

USING COALESCENT SIMULATIONS TO TEST THE IMPACT OF QUATERNARY CLIMATE CYCLES ON DIVERGENCE IN AN ALPINE PLANT-INSECT ASSOCIATION

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Abstract.—The Quaternary climate cycles forced species to repeatedly migrate across a continually changing landscape. How these shifts in distribution impacted the evolution of unrelated but ecologically associated taxa has remained elusive due to the stochastic nature of the evolutionary process and variation in species-specific biological characteristics and environmental constraints. To account for the uncertainty in genealogical estimates, we adopted a coalescent approach for testing hypotheses of population divergence in coevolving taxa. We compared genealogies of a specialized herbivorous insect, *Parnassius smintheus* (Papilionidae), and its host plant, *Sedum lanceolatum* (Crassulaceae), from the alpine tundra of the Rocky Mountains to null distributions from coalescent simulations to test whether tightly associated taxa shared a common response to the paleoclimatic cycles. Explicit phylogeographic models were generated from geologic and biogeographic data and evaluated over a wide range of divergence times given calibrated mutation rates for both species. Our analyses suggest that the insect and its host plant responded similarly but independently to the climate cycles. By promoting habitat expansion and mixing among alpine populations, glacial periods repeatedly reset the distributions of genetic variation in each species and inhibited continual codivergence among pairs of interacting species.

Key words.—Climate change, coalescent simulations, comparative phylogeography, *Parnassius smintheus*, Pleistocene glacial cycles, Rocky Mountain alpine tundra, *Sedum lanceolatum*.

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The field of comparative phylogeography attempts to infer the evolutionary history of an ecosystem or an ecological association by comparing genealogical estimates of history from multiple species and thus provides a foundation for understanding the assemblage, structure, and evolution of communities (Bermingham and Moritz 1998; Avise 2000; Arbogast and Kenagy 2001). In most cases, however, analyses have relied on qualitative comparisons, such as describing the history and underlying processes of an ecosystem from common patterns exhibited by several related taxa (e.g., Soltis et al. 1997; Calsbeek et al. 2003). By its very nature, this descriptive approach precludes statistical tests of a priori hypotheses concerning the history of the region in question (Carstens et al. 2005a). In cases where the histories of two distantly related but ecologically associated species (i.e., coevolving hosts and parasites) are of interest, tests have typically taken the form of genealogical comparisons and hunts for evidence of concordance or cospeciation (reviewed in Page and Charleston 1998). However, species-specific biological characteristics have likely prompted independent migrations of species (West 1964, 1980; Bennett 1990, 2004; Coope 1995; Elias 1996; Whitlock and Bartlein 1997; Taberlet et al. 1998; Hewitt 1999; Jackson and Overpeck 2000; Barnosky 2001; Davis and Shaw 2001; Stewart and Lister 2001; Kropf et al. 2003; Thompson and Cunningham 2002) that in turn inhibited strict codivergence of taxa (Michalakakis et al. 1993; Martinez et al. 1999; Anderson et al. 2004; Baer et al. 2004), except in special cases where life histories were tightly bound (e.g., codispersal; Dybdahl and Lively 1996; Jerome and Ford 2002). Moreover, variation in the topology and divergence times in gene trees, which are the basis for all interspecific comparisons, exists due to the stochastic nature of the coalescent process (reviewed in Nordburg 2001).

To evaluate the evolutionary history of distantly related but ecologically associated taxa analyses must accommodate the stochastic variation in genealogies caused by the coalescent process and life-history strategies of each species. So far, only a few comparative studies have taken this approach (e.g., Carstens et al. 2005a; Hickerson and Cunningham 2005). Parameters associated with different aspects of genetic variation, such as mutation rates, effective population size, and divergence time, can vary widely among genes and across taxa. In a coalescent approach, gene trees are viewed backward in time, such that the likelihood of certain events (e.g., coalescence of genes into a common ancestor, mutation, and migration) is estimated over the parameter space (Kingman 1982). Thus, coalescent methods explicitly account for the uncertainty in the estimates of historical events and are specifically tuned to the focal taxa through the use of probabilistic models to generate expected distributions for relevant parameters. These estimates can then be used to test a priori phylogeographic hypotheses that were developed from independent data (e.g., geological or paleoclimatic records). Testing hypotheses with different taxa gives an understanding of community assemblage and structure and the evolution of interspecific interactions, while accounting for temporal and spatial scale of the processes underlying the association.

We applied a comparative, coalescent approach to evaluate how the climate cycles of the Quaternary (the last two million years) impacted the evolution of specialized plant-insect associations in the alpine tundra, where plants and insects comprise the majority of community diversity and the effects of climate change are particularly severe. Because alpine taxa responded to historic climate changes locally through repeated cycles of down-slope habitat expansion during glacial periods and up-slope contraction onto many isolated peaks

as the climate warmed (Hewitt 1996; DeChaine and Martin 2004, 2005a), high elevation plant-insect associations may be more likely to have experienced similar histories of isolation and divergence than low-elevation species that underwent long-distance migration. Indeed, studies have demonstrated that alpine plants and insects in the Rocky Mountains of North America diverged during the Quaternary due to the insular nature of multiple sky island refugia (Golden and Baine 2000; Knowles 2001; Knowles and Richards 2005). Although recent evidence demonstrated that two high-elevation butterflies fit the same model of population divergence in the Rocky Mountains during the Pleistocene (DeChaine and Martin 2005b), it remains to be determined whether insects and their host plants responded to the same environmental changes associated with the glacial cycles, and whether they did so in a similar fashion. Determining how historic climate cycles impacted the evolution of specialized associations provides a historical framework for understanding current geographic patterns of diversification and local adaptation for coevolving species.

