

## Insights into the *Witheringia solanacea* (Solanaceae) Complex in Costa Rica. II. Insect Visitors and Pollination Biology of *W. asterotricha* and *W. meiantha*<sup>1</sup>

Lynn Bohs<sup>2</sup>

Department of Botany, Duke University, Durham, North Carolina 27708, U.S.A.

### ABSTRACT

Isolating mechanisms are important in maintaining the taxonomic integrity of closely related sympatric taxa. A previous study found strong post-zygotic isolating barriers between two species, *Witheringia asterotricha* and *W. meiantha*, of the *W. solanacea* (Solanaceae) species complex in Costa Rica. This study examines the presence of pre-zygotic barriers between the two species at La Selva Biological Station in Costa Rica. Both species offer pollen and nectar as floral rewards and are visited primarily by solitary or semi-social bees, some of which sonicate (“buzz”) the anthers to discharge pollen. No evidence was found for phenological differences in flowering time between *W. asterotricha* and *W. meiantha*, but pre-zygotic factors, such as ethological isolation and possibly fine-scale ecological or geographic barriers, may be responsible for restricting gene flow between the two species.

### RESUMEN

Los mecanismos de aislamiento son importantes para mantener la integridad taxonómica de especies estrechamente relacionadas que ocurren simpátricamente. Un estudio anterior halló fuertes barreras reproductivas poscigóticas entre dos especies, *Witheringia asterotricha* y *W. meiantha*, del complejo *W. solanacea* (Solanaceae) en Costa Rica. Este trabajo examina la presencia de barreras precigóticas entre las dos especies en la Estación Biológica La Selva, Costa Rica. Las flores de ambas especies ofrecen polen y néctar como recompensas florales, y son visitadas principalmente por abejas solitarias y semi-sociales, algunas de ellas “zumban” las anteras para descargar el polen. No se halló evidencia de barreras de aislamiento fenológicas entre *W. asterotricha* y *W. meiantha* en La Selva, pero factores precigóticos como barreras etológicas y posiblemente un aislamiento ecológico o geográfico de escala pequeña son responsables de restringir el flujo genético entre las dos especies.

*Key words:* bees; Costa Rica; La Selva; pollination; Solanaceae; *Witheringia*.

ISOLATING MECHANISMS LIMITING GENE FLOW between closely related taxa are important in taxonomic diversification and the maintenance of species identity (Dobzhansky 1937, Stebbins 1950, Mayr 1963, Levin 1978, Grant 1981). Isolating mechanisms can be classified as pre-zygotic or post-zygotic, and include spatial, temporal, ethological, and mechanical factors, as well as genetic incompatibility and hybrid inviability, sterility, and breakdown (Levin 1978, Avise 1994). Although more than one type of isolating mechanism may be operating in a given system, there are many examples in plants of one predominant factor leading to genetic isolation between sympatric taxa. Closely related sympatric taxa lack obvious geographic barriers to inter-taxon gene

flow, and are interesting systems in which elucidation of isolating mechanisms can lead to insights into processes of evolutionary differentiation. Knowledge of actual or potential gene exchange among morphologically differentiated population systems also can inform taxonomic decisions (Anderson 1995).

The *Witheringia solanacea* complex (Solanaceae) affords an opportunity to examine reproductive isolating barriers in a group of closely related sympatric taxa. The complex consists of three morphologically similar species [*W. asterotricha* (Standl.) Hunz., *W. meiantha* (Donn. Sm.) Hunz., and *W. solanacea* L'Her.] with ranges that overlap in Costa Rica. The group has been “taxonomically difficult” due to real or perceived complexities in morphological variation that have led to varying opinions of species delimitation (for a review of previous taxonomic concepts in the complex, see Bohs 2000). The morphology and distribution of the taxa in the complex are described in Bohs (2000).

<sup>1</sup> Received 15 January 1998; revision accepted 9 November 1998.

<sup>2</sup> Current address: Department of Biology, University of Utah, Salt Lake City, Utah 84112, U.S.A.; e-mail: bohs@biology.utah.edu.

TABLE 1. Study sites at La Selva Biological Station. Numbers in parentheses correspond to approximate grid coordinates on La Selva map (January 1996 version).

---

Site 1.	Sendero El Atajo (SAT) 550, secondary vegetation on sides of trail in full sun (900 × 000).
Site 2.	Lab clearing behind air-conditioned lab (600 × 800).
Site 3.	Successional plots, margin of four- to five-year-old plot (1000 × 1800).
Site 4.	On Sendero Occidental (SOC) in "Discovery Woods," margin of large treefall gap (300 × 300).
Site 5.	Sendero Occidental (SOC) 250–300, margin of trail near forest gap (400 × 700).
Site 6.	Sendero Las Vegas (SLV) 0 (junction of SLV and SOC), border of agricultural plot (200 × 500).
Site 7.	Next to trail between lab clearing and river station just over bridge (650 × 950).
Site 8.	Sendero Surá (SUR) at entrance to arboretum, edge of light gap (800 × 600).

---

Previous authors (Hunziker 1969, D'Arcy 1973) have hypothesized that inter-taxon hybridization contributed to the taxonomic difficulty of the complex by blurring morphological distinctions. A previous study used artificial hybridizations to examine post-mating isolating mechanisms among Costa Rican accessions of the three taxa (Bohs 2000). Results indicated that crosses nearly always failed between accessions of *W. meiantha* and the other two taxa, but that accessions of *W. asterotricha* and *W. solanacea* could cross freely and produce viable and vigorous F<sub>1</sub> hybrids. This study also found that *W. asterotricha* and *W. meiantha* are self-incompatible (SI), and therefore depend upon intergenetic pollen movement for successful fruit and seed set.

