

EVOLUTION OF LONG-DISTANCE MIGRATION IN AND HISTORICAL BIOGEOGRAPHY OF *CATHARUS* THRUSHES: A MOLECULAR PHYLOGENETIC APPROACH

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ABSTRACT.—We addressed the evolution of long-distance migration in and the historical biogeography of *Catharus* thrushes within a phylogenetic framework. *Catharus* thrushes are a Nearctic–Neotropical genus consisting of five migrant and seven resident species. We reconstructed a molecular phylogeny using a combined analysis of cytochrome-*b* and ND2 genes. Phylogenetic reconstructions indicate the nonmonophyly of migratory *Catharus* species. The Neotropics are the most likely ancestral geographic area for the entire lineage, and migratory species are sister to resident taxa whose ranges are restricted to Central America, Mexico, or both. Resident behavior may be ancestral within the lineage, with migratory behavior evolving three times, although confidence in those reconstructions is equivocal in many cases. However, uncertainty in ancestral character states presents an interesting scenario including potential drop-offs of resident species from migratory ancestors. Received 3 January 2002, accepted 2 February 2003.

RESUMEN.—Estudiamos la evolución de la migración a larga distancia y la biogeografía histórica del género *Catharus* desde una perspectiva filogenética. *Catharus* es un género Neártico–Neotropical que incluye cinco especies migratorias y siete residentes. Construimos una filogenia molecular por medio de un análisis combinado de secuencias de los genes citocromo-*b* y ND2. Las reconstrucciones indican que las especies migratorias de *Catharus* no forman un grupo monofilético. El Neotrópico constituye el área ancestral más probable para todo el grupo y las especies migratorias son hermanas de taxa residentes cuyos rangos están restringidos a América Central y/o México. El comportamiento residente podría ser ancestral en el grupo y el comportamiento migratorio habría evolucionado tres veces, aunque estas reconstrucciones son inciertas en varios casos. Sin embargo, la incertidumbre en cuanto a los caracteres ancestrales representa un escenario interesante, incluyendo la posibilidad de que especies residentes se hubieran derivado de ancestros migratorios.

THE POTENTIAL FACTORS driving the evolution of long-distance migration in birds have been fodder for speculation for more than 100 years (reviewed in Gauthreaux 1982 and Rappole 1995). There are many theories to explain this phenomenon within ecological and geographical contexts (Cox 1985, Levey and Stiles 1992, Rappole 1995), but those theories generally lack an evolutionary framework to examine patterns of relationships between migrant and resident congeners. Several recent papers have addressed migration within an evolutionary context (Cicero and Johnson 1998, 2002; Joseph

et al. 1999), and here an evolutionary approach is employed to test several existing theories for the evolution of migration.

Theories for the evolution of long-distance migration, defined by the presence of disjunct breeding and wintering areas (Cox 1985, Dingle 1996), differ in the factors inducing migratory behavior and ecological and geographical contexts of the evolving species. In the past, theorists focused on creating a universal model for evolution of migratory behavior (reviewed in Rappole 1995), but that view has changed as extensive studies have revealed the ecological and evolutionary diversity of migrating birds (e.g. Levey and Stiles 1992, Poulin and Lefebvre 1996, Rappole 1995). Many theories are not mutually exclusive, particularly because theories often explain different levels of the evolution-

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ary process, and converge on the importance of displacement of breeding distributions.

Cox (1985) presented a stepping-stone model of range expansion and speciation for the evolution of long-distance migration. In brief, resident, tropical species expand their ranges into the seasonal subtropics. Further range expansion into temperate areas due to competition and other selective pressures creates a partially migratory species. The partially migratory birds continue to expand into higher latitudes, where they breed successfully; migratory behavior becomes fixed (Stiles 1980). Resident and short-distance migrants are eliminated with the continuing range expansion of resident birds into the seasonal subtropics. The long-distance migrants, however, are unable to compete with closely related residents in the Mexican and Central American tropics and are forced to winter in South America (Cox 1985). That model leads to several predictions. First, migratory species are derived from resident birds. Second, migratory species evolved from ancestors of a seasonal subtropical origin. An additional prediction from pervading ideas about the evolution of long-distance migration (Rappole 1995) is that migratory species may form a monophyletic group, indicating that migratory behavior led to a single adaptive radiation of migratory species as habitat became available.

Safriel (1995) and Rappole (1995) developed generalized models, essentially modifications of Cox's (1985) model, proposing that migrant species are tropical birds that evolved a successful breeding strategy by utilizing the resources of temperate areas. Those models make no explicit phylogenetic predictions other than that migrant species are possibly derived from tropical resident species.

Levey and Stiles (1992) proposed both a model and a specific mechanism leading to the evolution of long-distance migration. Resident species that track seasonal resources (e.g. fruits) routinely engage in elevational migratory behavior, and may experience a period of interspecific competition. Their search for food may lead them north, with resource-driven elevational migratory tendencies preadapting them for long-distance migratory behavior. Thus, through selection and breeding isolation, speciation of migrants occurs. This model predicts a relationship between diet and habitat on the nonbreeding grounds, and migration.

