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Historical Biogeography, Divergence Times, and Diversification Patterns of Bumble Bees (Hymenoptera: Apidae: **Bombus**)

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Abstract.—Bumble bees (*Bombus*) are a cold-adapted, largely alpine group that can elucidate patterns of Holarctic historical biogeography, particularly in comparison to the alpine plants with which they likely coevolved. A recently published molecular phylogeny of bumble bees provides uniquely comprehensive species sampling for exploring historical patterns of distribution and diversification. Using this phylogeny and detailed data on extant distributions, I reconstruct the historical distribution of bumble bees in a temporal framework, estimating divergence times using fossil data and molecular rates derived from the literature. The nearly comprehensive phylogeny allows assessment of the tempo of diversification within the bumble bees using lineage-through-time plots and diversification statistics, which have been performed with special consideration to confidence intervals. These analyses reveal movements of *Bombus* concordant with geographic and climatic events of the late Cenozoic. The initial diversification of extant bumble bee lineages was estimated at around 25 to 40 Ma, near the Eocene-Oligocene boundary 34 Ma, a period of dramatic global cooling. Dispersal-vicariance analysis (DIVA) predicted an Old World *Bombus* ancestor, with early diversification events largely restricted to the eastern Old World. The numerous intercontinental dispersal events occurred mostly in the direction of Old World to New World and North America to South America. Early movements from the Palearctic into the Nearctic most likely took place after 20 Ma and may have coincided with a period of Miocene cooling that gave rise to taiga habitat across Beringia. Subsequent dispersal between these regions is estimated to have occurred among boreal and tundra-adapted species mostly in the last 5 million years. Radiations are estimated in both Nearctic and Neotropical regions at ∼6 to 8 Ma and after 3.5 Ma, concordant with the opening of land corridors between the continents. [Alpine; bumblebees; Holarctic; lineage-through-time; molecular dating.]

Bumble bees (*Bombus* Latr.) are an excellent system for exploring historical biogeographic patterns of cold-adapted, alpine, or Holarctic groups. A long history of taxonomic research on *Bombus* has resulted in relatively well-defined species, with descriptions of currently accepted species plateauing over 50 years ago (Williams, 1998). The distributions of the species are also relatively well documented and encompass a wide geographic range across the cool temperate and cold regions of the Holarctic and beyond, including Eurasia, North America, Africa north of the Sahara, and much of South America (Williams, 1998). Although distributional patterns of extant bumble bees suggest possible scenarios for historical biogeography and fossil evidence provides approximate dates for these events, estimation of the ancestral biogeographic distributions of *Bombus* clades and the temporal framework of such divergences are best examined using a robust phylogeny. Much effort has been invested in resolving the branching patterns of species within *Bombus* (e.g., Plowright and Stephen, 1973; Ito, 1985; Williams, 1985, 1994; Pedersen, 2002; Cameron and Williams, 2003; Kawakita et al., 2004; Hines et al., 2006). This research has culminated in the recent publication of a comprehensive phylogeny including 218 taxa of the ∼250 species based on five genes (Cameron et al., 2007). This nearly complete species sampling allows a more accurate assessment of historical patterns and provides multiple well-supported clades for assessing temporal trends in biogeography and diversification.

Bombus Distributional Patterns

Cold climates have been instrumental in the evolutionary history of bumble bees (*Bombus*). These robust, hairy bees have thermoregulatory adaptations involving facultative endothermy (Heinrich, 1979) that enable them to live in some of the highest-elevation insect-inhabited alpine ecosystems and reach high diversity in the arctic, subarctic, and boreal regions of the world. The primitively eusocial bumble bees are an abundant pollinating force in cool temperate and cold habitats. Accordingly, many high latitude and alpine flowers have a floral syndrome suited for the long tongues and learning-based foraging of bumble bees (Heinrich, 1979; Macior, 1982).

Bombus species richness is highest in the mountains of central China, is generally high in montane areas or cool temperate latitudes of both the Orient and Palearctic (∼175 Old World species), and declines in North America (∼60 species) and increasingly in South America (∼22 species), particularly outside of mountainous areas (Williams, 1994, 1996, 1998). Bumble bees have been able to recolonize and establish diverse communities across areas depopulated by glaciation events in the last 3 million years, including Scandinavia (∼38 species; Løken, 1973, 1984) and Kamchatka (∼12 species; Ito and Kuranishi, 2000). Their patterns of distribution are heavily influenced by vegetation zones and the temperature isotherms that shape them (Medler and Carney, 1963; Løken, 1973). Outside of cooler regions, exceptional species occur in warm desertic climates (e.g., *B. sonorus*, *B. morrisoni*) and in warm, wet tropical lowlands (*B. atratus*, *B. pullatus*, and *B. transversalis*). Species also vary in their habitat flexibility, with some widespread species able to occupy diverse habitats, such as from tropical lowlands to cool highlands (e.g., *B. ephippiatus*, *B. atratus*), whereas others have locally restricted regimes (e.g., *B. gerstaeckeri* is an oligolectic pollinator of *Aconitum*, Ponchau et al., 2006; *B. transversalis* is mostly restricted

to tropical lowlands, Moure and Sakagami, 1962; three of five *Alpinobombus* species are arctic distributed).

Fossil History

Bombus is the sole extant genus of the tribe Bombini, a member of the corbiculate bees, which also includes the primarily tropical orchid bees (Euglossini), honey bees (Apini), and stingless bees (Meliponini). The corbiculate bees radiated prior to 65 million years ago (Ma), the age assigned to the oldest corbiculate bee fossil (Grimaldi and Engel, 2005), and after the origin of the bees (Anthophila) at ~125 Ma (Danforth et al., 2006). Given that the corbiculate bees are a more apical clade within the bees (Danforth et al., 2006), they likely radiated tens of millions of years after the initial bee diversification. Accordingly, using paleontological and morphological evidence, Grimaldi and Engel (2005) estimated an 80- to 90-Ma corbiculate bee diversification. *Bombus* has been considered of early Cenozoic origin (Williams, 1985) based on early fossils described under the genus from the Paleocene and Oligocene, but these fossils have since been considered incorrectly or unreliably placed (Engel, 2001; Zeuner and Manning, 1976; Rasnitsyn and Michener, 1991). The only reliable *Bombus* fossils are compression fossils dating to the Miocene (Cockerell, 1931; Rasnitsyn and Michener, 1991) and include *Bombus luianus* (Zhang, 1990) from the Shandong Province in China (15.5 Ma; cf. Moreau et al., 2006), *Bombus vetustus* (Rasnitsyn and Michener, 1991) from Pacific Russia (Upper Miocene; <14 Ma), and *Bombus proavus* (Cockerell, 1931) from the Miocene Latah Formation in Washington, USA (~12.1 to 21.3 Ma; Gray and Kittleman, 1967).

Diversification Rates

As species-level phylogenies become increasingly resolved and complete, they provide opportunities to more confidently explore the tempo of diversification (Barraclough and Nee, 2001). Using dated phylogenies, fluctuations in the rate of diversification can be visualized by plotting the log of the number of lineages accumulating across time (Nee et al., 1992, 1994). Statistics, such the gamma statistic of Pybus and Harvey (2000) and the survival-based models of Paradis (1997) have been developed to test whether and how these plots deviate from a constant rate of diversification. Lineage-through-time (LTT) plots and their associated statistics have been used to reveal evolutionary associations, such as the simultaneous radiation of ants with the angiosperms (Moreau et al., 2006) and the radiation of leaf beetles (Chrysomelidae: *Cephaloleia*) during Paleocene/Eocene global warming (McKenna and Farrell, 2006). Yet, these plots and statistics have yet to undergo empirical scrutiny regarding their sensitivity to the conditions of analyses, including variation resulting from phylogenetic uncertainty and to changes in the data.

In this article, I examine the historical biogeography of bumble bees using the comprehensive species phylogeny of Cameron et al. (2007). Patterns of dispersal are placed in an environmental context using divergence time estimates derived from fossil and ecological information and molecular rates from the literature. The nearly complete species sampling also provides an appropriate framework to make reliable assessments of temporal patterns of diversification, which are analyzed for various clades of interest using LTT plots and diversification statistics, with the stability of these results tested across a body of nearly optimal trees and using a slightly modified data set. In combination, these data lead to a better understanding of the scenario in which the cold-adapted bumble bees evolved and radiated and provide an example of dispersal patterns among more recently evolving Holarctic and alpine groups.

MATERIALS AND METHODS
Phylogenetic Analysis

Comprehensive data set.—Divergence times, historical biogeography, and diversification statistics were estimated using the *Bombus* phylogeny and data set published in Cameron et al. (2007). This data set contains 218 taxa with unique names, including representatives of all 38 subgenera (Williams, 1998). A simplified subgeneric system for the bumble bees upon the 38 subgenera employed here and in Cameron et al. (2007) has been proposed (Williams et al., 2008). The sequence data include ~3745 amplified nucleotides, including both intron and exon regions from five genes: mitochondrial 16S rDNA, elongation factor-1α F2 copy (EF-1α), long-wavelength rhodopsin (opsin), arginine kinase (ArgK), and phosphoenolpyruvate carboxykinase (PEPCK). Missing species are mostly from the mountains of Asia and fall rather evenly across the subgenera (Cameron et al., 2007), with the exception of *Psithyrus* (9 of the 29 species missing from the phylogeny presented here) and *Mendacibombus* (5 of 12 species missing). Only 3 of the ~80 New World species were not sampled. Outgroup taxa represent the other corbiculate bee tribes, including two Euglossini, two Apini, and six Meliponini. These taxa provide the nodes necessary for fossil placement and encompass all major stingless bee clades, including the root node (Rasmussen and Cameron, 2007). This data set included binary characters assigned for the length-variable gap regions (Simmons and Ochoterena, 2000), including only those regions that were parsimony informative. To assess the influence of the resulting 126 gap characters, divergence time analyses and diversification statistics were also performed on the phylogeny obtained from the data set without coded gap characters.

From these data, Cameron et al. (2007) estimated phylogenies using mixed-model Bayesian analyses in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Because the biogeographic analyses require a fully bifurcating tree, the most likely Bayesian tree across the independent runs was used for the DIVA analyses and data presentation. A subset of trees was evenly drawn from all saved post-convergence Bayesian trees and used for determining divergence time confidence intervals and calculating diversification statistics.

Balancing clade distribution using a reduced data set.— Unequal taxon sampling can result in a bias of longer branch lengths in better represented clades (Fitch and Bruschi, 1987), so divergence time estimates were also obtained using a reduced taxon data set with a more balanced clade distribution between Bombini and the sister group Meliponini. This included sequences from the five genes for 16 *Bombus* taxa representing all of the major clades, 12 stingless bees (including sequences of 16S, EF-1α, opsin, and ArgK from 6 additional stingless bee species from Rasmussen and Cameron [2007] not included in Cameron et al. [2007]: *Axestotrigona ferruginea* voucher 428, *Meliponula bocandei*, *Melipona grandis*, *Odontotrigona haematoptera*, *Tetragonisca angustula*, and *Tetragonula fuscobalteata* voucher 194 [Fig. 1]), one Apis, and two Euglossini. Autapomorphic gap regions were excluded to reduce the missing data represented by gaps, and gap-coded characters were not included. Phylogenies were obtained using Bayesian analyses run in MrBayes for the five-gene reduced data set (2 independent runs, 3,000,000 generations, saving every 100 trees, the first 5000 of 30,001 trees removed for each run—a conservative measure of burn-in) and for each gene independently (2,000,000 generations, saving every 100 trees, burn-in = 5000). Individual genes were run to test the concordance of relative branch lengths and divergence times across independent data sets. The combined data set was also analyzed excluding 16S (same conditions) because of concerns regarding the impact of poor resolution and support among basal groups in the 16S tree on branch lengths. As with the large data set, the reduced data sets were run with flat priors, with each gene partitioned by introns and exons when relevant, and partitions were allowed to have variable rates under different models specified by the Akaike information criterion (AIC) in ModelTest (Posada and Crandall, 1998). Models included: HKY+G for opsin exons; HKY+I+G for the EF-1α intron; GTR+I for PEPCK exons and introns; GTR+G for opsin introns and ArgK exons; GTR+I+G for 16S, EF-1α exons, and the ArgK intron. In addition to Bayesian analyses, parsimony support for the node uniting the stingless bees and bumble bees was obtained by bootstrapping under parsimony criteria with 500 replicates, TBR branch swapping, and 10 random additions per replicate. *Tetragonula fuscobalteata* was pruned before divergence time estimation due to its unusually long branch length for ArgK.

