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CROSSING STUDIES IN CYPHOMANDRA (SOLANACEAE) AND THEIR SYSTEMATIC AND EVOLUTIONARY SIGNIFICANCE¹

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A crossing program involving eight species of *Cyphomandra* was instituted to elucidate systematic relationships between the taxa and evolutionary mechanisms operating in the genus. The results show that gametophytic self-incompatibility is widespread in *Cyphomandra*. Pollen tubes were able to reach the ovules in nearly all interspecific crosses. Most of these crosses failed, however, indicating that postzygotic barriers are important in reproductive isolation. Only five of 44 interspecific combinations attempted have produced viable seeds. F₁ plants have been evaluated from three of the five hybrid combinations: *C. betacea* × *acuminata*, *C. uniloba* × *betacea*, and *C. corymbiflora* × *diploconos*. All three hybrid combinations show reduced fertility as compared to the parents, apparently caused in part by differences in chromosome structure between parent species. Crossing success was congruent with morphological similarity in *C. betacea*, *C. acuminata*, and *C. uniloba*, but not in *C. corymbiflora* and *C. diploconos*. External isolating mechanisms such as geography, ecology, phenology, and pollinator specificity may also operate to maintain species integrity in nature.

Cyphomandra (Solanaceae) is a Neotropical genus of about 30 species of shrubs and small trees. Most species are gap plants of the tropical rain forest, and some can attain heights of 10 m or more. A few species produce edible fruits, and one, *C. betacea* (Cav.) Sendtn., the tree tomato or tamarillo, is grown as a commercial fruit crop.

A considerable number of studies have focused on intra- and interspecific crossability patterns in the Solanaceae, particularly in such economically and experimentally important genera as *Lycopersicon*, *Capsicum*, *Petunia*, *Nicotiana*, *Datura*, and *Solanum*. Most of the groups that have been examined are annual or short-lived perennial herbs. Information about breeding systems and interspecific hybridization can often provide valuable insights into the evolutionary mechanisms operating within a plant group. However, with the exception of such studies as Bawa (1974) and Bawa, Perry,

and Beach (1985), few have examined breeding systems of tropical woody plants, presumably because of space and time limitations. Even less is known about interspecific hybridization and isolating mechanisms of tropical woody taxa.

Eight species of *Cyphomandra* were used in the crossing program. *Cyphomandra*s are difficult experimental subjects because of their large size, long generation time, and tropical requirements. They cannot be grown outdoors in areas where frost occurs, and their large size often imposes serious space problems in the greenhouse. Many species need 1 or 2 yr to reach reproductive maturity and 8 to 10 mo to mature fruits after pollination. Although these limitations have restricted the size and scope of the present study, I hope that the results will inspire further studies of the reproductive biology of *Cyphomandra*. This information will be useful in elucidating taxonomic relationships among wild species of the genus as well as providing insight into evolutionary mechanisms operating in these tropical woody plants. In addition, the increasing importance of *C. betacea* as a cultivated crop (National Research Council, 1989) underscores the usefulness of crossing data for the breeding and improvement of this and other species in the genus.

MATERIALS AND METHODS

Plants were grown from seed in pollinator-free greenhouses at Harvard University, the

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TABLE 1. Sources of plants used in artificial crosses

Species	Code ^a	Pollen fertility mean, range, (no. flowers, no. plants)	Collector and locality	Bohs voucher number
<i>Cyphomandra acuminata</i> Rusby	acu	86%, 75%–93%, (4, 1)	<i>Solomon & Escobar 12458</i> Bolivia: Nor Yungas, 8.7 km below Chuspipata on road to Yolosa	2338
<i>Cyphomandra betacea</i> (Cav.) Sendtn.	bet	86%, 50%–97%, (25, 4)	<i>Sperling s.n.</i> Ecuador: Pichincha, Quito, purchased in La Vincentina market	2274, 2275
		88%, 87%–89%, (5, 1)	<i>Bohs 1599</i> Colombia: Putumayo, valley of Sibundoy	
		82%, 73%–90%, (3, 1)	<i>Nee 30359</i> Bolivia: Cochabamba, purchased in Cochabamba market	
<i>Cyphomandra corymbiflora</i> Sendtn. subsp. <i>corymbiflora</i>	cor	98%, 95%–100%, (6, 2)	Southeast Brazil, exact provenance unknown; sent by G. Pringle, DSIR, New Zealand (NZ IP #27772)	2343
<i>Cyphomandra diploconos</i> (Mart.) Sendtn.	dip	Purple-flowered form: 94%, 83%–99%, (14, 2)	Brazil: Paraná, city of Curitiba; sent by A. Child, Yorkshire, England	2335
		White-flowered form: 48%, 40%–59%, (10, 1)		
<i>Cyphomandra diversifolia</i> (Dun.) Bitt. subsp. <i>diversifolia</i>	div	97%, 95%–99%, (26, 7)	<i>Benitez de Rojas 2744</i> Venezuela: Aragua, Parque Nacional Henri Pittier	2341
		97%, 96%–98%, (3, 1)	<i>Benitez de Rojas 3672</i> Venezuela: Aragua, Parque Nacional Henri Pittier	
<i>Cyphomandra hartwegii</i> (Miers) Walp. subsp. <i>ramosa</i> Bohs	har	81%, 19%–98%, (33, 6)	<i>Buch s.n.</i> Colombia: Huila, Fundación Merenberg	2277, 2301
<i>Cyphomandra obliqua</i> (R. & P.) Sendtn.	obl	56%, 53%–61%, (7, 1)	<i>Plowman & Schunke 11550</i> Peru: San Martín, Tocache Neuvo	
<i>Cyphomandra uniloba</i> Rusby	uni	95%, 91%–98%, (9, 3)	<i>Sperling & King 5500</i> Bolivia: La Paz, Larecaja, between Consata and Mapi	2283, 2284