Concurrently, a field study was initiated to examine pre-mating isolating barriers among the taxa in the complex. Possible pre-mating barriers to gene flow include temporal differences in flowering phenology and ethological isolation via specialized pollinators. Pre-mating reproductive barriers may be superimposed on a background of post-mating factors, potentially reinforcing isolation or rendering it more efficient by eliminating gamete wastage. In addition, the pollination biology of *Witheringia* was completely unknown; thus it was of interest to document flower visitors to the *W. solanacea* complex. For logistical reasons, this work was done at the La Selva Biological Station, Prov. Heredia, Costa Rica, where two of the three species of the complex, *W. asterotricha* and *W. meiantha*, are native. Thus far, *W. solanacea* is known only from higher elevations outside the station boundaries (for representative sites, see Bohs 2000).

## MATERIALS AND METHODS

The study site at the La Selva Biological Station near Puerto Viejo, Costa Rica, is described in McDade *et al.* (1994) and a previous publication on the *W. solanacea* complex (Bohs 2000).

Insect visitors were collected from *W. asterotricha* and *W. meiantha* during 26–31 March 1991, 29 March–3 April 1992, and 28 March–1 April 1993. Information on sites for observing insect visitors is given in Table 1. *Witheringia asterotricha* plants were observed at sites 1–4; *W. meiantha* plants were observed at sites 5–8. No site included both species.

In an initial survey, plants from all sites were examined at irregular intervals throughout the day from 0530 (just after sunrise) to 1745 h (dusk). Plants also were observed several times after dark. In the morning, no insect visitors were observed on any plant until anthers began to dehisce on open flowers; this occurred sporadically during the day, starting after *ca* 0700 to 0730 h in *W. asterotricha* and after 0900 h in *W. meiantha*. In general, the time of anther dehiscence seemed to correlate with amount of solar exposure of the plants, with anthers opening earlier in plants exposed to full morning sun, with dehiscence also occurring during cloudy or rainy weather. Only flowers with freshly dehisced anthers displayed an appreciable amount of pollen; older flowers had most pollen removed either by flower visitors or by rain or condensation. Although many flowers of *W. meiantha* and a few of *W. asterotricha* remained open in the evening, no visitors were observed. As a consequence, subsequent continuous observations of both species were spread out over the day to include all hours between *ca* 0700 and 1745 h. A total of 22 hours was spent on *W. asterotricha* at the four sites. *Witheringia meiantha* plants were observed for 29 hours.

Representative samples of insect visitors were captured from the plants and killed with cyanide, and other flower visitors were noted. Pollen loads were examined by submersing the bees in aniline blue–lactophenol stain on a microscope slide and scraping pollen from their bodies or scopae. Pollen loads were observed with a compound microscope to determine the number of polleno-

morphs (morphologically different types of pollen grains) per load and the presence of *Witheringia* pollen. All bees were identified by Dr. Terry Griswold of the National Bee Research Laboratory, Logan, Utah, and Dr. David Roubik of the Smithsonian Tropical Research Institute, Balboa, Panama. Bee vouchers were deposited at the Museo Nacional in San José, Costa Rica, at the Instituto de Biodiversidad in Heredia, Costa Rica, or in the National Bee Research Laboratory in Logan, Utah. Herbarium vouchers were deposited in the National Museum in San José (CR) and at the Garrett Herbarium at the University of Utah. Accession numbers and provenance data for greenhouse plants are given in Bohs (2000).

Calculations of percent similarity (based on bee species visiting each plant taxon) and similarity index (index of association based on bee species and frequency of visitation) follow Whitaker (1952).

Observations of flowering and fruiting phenology were made in the field and in the University of Utah greenhouse. To determine flower longevity, flowers of greenhouse plants were tagged with colored threads and observed daily. Flowers were classified as senescent if the corolla was completely closed, or if it was judged that a pollinator would not be able to reach the anthers because of the narrowness of the corolla opening.

Nectar was collected in calibrated capillary tubes (Kimble, Inc.) from greenhouse plants of *W. asterotricha*. Although nectar was present in *W. meiantha* flowers, quantities were so low that they defied collection. Nectar sugar content was measured with a handheld Bausch and Lomb refractometer on a weight to weight basis (g solute/100 g solution) and converted to mg sucrose per flower using the method of Bolten *et al.* (1979; Kearns & Inouye 1993). Nectar from several flowers was pooled for a refractometer reading.

To detect the presence of secretory tissues, flowers were stained in a solution of 0.01 percent aqueous neutral red for one to seven hours.

## RESULTS

**PHENOLOGY.**—Published accounts from Costa Rica indicate that *W. asterotricha* produces flowers and fruits year-round (Opler *et al.* 1980, Loiselle & Blake 1990). No published reports of *W. meiantha* phenology exist, but herbarium collections at CR and Duke University, Durham, North Carolina, have flowering specimens from all months of the year and fruiting specimens from all months except December. My observations were made near the

end of the dry season in March and April; abundant flowers and fruits were present on both species. Both taxa have a “steady-state” mode of flower production (Gentry 1974); each plant flowered over an extended period with some new flowers appearing each day. Typically, *W. asterotricha* has many more flowers per inflorescence and per plant than *W. meiantha*.

Flowers of *W. asterotricha* and *W. meiantha* open at various times during the day. Flowers of *W. asterotricha* last an average of 2.94 days in fresh condition ( $N = 149$  flowers, range = 1–6 d). Those of *W. meiantha* remain open for an average of 2.42 days ( $N = 171$ , range = 1–5 d). In both species, the corolla opens and the stigmas appear receptive before the anthers dehisce. Corollas of *W. meiantha* are smaller and deeper yellow than those of *W. asterotricha* and lack the basal maculations usually found in the latter species. In both species, most anthers dehisce on the second day following anthesis. Anthers begin to dehisce at the distal end, so that initially the anthers are open only at the tips and dehiscence proceeds proximally. The anthers do not necessarily dehisce simultaneously. At maturity, the anthers of both species split longitudinally, and the two halves of the thecae fold back to expose the pollen completely. The style exceeds the anthers at all times, but pollen shakes out of the anthers easily and may contact the stigma.