Here, we present a comparative framework for testing hypotheses using coalescent models of putative population history for two ecologically associated species, the stonecrop *Sedum lanceolatum* (Crassulaceae) and its specialist herbivore *Parnassius smintheus* (Papilionidae) in the alpine tundra of the Rocky Mountains. The null hypothesis of independence due to species-specific constraints predicts that demographic histories and genetic divergence of the two species will be incongruent. Alternatively, if the plant and insect shared a common demographic response to the Quaternary climate cycles, then both species will fit the same model of population divergence. We adopted two approaches to test these alternative hypotheses. First, we compared the population structure of the two species using classical population genetics. Second, we used regional topography (Fig. 1a), paleoclimatic records, and inferences of historic events from previous studies (DeChaine and Martin 2004, 2005a) to construct phylogeographic models of divergence that were tested using coalescent simulations (Knowles 2000; Knowles and Maddison 2002; Carstens et al. 2005b). For the coalescent simulations, three models were tested: (1) a model (H1) of fragmentation of one ancestral source population (Fig. 1b), (2) an alternative model (H2) of two regional refugia separated by the Wyoming Basin (Fig. 1c), and (3) a model (H3) of three regional refugia (Fig. 1d) associated with biogeographic provinces (Brouillet and Whetstone 1993) that are roughly defined by latitudinal breaks in the Rocky Mountain cordillera. By employing coalescent simulations for taxon-specific mutation rates and population sizes, we can accommodate the stochastic variation in genealogies for geographically structured populations and explicitly test biogeographic hypotheses.

METHODS

The Plant-Insect Association

The highly specialized association between the butterfly *P. smintheus* (Papilionidae) and its host plant *S. lanceolatum* (Crassulaceae) is a common component of the alpine tundra community in the Rocky Mountains (Scott 1986, 1995; Sperling and Kondla 1991). As with other plant-insect interactions

(Loxdale and Lushai 1999), the range of *P. smintheus* is restricted by the distribution of its host plant, *S. lanceolatum* (Scott 1986). Both species exhibit low dispersal (Clausen 1975; Keyghobadi et al. 1999, 2005), isolation among populations, and geographic structure associated with known biogeographic provinces in the Rocky Mountains (Keyghobadi et al. 1999; DeChaine and Martin 2004, 2005a). *Sedum lanceolatum* produces a cyanogenic glycoside, sarmentosin, as a secondary defensive compound that deters herbivores, but the caterpillar of *P. smintheus* feeds exclusively on *S. lanceolatum* and sequesters the toxin for its own protection (Sperling and Kondla 1991; Nishida and Rothschild 1995). Furthermore, the adult butterfly of *P. smintheus* pollinates the flowers of *S. lanceolatum* (Scott 1973; Clausen 1975). The previously inferred phylogeographies of both species (DeChaine and Martin 2004, 2005a) and the specificity of the interaction make this an excellent system for testing whether high elevation plant-insect associations codiverged in response to the Quaternary glacial cycles.

Sampling

Sample collection and molecular techniques were previously described (DeChaine and Martin 2004, 2005a). Briefly, plant and insect specimens were collected from eighteen sites (Fig. 1a) throughout their shared range from southern Colorado, where the effects of interglacial habitat fragmentation are most severe, to northern Montana (Clausen 1975; Scott 1986) that was completely covered by the Cordilleran and Laurentide ice sheets (Richmond 1965). Approximately 800 bp of chloroplast DNA (tRNA L-F, tRNA S-G) and mitochondrial DNA (COI) were sequenced from about 20 individuals of *S. lanceolatum* and *P. smintheus*, respectively, per site. From the sequence data, population parameters, θ and F_{ST} , were estimated in FLUCTUATE 1.4 (Kuhner et al. 1995) and ARLEQUIN version 2.0 (Schneider et al. 2000), respectively. All analyses for *P. smintheus* were performed on the 18 populations in this study, which is a subset of the populations used for a previous phylogeographic analysis (DeChaine and Martin 2004).

Interspecific Comparisons of Demographic Histories

Estimates of within- and among-population genetic variation were contrasted between *S. lanceolatum* and *P. smintheus* to test for evidence of similarities in population structure. To evaluate similarities between species at the within-population level, estimates of θ were compared using a *t*-test and a nonparametric Spearman rank correlation. Matrices of pairwise population F_{ST} -values for each species were compared using a Mantel test to examine patterns of population differentiation between the species.

