FINAL REPORT: NATURAL COMMUNITY CONSERVATION PLANNING

LOCAL ASSISTANCE GRANT

NAME THAT JEWELFLOWER: USING GENETICS AND FIELD SURVEYS TO DETERMINE TAXONOMIC BOUNDARIES AND DEFINE OCCURRENCES FOR THE MOST BEAUTIFUL JEWELFLOWER AND METCALF CANYON JEWELFLOWER ON COYOTE RIDGE

Grant Agreement Number Q2030901

(Left) Two separate jewelflower individuals representing subtle differences in sepal color (Rancho Canada del Oro, Spring 2022). (Right) Sepal pigment variation detected along Metcalf Rd. in May 2021. (J. Whittall)

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Santa Clara University

Justen Whittall, Ph.D.

CREEKSIDE SCIENCE

Stuart B. Weiss, Ph.D.
Christal Niederer

Summary

This project leads to delineating occurrences of the two covered jewelflower taxa present on Coyote Ridge and in the vicinity. Differentiating the Metcalf Canyon jewelflower (*Streptanthus albidus* ssp. *albidus* Greene) and most beautiful jewelflower (*Streptanthus albidus* ssp. *peramoenus* (Greene) Kruckeb.) is essential to species protection, recovery, and the achievement of the Santa Clara Valley Natural Community Conservation Plan's (NCCP/Valley Habitat Plan) biological goals and objectives. The Santa Clara Valley Habitat Plan covers these two rare jewelflowers distinguished by their white and pink flower color, respectively. At their range extremes, they are clearly differentiated by their sepal color. Where their ranges meet, there is substantial variation in color especially along Coyote Ridge. This zone of intergradation makes it difficult to confidently define occurrences as either most beautiful or Metcalf Canyon jewelflowers. Establishing a consistent, reliable, biologically-based method for delineating these taxa (and their intermediates) is essential to determining the nature of the apparent introgression between these two taxa, determining the number of protected occurrences, seedbanking, establishing new occurrences and is a required recovery action under the Valley Habitat Plan.

The main conclusions of this work are:

Genetics: Sepal color is genetically determined, and is a function of anthocyanin concentration. The genetic system appears to be incompletely dominant meaning that artificially created F1 hybrids between white inbred lines and dark pink inbred lines are intermediate in sepal pigmentation. Assuming there is a single locus with two alleles, white individuals would be homozygous for the loss of function allele, intermediate phenotypes are heterozygous with intermediate levels of anthocyanins, and the darkest pink/purple individuals are homozygous for the fully functional anthocyanin allele(s). The frequency of white phenotypes can be used to estimate the frequency of the non-functional allele by applying the Hardy-Weinberg Equilibrium equation from population genetics. There may be additional alleles at this locus and even additional modifier loci that produce the apparent continuous gradient of sepal color from white, through light pink, dark pink to dark purple.

Quantifying color in the field: The Munsell color system (Hue, Value, Chroma) used in Dunn-Edwards color cards provides a biologically relevant, repeatable, and efficient means to quantify sepal color. Anthocyanin concentration is strongly correlated with the Value (light to dark) of the color cards. Samples of up to 100 plants in a local population can be rapidly (~1 hr) scored for phenotype frequency with this method.

Spatial gradients: There is a geographic gradient of sepal color from white to pink on Coyote Ridge that runs primarily NW (white) to SE (increasing frequency of light pink and eventually dark pink flowered individuals), with additional dark pink and purple flowered populations west in isolated serpentine outcrops in the Santa Cruz Mountains and to the east in the Mt. Hamilton Range. Multiple phenotypes (color card matches) coexist within most populations along Coyote Ridge. A cluster analysis based on phenotype frequencies identified four clusters -- white, light pink, dark pink, and purple -- that strongly

sort out geographically. Cluster assignment of populations was mostly stable between the two sample years (2021 and 2022), with 3/38 sites (8%) changing cluster assignment but remaining very similar in multivariate space.

Pollination: Pollinators are primarily bumblebees in the genus *Bombus* and do not show a preference for sepal color when assessed in experimental arrays in a mixed population along Coyote Ridge. Therefore, we have no evidence of selection pressure by pollinators.

Population dynamics: As an annual plant, *Streptanthus* populations fluctuate widely by a factor of 10 or more. The geographic distribution expands during high abundance years, and contracts during low abundance years: occupancy rates on a 100m grid varied by a factor of two among sample years. Repeated observations of jewelflowers being absent in some locations in low abundance years only to reappear in subsequent high abundance years is consistent with previous evidence of a persistent seedbank. The absence of plants at a location in one year does not indicate extirpation of the population or occurrence. We note that no conservation goals are met by constantly redrawing population boundaries following the natural rhythm of jewelflower population fluctuations.

Introduction

Creekside Science and Dr. Justen Whittall have been studying jewelflowers in Santa Clara County for over a decade (i.e., Weiss et al. 2007, Whittall and Strauss 2011), including a successful reintroduction of the Metcalf Canyon jewelflower to Tulare Hill (Niederer et al. 2017). During the course of this reintroduction and while conducting baseline surveys of both Metcalf Canyon jewelflower and most beautiful jewelflower on the Mayyan ‘Ooyakma Coyote Ridge Open Space Preserve (CROSP) (CCEO 2018), they realized the difficulty differentiating between two taxa covered by the Santa Clara Valley Habitat Plan (Valley Habitat Plan) would potentially impede conservation goals. The Valley Habitat Plan’s objectives focus mainly on conserving given numbers of occurrences with minimum population sizes. Similarly, the Valley Habitat Plan relies heavily on defining the taxa and occurrences therein. The following are some relevant Goals, Objectives and Studies from the Valley Habitat Plan:

- Goal 20. Maintain viability, protect, and increase the size and number of populations of covered serpentine plant species, including [...] Metcalf Canyon jewelflower, most beautiful jewelflower within the study area.
- Objective 20.5. Protect at least three currently unprotected occurrences and adequate lands to create ten new occurrences of Metcalf Canyon jewelflower [...].
- Objective 20.7. Protect at least 17 occurrences of most beautiful jewelflower [...]
- Studies-12. Ensure seeds from natural occurrences are stored and maintained at a minimum of one Center for Plant Conservation certified botanic garden.
- Studies-17. Monitor Metcalf Canyon jewelflower and most beautiful jewelflower introgression and develop protocols to protect the genetic integrity of both species.

However, CNDDB records of outliers suggested people were either not observing color in a comparable way and/or were documenting an occurrence based on the presence of a single individual within a colony of plants dominated by another phenotype. To conserve these taxa (and any evolutionary processes associated with them), managers need to be able to define boundaries, quantify occurrences, and ensure zones of intermediacy fit into the Valley Habitat Plan’s conservation model.

Objectives

The Project aims to address the following questions at two scales: (1) On the individual scale, are there phenotypic, biochemical, and genetic boundaries that define these taxa? If so, can a method for field identification be developed that is both functional in the field and biologically relevant? (2) On the population scale, how is an occurrence defined when it is composed of individuals with a range of flower colors? Also, how is an occurrence defined when the frequency of phenotypes changes year to year? Because occurrences are based on geographic distribution and population structure, a fine-grained map of the phenotypic variation was produced. Eventually, objective criteria to treat mixed populations must be used, and then these occurrences must be protected. The interpretation of the frequency of phenotypes in a population is based on the current understanding of the underlying genotypes. Therefore, studying the genetics of color variation is essential in objectively interpreting the geographic distribution of phenotypes.

Specific objectives of this Project are to:

1. Investigate the genetic nature of the color differences between the two taxa and, specifically, the inheritance of flower color differences (and any additional morphological differences detected in common garden between these taxa) by following two generations of crosses to follow the recombination of one or more flower color genes (i.e., segregating F2 population).
2. Use a piloted color tool to map color frequencies at multiple locations on Coyote Ridge.
3. Apply the adaptive management plan by preparing a draft protocol and presenting it to land managers and other stakeholders based on the results from Objectives 1 & 2. The meeting was conducted on May 3, 2023 at the City Council Chamber Building, in Morgan Hill with an option to participate virtually. Invitees and attendees are listed in Appendix A. We have attempted to integrate their questions and suggestions into this report.

Methods

Crosses

In order to decipher the genetic basis for sepal anthocyanin pigmentation in these jewelflowers, we needed to start with inbred lines that are mostly homozygous. This facilitates the identification of genes responsible for color without being hindered by complications arising from heterozygous parents. The first generation of inbred seeds were created in the field (Kirby = pigmented and Metcalf = white populations) in Spring 2019 as part of an attempt to make crosses in the field. Although our crosses produced no seeds, many of our white, pink and purple bagged maternal plants automatically self-pollinated and fruits were collected in Summer 2019. These seeds were planted and grown in the SCU Greenhouse in Fall 2019, flowered in early 2020 and were allowed to self pollinate (pollinators are not present in the SCU Greenhouse). This created the second generation of inbred seeds (Weiss et al. 2020). Since each generation of selfing reduced heterozygosity by half, we now have a parental population with approximately 25% of the original standing heterozygosity. Furthermore, by selecting for offspring with extreme phenotypes (i.e. white and purple sepals), we have further reduced the chances that this 2nd generation of inbred parents are heterozygous at the relevant flower color gene(s).

After initial delays accessing SCU Greenhouses for faculty and student researchers due to COVID-related public health restrictions, we germinated the parental seeds. Using sterile technique, we created

germination chambers out of 100mm x 20mm petri dishes containing a 90mm diameter Whatman #1 filter paper. The filter paper was saturated with millipure water and 5-50 seeds (based on availability) were placed near the center (Photo 1A). The petri dishes were then placed in a larger vessel with wet paper towels and a sealed lid. Cold stratification was conducted at 4 degrees Celsius. After approximately 15 days, germinated seedlings were transferred to one gallon pots containing saturated potting soil mix and grown outdoors to flowering for making controlled crosses (Photos 1B & 1C).

Photos 1A-E. Seeds from parental inbred lines of white sepalled Metcalf Canyon jewelflower and most beautiful jewelflower were germinated (A), grown outdoors (B) to flowering (C) and crossed (D & E) to produce F1 seeds (F). Orange puff paint in (E) marks flowers used in the genetic crosses.

Crosses were made between *S. albidus* ssp. *albidus* (white sepals) and *S. albidus* ssp. *peramoenus* (dark pink) (Photo 1D). All inbred lines of *S. albidus* ssp. *albidus* had white sepals, so the most vigorous plant was selected for crosses. The *S. albidus* ssp. *peramoenus* plant with the darkest pink sepals among the inbred lines was selected to maximize the sepal color contrast. Flowers were collected for color quantification (digital images in visible and UV wavelengths, UV-vis spectra and concentration of anthocyanins in acidified methanol detected on a plate reader).

Flowers selected for pollination were marked with orange puff paint along the stem. Anthers were removed between 7-8AM to prevent self-pollination. After waiting 24-48 hours, pollen was transferred to receptive stigmas by brushing a freshly removed anther over the receptive stigma. Mesh bags were used to exclude pollinators after the crosses were made (Photo 1D & E). Siliques containing mature F1 seeds were collected 4-6 weeks after pollination (Photo 1F).

Viable seeds with dark brown seed coats were separated from fruit material and immediately germinated in petri dishes with wet filter paper as described above for the parentals (Photo 2A). Seedlings (Photo 2B) were planted into wet potting soil in one-gallon pots (Photo 2C) and reared in the SCU Greenhouse Facility from fall 2022 to summer 2023. Five seedlings per pot x 12 pots were established on sunny, south facing greenhouse benches (Photo 2D). A long-day light regime (16 hrs light/8 hrs dark) plus ambient sunlight in a south-facing room successfully mimicked spring/summer growth rates and initiated flower development (Photo 2E). Early on, very young plants were misted daily to avoid desiccation until Oct 21, 2022. Once the seedlings were established, plants were watered 3x per week. Throughout the growing period, plants were rotated to avoid any positional effects of the south-facing window exposure. F1 plants began bolting in Dec 2022 (initiation of flowering stems).

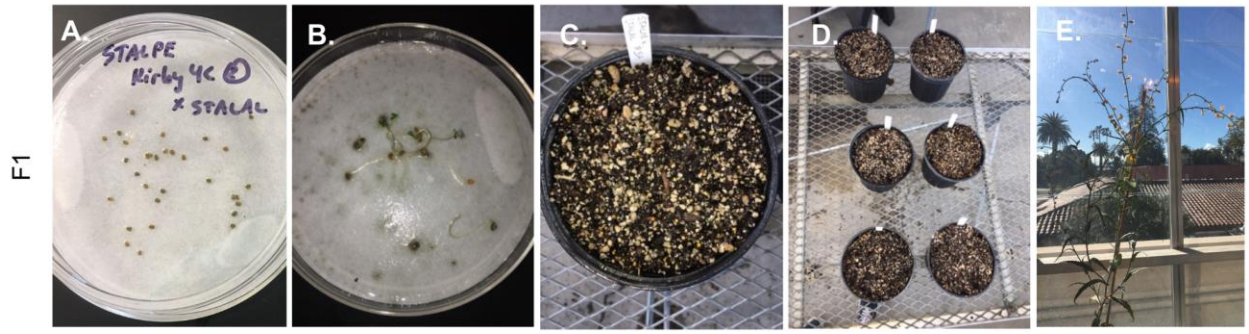


Photo 2. The F1 generation was raised starting with seeds in petri dishes stored at 4C (A). Seedlings (B) were planted into 1 gallon pots (C) and cultivated in the SCU Greenhouse facility (D). Long-day supplemental lighting conditions forced plants to flower early and profusely (E).

We measured the flower color of the F1 hybrids in Spring 2023, forced self-pollination of F1 flowers and eventually reduced the watering (May 2023) and then completely stopped watering to allow siliques to mature (June 2023). Siliques containing F2 seeds continue to mature and will be collected, germinated, planted and phenotyped in the coming months. Unfortunately, due to COVID-related delays at the onset of this project, we were not able to assess the phenotypes of the F2 generation within the timeline of this grant.

We developed a high-throughput flower color quantification technique necessary to phenotype large numbers of individuals in the eventual F2 population (Photo 3). This includes purchasing and setting-up a UV-Visible spectrophotometer (FLAME, OceanOptics, FL). In addition, we now have access to a UV digital camera to see reflectance patterns in wavelengths invisible to humans, but important to bees – the primary pollinators are bumblebees that can see in the UV. Finally, we have perfected the biochemical assays necessary to quantify the pigments conferring the flower color differences.

Photo 3. Flower color quantification for high throughput phenotyping. From left to right: UV light sources, UV camera and accessories (e.g., tripod), FLAME UV-Vis spectrophotometer and light source (gray box with white face) and detection probe (blue circular fiber optic cable).

Pollinator Arrays

In order to determine if pollinators are selective agents on flower color, we performed a pollinator array experiment at Malech Rd. This site contains both pure white and pink sepalled plants. We established six hexagonal arrays each composed of 7 inflorescences (Photo 4A) kept fresh in florist water picks (Photo 4B). Each inflorescence had the same number of open flowers (3-4) to control for overall attractiveness. The arrays were “observed” with digital cameras running non-stop for several hours throughout the day (Photo 4C). No nocturnal pollinators have been observed on these jewelflowers (Whittall, personal observation).

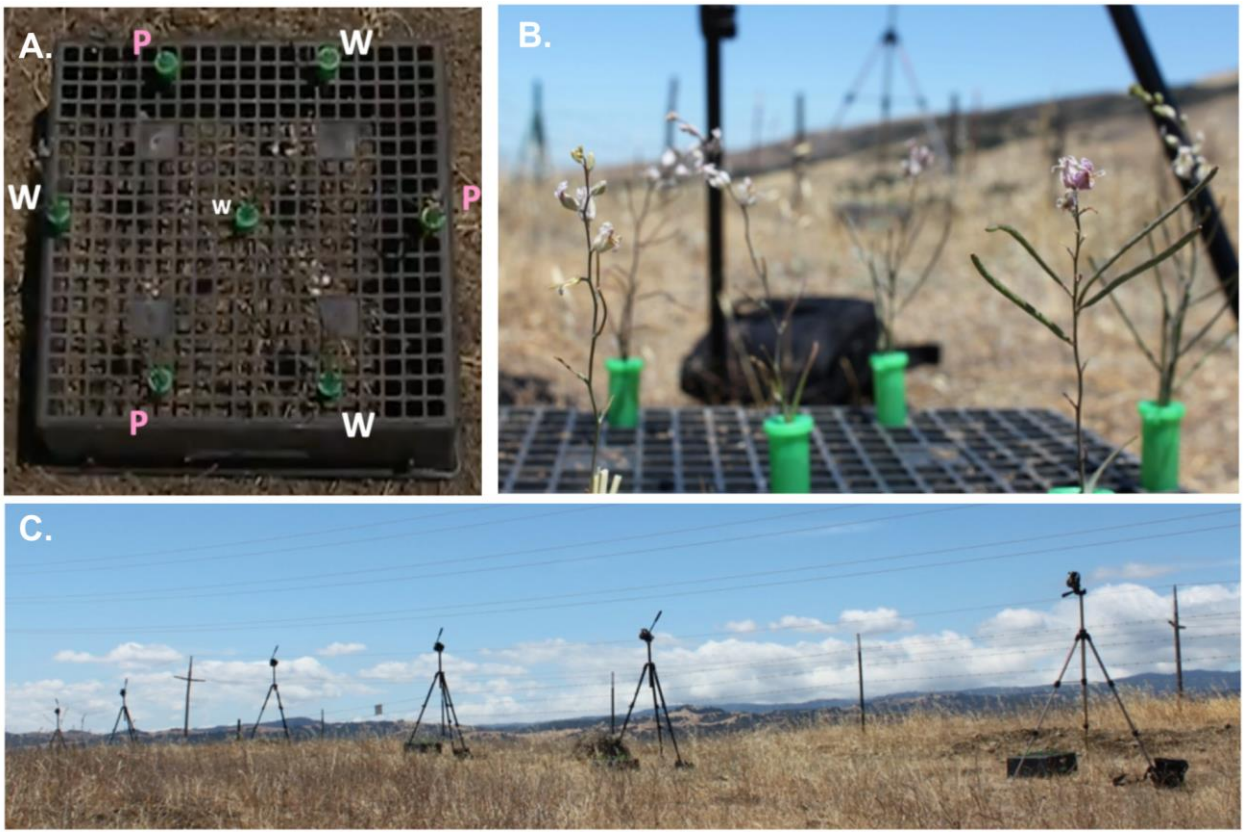


Photo 4. Pollinator arrays with white and pink sepalled jewelflowers consisted of seven plants per array (A) kept fresh in florist water picks (B). Six arrays were observed with digital cameras (C) throughout the day.

Dunn-Edwards Color Chips

The Dunn-Edwards color chips use the Munsell System of Hue, Value, and Chroma (Figure 1), giving a system of quantifying color. Hue is the spectral color, Value scales from black (low) to white (high), and Chroma is color saturation. The numerical values assigned to each color allow statistical color comparisons. The broad selection of Dunn-Edwards colors was narrowed to those colors detected in pilot field trials of Metcalf Canyon jewelflower (*Streptanthus albidus* ssp. *albidus*) (STALAL), most beautiful jewelflower (*Streptanthus albidus* ssp. *peramoenus*) (STALPE) and bristly jewelflower (*Streptanthus glandulosus*) (STGL) in southern Santa Clara County in Spring 2020 (Weiss et al. 2020). We used a field set consisting of 21 colors that can be ordered from white to pink to purple according to Value (Photo 5). In this set, Value and Chroma are negatively correlated ($R^2 = 0.79$) (Figure 2), and Hues varied from purple through red with some green showing up in the high Value (white) colors (Figure 3). Dunn-Edwards color chip Value correlated most strongly with the concentration of anthocyanins as determined biochemically (Figure 4). Therefore, all subsequent analyses were conducted using the Value from the Dunn-Edwards color cards.

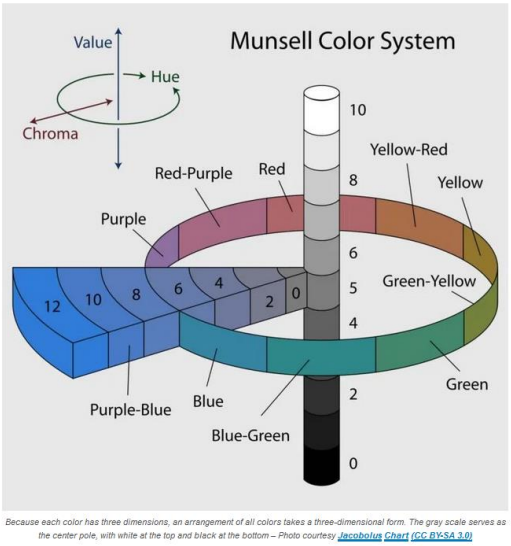


Figure 1. The Munsell color system uses three dimensions to distinguish human visible colors -- Value, Chroma and Hue.

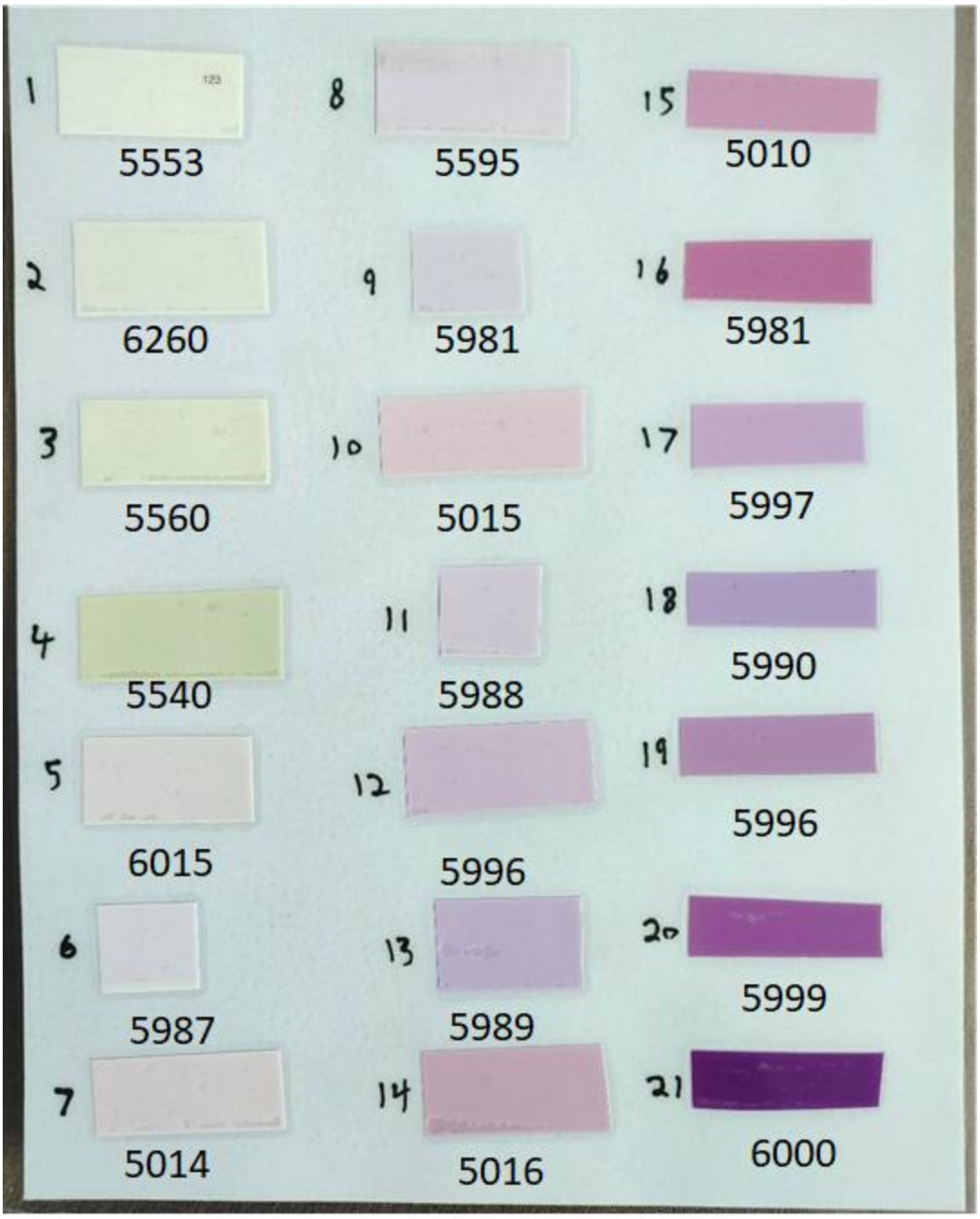


Photo 5. Field ready laminated Dunn-Edwards color tool representing the range of colors observed in preliminary studies of Metcalf Canyon jewelflower (*Streptanthus albidus* ssp. *albidus*) (STALAL), most beautiful jewelflower (*Streptanthus albidus* ssp. *peramoenus*) (STALPE) and bristly jewelflower (*Streptanthus glandulosus*) (STGL) (Weiss et al. 2020). Field-use numbers are to the left of swatches, and Dunn-Edwards numbers are below.

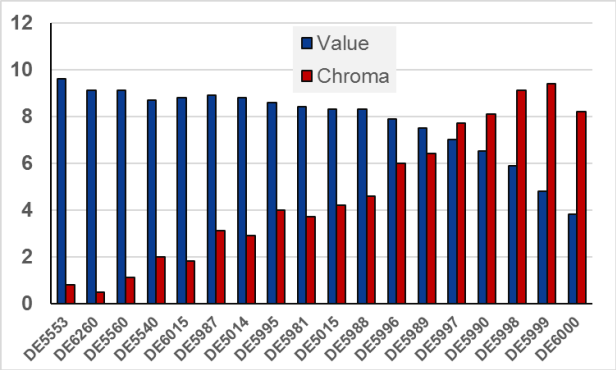


Figure 2. Value and Chroma of the Dunn-Edwards colors used in this study. Since these two measures are strongly negatively correlated (and Value correlates with the concentration of anthocyanins per Weiss et al. 2020), we chose to focus on the Value for all subsequent analyses.

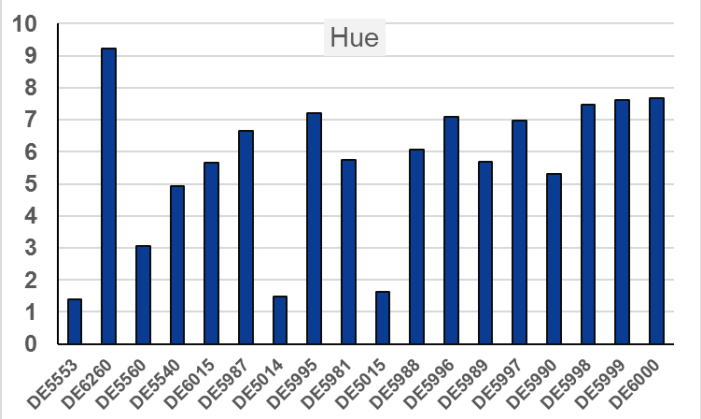


Figure 3. The Hue of the same Dunn-Edwards color cards in the same order as above further differentiates the colors, but doesn't follow the gradient observed for Value and Chroma.

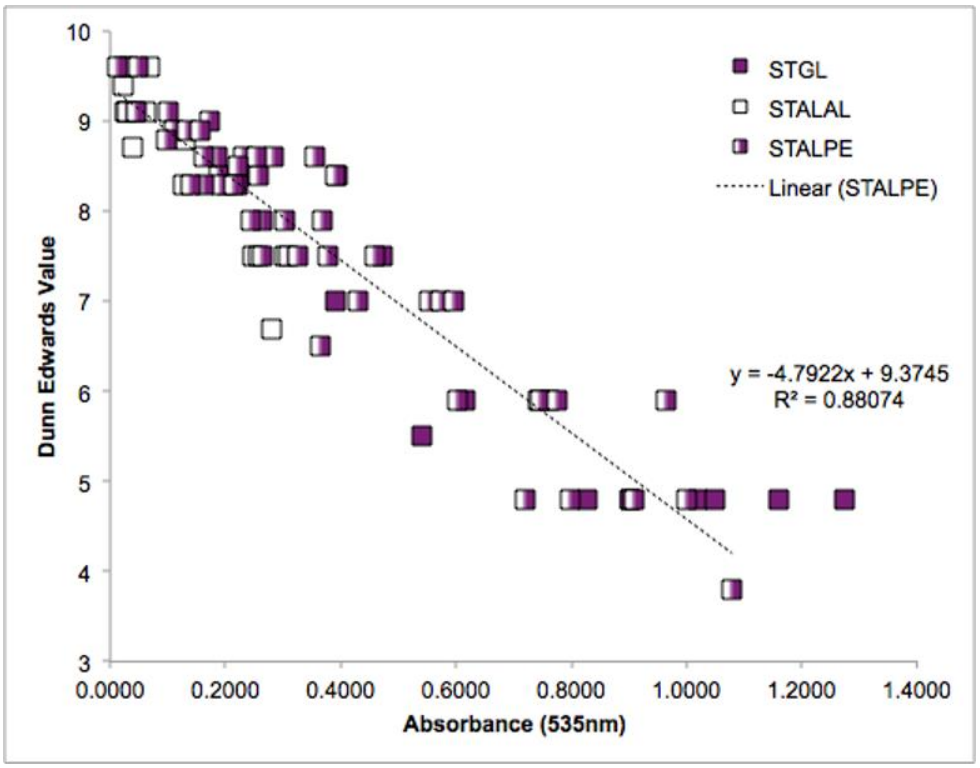
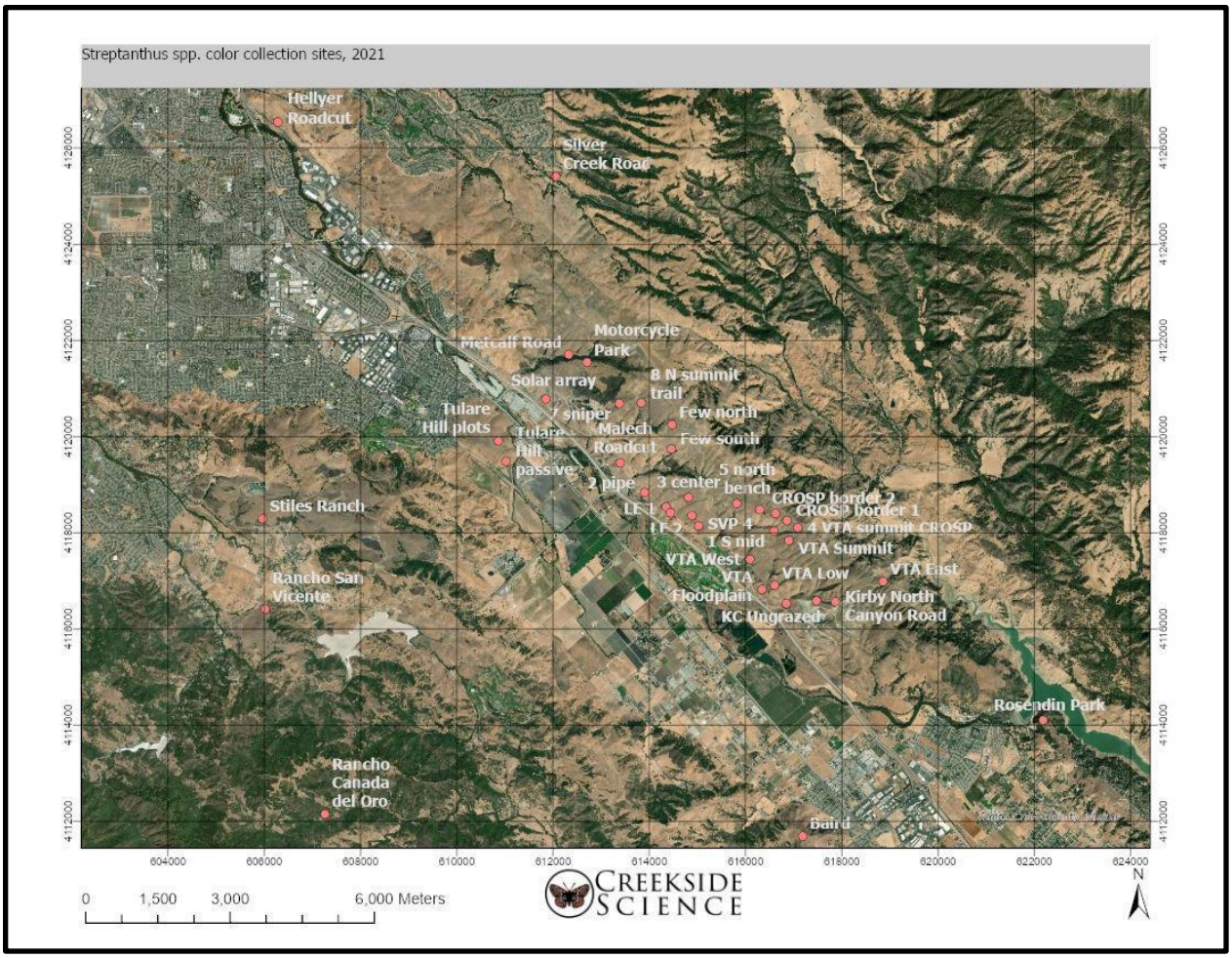


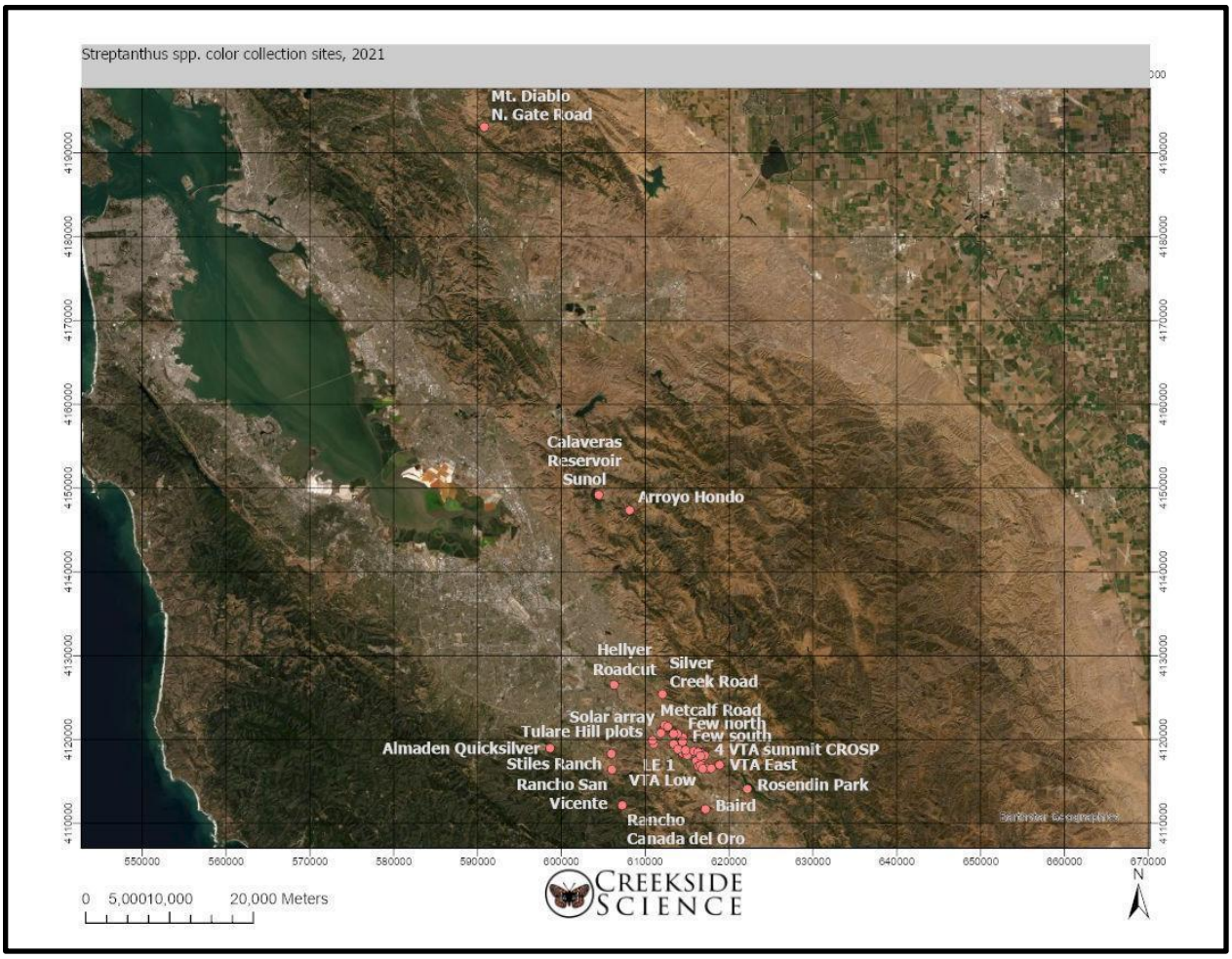
Figure 4. Dunn Edwards paint chip Value is strongly correlated with the anthocyanin concentration in the sepals. Jewelflower sepal samples representing as many color cards as possible were carded in the field. Then, anthocyanins were extracted in 99% methanol: 1% water and absorbance was measured on a plate reader at 535 nm (the peak absorbance for these anthocyanin pigments) (Weiss et al. 2020).

Field Surveys

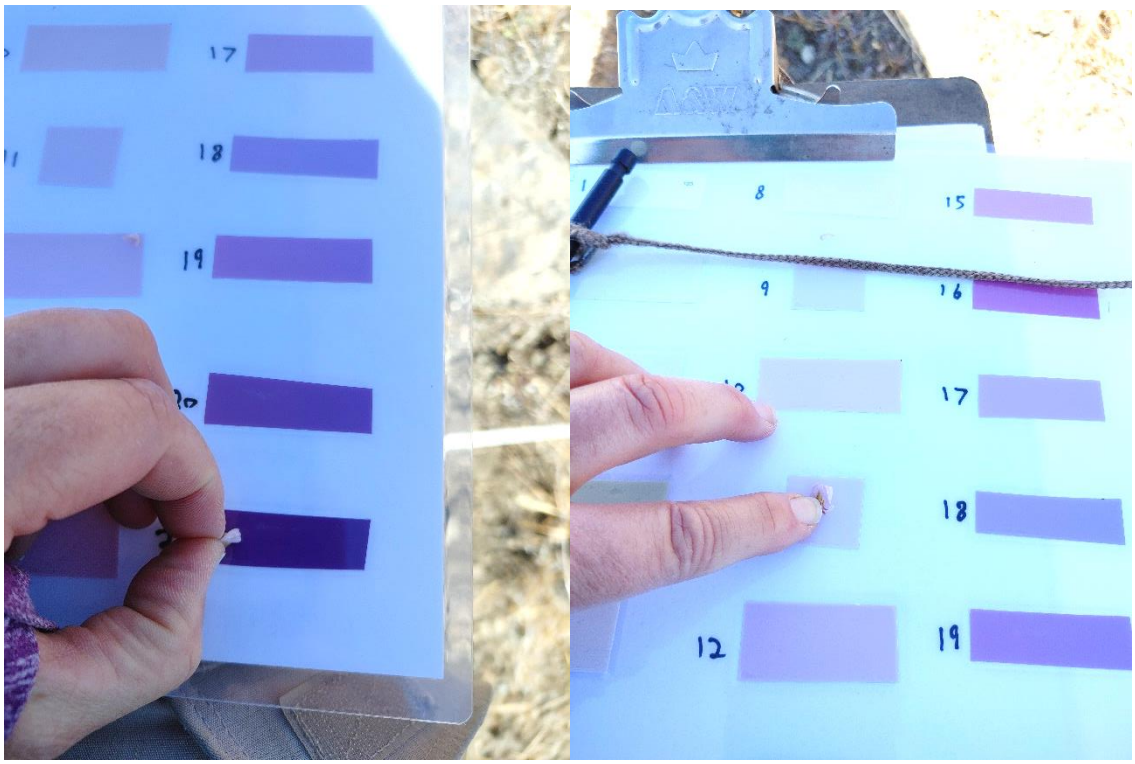
Color collection sites were first visited May 2021. Data were collected on 50 individuals at 36 sites in Santa Clara County (Maps 1 & 2). Five additional sites had fewer than 50 individuals (range 25-37 plants). Seven sites in Santa Clara County are west of Highway 101. At each site, a 100 m transect was placed and color data was collected on the flowering jewelflower closest to each 2-meter mark. Because flower color can vary slightly even on the same plant, the flower nearest the tip in full anthesis (anthers exserted) was selected. Color was scored to a Dunn-Edwards paint chip that most closely matched the sepal color (Photos 6a & 6b). Appropriate Dunn-Edwards paint chip colors had been chosen the previous year during pilot color data collection (Weiss et al. 2020). Observer records the number of plants assigned to each color on the tool. These color frequencies allowed us to thoroughly map the color variation present on Coyote Ridge in Spring 2021. The same sites were visited in May 2022 to document whether the color frequencies changed. Colors from fewer plants were collected from an additional three sites in Contra Costa and Alameda Counties representing an unusually dark purple/black sepalled form (Map 2, Appendix B).



Map 1. Color collection sites in Santa Clara County. Almaden Quicksilver in the southwestern corner is not shown on this map.



Map 2. All color collection sites including Alameda, Contra Costa, and Santa Clara Counties.



Photos 6a and 6b. Sepals are compared to the color tool in shade to avoid glare from the lamination. Incorrect matches (left) are skipped over and the most similar match (right) is selected.

Statistical Analysis

The goal of the analysis is to find natural groupings of sites, based on the composition of Dunn-Edwards colors scored in the field. The raw data are a matrix of Sites x Counts of each Dunn-Edwards color (Appendix B). The primary statistical method is agglomerative hierarchical clustering, executed in JMP 16.1 (SAS Institute). Hierarchical clustering builds up a dendrogram based on multivariate distances between sites, starting at the finest scale with many small clusters, and progressively agglomerating sites into larger clusters using the Ward method of minimal variance. The dendrogram can be cut at any number of clusters, but there are diagnostics for determining an optimal number of clusters. Both 2021 and 2022 data were combined in the analysis.

Principal Component Analysis (PCA) is applied in order to view the sites and clusters in multivariate space. This analysis allows a close examination of sites that may have switched cluster assignment.

Other Map Data

Fog and low cloud cover (FLCC) maps (Torregrosa et al. 2016) were downloaded via the Conservation Lands Network (www.bayarealands.org).

Results

Crosses

The inbred lines of white flowered *S. albidus* ssp. *albidus* (Metcalf) parents were consistently 100% white sepalled. However, the inbred lines of pink flowered *S. albidus* ssp. *peramoenus* parents still exhibited some variation in flower color (Photo 7).

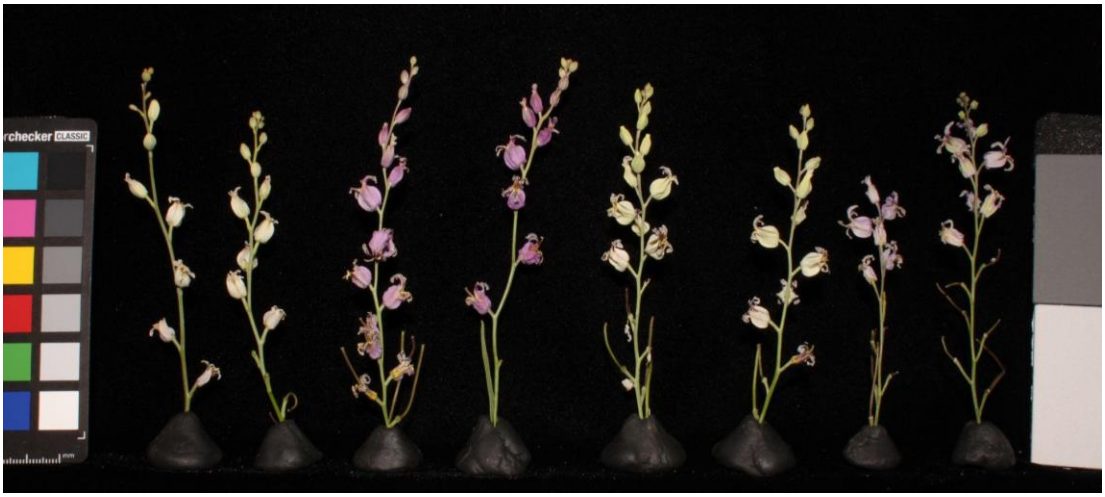


Photo 7. Inbred lines of parental *Streptanthus albidus* ssp. *albidus* (plants 1, 2, 5, 6) and *S. albidus* ssp. *peramoenus* (plants 3,4,7,8). Color checker (left) and UV standards (right) are included. Pure white and dark pink flowered parentals were chosen for crosses.

Only four out of 18 crosses produced F1 seeds (~22% with siliques >8 cm long), the remaining crosses produced siliques with no viable seeds (<2 cm long). All four successful seed producing crosses had the dark pink *S. albidus* ssp. *peramoenus* (Kirby) as the maternal parent. Two F1 plants survived to flowering. They show intermediate sepal color (light pink/purple, fading to cream/green with tinges of pigment particularly at the apex of the sepal) (Photo 8 & Photo 9). The intermediate pigmentation levels in the F1 confirm incomplete dominance (i.e. heterozygotes have phenotypes intermediate to the parents) (Figure 5).

Photo 8. Example of an F1 plant grown in the SCU Greenhouse which exhibits intermediate sepal pigmentation (January 2023).

Photo 9. F1 hybrids and their inbred parental lines were quantified for flower color. White sepalled Metcalf Canyon jewelflower on the left and dark pink most beautiful jewelflower on the right. Two F1 hybrids are in the center. Top image = visible; Bottom image = UV.

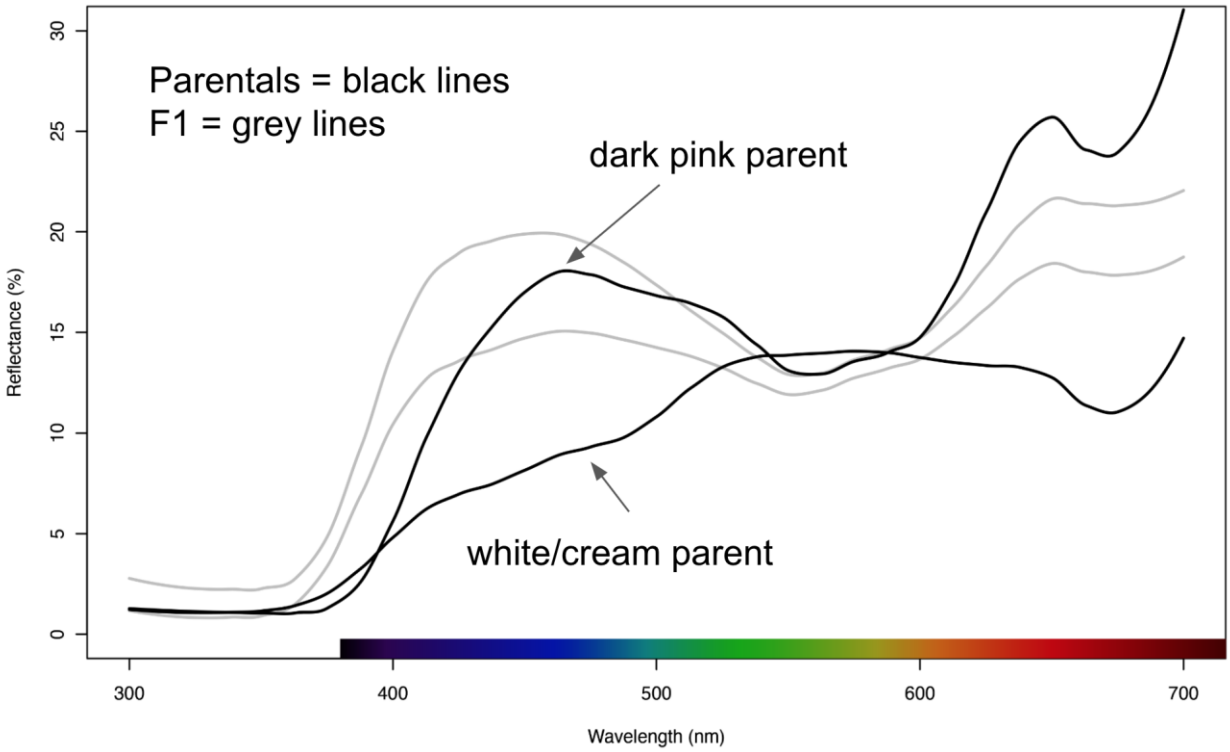


Figure 5. Reflectance spectra of F1 sepals (grey curves) and inbred parental lines (black curves). There are no significant differences in the amount of UV reflectance (< 400nm). The human perception of colors >400 are indicated along the x-axis.

Self-pollination of the flowers on the two F1 plants produced numerous fruits containing the F2 seeds. These will be grown to flowering to determine the ratio of white, light, and dark sepal color phenotypes in order to confidently determine the number of genes responsible for the flower color differences.

Pollinator Arrays

Overall, we documented 200 visits to the six arrays. Ninety-three percent of array visits were by bumblebees (*Bombus vosnesenskii*) (Figure 6A). The remaining visitors included honeybees (*Apis mellifera*, n=6 visits) and flies (unidentified Diptera, n=8 visits). Overall, there was no significant preference for either color (Binomial Test, $p > 0.05$, Figure 6A). Since most of the visits were by bumblebees, we could not assess honeybee and fly preferences individually to pink or white flowers (bumblebee = 89 pink & 97 white; honeybees = 3 pink & 3 white; flies = 7 pink and 1 white).

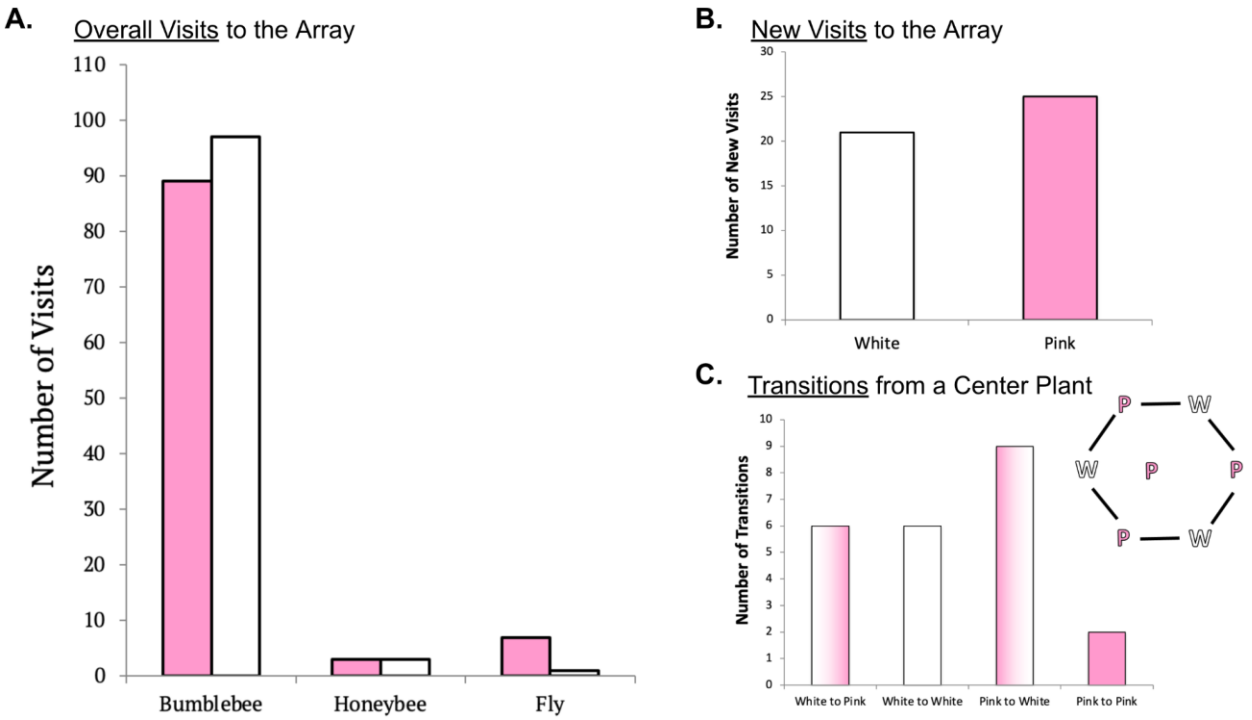


Figure 6. Pollinator arrays indicate no flower color preference and frequent transitions between colors. There were 200 overall visits in the arrays by three pollinator guilds (A). Of these 200 visits, 46 were new visits to the array (B). Once in the array, we documented the four types of flower color transitions while moving around the array (C).

Although the hexagon isn't perfectly balanced because of the central plant, we had an equal number of camcorders recording pink-centered arrays as we did white-centered arrays. Of these 200 visits, 46 were new visits to the array – 21 visitors chose white as their first flower and 25 chose pink (Figure 6B). There was no significant preference for one color over the other based on their first flower visited (Binomial Test, P = 0.33, Figure 6C). Although pollinators may visit both colored flowers, potentially they restrict themselves to a single color within a bout of pollinations. Since the array is designed with equal distances between all flowers, we tested if transitions between the same colors (n=8) were greater than transitions to a different color (n=15) (Figure 6C). Yet, we found that transitions to a different color were ~2x greater than transitions to the same color, yet the difference was not significant (Binomial Test, p = 0.11, Figure 6C). Pollinators do not have an overall preference for white or pink as assessed from multiple aspects of these pollinator arrays. Pollinators visit both colors with similar frequencies and move between flower colors readily.

Field Surveys

Three of the 21 previously determined colors (14, 15, and 16) were not selected either year and only one plant was coded a 10 so it was removed. Therefore, we simplified the color tool by reducing it to 17 color cards. The technique of collecting color frequencies using the laminated Dunn-Edwards tool was found to be simple, objective, and therefore consistent and repeatable among observers. Because of the direct side by side comparison, the method is relatively robust regardless of lighting conditions (Photos

6a & 6b). Color frequencies (raw data) are shown in Appendix B and are used for the hierarchical clustering below.

Hierarchical Clustering

Two sites (Calaveras Reservoir and Arroyo Hondo) were major outliers in the cluster analysis because they did not share any Dunn-Edwards colors with the main body of sites. All plants from these two sites exhibited nearly completely black sepals like those previously documented atop Mt. Hamilton (Whittall and Strauss, 2011). These have been dropped from the cluster analysis, since they form their own unique cluster and do not contribute to the differentiation of the target sites in southern Santa Clara County. These flowers were initially included in the sample to demonstrate a geographical boundary to the phenotypes found more locally in the Coyote Ridge area. Mt. Diablo (purple = *S. glandulosus*) plants were outside of our geographical boundary and removed as well.

The hierarchical clustering analysis is summarized with a dendrogram depicting shared color card frequencies (Figure 7). The names of the field sites are on the far left, color coded by cluster. In this analysis, both 2021 and 2022 data are included to demonstrate the year-to-year consistency of the cluster assignments.

The Dunn-Edwards colors (n=17 in this analysis) are listed by their number along the bottom, in left to right order of Value from lightest to darkest. The blue-gray-red colormap above the Dunn-Edwards numbers shows the relative frequency of the Dunn-Edwards colors in each site, with increasing frequency from dark blue (absent) through gray to dark red (very common). The dendrogram showing the hierarchical structure is on the right, scaled by statistical distance. At the lower right is a graph showing the variation explained as the number of clusters increases from right to left.

The progressive variance explained (lower right) shows a break at four clusters (vertical line), indicating that this is a near optimal number – the marginal increase in variance explained with each additional cluster beyond does not improve the clusters depicted. It is very important to not overinterpret the branches beyond 4 clusters – there is very little variance explained with 5, 6, 7 or more clusters (note the plateau in the progressive variance explained).

The top cluster (purple, Cluster 1) contains the darkest populations, all west of the Santa Clara Valley except Rosendin Park, south of Coyote Ridge and the Anderson Dam. Note the preponderance of Dunn-Edwards colors on the right (darker) end of the gradient.

The second cluster (light gray) contains the nearly uniformly white populations in the Silver Creek Hills, Metcalf Canyon, Motorcycle Park, and extending south to the northern part of CROSP. 85% of all plants in this cluster (range 64%-100% among sites) are the first four Dunn-Edwards colors -- white phenotypes with varying amounts of green, no pink (See Photo 5, colors 1-4). The summed frequency of these four colors is considered the “white fraction” (which becomes important in interpreting the allele frequencies later). The fifth color (6015) is the lightest pink (Photo 5, color 5) and appears consistently at low frequency (12% of the all plants, samples range from 0-24% among sites) in the lower tier of this cluster. A low frequency (<3%) of slightly darker pink phenotypes can be found among the white phenotypes. The reintroduced population on Tulare Hill (Niederer et al. 2017) is in this cluster, as expected since the source populations for the seeds used in the reintroduction were all from pure white sepalled populations.

Overall, the sites along Coyote Ridge (light and dark pink clusters) are organized into a strong color gradient (note left to right progression to darker phenotypes as you pass downward on the graph) with a spread of colors within populations and almost continual overlap between populations.

The third cluster (light pink) starts at the Malech Roadcut and upslope areas at the same latitude, and extends south to the CROSP-VTA border approximately. These populations represent a wide range of flower color phenotypes including low fractions of white phenotypes (colors 1-4), the bulk in the light and mid-pink (colors 5-12), and a very low frequency of dark pink and purple (colors 13-17).

The fourth cluster (dark pink) starts around the CROSP-VTA border and extends south past the Kirby Canyon Landfill. Few plants were in the light pink (colors 5-7), the bulk are in the dark pink (colors 9-14 & 16). Only one plant with a truly white phenotype (colors 1-4) was observed in this cluster at 2022 VTA low.

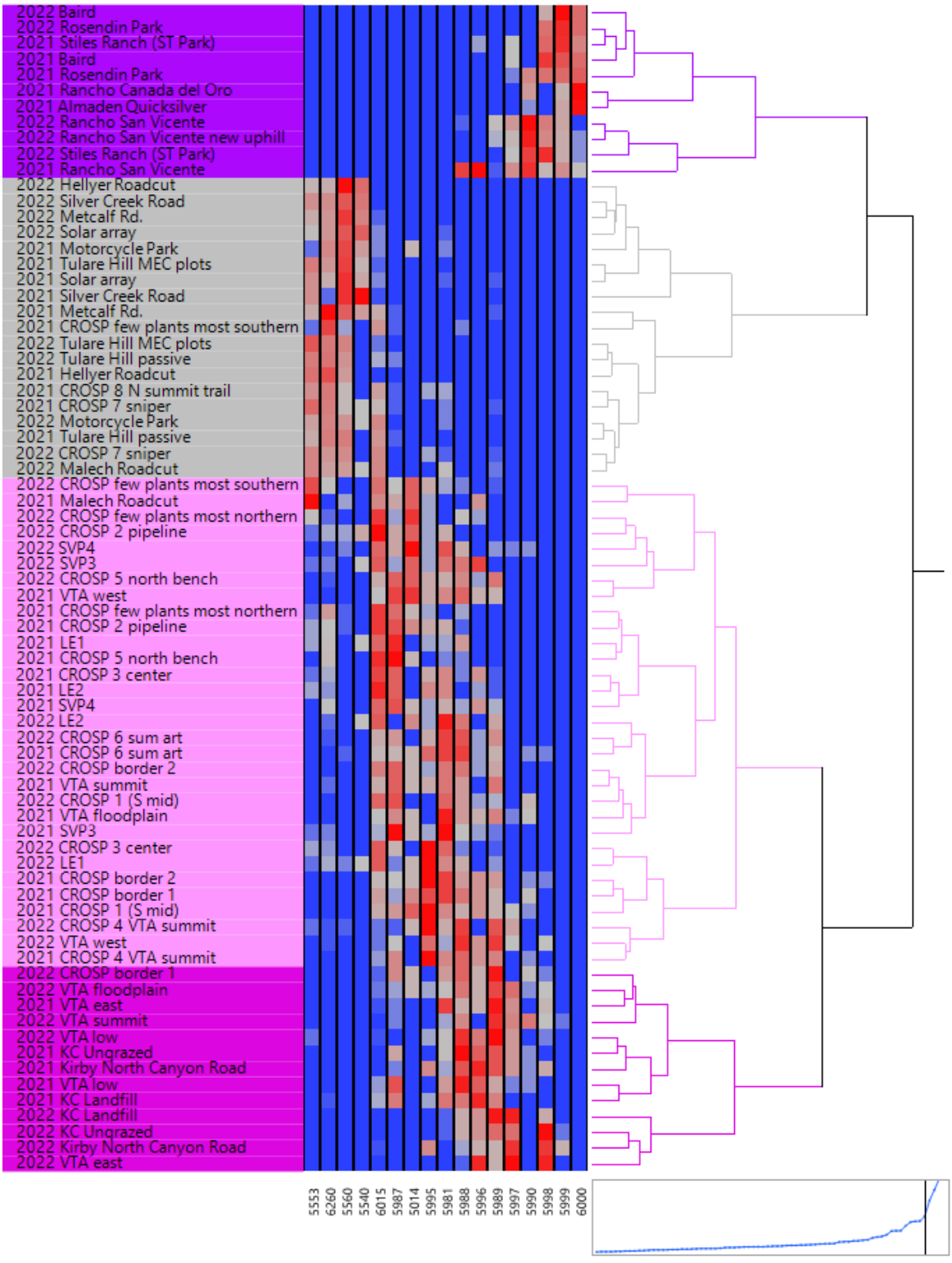


Figure 7. Dendrogram and associated graphical output (distance graph) from JMP 16.1. Clusters are color coded along a white-pink-purple gradient, but note that each population has a range of colors in the colormap section. Full explanation of the graphic layout is in the text.

This pattern can be seen more clearly in the parallel coordinate plots (Figure 8), where the frequencies of Dunn-Edwards colors within each population (lines) are grouped by clusters (different graphs). Each site is represented by one of the lines. The darkest plants are in their own cluster (purple). Note the gradient from south to north on Coyote Ridge, as populations transition from primarily white plants (gray), to mixed populations (light pink), to the loss of truly white plants among the pink plants (mid-pink), to a few dark plants appearing (dark pink). The geographic distribution of the four clusters is shown in Map 3. Remember that there are multiple phenotypes within each site, and the color coding reflects the four clusters described above. An initial biogeographic, genetic, and taxonomic interpretation of these patterns is presented in the Discussion section (below).

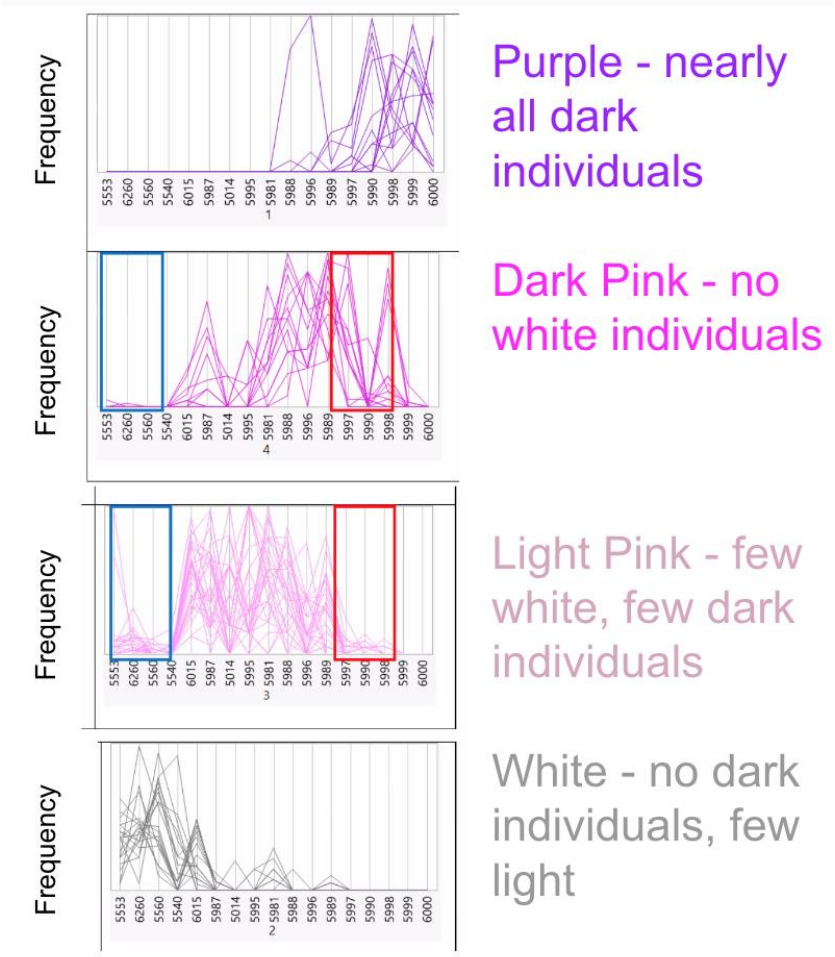


Figure 8. Parallel plots by cluster, arranged from darkest (top) to lightest (bottom) with the colors representing an ordinal ranking of the mean color of each cluster. The y-axis is the relative frequency (standardized to the maximum frequency in the cluster) of each Dunn-Edwards color (ordered by Value from left to right, light to dark as in Figure 7) in each site. Text describes the mixtures of colors present and the rectangles help delineate the white phenotypes (blue rectangle) and the dark pink phenotypes (red rectangle).

Another way to look at the data is with multivariate Principal Component Analysis (PCA), which takes into account the color correlations among sites. The positions of all the site-years in PCA space (Figure 9) shows how the clusters are separated roughly into quadrants in each score plot, but there is continuity and a small amount of overlap between the clusters in PCA space. Only three sites changed cluster assignment between years, a rate of 8% -- note how they are already at the borders between clusters. Site “Malech Roadcut” changed from light pink cluster to white cluster, “CROSP few plants southern” changed from white cluster to light pink cluster, and site “CROSP Border 1” changed from light pink to dark pink cluster. Note how close each year-over-year pair of samples that changes cluster are in the dendrogram (Figure 10 -- same as Figure 7, but with 2021 vs. 2022 pairs of samples that change color highlighted).

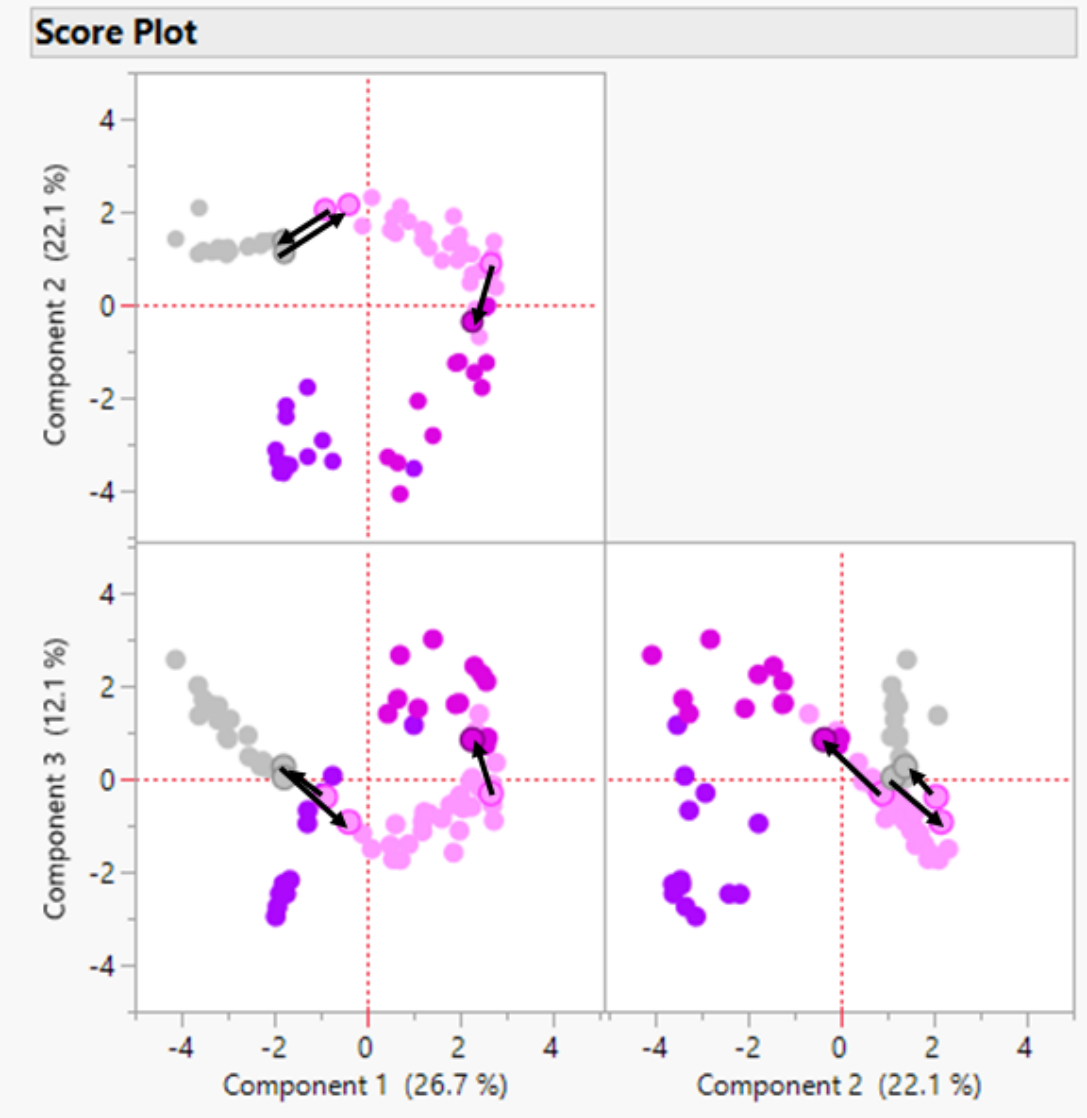


Figure 9. Principal component analysis (PCA) plots comparing color card frequencies at field sites. PC1 (26.7%), PC2 (22.1%), and PC3 (12.1%). Sites are colored based on the four major clusters in Figure 7 and described above. The three sites that changed cluster assignment between years are connected by black arrows.

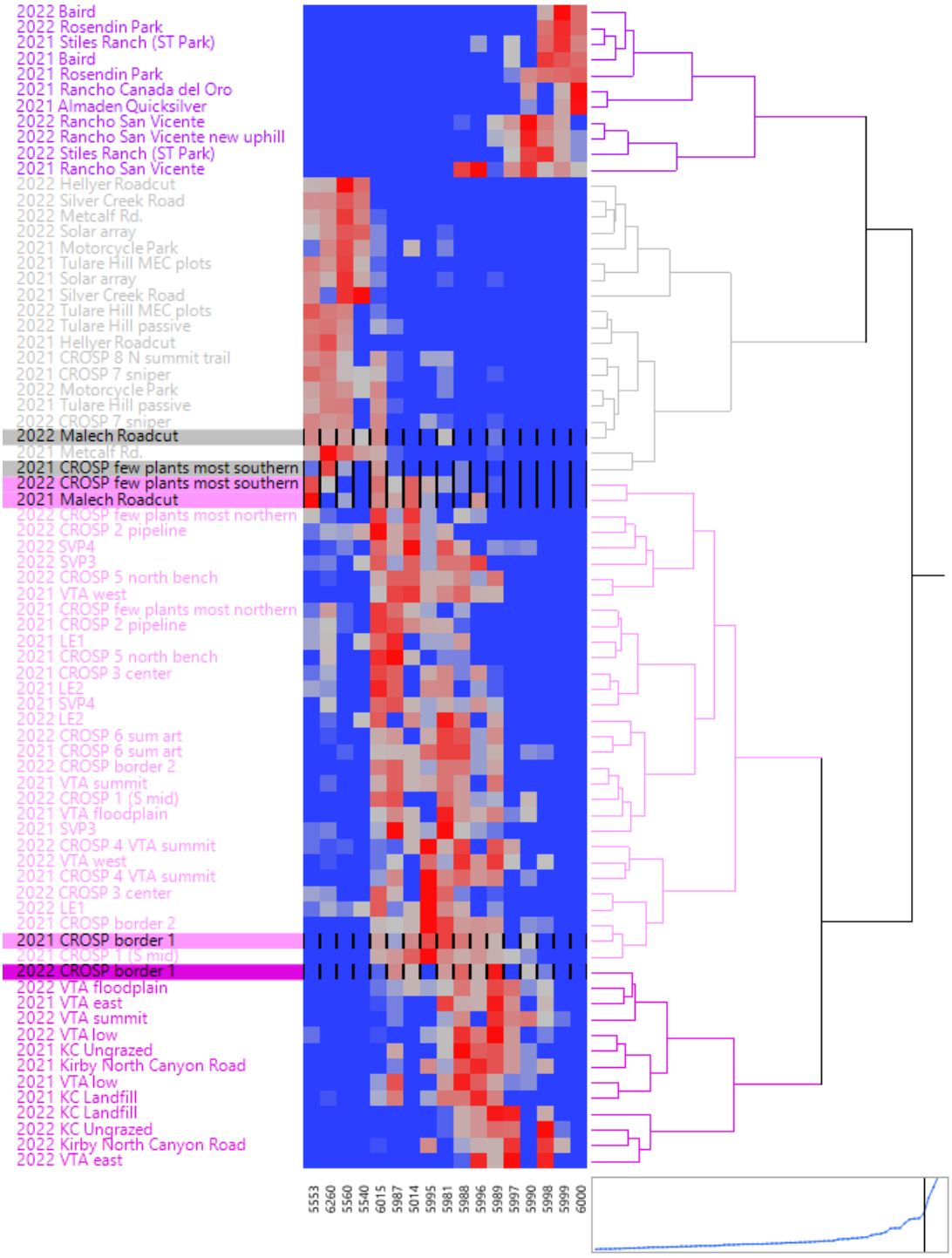
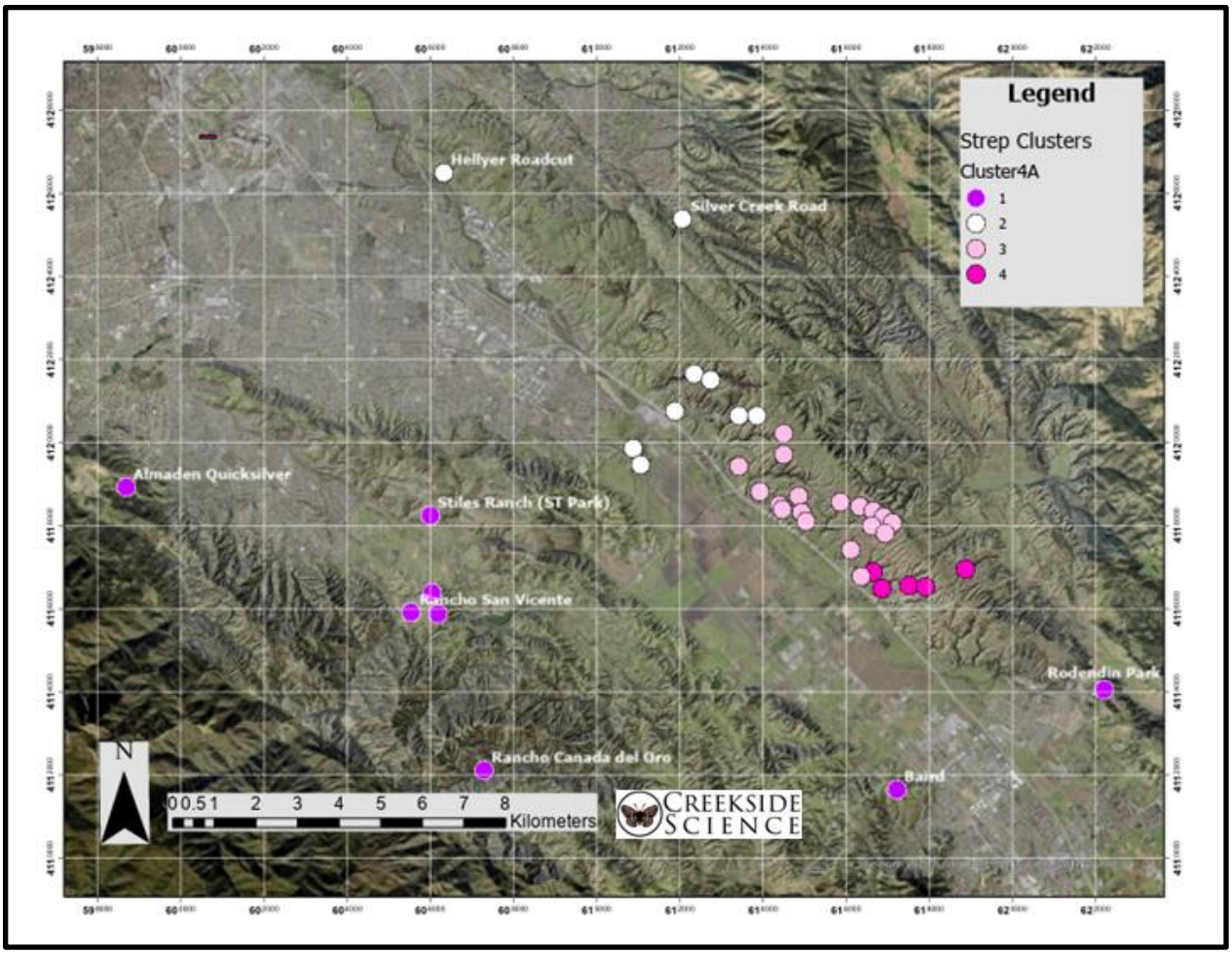
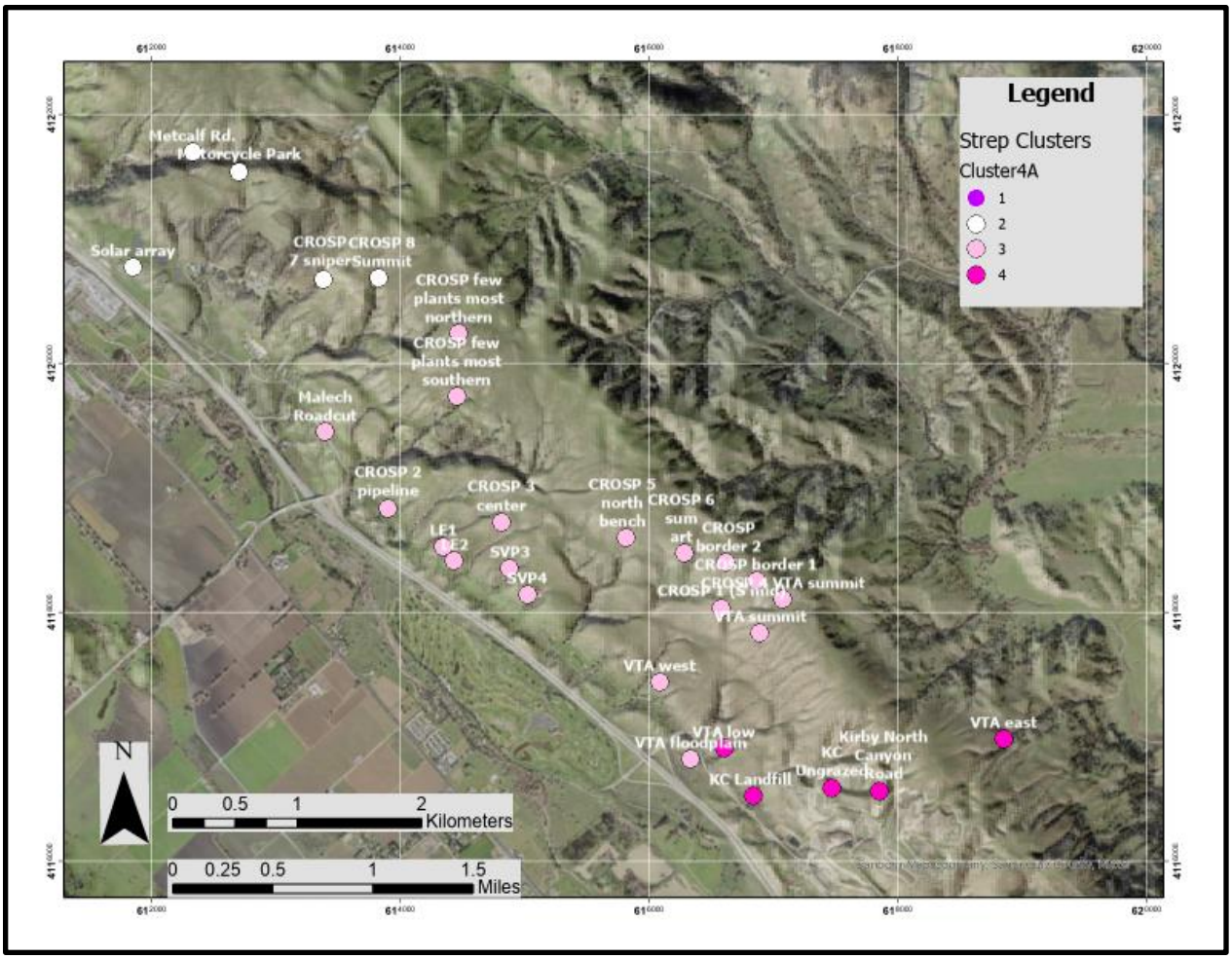


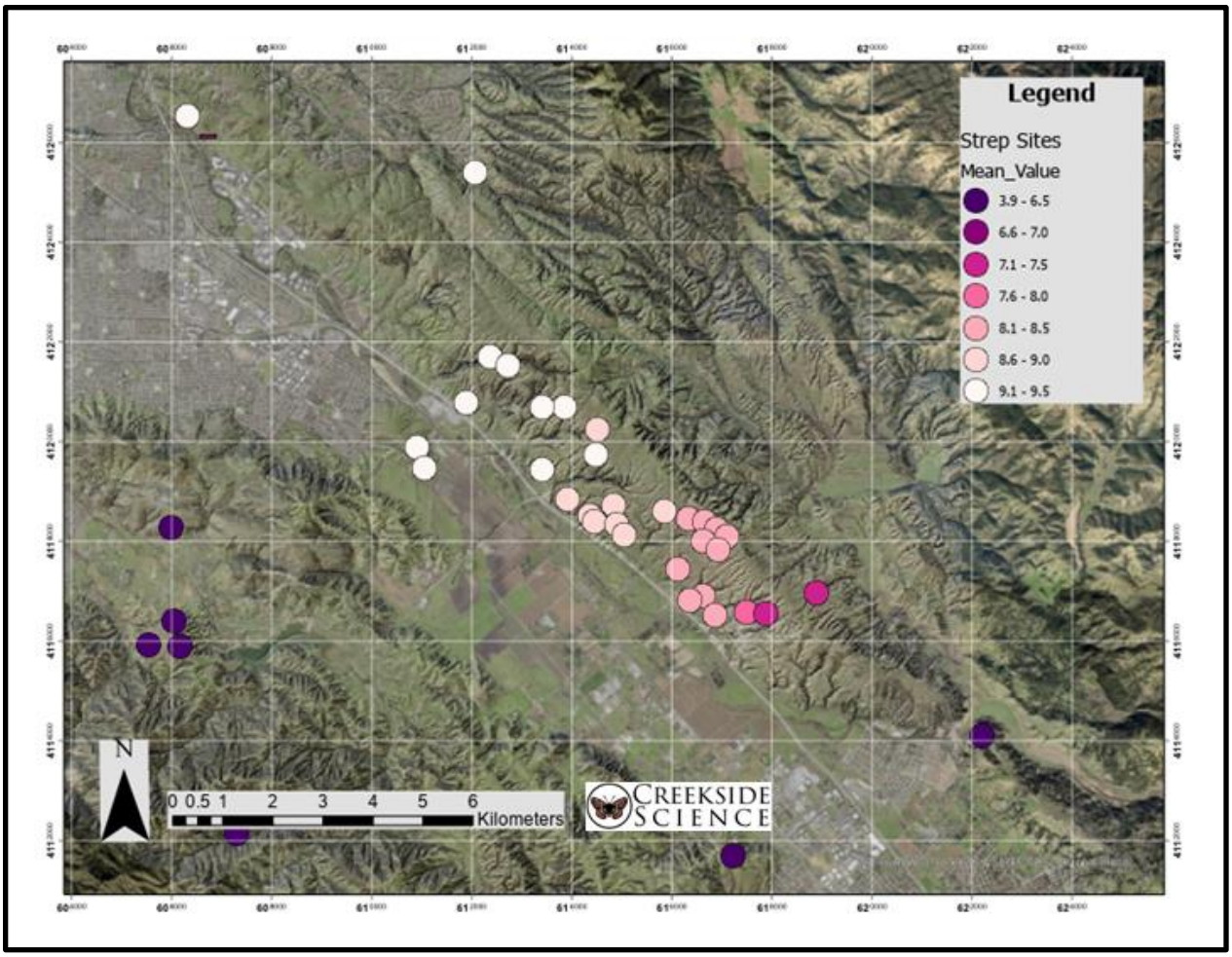
Figure 10. Dendrogram highlighting the three sites that switched cluster assignment between 2021 and 2022.



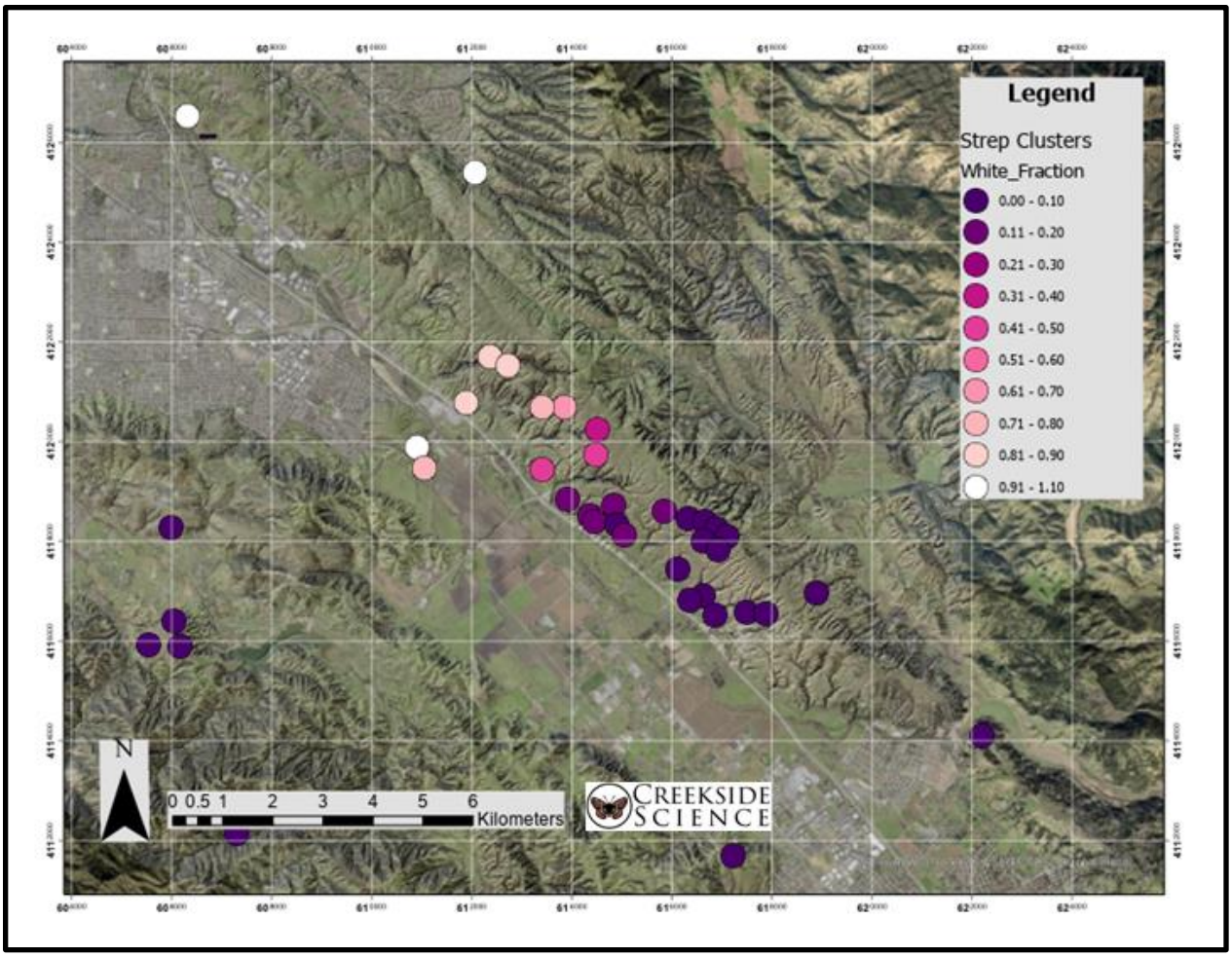
Map 3a. Geographic distribution of clusters shown in Figure 7, with the same gradient of pink distinguishing the clusters according to the order of light to dark. The northern sites are white flowered (Cluster 2), progressing south east along Coyote Ridge through light pink (Cluster 3) to dark pink (Cluster 4). Purple circles (Cluster 1) are farther south and west of Coyote Ridge. Note that each site and each cluster contain a range of Dunn-Edwards colors; review Figures 7 and 8 to see the amount of color variability in each site and cluster. Labels are left off on Coyote RIdge because of space limitations (site names shown on Map 3b). Data is from 2021.



Map 3b. Close up of Coyote Ridge with site names, showing gradients from white (Cluster 2) in the NW to light pink (Cluster 3) in the center and dark pink (Cluster 4) in the SE. Cluster 1 is off the map. Data is from 2021.



Map3c. Sites color coded by their mean Dunn-Edwards Value based on 2021 color card data. Almaden-Quicksilver is off the map to the west and would be dark purple (mean Value 3.9-6.5).



Map 3d. Fraction of white phenotypes among sampled sites from 2021 color card data.

Mean Dunn-Edwards Values of Sites

The mean Dunn-Edwards Value of each site in 2021 (with standard deviation) is organized along the gradient from lowest (darkest) to highest (lightest) (Figure 11). The cluster membership is shown as the gradient in white/pink/purple, as in Figure 7 and Map 3. Note that the clusters almost always correspond to the ordering by mean Value, but there are two exceptions, the Malech Roadcut (which is particularly variable in color, with a high frequency of white plants, but some mid- to dark-pink individuals), and the minor ordering difference in SVP 3 and 4 (which have nearly the same mean Value). Rancho San Vicente stands out with a unique mean Value between dark pink and purple, but it falls in with the purple cluster (Photo 10). The variability (SD) is highest at low values, and generally decreases as value increases.

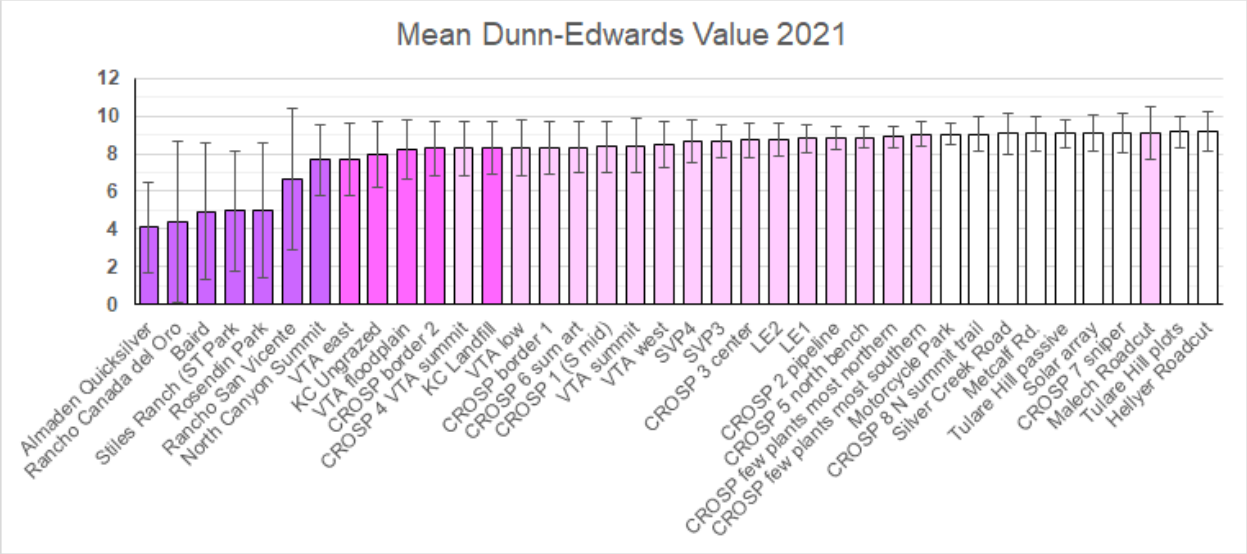
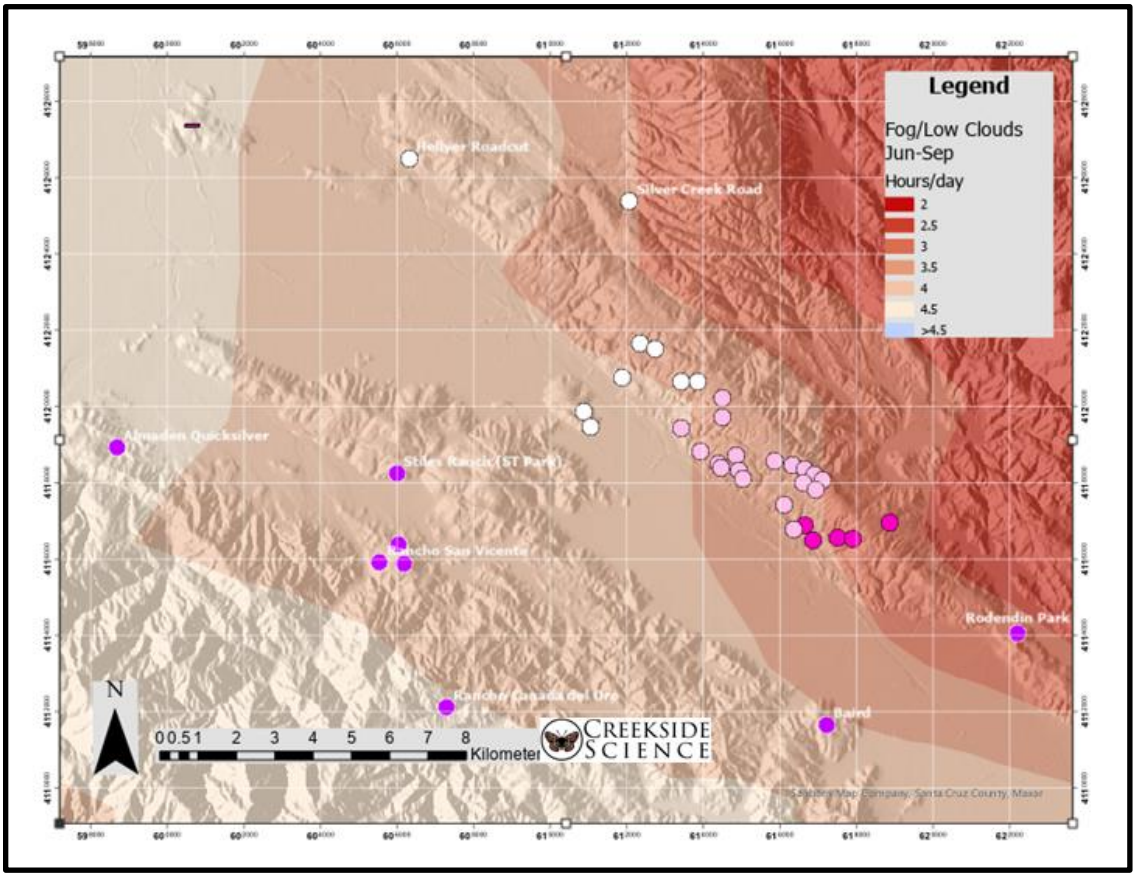


Figure 11. Cluster membership shown in a gradient of mean Value from the Dunn-Edwards color cards, with standard deviations (SD). Colored bars are determined by their cluster assignment as in Figure 7 above.

Photo 10. Flower color diversity at Rancho San Vicente from light pink (upper left) through purple (lower right).

Fog & Low Clouds

A map of the jewelflower color variation with summer-fall fog and low cloud cover superimposed (FLCC -- June through Sept., Torregrosa et al. 2016, data downloaded from www.bayarealands.org) indicates very little variation and no strong correlation with color. Specifically, the jewelflower sites along Coyote Ridge with the steepest cline in color covers a gradient of fog & low clouds of only ~1 hr/day (Map 4). The highest FLCC is at the purple flowered *S. glandulosus* site in Almaden-Quicksilver (4-4.5 hrs/day) and the lowest is in the southeastern tail of Coyote Ridge (i.e. Rosendin Park) which is also purple flowered. Although fog June through September may be a critical moisture source to prevent desiccation for some long-lived perennials like Coyote ceanothus (Hillman 2020) and *Arctostaphylos* spp., jewelflowers have largely completed their life cycles by June and are therefore less likely to be responding to this source of moisture. Therefore, FLCC alone is unlikely to be the driver of the color variation we see among these sites for jewelflowers, rather the short gradient is spatially correlated with the geographic/genetic gradient.



Map 4. Fog and low cloud cover June-September overlaid onto sample plots. Color coding of sites is based on color-card clusters as described in Figure 7 above.

Population Dynamics

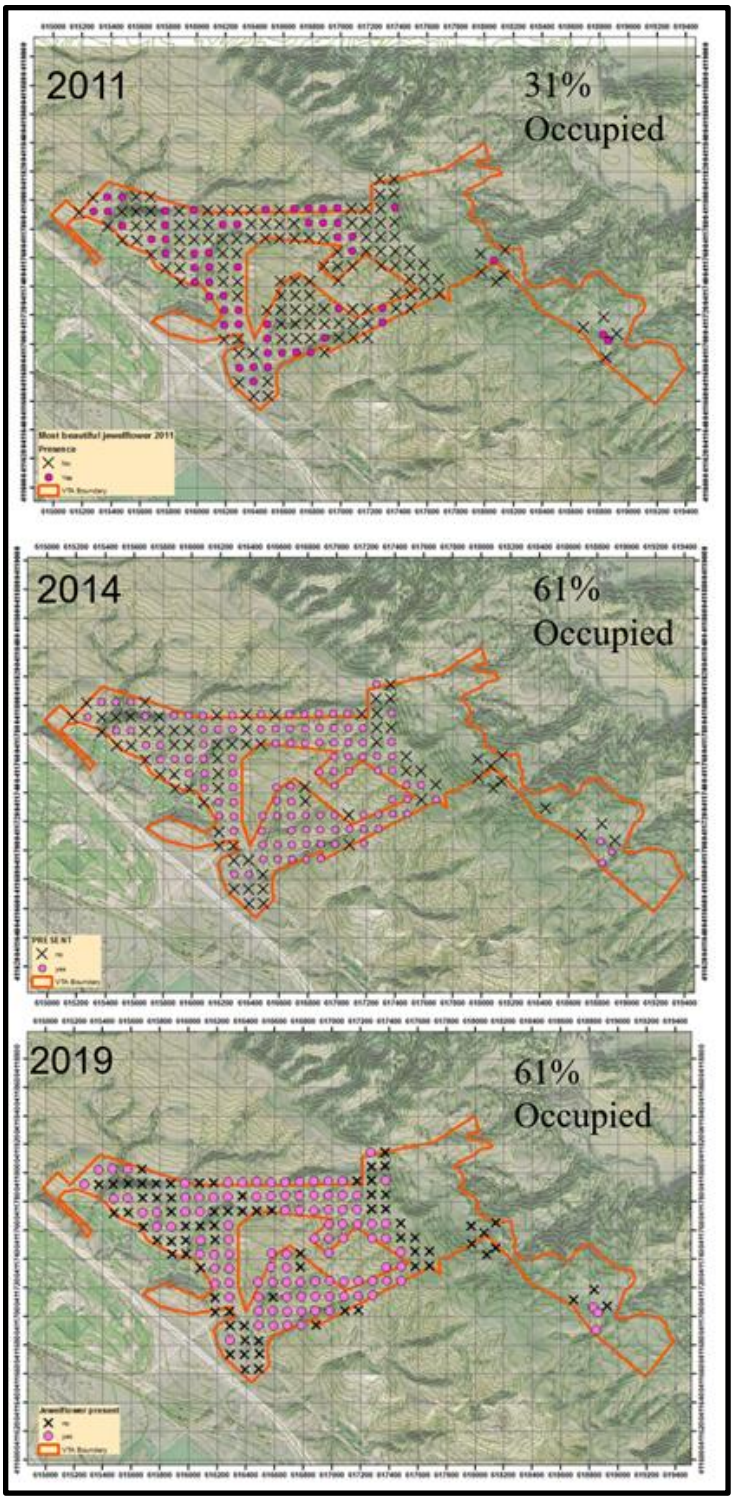
Annual plants have volatile population dynamics, which will affect the distribution of the taxa and the number of occurrences. From other projects, we have monitoring data from several local properties

where we can examine the range of population variability. The portions of these surveys that are relevant to this project are summarized in this section.

Occupancy Surveys

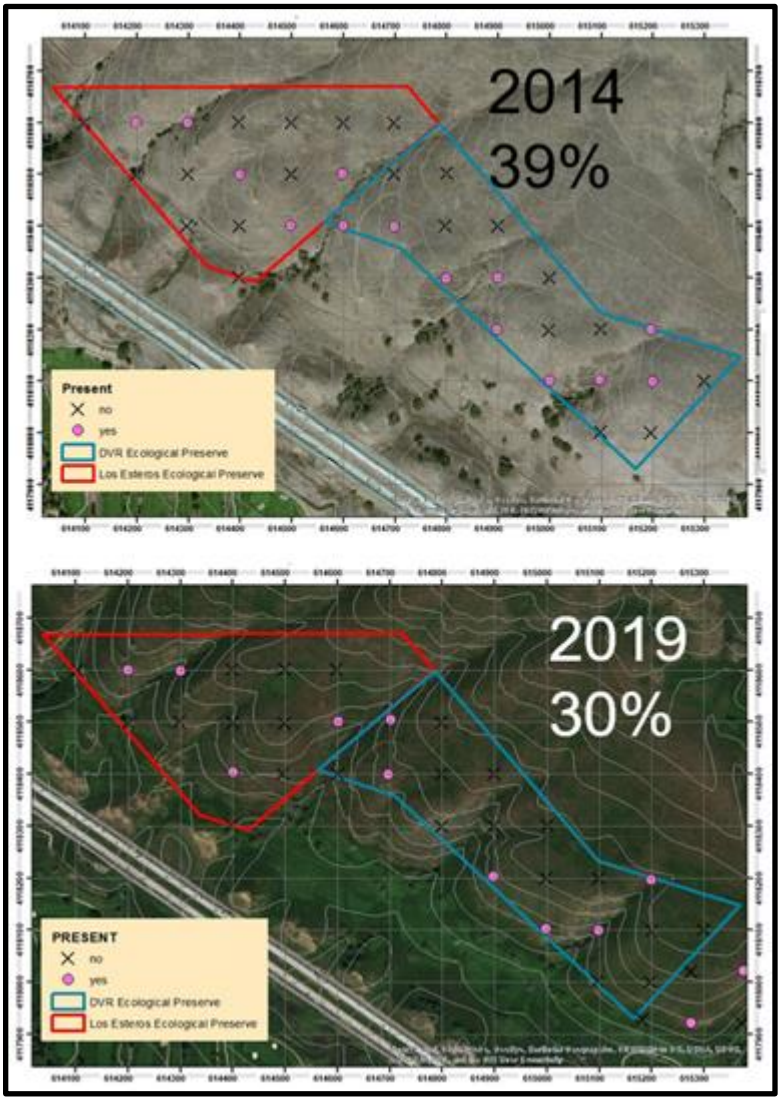
As part of the monitoring of several mitigation properties over the past two decades, the presence/absence of *Streptanthus* spp. at 100-meter grid points has been mapped. Repeated surveys document changes in distribution, and provide a basis for evaluating different definitions of occurrences for these protected taxa. The results from these surveys for three sites along Coyote Ridge are described below with relevant year-to-year maps for comparison in order to assess our definition of occurrences.

At Valley Transportation Authority (VTA, Maps 5a-c), surveys were done in 2011, 2014, and 2019 (the next survey is scheduled for 2024). Occupancy ranged from 31% in 2011, to 61% in both 2014 and 2019 (Creekside Science 2012, 2015, 2019a). There are subtle differences in the occupancy patterns in 2014 and 2019. In 2011, because of the separation distances, there would be several more occurrences than in the expanded distributions in 2014 and 2019 where only two can be discerned.



Maps 5 a, b, and c. Occupancy on 100 m grid on VTA in three years, 2011, 2014, and 2019 (Creekside Science 2019a).

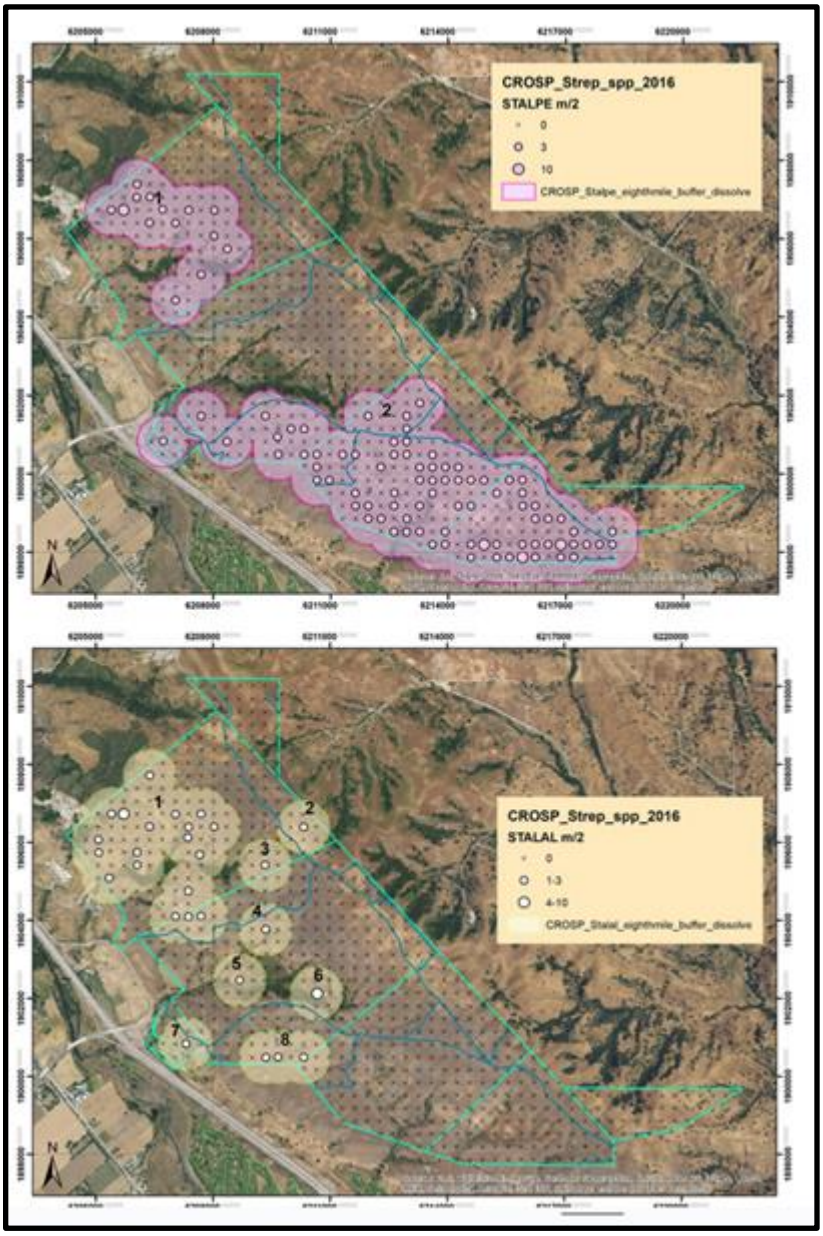
At Los Esteros and SIlicon Valley Power (LESVP, Maps 6a,b), occupancy over a much smaller area ranged from 39% in 2014, and 30% in 2019 (Creekside Science 2019b). The largest separation between occupied points was 233 m (0.14 mi), so all of the distribution would count as part of a single occurrence under the 0.25mi accepted default criteria in the Valley Habitat Plan and the CNDDB (ICF 2012). Note also that CROSP is the property immediately upslope (NE), so many of its populations of *Streptanthus* would be well within the 0.25 mi separation distance (see Map 7), emphasizing the need to work across property lines (i.e., the LESVP occurrence is a portion of at least one CROSP occurrence).



Maps 6 a, b. Occupancy on 100-m grid on Los Esteros and Silicon Valley Power (LESVP) parcels in two years, 2014 and 2019 (Creekside Science 2019b).

At CROSP in 2016 (Maps 7 a,b), the plants were visually (i.e., subjectively) classified into pink or white phenotypes at each grid point, and a log-scale abundance of each class estimated (size of circle). ⅛ mile

buffers around each point are included for each “taxon” (CCEO 2018). As mentioned above, this exercise demonstrates the inappropriate application of the current definition of occurrence because of the mixed populations.



Maps 7 a, b. Maps of occupancy and log-abundance of *Streptanthus* on CROSP in 2016, with ⅛ mile radius buffers according to definition of occurrences. Map 6a is for pink phenotypes, 6b is for white phenotypes (CCEO 2018). Color determination was done subjectively, before the Dunn-Edwards color cards were developed, yet produce sufficient granularity to clearly indicate that the traditional definition of an occurrence is not appropriate for these taxa in this region.

Population Numbers/Density

Jewelflower abundances can vary by more than an order of magnitude year-to-year thereby complicating the definition and persistence of occurrences. For example, abundances were estimated annually at six CNDDB occurrences from 2013-2016 (Figure 12 top). Over these four years, numbers fluctuated by a factor of 10 or more at each site. Population sizes at the smallest sites were below 100 in several years. The peak population year varied among sites, indicating some asynchronous population responses (Figure 12 bottom) (Niederer et al. 2017). Abundances have also been tracked at macroplots on VTA and LESVP properties showing similar fluctuations in population sizes (Creekside Science 2020, 2021). For example, over four sampled years (2012, 2014, 2015, and 2020) at VTA, population density fluctuated by a factor of ~10, with 2014 being the highest year (Figure 13 top). The highest densities were >10 plants/m^2. The Floodplain site crashed in 2015 to 0.2 plants/m^2, but had recovered to 10 plants/m^2 by 2020 (Figure 13 top). At LESVP, densities were much lower, and fluctuated by a factor 5 among years (Figure 13 bottom).

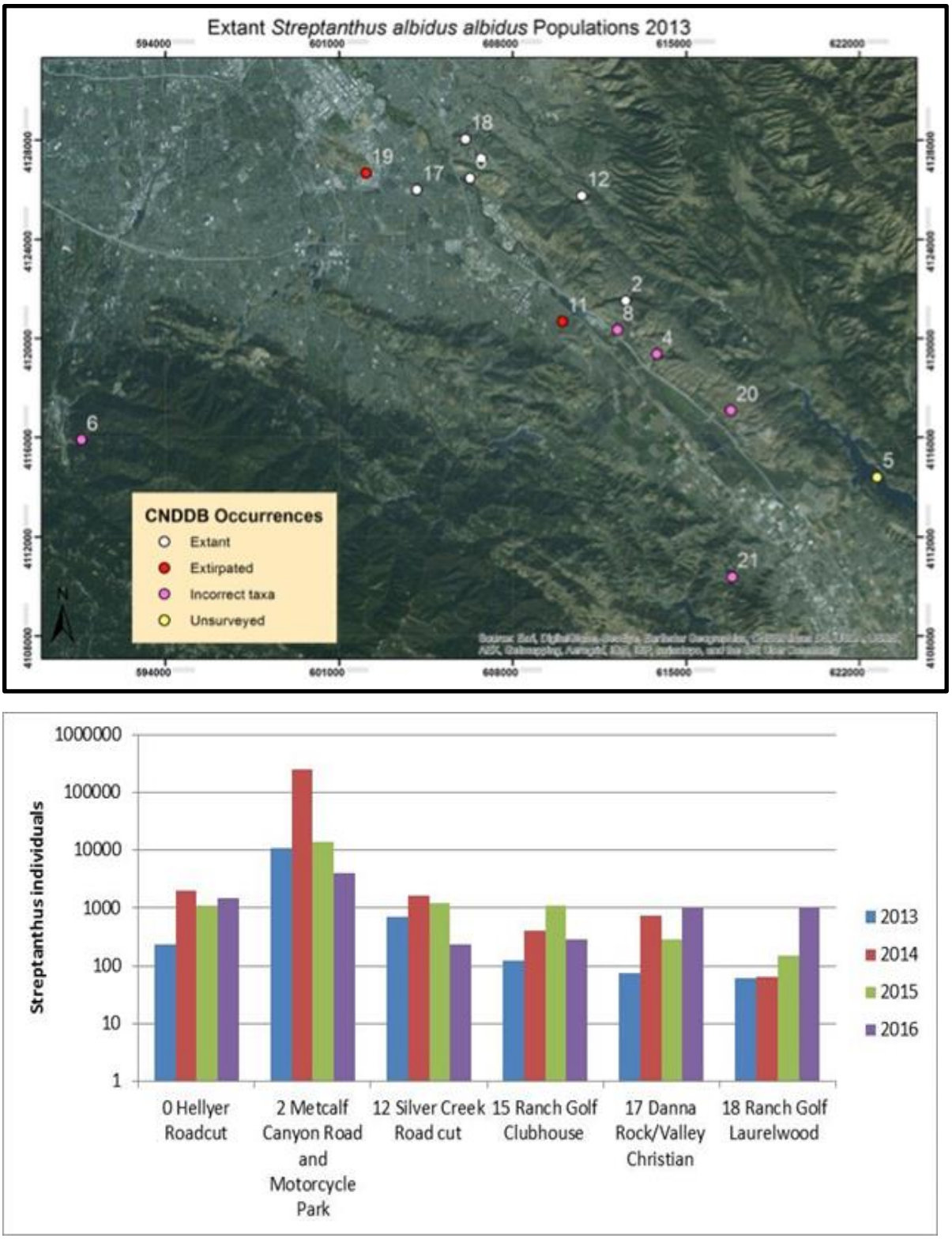
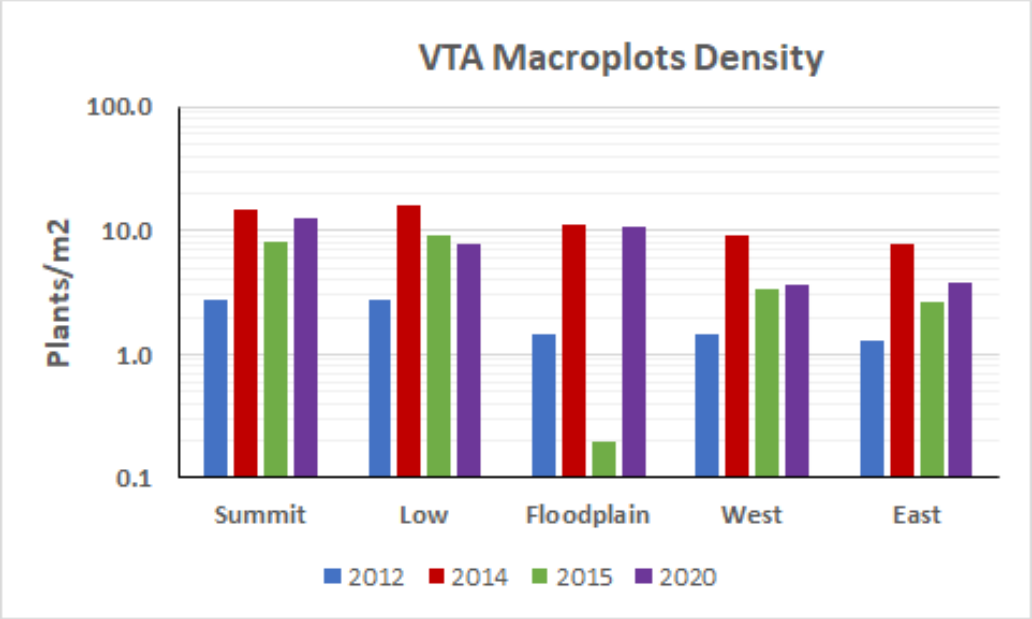


Figure 12. CNDDB occurrences (top), and four years of population estimates at five of these sites (bottom) (Niederer et al. 2017).



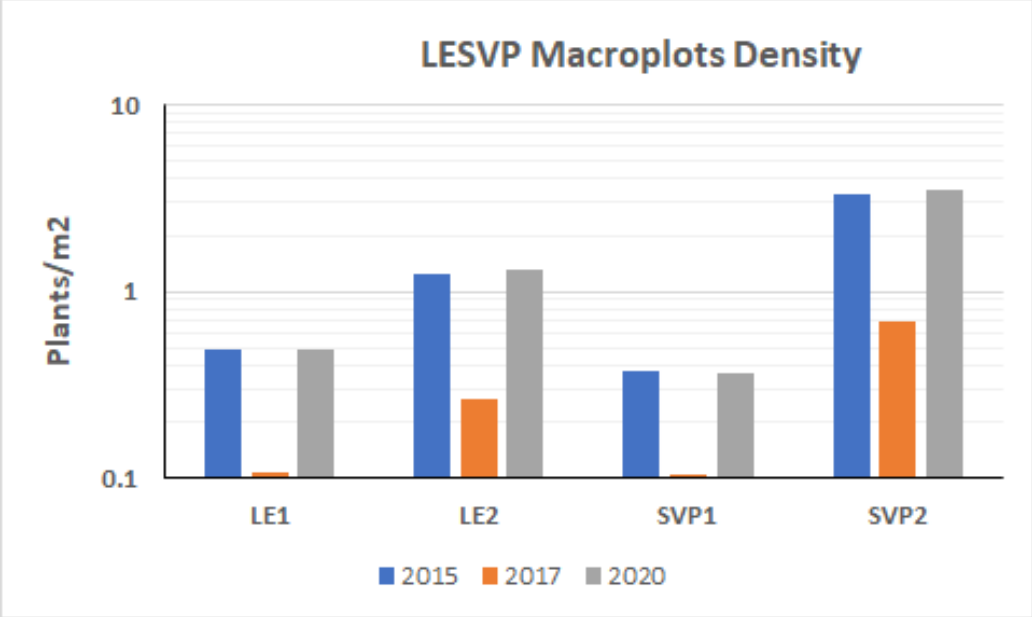


Figure 13. Population estimates in macroplots over time at VTA (top) and LESVP (bottom) properties. VTA was surveyed four times over nine years at five macroplots (Creekside Science 2021). LESVP was surveyed three times over six years at four macroplots (Creekside Science 2020).

Demographics

During a reintroduction project at Tulare Hill, detailed demographic monitoring across life stages was conducted (Niederer et al. 2017) which further informs how best to determine occurrences in these protected taxa. Some highlights from that study include:

1) Survival rates from seed to pre-fruiting adult was on the order of 1-3%, and varied by grazing, year planted, and site, but was not affected by seeding density (ranged from 100 to 400 seeds/m^2).
2) Mature plants suffered herbivory from grasshoppers and cattle in some years and sites.
3) Reference plots with no plants at the Motorcycle Park in 2013 were full of plants in 2014, indicating a large seed bank.
4) Large plants with multiple branches and fruits dominate reproductive output, although there can be many small plants present.
5) Despite it being the driest year of the decade, 2014 turned out to be the best jewelflower year across many sites. The late start of the rains (February) somehow gave the right cue for germination and allowed the plants to survive and thrive, perhaps through reduced competition with annual grasses.

Overall, the populations of jewelflowers on Coyote Ridge are widespread, and often diffuse across large areas. Dividing them up following traditional occurrence definitions (i.e. ¼ mile separation) is not a workable definition and does not reflect the complexity of the population dynamics. Large interannual fluctuations are normal for these annual taxa as we have documented in the past two decades. In low abundance jewelflower years, the distribution breaks up into more distinct populations separated by ¼ mile or more (yielding more, but smaller occurrences), only to merge and reduce the overall count during high abundance years (i.e. fewer, larger occurrences). **No conservation goals are met by constantly redrawing population boundaries following the natural rhythm of jewelflower population fluctuations.**

Discussion

Our combined ongoing genetic analysis and completed fieldwork has established that flower color is incompletely dominant leading to a range of flower color phenotypes. We have developed a quantitative method for scoring the color composition of *Streptanthus* around the Santa Clara Valley. The technique of collecting color frequencies using the laminated Dunn-Edwards tool was found to be simple, objective, and therefore consistent and repeatable among observers. This tool allows practitioners to assess sites with jewelflowers, count occurrences, and help guide conservation priorities.

This study has rigorously documented that there is a NW-SE gradient along the main axis of Coyote Ridge, with white-dominated populations at the northern end extending to just south of Metcalf Canyon. The remainder of the Coyote Ridge sites fall into a high-level cluster that exhibits a gradient from white/light pink in the northwest to pink-dark pink toward the southeast, with locally mixed white-pink populations in the middle. The cluster analysis did detect two fuzzy breaks among the populations as white plants fade away and pink predominates and where dark individuals start appearing. Three sites at the borders of these clusters switched cluster assignment from 2021 to 2022, but otherwise >90% of sites remained in the same cluster between years. Much darker pink-purple populations are on the western side of the Santa Clara Valley, as well as one just south of Coyote Ridge.

This is a classic cline of a phenotypic character on Coyote Ridge, without clear breaks – it is a matter of proportions of the different phenotypes in the populations. To determine conservation status,

taxonomy is assigned to the populations, not to individuals. It is possible to say that an individual plant has the white phenotype (or light pink, dark pink or purple as determined by the Dunn-Edwards colors), but saying that the individual plant is one subspecies or another is invalid since it is part of an interbreeding population that may contain other phenotypes. Therefore, we emphasize that **managers must assign populations to taxonomic units. Assigning individuals within a mixed population to taxonomic units is invalid for these taxa.**

Care should also be taken not to call intermediate phenotypes "hybrids" because the word hybrid suggests a combination of previously differentiated entities which has not been established here (see Whittall and Strauss 2011, Niederer et al. 2017).

Valley Habitat Plan Revision

It is our understanding that the Valley Habitat Plan will soon be revised, and the information in this project should be used to address the way these jewelflowers are conserved. To determine whether an occurrence of *Streptanthus* should count toward ssp. *albidus* or ssp. *peramoenus*, the key question becomes "what frequency of the white phenotype is required to be ssp. *albidus*?" The answer depends on the underlying genetics. Thus far, we know that artificially created F1 crosses produce intermediate levels of sepal pigmentation indicating that flower color is incompletely dominant (see Photos 8 & 9, Figure 5). Although we don't have the flower color phenotypes of the F2 population yet, for the remainder of this report, we will assume that the majority of the variation can be attributed to a one locus, two allele model similar to that determined in many other plant taxa (reviewed in Sobel and Streisfeld 2013; for specific examples, see work on *Parrya nudicaulis* by Dick et al. 2011; *Phlox drumondii* by Hopkins and Rausher 2011; *Ipomoea purpurea* by Zuffall and Rausher 2003; *Diplacus aurantiacus* by Streisfeld and Rausher 2009).

A very simple exploratory example, to illustrate the process, is a one locus, two allele system where the white allele is incompletely dominant. In this case, we hypothesize that the white allele is a loss of function allele that causes the heterozygotes to exhibit intermediate levels of pigmentation compared to the homozygotes. By assuming Hardy-Weinberg Equilibrium, we can then determine the frequency of the white allele from the proportion of white phenotypes (assuming they are homozygous for the white allele). The results are in Figure 14, showing that a cline in allele frequencies can produce a cline in phenotypes.

Figure 14. A cline in allele frequencies according to the Hardy-Weinberg equilibrium ($w^2 + 2pw + p^2 = 1$), w = white allele frequency, p = pigmented allele frequency.

A simple cline in allele frequencies in a two allele system with an incompletely dominant pink allele, and a white allele (loss of anthocyanin function) shows how such a system can produce a cline in phenotypes. The frequency of white phenotypes (w^2) represents the homozygous white fraction of the populations, and can be used to estimate the frequency of the white allele (w).

Note that a relatively high fraction of white alleles (i.e. 30%) will only produce 10% white phenotypes under Hardy-Weinberg conditions (Figure 14).

The actual field data on the proportion of white phenotypes transformed into allele frequencies assuming Hardy-Weinberg as indicated above, shows the strong geographic gradient. Populations in the white cluster all have white allele frequencies of >0.8 (or >80% white alleles). The light pink cluster extends from white allele frequencies of 0.75 down to 0.0 (75%-0%), and the dark pink cluster has very low frequencies of the white allele (<10%) (Figure 15).

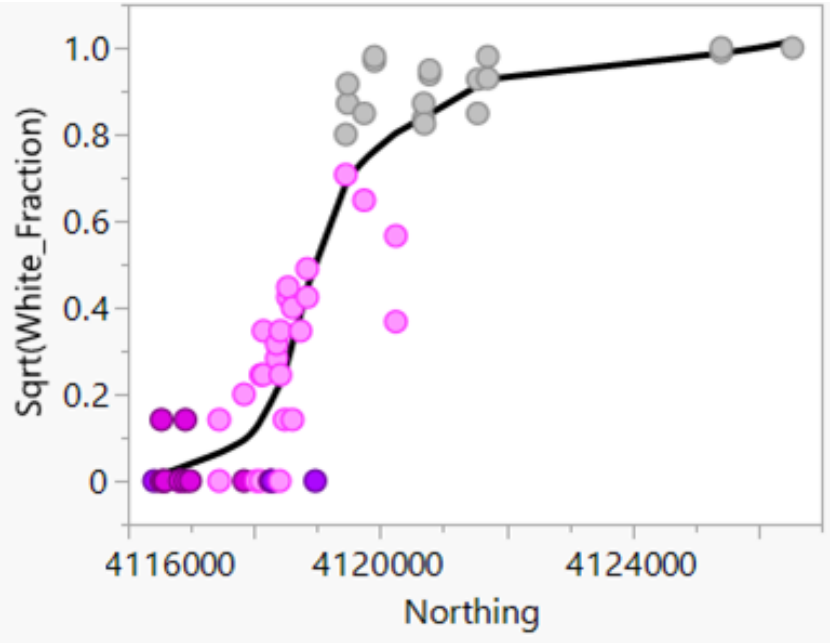


Figure 15. Field data on frequency of white phenotypes converted into white allele frequency assuming a one gene, two allele system and Hardy-Weinberg Equilibrium (i.e., as sqrt(white fraction)) plotted as a function of Northing coordinate (meters UTM Zone 10). White flowered sampled sites have higher allele frequency than sampled sites composed of pigmented individuals (>80%). The inability to discriminate between light pink, dark pink, and predominantly purple flowered sites at low allele frequencies is likely due to violations of the one gene, two allele assumption (i.e. one or more modifier loci).

A multi-allele system, and/or a multi-locus system will produce more complex patterns and more continuous phenotypic spectrum from white (still homozygous for loss of function) to varying shades of pink to purple, but the basic cline in phenotypes, like what is observed in the field, will remain evident (Map 3).

No matter what the details of the underlying genetics are, there is an active evolutionary process taking place on Coyote Ridge that should be the target of conservation. Whether the white phenotype is spreading south, or the pink phenotype is spreading north is an interesting biogeographic question, and the plant itself will eventually answer that question. Below we offer a hypothetical evolutionary history of these jewelflowers focused on the cline in flower color variation detected in southern Santa Clara County and Coyote Ridge in particular.

A Hypothesized History

How might the variation in flower color in Coyote Ridge jewelflowers have evolved? We speculate that at one point (say in the cool moist Pleistocene) the populations at the north end of Coyote Ridge were isolated by an increase in shrubs in the area just south of Metcalf Canyon (serpentine outcrops in wetter parts of Santa Clara County are dominated by leather-oak chaparral) -- there is already a bottleneck of serpentine in this area. These northern populations became fixed on the white allele by genetic drift (small population), inbreeding (small population), or some unidentified agent of selection (not present-day pollinators). When greater continuity of grassland was re-established with warming and drying in

the Holocene, gene flow between pink populations to the south and white populations to the north created the observed phenotypic gradient. Alternatively, the entirety of Coyote Ridge could have been fixed on white and the flow of darker alleles from purple populations south of Coyote Ridge (like present day Rosendin Park) entered the white flowered populations via pollinators and seed dispersal and the pigmented phenotypes progressively expanded north.

Original Valley Habitat Plan Treatment

In the original Valley Habitat Plan treatment, there were two distinct taxa, *Streptanthus albidus* ssp. *albidus* (Metcalf Canyon Jewelflower, MCJF) with 11 extant occurrences, and *Streptanthus albidus* ssp. *peramoenus* (Most beautiful jewelflower, MBJF) with 38 extant occurrences (Maps 8a, b). (This taxonomy was based on the first edition of the Jepson Manual, Hickman 1993). Some of the MCJF occurrences were likely based on the presence of a few white or light pink individuals in a larger population of darker plants. Darker populations in the Santa Cruz Mountains were treated as MBJF.

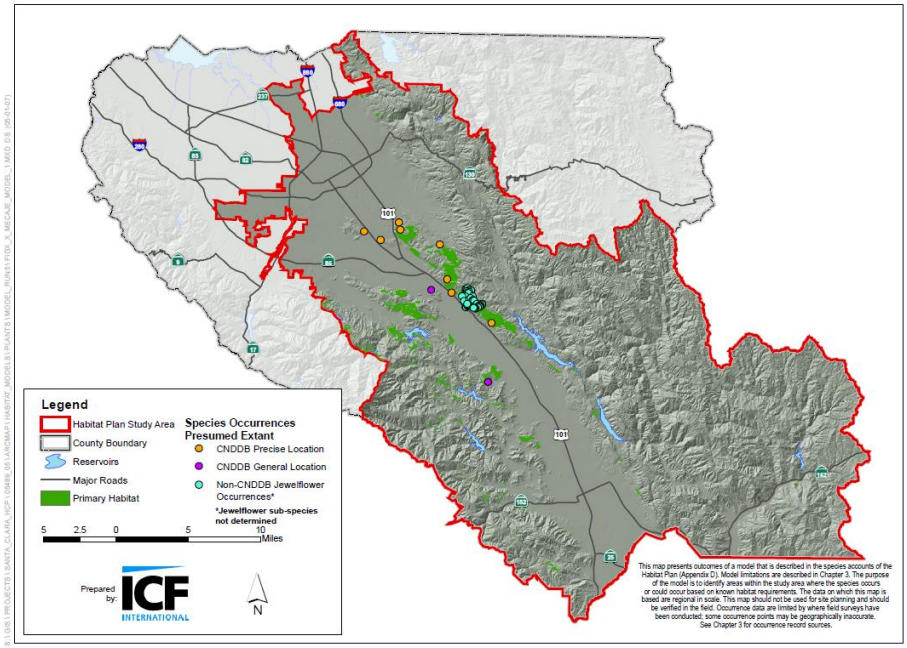


Figure 2
Metcalf Canyon Jewelflower Modeled Habitat Distribution - Santa Clara Valley Habitat Plan

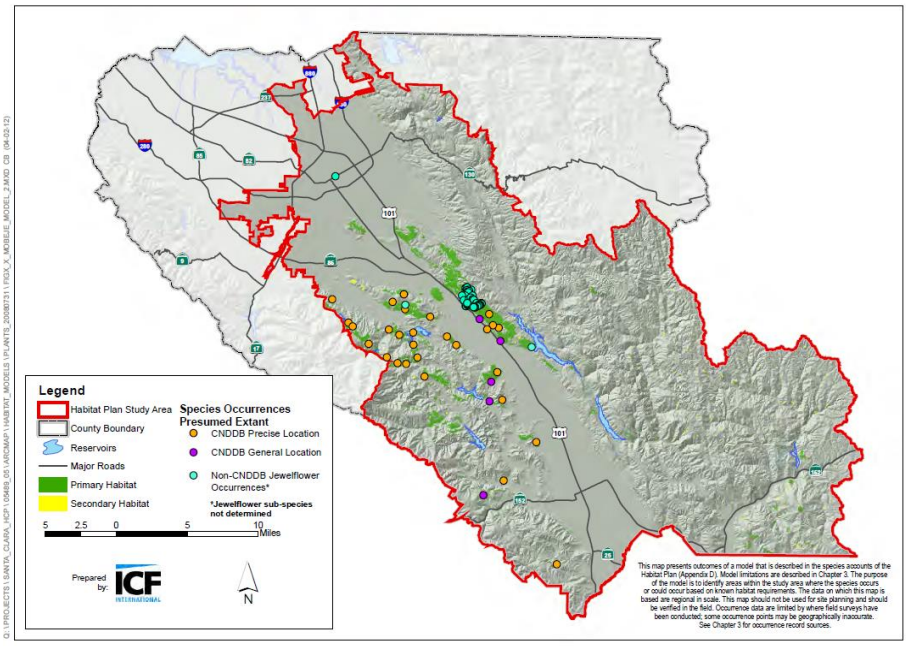


Figure 2
Most Beautiful Jewelflower Modeled Habitat Distribution - Santa Clara Valley Habitat Plan

Maps 8a, b. Santa Clara Valley Habitat Plan (ICF 2012) describes 11 extant Metcalf Canyon jewelflower occurrences (top) and 39 most beautiful jewelflower occurrences (bottom) based on CNDDB observations that did not account for natural variation in flower color.

A more current taxonomic treatment (the second edition of the Jepson Manual, Baldwin et al. 2012) recognizes two taxa, *Streptanthus glandulosus* ssp. *glandulosus,* (Bristly jewelflower BJF with purple and

pink phenotypes) and *S. glandulosus* ssp. *albidus* (MCJF, white phenotypes). This classification does not differentiate the pink phenotypes along Coyote Ridge and is based on insufficiently sampled, weakly supported nuclear ribosomal internal transcribed spacer molecular phylogeny for the Coyote Ridge sites and adjacent populations (Mayer & Beseda 2010).

A more recent molecular phylogenetic investigation uses AFLP markers scattered throughout the genome with dense sampling of southern Santa Clara County populations including many of those used in this color study reported herein. The results suggest that the white flowered individuals represent a distinct lineage of *Streptanthus albidus* ssp. *albidus* (STALAL) and the pink flowered individuals represent *S. albidus* ssp. *peramoenus* (STALPE) (Figure 16 top tree) (Whittall and Strauss 2011; Whittall et al. in prep). The pattern is consistent in both a phylogenetic context and in a population genetic analysis (Figure 16 bottom graph). Support values in the phylogenetic analysis are low and the presence of admixture in the population genetic analysis indicate that more markers may be necessary to disentangle these recently evolved, interfertile taxa that may be occasionally exchanging genes.

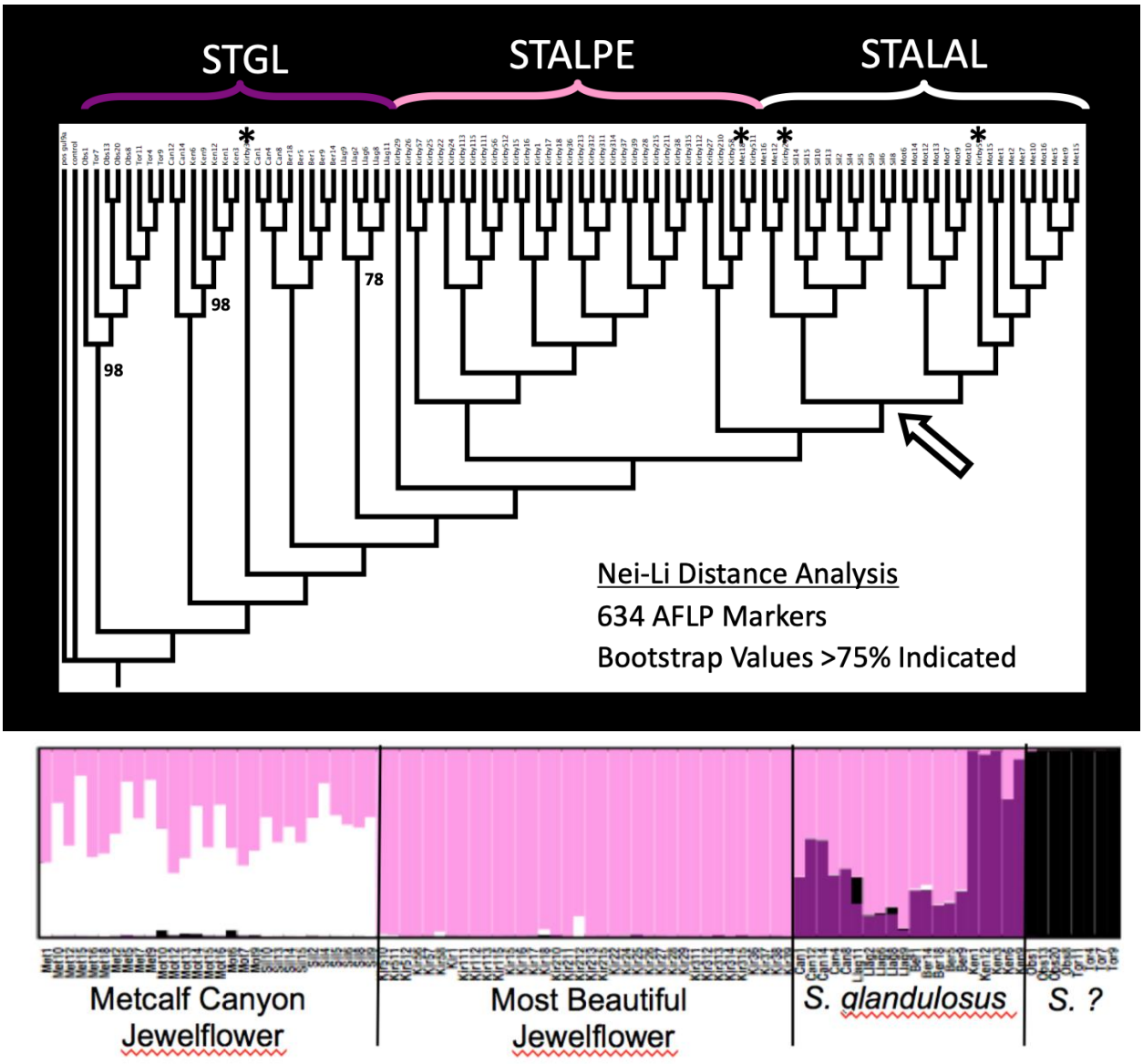


Figure 16. AFLP molecular markers were scored for several individuals per population for three populations of *S. albidus* ssp. *albidus* (STALAL), pink *S. albidus* ssp. *peramoenus* individuals spanning Coyote Ridge (STALPE) and four populations of *S. glandulosus* from west of HWY101 (STGL). Two populations with nearly black sepals from atop Mt. Hamilton are outgroups (*S. ?*). The phylogenetic analysis (A) with bootstrap values shown when >70% produced nearly completely monophyletic Metcalf Canyon jewelflower and most beautiful jewelflower (see asterisks for exceptions). In a population genetic analysis (B), STRUCTURE identified four clusters and although there is considerable admixture (likely due to recent common ancestry or introgression), there are three distinct groups plus the outgroup. Color coding reflects approximate human sepal pigmentation.

Defining occurrences and conservation targets and goals: *There is a dynamic evolutionary process taking place along Coyote Ridge where* Streptanthus *exhibits high interannual population variability and gene flow that produces a marked phenotypic gradient in sepal color. The full range of that process should be considered the conservation target.*

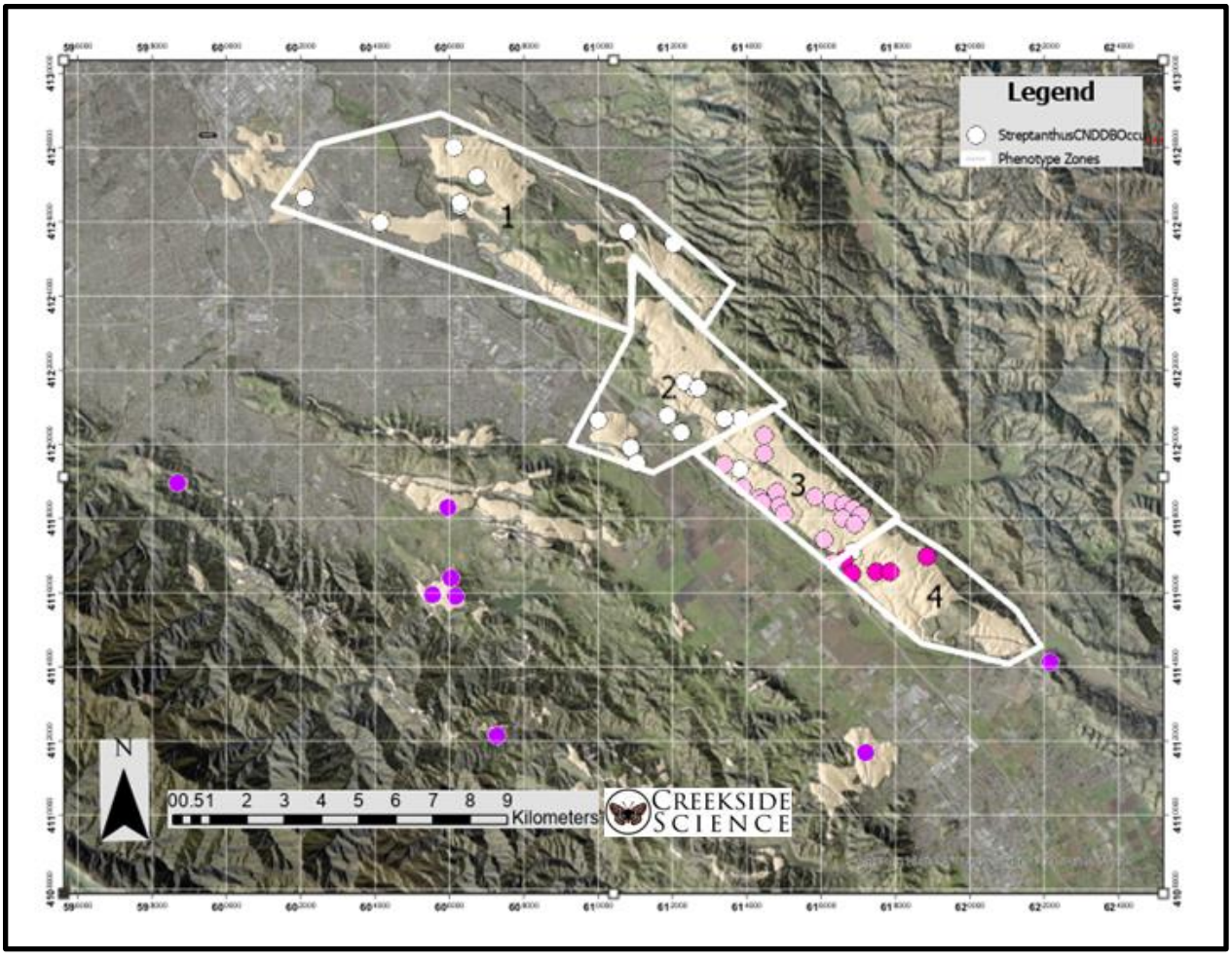
Securing and managing the full phenotypic gradient from white to dark pink and allowing it to evolve through time is the conservation goal. Under no circumstances should one taxon in a blended location be managed for lower numbers in order for the other to increase its numbers. The evolutionary processes taking place along Coyote Ridge are more complex than a typological taxonomic approach to conservation can currently account for.

Any definition of occurrences needs to reflect this process, and fully document success of the Valley Habitat Plan in meeting that goal. The original definition of occurrences is not usefully applicable along the semi-continuous distribution and phenotypic complexity on Coyote Ridge, where the bulk of the overall jewelflower population resides. We suggest the following outline for redefinition of jewelflower occurrences.

Delineate four phenotypic zones on Coyote Ridge to geographically distribute occurrences and represent the gradient. An initial suggestion, subject to modification is shown in Map 9:

1) The Silver Creek Hills and isolated populations north of Young Ranch including the Richmond Ranch where there are 100% white phenotypes.
    a) Conservation status: Two sites are within the Silver Creek Hills conservation parcels. Valley Christian site is privately owned. Roadcuts may provide opportunities for introductions of white phenotypes. Richmond Ranch is not yet conserved.
2) Young Ranch, Metcalf Canyon, and Motorcycle Park, with low frequencies (4-24%) of light pink phenotypes. This includes Tulare Hill. Breakline is the first canyon SE of Metcalf Canyon, and it corresponds to the SE limit of Cluster 2 (Figure 7, Map 3b).
    a) Conservation status: The Young Ranch supports large extensive populations, as does the Richmond Ranch. These parcels should account for multiple occurrences once mapped. The SE limit is conserved as part of CROSP.

3) Malech Roadcut SE to Coyote Creek Golf Drive, from base to the crest of the ridge, which corresponds to Cluster 3. At the NE end there are some white phenotypes, but fade to very low frequencies progressing SE.
    a) Conservation status: All of this area is conserved between CROSP, VTA, LESVP.
4) Coyote Creek Golf Drive SE to the end of Coyote Ridge (Anderson Dam), the darkest pink corresponding to Cluster 4.
    a) Conservation status: Valley Water and VTA are conserved, NE of Pigeon Point is SC County Parks, remainder is O'Connell Ranch (private). In 2021, an initial search of O'Connell Ranch did not find jewelflowers but additional searches in better jewelflower years should be done.
5) W of Coyote Valley and SE of Coyote Ridge, on more isolated serpentinite outcrops, the purple phenotype corresponding to Cluster 1.
    a) Many of the larger serpentine outcrops are now conserved. There are many unmapped purple jewelflower populations on smaller isolated serpentine outcrops in this region (i.e., Lakeview and Tilton).



Map 9. Proposed jewelflower conservation zones dividing up the flower color variation along Coyote Ridge (& northward). Serpentine soils indicated in tan. Colors match clustering as in Map 3 that accounts for natural variation in flower color.

