Exploring the Genetic Basis of Branching Variation in a Wild Mustard Boechera stricta

by

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Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biology in the Graduate School of Duke University

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ABSTRACT

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Abstract

Grazing herbivores can significantly reduce plant fitness by causing apical meristem damage and completely removing reproductive structures. Compensation is a type of tolerance to grazing herbivores by which plants replace the removed reproductive structures. However compensation is possibly costly because of resource allocation to new reproductive structures, which can cause a tradeoff in survival probability overwinter in perennial plants. We show that variation in compensation is heritable and correlated with the environment from which the accessions originated in *B. stricta*. Specifically, length of lateral branches produced upon apical meristem damage when flowering was associated with climatic variables that change along an elevation gradient. Genotypes originating from low elevations – where the temperature is warmer and growing seasons are longer – produce longer lateral branches. Genotypes from high elevation sites — where the temperature is cooler and growing seasons are shorter — produce shorter lateral branches. This study suggests that compensation in the form of lateral branch length is affected by natural selection, which is potentially the result of tradeoffs between survival and reproduction.

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1. Introduction

Plants damaged by herbivory can face significant reductions in fitness^{1,2}. Many studies have examined the evolution of chemical resistance and tolerance against herbivores³⁻⁶. They find that tradeoffs between resistance and tolerance traits constrain the evolution of these traits to reaching their phenotypic optimum^{4,7,8}. Fewer have looked at compensation^{1,9-11}, or induced growth of new reproductive structures such as inflorescences to mitigate the reduction of fitness by herbivory. Compensation is a type of tolerance to grazing commonly done by mammalian herbivores. Grazers can remove the entire apical meristems and/or primary inflorescence of an individual, completely reducing fecundity. Through compensation, plants can replace the removed reproductive structures through growth of lateral meristems because removal of the apical meristem interrupts the auxin signaling pathway¹²⁻¹⁴. While studies have begun to study the genetic basis for variation in compensation and tradeoffs between undamaged fitness and compensation potential^{15–17}, none have investigated the potential tradeoff between compensation and survival.

In perennial plants, segregation of variation in compensation may have been affected by a tradeoff between tolerance and survival. Typically perennials are dormant in the winter, reproduce in the summer, and then go back into dormancy for the winter. Thus if a perennial looses it's reproductive structures in the present reproductive season, it still has the ability to still reproduce next summer if it survives the winter. A perennial plant can also compensate in the present season if it loses its reproductive structures (Fig 1). Growth of meristems into inflorescences with enough time and resources can mitigate the reduction in fitness that an individual experiences when losing an inflorescence¹⁰. However without proper conditions to produce seeds, growth of lateral meristems may waste resources needed for dormancy in the winter. This may lead to a decrease in probability of survival to the next growing season without increasing fecundity in the present season. Thus, individuals that compensate highly without the correct environment would have a lower fitness than individuals that do not compensate as much. If this is so, variation in compensation would be the result of tradeoffs between fecundity and survival.



Dormancy without compensation

Figure 1 Perennial plant lifecycle with/without compensation.

To address whether variation in compensation can be affected by a tradeoff between compensation and survival, we used the model system *Boechera stricta*, which is a perennial plant that has variation in compensation. I used nearly 400 genotypes to characterize traits related to reproduction from *B. stricta's* native western North America range and over 100 of those genotypes (Fig 2) were used to characterize the intraspecific variation in compensation for a genome-wide association study (GWAS). This system exhibits the typical perennial lifecycle and compensation variation described previously (Fig 1). This experiment simulated grazing during reproduction by clipping the primary inflorescence at the day of flowering to induce compensation. We show that variation in compensation is heritable and correlated with the environment from which the accessions originated in *B. stricta*, suggesting that variation in compensation may have been affected by natural selection.



Figure 2 Map of 136 B. stricta genotypes used in compensation experiment

2. Materials and Methods

2.1 Plant Growth and Measurements

Genotypes included in this experiment come from the *B. stricta* GWAS reference panel in the Mitchell-Olds Lab. These genotypes were collected from natural populations throughout the Rocky Mountains in the United States.

