Pollinator composition, visitation rates, and seed set in flower-color polymorphic populations of a cedar glade endemic, Nashville Breadroot (*Pediomelum subacaule*).

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ABSTRACT

Nashville Breadroot *(Pediomelum subacaule*) is a flowering plant endemic to Cedar Glades of Northern Alabama, Northern Georgia, and Middle Tennessee. In middle Tennessee, populations of *P. subacaule* are characterized by a flower-color polymorphism in which individual plants produce exclusively purple or, very rarely, white flowers. The goal of this study was to test the hypothesis that the white flower variety suffers reduced fitness and is rare because of pollinator mediated selection. A series of field observation experiments was conducted to quantify pollinator composition, duration of pollinator visitation, and seed set in middle Tennessee populations of *P. subacaule*. My results suggest that flower color has a significant effect on pollinator preference, with different proportions of pollinator species visiting purple and white flowers (X^2=26.9, df=3, $p < 0.05$). Statistical analysis also reveals that seed production by white-flowered plants is less than that of the purple flower variety ($U = 12$, $p < 0.05$). A statistical analysis was not conducted on times pollinators spent on white vs purple flowers, as visitation to the white flower variety was limited to a single individual of the focal pollinator community. Taken together, these results are consistent with the hypothesis that white flowered individuals are less attractive to key pollinator species, and therefore produce fewer seeds. Hence, the white-flowered morph may suffer a reduction in fitness, which may account for its persistent low frequency of occurrence in the populations studied.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I: INTRODUCTION

Flower-color polymorphisms are characterized by variation in flower color within or between populations of the same species. Middle Tennessee populations of *Pediomelum subacaule* exhibit a polymorphism in which individuals with white flowers appear rarely among larger stands of plants with purple flowers. Understanding the evolutionary and ecological factors that maintain such polymorphisms in nature is an important question, especially when the traits of interest are strongly correlated with fitness. Numerous studies have shown that flower color functions as a signal to attract pollinators (Jones et al 2001), and pollinators are known to discriminate between flowers of different colors (Jones et al, 2001). It has been demonstrated, for example, that bees tend to prefer blue or purple flowers, whereas flies prefer white or yellow flowers (Thairu and Brunet 2015). Such preferences may result from pollinators learning and keying in on visual and chemical properties of flowers that provide high-quality rewards in the form of nectar and/or pollen. Moreover, the distribution of phenotypic variations within populations of flowers may be influenced by pollinator behavioral interactions, resulting in evolutionary divergence in color (Coetzee 2021). When flower color is heritable, strong pollinator preference for one color morph over others may result in directional selection and subsequently, divergent evolution. It seems reasonable to hypothesize that the flower color polymorphism observed in *P. subacaule* is maintained by pollinator mediated selection. Specifically, the rarity of the white color morph may result from a reduction in fitness if, for any reason, it is less attractive to pollinators. Hence, the purpose of this study was to test for differences in pollinator preference, pollinator visitation duration, and seed production in the two flower-color morphs of in *P. subacaule.*

(1.1) HABITAT AND DISTRIBUTION

The cedar glades of middle Tennessee are characterized by large areas of exposed limestone rock surrounded by shallow soils, amid which occasional pockets of deeper soils support stands of Eastern Red Cedar (*Juniperus virginiana*). This unique and endangered habitat is further characterized by extreme seasonal variation in temperature and rainfall (Baskin and Quarterman 2007), witch plants living having evolved to thrive under these harsh conditions. A total of nineteen species of endemic flora inhabit the Glades of the central, eastern United States, each exhibiting distinctive adaptations to the shallow-soiled terrain (Baskin and Quarterman 1970).

Pediomelum subacaule is common on the glades of Middle Tennessee, with a few populations known to inhabit areas in Georgia and Alabama (Baskin and Quarterman 1970). Middle Tennessee populations occur in large patches on open glades or as lone flowers dotting the perimeter. These populations are mainly composed of plants with purple flowers, but small numbers of plants with white flowers are occasionally found growing in their midst.

(1.2) LIFE HISTORY OF P. SUBACAULE

Pediomelum subacaule, commonly known as the Nashville Breadroot or White Scurfpea, is one of many tough, herbaceous perennials adapted to survive in the cracks of limestone on cedar glades. This glade endemic belongs to an aculescent subgenus of the Psoralea family (Torrey and Gray, 1838; Baskin 1970). Germination of seeds of *P. subacaule* is epigeal, such that developing cotyledons function as photosynthetic energy sources persisting until the first foliage appears at around three weeks (Baskin 1970). The primary tap root forms about four to six weeks after germination, and the seedlings, which become dormant in late June, sport a small bud on the root (Baskin 1970). The summer historically brings hot-dry conditions to the glades, and the shoots of mature *P. subacaule* die back, generating a small bud from which next year's shoots will emerge (Baskin 1990). Shoot development begins late October, and rapid elongation takes place in the months of February and March (Baskin 1970). *P. subacaule* produces floral apices throughout the winter with buds reaching maturity in late January (Baskin 1970). Pollen grain formation begins in early April (Baskin 1970), followed by flowering from late April to early May. (Baskin 1970). *P. subacaule* exhibits indeterminate flowering, and typically produces 10 – 60 flowers per raceme, and 10 – 15 racemes per plant (Baskin and Quarterman, 1970). Bumblebees and carpenter bees are reported to be primary pollinators, and flowers are occasionally visited by various species of flies and beetles (Baskin and

