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Exceptional longevity in songbirds is associated with high rates of evolution of cytochrome *b*, suggesting selection for reduced generation of free radicals

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Summary

In animals, longevity (maximal lifespan) is inversely related to mass-specific basal metabolic rates. However, contrary to expectation, in several mammalian taxa, exceptional longevity is associated with high basal metabolic rate, and also fast evolution of mtDNA-coded proteins. The association of these traits was suggested to result from adaptive selection of mutations in mtDNA-coded proteins, which accelerates basal respiration, thus inhibiting the generation of reactive oxygen species that constrain longevity. In birds, all the genera with high rate of cytochrome *b* evolution are songbirds (oscines). Within the songbirds group, both longevity residuals and lifetime expenditure of energy are positively correlated with the rate of cytochrome *b* evolution. Moreover, within the large songbirds family Fringillidae (true finches) mass-specific basal metabolic rates, longevity, longevity residuals and lifetime expenditure of energy are all positively correlated with the rate of evolution of cytochrome *b*. In *Serinus*, a genus of finches (canaries) that exhibits the highest rate of cytochrome *b* evolution, and the highest values of exceptional longevity and lifetime expenditure of energy in all birds, many of the substitutions in cytochrome *b* are clustered around Q_i, a ubiquinone binding site adjacent to the mitochondrial matrix, apparently selected to increase the rate of ubiquinone reduction. We therefore suggest that, in songbirds, the accelerated evolution of cytochrome *b* involved selection of mutations that reduce the generation of reactive oxygen species, thus contributing to the evolution of exceptional longevity, and possibly also exceptional long-term memory, which is necessary for learning songs.

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Key words: lifespan, songbirds, cytochrome *b*, energy expenditure, reactive oxygen species.

Introduction

The ‘free radicals’ theory of aging, a current paradigm of the biology of aging, postulates that the accumulated damage from metabolically generated free radicals constrains longevity (Harman, 1956; Sohal and Weindruch, 1996; Beckman and Ames, 1998; Barja, 2004; Sanz et al., 2006). Because the rate of generation of reactive oxygen species (ROS) is largely a function of metabolic rates (Ku and Sohal, 1993) this postulate is compatible with the fact that, in animals, longevity (L) is dependent on mass-specific basal metabolic rates (BMR_w). L is inversely related to BMR_w, obeying by the power law:

$$L = aBMR_w^{-b} , \quad (1)$$

where a and b are constants (b is the scaling exponent). In animals BMR_w is inversely related to body mass (M) obeying the power law:

$$BMR_w = cM^{-d} , \quad (2)$$

where c and d are constants. For the whole animal, basal metabolic rate, $BMR=BMR_wM$, and is therefore related to M by the power law:

$$BMR = cM^{\beta} , \quad (3)$$

where the scaling exponent $\beta=1–d$.

Since L is a function of BMRw, L is also a function of M, obeying the power law:

$$L=eM^{f} , \quad (4)$$

where e and f are constants.

From Eqn 1 and Eqn 2 it follows that e=a–(bc) and that the scaling exponent f=(bd) (for a review, see Speakman, 2005).

The ‘rate of living’ theory postulated that the mass-specific lifetime expenditure of energy (i.e. $L\times BMR_w$) is nearly constant in all animals (Pearl, 1928; Speakman, 2005). If L was a reciprocal function of BMR_w, i.e. b=1, then f=d and the lifetime expenditure of energy would be constant, since in this case $L\times BMR_w=(eM^{d})(cM^{-d})=ec=k$. In fact, both in birds and mammals, $b\leqslant 1$ and $d>f$ (Speakman, 2005), and the lifetime expenditure of energy should be inversely related to body mass, since from Eqn 4 and Eqn 2:

$$L\times BMR_w = eM^{f}cM^{-d} = ecM^{f-d}$$

or

$$L\times BMR_w = gM^{-h} , \quad (5)$$

where g=ec and h=d–f.

The relationships between longevity and body mass (power law 4), longevity and mass-specific basal metabolic rates (power law 1), and lifetime expenditure of energy and body mass (power law 5) could be explained very well by the free radical theory of aging if we accept that the ratio between the rate of generation of reactive oxygen species ($\dot{V}_{ROS}$) and the rate of oxygen consumption (and hence BMRw) is not necessarily the same in all animals. Thus, the fact that L is not a reciprocal function of BMRw, as well as the commonly observed large deviations (residuals) from the power laws 1, 4 and 5, could be accommodated by the theory if these were the result of the evolution of taxon-specific modulations of the relationship between metabolic rates and $\dot{V}_{ROS}$. Indeed, increasing evidence suggest that longevity correlates negatively with $\dot{V}_{ROS}$, and that this correlation is stronger than the negative correlation with BMRw (Ku et al., 1993; Perez-Campo et al., 1998; Herrero and Barja, 1998).

Recently, we showed that in a large clade of placental mammals the rate of evolution of cytochrome *b*, and most other mtDNA-coded proteins, is positively correlated with longevity (Rottenberg, 2006). Moreover, we showed later that, in the same clade the longevity residuals from power laws 1 and 4 are also positively correlated with the rate of evolution of cytochrome *b*, suggesting that longevity dependence on the rate of evolution of mtDNA-coded proteins is independent of body mass or mass-specific basal metabolic rates (Rottenberg, 2007). Since ROS production is largely a byproduct of mitochondrial electron transport, and cytochrome *b* and other components of the mitochondrial electron transport complexes, are coded by mtDNA, we suggested that the evolution of mtDNA-coded proteins in placental mammals is driven by adaptive selection of mutations that reduce $\dot{V}_{ROS}$. Anthropoid primates, as well as elephants, whales, dolphins and bats exhibit exceptionally high rates of evolution of cytochrome *b* and other mtDNA-coded proteins, and in addition to exceptional longevity (i.e. positive longevity residuals from power laws 1 and 4), exhibit also exceptionally high BMRw values (i.e. positive BMRw residuals from power law 2). These observations suggested that adaptive selection of mutations in cytochrome *b* and other mtDNA-coded proteins reduced $\dot{V}_{ROS}$, in part, by increasing mitochondrial proton leak (Rottenberg, 2007). Mitochondrial proton leak reduces the mitochondrial proton electrochemical potential difference and thus accelerates basal electron transport rate, thereby reducing $\dot{V}_{ROS}$ (Korshunov et al., 1997). Therefore, the free radical theory of aging predicts that an increase of mitochondrial proton leak should increase lifespan (Brand, 2000). We suggested that the observed association between positive L residuals from Eqn 1 and Eqn 4 with positive BMRw residuals from Eqn 2, that appear to be incompatible with the free radical theory of aging, actually reflects a lower degree of mitochondrial coupling that is associated with increased basal respiration rate, and consequently lower $\dot{V}_{ROS}$/BMRw ratio. A lower mitochondrial degree of coupling could accounts for the positive residuals from the power laws 1, 2, 4 and 5 observed in these taxa. Interestingly, most of these taxa also share exceptional sociality and cognitive abilities, and therefore it is not clear whether the selection for reduced $\dot{V}_{ROS}$ is driven by pressure to increase lifespan or sociality and cognitive abilities (see Discussion).

It is well-established that the power laws that describe the relationships between longevity, body mass and metabolic rates in birds and mammals are different: in the power laws 1, 2, 4 and 5 the coefficients a, c, e and g are larger in birds than in mammals, i.e. at equal body mass birds have higher mass-specific basal metabolic rates than mammals, and yet live longer than mammals of equal body mass, and their lifetime expenditure of energy is also larger (Holmes et al., 2001; Speakman, 2005). Birds and mammals share endothermic metabolism, but they evolved independently, and it should not be surprising that these coefficients, which are influenced by the anatomy, physiology and metabolism of these classes of animals are different. Moreover, there is direct evidence that birds live longer than mammals of equal mass or BMR_w because the ratio between $\dot{V}_{ROS}$ and oxygen consumption is smaller in birds than in mammals (Ku and Sohal, 1993; Barja et al., 1994; Barja, 1998). Similarly, the large deviations of some species from the birds' power laws most likely result from modulation of the relationships between BMRw and ROS generation in these species. We can therefore ask: do these modulations also result from adaptive selection in the evolution of mtDNA-coded proteins as observed in placental mammals?

