

Evidence from molecular systematics for decreased avian diversification in the Pleistocene Epoch

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ABSTRACT Pleistocene glaciations have been suggested as major events influencing speciation rates in vertebrates. Avian paleontological studies suggest that most extant species evolved in the Pleistocene Epoch and that species' durations decreased through the Pleistocene because of heightened speciation rates. Molecular systematic studies provide another data base for testing these predictions. In particular, rates of diversification can be determined from molecular phylogenetic trees. For example, an increasing rate of speciation (but constant extinction) requires shorter intervals between successive speciation events on a phylogenetic tree. Examination of the cumulative distribution of reconstructed speciation events in mtDNA phylogenies of 11 avian genera, however, reveals longer intervals between successive speciation events as the present time is approached, suggesting a decrease in net diversification rate through the Pleistocene Epoch. Thus, molecular systematic studies do not indicate a pulse of Pleistocene diversification in passerine birds but suggest, instead, that diversification rates were lower in the Pleistocene than for the preceding period. Documented habitat shifts likely led to the decreased rate of diversification, although from molecular evidence we cannot discern whether speciation rates decreased or extinction rates increased.

Temporal changes in rates of speciation and extinction result in variation in the net rate of organismal diversification through time (1). Documenting and explaining these rate changes represent major challenges in evolutionary biology. The Pleistocene Epoch presents such a challenge because of its marked environmental fluctuations and its recency, which permit detailed study of factors that influenced changes in species diversity. Many evolutionary biologists hypothesize accelerated vertebrate speciation in North America during the Pleistocene Epoch (2–5), owing to glacial advances and retreats that provided geographic barriers necessary for speciation (2, 3). Mayr (6) remarked, "Evolutionists agree on the overwhelming importance of Pleistocene barriers in the speciation of temperate zone animals." However, major Pleistocene extinctions are also known for some groups, including plants (7–9). The marked environmental effects of the Pleistocene Epoch clearly influenced rates of diversification, although the relative roles of speciation and extinction are unclear.

The relatively high passerine bird diversity in modern fauna is often attributed to a burst of Pleistocene speciation (2, 3). Selander (2) also predicted that most extant bird species originated in the Pleistocene Epoch. A correlate of accelerated Pleistocene speciation concerns the average duration of bird species in the fossil record. Brodkorb (10) proposed that passerine bird species persisted for an average of three million yr in the Pliocene Epoch but only 500,000–1,000,000 yr in the Pleistocene Epoch. Thus, he predicted that species' durations decreased as the present time was approached. Although these

predictions were historically difficult to test because of an incomplete fossil record for birds (D. Steadman, personal communication), tests are now possible based on evolutionary trees derived from molecular systematics (11–15).

Environmental variability clearly increased during the last 1 million yr (Fig. 1A), although previous models (3) were overly simplistic in envisioning only four discrete Pleistocene glacial cycles (4, 16). If this increased variability influenced speciation and extinction rates, specific predictions emerge concerning the net rate of diversification (Fig. 1B), which can be examined in a phylogenetic context (Fig. 1C; refs. 11–15). Evolutionary trees of different lineages based on the same molecule, such as mtDNA, can reveal the relative (and perhaps absolute) timings of speciation events (17) if all or most extant species in a lineage are sampled. Use of the same molecular region for all species mitigates the effects of rate differences among genes. To illustrate the approach, consider an evolutionary tree (Fig. 2) of extant species. At any time between its origin and the present, one could imagine a line drawn through the phylogeny, which gives an estimate of the number of species extant at that time. This estimate is termed the "reconstructed number" (RN; ref. 15), which will often be an underestimate of the actual number of species (AN) extant at that moment because extinct lineages will be "invisible." Nonetheless, the dynamics of the RN of species from phylogenies of extant taxa provide an estimate of combined speciation and extinction rates (i.e., diversification rate) over time (11–15), especially when summed over independent lineages that are each examined on the same molecular genetic scale.

