

# Phytochemistry

## Extensive population-level sampling reveals clinal variation in (R)(-)-linalool produced by the flowers of an endemic evening primrose, *Oenothera harringtonii* --Manuscript Draft--

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<b>Abstract:</b>	The study of floral trait diversity has a long history due to its role in angiosperm diversification. While many studies have focused on visual traits including morphology and color, few have included floral scent despite its importance in pollination. Of the studies that have included floral scent, sampling has been limited and rarely explores variation at the population level. We studied <i>Oenothera harringtonii</i> , which is primarily pollinated by hawkmoths and has flowers that open at dusk, produce nectar and are highly fragrant. We collected floral trait (morphology, scent chemistry and emission rates) data from 650 individuals from 19 wild populations and 49 individuals grown in a common environment. We identified 35 floral volatiles representing 5 biosynthetic classes. Population differentiation was stronger for floral scent chemistry than floral morphology. ( R )( - )-linalool was the most important floral trait differentiating populations, exhibiting a striking pattern of clinal variation across the distribution of <i>O. harringtonii</i> without any correlated shifts in floral morphology. Populations in the north and west produced ( R )( - )-linalool consistently, those in the east and south largely lacked it, and populations at the center of the distribution were polymorphic. Floral scent emissions in wild populations varied across years but chemical composition was largely consistent over time. Similarly, emission rates in greenhouse-grown plants were lower but chemical composition was consistent with the wild population of origin. Our data set represents the most extensive population-level sampling of floral scent to date and suggests that such sampling may be needed to accurately capture the nature of variation in wild populations.

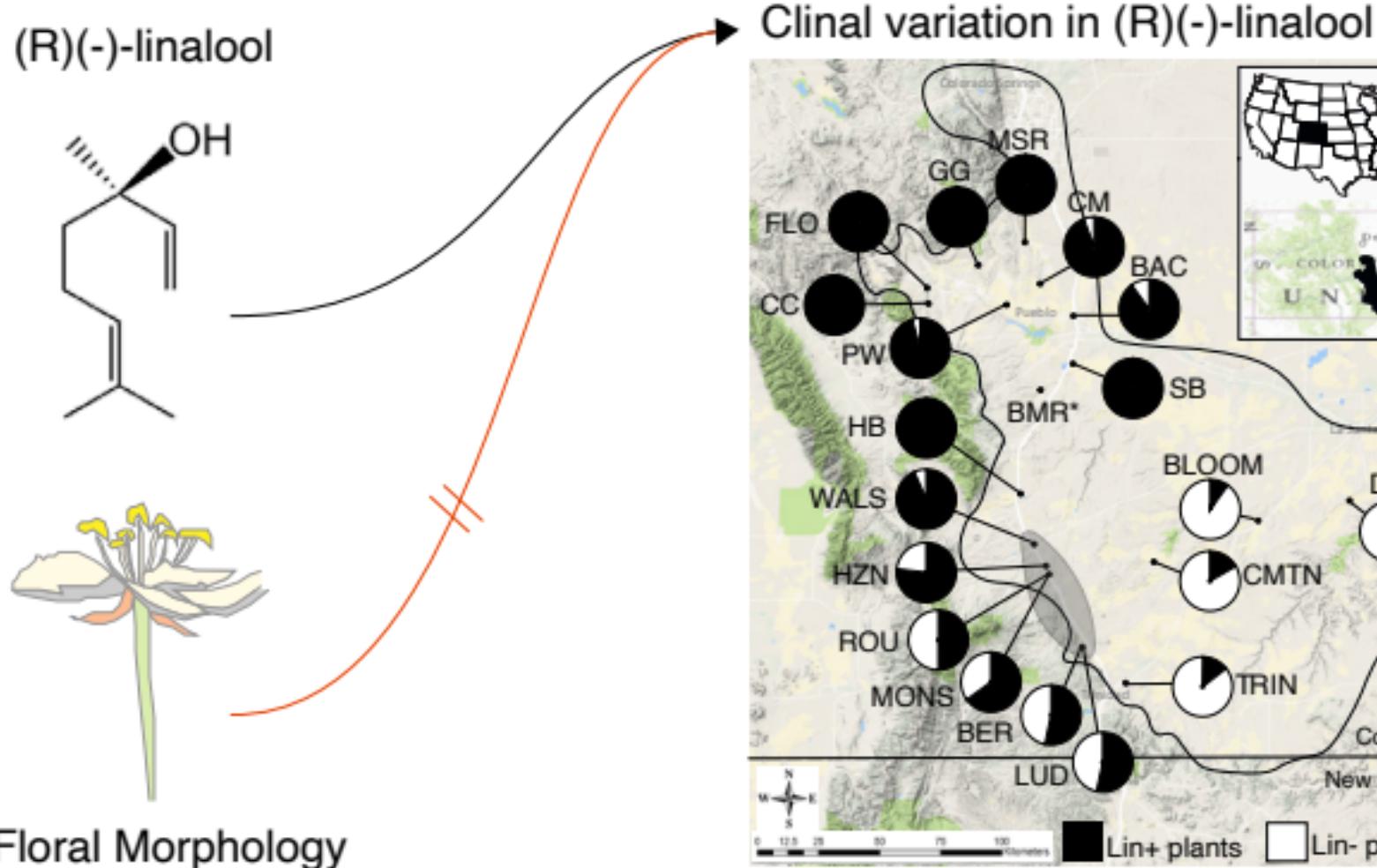
## Highlights

- Extensive sampling captures the nature of floral trait variation in wild populations
- Population differentiation was stronger for floral scent chemistry than morphology
- $(R)$ -linalool was the most important floral trait differentiating populations
- $(R)$ -linalool polymorphism exhibits a geographic cline with no shifts in morphology

# Graphical Abstract

*Oenothera harringtonii*

19 Populations  
650 Individuals



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Floral trait variation in  
*Oenothera harringtonii*

19 Populations  
650 Individuals



(R)(-)-linalool



Floral Morphology

Clinal variation in (R)(-)-linalool



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7     4 Extensive population-level sampling reveals clinal variation in (*R*(*-*)-linalool produced by the  
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9     5 flowers of an endemic evening primrose, *Oenothera harringtonii*  
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14     7 **Authors**  
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16     8 Krissa A. Skogen<sup>a,\*</sup>, Tania Jogesh<sup>a</sup>, Evan T. Hilpman<sup>a,b</sup>, Sadie L. Todd<sup>a,c</sup>, and Robert A.  
17  
18     9 Raguso<sup>d</sup>  
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21     10  
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23  
24     11 <sup>a</sup> Negaunee Institute for Plant Conservation Science and Action, Chicago Botanic Garden,  
25  
26     12 Glencoe, Illinois 60035 USA  
27  
28     13 kskogen@chicagobotanic.org  
29  
30  
31     14 <sup>b</sup> School of Biological Sciences, Washington State University, Pullman, Washington 99164 USA  
32  
33     15 <sup>c</sup> Iowa Department of Agriculture and Land Stewardship, Ankeny, IA 50023 USA  
34  
35     16 <sup>d</sup> Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853 USA  
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38     17  
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40  
41     18 **Corresponding Author**  
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43     19 Krissa Skogen, Negaunee Institute for Plant Conservation Science and Action, Chicago Botanic  
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45     20 Garden, Look Cook Road Glencoe, IL 60022. +1-847-835-6919.  
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48     21 kskogen@chicagobotanic.org  
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53     23 **Abstract**  
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55     24 The study of floral trait diversity has a long history due to its role in angiosperm diversification.  
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6    27 scent, sampling has been limited and rarely explores variation at the population level. We studied  
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9  
10    29 at dusk, produce nectar and are highly fragrant. We collected floral trait (morphology, scent  
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12    30 chemistry and emission rates) data from 650 individuals from 19 wild populations and 49  
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18 morphology. (*R*(*-*)-linalool was the most important floral trait differentiating populations,  
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34 floral scent to date and suggests that such sampling may be needed to accurately capture the  
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36 nature of variation in wild populations.

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44    **Keywords**

45    *Oenothera harringtonii*; Onagraceae, Arkansas River Valley evening primrose, mass  
46 spectrometry; pollination; floral scent; floral morphology; monoterpenes; linalool

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4 47 **1. Introduction**  
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7 48 The study of floral trait diversity has a long history due to its importance in  
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9 49 understanding angiosperm diversification (Fenster et al., 2004; Ollerton et al., 2011). Variation  
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11 50 in floral traits and the associated selective forces are considered a primary driver of speciation,  
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13 51 commonly attributed to pollinator-mediated selection (Grant and Grant 1965, Stebbins 1974).

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15 52 While floral morphology and color have been extensively studied, comparatively little is known  
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17 53 about the role that floral scent plays in mediating interactions with biotic agents of selection  
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19 54 (Delle-Vedove et al., 2017; Raguso, 2008; Whitehead and Peakall, 2009). Including floral scent  
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21 55 traits in studies of floral phenotypes is critical for developing a deeper understanding of  
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23 56 evolution and speciation in many angiosperm systems, particularly those for which scent plays  
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25 57 an integral role in pollinator attraction. The most detailed studies to date that provide evidence  
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27 58 for scent-mediated diversification focus on extreme pollination specialization, including sexually  
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29 59 deceptive pollination of orchids and brood-site mutualisms (reviewed in Raguso, 2004;  
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31 60 Whitehead and Peakall, 2009), but much remains to be learned from more generalized systems  
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33 61 and **those where visual and olfactory cues operate synergistically** (Byers et al., 2014; Larue et al.,  
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35 62 2016). More recent work has revealed that floral scent is not a fixed, species-level trait; rather, it  
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37 63 can be highly variable within a species, and can be explained by factors beyond pollinator-  
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39 64 mediated selection (reviewed in Delle-Vedove et al., 2017).

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41 65 Studies investigating variation in floral scent have tended to compare groups of related  
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43 66 taxa to understand the role that scent may play in reproductive isolation and speciation (Byers et  
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45 67 al., 2014; Chartier et al., 2011; Schiestl, 2015; Waelti et al., 2008). For example, work on the  
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47 68 genus *Lithophragma* and their pollinating floral parasites, *Greyia* moths, has revealed remarkable  
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49 69 floral scent variation among clades, species, and populations (Friberg et al., 2019). In other

systems, floral volatiles reflect taxonomic boundaries between otherwise morphologically similar taxa, including sexually deceptive *Chiloglottis* (Ebert et al., 2009) and *Orphys* orchids (Mant et al., 2005). Differences in floral scent among taxa have been shown to reduce the potential for introgression and to be an effective means of reproductive isolation between two cross-compatible species, *Silene latifolia* and *S. dioica* (Waelti et al., 2008). In other systems, hybrids have been shown to produce the same floral scents as parental taxa as well as transgressive compounds (those not produced by either parent; Chartier et al., 2016; Vereecken et al., 2010), providing opportunities for hybrids to attract new pollinators, enter unoccupied pollinator niches, and over time become reproductively isolated. While interspecific studies such as these provide important insight into broad patterns of diversification, fewer studies provide robust assessments of variation at the intraspecific level, which are needed to fully understand the proximate drivers of diversification.

Populations commonly experience different biotic and abiotic environments in space and time, giving rise to geographic mosaics of selection that can produce intraspecific variation in traits (Thompson, 2005, 1999). Geographic patterns in intraspecific variation may signal diversifying selection and reflect areas with conflicting selective pressures for specific traits. Recent studies have focused on intraspecific variation to better understand the drivers of diversity at larger scales and evidence for divergent/differential selection. To date, intraspecific variation in floral volatiles has been attributed to selection by different pollinators (Anderson et al., 2010; Chapurlat et al., 2018; Gross et al., 2016), differences in pollinator preference (Suinyuy et al., 2015), adaptation to heterogeneous pollinator communities (Szenteczki et al., 2021), and the impacts of larcenists or herbivores (Galen et al., 2011; Kessler et al., 2008; Theis, 2006), each with distinct and sometimes opposing impacts on plant fitness. Despite these

