Intersectional Hybridization among *Trichostema* Species

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Abstract. Controlled reciprocal crosses between *Trichostema lanatum* Benth. (section *Chromocephalum* F.H. Lewis) with *Trichostema arizonicum* A. Gray (section *Paniculatum* F.H. Lewis) and *Trichostema purpusii* Brandege (section *Rhodanthum* Lewis) were successful in generating the first artificial hybrids in the genus. Crosses where *T. lanatum* was used as the female were unsuccessful. Leaf and floral morphology among the hybrids was typically intermediate. Female sterility was seen in the *T. arizonicum* × *T. lanatum* hybrids, and these hybrids also produced abnormally small, nonviable pollen grains. Propagation procedures are also presented. Although these species may be in different sections, their crossability suggests that they are closely related.

Trichostema L., a North American genus in the Lamiaceae family comprised of annual and perennial species, is found growing across the continental United States and extending north into Canada and south into Mexico (Lewis, 1960). Of the species, the perennial *T. lanatum*, also known as romero or woolly blue curls, is probably the most recognizable species, as it is commercially available and is used in California landscapes (Clausen and Ekstrom, 1989; Crane 2007; Evans and Bohn, 2001; Wilson 2007). *Trichostema* spp. grows vigorously during the summer and early autumn dry season, making it an excellent plant for xeriscaping (Dalby, 2004; Heisey and Delwiche, 1984). The flowers are also reported to have a pleasant, aromatic fragrance, and are a favorite of hummingbirds and butterflies (Crane, 2007; Dalby, 2004). Not all species are of ornamental value, as a number of annual species, including *T. brachiatum* L., *T. dichotomum* L., and *T. setaceum* Houtt., are reported as weedy (Lewis, 1945).

The exact number of species has been unclear in the literature, although 16 species was reported as late as 1978 (Lewis and Rzedowski, 1978). Epling (1940) had suggested that *T. mexicanum* Epling could be separate from *T. arizonicum* based on reduced hairiness, flower size, and branching inflorescences. Lewis (1945) considered *T. mexicanum* to fall within the range of *T. arizonicum* and thus to be synonymous with *T. arizonicum*. Henrickson (1982) agreed with Epling that *T. mexicanum* is a distinct species based on corolla size and color, anther length, inflorescence habit, growth

habitat, and distribution. Lewis (personal communication) agreed that *T. mexicanum* should be considered a separate species. He recently described another species, *T. ruygtii* H. Lewis, and notes its close relationship to *T. lanceolatum* Benth. (Lewis, 2006). This brings the total to 18 species.

Originally, Lewis had proposed five sections (*Chromocephalum*, *Paniculatum*, *Rhodanthum*, *Orthopodium* Benth., and *Trichostema* Benth.) based on flower morphology, chromosome counts, and growth habit (Lewis, 1945). *Trichostema purpusii* (formerly the monotypic genera *Eplingia* L.O. Williams) was originally placed in sect. *Rhodanthum*, as Lewis (1945) notes only remote similarities with any of the other species. However, upon germination of some collected *T. purpusii* seed, Lewis and Rzedowski (1978) later reported that *T. purpusii* and *T. arizonicum* were more closely related to each other than any other species in the genus; therefore, he included *T. purpusii* with *T. arizonicum* in sect. *Paniculatum*. Huang (2002) agreed with Lewis's original decision to separate the species into their individual sections by reporting *ndhF* data showing sect. *Paniculatum* and *Rhodanthum* as being sisters to one another, and thus *T. purpusii* will be considered in sect. *Rhodanthum*.

Sections *Chromocephalum* and *Paniculatum* were reported to have $n = 10$ chromosomes, whereas other chromosome numbers within the genus vary among the species and even sections, ranging from $n = 7$ to $n = 19$ (Lewis, 1960). Two shrubby perennial California species are included in sect. *Chromocephalum*: *T. lanatum* and *T. parishii* Vasey.

Section *Trichostema* species observed in the wild generally grow in isolated populations; however, Lewis (1945, 2006) did report that some species can be found growing together where boundaries overlap, as in the case of *T. parishii* and *T. lanatum* populations, as well as *T. ruygtii* and *T. lanceolatum*. No intermediate hybrids have been

reported for sect. *Trichostema* from field observations, even though interspecific hybridization within Lamiaceae is reported to be common and has been seen in *Salvia* L., *Mentha* L., *Monarda* L., *Dicerandra* Benth., and *Hyptis* Jacq. (Huck, 1992). Lewis (personal communication) attempted crosses between *T. lanatum* and *T. parishii*, both sect. *Chromocephalum*, with no success.