Testing Phylogeographic Hypotheses Using Coalescent Simulations

We tested three simple phylogeographic models of population divergence through coalescent simulations (Fig. 1). We adopted an islandlike hypothesis (H1) of fragmentation from a single ancestral population (Fig. 1a,b) because plots of F_{ST} against distance showed no signal of isolation-by-

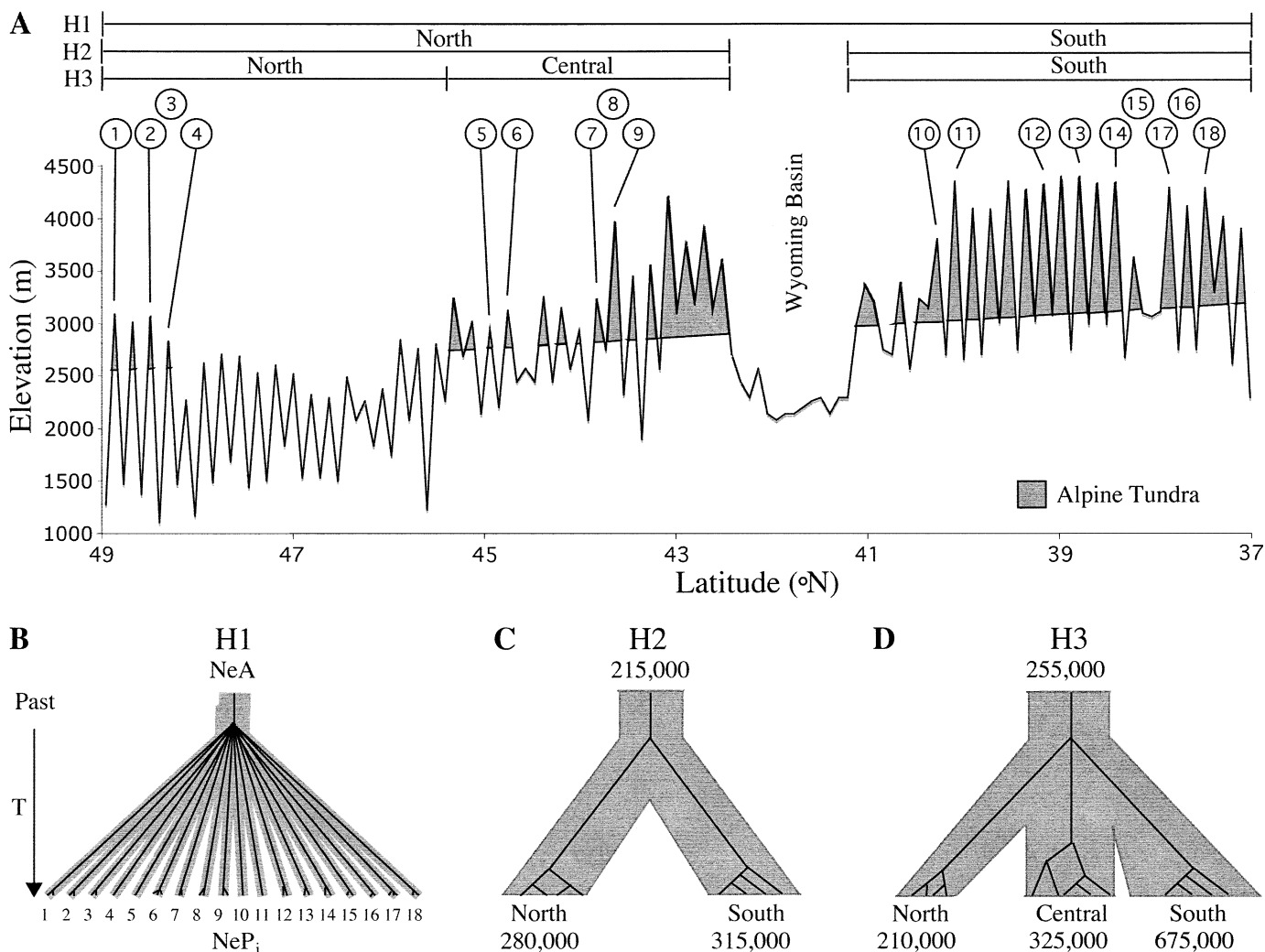


FIG. 1. Phylogeographic hypotheses for divergence of *Sedum lanceolatum* Torr. and *Parnassius smintheus* Doubleday. (A) Elevation profiles of alpine tundra in the Rocky Mountains during glacial cycles of the Quaternary. The north-south transect for the elevation profile of the Rocky Mountains basically follows the continental divide, including peaks from which specimens were collected (numbered as in Table 1). Sites for peaks that were not included in the profile (3, 8, 15, 16) are shown at their approximate latitude. The geographic breaks associated with the three phylogeographic models (H1, H2, and H3) as well as the Wyoming Basin are labeled for reference. The gray regions represent the current distribution of alpine habitat (from Küchler 1985). Latitude and elevation data were obtained from topographic atlases of the region (DeLorme 1998a,b,c,d). (B) The model of a single population (H1) that underwent fragmentation is shown with the ancestral effective population size, N_eA , and the current effective population sizes (N_eP_i , where P_i denotes each population). Each population corresponds to one of the study sites (see Table 1 for population details). (C) The two-refugia model (H2) divided by the Wyoming Basin is shown using the estimated ancestral and current Ne for *S. lanceolatum*. (D) The three-refugia model (H3) determined from latitudinal breaks in the Rocky Mountains associated with biogeographic provinces (Brouillet and Whetstone 1993) is illustrated using Ne estimates for *P. smintheus*. Gene trees (shown in black) are constrained within the gray population trees. A range of branch lengths was evaluated for divergence times (T) in the Quaternary.