To test predictions concerning Pleistocene diversification rates, we analyzed 11 passerine bird lineages in which evolutionary trees among extant species were inferred from mtDNA restriction site surveys (Table 1). These trees were selected because they are the most taxonomically complete surveys that use a common molecular technique, permitting comparison of relative rates of diversification across lineages. These lineages represent a diversity of avian passerine groups with varied habitat affiliations. We superimposed the 11 trees on a common mtDNA genetic distance scale and tabulated the distribution of the RNs of speciation events, which allowed a general test of the tempo of diversification and Selander's suggestion that most extant species originated in the Pleistocene Epoch.

The predicted increased speciation rates and shorter species' durations (2, 10) can be different aspects of the same phenomenon (Fig. 1C). If speciation rates were elevated in the Pleistocene Epoch, phylogenetic trees should reveal that Pleistocene species persisted for shorter intervals before subsequent speciation events than older species (assuming that each phylogenetic branch point results in one extinction and the origin of two new species). Alternatively, if extinction rates decrease toward the present time, with speciation rate remaining constant, a similar pattern can result (Fig. 2). Although in evolutionary trees of extant species one cannot distinguish increased speciation or decreased extinction as causes of net changes in diversification rate, the net rate for birds should

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Abbreviation: RN, reconstructed number.

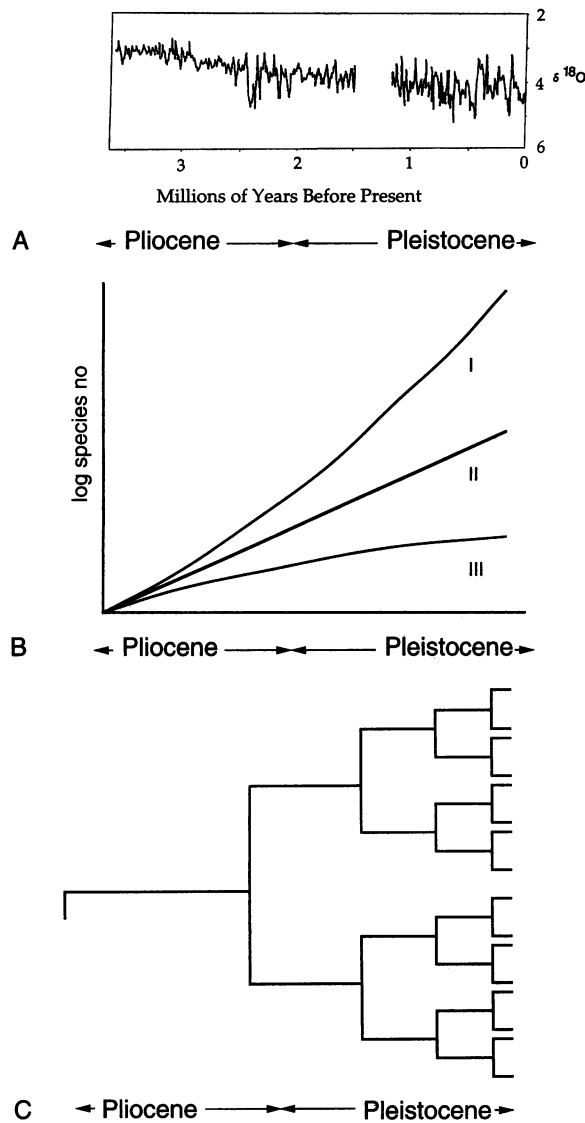


FIG. 1. (A) Oxygen isotope curve for the last 3.5 million yr (16), showing an increase in environmental variability as the present time is approached. Note that there is not evidence of four discrete glaciations. (B) Plot showing hypothetical changes in species diversity over time. The linear curve (II) represents a doubling of species at each time interval, with a constant probability of extinction. Most evolutionary predictions including that tested here involve rate increases that would result in an exponentially increasing curve (curve I) during the Pleistocene Epoch. Curve III depicts a decrease in diversification rate as the present time is approached. (C) Graphical depiction of Brodkorb's hypothesis that the duration of species (internodes between successive speciation events) decreased toward the present time. Note that the decrease is concomitant with an increase in speciation rate. We assume, as do systematists who deal with extant species, that the ancestral species becomes extinct at, or shortly after, speciation.

peak or be steadily increasing in the Pleistocene Epoch if previous evolutionary and paleontological predictions are correct.

