DNA sequences reveal phylogeographic similarities of Neotropical bats and birds*

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Abstract. *Ditchfield, A.D. & K. Burns. DNA sequences reveal phylogeographic similarities of Neotropical bats and birds. J. Comp. Biol. 3(2):164-xx.* The comparative phylogeography of unrelated species provides a powerful approach for identifying common historical influences on the geographic distribution of genetic structure among organisms. Levels of cytochrome b sequence differentiation for a total of 278 sequences representing 17 species of Neotropical bats are compared to those of birds and small nonvolant mammals. In contrast to patterns described for other Neotropical mammals, the bats studied show low levels of intraspecific divergence with little geographic structure. The phylogeographic patterns of these bats are remarkably similar to those of birds. Similar dispersal abilities of bats and birds are the most likely explanation.

Key words. Phylogeography; mitochondrial DNA; neotropics; Chiroptera; molecular variation; cytochrome *b*; birds.

Introduction

'Phylogeography' is an expression introduced by Avise *et al.* (1987), after observing that mtDNA (mitochondrial DNA) clones and clades within many species tend to be geographically localized. Phylogeography, hence, refers to "the study of the principles and processes governing the geographic distributions of genealogical lineages, including those at the intraspecific level" (Avise, 1994). Phylogeographic studies are based on two major aspects of intraspecific mtDNA variability: hierarchical structure, *i.e.*, the magnitude and pattern of mtDNA phylogenetic divergence; and geographic portioning, *i.e.*, the degree of geographic subdivision of the mtDNA phylogenetic groupings, or clades.

Birds in general have low intraspecific levels of genetic variation when compared to small vertebrates (Ward *et al.*, 1992). It has been proposed that there is a molecular clock slowdown for birds in relation to other small vertebrates such as rodents (Avise *et al.*, 1980a; Avise *et al.*, 1980b; Avise & Aquadro, 1982). This model was initially proposed to account for interspecific levels of divergence found among congeneric species of birds and small mammals. The model could however be extended to account for the phylogeographic pattern found for the vast majority of bird species, namely of comparatively lower within species levels of genetic divergence found in birds in relation to small mammals. An avian molecular clock slowdown would result in the potential accumulation of a smaller quantity of mutations for a given gene locus over time within and between populations of an avian species when compared to other small vertebrates.

An alternative explanation for this pattern is that birds have potentially greater facility to disperse than small, nonvolant mammals, for which field studies generally indicate short dispersal distances (see Chepko-Sade & Halpin, 1987). In fact, there is compelling evidence that gene flow is higher in birds than in small mammals (Ward & Skibinski, 1992; Barrowclough, 1983). If high gene flow is the primary mechanism for accounting for the differences in phylogeographic patterns between birds and nonvolant small mammals, then bats, which are also volant vertebrates, should present a phylogeographic pattern similar to birds. If an avian molecular clock slowdown is the principal motive accounting for these differences, then bats should present a highly structured pattern typical of other small mammals.

Cytochrome *b* sequences have been described from a large number of small Neotropical mammal species representing a broad spectrum of dietary and ecological requirements including marsupials, echymyid and murid rodents (Smith & Patton, 1991; Patton *et al.*, 1992; da Silva & Patton, 1993; Smith & Patton, 1993; Patton *et al.*, 1994; Lara *et al.*, 1996; Patton *et al.*, 1996a; Patton *et al.*, 1996b; Mustrangi & Patton, 1997). All these small terrestrial mammals exhibit high levels of

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* Research carried out while both authors were at the Museum of Vertebrate Zoology, University of California, Berkeley.

