Pleistocene effects on North American songbird evolution

John Klicka[*] **and Robert M. Zink**

J. F. Bell Museum of Natural History, University of Minnesota, 1987 Upper Buford Circle, St Paul, MN 55108, USA

Recent studies have used comparisons of mitochondrial DNA (mtDNA) sequence divergence among populations and species to test existing hypotheses about avian evolution during the Pleistocene epoch. In 1998, Avise and Walker concluded that the Pleistocene was an important time for avian evolution, including the initiation of phylogeographic separations and the completion of speciation events that began in the Pliocene. The study implied that these conclusions conflicted with the study, in 1997, by Klicka and Zink, which concluded that most species pairs previously thought to have originated in the past 250 000 years were much older. The two studies are complementary in the sense that Avise and Walker dealt primarily with phylogeographic (intraspecific) separations. Furthermore, Klicka and Zink concentrated on the inception of divergences whereas Avise and Walker focused on the timing of the completion of speciation. To accomplish this, Avise and Walker analysed 'phylogroups', geographically coherent subsets of biological species in which mtDNA haplotypes exhibit reciprocal monophyly. The study used the average inter-phylogroup mtDNA distance (0.027), calibrated at 2% per million years, to conclude that speciation required on average one million years to complete. Hence, speciation events begun in the Late Pliocene would have been completed in the mid- to late Pleistocene. Although we appreciate the extended nature of the speciation process and Avise and Walker's insightful attempt to estimate its duration, we conclude that their value was an overestimate by a factor of two. In particular we question whether phylogroups can be used in the novel evolutionary role that Avise and Walker envisioned, because of the vagaries of taxonomic practices and lack of consensus regarding species concepts. To extend their analysis of intraspecific, phylogeographic separations, we compiled previously analysed and newly available data for divergence times for North American songbird (order Passeriformes) phylogroups. More than 80% were initiated at least one million years ago, which is inconsistent with the late Pleistocene origins model previously rejected by Klicka and Zink. Although some divergence events can be traced to the late Pleistocene, the significance of the distribution must be judged with reference to a null model. Whether the Pleistocene was a profound time for avian phylogeographic differentiation is at present unknown.

Keywords: avian speciation; Pleistocene effects; recent glacial cycles; mitochondrial DNA; phylogeography

1. INTRODUCTION

The Pleistocene epoch has long been considered an important period of avian evolution. Earlier authors (e.g. Selander 1965; Udvardy 1969) believed that the Pleistocene was one million years in duration, marked by four major periods of glaciation. The two most recent glacial periods, the Wisconsinian (beginning *ca.* 100 000 years before present) and the Illinoian (*ca.* 250 000 years before present), were assumed to have played major roles in avian speciation (e.g. Mengel 1970; Hubbard 1973). Klicka & Zink (1997) termed this prevailing hypothesis of speciation the late Pleistocene origins (LPO) model. The model has been invoked by past authors who concluded that speciation events in a specific set of North American songbird species pairs resulted from isolation and allopatric speciation during these two most recent glacial advances. To test the LPO we compared mitochondrial DNA (mtDNA) for 35 such species pairs (Passerines), each of which had been postulated to have

evolved as a result of these two late Pleistocene glacial cycles. Assuming a molecular clock that ticks at *ca.* 2% per million years (Myr), a rate recently found appropriate for songbirds (Tarr & Fleischer 1993), divergences within the last 250 000 years should be less than 0.5%. The observed average pairwise mtDNA genetic distance, 5.1% ($\pm 3.0\%$), exceeded the LPO expectation tenfold. In addition, we found that the distribution of mtDNA distances for these 35 species did not differ significantly from a sample of 13 other sister species pairs not specifically hypothesized to have had late Pleistocene origins. These observations led us to conclude that speciation in these birds occurred much earlier than has been assumed.

In retrospect, the results of Klicka & Zink (1997) bring our understanding of recent avian evolution in line with current interpretations of palaeoecological data. It is now known that numerous glaciations have occurred during the last 2.5 million years in continental North America (Webb & Bartlein 1992). This modern view of the Pleistocene epoch predicts a more protracted history of glacial-induced diversification (speciation) events, consistent with Klicka

& Zink's (1997) findings, but not the LPO prediction that these species originated within the last 250 000 years.

