A comparison of pollination and herbivory between two chemotypes of *Oenothera harringtonii*

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Introduction

Floral scent is thought to play a role in facilitating plant-animal interactions across a wide range of plant taxa, mediating interactions with both mutualist and antagonist behavior (Knudsen et al. 2006, Cunningham et al. 2004; War et al. 2012). Evidence suggests that linalool plays a role in the attraction of pollinators in many plant taxa, but its role in influencing herbivore behavior is less understood (Raguso and Pichersky 1999). *Oenothera harringtonii*, provides an interesting system to study the effects of floral scent on plant-animal interactions. Hawkmoths serve both as pollinators (Fig. 1a and 1b) and as herbivores (Figs. 2, 3 & 8) and fruits are colonized by microlepidopteran moths (*Mompha*) that feed on seeds. Although its range is restricted in size and hawkmoth-mediated gene flow between populations is known, this species shows great variation in floral scent between populations (Skogen, unpublished). Populations in the southeast-portion of the range have been shown to produce flowers lacking S-(+)-linalool (DC) (n = 46), while populations further north produce the compound (FLO) (n = 34). It has also been observed that the presence of *Mompha* are more prevalent in populations that produce linalool than in populations that do not, while eggs and larvae of other moths (i.e. *Hyles lineata*) are more common in populations lacking linalool. As pollinators, however, hawkmoths tend to be attracted to linalool, which has been found to serve as a feeding cue in many plant taxa. By growing both chemotypes in a common garden, we can test for differences in pollination and herbivory between co-occuring chemotypes. Due to the spatial and temporal phenology differences of pollinators and herbivores, this is something that cannot be well observed in natural populations.

We hypothesize that S-(+)-linalool will positively influence pollinator visitation to flowers, and that plants producing it will therefore be pollinated more often, resulting in higher fitness, than linalool-deficient plants. We further hypothesize that linalool mediates interactions with herbivores, and that plants that do not produce the compound are under selection to reduce herbivory and should have lower levels of herbivory and fitness than plants that produce linalool. We tested these hypotheses in a common garden setting near Flagstaff, AZ.

Fig. 1b: *Hyles lineata* visiting *Oenothera harringtonii*
Photo credit: K. Skogen

Fig. 1b: *Manduca quinquemaculata* visiting *Oenothera harringtonii*
Photo credit: K. Skogen

Fig. 2: A *Hyles lineata* egg on *Oenothera harringtonii*.
Photo Credit: K Skogen

Fig. 3: *Hyles lineata* eating a bud of *Oenothera harringtonii*.
Photo Credit: K Skogen

Results

1) There is no significant difference in general insect herbivory or oviposition in either chemotype of *O. harringtonii* ($p > 0.05$).
2) There is no significant difference in *Mompha* sp. infection of either buds or fruits in either chemotype of *O. harringtonii* ($p > 0.05$).
3) There is no significant difference in the pollination success of either co-occuring chemotype of *O. harringtonii* ($p > 0.05$).
4) There is a significant difference in the estimated fitness of *O. harringtonii* chemotypes, with S-(+)-linalool producing plants having higher fitness on average ($p = 0.033$).

