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ORIGINAL ARTICLE

Low mitochondrial variability in birds may indicate Hill–Robertson effects on the W chromosome

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Interference among loci subject to selection (the Hill–Robertson effect) may considerably reduce levels of adaptation and variability in genomic regions that lack recombination. Y- or W chromosomes are particularly vulnerable to such effects, since they represent large, non-recombining blocks of genetic material. In birds, the W chromosome and mitochondrial genomes are both maternally transmitted, and hence fail to recombine with each other, whereas in mammals the Y chromosome is paternally transmitted. We show here that mitochondrial DNA sequence diversity is reduced in non-ratite birds compared with mammals. After considering possible confounding factors, such as differences in generation times, mutation rates and demography, we conclude that Hill–Robertson effects associated with the W chromosome provide the most likely explanation for this difference.
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Introduction

Population genetics theory predicts that genomic regions with low or reduced recombination will exhibit reduced levels of adaptation and variability, caused by the action of selection at closely linked sites (the Hill–Robertson effect) (Hill and Robertson, 1966; Gordo and Charlesworth, 2001). This effect arises from the fact that any locus linked to another that is under directional selection experiences a reduction in effective population size (N_e). Since the efficacy of selection on a mutation is a function of the product of N_e and the effect of the mutation on fitness (Kimura, 1983), such linkage affects the probability of fixation of new mutations; favourable mutations are less likely to reach fixation and the opposite is true for deleterious mutations. Hill–Robertson effects can be caused by the spread to fixation of advantageous mutations (hitchhiking: (Maynard-Smith and Haigh, 1974; Gillespie, 2000)), the elimination of recurrent deleterious mutations (background selection: (Charlesworth *et al.*, 1993)), the stochastic accumulation of deleterious mutations (Muller's ratchet: (Muller, 1964; Kim and Stephan, 2000)) and mutual interference between weakly selected mutations that spend long periods at intermediate frequencies (weak selection Hill–Robertson interference: (McVean and Charlesworth, 2000; Comeron and Kreitman, 2002)).

Recombination reduces the extent of this interference, increasing N_e and hence the efficacy of selection. We therefore expect lower rates of adaptive evolution, and

higher rates of fixation of deleterious mutations, in genomic regions with low levels of genetic recombination, compared with more highly recombining regions. In addition, the level of variability at neutral or nearly neutral sites will also be reduced in low recombination genomes, since such variability is proportional to the product of N_e and the mutation rate (Kimura, 1983).

This prediction is consistent with a number of observations on molecular evolution and variation, with evidence for reduced levels of variability and adaptation in genomic regions with low levels of recombination (Gordo and Charlesworth, 2001; Presgraves, 2005). In particular, the degeneration of Y- and W chromosomes (in species with male and female heterogamety, respectively) is thought to result from this effect, since these have evolved as permanently heterozygous, non-recombining units (Charlesworth and Charlesworth, 2000). Support for this hypothesis is provided by data showing accelerated rates of protein sequence evolution and reduced levels of silent site DNA sequence diversity in systems such as the neo-Y chromosome of *Drosophila miranda* (Bachtrog, 2004), the evolving Y chromosome of *Silene latifolia* (Nicolas *et al.*, 2005), and the W chromosome of the chicken (Berlin and Ellegren, 2004, 2006).

Here we ask whether this observation holds generally in birds by using the fact that the mitochondrial (mt) genome of birds appears to be strictly maternally transmitted, and is thus completely associated with the W chromosome (Berlin and Ellegren, 2001; Berlin *et al.*, 2004). If Hill–Robertson effects reduce W-chromosome diversity, then mtDNA diversity will also be reduced in birds. We test for such a reduction by comparing bird and mammalian mtDNA diversities, and evaluating possible confounding effects of differences in effective population size and mutation rates.

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It has recently been proposed that Hill–Robertson effects are so pervasive in animal mtDNA that its genetic diversity has little relation to population size (Bazin et al., 2006), in accordance with the theoretical proposal of Gillespie (2000). We show below that there are some patterns in DNA sequence variability in both bird and mtDNA that appear to be inconsistent with this idea; any Hill–Robertson effects that are acting seem to be too weak to entirely override the signature of demographic factors that influence effective population size.

Materials and methods

Sequences and statistical analyses

Mammalian and avian mitochondrial *cytochrome b* sequences were collected from NCBI (http://www.ncbi. nlm.nih.gov/). This gene was chosen because it is the most widely sequenced coding region of the mitochondrial genome. One hundred and four mammalian species from 69 genera and 88 avian species from 61 genera were included in the study. We did not include avian species from the superorder Palaeognathae (ratites and tinnamous) because the Z- and W chromosomes of many of these species have only differentiated to a limited extent (Tsuda et al., 2007). All raw data along with references to each data set can be found as Supplementary Information Tables S1–S4. We generated two data sets each for birds and mammals; one 'total' data set and one 'population' data set, the latter containing sequences collected from a single location. Thus, each data point in the 'total' data set represents sequence information from one species (regardless of sampling location), whereas each data point in the 'population' data set is from one population (sequences sampled from one location). If there was more than one species per genus in the 'total' data set, the average value of the sequence diversity estimates per genus was used; if there was more than one population per species in the 'population' data set, a population average was used, and species averages were then used to get the genus average.

