Genome size is not related to life-history traits in primates

Serge Morand and Robert E. Ricklefs

Abstract: Genome size (C value, the haploid DNA content of the nucleus) varies widely among eukaryotes, increasing through duplication or insertion of transposable elements and decreasing through deletions. Here, we investigate relationships between genome size and life-history attributes potentially related to fitness, including body mass, brain mass, gestation time, age at sexual maturity, and longevity, in 42 species of primates. Using multivariate and phylogenetically informed analyses, we show that genome size is unrelated to any of these traits. Genome size exhibits little variation within primates and its evolution does not appear to be correlated with changes in life-history traits. This further indicates that the phenotypic consequences of variation in genome size are dependent on the particular biology of the group in question.

Key words: age at maturity, body size, brain mass, C value, genome size, gestation time, life history, primate.

Résumé : La taille du génome (la valeur C ou le contenu en ADN d'un noyau haploïde) varie grandement au sein des eucaryotes. Il peut s'accroître par suite de duplications ou d'insertions d'éléments transposables et se contracter suite à des délétions. Les auteurs examinent ici la relation entre la taille du génome et des caractéristiques du cycle vital potentiellement liées à la vigueur dont la masse corporelle, la masse cérébrale, le temps de gestation, l'âge à la maturité sexuelle et la longévité chez 42 espèces de primates. À l'aide d'analyses multivariées et d'analyses s'appuyant sur la phylogénie, les auteurs montrent que la taille du génome n'est liée à aucune de ces caractéristiques. La taille du génome montre peu de variation parmi les primates et son évolution ne semble pas corrélée avec des changements au niveau des caractères étudiés. Ceci suggère que les conséquences phénotypiques associées à la variation pour la taille du génome sont dépendantes de la biologie propre du groupe à l'étude.

Mots clés : âge à la maturité, taille corporelle, masse du cerveau, valeur C, taille du génome, temps de gestation, cycle vital, primate.

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Introduction

Genome size (C value, picograms of DNA per haploid genome) varies more than 200 000 fold among eukaryotes and 3000 fold among animals. Most eukaryotic DNA is noncoding, which implies that a large genome does not imply a large number of genes (Gregory 2004). The existence of large quantities of noncoding DNA raises several questions in relation to effects on the organismal phenotype and to evolutionary origin of variation in genome size, i.e., the "C value enigma" (see Gregory 2001*a*). Genome size evolves through a balance between duplication and insertion of transposable elements, which increase C values, and deletions and DNA repair mechanisms, which might decrease the C value or allow it to increase when preventing large deletion errors (Petrov 2001). The origins and the evolutionary consequences of variation in genome size continue to be debated (Gregory and Hebert 1999; Petrov 2001). Because genome size and the number of protein-coding genes are unrelated, it has been established that variation in genome size results primarily from variation in the amount of silenced genes (pseudogenes) and selfish parasitic elements (Ohno 1972; Pagel and Johnstone 1992). Genome size is positively related to cell volume and cell division rate in several groups of organisms (Gregory 2001*a*). At high taxonomic levels, the relationships seem to be more complex and highly dependent on the biology of the studied group. Metabolism is important in mammals and birds (Gregory 2002*a*), but not in amphibians (Licht and Lowcock 1991; Gregory 2003), and developmental rate and organ or developmental complexity are relevant in amphibians, but not in mammals and birds (Gregory 2002*b*). Here, we investigate the relationship between genome size and body mass, brain mass, and several life-history traits of primates including

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gestation time, age at sexual maturity, and longevity. We use independent contrasts (Felsenstein 1985) to account for the non-independence of phylogenetically structured data.

