

Ice sheets promote speciation in boreal birds

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The premise that Pleistocene ice ages played an important role in generating present-day species diversity has been challenged by genetic data indicating that most of the youngest terrestrial species on Earth coalesced long before major glacial advances. However, study has been biased towards faunas distributed at low latitudes that were not directly fragmented by advancing ice sheets. Using mitochondrial sequence divergence and a molecular clock, we compared the coalescence times of pairs of avian species belonging to superspecies complexes from the high-latitude boreal forest with those of sub-boreal and tropical avifaunas of the New World. Remarkably, all coalescence events in boreal superspecies date to the Pleistocene, providing direct evidence that speciation was commonly initiated during recent glacial periods. A pattern of endemism in boreal superspecies plausibly links the timing of divergence to the fragmentation of the boreal forest by ice sheets during the Mid- and Late Pleistocene. In contrast to the boreal superspecies, only 56% of sub-boreal and 46% of tropical superspecies members coalesced during the Pleistocene, suggesting that avifaunas directly fragmented by ice sheets experienced rapid rates of diversification, whereas those distributed farther south were affected to a lesser extent. One explanation for the absence of pre-Pleistocene superspecies in boreal avifaunas is that strong selection pressures operated in boreal refugia, causing superspecies members to achieve ecological differentiation at an accelerated rate.

Keywords: boreal phylogeography; Pleistocene glaciation; speciation; glacial refugia

1. INTRODUCTION

The role of recent ice ages in vertebrate speciation is controversial (Zink & Slowinski 1995; Klicka & Zink 1997, 1999; Arbogast & Slowinski 1998; Avise & Walker 1998; Avise *et al.* 1998; Zink *et al.* 2004). Traditionally, Late Pleistocene glacial advances and their associated trends of global cooling were believed to have promoted speciation by fragmenting the geographical distributions of vertebrate faunas at both temperate and tropical latitudes (Mengel 1964; Haffer 1969; Hubbard 1974; Diamond & Hamilton 1980). Molecular investigations of temperate species have revealed numerous examples of phylogeographical structure concordant with segregation and subsequent genetic divergence in fragmented habitats during Pleistocene glacial cycles (Taberlet *et al.* 1998). Surprisingly few of these examples resulted in speciation (Drovetski 2003). Instead, the most recent speciation events in both temperate and tropical faunas, those involving present-day sister species, are now believed to have been initiated mostly before the Pleistocene, as indicated by their large values of sequence divergence (Klicka & Zink 1997; Avise & Walker 1998; Moritz *et al.* 2000). Although it remains true that speciation events initiated before the Pleistocene may have been completed during Pleistocene glacial events (Avise & Walker 1998; Avise *et al.* 1998), currently there is little evidence that many present-day species both diverged and speciated within Pleistocene glacial periods as originally held.

Recent studies have also failed to find support for increased rates of species proliferation during the Pleistocene (Zink & Slowinski 1995; Zink *et al.* 2004). Using a

series of North American sister taxa, Zink *et al.* (2004) found that rates of lineage accumulation through time best fit a model of constant diversification rather than indicating a recent burst of speciation during the Pleistocene.

These previous molecular investigations of ice-age speciation have studied faunas distributed largely to the south of the regions covered by the expanding and retracting ice sheets (Klicka & Zink 1997; Avise *et al.* 1998; Moritz *et al.* 2000). The geographical distributions of such faunas may have been fragmented periodically by habitat changes accompanying global cooling but not directly by advancing glaciers. The boreal forest of North America is a geographically extensive habitat zone that was directly fragmented by advancing glaciers into multiple refugia. To determine whether speciation was more prevalent near the advancing and retreating ice sheets we examined sequence divergence between members of avian superspecies complexes restricted to the boreal forest of North America. We compared these divergence events to those of North American superspecies not confined to, and distributed in habitats largely south of, the boreal forest and to those of neotropical lowland superspecies.