Quarterman 1970). By Seeds are fully developed by late May, at that time detaching from their fallen inflorescences and the pericarp that envelops them (Baskin 1970).

II: MATERIALS AND METHODS

Dense stands of *Pediomelum subacaule* are common on cedar glades in middle Tennessee, but individuals with white flowers are rare and difficult to locate. Plants typically begin to produce flowers in early spring (Baskin and Quarterman 1970), and in March of 2021 I reached out to amateur naturalists, via social media, requesting information on the location of white-flowered plants they may have encountered during their outdoor excursions. The response was positive, and I was able to locate three white-flowered plants using this approach. My own searching resulted in the location of an additional five plants, yielding a total of eight white-flowered individuals that were monitored over the course of this study. The locations of all plants were recorded with a hand-held Garmin GPS unit and flag-markers were placed at the sites.

Previous investigators have identified the most common insect visitors of *P. subacaule* flowers as carpenter bees (*Xylocopa virginica*) and bumblebees (*Bombus pennsylvanicus*) (Baskin and Quarterman 1970). My observations confirm this, but I also noted frequent visitation by hummingbird moths (*Hemaris diffinis*). For the purposes of this study, the three species mentioned above will be considered “primary” visitors and potential pollinators. Various other types of insects have also been reported to visit flowers (Baskin and Quarterman 1970), but no information exists concerning the species composition of this group. Insect visitors other than those in the primary group were therefore tabulated as "other".

Field observations were conducted during the month of April, 2021, during which time a total of eight white and twelve purple-flowered plants was monitored. Each observation session lasted for one hour and was restricted to the monitoring of a single focal plant. Observations were conduction in the late morning hours (9am-11am) or the early evening (36pm), as these seemed to be the peak times of pollinator activity. To facilitate quantification of pollinator composition and visitation duration, a 2' x 2' open frame made from 1/2" PVC pipe was placed on the ground and centered over the focal plant. Over the course of one hour, the identities of primary pollinators and the times they spent on flowers of the focal plant were recorded. Visitation to flowers by non-primary insect visitors was recorded, but visitation duration was not collected for this group.

Baskin (1970) reported fruit ripening to occur in mid-May, and it was at this time that I collected inflorescences from the plants used in this study. I was unable to collect inflorescences from plants that were severely damaged or destroyed by herbivory or human activity. Inflorescences from surviving plants were placed in plastic bags and taken to the laboratory for analysis. Here, the number of flowers with and without fruits, and the proportion of fruits containing viable seeds was determined for each plant. A fruit was considered to contain a viable seed if a clearly defined embryo was present, and seed set was calculated as proportion of viable seeds per flower.

III: RESULTS

(3.1) POLLINATOR COMPOSITION

Over the course of the study, individuals of each of the three primary pollinator species was observed visiting the purple flower morph. Eighteen bumblebees, twenty carpenter bees, and fifteen hummingbird moths were observed on purple flowers (table 1a). In contrast, white flowered plants were never visited by bumblebees or hummingbird moths, and there was only a single occurrence of visitation by a carpenter bee (table 1a). Insect classified as "other" in this study were observed visiting both purple and white flowers (Figure 1). A contingency table analysis revealed a statistically significant difference in the proportions of pollinators visiting the two flower varieties (Table 1b).

