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Pleistocene phylogeographic effects on avian populations and the speciation process

John C. Avise* **and DeEtte Walker**

Department of Genetics, University of Georgia, Athens, GA 30602-7223, USA

Pleistocene biogeographic events have traditionally been ascribed a major role in promoting speciations and in sculpting the present-day diversity and distributions of vertebrate taxa. However, this paradigm has recently come under challenge from a review of interspecific mtDNA genetic distances in birds: most sister-species separations dated to the Pliocene. Here we summarize the literature on intraspecific mtDNA phylogeographic patterns in birds and reinterpret the molecular evidence bearing on Pleistocene influences. At least 37 of the 63 avian species surveyed (59%) are sundered into recognizable phylogeographic units, and 28 of these separations (76%) trace to the Pleistocene. Furthermore, use of phylogroup separation times within species as minimum estimates of 'speciation durations' also indicates that many protracted speciations, considered individually, probably extended through time from Pliocene origins to Pleistocene completions. When avian speciation is viewed properly as an extended temporal process rather than as a point event, Pleistocene conditions appear to have played an active role both in initiating major phylogeographic separations within species, and in completing speciations that had been inaugurated earlier. Whether the Pleistocene was exceptional in these regards compared with other geological times remains to be determined.

Keywords: mtDNA; population structure; phylogeography; birds; speciation

1. INTRODUCTION

The Pleistocene epoch that began about two million years ago was a time of extraordinary oscillations in global climate (Berger 1984). Pronounced global cooling on a 100 000-year cycle spawned continental glaciers that extended far into Europe (to 52° N) and North America (to 40° N). Climatic warming with conditions more like those of Recent times (last 10 000 years) periodically interrupted the ice ages. Climatic sub-oscillations were nested within the main cycles, and similar fluctuations probably occurred in the Tertiary as well. The effect of such climatic changes on the geographic distributions of species was profound (Webb & Bartlein 1992).

Conventional wisdom is that Pleistocene climatic cycles precipitated a large proportion of speciation events between extant sister taxa. Under typical 'Pleistoscenarios', a widespread ancestral population became sundered into separate glacial refugia where allopatric divergence leading to speciation was initiated. Recently, Klicka & Zink (1997; see also Zink & Slowinski 1995) challenged this paradigm as it had been applied to avian species (for examples, see Rand 1948; Mengel 1964; Selander 1971; Gill 1995). In a review of mitochondrial (mt) DNA sequence divergences among North American songbirds, Klicka & Zink (1997) found that only 11 of 35 pairs of sister species (31%) dated to Quaternary separations under a conventional mtDNA clock calibration. The remaining pairs displayed genetic distances indicative of a protracted history of speciations over the past five million years. Klicka & Zink (1997) concluded that 'The most recent glaciations were not, it seems, the force driving songbird diversification so much as they functioned as an ecological obstacle course through which only some species were able to persist. The entrenched paradigm proclaiming that many North American songbird species originated as a consequence of these glaciations is flawed.'

Did Pleistocene environmental changes truly have little impact on extant avian diversity beyond an evolutionary filtration of pre-existing genetic variety? Here we extend the procedures of Klicka & Zink (1997) to an analysis of phylogeographic divergence among conspecific avian populations. Results indicate that Pleistocene biogeographic factors promoted substantial microevolutionary genetic diversification in birds.

2. MATERIALS AND METHODS

Studies included in this review met two criteria. First, they involved assaying of at least 200 base pairs (bp) of mtDNA sequence per individual, either as the sum of recognition sequences of multiple enzymes in restriction fragment length polymorphism (RFLP) assays of whole mtDNA, or as direct sequences of particular genes. Second, conspecific samples were taken from multiple widely spaced geographic locales. By these criteria, we found reports on a total of 63 avian species.

Sequence divergence estimates (p) between mtDNA haplotypes were taken from the original papers. Where it was possible from data provided, we also calculated 'net' sequence divergence between main phylogroups using the procedure

*Author for correspondence (avise@bscr.uga.edu).

