

Genetic methods to detect vertebrate herbivory on Tiehm's buckwheat (*Eriogonum tiehmi*): FINAL REPORT

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Executive Summary:

Environmental DNA (eDNA) was analyzed from damaged roots of Tiehm's buckwheat (*Eriogonum tiehmi*), undamaged control root samples taken from undamaged plants, soil tailings adjacent to damaged buckwheat plants, control soil collected from undamaged plants, and rodent scat found near damaged plants. The analysis revealed three genetic signatures of a rodent species belonging to tribe Marmotini, a taxon that includes antelope ground squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), marmots (*Marmotus*), Chinese rock squirrels (*Sciurotamias*), ground squirrels and rock squirrels (*Spermophilus*), and chipmunks (*Tamias*).

The genetic signatures range from 96.96 to 99.75% match ($e=0.0$) with the banked DNA sequence for Harris' antelope ground squirrel (*Ammospermophilus harrisi*) in the GenBank database maintained by the National Institutes of Health. The e-value is the number of expected DNA sequences of similar match quality that could be found just by chance. The closer e is to zero, the better the quality of the match. This does not mean that the DNA came from *A. harrisi*. The DNA most likely originated from the locally abundant white-tailed antelope ground squirrel (*A. leucurus*), which does not have a 12S DNA barcode banked in the GenBank or BOLD databases. These two antelope ground squirrels are expected to have extremely similar DNA sequences because they belong to the same genus.

These same *Ammospermophilus* genetic signatures were also found in soil samples and in the rodent pellets found within Tiehm's buckwheat subpopulation 2. The rodent pellets were also tested for plant eDNA, and found to contain DNA with a 100% match for the buckwheat genus, *Eriogonum*. A DNA barcode for Tiehm's buckwheat does not yet exist in GenBank or BOLD, which means that the nearest genetic match is *Eriogonum crosbyae* with which it has a 100% sequence match ($e=2 \times 10^{-65}$). No sciurid (squirrel family) eDNA was found in the control samples. These data strongly support the hypothesis that a diurnal rodent in the genus *Ammospermophilus* was responsible for damage to the Tiehm's buckwheat population at Rhyolite Ridge in the late summer and early fall of 2020.

Unidentified cervid (deer family) eDNA was present in five samples, and human DNA from sampling contamination was present in two samples.

Background: Damage to Tiehm's buckwheat

Tiehm's buckwheat (*Eriogonum tiehmi*) is a rare plant found on approximately 21 acres in the Silver Peak Range of Esmeralda County, Nevada (Reveal, 1985). On September 8, 2020

researchers from the Leger Lab at the University of Reno observed that an estimated 25-50% of plants distributed among six subpopulations had been damaged, cut, or dug up (McClinton, 2020). The damage was later noticed by biologists from other agencies who reported it to the media along with accusations of human malfeasance (Bahouth, 2020). Initial reports indicated that the damage was due to rodents (McClinton, 2020), but concern remained that humans may have been responsible. The work described below was undertaken to investigate potential causes of the damage.

Background: Environmental DNA

Environmental DNA (eDNA) is trace DNA found in soil, water, food items, or other substrates with which an organism has interacted. eDNA samples contain the DNA traces from multiple community members in the environment of interest. eDNA from residual saliva has been used to identify predator DNA from bites on clay models (Rößler *et al.* 2020), brown bear DNA left on salmon carcasses (Wheat *et al.* 2016), macaw DNA from fruits (Monge *et al.* 2020, and aye-aye DNA from feeding traces on trees (Aylward *et al.* 2018). These studies show that eDNA from residual saliva is a reliable way to detect when vertebrates have fed or attempted to feed on an item. Therefore, an eDNA analysis of damaged Tiehm's buckwheat roots could determine whether a vertebrate herbivore has left saliva on parts of plants still remaining in the field. Many rodent herbivores disturb the soil when they make burrows or shelters, which means that an eDNA analysis of disturbed soil near damaged plants may also reveal the genetic signature left behind by rodent herbivores.

Please note that Next Generation Sequencing (NGS) methods are extraordinarily sensitive, which means that human DNA contamination in the field-collected samples will be almost unavoidable.

