Letter to the Editor

Slow Rates of Molecular Evolution in Birds and the Metabolic Rate and Body Temperature Hypotheses

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It has long been hypothesized that the rate of molecular evolution in birds is slow relative to other tetrapods, particularly mammals, based on small genetic distances between bird taxa (e.g., Prager et al. 1974; Britten 1986). These studies rely heavily, however, on accurate dating of fossils or biogeographic events in estimating taxonomic divergence times. Adachi, Cao, and Hasegawa (1993) used a relative rate test, not dependent on dating, and found a significantly slower rate of mitochondrial amino acid substitution in chicken relative to human. Relative rate tests for birds have not yet been applied to either mitochondrial or nuclear DNAs.

The body temperature hypothesis (Prager et al. 1974; Avise 1983) pertains to birds and supposes that relatively small genetic distances between species stem from functional constraints imposed by higher average body temperatures. More recent studies suggest that vertebrates with higher whole body metabolic rates have faster rates of mitochondrial DNA evolution. The metabolic rate (MR) hypothesis (Martin and Palumbi 1993) suggests that this faster rate stems from oxygen radicals causing DNA damage during normal oxygen metabolism in animals. Predictions from the MR hypothesis are (1) that nonpasserine birds should have DNA sequence evolution rates similar to those of mammals, as both are warm-blooded and have similar specific metabolic rates, and (2) that all birds and mammals should have faster rates than cold-blooded reptiles with lower rates of oxygen metabolism. Here, we seek to assess the notion of a slow rate of molecular evolution in birds based on both mitochondrial and nuclear DNAs and place our findings in the context of the MR and body temperature hypotheses.

We obtained new mitochondrial 12S rDNA sequences for birds and a crocodylian using standard methods. We maintained alignment of conserved rDNA secondary structural features across taxa, and highly variable regions of uncertain alignment were excluded from analyses. To test for differences in rate of DNA sequence evolution among taxa, we conducted discrete character relative rate tests using parsimony (Mindell and Honeycutt 1990). These tests are independent of fossil or biogeographic event dating and are based on the expectation of equal amounts of character change in sister taxa relative to an outgroup, if rates of evolution in the sister taxa are equal. We use minimum branch lengths to estimate amounts of unambiguous character state change and a binomial test for departure from the expected 50% of all change being found in each of two ingroup taxa. The phylogeny used in assessing rate heterogeneity for mitochondrial 12S rDNAs (fig. 1) represents a consensus of recent hypotheses for birds with *Crocodylus* placed as sister to birds. Phylogenetic relationships for assessing potential rate differences in 13 mitochondrial and 6 nuclear protein-coding genes are (*Xenopus*,(*Gallus*,((*Mus, Rattus*),(*Bos, Homo*)))). Alternative placement of the two rodents as sister to *Homo* also was considered but did not significantly affect the results. Although we prefer analyses of branch lengths from trees including all the study taxa in order to provide a more accurate estimation of the actual history of character change, we also performed all possible pairwise analyses (not shown) including two ingroup taxa and the outgroup, and results were congruent with our findings in table 1.

Divergences among tetrapod classes are estimated to be 250–365 million years old, and in light of evidence that transitions (TIs) and third codon positions tend to become saturated with change in vertebrate mitochondrial DNAs over shorter time intervals we have excluded them, singly or in combination, from our relative rate tests to reduce homoplasy in the data set. We also use a likelihood approach in comparing relative rates of change across taxa for all characters as well as for TIs, transversions (TVs), and synonymous and nonsynonymous nucleotide substitutions with the program CODRATES (Muse and Gaut 1994). Synonymous and nonsynonymous rate comparisons, however, remain susceptible to saturation problems and rapidly changing third codon positions cannot be eliminated from analyses.

Key words: rates of evolution, relative rate tests, molecular evolution, metabolic rate hypothesis.

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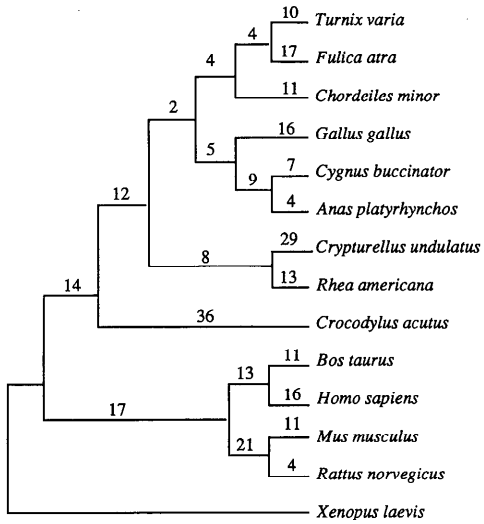

FIG. 1.—Phylogenetic tree topology used for discrete character relative rate tests in mitochondrial 12S rDNA. Numbers on branches denote minimum number of transversion substitutions for the most parsimonious distribution of unambiguous character change.