The nucleotide sequences were aligned by hand (no gaps). Intraspecific sequence variation was analysed in DnaSP 4.0 (Rozas et al., 2003). The pairwise nucleotide diversity, $\hat{\pi}$ (Nei, 1987), with Jukes Cantor correction (Jukes and Cantor, 1969), and Watterson's estimator (θ) (Watterson, 1975) were obtained for synonymous ($\hat{\pi}_S$, θ_S) and non-synonymous sites ($\hat{\pi}_A$, θ_A) separately for each species or population data set. These two estimates of the population parameter, $\theta = 4N\mu$, are expected to be the same for neutral sites at statistical equilibrium under mutation and genetic drift (Tajima, 1989). For alignments containing complex codons that differed by multiple substitutions, θ_S and θ_A were manually calculated, using the number of synonymous and replacement substitutions per synonymous and replacement sites respectively (S), from the formula

$$\hat{\theta} = S/a$$

where a is Watterson's correction factor, given by:

$$a = \sum_{i=1}^{n-1} 1/i$$

and n is the number of sequences in the sample (Watterson, 1975; Nei, 1987).

The variances of the values of $\hat{\pi}_S$ and $\hat{\pi}_A$ were obtained from the formula:

$$\text{var}\,\hat{\pi} = \frac{(n+1)\hat{\pi}_G}{3(n-1)k} + \frac{2(n^2+n+3)}{9n(n-1)}\hat{\pi}_G^2,$$

where k is the number of synonymous or non-synonymous sites, and $\hat{\pi}_G$ is the overall unweighted genus mean (Nei, 1987, p 257). We used the genus mean rather than the sample value, since this should be closer to the expected value of $\hat{\pi}$, and hence avoids biases caused by low estimates of $\hat{\pi}$ for individual samples (Bartolomé et al., 2005).

The variances of the corresponding θ values were obtained from the following formula (Nei, 1987, p 255):

$$\text{var}\,\hat{\theta} = (\hat{\theta}_G/ka) + (\hat{\theta}_G^2 b/a^2)$$

where θ_G is the overall unweighted genus mean θ for birds and mammals separately and b is given by:

$$b = \sum_{i=1}^{n-1} 1/i^2$$

Variance-weighted values of $\hat{\pi}_S$, $\hat{\pi}_A$ were then generated as the ratio of the means of $\hat{\pi}/\text{var}\,\hat{\pi}$ to the mean of $1/\text{var}\,\hat{\pi}$ (and similarly for θ), as described by Bartolomé et al. (2005). The standard errors of the mean diversity estimates across a set of genera were estimated as the standard deviation divided by the square root of the sample size (the number of genera).

The ratios $\hat{\pi}_A/\hat{\pi}_S$ and θ_A/θ_S were calculated as the mean values of the diversity estimates for each genus. The variances of these ratios were estimated using the above formulae and the relation:

$$\text{var}\,(\hat{\pi}_A/\hat{\pi}_S) \approx (\text{var}\,\hat{\pi}_A/\hat{\pi}_S^2) + (\hat{\pi}_A^2\,\text{var}\,\hat{\pi}_S/\hat{\pi}_S^4)$$

All estimates were statistically compared by non-parametric Mann–Whitney U-tests, in Mini-Tab 14.20.

Body masses

Species were classified according to body mass, with one category containing species with body masses up to 100 g and another category including species with body masses from 100 to 6175 g (the largest bird). Large mammals with body masses over 6175 g were not used in any comparisons. Body mass measurements were taken from the literature (Dunning, 1993; Silva and Downing, 1995; Morrow and Fricke, 2004; Pitcher et al., 2005). When the literature reported a range of body masses, the means of these were used: $\hat{\pi}_S$, $\hat{\pi}_A$, $\hat{\pi}_A/\hat{\pi}_S$, θ_S, θ_A and θ_A/θ_S were then compared for mammals and birds within each body mass category. These estimates were also compared between passerines and bats. The mean values of $\hat{\pi}_S$, $\hat{\pi}_A$, $\hat{\pi}_A/\hat{\pi}_S$, θ_S, θ_A and θ_A/θ_S were also reported for each order.

Population subdivision

Average F_{ST} values per genera for birds and mammals, respectively, were estimated in DnaSP 4.0 (Rozas et al., 2003), using equation 3 in Hudson et al. (1992).

