Introduction—Survival strategies used by North American ferns during the Last Glacial Maximum (LGM) are unknown. Like other plants, ferns had two options during the last glaciation when the Cordilleran and Laurentide ice sheets covered much of northern North America. They either could become locally extinct and persist beyond the southern terminus of the ice sheets until the ice retreated and recolonization was possible; or they could persist locally in isolated northern refugia and recolonize deglaciated areas from a much shorter distance. Of particular relevance to Alaskan ferns is the existence of the Beringian refugium, a large unglaciated area comprising much of northeastern Siberia and extreme northwestern North America (Hultén, 1937; Fig. 1). Beringia has been shown to serve as a refuge for a variety of Alaskan plants (Brubaker et al., 2005; Anderson et al., 2006; Beatty and Provan, in press). Although ferns have been shown to utilize glacial refugia in other regions of the world (Vogel et al., 1999; Trewick et al., 2002; Shepherd et al., 2007), the question has never been investigated in North America.

The genus *Cryptogramma*, commonly called the parsley ferns, consists of 7-10 species worldwide with a mostly circumboreal distribution (Alverson, 1989) and represents a potential model system for studying glacial refugia in seed-free plants. This small genus is in the basal clade of the Pteridaceae, the cliffbrake fern family (Schuettpelz et al., 2007; Fig. 2), and current efforts by the primary investigator are producing a comprehensive phylogenetic assessment of the genus based on molecular data (Metzgar, unpub.). Two species in section *Cryptogramma* are found in Alaska; *Cryptogramma acrostichoides* and *C. sitchensis* are closely related species with the diploid *C. acrostichoides* being one of the progenitors of the allotetraploid *C. sitchensis* (the other parent has been hypothesized to be the Asian taxon *C. raddeanna*; Alverson, 1989). The group is ideal to search for evidence of glacial refugia, as fossil remains show they were present on the Kenai Peninsula in the Late Miocene and are found here in the present day, leaving their behavior during the intervening time span open to investigation (Reinink-Smith and Leopold, 2007).

Knowledge of organismal behavior during previous episodes of dramatic climatic shifts would aid in projecting their response to ongoing global climate change. Niche modeling combines distribution data with climatic variables to project the current, past and future ranges of organisms. This method has predicted large potential shifts in the ranges of vascular plants due to global climate change (Loarie et al., 2008). It has not, however, been used to explore the response of ferns to global climate change, nor have potential benefits of polyploidy in a rapidly changing climate been examined in ferns. With overlapping ranges (Fig. 3A, Fig. 3B) and differing ploidy levels, this proposed *Cryptogramma* species pair would be an ideal test case.

Research objectives and approach—Both our efforts to identify glacial refugia and project future range shifts in *Cryptogramma* species will require collecting a large number of samples throughout much of each species respective range. Funding has already been obtained by the investigator from EPSCoR to collect and sequence samples of *C. sitchensis* populations located in southcentral Alaska this summer and funding has been requested to collect and sequence samples of both species from southeast Alaska and coastal Canada. Therefore, we ask for funding to allow highly accurate GPS locality data to be gathered for new collections, for laboratory expenses to sequence *C. acrostichoides* samples from southcentral Alaska and to expedite niche modeling for both taxa.

Localities will be selected to provide coverage throughout the range, as well as emphasizing hypothesized glacial refugia in coastal and southern regions of British Columbia (e.g., Haida Gwaii or Queen Charlotte Islands; Beatty and Provan, in press). Collaborators will collect specimens of *C. acrostichoides* from the Lower 48 and central Canadian portions of its range. Voucher specimens will be collected from each locality and deposited at the University of Alaska Museum Herbarium (ALA) and collection data will be entered into Arctos, an open access natural history collection database (http://arctos.database.museum/SpecimenSearch.cfm). Specimens collected will be used to ground-truth the current niche model for each species (Fig. 4). Leaf tissue from each voucher will be preserved on silica gel for DNA extraction and, when possible, up to 10 population samples will be collected.

