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Unusual mitochondrial DNA polymorphism in two local populations of blue tit *Parus caeruleus*

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Abstract

Mitochondrial DNA (mtDNA) from 25 blue tits *Parus caeruleus* sampled from two populations of the Grenoble region (France) was assayed for polymorphism with 17 restriction endonucleases. Nine genotypes were found. Several mtDNA genotypes were also analysed by amplification *via* the polymerase chain reaction (PCR) and direct sequencing of 903 bp of the cytochrome *b* gene. The mtDNA polymorphism is greater in *P. caeruleus* than in other comparable bird species and results from the presence of two clearly differentiated mitochondrial lineages. Using the data of restriction polymorphism, the mean sequence divergence between individuals of the two lineages is 1.23%. Therefore, *P. caeruleus* should fall into the category II of phylogeographic pattern *sensu* Avise *et al.* (1987): discontinuous mtDNA genotypes which co-occur in the same region. *P. caeruleus*, like humans and other mobile species with high gene flow, seems to have lost its geographic structure in terms of mtDNA phylogeny. This unusual mitochondrial polymorphism can be explained by the recent admixture of two long-term isolated populations. This could be accounted for by two different scenarios. One assumes a simultaneous post-glacial colonization of the Grenoble region by two isolated European populations of *P. caeruleus*. Alternatively, hybridization between *P. caeruleus* and *P. cyanus* could have caused the observed pattern of mtDNA variation.

Keywords: cytochrome *b* gene, evolutionary history, intraspecific phylogeny, mitochondrial DNA, *Parus caeruleus*, polymerase chain reaction

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Introduction

The first continent-wide survey of mtDNA variation in a bird, *Agelaius phoeniceus* (Ball *et al.* 1988) revealed very limited mtDNA variation across all of North America. The maximum sequence divergence between individuals was only 0.8%. The principal mtDNA genotypes were widely distributed and occurred frequently in the same locality. Another continent-wide survey, in *Colaptes auratus* (Moore, Graham & Price 1991) showed geographic variation in haplotype frequencies across North America, which may reflect past isolation of populations. In the same way, Zink (1991) found that two sympatric passerine birds (*Passerella iliaca* and *Melospiza melodia*) are characterized by very different geographic pattern of mtDNA variation, in relation to different probable evolutionary histories. In Sweden, Tegelström (1987a) found a large number of female lineages, but low mean sequence divergence (0.19%) within a regional population of *Parus major* (great tit). Other studies on avian mtDNA mostly concern systematic relationships between related species (e.g. Kessler & Avise 1984, 1985; Mack *et al.* 1986; Ovenden, Makinlay & Crozier 1987; Edwards & Wilson 1990; Zink & Avise 1990), between closely related species (Avise & Zink 1988), or between subspecies (Shields & Wilson 1987a; Avise & Nelson 1989; Shields 1990; Van Wagner & Baker 1990; Fleischer, Rothstein & Miller 1991). A summary of genetic distances for birds is given in Tegelström & Gelter (1990). As a general rule, avian species tend to have lower interspecific genetic distance than their non-avian counterparts in other vertebrate classes, both for mtDNA (Kessler & Avise 1985; Avise & Zink 1988) and for nuclear DNA or proteins (Avise & Aquadro 1982). The data of Ball *et al.* (1988) suggest that intraspecific genetic distances could also be lower in birds. However, Avise & Zink (1988) have found a high

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mtDNA genetic distance between two sibling species within the genus *Limnodromus* (8.2%). Similarly, Edwards & Wilson (1990) have detected very high mean sequence divergence among two allopatric populations of the species *Pomatostomus temporalis* (8.2%).

The *P. caeruleus* 'complex' (Vaurie 1957) includes a European and North African species, *P. caeruleus* (blue tit), and an Asiatic species, *P. cyanus* (azure tit). The *P. caeruleus* 'complex' has been re-examined by Martin (1991) using morphological and coloration characters. He proposed, following Mayr's (1963) concepts, that this 'complex' is a super-species consisting of four sister-species: European populations of *P. caeruleus*, African populations of *P. caeruleus*, and two allopatric populations of *P. cyanus*. Blue tits can hybridize with azure tits in a sympatric area which is localized in eastern Europe. Furthermore, the extent of this zone varies over time due to periodic westward expansion of *P. cyanus* (reviewed in Martin 1991).

Blue tits represent one of the most studied bird species in relation to their ability to breed in nestboxes. Therefore, many data concerning breeding biology, and variations in morphology and colour are available. In contrast, no genetic data have been published, except preliminary results on the biogeographical aspects of mtDNA polymorphism (Taberlet & Bouvet 1990). We investigated here the mtDNA variation among two local populations of *P. caeruleus* using a restriction site analysis. For comparison, we also generated sequence data from a large part of the cytochrome *b* gene (Genbank accession nos. M87527–M87531) from a small number of individuals.

Material and methods

Specimen collection

During the 1989 breeding season, 25 young *P. caeruleus* were collected at nesting boxes in two localities of the Grésivaudan valley, near Grenoble (Locality A: 3°85'W; 50°23'N; Locality B: 4°09'W, 50°52'N). The distance between these two localities is 30 km. Only one nestling was sampled in each nestbox. Nine individuals were from locality A and 16 from locality B.

