Interspecific Crossability of Selected *Salvia* Species and Potential Use for Crop Improvement

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ABSTRACT. The wide diversity in the genus *Salvia* represents an untapped genetic resource to improve and diversify *Salvia* grown as floriculture crops. Interspecific hybrids have formed naturally or by chance hybridization of cultivated plants, but the degree to which species are cross-compatible is largely unknown. The crossability of nine *Salvia* species selected to cover a wide range of the diversity in European and American species was evaluated in a full diallel mating scheme. Overall, crossability of the selected species was low with only five of 72 interspecific cross combinations producing viable seed, whereas all nine species were self-fertile. Successful crosses were mostly within close phylogenetic groupings. The majority of successful crosses were between species with different chromosome numbers, suggesting that chromosome number differences alone are not a major barrier to hybridization in this genus. A *Salvia nemorosa* × *Salvia transsylvanica* F₂ population exhibited transgressive segregation for several horticulturally important traits, including flower size, plant height, and time to flower. Plant height was correlated positively with flower length, inflorescence branch number, and time to flower. Time to flower was correlated positively with flower length. Individuals with desirable trait combinations were identified within the population.

With 900 species, native to every continent except Antarctica and Australia (Mabberly, 2008) and possessing a myriad of growth habits and flower colors, the genus *Salvia* represents an extraordinary range of diversity. Several species are widely used as ornamental plants. *Salvia splendens* and *S. farinacea* are widely grown seed-propagated annual bedding plants, whereas *S. nemorosa* is a popular herbaceous perennial species with both seed and vegetatively propagated cultivars. Although these and other species of *Salvia* are widely used horticulture crops, the diversity of the genus represents a largely untapped resource for crop improvement, which might be accessed by means of interspecific hybridization. There are a number of known interspecific hybrids in the genus. However, with the exception of intentional crosses between culinary sage (*S. officinalis*) and *S. lavandulifolia* (Sanchez-Gomez et al., 1995), *S. fruticosa*, and *S. tomentosa* (Putievsky et al., 1990), all reported hybrids have occurred spontaneously either in the wild or in cultivation. There is a paucity of information concerning the interspecific fertility of *Salvia* species.

The crossability of two species is often related to phylogenetic relationship and chromosome number. Crosses between closely related species with the same chromosome number are more likely to hybridize and produce fertile progeny, whereas crosses between distantly related species with different chromosome numbers are more likely to either not hybridize or to produce sterile offspring resulting from genetic incongruity or meiotic errors (Harlan and DeWet, 1971; Sybenga, 1992).

The phylogenetic grouping of species into the genus *Salvia* was based on a key morphological feature, namely the elongated connective between the two thecae of the anthers (Cläßen-Bockhoff et al., 2003). The validity of this grouping was called into question by Walker et al. (2004), who constructed a phylogeny based on *rbcL* and *trnL-F* gene sequences from 127 *Salvia* taxa. Based on these data, they concluded that *Salvia* is

not monophyletic, but rather composed of three separate monophyletic clades. Although the polyphyletic nature of the genus was unexpected, the clades they described largely corresponded with the most widely accepted infrageneric grouping of the genus based on Bentham (1848, 1876). Clades 1 and 3 comprise all European, Asian, and African *Salvia* species and one small, morphologically distinct group of American species belonging to Clade 1. Clade 1 is primarily native to Europe and includes members of subgenera *Salvia*, *Leoinia*, and *Sclarea*, whereas Clade 3 is largely East Asian and corresponds generally to section *Drymospace*. Clade 2 is comprised of species that are exclusively native to the Americas and is comprised of the subgenera *Audibertia* and *Calospatha*. Because the clades defined by Walker correspond largely with geographical distribution and with the subgenera and sections of Bentham, it is possible to extrapolate from the species included in Walker's study to classify most other *Salvia* species as probable members of one of the three clades. The wide divergence of these clades, especially Clades 1 and 2, lead to the hypothesis that crosses between the different clades will not be successful.