Dating the Age of Bombus

Two different techniques were used to assess the most likely time of diversification of extant *Bombus* lineages. First, molecular rates derived from other divergence time studies were used to estimate the date of the *Bombus* root node. Second, calibration points were fixed on the tree and divergence times were estimated using local clock, relaxed-clock penalized likelihood (Sanderson, 2002), and Bayesian methods (Thorne and Kishino, 2002).

Rates from the literature.—The rates of evolution for gene fragments used in the *Bombus* data set were esti-

mated from other divergence time studies of social Hymenoptera, including estimates from opsin and EF-1α sequences of <100-Ma halictid bees (Brady et al., 2006) and from opsin sequences of <175-Ma ants (Moreau et al., 2006). These molecular rates were obtained by plotting the divergence times for each node from these studies against the corrected pairwise distances of taxa united at these nodes, and calculating the rate (slope) using a weighted regression (corrects for Poisson-distributed variance, constrained to run through the origin) of the data points. Corrected pairwise distances were calculated in PAUP* using models selected in ModelTest (Posada and Crandall, 1998). Because the data set was so large, only nodes supported by a posterior probability of 1.0 were used from Moreau et al. (2006). Dates used from Moreau et al. (2006) were those based on the minimum dates for fossil calibration points (unpublished node dates obtained from C. Moreau) and dates from Brady et al. (2006) were those obtained when the root node was set at 80 ± 20 Ma.

Using these rates, the age of the *Bombus* root node was estimated from the average corrected pairwise distances of all *Bombus* species pairs united by the root node. Confidence intervals on the estimated root date of *Bombus* were obtained using the method of Hillis et al. (1996). This involved translating distance into number of substitutions, obtaining a 95% CI on the number of substitutions for a Poisson distribution, translating back to pairwise distances, and converting these distances to dates using the regression equation.

Using calibration points.—The second technique used for divergence time estimation involved fixing calibration points on the trees. Reliable *Bombus* fossils are too poorly preserved to reveal good morphological synapomorphies for placement within *Bombus* lineages. Although a minimum of 15 Ma could have been placed on the root node, it is uncertain whether these fossils diverged before or after the ancestor of extant lineages. Therefore, focus was placed on outgroups for calibrating a relaxed molecular clock. The node uniting *Liotrigona* and *Hypotrigona* was fixed at 44.1 Ma, the age of the Baltic amber fossils *Liotrigonopsis rozeni* Engel and *Kelneriapis* Engel, which are allied to these morphologically similar genera (Engel, 2001; C. Rasmussen, personal communication) and likely placed near the split of these genera. The node uniting *Plebeia* and *Trigona* was constrained to be a minimum of 15 Ma based on 15- to 20-Ma (Iturralde-Vinent and MacPhee, 1996) Dominican amber fossils of *Proplebeia* that belong within this clade (Camargo et al., 2000). The stingless bee *Cretotrigona prisca*, the oldest corbiculate bee fossil estimated at ~65 Ma, is still of questionable phylogenetic placement. The two genera it has been allied with, *Trigona* (Michener and Grimaldi, 1988) and *Dactylurina* (Engel, 2000), share a common ancestor at the basal node of the extant stingless bees (Rasmussen and Cameron, 2007), and it is possible the fossil evolved earlier on the stingless bee branch. For this reason, this fossil was not included in the final divergence time analyses. When included in earlier analyses, the *Bombus* dates were similar, as the root node for the stingless
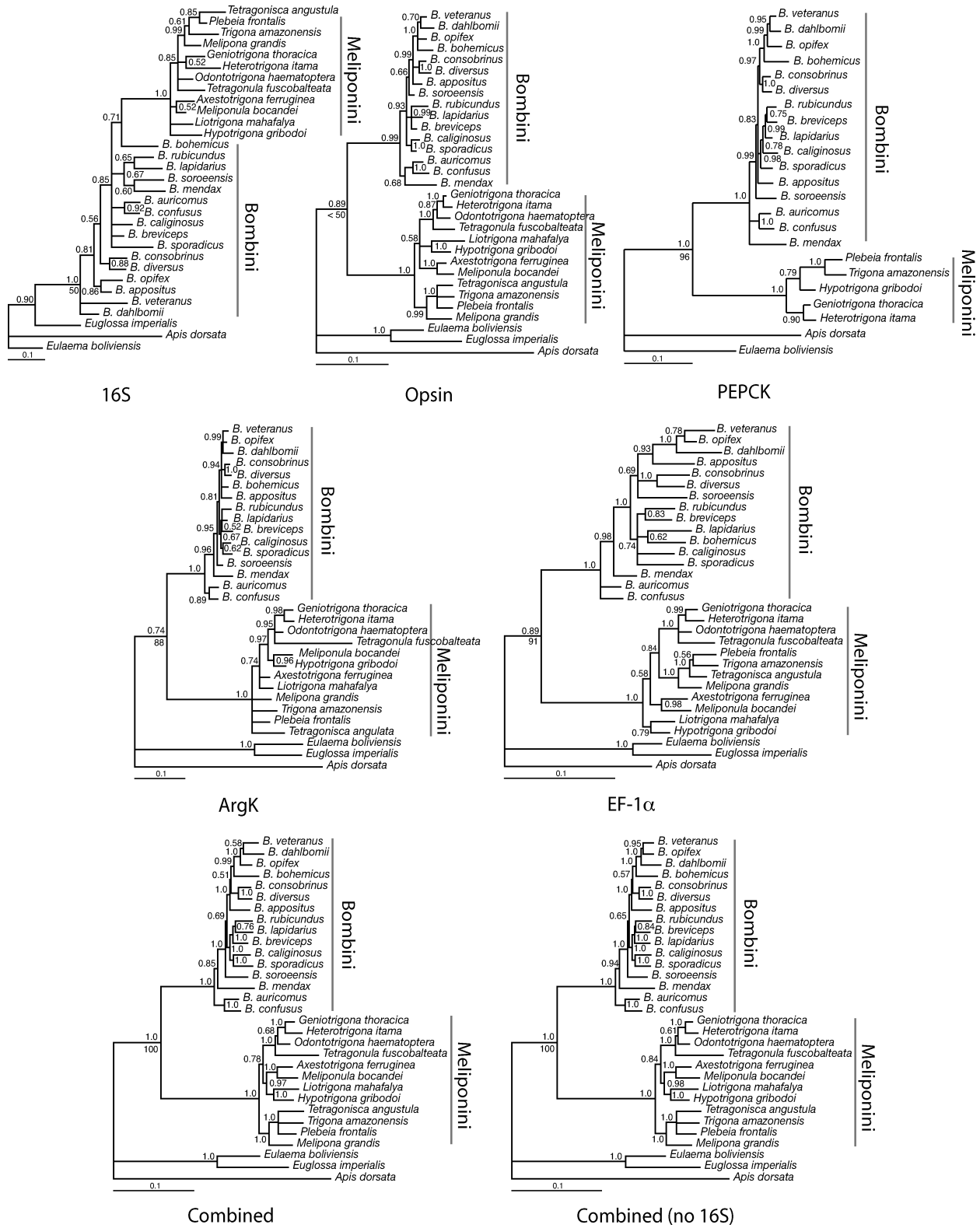
FIGURE 1.   Bayesian phylogenies generated from the reduced taxon data set for each gene separately, the combined data, and the combined data excluding 16S. These trees demonstrate the relative congruence of branch lengths across independent data sets.

bees is only slightly more recent using the remaining fossils.

Remaining calibration points involve nodes uniting the corbiculate bee lineages. Relationships among the corbiculate bees have been somewhat controversial, as morphological and behavioral data tend to unite the highly eusocial Meliponini and Apini (Schultz et al., 2001; Cardinal and Packer, 2007), whereas molecular data support a sister relationship between Meliponini and Bombini (Cameron and Mardulyn, 2001). Such conflicts may be a product of a relatively rapid early radiation of extant corbiculate bee lineages (Lockhart and Cameron, 2001). Additional calibration dates are here assigned to the Bombini and Meliponini sister-group bifurcation, the relationship overwhelmingly supported by the molecular data used in the analyses.

Separate analyses were run with the node uniting Bombini and Meliponini fixed across a range of alternative ages. The 65-Ma stingless bee fossil *Cretotrigona* represents an absolute minimum date for this node. The maximum date is the inferred origin of the bees, ~125 Ma. Therefore, separate analyses were run with the split between the Meliponini and Bombini set at 65, 70, 80, 90, 100, 110, or 125 Ma. Given that the stingless bees and bumble bees represent a highly derived divergence in bee evolution, the Bombini-Meliponini root node is likely considerably younger than 125 Ma. Because the 65-Ma fossil looks like an extant stingless bee and the extant stingless bee clade is separated from the node uniting Meliponini and Bombini by a considerable distance (Fig. 1), the date of this split is likely much older than 65 Ma. Therefore, the inferred most likely dates for the *Bombus* root node are derived from a Meliponini-Bombini split between 80 and 100 Ma.

Using these calibration points, relaxed clock methods were employed to smooth rates across the phylogeny in r8s v1.70 (Sanderson, 2003). This involved running penalized likelihood for all data sets (comprehensive gapped and ungapped, reduced, and individual genes separately) under a TN algorithm with a smoothing parameter set by cross-validation. Because a discrepancy in branch lengths was found between the stingless bee and bumble bee clades, a Langley Fitch local clock method was also performed in r8s treating each of the two clades under a separate rate. This was done in spite of both clades rejecting a molecular clock using a likelihood ratio test under the Langley-Fitch method in r8s for all analyses (except for the stingless bee clade for opsin; $P = 0.41$). Confidence intervals for penalized likelihood analyses were obtained using the profile command on 150 evenly spaced trees parsed from the converged Bayesian tree files. Finally, using the five-gene reduced data set and the same fossil calibration points, the divergence time estimates from r8s were compared to those acquired from the Bayesian dating method implemented in PAML/multidivtime (Yang, 1997; Thorne and Kishino, 2002) using standard procedures outlined in Rutschmann (2004). For this analysis the root prior for the Meliponini-Bombini split was fixed (constrained minimally and maximally ± 0.1 Ma from the date) at

either 65, 70, 80, 90, 100, 110, or 125 Ma. Apini and Euglossini outgroups were pruned from the trees for r8s and multidivtime analyses.

Biogeographic Analyses

Ancestral distributions were reconstructed on the phylogeny using dispersal-vicariance analysis in the program DIVA (Ronquist, 1996). This method reconstructs ancestral distributions on a phylogeny from current distributions by optimizing dispersal and vicariance events using a cost matrix. To address direction of movement between continents, DIVA was performed on species distributions categorized broadly into Old World, North American, and South American regions (Fig. 2). For examining movements within the continents, additional analyses were run with species assigned to the areas Palearctic, Oriental, Japan, Western Nearctic, Eastern Nearctic, Southern Nearctic, Northern Neotropical, Western Neotropical, Eastern Neotropical, and Southern Neotropical (Figs. 3 and 4). These regions are based on those defined by Williams (1996) using the TWINSPAN procedure, which in effect recognizes distinct areas of high *Bombus* endemicity. Distributions assigned to taxa for these regions were based on Williams (1998; available at http://www.nhm.ac.uk/research-curation/projects/bombus/regions.html). Taxa whose distribution only bordered on an area were not assigned to it. Taxa occurring in the Arctic are highlighted separately on the phylogeny (Fig. 2) to demonstrate patterns among the most cold-adapted taxa. To account for area proximity, the areas used in DIVA differed depending on the question. For example, to examine how taxa moved within North America, single regions for Old World and South America were used, and North American subregions were treated separately. This method was used for intracontinental movements in South America and the Old World as well. Results from all DIVA analyses were combined by assigning what broader region of the world the ancestor was in and then using the intraregional analyses to determine the more specific area of origin. For Old World analyses, DIVA was also run using two broader regions, Western Palearctic and E. Palearctic + Oriental (Fig. 3). Ambiguous area reconstructions were not distinguished from ancestors distributed across multiple areas, given that DIVA is likely to assign multiple areas when ancestry is ambiguous (Ronquist, 1996).

