The tempo of avian diversification during the Quaternary

Robert M. Zink[1*], John Klicka[2] and Brian R. Barber[1]

[1]*J. F. Bell Museum of Natural History, University of Minnesota, St Paul, MN 55108, USA*
[2]*Barrick Museum of Natural History, Box 454012, University of Nevada Las Vegas, Las Vegas, NV 89154, USA*

It is generally assumed that the Quaternary was a period of heightened diversification in temperate vertebrate organisms. Previous molecular systematics studies have challenged this assertion. We re-examined this issue in north temperate birds using log-lineage plots and distributions of sister-taxon distances. Log-lineage plots support earlier conclusions that avian diversification slowed during the Quaternary. To test plots of empirical sister-taxon distances we simulated three sets of phylogenies: constant speciation and extinction, a pulse of recent speciation, and a pulse of recent extinction. Previous opinions favour the model of recent speciation although our empirical dataset on 74 avian comparisons failed to reject a distribution derived from the constant and extinction models. Hence, it does not appear that the Quaternary was a period of exceptional rates of diversification, relative to the background rate.

Keywords: Quaternary; log-lineage plots; sister taxa; simulated phylogenies; diversification rates

1. INTRODUCTION

A major goal in evolutionary biology is to relate patterns of genetic differentiation to potential historical geographical isolating events. Studies can be at the level of deep history, such as discerning the effects of continental drift on evolutionary patterns. Other analyses deal with more recent events, such as Quaternary glaciations and their potential role in causing speciation. A reason for interest in more recent events is that genetic signatures of older events can decay or be overwritten. Consequently, much research has been directed at understanding how environmental perturbations during the Quaternary have shaped present patterns of genetic diversity.

Early models of the effects of the Pleistocene envisioned a few discrete glacial cycles that were predicted to have caused isolation and speciation (Mengel 1964; Hubbard 1973). It is now apparent that most extant north temperate species have been subjected to a succession of shifts in range with concomitant changes in population size and structure. Thus, genetic imprints left by successive glacial cycles might well be superimposed, with only the most dramatic or most recent being readily apparent. Because species can respond differently to the same events, it is necessary to examine a number of species to establish general patterns.

In this paper, we examine two aspects of the recent history of avian evolution. Zink & Slowinski (1995) evaluated log-lineage plots derived from species-level phylogenies of avian genera that had been constructed using mtDNA restriction site or sequence data. Analyses of these phylogenies indicated an apparent deceleration in the net rate

of diversification as the present time was approached (i.e. during the Late Quaternary). Whether this deceleration was a function of decreased speciation or increased extinction could not be determined with certainty. However, Zink & Slowinski (1995) suggested that the Late Quaternary might have been a time of increased extinction rather than speciation, because habitats were drastically compressed at the height of the last glacial maximum. That is, instead of envisioning recent glacial cycles as pistons in the engine of speciation, it is equally likely that they eliminated species by fragmenting the landscape and isolating small populations. New phylogenetic analyses of mtDNA sequences permit a more refined examination of the deceleration in net diversification noted by Zink & Slowinski (1995).

Klicka & Zink (1997) compiled mtDNA distances between pairs of species thought by previous authors to have originated during the last one or two glacial cycles (*ca*. 200 000 years before present), a phenomenon they termed the LPO model. The mean uncorrected sequence divergence among the 35 pairs examined was 5.1%, 10 times greater than expected under the LPO model. The distribution of sequence divergence values suggested a protracted history of isolation among these species pairs. Klicka & Zink (1997, 1999) did not discount the potential isolating effects of glacial cycles or the importance of the Pleistocene. Rather, they suggested that for this particular set of species, diversification times occurred over a much longer period of time than the last 200 000 years. In this paper, we evaluate new data and suggest strategies designed to extend the approach of Klicka & Zink (1999) and Avise & Walker (1998) to explore more fully the tempo of avian diversification during the Quaternary.

Our working hypothesis is that the Quaternary was a time of heightened net diversification caused by increased frequency of speciation. We believe that this captures the general sentiments of evolutionary biologists: 'Evolutionists

Phil. Trans. R. Soc. Lond. B (2004) **359**, 215–220
DOI 10.1098/rstb.2003.1392

215

agree on the overwhelming importance of Pleistocene barriers in the speciation of temperate zone animals' (Mayr 1970, p. 334). Thus, it is important to recognize that the question is not whether there were speciation events initiated or completed at any one time, but rather to ascertain whether the Quaternary experienced a level of diversification that exceeds the background rate.

