Conservation Implications of Spur Length Variation in Long-Spur Columbines (*Aquilegia longissima*)

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ABSTRACT: Populations of long-spur columbine (*Aquilegia longissima*) with spurs 10-16 cm long are known only from a few populations in Texas, a historical collection near Baboquivari Peak, Arizona, and scattered populations in Coahuila, Chihuahua, and Nuevo Leon, Mexico. Populations of yellow columbine with spurs 7-10 cm long are also found in Arizona, Texas, and Mexico, and are now classified as *A. longissima* in the recent Flora of North America. In a multivariate analysis of floral characters from 11 yellow columbine populations representing a continuous range of spur lengths, populations with spurs 10-16 cm long are clearly separate from other populations based on increasing spur length and decreasing petal and sepal width. The longer-spurred columbines generally flower after monsoon rains in late summer or fall, and occur in intermittently wet canyons and steep slopes in pine-oak forests. Also, longer-spurred flowers can be pollinated by large hawkmoths with tongues 9-15 cm long. Populations with spurs 7-10 cm long cluster with the common golden columbine (*A. chrysantha*), and may be the result of hybridization between *A. chrysantha* and *A. longissima*. Uncertainty about the taxonomic status of intermediates has contributed to a lack of conservation efforts for declining populations of the long-spur columbine.

The genus *Aquilegia* is characterized by a wide diversity of floral morphologies and colors that play a major role in isolating two species via differences in pollinator visitation or pollen transfer (Grant 1952, Fulton and Hodges 1999, Hodges et al. 2002). Since there are few post-zygotic reproductive barriers in the genus, populations with intermediate forms resulting from hybridization and introgression are very common (Payson 1918, Munz 1946, Taylor 1967, Whittemore 1997). In addition, many columbines occur in small, isolated populations, which results in reduced gene flow (Strand et al. 1996) and potentially increased morphological variability due to genetic drift and inbreeding.

Due to the complex nature of floral variation, many columbine populations are difficult to identify accurately in plant keys. For example, floral spurs lengths are a key character used to differentiate yellow columbine species in the Southwest. In early monographs of the genus by Payson (1918) and Munz (1946), yellow columbines with short spurs 4-7 cm long were included with *Aquilegia chrysantha* and yellow columbines with long spurs 10-16 cm long were included with *A. longissima*. Recently, yellow columbines with intermediate spurs 7-10 cm long have been collected. Some authors include these populations with the common *A. chrysantha* (Correll and Johnston 1979, Lott 1979). In the recent Flora of North America, these populations were grouped with *A. longissima* (Whittemore 1997).

Since the most recent taxonomy of southwestern yellow columbines groups

intermediate spur length populations with long-spur columbines, we examined floral variation among 11 yellow columbine populations in the Southwest. In particular, we selected populations representing short, intermediate and long spur lengths, and then identified floral characters that clearly differentiated populations using bivariate scatterplots and multivariate analyses. Finally, we describe differences among southwestern yellow columbine identified by previous researchers and discuss the taxonomic status of intermediate-spur length populations.

METHODS

Five floral characters were measured on 211 pressed flowers collected from 141 plants in 11 populations (table 1). The locations included seven populations with short-spurs (*A. chrysantha* Gray), two populations with long spurs (*A. longissima* Gray ex Watson), and two populations with intermediate spurs of uncertain taxonomic status. Floral measurements included the petal spur length, petal blade (lamina) length and width, and the sepal length and width. Lengths were measured from the point of attachment to the tip of each structure. Measurements from multiple flowers on a plant were averaged and statistical analyses where performed using R (R Development Core Team 2004). Bivariate scatterplots and principal components analyses were used to examine differences in floral characters among the 11 populations. The herbaria at New Mexico State University (NMC), University of Arizona (ARIZ) and Arizona State University (ASU) were visited to check for *A. chrysantha* hybrids and to measure specimens from noteworthy collections.

RESULTS

Spur lengths range from 4-16 cm long in southwestern yellow columbines and the only break in the distribution occurs at 10 cm (fig 1). The short-spur columbines in the San Andres and Bofecillos Mountains have spurs 4-7 cm long. This spur range is identical to the type locality of *A. chrysantha* in the Organ Mountains in southern New Mexico (based on 170 flowers measured over a three year period, unpublished data). In the Sierra Madres, Mexico, the spurs are 4.5-9.2 cm long and all three Mexico populations have some plants with spur lengths greater than the current 7.2 cm cutoff separating *A. chrysantha* and *A. longissima* in the Flora of North America.