distance for either species (not shown). Under the divergence model H2, populations were isolated in two refugia, north and south of the Wyoming Basin (Fig. 1a,c). The low elevation of the Wyoming Basin, which inhibits dispersal for high-elevation taxa (Noonan 2001) and the geographic structure inferred for *S. lanceolatum* through nested clade analyses (DeChaine and Martin 2005a) formed the basis for this hypothesis. The three-refugia hypothesis (H3) predicted divergence among the northern, central, and southern regions of the Rocky Mountains (Fig. 1a,d), which are delineated by latitudinal breaks in the cordillera (slightly modified from previously described biogeographic provinces of Brouillet

and Whetstone 1993). Thus, the three-refugia model was developed from the geology of the Rocky Mountains, previous analyses of molecular variance for both species, and phylogeographic patterns inferred for *P. smintheus* and another codistributed alpine butterfly, *Colias meadii* (DeChaine and Martin 2004, 2005b).

To begin, genetic diversity, θ ($=2N_e\mu$), was estimated within a species as well as within each population as defined by the models of divergence (Fig. 1). An overall measure of θ for each species was calculated using Watterson's method in ARLEQUIN 2.0 (Schneider et al. 2000) to provide an unbiased measure of ancestral diversity for use in each model.

TABLE 1. Estimates of within-population genetic variation and effective population size for the host plant *Sedum lanceolatum* (S) and its specialist herbivore *Parnassius smintheus* (P). Collection sites (numbered from north to south), numbers of individuals sampled per population (n), a genealogical, per-site estimate of genetic diversity (θ), and the corresponding estimate of N_e (based on the calibrated mutation rate for each species: *S. lanceolatum* $\mu = 8.24 \times 10^{-9}$, *P. smintheus* $\mu = 2.0 \times 10^{-8}$) are shown.

Location	n		θ		N_e	
	S	P	S	P	S	P
1. Numa Pk.	20	12	0.00084	0.00192	50,971	48,000
2. Gunsight Pass	19	27	0.00102	0.00087	61,893	21,750
3. Triple Divide	19	21	0.00070	0.00292	42,476	73,000
4. Dawson Pass	19	13	0.00043	0.00453	26,092	113,250
5. Amethyst Mtn.	17	20	0.00034	0.00142	20,631	35,500
6. Mt. Washburn	20	27	0.00000	0.00295	607 ¹	73,750
7. Moose Pass	21	20	0.00061	0.00631	37,015	157,750
8. Hyndman Pk.	17	32	0.00075	0.00258	45,510	64,500
9. Static Pk.	20	15	0.00040	0.00132	24,272	33,000
10. Sundance Mtn.	15	13	0.00149	0.00334	90,413	83,500
11. Long's Pk.	19	35	0.00192	0.00529	116,505	132,250
12. Quandary Pk.	20	10	0.00159	0.00054	96,481	13,500
13. Maroon Bells	18	10	0.00077	0.00251	46,723	62,750
14. Mt. Shavano	18	21	0.00091	0.00427	55,218	106,750
15. American Basin	17	33	0.00000	0.00450	607 ¹	112,500
16. San Luis Pk.	19	28	0.00078	0.00450	47,330	112,500
17. Humboldt Pk.	17	13	0.00026	0.00439	15,777	109,750
18. Iron Nipple	18	10	0.00059	0.00500	35,801	125,000
Average	19	20	0.00074	0.00329	40,716	82,166
Total ²	333	370	0.00354	0.01015	215,000	255,000
Among regions						
H2 northern	172	197	0.00462	0.02400	280,340	600,000
H2 southern	161	173	0.00516	0.02700	313,107	675,000
H3 northern	77	77	0.00420	0.00840	254,854	210,000
H3 central	95	120	0.00117	0.01300	70,995	325,000
H3 southern	161	173	0.00516	0.02700	313,107	675,000

¹ Estimates of N_e for populations with zero diversity were based on $\theta = 0.00001$. ² The estimates of θ (given on a per-site basis) for the total population, and thus the ancestral N_e , were calculated using Watterson's estimate in ARLEQUIN 2.0, all others are from FLUCTUATE 1.4.

For all individuals within a species, the calculated values of θ_w (per locus) were 3.1 and 8.0 for *S. lanceolatum* and *P. smintheus*, respectively. For each population and region, per site estimates of θ were estimated in the program FLUCTUATE 1.4 (Kuhner et al. 1995), which allows for population growth or decline within a single population.

