

Interspecific Associations between Circulating Antioxidant Levels and Life-History Variation in Birds

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ABSTRACT: Antioxidants play an important role in protecting tissues against aging-associated oxidative damage and are thus prime candidates for relating physiological mechanisms to variation in life histories. We measured total antioxidant capacity, antioxidant response to stress, and levels of uric acid, vitamin E, and four carotenoids in 95 avian species, mostly passerines from Michigan or Panama. We compared antioxidant measures to seven variables related to life histories (clutch size, survival rate, incubation period, nestling period, basal metabolic rate, body mass, and whether the species lived in a tropical or temperate climate). Life-history-related traits varied over at least three statistically independent axes. Higher antioxidant levels were generally characteristic of more rapid development, lower survival rate, smaller body size, larger clutch size, and

higher mass-adjusted metabolic rate, but the relationships of particular antioxidants with individual life-history traits showed considerable complexity. Antioxidant–life history associations differed between tropical and temperate species and varied with respect to taxonomic sampling. Vitamin E showed few relationships with life-history traits. Overall, our results partly support the hypothesis that antioxidant levels evolve to mirror free radical production. Clearly, however, the complex patterns of physiological diversification observed here result from the interplay of many factors, likely including not just investment in somatic maintenance but also phylogenetic constraint, diet, and other aspects of ecology.

Keywords: antioxidant, life history, carotenoid, survival, metabolism, uric acid.

Current theories about the evolution of aging and life span are mostly related to the disposable soma theory (Kirkwood 1977) and the related antagonistic pleiotropy theory (Williams 1957), which are both based on the optimal allocation of limited resources between physiological functioning, reproduction, and survival early and late in life. According to these theories, aging rate and its physiological mechanisms should evolve through the response of populations to selective factors in the environment, particularly the relative contribution to fitness of offspring produced early in life versus those produced later in life. A number of potential physiological mechanisms of aging have now been identified, most notably the free radical theory (Harman 1956), and many studies in model organisms have examined the relationships between these mechanisms and aging. However, few studies have examined variation in such a mechanism across a sample of many species (but see Haussmann et al. 2003).

Independently of the development of aging theory, ornithologists have endeavored to explain variation in life-history patterns of birds, focusing in particular on differences between temperate and tropical species in clutch size, growth rate, and survival rate (e.g., Moreau 1944; Lack 1948; Skutch 1949; Ricklefs 2000; Martin 2004). A number of theories have been proposed to explain these differences, from food limitation to variation in nest predation. Martin

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(2002) has suggested that much of the variation in life histories can be explained by risk of predation (either on adults or on offspring), which is consistent with both the disposable soma theory and the observed association between survival rates of young adults and the rate of aging (Ricklefs 1998). Recently, studies have also emphasized the importance of physiological variation as a determinant of life-history variation. Endocrine signaling pathways and immune functional capacities may respond to selection on life-history patterns, but they also constrain variation in life histories (Wikelski et al. 2000; Ricklefs and Wikelski 2002; Tieleman et al. 2004).

A study on variation in antioxidants across avian species in relationship to life-history variation has the potential to link aging and life-history research programs and to answer fundamental questions about the role of physiological evolution in determining broad patterns of variation in aging and life histories. Antioxidants protect against free radical damage (Beckman and Ames 1998; Halliwell 2000), which is the best-studied and most widely accepted aging mechanism to date, though there is still considerable debate as to its relative importance (e.g., Hulbert et al. 2007). Antioxidants also regulate various aspects of immune function (Surai 2002), and some antioxidants, notably carotenoids, are involved in secondary sexual traits and are supplemented in egg yolk in birds (Surai et al. 2001; McGraw and Ardia 2003). These relationships have generated considerable interest in the role that antioxidants might have in ecological and evolutionary contexts (e.g., Hōrak et al. 2006; Costantini et al. 2007). Recent studies of birds have examined associations between antioxidant defenses and immune function, development rate, stress, and individual quality (Costantini and Dell’Omo 2006; Alonso-Alvarez et al. 2007; Cohen et al. 2007; Isaksson et al. 2007). For measures of overall antioxidant capacity, results have been mixed; for example, antioxidant levels sometimes increase and sometimes decrease in response to an immune challenge (Costantini and Dell’Omo 2006; Hōrak et al. 2007). Such contradictory results preclude a framework for expected results in such experiments or for placing antioxidant capacity in a larger context. Our goal here was to relate antioxidants, including both carotenoids and other circulating micromolecules, such as uric acid and vitamin E, to life-history variation in birds in a broad comparative survey.

Exceptional variation in antioxidant capacity is not wholly unexpected in animals, given the complexity of antioxidant systems. Most endogenous free radical production occurs in the mitochondria, especially at complexes I and III, where enzymatic antioxidants such as superoxide dismutase are the primary defense (Brand et al. 2004; Huang and Manton 2004). Micromolecular antioxidants, such as vitamins C and E, carotenoids, and uric

acid, also have important roles, both in mitochondria and in circulating systems, where, for example, they protect against lipid oxidation that can lead to atherosclerosis (Neuzil and Stocker 1994). In general, antioxidants receive extra electrons from radicals, neutralizing the radical, but each antioxidant has a somewhat different function depending on its solubility (water vs. lipid) and reactivity with different types of free radicals. Some antioxidants can become pro-oxidant in some biochemical contexts, depending on the availability of other antioxidants (Surai 2002).

Experimental studies have pointed to the importance of controlling free radical protection in the electron transport chain, suggesting that antioxidants are a second line of defense and that effective control is more a function of lowering membrane gradients than upregulating antioxidants; in fact, high antioxidant levels can reflect less control of free radical production (Barja 2004; Brand et al. 2004). Such overall patterns need not be reflected in the levels of all components of the system; for carotenoids in particular, dietary limitation and highly specific functions—perhaps biochemical signaling as well as antioxidant protection (Hartley and Kennedy 2004)—suggest that patterns might differ from overall patterns of antioxidants. Birds excrete excess nitrogen via uric acid and can thus tolerate high levels of uric acid. It has been proposed that antioxidant properties of uric acid could account for the exceptional life spans of birds relative to mammals (Holmes et al. 2001), and several studies have purported to show antioxidant benefits of high uric acid levels in domestic fowl (e.g., Simoyi et al. 2002; Machin et al. 2004).

The few comparative studies on antioxidants have used a small number of species, often from different classes such as birds and mammals, on the assumption that the species chosen were representative (e.g., Lopez-Torres et al. 1993; Pérez-Campo et al. 1994). Unfortunately, strong inferences based on such distantly related species are problematic because physiology differs systematically and because there are not enough vertebrate classes to test hypotheses of antioxidant variation or to control for numerous confounding factors. Thus, studies that examine patterns of antioxidant protection across more closely related species in the context of life-history variation are clearly needed. It is not immediately apparent how closely the species should be related, so ideally the relationships could be tested at different taxonomic levels, such as within classes and orders. There is no reason to expect that relationships that are apparent at one taxonomic level can be generalized to others. Also, such patterns could vary with ecology, differing, for example, in relation to diet or climate. Thus, many factors could complicate relationships among antioxidants and life histories, including phylogeny, ecology,

and potential multidimensionality of both antioxidant variation and life-history variation.

We measured 13 antioxidant parameters in blood samples from 95 bird species and compared them to measurements of metabolic rate, clutch size, incubation period, nestling period, survival rate, and body mass. These data were collected as part of a large collaborative effort to understand avian physiology and life histories in the context of tropical-temperate differences; this study confines itself to antioxidant-specific aspects of that project. Avian life histories (and metabolic rate and mass) are generally considered to be distributed along a single strong axis of variation, often characterized by the extremes of “live fast, die young” versus “live slow, die old,” where the pace of life is closely associated with rate of reproduction and expected life span. Thus, birds with low survival rates tend to rear more offspring per nesting attempt, grow faster, and have higher metabolic rates and smaller body size (Wikelski and Ricklefs 2001; Ricklefs and Wikelski 2002). Differences in life histories of tropical and temperate bird species are well known (Martin 1996; Ricklefs and Wikelski 2002). Basal metabolic rate (BMR) is lower in tropical species than in temperate species (Wiersma et al. 2007), whereas survival rate patterns are less clear (Brawn et al. 1999; Ricklefs and Shea 2007). We sampled widely among both Neotropical and North American temperate species and incorporated the effect of climate zone both as a life-history covariate and as an environmental predictor.

Here, we attempt to place variation in serum antioxidant levels within the more general context of life-history variation. We hypothesized that higher antioxidant levels would be characteristic of birds with live fast–die young life-history strategies, confirming a secondary role for antioxidants in defense against free radical damage such that antioxidant levels tend to track free radical production levels. In particular, we expected high mass-adjusted metabolic rate to increase free radical production and thus to favor higher antioxidant levels (but see Ricklefs et al. 1996; Speakman et al. 2004). Among the antioxidants measured, vitamin E has the most clearly established role in free radical defense, so we expected antioxidant–life history associations to be clearest and strongest between vitamin E and BMR. Also, greater parental investment (proxied here by lower survival, larger clutches, and perhaps faster development) is associated with greater oxidative stress and thus should result in higher antioxidant levels (Alonso-Alvarez et al. 2004; Wiersma et al. 2004). However, knowledge of antioxidant systems in this context was insufficient for more detailed predictions, and the larger goal of this study was to characterize how antioxidants and life histories covary across evolutionary time. We thus describe the consistency and generality of antioxidant–life history relationships, attempt to determine which life-

history variables may be driving these patterns, and test the generality of our results across samples that are stratified ecologically (tropical vs. temperate) and phylogenetically (nine-primaried oscine, passerine, neoaves). We present several complex sets of results, which we believe represent an equally complex biological reality that defies either broad generalization or simple presentation.

