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Life History Traits and the Rate of Molecular Evolution in Galliformes (Aves)

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ABSTRACT: Rates of molecular evolution are known to vary widely among taxonomic groups. A number of studies, examining various taxonomic groups, have indicated that body size is negatively and clutch size is positively correlated with the rates of nucleotide substitutions among vertebrate species. Generally, either smaller body mass or larger clutch size is associated with shorter generation times and higher metabolic rates. However, this generality is subject to ongoing debate, and large-scale comparative studies of species below the Order level are lacking. In this study, phylogenetically independent methods were used to test for relationships between rates of the mitochondrial cytochrome *b* evolution and a range of life history traits, such as body mass and clutch size in the Order Galliformes. This analysis included data from 67 species of Galliformes birds and 2 outgroup species in Anseriformes. In contrast to previous studies, taxa were limited to within-Order level, not to Class or higher. I found no evidence to support an effect of life history traits on the rate of molecular evolution within the Galliformes. These results suggest that such relationship may be too weak to be observed in comparisons of closely related species or may not be a general pattern that is applicable to all nucleotide sequences or all taxonomic groups.

Key words: Galliformes, Life history, Molecular evolution, Phylogenetic comparative method

INTRODUCTION

Life history traits, such as body mass and clutch size, have been frequently used in studies of rate heterogeneity of evolutionary process as correlates to rates of molecular evolution, although a single particular factor of life histories may not control the molecular clock (e.g. Martin and Palumbi 1993, Mooers and Harvey 1994, Nunn and Stanley 1998, Bromham 2002, Gillooly et al. 2005). In general, two kinds of explanations have been proposed for the rate variation in molecular evolution among organisms with regard to life history: generation time hypothesis and metabolic rate hypothesis. Under the generation time hypothesis (Kohne 1970, Wu and Li 1985, Britten 1986, Bromham and Penny 2003), organisms with shorter generation times replicate germline DNA more often per unit time and therefore have more DNA replication errors. The metabolic rate hypothesis is that increased rates of DNA replication in organisms with higher metabolic rates should have higher mutation rates (Martin and Palumbi 1993, Bromham and Penny 2003, Gillooly et al. 2005). Accumulated metabolic byproducts, particularly free oxygen radicals, may cause more DNA damage that increases mutation rates. Body mass tends to co-vary with generation time and metabolic rate with smaller body mass being associated with shorter generation times and higher metabolic rates (Martin and Palumbi 1993, Nunn and Stanley 1998, Gillooly et al. 2005). Large clutch size or high fecundity may speed rates of molecular evolution by increasing the number of times the genome is replicated per generation (Britten 1986, Bromham 2002).

Although a negative correlation between body mass and molecular evolutionary rate has been frequently reported, many studies have analyzed pairs consisting of distantly related species, such as of different classes, orders or families (e.g. Bromham 2002, Thomas et al. 2006). There may have been a tendency, with this approach, to assume local homogeneity of evolutionary rates, in which closely related species have the same molecular clock rates, with variations at larger phylogenetic scale (Yoder and Yang 2000). However, to support the generality of life history effect on the rate of molecular evolution, comparisons even at closely related species should show the same pattern as the comparison being between families or orders. The objectives of this paper are to test for an association between life history traits and rates of molecular evolution at species- or genus-level, within an avian order, the Galliformes. The order provides an ideal opportunity for detailed study of rate heterogeneity because it offers many closely related species that display a wide variety of life history traits. Species within the Galliformes show more than 100-fold differences in body mass and 20-fold differences in clutch size (Dunning 1993, del Hoyo et al. 1994, Madge and McGowan 2002). In order to minimize the possibility of phylogenetic bias, my analyses were based on phylogenetically independent comparisons (Felsenstein 1985, Harvey and Pagel 1991,

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Barraclough et al. 1998).

MATERIALS AND METHODS

The data set integrates life history traits and molecular data, from 67 species of galliform birds and two anseriform species that act as outgroups. Morphological data (body mass, wing length, and tail length) as proxies for body size and reproductive (clutch size and incubation time) variables were primarily collected from Dunning (1993), del Hoyo et al. (1992), and Madge and McGowan (2002). Body mass for each species was estimated by the mean of males and females. For wing and tail length, female values were used because many available data in the literature are for females. Morphological data and clutch size were $\log_{10}$-transformed prior to analysis to minimize bias against normality. Species and their life history traits data are available upon request. Mitochondrial cytochrome *b* sequences were obtained from GenBank in order to construct phylogenetic trees of Galliformes. The sequences and phylogenetic trees were used to assess rates of molecular evolution and to choose phylogenetically independent species pairs in Galliformes. The cytochrome *b* sequence was chosen because it was most abundantly sequenced in this order. Also, I can directly compare my results with those from previous studies because many available studies have used the cytochrome *b* sequences to test for the life history effects on the rate of molecular evolution.

