DISTYLY, SELF-INCOMPATIBILITY, AND EVOLUTION IN *MELOCHIA*

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The genus *Melochia* of the subfamily Hermannieae, Sterculiaceae, includes about 60 species which are widely distributed through the tropics, and, less extensively, through the temperate zone. An encounter with a distylic species, *M. tomentosa* L., on the desert island of Mona, suggested this study of self- and cross-incompatibility. The occurrence of distyly in this genus evidently has been overlooked by specialists in the field (Crowe, 1964; Pandey, 1957; Brewbaker, 1957) and is not noted in floras of the Caribbean area. Heterostyly is considered to be an advanced system by Crowe (1964), because it occurs in 20 advanced families of the angiosperms, but not in primitive families. Its widely scattered occurrence suggests that it has arisen separately on a number of occasions.

One system of self-incompatibility has been described in the family Sterculiaceae. Knight and Rogers (1955) suggested that the system in *Theobroma cacao* L. is controlled by a series of incompatibility or *S* alleles, with or without dominance relations among them, and with control of phenotype of ovule and pollen expressed before meiosis. The site of the incompatibility reaction is the ovary. Incompatibility is due to the failure of fertilized ovules to develop (Knight and Rogers, 1955). Ovules are thus "sterilized" by fertilization, and cannot be stimulated by later compatible pollinations. The system is homomorphic, that is, not characterized by morphological variations of stigma, style, or pollen associated with the incompatibility phenotype. Cope (1958, 1962) found evidence that a portion (25, 50, or 100 per cent) of the ovules in the incompatibility pollinated ovary do not exhibit fusion of gametes, and that flowers absciss, even though gamete fusion occurs in other ovules as a result of compatible fertilization. Compatible matings are not characterized by such "non-fusion" ovules. These findings suggest a segregation of pollen types in some cases, and therefore a gametophytic or haploid determination of pollen phenotype. However, crossing reactions among parents and offspring indicate that sporophytic effects are also present.

Pandey (1960) and Cope (1962) have recently interpreted the physiological and genetic basis of incompatibility in the genus. The *Theobroma* system is considered unique in the plant kingdom (Crowe, 1964) although other systems, still not thoroughly investigated, resemble it (*Lilium*, *Freesia*, *Hemerocallis*, *Gasteria*, and *Narcissus*). Its characteristics are sufficiently dissimilar from other systems to suggest to Crowe (1964) the separate evolution of this system from a primitive, hypothetical system underlying incompatibility in flowering plants.

In addition to *Theobroma*, self-incompatibility has been found in the closely related genus *Herrania* (McKelvie, as reported by Fryxell, 1957). The details of the system have not been worked out. East (1940), in his survey of self-incompatibility in the flowering plants, did not report any other self-incompatible species in the family. Recently species of *Dombeya* have been found to be self-incompatible (Soderholm, personal communication, 1965). Because of the peculiarity of the *Theobroma* incompatibility system it is of great interest to encounter another system, distyly, in *Melochia*.

Materials and Methods

Four species of *Melochia* were observed in their native habitats on Puerto Rico and adjacent islands (Table 1). Counts were made of plants with long-style, short-sta-

TABLE 1. *Species of* Melochia *observed under field conditions, and some characteristics of the flowers.*

Species	Geographical site	Habitat	Pin : thrum ratio	Diameter of corolla (mm)	Note
M. nodiflora Sweet	Mayagüez, P.R.	Weedy fields	–	2–3	Not distylic
M. pyramidata L.	Guánica, P.R.	Roadside	2:3	5–6	
	Cabo Rojo, P.R.	Roadside	27:31	4–6	Uniform populations
	St. Croix, V. I.	Weedy fields	0:99	3–6	Thrum only
M. tomentosa L.	Mona Island, P.R.	Dry scrub	24:30	7–16	
	Guánica, P.R.	Dry scrub	5:4	9–12	
	St. Croix, V. I.	Grassy field	78:62	6–17	Weedy after clearing
	St. John, V. I.	Dry scrub	37:29	6–15	
M. villosa Fawc. & Rendle	Mayagüez, P.R.	Weedy fields	47:51	12–28	Prostrate in pastures