A superspecies is a monophyletic group of two or more allospecies (geographically allopatric species) or semi-species (species connected geographically by a narrow hybrid zone) that have just crossed the species threshold and are presumed to be the youngest species in an avifauna (Amadon 1966; Sibley & Monroe 1990). The ages at which superspecies members in an avifauna diverged give an approximation of the time frames needed for speciation to occur. If Pleistocene ice ages both initiated and completed the process of speciation in a fauna, then a high proportion of species belonging to superspecies in the fauna should date to glacial periods. Because the boreal forest was

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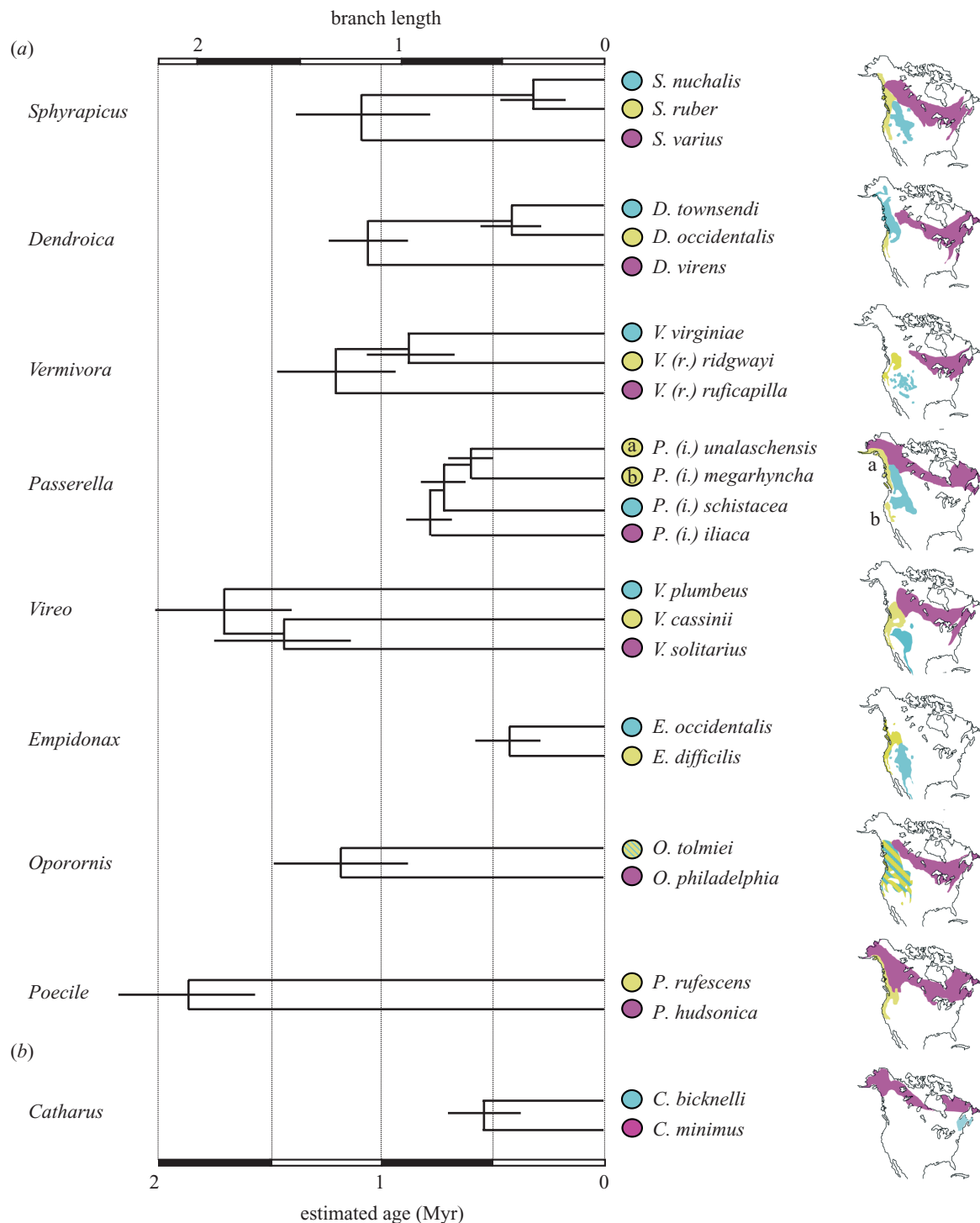


