Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoprers

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Abstract

There is a long-standing debate over whether or not the Pleistocene glaciations promoted speciation. While some models predict that extensive mixing of populations during interglacial expansion would have inhibited divergence, others postulate that divergence among allopatric glacial refuges or founder events during recolonization of previously glaciated areas would have promoted differentiation. Using a combination of traditional and coalescent based population genetic approaches, this study finds that the glaciations did not inhibit divergence among populations of the grasshopper *Melanoplus oregonensis*. Instead, drift associated with recolonization of previously glaciated areas, as well as divergence among multiple allopatric glacial refugia, have both contributed to differentiation in this montane grasshopper from the 'sky islands' of the northern Rocky Mountains. Significant population structure was detected by phylogenetic and F_{ST} analyses, including significant F_{ST} values among individual pairs of sky-island populations. In addition to clustering of haplotypes within populations, there is some evidence of regional phylogeographic structure, although none of the 'regional groups' form a monophyletic clade and there is a lack of concordance between the genealogical and geographical positions of some haplotypes. However, coalescent simulations confirm there is significant regional phylogeographic structure that most likely reflects divergence among multiple ancestral refugial populations, and indicate that it is very unlikely that the observed gene tree could have been produced by the fragmentation of a single widespread ancestral population. Thus, rather than inhibiting differentiation, the glaciations appear to have promoted population divergence in *M. oregonensis*, suggesting that they may have contributed to the radiation of *Melanoplus* species during the Pleistocene.

Keywords: coalescence, historical demography, phylogeography, Pleistocene, sky islands, speciation

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Introduction

The Pleistocene glaciations have indisputably had a major effect on many taxa as species' distributions shifted in response to climatic fluctuations (Huntley & Webb 1989; Pielou 1991; Webb & Bartlein 1992; Joseph *et al*. 1995; Hewitt 1996; Roy *et al*. 1996). However, whether these shifts promoted or inhibited divergence is a subject of debate, and thus the role of the glacial cycles in speciation is uncertain.

Different models provide conflicting predictions of the effect the glaciations would have on species divergence

(e.g. Mengel 1964; Coope 1979; Hewitt 1999). Moreover, these opposing views are each supported by empirical evidence. Estimates on the timing of species divergence from recent phylogenetic studies indicate that some species originated during the Pleistocene (e.g. Bermingham *et al*. 1992; Klicka & Zink 1997; Avise & Walker 1998; Knowles & Otte 2000; Knowles 2000). In contrast, other speciation events that had been ascribed to Pleistocene vicariance actually predate the Pleistocene (e.g. Cracraft & Prum 1988; Bush 1994; Riddle 1996). Although these studies indicate that speciation was not completely inhibited by the Pleistocene, and that the origin of some sister species did not coincide with the glaciations, they do not necessarily demonstrate that the glaciations affected diversification. Only by testing explicitly what effects the glaciations had on divergence will we understand what role, if any, the glaciations had in speciation (Knowles 1999).

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Comparing the rate of speciation during glaciated and unglaciated times can indicate whether the glaciations inhibited or promoted diversification (e.g. Zink & Slowinski 1995); however, not all taxa contain clades of Pleistocene and pre-Pleistocene origin. Similarly, inferences about the mode of species divergence from interspecific phylogenies (e.g. whether speciation occurred in allopatry or peripatry, e.g. Avise *et al.* 1983; Lynch 1987; Harrison 1991; Kliman & Hey 1993) may be inaccurate or limited for species originating during the Pleistocene. Because of the dynamic history of the Pleistocene and potential for multiple range shifts, the present geographical distributions of taxa may not accurately reflect the geography of speciation.

Alternatively, patterns of population variation can be used to test whether the glaciations promoted or inhibited divergence since intraspecific variation reflects recent as well as historical events (Barton & Wilson 1995; Templeton *et al.* 1995). For example, if divergence was associated with successive founder events during recolonization of previously glaciated areas, recently founded populations will be derived relative to the source population and exhibit reduced levels of diversity (e.g. Cwynar & MacDonald 1987; Green *et al.* 1996; Hewitt 1996, 1999; Alexandrino *et al.* 2000). Conversely, if divergence was primarily associated with glacial periods when populations were displaced to allopatric refuges, regional structuring of genetic variation may be evident (e.g. Joseph *et al.* 1995; Milot *et al.* 2000; Mitton *et al.* 2000). Thus, to the extent that the factors underlying population differentiation are representative of those leading to species divergences (Avise *et al.* 1987) this approach can potentially elucidate the role of the Pleistocene glaciations in speciation.

This paper adopts the latter approach and examines patterns of genetic variation in the montane grasshopper *Melanoplus oregonensis*. *M. oregonensis* is broadly distributed latitudinally, inhabiting previously glaciated areas in the northern Rocky Mountains (Knowles 1999), and thus offers an ideal opportunity to study what effects the glaciations had on population divergence. Moreover, *M. oregonensis*, and all of its close relatives in the Indigens species group, appear to have originated during the Pleistocene (i.e. within the last 2 Myr; Knowles & Otte 2000; Knowles 2000), and thereby provides a direct context in which patterns of population divergence can be used to make inferences about Pleistocene speciation.