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4 93 advances, our understanding of intraspecific variation in floral scent remains limited. To date,  
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6 94 most studies of floral scent include only a few (< 10) individuals per population and a small  
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8 95 number of populations and few of these studies investigate variation in space (sample the  
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10 96 complete geographic distribution of the taxa of interest) and time (multiple years) (Delle-Vedove  
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12 97 et al., 2017). Comprehensive sampling within populations and across space and time allows for a  
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14 98 fuller understanding of the nature of variation, which then can inform predictions of underlying  
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16 99 processes and mechanisms.  
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21 100 Here, we focus on a species with a limited global distribution, the flowers of which are  
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23 101 visited by nocturnal moths that rely on scent to locate floral resources. *Oenothera harringtonii*  
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25 102 (Onagraceae) W.L. Wagner, Stockh. & W.M. Klein is a self-incompatible annual that is endemic  
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27 103 to the arid shortgrass prairies of south and central Colorado and adjacent New Mexico, USA  
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29 104 (Fig. 1). The white lined sphinx moth, *Hyles lineata* (Sphingidae) is the primary pollinator of this  
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31 105 species (Skogen et al., 2016), is sensitive to and uses floral scent to locate flowers (Bischoff et  
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33 106 al., 2015; Raguso et al., 1996), and is also an herbivore as larvae consume flower buds and  
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35 107 leaves. Developing seeds are consumed by the larvae of another moth species, *Mompha*  
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37 108 *definitella* (Momphidae), whose females oviposit on ovaries at dusk and are thought to use floral  
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39 109 scent to locate flowers. From 2008-2012, we collected floral trait data (morphology, scent  
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41 110 chemistry and emission rates) from 650 individuals in 19 natural populations (70% of all known  
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43 111 occurrences; average of 30 individuals per population) and six populations grown under  
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45 112 controlled greenhouse conditions. This approach provided a robust characterization of variation  
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47 113 in floral traits both within and among populations, allowing us to assess the degree to which  
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49 114 plasticity contributes to chemical variation observed in natural populations.  
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4 116 **2. Results and Discussion**  
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7 117 **2.1. Species-wide Floral Scent Composition**  
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9 118 A total of 35 floral volatiles were identified from 19 natural populations of *O.*  
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11 119 *harringtonii* across the entirety of its natural distribution. These VOCs included 11  
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13 120 monoterpenoids, 11 sesquiterpenoids, 7 nitrogenous aldoximes and related compounds, 3  
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15 121 aromatics, two additional terpenoids and the fatty acid derived “jasmine lactone” (7-decen-5-  
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18 122 olide; Table 2). Most GC-MS traces were dominated by one or two monoterpenes, (*E*)- $\beta$ -  
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20 123 ocimene and (*R*(*–*)-linalool, combining for 80.7 to 97.2% of total ion chromatogram peak area  
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23 124 across all populations. Previous analyses using chiral GC-MS determined that linalool in *O.*  
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26 125 *harringtonii* and related species is exclusively attributable to the (*R*(*–*)-linalool enantiomer  
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29 126 (Bechen et al., In Review). Occasional individuals from southeastern populations (MONS,  
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31 127 WALS, DC, TRIN, CHAL/MAV) emitted large amounts of (*E,E*)- $\alpha$ -farnesene (Table 2).  
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34 128 Remarkably, none of the remaining 32 VOCs accounted for more than 5% of total emissions in  
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36 129 any population. These minor constituents included terpenoids related to the 3 dominant  
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38 130 compounds listed above (e.g. (*Z,E*)- $\alpha$ -farnesene, (*E*)- $\beta$ -farnesene and (*E,E*)-farnesol), along with  
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40 131 suites of biosynthetically linked metabolites, such as  $\beta$ -caryophyllene,  $\alpha$ -humulene and  
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42 132 caryophyllene oxide, and PHE-derived aromatics with (phenylacetonitrile, 1-nitro-2-  
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44 133 phenylethane and phenylacetaldoxime) or without N-atoms (2-phenylethanol,  
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46 134 phenylacetaldehyde)(Table 2). Nearly all these compounds are characteristic of night-blooming,  
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48 135 hawkmoth-pollinated plants worldwide (Kaiser, 1993; Knudsen and Tollsten, 1993; Levin et al.,  
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50 136 2003; Raguso et al., 2003), including other species of *Oenothera* (Raguso et al., 2007). Two  
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52 137 exceptions are methyl geranate and methyl farnesoate, representing terpenoid esters that are  
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54 138 better known from studies of insect sexual communication (Engel et al., 2016 see Schiestl and  
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4 139 Ayasse, 2001 for farnesyl esters). The original descriptions of *O. harringtonii* as a discrete  
5 taxonomic entity emphasized its distinctive “gardenia-like” floral fragrance (Gocken, 1968;  
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7 140 Wagner et al., 1985). Our analyses confirm that the combination of (E)- $\beta$ -ocimene, linalool,  
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9 141 methyl benzoate, (E,E)- $\alpha$ -farnesene, TMTT and jasmine lactone in floral headspace of *O.*  
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11 142 closely align with published scent profiles for *Gardenia jasminoides* (Cao et al.,  
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13 143 2020).  
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21 146 2.2. Population-Level Floral Variation  
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24 147 When all floral traits were considered together, population differentiation was strongest  
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26 148 for floral scent chemistry (ANOSIM:  $R = 0.32$ ,  $p < 0.001$ ) as compared with floral morphology  
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28 149 (ANOSIM:  $R = 0.10$ ,  $p < 0.001$ ). A Random Forest analysis showed that one compound in  
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30 150 particular, (*R*)(-)-linalool, was the most important floral trait differentiating populations (Fig. 2).  
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33 151 When present, (*R*)(-)-linalool was also a dominant contributor to total volatile emission rates  
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35 152 (Table 2, see 2.1), unlike other population-distinctive compounds such as jasmine lactone. For  
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37 153 these reasons, we focused our further analyses and discussion on (*R*)(-)-linalool, which is a  
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39 common floral volatile in night-blooming, hawkmoth-pollinated plants (Kaiser, 1993; Knudsen  
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41 154 and Tollsten, 1993; Raguso and Pichersky, 1999) and is a chiral compound (Raguso, 2016).  
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43 155  
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45 156 Linalool has been identified in other night-blooming members of the genus *Oenothera*, including  
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47 157 *O. acutissima* (sect. *Lavauxia*; (Raguso et al., 2007), *O. biennis* (sect. *Oenothera*; (Kawaano et  
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49 158 al., 1995), *O. californica* (sect. *Anogra*), *O. cespitosa* (sect. *Pachylophus*), *O. howardii* (sect.  
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51 159 *Megapterium*), *O. lavandulifolia* (sect. *Calylophus*) and *O. xylocarpa* (sect. *Contortae*); Jogesh,  
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53 159 Skogen, and Raguso unpublished data). Flowers of *O. harringtonii* emit only the (*R*(-)  
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55 160 enantiomer (Bechen et al., In Review), and the available evidence suggests that floral emission  
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57 161 enantiomer (Bechen et al., In Review), and the available evidence suggests that floral emission  
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4 162 of (*R*)(*-*)-linalool is a plesiomorphic trait in *Oenothera* sect. *Pachylophus*, inherited by the  
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6 163 ancestor of *O. harringtonii*. Hereafter, we refer to this compound as “linalool” for simplicity.  
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11 165 2.3. *Clinal Variation in (R)(-)-Linalool Emission*  
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14 166 Our data reveal a population-level linalool polymorphism, with a striking pattern of clinal  
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16 167 variation across the geographic range of *O. harringtonii* without any correlated shifts in floral  
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19 168 morphology. Emission of linalool was discrete and polymorphic; it was either completely absent  
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21 169 /emitted in small quantities or constituted up to 95% of total floral emissions (Tables 1, 2). In  
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23 170 northern and western populations, most plants (average of 86% per population) studied emitted  
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26 171 linalool (lin+ populations) compared with only 22% on average in southern and eastern  
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28 172 populations (lin- populations) (Fig. 1, Table 1). Emission rates varied between populations and  
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31 173 years (Fig. 3A). Floral volatile composition was significantly different between lin+ and lin -  
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34 174 populations ( $N_{lin+} = 361$ ,  $N_{lin-} = 289$ , ANOSIM  $R = 0.45$ ,  $P = 0.001$ ; Fig. 3B). Floral  
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36 175 volatile composition was significantly different between years in CC and DC but not in PW and  
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38 176 TRIN (CC ANOSIM  $R = 0.13$ ,  $P = 0.001$ , PW ANOSIM  $R = 0.01$ ,  $P = 0.35$ , TRIN ANOSIM  $R$   
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41 177  $= 0.14$ ,  $P = 0.03$ , DC ANOSIM  $R = 0.17$ ,  $P = 0.001$ ; Fig. 3C)  
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44 178 At the heart of this distribution are a few populations (HZN, MONS, ROU, BER, and  
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46 179 LUD) in close geographic proximity, which most clearly represent the sharp northwest to  
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48 180 southeast transition in floral scent chemotypes, as they included both lin+ and lin- plants in ratios  
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51 181 that change abruptly over c. 75 km. Among the few individuals that produced linalool in lin-  
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53 182 populations, emission rates were two orders of magnitude lower than those typical of lin+  
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56 183 populations (lin- populations have median linalool emission rates near zero, Table 1). Given that  
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58 184 populations of *O. harringtonii* that produce linalool are in the western portion of its distribution,  
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4 185 it is possible that linalool production may represent phylogenetic inertia (Raguso et al., 2006,  
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6 186 2003), as it is a local endemic species derived from a common ancestor of the widespread  
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8 187 species *O. cespitosa* subsp. *marginata* (Patsis et al., In Press), which occurs across the western  
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10 188 United States and is known to produce (*R*)-(−)-linalool (data not shown).  
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14 190 2.4. *Consistency Between Field vs Greenhouse-grown plants*  
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16 191 Other studies have documented plasticity in floral scent composition, emissions, and  
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18 192 rhythms in response to abiotic factors (Friberg et al., 2014; Hansted et al., 1994; Jakobsen and  
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20 193 Olsen, 1994; Majetic et al., 2007), prompting us to examine the potential for plasticity in *O.*  
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22 194 *harringtonii*. Total emissions per flower per hour were not different between field and  
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24 195 greenhouse grow plants (Fig. 4A). Floral scent phenotypes characterized from field grown plants  
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26 196 in lin+ populations were consistent in greenhouse grown plants from the same populations  
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28 197 (Table 3; Fig. 4), suggesting that variation in linalool production has a genetic basis and is not  
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30 198 plastic. Overall, floral volatile composition did not differ significantly between greenhouse  
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32 199 grown and field collected lin+ populations ( $N_{\text{field collected}} = 151$ ,  $N_{\text{greenhouse grown}} = 34$ , ANOSIM R =  
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34 200 0.05,  $p = 0.15$ ) nor between greenhouse grown and field collected lin- populations ( $N_{\text{field collected}} =$   
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36 201 80,  $N_{\text{greenhouse grown}} = 15$ , ANOSIM R = 0.07,  $p = 0.18$ ; Fig. 4B). Concerning minor scent  
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38 202 constituents, greenhouse-grown plants were noteworthy for the presence of two common  
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40 203 aromatic compounds not identified from field-grown plants (benzyl alcohol, methyl salicylate),  
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42 204 for the consistent presence of distinctive but low-abundance VOCs (methyl geranate, methyl  
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44 205 farnesoate, jasmine lactone) and for the complete absence of PHE-derived compounds (2-  
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46 206 phenylethanol, phenylacetaldehyde, nitrogenous aromatics)(Table 3).  
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4 208 2.5. *The Many Roles of Linalool*  
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6 209 2.5.1. *Linalool production and hawkmoth pollination*  
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8 210 Linalool is a common floral volatile in night-blooming, hawkmoth-pollinated plants  
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11 211 (Kaiser, 1993; Knudsen and Tollsten, 1993; Raguso and Pichersky, 1999), including long-  
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14 212 spurred orchids (Orchidaceae; (Tollsten and Bergström, 1993), including Darwin's orchid  
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16 213 *Angreacum sesquipedale* (Kaiser, 1993), members of the tomato family (Solanaceae; e.g.  
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19 214 *Datura wrightii*, Riffell et al., 2009 *Nicotiana* spp., Loughrin et al., 1990), and jasmines  
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21 215 (Oleaceae; *Jasminum* spp., Watanabe et al., 1993). Linalool has been shown to attract the  
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23  
24 216 widespread hawkmoth *Manduca sexta* in behavioral assays (Riffell et al., 2009), which uses *D.*  
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26 217 *wrightii* as both a nectar source and larval host. Variation in linalool has been documented in  
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29 218 other species pollinated by hawkmoths. For example, in South African *Gladiolus longicollis*  
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31 219 (Iridaceae), positive directional selection on tube length results in populations with long or short  
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33 220 morphs that are pollinated by long- and short-tongued hawkmoths, respectively (Anderson et al.,  
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36 221 2010). Floral scent data collected in one population in a contact zone with both floral morphs  
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38 222 revealed major differences in floral scent, whereby linalool was produced in plants with long  
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41 223 floral tubes but not those with short floral tubes. Additional population-level sampling is needed  
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43 224 to determine if floral scent differences are maintained beyond the contact zone and whether these  
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46 225 differences contribute to different pollination ecotypes. Geographic variation also has been  
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48 226 documented in floral VOCs derived from linalool. In moth pollinated *Linanthus dichotamus*  
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50 227 (Chess et al., 2008) and *Abronia umbellata* (Doubleday et al., 2013) in California and *Silene*  
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53 228 *latifolia* and *S. otites* (Dötterl et al., 2005a; Jhumur et al., 2007) in Europe, linalool is converted  
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55 229 to lilac aldehydes and alcohols, which are known to attract noctuid moths but are less common  
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58 230 than linalool in flowering plants (Dötterl et al., 2006). Linalool can function as a pollinator  
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4 231 attractant or as a defense compound when emitted from floral tissues (Boachon et al., 2015;  
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6 232 Okamoto, 2017; Raguso, 2016; Reisenman et al., 2010) and has been implicated in both direct  
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8 233 and indirect plant defenses when emitted from vegetative tissues (Raguso, 2016), addressed in  
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10 234 more detail below.  
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12 235  
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14 236 2.5.2. *Linalool and Floral Antagonists*  
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16 237 While many studies of floral trait variation have invoked pollinator-mediated selection,  
17  
18 238 floral antagonists use some of the same traits to locate resources, with opposing impacts on  
19  
20 239 fitness. The role of floral antagonists in generating floral trait diversity is relatively understudied  
21  
22 240 but has been documented (Suchet et al. 2011, Galen et al. 2011) and it is possible that variation  
23  
24 241 in scent in *O. harringtonii* may be the result of selection by antagonists that are attracted or  
25  
26 242 deterred by specific compounds or bouquets. *O. harringtonii* is a known larval host for *H. lineata*  
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28 243 (caterpillars consume flower buds and to a lesser extent leaves) and *Mompha definitella*  
29  
30 244 (caterpillars are seed predators), both of which are likely to locate floral resources via fragrance.  
31  
32 245 Female *H. lineata* have been observed to oviposit while nectaring and their larvae eat flower  
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34 246 buds and sometimes leaves of *O. harringtonii*, as has been shown for *O. cespitosa* in western  
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36 247 USA (Artz et al., 2010). Members of the Onagraceae are a known host for microlepidopteran  
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38 248 moths in the genus *Mompha*, and *M. definitella* females oviposit in immature fruits and larvae  
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40 249 consume developing seeds (Bruzze et al., 2019). Both *H. lineata* and *M. definitella* are  
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42 250 crepuscular and have been observed visiting flowers of *O. harringtonii* at dusk (data not shown).  
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48 252 2.5.3. *Factors influencing variation in linalool*  
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4 253 Intraspecific variation in floral scent has been attributed to many factors including  
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6 differences in biotic agents of selection (pollinators and herbivores), mating systems, and genetic  
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8 drift, among others (Delle-Vedove et al. 2017). In some systems, such as *Arum maculatum*, the  
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10 maintenance of intraspecific variation in floral odors is thought to be advantageous when  
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12 pollinator communities vary and do so over short time scales (Szenteczki et al., 2021). However,  
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14 257 geographic variation in floral scent in *O. harringtonii* appears unlikely to indicate pollination  
15  
16 ecotypes or differences in pollinator climate (Grant and Grant, 1965; Van der Niet et al., 2014).  
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18 260 *O. harringtonii* is self-incompatible, relying exclusively on pollination for reproduction. Our  
19  
20 previous studies revealed that *Hyles lineata* hawkmoths are the most consistent floral visitor and  
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22 most effective pollinator and are reliable in both space (range wide) and time (multiple years)  
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24 (Rhodes et al., 2017; Skogen et al., 2016), suggesting that the clinal variation in linalool is not  
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26 driven by local adaptation by pollinators to this particular compound. Intraspecific variation in  
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28 scent has been shown in other systems for which pollinators do not vary. Mant et al. (2005)  
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30 observed significant inter-regional differences in VOCs produced by flowers of the deceptive  
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32 orchid *Ophrys exaltata* despite all studied populations being pollinated by *Colletes cunicularis*  
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34 bees. They suggest that the pattern may be due to divergent selection resulting from differences  
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36 in local preferences of pollinators. Similarly, Suinyuy et al (2012) documented clinal variation in  
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38 volatiles emitted by cones of the cycad *Encephalartos villosus*, despite no difference in pollinator  
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40 assemblages, a pattern that was initially attributed to genetic drift or coevolution. Subsequent  
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42 work revealed that olfactory preferences of weevil pollinators matched cone volatiles in their  
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44 local regions, consistent with expectations for brood-site mutualisms (Suinyuy et al. 2015).  
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46 274 Lastly, variation in floral scent can play an important role in assortative mating (Dötterl et al.,  
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48 275 2005b; Huber et al., 2005; Plepys et al., 2002), especially for plant species for which scent is the  
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4 276 primary floral attractant, such as moths (Raguso, 2008; Waelti et al., 2008). While our  
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6 277 greenhouse data suggest linalool is heritable, a formal heritability study and fine scale  
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8 278 assessments of assortative mating in populations that are polymorphic for linalool would further  
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10 279 eliminate the role of pollinator-mediated selection for linalool in this system.  
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14 280 Though it remains possible that linalool influences the foraging behavior (floral  
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16 281 constancy or probing time) of *H. lineata* in a way that could contribute to the observed linalool  
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18 282 polymorphism, our prior genetic studies suggest that this is not the case. Hawkmoths use floral  
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20 283 visual and olfactory cues to locate floral resources (Raguso and Willis, 2005), are known to  
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22 284 travel over long distances (Alarcón et al., 2008; Finger et al., 2014; Stockhouse II, 1973), and  
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24 285 therefore have the potential to make meaningful contributions to gene flow and limit  
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26 286 differentiation via genetic drift. Our prior work in this system has documented extensive gene  
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28 287 flow range-wide, low genetic differentiation and weak isolation by distance (Skogen et al.,  
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30 288 2019). In addition, although there was a significant relationship between genetic and geographic  
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32 289 distance (Skogen et al., 2019), clinal variation in linalool was found to be related to geographic  
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34 290 distance (partial Mantel test:  $R^2 = 0.11$ , coefficient = 0.35,  $t = 4.54$ ,  $P < 0.01$ ) but not genetic  
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36 291 distance (partial Mantel test:  $R^2 = 0.11$ , coefficient = -2.49,  $t = -1.50$ ,  $P = 0.30$ ). These data  
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38 292 suggest that the clinal variation in linalool documented here for *O. harringtonii* is unlikely to be  
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40 293 explained by geographic variation in the foraging preferences of *H. lineata* for different floral  
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42 294 scent bouquets, or genetic drift, suggesting that other biotic or abiotic factors are at play.  
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45 295 Last, linalool can be present in different chiral configurations and ecological contexts, is  
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47 296 not restricted to hawkmoth-pollinated plants, and is not limited to a floral context (rev. by  
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49 297 Raguso, 2016; Raguso and Pichersky, 1999). For example, (S)(+)-linalool is a target of  
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51 298 phenotypic selection, increasing seed fitness in bee-pollinated *Penstemon digitalis*  
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4 299 (Parachnowitsch et al., 2012). Specific ratios of this enantiomer with other monoterpenes were  
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6 300 shown to mediate obligate mutualism between *Ficus hispida* and its pollinating wasps (Chen and  
7  
8 301 Song, 2008). Linalool is also a common vegetative volatile, either emitted constitutively by  
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10 302 glandular trichomes or as a component of systemically released blends induced by herbivory  
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12 303 (rev. by (Raguso, 2016). A transcriptome study of *O. harringtonii* revealed differential  
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14 304 expression of linalool, primarily due to differences in genes expressed in petals and not leaves  
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16 305 (Bechen et al., In Review). However, the extent to which linalool is an attractant or deterrent to  
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18 306 antagonists and whether it is constitutively expressed or induced in this system remain to be  
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20 307 determined.  
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29 309 **3. Conclusions**  
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31 310 We provide the most comprehensive dataset of intraspecific variation in floral volatiles to  
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33 311 date, which revealed clinal variation in linalool production in *O. harringtonii*. Such extensive  
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35 312 sampling may be needed to accurately capture the nature of variation in wild populations, which  
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37 313 has important implications for understanding floral trait evolution and for inferences of selective  
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39 314 pressures and adaptation within and among species and at higher taxonomic levels. Now that  
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41 315 floral scent is more commonly studied, it is clear that geographic variation in scent composition  
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43 316 is a widespread phenomenon with many potential causes (rev. by (Delle-Vedove et al., 2017).  
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46 317 When phenotypic variation is geographically structured, selection can lead to adaptive  
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48 318 divergence. Future work is needed to assess the genetic basis of linalool variation in *O.*  
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51 319 *harringtonii* and to explore the causal mechanisms that created the clinal pattern of linalool  
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54 320 variation, with special emphasis on the relative contributions of the mutualistic and antagonistic  
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57 321 roles of *Hyles lineata* and the antagonistic role of seed predation by *Mompha definitella* to  
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4 322 phenotypic selection. More broadly, additional detailed studies of intraspecific variation in floral  
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6 323 scent are needed to understand the extent to which this trait contributes to floral trait and  
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8 324 angiosperm diversification and the roles of biotic and abiotic agents of selection.  
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14 326 **4. Experimental**  
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16 327 *4.1. Study System*  
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19 328 *Oenothera harringtonii* Wagner, Stockhouse, and Klein (Onagraceae) is endemic to the  
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21 329 arid shortgrass prairies of south and south-central Colorado and adjacent New Mexico, USA.  
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24 330 (Wagner et al., 1985)(Fig. 1). It is a facultative annual with white, fragrant flowers that produce  
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26 331 copious nectar, open at dusk, and commonly last 14 -18 hours before fading the following  
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28 332 morning. Gene flow is high and population differentiation is low range-wide (Skogen et al.,  
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31 333 2019), likely facilitated by the primary pollinator, the white-lined sphinx moth, *Hyles lineata*  
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33 334 Fabricius (Sphingidae, Skogen et al., 2016). Solitary bees also visit and pollinate flowers in the  
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36 335 morning hours when most pollen has already been removed, and therefore make minimal  
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38 336 contributions to fitness. Herbivores include larvae of *H. lineata*, who consume flower buds and  
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41 337 to a lesser extent leaves, and *Mompha definitella* Zeller, who consume seeds and are host-  
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43 338 specific on *O. harringtonii* (Bruzze et al., 2019). Seeds are gravity dispersed and spatial  
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46 339 **genetic structure** has been documented (Rhodes et al., 2014). *O. harringtonii* is a member of  
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48 340 *Oenothera* section *Pachylophus* and is derived from the more widespread *O. cespitosa* (Patsis et  
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51 341 al., In Press).  
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56 343 *4.2. Floral Phenotype*