Methods

Collection of Avian Serum

We collected blood samples from wing veins of 744 birds from 95 species using nonheparinized microcapillary tubes (supplements A1, A2 in the online edition of the *American Naturalist*). Samples were centrifuged in a Zip-Spin portable centrifuge (LW Scientific), and serum was removed and kept on ice (1–6 h) until it could be frozen at -80°C . To assess potential effects of stress on antioxidant capacity, two samples were taken from each of 255 individuals, the first within 5 min of capture and the second after the subject had been held for 1 h in a cloth bag. Such stress causes large changes in all antioxidant parameters, but the direction and magnitude of the changes vary across both species and antioxidant type (Cohen 2007; Cohen et al. 2007).

Ninety-one (96%) of our study species were small forest and forest-edge species, mostly passerines (order Passeriformes), caught in mist nets in Panama or Michigan (suppl. A1). Netting was conducted at several locations in and around Gamboa, Panama, in March 2004 and March 2005, and at Kellogg Biological Station near Kalamazoo, Michigan, in June and July 2004 and July 2005. Tropical species sampled were all lowland species. The additional four species were savannah sparrows (*Passerculus sandwichensis*) and tree swallows (*Tachycineta bicolor*) sampled on Kent Island, New Brunswick, Canada, from June 18 to 25, 2005; Florida scrub jays (*Aphelocoma coerulescens*) caught at Archbold Biological Station, Lake Placid, Florida, throughout 2005 but mostly in January and February; and house sparrows (*Passer domesticus*) caught in Princeton, New Jersey, from September 1 to 5, 2005. In addition, one blue jay (*Cyanocitta cristata*), two northern cardinals (*Cardinalis cardinalis*), two eastern towhees (*Pipilo erythrophthalmus*), and 11 gray catbirds (*Dumetella carolinensis*) were caught in Princeton with the house sparrows, though these species are represented in greater number in the sampling from Michigan and Panama. Full antioxidant data on all individuals are provided in supplement A2.

Two species—the house wren (*Troglodytes aedon*) and the yellow warbler (*Dendroica petechia*)—have distinct subspecies in Panama and Michigan, and they show clear differences in most of the parameters we measured. We

thus include the subspecies separately in this analysis, providing a total of 97 taxa for analysis.

We attempted to minimize overlap of individuals for the four data collection programs incorporated into this study (blood sampling, metabolic measurements, mark-recapture studies, and observation of nesting success), despite use of the same study sites. We had less than 2% overlap between any two programs of the study. In all cases, efforts were made to sample during the breeding season, but degree of seasonality differs between tropical and temperate environments, and many individuals were of unknown breeding status. Appendix A in the online edition of the *American Naturalist* provides detailed methods for quantification of total antioxidant capacity, uric acid, vitamin E, carotenoids, survival rate, BMR, clutch size, incubation period, and nestling period, all of which follow generally accepted methods (e.g., Brawn et al. 1999; McGraw and Parker 2006; Cohen et al. 2007; Wiersma et al. 2007). Total antioxidant capacity is a measure of serum capacity to quench free radicals, and it detects the effects of micromolecular (rather than enzymatic) antioxidants (Cohen et al. 2007). Survival and metabolic rate data for tropical species were mostly measured directly for this study, but data for temperate species were taken mostly from published studies that used methods consistent with ours (e.g., the Monitoring Avian Productivity and Survivorship [MAPS] database for survival). Methods for data supplementation from the literature and phylogeny construction are also provided in appendix A; the phylogeny we used is provided in Newick format there. We attempted to use maximum recorded life span as a life-history variable but found that systematic bias in sampling intensity across species made it unsuitable for inclusion here.

Data Analysis

Total antioxidant capacity (TAC) and concentrations of uric acid, vitamin E, and all individual carotenoids were log transformed to improve normality. Three of the carotenoids—zeaxanthin, β -cryptoxanthin, and β -carotene—were lognormally distributed only when “0” values were excluded. Zero values indicate levels below the lower detection limit of 0.01 mg/L; accordingly, they were converted to 0.01 and log transformed with the rest of the data. Four carotenoids were present in enough species (>30 ; all other carotenoids ≤ 2 species) to be considered individually in these analyses: lutein, zeaxanthin, β -cryptoxanthin, and β -carotene. Total carotenoid concentrations were calculated, but results paralleled those for lutein, by far the most abundant carotenoid; given the considerable variation in patterns among carotenoids, total concentration is not an informative measure of overall variation (see Cohen 2007). Carotenoid number is the average

number of carotenoid types detected in the serum of each species. Overall variation in carotenoids is best represented by a carotenoid factor, a factor axis describing 70% of the variation in the four carotenoid types and carotenoid number, with factor loadings from 0.65 to 0.96 (Cohen 2007). Individual carotenoids show considerable heterogeneity in the analyses here; these results are presented in the online edition, and only the factor is used here.

Uric-acid adjusted TAC residuals (Res) were calculated following Cohen et al. (2007) and represent antioxidant capacity resulting from compounds other than uric acid. The response of uric acid (Δ UA) and TAC (Δ TAC) to stress was calculated as the logarithm of the value at 60 min minus the logarithm of the initial value on capture. Stress response of the residual (Δ Res) was calculated by subtracting the baseline value from the poststress value. In the context of this article, Δ UA and Δ Res behave similarly to Δ TAC, so their results are presented only in the article appendixes in the online edition. There were few sex differences in antioxidant levels within species, and the differences that were found showed no obvious patterns (Cohen 2007). Sex differences in life-history variables were generally unavailable and are not considered in this study.

All life-history variables except clutch size, survival rate, and climate zone were log transformed for normality. Clutch size was not normally distributed, due in part to the large number of tropical lowland species with a clutch size of 2, and was left untransformed. We distinguished climate zone in this analysis as a binary variable (1 = tropical; 0 = temperate). Because categorical variables can be problematic in multivariate analyses, analyses were checked by omitting climate zone and by using separate analyses for tropical and temperate species.

Phylogeny was accounted for with phylogenetic regressions (bivariate and multivariate) run in SAS using the v0.7 implementation provided by Grafen (1989, 2006). Intraspecific variation in antioxidants is high (Cohen 2007), and this could obscure patterns across species but not bias results. Variation within species (between sexes, across seasons, in relation to individual quality) will be addressed in forthcoming articles by our research group; here, we make the assumption that the species means are not overly biased by our sampling method or intraspecific variation and work only with these average values.

Multivariate Analyses

Principal components analysis (PCA) from the correlation matrix of transformed variables was used to examine associations among life-history variables (using the “princomp” function with R software v2.5.0 [R Development Core Team 2007]). We calculated a simple correlation matrix for all variables and factors using the CORR procedure

in SAS. Missing data prevented use of stepwise regression, a technique that also has been criticized as biased and unstable (Harrell 2001). As an alternative, we used bivariate partial correlations (SAS, GLM procedure, Type III sums of squares) to examine the dependence of antioxidant–life history associations on other life-history variables. We show these relationships in detail in appendix B in the online edition of the *American Naturalist*. We also developed a novel graphical representation for the full set of bivariate partial correlations, an association matrix with each association strength (r value) represented by an ellipse such as would be drawn around a field of points on a graph (R v.2.5.0, “ellipse” package; see fig. 1). Suites of associated variables can be identified because their uncontrolled correlations disappear when controlling for each other but not when controlling for other variables. For example, if variables A , B , and C were all tightly correlated with each other but not with D , the A – B correlation would disappear when we controlled for C but not when we controlled for D . Also, interactions and residual effects can be identified because the effect after controlling for a variable is stronger or in the opposite direction of the uncontrolled correlation. For example, if A and B were not correlated except when we controlled for C , then it would be the residual of B on C that correlates with A (or vice versa). Although this method does not incorporate models controlling simultaneously for multiple variables, we believe that in most cases, the covariance structure and dependency of a variable set can be accurately and intuitively understood with such a figure. It should also not suffer from the bias or instability of stepwise regression, and it is not affected by missing data (assuming that the data are missing at random).

Multiple Testing Issues

We performed a large number of statistical tests in this study. Whenever multiple tests are calculated, some are inevitably significant individually just by chance. On average, one out of 20 analyses will appear significant at $\alpha = 0.05$ even in the absence of an underlying pattern. In contrast, overcorrection for multiple testing can lower significance levels so far that many real patterns are obscured. For example, we present 126 simple correlations between antioxidant variables and life-history variables. The Bonferroni correction here would lower our $\alpha = 0.05$ significance threshold to 0.0004. Only five of 126 correlations are significant at $\alpha = 0.0004$, but 45 of them are significant at $\alpha = 0.05$ —far more than the 6.3 expected by chance. Moreover, inspection of these values (fig. 2) shows that they are not randomly distributed—mass tends to have more associations than other life-history variables, and TAC and UA tend to have more

associations than other antioxidant variables. These patterns strengthen our conclusion that these associations are real, not statistical artifacts. Thus, we do not formally correct for multiple testing issues but present our data with the caveat that some “significant” results might have been produced by chance.