Diversification Patterns

The tempo of diversification was assessed using both the combined comprehensive gap-coded and non–gap-coded Bayesian phylogenies across several key groups of bumble bees: (1) all bumble bee species; (2) the short-faced bumble bee clade (Fig. 2); (3) the long-faced bumble bee clade (Fig. 2); (4) the socially parasitic subgenus *Psithyrus*; (5) the clades containing most of the New World species, including the subgenera *Pyrobombus* and *Fervidobombus* (excluding the *dahlbomii* lineage, which renders *Fervidobombus* non-monophyletic; duplicate representatives of species removed) and the "New
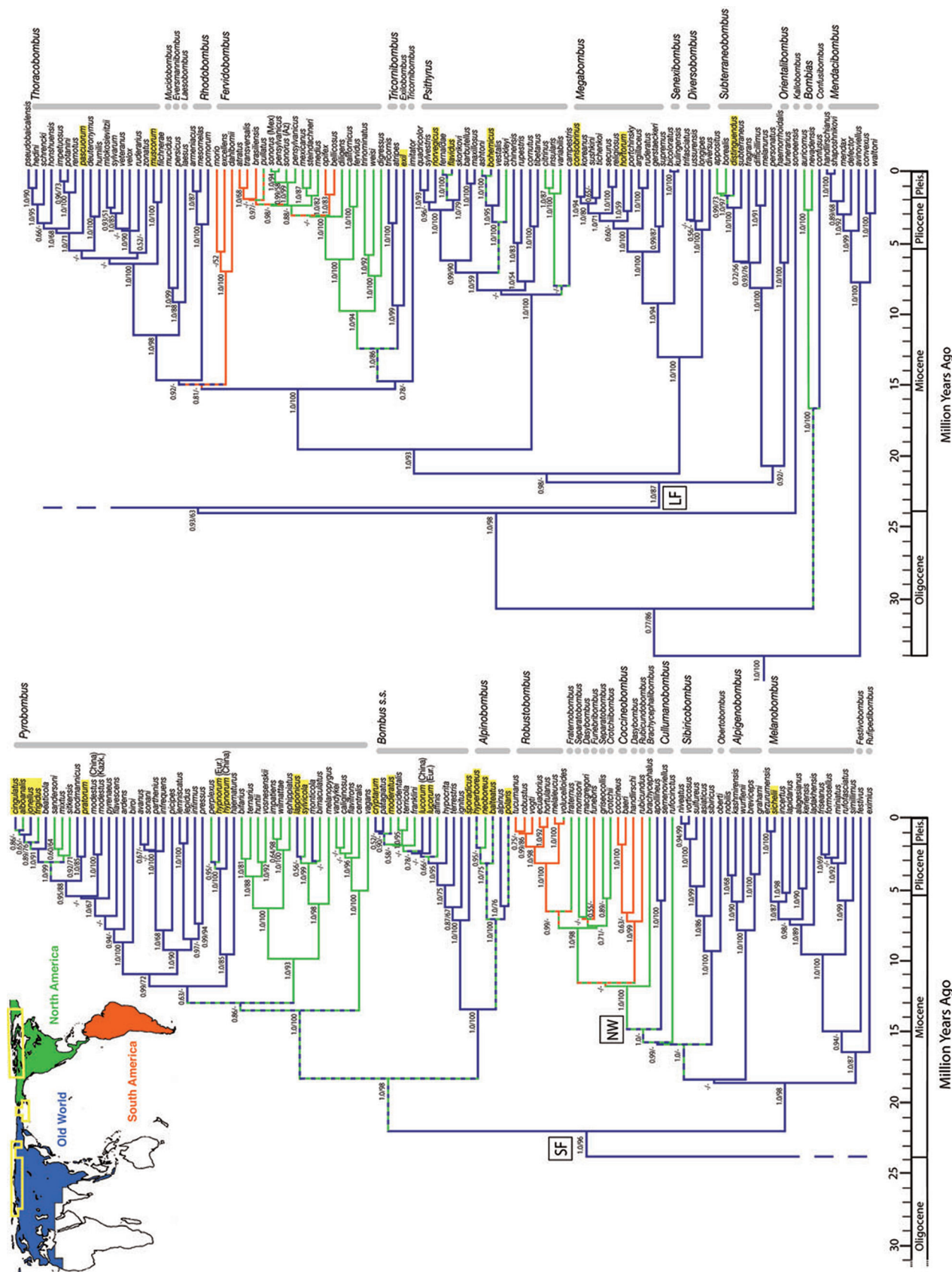
FIGURE 2. Chronogram (split into two parts; subgeneric groups indicated) for the full *Bombus* data set obtained in r8s, with extant and DIVA-reconstructed continental regions mapped onto branches. Multicolored branches are ambiguous or widespread for the respectively colored regions. Branches are colored based on the area reconstruction of the node they precede. Yellow highlighted species refer to arctic distributed taxa (see legend; defined based on Williams, 1996, and http://www.nhm.ac.uk/research-curation/projects/bombus/). Support values are Bayesian posterior probabilities from the majority-rule consensus tree followed by maximum parsimony bootstrap values. Dashes indicate clades with support values that are either less than 50% or support an alternative topology. SF = clade with mostly short-faced species; LF = clade with mostly long-faced species; NW = "New World" clade.

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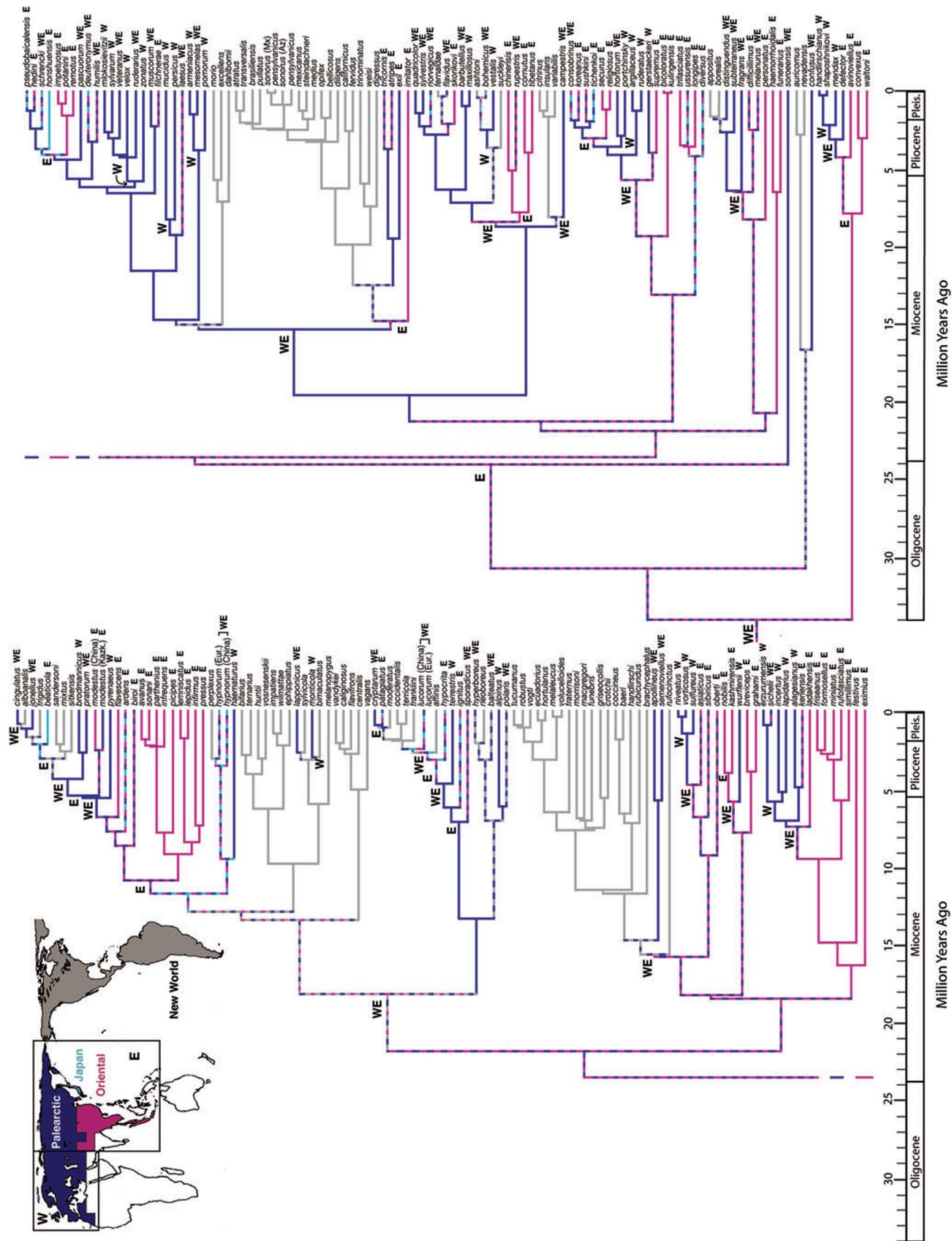
FIGURE 3. Chronogram highlighting Old World area reconstructions, including two ways of coding areas. Western and Eastern divisions are indicated by letters preceding nodes and apply to all areas after that node unless a new area is specified. Western/Eastern areas assigned to species are indicated to the right of the species name. Multicolored branches are colored based on the area reconstruction of the node they precede.
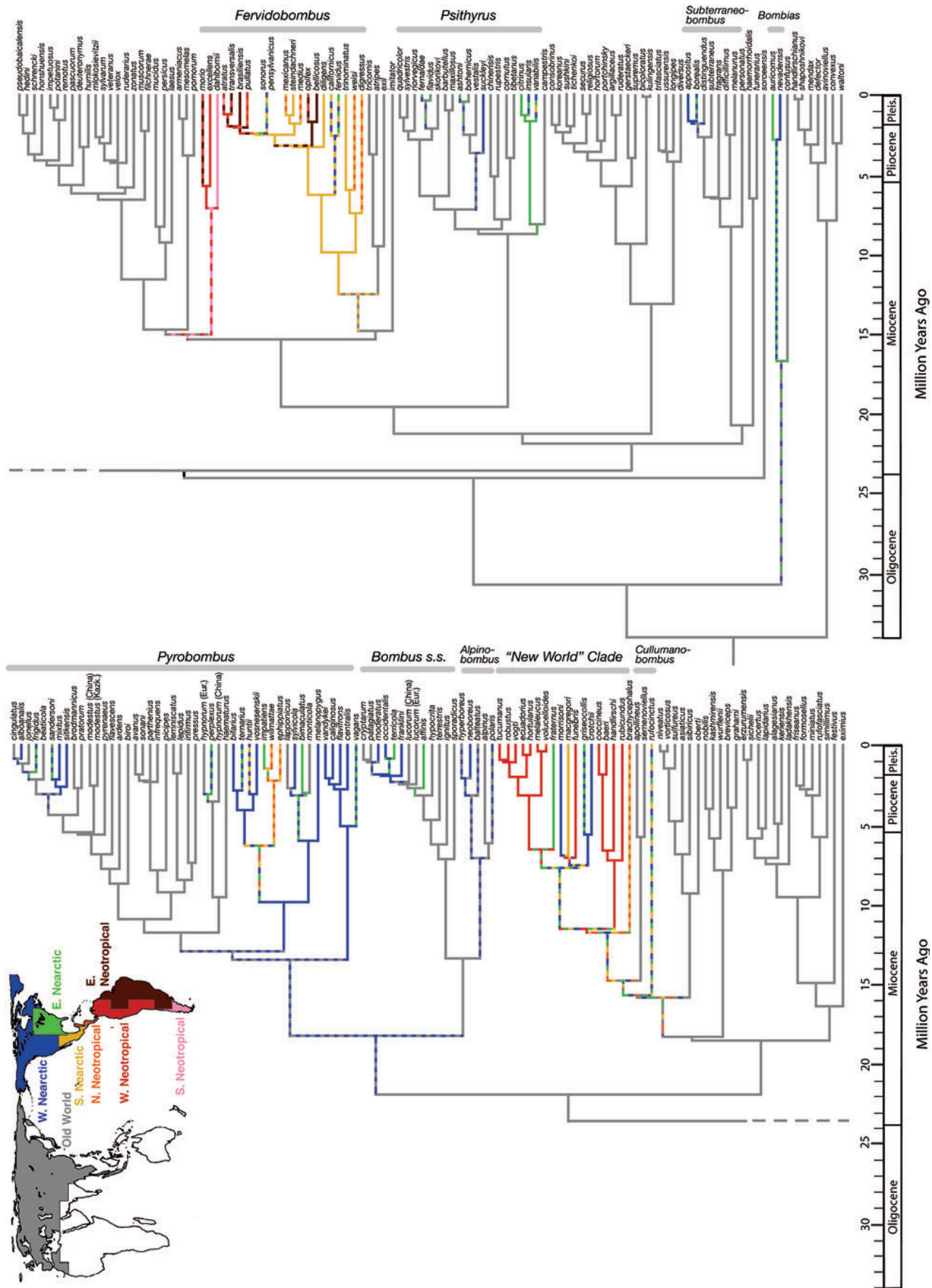
FIGURE 4. Chronogram highlighting New World area reconstructions. Multicolored branches are ambiguous or widespread for the respectively colored regions. Branches are colored based on the area reconstruction of the node they precede.