Material and methods

Data sources

We obtained mammal genome sizes from the Animal Genome Size Database (Gregory 2001*a*, 2001*b*) at http://www.genomesize. com. Primate life-history traits were obtained from Harvey and Clutton-Brock (1985). Additional values for maximum life span were obtained from Carey and Judge (2000) and we used the higher of the two when both sources provided values. We used the phylogeny of primates constructed by Purvis (1995). Altogether, we considered 43 species of primates (Table 1). For the purpose of conducting a nested analysis of variance based on taxonomic rank, we used the taxonomic arrangement of Fleagle (1999).

Data for *Macaca arctoides* were excluded because several life-history values were missing for this species. For other species, 3 values were missing for brain mass, 4 for age at sexual maturity, and 1 for life span. We replaced the missing values with dummy variables estimated from the available data by regression, using the independent variable explaining the largest amount of the variation in the dependent variable, beginning with brain mass, and continuing with age at sexual maturity, and life span, in that order. The equations for estimating the dummy variables were as follows (all variables $\log_{10}$ transformed):

$$\text{brain mass} = 1.2929\ (\pm 0.0440\ \text{SE}) + 0.82117\ (0.0521) \times \text{body mass}\ (R^2 = 0.867)$$

$$\text{age at maturity} = 0.8389\ (0.0750) + 0.4132\ (0.0413) \times \text{brain mass}\ (R^2 = 0.735)$$

$$\text{life span} = 1.0510\ (0.0487) + 0.2468\ (0.0273) \times \text{brain mass}\ (R^2 = 0.672),$$

all $P < 0.0001$. In the data set used in this analysis (N = 42 species), C values exhibited significant kurtosis (5.48) and 3 species had $\log_{10}$-transformed C values that were more than 3 standard deviations from the mean of C values for the other 39 species: *Callicebus torquatus* (–6.14), *Cercocebus torquatus* (4.56), and *Tarsius syrichta* (5.80). These outlying values were deleted in some analyses (kurtosis = 0.85, NS) to check the robustness of relationships. Unless otherwise noted, these outliers did not influence the relationships, or the absence of relationships, observed in this study.

Comparative analyses

Because species share varying degrees of evolutionary history, trait values for species are not fully independent and this can inflate the degrees of freedom used in statistical tests (Felsenstein 1985; Harvey and Pagel 1991). When variation resides at a low taxonomic level, i.e., when trait values vary primarily between closely related species, traits are evolutionarily labile and shared evolutionary history presents relatively little problem. In this case, one is justified in using species values. When variation resides at a high taxonomic level, traits are evolutionarily conservative and species tend to inherit their trait values from their ancestors. In this case, shared evolutionary history becomes an important consideration in comparative analysis and phylogenetically informed approaches should be used to analyse relationships among variables (see below).

We used random effects nested analysis of variance (SAS Proc Nested) to estimate the variance in traits at the levels of families within the order Primates, genera within families, and species within genera. As reported in the results section below, variation in genome size resides primarily at the level of genera within families, but other life-history traits are more conservative, with most of the variation being expressed among families. Thus, we conducted analyses of both species data and phylogenetically independent contrasts, which partition variation into evolutionarily independent sections of the phylogenetic tree (see below). When results from these approaches do not conflict, we can be more confident of conclusions from the analyses, whereas a conflict between both approaches may suggest phylogenetic inertia.

We used correlation analysis (SAS Proc Corr) and factor analysis (SAS Proc Factor) based on correlation matrices to examine the overall patterns of relationship of genome size to other life-history traits. We used multiple regression (SAS Proc GLM) to test the hypothesis that development time (gestation period, age at maturity) and life span are directly related to genome size when the effects of body mass (and brain mass) on development time and life span are accounted for. We also used stepwise regression (SAS Proc Stepwise) to determine the best predictive equation for genome size based on the life-history variables included in this analysis.

All variables were $\log_{10}$ transformed to produce more nearly normal distributions of the data and to make the variances more similar. All statistical analyses were carried out with SAS software, version 8.01 (SAS Institute Inc., Cary, N.C.).

