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Growing on a Scar: Genetic Variation of *Achillea Millefolium* Near Meeker, Colorado

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University of Northern Colorado
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GROWING ON A SCAR: GENETIC VARIATION OF *ACHILLEA MILLEFOLIUM*
NEAR MEEKER, COLORADO

A Thesis
Submitted in Partial
Fulfillment for Graduation with Honors Distinction and
the Degree of Bachelor of Science

Ace Spitzer

College of Natural and Health Sciences

December 2018

GROWING ON A SCAR

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Abstract

Mining operations deposit high concentrations of heavy metals such as lead, cadmium, arsenic and vanadium into the topsoil of an ecosystem. These toxic metals can affect flora species health and behavior, causing local extinction and shifts in ecosystem dynamics. However, genetic analyses of some plants growing on toxic soil conditions indicate higher genetic diversity than would be expected in that environment. Determining the effects of soil pollution on plants is important to determine a species' utility in conservation and restoration. This investigation compared the genetic diversity of western yarrow (*Achillea millefolium*, Asteraceae), growing near a mine site with vanadium and arsenic contamination to plants existing up to two kilometers away in undisturbed habitat. Leaf tissue from yarrow (N=88) was collected on and off the Butterfly-Burrell uranium mine northeast of Meeker, CO. Two microsatellite loci were scored for heterozygosity and allelic diversity, common measurements of a population's genetic diversity, which is correlated with the ability of organisms to adapt to selective pressures. While heterozygosity was highest at the mines, no significance was detected between them and off-site locations. Plants did not exhibit a reduction of genetic variability on the mine, indicating an adaptation or tolerance to the contaminated soils. This response establishes yarrow as a strong candidate for restoration and revegetation use at amended mines in the Rocky Mountains.

Keywords: *Achillea millefolium*, microsatellites, heterozygosity, mining reclamation

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Table of Contents

Introduction.....	6
Review of Related Literature	7
Environmental change and adaptation	7
Discourse on the impacts of mining pollution	10
Research Question	11
Methodology.....	11
Results.....	15
Primer success and allelic frequency	15
Heterozygosity	16
Discussion.....	17
Adaptive responses to metal stress.....	17
Conservation utility in the Rocky Mountains	20
Conclusion	20
References.....	22
Appendix A: PCR conditions for microsatellites.....	28
Appendix B: OUR summer 2017 research grant	29
Appendix C: OUR Spring 2018 Research Stipend.....	31

Table of Figures

Figure 1	12
Figure 2.....	13
Figure 3.....	14
Figure 4.....	15
Figure 5.....	16

List of Tables

Table 1.....	16
Table 2.....	17

Introduction

Human migration and economic activity impacts environments via habitat fragmentation, the introduction of non-native organisms into an ecosystem, and chemical pollution of soils and waterways. Heavy metals and hydrocarbons deposited by industry and mining can alter ecosystem interactions and are sources of environmental selection pressures. Colorado's Rocky Mountains have produced rare earth metals of economic value, but the process of extracting the valuable minerals deposits heavy metals in high concentrations into the soil and waterways surrounding mining operations (Bernd 2017). The xenobiotic compounds that are a byproduct of mining interfere with metabolic pathways, requiring plants to direct energy towards sequestering toxic compounds away from vital tissues. Consequently, novel anthropogenic conditions in an environment can select for genotypes that alter the native genetic structure of the population for its benefit or ill. Organisms have shown adaptations to anthropogenic environments, such as changing their generational period in the case of earthworms in a copper-saturated forest (Fisker et al. 2011).

Populations' adaptation to environmental stress informs our understanding of adaptation during natural and artificial climatic events and can aid in balancing economic activity with conservation (Carpenter 1976). For example, in a scenario where populations are unaffected by mining and subsequent reclamation procedures, focus and money can be precisely allocated to sites that require more intensive mitigation.

Review of Related Literature

Environmental change and adaptation

Soil chemistry is a fundamental variable that defines an ecosystem. Soil qualities such as acidity and nutrient content determine the plants and soil invertebrates are most likely to grow in those conditions. Mining results in extreme changes to acidity and deposition of toxins such as arsenic that disrupt enzyme activity in soil (Bhattacharyya et al. 2008). These novel changes can profoundly impact plant-community dynamics. The ability for many species to grow is greatly reduced by the presence of various heavy metals such as zinc, cadmium, and lead. Species richness in a community can decrease simply by existing adjacent to a mine or smelter, leading to the danger of an ecosystem collapsing or undergoing permanent dynamic change (Hernandez and Pastor 2008, Vidic et al. 2006). Soil organisms such as bacteria and micro-arthropods tolerate moderate pollution, but high enough concentrations such as those normally detected at mines cull populations before adaptation can occur (Salminen et al. 2001). Heavy metal concentrations are a significant selection force that can lead to altered plant-community dynamics.

In fertilization experiments on two flowering species, McNeilly and Antonovics (1968) revealed that the ability of the plants growing on a mine to mate with an isolated population was greatly reduced. This evidence supported barriers to migration because of the mine contaminants. Further evidence indicated that the barrier was genetic due to changes in flowering times between mine plants and non-mine plants (McNeilly and Antonovics 1968). This isolation of populations could lead to reduced genetic variation if only a minority of alleles are capable of existing on toxic soils.

Heavy metals absorbed by a flora can also radiate further through an ecosystem. Heavy metals were detected on bee pollinators near an exhausted mine in Italy (Satta et al. 2012). Metals accumulated on the exoskeleton of bees from the pollen they collect from flowers, and was transferred into the honey produced in their hives. Heavy metals can directly affect the immune response against pathogens and is believed to be a cause of the general decline of bees across Europe and North America (Polykretis et al. 2016).

