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Distribution of chemistry and sexual fecundity in the lichenized-fungi, Xanthoparmelia cumberlandia and Xanthoparmelia coloradoensis on Boulder Mountain, Aquarius Plateau, UT

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DISTRIBUTION OF CHEMISTRY AND SEXUAL FECUNDITY IN THE LICHENIZED-FUNGI, *XANTHOPARMELIA CUMBERLANDIA* AND *XANTHOPARMELIA COLORADOËNSIS*, ON BOULDER MOUNTAIN, AQUARIUS PLATEAU, UT

by

Heather Bird Jackson

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GRADUATE COMMITTEE APPROVAL

of dissertation submitted by

Heather Bird Jackson

This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the thesis of Heather Bird Jackson in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

DISTRIBUTION OF CHEMISTRY AND SEXUAL FECUNDITY IN THE LICHENIZED-FUNGI, *XANTHOPARMELIA CUMBERLANDIA* AND *XANTHOPARMELIA COLORADOËNSIS*, ON BOULDER MOUNTAIN, AQUARIUS PLATEAU, UT

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Three aspects of *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis* populations found at two elevations are explored: clustering of secondary chemicals and the resulting implications for taxonomic distinctions, the usefulness of thallus size as an indirect measure of sexual fecundity, and the frequency of sexual reproduction.

First, we use clustering of 46 chemicals produced by *X. cumberlandia* and *X. coloradoënsis* to evaluate the adequacy of the current taxonomic distinction between

them. Using principal components analysis and UPGMA, we find that the currently recognized species boundaries indicated by the presence of stictic acid in *X. cumberlandia* and salazinic acid in *X. coloradoënsis* are supported by distinct differences in their chemotypes (combinations of secondary chemicals). Norstictic acid, which the literature also associates with *X. cumberlandia,* is found frequently in both *X. cumberlandia* and *X. coloradoënsis,* and is not a good distinguishing characteristic. No chemical difference between sexually fecund and sterile individuals was found.

Second, we test the claim that thallus size can be used as an indirect measure of sexual fecundity. By comparing the number of apothecia, the total area of the apothecia, and the presence or absence of apothecia with thallus area, we found positive correlations between these measures of sexual fecundity and thallus size which are statistically significant. However, the total variation explained by these predictors is limited, and is significantly affected by elevation and micro-environmental features such as proximity to trees. We conclude that size is not a reliable synonym for sexual fecundity in *X. cumberlandia* and *X. coloradoënsis.*

Third, we make inferences concerning the frequency of sexual reproduction based on the frequency of sexual structures, rare chemicals, and unique chemotypes. We predicted that sexual reproduction would be more frequent at lower elevations, consistent with a common pattern found in plants and animals. The frequency of sexual structures indicates that sexual reproduction is more common at the lower elevation, while frequency of rare chemicals and chemotypes implies that outcrossing is more common at the upper elevation. Since these indicators lead to opposing

conclusions, we encourage the use of molecular markers to estimate the frequency of outcrossing directly.

ACKNOWLEDGEMENTS

My gratitude extends to Loreen Woolstenhulme and Leigh Johnson who as my committee members provided helpful recommendations for this project. I appreciate the lab expertise of Katy Knight, and the TLC work of Peter Ririe. Besides providing useful ideas and moral support throughout my thesis work, Nathan Jackson spent days helping to collect and organize samples. Thanks to Dennis Eggett for hours of statistical help. Special thanks to Larry St. Clair for his mentoring, advice and support.

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Can one chemical replacement distinguish between species of lichenized-fungi?

a comparison of a few *Xanthoparmelia* species

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Abstract. Boundaries between species of lichenized-fungi are sometimes defined by a replacement of one secondary chemical. We test to see if one chemical difference is consistent with differences in the full array of secondary chemicals produced by lichen populations. Specifically, we look at differences between putative species Xanthoparmelia cumberlandia *and* Xanthoparmelia coloradoënsis, *whose only official difference is the replacement of norstictic and stictic acids with salazinic acid in* X. coloradoënsis. *The chemical differences, if any, between these sexual species and their asexual morphs,* Xanthoparmelia plittii *and* Xanthoparmelia mexicana, *respectively, are also considered. PCA and UPGMA are used to identify chemically similar individuals. The replacement of salazinic acid with stictic is indeed associated with distinct combinations of secondary chemicals. Norstictic acid, however, was found in almost all individuals, and is not useful in distinguishing between species. No chemical difference was found between sexual, sterile, and asexual individuals.*

Few field lichenologists will be surprised to learn that a lichenized-fungus is difficult to identify. Slight morphological or chemical differences can denote a completely different species. Some phylogenetic species have no known morphological differences (Kroken and Taylor 2001), while some morphologically identified species have no significant phylogenetic differences (Ott et al. 2004).

Xanthoparmelia, a genus of lichenized-fungi, is no exception. On Boulder Mountain, we have found it particularly difficult to distinguish the species *Xanthoparmelia cumberlandia, Xanthoparmelia coloradoënsis, Xanthoparmelia plittii,* and *Xanthoparmelia mexicana* from each other. According to Hale's monograph of the *Xanthoparmelia* genus (1990), *X. cumberlandia* and *X. coloradoënsis* are distinguished from each other by the presence of norstictic and stictic acids (*X. cumberlandia*) or salazinic acid (*X. coloradoënsis*). Otherwise, these species are quite similar. The range of their morphological traits, such as spore size, lobe width, and rhizine length greatly overlap (Hale 1990). We know of no research which actually quantifies how significant morphological differences between them actually are. Neither does geography provide a way to distinguish between *X. cumberlandia* and *X. coloradoënsis* because they are often sympatric (Brodo et al. 2001; Hale 1990).

Both species have asexual sister taxa, *X. plittii* and *X. mexicana*, respectively, from which they are often difficult to tell apart. Sexual morphs (*X. cumberlandia* and *X. coloradoënsis*) are identified by the presence of apothecia, while the asexual morphs have isidia (asexual propagules). Frequently however, neither sexual or asexual reproductive structures are developed, as was the case with 79% of the samples found on Boulder Mountain, Aquarius Plateau, UT (Jackson 2004). Without reproductive structures, the sexual and asexual morphs cannot be distinguished.

In short, the only sure distinction recorded in the literature is the presence of salazinic acid in *X. coloradoënsis* and *X. mexicana* and the presence of norstictic and stictic acids in *X.*

cumberlandia and *X. plittii*. We question how well these putative boundaries are supported by the full array of chemicals produced by these lichens.

To assume that the replacement of one or two secondary chemicals alone can indicate a species boundary is to assume that the production of these chemicals is genetically determined. This is an old, though not uncontested, assumption in lichenology (Culberson 1986; Egan 1986). A recent molecular study showed general congruence between chemical and genetic variation in *Sticta* lichens (McDonald et al. 2003). Miadlikowska and Lutzoni (2000), found that chemicals increased statistical confidence in phylogenetic trees based on DNA, but alone, chemicals were somewhat uninformative. These studies are suggestive of a coarse-grained connection between chemistry and genetics. Research of greater breadth and depth among the 13,500+ species of lichenized-fungi must be conducted before the real value of chemicals in distinguishing among genetically distinct taxa can be established.

In the absence of molecular data, Hawksworth (1976) developed a protocol to make the process of species delineation based on chemistry more consistent. He described four inferences that could be made from chemical differences: 1) if a chemical replacement is correlated with a morphological and/or broad geographical difference, a species boundary can be inferred, 2) populations with chemical differences correlated with local geographical tendencies or microhabitat changes should be considered chemical varieties, 3) when no correlation with morphology or geography can be found, an inference of no genetic difference should be made, 4) changes in concentration of chemicals (as opposed to changes in the identity of chemicals) are not sufficient evidence of a genetic boundary.

We feel that the case for a genetic boundary can be made even stronger if it can be shown that the replacement of one chemical is accompanied by other chemical changes. The purpose of this study is to provide a glimpse into how well one chemical replacement can distinguish *X. cumberlandia* and *X. coloradoënsis*. We use all of the secondary chemicals identified using one dimensional thin-layered chromatography to answer this question. If many chemicals

consistently differ between stictic and salazinic morphs, the argument for a real genetic boundary is much stronger.

Not only do we look to see if there is a consistent whole-chemical difference between *X. cumberlandia/X. plittii* and *X. coloradoënsis/X. mexicana*, but we also consider the possibility that a whole-chemical approach may show some distinction between the sexual and asexual/sterile morphs. While we gathered only a few isidiate individuals, we did gather many sterile individuals. Also considered are the associations between chemotype and microhabitat characteristics, such as elevation, distance and direction from protection, and aspect.

Our study is limited to those individuals found at 2300-2400 m and 3300-3400 m on Boulder Mountain, Aquarius Plateau, UT, and therefore is not designed to answer the broader question of species boundaries, which would require sampling throughout the range of these organisms. Rather, we ask the more basic questions: 1) Given a population of these *Xanthoparmelia* taxa, how effectively does chemistry distinguish between them? 2) Is it reasonable to consider them genetically isolated? 3) Are these chemical distinctions correlated to reproductive mode and/or microhabitat characteristics?

MATERIALS AND METHODS

Species.—Lichens are symbiotic systems involving fungi, algae, and/or cyanobacteria. Members of the family Parmeliaceae, *X. cumberlandia* and *X. plittii* produce usnic, norstictic, stictic, and sometimes constictic acids, while *X. coloradoënsis* and *X. mexicana* produce usnic, salazinic, and sometimes consalazinic acids (Brodo et al. 2001; Hale 1990).

Sampling Strategy.—Individuals were sampled randomly at 2400-2500 m and 3300-3400 m on Boulder Mountain, Aquarius Plateau, UT. Lower elevation sites were limited in access, thus our collections were confined to the east and the west sides of the mountain.

Abiotic conditions.—The lower elevation is generally less moist and warmer than the upper, with the lower-west side receiving less rain and more heat than the lower-east (US Department of Agriculture et al. 1999; Western Regional Climate Center: www.wrcc.dri.edu).

Identification of secondary chemicals.— One dimensional thin-layer chromatography was performed using solvent G (Culberson et al. 1981). A non-apotheciate, dime-sized piece of each thallus was placed in a 1.5 dram vial 1/3 full of acetone. The vials were placed in a sonicator for approximately 5 minutes, after which the acetone was removed and stored in separate 1.5 dram vials. This process was repeated two more times. The vials full of acetone extract were placed on an evaporator until approximately one ml of fluid remained. With a one millimeter capillary tube, a spot of extract was placed 2 cm from the bottom of a 20 x 20 cm aluminum backed, F_{254}, silica gel plate. Each spot was 1 cm from the next sample and 2 cm from either edge of the plate. The plate was then placed in a Desaga tank containing Solvent G (Toluene - ethyl acetate - formic acid 139:83:8) (Culberson et al. 1981) for approximately 25 minutes, or until the solvent front reached a line 10 cm from the original spots of extract.

After drying under a fume hood, plates were inspected beneath white, short UV (254 nm), and long UV (366 nm) light. Color and distance from origin were recorded. Plates were then sprayed with 10% H_2SO_4 solution and baked at 110° C for 10 minutes. Colors and distances from origin were again noted using white, short UV, and long UV light.

Relative Rf values were calculated by dividing the distance of a chemical from the origin by the distance of the solvent front from the origin, and multiplying by 100.

Two to three samples from a previous plate were spotted on subsequent plates in order to standardize identification of chemicals. *Usnea sp.*, *Pseudevernia intensa*, *Lobaria pulmonaria* and *Parmelia saxatilis,* which contain known chemicals, were also spotted onto the plates so that comparisons could be made. Identification of substances was further supplemented by information from Culberson et al. (1981) and Huneck and Yoshimura (1996). Chemicals which showed a high range in Rf values were re-evaluated to assure that they were correctly identified

from plate to plate. If chemicals were a) not identified on the same plate, b) did not occur in the same individual, c) had a difference in Rf values of less than 5, and d) exhibited similar colors, they were considered the same chemical. Fifteen chemicals were renamed using this procedure. Sixty chemicals were identified, forty-six of which were from *X. cumberlandia* or *X. coloradoënsis*. The other fourteen chemicals were from four isidiate morphs (*X. plittii* or *X. mexicana*), *Pseudevernia intensa, Usnea sp.,* and/or *Parmelia saxatilis.*

Morphological and microhabitat measurements.—Morphological measurements included thallus area and presence of apothecia. Environmental measurements including elevation, aspect, direction of protection and distance from protection were also made.

Pictures of each thallus with a ruler for calibration were taken. The image size option in Adobe Photoshop CS (Adobe Systems Inc. 2003) was used to calibrate the size of the picture with the length of the ruler. Using the paintbrush tool, each thallus was outlined and then filled in with the paintbucket tool. After being saved as a tif file, the image was imported into NIH image 1.62 (freeware downloaded from NIH at rsb.info.nih.gov/nih-image/download.html) which was used to estimate thallus area from a measure of pixel count (Ruzin 2002; http://microscopy.berkeley.edu/).

The direction each lichen faced (aspect) was measured with a compass. Possible directions included each of the cardinal directions as well as their intermediates. Aspect was designated by "up" when the lichen was facing in that direction.

Since wind and sun could potentially affect our samples we measured the direction and distance from protective objects (usually a tree). The cardinal directions and their intermediates, as well as "0 m" and "> 30 m" were possible categories for direction of protection. "0 m" was assigned when the individual was directly under protection, and "> 30 m" was assigned when protection was over 30 m away, and the designation of any particular tree as the major source of protection became arbitrary.

Distance from protection categories included “0 m”, “0.5-4 m”, “4-10 m”, “10-30 m” and “> 30 m”. The limits of these categories were assigned based on our intuitive understanding of where different levels of protection from wind and sun have natural breaks.

Location as well as elevation was tested. Location is the same as elevation, except that location divides the lower elevation into lower-east and lower-west sides of the mountain to account for the possibility that differences in chemistry might occur on each side of the mountain.

Statistical analyses.— Principal component analysis, performed in S-PLUS (MathSoft Inc. 1999) was used to describe which chemicals represent most of the variation in chemistry.