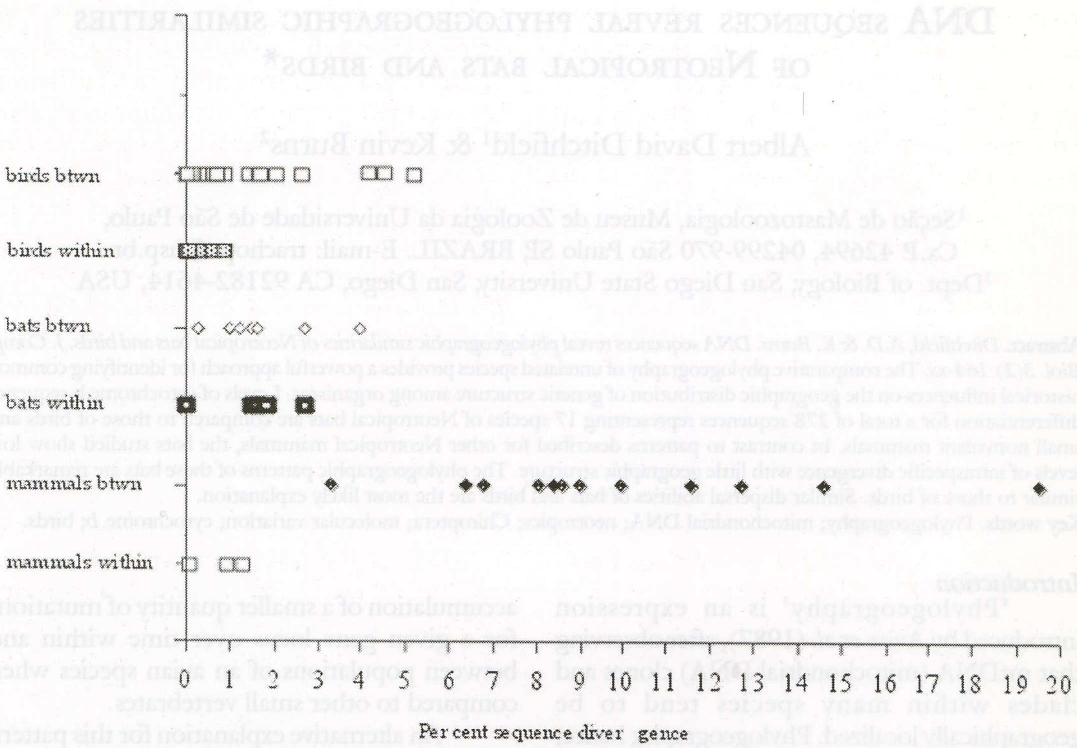

Figure 1. Average sequence divergence values for cytochrome *b* sequences plotted for intraspecific populations of bats, birds, and mammals. Values of divergence within and between localities are shown. The literature for birds included 46 references currently available; see text.

sequence divergence, ranging from 4 to 19% within a species, with strong geographic structure (Table IA). For example, in the Juruá river, localities less than 100 km apart frequently shared no haplotypes. Haplotype clusters were extremely localized and exhibited limited distribution, usually restricted to localities (da Silva & Patton, 1993; Patton *et al.*, 1996a). Thus, small nonvolant mammals show high values of sequence divergence and strong geographic structure. In contrast, studies of cytochrome *b* variation in birds show low levels of intraspecific divergence. We summarize in Figure 1 the cytochrome *b* divergence values for 46 avian species reported in the literature (see Johnson & Cicero, 1991; Birt-Friesen *et al.*, 1992; Hackett, 1992; Richman & Price, 1992; Wenink *et al.*, 1993; Murray *et al.*, 1994; Cicero & Johnson, 1995; Austin, 1996; Mariaux & Braun, 1996; Cicero, 1996; Nunn & Cracraft, 1996; Burns, 1998). Levels of sequence divergence within localities average 0.16%, whereas levels of divergence between localities within species average 0.86% (Fig. 1). Additionally, where reported, geographic structure is relatively weak within avian species when compared to patterns described in mammals.

The number of previous studies describing molecular geographic differentiation of bats is small. Most indicate high geographic structure for bats (Schmitt *et al.* 1995; Peterson & Heaney, 1993; Holman & Greenbaum, 1988; Pumo *et al.*, 1988; Pumo *et al.*, 1996), but all these studies were performed on island taxa. High geographic structuring between islands in an archipelago is not surprising, since presumably the ocean acts as a formidable barrier to dispersal. For continental taxa, available results are inconclusive. Some authors report little geographically structured variation (Sinclair *et al.*, 1996; Patterson *et al.*, 1992), while others show intermediate (Wilkinson & Fleming, 1996) to high (Worthington *et al.*, 1994) degrees of geographic structure. However, the molecular markers used differ among these studies, this fact alone being able to account for all these different results. A phylogeographic study with the sampling of co-distributed continental chiropteran taxa using the same molecular marker has so far not been attempted, though that is a prerequisite for an effective comparison to be made between bats, birds, and nonvolant small mammals. The bats sequenced here include a wide range of ecologically distinct species, representing a variety of different feeding strategies including species feeding on fruit, nectar, blood, frogs, and insects. Some of the results of this study were reported in a recent article (Ditchfield, 2000). The paper published here focuses on comparisons between bats and birds, contrasting their phylogeographic patterns with those of non-volant small mammals.

