Tania

Rob

Krissa

All

While your work is considered of interest - as indicated by the extensive comments from the reviewers - their comments indicate that the manuscript would benefit from some modifications and they make some suggestions for improving the presentation. There are also some queries raised in relation to the scientific content. All of these need to be addressed.

Please highlight your scientific modifications in the revised version as this helps both the editor and the reviewers to assess the revised paper more quickly and could help avoid a further review. Revisions without highlighting risk being returned to the authors, causing delay.

As you revise your paper, please check it very carefully for errors and that it conforms to the recommended Guidelines, available at:

<https://www.elsevier.com/journals/Phytochemistry/0031-9422/guide-for-authors>?

Closely following the Guidelines for articles helps to avoid delays in further processing.

Editor:

There are a small number of formatting issues: e.g. a sentence for the Graphical Abstract; Figures separate from text, etc.

Associate Editor:

Please check the references for formatting to Phytochemistry guidelines (abbreviate journals).

Reviewer #1: In this manuscript, Skogen et al. describe the floral scents of an evening primrose. The study is exceptional, considering the extremely high sample sizes: the authors sampled 650 individuals of 19 populations throughout the distribution range of the plant, and an additional 50 individuals in a common garden setting. They found that the scent varies among individuals and populations, mainly due to the presence or absence of R-linalool.

The manuscript is nicely written, and except some editorial issues, I only have a few suggestions: to include specific research questions, fig to also discuss why other compounds, such as ocimene, were less variable than linalool, and to reconsider the classification of lin+ and lin- populations.

All my specific comments are in the edited manuscript file.

Reviewer #2: In this study, intraspecific floral-scent- and floral-morphology variation was investigated in 70% of all known population of the species Oenothersa harringtonii, a species with a limited distribution range and in which floral scent is essential for its pollinators to locate the flowers. In addition, plants from a subset of the populations were grown in a greenhouse common-garden. It was found that there was considerable differentiation in floral traits among populations and that this differentiation was stronger for floral scent than for floral morphology. Thereby, the floral scent compound (R)-(−)-Linalool was the most important trait causing this differentiation and its emission showed a geographical cline. Moreover, floral scent composition was largely consistent across years and between plants in natural populations and plants in the greenhouse common-garden, indicating that variation in floral scent in general and in (R)-(−)-Linalool in particular have a genetic basis.

This study provides novel insights into intraspecific variation in floral traits, in particular in floral scent, which has, to date, not been studied to that extent on the within-species level. This study, thus, provides an important contribution to our understanding of the full extent of intraspecific floral scent variation in natural populations of a species. Overall, this study does not only address an important gap in our knowledge, but is also written in a nice, well understandable way, and, in general, is based on a soundly collected, large data set. However, I have the following major concerns especially regarding the way the study is framed and the data were analysed and discussed:

Firstly, a research question is lacking making it hard to grasp and judge the aim, major conclusion, and implication of the study.

We have provided additional context and goals of the study at the end of the Introduction.

Secondly, it is unclear how populations were grouped into lin+ and lin- populations especially because all populations contained at least a few lin+ individuals. As far as I understand, median linalool emission rate was probably used somehow, but the grouping into lin+ and lin- populations in Table 1 does not seem to be consistent with the grouping into lin+ and lin- populations in the first paragraph in 2.3.

We have removed the classification of populations as lin+ and lin- from Table 1 and from the text.

Thirdly, it is also unclear why populations were grouped into lin+ and lin- populations. As there are no known (at least so far) biological reasons to do so, a less restrictive approach would potentially provide better insights into the geographical pattern of floral scent in O. harringtonii and its biological implications. In addition, when you compared lin+ with lin- populations, it is not surprising that you found differences because mean linalool emission differs between lin+ and lin- populations. (Biologically) more interesting would be to assess whether floral scent also differed among populations apart from linalool emission. This could be addressed by once doing the analysis including linalool and once excluding linalool. In this context, I think the following question would be suitable to be able to draw biologically more meaningful conclusions: Do lin+ and lin- plants smell the same everywhere or are there additional population-specific differences in floral scent independent of linalool? See below for a suggestion how to address this.