The goals of this study were to determine: (i) if the glaciations inhibited divergence, and if they did not, whether divergence was; (ii) associated with founder events during the recolonization of previously glaciated areas; or (iii) the displacement of populations into allopatric glacial refugia. Phylogeographic and F_{ST} analyses in combination with coalescent simulations were used to elucidate the role of the Pleistocene glaciations in population differentiation, in the context of these explicit models of divergence (i.e. divergence in allopatric glacial refugia vs. during recolonization of previously glaciated areas).

Materials and methods

Study system

Melanoplus oregonensis Thomas (Orthoptera: Acrididae: Melanoplinae) is broadly distributed across the sky islands of the northern Rocky Mountains (Knowles 1999; D. Otte, in preparation). *M. oregonensis*, with extremely reduced wings, is flightless and restricted to patchily distributed meadows along mountain tops from northern Wyoming through western Montana to southern British Columbia. The northern part of its range was covered by the Cordilleran ice-sheet and a complex of mountain and valley glaciers influenced the region south of the ice-sheet margin in which it is also distributed today (Hollin & Schilling 1981; Mayewski *et al.* 1981; Pielou 1991; Elias 1996). Palynological evidence indicates that many plant species underwent repeated shifts in distribution, both latitudinal and elevational, in response to the glacial cycles (Wolfe 1987; Singh 1988; Huntley & Webb 1989; Pielou 1991; Elias 1996).

Collections, DNA amplification and sequencing

I collected and analysed 1275 bp of sequence data from cytochrome oxidase I (COI) from 124 individuals of *M. oregonensis* (Indigens group), and two outgroup species, *M. marshalli* (in the Indigens group) and the more distantly related *M. truchensis* (from the Dodgei group, which is distributed in the central Rockies) (Knowles 2000; Knowles & Otte 2000). Specimens were collected from 15 sky-island populations from different mountain ranges in western Montana and north-western Wyoming throughout the range of *M. oregonensis* (Fig. 1). Ten or more individuals were sequenced from each population except in four cases in which only one or two specimens were collected (see Appendix I). DNA amplification and sequencing methods are described by Knowles (2000).

Data analysis

Data were analysed using maximum-likelihood (ML), maximum-parsimony (MP), and neighbour-joining (NJ) methods (PAUP*, Swofford 1998). ML analyses were conducted using a model of among-site rate heterogeneity. ML was used to estimate the shape parameter of the gamma distribution ($\alpha = 0.16$) and the transition:transversion ratio (ts:tv = 2.68), assuming all sites were potentially variable (PAUP*, Swofford 1998). NJ analyses were conducted using the Kimura 2-parameter model to correct for multiple substitutions (Kimura 1980). There were no problems aligning
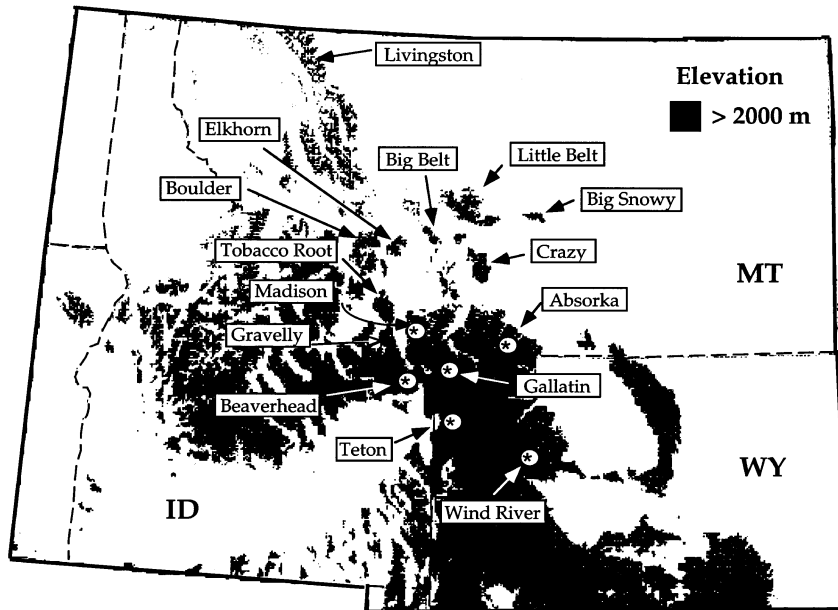
Fig. 1 Map of the sky islands of northern Rocky Mountains indicating where populations were collected (see Appendix I).

the sequences, given the absence of insertions and deletions. Unique sequences were determined using MacClade 3.0 (Maddison & Maddison 1992), and were used in all phylogenetic analyses.

Patterns of genetic variation and levels of genetic diversity within and among populations of *M. oregonensis* were analysed with HEAP BIG (Palumbi *et al.* 1997). Population-level differentiation was estimated using the F_{ST} approach of Hudson *et al.* (1992). Statistical significance of F_{ST} values was determined by 1000 Monte Carlo randomizations of mitochondrial DNA (mtDNA) sequences among populations, using HEAP BIG. Tests of significance of F_{ST} values for pairwise comparisons of individual populations were made after the level was adjusted using a Bonferroni correction (Sokal & Rohlf 1995). A Mantel test (Sokal & Rohlf 1995) was used to test for an association between F_{ST} and geographical distance separating populations among all pairs of populations (i.e. evidence of isolation by distance). Statistical significance of the Mantel test was determined by comparing the observed *r*-value to a distribution created from 1000 random permutations. Kimura 2-parameter model (Kimura 1980) was used to calculate pairwise genetic distances.