men (pin) and short-style, long-stamen (thrum) flowers. Measurements were made to assess variability. Pressed specimens of *Melochia* were observed in herbaria of the College of Agriculture, Mayagüez, Puerto Rico; Inter American University, San Germán, Puerto Rico; and Southern Methodist University, Dallas, Texas (Table 2). Living and pressed specimens of other species of the Sterculiaceae were observed when possible. Seeds of the *Melochia* species were collected, and plantings were established in Mayagüez. Pressed specimens were deposited in the herbarium, Southern Methodist University. Intraspecific crosses were made in the field or greenhouse at Mayagüez. When I became aware of the difficulty of isolating flowers in the field for controlled pollination, I rogued two widely separated plots of *M. villosa* to pin and thrum plants respectively, for additional pollinations. Flowering branches of the various species were brought to the laboratory for intra- and interspecific pollinations. Stigmas and styles from pollinated flowers were removed 8 hours after pollination, fixed in FAA (formalin, alcohol, acetic acid) and prepared for fluorescence microscopy (Martin, 1959). Pollen grains on the stigma and pollen tubes in the stigma, style, and ovary were counted (if less than 25) or estimated.

Pollen was germinated in a mineral solution recommended by Brewbaker (1957). Germination was checked with the microscope.

RESULTS

Habitat and Distribution

The four species of *Melochia* studied proved to be widely distributed through Puerto Rico and adjacent islands, but each occupied a somewhat different ecological niche. *Melochia tomentosa* is found almost solely in dry lands that receive 30 to 50 inches of rain a year, but that suffer from drought during a prolonged dry season. It is associated with a heavy chapparal, including species of cactus. Most of the terrain on which this species is found has not been cleared or farmed for many years, and plants have reached a height of 4 to 8 feet. On the other hand, in two instances the species was seen to have the ability to colonize disturbed habitats. On St. John, Virgin Islands, young plants were growing vigorously in a dirt road bulldozed three years previously. At Grapetree Bay, St. Croix, Virgin Islands, seedlings were established in a grass pasture developed after clearing away brush. In both habitats, plants grew best on well-drained slopes. All populations found were distylic with about an equal number of pin and thrum flowers.

The other three *Melochia* species thrived under wetter conditions. *Melochia villosa* Fawc. and Rendle was found chiefly in

TABLE 2. *Specimens of* Melochia *observed in herbaria.*

Species	Geographical source	Herbarium	Observations
M. anomala Griseb.	Córdoba, Argentina	Southern Methodist U.	Pin type
M. nodiflora	Mayagüez, P.R.	Inter American U.	Not distylic
	Rosario, P.R.	Inter American U.	Not distylic
	Vieques, P.R.	Inter American U.	Not distylic
	Rincón, P.R.	College of Agriculture	Not distylic
M. pyramidata	Guayanilla, P.R.	Inter American U.	Pin type
	La Parguera, P.R.	Inter American U.	Pin type
	Marisua, P.R.	Inter American U.	Pin type
	Rincón, P.R.	College of Agriculture	Pin and thrum
M. tomentosa	St. Croix, V. I.	Inter American U.	Pin type
	Magueyes Is., P.R.	Inter American U.	Pin type
	Vieques, P.R.	Inter American U.	Thrum type
	Yauco, P.R.	Inter American U.	Thrum type
	Guánica, P.R.	Inter American U.	Thrum type
	Mona Island, P.R.	Inter American U.	Pin type
	Tamaulipas, Mexico	Southern Methodist U.	Thrum type
M. villosa	Mayagüez, P.R.	College of Agriculture	Pin type

grass pastures with plenty of sun, in areas of about 80 inches of rain per year. This woody species has a prostrate habit, and spreads by rooting at the nodes. It quickly recovers from mowing, and sends up new branches with terminal inflorescences throughout the rainy season. It begins to die out when, in the normal course of plant succession, seedling trees begin to shade the pasture. I did not find this species under strictly wild conditions. All populations encountered were distylic. In contrast, *M. pryamidata* L., which is also weedy, woody, and prostrate, was more widespread, tolerated direct sun and shade, and was particularly abundant along roadsides, in overgrazed pasture, and in the stubble of sugar cane fields. In Puerto Rico, some populations were of pin and thrum types, whereas others were of thrum type flowers only. On St. Croix, Virgin Islands, three populations in distinct, well-separated habitats, consisted only of thrum-type plants.

The fourth species, *M. nodiflora* Sweet, was exceptionally weedy, and occurred chiefly in weedy fields after cultivation, but also occasionally along roadsides. It is annual with small, self-fertile, non-distylic flowers.

Observations of herbarium specimens (Table 2) extended my knowledge of the range of the species and confirmed the occurrence of distyly in the three species. In addition, flowers of *M. anomala* Griseb. from Córdoba, Argentina, were of the pin type, suggesting distyly in that species also. It appears likely that other species of the genus will prove distylic when carefully examined.