We evaluated the data in two ways. (i) We examined the behavior through time of the RN of species on each phylogeny. Harvey *et al.* (14) demonstrated that the expected RN of species on phylogenies of contemporaneous species generated by constant probabilities of speciation and extinction follows an exponential increase. We logarithmically transformed the number of reconstructed species present at intervals of 0.5% sequence divergence (beginning at the origin of each lineage) and plotted the transformed values against percentage of sequence divergence. The null hypothesis—that the phylog-

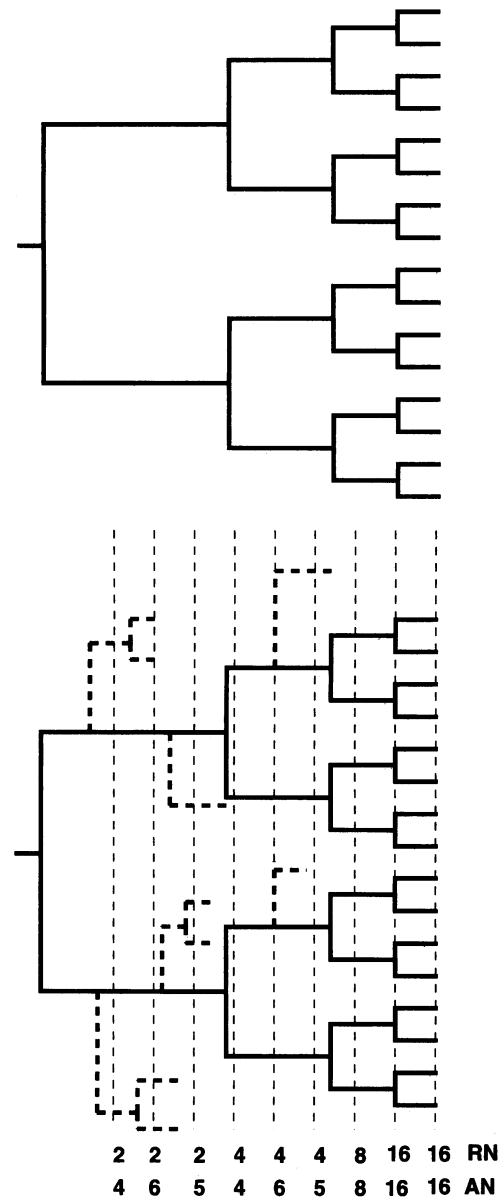


FIG. 2. (Upper) Hypothetical evolutionary tree with increased rate of speciation toward the present. (Lower) This tree exhibits a decreasing extinction rate toward the present. Dashed vertical lines drawn through the tree are used to estimate the RN and actual number (AN) of speciation events. Because extinctions are invisible on trees of extant taxa, the two trees would have the same reconstructed number of species, and both would indicate an increase in speciation toward the present time.

enies could have been generated by the constant parameter model—predicts that a plot of $\ln(\text{RN})$ at each time interval in a phylogenetic tree will be linear (ref. 15; Fig. 1B, curve II). The Brodkorb (10) hypothesis predicts that the plot of the distribution of speciation events should be an increasing exponential function as intervals between successive speciation events become shorter (Fig. 1B, curve I).