Seeds for each genotype were stratified on filter paper in the dark for 3 days at room temperature and then placed in a growth chamber at 22°C to germinate for 1 week. Ten germinated seedlings per genotype were transplanted into flats with a layer of Sun Gro Metro-Mix 200 on top of a layer of Sun Gro Fafard 4P. Five individuals of each genotype were assigned to the treatment group and the other 5 were assigned to be controls. Genotypes were arranged in a randomized block design and maintained in controlled greenhouse conditions for 8 weeks. At this point, rosette number was taken for all surviving individuals and then transferred to a 4°C vernalization treatment for 8 weeks.

After vernalization, plants were placed back in the greenhouse and length of the longest leaf (base of petiole to tip of blade) was measured in centimeters to the nearest 0.1cm. At flowering, date of first flower, height (base of rosette to top of tallest inflorescence) in centimeters to the nearest 0.1cm, and number of stalks were recorded. If plant was in the treatment group, scissors were used to remove the main inflorescence. If any axillary inflorescences were developing along the stalk, plants were clipped below the lowest growing axillary inflorescence. If assigned to the control group, plants were left untreated.

After growing for six weeks in the greenhouse, plants were harvested and imaged with a Canon EFS 18-55mm lens on a Canon EOS 50D camera. Lateral branch number and total length of lateral branches were extracted from the images manually for 138 of the 377 genotypes using ImageJ. The number of days from treatment to harvest was also recorded to use as a covariate in the statistical analysis.

2.2 Statistical Analysis

To obtain the heritability of the traits of interest, restricted maximum-likelihood (REML) linear mixed models were run for height at flowering, flowering time, and longest leaf, total lateral branch length without treatment, total lateral branch length with treatment, and total number of lateral branches with treatment. Maximumlikelihood generalized linear mixed models were used to analyze rosette number, stalk number, and total number of lateral branches without treatment since these data were non-normally distributed. For height at flowering, longest leaf and flowering time were run as fixed effect covariates to control for variation due to these variables. For lateral branch number in control and treatment, flowering time was used as a covariate. Height at flowering was not included in the model because when included the model did not converge and height at flowering in this model was not significant. For modeling the heritability of length of lateral branches, flowering time, height at flowering, and number of lateral branches were run as fixed effects in the model. For traits taken after treatment (lateral branch number and total lateral branch length), treatment was also included as a fixed effect. Both genotype and replicate were run as random effects, and the amount of variance in the data explained by genotype was used as the estimate for broad-sense heritability. All models were rerun with genotypes as fixed effects in order to obtain the least-squared means to use in the models to test for evidence of natural selection. All models were run using the package LME4 in the statistical program R.

In all models testing for evidence of natural selection on heritable traits, geographical parameters (longitude, latitude and elevation) of accession and population groups were fixed effects. Bartlett tests were used to confirm homogeneity of variances between population groups. Since the number of lateral branches in the control was nonnormal, a generalized linear model following a Poisson distribution was used. For height at flowering, flowering time, number of lateral branches in treatment, and total length of lateral branches in control and treatment, multiple linear regression models were run. We used the Shapiro-Wilk normality test to ensure that residuals for the multiple linear regression models did not violate the assumption of normality.

In order to analyze the correlation between the bioclimatic variables from the WorldClim- Global Climate Data to total lateral branch length. I ran a principle component analysis for the following variables at each accession location to obtain principle components for climatic variables: annual mean temperature, mean diurnal range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of coldest quarter, and mean temperature of warmest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter. I used the first four principle components and population group as fixed effects in a linear model with the genotypic means for total lateral branch length as the response variable. Principle component 1 (PC1) was significantly correlated with total lateral branch length. I ran each variable that was loading heavily on PC1 with latitude, longitude, and population group as fixed effects with total lateral branch length as the response variable to identify which climatic variable was correlated (model example).

Model Example:

Total length lateral branch ~ bioclimatic variable + latitude + longitude + population Maximum temperature of warmest month was the only variable on PC1 that was significantly correlated with total lateral branch length. Again we used the Shapiro-Wilk normality test to ensure that residuals did not violate the assumption of normality for multiple linear regressions.

