

## Insights into the *Witheringia solanacea* (Solanaceae) Complex in Costa Rica. I. Breeding Systems and Crossing Studies<sup>1</sup>

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### ABSTRACT

The *Witheringia solanacea* complex consists of three species, *W. asterotricha*, *W. meiantha*, and *W. solanacea*, native to Central and South America. The three taxa are morphologically similar, and their distinctions and relationships have been the subject of taxonomic controversy. To investigate breeding systems and potential for hybridization among the taxa of the complex, two Costa Rican accessions per species were used in a crossing program. All plants were self-incompatible except for one accession of *W. solanacea*. Hybrid plants resulted from all crosses among accessions of *W. asterotricha* and *W. solanacea*. Most crosses were unsuccessful using *W. meiantha* in combination with either of the other two taxa. It is suggested that *W. meiantha* and *W. solanacea* be recognized as separate taxa, but that *W. asterotricha* be considered a synonym of *W. solanacea*.

### RESUMEN

El complejo *Witheringia solanacea* consiste de tres especies de Centro y Suramérica, *W. asterotricha*, *W. meiantha*, y *W. solanacea*. Las tres especies son morfológicamente parecidas entre sí y han sido fuente de muchas controversias taxonómicas. Para investigar el sistema reproductivo y el potencial para hibridación entre las especies del complejo, se utilizaron seis colecciones de Costa Rica (dos por especie) en un programa de cruzamiento en invernadero. Todas las plantas eran auto-incompatibles salvo una colección de *W. solanacea*. Se formaron híbridos en todas las combinaciones entre las colecciones de *W. asterotricha* y *W. solanacea*. La mayoría de intentos entre *W. meiantha* y las otras dos especies fallaron. Se sugiera un esquema taxonómico para el complejo que reconoce dos especies, *W. meiantha* y *W. solanacea*, con *W. asterotricha* como sinónimo de *W. solanacea*.

*Key words:* breeding systems; Costa Rica; crossing studies; self-incompatibility; Solanaceae; Witheringia.

THE GENUS *WITHERINGIA* L'HER. (SOLANACEAE) INCLUDES ca 20 species of Neotropical herbs, shrubs, and small trees. The center of diversity for the genus is Costa Rica, where over half the species occur. *Witheringia* has been placed in subfamily Solanoideae, tribe Solaneae, by virtue of its flattened seeds with curved embryos, abundant endosperm, basal filament insertion, and valvate corolla aestivation (Hunziker 1979, D'Arcy 1991). *Witheringia* has been allied with the genera *Acnistus* Schott, *Athenaea* Sendtn., *Aureliana* Sendtn., *Brachistus* Miers, *Capsicum* L., *Cuatresia* Hunz., *Dunalia* H.B.K., *Iochroma* Benth., *Saracha* Ruiz & Pav., and *Vasobia* Rusby in traditional classification schemes (Hunziker 1984, 1987) and often has been considered as being closely related to *Capsicum* because of similarities in flower and fruit characters. Recent molecular studies, however, indicate that *Wither-*

*ingia* may be more closely related to the physaloid genera [*Physalis* L., *Margaranthus* Schlecht., *Leucophysalis* Rydb., *Chamaesaracha* (A. Gray) Benth.] and that the genus may not be monophyletic as currently circumscribed (Olmstead *et al.* 2000; Bohs, pers. obs.).

The genus *Witheringia* is distributed from Mexico to Bolivia, with the majority of species occurring in Mexico, Costa Rica, and Panama. Hunziker's (1969) revision treated 15 taxa, including 3 species of section *Brachistus*. He pointed out that 3 species, *W. asterotricha* (Standl.) Hunz., *W. meiantha* (Donn. Sm.) Hunz., and *W. solanacea* L'Her., form a tightly related group termed the *W. solanacea* complex. All are herbs or shrubs with truncate calyces and a ring of hairs inside the corolla tube, and with corolla lobes much longer than the tube. These species have very short (<5 mm) peduncles or lack them altogether, so that the flowers are arranged in fascicles in branch forks or opposite the paired leaves. *Witheringia solanacea* has the broadest geographical distribution, ranging from southern Mexico through Central America