Gallus gallus (domestic chicken) shows a significantly slower rate of change ($P \leq 0.05$) than each of four mammals for DNAs from all 13 mitochondrial protein-coding genes combined (table 1). This holds for three sets of analyses: (1) all codon position TVs only, (2) codon positions 1 and 2 with TIs and TVs combined, and (3) codon positions 1 and 2 TVs only (latter not shown). Slower rates in *Gallus* than in the mammals were highly significant ($P \leq 0.001$) in all the above comparisons except those involving *Mus* for codon positions 1 and 2.

Some genes analyzed separately differed from the pattern of rate variation seen for the 13 mitochondrial genes combined. Six of the 13 protein-coding genes are significantly slower in *Gallus* than in all four of the mammals; 1 gene shows no significant difference in rate from any of the mammals, and 6 genes are significantly slower in *Gallus* than in some but not all the mammals (table 1). None of the pairwise comparisons indicates a significantly faster rate of change in *Gallus*.

For 16S mitochondrial rDNA there were no significant differences in rates of change for *Gallus* relative to four mammals. For 12S there were no significant differences in rates of change between eight birds, four mammals, or *Crocodylus acutus,* with the exception of one bird, *Crypturellus undulatus,* which showed a significantly ($P < 0.05$) faster rate than two mammals and six of the other seven birds (see fig. 1 for taxa names). rDNAs have secondary structures that may impose functional constraints on evolutionary rate; however, separate analyses for hypothesized stems and loops were no different from analysis of stems and loops combined, as described above.

Birds (either *Gallus gallus* or *Anas platyrhynchos*) also had a significantly slower rate of sequence change relative to mammals (*Homo sapiens* and *Mus musculus* or *Rattus norvegicus*) considering all codon positions for up to six nuclear encoded genes combined (table 1). This slower rate for the combined nuclear genes was not observed in considering codon positions 1 and 2 only, likely due to the smaller number of variable characters at these positions within the more slowly evolving nuclear genome.

General agreement between the analyses of combined mitochondrial and nuclear protein-coding DNAs provides support for the hypothesized trend toward slower rates of molecular sequence change in birds. However, we also show that any particular taxon or sequence may contradict this trend, as expected given the independent evolutionary histories for different genes and taxa following divergence from common ancestors.

Relative rate comparisons using CODRATES and a likelihood approach (not shown) are congruent with results from the parsimony-based approach discussed above. Significant differences in rate were indicated between *Gallus* and four mammals for all 13 mitochondrial protein-coding genes combined. These rate differences are based on analyses of TVs and TIs combined, TVs alone, synonymous and nonsynonymous changes combined, and nonsynonymous changes alone. We also found significant rate differences over all characters and for TIs and synonymous substitutions alone for the five nuclear protein-coding genes combined in comparisons between *Gallus* or *Anas* and *Homo.* Only two sets of homologous nuclear sequences are available for comparisons involving birds and *Mus* or *Rattus,* and in the combined analysis TVs showed a significantly slower rate in birds.

It remains to be seen if *G. gallus,* our primary test taxon, is representative of other birds in regard to relative rate differences with mammals for these genes. We do not expect rates to be identical in any pair of taxa, and variable rates of evolution are already known among and within bird families based on immunological distances and DNA–DNA hybridization distances. However, variability in rate among birds and among mammals does not preclude larger or more generalized rate differences among birds and mammals. *G. gallus* is not significantly different in substitution rate from six of seven other bird taxa in our analyses of 12S mitochondrial rDNA. These additional bird taxa represent at least five different orders and a broad cross section of avian genetic diversity, suggesting that *G. gallus* is representative of rate differences for birds rel-

Table 1
Relative Rates of DNA Sequence Evolution for 13 Mitochondrial and 6 Nuclear Protein-Coding Genes for Birds (*Gallus gallus* or *Anas platyrhynchos*) and Mammals (*Homo sapiens, Bos taurus, Mus musculus, Rattus norvegicus*)

	ATP6	ATP8	COI	CO2	CO3	CYTB	ND1	ND2	ND3	ND4	ND4L	ND5	ND6	All 13 Combined
A. MT genes	*Gallus*													
All codon pos.														
TVs only														
Homo	−**	−*	−*	−*	−	−*	−**	−*	−**	−*	−	−**	−**	−**
Mus	−*	−*	−	+	−	+	−*	−*	−*	−	+	−*	−*	−**
Rattus	−*	−*	−	+	+	−	−*	−	−*	−	+	−**	−*	−**
Bos	−**	−**	−*	+	−*	−	−*	−*	−*	−	−	−*	−*	−**
Codon pos. 1 & 2														
TIs and TVs														
Homo	−**	−*	−	−*	−	−**	−*	−	−**	−	−	−**	−*	−**
Mus	−**	−	+	+	−	−	−*	−	−*	−	−	−**	−	−*
Rattus	−**	−	+	+	−	=	−*	−*	−*	−	−	−**	−	−**
Bos	−**	−**	+	+	−	−	−	−	−**	−	−	−*	−	−**