(ii) Our second analysis used a statistical analysis of trends for testing whether a series of events, such as ordered speciation events in a phylogeny, are randomly distributed through time (27). This analysis is used because of uncertainty over the calibration of mtDNA evolution (28), and it is designed to test for departures from randomness in ordered series of events, such as speciation events. For a phylogeny of n species with the interior nodes (speciation events) ordered through time, we denote the time of the k th speciation event as t_k . The first

Table 1. Lineages examined for diversification rates

Lineage*	Ref.	Species no.	μ (P)
1 Sparrows (<i>Zonotrichia</i>)	18	6	0.22 (0.587)
2 Towhees (<i>Pipilo</i>)	19	4	-1.29 (0.099)
3 Thrashers (<i>Toxostoma</i>)	†	11	-2.41 (0.008)
4 Sparrows (<i>Ammodramus</i>)	20	8	-0.73 (0.233)
5 Warblers (<i>Dendroica</i>)	4, 21	10	-1.13 (0.136)
6 Sparrows (<i>Melospiza</i>)	21	3	-0.38 (0.352)
7 Grackles (<i>Quiscalus</i>)	22	4	-0.54 (0.295)
8 Sparrows (<i>Spizella</i>)	23	6	-1.71 (0.044)
9 Titmice (<i>Parus</i>)	24	4	-0.02 (0.492)
10 Chickadees (<i>Parus</i>)	25	7	-2.24 (0.013)
11 Sparrows (<i>Passerella</i>)	26	4	-1.73 (0.042)

A negative μ value indicates a decrease in diversification as the present time is approached. P , probability.

*Each lineage is hypothesized to be monophyletic, even though some are parts of larger clades.

†R.M.Z., unpublished data.

speciation event (the root of the tree) is t_1 (which for convenience was set to 0), the second is t_2 , . . . , and the last is $t_n - j$; the present is denoted t_n . We used a stochastic birth process as our null model, in which the mean duration between successive speciation events when there are j lineages is $1/j \lambda$, where λ , the birth-rate parameter, is the mean number of speciation events occurring in one unit of time along a single lineage. Under this model, the statistic

$$\mu = \frac{\left(\frac{1}{n-2} \sum_{k=2}^{n-1} k t_k \right) - \frac{1}{2} t_n}{t_n \left(\frac{1}{12n-24} \right)}$$

follows a standard normal distribution. This statistic compares the centroid of the observed t_k to the midpoint of the duration of the phylogeny. Under the Brodtkorb hypothesis and, we argue, the collective judgment of evolutionary biologists, the times between successive speciation events in phylogenetic trees should become progressively shorter through the Pleistocene Epoch as the birth (speciation) parameter increases [Fig. 1 C, and B (curve I)]. In this case, μ will be positive as the centroid will be smaller than the midpoint. This is a conservative test because, under the model (14), the times between successive speciation events near the root will be longer than the times between more recent speciation events because of extinction effects. This hypothesis shifts the expected value of the test statistic μ into the positive range, favoring the Brodtkorb hypothesis. Lastly, we emphasize that this prediction is independent of any external calibration of evolutionary rate in mtDNA evolution.

To test the null hypothesis that the birth parameter has remained constant, we calculated μ for each of the 11 bird phylogenies and then found the area under the standard normal curve to the left of each value. The area is the probability of observing a value μ or less. These 11 independent probabilities were then tested collectively using the statistic $-2 \sum_{i=1}^m \ln(p_i)$, where p_i is the i th probability and m is the number of independent probabilities. This statistic is distributed as a χ^2 with $2m$ degrees of freedom (29).

If mtDNA evolves at a rate of 2% sequence divergence per million yr (30), any interior nodes of 4% or less [p (31)] are considered Pleistocene in origin, assuming 2 million yr for the Pleistocene Epoch. A histogram (Fig. 3) of the distribution of reconstructed speciation events for extant species confirms