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and the Antilles to South America. It is an extremely variable species, but is distinguished by its pubescence of unbranched or sparsely branched hairs and often tetramerous flowers. Hunziker (1969) listed one variety, *W. solanacea* var. *silvigaudens* (Standl. & Williams) Hunz., endemic to Dept. Morazán, Honduras. This variety is glabrous, has pentamerous corollas, and longer, narrower leaves than typical *W. solanacea*. *Witheringia asterotricha* is closely related to *W. solanacea*, and according to Hunziker (1969), probably should be considered a variety of the latter species. *Witheringia asterotricha*, thus far known only from Costa Rica and Panama, is morphologically distinguishable from *W. solanacea* by its dense pubescence of dendritically branched hairs and pentamerous flowers. *Witheringia meiantha* ranges from southern Mexico to western Panama. Plants of *W. meiantha* are glabrous, with small pentamerous flowers and fewer-flowered inflorescences than *W. solanacea* or *W. asterotricha*.

Unlike Hunziker (1969), D'Arcy (1973) recognized only two species in the complex. He maintained *W. asterotricha* as distinct, but indicated that hybrids with *W. solanacea* may occur when the two species come into contact. D'Arcy (1973) considered *W. meiantha* a synonym of *W. solanacea*, commenting that the reduced pubescence and slightly larger calyces characterizing *W. meiantha* were likely minor morphological variants of the widespread *W. solanacea*.

Nee (1986), treating only the taxa from Veracruz, Mexico, recognized *W. meiantha* as distinct from *W. solanacea* and considered *W. solanacea* var. *silvigaudens* a synonym of *W. meiantha*. According to Nee (1986), *W. meiantha* can be distinguished from *W. solanacea* by its larger calyx and seeds, pentamerous flowers, glabrous leaves, and few-flowered inflorescences.

To gain further insight into the relationships of these taxa, I carried out a crossing study in the greenhouse to determine if genetic isolating mechanisms were operating among members of the *W. solanacea* complex. It was also of interest to examine breeding systems of the three taxa, as no information of this kind is available for *Witheringia*, and the distribution of self-incompatibility (SI) among genera of the Solaneae is not well known. Greenhouse plants were grown from seed collections (accessions) of three sites in Costa Rica. Costa Rica, and perhaps Panama, are the most likely areas of sympatry or near sympatry for the three species, and therefore represent localities in which repro-

ductive interaction among the taxa is at least theoretically possible.

In addition to the crossing results, a brief key to the three entities is included at the conclusion of the paper. A complete systematic treatment of the genus *Witheringia* is being prepared by M. Sousa-Peña of the University of Connecticut.

## MORPHOLOGY

The three taxa of the complex are all erect herbs, weakly woody shrubs, or small trees reaching several meters in height. The leaves are entire, elliptic, unequal in size, and paired at the nodes (Fig. 1A). All three taxa lack obvious peduncles, and as a result, flowers are clustered in axillary fascicles (Fig. 1). The corollas are stellate and light yellowish to cream in color, with those of *W. meiantha* smaller and deeper yellow than those of *W. asterotricha* and *W. solanacea*. The latter two taxa often have greenish maculations in the corolla throat, and the corolla lobes are longer and less reflexed than in *W. meiantha*. The tapered anthers are connivent around the style, and begin to dehisce by large terminal pores that eventually open into longitudinal slits (Fig. 1B). After pollination, the corolla and attached stamens wither and fall, leaving only the calyx and gynoecium. As fruits develop, the pendulous flowering pedicels curve upward until the ripe fruits are held erect (Fig. 1A, C, D). At maturity, all three taxa have red, shiny, juicy, many-seeded fruits. *Witheringia meiantha* has glabrous fruits, those of *W. solanacea* are glabrous to very sparsely pubescent, and those of *W. asterotricha* are moderately to rather densely pubescent. Fruit color changes from green to orange-red very quickly, often in a single day. These attributes are characteristic of dispersal by birds, or ornithochory (van der Pijl 1982). Bird dispersal of *Witheringia* fruits has been documented at La Selva and Monteverde, Costa Rica, by Wheelwright *et al.* (1984), Murray (1987, 1988), Loiselle and Blake (1990), Blake and Loiselle (1992), and Murray *et al.* (1994).