Table 1. The number of plants collected and flowers measured in each population. The two long-spur populations are listed in bold and two populations of uncertain taxonomic status are listed in italics.

Mountain Range	Population	# Plants	# Flowers
Bofecillos, Texas	Madrid Falls	5	6
	Palo Amarillo Springs	5	17
Chisos, Texas	Cattail Falls	10	12
	Maple Canyon	14	19
	Pine Canyon	18	19
San Andres, New Mexico	Ash Springs	12	14
	Rope Springs	11	14
	San Nicholas Canyon	13	17
Sierra Madres, Chihuahua	Basaseachic Falls	8	18
	Cuarenta Casas	26	42
	El Salto de Babicora	19	33

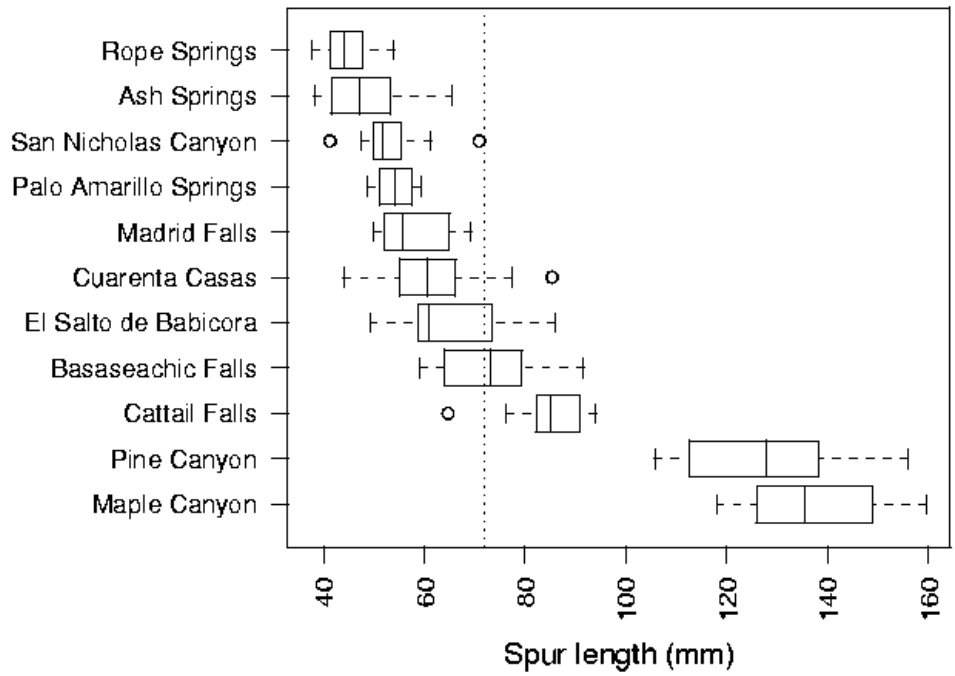

FIGURE 1. Side-by-side box plots displaying median spur length, interquartile range, smallest and largest spur lengths, and any outliers if present. The dotted line is the cutoff separating A. chrysantha and A. longissima in the Flora or North America.

A plot of spur length versus petal blade width clearly separates long-spur columbines at Pine and Maple Canyon from the remaining populations (Fig 2). For example, plants with intermediate-spur lengths at Cattail Falls cluster with other short-spur columbines due to the wider petals (mean = 1.5 cm wide, range = 1.0-1.7 cm) compared to Maple and Pine Canyon (mean=0.8 cm wide, range=0.4-1.2 cm). Also, spur lengths and petal widths are positively correlated in both groups, so the difference between petal widths is greatest when spur lengths from both groups are close to 10 cm long.

The principal component plot also separates the long-spur columbines from short- and intermediate-spur populations (fig. 3). The first principal component is primarily an indicator of floral size, and the second principal component separates long-spur columbines based on increasing spur length and decreasing petal blade width and sepal width (Table 2). Again, the intermediate-spur populations at Basasiachic and Cattail Falls cluster with other short-spur columbines, and in the principal components plot their overall shape is similar to a very large *A. chrysantha*

TABLE 2. Loadings on the first three principal components which incorporate 90.9 percent of the variance.

Character	PC1	PC2	PC3
Sepal length	-0.565	-0.065	-0.140
Sepal width	-0.322	0.559	0.762
Petal length	-0.568	0.042	-0.272
Petal width	-0.030	0.734	-0.571
Spur length	-0.502	-0.378	0.012
% Variance	51.5	30.7	8.7

flowers.