Neutrality index

The neutrality index (NI) (Rand and Kann, 1996) was estimated for sister taxa (more closely related to each other than to any other species in the data set) and then compared between mammals and birds. NI reflects the

extent to which the levels of non-synonymous diversity depart from a strict neutral model and can be calculated as follows: $(\hat{\pi}_A/\hat{\pi}_S)/(K_A/K_S)$. NI = 1, indicates neutrality; NI > 1, indicate an excess of amino acid replacements within species; and NI < 1 indicates an excess of amino acid changes between species.

$\hat{\pi}_A$ and $\hat{\pi}_S$ were estimated in DnaSP, as described above. K_A and K_S are the non-synonymous and synonymous pairwise sequence divergences between related taxa, calculated using the maximum likelihood method implemented in codeml (runmode = −2, seqtype = 1) in PAML 3.15 (Yang, 1997). PAML uses maximum likelihood to fit models of mutational changes among codons. Codeml implements the codon substitution model of Goldman and Yang (1994), which allows for arbitrary frequencies of all possible codons, a transition/transversion mutational bias, and a parameter equivalent to K_A/K_S that measures the relative rates of non-synonymous and synonymous changes. The substitution rates are estimated by using the nucleotide frequencies at each codon position (the F3 × 4 model of (Yang, 1997)) to obtain the expected codon frequencies at equilibrium under the substitution process.

In DnaSP, the total numbers of synonymous and non-synonymous sites are computed by the method of Nei and Gojobori (1986), with a Jukes–Cantor correction for multiple changes. In contrast to the method of Goldman and Yang (1994), this procedure assumes that all nucleotide changes are equally frequent; two-thirds of changes at twofold degenerate positions are thus assumed to be synonymous. The numbers of non-synonymous and synonymous sites in a sequence are estimated by weighting each site by the frequency with mutations are expected to result in the respective type of change, and the observed numbers of the two types of differences between two sequences are divided by the estimated numbers of sites to obtain the fractions of non-synonymous and synonymous changes, respectively. Despite the fact that the two methods may give differences in the estimates of K_A and K_S themselves, the ratio K_A/K_S is usually very similar, as we have established in a study of 22 genes in a comparison of *Drosophila americana* and *D. ezoana* (Maside and Charlesworth, unpublished). For either method, differences between species in levels of codon usage bias could result in differences in estimates of K_A/K_S or $\hat{\pi}_A/\hat{\pi}_S$, but we found little evidence for a difference in codon usage between birds and mammals for *cytochrome b* (data not shown).

Results and discussion

### Differences in mtDNA sequence diversity between mammals and birds

Mean pairwise diversity per synonymous nucleotide site ($\hat{\pi}_S$) for mammalian genera ranged from 0 to 0.452 with a mean of 0.086 ± 0.011, and non-synonymous site diversity ($\hat{\pi}_A$) ranged from 0 to 0.0259 with a mean of 0.0036 ± 0.0005 (Table 1). $\hat{\pi}_S$ in birds ranged between 0 and 0.1374, with a mean of 0.0266 ± 0.0045, and $\hat{\pi}_A$ ranged between 0 and 0.0158 with a mean of 0.0019 ± 0.0003 (Table 1). $\hat{\pi}_S$ was significantly higher in mammals than in birds (Mann–Whitney U-test, W = 2840.5, P < 0.0001), as was $\hat{\pi}_A$ (Mann–Whitney

Table 1 Total genetic diversity estimates for birds and mammals (±s.e.)