Facilities were not available for a detailed study of floral structure in other genera of the family. Nevertheless, the following species were inspected as fresh or dried specimens: *Ayenia insulicola* Cristobal, *Brachychiton populneus* R. Br., *Buettneria aculeata* Jacq., *Buettneria filipes* Mart., *Cola acuminata* (Beauv.) Schott. & Endl., *Commersonia fraseri* Gay, *Dombeya spectabilis*Bojer, *Firmiana simplex* (L.) W. F. Wight, *Fremontia napensis* Eastw., *Guazuma ulmifolia* Lam., *Helicteres jamaicensis* Jacq., *H. guazumefolia* H. B. K., *Hermannia texana* Gray, *Psysodium dubium* Hemsl. (*Melochia* sp. ?), *Pterospermum acerifolium* (L.) Willd., *Reevesia formo-*

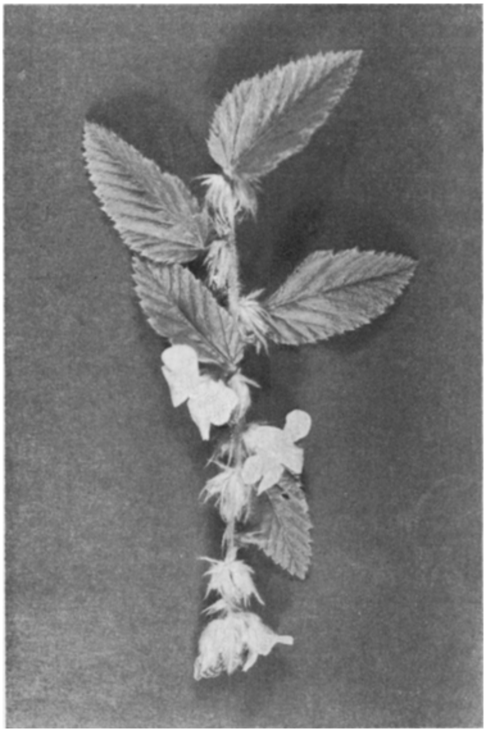

FIG. 1. Flowering branch of *Melochia villosa.*

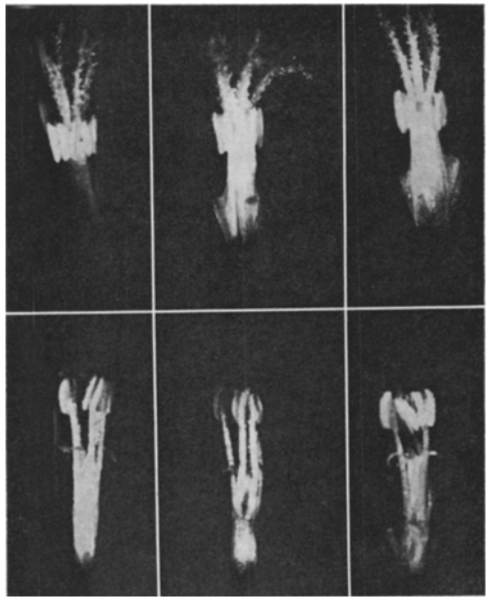

FIG. 2. A comparison of androgynoecia of *M. villosa.* Pin flowers above, and thrum flowers below.

sana Sprague, *Sterculia apetala* (Jacq.) Karst., *S. foetida* L., *Tarrietaria argyrodendron* Benth., *Theobroma cacao* L., and *Waltheria americana* L. In two of these species, *Hermannia texana* and *Psysodium dubium*, stamen and style lengths were sufficiently distinct to suggest distyly, but only pin flowers were seen of the first, and thrum flowers of the second species.

Floral Morphology

The four species of *Melochia* examined were quite similar in floral morphology: 5 separate sepals, 5 petals slightly united at base, 5 stamens attached to the corolla and in addition partially united at base into a tube surrounding the ovary, and pistil of 5 carpels, 5 locules, and 5 stigma lobes (Fig. 1). These characteristics are typical of the Sterculiaceae and are of an intermediate level of advancement. The species differed in flower size (Table 1). The relative length of the staminal tube varied among the species, but also from plant to plant within the species. In general, in pin type flowers, stamens were united almost to the anthers, whereas in thrum flowers, the column approached ⅓ the length of the stamens (Fig. 2). Longer tubes would have enclosed the branches of the short style. Sufficient measurements and observations were made to determine that all flowers of the three distylic species were of pin or thrum type, with very little morphological variation among them, and with no tendency towards monomorphism. Some attributes of *Melochia* are compared in terms of advancement with characteristics of the family in general in Table 3.