World" Clade (clade consisting of several exclusively New World species-poor subgenera; Fig. 1; Cameron et al., 2007); (6) New World species; and (7) South American species. Rate smoothed chronograms obtained from the Bayesian trees in r8s (same conditions as for divergence time estimations) were used to construct semilogarithmic LTT plots in APE v1.8-2 (Paradis et al., 2004). New World and South American regional LTT plots were constructed in Excel with the first splits in a new clade in these regions represented as a gain in two lineages.

Several diversification statistics were run on the rate-smoothed chronograms for each clade. To test whether diversification was constant as opposed to being concentrated near the base (declining rate of diversification) or the tips of the tree (increasing rate of diversification), the gamma statistic of Pybus and Harvey (2000) was employed, with adjustment for the number of missing taxa using their MCCR technique. This involved first obtaining a nonadjusted gamma statistic from the chronogram of a clade in APE. To adjust for missing taxa, the program Phyl-O-Gen (Rambaut, 2003) was first used to simulate 1000 trees using the default pure-birth parameters of the birth-death model and containing the actual number of species known from a clade. The number of species missing from the phylogeny were then pruned randomly from these trees. These simulated trees were then imported into the program GENIE (Pybus and Rambaut, 2002) to obtain the mean gamma statistic of the simulated trees (delta value). Whereas the simulated trees without missing taxa should have a mean gamma statistic of zero, the ones with missing taxa have some value less than zero, depending on the proportion of taxa missing. This negative value was then subtracted from the initial gamma statistic to obtain the adjusted statistic. An adjusted gamma statistic less than -1.645 indicates a significantly decreasing rate of diversification through time.

In addition to the gamma statistic, the fit of the chronograms to Paradis's (1997) three models of diversification was tested using the rather conservative (low type I error) hierarchical likelihood-ratio test executed in APE (AIC was used to compare models 2 and 3). The models of diversification include (1) a constant rate, (2) a variable rate through time (Weibull), and (3) two diversification rates split by a specified time point. For the third model, optimal time points for a shift in rate of diversification suggested by the LTT plot were tested (a recognized a posteriori bias). In none of these cases was the third model significantly better than model 1 or 2, unless the value was extremely near to the present (similar to McKenna and Farrell, 2006), which, given that populations were generally not sampled, may be an artifact of underrepresented population-level bifurcations. The gamma statistic was compared to the beta value of the Weibull model of Paradis (1997). Beta values >1 indicate declining diversification, whereas <1 indicates an increasing diversification rate. The mean rate of diversification across the clade was obtained from the delta value of the constant rate model of Paradis (1997) in APE. Both diversification pattern and rate statistics of Paradis (1997) assume com-
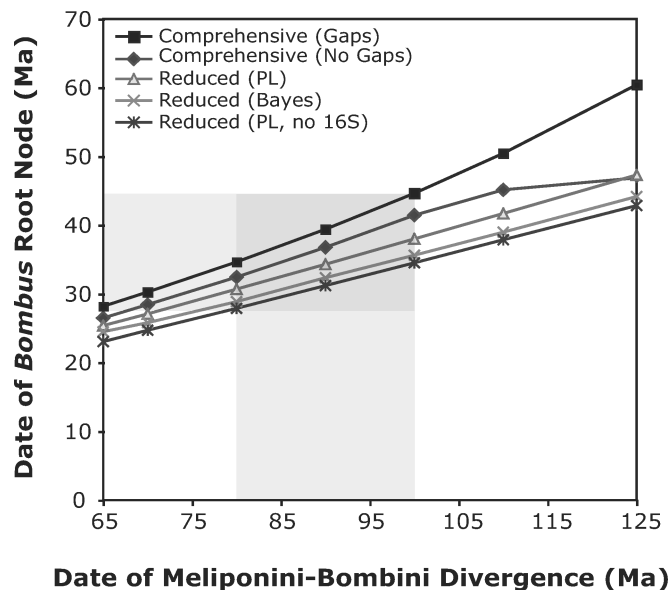

FIGURE 5. Summary of divergence time dates estimated for the *Bombus* root node for the primary data sets and types of analyses (Table 2) based on different calibration dates of the Meliponini-Bombini bifurcation.

plete taxon sampling, an assumption violated in this case to only a small extent.

To examine confidence in the resulting diversification patterns, confidence intervals for the statistics were obtained using 50 trees evenly parsed from the pool of converged Bayesian trees, and all 50 trees were plotted together in LTT plots. To assess the effects of slight data set modification, results were compared between gapped and ungapped data sets.

RESULTS

Phylogeny

The most likely Bayesian tree (Figs. 2 to 4) of the full data set is nearly identical to the majority rule Bayesian tree from Cameron et al. (2007), containing only seven topological differences. Meliponini is resolved as the sister group to the bumble bees in all analyses and is subtended by a particularly long branch relative to the total length of the bumble bee clade (Fig. 1). Relative branch lengths are similar across individual gene trees, providing a consistent signal for divergence time estimates (Fig. 1). As an exception, the 16S phylogeny is highly unresolved, with the stingless bee clade falling within the *Bombus* clade. In spite of this, the combined analyses with and without 16S yielded very similar relative branch lengths (Fig. 1). Trees and matrices are available in TreeBase (study accession number S1906; matrix accession numbers M3501 to M3509).

Divergence Times

Penalized likelihood analyses of the five-gene reduced data set estimated mean *Bombus* root node dates from 25.4 to 47.3 Ma (Fig. 5; Table 1) across the 65- to

TABLE 1. Age estimates of the *Bombus* root node. (a) Estimates using various data sets, calibrated based on Meliponini-Bombini split dates from 65 to 125 Ma and analyzed using penalized likelihood (PL), Bayesian (Bayes), or Langley-Fitch local clock (LF loc) methods. PL and LF local clock error values represent one standard deviation using 150 postconvergence Bayesian trees. The highlighted region indicates estimates inferred from a range of more likely Meliponini-Bombini split dates. (b) Molecular rates from the literature, including rates of opsin and EF-1α from Brady et al. (2006), and opsin sequences from Moreau et al. (2006) inferred using corrected (Corr. Dist.) distances. 95% Confidence intervals (95% CI) for opsin and EF-1α were obtained using the methods of Hillis et al. (1996).

Data set	65 PL (Bayes)	65 LF loc	70 PL (Bayes)	70 LF loc	80 PL (Bayes)	80 LF loc	90 PL (Bayes)	90 LF loc	100 PL (Bayes)	100 LF loc	110 PL (Bayes)	110 LF loc	125 PL (Bayes)	125 LF loc
(a) Meliponini-Bombini root age														
Comprehensive; no gaps	26.52 ± 2.84	26.16	28.44 ± 3.15	28.11	32.47 ± 3.90	32.07	36.80 ± 4.76	36.11	41.47 ± 5.72	40.15	45.17 ± 6.14	44.17	46.90 ± 13.41	50.19
Comprehensive; gaps	28.22 ± 3.10	26.90	30.28 ± 2.54	28.97	34.64 ± 3.04	33.14	39.40 ± 4.42	37.43	44.63 ± 4.33	41.31	50.45 ± 5.15	45.61	60.45 ± 6.51	51.76
Reduced; 5 gene	25.39 ± 2.43	25.60	27.12 ± 2.65	27.57	30.72 ± 3.06	31.50	34.32 ± 3.53	35.44	38.00 ± 3.97	39.37	41.71 ± 4.41	43.31	47.31 ± 5.08	49.21
	(24.50 ± 3.10)		(25.84 ± 3.14)		(28.88 ± 3.41)		(32.36 ± 3.75)		(35.65 ± 4.09)		(38.99 ± 4.44)		(44.19 ± 5.04)	
Reduced; 4 gene (no 16S)	23.07 ± 1.98	23.26	24.72 ± 2.14	25.02	27.91 ± 2.53	28.62	31.21 ± 2.90	32.2	34.54 ± 3.25	35.77	37.88 ± 3.54	39.36	42.84 ± 4.03	44.73
Reduced; EF1α	31.35 ± 4.78	32.02	33.62 ± 5.23	34.49	38.20 ± 6.12	39.42	42.90 ± 6.94	44.35	47.60 ± 7.76	49.27	52.29 ± 8.61	54.21	59.40 ± 9.82	61.57
Reduced; opsin	22.39 ± 6.55	21.57	23.97 ± 7.08	23.24	27.20 ± 7.92	26.56	30.36 ± 8.62	29.87	33.51 ± 9.03	33.18	36.42 ± 9.62	36.50	40.14 ± 10.95	41.49
Reduced; ArgK	24.06 ± 4.42	22.49	25.66 ± 5.21	24.20	28.72 ± 4.89	27.68	30.98 ± 5.31	31.11	33.17 ± 5.70	34.59	35.30 ± 6.16	38.05	38.31 ± 6.76	43.22
Reduced; PEPCK	19.28 ± 4.65	21.15	20.54 ± 5.00	23.10	23.07 ± 5.71	26.40	25.59 ± 6.42	29.70	28.12 ± 7.13	33.00	34.14 ± 9.17	36.30	46.03 ± 11.75	41.25

(b) Molecular rates

	Corr. Dist.	95% CI
Brady EF1α	18.18	13.31–28.76
Brady opsin	19.51	12.21–28.63
Moreau opsin	24.31	10.83–43.19

125-Ma Meliponini-Bombini root node calibration dates, whereas multidivtime analyses estimated a range of 24.5 to 44.2 Ma. Removal of the basally unresolved 16S from the combined data had little effect on the resulting dates (23.1 to 42.8 Ma). The comprehensive data set without gaps yielded nearly identical *Bombus* root dates to the reduced data set (26.5 to 46.9 Ma), which also contained no gaps, whereas the data set with gaps yielded somewhat older values (28.2 to 60.5 Ma), with the major noticeable difference when calibrating the Meliponini-Bombini split at 125 Ma. Results among individual genes were rather consistent, with EF-1α yielding the oldest estimates for the bumble bee root node, from 31.4 to 59.4 Ma, and PEPCK the youngest (19.3 to 46.0 Ma).