A UPGMA dendogram was created in PAUP (Swofford 1993) using presence or absence of chemicals for input. Sixty chemicals were used from 143 individuals, including three outgroups: *Pseudevernia intensa, Parmelia saxatilis,* and *Usnea sp.*, as well as four samples of *X. plittii* and *X. mexicana*.

Two linear regression models were developed using forward-selection logistic regression, with principal component 1 and principal component 2 scores as their response variables, respectively. Another model was created using forward-selection logistic regression in which cluster membership, as identified from the UPGMA dendogram, was the response. Thallus size, presence of apothecia, direction and distance from protection, elevation, location, and aspect were considered when selecting explanatory variables.

Some individuals were collected non-randomly from the upper elevation in an effort to collect more apotheciate lichens. Non-randomly collected individuals were included in the UPGMA dendogram, but not for PCA analysis or any regression model.

RESULTS

The UPGMA dendogram shows two well-supported clusters (Figure 1, Figure 2), which we refer to as Cluster A and Cluster B. Cluster A included one isidiate morph, while the other

three isidiate morphs were dispersed within Cluster B (Figure 1). Cluster B also contained outgroups *Usnea sp.* and *Parmelia saxatilis*, though they were somewhat distinct from the rest of the cluster (Figure 1). Almost all individuals produced usnic and norstictic acids (Figure 3). Stictic acid was produced by 98% of Cluster A individuals and only 3% of Cluster B. Less than 1% of Cluster A and 95% of Cluster B produced salazinic acid (Figure 3).

Location was a significant predictor of cluster membership (p-value < 0.0001, $F_{2,322}$ = 345.28; Table 1; Figure 4). Notably, reproductive mode (sexual vs. sterile) did not add to these models (p-value = 0.1702; drop in deviance $F_{1,321}$ = 1.89).

Populations in the lower elevations were almost exclusively comprised of the Cluster B chemotype (2400-2500 m west = 100.0%; 2400-2500 m east = 88.1% ± 7.8% (99% confidence); Figure 5), while 96.6% ± 1.4% (99% confidence) of the individuals from the upper elevation were in Cluster A.

One principal component explains 38.6% of the variation in chemical combinations (Figure 6). The values for the first principal component are best predicted by location (r^2 = 82%; p-value < 0.0001, $F_{2,322}$ = 728; Table 2; Figure 7), with chemicals produced by individuals from the upper elevation contrasted with chemicals from individuals in the lower elevation. The second principal component explains much less variation (only 9%). Its values are predicted by location and direction of protection (r^2 = 45%; Table 3; Figure 8). Principal component two highlights chemical differences between the lower-east and lower-west elevations. Individuals in the lower-west population show significant contrasts between those with protection directly above or to the east and those with protection to the north-east or north-west. For both components, location is the best predictor (Figure 9).

DISCUSSION

Using the whole-chemical approach does not give much more information than the few chemicals traditionally used by taxonomists to distinguish between *X. cumberlandia* and *X. coloradoënsis.* With 97.8% ± 2.2% (99% confidence) of its individuals producing stictic acid, Cluster A can be identified as *X. cumberlandia* and/or *X. plittii.* Ninety-five percent (± 3.5% with 99% confidence) of individuals in Cluster B produce salazinic acid, a pattern consistent with *X. coloradoënsis* and *X. mexicana* identifications. Norstictic acid, typically found in *X. cumberlandia* and *X. plittii* is produced by almost all individuals (97.5% ± 1.7% with 99% confidence), but appears to have reduced concentration in most individuals from Cluster B (the spot was lighter). At least one researcher has noticed that a small amount of norstictic acid production is common in other *X. coloradoënsis* individuals (Robert Egan, personal communication). We suggest that norstictic acid does not effectively distinguish between *X. cumberlandia* and *X. coloradoënsis.* On the other hand, these data suggest that salazinic and stictic acid production are indicative of a distinctive set of secondary chemicals (Figure 3).

The fact that a distinctive suite of chemicals is associated with the salazinic and stictic acid chemotypes adds weight to the claim that *X. cumberlandia* and *X. coloradoënsis* are indeed genetically distinct.

Surprisingly, individuals in Cluster B produced chemical arrangements that are more similar to those produced by supposed outgroups *Usnea sp.* and *Parmelia saxatilis* than they were to chemicals produced by Cluster A. Presence of an outgroup in a phylogenetic tree built with chemical characters has been noted elsewhere (Miadlikowska and Lutzoni 2000). If the chemical clusters are considered indicative of genetic associations, this would indicate that *X. cumberlandia* is more closely related to *Usnea sp. and Parmelia saxatilis* than to *X. coloradoënsis.* Since such a scenario is highly unlikely, given the overwhelming morphological similarities between *X. cumberlandia* and *X. coloradoënsis,* these data suggest that chemicals should not be used alone to identify genetic relationships, but rather in tandem with other distinguishing characters (e.g. molecular markers and morphology).

No distinctive chemical groups appeared which might indicate a genetic or environmental separation between sexual, asexual, and sterile morphs (Figure 1). Reproductive mode was unrelated to chemical cluster membership. If chemicals are indeed indicative of gene flow, these data imply that gene flow between sexual and asexual morphs is frequent. An alternative explanation, presented by Bowler and Rundel (1975), is that high similarity in chemicals produced by sexual and asexual sister species is due to very recent derivation of the asexual species from its sexual ancestor.

Principal component analysis indicated significant chemical differences between the upper and lower elevations, as well as some differences between lower-east and lower-west elevations. The fact that principal component two was associated with direction of protection also indicates that sunlight, wind, or some other correlate of proximity to trees may be affecting the secondary chemistry of these individuals. This possible effect will not be over-analyzed here, especially since the correlation with direction of protection was only significant in the lower-west population.

We conclude that stictic and salazinic acids can effectively distinguish between *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis* on Boulder Mountain, Aquarius Plateau, UT. The presence of these chemicals is associated with other chemical and environmental differences, which lends more credibility to the current species boundary between them. That the presence of stictic or salazinic acids represents a genetic difference between *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis* seems likely, but would benefit from molecular evidence. Furthermore, sexual morphs are chemically indistinguishable from sterile morphs.

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We extend our appreciation extends to Nathan Jackson for his help in the field and comments on our work. We thank Peter Ririe and Katy Knight for their assistance with TLC analysis. Loreen Woolstenhulme and Leigh Johnson provided valuable insights to this project.

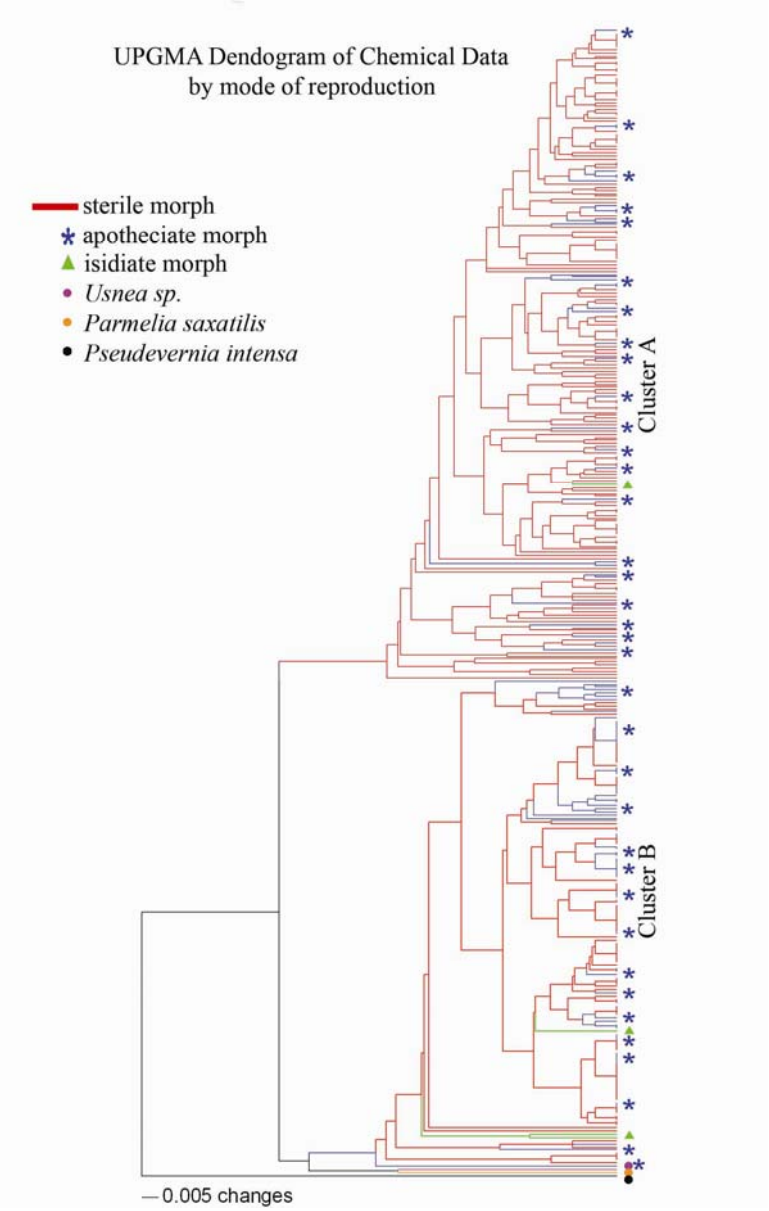

Figure 1. UPGMA dendogram based on presence/absence data for sixty chemicals.
Two clusters are well supported: Clusters A and B. Most individuals represented were either apotheciate (had sexual structures), which are by definition *X. cumberlandia* or *X. coloradoënsis*, or sterile, which cannot be identified to species. Four individuals had isidia (specialized asexual structures), and are by definition either *Xanthoparmelia plittii* or *Xanthoparmelia mexicana*. The isidiate morphs are not chemically distinguishable from the sterile or apotheciate morphs.

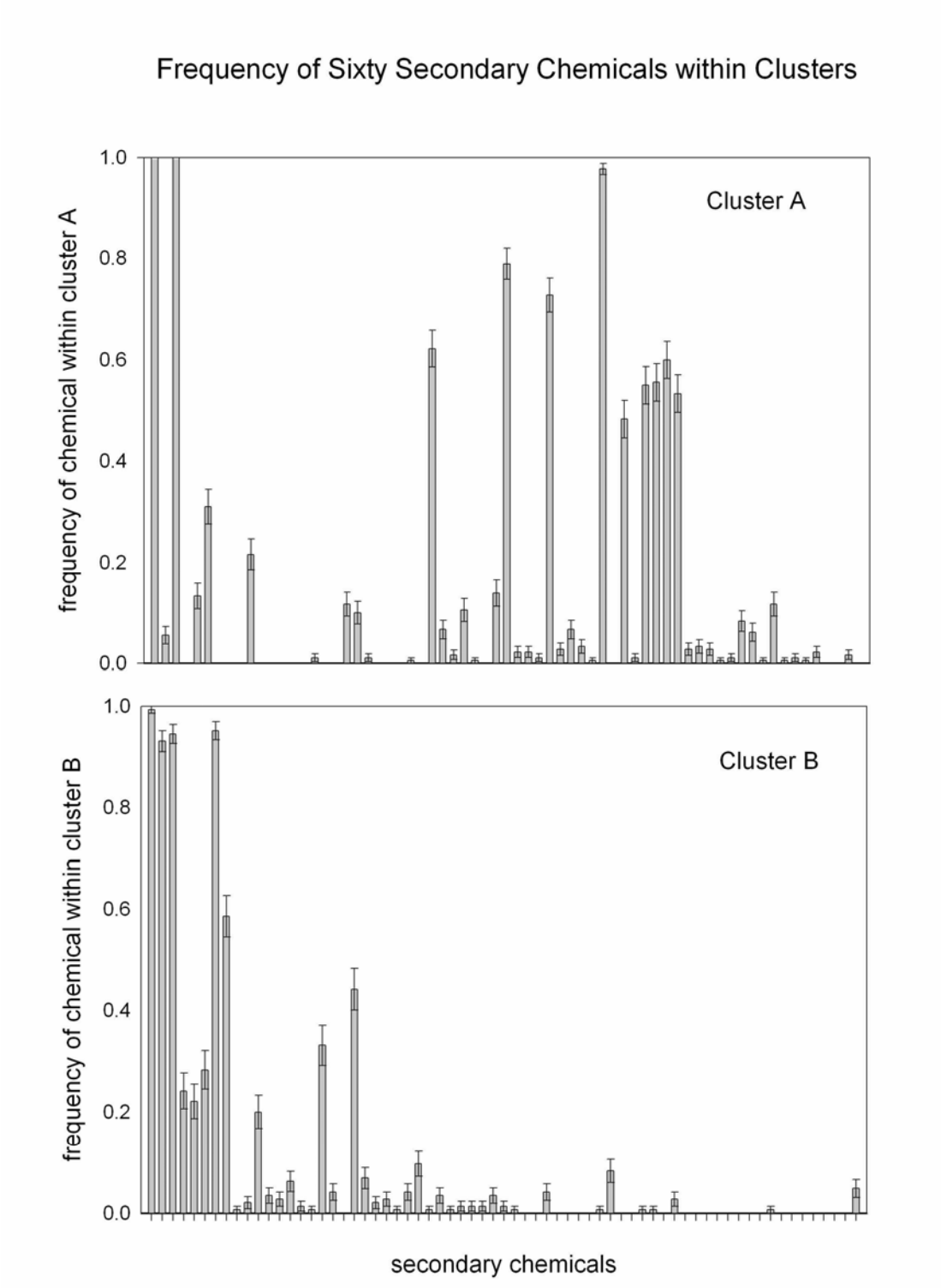

Figure 2. Distribution of secondary chemicals by cluster.

Most chemicals are unidentified. Secondary chemicals are in no particular order.