Such geographic stratification to capture gradients is a useful tool in conservation planning. For example the Bay Area Conservation Lands Network (Bay Area Open Space Council 2019) stratifies the region by mountain ranges to distribute conservation goals for vegetation types and individual taxa.

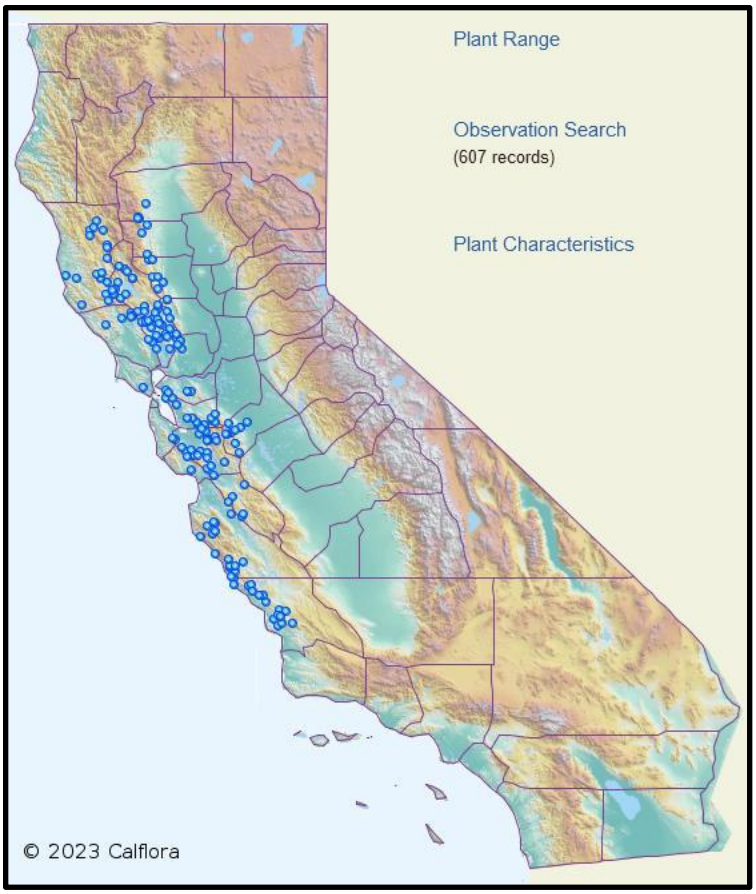
Taxonomic Nomenclature vs. Phenotypic Zones

In Map 9, Zone 4 corresponds most closely with the original description of the most beautiful jewelflower (Hickman 1993). However, Zone 3 represents a continuous cline in flower color variation with mixed populations containing white, pink, and dark pink individuals (see Figures 7 & 8). Zones 1 & 2 represent the pure white and nearly pure white sampling sites (>90% sampled individuals white flowers -- one of the four color cards with the highest Value) correspond to the originally defined Metcalf Canyon jewelflower (Hickman 1993).

In terms of the newer taxonomy (Mayer & Beseda 2010 integrated into Baldwin et al. 2012), Zones 1 and 2 are still *S. glandulosus* ssp. *albidus*, but 3, 4, and 5 (discussed above) become ssp. *glandulosus* (a non-listed taxon).

Aligning our phenotypic zones with either version of these currently available Streptanthus *taxonomies does not reflect the biological reality.*

We also note that the numerous populations with purple/dark pink phenotypes in the Santa Cruz Mountains and south of Coyote RIdge are part of a much larger distribution of *S. glandulosus* ssp. *glandulosus* extending across much of Central California and (Map 10). Whether the local populations of this bristly jewelflower in Santa Clara County deserve coverage under the Valley Habitat Plan should be addressed in the Valley Habitat Plan revision process.



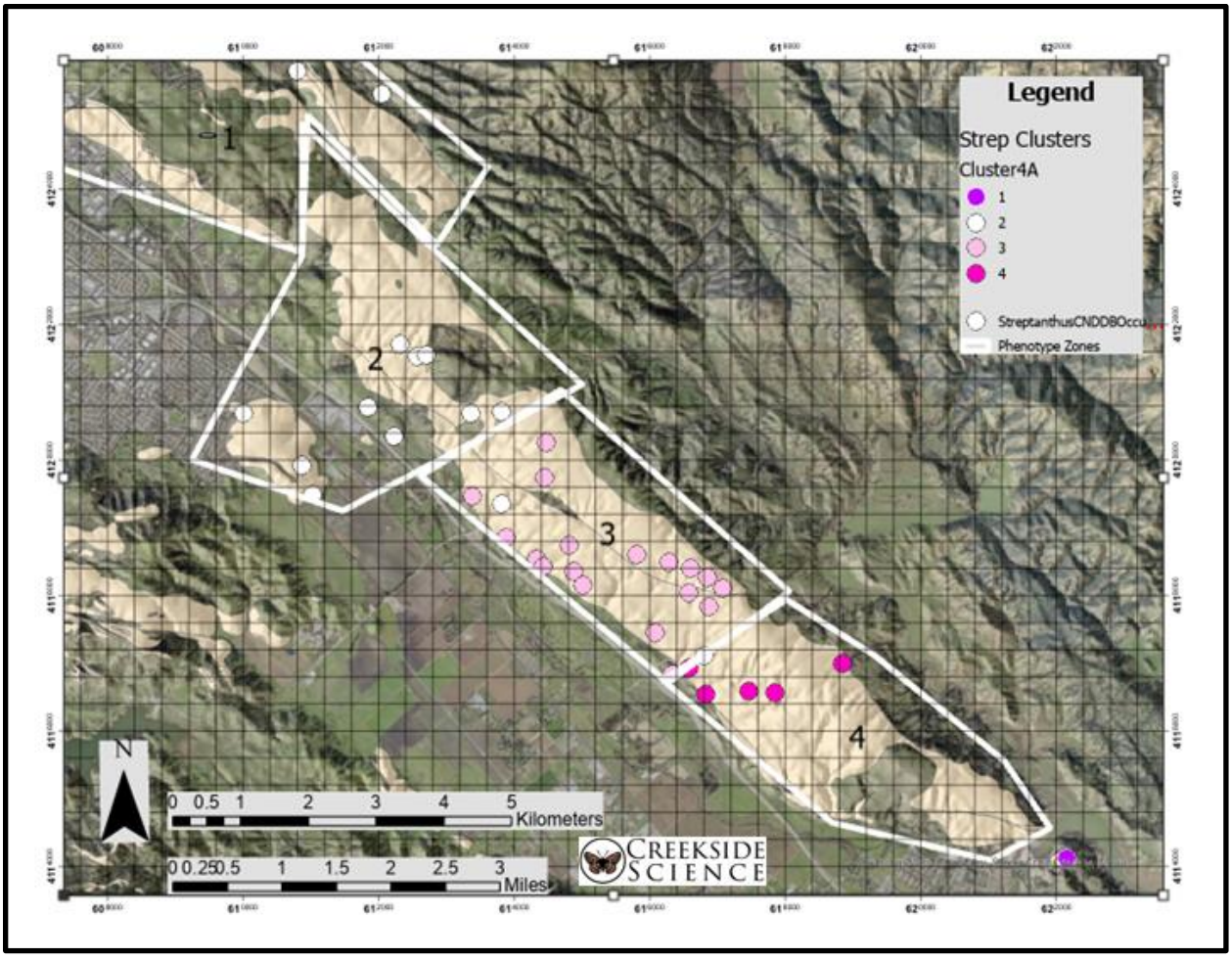
Map 10. Distribution of *Streptanthus glandulosus* ssp. *glandulosus* from CalFlora, ranging from San Luis Obispo County north to Mendocino County. https://www.calflora.org/app/taxon?crn=7837

Spatial Definitions of Occurrences

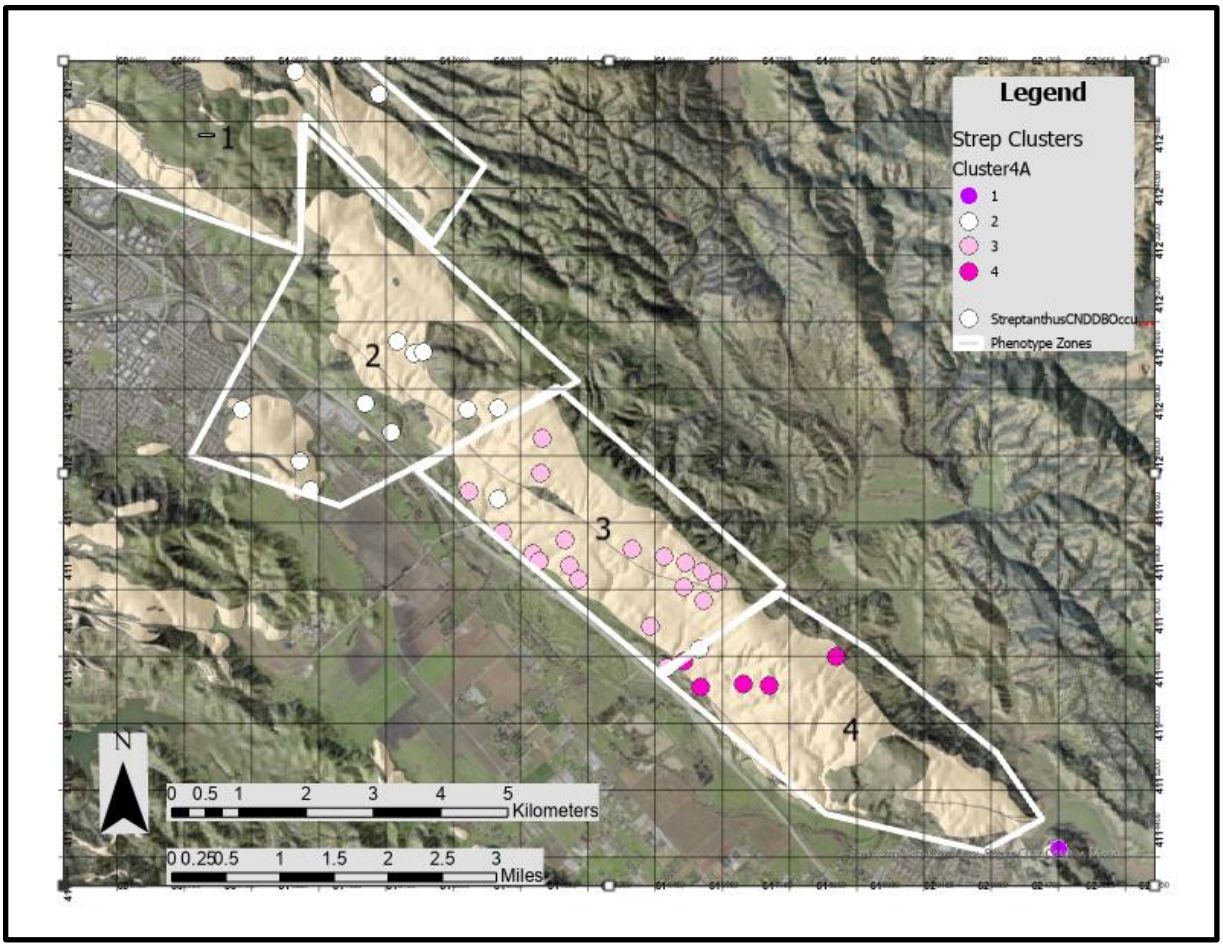
The semi-continuous distribution on Coyote Ridge that expands and contracts through time confounds the definition of an occurrence with a ¼ mile separation. If one were to use this rule, in high abundance years there would be one (or just a few) very large occurrence, while in low abundance years there would be a large number of small, spatially isolated occurrences. These changes would naturally occur based on interannual weather differences, and not necessarily reflect management or conservation actions. Therefore, breaking this distribution up into smaller units is desirable. The model for this procedure is the modification of Santa Clara Valley dudleya (*Dudleya abramsii* subsp. *setchellii*) occurrences on CROSP. This very large occurrence was broken into smaller zones to reflect the island-like rocky-outcrop habitat occupancy where the ¼ mile rule is inappropriate. It also better reflects the reality of conservation goals, in that one should get more credit for conserving one very large occurrence (by splitting it into smaller occurrences) than one very small occurrence (while still recognizing the importance of small, geographically isolated occurrences).

As an alternative, occupancy on a ¼ mile (400 m) grid is a starting point for discussion, since the ¼ mile corresponds to the separation distance considered in CNDDB. This procedure produces dozens of occurrences on Coyote Ridge (Map 11a). It also produces multiple occurrences within some of the

isolated populations -- for example, Tulare Hill, if fully occupied, could be considered ~10 occurrences (depending on what is done with the slivers at the edges). A larger separation distance would reduce the number of occurrences; i.e., a ½ mile separation distance (800 m) grid would produce four times fewer occurrences (Map 11b). This reduces Tulare Hill to 3 occurrences which is more realistic.



Map 11a. A ¼ mile grid (400 m) overlaid onto jewelflower sampling sites divided into four zones based on color frequencies (Figure 7, Map 3). Counting each ¼ mile grid cell as an occurrence, provided it is occupied, is one solution to dividing up the semi-continuous distribution. Note the jewelflower points here are sampling sites and that the populations are much larger and even more continuous (see Maps 5, 6, and 7 for examples of more fine-grained mapped occurrences).



Map 11b. A 1/2 mile grid (400 m) overlaid onto jewelflower sites divided into four zones based on color frequencies (Figure 7, Map 3) as an alternative example. Note the jewelflower points here are sampling sites; the populations are much larger and even more continuous (see Maps 5, 6, and 7 for examples of more fine-grained mapped occurrences).

In this method, conservation goals could be set by the fraction of occurrences in each phenotypic zone, so that the absolute number of occurrences is less important. Basically, this method is an area-based accounting of occupied habitat. We have offered these methods as suggestions, to be debated and resolved with the upcoming revision of the Valley Habitat Plan.

Specific Conservation Actions

Key Acquisition Targets

To secure the bulk of the white flowered phenotypes, the Young Ranch (Zone 2 in Maps 9 & 11) and Richmond Ranch (Zone 1 in Maps 9 & 11) are prime targets (for all covered serpentine grassland species). Some data from the Young Ranch may be available from WRA (who have been working on the site for many years). Once access is secured, the populations on these sites should be surveyed using the

100m grid method used by Creekside at VTA, CROSP, and LESVP, along with phenotype surveys using Dunn-Edwards color cards as described herein.

At this point in 2023, almost all of Zones 3 and 4 are conserved (see discussion of zones above).

Key Survey Targets

Various private lands north of Metcalf Road, including the long serpentine outcrop on the SW slope, are prime survey targets.

A thorough survey of Pigeon Point is needed to confirm absence. The eastern slope is owned by Santa Clara County Parks.

Key Restoration/Establishment Opportunities

Seeding pure white phenotypes into various unoccupied roadcuts and disturbed areas in Zone 1 would increase the number of occurrences in that area.

The final surface of the Kirby Canyon Landfill would be good jewelflower habitat, evidenced by the vigor of plants on filled slopes along the North Canyon Road, however, these areas are not expected to be available for several decades.

Points to Consider while Revising the Valley Habitat Plan

- Individuals within a mixed population will not be assigned a taxonomic unit; entire populations will be assigned taxonomic units based on the frequency of white phenotypes.
- The entire evolutionary process of the jewelflowers should be conserved. If an area becomes more pink or more white on its own, conservation goals are still being met.
- No conservation goals are met by constantly redrawing population boundaries following the natural rhythm of jewelflower population fluctuations.
- Coyote Ridge represents a zone of blending phenotypes. Four zones are suggested here to capture areas with specific phenotypic frequencies.
- We suggest a specific standard occurrence size within these zones, such as occupancy within a ½ mile grid.
- Objectives would drop the standard nomenclature and instead read something like “Establish X occurrences of at least X individual Coyote Ridge jewelflowers within Zone 1.” Such objectives would be established for all four zones.
- The purple phenotype west of Highway 101 and south of Coyote Ridge may be a more locally rare form of *Streptanthus glandulosus.* This could be investigated to see whether it deserves coverage under the Valley Habitat Plan.

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Appendix A Workshop Attendance

NAME THAT JEWELFLOWER – Stakeholder Workshop / Wednesday, May 3rd, 1:00 – 4:00

City of Morgan Hill – City Council Chamber Building

Confirmed:	**Tentative:**	**Declined:**
Matt Fogarty –Habitat in person	Amy Poopatanapong– ICF	Kristin Garrison - CDFW
Aaron Hebert – OSA	Robert Cain – SCC Plan	Ed Sullivan - Habitat
Denise Rosenberger – Habitat in person	Kraig Tambornini – CGil	Kathryn Gaffney - ICF
Torrey Edell – Grant PM, ICF	Julie King – Habitat-in person	Jill Mross - Habitat
Joe Chavez – in person	Gerry Haas – Habitat-in person	Lani Ho - VTA
Janell Hillman – VW	Peter Cowan - POST	
Christal Niederer – *Presenter in person		
Justen Whittall- *Presenter in person		
Stu Weiss - *Presenter in person		
Jeffrey Lewis – VW		
Nathan Hale – Habitat in person		
Joseph Terry – USFWS via teams		
Glen Tarr – USFWS via teams		
Don Arnold – Habitat in person		
Brenda Blinn – CDFW via teams		
Jimmy Quenelle-Creekside-attended in person		
Marissa Kent Creekside-via teams		
Chris Schwind—Creekside-via teams		
Kim Mancera-CMH-in person		
Ivan Carmona-Torres-CMH-in person		
Ann Calnan VW-in person		
Jeff Rosenberger-in person		

Green indicates people who attended the workshop

Appendix B Raw Color Field Data

Code	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18			
DE Color	5553	6260	5560	5540	6015	5987	5014	5995	5981	5988	5996	5989	5997	5990	5998	5999	6000	977	706	TOTAL
Hue	1.4	9.23	3.07	4.92	5.65	6.66	1.49	7.2	5.76	6.08	7.1	5.69	6.98	5.31	7.48	7.61	7.68	N/A	N/A	
Value	9.6	9.1	9.1	8.7	8.8	8.9	8.8	8.6	8.4	8.3	7.9	7.5	7	6.5	5.9	4.8	3.8	N/A	N/A	
Chroma	0.8	0.5	1.1	2	1.8	3.1	2.9	4	3.7	4.6	6	6.4	7.7	8.1	9.1	9.4	8.2	N/A	N/A	
Count	100	268	145	31	280	206	23	52	158	144	63	139	35	48	48	111	98	43	7	
Hellyer Roadcut	11	30	9																	50
Tulare Hill plots	10	15	21	2	2															50
Malech Roadcut	22		3		12	6	3	1			3									50
CROSP 7 sniper	12	20	5	1	8	1			2			1								50
Solar array	8	10	23	3	4				1			1								50
Tulare Hill passive	5	20	13		11	1														50
Metcalf Rd.	5	44	17	4	9	1						1								81
CROSP 8 N summit trail	8	21	5		10	1		2	3											50
Silver Creek Road	8	2	23	16	1															50
Motorcycle Park	1	18	20	4	4		1		2											50
CROSP few plants most southern	1	32	3		11	1				2										50
CROSP few plants most northern	1	14	1		19	10	1	1		3										50
CROSP 5 north bench		8			20	17	1		2	2										50

CROSP 2 pipeline	2	6	1		19	12	2	2	5	1										50
LE1	2	7		1	15	15		1	3	6										50
LE2	2	4			21	11		3	7		1	1								50
CROSP 3 center	1	5			18	11		2	7	2	3	1								50
SVP3	1	3			6	17	1	1	14	4	1	2								50
SVP4		6			15	12	1	1	5	3	3	4								50
VTA west					7	13	4	2	7	9	2	6								50
VTA summit		2			9	11	1	2	8	6		11								50
CROSP 1 (S mid)					9	7	3	7	7	5	3	6	2	1						50
CROSP 6 sum art			1		8	5	1	4	11	11	1	6		1	1					50
CROSP border 1					10	3	2	5	11	8	3	6		2						50
VTA low					5	12			6	13	5	7	1	1						50
KC Landfill		1			6	9		1	8	8	7	10								50
CROSP 4 VTA summit					5	8		7	7	9	3	10		1						50
CROSP border 2					8	4	1	7	11	6	3	8		1	1					50
VTA floodplain					7	8	1		13	6	2	10	1	2						50
Rancho San Vicente										11	8	1	7	18	3	11	2			61
KC Ungrazed						6			4	14	5	14	6	1						50
VTA east					1	2			11	5	2	19	7		3					50
North Canyon Summit						2		3	3	10	7	14	6	1	4					50
Rosendin Park													1	8	9	17	15			50
Stiles Ranch (ST Park											1		2		10	27	10			50
Baird													2		14	21	13			50
North Gate Rd. (Mt. Diablo)														4	3	23	7			37

Rancho Canada del Oro														6		5	26			37
Almaden Quicksilver														1		7	25			33
Sunol Form (Alameda Co.)																		22	3	25
Arroyo Hondo (Alameda Co.)																		21	4	25