Avise & Walker (1998) sought to extend the procedures of Klicka & Zink (1997), by asking 'Did Pleistocene environmental changes truly have little impact on extant avian diversity beyond an evolutionary filtration of pre-existing genetic variety?' (p. 457). They concluded that Pleistocene influences on avian intraspecific diversification 'clearly were profound' and that for avian sister species, 'Pleistocene environments must have permitted or facilitated continued differentiation of phylogeographic populations whose separations often had been initiated earlier' (Avise & Walker 1998, p. 462). In this paper we (i) address the implication that the conclusions of Avise & Walker (1998) conflict with those of Klicka & Zink (1997) by examining the methods and data Avise & Walker (1998) used to support their assertion, and (ii) extend their analysis of late Pleistocene songbird diversification in North America by considering additional evidence.

2. COMPARISON OF THE STUDIES OF AVISE & WALKER (1998) AND KLICKA & ZINK (1997) AND A CRITIQUE OF THE PHYLOGROUP CONCEPT

The studies of Avise & Walker (1998) and Klicka & Zink (1997) differ in geographic and temporal perspectives, taxonomic focus and evolutionary questions addressed. Avise & Walker (1998) presented a global perspective on avian intraspecific diversification throughout the Pleistocene (now commonly defined as the last two million years) whereas Klicka & Zink (1997) focused specifically on the evolution of a predefined set of North American passerine sister species over the last 250 000 years.

The species included in table 1 of Avise & Walker (1998) represent uneven sampling of both temperate and tropical avifaunas from around the world. The degree of influence of Pleistocene perturbations on local avifaunas certainly varied among the regions surveyed (Hewitt 1996), making the data set compiled by Avise & Walker (1998) inappropriate for inferring specific effects of particular Pleistocene events such as those invoked in the LPO model. For example, Klicka & Zink (1997) deliberately omitted comparisons between North American and Eurasian species comparisons (Zink et al. 1995) because of the ultimate difficulty of determining whether divergence was caused by glaciations in North America or by isolation on separate continents.

Klicka & Zink (1997) compared only passerine birds. Of the 41 species reported in table 1 of Avise & Walker (1998), 13 are non-passerines (such as the ostrich, penguin, shorebirds and seabirds). Differences in body size, generation times and metabolic rate are presumed to influence the rate of nucleotide substitution (Martin & Palumbi 1993; but see Slowinski & Arbogast 1999) as does between-taxon variation in constraints on mutation and fixation (Mindell & Thacker 1996). Passerines and non-passerines may not be directly comparable with respect to evolutionary rate.

Table 1 of Avise & Walker (1998) is also confounded by taxonomic issues. Several of their 'conspecific avian populations' are in fact also listed in Klicka & Zink's (1997) 'species' table. Indeed, species status has recently been conferred upon three of the ten North American

songbirds they listed and others will likely follow. Most of the Eurasian–North American comparisons involved taxa hypothesized to be species by the authors (Zink et al. 1995) of that study. Hence, Avise & Walker's (1998) 'intraspecific' value depends on which taxonomy is followed. Empirically, the database compiled by them has taxonomic and geographic biases that influence their interpretation of avian evolution in the Pleistocene.

An important theoretical difference between the two studies concerns the taxonomic unit employed and the actual question addressed. Avise & Walker (1998) created a new category which they termed the 'phylogroup'. In general, phylogroups are phenotypically and mitochondrially well-defined subsets of currently recognized biological species. Phylogroups in practice are extremely similar if not identical to the 'evolutionary significant unit' (ESU) of conservation genetics (Moritz 1994); we use the term phylogroup below to retain continuity with Avise & Walker (1998). Most of Avise & Walker's (1998) novel conclusions hinge upon the notion that phylogroups represent a valid evolutionary stage that is sandwiched between subspecies and species, and that comparison of phylogroups provides a measure of the duration (as opposed to the inception as in Klicka & Zink (1997)) of the speciation process. To clarify Avise & Walker's (1998) main conclusions as they pertain to Klicka & Zink's (1997) study, it is useful to recognize four distinct points on the evolutionary trajectory of diverging populations (figure 1): (i) initial divergence (i.e. coalescence) of haplotype lineages, (ii) the timing of the separation ('sundering') of the two organismal lineages, (iii) the evolution of phylogroups, and (iv) the time at which taxonomists recognize phylogroups as species.

