MOLECULAR PHYLOGENETICS OF THE BOMBYCILLIDAE AND *LIMOSA* (SCOLOPACIDAE)

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Abstract

The Bombycillidae and their allies and *Limosa* and their allies represent ideal groups in which to use phylogenetic reconstruction to examine historic patterns of intercontinental colonization between North America and Eurasia and the role of intercontinental colonization in diversification. Molecular phylogenetic reconstruction suggests a Neotropical origin for the Bombycillidae and a subsequent colonization of Eurasia via Beringia, which is an exception to the normal pattern of faunal exchange between these two continents. Molecular and morphological phylogenetic reconstructions suggest most relationships within *Limosa* are polytomous. Further analysis of the polytomous relationships indicates that the species of *Limosa* speciated relatively rapidly in relation to the average age of the lineages, and that intercontinental colonization probably played an important role in their diversification.

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Introduction

Elucidating historic patterns of intercontinental colonization can help explain continental biodiversity, current distributions of organisms, and provide insight into future faunal movements. Studies of intercontinental colonization between Eurasia and North America via Beringia have demonstrated a remarkable uniformity in the direction of faunal flow; with few exceptions (Steppan et al., 1999), the direction has been eastward from Eurasia to North America (Talbot and Shields, 1996; Lance and Cook, 1998; Voelker, 1999; Conroy and Cook, 2000). The avian family Bombycillidae (the waxwings) is a good group to study patterns of intercontinental colonization because two species occur in both the Old and New worlds. Where the family originated and in which direction they subsequently colonized is not immediately clear.

Also, large-scale environmental and geological phenomena are expected to promote rapid diversification of lineages through vicariance events (e.g., Vrba, 1993; Hoelzer and Melnick, 1994). The Charadriiformes are primarily coastal-dwelling birds and thus have great potential to be affected by major environmental and geological phenomena that can cause dramatic changes in sea level. Recent evidence suggests a pattern of rapid diversification promoted by major vicariance events (i.e., changes in sea level) at or near the genus level in the order Charadriiformes (*Cepphus* and auklets: *Aethia*, *Ptychoramphus*, and *Cerorhinca*; Kidd and Friesen, 1998; Walsh et al., 1999). *Limosa* (Charadriiformes: Scolopacidae) is a genus of large bodied, primarily coastal-dwelling migratory shorebirds that can be used to further explore this apparent pattern of rapid diversification at or near the genus level in the Charadriiformes.

This study uses DNA sequence data and phylogenetic reconstruction to elucidate the historical biogeography of the Bombycillidae and the pattern of diversification in *Limosa*. Molecular tests of phylogenetic relationships among the Bombycillidae and their putative closest relatives can resolve where the family originated and the direction of subsequent intercontinental colonization. Also, phylogenetic reconstruction of relationships among the species of *Limosa* using sequence data can help illuminate their biogeographic history and their temporal pattern of differentiation to determine whether it is similar to patterns observed in other genera of Charadriiformes.

LITERATURE CITED

Conroy, C. J., and Cook, J. A. 2000. Molecular systematics of a holarctic rodent (*Microtus*: Muridae). – Journal of Mammalogy 81: 344-359.

Hoelzer, G. A., and Melnick, D. J. 1994. Patterns of speciation and limits to phylogenetic resolution. – Trends in Ecology Evolution 9: 104-107.

Kidd, M. G., and Friesen, V. L. 1998. Sequence variation in the guillemot (Alcidae: *Cepphus*) mitochondrial control region and its nuclear homologue. - Molecular Biology and Evolution 15: 61-70.

Lance, E. W., and Cook, J. A. 1998. Phylogeography of tundra voles (*Microtus oeconomus*): Beringia region and southcoastal Alaska. - Journal of Mammalogy 79: 53-65.

Steppan, S. J., Akhverdyan, M. R., Lyapunova, E. A., Fraser, D. G., Vorontsov, N. N., Hoffmann, R. S., and Braun, M. J. 1999. Molecular phylogeny of the marmots

(Rodentia: Sciuridae): Tests of evolutionary and biogeographic hypotheses. - Systematic Biology 48: 715-734.

Talbot, S. L., and Shields, G. F. 1996. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. Molecular Phylogenetics and Evolution 5: 567-575.

Voelker, G. 1999. Dispersal, vicariance, and clocks: Historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). - Evolution 53: 1536-1552.

Vrba, E. S. 1993. Mammalian evolution in the African Neogene and a new look at the great American interchange. - In Goldblatt, J. (ed). Biological Relationships Between Africa and South America. Yale University Press, New Haven, Connecticutt, pp. 393-432.

Walsh, H. E., Kidd, M. G., Moum, T., and Friesen, V. L. 1999. Polytomies and the power of phylogenetic inference. - Evolution 53: 932-937.

Chapter 1.*

Intercontinental colonization and phylogenetic relationships in the avian family Bombycillidae

Abstract

The Bombycillidae and their allies represent an ideal group in which to use phylogenetic reconstruction to examine historic patterns of intercontinental colonization between North America and Eurasia. In this study, we reconstruct a phylogeny of all three species of Bombycillidae (*Bombycilla cedrorum*, *B. garrulus*, and *B. japonica*) and their allies based on 1050 bp of the mitochondrial cytochrome b gene. There is strong support (100% bootstrap values) for the monophyly of the Bombycillidae and strong support for the New World endemic families Dulidae and Ptilogonatidae being their closest relatives. Within the Bombycillidae, each species is monophyletic. A late Miocene (7.13 to 6.83 MYA) Neotropical origin for the Bombycillidae and a subsequent colonization of Eurasia via Beringia are inferred from the phylogeny. This direction of colonization seems to be an exception to the normal pattern of faunal exchange between these continents, which among other taxa is largely from Eurasia to North America.

* Spellman, G. M., and Winker, K. (in preparation). Intercontinental colonization and phylogenetic relationships in the avian family Bombycillidae. *Molecular Phylogenetics and Evolution.*

Introduction

Elucidating historic patterns of intercontinental colonization can help explain continental biodiversity, current distributions of organisms, and provide insight into future faunal movements. Studies of intercontinental colonization between Eurasia and North America via Beringia have demonstrated asymmetry in the direction of faunal flow; with few exceptions (Steppan et al., 1999), the direction has been eastward from Eurasia to North America (Shields et al., 1993; Talbot and Shields, 1996; Karafet et al., 1997; Lance and Cook, 1998; Voelker, 1999; Conroy and Cook, 2000).

With just three species, the avian family Bombycillidae (the waxwings) is a good group to study patterns of intercontinental colonization. All three species of Bombycillidae occur in the Northern Hemisphere, and the avifauna of Eurasia and North America each include two of these species (Fig. 1.1). Using morphological and behavioral data, Arvey (1951) reconstructed a phylogeny of the Bombycillidae and outgroups and suggested that the New World endemic families Dulidae and Ptilogonatidae were their closest relatives. From this phylogeny, Arvey (1951) inferred a Neotropical origin for Bombycillidae and a subsequent colonization of Eurasia, which seems to go against the pattern of colonization between Eurasia and North America observed among other organisms.

With two species occurring in both the Old and New worlds, it is not immediately clear where the family originated. This question can be resolved by a molecular test of phylogenetic relationships of the Bombycillidae and their putative closest relatives. If Dulidae and Ptilogonatidae are the closest relatives of Bombycillidae and the waxwings

are monophyletic, then a colonization of the Old World from the New World can be inferred.

Arvey (1951) also suggested that *Bombycilla garrulus* and *B. cedrorum* are sister taxa, and that the divergence of these taxa was a consequence of intercontinental colonization or isolation of *B. garrulus* in the Old World. On the contrary, Pielou (1991) suggested that *B. garrulus* and *B. cedrorum* diverged prior to colonization of the Old World, and that *B. garrulus* colonized the Old World during the glaciations of the late Pliocene or Pleistocene. Arvey (1951) provided no temporal estimates for colonization, only stating that Bombycillidae was present, in its modern form, by the late Pleistocene (Wetmore 1940). Additionally, Arvey (1951) hypothesized that *B. japonica* was an offshoot of an ancient bombycilline lineage, but no explanation was provided for how or why it came to reside in northeastern Asia. We use molecular phylogenetic reconstruction of relationships of the Bombycillidae and their relatives to determine a) whether *B. garrulus* and *B. cedrorum* are sister taxa, b) whether their divergence was the consequence of intercontinental colonization, and c) whether *B. japonica* was an offshoot of an ancient bombycilline lineage and when and how it came to inhabit its current range.

Methods

Outgroup taxa were selected based on Sibley and Alquist's (1990) suggestions that after Dulidae and Ptilogonatidae the cosmopolitan families Cinclidae, Sturnidae, and Turdidae are the next closest relatives of the Bombycillidae. To provide insight into species monophyly within Bombycillidae, multiple individuals were sequenced (Table 1.1). Redundant sequences were omitted from phylogenetic analyses.

DNA techniques

Whole genomic DNA was extracted from frozen or buffered tissue using a Qia-amp DNA extraction kit (Qiagen Laboratories) following the Qia-amp protocol for tissue extraction. DNA concentrations were quantified using a 260:280 test on an UV spectrophotometer and diluted with water to 20-ng/μL. Extractions and the setup of subsequent PCR amplifications were conducted in a PCR-free laboratory.

A 1050 bp section of the mtDNA cytochrome b gene was amplified via PCR in 50-μL reactions using the primers L14851 and H16064 (Table 1.2). Amplifications were conducted for 30 cycles under conditions of denaturation for 30 s at 94°C, annealing for 1 min at 48°C, and extension for 4 min at 72°C. A 5μl subsample of the amplification reactions was electrophoresed on a 2.5% agarose gel stained with ethidium bromide and checked over UV light for success. Negative controls were run with each reaction to detect any contamination (none was found). PCR products were isolated and cleaned by PEG (polyethylene glycol) precipitation (Kusukawa et al., 1990).

PCR products were used as template for 10-μL cycle sequencing reactions (ABI Prism® termination mix). Cycle sequencing conditions were denaturation for 30 s at 96°C, annealing for 15 s at 50°C, and extension for 4 min at 60°C. The primers used to cycle sequence were L14851, H15299 (H15424), L15350, and H16046 (Table 1.2). Cycle sequenced products were purified using Sephadex® columns, dried in a speed vac, and sequenced in both directions on an ABI model 373 automated sequencer. Sequences were manually aligned using Sequence Navigator (Ver. 1.01; ABI, 1994) and compared with other avian cyt b sequences on Genbank. Sequences were aligned with complete cyt

b sequence of *Gallus gallus* from Genbank to determine first, second, and third codon positions and then translated using MacClade 3.08 (Maddison and Maddison, 1999) to verify that all sequences were coding. All sequences have been deposited in Genbank.

Phylogenetic analyses

Phylogenetic analyses were conducted with PAUP* (Ver. 4.0b4; Swofford, 2000). Initially, a neighbor joining tree was generated. Maximum likelihood (ML) parameters for a number of models were estimated from the neighbor joining tree following Sullivan et al. (1997) and Waits et al. (1999), and a χ^2 test was conducted on the log likelihood scores to determine the simplest model that best explained the data. Maximum likelihood methods alone were used for the final analysis because within the entire data set saturation was observed at third base positions (not shown), and maximum likelihood techniques are more robust to the effects of saturation than parsimony methods (Swofford et al., 1996). The robustness of the phylogeny was tested by bootstrap analysis (100 replicates; Felsenstein 1985), and a Bremer's (1992) ancestral area analysis was applied to the bootstrapped phylogeny to address the biogeographic questions. When the hypothesis that molecular evolution had proceeded in a clocklike manner could not be rejected for a lineage, we used the measure of 2% sequence divergence per million years to estimate times of divergence, a value inferred for diverse avian lineages (Shields and Wilson, 1987; Tarr and Fleischer, 1993; Wood and Krajewski, 1996; Fleischer et al., 1998).

Results

The portion of the cyt b gene sequenced corresponds to positions 14941 to 15991 of the published *Gallus gallus* mitochondrial genome (Desjardins and Morais, 1990). There were 210 parsimony-informative characters in the sequence data. Mean base composition was similar across all taxa and among the bombycillines (0.281% A, 0.339% C, 0.132% G, and 0.248% T). There was an overall transition/transversion ratio of 2.06 (2.71 at first codon positions, 2.26 at second codon positions, and 1.72 at third codon positions), and saturation was detected at third codon positions (data not shown).

Phylogenetics

The likelihood model that best explained the data was GTR + I + Γ (-ln likelihood = 4395.45, I = 0.32, Γ = 0.46). This model was significantly better than the next best models (GTR + I and GTR + Γ; χ^2 = 38.2 and 4.2, d.f. = 1, $p > 0.05$), and was therefore used to reconstruct the phylogeny (Fig. 1.2). Corrected pairwise sequence divergence estimates made using the GTR + I + Γ model ranged from 0.2% to 2.7% within Bombycillidae species, from 6.8% to 15.1% among Bombycillidae species, and from 21.4% to 34.9% among the species of Bombycillidae and the outgroups (Table 1.3). The percent sequence divergence among families averaged 28.2% (range 20.0% to 34.9%).