Restriction fragment length polymorphism of mtDNA

Purified mtDNA from each individual was isolated by CsCl–ethidium-bromide gradient centrifugation (Lansman *et al.* 1981). Fresh liver and heart were used for mtDNA extraction. A sample (5–10 ng) of each individual DNA sample was then cleaved with restriction endonucleases according to manufacturer's specifications (Boehringer Mannheim). Seventeen restriction endonucleases were used: 16 with hexanucleotide recognition sites ($r = 6$ *Asn*I, *Asp*718, *Bam*HI, *Bgl*II, *Cla*I, *Eco*RI, *Hind*III, *Nde*I, *Nhe*I, *Sal*I, *Sno*I; $r = 16/3$ *Ban*I, *Dra*II, *Eae*I, *Sty*I, *Xho*II) and one with pentanucleotide recognition site ($r = 14/3$ *Ava*II). Digestion fragments were end-labelled with the appropriate α-^{32}P-labelled nucleotide, using the large fragment of *E. coli* polymerase I (Brown 1980), and then electrophoretically separated according to molecular weight in 0.6–1.5% horizontal agarose gels. These gels were vacuum-dried, and the fragments were visualized by autoradiography. *Hind*III digests of λDNA, run in adjacent lanes on all gels as molecular-weight markers, were used to estimate the sizes of the mtDNA fragments produced. No attempt was made to score fragments smaller than 300–500 bp.

Restriction patterns produced by a given enzyme were identified with the letters A, B, C and D in descending order of the pattern's frequency. Each tit was then assigned an observed composite mtDNA genotype of 17 letters. For convenience, any individuals sharing the same composite mtDNA genotype were assumed to belong to the same clone; however, the use of other endonucleases or the sequencing of any part of mtDNA would probably reveal several additional genotypes within such clones. A data matrix consisting of presence/absence information for each restriction fragment in each mtDNA clone was used to compute the overall proportion of shared restriction fragments (F) between pairs of clones and furthermore to estimate nucleotide sequence divergence (p) using Upholt's (1977) equation. Calculations for the three categories of endonucleases were computed separately, and weighted according to the total number of base pairs recognized by each type of enzyme. The minimum number of restriction site changes between all mtDNA genotype pairs corresponding to a given endonuclease were also estimated. After that, the different clones were interconnected in an unrooted phylogenetic network by the parsimony approach of Avise *et al.* (1979).

Amplification and sequencing of a part of the cytochrome b gene

Amplifications via the polymerase chain reaction (Saiki *et al.* 1988; White, Arnheim & Erlich 1989) were carried out as described by Kocher *et al.* (1989). Amplifications were carried out in 25 μl of 67-mM Tris (pH 8.8) containing 2-mM $MgCl_2$, 1-mM concentrations of each dNTP, 1-μM concentrations of each primer, 10–1000 ng of template DNA and 1.25 units of *Taq* polymerase (Cetus Corp.). The primers used are shown in Table 1. Methods by which the single-stranded DNA was obtained and sequences determined are detailed in Kocher *et al.* (1989). The DNA sequence data were analysed with a parsimony approach using PAUP (Swofford 1989).

Table 1 Primers used in the study

Primer	DNA sequence	Reference
L14841	5′-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3′	Kocher *et al.* (1989)
H15149	5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′	Kocher *et al.* (1989)
L15162	5′-GCAAGCTTCTACCATGAGGACAAATATC-3′	S. Pääbo (unpubl.)
L15424	5′-ATCCCATTCCACCCATACTACTC-3′	Edwards *et al.* (1991)
H15573	5′-AATAGGAAGTATCATTCGGGTTTGATG-3′	S. V. Edwards *et al.* (unpubl.)
H15915	5′-AACTGCAGTCATCTCCGGTTTACAAGAC-3′	Kocher *et al.* (1989)

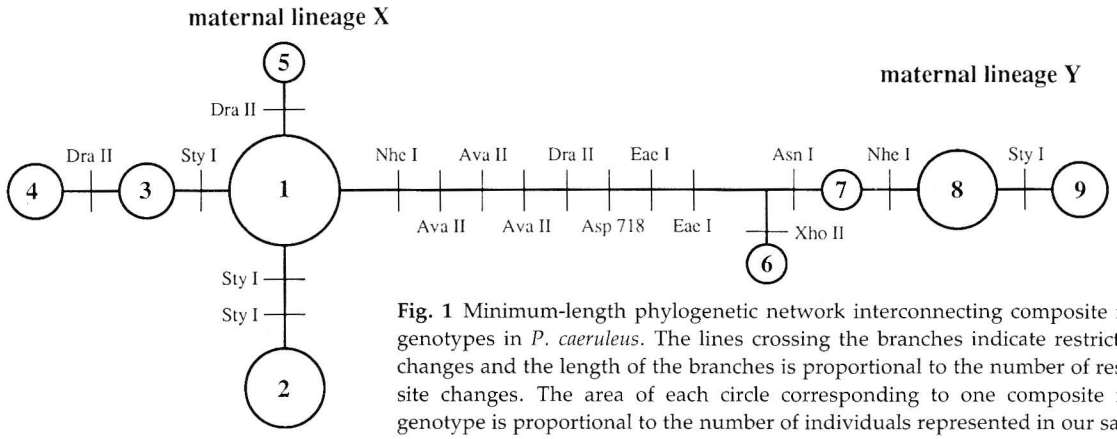


Fig. 1 Minimum-length phylogenetic network interconnecting composite mtDNA genotypes in *P. caeruleus*. The lines crossing the branches indicate restriction site changes and the length of the branches is proportional to the number of restriction site changes. The area of each circle corresponding to one composite mtDNA genotype is proportional to the number of individuals represented in our sample.