Figure 1. Cladograms derived from clock-like maximum-likelihood trees of superspecies complexes inhabiting the boreal forest zone. (a) Superspecies that contain two or more endemic taxa in the Taiga (magenta), Rocky Mountain (cyan) and Pacific Coast (yellow) regions of endemism. (b) The *Catharus minimus* superspecies displays a different geographical pattern of distribution. Standard errors are given for the dates of each node.

directly fragmented by expanding ice sheets whereas habitats farther south were not, we expect to find a higher proportion of superspecies dating to the Pleistocene in the boreal regions than in the sub-boreal and neotropical lowland regions.

2. METHODS

(a) Avifaunas compared

We compared the relative dates of origin for members of superspecies complexes in three avifaunas: the boreal forest avifauna,

the sub-boreal avifauna and the neotropical lowland avifauna (see electronic Appendix A, figure 4). The boreal avifauna here includes species restricted to boreal coniferous forests characterized by spruce (*Picea*), fir (*Abies*) Douglas-fir (*Pseudotsuga*), hemlock (*Tsuga*), cedar (*Thuja plicata*) or, more rarely, pine (*Pinus*), or to brushy habitat along the edges of these forests. The boreal coniferous forest biome stretches in a broad belt from Newfoundland to Alaska and south along the Pacific coast to California and along the Rocky Mountains into the mid-western USA and is