	N	Mean $\hat{\pi}_S$	Mean θ_S	Mean $\hat{\pi}_A$	Mean θ_A	$\hat{\pi}_A/\hat{\pi}_S$	θ_A/θ_S	Mean ($\hat{\pi}_S - \theta_S$)	Mean ($\hat{\pi}_A - \theta_A$)
All birds	61	0.02664±0.00450	0.02523±0.00354	0.00189±0.00031	0.00222±0.00032	0.0710±0.0168	0.0878±0.0177	0.00140±0.00261	−0.00033±0.00017
All mammals	69	0.08597±0.01097	0.07402±0.00932	0.00355±0.00046	0.00438±0.00071	0.0413±0.0075	0.0591±0.0122	0.00120±0.00500	−0.00083±0.00033
Mann–Whitney U-test		P<0.0001	P<0.0001	P=0.0001	P=0.0009	P=0.0427	P=0.0643	P=0.0360	P=0.2864
Birds <100 g	33	0.03362±0.00697	0.03124±0.00546	0.00222±0.00056	0.00265±0.00055	0.0660±0.0215	0.0847±0.0231	0.00237±0.00441	−0.00043±0.00029
Mammals <100 g	25	0.13223±0.02295	0.11194±0.01886	0.00410±0.00100	0.00542±0.00161	0.0310±0.0093	0.0484±0.0165	0.02029±0.01364	−0.00131±0.00072
Mann–Whitney U-test		P<0.0001	P<0.0001	P=0.0091	P=0.0125	P=0.0479	P=0.1623	P=0.1979	P=1.0000
Birds 100–6175 g	19	0.01536±0.00369	0.01508±0.00351	0.00165±0.00029	0.00184±0.00033	0.1072±0.0318	0.1217±0.0359	0.00028±0.00185	−0.00019±0.00022
Mammals 100–6175 g	19	0.07211±0.01690	0.06241±0.01526	0.00340±0.00081	0.00341±0.00076	0.0471±0.0158	0.0547±0.0181	0.00990±0.00533	0.00005±0.00025
Mann–Whitney U-test		P=0.0090	P=0.0058	P=0.1655	P=0.1746	P=0.0237	P=0.0169	P=0.0932	P=0.9884
Passerines <62 g	22	0.03848±0.00939	0.03661±0.00739	0.00165±0.00035	0.00225±0.00041	0.0428±0.0138	0.0614±0.0106	0.00190±0.00634	−0.00060±0.00039
Bats <62 g	13	0.12360±0.02434	0.09877±0.01543	0.00343±0.00070	0.00426±0.00081	0.0277±0.0079	0.0431±0.0167	0.02483±0.01476	−0.00083±0.00060
Mann–Whitney U-test		P=0.0004	P=0.0005	P=0.0537	P=0.0496	P=0.1124	P=0.4424	P=0.1887	P=0.5967
Mammals >6175 g	13	0.03455±0.01055	0.03672±0.0133	0.00266±0.00081	0.00376±0.00148	0.0769±0.0332	0.1024±0.0548	−0.00216±0.00489	−0.00110±0.00074

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Table 2 Total genetic diversity estimates for orders of birds and mammals (±s.e.)

	N	Mean $\hat{\pi}_S$	Mean θ_S	Mean $\hat{\pi}_A$	Mean θ_A	$\hat{\pi}_A/\hat{\pi}_S$	θ_A/θ_S	Mean ($\hat{\pi}_S - \theta_S$)	Mean ($\hat{\pi}_A - \theta_A$)
Rodents	24	0.09363±0.02229	0.08940±0.02209	0.00406±0.00106	0.00549±0.00182	0.0434±0.0153	0.0614±0.0254	0.00423±0.00844	−0.00143±0.00078
Insectivora	6	0.11040±0.05341	0.07346±0.02001	0.00283±0.00093	0.00278±0.00073	0.0256±0.0150	0.0379±0.0143	0.03694±0.03021	0.00004±0.00031
Primates	8	0.03770±0.01368	0.03450±0.01372	0.00251±0.00116	0.00378±0.00210	0.0666±0.0390	0.1097±0.0748	0.00320±0.00355	−0.00127±0.00096
Carnivora	7	0.04890±0.01400	0.04766±0.01952	0.00297±0.00075	0.00374±0.00147	0.0608±0.0232	0.07818±0.0444	0.00192±0.00897	−0.00072±0.00083
Cetartiodactyla	6	0.06720±0.03561	0.05687±0.02857	0.00439±0.00207	0.00395±0.00166	0.0653±0.0463	0.0695±0.0456	0.01033±0.00759	0.00043±0.00048
Chiroptera	14	0.12448±0.02301	0.09925±0.01516	0.00345±0.00071	0.00430±0.00086	0.0277±0.0077	0.0433±0.0110	0.02523±0.01358	−0.00085±0.00057
Galliformes	3	0.00769±0.00276	0.01043±0.00239	0.00123±0.00049	0.00241±0.00103	0.1601±0.0857	0.2314±0.1123	−0.00274±0.00115	−0.00118±0.00074
Strigiformes	5	0.01173±0.00359	0.01153±0.00552	0.00180±0.00035	0.00182±0.00067	0.1539±0.0560	0.1577±0.0953	0.00020±0.00216	−0.00001±0.00040
Psittaciformes	3	0.03980±0.01427	0.03295±0.01175	0.00180±0.00010	0.00191±0.00062	0.0452±0.0164	0.0581±0.0280	0.00685±0.00842	−0.00012±0.00052
Procellariiformes	3	0.02059±0.00851	0.01741±0.00945	0.00204±0.00161	0.00175±0.00140	0.0991±0.0882	0.1003±0.0970	0.00318±0.00198	0.00029±0.00021
Gruiformes	3	0.00198±0.00142	0.00211±0.00162	0.00076±0.00057	0.00074±0.00055	0.3875±0.03689	0.3609±0.3867	−0.00013±0.00022	0.00001±0.00014
Coraciiformes	2	0.02816±0.02166	0.01854±0.01187	0.00345±0.00158	0.00428±0.00158	0.1227±0.1104	0.1461±0.1263	0.00961±0.00979	0.00075±0.00004
Passeriformes	34	0.02859±0.00639	0.02952±0.00522	0.00135±0.00024	0.00190±0.00029	0.0471±0.0135	0.0675±0.0151	−0.00094±0.00422	−0.00055±0.00026
Apodiformes	2	0.06212±0.04300	0.04494±0.02907	0.01084±0.00493	0.01046±0.00588	0.1744±0.1445	0.2327±0.1995	0.01718±0.01393	0.00038±0.00096
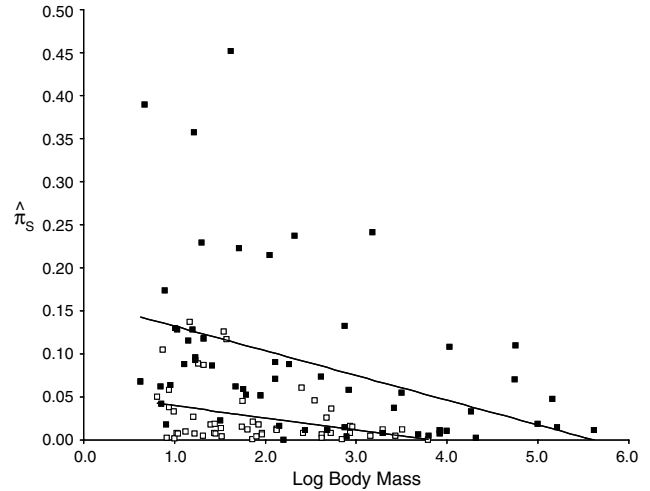
Figure 1 The mean values of synonymous diversity for genera of birds (open squares) and mammals (filled squares), plotted against the corresponding mean body masses. The regression lines, fitted as described in the text, are also shown.