Using the more probable range for the date of divergence of the Meliponini-Bombini split, between 80 and 100 Ma, yields a *Bombus* root node between 27.9 and 44.6 Ma across combined data sets (Fig. 5; Table 1). The reduced five-gene data set, which yields intermediate and stable values among the data sets, suggests dates between 30.7 and 38.0 Ma for r8s and 28.9 and 35.7 Ma for Bayesian divergence time estimation using these calibration points.

Fossil calibration points in Meliponini, in the absence of any other date constraints, result in unrealistically old dates (>160 Ma) for the Bombini-Meliponini split because of the unusually long branch subtending the stingless bees (Fig. 1). Correcting for branch length discrepancy on the *Bombus* root node by using a local clock separating these two clades resulted in similar values to penalized likelihood for the *Bombus* root node across Meliponini-Bombini root dates (Fig. 5; Table 1).

Results for the *Bombus* root date using molecular rates derived from Brady et al. (2006) and Moreau et al. (2006) were similar, with means ranging between 18.2 to 24.3 Ma. Values calculated from Brady et al. (2006) and Moreau et al. (2006) seem low given that a reliable North American *Bombus* fossil dates between 12 and 21 Ma and bumble bees are estimated to likely have arrived in the New World approximately half way through their diversification. Nevertheless, molecular rates from the literature suggest bumble bees evolved at the lower end of dates estimated using calibration points.

The age of the Eocene-Oligocene boundary, a time of dramatic climatic cooling (e.g., Zachos et al., 2001) and faunal turnover (e.g., Hartenberger, 1998), is an intermediate age among the variables tested and is an optimal middle estimate for the origin of *Bombus*. Therefore, the root node of the bumble bees was fixed at 34 Ma for the remaining biogeographic and divergence time assessments. This involved excluding non-*Bombus* and obtaining divergence dates and a chronogram using penalized likelihood in r8s with the same conditions used for divergence time estimation.

Biogeographic Analyses

The DIVA analysis reconstructs an Old World bumble bee ancestor (Fig. 2) and an Old World distribution for most of the first half of *Bombus* evolution. Eighteen phylogenetically independent dispersal events are estimated to have occurred in the direction of Old World to New World, most likely starting after 20 Ma, and three movements are estimated to have occurred in the opposite direction (Fig. 2), restricted to the last 3 to 4 million years. Because the ancestral distribution of the *Bombias* + *Confusibombus* clade is uncertain, a New World introduction is estimated to date as old as 31 Ma, but, given the paucity of species in this clade, a later movement after 20 Ma is likely. Most dispersal events between the Old World and New World appear to have taken place among the clades containing the most cold-adapted species (Fig. 2; highlighted taxa), mostly in the last 5 million years. The clades that apparently do not involve dispersal in highly cold-adapted ancestors include those with deeper Old World to New World divergences, such as *Fervidobombus* (New World arrival ~10 to 15 Ma), the "New World" clade (12 to 18 Ma), the *Psithyrus* subgroup *Citrinopsithyrus* (2 to 9 Ma), and *Bombias* (3 to 31 Ma) (Fig. 2).

Dispersal from North America into South America appears as six phylogenetically independent events (Fig. 2) (coded only as North American here, the Mexican and Central American species *B. ephippiatus* and *B. mexicanus* have been rarely reported from Ecuador and Venezuela and could be considered two additional phylogenetically independent dispersal events; Labougle, 1990). The results suggest that arrival in South America may have occurred as long as 15 Ma, but the first speciation events (i.e., the earliest inferred ancestral nodes) in South America are estimated at ~7.5 Ma in the *B. dahlbomii* and *B. handlirschi* species groups (Figs. 2 and 6). Dispersal from South America to North America is estimated to have occurred only in *B. volucelloides*, which extends from the Western Neotropics into Central America.

The Old World subregion of origin, and the historical distribution for the first third of *Bombus* evolution, is ambiguous using Oriental and Palearctic regions (Fig. 3). With Old World subregions Western Palearctic and Eastern Palearctic + Orient, most of the earlier bifurcations of the tree after 25 Ma were reconstructed as Eastern Palearctic + Orient. Although considerable movement between regions of the Old World is apparent, some Old World clades are regionally cohesive, such as clades of Oriental *Melanobombus* and *Pyrobombus*, and a clade of Western Palearctic *Thoracobombus*. Other clades, for instance, most of *Pyrobombus*, *Bombus s.s.*, and *Psithyrus*, are distributed widely even between sister species and within extant species.

Estimated ancestral distributions within the New World suggest relatively rapid transcontinental movement (e.g., the "New World" clade; Fig. 4). The subgenera *Pyrobombus*, *Bombus s.s.*, and *Subterraneobombus* include Western Nearctic ancestors that subsequently spread to the Eastern or Southern Nearctic regions. However, the large *Fervidobombus* clade and *B. impatiens* were reconstructed as being derived from Southern Nearctic ancestors.

Diversification Patterns

The null model for a constant rate of speciation is an exponential increase in the number of lineages through time. Therefore, a straight line in a semilogarithmic plot suggests a constant rate of diversification through time, whereas a downcurving plot indicates a declining rate and an upcurve signifies an increasing rate of diversification (Nee et al., 1992, 1994). Given this, the LTT plot for all bumble bee species (Fig. 6a) reveals a slow lineage accumulation for the first 10 million years (24 to 34 Ma), followed by a higher relatively constant rate of diversification thereafter. The plot failed Paradis's (1997) test for a constant rate of speciation in favor of decline in diversification rate over time, a pattern most evident in the plot from 10 to 25 Ma, but was not significantly different from a constant rate using the adjusted gamma statistic. An upturn in diversification occurs after 10 Ma, perhaps reflecting an increase in speciation in the New World. If a constant rate of diversification were assumed, the diversification rate for bumble bees is 0.175 lineages/million years (Table 2). The diversification patterns of the long-faced and short-faced bumble bee clades are similar (their diversification rates are 0.193 and 0.166 lineages/million years respectively; Table 2, Fig. 6b). The long-faced clade is estimated to have diversified at a constant rate (although Paradis's, model suggests a marginally significant declining rate), whereas the short-faced lineage better fits a declining rate of diversification based on Paradis (1997) and the adjusted gamma statistic. Significantly declining diversification was also inferred for the subgenus *Pyrobombus* using the gamma statistic and Paradis models. All plots, including those of individual subgenera, yield negative gamma statistics and positive beta statistics, so there is an overall trend towards a decreasing rate of diversification through time (i.e., a down curving plot; Fig. 6, Table 2).

New World speciation events appear to have occurred in two spurts, one from 6 to 8 Ma and another after 3.5 Ma, with a lack of speciation between 3.5 and 6 Ma (Fig. 6d). This trend is also apparent in the plots of the major New World clades, including *Fervidobombus*, the "New World" clade, and *Pyrobombus* (Fig. 6c), and in exclusively South American speciation events (Fig. 6d). The parasitic subgenus *Psithyrus* appears in the LTT plot to have rapid lineage accumulation after its initial diversification ~9 Ma followed by a relatively constant rate (Fig. 6b). In spite of appearances, the plot satisfied the null hypothesis of a constant rate of diversification.

The birth-death model run in the APE program indicated that no extinction was occurring across data sets. Most phylogenies likely fluctuate in speciation and extinction rates temporally, particularly in clades evolving under such extreme climate change like *Bombus*. Nonuniform curves exhibiting fluctuations in speciation and extinction rates violate the assumptions of the diversification statistics, therefore making it difficult to rule out the role of extinction in explaining diversification patterns. This is complicated by the reduced ability to detect extinction compared to speciation in LTT plots (Kubo and Iwasa, 1995).

Few studies have explored the uncertainty in inferred diversification patterns introduced by phylogenetic estimation and variation in signal across data sets. In these analyses, the LTT plots and statistics were similar across sets of likely Bayesian trees, fluctuating roughly ± ((date)/8), and statistics (Table 2) and LTT plots differed only slightly between gapped and ungapped data sets. General conclusions from diversification analyses were robust to comparisons among gapped, ungapped, and the body of nearly equally likely Bayesian trees (Table 2). Nevertheless, tests run against independent data sets would be likely to yield more variation in diversification patterns.

DISCUSSION

The numerous biogeographic and climatic events that have occurred during the Cenozoic have made Holarctic historical biogeographic patterns particularly perplexing (Sanmartin et al., 2001). In addition to providing historical information on a well-studied economically and ecologically relevant group, the historical biogeographic and divergence time results for bumble bees provide a lineage-rich example of how a Holarctic group has responded to climatic events across the late Cenozoic. The inferred biogeographic movements and best estimates of divergence dates of the bumble bees (results summarized in Fig. 7) are concordant with several notable geographic and climatic events.

Old World Origin and Diversification

The timing of the origin of the extant *Bombus* crown clade is estimated to have most likely occurred from the late Eocene to mid-Oligocene (25 to 40 Ma). The cold climate distribution of bumble bees makes them particularly well suited for radiation within this time period. The global greenhouse conditions of the mid-Eocene declined up to 34 Ma, the Eocene-Oligocene boundary, when a plummet in global temperatures caused extinction and a dramatic turnover of organisms, with warm tropical taxa being replaced by cooler temperate flora and fauna. One such recognized faunal turnover at this time period is that of the European *Grande Coupure* (Hartenberger, 1998), where Asian fauna, which appear to have undergone a remodeling themselves around this time period (Meng and McKenna, 1998), moved into Europe and replaced European fauna. *Bombus* may have behaved similarly, as DIVA reconstructed an ancestor within the Old World, with many early *Bombus* diversification events occurring in the eastern Old World.

Williams (1985) proposed that the initial diversification of *Bombus* was centered on the mountains of Asia, particularly because they reach their highest species richness there and because the primarily Asian montane *Mendacibombus* was resolved in his morphological analysis as paraphyletic at the base of the tree (Williams, 1985, 1994). In the phylogenetic tree presented here, *Mendacibombus* is a monophyletic sister clade to all other bumble bees, and extant species are estimated to have diversified only in the last 8 million years. Therefore,

TABLE 2. Diversification rate statistics for each clade. Statistics were estimated using data sets with (Gap) and without (No Gap) gap-coded characters. Paradis (1997) statistics include the diversification rate (div. rate) assuming a constant rate, the LRT test for a constant rate of diversification (asterisks indicate a variable rate), and the beta statistic (assesses pattern of diversification, with >1 indicating a declining rate). Pybus and Harvey (2000) statistics include the gamma statistic testing for a constant rate of diversification with and without adjustment (adj.) for number of missing taxa (asterisks indicate a variable rate). Error values are one standard deviation using 50 evenly parsed converged Bayesian trees.