Some exhausted mines and polluting industrial centers are found close to human populations or are situated in or adjacent to recreational and public lands. Public concern associated with the impact of industrial sites is often focused on the immediate health effects on iconic fauna rather than the whole community, prompting studies of mammals and birds at polluted sites. At the Rocky Mountain National Arsenal wildlife refuge, mule deer have grazed around the toxic waste site since before the refuge's inception in 1992, and long-term impacts on the effects of grazing is a cause for investigation (Creekmore et al. 1999). There was no evidence to support that grazing at the site was harming the deer, suggesting that the toxicity was not impacting the animal's fitness.

Organisms naturally evolve mechanisms for tolerating extremely toxic environments. Sulfur-compounds are a lasting threat to freshwater ecosystems and a common waste product from industrial operations and mining. Sulfides compete with oxygen in metabolic respiration in organisms, disrupting ATP production. However, sulfides occur naturally in rivers adjacent to volcanic activity, causing naturally acidic rivers and streams. In Mexico, *Poecilia mexicana* populations exist in rivers affected by volcanic sulfide run-off. Genetic analyses of different populations support that mitochondrial genes that decrease hydrogen sulfide binding affinity evolved more than once in regional *P. mexicana* populations (Pfenninger et al. 2014). Extreme

environments exist on earth, but natural evolution allows organisms to often adapt and thrive in those conditions.

Plants have also evolved physiological tolerance mechanisms to deal with extreme environments. Their roots passively absorb ions and other nutrients as part of their growth and unintentionally absorb heavy metals and other toxins. Physiological pathways have evolved in some species to sequester biologically harmful substances into nodules in the stems and leaves and away from areas vital to growth and reproduction (Dubey et al. 2014). The roots uptake heavy metals, where they are then moved to nodules in the roots or vacuoles in the cell. Some species deal with metals more effectively and will have greater success in polluted soils, while other plants experience severe damage to their roots and reproductive systems and exhibit stunted growth (Kazemeini et al. 2013). The energy and metabolism required for dealing with toxins takes energy from growth and reproduction and affects plants' overall fitness (Fomina and Gadd 2014).

Plants that are more tolerant of absorbing toxic compounds are used in a process called phytoremediation, where plants are allowed to absorb xenobiotic compounds in a contaminated location. After a sufficient treatment period, the plants are removed from the environment and disposed of properly, thereby removing contaminants from the area. Chehregani et al. (2009) notes that some species perform this more readily while resisting negative side-effects of absorbing heavy metals. Examining a species' phytoremediation ability indicates how well it will tolerate industrial activity and provides evidence about the adaptation potential of different plants as well as advises managers on which plants will be more successful in land management. Plants that are not as effective at absorbing toxins are not as useful for restoration, while plants with fast growing times and extensive root systems have been studied as potential tools in environmental

clean-up, such as in a pioneer study that showed successful phytoremediation of a water table by poplar trees (El-Gendy et al. 2009).

Discourse on the impacts of mining pollution

The genetic diversity of a species is commonly used in tracking evolutionary divergence and in assessing how likely a population will tolerate sudden changes to its environment. Heterozygosity is most commonly used because it indicates the presence of variable alleles. Populations can be quantitatively analyzed for heterozygosity by examining differences in multiple Single Strand Repeat (microsatellite) loci in a species. These regions are subject to natural mutation and are commonly used as a measure of population health (Kashi et al. 1997). Additionally, because microsatellite alleles can have variable sizes, the diversity and range of alleles can be an indicator of quantitative traits that can show graded adaptation to an environment (Vilas et al. 2015).

Environmental contaminants have generally been considered to be detrimental and that populations overall could not tolerate them. Using genetic markers, gene flow has been shown to be maintained between populations at contaminated sites in Canadian deciduous forests (Makela et al. 2016). While heavy metals were a factor in tree growth and health, heterozygosity and effective alleles appeared to be maintained by gene flow from beyond the contaminated sites, due to high mobility of the plant's pollen. This observation is explained by the selective pressure being too small to require adaptation before colonization.

Recent evidence suggests that plant populations growing on industrial sites such as mines and around refineries exhibit increased heterozygosity. Xie et al. (2016) reported greater genetic variation at industrial sites versus native habitat in grasses and admitted that their own previous studies have shown heterozygosity decreasing in other species. Genetic diversity can increase or

decrease in isolated populations under similar pressures, confounding the cause of the genetic change. This phenomenon leads to uncertainty regarding whether organisms will or will not recover from pollution.

Research Question

The goal of this study was to determine the impact of mine tailings on the genetic diversity of Yarrow (*Achillea millefolium*), a perennial wildflower in the Colorado Rocky Mountains. This work focused on identifying whether mining activity is changing the genetic makeup of plant populations. The study tested the general hypothesis that human-induced soil pollution is a significant selection pressure and impacts genetic variation in local flora. The main hypothesis was that the mine site selects for greater variability in *A. millefolium* growing on top of the reclaimed mine versus off the mine.

Methodology

Study site

The Butterfly-Burrell mine (Figure 1) is located northeast of Meeker, CO in the White River National Forest. This uranium/vanadium mine has been closed since the early 2000s and reclamation of the area by a private company started in 2005 and ended in 2006. Concentrations of vanadium and arsenic were detected up to 100 times the background concentration at the mines, and arsenic was detected at high levels downslope of mines and in the local watershed (AAK 2005). Radiation contamination was also detected, especially gamma radiation, which is a mutagen and profoundly effects biological processes (Hu et al. 2017). This particular mine was selected for this study because of the high abundance of the study organism in the region and for its accessibility.



