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Divergence in floral scent profiles among and within cultivated species of *Phlox*

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ABSTRACT

Variation in floral scent profiles within and among plant species may be attributed to a number of different phenomena, including selection by pollinators or enemies, direct or indirect artificial selection in horticulture, stochastic processes such as genetic drift, and pleiotropy between scent and other floral traits, including floral color. The genus *Phlox* represents a unique opportunity to study scent variation across multiple species using horticultural specimens derived, in part, to generate color diversity. In this study, we assessed floral scent variation among horticultural cultivars of nine different *Phlox* species and the related species *Polemonium caeruleum*, as well as differences in scent variation between wild-originated and horticultural *Phlox drummondii* color morphs using dynamic headspace extraction and gas chromatography–mass spectroscopy. We identified significant variation in floral scent among species of *Phlox*, between color morphs pooled across species, and significant scent variation between cultivars showing different floral colors within the species *P. subulata, P. stolonifera, P. carolina*, and *P. drummondii*. We also uncovered significant differences between the volatile profiles of wild-originated and horticultural specimens of *P. drummondii*. Color morphs of horticultural *P. drummondii* differed in their scent, while morphs from wild-originated plants did not. This study documents rampant variation in floral scent composition within and among *Phlox* species and their horticultural cultivars. Species-level differences are likely to have been shaped by differences in selection regimes (both artificial selection in cultivation and natural selection in wild progenitors) and/or stochastic processes among groups. Differences among color morphs suggest some possibility of pleiotropy between color and scent. Species with particularly high levels of between-cultivar variation further highlight the potential for pleiotropic interactions between color and scent phenotypes, although no clear patterns connecting pigment to production of particular scent compounds emerge. Our finding that horticultural *P. drummondii* shows greater variation in scent profiles among color morphs suggests that either selection in wild populations minimizes scent differences between color morphs or relaxation of selection/incidental artificial selection on scent in horticultural settings allows accumulation of variation between color morphs.

1. Introduction

Floral scent is well-established as a biologically relevant trait, particularly in the attraction of potential pollinators and/or floral enemies (e.g., Theis and Lerdau, 2003; Raguso, 2004; Schiestl, 2010). Floral scent varies within and among populations in several plant species (e.g., Dobson et al., 1997; Füssel et al., 2007; Majetic et al., 2008; Schlumpberger and Raguso, 2008), and at least some of this variation is attributable to the sensory biases and preferences of alternate floral visitors (Salzmann et al., 2007; Majetic et al., 2009a; Galen et al., 2011). Direct pollinator-mediated natural selection has long been considered to be a driver of floral scent divergence between congeneric species (e.g., Dobson et al., 1997; Raguso et al., 2003; Jürgens, 2004). Recent studies have increasingly recognized the role of floral enemies in shaping floral scent phenotype (e.g., Junker et al., 2011; Kessler et al., 2012; Theis and Adler, 2012).

Although much attention has been given to the likely role of pollinators or enemies in scent divergence, the possibility that genetic drift and/or correlated responses to selection on other floral or vegetative traits could drive divergence in scent composition has received less attention. For example, proof of concept for

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biosynthetic pleiotropy between specific floral volatiles and structurally related pigments that lend color to flowers was demonstrated in a gene-silencing study using anti-sense RNA to manipulate floral display in cultivated *Dianthus caryophyllus* (Zuker et al., 2002). Studies that have tested for volatile-pigment correlation in wild plant species have led to different outcomes, with some studies supporting (Knudsen and Stahl, 1994; Salzmann and Schiestl, 2007; Majetic et al., 2008) and others rejecting the biosynthetic pleiotropy hypothesis (Majetic et al., 2010; Wang et al., 2013).

Horticultural systems are prime vehicles to study indirect selection or stochastic processes on scent divergence, because observed differences between conspecific cultivars are likely outcomes of artificial selection for alternative flower color, size, and shape, or for divergent vegetative traits (Lavid et al., 2002; Nakamura et al., 2006; Cherri-Martin et al., 2007). It is noteworthy that cultivars of rose, lily, snapdragon, and petunia have scents which differ considerably regardless of breeding intent (Antonelli et al., 1997; Odell et al., 1999; Dudareva et al., 2000; Lavid et al., 2002; Verdonk et al., 2005; Nakamura et al., 2006; Kong et al., 2012). Cultivation for other marketable floral characteristics may also impact floral scent. Horticultural researchers have proposed that breeding for longer shelf-life may have led to unanticipated decreases in floral odor emission (Vainstein et al., 2001; Guterman et al., 2002; Lavid et al., 2002). While the exact link remains somewhat speculative, studies examining ethylene responses (a key focal area for increasing floral longevity) suggest that for species like sweet pea and petunia, modifying ethylene action also modifies floral scent gene activity and/or overall output (Sexton et al., 2005; Underwood et al., 2005; Dexter et al., 2008). Regardless of the underlying mechanisms of change, floral scent variation among cultivars suggests that species may contain a great deal of potential for variation in scent composition, and that scent divergence observed in wild variants of a species may not be driven only by visitor-mediated selection.