Phylogenetically independent contrasts

We calculated phylogenetically independent contrasts (PIC, Felsenstein 1985) to account for shared evolutionary history in the diversification of species traits (Harvey and Pagel 1991; Garland et al. 1992). Contrasts for a particular node in a phylogenetic tree are calculated as the difference between log-transformed values for the immediately descendant nodes or terminal taxa, and thus estimate evolution over a unique pair of branches. Each contrast is independent of all others. Nodal values are the means of the descendant species in the clade defined by a node, weighted by the branching arrangement within the clade (Harvey and Pagel 1991). We used the CAIC computer program (Purvis and Rambaut 1995) for independent contrast analyses. All regressions between contrasts were forced through the origin (Garland et al. 1992). To verify that contrasts were properly standardized we used CAIC to test whether the standard deviations of standardized contrasts were independent of their absolute values (Garland et al. 1992). We note that the calculation of contrasts potentially introduces additional sources of variation and bias owing to the estimation of nodal values from

Table 1. C values and life-history traits of primates investigated in our study.

Species	C value (pg)	Body mass (kg)	Gestation time (d)	Age at sexual maturity (months)	Lifespan (y)	Brain weight (g)
Alouatta palliata	3.63	5.7	187	42	25	55.1
Ateles geoffroyi	3.25	5.8	216	48	48	110.9
Callicebus torquatus	2.26	5.5	180	—	25	22.4
Callithrix jacchus	3.43	0.29	140	12	16.8	7.9
Cebuella pygmaea	3.48	0.14	140	12	18	4.2
Cebus albifrons	3.98	2.6	180	48	50	82
Cercocebus torquatus	4.9	1.1	173	56	30	109.6
Chlorocebus sabaeus	4.02	5.6	165	30	30	—
Colobus polykomos	3.61	8.4	175	24	30.5	76.7
Erythrocebus patas	3.52	5.6	167	30	23.9	106.6
Eulemur fulvus	3.36	1.9	117	18	36	25.2
Eulemur macaco	3.36	2.5	127	18	39	25.6
Eulemur mongoz	3.4	1.8	128	—	—	21.8
Galago senegalensis	4.08	0.21	131	10	18	4.8
Galgoides alleni	3.93	0.27	133	10	12	6.1
Gorilla gorilla	3.57	93	258	96	50	505.9
Hapalemur griseus	3.23	2	143	—	17.1	14.7
Homo sapiens	3.5	40.1	280	156	70	1250
Hylobates lar	3.08	5.3	225	102	40	107.7
Hylobates syndactylus	3.37	10.6	232	102	38	121.7
Lagothrix lagothricha	3.54	5.8	225	84	30	96.4
Lemur catta	3.28	2.5	136	18	33	25.6
Lepilemur mustelinus	3.25	0.64	135	18	12	14.7
Lophocebus albigena	3.76	6.4	174	48	32.7	99.1
Macaca fascicularis	3.41	4.1	165	36	38	69.2
Macaca fuscata	3.56	9.1	173	36	33	109.1
Macaca mulatta	3.29	3	166	36	36	95.1
Macaca nemestrina	3.56	7.8	174	36	34.3	106
Macaca silenus	3.33	5	180	60	40	85
Macaca sylvanus	3.5	10	165	36	22	93.2
Mandrillus sphinx	3.5	11.5	176	42	46	159.4
Microcebus murinus	3.12	0.084	60	19	15	1.8
Miopithecus talapoin	3.67	1.1	162	54	30.9	37.7
Nasalis larvatus	4.32	9.9	166	—	23	94.2
Nycticebus coucang	3.58	1.2	191	24	26.5	10
Otolemur crassicaudatus	3.61	2	133	20	18	—
Pan troglodytes	3.63	31.1	230	84	60	410.3
Papio hamadryas	3.53	9.4	172	60	37.5	142.5
Perodicticus potto	3.58	1.08	195	18	26	14.3
Pongo pygmaeus	3.66	37	245	84	59	413.3
Saimiri sciureus	3.3	0.58	162	36	30	24.4
Tarsius syrichta	5.36	0.12	178	18	15	4

species data on an imperfectly known phylogenetic tree (Ricklefs and Stark 1996; Price 1997).