o envision these stages of haplotype evolution (figure 1) one essentially 'looks backwards' from the present to the point (p_{AB}) where haplotypes coalesce to a single haplotype in the ancestral species. The coalescence point (or conversely the initial divergence) for extant haplotypes (p_{AB}) is estimated by computing distances between sister species' haplotypes (p_{AB}). Using a calibration (for example 2% Myr^{-1}), the coalescence of haplotypes represents a time at which the initial two sister haplotypes diverged. Correcting for saturation (multiple substitutions at single sites) can influence the estimation of p_{AB}, although its effects for passerine birds are unclear (Klicka & Zink 1998). The second point on the trajectory (figure 1), the timing of lineage sundering, occurs after initial haplotype divergence. This point ($P_{AB(net)}$) is estimated by applying a correction factor to the observed sequence divergence (p_{AB}) between sister taxa: $p_{AB(net)} = p_{AB} - 0.5(p_A + p_B)$ where p_A and p_B are values for the average haplotype diversity within taxa A and B, respectively. Avise (1994) reviewed why the time estimated for mtDNA lineage separation (p_{AB}) overestimates the sundering ($p_{AB(net)}$) of the ancestral lineage. Using empirical estimates, Moore (1995) computed a value of 350 000 years for the difference between the time of haplotype coalescence (p_{AB}) and the lineage sundering point ($p_{AB(net)}$). Evaluation of the distribution of $p_{AB(net)}$ values formed the basis for Klicka & Zink's (1997) rejection of the LPO (although the p_{AB} values were not formally corrected).

The third point, the evolution of phylogroups (reciprocal monophyly of haplotypes in each daughter
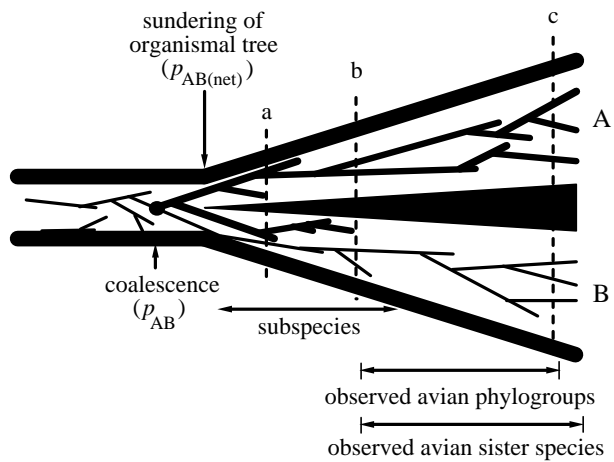
Figure 1. Diagrammatic relationship between gene tree, species tree and taxonomic levels. The dot represents the common haplotype ancestor for extant haplotypes in species A and B (p_{AB}). The ancestral species, containing several mtDNA haplotypes, is sundered ($p_{AB(net)}$). During the time between sundering and point b, typical of avian subspecies (Ball & Avise 1992), mtDNA haplotypes are paraphyletic with respect to the subspecies trinomial names (and estimate divergence values are low). For example, at point a haplotypes in species A and B are not reciprocally monophyletic (some haplotypes in lineage B (heavy lines) are more closely related to haplotypes in lineage A than to others in their own lineage, B). $4N_e$ generations after the sundering, phylogroups (*sensu* Avise & Walker 1998) are recognizable (point b) by possessing reciprocally monophyletic assemblages of mtDNA haplotypes. Point c is an arbitrary point that Avise & Walker (1998) assumed occurs one million years after point b, namely the point at which phylogroups are recognized as biological species by taxonomists. Evolutionarily, reciprocally monophyletic lineages between point b and the present simply differ in age and taxonomic status.

lineage; point b in figure 1), occurs on average $4N_e$ generations after the initial sundering of a single lineage, where N_e is the effective population size (Avise 1994). Given typical values of generation times (one year) and N_e ($\leqslant 50\,000$; Ball & Avise 1992) for songbirds, reciprocal monophyly should be achieved in $\leqslant 200\,000$ years on average. That is, diverging lineages become ESUs/phylogroups in this time frame. Estimation of these first three points on the evolutionary trajectory is well based in coalescence theory (Barton & Wilson 1996). The fourth point involves inferring the time required for phylogroups to become species (i.e. the extended speciation process), an interval influenced by taxonomy more so than evolutionary genetic theory.