The chromosome numbers of *Salvia* species are unusual in their extreme variability. Published counts range from a low of $2n = 12$ in *S. hispanica* (Harley and Heywood, 1992) to a high of $2n = 88$ in the octoploid *S. guaranitica* (Alberto et al., 2003). In addition to wide variation in ploidy level in *Salvia*, the basic number of chromosomes is also wide-ranging with x reported as 6, 7, 8, 9, 10, 11, and 15 for species within the genus (Goldblatt and Johnson, 1979; Harley and Heywood, 1992). Seven of 13 species studied by Alberto et al. (2003) had at least one known supernumerary chromosome (non-essential chromosomes absent in some individuals). It is possible that closely related species that appear to have different basic chromosome numbers might simply have supernumerary chromosomes that have not been properly identified as such.

To evaluate the potential of selected *Salvia* species as genetic sources for improvement of cultivated *Salvia* species and to provide information for plant breeders interested in

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developing hybrid *Salvia*, our objectives were to: 1) test the interspecific crossability of a select number of *Salvia* species from different clades and with different chromosome numbers; 2) establish chromosome numbers for selected *Salvia* species either lacking published counts or with inconsistently reported counts; and 3) evaluate inheritance of crop quality traits in the interspecific hybrid *S. nemerosa* × *S. transsylvanica*.

Materials and Methods

PLANT GROWTH AND POLLINATION CONDITIONS. Nine *Salvia* species native to Europe and North and South America from Clades 1 and 2 of the genus (as defined by Walker et al., 2004) were chosen to incorporate a wide range of native climates, growth habits, chromosome numbers, and flower colors (Table 1). One genotype per species was used. *Salvia transsylvanica*, *S. verticillata*, *S. azurea*, and *S. nemerosa* ‘Rosenwein’ were grown from seed, and a single representative individual was selected and clonally propagated for crossing. All other species were obtained as vegetative propagules. All plants were grown in a greenhouse from Oct. 2007 through May 2009 under a 16-h photoperiod achieved with ambient daylight extended as needed with supplemental high-pressure sodium lighting (50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 20 °C. All species in the study flowered under these conditions with the exception of *S. madrensis*. *Salvia madrensis* is a fall-flowering plant in the wild; therefore, plants were transferred to short-day conditions (a truncated 9-h photoperiod obtained by covering plants with opaque cloth from 1700 to 0800 HR daily), which induced flowering in *S. madrensis* after 3 weeks (data not shown).

Species were crossed in a full diallel mating scheme. In all cases, except for *S. nemerosa*, the same genotype was used both as the pollen parent and seed parent for all crosses. *Salvia nemerosa*, however, is gynodioecious with a male sterility gene and an accompanying complex of lethality and sublethality factors (Linnert, 1958). Therefore, for crosses involving *S. nemerosa*, the male-sterile ‘Caradonna’ was used as the seed parent, and the male-fertile ‘Rosenwein’ was used as the pollen parent. For each interspecific cross combination, 100 flowers were pollinated. Pollinations were conducted from Oct. 2007 through Jan. 2008 and replicated from Oct. 2008 through Jan. 2009 with 50 pollinations of each cross combination each year.

The effects of year were not significant, so data from both replications were subsequently pooled. Seed parent flowers were emasculated the day before anthesis. Once the style had fully elongated, pollen was applied to the stigma directly from the dehiscing anther of the pollen parent. Pollinated flowers were observed for partial or complete seed development. *Salvia* floral morphology is such that the developing seeds are exposed to view, and any growth of the developing seed is easily observed. Crosses were rated unsuccessful if no seed development was observed, successful if mature, viable seed was produced, and considered abortive if seeds partially developed (as observed by an increase in size after pollination but abortion before maturity). To determine the level of self-fertility of the parent plants, 50 flowers of each species were self-pollinated as described for the cross-pollinations (25 each year), although without emasculation. *Salvia nemerosa* was not self-pollinated as a result of male sterility as described previously and instead the cultivars Caradonna and Rosenwein were crossed. After pollination, seeds were observed and harvested as described for the interspecific crosses. All mature seed produced by successful crosses was harvested and sown under intermittent mist with bottom heat to observe germination.