To evaluate these models, to understand patterns of migrant evolution, and to understand the role of migration in speciation, it is critical that a well-supported phylogeny of a lineage consisting of both migrant and resident species be developed. Molecular phylogenetic analyses are fundamental to understanding the evolution of character states (Felsenstein 1985, Harvey and Pagel 1991, Maddison 1994). These techniques, coupled with advances in ancestral character state and ancestral geographic area reconstruction, allow theories for the evolution of long-distance migration to be addressed within an evolutionary context.

The *Catharus* thrushes provide a model system for evaluating theories of the evolution of long-distance migration. *Catharus* is a genus composed of twelve Nearctic and Neotropical species, seven of which are resident in Mexico and Central and South America, and five of which are migratory, breeding in North America and generally wintering south of the United States (Ridgely and Tudor 1989, Howell and Webb 1995, Clement 2000; Fig. 1). In addition to its potential utility for investigating the evolution of long-distance migration, this genus provides the opportunity to reconstruct the historical biogeography of a migrant and resident lineage, thus potentially elucidating patterns between ancestral area and migratory behavior.

An evaluation of the evolution of long-distance migration in *Catharus* requires a careful examination of its systematics and taxonomy. The morphological and ecological similarities between migrant species have led previous researchers to assume their monophyly; in fact, most taxonomic studies based on protein data of *Catharus* have included only migrant species (Hendrickson and Yow 1973, Avise et al. 1980, Winker and Rappole 1988). In light of the evolution of migration, that assumption (and inferences from previous studies) suggests that migrant *Catharus* may form a monophyletic group. A molecular phylogeny including all *Catharus* species is necessary to evaluate that assumption, as well as to ascertain the implications for the evolution of migration if that assumption is not true.

In addition to the interesting questions regarding the evolution of migratory behavior that *Catharus* poses, there are taxonomic questions in the genus that are unresolved. Until

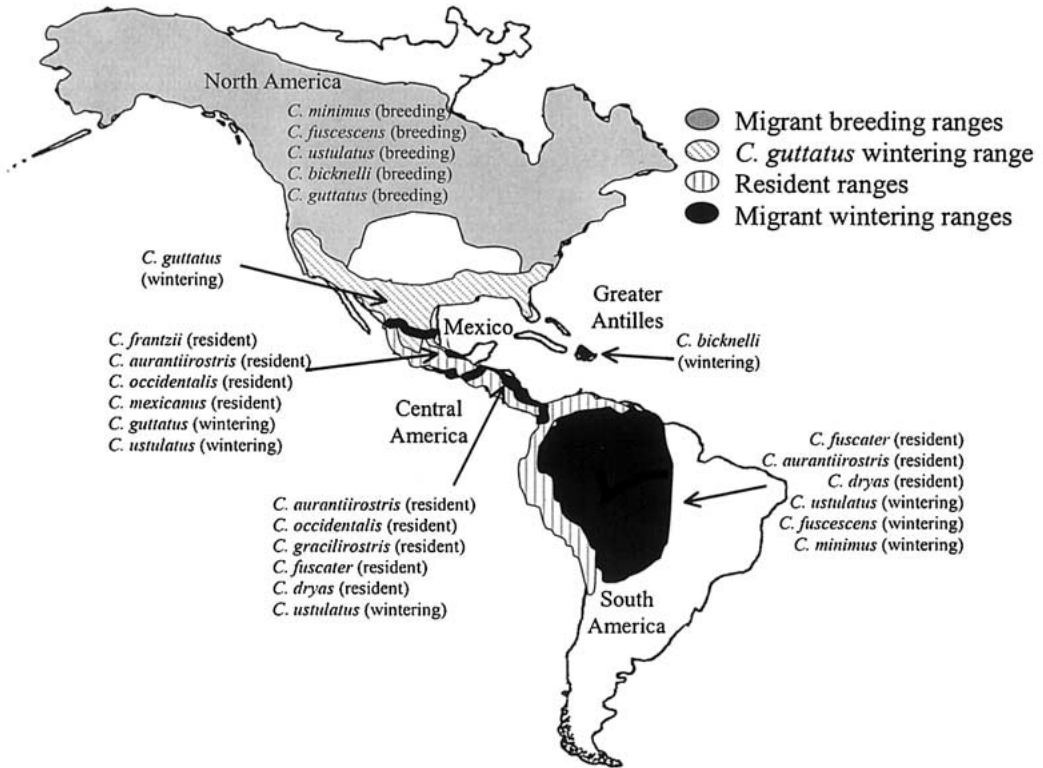


FIG. 1. Map of generalized *Catharus* distributions (Ridgely and Tudor 1989, Howell and Webb 1995, Clement 2000). Five areas are designated: North America, Mexico, Central America, South America, and the Greater Antilles; the species found in each area are listed, along with the stage of their life cycle with which each area is associated.

