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The Detection of Morphological Variation across Time in Two Roan Mountain Endemics: *Geum radiatum* and *Houstonia montana*.

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The Detection of Morphological Variation Across Time in Two Roan Mountain Endemics: *Geum radiatum* and *Houstonia montana*

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ABSTRACT

The Detection of Morphological Variation Across Time in Two Roan Mountain Endemics:
Geum radiatum and *Houstonia montana*

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Morphological variation between geographically distant populations has long been recognized. The primary objective of this study was to test whether nonrandom shifts in morphology have occurred across a 150-year time span in two rare, endangered plant species *Geum radiatum* and *Houstonia montana.* During the last century the vegetation on Roan Mountain has undergone numerous environmental pressures that may have produced morphological shifts.

A diverse suite of morphological characters was measured from both species. Characters included vegetative and reproductive structures. Herbarium specimens and direct field measurements were the sources of material used. Results indicated a significant increase in size across time in the majority of characters measured. Results of this study challenge standard taxonomic practices, present questions pertaining to the relationship between genetics and morphology, and raise issues concerning conservation and management strategies of endangered plant populations.

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CHAPTER 1

INTRODUCTION

Roan Mountain is an area of great botanical diversity and was among one of the most heavily botanized areas in the southeastern United States during the late 19th century. For over a century the great botanical diversity of Roan Mountain has attracted botanists from this country and from abroad. For many, Roan Mountain is considered to be the most beautiful and biologically interesting mountain east of the Rockies (Brown 1941).

This study concerns two plant species from this locality, *Geum radiatum* Michx. and *Houstonia purpurea* L. *var. montana* (Small) Terrell. *Geum radiatum,* [synonyms *Acomastylis radiata* (Michx.) Bolle, *Sieversia radiata* (Michx.) Greene] is a member of the Rosaceae family and is a perennial herb that begins flowering in late June and fruits in August and September. It grows 8-20 inches in height and is characterized by its large, toothed basal leaves with broad terminal lobes and its bright yellow flowers (USDA Forest Service 1983). *Houstonia montana* [synonym: (*Hedyotis purpurea var. montana* (Small) Fosberg)], a member of the Rubiaceae family, is also described as a perennial herb. It begins flowering in late May and fruits in August and September. *Houstonia montana* grows from 4-21 cm in height and is characterized by its opposite leaves with smooth margins and its small, reddish-purple flowers (Terrell 1996).

Geum radiatum and *H. montana* grow in similar habitats. Typically, they are found growing on rocky promontories, steep rock faces and narrow ledges, and in grassy balds and clearings at elevations of 5,000 feet or higher in the Southern Appalachians along the North Carolina-Tennessee border (Godt et al. 1996; USDA Forest Service 1983). Currently *G. radiatum* and *H. montana* are listed as federally endangered and are endemic to a very few high elevation rock outcrop habitats in the Southern Appalachians (Godt and Hamrick 1996). Roan

Mountain, which rises slightly above 6,000 feet, is located in the midst of these southern mountains (Brown 1941).

The focus of this study was to investigate population level variation among these 2 plant species, *G. radiatum* and *H. montana* across a time span of 150 years. Many rare and endangered plant species have recently experienced declines in their population number and size (Godt et al. 1996). Since the turn of the century, the Roan Mountain vegetation has sustained a great deal of environmental pressures due to changes such as clear-cut logging, residential and recreational development, climatic changes, community succession, and population bottlenecks. Because this is an area of scenic vistas at high elevations, these species are particularly vulnerable to human activities such as hiking, climbing, and sightseeing (Johnson 1995). It might seem unlikely that detectable changes in morphological features would occur in such a brief period of time as 150 years. However, given the rapid pace and degree of environmental and habitat changes, it is hypothesized that detectable morphological shifts within these species may have occurred in this relatively short time period.

This study was based on data collected from measurements of 175 herbarium specimens of *G. radiatum* and *H. montana,* collection dates for which ranged in time from the early 1840s to the mid to late 1990s. Additional data were collected from extant populations on Roan Mountain from June through September of 2000. A diverse suite of morphological characters were measured and used to compare groups of historic specimens to modern specimens, as well as to extant populations. This rather large sample size and the distribution of the specimens across a 150-year time span allowed for an investigation of populations across time.

The primary question of this research is: Have detectable morphological shifts occurred across a 150-year time span in Roan Mountain populations of the plant species *G. radiatum* and

H. montana? The null hypothesis for this study is that no detectable morphological shifts have occurred in these populations across this time period. There are two potential alternative hypotheses proposed in the study. The first is that detectable and statistically significant morphological shifts have occurred in these plants with a consistent pattern of change across a 150-year time span. The second alternative hypothesis is that detectable morphological shifts did occur across a 150-year time span but there was no pattern to the variation. Although morphological variation has frequently been observed in species that have geographically isolated populations, this study represents the first attempt to demonstrate within species morphological variation across time. If statistically significant shifts are detected, then this study will provide evidence that morphological variation can occur across relatively brief temporal spans.

The study also raises two related questions: (1) What relevance could population phenotypic variation have to genotypic variation? and (2) How can observed morphological shifts, if present, be accounted for in terms of environmental or genetic factors? If variation is due in part to genetics, then a change should be seen in morphological variation. These questions concern the biological processes underlying any observable morphological shift over time. They represent the broader issues raised by, but not tested, in this study.

There are several significant aspects to this study. It is the first attempt to demonstrate within species morphological variation over time at one locality. Previous research on within species variation have evaluated differences between geographically, rather that temporally isolated populations. Morphologically variant groups, when geographically isolated, are often recognized taxonomically at infraspecific levels. Temporally variant groups would challenge standard concepts and practices of taxonomic botany in which morphological traits are presumed to remain static over historical time periods.

A second significant aspect of this study is that it demonstrates a novel use of herbarium specimens. Herbarium specimens allow for testing of hypotheses that have an historical component. They offer a unique material basis to observe and gather information from historic plant populations for comparisons with extant populations. If this investigation of herbarium collections demonstrates temporal shifts in morphology, it will provide a novel method for examining population level processes in plant species.

A third significant aspect of this study is that it may give further insight into the consequences of reduced genetic diversity in rare and endangered plant species. Although molecular analyses were not used in this study, the morphological data could provide a basis for future research to examine the relationships between morphological and genetic diversity. An understanding of genetic diversity and distribution of genetic variation among these rare and endangered plant populations is essential for conservation and management strategies (Godt and Hamrick 1996). Herbarium resources may provide an untapped resource for examining and monitoring within population diversity and provide a valuable tool for the assessment and management of rare and endangered plant species.

CHAPTER 2

MATERIALS AND METHODS

To investigate whether morphological shifts occurred across a 150-year time span in the plant species *Geum radiatum* and *Houstonia montana,* measurements were taken for a variety of morphological characters from herbarium specimens and from extant populations. This study examined herbarium specimens collected from the summit of Roan Mountain beginning in the early 1840s and extending into the mid 1990s. Measurements were collected from 130 specimens of *G. radiatum* ranging from 1841-1994 and from 45 specimens of *H. montana* ranging from 1848-1994. Herbarium sheets were located by inquiries to over 50 herbaria, contacted via electronic correspondence. Index Herbariorum, an Internet resource, provided herbarium listings and addresses. Herbarium sheets were requested by both email and/or written correspondence from a total of 23 herbaria.

Measurements were also taken from live plants in the field during the months of June through September of 2000. Live plants were located on the summit of Roan Mountain along the Loop Road and the Cloudland Trail (Figure 1). Measurements were taken from 54 plants of *G. radiatum* and 79 plants of *H. montana.* Morphological characters, which provided reproducible, quantitative measurements, were selected for use. Characters were also selected on the basis that they were well presented on herbarium sheets and in the field. Morphological characters selected for measurement for *H. montana* were taken from a morphological comparison done by James Yelton in 1974 in his examination of differences between *Houstonia montana* and *Houstonia var. purpurea* (Terrell 1978). In a preliminary assessment 17 characters

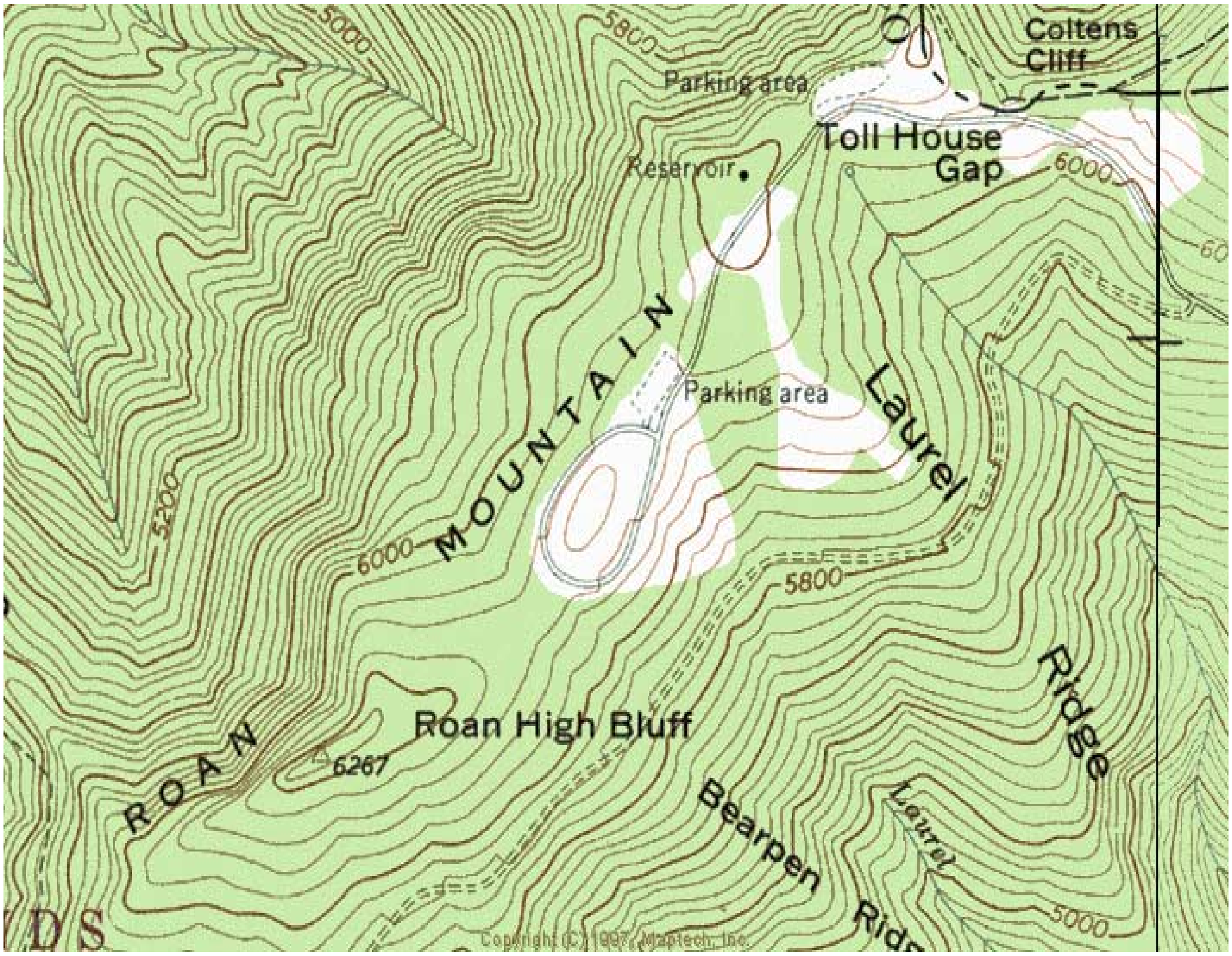


Figure 1. Locations of live plants measured on the summit of Roan Mountain along Loop Road and Cloudland trail. (Maptech:Terrain Navigator 1998)

were selected for measurement from *G. radiatum* and 12 were selected for measurement from *H. montana.* Several characters were not kept in the study due to difficulties in obtaining accurate measurements. Fourteen characters were measured for *G. radiatum* and 10 characters for *H. montana* were included in the final analysis.

Measurements of the morphological characters were taken directly from herbarium specimens. A standardized method of measurement was established for each character and was used throughout the measuring process. Characters and specific methods of measurement are listed in Table 1 for *G. radiatum* and Table 2 for *H. montana.*

Morphological data were divided into 3 groups based on the specimens' time periods: pre-logging, post-logging, and current. The pre-logging period ranges from 1840-1925 and is characterized as the period before the forests on Roan Mountain were clear-cut for timber. The post-logging time period ranges from 1932-1994 and is characterized as the period after Roan Mountain was deforested by clear-cut logging. Pre-logging and post-logging measurements were collected from herbarium specimens. The current period data set consists of measurements gathered from live plants on the summit of Roan from June through September of 2000. The current time period was incorporated into the study to assess the current morphological status of the plants, and to provide an additional test of any patterns observed between the 2 historical periods.