Dulidae and Ptilogonatidae were strongly supported as the closest relatives of Bombycillidae (96% bootstrap values, Fig. 1.2). This branch separates Bombycillidae, Dulidae, and Ptilogonatidae from the cosmopolitan families Cinclidae, Sturnidae, and Turdidae. Although the data cannot reliably distinguish whether Ptilogonatidae or Dulidae is the closest relative of the Bombycillidae (49% bootstrap values), the

monophyly of the Bombycillidae is strongly supported (100% bootstrap values). To explicitly test the hypotheses that Dulidae and Ptilogonatidae are the closest relatives of Bombycillidae and that the family Bombycillidae is monophyletic, topological constraints were enforced on the phylogeny, log-likelihood values were calculated for the reconstructed trees, and a Kishino-Hasegawa test (Kishino and Hasegawa, 1989) was applied (Table 1.4). A tree constructed with Cinclidae as the closest relative of Bombycillidae, rather than Dulidae or Ptilogonatidae, generated a significantly poorer likelihood score (Table 1.4). However, a tree constructed with Cinclidae positioned between Dulidae and Ptilogonatidae was not significantly different than the optimal tree (Table 1.4). The optimal tree placed Dulidae as the closest relative of the Bombycillidae, but a tree constructed placing Ptilogonatidae as the closest relative of the Bombycillidae was not significantly different from the optimal tree (Table 1.4). Finally, a tree constructed to break the monophyly of the Bombycillidae by placing Dulidae as sister to *B. cedrorum* also generated a significantly poorer likelihood value (Table 1.4).

Each species of Bombycillidae is monophyletic. *B. cedrorum* and *B. garrulus* are not sister taxa, and *B. japonica* is not an offshoot of an ancient bombycilline lineage. Instead, *B. garrulus* and *B. japonica* are sister taxa, and this clade is strongly supported (99% bootstrap values; Fig. 1.2). A tree constructed forcing *B. cedrorum* and *B. garrulus* to be sister taxa was not significantly worse than the optimal tree (Table 1.4); however, the Kishino-Hasegawa (1989) test used to compare the optimal tree (*B. japonica* and *B. garrulus* sister) with the constrained tree (*B. cedrorum* and *B. garrulus* sister) estimates and compares the variance in log-likelihood between the trees to determine whether they

are significantly different from each other. In the optimal tree (Fig. 1.2), most of the variance in log likelihood is contributed by the interfamilial relationships and not by interspecific relationships within Bombycillidae. Thus, it is not surprising that shuffling twigs around in this phylogeny can result in insignificant differences between trees. When the variance in log likelihood is removed from the analysis by using the likelihood ratio test, a tree forcing *B. cedrorum* and *B. garrulus* to be sisters is significantly different from the optimal phylogeny ($\chi^2 = 13.96$, d.f. = 10, $p < 0.05$).

Biogeography

An ancestral area analysis (Bremer, 1992) of the Bombycillidae and their closest relatives (Dulidae and Ptilogonatidae) suggests that a Neotropical origin for Bombycillidae is the most parsimonious explanation for the phylogenetic pattern observed (Fig. 1.3). The most parsimonious biogeographical reconstruction of the observed distributions of the species of Bombycillidae in relation to the phylogeny requires two intercontinental colonization events (Fig. 1.3). The first occurred from the New World to the Old World. To explicitly test whether such an initial colonization alone could explain the current diversity of Bombycillidae in the Old World, we forced the Old World bombycillids to be monophyletic. The resulting tree was significantly worse than the optimal phylogeny (Fig. 1.2; Table 1.4), causing us to reject the hypothesis that only a single intercontinental colonization event occurred in the Bombycillidae.

Divergence estimates

Only a single fossil of an identified Bombycillidae in its extant form exists (Wetmore, 1940), making estimation of divergence times for the family and the species within the family using fossil data impossible. Thus, a molecular clock was enforced on the ML tree (Fig. 1.2) to determine whether evolution could be assumed to have proceeded in a clocklike manner across the entire phylogeny, and a likelihood ratio test was performed. The hypothesis of rate heterogeneity was rejected ($\chi^2 = 17.66$, d.f. = 10, $p < 0.05$), causing us to accept a molecular clock. Dates were estimated using the corrected pairwise distances and standard errors of the corrected pairwise distances obtained using the GTR + I + Γ model (Table 1.3). Our molecular data suggest that the family Bombycillidae originated in the late Miocene (6.98 ± 0.15 million years ago, MYA), that an ancestor of *B. japonica* and *B. garrulus* diverged from *B. cedrorum* 3.42 ± 0.16 MYA, that *B. garrulus* and *B. japonica* diverged 1.88 ± 0.17 MYA, and that the Asian and North American populations of *B. garrulus* were split approximately 0.67 MYA. Although the application of a molecular clock in this case violates many of the assumptions of a perfect molecular clock, such as a phylogeny-specific calibration, an estimate of ancestral sequence polymorphism, and an estimate of ancestral effective population size (Hillis et al., 1996), the rough estimates of divergence times and times of colonization obtained from these analyses can help provide a relative temporal context for these events.

Discussion

Phylogenetics

Phylogenetic reconstruction and hypothesis testing generally support Arvey's (1951) suggestion that Dulidae and Ptilogonatidae are the closest relatives of the Bombycillidae (Fig. 1.2 and Table 1.4). There is a possible exception, however. A tree constructed with Cinclidae positioned between Dulidae and Ptilogonatidae was not significantly different than the optimal tree (Table 1.4), suggesting that Bombycillidae, Dulidae, and Ptilogonatidae may not comprise a monophyletic group as previously hypothesized (Arvey, 1951; Sibley and Alquist, 1991). Thus, these data do not support treatment of these three groups as a single family (Arvey, 1951).

The phylogenetic relationships within Bombycillidae are different from those proposed by Arvey (1951). *B. garrulus* is not sister to *B. cedrorum*, and *B. japonica* is not an off-shoot of an ancient bombycilline lineage. Instead, *B. garrulus* and *B. japonica* are sister taxa.

Biogeography

The direction of the initial colonization inferred for the Bombycillidae is from the New World to the Old World, and poses an exception to the normal pattern of faunal exchange between Eurasia and North America (Hopkins et al., 1982; Shields et al., 1993; Talbot and Shields, 1996; Karafet et al., 1997; Lance and Cook, 1998; Sher, 1999; Conroy and Cook, 2000). Most of these previous studies investigated relatively recent colonization events that probably occurred during mid- to late- Pleistocene glaciations, when North America and Eurasia were a contiguous land mass connected via Beringia. It has been suggested that most colonizations occurred from the Old World to the New World during this time because the North American (and not the Asian) border of

Beringia was usually blocked by glaciers, except during periods of warming and perhaps along coastal refugia (Hopkins et al., 1982; Pielou, 1991; Josenhans et al., 1995; Sher, 1999). However, the pattern inferred for Bombycillidae is deeper (between 3.5 and 1.8 MYA, Late Pliocene), and as a consequence may not reflect the same constraints on intercontinental movements occurring in the mid- to late- Pleistocene. In other words, the directional incongruity in this case may be temporally based, with an early intercontinental colonization event occurring when conditions were different from those predominating during a later period, when colonizations tended to go in the opposite direction.

By our reconstruction, the initial bombycilline intercontinental colonization event was from the New World to the Old World (Fig. 1.3). However, this initial colonization alone cannot explain the diversity of Bombycillidae in the Old World (Fig. 1.2; Table 1.4); more than one crossing of Beringia is necessary to explain the current distributions of the three bombycillid species. The most parsimonious explanation of the observed phylogenetic relationships and current distributions of the bombycillids is that they crossed Beringia twice. This second intercontinental colonization is needed to explain the occurrence of *B. garrulus* in the Old and New worlds. Thus, the initial colonization must be at one of two nodes on the phylogeny (Fig. 1.4): the node separating *B. cedrorum* from the *B. japonica*/*B. garrulus* clade or the node separating *B. japonica* from *B. garrulus*. Conversely, if intercontinental colonization is associated with both of these nodes, a third colonization must be added to the biogeographic reconstruction. The

addition of a third colonization event also implies that intercontinental colonization is associated with speciation, which is not always the case in birds (Zink et al., 1995).

Which of these two nodes is associated with colonization, and in which direction did the second colonization occur? If colonization is associated with the node separating *B. cedrorum* from the *B. japonica/B. garrulus* clade, then *B. japonica* and *B. garrulus* differentiated in the Old World, and *B. garrulus* subsequently recolonized the New World (Fig. 1.4A). On the other hand, if colonization is associated with the node separating *B. japonica* and *B. garrulus*, then an ancestor of *B. japonica* and *B. garrulus* differentiated from *B. cedrorum* in the New World and the Bombycillidae colonized the Old World twice (once by an ancestor of *B. japonica* and once by an ancestor of *B. garrulus centralasiae*; Fig. 1.4B). These explanations are equally parsimonious, each requiring two intercontinental colonizations and a speciation event not associated with intercontinental colonization. Given these considerations, we cannot infer which node is associated with the initial colonization event or in which direction the second colonization event occurred. Two additional considerations cause us to consider the first scenario marginally more probable. First, unlike New World *B. garrulus*, Old World *B. garrulus* has differentiated into two subspecies (*B. garrulus centralasiae* and *B. g. garrulus*), possibly indicating a longer presence. Secondly, under this scenario the direction of the second intercontinental colonization is in congruence with the normal direction of faunal flow at that time. Neither of these lines of evidence is particularily strong, however. Further phylogeographic study of the Asian and North American

populations of *B. garrulus* and of *B. japonica* may help determine which of these two scenarios is more likely.

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Literature Cited

Applied Biosystems (ABI). (1994). Sequence Navigator, Version 1.01. Perkin-Elmer, Foster City, CA.

American Ornithologist's Union (AOU). (1998). "Check-list of North American birds," 7th Ed. Amer. Ornithol. Union, Washington, D.C.

Arvey, M. D. (1951). Phylogeny of the waxwings and allied birds. *Univ. Kan. Pub.: Mus. Nat. Hist.* **3**: 473-530.

Brazil, M. A. (1991). "The Birds of Japan," Smithsonian Institution Press, Washington, D.C.

Bremer, K. (1992). Ancestral areas: a cladistic reinterpretation of the center of origin concept. *Syst. Biol.* **41**: 436-445.

Conroy, C. J., and Cook, J. A. (2000). Molecular systematics of a holarctic rodent (*Microtus*: Muridae). *J. Mammal.* **81**: 344-359.

Cramp, S., Brooks, D. J., Dunn, E., Gillmor, R., Hall-Crags, J., Hollom, P. A. D., Nicholson, E. M., Ogilvie, M. A., Roselaar, C. S., Sellar, P. J., Simmons, K. E. L., Voous, K. H., Wallace, D. I. M., and Wilson, M. G. (1988). "Handbook of the Birds of Europe, the Middle East, and North Africa: The birds of the Western Palearctic Volume V – Tyrant Flycatchers to Thrushes," Oxford University Press, Oxford.

Desjardins, P., and Morais, R. (1990). Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *J. Mol. Biol.* **212**: 599-634.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.

Fleischer, R. C., McIntosh, C. E., and Tarr, C. L. (1998). Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rate. *Mol. Ecol.* **7**: 533-545.

Flint, V. E., Boehme, R. L., Kostin, Y. V., and Kuznetsov, A. A. (1984). "A Field Guide to the Birds of the USSR," Princeton University Press, Princeton, New Jersey.

Hackett, S. J. (1996). Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Mol. Phylogenet. Evol.* **5**: 368-382.

Harshman, J. (1996). Phylogeny, evolutionary rates, and ducks. Ph.D. dissertation, University of Chicago, Chicago.

Hillis, D. M., Mable, B. K., and Moritz, C. (1996). Applications of molecular systematics: the state of the field and a look to the future. *In* "Molecular Systematics, 2nd Edition." (Hillis, D. M., Moritz, C., and Mable, B. K., Eds.), pp. 515-543. Sinaur Associates, Inc. Sunderland, Mass., U.S.A.

Hopkins, D. M., Matthews, J. V. Jr., Schweger, C. E., and Young, S. B. (1982). "Paleoecology of Beringia," Academic Press, Inc. New York, New York.

Josenhans, H. W., Fedle, D. W., Conway, K. W., and Barrie, J. V. (1995). Postglacial sea levels on the western Canadian continental shelf: evidence for rapid change, extensive subaerial exposure, and early human habitation. *Mar. Geol.* **125**: 73-94.

Karafet, T., Segura, S. L., Vuturo-Brady, J., Posukh, O., Osipova, L., Wiebe, V., Romero, F., Long, J. C., Harihara, S., Jin, F., Dashnyam, B., Gerelsaikhan, T., Omoto, K., and K. Hammer, K. (1997). Y chromosome markers and trans-Bering Strait dispersals. *Amer. J. Physical Anthropology.* **102**: 301-314.

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of Hominoidea. *J. Mol. Evol.* **29**: 170-179.

Klicka, J., and Zink, R. M. (1997). The importance of recent ice ages in speciation: a failed paradigm. *Science* **277**: 1666-1669.

Kornegay, J. R., Kocher, T. D., Williams, L. A., and Wilson, A. C. (1993). Pathways of lysozyme evolution inferred from the sequences of cytochrome b in birds. *J. Mol. Evol.* **37**: 367-379.

Kusukawa, N., Uemori, T., Asada, K., and Kato, I. (1990). Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. *Biotechniques* **9**: 66-72

Lance, E. W., and Cook, J. A. (1998). Phylogeography of tundra voles (*Microtus oeconomus*): Beringia region and southcoastal Alaska. *J. of Mammal.* **79**: 53-65.

