

Determining the optimal conditions for propagation and reproduction of the endangered serpentine endemic Metcalf Jewelflower (*Streptanthus albidus* ssp. *albidus*) of Santa Clara County and genetic assessment of its taxonomic status



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The following report summarizes research completed on the Department of the Interior grant entitled, “Determining the optimal conditions for propagation and reproduction of the endangered serpentine endemic Metcalf Jewelflower (*Streptanthus albidus* ssp. *albidus*) of Santa Clara County and genetic assessment of its taxonomic status.” This report is divided into the following sections:

- I. Permitting & Permissions
- II. Surveys (2007-2011)
- III. Flower Color Quantification
- IV. Pollinators
- V. Soil Chemical Analysis
- VI. Germination & Propagation
- VII. Local Adaptation Transplant Experiment
- VIII. AFLP Assessment of Taxonomic Status
- IX. Floral Anthocyanin Gene Survey
- X. Manuscript Preparation

Each section begins with the following components from the original proposal: **start date**, proposed objective, milestones, and **completion date**. This is followed by a description of methods and findings.

I. Permitting & Permissions

Start Date	Objective	Milestone	Completion Date
Summer 2007 (pre-funding tasks)	Secure proper permits and permission to work on private properties	Permits issued and permissions granted	Sept. 2007 (before the onset of funding)

Permits and permissions were granted for seed, tissue and soil collecting for the Metcalf Jewelflower (*Streptanthus albidus* ssp. *albidus*) and its close relatives (*S. albidus* ssp. *peramoenus* & *S. glandulosus*) in the following areas: Santa Teresa County Park, Metcalf Road County Motorcycle Park, Rancho Canada Del Oro Open Space Preserve, Kirby Canyon Butterfly Preserve, and the University of California property atop Mt. Hamilton. Access to these populations was granted in time to collect soil, tissue and seeds during summer, 2007.

II. Surveys (2007-2011)

Start Date	Objective	Milestone	Completion Date
May 2007	Survey extant, extinct and potential habitats for <i>S. albidus</i> ssp. <i>albidus</i>	Surveys completed	March 2011

A. 2007 Surveys

Multiple surveys for *S. albidus* ssp. *albidus* were conducted in spring and summer of 2007 – one year ahead of schedule. We verified that the two outlying populations previously thought to be *S. albidus* ssp. *albidus* (Lower Soda Springs & Llagas Rd.) are misidentifications and are *S. glandulosus*. The Lower Soda Springs population is near Lexington Reservoir outside of the town of Los Gatos. It has dark purple sepals and is glandular-hairy throughout, both characteristics of *S. glandulosus*. Similarly, the numerous small populations along Llagas Road from Morgan Hill to Chesbro Reservoir are also most likely *S. glandulosus* due to their similarly distinctive floral and vegetative characteristics. Further molecular analyses will help confirm these identifications. We did not find any white flowered individuals with glaucous upper-leaves suggestive of *S. albidus* ssp. *albidus* in either of these populations.

Extensive surveying of Kirby Canyon, immediately south of Metcalf Canyon, resulted in relocating many previously identified populations of *S. albidus* ssp. *peramoenus*. The primary distinction between this subspecies and *S. albidus* ssp. *albidus* is the light lavender to pink flower color of the former. Although there is considerable variation in the amount of floral pigment in most *S. albidus* ssp. *peramoenus* populations, no individuals with completely white sepals were found in Kirby Canyon.

Tulare Hill is a previously identified potential reintroduction site on the west side of Hwy101 immediately across from Metcalf Canyon. Dr. Stuart B. Weiss accompanied PI Whittall and Co-PI Strauss on a survey where we found no jewelflowers of any species. Previously, several collections of *S. albidus* ssp. *albidus* were collected there, but none have been seen for several decades. The three extirpated collections probably represented a historically continuous population occupying serpentine outcrops on the south-facing slopes of this hillside. We have included multiple soil samples from this area to determine whether its chemical and physical properties may be suitable for reintroduction of *S. albidus* ssp. *albidus*.

A series of *S. albidus* ssp. *albidus* collections immediately south of Metcalf Canyon were surveyed multiple times in summer of 2007. Although no plants were identified along Highway 101, nor along Malech Road, the frontage road on the east-side of Highway 1, we did find one small isolated population of predominantly white flowered individuals on UTC property, approximately 800 meters east of Malech Road between Metcalf and Kirby Canyons and we expect more plants of either subspecies of *S. albidus* on this property if additional surveys were performed. Soil and tissue samples were taken here to include in our soil analyses and molecular examination of genetic distinctiveness given their extended range outside of Metcalf Canyon itself. The site was inaccessible for seed collecting later in the season and therefore will not be included in the local adaptation transplant experiment.

Another noteworthy finding during our surveying was a single light-pink flowered individual found along the road-cut in Metcalf Canyon where the bulk of the otherwise pure white-flowered *S. albidus* ssp. *albidus* population occurs. This is exceptional since otherwise the population of several thousand individuals has entirely white to creamy-green sepals. This finding is also interesting because the most distinctive characteristic of *S. albidus* ssp. *albidus* is its flower color differences from *S. albidus* ssp. *peramoenus* (light pink to dark pink/lavender). We conducted another detailed survey in this particular area of Metcalf Canyon in spring, 2008 to determine whether the occasional presence of a pink flowered individual is a persistent phenomenon, likely representing introgression of *S. albidus* ssp. *peramoenus* alleles from the adjacent Kirby Canyon.

B. 2008 Surveys

We conducted a second year of surveys in spring 2008. These surveys include populations at Metcalf Rd., Motorcycle Park, Mt. Hamilton, and Los Gatos. We have identified another outlying population (on south facing serpentine chaparral slope near St. Joseph's Hill, 1 mi. north of Lexington Reservoir on Santa Clara County Park land) near one previously thought to be *S. albidus* ssp. *albidus* (Lower Soda Springs). Both populations are clearly *S. glandulosus* based on the heavy pubescence on the leaves and stems, relatively short stature (<40cm), and most distinctively, flowers with purple sepals. No pale purple, pink or white individuals were located in this population of approximately 200 individuals.

Additional surveying of the Metcalf Rd. and Motorcycle Park populations revealed several pink-flowered individuals (7 individuals along Metcalf Rd. and another 12 individuals at the primary population in Motorcycle Park). This is exceptional since otherwise the population of several thousand individuals has entirely white to creamy-green sepals. This finding is also interesting because the most distinctive characteristic of *S. albidus* ssp. *albidus* is its flower color differences from *S. albidus* ssp. *peramoenus* (light pink to dark pink/lavender). This is a substantial increase from the single pink flowered individual noted in last year's surveys. No correlations between flower color and leaf or stem color (which also varies from glaucous, grey-green to dark purple) were found. Furthermore, the primary pollinator, *Bombus vosnesenskii* (Fig. 1), does not appear to discriminate between white and pink individuals at these two sites going freely between the two color morphs in the few patches with individuals of mixed flower color. The presence of pink flowered individuals in *S. albidus* ssp. *albidus* populations two years in a row could represent introgression from *S. albidus* ssp. *peramoenus* from the adjacent Kirby Canyon via pollinator exchange or seed dispersal (although the mechanism is unclear since these plants do not seem to have any exceptional fruit or seed dispersal mechanisms).

Another noteworthy finding during our surveying was a new population of exceptionally dark flowered *S. glandulosus* plants on non-serpentine talus soils near the base of Mt. Hamilton (north side of Quimby Rd., approximately 0.5 miles southwest of



Fig. 1. The primary pollinator of the Metcalf Jewelflower, *Bombus vosnesenskii*.

the junction with Mt. Hamilton Rd.). Previously, these unusually dark flowered individuals were assumed to be restricted to Mt. Hamilton Peak and the immediate vicinity (ca. 4,200ft elevation). The distinctive flower color and potentially unique evolutionary lineage discovered in the Mt. Hamilton populations make these populations distinct from other *S. glandulosus* populations from this area of California, suggesting it may be a new taxon requiring conservation attention. Further surveying in this area of the Mt. Hamilton range (including Joseph D. Grant State Park, Henry Coe State Park, the newly acquired Blue Oak Canyon UC Reserve, and property owned by the University of California around the Mt. Hamilton summit) will be necessary to determine the geographic range of this lineage.

C. 2009 Surveys

In May 2009, we re-surveyed Metcalf and Kirby Canyons for *S. albidus* ssp. *albidus* and *S. albidus* ssp. *peramoenus*, respectively. Although the plants were very young (only two in flower in Metcalf Canyon and only one in flower in Kirby Canyon), it was obvious that the populations were substantially reduced compared to previous years (2007 & 2008). The persistence of the previous year's infructescences allowed for a direct comparison of plant densities between the two years. Along several transects walked along Metcalf Road, we found 1/2 the number of plants in 2009 compared to what was present in 2008. Relying on the barren infructescences from the previous year provides a minimum number of plants from 2008 since several were certainly dislodged over the winter and no infructescences survive two or more years making this a conservative estimate (the population is certainly *less than half* as large as the previous year's population). We had seen similar fluctuations in population densities in the past – 2006 was a very low density year relative to 2007 & 2008 (Sharon Strauss, personal communication).

