Population genetics of the widespread perennial wildflower *Phlox speciosa* using microsatellite markers

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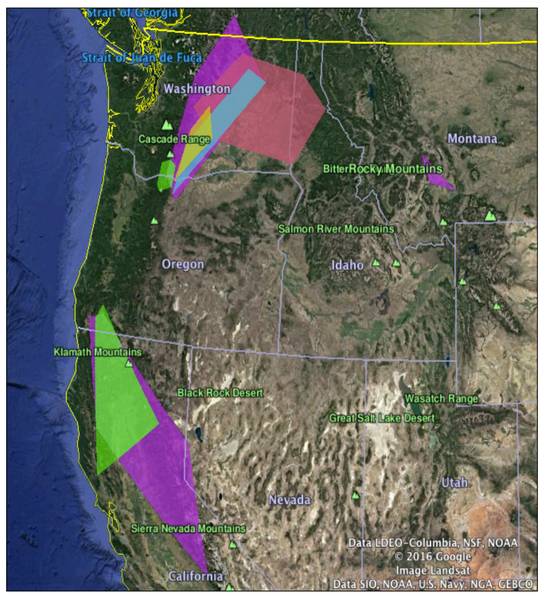
Introduction:

Phlox speciosa (showy phlox) is a perennial wildflower native to western North America. This species is widespread, ranging from the Sierra Nevada of California to British Columbia and into the mountains of Idaho and Montana. Individual plants are small herbs to subshrubs, with upright to trailing stems. *Phlox speciosa* has characteristic notched petals that range in coloration from white to bright pink (Fig. 1), and bloom during the months of April through June. It grows in several different types of environments, including dry rocky ridges, mixed conifer forests, and sagebrush slopes, on a variety of substrates (including basalt, granite, and serpentine soil) at low to medium elevations (75 - 2400 m) (Locklear 2011). Several subspecies and varieties have been identified by previous taxonomists based on morphological variation in the group, including plant habit, internode length, leaf pubescence and width, and petal notch depth (Fig. 2; Wherry 1955; Locklear 2011). This variation does not correspond well to geography, and the current Flora of North America and Jepson Manual taxonomic treatments have suspended the recognition of subspecific taxa in *P. speciosa* pending further investigation (Ferguson et al., 2016 and in prep.). Furthermore, many species of the genus *Phlox* have revealed variation in ploidy level when samples are obtained from across the species' range (e.g., Fehlberg and Ferguson 2012; Worcester et al. 2012; Zale et al. 2016), due to autopolyploidy or allopolyploidy.

Because this species is so wide-ranging, encompasses a great deal of morphological and ecological variation, and other species in the genus show variation in ploidy level, there is reason to suspect that *P. speciosa* may contain population structure or even cryptic species. The goal of this project is to investigate a sample of populations across the entire geographical range of *Phlox speciosa* cytotypically and genetically.

Figure 1. Images of *Phlox speciosa* from different localities, showing morphological differences in plant size, flower color, petal notch depth, and leaf shape. (a) Sierra National Forest, Fresno County, CA; (b) Grant County, WA; (c) Rogue River-Siskiyou National Forest, Josephine County, OR; (d) Rock Island, Douglas County, WA (corresponds to the type population of *P. speciosa* ssp. *lignosa*, shrubby habit highlighted). Photo credits: (a) and (d) Katherine Waselkov, © 2016; (b) Bill Harms, © 2013, (c) Walter Siegmund, © 2013.



Key to Figure 2
Purple: *Phlox speciosa* ssp. *occidentalis*,
Pink: spp. *α-speciosa*,
Green: ssp. *nitida*,
Blue: spp. *lanceolata*,
Yellow: ssp. *lignosa* (after Wherry 1955).

Figure 2. Map showing geographic ranges of subspecies, recognized by Wherry (1955), that are not currently recognized and instead treated as *Phlox speciosa* sensu lato (Ferguson et al. 2016). Map created in GoogleEarth.

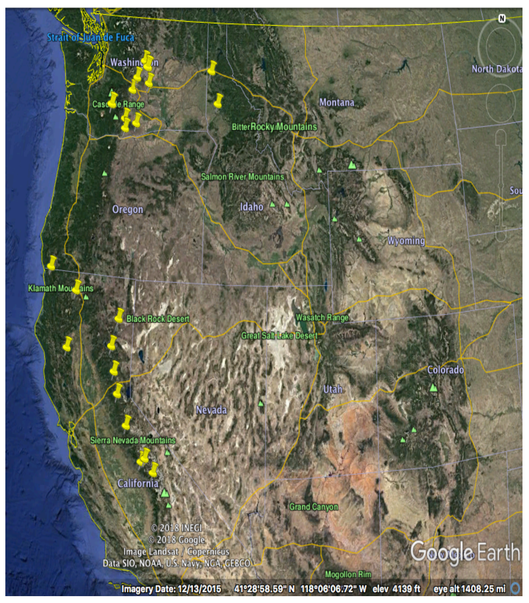


Figure 3. *Phlox speciosa* populations sampled for this project in May-June 2016 and April-May 2017. Map created in GoogleEarth.