More generally, our conclusions do not rely on the significance of any particular test but rather are based on the patterns of which results are significant and are confirmed by multiple analytical techniques and subsampling of data for various analyses. Thus, all P values presented here should be interpreted as indications of relative level of support and not as significance tests with a threshold. The approach we use here is favored for analysis of large, complex data sets, where formal correction for multiple testing is often inappropriate (Savitz and Olshan 1995; Perneger 1998). Hypothesis testing is not necessarily the most appropriate framework for such studies, especially when the goal is exploration or characterization of broad patterns rather than testing of specific relationships. In this case, the nature of complexity in the associations among antioxidants and life-history characters is a more important result than any specific relationships. However, this complexity is not a hypothesis that can be assessed with a single statistical test, and its qualitative characterization relies on synthesis of various lines of evidence.

Results

Principal Components Analysis

Complete data for all life-history variables were available for only 31 species, but excluding BMR and survival, we had complete data for 94 species. To examine robustness of patterns of subsampling and sample size, we generated principal component axes in four ways: (1) including BMR and survival rate ($n = 31$), (2) including survival rate but not BMR ($n = 58$), (3) including BMR but not survival rate ($n = 50$), and (4) including neither ($n = 94$; table 1). The analysis of all variables showed that clutch size, climate zone, and incubation period were tightly associated but nearly orthogonal to mass. Survival rate was orthogonal to BMR, and nestling period was only weakly associated with the first two axes (fig. 3). Tropical and temperate species partitioned cleanly on the plot. Three axes had eigenvalues greater than 1, together explaining 85% of the variance (table 1). The first represented small clutch (being from the tropics) and to some extent long incubation, high survivorship, and low BMR. The composition of the second and third axes changed somewhat depending on the inclusion of BMR in the model, probably because of the species composition of the subsample, but one axis was always strongly associated with body mass and the

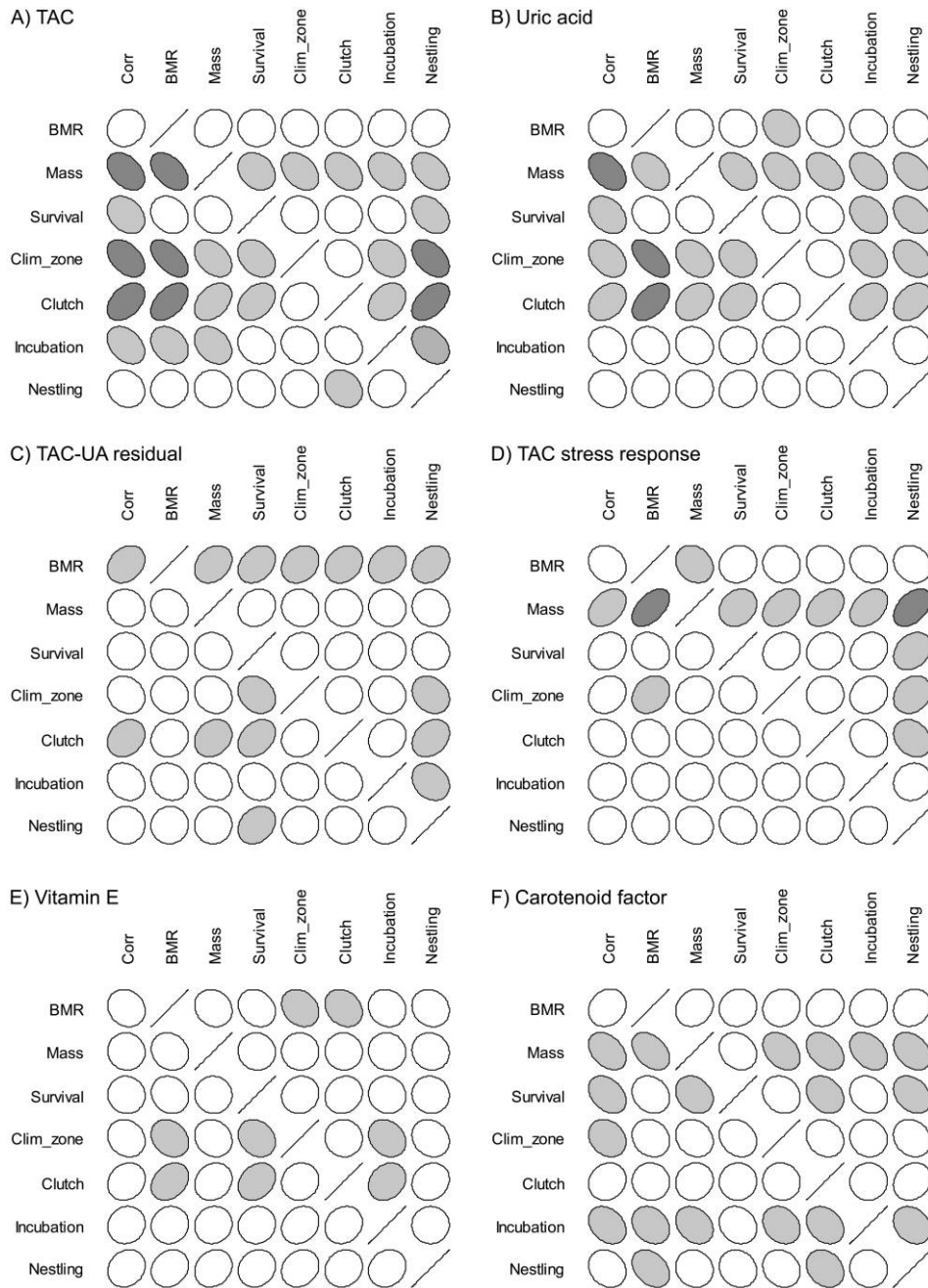


Figure 1: Associations of life-history variables with antioxidant variables, controlling individually for other life-history variables: total antioxidant capacity (*TAC*; A), uric acid (*UA*; B), *TAC-UA* residual (C), Δ *TAC* (D), vitamin E (E), and carotenoid factor (F). The basal metabolic rate (*BMR*) is mass adjusted. Each association strength (*r* value) is represented by an ellipse as would be drawn around a field of points on a graph. Narrower ellipses represent stronger associations; downward-sloping and upward-sloping ellipses show negative and positive associations, respectively. Darker shading also represents stronger associations. Each matrix shows a single antioxidant as a dependent variable. The first column of each matrix shows the Pearson correlation between the row life-history variable and the antioxidant variable for that matrix; each other column shows the partial correlation after controlling for the column variable. A row in which all associations are identical indicates that the row variable is associated with the antioxidant independent of all other life-history variables; a column with no associations shows that controlling for that variable makes all other associations disappear. This figure does not incorporate information on statistical significance; for more detail, see tables B7–B19 in the online edition of the *American Naturalist*. *Mass* = body mass, *Survival* = survival rate, *Clim zone* = climate zone, *Clutch* = clutch size, *Incubation* = incubation period, *Nestling* = nestling period, and *Corr* = correlation.