## STUDY SITES AND HABITATS

One accession of *W. asterotricha* and both accessions of *W. meiantha* used in the crossing study were collected at La Selva Biological Station near the confluence of the Sarapiquí and Puerto Viejo Rivers in Heredia Province (Table 1). Elevations at the station range from ca 35 to 140 m. La Selva has an annual mean temperature of 26°C and an annual mean rainfall of ca 4000 mm. Although

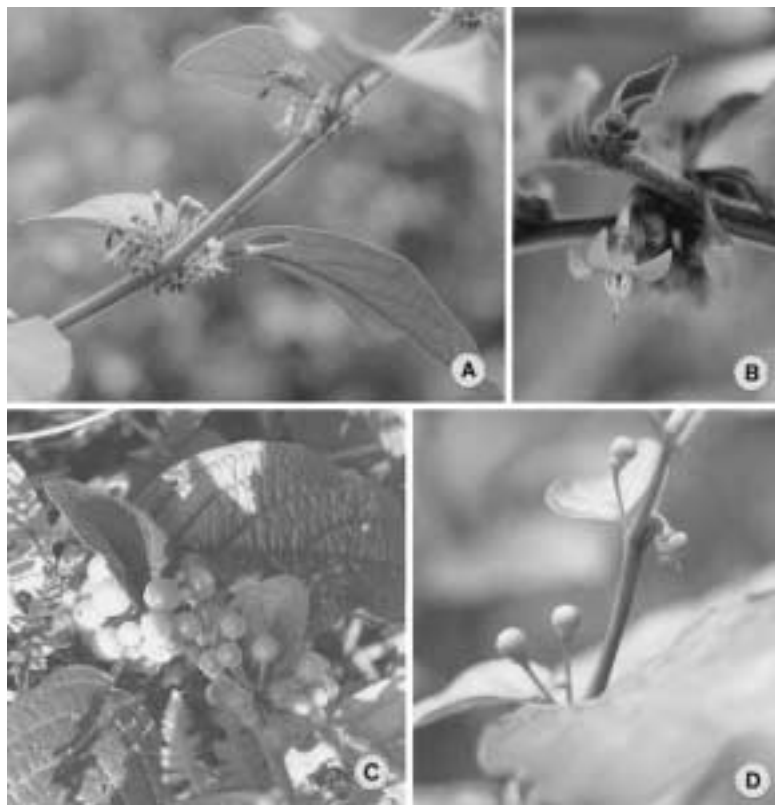


FIGURE 1. A. *Witheringia asterotricha* branch and inflorescence with flowers and young fruits. B. Flower of *W. solanacea*. C. Fruits of *W. asterotricha*. D. Flowers and fruits of *W. meiantha*.

rain falls at La Selva in all months of the year, there is a relatively wet season from late April to the beginning of August, with a smaller wet period from November through January. Detailed descriptions of the site can be found in McDade *et al.* (1994). *Witheringia asterotricha* and *W. meiantha* are fairly common on the station property. *Witheringia asterotricha* is found in open, sunny areas of secondary vegetation. *Witheringia meiantha* grows in more shady habitats in light gaps of the forest canopy, such as the margins of treefall gaps, trails, and streams. The two species were not observed growing together at La Selva.

One accession of *W. asterotricha* used in this study was collected near Cariari, ca 32 km SW of La Selva (Table 1). The climate in this area is similar to that of La Selva (J. Denslow, pers. comm.). *Witheringia asterotricha* was found in full sun by the roadside. No other *Witheringia* species were observed in the immediate vicinity.

Another field site was located above La Selva on the road between Puerto Viejo and Vara Blanca

(Route 9) near the Catarata de La Paz at an elevation of ca 1350 to 1400 m. Climatic data are not available for the site, but the average temperature is lower than at La Selva, and the annual rainfall is probably greater. One accession of *W. solanacea* was collected here at a disturbed site by the roadside in full sun. Other *Witheringia* species found in the immediate vicinity included *W. meiantha*, *W. cuneata* (Standl.) Hunz., *W. maculata* (Standl. & Morton) Hunz., and *W. fuscoviolacea* (Cufod.) Hunz.

