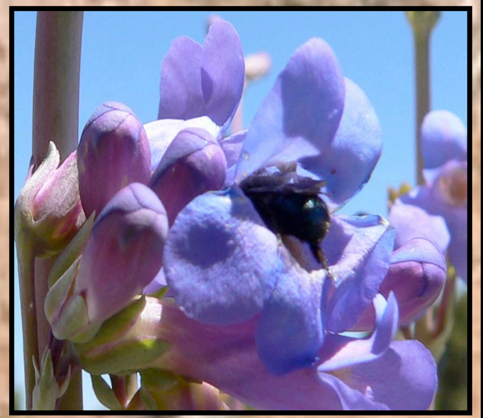

Intraspecific Variation in Penstemon Floral Characters

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INTRODUCTION

Penstemon is one of the largest plant genera endemic to the United States; it is comprised of over 270 species, with the vast majority located in the western portion of the country. This genus includes a variety of floral forms, and such diversity is thought to be the result of evolutionary specialization for different pollinator groups.[3] These floral forms can be assorted into pollinator syndromes or "a suite of traits associated with the attraction and utilization of a specific group of animal as pollinators."[1] The focal species of the current study, *Penstemon pachyphyllus* exemplifies the classic "bumblebee syndrome" "in being blue-violet in color, having a vestibular corolla, a lower lip in the position of a landing platform, and [relatively] included anthers."[4] Moreover, this particular species is predominately found in the area stretching from western Utah to eastern Nevada on the isolated mountain ranges characteristic of the Great Basin Region.

Consequently, the aim of this study is to examine color and morphological variation between discrete populations of *P. pachyphyllus*. Such intraspecific variation is of interest because both floral color and morphology largely influence the interactions between flower and pollinator, thus affecting the efficiency of pollen transfer and thereby influencing relative fitness. If variation in pollinator communities exists on separate mountain ranges, we expect a difference in flower color and shape between the *P. pachyphyllus* populations to be, at least in part, a reflection of the pollinator variation on associated ranges. Moreover, we expect to find the greatest degree of color and shape variation present in the floral regions most susceptible to the selective pressures of local pollinators, i.e. the petals and mouth of the flower.

MATERIALS AND METHODS

A. Color Quantification

- Three flowers were removed from wild individuals in Nevada's Snake Mountain Range (1) and along the East Shell Creek (2) as well as from individuals grown in the common garden, originating from populations in the previous two sites and from populations at high and low elevations in Zion National Park (3 & 4) (Fig. 1).
- Flowers were bilaterally dissected, separating upper and lower portions of the flower.
- Scanned red, blue, and green paint chips were used as color controls to account for possible light variation or discrepancies by scan.
- Saved scans were used to measure the color of the flowers' inner and outer face of the petals, top and bottom region of the mouth, and the corolla. Measurements were made using RGB color mode and the histogram instrument in Adobe Photoshop.
- The red, green, blue, and luminosity values were analyzed using the statistical program, JMP.
- Because blue is the dominant flower color, the statistical emphasis was placed upon the "Blue/Red" and "Blue/Green" values. Such ratios revealed the intensity of blue in regards to resultant reds and greens and because the red, green, and blue values collectively determine the luminosity, the ratios also assessed the blue value in the context of the flower's relative darkness/lightness.

B. Floral Morphology

- Three flowers were removed from different positions along inflorescence of at least 15 individuals per population.
- The flowers were gathered from population (1) occuring in the wild and from individuals of populations (3) and (4) grown in the common gardens and were preserved in labeled vials containing 70% ethanol solution.
- Fourteen floral dimensions were later measured using an electronic digital caliper: corolla length, anterior, central and posterior corolla width, the anther length, mouth diameter, upper lip length and width, lower lip length, entire width, and central segment width, staminode length, anther exertion, and bristle length.
- The data was recorded and again analyzed using JMP statistical software.