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4 344 We used unbiased methods to quantify chemical and morphological floral variation of *O.*  
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7 345 *harringtonii* range-wide by sampling plants *in situ* in more than 70% of known populations over  
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9 346 four years, 2009-2012 (650 individuals, 19 populations; Fig. 1 and Table 1). At each population,  
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11 347 we collected floral scent, morphology, and nectar data on one flower per plant from 9-30 plants.  
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13 348 We sampled floral scent immediately after anthesis, typically between 18:00 and 20:00 hrs, on  
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15 349 individual flowers using dynamic headspace collection methods (Raguso and Pellmyr, 1998).  
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17 350 Following scent collection, we excised the flowers from plants and measured floral morphology.  
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21 352 *4.2.1. Floral scent*  
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23 353 Individual flowers were enclosed in a Reynolds (nylon resin) oven bag (12 x 15 cm, 270  
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25 354 ml volume) affixed to the floral stem with plastic ties using protocols described by (Galen et al.,  
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27 355 2011). Floral volatiles were collected in a cartridge containing 10 mg of Super Q (80–100 mesh)  
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29 356 adsorbent (Alltech Associates, Waukegan, Illinois, U.S.A.), packed into a Pasteur pipette using  
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31 357 silanized quartz wool (Restek #24324). Air from the floral headspace, concentrated in the  
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33 358 enclosing bag, was pulled through the cartridge using a PAS-500 (Spectrex, Redwood City,  
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35 359 California, U.S.A.) personal air sampler vacuum pump, with a standardized flow rate of 200 ml  
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37 360 air/min. After 60 minutes of sampling, the cartridges were removed and volatiles were eluted  
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39 361 with 200 µl of GC-MS grade hexane (Honeywell Burdick and Jackson, Muskegon, Michigan,  
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41 362 U.S.A) into borosilicate glass “shell style” inserts placed within 1.5 ml amber glass autosampler  
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43 363 vials capped with silicone septa and polypropylene screw caps (Supelco, Bellefonte,  
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45 364 Pennsylvania, U.S.A.). Samples were stored at -20 °C until analysis. Prior to analysis, we  
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47 365 concentrated the samples to a uniform volume of 50 µl using gaseous N<sub>2</sub> and added 5 µl of  
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49 366 0.03% toluene in hexane (= 23 ng) as an internal standard.  
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4 367 One  $\mu$ l aliquots of each sample were injected into a Shimadzu GC-17A gas  
5 chromatograph equipped with a Shimadzu QP5000 quadrupole, electron ionization (EI) mass  
6 spectrometer (Shimadzu Scientific Instruments, Columbia, Maryland, U.S.A.) as a detector. All  
7 analyses were made using splitless injections on a polar GC column (diameter 0.25 mm, length  
8 370 30 m, film thickness 0.25  $\mu$ m (Econo Cap's carbowax coating, known as EC WAX); Alltech  
9 371 Associates), using ultra high purity (99.999%) helium as a mobile phase (split ratio 12:1,  
10 372 constant flow of 1ml/min.). The GC temperature and pressure parameters (injection port temp.  
11 373 240 °C, detector temp 260 °C, initial temp. 40 °C, hold time 2 min, increased at 15 °C/min to  
12 374 260 °C, hold time 2.38 min) were optimized to resolve linalool and other floral volatiles to  
13 375 baseline with a total run time of 19 min. per sample, allowing us to efficiently process high  
14 376 sample replicates.

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31 378 EI mass spectra (70 eV) were collected from m/z 40-350 (daltons) at a detector voltage of  
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33 379 70 eV, with scan speed of 1000 and a scan interval of 0.29 sec. Compounds were tentatively  
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36 380 identified using computerized mass spectral libraries (Wiley Registry of Mass Spectral Data,  
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38 381 National Institute of Standards and Technology, and Adams (> 120,000 mass spectra)). Peak  
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40 382 areas were integrated using Shimadzu's GCMSSolutions software, were normalized for slight  
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43 383 differences in final sample volume using the internal standard (see (Bruzzone et al., 2019) and  
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46 384 were quantified by comparison with 5-point (log scale) dose-response external standard curves  
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49 385 generated using serial dilutions (0.1 to 0.00001 mg/ml) of authentic standards for (R)(-)linalool.  
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51 386 Emission rates were expressed as ng per flower or per gram dry floral mass, per hour.

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53 387 To assess whether floral chemotypes were true breeding, we grew plants of *O.*  
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55 388 *harringtonii* from wild seeds collected from six focal populations (FLO, Bloom, BAC, BMR,  
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58 389 PW, and DC, Table 1) in 2008 and 2009 in the greenhouses at the Chicago Botanic Garden,

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4 390 Glencoe, Illinois, U.S.A. Seeds were sown in a 50:50 mix of field-collected soil and germination  
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6 potting soil. Seedlings with true leaves were transplanted individually to 11.4 cm x 11.4 cm x X  
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8 391 15.2 cm plastic pots. Plants were spaced evenly and randomly with respect to population of  
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10 origin and grown with supplemental lighting to achieve 14 hr day/6 hr night (25°C day, 22°C  
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12 393 night), and were fertilized weekly. Floral scent was sampled using the same methods described  
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14 394 above.  
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21 397 *4.2.2. Floral morphology*  
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24 398 Morphological measurements were made with digital calipers (nearest millimeter) and  
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26 399 included corolla diameter (averaged along the two longest perpendicular axes), floral tube length  
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28 400 (ovary to floral tube opening), floral flare (diameter of the floral tube opening), filament length  
29  
30 401 and style length. We calculated herkogamy (anther-stigma separation) as the difference between  
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32 402 the filament and style lengths. We then dried the flowers in silica and recorded dry weight to the  
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34 403 nearest milligram. Dry weights were used to calculate emission rates/ng dried flower/hour.  
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41 405 *4.2.3. Statistical Analyses*  
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43 406 We took two approaches to understand how floral traits vary between wild populations.  
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45 407 We first used a distance-based approach (ANOSIM) to understand if populations differed in their  
46  
47 408 overall scent and morphological composition. Emission rates and morphology were square-root  
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49 409 transformed and ANOSIM analysis was conducted using Bray-Curtis distances. We analyzed  
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51 410 floral scent and morphology separately and together. We then applied a Random Forest model to  
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53 411 determine the traits most important in differentiating the populations from each other  
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55 412 (Ranganathan and Borges, 2010). We tested for differences in linalool production between field  
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4 413 and greenhouse-grown plants using ANOSIM analyses and compared emission rates using t  
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6 414 tests. To determine if geographic patterns of linalool emission are driven by genetic  
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8 415 differentiation (using pairwise genetic distances from (Skogen et al., 2019), we conducted a  
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10 416 partial Mantel test using the multi.mantel function implemented in R package ‘phytools’ (Revell,  
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12 417 2012). All analyses were conducted in R version 4.0.2 (“R: The R Project for Statistical  
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14 418 Computing,” 2020).

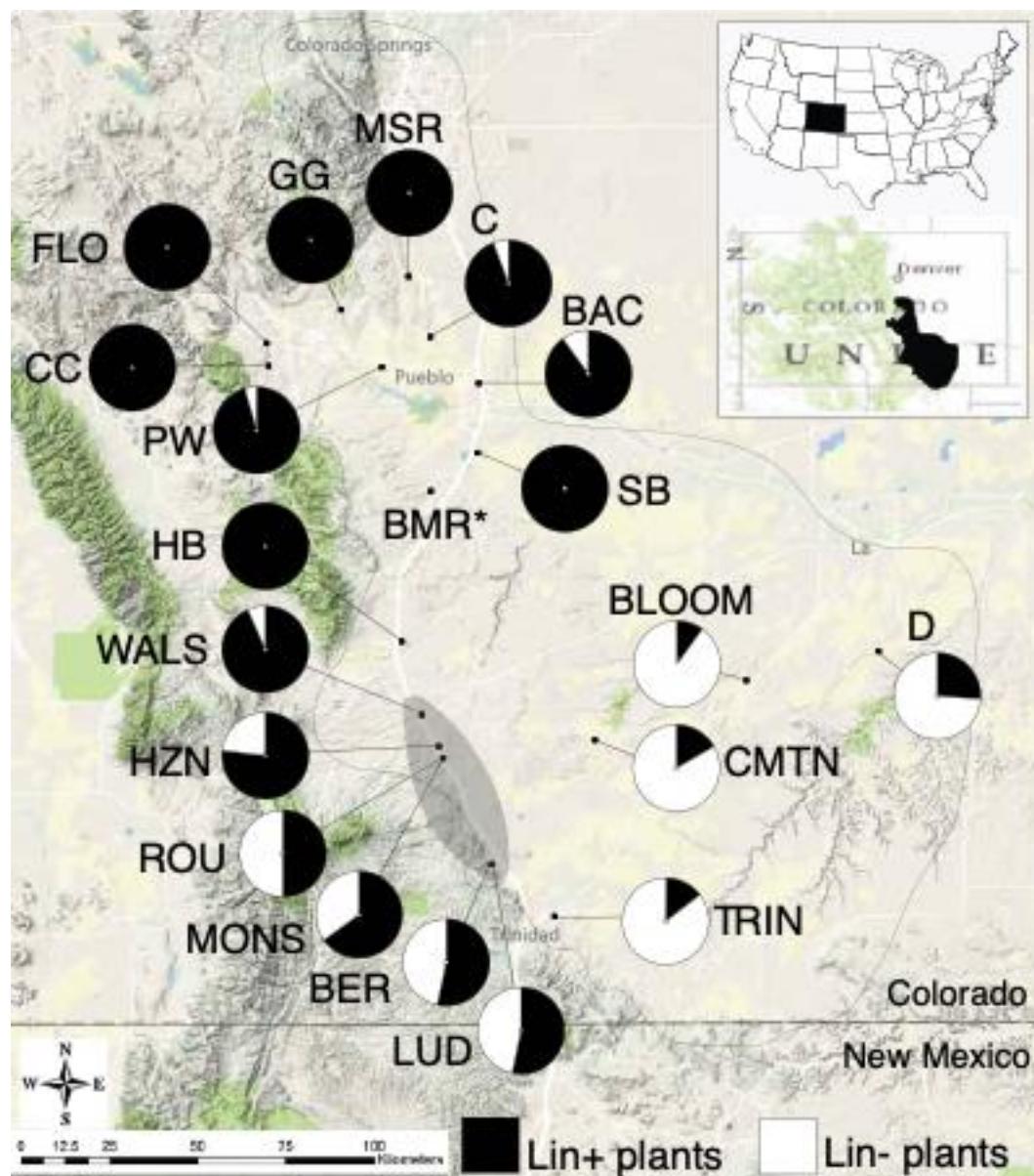
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21 420 **Declaration of competing interest**  
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23  
24 421 The authors declare no competing financial interest.  
25