| Clade	Paradis (1997)			Pybus and Harvey (2000)	
	Div. rate	LRT P value	Beta	Gamma	Adj. gamma
All					
Gap	0.175 ± 0.011	$0.015 \pm 0.011^*$	1.143 ± 0.019	-1.083 ± 0.327	-0.327 ± 0.327
No Gap	0.163 ± 0.009	$0.012 \pm 0.009^*$	1.146 ± 0.016	-1.136 ± 0.270	-0.360 ± 0.270
Short-faced					
Gap	0.166 ± 0.006	$0.002 \pm 0.002^*$	1.294 ± 0.034	$-2.132 \pm 0.308^*$	$-1.647 \pm 0.308^*$
No Gap	0.161 ± 0.006	$0.001 \pm 0.001^*$	1.308 ± 0.033	$-2.276 \pm 0.300^*$	$-1.791 \pm 0.300^*$
Long-faced					
Gap	0.193 ± 0.007	0.068 ± 0.045	1.165 ± 0.030	-0.787 ± 0.331	-0.189 ± 0.331
No Gap	0.196 ± 0.007	0.070 ± 0.037	1.161 ± 0.026	-0.736 ± 0.292	-0.139 ± 0.292
Psithyrus					
Gap	0.265 ± 0.017	0.276 ± 0.151	1.262 ± 0.095	-0.685 ± 0.306	-0.244 ± 0.306
No Gap	0.271 ± 0.018	0.266 ± 0.120	1.261 ± 0.080	-0.665 ± 0.260	-0.223 ± 0.260
Fervidobombus					
Gap	0.278 ± 0.017	0.054 ± 0.041	1.521 ± 0.098	-0.886 ± 0.339	-0.766 ± 0.339
No Gap	0.277 ± 0.018	0.055 ± 0.045	1.532 ± 0.199	-0.869 ± 0.420	-0.749 ± 0.420
Pyrobombus					
Gap	0.203 ± 0.008	$0.003 \pm 0.003^*$	1.481 ± 0.058	$-2.070 \pm 0.301^*$	$-1.854 \pm 0.301^*$
No Gap	0.173 ± 0.006	$0.002 \pm 0.002^*$	1.493 ± 0.044	$-2.144 \pm 0.227^*$	$-1.928 \pm 0.227^*$
"New World"					
Gap	0.190 ± 0.001	0.296 ± 0.139	1.271 ± 0.092	-0.889 ± 0.254	-0.763 ± 0.254
No Gap	0.192 ± 0.009	0.316 ± 0.121	1.253 ± 0.078	-0.840 ± 0.238	-0.714 ± 0.238
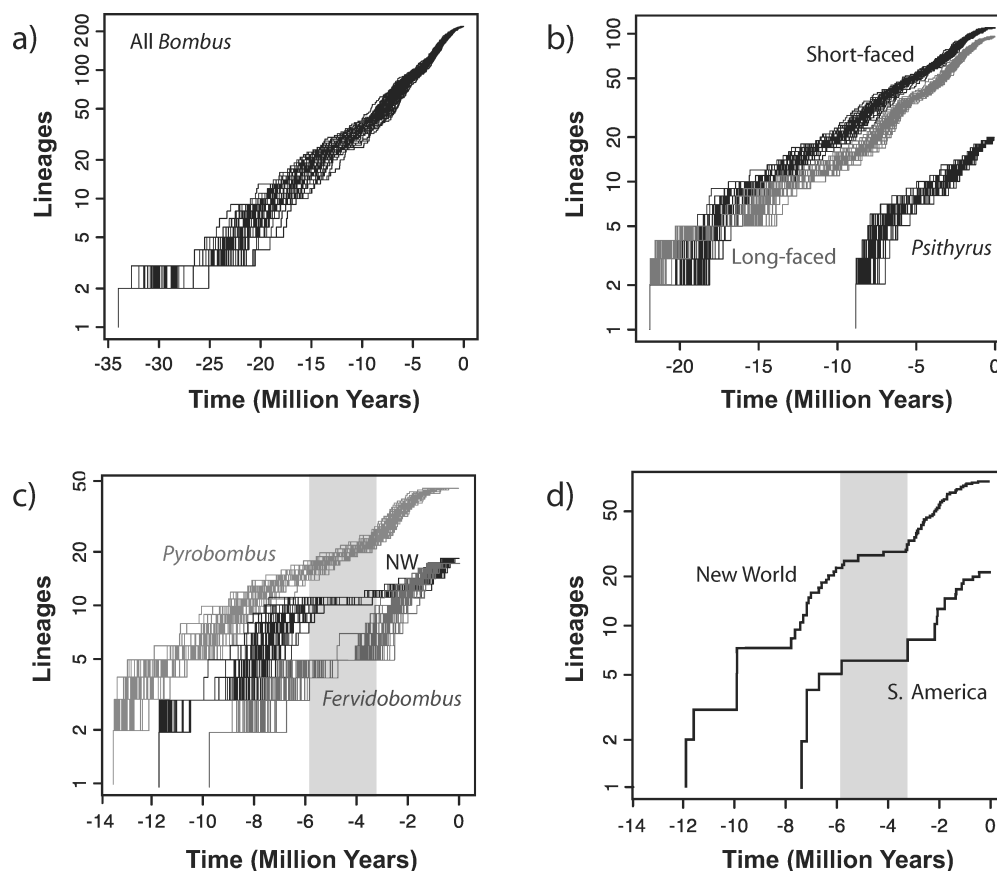
FIGURE 6. Overlayed semilogarithmic lineage-through-time (LTT) plots of chronograms estimated from 50 postconvergence Bayesian trees for several key clades, including (a) all *Bombus* species; (b) species of ecological interest, including the socially parasitic subgenus *Psithyrus*, and the short-faced and long-faced clades (Fig. 2); (c) the subgenera containing most of the New World species (NW = "New World" clade); and (d) regional plots, including all New World speciation events and South American events only. Shaded areas indicate an inferred period of low speciation across New World clades.

the ancestral condition of the represented *Mendacibombus* species does not alone indicate the geographic location of the early diversification of *Bombus*. An Asian montane *Bombus* origin is probable, given the primarily alpine distribution of *Bombus* and that much of the area outside of alpine regions lacked suitably cool climates 30 Ma (PALEOMAP project, www.scotese.com). Although most mountain ranges in Asia were not of high altitude during the Oligocene, resulting in more subtropical climates in southern Asia, mountain chains in northeastern Asia could have provided suitably cool climates, including the Greater Khinghan Range of northern China and other northeast Siberian mountain chains (D. Cunningham, personal communication, 2007). Nearly all missing lineages in the phylogeny are confined to the Old World. Further resolution of the relationships of these missing taxa may help resolve remaining uncertainties on the geographic distribution of earlier bifurcations.

Old World clades show considerable movement between the Palearctic and Oriental regions throughout the evolution of bumble bees. The mountains of Asia, which harbor the greatest *Bombus* diversity (Williams, 1998), were relatively low in elevation until the uplift of the Tibetan plateau that began with the initial collision of India 35 to 50 Ma but was most dramatic beginning ~21 Ma (Harrison et al., 1992). These mountains likely promoted speciation of taxa, contributing to some of the best examples of clades with restricted regional distribution. These mountains formed during a period of climatic stochasticity. Climatic cooling may have forced fauna to lower elevations, facilitating their dispersal, and subsequent warming could have lead to species isolation at higher altitudes (Williams, 1991).

Intercontinental Movements: Palearctic-Nearctic

Bumble bees likely began to diversify after the loss of major land connections across the Atlantic ocean from Europe to eastern North America ~39 Ma (Sanmartin et al., 2001). Therefore, much of the movement between these continents likely took place along the Bering continental connection, as suggested by Williams (1985). Initial dispersal into the New World is inferred to have occurred in as many as six separate clades between 10 and 20 Ma. These early dispersal events likely coincided with climatic cooling in the Miocene following a warming period that lasted from 15 to 25 Ma (Zachos et al., 2001). This cooling facilitated the transformation of forests on the Bering Land Bridge from mesophytic forest into a continuous belt of coniferous forest that was sustained from 3.5 to 14 Ma (Sanmartin et al., 2001). This taiga element may have been important in facilitating the early spread of bumble bees into the Nearctic.

Most of the remaining movements of *Bombus* between the continents are estimated to have occurred in the even cooler period of the last 5 million years and involve Old World sister taxa that are widespread and cold adapted (cf. Kawakita et al., 2004). For example, the sister species to the Nearctic *B. perplexus*, *B. hypnorum*, is exceptionally widespread across the Palearctic and Orient and also achieves a partially arctic distribution. Such a widespread climatically flexible Old World distribution is also achieved by *B. lucorum* and *B. pratorum*, which are each also sister to Nearctic clades. The Bering Strait separated these landmasses around 3.5 Ma, resulting in well-documented vicariant speciation of many taiga organisms (Sanmartin *et al.*, 2001). Vicariance events of bumble bees between the Old World and New World
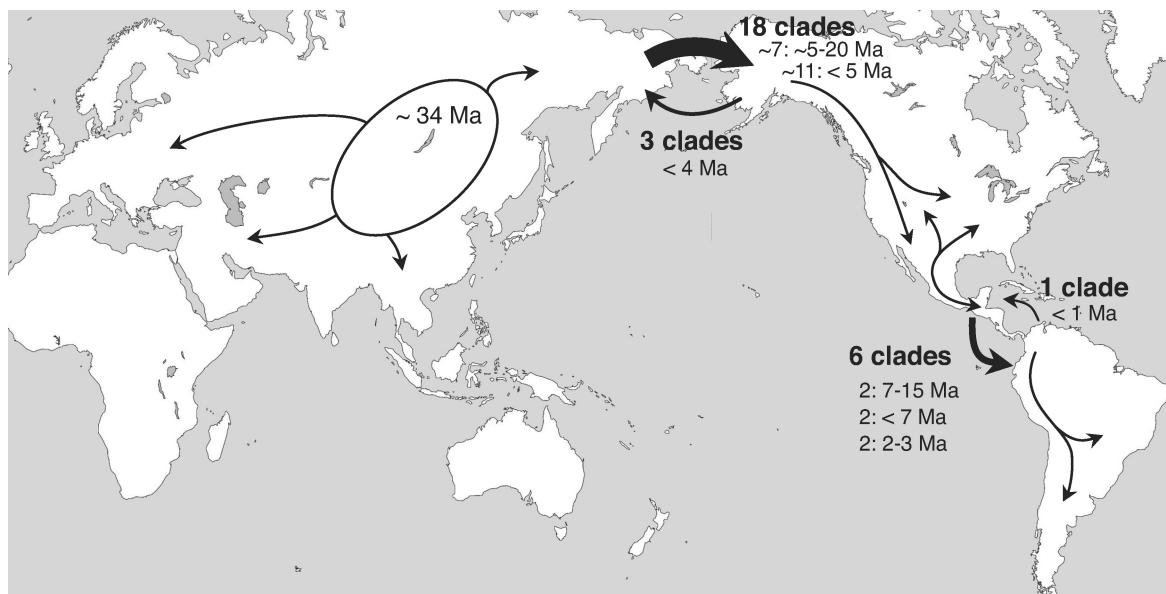

FIGURE 7.   Simplified summary of the historical dispersal events inferred across bumble bee lineages. Many of these events occurred at some point during a broad time range, so the timing of these events has been approximated (see Figs. 2 through 4). Arrow thickness between continents is scaled relative to the number of lineages that dispersed in the direction indicated.

estimated to have occurred around this time involve splits among species that retain a boreal distribution today, including species in the *B. lucorum* complex, *B. sitkensis–B. sandersoni* from its sister clade *B. cingulatus–B. beaticola*, *B. perplexus* from *B. hypnorum*, *B. lapponicus* and *B. monticola* from *B. sylvicola* and *B. bimaculatus*, and *B. appositus + B. borealis* from *B. distinguendus*. When this landmass reconnected again 1.0 to 1.5 Ma, it opened up as dry treeless tundra (Sanmartin et al., 2001). Therefore, some of the most recent dispersal events should involve tundra-adapted taxa, with very little species differentiation between Palearctic and Nearctic forms. The most likely candidate for this late migration is *B. alboanalis*. Nearly all of these Old World/New World species pairs involve species currently distributed on either side of the Bering Strait (Franklin, 1913; Ito and Kuranishi, 2000).

