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Noteworthy Collections: First Record of a Natural Hybrid Between *Phlox divaricata* ssp. *laphamii* (Alph. Wood) Wherry and *Phlox pilosa* ssp. *sangamonensis* (Levin & D.M. Sm.)

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ABSTRACT

Phlox pilosa ssp. *sangamonensis* is a state-endangered and narrowly endemic taxon only found in Champaign and Piatt counties, Illinois. Here, we present evidence of natural hybridization between *Phlox divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis*. In May 2018, we collected a putative F_1 hybrid of the two taxa in a sympatric population. We screened six microsatellites developed for North American *Phlox* species and measured eight morphological traits to test the specimen's hybrid status. The microsatellite data were analyzed using a Bayesian clustering technique to infer genetic groupings. The eight morphological characteristics were analyzed using principal components analysis. We also measured the flowering phenology of *P. divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis* to assess the possibility of cross pollination. Bayesian clustering and the principal components analysis indicated that the sample was a *Phlox divaricata* ssp. *laphamii* × *Phlox pilosa* ssp. *sangamonensis*. There was modest phenological overlap between the two taxa, suggesting that gene flow is possible—though likely uncommon—in sympatric populations. We then discuss the effects of this hybridization on *P. pilosa* ssp. *sangamonensis* conservation and genetic composition.

Key words: Endemic taxon, Hybridization, Microsatellites, Phenology

Phlox divaricata* ssp. *laphamii* × *Phlox pilosa* ssp. *sangamonensis
Champaign County, Illinois: Approximately 5 miles southwest of Mahomet, Illinois.

INTRODUCTION

On May 22, 2018, the first and senior author sampled the "S-S" population of *Phlox pilosa* ssp. *sangamonensis*. S-S represents one of the largest remaining populations of the state-endangered *P. pilosa* ssp. *sangamonensis* by number of individuals. It is on private property which includes a semi-shaded yard that is nested within a mosaic of woodland, residential, and agricultural land. To the north of the sampling site is a mesophytic woodland and a house, whereas the area south of the canopy openings includes a thicket-like roadside and corn-soy farmland. At this site, there is close sympatry between populations of *Phlox divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis,* which intergrade within the woodland-thicket.

We surveyed all known *P. pilosa* ssp. *sangamonensis* populations to sample leaves for microsatellite

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genetic analyses and survey reproductive success of individuals. During counting of *P. pilosa* ssp. *sangamonensis* individuals, one author found a putative F_1 hybrid individual between *P. divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis* (Figure 1). We observed a single large plant growing under a 6 m tall *Quercus macrocarpa* Michx. We suspected that it was of hybrid origin due to the close flowering proximity of the two taxa and almost perfectly intermediate characteristics, specifically its leaf shape, calyx, and its faint blue-pink corolla color that was pale compared to its putative parents (Table 1).

Phlox specimens are notoriously variable in appearance, even within populations (Zale 2014). Therefore, we tested the hypothesis that it was an F_1 hybrid by combining genetic and morphological measures.

MATERIALS AND METHODS

We screened six microsatellites that were developed for North American *Phlox* species (Fehlberg et al. 2008; Fehlberg and Ferguson 2012). Collection of microsatellite data followed the protocol from Fehlberg et al. (2008). We recorded five microsatellite loci that were developed by Fehlberg et al. (2008) for *Phlox pilosa*; Phl-33, Phl-84, Phl-113, Phl-115, and Phl-137. We also used an additional microsatellite locus, Phl-28, from Fehlberg and Ferguson (2012). We scored microsatellites manually using Geneious Prime® (v. 2021.1.1; Biomatters, Auckland, New Zealand). We sampled a total of 250 individuals: 212 were *P. pilosa* ssp. *sangamonensis*, 37 were *P. divaricata* ssp. *laphamii*, and the last sample was the putative hybrid. We used a Bayesian clustering approach using the program Structure v. 2.3.4 (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009) to assess the genetic composition of the putative hybrid. Structure estimates the proportion of an individual's genome that belongs to a particular cluster. The program uses Bayesian clustering and Markov chain and Monte Carlo simulations to estimate an individual's identity using posterior probabilities. The user defines the number of clusters to be tested, *K*. No prior information about taxonomic or population groupings is included in Structure. We averaged data from 10 runs, each with 200,000 iterations after an initial burn-in of 50,000 iterations. We determined the optimal number of clusters by testing K=1–13 and used the admixture model assuming correlated allele frequencies. We chose the best-supported number of clusters following Evanno et al. (2005) and using structure harvester (Earl and vonHoldt 2012). We considered individuals hybrids if q<0.85 (see Abraham et al. 2011); q represents an estimate of posterior probability of an individual genotype.