U-test, $W = 3169.5$, $P = 0.0001$) (Table 1). Similar results are obtained with the θ estimates. Overall, therefore, there appears to be highly significantly lower mtDNA variability in birds than in mammals. Similar results are obtained when diversity is compared between bird and mammalian orders; both mean synonymous and non-synonymous diversity are significantly lower for bird orders compared to mammalian orders ($\hat{\pi}_S$: $W = 117.0$, $P = 0.017$; $\hat{\pi}_A$: W: 123.0, $P = 0.044$) (Table 2).

Generation time effects on genetic diversity

Difference in generation times are likely to affect differences in mtDNA variability between birds and mammals, since the neutral diversity for a given effective population size (N_e) is proportional to mutation rate per generation (Kimura, 1983), which is the product of generation time and mutation rate per year. To examine this effect, we divided the groups of species into two categories with respect to body size (less than 100 g and 100–6175 g) (Table 1). Body size correlates strongly with generation time (Finch, 1990; Charnov, 1993), and reliable data are more widely available for body size than generation time.

We found a significantly negative correlation between the genus averages synonymous diversity and mammalian body mass (Spearman's rank correlation: $\rho = -0.499$, $P < 0.0001$). The correlation for birds is in the same direction, but not as strong ($\rho = -0.295$, $P = 0.034$). We also fitted least squares regression lines to the bird and mammals separately, and used bootstrapping to obtain confidence intervals (CI), as described by Haddrill et al. (2005). Figure 1 shows that birds have a lower intercept than mammals (0.030 with 95% CI 0.021/0.040 versus 0.095 with 95% CI 0.071/0.122), but a larger magnitude of regression coefficient (-7.39×10^{-6} with 95% CI $-2.21 \times 10^{-5}/-4.01 \times 10^{-6}$ versus -2.94×10^{-7} with 95% CI -1.06×10^{-6} versus -1.66×10^{-7}).

Overall, the results show that birds have significantly less synonymous site variability than mammals

Table 3 Pairwise divergence and diversity estimates for birds

Order	Species 1	Species 2	$\hat{\pi}_A/\hat{\pi}_S$	K_A/K_S	NI
Apodiformes	Aerodramus maximus lowi	Collocalia esculenta cyanoptila	0.1744	0.0153	11.4015
Coraciiformes	Atelornis pittoides	Todus todus	0.1227	0.0173	7.0917
Galliformes	Lagopus mutus	Tetraogallus himalyensis	0.2292	0.0078	29.3843
Gruiformes	Ardeotis arabs	Chlamydotis undulata macqueenii	0.3477	0.0016	217.3405
Passeriformes	Acrocephalus bistrigiceps	Seicercus omeiensis	0.0720	0.0159	4.5301
Passeriformes	Andropadus tephrolaemus	Hypsipetes amaurotis	0.0117	0.0195	0.6009
Passeriformes	Basileuterus flavicauda	Vermivora ruficapilla	0.0480	0.0170	2.8253
Passeriformes	Cacicus cela cela	Icterus galbula	0.0493	0.0120	4.1100
Passeriformes	Calcarius lapponicus	Plectrophenax nivalis	0.1031	0.0091	11.3295
Passeriformes	Corvus corax	Cyanopica cyanus pallescens	0.1470	0.0117	12.5668
Passeriformes	Erithacus rubecula	Stiphrornis sanghensis	0.0566	0.0166	3.4112
Passeriformes	Melospiza melodia	Spizella breweri	0.0783	0.0241	3.2494
Passeriformes	Xiphorhynchus elegans	Empidonax traillii	0.1388	0.0187	7.4240
Procellariiformes	Calonectis diomedea	Puffinus yelkouan	0.1399	0.0137	10.2147
Psittaciformes	Cactua sulphurea	Pionopsitta barrabandi	0.0641	0.0204	3.1418
Strigiformes	Aegolius funerus	Glaucidium brasilianum	0.1477	0.0212	6.9658
Strigiformes	Bubo bubo	Strix aluco	0.1412	0.0136	10.3790
Mean			0.1219±0.0194	0.0150±0.0013	20.3510±12.4144