In addition to θ , calibrated mutation rates were used to estimate the effective population sizes, N_e , in the models of population divergence. Based on comparisons between closely related species of insects (including Lepidoptera), the widely accepted rate estimate for mitochondrial cytochrome oxidase genes is 2% per million years pairwise divergence (DeSalle et al. 1987; Brower 1994; Megens et al. 2004), but ranges from 0.5% to 3.1% (e.g., Farrell 2001). Although this estimate holds for many insect taxa, the widespread AT bias in insects (Wirth et al. 1999) may cause saturation in the mitochondrial DNA (Moriyama and Powell 1997) and thus an underestimation of the actual level of divergence. Moreover, recent evidence suggests that substitution rates based on divergence of species underestimate mutation rates and, thus, it may be inappropriate to extrapolate molecular rates of change across different evolutionary timescales (Ho et al. 2005; Ho and Larson 2006). By employing a rate estimate that has been calibrated for many taxa and testing divergence over a broad timeframe (20,000 to 1,800,000 years), we have attempted to account for this discrepancy, but note that the timing of events is more likely recent (faster rates) than ancient (slower rates).

From the estimates of θ and the calibrated mutation rates, the N_e for each species was calculated for use in testing the phylogeographic models. We adopted a rate of 8.24×10^{-9} that was calibrated for the trnL-F intergenic spacer in the related perennial herb *Aichryson* (Crassulaceae) (Richardson et al. 2001) to estimate the ancestral N_e (= 215,000) for *S. lanceolatum*. Applying a molecular clock of 2% per million years for mitochondrial cytochrome oxidase I in insects (DeSalle et al. 1987; Brower 1994) to the butterfly data resulted in an ancestral N_e of 255,000. In addition, the current N_e for each population and region were calculated from estimates of θ , respectively (Table 1).

We tested the data for both taxa against a wide range of branch lengths, for each phylogeographic model. Because our aim was to test the fit of both species to models of population divergence over the same time periods, and because the MESQUITE 1.05 model (Maddison and Maddison 2003) uses the number of generations for branch lengths, we first had to convert time from years to generations. For *P. smintheus*, one generation is equal to one year. Since *S. lanceolatum* is a biennial though potentially long-lived herb, we estimated one generation to be two years. The divergence times that were tested for each model corresponded to paleoclimatic episodes of the Pleistocene (reviewed in Gradstein et al. 2004) as follows: the Late Wisconsin Glacial at 20,000 years ago (T_1), the Sangamon Interglacial at 135,000 years ago (T_2), a mid-Pleistocene interglacial at 610,000 years ago (T_3), and the Pleistocene-Pliocene boundary at 1,800,000 years ago (T_4).

For all tests, the branch length of the root was set to 10,000 generations. Thus, we were able to determine a range of branch lengths over which the observed, reconstructed species' genealogy did or did not reject a phylogeographic model.

Coalescent simulations of genealogies constrained within models of population divergence (see Fig. 1) were performed with MESQUITE 1.05 (Maddison and Maddison 2003). First, 1000 gene matrices (based on the haplotypes of each species, 45 for *S. lanceolatum* and 73 for *P. smintheus*) were simulated under a coalescent model for each of the four hypothetical divergence times (where branch length is the number of generations), using the model of DNA substitution determined by MODELTEST 3.6 (Posada and Crandall 1998) and an estimate of N_e from the calibrated mutation rate for both species. From the simulated gene matrices, genealogies were reconstructed in PAUP* 4.10b (Swofford 2003) using maximum likelihood.

The amount of discordance between a gene tree and the population model was measured by S , the minimum number of sorting events required to produce the genealogy within a given model of divergence (Slatkin and Maddison 1989). The S -value is a measure of the number of parsimony steps in a character (where the source population for the haplotype is the character state) for a reconstructed gene tree, such that more discordance between the population and gene trees leads to a higher S -value. If it is assumed that the populations have been separated for a long time (and $N \gg$ sample size), the only process causing discordance is migration; otherwise, incomplete lineage sorting may contribute to the differences. To obtain a distribution of S -values, the trees reconstructed from all coalescent simulations were constrained within the two-refugia model of population divergence in MESQUITE 1.05 (Maddison and Maddison 2003), and the amount of discord between the simulated genealogy and the population model was determined. Constraining all genealogies within the focal model (H2, derived from the geographic pattern of variation exhibited by *S. lanceolatum*) is important since the S -value increases almost linearly with the number of populations (Slatkin and Maddison 1989). Overall, this produced a null distribution of 1000 S -values based on reconstructed genealogies for each of the three models of population divergence, over the four divergence times, given the mutation rates for both species.

The S -value for the observed genealogy constrained within the two-refugia model was calculated and compared to the distribution of S -values from the coalescent simulations to determine whether the observed genealogy could have been generated under a given model (Fig. 1). Finally, due to the relationship between the mean S and T/N_e (divergence time divided by the current effective population size; Slatkin and Maddison 1989), the range of times over which a species might fit a model of divergence were predicted (roughly due to variation in the standard deviation with respect to T/N_e) and tested through further coalescent simulations. This approach permitted statistical tests ($\alpha = 0.05$) of whether observed genealogies could have been generated under a population model, given the mutation rates for the taxa and time-frames over which population divergence could have occurred.