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Table 1. *Estimates of mtDNA sequence divergence for phylogeographically distinct pairs of conspecific avian populations*

species	sequence divergence	no. units	estimated separation date[c]	data type[d]	no. of individuals, populations	source
Phylogeographic category I						
Fringilla coelebs (common chaffinch)	0.015	2	75 000	sequence, *CR* 300 bp	42, 4	Marshall & Baker 1997
Parus bicolor (tufted, black-crested titmice)	0.004	2	200 000	RFLP, 15 enzymes 83 restriction frag.	8, 2	Avise & Zink 1988
Poecilodryas albispecularis (grey-headed robin)	0.005[a]	2	250 000	RFLP, 13 enzymes 68 restriction sites	13, 4	Joseph & Moritz 1994
Thraupis episcopus (blue-grey tanager)	0.005[a]	2	250 000	RFLP, 14 enzymes 55 restriction sites	24, 8	Brawn *et al*. 1996
Uria aalge (common guillemot)	0.006	2	300 000	sequence, *cyt b* 204 bp	160, 10	Friesen *et al*. 1996
Branta bernicla (brant)	0.007	2	350 000	RFLP, 11 enzymes 88 restriction frag.	19, 5	Shields 1990
Dendroica nigrescens (black-throated gray warbler)	0.008	2	400 000	RFLP, 14 enzymes	11, ?	Bermingham *et al*. 1992
Pipilo erythrophthalmus (rufous-sided towhee)	0.008	2	400 000	RFLP, 18 enzymes	19, 4	Ball & Avise 1992
Passerella iliaca[b] (fox sparrow)	0.009	2	450 000	RFLP, nine enzymes 159 restriction frag.	46, 9	Zink 1991
Calidris alpina[b] (dunlin)	0.091[a]	2	455 000	sequence, *CR* 295 bp	73, 10	Wenink *et al*. 1993
Ammodramus maritimus (seaside sparrow)	0.010[a]	2	500 000	RFLP, 18 enzymes 110 restriction sites	40, 10	Avise & Nelson 1989
Ramphocelus dimidiatus (crimson-backed tanager)	0.011[a]	2	550 000	RFLP, 14 enzymes 59 restriction sites	51, 8	Brawn *et al*. 1996
Ammodramus caudacutus (sharp-tailed sparrow)	0.012[a]	2	600 000	RFLP, 17 enzymes 96 restriction sites	107, 14	Rising & Avise1993
Geothlypis trichas (common yellowthroat)	0.012	2	600 000	RFLP, 19 enzymes	21, 5	Ball & Avise 1992
Hirundo rustica (barn swallow)	0.015	2	750 000	RFLP, 12 enzymes	4, 2	Zink *et al*. 1995
Orthonyx spaldingii (chowchilla)	0.015[a]	2	750 000	RFLP, 13 enzymes 56 restriction sites	5, 3	Joseph & Moritz 1994
Sericornis citreogularis (yellow-throated scrubwren)	0.016[a]	2	800 000	RFLP, 11 enzymes 52 restriction sites	20, 6	ibid.
Apteryx australis (brown kiwi)	0.018	2	900 000	sequence, *cyt b* 654 bp	60, 9	Baker *et al*. 1995
Zosterops lateralis (Australian white-eye)	0.019[a]	2	950 000	RFLP, 13 enzymes 62 restriction sites	24, 17	Degnan & Moritz 1992
Leucosticte arctoa (rosy finch)	0.019	2	950 000	RFLP, 12 enzymes	6, 2	Zink *et al*. 1995
Larus canus (mew gull)	0.020	2	1 000 000	RFLP, 12 enzymes	4, 2	ibid.
Branta canadensis (Canada goose)	0.022[a]	2	1 100 000	RFLP, 21 enzymes 230 restriction frag.	47, 10	Van Wagner & Baker 1990
Sericornis magnirostris (large-billed scrubwren)	0.022[a]	2	1 100 000	RFLP, 11 enzymes 60 restriction sites	27, 7	Joseph & Moritz 1994
Parus carolinensis (Carolina chickadee)	0.024[a]	2	1 200 000	RFLP, 11 enzymes 56 restriction sites	52, 6	Gill *et al*. 1993
Coereba flaveola (bananaquit)	0.029	2	1 450 000	RFLP, 16 enzymes 108 restriction sites	170, 18	Seutin *et al*. 1994
Anthus rubescens (American pipit)	0.029	2	1 450 000	RFLP, 12 enzymes	7, 2	Zink *et al*. 1995
Toxostoma lecontei (Le Conte's thrasher)	0.035	2	1 750 000	sequence, *cyt b*, *ND6* 619 bp	14, 9	Zink *et al*. 1997
Pica pica (black-billed magpie)	0.039	2	1 950 000	RFLP, 12 enzymes	6, 2	Zink *et al*. 1995
Hemignathus virens (honeycreeper)	0.043[a]	2	2 150 000	RFLP, 15 enzymes 73 restriction sites	30, 13	Tarr & Fleischer 1993
Numenius phaeopus (whimbrel)	0.047	2	2 350 000	RFLP, 12 enzymes	6, 2	Zink *et al*. 1995