Thus, little is known of the breeding compatibility among the species. Artificial hybridization yields information on crossability, hybrid fertility, and the genetic contributions to a phenotype, all of which give insight into the relationship among species (Kruckeberg, 1962). The main objective of this work was to assess the genetic relationship through interspecific hybridization between three ornamental perennial *Trichostema* species: *T. lanatum* (sect. *Chromocephalum*), *T. purpusii* (sect. *Rhodanthum*), and *T. arizonicum* (sect. *Paniculatum*).

Materials and Methods

Hybridization. A single plant of *T. lanatum* was raised from seed obtained from Southwestern Native Seeds (Tucson, AZ). Seed of *T. arizonicum* was also obtained from the same source. Seed of *T. purpusii* was obtained from Seedhunt (Freedom, CA). All three species of *Trichostema* were maintained under greenhouse conditions. *T. arizonicum* and *T. purpusii* are dormant in winter and were kept cool (minimum winter temperature 50 °F) and dry. *Trichostema lanatum* grows throughout the year, with most flowers occurring in the spring, summer, and early fall. Controlled reciprocal pollinations between *T. arizonicum* × *T. lanatum* were made beginning on 24 Aug. 2004. Controlled reciprocal pollinations between *T. purpusii* × *T. lanatum* were made beginning on 12 Sept. 2006. Flowers were emasculated using tweezers before anthesis. Pollination occurred once a day for 3 d after emasculation. At least five flowers were used in each crossing experiment.

Mature seeds from the *T. arizonicum* × *T. lanatum* cross were collected on 5 Nov. 2004, and were subsequently sown on 1 Dec. 2004. Mature seeds from the *T. purpusii* × *T. lanatum* cross were collected on 10 Nov. 2006, and were subsequently sown on 12 Nov. 2006. All seeds were planted in pots using a 1:1 ratio of Fafard #2 (Portland, OR) mix and perlite that had been autoclaved and pretreated with boiling water. Pots were placed in a Park Seed Biodome™ (Greenville, SC) until seeds germinated.

Pollen viability. Pollen comparisons were made on pollen collected from mature anthers on greenhouse specimens of *T. arizonicum* × *T. lanatum* hybrids. Anthers from at least five flowers were used. Anthers were squashed on a microscope slide containing two to three drops of 1% acetocarmine. Measurements (based on 10 pollen grains per species) of the polar and equatorial axes were made with a light microscope (×40) using Motic Image Plus 2.0 program from

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Motic Instruments, Inc. (Richmond, British Columbia, Canada).

Propagation. Asexual reproduction was attempted using softwood cuttings and plant tissue culture. Softwood cuttings were taken in the spring or summer, dipped in 1000 ppm K-IBA, and placed under intermittent mist in perlite or other similar medium. To introduce the hybrids into tissue culture, shoot tips 2 to 3 cm in length were collected and mature leaves were removed. The shoots were rinsed under running water for 1 h and were then surface-sterilized in a 0.53% solution of sodium hypochlorite and subsequently rinsed three times in sterile water under a laminar flow hood. A 3- to 5-mm shoot tip was then excised and placed into a test tube containing 15 mL of Linsmaier and Skoog media (Linsmaier and Skoog, 1965), pH adjusted to 5.7, without added plant growth hormones. To multiply in vitro plants, individual shoots were sectioned into two- to four-node pieces and placed on new media under the laminar flow hood. Axillary buds then formed new shoots for additional subcultures. Subcultures were accomplished monthly. Developing

shoots were then rooted and established as described for *Itea virginica* L. (Lindstrom and Pelto, 2003).

Results and Discussion

Five viable seedlings were obtained from the *T. arizonicum* (female) $\times$ *T. lanatum* (male) cross, of which two later died. One viable seedling was obtained from the *T. purpusii* (female) $\times$ *T. lanatum* (male) cross. For both crosses, no seeds resulted when *T. lanatum* was used as the female. This could be from the lack of pollen germination or insufficient pollen tube growth through the highly exerted style found on *T. lanatum* compared with the relatively shorter styles on *T. arizonicum* and *T. purpusii*.

Keeley and Fotheringham (1998) reported that *T. lanatum* seed would not germinate without coupling a year-long soil contact and smoke treatment, resulting in $\approx 30\%$ germination. The smoke, having the active compound butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one, is thought to allow a germination block to be overcome (Flematti et al.,

2004). No smoke or year-long soil contact treatment was administered to either group of hybrid seed (≈ 15 seeds total), and six seedlings resulted, indicating that extended soil and smoke treatments may be species specific. A smoke treatment may have increased the germination percentage. After initial seedlings had developed, a smoke treatment was applied to ungerminated seeds, with no success.