To examine patterns of life history effects on the rate of molecular evolution in Galliformes, I conducted relative rate tests, which compare the genetic distances between two related taxa in comparison with a third, more distantly related outgroup (e.g., Nunn and Stanley 1998, Bromham 2002). Although it requires a large number of taxa and variable sites, the relative rates test, without any knowledge of the divergence times of the taxa in question, provides a simple and useful approach for testing the molecular clock and for exploring possible patterns of rate heterogeneity among taxonomic groups (Page and Holmes 1998, Robinson et al. 1998). I selected phylogenetically independent pairs from the phylogeny obtained from maximum parsimony in this study to compare the branch lengths of species. Differences in the number of synonymous and non-synonymous substitutions for each comparison were estimated using the program MEGA3 (Kumar et al. 2004). Three different distance matrices were used to evaluate rate heterogeneity. To assess the relationship of differences in branch lengths (i.e. rate heterogeneity of cytochrome *b* substitution) to life history traits, I used a non-parametric correlation approach with all possible species pairs. Then, I restricted the analyses to inter- and intra-genus species pairs, respectively, to see whether the taxonomic level affected the results.

Additionally, I applied the sign test where a positive sign was allocated to the pair when the larger body mass (or the larger clutch size) species had the longer branch. In contrast, a negative sign was given to the pair when the smaller body mass (or the smaller clutch size) species was associated with the longer branch. I assumed that the direction of results would be toward positive for clutch size and toward negative for body mass, wing and tail length, and incubation time, on the premise that there is the life history effect on the rate heterogeneity as frequently reported in other studies (e.g., Nunn and Stanley 1998, Bromham 2002). Thus, I used a one-tailed sign test under the null hypothesis of no relationships between life history traits and rates of molecular evolution (i.e. equal proportion of the positive and the negative signs). For the sign tests, first, I limited my analysis to the terminal sister-taxa pairs only. I also restricted my analysis to the pairs in which there were differences in life history traits. These restricted methods can reduce bias in selecting species for each taxa and minimize possible saturation effects (Nunn and Stanley 1998). However, given restriction of the data set, the number of pairs examined would be relatively small to make a conclusion statistically. Thus, I also used all phylogenetically independent pairs of species that differ in life history traits.

RESULTS

The strict consensus maximum parsimony tree from 67 galliform and two anseriform cytochrome *b* sequences (1143 bp) yielded a total of 34 species-pairs for phylogenetically independent comparisons (Fig. 1). For each of the five life history traits, three different branch lengths from maximum parsimony (Pars), synonymous (Syn), and non-synonymous (Non-syn) substitutions were analyzed separately. This test set contained a total of 15 relative rate tests, only one of which, wing length and synonymous substitutions, showed significant correlation between life history traits and the rate of cytochrome *b* substitution (Spearman's $r = -0.37$, $p = 0.04$; Fig. 2k). Of 32 pairs in the correlation analysis of WL and Syn, however, reanalysis with 20 intra-genus pairs (i.e. I restricted the analysis to more closely related species) did not show the expected association (Spearman's $r = -0.137$, $p = 0.57$; Table 1). In the remaining 14 tests, no evidence for life history effects on the rates of molecular evolution was observed (Fig. 2). In 15 non-parametric correlation analyses of inter-genus pairs, there were no significant relationships ($n = 9$ to 13, $p = 0.06$ to 0.99; Table 1). 14 of 15 analyses restricted to intra-genus pairs showed no relationships ($n = 16$ to 20, $p = 0.07$ to 0.87). Only the analysis of body mass (BM) and Pars exhibited a positive correlation ($n = 18$, $r = 0.556$, $p = 0.02$).