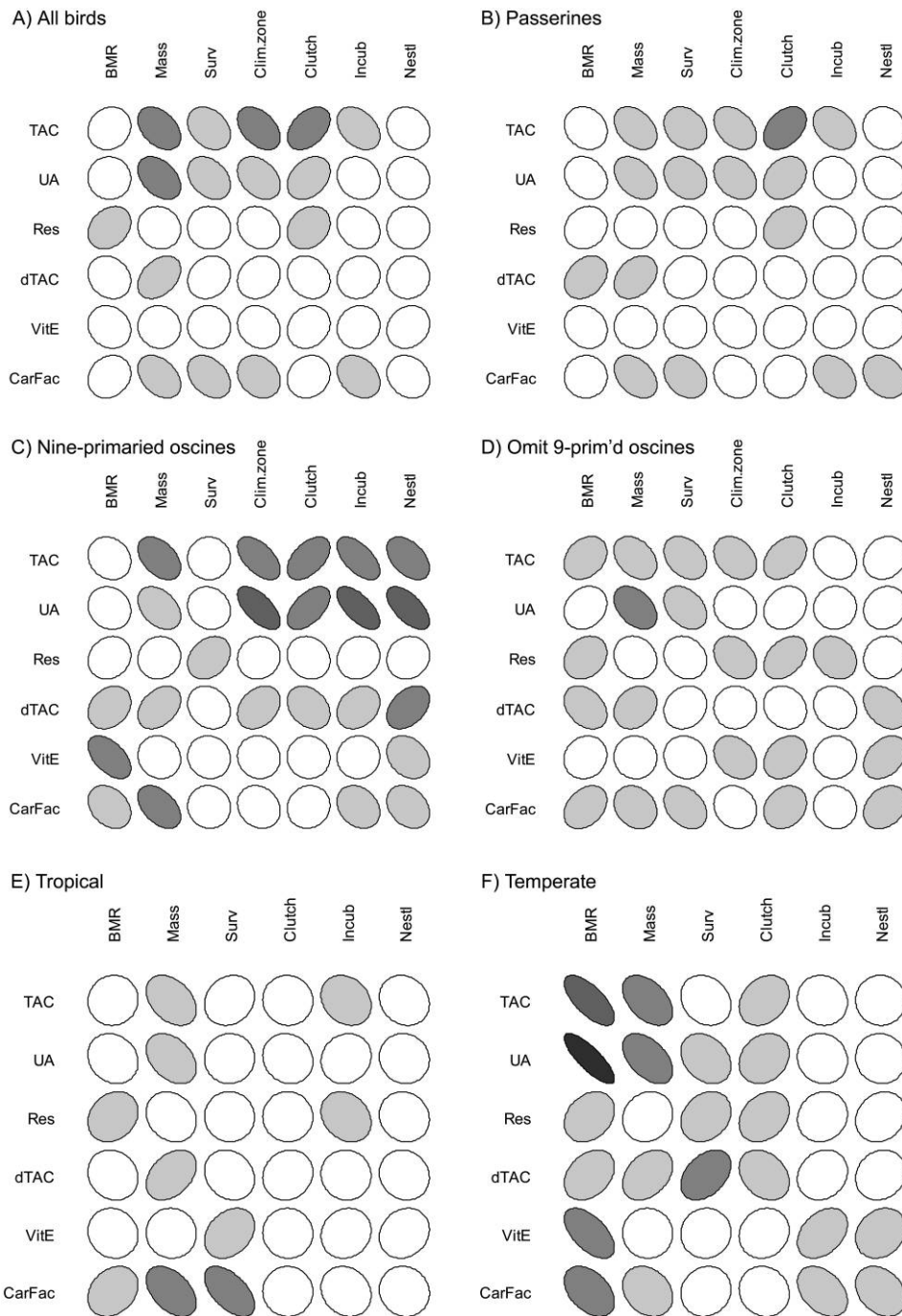


Figure 2: Correlations among main antioxidant and life-history variables. including all 97 taxa (A), only passerines (B), only nine-primaried oscines (C), omitting the nine-primaried oscines (D), only tropical species (E), and only temperate species (F). The basal metabolic rate (BMR) is mass adjusted. Each Pearson correlation (r) is represented by an ellipse as would be drawn around a field of points on a graph. Narrower ellipses represent stronger associations; downward-sloping and upward-sloping ellipses show negative and positive correlations, respectively. Darker shading also represents stronger correlations. This figure does not incorporate information on statistical significance; for more detail, see tables B1–B6 in the online edition of the *American Naturalist*. TAC = total antioxidant capacity, UA = uric acid, Res = UA-adjusted TAC residuals, dTAC = response of TAC to stress, VitE = vitamin E, CarFac = carotenoid factor, Mass = body mass, Surv = survival rate, Clim zone = climate zone, Clutch = clutch size, Incub = incubation period, and Nestl = nestling period.

Table 1: PCA loadings (eigenvectors) for first three axes using four life-history models

	Model with survival rate			Model without survival rate		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Model with BMR:						
<i>N</i>	94	94	94	50	50	50
Variance explained	46%	21%	18%	39%	24%	18%
Clutch size	-.51	-.07	.16	-.55	.34	-.15
Climate zone	.52	-.05	-.03	.59	-.15	.05
Incubation period	.40	-.13	.50	.43	.47	-.17
Survival rate	.42	.31	.01
Body mass	.12	.78	-.05	.17	.15	.85
BMR	-.34	.52	.06	-.37	-.01	.48
Nestling period	-.08	.10	.84	.05	.79	.01
Model without BMR:						
<i>N</i>	58	58	58	33	33	33
Variance explained	50%	23%	16%	47%	27%	18%
Clutch size	-.51	.33	.12	-.53	.42	-.21
Climate zone	.52	-.15	.13	.59	-.24	.05
Incubation period	.46	.06	.51	.54	.23	-.33
Survival rate	.46	.20	-.25
Body mass	.21	.52	-.66	.13	.45	.87
Nestling period	.02	.75	.46	.26	.72	-.30

Note: BMR = basal metabolic rate, PCA = principal components analysis, and PC = principal component. Bold indicates eigenvectors > 0.3.

other with long nestling period (table 1). If we remove body mass and nestling period from the analysis, the first axis describes 63% of the variation.

Correlations between Antioxidants and Life-History Traits

Antioxidant measures often correlated significantly with life-history variables such that large, long-lived, slow-reproducing, and slow-growing species had lower antioxidant levels (fig. 2A; table B1). Five of seven life-history variables were correlated with TAC. Vitamin E was not significantly correlated with any life-history parameter. In the full data set, the direction of associations for all variables was consistent with the overall pattern; all correlations between antioxidants and life-history variables were negative, except for those with clutch size and mass-adjusted BMR, which were always positive. Antioxidant stress response was always negatively associated with baseline levels, and accordingly, its associations with life-history variables were in the opposite direction of baseline measures. However, many correlations were not significant.

Robustness of Correlations to Ecological and Phylogenetic Subsampling

We conducted multiple analyses to check the robustness and generality of the correlations found in the full data set. We controlled for phylogeny using a phylogenetic re-

gression with no control variable (Grafen 1989), and the resulting *P* values mirror nearly exactly those for Pearson correlations (tables B1–B6). We conducted the analysis on three smaller data sets consisting of phylogenetic subsets: passerines only, nine-primaried oscines only, and omitting all nine-primaried oscines. The correlations for the phylogenetic subsets were similar to those for the full data set (fig. 2A–2D; tables B1–B4). The average differences in *r* between the full set and subsets were 0.06, 0.17, and 0.13, respectively. We tested for differences in *r* using a *t*-test on a *z* transformation. Out of 104 correlations in each subset matrix, 0, 3, and 3, respectively, were significantly different from the full set at $\alpha = 0.05$, which was fewer than the five or six expected at random. However, some correlations within nine-primaried oscines differed from those in the complementary set, that is, non-nine-primaried oscines (fig. 2C, 2D; tables B3, B4). The average difference in *r* was 0.29, and then 15 of 104 correlations were significantly different. Nine of these 15 were correlations with nestling period; also, nine-primaried oscines show strong associations between TAC, UA, and incubation.

When tropical and temperate species were analyzed separately, many effects emerged that were particular to one group or the other or to the full data set (fig. 2E, 2F; tables B5, B6). The average difference in *r* was 0.29, and 11 of 91 correlations were significantly different. Correlations between antioxidants and clutch size disappear among tropical birds, probably because there is so little variation in clutch size in the tropics (see also partial correlations

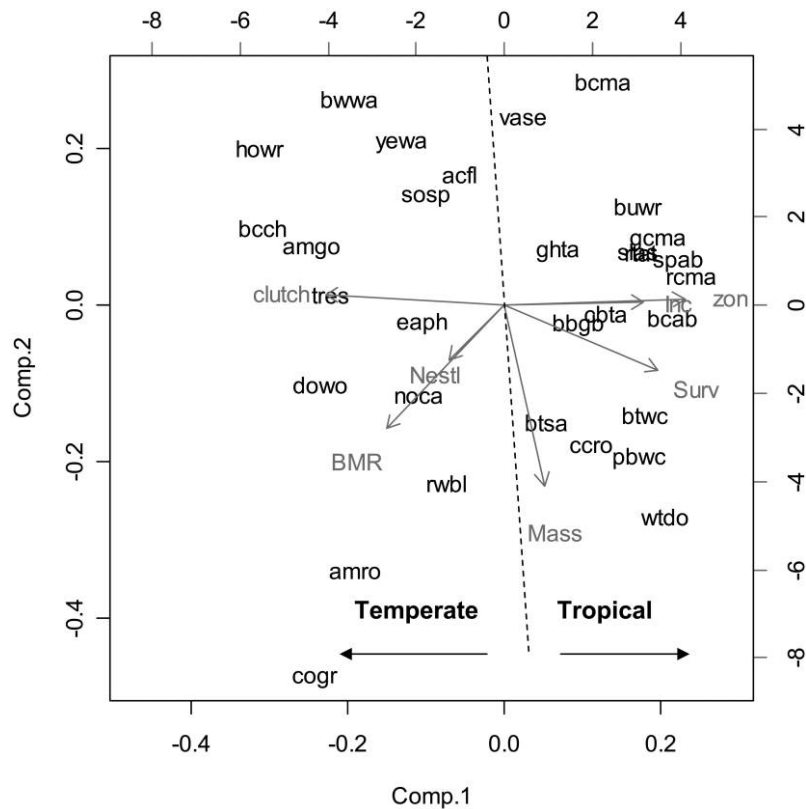


Figure 3: Biplot of first two principal component axes for 31 species with all life-history data. Species codes are listed in supplement A1 in the online edition of the *American Naturalist*.

in the next section and in tables B7–B19). BMR is strongly negatively correlated with TAC and UA but only in temperate birds. There appears to be a strong negative association between survival and carotenoid levels in tropical birds but no association in temperate birds. In both the phylogenetic and ecological subsets, a few of the significant correlations were in the opposite direction of the full data set, with low antioxidants characteristic of species with life-history variables indicating live fast–die young strategies. The exceptions with $P < .05$ include BMR with TAC, UA, and vitamin E and incubation with vitamin E in temperate birds; all associations with nestling period in the set excluding nine-primaried oscines; and vitamin E with BMR in nine-primaried oscines, a total of 10 out of 162 such relationships in tables B1–B6. Despite the small number of these exceptions, they appear nonrandomly distributed and thus may indicate real associations.