Fourthly, the discussion is rather unspecific and unclear. This is probably mainly due to the fact that there is not really a research question stated in this study. The major question that arises and I am expected to be discussed is: What causes this clinal linalool pattern and how is this linked to other trait variation? For example, the role of linalool in the interaction with pollinators, in particular hawkmoths, and with floral antagonists is discussed in some length, but not really how that might be related to the clinal linalool pattern. And then, the discussion implies that pollinators and floral antagonists may not even play a big role in the clinal linalool pattern. In addition it would be important to discuss why other floral traits than linalool do or do not differ among populations/along this geographic cline, because linalool is unlikely the sole scent compound playing a role in the plant's interaction with its pollinators and/or antagonists. In addition, differences in abiotic factors could also play a role. What might these be? In general, it is fine and interesting to focus on linalool but to get a biological understanding it should be discussed in the context of other floral scent compounds and floral morphological traits.

Fifthly, in the description of floral scent collection and analyses it is not mentioned whether negative (air) controls were collected. Based on the data presented, I think they were. However, since such negative controls are essential to discriminate between the floral scent compounds and contaminating volatile compounds in the samples, this information should be added.

We indeed collected ambient controls and data on contamination, this information was inadvertently omitted but is now included.

Lastly, labels and legends of some figures and tables are incomplete and/or unclear and there are several small mistakes and/or inconsistencies, which should be addressed (for details, see below).

Following, I detail these and additional more minor concerns and provide some suggestions. I refer to the line numbers in the PDF file.

**Abstract**

An actual research question is missing. Adding one will considerably improve the understandability and purpose of the study.

**Introduction**

In general, the introduction nicely provides the relevant background, but an actual research question is missing. Adding one will considerably improve the understandability and purpose of the study.

We have incorporated goals in the last paragraph of the Introduction.

**Results and Discussion**

Lines 152-153: I agree that it is fine and interesting to focus the remaining part of the analyses (mainly) on (R)-(−)-Linalool particularly because linalool is either (almost) absent or dominantly present. However, linalool is far from the only important compound explaining population differentiation and unlikely the only biologically relevant compound. Thus, for a better understanding of the entire floral trait variation and its implications, it would be essential to at least briefly mention and discuss (really nothing very long and as detailed as for linalool!) the other traits that seem to be almost as important for population distinction. According to Figure 2, this is at least jasmine lactone and beta-myrcene. In addition, I think it would be interesting to note that, when just focusing on floral morphology traits, herkogamy seems to be less important for population differentiation than the other traits, maybe as it has important implications for precise pollen deposition.

Following, I list several comments regarding the distinction into lin+ and lin- plants/populations. I am actually not sure whether the problem is that you do not describe detailed enough how the distinction was done so that I might have understood it wrongly or whether I do not fully agree with how the distinction was done. A clear and detailed description of how you did the distinction into lin+ and the lin-plants/populations and why you used this way to distinguish between lin+ and lin- plants/populations will be very helpful for an easier understanding of your study. This content has been removed/edited, see above.

Lines 168-169: Here, you write that linalool is either completely/almost absent in individuals or it accounts for a very high proportion of the total scent emission. Thus, I understand you have 2 distinct linalool morphs. Or do you discriminate among 3 linalool morphs, i.e. (1) no linalool emitted, (2) low linalool concentrations emitted, and (3) high linalool concentrations emitted? Please describe it in more detail.

Lines 170-172: As I mentioned above in my major concerns, it is not really clear to me how you defined what is a lin+ population and what is a lin- population; especially because there are no lin- populations since all populations contain at least some lin+ plants. Please describe this here. In addition, the grouping in Table 1 (column "Chemotype") does not match the average percentage of lin+ plants as it is given here in the text and in the column "Proportion emitting linalool" of Table 1. At least, I do not understand how the mean proportion of lin+ plants per lin+ population can be 86% when this percentage varies between 89.8% and 100% for lin+ populations according to Table 1 and how the mean proportion of lin+ plants per lin- population can be 22% when this percentage varies between 10.0% and 76.9% for lin- populations and is lower than 22% in only 3 out of 9 lin- populations according to Table 1. Please elaborate.

This content has been removed/edited, see above.

Lines 173-174: Is linalool included or excluded in this analysis? If linalool is included, it would very be surprising, if you did not find any difference. In my opinion, an interesting question would be whether populations differed in floral scent also independent of linalool? Please elaborate on this. It would probably make sense to conduct these analyses with and without linalool.