In mammals the relative rate of evolution of most mtDNA-coded proteins is very different in different taxa, and this fact and other independent evidence, suggest that in mammalian taxa the evolution of mtDNA-coded proteins is driven by adaptive (positive) selection (Gissi et al., 2000). There is also evidence that adaptive selection drives the evolution of mtDNA in most other classes of animals (Bazin et al., 2006). However, there are no studies of the relative rates of evolution of mtDNA-coded proteins in birds, and there are relatively few complete sequences of mtDNA of birds that are available for such studies. The exception is cytochrome *b*, the core peptide of complex III, which has been sequenced extensively for phylogenetic studies of birds. Cytochrome *b* is a major source of ROS (Demin et al., 1998), and its rate of evolution correlates strongly with longevity in placental mammals (Rottenberg, 2006; Rottenberg, 2007). It was also suggested that complex III (together with complex I) is responsible for the low $\dot{V}_{ROS}$ in birds (Herrero and Barja, 1997; Herrero and Barja, 1998). Therefore, in this study, we determined the relative rate of cytochrome *b* evolution in modern birds and investigated the relationships between the rate of cytochrome *b* evolution and longevity, basal metabolic rates and lifetime expenditure of energy.

Materials and methods

The database

We used the AnAge databank of the Human Aging Genomic Resources Databank (HAGR) [http://genomics.senescence.info/species/ (de Magalhaes et al., 2005)] to obtain the longevity (L, years) and body mass (M; g) of 809 species of birds belonging to 287 genera. We also collected

all the available values of mass-specific basal metabolic rates (BMR_w; mW g^{-1}) for these species from the AnAge databank. Data collection was terminated in February 2006. We computed the genera averages of L, M and BMR_w, and these average values were used for the analysis (Table S1 in supplementary material). Using the Entrez/protein search engine (http://www.ncbi.nlm.nih.gov/entrez/) we searched for complete cytochrome *b* sequences for the 287 genera. If more than one complete sequence was found for a genus we used the sequence of the species with life history traits that was closer to the genus average. In a few cases, where several complete sequences were available for a genus, we aligned them and used the one that was closest to the genus consensus. In most cases there was no significant difference between the values of substitution per site calculated for species of the same genus (see below). Analysis of the data at the genera level rather than at the species level provides several advantages. Firstly, it allowed us to increase the size of our database since very few species that have BMR_w value in the AnAge databank also have a complete cytochrome *b* sequence. Secondly, the error in estimating species L values is quite large, and the genus average is therefore more reliable. Thirdly, a few genera are represented by a large number of species in the AnAge databank, whereas most other genera are represented by a few, often a single, species; therefore, analysis at the species level would give unduly large weight to a few genera.

The computation of cytochrome b substitution per site

The complete cytochrome *b* sequences of 122 genera of birds were aligned using the CLUSTALX program (http://bips.u-strasb.fr/fr/Documentation/ClustalX) and submitted for genetic distance determination by the PRODIST program at the phylogeny site PHYLIP (http://bioweb.pasteur.fr/seqanal/interfaces/prodist.html). The Dayhoff PAM matrix distance model with the default setting was used. The distance matrix of the 122 cytochrome *b* sequences was used to compute the relative rates of cytochrome *b* evolution in modern birds. The detailed phylogeny of extant bird species has not been fully elucidated as yet. Nevertheless, it is accepted that the clade of Neognathae (modern birds) that contains most extant bird species diverged from the Paleognathae clade early on and later split into the Neoaves and the Galloanserae branches (Cracraft et al., 2004). Therefore, we use the three sequences of the Paleognathae clade as an outgroup to calculate the relative rate of evolution (i.e. substitution per site) of the 119 genera of modern birds from the node of divergence of the neoaves and the Galloanserae branches. To compute the substitution per site for a species i (Si) belonging to the Neoaves branch we used the formula: $Si=(D_{i,p}+D_{i,g}-D_{p,g})/2$ where $D_{i,p}$ is the distance from species i to p, a species that belongs to the Paleognathae branch; $D_{i,g}$ is the distance from i to g, a species that belongs to the Galloanserae branch; and $D_{p,g}$ is the distance from p to g. For each species we computed Si with the three different Paleognathae species (Table S1 in supplementary material) and three different Galloanserae species (*Gallus gallus*, *Anser albifrons*, *Ortalis vetula*), in all possible combinations (3×3), and averaged the nine values. To compute the substitution per site for species that belong to the Galloanserae branch we used the formula $Si=(D_{i,p}+D_{i,n}-D_{p,n})/2$ where $D_{i,n}$ is the distance between species i and n, a species that belong to the Neoaves branch, and $D_{p,n}$ is the distance from p to n. For each species we computed Si with the three different Paleognathae species and three Neoaves species belonging to different orders, in all possible combination, and averaged the nine values. The results of these computations are listed in Table S1 in supplementary material.

Statistical analysis (e.g. linear regression, multiple linear regression, Student's *t*-test) was carried out with the SigmaStat3.1 Statistical analysis package (Systat Software inc.)

Results

We found complete cytochrome *b* sequences for 122 genera of birds that also have longevity and body mass records. Using the three genera that belong to the Paleognathae branch as an outgroup to the 119 genera of the Neognathae clade we calculated the relative rate of cytochrome *b* evolution (i.e. substitution per site, Scytb) in Neognathae as described in the Materials and methods. The results of these calculations are given in Table S1 in supplementary material. There were large differences in the values of Scytb of these genera, ranging from 0.045 (*Ortalis*) to 0.163 (*Serinus*), a nearly fourfold difference, similar to the range of values that was observed in placental mammals (Rottenberg, 2006), suggesting adaptive selection of mutations in taxa that exhibit accelerated evolution of cytochrome b. The computed value of substitution per site, Scytb, is a sum of both neutral mutations and selected mutations. Assuming that the rate of neutral mutations is the same in all species [the Molecular Clock assumption (Page and Holmes, 1998)], genera with exceptionally low value of Scytb represent genera with relatively few positively selected mutations. By contrast, genera with exceptionally high values of Scytb, represent genera where most of the mutations were selected. Of the 119 genera of Neognathae that have a complete cytochrome *b* sequence, only 59 also have BMR_w values listed in the AnAge database (Table S2 in supplementary material). To find out if there are correlations between life history traits and selection of cytochrome *b* mutations we compared the relationship between body mass (M), basal metabolic rates (BMR), mass-specific basal metabolic rates (BMR_w), and longevity (L) in a group of genera with exceptionally high Scytb (the upper quadrant of the rates distribution, Scytb>0.1345, N=15) and a group with exceptionally low Scytb (the lower quadrant, Scytb<0.069, N=15, see Table S2 in supplementary material).