RESULTS

A. Color Quantification

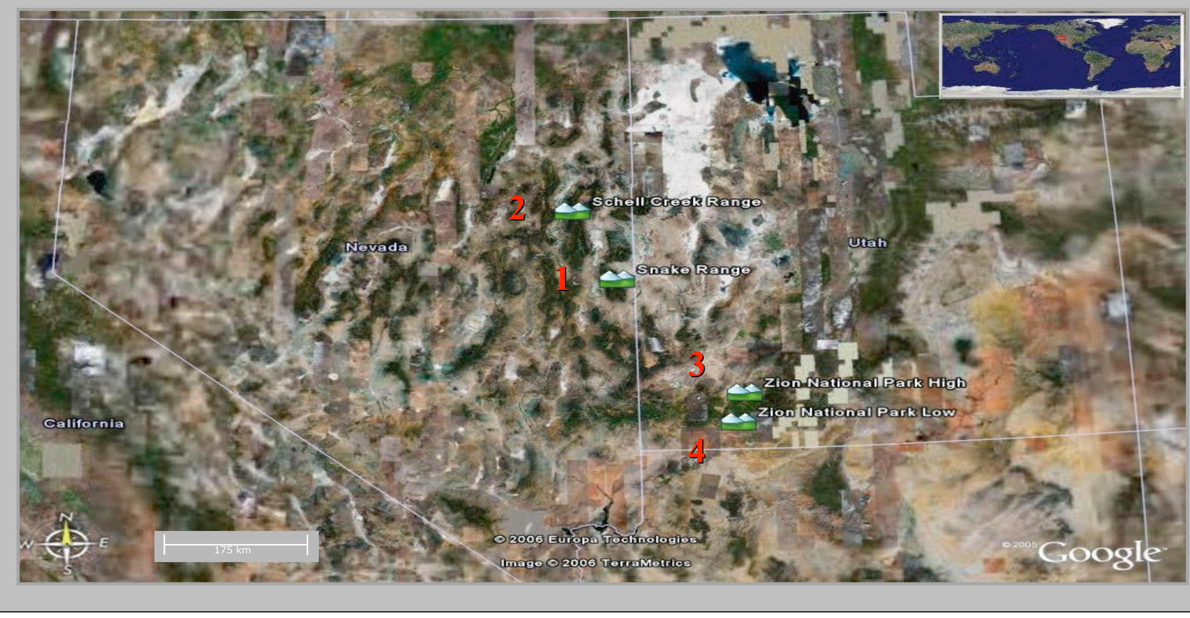
Significant variation was found between the four studied populations: East Schell Creek, Snake Range, and Zion National Park at high and low elevations. Moreover, the results show that largest degree of variation exists in the floral regions most susceptible to the selective pressures of local pollinators. As expected, of the six positions analyzed, the areas of the flower that exhibited the most color disparity between the populations were the outer face of the petal and the inside of the petal, followed by the regions of the mouth, and lastly, the corolla (Table 2). The average color of *P. pachyphyllus* flowers ranged from an intense blue, most characteristic of the petals in population "Snake", to various shades of purples, best demonstrated in the petals of the Zion populations and population "Schell", to vibrant magentas characterizing many of the mouth regions, and finally rosy pink colors typifying the corollas in all four populations (Table 1).

B. Floral Morphology

Significant variation in flower size and shape was determined from the morphological measurements. Moreover, the parts of the flower that exhibited the greatest variation between populations were the corolla length, upper lip length and width, the entire lower lip width, the width of the central segment, and the anther exsertion. As expected and illustrated in the PCA (Fig. 3), the 2006 morphology of population "Snake" is especially distinct from the high and low Zion populations. (cont'd)

CHARTS & TABLES

Fig 1: Site Map



A. Color Quantification

Table 1: Average Color of Flower Positions

	Petal Outside	Petal Inside	Mouth Top	Mouth Bottom	Corolla
Snake					
Schell					
Zion High					
Zion Low					

Table 2: Significant Differences in Coloration

	Outer Petal		Inner Petal		Upper Mouth		Lower Mouth		Corolla	
	B/R	B/G	B/R	B/G	B/R	B/G	B/R	B/G	B/R	B/G
Snake	A	A	A	A	A	A	A	A	A	B C
Schell	B	B	B	B	C	A	C	A B	C	C
Zion High	C	C	C	C	B	B	B	B C	B C	A
Zion Low	D	C	C	C	B C	B	B C	C	A B	A B

