NEW *PHLOX* HYBRIDS: Verification of the Origins of Horticultural Hybrids through Molecular and Morphological Techniques

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Abstract

Molecular and genetic analysis was used to confirm and or reject the paternity of horticulture hybrids. Morphological surveys of prominent characteristics were used to compare and contrast the assumed parents from their progeny. The data was analyzed suing discriminant statistical analysis. Genetic investigation using microsatellite loci were used to validate the conclusions reached through the statistical analysis obtained from the morphological data. From the data we were able to confirm and reject the parental lineages of thought to be *Phlox* hybrids as well as gain an understanding of the arising importance and advantage of using molecular genetic analysis in the horticulture trade as a validation method.

Introduction and Background

Phlox, a genus of the Polemoniaceae family, is comprised of over sixty species of flowering plants native to North America and Asia. A large number of these are being used in horticulture through methods such as: selection, crosses and hybridization, by which many different hybrids are being produced. Phlox are currently one of the focal genera in the horticultural breeding program at the Chicago Botanic Garden. Different hybrids produced by crosses were investigated using both morphological measurements and DNA fingerprinting techniques to verify the parental lines of the hybrids in question.

Objectives/ Hypothesis

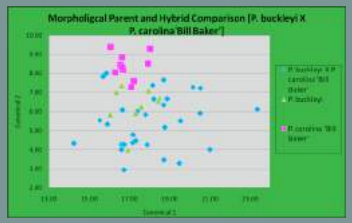
It is hypothesized that all the hybrids in question are true hybrids and that the morphological and molecular data will support this assumption.

The study will serve as a method of verifying whether the hybrids in question are true or not and to attempt to confirm or reject the assumed parent lines.

Methods and Materials

Morphological data was obtained from eight hybrids and their putative and parental lines. Sample measurements of: leaf width, leaf height, corolla face diameter, corolla tube length, petal width, and overall plant height were taken from six individuals and five different sub-samples of the individuals sampled. Calipers were used to measure all except for the final height. All measurements were compared using Principal component analysis using the statistical software JMP. Genetic analysis was then conducted to confirm the parentage of the progeny. Leaf material was obtained from each of the hybrids and the parents and stored in -80 C° until used. DNA extraction was done using a Qiagen DNeasy Plant Mini Kit. We used microsatellite loci and PCR conditions developed for *Phlox pilosa*[1]. We found primers, Phl 68, Phl 98, Phl 113, Phl 137 worked best for the study. The PCR products were scored using a Beckman Coulter CEQ Genetic Analysis System.

Results



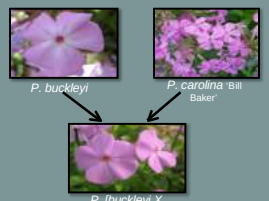
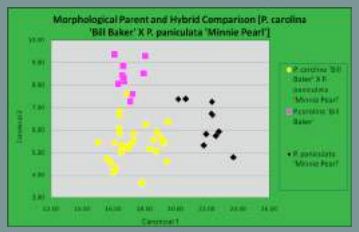
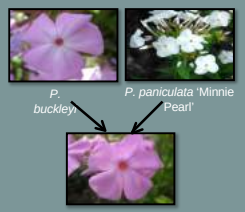


Figure 1.2: Discriminant Analysis of : final height , corolla tube length, leaf width, and petal width of parents and progeny





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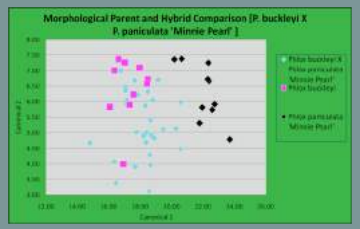

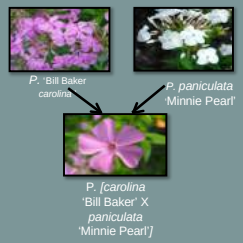

Figure 1.1: Discriminant Analysis of : final height , corolla tube length, leaf width, and petal width of parents and progeny

Cross	Percent alleles unique to pollen donor	Percent of alleles which can only be derived from pollen donor	Hybridization
P.borealis X P. kelseyi ' Lemhi Purple'	63%	4%	Likely self pollinated
P.borealis X P. kelseyi ' Lemhi Purple'	50%	3%	Likely self pollinated
P. buckleyi X P. subulata 'McDaniel's Cushion'	50%	0%	Likely self pollinated
P. carolina 'Bill Baker' X P. paniculata 'Minnie Pearl'	67%	0%	Likely self pollinated
P. buckleyi X P. carolina 'Bill Baker'	83%	22%	Possible hybridization
P. nivalis X P. kelseyi	75%	23%	Possible hybridization
P. kelseyi X P. nivalis	67%	22%	Possible hybridization
P. bifida X P. kelseyi 'Lemhi Purple'	83%	8%	Likely self pollinated

Table 1.0: Heritability of genotype from pollen donor

Discussion and Conclusion

Morphological data indicate that there are differences at the macro levels in each of the parents when compared to one another. When the morphological traits of the parents are compared with that of their potential progeny, it is indicated that the offspring have distinct differences from P. 'Minnie Pearl' and tend to be more similar to the other parent involved in the cross. After genetic analysis was conducted on the parents and the offspring, it was observed that both crosses involving, *P. paniculata* 'Minnie Pearl', are possible selfings of the other parent involved in the cross. This could indicate that *P. paniculata* 'Minnie Pearl' might not produce viable pollen, given that it is a natural occurring hybrid itself. In regards to the cross between *P. buckleyi* and *P. carolina* 'Bill Baker' both the morphological data as well and the genetic analysis indicate that the cross produced a true hybrid that is more similar to its seed parent.

Genetic analysis was carried out for five other crosses involving other parent lines. Morphological surveys were not conducted on these hybrids since not enough morphological characteristics were able to be obtained at the time of the study. Genetic analysis confirmed or rejected the parentage of the given hybrids in question. For future research it is advised that morphological surveys be taken for the other crosses on both the parents and the progeny. Further molecular techniques could also be used, such as sequencing, in order to confirm or reject the paternity of the hybrids studied. This study further confirmed the advantage and the importance of using molecular techniques to confirm the paternity of new hybrids

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