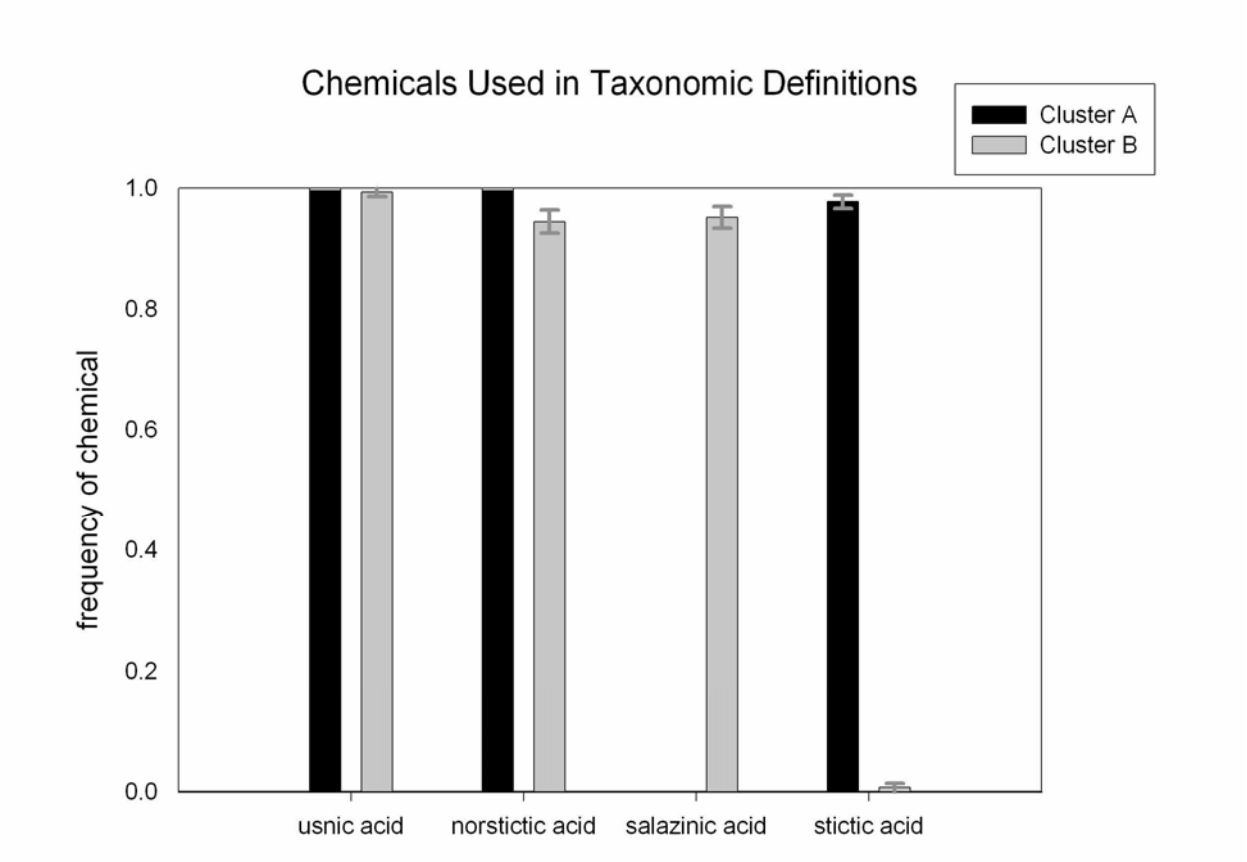

Figure 3. Chemicals used in taxonomic definitions for *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis.*

Individuals in Cluster A would traditionally be called *X. cumberlandia* due to the prevalence of stictic acid. The presence of salazinic acid in most individuals in Cluster B identifies them with *X. coloradoënsis.* Though norstictic acid is, by definition, found in *X. cumberlandia* and not *X. coloradoënsis,* almost all individuals contain norstictic acid, regardless of species identification.

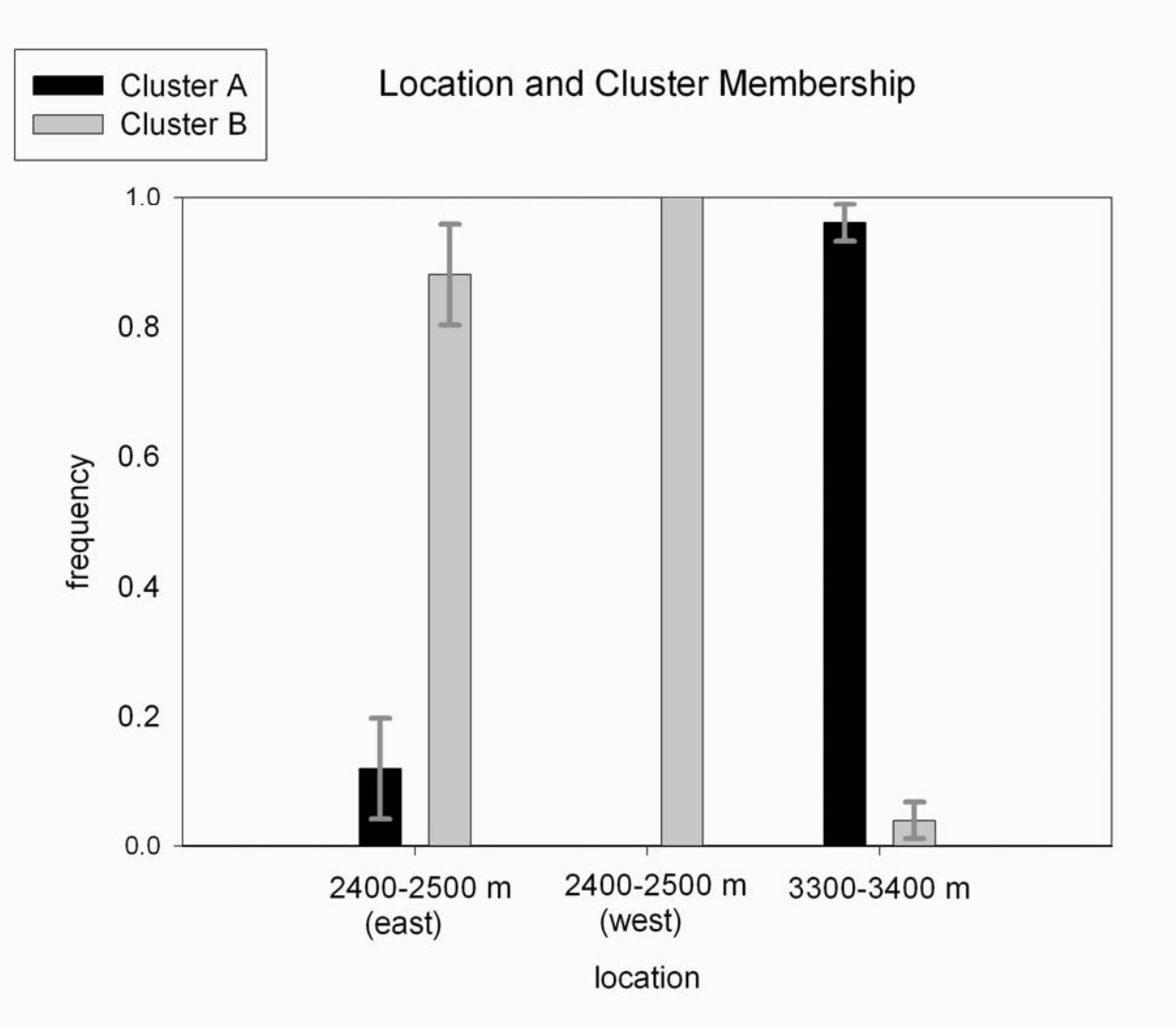

Figure 4. Location and cluster membership.

A forward-selection logistic regression model indicates that both location and direction of protection are significant predictors of cluster membership (See Table 1). Individuals in Cluster A were found primarily at 3300-3400 m, while Cluster B individuals were generally found between 2400-2500 m.

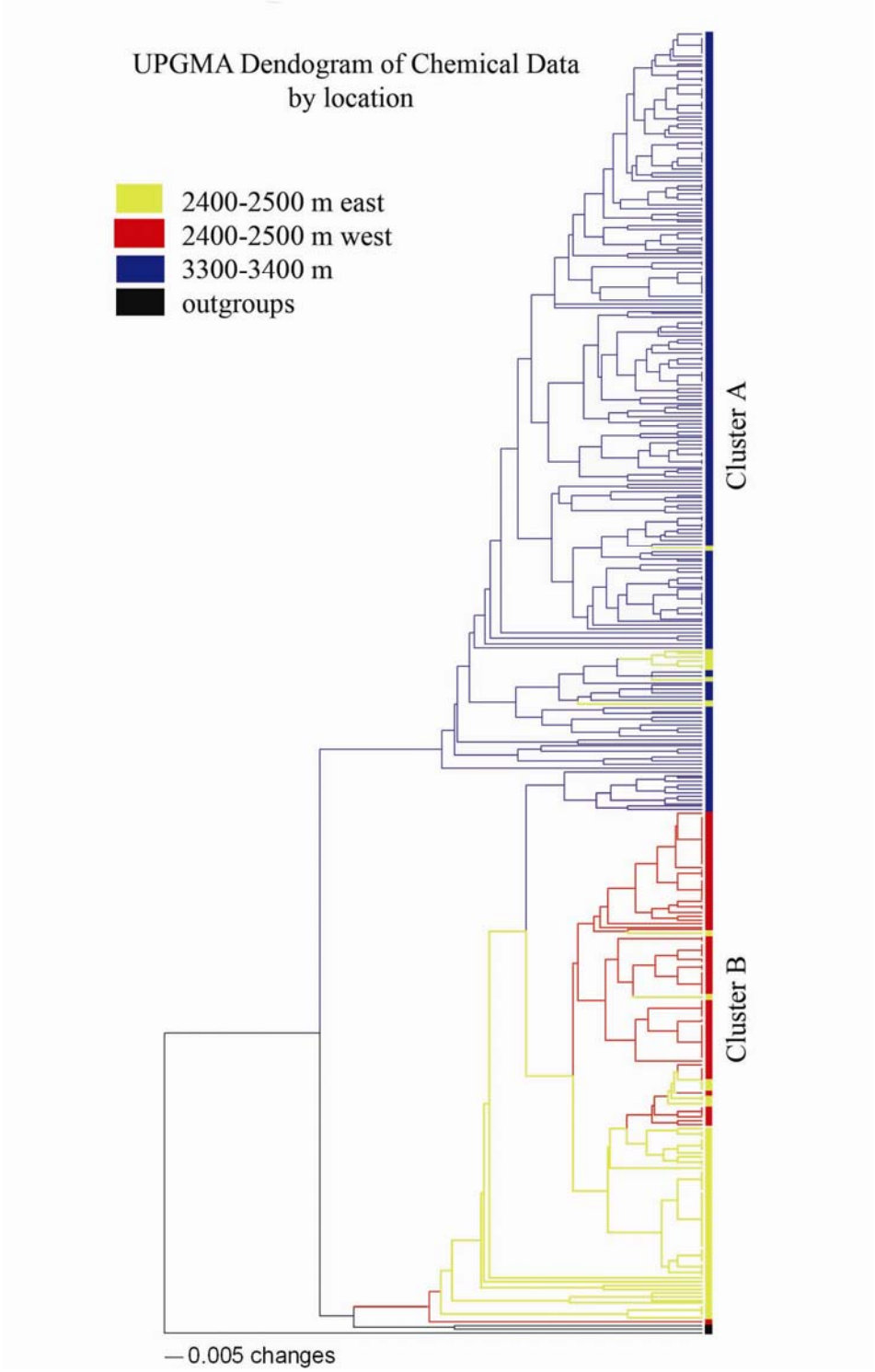

Figure 5. UPGMA dendogram of chemical data by location.

Almost all individuals in Cluster A were found between 3300-3400 m, while Cluster B is almost exclusively comprised of individuals from 2400-2500 m.

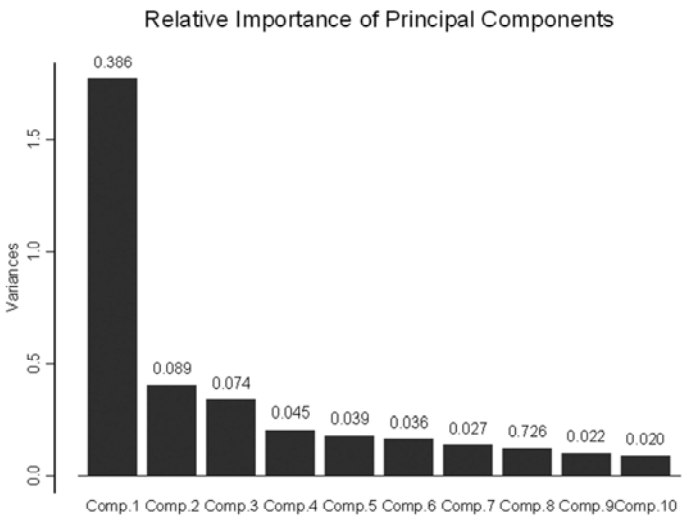

Figure 6. Relative importance of principal components.

Principal component analysis indicates that one linear combination of chemicals can summarize 38% of the variation in chemical combinations.

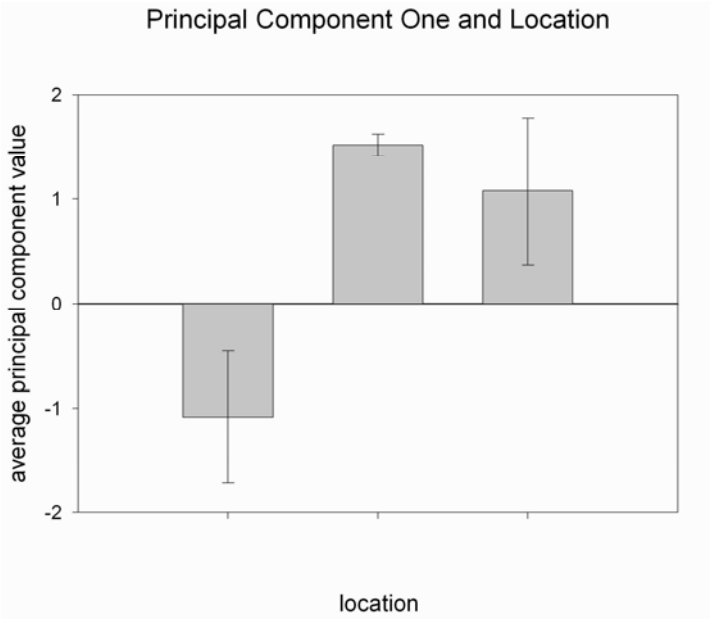

Figure 7. Principal component one and location.