When plotted phylogenetically, the trends in clutch size and body mass across Galliformes birds were not remarkable. Branch

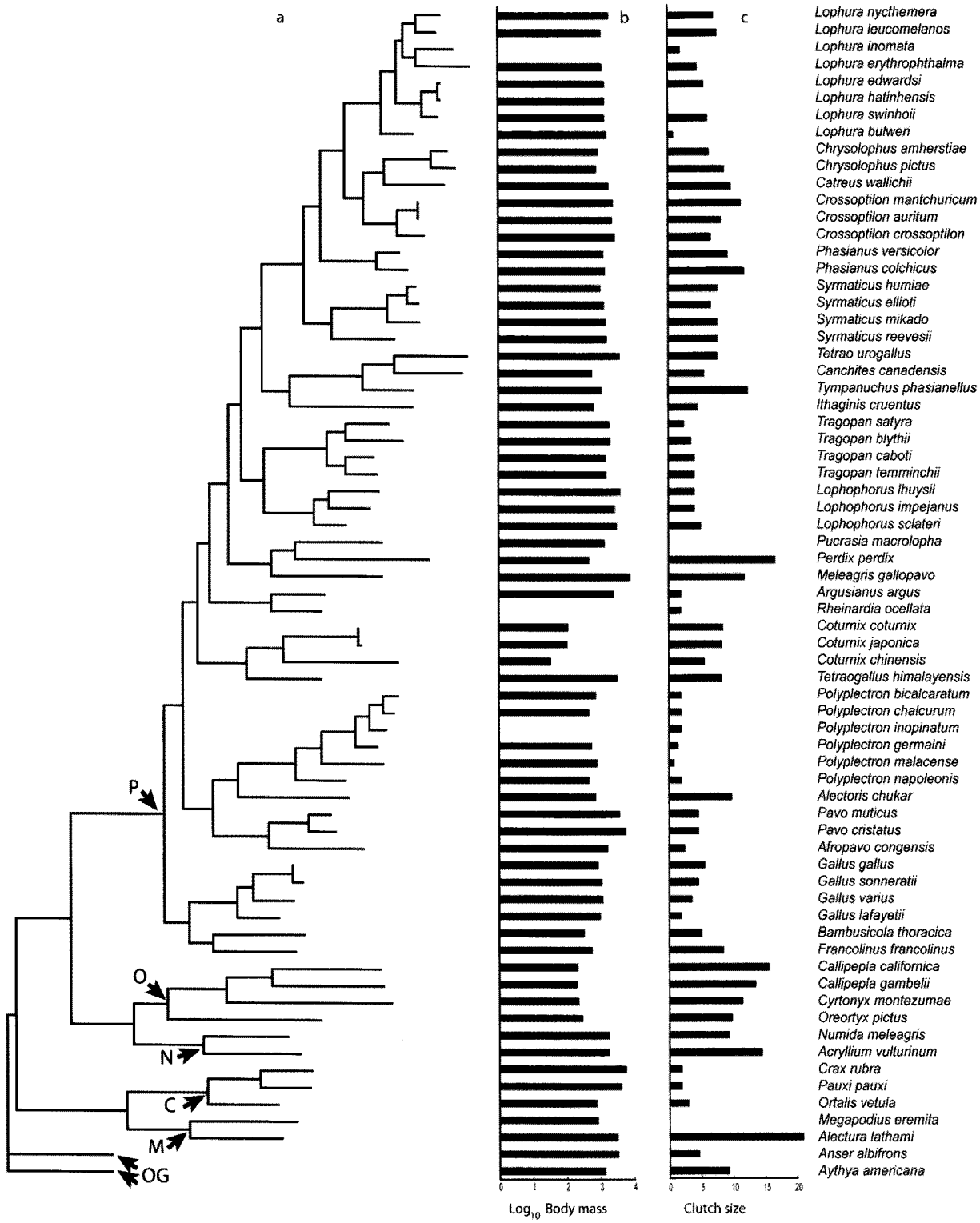

Fig. 1. One of two most parsimonious trees (a) of 67 mitochondrial cytochrome *b* genes from Galliformes, including those from two Anseriformes outgroup, shown wit[h branch lengths proportional. Each of the five traditionally recognized families within Galliformes is monophyletic. P, Phasianidae; O, Odontophoridae; N, Numididae; C, Cracidae; M, Megapodiidae; OG, outgroup. The bar graphs represent $\log_{10}$ transformed body mass (b) and clutch size (c), respectively, for each species on the tree. Branch lengths are not positively correlated with clutch size nor are they negatively correlated with body mass.

