

Microsatellite patterns in *Castilleja affinis* ssp. *neglecta*

CBG REU Summer Intern Project

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Castilleja affinis ssp. *neglecta*

- Family: Orobanchaceae
 - Common Name: Tiburon paintbrush
 - Previously in Scrophulariaceae
 - Many species were in this family but molecular work on plastid genes led to a reclassification (Olmstead et al., 2001)
- Ecological:
 - Hemiparasitic
 - Grows to be 3-6 dm tall
 - Flowers brightly hued yellow to peach bracts
 - Blooming period April to June
- Habitat:
 - Serpentine grassland endemic

Current Status

- Critically imperiled
 - USFWS federal and CA State listed
 - Confined to serpentine soils in the San Francisco Bay Area
 - Only 7 known sites
- Major Threats:
 - Habitat loss such as rapid residential development, gravel mining, and grazing

Previous work

- In the early 80's Chuang and Heckard offered a new mode of nomenclature for the *Castilleja* species found in California (Chuang & Heckard et al., 1982)
- The naming systematic was based on leaf size and shape, corolla pubescence, etc.

Current Work

- Microsatellites were developed to help characterize *Castilleja sessiliflora*
- These microsatellites have been shown to work in the other 23 major species (Fant and Skogen et al., 2013)
- Now we are using these for *C. affinis* ssp. *neglecta*

Why Molecular Vs. Morphological?

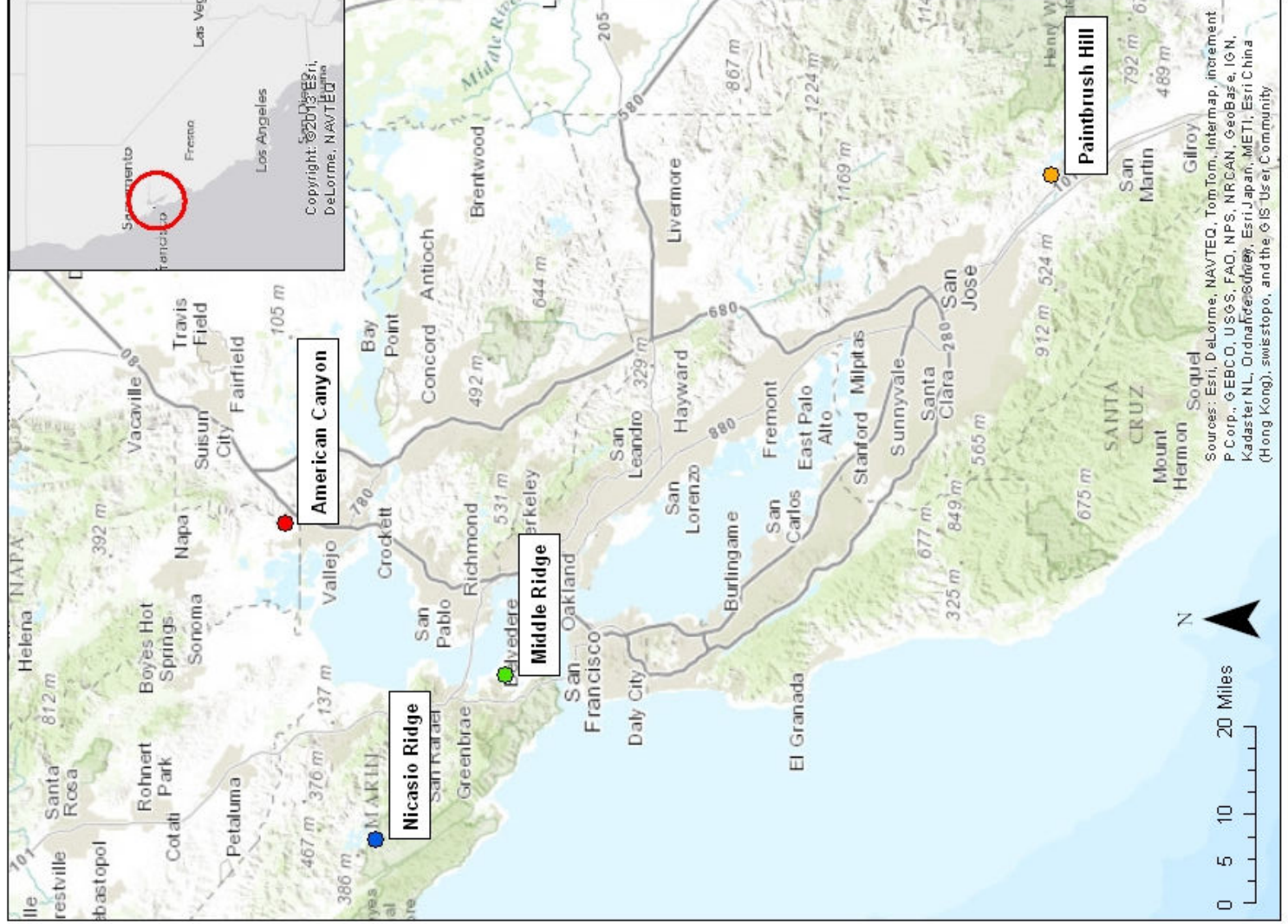
Castilleja is polyploidy and has many nuances to its morphology making it hard to correctly identify, now we can use molecular tools