Statistical analyses conducted on the measurements were subdivided into pre-logging, post-logging, and current time periods. A series of statistical analyses was chosen to address the primary focus of the study, whether morphological shifts had occurred over time. To accurately compare "live" measurements taken in the field to dry measurements taken from herbarium specimens a preliminary analysis was conducted to estimate the percentage of shrinkage, or

Table 1. Morphological characters and details of measurements used in analyses of morphological variation across time in *Geum radiatum.*

Characters:	Details of measurement:
1. Length of leaf blade (mm)	The largest basal leaf was selected for measurement. The measurement was taken from the base of the leaf where the petiole is attached to the base of the leaf blade to the outer edge of the leaf blade.
2. Width of leaf blade (mm)	The largest basal leaf was selected for measurement. The leaf blade was measured at its widest point.
3. Number of teeth found in a 30° angle on a basal leaf	The protractor was placed on the largest basal leaf. It was aligned at the base of the leaf where the petiole is attached to the leaf blade. Teeth were counted between 90° and 120°. The character was measured from herbarium sheets only.
4. Number of teeth found in 2 cm on a basal leaf	The ruler was placed along the leaf margin of the largest leaf. The numbers of teeth within 2 cm were counted.
5. Number of teeth found in a 20° angle on a stem leaf.	The protractor was placed on the largest stem leaf. The protractor was aligned at the base of the leaf, which clasps to the stem. The 90° mark was placed at the apex of the stem leaf. The teeth between 90° and 110° were counted. The character was measured from herbarium sheets only.
6. Number of teeth in 1 cm of stem leaf	The ruler was placed along the leaf margin of the largest stem leaf. The number of teeth within 1 cm were counted.
7. Internode distance between bottom and second to the bottom internodes on the floral stem (mm)	The ruler was placed parallel to the floral stem. The distance between the two internodes was measured.

Table 1 (continued)

8. Sepal length (mm)	Sepals were randomly chosen and were measured from their point of attachment to their tip.
9. Petal length (mm)	Petals from the largest and most well presented flower were measured from their point of attachment to the corolla to the outer edge of the petal.
10. Petal width (mm)	Petals from the largest and most well presented flower were measured at their widest point.
11. Depth of petal sinus (mm)	Petal sinuses from the largest and most well presented flower were measured from the inner point of the sinus to the outer edge of the petal.
12. Width of floral stem (mm)	Stems (peduncles) were measured below the bottom internode at the widest visible point.
13. Maximum inflorescence length (mm)	The length of the inflorescence was measured from the base of the inflorescence to the bottom of the largest cyme.
14. Petiole length of basal leaf (mm)	The petiole was measured from the point of attachment to the basal rosette to the point of attachment to the leaf blade.

Table 2. Morphological characters and details of measurements used in analyses of morphological variation across time in *Houstonia montana.*

Characters measured:	Details of measurement:
1. Stem length (mm)	Stems were measured from their base to the base of the tallest cyme.
2. Median internode distance (mm)	The distance between two internodes was measured midway up the stem.
3. Length of leaf blade (mm)	The largest leaf was selected for measurement. The leaf was measured from its base to its apex.
4. Width of leaf blade (mm)	The largest leaf was selected for measurement. The leaf blade was measured at its widest point.
5. Sepal length (mm)	Sepals were measured from their point of attachment at their base to their tip.
6. Sepal width (mm)	Sepals were measured at their widest point.
7. Capsule length	Capsules were measured from base to tip.
8. Capsule width	Capsules were measured at their widest point.
9. Corolla length	Corolla was measured from base of calyx to outer edge of petals.
10. Number of flowers in a cyme	Number of flowers were counted in the largest cyme.

drying effect' between live and dry specimens. The drying effect was tested by locating and collecting measurements from 2 living species of plants that are taxonomically closely related and are morphologically similar to *G. radiatum* and *H. montana.* Substitution of similar species was necessary due to the protected status of *G. radiatum* and *H. montana,* which prohibits removal of any plant parts. The surrogate species were *Geum canadense* Jacq. and *Houstonia purpurea* L. which occur locally and are taxonomically similar to their congeners, *H. montana* and *G. radiatum.* Seventeen plants of *G. canadense* and 16 plants of *H. purpurea* were measured. The *G. canadense* plants were collected in Unicoi County, TN and the *H. purpurea* plants were collected in Mitchell County, NC. After live measurements were taken, the plants were collected and placed in a dryer for 7 days. Voucher specimens are deposited in the ETSU herbarium. After 7 days the plants were removed from the dryer and the same characters were re-measured. Ten characters were selected for measurement from *G. canadense* and *H. purpurea.* These characters represent features which are similar to those measured from *G. radiatum* and *H. montana.* Characters and details on how they were measured are listed in Table 3 for *G. canadense* and in Table 4 for *H. purpurea.*

T-tests were used to test for significant differences between live versus dry measurements (Minitab 12.01) and percent changes between live and dry measurements were calculated for each character. Percent changes for each of the 3 time periods were calculated for *G. radiatum* and *H. montana*. These percent changes were calculated by dividing the means of current measurements by the means of pre-logging and post-logging measurements, and post-logging measurements by pre-logging measurements. The percent changes between live and dry measurements calculated from *G. canadense* and *H. purpurea* were then compared to the 3 calculated percentages for the corresponding characters measured from *G. radiatum* and *H.*

Table 3. Characters measured from *Geum canadense* to test for a drying effect.

Characters measured:	Details of measurement:
1. Length of leaf blade	The largest basal leaf was selected for measurement. The measurements was taken from the base of the leaf where the petiole is attached to the base of the leaf blade to the outer edge of the leaf blade.
2. Width of leaf blade (mm)	The largest basal leaf was selected for measurement. The leaf blade was measured at its widest point.
3. Petiole length on basal length (mm)	The petiole was measured from the point of attachment to the basal rosette to point of attachment to the leaf blade.
4. Internode distance (mm)	The ruler was placed parallel to floral stem. The distance was measured between the bottom and the 2^{nd} to the bottom internode.
5. Petal length (mm)	Petals were measured from their point of attachment to the corolla to their outer edge.
6. Petal width (mm)	Petals were measured at their widest point.
7. Width of floral stem (mm)	Stems were measured below the bottom internode at the widest visible point.

Table 4. Characters measured from *Houstonia purpurea* to test for a drying effect.

Characters:	Details of measurement:
1. Stem length (mm)	Stems were measured from their base to the base of the largest cyme.
2. Median internode distance (mm)	The distance between two internodes was measured midway up the stem.
3. Length of leaf blade (mm)	The largest leaf was selected for measurement. The leaf was measured from its base to its apex.
4. Width of leaf blade (mm)	The largest leaf was selected for measurement. The leaf blade was measured at its widest point.
5. Sepal length (mm)	Sepals were measured from their point of attachment at their base to their tip.
6. Sepal width (mm)	Sepals were measured at their widest point.
7. Capsule length (mm)	Capsules were measured from base to tip.
8. Capsule width (mm)	Capsules were measured at their widest point.
9. Number of flowers in a cyme	Number of flowers were counted in the largest cyme.

montana. If the percent found from the live versus dry measurements from the *G. canadense* and *H. purpurea* was significantly less than the other 3 percent changes calculated for the corresponding characters from *G. radiatum* and *H. montana* then a shift in morphology detected in the character could not be attributed to the drying effect but would be indicative of some other factor. Table 5 shows a summary of percent changes for each character tested for a drying effect.

Two-way ANOVAs were used to test 2 main effects: collector, which refers to the various individuals who collected the herbarium specimens over time, and month (June, July, August, and September) referring to the month in which the plants were collected. An interaction between collector and month was also tested. SAS version 6.12 was used to test for the collector and month effect. The 2-way ANOVA tested for significant differences in the means and variances for each character after excluding the effects of collector and month. Results of the 2-way ANOVA are listed in Table 6 for *G. radiatum* and Table 7 for *H. montana*. Although collector effects on morphological variation were tested it was not possible to separate the collector effect from the period effect, since collectors did not span time periods. There were 64 collectors tested for *G. radiatum* and 26 tested for *H. montana.* Collectors and their collection dates are listed in Table 8 for *G. radiatum* and Table 9 for *H. montana.* A significant collector effect would indicate that differences in a measurement for various specimens were dependant upon the individual who collected the specimen. If the collector effect was not significant, then the identity of the collector had no clear influence upon the observed measurement for a specimen or a group of specimens.

Table 5. Summary of percent changes calculated for each character tested for a drying effect for *Geum radiatum* and *Houstonia montana.* Characters showing a significant drying effect are noted by *.

Geum radiatum

Character:	Percentage shrinkage:
Leaf length	1.3 %
Leaf width	11.1%
Length of petiole	2.5 %
Internode distance	1.7 %
Width of floral stem	5.6%
Petal length	36.1 %*
Petal width	36.1 %*

Houstonia montana

Stem length	0.7 %
Median internode distance	2.5 %
Sepal width	17.7 %

Table 6. Two-way ANOVAs testing for collector and month effects and for interactions between collector and month for characters measured for Geum radiatum.

1. Length of leaf blade

Source	DF	Type III SS	MS	F	P
Collector	35	7675.75	219.30	0.78	0.79
Month	3	1230.97	410.32	1.46	0.22
Coll*Month	7	2109.97	301.43	1.07	0.39
Error	106	29775.80	280.90		
Total	153	61597.35			

2. Width of leaf blade

Source	DF	Type III SS	MS	F	P
Collector	30	19368.27	645.61	0.75	0.81
Month	3	7510.55	2503.52	2.90	0.04
Coll*Month	7	11222.13	1603.16	1.86	0.08
Error	96	82875.85	863.29		
Total	138	199762.74			

3. Number of teeth in a 30° angle of a basal leaf

Source	DF	Type III SS	MS	F	P
Collector	38	1734.65	45.6	1.40	0.11
Month	3	74.19	24.73	0.76	0.52
Coll*Month	4	204.82	51.2	1.58	0.19
Error	63	2047.33	32.50		
Total	109	4454.76			

4. Number of teeth in 2 cm of a basal leaf

Source	DF	Type III SS	MS	F	P
Collector	38	369.38	9.72	1.40	0.08
Month	3	27.17	9.05	1.31	0.28
Coll*Month	7	99.92	14.27	2.06	0.06
Error	112	776.91	6.94		
Total	162	2030.07			

5. Number if teeth in stem leaf in a 20° angle

Source	DF	Type III SS	MS	F	P
Collector	35	225.14	6.43	0.64	0.91
Month	3	17.05	5.68	0.56	0.64
Coll*Month	4	31.26	7.81	0.78	0.54
Error	55	553.40	10.1		
Total	98	903.29			

Table 6 (continued)

6. Number if teeth in 1 cm of stem leaf

Source	DF	Type III SS	MS	F	P
Collector	35	122.32	3.50	0.71	0.87
Month	3	0.88	0.29	0.06	0.98
Coll*Month	7	49.42	7.06	1.44	0.19
Error	107	523.66	4.90		
Total	154	744.67			

7. Petiole length of basal leaf

8. Internode distance between the second to the bottom and the bottom internode

Source	DF	Type III SS	MS	F	P
Collector	36	21059.26	584.98	0.79	0.79
Month	3	1249.55	416.52	0.56	0.64
Coll*Month	6	482.73	80.45	0.11	0.99
Error	109	80715.56	740.51		
Total	156	180476.31			

9. Sepal length

Source	DF	Type III SS	MS	F	P
Collector	40	137.20	3.43	0.95	0.56
Month	3	1.24	0.41	0.12	0.95
Coll*Month	6	11.2	1.87	0.52	0.79
Error	105	379.46	3.61		
Total	156	539.24			

10. Petal length

Source	DF	Type III SS	MS	F	P
Collector	29	186.71	6.44	0.56	0.95
Month	3	11.85	3.94	0.34	0.79
Coll*Month	4	46.69	11.67	1.01	0.40
Error	59	679.91	11.52		
Total	97	1145.19			

11. Petal width

Source	DF	Type III SS	MS	F	P
Collector	29	223.32	7.70	1.84	0.02
Month	3	53.83	17.94	4.29	0.01
Coll*Month	5	65.33	13.06	13.06	3.12
Error	59	246.80	4.18		
Total	98	844.51			

Table 6 (continued)

12. Depth of petal sinus

Source	DF	Type III SS	MS	F	P
Collector	30	7.22	0.24	0.41	0.99
Month	3	1.57	0.52	0.90	0.44
Coll*Month	5	1.44	0.28	0.50	0.77
Error	58	33.76	0.58		
Total	98	49.41			

13. Width of floral stem

Source	DF	Type III SS	MS	F	P
Collector	39	11.81	0.30	0.71	0.88
Month	3	0.42	0.13	0.33	0.80
Coll*Month	7	1.03	0.14	0.35	0.93
Error	116	49.30	0.42		
Total	167	83.85			

14. Maximum inflorescence length

Source	DF	Type III SS	MS	F	P
Collector	39	485080.53	12437.96	1.31	0.14
Month	3	240670.64	80223.55	8.43	0.00
Coll*Month	7	88343.08	12620.44	1.33	0.24
Error	114	1084767.06	9515.50		
Total	165	4430634.25			

Table 7. Two-way ANOVAs testing for collector and month effects and for interactions between collector and month for characters measured for *Houstonia montana*..