In Kirby Canyon, a similar set of transects indicated that in 2009 there was less than 1/4 the number of *S. albidus* ssp. *peramoenus* plants than were present in 2008. Dramatic fluctuations have been noted previously for these taxa and other species of *Streptanthus*. Determining the climatic factors that control population size fluctuations could be valuable for predicting future population viability and especially helpful in choosing successful reintroduction sites (i.e. the Tulare Hill site).

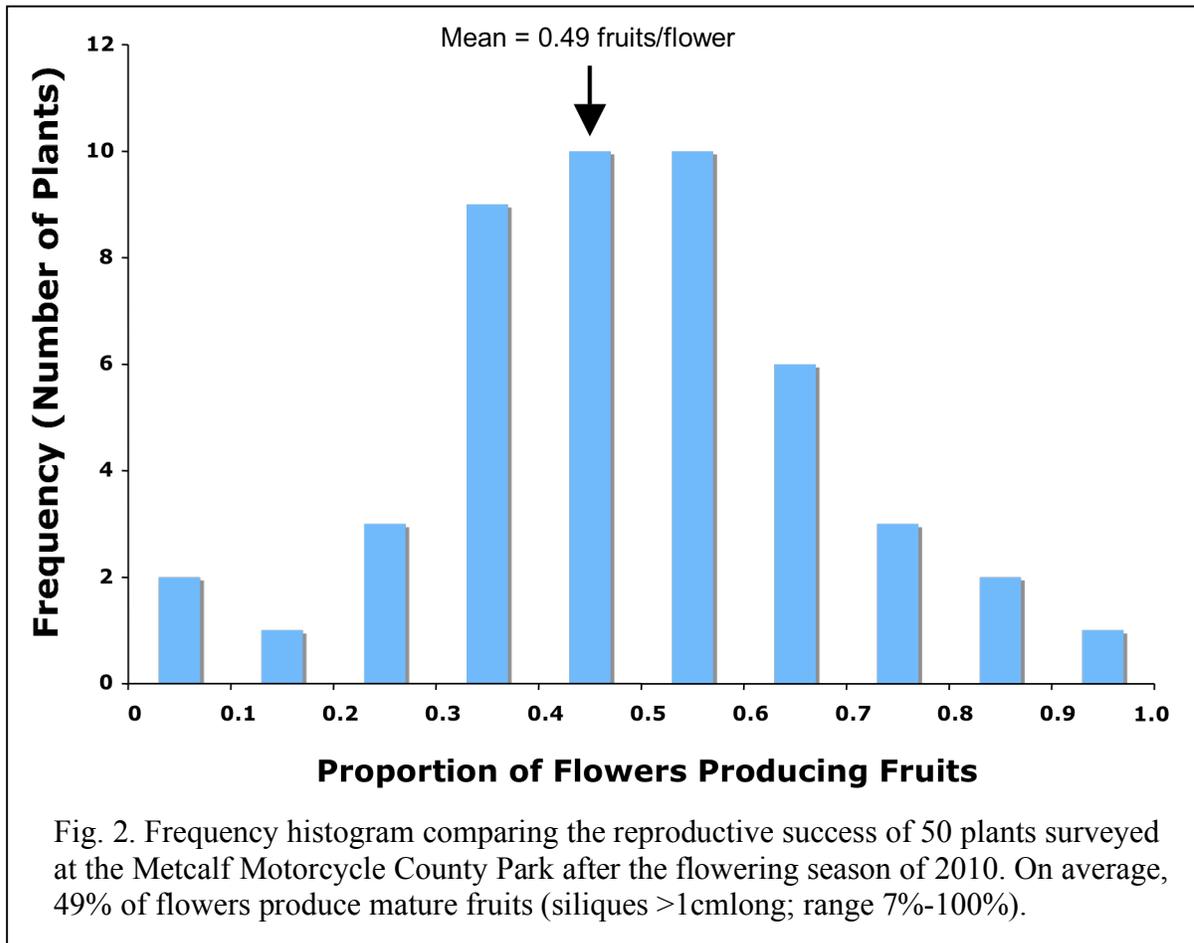
D. 2010 Surveys

In a survey of the Metcalf Rd. population of *Streptanthus albidus* ssp. *albidus* in March and April of 2010, we found very few young individuals (<100 along the length of the road-side population). Although more individuals may appear as the season progresses, seeds germinate in winter and should have been visible as basal rosettes by this time. This was the second year in a row with very few individuals appearing along Metcalf Rd. (usually the largest and densest population of *S. albidus* ssp. *albidus*).

Later that fall, we returned to these populations to compare total population sizes. Surveys of extant populations along Metcalf Road and at the Metcalf Motorcycle County Park revealed surprisingly low census counts for 2010 compared to 2007 & 2008 (~5%). A similar decline in population size was documented in 2006 (Sharon Strauss, personal communication). Comparable population fluctuations have been documented for another annual, serpentine endemic, *Camissonia benitensis*, where the population crashes

predictably every three years. The cause of the periodic declines is unknown in both *Camissonia* & *Streptanthus*, but may fluctuate with ecological antagonists such as invertebrate herbivores and/or pathogens (R. O'Dell, Hollister BLM, personal communication).

After documenting an exceedingly low count in spring/summer 2010, we conducted a survey to estimate reproductive success among the few plants in the population to compare to reproductive success in years with >20x the number of individuals. After the majority of plants had finished flowering, we compared the number of mature fruits (siliques < 2cm long) to the number of flowers that did not set fruit (only the pedicel remained) at the Metcalf Motorcycle County Park. From the fruit vs. flower counts, we estimated the proportion of fruits produced on 50 individuals. The mean percent of fruits set per flower was 49% (range 7%-100%; Fig. 2). The mean number of fruits per plant was 14.2. Since each fruit holds ~20 seeds, we estimate each plant can produce approximately 284 seeds.



We also quantified herbivory levels on any remaining green leaves. Although the survey was limited to the 14 individuals at the Metcalf Motorcycle Country Park with green leaves remaining this late in the season, we still found 57% of plants with clear signs of herbivory. Of the plants with herbivory, approximately half of the leaves within a plant showed signs of herbivory (mean = 53% of leaves within a plant with herbivory).

This substantial level of herbivory was most likely due to invertebrate herbivores since aphids and Pierid caterpillars have been observed in these populations previously. Large mammal herbivores (deer & elk) typically remove inflorescences and were unlikely the cause of the herbivory measured here.

E. 2011 Surveys

On March 29, 2011, we surveyed along Metcalf Rd from the serpentine seep to the 1st powerpole on the north side of the road. We counted only 95 individuals that could be positively identified from the roadside. Most were at the 5-10 leaf stage and still in a basal rosette (no inflorescences present). Approximately 10% of these plants had visible signs of damage to the leaves, likely from invertebrate herbivores, but possibly also from the recent hail storms (March, 2011). In a good year (i.e. 2007), we could find 1000's of individuals along this stretch of road. Since the population size here (and at Motorcycle Park) were very low in 2009 and 2010, there were very few infructescences remaining on the barren serpentine road-cut.

III. Flower Color Quantification

To determine the distinctiveness of the flower color of *Strepanthus albidus* ssp. *albidus* compared to its closest relatives, we collected samples from the three existing populations of the Metcalf Jewelflower and six additional populations representing its closest relatives (*S. albidus* ssp. *peramoenus* and *S. glandulosus*). Sepals were removed from 5-20 plants per population (Table 1) and stored at 4°C in zip lock bags until their flower color could be quantified. Reflectance of the outer-sepal surface was quantified for wavelengths across the UV and visible spectrum using an Ocean Optics USB2000 portable spectrometer with a pulsed Xenon light source. For each sample, an average of 3 measurements per flower were taken every 9 seconds.

Table 1. Samples used in flower color quantification.