One of the more interesting results concerning movements between the continents is the apparent directional asymmetry, with the vast majority of species moving from Old World to New World rather than in the opposite direction, a result also reported by Kawakita et al. (2004). Clearly reconstructed dispersal events from the New World to the Old World are estimated to have occurred in the last 4 million years in three clades. Two of these taxa involve the clade including *B. lapponicus*, *B. sylvicola*, *B. bimaculatus*, and *B. monticola*. These species could represent a hard polytomy involving vicariant speciation from a widespread taxon, given that their inferred split ~3 Ma corresponds to the onset of glaciation events and the formation of the Bering Strait. The Old World *B. lapponicus* retains the Western Nearctic montane mimetic color pattern in parts of its range. The predominantly Palearctic to Nearctic spread of bumble bee species may be an artifact of the species diversity on either side of the Bering Land Bridge. It was not until the Nearctic species had begun to establish and speciate that the reverse trend would have become as probable. This argument can also explain why the majority of reconstructed movements between North America and South America are from north to south.

Nearctic Movements

A spread within North America through the Nearctic mountain ranges and subsequently to the east and south is suggested in several instances, but the exclusively Nearctic subgenus *Fervidobombus* is a notable exception. Among the bumble bees, *Fervidobombus* contains some of the most warm-adapted taxa, including species that are distributed in warm dry regions of Mexico and in the lowland tropical rainforest (Moure and Sakagami, 1962; Labougle, 1990). The subgenus is reconstructed to have diversified in the Southern Nearctic and subsequently spread both north and south in separate lineages, as in the DIVA reconstruction by Cameron and Williams (2003). This suggests that the likely conspecific (Cameron et al., 2007) *B. sonorus + B. pensylvanicus*, which have a southern distribution in the United States, may have spread into both regions from a Mexican ancestor and undergone some vicariant divergence in their re-

spective regions. A similar situation may apply for the widespread more northern *B. californicus + B. fervidus*.

Movements into and within South America

While the timing of mammalian dispersal events into South America across Panamanian land connections in the late Cenozoic has been more thoroughly examined, relatively few papers have explored the dispersal of insects across the isthmus. Early mammalian migrations from North America to South America occurred around 9 to 10 Ma for the South American ground sloth and around 6 Ma for raccoons (Marshall et al., 1979), but the majority of the interchange between these continents occurred after the well-documented formation of the isthmus of Panama between 3 and 3.5 Ma, which created a permanent land corridor (Coates et al., 1992; Coates and Obando, 1996). The island arc between North America and South America began to form around 15 Ma and was a significant barrier to marine organisms by 6 Ma. This barrier may have even resulted from a temporary land connection between the continents between 6 and 8 Ma (Collins et al., 1996). Data from the poorly dispersing pseudoscorpions suggested two major movements and radiations into South America, one between 5 and 7 Ma and another after the 3 Ma formation of the Panamanian isthmus (Zeh et al., 2003).

Results from DIVA place the first S. American ancestral nodes at ~7.5 Ma. Early South American diversification events were estimated between 6 and 7.5 Ma and most remaining speciation events appear to have taken place after 3.5 Ma. For the first speciation events within South America to have occurred after the 3- to 3.5-Ma formation of the isthmus would require an age younger than 15 Ma for the *Bombus* root node, a date younger than all mean predicted divergence dates and the *Bombus* fossil record. These results provide additional support for the occurrence of an earlier land or substantial island route to South America around 6 to 8 Ma and are concordant with the migration of additional taxa after the initial opening of the isthmus 3.5 Ma.

Aside from land connections, bumble bee dispersal into South America would have required the availability of suitable temperate habitats. The major cooling event of the mid-Miocene (~15 Ma), which led to the formation of a continuous coniferous forest across the Bering Land Bridge, also promoted drier grassland regimes in North America and opened up the southern Nearctic and Central America to temperate savanna-like vegetation (Burnham and Graham, 1999). Around 15 Ma, the Central American Gatun flora showed the first indication of temperate plants but these flora were not common until 5 to 10 Ma (Burnham and Graham, 1999). The occurrence of temperate flora in South America was heavily influenced by the uprise of the Andes. Although the Andes began to rise around 40 Ma, half of the present elevation of the Central and Eastern Andes was obtained after dramatic uplifting began around 10 Ma (Ghosh et al. 2006, Lamb and Davis, 2003), and elevational gain of the Colombian Andes occurred mostly after 5 Ma (Gregory-Wodzicki,

2000). The data presented here, therefore, suggest that the bumble bees may have spread in the late Miocene with the first predominantly temperate habitats in Central America and arrived in South America around the time when the dramatic uprise of the Andes opened new patches of temperate habitats.

During the formation of the isthmus 3 to 3.5 Ma, a corridor of savanna and temperate elements between the continents was available and the Andean mountains had reached a substantial elevation to promote the spread of cold-adapted fauna, such as bumble bees, southward (Burnham and Graham, 1999). During this time, numerous northern grassland species were successful in establishing at high altitudes (>3000 m) across the Andes (Burnham and Graham, 1999). Hughes and Eastwood (2006) provide evidence for a massive radiation of lupines after 2 Ma along this route. Lupines and other highly successful northern migrants, including *Rubus* (Rosaceae) and *Scutellaria* (Lamiaceae), are herbaceous plants pollinated to a great part by bumble bees. By 3 Ma, bumble bee fauna already in South America appear to have established a widespread distribution. This earlier arrival could have facilitated the spread of later arriving flora particularly suited to pollination by the generalist bumble bees.

The two separate episodes of speciation around 6 to 8 Ma and after 3.5 Ma are inferred not only in South America but across the New World. The New World was influenced by three separate but related events around 3.5 Ma that could have caused an increase in speciation: (1) the disconnection of the Palearctic from the Nearctic through the Bering Strait, which promoted vicariant speciation of taxa distributed across the two landmasses; (2) the beginning of glaciation events, which pushed taxa southward into refugia during glacial maxima and likely northward during interglacials; and (3) the formation of the isthmus of Panama. The 6- to 8-Ma speciation events may have been influenced by the opening of a grassland corridor and temperate habitats, higher Andean elevations to the south, and a more substantial land connection to South America during this time. Interpretation of the climatic framework for these patterns is impeded by the low precision of the divergence time estimates. Although estimates here are concordant with geographic and climatic events, the reliability of these dates could be improved with new fossil data.

Psithyrus *Evolution*

Psithyrus, which was once recognized as a separate genus, is socially parasitic on other bumble bee subgenera. This parasitic lifestyle is inferred to have arisen ~9 Ma, relatively recently in bumble bee evolution. The LTT plot for *Psithyrus* (Fig. 6b) suggests a rapid initial diversification of these parasitic lineages, but diversification statistics support a constant rate of diversification. This may be partially expected, as Paradis (1997) reported a decline in power in these statistics with decreasing number of species tested. *Psithyrus* species vary in host specificity, with records of Western Palearctic species

suggesting more specificity than New World *Psithyrus*. *Psithyrus* tend not to occur in the extremes of bumble bee distribution, such as above the tree line or in tropical areas, perhaps because of restrictions posed by their late seasonal emergence or the low concentration of hosts in these areas. In spite of these restrictions, *Psithyrus* has managed to be one of the most successfully dispersing *Bombus* subgenera. Four phylogenetically independent movements into the Nearctic from the Palearctic are inferred over their evolution. Of these, each of the Palearctic-Nearctic species pairs *B. fernaldae–B. flavidus* and *B. ashtoni–B. bohemicus* were revealed to be so genetically similar they are likely conspecific (Cameron *et al.*, 2007). Such a widespread distribution, traversing across the Palearctic and northern Nearctic, is achieved by few other bumble bee species. Maintaining some flexibility in host specificity may facilitate the rapid spread of these species with host lineages.

Ecological Correlates

Bumble bee communites usually attain between 4 and 12 species (e.g., Pyke, 1982; Williams, 1986, 1991; Svensson et al., 2000; Hines and Hendrix, 2005) that may partition floral resources via morphological differences in body size and tongue length, floral preference, phenology, and habitat preference (Heinrich, 1976, 1979; Pekkarinen, 1984). If competition were to limit the number of species that can be supported in a region, it could result in less speciation through time. The declining rate of diversification exhibited across the LTT plots is consistent with such density dependent cladogenesis in bumble bees. Furthermore, the plots have a leap in diversification following the arrival of *Bombus* lineages into the New World, when the opening of an "empty" niche could have lowered competitive constraints. Alternatively, the declining diversification in the plots could result from increasing saturation deeper in the tree unaccounted for by models. The longer-tongued bumble bee clade ("long-faced") did not differ markedly in either pattern or rate of speciation from the shorter-tongued sister clade ("short-faced"), indicating that both long- and shorter-tongued species have been similarly successful across time.

Numerous alpine floral groups rely on bumble bees for pollination. One of the angiosperm genera most recognized for a strict reliance on bumble bees is *Pedicularis* (Macior, 1982; Macior et al., 2001). Like *Bombus*, *Pedicularis* reaches its highest species richness and morphological diversity in the Qinghai-Tibetan plateau and is distributed across arctic and alpine temperate regions of the Holarctic, including some of the highest zones of plant life (Li, 1948; Macior, 1982). Many other floral genera, such as the largely bumble bee pollinated *Gentiana* and *Rhododendron*, also reach their highest diversity in southwestern China (Qian, 2002). Future estimates of divergence times and historical biogeography in these groups could be compared with bumble bee evolutionary history to further illuminate general patterns in alpine Holarctic biogeography.

REFERENCES

Barraclough, T. G., and S. Nee. 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16:391–399.

Brady, S. G., S. Sipes, A. Pearson, and B. N. Danforth. 2006. Recent and simultaneous origins of eusociality in halictid bees. Proc. R. Soc. Lond. B Biol. Sci. 273:1643–1649.

Burnham, R. J., and A. Graham. 1999. The history of Neotropical vegetation: New developments and status. Ann. Mo. Bot. Gard. 86:546–589.

Camargo, J. M. F., D. A. Grimaldi, and S. R. M. Pedro. 2000. The extinct fauna of stingless bees (Hymenoptera: Apidae: Meliponini) in Dominican amber: Two new species and redescription of the male of *Proplebeia dominicana* (Wille and Chandler). Am. Mus. Novit. 3293:1–24.

Cameron, S. A., H. M. Hines, and P. H. Williams. 2007. A comprehensive phylogeny of the bumble bees (*Bombus*). Biol. J. Linn. Soc. 91:161–188.

Cameron, S. A., and P. Mardulyn. 2001. Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera: Apinae). Syst. Biol. 50:194–214.

Cameron, S. A., and P. H. Williams. 2003. Phylogeny of bumble bees in the New World subgenus *Fervidobombus* (Hymenoptera: Apidae): Congruence of molecular and morphological data. Mol. Phylogenet. Evol. 28:552–563.

Cardinal, S., and L. Packer. 2007. Phylogenetic analysis of the corbiculate Apinae based on morphology of the sting apparatus (Hymenoptera: Apidae). Cladistics 23:99–118.

Coates, A. G., J. B. C. Jackson, L. S. Collins, T. M. Cronin, H. J. Dowsett, L. M. Bybell, P. Jung, and J. A. Obando. 1992. Closure of the Isthmus of Panama: The near-shore marine record of Costa Rica and Western Panama. Geol. Soc. Am. Bull. 104:814–828.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American isthmus. Pages 21–56 *in* Evolution and Environment in Tropical America (J. B. C. Jackson, A. F. Budd, and A. G. Coates, eds). University of Chicago Press, Chicago, Illinois.

Cockerell, T. D. A. 1931. Insects from the Miocene (Latah) of Washington: II. Hymenoptera and Hemiptera. Ann. Entomol. Soc. Am. 24:309–312.

Collins, L. S., A. G. Coates, W. A. Berggren, M. Aubry, and J. Zhang. 1996. The late Miocene Panama isthmian strait. Geology 24:687–690.

Danforth, B. N., S. Sipes, J. Fang, and S. G. Brady. 2006. This history of early bee diversification based on five genes plus morphology. Proc. Natl. Acad. Sci. USA 103:15118–15123.