1. Stem length

Source	DF	Type I SS	MS	F	P
Collector	16	26243.22	1640.20	1.36	0.17
Month	3	8694.29	2898.09	2.41	0.07
Coll*Month	0	0.0000	*	*	*
Error	98	118060.23	1204.69		
Total	119	169241.99			

2. Median Internode Distance

Source	DF	Type I SS	MS	F	P
Collector	16	690.23	43.13	0.98	0.48
Month	3	67.8	22.6	0.52	0.67
Coll*Month	0	0.0000	*	*	*
Error	99	4344.24	43.88		
Total	120	5491.90			

3. Leaf length

Source	DF	Type I SS	MS	F	P
Collector	16	244.37	15.27	2.48	0.00
Month	3	56.39	18.80	3.06	0.03
Coll*Month	0	0.0000	*	*	*
Error	99	608.68	6.15		
Total	120	919.88			

4. Leaf width

Source	DF	Type I SS	MS	F	P
Collector	16	67.18	4.20	1.15	0.32
Month	3	36.14	12.05	3.30	0.02
Coll*Month	0	0.000	*	*	*
Error	99	361.17	3.64		
Total	120	473.60			

5. Sepal length

Source	DF	Type I SS	MS	F	P
Collector	15	3.87	0.25	0.80	0.66
Month	3	2.34	0.78	2.44	0.07
Coll*Month	0	0.000	*	*	*
Error	94	30.14	0.32		
Total	114	36.99			

Table 7 (continued)

6. Sepal width

Source	DF	Type I SS	MS	F	P
Collector	15	0.0000	0.00	0.00	1.00
Month	3	2.47	0.82	4.46	0.00
Coll*Month	0	0.000	*	*	*
Error	95	17.60	0.19		
Total	115	23.24			

7. Number of blooms

Source	DF	Type I SS	MS	F	P
Collector	15	148.8	9.92	1.58	0.09
Month	3	156.8	52.2	8.30	0.00
Coll*Month	0	0.000	*	*	*
Error	87	547.6	6.29		
Total	107	1158.19			

8. Corolla length

Source	DF	Type I SS	MS	F	P
Collector	14	23.4	1.70	1.02	0.48
Month	0	0.0000	*	*	*
Coll*Month	0	0.0000	*	*	*
Error	14	23.35	1.67		
Total	29	47.37			

9. Capsule length

Source	DF	Type I SS	MS	F	P
Collector	7	0.000	0.00	0.00	1.00
Month	1	0.43	0.43	1.94	0.17
Coll*Month	0	0.0000	*	*	*
Error	42	9.34	0.22		
Total	52	11.55			

10. Capsule width

Source	DF	Type I SS	MS	F	P
Collector	7	1.56	0.22	2.68	0.02
Month	1	0.12	0.12	1.46	0.23
Coll*Month	0	0.000	*	*	*
Error	42	3.48	0.08		
Total	52	5.92			

Table 8. Collectors, collections dates, and number of specimens for *Geum radiatum.*

Collectors	Collection dates	Number of Specimens
Gray	1841, -43	1
Gray, Carey	1841, -43	3
Canby	1868, -76, -78, -79, 88	8
Chickering	1877, -80	10
Vasey	1878, 1905	3
(Gray, Sargent, Redfield, Canney)	1879	1
Smith	1880, -84	3
(Meehan, Porter, Leidy, Wilcox)	1880	1
Ball	1884	1
Stubbs	1884	1
Britton	1883, -85	2
EGB	1883	1
Thaxler	1887	1
Hyams	1878, -83, -88	3
Heller	1890, -91	3
Jouy	1890	1
Small/Heller	1891	6
Merriam	1892	4
Edston	1893	1
Mohr	1894	1
Ashe	1885	1
Gibbes	1898	1
Cannon	1902	3
Rydberg	1908, -25	5
JTP	1925	1
Blomquist	1932	1
Oosting	1932	1
Hunnewell	1933	1
Brown	1934, -37	19
Hill	1934	1
Pyron	1936	1
Jennison	1937	1
Clausen	1941	1
Clausen, Trapido	1938	1
Alexander	1939	2
Shanks	1947	1
(Fairchild, Clebsch, Sharp, Hernandez)	1948	1
Barrell	1953	1

Table 8 (continued)

Sargent	1954	5
Ramseur	1956	3
Bartley	1956	1
Mark	1957	2
Radford	1966	1
Henry	1966	1
(Leonard, Radford, Moore)	1968	6
Churchill	1970, -94	4
Boufford,Wood	1975	1
Kral	1977, -79	2
(Hill, Myrick, Saunders)	1980	1
Unknown	1868, -71, -76, -94, 1903, -04	6

Table 9. Collectors, collections dates, and number of specimens for *Houstonia montana.*

Collectors	Collection dates	Number of Specimens
Vasey	1878	2
(Gray, Sargent Redfield, Canby)	1879	2
(Meehan, Porter Leidy, Wilcox)	1880	1
Chickering	1880	1
Ball	1884	2
Heller	1890	1
Rydberg	1925	2
Blomquist	1932	1
Oosting	1932	1
Shaver	1940	6
Stewart	1940	3
Shanks	1946	8
(Fairchild, Hernandez Clebsch, Sharp)	1948	1
Sargent	1954	4
Hermann	1959	1
Anderson	1964	2
Churchill	1968, 1994	3
Kral	1977, 1979	2
Wofford	1979	1
Somers	1979	1

If no collector effects were detected the next step was to test for period and month effects. Two-way ANOVAs were used to test for 2 main effects: period, which grouped samples into pre-logging, post-logging, and current time sets, and month referring to the month in which the plants were collected. These 2-way ANOVAs tested for significant differences in the means and variances for each character after excluding the effects of collection period and month. A Tukey's pairwise comparison was run for characters that displayed a significant period effect. This test was used to determine where significant differences occurred between the three periods. Results of the 2-way ANOVAs are listed in Table 10 for *G. radiatum* and Table 11 for *H. montana*. An interaction between period and month was also tested. Because the sample sizes were unbalanced a General Linear Model command was used. In the case of *H. montana* the data was not only unbalanced but was also not full rank (Ryan and Joiner 2001). The data were missing an observation from the month of September in the post-logging period. It was necessary for each of the characters measured for *H. montana* to estimate a missing value. The missing value was estimated by using the following equation: $\underline{Y_{ij} = aT_i + bT_j + T..}/(a-1)(b-1)$ and by reducing the residual degrees of freedom by one (Dowdy and Wearden 1983). The calculated value was then inserted into the missing cell and the 2-way ANOVA was completed using Minitab 12.21.

The final analyses run were 1-way ANOVAs. Results of the 1-way ANOVAs are listed in Table 12 for *G. radiatum* and Table 13 for *H. montana.* If a significant period effect was significant for a character, then the means and variances for that character were analyzed (Figure 2) for each time period in order to test the stated hypotheses. No change in the mean or the variance indicates that the population was unchanged over the time span studied. An increase in the mean and an increase in variance indicate an increase in size or number

Table 10. Two-way ANOVAs testing for period and month effects and interactions between period and month for characters measured for *Geum radiatum.*

1. Length of leaf blade

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Period	2	17783.7	18227.0	9113.5	24.60	0.000
Month	3	1907.3	2364.4	788.1	2.13	0.099
Period*Month	6	3071.2	3071.2	511.9	1.38	0.226
Error	148	54829.8	54829.8	370.5		
Total	159	77592.0				

2. Width of leaf blade

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period1	2	78731.6	71338.5	35669.2	46.25	0.000
month1	3	8938.2	9321.3	3107.1	4.03	0.009
period1*month1	6	11632.1	11632.1	1938.7	2.51	0.025
Error	132	101803.2	101803.2	771.2		
Total	143	201105.1				

3. Number of teeth in a 30° angle of a basal leaf

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period11	1	171.44	107.80	107.80	2.95	0.089
month11	3	249.77	145.02	48.34	1.32	0.271
period11*month11	3	197.87	197.87	65.96	1.80	0.151
Error	108	3949.93	3949.93	36.57		
Total	115	4569.00				

4. Number of teeth in 2 cm of a basal leaf

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period2	2	696.800	663.328	331.664	35.39	0.000
month2	3	70.276	69.472	23.157	2.47	0.064
period2*month2	6	54.311	54.311	9.052	0.97	0.450
Error	158	1480.902	1480.902	9.373		
Total	169	2302.288				

5. Number if teeth in stem leaf in a 20° angle

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period12	1	0.175	12.045	12.045	1.25	0.267
month12	3	58.358	54.648	18.216	1.89	0.137
period12*month12	3	41.923	41.923	13.974	1.45	0.234
Error	97	936.077	936.077	9.650		
Total	104	1036.533				

Table 10 (continued)

6. Number of teeth in 1 cm of stem leaf

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period3	2	36.756	37.344	18.672	3.97	0.021
month3	3	7.247	5.826	1.942	0.41	0.744
period3*month3	6	46.685	46.685	7.781	1.65	0.136
Error	149	700.704	700.704	4.703		
Total	160	791.391				

7. Petiole length of basal leaf

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period4	2	190503	188534	94267	33.57	0.000
month4	3	19850	22148	7383	2.63	0.052
period4*month4	6	27444	27444	4574	1.63	0.143
Error	154	432402	432402	2808		
Total	165	670199				

8. Internode distance between the second to the bottom and the bottom internode

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period5	2	71350.6	58395.4	29197.7	39.89	0.000
month5	3	7497.0	6810.7	2270.2	3.10	0.029
period5*month5	6	1496.4	1496.4	249.4	0.34	0.914
Error	151	110520.6	110520.6	731.9		
Total	162	190864.5				

9. Sepal length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period6	2	10.462	6.195	3.098	0.86	0.424
month6	3	2.699	4.734	1.578	0.44	0.725
period6*month6	6	16.020	16.020	2.670	0.74	0.615
Error	151	541.384	541.384	3.585		
Total	162	570.564				

10. Petal length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period0	2	225.701	167.694	83.847	18.15	0.000
month0	3	18.092	16.770	5.590	1.21	0.311
period0*month0	6	24.812	24.812	4.135	0.90	0.502
Error	91	420.424	420.424	4.620		
Total	102	689.029				

Table 10 (continued)

11. Petal width

Source	DF	Seq SS	Adj SS	Adj MS	F	P
periodX	2	247.523	196.188	98.094	18.53	0.000
monthX	3	51.783	37.375	12.458	2.35	0.077
periodX*monthX	6	45.286	45.286	7.548	1.43	0.213
Error	92	486.956	486.956	5.293		
Total	103	831.547				

12. Depth of petal sinus

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period8	2	3.8269	2.5732	1.2866	1.43	0.245
month8	3	0.8360	0.5059	0.1686	0.19	0.905
period8*month8	6	3.0556	3.0556	0.5093	0.57	0.756
Error	92	82.7599	82.7599	0.8996		
Total	103	90.4784				

13. Width of floral stem

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period9	2	21.1225	17.4696	8.7348	23.20	0.000
month9	3	3.8065	3.8227	1.2742	3.38	0.020
period9*month9	6	2.8646	2.8646	0.4774	1.27	0.275
Error	163	61.3799	61.3799	0.3766		
Total	174	89.1736				

14. Maximum inflorescence length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period10	2	1781100	1492697	746349	69.33	0.000
month10	3	772900	730091	243364	22.61	0.000
period10*month10	6	128563	128563	21427	1.99	0.070
Error	160	1722466	1722466	10765		
Total	171	4405029				

Table 11. Two-way ANOVAs testing for period and month effects and for interactions between period and month for characters measured for *Houstonia montana.*