NI is the neutrality index: $(\hat{\pi}_A/\hat{\pi}_S)/(K_A/K_S)$, where K_A and K_S denote interspecies sequence divergences for non-synonymous and synonymous sites, respectively.

Table 4 Pairwise divergence and diversity estimates for mammals

Order	Species 1	Species 2	$\hat{\pi}_A/\hat{\pi}_S$	K_A/K_S	NI
Carnivora	Martes americana	Mustela erminea	0.0303	0.0138	2.1918
Carnivora	Panthera leo	Lynx canadensis	0.1318	0.0283	4.6565
Cetartiodactyla	Bubalus bubalis	Pseudois nayaur	0.0683	0.0268	2.5501
Cetartiodactyla	Camelus bactrianus	Lama guanicoe huanacus	0.0709	0.0219	3.2354
Chiroptera	Lionycteris spurrelli	Lonchophylla robusta	0.0206	0.0164	1.2580
Chiroptera	Mesophylla macconnelli	Vampyressa pusilla	0.0275	0.0104	2.6399
Chiroptera	Myotis aurascens	Barbastella barbastellus	0.0530	0.0198	2.6781
Insectivora	Blarina brevicauda	Sorex caecutiens	0.0316	0.0119	2.6581
Primates	Alouatta caraya	Saimiri sciureus sciureus	0.1837	0.0458	4.0115
Primates	Mandrillus sphinx	Macaca nemestrina nemestrina	0.0605	0.0328	1.8450
Primates	Microcebus griseorufus	Lemur catta	0.0393	0.0345	1.1396
Rodentia	Apodemus draco	Rhabdomys pumilio	0.0507	0.0136	3.7243
Rodentia	Calomys fecundus	Scapteromys tumidus	0.1861	0.0159	11.7061
Rodentia	Cavia tschudii	Dasy proctaleporina	0.0714	0.0341	2.0932
Rodentia	Cratogeomys fumosus fumosus	Geomyspersonatus megapotamus	0.0537	0.0154	3.4888
Rodentia	Glaucomyssabrinus griseifrons	Pteromys volans	0.0476	0.0151	3.1501
Rodentia	Microtus oeconomus	Clethrionomys gapperi	0.0216	0.0090	2.4017
Mean			0.0676±0.0125	0.0215±0.0025	3.2605±0.5735

with comparable body sizes. A similar pattern is observed for non-synonymous variability, although the difference is not as pronounced. The most conservative comparison is probably that between small bats and small passerines, which are the species groups with the most similar generation times. Synonymous diversity is significantly lower in the passerines compared to the bats ($W = 292.0$, $P = 0.0004$), and non-synonymous diversity is also lower ($W = 339.0$, $P = 0.0537$) (Table 1). The relation between body size and diversity presumably reflect smaller N_e values for species with larger body size; this casts considerable doubt on the recent proposal that mtDNA is not affected by population size due to the prevalence of Hill–Robertson effects in the non-recombining mtDNA genome (Bazin et al., 2006).

Mutation rate differences between mammals and birds

It is possible that mutation rates in mammalian mtDNA are intrinsically higher than in birds. Since there are no direct estimates of these mutation rates in birds, we have to rely on comparisons of estimates of synonymous or non-coding sequence divergence per year from mammals and birds, assuming these to be neutral (Kimura, 1983). It is currently unclear whether birds or mammals have higher rates of mtDNA sequence divergence (Garcia-Moreno, 2004; Ho et al., 2005; Lynch et al., 2006). A 2% rate of sequence divergence per million years is commonly used for mitochondrial coding genes for both mammals and birds (Brown et al., 1979), but there are many factors that could violate this general rule. Overall, there seems to be no evidence for differences that are large enough to overcome the

differences in generation time between otherwise similar birds and mammals, given the fact that birds tend to have much longer generation times than mammals of comparable sizes (Finch, 1990). Other things being equal, this would tend to cause higher levels of variability in birds compared with mammals.