Partial Correlations

Correlations and bivariate partial correlations are summarized in figure 1 (see “Methods” for interpretation); for

full information on all correlations and bivariate partial correlations, see appendix B. The associations of life-history variables with TAC and UA are similar to each other. In both cases, mass is associated independent of (i.e., even when controlling for) other life-history variables, and clutch size and climate zone associations are dependent on each other but not on other variables. The association of survival weakened or disappeared when controlling for other variables except nestling period. Incubation period was associated only with TAC, and the association weakened or disappeared when controlling for survival, clutch, and climate zone. The TAC-UA residual (i.e., non-uric-acid antioxidant capacity), TAC stress response, and vitamin E are all much less closely associated with life histories. The residual is consistently positively associated with BMR when controlling for other variables, but the significance values are marginal ($.03 < P < .13$). Mass is robustly associated with TAC stress response, but the effect size weakens considerably when controlling for survival rate. None of the vitamin E associations can be supported with any confidence, considering both high P values and multiple testing issues. Mass and survival are

both associated with the carotenoid factor in a reciprocally dependent fashion, but the association with survival is also dependent on most of the other life-history variables. Incubation period was robustly negatively associated with carotenoids when controlling for other life-history variables. Individual carotenoids often show distinct patterns—for example, zeaxanthin is associated with BMR and β -carotene with nestling period (see tables B14–B19). Results from phylogenetic regression were equivalent to those from analyses without phylogenetic control.

Discussion

Although not all antioxidant variables were associated with all life-history variables, the direction of the observed associations was consistent with high antioxidant levels being characteristic of the live fast–die young life-history strategy. Antioxidant levels were negatively associated with body mass, nestling period, incubation period, and survival rate but positively associated with clutch size and mass-adjusted BMR. Although one might expect that live fast–die young species would have lower levels of antioxidants to support greater investment in current reproduction, recent work on model organisms suggests that antioxidant levels track free radical production, which might be elevated in species with high metabolism and short life spans (Van Remmen et al. 2003; Barja 2004).

At the broadest level, our results support the disposable soma theory of aging (Kirkwood 1977), the free radical theory of aging (Harman 1956), and general theories about life-history trade-offs and physiology (Wikelski and Ricklefs 2001). Closer examination of the results shows that although these theories have an element of truth, they fail to capture the interplay of many factors or the resulting complexity that resists generalization. Presumably, birds with high metabolic rates have higher free radical production; if antioxidant levels largely track free radical production as a result of direct need, we would expect small, short-lived birds with high metabolic rates to have higher antioxidant levels, as observed here. However, in contrast to the predictions of this hypothesis, vitamin E was less strongly associated with life histories than other antioxidants were, and the BMR was less strongly associated with antioxidants than other life-history variables were. BMR should have correlated positively with vitamin E, and this relationship should have been clearer than others we tested, but we found no association in most cases and a negative association in a few subsets of species.

Much of the literature on antioxidants and life span has focused on enzymatic antioxidants, primarily located in the mitochondria (but see Lopez-Torres et al. 1993; Sun and Tower 1999; Van Remmen et al. 2003). Here we show that similar trends appear to apply to circulating levels of

micromolecular antioxidants as well, across a wide range of bird species. This result is consistent with recent intraspecific work suggesting that high antioxidant capacity and uric acid are likely indicators of recent stress (e.g., oxidative or immune), perhaps because they are upregulated to deal with oxidative crises (Liu and Mori 1994; Oishi et al. 1999; Gümüslü et al. 2002; Tsahar et al. 2006; Hōrak et al. 2007; Cohen et al. 2008). However, given the complexity of our results, we cannot predict what we would have found had we studied other antioxidants, such as selenium and vitamin C, or measured tissue levels of antioxidants in these birds. In particular, circulating levels of antioxidants might indicate mobilization of stored molecules and thus reflect short-term changes in condition in ways that do not parallel tissue levels. At the species level, most of these fluctuations should have averaged out, but differences could still reflect species-specific patterns of mobilization.

Multidimensional Life Histories

We had expected a single axis of correlated variables to dominate life-history variation (Ricklefs and Wikelski 2002), but principal components analysis showed that at least three axes are necessary to represent the life-history variables included in this study. This challenges the widely held view that most life-history variation is evolutionarily intertwined with the trade-off between current and future reproduction, and this complicates the interpretation of our results. A recent study of mammalian life histories suggests that body size and lifestyle form independent axes driving evolution independently of the slow-fast continuum (Sibly and Brown 2007). In the context of our study, it is not clear that body mass and nestling period should be considered life-history variables; for example, body mass is related to foraging and nestling period to nest safety, so they might not associate with pace of life. Additionally, the body mass associations with antioxidants could result from allometric scaling independent of optimization of investment. When body mass and nestling period are removed, a single strong life-history axis emerges. These issues require further analysis beyond the scope of this study. Still, if antioxidant levels track free radical production and free radical production is a mechanism of the current versus future reproduction trade-off, it is not clear which life-history variables we should be comparing to antioxidants. The most consistent associations were between antioxidants and body mass, perhaps fortuitously indicating the impact of diet on antioxidant levels (see next section, final paragraph).

*Associations between Antioxidant and
Life-History Variables*

Most antioxidant variables showed strong associations with body mass, and this relationship was generally independent of other relationships. If this is a physiological scaling phenomenon that is similar to that known for BMR, it would be proper to use a mass-corrected antioxidant residual in analyses. However, because body size also associates with diet and ecology, it would be premature to assume that physiological scaling determines this relationship. Many antioxidants also showed associations with clutch size and climate zone. Nestling period and BMR were mostly unassociated with antioxidants, with one or two exceptions. Incubation period and survival rate showed associations that were generally not independently significant after controlling for other variables. However, controlling for survival rate made the effect of many other associations weaken or disappear, consistent with its split association with the first two principle component axes (see tables B7–B19). Thus, although survival rate cannot be viewed as independently driving the associations, its association with antioxidant levels was tightly tied to the effects of the other variables.

This survival-rate dependence is important for interpreting our results because we wished to assess interspecific variation in antioxidants in the context of physiological self-maintenance, the long-term effect of which is to mitigate the accumulation of oxidative damage with age. Rate of aging itself is difficult to measure, and our best proxy here is annual adult survival rate. Rate of aging (as measured by age-dependent mortality) is directly related to annual adult mortality rate in natural populations, implying that selection on aging rate is driven by expected future reproduction (Ricklefs 1998). However, aging-related mortality is nonetheless most prevalent among long-lived species such as pelagic seabirds, indicating that evolutionary responses to extend life span in long-lived species might be limited by lack of suitable genetic variation. Antioxidant levels were inversely related to survival rate in our analysis. That is, lower antioxidant levels seem to be associated with lower rate of aging and greater longevity. Accordingly, high uric acid levels are unlikely to explain the longer life spans observed in birds relative to mammals (Holmes et al. 2001). While this study does not directly address the potential health benefits of uric acid within species, the low levels of uric acid observed in some of our longest-lived species contradict the suggestion that high uric acid levels are a necessary component of proper avian health (Simoyi et al. 2002).

Vitamin E was not consistently associated with any life-history variation—a striking result, given the wide range of values present in our sample. Of the antioxidants mea-

sured here, vitamin E has the most clearly established physiological role as an antioxidant (Surai 2002), so the lack of associations between vitamin E and life histories somewhat undermines a functional interpretation of the observed associations. The carotenoid factor showed associations with body mass, survival rate, and incubation period, but individual carotenoid types often showed different patterns (see app. B). Nestling period was strongly associated with serum β -carotene concentration, and BMR was associated with zeaxanthin. However, it is unlikely that our current understanding of either antioxidant biochemistry or life-history evolution is sufficient to understand these patterns.

The general lack of associations between BMR and antioxidants is surprising. Only zeaxanthin showed a strong (positive) relationship with BMR; it might also be associated with the TAC-UA residual. BMR is the parameter most directly related to antioxidant measures mechanistically, and we expected the association here to be particularly strong. According to the theory that life span is inversely related to the “rate of living,” higher mass-adjusted metabolic rate should result in greater free radical production and thus higher antioxidant levels (Sohal 2002; Speakman 2005). That we did not find such an association suggests the interplay of other factors.

We measured metabolic rate as oxygen consumption, but the efficiency of energy production per unit oxygen consumed depends on the inner mitochondrial membrane gradient, which can be adjusted by opening or closing proton channels (Brand 2000). Since energy production efficiency trades off with free radical production (Echtay et al. 2002), for a given level of energy demand, mechanisms for reducing free radical production might result in increased oxygen consumption (Speakman et al. 2004). The way that this “uncoupling to survive” trade-off is modulated across species could obscure any independent relationship between antioxidants and energy production. Smaller birds show greater proton conductance than large birds, suggesting higher free radical production in species with high mass-specific energy expenditures (Brand et al. 2003), and free radical production rates may partially determine longevity. Also, higher rates of evolution of cytochrome *b* (a mitochondrial protein associated with energy and free radical production) in longer-lived songbirds appear to result from selection for amino acid substitutions that reduce free radical production (Rottenberg 2007). Unfortunately, we cannot distinguish whether the lack of association observed here between BMR and antioxidants is because of the confounding of this relationship by mitochondrial membrane gradients, other types of noise in the system (e.g., imperfect measures), or a general failure of the rate-of-living theory.