HYBRID CONFIRMATION AND FERTILITY. The hybridity of the germinated seedlings was first observed based on intermediate phenotype and then confirmed using random amplified polymorphic DNA (RAPD) markers. DNA was isolated from leaf tissue of each putative hybrid using the DNeasy Plant Mini kit (Qiagen, Valencia, CA) following the manufacturer’s protocol. Primer 3 (5′-agtcagccac-3′) from an Operon RAPD 10mer primer kit A (Eurofins MWG Operon, Huntsville, AL) was used to amplify DNA for the F₁s *S. transsylvanica* × *S. nemerosa*, *S. nemerosa* × *S. transsylvanica*, *S. transsylvanica* × *S. involucrata*, *S. guaranitica* × *S. involucrata*, and their parent species. Primer 5 (5′-agggtcttg-3′) from the same kit was used to amplify DNA from the F₁ *S. greggii* × *S. involucrata* and its parent species. Polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 94 °C for 2 min followed by 40 cycles of 94 °C for 2 min, 40 °C for 2 min, and 72 °C for 2 min with a final elongation step at 72 °C for 10 min. PCR products were separated on a 1% agarose gel containing ethidium bromide and visualized under ultraviolet light.

The fertility of the F₁ hybrids was measured by self-pollinating 50 flowers from each hybrid population. Hybrid

Table 1. Parental genotypes, native range, flower color, phylogenetic clade, and accession information for *Salvia* species used in a full diallel mating scheme.

Species	Native range	Flower color	Clade	Accession/source
<i>Salvia azurea</i>	Central United States	Blue	2	W6 17691 ^z
<i>Salvia greggii</i> ‘Lipstick’	Northern Mexico	Red	2	PDN ^y
<i>Salvia guaranitica</i> ‘Black and Blue’	Central South America	Blue	2	PDN
<i>Salvia involucrata</i> ‘Hadspen’	Mexico	Magenta	2	AWS ^x
<i>Salvia madrensis</i> ‘Red Neck Girl’	Mexico	Yellow	2	PDN
<i>Salvia nemerosa</i> ‘Caradonna’	Central Europe	Purple	1	PDN
<i>Salvia nemerosa</i> ‘Rosenwein’	Central Europe	Pink	1	BTWS ^w
<i>Salvia roemeriana</i>	Northern Mexico	Red	1	AWS
<i>Salvia transsylvanica</i>	Eastern Europe	Purple	1	BTWS
<i>Salvia verticillata</i>	Central Europe	Purple	1	PI 380900 ^z

^zU.S. Department of Agriculture National Plant Germplasm System accession number.

^yPlant Delights Nursery, Raleigh, NC.

^xA World of Salvias, Candor, NC.

^wB&T World Seeds, Pagnuignan, Aigues-Vives, France.

plants were also back-crossed to both of the parent species with 50 pollinations made for each reciprocal combination with each parent. Like with the original crosses, seed development was observed and any mature seed that developed was sown to observe germination.

TRAIT ANALYSIS AND INHERITANCE. Morphological traits were measured on the F_1 hybrid plants and parents at the time of first flowering. Length and width of rosette leaves was measured, and height and length of individual flowers were measured. Measurements were made on six independent plants vegetatively propagated from a representative F_1 individual and the parental species. Inheritance of horticulturally important traits in the *S. nemorosa* × *S. transsylvanica* hybrid was evaluated on a family of 124 F_2 plants derived from a single representative F_1 individual, parent species, and F_1 plants. Total plant height, flower length, number of branches in the inflorescence, time to flower, leaf length, and number of leaves produced before flowering were measured at the time of first flowering.