RESULTS

Comparisons of Estimates Using Classical Population Genetics

The geographic distribution of genetic variation differed between the two species. For the host plant, *S. lanceolatum*, and its specialist herbivore, *P. smintheus*, estimates of within-population genetic diversity (θ) (Table 1) were not correlated between the plant and the herbivorous insect (Spearman's rank correlation, $r_s = 0.117$, $P > 0.25$). Genetic diversity was significantly greater in *P. smintheus* (mean = 0.00329) than in *S. lanceolatum* (mean = 0.00074) based on a paired t -test ($t = 6.06$, $P < 0.001$). The frequency of private alleles was not correlated between species either (Spearman's rank correlation, $r_s = 0.08$, $P > 0.5$), and populations of *S. lanceolatum* harbored a higher frequency of private alleles than *P. smintheus*. Finally, the magnitudes and patterns of pairwise population genetic divergence (F_{ST}) were not congruent between species (Mantel test, $P = 0.33$), a result that reflects lack of significant correlation in levels of within-population genetic variation between species.

Tests of Phylogeographic Models

Genealogies were estimated for both species. The best model of DNA substitution for *S. lanceolatum* corresponded to a HKY + I model (Akaike information criterion [AIC] = 2817.4812; nucleotide frequencies: A = 0.3095, C = 0.1751, G = 0.1625, T = 0.3529; Ti/Tv ratio = 2.433; pinvar = 0.8526) and *P. smintheus* fit a K81uf + I + G model (AIC = 3423.8008; nucleotide frequencies: A = 0.2984, C = 0.1400, G = 0.1433, T = 0.4183; pinvar = 0.8439; gamma shape parameter = 0.9071; rA-C = 1, rA-G = 3.3568, rA-T = 0.3184, rC-G = 0.3184, rC-T = 3.3568, rG-T = 1). These models were used to reconstruct genealogies with and without enforcing a molecular clock. A likelihood ratio test (LRT; Felsenstein 1988) failed to reject the clock in both species (*S. lanceolatum* $\delta = 37.4$, $df = 43$, $P > 0.50$; *P. smintheus* $\delta = 79.2$, $df = 73$, $P > 0.25$).

To determine whether the observed data fit a given model of population divergence, S -values for *S. lanceolatum* and *P. smintheus* were calculated on their reconstructed genealogies and compared with the distribution of S -values determined from the coalescent simulations. The distributions of S -values from coalescent simulations for both species (Fig. 2) revealed that, in general, the amount of discordance is negatively correlated with divergence time, as is expected under the coalescent model. The host plant *S. lanceolatum* only fit the two-refugia model (H2) over divergence times of 610,000 ($P = 0.22$) and 1,800,000 ($P = 0.88$) years (Fig. 2b). Additional simulations determined the 95% confidence limit and thus the minimum divergence time (T_{min}) in *S. lanceolatum* (given the calibrated mutation rate) to be 400,000 years ($P = 0.06$). Because $S = 1$ in *S. lanceolatum*, a maximum divergence time could not be determined from the simulations. In contrast, the specialist herbivore *P. smintheus* fit both the two-refugia model (H2) and the three-refugia model (H3) for a divergence time of 135,000 years ($P = 0.4$) (Fig. 2e,f). Further coalescent simulations in *P. smintheus* estimated the temporal bounds on divergence at $T_{min} = 80,000$ ($P = 0.06$) and