A gene-tree population-tree approach (Fig. 2) (L. L. Knowles and W. P. Maddison, in preparation) and coalescent simulations were used to test alternative hypotheses about the demographic history of *M. oregonensis* using the program MESQUITE (Maddison & Maddison 2000). In this approach, different refugial models are represented as different population trees (see Fig. 3a,b). The gene tree is then contained within the population tree and the amount of discord between the trees due to the deep coalescence of
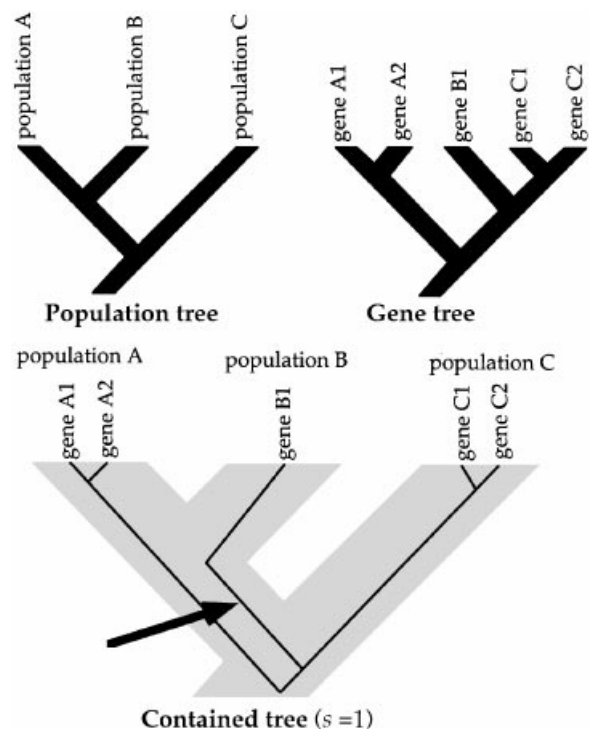

Fig. 2 A discordance between the gene and population tree is caused by the deep coalescence of a gene lineage, or the failure of a gene lineage to coalesce within the population lineage, as indicated by the arrow. The test statistic *s* (Slatkin & Maddison 1989) measures the degree of discordance between the trees, where the *s*-value represents the number of parsimony steps in a character for the genes whose states represent the source population of the genes. The *s*-value increases as the degree of discordance between the gene and population trees increases (i.e. concordant gene and population trees will have low *s*-values).
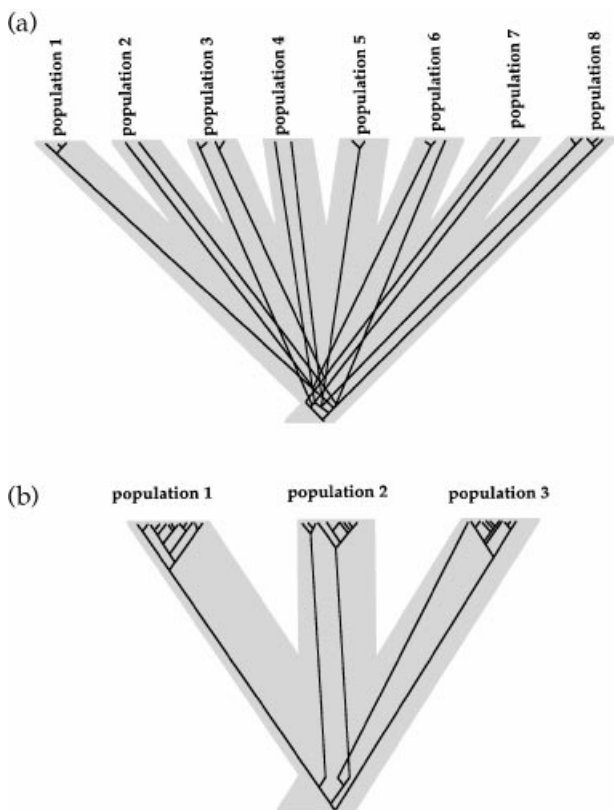
Fig. 3 Characterization of the two population trees representing the different population histories used in the coalescent simulations (a) the null model of the fragmentation of a widespread ancestor, and (b) the alternative multiple refugial model. In the null model (a), each of the populations corresponds to an individual sky-island population (not all populations are shown). Under the alternative model (b), each ancestral refugial population corresponds to one of the three regional groupings of populations identified from the phylogeographic analyses (see Figs 4 and 5).

a gene lineage, or the failure of a gene lineage to coalesce within the population lineage is measured (Fig. 2). The degree of concordance between an observed gene tree and population tree is compared to that of gene trees simulated by neutral coalescence to test the fit of the data to a specific model (L. L. Knowles and W. P. Maddison, in preparation).