Methods

Sample collection. On October 2, 2020 we obtained eDNA from three sources for this study: (1) depredated and control Tiehm's buckwheat plants at the site of root depredation, (2) soil from burrow tailings located directly next to depredated plants with soil from unaffected plants as a control, and (3) rodent scat found near depredated plants on the soil surface (Table 1, Figure A Appendix). Fifteen to twenty root samples were collected at Tiehm's buckwheat subpopulations 1, 2, 4, and 6. The root samples were combined into 5 pooled subsamples for subpopulations 1 and 6, and six pooled subsamples for subpopulations 2 and 4. Three intact plants were excavated from subpopulation 1 and subpopulation 6a, for a total of six control plants from which we expected little to no evidence of herbivore eDNA. The number of control plants was minimized to prevent further damage to the subpopulations.

In a separate analysis, fifteen to twenty samples of burrow tailings were collected at Tiehm's buckwheat subpopulations 1, 2, 4, and 6. These samples were pooled to produce six separate samples for analysis from each subpopulation. Four control soil samples that consisted of soil collected within a 30 cm radius of untouched plants were collected from subpopulations 1, 2, 4, and 6.

Rodent scat was found at subpopulation 2, where approximately 12 individual pellets were found near damaged plants. This pooled sample of pellets was divided into two subsamples to allow for an examination of eDNA from vertebrate herbivores and a separate analysis of plants in the diet.

Table 1. Five types of samples were collected for this study of environmental DNA (eDNA).

| Sample type | Rationale |
|---------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Chewed, damaged, and depredated roots | eDNA from herbivory should be present and might allow identification of a potential herbivore. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found on root samples. |
| 2. Untouched roots from intact <i>E. tiehmii</i> plants | Any eDNA present on intact plants should be background eDNA inherent to the site, which allows these samples to serve as controls. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found on control root samples. |
| 3. Soil taken from burrow tailings located within 30 cm of damaged <i>E. tiehmii</i> plants | eDNA in the soil tailings should be closely associated with the herbivore that dug the holes. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found in soil samples. |
| 4. Soil taken from within a 30 cm radius of untouched <i>E. tiehmii</i> plants | Any eDNA present in untouched soil should be background eDNA inherent to the site, which allows these samples to serve as controls. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found in control soil samples. |
| 5. Rodent scat in the form of pellets found within subpopulation 2 of Tiehm's buckwheat | <p>Pellets can be tested for the presence of vertebrate eDNA, which would indicate the taxon from which they originated. The <i>12S</i> mitochondrial rRNA barcode was used to sequence vertebrate eDNA in a sample of six pellets.</p> <p>Pellets can also be tested for the presence of plant eDNA, which would indicate whether or not DNA from <i>Eriogonum</i> had been ingested by the animal. The <i>trnl</i> barcode is specific to plants and was used to sequence plant eDNA in a sample of six pellets.</p> <p><i>E. tiehmii</i> does not yet have any DNA barcodes in the BLAST database that is coordinated by the National Institutes of Health (NIH), which means that any <i>Eriogonum</i> DNA found in our samples can only be identified to genus.</p> |

Sequencing. Samples were stored on ice in a portable cooler in the field and during shipping. Because our goal was to determine whether herbivore eDNA was present or absent in root and soil samples, we pooled root samples from 1-3 plants, and 2-4 soil subsamples per site (Appendix. Table A). Illumina Next Generation Sequencing (NGS) was performed by Jonah Ventures (Boulder, CO). Sequence data was matched to in-house libraries hosted by Jonah

Ventures and to sequences located in GenBank by using the Basic Local Alignment Search Tool (BLAST) web tool hosted by the NIH (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) on 11/10/20. The BOLD Systems Public Data Portal was checked on 11/12/20 for the existence of banked sequences for *Ammospermophilus leucurus* and *Eriogonum tiehmii*.