The linear combination of chemicals in principal component one (PCA1) is associated with the location from which individuals were collected (See Table 2), with the upper elevation showing significant differences from the lower elevations.

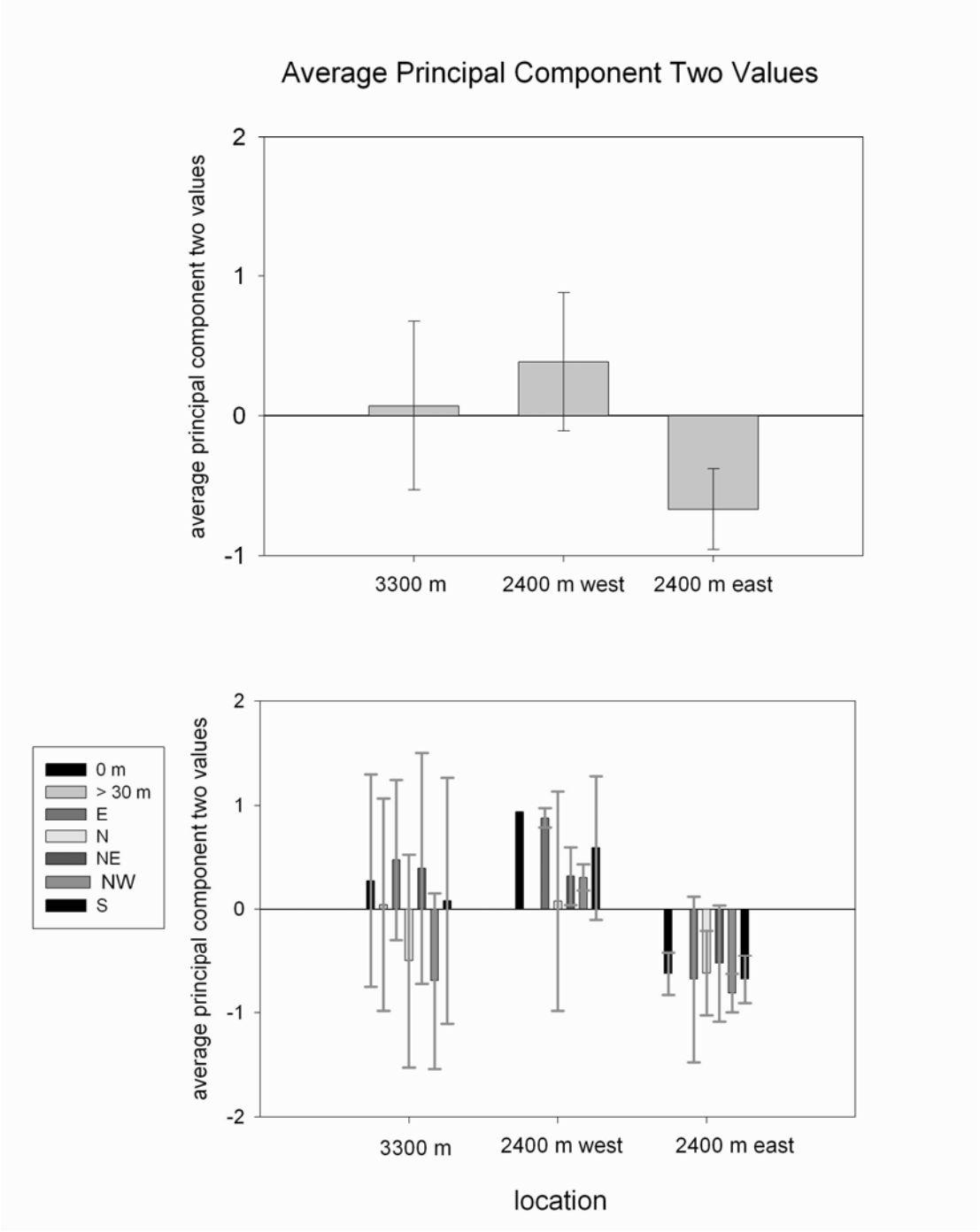

Figure 8. Principal component two (PCA2) by location and direction of protection.
Individuals in the lower-east and lower-west locations tend to have different PCA2 values. Also, within the lower-west location, individuals with protection directly above or to the east of them

have significantly different PCA2 values than those individuals with protection to their north-east and north-west. See Table 3.

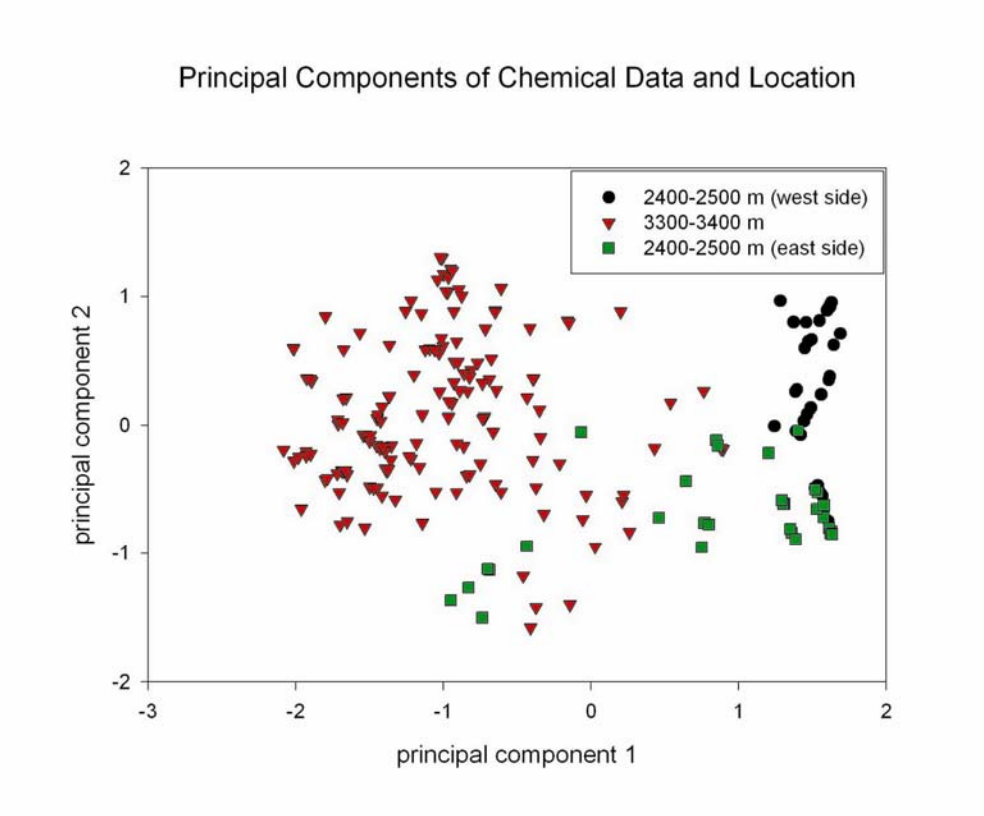

Figure 9. Principal components one and two by location.
Location is the best predictor of the two most powerful linear combinations of chemicals (See Table 2, Table 3, Figure 6, Figure 7).

Table 1. Cluster membership.

A forward-selection logistic regression model predicting the likelihood of cluster membership. Individuals from the lower-west and lower-east locations were more likely to be in Cluster B. "Location" includes two different locations at 2400-2500 m (east and west) as well as the upper plateau at 3300-3400 m on Boulder Mountain, Aquarius Plateau, UT. Direction of protection indicates the direction in which the nearest tree or protective structure lies in relation to the individual (See Figure 4).

Parameter	DF	Estimate	SE	Drop in Deviance $F_{2,322}$	p-value	z-stat	p-value
(Intercept)	1	-3.36	0.42			-8.08	0.00000
location	2			345.28	0.0000		
lower-west v. upper	1	14.56	18.4			0.79	0.21402
lower-east v. upper	1	5.35	0.56			9.55	0.00000

Table 2. Principle component one.

A forward-selection linear regression model indicates that location is a significant predictor of values for principal component one (See Figure 6). This PCA investigated linear combinations of chemicals which describe the variation in chemical combinations within individuals.

Parameter	DF	Sum of Squares	Mean Sum of Squares	F	p-value
location	2	471.86	235.93	727.89	3.42E-120
Residuals	322	104.37			
R-SQ =.8189					

Table 3. Principal component two.

The linear combination of chemicals described by principal component two is correlated with location, direction of protection, thallus size, and distance from protection (See Figure 7).

Parameter	DF	Sum of Squares	Mean Sum of Squares	F	p-value
location	2	42.70	21.35	93.64	1.16E-32
direction of protection	6	17.17	2.86	12.55	1.06E-12
Residuals	316	72.01	0.23		
R-SQ =.454					

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Size is not a reliable measure of sexual fecundity in *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis* on Boulder Mountain, Aquarius Plateau, UT

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Abstract. Since a number of studies have shown a strong positive correlation between apothecia production and lichen thallus size, it has been suggested that size can serve as an easy measure of lichen sexual fecundity. The consistency of the relationship between size and apothecia production among different environments has not been studied. We measured apothecia development and thallus area in populations of the lichenized fungi Xanthoparmelia cumberlandia *and* Xanthoparmelia coloradoënsis *located at different elevations on Boulder Mountain, Aquarius Plateau, UT. Our data suggest the relationship between size and apothecia production is not consistent among various environments, nor does size adequately explain variation in apothecia production.*

Many ecological studies require an estimate of fitness. Since a direct measurement of reproductive output is often difficult, other more easily measured correlates have been sought.

In lichenology, the possibility that size can be used as an estimate of fitness has been explored. Because height can be ignored in basically two-dimensional organisms like crustose and most foliose lichenized-fungi, size is a fairly simple measurement. In contrast, measuring more direct correlates of fitness, such as competitive success or sexual fecundity can be cumbersome, if not impossible.

Some studies suggest that, in a few species, larger lichen thalli may be better equipped to survive (Gauslaa and Solhaug 1998; Hestmark 1997). The ability of larger lichen individuals to hold more water longer gives them a competitive edge. Due to increased water retention, larger individuals of the lichenized-fungal species *Degelia plumbea* and *Lasallia pustulata* tend to remain photosynthetically active longer than smaller thalli (Gauslaa and Solhaug 1998; Hestmark 1997). Hestmark (1997) found that better water retention gives larger thalli an advantage, in that they more frequently expand over other individuals, thereby gaining more access to sunlight.

In addition to offering competitive advantages, size appears to be related to the onset of sexual reproductive ability (Hestmark 1992; Pringle et al. 2003; Ramstad and Hestmark 2001). For example, *Lasallia pustulata, Xanthoparmelia cumberlandia,* and *Umbilicaria spodochroa* appear to have a lower size limit above which most individuals have apothecia (sexual spore-bearing structures) (Hestmark 1992; Pringle et al. 2003; Ramstad and Hestmark 2001).

Beyond onset of sexual maturity, size exhibits a positive correlation with the number of apothecia produced. A study of the species *Xanthoparmelia cumberlandia* found that the number of apothecia produced by individuals shows a positive correlation with size (Pringle et al. 2003). Apothecia and size show a similar relationship in *Umbilicaria spodochroa* (Ramstad and Hestmark 2001). *Lasallia pustulata* showed a positive correlation between size-class and the presence of apothecia (Hestmark 1992).

These studies describing a strong relationship between size and apothecia production have been limited to homogenous environments, raising the question of how robust this relationship between size and fecundity really is. The *X. cumberlandia* study was conducted in Berkeley's Botanical Garden (Pringle et al. 2003), while *U. spodochroa* was observed within a narrow range of habitats in Norway (Ramstad and Hestmark 2001). Though the relationship between size and sexual reproduction in *Lasallia pustulata* was persistent when sampled over a variety of microhabitats (Hestmark 1992), variation in this trend was not reported. Thus, while an average trend has been noted in these organisms, the degree of error which can be expected when this trend is viewed across habitats is unknown.

We must also emphasize that this relationship between size and sexual fecundity is different among various genera. *Xanthoparmelia cumberlandia* showed a non-linear relationship between size and apothecia number, in that larger thalli produced disproportionately more apothecia (Pringle et al. 2003). *Umbilicaria spodochroa*, on the other hand, exhibited a simple linear relationship between size and sexual fecundity (Ramstad and Hestmark 2001). Rather than increasing in size and fecundity with time, *Cladonia furcata*, *Cetraria islandica*, and *Peltigara canina* stop growth altogether after

onset of sexual maturity (Jahns and Frey 1982; Jahns et al. 1978; Jahns and Schuster 1981).

We assess the validity of the hypothesis that size is an adequate predictor of sexual fecundity in *X. cumberlandia* and *X. coloradoënsis* by studying the reliability of this relationship when faced with elevation and microhabitat differences. Like the studies previously mentioned which compare size and sexual fecundity, we will only indirectly address the relationship between size and asexual reproductive effort.

MATERIALS AND METHODS

Study organism.—A lichen consists of two or more species: a fungus and its photosynthetic partner(s) (an alga and/or a cyanobacterium).

When measuring sexual reproductive fitness of a lichen, one is usually referring to the fitness of the fungus. The photosynthetic partner is generally understood to be asexual, though recent studies have indicated that cryptic recombination is occurring within putatively clonal algal populations (Kroken and Taylor 2000). Sexual reproductive structures (apothecia) are essentially permanent once formed, and so provide a record of the sexual effort of an individual.