2. MATERIAL AND METHODS

(a) *Log-lineage plots*

We constructed log-lineage plots for eight New World temperate clades of birds. In six of these lineages, likelihood ratio tests suggested some degree of rate heterogeneity—that is, evolving in a non-clocklike manner. Therefore, we used the program TREEEDIT (Rambaut & Charleston 2001) and its non-parametric rate-smoothing function (weighting rate differences at all nodes with the mean), to smooth trees. We then plotted the lineage diversification rates from each tree topology on a standardized scale to detect shifts in lineage diversification patterns.

(b) *Sister-taxon analysis*

To develop null expectations against which to compare an empirical set of sister-taxon distances, we simulated phylogenies under three explicit evolutionary models, which we refer to as constant, extinction and speciation, using the program PHYL-O-GEN v. 1.1 (Rambaut 2002). In the constant model, phylogenies were generated with equal speciation and extinction rates (birth $b = 0.2$ and death $d = 0.2$). Under the extinction model we used the constant parameters initially and added a burst of extinction in the last one-tenth of the simulation, which removed 50% of lineages (to represent a pulse of Late Pleistocene extinction envisioned by Zink & Slowinski (1995)). The speciation model was similar to the extinction model but with a pulse of lineage diversification ($b = 0.6$ or 0.4, $d = 0.2$), occurring in place of the extinction event (to represent the prevailing model of heightened Late Pleistocene diversification). In total we generated 20 phylogenies with 50 extant taxa per phylogeny for each of the three models. We manually compiled the distributions of branch lengths for sister taxa for each set of simulations.

We constructed a plot of empirical sister-taxon divergences from available data. We limited our comparisons to north-temperate passerine songbirds, to reduce phylogenetic effects. We considered all splits between reciprocally monophyletic groups of mtDNA haplotypes irrespective of how they are classified taxonomically, because our goal was to reconstruct the tempo of diversification. The biological species concept can obscure recent evolutionary patterns by considering some evolutionarily independent lineages to be conspecific (Klicka & Zink 1999). We did not correct for ancestral polymorphism (Avise 2000) because our simulations represent coalescence points not times of lineage sundering (Avise & Walker 1998). It is likely that the empirical mean would be reduced by less than or equal to 0.5% if such a correction were employed (Klicka & Zink 1999). We recognize that there is variance around single-locus (e.g. mtDNA) estimates of divergence times (Edwards & Beerli 2000). In fact, one might question whether the distribution of sister-taxon divergence times was due solely to such variance around a single event that isolated all sisters contemporaneously. However, it is equally plausible that divergence events occur throughout the Quaternary and that the observed distribution is a direct consequence. Thus, although we appreciate the value of multiple gene estimates of divergence (Moore 1995), we

suggest that plots of mtDNA divergences provide an estimate of the history of diversification.

To compare distributions of sister-taxon branch lengths and empirical sequence divergence values we employed the Kolmogorov–Smirnov test as implemented in STATVIEW (SAS Institute 1998). To avoid an ascertainment bias, we eliminated entries of '0' because they do not appear in our empirical dataset, but are encountered in simulations from the three models. We selected a random sample of 74 branch lengths from each model to match the sample size of the empirical dataset. Because the means of the distributions of sister-taxon branch lengths differed in the three simulations, we adjusted the empirical distribution separately so that it had the same mean as that found for each model. Standardizing the means permitted us to test the empirical and model distributions. In a subsequent analysis we will simulate sequence evolution and test both the mean and distribution of sister-species divergences.

3. RESULTS

(a) *Log-lineage plots*

The log-lineage plots (figure 1) reflect a decrease in net diversification as the present time is approached; only that for thrushes even approaches linearity. If an approximate molecular clock is assumed, the deceleration begins at the beginning or middle of the Quaternary.

(b) *Sister-taxon divergences*

Plots of sister-taxon branch lengths show clear differences between models (figure 2); pairwise Kolmogorov–Smirnov tests indicate that the distributions are significantly different. The extinction and speciation models show a large number of values near the origin, whereas the constant model shows more evenly distributed values. The speciation model has a smaller mean (0.52 ± 0.047 s.e.) than the extinction model (0.95 ± 0.065), and both are less than that for the constant model (1.7 ± 0.13). Interestingly, both the extinction and speciation models have lower values than the constant model. Thus, our simulations produced clear and distinct differences between three models of Quaternary diversification.