Phylogroups exist for variable periods of time, usually identified as subspecies groups, before taxonomists recognize them as species. Avise & Walker (1998) suggested that sister-phylogroup distances offer a relevant measure of the approximate length of time it takes for phylogroups to become recognized as species. By averaging sister-phylogroup (intraspecific) distances and 'subtracting' them from sister-species distances, Avise & Walker (1998) believed they had computed the duration of the 'extended speciation process'. We contend that this procedure is biased.

From 41 putative sister-phylogroup comparisons (including passerines and non-passerines from around the world), Avise & Walker (1998) concluded that the average mtDNA sequence divergence (p_{AB}) among phylogroups within a sundered avian species is 0.027 (range 0.004–0.085) [Avise & Walker (1998) equated this value with the correction factor used to estimate $p_{AB(net)}$ from p_{AB}, which is incorrect]. By correcting for intraphylogroup variation (Moore 1995; see figure 1), their mean value ($p_{AB(net)}$) is reduced to 0.020 (admittedly one-third of Avise & Walker's (1998) values were already so corrected). Translating this value into time using a conventional clock calibration of $0.020\,\mathrm{Myr}^{-1}$, the average age of Avise & Walker's (1998) phylogroup pairs and, by extension, the length of time required to become a species is one million years after the initial sundering (1.35 million years without the correction for ancestral diversity). Thus, although phylogroups achieve reciprocal monophyly within 200 000 years of sundering, Avise & Walker's (1998) computation suggested that they continue to be unrecognized as species for at least 800 000 years more on average and for up to 4.25 million years (for *Phylloscopus bonelli* phylogroups). Put another way, Avise & Walker (1998) contended that phylogroups that are 2.7% divergent (on average) in mtDNA sequence are not recognized as species, whereas by the time they differ by 5.1% they are recognized. Avise & Walker (1998) correctly noted that this perspective has important implications for Klicka & Zink's (1997) analysis. For example, speciation (sundering) events that began one million years ago would have been completed during the late Pleistocene, if not during the Wisconsin glacial period. Hence, although the late Pleistocene might not have been an important period for the initiation of divergence, its environmental (e.g. glacial) perturbations might have facilitated completion of speciation events begun earlier.

We credit Avise & Walker (1998) for recognizing the extended nature of speciation and attempting to estimate its duration. For three reasons, we doubt whether the average sister-phylogroup distance is a relevant measure of the duration of speciation. First, the question addressed by Avise & Walker (1998) requires knowledge of mtDNA divergence values at the point in time at which phylogroups and species each originate. The reason is that all taxonomic groups, once recognized, continue to accrue molecular divergence without changing taxonomic status. For example, the phylogroups of *P. bonelli* have existed for four million years without attaining species status, according to Avise & Walker's (1998) view, whereas they were not 8.5% divergent at their inception. Thus, the average sister-phylogroup (if indeed they are sisters) distances will be overestimates of the times at which they originate. Second, because of the inconsistent taxonomic definition of phylogroups (figure 1), the average will be inflated by inclusion of phylogroups that are in fact species that are as yet unrecognized by taxonomists. Third, inclusion of species from faunas with different times of major isolating events will confound the estimated timing of the speciation process. For example, the values for only the North American passerines listed in Avise & Walker's (1998) table 1 average 0.017 ± 0.015. Thus, the average sister-phylogroup mtDNA genetic distance (0.027) leads to a substantial overestimate of the duration of speciation for North American passerines. [The wording of Avise & Walker's (1998) section on the
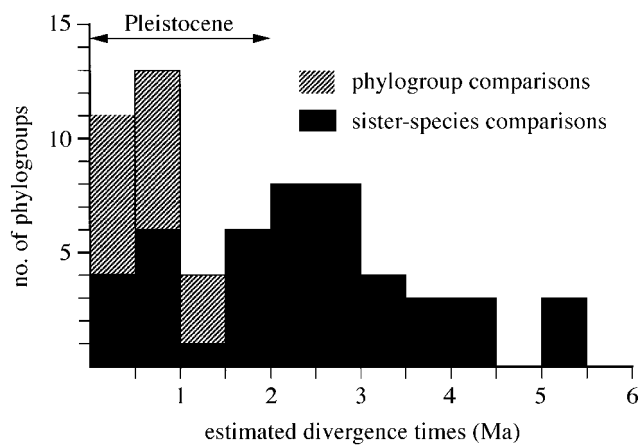
Figure 2. The distribution of mean corrected mtDNA divergence estimates for all North American songbird taxa for which data are available. We recognize that there are large variances associated with these distances; however, we maintain that they are sufficient to reject a preponderance of recent speciation events predicted by the LPO. Those currently recognized as subspecies (American Ornithologists' Union 1957, and subsequent supplements) are cross-hatched and those in black are formally (American Ornithologists' Union 1998) recognized as species. However, all taxon pairs depicted are likely phylogenetic species at different stages of evolutionary divergence.