Intra- and Interspecific Crosses

Crosses in the field demonstrated that *M. tomentosa* is definitely self-incompatible (Table 4). Isolated plants in field and greenhouse did not set seed. In the case of *M. villosa*, all crosses set fruit during first trials. However, after isolating plants of each type by rogueing out all plants of the

TABLE 3. *A comparison of some characteristics of* Melochia *to primitive and advanced characteristics of the family Sterculiaceae.*

Primitive	Advanced	*Melochia*
Perennial	Annual	Mixed
Trees	Shrubs, herbs	Shrubs, herbs
Petals large	Petals absent or reduced	Petals large or small
Many stamens	Five stamens	Five stamens
Filaments free	Filaments united	Filaments partially united
Filaments simple	Filaments specialized	Filaments simple
Ovary of 5–10 carpels	Ovary of 2 carpels	Ovary of 5 carpels

opposite type from a given area, only pollinations between distinct flower types succeeded. Flowers of *M. pyramidata* set fruit regularly with or without hand pollination, after selfing or crossing, and in field or greenhouse, even under conditions of isolation. Thus the species is self-fertile. *Melochia nodiflora* plants were not hand pollinated, but isolated plants in field or greenhouse set seed readily.

Styles and pollen tubes were exceptionally well differentiated. Callose deposits in the stigma and upper parts of style were heavy, and fluoresced brightly under ultraviolet light. In favorable preparations of *M. pyramidata* and *M. tomentosa* it was possible to trace tubes from pollen grain to ovule. Birefringence of hairs covering the lower part of the style and the ovary obscured pollen tubes in *M. villosa.* Counts of pollen grains and tubes after intraspecific pollinations are given in Table 4. These observations confirm the self-fertility of *M. pyramidata*, and the self-incompatibility of *M. tomentosa* and *M. villosa.* In the latter two species, excellent germination and pollen tube growth occurred in crosses between pin and thrum flowers. In *M. tomentosa* a moderate amount of pollen germinates after incompatible pollination, and tubes grow into the stigma branches. However, growth is restricted, and tubes seldom enter the main body of the style. In *M. villosa* pollen fails to germinate, or occasional grains germinate but do not penetrate through the style.

Interspecific crosses among the three species were usually characterized by reduced pollen germination and reduced tube penetration of style and stigma when flowers of similar morphology were cross-pollinated. When opposite flower types were crossed, pollen germination and tube

TABLE 4. *Results of intraspecific crosses and stylar observations. Pollen and tube counts are averages of 5 or more observations.*

				Number of				
Species	Combination	Pollination	Capsules	Pollen grains on stigma	Pollen tubes in stigma	Pollen tubes in upper style	Pollen tubes in lower style	Pollen tubes in ovary
M. pyramidata	Pin × Pin	135	96	25+	25+	25+	25+	25+
	Pin × Thrum	15	14	25+	25+	25+	25+	25+
	Thrum × Pin	15	11	25+	25+	25+	25+	25+
	Thrum × Thrum	39	28	25+	25+	25+	25+	25+
M. tomentosa	Pin × Pin	168	0	12	9	0.2	0.0	0.0
	Pin × Thrum	37	28	25+	25+	25+	25+	25+
	Thrum × Pin	45	15	25+	25+	25+	25+	25+
	Thrum × Thrum	40	0	25+	25+	0.3	0.0	0.0
M. villosa	Pin × Pin	23	0	2	2	.8	*	*
	Pin × Thrum	45	28	25+	25+	25+	*	*
	Thrum × Pin	45	21	11	9	6+	*	*
	Thrum × Thrum	42	0	1	1	0.0	*	*

* Accurate counts of pollen tubes in lower style and ovary were not possible with this species.

TABLE 5. *Pollen germination and pollen tube growth in crosses of three distylic* Melochia *species.*

		Species as male					
		M. pyramidata		*M. villosa*		*M. tomentosa*	
		Pin	Thrum	Pin	Thrum	Pin	Thrum
M. pyramidata	Pin	–	–	1	3	2	2
	Thrum	–	–	3	2	2–3	2
M. villosa	Pin	2	2–3	–	–	2	3
	Thrum	3	2	–	–	2	1–2
M. tomentosa	Pin	2	3	2	2–3	–	–
	Thrum	2–3	2	2	0–1	–	–

Key: 1 = Strongly reduced germination.
2 = Reduced germination and/or pollen tube growth in stigma.
3 = Excellent germination and pollen tube growth in stigma and style.

growth usually increased. The reactions of *M. Pyramidata* flowers were similar to those of the two self-incompatible species (Table 5). Thus the incompatibility barriers among species were somewhat less severe than those within species.