In this paper, two population models are used to test explicitly whether patterns of population differentiation are consistent with the fragmentation of a widespread ancestor (i.e. a single ancestral source population), or the alternative hypothesis of divergence in multiple glacial refugia (Fig. 3a,b). The null model of a single source population was represented as the fragmentation of a widespread ancestor (i.e. a star-phylogeny, Fig. 3a) rather than a stepping-stone model because the phylogeographic and F_{ST} analyses indicated that the latter model would not have been appropriate (see Results; Figs 4 and 7). Similarly,

postulated ancestral source populations in the multiple refuge model (Fig. 3b) were identified by regional phylogeographic patterning of haplotypes (see Results; Figs 4 and 5). Gene trees were first simulated by neutral coalescence within the population tree representing the fragmentation of a widespread ancestor (Fig. 3a) over a range of branch lengths representing different times since population splitting measured in generations ($N_e = 1000$; 100 gene trees were simulated for each set of parameters). These gene trees were then contained within the alternative model of a multiple-refuge population tree (Fig. 3b), and the amount of discordance between the trees was measured. This discordance was measured by s (Slatkin & Maddison 1989), where the s-value represents the number of parsimony steps in a character for the genes whose states represent the source population of the genes (Fig. 2). The s-value from the reconstructed gene tree was compared to the expected distribution of s-values generated from the simulated gene trees. If the s-value from the reconstructed tree was significantly lower than those from the simulated gene trees (i.e. less than 5% of the simulated gene trees had a s-value of equal or lesser value than the reconstructed gene tree) then the null model (Fig. 3a) was rejected.

Results

Phylogeographic patterns and tests of explicit refugial models

There were 292 variable sites (166 informative ones) and 104 unique haplotypes in the 1275-bp fragment sequenced in 124 individuals of *Melanoplus oregonensis*. The gene genealogy of *M. oregonensis* is monophyletic and estimates of the genealogical relationships among haplotypes were generally congruent across the ML, MP, and NJ analyses (Fig. 4). None of the individual sky-island populations form a monophyletic clade, excluding populations in which only one or two individuals were sequenced (Fig. 4). Nevertheless, the phylogenetic analyses indicate there is substantial geographical structuring. Across all populations, many of the haplotypes within a population cluster together even though they do not form monophyletic clades, as indicated by the bars in Fig. 4. No haplotypes are geographically widespread. Shared haplotypes occur within the same population or between phylogenetically closely related, but not necessarily geographically proximate, populations (Figs 1 and 4). For example, despite the geographical proximity of the Gravelly and Madison Ranges to the Tobacco Root Range (Fig. 1), haplotypes from the Tobacco Root mountains are phylogenetically more closely related to the haplotypes from the Gallatin, Teton, and Beaverhead mountains (Fig. 4). Moreover, even though the Gallatin and Teton mountains are about equidistant from the Tobacco Root and Absorka Ranges (Fig. 1), haplotypes from the Gallatin
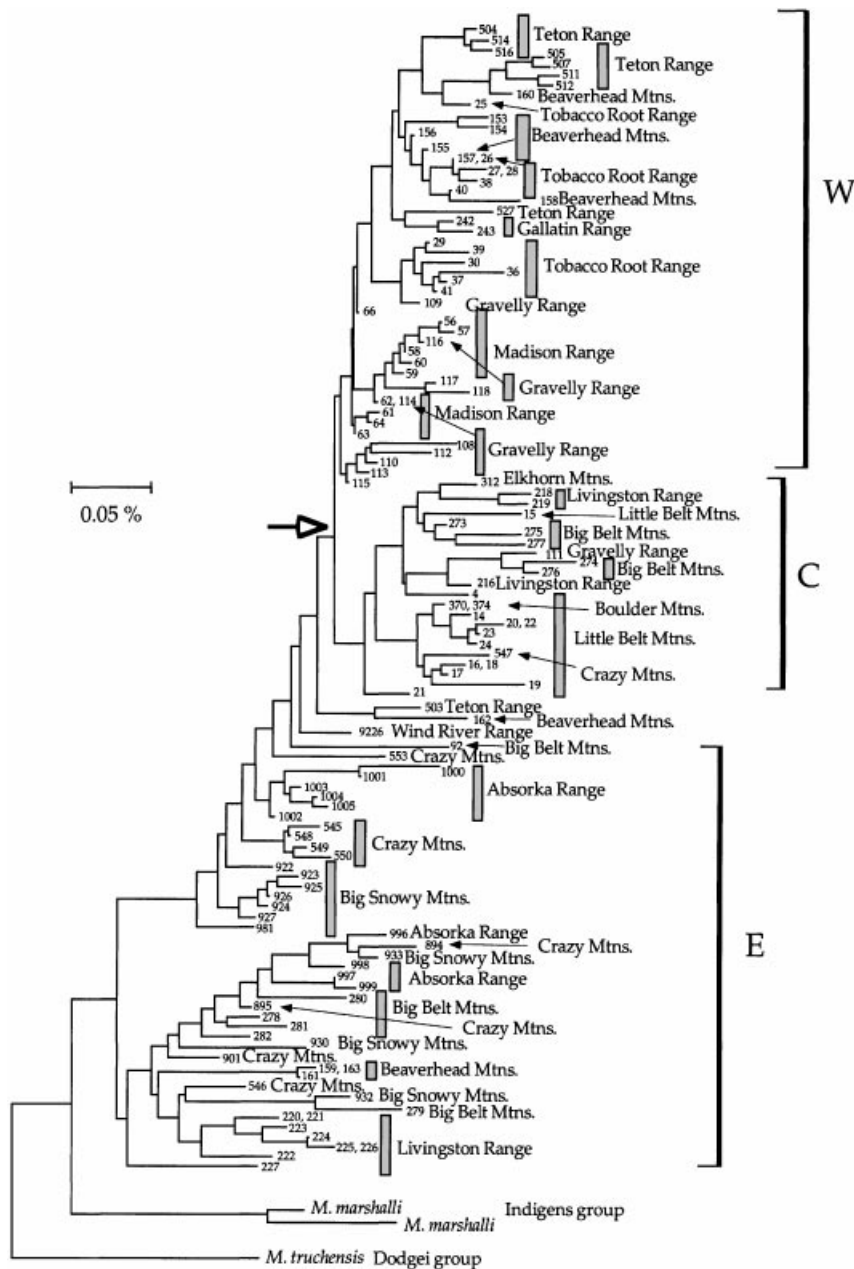
Fig. 4 Neighbour-joining tree with Kimura 2-parameter distances (Kimura 1980). Haplotype numbers and the population in which they occur are shown. Other than the rearrangement indicated by the open arrow, in which the regional group C occurred basal rather than sister to group W in the maximum-likelihood analysis, parsimony (CI = 0.97) and maximum-likelihood analyses gave very similar topologies with only minor differences in the relationships of some of the haplotypes within clades. Because these changes do not affect any of the conclusions, only the neighbour-joining tree is shown. Bars identify groups of haplotypes that are from the same population. Regional groupings marked E, C, and W are referred to in the text.