^a Codes refer to Figs. 1, 2 and Table 2.

University of Vermont, and the University of Utah. Due to limitations of space and seed availability, usually only one accession was grown of each species. Seed sources are given in Table 1. All species used in the study were diploids with $N = 12$ (Bohs, 1989b; Pringle and Murray, 1991). Voucher specimens of each accession have been deposited in the herbaria at Harvard University (GH) and/or the University of Vermont (VT).

Pollinations were effected by tapping pollen onto a clean glass slide which was rubbed against the stigma of the female parent. Self-compatible species were emasculated in the bud and the flowers were allowed to open fully before pollination.

Table 2 lists the number of pollinations per-

formed for each species in the crossability trials. Female parents are given first in all references to interspecific crosses. Pollinations were evaluated according to failure (flowers dropped off with little or no ovary development), fruit development, and seed number and viability. A cross was considered successful if it resulted in full-sized fruits with viable seeds. "Seedless" fruits were those that had very small or obviously underdeveloped seeds. Other fruits formed full-sized seeds, but they were either empty or inviable. Seeds were initially judged to be inviable based on their appearance, and plump or full-sized seeds were later planted in the greenhouse to determine their viability.

Pollen tube growth was observed using the

TABLE 2. *Intra- and interspecific pollinations in Cyphomandra. Intraspecific pollinations are outcrosses or sister crosses unless noted otherwise. Number of seeds per berry based on visual examination; only full-sized or nearly full-sized seeds are counted. For seed viability, see Fig. 1 and text*

	Female parent		Male parent						
	Selfed	acu ^a	bet	cor	dip	div	har	obl	uni
acu	No. Pollinations 60	0	10	29	25	27	16	0	10
	No. Fruits 0		4	5	2	9	5		1
	No. Seeds/fruit ^b 0		2-5 (1.75)	0	0	0	0		0
bet	No. Pollinations 20	10	36	20	36	41	31	13	25
	No. Fruits 12	5	21	9	20	5	7	1	11
	No. Seeds/fruit 0-192 (98)	15-103 (40)	0-166 (97)	0-1 (0.1)	0	0	0	0	0-3 (0.6)
cor	No. Pollinations 32	24	20	30	23	26	20	0	25
	No. Fruits 0	0	0	27	13	0	0		0
	No. Seeds/fruit 0	0	0	41-157 (105)	0-46 (13)	0	0		0
dip	No. Pollinations 108	0	23	22	119	97	24	0	34
	No. Fruits 0		0	15	78	5	0		0
	No. Seeds/fruit 0		0	0-5 (0.6)	0-265 (161)	0	0		0
div	No. Pollinations 80	71	70	63	70	164	42	20	31
	No. Fruits 0	0	0	0	3	14	10	3	3
	No. Seeds/fruit 0	0	0	0	0	0-170 (77)	0	0	0
har	No. Pollinations 38	20	40	20	33	41	29	11	24
	No. Fruits 0	4	1	4	3	6	18	6	3
	No. Seeds/fruit 0	0	0	0	0-38 (13)	0	0-94 (65)	0	0
obl	No. Pollinations 1	0	1	0	0	0	0	0	0
	No. Fruits 0		0						
uni	No. Pollinations 33	15	30	29	29	21	20	0	23
	No. Fruits 0	0	16	4	5	2	6		9
	No. Seeds/fruit 0	0	0-47 (15)	0	0	0	0		63-135 (106)

^a Codes refer to Figs. 1, 2 and Table 2.
^b Range (mean).