1969, *Catharus frantzii* and *C. occidentalis* were considered the same species (Phillips 1969). Their ranges are largely allopatric and where they are sympatric, ecology and behavior (e.g. song) operate as isolating mechanisms to maintain species identity (Raitt and Hardy 1970). *Catharus frantzii* and *C. occidentalis* are thought to have speciated from a common ancestor in allopatry via either the submergence of the Isthmus of Tehuantepec or their inability, as montane species, to cross the lowlands of the Isthmus of Tehuantepec (Raitt and Hardy 1970). Additionally, *Catharus bicknelli* was recently conferred species status (Rimmer et al. 1995), and may be a sister taxon to *C. minimus* (Ouellet 1993).

METHODS

Samples.—We sequenced two individuals from each species except for *Catharus occidentalis* where

only one sample was used (Table 1); samples were from regions well-separated within species' overall range. Four additional thrush species were included as outgroup taxa: *Zoothera piaggiae*, *Zoothera gurneyi*, *Turdus grayi*, and *Turdus migratorius*. Outgroup taxa from sister clades were selected from preliminary maximum-parsimony trees from cytochrome-*b* data from 39 species in over 10 thrush genera within the same family (J. Klicka and G. Voelker unpubl. data). Whole genomic DNA was extracted from blood and tissue samples using DNeasy protocols for animal tissues and whole-nucleated blood (Qiagen, Valencia, California).

DNA sequence data.—DNA sequence data were generated from mitochondrial cytochrome-*b* and NADH dehydrogenase subunit 2 (ND2) genes. Polymerase chain reaction was used to isolate and amplify portions of cytochrome *b* and ND2 using standard protocols (Saiki et al. 1988). For cytochrome *b*, a combination of primers was used: L14841, H15299 (Kocher et al. 1989); H4A (Harshman 1996); and LCBA (Klicka et al. 1999). For ND2, gene amplification and sequencing was performed with primers L5215 (Hackett 1996)

TABLE 1. Samples used for DNA sequence data.* Blood samples do not have voucher specimens.

Species	Sample	Location	Type
<i>Catharus aurantiirostris</i>	MBM 6630	Honduras	Tissue
<i>Catharus bicknelli</i>	SUNY 1531 48268	Vermont	Blood
<i>Catharus dryas</i>	MVZ 169692	Peru	Tissue
<i>Catharus frantzii</i>	LSU B28222	Panama	Tissue
<i>Catharus fuscater</i>	LSU B100003	Panama	Tissue
<i>Catharus fuscescens</i>	SUNY V209	New York	Blood
<i>Catharus gracilirostris</i>	LSU B2830	Panama	Tissue
<i>Catharus guttatus</i>	MVZ 177246	California	Tissue
<i>Catharus minimus</i>	SUNY G769	New York	Blood
<i>Catharus occidentalis</i>	SFSU 97N4116	Mexico	Blood
<i>Catharus ustulatus</i>	CAS 596	California	Tissue
<i>Catharus mexicanus</i>	MBM 7224	Honduras	Tissue
<i>Turdus migratorius</i>	MVZ 179227	California	Tissue
<i>Turdus grayi</i>	MBM 6620	Honduras	Tissue
<i>Zoothera piaggiae</i>	FMNH 355649	Uganda	Tissue
<i>Zoothera gurneyi</i>	FMNH 356762	Tanzania	Tissue

*FMNH: Field Museum of Natural History; SFSU: Center for Tropical Research, San Francisco State University; MBM: Marjorie Barrick Museum of Natural History; SUNY: State Universities of New York, Syracuse and Albany; MVZ: Museum of Vertebrate Zoology, University of California Berkeley; LSU: Museum of Natural Science, Louisiana State University; CAS: California Academy of Sciences.

and H6313 (Johnson and Sorenson 1998); all ND2 sequences included in this study were unidirectional due to a lack of conservative internal primers. All of the products were sequenced using dye-terminator labeled or Thermo Sequenase (Amersham Pharmacia, Buckinghamshire, United Kingdom) cycle sequencing reactions run on an automated ABI 377 DNA sequencer or Licor in the Barrick Museum of Natural History at the University of Nevada Las Vegas, the Conservation Genetics Laboratory at San Francisco State University, and the core-sequencing facility at Sonoma State University.

We aligned 975 basepairs of ND2 and 884 basepairs of cytochrome *b* for all species using SEQUENCHER 3.0 (Gene Codes Corporation, Ann Arbor, Michigan). Sequence data for both genes for all included samples were coding and consistent with published sequence data from those genes (e.g. Desjardin and Morais 1990). For cytochrome *b*, further confirmation was established by alignment to the complete published cytochrome-*b* sequence from *Catharus guttatus* as reported in Genbank (Helm-Bychowski and Cracraft 1993). Sequences have been deposited in Genbank (accession numbers AF529137–AF529164).

Phylogenetic analyses.—Conspecific sequence data with three or fewer basepair differences were eliminated from analyses to reduce computation time (thus leaving one sequence per species). We generated phylogenetic relationships among taxa using several approaches in PAUP* (Swofford 1999). First, we generated a neighbor joining tree using an uncorrected (*P*) distance matrix and default settings. Using that tree as a constraint tree, we estimated overall, gene-specific and codon position-specific values for the transition–transversion ratio, the number of invari-

able sites (*I*), transition bias (χ), and among-site rate variation (α) parameters via maximum likelihood (see Yang 1996; Table 2).