and Teton populations are closely related to those from the Tobacco Root mountains but are phylogenetically distant to haplotypes from the Absorka populations (Fig. 4).

In addition to the structuring of population genetic variation at the local geographical scale of individual sky islands, there also appears to be some regional phylogeographic structure (regional groups E, C, and W; Fig. 4). These regional groupings of haplotypes correspond roughly to populations of *M. oregonensis* from the eastern, central and western parts of its range (Fig. 5). However, none of the regional groups are monophyletic, and the haplotypes from some populations fall into more than one regional phylogeographic group (Figs 4 and 5). For example, several haplotypes from the Livingston and Big Belt mountains (identified by an asterisk in Fig. 5) are found in both the E and C regional groups. Likewise, while most of the haplotypes from the regional group W cluster with haplotypes from other south-western populations, a few haplotypes occur within the E and C regional groups (Figs 4 and 5). Despite this discordance, the coalescent simulations indicate that the observed regional phylogeographic pattern most likely reflects colonization of the sky-island populations from multiple allopatric refugial populations. The *s*-value from the reconstructed gene tree was
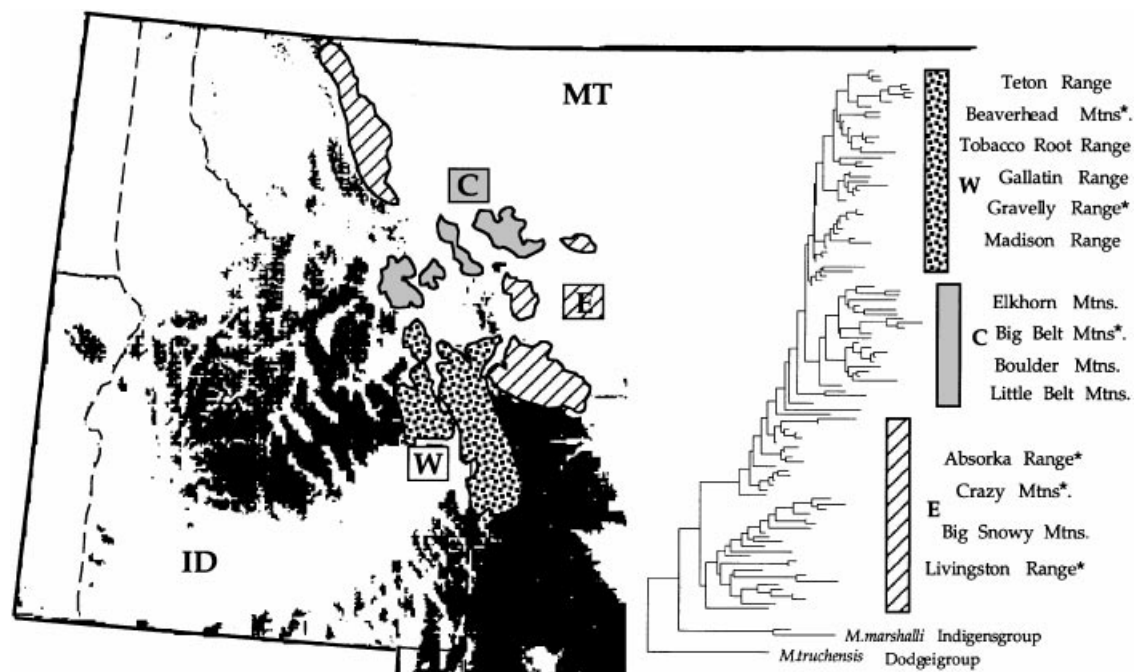
Fig. 5 Map of the sky islands and neighbour-joining tree showing the distribution of populations that correspond roughly to the E, C, and W regional phylogeographic groups. Populations marked with an asterisk contain haplotypes that occur in more than one regional phylogeographic group (see Fig. 4 for exact distribution of haplotypes). The Beaverhead and Gravelly mountains, as well as the Madison, Gallatin, and Teton ranges, are not delineated because they are not separated by any drops in elevation below 2000 m.

significantly lower than those values from the gene trees simulated by neutral coalescence, even when considering a range of different times of population splitting (Fig. 6). Thus, it is highly unlikely that the fragmentation of a widespread ancestor and the subsequent loss of gene lineages from some sky-island populations could have produced the observed geographical structuring of haplotypes.