Laboratory work will focus on PCR amplification and direct sequencing of several plastid loci (*trnL-trnF*, *rps4-trnS*, *trnP-petG*) that are of proven utility for population-level studies in ferns. Unlike many seed plants, fern plastid sequences are highly variable and these loci have been successfully used in population genetics studies to identify glacial refugia in other fern genera and study polyploidy complexes in the Pteridaceae (Vogel et al., 1999; Trewick et al., 2002; Rothfels et al., 2008; Grusz et al., 2009). This investigator has first-hand experience using these markers in reconstructing fern phylogenies (Metzgar et al., 2007; Metzgar et al., 2008).

Objective 1: Explore possible glacial refugia and recolonization in *Cryptogramma sitchensis* and *C. acrostichoides*. Colonization from glacial refugia into recently deglaciated areas has been hypothesized to have mainly comprised occasional long distance dispersal events by a single individual (Rowe et al., 2004). Thus, new populations would undergo a severe bottleneck event, resulting in low genetic diversity in more recent populations and high genetic diversity in refugial source populations.

By using plastid sequence data we generate from collection samples, we will reconstruct a phylogeny showing evolutionary relationships between samples. Bayesian inference (Ronquist and Huelsenbeck, 2003), maximum likelihood (Swofford, 2002) and maximum parsimony (Swofford, 2002) search algorithms will be used to estimate the phylogenetic tree. This phylogeny will allow us to identify any major geographical segregation between samples (i.e., northern vs. southern clades, or eastern vs. western clades). Haplotype networks will then be constructed using TCS 1.13 (Rowe et al., 2004). This haplotype network will show relationships between ancestral and derived populations, with a measure of mutational difference calculated. Ancestral populations would be internal nodes that are the source for many different derived tip node haplotypes. Using these results, we will be able to identify glacial refugia as source populations that were responsible for recolonizing deglaciated land.

Objective 2: Using niche modeling for *Cryptogramma sitchensis* and *C. acrostichoides* to calculate extinction probabilities under varying climate change scenarios. Niche modeling is a powerful geospatial analytical tool that allows users to model present day ranges and calculate sensitivity to changing environments. We have constructed preliminary niche models for both *C. sitchensis* (Fig. 4A) and *C. acrostichoides* (Fig. 4B) using specimen data from multiple DiGir providers in GBIF (http://www.gbif.org) and an openModeller tool that produces a current distribution niche model. These range maps predict a distribution for each taxon that is far larger than their actual range (Fig. 3A, Fig. 3B). This shortcoming is due to the extremely coarse scale of the GBIF model.

By extensively collecting samples from each species' range, we can produce a far more reliable data set that will overcome poor model performance stemming from insufficient number of data points (Loiselle et al., 2008) and wide geographic variability of *C. acrostichoides* range disjunctions (Godsoe, 2010). We will also use GARP (Stockwell and Peters, 1999), a more sophisticated algorithm that will perform geostatistical analyses that will correlate environmental predictors with presence/ absence data. Our final ecological niche models will be calculated using over 20 predictive layers, including mean annual temperature, mean annual precipitation, aspect and slope (Waltari and Guralnick, 2009). Presence data will be collected from GBIF and voucher specimens gathered in the field. A pseudo-absence layer will be generated and overlain in the construction of each niche model generated. In addition, we will calculate future distribution patterns using the niche models by incorporating three different climate scenarios projected by the IPCC (Solomon et al., 2007) and corresponding to mild, moderate and extreme temperature increases. This will allow us to make effective management recommendations.

Objective 3: Assess migration capacity of *Cryptogramma sitchensis* and *C. acrostichoides* based on results from glacial refugia analysis. Many glacial refugia studies have been performed for taxa that have slow dispersal rates such as deciduous trees (McLachlan et al., 2005) and chipmunks (Rowe et al., 2004). Few papers have looked at the post-glacial behavior of ferns, which have minute (60μM in *Cryptogramma*; Alverson, 1989), easily dispersed propagules, with no analysis of North American ferns yet conducted. These spores are commonly dispersed long distances, with 500 miles described as "not a significant barrier" to dispersal (Tryon, 1970). This has important implications for the ability of different taxa to adapt to changing climates. A beech tree that was only capable of migrating 100m/yr after the last glacial maximum (McLachlan et al., 2005) is unlikely to be able to move its range hundreds of miles northward in a century to survive a warming climate. However, if our glacial refugial analysis shows that *Cryptogramma* species were capable of migrating hundreds of miles in a short period of time, it would indicate a much higher probability that they would be able to adjust to new climatic conditions by colonizing new areas before going extinct in their present range.