Taxon	Population	No. of samples
<i>S. albidus</i> ssp. <i>albidus</i>	Metcalf Road	5
<i>S. albidus</i> ssp. <i>albidus</i>	Motorcycle Park	5
<i>S. albidus</i> ssp. <i>albidus</i>	Silver Creek Golf Course	5
<i>S. albidus</i> ssp. <i>peramoenus</i>	Kirby Landfill - 3 subpops	20
<i>S. glandulosus</i>	Canada del Oro	5
<i>S. glandulosus</i>	Kennedy Road	8
<i>S. glandulosus</i>	Llagas Road	5
<i>S. glandulosus</i>	Observatory Peak	8
<i>S. glandulosus</i>	Tortilla Flats	5

As depicted in Fig. 3, the most notable differences in the reflectance spectra comparing *S. albidus* ssp. *albidus* and *S. albidus* ssp. *peramoenus* involves reflectance between 500-600 nm. These are the wavelengths that are absorbed by the anthocyanin pigments that provide the pink and purple coloration to sepals of ssp. *peramoenus*. The white sepals of ssp. *albidus* produce no anthocyanins and therefore reflect most visible

wavelengths (400-700 nm) relatively evenly. When examining the variance around the average spectrum for these two subspecies, it is clear that lines encompassing ± 1 standard error easily distinguish these two subspecies with very little overlap. No samples produced substantial reflectance in the UV range in which some bees can see, yet are invisible to humans (350nm-400nm).

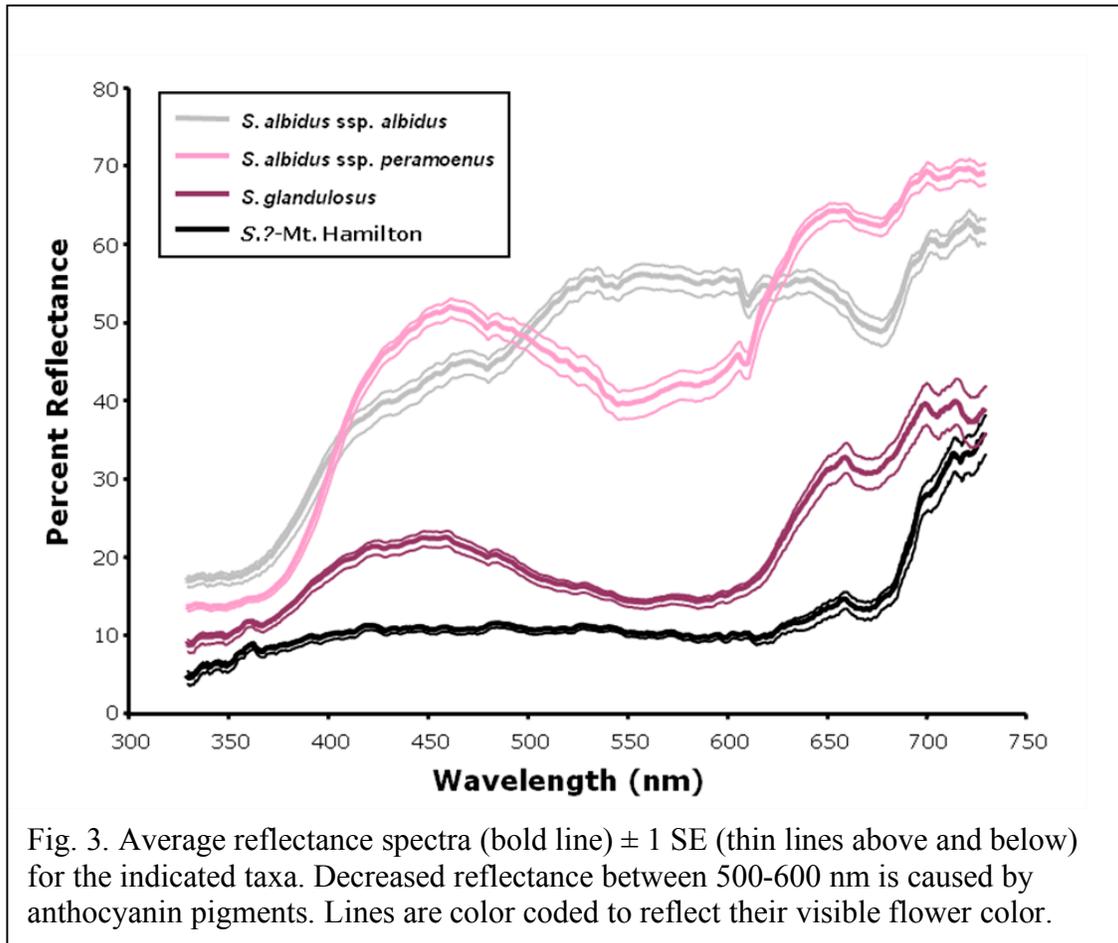


Fig. 3. Average reflectance spectra (bold line) ± 1 SE (thin lines above and below) for the indicated taxa. Decreased reflectance between 500-600 nm is caused by anthocyanin pigments. Lines are color coded to reflect their visible flower color.

In comparison, the UV-Vis spectra from the darker purple *S. glandulosus* have generally lower reflectance values consistent with the prediction that the darker purple coloration is due to increased anthocyanin concentrations in their sepals. Populations of an unidentified *Streptanthus* species atop Mt. Hamilton (*S. glandulosus?* - Observatory Peak and Tortilla Flats populations) have distinctively darker sepals (nearly black to the human eye). These sepals reflect very little light in the UV-visible spectrum and have an overall much lower percent reflectance across all wavelengths.

The flower color quantification using UV-Vis spectral analysis confirms the consistent differences in sepal reflectance among pigmented (*S. albidus ssp. peramoenus*, *S. glandulosus*) and non-pigmented (*S. albidus ssp. albidus*) taxa. The consistency of the flower color differences is a key component to justifying the taxonomic recognition of *ssp. albidus* as distinct from *ssp. peramoenus*.

IV. Pollinators

Start Date	Tasks	Milestones	Final Completion Date
May 2008	Survey extant, extinct and potential habitats for <i>S. albidus</i> ssp. <i>albidus</i> , conduct outcrossing experiment and make pollinator observations	Surveys completed and pollinators identified	July 2010

In order to determine the most common pollinators of *S. albidus* ssp. *albidus*, we recorded 33 hours of pollinator observations at the Motorcycle County Park on Metcalf Road on June 4, 2008. Between 7:30 am and 5:30 pm, we used six digital video cameras on tripods running simultaneously - each positioned to record pollinators of one to four inflorescences per plant for approximately 1.5 hours per digital videotape. Each camcorder was focused on between 16 to 27 open flowers per plant (average = 24). For each digital videotape, the number of open flowers, number of visits, length per visit, and pollinator identifications were recorded.

We documented 1429 visits. Nearly all pollinators recorded were *Bombus vosnesenskii* (Fig. 4) although a few honeybees (*Apis mellifera*) and flies (Diptera) were also documented pollinating the Metcalf Jewelflower. The length of visit is often proportional to the amount of pollen transferred, and thus provides an estimate of the effectiveness of the pollinator. The average visit duration for *B. vosnesenskii* was 2.26 seconds (± 0.1 sec) per flower. Visit durations ranged from 0.03 seconds to 120.9 seconds. There is no statistically significant correlation between visit duration and the time of day, but the longest visits did occur after 9am and before 12pm. Visits were divided into those occurring within the inflorescence and those visits occurring between plants. Approximately 2/3 of visits were documented within a plant (70.7%) and 1/3 of visits came from between plants (29.3%). Although visit duration was longer for between- compared to within-plant visits, this difference was not significant (Student's T-test, $p = 0.30$).