CHROMOSOME COUNTS. Chromosome counts were performed on *S. greggii*, *S. involucrata*, *S. madrensis*, and *S. transsylvanica* because published chromosome counts were unavailable or published counts were inconsistent. In addition, chromosomes were counted for plants from all hybrid populations produced in this study. Chromosome counting protocols were adapted from Singh (2003). For all species and hybrids, counts were performed on root tips. For *S. transsylvanica* and *S. transsylvanica* × *S. involucrata*, counts were also performed on anthers. To collect root tips, greenhouse-grown plants were removed from their pots, and exposed root tips were collected and placed directly into a pretreatment solution of 2 mM 8-hydroxyquinoline. For anther counts, flower buds between 0.75 and 1.5 mm long were removed from greenhouse-grown plants and placed directly in 2 mM 8-hydroxyquinoline. Root tips and flower buds in the 8-hydroxyquinoline pretreatment solution were placed in 18 °C for 3 to 4 h. Tissues were then transferred to 3:1 solution of 100% ethanol and glacial acetic acid for 24 h for fixation and then to 70% ethanol for storage. Tissues were stored in ethanol for 1 week before squashing. Root tips were then squashed in a drop of 2% acetocarmine stain and observed at 1000× magnification using phase contrast. The developing anthers were dissected out of the flower buds under a dissecting microscope at 10× magnification, then squashed and observed as described for the root tips. A minimum of five cells with clearly visible chromosomes was observed for each genotype.

DATA ANALYSIS. Analyses of variance, means separation (determined by Fisher's least significant difference at $P = 0.05$), and Pearson correlation coefficients were determined using SPSS (Version 17.0 for Windows; SPSS, Chicago, IL).

Results

Overall, crossability among the selected species was low with only five of 72 interspecific cross combinations producing viable seed. However, nearly half of the crosses produced some seeds showing partial development. The 37 cross combinations producing viable or partially developed seed are presented in Table 2. In most cases, only limited numbers of abortive seeds were produced, usually in 5% of pollinations or fewer, with the exception of *S. nemorosa* × *S. roemeriana* in which nearly all seeds began to develop and aborted (data not shown). Only one species combination, *S. nemorosa* and *S. transsylvanica*, was

Table 2. Interspecific crosses and self-pollinations from a full diallel mating scheme that yielded complete or partial seed development.^z

Cross	Successful crosses (%)	Avg seeds per cross (no.)
<i>Salvia. azurea</i> × self	46	1.3
<i>S. azurea</i> × <i>Salvia greggii</i>	pd ^y	0
<i>S. azurea</i> × <i>Salvia guaranitica</i>	pd	0
<i>S. azurea</i> × <i>Salvia madrensis</i>	pd	0
<i>S. azurea</i> × <i>Salvia verticillata</i>	pd	0
<i>S. greggii</i> × self	40	1.7
<i>S. greggii</i> × <i>S. guaranitica</i>	pd	0
<i>S. greggii</i> × <i>Salvia involucrata</i>	34	1.5
<i>S. greggii</i> × <i>S. madrensis</i>	pd	0
<i>S. greggii</i> × <i>Salvia roemeriana</i>	pd	0
<i>S. guaranitica</i> × self	32	2.4
<i>S. guaranitica</i> × <i>S. involucrata</i>	1	1
<i>S. guaranitica</i> × <i>S. madrensis</i>	pd	0
<i>S. guaranitica</i> × <i>S. roemeriana</i>	pd	0
<i>S. involucrata</i> × <i>S. azurea</i>	pd	0
<i>S. involucrata</i> × <i>S. greggii</i>	pd	0
<i>S. involucrata</i> × <i>S. guaranitica</i>	pd	0
<i>S. involucrata</i> × self	6	2.3
<i>S. madrensis</i> × <i>S. greggii</i>	pd	0
<i>S. madrensis</i> × <i>S. involucrata</i>	pd	0
<i>S. madrensis</i> × self	28	1.4
<i>S. madrensis</i> × <i>S. roemeriana</i>	pd	0
<i>S. madrensis</i> × <i>S. verticillata</i>	pd	0
<i>Salvia nemorosa</i> × <i>S. nemorosa</i> ^x	90	1.7
<i>S. nemorosa</i> × <i>S. roemeriana</i>	pd	0
<i>S. nemorosa</i> × <i>Salvia transsylvanica</i>	72	1.3
<i>S. nemorosa</i> × <i>S. verticillata</i>	pd	0
<i>S. roemeriana</i> × <i>S. azurea</i>	pd	0
<i>S. roemeriana</i> × <i>S. guaranitica</i>	pd	0
<i>S. roemeriana</i> × <i>S. involucrata</i>	pd	0
<i>S. roemeriana</i> × <i>S. nemorosa</i>	pd	0
<i>S. roemeriana</i> × self	100	3.8
<i>S. roemeriana</i> × <i>S. transsylvanica</i>	pd	0
<i>S. transsylvanica</i> × <i>S. involucrata</i>	1	1
<i>S. transsylvanica</i> × <i>S. nemorosa</i>	1	1
<i>S. transsylvanica</i> × self	100	2.6
<i>S. verticillata</i> × self	18	1