The genus *Phlox* and its close relatives in the Polemoniaceae are a favorable study system for an exploration of scent variation independent of pollinator preference because several species have multiple cultivars (Locklear, 2011) and because many of these species produce a noticeable scent (Grant and Grant, 1965; Harper, 2003; Junker et al., 2011; Majetic and Sinka, 2013). Within each species, the available cultivars generally vary in flower color (e.g., white, pink, purple, red), floral patterning (e.g., striped vs. solid), flower size, and/or plant height (e.g., dwarf vs. tall) (Locklear, 2011). Cultivars of perennial species typically are propagated through vegetative cloning, meaning that one can replicate a single "genotype" easily throughout an experiment. Moreover, the study of scent in horticultural systems has a great deal of practical application; changing or increasing scent production is seen as an area of significant interest (Clark et al., 2004). Working with a genus such as *Phlox* that is recognized in terms of horticulture and natural history allows us the opportunity to consider scent from both applied and basic research perspectives.

In this study, we explore variation in the chemical composition of floral scent in horticultural cultivars from nine species of *Phlox* and in *Polemonium caeruleum* (also a member of the family Polemoniaceae). First, we compare scent composition between *Phlox* accessions at the level of species, with the expectation of encountering species-specific volatile blends as is often observed in genus-level analyses (Dobson et al., 1997; Levin et al., 2003). Next, we focus on conspecific cultivars with differing pigmentation, and explore the potential for these cultivars to differ in their floral scent profiles due to scent-pigment pleiotropy. Finally, we compare the scent profiles of horticultural and wild *Phlox drummondii* color variants, which have likely been shaped by very different selective pressures.

2. Materials and methods

2.1. Plant material and floral scent collection

Between fall 2009 and spring 2011, *Phlox* (eighteen cultivars of nine different species) and *P. caeruleum* (blue and white cultivars) were ordered from several different nurseries and plant wholesalers (see Table 1 for a complete listing). Upon arrival, all plants were transplanted into 2.75 L pots (Magnum Square, Belden Plastics, Roseville, MN, USA) with Fafard #4 potting soil (Agawam, MA, USA), fertilized with Osmocote® Outdoor and Indoor Plant Food (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) and transferred to the Saint Mary's College greenhouse (Notre Dame, IN, USA). Plants were housed in the greenhouse and watered ad libitum until flowering. Plants that did not flower during the spring immediately following their arrival were overwintered for approximately four months in a cold frame adjacent to the greenhouse to stimulate flowering. Plants were returned to the greenhouse in March of the next year, fertilized again, and watered ad libitum until flowering. Representatives from some species (e.g., *P. maculata*, red *P. bifida*, and white *P. divaricata*) did not survive the overwintering period; others survived but overwintering was not sufficient to induce flowering. For these species, sample sizes were smaller than originally anticipated.

Upon flowering, floral scent was collected from fully opened (i.e. mature), attached flowers on each plant using dynamic headspace extraction for 1 h at ~250 ml/min air flow, as in Majetic et al. (2007). All available flowers on the inflorescence were enclosed with 0.5 L Reynolds Oven Bags (Reynolds Inc., Richmond, VA, USA) and traps containing 10 mg Porapak Super Q were eluted with 300 μL of GC–MS purity hexane and stored at −20 °C until chemical assessment. Ambient air and vegetative controls were collected periodically during the experiment to control for background volatile contaminants; all samples were collected between approximately 1000 and 1400 h. We recorded the mass of flowers sampled (to two significant digits) following collection to quantify sampled tissue (see Supplementary Appendix C).

In the spring and summer of 2011, the annual species *P. drummondii* was grown from seed collected from a wild population (2 mi. south of Bastrop, Bastrop Co., TX, USA) in the greenhouses at the University of Texas at Austin (Austin, TX, USA). Plants were grown in a standard greenhouse soil mix and were watered daily. Upon flowering, scent was collected from all available attached flowers on mature flowering plants using dynamic headspace extraction for 1 h, as described above. PAS-500 personal air sampler pumps (Spectrex Corporation, Redwood City, CA, USA) were used for scent collection; as before, traps were eluted with 300 μL of hexane and stored at -20 °C until chemical assessment. Ambient air and vegetative controls were again collected to control for background contamination and all samples were collected between 1000 and 1530 h. Floral mass was recorded following scent collection.

2.2. Floral scent chemical assessment

All floral scent samples were analyzed by gas chromatography–mass spectroscopy (GC–MS) at Cornell University (Ithaca, NY, USA) with a Shimadzu GC17A gas chromatograph with a QP mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan) as described in Majetic et al. (2008, 2010). Prior to analysis, all samples were concentrated to 75 μL with N_2 gas, and 5 μL of 0.03% toluene (16 μg) was added as an internal standard in order to normalize all compounds to toluene units (see description below). One microliter sample aliquots were injected (splitless) at 240 °C onto an EC-wax fused capillary GC column, exposed to a temperature ramp of 10 °C/min from 40 to 260 °C with a hold for 3 min at the beginning and 5 min at the end. A Shimadzu QP5000 quadropole