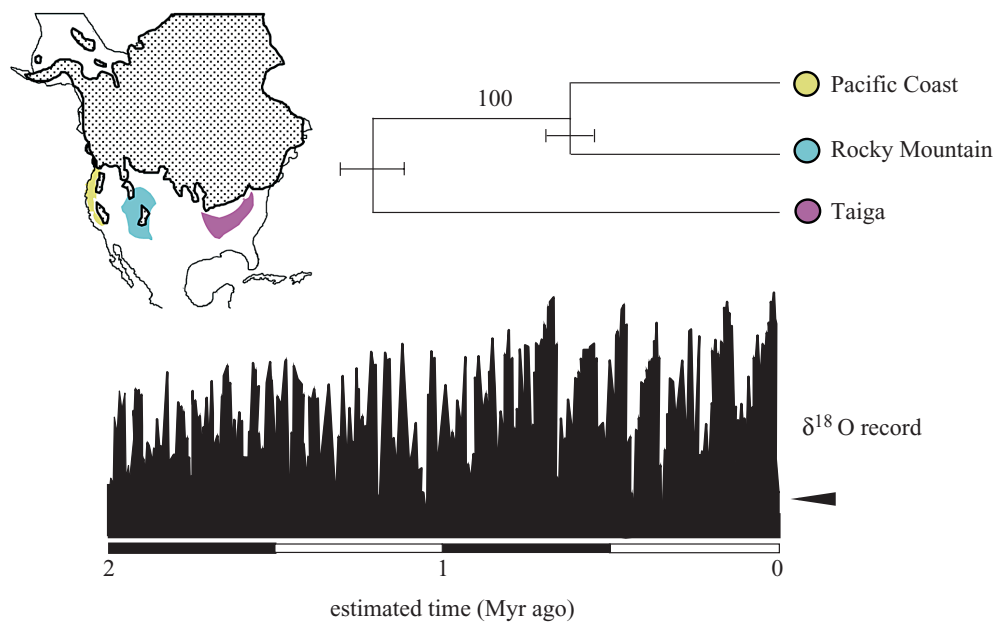


Figure 2. Consensus cladogram of boreal superspecies complexes, the Pleistocene $\delta^{18}\text{O}$ oxygen palaeotemperature record (redrawn from Barendregt & Irving 1998) and approximate distributions of boreal forest fragments during the last glacial maxima (Williams 2003; Lindbladh *et al.* 2003). Bootstrap support for the cladogram is indicated above the node, and standard errors are given for the dates of each node. All boreal forest superspecies were used to create the consensus cladogram except for the *Catharus minimus* superspecies, which displays a different biogeographical pattern. Peaks in the palaeotemperature record indicate cold periods of glacial advance, with seven major maxima occurring in the last 0.7 Myr. The present-day interglacial is represented by the black arrow.

present very locally at high elevations in northern Mexico. The sub-boreal avifauna includes species distributed in temperate habitats south of the boreal forest, including widespread species that extend their ranges up into the boreal zone but are not restricted to it. The neotropical avifauna includes species distributed in lowland habitats of tropical America from southern Mexico south to southern Brazil and Bolivia. Island endemics and oceanic and aquatic species were not included in any of the avifaunal comparisons.

We compared nine out of 10 boreal superspecies with a random sample of 20 sub-boreal and 21 neotropical superspecies. The boreal superspecies sampled are composed of 24 species, which represent a sizeable sample of the taxa restricted to the boreal zone. The only boreal superspecies that was not included was the alpine-inhabiting rosy-finch complex (*Leucosticte tephrocotis*), for which tissues were not available for genetic analysis. In addition, the boreal sparrows *Zonotrichia atricapilla* and *Z. albicollis* are believed to form a superspecies. Because mitochondrial DNA phylogenies do not support a sister-species relationship between these species, we excluded them from the analysis. Several boreal and neotropical lowland superspecies complexes contained a species distributed completely outside of their respective zones. Because we wished to compare only speciation events that occurred within each avifauna, we eliminated these taxa from our analysis.

Molecular phylogenetic studies have indicated that a number of traditionally held superspecies are not monophyletic and these have been excluded from this study. Such 'superspecies' do not represent taxonomic entities and their inclusion in this analysis would have the effect of creating artificially old dates. To be certain that superspecies were monophyletic, we included only superspecies from genera for which complete or nearly complete species-level phylogenies were available, or sister species whose relationship to each other is undisputed (i.e. they have been

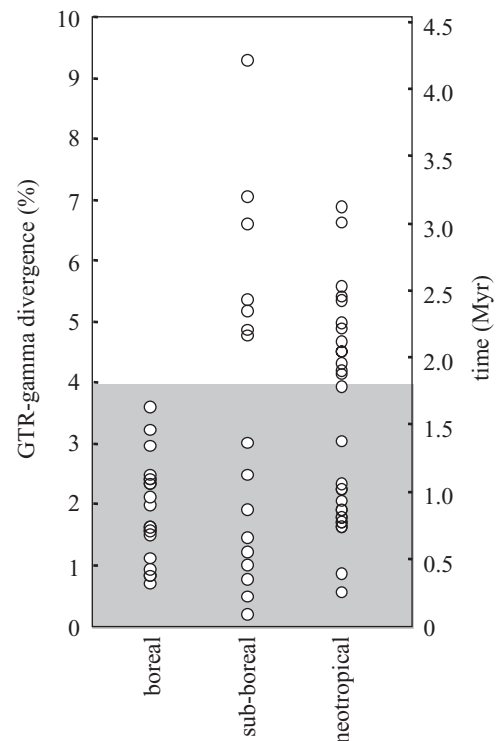


Figure 3. Genetic distances (GTR-gamma distances) and approximate dates of coalescence events between closely related species belonging to superspecies in boreal forest, sub-boreal and neotropical lowland avifaunas. The Pleistocene is indicated by the shaded area.