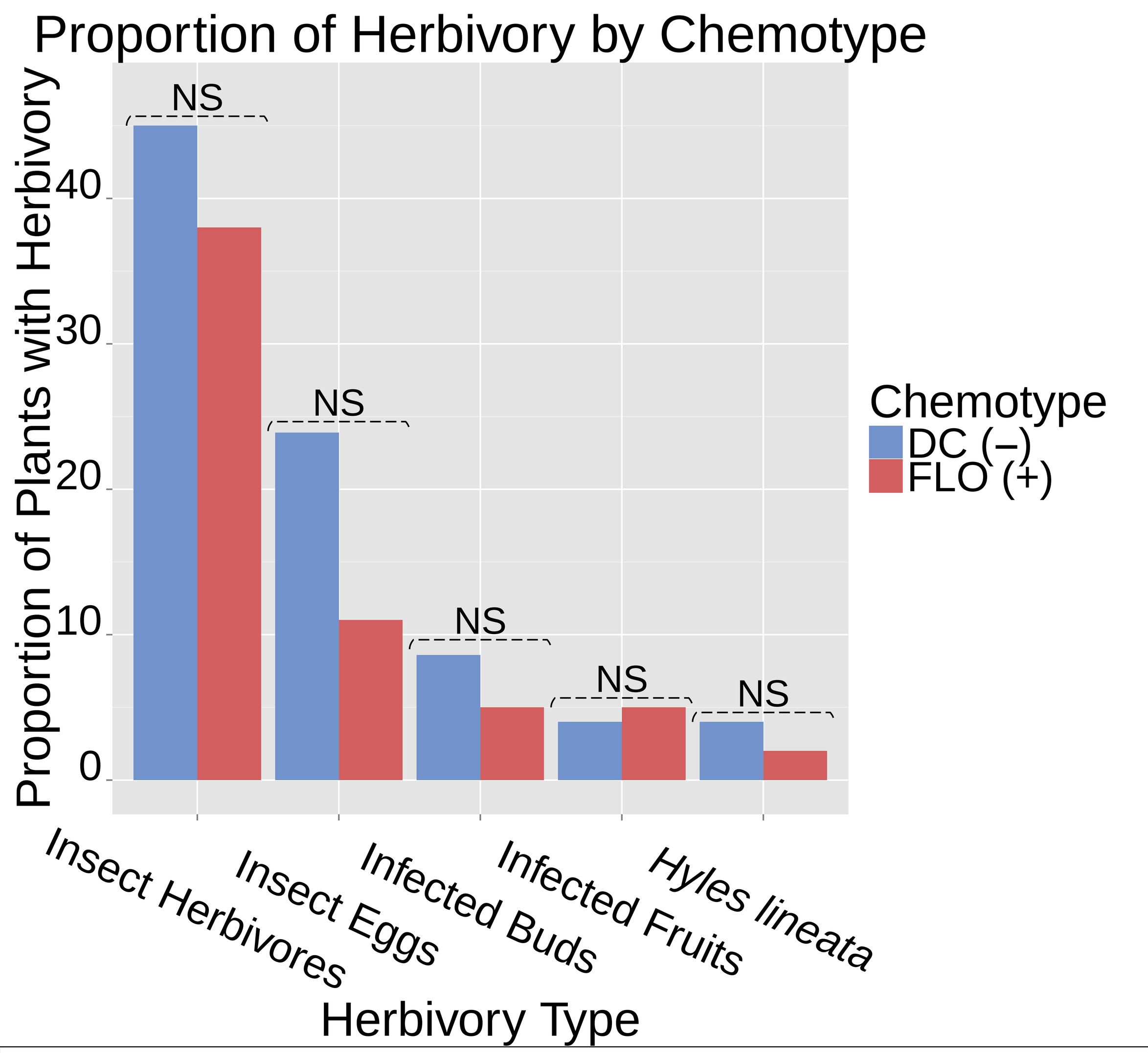

Fig. 4: A comparison of five categories of herbivory between two chemotypes of *O. harringtonii* in our common garden.
Fisher's Exact Test— $X^2 = 0.44, 0.107, 0.223, 0.097, 1.894$; $p = 0.507, 0.744, 0.637, 0.755, 0.169$