Lines 181-183: It is not surprising that the median linalool emission rate of all populations in a population is lower in populations with fewer lin+ individuals. In all populations with <50% lin+ individuals, median linalool emission will anyway be 0. More interesting would be to just include lin+ individuals in this comparisons.

Line 208: As there is no research question stated in the introduction, it is hard to figure out what the main focus of the discussion should be. As I understand it and what is not very well understood is what causes the geographical cline in linalool in particular or the divergence in floral scent among populations in general. What you discuss in 2.5.1. and 2.5.2. is not really new or something you found in your study. More interesting would be to discuss this in the context of the geographical variation in linalool emission.

Lines 236-250: Here, you discuss the relevance of floral scent in the interaction of O. harringtonii and its antagonists, but it does not become clear what the role of linalool is in this interaction. Please specify.

Line 294: Which other biotic and abiotic factors could play a role? A short discussion would be interesting. Additional information has been added

**Experimental**

Line 339: A short summary (1-2 sentences) of the genetic structuring found would be helpful to get a better idea how that could be related to the floral trait variation.

Line 364: Somewhere here it would be important what kind of negative control you collected. And further down it should be mentioned how it was included to assess which of the compounds were floral compounds and which were contaminations. Included information on how ambient controls and assessments of contaminants were collected.

Line 386: As it has not been mentioned so far that you quantified dry floral mass, its mentioning is confusing here. It would help, if you described this here or at least referred to the description below. Added content in 4.2 Floral Phenotype stating that flowers were dried and weighed for use in calculations of floral scent emission rates per unit mass. f461

**Figures and Legends**

Figure 1: Please add the following information to the legend: Why is there no pie chart for the population BMR and/or what does "\*" mean next to BMR?

Data presented in Figure 1 is from plants growing in the field, and the data provided for the BMR population are derived from greenhouse-grown plants (summarized in Table 1 and S2). The figure legend has been updated to be more explicit about this difference.

Lines 437-438: Now it gets even more confusing: in the main text you write about lin+ populations and lin- population, but here you suddenly also introduce polymorphic populations aside from the lin+ and lin- populations, with one of them being a lin+ and the others lin- populations according to Table 1. In addition, all populations with lin+ and lin- plants are per definition polymorphic populations. Please clarify.

This content has been removed/edited, see above.

Figure 3A: Please add the information what the meaning of the different grey scales is. In addition an interesting question would be: Is total scent emission related to the percentage of lin+ plants in a population? And related to that and essential to show in this manuscript: Does emission differ between lin+ and lin- plants within populations and does that differ between populations? This could be addressed for total emission as well as for linalool emission.

Figure 3B: Please add the information what the symbols represent (individuals?) and what the ellipses are. And again, it is not surprising that scent composition differs between lin+ and lin- population since linalool emission differs. I suggest the following, biologically more relevant way to look at the data: use different colours for lin+ and lin- plants and use different symbols for different populations. This would provide interesting insights into whether there is a general difference between lin+ and lin- plants (probably mainly do to the emission of linalool) and/or whether there are differences in the scent composition among populations. This should also be assessed in the statistical analyses. In addition, doing these analyses once including linalool and once excluding linalool may provide interesting insights into whether populations and/or lin+ and lin- plants also differ in the scent composition independent of linalool.

Figure 3C: Also here it would be very interesting and helpful to somehow indicate which plants were lin+ and which lin-.

Figure 4B: Also here it would be very interesting and helpful to somehow indicate which plants were lin+ and which lin-.

Line 461-463: Where is this visible? It is also not clear whether you are writing about total emission or linalool emission. Please describe this in more detail. If it were the latter, it would be essential for the understanding of the study to somewhere show the linalool emission rates for lin+ plants for each of the populations. These data are available in Tables S1 and S2.

**Tables**

Table 1: Again, I am not sure what the column "Median linalool emission rate (μg flower-1 hr-1)" tells us, because it is not surprising that median emission rate is lower in populations with fewer lin+ individuals. A more relevant information would be to show this only for the lin+ plants and including more decimal places would provide a better understanding of the entire extent of the variation in linalool.