Both species of *Witheringia* offer pollen and nectar as rewards to flower visitors. Droplets of nectar were observed on the outside of the ovaries of fresh flowers. Nectar secretion apparently originates from cells of the receptacle in a ring around the base of the ovary, as judged by staining in neutral red. Nectar quantity ranged from *ca* 0 to 3  $\mu$ l/flower. Nectar secretion began after anther dehiscence and continued until the flowers senesced. Thus, flowers were usually open for a day in which they offered no reward to visitors. Nectar sugar content ranged between 34 and 47 g sucrose equivalents/100 g solution and represented *ca* 0.76–0.95 mg sugar/flower.

In neutral red, both species showed a similar staining pattern, possibly indicating the presence of scent glands, or osmophores. Both abaxial and adaxial calyx surfaces had glandular hairs that stained in neutral red, but the epidermal tissues were otherwise unstained. The corolla margins are ringed by several-celled uniseriate unbranched and branched hairs; uptake of stain by these hairs was responsible for the positive staining response of the corolla margins. Microscopic examination of the adaxial corolla surfaces showed numerous epider-

mal papillae with a structure typical of osmophores (Vogel 1990). These took up neutral red stain, especially in older flowers. The papillate osmophores were located over the entire adaxial surface of the corolla lobes except at the base of the corolla, so that an older corolla stained in neutral red had deeply stained petals except for an unstained "star" at the base. Cells of the abaxial surface did not have a papillose morphology and took up stain only weakly, if at all. Part of the dark red color of the corollas visible abaxially and adaxially when exposed to neutral red was due to staining of unidentified inclusions in the cells within the corolla. The flowers have a dense ring of uniseriate, multicelled, branched and unbranched hairs within the corolla at the point of insertion of the filaments; these hairs did not stain. The anther thecae and anther connective tissue were generally darkly stained in older flowers, but often only weakly so in younger flowers with undehisced anthers. No papillate osmophores could be seen on the anthers or filaments. Pollen grains and stigmas always showed a positive staining reaction. No scent could be detected by the author.

**INSECT VISITORS.**—Bees were by far the most common insect visitors to *Witheringia* flowers. A list of bees captured on the two species is given in Tables 2 and 3. Other flower visitors to *W. asterotricha* included occasional nectar-drinking hummingbirds and beetles, but it is doubtful that these were effective pollinators. The grasshopper *Dryophilacris bimaculata* was a common herbivore of both *Witheringia* species, upon which it was seen eating flowers, pollen, and leaves. This grasshopper also was observed on other Solanaceae at La Selva, such as *W. cuneata* (Standl.) Hunz. and *Solanum lanceifolium* Jacq., and is apparently a specialist on Solanaceae (Rowell 1983); however, it played no evident role in pollination. Ants patrolled branches and inflorescences of both *Witheringia* species and were seen drinking nectar from the flowers. No extrafloral nectaries were evident on the plants. Although it is doubtful that the ants play a role in pollination, their effects on herbivory or competition for nectar in *Witheringia* remain to be elucidated.

*Witheringia asterotricha* was visited by at least 21 species of bees in nine genera and four families (Table 2). Most bees were collected from *W. asterotricha* between 0700 and 1100 h (Fig. 1). Bee visitors collected from *W. meiantha* belonged to approximately five species representing three genera

in two families (Table 3). With one exception, all bees were collected from *W. meiantha* between 1000 and 1130 h. Visitation rates calculated from both observed and captured bees are shown in Figure 1. Minimum visitation rates to roughly equivalent areas of plants (*ca* 1 m<sup>2</sup>) were highest in the morning, and ceased completely after *ca* 1700 h. No bees were seen on *Witheringia* plants during rainy periods. *Witheringia asterotricha* averaged 3.7 bee visits/h, whereas the average rate for *W. meiantha* was 0.5. Bee visits to *W. asterotricha* were thus on the order of seven times more frequent than those to *W. meiantha*. The most commonly collected visitors from both *Witheringia* species were female halictids and colletids. Three species of bees (*Caenaugochlora* sp. A, *Lasioglossum* sp. 3, and *Colletes* sp. NN) were collected from both *W. asterotricha* and *W. meiantha*. The percent similarity for bee visitors to the two *Witheringia* taxa was 16.9 percent and the similarity index was 0.129, indicating *ca* 13–17 percent overlap in species of bees that visited the two plant taxa.

Four genera of bees (*Augochloropsis*, *Caenaugochlora*, *Pseudaugochloropsis*, and *Colletes*) were observed sonicating flowers for pollen (Tables 2 and 3). The behavior observed was typical of that described for other buzzing bees, with the bee seizing the anther cone and rotating around the flower while vibrating its indirect flight muscles (Macior 1964; Buchmann, 1983, 1985). Other bees, such as *Lasioglossum*, did not buzz the anthers but gleaned pollen from the stamen and corolla surfaces. A few bees appeared only to drink nectar from the base of the corolla. Even very small bees, such as certain *Lasioglossum* species, crawled back and forth over the stigma when gleaning pollen, so they probably were effective pollinators in spite of their small size.

Pollen loads were generally homogeneous, with one to five pollenomorphs per load (Tables 2, 3). Pollen conforming to that of *Witheringia* was found in loads of all captured bees except *Epeolus* and one *Colletes*. It was neither possible to differentiate pollen from the two *Witheringia* species nor to distinguish with confidence *Witheringia* pollen from that of other common solanaceous taxa such as *Solanum*. Although pollen grains from various subfamilies and tribes of Solanaceae can be distinguished using light microscopy (Basak 1967; Gentry 1979, 1986), most genera within the tribe Solaneae have very similar spheroidal tricolporate pollen with little sculpturing (Murray & Eshbaugh 1971, Roubik & Moreno 1991). Detailed statistical analysis (Murray & Eshbaugh 1971) and/or ex-