In 2022, we measured eight morphological characteristics of the two taxa from 100 individuals of each taxon from the same populations, as well as two sets of measures from a single putative hybrid: leaf area, specific leaf area, leaf dry matter content, leaf length/width ratio, floral tube length, corolla lobe length/width ratio, flowering stem height, and relative corolla color measured as the ratio of R/B color values. Methods to process leaves followed Pérez-Harguindeguy et al. (2013). To calculate leaf area, and thereafter leaf dry matter content and specific leaf area, we measured leaves using the Leafscan smartphone app (Anderson and Rosas-Anderson 2017). To estimate floral color, we first photographed collected corollas under typical fluorescent light conditions in an office. Then we used the Image Color Picker (2022) selector tool for each corolla in the images to estimate the RGB (red, green, blue) values for each harvested corolla. From these values, we calculated the R/B ratio to assess reddishness of flowers. We used a clonal cutting grown in one author's garden for measurements because the wild plant was nonflowering and smaller in subsequent years, probably due to shading by *Lonicera maackii* (Rupr.) Herder. We conducted a principal component analysis (PCA) on these morphological traits to characterize the morphological features of the two parental taxa and the putative hybrid. The eight variables were scaled prior to the PCA, i.e., all were standardized to have a mean of zero and standard deviation of one. Visual inspection of transformed variables showed consistency with normal distributions. Phenology data for *Phlox divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis* were recorded in May–July 2019 in three sympatric populations (though not the S-S population) to assess if cross-pollination was feasible; the number of intact and senesced corollas were counted during the monitoring period for each individual.

Table 1. Morphological trait table of the putative hybrid, *Phlox divaricata* ssp. *laphamii*, and *P. pilosa* ssp. *sangamonensis*. For numeric measures in *P. divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis*, the given range represents the interquartile range calculated in 100 samples for each taxon, while the number in the parentheses represents the median trait value ($\tilde{x}$). The ranges for the hybrid were approximated from measurements from one individual. We show approximate colors for corolla and pollen color.

Trait	Hybrid	*P. divaricata* ssp. *laphamii*	*P. pilosa* ssp. *angamonensis*
Leaf shape	Ovate-lanceolate	Ovate	Linear-lanceolate
Leaf length/width ratio range	~6–8	2–3 (2.5)	7–10 (8.2)
Specific leaf area $cm^2\ g^{-1}$	~30–32	30–39 (34)	20–26 (23)
Leaf dry matter content (%)	~19	16–19 (18)	20–23 (22)
Calyx lobes	Somewhat reflexed	Somewhat reflexed	Erect
Flowering stem height (cm)	~32	27–34 (30)	34–41 (36)
Corolla lobe length/width ratio	~1.5–1.8	1.6–1.8 (1.7)	1.4–1.6 (1.5)
Floral tube length (mm)	~14.5–15.5	13–14.5 (14)	14–15.5 (15)
Corolla color	French lilac–Queen pink	Blue purple–Lavender	Dull pink–hot pink
Pollen Color	Bright yellow	Bright yellow	Orange yellow

Raw data can be obtained at (**https://castaneajournal.com/supplemental_data/03_CAST88%282%29_Zinnen_supplement.zip**).

RESULTS

We found robust evidence that the sample was an F_1 *Phlox divaricata* ssp. *laphamii* × *P. pilosa* ssp. *sangamonensis*. The PCA of morphological traits showed that the putative hybrid was intermediate to *P. divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis*, with relatively little overlap and clustering compared to the other 200 samples (Figure 2). The STRUCTURE analysis reinforced these findings; K=2 fit the data the best (Figure 3). For the hybrid, q=0.498 for cluster 1, and q=0.502 for cluster 2, suggesting nearly equal estimated probability of belonging to either the *P. divaricata* ssp. *laphamii* or *P. pilosa* ssp. *sangamonensis* cluster, respectively. No other genetic samples suggested significant hybrid status (max q>0.88).