Population genetic heterogeneity and diversity

Significant geographical structure in *M. oregonensis* is indicated by a highly significant F_{ST} of 0.46 ($P < 0.001$), which corresponds to the phylogeographic structure inferred from the genealogical reconstruction. Average pairwise divergences between populations range from 0.71 to 3.0% (Table 1). Variation among populations tends to be higher than variation within populations, averaging 1.90% and 1.01% sequence divergence, respectively, and consequently, most F_{ST} values among individual pairs of populations are also fairly high and significant (Table 1). However, there does not appear to be a relationship between F_{ST} values and geographical distances separating pairs of populations ($r = 0.27$, $P > 0.05$). For example, the F_{ST} value for the geographically proximate populations from the Tobacco and Gravelly Ranges is 0.32, whereas a lower and non-significant value of 0.19 was estimated for the much more geographically distant populations from the Crazy and
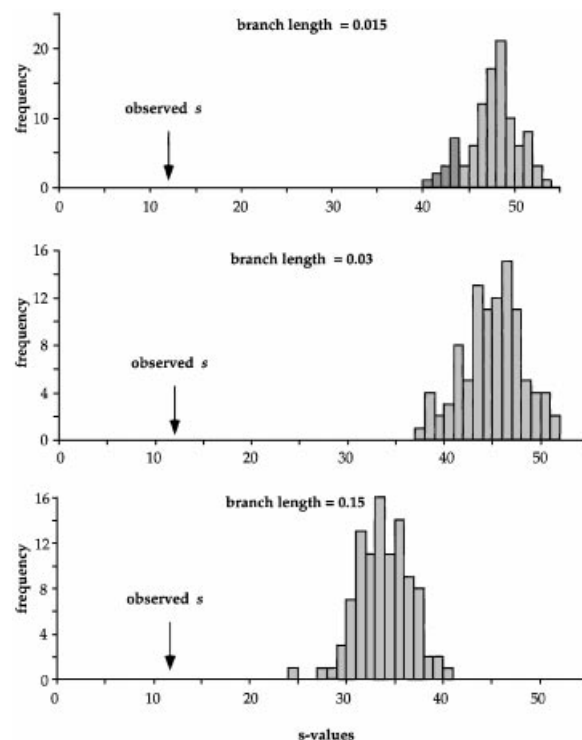

Fig. 6 Comparison of the *s*-value from the reconstructed gene tree to the expected distributions of *s* from gene trees simulated by neutral coalescence over a range of different times of population splitting (measured in generations).

Table 1 Cytochrome oxidase I (COI) sequence heterogeneity within and between populations of *Melanoplus oregonensis* (excluding populations in which fewer than 10 individuals were sequenced). Along the diagonal (italicized) are within-population, average pairwise sequence diversities. Above the diagonal are between-population values. Below the diagonal are estimates of F_{ST} from pairwise population comparisons. Significant F_{ST} values, $P < 0.05$ adjusted for multiple comparisons using a Bonferroni correction (Sokal & Rohlf 1995), are in boldface

	1	2	3	4	5	6	7	8	9	10	11
1. Teton Range (10)	*1.371*	2.597	1.651	2.204	1.731	1.988	2.583	2.712	2.054	2.942	3.003
2. Absorka Range (10)	**0.39**	*1.757*	2.153	2.394	2.596	2.304	1.957	1.862	2.481	2.349	2.174
3. Madison Range (10)	**0.49**	**0.52**	*0.309*	1.587	0.771	0.708	2.069	2.065	1.108	2.474	2.418
4. Beaverhead Mtns. (11)	**0.23**	0.21	**0.26**	*2.019*	1.740	1.901	2.373	2.348	2.091	2.647	2.457
5. Tobacco Root Range (12)	**0.40**	**0.53**	**0.36**	**0.22**	*0.674*	1.204	2.473	2.521	1.358	2.811	2.832
6. Gravelly Range (11)	**0.41**	**0.41**	0.10	0.21	**0.32**	*0.953*	2.224	2.187	1.374	2.542	2.581
7. Crazy Mtns. (10)	**0.36**	0.06	**0.46**	0.17	**0.48**	**0.36**	*1.889*	1.797	2.260	2.390	1.999
8. Big Snowy Mtns. (10)	**0.46**	0.11	**0.55**	0.24	**0.56**	**0.43**	0.04	*1.527*	2.264	2.284	1.957
9. Little Belt Mtns. (12)	**0.48**	**0.49**	**0.52**	0.33	**0.47**	**0.38**	**0.41**	**0.49**	*0.744*	2.522	2.523
10. Big Belt Mtns. (11)	**0.36**	0.12	**0.46**	0.17	**0.46**	0.35	0.11	0.15	0.18	*2.345*	2.261
11. Livingston Range (11)	**0.55**	**0.28**	**0.66**	**0.31**	**0.64**	**0.55**	0.19	**0.26**	**0.58**	0.18	*1.331*