2.3 Genome-wide Association Studies

Genome-wide association studies were conducted for flowering time, height at flowering, and total length of lateral branches in the treatment group. Least squared means for each genotype were obtained by running genotype as fixed effects in the models used to calculate heritability. We used the LSMEANS package in R on the models to generate the least square means and standard error for the means. We used the least square means in the Genomic Association and Prediction Integrated Tool (GAPIT) package in R²⁰. We used efficient mixed-model association (EMMAx) for mixed linear model for association^{21,22}. To control for population structure, we use the first 4 principle components of the genotypes as a fixed covariate in the model. The significant threshold for SNP-phenotype association uses a Bonferroni cut-off with alpha=0.01. The Bonferroni correction is stringent since most SNP-phenotype association tests are not completely independent.

3. Results and Discussion

3.1 Variation in reproductive traits within species

I recorded traits related to morphology and architecture for 377 *B. stricta* genotypes in 3142 individuals. Variation in rosette number before vernalization and stalk number did not appear to be heritable. This is possibly due to the lack of variation of plants exhibiting apical dominance with over half of the individuals observed producing only one rosette and one stalk. In addition, length of longest leaf did not show heritable variation for this experiment. However, variation in flowering time and height at flowering could be explained by genotype (H^2 = 0.11 and H^2 = 0.44 respectively).

Genome-wide association studies of flowering time did result in significant single nucleotide polymorphisms (Fig 3) on chromosome 7 but not for height at flowering (Appendix Fig 7). The region with significant SNPs on chromosome 7 contains a homologue to the gene *TWIN SISTER OF FT* (*TSF*, AT4G20370) in *Arabidopsis thaliana*, a close relative to *B. stricta*. In *A. thaliana*, this gene encodes a floral inducer and is homologous to *FLOWERING LOCUS T* (*FT*)¹⁸. This suggests that GWAS does work for this dataset of the *B. stricta* reference panel. Further molecular work to test for functionality of this gene would be needed to prove that this gene homologous to *TSF* causes variation in flowering time in natural populations of *B. stricta*.



Figure 3 Manhattan plot of SNPs from GWAS on flowering time.

3.2 Variation in compensation within species

I collected data on compensation related traits in 136 genotypes (Appendix Table 4) from throughout the natural range of *B. stricta*. I induced compensation by randomly removing the apical meristem from 50% of the plants from each genotype immediately following flowering. Consistent with the change in auxin signaling when apical meristem damage occurs^{12,13}, treated plants produced a larger number of lateral branches (Adj. R²=0.04689, F_{9,262}=2.648, p-value=0.0281) and longer total length of lateral branches (Adj. R²=0.679, F_{9,262}=64.68, p-value<2*10⁻¹⁶) compared to control plants (Fig 4). The variation in *number* of branches produced could not be attributed to genotype in either control or treatment groups. After controlling for flowering time, stalk height, and leaf length, the variation in *total length* of lateral branches was heritable (control group H² = 0.11 and treatment group H² = 0.34). A GWAS was only conducted on *total length* of lateral branches because of its high heritability but did not yield significant results

(Appendix Fig 8). This could be due to the lack of power from only using 138 genotypes rather than 377 or several minor affect loci that do not reach the significance threshold.





3.3 Variation in lateral branch length across environments

Variation in total length of lateral branches in the control and treatment groups is correlated to the elevation of which the accession was collected (Table 1-2). Population group, latitude, and longitude are not significantly associated with lateral branch length suggesting that this trend is not caused by isolation by distance. Accessions' elevations span the range of 1843-3548m. Genotypes originating from low elevations grew longer branches than those originating from high elevations (Fig 5). While not proving this trait is adaptive, this correlation between lateral branch length and elevation provides evidence that suggests both with and without apical meristem damage, growth of lateral meristems into branches is locally adapted¹⁹. This pattern could be driven by investment in lateral branch growth for more inflorescences increasing fecundity at low elevations but decreasing survival at high elevations.