B. Morphological Variation

Fig 2: PCA for 2005 Floral Characters

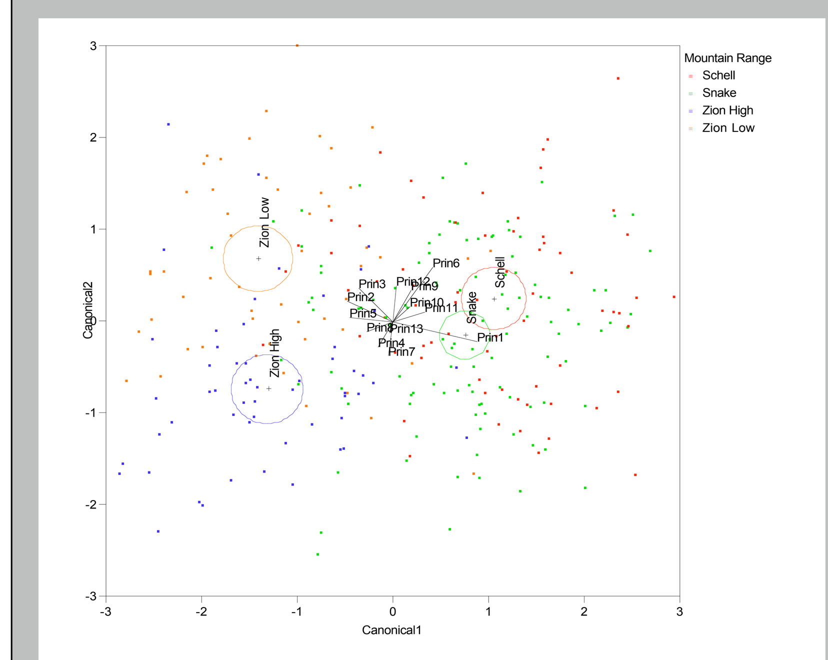
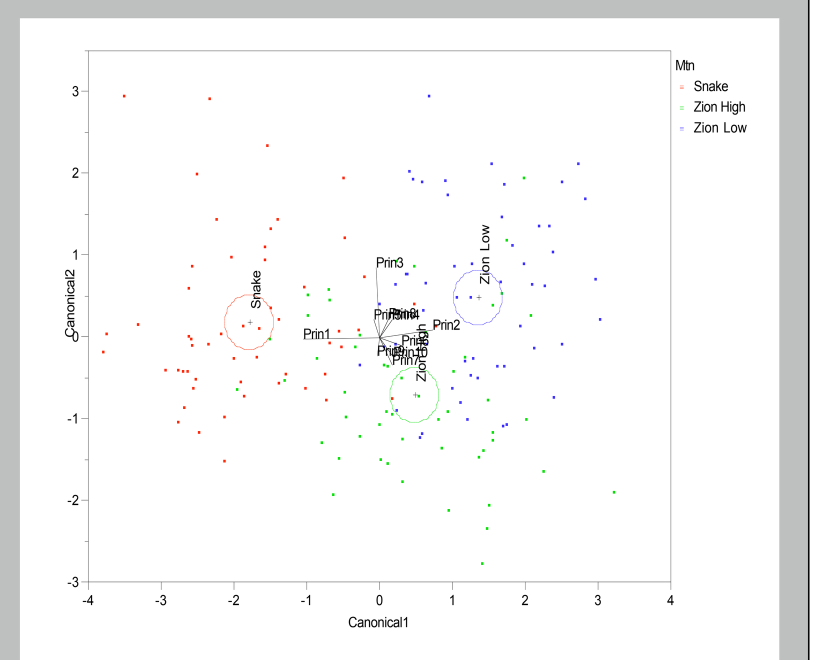


Fig 3: PCA for 2006 Floral Characters



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RESULTS CONT'D

Of the fourteen measurements taken, all but three - the average bristle length and the anterior and posterior corolla widths - were significantly different from the Zion populations. However, although consistent with expectation, our inability to collect from population-specific individuals occuring both naturally in the wild as well as in our common gardens certainly introduced experimental discrepancies. However, a comparison with 2005 morphological data, in which all flowers measured were from individuals exclusively grown in the common gardens, indicated a very similar degree of morphological variation (Fig. 2 & 3). Thus, we are confident that the environmental incongruities did not significantly influence the extent of morphological variation measured between the three populations.

DISCUSSION

While others have analyzed interspesific distinctions in Penstemon floral morphology, the current study is unique in that it is the first to examine intraspecifc variation, as well as, the only known to quantify, as opposed to, qualify floral color differences. The discerned intraspecific color and morphological variation of *P. pachyphyllus* is thought to proceed from adaptations to local pollinator communities, otherwise known as "pollinator shifts". Because the studied *P. pachyphyllus* populations reside in remote areas on isolated mountain ranges, we believe the assemblage of pollinators may vary with site. Consequently, it is thought that the *P. pachyphyllus* populations have responded to local pollinator pressures by "shifting" to make "the flowers more attractive to the abundant visitor which makes the visitor a more effective pollinator."[3]

Certainly such intraspecific shifts are subtle and thus, the prominent floral characters associated with all *P. pachphyllus* populations continue to satisfy the classic "bee-pollination syndrome" however, the degree of variation found between the populations is significant enough to reason that the discrete populations have likely adapted to their local pollinator communities. Of the four populations studied, the two populations exhibiting the greatest color and morphological disparity are the Zion population at low elevation and the population in Snake Range. They differ in that population "Snake" most accurately adheres to the traditional bee-pollination syndrome, with intense blue-colored petals, a large lower lip (or landing platform), wide mouth diameter, and comparably short anther exertion, whereas the low elevation Zion population displays more intermediate floral characters and thus, showed the greatest deviation from the defining syndrome.

While collecting flowers in Snake Range, it was noted that the *P. pachyphyllus* individuals were widely dispersed amongst *Penestemon* species, *P. confusus* and *P. eatonii* whose floral colors range from deep reds to soft pinks. Thus, "Snake's" intense and distinctive blue color as well as attractive morphology may not only be an evolutionary response to the selective pressures posed by the principal pollinator, the bumblebee, but also a response to the fierce interspecific competition for pollinators. Consequently, the population may have "shifted" to the more specialized end of the bee-pollination spectrum in order to distinguish itself from the surrounding flowers. Exhibiting floral characters that explicitly attract bumblebee or very similar insect pollinators would better ensure bumblebee fidelity, thereby increase foraging efficiency, and ultimately improving the population's fitness.

Conversely, the Zion population at low elevations exhibited more intermediate floral characters resulting in a relatively looser correspondence to the classic bee-pollination syndrome. The "Blue/Red" color ratio measured in its petals was indeed the lowest, thus producing a soft purple color. Moreover, the morphological measurements indicated that the population on average had the smallest mouth diameter, lowest lip area, shortest corolla length and yet, the greatest protraction of the anthers. Considering the population's floral characters, especially the length of its anthers and its apparent "purple" color, the population appears to have slightly shifted away from the bee-pollination syndrome; vaguely displaying hummingbird-pollination traits . Thus, we expect the population to favor a less specialized adaptation to local pollinators, thereby, still seeking pollination by bees and wasps while divagating enough to potentially allure nearby hummingbird pollinators. This tendency toward generalization may be "attributed toward the evolutionary risk associated with dependence on another species and to the unpredictability of pollinator populations in both time and space."[2] It seems likely that highly diverse and impermanent pollinator assemblages would be found at easily assessable areas such as low elevations and thus, in areas characterized by capricious pollinators, a more generalized pollination syndrome may in fact be advantageous. Moreover, in assessing the floral characters of the remaining populations found along East Schell Creek and at high altitudes in Zion National Park, we would expect the populations to exhibit a more intermediary pollination syndrome, some where in between highly specialized and moderately generalized.

Based upon the pattern and extent of color and morphology variation found between populations, it is reasonable to consider the possibility, that the measured intraspecific phenotypic differences are significantly attributed to the local assemblage of pollinators. However, more pollinator observations and floral character quantifications are required before such pollinator-morphology associations can inferred with confidence.

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