Maddison, W. P., and Maddison, D. R. (1999). MacClade: Analysis of phylogeny and character evolution, version 3.08. Sinauer, Sunderland, Massachusetts.

Pielou, E. C. (1991). "After the Ice Age," The University of Chicago Press. Chicago, Illinois.

Peters, J. L. (1960). "Check-list of Birds of the World, Vol. IX," E. Mayr and J.C. Greenway, Jr., Eds. Museum of Comparative Zoology, Cambridge, Massachusetts.

Sher, A. (1999). Traffic lights at the Beringian crossroads. *Nature* **397**: 103-104.

Shields, G. F., and Wilson, A. C. (1987). Calibration of mitochondrial DNA evolution in geese. *J. Mol. Evol.* **24**: 212-217.

Shields, G. F., Schmiechen, A. M., Frasier, B. L., Redd, A., Voevoda, M. I., Reed, J. K., and Ward, R.H. (1993). MtDNA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. *Am. J. Hum. Genet.* **53**: 549 –562.

Sibley, C. G., and Alquist, J. E. (1990). "Phylogeny and Classification of Birds: A Study in Molecular Evolution," Yale University Press, New Haven, Connecticut.

Steppan, S. J., Akhverdyan, M. R., Lyapunova, E. A., Fraser, D. G., Vorontsov, N. N., Hoffman, R. S., and Braun, M. J. (1999). Molecular phylogeny of the Marmots (Rodentia: Sciuridae): Tests of evolutionary and biogeographic hypotheses. *Syst. Biol.* **48**: 715-734.

Sullivan, J., Markert, J. A., and Kilpatrick, C. W. (1997). Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. *Syst. Biol.* **46**: 426-440.

Swofford, D. L. (2000). PAUP*: Phylogenetic analysis using parsimony, version 4.0b4. Sinauer Press, Sunderland, Massachusetts.

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. *In* "Molecular Systematics, 2nd Edition." (Hillis, D. M., Moritz, C., and Mable, B. K., Eds.), pp. 407-514. Sinaur Associates, Inc. Sunderland, Mass., U.S.A.

Talbot, S. L., and Shields, G. F. (1996). A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. *Mol. Phylogenet. Evol.* **5**: 567-575.

Tarr, C. L., and Fleischer, R. C. (1993). Mitochondrial DNA variation and evolutionary relationships in the 'Amakihi' complex. *Auk* **110**: 825-831.

Voelker, G. (1999). Dispersal, vicariance, and clocks: Historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). *Evolution* **53**: 1536-1552.

Waits, L. P., Sullivan, J., O'Brien, S. J., and Ward, R. H. (1999). Rapid radiation in the family Ursidae indicated by likelihood phylogenetic estimation from multiple fragments of mtDNA. *Mol. Phylogenet. Evol.* **13**: 82-92.

Wetmore, A. (1940). A check-list of the fossil birds of North America. *Smithson. Misc. Coll.* **94**: 1-88.

Wood, T. C., and Krajewski, C. (1996). Mitochondrial DNA sequence variation among subspecies of Sarus Crane (*Grus antigone*). *Auk* **113**: 655-663.

Zink, R. M., Rohwer, S, Andreev, A., and Dittmann, D. L. 1995. Trans-Beringian comparisons of mitochondrial DNA differentiation in birds. - Condor 97: 639-649.

Table 1.1. Specimens used in this study. *Turdus migratorius* and *Catharus guttatus* sequences were obtained from Genbank (accession numbers AF197835 and X74261, respectively).

Species	Locality	Voucher[1]
Bombycilla cedrorum	Minnesota	UAM 9948
B. cedrorum	Minnesota	UAM 8483
B. cedrorum	Alaska	UAM 10056
B. garrulus pallidiceps	Alaska	UAM 9901
B. g. pallidiceps	Alaska	UAM 8481
B. g. centralasiae	Japan	UMMZ 234981
B. g. centralasiae	Japan	UMMZ 234982
B. japonica	Japan	UMMZ 234979
B. japonica	Japan	UMMZ 234833
Outgroups:		
Dulus dominicus (Dulidae)	Hispaniola	UAM 8874
Phainopepla nitens (Ptilogonatidae)	New Mexico	UAM 10129
Cinclus mexicanus (Cinclidae)	Alaska	UAM 10133
Sturnus vulgaris (Sturnidae)	Minnesota	UAM 9964

[1] UAM = University of Alaska Museum; UMMZ = University of Michigan Museum of Zoology.

Table 1.2. Primer names, sequences, and sources used for PCR and sequencing reactions.

Primer name	Sequence	Source
L14851	5'-CCTACTTAGGATCATTCGGCCT-3'	Kornegay et al. (1993)
H15424	5'-GGAGGAAGTGCAGGGCGAAGAATCG-3'	Hackett (1996)
L15350	5'-TTACAAACCTATTCTCAGC-3'	Klicka and Zink (1997)
H16064(H4A)	5'-CTTCAGTCTTTGGTTTACAAGACC-3'	Harshman (1996)

Table 1.3. Total number of nucleotide differences between taxon pairs (above diagonal) and corrected pairwise distances calculated from the ML model (GTR + I + Γ) used to reconstruct the phylogeny (below diagonal).

	1	2	3	4	5	6	7	8	9	10	11
1 *Bombycilla japonica*	-	51	60	77	78	144	147	153	158	136	157
2 *B. garrulus* (North America)	0.068	-	25	86	87	142	146	161	156	141	161
3 *B. garrulus* (Japan)	0.082	0.027	-	91	92	147	152	163	166	146	162
4 *B. cedrorum* (Minnesota)	0.116	0.143	0.150	-	2	149	154	160	157	130	145
5 *B. cedrorum* (Alaska)	0.118	0.145	0.151	0.002	-	148	156	162	159	131	145
6 *P. nitens*	0.260	0.253	0.268	0.281	0.277	-	164	158	168	149	154
7 *D. dominicus*	0.269	0.265	0.288	0.291	0.299	0.319	-	152	167	156	161
8 *C. guttatus*	0.281	0.320	0.324	0.308	0.316	0.290	0.265	-	122	124	134
9 *T. migratorius*	0.31	0.309	0.349	0.305	0.314	0.349	0.333	0.203	-	138	142
10 *C. mexicanus*	0.235	0.259	0.269	0.215	0.217	0.271	0.294	0.200	0.246	-	127
11 *S. vulgaris*	0.307	0.323	0.319	0.255	0.256	0.284	0.312	0.227	0.248	0.210	-

Table 1.4. Results of Kishino-Hasegawa tests.

Hypothesis	Topological constraint	Difference in –ln likelihood	s.d.	p
Bombycillidae monophyletic	((*B. cedrorum*)Dulidae)	53.59	11.8	0.0001
Dulidae and Ptilogonatidae closest relatives of Bombycillidae	((Bombycillidae)Cinclidae)	24.13	10.1	0.017
Dulidae, Ptilogonatidae, Bombycillidae are a monophyletic group	((Bombycillidae Dulidae Cinclidae)Ptilogonatidae)	13.68	7.64	0.074
Dulidae, Ptilogonatidae, Bombycillidae are a monophyletic group	((Bombycillidae Dulidae Sturnidae)Ptilogonatidae)	16.32	7.93	0.04
Ptilogonatidae, as opposed to Dulidae, closest relative of Bombycillidae	(((Bombycillidae)Ptilogonatidae) Dulidae)	0.66	4.03	0.87
B. garrulus and *B. cedrorum* sister taxa	((*B. garrulus B. cedrorum*)*B. japonica*)	6.98	4.29	0.10
Single crossing of Beringia by the Bombycillidae	((*B. garrulus centralasiae B. japonica*))	16.38	6.9	0.018

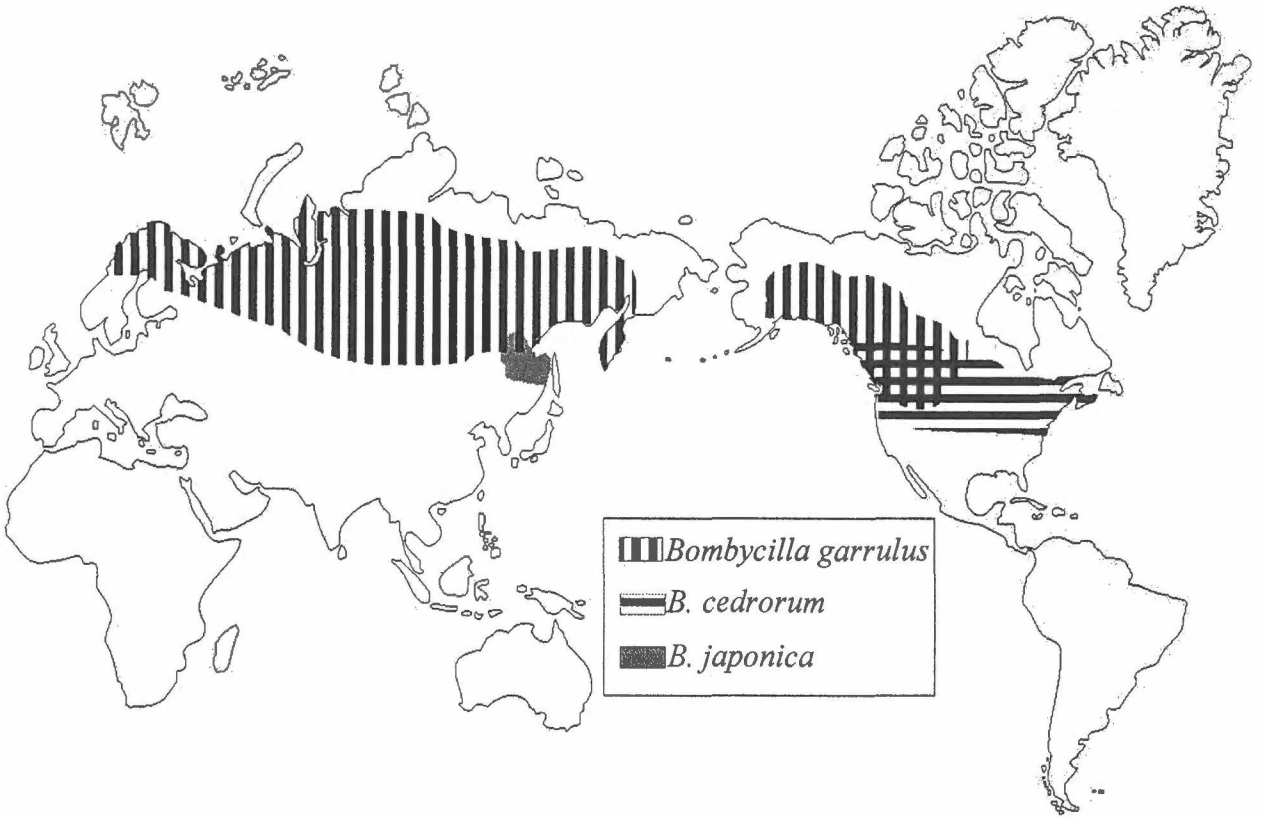

Figure 1.1. Breeding distributions of the species of Bombycillidae (Flint et al., 1984; Cramp et al., 1988; Brazil, 1991; AOU, 1998).

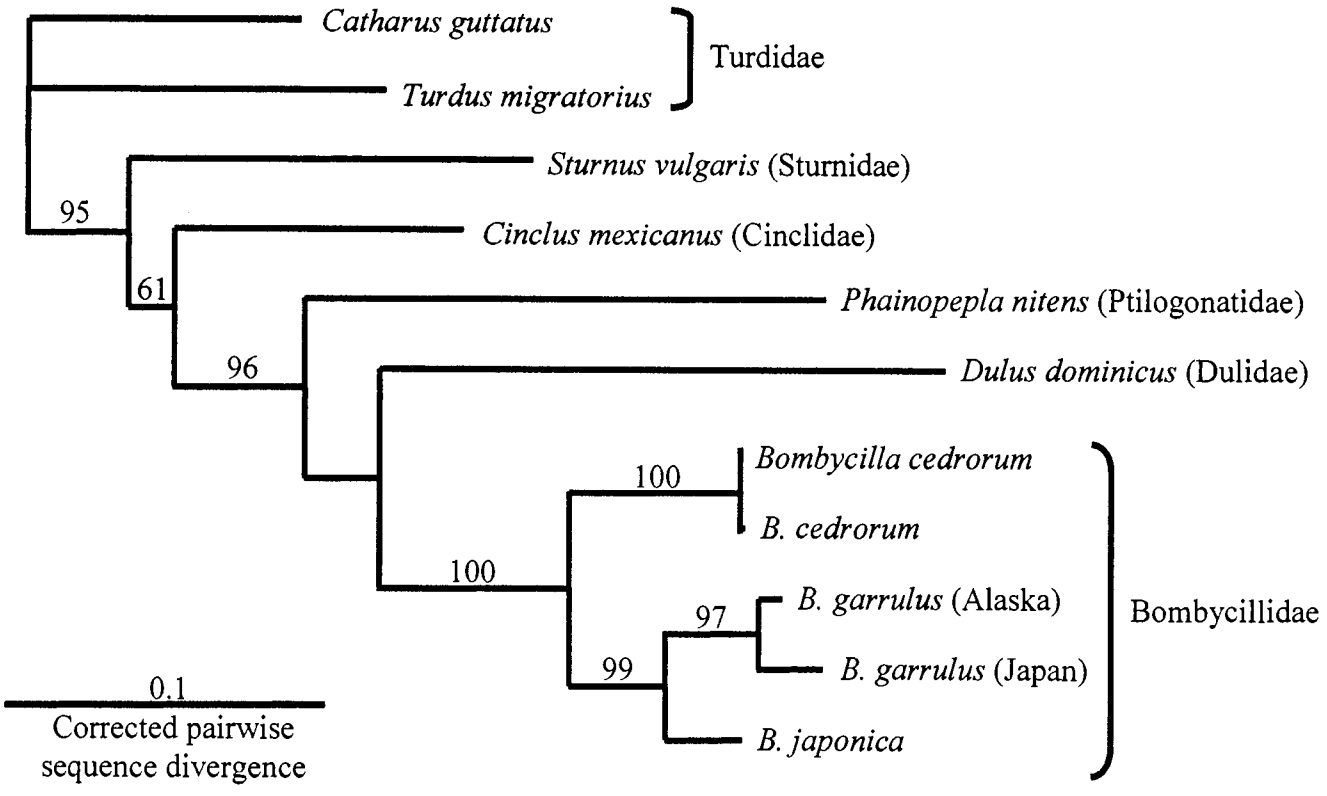

Figure 1.2. Maximum likelihood phylogeny of the Bombycillidae and their allies based on a GTR + I + Γ model of molecular evolution. Numbers above branches represent bootstrap support values greater than 50 (100 replicates).