26 422  
27  
28 423 **Acknowledgements**  
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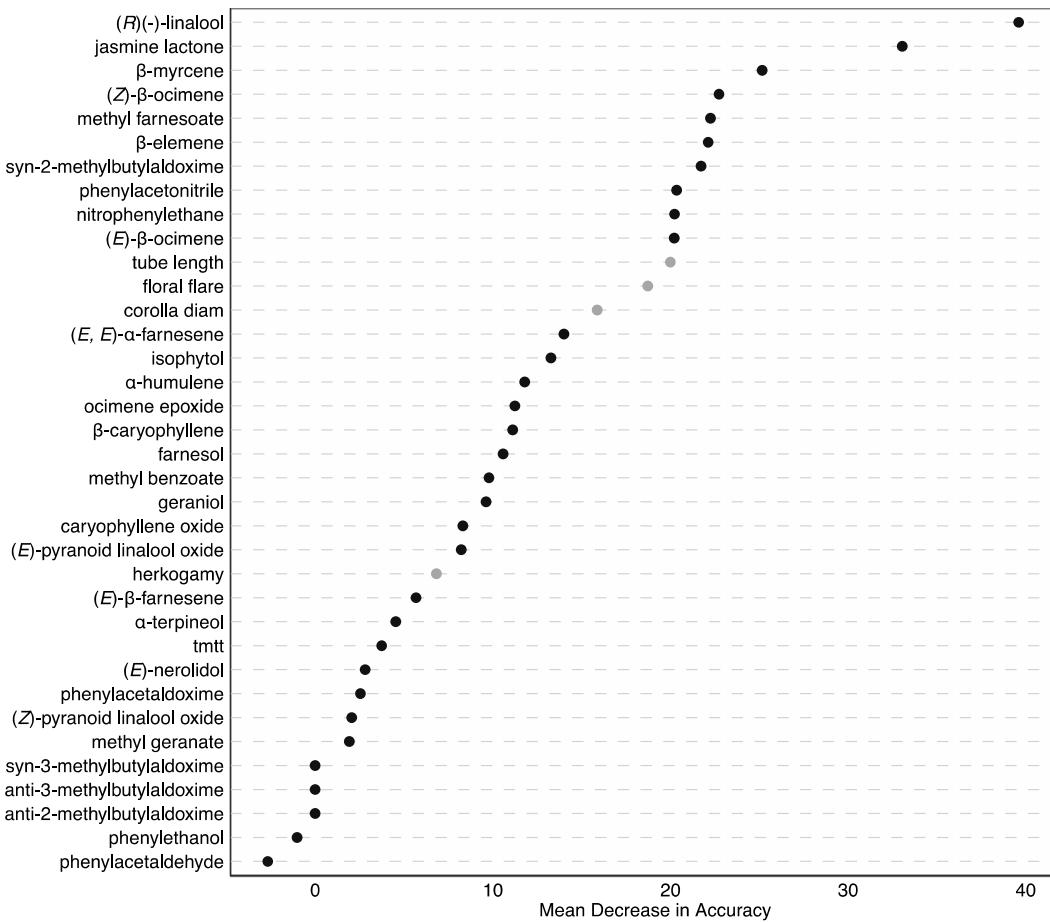
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38  
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42  
43 430 olide, respectively. This work was supported by the National Science Foundation [DEB 1342873  
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4 433 Figures and Legends

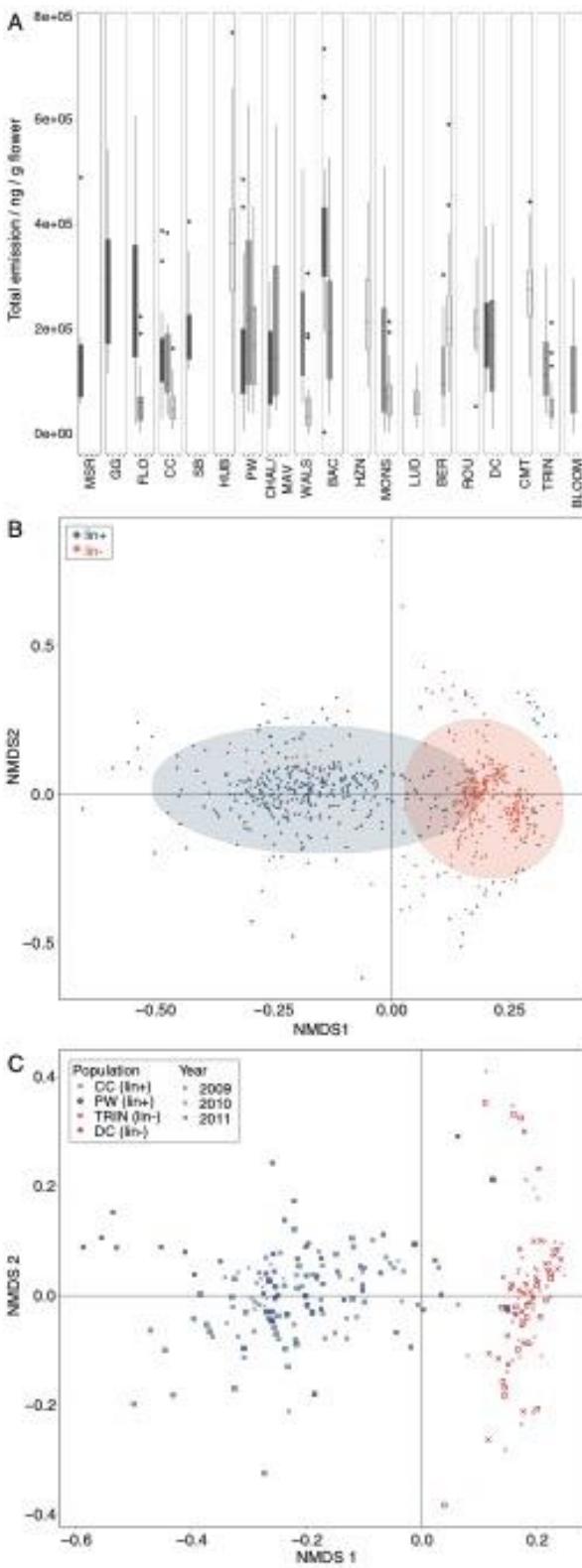


434  
435 **Figure 1.** Geographic distribution of the cline in linalool production across *O. harringtonii*  
436 populations. Pie charts depict the proportion of sampled individuals that emit linalool (lin+,  
437 black) and the proportion that do not (lin-, white) (Table 1). The gray oval indicates the portion  
438 of the distribution where plants are polymorphic for linalool production. Note that for the few  
439 linalool-emitting individuals in the lin- populations, emission rates are two orders of magnitude

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4 440 lower than those typical of lin+ populations (lin- populations have median linalool emission rates  
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6 441 near 0, Table 1).  
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**Figure 2.** Ranking of the relative importance of each trait based on the Random Forest classification algorithm for differentiating populations using data collected *in situ* ( $N = 650$  plants). Gray circles represent morphological traits and black circles represent floral volatile traits. The importance (mean decrease in Gini coefficient) indicates the extent to which populations are not correctly differentiated if the trait is not included in the model. Thus, the higher the mean decrease in Gini, the more important the trait is for distinguishing populations.



**Figure 3.** Floral scent data. (A) Total emission rates (ng/flower/hr  $\pm$  SEM) of volatile headspace from single flowers of *Oenothera harringtonii* collected in 19 populations across 4 years, (B)

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4 452 Non-metric multidimensional scaling plot (NMDS) of floral volatile composition. Floral  
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6 453 volatile composition was significantly different between lin+ and lin - populations ( $N_{lin+} = 361$ ,  
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8 454  $N_{lin-} = 289$ , ANOSIM  $R = 0.45$ ,  $P = 0.001$ ); (C) NMDS floral volatile composition of populations  
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10 455 sampled in more than one year. Floral volatile composition was significantly different between  
11  
12 456 years in CC and DC but not in PW and TRIN (CC ANOSIM  $R = 0.13$ ,  $P = 0.001$ , PW ANOSIM  
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14 457  $R = 0.01$ ,  $P = 0.35$ , TRIN ANOSIM  $R = 0.14$ ,  $P = 0.03$ , DC ANOSIM  $R = 0.17$ ,  $P = 0.001$ ).  
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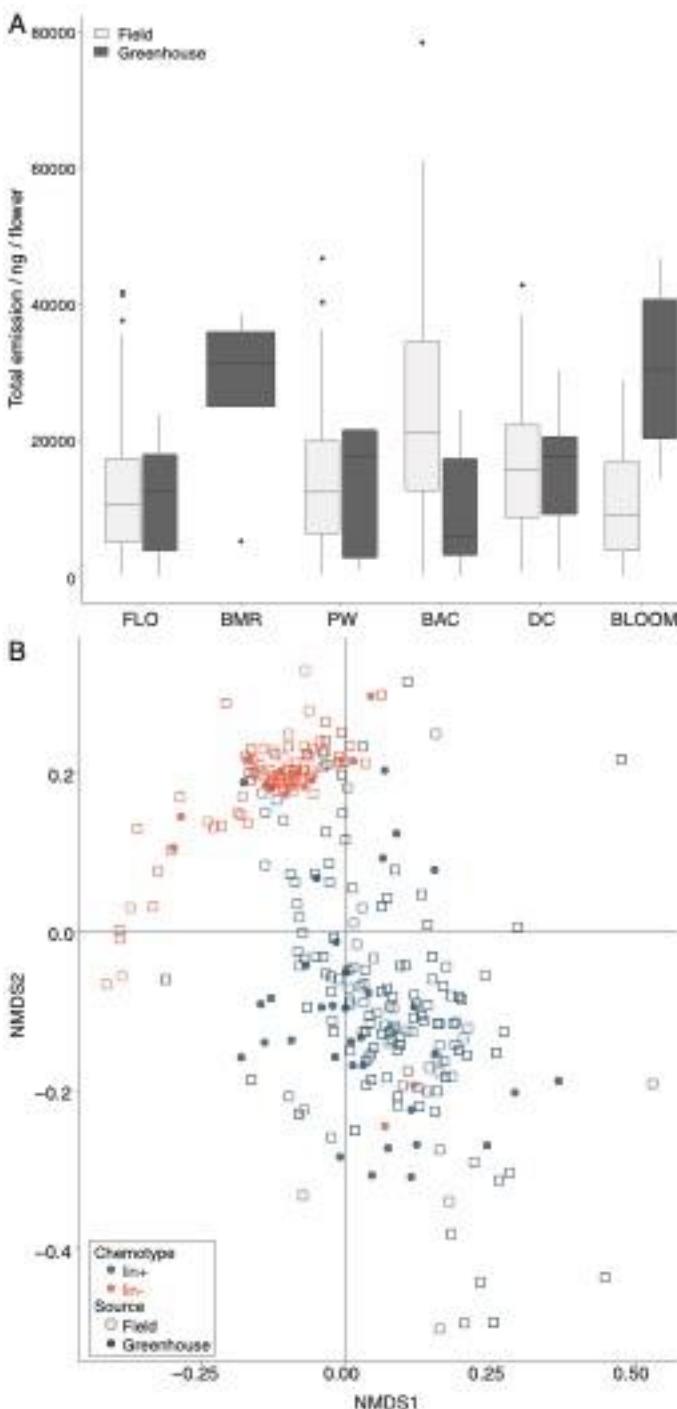


Figure 4. (A) Total emission rates ng/flower/hour  $\pm$  SEM between field-collected and greenhouse-grown plants from four lin+ (FLO, BAC, PW, BMR) and two lin- populations (DC and Bloom), which nevertheless contained some plants producing low-levels of linalool (mean emission rates for DC were 100-fold (field) or 10-fold (greenhouse) lower, respectively than

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4 463 those of plants from FLO); N<sub>FLO</sub>, Field = 34, N<sub>FLO</sub>, Greenhouse = 15, N<sub>PW</sub>, Field = 68, N<sub>PW</sub>, Greenhouse = 5,  
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7 464 N<sub>BAC</sub>, Field = 49, N<sub>BAC</sub>, Greenhouse = 9, N<sub>BMR</sub>, Greenhouse = 5, N<sub>DC</sub>, Field = 50, N<sub>DC</sub>, Greenhouse = 11, N<sub>BLOOM</sub>,  
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9 465 Field = 30, N<sub>BLOOM</sub>, Greenhouse = 4. Note that emission rates are per flower per hour and not per gram  
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11 466 of dry flower as in Fig. 3A. (B) Non-metric multidimensional scaling plot (NMDS) of volatile  
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13 467 headspace from single flowers of *Oenothera harringtonii*. Floral volatile composition was not  
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15 468 significantly different between greenhouse grown and field collected lin+ populations (N<sub>field</sub>  
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17 469 collected = 151, N<sub>greenhouse grown</sub> = 34, ANOSIM  $R = 0.05, P = 0.15$ ) nor between greenhouse grown  
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19 470 and field collected lin- populations (N<sub>field collected</sub> = 80, N<sub>greenhouse grown</sub> = 15, ANOSIM  $R = 0.07, P$   
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21  
22 471 = 0.18). Data are square root transformed chromatographic peak areas expressed as relative  
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24 472 percentages of total emissions. The NMDS plot compares chemical space loci for volatile blends  
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26 473 from the flowers of greenhouse grown (filled symbols) plants with seeds collected from lin+  
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28 474 (blue: FLO, BAC, PW, BMR) and lin- (red: DC and Bloom) populations to emissions from  
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30 475 flowers sampled in the field from the same six populations, except for BMR (data collected in  
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32 476 the greenhouse only).

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 22 478 **Table 1.** Population name, code, chemotype and location for all populations included in this study. Sample sizes are provided for the  
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 25 479 following data collected *in situ*: floral traits (floral scent and morphology data), for the years data were collected. \*Floral scent for the  
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 27 population BMR were collected from plants grown under common greenhouse conditions and were not collected *in situ*. Populations  
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 29 are arranged by proportion of individuals emitting linalool (highest at top) and then by latitude for populations where linalool was  
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 31 produced in all individuals.  
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Population Name	Code	Collector Number		Latitude	Longitude	Chemotype	Median linalool emission rate ( $\mu\text{g flower-1 hr-1}$ )	Proportion emitting linalool	Sample Sizes for Scent, Morphology, Nectar				Number of Greenhouse-grown plants sampled
		(Herbarium Code)							2009	2010	2011	2012	
Military Service Road	MSR	LOL_787 (CHIC)	38.51209	-104.74081	lin+	4.93	100.0	9					
Green Gulch	GG	LOL_988 (CHIC)	38.4444	-104.9166	lin+	9.87	100.0	10					
Florence	FLO	LOL_733 (CHIC)	38.37695	-105.10983	lin+	3.99	100.0	20	14				15
Cockleburr Creek	CC	LOL_737 (CHIC)	38.33025	-105.10507	lin+	2.93	100.0	29	15	30			
Stem Beach	SB	LOL_770 (CHIC)	38.18185	-104.59006	lin+	2.36	100.0	10					
Burnt Mill Road	BMR*	LOL_731 (CHIC)	38.09803	-104.73043	lin+	7.99	100.0						5
Huerfano Butte	HB	LOL_757 (CHIC)	37.76433	-104.8336	lin+	8.76	100.0					27	
Pueblo West	PW	LOL_756 (CHIC)	38.33421	-104.81546	lin+	4.08	95.6	38	15	15			5
ChalMav	CM	LOL_734 (CHIC)	38.38919	-104.68282	lin+	3.78	94.3	20	15				
Walsenburg	WALS	LOL_746 (CHIC)	37.61947	-104.75932	lin+	1.94	93.0	15	30				
Baculite Mesa	BAC	LOL_989 (CHIC)	38.293916	-104.557661	lin+	4.24	89.8	19	30				9
Hezron	HZN	LOL_738 (CHIC)	37.5484	-104.7142	lin-	0.00	76.9					12	
Monson	MONS	LOL_742 (CHIC)	37.52425	-104.6972	lin-	0.03	65.0		29	29			
Ludlow	LUD	LOL_740 (CHIC)	37.317598	-104.554034	lin-	0.02	53.3				15		
Berwind	BER	LOL_730 (CHIC)	37.310891	-104.54689	lin-	0.00	53.3				15	29	
Rouse	ROU	LOL_956 (CHIC)	37.529468	-104.703226	lin-	0.00	50.0					8	
David Canyon	DC	LOL_737 (CHIC)	37.75612	-103.5981	lin-	0.00	26.0	20	30				11
Cone Mountain	CMTN	LOL_736 (US)	37.566782	-104.299439	lin-	0.00	16.7					30	
Trinidad	TRIN	LOL_710 (CHIC)	37.227249	-104.413306	lin-	0.00	14.3		14	28			
Bloom	BLOOM	LOL_727 (US)	37.69002	-103.95087	lin-	0.00	10.0	30					4