1. Stem length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Period	2	15087	13563	6781	5.31	0.006
Month	3	6054	6165	2055	1.61	0.191
Period*Month	6	10541	10541	1757	1.38	0.231
Error	108	137907	137907	1277		
Total	119	169589				

2. Median internode distance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period1	2	340.16	297.38	148.69	3.27	0.042
month1	3	144.48	192.72	64.24	1.41	0.243
period1*month1	6	96.92	96.92	16.15	0.36	0.905
Error	108	4912.02	4912.02	45.48		
Total	119	5493.59				

3. Leaf length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period2	2	10.400	12.446	6.223	0.88	0.418
month2	3	17.339	33.677	11.226	1.59	0.196
period2*month2	6	105.649	105.649	17.608	2.49	0.027
Error	108	763.412	763.412	7.069		
Total	119	896.800				

4. Leaf width

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period3	2	4.407	12.521	6.260	1.72	0.185
month3	3	23.782	9.481	3.160	0.87	0.461
period3*month3	6	37.949	37.949	6.325	1.73	0.120
Error	108	394.228	394.228	3.650		
Total	119	460.367				

5. Sepal length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period4	2	0.4473	0.1507	0.0754	0.24	0.788
month4	3	2.7121	0.6608	0.2203	0.70	0.556
period4*month4	6	0.2973	0.2973	0.0496	0.16	0.987
Error	103	32.5434	32.5434	0.3160		
Total	114	36.0000				

Table 11 (continued)

6. Sepal width

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period5	2	2.2610	1.1812	0.5906	4.40	0.015
month5	3	1.1369	0.1074	0.0358	0.27	0.849
period5*month5	6	0.7294	0.7294	0.1216	0.90	0.495
Error	103	13.8379	13.8379	0.1343		
Total	114	17.9652				

7. Number of blooms

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period6	2	134.221	10.457	5.229	0.63	0.535
month6	3	20.717	25.899	8.633	1.04	0.379
period6*month6	6	25.178	25.178	4.196	0.51	0.801
Error	70	579.945	579.945	8.285		
Total	81	760.061				

8. Corolla length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period7	2	31.320	31.889	15.945	12.54	0.000
month7	1	0.123	0.222	0.222	0.17	0.678
period7*month7	2	0.965	0.965	0.483	0.38	0.686
Error	50	63.575	63.575	1.271		
Total	55	95.982				

9. Capsule length

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period8	2	0.5069	0.3804	0.1902	1.65	0.204
month8	1	0.1278	0.0052	0.0052	0.04	0.834
period8*month8	2	0.0188	0.0188	0.0094	0.08	0.922
Error	42	4.8428	4.8428	0.1153		
Total	47	5.4963				

10. Capsule width

Source	DF	Seq SS	Adj SS	Adj MS	F	P
period9	2	0.00938	0.00797	0.00398	0.15	0.863
month9	1	0.05420	0.00219	0.00219	0.08	0.777
period9*month9	2	0.00797	0.00797	0.00398	0.15	0.863
Error	42	1.13158	1.13158	0.02694		
Total	47	1.20313				

Table 12. One-way ANOVAs testing for significant differences between time periods for *Geum radiatum.*

1. Length of leaf blade

```
Source     DF        SS        MS        F        P
C5          2     20523     10261    29.80    0.000
Error     162     55785       344
Total     164     76308
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ------+---------+---------+---------+
1          61     59.25     21.71   (---*----)
2          50     73.28     17.81              (----*----)
3          54     85.96     15.03                          (----*----)
                                   ------+---------+---------+---------+
Pooled StDev =    18.56                 60        70        80        90
```

2. Width of leaf blade

```
Source     DF        SS        MS        F        P
C11         2     89290     44645    52.10    0.000
Error     147    125968       857
Total     149    215258
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
1          56     92.23     29.53  (---*---)
2          40    117.72     30.34              (----*---)
3          54    149.17     28.19                               (---*---)
                                   --------+---------+---------+--------
Pooled StDev =    29.27                  100       120       140
```

3. Number of teeth in a 30° angle of a basal leaf

```
Analysis of Variance for C15
Source     DF        SS        MS        F        P
C16         1      81.6      81.6     1.78    0.185
Error     122    5599.6      45.9
Total     123    5681.2
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
1          65    21.308     5.609   (----------*----------)
2          59    22.932     7.863            (-----------*-----------)
                                   ----------+---------+---------+------
Pooled StDev =    6.775                   21.0      22.5      24.0
```

Table 12 (continued)

4. Number of teeth in 2 cm of a basal leaf

```
Source     DF        SS        MS        F        P
C22         2    779.79    389.90    45.77    0.000
Error     172   1465.15      8.52
Total     174   2244.94
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -+---------+---------+---------+-----
1          64    15.891     3.568                             (--*---)
2          57    14.158     2.795                    (---*---)
3          54    10.778     2.062   (---*---)
                                   -+---------+---------+---------+-----
Pooled StDev =    2.919          10.0      12.0      14.0      16.0
```

5. Number of teeth in stem leaf in a 20° angle

```
Source     DF        SS        MS        F        P
C28         2     24.01     12.01     1.47    0.233
Error     173   1415.78      8.18
Total     175   1439.80
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ---+---------+---------+---------+---
1          65     6.908     2.957    (---------*---------)
2          47     6.979     3.467   (-----------*----------)
3          64     7.703     2.194               (---------*---------)
                                   ---+---------+---------+---------+---
Pooled StDev =    2.861             6.30      7.00      7.70      8.40
```

6. Number of teeth in 1 cm

```
Source     DF        SS        MS        F        P
C34         2     40.98     20.49     4.22    0.016
Error     165    801.01      4.85
Total     167    841.99
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+---------+-
1          64     7.703     2.194                    (-------*-------)
2          50     7.440     2.533               (--------*--------)
3          54     6.556     1.860  (--------*-------)
                                   -----+---------+---------+---------+-
Pooled StDev =    2.203               6.30      7.00      7.70      8.40
```

Table 12 (continued)

7. Petiole length of basal leaf

```
Source      DF        SS        MS        F        P
C40          2    215878    107939    39.23    0.000
Error      167    459460      2751
Total      169    675337
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level        N      Mean     StDev  -+---------+---------+---------+-----
1           63    101.42     48.66  (----*---)
2           54    163.80     62.42                      (----*---)
3           53    183.28     45.19                            (----*----)
                                    -+---------+---------+---------+-----
Pooled StDev =     52.45            90        120       150       180
```

8. Internode distance between the second to the bottom internode

```
Source      DF        SS        MS        F        P
C46          2     74713     37356    51.69    0.000
Error      166    119966       723
Total      168    194679
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level        N      Mean     StDev  ----+---------+---------+---------+--
1           64     79.36     25.57  (---*--)
2           51     98.73     29.22            (--*---)
3           54    129.70     26.09                           (---*--)
                                    ----+---------+---------+---------+--
Pooled StDev =     26.88               80       100       120       140
```

9. Sepal length

```
Source      DF        SS        MS        F        P
C52          2      9.09      4.54     1.24    0.292
Error      166    609.04      3.67
Total      168    618.13
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level        N      Mean     StDev  -----+---------+---------+---------+-
1           63     9.825     1.939              (---------*--------)
2           55     9.382     2.198    (----------*---------)
3           51     9.314     1.516   (---------*----------)
                                    -----+---------+---------+---------+-
Pooled StDev =     1.915               9.00      9.50     10.00     10.50
```

Table 12 (continued)

10. Petal length

```
Source     DF        SS        MS        F        P
C64         2    173.73     86.86    13.45    0.000
Error     105    678.01      6.46
Total     107    851.74
                                 Individual 95% CIs For Mean
                                 Based on Pooled StDev
Level       N      Mean     StDev  ---+---------+---------+---------+---
1          50    10.280     2.399         (----*---)
2          29     9.552     2.028  (------*-----)
3          29    12.793     3.167                       (-----*------)
                                   ---+---------+---------+---------+---
Pooled StDev =    2.541               9.0      10.5      12.0      13.5
```

11. Petal width

```
Source     DF        SS        MS        F        P
C70         2    197.42     98.71    17.20    0.000
Error      92    528.11      5.74
Total      94    725.54
                                 Individual 95% CIs For Mean
                                 Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+---------+-
1          39    12.744     2.359                (----*----)
2          28    10.714     2.355  (-----*-----)
3          28    14.464     2.487                          (-----*-----)
                                   -----+---------+---------+---------+-
Pooled StDev =    2.396                10.5      12.0      13.5      15.0
```

12. Depth of petal sinus

```
Source     DF        SS        MS        F        P
C76         2     3.345     1.673     3.31    0.040
Error     106    53.595     0.506
Total     108    56.940
                                 Individual 95% CIs For Mean
                                 Based on Pooled StDev
Level       N      Mean     StDev  --+---------+---------+---------+----
1          53    1.3396    0.6007           (------*-----)
2          29    1.1034    0.4749  (--------*--------)
3          27    1.5926    1.0473                 (--------*--------)
                                   --+---------+---------+---------+----
Pooled StDev =   0.7111            0.90      1.20      1.50      1.80
```

Table 12 (continued)

13. Width of floral stem

```
Source     DF        SS        MS        F        P
C82         2    24.175    12.087    28.07    0.000
Error     181    77.945     0.431
Total     183   102.120
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
1          71    1.9824    0.6315  (----*---)
2          59    2.0720    0.6680    (----*----)
3          54    2.8148    0.6750                         (----*----)
                                   --------+---------+---------+--------
Pooled StDev =   0.6562                  2.10      2.45      2.80
```

14. Maximum inflorescence length

```
Source     DF        SS        MS        F        P
C88         2   2102780   1051390    53.00    0.000
Error     181   3590440     19837
Total     183   5693220
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ---+---------+---------+---------+---
1          71     310.7     132.5   (--*--)
2          59     433.9     183.7               (--*---)
3          54     572.3      87.3                            (---*---)
                                   ---+---------+---------+---------+---
Pooled StDev =    140.8             300       400       500       600
```

Table 13. One-way ANOVAs testing for significant differences between time periods for *Houstonia montana*.

1. Stem length

```
Source     DF        SS        MS        F        P
C5          2     17714      8857     6.86    0.002
Error     120    154851      1290
Total     122    172565
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
1          10    124.00     37.27   (----------*----------)
2          34    141.47     34.59                  (-----*-----)
3          79    160.81     36.31                             (---*---)
                                   ----------+---------+---------+------
Pooled StDev =    35.92                    120       140       160
```

2. Median Internode Distance

```
Source     DF        SS        MS        F        P
C10         2     459.2     229.6     4.77    0.010
Error     121    5824.2      48.1
Total     123    6283.4
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
1          11    18.273     6.784  (-----------*-----------)
2          34    21.735     5.316                 (------*------)
3          79    24.430     7.537                           (----*---)
                                   ----------+---------+---------+------
Pooled StDev =    6.938                   17.5      21.0      24.5
```

3. Leaf length

```
Source     DF        SS        MS        F        P
C16         2     15.58      7.79     1.01    0.366
Error     121    929.90      7.69
Total     123    945.48
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+---------+-
1          11    15.182     2.892  (----------------*---------------)
2          34    15.794     2.962                (--------*--------)
3          79    16.304     2.672                        (-----*-----)
                                   -----+---------+---------+---------+-
Pooled StDev =    2.772              14.0      15.0      16.0      17.0
```

Table 13 (continued)

4. Leaf width

```
Source     DF        SS        MS        F        P
C22         2      6.45      3.22     0.81    0.445
Error     121    479.04      3.96
Total     123    485.48
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
1          11     8.364     2.541   (---------------*----------------)
2          34     9.029     1.834                   (---------*---------)
3          79     9.177     1.973                         (-----*-----)
                                   --------+---------+---------+--------
Pooled StDev =    1.990                  7.70      8.40      9.10
```

5. Sepal length

```
Source     DF        SS        MS        F        P
C28         2     0.576     0.288     0.90    0.409
Error     120    38.415     0.320
Total     122    38.992
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
1          10    2.0000    0.0000  (----------------*-----------------)
2          34    1.8824    0.5911     (--------*---------)
3          79    2.0380    0.5871              (-----*-----)
                                   --------+---------+---------+--------
Pooled StDev =   0.5658                  1.80      2.00      2.20
```

6. Sepal width

```
Source     DF        SS        MS        F        P
C34         2     2.212     1.106     8.33    0.000
Error     119    15.798     0.133
Total     121    18.010
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ---------+---------+--------+-------
1          10    0.9000    0.2108   (-------------*--------------)
2          33    0.8939    0.2076         (-------*-------)
3          79    1.1772    0.4239                              (----*----)
                                   ---------+---------+--------+-------
Pooled StDev =   0.3644                   0.80      0.96      1.12
```