We will study the timing and tempo of migration from glacial refugia using a phylogeographic Bayesian stochastic search variable selection approach that will allow us to calculate estimates of population age based on the data from our phylogeny and a relaxed molecular clock (Lemey et al., 2009). With this approach implemented in BEAST (Drummond and Rambaut, 2007), we will be able to reconstruct the timing and pattern of post-glacial dispersal in each *Cryptogramma* species. These results will allow us to determine if there is a historical precedent in *Cryptogramma* for rapid, widespread colonization of new suitable habitat created by global climate change.
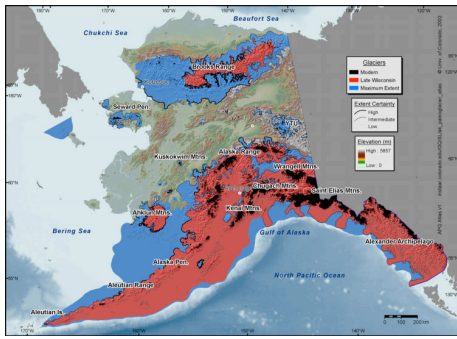
Fig. 1. Extent of glaciation and refugia in Alaska during the LGM. Modified from Manley and Kaufman, 2002.
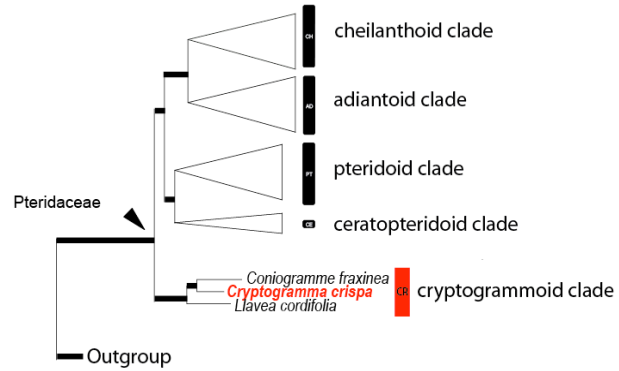
Fig. 2. Phylogeny of the Pteridaceae generated using a combined data set of *rbcL*, *atpB* and *atpA*; *Cryptogramma* is shown in red. Modified from Schuettpelz et al. 2007.
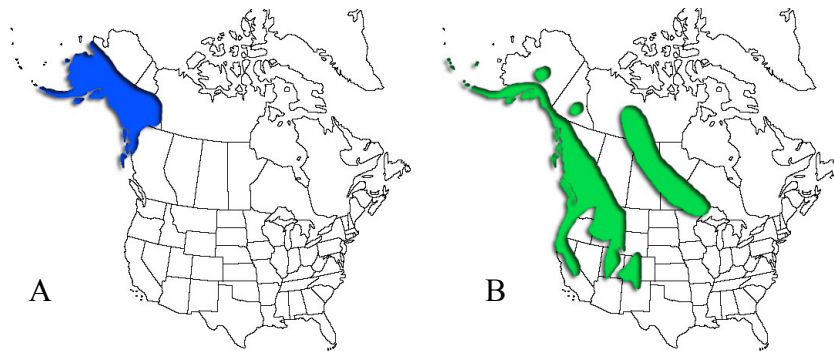
Fig. 3. Range maps for selected *Cryptogramma* species. Modified from Alverson, 1989. A) tetraploid *Cryptogramma sitchensis*. B) diploid *Cryptogramma acrostichoides*.
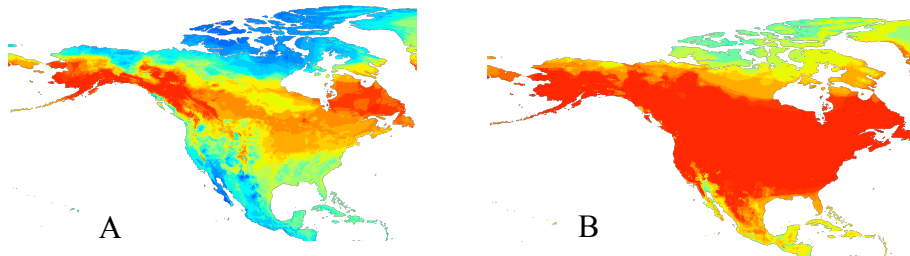
Fig. 4. Preliminary niche model for selected *Cryptogramma* species. Red indicates highest probability and blue indicates lowest probability of suitable habitat. A) *Cryptogramma sitchensi*s. B) *Cryptogramma acrostichoides*.