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20 484 Table 2: Floral scent collected in situ from wild populations of *Oenothera harringtonii*, during the first hour of anthesis (single  
 21 flowers). Compounds shown in bold font were confirmed through co-injection with authentic standards. Other compound identities are  
 22 inferred from mass spectral library match and co-occurrence with related compounds or isomers. Enantiomeric configuration of  
 23  
 24 486 linalool was determined using chiral GC-MS. Data shown are mean +/- std. error (s.e.m.) % of total emissions (TIC peak areas) for  
 25  
 26 487 linalool was determined using chiral GC-MS. Data shown are mean +/- std. error (s.e.m.) % of total emissions (TIC peak areas) for  
 27  
 28 488 each compound. Population abbreviations and order (proportion of plants emitting linalool) follow those presented in Table 1.  
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Population	ret time	MSR	2009 mean	2009 s.e.m.	GG	2009 mean	2009 s.e.m.	FLO	2009 mean	2009 s.e.m.	CC	2009 mean	2009 s.e.m.	CC	2010 mean	2010 s.e.m.	SB	2009 mean	2009 s.e.m.	HB	2012 mean	2012 s.e.m.	PW	2009 mean	2009 s.e.m.	PW	2010 mean	PW	2010 mean	PW	2011 mean	PW	2011 mean	CHAL/MAV	2008 mean	CHAL/MAV	2009 mean	CHAL/MAV	2009 mean					
<b>Volatile Organic Compounds (35)</b>																																												
<b>Monoterpeneoids(11)</b>																																												
$\beta$ -myrcene	6.07	0.263	0.048	0.321	0.035	0.419	0.036	0.150	0.024	0.340	0.023	0.176	0.020	0.093	0.017	0.424	0.047	0.358	0.040	0.285	0.030	0.177	0.027	0.318	0.038	0.315	0.027	0.298																
(2) $\beta$ -ocimene	6.85	0.788	0.151	0.841	0.099	1.152	0.101	0.470	0.075	0.890	0.060	0.558	0.068	0.287	0.034	1.202	0.151	1.203	0.120	0.791	0.092	0.869	0.108	0.511	0.071	0.807	0.114	0.867	0.070	0.882														
(E)- $\beta$ -ocimene	7.08	55.436	6.215	53.191	6.076	59.683	4.060	55.270	5.569	55.072	3.045	45.581	4.253	44.109	3.021	69.957	5.355	69.190	3.701	49.588	3.944	63.438	4.553	55.405	4.593	62.323	6.463	57.841	4.253	58.702														
ocimene epoxides	8.53	0.121	0.065	0.198	0.044	0.511	0.096	0.250	0.098	0.333	0.049	0.308	0.056	0.194	0.063	0.450	0.108	0.483	0.083	0.325	0.088	0.228	0.061	0.411	0.073	0.288	0.200	0.291	0.093	0.394														
ocimene epoxide (myroxide isomer)2	9.37	0.005	0.003	0.011	0.003	0.012	0.003	0.004	0.002	0.007	0.002	0.006	0.005	0.014	0.007	0.043	0.007	0.013	0.003	0.008	0.003	0.001	0.001	0.005	0.011	0.003	0.006																	
(R)-linalool	9.85	38.969	6.147	43.119	6.144	31.568	3.948	40.044	5.535	39.169	2.747	48.738	4.285	43.114	2.553	24.872	5.683	25.132	3.957	45.216	4.237	30.717	4.306	36.169	4.725	26.325	4.469	35.931	4.104	29.203														
$\alpha$ -terpineol	11.22							0.353	0.340			0.024	0.016	0.018	0.016			0.042	0.005	0.002	0.001	0.005					0.003	0.002																
methyl geranate	11.25							0.013	0.008	0.005	0.003	1.057	0.269	0.009	0.003	2.168	0.429	6.282	1.450			0.036	0.005	0.003	0.002	1.019	0.420	3.442	0.645															
(Z)-pyranoid linalool oxide	11.51											<0.001																																
(E)-pyranoid linalool oxide	11.67											<0.001																																
geranial	12.33							0.012	0.008		0.648	0.634			0.010	0.003		0.023	0.018	0.003	0.003	0.032	0.007	0.002	0.001					0.053	0.028													
<b>Sesquiterpenoids(11)</b>																																												
$\beta$ -elemene	10.40																																											
$\beta$ -caryophyllene	10.53	0.107	0.020	0.198	0.090	0.256	0.068	0.141	0.050	0.301	0.070	0.191	0.069	0.273	0.102	0.283	0.094	0.276	0.123	0.160	0.049	0.009	0.008	0.075	0.029	0.107	0.048	0.149	0.033	0.061														
(E)- $\beta$ -farnesene	10.97	0.006	0.006	0.001	0.001	0.006	0.002					0.006	0.002					0.003	0.003	0.020	0.002	0.011	0.003			0.012	0.005	0.005	0.003															
$\alpha$ -humulene	11.19	0.002	0.001	0.010	0.005	0.021	0.007					0.022	0.006	0.002	0.002	0.003	0.013	0.007	0.061	0.011	0.009	0.004					0.001	0.001	0.008	0.003														
(Z,E)- $\alpha$ -farnesene	11.44	0.006	0.006					0.002	0.001			0.001	0.000					0.060									0.008	0.005	0.005	0.004														
(E,E)- $\alpha$ -farnesene	11.64	3.065	3.065					0.005	0.002	0.018	0.017	0.001	0.000	0.083	0.063	0.006	0.006	0.005	0.003	11.620	3.160	1.881	1.044					9.298	2.874	2.779	1.826													
Caryophyllene oxide	13.60	0.016	0.015	0.002	0.002	0.057	0.031					0.040	0.015					0.062	0.045	0.012	0.008	0.149	0.025	0.023	0.015			0.001	0.001	0.012	0.010													
(E)-nerolidol	13.73	0.001	0.001					0.001	0.001			0.008	0.003					0.035	0.007	0.003	0.003					0.108	0.059	0.766	0.185															
Methyl farnesate	14.97	0.632	0.315	0.878	0.225	2.277	0.947					1.788	0.519							1.018	0.186	0.823	0.205	0.921	0.192																			
$\beta$ -farnesol	15.25																																											
(E,E)-farnesol	15.78	0.342	0.158	0.356	0.065	1.417	0.748	1.103	0.414	0.627	0.123	0.956	0.236	2.144	0.427	0.603	0.146	0.576	0.115	0.289	0.052	1.631	0.969	1.794	0.422	0.113	0.046	0.291	0.066	1.276														
<b>Other terpenoids(2)</b>																																												
TMTT <sup>2</sup>	12.08																																											
$\gamma$ -sophytol	15.34	0.075	0.023	0.055	0.024	0.184	0.085	0.326	0.139	0.030	0.014	0.093	0.016	0.296	0.059	0.105	0.032	0.075	0.009	0.050	0.013	0.194	0.080	0.257	0.047	0.100	0.051	0.093	0.036	0.154														
<b>Aromatics(3)</b>																																												
Methyl benzoate	10.68	0.011	0.005	0.005	0.002	0.016	0.006					0.026	0.012	0.130	0.081					0.007	0.004	0.063	0.017	0.006	0.003					0.004	0.004	0.026	0.025	0.012	0.004	0.022								
B-phenylacetaldehyde	10.84																																											
2-phenylethanol	12.88																																											
<b>N-volatiles(7)</b>																																												
syn-2-methylbutyraldoxime	9.36																																											
syn-3-methylbutyraldoxime	9.41																																											
anti-2-methylbutyraldoxime	9.54																																											
anti-3-methylbutyraldoxime	9.75																																											
B-phenylacetonitrile	13.08							0.016	0.016			0.008	0.004	0.006	0.004	0.004																												

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20 492 2. m/z 79(100), 81(37), 41(28), 77(26), 43(24), 39(19), 53(13), 72(11), 93(11), 91(9)  
21  
22 493 3. TMTT = 4,8,12-trimethyl-1,3,7,11-tridecatetraene; stereo configuration not determined without an authentic standard  
23  
24 494 4. Jasmine lactone = 7-decen-5-olide

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4 498 Table 3: Floral scent collected *in situ* from wild populations of *Oenothera harringtonii*, during  
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6 499 the first hour of anthesis (single flowers). Compounds in bold font were confirmed through co-  
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8 500 injection with authentic standards. Other compound identities are inferred from mass spectral  
9  
10 501 library match and co-occurrence with related compounds or isomers. Enantiomeric configuration  
11  
12 502 of linalool was previously determined using chiral GC-MS. Data shown are mean +/- std. error  
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14 503 (s.e.m.) % of total emissions (TIC peak areas) for each compound. Population abbreviations and  
15  
16 504 order (proportion of plants emitting linalool) follow those presented in Table 1.  
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4	Parent population	ret time	FLO	BMR	PW	BAC	DC	BLOOM
5	Compound name	(min)*	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
<b>Monoterpenoids</b>								
6	<b>β-myrcene</b>	6.07	0.205	0.016	0.322	0.062	0.201	0.034
7	<b>(Z)-β-ocimene</b>	6.85	0.612	0.041	0.985	0.143	0.514	0.091
8	<b>(E)-β-ocimene</b>	7.08	53.673	2.984	59.287	7.020	56.781	5.603
9	ocimene epoxide <sub>1</sub>	8.53	0.994	0.876	0.251	0.053	0.199	0.084
10	(Z)-furanoid linalool oxide	9.06					0.728	0.358
11	(E)-furanoid linalool oxide	9.29	0.001				0.001	
12	ocimene epoxide (myroxide isomer) <sub>2</sub>	9.37	0.003	0.001	0.008	0.003	0.009	0.003
13	<b>(R)-(-)-linalool</b>	9.85	36.927	2.804	31.102	4.308	32.103	3.846
14	<b>α-terpineol</b>	11.22	0.001		0.001	0.001	0.001	
15	<b>methyl geranate</b>	11.25	0.054	0.049	0.002	0.002	0.125	
16	<b>(Z)-pyranoid linalool oxide</b>	11.51						
17	<b>(E)-pyranoid linalool oxide</b>	11.67						
18	<b>geraniol</b>	12.33	0.001					0.006
<b>Sesquiterpenoids</b>								
19	<b>β-elemene</b>	10.40						
20	<b>β-caryophyllene</b>	10.53	2.717	1.156	1.218	0.450	1.390	1.122
21	<b>(E)-β-farnesene</b>	10.97			0.001	0.001	0.000	0.000
22	<b>α-humulene</b>	11.19	0.103	0.040	0.042	0.018	0.074	0.061
23	(Z,E)-α-farnesene	11.44			0.008	0.008		<0.001
24	(E,E)-α-farnesene	11.64	0.096		1.015	1.015		
25	<b>Caryophyllene oxide</b>	13.60	0.165	0.131	0.079	0.048	0.049	0.031
26	<b>(E)-nerolidol</b>	13.73			0.007	0.007	0.029	0.019
27	Methyl farnesoate	14.97	2.109	0.886	0.596	0.330	3.912	1.422
28	<b>(E,E)-farnesol</b>	15.78	0.739	0.260	0.174	0.039	0.768	0.265
<b>Other terpenoids</b>								
29	TMTT <sub>3</sub>	12.08						
30	Isophytol	15.34	0.195	0.058	0.039	0.016	0.311	0.127
<b>Aromatics</b>								
31	<b>Methyl benzoate</b>	10.68	0.040		0.001	0.001		0.017
32	<b>Phenylacetaldehyde</b>	10.84						
33	<b>Methyl salicylate</b>	12.09	0.001					
34	<b>Benzyl alcohol</b>	12.82					0.001	
35	<b>2-phenylethanol</b>	12.88						
<b>N-volatiles</b>								
36	<b>syn-2-methylbutyraldoxime</b>	9.36						
37	<b>syn-3-methylbutyraldoxime</b>	9.41						
38	<b>anti-2-methylbutyraldoxime</b>	9.54						
39	<b>anti-3-methylbutyraldoxime</b>	9.75						
40	<b>Phenylacetonitrile</b>	13.08						
41	Nitrophenylethane	14.45						
42	<b>Phenylacetaldoxime</b>	15.57						
<b>Fatty acid derivatives</b>								
43	<b>Jasmine lactone<sub>4</sub></b>	15.37	1.228	0.509		2.375	1.020	1.411
44								0.505
45	<b>N plants sampled</b>		52		11		33	
46	cumulative # VOCs		20		19		18	
47	mean +/- s.e.m. # VOCs		9.0	0.4	9.5	1.1	9.1	0.4
48	dry floral mass, g		n/a		n/a		n/a	
49	total scent ng/fl/hr		16883.21	2197.36	32657.98	6773.92	9975.54	2250.93
50	total scent ng/g flw/hr		n/a		n/a		n/a	
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\* original GC oven program was 10C/min, rather than 15C/min. Retention times provided here are translated to the faster program for consistency with field-collected data

n/a: floral masses not recorded, emission rates per floral mass not calculated

1. m/z 41(100), 67(73), 83(45), 43(43), 69(35), 55(33), 82(25), 53(21), 54(14), 81(14)

2. m/z 79(100), 81(37), 41(28), 77(26), 43(24), 39(19), 53(13), 72(11), 93(11), 91(9)