My project

- Using microsatellites to look at genetic differences and similarities of four separate populations of *C. neglecta*
- This is important for recovery efforts and restoration by Creekside Center for Earth Observation
- <http://creeksidescience.com/>

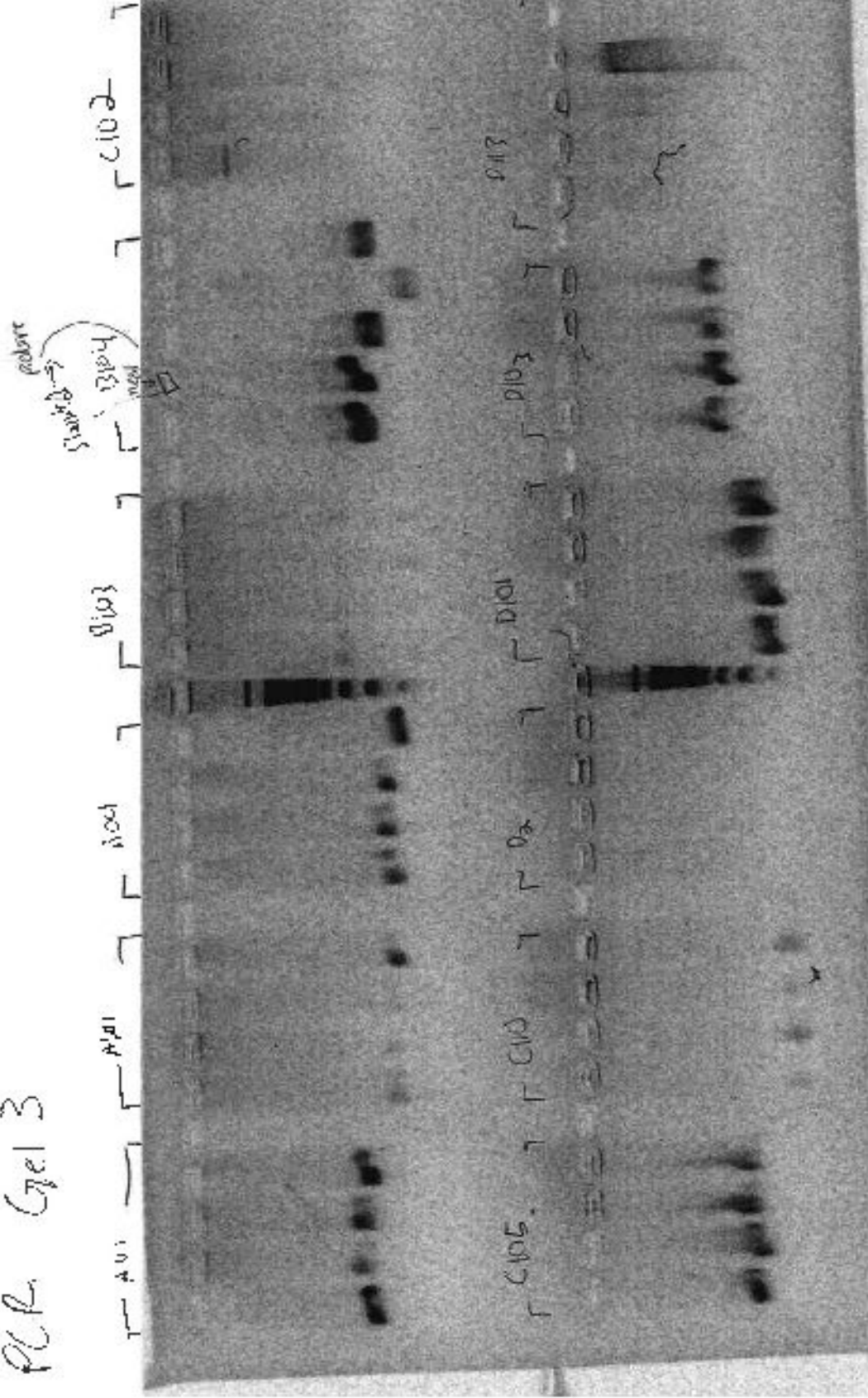