considered conspecific). Superspecies boundaries were taken from Sibley & Monroe (1990) and the checklist of North American birds (American Ornithologists Union 1998) with several

exceptions where recent phylogenetic studies challenged the boundaries of traditionally held superspecies. We consider *Piranga bidentata* and *P. ludoviciana* to represent a superspecies rather than *P. olivacea* and *P. ludoviciana*. We also treated the *Passerella iliaca* complex as a superspecies because it contains four genetically and morphologically distinct taxa that, although traditionally classified as a single species, have evolved a degree of reproductive isolation and are on the threshold of becoming distinct biological species (Zink 1994; Zink & Weckstein 2003). Excluding *Pa. iliaca* did not significantly alter our results.

(b) Sequencing and phylogenetic analysis

Cytochrome *b* was sequenced using standard protocols or obtained from GenBank and the literature for all superspecies and outgroups analysed (see electronic Appendix A, materials). Sequences from multiple individuals representing separate subspecies or geographical regions were included where available. To increase phylogenetic accuracy in the *Pa. iliaca* superspecies we added sequences of the mitochondrial ND2 (NADH dehydrogenase subunit 2) gene to the 450 bp sequences of cytochrome *b*. Both gene regions were evolving at similar rates. We performed all phylogenetic analyses in PAUP v. 4.0b10 (Swofford 2002). The general time reversible (GTR) model of sequence evolution (Kimura 1980) with among-site rate variation following a discrete gamma distribution (Yang 1993) was used in all maximum-likelihood analyses because this model provides a more realistic estimation of branch length and genetic distance, which are often underestimated by simpler models even for very recently diverged taxa (Arbogast *et al.* 2002). Parameters of the GTR-gamma model were estimated for the combined boreal, sub-boreal and neotropical dataset of 115 species ($-\ln$ likelihood = 23 146.72; $\alpha = 0.34$). GTR-gamma distances between species were generated using these parameters and were dated using molecular clocks.

The passerine molecular clock has been previously calibrated for Hawaiian honeycreepers (Drepanidinae) using gamma-corrected Kimura 2-parameter distances generated from 675 bp of cytochrome *b* (Fleischer *et al.* 1998). We used similar methods and the same dataset to calibrate this clock using GTR-gamma distances. Parameters for the GTR-gamma model of sequence evolution were estimated from a parsimony tree ($-\ln$ likelihood = 2651.58; $\alpha = 0.16$). A corrected divergence of 0.0352 was generated for the species pair *Hemignathus flavus* and *H. virens wilsonia* inhabiting Oahu and Maui, respectively. The latter species is believed to have colonized the island of Maui shortly after it formed 1.6 Myr ago (Fleischer *et al.* 1998). A minimum calibration of $2.2\% \text{ Myr}^{-1}$ (0.011 substitutions site⁻¹ lineage⁻¹ Myr⁻¹) is derived by dividing the gamma-GTR divergence by the age of Maui. An identical rate was obtained for the species pair *H. virens wilsonia* and *H. v. virens*; the latter is thought to have colonized Hawaii from Maui shortly after its formation 0.43 Myr ago (corrected divergence = 0.0096).

Our calibration of the passerine molecular clock is slightly older than the traditional molecular clock of $2.0\% \text{ Myr}^{-1}$ (see note 11 in Klicka & Zink (1997)). Most attempts to calibrate the molecular clock have used simple models of sequence evolution, which underestimate the true number of substitutions separating species pairs (Arbogast *et al.* 2002). The GTR-gamma model that we used corrects for homoplasy in sequences and provides a better estimate of pairwise divergence. We applied this passerine clock of $2.2\% \text{ Myr}^{-1}$ throughout this study. However, although many mitochondrial-clock calibrations for diverse groups of land birds seem consistent with a rate of *ca.* $2\% \text{ Myr}^{-1}$ (Klicka & Zink 1997),

a global molecular clock consistent across avian orders may not exist. Excluding non-passerine taxa from our analysis did not significantly alter our results.