Table 1 Analysis of variance table for model on total length of lateral branches in the control group by geographical parameters and population (Adj R²=0.00684, Significance code: 0.05 "*")

	Df	Sum Sq.	Mean Sq.	F-value	Pr(>F)
Latitude	1	1.6	1.57	0.0099	0.92106
Longitude	1	66.8	66.81	0.4186	0.51885
Elevation	1	650.6	650.62	4.0763	0.04568*
Population	5	91.8	18.37	0.1151	0.98885
Elevation x Population	5	706.3	141.26	0.8850	0.49341
Error	122	19472.3	159.61		
Total	136	20989.4			

Table 2 Analysis of variance table for model on total length of lateral branch inthe treatment group by geographical parameters and population (Adj R²=0.120,Significance code: 0.001 "**")

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)
Latitude	1	926	926.34	3.2704	0.073003
Longitude	1	157	156.68	0.5531	0.458465
Elevation	1	2495	2495.13	8.8089	0.003609**
Population	5	2525	505.10	1.2503	0.121201
Elevation x Population	5	1771	354.15	1.2503	0.289942
Residuals	122	34556	283.25		
Total	136	42430			



Figure 5 Total length in lateral branches decreases as elevation of the accession increases in control (F_{1,122}=4.0763, p-value=0.04568) and treatment (F_{1,122}=8.8089, p-value=0.003609). Grey region is 95% confidence interval.

Environmental variables crucial for growth, such as temperature and precipitation vary by elevation. Bioclimatic variables from WorldClim- Global Climate Data were used to analyze how these variables from which the *B. stricta* accessions were collected are correlated with lateral branch length. Bioclimatic variable were only available for 124 out of 136 of the genotypes phenotyped for compensation. While precipitation variables and most temperature variables were not correlated, maximum temperature during the warmest month is significantly correlated with lateral branch length in the treatment group (Table 3). Locations that experience higher temperatures in the summer have genotypes that produce longer branches upon apical meristem

damage (Fig 6).

Table 3 Analysis of variance table of model on total length of lateral branch length in the treatment group by latitude, longitude, population, and maximum temperature in warmest month. (Adj. R²=0.0972, Significance code: 0.001 "**")

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)
Latitude	1	216.9	216.92	0.7729	0.381213
Longitude	1	282.5	282.49	1.0066	0.317904
Max. Temp.	1	2471.5	2471.55	8.8067	0.003677*
Population	5	2030	406.06	1.4469	0.213157
Population x Max. Temp.	5	941.4	188.28	0.6709	0.646353
Error	111	31151	280.64		
Total	124	37094.1			



Total Lateral Branch Length by Maximum Temperature in Warmest Month

Figure 6 Genotypes from environments that have a higher maximum temperature during the warmest month of the year produce longer lateral branches (F_{1,111}=8.8067, p-value=0.003677). Grey region is 95% confidence interval.

This could be due to genotypes that grow longer lateral branches when compensating being favored in environments with higher temperatures. Areas with higher temperatures may experience a longer growing season, such as low elevation sites where snowmelt is earlier in the year and snow pack begins later relative to high elevation sites. In this environment, these genotypes have time and resources to produce seeds from lateral branches^{10,11}. These genotypes may not be favored in environments with lower temperatures, such as high elevation sites. At high elevation, compensation may not increase fitness due to a shorter growing season, with scarcer resources limiting seed maturation. The higher allocation of resources into larger reproductive structures may reduce overwinter survival or lower seed production in the next year. Less compensation may be selected for at those sites because there is possibly a tradeoff between compensation and survival. To fully test this hypothesis, further work will need to be done on testing the relationship between lateral branch lengths and seed production in the field. In addition, more work will need to be done to access the effect of compensation on overwinter survival in *B. stricta*.

4. Conclusion

Compensation along the inflorescence is important for plants. It alleviates the reduction in fitness if there is removal of reproductive structure such as primary inflorescences^{10,11}, which is commonly done by grazing herbivores. Similar to reproductive traits unrelated to herbivory, variation in compensation can be heritable. This variation may be influenced by natural selection. Specifically, this study demonstrated that compensation in the form of lateral branch length from apical meristem removal during reproduction varies by accession locations' elevation and temperature. This pattern may be due to a tradeoff between compensation and survival in different environments. While further work will need to be done to test whether natural selection on compensation is the result of a tradeoff, this study begins to show that variation in response to herbivory in the form of growth is possibly adaptive.

Appendix



Figure 7 Manhattan plot for GWAS on height at flowering for 377 genotypes



Figure 8 Manhattan plot for GWAS on total length of lateral branches with treatment for 136 genotypes.