Castilleja affinis ssp. neglecta Sites



Materials and Methods

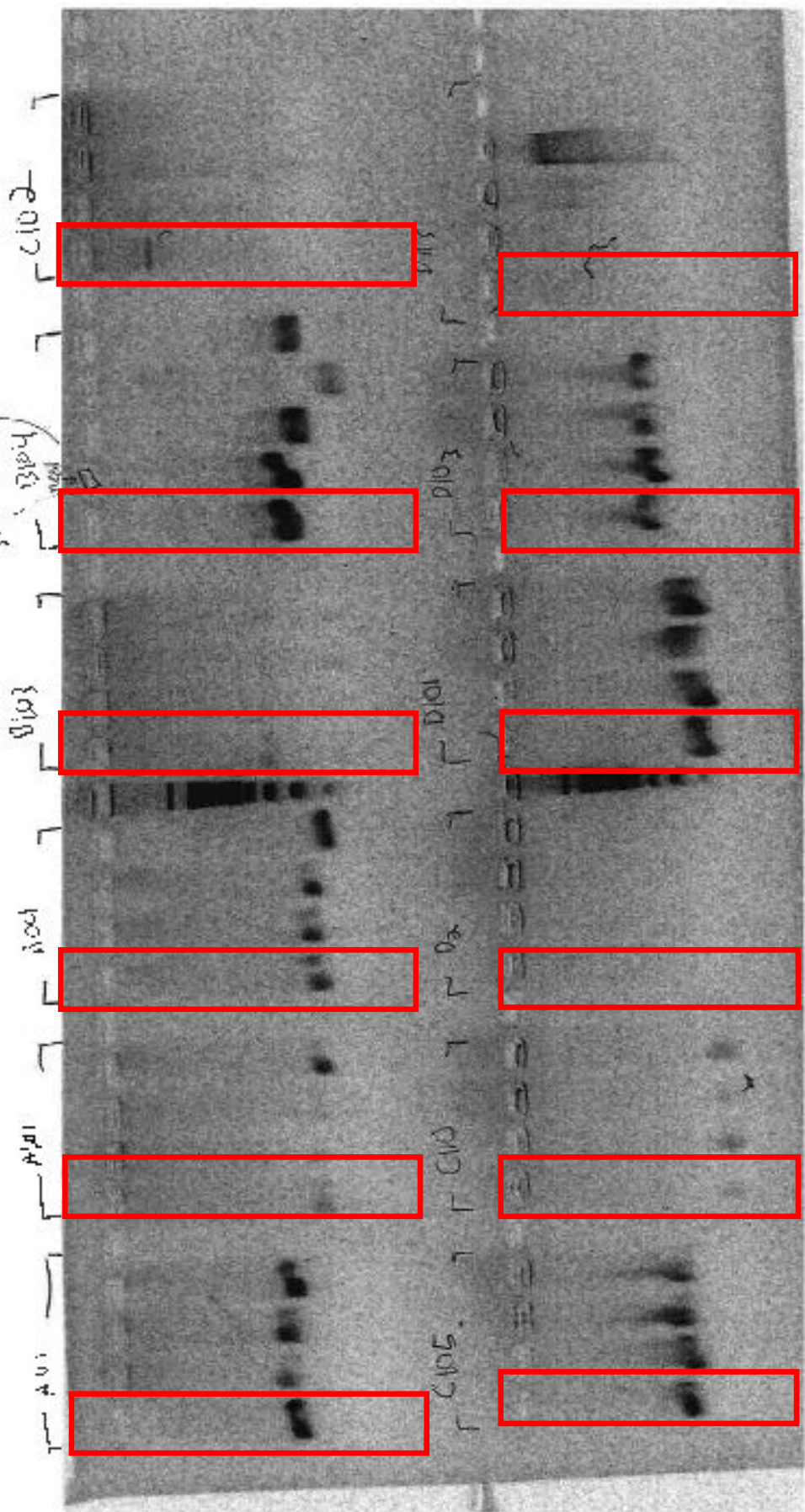
- CTAB DNA extraction
 - Adapted from (Doyle and Doyle, 1987)
 - Dried Leaf samples
- Gel Electrophoresis
 - Used to visualize DNA
 - Assure DNA was extracted
 - Shows which primers worked