Engel, M. S. 2000. A new interpretation of the oldest fossil bee (Hymenoptera: Apidae). Am. Mus. Novit. 3296:1–11.

Engel, M. S. 2001. A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). Bull. Am. Mus. Nat. Hist. 259:1–192.

Fitch, W. M., and M. Bruschi. 1987. The evolution of prokaryotic ferredoxins—With a general method correcting for unobserved substitutions in less branched lineages. Mol. Biol. Evol. 4:381–394.

Franklin, H. J. 1913. The Bombidae of the New World. Trans. Am. Entomol. Soc. (Phila.) 38:177–486.

Ghosh, P., C. N. Garzione, and J. M. Eiler. 2006. Rapid uplift of the altiplano revealed through ^{13}C-^{18}O bonds in paleosol carbonates. Science 311:511–515.

Gregory-Wodzicki, K. M. 2000. Uplift history of the Central and Northern Andes: A review. Geol. Soc. Am. Bull. 112:1091–1105.

Gray, J., and L. R. Kittleman. 1967. Geochronometry of the Columbia River Basalt and associated floras of eastern Washington and western Idaho. Am. J. Sci. 265:257–291.

Grimaldi, D., and M. S. Engel. 2005. Evolution of the insects. Cambridge University Press, New York.

Harrison, T. M., P. Copeland, W. S. F. Kidd, and A. Yin. 1992. Raising Tibet. Science 255:1663–1670.

Hartenberger, J. 1998. An Asian *Grande Coupure*. Nature 394:321.

Heinrich, B. 1976. Resource partitioning among some eusocial insects: Bumblebees. Ecology 57:874–899.

Heinrich, B. 1979. Bumblebee economics. Harvard University Press, Cambridge, Massachusetts.

Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular systematics. Sinauer Associates, Inc., Sunderland, Massachusetts.

Hines, H. M., S. A. Cameron, and P. H. Williams. 2006. Molecular phylogeny of the bumble bee subgenus *Pyrobombus* (Hymenoptera: Apidae: *Bombus*) with insights into gene utility for lower-level analysis. Invertebr. Syst. 20:289–303.

Hines, H. M., and S. D. Hendrix. 2005. Bumble bee (Hymenoptera: Apidae) diversity and abundance in tallgrass prairie patches: Effects of local and landscape floral resources. Environ. Entomol. 34:1477–1484.

Hughes, C., and R. Eastwood. 2006. Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. Proc. Natl. Acad. Sci. USA 103:10334–10339.

Ito, M., 1985. Supraspecific classification of bumblebees based on the characters of male genitalia. Contr. Instit. Low Temp. Sci. Hokkaido Univ. B 20:1–143.

Ito, M., and R. B. Kuranishi. 2000. Bumble bees (Hymenoptera: Apidae) occurring in the Kamchatka Peninsula and the North Kuril Islands. Nat. Hist. Res. Special Issue No. 7 7:281–289.

Iturralde-Vinent, M. A., and R. D. E. MacPhee. 1996. Age and paleogeographical origin of Dominican amber. Science 273:1850–1852.

Kawakita, A., T. Sota, M. Ito, J. S. Ascher, H. Tanaka, M. Kato, and D. W. Roubik. 2004. Phylogeny, historical biogeography, and character evolution in bumble bees (*Bombus*: Apidae) based on simultaneous analysis of three nuclear gene sequences. Mol. Phylogenet. Evol. 31:799–804.

Kubo, T., and Y. Iwasa. 1995. Inferring the rates of branching and extinction from molecular phylogenies. Evolution 49:694–704.

Labougle, J. M. 1990. *Bombus* of México and Central America (Hymenoptera, Apidae). Univ. Kans. Sci. Bull. 54:35–73.

Lamb, S., and P. Davis. 2003. Cenozoic climate change as a possible cause for the rise of the Andes. Nature 425:792–797.

Li, H. 1948. A revision of the genus *Pedicularis* in China. Part I. Proc. Acad. Nat. Sci. Phila. 100:205–378.

Lockhart, P. J., and S. A. Cameron. 2001. Trees for bees. Trends Ecol. Evol. 16:94–98.

Løken, A. 1973. Studies on Scandinavian bumble bees (Hymenoptera, Apidae). Nor. Entomol. Tidsskr. 20:1–218.

Løken, A. 1984. Scandinavian species of the genus *Psithyrus* Lepeletier (Hymenoptera: Apidae). Entomol. Scand. Suppl. 23:1–45.

Macior, L. W. 1982. Plant community and pollinator dynamics in the evolution of pollination mechanisms in *Pedicularis* (Scrophulariaceae). Pages 29–45 *in* Pollination and evolution (J. A. Armstrong, J. M. Powell, and A. J. Richards, eds.). Royal Botanic Gardens, Sydney, Australia.

Macior, L. W., T. Ya, and J. Zhang. 2001. Reproductive biology of *Pedicularis* (Scrophulariaceae) in the Sichuan Himalaya. Plant Species Biol. 16:83–89.

Marshall, L. G., R. F. Butler, R. E. Drake, G. H. Curtis, and R. H. Tedford. 1979. Calibration of the Great American Interchange. Science 204:272–279.

McKenna, D. D., and B. D. Farrell. 2006. Tropical forests are both evolutionary cradles and museums of leaf beetle diversity. Proc. Natl. Acad. Sci. USA 103:10947–10951.

Medler, J. T., and D. W. Carney. 1963. Bumblebees of Wisconsin (Hymenoptera: Apidae). Res. Bull. Univ. Wisc. Agric. Exp. Sta. 240:1–47.

Meng, J., and M. C. McKenna. 1998. Faunal turnovers of Palaeogene mammals from the Mongolian Plateau. Nature 394:364–366.

Michener, C. D., and D. A. Grimaldi. 1988. A *Trigona* from Late Cretaceous amber in New Jersey (Hymenoptera: Apidae: Meliponinae). Am. Mus. Novit. 2917:1–10.

Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. Science 312:101–104.

Moure, J. S., and S. F. Sakagami. 1962. As mamangabas sociais do Brasil (*Bombus* Latreille) (Hymenoptera, Apoidea). Stud. Entomol. 5:65–194.

Nee, S., R. M. May, and P. H. Harvey. 1994. The reconstructed evolutionary process. Phil. Trans. R. Soc. Lond. B Biol. Sci. 344:305–311.

Nee, S., A. O. Mooers, and P. H. Harvey. 1992. Tempo and mode of evolution revealed from molecular phylogenies. Proc. Natl. Acad. Sci. USA 89:8322–8326.

Paradis, E. 1997. Assessing temporal variations in diversification rates from phylogenies: Estimation and hypothesis testing. Proc. R. Soc. Lond. B Biol. Sci. 264:1141–1147.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.

Pedersen, B. V. 2002. European bumblebees (Hymenoptera: Bombini)—Phylogenetic relationships inferred from DNA sequences. Insect Syst. Evol. 33:361–386.

Pekkarinen, A. 1984. Resource partitioning and coexistence in bumblebees (Hymenoptera, Bombinae). Ann. Entomol. Fenn. 50:97–107.

Plowright, R. C., and W. P. Stephen.1973. A numerical taxonomic analysis of the evolutionary relationships of *Bombus* and *Psithyrus* (Apidae: Hymenoptera). Can. Entomol. 105:733–743.

Ponchau, O., S. Iserbyt, J-C Verhaeghe, and P. Rasmont. 2006. Is the caste-ratio of the oligolectic bumblebee *Bombus gerstaeckeri* Morawitz (Hymenoptera: Apidae) biased to queens? Ann. Soc. Entomol. Fr. 42:207–214.

Posada, D., and K. A. Crandall. 1998. ModelTest: Testing the model of DNA substitution. Bioinformatics 14:817–818.

Pybus, O. G., and P. H. Harvey. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. Proc. R. Soc. Lond. B Biol. Sci. 267:2267–2272.

Pybus, O. G., and A. Rambaut. 2002. GENIE: Estimating demographic history from molecular phylogenies. Bioinformatics 18:1404–1405.

Pyke, G. H. 1982. Local geographic distributions of bumblebees near Crested Butte, Colorado: Competition and community structure. Ecology 63:555–573.

Qian, H. 2002. A comparison of the taxonomic richness of temperate plants in East Asia and North America. Am. J. Bot. 89:1818–1825.

Rambaut, A. 2003. Phylogen v1.1, available from http://evolve.zoo.ox.ac.uk/.

Rasmussen, C. and S. A. Cameron. 2007. A molecular phylogeny of the Old World stingless bees (Hymenoptera: Apidae: Meliponini) and the monophyly of the large genus *Trigona*. Syst. Entomol. 32:26–39.

Rasnitsyn, A. P., and C. D. Michener. 1991. Miocene fossil bumble bee from the Soviet Far East with comments on the chronology and distribution of fossil bees (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 84:583–589.

Ronquist, F. 1996. DIVA version 1.1. Computer program and manual available by anonymous FTP from Uppsala University (ftp.uu.se or ftp.systbot.uu.se).

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rutschmann, F. 2004. Bayesian molecular dating using PAML/multidivtime. A step-by-step manual. University of Zurich, Switzerland.

Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. Mol. Biol. Evol. 14:1218–1231.

Sanderson, M. J. 2003. r8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.

Sanmartín, I., H. Enghoff, and F. Ronquist. 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc. 73:345–390.

Schultz, T. R., M. S. Engel, and J. S. Ascher. 2001. Evidence for the origin of eusociality in the corbiculate bees (Hymenoptera: Apidae). J. Kans. Ent. Soc. 74:10–16.

Simmons, M. P., and H. Ochoterena, 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49:369–381.

Svensson, B., J. Lagerlöf, and B. G. Svensson. 2000. Habitat preferences of nest-seeking bumble bees (Hymenoptera: Apidae) in an agricultural landscape. Agric. Ecosys. Environ. 77:247–255.

Thorne, J. L., and H. Kishino. 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51:689–702. (http://statgen.ncsu.edu/thorne/multidivtime.html)

Williams, P. H. 1985. A preliminary cladistic investigation of relationships among the bumble bees (Hymenoptera, Apidae). Syst. Entomol. 10:239–255.

Williams, P. H. 1991. The bumble bees of the Kashmir Himalaya (Hymenoptera: Apidae, Bombini). Bull. Br. Mus. (Nat. Hist.) Entomol. 60:1–204.

Williams, P. H. 1994. Phylogenetic relationships among bumble bees (*Bombus* Latr.): A reappraisal of morphological evidence. Syst. Entomol. 19:327–344.

Williams, P. H. 1996. Mapping variations in the strength and breadth of biogeographic transition zones using species turnover. Proc. R. Soc. Lond. B Biol. Sci. 263:579–588.

Williams, P. H. 1998. An annotated checklist of bumble bees with an analysis of patterns of description (Hymenoptera: Apidae, Bombini). Bull. Nat. Hist. Mus. Lond. (Ent.) 67:79–152.

Williams, P. H., S. A. Cameron, H. M. Hines, B. Cederberg, and P. Rasmont. 2008. A simplified subgeneric classification of the bumblebees (genus *Bombus*). Apidologie 39.

Yang, Z. 1997. PAML: A program package for phylogenetic analysis by maximum likelihood. CABIOS 13:555–556. (http://abacus.gene.ucl.ac.uk/software/paml.html)

Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.

Zeh, J. A., D. W. Zeh, and M. M. Bonilla. 2003. Phylogeography of the harlequin beetle-riding pseudoscorpion and the rise of the Isthmus of Panama. Mol. Ecol. 12:2759–2769.

Zeuner, F. E., and F. J. Manning. 1976. A monograph on fossil bees (Hymenoptera: Apoidea). Bull. Br. Mus. (Nat. Hist.) Geol. 27:149–268.

Zhang, J. 1990. New fossil species of Apoidea. Acta Zootax. Sin. 15:83–91. (In Chinese, English summary.)