			Elevation		Control	Treatment
Genotype	Latitude	Longitude	(m)	State	(N)	(N)
RP003	44.62	-114.5141	2632	ID	4	3
RP009	43.966	-114.6894	2608	ID	5	2
RP012	40.684	-110.9318	3111	UT	4	5
RP014	41.844	-115.4473	2387	NV	2	1
RP019	40.01938	-105.34722	2001	CO	5	4
RP026	40.693	-109.4946	2563	UT	2	1
RP035	44.61562	-114.51843	2672	ID	2	2
RP042	40.001	-105.4681	2528	CO	4	5
RP053	44.288	-115.4813	2018	ID	5	5
RP058	40.309	-106.0621	2743	CO	1	3
RP070	45.71	-113.9981	2370	MT	5	3
RP088	42.139	-114.2085	2370	ID	3	2
RP089	45.2	-115.9902	1990	ID	2	3
RP091	42.163	-114.2638	2102	ID	5	5
RP095	42.596	-110.5083	2525	WY	2	1
RP101	44.027	-113.453	2312	ID	3	3
RP114	45.336	-114.0039	2273	ID	4	5
RP115	45.141	-113.4695	2181	MT	5	4
RP116	44.642	-114.529	2484	ID	4	4
RP121	44.3745	-113.3883	2160	ID	1	3
RP122	44.8413	-114.2723	2349	ID	4	4
RP127	43.804	-114.6431	2213	ID	3	3
RP135	45.551	-113.7742	2047	MT	5	4
RP142	45.151	-111.8047	2233	MT	5	4
RP144	43.937	-114.6904	2377	ID	2	3
RP145	40.612	-115.3709	2663	NV	4	5
RP149	43.768	-113.9014	2449	ID	5	2
RP151	38.85	-107.1009	3052	CO	5	3
RP155	42.506	-110.6781	2620	WY	3	5
RP157	44.535	-115.5688	2178	ID	5	5
RP161	46.268	-111.9275	2079	MT	5	5
RP166	44.41	-114.4077	2650	ID	5	5
RP173	41.801	-115.479	2496	NV	5	5
RP174	45.1564	-114.1517	2058	ID	4	5
RP176	42.475	-110.6166	2521	WY	5	5

Table 4 Table of genotypes used in analysis of compensation

RP178	38.9805	-106.9917	3009	CO	2	2
RP179	44.791	-113.3126	2523	ID	5	5
RP180	38.2566	-107.3697	3170	CO	5	5
RP181	37.612	-112.8303	3132	UT	5	5
RP187	41.746	-115.4676	2270	NV	4	4
RP190	41.922	-111.4783	2418	UT	5	4
RP191	44.964	-114.0805	2623	ID	5	5
RP192	44.411	-115.372	1955	ID	5	5
RP195	37.8076	-109.8187	2660	UT	5	5
RP198	44.421	-112.876	2477	MT	2	1
RP200	44.296	-115.4799	2015	ID	5	5
RP213	44.536	-112.618	2630	MT	5	5
RP214	38.966	-111.5722	2564	UT	5	5
RP215	44.556	-112.639	2355	MT	5	5
RP223	44.37	-115.397	1976	ID	4	4
RP235	44.3763	-113.4127	2244	ID	4	3
RP239	44.9643	-114.1931	2663	ID	4	3
RP242	44.6928	-114.3353	2142	ID	5	5
RP251	44.7414	-114.2641	1843	ID	4	5
RP254	45.17	-113.5205	2260	MT	3	3
RP259	44.6211	-114.5079	2711	ID	5	5
RP264	44.5897	-114.448	2475	ID	5	2
RP273	42.74451	-106.3251	2408	WY	4	5
RP275	41.22667	-105.38292	2528	WY	4	5
RP279	41.33122	-106.50222	2589	WY	5	5
RP280	43.54241	-109.837	2725	WY	5	5
RP285	38.53158	-106.32417	3165	CO	1	2
RP304	38.99427	-107.06551	3548	CO	3	5
RP308	38.8788	-106.99912	2737	CO	1	1
RP311	44.18767	-113.75333	2513	ID	5	4
RP313	44.18842	-113.74665	2465	ID	5	5
RP317	44.18177	-113.75175	2495	ID	2	2
RP319	44.17648	-113.77137	2658	ID	1	1
RP322	44.18517	-113.766	2627	ID	5	5
RP323	44.17983	-113.77517	2684	ID	3	4
RP327	39.88347	-110.77562	2744	UT	4	3
RP337	37.94522	-112.20185	2813	UT	5	5
RP338	40.21893	-111.25043	2621	UT	3	5