PCR Gel 3



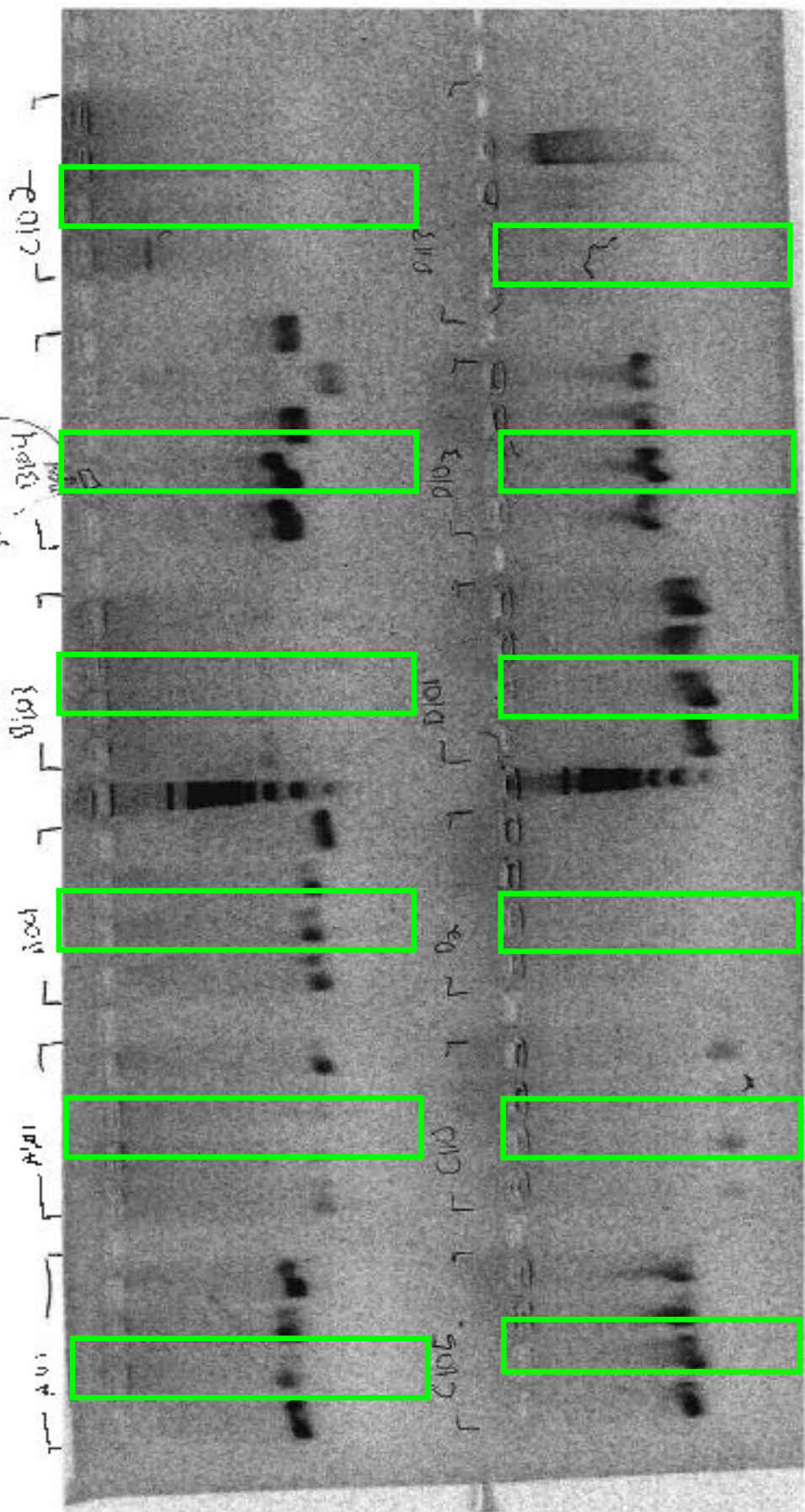
PCR Gel 3

probe
5' →
3' →



PCR Gel 3

probe
5' → 3'
1384
1384



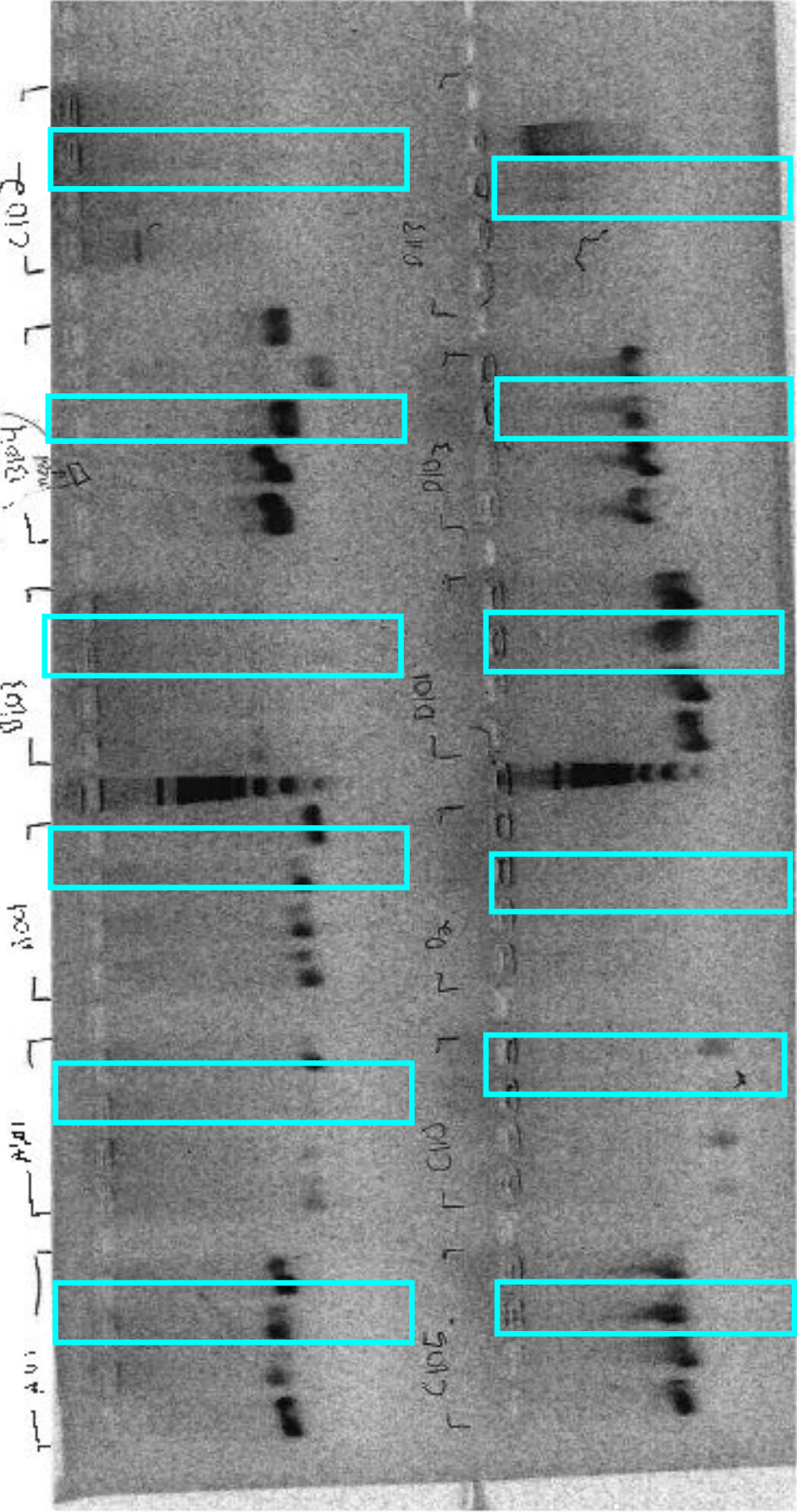
CNMR8

KODAK

Hasin2.tif

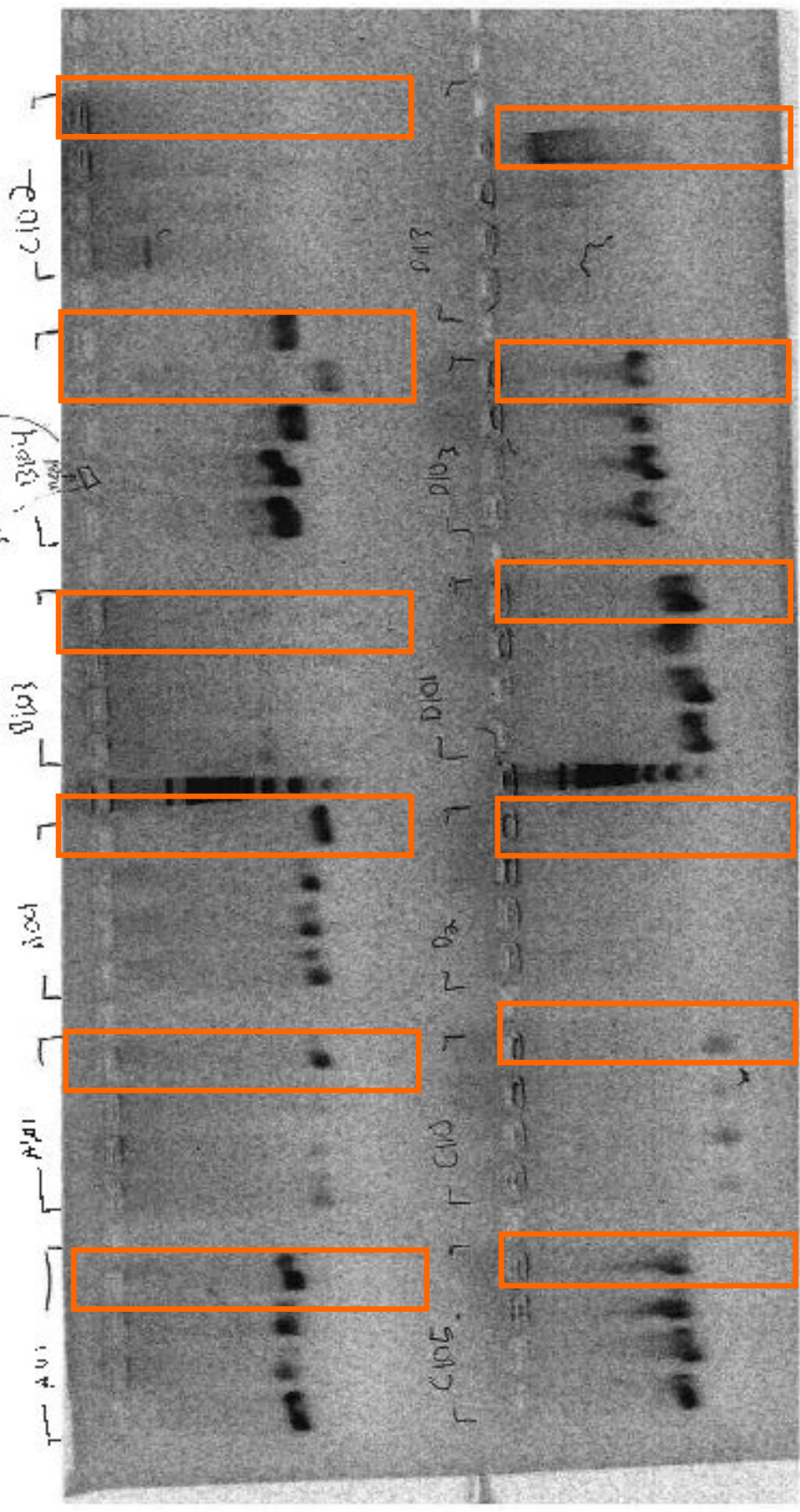
PCR Gel 3

probe
5' → 3'
1384
1384
1384



PCR Gel 3

probe
5' →
3' ←
1384
1000



DNA fragment analysis

- Beckman Coulter CEQ 8000
 - The lengths of DNA fragmented and selected by the primers are measured using dyes
 - The number of peaks and intensity of peaks shows how many alleles are present