Fig. 1A (solid line) shows the linear regression of power law (3) that describe BMR as a function of body mass in the 59 Neognathae genera (Table S2 in supplementary material): $BMR=3.76*M^{0.663}$. The scaling exponent 0.663±0.014 is similar to previously reported value for birds, 0.671

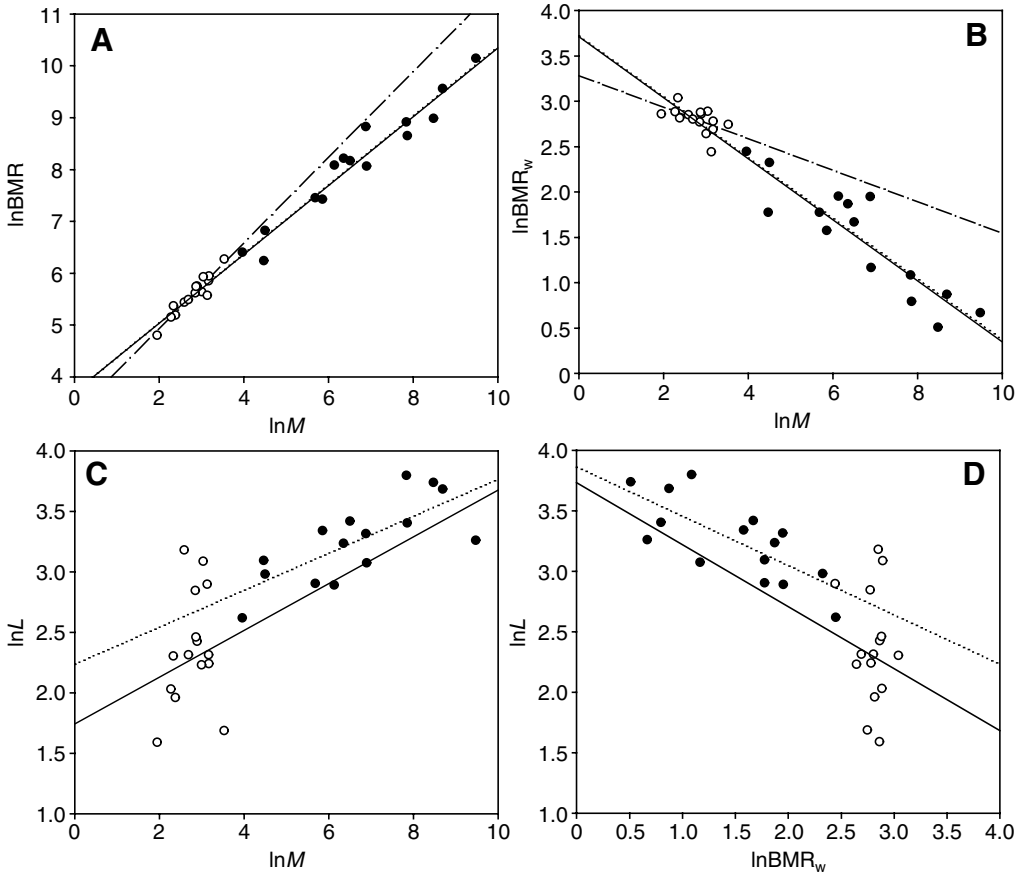

Fig. 1. The rate of cytochrome *b* evolution (Scytb) modulates the relationships between body mass (*M*), basal metabolic rate (BMR), mass-specific basal metabolic rate (BMR_w) and longevity (*L*) in Neognathae birds. Data are from Table S2 in supplementary material. Linear regressions of the power laws for the 59 genera of Neognathae birds for which there are complete cytochrome *b* sequences available, and the *M*, *L* and BMR_w values, are shown by solid lines. Linear regressions of the genera with the lowest values of Scytb (the lower quadrant of the rate distribution, Scytb<0.069, *N*=15, full symbols) are shown by dotted lines. The linear regressions of the genera with the highest values of Scytb (the upper quadrant of the rate distribution, Scytb>0.1345, *N*=15, empty symbols) are shown by dash–dot–dash lines. When the regression was not significant (*P*>0.05) the lines were omitted. (A) lnBMR as a function of ln*M*: for the Neognathae clade lnBMR=3.712+0.6633ln*M* (*N*=59, r^2=0.973, *P*<0.001); for the high Scytb group lnBMR=3.283+0.8263ln*M* (*N*=15, r^2=0.903, *P*<0.001); for the low Scytb group lnBMR=3.715+0.6663ln*M* (*N*=15, r^2=0.942, *P*<0.001). The difference in the slopes between the low Scytb group 0.666±0.046 and the high Scytb group 0.826±0.075 is significant (*P*<0.001). The curves for the low Scytb and the whole clade are nearly identical. (B) *L*nBMR_w as a function of *M*: for the whole clade lnBMR_w=3.712–0.3373ln*M* (*N*=59, r^2=0.904, *P*<0.001); for the high Scytb group lnBMR_w=3.283–0.1743ln*M* (*N*=15, r^2=0.291, *P*=0.038); for the low Scytb group lnBMR_w=3.715–0.3343ln*M* (*N*=15, r^2=0.804, *P*<0.001). The difference in the slopes between the low Scytb group, –0.334±0.046, and the high Scytb group, –0.175±0.075, is significant. The curves for the low Scytb and the whole clade are nearly identical. (C) ln*L* as a function of ln*M*: for the whole clade ln*L*=1.742+0.193×ln*M* (*N*=59, r^2=0.473, *P*<0.001); for the high Scytb group ln*L*=1.616+0.27×ln*M* (*N*=15, r^2=0.058, *P*=0.384); for the low Scytb group ln*L*=2.23+0.153×ln*M* (*N*=15, r^2=0.551, *P*=0.002). (D) ln*L* as a function of BMR_w: for the whole clade ln*L*=3.733–0.513×lnBMR_w (*N*=59, r^2=0.417, *P*<0.001); for the high Scytb group ln*L*=3.63–0.451×lnBMR_w (*N*=15, r^2=0.017, *P*=0.643); for the low Scytb group ln*L*=3.859–0.408×lnBMR_w (*N*=15, r^2=0.547, *P*=0.002).

(Speakman, 2005). However, when plotted separately there was a significant difference between the scaling exponent of the high Scytb group, that is composed entirely of small birds (empty symbols), and the scaling exponent of the whole group (0.826±0.075 compared to 0.663±0.014; d.f.=70, *t*=2.09, *P*=0.02). By contrast, the scaling exponent of the low Scytb group (0.666±0.0458) was not significantly different from the exponent of the entire Neognathae group. This difference between the high and low Scytb groups is shown more clearly in Fig. 1B, in which the dependence of BMR_w on *M* (power law 2) is shown. In the Neognathae clade $BMR_w=3.712M^{-0.337}$ (solid line) the scaling exponent of the low Scytb group (–0.334±0.0458) is nearly identical to the scaling exponent of the whole clade (–0.337) but the scaling exponent of the high Scytb is only half as much (–0.174±0.0753); the coefficient of determination was also much higher in the low Scytb group than in the high Scytb group (r^2=0.804 and r^2=0.291, respectively), indicating a much weaker dependence of BMR_w on *M* in the high Scytb group. These results suggest that accelerated evolution of cytochrome *b* is associated with taxon-specific modulation of BMR_w.

There is also a large difference between the two groups in the dependence of *L*, on BMR_w (power law 1), or on *M* (power law 4). Fig. 1C shows that within the low Scytb group *L* increases with *M* with a slope that is similar to the dependence in the whole clade. However, within the high Scytb group, *L*, which is highly variable, is not dependent on *M* at all. Fig. 1D shows that within the low Scytb group *L* decreases with BMR_w, with a slope that is similar to the dependence in the whole clade, but within the high Scytb group *L* is not dependent on BMR_w at all (Fig. 1D).