3. TMTT = 4,8,12-trimethyl-1,3,7,11-tridecatetraene; stereo configuration not determined without an authentic standard

4. Jasmine lactone = 7-decen-5-olide

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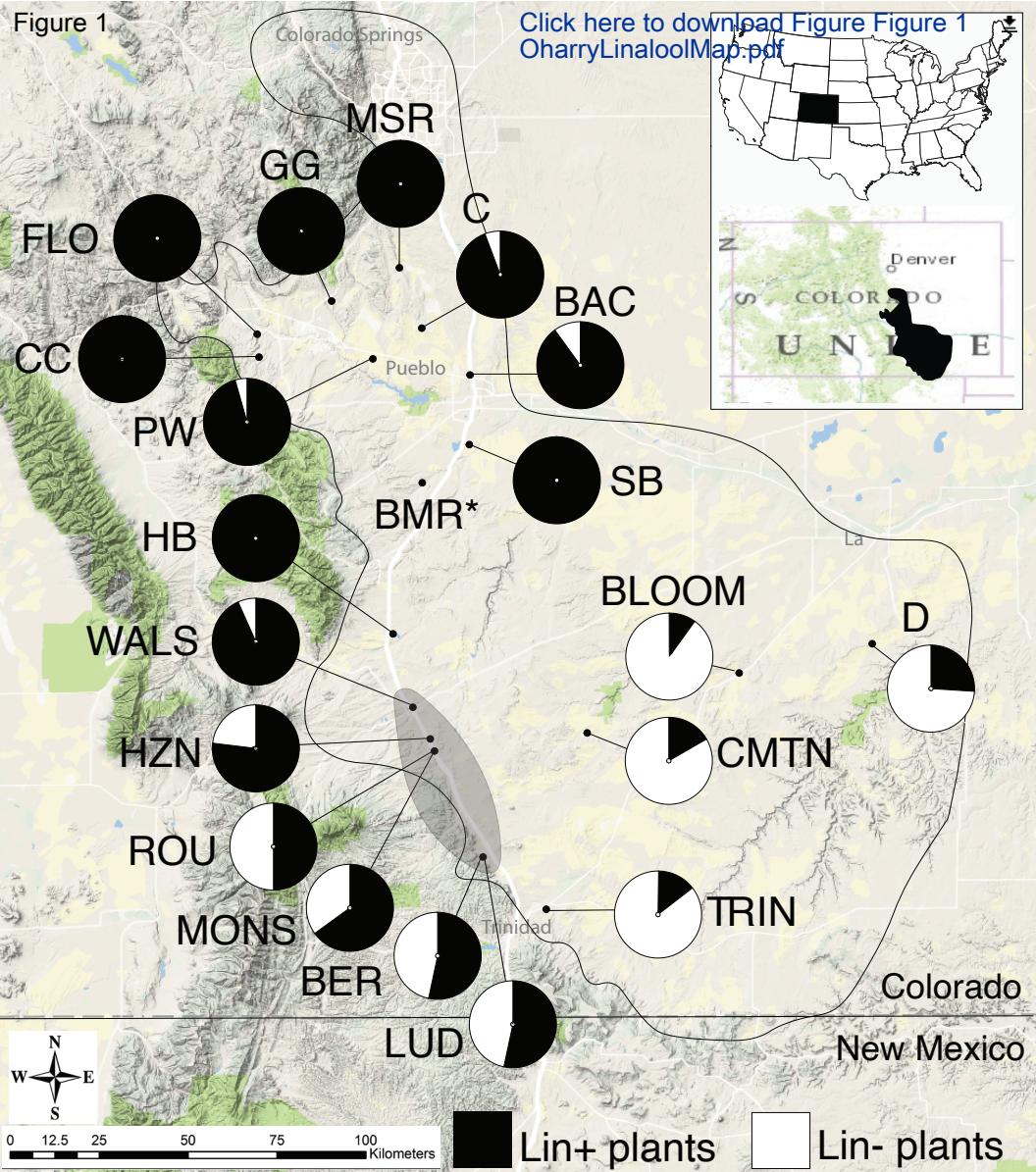
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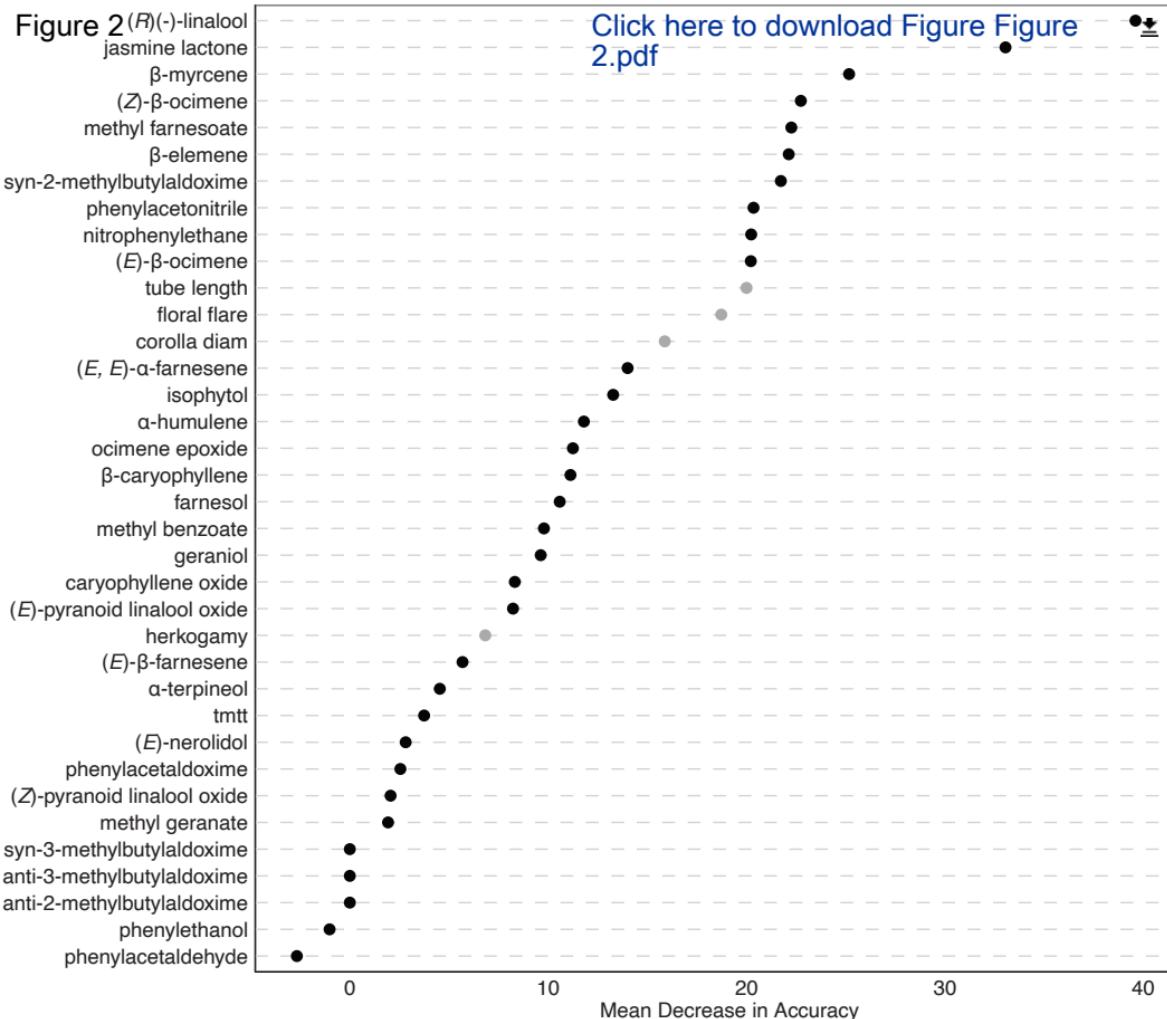
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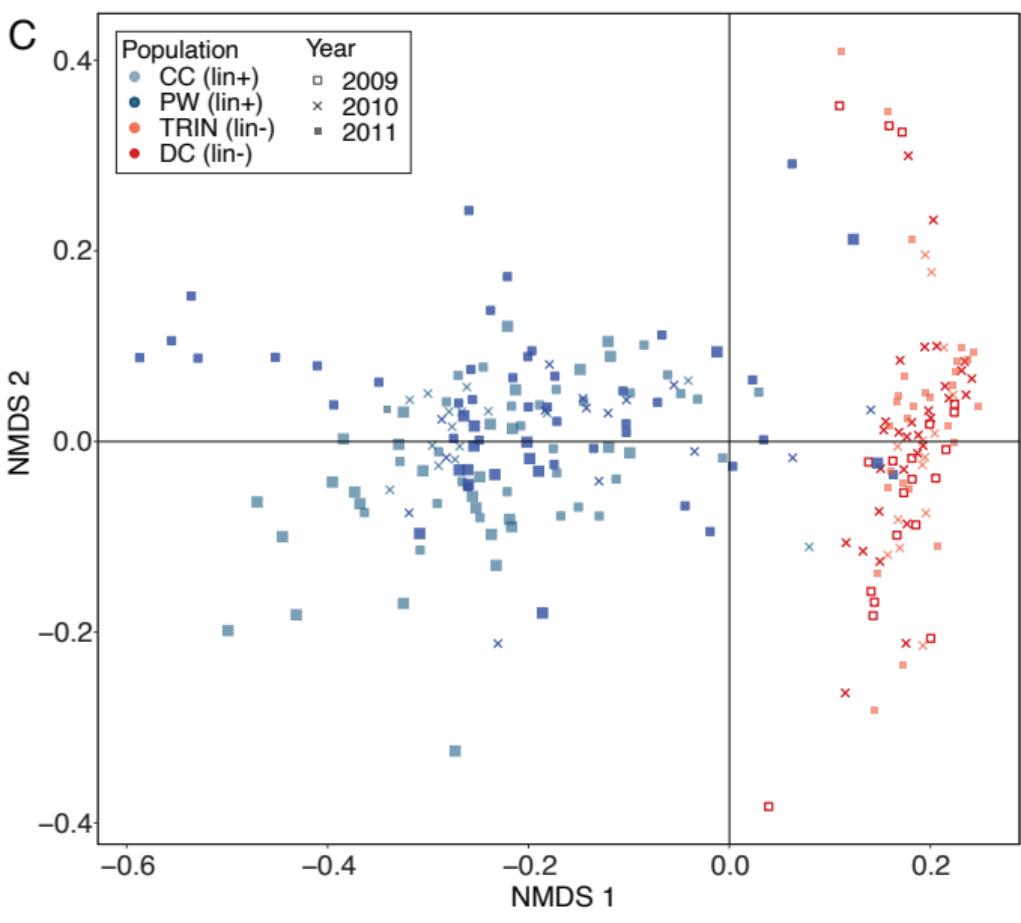
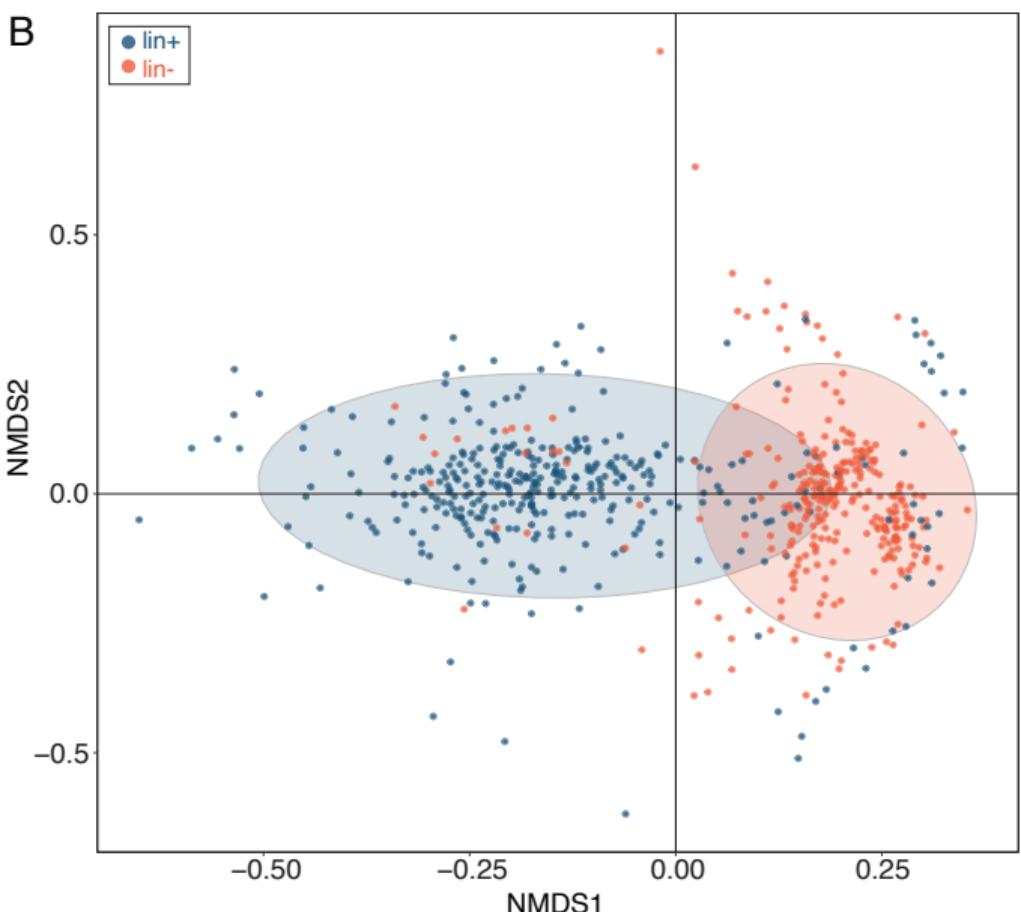
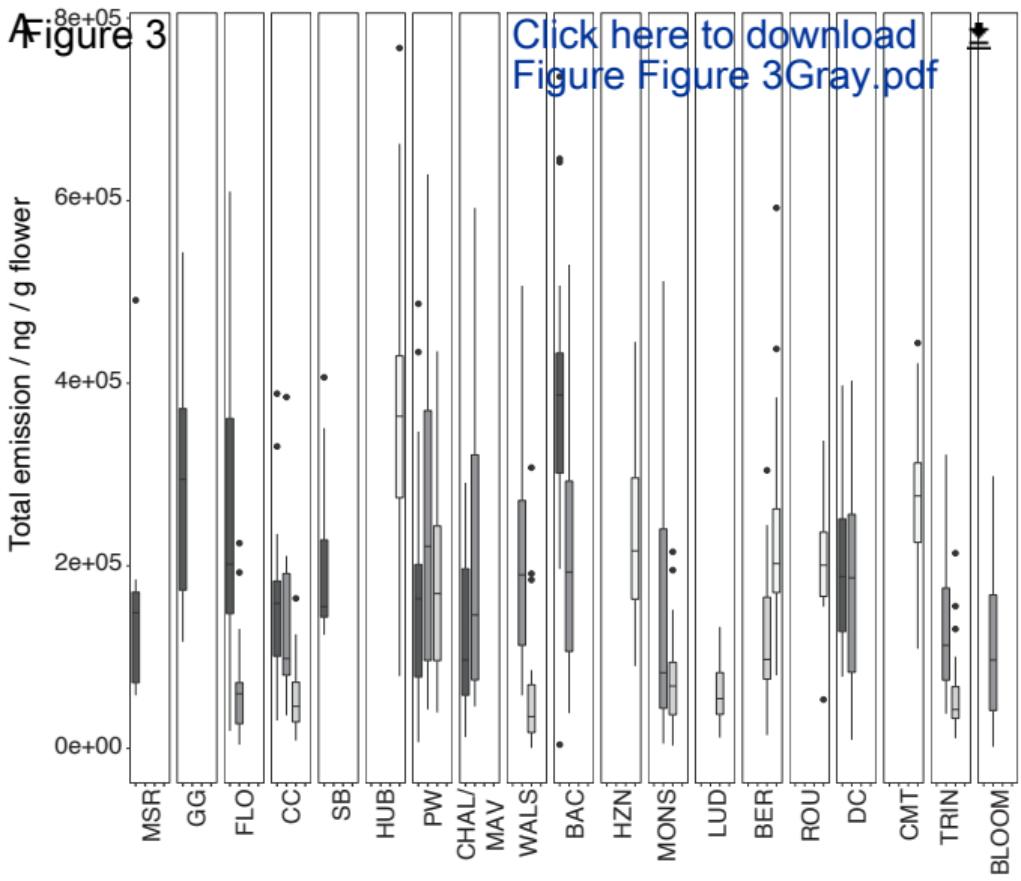
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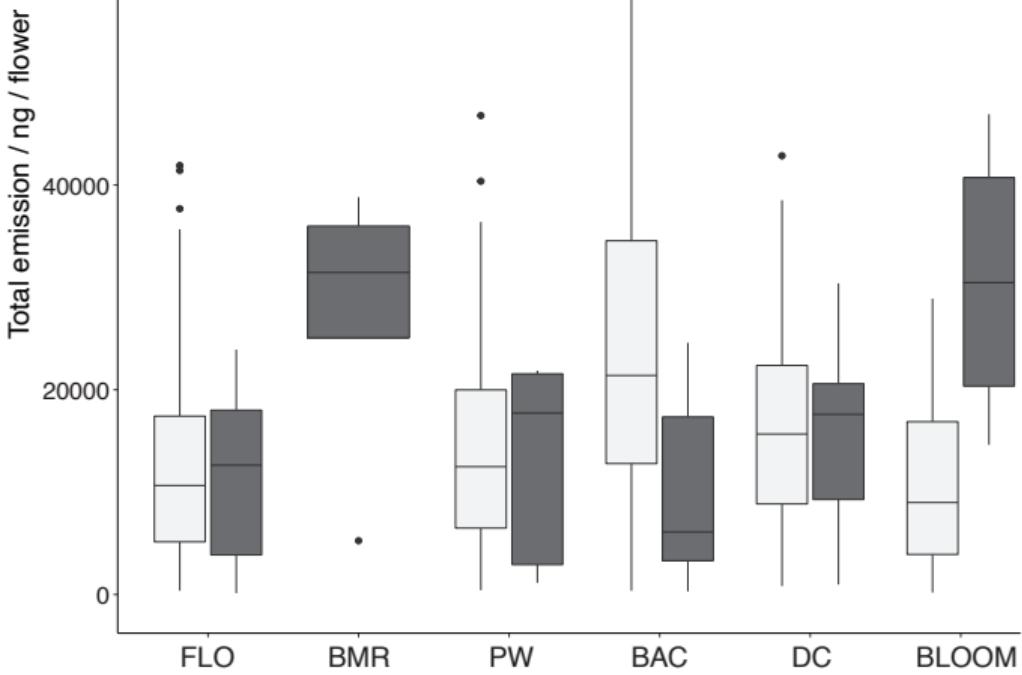
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4 762 **Data, code, and materials**  
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7 763 Original data and code created for the study will be available in a persistent repository upon  
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9 764 publication.  
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Figure 1.