Table 2 and 3 or Table S1 and S2, respectively: There are several issues with these tables. First, Table 2 and Table S1 are the same as far as I can see, and Table 3 and Table S2 are also the same as far as I can see. Thus, it should just be the one or the other. Since the tables are very large, I suggest not including them in the main manuscript but rather in the Supplementary Information as Table S1 and Table S2. Second, in the main text there is only a reference to Table 2 and Table 3 but not to Table S1 and Table S2. This is probably connected to the first point and should be addressed accordingly. Last, the table headings of Table S1 (Table 2) and Table S2 (Table 3) are with a few minor linguistic differences the same regarding the content/meaning. I am not sure but is Table S1 about the plants collected in natural populations and Table S2 about plants grown in the greenhouse? Please specify. The submitted as Tables 2 and 3 had errors in their titles in the excel file. Both have been revised and are not provided as Tables S1 and S2, respective. We have updated and clarified the title for both tables as well.

More minor linguistic comments

Line 64: The bracket between "in" and "Delle-Vedove" should be deleted.

Line 122: "Table 2" comes before "Table 1" in the text. Thus, the numbering should be changed. But the numbering of the tables is anyway confusing and should be adjusted (see my comment above). It probably should be "Table S1". Table 1 has been referenced at the end of the Introduction. Tables 2 and 3 have been changed to Tables S1 and S2.

Line 126: "In Review" should not be capitalised. Changed to lower case and to “in press”

Line 138: There should be a semicolon after "Engel et al. 2016". Added semicolon

Line 142: Please include the full name of TMTT the first time it is mentioned in the text. Added

Line 169: Do you mean Table S1 and Table S2? But anyway none of the tables shows that linalool emission constitutes up to 95% of the total scent emission, probably because the tables show mean emission and not the values for every individual separately. Please specify.

Lines 212-215: Please double-check the punctuation marks here. Some are missing, some make it confusing which reference belongs to what.

Line 260: This sentence is a bit out of context here. It might better fit into the species description part. And what is the reference? Moved to species description, added reference (Skogen et al. 2016)

Line 344: What do you mean with "unbiased methods"? What are they exactly?

Line 431: Something is linguistically weird here. Should it be "and DEB 1342792 to R.A.R"? Corrected

Table 1: The population codes in the column "code" are not always consistent with those in Figure 1, in particular for the following two populations: (1) C in Figure 1 but CM in Table 1 and (2) D in Figure 1 but DC in Table 1. Please correct it in either Figure 1 or in Table 1 (and the graphical abstract). Corrected

What does the information in column "Collector Number (Herbarium Code)" refer to? Please describe. If it is not relevant to the study, maybe it could be excluded? Herbarium voucher information is necessary for open and repeatable science and its inclusion is standard practice in studies of organismal diversity. Collector number is a unique identifier for the herbarium, which vouches for the fact that the species was found in a specific location on a specific date. The herbarium code indicates where vouchers are deposited. Together, this information connects the voucher to the population, allowing others to locate them in perpetuity.

Table caption has been updated accordingly.

References: Please double-check the references and make sure that they are consistent. Some genera and species names are not in italics. Some titles are capitalised others are not.

**Track-changes feedback**

Line 29 – somehow, the research question is unclear;

whether morphology and scent varies in concerted manner or not?

Line 31 – research question is unclear

Line 37 – I suggest to include information above about how often (n years) were populations sampled Added ‘four’

Line 41 – well, I guess that more variation would even be detected in this species when still increasing the sampling size....

why not speculating about the evolutionary drivers or the ecological implication of the findings?

Line 61 – visual and olfactory cues also work in in several sexually deceptive systems....Revised to state ‘… visual and olfactory cues function in concert (Spaethe et al. 2007).’

Line 87 – please also consider to cite a recently published paper on this topic: <https://doi.org/10.3389/fpls.2021.719092>

Added reference

Line 112 – I suggest to formulate specific research questions, and explain, why data on phenotypic plasticity can be obtained. We have incorporated goals and clarified the content in the last paragraph of the Introduction.