Results & Discussion

Root eDNA analysis. Cervid eDNA (Appendix Table B) was found in one control root sample out of six tested (16%). A variety of DNA sequences (sciurid, cervid, hominid, and unmatchable) was detected in eleven of twenty two damaged root samples (50%). Three taxa were detected in the damaged roots, and their eDNA has a >90% match to known DNA sequences: Cervidae (deer family of mammals), Sciuridae (squirrel family of mammals), and Hominidae (human DNA resulting from contamination during sampling).

Soil eDNA analysis. Cervid eDNA was found in two soil tailings samples out of 24 tested (8.3%). A variety of DNA sequences was detected in ten of 24 soil tailings samples (41.7%). Three matchable taxa were detected in soil tailings, and these eDNA sequences had a >90% match to known DNA sequences: Cervidae (deer family of mammals), Sciuridae (squirrel family of mammals), and Hominidae (human DNA resulting from contamination during sampling).

With 825 identifiable reads in both soil and root samples, the combination of cervid (471 reads) and sciurid (354 reads) DNA sequences far outnumbered human DNA (19 total reads). Note that read-number cannot be perfectly correlated with species abundance in a sample because the DNA from each species interacts with the sequencing process in a slightly different way. Therefore, these data should be considered as providing a measure of presence or absence, not abundance.

The analysis revealed three genetic signatures of a rodent species belonging to tribe Marmotini, a taxon that includes antelope ground squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), marmots (*Marmotus*), Chinese rock squirrels (*Sciurotamias*), ground squirrels and rock squirrels (*Spermophilus*), and chipmunks (*Tamias*).

The genetic signatures range from 96.96 to 99.75% match ($e=0.0$) with the banked DNA sequence for Harris' antelope ground squirrel (*Ammospermophilus harrisi*) in the GenBank database maintained by the National Institutes of Health. The e-value is the number of expected DNA sequences of similar match quality that could be found just by chance. The closer "e" is to zero, the better the quality of the match. This does not mean that the DNA came from *A. harrisi*. The DNA most likely originated from the locally abundant white-tailed antelope ground squirrel (*A. leucurus*), which does not have a 12S DNA barcode banked in the GenBank or BOLD databases. These two antelope ground squirrels are expected to have extremely similar DNA sequences because they belong to the same genus.

These same *Ammospermophilus* genetic signatures were also found in soil samples and in the rodent pellets found within Tiehm's buckwheat subpopulation 2. The rodent pellets were also tested for plant eDNA, and found to contain DNA with a 100% match for the buckwheat genus, *Eriogonum*. A DNA barcode for Tiehm's buckwheat does not yet exist in GenBank or BOLD,

which means that the nearest genetic match is *Eriogonum crosbyae* with which it has a 100% sequence match ($e=2 \times 10^{-65}$). Plant eDNA from the following genera were also found in the rodent pellets: *Silene*, *Salsola*, *Haloxylon*, and *Atriplex*. No sciurid (squirrel family) eDNA was found in the control samples. These data strongly support the hypothesis that a diurnal rodent in the genus *Ammospermophilus* was responsible for damage to the Tiehm's buckwheat population at Rhyolite Ridge in the late summer and early fall of 2020. The genetic analysis is supported by morphological evidence consisting of rodent incisor marks on roots of damaged plants found in subpopulations 1, 2, 4, and 6 (Figure 1).



Depredated root that shows clear incisor marks along its length.



Typical damage found in cut plants that were found laying on the soil.



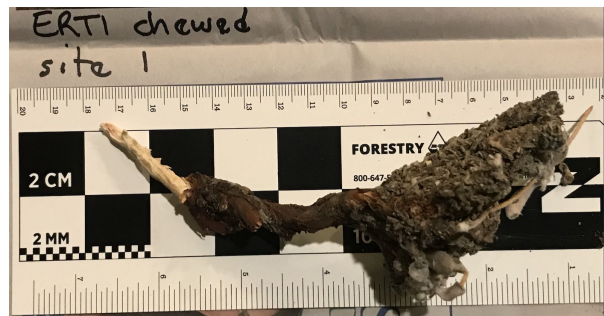
A beautiful specimen of *E. tiehmii* in which two areas of depredation can be seen at the top of the photo near the notebook. In these two areas, the roots appeared to be excavated by a non-human vertebrate. The rest of the plant was intact and healthy. The appearance of this plant does not support the hominid poaching hypothesis.



