

HOLARCTIC PHYLOGEOGRAPHY AND SPECIES LIMITS OF THREE-TOED WOODPECKERS

ROBERT M. ZINK^{1,3}, SIEVERT ROHWER², SERGEI DROVETSKI², RACHELLE C. BLACKWELL-RAGO¹ AND SHANNON L. FARRELL¹

¹J. F. Bell Museum of Natural History, University of Minnesota, St. Paul, MN 55108

²Burke Museum and Department of Zoology, University of Washington, Seattle, WA 98195-3010

Abstract. We compared mitochondrial DNA sequences of Three-toed Woodpeckers (*Picoides tridactylus*) within and between Eurasia and North America. Samples from the two continents are each reciprocally monophyletic, and differ by approximately 4% sequence divergence, suggesting that a separate species exists on each continent. We recommend formal taxonomic action. Within continents, no phylogeographic structuring was found, suggesting that both species have recently expanded their ranges following the end of the last ice age.

Key words: mtDNA, *Picoides tridactylus*, population expansions, species limits, Three-toed Woodpecker.

Filogeografía Holártica y Límites de la Especie *Picoides tridactylus*

Resumen. Comparamos secuencias de ADN mitocondrial en *Picoides tridactylus*, dentro y entre Eurasia y América del Norte. Las muestras de cada uno de los dos continentes son recíprocamente monofiléticas y difieren aproximadamente en un 4% de divergencia secuencial, sugiriendo que existe una especie distinta en cada continente. Recomendamos acción taxonómica formal. No encontramos estructura filogeográfica dentro de los continentes, sugiriendo que ambas especies han expandido recientemente sus rangos luego del final de la última era glacial.

Zink et al. (1995) reported that small samples of Three-toed Woodpeckers (*Picoides tridactylus*) from Eurasia and North America differed substantially in mitochondrial DNA (mtDNA) restriction site patterns. In particular, samples of *P. t. tridactylus* and *P. t. albidior* from Russia differed from samples of *P. t. fasciatus* and *P. t. dorsalis* (North America) by an estimated 5.5% sequence divergence, a level consistent with that observed between other woodpecker species (DeFillipis and Moore 2000). The observed sequence divergence parallels the morphological and call-note differences between taxa from the two continents (Winkler et al. 1995). In this paper, we compare

mtDNA sequence divergence within and between Eurasia and North America to augment the restriction site study.

METHODS

We collected small numbers of individuals from nine sites in Eurasia (Fig. 1) and three sites in North America. GenBank entries (AF424985–425071) provide information on museums and catalogue numbers where voucher specimens are housed. General localities (and sample sizes) for *P. t. tridactylus* were Oulu (1), Vologda (1), Irkutsk (1), Khabarovsk (1), Sakhalin Island (2); for *P. t. crissoleucus* were Noyabr'sk (5), Krasnoyarsk (1), Magadan (8); for *P. t. albidior* Kamchatka (1); for *P. t. fasciatus* Alaska (2), Washington (3); and for *P. t. bacatus* Quebec (3). We extracted DNA following standard methods, and used the polymerase chain reaction to amplify parts of three mitochondrial genes, ND2 (383 base pairs), ND3 (418 bp), and cytochrome *b* (433 bp). Primers and laboratory methods are described in Zink et al. (2000). We used Paup* (Swofford 1999) to estimate the phylogenetic relationships among haplotypes, summarizing equally parsimonious trees as a strict consensus. Base positions were weighted equally. We also estimated a phylogenetic tree using maximum likelihood; likelihood-ratio tests were used to choose the most appropriate model (see Posada and Crandall 1998). Trees were rooted with a single sequence from the Black-backed Woodpecker (*P. arcticus*). We also used Paup* to compute HKY distances. We used Arlequin (Schneider et al. 2000) to calculate F_{st} nucleotide diversity (π), and mismatch distributions (which reveal the history of population growth; Harpending 1994). DnaSP (Rozas and Rozas 1999) was used (1) to compute the average number of nucleotide differences (k), haplotype diversity, Fu and Li's (1993) F -test for selective neutrality, and Nm (general immigration rate) and, (2) to verify values computed with Arlequin.