Using MODELTEST (all options; 56 models compared; Posada and Crandall 1998, 2001), we identified the general time reversible model, with the number of invariable sites and an alpha shape parameter describing rate heterogeneity across the sequence data (GTR + *I* + Γ), as the most appropriate model of nucleotide substitution. However, two other simpler models, Felsenstein 1981 and Hasegawa-Kishino-Yano 1985, both with corrections for invariable sites and a gamma distribution (Felsenstein 1981, Hasegawa et al. 1985), were examined manually (in PAUP*) and compared to GTR + *I* + Γ via likelihood ratio tests (Huelsenbeck and Rannala 1997) to more fully address information in the data. Site-specific rates (GTR + SS; Buckley et al. 2001) were also examined, although performance of those methods has been shown to be inferior to incorporating rate heterogeneity and invariable sites (Buckley et al. 2001; J. Sullivan pers. comm.). A maximum-likelihood tree was constructed using GTR + *I* + Γ with the estimated overall base substitution rates and α and *I* parameters (Yang 1994).

We incorporated the codon position-specific transition–transversion ratios into maximum-parsimony analyses via codon-position-specific step-matrices. We produced a 50% majority-rule bootstrap consensus tree (1,000 replicates) via a full heuristic search using TBR branch swapping with random taxon addition replicates. Finally, we performed a full heuristic maximum-likelihood bootstrap analysis (100 replicates).

Systematics and taxonomic issues.—Many studies

TABLE 2. Cytochrome *b* and ND2 gene dynamics, both overall and codon position-specific for all taxa. Transition–transversion (Ti/Tv) ratio values, relative rate (χ) values, rate heterogeneity (α) values, and the proportion of invariable sites (*I*) were estimated via maximum-likelihood from an uncorrected (*p*) neighbor-joining constraint tree in PAUP* using empirical base frequencies.

Codon position	Number of sites	Variable sites	Parsimony informative	Ti/Tv	<i>I</i>	χ	α
Cytochrome <i>b</i>							
All	884	264	166	4.09	0.44	8.16	0.52
1st	295	43	20	5.80	0.71	11.69	0.58
2nd	295	12	4	1.80	0.86	3.02	–
3rd	294	209	142	6.32	0.03	25.72	1.87
ND2							
All	975	423	297	6.80	0.35	14.11	0.76
1st	325	101	62	8.66	0.26	18.57	0.38
2nd	325	49	32	7.50	0.68	10.06	1.39
3rd	325	273	203	6.94	0	21.51	2.90

have attempted to quantify the relationships between the North American temperate *Catharus* species as well as between the North American *Catharus* species and *Hylocichla mustelina*, a species that many have considered to belong within *Catharus* (Hendrickson and Yow 1973, Avise et al. 1980, Winker and Rappole 1988, but see Gibson et al. 1976). Preliminary analyses of cytochrome-*b* data from most currently recognized genera within Turdidae suggests that *Hylocichla* is not in fact a *Catharus* (J. Klicka and G. Voelker unpubl. data). Given this preliminary finding, we do not include *Hylocichla* in this study, but will address the taxonomic position of this long-contentious genus (Dilger 1956 a, b) elsewhere.

Traditional (*a priori*) hypotheses of phylogenetic relationships within *Catharus* were tested via Shimodaira-Hasegawa tests (Shimodaira and Hasegawa 1999, Goldman et al. 2000; Table 3; RELL approximation with 1,000 bootstrap replicates). Three relationships were tested against the maximum-likelihood tree: (1) *Catharus frantzii* and *C. occidentalis* as sister taxa (Phillips 1969); (2) *Catharus minimus* and *C. bicknelli* as sister taxa; and the monophyly of migrant *Catharus* species. Monophyly of migrants was generated with the fewest possible tree rearrangements in MACCLADE (Maddison and Maddison 1992).

TABLE 3. Shimodaira-Hasegawa (1999) tests comparing the best maximum-likelihood tree to alternative trees reflecting traditional taxonomic hypotheses. Values of $P \leq 0.05$ indicate that alternative trees are significantly worse estimates of phylogeny.

Tree topology	–ln L	<i>P</i>
“Best” maximum-likelihood tree	9341.16	best
<i>frantzii</i> – <i>occidentalis</i> sisters	9376.80	<0.05
<i>bicknelli</i> – <i>minimus</i> sisters	9342.04	>0.670
Migratory species monophyletic	9401.61	<0.05

Molecular clock.—Despite the controversies that surround the use of a molecular clock, it can be a useful tool for estimating speciation timing (Swofford et al. 1996). We have used the estimate of 1.9% average pair-wise sequence divergence (cytochrome *b* for passerine birds) between taxa, per million years (Fleischer et al. 1998) on only the cytochrome-*b* data. To examine whether lineages within *Catharus* were evolving in a clocklike manner, we applied the two-cluster test (Takezaki et al. 1995) to the maximum-likelihood topology using uncorrected (*P*) distances (cytochrome-*b* data only). That test determines if the length difference of two sister lineages is significantly different from zero (δ), and provides a confidence probability (CP) with values over 95% denoting absolute rejection of a molecular clock (Takezaki et al. 1995; Table 3). Where a clock was not rejected, branch height ($h \pm SE$)—the mean branch length of two sister lineages—was used to date clocklike nodes within the tree (Table 3). However, nonclocklike nodes were also dated for speculative purposes.