Table 13 (continued)

7. Number of blooms

```
Source     DF        SS        MS        F        P
C59         2    170.41     85.20     9.15    0.000
Error      82    763.55      9.31
Total      84    933.95
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+---------+-
1          11     5.727     3.289   (-----------*-----------)
2          34     4.853     2.956  (------*------)
3          40     7.850     3.068                      (-----*------)
                                   -----+---------+---------+---------+-
Pooled StDev =    3.051                4.5       6.0       7.5       9.0
```

8. Corolla length

```
Source     DF        SS        MS        F        P
C40         2     33.97     16.98    12.27    0.000
Error      60     83.02      1.38
Total      62    116.98
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+--
1           9     9.889     1.167      (----------*----------)
2          25     9.320     1.282  (------*------)
3          29    10.897     1.081                        (------*-----)
                                   ----+---------+---------+---------+--
Pooled StDev =    1.176               9.10      9.80     10.50     11.20
```

9. Capsule length

```
Source     DF        SS        MS        F        P
C47         2     0.698     0.349     3.44    0.040
Error      49     4.975     0.102
Total      51     5.673
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+--
1           6    2.0000    0.0000   (------------*------------)
2           6    2.0000    0.0000   (------------*------------)
3          40    2.2750    0.3572                        (----*----)
                                   ----+---------+---------+---------+--
Pooled StDev =   0.3186               1.80      2.00      2.20      2.40
```

Table 13 (continued)

10. Capsule width

```
Source     DF        SS        MS        F        P
C53         2    0.7289    0.3644     5.34    0.008
Error      51    3.4795    0.0682
Total      53    4.2083
                                   Individual 95% CIs For Mean
                                   Based on Pooled StDev
Level       N      Mean     StDev  -----+---------+---------+---------+-
1           7    2.8571    0.3780          (---------*---------)
2           7    2.7143    0.4880   (---------*---------)
3          40    3.0375    0.1750                       (---*---)
                                   -----+---------+---------+---------+-
Pooled StDev =   0.2612               2.60      2.80      3.00      3.20
```

accompanied by an increase of variation over the time periods studied. An increase in the mean with a decrease in the variance indicates an increase in size or number accompanied by a decrease in variation. A decrease in mean accompanied by an increase in variance indicates that size (number) has decreased but that variation has increased. A decrease in mean accompanied by a decrease in variance indicates that both size (number) and variation have decreased across time.

		Variance: Increase	Variance: Decrease
Mean	Increase	• Plants parts get larger • Variation increases	• Plants parts get larger • Variation decreases
	Decrease	• Plants parts get smaller • Variation increases	• Plants parts get smaller • Variation decreases

Figure 2: Method for analyzing means and variances for characters showing significant differences among time periods.

Chapter 3

RESULTS

Data Analysis

A total of 175 herbarium specimens and 133 live plants of *G. radiatum* and *H. montana* were measured for this study. The null hypothesis-that no significant morphological shifts have occurred over time-- was rejected for 18 out of 24 characters measured. The first alternative hypothesis-that detectable and statistically significant morphological shifts have occurred in these plants across a 150-year time span with a consistent pattern of change over time--is supported for most of the characters examined.

Period Effects

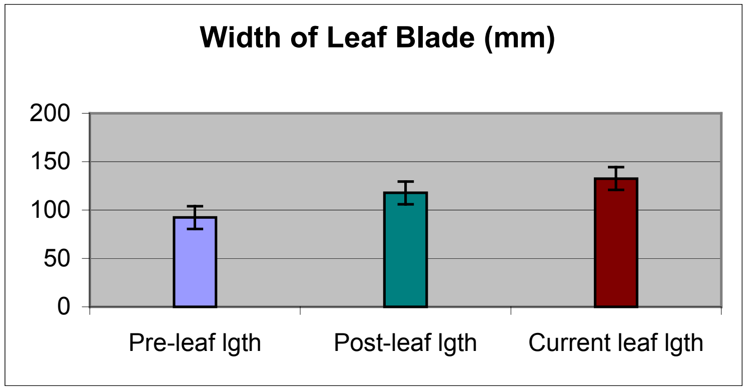
Five out of 14 characters measured for *G. radiatum* and 3 out of 10 characters measured for *H. montana* support the alternative hypothesis: detectable and statistically significant morphological shifts have occurred in both plant species across a 150-year time span with a consistent pattern of change over time. These characters, which include both vegetative and reproductive structures, all showed the same pattern: for each character there was a significant increase in the mean and a decrease in variance (Table 14). This "Type I" pattern of change showed that many measured features grew larger and presented a reduction in size variation across the 150-year time span. Figure 3, graphs A through F for *G. radiatum* and figure 4 graphs A through C for *Houstonia montana* show the predominant pattern of change across time.

Another pattern of statistically significant change across periods is evident in one character, sepal width, for *H. montana*. In this character there was a significant increase in the mean sepal width with an increase in variance as well. (Figure 5, graph A) This "Type II"

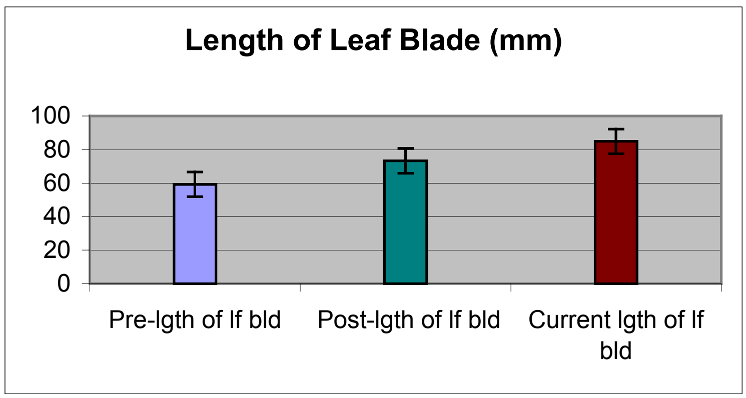
Table 14. Characters that show Type I morphological shifts across time. Each character shows an increase in size accompanied by a decrease in variation. Characters include vegetative and reproductive structures.

Geum radiatum		
	Vegetative structures:	Leaf length
		Leaf width
		Petiole length
		Internode distance on primary axis of inflorescence
		Inflorescence width
Houstonia montana		
	Vegetative structures:	Stem length
		Median internode distance
	Reproductive structures:	Corolla length

A.



B.



C.

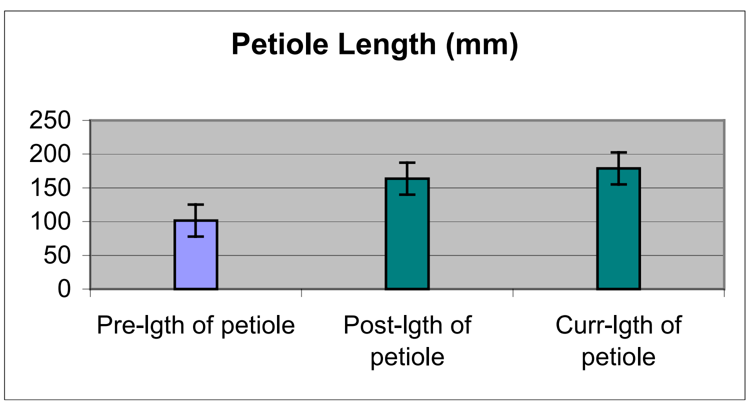
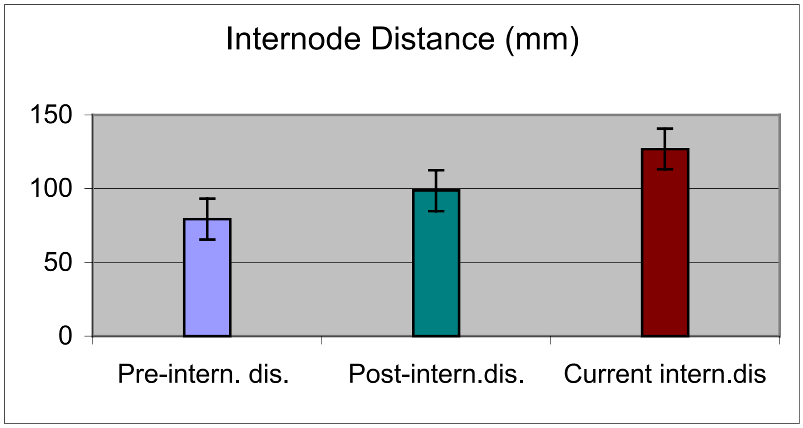


Figure 3 (Graphs A-E): Characters measured from *Geum radiatum* displaying a Type I period effect. These characters depict the predominant pattern of change between time periods: an increase in size accompanied by a decrease in variation across time. Different bar colors indicate significant differences between periods. Mean measurements are shown with error bars indicating a 95% confidence interval with respect to the mean.

D.



E.

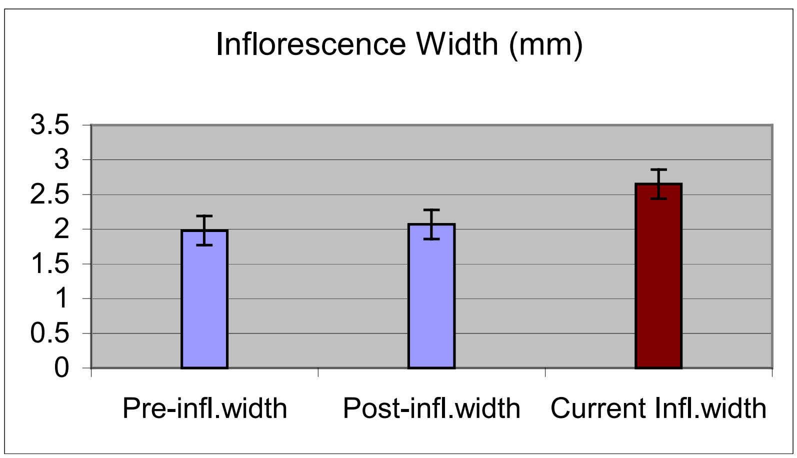
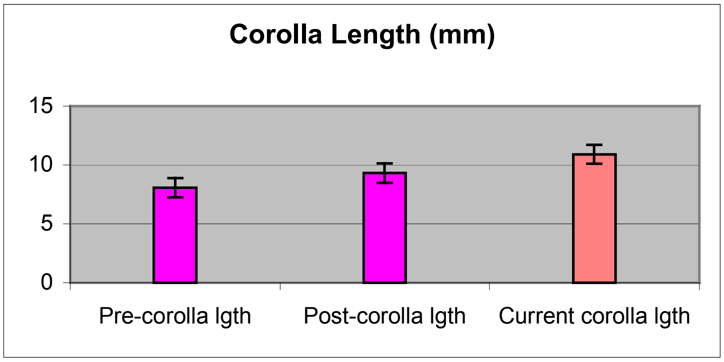
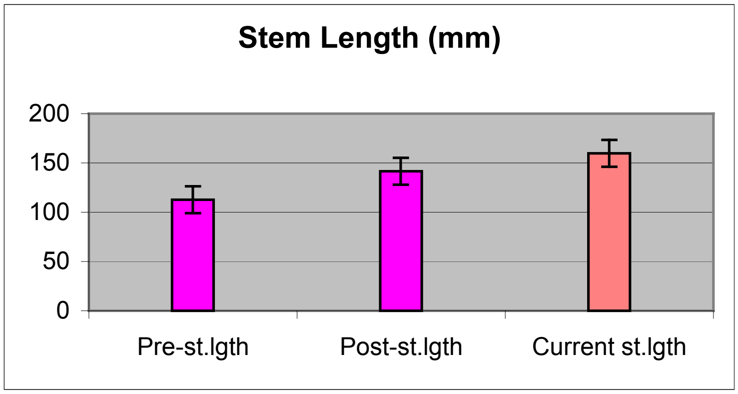


Figure 3 (continued).

A.



B.



C.