RP340	38.63022	-111.97983	3045	UT	5	4
RP345	38.07008	-112.1417	2883	UT	5	4
RP350	38.80653	-111.68993	2362	UT	4	5
RP353	37.83053	-111.88277	2908	UT	5	4
RP354	40.53782	-110.35575	2521	UT	3	6
RP362	38.55042	-111.97152	2838	UT	4	5
RP366	40.29412	-111.2513	2415	UT	4	5
RP370	40.53378	-110.46438	2334	UT	5	5
RP373	38.72395	-111.67807	3072	UT	4	5
RP374	39.8126	-111.69457	2512	UT	5	4
RP375	39.83448	-111.72215	2759	UT	4	5
RP377	39.87908	-111.68562	2662	UT	5	5
RP380	38.69638	-111.9379	2392	UT	4	4
RP381	39.3141	-111.36673	2664	UT	4	5
RP389	39.30538	-111.38982	2723	UT	3	4
RP400	40.58565	-110.33055	2451	UT	3	4
RP408	38.22568	-112.43942	2807	UT	5	5
RP411	40.93557	-110.70413	2876	UT	5	5
RP418	40.94707	-110.5829	2722	UT	5	5
RP419	40.93273	-110.56252	2755	UT	5	5
RP420	40.90998	-110.54825	2829	UT	5	5
RP421	40.9567	-110.55885	2892	UT	5	5
RP423	40.95303	-110.46135	2954	UT	5	5
RP425	40.92312	-110.40498	2881	UT	5	5
RP441	44.49355	-111.7673	2113	ID	5	5
RP449	45.14195	-111.83497	2325	MT	4	5
RP450	45.12525	-111.862	2453	MT	5	5
RP456	44.89812	-111.78012	2567	MT	4	5
RP459	44.86222	-111.62193	2163	MT	5	5
RP461	44.5627	-112.143	2181	MT	5	5
RP462	44.54178	-112.12652	2335	MT	5	4
RP466	44.49063	-111.65457	2042	ID	4	3
RP467	44.41103	-112.00128	2280	ID	3	3
RP470	41.41775	-111.52133	2714	UT	4	5
RP475	37.74395	-119.03577	2629	CA	2	2
RP476	44.2524	-113.12078	2332	ID	5	4
RP477	44.58915	-113.45617	2246	ID	5	5
RP480	44.60812	-113.57627	2298	ID	4	4

RP483	40.19816	-105.54125	2653	CO	5	5
RP485	40.2572	-105.53355	2660	CO	4	3
RP495	39.19892	-106.26055	3157	CO	5	5
RP498	39.89079	-105.48635	2739	CO	5	5
RP513	39.85218	-105.57111	2936	CO	5	4
RP514	39.44145	-106.15763	3234	CO	5	5
RP528	39.36264	-106.31117	3179	CO	5	5
RP538	38.46048	-108.54037	2220	CO	3	5
RP558	39.8351	-105.41198	2577	CO	5	4
RP561	39.8351	-105.41198	2577	CO	4	5
RP563	39.8351	-105.41198	2577	CO	4	4
RP564	39.8351	-105.41198	2577	CO	5	4
RP569	39.79634	-105.77581	3460	CO	4	5
RP584	44.96617	-114.08133	2639	ID	5	5
RP591	38.44063	-107.64228	2453	CO	5	5
RP596	37.36942	-108.07867	2689	CO	5	5
RP601	37.78007	-106.81818	2843	CO	3	1
RP603	37.75487	-108.00705	2787	CO	3	5
RP604	37.61628	-106.67578	2581	CO	4	4
RP605	38.33233	-108.10748	2779	CO	5	5
RP608	38.5211	-108.60332	2841	CO	5	3
RP609	38.59662	-108.6557	2808	CO	5	5
RP645	39.8251	-111.35645	2693	UT	4	4
RP648	38.2235	-112.3766	2982	UT	5	5
RP649	44.57703	-113.47208	2318	ID	5	5

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