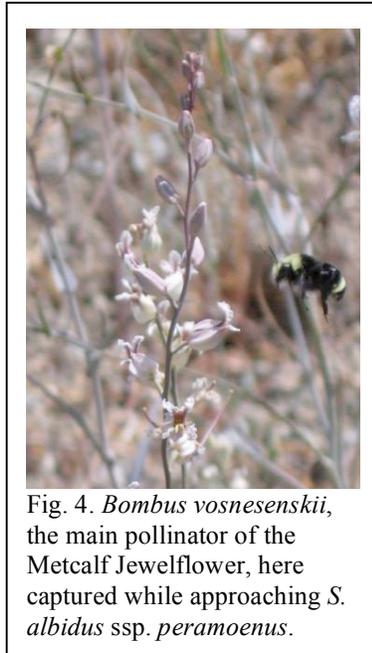
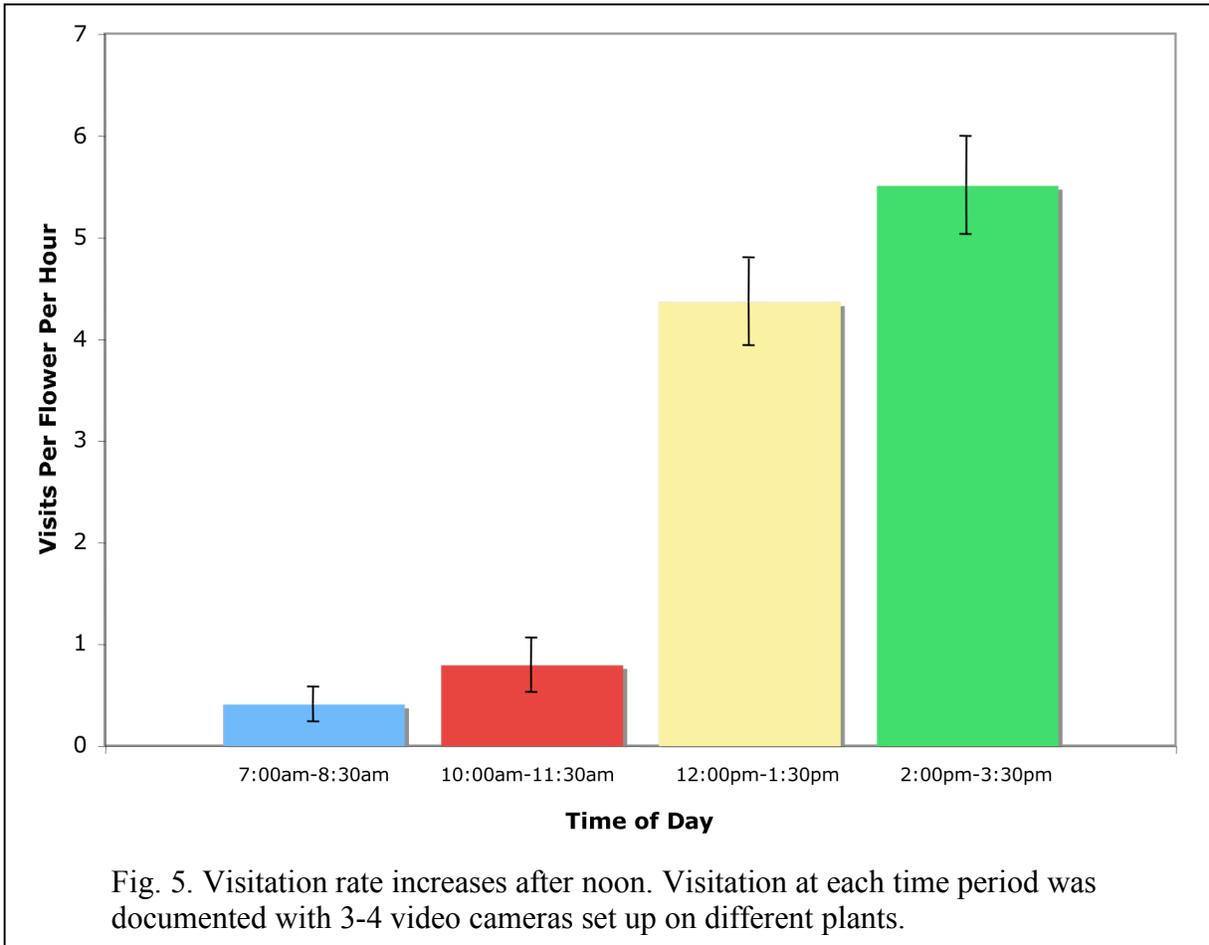


Fig. 4. *Bombus vosnesenskii*, the main pollinator of the Metcalf Jewelflower, here captured while approaching *S. albidus* ssp. *peramoenus*.

When taken collectively, we recorded an average of ~2.92 (range 0.11 - 6.51) visits per flower per hour. Since we had multiple video cameras documenting pollination at 1.5 hr time periods, we compared visitation rate throughout the day. Visitation is >8x higher after 12pm than before 12pm (Fig. 5; Student's T-test, $p < 0.0001$). These may seem like relatively low visitation rates, but when you consider the lifespan of each flower (2 days) and the number of hours per day that pollinators are active (at least 9 hrs), then each flower could easily be visited >50 times in its lifetime ($2.92 \times 9\text{hrs} \times 2\text{ days}$).



This would be more than sufficient visitation to produce the exceptionally high fruit set which we are not able to replicate in greenhouse and lathhouse conditions (lacking pollinators).

When multiple flowers on a plant are open at the same time, pollinators can cause self-fertilization through movement of pollen within a plant (also known as geitonogamy). Since we had numerous flowers of the same plant within view of each camcorder, we could quantify the lower range of geitonogamy (often we did not have all inflorescences of a plant within the frame). In general, many bumblebees visited multiple flowers on the same plant in succession potentially leading to geitonogamy. In the seven tapes viewed so far (approximately 15 hours), there were 10 bumblebees that visited flowers on the same inflorescence 8-10 times before flying out of frame. This is certainly an underestimate of the potential for geitonogamy, but is an essential consideration when

predicting the genetic consequences of the relatively high frequency of pollinators and the dynamics of gene flow in these populations.

We have collected a substantial amount of pollinator observation data using digital video technology. *Bombus vosnesenskii* is the primary pollinator of *ssp. albidus*. At nearly three visits per flower per hour, each individual flower should be visited an average of 52 times during the flowers receptive stages. This high frequency of visitation may account for the high fruit set in *ssp. albidus* in the wild. Conservation measures must include accounting for preservation of the pollinator fauna since the pollinators of *ssp. albidus* appear to be essential for any significant fruit set in this taxon. Although this species of bumblebee is common in California, local population densities should be monitored to maintain sufficient pollinator services for *S. albidus ssp. albidus*. Additional observations throughout the flowering season and across years would also be helpful in determining the generality of these findings.

V. Soil Chemical Analysis

Start Date	Tasks	Milestones	Final Completion Date
Sept. 2007	Collect soils for chemical analysis, collect seeds for local adaptation study	Soils and seed collected, soils analyzed for chemical components	Sept. 2009

Soil samples and seeds were collected from numerous populations of the Metcalf Jewelflower and its closest relatives. In summer of 2007, we collected 38 soil samples representing 2-5 samples per population from multiple populations of *S. albidus ssp. albidus* (along Metcalf Road, at the Metcalf Road County Motorcycle Park, at the Silver Creek Golf Course, and the UTC population near Malech Road south of Metcalf Road). In addition, we collected soil from three populations of *S. albidus ssp. peramoenus* (multiple sites along Kirby Canyon, Rancho Canada Del Oro Open Space Preserve, and Santa Teresa County Park). Lastly, we included soil samples from three populations of the closely related *S. glandulosus* (Llagas Road, Kennedy Road, and atop Mt. Hamilton) and the potential reintroduction site at Tulare Hill. Since plants were still green and flowering at this time, flowers and leaves were collected from 20-40 individuals from each population. Flower color was quantified with a UV-VIS spectrometer and leaves were preserved for subsequent molecular analyses. Each leaf was transported on ice back to the laboratory where 100mg of actively growing material (young leaves and flower buds) were frozen in liquid nitrogen and stored at -80C until further analysis.

Each population was revisited in late summer/early fall (2007) when multiple fruits from 20-40 individuals per population were collected. Maternal half-sibships were kept separate and seeds from each maternal plant were crushed yielding greater than 50 seeds per individual. Seeds would be later used for determining appropriate germination protocols, seed banking and the greenhouse transplant experiment.

Soil analysis indicates that serpentine soils have much lower Ca:Mg ratios than non-serpentine soils (Mt. Hamilton; Fig. 6). Although there is a range of Ca:Mg ratios among the serpentine-derived samples, there is no obvious differences that correlate with the taxa.

To determine if the serpentine soils of the Metcalf Jewelflower were unique compared to the serpentine soils of its closest relatives, we collected soils and determined soil chemistry and physical properties from 32 sites harboring populations of *Streptanthus albidus* ssp. *albidus* (N=12), *S. albidus* ssp. *peramoenus* (N=8), and serpentine populations of *S. glandulosus* (N=6). In addition, we included six samples from the Tulare Hill site – an extirpated population of ssp. *albidus* and under consideration as a potential reintroduction site for this taxon. In a principle components analysis of all 24 soil chemical and physical measurements, the first two axes accounted

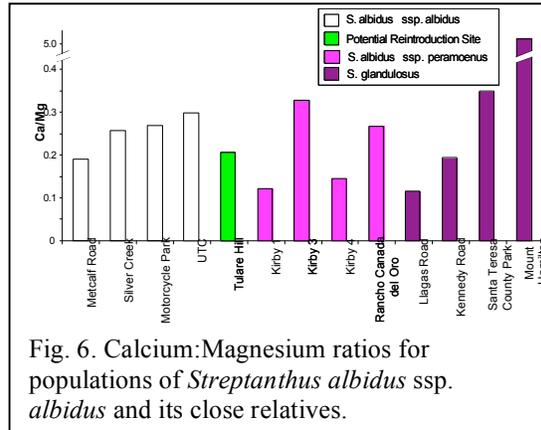


Fig. 6. Calcium:Magnesium ratios for populations of *Streptanthus albidus* ssp. *albidus* and its close relatives.