The Las Cruces Biological Station is located near San Vito in the Coto Brus region of Puntarenas Province in southeastern Costa Rica. Elevations range from ca 1100 to 1500 m. Las Cruces is more seasonal than La Selva, with the dry season extending from January to March, when there is little or no rain. Annual rainfall averages ca 4000 mm, and the average daytime temperature is between 21 and 26°C (Organization for Tropical Studies, pers. comm.). One accession of *W. solanacea* was collected at Las Cruces from disturbed areas in full sun along the access road and trails.

TABLE 1. Sources of *Witheringia* plants used in this study. Seeds were collected from a single plant. Number of plants used in crosses is listed under each accession.

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<i>Witheringia asterotricha</i> . Bohs 2377. Costa Rica: Prov. Limón, Portico logging project near Cariari, ca 10°20'N, 83°45'W, ca 50 m elev. Seed accession no. 91-13; 5 plants.
<i>Witheringia asterotricha</i> . Bohs 3007. Costa Rica: Prov. Heredia, La Selva Biological Station, 10°26'N, 83°59'W, Sendero La Chanchera (SCH) near junction with Camino Experimental Norte (CEN) (approx. grid coordinates 600 × 100), ca 50–60 m elev. Seed accession no. 90-3; 3 plants.
<i>Witheringia meiantha</i> . Bohs 3015. Costa Rica: Prov. Heredia, La Selva Biological Station, 10°26'N, 83°59'W, Sendero Oriental (SOR) just past waterfall (approx. grid coordinates 600 × 1300), ca 40–60 m elev. Seed accession no. 90-7; 5 plants.
<i>Witheringia meiantha</i> . Bohs 2387. Costa Rica: Prov. Heredia, La Selva Biological Station, 10°26'N, 83°59'W, Sendero Surá (SUR) at entrance to arboretum (approx. grid coordinates 800 × 600), ca 40–60 m elev. Seed accession no. 91-22; 2 plants.
<i>Witheringia solanacea</i> . Bohs 2416. Costa Rica: Prov. Alajuela, Rio La Paz Pequeña, ca 500 m N of Catarata de La Paz next to road between Puerto Viejo and Vara Blanca, ca 10°13'N, 84°10'W, ca 1350–1400 m elev. Seed accession no. 92-8; 10 plants.
<i>Witheringia solanacea</i> . Bohs 2427. Costa Rica: Prov. Puntarenas, vicinity of Las Cruces Biological Station near San Vito, margin of road between station and Agua Buena, ca 8°50'N, 83°05'W, ca 1100 m elev. Seed accession no. 92-13; 10 plants.

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*Witheringia meiantha* also was found at Las Cruces, where it was uncommon in light gaps of the primary forest.

## MATERIALS AND METHODS

Seeds were gathered from ripe fruits at the study sites described above and plants were grown in pollinator-free greenhouses at the University of Utah. Sources of plants and voucher information are given in Table 1.

For the artificial crosses, pollen was tapped onto a clean glass slide and rubbed on the stigma of the maternal parent. Plants suspected or determined to be self-compatible were emasculated in the bud before pollination. Numbers of crosses performed, number of fruits set, and number of seeds per fruit are listed in Table 2. "Sib crosses" refer to crosses involving individuals from a single seed accession, whereas "outcrosses" refer to crosses between individuals from different seed accessions. In crosses yielding fruits, the fruit size, color, and seed number and appearance were recorded. Seeds subsequently were planted in the greenhouse to determine their viability and to assess the vitality of the F<sub>1</sub> plants.

Pollen viability was determined by shaking grains onto a clean glass slide and staining in aniline blue-lactophenol (Hauser & Morrison 1964). Pollen grains were allowed to stain for at least one hour before scoring. The first 300 grains encountered were scored, and unshriveled grains staining blue in the preparation were presumed to be viable. Pollen stainability was measured in all parent

plants, but only a subset of the F<sub>1</sub> plants reached flowering maturity.

Pollen tube growth was observed using the technique of Martin (1959) with the following modifications. Flowers were harvested and fixed 48–72 hours after pollination. Gynoecia were then rinsed and cleared in 0.8 N NaOH at 60°C for 1 to 2 hours, rinsed briefly, and stained. At least three flowers were examined per crossing combination.