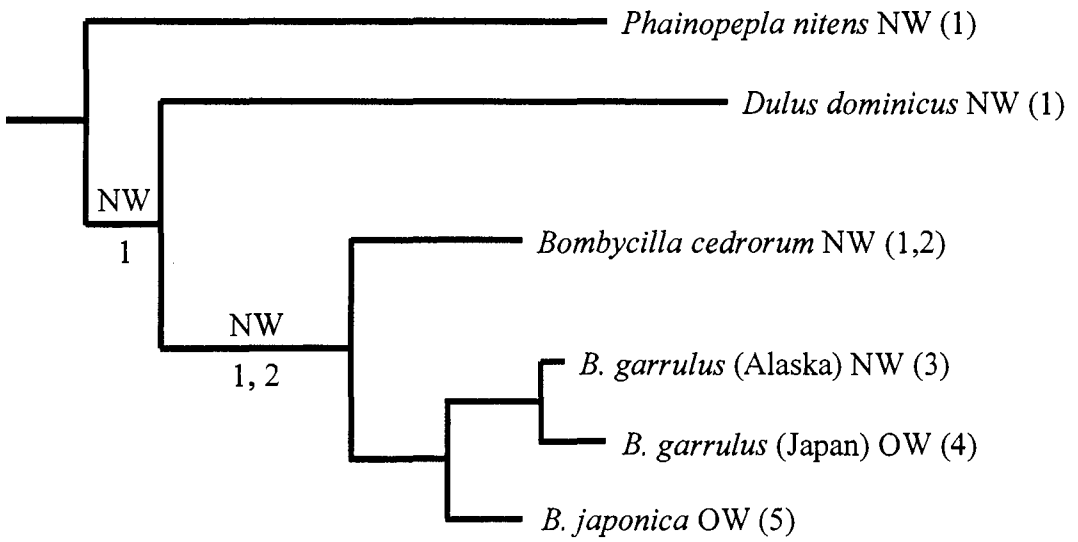

Figure 1.3. Area cladogram (based on the ML phylogeny) used in the ancestral area analysis (Bremer, 1992) and ancestral area reconstructions. Area designations are the following: NW = New World, OW = Old World; 1 = Neotropic, 2 = Neotemperate, 3 = Nearctic, 4 = Palearctic, and 5 = Paleotemperate.

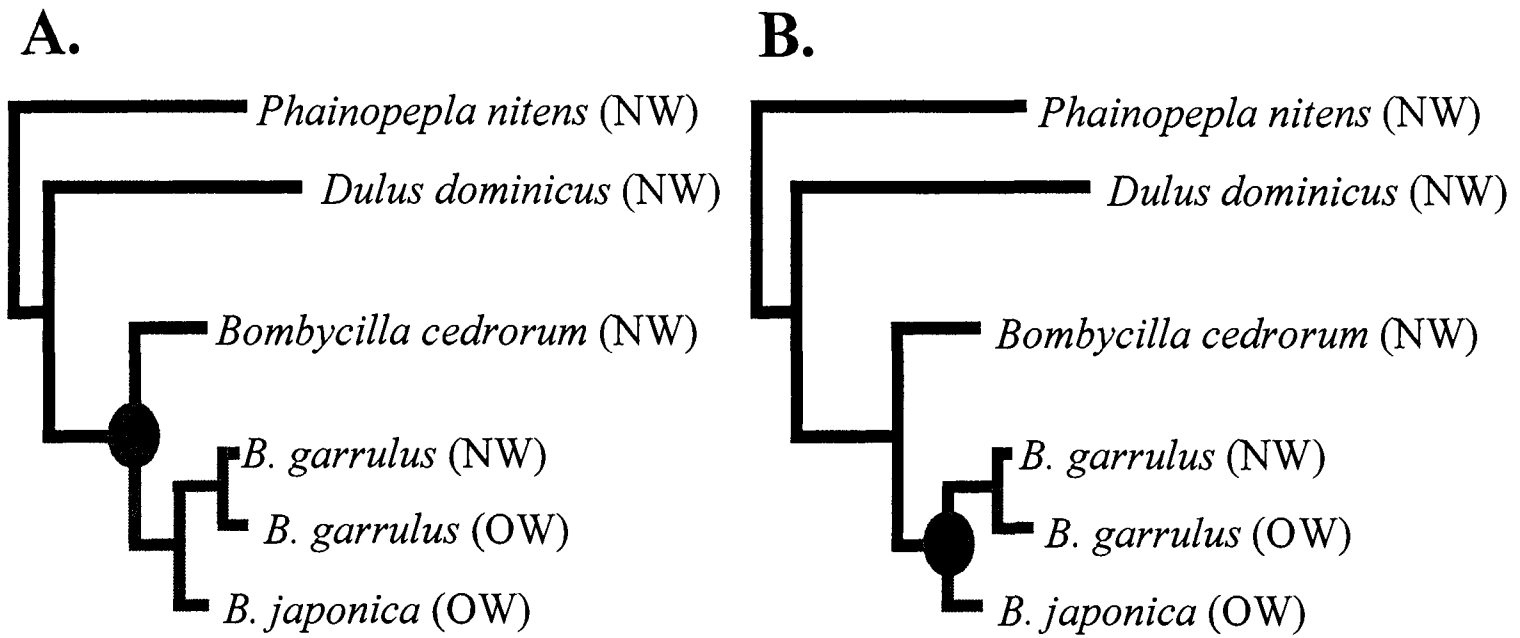

Figure 1.4. Two equally parsimonious scenarios for colonization between the Old and New worlds. The gray dot represents the initial colonization of the Old World, and the gray color indicates lineages in the Old World. A) Indicates an initial colonization of the Old World followed by a recolonization of the New World. B) Indicates two independent colonizations of the Old World.

Chapter 2.*

Systematics of the godwits (Scolopacidae: *Limosa*): rapid diversification and intercontinental colonization.

Abstract

Phylogenetic relationships among the four species of the avian genus *Limosa* (Charadriiformes: Scolopacidae) and outgroups (*Numenius phaeopus, Limnodromus scolopaceus,* and *Tringa melanoleuca*) were analyzed using data from the mitochondrial genes cytochrome b (1019 base pairs) and 12S rRNA (462 base pairs) and from 22 external morphological characters. Maximum likelihood analyses of sequence data and maximum parsimony analysis of morphological data suggest the genus *Numenius* is the closest relative of *Limosa*. *Limosa haemastica* and *L. limosa* are supported as sister taxa on the morphological phylogeny. There is relatively strong support on the cyt b phylogeny that the primarily Old World species *L. lapponica* was the first *Limosa* lineage to diverge, suggesting a possible Old World origin and subsequent New World colonization. In all phylogenies, most relationships within *Limosa* remain equivocal and result in an unresolved polytomy. Further analysis of the polytomous relationships suggests that the species of *Limosa* speciated relatively rapidly (<0.09 MY) in relation to the average age of the lineages(~6.27 ± 0.61 MY old), and that intercontinental colonization probably played an important role in their diversification.

* Spellman, G. M., and Winker, K. (in preparation). Systematics of the godwits (Scolopacidae: Limosa): rapid diversification and intercontinental colonization. *Journal of Avian Biology.*

Introduction

Large-scale environmental and geological phenomena are expected to promote the rapid diversification of lineages through vicariance events (e.g., Vrba 1993, Hoelzer and Melnick 1994). The Charadriiformes are primarily coastal-dwelling birds and thus have great potential to be affected by major environmental and geological phenomena that can cause dramatic changes in sea level. Recent evidence suggests a pattern of rapid diversification promoted by major vicariance events (i.e., changes in sea level) at or near the genus level in the order Charadriiformes (among the guillemot genus *Cepphus* and the auklet genera: *Aethia*, *Ptychoramphus*, and *Cerorhinca*; Kidd and Friesen 1998, Walsh et al. 1999).

Limosa (Charadriiformes: Scolopacidae) is a genus of large bodied, primarily coastal-dwelling migratory shorebirds that can be used to further explore this apparent pattern of rapid diversification at or near the genus level in the Charadriiformes. *Limosa* is comprised of four species: *Limosa limosa*, *L. lapponica*, *L. haemastica*, and *L. fedoa.* Phylogenetic reconstruction of relationships among these species using sequence data can help illuminate biogeographic history and the temporal pattern of differentiation to determine whether it is similar to patterns observed in other genera of Charadriiformes.

Also, the distributions of the species of *Limosa* are conducive to exploring the role in diversification of intercontinental colonization between North America and Eurasia (Fig. 2.1). With two species occurring in the Old World and three in the New World, it is not immediately clear whether the genus originated in the New or Old worlds, or whether intercontinental colonization played an important role in its

diversification. The role of intercontinental colonization in the diversification of *Limosa* can be addressed by molecular and morphological reconstructions of phylogenetic relationships of *Limosa* and their closest relatives. Two previous attempts to reconstruct the phylogenetic relationships of the Charadriiformes using osteological characters failed to provide resolution within the genus *Limosa* (Strauch 1978, Chu 1996), and these relationships remain unknown.

Methods

Outgroup taxa were selected based on the suggestion that the genera *Limnodromus*, *Numenius*, and *Tringa* are the closest relatives of *Limosa* (Peters 1934, Sibley and Alquist 1990, Chu 1996, AOU 1998). *Numenius phaeopus hudsonicus* and *N. p. variegatus* were both sequenced to compare with previously recorded mitochondrial DNA divergence between these two subspecies (Zink et al. 1995). To provide insight into species monophyly and evolution within the genus, multiple individuals of each species of *Limosa* were sequenced (Table 2.1). Sample availability precluded the inclusion of all subspecies. Redundant sequences were omitted from the phylogenetic analyses.

DNA methods

Whole genomic DNA was extracted from frozen or buffered tissue using a diatomaceous earth extraction method (Carter and Milton 1993). DNA was extracted from skin samples for *L. fedoa* using a Qia-amp DNA extraction kit (Qiagen Laboratories) following the Qia-amp protocol for tissue extraction. DNA concentrations were quantified using a 260:280 test on a spectrophotometer and diluted with water to 20-

ng/μL. Extractions and the setup of subsequent PCR amplifications were conducted in a PCR-free laboratory.

A 1019 base pair section of the cytochrome b gene and a 462 base pair section of the 12S rRNA gene of the mtDNA were amplified via PCR in 50-μL reactions using the primer pairs L14841/H16065 and 12S L281/12S H745 (Table 2.2). The cyt b gene for the *L. fedoa* samples taken from museum skins was amplified via PCR in smaller fragments (705 bp and 637 bp) using the following primer pairs: L14841/H637 and L519/H16065 (Table 2.2). Amplifications were conducted for 30 cycles under conditions of denaturation for 30 s at 94°C, annealing for 1 min at 55°C (for tissue extractions) or 48°C (for skin extractions), and extension for 4 min at 72°C. A 5-μL subsample of the amplification reactions was electrophoresed on a 2.5% agarose gel stained with ethidium bromide and checked over UV light for success. Negative controls were run with each set of reactions to detect any contamination (none was detected). Amplifications from fresh tissue were isolated and cleaned by PEG (polyethylene glycol) precipitation (Kusukawa et al. 1990). Amplified products from skin samples were isolated, cleaned, and concentrated using a Qiagen DNA Purification Kit (Qiagen Laboratories) following the microcentrifuge protocol to limit loss of amplified product.

PCR products were used as template for 10-μL cycle sequencing reactions (ABI Prism® termination mix). Cycle sequencing conditions were denaturation for 30 s at 96°C, annealing for 15 s at 50°C, and extension for 4 min at 60°C. Primers used for cycle sequencing reactions were L14841, H519, L637, and H16065 for cytochrome b and 12S L281 and 12S H745 for 12S rRNA (Table 2.2). Cycle sequenced products were

purified in Sephadex® columns, dried in a speed vac, and sequenced in both directions on an ABI model 373 automated sequencer. Sequences were manually aligned using Sequence Navigator (Ver. 1.01 ABI 1994). Cyt b sequences were aligned with a cyt b sequence of *Gallus gallus* from Genbank to determine first, second, and third codon positions, then checked on MacClade (Ver. 3.08 Maddison and Maddison 1999) to ensure that all sequence was coding. The 12S sequences were aligned with 12S sequences from *Scolopax minor* and *Gallus gallus* from Genbank to determine homology. All sequences were deposited on Genbank.