If longevity in the high Scytb group, a group of taxa, where most of the cytochrome *b* mutations were selected, is not strongly dependent on either *M* or BMR_w does it depend on

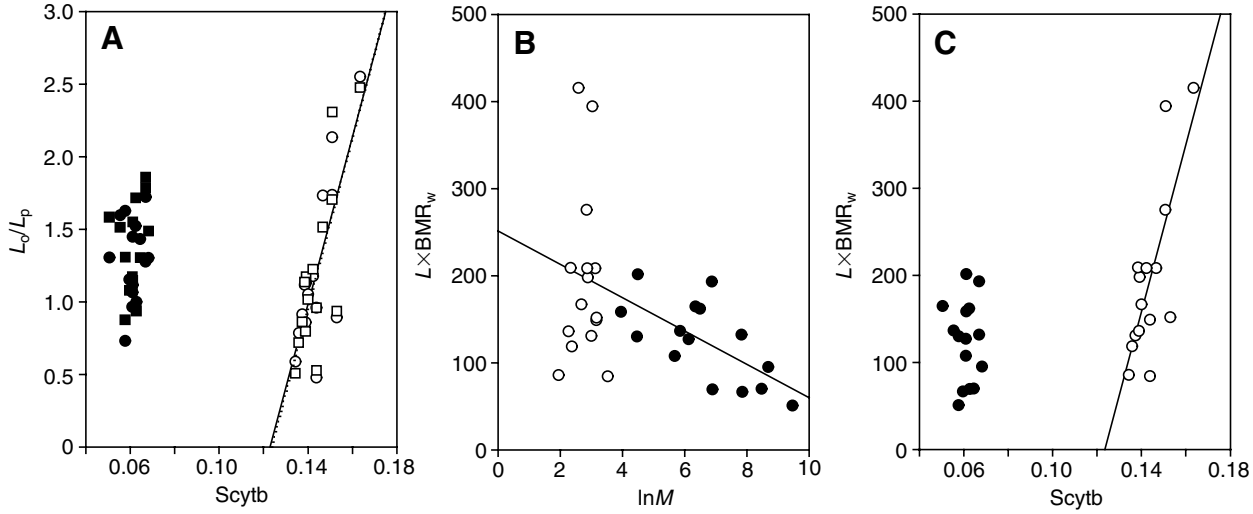

Fig. 2. In the group of genera with accelerated evolution of cytochrome *b* exceptional longevity and the lifetime expenditure of energy are strong functions of the rate of evolution of cytochrome *b*. Data are from Table S2 in supplementary material. The genera with high Scytb values (the upper quadrant, Scytb>0.1345) are indicated by open symbols; the genera with the low Scytb values (the lowest quadrant, Scytb<0.069) are indicated by solid symbols. When the linear regression was not significant the regression line was omitted. (A) Exceptional longevity as a function of Scytb. The ratio of observed longevity (L_o) to predicted longevity (L_p), L_o/L_p, is derived from the residuals of the linear regressions (solid curves) of the Neognathae clade shown in Fig. 1C,D. The squares indicate the residuals from $L(BMR_w)$ (Fig. 1D) and the circles indicate the residuals from $L(M)$ (Fig. 1C). For the high Scytb group, $L_o/L_p(M)$=–7.068+57.5×Scytb (N=15, r^2=0.605, P<0.001) and $L_o/L_p(BMR_w)$=–7.258+58.7×Scytb (N=15, r^2=0.618, P<0.001). For the low Scytb group, $L_o/L_p(M)$=0.869+6.8×Scytb (N=15, r^2=0.013, P=0.689), and $L_o/L_p(BMR_w)$=0.347+16.6×Scytb (N=15, r^2=0.06, P=0.371). For the whole clade, $L_o/L_p(M)$=1.02+0.55×Scytb (N=59, r^2=0.0021, P=0.731) and L_o/L_p(BMRw)=1.13–0.419×Scytb (N=59, r^2=0.001, P=0.804). (B) Lifetime energy expenditure as a function of body mass. The linear regression for the high Scytb group is $L\times BMR_w$=153+15×ln$M$ (N=15, r^2=0.004, P=0.854). The linear regression for the low Scytb group is $L\times BMR_w$=250–19×ln$M$ (N=15, r^2=0.436, P=0.007). For the whole clade, $L\times BMR_w$=218–17.2×ln$M$ (N=59, r^2=0.217, P<0.001). The correct value of lifetime expenditure of energy can be obtained by multiplying $L\times BMR_w$ by 31 536 000 (the number of seconds in one year). (C) Lifetime expenditure of energy as a function of Scytb. For the high Scytb group, $L\times BMR_w$=–488+9612×Scytb (N=15, r^2=0.579, P<0.001). For the low Scytb group, $L\times BMR_w$=155–514×Scytb (N=15, r^2=0.0026, P=0.857). For the whole clade, $L\times BMR_w$=39.3+982×Scytb (N=59, r^2=0.206, P<0.001).

Scytb? To answer this question we computed the longevity residuals from power laws 1 and 4, to obtain the extent to which the L value of each genus deviates from the prediction of the birds' power laws, and plotted the residuals of the two groups against theirs Scytb values (Fig. 2A). Within the low Scytb group there was no significant correlation between Scytb and the L residuals, but within the high Scytb group there was a very strong positive correlation between the L residuals and Scytb. These results indicate that Scytb does not have strong effect on longevity in the low Scytb group, in which few mutations were selected and L depend strongly on the magnitude of BMR_w or M (Fig. 1C,D). By contrast, in the high Scytb group, in which most of the mutations were selected, and BMR_w (or M) have little effect on L; the L residuals are strongly dependent on the value of Scytb. These results suggest that the selection of mutations in cytochrome *b* increased longevity independently of M or BMR_w.

An alternative measure of the effect of BMRw on L is the lifetime expenditure of energy, $L\times BMR_w$. In general $L\times BMR_w$ decrease with increasing M (power law 5), and this holds true for the low Scytb group; however, in the high Scytb group $L\times BMR_w$ varies greatly but shows no significant dependence on M (Fig. 2B). By contrast in the high Scytb group, $L\times BMR_w$ depends strongly on Scytb, but this is not true for the low Scytb group (Fig. 2C). Thus, the selected mutations in cytochrome *b* increased the lifetime expenditure of energy. The genera with the highest values of Scytb (i.e. *Serinus* and *Fringilla*, Table S2 in supplementary material), also exhibit the highest values of $L\times BMR_w$ (Fig. 2C) and the highest L residuals (Fig. 2A).

The averaged value of Scytb is also different in taxonomic groups of higher order. The average Scytb for all neognath genera is 0.096±0.034, but for oscines (song birds) a branch of the order Passeriformes (passerines, or perching birds) the average Scytb value (0.134±0.011) is nearly twice as large as that of all other neognaths (0.073+0.019, t=19.6, P<0.001). The phylogeny of neognaths is not fully resolved yet, but the average Scytb of each non-oscine family (and therefore any ordinal level clade of non-oscine birds) is significantly lower than that of songbirds. All the genera in the high Scytb group (Scytb>0.1345) are oscine birds; but the low Scytb group (Scytb<0.069) includes genera from several families that belong to the Neoaves branch, and from the Galliformes order that belong to the Galloanserae branch (Table S2 in supplementary material). Therefore, the differences between the high and low Scytb groups, described above (Figs 1, 2), are to a considerable extent the differences between oscine birds and other orders of modern birds.