Maximum likelihood was used to generate clock-like phylogenies with the GTR-gamma model of sequence evolution for each boreal superspecies complex (figure 1). Each complex was rooted with one or more closely related outgroups (see electronic Appendix A, materials). Likelihood ratio tests (Huelsenbeck & Rannala 1997) failed to reject the molecular clock in all boreal analyses.

Owing to the presence of ancestral polymorphism in DNA sequences, the time at which a population splits into two (population divergence) often postdates the coalescence times of DNA markers, resulting in species being younger than they appear. Coalescence theory predicts that if daughter populations have each maintained the same mean effective population size as the ancestral population that gave rise to them then the levels of polymorphism within each daughter population can be used as an estimate of ancestral polymorphism. Using this approach, a mean correction factor of 0.18 Myr for birds was derived by calculating the mean intraspecific variation within present-day species (Moore 1995; Edwards & Beerli 2000). Although a large variance was associated with this value, it suggests that, on average, coalescence dates are only a few hundred thousand years older than the time at which population divergence occurs. While this correction cannot be used to adjust individual coalescence dates it provides a yardstick whereby mean divergence times for an avifauna can be approximated. Throughout this paper we report uncorrected dates.

3. RESULTS

Coalescence times for all members of boreal forest superspecies fall completely within the Pleistocene (figure 1), suggesting that habitat fragmentation associated with recent ice ages played a major role in initiating speciation. A pattern of endemism is shared by eight out of the nine boreal superspecies (the *Catharus minimus* superspecies did not conform to this pattern). These eight superspecies each have two or three differentiated species presently restricted to the Eastern Taiga, Pacific Coast and Rocky Mountain regions of the boreal zone. Phylogenetic analysis of these superspecies revealed similar branching orders in cladograms (figure 1). The taxa inhabiting the Rocky Mountain and Pacific Coast endemism regions tend to be most closely related, and the taxa endemic to the Taiga tend to be basal. Dates of divergence in each of these complexes are similar, assuming that the molecular rate of evolution is approximately the same in all lineages. The regularity of the timing and order of splitting events in so many clades points to a common basis in range fragmentation as the initial cause of speciation events.

To explore the potential role of glaciation in forming this pattern of endemism, a consensus cladogram was generated by creating a clock-like maximum-likelihood tree with molecular sequences from each superspecies, complex combined (excluding the *C. minimus* superspecies, which does not display this pattern of endemism). This was achieved by combining a single DNA sequence from each species within an endemism region into a composite sequence. If a representative of a superspecies was not present in an endemism region then a series of *n*'s of appropriate length was inserted to represent the missing

sequence. Composite sequences were generated for each of the Tiaga, Pacific Coast and Rocky Mountain endemism regions with representatives of each superspecies added in the same order. Character weights were assigned to each base pair such that sequences from each species contributed equally to the cladogram. This was necessary because sequence length varied between 711 and 1463 bp between different species. A consensus cladogram of 100 bootstrap replicates was generated with the gamma-GTR model. If a single vicariant history affected each superspecies complex in a similar fashion then a high bootstrap support in the combined analysis would be generated. This method is similar to creating an area cladogram (Page 1988), except it assigns the actual sequence data to geographical areas and thus provides branch-length information, which can be used to date divergence events.

The resulting consensus cladogram was robust with 100% bootstrap support for the initial segregation of the boreal forest avifauna into eastern and western fragments and later divergence of the western fragment into Pacific Coast and Rocky Mountain refugia (figure 2). Randomly choosing a second set of sequences from each species did not alter the results. Dates of branching events on the consensus cladogram suggest that the earlier coalescence event in these complexes occurred on average 1.20 Myr ago (± 0.10) and the later 0.61 Myr ago (± 0.07). These approximate dates are based on the rate of 2.2% Myr⁻¹ calibrated for Hawaiian honeycreepers (see § 2b). Applying the traditional molecular clock of 2.0% Myr⁻¹ does not change these results greatly. These coalescence times are expected to be slightly older than actual population splitting times (see § 2b).