Figure 1

Burrell Mine, White River National Forest, June 30th, 2017. The fencing in the background is where imported plant seeds were sowed

Study organism

Yarrow (*Achillea millefolium* L., Asteraceae, Figure 2) is a perennial herbaceous plant with varying flower color. Its seeds are highly mobile, and the species is distributed across the northern hemisphere with only minor differences in its morphology. Yarrow grows to a maximum height of one meter and has compound, pubescent leaves (Weatherby 2002). In North America, yarrow is used medicinally in most indigenous tribes by steeping its leaves in water to make tea or by using leaf pulp to treat cuts and sores. Yarrow is reported to contain over 200 secondary compounds with various physiological effects and is studied as a potential source for novel treatments to ulcers, cancer, and diabetes (Applequist & Moerman 2011). Syso et al. (2016) demonstrated that *Achillea millefolium* sequesters pollutants such as Lead, Cadmium, and Zinc in its leaves and stems along roads and industrial parks where disturbances to the soil are high, making it a candidate for use in phytoremediation.



Figure 2.

Yarrow (*Achillea millefolium*) in bloom. Photo taken from the Smithsonian Institution, Department of Botany

Data collection and analysis

On June 30, 2017, the researchers collected leaf tissue from 88 individual plant specimens at both mine sites and systematically along the forest access road at 100 meters, 1000 meters, and 2000 meters distance from the mine (Table 1). Each individual's location was recorded using a GPS positioner and later uploaded into ArcMap (Figure 3). Leaf tissue was ground in liquid nitrogen with a mortar and pestle and genomic DNA was extracted using Qiagen DNEasy Kits. Presence of DNA was confirmed with gel electrophoresis.

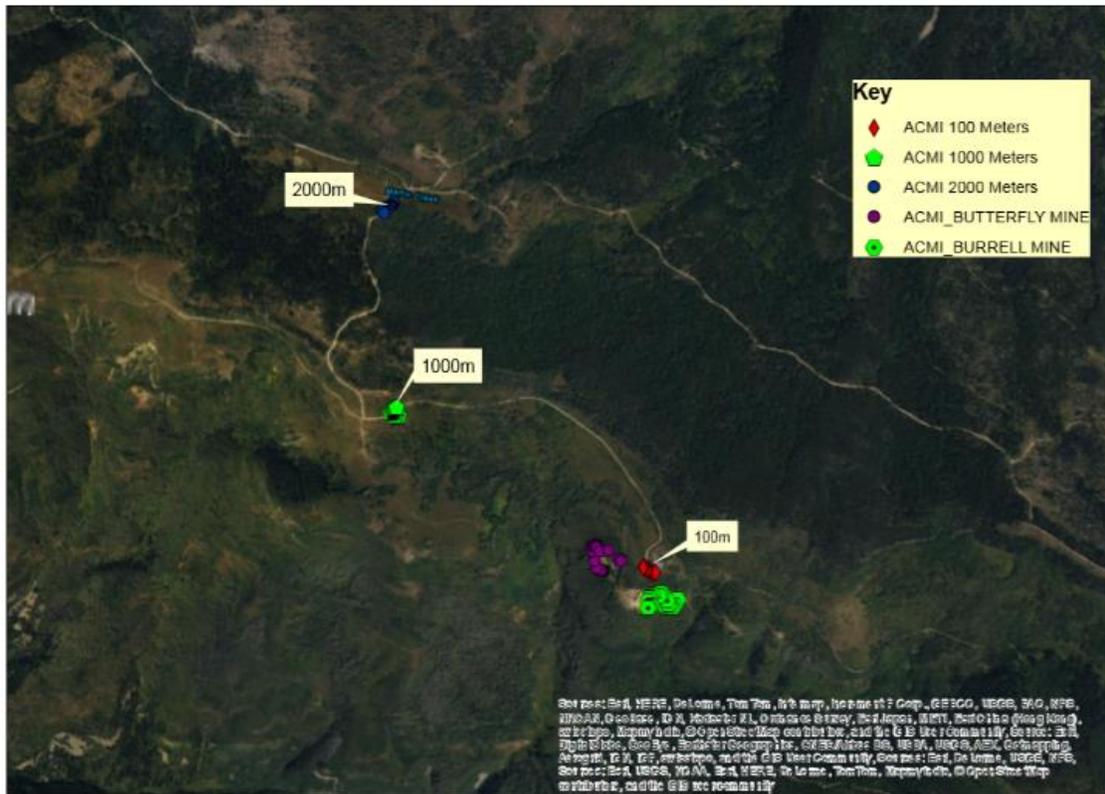


Figure 3.

Individuals GPS position of plants sampled for this study.

Microsatellite amplification

Microsatellite loci were selected from Rahimmalek et al. (2011) and adapted for a florescent tag technique (Appendix 1). The technique involves adding a “tag” sequence to the 5’ end of either the forward or reverse primer. This modification allows for a 3 primer PCR reaction with the forward and reverse primers, plus a fluorescently labeled primer that is complementary to the known sequence (Boutin-Ganache et al. 2001). Additional optimization of the primers was done by modifying anneal temperatures and Magnesium concentrations during PCR reactions (Appendix 1). Successful fragment amplification was confirmed with gel electrophoresis and loci were sent to Arizona State University DNA Lab for fragment analysis.

Data analysis

Microsatellite loci were scored in Geneious™ and analyzed using GenAlEx (Peakall and Smouse, 2012). Heterozygosity of loci in populations is presented as Mean Observed Heterozygosity \pm SE. The unpaired T-test was performed between the mine populations and the off-site plants. Allelic frequency, range, and average allele of each locus was collected and compared between the Butterfly mine and 100 meters.