Results

The 17 restriction endonucleases used in this study gave a mean of 74 DNA fragments per individual, representing 403 nucleotides, or 2.4% of the mitochondrial genome. Table 2 provides a summary of the restriction morphs produced by all digests.

If endonucleases *Eae*I and *Sty*I are not included because of numerous small fragment losses, the estimated total number of base pairs in the *P. caeruleus* mtDNA molecule varies between 16 000 and 17 190. This estimate agrees with values obtained for other birds.

Nine different mtDNA clones were resolved with restriction analysis of the 25 *P. caeruleus* assayed. Table 3 shows their observed composite mtDNA genotype.

Table 4 gives the fraction of shared fragments (F) and the nucleotide sequence divergence (p) for all pairs of mtDNA clones. The greatest sequence divergence found is 1.49 ± 0.60%. The phylogenetic network (Fig. 1) and the UPGMA phenogram (Fig. 2) indicate the presence of two well-differentiated maternal lineages (X and Y) which co-occur in the two localities. The mean sequence divergence is 1.23% between individuals of the two lineages, and 0.12% between individuals of the same lineage.

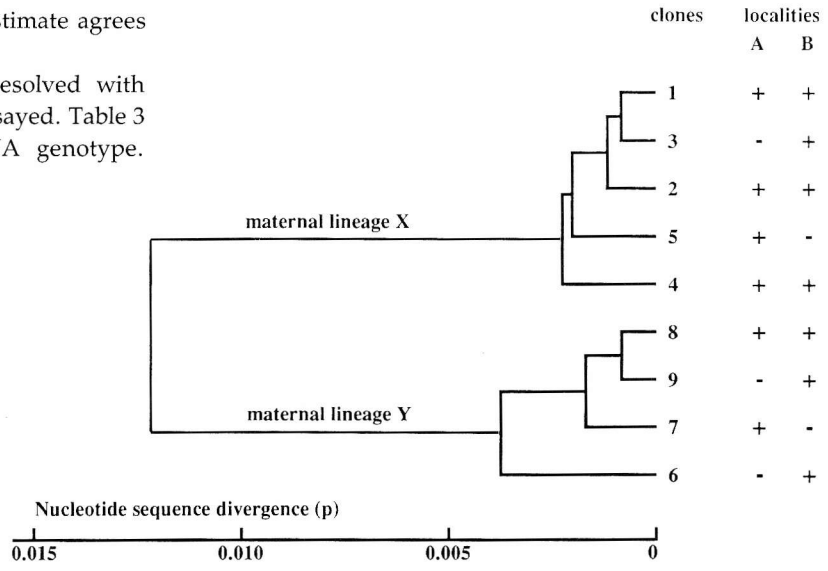


Fig. 2 Phenogram for the nine composite mtDNA genotypes of *P. caeruleus* generated by UPGMA cluster analysis of a matrix of nucleotide sequence divergences. The matrix of nucleotide sequence divergences was estimated from restriction fragment data according to Upholt (1977).

Table 2 Restriction fragment sizes (kilobase pairs) of *P. caeruleus* mtDNA produced by each of the 17 restriction endonucleases. Only differences with restriction morph A are indicated in restriction morphs B, C and D

Enzyme	*r*	Restriction morph	Fragment size (kb) of differences with restriction morph A	Σ
*Asn*I	6	A	4.1, 3.6, 3.0, 2.65, 2.15, 0.5	16.00
		B	−4.1, +2.85, +1.25	16.00
*Asp*718	6	A	15–8, 1–15	16.95
		B	−15.8, −1.15, +16.95	16.95
*Bam*HI	6	A	17.0	17.00
*Bgl*II	6	A	11.2, 5.3	16.50
*Cla*I	6	A	15.0, 1.55	16.55
*Eco*RI	6	A	17.0	17.00
*Hind*III	6	A	10.5, 6.2	16.70
*Nde*I	6	A	11.5, 3.15, 2.3	16.95
*Nhe*I	6	A	15.5, 1.5	17.00
		B	−15.5, +13.3, +2.2	17.00
		C	−15.5, +10.0, +3.3, +2.2	17.00
*Sal*I	6	A	8.2, 7.3, 1.25	16.75
*Sno*I	6	A	14.0, 2.8	16.80
*Ban*I	16/3	A	7.7, 4.7, 2.7, 1.15, 0.45	16.70
*Dra*II	16/3	A	4.1, 3.3, 2.9, 1.7, 1.67, 1.65, 0.9	16.22
		B	−1.67, +1.55	16.10
		C	−4.1, −3.3, +7.4	16.22
		D	−3.3, +2.7, +0.63	16.25
*Eae*I	16/3	A	4.0, 3.5, 2.4, 1.55, 1.3, 1.1, 0.96, 0.59	15.40
		B	−1.1. −0.96, +1.08, +0.98	15.40
*Sty*I	16/3	A	3.3, 2.35, 1.8, 1.55, 0.77, 0.75, 0.67, 0.61	11.80
		B	−0.75, −0.67, +0.64	11.02
		C	−0.75, +0.9	11.95
		D	−2.35, +2.15	11.60
*Xho*II	16/3	A	8.4, 5.3, 1.55, 1.03, 0.47	16.75
		B	−8.4, +5.1, +3.3	16.75
*Ava*II	14/3	A	3.8, 3.8, 1.6, 1.25, 1.1, 1.1, 0.9, 0.78. 0.63, 0.63, 0.55, 0.36, 0.36, 0.33	17.19
		B	−3.8, −1.25, −1.1, −0.63, −0.36, +4.2, +2.35, −0.5	17.10