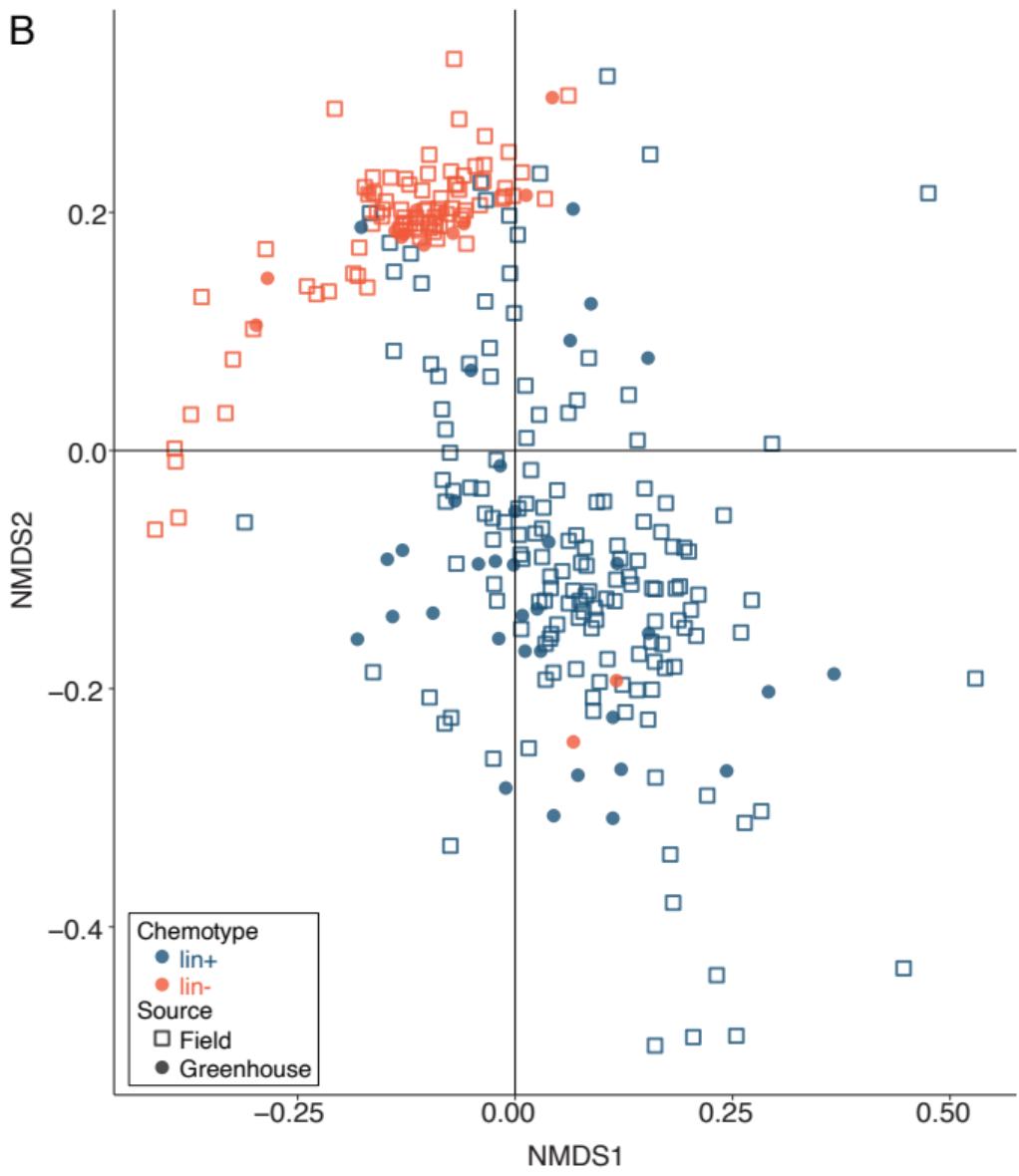








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5 Table 1. Population name, code, chemotype and location for all populations included in this  
6 traits (floral scent and morphology data), for the years data were collected. \*Floral scent for  
7 greenhouse conditions and were not collected in situ. Populations are arranged by proportion  
8 of populations where linalool was produced in all individuals.  
9

Population Name	Code	Collector Number			
		(Herbarium Code)	Latitude	Longitude	Chemotype
Military Service Road	MSR	LOL_787 (CHIC)	38.51209	-104.74081	lin+
Green Gulch	GG	LOL_988 (CHIC)	38.4444	-104.9166	lin+
Florence	FLO	LOL_733 (CHIC)	38.37695	-105.10983	lin+
Cockleburr Creek	CC	LOL_737 (CHIC)	38.33025	-105.10507	lin+
Stem Beach	SB	LOL_770 (CHIC)	38.18185	-104.59006	lin+
Burnt Mill Road	BMR*	LOL_731 (CHIC)	38.09803	-104.73043	lin+
Huerfano Butte	HB	LOL_757 (CHIC)	37.76433	-104.8336	lin+
Pueblo West	PW	LOL_756 (CHIC)	38.33421	-104.81546	lin+
ChalMav	CM	LOL_734 (CHIC)	38.38919	-104.68282	lin+
Walsenburg	WALS	LOL_746 (CHIC)	37.61947	-104.75932	lin+
Baculite Mesa	BAC	LOL_989 (CHIC)	38.293916	-104.557661	lin+
Hezron	HZN	LOL_738 (CHIC)	37.5484	-104.7142	lin-
Monson	MONS	LOL_742 (CHIC)	37.52425	-104.6972	lin-
Ludlow	LUD	LOL_740 (CHIC)	37.317598	-104.554034	lin-
Berwind	BER	LOL_730 (CHIC)	37.310891	-104.54689	lin-
Rouse	ROU	LOL_956 (CHIC)	37.529468	-104.703226	lin-
David Canyon	DC	LOL_737 (CHIC)	37.75612	-103.5981	lin-
Cone Mountain	CMTN	LOL_736 (US)	37.566782	-104.299439	lin-
Trinidad	TRIN	LOL_710 (CHIC)	37.227249	-104.413306	lin-
Bloom	BLOOM	LOL_727 (US)	37.69002	-103.95087	lin-

study. Sample sizes are provided for the following data collected in situ: floral  
the population BMR were collected from plants grown under common  
on of individuals emitting linalool (highest at top) and then by latitude for

Median linalool emission rate ( $\mu\text{g flower}^{-1} \text{hr}^{-1}$ )	Proportion emitting linalool	Sample Sizes for Scent, Morphology, Nectar				Number of Greenhouse- grown plants sampled
		2009	2010	2011	2012	
4.93	100.0	9				
9.87	100.0	10				
3.99	100.0	20	14			15
2.93	100.0	29	15	30		
2.36	100.0	10				
7.99	100.0					5
8.76	100.0			27		
4.08	95.6	38	15	15		5
3.78	94.3	20	15			
1.94	93.0		15	30		
4.24	89.8	19	30			9
0.00	76.9			12		
0.03	65.0		29	29		
0.02	53.3			15		
0.00	53.3			15	29	
0.00	50.0				8	
0.00	26.0	20	30			11
0.00	16.7			30		
0.00	14.3		14	28		
0.00	10.0		30			4

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 3 Table S1: Floral scent collected *in situ* from wild populations of *Oenothera harringtonii*, durin  
 4 Compounds shown in bold font were confirmed through co-injection with authentic standards.  
 5 and co-occurrence with related compounds or isomers. Enantiomeric configuration of linalool v  
 6 Data shown are mean +/- std. error (s.e.m.) % of total emissions (TIC peak areas) for each comp  
 7 Population abbreviations and order (proportion of plants emitting linalool) follow those present  
 8  
 9

Parent population	ret time (min)	MSR mean
<b>Volatile Organic Compounds (35)</b>		
Monoterpenoids (11)		
<b>β-myrcene</b>	6.07	0.263
<b>(Z)-β-ocimene</b>	6.85	0.788
<b>(E)-β-ocimene</b>	7.08	55.436
ocimene epoxide <sup>1</sup>	8.53	0.121
ocimene epoxide (myroxide isomer) <sup>2</sup>	9.37	0.005
<b>(R)-(-)-linalool</b>	9.85	38.969
<b>α-terpineol</b>	11.22	
<b>methyl geranate</b>	11.25	
<b>(Z)-pyranoid linalool oxide</b>	11.51	
<b>(E)-pyranoid linalool oxide</b>	11.67	
<b>geraniol</b>	12.33	
Sesquiterpenoids (11)		
<b>β-elemene</b>	10.40	
<b>β-caryophyllene</b>	10.53	0.107
<b>(E)-β-farnesene</b>	10.97	0.006
<b>α-humulene</b>	11.19	0.002
<b>(Z,E)-α-farnesene</b>	11.44	0.006
<b>(E,E)-α-farnesene</b>	11.64	3.065
<b>Caryophyllene oxide</b>	13.60	0.016
<b>(E)-nerolidol</b>	13.73	0.001
Methyl farnesoate	14.97	0.632
Farnesol isomer	15.25	
<b>(E,E)-farnesol</b>	15.78	0.342
Other terpenoids (2)		
<b>TMTT<sub>3</sub></b>	12.08	
<b>Isophytol</b>	15.34	0.075
Aromatics (3)		
<b>Methyl benzoate</b>	10.68	0.011
<b>Phenylacetaldehyde</b>	10.84	
<b>2-phenylethanol</b>	12.88	
N-volatiles (7)		
<b>syn-2-methylbutylaldoxime</b>	9.36	
<b>syn-3-methylbutylaldoxime</b>	9.41	
<b>anti-2-methylbutylaldoxime</b>	9.54	

<b>anti-3-methylbutylaldoxime</b>	9.75	
<b>Phenylacetonitrile</b>	13.08	
<b>1-nitro-2-phenylethane</b>	14.45	
<b>Phenylacetaldoxime</b>	15.57	

Fatty acid derivatives (1)

<b>Jasmine lactone4</b>	15.37	0.155
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<b>N plants sampled</b>	10
<b>cumulative # VOCs</b>	18
<b>mean +/- s.e.m. # VOCs</b>	10.3
<b>dry floral mass, g</b>	0.0857
<b>total scent ng/fl/hr</b>	14214.67
<b>total scent ng/g flw/hr</b>	165545.79

1. m/z 41(100), 67(73), 83(45), 43(43), 69(35), 55(33), 82(25), 53(21), 54(14), 81(14)

2. m/z 79(100), 81(37), 41(28), 77(26), 43(24), 39(19), 53(13), 72(11), 93(11), 91(9)

3. TMTT = 4,8,12-trimethyl-1,3,7,11-tridecatetraene; stereo configuration not determined without an authentic standard

4. Jasmine lactone = 7-decen-5-olide

ing the first hour of anthesis (single flowers).

Other compound identities are inferred from mass spectral library match was determined using chiral GC-MS.

ound.

ed in Table 1.

2009 s.e.m.	GG mean	2009 s.e.m.	FLO mean	2009 s.e.m.	FLO mean	2010 s.e.m.	CC mean	2009 s.e.m.
0.048	0.321	0.035	0.419	0.036	0.150	0.024	0.340	0.023
0.151	0.841	0.099	1.152	0.101	0.470	0.075	0.890	0.060
6.215	53.191	6.076	59.683	4.060	55.270	5.569	55.072	3.045
0.065	0.198	0.044	0.511	0.096	0.098	0.033	0.250	0.049
0.003	0.011	0.003	0.012	0.003	0.004	0.002	0.007	0.002
6.147	43.119	6.144	31.568	3.948	40.044	5.535	39.169	2.747
			0.353	0.340			0.024	0.016
			0.013	0.008	0.005	0.003	1.057	0.269
							<0.001	<0.001
			0.012	0.008	0.648	0.634		0.010
								0.003
0.020	0.198	0.090	0.256	0.068	0.141 0.018 0.017	0.050 0.017 0.414	0.301	0.070
0.006	0.001	0.001	0.006	0.002			0.006	0.002
0.001	0.010	0.005	0.021	0.007			0.022	0.006
0.006			0.002	0.001			0.001	0.000
3.065			0.005	0.002			0.001	0.000
0.015	0.002	0.002	0.057	0.031			0.040	0.015
0.001			0.001	0.001			0.008	0.003
0.315	0.878	0.225	2.277	0.947			1.788	0.519
0.158	0.356	0.065	1.417	0.748	1.103	0.414	0.627	0.123
0.023	0.055	0.024	0.001	0.001				
			0.184	0.085	0.326	0.139	0.030	0.014
0.005	<0.001	0.002	0.016	0.006			0.026	0.012
							<0.001	
			0.001	0.001			0.002	0.001
							0.010	0.010
							0.011	0.011
							0.002	0.002

	0.016	0.016					0.004	0.004
	0.002	0.002					0.008	0.004
							0.003	0.002
0.075	0.770	0.118	1.413	0.448	1.318	0.317	1.339	0.259
	10		20		15		28	
	21		22		12		31	
0.8	12.3	0.9	13.6	0.6	8.7	0.4	13.1	0.8
0.0019	0.0686	0.0026	0.0818	0.0029	0.0945	0.0031	0.1001	0.0027
3445.69	20056.13	2931.36	19373.58	2836.84	6947.30	1587.62	14904.33	1555.45
39211.33	291480.05	42613.93	248338.71	38445.19	71230.59	16006.18	150780.24	15457.25

<b>CC</b>	<b>2010</b>	<b>CC</b>	<b>2011</b>	<b>SB</b>	<b>2009</b>	<b>HB</b>	<b>2012</b>	<b>PW</b>
mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean
0.176	0.020	0.093	0.017	0.424	0.047	0.358	0.040	0.285
0.558	0.068	0.287	0.034	1.202	0.151	1.203	0.120	0.791
45.581	4.253	44.109	3.021	69.957	5.355	69.190	3.701	49.588
0.308	0.056	0.194	0.063	0.450	0.108	0.483	0.083	0.325
0.006	0.005			0.014	0.007	0.043	0.007	0.013
48.738	4.285	43.114	2.553	24.872	5.683	25.132	3.957	45.216
0.018	0.016					0.042	0.005	0.002
2.168	0.429	6.282	1.450			0.036	0.005	0.003
		0.023	0.018	0.003	0.003	0.032	0.007	0.002
0.191	0.069	0.273	0.102	0.283	0.094	0.776	0.123	0.160
0.002	0.002	0.003	0.003	0.003	0.003	0.020	0.002	0.011
0.083	0.063	0.006	0.006	0.005	0.003	0.061	0.011	0.009
		0.062	0.045	0.013	0.007	0.060		0.004
0.956	0.236	2.144	0.427	0.005	0.003	11.620	3.160	1.881
		0.062	0.045	0.012	0.008	0.149	0.025	0.023
				1.018	0.186	0.035	0.007	0.003
						0.823	0.205	0.921
						0.020	0.000	
						0.576	0.115	0.289
0.093	0.016	0.296	0.059	0.105	0.032	0.110		
0.130	0.081			0.007	0.004	0.075	0.009	0.050
						0.063	0.017	0.006
						0.018	0.002	
						0.047	0.007	
						0.130	0.011	
						0.170	0.022	
						0.035	0.001	

0.006	0.004	0.004	0.004			0.075	0.015		0.001
0.002	0.002					0.125	0.015		
						0.044	0.003		
						0.171	0.021		
0.984	0.227	3.183	0.529	1.029	0.229	0.440		0.428	
15		30		10		27		38	
17		15		17		32		22	
10.5	0.5	8.0	0.3	11.9	0.7	17.0	0.7	10.3	
0.1034	0.0049	0.0858	0.0032	0.0633	0.0032	0.0878	0.0019	0.0840	
14152.34	2247.33	4619.75	550.23	13068.03	2045.27	32973.78	2932.00	13677.74	
141129.50	22822.08	54836.35	6735.41	206264.98	30746.96	370208.89	29441.66	159715.17	