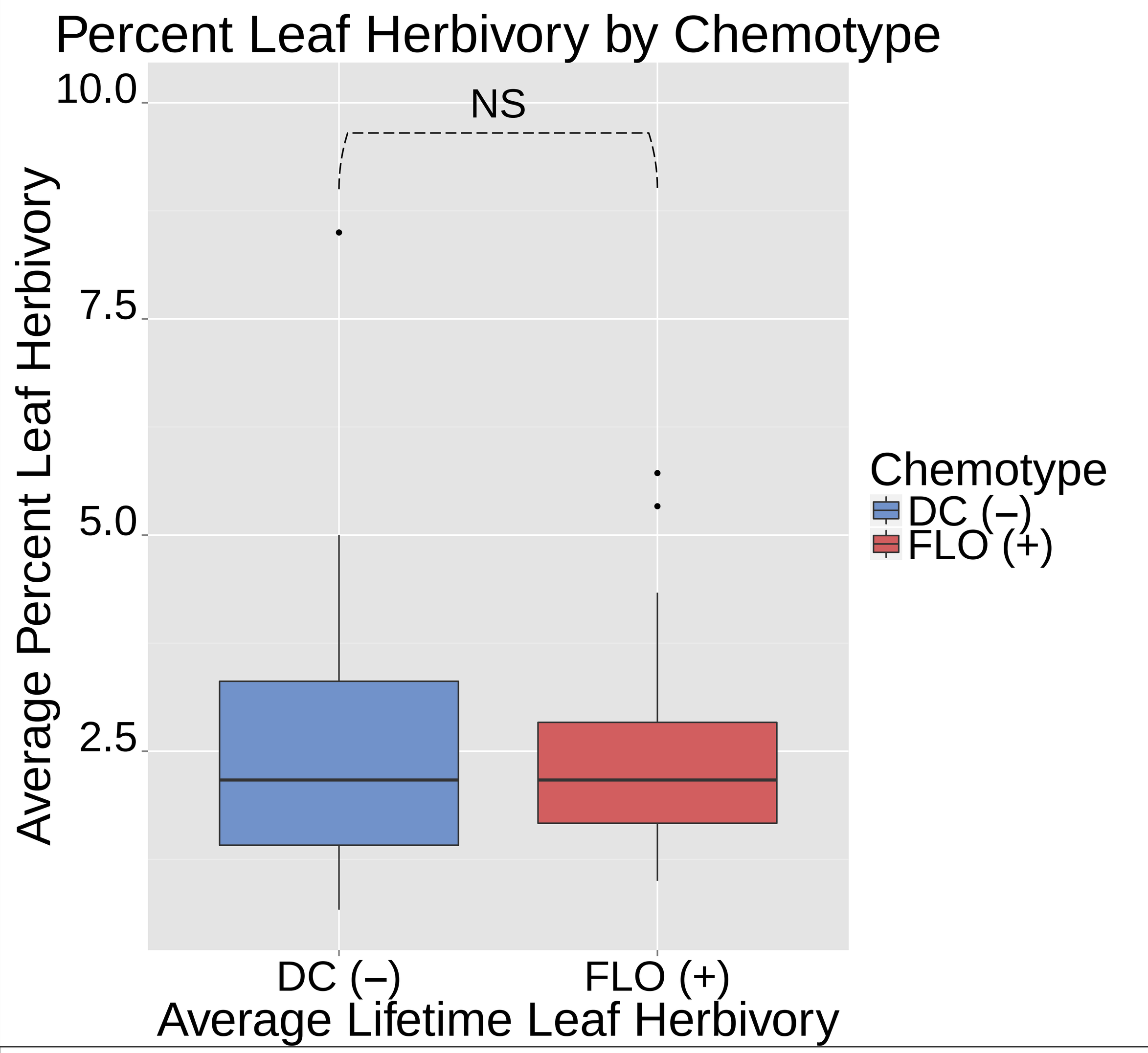

Fig. 5: A comparison of the average percent of total leaf herbivory present between two chemotypes of *O. harringtonii*.
Kruskal-Wallis Rank Sum Test— $X^2 = 0.897$; $df = 1$; $p = 0.334$