Line 117 – what about morphology? Changed section subheader to “*Species-Wide Variation in Floral Traits”*

Line 123 – please use I-(-)-linalool throughout the manuscript; or just I-linalool All have been changed to I-(-)-linalool

Line 127 – Table 1 should be mentioned earlier Table 1 is now referenced in the Introduction

Line 129 – related in which sense? Revised to ‘that were structurally similar’

Line 138 – methyl geranate is not that rare among flower scents, e.g., Knudsen et al 2006 Revised to state ‘esters which are occasionally emitted by flowers but are better known from studies of insect sexual communication’

Line 147 – not appropriate in the present sentence, as the ANOSIM analyses were calculated separately for scent and morphology

Line 149 – scent and morphology included? Clarified text

Line 150 – I suggest to use the same compound names as in Table 2; it is very interesting. And might be worth to discuss, that one aldoxime isomer behaves very different than the others

Line 153 – this is easonable; however, it would at least be interesting to discuss why other compounds, especially E-beta-ocimene, were not that variable

Line 167-168 – needs statistical support

Line 171 – see comment on classification at Table 1. This content has been removed/edited, see above.

Line 172 – statistical support is needed

Line 176 – 177 – in my opinion, it would be enough to present the detailed statistical outcomes just once....either in the text or in the legend

Line 180 – in all these populations, most of the plants released linalool! See comment on classification also elsewhere. This content has been removed/edited, see above, as well as Figure 1B and C.

Line 194 – statistical support is needed

Line 197 – and also of other compounds

Line 249 – does not oviposit in flowers, only later in developing fruits? Clarified text

Line 250 – somehow, a conclusion of this chapter is missing; can antagonists explain the variation?

Line 257 – please also consider: <https://doi.org/10.3389/fpls.2021.719092> Added reference

Line 280 – 282 – context is unclear; differences in foraging behavior influence pollination efficiency? Why should different populations depend on different effectiveness?

Line 289 – original data of present manuscript? Clarified text

Line 301-305 – is somehow off-topic; and not related to the different enantiomers

Line 339 – conflict to above?

Line 347 – no results presented...either include results or delete information here. Removed all mention of nectar

Line 372 – above: splitless Content was updated for clarity

Line 375 – unclear; volatiles that eluted nearby linalool should be specified Content was updated for clarity, focus on linalool was removed

Line 388 – seeds of one or several mothers per population were used? Information of seed family available? Clarified text in two places – ‘wild seeds collected from multiple maternal plants from each of six focal populations’ and end of paragraph ‘from different maternal lines (unrelated individuals).

Line 408 - ? Removed extra ‘X’

Line 393 – and the other 4 hrs? Corrected hours

Line 408 – ER: relative amounts, as stated before?

M: I guess that data were normalized before analyses?...to avoid that “apples are compared with pears”...

Line 409 – 410 – why?

Line 412 – scent composition? See results

Line 413-414 – assumptions of normality and heterogeneity of variances tested? I have not seen these tests in the Results

Line 416 – scents were also tested against geographic distance

Line 450 – please indicate the different years of sampling in the Fig.; also, which values are shown? Mean, median, SE, Quartiles,....

Line 452 – please indicate the stress values in B and C

Line 452-454 – I do not see that this Figure and the corresponding analyses are needed; when classifying the populations according the occurrence of the compound that is most responsible for population differentiation, it is not surprising that differences are found

Line 456 – the year effects are somehow not obvious in the Figure; the dispersion seems also to be similar

Line 465 – why different? The following text was added to the end of the sentence “because dry floral masses were not collected for the greenhouse plants.”

Line 478-479 – Table 1- please rephrase, and simplify; also, why is nectar given in the Table? It was not mentioned before that nectar was studied Table title was revised; references to nectar was removed as these data were not included

Line 483 – Table 1 – please explain, what lin+ and lin- indicates; also, most populations were polymorphic, which makes classification as lin+ or lin- problematic; this is most obvious for BAC and HZN: though most individuals released linalool, BAC is classified as lin+, HZN as lin-; lin+ and lin- have been removed from Table 1

please discriminate between "true 0" and < 0.005

line 486 – Table 2 - if possible, please replace retention time by kovats retention index;

These are now provided in Table S1 (field data) and Table S2 (greenhouse data), they have retention times and calculated KI for all compounds and the range of published KI for as many as possible. The absolute retention times were not replaced because those are data, not a derived calculation.

Line 498 – Table 3 - I guess that these are green house data?

If yes, I suggest to indicate the compounds that were found both in the wild and the green house, or only once

Yes, these are greenhouse data. We have clarified the Table legend and data from field- and greenhouse-grown plants are now color coded. We have also indicated which compounds were novel to the greenhouse samples and which were missing (in comparison to field collected samples).