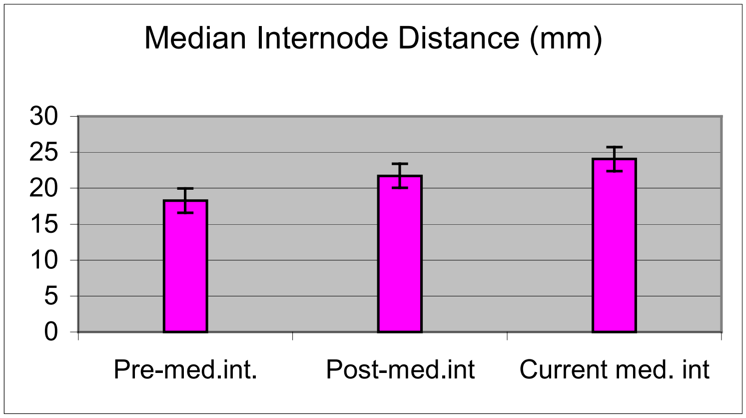


Figure 4 (Graphs A-C): Characters measured from *Houstonia montana* displaying a Type I period effect. These characters depict the predominant pattern of change: an increase in size accompanied by a decrease in variation across time. Different bar colors indicate significant differences between periods. Mean measurements are shown error bars indicating a 95% confidence interval with respect to the mean. Graph C displays a trend. The difference between any 2 time periods were not significant. Differences across all 3 periods were significant. The means changed in a consistent direction.

A.

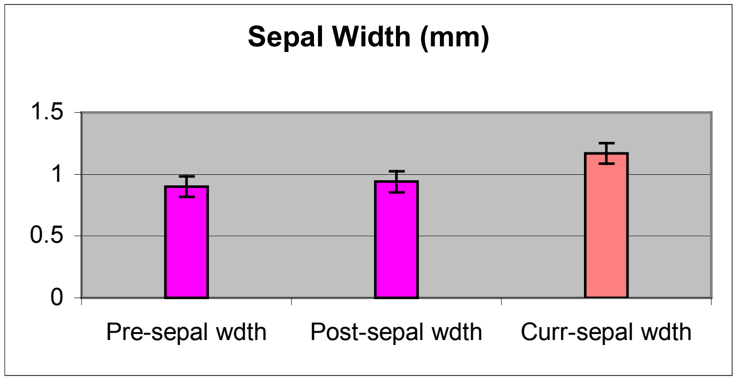


Figure 5 (Graph A): Character measured from *Houstonia montana* displaying a Type II period effect. This character depicts the predominant pattern of change between time periods: an increase in size accompanied by an increase in variation. Different bar colors indicate significant differences between periods. Mean measurements are shown with error bars indicating a 95% confidence interval with respect to the mean.

pattern of change is consistent with the Type I pattern of change in that the structure gets larger across time and also supports the first alternative hypothesis that statistically significant changes occur between periods. The observed increase in variance may be due to the larger size of the character itself or may be due to this character lacking the overall size increase observed in the mean.

There are 3 other types of results that are consistent with the overall trend of larger plants across time. The 1st type only occurs in 2 characters, the number of teeth found in 2 cm of a basal leaf and the number of teeth found in 1 cm of a “stem leaf” (or inflorescence bract), for *G. radiatum.* These characters display a reduction in number of teeth across time periods and reduced variance in mean tooth number. This pattern of change is consistent with the Type I pattern, since it indicates a shift towards larger teeth accompanied by reduced variation. The number of teeth in 2 cm of a basal leaf was also confounded with a collector effect. The type of change displayed in this character may have been affected by a collector bias, perhaps due to selection for larger leaves by certain collectors.

One character, maximum inflorescence length for *G. radiatum,* showed an increase in size with a fluctuation in variance. This character showed an increase in mean and a fluctuation in variance in which the variance fluctuates from initially increasing to decreasing across the 150-year time span. This type of change still confirms the general pattern of increase in size over time. The fluctuating pattern observed in the variance can be attributed to an interaction with a collector effect, which is significant for this character. The overall trend of the larger plants across time remains unaffected, but the narrowing of variation was obscured by a collector bias.

One character, petal width, displayed a decrease in size from pre-logging and post-logging periods, followed by an increase in current specimens. This type of change occurs in only 1 character, petal width for *G. radiatum.* In this case the petal width could be variably affected by petal length. In other words in some cases longer petals may grow more narrow. In this case this change was also accompanied by a significant collector effect. This result might indicate that some collectors may sample more carefully than others for the largest flowering specimens.

Collector Effects

Significant collector effects were observed in only 5 out of 24 characters, 3 from *G. radiatum* and 2 for *Houstonia montana* (Table 15). These observations argue against the apparently widespread expectation that individual collectors' behavior would be so variable and unpredictable that data taken from herbarium specimens would be unreliable. The large number of characters demonstrating statistically significant changes across time strongly refutes the dismissal of herbarium specimens in studies of temporal variation in morphology. For the majority, 19 of 24 characters analyzed there is no significant collector effect.

Even in the relatively few cases where collector effects occur, the overall trend of increase in size remains evident. Collector effects are clearly peripheral to the overall pattern of an increase in size over time, which is documented for 9 characters, and the broader pattern of larger sizes and numbers, which is documented for 17 of 24 characters showing significant change.

Table 15. Characters displaying a significant collector effect. Characters include vegetative and reproductive structures.

Geum radiatum

Vegetative structures: Maximum inflorescence length
Number of teeth in 2 cm of basal leaf

Reproductive structures: Petal width

Houstonia montana

Vegetative structures: Leaf length

Reproductive structures: Capsule width

Month Effects and Interactions

Significant month effects occurred in 7 of the 14 characters from *G. radiatum* and 3 of the 10 characters from *H. montana.* These characters are listed in Table 16. Month effects occurred in seasonal characters and show the progressive growth and development of various structures from spring to autumn. Seasonal changes are not responsible for period effects. There was usually not an interaction between period and month effects. Only 2 characters, 1 from each species, displayed an interaction between period and month, leaf width for *G. radiatum* and leaf length for *H. montana.* Only 1 character, petal width for *G. radiatum,* displayed an interaction between collector and month.

Drying Effects

Only 2 out of 7 characters (petal length and width) tested for a drying effect from *Geum radiatum* showed a significant drying effect. When drying petals became significantly smaller in size. None of the 3 characters tested for a drying effect from *Houstonia montana* showed a significant drying effect.

No Change

Four out of 14 characters measured from *Geum radiatum* and 2 out of 10 characters measured from *Houstonia montana* showed no significant change throughout the time periods studied. These characters are listed in Table 17.

Table 16. Characters displaying a month effect. Characters include both vegetative and reproductive structures.

Geum radiatum

Vegetative structures: Leaf width
Number of teeth in 2 cm of basal leaf
Number of teeth in 1 cm of stem leaf
Maximum inflorescence length
Internode distance on primary axis of inflorescence
Inflorescence width

Reproductive structures: Petal width

Houstonia montana

Vegetative structures: Leaf length
Leaf width

Reproductive structures: Number of blooms

Table 17. Characters showing no change in morphology across time. Characters include vegetative and reproductive structures.

Geum radiatum

Vegetative structures: Number of teeth in a 30° angle of a basal leaf
Number of teeth in a 20° angle of a stem leaf

Reproductive structures: Sepal length
Petal sinus

Houstonia montana

Reproductive structures: Sepal length
Capsule width

Chapter 4

DISCUSSION

Nonrandom morphological variation was detected among the 2 plant species, *G. radiatum* and *H. montana* across a time span of 150-years. Morphological variation has frequently been observed in species that have geographically isolated populations (Huang and Dane 1998, Boyd 2000, Menges and Dolan 1998). This study is the 1st to demonstrate within species morphological variation across time. Statistically significant shifts detected through this study provided evidence that morphological variation can occur across relatively brief temporal spans.

A series of statistical analyses were selected and conducted. In addition to testing for period effects these analyses tested for drying effects, collector effects, and interactions between collector and month effects and period and month effects. These analyses were chosen in order to clarify the primary focus of this study, which was to detect shifts in morphology across time, as well as to eliminate arguments and criticisms that shifts in morphology could be attributed to effects other than time. Collector effects, month effects, and interactions proved to be peripheral to the overall trend of an increase in size across time in the majority of characters measured.

The null hypothesis, which stated that no detectable morphological shifts have occurred within these plant species across a 150-year time span, was rejected for the majority of characters. In most cases floral and fruit characters were consistent with the null hypothesis and did not show shifts in morphology. The first alternative hypothesis--that detectable and statistically significant morphological shifts have occurred in the plants across a 150-year time span with a consistent pattern of change over time--was supported in many cases.

The most notable outcome was the finding of a distinct “Type I” pattern of change-an increase in size accompanied by a decrease in variation across time. The strongest results were found in *G. radiatum,* the larger of the 2 species. Five out of the 14 characters measured from *G. radiatum* displayed Type I shifts in morphology across time. These 5 characters were all vegetative structures. The floral structures measured from *G. radiatum* showed no significant change in morphology over the 150-year time span.

Three out of 10 characters measured from *H. montana* displayed Type I shifts in morphology. These 3 characters include 2 vegetative (stem length and median internode distance) and 1 reproductive structure (corolla length). It is important to mention that even though a change was observed in corolla length, a reproductive structure for *H. montana,* a drying effect was not tested for this character. Blooms were absent when *H. purpurea* was collected in the field in the fall of 2000. Because petal characters were the only characters to show drying effects in *G. radiatum* it is likely that corolla length for *H. montana* may also have shown a significant drying effect. The inability to test for a drying effect for corolla length in *H. montana* may have produced unreliable results for this character.

The most decisive results were characters that showed significant differences between all 3 time periods: pre-logging, post-logging, and current. Of particular importance were significant differences found between the first 2 time periods: pre-logging and post-logging. A key question addressed by this study was; can herbarium specimens be used as a reliable source for detecting population level processes across time? Measurements from pre-logging and post-logging periods were taken strictly from herbarium specimens. Because other possible effects, (drying, collector, month, and interactions) were ruled out in advance, statistically significant differences between pre-logging and post-logging time periods confirms that herbarium specimens are indeed a reliable source of material for detecting population level processes across time. Significant differences occurring between the current period and pre-logging and post-logging periods mainly served to confirm that these plants have undergone change and have continued to change across time. Characters that show changes between pre-logging and current periods only or between post-logging and current time periods only do not directly address the issue of herbarium specimens providing a reliable material basis for monitoring population level processes across time. However, these changes are still of importance because they also demonstrate statistically significant changes that may be ongoing.

Three out of 5 characters, from *G. radiatum* displaying a Type I shift in morphology, (width of leaf blade, length of leaf blade, and internode distance (on primary axis of inflorescence) showed that significant differences occurred between all three-time periods. Petiole length for *G. radiatum* showed differences between pre-logging and post-logging and between pre-logging and current periods, and inflorescence width showed differences between the pre-logging and current period and between the post-logging and current period. It is important to note that four out of five characters from *G. radiatum* displaying a Type I pattern of change showed that significant differences occurred between pre-logging and post-logging periods.

Two out of 3 characters, corolla length and stem length, from *H. montana* displaying Type I shifts in morphology showed significant differences between only pre-logging and current periods. One character, median internode distance displayed a trend, meaning that the differences detected between any two periods were not significant; significance occurred only when all 3 time periods were considered. Also, the means for internode distance steadily increased across time indicating that a trend had occurred in this character. The results from *H.*

montana were not as significant as those from *G. radiatum* in that no significant differences were detected between pre-logging and post-logging periods in characters displaying a period effect. These results while showing that changes in morphology have occurred do not address the issue of the usefulness of herbarium specimens in detecting population level processes across time.

In addition to the Type I pattern of change, an increase in mean accompanied by a decrease in variation, there were 4 other types of changes that occurred in several characters that also provided evidence that plant structures have grown larger across time. The 1st of these changes, a "Type II" change--an increase in mean and variance-- was found in only one character, sepal width, for *H. montana.* A 2nd type of change found in 2 characters, the number of teeth in 2 cm of a basal leaf and the number of teeth in 1 cm of a "stem leaf" (inflorescence bract) from *G. radiatum* showed a reduced number of teeth and reduced variance. The reduced number of teeth per unit length indicates larger tooth size, thus, reduced teeth per length of leaf margin represents an increase in size, as found in many other Type I character changes. A 3rd type of change detected in only 1 character, maximum inflorescence length from *G. radiatum*, showed an increase in mean accompanied by a fluctuation in variance. The 4th type of change also detected in only one character, petal width, showed a fluctuation in the mean accompanied by a decrease in variation. These different types of changes, although showing less robust results than the many Type I changes, all confirm the general pattern of an increase in size across time.

<u>Possible Biological Processes Contributing to Changes in Morphology</u>

There are several possible biological processes that may have contributed to the observed changes in morphology. For example, a warmer climate (Crawford and Abbott 1994, Chaloner and McElwain 1997, Arft et al. 1999), exposure to more sunlight (Wiser et al. 1998), relief from the impacts of grazing (Bock et al. 1995), increases in soil fertility (Wiser 1998), and other anthropogenic influences may have contributed to the general trend of an increase in size over time observed in many characters. Changes in morphology have been reported from experimental studies of plants subjected to altered growing conditions, such as elevated CO_2 levels (Fischer et al. 1997, Pregitzer et al. 2000, Tischler et al. 2000) ultraviolet radiation (Visser et al. 1997), or soil nutrients (Wiser et al. 1998). Whatever the underlying environmental factors, the changes in morphology observed in *G. radiatum* and *H. montana* could have resulted from phenetic changes in expressions or accumulated genetic shifts, or both.