Chromosome observations were made from fresh flower buds gathered from greenhouse-grown plants. Anthers were macerated in a 1.0 percent acetocarmine solution or in LP orcein (W. Bloom, pers. comm.), a cover slip was added, and the preparation was squashed. Herbarium vouchers were deposited at the National Museum in San José, Costa Rica, and at the Garrett Herbarium at the University of Utah, Salt Lake City. Type specimens of *Acnistus lehmanni* Damm., *Capsicum asterotrichum* Standl., *C. costaricense* Standl. & Morton, *C. isothrix* Standl., *C. multiflorum* Standl. & Morton, *C. silvigaudens* Standl. & Williams, *C. stenophyllum* Morton & Standl., *C. tetramerum* Standl. & Morton, *Sicklera tetrandra* A. Braun & Bouché, and *Solanum panamense* van Heurck & Muell.-Arg. were examined to ensure that my taxonomic concepts were congruent with those of earlier authors. These names and types include a majority of the nomenclatural and taxonomic synonyms that have been used for the taxa of the *W. solanacea* complex.

## RESULTS

CHROMOSOME NUMBERS.—Meiotic squashes from accessions of all three taxa (*W. asterotricha*: Bohs

TABLE 2. *Intra- and intertaxon crosses in Witheringia. Taxon abbreviations follow Figures 2 and 3. Intertaxon crosses are outcrosses or sib crosses unless noted otherwise. All flowers of sol 2416 were emasculated in the bud before pollination. Number of seeds/fruit was based on visual examination; only full-sized seeds were counted. For seed viability, see Figure 2 and text.*

Female parent		Selfed	Male parent					sol 2416
			ast 3007	ast 2377	mei 3015	mei 2387	sol 2427	
ast 3007	No. pollinations	20	18	19	20	20	25	22
	No. fruits	0	16	18	0	9	23	18
	No. seeds/fruit <sup>a</sup>	0	32–69 (55)	14–28 (24)	0	8–24 (14)	10–29 (19)	13–54 (36)
ast 2377	No. pollinations	30	18	37	28	24	19	21
	No. fruits	0	17	11	0	3	6	14
	No. seeds/fruit	0	30–58 (41)	19–77 (54)	0	45–48 (46)	29–63 (46)	6–64 (38)
mei 3015	No. pollinations	26	22	50	30	22	25	26
	No. fruits	0	0	0	12	14	0	0
	No. seeds/fruit	0	0	0	2–47 (16)	2–36 (22)	0	0
mei 2387	No. pollinations	23	29	21	22	23	24	20
	No. fruits	0	0	0	11	12	0	0
	No. seeds/fruit	0	0	0	10–34 (21)	16–32 (24)	0	0
sol 2427	No. pollinations	71	30	25	34	24	21	24
	No. fruits	0	17	14	11	2	11	14
	No. seeds/fruit	0	6–93 (50)	7–78 (37)	5–59 (29)	40–51 (46)	16–87 (50)	32–104 (75)
sol 2416	No. pollinations	31	23	18	23	19	20	15
	No. fruits	6	19	5	3	2	16	2
	No. seeds/fruit	33–75 (59)	30–76 (56)	7–85 (39)	7–37 (22)	19 (19)	12–86 (56)	18–77 (48)

<sup>a</sup> Range ( $\bar{x}$ ).

TABLE 3. *Pollen stainability of Witheringia parents and hybrids. Abbreviations for accessions follow Figures 2 and 3.*

	No. flowers	No. plants	Mean percent (SD)	Range (%)
<b>Parents</b>				
ast 3007	7	4	90.7 (6.8)	83-99
ast 2377	6	3	93.9 (2.4)	90-97
mei 3015	25	5	80.9 (13.7)	27-94
mei 2387	10	2	94.3 (7.3)	77-99
sol 2427	9	8	84.3 (12.6)	54-97
sol 2416	6	3	83.7 (12.8)	68-99
Overall	63	25	86.1 (12.2)	27-99
<b>Hybrids</b>				
ast 3007 × sol 2427	10	5	57.3 (5.2)	50-66
ast 2377 × sol 2427	4	4	71.6 (5.9)	65-79
sol 2427 × ast 3007	5	5	57.9 (10.5)	42-66
sol 2427 × ast 2377	5	4	67.0 (6.0)	58-75
sol 2427 × mei 2387	5	1	62.6 (2.8)	60-66
Overall	29	19	61.9 (8.0)	42-79

3007; *W. meiantha*: Bohs 3015; *W. solanacea*: Bohs 2427) had  $N = 12$  chromosomes. Chromosomes at meiotic metaphase ranged from *ca* 1.5 to 3.5  $\mu\text{m}$  long.