Plant showing damaged, but intact roots still connecting plant to substrate in a way unlikely to have been achieved by hominid tool use.



Close up view of plant pictured directly above. Roots are clearly damaged almost 360° around root, which supports rodent herbivory hypothesis.



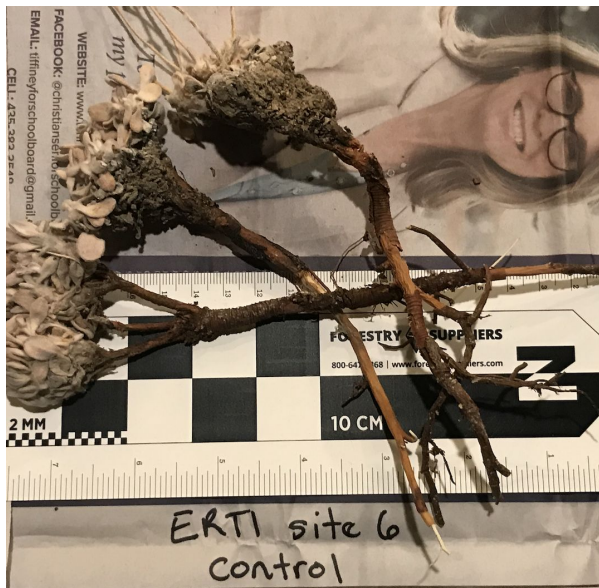
Stripped roots (top left and right) from plants at subpopulation 1. By comparison note dark brown root covering still intact on control plant (lower right) that was excavated by humans during sample collection on 10/2/20.



Stripped roots on plants from subpopulation 2.



Stripped roots on plants from subpopulation 4.



Intact roots from plants excavated by humans to be used as an eDNA control from subpopulation 6.



Stripped roots on plants from subpopulations 6a and 6b.

Figure 1. Photographs of depredated roots and control (untouched) roots used for genetic analysis in this study.

END OF REPORT. APPENDIX WITH TWO TABLES FOLLOWS.

APPENDIX

Table A. Samples tested for presence of eDNA from herbivores, identification of herbivore DNA from scat, and presence of *Eriogonum* DNA in scat collected from Tiehm’s buckwheat subpopulation 2.

| Subpopulation | Sample Type | Description of sample | Pooled sample info | DNA barcode: |
|---------------|-------------|---------------------------------------------------------|--------------------|--------------|
| 1 | control | roots from 1 untouched plant in 10X TE buffer | NA | vertebrates |
| 1 | control | roots from 1 untouched plant in 10X TE buffer | NA | vertebrates |
| 1 | control | roots from 1 untouched plant in 10X TE buffer | NA | vertebrates |
| 1 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 1 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 1 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 1 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 1 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 2 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 2 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 2 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 2 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 2 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 2 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 4 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 4 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 4 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 4 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 4 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 4 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 6 | control | roots from 1 untouched plant in 10X TE buffer | NA | vertebrates |
| 6 | control | roots from 1 untouched plant in 10X TE buffer | NA | vertebrates |
| 6 | control | roots from 1 untouched plant in 10X TE buffer | NA | vertebrates |
| 6 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 6 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 6 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 6 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 6 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 6 | roots | mixed damaged roots from 1-3 plants in 1X TE buffer | 1-3 plants | vertebrates |
| 1 | soil | soil from 4 control sites with undisturbed soil | controls 1-4 | vertebrates |
| 1 | soil | Soil tailings from within 30 cm radius of damaged plant | 1-4 | vertebrates |