		Male parent							
		1	2	3	4	5	6	7	8
Female parent	acu 1	-	⊖	⊖	⊖	⊖	⊖		⊖
	bet 2	●	●	○	○	⊖	⊖	○	○
	cor 3	-	-	⊖	●	-	-		-
	dip 4		-	○	-	●	-		-
	div 5	-	-	-	⊖	-	●	○	⊖
	har 6	⊖	-	⊖	⊖	○	-	●	○
	obl 7								
	uni 8	-	●	⊖	⊖	-	⊖		●

Fig. 1. Results of crossing experiments in *Cyphomandra*. Intraspecific pollinations fall along diagonal line: above line = sister crosses or outcrossed, below line = selfed. Empty squares = cross not attempted; minus sign (-) = cross not successful (no fruits or fruits less than 10 mm long); divided circle (⊖) = small- to medium-sized seedless fruit; open circle (○) = full-sized fruits, seedless or without viable seeds; half-filled circle (◐) = full-sized fruits with a few viable seeds; filled circle (●) = full-sized fruits with many viable seeds.

procedure of Martin (1959) with the following modifications. Flowers were harvested approximately 48 hr after pollination. After fixation, gynoecia were rinsed and cleared in 0.8 N NaOH at 60 C for 1–2 hr, rinsed briefly, then stained. Gynoecia were mounted on slides in glycerin and observed with a fluorescence microscope. Pollen tube growth was considered successful if pollen tubes were seen in the ovary and around the ovules. Except where noted, tubes from nearly all the germinated grains reached the ovules in successful crosses. At least three flowers were examined per cross, but in ambiguous cases flowers were examined repeatedly until a consistent conclusion could be reached.

Pollen stainability was measured using aniline blue-lactophenol as described by Hauser and Morrison (1964). The pollen tapped onto a glass slide was allowed to stain for at least 1 hr before the first 300 grains encountered were scored. Unshriveled pollen grains staining blue in the preparation were presumed to be viable. Pollen grains of all species were stained with 1%–2% acetocarmine to observe the number of nuclei in the grains.

Meiotic chromosome observations were made using fresh flower buds or buds fixed in freshly prepared Farmer's solution (3:1 absolute ethanol : glacial acetic acid). Anthers were

		Male parent						
		1	2	3	4	5	6	7
Female parent	acu 1	-	+	+	+	+	+	
	bet 2	+	+	+	+	+	+	+
	cor 3	(+)	+	-	+	(+)	-	+
	dip 4	+	+	+	-	+	+	+
	div 5	+	+	+	+	-	+	+
	har 6	+	+	+	+	+	-	+
	uni 7		+	+	+	+		(+)

Fig. 2. Results of pollen tube observations in *Cyphomandra*. Intraspecific pollinations are selfs. + = most pollen tubes reach ovary and ovules; (+) = a few pollen tubes occasionally seen in ovary; - = all pollen tubes arrested in stigma or style.

stained in 1%–2% acetocarmine, heated gently, and squashed. Mitotic chromosomes were observed from root tips that were pretreated for up to 24 hr in a saturated aqueous solution of paradichlorobenzene. Roots were then fixed in Farmer's solution for up to 24 hr and stored in 70% ethanol until use. Preparations were hydrolyzed in 1 N HCl for 10 min at 60 C and stained as above.

Cyphomandra acuminata was not outcrossed because only one plant of this species was available. Similarly, only a single individual of *C. obliqua* survived until flowering, and this plant died before it could serve as the female parent for any crosses.

RESULTS

Intraspecific crosses—Results of the intraspecific pollinations are given in Table 2, Figs. 1, 2, and in Bohs, 1989c. No fruits or seeds were produced in self-pollinations with any *Cyphomandra* species except *C. betacea*. Pollen tubes from nearly all the germinated grains reached the ovary in self-pollinations of *C. betacea*. In selfs of *C. uniloba*, occasionally a few pollen tubes could be seen entering the ovary. Pollen tube growth was arrested in the style in self-pollinations of *C. acuminata*, *C. corymbiflora*, *C. diploconos*, *C. diversifolia*, and *C. hartwegii*.

The results from fruit set and pollen tube observations indicate that *C. acuminata*, *C. corymbiflora*, *C. diploconos*, *C. diversifolia*, and

C. hartwegii are self-incompatible (SI). Although *C. acuminata* set no fruits in the selfing experiments, a spontaneous fruit of unknown parentage yielded viable seeds. As only one plant of *C. acuminata* was available, this indicates that it is either self-compatible (SC), apomictic, or can hybridize with one of the other species present.

Cyphomandra betacea may be SC or apomictic. In this species, spontaneous fruits were very seldom produced in the greenhouse, and they could be explained by shaking of the plants by the wind or by experimental manipulations. Alternatively, apomixis could cause spontaneous fruit production, but if this is occurring, it happens infrequently. However, the possibility exists that this species may be apomictic and pseudogamous.

According to fruit set data, *C. uniloba* is also SI, but in a few cases pollen tubes could be seen entering the ovary. This behavior was also observed by G. Pringle (personal communication) in *C. uniloba* growing in New Zealand. The significance of this finding is unclear; perhaps these represent contamination by foreign pollen, or rare cases of nonrejection of self-pollen in this species.