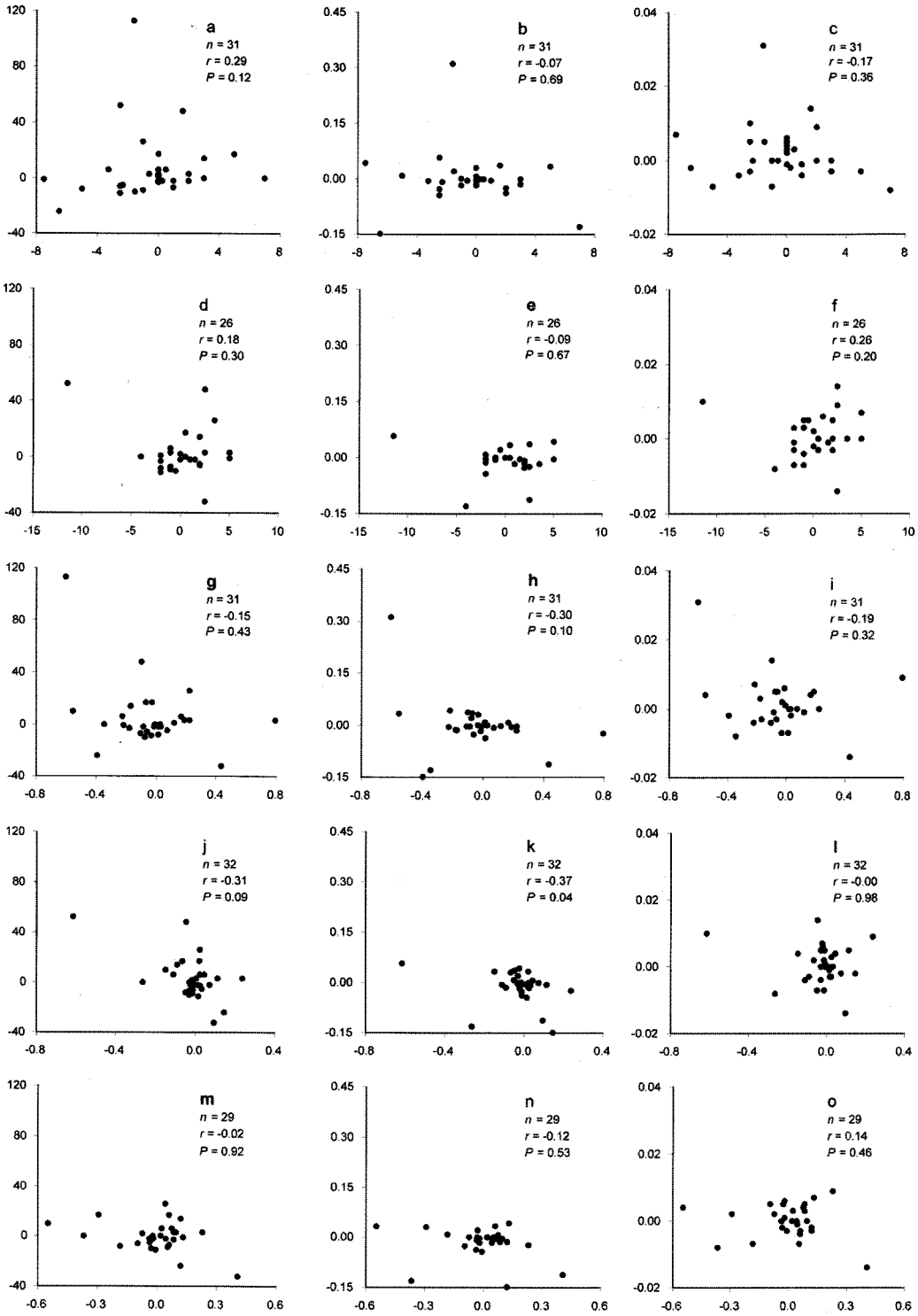

Fig. 2. Correlation analysis between differences in life history traits (X-axis) and differences in relative rates of cytochrome *b* substitution (Y-axis) in Galliformes species. For five life history traits (clutch size (CS), incubation time (IT), body mass (BM), wing length (WL), and tail length (TL), three measures of branch lengths were calculated from maximum parsimony (Pars), synonymous substitutions (Syn), and non-sysnonymous susbstitutions (Non-syn). Statistics of Spearman's rank correlation were given in each panel: (a) CS and Pars; (b) CS and Syn; (c) CS and Non-syn; (d) IT and Pars; (e) IT and Syn; (f) IT and Non-syn; (g) BM and Pars; (h) BM and Syn; (i) BM and Non-syn; (j) WL and Pars; (k) WL and Syn; (l) WL and Non-syn; (m) TL and Pars; (n) TL and Syn; (o) TL and Non-syn.