| | | | | |
|----|------|-----------------------------------------------------------|--------------|-------------|
| 1 | soil | Soil tailings from within 30 cm radius of damaged plant | 5-8 | vertebrates |
| 1 | soil | Soil tailings from within 30 cm radius of damaged plant | 9-11 | vertebrates |
| 1 | soil | Soil tailings from within 30 cm radius of damaged plant | 12-14 | vertebrates |
| 1 | soil | Soil tailings from within 30 cm radius of damaged plant | 15-17 | vertebrates |
| 1 | soil | Soil tailings from within 30 cm radius of damaged plant | 18-20 | vertebrates |
| 2 | soil | soil from 4 control sites with undisturbed soil | controls 1-4 | vertebrates |
| 2 | soil | Soil tailings from within 30 cm radius of damaged plant | 1-3 | vertebrates |
| 2 | soil | Soil tailings from within 30 cm radius of damaged plant | 4-6 | vertebrates |
| 2 | soil | Soil tailings from within 30 cm radius of damaged plant | 7-9 | vertebrates |
| 2 | soil | Soil tailings from within 30 cm radius of damaged plant | 10-11 | vertebrates |
| 2 | soil | Soil tailings from within 30 cm radius of damaged plant | 12-13 | vertebrates |
| 2 | soil | Soil tailings from within 30 cm radius of damaged plant | 14-15 | vertebrates |
| 4 | soil | soil from 4 control sites with undisturbed soil | controls 1-4 | vertebrates |
| 4 | soil | Soil tailings from within 30 cm radius of damaged plant | 1-3 | vertebrates |
| 4 | soil | Soil tailings from within 30 cm radius of damaged plant | 4-6 | vertebrates |
| 4 | soil | Soil tailings from within 30 cm radius of damaged plant | 7-9 | vertebrates |
| 4 | soil | Soil tailings from within 30 cm radius of damaged plant | 10-11 | vertebrates |
| 4 | soil | Soil tailings from within 30 cm radius of damaged plant | 12-13 | vertebrates |
| 4 | soil | Soil tailings from within 30 cm radius of damaged plant | 14-15 | vertebrates |
| 6 | soil | soil from 4 control sites with undisturbed soil | controls 1-4 | vertebrates |
| 6a | soil | Soil tailings from within 30 cm radius of damaged plant | S1, 1-4 | vertebrates |
| 6a | soil | Soil tailings from within 30 cm radius of damaged plant | S1, 5-8 | vertebrates |
| 6a | soil | Soil tailings from within 30 cm radius of damaged plant | S1, 9-11 | vertebrates |
| 6a | soil | Soil tailings from within 30 cm radius of damaged plant | S1, 12-14 | vertebrates |
| 6b | soil | Soil tailings from within 30 cm radius of damaged plant | S1, 15-17 | vertebrates |
| 6b | soil | Soil tailings from within 30 cm radius of damaged plant | S1, 18-20 | vertebrates |
| 2 | scat | 6 rodent pellets to examine for vertebrate DNA | NA | vertebrates |
| 2 | scat | 6 rodent pellets to examine for presence of buckwheat DNA | NA | plants |

Table B. DNA barcode sequence data and most closely matching taxon for all samples in which >10 sequence reads were present. Matches between the sample DNA and database DNA were at least 90% similar. A single sample may contain more than one genetic sequence from more than one taxon. Unknown DNA sequences usually can be attributed to DNA sequences that contain shared, common sequence information that make them indistinguishable between taxa. The number of reads is the number of DNA sequences that were detected in the sample.