2009 s.e.m.	PW mean	2010 s.e.m.	PW mean	2011 s.e.m.	CHAL/MAV mean	2008 s.e.m.	CHAL/MAV mean
0.030	0.285	0.036	0.177	0.027	0.318	0.038	0.315
0.092	0.869	0.108	0.511	0.071	0.807	0.114	0.867
3.944	63.438	4.553	55.405	4.593	62.323	6.463	57.841
0.088	0.228	0.061	0.411	0.073	0.288	0.200	0.291
0.003	0.008	0.003	0.001	0.001	0.005	0.005	0.011
4.237	30.717	4.306	36.169	4.725	26.325	4.469	35.931
0.001	0.005	0.005					0.003
0.002	1.019	0.420	3.442	0.645			0.003
0.001			0.053	0.028			0.002
0.049	0.009	0.008	0.075	0.029	0.107	0.048	0.149
0.003					0.012	0.005	0.005
0.004					0.001	0.001	0.008
0.002					0.008	0.005	0.005
1.044					9.298	2.874	2.779
0.015					0.001	0.001	0.012
0.003					0.108	0.059	0.766
0.192							
0.052	1.631	0.969	1.794	0.422	0.113	0.046	0.291
0.013	0.194	0.080	0.257	0.047	0.100	0.051	0.093
0.003			0.004	0.004	0.026	0.025	0.012

0.001	0.001	0.001					
0.075	1.598	0.563	1.719	0.498	0.159	0.066	0.619
	15		15		13		20
	13		13		17		20
0.5	9.2	0.3	9.3	0.4	9.2	0.7	11.2
0.0023	0.0716	0.0030	0.0737	0.0038	n/a	n/a	0.0860
1643.79	18021.28	3461.50	13864.13	2190.36	8683.28	1471.60	10682.57
18413.53	248407.65	46498.26	185126.26	28265.88	n/a	n/a	124638.20

2009 s.e.m.	CHAL/MAV mean	2010 s.e.m.	WALS mean	2010 s.e.m.	WALS mean	2011 s.e.m.	BAC mean	2009 s.e.m.
0.027	0.298	0.035	0.219	0.024	0.042	0.010	0.719	0.057
0.070	0.882	0.105	0.715	0.075	0.288	0.045	4.331	2.320
4.253	58.702	4.395	55.516	4.104	49.355	3.562	64.133	4.779
0.093	0.394	0.093	0.234	0.034	0.233	0.078	0.397	0.123
0.003	0.006	0.002	0.011	0.004			0.028	0.007
4.104	29.203	4.116	32.961	3.941	38.527	4.298	25.801	3.243
0.002			0.001	0.001			0.004	0.002
0.002	2.542	0.984	0.261	0.067	0.249	0.156	0.001	0.001
0.001					0.005	0.003	0.005	0.003
0.033	0.061	0.023	0.479	0.174	0.445	0.112	0.248	0.083
0.003			0.020	0.011	0.018	0.011	0.011	0.004
0.003					0.014	0.007	0.015	0.006
0.004					8.569	2.623	0.007	0.003
1.826	5.731	2.548	8.701	3.320	0.081	0.057	2.632	1.441
0.010							0.019	0.009
0.185							0.508	0.165
0.066	1.276	0.320	0.390	0.108	0.472	0.216	0.556	0.184
0.036	0.154	0.036	0.071	0.018	0.778	0.671	0.084	0.022
0.004	0.022	0.020	0.040	0.020	0.004	0.004	0.005	0.004
			0.004	0.003				

	0.001	0.001	0.162	0.040	0.918	0.755	0.002	0.002
			0.036	0.009	0.000	0.000	0.001	0.001
			0.025	0.025				
0.137	0.728	0.140	0.153	0.140			0.523	0.128
	15		15		30		19	
	14		19		17		22	
0.7	9.7	0.4	11.6	0.6	6.8	0.6	13.1	0.9
0.0038	0.0905	0.0057	0.0879	0.0038	0.0849	0.0030	0.0845	0.0054
1686.75	19611.26	4058.50	17402.25	2687.35	4749.01	1060.30	34469.18	3980.00
19585.27	217479.40	42810.21	200940.78	30454.32	55557.30	12017.42	451444.87	74263.92



0.005	0.003	0.292	0.089	0.449	0.205	0.527	0.272
0.001	0.001	0.076	0.015	0.073	0.028	0.014	0.010
		0.390	0.110	0.128	0.053	0.061	0.047

0.797 0.174 2.890 0.049 0.041 1.009 0.904

30		12		29		29	
16		25		19		19	
9.5	0.3	16.3	1.0	10.6	0.6	8.8	0.4
0.0861	0.0028	0.0832	0.0034	0.0830	0.0022	0.0893	0.0037
18105.56	1912.70	20884.84	3250.91	13301.98	2273.68	6280.92	822.80
219346.40	24589.69	244203.96	33070.55	155918.55	26846.19	73341.32	9576.80

LUD	2011	BER	2011	BER	2012	ROUS	2012
mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
0.177	0.034	0.488	0.062	0.516	0.024	0.250	0.027
0.572	0.083	1.293	0.200	1.735	0.073	0.906	0.113
72.481	7.031	80.521	4.463	88.351	2.091	84.243	7.283
0.370	0.070	0.646	0.118	0.988	0.129	0.905	0.448
0.001		0.020	0.007	0.061	0.004	0.028	0.009
24.713	7.179	8.911	4.491	6.919	2.780	23.718	9.680
				0.040	0.005		
0.376	0.133	1.239	0.489	0.180			
0.009	0.008	0.098	0.023	0.101	0.043	0.030	
0.261	0.071	0.996	0.302	1.361	0.233	1.036	0.503
				0.080	0.021		
0.001		0.070	0.027	0.094	0.020	0.056	0.028
		0.003	0.003	0.150			
0.083		2.489	2.483	8.338	3.008		
0.131	0.046	0.214	0.099	0.269	0.055	0.102	0.035
				0.762	0.136	0.216	0.046
				0.030			
0.525	0.190	1.763	0.637	0.876	0.118	0.276	0.038
0.237	0.068	0.674	0.269	0.085	0.012		
				0.164	0.034	0.043	0.008
		0.005	0.004	0.023	0.005	0.037	0.005
				0.008	0.001	0.007	0.002
				0.025	0.005	<0.001	

0.060	0.027	0.020	0.009	0.115	0.022	0.115	0.031
0.001		0.001	0.001	0.129	0.059	0.015	0.003
0.001		0.550	0.503	0.331	0.072	0.170	

0.290

15		15		29		8	
17		19		27		19	
8.5	0.4	10.4	0.5	15.0	0.5	13.0	0.9
0.0881	0.0037	0.0939	0.0031	0.1027	0.0034	0.0849	0.0064
5345.57	802.93	11856.22	1755.02	23758.20	2092.89	17823.54	3378.86
62368.74	10207.67	130001.59	20729.81	233562.42	18815.65	204672.95	31175.55

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DC	2009 mean	DC	2010 mean	CNMT	2012 mean	TRIN	2010 mean	TRIN mean
	s.e.m.		s.e.m.		s.e.m.		s.e.m.	
0.586	0.035	0.440	0.034	0.668	0.040	0.376	0.022	0.223
1.816	0.115	1.345	0.091	2.096	0.099	1.294	0.090	0.726
86.130	3.345	93.011	1.389	90.398	1.173	91.564	1.448	92.276
0.197	0.056	0.355	0.105	0.546	0.071	0.870	0.312	0.168
0.020	0.003	0.015	0.004	0.083	0.008	0.009	0.005	0.001
0.028	0.013	0.030	0.022	0.026	0.002	0.014	0.005	
0.010	0.004	0.003	0.002	0.035	0.005	0.002	0.002	
0.002	0.002	0.696	0.180	0.020		0.163	0.083	0.240
0.004	0.004	0.001	0.001	0.040	0.005			0.023
0.004	0.002							
1.184	0.338	0.460	0.097	0.568	0.104	1.605	0.448	0.320
0.023	0.009			0.070				
0.120	0.037	0.017	0.007	0.049	0.007	0.093	0.040	0.015
0.021	0.010			0.077	0.010			0.009
7.945	3.686	2.891	1.424	7.021	2.150	1.947	1.333	4.876
0.318	0.106			0.145	0.019			0.105
1.017	0.362			1.988	0.380			
0.480	0.122	0.581	0.224	1.051	0.178	1.606	0.592	0.809
0.005	0.004			0.160				
0.050	0.011	0.152	0.059	0.160	0.018	0.196	0.054	0.191
0.005	0.002	0.003	0.003	0.104	0.023	0.002	0.002	
0.001	0.001			0.040				
0.002	0.002			0.025	0.001			

0.015	0.015			0.143	0.016	0.256	0.228	0.018
0.005	0.005	0.001	0.001	0.053	0.006	0.001	0.001	0.001
0.017	0.017			0.257	0.038			
0.002	0.002			0.140				
20		30		30		14		28
27		16		26		16		16
12.7	0.7	7.7	0.5	13.1	0.4	8.9	0.4	7.1
0.0858	0.0041	0.1103	0.0236	0.1054	0.0027	0.1050	0.0062	0.1085
16816.12	1676.41	16145.46	2021.07	28000.80	1464.62	13608.57	2333.08	6606.68
201005.09	19274.36	179078.33	20641.39	268606.15	14472.15	133811.55	22715.98	58706.73

2011	BLOOM	2010
s.e.m.	mean	s.e.m.
0.021	0.355	0.037
0.059	1.099	0.104
2.126	95.767	0.659
0.042	0.337	0.062
0.001	0.036	0.017
	0.002	0.001
0.062	0.813	0.242
0.012	0.002	0.002
0.086	0.188	0.054
0.009	0.002	0.002
0.004		
2.238		
0.044		
0.244	1.134	0.371
0.039	0.252	0.128
	0.005	0.003

4	0.010	0.005	0.005
5	0.001	0.002	0.001
6			
7			
8			
9			
10		0.001	0.001
11			
12			
13		30	
14		16	
15	0.5	7.3	0.5
16	0.0031	0.0985	0.0033
17	1051.69	10655.82	1517.99
18	8688.45	109290.12	15455.42
19			
20			

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3 Table S2: Floral scent collected *in situ* from wild populations of *Oenothera harringtonii*, during th  
4 Compounds shown in bold font were confirmed through co-injection with authentic standards. Other  
5 and co-occurrence with related compounds or isomers. Enantiomeric configuration of linalool was j  
6 Data shown are mean +/- std. error (s.e.m.) % of total emissions (TIC peak areas) for each compound.  
7 Population abbreviations and order (proportion of plants emitting linalool) follow those presented in  
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Parent population	ret time	FLO	BMR		PW	
Compound name	(min)*	mean	s.e.m.	mean	s.e.m.	mean
Monoterpenoids						
<b>β-myrcene</b>	6.07	0.205	0.016	0.322	0.062	0.201
<b>(Z)-β-ocimene</b>	6.85	0.612	0.041	0.985	0.143	0.514
<b>(E)-β-ocimene</b>	7.08	53.673	2.984	59.287	7.020	56.781
ocimene epoxide <sup>1</sup>	8.53	0.994	0.876	0.251	0.053	0.199
(Z)-furanoid linalool oxide	9.06					
(E)-furanoid linalool oxide	9.29	0.001				
ocimene epoxide (myroxide isomer) <sup>2</sup>	9.37	0.003	0.001	0.008	0.003	0.003
<b>(R)-(-)-linalool</b>	9.85	36.927	2.804	31.102	4.308	32.103
<b>α-terpineol</b>	11.22	0.001		0.001	0.001	
<b>methyl geranate</b>	11.25	0.054	0.049	0.002	0.002	0.125
<b>(Z)-pyranoid linalool oxide</b>	11.51					
<b>(E)-pyranoid linalool oxide</b>	11.67					
<b>geraniol</b>	12.33	0.001				
Sesquiterpenoids						
<b>β-elemene</b>	10.40					
<b>β-caryophyllene</b>	10.53	2.717	1.156	1.218	0.450	1.390
<b>(E)-β-farnesene</b>	10.97			0.001	0.001	
<b>α-humulene</b>	11.19	0.103	0.040	0.042	0.018	0.074
(Z,E)-α-farnesene	11.44			0.008	0.008	
<b>(E,E)-α-farnesene</b>	11.64	0.096		1.015	1.015	
<b>Caryophyllene oxide</b>	13.60	0.165	0.131	0.079	0.048	0.049
<b>(E)-nerolidol</b>	13.73			0.007	0.007	0.029
Methyl farnesoate	14.97	2.109	0.886	0.596	0.330	3.912
<b>(E,E)-farnesol</b>	15.78	0.739	0.260	0.174	0.039	0.768
Other terpenoids						
<b>TMTT<sub>3</sub></b>	12.08					
<b>Isophytol</b>	15.34	0.195	0.058	0.039	0.016	0.311
Aromatics						
<b>Methyl benzoate</b>	10.68	0.040		0.001	0.001	
<b>Phenylacetaldehyde</b>	10.84					
<b>Methyl salicylate</b>	12.09	0.001				
<b>Benzyl alcohol</b>	12.82					
<b>2-phenylethanol</b>	12.88					
N-volatiles						
<b>syn-2-methylbutylaldoxime</b>	9.36					
<b>syn-3-methylbutylaldoxime</b>	9.41					
<b>anti-2-methylbutylaldoxime</b>	9.54					

<b>anti-3-methylbutylaldoxime</b>	9.75			
<b>Phenylacetonitrile</b>	13.08			
Nitrophenylethane	14.45			
Phenylacetaldoxime	15.57			
<b>Fatty acid derivatives</b>				
<b>Jasmine lactone<sup>4</sup></b>	15.37	1.228	0.509	2.375

<b>N plants sampled</b>	52		11	14
<b>cumulative # VOCs</b>	20		19	15
<b>mean +/- s.e.m. # VOCs</b>	9.0	0.4	9.5	1.1
<b>dry floral mass, g</b>	n/a		n/a	n/a
<b>total scent ng/fl/hr</b>	16883.21	2197.36	32657.98	6773.92
<b>total scent ng/g flw/hr</b>	n/a		n/a	n/a

\* original GC oven program was 10C/min, rather than 15C/min. Retention times provided here are translated to the faster program for consistency

n/a: floral masses not recorded, emission rates per floral mass not calculated

1. m/z 41(100), 67(73), 83(45), 43(43), 69(35), 55(33), 82(25), 53(21), 54(14), 81(14)

2. m/z 79(100), 81(37), 41(28), 77(26), 43(24), 39(19), 53(13), 72(11), 93(11), 91(9)

3. TMTT = 4,8,12-trimethyl-1,3,7,11-tridecatetraene; stereo configuration not determined without an authentic standard

4. Jasmine lactone = 7-decen-5-oxide

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3      the first hour of anthesis (single flowers).  
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5      Major compound identities are inferred from mass spectral library match  
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7      previously determined using chiral GC-MS.

8      id.

9      n Table 1.

	BAC		DC		BLOOM	
s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
0.034	0.245	0.031	0.472	0.037	0.513	0.101
0.091	0.763	0.076	1.301	0.109	1.455	0.236
5.603	59.748	3.789	88.451	4.067	91.003	3.789
0.084	0.728	0.358	0.212	0.070	0.150	0.105
	0.001					
	0.001					
0.002	0.009	0.003	0.022	0.007	0.015	0.005
3.846	32.189	3.420	7.178	4.086	0.018	0.009
	0.001				0.003	0.003
	0.002		0.006	0.005		
		0.006				
1.122	1.775	0.940	0.318	0.076	0.235	0.215
	0.000	0.000	0.001		0.008	0.005
0.061	0.091	0.050	0.016	0.005	0.015	0.015
		< 0.001				
	2.269	1.427	0.684	0.472	5.935	3.440
0.031	0.074	0.029	0.039	0.019	0.115	0.115
0.019						
1.422	0.630	0.129	0.499	0.296	0.163	0.068
0.265	0.654	0.146	0.304	0.073	0.293	0.269
0.127	0.175	0.041	0.071	0.023	0.065	0.017
	0.017	0.013				
	0.001					

	1.020	1.411	0.505	0.122	0.076	0.003
	2250.93	16530.92	2509.79	20167.80	2513.80	30617.36
	n/a			n/a		n/a
	0.4	9.1	0.4	9.4	0.6	10.5
				n/a		1.9
	33			18		4
		21		17		16

with field-collected data

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: