BIOGEOGRAPHY AND EVOLUTION OF FLOWERING PLANTS IN THE AMERICAN WEST: *GAILLARDIA* (ASTERACEAE) AND *SYNTHYRIS* (PLANTAGINACEAE)

By

KAROL MARLOWE

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of KAROL MARLOWE find it satisfactory and recommend that it be accepted.

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Chair

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BIOGEOGRAPHY AND EVOLUTION OF FLOWERING PLANTS IN THE AMERICAN WEST: *GAILLARDIA* (ASTERACEAE) AND *SYNTHYRIS* (PLANTAGINACEAE)

Abstract

by Karol Marlowe, Ph.D.
Washington State University
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Chair: Larry D. Hufford

This study explores the biogeography of flowering plants in the American West. We examine the biogeography of both the low elevation, largely xeric-adapted *Gaillardia* (Asteraceae) that radiated out of the Chihuahuan Desert and two clades of the cool-adapted *Synthyris* (Plantaginaceae) that diversified in mountain environments. We examine evolutionary processes concerning range shifts and consider how climate changes have influenced the distribution of organisms in the American West.

We used phylogeny reconstructions based on DNA sequences from nuclear ribosomal internal and external transcribed spacers and plastid *trnT-trnF* regions to infer sister group relationships and identify the geographic origin and avenues of range expansion in North American *Gaillardia*. Phylogenetic analyses recovered three previously hypothesized sections and well supported clades associated with particular geographic regions. Dispersal-Vicariance Analysis reconstructed a Chihuahuan Desert origin and major range expansions eastward into central Texas and along the Gulf Coast, northwest into Arizona and the Intermountain region, and northward onto the Great Plains and along the Rocky Mountains.

We used phylogeography to infer modes of speciation and migration patterns in two clades of *Synthyris*. The Southern Rocky Mountain clade of *S.* sect. *Besseya* is distributed in the Southern Rocky Mountains and *S.* sect. *Dissecta* is centered in the Northern Rocky Mountains with Olympic Mountains disjuncts. Both clades have species currently restricted to high elevation interglacial refugia. We used plastid DNA *trnT-trnL* and *psbA-trnH* intergenic spacer sequences to infer haplotypes for phylogeographic analyses. Haplotype distributions in both clades were consistent with broad scale gene flow in their ancestral lineages with subsequent random capture of haplotypes in widespread populations. Disjunctions in both sections were likely caused by fragmentation of formerly widespread populations. Genetic signatures were consistent with widespread migration in the southern clade especially in the lower elevation species, whereas we found evidence of more isolation in *S.* sect. *Dissecta*. In both clades we found evidence consistent with speciation following peripheral isolation, and in the southern clade we find evidence consistent with a hypothesis of allopolyploid speciation. We infer that Pleistocene glaciations played an important role in species origins and shaping extant distributions in alpine *Synthyris*.

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Dedication

This dissertation is dedicated to Alan

who generously provided endless encouragement and support.

CHAPTER ONE: TAXONOMY AND BIOGEOGRAPHY OF *GAILLARDIA* (ASTERACEAE): A PHYLOGENETIC ANALYSIS

INTRODUCTION

Gaillardia are New World Asteraceae of the tribe Helenieae. *Gaillardia* share with other Helenieae basally non-caudate anthers, a paleaceous pappus, and herbaceous involucral bracts (Biddulph 1944) but differ from other members of the tribe in having setae or dentiform fimbrillae among the flowers rather than naked receptacles. Phylogenetic studies of Baldwin and Wessa (2000) found *Gaillardia* to be most closely related to *Helenium* and *Balduina*, and they placed these three genera in Helenieae subtribe Gaillardiinae.

Since *Gaillardia* was described by Fougeroux (1786), it has been the subject of three comprehensive systematic revisions (Gray 1884; Rydberg 1915; Biddulph 1944; Table 1). The most recent monograph of *Gaillardia* by Biddulph (1944) recognized 18 species, notably reducing several of Rydberg's (1915) species to synonymy. Biddulph raised questions about selected circumscriptions presented in her monograph because of limited availability of material. These questions centered largely on species she recognized as geographically widespread. For example, her concept of a polymorphic *G. pulchella* included as synonyms the previously recognized *G. picta*, *G. drummondii*, and *G. villosa*, while she opted to recognize the narrowly distributed *G. neomexicana* in Arizona. Subsequent workers have treated *G. neomexicana* as a synonym of *G. pulchella* (Kearney and Peebles 1960; Turner and Whalen 1975; Table 1). Circumscription of the widespread *G. pinnatifida* was also a concern. Biddulph was unsure whether the narrow endemic *G. flava*, known at the time of her monograph from only two specimens, was synonymous with *G. pinnatifida.* She recognized *G. multiceps* as separate from

G. pinnatifida but was later criticized by Kearney and Peebles (1960) for this decision. The diversity of *Gaillardia* along the Gulf Coast and in Texas also presented problems for Biddulph, and populations from these areas have received the attention of subsequent workers (Rock 1956; Correll and Johnston 1970; Waterfall 1971; Averett and Beaman 1975; Cronquist 1977; Turner 1979; Table 1). Biddulph (1944) also proposed the first formal subgeneric taxonomy for *Gaillardia*, in which the three sects. *Gaillardia*, *Agassizia*, and *Hollandia* were recognized. The new sect. *Austroamericania* was segregated recently from sect. *Agassizia* by Petenatti and Ariza Espinar (1995; Table 1) to accommodate taxa restricted to South America, including *G. cabrerae*, *G. megapotamica*, and *G. tontalensis* (Petenatti and Ariza Espinar 1993; Petenatti et al. 1996).

The distribution of *Gaillardia* raises several questions relevant to New World biogeography, especially evolutionary radiations in the North American West. *Gaillardia* is among the substantial number of taxa that have an amphitropical disjunction between North and South America (Constance 1963; Raven 1963; Solbrig 1972; Graham 1999). The South American populations are restricted to Uruguay and Argentina (Petenatti et al. 1996). In North America, the genus is found near the coasts in the Southeast, in the Chihuahuan Desert, throughout the Great Plains, Rocky Mountains and Intermountain region. Biddulph suggested that the sections of *Gaillardia* were differentiated geographically and hypothesized a geographic radiation of the genus from a “center of dispersal” in Texas. She envisioned sect. *Hollandia* radiating eastward through Texas along the Gulf Coast, sect. *Agassizia* radiating into both southeastern Texas and the southern Great Plains, and the more taxon rich sect. *Gaillardia* radiating into southwestern North America, the Rocky Mountains, and southern Great Plains. Biddulph hypothesized a secondary center of dispersal for sect. *Gaillardia* in Arizona and Utah

to account for taxic diversity in that region. Stoutamire (1977) questioned Biddulph's hypothesized out-of-Texas radiation of *Gaillardia*, hypothesizing instead an origin of the genus in northeastern Mexico, with subsequent spread to the northeast and northwest.

Our objective is to use phylogenetic systematics to address taxonomic issues and historical biogeography. We apply nuclear ribosomal and plastid DNA sequences for phylogeny reconstruction to address questions of circumscription and infer sister group relationships that will resolve alternative hypotheses of relationships. We address the hypotheses posed by Biddulph (1944) and Stoutamire (1977) for the geographic origin of *Gaillardia* and associations of clades with geographical regions. We propose hypotheses for potential geographic avenues of range expansion and historical processes that may have been involved in cladogenesis in the genus.

MATERIALS AND METHODS

***Taxon Sampling*.** Our sampling emphasized the inclusion of taxa that have been recognized in *Gaillardia* by diverse workers. All material we sampled from South America, available only as herbarium specimens, suffered degraded DNA, which has prevented the inclusion of populations from that region and left us unable to address questions about the origin of the amphitropical disjunction. Most taxa were sampled from more than one population across their geographic ranges to test for monophyly as well as phylogenetic differentiation within their ranges. We were only able to amplify both nuclear ribosomal and plastid DNA for one individual of *G. suavis*, therefore the geographic range of this entity is not fully represented.

Outgroup selection was based on phylogeny reconstructions of Helenieae that had included exemplars from *Gaillardia* (Baldwin and Wessa 2000; Baldwin et al. 2002). For the

internal transcribed spacer (ITS), 15 outgroups, including species of *Amblyolepis*, *Baileya*, *Balduina*, *Helenium*, *Hymenoxys*, *Pelucha*, *Psilostrophe,* and *Psathyrotes*, were used (Appendix). External transcribed spacer (ETS) sequences were not available from GenBank for these same species and we were only able to amplify the ETS region for one individual of *Helenium arizonicum*. For analyses of the plastid dataset and the datasets that combined all phylogenetic markers we used as outgroups *Balduina atropurpurea*, *Helenium arizonicum* and *H. bigelovii*, although ETS data were available only for *H. arizonicum*.

DNA Isolation and Sequencing. DNA sequences for ITS and ETS of nuclear ribosomal DNA as well as the plastid *trnT-trnF* region (including the *trnT-trnL* intergenic spacer, *trnL* intron, and *trnL-trnF* intergenic spacer) were obtained from GenBank or generated as part of this study (Appendix). For the new sequences, total genomic DNA was isolated from approximately 10 mg silica-gel dried or herbarium specimen leaf material using the CTAB procedure of Doyle and Doyle (1987). The ITS region (ITS-1, 5.8S, ITS-2) was amplified by polymerase chain reaction (PCR) using primers N-nc18s10 and C26A (Wen and Zimmer 1996). The ETS region was amplified using primers AST8 (Markos and Baldwin 2001) and 18S-ETS (Baldwin and Markos 1998). The plastid region was amplified using primers a and b (*trnT-trnL* intergenic spacer) and c and f (*trnL* intron and *trnL-trnF* intergenic spacer; Taberlet et al. 1991). The 25 μl PCR reactions contained 12.05 μl (10.8 μl for chloroplast reaction) sterile water, 1.25 μl (2.5 μl for chloroplast reaction) 10× reaction buffer (Promega), 2.5 μl 5′ 5μM primer, 2.5 μl 3′ 5 μM primer, 3.0 μl $MgCl_2$, 1.5 μl dNTP, 0.2 μl Taq polymerase (Promega), and 2.0 μl diluted DNA template (approximately 1.8 μl sterile water to 0.2 μl DNA template). PCR reaction conditions in a Biometra® thermocycler (Whatman) included initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final

extension at 72°C for 7 min. PCR products were visualized by 2% agarose gel electrophoresis and purified using 20% polyethylene glycol 8000 in 2.5 M NaCl. The 20 μl cycle sequencing reactions contained 10.7 μl sterile water, 0.5 μl DMSO, 0.8 μl 5 μM primer, 2.0 μl 5× sequencing buffer, 4.0 μl BigDye® Terminator v3.1 (Applied Biosystems), and 2.0 μl diluted clean PCR product. Cycle sequence reaction conditions began at 95°C for 2 min, followed by 25 cycles at 95°C for 30 sec, 51°C for 1 min, with a final extension at 60°C for 4 min. Cycle sequence products were purified using 75% isopropanol precipitation, and DNA sequences were obtained on polyacrylamide gels using an Applied Biosystems 377 automated sequencer (Applied Biosystems). Contigs were assembled and edited using Sequencher™ ver. 4.1 (Gene Codes Corporation) and sequences were aligned using Se-Al ver. 2.0a11 (Rambaut 1996-2002). Alignment was unambiguous within the ingroup taxa.

Phylogenetic Analysis. Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses were conducted to explore phylogenetic signals in the data (TreeBASE study accession number SN2745). MP and ML analyses were conducted on the independent ITS, ETS, and plastid data partitions as well as a dataset that combined the data partitions (= combined dataset). BI was used to analyze only the combined dataset. The combined dataset included only the 31 accessions for which sequences were available from all four data partitions.

The Partition Homogeneity Test was implemented in PAUP* ver. 4.0b10 (Swofford 2002) to examine potential topological incongruences in trees obtained from independent analyses of the ITS, ETS, *trnT-trnL* and *trnL-trnF* data partitions, respectively. These analyses applied 100 test replicates, each with 100 random order entry heuristic searches and one tree saved per replicate. Based on incongruent signals between the nuclear and plastid partitions, we

conducted taxon deletion experiments for a series of datasets that lacked one or more *Gaillardia* accessions to determine whether specific taxa were sources of significant incongruence between markers. Because the taxon deletion experiments identified *G. pulchella* var. *drummondii* and *G. suavis* as sources of incongruence between plastid and nuclear partitions, we conducted MP analyses of the combined dataset with these two taxa removed as well as with them included.

All characters were weighted equally, and gaps were treated as missing data for MP analyses. Heuristic MP searches used random taxon addition with branch-swapping by tree bisection-reconnection (TBR), saving 1 tree per replicate; 1,000 replicated searches were conducted to search for islands of equally most parsimonious trees. For searches that did not run to completion, MaxTrees was set at 40,000.

For ML analyses, models of molecular evolution were evaluated using Modeltest ver. 3.06 (Posada and Crandall 1998), which uses likelihood ratio tests to compare 56 alternative models for DNA substitution. Models were evaluated for each dataset and selected using likelihood ratio tests (LRTs) and the Akaike information criterion (AIC) provided by Modeltest. The selected models and specified parameters were applied in ML searches of the independent datasets in which starting trees were obtained by random taxon addition. One thousand replicated tree searches used TBR branch swapping with one tree held at each step.

Bootstrap analysis (Felsenstein 1985) was used to assess support for clades recovered by MP and ML using PAUP* ver. 4.0b10 (Swofford 2002). MP and ML bootstrap analyses used 1,000 random taxon addition replicates and TBR branch swapping. For bootstrap searches that did not run to completion, MaxTrees was set at 10,000; otherwise MaxTrees automatically increased by 100.

BI analyses used MrBayes ver. 3.0B4 (Huelsenbeck and Ronquist 2001). Different DNA

substitution models selected using the LRT results from Modeltest ver. 3.06 (Posada and Crandall 1998) for the ITS, ETS, *trnT-trnL*, and *trnL-trnF* were applied in the Bayesian analyses that were initiated with random starting trees and run for 1,000,000 generations. Four Markov chains (three incrementally heated, one cold) were initiated, and were sampled every 100 generations. The number of trees required to reach stationarity ("burn-in") in the Markov chain Monte Carlo was estimated by visual examination of the log likelihood results for each chain. The first 20,000 trees of the burn-in were excluded from the tree set. A 50% majority rule consensus tree was calculated from the remaining collection of trees to represent the posterior probabilities that the nodes are correct. To ensure that the posterior probability values had not become fixed on local optima, we performed the BI analyses four times using different starting trees to ensure that the runs converged on the same topology.

Alternative Phylogenetic Hypotheses. Prior hypotheses of relationships were modeled using topological constraints (Table 2). We tested whether these models were significantly worse than the most likely trees obtained from our ML analyses. Maximum likelihood searches (100 replicates) were used to find the best topology under each constraint (Table 2) using the TrN+I+G model of nucleotide substitution. This model of molecular evolution was selected by Modeltest for the combined dataset. The ML score was estimated from the three MP trees using PAUP*. Then likelihood scores of the MP trees were compared with the ML score of the constrained tree using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) as implemented in PAUP*, with the resampling-estimated log-likelihood (RELL) optimization.

Historical Biogeography. Ancestral geographic regions for clades and dispersal or vicariance as modes of geographic change were inferred by Dispersal-Vicariance Analysis ver. 1.1 (DIVA; Ronquist 1996) using a topology based on the MP analysis of the combined dataset.

Because a fully-resolved topology is required in order to implement DIVA, selected accessions were eliminated from the consensus tree to leave only one exemplar for each species. The resulting topology was consistent with the topologies of the three equally most parsimonious trees, and differed from the ML and BI topology only in the placement of *G. amblyodon*. The reliability of optimizations in DIVA can decrease as the root node is approached (Ronquist 1996); therefore, we included in our topology for the DIVA analyses seven outgroup genera whose placements were based on prior phylogenetic analyses (Baldwin and Wessa 2000; Baldwin et al. 2002).

Seven geographical areas that encompass the current distributions of the *Gaillardia* species and outgroups were applied in the DIVA analysis; these areas included the following: Gulf Coast (A), Great Plains (B), Chihuahuan (C), Sonoran (D), Colorado Plateau (E), Great Basin-Columbia Plateau (F), and Rocky Mountains (G). We used Takhtajan's (1986) floristic areas to delimit the Gulf Coast, Great Plains, and Rocky Mountains; MacMahon and Wagner (1985) to delimit the Chihuahuan and Sonoran areas; and McLaughlin's (1992) floristic areas to delimit the Colorado Plateau and Great Basin-Columbia Plateau. Each species was coded for presence in the geographical areas where extant representatives are found. Outgroup genera were coded as present in a geographical area if any of their constituent species were found in the region. Ronquist (1996) cautioned against coding higher taxa, such as our outgroup genera, as being distributed in all areas where descendents occur, because it can potentially obscure more restricted distributions that are critical for the optimization of ancestral states. Therefore, we used the maxareas option in DIVA to restrict the number of unit areas that may have been occupied by any ancestral species (as recommended by Ronquist). Analyses were run with maxareas = 2, maxareas = 3, and with an unrestricted maximum number of ancestral areas.

Gypsophily Optimization. MacClade 4.0 (Maddison and Maddison 2000) was used to examine the evolution of gypsophily in *Gaillardia*. We applied the same resolved topology based on the MP analysis of the combined dataset that was used in the DIVA reconstruction. Both ACCTRAN (homoplasies maximized as reversals) and DELTRAN (homoplasies maximized as parallelisms) optimizations were applied.

RESULTS

Phylogenetic Data. The aligned DNA sequences for the data partitions ranged from 518 to 767 bases in length with the nuclear DNA markers providing far more parsimony informative characters than the plastid DNA (Table 3). No polymorphisms were found in any of the sequences. The likelihood (-ln *L*) scores of topologies resulting from the models suggested by hLRT (Table 3) were better than those resulting from models suggested by AIC. Therefore, we used the hLRT criteria.

Congruence of the Data Partitions. Topologies resulting from MP analyses of the *trnT-trnL* and *trnL-trnF* regions of the plastid data were congruent (Table 4) but had little resolution (not shown); thus, we present only results of an analysis of a combined plastid dataset (= *trnT-trnF*). The MP analysis of *trnT-trnF* resulted in 47,293 equally parsimonious trees of 67 steps, and the strict consensus of these trees (Fig. 1; summary statistics in Table 3) was identical in topology to the most likely tree from the ML analysis (not shown).

The MP analysis of ITS resulted in 12 equally parsimonious trees of 641 character state changes (strict consensus topology in Fig. 2; tree statistics in Table 3). The MP analysis of ETS resulted in 12 equally parsimonious trees of 241 character state changes (strict consensus topology in Fig. 3; tree statistics in Table 3). The results of ML analyses of the ITS and ETS

(not shown) were congruent with their respective topologies from the MP analyses, except that ITS MP and ML topologies differed in the placement of the outgroups *Psilostrophe* and *Amblyolepis*, and the ETS ML analysis further resolved one additional sister relationship between two *G. pulchella* accessions and resolved the *G. henricksonii-G. powellii* clade as the sister to the *G. amblyodon-G. aristata-G. pulchella* clade.

Topologies based on these different data partitions were largely congruent. Results of the Partition Homogeneity test (Table 4) showed that topologies from the ITS and ETS data were not significantly different ($P = 0.29$; $\alpha = 0.05$). The Partition Homogeneity tests (Table 4) found no significant differences between ETS and *trnL-trnF* ($P = 0.05$) or the combined plastid datasets ($P = 1.00$), but ETS and *trnT-trnL* resulted in significantly different topologies ($P = 0.01$). The incongruence between ETS and *trnT-trnL* was centered in the placement of *G. suavis* and *G. pulchella* var. *drummondii*. Similarly, incongruences found in our topologies and the Partition Homogeneity test results between ITS and *trnT-trnL* ($P = 0.07$) and ITS and *trnL-trnF* ($P = 0.36$) were centered in *G. suavis*.

Analyses of the Combined Dataset. The MP analysis of the combined dataset resulted in three equally most parsimonious trees of 578 steps (strict consensus in Fig. 4; summary statistics in Table 3). Because our independent analyses of the different data partitions found *G. pulchella* var. *drummondii* and *G. suavis* as sources of incongruence between plastid and nuclear partitions, we conducted MP analyses of the combined dataset with these two taxa removed. Analyses with and without *G. pulchella* var. *drummondii* and *G. suavis* resulted in identical placements of all other sampled accessions (results not shown). Based on the consistency of topologies whether or not *G. pulchella* var. *drummondii* and *G. suavis* were included, we conducted subsequent analyses, including ML, BI, tests of alternative phylogenetic hypotheses,

and DIVA, using datasets that included both accessions.

The four separate BI runs with separate random starting trees converged on similar log likelihood values after about 9,000 generations, and topologies were identical. Thus, the posterior probability values did not become fixed on local optima in separate runs. The BI topologies were identical to the best tree from the ML analysis (Fig. 5).

The most likely tree from the ML and BI analyses (Fig. 5; models and summary statistics in Table 3) differed from the strict consensus of the MP topologies (Fig. 4) in the placement of *G. amblyodon* and resolution of *G. coahuilensis-G. mexicana* as the sister of *G. amblyodon-G. pulchella-G. henricksonii-G. powellii*. In the MP analysis (Fig. 4), *G. amblyodon* was placed as sister to a clade consisting of accessions of both *G. aristata* and *G. pulchella* (= *G. pulchella* complex); whereas, the BI and ML analyses nested *G. amblyodon* among accessions of *G. aristata* and *G. pulchella* in a lineage designated as clade A (Fig. 5).

The different phylogenetic analyses of the combined dataset recovered several well supported clades that were consistent across the analyses (Figs. 4, 5). Each analysis recovered a strongly supported, monophyletic *Gaillardia*. *Gaillardia comosa*, *G. gypsophila* and *G. suavis*, forming sect. *Agassizia*, were placed sister to the rest of *Gaillardia*, in which the two major clades correspond to sects. *Gaillardia* and *Hollandia*. The Gulf Coast *G. aestivalis* complex (sect. *Hollandia*) was placed as the sister of sect. *Gaillardia*. In sect. *Gaillardia*, *G. pulchella*, *G. aristata*, *G. amblyodon*, *G. coahuilensis-G. mexicana,* and *G. henricksonii-G. powellii* clades were monophyletic (= clade B). Clade B was placed as the sister of the *G. pinnatifida-G. arizonica* clade (= *G. pinnatifida* complex). *Gaillardia multiceps* and *G. turneri* were sister to a clade that consisted of *G. parry*, *G. spathulata*, and *G. flava* (= Intermountain clade), together forming a monophyletic group (= clade C). Clade B was consistently placed as the sister of the

G. pinnatifida complex, both of which were consistently placed as sister to clade C.

Alternative Phylogenetic Hypotheses. SH tests indicated that the difference in –ln *L*'s between constrained and unconstrained topologies was significant for all relationship hypotheses tested (Table 2). Thus we can reject all alternative relationship hypotheses tested in favor of our ML trees.

DIVA. The optimal DIVA reconstruction without limiting the maximum number of areas for ancestral distribution offered the same reconstructions for dispersal and vicariance events as the reconstructions in which we restricted the number of ancestral areas. We therefore present our results of the analysis based on unrestricted maximum number of ancestral areas (Fig. 6).

DIVA indicated that the ancestral area for *Gaillardia* was the Chihuahuan region. Dispersal was inferred to result in the Gulf Coast/Great Plains/Chihuahuan distribution of *G. suavis*, within sect. *Agassizia*, which is centered primarily in the Chihuahuan region. Three alternative distributions (Gulf Coast + Chihuahuan, Great Plains + Chihuahuan, or Gulf Coast + Great Plains + Chihuahuan) were inferred for the common ancestor of sects. *Hollandia* and *Gaillardia*. In sect. *Gaillardia*, dispersal was inferred to expand the distribution of sect. *Gaillardia* to the Colorado Plateau (clade C) and Sonoran Desert (clade B and *G. pinnatifida* complex). A vicariance event was inferred to separate the ancestor of clade C from the Intermountain clade. Dispersal events were inferred to result in the present Sonoran/Colorado Plateau distribution of *G. parryi* and the present disjunct Great Plains/Chihuahuan and Colorado Plateau distribution of *G. multiceps*. The common ancestor of clade B and the *G. pinnatifida* complex was suggested to have either a Chihuahuan or Sonoran distribution. Multiple possible distributions were inferred for the ancestor to *G. arizonica* and *G. pinnatifida*. The common ancestor to *G. mexicana* + *G. coahuilensis* and the rest of *Gaillardia* was inferred to have a

Chihuahuan distribution. Possible distributions inferred for the common ancestor of the *G. henricksonii* + *G. powellii* clade and clade A included Chihuahuan, Gulf Coast + Chihuahuan, Great Plains + Chihuahuan, or Gulf Coast + Great Plains + Chihuahuan. The common ancestor to *G. henricksonii* and *G. powellii* was inferred to have a Chihuahuan distribution. The inferred distribution for the common ancestor to clade A, and the common ancestor to the *G. pulchella* complex included combinations of Gulf Coast, Great Plains, Chihuahuan, Great Basin-Columbia Plateau and Rocky Mountains. We found that different outgroup placements did not affect the DIVA results for the ingroup.

Gypsophily Evolution. MacClade 4.0 (Maddison and Maddison 2000) reconstructions of gypsophily in *Gaillardia* were identical under ACCTRAN and DELTRAN for the MP tree (Fig. 7). Three independent origins of gypsophily were inferred, each characterizing a clade that subsequently underwent speciation. We infer one origin of gypsophily in sect. *Agassizia* (*G. comosa* + *G. gypsophila*) and two in sect. *Gaillardia* (*G. multiceps* + *G. turneri* and *G. henricksonii* + *G. powellii*).

DISCUSSION

Sectional Taxonomy. The results of our phylogenetic analyses of combined plastid and nuclear markers provided strong support for the subgeneric sections circumscribed by Biddulph (1944; Fig. 4). Independent phylogenetic analyses of the plastid data alone, however, recovered a paraphyletic sect. *Agassizia*, in which *G. suavis* was placed as the sister to sects. *Gaillardia* and *Hollandia*. *Gaillardia suavis* was a major source of incongruence between the plastid and nuclear markers, which may be an indication of either incomplete lineage sorting of plastid haplotypes among species or chloroplast capture via hybridization. *Gaillardia suavis* is found on

the Great Plains and in the Chihuahuan Desert, where other species of *Gaillardia* are also common and interspecific hybridization with species outside of sect. *Agassizia* could have occurred. Our ability to amplify plastid DNA for only one of several sampled herbarium specimens of *G. suavis* limited opportunities to test these alternatives. Sampling additional individuals from the entire geographic range might provide more evidence for either a lineage sorting or hybridization hypothesis.

Since Biddulph's (1944) monograph, Petenatti and Ariza Espinar (1995) have described the new sect. *Austroamericania* for the South American species. These South American species are not well represented in North American herbaria, and our attempts to amplify DNA from available samples were not successful. At the time of Biddulph's (1944) monograph only a single species of *Gaillardia* was recognized in South America. This species, *G. megapotamica*, was placed in sect. *Agassizia* by Biddulph (1944), although Turner et al. (1979) suggested it is closely related to *G. pulchella*, a member of sect. *Gaillardia*. More recently three species, *G. cabrerae*, *G. tontalensis*, and *G. megapotamica*, including three varieties of the latter, have been recognized in South America on the basis of differing structures in the fibrous layers of the phyllaries (Petenatti and Ariza Espinar 1995) and chemotaxonomy (Petenatti et al. 1996). The monophyly of sect. *Austroamericania* and its relationship to the other sections of *Gaillardia* remain to be tested.

***Sect.* Agassizia *Taxonomy*.** Biddulph (1944) distinguished sect. *Agassizia* on the basis of long receptacular setae, short style branches that are glabrous, and short lobes on disk corollas. Turner (1972) noted that receptacular setae in sect. *Agassizia* are highly reduced, if present at all, rather than long as indicated by Biddulph; our observations were consistent with those of Turner.

Biddulph (1944) recognized sect. *Agassizia* as consisting of *G. comosa*, *G. suavis*, and *G.*

megapotamica. As discussed above, *G. suavis* may have hybridized with species from either sects. *Gaillardia* or *Hollandia*, at least in the northern part of its range from which we sampled a population for plastid DNA. Despite this possible capture of plastids from another lineage of *Gaillardia*, *G. suavis* exhibits the diagnostic attributes of sect. *Agassizia* as emended by Turner (1972). *Gaillardia suavis* is the only North American species of *Gaillardia* reported to lack ray flowers (when present they are relatively short), although the South American *G. megapotamica* was also reported by Biddulph (1944) to be polymorphic for presence or absence of ray flowers. The more recently described *G. gypsophila* was suggested by Turner (1972) to be most closely related to *G. comosa*, which was consistent with our results.

Sect.* Hollandia *Taxonomy. Biddulph (1944) treated sect. *Hollandia* as consisting of the three species *G. lanceolata*, *G. fastigiata*, and *G. lutea*, although these names were affected by Rock's (1956) combination of *Helenium aestivale* and *H. serotinum* with *Gaillardia*. Rock (1956) treated *G. lanceolata* as a synonym of *G. aestivalis* (based on *H. aestivale*) and *G. lutea* as a synonym of *G. serotina* (based on *H. serotinum*) because the epithets from *Helenium* had priority. The taxonomy of the *G. aestivalis* complex has been treated differently by various authors (Biddulph 1944; Rock 1956; Correll and Johnston 1970; Waterfall 1971; Averett and Beaman 1975; Cronquist 1977; Barkley 1986; Table 1).

Our sampling of the *G. aestivalis* complex was most extensive for ETS, which provided some resolution among the accessions (Fig. 3). Our results showed that accessions corresponding to *G. fastigiata* (*Carr 13156* and *Stratton s.n.*), *G. serotina*/*lutea* (*Cory 49879*), and *G. aestivalis* var. *winkleri* (*Orzell & Bridges 8596*, *Cory 56654*, and *Marlowe 45*) were nested within the broadly conceived *G. aestivalis* complex; however, our results do not provide sufficient insights to determine whether some of these entities warrant taxonomic recognition as

subspecific taxa in *G. aestivalis*. We call attention to coastal Texas populations that were distinguished by the ETS sequences as distinct from other Texas and Oklahoma populations. We also recovered a previously unrecognized Oklahoma clade that received modest support. These clades in the *G. aestivalis* complex call attention to lineages that share derived attributes and may warrant taxonomic recognition. They warrant further sampling with more variable markers to test whether they are reproductively isolated.

Sect.* Gaillardia *Taxonomy. At the time of her monograph, Biddulph (1944) recognized that the delimitations of particular species, namely *G. pulchella* and *G. pinnatifida*, presented considerable problems. She treated both as polymorphic species but accepted the segregation of selected morphological and geographical variants as separate species.

The first two names published in *Gaillardia*, *G. pulchella* and *G. aristata*, led to consternation among those who regarded the latter as merely a perennial form of the former (Biddulph 1944). Biddulph (1944) did not question in her monograph whether *G. pulchella* and *G. aristata* should be treated as conspecific. We identify a *G. pulchella* complex that includes sampled accessions of both *G. pulchella* and *G. aristata*. The *G. pulchella* complex was monophyletic in our MP results (Fig. 4) but paraphyletic to *G. amblyodon* in our ML and BI results (Fig. 5). The *G. pulchella* complex is a polytomy that includes well supported clades composed of Arizona and Texas populations for *G. pulchella* and Central Rocky Mountain populations of *G. aristata*. The *G. pulchella* complex includes a representative (*G. pulchella*; *Marlowe 13*) that corresponds to the entity treated as *G. neomexicana* by Biddulph (1944).

The polytomy of the *G. pulchella* complex may indicate that our phylogenetic markers are insufficiently variable to detect clades. The polytomy could also indicate that lineages of both annual and perennials have experienced insufficient isolation to acquire uniquely derived

states and/or it is a weakly differentiated complex in which annuals, assuming the perennial habit is plesiomorphic, have evolved independently more than once. The polytomy could also be a signature of initially divergent annual and perennial lineages that experienced extensive secondary contact and hybridization, such as could have occurred in a glacial refugium; however, Stoutamire (1977) argued against any recent secondary contact between *G. pulchella* and *G. aristata*.

Gaillardia amblyodon has been recognized since the mid 19th century as distinct from, but closely related to, *G. pulchella* (Gay 1839; Rydberg 1915; Biddulph 1944). Correll and Johnston (1970) suggested that the distinction between *G. amblyodon* and *G. pulchella* probably did not deserve formal recognition. Turner and Whalen (1975) agreed with earlier workers in distinguishing *G. amblyodon* from *G. pulchella* on the basis of ray color and dimorphic achenes. Our data lack a strong phylogenetic signal to distinguish between the alternatives found in our MP (*G. amblyodon* sister of *G. pulchella* complex) and ML/BI (*G. amblyodon* nested in *G. pulchella* complex) results, an ambiguous result that points to the need for more variable markers to infer the relationships of *G. amblyodon*.

The clade that consists of *G. amblyodon* and the *G. pulchella* complex formed a polytomy in our parsimony results (Fig. 4) with the clades *G. coahuilensis* + *G. mexicana* and *G. henricksonii* + *G. powellii*. Both ML and BI resolved *G. henricksonii* + *G. powellii* as the sister of *G. amblyodon* + *G. pulchella* complex. Turner (1977, p. 541) hypothesized that *G. coahuilensis* and *G. mexicana* “arose from the same ancestral stock,” which is consistent with our phylogenetic results that show the latter nested in a paraphyletic *G. coahuilensis*. Turner’s (1977) original description of *G. coahuilensis* distinguished it from the perennial *G. mexicana* as a tap-rooted annual but largely similar in other attributes. Our results were also consistent with

Turner's (1976) hypothesis that *G. henricksonii* was most closely related to *G. powellii*. Turner's (1972) original description of *G. powellii* was based on populations from Coahuila, Mexico, that Biddulph (1944) had treated as part of *G. multiceps*. Subsequently, Turner (1976) recognized *G. henricksonii*, which is also restricted to Coahuila, Mexico, as distinct from *G. powellii* on the basis of several morphological attributes.

One of the sources of incongruence between the nuclear and plastid data partitions involved the placement of *G. pulchella* var. *drummondii*, which was nested in the *G. coahuilensis* + *G. mexicana* clade in the trees based on plastid data rather than the *G. pulchella* complex where it was placed by the nuclear data. As discussed above for *G. suavis*, these incongruent placements for *G. pulchella* var. *drummondii* may be a signal of either incomplete lineage sorting of plastid haplotypes among species or chloroplast capture via hybridization. The current distribution of the *G. pulchella* complex is adjacent to, if not slightly sympatric with, that of *G. coahuilensis* + *G. mexicana* clade in southwest Texas and northeastern Mexico, which may have provided ample geographic opportunity for gene flow.

Gaillardia pinnatifida is a perennial that has pinnatifid leaves and inflorescences that have yellow ray corollas and purplish-burgundy disk corollas (the bases of disk corollas yellowish). The type was collected on the Great Plains, and Nelson (1904) and Rydberg (1915) later described new species from Utah and Arizona-New Mexico that fit the general description of *G. pinnatifida* as *G. gracilis* A. Nels. and *G. crassa* Rydb., respectively. Biddulph (1944) treated *G. pinnatifida* as broadly distributed and polymorphic, encompassing the variants that Nelson (1904) and Rydberg (1915) had proposed. Our results were consistent with Biddulph's treatment of *G. pinnatifida*. We recovered a clade with populations from the geographic range that she recognized for *G. pinnatifida*, including the Utah and Arizona-New Mexico region

recognized by Nelson and Rydberg as different species, although the clade included also *G. arizonica*. *Gaillardia arizonica* is restricted largely to the Sonoran and Mohave deserts, where it extends beyond the periphery of the *G. pinnatifida* range. *Gaillardia arizonica* is distinguished from *G. pinnatifida* both by its annual habit and yellow rather than purplish disk corollas.

Biddulph (1944) treated *G. multiceps* as distinct from *G. pinnatifida*, although she was later criticized by Kearney and Peebles (1960) for this decision. Turner (1972, p. 187) also considered *G. pinnatifida* "undoubtedly closely related to *G. multiceps* and the latter perhaps arose from stocks ancestral to that species." Averett and Powell (1976) had similarly suggested that *G. turneri* was closely related to *G. pinnatifida*. Our results indicated that *G. multiceps* is more closely related to *G. turneri* than to *G. pinnatifida*, although we modeled Turner's (1972) and Averett and Powell's (1976) hypotheses of "close relationships" to *G. pinnatifida* using constraint trees that forced (1) the monophyly of *G. pinnatifida* and *G. multiceps*, (2) the monophyly of *G. pinnatifida* and *G. turneri* and (3) the monophyly of *G. pinnatifida*, *G. multiceps*, and *G. turneri* (Table 2). The best trees under these constraints had significantly different likelihood scores than our best ML topologies based on the SH test (Table 2), which leads us to consider the alternative hypotheses for relationships of *G. multiceps* and *G. turneri* to be significantly worse than that recovered in our best topologies.

Our results placed *G. multiceps* and *G. turneri* as the sister of a clade that consisted of *G. parryi*, *G. spathulata*, and *G. flava* (= Intermountain clade). The alliances in this clade of five species had not been suggested previously. Biddulph (1944) was particularly concerned that *G. flava*, an endemic to Desolation and Gray's canyons of the Green River in central eastern Utah, which she chose to recognize on the basis of the two collections known at the time of her monograph, was not distinct from *G. pinnatifida*. The ranges of *G. pinnatifida* and *G. flava*

overlap slightly at the southern end of the range of the latter (the mouth of Gray's Canyon, Emery and Grand counties, Utah), but we have not observed any morphological intermediates suggestive of hybridization. *Gaillardia flava* has pinnatifid leaves that are markedly similar to those of *G. pinnatifida*, but it is distinct from the latter in having disk corollas that are yellow rather than purplish. Our results indicate that *G. flava* is more closely related to *G. spathulata*, with which it shares disk corollas that are yellow but differs in leaf form, than to *G. pinnatifida*. When we modeled the hypothesis that *G. flava* and *G. pinnatifida* are monophyletic using topology constraints, the best trees had significantly different likelihood scores than our best ML topologies based on the SH test (Table 2), which leads us to reject the alternative hypothesis that *G. flava* and *G. pinnatifida* are monophyletic. We infer that the pinnatifid leaf forms shared by *G. flava* and *G. pinnatifida* are homoplastic.

Biogeography and Evolution. Phylogeographic processes, including allopatric speciation, rather than stochastic lineage sorting, appear to be the major determinant of current patterns of DNA sequence variation in North American *Gaillardia*. Our results recover a strong association of clades with geographic regions. Environmental change has long been seen as a stimulus for biogeographic processes in western North America (e.g., Axelrod 1958; Stebbins 1952) and has been implicated in the evolution of *Gaillardia* (Stoutamire 1977). The responses of populations, however, can vary in the face of environmental change in ways that include (1) geographic change that may be local and/or more extensive in the face of little or no adaptive evolution (= niche conservatism), (2) adaptive change that results in a niche shift to accommodate the environmental change, or (3) extinction (Jackson and Overpeck 2000; Ackerly 2003). Given the absence of fossils of *Gaillardia*, we cannot assess the importance of extinction as a response to environmental change in the genus. Adaptive change, inferred from divergent

habitat preferences and/or morphology, has played a role in the evolution of *Gaillardia*, but so also, we suggest, has niche conservatism.

Biddulph (1944) and Stoutamire (1977) presented contrasting hypotheses for the geographic origin of *Gaillardia*. Biddulph (1944) hypothesized that *Gaillardia* had radiated geographically from a "center of dispersal" in Texas. In contrast, Stoutamire (1977) suggested the genus originated in northeastern Mexico. Rather than using countries and states to examine biogeography, we applied floristic areas proposed by MacMahon and Wagner (1985), Takhtajan (1986), and McLaughlin (1992). This is important because states, such as Texas, can overlap with multiple floristic areas. Floristic areas can be more representative of the historical, climatic, and topographic factors that are important in biogeographic inferences of range expansion, vicariance, and comparisons among clades. Our DIVA results indicated a Chihuahuan desert origin for *Gaillardia*, an inference that could be consistent with the hypotheses of either Biddulph (1944) or Stoutamire (1977). Although in regard to the Biddulph (1944) hypothesis, it would restrict our attention to that part of Texas that is part of the Chihuahuan desert.

Several extant species of *Gaillardia* are either endemic to or have populations in the Chihuahuan Desert (Figs. 8, 9), and these are groups we hypothesize to have remained in the inferred ancestral area. Notably, many Chihuahuan desert species of *Gaillardia* are restricted to gypsum substrates in contrast to species in arid regions outside of this desert. Indeed, three clades of *Gaillardia* (*G. multiceps* + *G. turneri*, *G. comosa* + *G. gypsophila*, and *G. henricksonii* + *G. powellii*) in the Chihuahuan Desert are characterized by gypsophily. We reconstruct gypsophily to have three independent origins following early cladogenesis in *Gaillardia* in the Chihuahuan Desert (Fig. 7). These shifts onto gypsum substrates may have occurred with aridification in the Chihuahuan Desert, particularly as a response to heightened competition

caused by the increasing aridity. Gypsum substrates, on which surface crusts preclude seedling establishment for many plants (Meyer 1986; Borselli et al. 1996), can provide local refuges that allow for persistence with lower competition (Solbrig 1979). Gypsum crusts can also provide strong thermal insulation (Muller-Stoll and Lerch 1963), and gypsum rich soils retain water better than nongypsum substrates, which may have permitted the persistence of *Gaillardia* populations in the face of insufficient adaptation to aridification. Thus, gypsophily in Chihuahuan Desert gaillardias may be a consequence—at least initially—of local geographic change spurred by niche conservatism. We cannot discount completely a role for adaptation in the shift to gypsophily; for example, if any clades are obligate gypsophiles then adaptive changes are likely to have occurred.

Range expansion is a prominent feature of the biogeography of major clades of *Gaillardia*, each of which, except the *G. aestivalis* complex (sect. *Hollandia*; see below), has species in the Chihuahuan desert. Environmental change, especially the aridification of northern Mexico and the American West, is cited generally as the cause of these kinds of range expansions (Axelrod 1958). Wolfe (1987), for example, noted that the geographic radiation of Asteraceae with "southern affinities" into the Rocky Mountains and western North America was rapid following climatic drying that began in the Miocene. We anticipate that the geographic radiations in *Gaillardia* are likely to have occurred in the last 10 million years when the uplift of the Rocky Mountains and Sierra Nevada-Cascade ranges led to significant aridification of the mid-continent and western regions of North America (Graham 1999). The major range expansions that occurred in *Gaillardia* included the following: (1) eastward range expansion into central Texas and along the Gulf Coast, resulting in the *G. aestivalis* complex (sect. *Hollandia*); (2) northwestern range expansion, resulting ultimately in the Intermountain clade,

but including also *G. multiceps* and *G. turneri*; (3) the northward range expansion of the *G. pinnatifida* complex; (4) northward range expansion of the *G. pulchella* complex (giving rise to *G. amblyodon*, *G. pulchella*, and *G. aristata*) and, possibly, (5) northward expansion onto the Great Plains by *G. suavis* (we note the caveat that the potential hybrid origin of this taxon could influence this interpretation).

All three North American species of sect. *Agassizia* (*G. comosa*, *G. gypsophila*, and *G. suavis*) have populations in the Chihuahuan Desert (Fig. 8). Both *G. comosa* and *G. gypsophila* have relatively narrow distributions in northeastern Mexico. Turner (1972) characterized *G. gypsophila* as restricted to gypsum dunes in Coahuila and *G. comosa* as having somewhat broader habitat preferences on either gypsum or saline soils. These two species also differ in chromosome numbers; *G. gypsophila* is a diploid (n = 17) and *G. comosa* tetraploid (n = 34) (Turner 1972). This suite of differences between the two species makes it difficult to infer whether a particular evolutionary force—ploidy change, geographic isolation, or ecological differentiation—had a primary role in speciation with other changes resulting from anagenesis after speciation. *Gaillardia suavis*, which our nuclear data place as the sister of *G. comosa* and *G. gypsophila*, has a broad distribution, and we infer that it has experienced range expansion from the Chihuahuan region onto the Great Plains. As discussed above, the plastid and nuclear data have conflicting phylogenetic signals for *G. suavis*: nuclear data place it as the sister of *G. comosa* and *G. gypsophila*, whereas the plastid data place it as the sister of the sect. *Hollandia* + sect. *Gaillardia* clade. We sampled only Great Plains accessions of *G. suavis*, which prevents us from addressing whether populations throughout its range have conflicting phylogenetic signals in their nuclear and plastid DNA partitions. If the conflicting signal in *G. suavis* has resulted from hybridization with a member of either sects. *Hollandia* or *Gaillardia* then this could have

occurred at differing times in its geographic radiation. If *G. suavis* had a distribution on the Great Plains prior to Pleistocene glaciations, then range restriction and shift to a southern refugium could have brought the species into contact with populations of either sects. *Hollandia* or *Gaillardia*. Because the phylogenetic signal from the plastid data place *G. suavis* as sister only to the clade that consists of both *Hollandia* and *Gaillardia* rather than to a particular species of either section, we infer that any hybridization involving *G. suavis* would have been relatively early in its history and involved only an ancestral taxon on the stem lineage of sects. *Hollandia* and *Gaillardia* rather than more recent species that would have been present during the Pleistocene.

The divergence of the sect. *Hollandia* and sect. *Gaillardia* clades is one of the deeper phylogenetic events in *Gaillardia*. Section *Hollandia* is distributed today from central Texas along the Gulf Coast to Florida and southern Georgia (Fig. 8). Much of the distribution of sect. *Hollandia* is centered in the West Gulf Coastal Plain (WGCP) area of endemism (MacRoberts and MacRoberts 2003; cf. also Takhtajan 1986). MacRoberts and MacRoberts (2003) suggested that Pleistocene and subsequent climatic changes were significant in invasion of the WGCP region by taxa centered earlier either to the east or the west. It may have been the origin of dry, sand barrens (MacRoberts and MacRoberts 2003), which are widespread but patchy in the WGCP, that facilitated the eastern expansion of sect. *Hollandia* into habitats that required little niche shift to accommodate. Although the varieties of *G. aestivalis* that compose sect. *Hollandia* are restricted to these sandy habitats, Turner (1979) reported edaphic differentiation and habitat selection among varieties, which include the narrow endemic *G. aestivalis* var. *winkleri*. Thus, some local adaptation to environments encountered during the range expansion seems likely to have occurred. We infer that evolutionary differentiation in sect. *Hollandia* arose with the

isolation of populations during Pleistocene glacial/interglacial cycles or with geographic radiations that began at the end of the last glaciation, which have been dated to have begun approximately 18,000 years ago in the region of central Texas where sect. *Hollandia* has its western margin and greatest diversity. The divergence of the sect. *Hollandia* and sect. *Gaillardia* clades may, however, have preceded the end of the Pleistocene and diversification within sect. *Hollandia* by a few million years.

Johnston (1941) called attention to the disjunct distribution of *G. multiceps* between an eastern region in the Chihuahuan Desert of Texas and adjacent eastern New Mexico and a western region in east central Arizona (Fig. 9), which he regarded as unusual given its gypsophily. DIVA indicated that the *G. multiceps* disjunction was a consequence of dispersal, but vicariance seems an equally likely possibility. If vicariance was the cause of the disjunction in *G. multiceps*, then this species may have been most widespread before at least the latest uplift of the Rocky Mountains, which largely ceased about 4 million years ago, and possibly extends back as far as 27-21 million years ago when the first phase of the uplift was underway. The gypsophily of *G. multiceps* is shared also by its sister *G. turneri*, which has a restricted distribution in Chihuahua, Mexico (Fig. 9). It is most parsimonious to infer that the gypsophily of this clade preceded the origin of the two extant species and its range expansion to the north. Based on current distribution patterns we hypothesize that speciation leading to the origin of *G. multiceps* and *G. turneri* was allopatric; however, polyploidy may have been one of the forces driving speciation and the continuing differentiation between the disjunct regions of *G. multiceps*. *Gaillardia turneri* is diploid (n = 17; Averett and Powell 1976), the eastern populations of *G. multiceps* are tetraploid (n = 34), and the western populations of *G. multiceps* are hexaploid (n = 51; Turner 1972).

Gaillardia turneri and *G. multiceps* are most closely related to a clade that lacks gypsophily and is restricted to the Intermountain region. In the Intermountain clade, the sister species *G. flava* and *G. spathulata* are found only east of the Wasatch-Utah Plateaus axis and *G. parryi* only to the west (Fig. 9). DIVA inferred the distribution of *G. parryi* to be a consequence of dispersal (Fig. 6), but its allopatry relative to the *G. flava* + *G. spathulata* clade may be consistent with vicariance caused by mountain-building in the Wasatch-Utah Plateaus axis. If the uplift of the Wasatch-Utah Plateaus axis was responsible for this vicariance, then we could expect the ancestor of this clade to have been more widespread prior to the origin of this high elevation barrier, which began to uplift approximately 12 to 17 million years ago (Milligan 2000). In contrast, dispersal via the so-called Dixie Corridor at the southern end of the Utah Plateaus region could have occurred subsequent to the mountain-building. The Dixie Corridor has been implicated in the dispersal of various taxa that are found in southwestern Utah (e.g. San Juan and Kane counties) and areas to the west (Cronquist et al. 1972; Gross et al. 2003).

The distributions of the sister species *G. flava* and *G. spathulata* are nearly continuous (Fig. 9). *Gaillardia spathulata* is centered in the San Rafael desert and surrounding regions, where it grows on dry desert plains and areas where blown sand impacts the vegetation. *Gaillardia flava* is found in sandy to gravelly habitats, especially at wash margins where it is restricted to Desolation and Gray's Canyons, which extend from the northeastern periphery of the *G. spathulata* distribution, in the Tavaputs Plateau of eastern Utah. The origin of *G. flava* at the periphery of *G. spathulata* (if current distributions reflect those at the time of their divergence) may have involved either peripatric or parapatric speciation. The two species have largely identical inflorescences and flowers, and this may indicate that their current geographic proximity has resulted from secondary range expansions following greater isolation during the

speciation process. The two species differ notably in leaf shape and *G. flava* has a more pronounced resinous odor than *G. spathulata*, which may be indicative of ecophysiological differences that have resulted from adaptive evolution. The DNA sequences of *G. flava* and *G. spathulata* are weakly differentiated, from which we infer that speciation has been relatively recent. Given the restriction of *G. flava* to Desolation and Gray's Canyons, which are thought to have begun to erode with the uplift of the Tavaputs Plateau beginning 10 million years ago (Milligan 2000), we consider this to be a maximum age for the divergence of the two species, although most erosion of these canyons has occurred in the last 5.5 million years and this may provide a better time frame estimate for the origin of *G. flava*.

Gaillardia arizonica is derived within a paraphyletic *G. pinnatifida*, which is one of the most widespread species of *Gaillardia*. *Gaillardia pinnatifida* extends from the Chihuahuan Desert onto the Great Plains and across the Rocky Mountains into Arizona and Utah (Fig. 9). *Gaillardia arizonica*, associated with both the Sonoran and Mojave Deserts, extends westward from a zone of sympatry along the northwestern periphery of the distribution of *G. pinnatifida* (Fig. 9). There are no geographic barriers to gene flow between the current distributions of these two taxa, which now overlap slightly. We infer that speciation was ecological (sensu Coyne and Orr 2004). Either reproductive or life history changes or both may have been forces driving the origin of *G. arizonica*. *Gaillardia arizonica* has yellow disk flowers in contrast to the purplish disk flowers of *G. pinnatifida*, and it is a relatively diminutive annual compared to the larger, more widespread perennial form of *G. pinnatifida*.

Gaillardia arizonica and *G. pinnatifida* are sister to a group we have designated clade B (including *G. coahuilensis* + *G. mexicana*, *G. henricksonii* + *G. powellii*, and the *G. pulchella* complex + *G. amblyodon*; Fig. 6). DIVA reconstructs the ancestral area for clade B to be the

Chihuahuan Desert. The clades *G. coahuilensis* + *G. mexicana* and *G. henricksonii* + *G. powellii* are restricted to the Chihuahuan Desert (Fig. 9). The species pairs in both clades are allopatric. Both *G. henricksonii* and *G. powellii* are gypsophiles restricted to Coahuila, Mexico (Figs. 7, 9). We infer this to be another instance in which gypsophily has preceded a more recent speciation in *Gaillardia*. The two species are only modestly differentiated morphologically (Turner 1972, 1976), which may be indicative of little adaptive differentiation. In contrast, *G. coahuilensis* and *G. mexicana*, the other species pair restricted to the Chihuahuan Desert in clade B, display variation that we infer reflects ecological selection in their evolutionary differentiation. For example, *G. coahuilensis* is an annual and *G. mexicana* a perennial, and, Turner (1977), although he did not provide data on the habitat of *G. coahuilensis*, emphasized that it differed from the higher elevation pine and oak woodland habitat where *G. mexicana* was found.

We discussed above the equivocal resolution of *G. amblyodon* relative to the *G. pulchella* complex (Figs. 4, 5), although both artificial hybridization studies of Stoutamire (1977) and allozyme diversity studies of Heywood and Levin (1984) indicated that *G. amblyodon* was reproductively isolated from *G. pulchella*, with which it is sympatric in southeastern Texas. Heywood (1986a, 1986b) characterized *G. amblyodon* as ecologically differentiated from populations of *G. pulchella* with which it is sympatric. Without phylogeographic studies that provide much greater resolution of relationships among relevant lineages, it is difficult to infer whether this ecological differentiation is a signature of speciation or a consequence of ecological exclusion that has resulted following secondary contact between populations of the two species. Provisionally, however, we accept the ecological differentiation as a sign of limited niche conservatism in the radiation of the clade that consists of *G. amblyodon* and the *G. pulchella* complex.

Heywood and Levin (1984) also characterized *G. pulchella* as having limited genetic differentiation, which is consistent with our observations for the broader *G. pulchella* complex. The *G. pulchella* complex, including the sampled accessions of both the annual *G. pulchella* and the perennial *G. aristata*, as discussed above may be a weakly differentiated assemblage in which the annual and perennial lineages have evolved recently multiple times. The lineages may have experienced insufficient isolation to acquire uniquely derived states, although Stoutamire (1977) indicated that *G. pulchella* and *G. aristata* had limited interfertility. We note, however, Stoutamire (1958) earlier also reported limited interfertility among some populations of *G. pulchella*. Heywood and Levin (1984) reported allozymic patterns consistent with isolation by distance in *G. pulchella*, and it is through such a process that we might understand the ongoing differentiation of the perennial *G. aristata* form, which composes largely the northern part of the range of the complex, from the annual *G. pulchella* form, which composes the southern part of the range of the complex. As Stoutamire (1977) recognized, much of the montane and northern Great Plains areas now occupied by *G. aristata* would have been ice free for no more than 12,000-15,000 years, and much of the range of the species must be a consequence of post-Pleistocene range expansion.

The current distribution of *Gaillardia* in North America and the observed patterns of genetic variation within these populations are indicated to have resulted from a combination of vicariance and dispersal events. In interpreting the DIVA reconstruction, however, it is important to recognize that DIVA assumes current distributions were the result of vicariance events. It assigns cost only for dispersal events, thus the results may underestimate the number of dispersal events. The method also assumes biogeographic change is parsimonious. Our inferences build on the earlier 'center of dispersal' hypotheses of Biddulph (1944) and

Stoutamire (1977) to provide a more comprehensive framework for the biogeography of *Gaillardia*. In this biogeographic setting, we infer that fragmentation of populations associated with either niche conservatism or adaptive changes have been important in the differentiation of species in the Chihuahuan Desert. With range expansion outside the Chihuahuan Desert, especially to the north, adaptive changes appear to have played an increasingly important role in speciation. While polyploidization has been documented and we recover some evidence consistent with interspecific hybridization, these phenomena have played relatively minor roles in evolutionary differentiation and speciation in *Gaillardia*.

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APPENDIX

Taxa and accessions sampled in this study. GenBank accession numbers for ITS, ETS, *trnT-trnF*; – = sequence not obtained. An asterisk (*) indicates ITS accessions that were obtained from GenBank, and were generated by Baldwin and Wessa (2000). To facilitate data location, taxa are listed in alphabetical order by genus and species:

Ingroup:

Gaillardia aestivalis (Walt.) H. Rock, Cleveland County, OK, *Benesh 103* (OKL); DQ391190, DQ391230, DQ395144; *G. aestivalis* (Walt.) H. Rock, Tarrant County, TX, *Carr 13156* (TEX);–, DQ391234,–; *G. aestivalis* (Walt.) H. Rock, Austin County, TX, *Mayfield 1492* (TEX);–, DQ391231,–; *G. aestivalis* (Walt.) H. Rock, San Patricio County, TX, *Mayfield 2166* (TEX);–, DQ391233,–; *G. aestivalis* (Walt.) H. Rock, Caddo County, OK, *Pyzner s.n.* (TEX); DQ391189, DQ391232,–; *G. aestivalis* (Walt.) H. Rock, Payne County, OK, *Stratton s.n.* (WS); DQ391191, DQ391235,–; *G. aestivalis* var. *winkleri* (Cory) Turner, Hardin County, TX, *Cory 49879* (WS);–, DQ391236,–; *G. aestivalis* var. *winkleri* (Cory) Turner, Hardin County, TX, *Cory 56654* (WS);–, DQ391239,–; *G. aestivalis* var. *winkleri* (Cory) Turner, *Marlowe 45* (WS), grown from seedlings in WSU greenhouse; seedlings from Stephen F. Austin State University Mast Arboretum, Nacogdoches, TX; DQ391192, DQ391237, DQ395145; *G. aestivalis* var. *winkleri* (Cory) Turner, Hardin County, TX, *Orzell & Bridges 8596* (TEX);–, DQ391238,–; *G. amblyodon* Gay, Jim Hogg County, TX, *Simpson 4-V-00-7* (TEX); DQ391193,–,–; *G. amblyodon* Gay, Leon County, TX, *Turner & Turner 95-88* (TEX); DQ391194, DQ391240, DQ395146; *G. aristata* Pursh, Uintah County, UT, *Huber 1247* (BRY); DQ391196,–, DQ395148; *G. aristata* Pursh, Park County, WY, *Kirkpatrick 1969* (BRY); DQ391197,–, DQ395149; *G. aristata* Pursh, Benton County, WA, *McKinnon & Smith 354* (BRY); DQ391195,

DQ391241, DQ395147; *G. arizonica* A. Gray, Washington County, UT, *Baird 2241* (BRY); DQ391198, DQ391242,–; *G. arizonica* A. Gray, Pima County, AZ, *Felger & Seminoff 93-378* (TEX); DQ391199,–, DQ395150; *G. coahuilensis* B.L. Turner, Coahuila, Mexico, *Phillips JTM/ARP 84S-8* (ARIZ); DQ391205, DQ391251, DQ395156; *G. coahuilensis* B.L. Turner, Kinney County, TX *Turner 96-11* (TEX); DQ391200, DQ391243, DQ395151; *G. comosa* A. Gray, San Luis Potosi, Mexico, *Johnston 8217* (ARIZ);–, DQ391245,–; *G. comosa* A. Gray, Coahuila, Mexico, *Snow & Valdez-Reyna 6682* (TEX); DQ391201, DQ391244, DQ395152; *G. flava* Rydb., Grand County, UT, *Atwood & Evenden 24413* (BRY); DQ391202, DQ391246, DQ395153; *G. flava* Rydb., Emery County, UT, *Atwood 25805b* (BRY);–, DQ391247,–; *G. gypsophila* B.L. Turner, Coahuila, Mexico, *Carranza & Carranza 640* (ARIZ); DQ391203, DQ391249, DQ395154; *G. gypsophila* B.L. Turner, Coahuila, Mexico, *Gonzalez-Ledesma & Koch 239* (TEX);–, DQ391248,–; *G. henricksonii* B.L. Turner, Coahuila, Mexico, *Henrickson 15223* (TEX); DQ391204, DQ391250, DQ395155; *G. mexicana* A. Gray, Coahuila, Mexico, *Carranza & Villarreal 1440* (ARIZ);–, DQ391252, DQ395157; *G. mexicana* A. Gray, Coahuila, Mexico, *Hinton 20292* (ARIZ); DQ391206,–,–; *G. multiceps* Greene, Coconino County, AZ, *Bierner 89-27* (ARIZ);–,–, DQ395159; *G. multiceps* Greene, Culberson County, TX, *Turner & Turner 93-5* (TEX); DQ391207, DQ391253, DQ395158; *G. multiceps* Greene, Chaves County, NM, *Van Devender & Van Devender 85-124* (ARIZ); DQ391208,–,–; *G. parryi* Greene, Mohave County, AZ, *Cronquist & Neese 11891* (WS); DQ391210, DQ391254, DQ395160; *G. parryi* Greene, Kane County, UT, *Franklin 6406* (BRY); DQ391209,–,–; *G. pinnatifida* Torr., Cochise County, AZ, *Cohen 26* (ARIZ); DQ391211, DQ391255,–; *G. pinnatifida* Torr., Harmon County, OK, *McCarty & Benesh ADZ0134* (OKL); DQ391213, DQ391257, DQ395162; *G. pinnatifida* Torr., Wayne County, UT, *McLaughlin 7935* (ARIZ); DQ391214, DQ391258,–; *G. pinnatifida*

Torr., Chihuahua, Mexico, *Spencer & Atwood 41* (ARIZ); DQ391212, DQ391256, DQ395161; *G. pinnatifida* Torr., Catron County, NM, *Van Devender & Betancourt 84-314* (ARIZ);–, DQ391259, DQ395163; *G. powellii* B.L. Turner, Coahuila, Mexico, *Villarreal & Vazquez 4848* (TEX); DQ391215, DQ391260, DQ395164; *G. pulchella* (Foug.), Sonora, Mexico, *Baker s.n.* (ARIZ); DQ391219, DQ391265,–; *G. pulchella* (Foug.), Pima County, AZ, *Chamberland 1558* (ARIZ); DQ391216, DQ391261,–; *G. pulchella* (Foug.), Gila County, AZ, *Marlowe 13* (WS);–,–, DQ395167; *G. pulchella* (Foug.), Sandoval County, NM, *Heil 4737* (BRY); DQ391218, DQ391263, DQ395166; *G. pulchella* (Foug.), Coconino County, AZ, *Higgins 23788* (BRY);–, DQ391264,–; *G. pulchella* (Foug.), Moore County, TX, *Watson 1868* (ARIZ); DQ391217, DQ391262, DQ395165; *G. pulchella* var. *drummondii* (Hook.) B.L. Turner, Aransas County, TX, *Watson 1888* (TEX); DQ391220, DQ391266, DQ395168; *G. spathulata* A. Gray, Emery County, UT, *Biddulph 3* (WS);–, DQ391267, DQ395169; *G. spathulata* A. Gray, Emery County, UT, *Hufford 3944* (WS); DQ391221, DQ391268, DQ395170; *G. suavis* (A. Gray & Engelm.) Britton & Rusby, Noble County, OK, *Buthod & Fagin AB-2222* (OKL); DQ391223, DQ391272, DQ395171; *G. suavis* (A. Gray & Engelm.) Britton & Rusby, Tillman County, OK, *Hoagland & McCarty BLM075* (OKL);–, DQ391269,–; *G. suavis* (A. Gray & Engelm.) Britton & Rusby, Coahuila, Mexico, *Neson & Mayfield 7370* (TEX); DQ391224,–,–; *G. suavis* (A. Gray & Engelm.) Britton & Rusby, Val Verde County, TX, *Watson 1953* (TEX); DQ391222, DQ391270,–; *G. suavis* (A. Gray & Engelm.) Britton & Rusby, Frio County, TX, *Watson 1954* (TEX);–, DQ391271,–; *G. turnerii* Averett & A.M. Powell, Chihuahua, Mexico, *Henrickson 13018* (TEX); DQ391225, DQ391273, DQ395172.

Outgroup:

Amblyolepis setigera DC., AF229281*; *Baileya multiradiata* Harv. & A. Gray, AF229273*; *B. pauciradiata* Harv. & A. Gray, AF229275*; *B. pleniradiata* Harv. & A. Gray, AF229274*; *Balduina angustifolia* (Pursh) B.L. Rob., Coffee County, GA, *Norris & Hopkins, Jr. 5896* (GA); DQ391226,–,–; *B. atropurpurea* R.M. Harper, Liberty County, GA, *Zebryk 0580* (GA); DQ391227,–, DQ395173; *B. uniflora* Nutt., AF229270*; *Helenium arizonicum* S.F. Blake, Coconino County, AZ, *Mittleman & Hodgson H828* (WS); DQ391228, DQ391274, DQ395174; *H. bigelovii* A. Gray, AF229269*; *H. bigelovii* A. Gray, Butte County, CA, *Bjork 5694* (WS); DQ391229,–, DQ395175; *Hymenoxys hoopesii* (A. Gray) Bierner, AF229279*; *H. lemmonii* (Greene) Cockerell, AF229280*; *Pelucha trifida* S. Watson, AF229267*; *Psathyrotes annua* (Nutt.) A. Gray, AF229264*; *Psilostrophe cooperi* (A. Gray) Greene, AF229276*.

Table 1. Major taxonomic treatments of *Gaillardia*.

Gray 1884	Rydberg 1915	Biddulph 1944	Miscellaneous treatments of selected species (citations included)
		Section *Gaillardia*	
G. acaulis A. Gray	Synonym of *G. parryi*		
G. amblyodon Gay	*G. amblyodon*	*G. amblyodon*	
G. aristata Pursh.	*G. aristata*	*G. aristata*	
G. arizonica A. Gray	*G. arizonica*	*G. arizonica*	
			G. coahuilensis Turner (Turner 1977)
	G. crassa Rydb.	Synonym of *G. pinnatifida*	
	G. crinita Rydb.	Synonym of *G. arizonica*	
	G. drummondii (Hook.) DC.	Synonym of *G. pulchella*	
	G. flava Rydb.	*G. flava*	
	G. gracilis A. Nelson	Synonym of *G. pinnatifida*	
	G. hallii Rydb.	Synonym of *G. aristata*	
			G. henricksonii B. L. Turner (Turner 1976)
	G. linearis Rydb.	Synonym of *G. pinnatifida* var. *linearis*	
	G. mearnsii Rydb.	*G. mearnsii*	Synonym of *G. pinnatifida* (Kearney and Peebles 1960)
G. mexicana A. Gray	*G. mexicana*	*G. mexicana*	
		G. mexicana var. *trifida* Biddulph	
	G. multiceps Greene	*G. multiceps*	*G. multiceps* var. *multiceps* (Turner 1972)
			G. multiceps Greene var. *microcephala* B. L. Turner (Turner 1972)
		G. neomexicana A. Nels.	Synonym of *G. pulchella* var. *pulchella* (Turner and Whalen 1975)
	G. parryi (Greene)	*G. parryi*	
	G. picta D. Don	Synonym of *G. pulchella*	
G. pinnatifida Torr.	*G. pinnatifida*	*G. pinnatifida*	
		G. pinnatifida var. *linearis* (Rydb.) Biddulph	
			G. powellii B. L. Turner (Turner 1972)
	G. pringlei Rydb.	Synonym of *G. arizonica*	

G. pulchella Foug.	*G. pulchella*	*G. pulchella*	*G. pulchella* var. *pulchella* (Turner and Whalen 1975)
			G. pulchella Foug. var. *australis* Turner & Whalen (Turner & Whalen 1975)
			G. pulchella Foug. var. *drummondii* (Hook.) B. L. Turner (Turner 2003)
G. pulchella var. *picta* (D. Don) A. Gray	Synonym of *G. picta*	Synonym of *G. pulchella*	*G. pulchella* Foug. var. *picta* (Sweet) Gray (Turner and Whalen 1975)
G. spathulata A. Gray	*G. spathulata*	*G. spathulata*	
			G. turneri Averett & Powell (Averett & Powell 1976)
	G. villosa Rydb.	Synonym of *G. pulchella*	
		Section *Hollandia*	
			G. aestivalis (Walt.) H. Rock (Rock 1956)
			G. aestivalis var. *winkleri* (Cory) Turner (Turner 1979)
	G. chrysantha Small	Synonym of *G. lutea*	Synonym of *G. aestivalis* (Correll and Johnston 1970)
	G. fastigiata Greene	*G. fastigiata*	Synonym of *G. aestivalis* (Correll and Johnston 1970)
	G. lanceolata	*G. lanceolata*	Synonym of *G. aestivalis* (Rock 1956)
	G. lutea Greene	*G. lutea*	Synonym of *G. aestivalis* (Correll and Johnston 1970)
G. lanceolata Michx.	*G. rigida* Small ex Rydb.	Synonym of *G. fastigiata*	
		Section *Agassizia*	
G. comosa A. Gray	*G. comosa*	*G. comosa*	
			G. gypsophila B. L. Turner (Turner 1972)
	G. nervosa Rydb.	Synonym of *G. comosa*	
G. simplex Scheele	Synonym of *G. suavis*		
	G. suavis (A. Gray & Engelm.)	*G. suavis*	
			Section *Austroamericania* (Petenatti and Ariza Espinar 1995)
			G. cabrerae Covas (Petenatti et al. 1996)
		G. megapotamica (Spreng) Baker	*G. megapotamica* var. *megapotamica*

	G. megapotamica var. *radiata* (Griseb.) Baker (Petenatti et al. 1996)
	G. megapotamica var. *scabiosoides* (Arn. ex DC.) Baker (Petenatti et al. 1996)
	G. tontalensis Hieron. (Petenatti and Ariza Espinar 1993)

Table 2. Shimodaira-Hasegawa (SH) tests comparing the optimal tree obtained from searches using maximum likelihood (TrN+I+G) where selected taxa were forced to be monophyletic using constrained topologies. The -ln *L* scores for MP trees 1, 2, and 3, and the ML tree were respectively: 7219.61202, 7219.61202, 7220.60416, and 7219.61202. Asterisks next to *P*-values indicate significance at the α = 0.05 level.

Taxa constrained to be monophyletic	Rationale	Constrained tree -ln *L*	-ln *L* difference	SH-test *P*-value
G. pinnatifida *G. flava*	Close relationship suggested by Biddulph (1944).	7543.32315	323.71113	0.000*
G. pinnatifida *G. turneri*	Close relationship suggested by Turner (1976), Averett and Powell (1976).	7490.94232	271.33030	0.000*
G. powellii *G. multiceps*	Close relationship suggested by Turner (1972).	7546.07084	326.45882	0.000*
G. pinnatifida *G. multiceps*	Kearney and Peebles (1960) do not recognize as distinct.	7473.24911	253.63709	0.000*
G. powellii *G. multiceps* *G. pinnatifida*	Close relationship suggested by Turner (1972).	7715.47353	495.86151	0.000*

Table 3. Statistics for datasets used, including results from MP and ML searches, and likelihood model parameters.

	ITS	**ETS**	***trnT-trnL***	***trnL-trnF***	**Combined**
Number of accessions sampled	52	45	27	26	31
Number of characters	655	477	518	767	2438
Percentage of data matrix cells scored as missing data	0.003%	0.005%	0.000%	0.004%	1.462%
Parsimony-informative characters	241	113	13	19	257
MaxTree setting in PAUP*	auto-increased	auto-increased	40,000	auto-increased	auto-increased
Swapped to completion?	Yes	Yes	No	Yes	Yes
Number of equally parsimonious trees	12	12	40,000	1	3
Length of shortest trees	641	241	31	36	578
CI (excluding uninformative characters)	0.679	0.805	0.936	1.0	0.811
RI	0.873	0.941	0.949	1.0	0.892
RC	0.592	0.758	0.888	1.0	0.724
HI (excluding uninformative characters)	0.321	0.195	0.133	0.0	0.248
Molecular evolution model (hLRT criterion)	TrN+G	HKY+G	F81	K81uf	TrN+I+G
Likelihood score (-ln L)	4290.85415	2005.29131	885.91989	1296.67004	6804.82193
Length of ML tree	642	243	31	36	579
Likelihood CI	0.678	0.798	0.935	1.0	0.810
Likelihood RC	0.872	0.939	0.949	1.0	0.891
Likelihood RI	0.591	0.750	0.888	1.0	0.722
Proportion invariant sites (I)	None	None	None	None	0.5134
Gamma distribution shape parameter	0.6933	0.5637	N/A	N/A	0.8481

Table 4. Results of 100 Partition-Homogeneity test replicates as implemented in PAUP* to examine potential sequence incongruence between ITS, ETS, t*rnT-trnL*, and *trnL-trnF* datasets. No maximum trees were set (except for comparisons including t*rnT-trnL* where a maximum of 40,000 trees was set). Asterisks next to *P*-values indicate significance at the α = 0.05 level.

	P-values			
Markers	ITS	ETS	*trnT-trnL*	*trnL-trnF*
ITS - complete dataset	-	0.29	0.01*	0.03*
excluding *G. suavis* AB2222	-	0.81	0.07	0.36
excluding *G. pulchella* var. *drummondii* W1888	-	0.37	0.02*	0.03*
excluding *G. suavis* and *G. pulchella* var. *drummondii*	-	0.87	0.11	0.37
ETS - complete dataset	0.29	-	0.01*	0.05
excluding *G. suavis* AB2222	0.81	-	0.01*	0.08
excluding *G. pulchella* var. *drummondii* W1888	0.37	-	0.01*	0.03*
excluding *G. suavis* and *G. pulchella* var. *drummondii*	0.87	-	0.01*	0.10
trnT-trnL - complete dataset	0.01*	0.01*	-	1.00
excluding *G. suavis* AB2222	0.07	0.07	-	1.00
excluding *G. pulchella* var. *drummondii* W1888	0.02*	0.02*	-	1.00
excluding *G. suavis* and *G. pulchella* var. *drummondii*	0.11	0.11	-	1.00
trnL-trnF - complete dataset	0.03*	0.05*	1.00	-
excluding *G. suavis* AB2222	0.36	0.08	1.00	-
excluding *G. pulchella* var. *drummondii* W1888	0.03*	0.03*	1.00	-
excluding *G. suavis* and *G. pulchella* var. *drummondii*	0.37	0.10	1.00	-

Figs. 1-2. 1. Strict consensus of 47,293 equally parsimonious trees obtained from analysis of the plastid *trnT-trnF* DNA sequences. Numbers above branches are bootstrap values. 2. Strict consensus of 12 equally parsimonious trees obtained from analysis of nuclear ribosomal ITS DNA. Numbers above branches are bootstrap values.

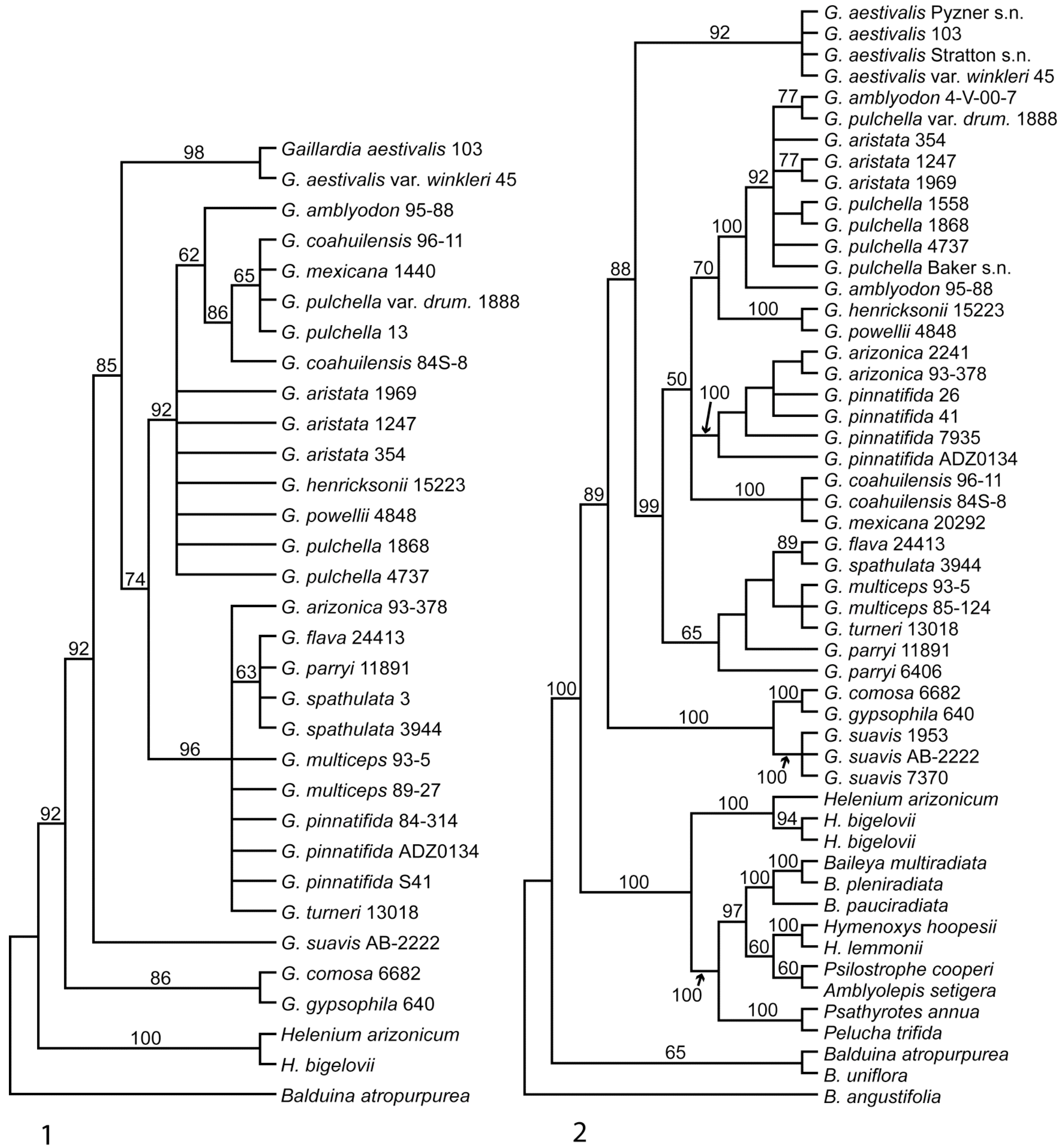
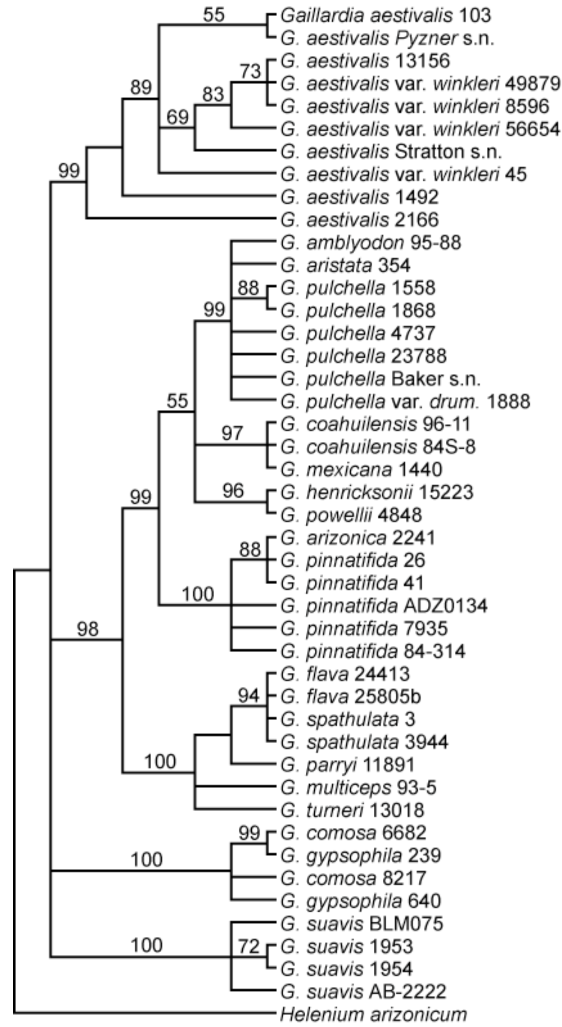
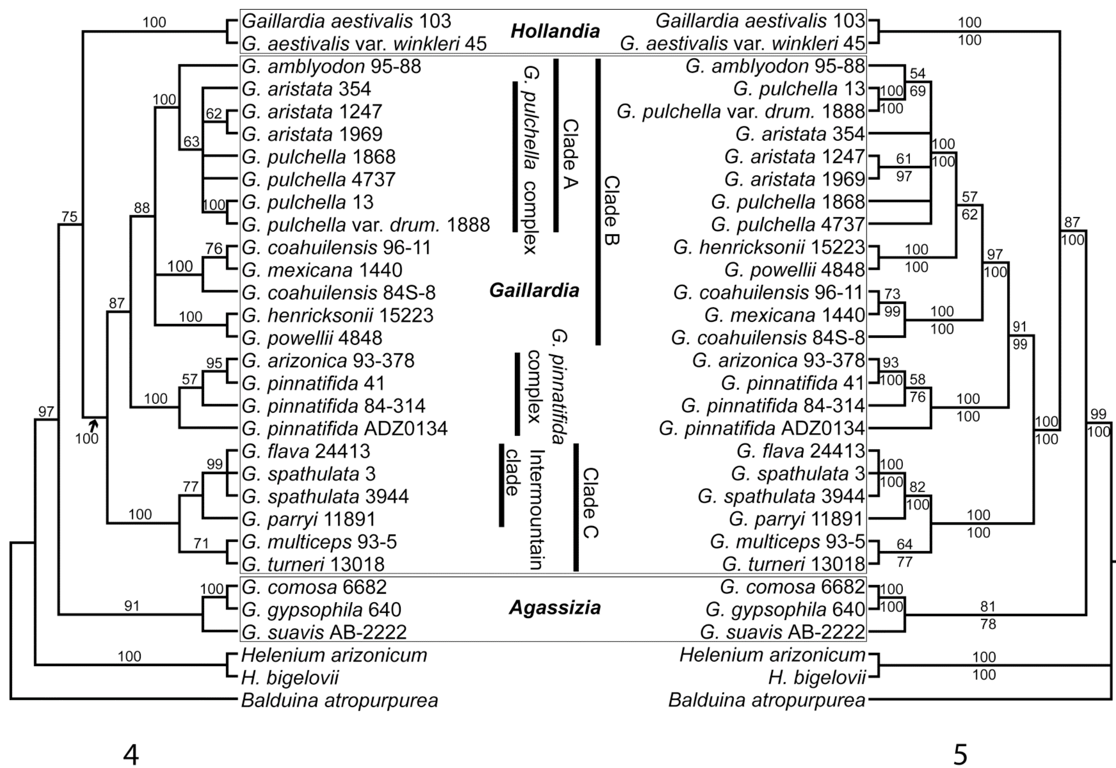


Fig. 3. Strict consensus of 12 equally parsimonious trees obtained from analysis of nuclear ribosomal ETS DNA sequences. Numbers above branches are bootstrap values.



FIGS. 4-5. 4. Strict consensus of three equally parsimonious trees obtained from analysis of combined nuclear ribosomal ITS and ETS and plastid *trnT-trnF* DNA sequences. Numbers above branches are bootstrap values. Boxes correspond to Biddulph's (1944) subgeneric sections. 5. Topology resulting from analyses of combined nuclear ribosomal ITS and ETS and plastid *trnT-trnF* DNA sequences using maximum likelihood (ML; TrN+I+G model of nucleotide substitution) and Bayesian inference (50% majority rule consensus). Numbers above branches are ML bootstrap values; numbers below branches are Bayesian posterior probabilities. Boxes correspond to Biddulph's (1944) subgeneric sections.



Figs. 6-7. 6. Dispersal-Vicariance Analysis results. DIS = dispersal event; VIC = vicariance event. A = Gulf Coast, B = Great Plains, C = Chihuahuan Desert, D = Sonoran Desert, E = Colorado Plateau, F = Great Basin-Columbia Plateau, G = Rocky Mountains, * = Multiple possibilities. Boxes correspond to Biddulph's (1944) subgeneric sections. 7. Gypsophily optimized onto the *Gaillardia* phylogeny using MacClade. Gypsophiles are indicated by thickened branches. Boxes correspond to Biddulph's (1944) subgeneric sections.

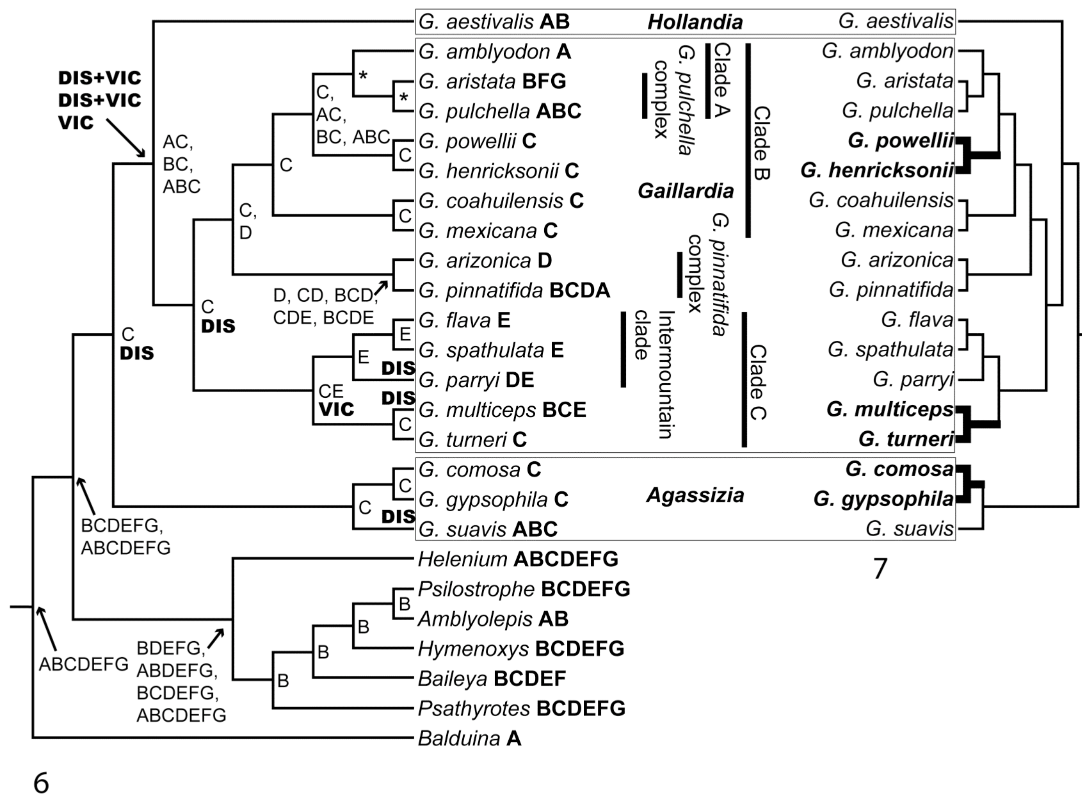


Fig. 8. Geographic distribution of species in *Gaillardia* sects. *Agassizia* (solid line) and *Hollandia* (dashed line) based on Biddulph (1944) and Turner (1979).

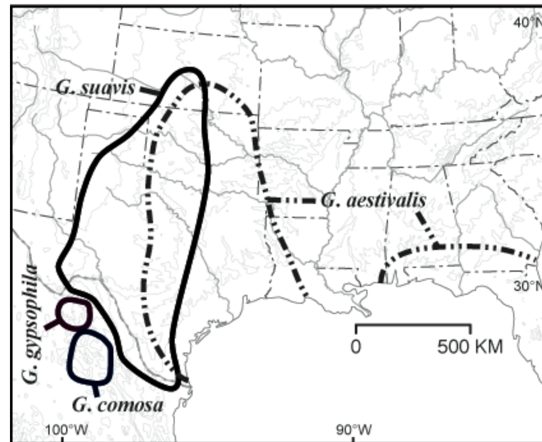
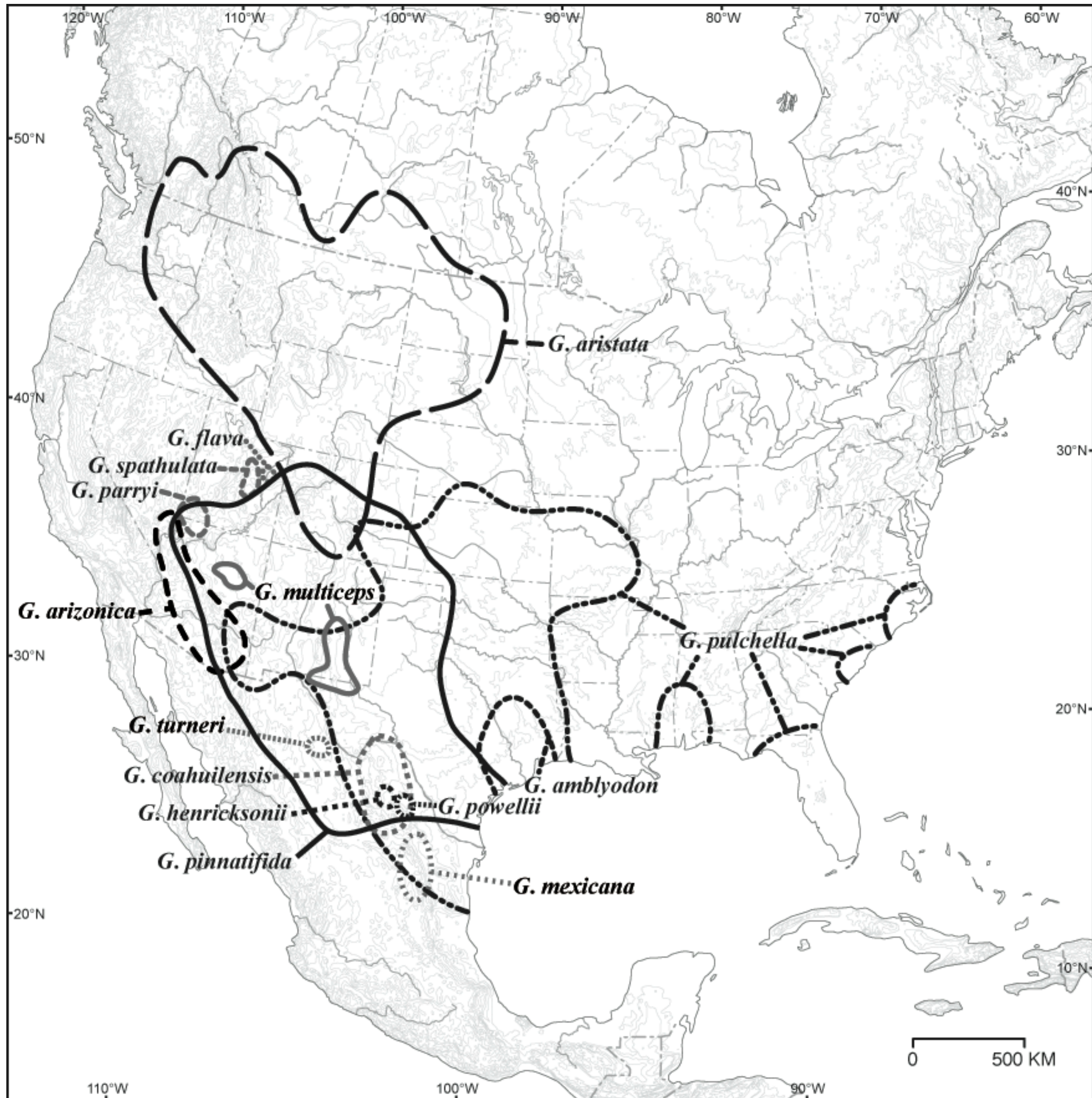


Fig. 9. Geographic distribution of species in *Gaillardia* sect. *Gaillardia* based on Biddulph (1944), Turner (1972; 1976; 1977), and Averett and Powell (1976).



CHAPTER TWO: GEOGRAPHIC RADIATIONS AND SPECIATION IN *SYNTHYRIS* (PLANTAGINACEAE) OF THE SOUTHERN ROCKY MOUNTAINS

INTRODUCTION

The topographic and ecological complexity of mountain landscapes in the American West can present considerable potential for evolutionary diversification. These opportunities are altered by climate changes, such as glacial-interglacial cycles, that can alternatively induce range expansion or contraction that impact population demography and gene flow. These repeated range shifts in response to climate change are hypothesized to rapidly bring about evolutionary change (Stebbins 1984; Huntley and Webb 1989). As populations are partitioned based on genetic preadaptations to diverse environments, we would expect relatively high levels of organismal diversity and endemism. One signature of evolutionary diversity is the relatively high level of endemism noted for mountain ranges (Billings 1974; Kruckeberg and Rabinowitz 1985).

Organisms respond to environmental change either with migration, evolution, or extinction (Levin 2003; Ackerly 2003). Davis and Shaw (2001), however, argue the importance of the interplay between evolution and migration in how plant taxa respond to climate change. Migration occurs when plant taxa passively track areas of favorable environmental conditions to which they are preadapted (Huntley and Webb 1989) and can be thought of as a way for organisms to avoid evolution. However, migration can also be viewed as a way for plants to encounter ecologically diverse environments which would promote diversification. Changes in the ecological attributes of populations are an important component of speciation in most flowering plant lineages (Levin 2000).

Plant distributions shift regularly in response to global and regional climatic changes (Huntley and Webb 1989). Phylogeographic studies have called attention especially to the roles of glacial-interglacial cycles. Plant distributions have shifted latitudinally, with populations retreating to lower latitudes in response to glacial advances, and expanding into higher latitudes as glacial ice retreats. These distribution shifts also involve elevational shifts. One estimate important to this study is that the late-glacial treeline in the Rocky Mountains may have been 1200m lower than the present treeline (Baker 1983). Many taxa alternate between having large widespread populations, and having reduced or fragmented populations (Webb 1987). During glaciations when plant populations are widespread at lower elevations, species ranges potentially overlap resulting in secondary contact, facilitating gene flow, and become fragmented into isolated higher elevation refugia during interglacials (Hewitt 2000).

Quaternary climatic oscillations had a strong influence on plant geographical distributions (Hewitt 1999, 2000), and we would expect genetic subdivision associated with such changes in geographical distribution to be an important component of evolutionary diversification (Davis and Shaw 2001; Huntley and Webb 1989; Kadereit et al. 2004; Raven 1973). Several studies of western North American taxa have suggested that Pleistocene glacial/interglacial cycles have greatly affected population genetic and biogeographic structure within species (DeChaine and Martin 2004; Mitton et al. 2000; Good and Sullivan 2001; Nielson et al. 2001; Findley 1969). Few studies, however, have focused on alpine taxa which may respond differently to climate change as compared to lower-elevation taxa.

Our aim is to look for evidence that range shifts – expansion, contraction, and fragmentation – have played evolutionary roles in speciation of the endemic alpine flora of the Southern Rocky Mountains in a phylogeographic study of the flowering plant genus *Synthyris*

(Plantaginaceae). The concept of *Synthyris* applied here follows Hufford and McMahon (2004) and includes species treated formerly as *Besseya* (e.g., Rydberg 1903; Pennell 1933; Schaack 1983a). *Synthyris* consists of 20 species of rhizomatous, herbaceous perennials restricted largely to the mountainous North American West. The plants have capsular fruits and small discoid seeds, and in most species these structures are not specialized for dispersal by wind or animals. Several life history traits in this clade, including perennial habit, slow growth, local outcrossing through insect pollination, vegetative reproduction, and limited or short-distance seed dispersal, probably influenced the high degree of isolation of this group. Hufford and McMahon (2004) used DNA sequence data from the nuclear ribosomal internal transcribed spacer (ITS) and 5.8S coding region alone and in combination with morphological data to reconstruct the phylogeny of *Synthyris* and found support for several clades defined by unique biogeographical and/or morphological features. In this study we use phylogeography to examine evolutionary processes in the Southern Rocky Mountain clade of *Synthyris* section *Besseya* (Figs. 1-2).

The Southern Rocky Mountain clade of *S.* sect. *Besseya* consists of *S. alpina* + *S. ritteriana* as the sister of *S. oblongifolia* + *S. plantaginea* (Fig. 1). The four species are well differentiated by leaf and corolla forms as well as corolla color (Schaack 1983a; Hufford 1992a, 1992b), and populations of the different species are currently allopatric and not known to hybridize. *Synthyris alpina* and *S. oblongifolia* are restricted to alpine tundra (3200-3900m and 3500m, respectively), *S. ritteriana* to subalpine meadows (3000-3400m), and *S. plantaginea* is found at lower elevations (1800-2700m) in conifer savannas and grasslands (Fig. 2).

The differences in elevation among species of this clade provide an opportunity to examine evolutionary processes and genetic consequences of geographic and elevational shifts associated with speciation. Although the distributions of *S. alpina* and *S. plantaginea* are

broadly overlapping (Fig. 2), the populations of these two species are widely separated by elevational difference. We test the hypothesis that the initial speciation in this clade was between *S. alpina* and *S. plantaginea* ancestral lineages, and that speciation was due to isolation due to elevational difference. *Synthyris oblongifolia* is restricted to one population at the southernmost point of the range. We explore whether there is evidence to support the idea that this isolated species evolved via peripheral isolation when formerly widespread ancestral lineages shifted upward in elevation during interglacial warming. *Synthyris ritteriana*, a tetraploid, is hypothesized (Schaack 1983a) to have originated by allopolyploid hybridization between *S. alpina* and *S. plantaginea*. We look for molecular signals that support this hypothesis. Because the two putative parental species currently do not occur in sympatry and are separated by elevational difference, we look for evidence of past elevation and range shifts that would have enabled hybridization.

Synthyris plantaginea, the most widespread species of this clade, extends through intermountain valleys from southern Wyoming to New Mexico and is disjunct in mountains of Arizona, western New Mexico, and western Colorado. We explore whether disjunctions can be linked to vicariance of once continuous populations that were at lower elevations during glaciations. We consider whether previously hypothesized migration routes or others can be inferred for this clade. Information about migration routes and secondary contact among populations adds to our understanding of the shaping of organismal distributions of other Southern Rocky Mountain taxa. For *S. alpina*, the highest elevation species of this clade, we test for evidence of gene flow that could be indicative of population expansion from sky islands.

We address the hypotheses for the phylogeography of the Southern Rocky Mountain clade of *S.* section *Besseya* presented above to enhance our understanding of alpine

biogeography in the Southern Rocky Mountains. This research will contribute new information on the origin of species and genetic diversity in alpine plants. Knowledge of biogeographic changes associated with past climate changes are especially relevant in the face of current climate warming, which is predicted to affect dramatically the diversity of alpine organisms (Halloy and Mark 2003). Organisms limited currently to refugial sky islands are likely to become even more geographically restricted and, consequently, experience greater threat of extinction as the climate warms (Thuiller et al. 2005). Two species (*S. oblongifolia* and *S. ritteriana*) of this clade have relatively small geographic ranges, and this research will make available data on genetic diversity of populations for application in management strategies. Understanding the sources of genetic diversity in this clade may be important for the conservation of populations where they are relatively rare and may be at risk of extinction or extirpation.

We use plastid haplotype data based on DNA sequences of *trnT-trnL* and *psbA-trnH* intergenic spacers to examine the geographic structure of genetic diversity in this clade. These genetic regions have been found to be variable within and among populations of *Synthyris* (Streit 2004; Brustkern 2006), and *psbA-trnH* was found to be valuable for intraspecific investigation in arctic-alpine New Zealand taxa (Holdregger and Abbott 2003). Incomplete lineage sorting of plastid haplotypes among the species in this clade combined with haplotypes that are unique to each species provides us with a system to explore geographic and genetic signatures that occurred during and subsequent to the differentiation of species.

MATERIALS AND METHODS

Taxon Sampling. We collected from populations across the distributional and

elevational ranges of the species of the Southern Rocky Mountain clade of *Synthyris* sect. *Besseya*, sampling the following number of populations for each species: *S. alpina*, 9; *S. plantaginea*, 15; *S. oblongifolia*, 1 (only population), and *S. ritteriana*, 4. We collected leaves from 20 ramets per population for 29 populations and sampled ultimately 12 individuals per population (except for populations where individuals were spaced very closely or where fewer than 20 individuals were present), encompassing 334 individuals (Table 1). Because *Synthyris* have short subterranean rhizomes that permit asexual reproduction, individuals sampled within each population were at least 6m apart. We attempted to collect over the range of the population unless it was geographically very widespread. Vouchered specimens for all sampled populations were deposited in the Marion Ownbey Herbarium (WS; leaf and inflorescence/infructescence samples only for rare taxa). GenBank accession numbers for all individuals sampled are listed in the appendix.

DNA Isolation and Sequencing. Haplotypes were inferred from concatenated sequences of the *trnT-trnL* and *psbA-trnH* intergenic spacers of plastid DNA that were generated as part of this study (Table 1). Total genomic DNA was isolated from approximately 10 mg silica-gel dried leaf material using the CTAB procedure of Doyle and Doyle (1987). The *trnT-trnL* intergenic spacer regions were amplified using primers a and b (Taberlet et al. 1991), and the *psbA-trnH* intergenic spacer regions were amplified using primer *psbA-trnH* (GUG; Hamilton 1999). Each 25 μl PCR contained 10.8 μl water, 2.5 μl 10× reaction buffer (Promega), 2.5 μl 5′ 5μM primer, 2.5 μl 3′ 5 μM primer, 3.0 μl $MgCl_2$, 1.5 μl dNTP, 0.2 μl Taq polymerase (Promega), and 2.0 μl diluted DNA template (1.5 μl water + 0.5 μl ~20ng DNA concentration). PCR conditions included initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 7 min. PCR

products were visualized by 1.5% agarose gel electrophoresis and purified using 20% polyethylene glycol 8000 in 2.5 M NaCl. The 10 μl cycle sequencing reactions contained 3.67 μl water, 0.33 μl 10 μM primer, 1.0 μl 5× sequencing buffer (Applied Biosystems), 1.0 μl BigDye® Terminator v3.1 (Applied Biosystems), and between 1.0 and 4.0 μl clean PCR product. Cycle sequence reaction included 25 cycles of 96°C for 10 sec and 50°C for 5 sec, with a final extension at 60°C for 4 min. Cycle sequence products were purified using 75% isopropanol precipitation, and DNA sequences were obtained on a 48-capillary 3730 DNA Analyzer (Applied Biosystems). Contigs were assembled and edited using Sequencher™ ver. 4.2.2 (Gene Codes Corporation) and sequences were aligned manually using Se-Al ver. 2.0a11 (Rambaut 1996-2002). Sequence alignment was unambiguous. PCR, cycle sequencing, and automated sequencing were repeated for individuals with questionable or unique sequences (six *trnT-trnL*, three *psbA-trnH*) to rule out PCR error.

Phylogenetic Analysis. A phylogenetic analysis was used to infer evolutionary relationships among haplotypes. Redundant haplotypes were removed and models of molecular evolution were evaluated using DT-ModSel (Minin et al. 2003), which uses decision theory to incorporate estimates of branch length error along with a Bayesian information criterion, and includes a penalty for over-parameterization. The specified parameters of a K81uf+I model (six substitution rates: rAC = 1.00, rAG = 1.16, rAT = 0.18, rCG = 0.18, rCT = 1.16, rGT = 1.00; base frequencies: A = 0.3802, C = 0.1168, G = 0.1462, T = 0.3568; proportion of invariable sites = 0.85) were applied in maximum likelihood (ML) searches of the combined dataset. ML analyses were conducted in PAUP* ver. 4.0b10 (Swofford 2002) with all characters weighted equally using heuristic ML searches (1000 replicates), including random taxon addition and branch-swapping by tree bisection-reconnection, saving one tree per replicate. Bootstrap

analysis (Felsenstein 1985) was used to assess support for branches recovered by ML using PAUP*. Maximum likelihood bootstrap analyses used the same model of evolution and random addition of haplotypes as the heuristic search for 1000 replicates.

Phylogeographic Analyses. Haplotype networks were constructed using TCS ver. 1.21 (Clement et al. 2000) under the criterion of a parsimony network in which connections have a probability of at least 95%. Ten insertion-deletion gaps resulting from a duplication of adjacent sequence were coded as single evolutionary events following Simmons and Ochoterena (2000). Coding these areas as "missing data" would lead to branch length inflation, and if excluded, would lead to possible loss of resolution (Cox and Chase 1995). Gaps were treated as a fifth character state. Most closed loops in the network were resolved using procedures from Templeton et al. (1992) and Templeton and Sing (1993), in combination with the ML topology (haplotype G is closer than haplotype L to haplotype Q; haplotypes B, C, and D are closer to haplotype AD than to haplotype G). Because the resolution of one loop was ambiguous, we performed the NCA using the two possible resolutions. In the haplotype network based solely on data for *S. alpina*, one closed loop was resolved using an ML topology for *S. alpina* alone (phylogeny not shown), in which haplotype G was recovered as more closely related to haplotype M than haplotype L.

Haplotype networks from TCS were used for nested clade analysis (NCA; Templeton et al. 1995; Templeton 1998) to assess geographic associations among haplotypes. The guidelines of Crandall (1996), Templeton et al. (1987, 1992), and Templeton and Sing (1993) were used to infer nested clades based on the TCS network. NCA was performed using GeoDis ver. 2.5 (Posada et al. 2000). Significance in the NCA was determined by using an alpha = 0.05 for clade distances. Posada and Templeton's (2005) inference key was used to infer geographic processes

associated with statistically significant patterns determined by NCA. The haplotype network reconstruction and NCA were performed using the four species to identify possible evolutionary processes that led to speciation within the group. NCA was performed separately on haplotype networks inferred for *S. plantaginea* and *S. alpina* to examine whether different evolutionary geographic processes would be identified from intraspecific analyses.

Because of their geographic breadth and numerous populations, *S. alpina* and *S. plantaginea* were analyzed in greater detail than *S. oblongifolia* and *S. ritteriana*. Evidence for range expansion for *S. plantaginea* and *S. alpina* was tested under the expansion model of Rogers and Harpending (1992) by examining pairwise mismatch distributions. The mismatch distribution of a population that has been constant in size for a long time is expected to be ragged and erratic, whereas a population that has undergone recent expansion is expected to generate a smooth distribution with a peak (Harpending 1994). Mismatch distributions were calculated (1000 replicates) using Arlequin ver. 3.0 (Excoffier and Schneider 2005) for samples contained in each of the clades identified in the separate NCAs for *S. alpina* and *S. plantaginea*. The Harpending (1994) raggedness index was used to evaluate deviation from a model of population expansion. A Mantel test (Sokal & Rohlf 1995) was performed each on *S. alpina* and *S. plantaginea* populations using Arlequin to look for evidence of isolation by distance. A distribution was created from 1000 random pairwise permutations, and statistical significance was determined by comparing the observed *r*-value to this distribution. Estimated pairwise F_{ST} values were plotted against corresponding geographical distance in order to illustrate the distribution of pairwise comparisons.

Estimates of Genetic Diversity. Arlequin was used to conduct analyses of molecular variance (AMOVA), based on pairwise distance, to assess the partitioning of genetic variation

within and among populations, and within and among recognized species. In addition, AMOVA was performed separately for *S. plantaginea* and *S. alpina* haplotypes in order to assess genetic variation partitioning solely within these widespread species. AMOVA was not conducted separately for either *S. oblongifolia* or *S. ritteriana*, which have relatively narrow distributions that include a low number of populations. Nei's (1987) haplotypic diversity (the mean number of differences between all pairs of haplotypes in the sample) and nucleotide diversity (the probability that two randomly chosen homologous nucleotides are different; Nei and Tajima 1981) were calculated also using Arlequin.

RESULTS

Haplotypes. The aligned DNA sequences for the *trnT-trnL* and *psbA-trnH* data partitions were 738 and 346 bp, respectively (before indel coding; after indel coding, 680 and 310), 37 of which were variable and 26 parsimony informative. We recovered 34 haplotypes based on the concatenated *trnT-trnL* and *psbA-trnH* sequences (Table 1). Ten populations consisted of individuals that shared multiple unique haplotypes (*Marlowe 46*, *51*, *58*, *60*, *63*, *65*, *88*, *91*, *95*, and *96*), three of which consisted of haplotypes that were unique to the population (*Marlowe 60*, *91*, and *96*). Haplotypes A-L, N and O were exclusive to *S. alpina*; haplotype P was exclusive to *S. oblongifolia*; haplotypes S, U-Z, and AA-AE were exclusive to *S. plantaginea*; and haplotypes AF-AH were exclusive to *S. ritteriana*. Haplotype M was shared by *S. alpina* (15 individuals) and *S. ritteriana* (three individuals). Haplotype Q was shared by *S. oblongifolia* (two individuals) and *S. plantaginea* (12 individuals). Haplotype R was shared by *S. oblongifolia* (nine individuals) and *S. ritteriana* (one individual). The most common haplotype (T) was shared by 46 individuals (37 *S. plantaginea*; 9 *S. ritteriana*) and was present in seven of the 29

localities. The geographically widespread haplotype Q was found in populations from southern Wyoming (*Marlowe 66*), central and southern Colorado (*Marlowe 89* and *99*), southern New Mexico (*Marlowe 50*), and eastern Arizona (*Marlowe 87*). To illustrate the geographic spread of genetic diversity, haplotypes were mapped onto the distribution maps (Figs. 3-4). The ML tree (Fig. 5; -ln 1718.6645) has nonexclusive haplotype phylogenies of the four species. The species are intermixed throughout the phylogeny.

Phylogeography. All haplotypes were connected to the networks reconstructed by TCS using 95% parsimony (Figs. 6-8). We apply the term "missing haplotypes" to intervening steps between haplotypes, which could represent haplotypes lost due to extinction, extant haplotypes not sampled, or haplotypes that never existed.

Twenty-one significant associations between clades and geography were indicated in the NCA using the four species (Fig. 6, Tables 2-3). There were eight inferences of contiguous range expansion, three inferences of allopatric fragmentation, and two inferences of restricted gene flow with isolation by distance (Fig. 6, Table 2). Clade 3-1 had ambiguous loops and was resolved using two alternatives (Fig. 6a, b). The alternatives were inferred to be allopatric fragmentation (Fig. 6a) and contiguous range expansion (Fig. 6b), respectively. The mismatch distributions failed to reject a model of population expansion for all clades except 1-13 (P = 0.023), 2-7 (P = 0.041), and 4-2 (P = 0.012).

The NCA of the 14 *S. plantaginea* haplotypes alone revealed seven significant associations between clades and geography (Fig. 7, Tables 4-5). There were three inferences of contiguous range expansion (clades 1-2, 1-6, and 3-1), one inference of allopatric fragmentation (clade 1-4), one inference of allopatric fragmentation or restricted gene flow with isolation by distance (depending on the inference chain; clade 2-3), and one inference of past fragmentation

or long-distance dispersal, or restricted gene flow with some long-distance dispersal (depending on the inference chain; clade 2-1), and one inference that was inconclusive (clade 2-5). The population expansion model was rejected for clade 1-4 (P = 0.042) based on the analysis of mismatch distributions.

The NCA of the 15 *S. alpina* haplotypes alone revealed eight significant associations between clades and geography (Fig. 8, Tables 6-7). There were two inferences of contiguous range expansion (clades 2-1 and 2-3), two inferences of allopatric fragmentation (clades 2-6 and 4-1), one inference of restricted gene flow with isolation by distance (clade 4-2), one inference of past fragmentation followed by range expansion (clade 3-1), one inference of past fragmentation and/or long-distance colonization (clade 1-2), and one inference that was inconclusive (clade 1-1). The population expansion model was rejected for clades 2-6, 3-3, 4-1, and the total cladogram (P = 0.023, 0.034, 0.002, and 0.001, respectively) based on the analysis of mismatch distributions.

The Mantel test revealed a nonsignificant relationship between F_{ST} values and geographical distances separating pairs of *S. plantaginea* populations ($r^2 = 0.0567$; P = 0.0650). The Mantel test performed on *S. alpina* populations revealed a significant relationship between F_{ST} values and geographical distances separating pairs of *S. alpina* populations ($r^2 = 0.1497$; P = 0.0210). The plots of geographical distance versus pairwise F_{ST} values (Figs. 9-10, Tables 8-9) illustrate the results of the Mantel test.

Genetic Diversity. AMOVA of all four species combined found that genetic variance in the Southern Rocky Mountain group of sect. *Besseya* was higher among (79.19%) than within populations (20.81%; Table 10). Haplotypic structure among populations (F_{ST}) was high (0.79191, P < 0.0001; Table 10). When populations were grouped by species, little genetic

variance was centered among species (11.86%; Table 10). Correspondingly, genetic subdivision among species was low (F_{CT} = 0.11865, P < 0.0001; Table 11) relative to subdivision among populations within species (F_{SC} = 0.77372, P < 0.0001). Variance among populations when not grouped by species was high (F_{ST} = 0.80057, P < 0.0001).

AMOVA conducted separately for *S. plantaginea* and *S. alpina* found that genetic variance was higher among (81.05% and 80.13%, respectively) than within populations (18.95% and 19.87%, respectively; Table 10). Population specific F_{ST} indices were very similar, ranging from 0.74141 to 0.82766 (Table 8) for *S. plantaginea*, and from 0.76017 to 0.81786 for *S. alpina* (Table 9).

Populations with the greatest haplotypic diversity (Table 1) included Mt. Evans along the central Colorado Front Range (*S. alpina*, *Marlowe 98*; 7.4091 +/- 3.7274), Red Hill Pass in central Colorado (*S. plantaginea*, *Marlowe 64*; 6.0000 +/- 3.4429), Stony Pass in southwestern Colorado (*S. alpina*, *Marlowe 93*; 4.4849 +/- 2.3750), and Lizard Head Pass in southwestern Colorado (*S. ritteriana*, *Marlowe 59*; 4.0606 +/- 2.1780). Each of these populations also had relatively high nucleotide diversity (Table 1). As haplotypic diversity decreased, there was a corresponding decrease in nucleotide diversity among all individuals sampled. Three populations of *S. alpina* and seven populations of *S. plantaginea* (Table 1) had haplotypic and nucleotide diversities of zero.

DISCUSSION

Speciation. Our results find the four species of the Southern Rocky Mountain clade of sect. *Besseya* have nonexclusive plastid haplotype phylogenies, which might be attributed to either hybridization or incomplete lineage sorting. These species are not known to hybridize

currently, although hybridization has been implicated in the origin of *S. ritteriana* (Schaack 1983a). The four extant species have distinct floral forms, no sympatric populations, and are separated by elevational and/or geographic distances, all of which are likely to prevent interspecific gene flow. No intermediate forms have been identified that would be indicative of recent hybridization. Plastid haplotype polymorphism is common throughout *Synthyris* (e.g. Streit 2004; Hufford unpublished data). Based on the factors that limit gene flow in this clade and the extent of haplotype polymorphism among species of *Synthyris*, we contend that most of the sharing of haplotypes among species of the Southern Rocky Mountain clade of sect. *Besseya*, with the exception of *S. ritteriana*, can be attributed to the persistence of ancestral polymorphism as a consequence of incomplete lineage sorting.

Although there are haplotypes shared between *S. alpina* and *S. ritteriana*, *S. alpina* and *S. oblongifolia*, *S. plantaginea* and *S. ritteriana*, and *S. plantaginea* and *S. oblongifolia*, no haplotypes are shared between *S. alpina* and *S. plantaginea*. This is consistent with inferences from the phylogenetic study based on ITS sequences (Hufford and McMahon 2004) that the initial cladogenic event in the Southern Rocky Mountain clade was between the ancestral lineages of *S. alpina* and *S. plantaginea*. As we discuss below, we infer that both *S. oblongifolia* and *S. ritteriana* are derived in the clade. Our NCA recovered significant associations in nested groups (Fig. 6, Table 2; groups 2-3, 3-1, 3-2, 3-4, 4-1, and 4-2) of haplotypes that include populations of both *S. alpina* and *S. plantaginea*. If these nested groups represent haplotypic differences arising from events that caused speciation, then we should be able to use inferences from the NCA to provide insights on speciation. We obtain congruent evolutionary signals from the nested groups 3-2 and 4-1 of restricted gene flow with isolation by distance, and allopatric fragmentation is inferred for nested groups 2-3 and 3-1. Both these inferences are consistent

with an initial speciation event resulting from elevational differentiation between early *S. alpina*-like and *S. plantaginea*-like lineages. Differences in flower color between these two species (*S. alpina* = blue; *S. plantaginea* = white) might be a signature of pollinator selection. The current ranges of *S. alpina* and *S. plantaginea* overlap, but they are separated by about 500m in elevation. Isolation of *S. alpina* and *S. plantaginea* resulting from elevational differentiation could have occurred not only due to treeline fluctuations throughout glaciations, but also due to the uplift of the Rocky Mountains, which occurred from middle and late Tertiary through the Pliocene (Richmond 1965) during which time the entire Rocky Mountain area was raised over 1,000m (Stokes et al. 1978).

The phylogenetic analysis of ITS sequences and morphological data, found *S. plantaginea* paraphyletic to *S. oblongifolia* (Fig 1; Hufford and McMahon 2004). *Synthyris oblongifolia* is restricted to a single ridge of the Sierra Blanca massif in southern New Mexico, the southernmost mountain in the U.S. that extends upward into the arctic-alpine life zone. The isolation of *S. oblongifolia* to this peak could be explained by long-distance dispersal or expansion of an ancestral range followed by fragmentation. As mentioned previously, a long-distance dispersal event is unlikely for this clade. Martin and Mehringer (1965) hypothesized a continuous spruce/fir/pine forest that extended south from the Colorado Rockies to include Sierra Blanca Peak from 17,000-23,000 years ago based on fossil pollen data, which is congruent with both Richmond's (1965) proposal that the actual snow line of the last glacial maximum 24,000 years ago was between 670 and 762m lower than at present and Antevs' (1954) less conservative proposal of a 914-1219m snow line depression. This opportunity for range expansion of mountain taxa, including the *S. plantaginea* lineage, across relatively low elevation continuous landscape may account for the southern distribution of *S. oblongifolia*. Climatic warming since

the last glacial maximum may have resulted in the fragmentation of the ancestral *S. plantaginea* lineage and isolation of populations in Sierra Blanca as the snow line rose in elevation. Our sampling recovered for *S. oblongifolia* three plastid haplotypes (Fig. 6, Table 1), one (P) of which is unique to the species and present in only one individual, one (R) of which is shared between one individual of *S. ritteriana* and nine individuals of *S. oblongifolia* and one (Q) of which is shared among 12 individuals from four widespread *S. plantaginea* populations and two individuals of *S. oblongifolia*. Haplotype Q is widespread throughout *S. plantaginea* populations along the Colorado Front Range, and haplotype R, although only found in *S. oblongifolia*, is in the same nesting clade as other haplotypes widespread throughout *S. plantaginea* populations. The distribution of haplotypes (Fig. 3) is consistent with fragmentation of a once widespread range of *S. plantaginea*. The sharing of haplotype R by *S. oblongifolia* and *S. ritteriana* could be a consequence of homoplasy or a haplotype independently isolated in these two species from ancestral *S. plantaginea* populations, in which it has subsequently been lost by genetic drift. The unique haplotype in one individual sampled from the *S. oblongifolia* population might represent a mutation that occurred since isolation of the population. Therefore enough time has elapsed since the isolation for the development of new haplotypes, but not enough time has elapsed to remove the ancestral haplotypes via genetic drift. The one individual with haplotype P is in nested clade 1-18, from which NCA failed to detect significance (Fig. 6, Table 3), probably because the *S. oblongifolia* population still maintains the ancestral haplotypes. Printzen et al. (2003), in their study of the widespread lichen *Cavernularia hultenii*, also demonstrated NCA's limited ability to detect past range fragmentations because of their shallow haplotype network and widespread ancestral haplotypes. NCA does detect a signal for contiguous range expansion

in clade 1-10 (Fig. 6, Table 3) which consists of haplotypes R (*S. oblongifolia* and *S. ritteriana*) and AF (*S. ritteriana*), but fails to detect the present fragmentation.

Synthyris ritteriana is restricted geographically to the San Juan Mountains, a discontinuous section of the Southern Rocky Mountains situated along the Continental Divide of southwestern Colorado (Hartman and Rottman 1985) that is especially rich in disjunct and endemic organisms (Weber 1965; Shaffer 1999; Shaw and Schneider 1995). This species is a tetraploid, putatively arising via unreduced gamete formation between diploids *S. alpina* and *S. plantaginea* (Schaack 1983a), and is the only case of potential allopolyploidy in *Synthyris* (occasional polyploids in *S. wyomingensis* and *S. missurica* are likely autopolyploids). *Synthyris ritteriana* is more mesic than *S. plantaginea* and more subalpine than *S. alpina*, and Schaack (1983a) suggested it is intermediate in form and flavonoid profiles to *S. plantaginea* and *S. alpina*. Both putative parents have current populations in the San Juan Mountains; however, *S. alpina* and *S. plantaginea* do not currently have any known sympatric populations. Our ML tree (Fig. 5) and plastid haplotype network (Fig. 6) place *S. ritteriana* among haplotypes found in *S. alpina* and also among haplotypes found in *S. plantaginea* and *S. oblongifolia*, a signal that is consistent with multiple allopolyploid hybridization events between *S. alpina* and *S. plantaginea*. These placements for the cpDNA haplotypes of *S. ritteriana* are the first genetic-based results consistent with Schaack's (1983a) hypothesis of hybridization between *S. alpina* and *S. plantaginea*.

If *S. ritteriana* originated via hybridization of *S. alpina* and *S. plantaginea*, then hybrid speciation may have occurred during a glaciation when populations of *S. alpina* would have expanded into valley areas where *S. plantaginea* had persisted. Andrews et al. (1975) provide evidence of significant treeline movement during the Holocene in the San Juan Mountains, as

well as a treeline retreat over the last 5,000 years. High elevation areas of the San Juan Mountains are estimated to have become ice free most recently 11,000-9,000 years ago (Andrews et al. 1975; Carrara et al. 1984) and this may be a time when *S. ritteriana* moved into its current habitats. The absence of *S. ritteriana* from areas outside of the San Juan Mountains suggests that the species was not widespread during the glaciations when treelines were lowered. Tetraploidy in *S. ritteriana* would have served as an isolating mechanism from its parental lineages. Evolution of higher plants via polyploidy has been especially important in areas affected by Pleistocene glaciations or areas enduring the effects of periglacial climates (Johnson et al. 1965).

***Phylogeography within* Synthyris plantaginea.** Of the four species in this group, *S. plantaginea* has the most widespread distribution, extending through intermountain valleys from southern Wyoming to New Mexico with several disjunct populations in mountains of Arizona, western New Mexico, and western Colorado (Fig. 2). Several of its haplotypes also have widespread distributions. *Synthyris plantaginea* has greater haplotype diversity in Colorado in the more northern part of its range than in New Mexico and Arizona (Fig. 3). Haplotypes Q and T are common and geographically widespread (Fig. 3), ranging from southern Wyoming, the Colorado Front Range, northern New Mexico, and eastern Arizona. This disjunct distribution with widespread haplotypes is consistent with fragmentation of a once continuous population.

Schaack (1983b) hypothesized that southern disjunct *S. plantaginea* populations are relicts of a southward migration corridor. In contrast, Nice and Shapiro (2001) suggested that *Oeneis* butterflies of the Rocky Mountains had a single southern refugium during the Pleistocene. If there was northward expansion from a southern refugium, we would expect greater haplotype diversity in the southern part of its range than in the north. However, the

distribution of *S. plantaginea* haplotypes (Fig. 3) illustrates greater haplotype diversity in the northern part of the range than in the disjunct New Mexico and Arizona populations. This pattern is more consistent with the persistence of refugia in the northern part of the range and southward range expansion of *S. plantaginea*, rather than a single, southern refugium for this species. The southern disjunct populations have genetic affinities to nearby populations extending south from the Colorado Front Range, which is consistent with founder events associated with southward migration from nearby source populations. The distribution of widespread haplotypes S and T (Fig. 3) is consistent with contiguous range expansion from central Colorado and north central New Mexico southwest into western New Mexico and the San Francisco Mountains. Contiguous range expansion was inferred by NCA for *S. plantaginea* for three clades (Fig. 7, Table 4; clades 1-2, 1-6, and 3-1), including populations from Wyoming, central Colorado Front Range, northern and northwestern New Mexico, and the White Mountains of Arizona. Mismatch distributions for *S. plantaginea* clades fail to reject a model of range expansion for most clades. It is curious though that the mismatch distribution test rejects a model of range expansion for clade 1-2 considering the classic signal of range expansion illustrated on the haplotype distribution map (Fig. 3) and the NCA inference. Past fragmentation/long-distance dispersal or restricted gene flow with some long-distance dispersal is inferred for clade 2-1 (Fig. 7, Table 4). These inferences are consistent with southern range expansion along a former migration corridor, followed by isolation upon climate warming. The nonsignificant Mantel test performed on *S. plantaginea* populations (Fig. 9) does not support isolation by distance, which is expected when haplotypes are so widespread throughout the distribution.

Mountain corridors between the main axis of the Southern Rocky Mountains and outlying ranges in western New Mexico and Arizona have been hypothesized to account for the migration of alpine taxa to San Francisco Peaks (Schaack 1983b). At least 90% of the San Francisco Mountain's present alpine tundra vascular flora is believed to have migrated southward to the mountain during Pleistocene time when the treeline was greatly depressed and alpine environments were more continuous (Little 1941; Moore 1965; Schaack 1983b). These corridors may have been present during intervals between glacial maxima when high elevation areas would have been covered by ice. Migration of Rocky Mountain taxa to the San Francisco peaks has been suggested at 75,000-65,000 years (Moore 1965) and 20,000 years ago (Martin and Mehringer 1965). Based on the southern distribution of *S. plantaginea*, Schaack (1983b) hypothesized that the primary dispersal corridor used by alpine taxa to reach the San Francisco Mountain was the continuous area of high elevation to the east and north beginning with the main Rocky Mountain chain of Colorado and New Mexico. The Chuska Mountains were not affected by glaciation (Carrara et al. 1984), and could have represented a northern part of this migration corridor. If *S. plantaginea* had a more continuous distribution between the Rocky Mountains and Colorado Plateau, fragmentation leading to the current disjunction could have been established with the altithermal beginning 9,000 years ago (Hevly and Karlstrom 1974; Hadley 1987). Being the lowest elevation species in the clade, *S. plantaginea* was likely able to take the most advantage of these ephemeral migration corridors, and this might explain its broad distribution.

In the western part of the range, *S. plantaginea* populations have a single haplotype, whereas populations along the Front Range have multiple haplotypes (Fig. 3, Table 1). This distribution of haplotypes is consistent with a hypothesis of a westward migration of *S.*

plantaginea from more ancestral populations that persist along the Front Range. NCA infers allopatric fragmentation for clade 1-4 which consists of individuals from East Naturita Creek and West Rifle Creek of the western slope of Colorado, each population having its own unique haplotype. No other population shares haplotypes with either of these, but we can infer from the ML tree (Fig. 5) that haplotypes AA and AB (of East Naturita Creek and West Rifle Creek, respectively; Figs. 3, 6) are more closely related to the eastern *S. plantaginea* populations than to those to the south. The origin of these western Colorado populations could have resulted from westward spread during glacial treeline depression, followed by allopatric fragmentation that allowed the evolution of unique haplotypes.

***Phylogeography within* Synthyris alpina.** Populations of *Synthyris alpina* are currently found on high elevation (~3,000-4,000m) peaks extending along the Front Range from southern Wyoming to northern New Mexico and westward throughout high elevation areas of Colorado and into eastern Utah. Based on the distribution of haplotypes (Fig. 4), populations can be divided into three regions: northern (Front Range), southwestern (San Juan and La Sal Mountains, and Grand Mesa), and southern (Sangre de Cristo Range). These three regions each have several haplotypes that are unique to a region, with only two haplotypes (G and I) found in more than one region (northern and southwestern). The northern, southwestern, and southern parts of the distribution therefore might represent three general areas of range expansion during glaciation. We hypothesize *S. alpina* populations are currently in interglacial refugia on high-elevation sky islands, but were once widespread at lower elevations during glaciations, permitting broader gene flow within the three regions but limited gene flow among the regions. F_{ST} values for *S. alpina* and *S. plantaginea* (0.80 and 0.81, respectively; Table 10) are both consistent with populations that are isolated. The significant Mantel test (Fig. 10) for *S. alpina*

could be an indication of isolation by distance which would be consistent for an organism with limited migration followed by fragmentation. Because *S. alpina* is limited to discontinuous alpine habitats restricted to peaks, we hypothesize its populations may have been isolated from one another longer than have populations of the lower elevation *S. plantaginea*. Signatures of range expansion were detected in the NCA. Two inferences of contiguous range expansion were inferred by NCA (clades 2-1 and 2-3; Fig. 8, Table 6), and mismatch distributions for *S. alpina* clades fail to reject a model of range expansion for all but four clades (clades 2-6, 3-3, 4-1, and the total cladogram). NCA inferred contiguous range expansion as the process that distinguishes clade 1-1 from clade 1-2 (individuals from La Sal Mountains, Mt. Evans, Stony Pass; Fig. 8, Table 6). The alpine zone encompassing these populations was contiguous during Wisconsin glaciations (Péwé 1983), and this would have facilitated range expansion.

We recovered the greatest number of haplotypes (5; G, I, J, K, M; Fig. 4, Table 1) from the Mt. Evans population. Haplotype M is found only in the northern Front Range populations (Mt. Evans, Rocky Mountain National Park, and Medicine Bow Peak) among sampled populations of *S. alpina*, although the presence of this haplotype in *S. ritteriana* leads us to infer that this haplotype was more widespread prior to the origin of the latter species. Medicine Bow Peak, the northernmost population, has three haplotypes (M, N, O). Haplotypes N and O are unique to Medicine Bow Peak. We hypothesize that haplotype M was locally widespread along the northern Colorado Front Range, where alpine habitat is continuous at elevations subjacent to peaks. Unique haplotypes O and N (in the Medicine Bow Peak population), L (in the Rocky Mountain National Park population), and J and K (in the Mt. Evans population) either appeared following isolation of these populations, or are ancient haplotypes.

The three haplotypes (B, C, and D; Fig. 4, Table 1) recovered from the Sangre de Cristo Mountain Range populations of the southern region are very distinct from the remaining sampled haplotypes, being separated by many intervening missing haplotypes (Fig. 8). For clade 2-3 (Fig. 8), which contains haplotypes from the two populations in the southern region, NCA infers contiguous range expansion (Table 6). This inference is consistent with the topography, as the Sangre de Cristo Mountain Range extends south from the Lily Lake population, terminating near Pecos Baldy Peak (Fig. 2). Range expansion could have occurred during glaciations when populations migrated downward in elevation. Although the high peaks of the Sangre de Cristo Mountains supported small glaciers in late Pleistocene time, the glaciers were not large enough to descend from the high mountains to coalesce into the valleys (Ray 1940). The lowest point at which glacial ice has ever been reported in New Mexico is 2590m (Ray 1940), so there are high mountain areas in the Sangre de Cristo Mountain range that were not glaciated where range expansion of *S. alpina* would have been facilitated.

Conservation Implications. This century is predicted to show a rate of temperature rise among the highest in the world's history (Halloy and Mark 2003). Range-restricted species, especially those on mountaintops, have been observed to experience severe range contractions and extinction due to recent climate warming (Parmesan 2006). Understanding the historical biogeography of the alpine is gaining urgency because of the profound effect of impending climate change on alpine organisms (DeChaine and Martin 2004). The life history traits of the Southern Rocky Mountain group of *S.* sect. *Besseya* (perennial habit, slow growth, and short-distance seed dispersal) and the high-elevation isolation of many populations may make them vulnerable to climate warming. *Synthyris oblongifolia* is currently a conservation concern because of restriction to Sierra Blanca Peak and low number of individuals

(http://nmrareplants.unm.edu/agency.php#Section5). Our data suggest this species has relatively low genetic diversity (as inferred from plastid haplotypes) and is a relatively recent adaptation to the alpine environment. Three populations of *S. alpina* (Grand Mesa, Pecos Baldy Peak, and Cumberland Pass; Fig. 2, Table 1) in the western and southern parts of its range have only one haplotype, with the Cumberland Pass population having a unique haplotype. Such populations that are already at or near the top of high elevation peaks have no means of escaping climate warming and would be vulnerable to local extirpation (Halloy and Mark 2003), thus reducing the overall genetic diversity and/or the geographic range of their respective species.

Of possible greater conservation concern are the disjunct populations of *S. plantaginea* that have low genetic diversity following southward migration as well as the Colorado western slope populations (East Naturita Creek and West Rifle Creek; Fig. 2, Table 1) that each harbor unique haplotypes. These disjunct populations have little to no opportunity for gene flow among populations, and may face habitat loss with global warming. Populations of *S. plantaginea* along the Colorado Front Range, depending on local topology, may be able migrate up in elevation to escape climate warming if such warming is not too rapid.

A conservation strategy that focuses on preserving populations with high levels of genetic diversity might not consider the southwestern disjunct populations of *S. plantaginea* as having conservation value because their haplotypes are also present in other widespread populations. Because of their isolation, however, such peripheral populations can be viewed as areas likely to generate future evolutionary diversity (Lesica and Allendorf 1995) as mutations arise via genetic drift, introducing new unique haplotypes and potentially forming new species.

Conservation strategies must focus not only on retaining *patterns* of genetic diversity, but also on ecological and evolutionary *processes* that are important in maintaining and promoting

diversification (Cowling and Pressey 2001). We infer from this study that gene flow among populations has been important for maintaining genetic diversity, and in one example, the origin of a hybrid species. We also infer that preserving both ecological and topographic complexity of the landscape is vital for maintaining genetic diversity and promoting diversification in plant taxa of the American West.

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APPENDIX

GenBank accession numbers for *trnT-trnL* and *psbA-trnH* intergenic spacer regions for haplotypes of the Southern Rocky Mountain clade of *Synthyris* sect. *Besseya*.

A: EF550087, EF550053; B: EF550088, EF550054; C: EF550089, EF550055; D: EF550090, EF550056; E: EF550091, EF550057; F: EF550092, EF550058; G: EF550093, EF550059; H: EF550094, EF550060; I: EF550095, EF550061; J: EF550096, EF550062; K: EF550097, EF550063; L: EF550098, EF550064; M: EF550099, EF550065; N: EF550100, EF550066; O: EF550101, EF550067; P: EF550102, EF550068; Q: EF550103, EF550069; R: EF550104, EF550070; S: EF550105, EF550071; T: EF550106, EF550072; U: EF550107, EF550073; V: EF550108, EF550074; W: EF550109, EF550075; X: EF550110, EF550076; Y: EF550111, EF550077; Z: EF550112, EF550078; AA: EF550113, EF550079; AB: EF550114, EF550080; AC: EF550115, EF550081; AD: EF550116, EF550082; AE: EF550117, EF550083; AF: EF550118, EF550084; AG: EF550119, EF550085; AH: EF550120, EF550086.

Table 1. Locality and collection information, number of individuals, and genetic diversity (Nei 1987) for individuals of *Synthyris alpina*, *S. oblongifolia*, *S. plantaginea*, and *S. ritteriana* sequenced for *trnT-trnL* and *psbA-trnH*. Locality numbers correspond to those in Figure 2.

Locality #	Taxon	*n*	Collection	Locality	Elev. (m)	Coord.	Haplotypes (# of individuals)	Haplotypic diversity (h)	Nucleotide diversity (π)
1	*S. alpina* A. Gray	12	Marlowe 86	Manti-La Sal National Forest, San Juan County, UT	3629	N38°27 W109°14	A (11), I (1)	0.1667+/- 0.2401	0.0002+/- 0.0003
2		12	Marlowe 88	Pecos Baldy Peak, Mora County, NM	3774	N35°54 W105°39	B (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
3		12	Marlowe 90	Lily Lake, Sangre de Cristo Mountains, Huerfano County, CO	3717	N37°35 W105°29	B (3), C (5), D (4)	3.4848+/- 1.9098	0.0035+/- 0.0022
4		12	Marlowe 91	Cumberland Pass, Gunnison County, CO	3799	N38°41 W106°28	H (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
5		12	Marlowe 93	Stony Pass, San Juan County, CO	3856	N37°47 W107°32	A (1), E (2), F (2), G (7)	4.4848+/- 2.3750	0.0045+/- 0.0027
6		12	Marlowe 95	Cottonwood Lake #1, Grand Mesa, Mesa County, CO	3231	N39°03 W107°58	A (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
7		12	Marlowe 97	Ute Trail, Rocky Mountain National Park, Larimer County, CO	3536	N40°22 W105°41	L (1), M (11)	1.3333+/- 0.8887	0.0013+/- 0.0010
8		12	Marlowe 98	Summit Lake, Mt. Evans, Denver Mountain Parks, Clear Creek County, CO	3917	N39°35 W105°38	G (2), I (4), J (2), K (1), M (3)	7.4091+/- 3.7274	0.0075+/- 0.0042

9		12	Marlowe 100	Medicine Bow Peak, Albany County, WY	3414	N41°21 W106°18	M (1), N (10), O (1)	2.2576+/- 1.3333	0.0023+/- 0.0015
10	*S. oblongifolia* (Pennell) L. Hufford & M. McMahon	12	Marlowe 50	Sierra Blanca Peak, Lincoln County, NM	3450	N33°23 W105°48	P (1), Q (2), R (9)	2.5303+/- 1.4623	0.0026+/- 0.0017
11	*S. plantaginea* Benth.	12	Marlowe 46	San Francisco Mountains, Coconino County, AZ	2593	N35°21 W111°44	S (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
12		11	Marlowe 51	Pecos River, San Miguel County, NM	2368	N35°45 W105°40	T (11)	0.0000+/- 0.0000	0.0000+/- 0.0000
13		12	Marlowe 55	Cimarron Canyon, Colfax County, NM	2449	N36°32 W105°12	S (1), T (11)	1.6667+/- 0.2401	0.0002+/- 0.0003
14		12	Marlowe 58	Chuska Mountains, San Juan County, NM	2278	N36°08 W109°01	S (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
15		12	Marlowe 60	East Naturita Creek, San Miguel County, CO	2467	N37°59 W108°18	AA (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
16		12	Marlowe 61	Trout Creek Pass, Chaffee County, CO	2832	N38°53 W105°59	U (6), V (6)	3.8182+/- 2.0652	0.0039+/- 0.0023
17		12	Marlowe 62	Divide, Teller County, CO	2738	N38°56 W105°11	U (2), X (9), Z (1)	3.0606+/- 1.7115	0.0031+/- 0.0019
18		12	Marlowe 63	Kenosha Pass, Park County, CO	3101	N39°24 W105°45	U (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
19		5	Marlowe 64	Red Hill Pass, Park County, CO	3045	N39°15 W105°57	S (2), U (3)	6.0000+/- 3.4429	0.0061+/- 0.0041

20		12	Marlowe 65	Poudre Canyon, Larimer County, CO	1824	N40°41 W105°20	T (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
21		12	Marlowe 66	Crow Creek, Albany County, WY	2448	N41°09 W105°21	Q (1), AD (10), AE (1)	2.2121+/- 1.3118	0.0022+/- 0.0015
22		6	Marlowe 87	White Mountains, Apache County, AZ	2749	N34°02 W109°35	Q (4), W (2)	0.5333+/- 0.5077	0.0005+/- 0.0006
23		12	Marlowe 89	La Veta Pass, Sangre de Cristo Canyon, Costilla County, CO	2545	N37°30 W105°17	Q (3), T (3), Y (6)	1.7727+/- 1.1020	0.0018+/- 0.0013
24		12	Marlowe 96	West Rifle Creek, Garfield County, CO	2232	N39°43 W107°53	AB (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
25		12	Marlowe 99	Golden Gate Canyon, Gilpin County, CO	2469	N39°49 W105°25	Q (4), AC (8)	0.9697+/- 0.7070	0.0010+/- 0.0008
26	*S. ritteriana* Eastw.	12	Marlowe 56	Red Mountain Pass, San Juan County, CO	3358	N37°53 W107°48	T (1), AF (11)	0.3333+/- 0.3564	0.0003+/- 0.0004
27		12	Marlowe 57, 92	Little Molas Lake, San Juan County, CO	3308	N37°44 W107°42	T (7), AF (4), AG (1)	1.1364+/- 0.7911	0.0011+/- 0.0009
28		12	Marlowe 59	Lizard Head Pass, Delores County, CO	3080	N37°48 W107°54	R (1), T (1), AF (1), AG (4), AH (5)	4.0606+/- 2.1780	0.0041+/- 0.0025
29		12	Marlowe 94	Yankee Boy Basin, Ouray County, CO	3365	N37°59 W107°45	M (2), AF (10)	2.4242+/- 1.4122	0.0024+/- 0.0016

Table 2. Results of nested clade analysis on haplotypes of all four species of the Southern Rocky Mountain group of *Synthyris* sect. *Besseya*. Nested clades without significant geographic associations are not listed. The inference chain is based on Posada and Templeton's (2005) inference key. The asterisk indicates an alternative inference for clade 3-1 based on alternative resolution of a closed loop.

Clade	χ^2 statistic	Probability	Inference chain	Inferred pattern
1-1	7.000	0.046	1-19-20-2-11-17-No	Inconclusive outcome
1-2	36.000	0.000	1-19-20-2-3-5-15-No	Past fragmentation and/or long-distance colonization between **A** (La Sal, Stony Pass, Grand Mesa) and **H** (Cumberland Pass)
1-6	22.000	0.000	1-19-20-No	Inadequate geographic sampling between **M** (Rocky Mountain National Park, Mt. Evans, Medicine Bow Peak, Yankee Boy Basin) and **AH** (Lizard Head Pass)
1-8	20.000	0.000	1-19-20-2-Tip/interior could not be determined	Inconclusive outcome
1-10	33.507	0.000	1-2-11-12-No	Contiguous range expansion between **R** (Sierra Blanca Peak, Lizard Head Pass) and **AF** (Red Mountain Pass, Little Molas Lake, Lizard Head Pass, Yankee Boy Basin)
1-11	69.067	0.000	1-2-11-12-No	Contiguous range expansion between **S** (San Francisco Mountains, Cimarron Canyon, Chuska Mountains, Red Hill Pass) and **T** (Pecos River, Cimarron Canyon, Poudre Canyon, La Veta Pass, Red Mountain Pass, Lizard Head Pass, Little Molas Lake)
1-12	11.000	0.002	1-2-11-12-No	Contiguous range expansion between **Y** (La Veta Pass) and **AG** (Lizard Head Pass, Little Molas Lake)
1-13	24.000	0.000	1-19-No	Allopatric fragmentation between **AA** (East Naturita Creek) and **AB** (West Rifle Creek)

2-1	31.383	0.000	1-2-11-12-No	Contiguous range expansion between **1-1** (Stony Pass, La Sal Mountains, Mount Evans) and **1-2** (La Sal Mountains, Stony Pass, Grand Mesa, Cumberland Pass)
2-3	11.000	0.016	1-19-No	Allopatric fragmentation between **1-4** (Divide) and **1-5** (Mount Evans)
2-5	14.933	0.005	1-2-3-4-No	Restricted gene flow with isolation by distance between **1-7** (Golden Gate Canyon) and **1-8** (Sierra Blanca Peak, Crow Creek, White Mountains, La Veta Pass, Golden Gate Canyon, Trout Creek)
2-6	201.163	0.000	1-2-3-4-No	Restricted gene flow with isolation by distance among **1-9** (Divide) and **1-10** (Sierra Blanca Peak, Lizard Head Pass, Red Mountain Pass, Little Molas Lake, Yankee Boy Basin) and **1-11** (San Francisco Mountains, Cimarron Pass, Chuska Mountains, Red Hill Pass, Pecos River, Poudre Canyon, La Veta Pass, Red Mountain, Lizard Head Pass, Little Molas Lake)
2-7	35.000	0.000	1-19-20-2-3-4-9-No	Allopatric fragmentation between **1-12** (La Veta Pass, Lizard Head Pass, Little Molas Lake) and **1-13** (East Naturita Creek, West Rifle Creek)
2-10	35.000	0.000	1-19-20-2-Tip/interior could not be determined	Inconclusive outcome
2-13	10.000	0.003	1-2-11-12-No	Contiguous range expansion between **1-22** (Pecos Baldy Peak, Lily Lake) and **1-23** (Lily Lake)
3-1	45.000	0.002	1-19-20-2-3-4-9-No	Allopatric fragmentation between **2-1** (La Sal Mountains, Stony Pass, Grand Mesa, Cumberland Pass, Mount Evans) and **2-2** (White Mountains)
3-1*	45.780	0.000	1-2-11-12-No	Contiguous range expansion between **2-1** (La Sal Mountains, Stony Pass, Grand Mesa, Cumberland Pass, Mount Evans) and **2-2** (Mount Evans, Divide)

3-2	112.018	0.000	1-2-3-4-No	Restricted gene flow with isolation by distance among **2-3** (Mount Evans, Divide) and **2-4** (Rocky Mountain National Park, Mount Evans, Medicine Bow Peak, Yankee Boy Basin, Lizard Head Pass) and **2-5** (Sierra Blanca Peak, Crow Creek, White Mountains, La Veta Pass, Golden Gate Canyon, Trout Creek)
3-3	119.710	0.000	1-2-11-12-No	Contiguous range expansion between **2-6** (Sierra Blanca Peak, Lizard Head Pass, Red Mountain Pass, Little Molas Lake, Yankee Boy Basin, Divide, San Francisco Mountains, Cimarron Canyon, Chuska Mountains, Red Hill Pass, Pecos River, Poudre Canyon, La Veta Pass) and **2-7** (La Veta Pass, Lizard Head Pass, Little Molas Lake, East Naturita Creek, West Rifle Creek)
3-4	58.121	0.014	1-2-11-12-No	Contiguous range expansion among **2-8** (Stony Pass, Mount Evans, Rocky Mountain National Park, Medicine Bow Peak) and **2-9** (Mount Evans) and **2-10** (Sierra Blanca Peak, Trout Creek, Divide, Kenosha Pass, Red Hill Pass, Medicine Bow Peak, Crow Creek)
4-1	421.765	0.000	1-2-3-4-No	Restricted gene flow with isolation by distance among **3-1** (La Sal Mountains, Stony Pass, Grand Mesa, Cumberland Pass, Mount Evans, White Mountains) and **3-2** (Mount Evans, Divide, Sierra Blanca, Crow Creek, White Mountains, La Veta Pass, Golden Gate Canyon, Trout Creek, Rocky Mountain National Park, Medicine Bow Peak, Yankee Boy Basin, Lizard Head Pass) and **3-3** (Sierra Blanca Peak, Lizard Head Pass, Red Mountain Pass, Little Molas Lake, Yankee Boy Basin, Divide, San Francisco Mountains, Cimarron Canyon, Chuska Mountains, Red Hill Pass, Pecos River, Poudre Canyon, La Veta Pass, East Naturita Creek, West Rifle Creek)
4-2	79.241	0.000	1-2-11-12-No	Contiguous range expansion between **3-4** (Stony Pass, Mount Evans, Rocky Mountain National Park, Medicine Bow Peak, Sierra Blanca Peak, Trout Creek, Divide, Kenosha Pass, Red Hill Pass, Crow Creek) and **3-5** (Pecos Baldy Peak, Lily Lake, Crow Creek)

Total cladogram	258.8547	0.000	1-2-Tip/interior could not be determined	Inconclusive outcome
Total cladogram *	258.8547	0.000	1-2-Tip/interior could not be determined	Inconclusive outcome

Table 3. Nested clade analysis of the haplotype network for all four species of the Southern Rocky Mountain group of *Synthyris* sect. *Besseya* with average clade distance (D_C) and nested clade distance (D_N) for each haplotype and clade and interior-tip (I-T) distances for each clade. Distances that were significantly large or small via permutation tests are labeled ^{L} and ^{S}, respectively. The asterisk indicates an alternative result for clade 3-1 based on alternative resolution of a closed loop.

	Topology	Haplotype/Clade	D_C	D_N
Clade 1-1	Tip	F	0.0000	144.8380
	Interior	I	107.0576	139.6239
	I-T		107.0576	-5.2141
Clade 1-2	Interior	A	67.3012^S	81.0563^S
	Tip	H	0.0000^S	119.2491^L
	I-T		67.3012^L	-38.1928^S
Clade 1-6	Interior	M	80.5427^S	121.2812^S
	Tip	AH	0.0000^S	229.9400^L
	I-T		80.5427	-108.6588^S
Clade 1-8	Interior	Q	346.1738^L	353.6506^L
	Interior	V	0.0000^S	237.8808^S
Clade 1-10	Interior	R	94.8182^S	371.8543^L
	Tip	AF	8.6108^S	132.8955^S
	I-T		86.2075	238.9588^L
Clade 1-11	Tip	S	196.9301^S	306.9727^L
	Interior	T	208.9523^S	234.7847^S
	I-T		12.0222	-72.1880^S
Clade 1-12	Tip	Y	0.0000^S	104.1428^S

	Interior	AG	6.0989^{S}	124.7504^{L}
	I-T		6.0989	20.6076^{L}
Clade 1-13	Tip	AA	0.0000^{S}	97.9703^{L}
	Tip	AB	0.0000^{S}	97.9299^{S}
Clade 1-16	Tip	L	0.0000	60.4925
	Interior	O	0.0000	60.4101
	I-T		0.0000	-0.0824
Clade 1-18	Interior	P	0.0000	624.5449
	Tip	U	26.1145	35.7988
	I-T		-26.1145	588.7462
Clade 1-19	Tip	N	0.0000	7.4810
	Interior	AE	0.0000	74.9161
	I-T		0.0000	67.4351
Clade 2-1	Tip	Clade 1-1	141.1136^{L}	163.5968^{L}
	Interior	Clade 1-2	93.7873^{S}	94.9428^{S}
	I-T		-47.3264^{S}	-68.6539^{S}
Clade 2-3	Interior	Clade 1-4	0.0000^{S}	14.9087^{S}
	Tip	Clade 1-5	0.0000	67.0197^{L}
	I-T		0.0000	-52.1110^{S}
Clade 2-5	Tip	Clade 1-7	0.0000^{S}	264.0141
	Interior	Clade 1-8	324.7082	312.8404
	I-T		324.7082^{L}	48.8263
Clade 2-6	Tip	Clade 1-9	0.0000	272.1401
	Tip	Clade 1-10	199.2730^{S}	182.9361^{S}
	Interior	Clade 1-11	270.4300^{L}	270.1244^{L}
	I-T		76.5428^{L}	84.7774^{L}
Clade 2-7	Interior	Clade 1-12	113.5099	160.5109^{L}

	Tip	Clade 1-13	97.9501^{S}	112.0945^{S}
	I-T		15.5598	48.4163^{L}
Clade 2-8	Tip	Clade 1-14	0.0000	95.2933
	Interior	Clade 1-15	89.6886	111.0226
	Tip	Clade 1-16	60.4513	275.8139
	I-T		59.4630	-74.5310
Clade 2-10	Tip	Clade 1-18	56.6763^{S}	75.7107^{S}
	Tip	Clade 1-19	13.6115^{S}	188.9709^{L}
Clade 2-13	Interior	Clade 1-22	60.0295^{S}	82.5407^{S}
	Tip	Clade 1-23	0.0000	112.5499^{L}
	I-T		60.0295	-30.0092^{S}
Clade 3-1	Tip	Clade 2-1	106.1191^{S}	117.6983^{S}
	Interior	Clade 2-2	0.0000	503.9668^{L}
	I-T		-106.1191	386.2684^{L}
Clade 3-1*	Tip	Clade 2-1	106.1191^{S}	112.2122^{S}
	Interior	Clade 2-2	24.3834^{S}	169.6108^{L}
	I-T		-81.7356^{S}	57.3986^{L}
Clade 3-2	Tip	Clade 2-3	24.3834^{S}	112.2024^{S}
	Tip	Clade 2-4	145.9764	188.6786
	Interior	Clade 2-5	300.6338^{L}	273.8971^{L}
	I-T		195.1885^{L}	110.7106^{L}
Clade 3-3	Tip	Clade 2-6	238.3483^{L}	233.7983^{L}
	Interior	Clade 2-7	127.3111^{S}	167.7618^{S}
	I-T		-111.0372^{S}	-66.0364^{S}
Clade 3-4	Interior	Clade 2-8	133.9552	181.2135^{L}
	Tip	Clade 2-9	0.0000	52.1782
	Interior	Clade 2-10	107.4929	108.0889

	I-T		114.6598^{S}	75.7153
Clade 3-6	Interior	Clade 2-12	0.0000	93.7910
	Tip	Clade 2-13	90.0430	93.7948
	I-T		-90.0430	-0.0039
Clade 4-1	Tip	Clade 3-1	123.9045^{S}	164.8467^{S}
	Interior	Clade 3-2	275.9491^{L}	287.7773^{L}
	Interior	Clade 3-3	218.2655	217.7717
	I-T		109.5871^{L}	71.4037^{L}
Clade 4-2	Interior	Clade 3-4	124.9067^{S}	136.0824^{S}
	Tip	Clade 3-5	203.5535	244.2391^{L}
	I-T		-78.6467^{S}	-108.1567^{S}
Total cladogram	Tip	Clade 4-1	222.3820	224.0099
	Tip	Clade 4-2	178.2536^{S}	217.9020
Total cladogram*	Tip	Clade 4-1	222.3820	224.0099
	Tip	Clade 4-2	178.2536^{S}	217.9020

Table 4. Results of nested clade analysis on haplotypes of *Synthyris plantaginea*. Nested clades without significant geographic associations are not listed. The inference chain is based on Posada and Templeton's (2005) inference key.

Clade	χ^2 statistic	Probability	Inference chain	Inferred pattern
1-2	60.242	0.000	1-2-11-12-No	Contiguous range expansion between **S** (San Francisco Mountains, Cimarron Canyon, Chuska Mountains, Red Hill Pass) and **T** (Pecos River, Cimarron Canyon, Poudre Canyon, La Veta Pass)
1-4	24.000	0.000	1-19-No	Allopatric fragmentation between **AA** (East Naturita Creek) and **AB** (West Rifle Creek)
1-6	24.444	0.000	1-2-11-12-No	Contiguous range expansion among **Q** (Crow Creek, White Mountains, La Veta pass, Golden Gate Canyon), **W** (White Mountains), and **V** (Trout Creek)
2-1	116.115	0.000	1-2-3-5-16-No; or 1-2-3-5-6-7-Yes	Past fragmentation or long-distance dispersal among **1-1** (Divide), **1-2** (San Francisco Mountains, Cimarron Canyon, Chuska Mountains, Red Hill Pass, Pecos River, Poudre Canyon, La Veta Pass), and **1-3** (La Veta Pass); or restricted gene flow with some long-distance dispersal
2-3	56.733	0.000	1-2-3-4-9-No; or 1-2-3-4-No	Allopatric fragmentation between **1-5** (Golden Gate Canyon) and **1-7** (Divide); or restricted gene flow with isolation by distance
2-5	33.000	0.000	1-19-20-2-Tip/Interior status cannot be determined	Inconclusive outcome
3-1	246.906	0.000	1-2-11-12-No	Contiguous range expansion among **2-1** (Divide, San Francisco Mountains, Cimarron Canyon, Chuska Mountains, Red Hill Pass, Pecos River, Poudre Canyon, La Veta Pass), **2-2** (West Rifle Creek, East Naturita Creek), and **2-3** (Golden Gate Canyon, Crow Creek, White Mountains, La Veta Pass, Trout Creek Pass, Divide)

Total cladogram	124.351	0.000	1-2-Tip/Interior status cannot be determined	Inconclusive outcome

Table 5. Nested clade analysis of the haplotype network for *Synthyris plantaginea* with average clade distance (D_C) and nested clade distance (D_N) for each haplotype and clade and interior-tip (I-T) distances for each clade. Distances that were significantly large or small via permutation tests are labeled ^{L} and ^{S}, respectively.

	Topology	Haplotype/Clade	D_C	D_N
Clade 1-2	Tip	S	196.9301^S	308.0627
	Interior	T	212.1995^S	267.9061
	I-T		15.2694	-40.1566
Clade 1-4	Tip	AB	0.0000^S	97.9299^S
	Tip	AA	0.0000^S	97.9703^L
Clade 1-6	Interior	Q	346.0649	346.7606
	Tip	W	0.0000	355.0329
	Interior	V	0.0000^S	273.1198^S
	I-T		230.7099	-32.8192
Clade 2-1	Tip	Clade 1-1	0.0000	255.4595
	Interior	Clade 1-2	285.5560^L	284.6950^L
	Interior	Clade 1-3	0.0000^S	158.0274^S
	I-T		261.0798^S	18.3783
Clade 2-3	Tip	Clade 1-5	0.0000^S	250.6583
	Interior	Clade 1-6	331.0392	326.4045^L
	Tip	Clade 1-7	0.0000^S	179.6812^S
	I-T		331.0392^L	113.3223^L
Clade 2-5	Tip	Clade 1-9	26.1145^S	60.7061^S
	Tip	Clade 1-10	0.0000^S	159.9944^L
Clade 3-1	Tip	Clade 2-1	274.1436	282.9782

	Interior	Clade 2-2	97.9501[S]	179.4394[S]
	Tip	Clade 2-3	281.6026	297.3533
	I-T		-178.7489[S]	-108.4636[S]
Clade 3-2	Interior	Clade 2-4	0.0000	155.8047
	Tip	Clade 2-5	87.3965	89.2702
	I-T		-87.3965	66.5345
Total cladogram	Tip	Clade 3-1	269.8132[L]	266.8853[L]
	Tip	Clade 3-2	91.0120[S]	213.1504[S]

Table 6. Results of nested clade analysis on haplotypes of *Synthyris alpina*. Nested clades without significant geographic associations are not listed. The inference chain is based on Posada and Templeton's (2005) inference key.

Clade	χ^2 statistic	Probability	Inference chain	Inferred pattern
1-1	7.000	0.046	1-19-20-2-11-17-No	Inconclusive outcome
1-2	36.000	0.000	1-19-20-2-3-5-16-No	Past fragmentation and/or long-distance colonization between **A** (La Sal, Stony Pass, Grand Mesa) and **H** (Cumberland Pass)
2-1	31.383	0.000	1-2-11-12-No	Contiguous range expansion between **1-1** (Stony, La Sal, Mount Evans) and **1-2** (Cumberland Pass, La Sal, Stony Pass, Grand Mesa)
2-3	10.000	0.003	1-2-11-12-No	Contiguous range expansion between **1-11** (Pecos Baldy Peak, Lily Lake) and **1-12** (Lily Lake)
2-6	22.037	0.001	1-2-3-4-9-No	Allopatric fragmentation between **1-7** (Stony Pass, Mount Evans) and **1-8** (Medicine Bow Peak)
3-1	49.059	0.000	1-2-11-12-13-Yes	Past fragmentation followed by range expansion between **2-1** (Stony Pass, La Sal, Mount Evans, Cumberland Pass, Grand Mesa) and **2-3** (Rocky Mountain National Park, Mount Evans, Medicine Bow Peak)
4-1	82.000	0.000	1-19-No	Allopatric fragmentation between **3-1** (Stony Pass, La Sal, Mount Evans, Cumberland Pass, Grand Mesa, Rocky Mountain National Park, Medicine Bow Peak) and **3-5** (Lily Lake, Pecos Baldy Peak)
4-2	9.798	0.025	1-2-3-4-No	Restricted gene flow with isolation by distance between **3-3** (Mount Evans, Rocky Mountain National Park, Medicine Bow Peak) and **3-4** (Stony Pass, Mount Evans, Medicine Bow Peak)
Total cladogram	69.704	0.000	1-2-Tip/Interior status cannot be determined	Inconclusive outcome

Table 7. Nested clade analysis of the haplotype network for *Synthyris alpina* with average clade distance (D_C) and nested clade distance (D_N) for each haplotype and clade, and interior-tip (I-T) distances for each clade. Distances that were significantly large or small via permutation tests are labeled ^{L} and ^{S}, respectively.

	Topology	Haplotype/Clade	D_C	D_N
Clade 1-1	Tip	F	0.0000	144.8380
	Interior	I	107.0576	139.6239
	I-T		107.0576	-5.2141
Clade 1-2	Tip	H	0.0000^S	119.2491^L
	Interior	A	67.3012^S	81.0563^S
	I-T		67.3012^L	-38.1928^S
Clade 1-11	Interior	L	0.0000	60.4925
	Interior	O	0.0000	60.4101
Clade 2-1	Tip	Clade 1-1	141.1136^L	163.5968^L
	Interior	Clade 1-2	93.7873^S	94.9428^S
	I-T		-47.3264^S	-68.6539^S
Clade 2-3	Tip	Clade 1-4	0.0000	112.5499^L
	Interior	Clade 1-5	60.0295^S	82.5407^S
	I-T		60.0295	-30.0092^S
Clade 2-6	Interior	Clade 1-7	89.6886^S	191.2466
	Tip	Clade 1-9	0.0000^S	192.5490
	Tip	Clade 1-10	0.0000	218.1294
	I-T		89.6886^L	-5.5657
Clade 3-1	Tip	Clade 2-1	106.1191^S	119.8657^S
	Interior	Clade 2-3	31.7191^S	180.9321^L

	I-T		-74.4000^{S}	61.0665^{L}
Clade 3-2	Tip	Clade 2-3	90.0430	93.7948
	Interior	Clade 2-4	0.0000	93.7910
	I-T		-90.0430	-0.0039
Clade 3-3	Tip	Clade 2-5	0.0000	92.7429
	Interior	Clade 2-6	194.4271	194.2303
	I-T		194.4271	101.4873
Clade 3-4	Interior	Clade 2-7	60.4513	75.7605
	Tip	Clade 2-8	0.0000	72.4468
	I-T		60.4513	3.3137
Clade 4-1	Tip	Clade 3-1	135.6587^{S}	153.3323^{S}
	Tip	Clade 3-2	93.7942^{S}	219.1204^{L}
Clade 4-2	Interior	Clade 3-3	189.6172^{L}	188.4088^{L}
	Tip	Clade 3-4	74.1037	112.0929^{S}
	I-T		115.5135	76.3158^{L}
Total cladogram	Tip	Clade 4-1	172.5873	171.1848
	Tip	Clade 4-2	176.6679	200.6942

Table 8. Pairwise comparisons between populations of *Synthyris plantaginea*. Along the diagonal are population specific F_{ST} indices (regular font). Above the diagonal are estimates of F_{ST} from pairwise population comparisons (bold). Below the diagonal are geographic distances between populations (km; italic).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. San Fran. Pks., AZ	0.83	**1.00**	**0.91**	**0.00**	**1.00**	**0.75**	**0.73**	**1.00**	**0.68**	**1.00**	**0.89**	**0.96**	**0.68**	**1.00**	**0.91**
2. Pecos River, NM	*551.3*	0.83	**0.00**	**1.00**	**1.00**	**0.70**	**0.66**	**1.00**	**0.65**	**0.00**	**0.87**	**0.95**	**0.48**	**1.00**	**0.88**
3. Cimarron Cyn., NM	*603.0*	*97.0*	0.82	**0.91**	**0.98**	**0.70**	**0.66**	**0.99**	**0.64**	**0.00**	**0.87**	**0.92**	**0.47**	**0.98**	**0.87**
4. Chuska Mtns., NM	*260.5*	*305.0*	*344.8*	0.83	**1.00**	**0.75**	**0.73**	**1.00**	**0.68**	**1.00**	**0.89**	**0.96**	**0.68**	**1.00**	**0.91**
5. E. Naturita Creek, CO	*424.4*	*341.8*	*317.9*	*215.7*	0.83	**0.75**	**0.74**	**1.00**	**0.78**	**1.00**	**0.89**	**0.96**	**0.81**	**1.00**	**0.91**
6. Trout Creek, CO	*644.4*	*351.1*	*271.2*	*407.2*	*225.3*	0.76	**0.26**	**0.45**	**0.07**	**0.71**	**0.52**	**0.47**	**0.53**	**0.75**	**0.59**
7. Divide, CO	*704.1*	*357.0*	*266.9*	*459.5*	*290.6*	*69.2*	0.78	**0.75**	**0.29**	**0.67**	**0.64**	**0.52**	**0.47**	**0.74**	**0.62**
8. Kenosha Pass, CO	*631.4*	*296.6*	*214.5*	*384.0*	*226.9*	*57.0*	*76.4*	0.83	**0.46**	**1.00**	**0.79**	**0.98**	**0.90**	**1.00**	**0.95**
9. Red Hill Pass, CO	*671.0*	*391.5*	*310.1*	*439.8*	*247.8*	*40.8*	*75.5*	*95.7*	0.74	**0.66**	**0.51**	**0.55**	**0.48**	**0.78**	**0.66**
10. Poudre Canyon, CO	*815.5*	*549.9*	*461.7*	*598.8*	*393.2*	*206.7*	*195.1*	*255.2*	*166.9*	0.83	**0.88**	**0.95**	**0.49**	**1.00**	**0.89**
11. Crow Creek, WY	*853.6*	*603.1*	*515.0*	*643.8*	*434.5*	*258.3*	*248.3*	*308.0*	*218.0*	*53.3*	0.79	**0.80**	**0.78**	**0.89**	**0.83**
12. White Mtns., AZ	*246.6*	*404.9*	*485.3*	*239.8*	*455.2*	*629.3*	*672.2*	*596.0*	*665.5*	*829.4*	*876.7*	0.82	**0.50**	**0.96**	**0.52**
13. La Veta Pass, CO	*608.0*	*199.2*	*109.0*	*365.6*	*269.5*	*165.1*	*158.4*	*108.1*	*202.7*	*352.9*	*406.1*	*547.5*	0.80	**0.81**	**0.62**
14. E. Rifle Creek, CO	*593.3*	*483.2*	*425.0*	*411.5*	*196.5*	*187.5*	*247.8*	*234.2*	*173.1*	*240.6*	*267.7*	*651.2*	*333.3*	0.83	**0.91**
15. Golden Gate Cyn., CO	*746.9*	*454.2*	*366.6*	*518.1*	*322.7*	*114.8*	*101.1*	*160.0*	*78.3*	*95.7*	*148.9*	*743.5*	*257.6*	*211.2*	0.81

Table 9. Pairwise comparisons between populations of *Synthyris alpina*. Along the diagonal are population specific F_{ST} indices (regular font). Above the diagonal are estimates of F_{ST} from pairwise population comparisons (bold). Below the diagonal are geographic distances between populations (km; italic).

	1	2	3	4	5	6	7	8	9
1. La Sal Mountains, UT	0.82	**1.00**	**0.90**	**0.92**	**0.65**	**0.00**	**0.91**	**0.36**	**0.88**
2. Pecos Baldy Peak, NM	*425.6*	0.82	**0.30**	**1.00**	**0.85**	**1.00**	**0.96**	**0.78**	**0.92**
3. Sangre de Cristo Rng., CO	*342.6*	*188.0*	0.79	**0.91**	**0.74**	**0.91**	**0.86**	**0.68**	**0.81**
4. Cumberland Pass, CO	*242.2*	*318.2*	*149.8*	0.82	**0.71**	**1.00**	**0.93**	**0.46**	**0.90**
5. Stony Pass, CO	*165.3*	*269.0*	*182.8*	*137.3*	0.78	**0.66**	**0.65**	**0.15**	**0.39**
6. Grand Mesa, CO	*128.7*	*406.5*	*272.1*	*136.7*	*146.1*	0.82	**0.92**	**0.37**	**0.88**
7. Rocky Mtn. Nat. Pk., CO	*373.2*	*497.7*	*310.7*	*199.5*	*329.8*	*245.0*	0.81	**0.34**	**0.79**
8. Mt. Evans, CO	*336.1*	*410.1*	*223.1*	*123.4*	*260.1*	*209.9*	*87.7*	0.76	**0.43**
9. Medicine Bow Pk., WY	*408.8*	*609.2*	*425.0*	*297.0*	*410.8*	*292.3*	*120.9*	*204.5*	0.80

Table 10. Results of analyses of molecular variance examining genetic variation in the Southern Rocky Mountain group of *Synthyris* sect. *Besseya*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variance
Partitioned by population (4 species)				
Among populations	28	996.289	3.02269 Va	79.19
Within populations	305	242.250	0.79426 Vb	20.81
Total	**333**	**1238.539**	**3.81696**	
Partitioned by taxonomic units (4 species)				
Among species	3	198.353	0.47253 Va	11.86
Among populations within species	25	797.936	2.71587 Vb	68.19
Within populations	305	242.250	0.79426 Vc	19.94
Total	**333**	**1238.539**	**3.98265**	
Within *Synthyris plantaginea*				
Among populations	14	354.480	2.24685 Va	81.05
Within populations	151	79.333	0.52539 Vb	18.95
Total	**165**	**433.813**	**2.77223**	
	F_{ST}: 0.810			
Within *Synthyris alpina*				
Among populations	8	420.102	4.28747 Va	80.13
Within populations	99	105.250	1.06313 Vb	19.87
Total	**107**	**525.352**	**5.35060**	
	F_{ST}: 0.801			

Table 11. Average F-statistics measuring genetic subdivision in the Southern Rocky Mountain group of *Synthyris* sect. *Besseya*. F_{ST} measures genetic subdivision among populations; F_{CS} measures genetic subdivision among populations within each defined group; F_{CT} measures genetic subdivision among each defined group.

F-Statistic	Value	Significance
Among populations		
F_{ST}	0.79191	P < 0.0001
Among taxonomic units		
F_{ST}	0.80057	P < 0.0001
F_{SC}	0.77372	P < 0.0001
F_{CT}	0.11865	P < 0.0001

Fig. 1. Phylogenetic relationships in the Southern Rocky Mountain clade of *Synthyris* sect. *Besseya* found by Hufford and McMahon (2004).

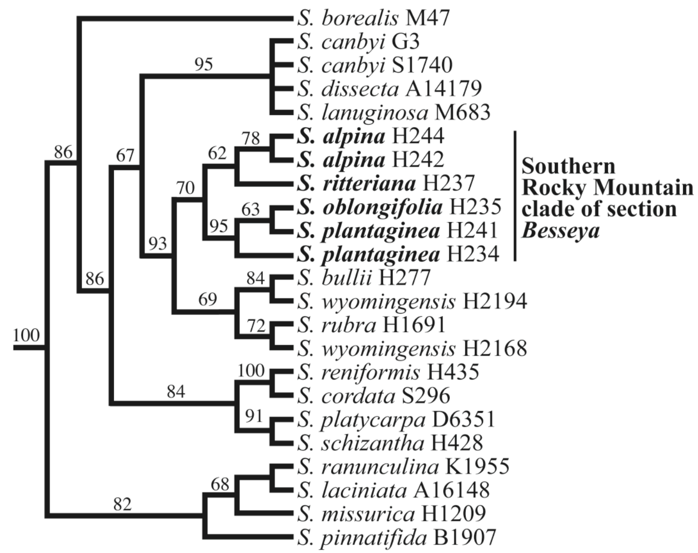


Fig. 2. Geographic distribution of species in the Southern Rocky Mountain group of *Synthyris* sect. *Besseya*. Numbers correspond to sampled localities in Table 1.

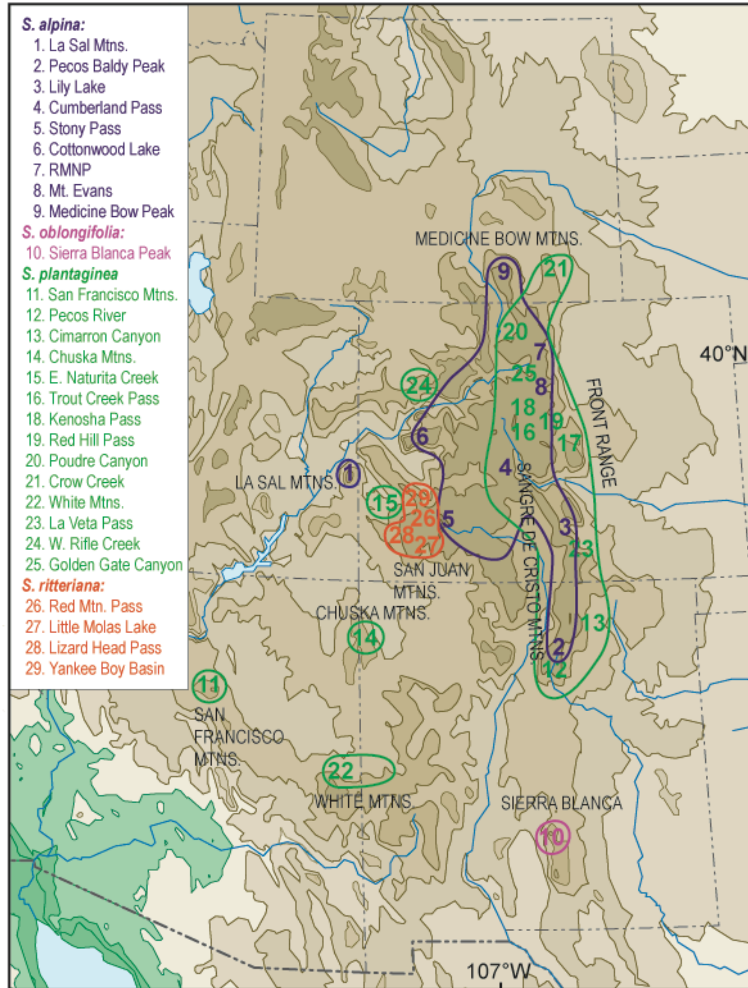


Fig. 3. Geographic distribution of 14 plastid haplotypes recovered in *Synthyris plantaginea* and two additional haplotypes recovered in *S. oblongifolia*. The sectors of the pie indicate the frequency of a particular haplotype.

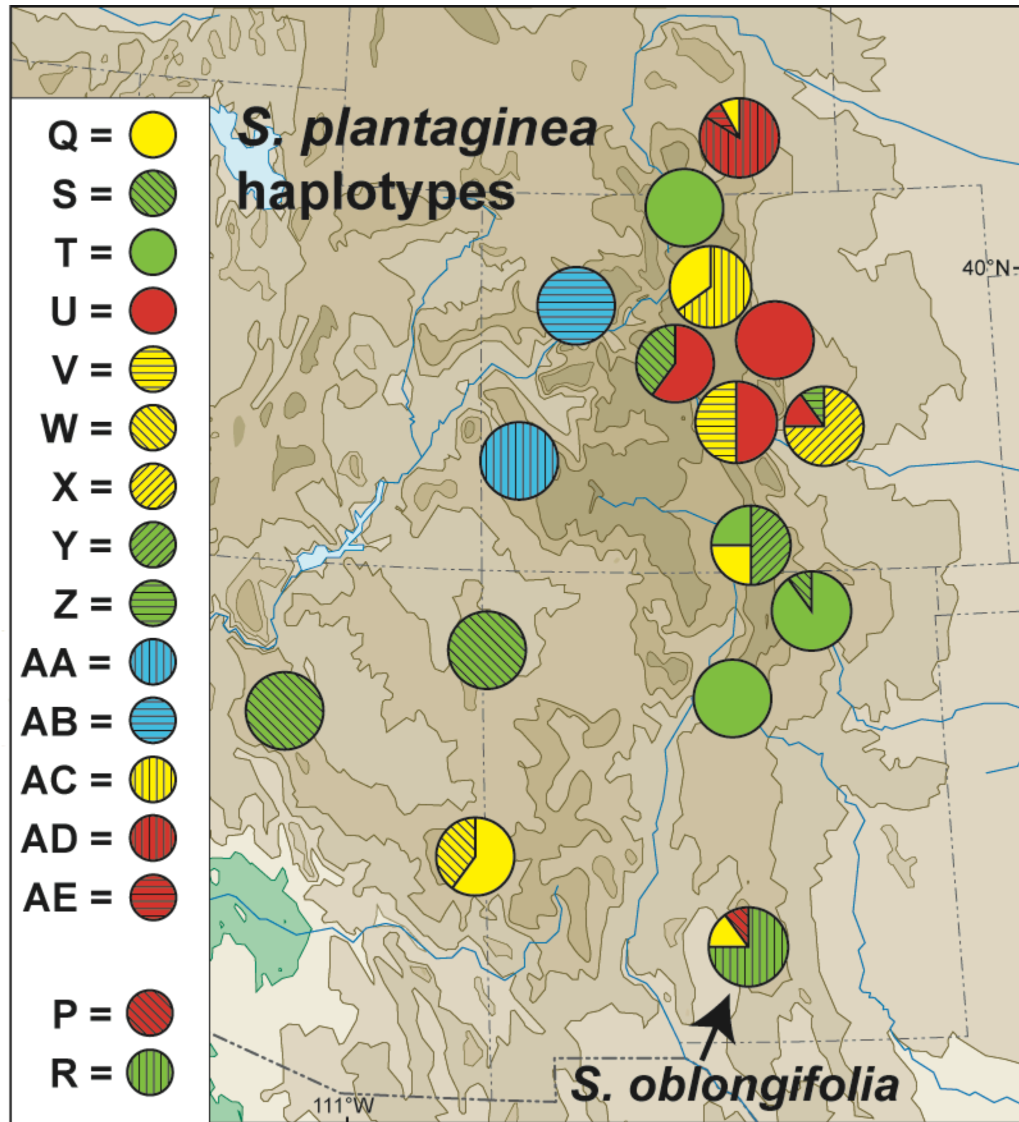


Fig. 4. Geographic distribution of 15 plastid haplotypes recovered in *Synthyris alpina*. The sectors of the pie indicate the frequency of a particular haplotype. Three regions are circled based on the distribution of haplotypes to include northern (Front Range), southwestern (San Juan and La Sal Mountains, and Grand Mesa), and southern (Sangre de Cristo Range).

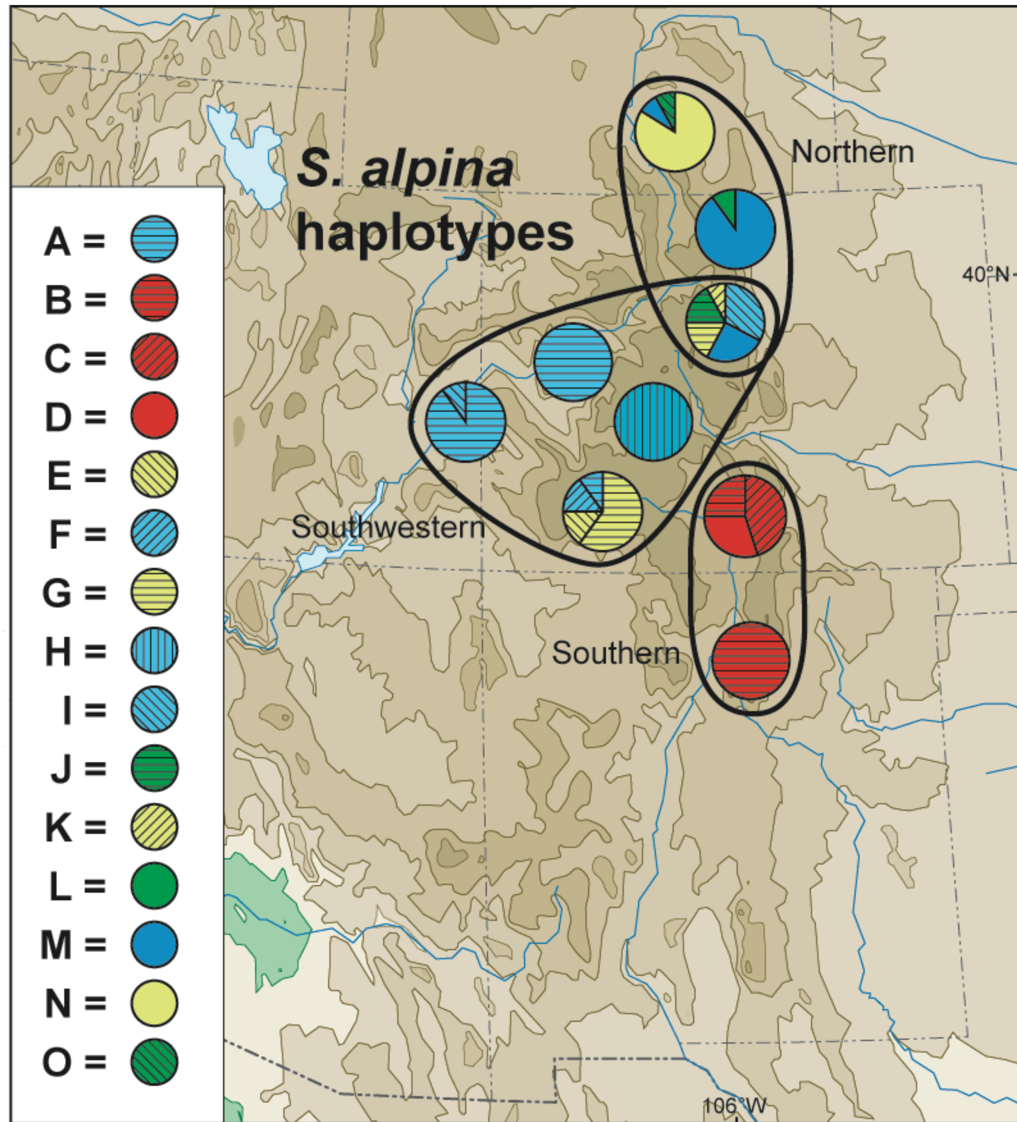


Fig. 5. Topology for the Southern Rocky Mountain clade of *Synthyris* sect. *Besseya* resulting from analyses of combined plastid *trnT-trnL* and *psbA-trnH* DNA sequences using maximum likelihood (ML; -ln 1718.6645; K81uf+I model of nucleotide substitution). Letters on tips correspond to haplotypes in Table 1. Numbers on branches are ML bootstrap values.

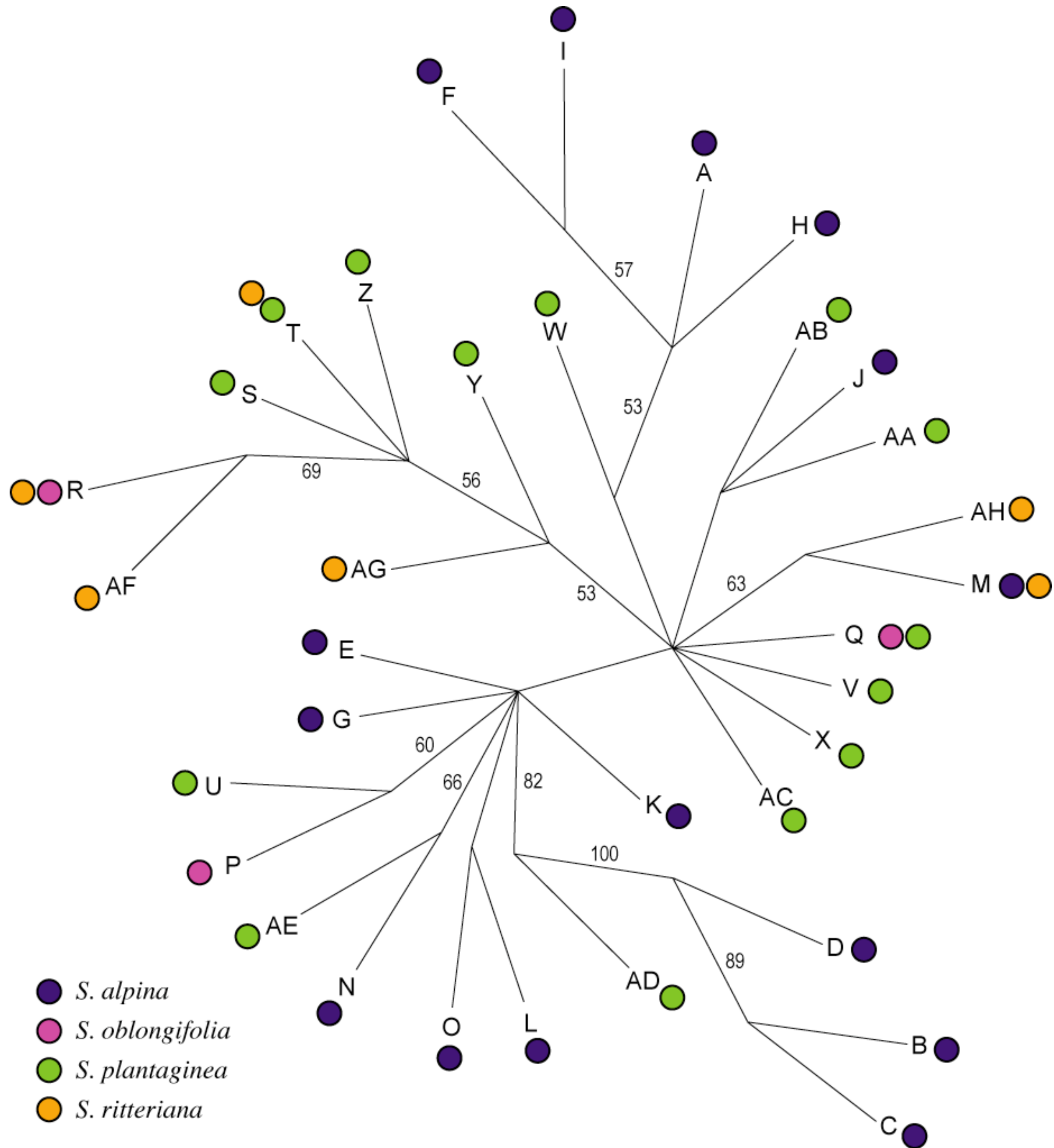


Fig. 6. Haplotype network of the Southern Rocky Mountain group of *Synthyris* sect. *Besseya*. Letters correspond to haplotypes in Table 1. Solid lines connecting haplotypes represent a single substitution. Dashed lines represent alternative resolutions of loops. Dots represent missing haplotypes. Nesting levels for the nested clade analysis (NCA) are shown, with one-step clades in white, and with two-, three-, and four-step clades indicated by successively darker shading. Numbered clades are those found to be significant by NCA and are referred to in the text and in Tables 2 and 3.

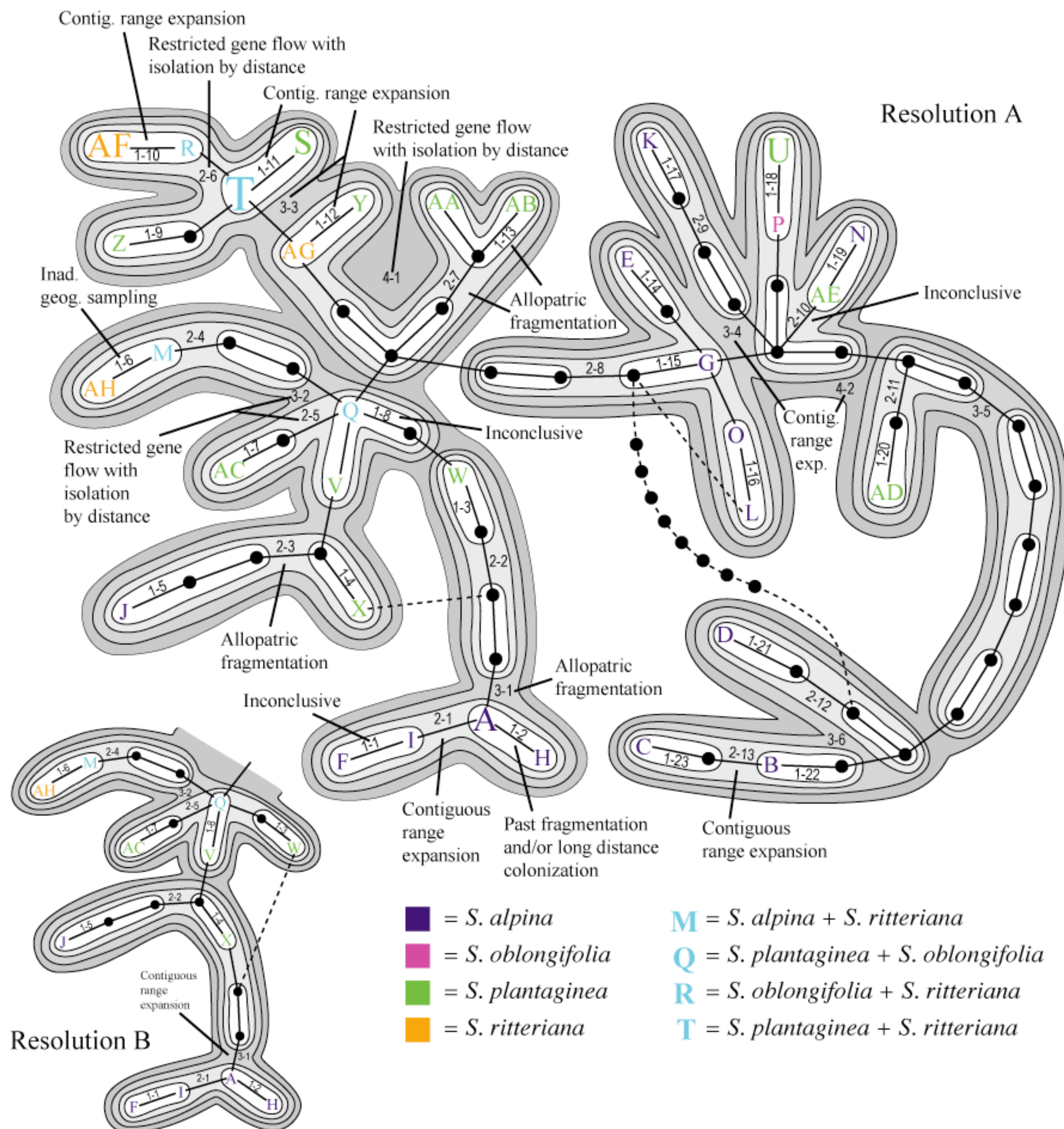


Fig. 7. Haplotype network of *Synthyris plantaginea*. Letters correspond to haplotypes in Table 1. Solid lines connecting haplotypes represent a single substitution. Dots represent missing haplotypes. Nesting levels for the nested clade analysis (NCA) are shown, with one-step clades in white, and with two-, and three-step clades indicated by successively darker shading. Numbered clades are those found to be significant by NCA and are referred to in the text and in Tables 4 and 5. Asterisks indicate clades for which a model of population expansion was rejected by mismatch distribution analysis.

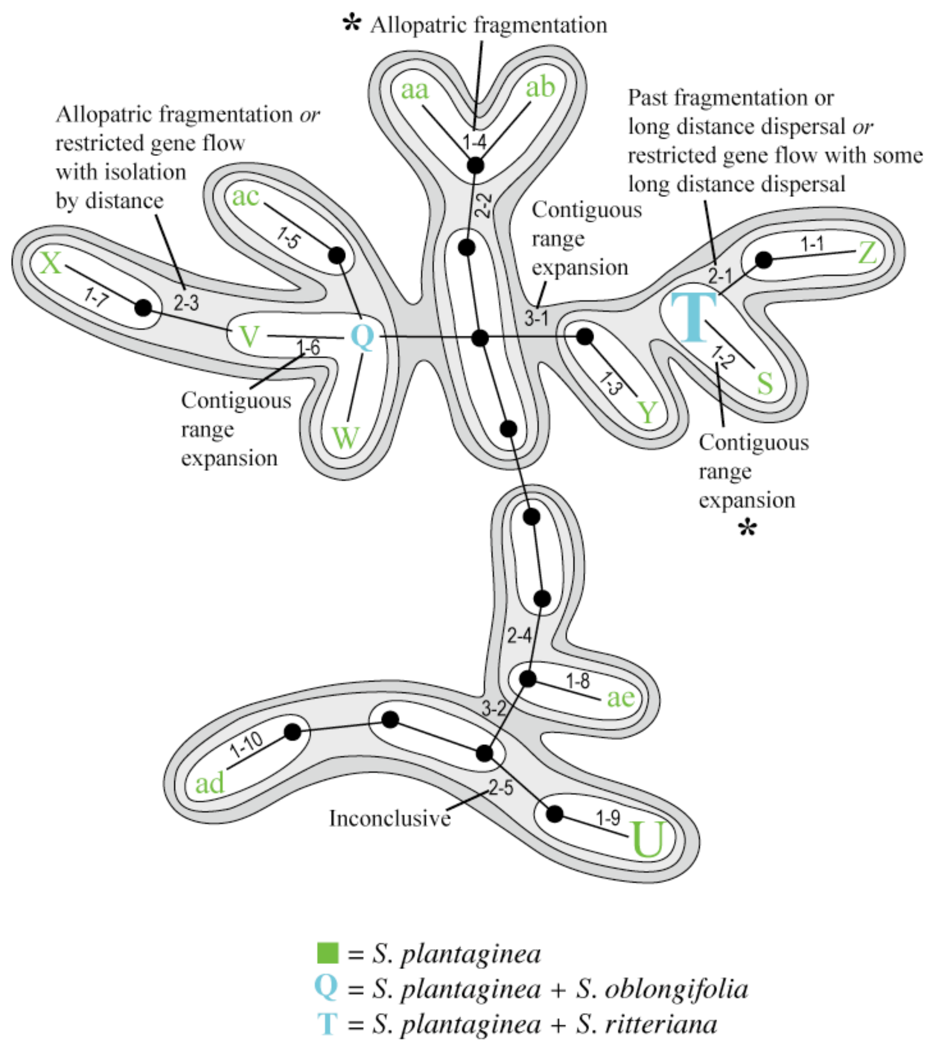


Fig. 8. Haplotype network of *Synthyris alpina*. Letters correspond to haplotypes in Table 1. Solid lines connecting haplotypes represent a single substitution. The dashed line represents an alternative loop resolution. Dots represent missing haplotypes. Nesting levels for the nested clade analysis (NCA) are shown, with one-step clades in white, and with two-, three-, and four-step clades indicated by successively darker shading. Numbered clades are those found to be significant by NCA and are referred to in the text and in Tables 6 and 7. Asterisks indicate clades for which a model of population expansion was rejected by mismatch distribution analysis.

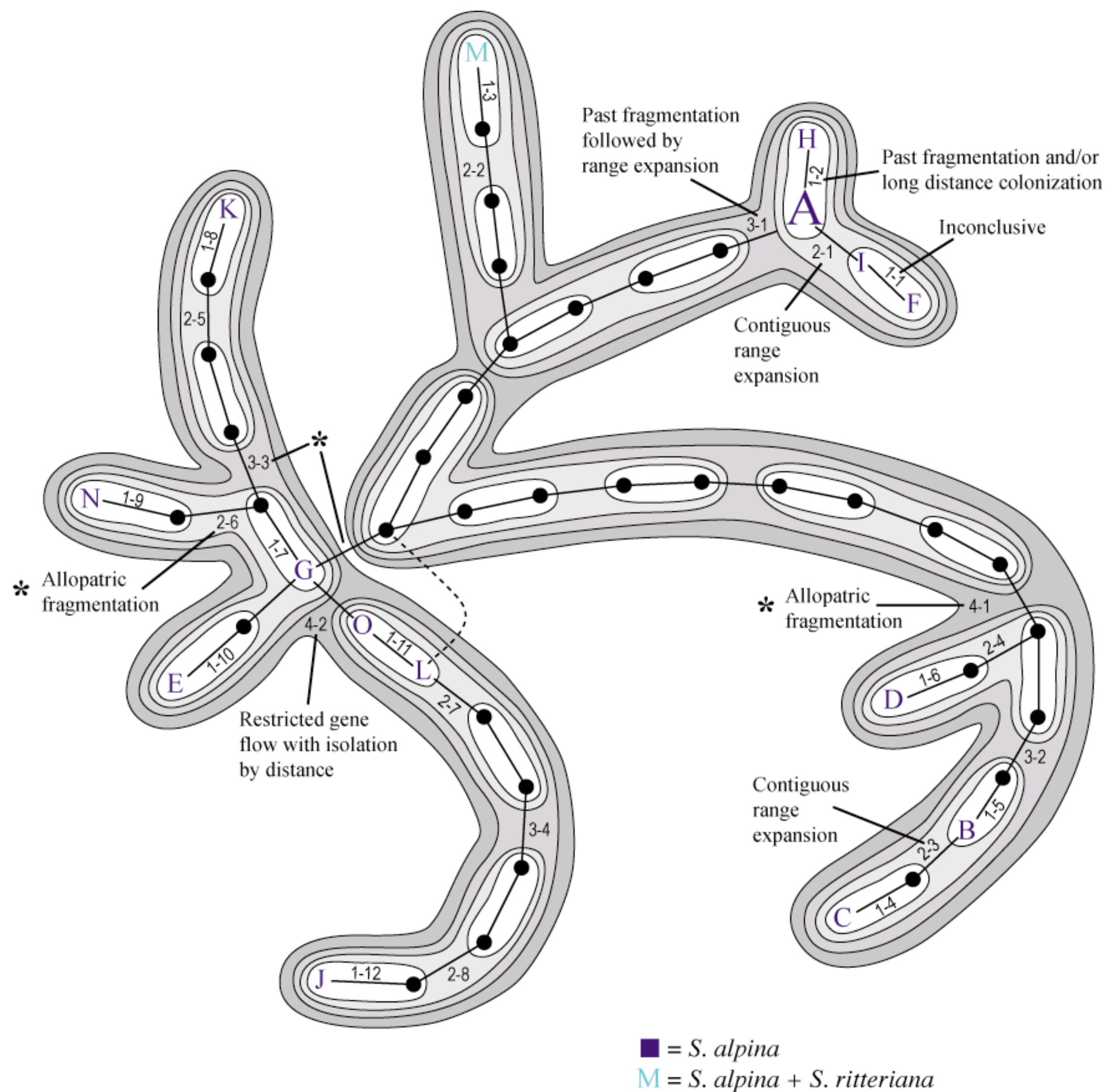


Fig. 9. Pairwise F_{ST} vs. geographic distance (km) for *Synthyris plantaginea*. The line is a simple linear regression. Statistical significance was based on a Mantel test (P = 0.0650).