^zPercentage of successful crosses between species is out of 100 pollinations, whereas percentage of successful self-pollinations is out of 50 pollinations. Average seeds per cross is out of a maximum potential of four seeds.

^ypd indicates crosses that showed partial development (an increase in size) but aborted before seed maturation.

^x*Salvia nemorosa* 'Caradonna' × *S. nemorosa* 'Rosenwein'.

successful in both reciprocal crosses (Table 2). Success rate of this cross combination was dramatically lower with *S. transsylvanica* as the seed parent than as the pollen parent. For the other three successful hybrid combinations (*S. transsylvanica* × *S. involucrata*, *S. greggii* × *S. involucrata*, *S. guaranitica* × *S. involucrata*), the reciprocal crosses produced no viable seed. Self-pollination of the parent species revealed significant variability in self-fertility. Although most species showed a high degree of fertility when self-pollinated, *S. verticillata* and *S. involucrata* had very low seed set (Table 2). For successful cross combinations, all of the F_1 seeds germinated (data not shown).

In all hybrids, at least one phenotypic trait varied significantly from the parent species (Table 3). In most cases, hybrid traits were intermediate between the two parents, although in the crosses *S. transsylvanica* × *S. involucrata* and *S. transsylvanica* × *S. nemorosa*, leaf length and width of the F₁ were significantly larger than either parent plant. Hybridity was confirmed based on RAPD banding pattern (Fig. 1).

Of all hybrids produced, *S. nemorosa* × *S. transsylvanica* showed the highest fertility when used as the female parent and back-crossed to either parent species (Table 4). *Salvia transsylvanica* × *S. involucrata* was highly fertile when self-pollinated but failed to back-cross with either parent species. The *S. transsylvanica* × *S. nemorosa* hybrids exhibited low self-fertility but moderate fertility when back-crossed to either parent species. All harvested mature seeds for each cross combination germinated successfully (data not shown). The other two hybrids produced, *S. greggii* × *S. involucrata* and *S. guaranitica* × *S. involucrata*, were completely sterile when selfed or back-crossed to either parent and are therefore not included in Table 4. The F₁ hybrid plants had chromosome numbers as expected based on parental chromosome numbers with the exception of *S. transsylvanica* × *S. involucrata* (Table 5). With 38 chromosomes, this hybrid appears to be an allopolyploid, possibly formed from the combination of restitution gametes or spontaneous chromosome doubling of the zygote.

The *S. nemorosa* × *S. transsylvanica* F₂ population exhibited transgressive segregation for a wide range of phenotypic traits (Fig. 2). For example, time to flower varied from 13 to 29 weeks compared with 15 and 19 weeks to flower for *S. nemorosa* and *S. transsylvanica*, respectively. Similarly, plant height at flowering ranged from 20 to 85 cm for F₂ individuals compared with 30 and 55 cm for *S. nemorosa* and *S. transsylvanica*, respectively. In contrast, only three of the 124 F₂ individuals had flower lengths less than *S. nemorosa* or greater than *S. transsylvanica* (Fig. 2). Many of the measured traits were significantly correlated in the F₂ population. Plant height was correlated positively with flower length, leaf length, inflorescence branch number, and time to flower. Time to flower was correlated positively with flower length (Table 6). Although many of these traits were significantly correlated, individuals with combinations of desirable trait values (e.g., large flowers with compact growth habit) were identified within the population (Fig. 3) showing the associations may be relatively weak.