| Sample ID | Subpopulation | Type | DNA barcode sequence | Taxon/# reads |
|-----------|---------------|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| 26055 | 1 | control | Available upon request | Unknown/ 13 |
| 26056 | 1 | control | none | none |
| 26057 | 1 | control | none | none |
| 26058 | 1 | roots | none | none |
| 26059 | 1 | roots | CTTAGCCCTAAACATAAACATCCAATAAACAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT CACCACCTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGGCTACATTTTC TATTTTTCAGAATAAAATACTCAGGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC | Sciuridae/ 50 |
| 26059 | 1 | roots | Available upon request | Unknown/ 67 |
| 26059 | 1 | roots | Available upon request | Unknown/ 13 |
| 26060 | 1 | roots | Available upon request | Unknown/ 77 |
| 26060 | 1 | roots | CTTAGCCCTAAACACAAATAATTACATCAACAAAATTATTCGCCAGA GTACTACCGCAATAGCTTAAAACCTCAAAGGACTTGGCGGTGCTTTA TACCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACC TTACCACCCCTTGCTAATTCAGTCTATATACCACCATCTTCAGCAAAC CCTAAAAAAGGAATAAAAGTAAGCTCAATCATATTACATAAAAACGT TAGGTCAAGGTGTAACCTATGGGATGGAAAGAAATGGGCTACATTTT CTAACTTAAGAAAATCCATTATGAAAATTATTATGAAATTAATAATTA AAGGAGGATTTAGCAGTAAACTAAGAATAGAGTGCTTAGTTGAATTA GGCCATGAAGCACGCAC | Cervidae/ 27 |
| 26061 | 1 | roots | CTTAGCCCTAAACATAAACACTCAATAAACAGAATGTTCCGCCAGAA TACTACTAGCAATGGCCTAAAACCTCAAAGGACTTGGCGTTGCTTTACA TCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATACATCTC ACCACCTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAACC CTAATAAGGTCTAACAGTAAGCAGGATAATTTACATTAATACGTTA GGTCAAGGTGTAGCCTATAAGGTAGGAAGAAATGGGCTACATTTTCT ATTTTTCAGAATAAAATACTCAGGATAGCTTTTATGAAACTTAAAGCAT AAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATTG GGCAATAAAGCACGCAC | Sciuridae/ 58 |
| 26062 | 1 | roots | Available upon request | Unknown/ 16 |
| 26063 | 2 | none | none | none |
| 26064 | 2 | roots | none | none |
| 26065 | 2 | roots | none | none |
| 26066 | 2 | roots | none | none |

| | | | | |
|-------|---|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| 26067 | 2 | roots | Available upon request | Unknown/ 118 |
| 26068 | 2 | roots | none | none |
| 26069 | 4 | roots | none | none |
| 26070 | 4 | roots | none | none |
| 26071 | 4 | roots | none | none |
| 26072 | 4 | roots | none | none |
| 26073 | 4 | roots | CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA GTA CTACTACCGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACC TTACCACCCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAGTAAGCTTAATCATTTCATATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGGCTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC | Cervidae/ 130 |
| 26074 | 4 | roots | Available upon request | Unknown/ 13 |
| 26074 | 4 | roots | Available upon request | Unknown/ 15 |
| 26075 | 6 | control | none | none |
| 26076 | 6 | control | CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA GTA CTACTACCGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACC TTACCACCCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAGTAAGCTTAATCATTTCATATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGGCTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC | Cervidae/ 263 |
| 26077 | 6 | control | none | none |
| 26078 | 6 | roots | CTTAACCCTAAACTCGAATAGTTAGATCAACAAAATGTTACCCAGA ACACTACAAGCAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA TATCCCTCTAAAGGAGCCTGTTCTATAATCGATAAACCCCAATTTACC TCACCACCTCTTGCCAGCCTATATACCTCCATCTTCAGCAAACCTG GAAAGGCCACAGAGTAAGCACAAGTATCTACATAAAAAACGTTAGGTC AAGGTGTAGCCCATGAGGTGGCAAGAAATAGGTATGTTTTCTACATC CAGAAAAATCTCGCACAACCGTTATGAAATCTAAGGGCTCAAGGAG GATTTAGCAATAAATTGAGAGCAGAGTGTTAATCGAATAAGGCCAT GAAGCACGCAC | Human/ 11 |
| 26079 | 6 | roots | none | none |
| 26080 | 6 | roots | none | none |
| 26081 | 6 | roots | none | none |
| 26082 | 6 | roots | none | none |
| 26083 | 1 | Soil control | none | none |
| 26084 | 1 | Soil tailings | none | none |
| 26085 | 1 | Soil | CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA | Cervidae/ 35 |