Table 1. Correlation analysis with inter- and intra-genus pairs comparisons between life history traits and relative rates of molecular evolution in Galliformes species. For each life history trait, three measures of branch lengths were calculated from maximum parsimony (Pars), synonymous substitutions (Syn), and non-sysnonymous susbstitutions (Non-syn). n is the number of comparisons and r is Spearman's correlation coefficient

Branch length	Inter-genus pairs comparison			Intra-genus pairs comparison		
	n	r	p	n	r	p
Clutch size (CS)						
Pars	12	0.368	0.24	19	0.428	0.07
Syn	12	−0.333	0.29	19	0.329	0.17
Non-syn	12	−0.046	0.89	19	−0.236	0.33
Incubation time (IT)						
Pars	10	−0.197	0.59	16	0.403	0.12
Syn	10	−0.006	0.98	16	−0.114	0.68
Non-syn	10	0.178	0.62	16	0.186	0.49
Body mass (BM)						
Pars	13	−0.467	0.11	18	0.556	0.02
Syn	13	−0.385	0.19	18	−0.141	0.58
Non-syn	13	−0.319	0.29	18	0.108	0.67
Wing length (WL)						
Pars	12	−0.552	0.06	20	0.271	0.25
Syn	12	−0.399	0.20	20	−0.137	0.57
Non-syn	12	0.042	0.90	20	0.040	0.87
Tail length (TL)						
Pars	9	−0.283	0.46	20	0.212	0.37
Syn	9	−0.183	0.64	20	0.124	0.60
Non-syn	9	0.083	0.83	20	−0.148	0.53

Table 2. Sign tests for life history traits variation in rates of molecular evolution in Galliformes species, using phylogenetically independent comparisons with the terminal sister-taxa pairs only. For the life history traits, three measures of branch lengths were calculated from relative rate tests on maximum parsimony (Pars), synonymous substitutions (Syn), and non-sysnonymous susbstitutions (Non-syn). n_c and n_e are the numbers of total comparisons and expected sign comparisons

Branch length	Phylogenetically independent comparison			Terminal sister-species comparison only		
	n_c	n_e	p	n_c	n_e	p
Clutch size (CS)						
Pars	24	13	0.42	13	6	0.71
Syn	24	9	0.92	13	5	0.87
Non-syn	24	8	0.97	13	5	0.87
Incubation time (IT)						
Pars	24	10	0.85	15	7	0.70
Syn	24	12	0.58	15	8	0.50
Non-syn	24	9	0.97	15	4	0.90
Body mass (BM)						
Pars	31	13	0.86	20	7	0.94
Syn	31	17	0.36	20	9	0.75
Non-syn	31	15	0.64	20	9	0.75
Wing length (WL)						
Pars	32	14	0.81	21	9	0.81
Syn	32	18	0.30	21	11	0.50
Non-syn	32	16	0.57	21	9	0.81
Tail length (TL)						
Pars	29	10	0.97	20	7	0.94
Syn	29	14	0.64	20	9	0.75
Non-syn	29	14	0.64	20	9	0.75

lengths were not negatively associated with body mass or positively associated with clutch size of the species (Fig. 1). Of 30 sign test sets, including 15 sets for terminal sister-taxa pairs only, none demonstrated significant relationships between life history traits and cytochrome *b* evolutionary rates (Table 2).

DISCUSSION

Previous tests for relative rates of molecular evolution in vertebrates have concentrated primarily on differences between distantly related taxa. To demonstrate the generality of life history effects on the rate of molecular evolution, the association should also be applicable within groups of closely related species. In contrast to most previous analyses, I found little evidence to support the predicted association in the avian order Galliformes. The present analyses suggest that such relationship may be too weak to be observed in comparisons of closely related species or may not be a general pattern that is applicable to all nucleotide sequences or all taxo-

nomic groups.