RESULTS

We obtained 1234 bp of sequence from each of 29 individuals. Similar patterns among gene regions suggest that no nuclear copies were amplified. For the two largest samples, π was 0.0014 (Magadan) and 0.0003 (Noyabr'sk). For the 21 Eurasian samples combined, there were nine haplotypes, π was 0.0011 (\pm 0.0008 [SD]), k was 1.32, haplotype diversity was 0.63 and



FIGURE 1. Approximate location of sample sites for Three-toed Woodpeckers in Eurasia.

Fu and Li's (1993) F was -3.2 ($P < 0.02$). One haplotype ("1 Irkutsk") was widespread, occurring in the samples from Irkutsk, Vologda, Magadan, Kamchatka, Sakhalin, Khabarovsk, and Noyabr'sk. For the eight individuals from North America, there were eight haplotypes, π was 0.002 (± 0.0014 [SD]), k was 2.43 , haplotype diversity was 0.96 , and Fu and Li's F was -1.66 ($P > 0.05$). The mismatch distributions (Fig. 2) for the combined Eurasian samples and North American samples were unimodal, suggesting population

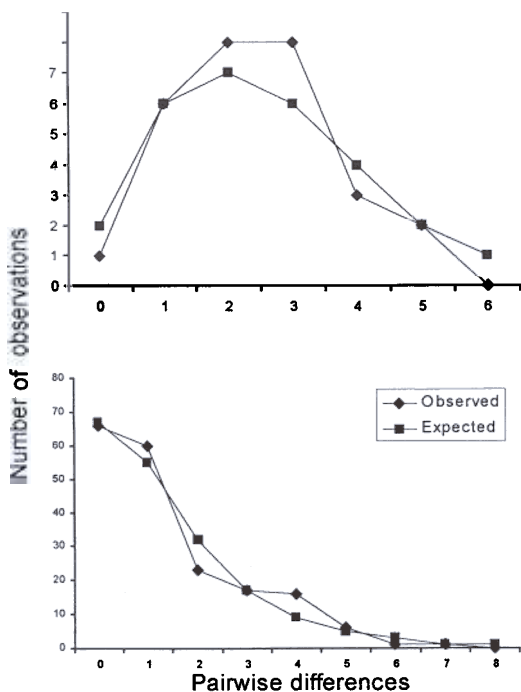


FIGURE 2. Mismatch distribution for haplotypes found in Three-toed Woodpecker population samples from North America (top) and Eurasia (bottom). Expected distributions are those expected for exponentially growing populations (Rogers 1995), and do not differ from observed distributions.

growth. One hundred thirty-five base positions were variable and 48 were parsimony informative. Parsimony analysis yielded 12 equally parsimonious trees (length 194, consistency index 0.97, rescaled consistency index 0.95). The consensus tree (not shown) as well as the maximum-likelihood tree (Fig. 3), shows two polytomies, one including haplotypes from Eurasia, and the other including haplotypes from North America. Thus, no geographic structuring was evident within either continent. Uncorrected and HKY85 sequence divergence between the two groups of haplotypes averaged 3.8%; the HKY85 distance to the out-group was 0.14. F_{st} was 0.97 ($P < 0.001$), with 94% between continents, 0.01% among populations within regions, and 3% within populations.

DISCUSSION

Haplotypes from each continent are reciprocally monophyletic (Fig. 3), and the two continental clusters of haplotypes differ by 3.8%, similar to that estimated by Zink et al. (1995) from restriction site analysis. Because the two taxa are allopatric, we prefer not to speculate on whether they are biological species (Klicka et al. 2001). The two continental populations should thus be treated as separate phylogenetic species. Linnaeus