The empirical distribution (figure 3) has a mean of 0.04% uncorrected sequence divergence, and shows a considerable spread of values. The scaled empirical distributions (not shown) differed from the speciation model ($p = 0.002$), but not from the constant model ($p = 0.52$) or the extinction model ($p = 0.14$).

4. DISCUSSION

Log-lineage plots (figure 1), which have an explicit null expectation, suggest a decrease in net diversification as the present time is approached. It is possible that a bias against recognition and inclusion of recent evolved taxa that are not yet recognized as species partly accounts for the appearance of a decelerating rate of diversification (C. Orme and A. Purvis, unpublished data). However, even if log-lineage plots were linear, it would not support the traditional model of accelerating divergence. Hence, we conclude that our analysis supports the general conclusions of Zink & Slowinski (1995) that the tempo of diversification slowed during the Quaternary (see also
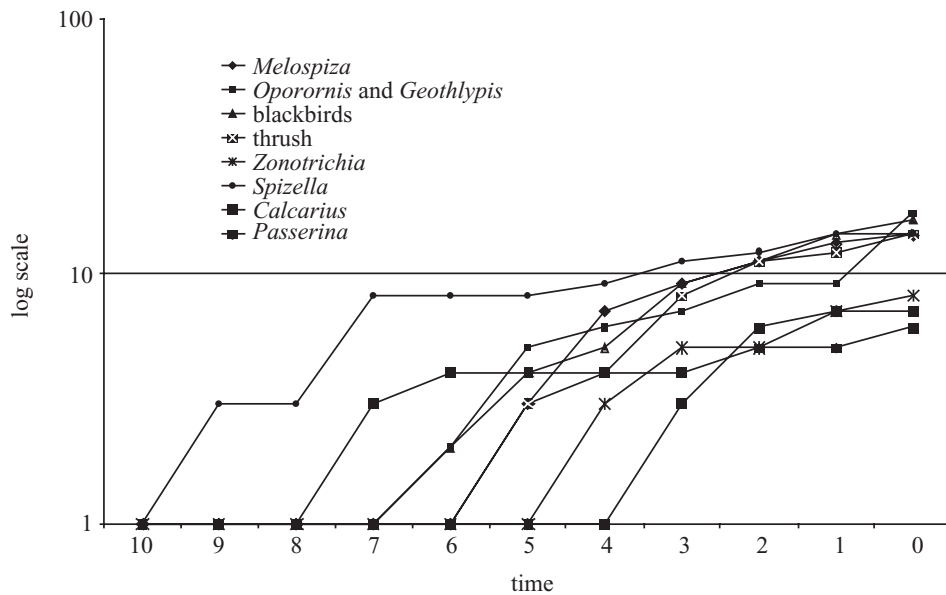
Figure 1. Log-lineage plots for eight avian lineages (J. Klicka, unpublished data). These plots show a characteristic decay in net diversification as the present time (value 0) is approached.

Lovette & Bermingham 1999). However, it is still not clear whether this is attributable to a Late Pleistocene increase in extinction rate or a decreasing speciation rate.

Inferences from plots of sister-taxon distances (Klicka & Zink 1997; Avise & Walker 1998; Klicka & Zink 1999) have played a major role in assessing the tempo of diversification. A deficiency of these past studies is the lack of a clear null model. We therefore set out to begin investigations based on a null model that incorporated constant speciation and extinction rates. In addition, we generated expectations from models that reflect two opposing evolutionary histories, one of a burst of speciation (Mayr 1970) and another including a burst of extinction (Zink & Slowinski 1995), each occurring late in the simulations, to represent differing views of Quaternary events. Thus, the question is not whether any divergences were initiated or completed during the Late Pleistocene, but whether they occurred at a greater-than-expected frequency.

The distributions of simulated sister-taxon branch lengths (figure 2) show that there are both very close and distant genetic differences under all three evolutionary scenarios. For example, 20% of the values in the constant model are within the first interval. Thus, simply finding a large number of very similar empirical distances between sister taxa (Avise & Walker 1998) permits no inferences about the relative role of Quaternary isolating barriers, unless the empirical distribution differs significantly from background expectation. It is also tempting to speculate that large distances between sister taxa (figure 3) result from recent extinctions of 'true' sister taxa. However, our simulations show that some large distances are to be expected by chance (figure 2). These observations reveal the danger in making inferences about diversification rates from simple plots (figure 3) without null models (figure 2).