Results

The standard deviations of genome size and each of the life history traits, as well as the distribution of their variation at the level of species, genus, and family, are presented in Table 2. Variation among species was greatest in body and brain mass (SD equivalent to factors of 5.0 and 4.1, respectively), intermediate for gestation period (1.30), age at maturity (1.97), and life span (1.53), and least for genome size (1.14). Most of the relatively small amount of variation in genome size resided at the level of genera within families, whereas that of the other life history traits resided primarily at the level of families within the order Primates. Variance in life span was more evenly spread among the taxonomic levels; however, this trait is poorly estimated and much of the variation at lower taxonomic levels undoubtedly represents measurement error. Relatively few genera were represented in the dataset by more than one species (*Hylobates*, 2; *Eulemur*, 3; *Macaca*, 6), so the estimate of variation among species within genera was based on a small sample. The distribution of most of the variance in life-history traits at high

Table 2. Standard deviations among species of the trait values used in this analysis.

Source	DF	Genome size	Body mass	Brain mass	Gestation period	Age at maturity	Life span
Standard deviation	41	0.056	0.697	0.614	0.114	0.294	0.186
Family (%)	12	23.4	81.9	82.2	94.1	71.5	58.4
Genus (%)	21	69.5	11.8	16.9	4	21.6	18.8
Species (%)	8	7.1	6.3	0.9	1.9	6.9	22.8

Note: Units of the standard deviations are logarithms to the base 10 of the original measurements. DF, degrees of freedom. $N = 42$ species.

Table 3. Correlations of genome size with other life-history traits at the hierarchical levels of species, genus, and family, and among species within the order Primates (total) based on mean

		Mean square correlations with genome size				
Source	DF	Body mass	Brain mass	Gestation period	Age at maturity	Life span
Total	41	–0.169	–0.001	0.09	–0.016	–0.110
Family	12	–0.204	–0.010	0.074	–0.195	–0.203
Genus	21	–0.195	0.264	0.267	0.381	0.061
Species	8	0.847	0.469	0.202	–0.263	–0.382

Note: The analysis was conducted using SAS Proc Nested. $N = 42$.

taxonomic levels indicates that phylogenetically independent contrasts are most appropriate for these data. However, the mismatch in the distribution of the variance in genome size compared with that in other life-history traits suggests that one should not expect close coevolution among these traits.

Product–moment (Pearson) correlations of genome size with other life-history traits at each level in the taxonomic hierarchy are presented in Table 3. The only significant correlation in the table was the relationship between genome size and body mass at the level of species within genera ($P < 0.01$). This relationship is driven by the correlation within the genus *Macaca* ($r = 0.883$, $P = 0.020$, $N = 6$). No other correlations were significant (all $P > 0.28$).

To further explore the relationship among the variables, we subjected the correlation matrix of the log-transformed trait variables to a factor analysis, which constructs derived, orthogonal, uncorrelated axes (equivalent to principal components) that are linear combinations of the original variables. The axes are normalized by the standard deviations of the variables and the total variance in the dataset is equal to the number of variables ($N = 6$). The eigenvalues of the first two axes (4.10 and 1.05) represented 85.7% of the total variance. The factor pattern in Table 4 represents the correlation of each original variable with the first two derived axes, or factors. The factor analysis shows that the life-history variables are highly intercorrelated, but also that they are independent of genome size. A varimax factor rotation (not shown), which maximizes the contrast in the distribution of variance among the first n factors, did not alter this pattern. The factor analysis also suggests that most of the variation in primate life histories is associated with body size and that orthogonal variation in development time, time to maturity, and life span is minimal.

