

# Phylogeographic patterns in the great spotted woodpecker *Dendrocopos major* across Eurasia

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We sequenced parts of three mitochondrial genes in 67 great spotted woodpeckers *Dendrocopos major* taken from 17 sites across Eurasia and Japan. Two groups, differing by ca. 3% sequence divergence, were determined to have had independent evolutionary histories: samples from the southeast (Sakhalin, Primor'ye, Hokkaido), and the rest of Eurasia. The southeastern group corresponds to the subspecies *D. m. japonicus*, which will likely merit species status upon study of all subspecies; at present it should be considered an evolutionarily significant unit. From the United Kingdom to the Russian North Pacific, no phylogeographic divisions were found, which is likely a result of recent range expansion following retreat of permafrost after the last ice age.

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The great spotted woodpecker *Dendrocopos major* is widely distributed across Eurasia, from the United Kingdom to the Russian North Pacific, south to Japan, China, Morocco, Algeria, Iran, and Tunisia, as well as the Canary Islands. It occurs primarily in forests, where it is assumed to be territorial throughout the year. Based on plumage coloration and size, up to 27 subspecies are recognized (e.g., Peters 1948), whereas some (e.g., Winkler et al. 1995) recognize 14. Some taxonomists (e.g., Dement'ev and Gladkov 1954) further lump the three races typically identified as part of *D. leucopterus* (white-winged woodpecker) into *D. major*. We analyzed phylogeographic patterns (Avisé 2000) in mitochondrial DNA (mtDNA) from population samples of *D. major* taken across much of the species' Eurasian range, a region for which little is known about patterns of genetic differentiation (Bensch and Hasselquist 1999, Fedorov et al. 1999, Hewitt 2000, Kryukov and Suzuki 2000). Our goal was not a complete analysis of intraspecific or taxonomic variation, rather we discerned whether major phylogeographic gaps existed. The analysis represents one of the largest geographic expanses surveyed for phylogeographic structure. Furthermore, our study reports the most

geographically extensive phylogeographic analysis of an essentially sedentary bird species (Ball and Avisé 1992), and provides a contrast to studies of migratory species.

## Methods

We obtained 67 individuals from 17 sampling localities (Fig. 1, Table 1). Museum vouchers accompany tissue samples from most individuals; exceptions are the eight individuals from Japan, United Kingdom, Finland and Switzerland, where only tissue samples were obtained.

Genomic DNA isolation and purification followed either a modified Chelex (Ellegren 1992) or phenol-chloroform protocol (Hillis et al. 1996). Using standard PCR methods (Saiki et al. 1988), we amplified parts of three mitochondrial genes, ND2, ND3, and Cyt *b*. Primers for each region are available from the authors. PCR products were cleaned using a Qiaquick PCR Purification Kit (Qiagen). Sequencing reactions were cleaned using standard Sephadex columns and sequenced on an ABI 310 automated sequencer using the Dideoxy Terminator kit. PCR products were sequenced using the same primers as used for amplifications.

Fig. 1. Approximate location of sample sites. Not shown is the site in the United Kingdom and the site in Switzerland.



Sequences were aligned by eye because no gaps occurred in the three gene-coding regions. The sequences were concatenated and analyzed simultaneously. The Arlequin (Schneider et al. 2000) software was used to calculate the number of haplotypes, nucleotide diversity ( $\pi$ ),  $F_{ST}$ ,  $F_u$ 's (1997) F-statistics, and mismatch distributions. PAUP\* (Swofford 1999) was used to conduct maximum parsimony searches as well as a maximum likelihood analysis (using parameters estimated from the sequences) of unique haplotypes. A single individual of *D. leucotos* (white-backed woodpecker) was used to root the haplotypes of *D. major*.