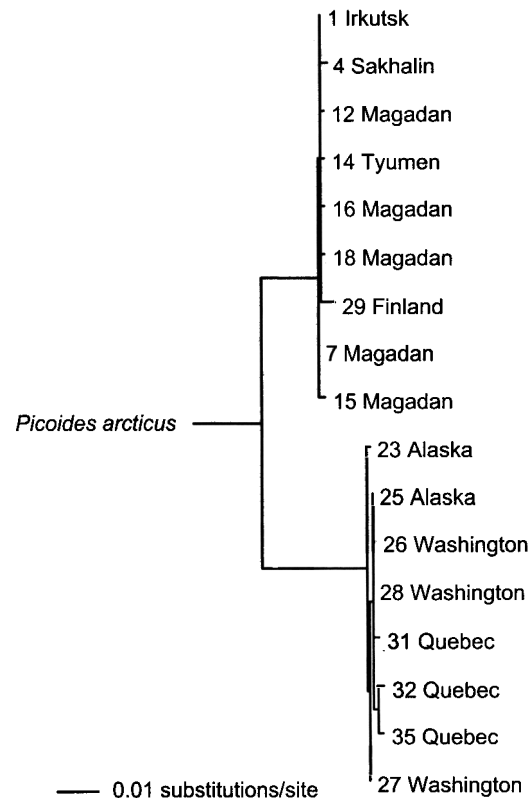


FIGURE 3. Maximum-likelihood tree for unique haplotypes of Three-toed Woodpeckers from Eurasia and North America, rooted using a single sequence from Black-backed Woodpecker (*Picoides arcticus*).

described *Picoides tridactylus* from Europe in 1758, whereas the first three-toed woodpecker from North America was described as *Picoides dorsalis* by Baird in 1858 (see AOU 1957). Thus, the American Three-toed Woodpecker should be reclassified as *Picoides dorsalis* and the Eurasian species should remain *P. tridactylus*.

The differences between continental populations of Three-toed Woodpeckers parallel those detected using mtDNA restriction sites in Old and New World samples of several other taxa (Zink et al. 1995), including Marbled Murrelet (*Brachyramphus marmoratus*; now considered specifically distinct from Long-billed Murrelet [*B. perditus*], AOU 1998), Whimbrel (*Numenius phaeopus*), Mew Gull (*Larus canus*), Black-billed Magpie (*Pica hudsonia*, now considered specifically distinct from Eurasian Magpie [*P. pica*]; AOU 2000), American Pipit (*Anthus rubescens*; now considered specifically distinct from Water Pipit [*A. spinoletta*]; AOU 1998), and Gray-crowned Rosy-Finch (*Leucosticte tephrocotis*, now considered specifically distinct from Rosy Finch [*L. arctoa*]; AOU 1998). These taxon pairs differ by at least 2% sequence divergence, suggesting a relatively ancient separation (Zink and Klicka 1999), and not one consistent with the most recent closure of the Bering land bridge.

Despite the large differences between continental populations, we observed little variability and no phylogeographic structure within Eurasia. This result parallels that found for phylogeographic surveys of the Great Spotted Woodpecker (*Dendrocopos major*; Zink et al., in press), Ravens (*Corvus corax*; Omland et al. 2000), and crows (*Corvus* spp., Kryukov and Suzuki 2000) across Eurasia. The lack of structure over a large section of Eurasia, the widespread occurrence of haplotype 1, the apparent population expansion inferred from the mismatch distribution, and the negative value of Fu and Li's (1993) *F* suggest that the species has recently undergone range expansion on a scale sufficient to preclude differentiation. Unlike North America, only northwestern Eurasia was glaciated during the last glacial cycle (Hewitt 2000), and likely unforested. However, much of the current range of *P. tridactylus* was permafrost 15 000 years ago (Hewitt 2000), which probably excluded forest suitable for this species. Thus, range expansion is consistent with reforestation following the last ice age. However, even populations as geographically proximate as Kamchatka and Alaska are genetically different, showing that range expansion did not result in intercontinental gene flow. In North America, the lack of phylogeographic structure parallels that documented for many, but not all, bird species (Zink 1997), including the Downy Woodpecker (*Picoides pubescens*; Ball and Avise 1992). The lack of geographic differentiation across large continental regions suggests that although woodpeckers are largely sedentary, they possess the potential for rapid population and range expansion given suitable conditions.

In North America, nucleotide diversity was approximately twice that in Eurasia. If this holds for larger samples, it might suggest that Eurasia was colonized from North America. However, because the two continental populations appear to have been separated for a long period, current nucleotide diversity might not

represent that which existed at the time of colonization (e.g., Eurasia might have experienced a more recent bottleneck). Stronger inference about a consistent direction of colonization will be possible when nucleotide diversities are available for many sister taxa on both continents.