Results

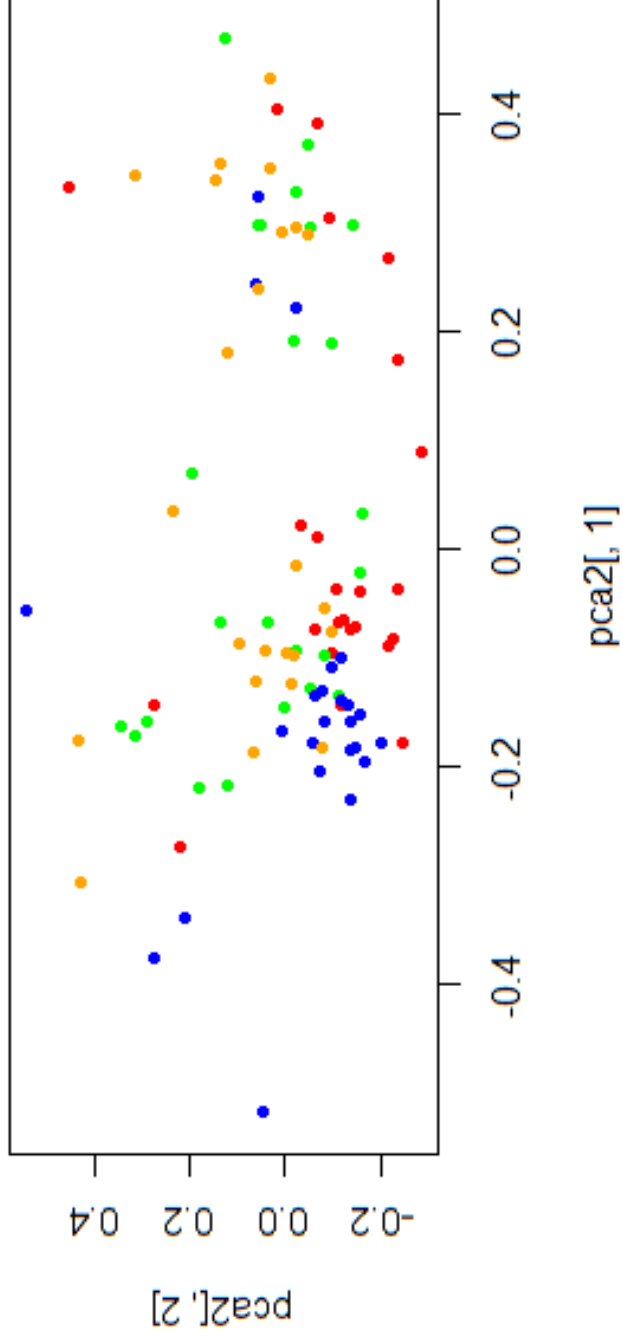
- *Castilleja affinis* ssp. *neglecta* is hexaploid
- R – statistical software
 - Resulting fragments came in hexaploid form
 - Combinat & Polysat programs were used to generate a Principle Component Analysis plot with Lynch distance

PCA Plot

- A graphical representation of how similar or dissimilar samples are
- Depicts how related samples from the same population are
- Represents how similar separate populations are to one another