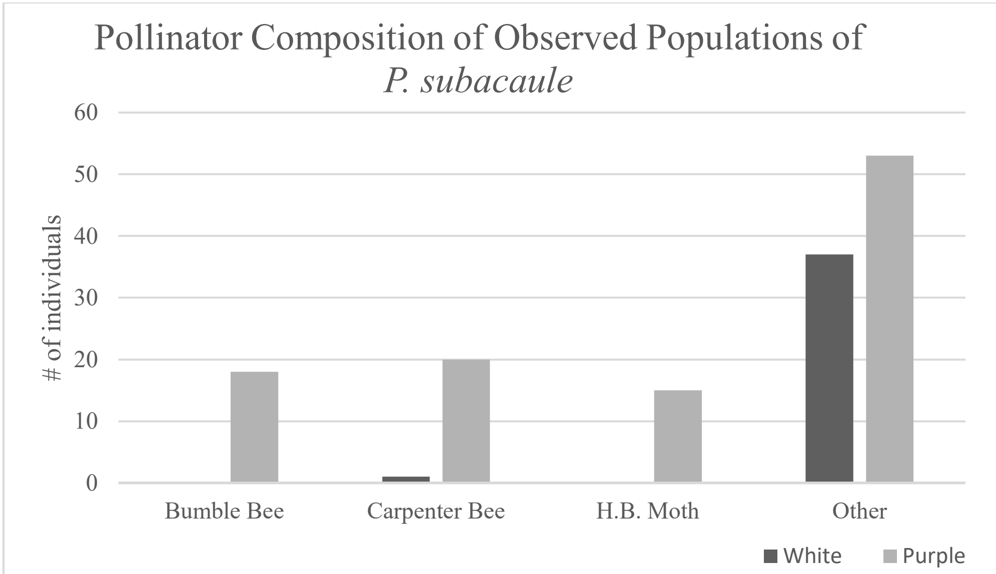

Figure 1: OBSERVED POLLINATOR COMPOSITION. A visual comparison of pollinator composition for individuals of *P. subacaule* displaying each color variation; pollinator species include bumble bees, carpenter bees, hummingbird moths, and other (such as flies, butterflies, etc.).

Table 1a: POLLINATOR COMPOSITION. Pollinator species type and quantities recorded during each observational period. Other category describes non-primary pollinators, such as flies, butterflies, etc.

TOTAL POLLINATOR COMPOSITION

	Bumble Bee	Carpenter Bee	H.B. Moth	Other	Total
White	0	1	0	37	38
Purple	18	20	15	53	106
Total	18	21	15	90	144

Table 1b: CONTINGENCY TABLE ANALYSIS. Chi-squared Analysis data for Pollinator Composition (X^2 =26.9287558 (df=3; N=144), $p<0.05$).

CHI-SQUARED ANALYSIS FOR POLLINATOR COMPOSITION

X^2	26.9287558
df	3
p-value	6.0933E-06
alpha	0.05

(3.2) POLLINATOR VISITATION DURATION

Purple flowered plants were visited by eighteen bumblebees with an average visitation duration of 29.8 seconds. Fifteen hummingbird moths spent an average of 14.4 seconds on the flowers, and twenty carpenter bees had an average visitation time of 37.9 seconds. In contrast, visitation to white flowered plants was limited to that of a single carpenter bee which spent 344 seconds on the flowers (Figure 2). Visitation time of "other" insects was not monitored.

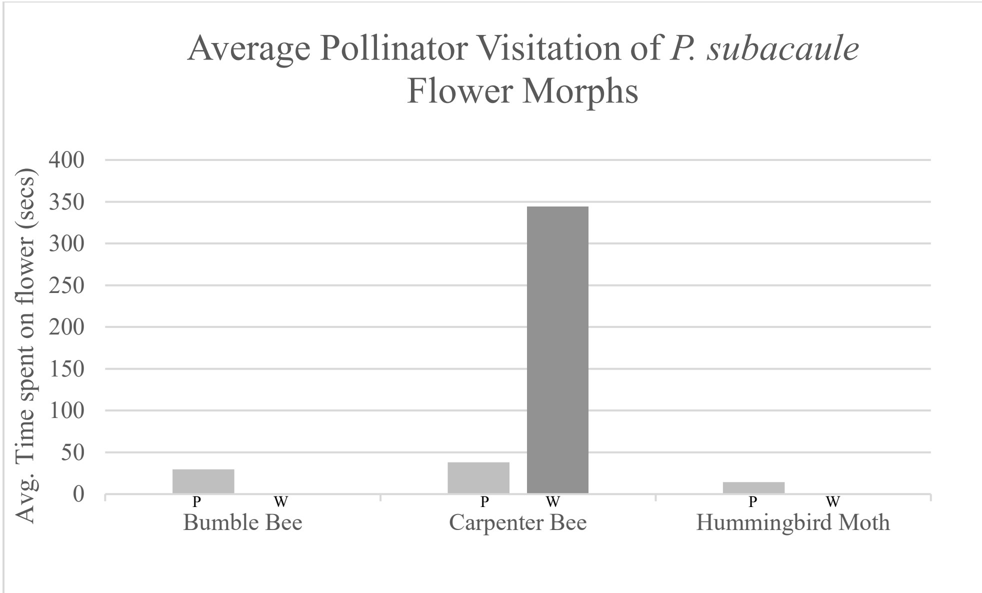

Figure 2: AVERAGE POLLINATOR VISITATION DURATION: A comparison of the duration of pollinator visitation rates (seconds) for each color polymorph of *P. subacaule*; includes Bumble Bee, Carpenter Bee, and Hummingbird moth species. It is worth noting that the White morph Carpenter Bee Visitation represents a single individual pollinator. The pollinators classified as other were not included when recording rates of visitation.