## Results

The three gene regions yielded 1365 base pairs of mtDNA sequence (ND3-550, ND2-382, Cyt *b*-433). Thirty-one haplotypes were distinguished based on 60 polymorphic sites. Haplotype diversity was 0.81 and  $\pi$  was 0.0056. One haplotype was found in 26 individuals sampled from the following localities (Table 1, Fig. 1): Irkutsk, Krasnodar, Noyabr'sk, Kursk, Vologda, Magadan, Khabarovsk, Mongolia, Astrakhan', and Oulu. Parsimony analysis of unique haplotypes found 50 equally parsimonious trees (length 141), an example of which is shown in Fig. 3, which revealed two groups of samples: those from Japan, Sakhalin and Primor'e, and the rest of the range. Each group received at least 97% bootstrap support. There was no phylogeographic structure evident within either group – that is, no haplotypes from a sample site or region were reciprocally monophyletic at greater than 50% bootstrap support. The same result was obtained with a maximum likelihood analysis employing the GTR + G + I model. The uncorrected sequence divergence between the two groups averaged 3.0% and the distance to the outgroup averaged 6.5%.

Hierarchical F-statistics reinforced the recognition of two groups, with 95% of the variance among groups, 1% among samples within groups, and 4% within samples. A mismatch distribution (not shown) for all non-southeastern samples did not differ significantly from the expectation of sudden population expansion (Schneider and Excoffier 1999), nor did one (not shown) for the six southeastern individuals. The mismatch distribution (Fig. 2) for individuals from Krasnodar, our largest sample, did not differ from the expectation for sudden population expansion.

Table 1. Sample sites, subspecies, numbers of individuals (N) and haplotypes (H), and nucleotide diversity ( $\pi$ ) of great spotted woodpeckers. See Fig. 1 for location of sample sites. Subspecies designations from Winkler et al. (1995), although we have left the eastern populations as *major* and not *brevirostris* (which is poorly defined according to the authors). Genbank numbers AF430848-431048. NC = not computed because of small sample size.

Sample site	Subspecies	N	H	$\pi$
		2	2	NC
		2	1	NC
		2	2	NC
		1	1	NC
		2	2	NC
		15	9	0.0011
		7	7	0.0021
		4	4	NC
		3	3	NC
		6	3	0.0007
		6	4	0.0011
		2	1	NC
		2	2	NC
		7	3	0.0006
		2	2	NC
		3	1	NC
		1	1	NC

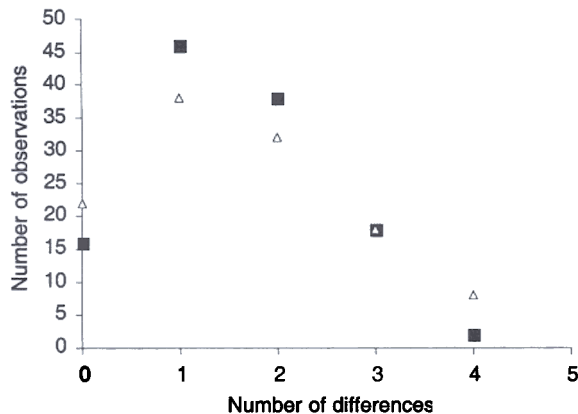


Fig. 2. Mismatch distribution for population sample from Krasnodar. Observed (squares) and expected (triangles).

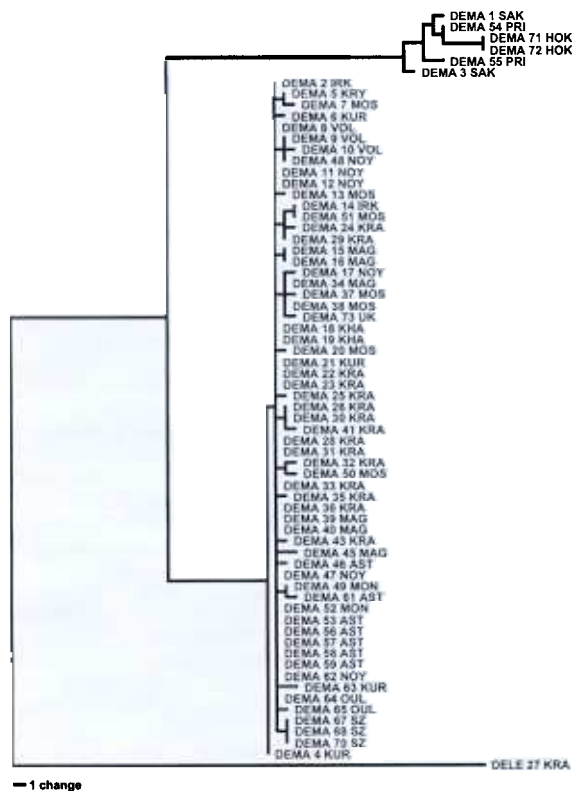


Fig. 3. Parsimony phylogram showing existence of two major groups, with a lack of phylogeographic structure in each group. Locality abbreviations correspond to names given in Fig. 1.