Historical biogeography.—Ancestral geographic areas were reconstructed with DIVA (Ronquist 1996, 1997). Within a parsimony framework, DIVA uses the areas in which species are currently found to reconstruct the ancestral area for each node within the tree. DIVA’s primary function is to compare the relative importance of vicariance and dispersal in generating the modern distributions of taxa, but the secondary utility of reconstructing ancestral areas was employed here. The ancestral areas were reconstructed using all areas (breeding and wintering) of species.

We assigned geographic areas using the distributions of allopatric and sympatric species as a guide: North America, Mexico, Central America, South America, and the Greater Antilles (Fig. 1; Ridgely and Tudor 1989, Howell and Webb 1995, Clement 2000). All migratory species breed in North America, whereas none of the resident species fall within that

area (Fig. 1). However, *Catharus guttatus* winters in southern North America and Mexico. Based on the breeding distributions of the migrants (and the wintering range of *C. guttatus*), North America was designated as one area. Several resident species have ranges within Mexico. However, two resident species, *C. gracilirostris* and *C. dryas*, are not found within Mexico, supporting the division of Mexico and Central America as separate areas. Two migrant species, *C. ustulatus* and *C. guttatus* winter in Mexico (Fig. 1). Within Central America, six resident species have ranges: *C. gracilirostris* exclusively (Fig. 1), whereas *C. occidentalis* is not found within Central America. *Catharus ustulatus*, a migrant, winters in Central America (Fig. 1). Three resident species have South American ranges, although none of those species is exclusive to South America (Fig. 1). However, the reason for including a South American area is that three migrant species winter there, two exclusively so (Fig. 1). *Catharus bicknelli* only winters in the Greater Antilles.

Using the five areas within DIVA, we calculated ancestral geographic areas with no restrictions on the number of areas, and with the maximum areas numbering two, the minimum number of areas allowed (Ronquist 1997).

Reconstruction of ancestral character states.—We defined a migratory species as one that engages in an annual long-distance migration (*Catharus ustulatus*, *C. guttatus*, *C. minimus*, *C. fuscescens*, and *C. bicknelli*; Clement 2000); those species were assigned a character state of one (1). Resident species may move intratropically but do not migrate long distances. Several resident *Catharus* species move intratropically, but do not undergo a major shift in range. Resident species (*Catharus aurantiirostris*, *C. mexicanus*, *C. dryas*, *C. fuscater*, *C. occidentalis*, and *C. frantzii*; Clement 2000) were assigned a character state of zero (0). We reconstructed ancestral character states within *Catharus* using two approaches. First, we used MACCLADE (Maddison and Maddison 1992) to trace evolution of migratory behavior onto the phylogeny within a parsimony framework. Second, we used DISCRETE 4.0 (M. Pagel in litt. [see Acknowledgments]), which reconstructs ancestral character states within a maximum likelihood framework. Outgroups were not used in ancestral character-state reconstruction (Mooers and Schluter 1999) because one purpose was to assess the most likely character state at the root of the *Catharus* tree.

DISCRETE 4.0 calculates character transition rates (the model of trait evolution) against a background of the tree, the branch lengths, and the character states of the extant taxa. We used two methods in DISCRETE 4.0 to reconstruct ancestral character states: first, over the entire tree and second, at each node separately. Over the entire tree, global reconstruction of ancestral character states was performed using the ancestral

states function, which maximizes the likelihood of ancestral character states using the topology, branch lengths, and transition rates, but does not provide measures of confidence.

To assess confidence in ancestral character states in maximum-likelihood, we also reconstructed ancestral character states for each node. This method calculates the likelihoods and probabilities for each state at each node using only the transition rates and branch lengths. Therefore, it ignores ancestral character states at adjacent nodes. However, that method is the only function that provides measures of confidence at each node.

We performed several tests to choose between evolutionary models within DISCRETE 4.0. A two transition-rate model ($-\ln = 11.48$) was not significantly better than a one-rate model ($-\ln = 10.63$) in a Monte Carlo simulation (100 iterations, $P > 0.10$). The two rate models are not comparable via a likelihood-ratio test because the number of parameters does not change from one model to the other. The one-rate model invokes fewer assumptions about the nature of migrant evolution. Mooers and Schluter (1999) have suggested that a one-rate model may be the most appropriate for a small data set. Therefore, we used a one-rate model to reconstruct ancestral character states at all nodes. Global estimates (Mooers and Schluter 1999, Pagel 1999) have been used because of the restriction of a one-rate model. Differences in log-likelihoods were used to assess if one character state was significantly more supported at each node (Edwards 1972, Pagel 1999).