The taxonomic level may be one potential explanation for the lack of a significant relationship between life history traits and substitution rates. It may be difficult for closely related species to have sufficient time to accumulate a sufficient number of substitutions to allow life history effects to be observed (Bromham and Cardillo 2003) and, as a result, it would underestimate the effects. I examined a total of 15 correlation analyses, only one of which indicated a significant association (WL and Syn). However, reanalysis in WL and Syn, which is limited to more closely related species pairs (i.e. 20 intra-genus pairs), did not show the expected association. In contrast, some tests using only the inter-genus pairs increased the likelihood of observing the expected patterns (e.g. for WL and Pars, p = 0.06 to 0.09; for BM and Pars, p = 0.11 to 0.43). Nevertheless, these results do not allow us to draw any general conclusion because of the lack of statistical significance and relatively small number of samples. Moreover, there was a significant positive relationship between body size (BM) and evolutionary rate (Pars). This positive association is inconsistent with previously observed negative relationships (e.g., Martin and Palumbi 1993, Nunn and Stanley 1998, Bromham 2002, Gillooly et al. 2005). To assess whether the taxonomic level affects explain the expected hypothesis, it will be necessary to collect more data on a wider range of species both across and within genera.

The nucleotide sequences chosen for this study could also influence the test. I used the mitochondrial cytochrome *b* sequence because it was the most widely available for the group and for comparability with other studies. However, I found no evidence to support the life history effects on the molecular evolutionary rate. The explanations for correlations between life history traits and evolutionary rates have mainly originated from generation time, metabolic rate, and population size effects. First, mitochondrial genes such as the cytochrome *b* gene may not be expected to show the generation time effect like nuclear genes. The replication of nuclear genome is linked to cell division whereas mitochondrial genome can replicate independent of the cell cycle (Martin and Palumbi 1993, Rand 1994). Unfortunately, I cannot resolve the relationships between nuclear genome and generation time because it is currently not possible to obtain sufficient nuclear samples for Galliformes birds. Additionally, evolutionary features of the cytochrome *b* gene may not permit the detection of rate differences of a taxonomic group with long evolutionary history. The rapid rate of cytochrome *b* gene substitution is appropriate for the phylogenetic study for species-level phylogeny. However, this feature contributes to potentially high levels of homoplasy in this gene, particularly for genus- or higher-level relationships or for taxa that have long evolutionary history (Naylor and Brown 1998, Garcia-Machado et al. 1999, Wiens and Hollingsworth 2000). In this analysis, the cytochrome *b* sequences were analyzed at relatively close relationships, but this was based on the Galliformes that has long evolutionary history. Thus, it is less likely to detect a significant relationship between life history and the rate of nucleotide substitutions because of the increased chance of multiple substitutions in the Galliformes cytochrome *b* sequences. In addition, the use of nuclear sequences as well as mitochondrial genes may help refine the hypotheses. In contrast to mitochondrial genes, the nuclear genome is less prone to excessive homoplasy (Engstrom et al. 2004). Moreover, nuclear introns may be informative because they show little base compositional bias, relatively low transition-transversion ratio, and little among-site rate heterogeneity (Amstrong et al. 2001, Prychitko et al. 2003, Fujita et al. 2004). Additional studies that include more extensive sampling from both nuclear and mitochondrial sequences will be useful in examining possible effects of specific nucleotide sequences on the expected association.

Numerous studies have shown the importance of not only life history of species, but also ecological, behavioral, or environmental factors on the rate heterogeneity among taxonomic groups (e.g., Schmitz and Moritz 1998, Held 2001, Rowe and Honeycutt 2002). The concept of rate heterogeneity across species has allowed us to test other similar, but significant hypotheses, for example, the link of molecular clock, latitude (temperature), and speciation (Bromham and Cardillo 2003). Some studies have extended these relationships to non-vertebrate animals and plants (e.g., Barraclough et al. 1996, Schmitz and Moritz 1998, Barraclough and Savolainen 2001, Zhong et al. 2002, Bromham and Leys 2005, Thomas et al. 2006), and demonstrated that the expected patterns exist in those taxonomic groups.

I found no evidence that life history traits influence rates of molecular evolution in Galliformes birds. Although most available studies have supported the expected patterns, there are some examples of taxonomic groups in which molecular substitution rates are not significantly correlated with the environmental variables (e.g., Held 2001, Bromham and Cardillo 2003). This suggests that such relationship may not be a general pattern that is applicable to all taxonomic groups, at least not to the order Galliformes. To evaluate whether there are taxon-specific life history effects relating to rate heterogeneity, it will be necessary to collect sufficient data on species of not only Galliformes birds, but other orders as well.

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