Low pollen fertility was an unlikely explanation for failure of crosses because pollen stainability was generally high during the crossability trials (Table 1). Pollen fertility and seed set in the white-flowered form of *C. diploconos* was about half that of the purple-flowered form. Pollen stainability was also rather low in *C. obliqua* ($\bar{X} = 56\%$) and in one plant of *C. hartwegii* ($\bar{X} = 58\%$).

There was no evidence of dioecy, andromonoecy, or other differential sex expression in the *Cyphomandra* species studied. All flowers were homomorphic with normal-looking pollen grains. Pollen grains of all species were shed at the binucleate stage.

Interspecific crosses—Of the 44 interspecific combinations attempted, 28 resulted in fruit development, but only five combinations have produced viable hybrid seeds. Of these five successful combinations, one (*C. hartwegii* × *diploconos*) has been obtained only once. In all cases of hybrid seed production, the number of seeds per berry was low when compared to seed production in intraspecific crosses. Results of the interspecific pollinations are given in Table 2 and Figs. 1, 2.

In almost all interspecific crosses, pollen germination was high (usually greater than 75%) and nearly all germinated grains produced tubes that reached the ovary and ovules. In many cases, tubes could be seen entering the ovular

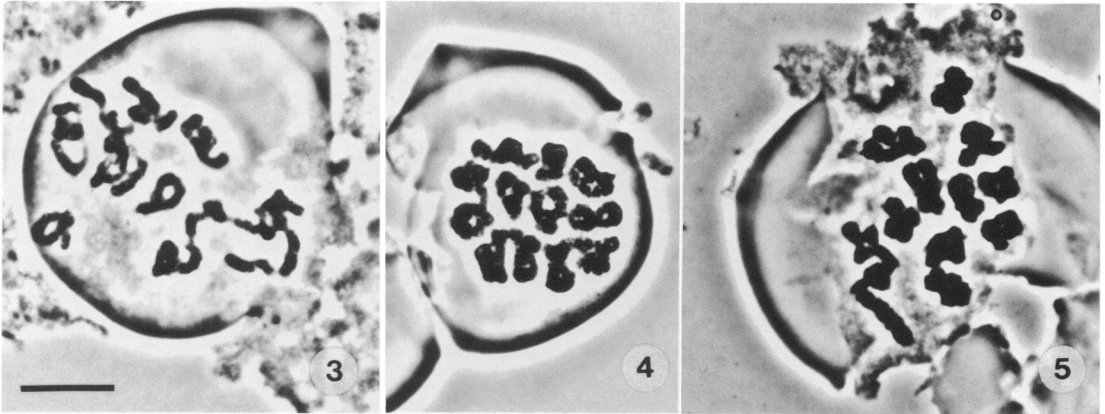
micropyle. When *C. diversifolia* was used as the female parent, numerous pollen tubes could usually be seen in the ovary, but they were faint. In pollinations of *C. corymbiflora* with *C. acuminata* and *C. diversifolia*, most pollen tubes were inhibited in the style and just a few were observed in the ovary.

In spite of generally good pollen tube growth, 85% (1,134 out of 1,331) of the interspecific crosses failed to produce fruits. Presumably most of these crosses failed soon after fertilization. Fruits with no viable seeds resulted from 12% of the crosses (158 out of 1,331). Only 3% (39 out of 1,331) of the crosses attempted resulted in fruits with full-sized seeds, and not all of these germinated. In nearly all cases, successful hybrids were obtained in one direction only, the reciprocal cross failing to set viable seeds.

Fruits were produced in only 15% of the crosses, and most of them were seedless or the seeds failed to germinate. Seed abortion followed two main patterns similar to those outlined by Whalen, Costich, and Heiser (1981) for *Solanum* sect. *Lasiocarpa*. In the first case, seeds aborted at an early stage, and were very small and underdeveloped. Often the fruits were also smaller than usual, but in some cases the fruits reached full size. In other cases, the testa grew to nearly full size, but the seeds were empty. Apparently, hybrid seed abortion occurred at a relatively late stage in these crosses.

Full-sized fruits with a few large seeds were produced in the cross of *C. acuminata* × *C. betacea*. Most of the seeds were empty and did not germinate. The single seed that sprouted died as a seedling.