Discussion

It is often assumed that crosses between organisms with equal chromosome numbers will be more successful, but

Table 3. Mean leaf length, leaf width, flower height, and flower length for parental species and interspecific F₁ hybrids (F₁) among *Salvia* species.

Genotype	Leaf length (mm)	Leaf width (mm)	Flower ht (mm)	Flower length (mm)
<i>Salvia greggii</i> × <i>Salvia involucrata</i>				
<i>S. greggii</i>	36 a ^z	10 a	32 a	22 c
<i>S. involucrata</i>	110 b	75 c	40 b	9 a
F ₁	48 a	29 b	33 a	16 b
<i>Salvia guaranitica</i> × <i>S. involucrata</i>				
<i>S. guaranitica</i>	107 a	64 a	43 a	23 c
<i>S. involucrata</i>	110 a	75 a	40 a	9 a
F ₁	125 a	71 a	42 a	17 b
<i>Salvia transsylvanica</i> × <i>S. involucrata</i>				
<i>S. transsylvanica</i>	120 a	75 a	20 a	14 a
<i>S. involucrata</i>	110 a	75 a	40 b	9 a
F ₁	147 b	94 b	23 a	15 a
<i>S. transsylvanica</i> × <i>Salvia nemorosa</i>				
<i>S. transsylvanica</i>	120 b	75 b	20 c	14 b
<i>S. nemorosa</i>	69 a	27 a	9 a	6 a
F ₁	206 c	93 c	15 b	15 b
<i>S. nemorosa</i> × <i>S. transsylvanica</i>				
<i>S. nemorosa</i>	69 a	27 a	9 a	6 a
<i>S. transsylvanica</i>	120 b	75 c	20 c	14 b
F ₁	113 b	49 b	14 b	13 b

^zNumbers followed by the same letter are not significantly different as determined using analysis of variance with means separation by Fisher's least significant difference at *P* = 0.05.

Fig. 1. Random amplified polymorphic DNA banding patterns for parental *Salvia* species and F₁ hybrids.

notably in this study, three of the five successful crosses were between species with different basic chromosome numbers. Of those crosses, the hybrid between species with the same basic chromosome numbers (*S. greggii* × *S. involucrata*, *S. guaranitica* × *S. involucrata*) were sterile, whereas the crosses between individuals with different basic chromosome numbers (*S. transsylvanica* × *S. involucrata*, *S. transsylvanica* × *S. nemorosa*, *S. nemorosa* × *S. transsylvanica*) were fertile, results opposite from what would be expected. In the case of *S. transsylvanica* × *S. involucrata*, the fertility may be explained by the likely allopolyploid nature of this hybrid (based on chromosome counts for the parents and hybrid), but no such

Table 4. Fertility of interspecific *Salvia* hybrids after self-pollination or back-crossing to parental species.^z