The associations among life-history variables, as char-

acterized by PCA, tended to break down when applied to carotenoids. Most notably, carotenoids exhibited associations with climate zone but not with clutch size. Also, body mass was associated with carotenoids, but whole-animal BMR was not. The axes identified by PCA thus appear to change depending on the context of their application. Tella et al. (2004) also found an association between total plasma carotenoids (presumably mostly lutein) and body mass, and their relationship was robust to phylogenetic control in their taxonomically more diverse sample. Phylogeny does matter, however. For example, galliforms have different carotenoid absorption rates than passerines for a given dietary level, suggesting phylogenetic variation not just in diet but in physiology (McGraw 2005).

Diet could drive some of the patterns shown here. For example, large birds might consume different types of foods or have more diverse diets than small birds. Of the 15 heaviest species in our sample, only two are insect specialists—a woodcreeper and a woodpecker—and these and a blackbird have the highest TAC and uric acid levels among the large species. Among the 15 smallest species, 11 eat primarily insects, and many, particularly the temperate insectivores, have high TAC and UA levels. Because of their complexities, analyses of diet were not incorporated into this article, but diet underlies much of the variation in antioxidants in this sample (A. A. Cohen, unpublished data). However, because the antioxidant variation is multidimensional, particularly in relation to life histories, it is unlikely that diet can fully explain our observations. The relationship between diet and antioxidant levels will be explored in future publications.

Generality of Antioxidant–Life History Associations

Although some relationships between antioxidant and life-history variables were evident in all analyses, many of them depended on which species were included. Phylogenetic subsampling and division into tropical and temperate subsets both produced changes in the antioxidant–life history correlation structure. For example, nestling period was strongly negatively correlated with TAC and UA only in nine-primaried oscines, and BMR was negatively associated with these variables only among temperate species. Thus, both phylogeny and ecology appear to influence relationships between physiology and life histories, although phylogeny in this context could simply reflect similar ecology among related species. Tropical–temperate differences could reflect fruit availability, temperature, time of year, degree of seasonality, parasite load, migration intensity, or any number of other factors. Our results confirm the importance of relationships between physiology and life histories but suggest that the particulars of the relationships often depend on so many factors that con-

sistent patterns might not be present. One exception is the negative association of body mass with TAC, UA, and carotenoids, which remains largely unchanged in all subsets and despite controlling for other life-history variables.

Most of our data were obtained during the (loosely defined) breeding season. However, even within the breeding season, we observed temporal variation in antioxidant levels (A. A. Cohen, unpublished data); we were unable to control for this or for the breeding status of individual birds. Sex differences in antioxidant levels are also species specific and often are absent (Cohen 2007). However, because of the relative homogeneity of sampling during breeding season, it seems unlikely that omitting these factors has biased our overall conclusions. The magnitude of the tropical–temperate difference seems too large to attribute to a seasonal bias alone, and it was accounted for in our analyses by stratifying according to climate zone.

A Hypothesis Relating Antioxidants and Homeostasis

Traditional hypotheses about the relationship of physiology to life-history trade-offs predict that high investment in physiological maintenance comes at a cost to reproduction. Our results provide some support for this but also for a second hypothesis, that investment in long-term physiological homeostasis comes at the expense of responsiveness to external stimuli. In this study, live slow-die old species not only had low constitutive antioxidant levels but also showed increases in these levels in response to handling stress. Conversely, live fast-die young species showed large decreases in response to stress (table B11). One explanation is that large species with constitutively low levels produce/mobilize antioxidants in response to a specific stress; smaller species may have high constitutive levels in anticipation of stress. In other words, small, short-lived species experience repeated stresses on a day-to-day basis, and they thus invest in strategies based on the assumption that stress is unavoidable (see Wingfield et al. 1998). In this sense, antioxidant levels might be related to life span through the temporal pattern of stress, which is influenced by body size and environment, rather than as a physiological function optimized with respect to longevity per se. Longer-lived species probably experience more constant environments and can invest in the tight regulation of their internal physiological environment, which is probably a prerequisite for the physiological maintenance necessary to live for decades.

Homeostasis can be considered a lack of variance in physiological parameters over time (R. Varadhan, personal communication). Physiological systems are highly complex and tightly interwoven, and a change in one system affects the others (e.g., Casto et al. 2001). Accrual of damage associated with aging and physiological decline would

be greater in species that allowed more external influence on their physiological processes. Increasing sensitivity of physiological systems to extrinsic factors could reduce the stability and predictability of internal processes, including those related to protection against damage. However, immediate response to extrinsic factors could also have benefits, including greater adaptability to changing conditions. Tight regulation of homeostasis could be costly due to inability to adjust each system optimally to external conditions and due to the direct cost of producing and maintaining molecules and systems involved in homeostatic regulation. Thus, homeostasis might trade off with adaptability in the evolution of life-history strategies. Resident tropical lowland forest bird species have more stable environments than temperate species and might be subject to less selection for adaptability at the expense of homeostasis. High constitutive antioxidant levels in temperate species might reflect both greater need for an acute response to stress and higher baseline levels of free radical production in species selected to maximize current over future reproduction. In this sense, the trade-off between current and future reproduction, which underlies differences in life span according to life-history theory and the disposable soma theory, could be complemented by a physiological trade-off between tight regulation of the internal environment and responsiveness to the external environment (stability versus flexibility).

Overall Conclusions

This study has several implications beyond the association between high antioxidant levels and live fast–die young life-history strategies. First, the complexity underlying this relationship is remarkable. Clearly, antioxidants are not unified in their relationship to avian life histories, suggesting that the functional classification “antioxidant” may not be appropriate in this context. Other roles, such as signaling and use in the immune respiratory burst, could affect these patterns. Similarly, variation in immune system responses also cannot be condensed into a single, easily understood axis (Matson et al. 2006), and hormone action is also species specific (e.g., Wingfield et al. 1998). Our results thus support the general hypothesis that life-history variation is associated with and at least partly determined by the evolution of physiological systems (Wikelski and Ricklefs 2001), but they suggest that we may not be able to understand these relationships easily. Second, survival rate plays a unifying role relating antioxidants to life histories; antioxidant correlations with survival were weaker than they were for some other life-history variables, but controlling for survival made most of the other relationships disappear or weaken, consistent with the loading of survival rate on both the first and the second principle

component axes. Thus, despite its complex relationship to environment and fitness (Ricklefs 2000), survival rate is the only life-history trait measured here that seems to fully capture the main axis of life-history variation as broadly discussed in the literature (Ricklefs and Wikelski 2002). We are just beginning to understand how antioxidant levels and their regulation are integrated into the life history of birds.

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Literature Cited

- Alonso-Alvarez, C., S. Bertrand, G. L. Devevey, J. Prost, B. Faivre, G. Sorci, and V. Loeschcke. 2004. Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecology Letters* 7:363–368.
- Alonso-Alvarez, C., S. Bertrand, B. Faivre, and G. Sorci. 2007. Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Functional Ecology* 21:873–879.
- Barja, G. 2004. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical–DNA damage mechanism? *Biological Reviews of the Cambridge Philosophical Society* 79:235–251.
- Barker, F. K. 2004. Monophyly and relationships of wrens (Aves: Troglodytidae): a congruence analysis of heterogeneous mitochondrial and nuclear DNA sequence data. *Molecular Phylogenetics and Evolution* 31:486–504.
- Barker, F. K., A. Cibolis, P. Schickler, J. Feinstein, and J. Cracraft. 2004. Phylogeny and diversification of the largest avian radiation. *Proceedings of the National Academy of Sciences of the USA* 101:11040–11045.
- Beckman, K. B., and B. N. Ames. 1998. The free radical theory of aging matures. *Physiological Reviews* 78:541–581.
- Bent, A. C. 1963*a*. Life histories of North American flycatchers, larks, swallows and their allies. Dover, New York.
- . 1963*b*. Life histories of North American wood warblers. Dover, New York.
- . 1964*a*. Life histories of North American birds nuthatches, wrens, thrashers, and their allies. Dover, New York.
- . 1964*b*. Life histories of North American jays, crows, and titmice. Dover, New York.
- . 1965. Life histories of North American blackbirds, orioles, tanagers, and allies. Dover, New York.
- . 1968. Life histories of North American thrushes, kinglets, and their allies. Dover, New York.