Most of the characters that demonstrated significant changes were from vegetative structures. This suggests that phenetic, rather than genetic processes may be responsible for the observed morphological shifts. Reproductive structures are assumed to be under more stringent genetic control of their size morphology compared to vegetative structures due to their critical reproductive function. Therefore, vegetative structures may have a much greater capacity to change in response to environmental changes. Taxonomic distinctions are primarily based on reproductive traits rather than solely on vegetative traits for this reason. Because changes in morphology occurred primarily in vegetative characters, this may suggest that changes in morphology are due to simple growth factors such as sunlight, nutrients, water, and temperature and are, therefore, more likely the result of phenetic rather than genetic changes.

Genetic changes within the plant populations may also cause morphological changes. Many rare and endangered plant species, including *G. radiatum* and *H. montana* have recently experienced declines in population size and number (Godt et al. 1996). The maintenance of genetic diversity has often been associated with population size. Population genetic theory predicts the loss of genetic diversity in populations that remain small for generations, in populations initiated from a small number of colonies, and in populations that suffer rapid declines in size (Barrett and Kohn 1991). All 3 of these predictions apply to *G. radiatum* and *H. montana* their ranges are limited, their populations are restricted and isolated, and they have recently undergone declines in populations. The predominant change observed in the majority of characters was a Type I pattern of change in which an increase in mean was accompanied by a decrease in variation. These results suggest that as population size and numbers have decreased over time remaining plants have increased in size, but the reduction in variance for size measurements indicates they may have experienced a loss in genetic diversity.

The results of this study raise, but do not resolve, 2 fundamental questions: (1) are observed morphological shifts due to phenotypic and/or genotypic changes in the populations and (2) what environmental and historical factors have produced these changes in morphology? If variation is due in part to genetics than a change in morphological variation would be expected. These questions address the broader issues raised by, but not tested in this study, which future studies could investigate in detail.

Common garden experiments could be used to assess the degree of variation due to growth conditions. This could be accomplished by growing the same plants or seeds or cuttings from the same plants under different treatments such as amount of sunlight, soil nutrients, "grazing" regimes, and other growth influencing factors. The null hypothesis for these common garden experiments would state that variation is phenetic, due to simple growth factors, which would be supported by treatments producing a high degree of variation among all plants. The alternative hypothesis would state that the differences detected in morphology are due to genetic factors, and that genetically different individuals would show differences regardless of growing conditions. The results obtained from common garden experiments would reveal whether observed morphological changes were the result of different growth conditions, and thus phenetic rather than genetic.

Genetic experiments could be used to test the hypothesis that there have been genetic changes associated with the morphological changes observed in the results. Genetic markers to be used would be DNA characters such as sequence data or DNA microsatellite patterns. The null hypothesis would state that there are no significant differences among the morphologically different groups or between the different time periods: pre-logging, post-logging, and current. Finding significant differences in the distribution of genetic markers across the time groups would support the alternative hypothesis. There could be a change in variation and/or a shift in the genetic types present in each period. Genetic data could be collected from herbarium sheets and from field collected leaves. Micro-preps, which require very little material, could be used to collect samples, followed by PCR, and last sequencing or microsatellite analyses. The genetic experiments could also be combined with common garden experiments to confirm that there are no genetic differences among the treatment groups.

Final suggestions for future research would be to continue the study but with a more widespread species growing in the same area, such as *Potentilla tridentata* or with a woody plant species or weeds. A disadvantage to using a woody species would be that the whole plant would not be present on herbarium sheets, which would lead to many characters being unaccounted for in historical periods. By continuing the study using a widespread species or a species of a different habit you would be able to determine if the same types of shifts have occurred within more stable populations or if the detected shifts in morphology are unique to species that have experienced reductions in population size and number. The types of shifts detected using widespread or woody plant species would give further insight into the relationship between morphological and genetic diversity.

In this study I demonstrated that statistically significant changes in morphology have occurred over a brief, 150-year span, in plants from a single Southern Appalachian locality. This study is unique in that it demonstrated the use of herbarium specimens to document changes in morphology thus confirming that herbarium specimens provide an untapped resource for examining and monitoring within population diversity. It also demonstrated that herbarium resources provide a valuable tool for the assessment and management of rare and endangered plant species.

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APPENDIX A

GEUM RADIATUM SPECIMENS USED

Collector	Collector #	Date	Location	Herbarium #	Herbarium
Alexander	s.n.	23-Jun-1939	Roan Mtn	32742	NY
Ashe, W.W.	s.n.	July 17 1895	Roan Mtn	32780	NY
Ball	s.n.	Sept 15,1884	Roan Mtn,NC	587447	US
Barrell	s.n.	June, 1953	Top of Roan	985995	JEPS
Bartley	2261	3-Aug-1956	Roan Mtn	587453	US
Blomquist	3831	16-Jul-1932	Top of Roan	3831	DUKE
Boufford, Wood	17721	25-Jul-1975	Roan Mtn	203108	CM
Britton	s.n.	Sept 9 1883	Roan Mtn	32760	NY
Britton	s.n.	Sept 9,1885	Roan Mtn, NC	587464	US
Brown, D.M.	70	21-Jun-1934	Roan Mtn	40034	DUKE
Brown, D.M.	1057	27-Aug-1937	Top of Roan	108703	ARIZ
Brown, D.M.	1057	27-Aug-1937	Top of Roan	717686	JEPS
Brown, D.M.	1057	27-Aug-1937	Top of Roan	67430	DUKE
Brown, D.M.	1057	27-Aug-1937	Top of Roan	71814	FLAS
Brown, D.M.	1057	27-Aug-1937	Top of Roan	151220	IA
Brown, D.M.	1057	27-Aug-1937	Top of Roan	489660	MSC
Brown, D.M.	1057	27-Aug-1937	Top of Roan		MICH
Brown, D.M.	1057	27-Aug-1937	Top of Roan	4636	NCSC
Brown, D.M.	1057	27-Aug-1937	Top of Roan	32801	NY
Brown, D.M.	1057	27-Aug-1937	Top of Roan	75982	OK
Brown, D.M.	1057	27-Aug-1937	Top of Roan	198758	TEX
Brown, D.M.	1057	27-Aug-1937	Top of Roan	68277	TEX
Brown, D.M.	1057	27-Aug-1937	Top of Roan	93783	WVA
Brown, D.M.	1057	27-Aug-1937	Roan Mtn		BRIT
Brown, D.M.	1057	27-Aug-1937	Roan Mtn	277548	CAS
Brown, D.M.	1057	27-Aug-1937	Roan Mtn	284486	CAS
Brown, D.M.	1057	27-Aug-1937	Summit Roan	587471	US
Brown,D.M.	1057	27-Aug-1937	Top of Roan	21631	GA
Canby, WM.M.	s.n.	August 1871	Roan Mtn	587467	US
Canby, WM.M.	s.n.	July 1878	Roan Mtn	32782	NY
Canby, WM.M.	s.n.	Jun 1888	Roan Mtn	32749	NY
Canby, WM.M.	s.n.	June 1868	Roan Mtn	122203	CAS
Canby, WM.M.	s.n.	June 1879	Top of Roan		VT
Canby, WM.M.	s.n.	June 1888	Roan Mtn	32767	NY
Canby, WM.M.	s.n.	Sept. 1876	Roan Mtn	32766	NY
Canby, WM.M.	s.n.	Sept.1876	Summit Roan	587468	US
Cannon, W.A.	20	27-Jun-1902	Roan Mtn		NY
Cannon, W.A.	s.n.	27-Jun-1902	Roan High	587451	US
Cannon, W.A.	20	27-Jun-1966	Roan Mtn	43033	MSC
Chas. Mohr	s.n.	July 1894	Roan Mtn	587449	US
Chickering	s.n.	July 5 1880	Roan Mtn	32789	NY
Chickering	s.n.	July 5, 1880	Roan Mtn	24106	GA
Chickering	s.n.	July 5, 1880	Roan Mtn	113661	DUKE
Chickering	s.n.	July 5, 1880	Roan Mtn,NC	587461	US
Chickering	s.n.	July 5, 1880	Roan Mtn,NC	587460	US

Chickering	s.n.	July 5, 1880	Roan Mtn,NC	587462	US
Chickering	s.n.	July 5, 1880	Roan Mtn,NC		VT
Chickering	s.n.	Sept 12 1877	Roan Mtn	32751	NY
Chickering	s.n.	Sept 12 1877	Roan Mtn		VT
Chickering	s.n.	Sept 12 1877	Roan Mtn,NC	587459	US
Churchill, J.	84145	4-Jul-1970	Top of Roan	230602	MSC
Churchill, J.	s.n.	4-Jul-1970	Roan Mtn	289964	MSC
Churchill, J.	s.n.	4-Jul-1970	Roan Mtn		BRIT
Churchill, J.	94149	6-Jul-1994	Roan Mtn	907597	CAS
Clausen R.T.	5597	6-Sep-1941	Roan Mtn	32790	NY
Clausen, Trapido	3703	23-Sep-1938	Top of Roan	32800	NY
Clausen, Trapido	3703	23-Sep-1938	Top of Roan	860098	JEPS
E.G.B.	s.n.	Sept. 9 1883	Roan Mtn	32774	NY
Edston	s.n.	1893	Roan Mtn	587450	US
Fairchild, Hernandez, Clebsch, Sharp	11713	22-Jul-1948	Roan Mtn	80551	GA
Gibbes, L.R.	s.n.	1898	Roan Mtn	32771	NY
Gray, A, Carey,J	s.n.	July 1841	Roan Mtn	68794	GA
Gray, A, Carey,J	s.n.	July 1841	Roan Mtn	32745	NY
Gray, A, Carey,J	s.n.	July 1841	Roan Mtn	32792	NY
Gray, A.	s.n.	July 1841	Roan Mtn	32762	NY
Gray, Sargent, Redfield, Canby	s.n.	June 17 1879	Top of Roan	32747	NY
Heller, A.A.	s.n.	Aug 13, 1890	Roan Mtn	12281	JEPS
Heller, A.A.	s.n.	Aug 13, 1890	Roan Mtn	32764	NY
Heller, A.A.	43	Aug 13, 1890	Roan Mtn		VT
Henry, L.K.	s.n.	23-Jun-1966	Roan Mtn	456106	CM
Hill, C.O.	1795	30-Jun-1934	Roan Mtn	32743	NY
Hill, Myrick, Saunders	628	3-Aug-1980	Roan Mtn	32758	NY
Hunnewell	12837	25-Jul-1925	Top of Roan	32754	NY
Hyams, M.E.	s.n.	June 1878	Roan Mtn, NC	587462	US
Hyams, M.E.	s.n.	June 1883	Roan Mtn	32772	NY
Hyams, M.E.	s.n.	June 1888	Roan Mtn	42007	MSC
J.T.P	2323	7-Jul-1925	Roan Mtn	KAN00237415	KANU
Jennison	s.n.	22-Aug-1937	Top of Roan	860097	JEPS
Jouy, PL	479	July 22, 1890	Roan Mtn, NC	587466	US
Kral, R	60793	3-Aug-1977	Roan Mt		BRIT
Kral, R	64227	7-Aug-1979	Roan Mt		BRIT
Leonard, Radford, Moore	1815	7-Jul-1968	Top of Roan	15793	AUA
Leonard, Radford, Moore	1815	25-Jul-1968	Top of Roan	272551	TEX
Leonard, Radford, Moore	1815	25-Jul-1968	Top of Roan	93784	WVA
Leonard, Radford, Moore	1815	25-Jul-1968	Roan Mtn		MISS
Leonard, Radford, Moore	1815	25-Jul-1968	Top of Roan	UNA00015055	UNA
Leonard, Radford, Moore	1815	25-Jul-1968	Roan Mtn	173621	ARIZ
Mark, A.F.	s.n.	7-Feb-1957	Roan High	139758	DUKE
Meehan, Porter, Leidy, Willcox	s.n.	July 1880	Roan Mtn	32748	NY
Merriam, C.H.	s.n.	Aug 8,1892	Roan Mtn,NC	587444	US
Merriam, C.H.	s.n.	Aug.30 1892	Roan Mtn, NC	587443	US
Merriam, C.H.	s.n.	Aug 30, 1892	Roan Mtn	32763	NY
Merriam, C.H.	s.n.	Aug 8,1892	Roan Mtn	587444	US
Oosting, H.J.	3614	15-Jun-1936	Roan Mtn	35585	DUKE