Our analyses suggest that neither a model of constant speciation/extinction nor accelerated extinction can be rejected. Importantly, the empirical data were able to reject a model that incorporated a pulse of recent diversification (speciation). These results, and the log-lineage plots, are inconsistent with past opinions of heightened diversification rates in the Quaternary. We suggest that past opinions were influenced by a problem in 'evolutionary depth perception'. One expects more recent events to be 'visible' in phylogenies of extant species because extinction has erased more older lineages. Because the record is biased towards more recent events, it is easy to overestimate the significance of the most recent time period(s) in evolutionary diversification. Null models are essential (Harvey *et al.* 1994; Nee *et al.* 1995, 1996) in establishing baseline expectations.

Log-lineage plots and the results of sister-taxon analyses appear to conflict, as the former appear inconsistent with a constant rate of diversification. However, log-lineage plots take into account more than just the most recent divergence events, and it is possible to interpret our results as being consistent with a hypothesis of heightened extinction in the Late Quaternary. This could be a result of fragmentation, displacement and compression of habitats, which would have reduced refugial populations and made them vulnerable. One might predict that rare (and potentially 'old') species, probably those with specific habitat requirements, might have been especially extinction prone when isolated in small refugia with potentially many new competitors. Common, recently evolved species with relatively broad habitat requirements might have been better able to survive habitat compression and displacement, and perhaps competitively exclude specialists.

It is necessary to be clear about the questions being asked in studies of Quaternary diversification. For example, Klicka & Zink (1997) concluded that a specific set of species previously hypothesized by other authors to have originated very recently were mostly much older. There is nothing in this analysis, or any other that we are aware of, that changes that interpretation. Klicka & Zink (1997) did not address the tempo of diversification, because taxa were chosen *a priori* and there was no null model. Avise & Walker (1998) introduced two additional questions, namely that of the tempo of speciation throughout
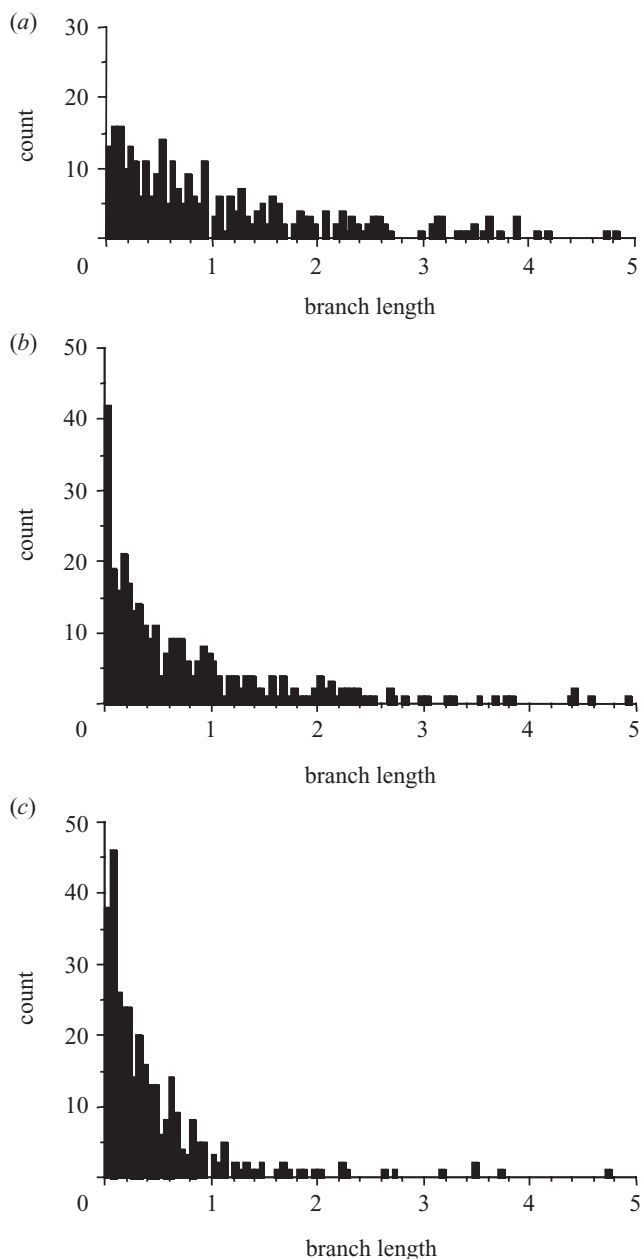
Figure 2. Plots of sister-species branch lengths (magnification ×0.5) derived from PHYL-O-GEN (Rambaut 2002) for three evolutionary models. (*a*) Constant; (*b*) extinction and (*c*) speciation.
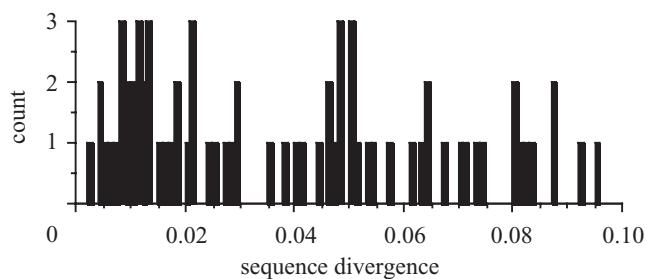