	Enolase	IGF	HSP	Ferritin	C-myc	18S rDNA	All 6 combined
B. Nuclear genes	*Gallus/Anas*						
All codon pos.							
Homo	−	−*	−*	−*	−	−	−**
Mus/Rattus	NA	NA	NA	−*	−*	−	−**
Codon pos. 1 and 2							
Homo	−*	−*	+	=	−	NA	−
Mus/Rattus	NA	NA	NA	=	−*	NA	−

NOTE.—Tests are based on numbers of unambiguous discrete character changes calculated from branch lengths (see text for tree topology), and binomial tests for departure from an expectation of equal amounts of character change in any two lineages relative to the outgroup *Xenopus laevis*. Whether *Gallus gallus* or *Anas platyrhynchos* has experienced fewer, equal, or more nucleotide character changes relative to taxa listed in the first column on the left is designated by −, =, or +, respectively; * $P \leq 0.05$; ** $P \leq 0.001$. For nuclear genes the avian sequence used was *Gallus* for all genes except enolase, and the rodent sequence used was *Mus* for 185 ferritin. IGF = insulin-like growth factor; HSP = heat shock protein; NA = no sequence available.

ative to mammals for this gene; however, more data are needed. Ultimately, as more sequence data are obtained it will be possible to compare degrees of rate heterogeneity within and between vertebrate classes for sets of homologous genes.

Our findings regarding *G. gallus* do not support predictions of the MR hypothesis using whole body metabolic rate measures, as *Gallus* has slower rates of mitochondrial and nuclear DNA sequence evolution relative to four mammals, despite similarly high specific metabolic rates. Our data regarding cold-blooded reptiles are limited to that for the 12S mitochondrial rDNA gene, but they are also counter to the MR hypothesis predictions, as we found that *C. acutus* does not have a slower rate of change for 12S mitochondrial rDNA relative to birds or mammals.

There are many potential influences on rates of sequence evolution, and no single mechanism is expected to dominate in all cases. The MR hypothesis is primarily concerned with differences across taxa in the mutation rate (due to oxidative damage to DNAs) and presumes the likelihood of fixation of mutations to be similar across taxa, similar to a neutral evolution model. Yet differences across taxa in natural selection pressure and functional constraints on sequences also influence rates of change. Selective effects may potentially be controlled by examining rates at synonymous sites only. Divergences between birds and other tetrapods are not recent enough to avoid saturation problems at synonymous sites in the mitochondrial protein-coding genes examined; however, we did find (using CODRATES) significant differences in rate between birds and *Homo* for synonymous sites in the more slowly evolving nuclear genes combined, suggesting that the slower rate in birds cannot be attributed to selective effects alone. Further, proponents of neutral theory do not limit neutral evolution to synonymous sites, and metabolic rate effects might be expected to have some influence on nonsynonymous sites as well.

Following Thomas and Beckenbach (1989), Adachi, Cao, and Hasegawa (1993) attributed higher rates of amino acid substitution in a bird and several mammals relative to a fish and an amphibian, in part, to relaxation of selective constraints operating on proteins in cold-blooded vertebrates. Although they discuss this relaxation as a shared phenomenon for birds and mammals, birds may have greater constraints on molecular sequence evolution than mammals due to their generally higher body temperatures. This hypothesis supposes that relatively high body temperatures for birds present an extreme physiological environment for which fewer alternative forms of a protein are well adapted (Avise 1983) and is consistent with our findings for protein-coding genes. Negative effects of high temperatures on chemical bonds for protein structures and chemical reactions have been described by Somero (1978). Our finding that 12S mitochondrial rDNA has *not* experienced a relatively slow rate of change in eight bird species compared to four mammal species is also consistent with the body temperature hypothesis. If high body temperatures do have a greater effect on the weak chemical bonds of protein structure and efficiency of enzyme operation than on any functions of nonprotein-coding genes, then higher body temperature can influence rates in protein-coding sequences but not necessarily in nonprotein-coding DNA sequences. Prager et al. (1974) report slower rates of serum albumin evolution in birds than in mammals, iguanids, and crocodylians based on microcomplement fixation distances and use of fossils for estimating divergence times. This is also consistent with the body temperature but not the MR hypothesis regarding molecular evolution rates in birds.

We do not interpret lack of support for the MR hypothesis by our analyses or analyses of others to mean that metabolic rate has no or little impact on rates of molecular evolution, as no single variable is expected to dominate rates of sequence evolution in all cases. More importantly, it is the absolute rate of oxygen radical production in gonad cells, the site of potential gametic DNA damage, that is most relevant to the issue of potential oxidative damage, and given the current absence of data on this, we cannot assume that relative rates of oxygen radical production in gonad cells is effectively measured by whole body metabolic rate estimates. Further assessment of the significance of a slower rate of sequence evolution in birds for the MR hypothesis would benefit from comparative data on rates of oxygen radical production and decoupling of whole body and gonad-specific measurements.

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