All interspecific combinations using *C. betacea* as the female parent resulted in some fruit development, but F_1 hybrids have been obtained only in crosses with *C. acuminata*. Hybrids were originally reported from crosses of *C. betacea* with *C. obliqua* (Bohs, 1986), but the resulting plants resembled *C. betacea* in all vegetative and floral features and were apparently the products of contamination with *C. betacea* pollen. The *C. betacea* × *acuminata* hybrids are diploids ($2n = 24$) and are intermediate in morphology between the two parental species. The hybrids are self-compatible, as judged by both pollen tube growth and fruit set after selfing. Fruits were set in backcrosses with *C. betacea* in both directions and in backcrosses with *C. acuminata* as the male parent. Pollen tubes reached the ovaries in at least a few crosses in every combination of the hybrid and parents, but in general the hybrids had very low pollen germination and the tubes showed frequent abnormalities. Pollen stain-



Figs. 3–5. Chromosomes of *Cyphomandra* hybrids at meiosis I. Bar = 10 μ m. 3. *C. betacea* \times *acuminata*. 4. *C. uniloba* \times *betacea*. 5. *C. corymbiflora* \times *diploconos*.

ability of the hybrid was very low (\bar{X} = 6%, range 0–18%, measured from 22 flowers on 18 plants). Examination of chromosomes in pollen mother cells at meiotic metaphase I showed many irregularities, including unpaired segments, univalents, and chains (Fig. 3). Micronuclei resulting from unincorporated univalents or fragments were seen at later stages. Two fruits resulting from F_1 self-pollinations ripened. One contained four and the other five full-sized seeds. None germinated.

Vigorous hybrid plants resulted from the cross *C. uniloba* \times *C. betacea*. The F_1 plants were intermediate between the two parents in floral characters. Both parent species and the hybrids were similar vegetatively. The hybrids were diploids ($2n = 24$). Good pollen tube growth into the ovaries and abundant fruit set upon selfing demonstrated that the hybrids were self-compatible. Pollen stainability of the hybrids was high (\bar{X} = 64%, range 2%–88%, measured from 11 flowers on nine plants). The chromosomes of *C. uniloba* are roughly twice the size as those of *C. betacea* (Pringle and Murray, 1991), and some of these size differences could be seen at diakinesis in pollen mother cells. Pairing appeared to be normal at metaphase I (Fig. 4). Micronuclei resulting from excluded chromosome fragments or unpaired univalents were seen at a frequency of approximately 11% in anaphase I and later stages (619 PMCs examined), but most divisions appeared normal. Numerous fruits resulted from selfing the F_1 s, but they are not yet ripe. Backcrosses to *C. betacea* produced full-sized fruits containing an average of 15.5 seeds per fruit (range 0–30, $N = 8$ fruits). A few germinated. All backcrosses to *C. uniloba* failed (20 attempts). Pollen tubes were observed in the ovary in backcrosses with *C. betacea* in both di-

rections. The reciprocal cross, *C. betacea* \times *C. uniloba*, yielded full-sized but seedless fruits.

Hybrid plants were also obtained from the cross *C. corymbiflora* \times *C. diploconos*. The F_1 plants had good pollen stainability (\bar{X} = 63%, range 23%–77%, measured from 12 flowers on 12 plants). They were intermediate between the parents in vegetative and floral characters. Mitotic observations indicated that they were diploids ($2n = 24$). Most cells had ten bivalents and one chain quadrivalent at meiotic metaphase I (Fig. 5), although the following arrangements were occasionally observed: 10 II + 1 I + 1 III, 11 II + 2 I, and 9 II + 2 I + 1 IV. The chain quadrivalent configuration is diagnostic for a translocation heterozygote. A few of the bivalents were unequal. Most anaphase I and II stages appeared normal, but occasionally lagging chromosomes or one to two micronuclei were seen in the tetrads. Observations of pollen tube growth and fruit set after selfing indicate that the F_1 plants are self-incompatible, like the parents. Three fruits from F_1 sib pollinations contained an average of 13 seeds (range 11–14), and a few germinated to produce F_2 seedlings. Backcrosses to *C. corymbiflora* also yielded fruits with viable seeds. Backcrosses to *C. diploconos* resulted in full-sized but still unripe fruits. Pollen tubes reached the ovary in all combinations of the hybrids and parents. The reciprocal cross, *C. diploconos* \times *C. corymbiflora*, produced full-sized fruits. Most had very small and underdeveloped seeds, and the few large seeds that developed failed to germinate.

Although three trials of the cross *C. hartwegii* \times *C. diploconos* resulted in fruit set, only one contained well-developed seeds. These produced F_1 plants that vegetatively resembled *C. hartwegii*. Unfortunately, these putative hy-

brids did not survive long enough to flower or to be evaluated cytologically, and this result has not been reproduced in other crosses of *C. hartwegii* with *C. diploconos*.

DISCUSSION

The data indicate that SI is widespread in *Cyphomandra*. According to Pandey (1957) and Lewis (1979), SI in the Solanaceae operates by a one- or two-locus, multiallelic homomorphic gametophytic system. It can be inferred that *Cyphomandra* also exhibits gametophytic self-incompatibility (GSI) based on knowledge from other solanaceous groups (de Nettancourt, 1977, 1984; Haring et al., 1990) as well as on characters associated with GSI species (i.e., homomorphic flowers, binucleate pollen, wet stigmatic surfaces, and pollen tube growth arrested in the style in incompatible crosses) (Brewbaker, 1957; Pandey, 1960).