**POLLEN FERTILITY.**—Results of the pollen stainability tests are given in Table 3. In general, all parent plants had high percentages of stainable pollen throughout the study. Mean pollen stainability in the hybrids was consistently lower than in the parental accessions (Table 3).

**BREEDING SYSTEM.**—Both accessions of *W. asterotricha* and *W. meiantha* were SI, as judged by the results of artificial pollinations and by pollen tube growth observations (Table 2; Figs. 2 and 3). No fruits were set by any plant upon self-pollination (Table 2; Fig. 2), although fruits and seeds frequently resulted from sib crosses or outcrosses. Several *W. asterotricha* and *W. solanacea* plants spontaneously developed small fruits that occasionally contained a few seeds. Numerous pollen tubes grew into the ovary and around the ovules in sib crosses and outcrosses in *W. asterotricha* and *W. meiantha*, but all tubes were inhibited in the style in self-

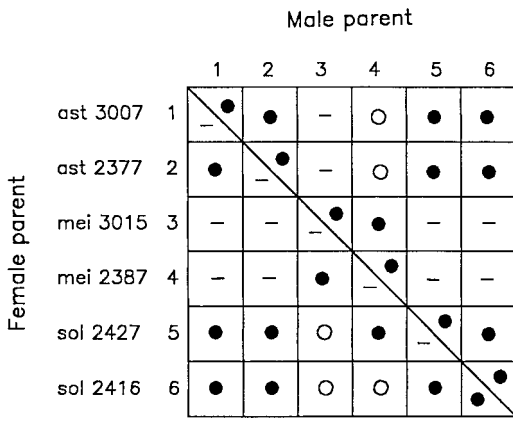


FIGURE 2. Results of crossing study. Taxon abbreviations correspond to accessions listed in Tables 1 and 2; numbers 1 through 6 refer to same taxa in rows and columns. Minus sign = no fruits produced; open circle = full-sized fruits, but seeds not viable; filled circle = full-sized fruits with viable seeds that subsequently produced  $F_1$  plants. Intraspecific pollinations fall along diagonal line; space above line = sib crosses or outcrossed, space below line = selfed.

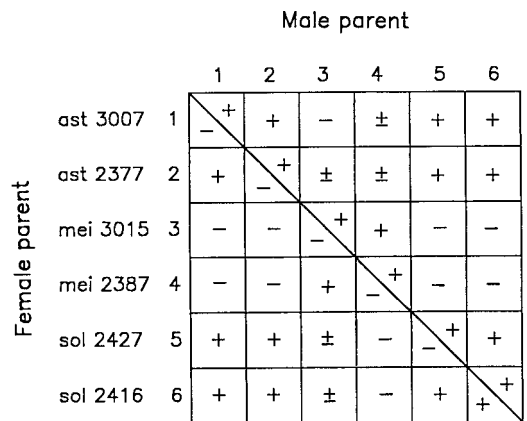


FIGURE 3. Results of pollen tube observations. Taxon abbreviations as in Figure 2. Plus sign = most pollen tubes reach ovary and ovules; plus/minus sign = a few pollen tubes occasionally seen in ovary; minus sign = all pollen tube growth arrested in style. Intraspecific pollinations are selfs. See Figure 2 for explanation of diagonal.

pollinations (Fig. 3). The site of inhibition of pollen tube growth in selfed pollinations was in the upper style, consistent with the system of gametophytic SI found in many other Solanaceae.

All plants of accession 2427 of *W. solanacea* appeared to be SI. In contrast, several individuals of accession 2416 set fruits and seeds after self-pollination. Pollen tube observations confirmed that self pollen was able to germinate and grow down the styles into the ovaries in these plants. Other individuals of accession 2416 did not exhibit this response, and instead behaved like SI plants.