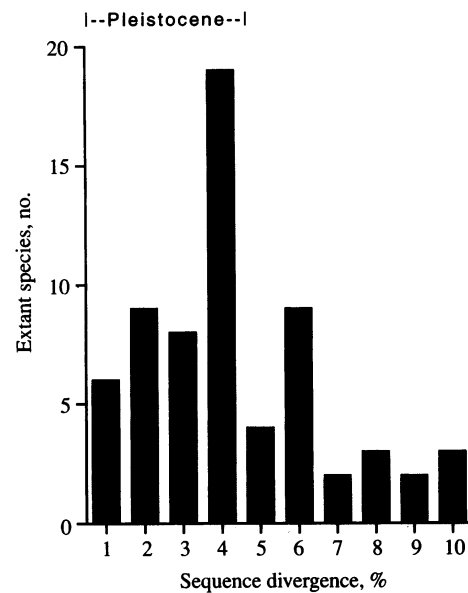


FIG. 3. Distribution of occurrence of speciation events for extant bird species. Note that this plot does not include speciation events in evolutionary trees that were followed by subsequent speciation events. Duration of Pleistocene assumes a rate of sequence divergence of 2% per million yr and a length of 2 million yr (some view the Pleistocene Epoch as the last 1.6 million yr). Also, we assume that molecular divergence is roughly constant through time and is independent of the number of species present.

Selander's (2) suggestion that extant species originated in the Pleistocene Epoch (although perhaps not as recently as Selander expected). However, this analysis excludes Pleistocene speciation events that are not "terminal" and is, therefore, not an adequate test of overall rates of diversification.

Extant species should have evolved relatively recently because the probability of extinction increases with time before the present, the feature our next analyses take into account. Considering all data, plots of the numbers of lineages against time indicate a decrease in diversification rate in the Pleistocene Epoch (Fig. 4). Only 10 evolutionary trees were examined using curvilinear regression. In nine cases the quadratic coefficient was negative ($P < 0.02$, two-tailed sign test), consistent with a decrease in diversification rate toward the present time. In the second analysis (Table 1), 10 of 11 phylogenies resulted in negative μ values (combined $\chi^2 = 49.42$, $P < 0.001$, $df = 22$), strongly implying a reduction in the diversification rate. Thus, the lengths of times between successive speciation events have not been independent of geological time but, rather, have increased through the Pleistocene Epoch, contrary to previous predictions (2, 3, 10).

Our conclusions about the absolute timing of speciation events (Fig. 3) depend on a constant rate of molecular evolution among lineages and a particular calibration of mtDNA diversification (30). If mtDNA evolves at a higher or lower rate, which some have suggested (28, 32), the distribution of speciation events would be shifted earlier or later. Several studies have supported the 2% per million yr rate, in general (33), and for birds, in particular (30, 34). Using this calibration, Bermingham *et al.* (4) also suggested pre-Pleistocene diversification for many other avian species. Our statistical analysis of trends suggests a consistent decline in net diversification rate as the present time is approached, irrespective of the rate calibration. This decline was evident in multiple lineages of birds, suggesting that the phenomenon is a general one. A logical question is what caused the decline—reduced speciation or heightened extinction?

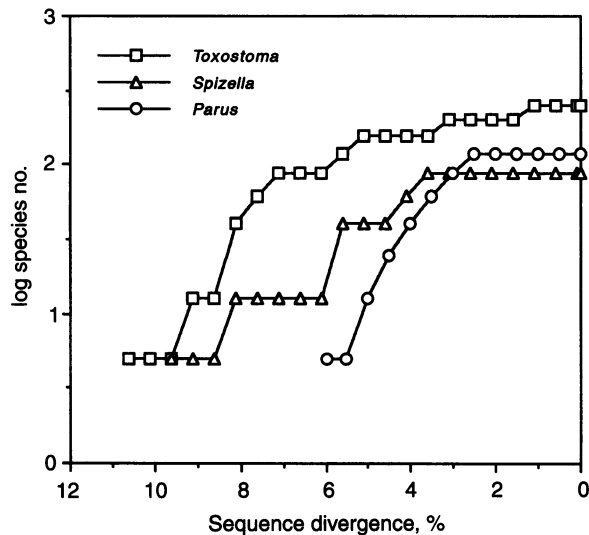


FIG. 4. Percentage sequence divergence in mtDNA versus the natural logarithm of the RN of species for the three passerine bird clades with the highest μ values. Logarithm-linear plots of the RN of species should be generally linear for clades that developed through stochastic speciation and extinction. The clades graphed here show a slowing of the diversification rate through the Pleistocene Epoch. Duration of the Pleistocene is based on the assumption that mtDNA sequence divergence occurs at 2% per million yr; thus any event $\leq 4\%$ sequence divergence is considered Pleistocene in origin.