There was modest phenological overlap between *P. divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis*, with overlap occurring for about one week from May 17 through May 26 (Figure 4). Within this time period, the percentage of the flowers that bloomed was relatively low for both taxa. However, on May 23 there was a similar percentage (~5%) of blooming flowers for both taxa. Around this time, blooming of *P. divaricata* ssp. *laphamii* is steeply in decline, coinciding with a brief period of scattered *P. pilosa* ssp. *sangamonensis* blooming before a sharp increase at the end of May.

The hybrid had several notable physical features that distinguish it from the parental taxa. The hybrid corolla colors have hues of both blue-purple and pink that do not appear consistent with either parental taxon. Like *P. divaricata* ssp. *laphamii*, the hybrid has somewhat reflexed calyx lobes, and its overall corolla size was larger than those of *P. pilosa* ssp. *sangamonensis*. However, the hybrid had ovate-lanceolate leaves that have length/width ratios greater than five and floral tube lengths >14.5 mm, which were closer in appearance to *P. pilosa* ssp. *sangamonensis*.

After confirming the specimen was a hybrid, we deposited the specimen to the Illinois Natural History Survey Herbarium, ILSS (Figure S1).

Figure 1. The hybrid *Phlox divaricata* ssp. laphamii × *Phlox pilosa* ssp. *sangamonensis* at the S-S site (May 2018).

Significance

This is the first documented wild occurrence of hybridization event with *P. pilosa* ssp. *sangamonensis*. *Phlox pilosa* ssp. *sangamonensis* is a state-endangered and narrowly endemic taxon, found only in Champaign and Piatt counties (east-central Illinois) near the Sangamon River. *Phlox pilosa* ssp. *sangamonensis* is recognized as a distinct taxon that is reproductively incompatible with other *P. pilosa* individuals found in Illinois (Levin and Smith 1965; Levin 1966; Levin 1984).

The relationships among eastern *Phlox* taxa, including for *P. pilosa* taxa, are convoluted. Historical and recent evidence may suggest that *P. pilosa* ssp. *sangamonensis* represents a disjunction of a genetic cluster of *P. pilosa* populations from the southeastern United States–specifically *P. pilosa* ssp. *detonsa* (A. Gray) Wherry (Levin and Smith 1965, Levin 1984). However, interpreting *P. pilosa* taxonomy is complicated because multiple studies suggest that many eastern United States *Phlox* taxa are polyphyletic. Ferguson and Jansen (2002) produced phylogenies of eastern *Phlox* species using chloroplast DNA. These authors found a lack of monophyly among many *Phlox* taxa, including subspecies of *P. pilosa*. Furthermore, recent evidence using single nucleotide polymorphisms suggests some southeastern *Phlox pilosa* ssp. *pilosa* L. populations are a monophyletic clade that is distinct from northern *P. pilosa* ssp. *pilosa* populations (Garner et al. 2022, preprint).

