Is flower position within the inflorescence related to pollination success in *Physaria globosa*?

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Introduction

Physaria globosa (Desv.) O'Kane & Al-Shehbaz (Short's bladderpod) is a federally endangered species limited to 33 populations in Tennessee, Kentucky, and Indiana. The species is prone to grow in limestone beds and can be found along railroad tracks, steep slopes next to highways and tucked away next to gravel roads. This species is primarily found on steep limestone bluffs, although anthropogenic changes and habitat fragmentation have altered several of the critical habitats *P. globosa* occupies. Threats such as geographical isolation, non-native invasive species, poorly timed herbicide spraying, rights-of-way maintenance, construction, erosion, and overstory shading negatively impact the long-term survival of this species. Despite its current threat of endangerment, *P. globosa* has a "high recovery potential" (USFWS) though it faces natural limitations.

The extent to which *P. globosa* relies on pollinators, how floral position impacts fecundity, and which factors limit reproductive success is largely unknown. *Physaria globosa* must be pollinated via outcrossing as it is not self-compatible. This species typically flowers from late February through June. Each plant may possess numerous indeterminate racemes consisting of primary and secondary axes with hundreds of flowers per inflorescence. Each plant may possess between one and 40 compound racemes, each with anywhere from 10 to 96 flowers per raceme. Flowering in a single raceme proceeds over many weeks, with the youngest flowers produced at the top of each branch.

Previous studies on other species have shown that flowers produced earlier in the season or at specific positions within the inflorescence may experience different probabilities of successful pollination (Diggle 1995, Ehrlén 1992). Resource availability may negatively affect younger flowers when resources are sent to older flowers that are developing fruit. These studies suggest that the plant's total resource availability could limit seed development. Alternatively, timing of flowering could also affect the likelihood of successful pollination.

Since *P. globosa* is known to exhibit varying degrees of branching within numerous flowering stems, an investigation into how resource availability impacts successful fertilization is essential to the long-term success of this species. This study aims to examine rates of successful pollination in *P. globosa* to better understand the potential impacts of flower position in a compound raceme. To facilitate the conservation of *P. globosa*, findings from these data will contribute to a better understanding of the reproductive dynamics in this species. By calculating pollen tube presence in the ovaries as it relates to inflorescence position, insights into resource production and allocation can guide future management decisions. The hypothesis being evaluated states that flowers along the primary branch (Fig. 1, "P"; Fig. 2) are more likely to be successfully pollinated in comparison to secondary branches.

Methods

Inflorescences were collected from five populations: two in Tennessee, two in Kentucky, and one in Indiana. For the work presented here, five plants each from KY and TN and four plants from Indiana were used. One flowering stem from each plant was collected and stored in FAA, transferred to ethanol, and then placed in softening solution and heated at 60 ºC for 2 hours. Pistils were numbered based on their location on the primary raceme and their position along each branch (Fig. 1). Given that *P. globosa* exhibits sporophytic self-incompatibility, the presence of pollen tubes in the ovary was scored as a measure of successful (out-crossed) pollination. Softened pistils were removed and examined under fluorescence using aniline blue stain (Fig. 3). Pistils were sampled every 10 flowers along the primary axis starting with the oldest flower (P-1) and moving upward (P-11, P-21, P-31, P-41). On secondary branches, only the oldest pistils were examined for pollen tube presence. A total of 98 pistils were analyzed across 14 plants. To analyze the significance of flower position as it relates to reproduction, a chi-squared test was performed to compare primary vs. secondary branches with respect to their percentage of successful pollination (visualized as pollen tube presence in ovary). The null hypothesis for the Chi-square test is that there is no difference between the primary and secondary axes in terms of the likelihood for successful pollination.