Material and methods

A total of 278 sequences were analyzed; 265 individuals comprising seventeen species of bats from the Neotropics (Mexico to the Atlantic forest of Brazil), sequenced in this study, and an additional 13 sequences obtained from Genbank. Total genomic DNA was extracted from frozen or ethanol-preserved (95%EtOH) liver tissues using the salt extraction method (Miller *et al.*, 1988). All tissue was collected from vouchered specimens deposited at the Museum of Vertebrate Zoology at Berkeley, and the Museum of Zoology, University of São Paulo. The initial sequence for the mitochondrial cytochrome *b* gene was amplified via PCR (Saiki *et al.*, 1988) using the Museum of Vertebrate Zoology (MVZ) primers 05 and 16, whose sequence has been published elsewhere (Kocher *et al.*, 1989).

Double stranded amplification was performed for 38 cycles in a Techne Programmable Heat/Cool Dri-Block PHC-2 thermocycler. Each cycle consisted of three segments: denaturation at 92° C for one minute, followed by annealing at 45° C for one minute, and extension at 72° C for one and a half minutes. Single strand product was generated by asymmetrical (relative concentrations of 1:50). The limiting primer was MVZ05 at 0.2μM concentration. Sequencing was carried out following Sanger *et al.* (1977) with the Sequenase DNA.

MVZ 05 was used in the sequencing reactions. The first 402 bp of cyt b for all species were obtained, save for *Desmodus rotundus*, where only 300bp were sequenced. The products of each individual with the S35-labelled sequences were run electrophoretically in polyacrylamide gels, dried, and exposed to x-ray films yielding a permanent record of the DNA sequences. Since other researchers were sequencing the entire cytochrome b for inferring the phylogeny of phyllostomid bats (Van Den Bussche *et al.*, 1993; Sudman *et al.*, 1994; Baker *et al.*, 1994), no attempt was made to duplicate this effort and sequence more than a short stretch of 402 bp. This decision permitted the sequencing of the largest number of individuals given the amount of funding available. Sequencing the entire cytochrome b gene would not help to find the relationships among the haplotypes, when the sequence divergence values found within a species are very low. A case in point is the human species, where the entire mitochondrial genome has been sequenced for a large number of haplotypes, with little resolution among trees, see the discussion in Templeton (1993, 1997).

Phylogenetic analyses of the sequences were performed employing maximum parsimony using the program PAUP (Swofford, 1993), version 3.1.1. All characters were included and weighted equally (unweighted parsimony analysis). The repeatability of the maximum parsimony tree generated was evaluated by 100 bootstrap replicates (Felsenstein, 1985). All the maximum parsimony trees presented in this paper are the 50 % majority rule consensus tree from the bootstrap analyses except when stated otherwise. The consensus tree from the bootstrap analysis "can be considered to be an overall estimate of the phylogeny" (Felsenstein, 1985).

Results

The phylogeographic pattern found to be typical for bats is rather well displayed by the fruitbat *Artibeus lituratus* best displays (Fig. 2). For this species, 14 localities from Mexico to southern Brazil were sampled, a distance between localities of over 8000 km. 26 haplotypes were identified in 34 individuals. Haplotype variation averages 1.5 % sequence divergence. Of the 10 individuals sequenced for one locality, Itaipu, 9 haplotypes were identified. Four haplotypes were shared with other distant localities (AL8, AL9, AL17, AL20). Local haplotype variation as found in Itaipu is essentially the same as that found throughout the species range. This pattern is not unique to *Artibeus lituratus. Glossophaga soricina*, *Sturnira lilium* and *Carollia perspicillata*, species for which large numbers of specimens (30-50/sp) were available, show the same pattern. In these four species, there are broadly distributed haplotype clades which are clearly not geographically structured (Table IB). At a given locality, the number of haplotypes present was invariably high (8 or 9/10). Haplotypes were frequently shared among localities separated by