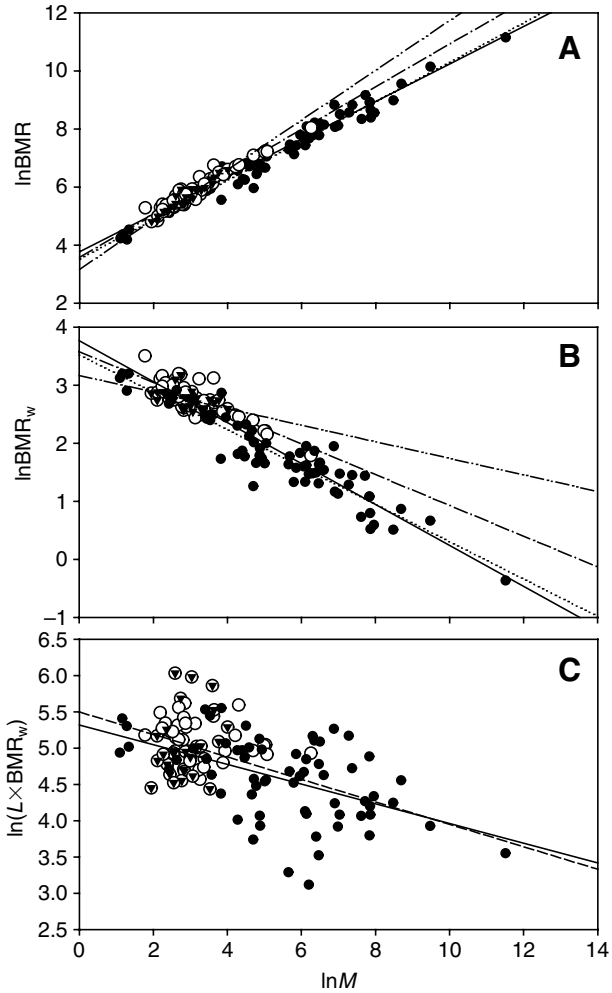

Fig. 3. The dependence of basal metabolic rates (BMR), mass-specific basal metabolic rate (BMR_w), and lifetime expenditure of energy ($L\times BMR_w$) on body mass (M) in songbirds (oscines) is different from that of all other birds. The oscines birds genera are indicated by open circles, and all other bird genera are indicated by solid circles; the oscines family Fringillidae (true finches) genera are indicated by inverted triangles. The regression lines for the whole Neognathae clade (N=123) are solid, the lines for all other birds (N=64) are dotted, the oscine birds lines (N=59) are dash–dot–dash, and the Fringillidae lines (N=28) are dash–dot–dot–dash. If the regression was not significant the line was omitted. (A) lnBMR as a function of ln$M$. For the whole clade, lnBMR=3.765+0.648×ln$M$ (r^2=0.966, P<0.001). For oscine birds, lnBMR=3.577+0.735×ln$M$ (r^2=0.934, P<0.001). For Fringillidae, lnBMR=3.167+0.857×ln$M$ (r^2=0.920, P<0.001); and for all other birds, lnBMR=3.519+0.678×ln$M$ (r^2=0.966, P<0.001). The slopes of the three curves were significantly different from each other. (B) $lnBMR_w$ as a function of ln$M$. For the whole clade, $lnBMR_w$=3.765–0.352×ln$M$ (r^2=0.893, P<0.001). For oscines, $lnBMR_w$=3.577–0.256×ln$M$ (r^2=0.648, P<0.001). For Fringillidae, $lnBMR_w$=3.167–0.143×ln$M$ (r^2=0.243, P=0.008). For all other birds, $lnBMR_w$=3.519–0.322×ln$M$ (r^2=0.866, P<0.001). The difference in slopes between oscines, –0.256±0.026, and all birds, –0.352±0.011, is highly significant (t=3.13, P=0.001), but the difference in intercept is not significant. (C) ln($L\times BMR_w$) as a function of ln$M$. For the whole clade, ln($L\times BMR_w$)=5.495–0.155×ln$M$ (r^2=0.323, P<0.001). For oscines, ln($L\times BMR_w$)=5.136–0.0139×ln$M$ (r^2=0.001, P=0.805). For Fringillidae, ln($L\times BMR_w$)=4.411+0.212×ln$M$ (r^2=0.066, P=0.188); and for all other birds: ln($L\times BMR_w$)=5.317–0.136×ln$M$ (r^2=0.245, P<0.001). The average value of ln($L\times BMR_w$) was significantly larger for oscines birds, 5.093±0.368, than that of all other birds 4.567±0.562 (t=6.082, P<0.001).

This is demonstrated in the following analysis of the birds' database (123 genera for which there are L, M and BMR_w values; Table S1 in supplementary material). Fig. 3A shows that for the whole class, BMR=$3.7658M^{0.648}$, but for oscine birds the scaling exponent is 0.735±0.0255, which is significantly larger than the scaling exponent for the class, 0.648±0.0111 (t=3.13, P=0.001). This difference in the scaling exponents can be attributed to the life history traits of the true finches family, Fringillidae (averages Scytb=0.139±0.001), which includes half of the oscines genera in this database. When plotted separately the scaling exponent for this family alone is very high, 0.857±0.049 and was significantly different from the scaling exponent of oscines (t=2.194, P=0.0154) and all other groups of birds. To see more clearly this difference between oscine birds, particularly true finches, and other birds we plotted the linear regression of BMR_w as a function of M (Fig. 3B). For all birds $BMR_w=3.76M^{-0.352}$, but when oscine birds were analyzed separately from other birds they exhibited significantly smaller scaling exponent, 0.256±0.026, compared to 0.322±0.016, for all other birds. The scaling exponent for the Fringillidae family alone was less than half of that of the whole clade, 0.143±0.049, and the correlation between BMR_w and M was very weak (r^2=0.243), suggesting that another variable contribute to the determination of BMR_w in this family. The dependence of L on M or BMR_w in oscine birds, and particularly in the Fringillidae family is also different from that of other birds: the correlation between L and BMR_w or M in oscine birds is much weaker than in other birds. In fact, within the Fringillidae family there was no correlation at all between L and M or L and BMR_w. However, in the oscine clade, which excludes the Fringillidae family, the correlation between L and M and L and BMRw was significant (result not shown). Apparently, within the Fringillidae family another variable determines L. This difference is also observed when we examine the lifetime expenditure of energy, $L\times BMR_w$ (Fig. 3C). The average value of $L\times BMR_w$ (179±77) in oscine birds is significantly larger (t=4.273, P<0.001) than that of the other birds group (106±45). This is partially because oscine birds are small and $L\times BMR_w$ is inversely related to M (power law 5). However, within the oscine birds group $L\times BMR_w$ does not show any dependence on M. Moreover, the average value of $L\times BMR_w$ of the Fringillidae family is not different from that of oscines birds (165±98, P=0.427) but the variability of the value of $L\times BMR_w$ within the Fringillidae is very large despite the narrow range of M values. The highest values of $L\times BMR_w$ in birds are exhibited by genera that belong to the Fringillidae

family. It is apparent that another variable, other than M, determines the value of $L\times BMR_w$ within this family.

We, therefore, examined the relationships between Scytb and life history traits in oscine birds, and particularly in the Fringillidae family, in comparison with all other neognaths. Within the Fringillidae family BMR_w was rather a weak function of M (Fig. 3B), and the residuals of BMR_w of the family genera (from the Neognathae power law $BMR_w=3.712M^{-0.337}$) were positively correlated with Scytb: residuals. $\ln BMR_w(M)=-1.244+8.74\text{Scytb}$ (N=16, r^2=0.406, P=0.008). In fact in this family both M and Scytb are independent variables that determine the value of BMR_w as shown by the multiple linear regression: $\ln BMR_w=2.304-0.157\times\ln M+6.37\text{Scytb}$ (N=16, r^2=0.706, P<0.001; Fig. 4A). Therefore, in this family, cytochrome b mutations were apparently selected to increase basal respiration and thus the increased BMR_w. As a result, within the true finches family L is not dependent significantly on either M or BMR_w but is dependent on Scyb: $L=-40+368\text{Scytb}$ (N=16, r^2=0.459, P=0.004).

The linear regression of L as a function of BMR_w in the Neognathae group of 59 genera that have Scytb, L, M and BMR_w values: $\ln L=3.73-0.513\times\ln BMR_w$ (N=59, r^2=0.417, P<0.001, Fig. 1D) is nearly the same as the linear regression for all birds: $\ln L=3.73-0.511\times\ln BMR_w$ (N=123, r^2=0.47, P<0.001). Similarly the dependence of L on M in this group: $\ln L=1.74+0.193\times\ln M$ (N=59, r^2=0.473, P<0.001, Fig. 1C) is nearly the same as in all birds: $\ln L=1.73+0.198\times\ln M$ (N=123, r^2=0.505, P<0.001). The dependence of the longevity residuals (L_o/L_p), from the Neognathae power laws (N=59), on Scytb, plotted separately for oscine birds, the Fringillidae family, and for all other neognaths is shown in Fig. 5B. It is observed that in oscine birds both the $L(M)$ residuals and the L(BMRw) residuals are positively correlated with Scytb. Within the Fringillidae family the dependence of the residuals on Scytb was much stronger than in the oscine clade (Fig. 4B). Without the Fringillidae family the residuals in the oscine clade showed a weaker dependence on Scytb, which was statistically significant only for the $L_o/L_p(M)$ residuals (not shown). In the Neognathae group that excludes oscine birds, exceptional longevity is negatively correlated with Scytb (Fig. 4B). Therefore in most orders of birds, in contrast to oscine birds, exceptional longevity actually decreases with increased Scytb. However, the negative scaling exponent is small compared to the positive scaling exponent of the oscines clade, suggesting only a weak dependence of the longevity residuals on Scytb in most orders of birds (see Discussion).