We thank the Burke Museum for curatorial assistance; S. Birks subsampled tissues. We thank T. Line for laboratory assistance. C. Wood, B. Schmidt, G. Voelker, D. Banin, A. Andreev, I. Fadeev, E. Nesterov, and I. Karagodin provided logistical help with expeditions. R. Winker, E. Milot, and M. Orell provided some tissues. G. Eddy supported expeditions; additional laboratory support came from the NSF (DEB 9707496). A. Jones, A. Pavlova, and A. Kessen provided helpful assistance with manuscript preparation. SVD was supported by G. Eddy and by Leaders Five Fellowships from the Burke Museum.

LITERATURE CITED

- AMERICAN ORNITHOLOGISTS' UNION. 1957. Check-list of North American birds. 5th ed. Lord Baltimore Press, Baltimore, MD.
- AMERICAN ORNITHOLOGISTS' UNION. 1998. Check-list of North American birds. 7th ed. American Ornithologists' Union, Washington, DC.
- AMERICAN ORNITHOLOGISTS' UNION. 2000. Forty-second supplement to the American Ornithologists' Union check-list of North American birds. *Auk* 117:847–858.
- BALL, R. M., JR., AND J. C. AVISE. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* 109:626–636.
- DEFILIPPIS, V. R., AND W. S. MOORE. 2000. Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: an empirical study based on woodpeckers (Aves: Picidae). *Molecular Biology and Evolution* 16:143–160.
- FU, Y.-X., AND W.-H. LI. 1993. Statistical tests of neutrality of mutations. *Genetics* 133:693–709.
- HARPENDING, H. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* 66:591–600.
- HEWITT, G. M. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405:907–913.
- KLICKA, J., R. M. ZINK, J. C. BARLOW, W. B. MCGILLIVRAY, AND T. J. DOYLE. 2001. The taxonomic rank of *Spizella taverneri*: a response to Mayr and Johnson. *Condor* 103:420–422.
- KRYUKOV, A. P., AND H. SUZUKI. 2000. Phylogeography of Carrion, Hooded, and Jungle Crows (Aves, Corvidae) inferred from partial sequencing of the mitochondrial cytochrome *b* gene. *Russian Journal of Genetics* 36:922–929.
- OMLAND, K. E., C. L. TARR, W. I. BOARMAN, J. M. MARZLUFF, AND R. C. FLEISCHER. 2000. Cryptic genetic variation and paraphyly in Ravens. *Proceedings of the Royal Society of London Series B* 267:2475–2482.
- POSADA, D., AND K. A. CRANDALL. 1998. MODEL-

- TEST: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- ROGERS, A. R. 1995. Genetic evidence for a Pleistocene population explosion. *Evolution* 49:608–615.
- ROZAS, J., AND R. ROZAS. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* 15:174–175.
- SCHNEIDER, S., J.-M. DUEFFER, D. ROESSLI, AND L. EXCOFFIER [ONLINE]. 2000. Arlequin ver. 2.0: a software for population genetic data analysis. Genetics and Biometry laboratory, University of Geneva, Switzerland. (<http://anthropologie.unige.ch/arlequin/>) (15 September 2000).
- SWOFFORD, D. L. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b2. Sinauer and Associates, Sunderland, MA.
- WINKLER, H., D. A. CHRISTIE, AND D. NURNEY. 1995. Woodpeckers: an identification guide to the woodpeckers of the world. Houghton Mifflin, New York.
- ZINK, R. M. 1997. Phylogeographic studies of North American birds, p. 301–324. *In* D. P. Mindell [ED.], *Avian molecular evolution and systematics*. Academic Press, San Diego, CA.
- ZINK, R. M., G. F. BARROWCLOUGH, J. L. ATWOOD, AND R. C. BLACKWELL-RAGO. 2000. Genetics, taxonomy and conservation of the threatened California Gnatcatcher. *Conservation Biology* 14:1394–1405.
- ZINK, R. M., S. DROVETSKI, AND S. ROHWER. In press. Phylogeographic patterns in the Great Spotted Woodpecker (*Dendrocopos major*) across Eurasia. *Journal of Avian Biology*.
- ZINK, R. M., AND J. KLICKA. 1999. Avian evolution during the Pleistocene in North America, p. 275–288. *In* M. Kato [ED.], *The biology of biodiversity*. Springer-Verlag, Tokyo.
- ZINK, R. M., S. A. ROHWER, AND D. L. DITTMANN. 1995. Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. *Condor* 97: 639–649.