Per cent sequence divergences among COI haplotypes corrected for multiple hits using Kimura 2-parameter model (Kimura 1980). Number of specimens sequenced given in parentheses.
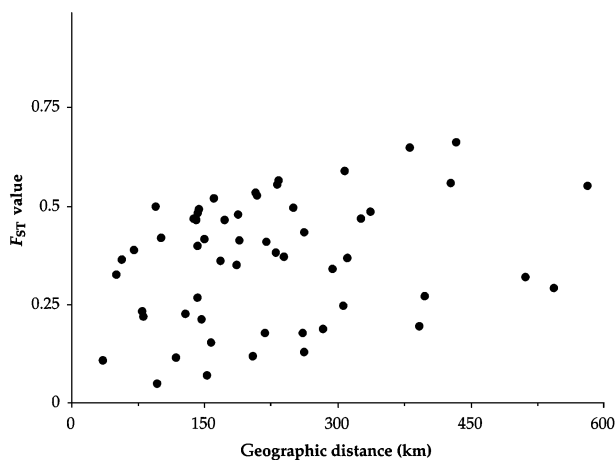

Fig. 7 Plot of the estimated F_{ST} values and corresponding geographical distance from pairwise population comparisons.

Livingston mountains. Furthermore, the variance in F_{st} values is fairly large and consistent across all geographical distances separating pairs of populations (Fig. 7).

In addition to the lack of evidence for isolation by distance, there also does not appear to be any latitudinal cline of decreasing genetic diversity in northern populations (as measured by the average pairwise divergences within populations, Table 1). Although the sky islands from the Livingston mountains were previously glaciated, the diversity from this population is not low, and is actually higher than four other populations (Table 1), including the southerly distributed populations from the Madison and Gravelly Ranges (Fig. 1).

Discussion

Effects of Pleistocene glaciations on population divergence

Contrary to the hypothesis that the Pleistocene glaciations inhibited divergence (e.g. Coope 1979; Zink & Slowinski 1995), *Melanoplus oregonensis* exhibits significant population structure. Moreover, there is no trend towards reduced diversity in northern populations or evidence that northern populations were established by successive founder events (i.e. no progressive isolation-by-distance) as has been described for other species that inhabit previously glaciated areas (e.g. Hewitt 1996, 1999), including taxa from northwestern North America (e.g. Cwynar & MacDonald 1987; Green *et al*. 1996; Conroy & Cook 2000). Instead, the effects of the glaciations on patterns of population variation in *M. oregonensis* are evident at two different geographical and temporal scales: recent divergences associated with the colonization of individual sky islands and historical divergences associated with displacement to multiple glacial refugia. Drift, rather than gene flow, appears to be the dominant force structuring genetic variation in *M. oregonensis*.

Many haplotypes within populations cluster together in the genealogical estimate (Fig. 4) and are not found in any other populations, suggesting they arose *in situ*. Furthermore, none of the haplotypes are geographically widespread. All shared haplotypes occurred between phylogenetically closely related populations or, in most cases, occurred within the same population (Fig. 4), indicating a lack of current gene flow. This is in accord with the natural history of the grasshoppers (i.e. *M. oregonensis* is flightless and restricted to isolated montane meadows). These results

indicate that the divergence associated with colonization of individual sky islands is drift dominated rather than gene flow. This conclusion is also supported by the F_{ST} analyses. Not only are many of the F_{ST} values between pairs of population significant (Table 1), but the substantial amount of variation in the F_{ST} values across all geographical distances (Fig. 7) is consistent with a drift dominated system (Hutchison & Templeton 2000).

Patterns of population differentiation in *M. oregonensis* also appear to reflect historical differentiation in multiple refugia (e.g. Conroy & Cook 2000; Mitton *et al*. 2000), in contrast to some other recently expanded populations (e.g. Zink & Dittmann 1993; Avise 1994). The coalescent simulations confirm that the discordance between the gene tree and the three regional geographical groups (Figs 4 and 5) is not consistent with the fragmentation of a single ancestral source population. Although this test assumes that the discord between the reconstructed gene tree and the multiple refugial population tree reflects the retention of ancestral variation, the discord could also result from migration between sky islands. However, the lack of evidence for gene flow as discussed above, and the observation that phylogenetically closely related populations are not necessarily geographically proximate, suggest that incomplete lineage sorting is a more likely explanation. While the data appear to be consistent with a three-refuge model, alternative models (e.g. a four-refuge model) need to be explored. Such models are currently being investigated, and include the possibility of using palaeoclimatological data to generate such models. Despite these limitations, the analyses do demonstrate that a multiple refuge model is more appropriate than a model of the fragmentation of a single ancestral source population.

Inferences about Pleistocene speciation

The intraspecific data in this study explicitly test models of evolution that have been postulated to lead to speciation (e.g. Mengel 1964; Hewitt 1996, 1999). These results are especially relevant given that *M. oregonensis* and its close relatives originated during the Pleistocene (Knowles 2000).