Fig. 4C show that the lifetime expenditure of energy is also a strong function of Scytb in oscine birds, and that this dependence is entirely due to the strong effect of Scytb on $L\times BMR_w$ in the Fringillidae family. Combining these results with the results of Fig. 3C clearly shows that whereas in all other birds $L\times BMR_w$ is a function of body mass, within the Fringillidae family it is only a function of Scytb. In summary, the results of Fig. 4 show that in the whole oscines clade the longevity residuals are positively correlated with Scytb, but

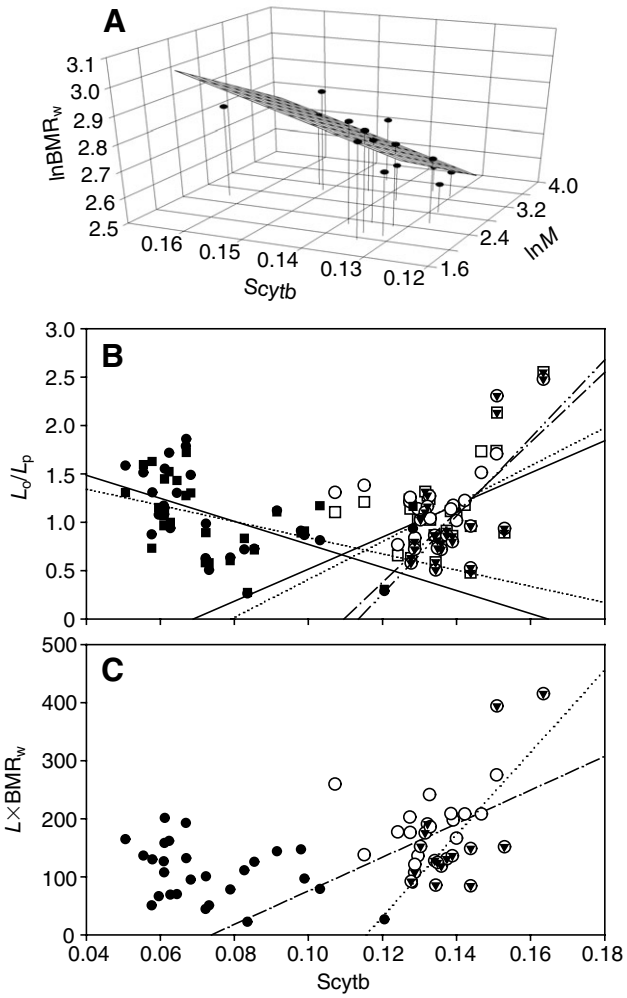

Fig. 4. Mass-specific basal metabolic rates (BMR_w), exceptional longevity (L_o/L_p), and lifetime expenditure of energy ($L\times BMR_w$) are function of the rate of cytochrome b evolution. Data are from Table S2 in supplementary material. (A) Mass-specific basal metabolic rates (BMR_w) are function of both body mass (M) and the rate of cytochrome b evolution (Scytb) in the Fringillidae family. Multiple linear regression of $\ln BMR_w$ as a function of both $\ln M$ and Scytb: $\text{Ln}BMR_w=2.304-0.157\times\ln M+6.373\times\text{Scytb}$ (N=16, r^2=0.706, P<0.001, VIF=1.07, $P(\ln M)$<0.001, P(Scytb)=0.002). (B) L_o/L_p as function of Scytb. Open symbols indicate oscines (N=31), inverted triangles indicate Fringillidae (N=16), and solid symbols indicate all other birds (N=28). L_o/L_p (BMR_w), the residuals from power law 1, are indicated by squares; L_o/L_p (M), the residuals from power law (3) are indicated by circles. For oscine birds $L_o/L_p(BMR_w)=-1.143+16.5\times\text{Scytb}$ (r^2=0.170, P=0.021, and $L_o/L_p(M)=-1.547+19.6\times\text{Scytb}$ (r^2=0.243, P=0.005). For Fringillidae; $L_o/L_p(M)=-3.964+36.2\times\text{Scytb}$ (r^2=0.42, P=0.007); $L_o/L_p(BMR_w)=-4.575+40.35\times\text{Scytb}$ (r^2=0.489, P=0.003). For all other birds: $L_o/L_p(BMR_w)=1.96-11.9\times\text{Scytb}$ (N=28, r^2=0.300, P=0.003) and $L_o/L_p(M)=1.676-8.37\times\text{Scytb}$ (r^2=0.186, P=0.022). (C) $L\times BMR_w$ as a function of Scytb. Symbols are as in B. For oscine birds $L\times BMR_w=-213+2893\times\text{Scytb}$ (r^2=0.171, P=0.021); for Fringillidae $L\times BMR_w=-817+7079\times\text{Scytb}$ (r^2=0.507, P=0.002); for all other birds $L\times BMR_w=164-778\times\text{Scytb}$ (r^2=0.104, P=0.095). The difference in $L\times BMR_w$ between oscines, 178.7±77, and all other birds 106±47 was significant (t=4.273, P<0.001).

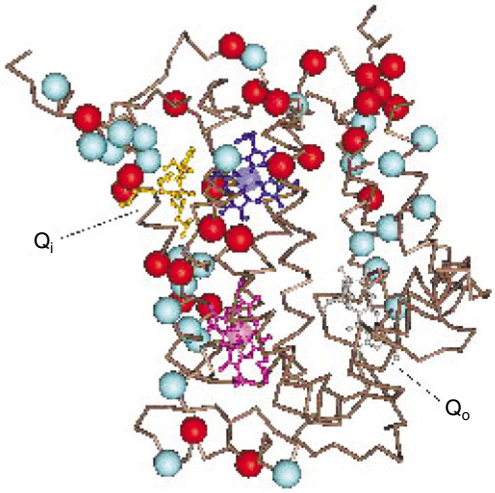

Fig. 5. The selected mutations in *Serinus* are concentrated on the matrix side of cytochrome *b* in the vicinity of the ubiquinone binding site Q_i. Sixteen complete or nearly complete cytochrome *b* sequences of species of the oscine genus *Serinus* were aligned and used to derive a consensus sequence, which was aligned with the sequence of the non-oscine genus *Empidonex*. The location of the substitutions was mapped onto the chicken cytochrome *b* structure (3BCC) (Zhang et al., 1998), which is shown as a three-dimensional model. The location of a conserved substitution is indicated by a blue ball on the protein backbone, and that of a non-conserved substitution is indicated by a red ball. The top of the structure faces the mitochondrial matrix and includes the Q_i ubiquinone binding site (with bound ubiquinone) and the b_H heme (blue). The bottom of the structure faces the intermembrane space and includes the Q_o ubiquinone binding site (with bound Stigmatellin) and the b_L heme (magenta).

only in the Fringillidae family is there also positive correlation between BMR_w and Scytb, and *L* and Scytb.