| | | | | |
|-------|---|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| | | tailings | GTACTACCGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAAC TTACCACCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAAGTAAGCTTAATCATTTTACATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGCTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC | |
| 26086 | 1 | Soil tailings | Available upon request | Unknown/ 15 |
| 26087 | 1 | Soil tailings | Available upon request | Unknown/ 46 |
| 26088 | 1 | Soil tailings | CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA GTACTACCGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAAC TTACCACCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAAGTAAGCTTAATCATTTTACATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGCTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC | Cervidae/ 16 |
| 26088 | 1 | Soil tailings | CTTAGCCCTAAACACAAATAATTACACAAAACAAAATTGTTGCCAGA GTACTACTAGCGCAACAGCTTAAAACTCAAAGGACTTGGCGGTGCT TTATACCCTTCTAGAGGAGCCTGTTCTATAATCGATGAACGCTGATAA ACCGCAGCAGTCCTTGCTAATACAGTCTATACACCACCTCTTCAGCA AACCTGAAAAGAAAACAAAAGTAAGCAACATCATAGTACATAAAAA CATTAGGTCAAGGTGTAACCTATGGAGTGGGAAGAAATGCACTACAT CTTCTAATTTAAGAAAACCAATACGGAAGTACTATGAACTAATA ACCAAAGGAGGATTTAGTAGTAACTAAGAATAGAGTGCTTAGTTGA ACTAGGCCATGAAGCATGCAC | Cervidae/ 34 |
| 26089 | 1 | Soil tailings | none | none |
| 26090 | 2 | Soil control | Available upon request | Unknown/ 13 |
| 26091 | 2 | Soil tailings | none | none |
| 26092 | 2 | Soil tailings | none | none |
| 26093 | 2 | Soil tailings | none | none |
| 26094 | 2 | Soil tailings | none | none |
| 26095 | 2 | Soil tailings | Available upon request | Unknown/ 23 |
| 26095 | 2 | Soil tailings | Available upon request | Unknown/ 19 |
| 26096 | 2 | Soil tailings | none | none |

| | | | | |
|-------|----|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| 26097 | 4 | Soil control | Available upon request | Unknown/ 98 |
| 26098 | 4 | Soil tailings | none | none |
| 26099 | 4 | Soil tailings | none | none |
| 26100 | 4 | Soil tailings | Available upon request | Unknown/ 14 |
| 26100 | 4 | Soil tailings | Available upon request | Unknown/ 22 |
| 26101 | 4 | Soil tailings | Available upon request | Unknown/ 14 |
| 26102 | 4 | Soil tailings | Available upon request | Unknown/ 25 |
| 26102 | 4 | Soil tailings | Available upon request | Unknown/ 16 |
| 26102 | 4 | Soil tailings | Available upon request | Unknown/ 11 |
| 26103 | 4 | Soil tailings | none | none |
| 26104 | 6 | Soil control | none | none |
| 26105 | 6a | Soil tailings | none | none |
| 26106 | 6a | Soil tailings | none | none |
| 26107 | 6a | Soil tailings | none | none |
| 26108 | 6a | Soil tailings | none | none |
| 26109 | 6b | Soil tailings | CTTAGCCCTAAACATAAACATCCAATAAACAAAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT CACCACCTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAACTCAGGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC | Sciuridae/ 69 |
| 26109 | 6b | Soil tailings | Available upon request | Unknown/ 15 |
| 26110 | 6b | Soil tailings | CTTAGCCCTAAACATAAACATCCAATAAACAAAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT | Sciuridae/ 21 |

| | | | | |
|-------|---|----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| | | | CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC | |
| 26111 | 2 | rodent pellets | CTTAGCCCTAAACATAAACATCCAATAAACAAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC | Sciuridae/ 133 |
| 26111 | 2 | rodent pellets | CTTAGCCCTAAACATAAACATCCAATAAACAAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATT GGGCAATAAAGCACGCAC | Sciuridae/ 14 |
| 26112 | 2 | rodent pellets | GACTTAATTGGTTGAGCCTTAGTATGGAAACCTACTAAGTGAGAACTT TCAAAATCAGAGAAACCCTGGAATTAATAAATAAATGGGCAATCCTGAG CCAACCTCTGCTTTCCAAACTTTCCAAAAGGGAGAATAAATAAAG | <i>Eriogonum</i> / 111 |
| 26112 | 2 | rodent pellets | Plant eDNA from the following genera were also found in the rodent pellets: <i>Silene</i> , <i>Salsola</i> , <i>Haloxylon</i> , and <i>Atriplex</i> . Sequence data and read numbers available upon request. | |