Xanthoparmelia cumberlandia, is generally distinguished from its closely related sister taxon, *X. plitii,* by the presence of apothecia and lack of isidia. Isidia are specialized asexual structures which disperse viable cells of both symbionts. *X. cumberlandia* is facultatively sexual, meaning that it can engage in both sexual and asexual reproduction; this lichen can produce offspring through spore production or

unspecialized asexual thallus fragments. More information concerning *X. cumberlandia* and *X. plitii* can be found in Hale (1990) and Brodo et al. (2001).

X. cumberlandia is morphologically indistinguishable from *X. coloradoënsis*. Vague differences are discussed in Hale's monograph of *Xanthoparmelia* genus (Hale 1990), which describes overlapping ranges of rhizine length, and size of thallus, lobes, conidia, apothecia, and spores. Thalli, medullaea, lower surfaces, and rhizines are reportedly the same color, except for the hazy distinction that the lower surface of *X. cumberlandia* is "pale brown to darkening", while *X. coloradoënsis'* lower surface is just "pale brown". The only solid difference described by Hale involves the secondary chemicals produced by these two lichens. *X. cumberlandia* purportedly produces stictic, constictic, norstictic, and usnic acids, while *X. coloradoënsis* produces salazinic, usnic, and possibly consalazinic acids.

In order to ensure correct species identification, thin layer chromatography (TLC) was performed. Both *X. cumberlandia* and *X. coloradoënsis* were present in our sample (see Jackson 2004 for more details). We assume the relationship between size and sexual fecundity is comparable in these closely related species. This assumption was tested by considering species identity during the model building process, as described below.

Sampling strategy.—Due to the abundance of *X. cumberlandia* and *X. coloradoënsis* covered boulders, Boulder Mountain, Aquarius Plateau, UT was the site selected for this investigation. With approximately ten individuals measured in each population, fifteen populations were observed at an elevation of 2400-2500 m, and eighteen populations at 3300-3400 m (Table 4). The resulting 325 samples provided us with ample statistical power. Eight of the lower elevation populations occurred on the

western slope of Boulder Mountain, while the other seven occurred on the eastern slope. Poor access to the northern and southern slopes prevented observations in those areas. The higher elevation observations were made along RD 178 which effectively transects the upper plateau from northwest to southeast. Populations were chosen at random distances from the road.

Abiotic conditions.—In general, the lower elevations of Boulder Mountain can be characterized as warm and dry, while the upper elevations are cooler and more moist (US Department of Agriculture et al. 1999). Weather sensors near the study sites indicate that average yearly temperature near the upper elevation (Donkey Reservoir, 3064 m) is more than 4° C cooler than the lower-west elevation site (Boulder City, 2150 m) and 7° C cooler than the lower-east elevation (Loa City, 2035 m) (Western Regional Climate Center: www.wrcc.dri.edu). Average yearly precipitation at the higher elevation sensor is 75 cm, while Boulder City (lower-east) receives only 27 cm per year, and Loa (lower-west) receives only 6.3 cm per year (National Water and Climate Center: www.wcc.nrcs.usda.gov).

Measurement.— Thallus area was measured by taking a picture of each lichen thallus with a ruler, and then estimating area using NIH image 1.62 (freeware downloaded from NIH at rsb.info.nih.gov/nih-image/download.html). When more than one picture was required due to large size or extension of the thallus around the edge of a rock, a straight object was used to mark the cutoff between the two pictures. The pictures were later opened in Adobe Photoshop CS (Adobe Systems Inc. 2003) where the photographed ruler was used to calibrate the size of the picture using the "image size" menu. The paintbrush tool was used to outline the thallus, and the paintbucket tool was

used to fill it in. Saved as a TIF file, these pictures were opened in NIH Image 1.62, and thallus area was calculated. More instructions for using NIH image are found in Ruzin (2002; http://microscopy.berkeley.edu/).

Three sexual fitness correlates were considered: presence of apothecia, number of apothecia, and total area of apothecia.

Apothecia were counted according to the procedure found in Pringle et al. (2003). Total apothecia area was measured in the same way as thallus area; each apothecium was painted and the sum of all apothecial areas for each individual was calculated.

In order to see how consistent spore production was, average ascospore production per ascus was measured. In *X. cumberlandia* and *X. coloradoënsis,* eight ascospores are generally found in each ascus (a sac-like structure). Thousands of asci make up a single apothecium. A sample from each thallus was collected in the field, and ascospores were counted using an Olympus microscope. Three apothecia from each thallus were selected and sectioned. Ascospores from three asci from each apothecium were then counted. When available, apothecia > 5 mm in diameter were selected for ascospore counts.

Morphological and environmental covariates were also measured. These included aspect, direction of protection, distance from protection, and as previously mentioned, species identification.

With a compass we measured the aspect towards which each individual faced. Potential categories included the cardinal directions and their intermediates. Those lichens facing straight up were placed in the “up” category.

Any large object (usually a tree or clump of trees) that could potentially block wind or sun was considered protection. Categories for direction of protection were the same as for aspect. Those with protection directly above them (i.e. located beneath a tree), were placed in the < 0 m category. Due to the difficulty of determining which clump of trees was providing the most protection beyond 30 meters, we simply placed those lichens with no protection within 30 meters in a separate category.

In addition, we measured the distance to the nearest protective feature. Categories for this measurement included "0 m", "0.5-4 m", "4-10 m", "10-30 m" and "> 30 m". Category ranges were assigned based on logical increments in exposure to wind and sun.

Statistical analyses.—Models were selected after using forward-selection regression in SAS (SAS Institute Inc. 2001) and S-PLUS (MathSoft Inc. 1999).

All randomly collected data were used for the model investigating the probability of the presence of apothecia using binary logistic regression. Because most lichens had no apothecia, the response variables number of apothecia, area of apothecia, and ascospores per ascus were evaluated *after* removing non-apotheciate individuals from the model. On a follow-up visit, additional apotheciate individuals were non-randomly collected from the upper elevation in order to increase sample size for these analyses. Thus, these models attempt to answer the question: *if* apothecia are present, how well does size predict the number and area of apothecia?

The distribution of both number of apothecia and area of apothecia were heavily skewed, even when zeros were removed. These responses were log-transformed before evaluation using linear regression. Using Bonferonni's correction for multiple analyses,

we accepted as significant those explanatory variables with p-values less than or equal to 0.010.

The adequacy of parameters included in the final models was evaluated using drop-in-deviance and extra sum of squares F-tests for logistic and linear regressions, respectively.

RESULTS

Thallus size was a significant predictor of presence of apothecia, number of apothecia and size of apothecia (Table 5; Figure 10, Figure 11, Figure 12). However, much of the variation in apothecia production remains unexplained with only 15.83% of the variation in apothecia number explained by thallus size and elevation (Table 7; Figure 11). Thallus size and elevation explain more of the variation in apothecia size (r^2 = 37.00%; Table 8; Figure 12). None of the variables measured significantly predicted variation in ascospore production.

The effect of elevation was less clear than the effect of thallus size on apothecia production. Elevation significantly predicted the presence of apothecia (Figure 13) and number of apothecia, but was only marginally significant in predicting total area of apothecia on an individual (Table 5).

Distribution of species is strongly correlated with elevation (r=.91); *X. coloradoënsis* was generally located at 2400-2500 m (95.20% were in the lower elevation) and *X. cumberlandia* was commonly found at 3300-3400 m (95.53% were in

the upper elevation). Because species and elevation were so highly correlated, the effects of species are impossible to separate from the effects of elevation.

Habitat variables distance from protection and direction of protection helped to predict the occurrence of apothecia (Table 6), but were not significant in predicting number or total area of apothecia.

The average number of ascospores per ascus was consistent no matter what variable was considered.

DISCUSSION

While thallus size was significantly associated with all measures of sexual fecundity, the relationship between the two variables was not consistent across habitats. Elevation as well as distance and direction of protection also affected the relationship between size and apothecia production.

Though statistically significant, the correlation between thallus area and apothecia production explains little of the variance in sexual fecundity. Large amounts of variation in apothecia production remain unexplained in our models (number of apothecia: r^2 = 15.83%; Table 7; area of apothecia: r^2 = 37.00%; Table 8; Figure 11, Figure 12).

The onset of sexual reproduction was not reliably predicted by thallus size. Pringle et al. (2003) found a distinct size cutoff at 10 cm^2 after which most *X. cumberlandia* individuals produced apothecia; however, we found that *most* individuals had *no* apothecia, regardless of size (Figure 14). We did, however, find a threshold at 15 cm^2 after which roughly 37% of all individuals had apothecia (Figure 14).

The effects of protection on sexual fecundity are only roughly explored in this paper. While the model predicting the presence of apothecia (Table 6) includes distance from and direction of protection, the particular reasons for these correlations are not clear. Improved data describing spatial patterns of protection, microhabitat wind patterns, and solar exposure are needed to understand the relationship between protection and sexual fecundity in *X. cumberlandia* and *X. coloradoënsis.*

Though the correlation between size and fecundity in *X. cumberlandia* is strong in the homogeneous environment of a botanical garden, we conclude that this relationship is not reliable in natural environments. Apothecia production is simply too variable across the landscape to allow for size and fecundity to be used interchangeably.

ACKNOWLEDGEMENTS

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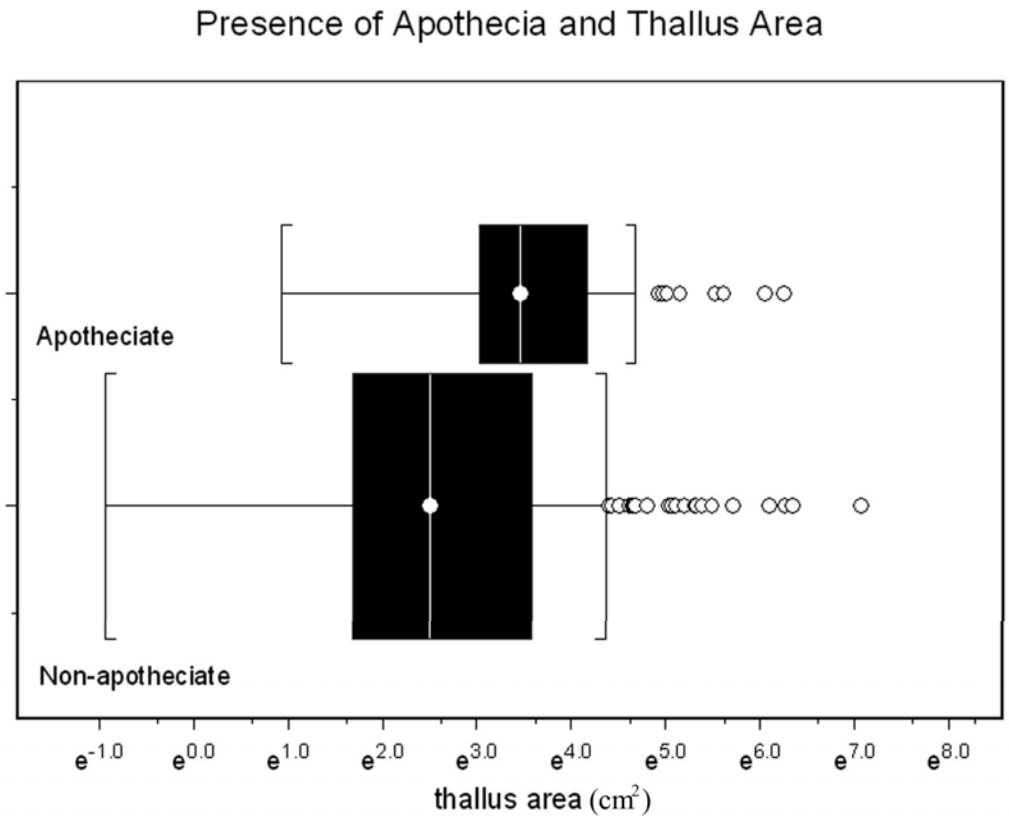

Figure 10. Predicting the presence of apothecia using thallus size.

On average, the larger the thallus, the more likely apothecia will be present.

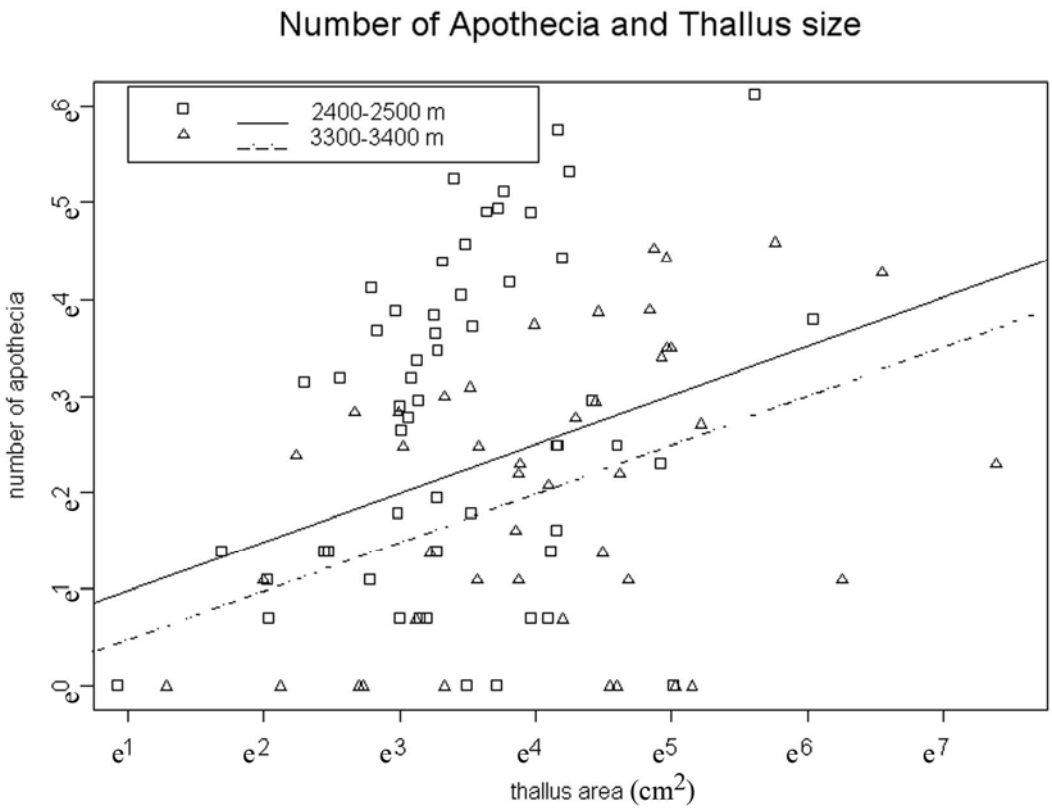

Figure 11. Number of apothecia and thallus size.