Morphology

External morphological characters were compared across ingroup and outgroup species. From this comparison, 22 characters were defined and scored (Appendices 1 and 2). Comparisons were made between adult females in breeding plumage, except for characters 9, 10, and 17, which are heterosexual and thus were scored between females and males in breeding plumage.

Phylogenetic analyses

Phylogenetic analyses were done using PAUP* (Ver. 4.0b4 Swofford 2000). First the cyt b sequences were analyzed alone, then a combined analysis of both the cyt b and 12S sequences was performed using the same process: a neighbor-joining tree was generated using Tamura-Nei (1993) distances, then maximum likelihood parameters for a number of models were estimated from this tree (Sullivan et al. 1997, Waits et al. 1999), and a χ^2 test was conducted to establish the simplest model that best explained the data. The 22 morphological characters were analyzed using unweighted maximum parsimony

methods (heuristic search with 10 random seed replicates). The robustness of all trees was tested by bootstrap analyses with 100 replicates for the molecular trees and 10,000 replicates for the morphological tree (Felsenstein 1985).

Results

The portion of the cyt b gene amplified corresponds to positions 14995 to 16014 of the published *Gallus gallus* mitochondrial genome (Desjardins and Morais 1990). There were 230 parsimony-informative characters in the cyt b data and 86 parsimony-informative characters in the 12S data. Base composition of the cyt b data was similar across taxa and among the *Limosa* (0.275% A, 0.328% C, 0.135% G, and 0.262% T). The overall transition/transversion ratio was 3.89 for the cyt b data and 4.20 for the 12S data. Saturation was detected at third codon positions for the cyt b data (not shown).

Molecular Phylogenetics

The model with fewest variable parameters that best explained the cyt b data was the general time reversible model plus a discrete gamma distribution (GTR + Γ; Γ = 0.279, -ln likelihood = 3878.37). This model was significantly better than the most complex model (GTR + I + Γ; $\chi^2 = 2.4$, d.f. = 1, $p < 0.05$). Corrected pairwise sequence divergence calculated using this model ranged from 0.1% to 6.2% within species, 11.7% to 19.0% among species of *Limosa*, and 26.1% to 36.9% among genera (Table 2.3). The model that best explained the cyt b + 12S data was the general time reversible model plus the proportion of invariable sites plus a discrete gamma distribution (GTR + I + Γ; I = 0.415, Γ = 0.698, -ln likelihood = 5305.51); this model was significantly better than the next less-complex models GTR + I and GTR + Γ ($\chi^2 = 38.2$ and 4.2 respectively, d.f. = 1,

$p > 0.05$). Corrected pairwise sequence divergence calculated using this model ranged from 0.1% to 4.9% within species, 10.6% to 18.1% among species of *Limosa*, and 24.2% to 36.1% among genera (Table 2.3). The two subspecies of *Numenius phaeopus* were 6.2% and 4.9% divergent, respectively, in the two analyses, which corroborates previous findings using RFLP methods (Zink et al. 1995). The mean difference between the corrected pairwise distances of the two models and analyses (cyt b and cytb + 12S) was 2.7% ± 0.23. These differences between the distance measurements are probably due to the extra parameter (I) in the model used to calculate the pairwise distances for the combined analysis of cyt b and 12S data and the slower evolutionary rate of the 12S rRNA gene (Moum et al. 1994).

Bootstrapped phylogenies from both models (GTR + Γ and GTR + I + Γ) strongly support the globally distributed genus *Numenius* as the closest relative of *Limosa* (98% and 100% bootstrap values, respectively), which is contrary to other recent hypotheses (Sibley and Alquist 1990, Chu 1996; Figs. 2.2 and 2.3). A tree constructed to directly violate this relationship (e.g., *Limnodromus* as the closest relative of the *Limosa*) using the GTR + Γ model and compared to the optimal tree using a Kishino-Hasegawa (1989) test was not significantly different (difference in –ln likelihood = 7.35, s.d. = 4.52, p = 0.1). Conversely, the same constraint enforced using the GTR + I + Γ model produced a tree that was significantly poorer than the optimal tree (difference in –ln likelihood = 25.77, s.d. = 9.02, p = 0.004). Thus, even though there is some discrepancy between tests, there is relatively strong support that *Numenius* is the closest relative of *Limosa*.

There is strong support for the monophyly of *Limosa* (100% and 99% bootstrap values). Trees constructed to directly violate this relationship (e.g., *Numenius* within the genus *Limosa*) were significantly poorer than the optimal trees produced by both models (GTR + Γ, difference in –ln likelihood = 140.46, s.d. = 18.03, $p < 0.0001$; GTR + I + Γ, difference in –ln likelihood = 44.45, s.d. = 11.17, $p = 0.0001$). The cyt b phylogeny suggests that *L. lapponica* was the first to diverge, with the branch supporting the three remaining species (*L. limosa*, *L. haemastica*, and *L. fedoa*) being short and moderately well supported (66% bootstrap values; Fig. 2.2). Each species of *Limosa* is highly divergent from the other species, as evidenced by the long branch lengths and high pairwise sequence divergences (10.6% to 18.1%).

Most relationships within *Limosa* remain equivocal and result in an unresolved polytomy for either three species of *Limosa* (*L. limosa*, *L. haemastica*, and *L. fedoa*) using just the cyt b data, or for the entire genus using the combined cyt b and 12S data (Figs. 2.2 and 2.3). To further examine these polytomies, we applied a molecular clock (because of a lack of fossil data for the *Limosa*) to attempt to correlate the divergences with environmental events and synchronous divergences with other taxa (Vrba 1993, Hoelzer and Melnick 1994). We also performed an *a posteriori* power analysis to assess the power of the sequence data to resolve the phylogenetic relationships within *Limosa* (Walsh et al. 1999).

Molecular clock

A molecular clock was enforced on the ML trees, rooted at the outgroup *Tringa melanoleuca*, and a likelihood ratio test was performed to see if the hypothesis of rate

heterogeneity could be rejected for both full phylogenies. Rate heterogeneity was not rejected for either full phylogeny. Because we were only interested in dating the divergences of the species within *Limosa*, we also tested for rate heterogeneity within the genus using one representative from each species of *Limosa* and one representative from *Numenius*. The hypothesis of rate heterogeneity for the species within the genus *Limosa* was rejected for the cyt b data ($\chi^2 = 8.86$, d.f. = 4, $p < 0.05$), but was not for the cyt b + 12S data.

This difference between the cyt b and cyt b + 12S data sets is most likely a residual effect of the evolution of the 12S rRNA gene, which has hypervariable loop regions where substitutions and indels are frequent as well as extremely conservative regions between the loops, precluding rate constancy across the gene (Moum et al. 1994). Thus, clocklike evolution across the 12S gene is highly unlikely. To date the divergences of the species of *Limosa*, we accepted a molecular clock for the cyt b data alone and using the corrected pairwise distances obtained from the cyt b data (Table 2.3), used the estimate of 2% sequence divergence per million years, a value inferred for many diverse avian lineages (Shields and Wilson 1987, Tarr and Fleischer 1993, Wood and Krajewski 1996, Fleischer et al. 1998). Although the application of a molecular clock in this case violates many of the assumptions of a perfect molecular clock, such as a phylogeny-specific calibration, an estimate of ancestral sequence polymorphism, and an estimate of ancestral effective population size (Hillis et al. 1996), the rough estimates of divergence times obtained from this analysis can help provide a relative temporal context for these events. Divergence time estimates for *L. limosa*, *L. haemastica*, and *L. fedoa* (stemming

from the cyt b polytomy; Fig. 2.2) suggest that these species diverged 6.3 ± 0.61 million years ago (MYA), and that *L. lapponica* diverged 8.3 ± 0.34 MYA.

Power analysis

Power analysis can be used to determine the amount of sequence data needed to resolve phylogenetic relationships among taxa in which speciation occurred within a certain period (years) at a certain time in the past (Walsh et al. 1999). In other words, this test attempts to distinguish between a lack of effect (i.e., rapid or nearly simultaneous speciation) and the effect of insufficient data (Walsh et al. 1999). The analysis can be used to construct a curve plotting the relationship between the amount of sequence data equivalent to the existing data and estimated divergence time. We used the equations for difference of means using maximum likelihood distances to assess the resolving power of the data and to construct the corresponding curve (because these equations were shown to outperform other equations; Walsh et al. 1999). The analysis requires preliminary sequence data and a time estimate for the divergences of the taxa being studied. Divergence times based on fossil evidence were not available, so we used the 1019 bp of the cyt b distance data (Table 2.3) and a divergence time estimate of 6.27 MYA (taken from the molecular clock analysis of the cyt b data) for the species stemming from the polytomy (*L. limosa*, *L. haemastica*, and *L. fedoa*; Fig. 2.2).

The resulting curve demonstrates an exponential relationship between the period of divergence and the amount of sequence data necessary to resolve the corresponding phylogenetic relationships (Fig. 2.4). Also, the curve indicates that 1019 bp of cyt b should be enough to resolve relationships among the three *Limosa* species if they

diverged within a time period greater than or equal to 90,000 years. This value is very small, only 1.4% of the age of the lineages, in relation to the estimated origin of these three species of *Limosa* (6.27 ± 0.61 MYA).

Morphological phylogenetics

There were 13 parsimony-informative characters in the external morphological data. The heuristic search produced three equally parsimonious trees. These trees were combined in a strict consensus tree, and bootstrap analysis was performed on the consensus tree (Fig. 2.5). The bootstrapped phylogeny corroborates the molecular data, with support for *Numenius* as the closest relative of *Limosa* and for the monophyly of the godwits (63% and 68% bootstrap values respectively; Fig. 2.5). There is strong support that *L. limosa* and *L. haemastica* are sister taxa (81% bootstrap values). However, the other relationships within *Limosa* are polytomous.

Biogeography

The polytomous relationships within *Limosa* in both the molecular and morphological phylogenies make biogeographical analysis difficult. However, one inference can be made. The initial split between *L. lapponica* and the remaining *Limosa* species on the cyt b phylogeny (Fig. 2.2) suggests that *Limosa* may have originated in the Old World and subsequently colonized the New World.

Discussion

Rapid diversification in Limosa

There is an obvious lack of resolution within the *Limosa* in all phylogenies (Figs. 2.2, 2.3, and 2.5). Is this an artifact of the poor resolving power of the sequence data

(i.e., a 'soft' polytomy; Maddison 1989, DeSalle et al. 1994), or was speciation within *Limosa* rapid (i.e., a 'hard' polytomy; Maddison 1989)? A polytomous relationship is potentially falsifiable with the addition of more data (Hoelzer and Melnick 1994). In this study, the addition of the 12S data to the cyt b data resulted in reduced resolving power. The most likely reason for this is that 12S is a slower-evolving gene than cyt b, and probably contributed very little to resolving the relationships within the genus while at the same time reinforcing the long branches in the phylogeny. Long branch attraction (i.e., the "Felsenstein Zone;" Felsenstein 1978, Huelsenbeck and Hillis 1993) could also account for the polytomous relationships. However, unlike parsimony methods, the maximum likelihood methods used to analyze the sequence data are more robust to long branch attraction and more likely to produce the correct tree (Swofford et al. 1996). As for the morphological data, all branch lengths were relatively short, making long branch attraction improbable.

A 'hard' polytomy is an alternative hypothesis to a 'soft' polytomy (Maddison 1989, Walsh et al. 1999). It implies simultaneous or very rapid diversification of three or more lineages (Maddison 1989). Unfortunately, in phylogenetic reconstruction a polytomy (all taxa equally related) is the null hypothesis and therefore cannot be proven (Walsh et al. 1999). However, recently researchers have proposed methods to distinguish between 'hard' and 'soft' polytomies (Vrba 1993, Hoelzer and Melnick 1994, Lara et al. 1996, Walsh et al. 1999). One method is to assess the strength of the phylogenetic signal above and below the polytomy (Lara et al. 1996, Lessa and Cook 1998), and suggests that strong support for branches both above and below the polytomy indicate a 'hard'

polytomy, but they caution that saturation could blur the phylogenetic signal. There is strong support for branches below and above the polytomies using both models (i.e., the higher level relationships, *Numenius* as the closest relative of *Limosa* and monophyly of the *Limosa* and those supporting the monophyly of the species of *Limosa*; Figs. 2.2 and 2.3), seeming to support the hypothesis of a 'hard' polytomy. Also, it is likely the polytomies are not due to saturation, because the maximum likelihood models (GTR + Γ and GTR + I + Γ) employed in the analyses account for the effects of saturation (Swofford et al. 1996). In the morphological phylogeny there is also support above and below the polytomy (Fig. 2.5), but whether this is enough to support the polytomy as 'hard' is debatable.