Cross	Successful crosses (%)	Avg seeds per cross (no.)
(<i>Salvia transsylvanica</i> × <i>Salvia involucrata</i>) × self	60	1.8
(<i>S. transsylvanica</i> × <i>S. involucrata</i>) × <i>S. transsylvanica</i>	0	0
(<i>S. transsylvanica</i> × <i>S. involucrata</i>) × <i>S. involucrata</i>	0	0
<i>S. transsylvanica</i> × (<i>S. transsylvanica</i> × <i>S. involucrata</i>)	0	0
<i>S. involucrata</i> × (<i>S. transsylvanica</i> × <i>S. involucrata</i>)	0	0
(<i>Salvia nemorosa</i> × <i>S. transsylvanica</i>) × self	46	1.0
(<i>S. nemorosa</i> × <i>S. transsylvanica</i>) × <i>S. nemorosa</i>	76	1.5
(<i>S. nemorosa</i> × <i>S. transsylvanica</i>) × <i>S. transsylvanica</i>	64	1.7
<i>S. nemorosa</i> × (<i>S. nemorosa</i> × <i>S. transsylvanica</i>)	40	1.2
<i>S. transsylvanica</i> × (<i>S. nemorosa</i> × <i>S. transsylvanica</i>)	20	1.2
(<i>S. transsylvanica</i> × <i>S. nemorosa</i>) × self	8	1.0
(<i>S. transsylvanica</i> × <i>S. nemorosa</i>) × <i>S. transsylvanica</i>	24	1.0
(<i>S. transsylvanica</i> × <i>S. nemorosa</i>) × <i>S. nemorosa</i>	44	1.0
<i>S. transsylvanica</i> × (<i>S. transsylvanica</i> × <i>S. nemorosa</i>)	52	2.0
<i>S. nemorosa</i> × (<i>S. transsylvanica</i> × <i>S. nemorosa</i>)	48	1.3

^zSuccessful cross percentages are out of 50 total pollinations for each cross. Average seeds per cross is out a maximum potential of four seeds.

Table 5. Chromosome numbers of *Salvia* species and interspecific hybrids.

Species	2n =	Reference:
<i>Salvia azurea</i>	20	Harley and Heywood, 1992
<i>Salvia guaranitica</i>	88	Alberto et al., 2003
<i>Salvia greggii</i>	23 ^z	This study
<i>Salvia involucrata</i>	22	This study
<i>Salvia madrensis</i>	20	This study
<i>Salvia nemorosa</i>	14	Goldblatt and Johnson, 1979
<i>Salvia roemeriana</i>	28	Walker and Elisens, 2001
<i>Salvia transsylvanica</i>	16	This study
<i>Salvia verticillata</i>	16	Goldblatt and Johnson, 1979
F ₁ hybrids		
<i>S. greggii</i> × <i>S. involucrata</i>	23 ^z	This study
<i>S. nemorosa</i> × <i>S. transsylvanica</i>	15	This study
<i>S. transsylvanica</i> × <i>S. nemorosa</i>	15	This study
<i>S. guaranitica</i> × <i>S. involucrata</i>	55	This study
<i>S. transsylvanica</i> × <i>S. involucrata</i>	38	This study

^zLikely 2n = 22 + 1 supernumerary chromosome.

explanation is possible with the *S. transsylvanica* × *S. nemorosa* and *S. nemorosa* × *S. transsylvanica* hybrids.

Hybrids between *Salvia* species with unequal chromosome numbers have been reported in the past. *Salvia nemorosa*, with 14 chromosomes, has been reported to form natural, fertile hybrids with *S. pratensis* (Kerner con Marilaun, 1891) with 18 chromosomes (Goldblatt and Johnson, 1979). Epling (1938) observed natural hybrids between *S. columbaria* (2n = 26) and *S. mellifera* (2n = 30) (Epling et al., 1962). *Salvia farinacea* [2n = 18 (Haque and Ghoshal, 1980) or 2n = 20 (Alberto

et al., 2003)] and *S. longispicata* (2n = 22; Goldblatt and Johnson, 1979) have formed a chance sterile hybrid in cultivation (Clebsch, 2003). Together, these data suggest that *Salvia* is highly tolerant of aneuploidy in its hybrids and that different chromosome numbers in this genus are not an absolute barrier to hybrid formation.

There are other genera in which species show a high level of crossability between different basic chromosome numbers. In *Brassica*, *B. nigra* (2n = 16), *B. oleracea* (2n = 18), and *B. campestris* (2n = 20) have all hybridized naturally to form the allotetraploid species *B. carinata* (2n = 34) *B. juncea* (2n = 36), and *B. napus* (2n = 38) and can be hybridized artificially producing F₁ plants that have low fertility but are not completely sterile (U, 1935). Such successful hybridization between different chromosome num-

bers is the exception rather than the rule, however. Ladizinsky (1992) reviewed efforts to produce interspecific hybrids in 11 genera of important crop plants. Comparing Ladizinsky's crossability data with chromosome numbers compiled by Goldblatt and Johnson (1979) reveals that although a few hybrids were between species of different ploidy, none were between species with different basic chromosome numbers.