If the accelerated evolution of cytochrome *b* in birds involved positive selection of mutations, the sites and nature of these mutations should relate to their effect on the protein function. The results presented in Figs 3 and 4 suggest that in the Fringillidae family these mutations increase both BMR_w and *L* (hence also $L\times BMR_w$), most likely by increasing mitochondrial proton leak, which accelerates metabolic rate (Porter and Brand, 1993; Rolfe et al., 1999), and inhibits $\dot{V}_{ROS}$ (Korshunov et al., 1997). The Fringillidae genus *Serinus* has the highest values of Scytb (0.163), $L\times BMR_w$ (415), $L_o/L_p(M)$ (2.55) and $L_o/L_p(BMR_w)$ (2.48) of all birds (Table S2 in supplementary material), which suggests that in this genus the modulation of cytochrome *b* function is the most extensive. Indeed it has been shown that in canaries (*Serinus canaria*) the ratio between $\dot{V}_{ROS}$ and oxygen consumption is very low and involves inhibition of ROS generation at both complex I and cytochrome *b* (Herrero and Barja, 1998). Fig. 5 shows the sites of the cytochrome *b* substitutions in this genus, in comparison to the sequence of the Tyrannidae family (suboscine) genus *Empidonax* (Scytb=0.083). It is apparent that the substitutions are not distributed randomly in the protein. Most of the substitutions are on the mitochondrial matrix side of the protein and many of these are in the vicinity of the Q_i ubiquinone binding site. There are large domains of the protein with no substitutions at all whereas in others, e.g. helices A and E, which flank the Q_i binding site, approximately half of the residues have been substituted. Moreover, the substituted residues in *Serinus* are, on average, smaller and less hydrophobic than in *Empidonax*. This pattern supports the suggestion that most of these mutations were selected to accelerate the reduction of ubiquinone at the Q_i site (see Discussion).

Discussion

Lifetime expenditure of energy and the mitochondrial degree of coupling in birds

It is possible to explain the inverse relationship between maximal lifespan (*L*) and mass-specific basal metabolic rates (BMR_w) within the paradigm of the free radical theory of aging by postulating that $\dot{V}_{ROS}$ depends on BMR_w. If $\dot{V}_{ROS}$ was the same fraction of the rate of oxygen consumption in all genera of birds we would expect that $L=K/BMR_w$ or $L\times BMR_w=K$. In fact, in birds, the linear regression of *L* as a function of BMR_w exhibits a slope that is approximately half of that expected for reciprocal relationship (cf. Fig. 1D) and consequently $L\times BMR_w$ is not constant but is inversely related to body mass (power law 5 and Fig. 3C). The free radical theory of aging could accommodate this fact if the ratio $\dot{V}_{ROS}/BMR_w$ in birds with high BMR_w values was lower than the ratio in birds with low BMRw values. Since BMR_w is inversely related to body mass (power law 2), this is equivalent to the requirement that the ratio $\dot{V}_{ROS}/BMR_w$ would be lower in small birds than in large birds. In fact, it was reported recently that liver mitochondria from small birds have increased proton conductance, and are therefore less coupled, than liver mitochondria from large birds (Brand et al., 2003). A similar finding was reported earlier for mammalian liver mitochondria (Porter and Brand, 1993). Thus, to the extent that the degree of coupling of liver mitochondria represents mitochondrial coupling in all tissues, and that the small number of studied species represents these classes of animals, these data support the suggestion that in birds and mammals mitochondria of large animals are better coupled than mitochondria from small animals. All the small birds in the study of Brand et al. (Brand et al., 2003) were passerines, which as we show here, share exceptionally high rate of cytochrome *b* evolution, and it is therefore possible that the modulation of coupling in mtDNA-coded proteins contribute to the observed difference between small and large birds. However, it is quite likely that the pressure to reduce the mitochondrial degree of coupling or $\dot{V}_{ROS}$ resulted in selective adaptation of many other proteins, as well as changes of the mitochondrial membrane properties (Porter and Brand, 1993; Brand et al., 2003).

Additional support for the conclusion that the mitochondrial degree of coupling in small animals is lower than in large animals, could be obtained from studies of the relationships between metabolic rates (MR) and body mass. The scaling exponent β, for the power law $MR=cM^{\beta}$ is larger for maximal

metabolic rates, MMR, than for basal metabolic rates, BMR (~0.9 and ~0.7, respectively) (Bishop, 1999; Weibel et al., 2004). Since MMR is always higher than BMR [i.e. c(MMR) is larger than c(BMR)], the higher scaling exponent of MMR means that the ratio MMR/BMR is higher in large animals compared with small animals. The ratio MMR/BMR reflects to a large extent the ratio between oxygen consumption during intense exercise, when the rate of ATP synthesis (and consumption) is very high, and oxygen consumption during rest where the rate of ATP synthesis is minimal. This ratio therefore may reflect the mitochondrial respiratory control ratio, which is a classic indicator of the mitochondrial degree of coupling.

We, therefore, suggest that the fact that the scaling exponent b of power law 1 is less than 1 reflects the body mass dependence of the mitochondrial degree of coupling. Consequently the body mass dependence of $L\times BMR_w$ (power law 5) also results from the body mass dependence of the mitochondrial degree of coupling.

The dependence of $L\times BMR_w$ on M accounts for some, but not all, of the variability in $L\times BMR_w$ in birds (Fig. 3C). The residuals of $L\times BMR_w$ could result from taxon-specific modulation of mitochondrial coupling as demonstrated by the case of the Frangillidae family. In this family of small birds, $L\times BMR_w$ varies from 84 to 415, a fivefold range, but it is not dependent on M at all. In contrast, $L\times BMR_w$ depends on Scytb (Fig. 4C). Since mitochondrial uncoupling can increase both L and BMR_w, and in this family, BMR_w, L and $L\times BMR_w$ all depend on Scytb (Fig. 4A–C), it is likely that the increase of $L\times BMR_w$ within this family is due to a decrease in the mitochondrial degree of coupling as a result of the evolution of cytochrome *b* (and probably other mtDNA-coded proteins).

The results of this study suggest that BMR_w, and therefore also BMR, are not only a function of body mass but also a function of the mitochondrial degree of coupling. To the extent that mitochondrial coupling itself depends on body mass this dependence is already embedded in the power laws 2 and 3. However, mitochondrial coupling is not dependent only on body mass and there are large taxon-specific deviations from this dependence (cf. Dobson, 2003). The best example in birds is provided by the Fringillidae family in which the value of BMRw (and hence BMR), and presumably mitochondrial coupling, depend strongly on the rate of cytochrome *b* evolution (and presumably other mtDNA-coded proteins), independently of body mass (Fig. 4A). As a result, the scaling exponent of power law 3 in the Fringillidae family is 0.857, compared with 0.648 in all birds (Fig. 3A). Similar effects of the accelerated evolution of cytochrome *b* on the scaling exponent of power law 3 were observed in mammals (Rottenberg, 2007).

There is a longstanding controversy regarding the 'true' value of the scaling exponent of power law 3 and its biological significance (West et al., 2002; Darveau et al., 2002; White and Seymour, 2003). Our analysis suggest that in addition to many other factors (Darveau et al., 2002) the observed value is influenced by the body-mass dependence, as well as the taxon-specific evolution, of the mitochondrial degree of coupling.

A possible modulation of cytochrome b function in Serinus

It has been demonstrated previously that the exceptionally low rate of ROS generation in *Serinus canaria* resulted, in part, from inhibition of ROS generation in the bc1 complex (Herrero and Barja, 1998). In coupled mitochondria, the proton electrochemical gradient inhibits the oxidation of ubihydroquinine at the Q_o site by inhibiting the transfer of the second electron from the b_L heme, to the b_H heme, and the subsequent reduction of ubiquinone at the Q_i site (Crofts, 2004). The inhibition of this segment of the Q cycle increases the steady state concentration of the radical semiquinone at the Q_o site, which interacts with oxygen to generate superoxide (Demin et al., 1998). Therefore, the rate of ROS generation could be inhibited, in coupled mitochondria, by accelerating the reduction of ubiquinone at the Q_i site. A modulation of the dielectric environment surrounding Q_i, b_L and b_H may result in acceleration of this segment of the Q cycle. Acceleration may also result from increasing internal proton leak. If protons can find a leak path from the outer surface (where the proton electrochemical potential is high) to the inner surface the proton electrochemical gradient will decrease and the reduction of the quinone at the Q_i site will be accelerated. The distribution of substitutions in *Serinus* cytochrome b, and the fact that the substituted residues are, on average, smaller and less hydrophobic, is compatible with increased proton leak and/or an increase of the dielectric constant at the protein core (Fig. 5).