Results

Primer success and allelic frequency

Three primers were optimized with the molecular tags. Of these three, Amk54 and Amk439 amplified reliably and were variable. 88 individuals were measured. The average allele size of Amk54 is 220-242 with a mean size of 236 and the average size of Amk439 is 255-267 with a mean size of 263 (Figure 4). Amk54 and Amk439 have an average number of alleles of 4.8 and 4.2, respectively.

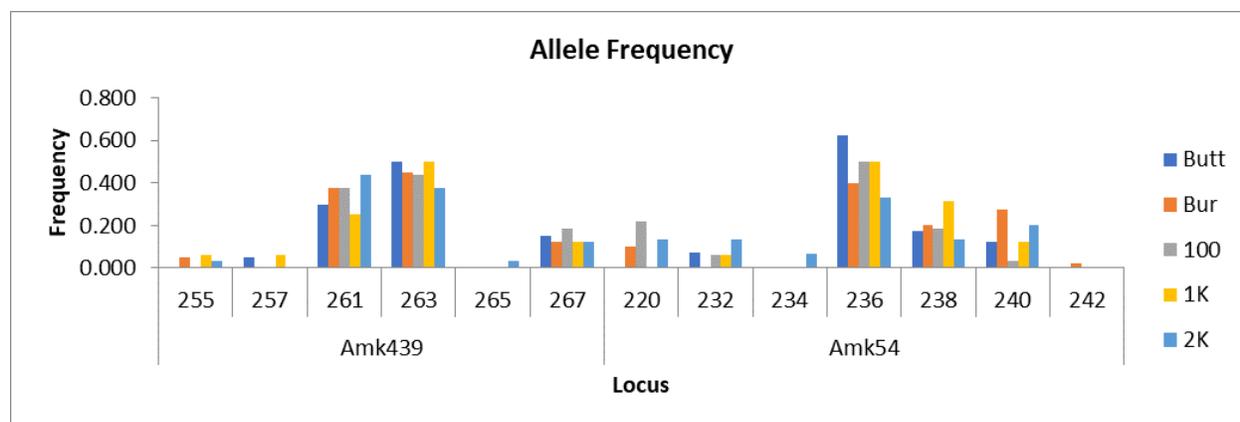


Figure 4.

This figure describes the frequency of various alleles in each locus. Butt refers to the Butterfly mine population. Bur refers to Burrell. The additional populations are 100 = 100 meters, 1k = 1 kilometers, 2k = 2 kilometers from the mine, respectively.

Heterozygosity

Heterozygosity varied only slightly between the mines, likely due to their proximity to each other. The highest measured heterozygosity was recorded at Burrell mine ($H_0 = 0.675 \pm 0.100$) and the lowest was measured at the 2 kilometer site ($H_0 = 0.473 \pm 0.340$) (Figure 5, Table 1). Overall, heterozygosity is high across all populations (Table 1). No significant difference in heterozygosity was detected between the mine and off-site populations (Table 2).

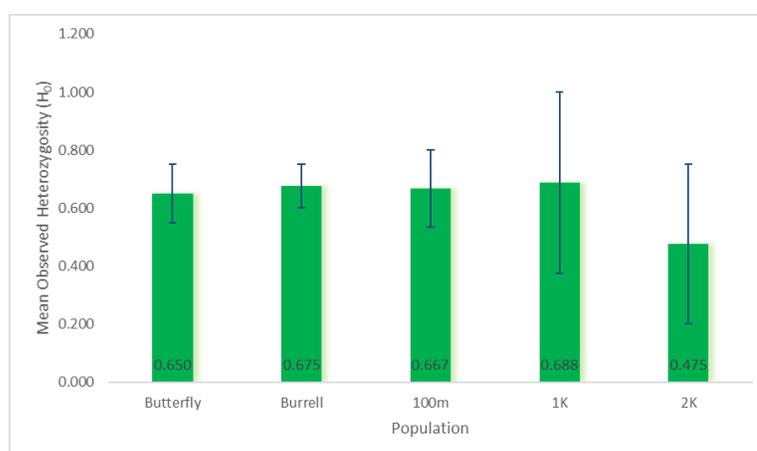


Figure 5.

Mean Observed Heterozygosity of *Achillea millefolium* using two loci with Standard Error.

Table 1.

Number of individuals successfully analyzed for heterozygosity, including mean heterozygosity and standard error and average number of alleles

Population	Number of individuals	Mean Ho	Mean Number of Alleles
Butterfly	20	0.650 ± 0.100	4.0 ± 0.0
Burrell	20	0.675 ± 0.075	4.5 ± 0.5
100 meters	16	0.688 ± 0.125	4.0 ± 1.0
1 Kilometer	16	0.625 ± 0.250	4.5 ± 0.5
2 Kilometers	16	0.473 ± 0.340	5.5 ± 0.5

Table 2.

Mine population mean observed heterozygosity compared to variable populations using an unpaired t-test. No difference in heterozygosity could be detected.

<i>Populations</i>	<i>df</i>	<i>T-statistic</i>	<i>P-value</i>
<i>Butterfly-100</i>	34	0.2404	0.8114
<i>Burrell-100</i>	34	0.0848	0.9332
<i>Butterfly-1k</i>	34	0.1002	0.9207
<i>Burrell-1k</i>	34	0.21	0.8349
<i>Butterfly-2k</i>	34	0.7097	0.4837
<i>Butterfly-2k</i>	34	0.6424	0.5249

Discussion

Adaptive responses to metal stress

This research asked if the contaminated soil of a Uranium/Vanadium mine in the Rocky Mountains impacted local genotypes in a way that would impact the long-term viability of populations. The general concern of conservationists and environmentalists is that intense pollution selects for lower heterozygosity by selecting for stabilizing traits. Mining in general acts as primary succession because the habitat is destroyed to access underground ore and organisms must recolonize once mining is completed.