The *T. purpusii* $\times$ *T. lanatum* hybrid, which flowered for the first time on 14 May 2007, unfortunately did not retain the unique pink corolla lobe color of *T. purpusii*. Corolla lobes were actually violet, which is intermediate in color between the two parents. This plant did show hybrid vigor in the anterior corolla lobe, as its size was greater than either parent (Fig. 1). Leaf, stamen, and pistil size were intermediate between the parents (Fig. 1; Table 1). Leaf and pistil morphology were also intermediate for the *T. arizonicum* $\times$ *T. lanatum* hybrids, which flowered for the first time 15 May 2005; however, stamen length was shorter than either parent (Fig. 2; Table 1). Corolla lobe color for *T. arizonicum* $\times$ *T. lanatum* hybrids were purple, and thus resemble the purple-blue flowers of *T. lanatum*.

The *T. purpusii* $\times$ *T. lanatum* hybrid did set limited seed, whereas no seed has been produced by *T. arizonicum* $\times$ *T. lanatum* hybrids for two consecutive years despite attempts to self and sib-cross the hybrids. The *T. arizonicum* $\times$ *T. lanatum* hybrids were observed to have limited pollen production. Anthers were nondehiscent and uncharacteristically white, turning to brown upon maturity. Similar intermediate male sterile flowers, having white indehiscent anthers that turn brown, unlike fertile purple anthers, were noted in *Rosmarinus officinalis* L. (Uberta-Jiménez and Hidalgo-Fernández, 1992).

Microscopic examination revealed that pollen from *T. arizonicum* $\times$ *T. lanatum* hybrids were roughly half the size of pollen from either parent. Additionally, the pollen of *T. arizonicum* $\times$ *T. lanatum* hybrids were flaccid, ranging from elliptical to crescent-shaped, indicating lack of maturity (Fig. 3,

Fig. 1. Leaf and flower comparisons between *T. lanatum* (left), *T. purpusii* $\times$ *T. lanatum* (center), and *T. purpusii* (right). Bar = 7 mm.

Table 1. Morphological comparisons among *T. arizonicum*, the intersectional hybrid *T. arizonicum* $\times$ *T. lanatum*, *T. lanatum*, an intersectional hybrid *T. purpusii* $\times$ *T. lanatum*, and *T. purpusii*. Numeric data from at least five different samples.

Characteristics	<i>T. arizonicum</i>	<i>T. arizonicum</i> $\times$ <i>T. lanatum</i>	<i>T. lanatum</i>	<i>T. purpusii</i> $\times$ <i>T. lanatum</i>	<i>T. purpusii</i>
Growth habit	Subshrub	Shrub	Shrub	Shrub	Shrub
Leaves ^a	1.7–2.3	4.6–5.5	11.7–24.1	3.7–5.6	1.6–2.3
Leaf shape	Ovate	Lanceolate	Linear/lanceolate	Linear	Ovate
Leaf margin	Undulate	Entire	Entire	Entire	Entire
Posterior corolla lobe ^a	1.6–3.2	1.4–2.3	1.3–2.6	1.2–2.0	1.5–2.0
Anterior corolla lobe (lip) ^a	1.0–1.8	1.1–1.6	1.7–2.4	0.9–1.8	1.6–1.9
Petal color ^a	155B (white)	86B (purple)	93B (purple-blue)	83B (violet)	70B (pink)
Lip color ^a	88A/155B (violet/white)	89B (royal purple)	89B (royal purple)	83B (violet)	70B (pink)
Pubescence	Sparse	Sparse	Dense	Sparse	Sparse
Stamen length (mm)	16–20	9–15	25–29	14–20	10–18
Anther color ^a	155A (off-white)	1C (green-yellow)	101B (blue)	195A (gray-green)	161A gray-yellow
Filament color ^a	155D (off-white)	76A light purple	77B (wine)	76A (pink-violet)	155C (white)
Pistil length (mm)	23	27	33	29	20
Style color ^a	76D (light lavender)	76A (light purple)	83D (purple)	76A (light purple)	75D (white-purple)

^aValue given as a ratio of length/width.

^bColor ratings based on the Royal Horticultural Society Color Chart (Royal Horticultural Society, 1966).

Fig. 2. Leaf and flower comparisons between *T. lanatum* (left), *T. arizonicum* × *T. lanatum* (center), and *T. arizonicum* (right). Bar = 10 mm.

Fig. 3. Pollen comparisons of *T. lanatum* (left), *T. arizonicum* × *T. lanatum* hybrid (center), and *T. arizonicum* (right). Bar = 50 µm.