There is also indirect evidence that mutation rate differences cannot explain the difference in synonymous site diversity between birds and mammals that we have described. This comes from the results on non-synonymous variability (Tables 1 and 3). These estimates are much more similar for birds and mammals than the corresponding synonymous site variability estimates. In addition, θ_A is nearly always greater than $\hat{\pi}_A$, consistent with the action of purifying selection against deleterious amino acid site mutations that has been extensively documented before (Table 1) (Nachman, 1998; Fry, 1999; Weinreich and Rand, 2001). With such selection, a larger N_e has a much smaller effect on $\hat{\pi}_A$ than $\hat{\pi}_S$, since the former is more dependent on the ratio of mutation rate to selection coefficient than on N_e, whereas neutral or nearly neutral diversity is proportional to N_e (Loewe et al., 2006).

If a difference in mutation rate were involved in the mammal–bird difference, both non-synonymous and synonymous diversity would be affected proportionately. In fact, overall $\hat{\pi}_A/\hat{\pi}_S$ is significantly higher in birds than mammals, and individual comparisons are always nominally higher (Table 1). This pattern is what is expected on the hypothesis of a reduction in the efficacy of selection in birds, due to Hill–Robertson effects that reduce N_e (Charlesworth and Charlesworth, 2000; Gordo and Charlesworth, 2001). Significantly higher NIs in birds (Table 3) (mean: 20.35 ± 12.41) compared to mammals (mean: 3.26 ± 0.57) (Table 4) (W: 205.0, $P < 0.0015$) further support this conclusion. A high NI reflects less efficient selection against slightly deleterious mutations, which therefore become more abundant in the population but still have a low probability of fixation (Charlesworth, 1994; Rand and Kann, 1996). The average K_A/K_S ratio is non-significantly higher in mammals than in birds (U: 103, $P = 0.15$). The theory of selection against slightly deleterious mutations predicts a much smaller effect of a reduction in N_e on $\hat{\pi}_A/\hat{\pi}_S$ than on K_A/K_S (Charlesworth, 1994; Loewe et al., 2006), consistent with the lack of a significant difference in K_A/K_S between birds and mammals.

Demography and sequence variation

The last possible confounding factor is the operation of demographic factors that cause lower N_e values in birds. Comparisons between nuclear gene diversities for birds and mammals should shed light on this possibility. Unfortunately, there are relatively few data on putatively neutral DNA sequence diversity in birds. The evidence suggests that birds have larger N_e values for nuclear genes than mammals. The ancestral N_e in a passerine species was recently estimated as several hundred thousand (Jennings and Edwards, 2005). The recent effective population size (N_e) in chicken is well above one million, using a mutation rate of 1.2×10^{-9} (based on divergence data between chicken and turkey (Axelsson et al., 2004), a time of divergence of 38×10^6 (Dimcheff et al., 2002; van Tuinen and Dyke, 2004), and a generation

Table 5 Genetic diversities within populations for birds and mammals (±s.e.)

	N	Mean $\hat{\pi}_S$	Mean θ_S	Mean $\hat{\pi}_A$	Mean θ_A	$\hat{\pi}_A/\hat{\pi}_S$	θ_A/θ_S	Mean $(\hat{\pi}_S - \theta_S)$	Mean $(\hat{\pi}_A - \theta_A)$
All birds	36	0.00444 ± 0.00069	0.00454 ± 0.00067	0.00049 ± 0.00009	0.00052 ± 0.00009	0.1104 ± 0.02622	0.1144 ± 0.0194	-0.00010 ± 0.00024	-0.00003 ± 0.00002
All mammals	35	0.01172 ± 0.00184	0.01336 ± 0.00267	0.00117 ± 0.0002	0.00130 ± 0.00021	0.0996 ± 0.0235	0.0970 ± 0.0002	-0.00164 ± 0.00114	-0.00013 ± 0.00009
Mann–Whitney U-test		$P = 0.0001$	$P = 0.0005$	$P = 0.0003$	$P = 0.0072$	$P = 0.7969$	$P = 0.8164$	$P = 0.4109$	$P = 0.8225$
Birds <100 g	13	0.00524 ± 0.00070	0.00483 ± 0.00062	0.00053 ± 0.00018	0.00056 ± 0.00018	0.1010 ± 0.0367	0.1160 ± 0.0404	0.00042 ± 0.00018	-0.00003 ± 0.00003
Mammals <100 g	22	0.01528 ± 0.00274	0.01860 ± 0.00413	0.00135 ± 0.00058	0.00155 ± 0.00030	0.0883 ± 0.0244	0.0833 ± 0.0245	-0.00332 ± 0.00188	-0.00020 ± 0.00015
Mann–Whitney U-test		$P = 0.0044$	$P = 0.0028$	$P = 0.0302$	$P = 0.0358$	$P = 0.4785$	$P = 0.6787$	$P = 0.2508$	$P = 0.5502$
Birds 100–6175 g	13	0.00312 ± 0.00100	0.00359 ± 0.00115	0.00050 ± 0.00015	0.00053 ± 0.00014	0.1607 ± 0.0695	0.1469 ± 0.0613	-0.00047 ± 0.00023	-0.00003 ± 0.00003
Mammals 100–6175 g	8	0.00632 ± 0.00133	0.00640 ± 0.00179	0.00100 ± 0.00038	0.00112 ± 0.00040	0.1286 ± 0.0688	0.1752 ± 0.0801	-0.00008 ± 0.00078	-0.00012 ± 0.00017
Mann–Whitney U-test		$P = 0.0760$	$P = 0.1195$	$P = 0.3653$	$P = 0.3282$	$P = 0.6251$	$P = 0.7558$	$P = 0.1803$	$P = 0.8563$
Passerines <62 g	9	0.00602 ± 0.00084	0.00562 ± 0.00072	0.00075 ± 0.00022	0.00080 ± 0.00022	0.1241 ± 0.0410	0.1417 ± 0.0431	0.00040 ± 0.00004	-0.00005 ± 0.00004
Bats <62 g	12	0.01736 ± 0.00438	0.02413 ± 0.00701	0.00123 ± 0.00030	0.00170 ± 0.00036	0.0711 ± 0.0861	0.0706 ± 0.0943	-0.00676 ± 0.00309	-0.00047 ± 0.00022
Mann–Whitney U-test		$P = 0.0428$	$P = 0.0507$	$P = 0.2864$	$P = 0.2555$	$P = 0.4941$	$P = 0.2545$	$P = 0.0817$	$P = 0.5224$