Many authors since Darwin (1876) and East and Park (1917) have emphasized the importance of SI as a mechanism preventing selfing and/or ensuring outcrossing. *Cyphomandra*s are relatively long-lived trees with low population densities and patchy distributions in light gaps. Reproductive success in these SI species may depend on pollinators that fly between populations or gaps. Although data on pollinators of *Cyphomandra* are inadequate, the few reports available indicate that large bees, especially euglossines, may be the primary pollen vectors in the genus (Soares et al., 1989). These bees, which can fly long distances between resource patches (Janzen, 1971), may ensure the success of the SI strategy. Further investigations of pollinators in *Cyphomandra* may shed light on this question. In any case, the frequent occurrence of SI or other mechanisms favoring outcrossing (dioecy, andromonoecy, enantiostyly) in the Solanaceae (Whalen, 1979; Whalen and Costich, 1986; Anderson and Symon, 1989) indicate that such systems are important in the evolutionary history of these organisms.

SI is widely distributed in angiosperms (Whitehouse, 1950; Fryxell, 1957; de Nettancourt, 1977; Charlesworth, 1985), and can rather easily be broken by mutations in the sterility genes (de Nettancourt, 1977; Hogenboom, 1979; Lewis, 1979). However, reversions to SI from SC are rare (Stebbins, 1957; de Nettancourt, 1977; Lewis, 1979). For these reasons, SC is usually considered as being derived from SI. SI has been previously reported in at least six solanaceous genera (*Lycopersicon*, *Lycium*, *Nicotiana*, *Petunia*, *Physalis*, and

Solanum; Fryxell, 1957; Whalen and Anderson, 1981; Charlesworth, 1985). Some of these genera are rather distant phylogenetically and most contain some SC species. If SI is primitive in the Solanaceae, SC has evolved many times in the family.

Likewise, SC is probably derived in *C. betacea*, and may be related to its domesticated status. There are many examples of SC crop species that have been derived from SI relatives (Rick, 1988). The switch from SI to SC accompanying domestication in these species may have been selected for in order to promote establishment of the species outside its native area where outcrossing might not be possible (Baker, 1955), to ensure genetic uniformity in the crop, or to increase fruit yield (Rick, 1988). The latter is probably most important in the case of *C. betacea*. The increased fruit set brought about by the switch to SC in this species has probably been reinforced by human selection, and is perhaps one of the reasons that *C. betacea* has been much more commonly cultivated than the other SI species of the genus (Bohs, 1989a).

The SC species *C. betacea* exhibits none of the morphological characters often associated with obligate or predominant autogamy, such as reduced corollas, shorter styles, or scentless flowers (Ornduff, 1969; Wyatt, 1988), as compared to other SI species in the genus. Shortening of the style so that the stigma is equal to or shorter than the anthers is correlated with a switch from outcrossing to selfing in domesticated tomato and eggplant (Rick, 1988). Anderson (1977, 1979; Whalen and Anderson, 1981) found that SC and autogamous species of *Solanum* sect. *Basarthurum* had pistils that nearly equaled the length of the staminal column, whereas the SI species had exerted styles. Probably *C. betacea*, although SC, is predominantly xenogamous and has not evolved adaptations ensuring self-pollination.

Lewis and Crowe (1958) proposed that the breeding system of a species can influence its behavior in interspecific crosses. Using species from the Solanaceae and other families, they presented evidence to show that pollen tubes from SC plants cannot penetrate the styles of SI species, whereas all other combinations were successful. This behavior, which they termed unilateral interspecific incompatibility, has been frequently reported in SI species of Solanaceae (e.g., Hogenboom, 1972 and references therein). Lewis and Crowe (1958) interpret this pattern as signifying that the S-locus can influence not only intraspecific (self-) incompatibility but also interspecific crossing failure. According to Lewis and Crowe (1958),

the main exception to this pattern occurs in the case of SC species which they regard as having recently mutated from SI. In this case, pollen from SC species can penetrate SI styles. If Lewis and Crowe are correct, the pollen tube data from *Cyphomandra* support the contention that *C. betacea* has only recently evolved SC from SI ancestors. No evidence of inhibition of *C. betacea* pollen tubes was seen in any of the SI *Cyphomandra* species.

On the other hand, exceptions to Lewis and Crowe's unilateral incompatibility rule have been reported (e.g., Pandey, 1962; Grun and Aubertin, 1966; Abdalla and Hermesen, 1972; Hogenboom, 1973), including cases of inhibition of SC \times SC, SC \times SI, and SI \times SI crosses as well as success in SI \times SC combinations. The role of the S-locus in interspecific incompatibility is a controversial issue (de Nettancourt, 1984; Hogenboom, 1984). Unilateral interspecific incompatibility was not seen in the present study, supporting the hypothesis that interspecific crossing failure is controlled by genes other than the S-alleles governing SI. However, more evidence is needed to resolve this question.