Pollen of all species and both flower types germinated freely in Brewbaker's solution (Brewbaker and Kwack, 1963). In some exceptional trials which resulted in poor germination, the pollen of *M. villosa* was found to be infested by a fungus. Later observations in the field showed this disease to be of frequent occurrence, and the *in vivo* tests (Tables 4 and 5) may have been affected in some cases.

DISCUSSION

Two systems of self-incompatibility have been reported in Solanaceae (Pandey, 1957) and Rubiaceae (Crowe, 1964). In Solanaceae the incompatibility of the genus *Physalis* is based on two interacting loci, a condition that could have arisen by duplication of the *S* locus common to other genera of the family. In Rubiaceae, heterostyly is common, but in the genus *Galium* the incompatibility system, while controlled by a similar genetic system, is free of flower heteromorphy. The direction of change in this family is not clear, but from other evidence Crowe (1964) hypothesizes that evolution proceeds from homostyly to heterostyly. Sterculiaceae is the third family discovered to have two systems of self-incompatibility, and the differences between the two systems (distyly in *Melochia*; homostyly in *Theobroma*) are more difficult to reconcile. The *Melochia* system, with two distinct flower types between which crosses are possible, is probably a sporophytically controlled system, as are all other heterostylic systems. I am now working on the genetic control to clarify this point. The system in *Theobroma* is also sporophytically controlled but by a series of multiple alleles (Knight and Rogers, 1955). In addition, the site of incompatibility is different in the two genera. Whereas incompatibility in *Theobroma* occurs within the ovule after fertilization, incompatibility in *Melochia* is expressed both as failure of pollen germination and as restriction of pollen tube growth in stigma and style. More study is needed in the Sterculiaceae to elucidate the evolutionary mechanisms which have produced the two systems.

The four species of *Melochia* studied appear to be evolving toward annualism, weediness, self-fertility, and homostyly. It is not now clear whether *M. nodiflora* is a primitively self-fertile species, as would be suggested from its small flowers, or an end product of the postulated evolutionary sequence. However, *M. pyramidata* has clearly lost its system of incompatibility while retaining distyly as a relic. The uniform, thrum-type populations of this species on St. Croix suggest that the controlling *S* gene is homozygous, but do not suggest whether thrum is controlled by the recessive or dominant phase. Again, additional studies are necessary. *Melochia villosa* is clearly self-incompatible but inhabitats weedy places and may be thus exposed to strong selective pressures toward self-fertility. Finally, although self-incompatible *M. tomentosa* normally occurs in undisturbed areas, it also shows the ability to colonize new areas, particularly those disturbed by man. This evolutionary pattern appears to be typical of other genera (Lewis and Crowe, 1958).

The self-fertility of *M. pyramidata* has not been accompanied by development of unilateral barriers to interspecific hybridization, similar to those in other species (Lewis and Crowe, 1958). The mode of action of pin and thrum type flowers in interspecific crosses resembles that of the corresponding flowers of the two self-incompatible species. In view of the fact that two functions of the *S* allele have been retained, heterostyly and restriction of pollen tube growth in interspecific crosses of similar flower types, it is not certain that the *S* allele itself has been changed, and the possibility remains that self-fertility is due to changes in the background genotype of the species.

In addition to further studies with *Melochia*, an important area of study is the comparative morphology of flowers of other genera of the Sterculiaceae. My brief survey of materials available to me suggests that other cases of distyly in this family may have also been overlooked.

Summary

Four species of *Melochia* from Puerto Rico and adjacent islands were examined for distyly and for self-incompatibility. Two species (*M. tomentosa* and *M. villosa*) were distylic, self-incompatible, and cross-compatible between pin and thrum type flowers. *Melochia pyramidata* was distylic but self-fertile. *Melochia nodiflora* was homostylic and self-fertile. All species have certain weedy tendencies, which appear to be correlated with an evolutionary trend toward annualism, homostyly and self-fertility. Interspecific crosses revealed that *M. pyramidata* retains a relic of its *S* allele functions. The self- and cross-incompatibility reactions were due to inhibition of pollen germination and/or tube growth in the style. The finding of distyly in the family Sterculiaceae raises problems as to how such diverse systems as distyly and the peculiar sporophytic system of *Theobroma* could have evolved.

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Recent Literature

After completion of the research reported in this paper, the following article appeared which describes briefly the heterostyly of *Melochia* and *Waltheria*: Brizicky, G. K. 1966. The genera of Sterculiaceae in the Southeastern United States. J. Arnold Arboretum 47: 60–74.