Pyron, J.H.	s.n.	15-Jun-1936	Roan Mtn	11944	GA
Radford, A.E.	45003	17-Jul-1966	Roan Mtn		BRIT
Ramseur G.	1352	6-Aug-1956	Roan Mtn	63788	GA
Ramseur G.	1217	6-Aug-1956	Roan Mtn	63745	GA
Ramseur G.	1155	7-Aug-1956	Roan Mtn	62061	FSU
Rydberg, P.A.	8257	30-Aug-1908	Roan Mtn	32778	NY
Rydberg, P.A.	8257	30-Aug-1908	Roan Mtn	32779	NY
Rydberg, P.A.	9304	7-Jul-1925	Roan Mtn	280758	JEPS
Rydberg, P.A.	9304	7-Jul-1925	Roan Mtn	32773	NY
Rydberg, P.A.	4304	7-Jul-1925	Roan Mtn	587445	US
Sargent, F.H.	s.n.	28-Jun-1954	Roan Mtn	75981	OK
Sargent, F.H.	s.n.	28-Jun-1954	Roan Mtn	KAN00237416	KANU
Sargent, F.H.	6841	28-Jun-1954	Roan Mtn		BRIT
Sargent, F.H.	6841	28-Jun-1954	Roan Mtn.	392979	CAS
Sargent, F.H.	s.n.	28-Jul-1954	Roan Mtn	93785	WVA
Shanks, R.E.	3120	5-Jul-1947	Roan Mtn	587472	US
Small, Heller	s.n.	July 16, 1891	Roan Mtn	32759	NY
Small, Heller	s.n.	July 16, 1891	Roan Mtn		VT
Small, Heller	43	July 16, 1891	Roan Mtn,NC	587465	US
Small, Heller	s.n.	July 16, 1891	Top of Roan	42006	MSC
Small, Heller	s.n.	July 16, 1891	Top of Roan	50693	ARIZ
Small, Heller	s.n.	July 16, 1891	Top of Roan	249762	DUKE
Smith, J.D.	s.n.	July 13, 1880	Summit Roan	587442	US
Smith, J.D.	s.n.	July 15, 1880	Roan Mtn	456104	CM
Smith, J.D.	835	Sept 11,1884	Summit Roan	587441	US
Stubbs, A.A.	s.n.	Aug 1884	Roan Mtn	151219	IA
Thaxler, Roland	s.n.	Aug.18, 1887	Summit Roan	587446	US
Unknown	3942	14-Jul-1903	Summit Roan	587470	US
Unknown	3942	17-Sep-1904	Roan Mtn,TN	587469	US
Unknown	s.n.	?1894	Roan Mtn	587448	US
Unknown	s.n.	August 1871	Roan Mtn,NC	587467	US
Unknown	s.n.	June 1868	Roan Mtn	32767	NY
Unknown	s.n.	?1876	Roan Mtn	32776	NY
Vasey, G.R.	s.n.	20-Feb-1905	Roan Mtn	32756	NY
Vasey, G.R.	s.n.	?1878	Roan Mtn		VT
Vasey, G.R.	s.n.	?1878	Roan Mtn	32757	NY

APPENDIX B

HOUSTONIA MONTANA SPECIMENS USED

Collector	Collector #	Date	Location	Herbarium	Herbarium #
Anderson	s.n.	9-Jul-1964	Roan Mtn	MICH	
Anderson,Lewis	s.n.	9-Jul-1964	Roan Mtn	DUKE	168164
Ball, John	s.n.	Sept 15,1884	Roan Mtn	IA	151221
Ball, John	s.n.	Sept 15,1884	Roan Mtn	IA	151222
Blomquist	4961	16-Jul-1932	Roan Mtn	DUKE	18765
Wofford	79-197	16-Jul-1979	Roan Mtn	NY	
Chickering, J.D.	s.n.	July 5, 1880	Roan Mtn	CM	160633
Churchill, J.	94040	6-Jul-1994	Roan Mtn	CAS	909441
Churchill, J.	94040	6-Jul-1940	Roan Mtn	MSC	346299
Churchill, J.	680114	5-Jul-1968	Roan Mtn	MSC	290206
Fairchild, Hernanadez, Clebsch, Sharp	11709	22-Jul-1948	Roan Mtn	GA	80686
Gray, Sargent, Redfield, Canby	2116	June 17,1879	Top of Roan	CM	160634
Gray, Sargent, Redfield, Canby	s.n.	June 1879	Top of Roan	VT	
Heller, A.A.	s.n.	Aug 13, 1890	Roan Mtn	JEPS	28406
Hermann, F.J.	15207	11-Jul-1959	Roan Mtn	NY	
Kral, R	60740	3-Aug-1977	Roan Mtn	BRIT	
Kral, R	64226	7-Aug-1979	Roan Mtn	BRIT	
Meehan, Porter, Leidy, Willcox	s.n.	July 1880	Roan Mtn	CM	160632
Oosting, H.J.	4961	16-Jul-1932	Top of Roan	DUKE	18765
Rydberg, P.A.	9306	7-Jul-1925	Roan Mtn	JEPS	280724
Rydberg, P.A.	9306	7-Jul-1925	Roan Mtn	CAS	138711
Sargent, F.H.	6860	28-Jun-1954	Roan Mtn	GA	64062
Sargent, F.H.	6860	28-Jun-1954	Roan Mtn	KANU	KAN00239955
Sargent, F.H.	6860	28-Jul-1954	Roan Mtn	WVA	
Sargent, F.H.	6860	28-Jun-1954	Roan MT, NC	BRIT	
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	GA	64049
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	MSC	180275
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	CM	160631
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	DUKE	159775
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	FLAS	68229
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	TEX	
Shanks, R.E.	3008	16-Jun-1946	Roan Mtn	WVA	
Shanks, R.E.	3008	16-Jul-1946	Roan Mtn	CAS	593831
Shaver, Jesse	8786	3-Aug-1940	Roan Mtn	BRIT	
Shaver, Jesse	8786	3-Aug-1940	Roan Mtn	BRIT	
Shaver, Jesse	8761	3-Aug-1940	Roan Mtn	BRIT	
Shaver, Jesse	8761	3-Aug-1940	Roan Mtn	BRIT	
Shaver, Jesse	8761	3-Aug-1940	Roan Mtn	BRIT	
Shaver, Jesse	8761	3-Jul-1940	Roan Mtn	BRIT	
Somers	1812	3-Jul-1979	Roan Mtn	BRIT	
Stewart, Laurie	1526	2-Jul-1940	Top of Roan	TEX	138517
Stewart, Laurie	1526	2-Jul-1940	Roan Mt	BRIT	
Stewart, Laurie	1526	2-Jul-1940	Roan Mtn, TN	JEPS	889257
Vasey, G.R.	219	July 1878	Roan Mtn	NY	

Vasey, G.R.	s.n.	1878	Roan Mtn	VT

APPENDIX C

GEUM RADIATUM SPECIMENS EXAMINED

Collector	Collector #	Date	Location	Herbarium #	Herbarium
Alexander	s.n.	22-Jun-1939	Grandfather	32802	NY
Ashe, W.W.	s.n.		Roan Mtn	32777	NY
Buckley, S.B.	s.n.		Roan Mtn	32741	NY
Buckley, S.B.	s.n.		Roan Mtn	32740	NY
Curtiss, A.H.	s.n.	July	Roan Mtn	456105	CM
Curtiss, A.H.	s.n.	July	Roan Mtn	37420	GA
Curtiss, A.H.	s.n.	July	Roan Mtn	KAN00237417	KANU
Curtiss, A.H.	s.n.	July	Roan Mtn		MICH
Curtiss, A.H.	s.n.	July	Roan Mtn		MICH
Curtiss, A.H.	s.n.	July	Roan Mtn	32750	NY
Curtiss, A.H.	s.n.	July	Roan Mtn	32744	NY
Curtiss, A.H.	s.n.	July	Roan Mtn		VT
Curtiss, A.H.	s.n.	July	Roan Mtn	32765	NY
Harshberger, JW	94	summer	Grandfather	32781	NY
Huger, A.M.	s.n.	July 1892	Ashe Co., NC	32761	NY
Hyams, M.E.	s.n.	June 1879	Statesville	32752	NY
Hyams, M.E.	s.n.		Roan Mtn		JEPS
Hyams, M.E.	s.n.		Roan Mtn		MICH
Hyams, M.E.	s.n.	1916	Roan Mtn	121075	CAS
Mark, A.F.	s.n.	5-Aug-1956	Bald mtn	140099	DUKE
Miss Andrews	s.n.		Roan Mtn	587452	US
P.O.S.	7313	16-Jun-1923	Linville Falls	7812	DUKE
Radford, A.E.	44913	7-Jul-1966	Bluff Mtn	310368	JEPS
Radford, A.E.	44913	7-Jul-1966	Bluff Mtn	32784	NY
Unknown	s.n.		Roan Mtn	32753	NY
Unknown	s.n.		Roan Mtn	32746	NY
Unknown	s.n.		Roan Mtn	32770	NY

APPENDIX D

HOUSTONIA MONTANA SPECIMENS EXAMINED

Collector	Collector #	Date	Location	Herbarium	Herbarium #
Churchill, J.	s.n.	3-Jul-1970	Grandfather	BRIT	
Heller, A.A.	s.n.	Aug 11, 1890	Grandfather	JEPS	28408
Henry, L.K.	s.n.	6-Jun-1966	Grandfather	CM	160598
Mark, A.F.	s.n.	6-Aug-1956	Bald Mtn	DUKE	139764
Welch, Winona	s.n.		Grandfather	NY	19479
Unknown	3698	23-Jun-1935	Roan Mtn	GA	80698
Unknown	s.n.	1844	Roan Mtn	CAS	456535

APPENDIX E

GEUM CANADENSE VOUCHER SPECIMENS

Collector	Collector #	Location	Date
Medford, D	1	Unicoi Co.	September, 2000
Medford, D	2	Unicoi Co.	September, 2000
Medford, D	3	Unicoi Co.	September, 2000
Medford, D	4	Unicoi Co.	September, 2000
Medford, D	5	Unicoi Co.	September, 2000
Medford, D	6	Unicoi Co.	September, 2000
Medford, D	7	Unicoi Co.	September, 2000
Medford, D	8	Unicoi Co.	September, 2000
Medford, D	9	Unicoi Co.	September, 2000
Medford, D	10	Unicoi Co.	September, 2000
Medford, D	11	Unicoi Co.	September, 2000
Medford, D	12	Unicoi Co.	September, 2000
Medford, D	13	Unicoi Co.	September, 2000
Medford, D	14	Unicoi Co.	September, 2000
Medford, D	15	Unicoi Co.	September, 2000
Medford, D	16	Unicoi Co.	September, 2000
Medford, D	17	Unicoi Co.	September, 2000

APPENDIX F

HOUSTONIA PURPUREA VOUCHER SPECIMENS

Collector	Collector #	Location	Date
Medford, D	1	Mitchell Co.	September, 2000
Medford, D	2	Mitchell Co.	September, 2000
Medford, D	3	Mitchell Co.	September, 2000
Medford, D	4	Mitchell Co.	September, 2000
Medford, D	5	Mitchell Co.	September, 2000
Medford, D	6	Mitchell Co.	September, 2000
Medford, D	7	Mitchell Co.	September, 2000
Medford, D	8	Mitchell Co.	September, 2000
Medford, D	9	Mitchell Co.	September, 2000
Medford, D	10	Mitchell Co.	September, 2000
Medford, D	11	Mitchell Co.	September, 2000
Medford, D	12	Mitchell Co.	September, 2000
Medford, D	13	Mitchell Co.	September, 2000
Medford, D	14	Mitchell Co.	September, 2000
Medford, D	15	Mitchell Co.	September, 2000
Medford, D	16	Mitchell Co.	September, 2000

VITA

DALENIA S. MEDFORD

Personal Data:	Date of Birth: July 1, 1976 Place of Birth: Spruce Pine, North Carolina Marital Status: Married
Education:	Public Schools, Spruce Pine and Marion, North Carolina University of North Carolina at Asheville, North Carolina Biology, B.A., 1998 East Tennessee State University, Johnson City, Tennessee; Biology, M.S., 2001
Professional Experience:	Graduate Assistant, East Tennessee State University, College of Arts and Sciences, 1999 – 2000
Honors and Awards:	The National Dean's List Departmental Distinction, Department of Biology, University of North Carolina at Asheville.