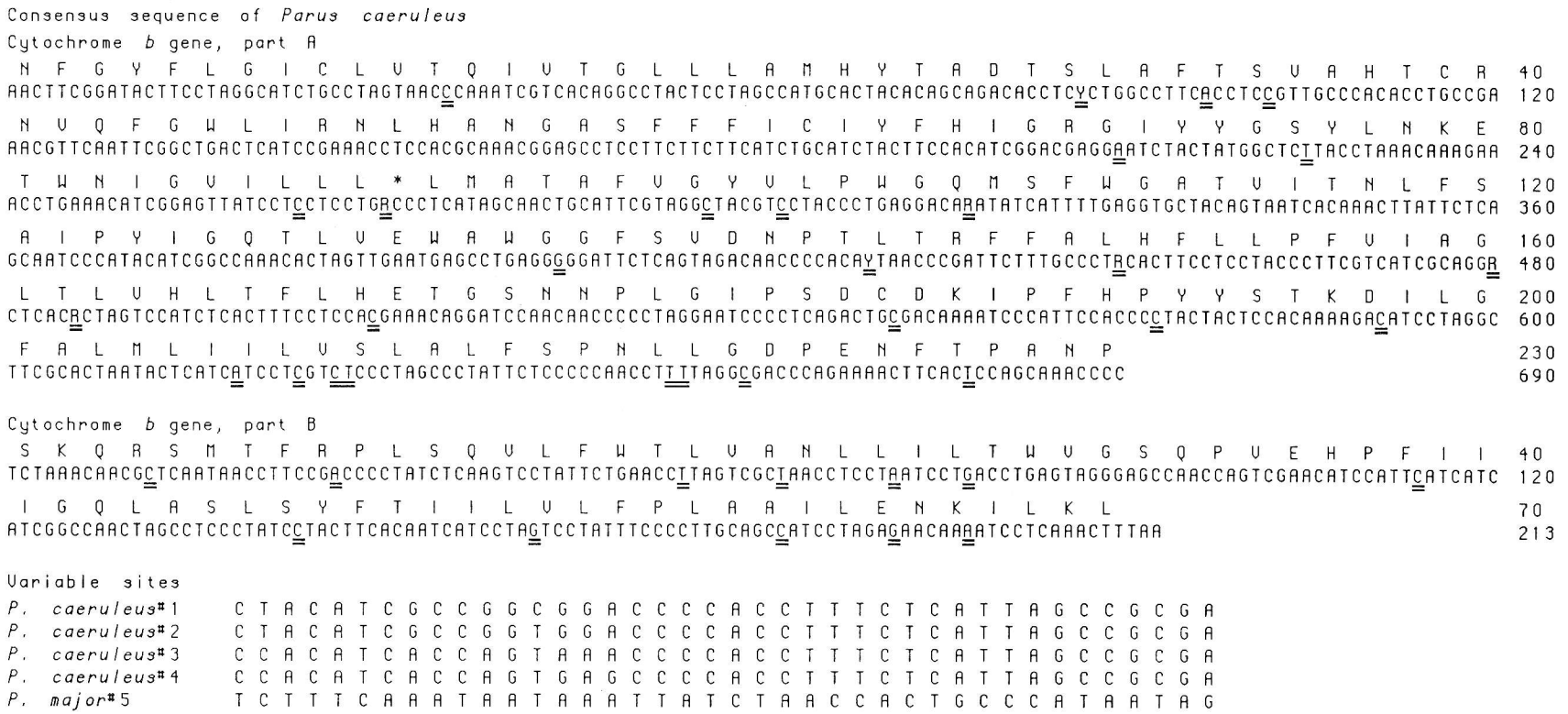
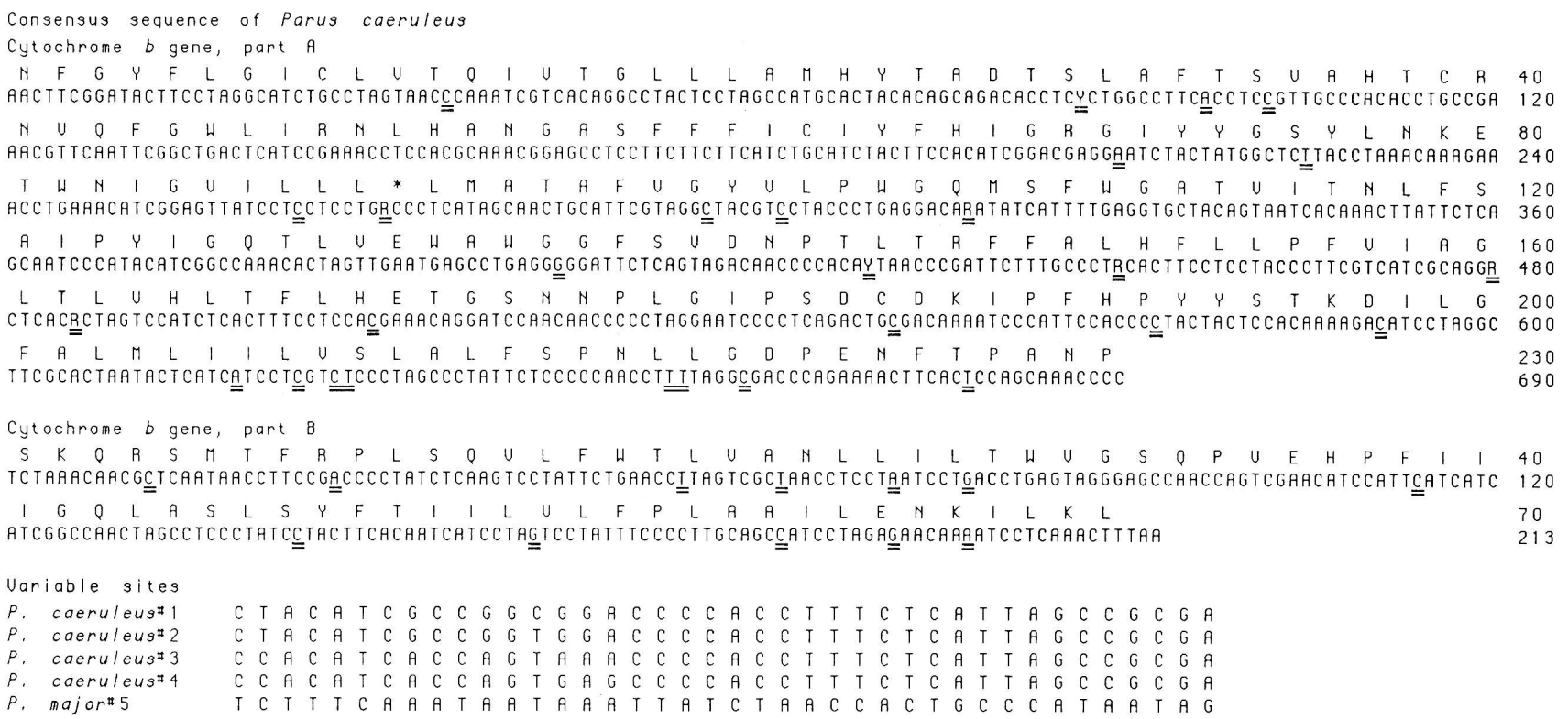