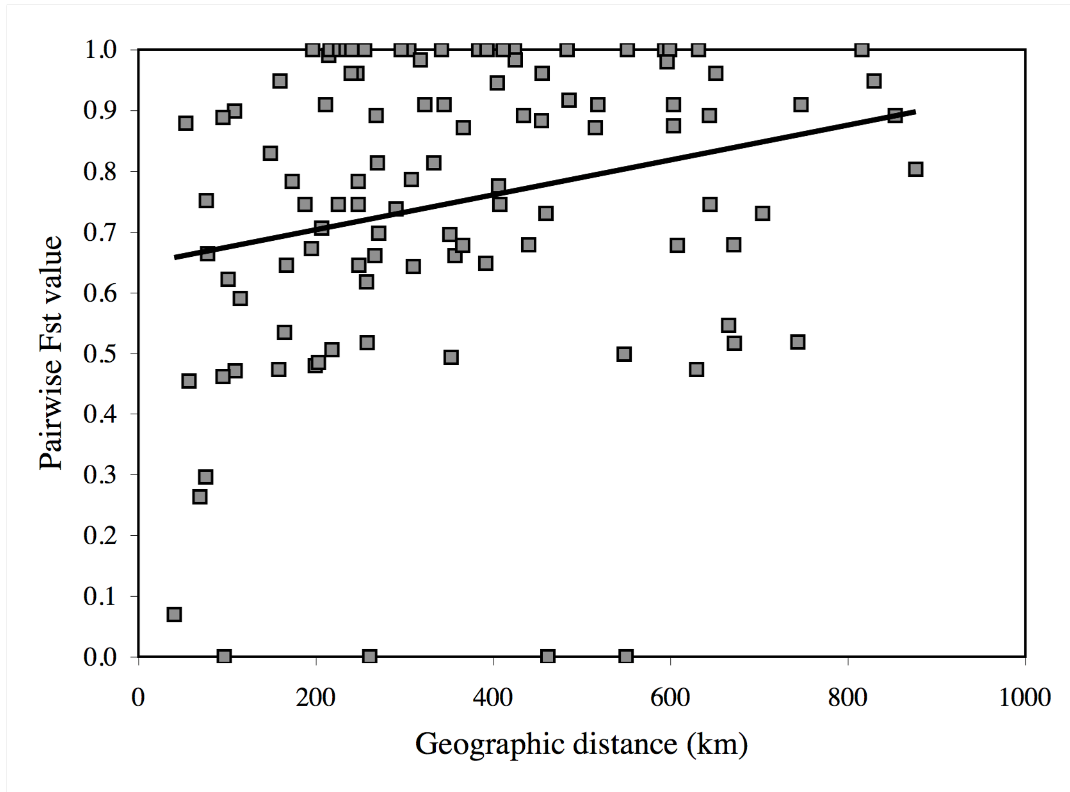
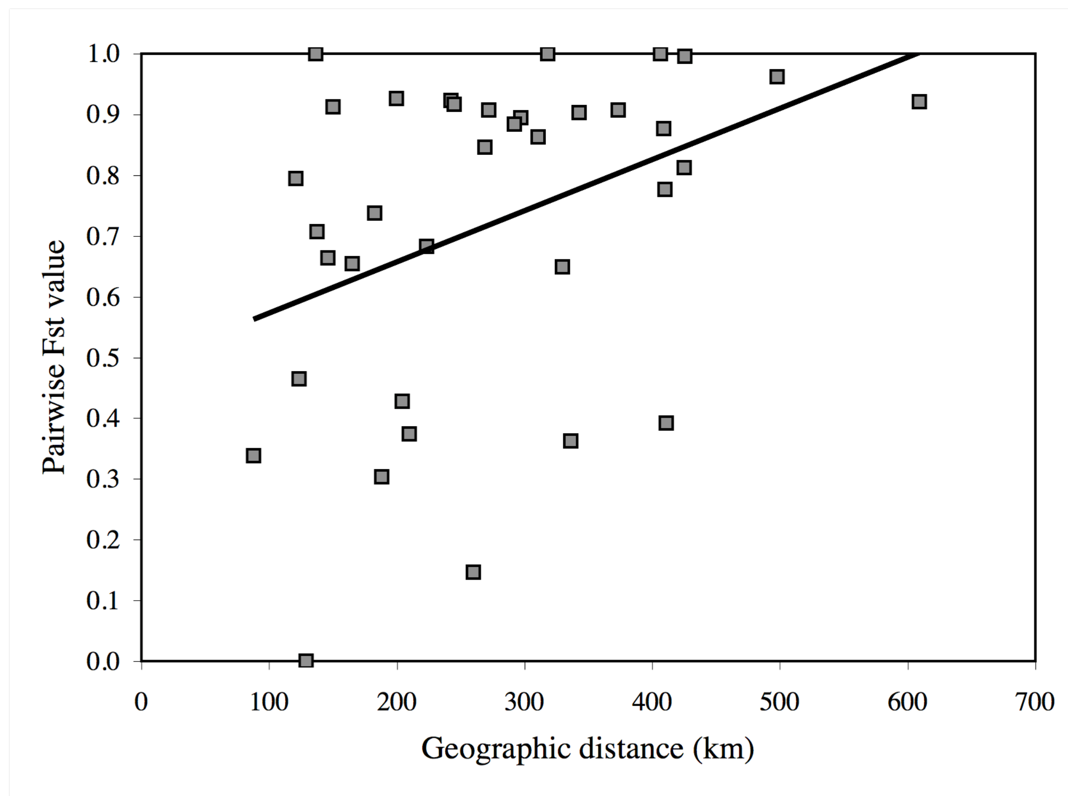


Fig. 10. Pairwise F_{ST} vs. geographic distance (km) for *Synthyris alpina*. The line is a simple linear regression. Statistical significance was based on a Mantel test (P = 0.0210).



CHAPTER THREE: EVOLUTION OF *SYNTHYRIS* SECT. *DISSECTA* (PLANTAGINACEAE) ON NORTHERN ROCKY MOUNTAIN SKY ISLANDS

INTRODUCTION

Sky islands are landscapes, such as mountain peaks, that have sharp ecological differentiation associated with elevation. The community dissimilarities at different elevations in sky island environments are analogous to the differences between land and water environments in oceanic archipelagos in the limitations they present for the migration of some organisms. Sky islands, because they are situated in continental environments, can differ from oceanic islands when global climate changes periodically shift the elevational associations of ecological communities, resulting in the lowering of high elevation communities, such as alpine tundra, to elevations where they are on continuous landscapes rather than isolated on peaks. Sky islands, thus, differ typically from oceanic islands not only in the ease with which the area of the island can shift with climatic fluctuations but also in the incorporation of the sky islands with a continent's biotic landscape. Thus, migration opportunities for organisms, such as plants, in a sky island landscape will vary temporally depending on regional climatic conditions.

Continental glaciations, associated with long-term Milankovitch cycles (Hays et al. 1976) as well as other shorter-term global cycles (Hewitt 1996), have shifted the elevational associations of various communities and organisms, especially at middle to high northern latitudes. Continental glaciations have been implicated in southward migration and population fragmentation in many lineages (Axelrod and Raven 1985; Hewitt and Ibrahim 2001). Pleistocene glaciations have been especially important in shaping current organismal

distributions and spatial patterns of genetic diversity (DeChaine and Martin 2004, 2005a, 2005b; Knowles 2001; Knowles and Richards 2005; VanDyke et al. 2004) and may also have played an important role in speciation in many clades (Green et al. 1996; Barber 1999; Nielson et al. 2001; Demboski and Sullivan 2003). The continental and associated glaciations of mountain ranges during the Pleistocene forced many alpine organisms to migrate to lower elevations, where communities could expand across broader contiguous landscapes (Billings 1974). The range expansions of alpine organisms during glaciations may have had various consequences, including opportunities to found populations in different mountain ranges (DeChaine and Martin 2004) and for secondary contact with other formerly isolated populations that would permit gene flow (Arbogast et al. 2001). Conversely, interglacial climate warming is seen as driving alpine organisms into high elevation refugia (DeChaine and Martin 2004, 2005a, 2005b; Hewitt 1996), where populations may have been reproductively isolated. Isolation of alpine taxa in interglacial refugia may have allowed for differentiation via genetic drift in small mountain top populations (Knowles and Richards 2005) and/or speciation (Green et al. 1996; Barber 1999; Nielson et al. 2001; Demboski and Sullivan 2003).

The Northern Rocky Mountains of the United States are largely south of the maxima of Pleistocene continental glaciations but were subject to cordilleran glaciations. The Northern Rocky Mountains have sharp elevational differentiation in many ranges, creating sky islands that are often separated during interglacials by vast tracts of montane forest and meadows that reflect ecological conditions unsuitable for alpine organisms. Therefore, depending on the dispersability of the organism, gene flow among alpine organisms may be limited during interglacials. Recent studies on alpine organisms, including grasshoppers (Knowles 2001; Knowles and Richards 2005; VanDyke et al. 2004), butterflies (DeChaine and Martin 2004,

2005a), and plants (DeChaine and Martin 2005b), find evidence that Pleistocene climate fluctuations played an important role in isolating alpine populations on Northern Rocky Mountain sky islands. Organisms limited currently to refugial sky islands are likely to become even more geographically restricted (Hewitt 2000) and, consequently, may face greater threat of extinction as the climate warms (Thuiller et al. 2005).

Our phylogeographic study of *Synthyris* section *Dissecta* (Plantaginaceae) examines evolutionary processes in the Northern Rocky Mountains. Section *Dissecta* is a clade (Figs. 1-2; Hufford and McMahon 2004) restricted to alpine environments of the Northern Rocky Mountains (2600-3400m) and the Olympic Mountains (1500-2000m). These plants are rhizomatous, herbaceous perennials that have capsular fruits and small discoid seeds that can be distributed over short distances by wind (Schaack 1983; Hufford 1992b; Hufford and McMahon 2004). Although taxon circumscriptions in sect. *Dissecta* have been controversial, we follow Hufford and McMahon's (2004) recognition of the three species *S. canbyi*, *S. dissecta* (= *S. dissecta* var. *dissecta* sensu Schaack 1983), and *S. lanuginosa* (= *S. pinnatifida* ssp. *lanuginosa* sensu Piper (1906) and *S. dissecta* var. *lanuginosa* sensu Schaack 1983). These species are differentiated by leaf and capsule forms (Hufford 1992a, b) have allopatric distributions (Fig. 2) and are currently not known to hybridize. *Synthyris canbyi* is restricted to the Mission, Swan, and Jocko mountains of western Montana. *Synthyris lanuginosa*, an Olympic Mountains endemic, is geographically disjunct from the rest of the section, and has distinctive densely tomentose shoots. Both *S. canbyi* and *S. lanuginosa* are conservation concerns because of their small population sizes. *Synthyris dissecta* is the most widespread species of the section, consisting of populations in southwestern Montana and adjacent Idaho. Following Schaack (1983), we provisionally recognize *S. dissecta* as including populations in Idaho that Pennell

(1933) described as *S. hendersonii* Pennell and *S. cymopteroides* Pennell, although these taxonomic differences may indicate possible cryptic species. Tomentose pubescence on the shoots of variant Idaho populations calls attention to their possible genetic differentiation from other populations of *S. dissecta*. This tomentose pubescence is, however, similar to that found in *S. lanuginosa*, and this raises the possibility that populations of *S. dissecta* in Idaho are the closest relatives of the disjunct populations in the Olympic Mountains of northwestern Washington State.

Sky islands are by definition disjunct landscapes. In sect. *Dissecta*, we have different degrees of disjunction among the species. The largest disjunction is between *S. lanuginosa* of the Olympic Mountains and *S. dissecta* and *S. canbyi* of the Northern Rocky Mountains. This disjunction, which spans the relatively arid, low elevation Columbia Basin is relatively common and has been inferred to result especially from aridification of the inland Northwest in association with the uplift of the Cascade Mountains (Glew 1994; Buckingham et al. 1995; Brunsfeld et al. 2001; Demboski and Sullivan 2003). We test whether genetic signatures are consistent with a hypothesis of vicariance rather than dispersal as the source of this disjunction in sect. *Dissecta*. Although the disjunction between *S. canbyi* and *S. dissecta* is no greater than among populations of the latter more widespread species, we test again for signatures of relatively deep vicariance versus fragmentation following a relatively recent northward range expansion in the origin of *S. canbyi*, which we hypothesize evolved as a peripheral isolate of *S. dissecta* or a common ancestor of these two species. We test also for similar processes in the possible peripheral isolation of *S. dissecta* populations in the southwest part of its range, where Pennell had recognized the species *S. hendersonii* and *S. cymopteroides*.

Pleistocene climatic fluctuations are hypothesized to have significant impacts on the ranges of alpine organisms (Billings 1974). We test for possible effects of Pleistocene glaciations within species of sect. *Dissecta*. For example, if glaciations resulted in range expansions that allowed populations to expand out of mountain ranges across intermountain valleys, then we would expect relatively little genetic geographic structure within species. In contrast, if populations have been restricted largely to mountain ranges through recent glacial cycles, then we would expect stronger signatures of geographic isolation and greater geographic structure within species. Because of the small area and high relief of many Montana mountain ranges, most of the ranges experienced only cirque and valley glaciers (Locke 1990). Thus we expect to see strong signals of geographic isolation in *S. dissecta* and *S. canbyi*. Alpine species can also remain in mountain top refugia (nunataks) during glaciations (Schönswetter et al. 2005; Brubaker and McLachlan 1996; DeChaine and Martin 2005b), and this can be associated with population crashes and genetic drift (Knowles and Richards 2005). Nunataks may have been important in the Olympic Mountains where all but one endemic species—including *S. lanuginosa*—are found above the maximum height of the continental ice sheets and survived on nunataks exposed above the ice (Peterson et al. 1997). We test especially for genetic signatures of nunatak survival in *S. lanuginosa*. We use plastid haplotype data based on DNA sequences of *trnT-trnL* and *psbA-trnH* intergenic spacers to examine the geographic structure of genetic diversity in this clade. These genetic regions have been found to be variable within and among populations of *Synthyris* (Marlowe, chapter 2; Streit 2004; Brustkern 2006).

MATERIALS AND METHODS

Taxon Sampling. We collected from populations across the distributional and

elevational ranges of the species of *Synthyris* sect. *Dissecta*, including 10 populations of *S. dissecta*, two of *S. canbyi*, and four of *S. lanuginosa*. We collected leaves from 20 ramets per population for 16 populations, and sampled 12 individuals per population (except for populations where individuals were spaced very closely or where fewer than 20 individuals were present), encompassing 302 individuals (Table 1). Two additional individuals of *S. canbyi* were sampled using herbarium specimens. Because *Synthyris* have short subterranean rhizomes that permit asexual reproduction, individuals sampled within each population were at least 6m apart. We attempted to collect over the range of the population unless it was geographically very widespread. In order to represent the diversity of leaf pubescence in *S. dissecta* our sampling included one population (White Cloud Range; Fig. 2) with tomentose shoots. All populations sampled are vouchered by collections deposited in the Marion Ownbey Herbarium (WS; leaf and inflorescence/infructescence sample only for Olympic National Park taxa). GenBank accession numbers for all individuals sampled are listed in the appendix.

DNA Isolation and Sequencing. Haplotypes were inferred from concatenated DNA sequences of the *trnT-trnL* and *psbA-trnH* plastid regions which were generated as part of this study (Table 1). Total genomic DNA was isolated from approximately 10 mg silica-gel dried leaf material using the CTAB procedure of Doyle and Doyle (1987). The *trnT-trnL* intergenic spacer regions were amplified using primers a and b (Taberlet et al. 1991), and the *psbA-trnH* intergenic spacer regions were amplified using primer *psbA-trnH* (GUG; Hamilton 1999). Each 25 μl PCR contained 10.8 μl water, 2.5 μl 10× reaction buffer (Promega), 2.5 μl 5′ 5μM primer, 2.5 μl 3′ 5 μM primer, 3.0 μl $MgCl_2$, 1.5 μl dNTP, 0.2 μl Taq polymerase (Promega), and 2.0 μl diluted DNA template (1.5 μl water + 0.5 μl ~20ng DNA concentration). PCR conditions in a Biometra® thermocycler (Whatman) included initial denaturation at 94°C for 5 min, followed by

30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 7 min. PCR products were visualized by 1.5% agarose gel electrophoresis and purified using 20% polyethylene glycol (PEG) 8000 in 2.5 M NaCl. The 10 μl cycle sequencing reactions contained 3.67 μl water, 0.33 μl 10 μM primer, 1.0 μl 5× sequencing buffer (Applied Biosystems), 1.0 μl BigDye® Terminator v3.1 (Applied Biosystems), and between 1.0 and 4.0 μl clean PCR product. Cycle sequence reaction included 25 cycles of 96°C for 10 sec and 50°C for 5 sec, with a final extension at 60°C for 4 min. Cycle sequence products were purified using 75% isopropanol precipitation, and DNA sequences were obtained on a 48-capillary 3730 DNA Analyzer (Applied Biosystems). Contigs were assembled and edited using Sequencher™ ver. 4.2.2 (Gene Codes Corporation). Sequences were aligned manually using Se-Al ver. 2.0a11 (Rambaut 1996-2002). Sequence alignment was unambiguous.

Phylogenetic Analysis. A phylogenetic analysis was used to infer evolutionary relationships among haplotypes. Redundant haplotypes were removed and models of molecular evolution were evaluated using DT-ModSel (Minin et al. 2003), which uses decision theory to incorporate estimates of branch length error along with a Bayesian information criterion and includes a penalty for over-parameterization. The selected model was chosen based on the data set in which the indels were not coded as single evolutionary events. The specified parameters of a K81uf model (six substitution rates: rAC = 1.00 rAG = 1.48, rAT = 0.19, rCG = 0.92, rCT = 1.48, rGT = 1.00; base frequencies: A = 0.3882, C = 0.1176, G = 0.1499, T = 0.3443) were applied in maximum likelihood (ML) searches of the combined dataset. ML analyses were conducted in PAUP* ver. 4.0b10 (Swofford 2002) with all characters weighted equally using heuristic ML searches (1000 replicates), including random taxon addition and branch-swapping by tree bisection-reconnection, saving one tree per replicate. Bootstrap analysis (Felsenstein

1985) was used to assess support for branches recovered by ML using PAUP*. Maximum likelihood bootstrap analyses used the same model of evolution and random addition of haplotypes as the heuristic search for 1000 replicates.

Phylogeographic Analyses. Nested clade analysis (NCA; Templeton et al. 1995, Templeton 1998) was used to assess geographic associations among haplotypes. Haplotype networks were constructed using TCS ver. 1.21 (Clement et al. 2000) under the criterion of a parsimony network in which connections have a probability of at least 95%. Four indels resulting from a duplication of adjacent sequence were coded as single evolutionary events following Simmons and Ochoterena (2000). Coding these areas as "missing data" would lead to branch length inflation, and if excluded, would possibly lead to loss of resolution (Cox and Chase 1995). One region of 35 nucleotides had two different sequences and was coded alternatively as each for analyses. Without recoding this region as such, the number of nucleotide changes was inflated. By recoding this region the haplotypes were able to be combined into one network. Gaps were treated as a fifth character state. Closed loops in the network were resolved using procedures from Templeton et al. (1992) and Templeton and Sing (1993) in combination with the ML topology. On the ML topology, haplotype D is closer to haplotype B than to haplotype C; haplotype D is closer to haplotypes E, I, and K than to haplotypes T and U; haplotype U is closer to haplotypes K, M, and N than to haplotype P (evident from phylogram; not shown). The guidelines of Crandall (1996), Templeton et al. (1987, 1992), and Templeton and Sing (1993) were used to infer nested clades based on the TCS network. NCA was performed using GeoDis ver. 2.5 (Posada et al. 2000). Significance in the NCA was determined by using an alpha = 0.05 for clade distances. Posada and Templeton's (2005) inference key was used to infer geographic processes associated with statistically

significant patterns determined by NCA. The haplotype network reconstruction and NCA were performed using the three species to identify possible evolutionary processes that led to speciation within the group, as well as differentiation with species.

Evidence for range expansion was tested under the expansion model of Rogers and Harpending (1992) by examining pairwise mismatch distributions using Arlequin ver. 3.0 (Excoffier and Schneider 2005). The mismatch distribution of a population that has been constant in size for a long time is expected to be ragged and erratic, whereas a population that has undergone recent expansion is expected to generate a smooth distribution with a peak (Harpending 1994). Mismatch distributions were calculated (1000 replicates) using Arlequin for samples in each of the clades identified by the NCA. The Harpending (1994) raggedness index was used to evaluate deviation from a model of population expansion.

Because of its geographic breadth and numerous populations, *S. dissecta* was analyzed in greater detail than *S. canbyi* and *S. lanuginosa*. A Mantel test (Sokal and Rohlf 1995) was performed on *S. dissecta* populations using Arlequin to look for evidence of isolation by distance. A distribution was created from 1000 random pairwise permutations, and statistical significance was determined by comparing the observed *r*-value to this distribution. Estimated pairwise F_{ST} values were plotted against corresponding geographical distance in order to illustrate the distribution of pairwise comparisons.

Estimates of Genetic Diversity. Arlequin was used for analyses of molecular variance (AMOVA), based on pairwise distances, to assess the partitioning of genetic variation within and among populations, within and among recognized species, and within and among regions (Rocky Mountains versus Olympic Peninsula). Nei's (1987) haplotypic diversity (the mean number of differences between all pairs of haplotypes in the sample) and nucleotide diversity (the

probability that two randomly chosen homologous nucleotides are different; Nei and Tajima 1981) were calculated also using Arlequin. In addition, AMOVA was performed separately for *S. dissecta* haplotypes in order to assess genetic variation partitioning solely within this widespread species.

RESULTS

Haplotypes. The aligned DNA sequences for the *trnT-trnL* and *psbA-trnH* data partitions were 687 and 312 bp, respectively (before indel coding; after indel coding, 679 and 271), 28 of which were variable and nine parsimony-informative. We recovered 22 haplotypes based on the concatenated *trnT-trnL* and *psbA-trnH* sequences (Table 1). Three haplotypes (A, B, and M) were recovered from the four populations of *S. lanuginosa* and were exclusive to this species; haplotype A, the most common haplotype, was shared by 45 individuals among the four *S. lanuginosa* populations. Haplotypes C, E-J, L, and N-V were exclusive to and shared among *S. dissecta* populations; haplotype D was found in only one *S. dissecta* population (*Marlowe 79*). The two populations of *S. canbyi* (*Marlowe 84/104*, *Marlowe 85/Gilbert 3*) were represented by only one haplotype (K), which was shared with two populations of *S. dissecta* (*Marlowe 74*, *Thomson 110*). Haplotype C was exclusive to the tomentose Idaho *S. dissecta* population; haplotype L this population was also found in individuals from the Tobacco Root population. To illustrate the geographic spread of genetic diversity, haplotypes were mapped onto the distribution maps (Fig. 3). The ML tree (Fig. 4; -ln 1480.8501) has nonexclusive haplotype phylogenies of the three species. The species are intermixed throughout the phylogeny.

Phylogeography. All haplotypes were connected to the networks reconstructed by TCS using 95% parsimony (Fig. 5). We apply the term "missing haplotypes" to intervening steps

between haplotypes, which could represent haplotypes lost due to extinction, extant haplotypes not sampled, or haplotypes that never existed. Eight significant associations between clades and geography were indicated in the NCA using the three species (Fig. 5, Tables 2-3). The mismatch distributions failed to reject a model of population expansion for all clades except 2-2 ($P = 0.0090$; $\alpha = 0.05$) and 2-3 ($P = 0.0430$).

The Mantel test revealed a significant relationship between F_{ST} values and geographical distances separating pairs of *S. dissecta* populations ($r^2 = 0.1076$; $P = 0.0270$; Fig. 6, Table 4). Although the Mantel test was significant, five population comparisons (Anaconda-Northern Bitterroot, Anaconda-Central Bitterroot, Anaconda-Southern Bitterroot, Central Bitterroot-Northern Bitterroot, and Central Bitterroot-Southern Bitterroot) contrast with the pattern of highest F_{ST} values among geographically closest populations in having small geographic distances with large pairwise F_{ST} values.

Genetic Diversity. AMOVA of all three species combined found that genetic variance in sect. *Dissecta* was higher among (70.48%) than within populations (29.52%; Table 5). Haplotypic structure among populations (F_{ST}) was high and significant (0.70478, $P < 0.0001$; Table 6). When populations were grouped by species, a moderate amount of genetic variance was centered among species (37.42%; Table 5), with a similar value centered among populations within species (38.23%; Table 5). When populations were grouped by region (Rocky Mountains versus Olympic Peninsula), genetic variance was higher between regions (41.77%; Table 5) than among populations within regions (35.97%; Table 5). AMOVA performed on *S. dissecta* haplotypes alone found that genetic variance was higher among (60.80%) than within populations (39.20%; Table 5). F_{ST} values for *S. dissecta* populations ranged from 0.54725 (Tobacco Root Mountains) to 0.64042 (Anaconda-Pintler Range; Table 4).

Genetic subdivision among species was moderate (F_{CT} = 0.37419, P < 0.0001; Table 6) relative to higher subdivision among populations within species (F_{SC} = 0.61082, P < 0.0001). Variance among populations when not grouped by species (F_{ST} = 0.75645, P < 0.0001) was high. Population specific F_{ST} indices were very similar, ranging from 0.55 to 0.64 (Table 6). Populations with the greatest haplotypic diversity (Table 1) included the Tobacco Root Mountains in the central part of the *S. dissecta* range (*S. dissecta*, *Marlowe 106*; 5.8182 +/- 2.9926) and Meadow Lake in the Lemhi Range of east central Idaho (*S. dissecta*, *Marlowe 83*; 4.3485 +/- 2.3117). Each of these populations also had relatively high nucleotide diversity (Table 1). As haplotypic diversity decreased, there was a corresponding decrease in nucleotide diversity among all individuals sampled. Two populations of *S. canbyi*, one population of *S. dissecta*, and two populations of *S. lanuginosa* (Table 1) had haplotypic and nucleotide diversities of zero.

DISCUSSION

***Speciation*.** The three currently recognized species have nonexclusive plastid haplotype phylogenies (Figs. 4 and 5), which might be attributed to hybridization or incomplete lineage sorting. These species are not known to hybridize, and no intermediate forms have been identified that would be indicative of hybridization. The three extant species have distinct floral and leaf characteristics, and no sympatric populations, all of which are likely to prevent interspecific gene flow. *Synthyris* are perennials that can propagate vegetatively (clonally), so some of these clones may be of very old age. Plastid haplotype polymorphism is common throughout *Synthyris* (e.g. Streit 2004; Hufford unpublished data). Based on the factors that limit gene flow in this clade and the extent of haplotype polymorphism among species of *Synthyris*,

we contend that the sharing of haplotypes among species of *S.* sect. *Dissecta* can be attributed to the ancient gene flow in early panmictic ancestors prior to population fragmentation, and that persistence of ancestral polymorphism is a consequence of incomplete lineage sorting.

Our AMOVA found that a high portion (70.48%; Table 4) of genetic variation could be explained by differences among populations. This distinct geographical partitioning of molecular variance is typical for glacial relict populations that were subjected to geographic isolation and, potentially, genetic drift in populations restricted to refugia (Reisch et al. 2003). We would expect plants that have undergone recent dispersal to instead show low levels of differentiation between populations. The strong geographical differentiation of *S.* sect. *Dissecta* is therefore most likely due to genetic drift in isolated populations associated with glaciation, especially warm interglacials. The high level of differentiation between *S.* sect. *Dissecta* populations is not associated with a strong loss of genetic diversity within populations (Table 4). Special life-history traits of *Synthyris* such as the long life span, the vegetative propagation, and the ability to both self and outcross (McKone 1995), may contribute to this genetic diversity within populations.

Synthyris lanuginosa *Disjunction.* Several organisms, including plants (Brunsfeld and Sullivan 2006; Buckingham et al. 1995;), lichens (Glew 1994), and animals (Demboski and Cook 2001; Good and Sullivan 2001; Wilke and Duncan 2004; Carstens et al. 2005) are disjunct between the Olympic and/or Cascade Mountains on the western side of the Pacific Northwest and the Northern Rocky Mountain populations. Brunsfeld et al. (2001) identified both ancient vicariance and more recent long-distance dispersal as possible causes for the disjunction. *Synthyris lanuginosa*, an Olympic Mountains endemic, was found by Hufford and McMahon (2004) to be the sister species of *S. dissecta* and *S. canbyi* of the Northern Rocky Mountains of

western Montana and adjacent Idaho. *Synthyris lanuginosa* has relatively limited genetic diversity compared to its sister clade, which can be a signature of relatively recent dispersal, but the absence of shared haplotypes between the Olympic and the Northern Rocky Mountains points toward more a ancient source of the disjunction. NCA inferred allopatric fragmentation as the evolutionary process that led to the differentiation between *S. dissecta* and *S. lanuginosa* haplotypes (clades 1-1 and 1-10; Fig. 5, Table 2), and we hypothesize this occurred in association with vicariance that resulted in the capture of at least two different haplotype lineages in the Olympic Mountains populations (Figs. 3, 5). Our AMOVA, when partitioned by region (Olympic Mountains versus Northern Rocky Mountains; Table 4), recovered a higher amount of genetic variation associated with differences among regions (42%) than among populations within regions (36%), likely due to a relatively deep split between the two regions.

The Cascade Range was probably rising during mid-Miocene time (McKee 1972), but major uplift occurred in the Pliocene (ca 5-2 mya; Graham 1999). This uplift produced a rain shadow that resulted in the aridification of the inland Northwest, where there was substantial biotic change (Daubenmire 1975), especially after 4.5 mya (Leopold and Denton 1987) and this has been associated with disjunction in various taxa in the Northwest (Nielson et al. 2001; Buckingham et al. 1995). This biotic change associated with aridification could be the source also of the major east-west disjunction in sect. *Dissecta*, although given the limitation of the clade to high elevation environments we anticipate that another causal factor that affected high mountain environments may have played a more significant role in the east-west fragmentation. The high mountain region between the Olympics and the Northern Rocky Mountains in Montana was heavily glaciated during the Pleistocene (Crandell 1965) with the earliest record of alpine glaciation dating to approximately 1.75 million years ago (Kaufman et al. 2004). Alpine

glaciation in the Canadian Rockies and especially high elevation glaciation in the U.S/Canada border region represents a possible force in the disjunction of Olympic *S. lanuginosa* from its sister clade in the Northern Rocky Mountains due to extirpation of intervening populations.

The unique haplotypes and low haplotypic diversity of *S. lanuginosa* are consistent also with its likely persistence in nunatak refugia in the Olympic Mountains. Glacial refugia have previously been hypothesized for the northeastern portion of the Olympic Peninsula, where *S. lanuginosa* is distributed, (Brubaker and McLachlan 1996; Heusser and Heusser 1990; Heusser 1977). During the last glacial period, a portion of the Buckhorn Mountain area was not covered by ice and is considered a refugium for numerous rare plants (Glew 1994). Among the species endemic to the Olympic Peninsula, all (including *S. lanuginosa*) but one are found at elevations exceeding the maximum height of the Cordilleran ice sheet, which has led to inferences that glacial survival of these endemics was in refugia on mountain peaks exposed above the ice (Peterson et al. 1997; Buckingham et al. 1995). Persistence of *S. lanuginosa* in small populations in nunatak refugia, in which genetic drift occurred, could account for both the low genetic diversity of the populations and the origin of the unique haplotypes B and M.
The long-term separation of *S. lanuginosa* from its sister clade in the Northern Rocky Mountains and its persistence in small, isolated refugia are consistent with our data.

From the population of tomentose *S. dissecta* (White Cloud Range; Fig. 2) that represents the formerly recognized *S. hendersonii* or *S. cymopteroides*, we recovered two haplotypes, C and L (Figs. 3, 5). Haplotype C is unique to this population, and L is also found in the Tobacco Root population. Both the haplotype network (Fig. 5) and haplotype distribution map (Fig. 3) illustrate that tomentose populations of *S. dissecta* are in the same clade of haplotypes with *S. lanuginosa*, which is a signal indicating the tomentose *S. dissecta* variants may represent

homology of tomentose vestiture in the Olympic Mountains and Northern Rocky Mountain regions. The vestiture on *S. lanuginosa* and the tomentose *S. dissecta* individuals, however, is not identical (pers. obs.). The leaves of White Cloud Range *S. dissecta* individuals are much less tomentose than those of the densely woolly *S. lanuginosa*. Vestiture among *S. dissecta* populations is highly variable with leaves ranging from glabrous to villous (Schaack 1983; pers. obs.). Three possible scenarios might explain the geographic distribution of *S. lanuginosa* and tomentose *S. dissecta* haplotypes. First, tomentose leaves could have arisen in some *S. dissecta* populations with subsequent long-distance dispersal to the Olympic Mountains, in which case tomentose leaves would be synapomorphic for *S. lanuginosa* and some individuals of *S. dissecta*. Second, tomentose individuals of *S. dissecta* might represent relict intermediates between *S. lanuginosa* and *S. dissecta*, in which case tomentose leaves would be symplesiomorphic for the clade. Lastly, tomentose leaves could have arisen independently in *S. lanuginosa* and certain populations of *S. dissecta*, in which case tomentose leaves would be homoplastic. As mentioned previously we do not find signals of long-distance dispersal and we consider such an event unlikely for this clade, however, studies of leaf vestiture would be beneficial in evaluating the possibility of each of these scenarios.

Origin of *Synthyris canbyi*. Populations of *S. canbyi* are restricted to calcareous substrate (Schaack 1983) in the Jocko Mountains, and Mission and Swan Ranges, a distribution continuous with but north of *S. dissecta*. *Synthyris canbyi*, which has scattered populations on rugged terrain, has not been well collected. Our sampling includes 14 individuals from the Mission Range, only two individuals from the Swan Range, and none from the Jocko Mountains populations, which could have affected the diversity of haplotypes recovered. All sampled individuals of *S. canbyi* had the same haplotype (K; Table 1), which is also found in Central

Bitterroot Range and the Gravelly Range populations of *S. dissecta*. This haplotype was also recovered in *S. plantaginea* of the Southern Rocky Mountain clade of *Synthyris* sect. *Besseya* (Marlowe, chapter 2), and, thus, may be one of the more common and ancient haplotypes in *Synthyris*.

The isolation of northern individuals of haplotype K could be the result of a ploidy event or fragmentation of a once continuous population. *Synthyris dissecta* and *S. lanuginosa* are diploid, but a single tetraploid count is the only chromosome count known for *S. canbyi* (Schaack 1983). Polyploidy has been one of the important evolutionary mechanisms in higher plants (Johnson et al. 1965), but without more chromosome counts for *S. canbyi* we cannot say whether or not polyploidy has played a role in its speciation. A likely explanation for the isolation and differentiation of *S. canbyi* populations is fragmentation of a once widespread *S. canbyi/S. dissecta* ancestral population. Valley glaciers were common during Pleistocene glaciations throughout western Montana and central Idaho (Davis 1920), which would have prevented populations from migrating downward in elevation during glacial maxima that would have allowed gene flow. Many areas in the Mission and Swan Ranges and Jocko Mountains were unaffected by glaciation (Davis 1920), with glacial reconstructions inferring several nunataks in the Swan and Mission Ranges (Locke 1995). These unglaciated areas would have served as refugia, isolating populations and ultimately leading to speciation of *S. canbyi*. NCA did not find significance in order to infer an evolutionary process to explain the distribution of *S. canbyi* and populations of *S. dissecta*, however, the low haplotype and nucleotide diversity of *S. canbyi* is consistent with newly founded populations experiencing a genetic bottleneck (Nei et al. 1975). Knowles (2001), who studied montane grasshoppers with the same Northern Rocky Mountain distribution, found evidence of a refugium that includes the distribution of *S. canbyi*.

However, with *S. canbyi* we find a pattern consistent with more recent peripheral isolation of northern *S. dissecta* populations.