Though thallus size is positively correlated with the number of apothecia, substantial variation still exists in the data (r^2 = 15.83%; Table 7). The relationship between thallus size and apothecia number is negatively affected by increasing elevation.

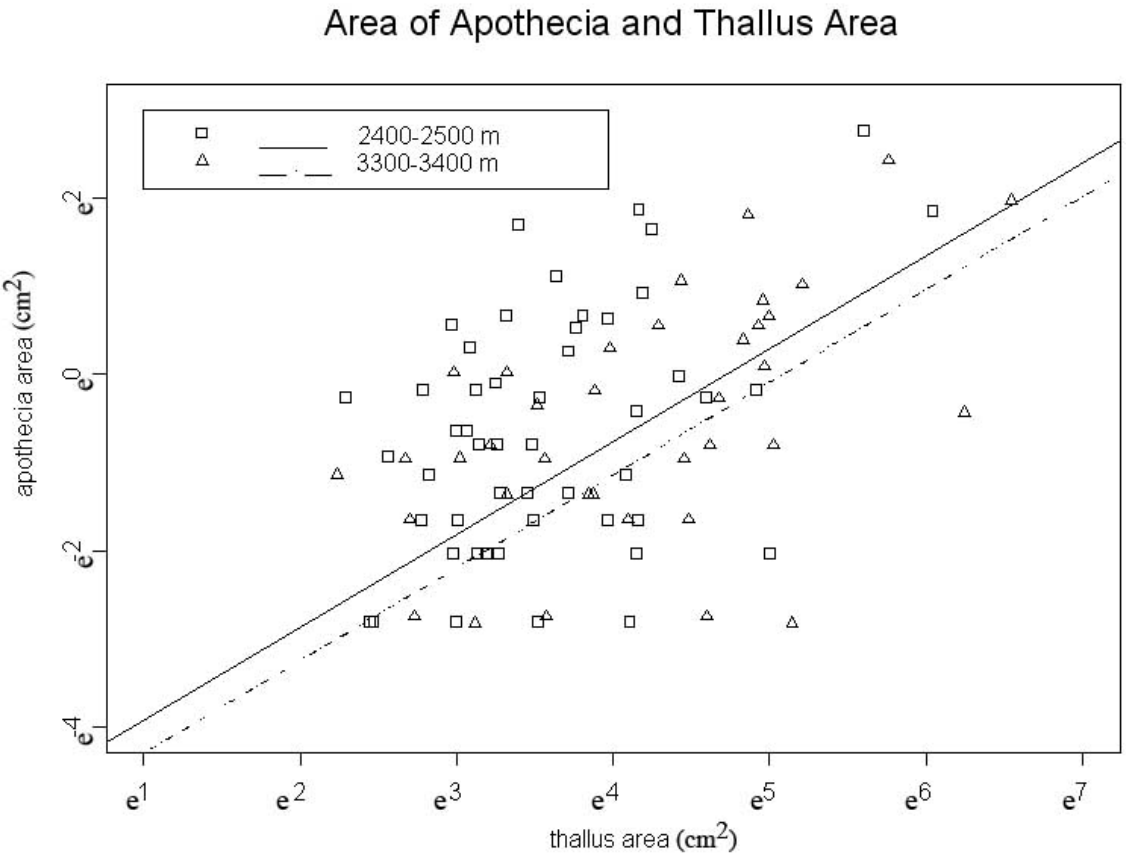

Figure 12. Area of apothecia and thallus size.

Only 37% of the variation in area of apothecia can be explained by thallus size and elevation.

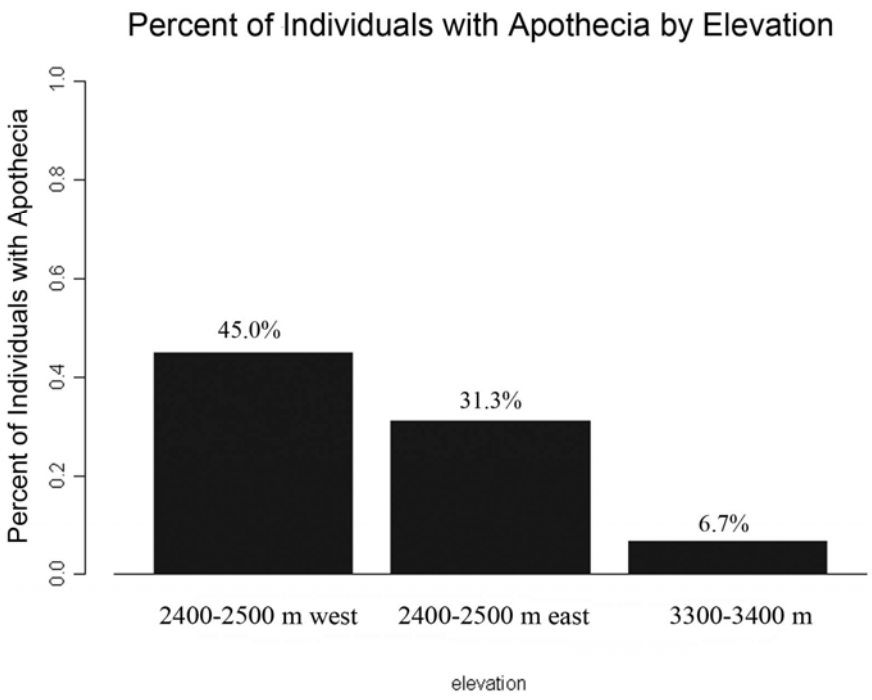

Figure 13. Predicting the presence of apothecia with elevation.

After taking into account direction to protection, distance from protection, and thallus area, individuals from the upper elevation (3300-3400 m) are less likely to have apothecia than those from lower-west and lower-east locations. Both lower-west and lower-east locations are situated at 2400-2500 m on Boulder Mountain.

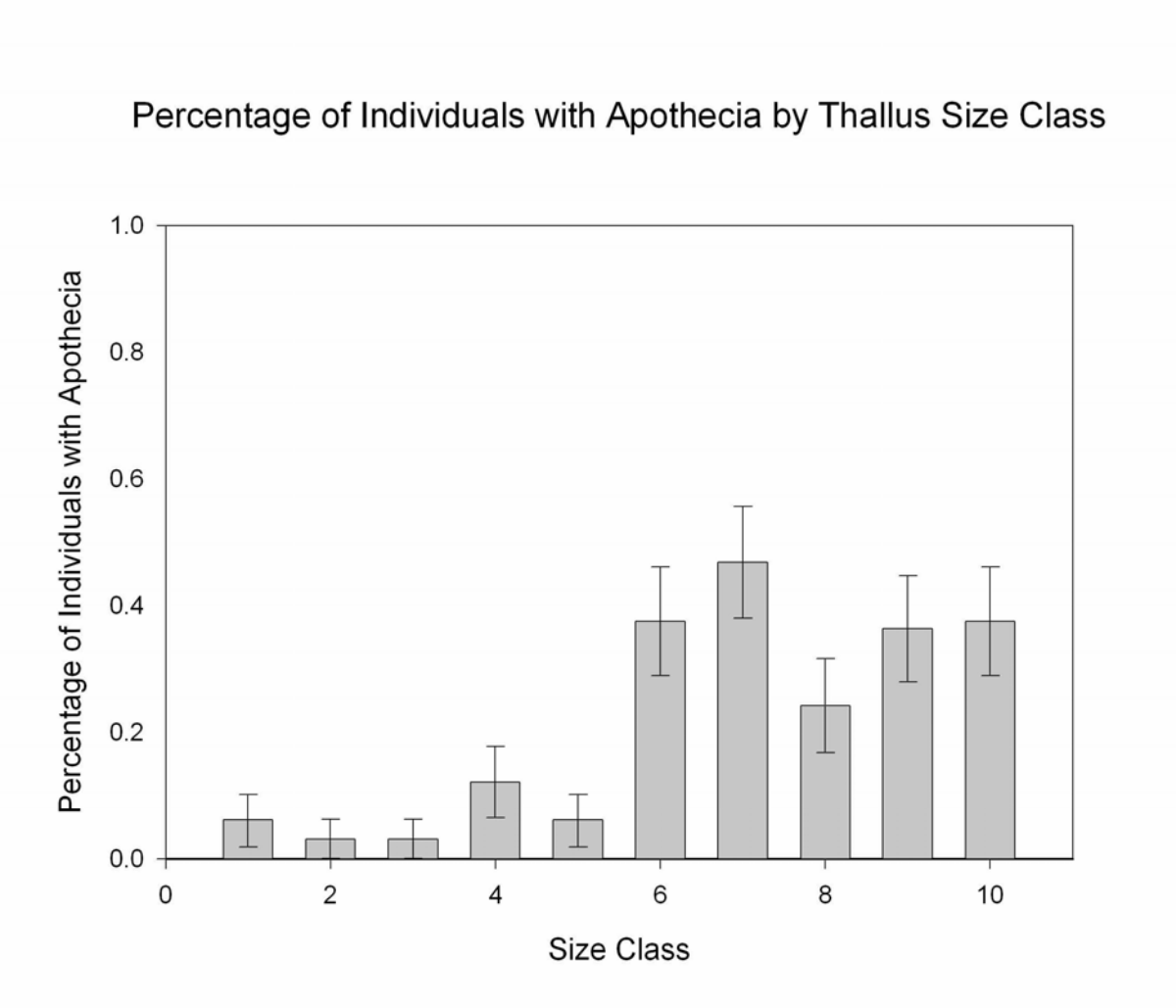

Figure 14. Presence of apothecia by thallus size class.

Though most individuals in all size classes have no apothecia, a distinct difference in the proportion of individuals with apothecia is seen between size classes above and below 15 cm^2. Size classes were chosen by percentiles, with size class one containing individuals in the 10^{th} percentile and lower and so on. Sizes in each size class are as follows: 1: 0.39-3.35 cm^2, 2: 3.42-5.10 cm^2, 3: 5.10-7.42 cm^2, 4: 7.61-11.74 cm^2, 5: 11.87-15.81 cm^2, 6: 15.94-22.77 cm^2, 7: 22.90-33.03 cm^2, 8: 33.35-51.42 cm^2, 9: 52.90-91.16 cm^2, 10: 93.94-1174.26 cm^2.

Table 4. Collection sites.

Approximately ten individuals were collected at each site. In order to have enough apotheciate lichens to include in a regression model, extra apotheciate individuals were collected non-randomly from the upper population.

Location	N	elev	Latitude	Longitude
lower-east	12	2467	38.1189	-111.531
lower-east	10	2433	38.1300	-111.361
lower-east	10	2400	38.1764	-111.633
lower-east	10	2367	38.1800	-111.527
lower-east	10	2367	38.2850	-111.407
lower-east	10	2367	38.3378	-111.506
lower-east	10	2367	38.3575	-111.462
lower-west	8	2433	38.2336	-111.622
lower-west	2	2400	38.2356	-111.627
lower-west	10	2433	38.2397	-111.626
lower-west	10	2433	38.2400	-111.644
lower-west	10	2433	38.2403	-111.624
lower-west	10	2400	38.2431	-111.640
lower-west	10	2433	38.2456	-111.653
lower-west	10	2400	38.2725	-111.698
lower-west	10	2433	38.2894	-111.706
upper	10	3333	38.1208	-111.677
upper	10	3367	38.1439	-111.481
upper	10	3363	38.1508	-111.759
upper	7	3400	38.1614	-111.161
upper	10	3367	38.2103	-111.714
upper	10	3367	38.2175	-111.505
upper	10	3367	38.2194	-111.729
upper	10	3367	38.2217	-111.708
upper	10	3300	38.2269	-111.556
upper	10	3433	38.2375	-111.701
upper	10	3367	38.2717	-111.548
upper	3	3367	38.2897	-111.567
upper	9	3367	38.3083	-111.592
upper	10	3367	38.3108	-111.689
upper	10	3367	38.3133	-111.707
upper	10	3433	38.3192	-111.711
upper	10	3333	38.3303	-111.594
upper	10	3367	38.3314	-111.516
upper	10	3367	38.3625	-111.579
upper-extra	10	3367	38.1408	-111.665
upper-extra	5	3367	38.1583	-111.759
upper-extra	6	3367	38.2236	-111.760
upper-extra	10	3367	38.2864	-111.583
upper-extra	2	3333	38.3303	-111.594

Table 5. Summary of models predicting sexual fecundity.

On Boulder Mountain, apothecia production in *X. cumberlandia* and *X. coloradoënsis* is generally correlated with thallus size and elevation. R^2 values indicate that this correlation is not strong. All models were developed using forward-selection regression.

Linear Regression Models						
Response	**Model**	**F**	**d.f.**	**d.f**	**p-value**	**r^2**
number of apothecia	N = A + E	9.03	2	96	2.56E-04	15.83%
area of apothecia (cm^2)	S = A + E	28.20	2	96	2.33E-10	37.00%

Maximum Likelihood Models					
Response	**Model**	**F**	**d.f.**	**d.f.**	**p-value**
presence of apothecia	logit (π) = L + D + P + A	23.82	11	313	2.15E-35
ascospores per ascus	no model significant				

Parameters

A = ln (thallus area) (cm^2)
D = distance from protection (0 m, 0.5-4.0 m, 4.0-10.0m, 10-30m, >30m)
E = elevation (2400-2500 m, 3300-3400 m)
L = location (lower-east, lower-west, upper)
N = ln (number of apothecia)
P = direction of protection (E, N, NE, S, NW, >30 m, 0 m)
S = ln (area of apothecia) (cm^2)
π= probability of apothecia

Table 6. Presence of apothecia.