## Discussion

### Taxonomic implications

The phylogeographic break (Fig. 3) between samples from Hokkaido, Sakhalin and Primor'e corresponds to an apparent shift in plumage patterns. The southeastern group corresponds to *D. m. japonicus*, which was origi-

nally described as a species (*Picus japonicus*) by Seebom (1883, in Peters 1948) based on its darker lower body. The darker body is caused by infusion of rufous or cream coloration, and the white spots on the remiges have been described as large and as extending to the inner parts of the majority of secondaries (Stepanyan 1990). However, although the voucher specimens at the Burke Museum do have buffier bellies, variation in ventral coloration does not appear to be discontinuous, rather it is clinal and related to latitude. SR and SVD could see no association between spot size variation and haplotype group. Indeed, one of our three specimens from Primor'e has smaller spots than were found in any of the other specimens from eastern Eurasia. The most impressive trend was for specimens from northern areas (e.g., the six from Magadan) to have large spots in their secondaries.

Because our samples are reciprocally monophyletic in mtDNA, it would appear that *japonicus* has had a distinct evolutionary history that merits taxonomic recognition. Better samples will be required to determine whether these mitochondrial groups can be characterized morphologically. Given the lack of structure across most of northern Eurasia (Table 1), relative to the sharp mtDNA division in the southeast, we suggest that *japonicus* will be returned to species status upon study of samples from the rest of the range. Authors studying these forms should be aware of the existence of significant DNA differences between these groups, as they no doubt correspond minimally to evolutionarily significant units (Moritz 1994).

Numerous authors have commented on the potential disparity between mtDNA gene and organismal trees (Avice 2000). In birds, few examples have been found in which a mtDNA tree apparently conflicts with the organismal tree. Because of the relatively short coalescence time of avian mtDNA genomes (Moore 1995), mtDNA gene trees tend to be accurate predictors of phylogeny. Palumbi et al. (2001) reviewed why nuclear gene trees are deeper than mtDNA gene trees, making recovery of short internodes difficult, and it is unlikely that analysis of nuclear genes would challenge the mtDNA support reported here for the two groups of *D. major*.

### Phylogeographic conclusions

The large expanse of Eurasia occupied by undifferentiated populations of *D. major* reveals no effects of major isolating barriers. A similar result was obtained in a mtDNA restriction site study of the downy woodpecker *Picoides pubescens* across much of North America (Ball and Avice 1992), and the three-toed woodpecker *Picoides tridactylus* across Eurasia (Zink et al. 2002). Hence, although these woodpeckers are largely sedentary, which might be expected to enhance differentia-

tion, phylogeographic structure is absent across most of Eurasia. *Dendrocopos major* is therefore one of the most widely distributed genetically homogeneous species of birds yet discovered. Inspection of paleoecological reconstructions (Hewitt 2000) shows that much of the current range was permafrost 18 000 years ago. Although stunted vegetation can grow in such conditions, it is unlikely that forest appropriate for *D. major* was present. Hence, the species likely only recently recolonized much of the current Siberian and northern European range following post ice-age expansion of forest (see also Hewitt 2000). Such a history would explain the lack of phylogeographic structure across such a broad region; a similar hypothesis could explain the lack of phylogeographic structure in the North American *Picoides pubescens*.