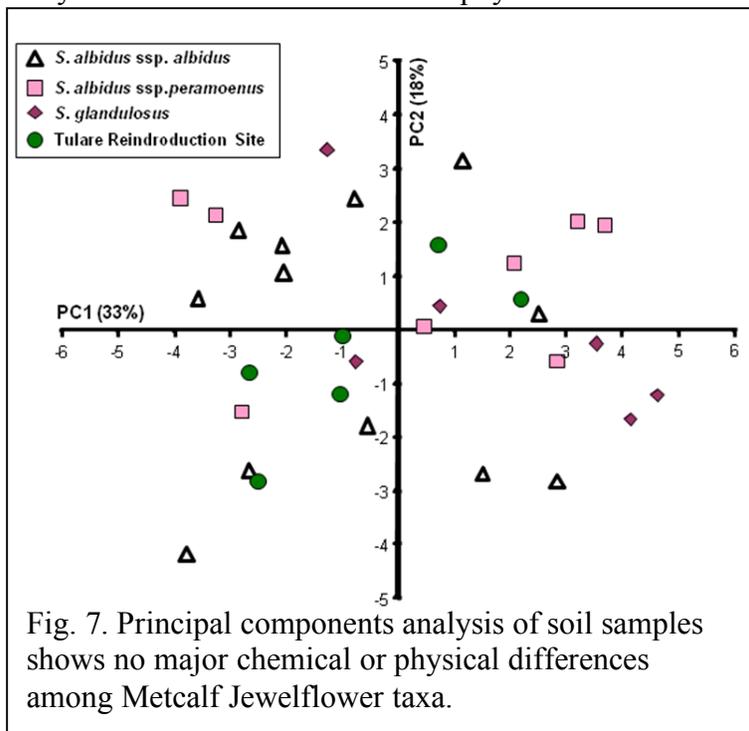


Fig. 7. Principal components analysis of soil samples shows no major chemical or physical differences among Metcalf Jewelflower taxa.

for >51% of the variation. Principal component 1 was negatively correlated with organic matter and Ca:Mg ratio and positively correlated with soil pH and Molybdenum. Principal component 2 was negatively correlated with Zinc and percentage soil and positively correlated with Magnesium, cation exchange and percentage gravel. We see few differences in soil chemistry among *S. albidus* ssp. *albidus* and its close relatives (Fig. 7).

By comparing the mean PC values of each taxon (± 1 SE) in Fig. 8, it becomes readily apparent that the soil harboring ssp. *albidus* is not dramatically different from any of its closest relatives (especially when considering PC1 which explains the most variation in soil chemistry). Although the variance for each taxon is relatively large, there may be a weak difference in PC1 between ssp. *albidus* and ssp. *peramoenus*.

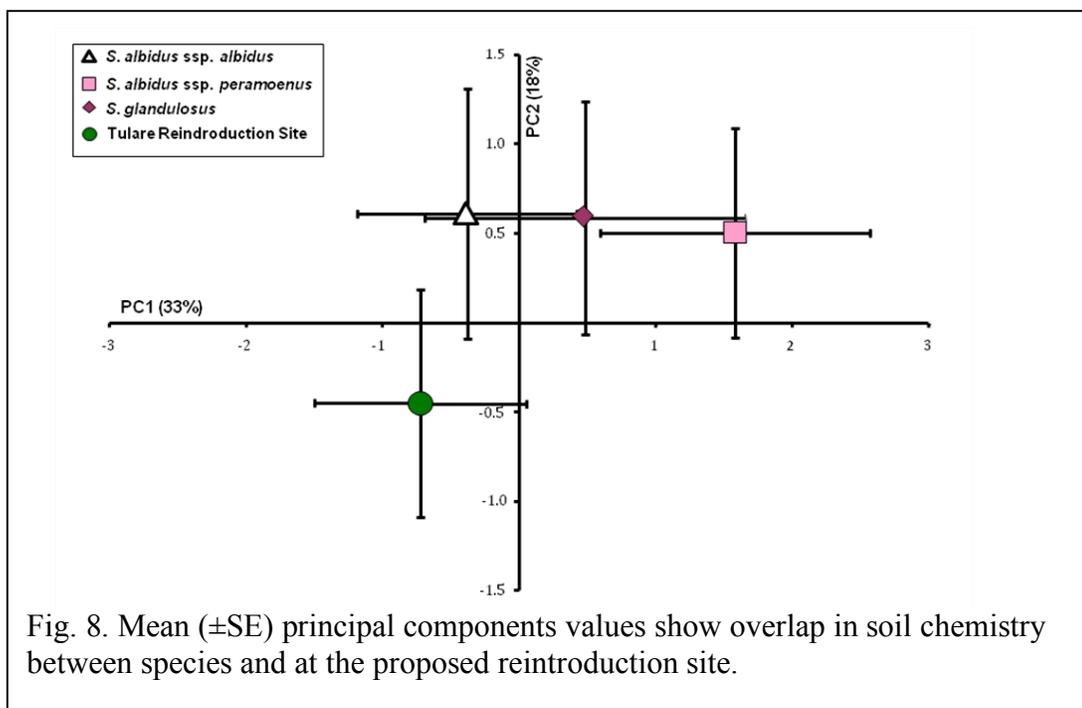


Fig. 8. Mean (\pm SE) principal components values show overlap in soil chemistry between species and at the proposed reintroduction site.

The soil chemistry at Tulare Hill, a potential reintroduction site for *S. albidus* ssp. *albidus*, falls within the range of the soil chemistry for extant populations of ssp. *albidus*. This suggests that Tulare Hill soil may be able to support a viable ssp. *albidus* population if reintroduction/repatriation efforts were pursued. The probability of establishing a successful population at Tulare Hill will also depend on the ability of the plants to grow on these soils. The results from a transplant study to address this issue are described below.

VI. Germination & Propagation

Start Date	Tasks	Milestones	Final Completion Date
Oct. 2007	Determine appropriate germination and propagation protocols, perform local adaptation greenhouse study on serpentine soils	Successful germination and propagation in the greenhouse (by Dec. 2007), completed local adaptation study (by April 2008), data analysis completed (Sept. 2009)	Sept. 2009

A. Germination Protocol

To determine optimal germination and propagation techniques for *S. albidus* ssp. *albidus* and its close relatives, five individuals per population were randomly selected

from each population. Ten to twenty seeds per individual were plated on wet filter paper in sterile petri dishes, sealed and refrigerated at 4°C. All populations began germinating in the refrigerator by the tenth day of incubation. Over 95% germination success was found among all populations. Interestingly, several fruits from the Metcalf Motorcycle Park population produced polymorphic seed coloration: black vs. tan. Only black seeds were found in all other jewelflower populations. As these seeds germinated, it became apparent that the seed color was primarily reflecting the underlying cotyledon pigmentation. After the cotyledons emerged from the seed coat, black seeds had dark purple cotyledons while the tan seeds had vibrant green cotyledons (Fig. 9). Interestingly, the observed seed coat and cotyledon pigment differences are caused by anthocyanins and their precursors – the same pigment responsible for the flower color differences between *S. albidus* ssp. *albidus* (white flowered) and its closest relative *S. albidus* ssp. *peramoenus* (pink/lavender)

Three to seven day old seedlings were planted into 15cm diameter plastic pots containing a 1:1 mixture of standard potting soil and sterile beach sand amended with 8 Osmocoat granules at the SCU greenhouse facilities (3 seedlings planted per wild collected individual). Plants were grown under 16 hour days (natural lighting amended with greenhouse lights), at 80F days/60F nights, bottom watered 2x per week for 1 hr. Nearly 100% of seedlings survived transplantation. Most species were flowering within two months after germination. As plants began flowering, they lost their positive phototropism and their inflorescences began sprawling thus requiring staking. The greenhouse is free of pollinators, thereby allowing for an assessment of the ability of *S. albidus* ssp. *albidus* to self-fertilize. The large majority of flowers across multiple individuals and multiple populations show no fruit development. Powdery mildew appeared after about 3 weeks of flowering, but three treatments over 1 week with organic oil/soap solution (Dr. Earth) eliminated most signs of fungal infection. Unfortunately, soon thereafter, many plants began showing signs of stress with curled leaves and discolored, dry and dying apical growing tips (grey/brown). In the end, most plants survived, but showed substantial signs of stress. This same phenomenon has been documented previously by Sharon Strauss at UC Davis greenhouse facilities when trying to grow these species by bottom watering in overly-moist soil conditions. Generally speaking, normal greenhouse culture has not been successful for cultivation and seed production in this species. We suspect that using soil within improved draining capacity and watering 1x per week once plants are established would increase the health of greenhouse-grown plants. We decided to conduct the greenhouse transplant study in a lath-house with much higher levels of air flow, cooler/drier temperatures, and restricted water availability which prevented the aforementioned greenhouse symptoms.