TABLE 2. *Floral visitors to Witheringia asterotricha.*

Taxon <sup>a</sup>	Number collected	Site(s) <sup>b</sup>	Behavior <sup>c</sup>	Number of pollenomorphs
F. Anthophoridae				
SF. Anthophorinae				
Tr. Exomalopsini				
<i>Paratetrapedia</i> ( <i>Paratetrapedia</i> ) sp. 6 ♀	3	1, 2	G?	1–4
SF. Nomadinae				
Tr. Epeolini				
<i>Epeolus</i> sp. ♀	1	1	—	2
F. Apidae				
SF. Meliponinae				
Tr. Trigonini				
<i>Plebeia jatifformis</i> ♀	1	1	G	1
<i>P. frontalis</i> ♀	1	1	G	3
<i>Trigona</i> ( <i>Tetragonisca</i> ) <i>angustula</i> ♀	1	1	G	1
F. Halictidae				
SF. Halictinae				
Tr. Augochlorini				
<i>Augochloropsis auriferia</i> ♀	1	1	—	2
<i>A. ignita</i> ♀	3	1, 2	B	1
<i>Augochloropsis</i> sp. ♀	1	1	B	2
<i>Augochloropsis</i> sp. 1 ♀	1	1	G	1
<sup>d</sup> <i>Caenaugochlora</i> sp. A ♀	1	1	—	2
<i>Caenaugochlora</i> sp. B ♀	1	1	—	1
<i>Caenaugochlora</i> ( <i>Caenaugochlora</i> ) sp. 5 ♀	1	3	N	2
<i>Pseudaugochloropsis graminea</i> ♀	1	1	B	2
Tr. Halictini				
<i>Lasioglossum</i> ( <i>Dialictus</i> ) sp. ♀	3	1, 2	G, N	1–4
<i>Lasioglossum</i> ( <i>Evyllaes</i> ) sp. ♀	1	1	G	2
<i>Lasioglossum</i> ( <i>Evyllaes</i> ) sp. 2 ♀	9	3	G, N	1–2
<sup>d</sup> <i>Lasioglossum</i> ( <i>Evyllaes</i> ) sp. 3 ♀	4	2	G	1
F. Colletidae				
SF. Colletinae				
Tr. Colletini				
<i>Colletes punctipennis</i> ♀	1	3	—	1
<i>C. rugicollis nigrrior</i> ♀	9	2	B, G	1–2
<i>C. ?rugicollis nigrrior</i> ♂	1	2	G?	1
<i>Colletes</i> sp. LL ♀	1	1	B	2
<sup>d</sup> <i>Colletes</i> sp. NN ♀	1	4	B	1
Total collected	47			

<sup>a</sup> Taxa not identified to species follow provisional species notations of T. Griswold, National Bee Research Laboratory, Logan, Utah. F = family; SF = subfamily; Tr = tribe; parentheses = subgenus.

<sup>b</sup> See Table 1.

<sup>c</sup> B = buzzing; G = gleaning; N = drinking nectar; — = no information.

<sup>d</sup> Species also captured on *W. meiantha*.

amination of fine surface features using scanning electron microscopy (SEM; e.g., Anderson & Gensel 1976, Edmonds 1984) may aid in distinguishing pollen grains of *W. asterotricha* and *W. meiantha*, but these methods were outside the scope of this study. In general, there was little evidence that the bees were carrying pollen loads from taxa other than those of the tribe Solaneae.

## DISCUSSION

Whether or not pre-zygotic isolation via pollinator specialization plays a major role in preventing pollen exchange between *W. asterotricha* and *W. meiantha* is an open question. The records of insect visitors to both *Witheringia* species show at least three bee species in common; however, the values for

TABLE 3. *Floral visitors to Witheringia meiantha.*

Taxon <sup>a</sup>	Number collected	Site(s) <sup>b</sup>	Behavior <sup>c</sup>	Number of pollenomorphs
F. Halictidae				
SF. Halictinae				
Tr. Augochlorini				
<sup>d</sup> <i>Caenaugochlora</i> ( <i>Caenaugochlora</i> ) sp. A ♀	3	5	B	2-3
<i>Caenaugochlora</i> ( <i>Caenaugochlora</i> ) sp. C ♀	1	5	—	1
<i>Caenaugochlora</i> ( <i>Caenaugochlora</i> ) sp. 6 ♀	3	5, 7	B	1-4
Tr. Halictini				
<sup>d</sup> <i>Lasioglossum</i> ( <i>Evyllaesus</i> ) sp. 3 ♀	3	5, 7	G	1
F. Colletidae				
SF. Colletinae				
Tr. Colletini				
<sup>d</sup> <i>Colletes</i> sp. NN ♀	2	5	B	1-5
Total collected	12			

<sup>a</sup> Taxa not identified to species follow provisional species notations of T. Griswold, National Bee Research Laboratory, Logan, Utah. F = family; SF = subfamily; Tr = tribe; parentheses = subgenus.

<sup>b</sup> See Table 1.

<sup>c</sup> B = buzzing; G = gleaning; N = drinking nectar; — = no information.

<sup>d</sup> Species also captured on *W. asterotricha*.

percent similarity and association of samples of bee visitors to the plants were low (*ca* 13–16%), indicating relatively little overlap in species visiting both *W. asterotricha* and *W. meiantha*. Such data are difficult to interpret without more information on insect visitors over longer periods, movements

of individual bees, and precise identification of the pollen they carry.

On one hand, it seems possible for the guild of bee visitors to move between the two plant species. This perhaps is expected, given the spatial and temporal complexity of plant–pollinator assemblages noted by other investigators (*e.g.*, Horvitz & Schemske 1990, Thompson & Pellmyr 1992) and the constraints on the evolution of specialized co-evolved plant–pollinator mutualisms (Feinsinger 1983, Schemske 1983, Howe 1984). On the other hand, the probability of an individual visitor moving pollen between the two plant taxa may be very low, based on the spatial separation of plants, temporal differences in observed bee activity, and relatively little overlap of bee species that visit both *Witheringia* taxa.