To test the hypothesis that development time is influenced by genome size, when the effect of body mass is factored out, we performed multiple regressions, relating gestation period, age at sexual maturity, and life span to body mass, brain mass, and genome size. C value was not a significant effect for any of the dependent time-related variables $P > 0.25$ for all).

Table 4. Factor pattern based on the correlation matrix for genome size and 5 life-history variables.

Trait	Factor 1	Factor 2
Genome size	–0.058	0.989
Body mass	0.927	–0.141
Brain mass	0.966	0.039
Gestation period	0.836	0.201
Age at maturity	0.909	0.052
Life span	0.879	–0.074

Note: The values in the table are the correlations of each of the original variables with the first 2 derived axes.

We performed a stepwise multiple regression of genome size on the other variables to determine if genome size can be predicted by some combination of primate traits. We found that genome size is significantly related ($F_{2,39} = 5.3$, $P = 0.009$, $R^2 = 0.215$) to body mass (slope of the regression, $b = –0.102 \pm 0.031$ SE, $F = 10.7$, $P = 0.0023$) and brain mass ($b = 0.108 \pm 0.036$ SE, $F = 9.24$, $P = 0.0042$). When 3 outlying values for genome size were deleted from the analysis, stepwise regression revealed no significant relationships between genome size and the independent variables.

Independent contrasts

Independent contrasts were calculated for 29 nodes in the phylogenetic tree relating the primate species in this analysis. The correlation of contrasts for genome size with con-

Table 5. Pearson and Spearman correlation coefficients relating contrasts for genome size to contrasts for body mass, brain mass, gestation period, age at sexual maturity and life span.

Correlation	Sample size	Body mass	Brain mass	Gestation period	Age at maturity	Life span
Pearson	29	–0.534	–0.215	0.261	–0.081	–0.249
Probability		0.003	0.263	0.172	0.676	0.193
Spearman	29	–0.156	–0.045	0.126	–0.168	–0.076
Probability		0.419	0.818	0.516	0.382	0.695
Pearson (outliers removed)	27	–0.013	0.122	0.266	–0.063	0.058
Probability		0.95	546	0.18	0.756	0.773

trasts for the other life-history variables revealed significant negative relationships with product–moment (Pearson) correlations between genome size and body mass and brain mass (Table 5). However, Spearman rank correlations were not significant for any of the life-history variables (all $P >$ 0.38), suggesting that the significant Pearson correlations were driven by outliers. When two outlier values (>3 standard deviations from the mean) for contrasts for C values were removed, none of the Pearson correlations, or any of the Spearman correlations, was significant.

Discussion

Recent studies have attempted to clarify the respective roles of natural selection and stochastic changes in genome evolution (Gregory 2002*a*, 2002*b*, 2004). It appears that genome size is related to cell volume and cell division in the 5 classes of vertebrates (Gregory 2001*a*, 2001*b*). However, the organism-level expression of these cytological effects vary among groups with differing metabolic and developmental characteristics (Gregory 2002*a*, 2003).

If genome size influenced such important features of an organism as cell development rate and metabolic rate, this would have fitness consequences for the organism and presumably would constrain the evolution of genome size. Accordingly, one would expect genome size to be related to variation in life-history traits among species. In support of Gregory's (2002*b*) analysis of genome size and development time in birds and mammals, we have failed to produce compelling results to support a relationship between genome size and several life-history traits in primates. Most of the variation in genome size among primates was concentrated at the taxonomic level of genera within families. Although variation among species within genera was low, this is a consequence of the fact that most of the genera were represented by single species. Thus, some of the variation in genome size among primates expressed at the level of genera within families might actually reside at the level of differences among species within genera. This raises the possibility that some of the variation in genome size might be measurement error, thereby reducing the biologically interesting variation in genome size among primates even further than it is already.