Paleoecological reconstructions of Pleistocene habitats suggest severe reductions in size and marked geographic displacements of many widespread habitats, such as deciduous forest (9, 35, 36). Such habitat alterations could have accelerated extinction rate in the birds we studied, as it did for many large mammals and birds (7, 8). Alternatively, reduced and displaced habitats might have decreased speciation rates. Simulations (M. Foote, personal communication) suggest that the pattern we observed in the RN of species is most likely due to decreased speciation rate rather than heightened extinction rate. Furthermore, $<10\%$ of passerine bird species known from the Pleistocene Epoch are extinct (D. Steadman, personal communication). Although it is premature to make definitive conclusions about the mechanism(s) causing the decline in net diversification, it seems possible that the habitat alterations of the Pleistocene reduced rather than accelerated speciation rates in passerine birds.

Although some avian species almost certainly evolved as a result of Pleistocene glaciations (2, 3, 37), the pervasive notion of increased avian speciation rates might have been based on too few taxa [such as those taxa with current east-west divisions (37)] or undue attention to extant species only (e.g., Fig. 3). Our results mirror those of Nee *et al.* (12), who studied patterns of diversification represented in the relatively comprehensive, although controversial (38, 39), DNA-DNA hybridization-based phylogeny of birds derived by Sibley and Ahlquist (40). Nee *et al.* (12) found that the phylogeny supported a continuous diminution in the rate of cladogenesis of birds, although the effect seemed lacking for passerine birds. However, they did not analyze species-level taxa (only higher-level or more inclusive taxonomic groups), which in our analysis show a decreasing pattern of diversification as the present time is approached. Perhaps the rate of cladogenesis is diversity-dependent, explaining the patterns found both on a recent time scale within avian genera (this paper) and throughout much of avian diversification (12). Further molecular studies will prove fruitful with birds because of the diversity of lineages of different sizes (40).