Nonetheless, pollinator isolation may be a relatively unimportant issue, because the two plant species are isolated by internal genetic barriers to crossing (Bohs 2000). In fact, the similarity in flower morphology of *W. asterotricha* and *W. meiantha* may promote pollinator sharing and increase visitation rates, as has been suggested for Solanaceae and other buzz-pollinated taxa (Michener & Kerfoot 1967; Macior 1968, 1971; Linsley & Cazier 1970; Bowers 1975; Thorp & Estes 1975). Schemske (1981) described a similar case in *Costus* (Zingiberaceae) and proposed that pollinator sharing may be tolerated or even selected for when sharing increases the rate of pollinator

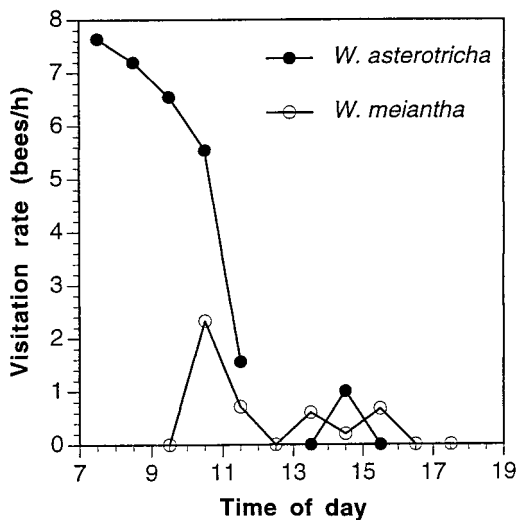


FIGURE 1. Visitation rate (bees/h) versus time of day for flower visitors to *W. asterotricha* and *W. meiantha* at all study sites. Data are from both observed and collected bees.

visitation in plant taxa that have strong genetic or environmental barriers to hybridization. *Witheringia asterotricha* and *W. meiantha*, however, have not been observed growing intermixed at any site, and it is not known if individual bees actually effect interspecific pollen transfer. Depending on the flight distances and behaviors of flower visitors, spatial separation of plants, possibly due to differences in microhabitat preferences between *W. asterotricha* and *W. meiantha*, may effectively prevent interspecific gene flow.

Although *Witheringia* flowers offer both pollen and nectar, most captured bees probably were collecting pollen to some extent, as reflected by the presence of *Witheringia* pollenomorphs in nearly all pollen loads examined. Exceptions include male bees, which do not collect pollen, that probably were visiting the flowers for nectar, and parasitic bees such as *Epeolus*, which have larvae that feed on the nest provisions of other bees (Michener 1974, Roubik 1989). *Epeolus* has no morphological adaptations for pollen manipulation and is only sparsely hairy, unlike the other bees collected from *Witheringia*. The single *Epeolus* captured from *W. asterotricha* had just a few pollen grains recovered from its body, and none conformed in morphology to *Witheringia* pollen. It is likely that the pollen recovered from *Epeolus* was placed there only incidentally in the course of its flower visits for other purposes. For instance, *Epeolus* may feed on *Witheringia* nectar, as do other bees. Parasitic bees may lie in wait on flowers for host bees, which they follow back to the nest (Roubik 1989). *Epeolus* is known to parasitize nests of *Colletes* (Linsley 1958), a common visitor to *W. asterotricha*. Also, bees may mate in or near flowers (Linsley 1958, Roubik 1989); thus the *Epeolus* and the male *Colletes* collected at *W. asterotricha* may have been waiting there for mates.

It is not known how bees locate *Witheringia* flowers. Both species have small, pendant, and rather dull-colored flowers which in many cases are hidden beneath the foliage. Although they exude no discernible scent, the positive staining reaction of the flowers in neutral red indicates that the corolla, anthers, or pollen may emit volatile compounds detected by bees. D'Arcy *et al.* (1990) found that anthers (including pollen) of two other *Witheringia* species emitted an odor detectable by humans after flowers were concentrated in jars. Evidence has shown that *Colletes* can use olfactory cues from pollen and floral tissues to discriminate among pollen sources (Dobson 1987). Putative floral osmophores and scented pollen have been described in other solanaceous taxa (Buchmann *et al.* 1977, Buchmann 1983, D'Arcy *et al.* 1990, Sazima

*et al.* 1993, Dean 1995), but much remains to be discovered regarding their systematic distribution and evolutionary importance. In particular, the presence and role of floral scents in pollinator attraction for *Witheringia* species needs to be tested experimentally.

Pollen loads recovered from bees were largely homogeneous, but this does not necessarily imply oligolecty (reliance on one or very few plant species as pollen sources). Flower constancy (visitation of only a few plant taxa) is a general behavioral characteristic of many foraging bees (Grant 1950, Linsley 1958, Michener 1974). Even polylectic bees often visit flowers of a particular plant or plant species on a given foraging trip, and some bees prefer returning to localized foraging areas on consecutive trips or throughout the duration of the plant's flowering season (Linsley 1958, Michener 1974). Although oligolecty is well known in solitary bees from temperate regions (Linsley 1958), most tropical bees are polylectic, as are most social bees from temperate and tropical regions (Linsley 1958, Wille 1963). Thus, the nearly homogeneous pollen loads on captured bee visitors do not necessarily imply that they are oligolectes on *Witheringia*, or on Solanaceae. Detailed studies on foraging behavior of individual bees in many areas and examinations of colony diets are needed to establish pollen collecting preferences conclusively.

Despite the possible polylecty of *Witheringia*-visiting bees, they are probably effective pollinators. Nearly all the bee visitors, even very small gleaning bees, contacted the stigma surface at some time during their visit and probably deposited pollen there. Most bees visited several open flowers on a single plant, and therefore may have contaminated the stigma with self pollen. The extent of movement among genetically different individuals is not known, but judging from the abundant fruit set of both self-incompatible *Witheringia* species, enough intergenetic movement of pollinators must be occurring to effect pollination on a regular basis. Roubik (1989) suggested that pollen also may be exchanged among bees in the nest, and thus interplant pollen transport may take place by mechanisms other than foraging trips.