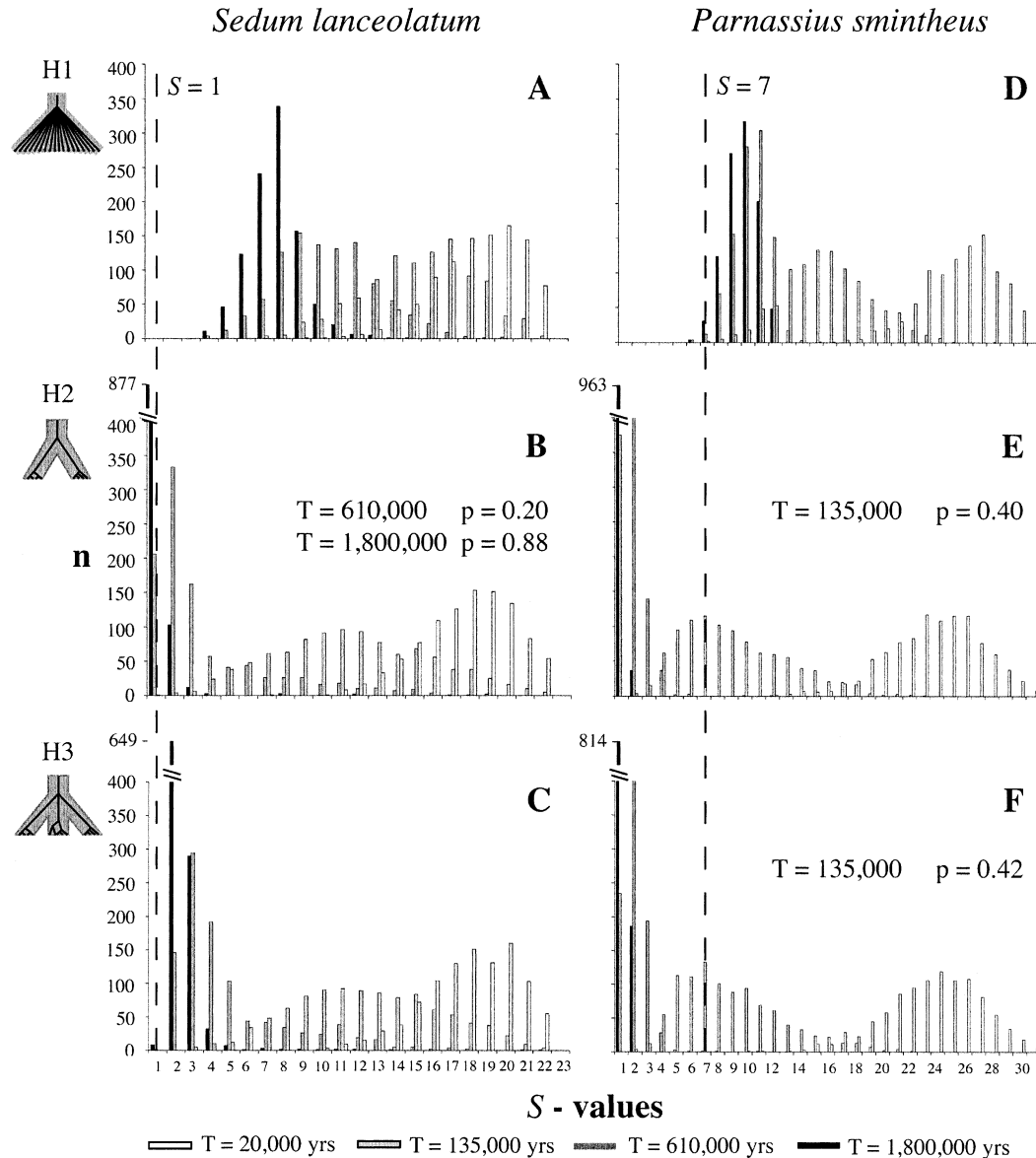


FIG. 2. Tests of phylogeographic models comparing the observed measure of discordance, S , with a distribution of S -values from simulated genealogies constrained within models of population divergence. The observed S -values (dashed line) compared with the S -distributions from simulated data (shaded bars) are shown for *Sedum lanceolatum* Torr. and *Parnassius smintheus* Doubleday, given the calibrated mutation rate for each species (*S. lanceolatum* $\mu = 8.24 \times 10^{-9}$, *P. smintheus* $\mu = 2.0 \times 10^{-8}$). The outcomes for each of the three models of population divergence are shown (H1 in A and D; H2 in B and E; H3 in C and F). S -distributions for the different divergence times are delineated by shading: 20,000 years (white), 135,000 years (light gray), 610,000 years (dark gray), and 1,800,000 years (black). A model was rejected if the observed S -value did not significantly fit ($\alpha = 0.05$) the expected S -distribution generated by coalescent simulations. The P -values for models and divergence times (T) that could not be rejected are shown.

$T_{max} = 300,000$ ($P = 0.06$) years ago. The null model of fragmentation from a single source population was rejected for both species for all time periods tested (Fig. 2a,d). Thus, the coalescent simulations support divergence among multiple refugia during the Pleistocene for both taxa, but over different time periods.

DISCUSSION

Divergence in the Alpine Plant-Insect Association

Although paleoclimatic cycles probably influenced the distribution of both species in a similar way, through repeated

rounds of up-slope contraction during interglacials and down-slope expansion during cold glacial periods (DeChaine and Martin 2005a,b), the specialist herbivore and its host plant fit different models of divergence among refugia. Incongruent patterns of genetic variation were first revealed through comparative analyses of within-population genetic variation and among-population differentiation. Notably, higher frequencies of private alleles and pairwise F_{ST} -values in *S. lanceolatum* suggested that geographic isolation is more pronounced for the host plant than for *P. smintheus*.

Despite convincing evidence for discordant genealogical

histories, the data for both species were compatible with a two-refugia phylogeographic model, implying both species experienced regional isolation during the Quaternary. Support for a multiple refugia model is not unexpected; multiple refugial populations have been hypothesized for several North American species (Zink 1996; Tremblay and Schoen 1999; Yates et al. 1999; Abbott et al. 2000; Good and Sullivan 2001; Demboski and Sullivan 2003) and particularly alpine insects (Knowles 2000). Paradoxically, even though *S. lanceolatum* exhibited a greater degree of isolation than *P. smintheus*, the host plant only fit the more general two-refugia model. However, we could not differentiate between the two- and three-refugia hypotheses for the butterfly, given the genetic data and models of DNA substitution. The xeric, low-elevation Wyoming Basin that bisects the Rocky Mountain cordillera at approximately 42°N (Fig. 1a), has been shown to inhibit dispersal of other high-elevation taxa (Britten and Brussard 1992; Noonan 1992, 1999, 2001; Reiss et al. 1999; Nice and Shapiro 2001) and obviously had an impact on genetic divergence in this plant-insect pair. Although the impact of the Pleistocene climate cycles was readily apparent in the genealogies of both species, populations of *P. smintheus* diverged approximately 80,000 to 300,000 years ago, but the coalescent times for the host plant were more ancient (>400,000 years ago). Interestingly, *P. smintheus* and another codistributed alpine butterfly, *Colias meadii*, both underwent divergence during the same time frame (DeChaine and Martin 2005b). The difference in divergence times between *P. smintheus* and *S. lanceolatum*, but similarities between the two butterflies, underscore the role that life-history strategies might play in genetic divergence. Thus, despite evidence that both alpine species were sundered in multiple refugia, the implied climatic and geographic constraints were not sufficiently pronounced that the interacting plant and insect experienced similar demographic histories.