Methods:

Sample Collection and Flow Cytometry:

- 23 populations of *Phlox speciosa* were sampled for this project from May 19 to June 28, 2016 and April 26 to May16, 2017 (Fig. 3). The sampled populations cover the geographical ranges of all five of Wherry's previously-recognized subspecies (compare to Fig. 2), with denser sampling in Washington because of the wide range of morphological and ecological diversity found in the Columbia Plateau region.
- Leaf material was preserved in silica gel for DNA analysis from 10 individuals from each population.
- One sample from each population was sent to Kansas State University for flow cytometry analysis.

DNA Extraction and Microsatellite Analysis:

- DNA has been extracted for population genetic analysis from all 23 populations collected.
- Leaf tissue was ground with steel beads in a tissue homogenizer and then extracted following a modified CTAB protocol (Doyle and Doyle 1987).
- Six microsatellite markers previously designed for *Phlox amabilis, P. pilosa*, and *P. nana* (Fehlberg et al. 2008; S. Fehlberg, unpubl. data) were selected for analysis in *Phlox speciosa* based on successful amplification and polymorphism in this species.
- Currently, all populations are being genotyped for these six microsatellites. Fluorescent dye labels are attached to each marker during PCR to enable multiplexing and genotyping on a fragment length analysis platform provided by Laragen, Inc. (Culver City, CA).
- Fragment size analysis (reading the microsatellite size peaks, or alleles) is performed using GeneMarker software (SoftGenetics). Thus far, data has been obtained for five of the six microsatellite loci, for some individuals in 22/23 populations, although much data is still missing.
- To calculate population genetic (diversity and distance) statistics (including principal coordinates analysis), the program GenAlEx (Peakall and Smouse 2006) was used, and to assess population structure, the programs STRUCTURE, StructureHarvester, and CLUMPAK were used (Pritchard et al. 2002; Earl and vonHoldt 2012; Kopelman et al. 2015).

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Results to Date: Very little genetic structure or cytotypic variation

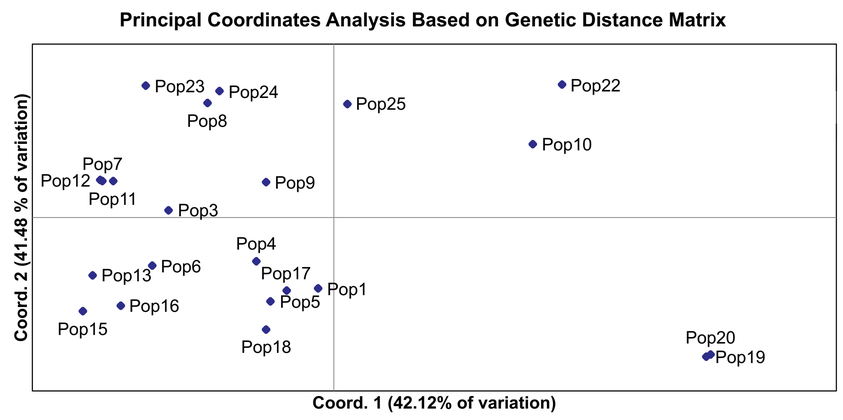

Figure 4. Principal coordinates analysis based on a pairwise genetic distance matrix from 5 microsatellites. Genotyping data was scored as dominant/haploid to control for possible polyploidy.

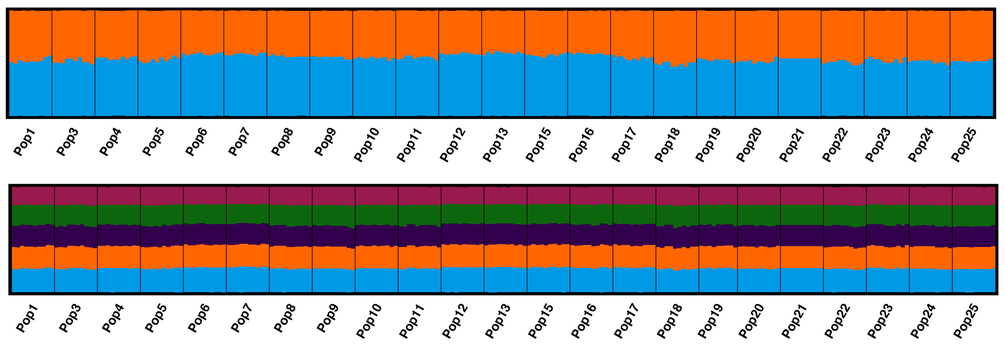

Figure 5. STRUCTURE bar graphs for K=2 and K=5, the two highest-likelihood number of genetic clusters based on Evanno's *ΔK* method (Evanno et al. 2005).