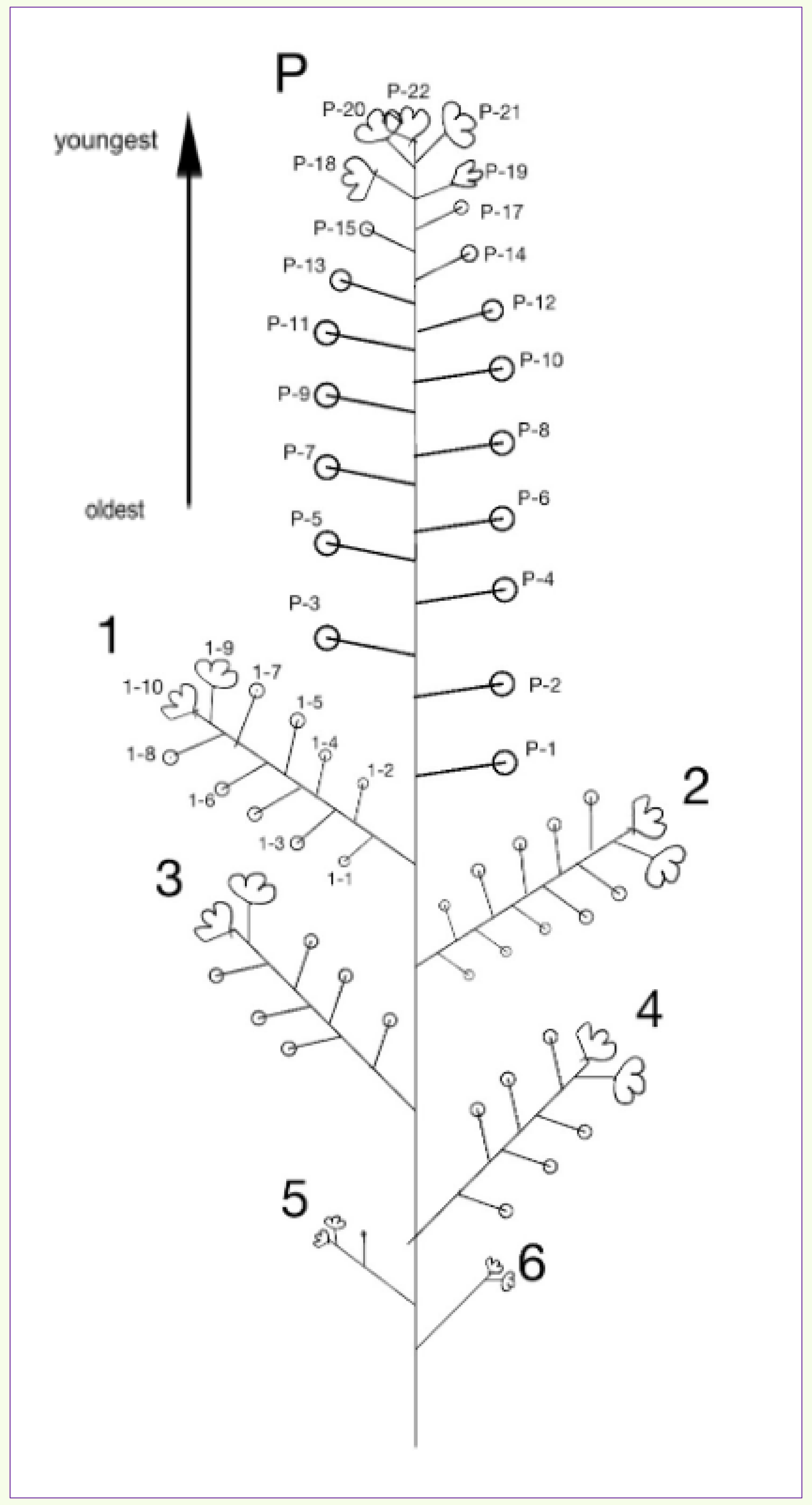

Figure 1: Illustration of a typical inflorescence in *P. globosa* with primary (P) and secondary axes (1-6) labeled.

Figure 2: *Physaria globosa* growing in the Native Plant Garden at Tennessee Tech.