While the effects of drift associated with the recolonization of sky islands is obvious, its role in speciation is less clear. Founding of *M. oregonensis* populations, in general, does not appear to have been associated with extreme bottlenecks. Most population diversities are not very low (Table 1), and none of the sky-island populations are monophyletic (Fig. 4). This suggests that speciation by rapid drift-induced divergence such as postulated by founder-effect models (e.g. Mayr 1954; Templeton 1980; Carson 1982; Gavrilets & Hastings 1996) is unlikely. The lack of such a bottleneck at speciation has also been confirmed in eight *Melanoplus* species using coalescent simulations (Knowles 2001).

Similarly, divergence among refugial populations is evident in patterns of population variation, but what role these ancestral allopatric populations played in the formation of new species is somewhat equivocal. The lack of monophyly of the regional groups and the observation that some sky-island populations contain haplotypes that occur in more than one regional phylogeographic group indicates the potential for gene flow between refugial populations, or at the least, insufficient time for sorting of ancestral variation by drift. Neither interpretation is surprising considering the frequency of glacial cycles (Bartlein & Prentice 1989; Dansgaard *et al.* 1993), and the consequently short-time interval separating shifts in species distributions (Roy *et al.* 1996). However, the implication is that differences accumulated between refugial populations may be lost.

So what conclusions can be drawn? While the glaciations appear to have promoted divergence, neither drift during colonization of sky-island populations nor drift among allopatric, refugial populations appears to be sufficient by itself to generate new species. Nonetheless, *M. oregonensis* and related melanoplines underwent a radiation during the Pleistocene (Knowles & Otte 2000; Knowles 2000). Thus, some other mechanism, in addition to drift, must have contributed to the speciation process. In *Melanoplus* grasshoppers, that mechanism may very well be sexual selection (L. L. Knowles, in preparation). However, irrespective of the particular mechanism, one inescapable effect of the glaciations on species diversification had to have been the restriction of speciation to modes of divergence that involved the rapid evolution of reproductive isolation.

Conclusions

This study demonstrates that the Pleistocene glaciations did not inhibit divergence, and that both the colonization of sky islands and displacement to multiple glacial refugia has contributed to population divergence in *Melanoplus oregonensis*. When these results are considered in conjunction with previous work on the timing of species divergence (Knowles & Otte 2000; Knowles 2000), they suggest that for *Melanoplus* species originating during the Pleistocene, speciation did not simply coincide with the glaciations, but that the glaciations played an active role in diversification. However, these results also suggest that while drift among refugial populations or during the colonization of previously glaciated areas affects patterns of population divergence, some other mechanism must be invoked to explain the maintenance of the differences in the face of shifting population distributions. Consequently, this paper offers important insight about the effects of the glaciations on speciation.

Moreover, this paper demonstrates how explicit population models can be used to distinguish between alternative population histories (i.e. different models of Pleistocene

divergence). The gene-tree population-tree framework used here is not only statistically powerful (the coalescent simulations provide expected distributions for test statistics under specific population models), but it is also incredibly versatile, allowing a diversity of population histories to be modelled. The use of explicit population models promises to be an important development in the general field of phylogeography.

Acknowledgements

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This study complements my early work on the timing of species divergence in *Melanoplus* (Knowles 1999, 2000; Knowles & Otte 2000). I take an hierarchical approach in my research, studying both patterns of population variation and patterns of species splitting, and use a combination of phylogenetic and population genetic techniques to try to understand what factors contribute to divergence and ultimately speciation. In collaboration with Wayne Maddison, I am currently exploring how the structure of a gene tree can be used to test for drift in ancestral refugial populations.

Appendix I

Sampling localities with number of individuals sampled at each site (*n*). The geographical position of sample localities are indicated in Fig. 1

Species		*n*
Indigens group		
Melanoplus oregonensis	Wind River Range, Teton Co., Wyoming	1
	Teton Range, Rendevous Mtn, Teton Co., Wyoming	1
		0
	Gallatin Range, Teton Co., Wyoming	2
	Absorka Range, Beartooth Plateau, Carbon Co., Montana	1
		0
	Beaverhead Mtns., Sawtelle Peak, Fremont Co., Idaho	1
		1
	Madison Range, Lone Mtn, Madison Co., Montana	1
		0
	Gravelly Range, Madison Co., Montana	1
		1
	Tobacco Root Mtns., Madison Co., Montana	1
		2
	Crazy Mtns., Sweet Grass Co., Montana	1
		0
	Big Snowy Mtns., Fergus Co., Montana	1
		0
	Little Belt Mtns., Cascade Co., Montana	1
		2
	Big Belt Mtns, Meagher Co., Montana	1
		1
	Elkhorn Mtns., Jefferson Co., Montana	1
	Boulder Mtns., Jefferson Co., Montana	2
	Livingston Range, Glacier Co., Montana	1
		1
M. marshalli	Mt. Nebo, Wasatch Range, Juab Co., Utah	1
	Uinta Mtns., Summit Co., Utah	1
Dodgei group		
M. truchensis	Sangre de Cristo Mtns., Sante Fe Co., New Mexico	1