**INTERSPECIFIC CROSSES.**—Most crosses between *W. meiantha* and the other two *Witheringia* species were unsuccessful. All crosses failed when either accession of *W. meiantha* was used as the female parent in combination with *W. asterotricha* or *W. solanacea* pollen (Fig. 2). In these crosses, large numbers of pollen grains germinated to produce pollen tubes, but all pollen tube growth was inhibited in the style (Fig. 3). Occasional full-sized fruits resulted from pollinations of *W. asterotricha* and *W. solanacea* with *W. meiantha* pollen, but the seeds from these fruits were smaller than usual and failed to germinate. F<sub>1</sub> hybrid plants were produced in only one interspecific crossing combination using *W. meiantha* (sol 2427 X mei 2387), and this result was obtained with only one individual from each accession. Hybrid plants were sparsely to moderately pubescent with unbranched hairs. One plant flowered, and had 5-merous (rarely 4-merous) corollas and the uniform corolla coloration of the *W. meiantha* parent. Pollen stainability was somewhat lower in the hybrid than in the parent plants (Table 3). Pollen tube growth in crosses using *W. meiantha* pollen with *W. asterotricha* or *W. solanacea* was variable, with most pollen tubes inhibited in the style; occasionally, however, a few grew to the ovules (Fig. 3).

In contrast, crosses between all accessions of *W. asterotricha* and *W. solanacea* were successful in both directions (Fig. 2). The resulting hybrid plants grew vigorously and flowered profusely. A subset of the hybrid plants was examined for pollen fertility, and the hybrids on average had lower pollen stainability than the parental accessions (Table 3). Nearly all of the F<sub>1</sub> hybrid plants had both unbranched and branched pubescence, and most had both 4-merous and 5-merous flowers on the same plant. Although I have only done limited studies of herbarium material, specimens have been seen that conform in morphology to the synthesized F<sub>1</sub>s (e.g., Donnell Smith 6672, the type of *C.*

*costaricense* Standl. & Morton), pointing to the likelihood of natural hybridization between *W. asterotricha* and *W. solanacea*.

## DISCUSSION

**BREEDING SYSTEM.**—All three *Witheringia* species were found to be SI, thus increasing to nine the number of solanaceous genera for which SI has been reported (the others are *Brugmansia*, *Brunfelsia*, *Capsicum*, *Lycium*, *Nicotiana*, *Petunia*, *Physalis*, and *Solanum* [including *Cyphomandra* and *Lycopersicon*]) (Fryxell 1957; Heiser & Smith 1958; Lockwood 1973a, b; Plowman 1973; Whalen & Anderson 1981; Charlesworth 1985; Bohs 1991; Preißel & Preißel 1991). *Brunfelsia*, *Nicotiana*, and *Petunia* belong to subfamily Cestroioideae, which occupies a basal position in the family according to phylogenetic analyses of molecular data (Olmstead & Palmer 1992, Olmstead *et al.* 2000). *Brugmansia*, *Capsicum*, *Lycium*, *Physalis*, *Solanum*, and *Witheringia* belong to the more derived subfamily Solanoideae. More data on the distribution of breeding systems in Solanaceae are needed to determine whether SI is the ancestral condition within the family, or whether SI evolved from self-compatible (SC) ancestors (*cf.* Weller *et al.* 1995). Similarly, better knowledge of breeding systems and phylogenetic relationships is needed to ascertain the basal condition in subfamily Solanoideae and tribe Solaneae to which *Witheringia* belongs. Furthermore, given the possibility that *Witheringia* itself may not be monophyletic, phylogenetic relationships and breeding systems of *Witheringia* and its putative relatives should be examined in detail to determine the evolutionary directions and constraints on the development of SI in this clade.

Plants of *W. solanacea* 2416 showed polymorphism in breeding system, with some individuals exhibiting SI and others SC. Changes in breeding system, usually from SI to SC, have been reported for other solanaceous plants (Nettancourt 1977), and have occurred many times over the course of angiosperm evolution (e.g., Jain 1976, Weller *et al.* 1995). *W. solanacea* 2416 may represent the early stages of SI breakdown. Investigations of additional populations throughout its range are needed to ascertain the predominant breeding system in this taxon.