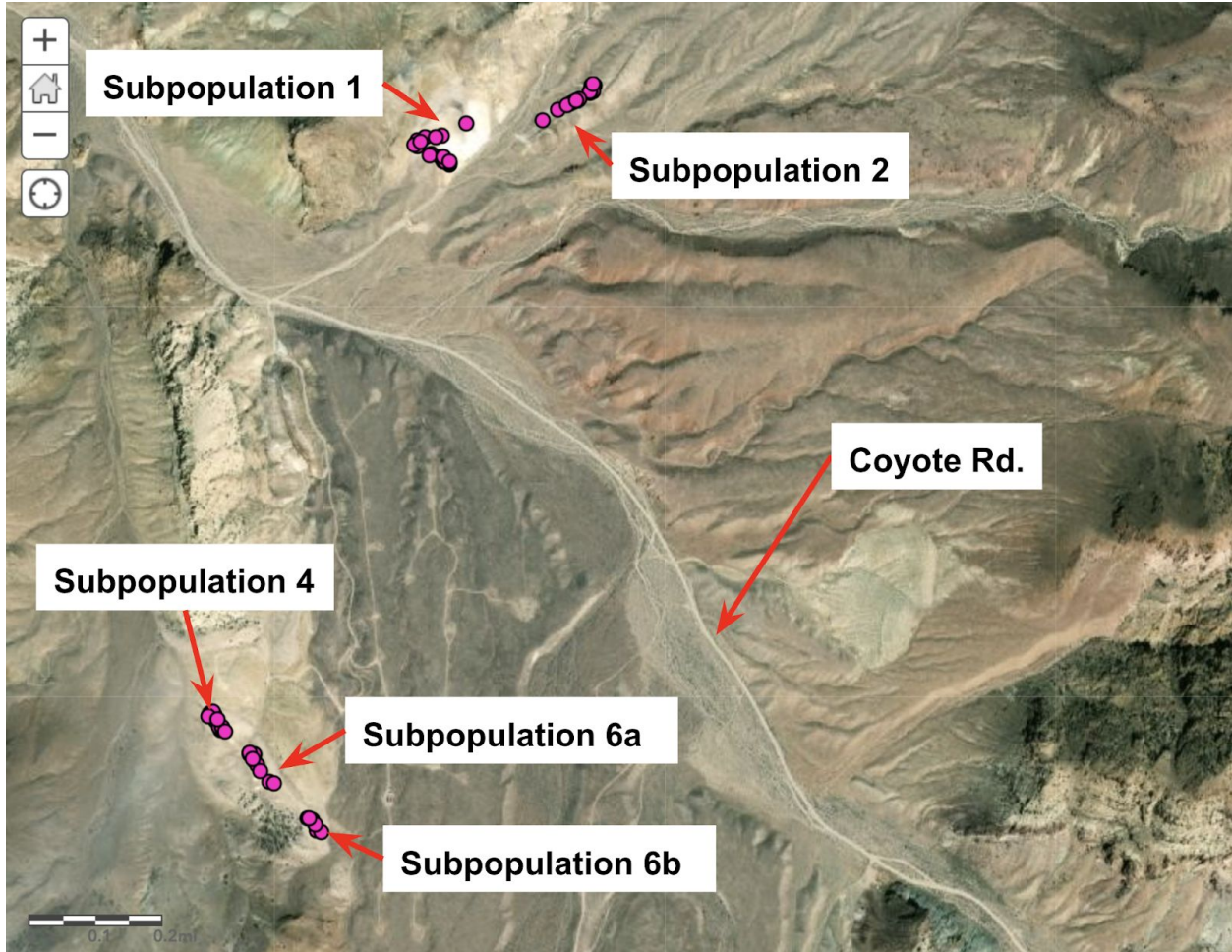


Figure A. eDNA collection sites at Tiehm's buckwheat (*Eriogonum tiehmi*) subpopulations 1, 2, 4, 6a and 6b. Pink dots indicate where soil samples and roots were collected on 10/2/20.