The Animal Genome Size Database maintained by T.R. Gregory (http://www.genomesize.com/) lists 111 genome size values for primates, representing 65 species in 36 genera, 11 of which include more than 1 species in the database. C values range from 2.26 to 5.26 pg with an average of 3.61 and a standard deviation of 0.55. No outliers within this range are indicated. The $\log_{10}$ of genome size has a mean of 0.553, a standard deviation of 0.065, with minimum and maximum values of 0.354 and 0.721, respectively. The skewness of $\log_{10}$ genome size (0.022) suggests that the distribution has no asymmetry, and the kurtosis (1.24) indicates mild leptokurtosis, with 74 of 111 values between 0.50 and 0.60. The database has multiple values for many species, and so it is possible to examine the distribution of variation, including the within-species (repeatability or measurement error) component. In this analysis the variation is distributed as follows: families within Primates (18.2%, 9 degrees of freedom (DF)), genera within families (36.0%, 26 DF), species within genera (0.0%, 35 DF), and measurements within species (45.8%, 40 DF). Thus, a substantial part of the variation in genome size at the species level is measurement error (or, less likely but still possibly, real intraspecific variation); variation among species within genera is not associated with an additional component of the variance. Thus, the biologically meaningful variation in genome size in primates is no more than 54% of the total (equivalent to a standard deviation of 0.048, or ± ~12%), about two thirds of which resides at the level of genera within families and one third at the level of families within the order Primates.

The reason that genome size may be unrelated to life history evolution in primates is its low variability. Outside of the outliers for C values in our dataset (though not necessarily among all primates), the range of genome sizes among 42 species of primates was only 3.08 (*Hylobates lar*) to 4.32 (*Nasalis larvatus*), a factor of about 1.4. To fully appreciate the narrowness of this range, it can be compared with that of body size, which varied from 0.084 kg in *Microcebus murinus* to 93 kg in *Gorilla gorilla*, a factor of more than 1000. By comparison with some other orders of vertebrates, genome sizes in salamanders (order Urodela) vary between 14 pg in *Desmognatuis wrighti* to 120 pg in *Necturus* spp. (9 fold) and genome sizes in frogs (order Anura) vary between 0.95 pg in *Limnodynastes ornatus* to 11.5 pg in *Bombina bombina* (12 fold). Evidently, considerable variation in body mass and associated life-history traits can occur within a clade of mammals with little change in genome size (Gregory 2002*b*). It is also relevant that most of the variation in primate life-history traits resides at the level of families within the order Primates. The one exception to this is life span, which is so poorly estimated in many species that much of the variation at lower taxonomic levels may actually represent measurement error. With a serious mismatch in the levels at which variation in genome size and life-

history traits are expressed, a close connection between their evolution and certainly strong statistical relationships between these traits seems unlikely.

Recent studies have challenged some ideas about genome size evolution. For example, a relationship between genome size and longevity in birds was proposed by Monaghan and Metcalfe (2000) in a family-level analysis, but rejected by Ricklefs and Scheuerlein (2001) in a species-level analysis using a measure of actuarial senescence (the Weibull aging parameter), by Morand and Ricklefs (2001) in an independent contrasts analysis of species-level data of maximum life span, and by Gregory (2002*a*) in an analysis using Pearson correlations performed independently at the species, genus, family, and order levels on species values for maximum life span. Furthermore, Gregory (2002*b*) showed that the dataset of Monaghan and Metcalfe was not reliable. Gregory (2002*b*) also emphasized that relationships of genome size to life-history traits vary among groups. For example, although genome size varies in relation to body mass in mammals as a whole, it does not in Primates, as we have found in this study, and in several other orders of mammals.

Metabolic rate correlates with genome size in birds and mammals in relation with erythrocyte size (Vinogradov 1995; Gregory 2002*a*), which suggests that the effect of the surface-to-volume ratio of erythrocytes on gas exchange translates to the organism level in endotherms. The fact that genome size varies so little in primates confirms that life-history traits, which exhibit much greater variation, are not influenced by C value in this group, although they are in others such as amphibians. It also suggests that additional constraints may regulate genome size and that metabolism likely plays a role.