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Permit
Research on Federal, State, and some private land may require a permit.
Do you (or your advisor) have the required permit for your proposed research?   **YES**

If you do not have the required permit:
   • Provide the name of the issuing agency and person in charge of permitting (and their contact info.).
   • Describe at what stage you are in the permitting process (i.e., the application is submitted, but no response, or you have a verbal assent, but not in writing, etc.), and the likelihood that you will receive the permit.
   • *Applicants that have not started the permitting process at the time of proposal submission may be at a disadvantage during proposal evaluation.*

Agency: Agency contact person and their contact information (phone and/or e-mail address):   Stage of the permitting process and likelihood of receiving the permit:

Budget

Please include an itemized budget and budget justification being as detailed as possible (add or delete rows as needed):		
Description	**Amount**	**Source of Funding**
Field work		
Collecting expenses (food, lodging, vehicle rental): southcentral Alaska collecting	$2,426	Alaska EPSCoR
Collecting expenses (food, lodging, vehicle rental): southeastern Alaska and coastal Canada collecting	$4,173	CGC (requested)
Garmin GPS unit	$ 350	AQC (requested)
Lab work		
DNA sequencing and laboratory reagents for southcentral Alaska *C. sitchensis* specimens:	$2,519	Alaska EPSCoR
DNA sequencing (3 loci) and laboratory reagents for southcentral Alaska *C. acrostichoides* specimens (55 samples at $10.90/ea):	$ 600	AQC (requested)
DNA sequencing and laboratory reagents for southeastern Alaska and coastal Canada specimens (both species):	$5,808	CGC (requested)
GIS site license for ArcGIS software (to be purchased from Geophysical Institute)	$ 250	AQC (requested)
Amount requested from AQC	**$1,200**	
TOTAL Project:	**$16,126**	

Budget Justification:

Student investigator has obtained $4,945 to collect and sequence *Cryptogramma sitchensis* from southcentral Alaska in 2010 for three plastid DNA markers and applied for $9,981 to collect and sequence both *C. sitchensis* and *C. acrostichoides* from southeastern Alaska and western Canada. These funds will cover basic collecting expenses (food, lodging, vehicle rental) and all laboratory expenses to generate sequences from *C. sitchensis*. However, these funds do not provide laboratory expenses for *C. acrostichoides* samples from southcentral Alaska.

Therefore, AQC funds are requested to generate sequences from *C. acrostichoides* samples from southcentral Alaska ($600). In addition, AQC funds are requested for a Garmin GPS unit to record highly accurate GPS locality data for all new collections ($350) and for a GIS site license for the time-intensive process of generating niche models for both species ($250). If fully funded, this study will provide the most complete picture worldwide of fern responses to glacial maxima. If CGC funding is not received, sampling of southcentral Alaska populations will be reduced and samples from exemplar populations collected by collaborators in southeast Alaska and coastal Canada will be used to complete the project. I am transferring from UAF's master's program to its Ph.D. program, so the results of this project will also be used to apply for a Doctoral Dissertation Improvement Grant from NSF to obtain funding for additional studies on glacial refugia and climate change resilience in other species of *Cryptogramma* worldwide.