Elevation (including side of mountain), direction of protection, distance from protection, and thallus size help to estimate the probability that an individual will have apothecia. This analysis includes all randomly selected individuals.

Parameter	DF	Estimate	SE	Drop in Deviance	p-value	Odds Ratio	99% confidence interval min	99% confidence interval max	z-stat	p-value	
(Intercept)	1	-10.35	1.7045458						-6.07	0.0000	***
location	2			43.21	0.0000						
lower-west v. upper		3.88	0.7101703			48.48	7.82	300.77	5.47	0.0000	***
lower-east v. upper		1.88	0.616464			6.54	1.34	31.90	3.05	0.0012	***
distance from protection	2			32.53	0.0000						
0 m v. > 10-30 m		1.36	1.6517646			3.90	0.06	272.39	0.82	0.2048	
0.5-4 m v. > 10-30 m		3.72	0.8168541			41.34	5.07	337.32	4.56	0.0000	***
4-10 m v. > 10-30 m		1.64	0.7657091			5.13	0.72	36.72	2.14	0.0163	
> 30 m v. 10-30 m		3.21	1.5759738			24.88	0.43	1428.45	2.04	0.0207	
direction of protection	4			16.10	0.0000						
east v. northwest		1.61	1.0159038			4.99	0.37	67.86	1.58	0.0569	
north v. northwest		2.73	1.1216853			15.26	0.85	272.57	2.43	0.0076	***
northeast v. northwest		0.23	1.1867112			1.26	0.06	26.69	0.20	0.4218	
south v. northwest		0.52	1.0655954			1.68	0.11	25.97	0.49	0.3133	
> 30 m and 0 m excluded due to collinearity with distance from protection categories											
lnthallus	1	1.09	0.1950789	42.74	0.0000	2.97	1.80	4.91	5.58	0.0000	***

*** highly significant

Table 7. Number of apothecia.

Both thallus size and elevation help to predict the number of apothecia on an individual. The r^2 value indicates that much variation is left unexplained. Non-apotheciate individuals were not considered in this model.

				99 % C. I.					
Parameter	**DF**	**Estimate**	**SE**	**min**	**max**	**Z**	**p-value**	**F**	**p-value**
Intercept		0.49	0.56			0.88	0.1885		
ln(thallus area) cm^2	1	0.50	0.14	0.14	0.87	3.53	0.0002	7.22	0.0085
elevation	1	-0.52	0.16	-0.92	-0.11	-3.29	0.0005	10.84	0.0014
r^2 = .1583									

Table 8. Area of apothecia.

Thallus size and elevation predict the area of the apothecia produced by an individual lichen. Non-apotheciate individuals were not considered in this model.

				99 % C. I.					
Parameter	**DF**	**Estimate**	**SE**	**min**	**max**	**Z**	**p-value**	**F**	**p-value**
Intercept		-4.90	0.54			-9.06	0.0000		
ln(thallus area) cm^2	1	1.04	0.14	0.68	1.39	7.50	0.0000	50.50	0.0000
elevation	1	-0.37	0.15	-0.76	0.02	-2.43	0.0076	5.89	0.0171
r^2 = .3700									

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Chemical distribution is contrary to pattern of geographic parthenogenesis in the lichenized-fungi, *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis*

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Abstract. A pattern in which the range of asexual species tends to reach higher elevations and latitudes than closely related sexual species, called geographic parthenogenesis, has been observed in vascular plants and animals. Little has been done to see if this pattern holds with fungi. We report the presence of rare chemicals and chemotypes within populations of the lichenized-fungi, Xanthoparmelia cumberlandia *and* Xanthoparmelia coloradoënsis, *and discuss the implications of these findings for the distribution of sexual reproduction. The proportion of individuals with rare chemicals, a possible indicator of outcrossing, did not vary with elevation, but appears to be affected by the quality of protection from wind or sun. Unique chemotypes were more common in upper elevation populations, possibly indicating that more outcrossing is occurring at the high elevation sites. Our findings indicate that the distribution of sexual reproduction within these fungal species does not follow the general pattern of geographic parthenogenesis, which may*

imply that selection pressure on sex in fungi operates differently than in other organisms.

Observations of vascular plants and animals reveal that asexual species tend to range into higher latitudes, and higher elevations (Bierzychudek 1987) and in more marginal areas (Bell 1982) than do their sexual sister species. This geographic pattern, frequently called geographic parthenogenesis, has provoked the development of various evolution of sex theories (reviewed in Bell 1982; Bierzychudek 1987; Hurst and Peck 1996).

Most explanations depend on the idea that, all else being equal, asexuality is more cost efficient because each offspring contains 100% of the parents genetic information (Maynard Smith 1971; Maynard Smith 1978). Sexuality will be favored only when the benefits of outcrossing and recombination outweigh the cost.

Some argue that the benefits of sex will be most helpful when the main selective pressures are biological (predators, competitors, and parasites) (Glesener and Tilman 1978), with parasites generally considered the most important (Glesener and Tilman 1978; Hurst and Peck 1996; Lively and Howard 1994). Due to the larger genetic pool available to sexuals through recombination, sexual populations are able to reduce intra-specific competition as well as create rare genotypes that avoid problems with coevolving organisms. On the other hand, when an organism's surroundings are not so rapidly and unpredictably changing, such as when the major selection forces are abiotic, recombination is not as necessary and the cheaper option, asexuality, is favored (Glesener and Tilman 1978).

Other hypotheses focus more on the asexuals ability to reproduce without a mate (Gerritsen 1980). In marginal habitat, where growing season is shorter and reproduction is slower, migration is more frequent and mates are rarer, so asexuals have an advantage (Peck et al. 1998). Advantages for colonization also include the fact that once established, asexuals are expected to reproduce faster because all their reproductive energy can be devoted to the production of female progeny avoiding the cost of producing males (Bierzychudek 1987). A similar idea maintains that asexuals have an advantage in areas with low population density where mates are limited, but that in areas of high density, sexuals dominate (Bell 1982; Gerritsen 1980). This view depends on the non-equilibrium view of the environment. In other words, sexuals can live in places where asexuals thrive, but there is such frequent disturbance that asexuals consistently have the advantage during recolonization (Bierzychudek 1987).

While these arguments make intuitive sense, it is possible that the advantages of sex are different for different organisms. Lichenized-fungi, for example, are known to thrive in marginal habitats. They are one of the few organisms found in central Antarctica, tundra, and desert soils. Perhaps competition for these organisms is just as great in higher elevations and latitudes. Or perhaps, as slow-growing organisms, other pressures such as deleterious mutations (Hurst and Peck 1996) are more important than biotic/abiotic pressures in the maintenance of sex.

Most fungi have the option of both sexual and asexual reproduction, which makes them facultatively sexual. Little research has been focused on the possibility that either sex or asex may be preferred *within* a species at different latitudes and elevations, as is seen *between* species.

Some studies have noted that species of lichenized-fungi with sexual structures (apothecia), are in the majority in marginal habitats (Fahselt et al. 1989; Ott 1987; Sancho and Valladares 1993). This suggests that, contrary to the pattern observed in vascular plants and animals, lichenized-fungi may actually favor a sexual strategy in marginal areas.

One explanation for this paradox depends on the recent studies showing that even putatively obligate sexuals have been shown to self-fertilize (Marra and Milgroom 2001; Murtagh et al. 2000). Murtagh et al. suggest this discovery of homothallism and lichens' ability to self-fertilize could mean that frontier lichens are engaging in asex with sexual structures (Murtagh et al. 2000). Perhaps, the key factor in marginal habitat is not outcrossing, but propagule size. Since the sexual spores disperse further (Hestmark 1992), perhaps this explains why apotheciate lichen are the first to appear in disturbed and/or marginal habitats. Or, as previously suggested, maybe different selective pressures than are common to animals and vascular plants are important to these slow-growing organisms.

On Boulder Mountain, UT, *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis* were more likely to have apothecia (spore-producing sexual structures) at lower elevations (Jackson 2004), a pattern consistent with the general pattern of geographic parthenogenesis.

In this paper, we take the same individuals that were shown to have a higher frequency of sexual reproductive structures (apothecia) at lower elevations on Boulder Mountain, Aquarius Plateau, UT (Jackson 2004), and examine their secondary chemistry for clues concerning the distribution of outcrossing and recombination.

Some researchers have suggested that the amount of chemical variation as evidenced by the proportion of individuals with rare chemicals or chemotypes is an indication of sexual reproduction, since outcrossing and recombination are likely to produce rare phenotypes (Culberson et al. 1988; Peck et al. 1998; Porter and St. Clair in review). *Rhizoplaca melanopthalma* located along a 1200 m elevation gradient in southern Utah showed a chemotype gradient, with more secondary compounds and rare chemotypes located at lower elevations (Porter and St. Clair), a finding consistent with more outcrossing at lower elevations.

We test the hypothesis that more rare chemicals and unique chemotypes will be found at the lower elevation, thus indicating increased rates of outcrossing at those locations.

This study has important implications for the maintenance of facultative sex. Furthermore, this study investigates the distribution of sexual reproduction in lichenized-fungi, a group that has been largely overlooked in this context.

MATERIALS AND METHODS

Species.—As complex symbiotic systems, lichens consist of a fungus, an alga, and/or a cyanobacterium. We focus on the fungal partner within this system, specifically species *Xanthoparmelia cumberlandia* and *Xanthoparmelia coloradoënsis.* Because these two species are indistinguishable in the field, samples from both were collected. The only definite character that distinguishes the two species is the presence of stictic acid in *X. cumberlandia,* and the presence of salazinic acid in *X. coloradoënsis.* This

study was made with the assumption that any possible genetic differences between the two species will not influence the production of rare chemicals and unique chemotypes.

Seventy-nine percent of the individuals we sampled were sterile, bearing no specialized sexual or asexual structures. We assume, therefore that the only means of reproduction available to these individuals is thallus fragmentation, a rudimentary form of asexual reproduction. It is these individuals to which we refer when we discuss asexuals in our sample.

Sampling Strategy.—Sampled at random distances from the road, approximately ten individuals were collected at each site. Eight sites were selected in a lower west elevation area (2400-2500 m), nine sites in a lower-east elevation area (also 2400-2500 m), and seventeen sites in an upper elevation area (3300-3400 m). Due to limited access at the lower elevations, north and south sites were not investigated.

Abiotic conditions.—The upper elevation is generally more moist and colder. The lower-west side receives less rain and more heat than the lower-east populations (US Department of Agriculture et al. 1999, Western Regional Climate Center, www.wrcc.dri.edu).

Identification of secondary chemicals.— Chemicals were extracted and identified using solvent G (Culberson et al. 1981) using one dimensional thin-layer chromatography. We were careful not to include apothecia, which sometimes produce unique chemicals (Hyvärinen et al. 2000) in our chemical analysis. A dime-sized piece of each thallus was placed in a 1.5 dram vial 1/3 full of acetone. After sitting in a sonicator for approximately 5 minutes, the acetone was removed and stored in separate 1.5 dram vials. We repeated this process a total of three times. The acetone vials were

then evaporated until approximately one ml of fluid remained. A spot of extract was placed 2 cm from the bottom of a 20 x 20 cm aluminum backed, F_{254}, silica gel plate using a one mm capillary tube. Each spot was at least 2 cm from the edge and 1 cm from the next sample. Solvent G (Toluene - ethyl acetate - formic acid 139:83:8) was prepared in a Desaga tank (Culberson et al. 1981). Plates were then placed in the tank for approximately 25 minutes, or until the solvent front reached a line 10 cm from the original spots of extract. Plates were then dried under a fume hood.

Color and distance from origin of each chemical spot was recorded while inspecting plates under white, short UV (254 nm), and long UV (366 nm) light. After spraying the plates with 10% H_2SO_4 solution, and baking them at 110° C for 10 minutes, descriptions of spots were again recorded under the same wavelengths of light. By dividing the distance of a specific chemical from its point of origin by the distance of the solvent front from its point of origin, and multiplying by 100, relative Rf values were calculated.

In order to standardize identification of chemicals, two to three samples from a previous plate were spotted on subsequent plates. Comparisons of spots were made with known chemicals in *Usnea sp., Pseudevernia intensa, Lobaria pulmonaria* and *Parmelia saxitalis*. Information from Culberson et al. (1981) and Huneck and Yoshimura (1996) were also used to identify chemicals. In order to make the most parsimonious identification of chemicals, chemicals which showed a high range in Rf values were re-evaluated. If chemicals were a) not identified on the same plate, b) did not occur in the same individual, c) had a difference in Rf values of less than 5, and d) exhibited similar colors they were combined and considered the same chemical. We renamed fifteen

chemicals using this procedure. A total of sixty chemicals were identified, forty-six of which were from *X. cumberlandia* or *X. coloradoensis*. The other fourteen chemicals were from an isidiate morph (*X. plittii* or *X. mexicana*), *Pseudevernia intensa, Usnea sp.,* and/or *Parmelia saxatilis.*

Habitat and morphological measurements.—Besides elevation, aspect, direction of protection, and distance from protection were also considered for their affect on outcrossing and reproduction. We also considered morphological traits such as thallus area and the presence of sexual structures (apothecia).

Size was determined by taking picture of each thallus with a ruler included. Pictures were later adjusted for size in Adobe Photoshop CS (Adobe Systems Inc. 2003). We used the paintbrush tool to outline each thallus, and the paintbucket tool to fill it in. Saved as a TIF file, these pictures were imported into NIH image 1.62 (freeware downloaded from NIH at rsb.info.nih.gov/nih-image/download.html) where the number of pixels was used to estimate thallus area (Ruzin 2002; http://microscopy.berkeley.edu/).

Aspect, or the direction a lichen faces, was measured with a compass and recorded as one of the following: N, S, E, W, NW, NE, SW, SE, and up. "Up" refers to an individual which lies on top of a rock.