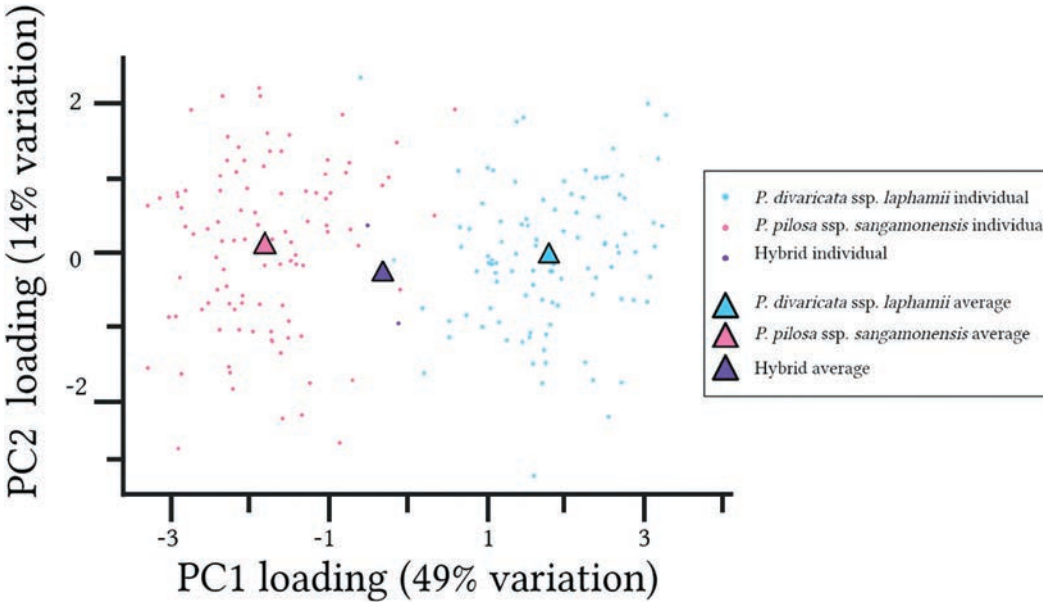

Figure 2. Principal component analysis of eight morphometric variables for *Phlox divaricata* ssp. *laphamii* (n=100), *P. pilosa* ssp. *sangamonensis* (n=100), and the putative hybrid individual (two sets of measurements from one plant). Two PCs explained 63% of the total variance. Variables that were the strongest contributors to PC1 (49% variation explained) were R/B ratio (-0.44), leaf length/width ratio (-0.43), and specific leaf area (0.41); the strongest contributors to PC2 (14% variation) included R/B ratio (-0.66), leaf area (0.46), and flowering stem height (0.40).

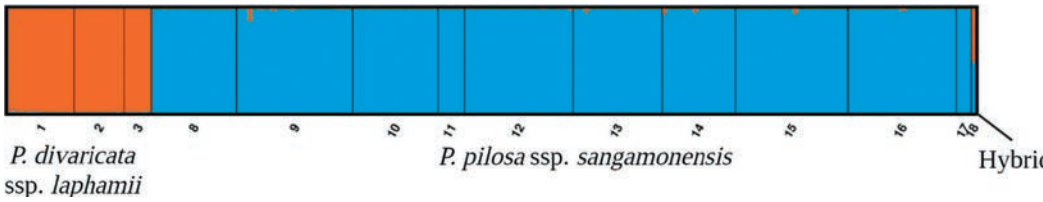

Figure 3. The results of the structure analysis (*K*=2) showing strong evidence for hybridization between *Phlox divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis*. Cluster 1 (orange) associated strongly with the 37 *P. divaricata* ssp. *laphamii* populations 1–3; cluster 2 (blue) associated with the 212 *P. pilosa* ssp. *sangamonensis* samples in populations 8–17. No a priori information about population or taxonomic status was included in the structure algorithm. This figure was created using StructureSelector (Li and Liu 2018).

In the Garner et al. (2022 preprint) study, *P. pilosa* ssp. *sangamonensis* was closely related to *P. pilosa* ssp. *detonsa*, *Phlox pilosa* ssp. *ozarkana* (Wherry) Wherry, and the southeastern *P. pilosa* ssp. *pilosa* populations, but not northern *P. pilosa* ssp. *pilosa* populations or other *P. pilosa* subspecies. This lack of monophyly could be in part due to introgression, or because some *Phlox* taxa (e.g., *P. pilosa* ssp. *pilosa*) combine separate, cryptic taxonomic units.

There is precedence for hybridization between *P. pilosa* and *P. divaricata*. Wherry (1955) described the hybrid *Phlox ×glutinosa* Buckley, which were putative hybrids of *P. pilosa* and *P. divaricata* that were found in Alabama and Arkansas. The intraspecific taxonomic designations of the *P. pilosa* parents for the *Phlox ×glutinosa* collections were unspecified. Nonetheless, our collection represents the first confirmed wild hybrid involving *P. pilosa* ssp. *sangamonensis* using genetic, morphological, and phenological data.