The yarrow likely established on the mines by secondary succession in the previous decade maintained high heterozygosity. Yarrow as a generalist species could naturally have more allelic diversity which would allow for variable tolerance mechanisms to deal with the variety of habitats it encounters. Generalist plants can thrive in varying light availability and soil quality and may have more genetic variation, allowing them to tolerate disturbances (Theriault et al. 2013).

Generalists may have a higher frequency of alleles. Another possibility is that the reclamation activity in the mid-2000s effectively reduced the contamination present at the mine, reducing the presence of selective pressures on colonization and growth. Yarrow was the most abundant plant at the mines, apart from planted grasses. There is evidence that seeds were planted to facilitate succession on the mines, however, no plants were observed inside of the reclamation fences except for yarrow.

Extreme environments are common in Earth's natural history, and the dynamic nature of populations allows organisms to respond to disruptions in the environment. A beneficial mutation may survive initial population disruption, then thrive and propagate throughout the population. In contrast, populations with few or no variable mutations are more likely to result in local extinction. Little evidence exists that suggests this yarrow population is lacking in variable alleles. One explanation is that as a disturbance specialist, yarrow evolved greater genetic diversity represented by its high frequency of alleles (Figure 2) to a wide variety of soil conditions before being exposed to them. Given the species' global distribution, it is likely Yarrow is able to colonize a wide variety of soil types and levels of contamination.

A second possibility is that the population has experienced selection removing non-tolerant genotypes from the population in the decade it has been able to access the mines. Through several seasons of colonization events, only plants with selected genotypes would succeed in establishing so that only certain genotypes are existing on the mine. High heterozygosity after exposure to various heavy metals was reported in invertebrate communities and represents natural adaptation (Hochmuth et al. 2015). Tolerance and high heterozygosity in an experimental population of *Daphnia magna* occurred after only eight generations. The authors also noted that while their populations recovered, the dynamics of population size and

reproductive output changed as a response to the metal it was exposed to and never returned to the conditions of the control group (Hochmuth et al. 2015). Genetic variation was not recorded in this study's yarrow population before mine closure, therefore we are unable to determine if heterozygosity and allelic diversity were different before the environmental change. The role yarrow or any organism plays in an ecosystem after selection would change if its abundance or proliferation is altered by a selective pressure such as soil chemistry, so its interactions with other organisms may not be what is expected under less extreme environmental change.

Greater genetic variation in tolerant plant populations is commonly reported regardless of the genetic technique which focuses on different parts of the genome (Mengoni et al. 2000). Researchers generally focus on heterozygosity to determine a population's tolerance to polluted soils. However, plant responses to metal stress can involve changes in a plant's growth (Kazemeini et al. 2013) due to the siphoning of energy to upregulate pathways responsible for producing anti-oxidant metabolites and removing metals from vital tissues (Dubey et al. 2014). These traits may be more accurately represented in markers by allelic diversity because they relate to quantitative traits in coding genes (Vilas et al. 2015). Allelic diversity may also be a more accurate diversity measurement than heterozygosity in situations where the number of available loci for a species is limited, such as this study. Greater rates of mutations could be induced in stressed populations, thus increasing the likelihood of maladaptive mutations becoming common. To this author's knowledge, no study has been performed on plant populations exploring associations between greater microsatellite heterozygosity and maladaptive mutations.

Conservation utility in the Rocky Mountains

Mining as an economic activity has existed at least since ancient Greek society, but its implications on the physical environment and organisms have been of concern in recent history (Kelepertsis and Bibou, 1991). The impacts of metal pollution are a concern for fears of loss of a pristine environment that no longer supports the same flora and fauna as in its past.

An aim of this current study was to assess whether yarrow would be a candidate for remediating disturbed sites such as mines, indicated by whether the population growing on the mine is low in genetic diversity. Since the mine does not appear to threaten the local yarrow, the findings would support the use of the species as a remediation organism. Amendments to soils polluted with arsenic, such as those at Butterfly-Burrell, have the potential to restore soil microbial activity (Koo et al. 2012). Soil amendments combined with reseeded of appropriate plants can rehabilitate ecosystem functions and biodiversity (Kneller et al. 2018). The yarrow in this investigation represents a naturally occurring population in the Rocky Mountains. Yarrow's phytoremediation properties and tolerance to disturbed sites makes it a candidate for rehabilitating biodiversity at disturbed locations. Yarrow is currently grown by conservation agencies in habitat restoration and could represent an additional cost-effective method in rehabilitating soils (Winslow and USNRCS 2014).

Conclusion

This study focused on microsatellite variation in the genome of yarrow. While analyses of population genetic diversity provides an understanding of a species' adaptation to novel selection pressures, the value of a comprehensive approach should not be ignored. The relationship between yarrow and its pollinators and herbivores was not considered in this study

and could provide a community wide understanding of how mine tailings will impact the larger ecology of a habitat.

An additional issue that arose during this investigation came from the use of the published microsatellite primers. Only two of the four primers purchased successfully amplified. The microsatellites in the paper were designed from Yarrow from Eurasia. The possibility of divergence and speciation beyond subspecies between Eurasian and American yarrow has never been explored, nor the dispersal of yarrow seeds across the globe. The number of studies using these primers is limited and remains focused on Eurasian populations (Lopez-Vinyallonga et al. 2015). Comparing *Achillea* genomes across continents would identify divergence events and phylogenies.