Figure 9. Seed color polymorphism in *S. albidus* ssp. *albidus* from Metcalf Motorcycle Park is caused by the underlying cotyledon pigmentation. Tan seeds have light green cotyledons (left) vs. black seeds have dark green/purple cotyledons (right).

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As these plants finished flowering in the greenhouse, we quantified the number of flowers produced and the number of fruits matured per plant. There was no significant difference in the number of flowers produced per plant among populations in the

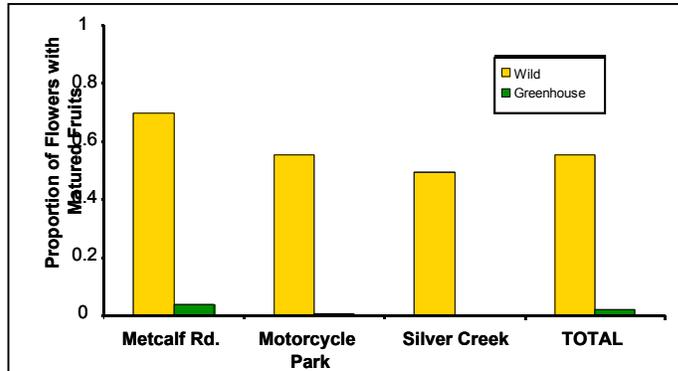


Fig. 10. A comparison of fruit production under wild and greenhouse conditions shows the effects of genetic self-incompatibility expressed in the greenhouse due to a lack of pollinators.

greenhouse, nor was there a significant difference in the number of flowers produced in the greenhouse compared to wild conditions (data not shown). Yet, there was <1% fruit production per flower in the greenhouse which was significantly less than wild fruit production (>50% measured across several populations; $p < 0.05$; Fig. 10) suggesting that greenhouse culture is NOT an efficient approach to generating seeds for reintroduction.

This could be due to genetic self-incompatibility since the greenhouse grown plants were prevented from cross-fertilization because there are no pollinators were allowed in the greenhouse. Genetic self-incompatibility is a common mechanism to maintain high genetic diversity in many genera of the mustard family, yet no jewelflowers have ever been directly examined for this. Alternatively, the low fruit set compared to levels in the wild could be due to some unknown factor associated with growing these plants under controlled conditions. Similarly low fruit set occurred in the UC Davis Lath-House when plants were grown in different soil types reducing the likelihood that it is a greenhouse specific effect. This result has now been verified in a second attempt at growing these plants in the SCU greenhouses (fall 2008). This is consistent with the self-incompatibility conclusion, yet needs specific testing with a pollinator exclusion experiment in the field.

VII. Local Adaptation Transplant Experiment

To determine whether there are additional soil factors that may contribute to the distinctiveness of *S. albidus* ssp. *albidus*, we conducted a reciprocal transplant study in the lathhouse (a partially enclosed greenhouse without cooling or heating capacity) at UC Davis from winter to spring of 2008. First, seeds were collected from twenty individuals from seven populations of *S. albidus* ssp. *albidus*, *S. albidus* ssp. *peramoenus* and *S. glandulosus* from the wild. They were stored at room temperature in coin envelopes for approximately six months until they were planted in a diversity of soils.

Second, soil was collected from existing *Streptanthus* populations to determine the degree of local adaptation. We removed the upper 10 cm of soil from five or more locations within the population which were immediately adjacent to a large number of plants in hopes of harvesting soil representative of each population. Soil was collected from *S. albidus* ssp. *albidus* at Metcalf Rd., for *S. albidus* ssp. *peramoenus* at Kirby Canyon (subpops #2,3&4), for *S. glandulosus* along Llagas Rd. (near junction with Castle) and for the unusual, non-serpentine *Streptanthus* populations atop Mt. Hamilton,

located ca. 100m south of the main Observatory Dome and Visitors Center. Additional soils from a potential reintroduction site at Tulare Hill were collected at three locations ca. 50m, 200m and 400m from the canal crossing.

Third, soil samples within each site were thoroughly mixed and then used to fill 25 cm tall conetainers (Fig. 11). In each conetainer, four seeds were planted from the same maternal family. After approximately two weeks, all conetainers were thinned to one seedling each and if no seedlings emerged, another set of seeds were planted. After approximately four months of growth, plant fitness was estimated using three measures: the total number of flowers per plant, total plant height, and the aboveground dry biomass. Although typical fitness estimates include components of seed or fruit production, this was not appropriate since <1% of plants produced fruits due to the absence of pollinators in the lathhouse.

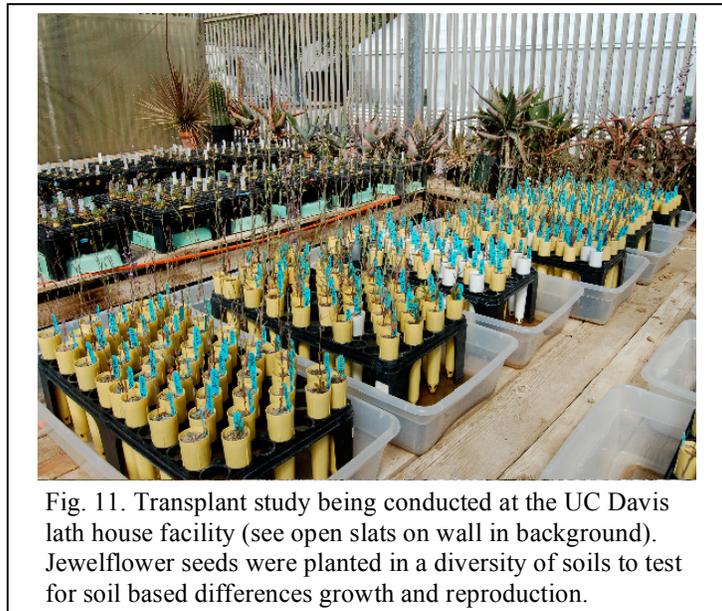
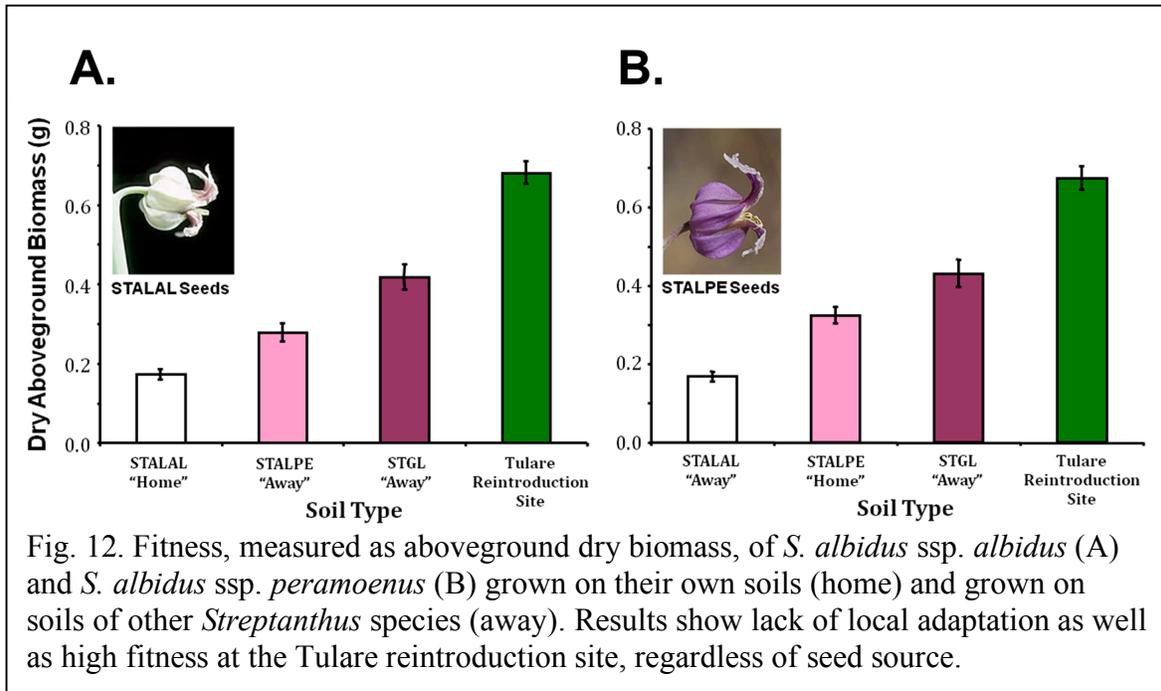


Fig. 11. Transplant study being conducted at the UC Davis lath house facility (see open slats on wall in background). Jewelflower seeds were planted in a diversity of soils to test for soil based differences growth and reproduction.