- Bent, A. C., and O. L. Austin. 1968. Life histories of North American cardinals, grosbeaks, buntings, towhees, finches, sparrows, and allies. Dover, New York.
- Bent, A. C., and W. Zimmerman. 1992. Life histories of North American woodpeckers. Indiana University Press, Bloomington.
- Brand, M. D. 2000. Uncoupling to survive? the role of mitochondrial inefficiency in ageing. *Experimental Gerontology* 35:811–820.
- Brand, M. D., N. Turner, A. Ocloo, P. L. Else, and A. J. Hulbert. 2003. Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds. *Biochemical Journal* 376:741–748.
- Brand, M. D., C. Affourtit, T. C. Esteves, K. Green, A. J. Lambert, S. Miwa, J. L. Pakay, et al. 2004. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radical Biology and Medicine* 37:755–767.
- Brawn, J. D., J. R. Karr, J. D. Nichols, and W. D. Robinson. 1999. Demography of tropical forest birds in Panama: how do transients affect estimates of survival rates? *Proceedings International Ornithological Congress* 22:297–305.
- Burns, K. J. 1998. A phylogenetic perspective on the evolution of sexual dichromatism in tanagers (Thraupidae): the role of female versus male plumage. *Evolution* 52:1219–1224.
- Burns, K. J., S. J. Hackett, and N. K. Klein. 2003. Phylogenetic relationships of Neotropical honeycreepers and the evolution of feeding morphology. *Journal of Avian Biology* 34:360–370.
- Carson, R. J., and G. S. Spicer. 2003. A phylogenetic analysis of the emberizid sparrows based on three mitochondrial genes. *Molecular Phylogenetics and Evolution* 29:43–57.
- Casto, J. M., V. Nolan Jr., and E. D. Ketterson. 2001. Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). *American Naturalist* 157:408–420.
- Chesser, R. T. 2004. Molecular systematics of New World suboscine birds. *Molecular Phylogenetics and Evolution* 32:11–24.
- Cohen, A. A. 2007. The role of antioxidants in the physiology, ecology, and life histories of wild birds. PhD diss. University of Missouri, St. Louis.
- Cohen, A. A., K. C. Klasing, and R. E. Ricklefs. 2007. Measuring circulating antioxidants in wild birds. *Comparative Biochemistry and Physiology B* 147:110–121.
- Cohen, A. A., M. Hau, and M. Wikelski. 2008. Stress, metabolism, and antioxidants in two wild passerine bird species. *Physiological and Biochemical Zoology* 81:463–472.
- Costantini, D., and G. Dell’Omo. 2006. Effects of T-cell-mediated immune response on avian oxidative stress. *Comparative Biochemistry and Physiology A* 145:137–142.
- Costantini, D., M. Cardinale, and C. Carere. 2007. Oxidative damage and anti-oxidant capacity in two migratory bird species at a stop-over site. *Comparative Biochemistry and Physiology* 144:363–371.
- Cracraft, J., F. K. Barker, M. J. Braun, J. Harshman, G. J. Dyke, J. Feinstein, S. Stanley, et al. 2004. Phylogenetic relationships among modern birds (Neornithes): toward an avian tree of life. Pages 468–489 in J. Cracraft and M. J. Donoghue, eds. *Assembling the tree of life*. Oxford University Press, New York.
- del Hoyo, J. A., A. Elliott, and J. Sargatal. 1992–2006. *Handbook of birds of the world*. Lynx, Barcelona.
- Dunning, J. B. 1992. *CRC handbook of avian body mass*. CRC, Boca Raton, FL.
- Echtay, K. S., D. Roussel, J. St-Pierre, M. B. Jekabsons, S. Cadenas, J. A. Stuart, J. A. Harper, et al. 2002. Superoxide activates mitochondrial uncoupling proteins. *Nature* 415:96–99.
- Ehrlich, P., D. S. Dobkin, and D. Wheye. 1988. *The birder’s handbook: a field guide to the natural history of North American birds*. Simon & Schuster, New York.
- Ericson, P. G. P., and U. S. Johansson. 2003. Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetics and Evolution* 29:126–138.
- Ericson, P. G. P., A.-L. Jansen, U. S. Johansson, and J. Ekman. 2005. Inter-generic relationships of the crows, jays, magpies and allied groups (Aves: Corvidae) based on nucleotide sequence data. *Journal of Avian Biology* 36:222–234.
- Grafen, A. 1989. The phylogenetic regression. *Philosophical Transactions of the Royal Society B: Biological Sciences* 326:119–157.
- . 2006. Phylogenetic regression SAS implementation. Version v0.7. <http://users.ox.ac.uk/~grafen/phylo/index.html>.
- Grapputo, A., A. Pilastrò, A. J. Baker, and G. Marin. 2001. Molecular evidence for phylogenetic relationships among buntings and American sparrows (Emberizidae). *Journal of Avian Biology* 32:95–101.
- Gümüşlü, S., S. B. Sarıkçıoğlu, E. Sahin, P. Yargıçoğlu, and A. Agar. 2002. Influences of different stress models on the antioxidant status and lipid peroxidation in rat erythrocytes. *Free Radical Research* 36:1277–1282.
- Halliwell, B. 2000. The antioxidant paradox. *Lancet* 355:1179–1180.
- Harman, D. 1956. Aging: a theory based on free radical radiation chemistry. *Journal of Gerontology* 11:298–300.
- Harrell, F. E., Jr. 2001. *Regression modeling strategies*. Springer, New York.
- Hartley, R. C., and M. W. Kennedy. 2004. Are carotenoids a red herring in sexual display? *Trends in Ecology & Evolution* 19:353–354.
- Hausmann, M. F., D. W. Winkler, K. M. O’Reilly, C. E. Huntington, I. C. T. Nisbet, and C. M. Vleck. 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society B: Biological Sciences* 270:1387–1392.
- Hill, R. N. 1972. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *Journal of Applied Physiology* 33:263.
- Holmes, D. J., R. Fluckiger, and S. N. Austad. 2001. Comparative biology of aging in birds: an update. *Experimental Gerontology* 36:869–883.
- Hörak, P., M. Zilmer, L. Saks, I. Ots, U. Karu, and K. Zilmer. 2006. Antioxidant protection, carotenoids and the costs of immune challenge in greenfinches. *Journal of Experimental Biology* 209:4329–4338.
- Hörak, P., L. Saks, Z. Zilmer, U. Karu, and K. Zilmer. 2007. Do dietary antioxidants alleviate the cost of immune activation? an experiment with greenfinches. *American Naturalist* 170:625–635.
- Huang, H., and K. G. Manton. 2004. The role of oxidative damage in mitochondria during aging: a review. *Frontiers in Bioscience* 9:1100–1117.
- Hulbert, A. J., R. Pamplona, R. Buffenstein, and W. A. Buttemer. 2007. Life and death: metabolic rate, membrane composition, and life span of animals. *Physiological Reviews* 87:1175–1213.
- Irestedt, M., U. S. Johansson, T. S. Parsons, and P. G. P. Ericson. 2001. Phylogeny of major lineages of suboscines (Passeriformes) analysed by nuclear DNA sequence data. *Journal of Avian Biology* 32:15–25.