continued

Table 1. (*continued*)

species	sequence divergence	no. units	estimated separation date[c]	data type[d]	no. inds., pops.	source
Parus inornatus (plain titmouse)	0.050	2	2 500 000	RFLP, 15 enzymes 54 restriction sites	17, 2	Gill & Slikas 1992
Picoides tridactylus (three-toed woodpecker)	0.055	2	2 750 000	RFLP, 12 enzymes	9, 2	Zink *et al*. 1995
Brachyramphus marmoratus (marblet murrelet)	0.060	2	3 000 000	RFLP, 12 enzymes	6, 2	ibid.
Saltator albicollis (streaked saltator)	0.063	2	3 150 000	RFLP, 13 enzymes 62 restriction sites	81, 7	Seutin *et al*. 1993
Pomatostomus temporalis (grey-crowned babbler)	0.064	2	3 200 000[e]	RFLP, 8 enzymes 53 restriction sites	35, 5	Edwards & Wilson 1990
Struthio camelus (ostrich)	0.073[a]	2	3 650 000	RFLP, 15 enzymes 67 restriction sites	97, 26	Freitag & Robinson 1993
Phylloscopus bonelli (Bonelli's warbler)	0.085	2	4 250 000	sequence, *cyt b* 1038 bp	9, 3	Helbig *et al*. 1995
Phylogeographic category II						
Pygoscelis adeliae (Adelie penguin)	0.050	2	250 000	sequence, *CR* 300 bp	81, 3	Monehan 1994
Anas platyrhynchus (mallard)	0.007[a]	2	350 000	RFLP, 17 enzymes	20, 2	Avise *et al*. 1990
Chen caerulescens (snow goose)	0.012	2	600 000	RFLP, 18 enzymes	129, 6	Avise *et al*. 1992
Parus caeruleus (blue tit)	0.012	2	600 000	RFLP, 17 enzymes	25, 2	Taberlet *et al*. 1992

[a] Net sequence divergence, corrected for within-phylogroup variation. Let p_A and p_B be the mean sequence divergence among individuals within mtDNA phylogroups A and B, respectively, and p_{AB} be mean sequence divergence between individuals of these two groups. Then, net sequence divergence between phylogroups A and B is estimated as $p_{AB(net)} = p_{AB} - 0.5\ (p_A + p_B)$.
[b] Additional intraspecific mtDNA phylogroups were reported but sequence divergence information was not available for all.
[c] From the mtDNA clock calibrations described in §2.
[d] RFLP, restriction fragment length polymorphisms; *CR*, control region; *cyt b*, cytochrome *b* gene; *ND6*, NADH subunit 6.
[e] A much more recent date of separation was estimated in a subsequent survey based on control-region sequences (Edwards 1997).