use of the average phylogroup distance further confounds this issue. Avise & Walker (1998) stated that the 'inferred separations' of 14 species pairs are '...''bumped'' sufficiently to the left...' to be of Pleistocene origin (their figure 2a) by subtracting the average interphylogroup distance from each sister-species value. However, by inferred separations they are referring to the completion of speciation events, not to the initial divergence of haplotypes (p_{AB}), the sundering point ($p_{AB(net)}$) or the origin of phylogroups.] We suggest that speciation occurs more rapidly than Avise & Walker (1998) estimated, probably less than 500 000 years. Whether this figure will prove general requires additional data and consideration of species concepts. This value, however, takes into account the time required for reciprocal monophyly and the fact that few avian species differ by $\leqslant 1\%$ sequence divergence. In our view, the number of speciation events completed in the Pleistocene is certainly less than that estimated by Avise & Walker (1998).

Much of our concern about the significance of phylogroups represents a difference of opinion as to when speciation occurs (Zink & McKitrick 1995; Avise & Wollenberg 1997). From a systematics perspective, divergent taxa become evolutionary species (Wiley 1978) at the point at which gene flow desists and phylogenetic species (Cracraft 1983) are at (or near) the point at which reciprocal monophyly is achieved. This latter stage clearly does not require one million years. Avise & Walker (1998) estimated the time required for avian systematists to recognize phylogroups as biological species (*sensu* Mayr 1970). It is likely that biological species take longer to evolve than phylogenetic species because of the requirement that reproductive isolating mechanisms exist. However, even under the biological species concept, equating phylogroups with a real sub-specific stage of evolutionary divergence suffers from the vagaries of

inconsistent taxonomic practices. A phylogroup to one taxonomist is a species to another. The near complete overlap of the distributions of interphylogroup and interspecies distances in Avise & Walker's (1998) figure 2 reveals such taxonomic differences. Hence, we believe that Avise & Walker's (1998) novel use of phylogroups as a window on the duration of the speciation process requires a more refined description of phylogroups and an explicitly stated species concept.

Thus, the studies of Avise & Walker (1998) and Klicka & Zink (1997) provide different perspectives on avian evolution. They do not conflict because each tested a different hypothesis using different species, methods and taxonomic levels. Both studies agree that those species pairs of North American songbirds thought to have evolved in the last 250 000 years are generally much older. Furthermore, we agree with Avise & Walker's (1998) comment that whether phylogroups tended to remain isolated and become species more often in the late Pleistocene than at other times is unknown. We now expand on Avise & Walker's (1998) analysis of intraspecific divergence, which considers a hypothesis suggested by Klicka & Zink (1997): 'evidence of Late Pleistocene diversification for songbirds will more likely be found among geographically segregated conspecific populations and subspecies...' (p. 1668).

3. EVIDENCE, THEORY AND PLEISTOCENE DIVERSIFICATION OF NORTH AMERICAN PASSERINE BIRDS

In their summary of 63 avian species, Avise & Walker (1998) discerned that 44% have divided into phylogroups with Pleistocene origins. They interpreted this finding as evidence for the importance of this time for avian diversification. Here we investigate this topic more thoroughly, restricting our analysis to North American passerine birds, which constitutes the largest phylogenetically and geographically coherent sample. Of the 41 species that Avise & Walker (1998) considered, only ten are passerines from North America and, therefore, relevant for testing the LPO in North America (*Parus bicolor*, *Dendroica nigrescens*, *Pipilo erythrophthalmus*, *Passerella iliaca*, *Ammodramus caudacutus*, *Ammodramus maritimus*, *Geothlypis trichas*, *Parus carolinensis*, *Toxostoma lecontei* and *Parus inornatus*). To these we add the non-redundant taxon pairs listed in Klicka & Zink (1997, table 1 and reference 21) and 14 species that were either not listed by Avise & Walker (1998) or are from currently unpublished studies (table 1).