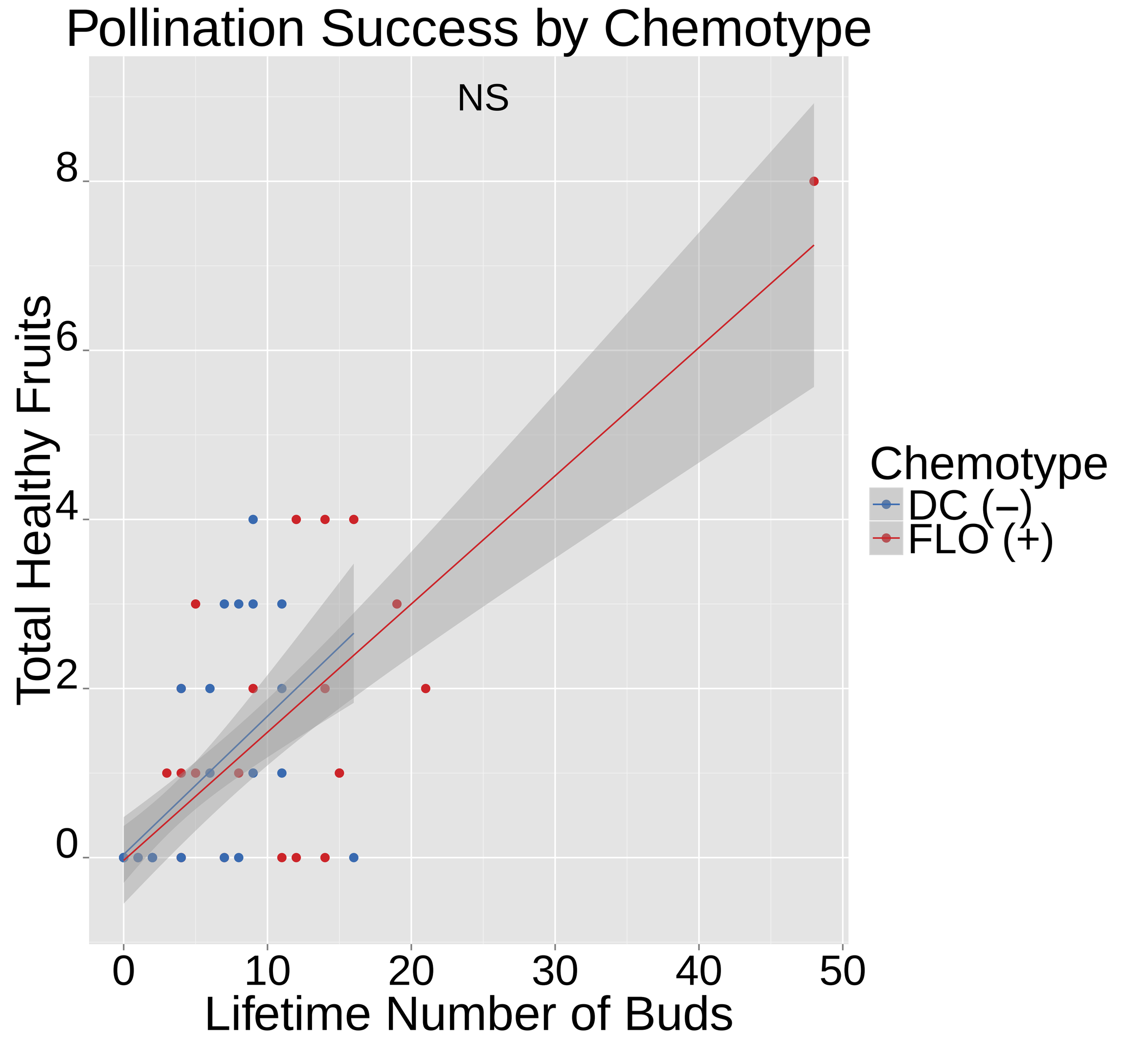

Fig. 6: A comparison of pollination success (as a ratio of healthy fruit to bud number) between two chemotypes of *O. harringtonii*.
ANCOVA— $F = 0.497$, $df = 1$, $p = 0.4634$