From an ecological perspective, SI is common in Neotropical trees from lowland rain forest (Bawa 1979, Bawa, Perry, & Beach 1985), semi-deciduous forest (Bawa 1974), and deciduous forest (Ruiz Zapata & Arroyo 1978, Bullock 1985). At La Selva, Kress and Beach (1994) reported that 88 per-

cent of the hermaphrodite tree species that have been studied are SI. Fewer studies are available on breeding systems of tropical herbs and shrubs, but SC appears to be more common than in tropical trees (Schemske 1981, Whalen & Anderson 1981, Kress 1983, McDade 1985, Renner 1989). Kress and Beach (1994) emphasized the disparity between predominant breeding systems in understory versus canopy and subcanopy taxa at La Selva, with 34 percent SI species in the understory and 84 percent SI species in the upper forest strata. They cited this pattern as evidence supporting the vertical stratification of breeding systems in a fashion similar to the vertical stratification of pollination mechanisms described by Bawa, Bullock *et al.* (1985). The abundance of SI species in the upper forest strata as compared to the understory may be a real pattern, or it may prove to be a sampling artifact. Most of the SI understory species at La Selva (10 out of 13) belong to a single family, the Rubiaceae (Bawa & Beach 1983, Kress & Beach 1994). The proportion of SI understory species at La Selva may change radically when more taxa are investigated, especially in families in which SI is known to be common (*e.g.*, Solanaceae, Rubiaceae, Commelinaceae, Poaceae, Asteraceae, Melastomataceae; Fryxell 1957, Charlesworth 1985, Renner 1989). Two solanaceous species now may be added to the list of SI understory taxa at La Selva, raising the total percentage to 37.5.

INTERTAXON CROSSES.—Genetic isolation appears to be nearly complete between *W. meiantha* and both *W. asterotricha* and *W. solanacea*. Failure of crosses between these taxa cannot be explained by differences in chromosome number or by low pollen fertility of the parents. Crossing barriers take the form of pollen tube growth inhibition, early abortion of hybrid seed, and nonviable seed formation.

In contrast, hybrids were produced easily between *W. asterotricha* and *W. solanacea*. Although pollen stainability of the hybrid plants was somewhat lower than that of the parents, the F<sub>1</sub> plants were vegetatively vigorous and flowered profusely. There is some evidence from limited herbarium and field studies that natural hybridization takes place when pollen exchange between the two taxa can occur. These two taxa are examples of semi-species (*sensu* Grant 1963, Mayr 1963, Grant 1981), which are morphologically or geographically differentiated entities that retain the ability to exchange genes. These may represent species in the early stages of genetic isolation and differentiation,

or they may result from distinct allopatric entities that have come back into contact secondarily.

The studies reported here involved a small number of collections of the *W. solanacea* complex from a limited part of its range. Whether or not these results can be extrapolated to the *W. solanacea* complex over its entire distribution is unclear, but future taxonomic conclusions should take the following points into account: (1) all three entities are morphologically distinguishable, although the differences between *W. asterotricha* and *W. solanacea* are slight and may become blurred in areas of possible hybridization; (2) all accessions of *W. asterotricha* and *W. solanacea* tested were completely interfertile in greenhouse crosses; and (3) *W. meiantha* was genetically isolated from the other two taxa. Perhaps the most rational scheme for dealing with these issues is to recognize *W. solanacea* and *W. meiantha* as distinct taxa, and to subsume *W. asterotricha* within *W. solanacea*. Such taxonomic decisions must await a complete systematic study of the complex throughout its range.

For convenience, a key to the three taxa of the complex is included below. This includes observations of the plants used in the study as well as herbarium specimens from Duke University and Costa Rica, but is based on Costa Rican material only.

Key to the *Witheringia solanacea* complex  
in Costa Rica

- A. Plants glabrous; flowers in fascicles of 10 (–15) or fewer; plants of forest light gaps . . . . . *W. meiantha*
- A. Plants pubescent; flowers in fascicles of generally more than 10; pioneer plants of disturbed areas and secondary vegetation . . . . . B
- B. Pubescence of dendritically branched hairs; flowers pentamerous; fruits pubescent . . . . . *W. asterotricha*
- B. Pubescence of unbranched or rarely forked hairs; flowers often tetramerous; fruits glabrous or very sparsely pubescent . . . . . *W. solanacea*

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