Another method of distinguishing between 'hard' and 'soft' polytomies is linking a burst of speciation (hypothesized by the polytomy) to a large scale vicariance event (e.g., glaciations or changes in sea level; Hoelzer and Melnick 1994, Kidd and Friesen 1998, Lessa and Cook 1998). To do this, one needs an estimate of the divergence time for the burst of speciation. The molecular clock analysis of the cyt b data provides a rough estimate for the time of divergence for the three species of *Limosa* stemming from the polytomy in the GTR + Γ phylogeny (Fig. 2.2) of 6.27 MYA, but this suffers from a relatively large standard error (± 0.61 MY) inherent in all molecular clocks (Hillis et al. 1996). It seems futile to attempt to link the divergence of the *Limosa* with a single historical vicariance event when the hypothesized divergence time spans many major environmental and geological phenomena (Hallam 1983, 1994, Marinkovich and Gladenkov 1999).

The most recently proposed method for distinguishing between 'hard' and 'soft' polytomies is an *a posteriori* power analysis of the sequence data (Walsh et al. 1999). The curve constructed from the power analysis (Fig. 2.4) suggests that the amount of data collected should be enough to resolve the dichotomous branching pattern of the *Limosa* if they diverged within a period longer than or equal to 90,000 years. However, if the *Limosa* diverged within a smaller time period, say half of this (e.g., over 45,000 years), the amount of mitochondrial sequence data equivalent to the cyt b data needed to resolve the phylogenetic relationships is 5,072 bp. If more sequence data could improve resolution, then why did the addition of the 12S sequence data reduce resolving power? The most likely explanation is that the tempo of evolution and the evolutionary dynamics of the 12S gene (Moum et al. 1995) are probably not as appropriate for addressing this question as those of cyt b. Nevertheless, the power analysis suggests that the diversification of three species of *Limosa* (*L. limosa*, *L. haemastica*, and *L. fedoa*) was very rapid in relation to the age of these three species. This is consistent with patterns observed within and among other genera of Charadriiformes (the guillemot genus *Cepphus* and the auklet genera: *Aethia*, *Ptychoramphus*, and *Cerorhinca*; Kidd and Friesen 1998, Walsh et al. 1999). However, the rapid diversification in relation to the ages of the lineages within and among these other genera was attributed to relatively recent vicariance events (glaciations and changes in sea level in the Pleistocene; Kidd and Friesen 1998, Walsh et al. 1999). This is not the case for *Limosa*. *Limosa* is an old avian genus whose species appear to have diversified prior to the glaciations and changes in sea level of the late Pliocene and early Pleistocene. Thus, the forces promoting the

diversification of the *Limosa* were not the same as those resulting in the diversification among and within these other Charadriiforme genera. For *Limosa*, there is another plausible explanation for their diversification: intercontinental colonization (or the intercontinental distribution) of an anscestral *Limosa*.

Intercontinental Colonization

Although each species of *Limosa* is widely distributed, each has a distributional limit terminating in Beringia (Fig. 2.1). This suggests that Beringia probably played a pivotal role in the diversification of the species of *Limosa*. Most faunal intercontinental colonizations between the Old and New worlds have occurred via Beringia (e.g., Repenning 1980, Hopkins 1982, Rausch 1994, Talbot and Shields 1996, Lance and Cook 1998, Sher 1999, Steppan et al. 1999, Voelker 1999, Conroy and Cook 2000). The cyt b phylogeny (Fig. 2.2) suggests that *Limosa* may have originated in the Old World and subsequently colonized the New World. However, regardless of which side *Limosa* originated upon, the intercontinental diversity pattern (Fig. 2.1) of the extant species of the genus suggests that intercontinental colonization or a trans-Beringian distribution following colonization played a strong role in speciation within the genus.

Acknowledgments

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Literature cited

Applied Biosystems (ABI). 1994. Sequence Navigator, Version 1.01. - Perkin-Elmer, Foster City, CA.

American Ornithologists Union (AOU). 1998. The A.O.U. Checklist of North American Birds, 7th Ed. - Amer. Ornithol. Union, Washington, D.C.

Carter, M. J., and Milton, I. D. 1993. An inexpensive and simple method for DNA purifications on silica particles. - Nucleic Acids Research 21: 1044.

Chu, P. C. 1996. Phylogenetic reanalysis of Strauch's osteological data set for the Charadriiformes. - Condor 97: 174-196.

Conroy, C. J., and Cook, J. A. 2000. Molecular systematics of a holarctic rodent (*Microtus*: Muridae). - J. Mammal. 81: 344-359.

Cramp, S., Simmons, K. E. L., Brooks, D. J., Collar, N. J., Dunn, E., Gillmor, R., Hollom, P.A.D., Hudson, R., Nicholson, E. M., Ogilvie, M. A., Olney, P. J. S., Roselaar, C. S., Voous, K. H., Wallace, D. I. M., Wattel, J., and Wilson, M. G. 1983. Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic, Volume III – Waders to Gulls. – Oxford, Oxford University Press.

Dement'ev, G. P., and Gladkov, N. A. 1969. Birds of the Soviet Union: Volume III. - Translated by Birron, A., Cole, Z. S., and Gordon, E. D. Israel Program for Scientific Translations, Jerusalem.

DeSalle, R., Absher, R., and Amato, G. 1994. Speciation and phylogenetic resolution. - Trends Ecol. Evol. 9: 297-298.

Desjardins, P., and Morais, R. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. - J. Miol. Biol. 212: 599-634.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. - Syst. Biol. 27: 401-410.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. - Evolution 39: 783-791.

Fleischer, R. C., McIntosh, C. E., and Tarr, C. L. 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rate. - Mol. Ecol. 7: 533-545.

Gibson, D. D., and Kessel, B. 1997. Inventory of the species and subspecies of Alaska birds. - Western Birds 28: 45-95.

Hallam, A. 1983. Plate tectonics and evolution. - In: Bendall, D. S. (ed.), Evolution From Molecules to Men. Cambridge, Cambridge University Press, pp. 367-386.

Hallam, A. 1994. Outline of Phanerozoic Biogeography. Oxford Biogeography Series, 10. - Oxford, Oxford University Press.

Helm-Bychowski, K., and Cracraft, J. 1993. Recovering phylogenetic signal from DNA sequences: relationships within the Corvine assemblage (class Aves) as inferred from the complete sequences of the mitochondrial DNA cytochrome b gene. - Mol. Biol. Evol. 10: 1196-1214.

Hillis, D. M., Mable, B. K., and Moritz, C. 1996. Applications of molecular systematics: the state of the field and a look to the future. - In: Hillis, D. M., Moritz, C., and Mable, B. K., (eds). Molecular Systematics, 2nd Edition. Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A., pp. 515-543.

Hoelzer, G. A., and Melnick, D. J. 1994. Patterns of speciation and limits to phylogenetic resolution. - Trends Ecol. Evol. 9: 104-107.

Hopkins, D. M., Matthews, Jr., J. V., Schweger, C. E., and Young, S. B. 1982. Paleoecology of Beringia. - Academic Press, New York, U.S.A.

Huelsenbeck, J. P., and Hillis, D. M. 1993. Success of phylogenetic methods in the four-taxon case. - Syst. Biol. 42: 247-264.

Kidd, M. G., and Friesen, V. L. 1998. Sequence variation in the guillemot (Alcidae: *Cepphus*) mitochondrial control region and its nuclear homologue. - Mol. Biol. Evol. 15: 61-70.

Kishino, H., and Hasegawa, M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of Hominoidea. - J. Mol. Evol. 29: 170-179.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., and Wilson, A. C. 1989. Dynamics of the mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. - Proc. Natl. Acad. Sci. *USA* 86: 6196-6200.

Kusukawa, N., Uemori, T., Asada, K., and Kato, I. 1990. Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. - Biotechniques 9: 66-72

Lance, E. W., and Cook, J. A. 1998. Phylogeography of tundra voles (*Microtus oeconomus*): Beringia region and southcoastal Alaska. - J. of Mammal. 79: 53-65.

Lara, M. C., Patton, J. L., and da Silva, M. N. F. 1996. The simultaneous diversification of South American echimyid rodents (Hystrieognathi) based on complete cytochrome b sequences. - Mol. Phylogenet. Evol. 5: 403-413.

Lessa, E. P., and Cook, J. A. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. - Mol. Phylogenet. Evol. 9: 88-99.

Maddison, W. 1989. Reconstructing character state evolution on polytomous cladograms. - Cladistics 5: 365-377.

Maddison, W. P. , and Maddison, D. R. 1999. MacClade: Analysis of phylogeny and character evolution, version 3.08. - Sinauer, Sunderland, Massachusetts.

Marincovich, L. Jr., and Gladenkov, A. Y. 1999. Evidence for an early opening of the Bering Strait. - Nature 397: 149-151.

Moum, T., Johansen, S., Erikstad, K. E., and Piatt, J. F. 1994. Phylogeny and evolution of the auks (subfamily Alcinae) based on mitochondrial DNA sequences. - Proc. Natl. Acad. Sci. USA 91: 7912-7916.

Peters, J. L. 1934. Check-list of Birds of the World, Vol. II. - Museum of Comparative Zoology, Cambridge, Massachusetts.

Rausch, R. L. 1994. Transberingian dispersal of cestodes in mammals. - International J. Parasitology 24: 1203-1212.

Repenning, C. A. 1980. Faunal exchanges between Siberia and North America. - Can. J. Anthro. 1: 37-44.

Sher, A. 1999. Traffic lights at the Beringian crossroads. - Nature 397: 103-104.

Shields, G. F., and Wilson, A. C. 1987. Calibration of mitochondrial DNA evolution in geese. - J. Mol. Evol. 24: 212-217.

Sibley, C. G., and Alquist, J. E. 1990. Phylogeny and Classification of Birds: A Study in Molecular Evolution. - Yale University Press, New Haven, Connecticut.

Steppan, S. J., Akhverdyan, M. R., Lyapunova, E. A., Fraser, D. G., Vorontsov, N. N., Hoffmann, R. S., and Braun, M. J. 1999. Molecular phylogeny of the marmots (Rodentia: Sciuridae): Tests of evolutionary and biogeographic hypotheses. - Syst. Biol. 48: 715-734.

Strauch, J. G., Jr. 1978. The phylogeny of the Charadriiformes (Aves): a new estimate using the methods of character compatibility analysis. - Trans. Zool. Soc. Lond. 34: 263-345.

Sullivan, J., Markert, J. A., and Kilpatrick, C.W. 1997. Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. - Syst. Biol. 46: 426-440.

Swofford, D. L. 2000. PAUP*: Phylogenetic analysis using parsimony, version 4.0b4. - Sinauer, Sunderland, Massachusetts.

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. 1996. Phylogenetic inference. - In Hillis, D. M., Moritz, C., and Mable, B. K. (eds). Molecular Systematics, 2nd Edition. Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A., pp. 407-514.

Talbot, S. L., and Shields, G. F. 1996. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. Mol. Phylogenet. Evol. 5: 567-575.

Tamura, K., and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. - Mol. Biol. Evol.10: 512-526.

Tarr, C. L., and Fleischer, R. C. 1993. Mitochondrial DNA variation and evolutionary relationships in the 'Amahiki' complex. - Auk 110: 825-831.

Voelker, G. 1999. Dispersal, vicariance, and clocks: Historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). - Evolution 53: 1536-1552.

Vrba, E. S. 1993. Mammalian evolution in the African Neogene and a new look at the great American interchange. - In Goldblatt, J. (ed). Biological Relationships Between Africa and South America. Yale University Press, New Haven, Connecticutt, pp. 393-432.

Waits, L. P., Sullivan, J., O'Brien, S. J, and Ward, R. H. 1999. Rapid radiation in the family Ursidae indicated by likelihood phylogenetic estimation from multiple fragments of mtDNA. - Mol. Phylogenet. Evol. 13: 82-92.

Walsh, H. E., Kidd, M. G., Moum, T., and Friesen, V. L. 1999. Polytomies and the power of phylogenetic inference. - Evolution 53: 932-937.

Wood, T. C., and Krajewski, C. 1996. Mitochondrial DNA sequence variation among subspecies of Sarus Crane (*Grus antigone*). - Auk 113: 655-663.

Zink, R. M., Rohwer, S, Andreev, A., and Dittmann, D. L. 1995. Trans-Beringian comparisons of mitochondrial DNA differentiation in birds. - Condor 97: 639-649.

Table 2.1. Specimens used in this study.

Species	Locality	Voucher[1]
Limosa haemastica	Fairbanks, Alaska	UAM 8259
L. haemastica	Fairbanks, Alaska	UAM 8804
L. fedoa	Alaska Peninsula, Alaska	UAM 4870
L. fedoa	Alaska Peninsula, Alaska	UAM 4871
L. limosa	Adak Island, Alaska	UAM 8216
L. limosa	Attu Island, Alaska	UAM 8400
L. lapponica	Germany	NCSM 13192
L. lapponica	Seward Peninsula, Alaska	UAM 8832
L. lapponica	Seward Peninsula, Alaska	[2]
Outgroups		
Numenius phaeopus variegatus	Buldir Island, Alaska	UAM 8212
N. phaeopus hudsonicus	Fairbanks, Alaska	UAM 10237
Limnodromus scolopaceus	Alaska Peninsula, Alaska	UAM 8849
Tringa melanoleuca	Alaska Peninsula, Alaska	UAM 8970

[1] UAM = University of Alaska Museum; NCSM = North Carolina State Museum of Natural Science.

[2] No voucher preserved (mangled road kill).