RESULTS AND DISCUSSION

Phylogenetic relationships and taxonomic issues.—Although phylogenetic analyses confirm the monophyly of *Catharus*, the relationships within *Catharus* are more complex than expected (Fig. 2). *Catharus* consists of two clades (Fig. 2), which are relatively divergent from one another, (average uncorrected pairwise sequence divergence $\sim 11\%$).

With regard to traditional taxonomy, both maximum-likelihood and maximum-parsimony analyses place *Catharus occidentalis* as sister to *C. guttatus*, and *C. frantzii* as sister to a clade containing *C. bicknelli*, *C. minimus*, and *C. fuscescens* (hereafter, "the *C. bicknelli* clade"). These relationships are supported by the Shimodaira-Hasegawa (1999) test, which rejects as significantly worse a tree on which *C. frantzii* and *C. occidentalis* were forced to be sisters (Table 3).

Catharus bicknelli was historically considered conspecific with *C. minimus*. Ouellet (1993) suggested that *C. bicknelli* and *C. minimus* were

close relatives, and although the results of all our analyses suggest that *C. bicknelli* and *C. minimus* are not in fact sisters (Fig. 2), these results can not be rejected via the Shimodaira-Hasegawa (1999) test (Table 3).

A clear result of the phylogenetic hypothesis presented here is the nonmonophyly of the migrant *Catharus* species (Fig. 2). Topology tests forcing the monophyly of the migrant species result in a significantly worse estimate of the data (Table 3).

Historical biogeography and speciation timing of migrant and resident Catharus.—Estimates of speciation timing place divergence times between *Catharus* species ranging from the early Pliocene to mid-Pleistocene (Table 4). Applying a correction for intraspecific variation (~350,000 years; Moore 1995, Edwards 1997) does not substantially change those estimates given the standard errors associated them (Table 4), except to place the divergence times within the *C. bicknelli* clade in the late Pleistocene–Holocene (Fig. 2; Table 4).

With no restrictions on the number of ancestral geographic areas, all areas except the Greater Antilles are reconstructed as the ancestral areas of *Catharus* (not shown). That implies a widespread ancestor. Although we cannot rule that possibility out, we restricted the number of possible ancestral areas to two, which reconstructs possible ancestral areas if the ancestors' ranges were limited, an assumption we feel is not unreasonable (Voelker 1999).

Catharus originated in either Central America, Mexico–Central America, Mexico–South America, or Central America–South America ~5 mya

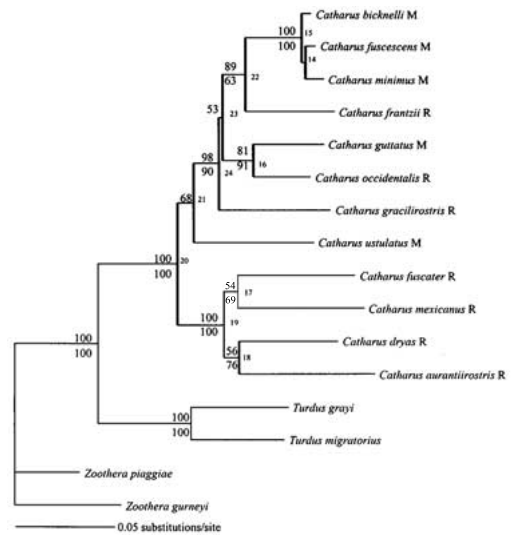


FIG. 2. Maximum-likelihood tree of phylogenetic relationships among *Catharus* species ($-ln= 9341.16$), with migrants designated “M” and residents “R.” Maximum-likelihood bootstrap values are indicated above nodes and maximum-parsimony bootstrap values are indicated below nodes. Node numbers correspond to nodes used in the two-cluster test (Takezaki et al. 1995; Table 4) to determine clocklike daughter lineages. Bold nodes denote those that are evolving in a clocklike manner.

(Fig. 3, Table 4). Resident species expanded their ranges north into Mexico, probably several times in the last 3 to 5 Ma, although one of those nodes is not clocklike (Fig. 2, Table 4). Migrant species expanded from Mexico, the range of their resident sister species (Fig. 3).

Catharus ustulatus, a migrant, diverged ~4 mya

TABLE 4. Results of a two-cluster test (Takezaki et al. 1995) for *Catharus* cytochrome *b* sequence data, based on the maximum-likelihood topology, using uncorrected (*P*) distances. Node numbers correspond to nodes within the maximum-likelihood tree depicted in Figure 2. Branch height (*h*) is the average branch length above internal nodes; this value plus the standard error ($h \pm SE$) was used assuming 1.9% sequence divergence per million years (Fleischer et al. 1998) to estimate speciation timing.