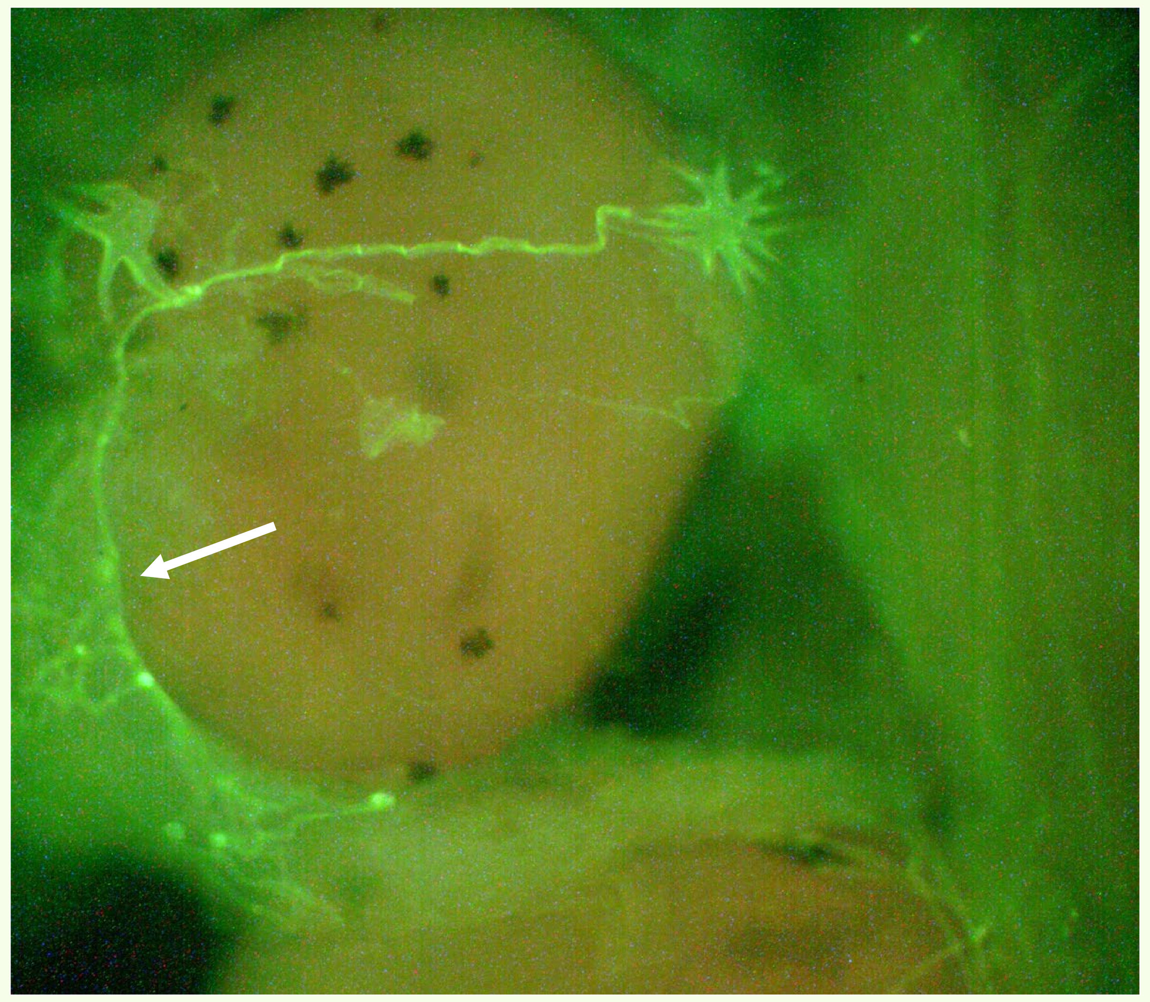

Figure 3: An example of a pollen tube near an ovule (sample 1478 from Indiana). Arrow indicates pollen tube running along the side of an ovule (orange).

Results

In total, 14 flowering stems were examined. The average number of pistils examined for each flower position was 10.88 (min 8, max 13; Table 1). For Kentucky samples examined, 29 out of 34 total pistils had pollen tubes (85%). In Tennessee, 17 of 37 (46%) had pollen tubes, and for Indiana 23 out of 27 (85%) had pollen tubes. Across all three states, 77% of pistils were successfully pollinated on the primary stem, while 61.9% were successfully pollinated on secondary stems. However, this difference is not statistically significant ($p = 0.2001$).

Flower position	Number pistils with pollen tubes	Total number of pistils
P-1	6	12
p-11	9	13
p-21	12	12
p-31	10	11
p-41	6	8
4-1	5	9
3-1	5	9
2-1	10	12
1-1	6	12

Table 1: Comparison between flowers on primary stems (p1-p-41) vs. secondary stems (4-1 through 1-1), including samples from all three states.

Discussion

The Chi-square results indicate that there is not a significant difference between the pollination success of primary and secondary branches. However, pollination success is slightly higher on flowers along the primary branch. With a larger sample size, it is possible this trend may become significant. Earlier in the season, the plant's resources may be more available to the first flowers to appear, thus optimizing conditions for fertilization and seed production. Alternatively, pollinators might be more available or more attracted to early flowers versus those appearing later along the flowering stem. Other studies have suggested that successful pollination is affected by flower position in the inflorescence (Diggle 1995, Yuan 2016), though this is not supported by the preliminary data shown here. Pollinator attraction requires just as many resources by the plant as does seed production (Ehrlén 1992, Horvitz 2010), which could serve as an explanation as to why the secondary branches are less likely to be pollinated. To expand on this study, the sample size will be increased and will include inflorescences collected from different stages during the full flowering season. This information can be used to inform conservation strategies for *P. globosa*.

Acknowledgements

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