Fig. 3 Consensus partial sequences of cytochrome b gene of *P. caeruleus* and variable sites among one individual of *P. major* (which was included as an out-group) and four individuals of *P. caeruleus* (individuals 1 and 2 are from clone 8 and individuals 3 and 4 from clone 1, see Fig. 1). The two parts (A and B) of the consensus sequences correspond respectively to bases 14840–15529 and 15674–15886 of the human mtDNA sequence (Anderson *et al.* 1981) or to bases 14989–15678 and 15823–16035 of the chicken mtDNA sequence (Desjardins & Morais 1990). The inferred amino-acid sequence (in one-letter code) of *P. caeruleus* is shown on the upper line. *Indicates an amino-acid change among the four individuals of *P. caeruleus*. R and Y represents, A or G, C or T respectively. The variable sites below correspond to underlined ones in the consensus sequence.



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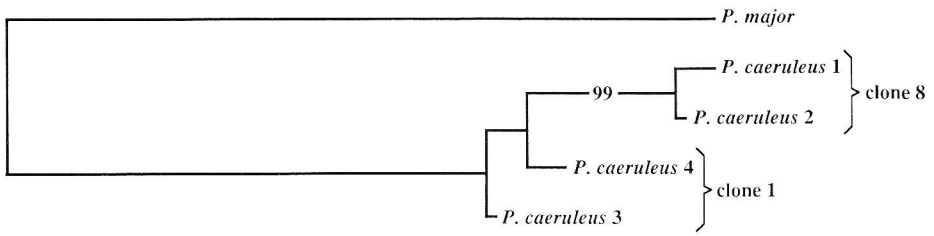

Fig. 4 Phylogenetic tree based on a parsimony analysis using PAUP (Swofford 1989) using the DNA sequence data from Fig. 3. The bootstrap value is indicated on the branch that connects the two clones (99 out of 100 replicates gave this branching order). Assuming a sequence divergence of about 2% per million years (Wilson *et al.* 1985) the phylogenetic depth of this branch is about 300 000 years. Based on this rate and the amount of divergence (see Table 5) it appears that the split between *P. major* and *P. caeruleus* took place about 2.5 million years ago.