Since wind and sun can potentially affect the chemicals produced by lichens we measured the direction and distance from protective objects (usually a tree). The same categories were possible as for aspect, with the addition of "> 30 m", which was assigned to those individuals with protective structures so far away that a decision about which tree was providing the most protection became arbitrary. Distance from protection categories included 0 m, 0.5-4 m, 4-10 m, 10-30 m and > 30 m. Distance categories

were chosen based on our best guess as to how distance from a tree affects exposure to wind and/or sun.

Since the lower elevation populations were either on the east or west sides of the mountains, location was also considered in model building.

Statistical analyses.—In order to account for the different spatial scales at which samples were collected, we simply compared chemicals and chemotypes within sites only. Thus we assumed that rare chemicals and unique chemotypes found within a site were indications of outcrossing and recombination.

Chemicals were considered rare if they were found with a frequency less than 10% within their site. By this definition, a chemical that is rare in one site may be common in another. Immigration is ignored, though we understand that a more realistic scenario would include the possibility that a rare chemical could appear in a population through immigration rather than outcrossing and recombination.

Chemotype was defined as the particular combination of chemicals an individual produces. Chemotypes were considered unique if no other individual within a site had the same chemotype. Again, a chemotype may be unique within a site, while common in the global population.

Individuals were categorized in a binary fashion as producing or not producing rare chemicals, and possessing or not possessing a unique chemotype. In Splus, (MathSoft Inc. 1999) forward-selection logistic regression was used to identify which environmental and/or morphological variables best predicted the presence of rare chemicals or unique chemotypes.

RESULTS

Distance from protection and direction of protection significantly predicted the presence of rare chemicals within an individual (distance from protection: p-value = 0.0116, $F_{2,319} = 4.53$; direction of protection: p-value = .0001, $F_{4,319} = 5.89$; Table 9, Figure 15). Individuals located at medium distances from protection (4-10 m) were most likely to have no rare chemicals, while individuals located over 30 m from protection were the most likely to have rare chemicals (p-value = 0.0015, Z = 2.96; Table 1, Figure 15).

Direction of protection as a whole significantly reduced the residual variation in the model predicting the presence of rare chemicals (p-value = 0.0001, F = 5.89). Lichens with protection from the east were most likely to have rare chemicals, while lichens with protection from the north-east were least likely to have rare chemicals, though this difference was only marginally significant (p-value = 0.0278, Z = 1.91; Table 9, Figure 15).

Elevation was the most significant predictor of the presence of unique chemotypes, along with distance from protection (elevation: p-value < 0.0001, $F_{1,319} = 72.82$; distance from protection: p-value < 0.0001, $F_{4,319} = 22.88$; Table 10, Figure 16). An individual in the upper elevation was 17 times more likely to have a unique chemotype than was an individual in the lower elevation (p-value <0.0001, Z = 7.23; Table 10; Figure 16). Furthermore, individuals located less than 4 meters from protection are more likely to have a unique chemotype than are individuals located between 4-10 m (p-value = 0.0073, Z = 2.44; Table 10, Figure 16).

DISCUSSION

These findings present an ambiguous picture concerning the amount of outcrossing and recombination that is occurring in these populations. Though individuals in the lower elevation populations are more likely to have apothecia (Jackson 2004), those same individuals are *less* likely to have unique chemotypes. Additionally, elevation does not significantly change the odds of rare chemicals being present when direction and distance of protection are taken into account.

Clearly, we are not sure how well either sexual reproductive structures or chemical variation indicate outcrossing and recombination. To have a clear picture of outcrossing rate, we must look at more direct measures such as those described by Kroken and Taylor (Kroken and Taylor 2001), which make use of molecular genetic tools.

An alternative hypothesis explaining the increase in the number of chemotypes at higher elevations might consider the haploid nature of lichenized-fungi, in which mutations are easily expressed. Perhaps the greater amount of radiation experienced by individuals at high elevations can explain the diversity of chemicals within populations. Our observation that individuals located furthest from protection, regardless of elevation, are more likely to have rare chemicals supports the idea that increased radiation will cause more mutations that will in turn lead to unique chemotypes. Given this hypothesis, we would also expect that individuals with protection on their south side would be least likely to have rare chemicals. Our data are not clear on this point, since the most

significant difference in the proportion of individuals with rare chemicals was between individuals with protection to the east and north-east. If increased mutation rate is the reason for greater probability of rare chemicals and chemotypes, our data suggest that solar radiation is not the only important mutagen to consider.

The fact that *X. cumberlandia* exhibits a pattern of chemical variation opposite that of *Rhizoplaca melanopthalma,* for which more rare chemotypes were found at lower elevations, implies that chemical variation is a taxon specific process. Other studies have shown that the concentration of some chemicals, such as usnic acid, does increase with elevation (Rundel 1969), but few studies have recorded how the diversity of chemicals changes with elevation.

If chemical variation is indicative of outcrossing rate, lichens from our study have an increased outcrossing rate in the higher elevation locations, a pattern opposite to the trend found in vascular plants and animals. The presence of sexual structures does not necessarily correlate with outcrossing rate in these organisms. This study emphasizes the need to further investigate outcrossing rate in facultative organisms including fungi.

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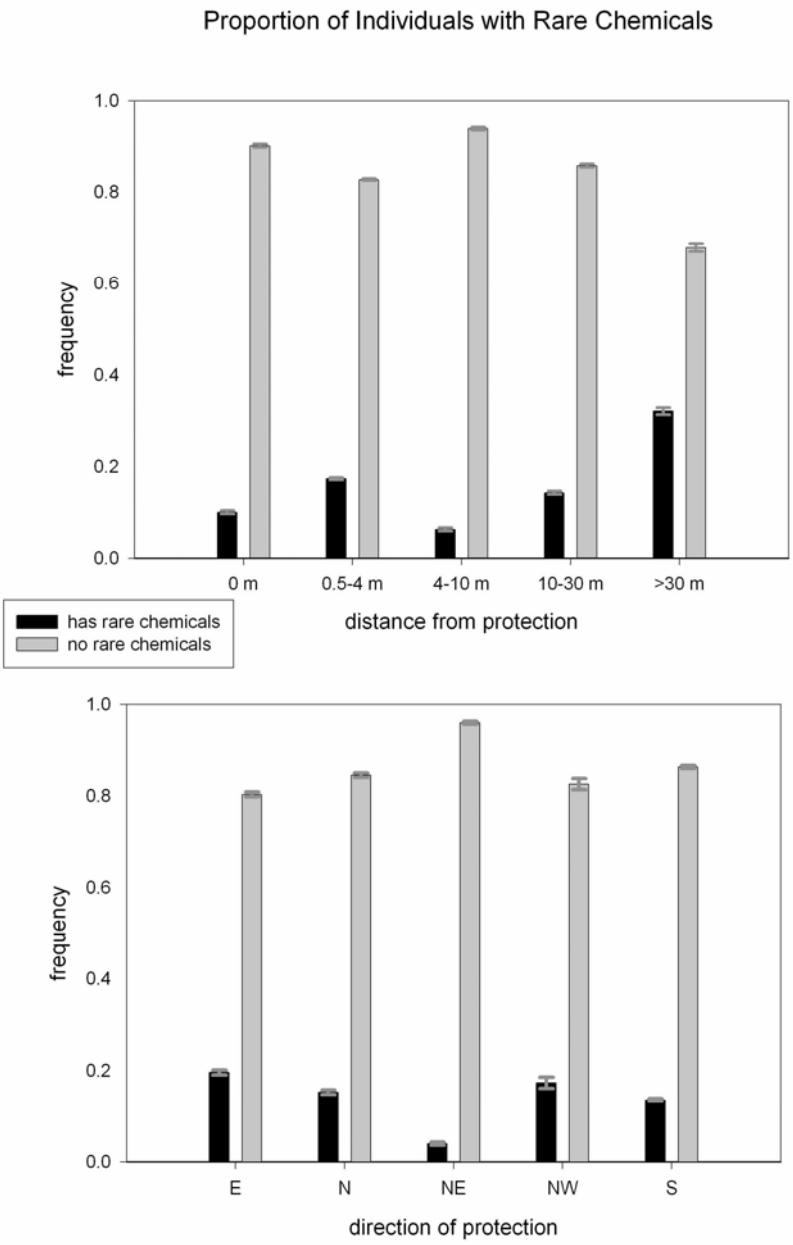

Figure 15. Rare chemicals.

The distance of an individual from protection is a strong predictor of the probability that an individual has a rare chemical. Individuals located at a mid-distance from protection

are least likely to have rare chemicals. Individuals with protection to the northeast of them are least likely to have rare chemicals.

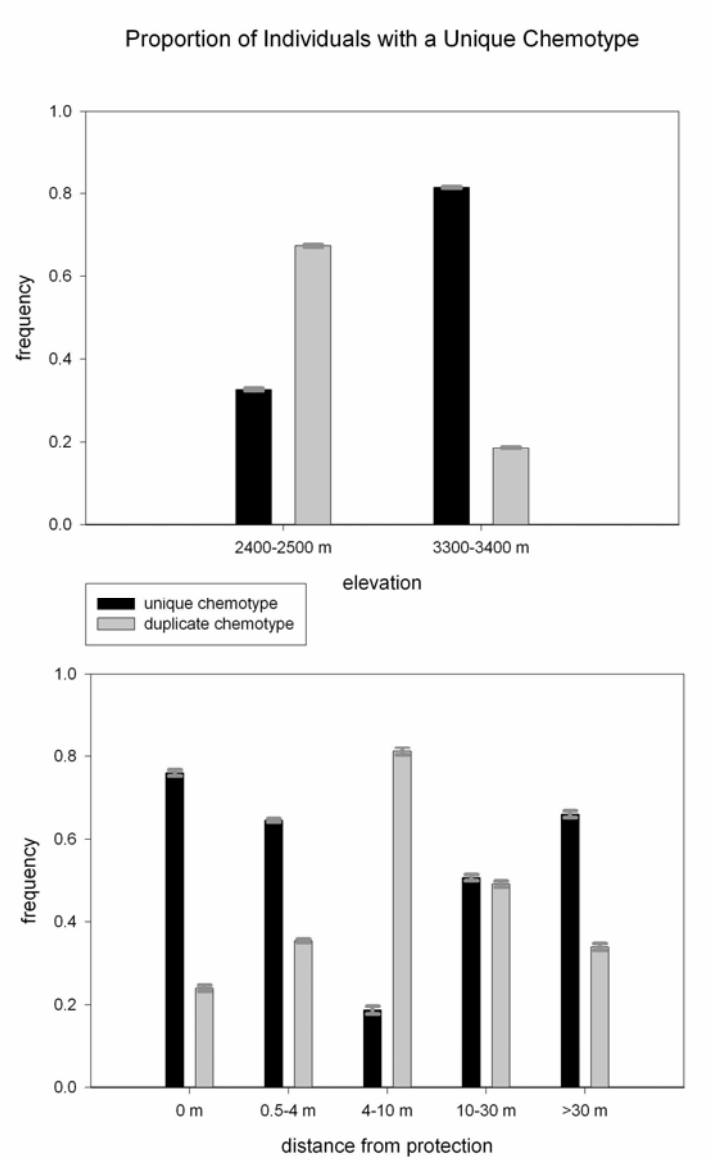

Figure 16. Unique chemotypes.

Elevation is a significant predictor of the presence of a unique chemotype. Individuals located at 3300-3400 m on Boulder Mountain are 17 times more likely to have a unique chemotype than are individuals from 2400-2500 m. As with the probability that an individual will have a rare chemical, distance from protection is a significant predictor of

the probability that an individual has a unique chemotype. Individuals mid-distanced from protection (4-10 m) are least likely to have a unique chemotype.

TABLES

Table 9. Probability that an individual will have rare chemicals.

Distance from and direction of protection from wind or sun were better predictors of the presence of rare chemicals in an individual than was elevation.

							99% C.I.				
Parameter	DF	Estimate	SE	Drop in Deviance	p-value	Odds Ratio	min	max	z-stat	p-value	
(Intercept)	1	-4.51	1.23						-3.65	0.00013	***
distance from protection	2			4.53	0.0116						
0 m v. 4-10 m		2.31	1.32			10.08	0.34	300.70	1.75	0.04014	
0.5-4 m v. 4-10 m		1.44	0.77			4.24	0.58	31.04	1.86	0.03119	
10-30 m v. 4-10 m		1.15	0.82			3.15	0.38	26.21	1.39	0.08210	
> 30 m v. 4-10 m		3.76	1.27			42.85	1.64	1117.99	2.96	0.00153	***
direction of protection	4			5.89	0.0001						***
east v. northeast		1.99	1.04			7.28	0.51	104.84	1.91	0.02783	
north v. northeast		1.71	1.06			5.53	0.37	83.48	1.62	0.05269	
northwest v. northeast		1.72	1.12			5.56	0.31	99.13	1.53	0.06295	
south v. northeast		1.31	1.05			3.71	0.25	54.42	1.25	0.10489	

> 30 m and 0 m excluded due to collinearity with distance from protection categories

*** highly significant

Table 10. Probability that an individual will have a unique chemotype.

Elevation and distance from protection significantly predicted whether an individual would have a unique chemotype.

							99% C.I.				
Parameter	DF	Estimate	SE	Drop in Deviance	p-value	Odds Ratio	min	max	z-stat	p-value	
(Intercept)	1	-1.47	0.45						-3.24	0.00060	***
location	1			72.82	5.83E-16						***
3300-3400 m v. 2400-2500 m		2.83	0.39			16.87	6.18	46.08	7.23	0.00000	***
distance from protection	4			22.88	1.24E-16						***
0 m v. 4-10 m		0.42	0.67			1.52	0.27	8.42	0.63	0.26544	
0.5-4 m v. 4-10 m		1.23	0.51			3.43	0.94	12.56	2.44	0.00734	***
10-30 m v. 4-10 m		0.20	0.58			1.22	0.28	5.37	0.34	0.36752	

> 30 m v. 4-10 m	-0.69	0.66		0.50	0.09	2.76	-1.04	0.14819

*** highly significant

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