(3.3) SEED SET

Statistical analysis reveals that purple flowered plants produce more viable seeds per flower (0.27) than do white flowered plants (0.07) (table 2; Figure 3). A Mann-Whitney U test was also used to examine the effect of color variation on seed production ($U=12$; $p<0.05$).

Table 2: SUMMARY TABLE OF TOTAL SEED AND FLOWER COLLECTION; Average Flowers, Fruit Collected, Seedless flowers per inflorescence, and the proportion of seeds collected (or lack thereof) to flowers collected.

SEED COUNT SUMMARY TABLE

	Purple	**White**
Avg. Flowers	19.00	16.294
Avg. Seeds Present	5.22	1.529
Avg. Seedless	13.78	14.764
Seedless Fruits Per Flower	0.725	0.906
Viable Seeds Present Per Flower	0.275	0.0939

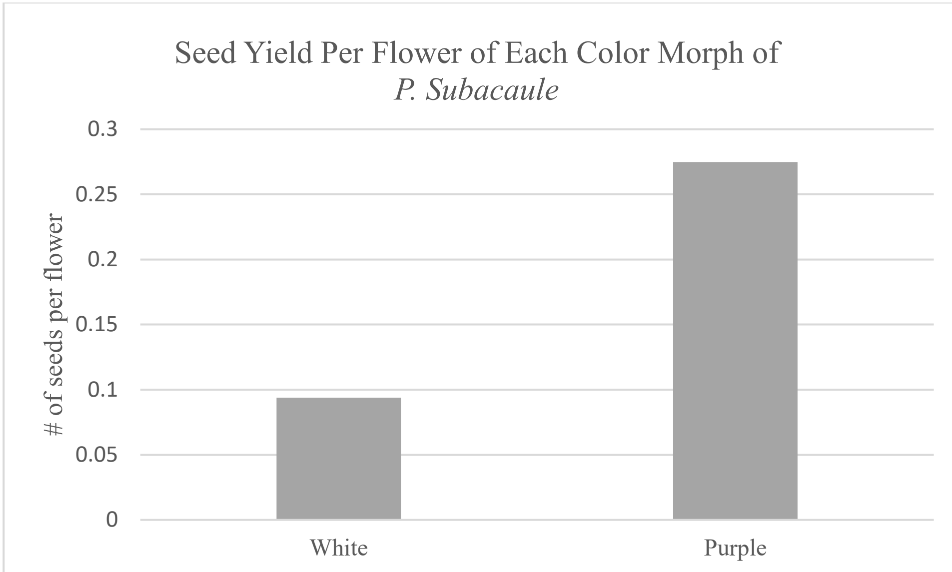

Figure 3: SEED SET OF *P. SUBACAULE*. Viable seed yield per flower for white and purple color morphs.

IV: DISCUSSION

My results are consistent with the hypothesis that white flowered *P. subaculae* suffer reduced fitness and remain rare due to pollinator mediated selection. Indeed, with a single exception, insects known to be key pollinators of this plant never visited an individual with white flowers. A pollinator not identified by previous investigators, the hummingbird moth, was a frequent visitor to purple flowered plants over the course of my study. It is possible that changing environmental conditions might have led to a shift in this species away from other plants, but I could find no information on its role as a pollinator in cedar glade communities.

The question also remains as to *why* pollinators prefer purple over white flowers. One possibility is that purple flowers provide superior rewards in the form of nectar and/or pollen. Previous studies have shown that pollinators discriminate between flowers when color provides a cue to the quality of the reward (Coetzee, A et al 2021). Another possibility is that pollinators develop a search image for the most common flower color, bypassing flowers of other colors (Bahlai and Landis 2016). More research must be done, however, before these questions can be answered with respect to *P. subaculae*.

The low number of seeds produced by the white flower-color morph is likely due to this deficiency of pollinators, but a direct connection can be established only through future research on the relationship between specific pollinators and seed set. It is worth noting that despite almost no visitation by key pollinators, white-flowered plants still managed to produce some viable seeds. This may result from successful pollination by other insects, such as flies or beetles, or from self-fertilization.

V: CONCLUSION

Results from this study may explain why the white-flowered variety remains at persistently low frequencies in middle Tennessee populations of *P. subacaule*, but key questions remain unanswered. In particular, studies focusing on the relationship between flower color and reward are needed to explain the strong preference of pollinators for purple over white-colored flowers. This may provide further insight into the evolutionary dynamics and long-term persistence of the flower-color polymorphisms observed in this plant.

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