Kress and Beach (1994) reported that small bees were the predominant flower visitors of 16 percent of the understory species studied at La Selva, making them the fourth largest pollinator guild behind hummingbirds, medium to large bees, and beetles; however, further research on pollination systems in understory families such as the Solanaceae, Melastomataceae, and Piperaceae may in-

crease the proportion of taxa visited by small bees. This study highlights the importance of small solitary bees as pollinators of tropical understory plants.

The patterns of bee activity observed in this study (Fig. 1) are similar to those reported for other bees. Peak foraging for both pollen and nectar occurs in the morning, often with a smaller peak in the late afternoon (Roubik 1989). Bee foraging patterns are affected by light, temperature, and weather conditions, with most bees showing restricted foraging activity in cloudy, cool, or rainy weather (Linsley 1958, Roubik 1989). The timing and rate of nectar secretion also may be affected by light and temperature (Percival 1965), and thus may affect bee visitation rates. The first bees were not collected from *W. asterotricha* until an hour or two after sunrise, and the earliest bees at *W. meiantha* were collected after 1000 h. Although this may be a function of the time when intensive observations started, preliminary surveys showed that bees were not present on the plants before new anthers dehisced on *W. asterotricha* (after ca 0700 h) and *W. meiantha* (after ca 0900 h; see Materials and Methods). *Witheringia asterotricha* occurs in more open habitats than *W. meiantha*, and sunlight strikes the plants earlier. It is probable that bee visitation patterns to *Witheringia* parallel flowering phenology.

Bee visitation rates to *W. asterotricha* were much higher than those to *W. meiantha*. This pattern may reflect the greater number of flowers present on *W. asterotricha* at any given time. Other workers have noted that pollinator visitation increases as flower density increases (e.g., Willson & Price 1977, Schaal 1978, Augspurger 1980, Schemske 1980, Thomson 1981).

Over 50 genera of bees in at least seven families are known to buzz flowers (Michener 1962; Buchmann 1983, 1985; Buchmann & Cane 1989). These include both solitary and social bees, and both polylectic and oligolectic taxa. All four bee genera observed to buzz *Witheringia* flowers in the present study are known to buzz other plant taxa. Buzz pollination is usually associated with poricidal anther dehiscence, although there are cases in which bees buzz non-poricidally dehiscent taxa and examples of plants with poricidal anthers that are not buzz pollinated (Buchmann 1983, 1985).

Flowers that are buzzed by bees often have a "shooting star" flower form (*sensu* Vogel 1978, Procter *et al.* 1996), with few stamens and anthers connivent around the style. Anther dehiscence in these flowers generally begins apically; the small

apical openings may remain as anther pores or enlarge into longitudinal slits as the flowers age. Examples include *Dodecatheon* (Primulaceae; Macior 1964), *Vaccinium* (Ericaceae; Cane *et al.* 1985), *Borago*, *Onosma*, *Symphytum*, *Trichodesma* (Boraginaceae; Corbet *et al.* 1988, Dukas & Dafni 1990), and *Solanum* (including *Lycopersicon*), *Lycianthes*, and *Datura* (Solanaceae; e.g., Rick 1950; Linsley 1962; Michener 1962; Linsley & Cazier 1963; 1970; Macior 1964; Bowers 1975; Buchmann *et al.* 1977; Symon 1979; Buchmann 1983; de Nevers 1986; Knapp 1986; Anderson & Symon 1988; Dean 1995). *Witheringia asterotricha* and *W. meiantha* have flowers that belong to this floral form, although they differ from those of most *Solanum* and *Lycianthes* species by producing nectar and having anthers that are longitudinally dehiscent at maturity but are functionally poricidal in early stages. *Witheringia* anthers, particularly those of *W. meiantha*, dehisce at various times, so young flowers may be available throughout the day. In the flowers observed during this study, buzzing took place throughout the day, but it was not determined if buzzing occurred only on young flowers. Buzzing is probably the most efficient method of removing pollen from poricidal anthers (Buchmann 1983), and it also may be effective in gathering pollen from fully dehisced longitudinal anthers.

In summary, there is some evidence for the existence of pre-zygotic isolating mechanisms restricting gene flow between *W. asterotricha* and *W. meiantha* at La Selva. Flowering phenologies of the two species overlap, but ethological isolation via specialized pollinators is possible, given the low percentage of shared flower visitors to the two plant species. Although both *Witheringia* species are found at La Selva, fine-scale geographic differences or microhabitat preferences may prevent their coexistence at any one site; thus, ecogeographic factors may be involved as reproductive barriers. Investigations at sites where the two, or even all three, species of the *W. solanacea* complex co-occur (if they exist) will be useful in elucidating the presence of pre-mating isolating mechanisms in this group of plants.

## ACKNOWLEDGMENTS

I thank T. Griswold, D. Roubik, and V. Tepedino for their contagious enthusiasm for bees, C. LeBlanc for help with the nectar measurements, W. Pockman for assistance with the Figure, J. Sperry for laboratory facilities, M. A. Jaramillo for correcting the Spanish abstract, the staff of the La Selva Biological Station, particularly D. and D. Clark, for facilitating fieldwork in Costa Rica, and the



greenhouse staff of the University of Utah Biology Department for maintaining my living collections. Financial

assistance to C. LeBlanc was provided by grants from the University of Utah Undergraduate Research Initiative.