The most unexpected result of the crossability study was the production of a successful hybrid between *S. transsylvanica* and *S. involucrata*. These species have been placed in different

Fig. 2. Population distributions of (A) plant height at first flower, (B) time to flower, (C) branch number of inflorescence, and (D) flower length in the *Salvia nemorosa* × *Salvia transsylvanica* F₂ population; n, t, and F₁ indicate values for *S. nemorosa*, *S. transsylvanica*, and their F₁ hybrid, respectively.

Table 6. Pearson's correlation coefficients of selected phenotypic traits in a *Salvia nemorosa* × *Salvia transsylvanica* F₂ population.

	Time to flower	Plant ht	Branch no.	Leaf no.	Leaf length
Flower length	0.23*	0.25**	-0.02	0.14	0.53**
Time to flower		0.20*	-0.17	0.88***	-0.02
Plant ht			0.24*	0.16	0.43***
Branch no.				-0.15	0.23*
Leaf no.					-0.21*

*, **, *** denote significance at $P < 0.05$, 0.01 , and 0.001 , respectively.

clades of the genus, which are thought to be widely divergent. However, the conclusions of a recent phylogenetic study indicate that these two clades are only classified as members of the same genus as a result of chance convergent evolution of the key morphological traits used to define the genus *Salvia* (Walker et al., 2004). This successful hybrid between the two species indicates that the two clades may be more closely related than is indicated by Walker's phylogeny of the genus.

Most interspecific hybridization efforts start with the assumption that closely related species with the same chromosome number will be the most likely to successfully hybridize. Our data conflict with those assumptions, indicating that in *Salvia*, chromosome number is not an accurate predictor of interspecific hybrid formation potential and that hybrids are possible between extremely divergent parts of this genus.

Furthermore, our data likely underestimate the potential for successful interspecific hybridization among *Salvia* species. It is possible that many of the crosses that failed to produce seed in this study would be successful with other genotypes or in other conditions. These data, unfortunately, do not indicate any systematic method to predict interspecific hybridity potential within the genus, meaning breeders will have to explore possible hybrids largely through trial and error.

Analysis of the *S. nemorosa* × *S. transsylvanica* F₂ family revealed a strong potential to integrate valuable traits from the wild species for improvement of *S. nemorosa* (Fig. 2), a popular herbaceous perennial plant. The F₂ family exhibited significant transgressive segregation for numerous traits, likely as a result of heterozygosity of the parent plants, including many individuals with significantly larger flowers than *S. nemorosa* and a more profuse floral display resulting from greater branching of the inflorescence. Unfortunately, flower size was correlated positively with plant height and time to flower (Table 6), and inflorescence number was correlated positively with plant height. Excessive plant height and delayed flowering are undesirable traits for ornamental crops. However, these correlations were relatively weak and highly ornamental individuals combining the desirable growth habit of *S. nemorosa* with the improved floral characteristics of *S. transsylvanica* existed in this population (Fig. 3). Because most herbaceous perennial *Salvia* are asexually propagated, improved *Salvia* cultivars lacking the negative traits of the wild species can be selected directly from this hybrid population.

Fig. 3. Population distributions for morphological traits and time to flower for *Salvia nemorosa* × *Salvia transsylvanica* F₂ generation individuals: (A) inflorescence branch number versus time to flower, (B) inflorescence branch number versus plant height, (C) plant height versus flower length, and (D) flower length versus time to flower.

In conclusion, crossability among the selected *Salvia* species was low. However, almost half of the cross combinations produced partially developed seeds. Embryo rescue techniques may be successful for recovering hybrid plants of these cross combinations. *Salvia transsylvanica* may be a useful genetic source for improving floral size of the popular herbaceous perennial *S. nemorosa*.

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