Node	Daughter lineages (L, R)	δ	CP (%)	<i>h</i>	SE	Time (mya) \pm SE
18	<i>dryas</i> < <i>aurantiirrostris</i>	0.0036	57.04	0.0459	0.0049	4.83 \pm 516,000
17	<i>fuscater</i> > <i>mexicanus</i>	0.0033	57.62	0.0278	0.0039	2.93 \pm 411,000
19	17 < 18	0.0109	99.88	0.0402	0.0024	4.23 \pm 253,000
14	<i>fuscescens</i> > <i>minimus</i>	0.0002	40.38	0.0034	0.0014	0.36 \pm 147,000
15	<i>bicknelli</i> > 14	0.0025	54.08	0.0045	0.0011	0.47 \pm 116,000
22	15 < <i>frantzii</i>	0.0018	38.30	0.0248	0.0022	2.61 \pm 232,000
16	<i>guttatus</i> > <i>occidentalis</i>	0.0012	23.58	0.0249	0.0037	2.62 \pm 389,000
23	16 > 22	0.0043	81.64	0.0316	0.0016	3.33 \pm 168,000
24	<i>gracilirostris</i> > 23	0.0061	84.14	0.0333	0.0019	3.51 \pm 200,000
21	24 < <i>ustulatus</i>	0.0079	90.10	0.0377	0.0019	3.97 \pm 200,000
20	21 < 19	0.0063	68.76	0.0423	0.0012	4.45 \pm 126,000

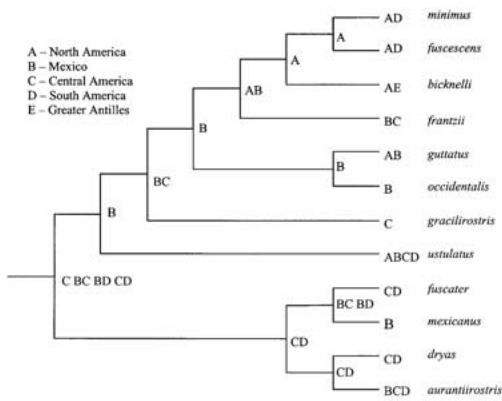


FIG. 3. Cladogram of the reconstruction of ancestral geographic areas using DIVA (max areas = 2). All areas in which species are currently found occur at the tips of the tree and all were used in the analysis. Letters defines areas.

(Table 4), most likely in Mexico (Fig. 3). This old migrant begs the question of its spatial and temporal distributions within the last four million years, as well as its behavior. Has *Catharus ustulatus* been a migrant during its entire species lifetime? If so, how did it remain migratory at the heights of glacial maxima? While these questions are unanswerable, Williams and Webb (1996) suggested that suitable habitat for migratory species remained during glacial maxima during the Pleistocene, although little data is available for the Pliocene. *Catharus ustulatus* may simply have adjusted as its breeding habitat shifted. Interestingly, recent species-level data from *Catharus ustulatus* (Rhuegg and Smith unpubl. data) suggest that sequence divergence within *C. ustulatus* is very low, which may imply a recent range expansion.

Catharus guttatus and *C. occidentalis* split from a common ancestor within the last three million years (Table 4), and the most likely ancestral area for these two species is Mexico (Fig. 3). However, the same questions that pertain to *Catharus ustulatus*, particularly about its behavior at glacial maxima, also apply to *C. guttatus*.

Two species within the migrant *C. bicknelli* clade are estimated to have originated within the last 1 Ma (Fig. 2, Table 4). The putative ancestral geographic area for this clade is North America (Fig. 3). That supports the scenario of a refugial hypothesis for the origin of the recent migrant species. However, given that their sister taxon is a Mexican–Central American resident

(Fig. 3), it is likely that this lineage originated in Mexico–Central America and expanded its range into North America with the wintering distributions evolving at a later time.

Ancestral character states and the evolution of migratory behavior.—Parsimony analyses (Maddison and Maddison 1992) reconstruct the ancestral character state within *Catharus* to be resident behavior, with migratory behavior evolving three times (Fig. 4). In maximum-likelihood analysis, global ancestral character state reconstruction (using the ancestral states function) suggests that resident behavior is ancestral at every node with the exception of nodes 14 and 15 (Fig. 4), although this method provides no estimate of confidence in those reconstructions. Using a node-by-node approach in maximum-likelihood analysis, the possibility exists of migratory behavior being ancestral within the lineage; pie charts represent the relative probabilities of character states at each node and are equivocal at virtually every node (Fig. 4). Without the ability to test the likelihoods against one another via a likelihood-ratio test, we relied on Edwards (1972) suggestion that ~ 2 log units between likelihoods be used to suggest that they are significantly different. With that criterion, only nodes 14 and 15 show significant support for one ancestral character state being more likely than another (Fig. 4).

Interestingly, although we could not statistically justify employing a two-rate model in DISCRETE 4.0, the transition rates may provide some information about the nature of the evolution of migration, although Mooers and Schluter (1999) caution against interpretation of two-rate models with a small data set. The forward and backward transition rates for migration are 0.00136 and 13.0448, respectively; the higher backward transition rate indicates that migration is much easier to lose than it is to gain. If this model were statistically supported, its results would suggest that migration evolved once at the base of *Catharus*, with several losses. That would not refute Cox's (1985) model, because the impetus for migration still seems to be seasonal areas within Central America, with drop-offs of migration occurring in habitats unoccupied by resident *Catharus* species. When restricted to equal forward and backward rates, the transition rate is 16.37766, which confers a model of rapid gain and loss of migration on the tree.