Discussion

Two clearly differentiated mitochondrial lineages were found in the local populations of *P. caeruleus*, both by restriction analysis and by direct sequencing. One and two nucleotide substitutions of 903 bp of cytochrome *b* were found between the two individuals of each clone analysed by direct sequencing. This points out one of the advantages of PCR and direct sequencing over a restriction analysis: a higher resolution can be obtained. Based on restriction analysis for the whole mitochondrial genome, the clones 1 and 8 differ by 1.13 ± 0.53% ($p \pm$ SD). But they differ only by 0.55–0.66% for the cytochrome *b* gene. Apart from the variations due to the standard errors of the estimates, one possible explanation for this discrepancy is that the cytochrome *b* gene might be slower in its evolution than the mitochondrial genome as a whole.

The maximum genetic distance observed for mtDNA in a local population of *P. caeruleus* (1.49%) is approximately twice as high as that of *Agelaius phoeniceus* across the North American continent (Ball *et al.* 1988) and three times greater than that of *Parus major* (Tegelström 1987a). It appears the local polymorphism of mtDNA is greater in *P. caeruleus* than in the two above-mentioned bird species. This result stems from the presence of two well-differentiated maternal lineages. The phylogenetic network of each of these two lineages has the same basic structure as that found in *Agelaius phoeniceus* (Ball *et al.* 1988), i.e. a central clone linked to the others only by a limited number of losses or gains of restriction sites. The average genetic distance between individuals of the same lineage is almost identical to that found in *P. major* (Tegelström 1987a). As a result, both the diversity and the genetic structure of each of these two lineages of *P. caeruleus* correspond approximately to that which occurs in other comparable bird species.

The genetic distance between these two lineages (1.23%) is three times greater than that found for two sibling species within the genus *Parus* (0.4%) (Avise & Zink 1988), is comparable to that for two groups of clearly differentiated subspecies of *Branta canadensis* (Shields & Wilson 1987a), and is smaller than that found by Mack *et al.* (1986) for *Parus atricapillus* and *Parus carolinensis* (4%). Compared with other species of birds, the two lineages within the populations of *P. caeruleus* have diverged as much in their mtDNA sequences as two clearly demarcated subspecies, or two closely related sibling species. Does this imply that the individuals belonging to the two lineages are genetically isolated ? This seems not to be the case as no previous study of *P. caeruleus* suggests such isolation based on behavioural or morphological differences; the two lineages also co-occur in the same environment.

The co-occurrence within the same population of two very distinct mitochondrial lineages has been previously reported for other species. For humans, each geographic area studied by Cann *et al.* (1987) and by Vigilant *et al.* (1989) contains clones that are specific to the area, and clones whose nearest relatives are on other continents. Stoneking and colleagues (Stoneking, Bhatia & Wilson 1986; Stoneking *et al.* 1990; Stoneking & Wilson 1989), as well as Cann, Stoneking & Wilson (1987) and Wilson *et al.* (1987), offered the following interpretation: areas like New Guinea were colonized by multiple females, each of whom founded a maternal lineage, some of which flourished and survive today. A similar situation has been also reported for house mice (Ferris *et al.* 1983) for which the same lineages can occur in two distant areas, in relation to the human displacements.

The lack of macrogeographic structuring in mtDNA clones seems to be a feature of species with high gene flow. However, for humans and for house mice there are no genetic discontinuities (*sensu* Avise *et al.* 1987) in mtDNA at the species level. The situation seems to be somewhat different in *P. caeruleus*: a genetic discontinuity is apparent between the maternal lineages X and Y because the observed sequence divergence is high compared to other *Parus* species. Therefore *P. caeruleus* should fall into the category II of phylogeographic pattern *sensu* Avise (Avise *et al.* 1987; Avise 1989): discontinuous mtDNA

genotypes which co-occur in the same geographic region. Until now, only the East African black-backed jackal *Canis mesomelas elongae* has been reported to exhibit such a phylogeographic pattern (Wayne *et al.* 1990). Avise *et al.* (1987) explain this type of phylogeographic pattern either by the presence of recent secondary admixture zones for two populations that have been previously isolated, or by an intrinsic barrier, e.g. reproductive isolation, between two sympatric sibling species. Also, bearing in mind the stochastic extinction of mitochondrial lineages, these are the two most plausible explanations, as survival since the period of divergence of two so totally different lineages appears very unlikely within a single population (Neigel & Avise 1986; Avise, Neigel & Arnold 1984). Nevertheless, the persistence of an ancestral polymorphism in the same population cannot be definitively excluded because of the high variance in time of maternal lineage extinctions (Ball, Neigel & Avise 1990). Reproductive isolation between two subspecies is highly implausible, but this possibility could be tested by the study of nuclear genes.

Recent admixture of two isolated populations from two different refuges during Pleistocene cold periods would appear to be the most likely explanation (Taberlet & Bouvet 1990). This is supported by recent analyses showing that intraspecific mtDNA variations appear to correlate with the historical biogeography of a taxa (Moore *et al.* 1991; Zink 1991). This could be accounted for by two different scenarios. The first would be based on a simultaneous post-glacial colonization of the Grenoble region by two isolated European populations of *P. caeruleus*. The current pattern of variation in mtDNA would be a reflection of this past event. The second scenario involves the possibility of hybridization with another species. The introgression of the mitochondrial genome of one species into another has already been observed in mammals (Ferris *et al.* 1983; Carr *et al.* 1986; Tegelström 1987b; Gyllensten & Wilson 1987; Lehman *et al.* 1991; Wayne & Jenks 1991), and amphibians (Spolsky & Uzzell 1984). The species most likely to be involved in this hybridization is *P. cyanus*. The hybridization zone for *P. caeruleus* and *P. cyanus* varies in accordance to westward invasions of *P. cyanus*. The mtDNA of this oriental species could have been maintained in *P. caeruleus* populations, thereby explaining the two distinct mitochondrial lineages found in the Grenoble region. We are planning to sequence *P. cyanus* and *P. caeruleus* from a wider geographic range to put the Grenoble populations into a more complete context.