The histogram in figure 2 depicts corrected mtDNA sequence divergence values ($p_{AB(net)}$) for 63 sister-phylogroup pairs (the fact that Avise & Walker (1998) and our study have 63 data points is coincidental). All are songbirds from North America and, hence, relevant for testing the LPO model. The plot shows that 80% of all phylogroups (whether within or between biological species) have divergence dates older than 500 000 years, a result inconsistent with the LPO model (see also Bermingham *et al.* 1992; Gill *et al.* 1993; Joseph *et al.* 1995). Nonetheless, given the large number of values in the first two columns, one might interpret figure 2 as support for Avise & Walker's (1998) assertion that the Pleistocene as a whole was a profound time for avian phylogeographic diversification. We believe such an inference is unfounded.

Table 1. *Additional North American passerine species surveyed for mtDNA divergence (not in table 1 of Avise & Walker (1998))*

(Numbers of subspecies are from the American Ornithologists' Union (1957) *Check-list of North American birds.*)

species	number of subspecies	number of phylogroups	splits < 1 Myr	estimated age of[a]	source
Melospiza melodia	34	1	no	—	Zink & Dittmann (1993*a*)
Passerella iliaca	18	4	yes	900 000	Zink (1994)
				750 000	
				550 000	
Agelaius phoeniceus	14	1	no	—	Ball *et al.* (1988)
Parus atricapillus	9	2	yes	< 250 000	Gill *et al.* (1993)
Spizella passerina	7	1	no	—	Zink & Dittmann (1993*b*)
Toxostoma curvirostre	7	3	yes	650 000	R. M. Zink (unpublished data)
				800 000	
Pipilo fuscus	7	3	yes	400 000	R. M. Zink (unpublished data)
Campylorhynchus brunneicapillus	7	2	no	1 000 000	R. M. Zink (unpublished data)
Zonotrichia leucophrys	5	1	no	—	R. M. Zink (unpublished data)
Parus hudsonicus	5	2	yes	< 250 000	Gill *et al.* (1993)
Auriparus flaviceps	3	1	no	—	R. M. Zink (unpublished data)
Molothrus ater	3	1	no	—	Ball & Avise (1992)
Quiscalus quiscula	3	1	no	—	Zink *et al.* (1991)
Spizella breweri	2	2	yes	< 250 000	Klicka & Zink (1997)

[a]Sequence divergence estimates (not shown) were corrected for intraspecific variation using the method employed by Avise & Walker (1998). Net sequence divergence estimates were then used to calculate approximate times of divergence using the standard mtDNA clock calibration of 2% Myr^{-1}.

Rather than asking whether there are some divergence dates that trace to the late Pleistocene, which has never been in doubt, a more appropriate question is, given some background rate of speciation and extinction, was the late Pleistocene a time of exceptional change in net diversification (Zink & Slowinski 1995)? We think this latter view captures the sentiments of evolutionary biologists such as Mayr (1970) who stated that 'Evolutionists agree on the overwhelming importance of Pleistocene barriers in the speciation of temperate zone animals' (p. 334). The question then is, what is meant by 'overwhelming importance'? Does the plot of intra- and interphylogroup divergences suggest a profound or overwhelming role for the late Pleistocene? Avise & Walker (1998) answered this question at least twice in their paper, but in contradictory ways (e.g. pp. 461 and 462).

The issue is to decide what one would expect in a plot of distances between phylogroups at different stages of evolutionary divergence. A model with a constant rate of extinction and origination of phylogroups might suggest a distribution such as that depicted in figure 2 (Nee *et al.* 1994). That is, if we view each bar of the histogram as an 'age class' or cohort, we would expect to see the highest values (i.e. largest cohorts) near zero with values diminishing through time as extinctions increase in frequency, resulting in loss of one sister species. Furthermore, one expects diminishing numbers of older sister phylogroups because subsequent speciation will result in their replacement by new (younger) daughter species. Hence, the singular role of the late Pleistocene in accelerating divergence is not yet clear. If ultimately the distribution in figure 2 is explained by a constant rate of origin and extinction of phylogroups (Nee *et al.* 1994), it clearly would contradict the widely held notion of the late Pleistocene as a period of accelerated net diversification.