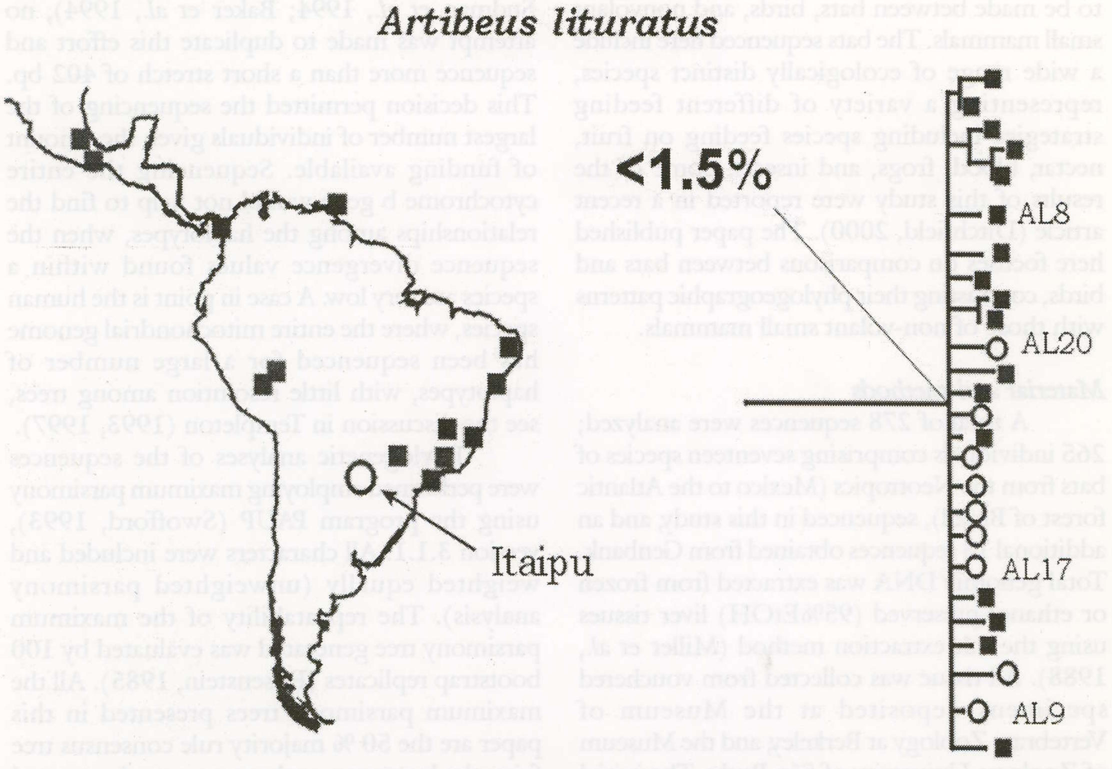

Figure 2. Distribution of haplotypes per localities in *Artibeus lituratus*. This is a consensus bootstrap maximum parsimony tree with a 98% bootstrap support level. Branch lengths are drawn proportionally to the number of nucleotide changes along each branch. The average percent sequence divergence value for the node is 1.5%. Outgroups used to root this tree were the other species of the genus sequenced. In this large frugivorous bat, as in the other species in this study, an avian phylogeographic pattern is revealed, with broad sharing of haplotypes and low sequence variation across large distances.

thousands of kilometers. These broadly shared haplotypes could either be evidence for the retention of ancestral haplotypes after expansion and subsequent local diversification, or evidence of occasional and active dispersal between populations.

Discussion

Shared haplotypes between localities could of course be the result of an extremely recent origin, without time for subsequent local diversification. We reasoned, however, that if low levels of sequence divergence were found within a progressively larger number of bat species, recency of origin would become a progressively less likely explanation for these differences. To test the generality of the pattern found, a few bats were sequenced for thirteen additional species (Table IB). In almost all cases low levels of sequence divergence were found between localities separated by thousands of kilometers. This pattern contrasts sharply with that found for Neotropical rodents and marsupials, which almost invariably had high levels of sequence divergence (>10%) between much shorter distances (<1000 km). Thus an avian phylogeographic pattern of low levels of intraspecific divergence found across a continent is common within Neotropical bat species. The one exception, *Trachops cirrhosus*, under a subsequent morphological study has proven to be a composite species (Ditchfield, 1998).

Conclusions

The geographic grain of intraspecific variation is much finer in small rodents and marsupials than it is in volant vertebrates. This conclusion is robust regardless of the species concept applied. Independent of where species boundaries are placed, bats and birds show low levels of differentiation in clades that are distributed on a continent sized scale. None of the small nonvolant mammals cited above do so. The phylogeographical pattern observed within bat and bird species most likely reflects comparatively higher levels of gene flow in these volant vertebrates. Further support for this conclusion comes from a study on the kiwi, a flightless bird that shares ecological similarities with mammals, and has an extremely subdivided population structure (Baker *et al.*, 1995). An island population structure (Wright, 1978) with alleles being actively exchanged between widely distributed localities is probably the best model for describing population structure in small volant vertebrates. A stepping stone model, with a marked effect of isolation by distance, would in turn be better at describing the population structure of small nonvolant vertebrates.

Acknowledgements. The research was carried out during the Ph.D. thesis of the senior author at the Museum of Vertebrate Zoology's molecular systematics laboratory at the University of California, Berkeley. We thank James L. Patton, A. and J. M. Bates (Field Museum of Natural History), for comments on the manuscript. Funding was provided by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Organization of American States (OAS).

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