The mtDNA sequence divergence rate for geese calculated by Shields & Wilson (1987b) is approximately 2% per million years. The same rate that has been advanced for mammals (Brown, George & Wilson 1979; Ferris *et al.* 1983; Higuchi *et al.* 1984). The restriction data suggest that the two maternal lineages diverged approximately 600 000 years ago (Taberlet & Bouvet 1990). Because of a large standard error in estimates of sequence divergence and also the uncertainty of the rate of nucleotide substitution, this dating of lineage divergence is imprecise. Nevertheless, these estimations are in agreement with the three major cold periods (between 800 000 and 450 000 years ago) after the mid-Pleistocene climatic change (Williams *et al.* 1988; Shackleton & Imbrie 1990).

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References

Anderson S, Bankier AT, Barrell GG *et al.* (1981) Sequence and organization of the human mitochondrial genome. *Nature*, **290**, 457–465.

Avise JC (1989) Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution*, **43**, 1192–1208.

Avise JC, Aquadro CF (1982) A comparative summary of genetic distances in the vertebrate. *Evolutionary Biology*, **15**, 151–185.

Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

Avise JC, Giblin-Davidson C, Laerm J, Patton JC, Lansman RA (1979) Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. *Proceedings of the National Academy of Sciences of the USA*, **76**, 6694–6698.

Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution*, **20**, 99–105.

Avise JC, Nelson WS (1989) Molecular genetic relationships of the extinct Dusky Seaside Sparrow. *Science*, **243**, 646–648.

Avise JC, Zink RM (1988) Molecular genetic divergence between avian sibling species: king and clapper rails, long-billed and short-billed dowitchers, boat-tailed and great-tailed grackles, and tufted and black-crested titmice. *Auk*, **105**, 516–528.

Ball RM, Freeman S, James FC, Bermingham E, Avise JC (1988) Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. *Proceedings of the National Academy of Sciences of the USA*, **85**, 1558–1562.

Ball RM Jr, Neigel JE, Avise JC (1990) Gene genealogies within the organismal pedigrees of random-mating populations. *Evolution*, **44**, 360–370.

Brown WM (1980) Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. *Proceedings of the National Academy of Sciences of the USA*, **77**, 3605–3609.

Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the USA*, **76**, 1967–1971.

Cann RL, Stoneking M, Wilson AC (1987) Mitochondrial DNA and human evolution. *Nature*, **325**, 31–36.

Carr SM, Ballinger SW, Derr JN, Blankenship LH, Bickham JW (1986) Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas. *Proceedings of the National Academy of Sciences of the USA*, **83**, 9576–9580.

Desjardins P, Morais R (1990) Sequence and gene organization of the chicken mitochondrial genome, a novel gene order in higher vertebrates. *Journal of Molecular Biology*, **212**, 599–634.

Edwards SV, Arctander P, Wilson AC (1991) Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. *Proceedings of the Royal Society of London, Series B*, **243**, 99–107.

Edwards SV, Wilson AC (1990) Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostopus*). *Genetics*, **126**, 695–711.

Ferris SD, Sage RD, Huang C-M, Nielsen JT, Ritte U, Wilson AC (1983) Flow of mitochondrial DNA across a species boundary. *Proceedings of the National Academy of Sciences of the USA*, **80**, 2290–2294.

Fleischer RC, Rothstein SI, Miller LS (1991) Mitochondrial DNA variation indicates gene flow across a zone of known secondary contact between two subspecies of the brown-headed cowbird. *Condor*, **93**, 185–189.

Gyllensten U, Wilson AC (1987) Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. *Genetic Research*, **49**, 25–29.

Higuchi RG, Wrischnik LA, Oakes E, George M, Tong B, Wilson AC (1987) Mitochondrial DNA of the extinct quagga: relatedness and extent of postmortem change. *Journal of Molecular Evolution*, **25**, 283–287.

Kessler LG, Avise JC (1984) Systematic relationships among waterfowl (*Anatidae*) inferred from restriction endonuclease analysis of mitochondrial DNA. *Systematic Zoology*, **33**, 370–380.

Kessler LG, Avise JC (1985) A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. *Molecular Biology and Evolution*, **2**, 109–125.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196–6200.

Lansman RA, Shade RO, Shapira JF, Avise JC (1981) The use of restriction endonuclease to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. *Journal of Molecular Evolution*, **17**, 214–226.

Lehman N, Eisenhawer A, Hansen K *et al.* (1991) Introgression of coyote mitochondrial DNA into sympatric north American gray wolf populations. *Evolution*, **45**, 104–119.

Mack AL, Gill FB, Colburn R, Spolsky C (1986) Mitochondrial DNA: a source of genetic markers for studies of similar passerine bird species. *Auk*, **103**, 676–681.