time of 1.5 years and average $\theta = 7.7 \times 10^{-3}$ (Sundstrom et al., 2004)). In contrast, N_e in higher primates has been estimated as 10 000–20 000, and in rodents as 160 000 (Eyre-Walker et al., 2002).

Population subdivision could, however, result in very different total N_e values for mtDNA and nuclear genes, as the N_e that controls diversity among genes sampled randomly from the species as a whole is inversely related to the rate of migration among local populations (Charlesworth et al., 2003). MtDNA is affected only by migration of females, and there is evidence that female mammals mostly disperse less than males, whereas the reverse is true for birds (Greenwood, 1980; Prugnolle and de Meeus, 2002). This difference in dispersal may contribute to the patterns we have reported. In order to examine this possibility, we estimated synonymous and non-synonymous diversities for sequences collected at the same locality. This provides estimates of genetic diversities within local populations, which are expected to be much less sensitive to migration rates than species-wide diversity levels (Charlesworth et al., 2003).

One hundred and thirty-four mammalian populations from 35 genera and 127 bird populations from 36 genera were analysed (Supplementary Material). Mean within-population $\hat{\pi}_S$ for mammalian genera ranged from 0.0017 to 0.0521, with a mean of 0.0117 ± 0.0018; $\hat{\pi}_A$ ranged from 0 to 0.0050 with a mean of 0.0012 ± 0.0002 (Table 5). Mean within-population $\hat{\pi}_S$ in bird genera ranged between 0 and 0.0209, with a mean of 0.0044 ± 0.0007; and $\hat{\pi}_A$ ranged between 0 and 0.0022, with a mean of 0.0005 ± 0.00009. Both synonymous and non-synonymous diversities were significantly lower in birds than in mammals ($\hat{\pi}_S$: $W = 955.0$, $P = 0.0001$, $\hat{\pi}_A$: $W = 1064.0$, $P = 0.0078$). We also grouped species by body mass; in most comparisons, $\hat{\pi}_S$ was significantly lower in birds compared to mammals. $\hat{\pi}_A$ was always lower in birds compared to mammals, although these differences were not statistically significant. We also compared passerines with bats, and again found lower $\hat{\pi}_S$ in the passerines compared to the bats.

We compared population structures in the mammals and the birds by estimating F_{ST} (Hudson et al., 1992; Charlesworth et al., 2003) for mammalian and bird species (averaging over species within genera). The average mammalian F_{ST} was 0.563 ± 0.067 and average bird F_{ST} was 0.384 ± 0.068 ($P = 0.077$). These figures suggest that mammals may have a more pronounced population structure than birds with respect to mtDNA, although the difference is not statistically significant. A lower N_e for local populations, as expected if there are more intense Hill–Robertson effects on bird than mammalian mtDNA, would lead to increased F_{ST} (Charlesworth et al., 2003), so that this apparent difference is consistent with our hypothesis.

Conclusions

Overall, the finding that birds have lower mtDNA variability than mammals is most easily explained by Hill–Robertson effects acting on the W chromosome. We recognize that this conclusion is only tentative, since this difference could be due to confounding factors for which we have not allowed. Further data on effective population sizes based on autosomal genes in birds and mammals, as well as direct measurements of W-chromosome variability in a range of species, will provide additional data for testing this explanation further. Similar comparisons between other taxonomic groups with male and female heterogamety would also help to explore this question further.

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