described in a footnote to table 1. Estimates of p were converted to absolute time by using a conventional avian mtDNA clock: 2% sequence evolution between a pair of lineages per million years (Brown *et al*. 1979; avian references reviewed in Klicka & Zink 1997). For studies that assayed a hypervariable portion of the control region, a ten-fold faster calibration was employed following Quinn (1992) and Baker & Marshall (1997). Molecular clocks are fraught with uncertainties (for examples, see Martin & Palumbi 1993; Rand 1994; Mindell & Thacker 1996), and rates can vary even among related taxa (Zhang & Ryder 1995). In the future, it may be useful to revisit the issues raised in this paper using detailed molecular-clock appraisals of mtDNA sequences both within and between the species under scrutiny. For now, the clock calibration employed is identical to that used by Klicka & Zink (1997) and, thus, permits direct comparisons between the two summaries with regard to any general trends.

3. RESULTS

A total of 37 of the 63 avian species analysed (see table 1) displayed a 'category I' phylogeographic pattern as defined by Avise *et al*. (1987). In this pattern, significant mtDNA phylogroups (as gauged by bootstrapping, for example) exist in the intraspecific matrilineal gene tree, and these phylogroups display a strong geographic orientation. In most cases, the assignment of a species to phylogeographic category I was unambiguous. In figure 1, two examples involving *Ammodramus* sparrows are illustrated.

For four avian species surveyed (table 1), recognizable phylogroups in the intraspecific mtDNA gene tree were broadly sympatric (phylogeographic category II; Avise *et al*. 1987). The remaining 22 surveyed species (35%) displayed either 'shallow' (categories III, V) or no (category IV) mtDNA phylogeographic population structure across the species' monitored range.

For avian species in phylogeographic categories I and II, sequence divergence estimates between the intraspecific phylogroups (when based on RFLP or *cyt b* analyses) ranged from $p = 0.004$ to $p = 0.085$ (table 1). Under the above-mentioned clock calibrations, these translate into population-separation times ranging from about 0.2 to 4.2 million years BP. A histogram of divergence times between these intraspecific phylogroups is compared with a similar plot of sister-species divergence times in figure 2. Out of 37, a total of 28 pairs of intraspecific phylogroups (76%) show evidence of separation dates that fall within the Pleistocene. The difference between this proportion and the corresponding fraction of sister-species pairs that by the same criteria date to Pleistocene origins (31%; Klicka & Zink 1997) is highly significant ($\chi^2 = 14.4$, d.f. $= 1$, $p < 0.001$).