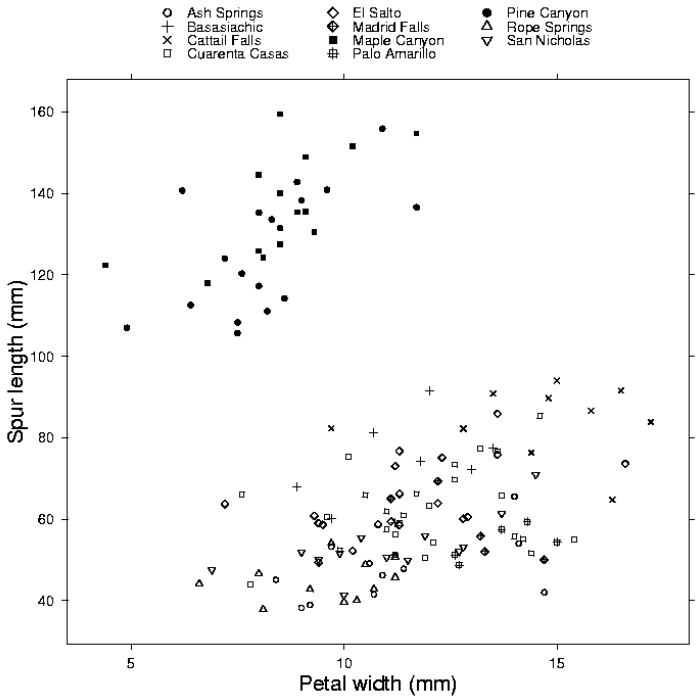

FIGURE 2. Bivariate scatterplot of spur length versus petal blade width.

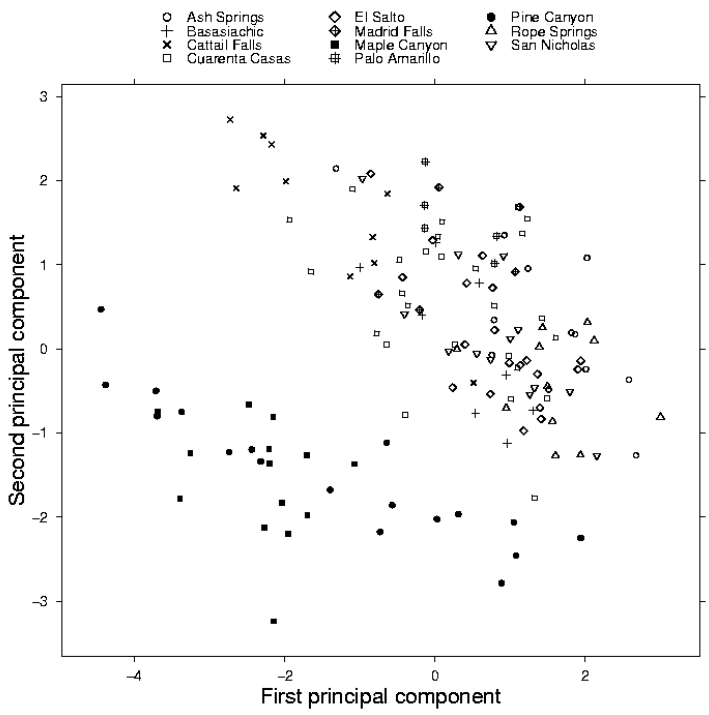

FIGURE 3. The first two principal components of all floral characters combined plotted for individual plants.

DISCUSSION

In the original species descriptions (Watson 1882 and Gray 1883), *A. longissima* is distinguished from *A. chrysantha* based on the extremely long, slender spur over 10 cm long, the narrow spatulate petals, and the very narrow opening at the base of the spur. In our multivariate analysis the combination of long spurs and narrow petal widths also differentiates long-spur columbines from other yellow columbines in the Southwest. Also, long-spur columbines at Pine and Maple Canyon have a very constricted opening at the base of the spur (mean= 2.4 mm wide, range=1.0-3.1 mm). Due to the narrow opening, the slender spurs usually hang straight down and the flower position is erect. In short- and intermediate-spur columbines, the spur opening is much wider and tapered. Due to the tapered opening, the spurs are often spreading and the position of the flower may vary from horizontal to erect.