For estimating aboveground dry biomass, plants were clipped at the soil surface, dried at 20C for one week and then weighed to the nearest milligram on a microbalance.

All three measures of fitness were highly correlated across seed sources and soil types so we present only the aboveground dry biomass results here. Surprisingly, when *S. albidus* ssp. *albidus* seeds were grown on a diversity of soils, the home soil type (Metcalf Rd.) had the poorest growth (Fig. 12A). This suggests no evidence of a home-site advantage. Soil type had a substantial effect on the growth of ssp. *albidus*. These seeds performed best on the soil from Tulare Hill – the potential reintroduction site. In general, this suggests that the ability to grow in neighboring soils is not the limiting factor in keeping ssp. *albidus* from expanding into neighboring species' habitats.

Seeds of *S. albidus* ssp. *peramoenus* had the lowest aboveground dry biomass in the Metcalf soil of ssp. *albidus*, but also grew relatively poorly on its home soil (Fig. 12B). Fitness was moderately high when grown on soil from *S. glandulosus* populations, but like ssp. *albidus*, it had highest fitness in soil from the Tulare Hill reintroduction site. The similar profiles of fitness across soil types for ssp. *albidus* vs. ssp. *peramoenus* suggest that these two lineages are not distinguished by their ability to grow on a diversity of serpentine soils.



When the soil chemistry for these populations is compared with plant performance, the three soil components that correlate with plant fitness are soil iron, available nitrogen, and organic matter. Soil iron is rarely suggested as a limiting factor of serpentine soils, yet may affect *Streptanthus* spp. growth rates.

Interestingly, only 20% of *S. glandulosus* seeds from the non-serpentine population atop Mt. Hamilton survived on serpentine soil while 71% survived on their own non-serpentine soils (data not shown). However, the reverse was not true as 56% of *S. albidus* seeds survived on Mt. Hamilton soil while 65% survived on their own serpentine soils. This suggests that our lathhouse reciprocal transplant experiment was capable of detecting significant differences in grossly different soil types, yet no clear distinctions between ssp. *albidus* and ssp. *peramoenus* were detected.

Chemical analyses have revealed very few differences in soil characteristics among several *Streptanthus* species including the Metcalf Jewelflower. At the same time, the soil chemistry at the Tulare Hill potential reintroduction site contains soils seemingly capable of sustaining a viable population of the Metcalf Jewelflower. The local adaptation study showed plants rarely grow best on their own soil. In fact, both ssp. *albidus* and ssp. *peramoenus* grew poorest on the Metcalf soil. The highest fitness of both subspecies was obtained when growing on the Tulare Hill soil providing further evidence that this site has the potential soil characteristics to promote a viable population. This suggests that from a soil chemistry and local adaptation perspective, Tulare Hill should be a successful reintroduction site.

VIII. AFLP Assessment of Taxonomic Status

Start Date	Objective	Milestone	Completion Date
Sept. 2008	Conduct molecular analyses to determine taxonomic status	DNA extractions (by Oct. 2008), AFLP data collection completed (Dec. 2009)	Dec. 2008

The Metcalf Jewelflower, *S. albidus ssp. albidus*, is distinguished from its more widespread relatives, *S. albidus ssp. peramoenus* and *S. glandulosus*, primarily by its flower color. In order to assess its evolutionary distinctiveness at the molecular level, we have extracted DNA for AFLP analysis from the following 95 individuals (Table 2).

Table 2. Samples used in molecular analysis of taxonomic distinctiveness.		
Taxon	Population	No. of Individuals
<i>S. albidus ssp. albidus</i>	Metcalf Road (Met)	10
<i>S. albidus ssp. albidus</i>	Silver Creek Golf Course (Sil)	10
<i>S. albidus ssp. albidus</i>	Motorcycle Park (Mot)	10
<i>S. albidus ssp. peramoenus</i>	Kirby Landfill (Kirby)	36
<i>S. albidus ssp. peramoenus</i>	Llagas Rd. (Llag)	5
<i>S. albidus ssp. peramoenus</i>	Canada del Oro (Can)	5
<i>S. glandulosus</i>	Kennedy Road (Ken)	5
<i>S. glandulosus</i>	Bernal Road (Ber)	5
<i>S. glandulosus</i>	Observatory Peak (Obs)	4
<i>S. glandulosus</i>	Tortilla Flats (Tor)	4
TOTAL		94

After extracting DNA from these 94 individuals, we determined the concentration and quality of the genomic DNA using agarose gel electrophoresis (Fig. 13). The DNAs were of sufficient concentration and quality to proceed with the AFLP procedure in order to determine if there are molecular markers that also distinguish these lineages.

The Metcalf Jewelflower, *S. albidus ssp. albidus*, is distinguished from its more widespread relatives primarily by its flower color. In order to assess its evolutionary distinctiveness, we conducted an AFLP survey of the genomes of 94 individuals. In a neighbor-joining, Nei-Li distance analysis of 634 scored bands, we recovered a nearly exclusive lineage of *S. albidus ssp. albidus* amongst the remaining samples from *S. albidus ssp. peramoenus* and *S. glandulosus* (Fig. 14). This lineage also contains two

Kirby samples (Kirby510 and Kirby212), and there is one Metcalf sample of *S. albidus* ssp. *albidus* that is not present in this lineage (Met8). A nearly complete clade of *S. albidus* ssp. *albidus* suggests this taxon may be a unique evolutionary lineage. Yet, this branch in the phylogeny is supported by less than 50% in a bootstrap analysis (i.e. weakly supported, at best). This result suggests either (1) a relatively recent evolutionary split among these closely related taxa, or (2) occasional introgression/hybridization between the populations via gene flow from seeds and/or pollinators. These two evolutionary scenarios are very difficult to distinguish based on the AFLP data alone and are not mutually exclusive. We will be conducting a Bayesian analysis on the AFLP data to further assess the distinctiveness of this lineage composed nearly entirely of *S. albidus* ssp. *albidus*.

It is also noteworthy that after doubling the number of AFLP bands scored since the preliminary analysis (not shown), the Mt. Hamilton samples from Tor and Obs remain evolutionarily distinctive (see Mt. Hamilton arrow in Fig. 14). This branch is supported by a bootstrap value of 98% indicating very strong support. These two populations are also distinguished by having unusually dark purple/black flowers reminiscent of *S. insignis* from >75 km south in the Diablo Range (the San Benito or Plumed Jewelflower), yet the latter taxon is noted for its sterile terminal flowers which are not present in the plants from Mt. Hamilton. The genome-wide divergence and morphological distinctiveness of these populations strongly suggest that this taxon is in need of further taxonomic and conservation attention.



Fig. 13. A representative collection of seven DNAs indicates that genomic DNA is of high enough quality and concentration to proceed with the AFLP assessment of the molecular distinctiveness of the Metcalf Jewelflower. MW = molecular weight standard.