However, examining the nature of Pleistocene diversification is further complicated by a common observation in biogeographic analysis—the fact that not all species respond similarly to isolating barriers (Avise 1994; Hewitt 1996). In our opinion, views about late Pleistocene evolution refer not only to a preponderance of recent phylogeographic splits, but also to a disproportionate number of established species undergoing diversification into phylogroups. Thus, to examine the tempo of Pleistocene diversification fairly, we need to consider all species that have been studied, including those currently lacking phylogeographic structure. Such species were omitted by Avise & Walker (1998). Of course, as noted by Avise & Walker (1998), species currently without phylogeographic structure simply might not have had enough time for lineage sorting to have revealed evidence (reciprocally monophyletic groups) of late Pleistocene sundering. However, many studies of such species suggest not only a lack of current phylogeographic structure, but high levels of gene flow (Zink & Dittmann 1993*a*), which will prevent the eventual origin of phylogroups. Thus, it might be useful to consider the proportion of species undergoing diversification as well as the rate of diversification for those that are.

To focus on recent diversification events, we examined 23 North American species that have been surveyed widely for phylogeographic structure. These include the species in table 1 and those listed above from table 1 of Avise & Walker (1998) (*P. iliaca* is on both lists). Sixteen of the 23 (70%) species contain multiple phylogroups and only five species show evidence of a phylogroup originating within the last 250 000 years. Does this then lead to a general conclusion about the late Pleistocene origin of phylogroups within biological species? Here the nature of the database becomes important. For these 23 species, the average number of named subspecies per species is 8.1 (±7.7), whereas the value for North American passerine birds as a whole is 3.3 ± 3.9 (*n* = 234; J. Klicka and R. M. Zink, unpublished data). Furthermore, in North America, 51% of passerine birds are taxonomically monotypic

(Mayr & Short 1970) and no thorough mtDNA phylogeographic study has been done on any monotypic species of North American passerine bird. Although molecular methods might reveal some morphologically cryptic phylogroups (e.g. Gill *et al.* 1993), we know of no evidence that would indicate this is a major source of new taxa. Alternatively, we might observe more recent divergence events if more phenotypically uniform species were assayed. In any event, currently available mtDNA phylogeographic surveys have been biased towards those species that contain multiple phylogroups. Without random sampling of species one cannot judge how pervasive the effects of the late Pleistocene were or whether the distribution we obtained (figure 2) is representative.

Therefore, the true percentage of avian species that display phylogeographic structure that can be linked to late Pleistocene glaciations is currently unknown. Granted, the distributions of interphylogroup and sister-species distances will form a continuum, as Avise & Walker (1998) documented. This fact, however, is not what has captured the attention of evolutionary biologists. There is some unknown background level of speciation and extinction and the task is to show a disproportionate level of net diversification concomitant with the last few glacial cycles. Given that Avise & Walker (1998) acknowledged this in the last two sentences of their abstract, we were surprised to see them assert that 'At intraspecific levels in existing taxonomies, Pleistocene influences on the phylogeographic architectures of extant populations clearly were profound' (p. 462). Certainly there were influences but whether they were profound awaits a valid database.

REFERENCES

American Ornithologists' Union 1957 *Check-list of North American birds*, 5th edn. Washington, DC: American Ornithologists' Union.

American Ornithologists' Union 1998 *Check-list of North American birds*, 7th edn. Washington, DC: American Ornithologists' Union.

Avise, J. C. 1994 *Molecular markers, natural history and evolution.* New York: Chapman & Hall.

Avise, J. C. & Walker, D. 1998 Pleistocene phylogeographic effects on avian populations and the speciation process. *Proc. R. Soc. Lond.* B **265**, 457–463.

Avise, J. C. & Wollenberg, K. 1997 Phylogenetics and the origin of species. *Proc. Natl Acad. Sci. USA* **94**, 7748–7755.

Ball, R. M. Jr & Avise, J. C. 1992 Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**, 626–636.

Ball, R. M. Jr, Freeman, S., James, F. C., Bermingham, E. & Avise, J. C. 1988 Phylogeographic structure of red-winged blackbirds assessed by mitochondrial DNA. *Proc. Natl Acad. Sci. USA* **85**, 1558–1562.

Barton, N. H. & Wilson, I. 1996 Genealogies and geography. In *New uses for new phylogenies* (ed. P. H. Harvey, A. J. L. Brown, J. M. Smith & S. Nee), pp. 23–56. Oxford University Press.