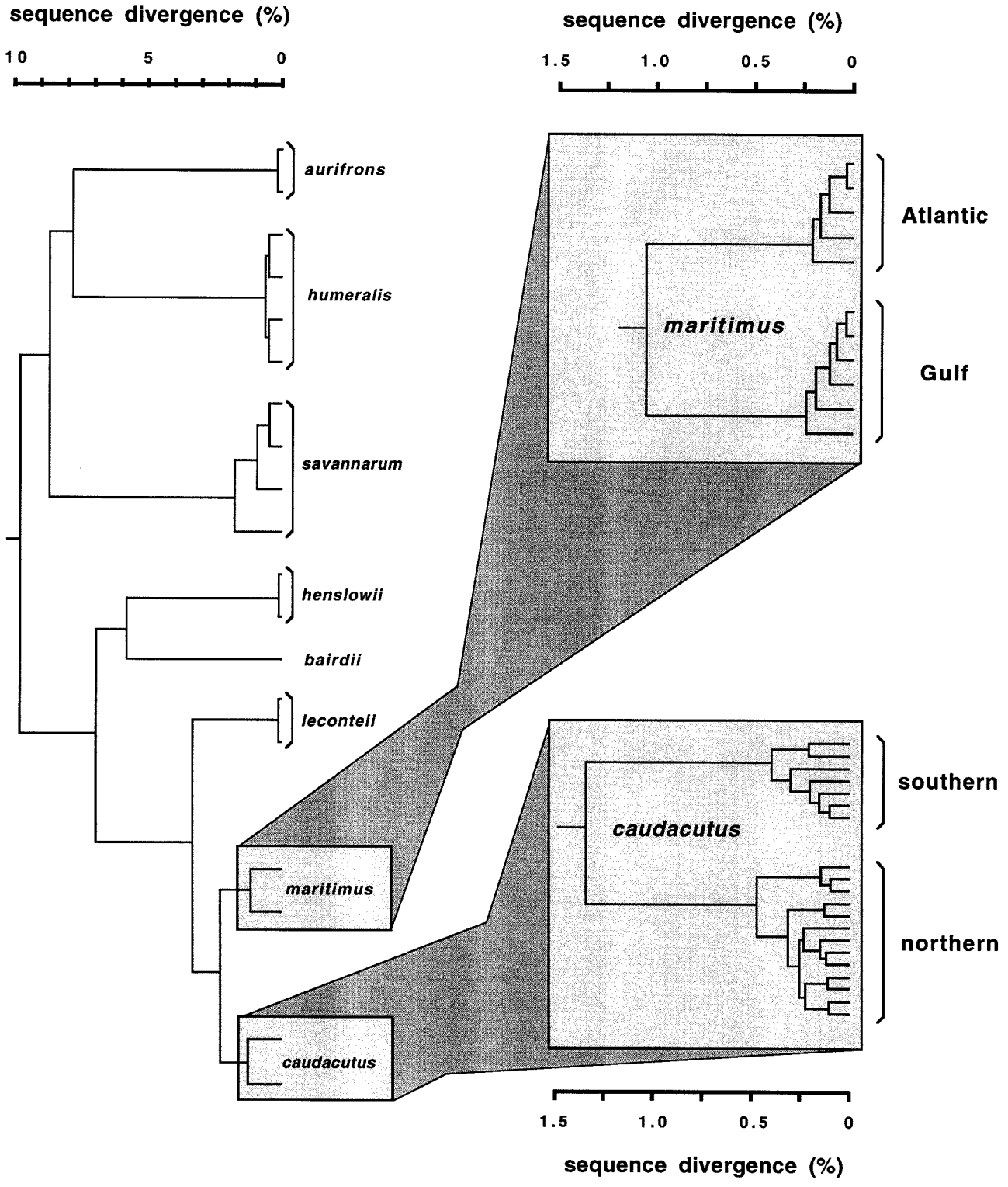

Figure 1. MtDNA phylogenies in *Ammodramus* sparrows. Left: matrilineal phylogeny for eight congeneric species as estimated by Zink & Avise (1990). Right: magnified view of matrilineal relationships within each of two species surveyed across their ranges (*A. maritimus* from Avise & Nelson (1989); *A. caudacutus* from Rising & Avise (1993)).

4. DISCUSSION

(a) *Pleistocene intraspecific phylogeography*

The current data compilation indicates that the main phylogeographic subdivisions within avian species frequently date to Pleistocene population separations. Indeed, in several studies that identified the main intraspecific phylogroups, the authors invoked explicit Pleistoscenarios to account for the phylogeographic outcomes. For example, from an integration of mtDNA data (see figure 1) with evidence from morphology and behaviour, Rising & Avise (1993) hypothesized glacial refugia and subsequent range expansions of two main phylogeographic units in the sharp-tailed sparrow.