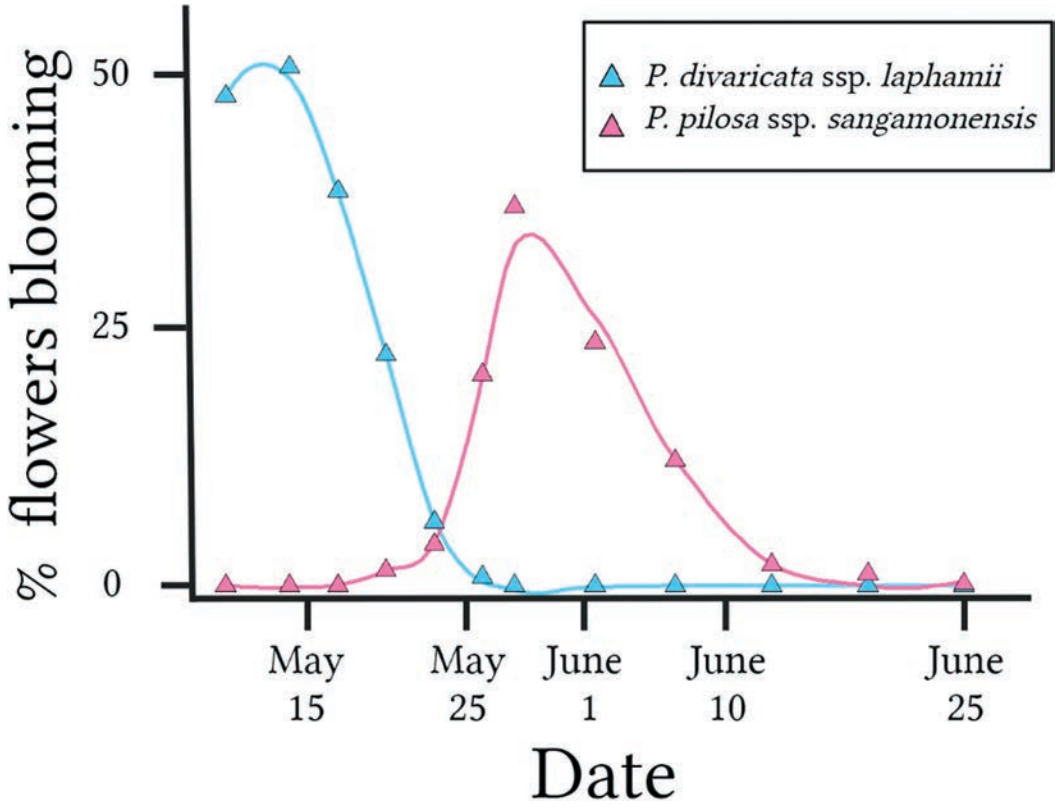

Figure 4. Flowering density of *Phlox divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis* from May–June. Percentage of flowers blooming (viz., shedding pollen) are shown as averages across three populations of *P. divaricata* ssp. *laphamii* (n=34) and *P. pilosa* ssp. *sangamonensis* (n=32). Blue and pink lines show the localized fit of sample points.

Previously, Levin (1966) showed that interspecific crosses of *P. divaricata* ssp. *laphamii* × *P. pilosa* ssp. *sangamonensis* can produce seeds, though crossings faced moderate to strong reproductive barriers with a substantial reduction (nearly 50%) in seed set for both taxa. Our data show that such pollination events can feasibly occur in natural settings. In addition to the moderate incompatibility barriers shown by Levin (1966), we note that the incongruent phenology between *P. divaricata* ssp. *laphamii* and *P. pilosa* ssp. *sangamonensis* is probably a strong hybridization barrier. Due to partial incompatibility and incongruent phenology, we suspect hybridization events are uncommon in nature. However, *P. divaricata* ssp. *laphamii* is sympatric or within close pollination distance (viz.,<25 m between flowering individuals) in half of the known existing populations of *P. pilosa* ssp. *sangamonensis*. Furthermore, in two of the four largest populations, individuals of the taxa are directly adjacent (<2 m distance). Because *Phlox* taxa are protandrous, we speculate that asymmetric pollination may occur; the relatively few *P. pilosa* ssp. *sangamonensis* that bloom ~May 20 may have a high probability of pollinating receptive *P. divaricata* ssp. *laphamii* flowers.

We suggest two potential consequences of natural hybridization for the conservation of state-endangered *P. pilosa* ssp. *sangamonensis*. First, the work of Levin (1966) suggests that pollen flow from *P. divaricata* to *P. pilosa* ssp. *sangamonensis* could interfere with the reproduction of *P. pilosa* ssp. *sangamonensis* at several populations during the initial blooming stages. However, because of protandry of the two taxa, it is unlikely that reproductive interference through ovule loss

is occurring at large scales in *P. pilosa* ssp. *sangamonensis*; for *P. divaricata* ssp. *laphamii*, large population sizes mean that any ovule loss is insignificant. Second, backcrossing may bilaterally introduce novel genetic diversity to the taxa. Levin (1966) documented similar hybrids of eastern *Phlox* taxa have viable pollen, though at reduced fitness.

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