Pennell (1933) suggested that the range of *Synthyris* was probably widespread throughout western North America and western Canada prior to the Pleistocene. The uplift of the Rocky Mountains, which continued sporadically from Oligocene to Pleistocene time (Richmond 1965), could have initiated isolation of the northern peripheral populations that may have included haplotype K. Pleistocene glaciations would have further isolated these populations, allowing allopatric speciation of *S. canbyi*. Ecological specialization may also have played a role because *S. canbyi* is limited to calcareous substrate (Schaack 1983). The bedrock material from the Swan and Mission Range populations of *S. canbyi* is derived from the same calcareous geologic formation (Schaack and Stickney 1981). *Synthyris dissecta* is also sometimes found on limestone but is usually found in igneous intrusive material (Schaack 1983). Plants found on unusual soil types almost inevitably involves the formation of genetically distinct races, and marginal populations often are found on soil types unusual for the species as a whole (Raven 1964).

***Phylogeography within* Synthyris dissecta.** *Synthyris dissecta* (= *S. dissecta* var. *dissecta sensu* Schaack 1983) is the most widespread species of this section, consisting of populations at or above treeline (2600-3400m) in southwestern Montana and adjacent Idaho. Following Schaack (1983), we provisionally include Idaho populations that Pennell (1933) had described as *S. hendersonii* Pennell and *S. cymopteroides* Pennell.

If range expansion was an important factor in the current distribution of *S. dissecta*, we would expect to find phylogenetically closely related haplotypes in close proximity. In general, closely related haplotypes are not necessarily geographically proximate (exception: haplotype T

occurs in two populations in close proximity; Fig. 3). Several populations have haplotypes that are phylogenetically distantly related (e.g. Northern Bitterroot, Tobacco Root, Gravelly Range, and White Cloud Range populations; Fig. 3). Some haplotypes (e.g. E, K, and L; Fig. 3) are somewhat widespread, which is consistent with incomplete lineage sorting and random capture of haplotypes in refugia. Some populations (e.g. Crazy Mountains, Bridger Range, and Central Bitterroot populations) contain haplotypes that are one or two mutational steps from one another (Fig. 5), which we would expect as random mutations occur in isolated populations. For example, the Central Bitterroot population contains the putatively ancient haplotype K which is centrally located on the haplotype network (Fig. 5). The population also contains closely related haplotypes N and P, which possibly represent mutations that arose after the isolation of haplotype K. Genetic variation in *S. dissecta* is highly structured, as indicated by F_{ST} analyses (Table 5), so drift, rather than gene flow, appears to be the dominant force structuring genetic variation in *S. dissecta*. Population range expansion out of sky islands during glaciations may have been insufficient to permit gene flow among populations of different mountain ranges. This is consistent with data from DeChaine and Martin (2004, 2005a) who found little population expansion in alpine butterflies among mountain ranges during glaciations in the Northern Rocky Mountains. Contiguous range expansion was inferred by NCA as the process leading to genetic variation for the total cladograms as well as for two other clades consisting of *S. dissecta* populations (clades 2-2 and 2-3; Fig. 5, Table 2). NCA, however, may be picking up a signal of ancient range expansion while failing to detect the fragmentation of currently isolated populations. When ancestral haplotypes from the pre-fragmentation era persist in the fragmented subpopulations, NCA cannot detect fragmentation due to the shallow genealogy (Printzen et al. 2003). The mismatch distribution rejects a hypothesis of range expansion for these clades, in

conflict with the NCA results. The phylogeographic pattern for *S. dissecta* most likely appears to have occurred via the stochastic partitioning of ancestral variation into isolated populations, followed by genetic drift associated with isolation of populations in interglacial refugia. *Synthyris dissecta* populations exhibit high haplotype diversity with populations harboring between two and four haplotypes (although the Anaconda Range population has only one haplotype). Nucleotide diversity for these populations is generally low (Table 1). High haplotype diversity combined with low nucleotide diversity is characteristic of rapid population expansion after a bottleneck.

Many nunataks existed across the present distribution of *S. dissecta* (Pierce 2004; Daubenmire 1952; Davis 1920) where populations could have persisted throughout the climate cycles, and we might expect the genetic signature to reflect this isolation by showing little or no evidence of past gene flow. The significant Mantel test (Fig. 6) could be an indication of past isolation by distance within *S. dissecta*, which would be consistent for an organism such as *S. dissecta* that experienced limited migration followed by fragmentation. The five population comparisons that were contradictory to the significant Mantel test include comparisons between the Anaconda Range and the three Bitterroot Range populations. We might expect an easy migration along the Bitterroot Range, but instead our results find isolated populations in alpine refugia. Although the Bitterroot Range is north-south oriented, it is dissected by numerous east-west trending deep canyons which would serve as isolating features among high-elevation populations. The Anaconda Range population is in close geographic proximity to the Bitterroot Range but the two areas are separated by a wide valley that experienced multiple glaciations during the Pleistocene (Waitt, Jr. and Thorson 1983).

Comparison between* Synthyris *Clades. Similar analyses were performed on the

Southern Rocky Mountain clade of *S.* sect. *Besseya* (Marlowe, chapter 2), which also has populations currently isolated on sky islands, and one species, *S. plantaginea*, that occurs in lower elevation montane forests. Our analyses point to the idea that *S.* sections *Besseya* and *Dissecta* both had panmictic widespread ancestral lineages, and that disjunctions in both sections were caused by fragmentation of those widespread populations. Our results for the Southern Rocky Mountain clade revealed more genetic signal consistent with migration during glacial episodes followed by interglacial isolation, whereas populations of *S.* sect. *Dissecta* appear to have experienced relatively greater isolation. The greater amount of isolation of the northern populations may have been due to the more extensive valley glaciers in the Northern Rocky Mountains (Davis 1920). Our results parallel those of DeChaine and Martin (2005b) for *Sedum lanceolatum* where northern populations appear to have been isolated not only on nunataks during glaciations, but also in sky islands during interglacials. Lastly, we have seen that Pleistocene glaciations probably played an important role in species origins and shaping extant distributions in these two clades of *Synthyris*.

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APPENDIX

GenBank accession numbers for *trnT-trnL* and *psbA-trnH* intergenic spacer regions for haplotypes of *Synthyris* sect. *Dissecta*.

A: EF550031, EF550009; B: EF550032, EF550010; C: EF550033, EF550011; D: EF550034, EF550012; E: EF550035, EF550013; F: EF550036, EF550014; G: EF550037, EF550015; H: EF550038, EF550016; I: EF550039, EF550017; J: EF550040, EF550018; K: EF550041, EF550019; L: EF550042, EF550020; M: EF550043, EF550021; N: EF550044, EF550022; O: EF550045, EF550023; P: EF550046, EF550024; Q: EF550047, EF550025; R: EF550048, EF550026; S: EF550049, EF550027; T: EF550050, EF550028; U: EF550051, EF550029; V: EF550052, EF550030.

Table 1. Locality and collection information, number of individuals, and genetic diversity (Nei 1987) for individuals of *Synthyris canbyi*, *S. dissecta*, and *S. lanuginosa* sequences for *trnT-trnL* and *psbA-trnH*. Locality numbers correspond to those in Figure 2.

Locality #	Taxon	*n*	Collection	Locality	Elev. (m)	Coord.	Haplotypes (# of individuals)	Haplotypic diversity (h)	Nucleotide diversity (π)
1	*S. canbyi* Pennell	2	Marlowe 84, 104	Swan Range, Missoula County, MT	2439	N47°34 W113°37	K (2)	0.0000+/- 0.0000	0.0000+/- 0.0000
2		14	Marlowe 85, Gilbert 3 (MONTU)	Mission Range, Lake County, MT	2292	N47°17 W113°54	K (14)	0.0000+/- 0.0000	0.0000+/- 0.0000
3	*S. dissecta* Rydb.	12	Marlowe 68	St. Mary's Peak, North Bitterroot Range, Ravalli County, MT	2804	N46°30 W114°14	E (11), V (1)	1.0000+/- 0.7224	0.0011+/- 0.0009
4		12	Marlowe 70	Fairy Lake, Bridger Range, Gallatin County, MT	2499	N45°54 W110°58	O (1), R (1), T (9), U (1)	0.9697+/- 0.7070	0.0010+/- 0.0008
5		12	Marlowe 72	Sunlight Lake, Crazy Mountains, Park County, MT	2742	N46°06 W110°23	S (1), T (11)	0.3333+/- 0.3564	0.0004+/- 0.0004
6		12	Marlowe 74	Black Butte, Gravelly Range, Madison County, MT	2829	N44°54 W111°50	G (5), K (7)	2.6515+/- 1.5194	0.0028+/- 0.0018

7	12	Marlowe 79	Goat Flat, Anaconda Range, Deer Lodge/Granite Counties, MT	2810	N46°03 W113°16	D (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
8	12	Marlowe 106	Tobacco Root Mountains, Madison County, MT	3008	N45°31 W111°59	J (6), L (4), Q (2)	5.8182+/- 2.9926	0.0061+/- 0.0036
9	12	Marlowe 108	Ajax Lake, South Bitterroot Range, Beaverhead County, MT	2734	N45°19 W113°43	E (2), I (10)	1.2121+/- 0.8288	0.0013+/- 0.0010
10	13	Thomson 110	Trapper Peak, Central Bitterroot Range, Ravalli County, MT	2975	N45°53 W114°17	K (2), N (5), P (6)	1.1026+/- 0.7700	0.0012+/- 0.0009
11	12	Marlowe 82	Washington Peak, White Cloud Mountains, Custer County, ID	3019	N44°02 W114°37	C (9), L (3)	2.8636+/- 1.6191	0.0030+/- 0.0019
12	12	Marlowe 83	Meadow Lake, Lemhi Range, Lemhi County, ID	2824	N44°25 W113°19	F (5), G (1), H (6)	4.3485+/- 2.3117	0.0046+/- 0.0027

13	*S. lanuginosa* (Piper) Pennell & J.W. Thomps.	12	Marlowe 101	Obstruction Point, Olympic National Park, Clallam County, WA	1951	N47°54 W123°21	A (10), M (2)	2.1212+/- 1.2686	0.0022+/- 0.0015
14		12	Marlowe 102	Eagle Point, Olympic National Park, Clallam County, WA	1798	N47°55 W123°24	A (12)	0.0000+/- 0.0000	0.0000+/- 0.0000
15		12	Marlowe 103	Dinosaur Back Ridge, Olympic National Park, Clallam County, WA	1737	N47°57 W123°15	A (11), B (1)	0.16667+/- 0.2401	0.0002+/- 0.0003
16		12	Marlowe 105	Marmot Pass, Buckhorn Wilderness, Olympic National Forest, Jefferson County, WA	1829	N47°49 W123°08	A (12)	0.0000+/- 0.0000	0.0000+/- 0.0000

Table 2. Results of nested clade analysis on haplotypes of three species of *Synthyris* sect. *Dissecta*. Nested clades without significant geographic associations are not listed. The inference chain is based on Posada and Templeton's (2005) inference key.

Clade	χ^2 statistic	Probability	Inference chain	Inferred pattern
1-1	54.000	0.000	1-19-No	Allopatric fragmentation between **A** (all four Olympic National Park, WA, populations of *S. lanuginosa*) and **C** (White Cloud Mountains, ID, *S. dissecta*)
1-5	8.571	0.015	1-2-3-4-9-No or 1-2-3-4-No	Allopatric fragmentation or restricted gene flow with isolation by distance between **F** (Lemhi Range, ID) and **G** (Lemhi Range, ID, and Gravelly Range, MT)
1-10	27.000	0.010	1-19-No	Allopatric fragmentation between **K** (both populations of *S. canbyi*, Central Bitterroot Range and Gravelly Range *S. dissecta*) and **M** (one population of *S. lanuginosa*)
2-2	70.000	0.000	1-19-20-2-11-12-No	Contiguous range expansion among **1-3** (Anaconda-Pintler Range), **1-4** (North Bitterroot Range, South Bitterroot Range), and **1-5** (Lemhi Range and Gravelly Range)
2-3	16.000	0.000	1-19-20-2-11-12-No	Contiguous range expansion between **1-6** (South Bitterroot Range) and **1-7** (Tobacco Root Mountains)
3-2	122.252	0.000	1-2-Tip/interior status could not be determined	Inconclusive outcome
3-3	17.094	0.008	1-2-11-17-No	Inconclusive outcome
Total cladogram	281.539	0.000	1-2-11-12-No	Contiguous range expansion (all clades)

Table 3. Nested clade analysis of the haplotype network for *Synthyris* sect. *Dissecta* with average clade distance (D_C) and nested clade distance (D_N) for each haplotype and clade, and interior-tip (I-T) distances for each clade. Distances that were significantly large or small via permutation tests are labeled ^{L} and ^{S}, respectively.

	Topology	Haplotype/Clade	D_C	D_N
Clade 1-1	Interior	A	8.9441^{S}	428.5037^{L}
	Tip	C	0.0000^{S}	366.0198^{S}
	I-T		8.9441	62.4840^{L}
Clade 1-5	Tip	F	0.0000^{S}	24.8621^{S}
	Interior	G	60.4070	74.3614^{L}
	I-T		60.4070^{L}	49.4994^{L}
Clade 1-10	Interior	K	114.5180^{S}	111.1217^{S}
	Interior	M	0.0000	716.0603^{L}
Clade 1-11	Interior	N	0.0000	22.9954
	Tip	O	0.0000	233.4428
	Tip	P	0.0000	22.9954
	I-T		0.0000	-30.0639
Clade 1-13	Interior	T	24.8471	24.9785
	Interior	U	0.0000	26.3133
Clade 2-1	Tip	Clade 1-1	394.4216	394.8158
	Interior	Clade 1-2	0.0000	425.4172
	I-T		-394.4216	30.6014
Clade 2-2	Interior	Clade 1-3	0.0000^{S}	72.9717^{S}
	Interior	Clade 1-4	35.7586^{S}	120.4867^{L}
	Tip	Clade 1-5	38.3994^{S}	114.2597
	I-T		-19.8050	-16.5802^{S}

Clade 2-3	Interior	Clade 1-6	0.0000[S]	51.4217[S]
	Tip	Clade 1-7	0.0000[S]	85.5547[L]
	I-T		0.0000	-34.1331[S]
Clade 3-2	Interior	Clade 2-3	64.2216[S]	66.9273[S]
	Interior	Clade 2-4	0.0000[S]	129.1827
	Interior	Clade 2-5	175.8291[L]	182.7476[L]
Clade 3-3	Interior	Clade 2-6	37.4054	37.9591
	Tip	Clade 2-7	39.8531	69.4338
	I-T		-2.4477	-31.4747
Total cladogram	Tip	Clade 3-1	357.7650[L]	330.7565[L]
	Interior	Clade 3-2	155.1271[S]	200.5132[S]
	Tip	Clade 3-3	41.5908[S]	334.4555
	I-T		-132.3770[S]	-131.0653[S]

Table 4. Pairwise comparisons between populations of *Synthyris dissecta*. Along the diagonal are population specific F_{ST} indices (regular font). Above the diagonal are estimates of F_{ST} from pairwise population comparisons (bold). Below the diagonal are geographic distances between populations (km; italic).

	1	2	3	4	5	6	7	8	9	10
1. No. Bitterroot, MT	0.62	**0.83**	**0.89**	**0.52**	**0.84**	**0.69**	**0.48**	**0.53**	**0.69**	**0.80**
2. Bridger Range, MT	*261.1*	0.62	**0.02**	**0.57**	**0.85**	**0.66**	**0.66**	**0.45**	**0.82**	**0.69**
3. Crazy Mountains, MT	*299.6*	*50.3*	0.64	**0.66**	**0.95**	**0.70**	**0.71**	**0.50**	**0.87**	**0.80**
4. Gravelly Range, MT	*258.8*	*130.7*	*175.8*	0.60	**0.63**	**0.54**	**0.31**	**0.27**	**0.44**	**0.47**
5. Anaconda Range, MT	*89.9*	*179.5*	*223.1*	*170.6*	0.64	**0.56**	**0.56**	**0.45**	**0.80**	**0.87**
6. White Cloud Rng., ID	*277.3*	*355.4*	*405.4*	*241.6*	*248.7*	0.59	**0.54**	**0.42**	**0.65**	**0.71**
7. Lemhi Range, ID	*242.4*	*247.0*	*296.1*	*128.5*	*180.8*	*113.1*	0.57	**0.31**	**0.32**	**0.63**
8. Tobacco Root, MT	*205.2*	*90.1*	*140.3*	*71.0*	*115.5*	*266.3*	*160.8*	0.55	**0.31**	**0.48**
9. So. Bitterroot, MT	*137.0*	*224.4*	*273.8*	*156.2*	*87.8*	*160.9*	*105.4*	*137.4*	0.62	**0.79**
10. Ctr. Bitterroot, MT	*69.3*	*257.5*	*302.7*	*221.0*	*80.5*	*208.2*	*179.3*	*182.9*	*75.8*	0.62

Table 5. Results of analysis of molecular variance examining genetic variation in *Synthyris* sect. *Dissecta*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variance
Three species partitioned by population				
Among populations	15	315.813	1.76264 Va	70.48
Within populations	169	124.782	0.73836 Vb	29.52
Total	**184**	**440.595**	**2.50100**	
Three species partitioned by taxonomic units				
Among species	2	134.249	1.13438 Va	37.42
Among populations within species	13	181.564	1.15885 Vb	38.23
Within populations	169	124.782	0.73836 Vc	24.36
Total	**184**	**440.595**	**3.03159**	
Partitioned by region (Olympic Mountains vs. Northern Rocky Mountains)				
Among regions	1	113.581	1.38524 Va	41.77
Among populations within regions	14	202.231	1.19303 Vb	35.97
Within populations	169	124.782	0.73836 Vc	22.26
Total	**184**	**440.595**	**3.31662**	
***Synthyris dissecta* partitioned by population**				
Among populations	9	179.834	1.56794 Va	60.80
Within populations	111	112.199	1.01080 Vb	39.20
Total	**120**	**292.033**	**2.57874**	

Table 6. Average F-statistics measuring genetic subdivision in *Synthyris* sect. *Dissecta*. F_{ST} measures genetic subdivision among populations; F_{CS} measures genetic subdivision among populations within each defined group; F_{CT} measures genetic subdivision among each defined group.

F-Statistic	Value	Significance
Among populations		
F_{ST}	0.70478	$P < 0.0001$
Among taxonomic units		
F_{ST}	0.75645	$P < 0.0001$
F_{CS}	0.61082	$P < 0.0001$
F_{CT}	0.37419	$P < 0.0001$

Fig. 1. Phylogenetic relationships in *Synthyris* sect. *Dissecta* found by Hufford and McMahon (2004).

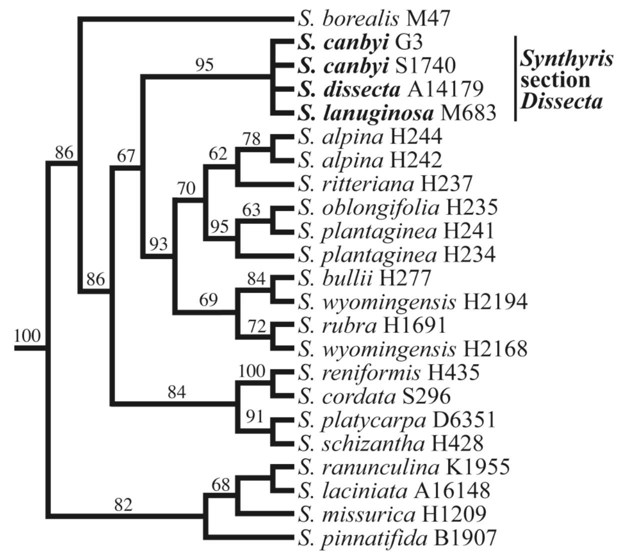

Fig. 2. Geographic distribution of species in *Synthyris* sect. *Dissecta*. Numbers correspond to sampled localities in Table 1.

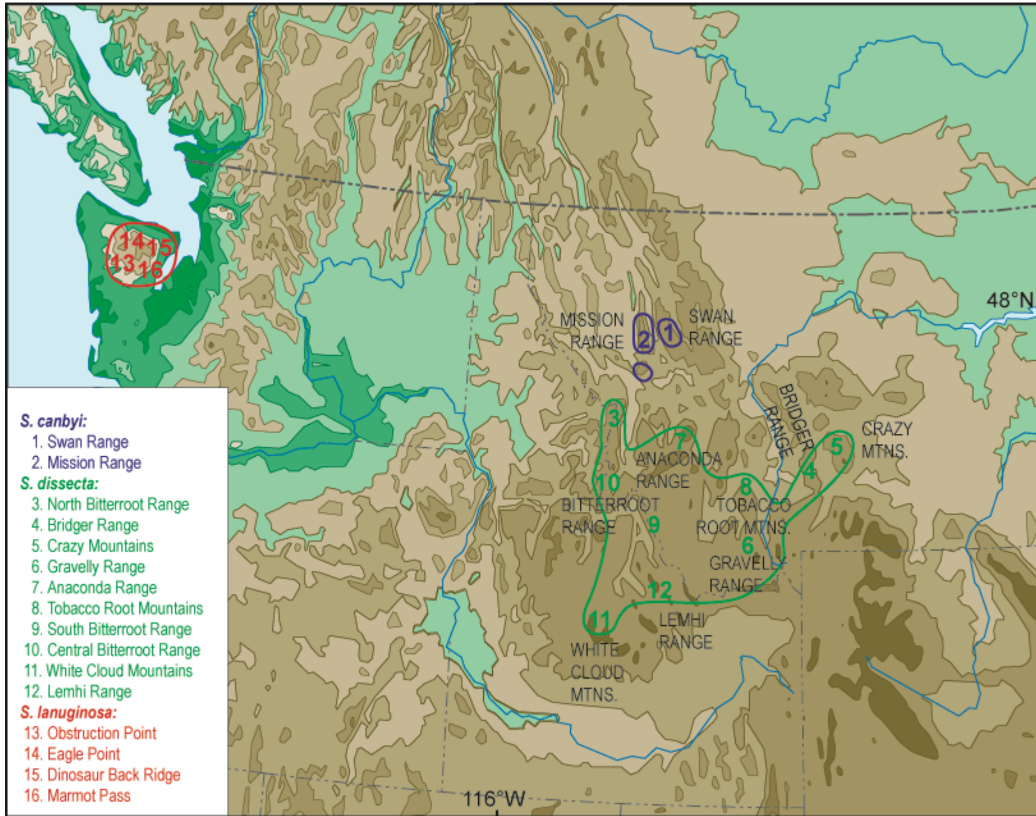

Fig. 3. Geographic distribution of 22 plastid haplotypes recovered in *Synthyris* sect. *Dissecta*. The sectors of the pie indicate the frequency of a particular haplotype.

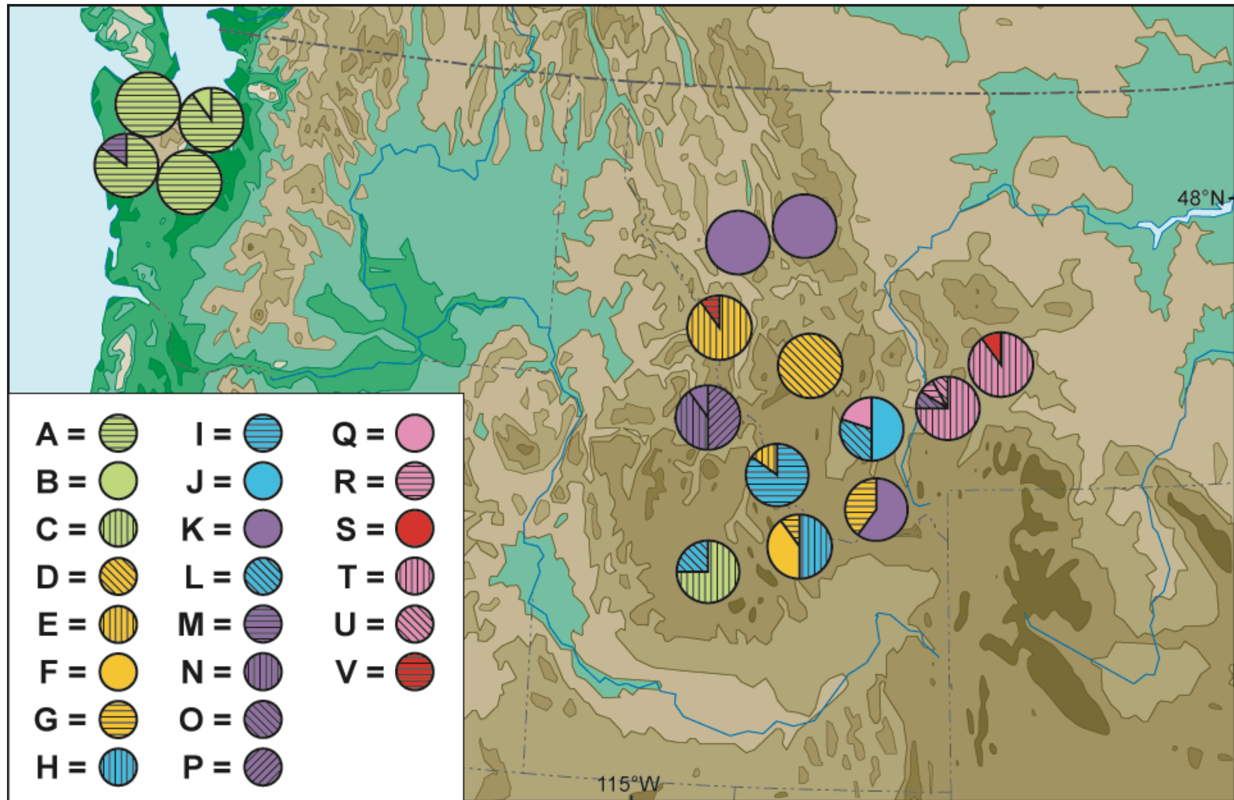

Fig. 4. Topology for *Synthyris* sect. *Dissecta* resulting from analyses of combined plastid *trnT-trnL* and *psbA-trnH* DNA sequences using maximum likelihood (ML; -ln 1480.8501; K81uf model of nucleotide substitution). Letters on tips indicate correspond to haplotypes in Table 1. Numbers on branches are ML bootstrap values.

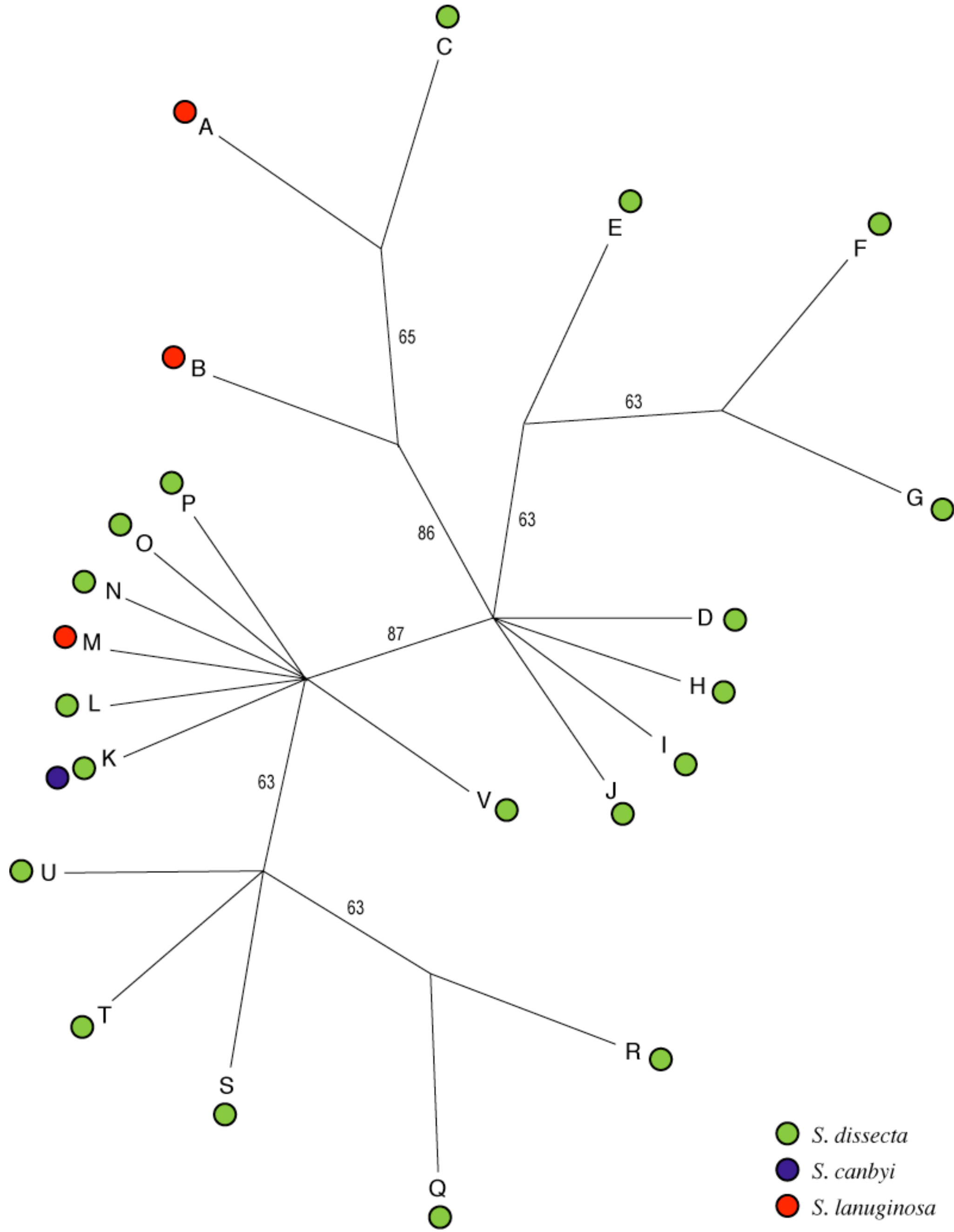

Fig. 5. Haplotype network of *Synthyris* sect. *Dissecta*. Letters correspond to haplotypes in Table 1. Solid lines connecting haplotypes represent a single substitution. Dashed lines represent alternative connections of haplotypes prior to resolution of loops. Dots represent missing haplotypes. Nesting levels for the nested clade analysis (NCA) are shown, with one-step clades in white, and with two-, and three-step clades indicated by successively darker shading. Numbered clades are those found to be significant by NCA, and are referred to in the text and in Tables 2 and 3.

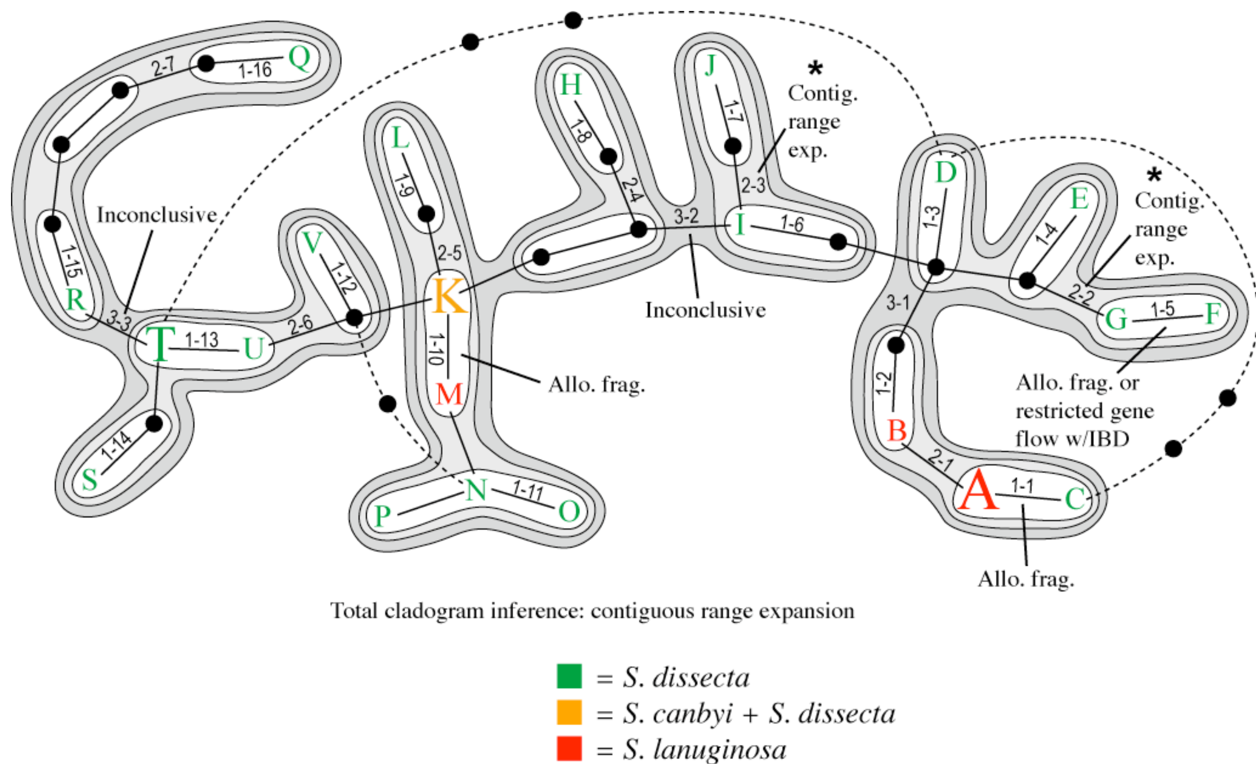

Fig. 6. Pairwise F_{ST} vs. geographic distance (km) for *Synthyris dissecta*. The line is a simple linear regression. Statistical significance was based on a Mantel test (P = 0.0270). The circle surrounds four populations (Anaconda, Northern Bitterroot, Central Bitterroot, and Southern Bitterroot Ranges) for which pairwise comparisons reveal a high degree of isolation (high pairwise F_{ST} values) in spite of short geographical distance between populations.