## LITERATURE CITED

- ANDERSON, G. J. 1995. Systematics and reproductive biology. *In* P. C. Hoch and A. C. Stephenson (Eds.), *Experimental and molecular approaches to plant biosystematics*, pp. 263–272. *Monographs in Systematic Botany from the Missouri Botanical Garden*, St. Louis, Missouri.
- , AND P. G. GENSEL. 1976. Pollen morphology and the systematics of *Solanum* section *Basarthrum*. *Pollen et Spores* 18: 533–552.
- , AND D. SYMON. 1988. Insect foragers on *Solanum* flowers in Australia. *Ann. Mo. Bot. Gard.* 75: 842–852.
- AUGSPURGER, C. K. 1980. Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. *Evolution* 34: 475–488.
- AVISE, J. C. 1994. *Molecular markers, natural history, and evolution*. Chapman & Hall, New York, New York.
- BASAK, R. K. 1967. The pollen grains of Solanaceae. *Bull. Bot. Soc. Bengal* 21: 49–58.
- BOHS, L. 2000. Insights into the *Witheringia solanacea* complex in Costa Rica. I. Breeding systems and crossing studies. *Biotropica* 32(1): 70–79.
- BOLTEN, A. B., P. FEINSINGER, H. G. BAKER, AND I. BAKER. 1979. On the calculation of sugar concentration in flower nectar. *Oecologia (Berl.)* 41: 301–304.
- BOWERS, K. A. W. 1975. The pollination ecology of *Solanum rostratum* (Solanaceae). *Am. J. Bot.* 92: 633–638.
- BUCHMANN, S. L. 1983. Buzz pollination in angiosperms. *In* C. E. Jones and R. J. Little (Eds.), *Handbook of experimental pollination biology*, pp. 73–113. Van Nostrand Reinhold, New York, New York.
- . 1985. Bees use vibration to aid pollen collection from non-poricidal flowers. *J. Kans. Entomol. Soc.* 58: 517–525.
- , AND J. H. CANE. 1989. Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia (Berl.)* 81: 289–294.
- , C. E. JONES, AND L. J. COLIN. 1977. Vibratile pollination of *Solanum douglasii* and *S. xanti* (Solanaceae) in southern California. *Wasmann J. Biol.* 35: 1–25.
- CANE, J. H., G. C. EICKWORT, F. R. WESLEY, AND J. SPIELHOLZ. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae). *Am. J. Bot.* 72: 135–142.
- CORBET, S. A., H. CHAPMAN, AND N. SAVILLE. 1988. Vibratory pollen collection and flower form: bumble-bees on *Actinidia*, *Symphytum*, *Borago*, and *Polygonatum*. *Funct. Ecol.* 2: 147–155.
- D'ARCY, W. G. 1973. Solanaceae. *In* R. E. Woodson and R. W. Schery (Eds.), *Flora of Panama*, pp. 573–780. *Ann. Mo. Bot. Gard.* Vol. 60.
- , N. S. D'ARCY, AND R. C. KEATING. 1990. Scented anthers in the Solanaceae. *Rhodora* 92: 50–52.
- DEAN, E. A. 1995. Systematics and ethnobotany of *Lycianthes* series *Meizonodontae*. Ph.D. dissertation, University of California, Berkeley, California.
- DE NEVERS, G. 1986. Pollination of *Lycianthes amatitlanensis* in eastern Panama. *Solanaceae Newsl.* 2: 36–38.
- DOBSON, H. E. M. 1987. Role of flower and pollen aromas in host-plant recognition by solitary bees. *Oecologia (Berl.)* 72: 618–623.
- DOBZHANSKY, T. 1937. *Genetics and the origin of species*. Columbia University Press, New York, New York.
- DUKAS, R., AND A. DAFNI. 1990. Buzz-pollination in three nectariferous Boraginaceae and possible evolution of buzz-pollinated flowers. *Plant Syst. Evol.* 169: 65–68.
- EDMONDS, J. M. 1984. Pollen morphology of *Solanum* L. section *Solanum*. *Bot. J. Linn. Soc.* 88: 237–251.
- FEINSINGER, P. 1983. Coevolution and pollination. *In* D. J. Futuyma and M. Slatkin (Eds.), *Coevolution*, pp. 282–310. Sinauer Associates, Sunderland, Massachusetts.
- GENTRY, A. H. 1974. Flowering phenology and diversity in tropical Bignoniaceae. *Biotropica* 6: 64–68.
- GENTRY, J. L., JR. 1979. Pollen morphology of the Salpiglossideae (Solanaceae). *In* J. G. Hawkes, R. N. Lester, and A. D. Skelding (Eds.), *The biology and taxonomy of the Solanaceae*, pp. 327–332. Academic Press, London, England.
- . 1986. Pollen studies in the Cestreae (Solanaceae). *In* W. G. D'Arcy (Ed.), *Solanaceae: biology and systematics*, pp. 138–158. Columbia University Press, New York, New York.
- GRANT, V. 1950. The flower constancy of bees. *Bot. Rev.* 16: 379–398.
- . 1981. *Plant speciation*, 2nd edition. Columbia University Press, New York, New York.
- HORVITZ, C. C., AND D. W. SCHEMSKE. 1990. Spatiotemporal variation in insect mutualists of a Neotropical herb. *Ecology* 71: 1085–1097.
- HOWE, H. F. 1984. Constraints on the evolution of mutualisms. *Am. Nat.* 123: 764–777.
- HUNZIKER, A. T. 1969. Estudios sobre Solanaceae. V. Contribución al conocimiento de *Capsicum* y géneros afines (*Witheringia*, *Acnistus*, *Athenaea*, etc.) Primera parte. *Kurtziana* 5: 101–179.
- KEARNS, C. A., AND D. W. INOUE. 1993. *Techniques for pollination biologists*. University Press of Colorado, Niwot, Colorado.
- KNAPP, S. D. 1986. Reproductive biology of *Solanum* section *Geminata* in a Costa Rican cloud forest. *In* W. G. D'Arcy (Ed.), *Solanaceae: biology and systematics*, pp. 253–263. Columbia University Press, New York, New York.