Coalescence times for boreal birds have a very different distribution from those for birds from North American sub-boreal and neotropical lowland regions (figure 3 and electronic Appendix A, tables). In contrast to boreal superspecies members, which coalesce exclusively during the Pleistocene (mean = 0.90 \pm 0.4 Myr ago), superspecies from sub-boreal and neotropical avifaunas speciated throughout the Pleistocene and Pliocene, with mean coalescence dates of 1.75 Myr ago (± 1.36) and 1.89 Myr ago (± 0.97), respectively.

4. DISCUSSION

Estimated dates of splitting in the boreal avifauna correspond to important events of the Pleistocene ice age. The intensities and durations of Pleistocene glacial advances are recorded in the oxygen isotope record preserved in deep-sea foraminiferan deposits (figure 2; Barendregt & Irving 1998). A high oxygen δ^{18} to δ^{16} ratio in these deposits indicates environmental cooling. During the early Pleistocene, weak peaks in the δ^{18} palaeotemperature record indicate that glacial advances were initially mild and short in duration. During this period, major ice sheets were initially confined to the Pacific northwest, high arctic and Atlantic northeast and did not unite to form a single ice mass until the second half of the Pleistocene (Barendregt & Irving 1998). Consequently, it is likely that prolonged fragmentation of the boreal zone into eastern and western sectors occurred sometime during the Early to Mid-Pleistocene (1.8–0.8 Myr ago) when the intensities of glacial advances began steadily to increase (figure 2). This period corre-

sponds with the earliest splits observed in most boreal superspecies. As indicated by the δ^{18} palaeotemperature record, a series of major glacial advances southward began 0.7 Myr ago and continued to the Recent. These major glacial advances probably pushed the eastern and western boreal forest fragments farther south and divided the western forest fragment into Pacific Coast and Rocky Mountain fragments (figure 2). This corresponds to the later split in the lineages of most boreal superspecies. Although there is uncertainty in rates of molecular evolution and in phylogenetic trees, we suggest that this pattern of habitat fragmentation is responsible for the similarity in the branching patterns and timings of splits in boreal birds.

These splits persist in the molecular record even though there were multiple retreats and advances of the ice sheets over the subsequent 0.7 Myr, suggesting limited opportunity for excessive gene flow after the first major advance. Whether boreal populations reconnected as they expanded their ranges during the brief interglacials is not known, but it is probable, suggesting that speciation occurred in spite of repeated periods of secondary contact. It is unlikely that reproductive isolation evolved to completion during single glacial advances, because most sister-species pairs in the western sector of the boreal zone that currently come into contact still hybridize occasionally, even after 0.7 Myr. Though it is not known when reproductive isolation was achieved, the most recent glacial advances of the Late Pleistocene may have been instrumental in completing the speciation process for many boreal species.

Did Pleistocene ice ages promote speciation exclusively in high-latitude faunas fragmented by ice? Coalescence dates for many members of superspecies distributed in sub-boreal North America and in the neotropics also fall within the Pleistocene (figure 3), suggesting that fragmentation resulting from global cooling associated with glaciation may have promoted speciation in these avifaunas as well. However, in contrast to 100% of boreal superspecies, only 56% of sub-boreal and 46% of tropical superspecies coalesced during the Pleistocene. The remainder of the coalescence events in superspecies of these avifaunas date to the Pliocene and Miocene. Therefore, the key difference between boreal and other avifaunas is not that Pleistocene speciation was rare elsewhere but that such a high proportion of superspecies members in the boreal zone date to the Pleistocene rather than earlier.