Ancestral character state reconstruction and

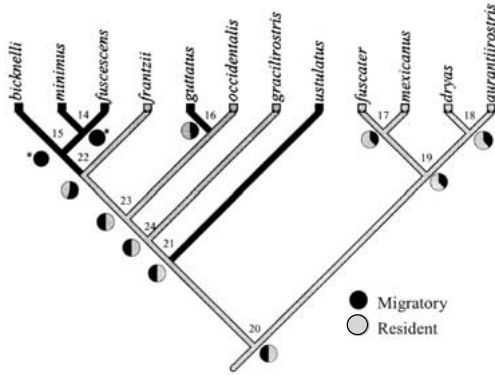


FIG. 4. Cladogram indicating the most parsimonious reconstruction of migratory behavior on the phylogeny using MACCLADE (Maddison and Maddison 1992). Pie charts represent the relative probability of each character state in node-by-node estimations using DISCRETE 4.0 (Pagel 1999a), although global ancestral character state reconstruction gave the same results as parsimony analysis (see text for details). Pie charts with an asterisk represent reconstructions for which one state is significantly more likely to be the ancestral character state than the other. Node numbers correspond to those in Figure 2.

historical biogeographic analysis suggest that a tropical origin of migrant *Catharus* may be the most likely scenario (Figs. 3 and 4). Given the uncertainty in the maximum-likelihood reconstruction, however, we cannot eliminate the possibility that migratory behavior evolved relatively early. If this was the case, migrants could have evolved while temperate niches remained relatively open with subsequent losses of migratory behavior at later times (T. Price pers. comm.). Although the latter scenario and the model presented are statistically indistinguishable according to maximum-likelihood analysis (Fig. 4), parsimony, maximum-likelihood global ancestral character state reconstruction, and historical biogeographic analyses support a southern resident ancestor, with migratory behavior evolving several times.

Models of migrant evolution.—In topology tests, forcing the migrant *Catharus* species to be monophyletic results in a significantly worse tree (Table 3). Our results are unexpected given habitat usage of migrant species. It has been implicitly assumed that migrant *Catharus* species form a monophyletic group, therefore forming a foraging guild between closely related congeners (Noon 1981). Complicating that picture is

the result that migrant species are in some cases more closely related to resident species found in the tropics. That suggests that the migratory species may have converged in morphology and behavior, or if migration evolved only once, then resident species diverged ecologically.

We acknowledge the lack of confidence in the ancestral character state reconstructions, which may imply a migratory origin for the genus. The potential losses of migration (nodes 24, 16, and 22; Figs. 2 and 4) imply the importance of novel environments (where resident congeners are not found) in the loss of migration. If the maximum-parsimony and "entire-tree" maximum-likelihood ancestral state analyses are correct, however, the fact that most migrants do not winter where residents are found supports the potential influence of competition in migrant distributions. It is likely that variation existed in the distance south that migrants traveled, and those individuals that overlapped with resident species were eliminated via selection, leading to the evolution of longer migration distances. The two oldest migratory species, *Catharus ustulatus* and *C. guttatus*, do overlap with resident species, which may be attributable to time for ecological divergence.

Cox's (1985) model predicts that migratory species will be derived from resident species within the seasonal subtropics, and that migratory behavior is a derived character state. Although our data are not well supported, we believe that the *Catharus* lineage fits this model in many ways, particularly because most migrant species are closely related to the resident species found in the seasonal highlands of Mexico and Central America

With regard to other models for the evolution of long-distance migration, Levey and Stiles' (1992) model of a diet-habitat-migration relationship predicts that migrant species would be derived from resident frugivores, open habitat species, or both. That is not the case in the *Catharus* lineage; migrant *Catharus* species do consume more fruit in winter than during breeding (Levey and Stiles 1992), but none of the species is primarily frugivorous, and most occupy forests. As mentioned before, Safriel (1995) and Rappole (1995) provided no explicit predictions.

Conclusion.—An important result of the current study is that migratory species do not form a monophyletic group. The relationships between migrant and resident *Catharus* species

are more complex than we expected, and several interesting points arise from that complexity. Several methods of ancestral character state reconstruction indicate that migratory behavior may be derived, and subsequently that gains in migration outnumber losses. However, given that the confidence in those reconstructions is equivocal, we cannot ignore the possibility that migratory behavior was ancestral with several drop-offs of migration. That possibility is interesting in and of itself, because it indicates that the trait may not be tractable over substantial evolutionary time. Migratory species either evolved several times over the last 4 Ma, or once about 4 mya. The historical biogeographic analyses suggest that the migrants arise from common ancestors shared with resident species of the highlands of Mexico and Central America. The patterns within *Catharus* may be consistent with Cox's model of the seasonal subtropics as a staging area for the evolution of long-distance migration (Cox 1985).

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