Bermingham, E., Rohwer, S., Freeman, S. & Wood, C. 1992 Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Proc. Natl Acad. Sci. USA* **89**, 6624–6628.

Cracraft, J. 1983 Species concepts and speciation analysis. *Curr. Ornithol.* **1**, 159–187.

Gill, F. B., Mostrom, A. M. & Mack, A. L. 1993 Speciation in North American chickadees. I. Patterns of mtDNA genetic divergence. *Evolution* **47**, 195–212.

Hewitt, G. M. 1996 Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**, 247–276.

Hubbard, J. P. 1973 Avian evolution in the aridlands of North America. *Living Bird* **12**, 155–196.

Joseph, L., Moritz, C. & Hugall, A. 1995 Molecular support for vicariance as a source of diversity in rainforest. *Proc. R. Soc. Lond.* B **260**, 177–182.

Klicka, J. & Zink, R. M. 1997 The importance of recent ice ages in speciation: a failed paradigm. *Science* **277**, 1666–1669.

Klicka, J. & Zink, R. M. 1998 'Response' to Pleistocene speciation and the mitochondrial DNA clock. *Science* **282**, 1955a.

Martin, A. P. & Palumbi, S. R. 1993 Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl Acad. Sci. USA* **90**, 4087–4091.

Mayr, E. 1970 *Populations, species, and evolution.* Belknap Press, Harvard University.

Mayr, E. & Short, L. L. Jr 1970 Species taxa of North American birds. *Nuttall Ornithology Club* **9**, 1–27.

Mengel, R. M. 1970 The North American Great Plains as an isolating agent in bird speciation. *Univ. Kans. Dept Geol. Spec. Publ.* **3**, 279–340.

Mindell, D. P. & Thacker, C. E. 1996 Rates of molecular evolution: phylogenetic issues and applications. *A. Rev. Ecol. Syst.* **27**, 279–304.

Moore, W. S. 1995 Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49**, 718–726.

Moritz, C. 1994 Defining evolutionary significant units for conservation. *Trends Ecol. Evol.* **9**, 373–375.

Nee, S., May, R. M. & Harvey, P. H. 1994 The reconstructed evolutionary process. *Phil. Trans. R. Soc. Lond.* B **344**, 305–311.

Slowinski, J. B. & Arbogast, B. S. 1999 Is the rate of molecular evolution related to body size? *Syst. Biol.* (In the press.)

Tarr, C. L. & Fleischer, R. C. 1993 Mitochondrial-DNA variation and the evolutionary relationships in the Amakihi complex. *Auk* **110**, 825–831.

Udvardy, M. D. F. 1969 *Dynamic zoogeography.* New York: Van Nostrand Reinhold Co.

Webb, T. III & Bartlein, P. J. 1992 Global changes during the last 3 million years: climatic controls and biotic responses. *A. Rev. Ecol. Syst.* **23**, 141–173.

Wiley, E. C. 1978 The evolutionary species concept reconsidered. *Syst. Zool.* **27**, 17–26.

Zink, R. M. 1994 The geography of mitochondrial DNA variation, population structure, hybridization, and species limits in the fox sparrow (*Passerella iliaca*). *Evolution* **48**, 96–111.

Zink, R. M. & Dittmann, D. L. 1993a Gene flow, refugia, and evolution of geographic variation in the song sparrow (*Melospiza melodia*). *Evolution* **47**, 717–729.

Zink, R. M. & Dittmann, D. L. 1993b Population structure and gene flow in the chipping sparrow and a hypothesis for evolution in the genus *Spizella*. *Wilson Bull.* **105**, 399–413.

Zink, R. M. & McKitrick, M. C. 1995 The debate over species concepts and its implications for ornithology. *Auk* **112**, 701–719.

Zink, R. M. & Slowinski, J. B. 1995 Evidence from molecular systematics for decreased avian diversification in the Pleistocene epoch. *Proc. Natl Acad. Sci. USA* **92**, 5832–5835.

Zink, R. M., Rootes, W. L. & Dittmann, D. L. 1991 Mitochondrial DNA variation, population structure and evolution of the common grackle (*Quiscalus quiscula*). *Condor* **93**, 318–329.

Zink, R. M., Rohwer, S., Andreev, A. V. & Dittmann, D. L. 1995 Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. *Condor* **97**, 639–649.