Interspecific hybrids are seldom obtained in *Cyphomandra* with the species studied, and internal barriers to hybridization seem to be well-developed. These results are similar to those of Anderson (1975, 1977, 1979) for *Solanum* section *Basarthurum*, but differ from the easy hybridization between species of *Solanum* section *Petota* (Grun, 1961; Hawkes and Hjeriting, 1969, 1989; Hawkes, 1990) and *Solanum* section *Solanum* (Edmonds, 1977). Internal barriers also seem to play a role in restricting the success of some crosses in *Solanum* section *Androcera* (Whalen, 1979), *Solanum* section *Lasiocarpa* (Whalen, Costich, and Heiser, 1981), *Solanum* subgenus *Leptostemonum* (Rao, 1979), and in the genus *Lycopersicon* (Hogenboom, 1979; Rick, 1979).

Internal barriers to crossability can act at many stages: the failure of pollen tubes to penetrate the style, failure of fruit set or seed development (at both early and late stages), and hybrid mortality, sterility, or breakdown in succeeding generations. In this study, pollen tubes reached the ovaries in nearly all crosses between *Cyphomandra* species and in many cases could be seen entering the ovular micropyles. Although fertilization was not positively demonstrated, it is likely that it occurred. Postzygotic factors thus may be the most important internal barriers to hybridization within the genus. Over 80% of the crosses failed completely, and seedless fruits were produced in 12% of the crosses, especially using *C. acu-*

minata, *betacea*, *hartwegii*, and *uniloba* as female parents. In many crosses yielding seedless fruits, seed abortion occurs at a relatively late stage. Seed abortion in interspecific crosses was also observed in *Solanum* sections *Androcera* (Whalen, 1979), *Lasiocarpa* (Whalen, Costich, and Heiser, 1981), *Basarthurum* (Anderson, 1977) and *Petota* (Dionne, 1961; Johnston et al., 1980; Johnston and Hanneman, 1980, 1982), and in the genus *Datura* (Avery, Satina, and Rietsema, 1959). The factors governing hybrid seed abortion in interspecific crosses are not well understood, but Edmonds (1977) suggested that it is due to the inability of the endosperm to nourish the embryo, disharmony between parental genomes, or both. Endosperm failure was found responsible for hybrid seed abortion in some species of *Datura* and *Solanum* section *Petota* (Beamish, 1955; Lee and Cooper, 1958; Avery, Satina, and Rietsema, 1959; Johnston et al., 1980; Johnston and Hanneman, 1980, 1982).

Nonreciprocal crossing success was commonly seen in *Cyphomandra*. Nonreciprocal crossability was also noted in *Solanum* section *Basarthurum* by Anderson (1975), who implicated gene-cytoplasm interactions as the cause, and in *Solanum* section *Petota* by Dionne (1961), Grun (1961), Pandey (1962), Grun and Aubertin (1966), and Johnston and Hanneman (1980, 1982). Grun (1961) attributed crossing failure in these cases to unfavorable relationships between the endosperm and embryo. Johnston et al. (1980) and Johnston and Hanneman (1980, 1982) emphasized the importance of endosperm development in determining success in interspecific crosses, and demonstrated that the success of interspecific and interploidy crosses depends on the ratio of maternal to paternal genomes in the hybrid endosperm. All of these factors, as well as disharmony between the endosperm and maternal tissue have been found to cause nonreciprocal crossing success in various plant groups (Stebbins, 1958).

Other postfertilization barriers to hybridization include hybrid mortality, sterility, or breakdown in subsequent generations. Sterility is observed in the *Cyphomandra* hybrids. Plants of three interspecific combinations (*C. betacea* \times *acuminata*, *corymbiflora* \times *diploconos*, and *uniloba* \times *betacea*) are vegetatively vigorous, but all three have reduced pollen stainability as compared to the parents. Seed set and viability are also low in the hybrids and backcrosses as compared to the parents. Meiotic irregularities were seen in the hybrids, evidently caused in part by structural differences in the chromosomes of the parent species. The

vigor and fertility of the F_2 and backcross generations remain to be evaluated.

Because crossing success is dependent on many factors (e.g., temperature, light, season, pollen fertility, methodology involved in pollination, etc.), perhaps more importance should be attached to crossing success than failure. Other crosses at different times of the year or with different accessions might be successful. For this reason, only the successful crosses are discussed in detail here.