In conclusion, soil pollution was explored as a selective pressure that reduces genetic diversity and therefore increase the likelihood of local extinction in plants. Our species, *Achillea millefolium* did not show evidence that its frequency or diversity of alleles was affected. Additionally, because of this result, *A. millefolium* can be supported as a strong candidate for phytoremediation.

References

- Applequist, W. L., & Moerman, D. E. (2011). Yarrow (*Achillea millefolium* L.): A neglected panacea? A review of Ethnobotany, bioactivity, and Biomedical Research. *Economic Botany*, 65(2), 209-225. doi:10.1007/s12231-011-9154-3
- Au' Authum Ki, Inc. (AAK) (2005) Site Assessment Report: Butterfly and Burrell Mines; Rio Blanco County, Colorado; Order Number 43-82D7-4-0038.
https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5281117.pdf
- Bhattacharyya, P., Tripathy, S., Kim, K., & Kim, S. (2008). Arsenic fractions and enzyme activities in arsenic-contaminated soils by groundwater irrigation in west Bengal. *Ecotoxicology and Environmental Safety*, 71(1), 149-156. doi:10.1016/j.ecoenv.2007.08.015
- Boutin-Ganache, I., Raposo, M., Raymond, M., & Deschepper, C. (2001). M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, 31(1), 24-24.
- Carpenter, R. A. (1976). Tensions between materials and environmental quality. *Science*, 191(4228), 665-668. doi:10.1126/science.191.4228.665
- Chehregani, A., Noori, M., & Yazdi, H. L. (2009). Phytoremediation of heavy-metal-polluted soils: Screening for new accumulator plants in Angouran mine (Iran) and evaluation of removal ability. *Ecotoxicology and Environmental Safety*, 72(5), 1349-1353.
doi:10.1016/j.ecoenv.2009.02.012
- Creekmore, T. E., Whittaker, D. G., Roy, R. R., Franson, J. C., & Baker, D. L. (1999). Health status and relative exposure of mule deer and white-tailed deer to soil contaminants at the Rocky Mountain Arsenal. *Environmental Toxicology and Chemistry*, 18(2), 272-278. doi:10.1002/etc.5620180225

- Dubey, S., Shri, M., Misra, P., Lakhwani, D., Bag, S. K., Asif, M. H., . . . Chakrabarty, D. (2014). Heavy metals induce oxidative stress and genome-wide modulation in transcriptome of rice root. *Functional & Integrative Genomics*, *14*(2), 401-417. doi:10.1007/s10142-014-0361-8
- El-Gendy, A. S., Svingos, S., Brice, D., Garretson, J. H., & Schnoor, J. (2009). Assessments of the efficacy of a long-term application of a phytoremediation system using hybrid poplar trees at former oil tank farm sites. *Water Environment Research*, *81*(5), 486-498. doi:10.2175/106143008X357011
- Fisker, K. V., Sørensen, J. G., Damgaard, C., Pedersen, K. L., & Holmstrup, M. (2011). Genetic adaptation of earthworms to copper pollution: Is adaptation associated with fitness costs in *Dendrobaena octaedra*? *Ecotoxicology*, *20*(3), 563-573. doi:10.1007/s10646-011-0610-8
- Fomina, M., & Gadd, G. M. (2014). Biosorption: Current perspectives on concept, definition, and application. *Bioresource Technology*, *160*, 3-14. doi:10.1016/j.biortech.2013.12.102
- Hernández, A. J., & Pastor, J. (2008). Relationship between plant biodiversity and heavy metal bioavailability in grasslands overlying an abandoned mine. *Environmental Geochemistry and Health*, *30*(2), 127-133. doi:10.1007/s10653-008-9150-4
- Hochmuth, J., De Meester, L., Pereira, C., Janssen, C., & De Schamphelaere, K. (2015). Rapid adaptation of a *Daphnia magna* population to metal stress is associated with heterozygote excess. *Environmental Science & Technology*, *49*(15), 9298-9307. doi:10.1021/acs.est.5b00724
- Hu, S., Gao, Y., Zhou, H., Kong, F., Xiao, F., Zhou, P., & Chen, Y. (2017). New insight into mitochondrial changes in vascular endothelial cells irradiated by gamma ray. *International Journal of Radiation Biology*, *93*(5), 470-476. doi:10.1080/09553002.2017.1286048
- Kashi, Y., King, D., & Soller, M. (1997). Simple sequence repeats as a source of quantitative

- genetic variation. *Trends in Genetics*, 13(2), 74-78. doi:10.1016/S0168-9525(97)01008-1
- Kazemeini, F., Malayeri, B. E., Chehregani, A., Lorestani, B., & Kalvandi, R. (2013). Identification of the heavy metals accumulator plants in surrounding area of mine. *International Journal of Agriculture and Crop Sciences*, 6(10), 565.
- Kelepertsis, A., & Bibou, A. (1991). Heavy-metal contamination of soils at old mining sites on Thasos island, Greece. *Environmental Geochemistry and Health*, 13(1), 23-28. doi:10.1007/BF01783492
- Kneller, T., Harris, R. J., Bateman, A., & Muñoz-Rojas, M. (2018). Native-plant amendments and topsoil addition enhance soil function in post-mining arid grasslands. *Science of the Total Environment*, 621, 744-752. doi:10.1016/j.scitotenv.2017.11.219
- Koo, N., Lee, S., & Kim, J. (2012). Arsenic mobility in the amended mine tailings and its impact on soil enzyme activity. *Environmental Geochemistry and Health*, 34(3), 337-48. doi:http://dx.doi.org/10.1007/s10653-011-9419-x
- Lopez-Vinyallonga, S., Soriano, I., Susanna, A., Montserra, J., Roquet, C., & Garcia-Jacas, N. (2015). The polyploid series of the *Achillea millefolium* aggregate in the Iberian Peninsula investigated using microsatellites. *Plos One*, 10(6), e0129861. doi:10.1371/journal.pone.0129861
- Lottermoser, B. G. (2017;2016). *Environmental indicators in metal mining* (1st 2017 ed.). Switzerland: Springer. doi:10.1007/978-3-319-42731-7
- Makela, M., Michale, P., Theriault, G., & Nkongolo, K. K. (2016). High genetic variation among closely related red oak (*Quercus rubra*) populations in an ecosystem under metal stress: analysis of gene regulation. *Genes & Genomics*, 38(10), 967-76.
- McNeilly, T., & Antonovics, J. (1968). Evolution in closely adjacent plant populations. 4. Barrie