Also of interest to this analysis of Eurasian phylogeography is the large sample (Krasnodar) from the western Caucasus Mountains, a region suggested as a Late Pleistocene refugium in southwestern Europe (see Hewitt 2000). The sample did not show signs of isolation, although if very recent, it might be difficult to detect (Avice 2000). Although we lacked large samples from most localities, the Krasnodar sample shows the genetic signature of a population expansion (Fig. 2). However, this relatively southern sample (45° N) has the same nucleotide diversity as the sample from Magadan (60° N). Hence, whether there was a "leading edge expansion" typically seen in recent European post-glacial recolonizations (Merilä et al. 1997, Hewitt 2000) is unclear. It is possible that a refugium or refugia existed further south, and that all of our sites across Eurasia, including Krasnodar, were recently recolonized. Samples from the southern extent of the species' range are required to test this idea.

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## References

Avice, J. C. 2000. Phylogeography. Harvard Univ. Press, Cambridge, MA.  
 Ball, R. M., Jr. and Avice, J. C. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. – *Auk* 109: 626–636.

Bensch, S. and Hasselquist, D. 1999. Phylogeographic population structure of great reed warblers: an analysis of mtDNA control region sequences. – *Biol. J. Linn. Soc.* 66: 171–185.  
 Dement'ev, G. P. and Gladkov, N. A. (eds). 1954. Birds of the Soviet Union. Vol. 5. – Sovetskaya Nauka, Moscow.  
 Ellegren, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites – a new approach to studies of genetic relationships in birds. – *Auk* 109: 886–895.  
 Fedorov, V., Goropashnaya, A., Jarrell, G. H. and Fredga, K. 1999. Phylogeographic structure and mitochondrial DNA variation in true lemmings (*Lemmus*) from the Eurasian arctic. – *Biol. J. Linn. Soc.* 66: 357–371.  
 Fu, Y.-X. 1997. Statistical tests of neutrality against population growth, hitchhiking and background selection. – *Genetics* 147: 915–925.  
 Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. – *Nature* 405: 907–913.  
 Hillis, D. M., Mable, B. K., Larson, A., Davis, S. K. and Zimmer, E. A. 1996. Nucleic acids IV: sequencing and cloning. – In: Hillis, D. M., Moritz, C. and Mable, B. K. (eds). *Molecular systematics*. 2nd edn. Sinauer, Sunderland, MA, pp. 321–381.  
 Kryukov, A. P. and Suzuki, H. 2000. Phylogeography of carrion, hooded, and jungle crows (Aves, Corvidae) inferred from partial sequencing of the mitochondrial cytochrome b gene. – *Russian J. Genetics* 36: 922–929.  
 Merilä, J., Björklund, M. and Baker, A. J. 1997. Historical demography and present day population structure of the greenfinch *Carduelis chloris* – an analysis of mtDNA control-region sequences. – *Evolution* 51: 946–956.  
 Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. – *Evolution* 49: 718–726.  
 Moritz, C. 1994. Defining evolutionarily significant units for conservation. – *Trends Ecol. Evol.* 9: 373–375.  
 Palumbi, S. R., Cipriano, F. and Hare, M. P. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. – *Evolution* 55: 859–868.  
 Peters, J. L. 1948. Check-list of Birds of the World. Vol. VI. – Harvard Univ. Press, Cambridge, MA.  
 Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Ehrlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. – *Science* 239: 487–491.  
 Schneider, S., Dueffer, J.-M., Roessli, D. and Excoffier, L. 2000. Arlequin ver. 2.0: A software for population genetic data analysis. – Genetics and Biometry Laboratory, University of Geneva, Switzerland. URL: [anthropologie.unige.ch/arlequin/](http://anthropologie.unige.ch/arlequin/)  
 Schneider, S. and Excoffier, L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. – *Genetics* 152: 1070–1089.  
 Stepanyan, L. S. 1990. Conspectus of the ornithological fauna of the USSR. – "Nauka", Moscow. 728 pp. (In Russian.)  
 Swofford, D. L. 1999. PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). Version 4.0b2. – Sinauer, Sunderland, MA.  
 Winkler, H., Christie, D. A. and Nurney, D. 1995. Woodpeckers. Houghton Mifflin Co., Boston.  
 Zink, R. M., Rohwer, S., Drovetski, S. V., Blackwell-Rago, R. C. and Farrell, S. L. 2002. Holarctic phylogeography and species limits of three-toed woodpeckers. – *Condor* in press.

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