It would be helpful at this point to have a better understanding of the direct implications of genome size for cellular processes so that we can determine the mechanisms by which genome size influences individual fitness and how these influences might constrain life history evolution (Gregory 2001*a*).

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References

Carey, J.R., and Judge, D.S. 2000. Longevity records: life spans of mammals, birds, amphibians, reptiles, and fish. Monographs on population aging. Vol. 8. Odense University Press, Odense, Denmark.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. **125**: 1–15.

Fleagle, J.G. 1999. Primate adaptation and evolution. Academic Press, New York, N.Y.

Garland, T. Jr., Harvey, P.H., and Ives, A.R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Sys. Biol. **41**: 18–32.

Gregory, T.R. 2001*a*. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol. Rev. **76**: 65–101

Gregory, T.R. 2001*b*. The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates. Blood Cell Mol. Dis. **27**: 830–843.

Gregory, T.R. 2002*a*. A bird's-eye view of the C-value enigma: genome size, cell size, and metabolic rate in the class *Aves*. Evolution, **56**: 121–130.

Gregory, T.R. 2002*b*. Genome size and developmental parameters in the homeothermic vertebrates. Genome, **45**: 833–838.

Gregory, T.R. 2003. Variation across amphibian species in the size of the nuclear genome supports apluralistic, hierarchical approach to the C-value enigma. Biol. J. Linn. Soc. **79**(2): 329–339.

Gregory, T.R. 2004. Macroevolution, hierarchy theory, and the C-value enigma. Paleobiology, **30**: 179–202.

Gregory, T.R., and Hebert, P.D.N. 1999. The modulation of DNA content: proximate causes and ultimate consequences. Genome Res. **9**: 317–324.

Harvey, P.H., and Clutton-Brock, T.H. 1985. Life history variation in primates. Evolution, **39**: 559–581.

Harvey, P.H., and Pagel, M. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford, UK.

Licht, L.E., and Lowcock, L.A. 1991. Genome size and metabolic rate in salamanders. Comp. Biochem. Physiol. **100B**: 83–92.

Monaghan, P., and Metcalfe, N.B. 2000. Genome size and longevity. Trends Genet. **16**: 331–332.

Morand, S., and Ricklefs, R.E. 2001. Genome size, longevity and development time in birds. Trends Genet. **17**: 567–568.

Ohno, S. 1972. So much "junk" DNA in our genome. *In* Evolution of genetic systems. *Edited by* H.H. Smith. Gordon and Breach, New York, N.Y. pp. 366–370.

Pagel, M., and Johnstone, R.A. 1992. Variation across species in the size of the nuclear genome supports the junk-DNA explanation for the C-value paradox. Proc. R. Soc. Ser. B. **249**: 119–124.

Petrov, D.A. 2001. Evolution of genome size: new approaches to an old problem. Trends Genet. **17**: 23–28.

Price, T. 1997. Correlated evolution and independent contrasts. Philos. Trans. R. Soc. Lond. Ser. B. Biol. Sci. **352**: 519–529.

Purvis, A. 1995. A composite estimate of primate phylogeny. Proc. R. Soc. Ser. B. **348**: 405–421.

Purvis, A., and Rambaut, A. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. CABIOS, **11**: 247–251.

Ricklefs, R.E., and Scheuerlein, A. 2001. Comparison of age-related mortality among birds and mammals. Exp. Gerontol. **36**: 845–857.

Ricklefs, R.E., and Starck, J.M. 1996. The application of phylogenetically independent contrasts: a mixed progress report. Oikos, **77**: 167–172.

Vinogradov, A.E. 1995. Nucleotypic effect in homeotherms: body mass-corrected basal metabolic rate of mammals is related to genome size. Evolution, **49**: 1249–1259.