- KRESS, W. J., AND J. H. BEACH. 1994. Flowering plant reproductive systems. In L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn (Eds.). *La Selva: ecology and natural history of a Neotropical rain forest*, pp. 161–182. University of Chicago Press, Chicago, Illinois.
- LEVIN, D. A. 1978. The origin of isolating mechanisms in flowering plants. In M. K. Hecht, W. C. Steere, and B. Wallace, (Eds.). *Evolutionary biology*, Vol. 11, pp. 185–317. Plenum Press, New York, New York.
- LINSLEY, E. G. 1958. The ecology of solitary bees. *Hilgardia* 27: 543–599.
- . 1962. The colletid *Ptiloglossa arizonensis* Timberlake, a matinal pollinator of *Solanum*. *Pan-Pac. Entomol.* 38: 75–82.
- , AND M. A. CAZIER. 1963. Further observations on bees which take pollen from plants of the genus *Solanum*. *Pan-Pac. Entomol.* 39: 1–18.
- , AND ———. 1970. Some competitive relationships among matinal and late afternoon foraging activities of caupolicanine bees in southeastern Arizona (Hymenoptera: Colletidae). *J. Kans. Entomol. Soc.* 43: 251–261.
- LOISELLE, B. A., AND J. G. BLAKE. 1990. Diets of understory fruit-eating birds in Costa Rica: seasonality and resource abundance. *Stud. Avian Biol.* 13: 91–103.
- MACIOR, L. W. 1964. An experimental study of the floral ecology of *Dodecatheon meadia*. *Am. J. Bot.* 51: 96–108.
- . 1968. Pollination adaptation in *Pedicularis groenlandica*. *Am. J. Bot.* 55: 927–932.
- . 1971. Co-evolution of plants and animals—systematic insights from plant–insect interactions. *Taxon* 20: 17–28.
- MAYR, E. 1963. *Animal species and evolution*. Belknap Press of Harvard University Press, Cambridge, Massachusetts.
- MCDADE, L. A., K. S. BAWA, H. A. HESPENHEIDE, AND G. S. HARTSHORN. 1994. *La Selva: ecology and natural history of a Neotropical rain forest*. University of Chicago Press, Chicago, Illinois.
- MICHENER, C. D. 1962. An interesting method of pollen collecting by bees from flowers with tubular anthers. *Rev. Biol. Trop.* 10: 167–175.
- . 1974. *The social behavior of the bees: A comparative study*. Harvard University Press, Cambridge, Massachusetts.
- , AND W. B. KERFOOT. 1967. Nests and social behavior of three species of *Pseudaugochloropsis* (Hymenoptera: Halictidae). *J. Kans. Entomol. Soc.* 40: 214–232.
- MURRAY, L. E., AND W. H. ESHBAUGH. 1971. A palynological study of the Solaninae (Solanaceae). *Grana* 11: 65–78.
- OPLER, P. A., G. W. FRANKIE, AND H. G. BAKER. 1980. Comparative phenological studies of treelet and shrub species in tropical wet and dry forests in the lowlands of Costa Rica. *J. Ecol.* 68: 167–188.
- PERCIVAL, M. S. 1965. *Floral biology*. Pergamon Press, Oxford, England.
- PROCTER, M., P. YEO, AND A. LACK. 1996. *The natural history of pollination*. Timber Press, Portland, Oregon.
- RICK, C. M. 1950. Pollination relations of *Lycopersicon esculentum* in native and foreign regions. *Evolution* 4: 110–122.
- ROUBIK, D. W. 1989. *Ecology and natural history of tropical bees*. Cambridge University Press, Cambridge, England.
- , AND J. E. MORENO P. 1991. Pollen and spores of Barro Colorado Island. *Monographs in Systematic Botany from the Missouri Botanical Garden*, vol. 36. Missouri Botanical Garden, St. Louis, Missouri.
- ROWELL, H. F. 1983. *Drymophilacris bimaculata*. In D. H. Janzen, (Ed.). *Costa Rican natural history*, pp. 714–716. University of Chicago Press, Chicago, Illinois.
- SAZIMA, M., S. VOGEL, A. COCUCCI, AND G. HAUSNER. 1993. The perfume flowers of *Cyphomandra* (Solanaceae): pollination by euglossine bees, bellows mechanism, osmophores, and volatiles. *Plant Syst. Evol.* 187: 51–88.
- SCHAAL, B. A. 1978. Density dependent foraging on *Liatrix pycnostachya*. *Evolution* 32: 452–454.
- SCHEMSKE, D. 1980. Evolution of floral display in the orchid *Brasavola nodosa*. *Evolution* 34: 489–493.
- . 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62: 946–954.
- . 1983. Limits to specialization and coevolution in plant–animal mutualisms. In M. H. Nitecki (Ed.). *Coevolution*, pp. 67–109. University of Chicago Press, Chicago, Illinois.
- STEBBINS, G. L., JR. 1950. *Variation and evolution in plants*. Columbia University Press, New York, New York.
- SYMON, D. E. 1979. Sex forms in *Solanum* (Solanaceae) and the role of pollen collecting insects. In J. G. Hawkes, R. N. Lester, and A. D. Skelding (Eds.). *The biology and taxonomy of the Solanaceae*, pp. 385–397. Academic Press, London, England.
- THOMPSON, J. N., AND O. PELLMYR. 1992. Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology* 73: 1780–1791.
- THOMSON, J. D. 1981. Spatial and temporal components of resource assessment by flower-feeding insects. *J. Anim. Ecol.* 50: 49–59.
- THORP, R. W., AND J. R. ESTES. 1975. Intrafloral behavior of bees on flowers of *Cassia fasciculata*. *J. Kans. Entomol. Soc.* 48: 175–184.
- VOGEL, S. 1978. Evolutionary shifts from reward to deception in pollen flowers. In A. J. Richards (Ed.). *The pollination of flowers by insects*, pp. 89–96. Academic Press, London, England.
- . 1990. *The role of scent glands in pollination*. Smithsonian Institution, Washington, DC.
- WHITAKER, R. H. 1952. A study of summer foliage insect communities in the Great Smoky Mountains. *Ecol. Monogr.* 22: 1–44.
- WILLE, A. 1963. Behavioral adaptations of bees for pollen collecting from *Cassia* flowers. *Rev. Biol. Trop.* 11: 205–210.
- WILLSON, M. E., AND P. W. PRICE. 1977. The evolution of inflorescence size in *Asclepias* (Asclepiadaceae). *Evolution* 31: 495–511.