The interpretation of crossing behavior in a phylogenetic context is usually not straightforward. On the one hand, crossing success has been considered a primitive trait, as species that will successfully hybridize may not have evolved internal isolating mechanisms (Rosen, 1979). Similarly, species very closely related phylogenetically may have developed strong internal barriers to crossability. On the other hand, crossing success indicates at least some degree of genomic similarity, and may link genetically compatible taxa while separating them from genetically divergent taxa. Crossing relationships may or may not correspond with morphological similarity (Stebbins, 1950; Grant, 1981). In certain solanaceous groups, crossing relationships were sometimes congruent with those derived from morphological studies (e.g., Anderson, 1975; Edmonds, 1977), and in other cases they were not (Rao, 1979). For these reasons, it is often difficult to interpret complex patterns of interspecific crossability. In *Cyphomandra*, drawing phylogenetic conclusions from crossing data is even more difficult because no infrageneric classification schemes based on morphology or other data exist for comparison. Thus, few robust phylogenetic patterns can be deduced from the crossing results reported here, although they suggest several intriguing systematic and evolutionary hypotheses.

In the present study, crossing success was more or less congruent with morphological similarity in some cases, but in others, hybrids were produced between very dissimilar species. For instance, the success of the combination *C. corymbiflora* \times *diploconos* was unexpected on the basis of morphology. The two species differ in numerous vegetative and floral characters. Furthermore, their ranges overlap in southeastern Brazil, and one might expect them to have developed strong internal barriers to hybridization. Despite the ability to cross under artificial conditions, hybrids between these species are apparently rare in nature, for there are no instances of morphological intermediates in the herbarium material I have examined. Evidence given above indi-

cates that the two species differ in their chromosome structure. In addition, external isolating mechanisms such as microhabitat differences, differences in flowering times, or ethological isolation by pollinators may operate in nature to restrict gene flow between the two species. According to data from herbarium sheets, there are few consistent differences between the two species in time of flowering. Their habitat preferences also seem to overlap, although *C. diploconos* is in general more coastal and found at lower elevations and perhaps wetter habitats than *C. corymbiflora*. Very great differences in floral morphology between the two species may implicate pollinator specialization as an isolating mechanism. Recent work by Soares et al. (1989) on *Cyphomandra calycina* Sendtn. indicates that male glossine bees are the main pollinators of this Brazilian species. The bees brush the anther connectives with their forelegs in order to gather volatile odor substances. Presumably, the odor substances act as attractants for the bees. If the same type of pollination syndrome occurs in *C. corymbiflora* and *C. diploconos*, differences in the chemical constituents of the anther connectives could result in differences in pollinator preferences, and thus act as barriers to hybridization. This intriguing phenomenon needs further field study. In the reciprocal cross (*C. diploconos* \times *corymbiflora*), all the F_1 seeds failed to germinate, so internal factors may be a major factor limiting success of this cross.

Conversely, crossing success with *C. betacea*, *C. uniloba*, and *C. acuminata* correlates well with morphological similarity. All three species have exclusively simple leaves with cordate bases, coriaceous stellate corollas, short broad anthers, and rather thick cylindrical styles. *Cyphomandra acuminata* and *C. uniloba* are sympatric and endemic to Bolivia, and are so similar on herbarium sheets that they can scarcely be distinguished without mature fruits and seeds. *Cyphomandra betacea* is presently found only in cultivation. Its natural range, wild relatives, and place of origin are unknown, although wild populations have been reported in southern Bolivia and northwestern Argentina (Bohs, 1989a). Although *C. betacea* will cross with *C. acuminata* and *C. uniloba*, this is not strong evidence supporting the relatedness of the three species, given the uncertainties mentioned above. Also, we currently know nothing about the influence of artificial selection on the morphology or breeding behavior of *C. betacea* as compared with the wild species of the genus. Furthermore, without knowledge of the natural distribution of this species, it is difficult to surmise with which species crossing

barriers might have arisen or whether gene flow between the species is restricted by substantial geographic disjunctions. No evidence of natural hybridization has been noted from herbarium specimens, but, as stated above, the species are very similar morphologically and such hybrids may be easily overlooked.

On the other hand, the sympatric species *C. uniloba* and *C. acuminata* have well-developed internal barriers to hybridization. Present distributional data puts *C. acuminata* at higher elevations than *C. uniloba* (L. Bohs, unpublished data). The corolla color is very different in the two species (red to purple in *C. acuminata*, green or yellow-green in *C. uniloba*), and may serve to attract different pollinators. Volatile components of the anther connectives may also affect pollinator visitation, as proposed above for *C. corymbiflora* and *C. diploconos*. Thus, external isolating mechanisms may also play a role in enforcing reproductive isolation in these species.

The success of interspecific hybrids with *C. betacea* has positive implications for the improvement of the cultivated tree tomato and other species of *Cyphomandra* through breeding. Although many interspecific combinations are not successful, it seems possible to introduce at least some desirable characteristics into *C. betacea* and other species by conventional breeding methods. Embryo rescue may also be of use in obtaining hybrids from combinations in which endosperm failure is responsible for failure of the cross.

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