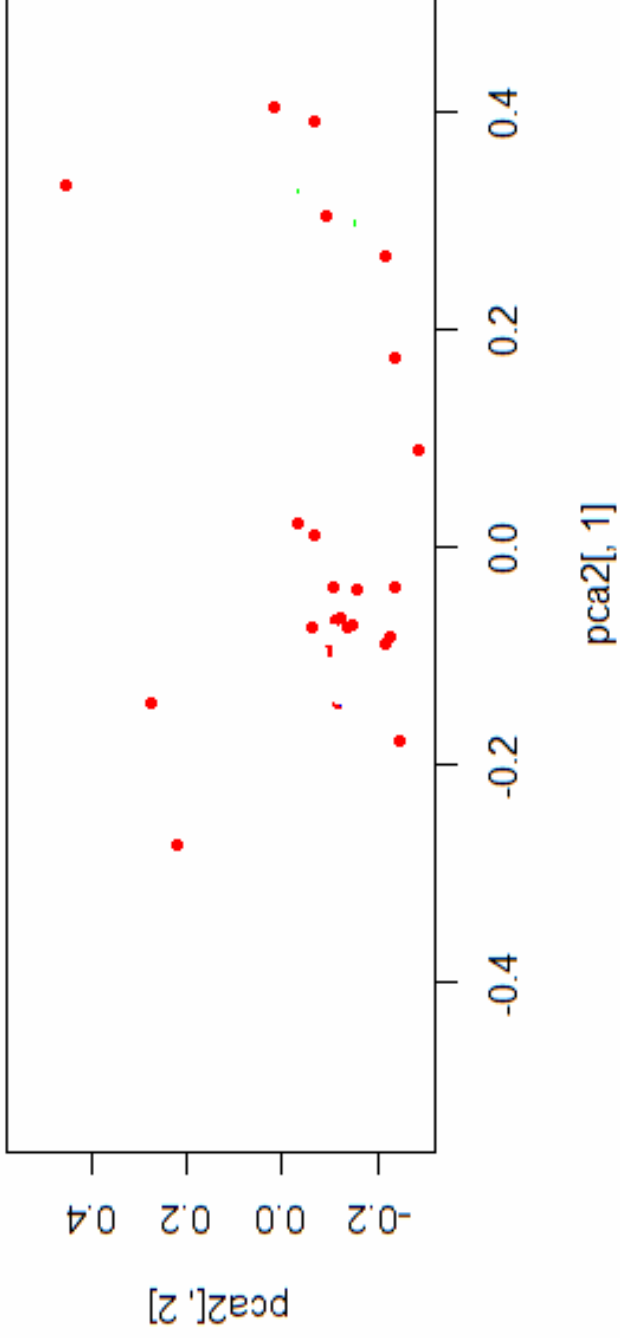
Relatedness between populations

Separation by Lynch distance



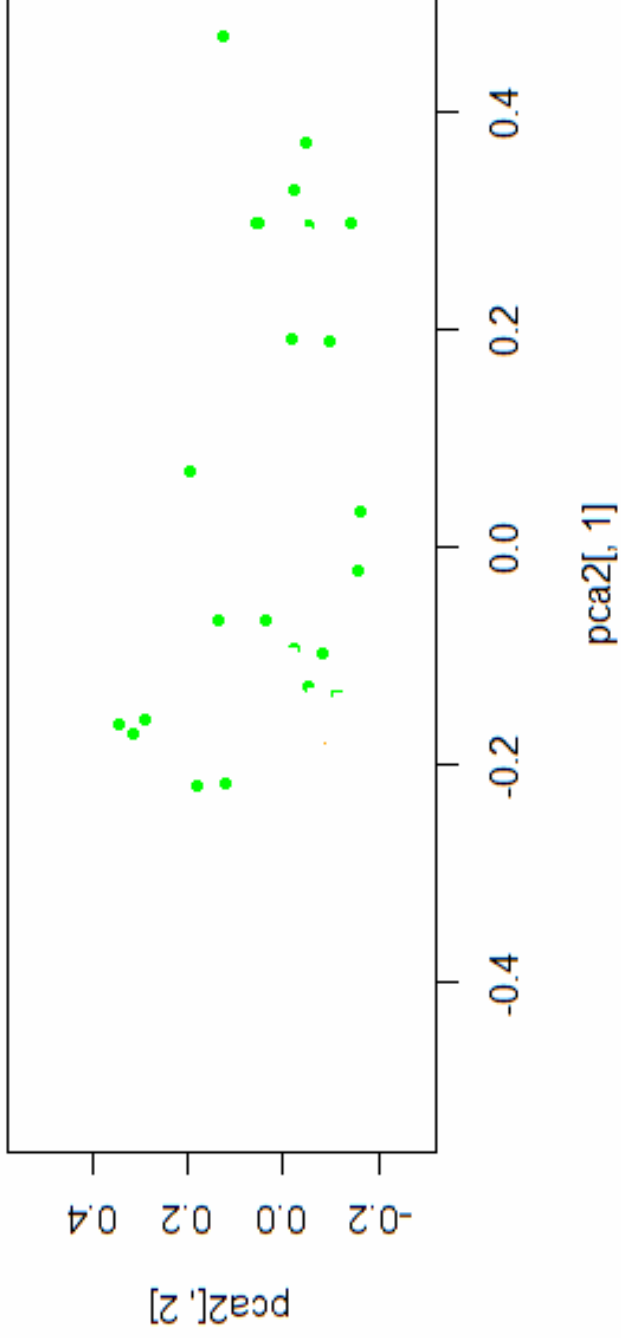
American Canyon

Separation by Lynch distance



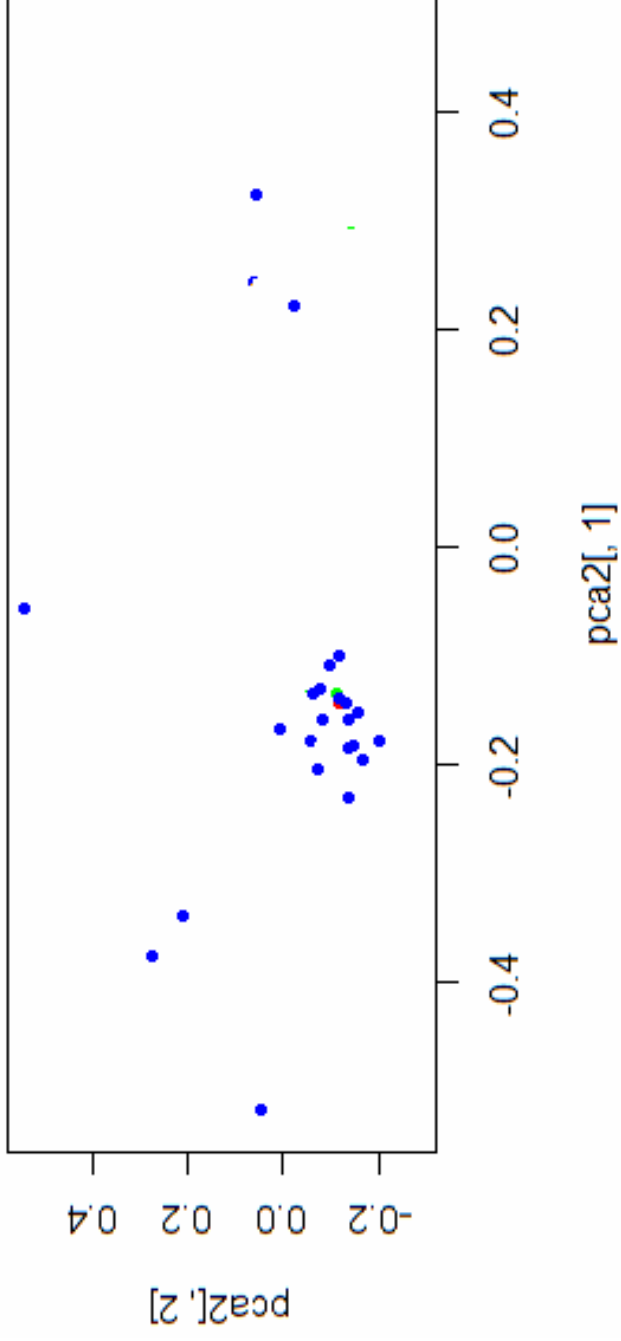
Middle Ridge

Separation by Lynch distance



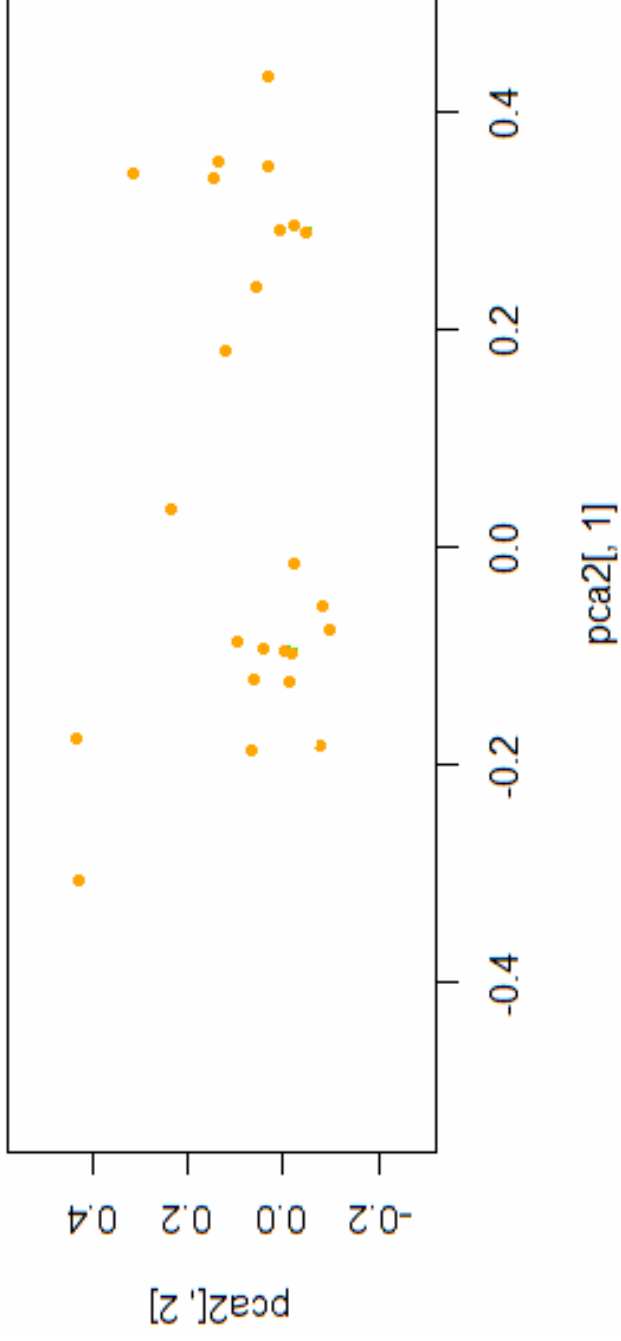
Nicasio Ridge

Separation by Lynch distance



Paintbrush Hill

Separation by Lynch distance



Allelic Diversity

	Shannon	Simpson	Average Alleles per pop
CNAC	3.18	1.00	15.50
CNMR	3.17	0.98	19.83
CNNR	2.61	0.92	15.83
CNPH	3.18	1.00	15.67

- We used 3 different measures of diversity
- Nicasio Ridge was the least diverse among the four populations

Conclusion

- Within each population their tends to be clustering with a few outliers
- Between each population there tends to be a separation
- In conserving this species land managers should be careful moving seeds or plant material for population enhancement purposes

Questions on Diversity

- Nicasio Ridge had the least diversity when compared to the other sites
- Possible reasons:
- Physical isolation since Nicasio Ridge is furthest west
 - Has many other rare plants that only grow in Nicasio Ridge
 - Pollination distance might be too far for other sites

Bibliography

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