Table 2.2. Primer names, sequences, and sources used for PCR and sequencing reactions.

Primer name	Sequence	Source
L14841	5'-CTTCCATCCAACATCTCAGCATGATGAAA-3'	Kocher et al. 1989
H637	5'-AGATGCCTAGGGGGTTGTTTGA-3'	This study
L519	5'-CCAACCCTTACCCGATTCTTCG-3'	This study
H16065	5'-GGAGTCTTCAGTCTCTGGTTTACAAGAC-3'	Helm-Bychowski and Cracraft 1993
12S L281	5'-CCGTAAACGGCGTAAAGAGTG-3'	This study
12S H745	5'-GTAGCCCATTTCTTCCACCCC-3'	This study

Table 2.3. Corrected pairwise distances calculated from the GTR + Γ model used to reconstruct the cyt b phylogeny (below diagonal), and corrected pairwise distances from the GTR + I + Γ model used to reconstruct the cyt b + 12S phylogeny (above diagonal).

	1	2	3	4	5	6	7	8	9	10	11
1 *Limosa haemastica*	--	0.106	0.097	0.112	0.112	0.110	0.127	0.273	0.259	0.286	0.263
2 *L. limosa* (Alaska)	0.117	--	0.008	0.151	0.151	0.149	0.181	0.272	0.249	0.249	0.297
3 *L. limosa* (Alaska)	0.101	0.010	--	0.148	0.148	0.146	0.176	0.269	0.242	0.249	0.297
4 *L. lapponica* (Alaska)	0.131	0.190	0.181	--	0.001	0.003	0.171	0.279	0.261	0.282	0.292
5 *L. lapponica* (Alaska)	0.131	0.189	0.181	0.002	--	0.003	0.171	0.278	0.261	0.282	0.290
6 *L. lapponica* (Germany)	0.130	0.188	0.179	0.001	0.002	--	0.173	0.269	0.254	0.275	0.284
7 *L. fedoa*	0.101	0.159	0.149	0.165	0.165	0.163	--	0.341	0.359	0.361	0.331
8 *Tringa melanoleuca*	0.291	0.338	0.326	0.309	0.307	0.307	0.322	--	0.320	0.332	0.276
9 *Numenius phaeopus hudsonicus*	0.261	0.285	0.270	0.290	0.290	0.288	0.341	0.341	--	0.049	0.366
10 *N. p. variegatus*	0.291	0.269	0.265	0.308	0.308	0.306	0.345	0.347	0.062	--	0.363
11 *Linmodromus scolopaceus*	0.315	0.351	0.345	0.342	0.339	0.339	0.369	0.340	0.349	0.357	--

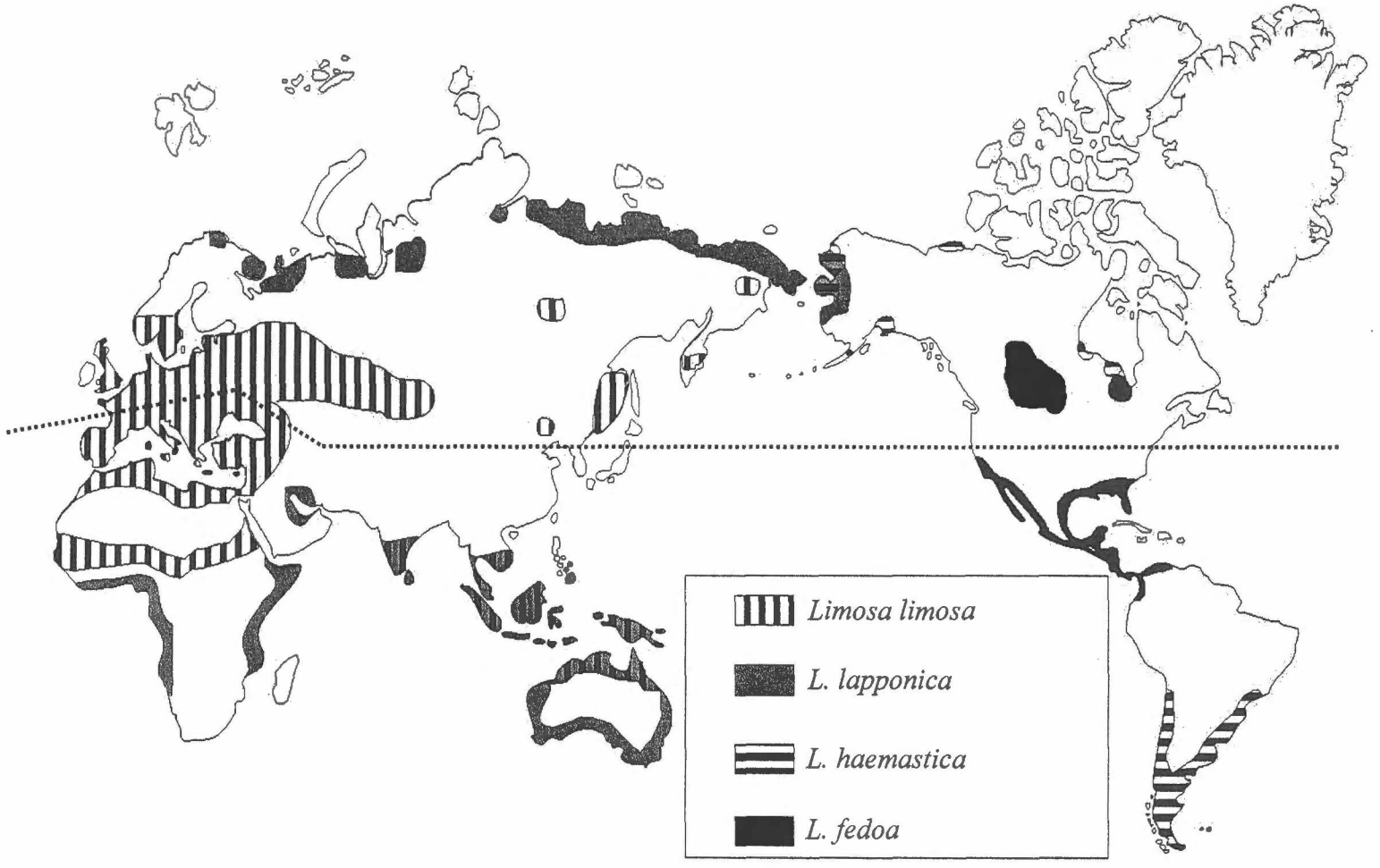

Fig. 2.1. Breeding (above line) and wintering (below line) distributions of the species of *Limosa* (Peters 1934, Dement'ev and Gladkov 1969, Cramp et al. 1983, Gibson and Kessel 1997, and AOU 1998).

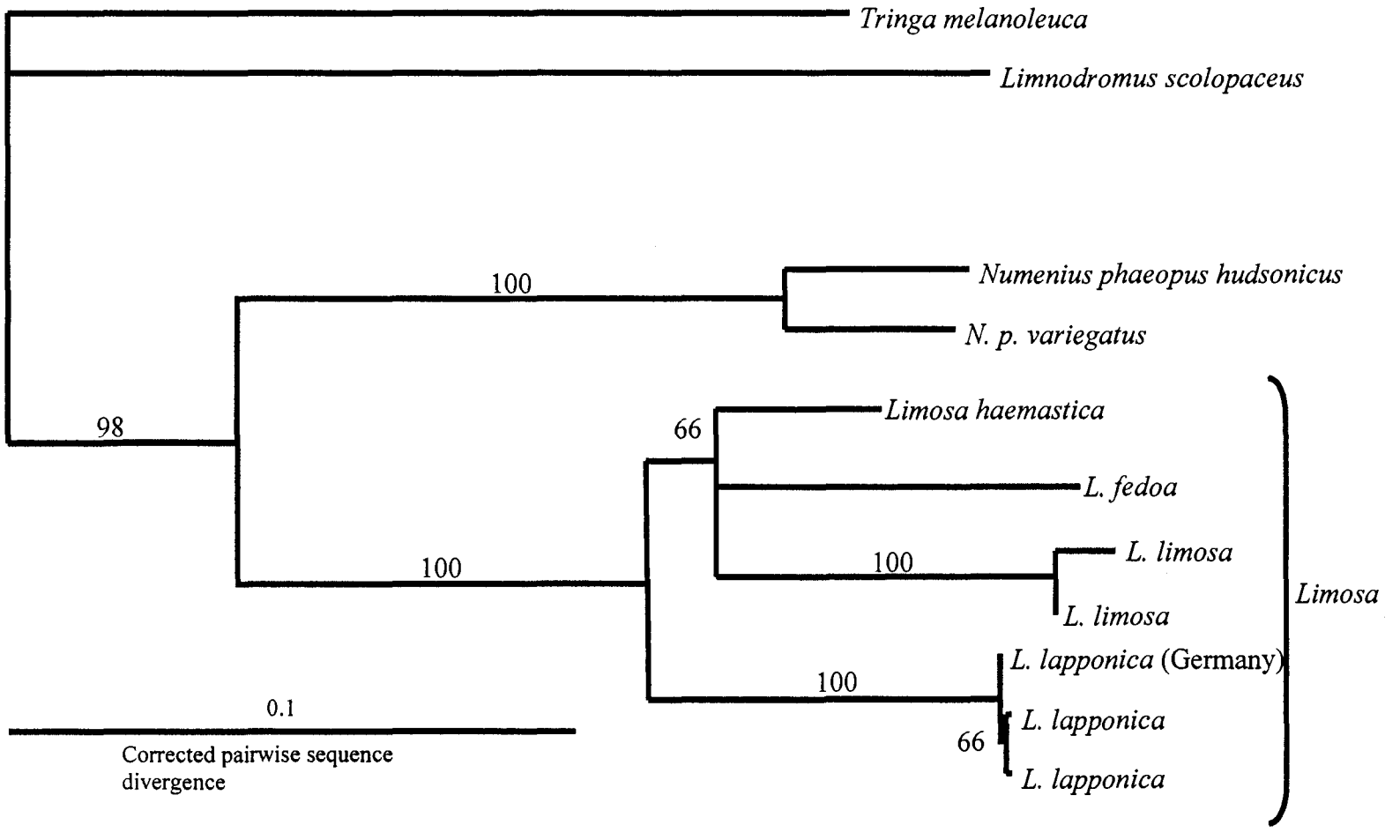

Fig. 2.2. Phylogeny for cytochrome b sequence data based on a GTR + Γ model of molecular evolution. Numbers above branches represent bootstrap support (100 bootstrap replicates). Nodes with <50% bootstrap support collapsed.

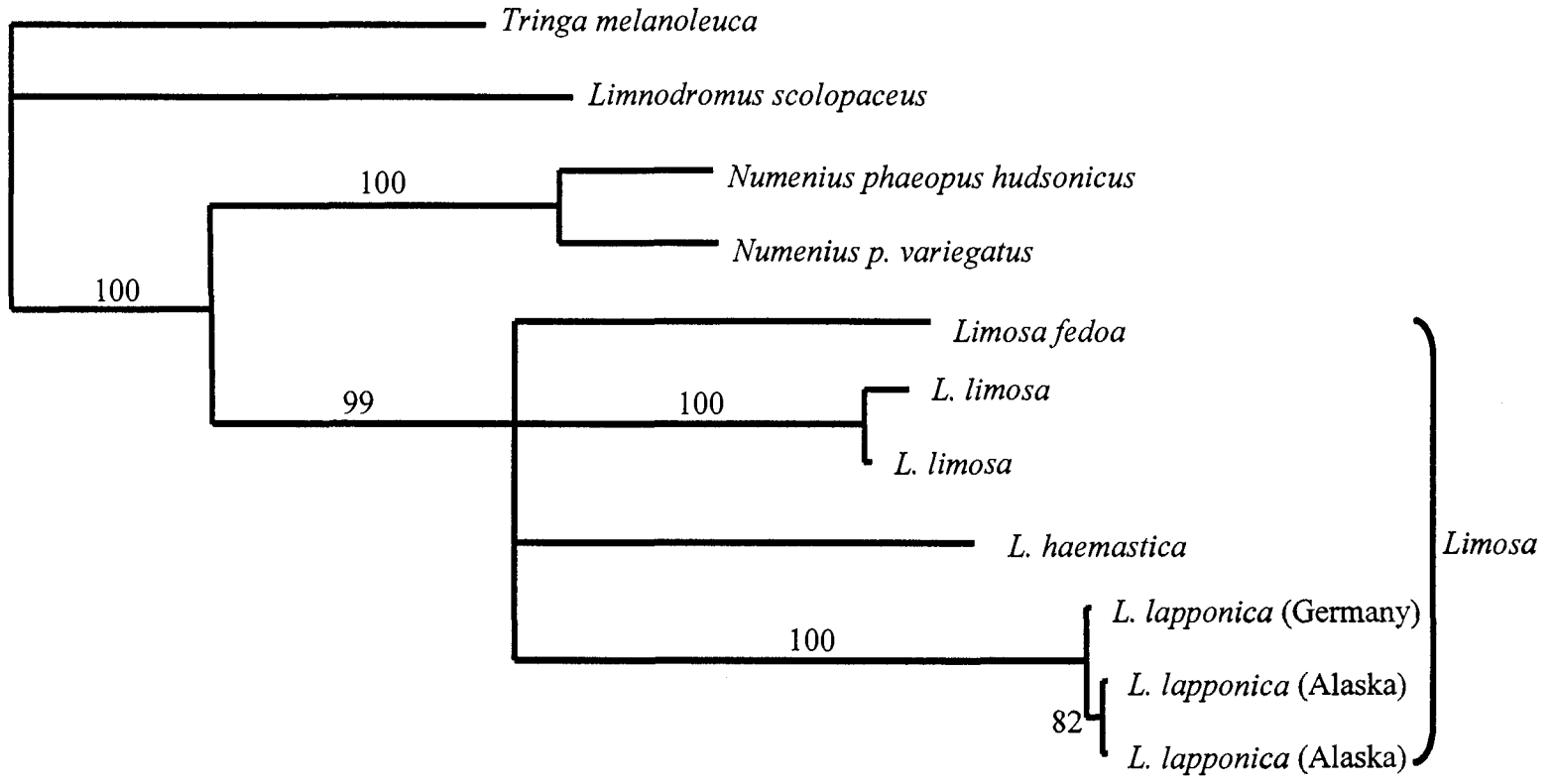

Fig. 2.3. Phylogeny for cytochrome b and 12S data based on a GTR + I + Γ model of molecular evolution. Numbers on branches represent bootstrap support (100 bootstrap replicates). Nodes with <50% bootstrap support collapsed.