rs to gene flow. *Heredity*, 23, 205-205

- Mengoni, A., Gonnelli, C., Galardi, F., Gabbrielli, R., & Bazzicalupo, M. (2000). Genetic diversity and heavy metal tolerance in populations of *Silene paradoxa* L. (Caryophyllaceae): a Random Amplified Polymorphic DNA analysis. *Molecular Ecology*, 9(9), 1319-1324. 10.1046/j.1365-294x.2000.01011.x
- Peakall, R. and Smouse P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28, 2537-2539.
- Pfenninger, M., Lerp, H., Tobler, M., Passow, C., Kelley, J. L., Funke, E., . . . Plath, M. (2014). Parallel evolution of cox genes in H₂S-tolerant fish as key adaptation to a toxic environment. *Nature Communications*, 5, 3873 Doi: 10.1038/ncomms4873
- Polykretis, P., Delfino, G., Petrocelli, I., Cervo, R., Tanteri, G., Montori, G., . . . Gulisano, M. (2016). Evidence of immunocompetence reduction induced by Cadmium exposure in honey bees (*Apis mellifera*). *Environmental Pollution*, 218, 826-834. doi:10.1016/j.envpol.2016.08.006
- Rahimmalek, M., Tabatabaei, B., Arzani, A., & Khorrami, M. (2011). Development and characterization of microsatellite markers for genomic analysis of yarrow (*Achillea millefolium* L.). *Genes & Genomics*, 33(5), 475-482. doi:10.1007/s13258-011-0045-x
- Salminen, J., van Gestel, C. A., & Oksanen, J. (2001). Pollution-induced community tolerance and functional redundancy in a decomposer food web in metal-stressed soil. *Environmental Toxicology and Chemistry / SETAC*, 20(10), 2287-2295. doi:10.1002/etc.5620201022
- Satta, A., Verdinelli, M., Ruiu, L., Buffa, F., Salis, S., Sassu, A., & Floris, I. (2012). Combination of beehive matrices analysis and ant biodiversity to study heavy metal

- pollution impact in a post-mining area (Sardinia, Italy). *Environmental Science and Pollution Research*, 19(9), 3977-3988. doi:10.1007/s11356-012-0921-1
- Syso, A. I., Syromlya, T. I., Myadelets, M. A., & Cherevko, A. S. (2016). Ecological and biogeochemical assessment of elemental and biochemical composition of the vegetation of anthropogenically disturbed ecosystems (based on the example of *Achillea millefolium* L.). *Contemporary Problems of Ecology*, 9(5), 643-651. doi:10.1134/S1995425516050164
- Therriault, G., Nkongolo, K. K., Narendrula, R., & Beckett, P. (2013). Molecular and ecological characterization of plant populations from limed and metal-contaminated sites in northern Ontario (Canada): ISSR analysis of white birch (*Betula papyrifera*) populations. *Chemistry and Ecology*, 29(7), 573-585. doi:10.1080/02757540.2013.820715
- Vidic, T., Jogan, N., Drobne, D., Vilhar, B., (2006). Natural Revegetation in the Vicinity of the Former Lead Smelter in Zerjav, Slovenia. *Environmental Science Technology*, 40, 4119-4125 doi:10.1021/es052339x
- Vilas, A., Pérez-Figueroa, A., Quesada, H., & Caballero, A. (2015). Allelic diversity for neutral markers retains a higher adaptive potential for quantitative traits than expected heterozygosity. *Molecular Ecology*, 24(17), 4419-4432. doi:10.1111/mec.13334
- Weatherby, C. (2002). *Peterson field guide to western medicinal plants and herbs*. Botanical Research Institute of Texas.
- Winslow, S. R., & United States. Natural Resources Conservation Service. (2014). Western yarrow, *Achillea millefolium* var. *occidentalis*: A native forb for conservation use in Montana and Wyoming. Bridger, Montana: United States Department of Agriculture, Natural Resources Conservation Service.
- Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., & Fu, J. (2016). Effect of heavy

metals pollution on soil microbial diversity and bermudagrass genetic variation. *Frontiers in Plant Science*, 7, 755. doi:10.3389/fpls.2016.00755

Appendix A: PCR conditions for microsatellites

1. Primer sequences were obtained from Rahimmalek et al. (2011) and modified for use with fluorescent molecular tags.

<i>Locus ID</i>	Repeat Motif	Anneal Temperature (°C)	25 nM MgCl ₂ (μL)
<i>Amk54</i>	TA	61.7	1
<i>Amk439</i>	(ATG)TG(ATG)	65.1	3

Appendix B: OUR summer 2017 research grant

Office of Undergraduate Research Grant proposal Su2017 (includes budget)

Climate change is most commonly discussed as habitat loss and global temperature variation but encompasses a wide range of anthropogenic effects from industrial activity and the introduction of human waste and pharmaceuticals to riparian ecosystems. Industries such as mining and fossil fuel production produce particulates that radiate from their source and deposit into top-soils. Plants roots and stems then uptake of these various xenobiotic compounds into their cell membrane space (Fomina and Gadd 2014). This process is called phytoremediation and is important in the discussion of environment conservation and management of soil contamination. The effect of these xenobiotic compounds can have varying consequences for the plant. Some species perform phytoremediation more effectively and will have greater success in polluted soils, while other plants exhibit damage to root systems and to reproductive systems (Kazemeini et al, 2013).