There are a number of other potential reproductive barriers that maintain species boundaries between *A. chrysantha* and *A. longissima*, including temporal and ecogeographic isolating mechanisms and pollinator behaviors. The flowering phenology, ecology, and pollinators of southwestern columbines were discussed in detail by Lott (1979) who collected from a larger sample of mountain ranges in the Southwest, including long-spur columbines in the Davis Mountains, Texas and near the type locality in Coahuila, Mexico, and an intermediate-spur population in the Chinati Mountains, Texas. First, the long-spur columbines typically flower in later summer or fall after monsoon rains, while short- and intermediate spur columbines flower in spring and early summer. In some years, a few long-spur columbines may flower early and some short-spur columbines may flower late during the monsoon rains. In our study the plants in the Chisos Mountains at Catttail Falls were collected in late March, while plants at Maple and Pine Canyon were collected in flower six months later in October. Second, long-spur columbines are usually found near intermittently wet substrates in oak-pine-juniper woodlands in shaded canyons or talus slopes. Short- and intermediate-spur columbines are usually found near permanent water by streams, pools, waterfalls, and springs.

The primary pollinators of *A. chrysantha* include small hawkmoths in the genus *Hyles*, *Eumorpha*, and *Sphinx* (Miller 1985, personal observations). The pollinators of *A. longissima* have not been recorded in the literature and we have not observed any pollinators on a number of visits to the Chisos Mountains. Trelease (1883) thought that the most likely pollinator of *A. longissima* would be the giant sphinx moth, *Cocytius antaeus*. The giant sphinx moth is a rare stray in the United States and has been collected near Pine Canyon (Van Pelt 1995). However, the most likely pollinators would be large hawkmoths in the genus *Agrius* and *Manduca* with tongues lengths 9-14 cm long (see Gregory 1964 for tongue measurements). These hawkmoths also pollinate other very long-tube flowers in the Southwest (Grant 1983, Grant and Grant 1983, Grant 1985).

There are three locations of intermediate-spur columbines in the United States and all have been collected near historical or recent collections of *A. longissima*. In Texas, a relatively large population is found at the base of Cattail Falls in the Chisos Mountains. There is a historical collection by Warnock (no

collection number at ARIZ) of a long-spur columbine in 'Upper Cattail Falls' collected August 8, 1937 with spurs 13.4 cm long Two small populations of long-spur columbine can still be found nearby in Maple and Pine Canyon. Lott (1979) also surveyed a population with intermediate spurs at Tinaja Prieta in the Chinati Mountains, Texas and recently collected long-spur columbines in the same area (personal communication).

In Arizona, a population with intermediate spurs is found in Thomas Canyon in the Baboquivari Mountains. A collection by Toolin (#358 at ARIZ) is typical. The plant was collected 'About 1.0 mile above ranch house, on slope at west edge of main wash, under *Quercus arizonica* Sarg., *Juglans major* (Torr.) Heller, with *Rubus arizonensis* Focke. Thomas Canyon, Baboquivari Mountains, April 22, 1979'. The spur lengths from various flowers measured 7.0, 7.3, 8.0, 8.5, and 8.6 cm long. There is only one known collection of *A. longissima* in Arizona. Gentry (#3418 at ARIZ) collected a long-spur columbine in flower 'Near peak of Baboquivari Mountains, moist shade side of cliff; oak belt. October 1937'. The two spurs are 12.3 and 12.4 cm long, with narrow petals and a very narrow opening at the base of the spur 2.7 mm wide. This collection was probably from the western slope of the Baboquivari Mountains near the saddle above Thomas Canyon.

Contrary to the recent description in the Flora of North America, populations with intermediate spurs 7-9 cm long should not be included with *A. longissima*. In addition, these populations are also outside the typical range of spur lengths found in *A. chrysantha.* Although additional studies are needed, it is possible that long-distance pollen flow from *A. chrysantha* populations into isolated mountain ranges having only long-spur populations has resulted in hybrid populations of *A. chrysantha* x *A. longissima* near permanent water at lower elevations than the long-spur parent. In our survey of local herbaria *A. chrysantha* forms hybrids with *A. Formosa* Fisch. ex DC. in Zion National Park, Utah, with *A. coerulea* James in the San Francisco Peaks, Arizona, with *A. desertorum* (M.E. Jones) ex Heller in the Chiricahua Mountains, Arizona, and with *A. skinneri* Hook near Basasiachic Falls, Mexico. In Mexico, the columbine populations have spurs 4-9 cm long, which may be the result of local adaptations to larger hawkmoths or extensive backcrossing and introgression between both species in the same range.

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