Table 2. Average pollen size (in micrometers) and shape of 10 pollen grains for *Trichostema lanatum*, *T. arizonicum*, and *T. arizonicum* × *T. lanatum* hybrids.

Species	Polar axis		Equatorial axis		P/E ratio		Pollen shape ^y
	Mean	Range	Mean	Range	Mean	Range	
<i>Trichostema</i>							
Sect. <i>Chromocephalum</i> ^z							
<i>T. lanatum</i>	72.1	69–75	60.1	55–65	1.2	1.1–1.4	Euprolate
Sect. <i>Paniculatum</i> ^z							
<i>T. arizonicum</i>	67.6	60–76	73.8	71–78	0.9	0.8–1.1	Prolate spheroidal-subprolate
<i>T. arizonicum</i> × <i>T. lanatum</i> hybrid	44.7	40–50	37.1	30–44	1.2	0.9–1.7	Prolate

^zSections with *n* = 10 chromosomes.

^yShapes classified according to Walker and Doyle (1975).

Table 2). Sarkissian and Harder (2001) indicated that amount and quality of pollen, including size and morphology, are often important components of fitness and relate to mating success. Attempts to germinate pollen in vitro on Brewbaker and Kwack (1963) media were not successful.

The lack of fertility in the F1 plants from *T. arizonicum* × *T. lanatum* crosses was not expected, as both plants are reported to have the same chromosome number (Lewis, 1960). It is known that hybrids resulting from taxonomically unrelated parents (such as those from different sections) tend to be sterile or of low fertility (Qureshi et al., 2002). Sterile hybrids were also seen in the genus *Calylophus* Spach., where the high degree of sterility in certain intrasectional crosses was found to be correlated with cytological differences between the parental plants (Towner, 1977).

In an effort to have fertile plants for future breeding, a solution of 7 µm oryzalin was

applied as a mist using a spray bottle on a few emerging seedlings, which were derived from repeating earlier crossing experiments between *T. arizonicum* and *T. lanatum*. Inducing polyploidy is a viable method for improving meiotic pairing and restoring fertility in wide crosses (Sanford, 1983). A single plant (likely mixoploid) resulted from this study. The main stem on this plant branched, so that half of the plant produced branches with flowers that had enhanced flower size, corolla lobe color, stamen length, pollen production, and fertility (Fig. 4). Crosses between this oryzalin-induced hybrid and *T. lanatum* produced nutlets, indicating that the sterility seen in the original *T. arizonicum* × *T. lanatum* crosses was likely from a meiotic imbalance.

Propagation of the hybrids was not difficult, as softwood cuttings rooted at high percentages within 2 to 3 weeks (data not shown). We were also successful in propagating both hybrids by plant tissue culture.

Fig. 4. Flower comparison between untreated (left) and oryzalin-treated *T. arizonicum* × *T. lanatum* seedling (right). Bar = 5 mm.

By establishing plants in tissue culture, large numbers of plants can be generated quickly without having to maintain a large number of stock plants.

The taxonomic significance of the intersectional hybrids *T. arizonicum* × *T. lanatum* and *T. purpusii* × *T. lanatum* provides genetic evidence that the genomes are sufficiently homologous to permit the formation of hybrids. In the case of *T. purpusii* × *T. lanatum*, these hybrids are fertile. The sterility and later restored fertility seen in the *T. arizonicum* × *T. lanatum* hybrids indicate that these two taxa are less related. We can also conclude that although *T. arizonicum* and *T. purpusii* are found in different sections than *T. lanatum*, these species may be more closely related than is apparent from their present classification in distinct sections. This goes against Huang (2002), who found that sect. *Chromocephalum* is a sister group to the remainder of the genus after she combined morphological and molecular data. These intersectional hybrids may be viewed as evidence for the feasibility of crossing other *Trichostema* species, having similar chromosome numbers, within and among sections. The fact that oryzalin was successful in restoring fertility further expands the breeding potential among wide crosses within *Trichostema*. An important result of continued hybridization studies may well be a greatly improved understanding of the taxonomy of this genus. The data we collected agrees with Spira (1978) who reported that flower size and reproductive parts vary greatly within *Trichostema* species. The *T. purpusii* × *T. lanatum* hybrid has not been selected for release as of yet; however, one *T. arizonicum* × *T. lanatum* hybrid was selected and released as ‘Blue Myth’ (Dunn and Lindstrom, 2007).