Thus, our conclusion that Pleistocene events had a profound impact on the phylogeographic architectures of

extant species is hardly novel (see, for example, Hewitt 1996). However, the current findings assume enhanced significance in light of the deeper evolutionary separations reported by Klicka & Zink (1997) for most pairs of avian sister species. According to these authors, phylogenetic separations leading to most of the extant sister species of birds were initiated in the Pliocene, whereas current analyses suggest that pronounced phylogeographic separations within avian species usually occurred within the last two million years. These outcomes mesh and, with hindsight, might have been anticipated: if species separations tend to be older than population separations, then most species-level divergences must have been initiated prior to the Quaternary to accommodate (time-wise) Pleistocene effects on the phylogeographic structures of conspecific avian populations.

In the RFLP and *cyt b* analyses, the most recent phylogeographic splits date to about 200 000 years ago (table 1). However, a severe bias operates against the detection of later population separations. In a typical assay, about 500 bp of mtDNA sequence were monitored. Under a standard mtDNA clock, only about one nucleotide substitution in a sequence of this length is expected to distinguish two matrilines that separated 100 000 years ago. Yet, at least three or four substitutions (uncompromised by homoplasy in the broader data) are required for robust statistical support of a putative clade in most phylogenetic analyses (Felsenstein 1985). Thus, available data cannot rule out the possibility that late-Pleistocene events also initiated many avian phylogeographic separations that remain undetected with conventional laboratory efforts.

In the current summary, Latin binomials from the original mtDNA publications were employed. Subsequent revisions (motivated in part by mtDNA findings) have sometimes altered these taxonomic assignments. For example, the two phylogeographic forms of the sharp-tailed sparrow now are afforded species status, as are those (table 1) of the rufous-sided towhee (AOU 1995). Whether such main phylogeographic units are deemed taxonomic species, subspecies, or populations, the lineage separations leading to these distinctive forms frequently date to the Pleistocene.

(b) *Speciation as an extended temporal process*

Our use of 'net' sequence divergence between mtDNA phylogroups is an attempt to correct for within-phylogroup diversity of mtDNA lineages. The procedure subtracts mean within-group variation from between-group differences, and thereby counteracts a tendency for splits in an mtDNA gene tree to predate population separations. What are the effects of analogous corrections applied to genetic distances between species? Mean distances between phylogroups (rather than intraspecific distances overall) provide a novel and relevant perspective. Main phylogeographic units are the likely evolutionary wellsprings of further evolutionary differentiation potentially leading to new species, and they are only one (rather than two) hierarchical level below the species category to which the correction is applied.

For the intraspecific phylogroups in table 1, mean mtDNA sequence divergence is 0.027 (clock translation: about 1.4 million years), a value 3.8 times higher than the intraspecific correction factor suggested by Klicka & Zink's (1997) interpretation of Edwards (1997). When this modified correction is subtracted from the between-species distance estimates, an additional 14 sister-species pairs are 'bumped' sufficiently to the left in figure 2*a* such that their inferred separations fall within the Pleistocene, and about ten other species pairs with previously inferred mid-or late-Pleistocene origins now have separation times indistinguishable from zero. This does not mean that speciations were initiated at these later times, but it does suggest that speciations were not completed much before then.

We interpret these results as follows. If, as Klicka & Zink (1997) suggest, Pliocene biogeographic-sundering agents on avian populations were at least as effective as those that were operative during the Pleistocene, then many species entering the Pleistocene epoch would have already been separated into distinctive intraspecific phylogeographic units (as are many extant bird species today). Such units would be likely candidates for subsequent evolutionary divergence during the Quaternary, eventually achieving a level of differentiation currently recognizable as taxonomic species.

Thus, if avian speciation is viewed properly as a gradual process rather than a point event in time, then Quaternary biogeographic factors must have been of considerable importance in promoting extensions of phylogeographic differences that often were initiated earlier (otherwise, the still-conspecific forms alive at that time would have gone extinct or reintegrated into single lineages). Population separations potentially leading to new species arise continually within any species that is not panmictic. The salient question is whether the Pleistocene was a key period of time with respect to fostering continued differentiation of avian populations to the species level. The mtDNA data are consistent with a main role for Pleistocene effects on related avian forms, but the current analyses have not determined whether the Pleistocene was unusual in these respects compared with other similar-length geological episodes.