Adaptive selection and the evolution of cytochrome b in birds

The results of this study suggest that, similar to placental mammals, the evolution of the mtDNA-coded protein cytochrome *b* in birds is driven by adaptive selection. It is likely that, similar to mammals, the evolution of other mtDNA-coded proteins is driven by the same forces. However, there are significant differences between the evolution of cytochrome *b* in birds and placental mammals. The positive correlation between the residuals from power laws 1 and 4 and the rate of evolution of cytochrome *b* was observed in a large clade of placental mammals that included all ordinal level taxa except rodents (Rottenberg, 2007). In birds, this positive correlation was observed only in songbirds, a suborder of the order Passeriformes. However, one should point out that the songbirds (oscines) group is a very large and diversified clade, containing nearly half of extant birds species. In mammals, the positive correlations that are observed between exceptional longevity and the rate of evolution of cytochrome b, and other mtDNA-coded proteins, at the species level are stronger at the genera level, become stronger when analyzed at the averaged family level values, and even stronger when analyzed at the ordinal level (Rottenberg, 2007). However, there were no significant correlations between longevity residuals and the rate of cytochrome *b* evolution within most families. This suggests that the selection for reduced ROS generation begun shortly after the divergence of placental mammals ordinal taxa, coinciding with the explosive radiation of mammals, about 65 million years ago.

By contrast, in songbirds, the correlations between the rate of cytochrome *b* evolution and exceptional longevity are

positive and significant at the averaged genera level but not at the averaged families level. However, the correlations are strong within the families. This is true not only within the Fringillidae family (Figs 3, 4), but also within the Muscicapidae family where the correlation between the longevity residuals from power law 4 and Scytb is very strong: $L_o/L_p(M)$=–2.25+26.2Scytb (L_o, observed longevity; L_p, predicted longevity; N=5, r^2=0.865, P=0.024). This observation suggests that the selection for reduced $\dot{V}_{ROS}$ is relatively recent, and begun only after the divergence of the oscines families. There is some evidence that this selection continued after the divergence of species, at least in some genera. For example, in the genus *Carduelis* (golden finches), the longevity of *Carduelis carduelis* (European golden finch) is 27 years compared to an average of 12.7 years in nine species of this genus. However, *Carduelis carduelis* body mass, 16 g, is the same as the genus average (15.5 g). That means that this species has exceptional longevity, not only relatively to all other birds, but also relatively to its sister species. The cytochrome *b* sequence of *Carduelis carduelis* has 10 nonconservative substitutions from the consensus sequence of the genus. No other *Cardulelis* species show more than two or three substitutions from the consensus (results not shown).

Outside the oscines clade, there are taxa that evolved to reduce ROS by a different mechanism. For example, the Psittacidae family exhibits an average Scytb that is only slightly higher that most other non-oscine Neognathae families, but exhibits positive $L(M)$ residuals for all its genera. However, the $BMR_w(M)$ residuals of all the genera of this order are all negative (results not shown). Thus, reduced $\dot{V}_{ROS}$ appears to result from reduction of BMR_w and not from mitochondrial uncoupling. Indeed, it was found that a species of this order has reduced $\dot{V}_{ROS}$, which is the result of a slow rate of oxygen consumption and not reduced $\dot{V}_{ROS}/BMR_w$ ratio (Herrero and Barja, 1998).

If the selection for reduced $\dot{V}_{ROS}$ is relatively recent, what was the driving force for cytochrome *b* evolution before the divergence to families? It appears that the unexpected answer to this question is that, in modern birds, in general, early evolution of cytochrome *b* selected mutations that increased ROS production. The longevity residuals in neognaths, when oscines are excluded, are inversely correlated with the rate of cytochrome *b* evolution (Fig. 4B). Unlike the positive correlations in oscines, these negative correlations, in other neognaths, are stronger on the family average level, but they are not significant within families (results not shown). This suggests that, early, but slow, evolution of cytochrome *b* involved adaptive selection that increased $\dot{V}_{ROS}$. Most likely the selection was for increased mitochondrial coupling, perhaps to allow birds to travel longer distances on a limited amount of stored fat.

We therefore suggest the following narrative for the evolution of mtDNA-coded proteins and the mitochondrial degree of coupling in birds: as birds evolved from reptiles and developed endothermic metabolism they increased their metabolic rates to increase heat production by various means including a reduction of their mitochondrial degree of coupling. The latter apparently did not happened in the endothermic evolution of mammals hence the generally higher metabolic rates and longevity in birds compared to mammals. Later, during the early evolution of modern birds, they perfected their flight capabilities and consequently many bird species selected mtDNA mutations that slightly increased their mitochondrial degree of coupling in order to improve their metabolic efficiency during flight. However, more recently, during the evolution of oscine birds, and particularly the small size birds of the Fringillidae family, many species reverse this trend, and selected mtDNA mutations that decreased the mitochondrial degree of coupling further, most likely to increase heat production (which was necessary when they decrease their body size), and possibly also to reduce $\dot{V}_{ROS}$ in order to increase longevity and/or to enable improved cognitive functions. Larger birds, which did not need to increase heat production (e.g. Psittacidae), reduced ROS production to increase longevity and/or cognitive function by lowering their metabolic rates.

Long-term memory, learning and ROS generation

A prerequisite for exceptional cognitive abilities is a large capacity for long-term memory (Fagot and Cook, 2006). Although the mechanism of the formation and maintenance of long-term memory is not yet clearly understood there is enough evidence to suggest that excess ROS production interferes with the formation and maintenance of long-term memory (Auerbach and Segal, 1997). There is also evidence that the age-dependent decline in cognitive functions in humans and non-human animals result from the increased damage to proteins, DNA and membrane lipids, from mitochondrial ROS (Beckman and Ames, 1998; Barja, 2004). It is therefore likely that ROS generation by mitochondria constrains the development of exceptional cognitive abilities. Although exceptional cognitive abilities appear to have evolved independently in several mammalian and bird taxa there is often convergence in aspects of brain anatomy, physiology and specific protein functions in these taxa (Emery and Clayton, 2004; Scharff and Haesler, 2005; Bolhuis and Gahr, 2006).

Anthropoid primates, elephants, whales and dolphins share exceptional cognitive abilities, and exceptional sociality. They also share exceptional longevity (i.e. positive longevity residuals from power laws 1 and 4), exceptionally high mass-specific basal metabolic rates (i.e. positive residuals from power law 2), and accelerated evolution of mtDNA-coded proteins (Rottenberg, 2007). The latter association suggests that they also share low $\dot{V}_{ROS}$.

In birds, the Psittacidae family (parrots) combines exceptional cognitive functions with exceptional longevity (i.e. positive residuals from power law 4, data not shown), and low rate of ROS generation (Herrero and Barja, 1998). The Corvidae family, which belongs to the oscines branch of the passerines, also combine exceptional longevity (positive residuals from power laws 1 and 4), and exceptionally high lifetime expenditure of energy, with exceptional cognitive ability (Emery and Clayton, 2004). Songbirds that exhibit exceptional capacity to memorize songs (Bolhuis and Gahar, 2006), e.g. *Serinus canaria*, exhibit exceptional longevity and

low rate of ROS generation (Herrero and Barja, 1998), which are also associated with exceptionally high mass-specific basal metabolic rates and accelerated evolution of cytochrome *b* (this study). Therefore, we suggest that, in mammals and birds, there is a convergence in the evolution of mitochondria with reduced rates of ROS production in taxa with high cognitive abilities.

List of abbreviations

BMR	basal metabolic rate
BMR_w	mass-specific basal metabolic rate
L	longevity (maximal lifespan)
$L \times BMR_w$	lifetime energy expenditure
L_o/L_p	ratio of observed longevity to predicted longevity
M	body mass
MMR	maximal metabolic rate
MR	metabolic rate
MtDNA	mitochondrial DNA
Q_i, Q_o	ubiquinone binding sites
ROS	reactive oxygen species
Scytb	cytochrome *b* substitution per site
$\dot{V}_{ROS}$	rate of generation of reactive oxygen species

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