Figure 3. *Passerina caerulea* versus *P. amoena*, *P. versicolor* versus *P. ciris* (Klicka *et al.* 2001), *Dendroica graciae* versus *D. nigrescens*, *D. occidentalis* versus *D. townsendi*, *D. pinus* versus *D. pityophila*, *D. castanea* versus *D. fuscus*, *D. petechia* versus *D. striata*, *D. caerulescens* versus *Setophaga ruticilla* (Lovette & Bermingham 1999), *Dendroica nigrescens*, *Pipilo erythrophthalmus*, *Passerella iliaca*, *Ammodramus maritimus*, *A. caudacutus*, *Geothlypis trichas*, *Poecile carolinensis*, *Coereba flaveola*, *Toxostoma lecontei* (phylogroups) (Avise & Walker 1998), *Poecile atricapillus*, *P. hudsonicus*, *Toxostoma curvirostre*, *Pipilo fuscus*, *Campylorhynchus brunneicapillus*, *Auriparus flaviceps*, (phylogroups) (Klicka & Zink 1999), *Baeolophus bicolor* versus *B. atricristatus*, *Sturnella magna* versus *S. neglecta*, *Polioptila melanura* versus *P. californica*, *Pipilo erythrophthalmus* versus *P. maculatus*, *Dendroica coronata* versus *D. auduboni*, *D. townsendi* versus *D. occidentalis*, *Ammodramus caudacutus* versus *A. nelsoni*, *Sialia sialis* versus *S. mexicana*, *Cardinalis cardinalis* versus *C. sinuatus*, *Oporornis philadelphia* versus *O. tolmiei*, *Pheucticus ludovicianus* versus *P. melanocephalus*, *Cyanocitta cristata* versus *C. stelleri*, *Pipilo aberti* versus *P. crissalis*, *Toxostoma rufum* versus *T. longirostre*, *T. lecontei* versus *T. crissale*, *T. bendirei* versus *T. cinereum* (Klicka & Zink 1997), *Plectrophenax nivalis* versus *Calcarius mccownii*, *Calcarius ornatus* versus *C. pictus*, *Agelaius humeralis* versus *A. xanthomus*, *A. tricolor* versus *A. phoeniceus*, *Molothrus ater* versus *M. bonariensis*, *Euphagus cyanocephalus* versus *E. carolinus*, *Quiscalus major* versus *Q. mexicanus*, *Hylocichla mustelina* versus *Ridgwayia pinicola* , *Entomodestes coracinus* versus *E. leucotis*, *Passerella iliaca* versus *Spizella arborea*, *Spizella pusilla* versus *S. breweri*, *Geothlypis semiflava* (phylogroup), *G. aequinoctialis* versus *G. poliocephala*, *G. flaveola* versus *G. trichas*, *Cyanocompsa brissoni* versus *C. cyanoides*, *Chondestes grammacus* versus *Calamospiza melanocorys*, *Amphispiza bilineata* versus *Aimophila quinquestriata*, *Ammodramus henslowii* versus *A. bairdii*, *Melospiza lincolnii* versus *M. georgiana*, *Pooecetes gramineus* versus *Amphispiza belli* (J. Klicka, unpublished data), *Empidonax affinis* versus *E. oberholseri*, *E. atriceps* versus *E. fulvifrons*, *E. traillii* versus *E. alnorum*, *E. d. difficilis* versus *E. d. insulicola*, *E. f. flavescens* versus *E. f. salvinii* (Johnson & Cicero 2002), *Piranga ludoviciana* versus *P. bidentata* (Burns 1998), *Catharus bicknelli* versus *C. minimus*, *C. aurantiirostris* versus *C. dryas*, *C. fuscater* versus *C. mexicanus*, *C. guttatus* versus *C. occidentalis* (Outlaw *et al.* 2003).