Independent biogeographic histories in the specialized plant-insect association involving *S. lanceolatum* and *P. smintheus* probably reflect repeated population mixing events promoted by habitat expansion during glacial periods of the Quaternary. We imagine that during warm interglacial periods, populations of the host plant and its specialist herbivore were isolated on multiple sky islands, and during these periods, the two species may have undergone some degree of codifferentiation and coadaptation consistent with the predictions of the geographic mosaic theory of coevolution (Thompson 1999). However, during the cool periods of glacial advance and down-slope spreading of the alpine tundra, populations probably also expanded, became connected, and mixed. During the expansion phase, local directional selection and genetic coadaptation may have been diluted by higher levels of gene flow and the immigration of novel genes. The shifting climate might have not only maintained a state of evolutionary stasis for any one species (Bennett 1990; 2004), but also achieved coevolutionary stasis by precluding continual directional changes in associated taxa. This supports the contention that, for high-elevation species, patterns of local adaptation, such as those outlined in the geographic mosaic theory of coevolution (Thompson 1994), arising over time periods shorter than the frequency of climatic shifts (e.g., within an interglacial period) have probably been erased

by the rapid population growth and extensive gene flow associated with each glacial episode (Bennett 1990; Jackson and Overpeck 2000; Barnosky 2001). Given the large number of sky islands in the Rocky Mountains, cycles of population isolation and expansion created an immense number of possible phylogeographic histories. Thus, the expanding-contracting archipelago model for sky islands (DeChaine and Martin 2004, 2005b) should yield individual species' phylogeographic histories that appear independent even though all species of the alpine community may have experienced very similar demographic histories and geographic constraints, and many species may be mutually dependent and involved in some sort of coevolutionary dynamic.

Methods for Comparative Phylogeography

Our coalescent approach to comparative phylogeography begins where many other methods have left off. Previous studies that have attempted to synthesize phylogeographic works on alpine taxa (e.g., Hewitt 1999; Schönswetter et al. 2005) have looked for concordance among genealogies, rather than testing hypotheses about the environmental processes that might have given rise to the patterns of genetic diversity. In contrast, the approach presented here uses a gene genealogy as a platform from which to test phylogeographic hypotheses about an association and an ecosystem, rather than as the endpoint of the analysis. By estimating a range of genealogies through coalescent simulations based on empirically estimated population parameters over several competing hypotheses of population divergence (as per Knowles 2001), we were able to account for the stochastic variation across taxa that might have existed due to population dynamics, coalescent stochasticity, incomplete lineage sorting, sampling effects, life-history parameters, and phylogenetic uncertainty (Hudson 1983; Moritz et al. 1987; Edwards and Beerli 2000; Felsenstein 2004). Thus, our comparative, coalescent approach provides a robust means for evaluating the processes that have given rise to the distribution and evolution of interactions and communities over relevant spatial and temporal scales (Schluter and Ricklefs 1993; Jackson and Overpeck 2000).

This comparative, coalescent approach to phylogeography has several advantages over the classical, descriptive means of examining the history of an ecosystem. First, our approach explicitly tests a priori hypotheses that were derived from independent information on the Rocky Mountain alpine tundra. More importantly, the coalescent tests account for the stochastic variation in genealogies due to the coalescent process and species-specific characteristics. For instance, coalescent tests allowed us to perform simulations on population divergence in two phylogenetically unrelated taxa while accounting for variation in the mutation rates of the genetic loci, effective population sizes, and a range of divergence times. By evaluating the fit of multiple species data to a given model of evolutionary history, we were able to test for the underlying processes affecting the ecosystem rather than simply looking for genealogical concordance.

This comparative approach is not without its limitations, however. The quality of the simulations depends on the a priori hypotheses of population structure, which requires a

substantial geological and/or paleoclimatic record for the region. Moreover, these tests require incorporating parameters that were estimated through various population genetic and phylogenetic methods, and different methods can lead to different estimates of θ , divergence times, or genealogies (under different models of DNA substitution) depending on the assumptions of the models. Furthermore, the analyses are computationally intensive and many simulations must be run for each model, given a set of population parameters for the species and potential time frames of divergence. Finally, because each gene tree is only one estimate of the evolutionary history of a species (as emphasized recently by Jennings and Edwards 2005), multiple genetic markers should be used to infer the demographic history of a species or interspecific association.

Conclusions

By adopting a comparative, coalescent approach, we were able to determine that both *S. lanceolatum* and its specialist herbivore, *P. smintheus*, underwent divergence among multiple refugia during the Pleistocene, but that the timing of divergence and structure of populations differed between the taxa. Furthermore, we demonstrated a statistical approach to comparative phylogeography, which accounts for the stochastic variation in genealogies that arises through the coalescent process and differences among organisms in life-history characteristics. Our analyses provide a foundation for studying the evolutionary history of the Rocky Mountain alpine tundra, where organisms that are currently marooned atop mountains in an archipelago of shrinking sky islands are particularly vulnerable to the impacts of climate change.

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