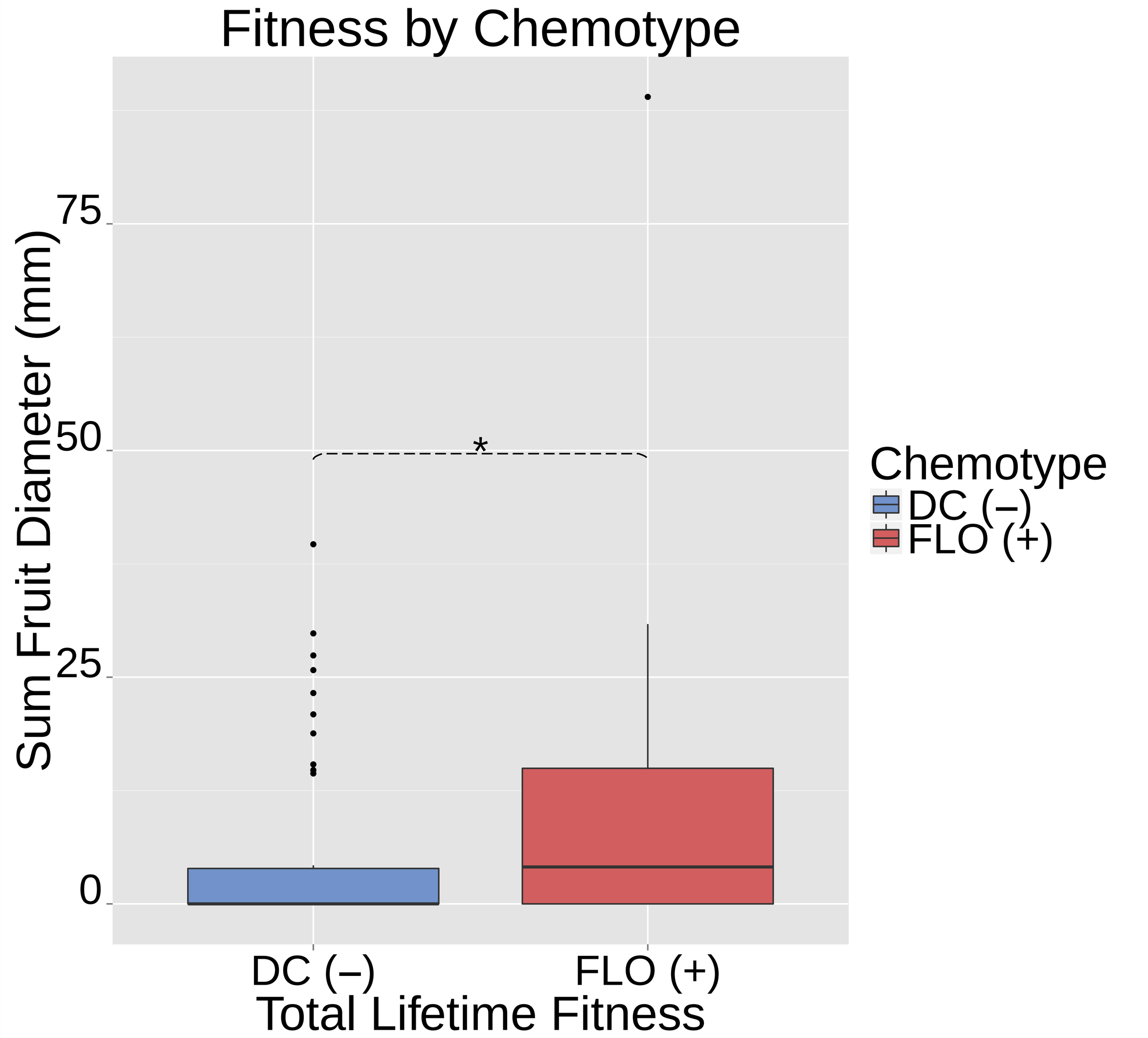

Fig. 7: A comparison of fitness (measured as the sum of fruit diameter per plant) between two chemotypes of *O. harringtonii*.
Kruskal-Wallis Rank Sum Test— $X^2 = 4.495$; $df = 1$; $p = 0.034$

Materials and Methods

Chemical profiles of *Oenothera harringtonii* were collected at all natural populations in Colorado using headspace methods (Raguso and Pellmyr 1998), and the chemical profiles of each chemotype were determined using GC-MS (Skogen, unpublished). A common garden containing both chemotypes of our study species was planted in conifer-enclosed glade in the Southwest Experimental Garden Array at the Merriam Powell Research Station in Flagstaff, Arizona, owned by Northern Arizona University. Visitation rates of dominant floral pollinators were determined by conducting one-hour pollinator observations in the evenings and thirty-minute observations in the mornings, documenting visitor species, as well as their movement between plants being observed. Herbivory was assessed via a weekly monitoring for the presence of herbivore eggs, larvae, and herbivore-induced tissue damage on all specimens of each chemotype within the common garden. Fitness was calculated by measuring the diameter of all fruits at their widest point for plants of each chemotype, and summing the results. All data were analyzed and graphs were created in R.

Discussion

No significant differences were found in herbivory or pollination success in either chemotype of *Oenothera harringtonii* (Fig. 4-6). However, we did find a trend toward less herbivory in plants that produce S-(+)-linalool. In addition, fitness was significantly higher in linalool-producing plants ($p < 0.05$) (Fig. 7). This suggests that when they co-occur, selection may favor plants that produce S-(+)-linalool over those that do not produce the compound. Were the selection to be driven primarily by mutualists, we would expect to see linalool-producing plants having higher levels of pollinator visitation and fitness and similar levels of herbivory compared to plants that do not produce linalool. Although pollinators and herbivores were not abundant, *O. harringtonii* is self incompatible, and therefore all fruit production occurred via insect-mediated cross pollination between plants. In the natural range of this species, co-occurrence of the two chemotypes may occur. If such is the case, we may see similar pollinator and herbivore behavior within this population, which may result in higher fitness in one of the two chemotypes– linalool (+) or linalool (-).

Fig. 8: *Oenothera harringtonii* with evidence of *Hyles lineata* damage
Photo credit: K. Skogen

Fig. 9: Hemiptera eggs on an *Oenothera harringtonii* leaf
Photo credit: A. Rork

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Acknowledgements

I would like to thank Drs. Jeremy Fant and Rick Overson for logistical support while on this REU, as well as Evan Levy for assistance with data collection. I would also like to thank Amy Whipple for allowing us to stay at MPRS and conduct research on their grounds. I also thank Dr. Kyra N. Krakos for allowing me to be a member of her lab and for allowing me to conduct research for the Chicago Botanic Garden this summer. I also thank the NSF (grant number DBI-1461007) for funding this REU.