Martin JL (1991) The *Parus caeruleus* "complex" revisited. *Ardea*, **79**, 429–438.

Mayr E (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, Mass.

Moore WS, Graham JH, Price JT (1991) Mitochondrial DNA variation in the Northern Flicker (*Colaptes auratus, Aves*). *Molecular Biology and Evolution*, **8**, 327–344.

Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: Nevo E, Karlin S (eds) *Evolutionary Processes and Theory*, pp. 515–534, Academic Press, New York.

Ovenden JR, Makinlay AG, Crozier RH (1987) Systematics and mitochondrial genome evolution of Australian rosellas (Aves: Platycercidae). *Molecular Biology and Evolution*, **4**, 526–543.

Saiki RK, Gelfand DH, Stoffel S *et al.* (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487–491.

Shields GF (1990) Analysis of mitochondrial DNA of Pacific Black Brant (*Branta bernicla nigricans*). *Auk*, **107**, 620–623.

Shields GF, Wilson AC (1987a) Subspecies of the Canada Goose (*Branta canadensis*) have distinct mitochondrial DNAs. *Evolution*, **41**, 662–666.

Shields GF, Wilson AC (1987b) Calibration of mitochondrial DNA evolution in geese. *Journal of Molecular Evolution*, **24**, 212–217.

Shackleton NJ, Imbrie J (1990) The $\delta^{18}O$ spectrum of oceanic deep water over a five-decade band. *Climatic Change*, **16**, 217–230.

Spolsky C, Uzzell T (1984) Natural interspecies transfer of mitochondrial DNA in amphibians. *Proceedings of the National Academy of Sciences of the USA*, **81**, 5802–5805.

Stoneking M, Bhatia K, Wilson AC (1986) Rate of sequence divergence estimated from restriction maps of mitochondrial DNAs from Papua New Guinea. *Cold Spring Harbor Symposia on Quantitative Biology*, **LI**, 433–439.

Stoneking M, Jorde LB, Bhatia K, Wilson AC (1990) Geographic variation in human mitochondrial DNA from Papua New Guinea. *Genetics*, **124**, 717–733.

Stoneking M, Wilson AC (1989) Mitochondrial DNA. In: Hill AVS, Serjeantson SW (eds) *The Colonization of the Pacific, a Genetic Trail*, pp. 215–245, Clarendon Press, Oxford.

Swofford DL (1989) *PAUP, Phylogenetic analysis using parsimony, version 3.0b*. Illinois Natural History Survey, Champaign, Ill.

Taberlet P, Bouvet J (1990) Phylogéographie de la Mésange bleue (*Parus caeruleus*): résultats préliminaires. *Comptes Rendus de l'Académie des Sciences Paris Série III*, **309**, 503–507.

Tegelström H (1987a) Genetic variability in mitochondrial DNA in a regional population of the Great Tit (*Parus major*). *Biochemical Genetics*, **25**, 95–110.

Tegelström H (1987b) Transfer of mitochondrial DNA from the Northern Red-Backed Vole (*Clethrionomys rutilus*) to the Bank Vole (*C. glareolus*). *Journal of Molecular Evolution*, **24**, 218–227.

Tegelström H, Gelter HP (1990) Haldane's rule and sex biassed gene flow between two hybridizing Flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). *Evolution*, **44**, 2012–2021.

Upholt WB (1977) Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. *Nucleic Acids Research*, **4**, 1257–1265.

Van Wagner CE, Baker AJ (1990) Association between mitochondrial DNA and morphological evolution in Canada Geese. *Journal of Molecular Evolution*, **31**, 373–382.

Vaurie C (1957) Systematic notes on the Palearctic birds, number 26. Paridae. The *Parus caeruleus* complex. *American Museum Novitates*, **1833**, 1–15.

Vigilant L, Pennington R, Harending H, Kocher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African population. *Proceedings of the National Academy of Sciences of the USA*, **86**, 9350–9354.

Wayne RK, Jenks SM (1991) Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. *Nature*, **351**, 565–568.

Wayne RK, Meyer A, Lehman N *et al.* (1990) Large sequence divergence among mitochondrial DNA genotypes within populations of East African black-backed jackals. *Proceedings of the National Academy of Sciences of the USA*, **87**, 1772–1776.

White TJ, Arnheim N, Erlich HA (1989) The polymerase chain reaction. *Trends in Genetics*, **5**, 185–189.

Williams DF, Thunell RC, Tappa E, Rio D, Raffi I (1988) Chronology of the Pleistocene oxygen isotope record: 0–1.88 m.y. B.P. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **64**, 221–240

Wilson AC, Cann RL, Carr SM *et al.* (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, **26**, 375–400.

Wilson AC, Stoneking M, Cann RL *et al.* (1987) Mitochondrial clans and the age of our common mother. In: Vogel F, Speling K (eds) *Human Genetics*, pp. 158–164, Springer-Verlag, Berlin.

Zink RM (1991) The geography of mitochondrial DNA variation in two sympatric sparrows. *Evolution*, **45**, 329–339.

Zink RM, Avise JC (1990) Patterns of mitochondrial DNA and allozyme evolution in the avian genus *Ammodramus*. *Systematic Zoology*, **39**, 148–161.

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