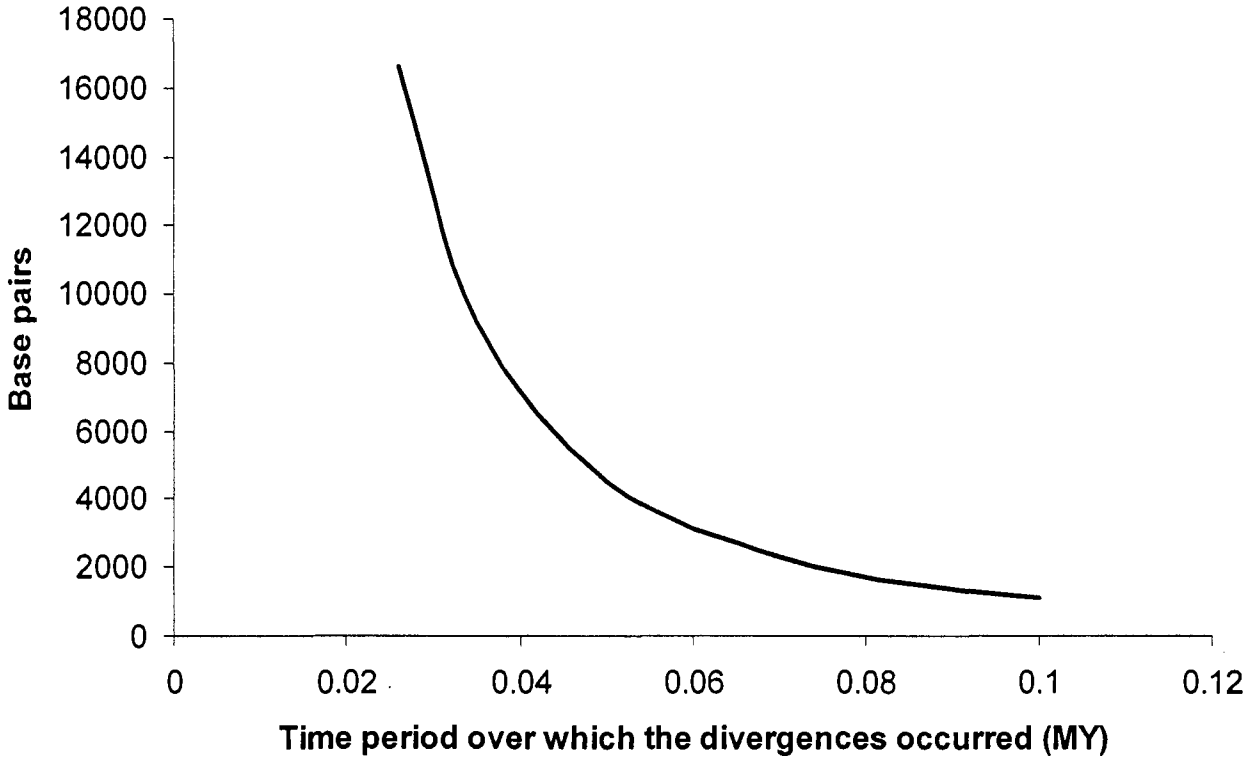

Fig. 2.4. Curve produced from the power analysis of the cyt b data set showing the relationship between the number of base pairs required to resolve phylogenetic relationships within *Limosa* and the time period over which the divergences occurred (in millions of years).

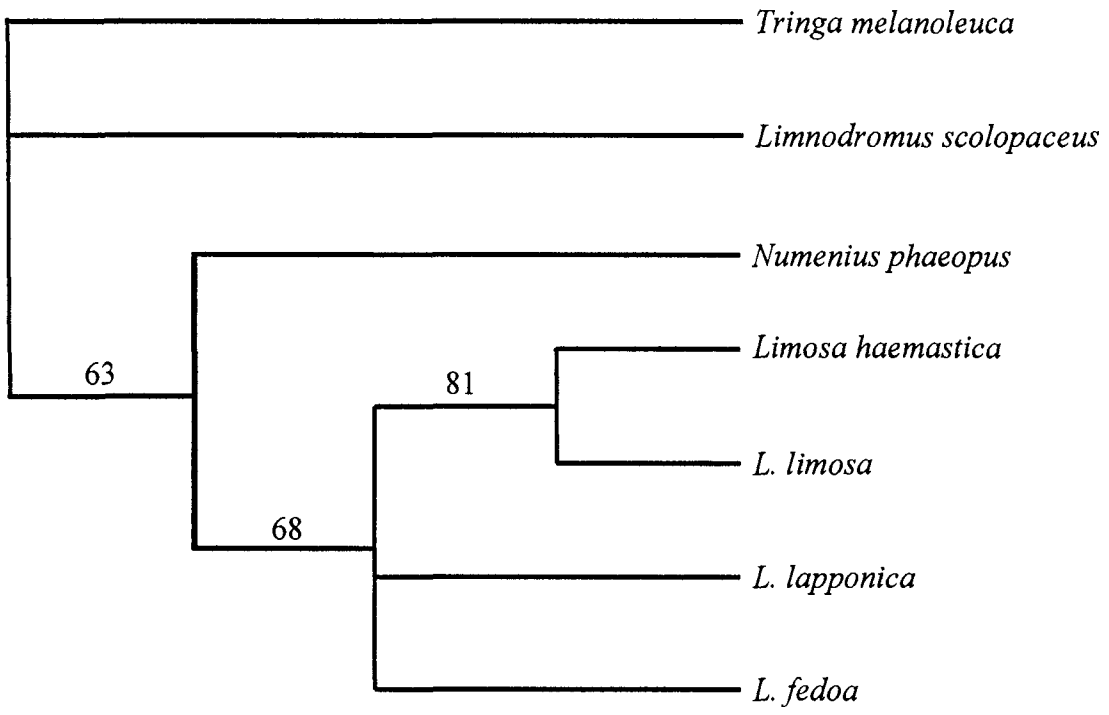

Fig. 2.5. Strict consensus of three equally parsimonious trees based on external morphology. Numbers above branches represent bootstrap support (10,000 bootstrap replicates). Nodes with <50% bootstrap support collapsed.

Appendix 1. External morphological characters used in the parsimony analysis.

1. Unstreaked white throat.
2. Rufous auriculars.
3. Barred abdomen.
4. Barred upper tail coverts.
5. Barred axillaries (1), Unbarred black (2), Unbarred white (3).
6. Pronounced white wing bar.
7. Rich brown nape and crown.
8. Heavily rich brown streaked back.
9. Breeding season males with richly brown venter, females lighter brown.
10. Males rich brown on dorsal feathers, females paler brown.
11. Rectrix patterning: Many distinct bars (1), Many indistinct bars (2), Two distinct bars (3).
12. Bi-colored bill.
13. Slightly upturned bill.
14. Heavy throat streaking/no throat streaking.
15. Heavy ventral neck streaking/little or no ventral neck streaking.
16. Dark legs.
17. Dorsal feather pattern sexually dimorphic during breeding season.
18. Pale loral streak (from upper ramphothecum to top of eye).
19. Primaries unbarred.
20. Inner web of primaries variegated.
21. Dorsal feather patterning: spotted (0), longitudinally oriented (1), laterally oriented (2), intermediate 45° angles (3).
22. Sharp contrast of patterning on dorsal feathers.

Appendix 2. Data matrix used in the parsimony analysis of external morphological characters. Characters 1-22 defined in Appendix 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Limosa limosa	1	1	1	0	3	1	1	1	0	0	3	1	1	0	0	1	0	1	0	0	3	1
L. lapponica	0	1	0	1	1	0	0	0	1	1	2	1	1	0	0	1	0	0	0	0	1	1
L. fedoa	1	0	0	1	1	0	0	0	0	0	2	1	1	0	1	1	0	1	0	1	2	1
L. haemastica	1	0	1	0	2	1	0	0	1	0	3	1	1	0	1	1	1	1	0	0	3	1
Numenius phaeopus	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	0	1	1	0	3	0
Tringa melanoleuca	0	0	0	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	1	0	1
Limnodromus scolopaceus	0	1	0	1	1	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	3	1

Conclusions

The Bombycillidae appear to demonstrate two intercontinental colonizations between Eurasia and North America. The direction of the initial colonization inferred for the Bombycillidae is from the New World to the Old World, posing an exception to the normal pattern of faunal exchange between Eurasia and North America (Hopkins et al., 1982; Shields et al., 1993; Talbot and Shields, 1996; Karafet et al., 1997; Lance and Cook, 1998; Sher, 1999; Conroy and Cook, 2000). However, most of these previous studies investigated relatively recent colonization events that probably occurred during mid- to late- Pleistocene glaciations, when North America and Eurasia were a contiguous land mass connected via Beringia. The pattern inferred for Bombycillidae is deeper (between 3.5 and 1.8 MYA, Late Pliocene), and as a consequence may not reflect the same constraints on intercontinental movements occurring during the mid- to late-Pleistocene. The direction of the second colonization cannot be inferred, but further phylogeographic study of the Eurasian and North American populations of *B. garrulus* and of *B. japonica* may help determine its direction.

The *Limosa* appear to demonstrate a rapid diversification (ca. 90,000 years) in relation to the age of the lineages within the genus (mean of 6.3 ± 0.61 MYA). This is consistent with patterns observed within and among other genera of Charadriiformes (the guillemot genus *Cepphus* and the auklet genera *Aethia*, *Ptychoramphus*, and *Cerorhinca*; Kidd and Friesen, 1998; Walsh et al., 1999). However, the rapid diversification in relation to the ages of the lineages within and among these other genera was attributed to relatively recent vicariance events (glaciations and changes in sea level in the

Pleistocene; Kidd and Friesen, 1998; Walsh et al., 1999). *Limosa*, on the other hand, is an old avian genus that appears to have diversified prior to the glaciations and changes in sea level of the late Pliocene and early Pleistocene. Thus, the forces promoting diversification in *Limosa* were not the same as those resulting in the diversification among and within these other Charadriiformes genera. The intercontinental diversity pattern (Fig. 1) of the extant species of *Limosa* suggests that intercontinental colonization or a trans-Beringian distribution following colonization played a strong role in the speciation within this genus.

LITERATURE CITED

Conroy, C. J., and Cook, J. A. 2000. Molecular systematics of a holarctic rodent (*Microtus*: Muridae). - Journal of Mammalogy 81: 344-359.

Hopkins, D. M., Matthews, Jr., J. V., Schweger, C. E., and Young, S. B. 1982. Paleoecology of Beringia. - Academic Press, New York, U.S.A.

Kidd, M. G., and Friesen, V. L. 1998. Sequence variation in the guillemot (Alcidae: *Cepphus*) mitochondrial control region and its nuclear homologue. - Molecular Biology Evolution 15: 61-70.

Lance, E. W., and Cook, J. A. 1998. Phylogeography of tundra voles (*Microtus oeconomus*): Beringia region and southcoastal Alaska. - Journal of Mammalogy 79: 53-65.

Sher, A. 1999. Traffic lights at the Beringian crossroads. - Nature 397: 103-104.

Shields, G. F., Schmiechen, A. M., Frasier, B. L., Redd, A., Voevoda, M. I., Reed, J. K., and Ward, R.H. 1993. MtDNA sequences suggest a recent evolutionary

divergence for Beringian and northern North American populations. - American Journal of Human Genetics 53: 549 –562.

Steppan, S. J., Akhverdyan, M. R., Lyapunova, E. A., Fraser, D. G., Vorontsov, N. N., Hoffman, R. S., and Braun, M. J. 1999. Molecular phylogeny of the marmots (Rodentia: Sciuridae): Tests of evolutionary and biogeographic hypotheses. - Systematic Biology 48: 715-734.

Talbot, S. L., and Shields, G. F. 1996. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. Molecular Phylogenetics Evolution 5: 567-575.

Voelker, G. 1999. Dispersal, vicariance, and clocks: Historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). - Evolution 53: 1536-1552.

Walsh, H. E., Kidd, M. G., Moum, T., and Friesen, V. L. 1999. Polytomies and the power of phylogenetic inference. - Evolution 53: 932-937.