- Irestedt, M., J. Fjeldsa, J. A. A. Nylander, and P. G. P. Ericson. 2004a. Phylogenetic relationships of typical antbirds (Thamnophilidae) and test of incongruence based on Bayes factors. *BMC Evolutionary Biology* 4:23.
- Irestedt, M., J. Fjeldsa, and P. G. P. Ericson. 2004b. Phylogenetic relationships of woodcreepers (Aves: Dendrocolaptinae): incongruence between molecular and morphological data. *Journal of Avian Biology* 35:280–288.
- Isaksson, C., P. McLaughlin, P. Monaghan, and S. Andersson. 2007. Carotenoid pigmentation does not reflect total nonenzymatic antioxidant activity. *Functional Ecology* 21:1123–1129.
- Johnson, K. P., and D. H. Clayton. 2000. Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves (Aves: Columbiformes). *Molecular Phylogenetics and Evolution* 14:141–151.
- Johnson, K. P., S. de Kort, K. Dinwoodey, A. C. Mateman, C. ten Cate, C. M. Lessells, and D. H. Clayton. 2001. A molecular phylogeny of the dove genera *Streptopelia* and *Columba*. *Auk* 118:874–887.
- Joseph, L., T. Wilke, E. Bermingham, D. Alpers, and R. E. Ricklefs. 2004. Towards a phylogenetic framework for the evolution of shakes, rattles, and rolls in *Myiarchus* tyrant-flycatchers (Aves: Passeriformes: Tyrannidae). *Molecular Phylogenetics and Evolution* 31:139–152.
- Karr, J. R., J. D. Nichols, M. K. Klimkiewicz, and J. D. Brawn. 1990. Survival rates of tropical and temperate forest birds: will the dogma survive? *American Naturalist* 136:277–291.
- Kirkwood, T. B. L. 1977. Evolution of ageing. *Nature* 270:301–304.
- Klicka, J., K. P. Johnson, and S. M. Lanyon. 2000. New World nine-primaried oscine relationships: constructing a mitochondrial DNA framework. *Auk* 117:321–336.
- Lack, D. 1948. The significance of clutch size. 3. Some interspecific comparisons. *Ibis* 90:25–45.
- Levy, A. 1964. The accuracy of the bubble meter for gas flow measurements. *Journal of Scientific Instruments* 41:449–453.
- Liu, J., and A. Mori. 1994. Involvement of reactive oxygen species in emotional stress: a hypothesis based on the immobilization stress-induced oxidative damage and antioxidant defense changes in rat brain, and the effect of antioxidant treatment with reduced glutathione. *International Journal of Stress Management* 1:249–263.
- Lopez-Torres, M., R. Perez-Campo, S. Cadenas, C. Rojas, and G. Barja. 1993. A comparative study of free radicals in vertebrates. II. Non-enzymatic antioxidants and oxidative stress. *Comparative Biochemistry and Physiology B* 105:757–763.
- Lovette, I. J., and W. M. Hochachka. 2006. Simultaneous effects of phylogenetic niche conservatism and competition on avian community structure. *Ecology* 87:S14–S28.
- Machin, M., M. F. Simoyi, K. P. Blemings, and H. Klandorf. 2004. Increased dietary protein elevates plasma uric acid and is associated with decreased oxidative stress in rapidly-growing broilers. *Comparative Biochemistry and Physiology B* 137:383–390.
- Mann, N. I., F. K. Barker, J. A. Graves, K. A. Dingess-Mann, and P. J. B. Slater. 2006. Molecular data delineate four genera of “*Thryothorus*” wrens. *Molecular Phylogenetics and Evolution* 40:750–759.
- Martin, T. E. 1996. Life history evolution in tropical and south temperate birds: what do we really know? *Journal of Avian Biology* 27:263–272.
- . 2002. A new view of avian life-history evolution tested on an incubation paradox. *Proceedings of the Royal Society B: Biological Sciences* 269:309–316.
- . 2004. Avian life-history evolution has an eminent past: does it have a bright future? *Auk* 121:289–301.
- Matson, K. D., A. A. Cohen, K. C. Klasing, R. E. Ricklefs, and A. Scheuerlein. 2006. No simple answers for ecological immunology: relationships among immune indices at the individual level break down at the species level in waterfowl. *Proceedings of the Royal Society B: Biological Sciences* 275:815–822.
- McGraw, K. J. 2005. Interspecific variation in dietary carotenoid assimilation in birds: links to phylogeny and color ornamentation. *Comparative Biochemistry and Physiology B* 142:245–250.
- McGraw, K. J., and D. R. Ardia. 2003. Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *American Naturalist* 162:704–712.
- McGraw, K. J., and R. S. Parker. 2006. A novel lipoprotein-mediated mechanism controlling sexual attractiveness in a colorful songbird. *Physiology and Behavior* 87:103–108.
- McKechnie, A. E., and B. O. Wolf. 2004. The allometry of avian basal metabolic rate: good predictions need good data. *Physiological and Biochemical Zoology* 77:502–521.
- Miller, N. J., C. Rice-Evans, M. J. Davies, V. Gopinathan, and A. Milner. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science* 84:407–412.
- Moreau, R. E. 1944. Clutch size: a comparative study, with reference to African birds. *Ibis* 86:286–347.
- Neuzil, J., and R. Stocker. 1994. Free and albumin-bound bilirubin are efficient co-antioxidants for α -tocopherol, inhibiting plasma and low-density lipoprotein lipid peroxidation. *Journal of Biological Chemistry* 269:16712–16719.
- Oishi, K., M. Yokoi, S. Maekawa, C. Sodeyama, T. Shiraishi, R. Kondo, T. Kuriyama, et al. 1999. Oxidative stress and haematological changes in immobilized rats. *Acta Physiologica Scandinavica* 165: 65–69.
- Patten, M. A., and M. Fugate. 1998. Systematic relationships among the emberizid sparrows. *Auk* 115:412–424.
- Pérez-Campo, R., M. López-Torres, C. Rojas, S. Cadenas, and G. Barja. 1994. Longevity and antioxidant enzymes, non-enzymatic antioxidants and oxidative stress in the vertebrate lung: a comparative study. *Journal of Comparative Physiology B* 63:682–689.
- Perneger, T. V. 1998. What’s wrong with Bonferroni adjustments. *British Medical Journal* 316:1236–1238.
- R Development Core Team. 2007. R: a language and environment for statistical computing. Version 2.5.0. R Foundation for Statistical Computing, Vienna.
- Ricklefs, R. E. 1998. Evolutionary theories of aging: confirmation of a fundamental prediction, with implications for the genetic basis and evolution of life span. *American Naturalist* 152:24–44.
- . 2000. Density dependence, evolutionary optimization, and the diversification of avian life histories. *Condor* 102:9–22.
- Ricklefs, R. E., and R. E. Shea. 2007. Estimating annual survival in sexually dimorphic species from proportions of first-year birds. *Ecology* 88:1408–1419.
- Ricklefs, R. E., and M. Wikelski. 2002. The physiology/life history nexus. *Trends in Ecology & Evolution* 17:462–468.
- Ricklefs, R. E., M. Konarzewski, and S. Daan. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *American Naturalist* 147:1047–1071.
- Rottenberg, H. 2007. Exceptional longevity in songbirds is associated

- with high rates of evolution of cytochrome b, suggesting selection for reduced generation of free radicals. *Journal of Experimental Biology* 210:2170–2180.
- Savitz, D. A., and A. F. Olshan. 1995. Multiple comparisons and related issues in the interpretation of epidemiologic data. *American Journal of Epidemiology* 142:904–908.
- Schmidt-Nielsen, K. 1997. *Animal physiology: adaptation and environment*. Cambridge University Press, Cambridge.
- Sibley, C. G., and J. E. Ahlquist. 1990. *Phylogeny and classification of birds: a study in molecular evolution*. Yale University Press, New Haven, CT.
- Sibly, R. M., and J. H. Brown. 2007. Effects of body size and lifestyle on evolution of mammal life histories. *Proceedings of the National Academy of Sciences of the USA* 104:17707–17712.
- Simoyi, M. F., K. van Dyke, and H. Klandorf. 2002. Manipulation of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. *American Journal of Physiology* 282:R791–R796.
- Skutch, A. F. 1949. Do tropical birds rear as many young as they can nourish? *Ibis* 91:430–455.
- . 1969. *Life histories of Central American birds*. III. Pacific Coast Avifauna no. 35. Cooper Ornithological Society, Berkeley.
- Sohal, R. S. 2002. Role of oxidative stress and protein oxidation in the aging process. *Free Radical Biology and Medicine* 33:37–44.
- Speakman, J. R. 2005. Body size, energy metabolism and lifespan. *Journal of Experimental Biology* 208:1717–1730.
- Speakman, J. R., D. Talbot, C. Selman, S. Snart, J. S. McLaren, P. Redman, E. Krol, et al. 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell* 3:87–94.
- Sun, J., and J. Tower. 1999. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Molecular and Cellular Biology* 19:216–228.
- Surai, P. F. 2002. *Natural antioxidants in avian nutrition and reproduction*. Nottingham University Press, Nottingham.
- Surai, P. F., B. K. Speake, N. A. R. Wood, J. D. Blount, G. R. Bortolotti, and N. H. C. Sparks. 2001. Carotenoid discrimination by the avian embryo: a lesson from wild birds. *Comparative Biochemistry and Physiology B* 128:743–750.
- Tella, J. L., J. Figuerola, J. J. Negro, G. Blanco, R. Rodríguez-Estrella, M. G. Forero, M. C. Blázquez, et al. 2004. Ecological, morphological and phylogenetic correlates of interspecific variation in plasma carotenoid concentration in birds. *Journal of Evolutionary Biology* 17:156–164.
- Tello, J. G., and J. M. Bates. 2007. Molecular phylogenetics of the tody-tyrant and flatbill assemblage of tyrant flycatchers (Tyrannidae). *Auk* 124:134–154.
- Tieleman, B. I., and J. B. Williams. 2000. The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiological and Biochemical Zoology* 73:461–479.
- Tieleman, B. I., J. B. Williams, and M. E. Buschur. 2002. Physiological adjustments to arid and mesic environments in larks (Alaudidae). *Physiology and Biochemical Zoology* 75:305–313.
- Tieleman, B. I., J. B. Williams, R. E. Ricklefs, and K. C. Klasing. 2004. Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. *Proceedings of the Royal Society B: Biological Sciences* 272:1715–1720.
- Tsahar, E., Z. Arad, I. Izhaki, and C. G. Guglielmo. 2006. The relationship between uric acid and its oxidative product allantoin: a potential indicator for the evaluation of oxidative stress in birds. *Journal of Comparative Physiology B* 176:653–661.
- Van Remmen, H., Y. Ikeno, M. Hamilton, M. Pahlavani, N. Wolf, S. R. Thorpe, N. L. Alderson, et al. 2003. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiological Genomics* 16:29–37.
- White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study* 46: 120–138.
- Wiersma, P., C. Selman, J. R. Speakman, and S. Verhulst. 2004. Birds sacrifice oxidative protection for reproduction. *Proceedings of the Royal Society B: Biological Sciences* 271:S360–S363.
- Wiersma, P., A. Muñoz-García, A. Walker, and J. B. Williams. 2007. Tropical birds have a slow pace of life. *Proceedings of the National Academy of Sciences of the USA* 104:9340–9345.
- Wikelski, M., and R. E. Ricklefs. 2001. The physiology of life histories. *Trends in Ecology & Evolution* 16:479–481.
- Wikelski, M., M. Hau, and J. C. Wingfield. 2000. Seasonality of reproduction in a Neotropical rain forest bird. *Ecology* 81:2458–2472.
- Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11:398–411.
- Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage.” *American Zoologist* 38:191–206.
- Yuri, T., and D. P. Mindell. 2002. Molecular phylogenetic analysis of Fringillidae, “New World nine-primaried oscines” (Aves: Passeriformes). *Molecular Phylogenetics and Evolution* 23:229–243.

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