Population number	DNA content in picograms for one individual (based on Zea)	Inferred ploidy level	County and State	Mean samples genotyped	Total number of alleles over 5 loci	Mean number of alleles per locus	Total number of private alleles over 5 loci	He (expected heterozygosity)
1	9.41	2x	Yakima, WA	7.231	15	1.26	0	0.075
3	9.39	2x	Douglas, WA	7.590	22	1.60	2	0.104
4	9.93	2x	Kootenai, ID	7.449	15	1.06	2	0.058
5	9.75	2x	Nez Perce, ID	8.000	20	1.14	5	0.091
6	10.95 (11.02)	2x	Tuolumne, CA	8.513	12	1.26	0	0.051
7	11.48	2x	El Dorado, CA	7.564	9	1.65	1	0.019
8	10.74	2x	Mendocino, CA	5.244	11	1.65	1	0.035
9	9.46	2x	Siskiyou, CA	6.218	15	1.48	2	0.057
10	11.07 (11.34)	2x	Del Norte, CA	2.603	6	0.90	1	0.029
11	12.79 (11.26)	2x	Fresno, CA	7.564	10	1.42	0	0.035
12	11.54	2x	Fresno, CA	7.564	10	1.65	0	0.033
13	11.01	2x	Fresno, CA	9.321	13	1.56	0	0.051
15	10-11	2x	Nevada, CA	10.000	15	1.68	0	0.054
16	10-11	2x	Plumas, CA	9.590	18	1.82	3	0.053
17	10-11	2x	Lassen, CA	7.372	11	1.42	0	0.036
18	9.73	2x	Wasco, OR	8.462	21	1.72	0	0.086
19	10.59	2x	Lane, OR	4.154	11	1.6	0	0.048
20	10.51	2x	Yakima, WA	4.615	15	1.70	0	0.060
22	20.78	4x	Chelan, WA	2.115	10	1.70	2	0.042
23	10.29	2x	Klickitat, WA	5.385	11	2.19	2	0.031
24	Unknown	N/A	Klickitat, WA	4.769	16	2.50	1	0.046
25	Unknown	N/A	Fresno, CA	3.654	8	2.90	1	0.006
			All populations	6.303	78	1.56	23	0.048

Table 1. Populations of *Phlox speciosa* sampled in 2016, with flow cytometry data (DNA content in picograms), ploidy level, county, and state, and population genetic statistics from GenAlEx. *Zea* = *Zea mays* (a flow cytometry standard). *From the type locality of ssp. *lignosa*, sensu Wherry (Figure 2d).

Discussion and Ongoing Investigation:

Evidently, the subspecific variation observed by Wherry and Locklear does not have a cytotypic basis. In the genus *Phlox*, where cryptic polyploidy is very common, it is actually somewhat surprising to find an almost uniformly diploid species. It is also entirely unexpected that this species, which covers about 13 degrees of latitude and has a large geographical disjunct between California and Washington, shows no population structure at all with 5 microsatellite markers, at least using the Bayesian inference methods of the program STRUCTURE. The even apportionment of the hypothesized number of ancestral genetic clusters within individuals in all populations is an indication of lack of genetic structure (Fig. 5).

Principal coordinates analysis shows that the populations are not genetically identical, as does the presence of private alleles in several populations (Table 1), and the Φ_{PT} value for all populations is 0.683, which shows a substantial amount of genetic differentiation among vs. within populations. However, genetic diversity overall is low, as shown by number of alleles and expected heterozygosity.
Based on field observations, we suspect that *Phlox speciosa* ssp. *lignosa*, one of the most distinctive subspecies described by Wherry (with thin, sticky leaves, shubby habit, and white flowers), may be a plastic phenotype associated with basalt soil substrate. Our genetic analysis thus far supports this hypothesis, as the Washington and northern California populations that show this morphology are not genetically distinctive.

Population genetic work is ongoing, with data missing for all populations, and some populations with very little data collected yet. At the conclusion of our investigation, the same population genetic programs will be used to evaluate genetic diversity, connectivity, and structure within and among populations of *Phlox speciosa* across its range. At this time, we will have a firmer basis for decisions about species limits and subspecific variation in *Phlox speciosa,* and more information about evolutionary processes in *Phlox*.

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