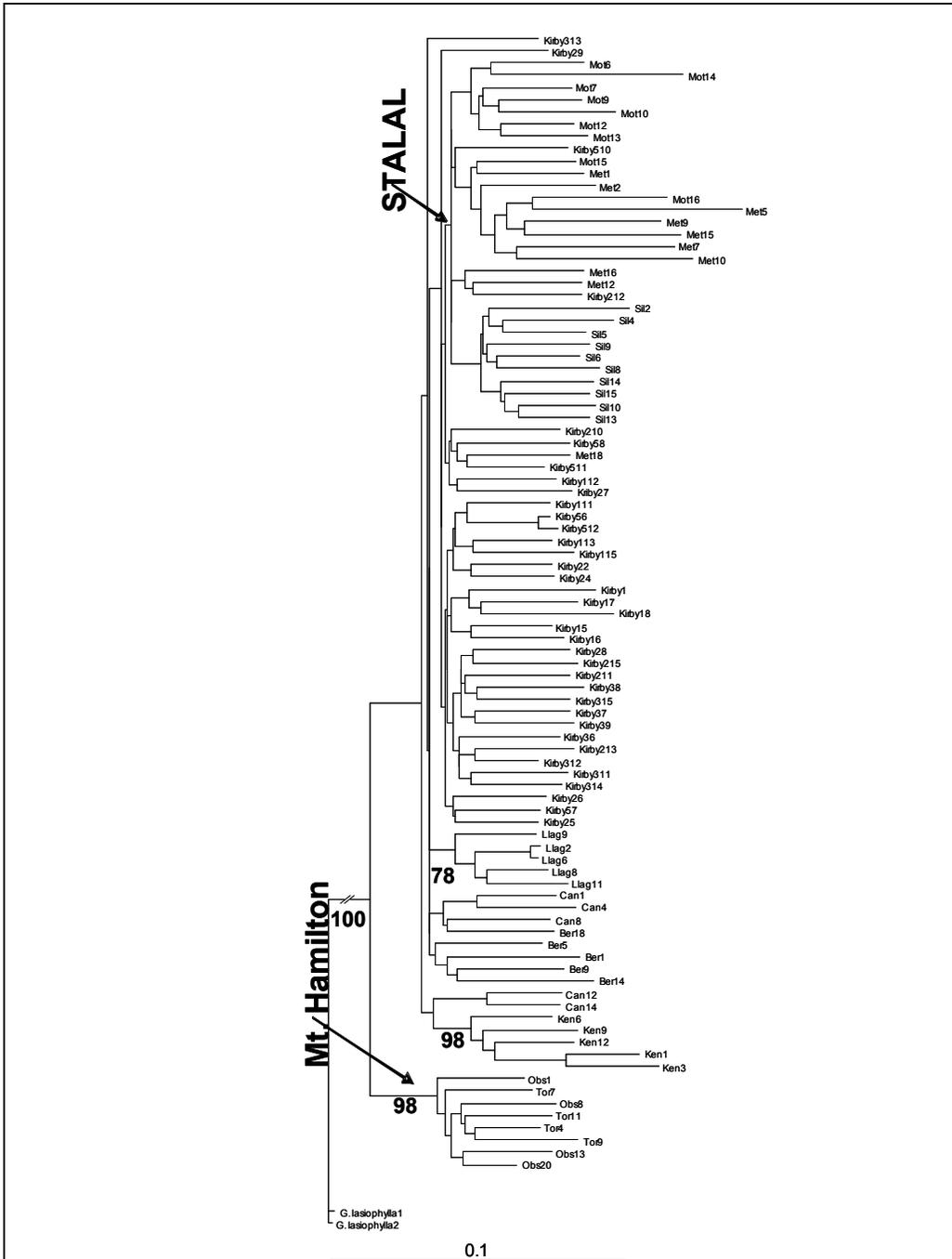


Fig. 14. A genetic distance analysis of 634 AFLP bands detects weak genomic distinctiveness of *S. albidus* ssp. *albidus* (Met, Mot & Sil pops.; STALAL with arrow) when compared to samples of closely related taxa. A unique lineage comprising two Mount Hamilton populations (Obs & Tor; Mt. Hamilton with arrow) is worthy of further taxonomic and conservation study. The tree is rooted with the more distantly related *Guillenia lasiophylla*. Bootstrap values >75% are indicated below branches with strong support.

IX. Floral Anthocyanin Gene Survey

Streptanthus albidus ssp. *albidus* is distinguished from its closest relatives nearly entirely by its white flower color (Fig. 15). Since there is only weak genomic distinctiveness of this lineage based on the AFLP results described above, we pursued the molecular basis for this single distinguishing trait. The difference in flower colors can be traced to their anthocyanin pigments (or lack thereof).

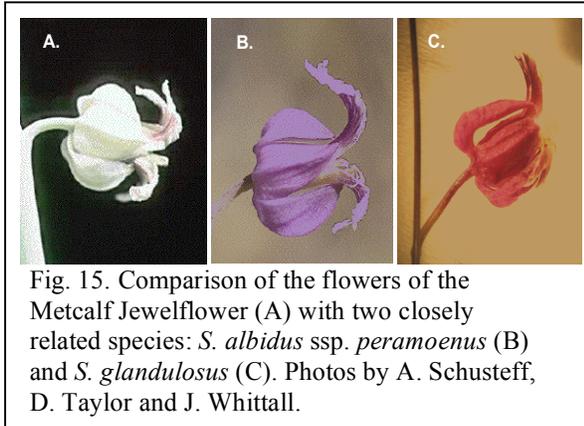


Fig. 15. Comparison of the flowers of the Metcalf Jewelflower (A) with two closely related species: *S. albidus* ssp. *peramoenus* (B) and *S. glandulosus* (C). Photos by A. Schusteff, D. Taylor and J. Whittall.

The anthocyanin biosynthetic pathway (ABP) is a linear, six-step pathway responsible for producing the anthocyanin pigments (Fig. 16). A blockage at any step in this pathway prevents biochemical intermediates from completing their journey to becoming visible anthocyanin pigments. Therefore, the molecular difference between purple and white flowered *plants* can be immediately targeted to the loss-of-function of one or more of these six genes. In previous studies, I have been able to easily and efficiently characterize all six ABP loci in taxa only distantly related to model species. Identifying the genes responsible for the sole difference between these two lineages would allow us to discern whether this flower color difference was driven by natural selection (suggestive of local adaptation) or through random processes such as genetic drift (accentuated in small populations). The difference between these two alternative evolutionary scenarios can be distinguished by the patterns of allelic diversity within and between populations. Evidence for natural selection would have substantial conservation implications indicating the white flower color reflects local adaptation of the plants to their pollinators or some other agent of selection (i.e. through interaction with the serpentine soils of these populations). Alternatively, evidence that the flower color difference accumulated due to genetic drift would argue that this trait was not a significant factor in the original differentiation of this lineage.

In order to characterize the six ABP loci in *Streptanthus*, we start with all the genes expressed in the petals (mRNAs) to ensure that the genes we isolate are expressed in the correct tissue at the appropriate developmental stage. To date, we have extracted total RNA from the white sepals of *S. albidus* ssp. *albidus* and purple sepals of the closely related *S. glandulosus*. Then, after converting the labile RNA to more stable cDNA, we have amplified, cloned and sequenced a large portion of all six ABP loci (Table 3). Although we find many DNA differences, some of which represent amino acid substitutions, we have yet to find a consistent difference between plants with

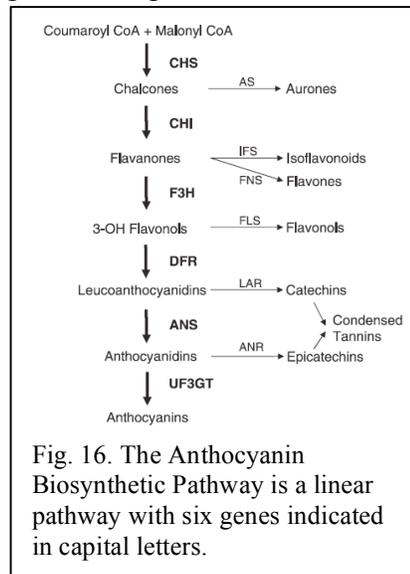


Fig. 16. The Anthocyanin Biosynthetic Pathway is a linear pathway with six genes indicated in capital letters.

purple vs. white sepals. This suggests that either (1) the blockage is in a portion of a gene(s) that we have not sequenced or (2) the blockage is due to loss of expression of the genes, rather than a structural mutation in the gene itself. We have used 5' and 3' RACE to characterize the entire sequence of the first gene in the ABP (CHS in Table 3).

Table 3. Sequencing results of ABP genes from cDNA of *Streptanthus glandulosus* and *S. albidus* ssp. *albidus*.

Gene	Amount Sequenced (bp)	Total Gene Length (bp)	Proportion Sequenced (%)
CHS	1200	1200	100
CHI	430	700	61
F3H	798	1096	73
DFR	665	1205	55
ANS	568	1084	52
UF3GT	1043	1380	76
AVERAGES	784	1110	71

X. Manuscript Preparation

Start Date	Tasks	Milestones	Final Completion Date
Jan. 2009	Analyze data, write reports and manuscripts describing results	Data analysis completed, scientific reports and manuscripts submitted	March 2011

We are now in the process of preparing a manuscript that describes these findings. Sharon Strauss and I have decided to prepare a manuscript describing our results for the *American Journal of Botany*. We are currently preparing figures and tables for the manuscript and expect submission soon after the submission of this final report.

Our findings have greatly broadened the baseline biological data for the Metcalf Canyon Jewelflower from propagation, pollination and soil preference perspectives. We hope to now utilize these findings to improve the status of the Metcalf Jewelflower. To this end, Dr. "Stu" Weiss of Creekside Center for Earth Observation and I have developed a reintroduction proposal submitted to the Bureau of Reclamation. Our hope is to experimentally reintroduce the Metcalf Jewelflower at several sites on Tulare Hill. Based on the findings in this report, we have the germination & propagation protocols to establish plants in a site with comparable soil chemistry that has already been shown to support robust *S. albidus* ssp. *albidus* plants in lath-house conditions. We envision an opportunity to define the reintroduction parameters necessary to establish viable populations of *S. albidus* ssp. *albidus* at Tulare Hill and beyond. The combined forces of the Creekside Center (especially Stu Weiss' expertise) with my research on pollination, soil chemistry, germination, propagation and distinctiveness of *S. albidus* ssp. *albidus* provides a unique opportunity for this plant to become a model for serpentine plant restoration and reintroduction.