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- Stanley, S. M. (1990) in *Causes of Evolution*, eds. Ross, R. M. & Allmon, W. D. (Univ. of Chicago Press, Chicago), pp. 103-127.
- Selander, R. K. (1971) in *Avian Biology*, eds. Farner, D. S. & King, J. R. (Academic, New York), Vol. 1, pp. 57-147.
- Mengel, R. M. (1964) *Living Bird* 3, 9-43.
- Bermingham, E., Rohwer, S., Freeman, S. & Wood, C. (1992) *Proc. Natl. Acad. Sci. USA* 89, 6624-6628.
- Wetmore, A. (1959) *Smithson. Misc. Collect.* 138, 1-24.
- Mayr, E. (1970) *Populations, Species, and Evolution* (Belknap Press/Harvard Univ. Press, Cambridge, MA).
- Webb, S. D. (1984) in *Quaternary Extinctions*, eds. Martin, P. S. & Klein, R. G. (Univ. of Arizona Press, Tucson), pp. 189-210.
- Steadman, D. W. & Martin, P. S. (1984) in *Quaternary Extinctions*, eds. Martin, P. S. & Klein, R. G. (Univ. of Arizona Press, Tucson), pp. 466-477.
- Watts, W. A. (1988) in *Vegetation History*, eds. Huntley, B. & Webb, T., III (Kluwer, Dordrecht, The Netherlands), pp. 155-192.
- Brodkorb, P. (1971) in *Avian Biology*, eds. Farner, D. S. & King, J. R. (Academic, New York), Vol. 1, pp. 19-55.
- Harvey, P. H., Nee, S., Mooers, A. O. & Partridge, L. (1991) in *Genes in Ecology*, eds. Berry, R. J., Crawford, T. J. & Hewitt, G. M. (Blackwell Scientific, Oxford), pp. 123-137.
- Nee, S., Mooers, A. O. & Harvey, P. H. (1992) *Proc. Natl. Acad. Sci. USA* 89, 8322-8325.
- Harvey, P. H., May, R. M. & Nee, S. (1994) *Evolution* 48, 523-529.
- Harvey, P. H., Holmes, E. C., Mooers, A. O. & Nee, S. (1994) in *Models in Phylogeny Reconstruction*, eds. Scotland, R. W., Siebert, D. J. & Williams, D. M. (Systematics Association, Oxford), Special Vol. 52, pp. 313-333.
- Nee, S., Holmes, E. C., May, R. M. & Harvey, P. H. (1994) *Philos. Trans. R. Soc. London B* 344, 77-82.
- Shackleton, N. J., Backman, J., Zimmerman, H., Kent, D. V., Hall, M. A., Roberts, D. G., Schnitker, D., Baldauf, J. G., Desprairies, A., Homrighausen, R., Huddleston, P., Keene, J. B., Kaltenback, A. J., Krumsiek, K. A. O., Morton, A. C., Murray, J. W. & Westberg-Smith, J. (1984) *Nature (London)* 307, 620-623.
- Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., Higuchi, R. G., Palumbi, S. R., Prager, E. M., Sage, R. D. & Stoneking, M. (1985) *Biol. J. Linn. Soc.* 26, 375-400.
- Zink, R. M., Dittmann, D. L. & Rootes, W. L. (1991) *Auk* 108, 578-584.
- Zink, R. M. & Dittmann, D. L. (1991) *Condor* 93, 98-105.
- Zink, R. M. & Avise, J. C. (1990) *Syst. Zool.* 39, 148-161.
- Kessler, L. G. & Avise, J. C. (1985) *Mol. Biol. Evol.* 2, 109-125.
- Zink, R. M., Rootes, W. L. & Dittmann, D. L. (1991) *Condor* 93, 318-329.
- Zink, R. M. & Dittmann, D. L. (1993) *Wilson Bull.* 105, 399-413.
- Gill, F. B. & Slikas, B. (1992) *Condor* 94, 20-28.
- Gill, F. B., Mostrom, A. M. & Mack, A. L. (1993) *Evolution* 47, 195-212.
- Zink, R. M. (1994) *Evolution* 48, 96-111.
- Cox, D. R. & Lewis, P. A. W. (1966) *The Statistical Analysis of Series of Events* (Methuen, London).
- Sheldon, F. H. & Bledsoe, A. H. (1993) *Annu. Rev. Ecol. Syst.* 24, 243-278.
- Sokal, R. R. & Rohlf, F. J. (1981) *Biometry* (Freeman, New York), 2nd Ed.
- Shields, G. F. & Wilson, A. C. (1987) *J. Mol. Evol.* 24, 212-217.
- Nei, M. & Li, W. H. (1979) *Proc. Natl. Acad. Sci. USA* 76, 5269-5273.
- Avise, J. C., Bowen, B. W., Lamb, T., Meylan, A. B. & Bermingham, E. (1992) *Mol. Biol. Evol.* 9, 457-473.
- Irwin, D. M., Kocher, T. D. & Wilson, A. C. (1991) *J. Mol. Evol.* 32, 128-144.
- Tarr, C. L. & Fleischer, R. C. (1993) *Auk* 110, 825-831.
- Webb, T., III (1988) in *Vegetation History*, eds. Huntley, B. & Webb, T., III (Kluwer, Dordrecht, The Netherlands), pp. 385-414.
- Bennett, K. D. (1990) *Paleobiology* 16, 11-21.
- Rising, J. D. (1993) *Curr. Ornithol.* 1, 131-157.
- Gill, F. B. & Sheldon, F. H. (1991) *Science* 252, 1003-1005.
- Siegel-Causey, D. (1992) *Auk* 109, 939-944.
- Sibley, C. G. & Ahlquist, J. E. (1990) *Phylogeny and Classification of Birds: A Study in Molecular Evolution* (Yale Univ. Press, New Haven, CT).