Perhaps the primary biological significance of molecular findings on closely related avian taxa concerns not species' 'origination' times *per se*, but rather the extended temporal durations of the avian speciation process. Evolutionary times associated with sequence divergences between main intraspecific phylogroups (figure 2*b*) and between sister species (figure 2*a*) can be interpreted as minimum and maximum durations, respectively, of the avian speciation process. Thus, both the review by Klicka & Zink (1997) and the current summary are consistent with (and combine to support) the notion that avian population divergences leading to species-level taxonomic recognition often entail substantial evolutionary time. Even if the minimal estimates of speciation durations obtained from extant faunas (figure 2*b*) apply also to the past, then many avian speciations initiated in the Pliocene must have continued as an extended process well into the Pleistocene.

(c) *Conclusion*

In summary, it is premature to dismiss Pleistocene biogeographic factors as important players in recent avian evolution, including extended speciation processes.

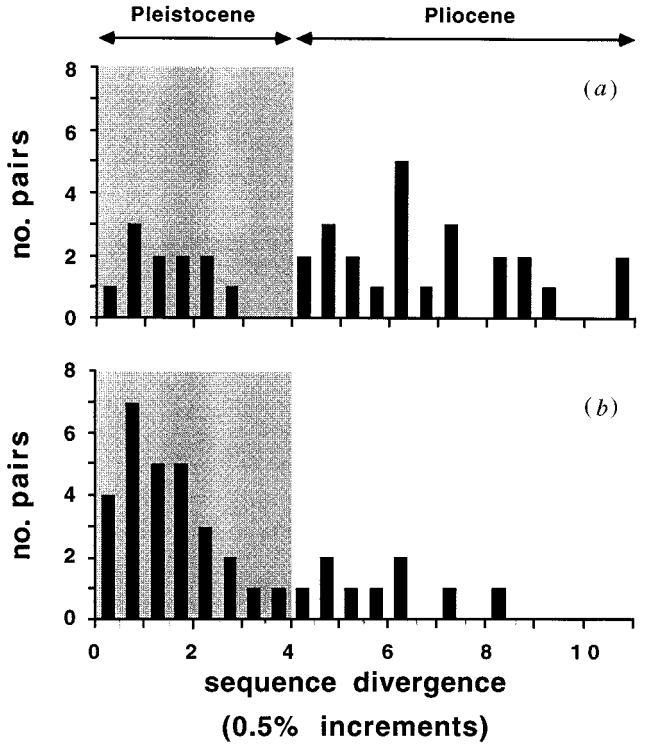

Figure 2. Histograms of estimated mtDNA sequence divergence between (*a*) 35 sister-species pairs of North American songbirds (after Klicka & Zink 1997); and (*b*) 37 pairs of major phylogeographic units within avian taxonomic species (table 1). In the latter histogram, two values derived from rapidly evolving control region sequences were adjusted (tenfold) to be consistent with the whole mtDNA estimates otherwise depicted.

At intraspecific levels in existing taxonomies, Pleistocene influences on the phylogeographic architectures of extant populations clearly were profound. For avian taxa now recognized as sister species, Pleistocene environments must have permitted or facilitated continued differentiation of phylogeographic populations whose separations often had been initiated earlier.

In two million years hence, the Pleistocene might be viewed as a time of active population differentiation that led to many speciations, but this will depend primarily on whether environmental conditions over the next two million years are conducive to fostering the survival and continued evolutionary divergence between the intraspecific phylogeographic assemblages so evident in many of today's avifauna. Such 'sliding-window' perspectives on the temporal framework of biological differentiation apply with equal force to the past. When viewed from contemporary time, the Pleistocene now can be appreciated as having played a primary role in sponsoring phylogeographic differentiation within many avian species, and also in further sculpting incipient phylogeographic variety into extant forms recognizable as today's sister taxonomic species.

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