Post-mining sites and industrial centers are often found near human recreation or situated in or adjacent to public lands. Public forums are often concerned with the immediate health effects on fauna, prompting studies of animals and birds at polluted sites. At the Rocky Mountain National Arsenal wildlife refuge, mule deer grazing occurs at disturbed sites, but the deer have not shown evidence of health impact from acute metal toxicity (Creekmore et al 1999).

Additionally, an understanding of the consequences of soil pollution remain unclear for flora. Until the early 21st century bloom in genetic techniques, soil studies on impacted sites remained focused on the species diversity at the ecosystem level. Repeated studies of floral and arthropod diversity provide consistent evidence that diversity decreases in impacted soils, and that only a few plant species are able to take advantage of the new contaminated niche and outcompete the historically established species (Salminen et al. 2001 & Vidic et al. 2006). At the level of genetic diversity, the impact of soil contamination becomes less clear. In Canada, Theriault and colleagues (2013) have found that soil contamination has not had a clear impact on deciduous trees and that gene flow is strong. In herbaceous ground level plants, studies have found variable results in genetic diversity from not affecting genetics at all to increased genetic diversity at an impacted site (Xie et al. 2016). The length of time between soil contamination and its effect on plant generations is conceded as a limiting factor in these situations.

Purpose of Research

The goal of this project is to contribute to the discussion of impacted soils on the genetic diversity and gene flow of a species. This research is to be conducted in a different climate from the previously discussed studies and will provide a different perspective by studying the genetic diversity and gene flow in Colorado. This study will test the theory that human-induced soil pollution contributes to mutation and genetic variation in local the flora. This study will compare the genome of *Achillea millefolium* (Western Yarrow), using microsatellite DNA markers described in Rahimmalek et al. 2011 in the White River National Forest, between two abandoned mining sites and two pristine sites in the same area.

Methods and protocol

Data collection will occur in July of 2017. A total of $N = 60$ individuals will be sampled at White River National Forest. Individual samples will be collected by random sampling within a contaminated or pristine area. Samples will be collected from each individual and tracked via GIS. Tissue samples will be transferred to the University of Northern Colorado and prepared via Polymerase Chain Reaction. Prepared samples will be analyzed using GenAIEx.

Expected Budget(Dollars)

Fuel Estimates	100
Ice (For tissue samples)	10
DNA Extraction Kit (N = 60) at \$3.50 per sample	210
Species specific primers	150
DNA analysis \$2 / DNA sample	120
Student Stipend (8.00/hr) projected 6 days	384
Total	974

Itinerary

Summer 2017: Purchase DNA extraction kit and *Achillea* microsatellite primers

June 25th-30th: Travel to Butterfly-Burrell Mines in White River National Forest. Collect field data

September-January: Prepare population DNA using PCR and check with gel electrophoresis. Send to Arizona State University for analysis.

2018: Prepare final thesis

Appendix C: OUR Spring 2018 Research Stipend

Proposal to the Office of Undergraduate Research
For the Spring 2018 Research Stipend
By Ace Spitzer

Since the summer of 2017, I have been exploring the impacts of environmental pollution on genetic diversity in Colorado sunflower populations. *Achillea millefolium*, western yarrow, is a cosmopolitan perennial plant with a history of use in folklore medicine and documented medically important metabolites. It also absorbs airborne and soil pollutants and sequesters toxic compounds in its leaves and stems. I am interested in this species' reaction to novel environmental changes because of its role as a pioneer species and in bioremediation. It is often found in environments undergoing primary succession and at sites disturbed by human industrial activity. I have been exploring the population genetics of *A. millefolium* that have been growing at an abandoned uranium ore mine near Meeker, CO compared it to plants growing outside of the mine's influence. I believe that if this species can resist novel changes we could use that information to advise management of public lands and explore how species adapt to rapidly changing environments.

This academic year I have tasked myself with extracting DNA and amplifying Single Strand Repeat (SSR) regions of *A. millefolium*, which are regions of DNA that can infer mutations in the genome and overall genetic health of a population. This species has been a challenge to produce results due to abundant secondary compounds present in its cells and to a lack of prominent literature on its SSRs. I have learned of many molecular biology troubleshooting techniques and we have made substantial recent progress in producing viable DNA and amplifying our SSRs. My advisor and I communicate bi-weekly and have agreed that I will work 6 hours per week during this semester on this project as well as assisting with ongoing projects in his lab. As an undergraduate pursuing original research, I will be presenting my research topic during Research Day on April 5th, 2018 for the College of Natural & Health Sciences and the Honors program. This gives me a timeline to produce and analyze my results and experience communicating my research to the public, which in turn will benefit my career in the natural sciences and academia. For this original research I am requesting a research stipend of \$960.00 at 10.00\$ per hour, the Colorado minimum wage. I will be working three hours on Tuesday morning and Thursday afternoon for a total of six hours per week during the 16-week semester as I collect results and prepare them for presentation at Research Day and ultimately for submission to *Ursidae: The Undergraduate Research Journal* at UNC.

Budget for Spring 2018

	Hours per week	Stipend (Dollars/hour)
	6	10.00
Weeks active in lab	16	
Total	80 hours	\$960.00