The absence of boreal superspecies members that predate the Pleistocene is surprising given that the boreal biome existed in North America throughout the Miocene and Pliocene (Brunsfeld *et al.* 2001). Diversification did occur in the boreal zone prior to the Pleistocene. For example, the closest sister taxa to boreal superspecies (J. T. Weir, unpublished data) and the radiation of *Dendroica* warblers (Lovette & Bermingham 1999) date primarily to the Pliocene and early Miocene. However, in contrast to many sub-boreal and tropical species, these pre-Pleistocene boreal species have evolved beyond the status of superspecies. Three explanations for this pattern are as follows. First, the older dates associated with tropical superspecies may be an artefact of current uncertainty regarding species boundaries in the tropics. However, the superspecies in the sub-boreal North American avifauna were also older on average than those in the boreal avifauna, and taxonomic treatment of species between

these two avifaunas is comparable. Therefore we suggest that a taxonomic artefact does not fully account for the pattern, even though it may play a role.

Second, the frequency of hybridization and complete mitochondrial introgression across species boundaries may be higher in the boreal zone than in other avifaunas. This would have the effect of making the members of boreal superspecies appear artificially younger. Evidence of extensive introgression has been found in two species of boreal bird (Rohwer *et al.* 2001; Weckstein *et al.* 2001). Although it cannot be ruled out, we do not regard this explanation as the most likely one because introgression has also been observed in sub-boreal taxa (Gill 1997; Lovette & Bermingham 2001).

Third, the divergence of boreal superspecies members may be confined to the Pleistocene because the rate of evolution may have been faster in the boreal avifauna. This faster rate of evolution would result in boreal species leaving the taxonomic category of superspecies much sooner than species in sub-boreal and tropical avifaunas. Species leave the taxonomic category of superspecies when they become sufficiently differentiated ecologically that they become sympatric, or when they achieve sufficient distinctness in morphology and ecology (Amadon 1966; Sibley & Monroe 1990). The rate at which ecological differentiation evolves could have been accelerated in the Pleistocene glaciations, perhaps because of stronger divergent selection resulting from greater ecological opportunities in depauperate boreal habitats. By contrast, fewer ecological opportunities in the more species-rich sub-boreal and tropical habitats could have led to weaker divergent selection pressures and longer waiting times in the superspecies state. We feel that this is the most likely explanation for our findings, although further tests are needed.

The data also suggest, but do not confirm, that a greater proportion of lineages entering the Pleistocene produced new species in the boreal zone than elsewhere. In other words, Pleistocene speciation rates were highest in the boreal avifauna. Confirming this would require estimating speciation and extinction rates in each of these avifaunas, for which the phylogenetic information is presently insufficient. Nevertheless, a high fraction of all passerine songbirds restricted to the boreal forest belt have been shown by this study to trace their origin to the Pleistocene (at least 24 out of 82 species). This fraction is almost certainly lower in avifaunas further south.

These results support the view that Pleistocene climatic changes were a major factor in the initiation and completion of speciation of New World birds, but suggest that they had the greatest impact on the boreal avifauna. Apparently, the effect of the ice ages in promoting diversification was strongest near the edges of the advancing glaciers. The contrast between boreal birds and more southern avifaunas suggests that direct fragmentation by ice was more important in promoting diversification than the global climatic changes accompanying the advances. North American fishes show a similar trend: clades occupying formerly glaciated areas date to recent Pleistocene glacial periods (Bernatchez & Wilson 1998; Taylor & McPhail 1999), while clades distributed south of the glaciated regions are older and most often predate the Pleistocene altogether (Near *et al.* 2003). Furthermore, there is an indication that the fragmentation of the boreal forest affected mammals in

a similar fashion. Many boreal species of mammal have genetically differentiated populations in the eastern and western sections of the boreal forest (Wooding & Ward 1997; Arbogast & Kenagy 2001; Demboski & Cook 2001; Stone *et al.* 2002), the origins of which appear to correspond with the first glacial vicariant event in birds, but, unlike birds, few of these populations are known to be reproductively isolated. Studies of this variation in the reproductive isolation exhibited by avian and mammalian faunas should yield a greater understanding of the mechanisms of speciation following fragmentation.

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