the Quaternary, and the duration of the speciation process. This requires consideration of pairwise distances at all taxonomic levels (Klicka & Zink 1999). This theme was pursued by Klicka & Zink (1999) and in this study. Far from showing that the Quaternary was an 'unimportant' time for the inception and completion of vertebrate speciation, many species trace their beginnings to the Late Pleistocene. However, the initial question was whether this period was of exceptional importance, which we believe molecular data show is not the case.

Avise & Walker (1998) suggest that events of the Late Quaternary might be too recent to be detectable, as yet, in mtDNA gene trees. This notion is based on the idea that if mtDNA evolves at 2% per million years, there has not been enough time elapsed since the last glacial cycle for mutations to have become fixed in nascent sister

phylogroups. However, this view relies on the origin of new mutations as a means for recognizing recent isolating events. There is no reason to assume that prior to the last rounds of glaciation events, ancestral populations were homogenous in their sequences and that only mutation would have induced variation that could then serve as markers of past isolation events. If an ancestral population were indeed sundered during the last 100 000 years, the relevant parameters include effective populations sizes, level of polymorphism in the ancestral population and generation lengths. It is well established that on average, isolated populations will reveal a pattern of reciprocal

monophyly in mtDNA gene trees in $2N_{ef}$ generations, where N_{ef} is the inbreeding effective size of the female population. Mutation rate does not play a role in this computation.

Estimates of effective population size are notoriously vague, but, if two populations were isolated 100 000 years ago, at the beginnings of the formation of the Laurentide Ice Sheet, and N_{ef} was on the order of 50 000 (one of the highest values calculated for a bird species (Avise *et al.* 1988)), one should see structured gene trees in songbirds provided that this was a period of pervasive isolation, irrespective of the occurrence and fixation of new mutations. More recent splits are probably below the level of resolution of many current techniques. However, in many phylogeographic studies (Zink 1996; Zink *et al.* 2001), there is little evidence for incipient (e.g. incomplete) diversification; rather, gene flow appears to play a major homogenizing role in north temperate birds.

5. CONCLUSIONS

It is no longer valid simply to examine plots of sister-species divergence values and make inferences about the tempo of diversification without a null model(s). However, there are many variations possible with the simulations used in this study, because one can use a variety of speciation and extinction rates. For instance, 'constant' speciation : extinction ratios that themselves differ (e.g. 0.2 : 0.2, 0.5 : 0.5) can be used. In fact, if both speciation and extinction rates were 'high', i.e. many speciation and extinction events, then perhaps all previous views of the Quaternary could be accommodated (simulations with such high values, however, usually lead to lineage extinction). Our goal was to introduce a new way of examining the tempo of speciation. Further simulations are underway to evaluate the conclusions reached in this study, and to extend the approach to birds from other regions as well as to other groups of organisms.

REFERENCES

Avise, J. C. 2000 *Phylogeography*. Cambridge, MA/London: Harvard University Press/University Press.

Avise, J. C., Ball, R. M. & Arnold, J. 1988 Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. *Mol. Biol. Evol.* **5**, 331–344.

Avise, J. C. & Walker, D. 1998 Pleistocene phylogeographic effects on avian populations and the speciation process. *Proc. R. Soc. Lond.* B **265**, 457–463. (DOI 10.1098/rspb.1998.0317.)

Burns, K. J. 1998 Molecular phylogenetics of the genus *Piranga*: implications for biogeography. *Auk* **115**, 621–634.

Edwards, S. V. & Beerli, P. 2000 Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**, 1839–1854.

Harvey, P. H., May, R. M. & Nee, S. 1994 Phylogenies without fossils. *Evolution* **48**, 523–529.