Literature Cited

Brewbaker, J.L. and B.Y. Kwack. 1963. The essential role of calcium ion in pollen germination

- and pollen tube growth. *Amer. J. Bot.* 50:859–865.
- Clausen, R.R. and N.H. Ekstrom. 1989. *Perennials for American gardens*. Random House, New York.
- Crane, K. 2007. Yerba Buena Nursery (*Trichostema lanatum*). 13 May 2007. http://www.yerbabuenanursery.com/online_album/0810.htm.
- Dalby, R. 2004. Blue Curls. *Amer. Bee J.* 144:127–128.
- Dunn, B.L. and J.T. Lindstrom. 2007. 'Blue Myth' *Trichostema*. *J. Environ. Hort.* 25:250–251.
- Epling, C. 1940. Supplementary notes of American Labiatae. *Bull. Torrey Bot. Club* 67:509–534.
- Evans, M. and J. Bohn. 2001. *Trichostema lanatum*, Woolly Bluecurls. 13 May 2007. http://www.treeofflifenursery.com/main/PDFs/Plant-Profiles/Trich_lana.pdf.
- Flematti, G.R., E.L. Ghisalberti, K.W. Dixon, and R.D. Trengove. 2004. A compound from smoke that promotes seed germination. *Science* 305:977.
- Heisey, R.M. and C.C. Delwiche. 1984. Phytotoxic volatiles from *Trichostema lanceolatum* (Labiatae). *Amer. J. Bot.* 71:821–828.
- Henrickson, J. 1982. On the recognition of *Trichostema mexicanum* Epling (Lamiaceae). *Madrono* 29:104–108.
- Huck, R.B. 1992. Overview of pollination biology in the Lamiaceae, p. 167–181. In: R.M. Harley and T. Reynolds (eds.). *Advances in Labiate science*. Royal Botanic Gardens, Kew, UK.
- Keeley, J.E. and C.J. Fotheringham. 1998. Smoke-induced seed germination in California Chaparral. *Ecology* 79:2320–2336.
- Kruckeberg, A.R. 1962. Intergeneric hybrids in the Lychnideae (Caryophyllaceae). *Brittonia* 14:311–321.
- Lewis, H. 1945. A revision of the Genus *Trichostema*. *Brittonia* 5:276–303.
- Lewis, H. 1960. Chromosome numbers and phylogeny of *Trichostema*. *Brittonia* 12:93–97.
- Lewis, H. 2006. *Trichostema ruygtii* (Lamiaceae): A new species from Napa county, California. *Madrono* 53:282–287.
- Lewis, H. and J. Rzedowski. 1978. The genus *Trichostema* (Labiatae) in Mexico. *Madrono* 25:151–154.
- Lindstrom, J.T. and M.C. Pelto. 2003. Micropropagation of Virginia sweetspire (*Itea virginica* 'Henry's Garnet'). *J. Environ. Hort.* 21:206–208.
- Linsmaier, E.M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant.* 18:100–128.
- Huang, H. 2002. Systematics of *Trichostema* L. (Lamiaceae) and phylogenetic relationships with its disjunct taxa in Asia. Ohio State University, Columbus, OH, PhD dissertation.
- Qureshi, S.J., A.G. Awan, M.A. Khan, and S. Bano. 2002. Study of pollen fertility of the genus *Launaea* from Pakistan. *Asian J. Plant Sci.* 1:73–74.
- Sarkissian, T.S. and L.D. Harder. 2001. Direct and indirect responses to selection on pollen size in *Brassica rapa* L. *J. Evol. Biol.* 14:456–468.
- Sanford, J.C. 1983. Ploidy manipulations, p. 100–123. In: J.N. Moore and J. Janick (eds.). *Methods in fruit breeding*. Purdue University Press, West Lafayette, IN.
- Spira, T.P. 1978. Floral parameters associated with breeding system and pollinator type in *Trichostema* (Labiatae). California State University, Chico, CA, MA thesis.
- Towner, H.F. 1977. The biosystematics of *Calylophus* (*Onagraceae*). *Ann. Mo. Bot. Gard.* 64:48–120.
- Uberta-Jiménez, J.L. and P.J. Hidalgo-Fernández. 1992. Temporal gynodioecy in *Rosmarinus officinalis*, p. 281–289. In: R.M. Harley and T. Reynolds (eds.). *Advances in Labiate science*. Royal Botanic Gardens, Kew, UK.
- Walker, J.W. and J.A. Doyle. 1975. The basis of angiosperm phylogeny. *Ann. Mo. Bot. Gard.* 62:664–723.
- Wilson, B. 2007. *Trichostema lanatum* Woolly Blue Curls. 1 May 2007. <http://www.laspilatas.com/plants/680.htm>.