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

Johnson, N. K. & Cicero, C. 2002 The role of ecologic diversification in sibling speciation of *Empidonax* flycatchers (Tyrannidae): multigene evidence from mtDNA. *Mol. Ecol.* **11**, 2065–2081.

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. 1999 Pleistocene phylogeographic effects on avian evolution. *Proc. R. Soc. Lond.* B **266**, 695–700. (DOI 10.1098/rspb.1999.0691.)

Klicka, J., Fry, A. J., Zink, R. M. & Thompson, C. W. 2001 A cytochrome-*b* perspective on *Passerina* bunting relationships. *Auk* **118**, 611–623.

Lovette, I. J. & Bermingham, E. 1999 Explosive speciation in the New World *Dendroica* warblers. *Proc. R. Soc. Lond.* B **266**, 1629–1636. (DOI 10.1098/rspb.1999.0825.)

Mayr, E. 1970 *Populations, species and evolution.* Cambridge, MA: Belknap Press of Harvard University.

Mengel, R. M. 1964 The probable history of species formation in some northern wood warblers (Parulidae). *Living Bird* **3**, 9–43.

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

Nee, S., Holmes, E. C., Rambaut, A. & Harvey, P. H. 1995 Inferring population history from molecular phylogenies. *Phil. Trans. R. Soc. Lond.* B **349**, 25–31.

Nee, S., Holmes, E. C., Rambaut, A. & Harvey, P. H. 1996 Inferring population history from molecular phylogenies. In *New uses for new phylogenies* (ed. P. H. Harvey, A. J. Leigh Brown, J. Maynard Smith & S. Nee), pp. 399–411. New York: Oxford University Press.

Outlaw, D. C., Voelker, G., Mila, B. & Girman, D. J. 2003 Evolution of long-distance migration in and the historical biogeography of *Catharus* thrushes: a molecular phylogenetic approach. *Auk* **120**, 299–310.

Rambaut, A. 2002 PHYL-O-GEN: *phylogenetic tree simulator package*, v. 1.1. See http://evolve.zoo.ox.ac.uk/software/PhyloGen/.

Rambaut, A. & Charleston, M. 2001 See http://evolve.zoo.ox.ac.uk/software/TreeEdit/.

SAS Institute 1998 STATVIEW *for Windows*, v. 5.0.1. Cary, NC: SAS Institute, Inc.

Zink, R. M. 1996 Comparative phylogeography of North American birds. *Evolution* **50**, 308–317.

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., Kessen, A., Line, T. V. & Blackwell-Rago, R. C. 2001 Comparative phylogeography of some aridland bird species. *Condor* **103**, 1–10.

Discussion

S. J. Oppenheimer (*Green College, University of Oxford, Oxford, UK*). If the Plio-Pleistocene change extinguished many species not adapted to the new climate, one might expect a lag period followed by increase in diversification followed by equilibrium.

R. M. Zink. This is possible, although it is equally likely that events that extinguished some species facilitated diversification in others. The increase in diversity would probably be in the future.

G. Rowe (*School of Biological Sciences, University of Sussex, Sussex, UK*). A speciation event, together with rapid lineage sorting, may have occurred as a consequence of

the last glacial cycle creating allopatric populations, whereas the genetic divergence you are looking at may have occurred much earlier, before the populations became isolated, owing to isolation by distance. How can you distinguish between these situations?

R. M. Zink. The pattern of reciprocal monophyly in haplotype trees is definitive to detecting isolating events in the past. Isolation by distance is not likely to produce reciprocally monophyletic groups.

E. Verheyen (*Royal Belgian Institute of Natural Sciences, Brussels, Belgium*). A question about the described lineage through time approach: how complete has a dataset to be to allow the current interpretation of increasing or decreasing rates of diversification? My concern is that if one uses phylogenies at and above the species level, the most recently originated diversity will not be detected, a fact that may certainly have a significant effect on the shape of this curve.

R. M. Zink. We agree that one must use all reciprocally monophyletic groups in log-lineage plots, irrespective of their taxonomic status. It is for this reason that I suggested that the biological species concept would hinder detection of recent patterns of diversification. In our plots of sister-taxon divergences, we include all reciprocally monophyletic groups, whether classified as species or not.

GLOSSARY

LPO: Late Pleistocene origin