Did Natural Selection or Genetic Drift Produce the Cranial Diversification of Neotropical Monkeys?

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abstract: A central controversy among biologists is the relative importance of natural selection and genetic drift as creative forces shaping biological diversification (Fisher 1930; Wright 1931). Historically, this controversy has been an effective engine powering several evolutionary research programs during the last century (Provine 1989). While all biologists agree that both processes operate in nature to produce evolutionary change, there is a diversity of opinion about which process dominates at any particular organizational level (from DNA and proteins to complex morphologies). To address this last level, we did a broadscale analysis of cranial diversification among all living New World monkeys. Quantitative genetic models yield specific predictions about the relationship between variation patterns within and between populations that may be used to test the hypothesis that genetic drift is a sufficient explanation for morphological diversification. Diversity at several levels in a hierarchy of taxonomic/phylogenetics relationship was examined from species within genera to families within superfamilies. The major conclusion is that genetic drift can be ruled out as the primary source of evolutionary diversification in cranial morphology among taxa at the level of the genus and above as well as for diversification of most genera. However, drift may account for diversification among species within some Neotropical primate genera, implying that morphological diversification associated with speciation need not be adaptive in some radiations.

Keywords: adaptation, morphological evolution, evolutionary processes, Platyrrhini, quantitative genetics.

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While biologists agree that the process of natural selection is the predominant source of morphological diversification, large-scale multivariate tests of this hypothesis have not been attempted. One notable exception is Lynch's work (1990) comparing univariate evolutionary rates for morphological characters in mammals. However, given that such rates are time dependent, the punctuated nature of morphological evolution attributable to the existence of adaptive landscapes results in low-rate magnitudes over large temporal scales, a problem difficult to circumvent in univariate tests (Lemos et al. 2001). Even if it is recognized that interspecific variation in complex morphological structures like the mammalian skull is often adaptive, there is always the possibility that neutral evolution by genetic drift might be responsible for observed morphological diversity. Therefore, instead of assuming that morphological variation is adaptive, we start by testing the null hypothesis that genetic drift is a sufficient explanation for observed multivariate morphological diversification. It is important to consider this question in a multivariate context so that genetic constraints on evolution (Arnold 1992) are accounted for in the analysis. Failure to reject the null hypothesis of genetic drift suggests that morphological diversification may be nonadaptive. In contrast, if the genetic drift model is rejected, the logical alternative is that natural selection was primarily responsible for morphological diversification. However, uniform differences in selection on all traits will result in patterns of interspecific divergence that mimic the pattern expected under drift, so failure to reject the null hypothesis of genetic drift as a cause of morphological diversification is not fully definitive of the absence of selection.

Quantitative evolutionary theory predicts that the amount of divergence in morphological features among groups produced by genetic drift should be proportional to their degrees of variation in the ancestral population (Clayton and Robertson 1955; Lande 1979), which are best estimated by the pooled-within-groups additive genetic variance/covariance (V/CV) matrix (**G** matrix; Lande

1979). This theoretical result is based on certain assumptions (Lande 1979, 1980; Turelli 1988; Barton and Turelli 1989), including the following: the absence of divergent selection; a constant input of additive pleiotropic mutations that maintain the mean phenotypes and the additive genetic variances and covariances, resulting in roughly constant or proportional **G** matrices; and the fact that changes in mean phenotype are caused by genetic changes rather than by direct phenotypic responses to changing environmental conditions (Lande 1979; Turelli et al. 1988). Accurate estimates of the **G** matrix require hundreds or even thousands of genealogically related animals for each population compared, an obstacle that has hampered large-scale tests of the processes involved in phenotypic evolution. However, phenotypic variance/covariance matrices (**P** matrices) may be a substitute for their associated **G** matrices if the **P** matrices are similar or proportional to their genetic counterparts. Meta-analyses of **G** and **P** matrices have indicated that they are typically proportional to one another for sets of morphological traits (Cheverud 1988, 1996; Roff 1995, 1997; Koots and Gibson 1996). We tested this assumption previously for New World monkey (NWM) cranial variation (Cheverud 1996; Marroig and Cheverud 2001), supporting our use of **P** matrices in this study of Platyrrhine cranial variation.

New World monkeys are anthropoid primates of the infraorder Platyrrhini, the sister group of the Old World monkeys (Catarrhini) that also includes apes and humans. The evolutionary diversification of NWM occurred over a period of more than 30 million years (at least since the Oligocene). They are particularly well suited for a broad-scale inquiry into evolutionary processes for several reasons. They are taxonomically diverse, including at least 110 species (Rylands et al. 2000), and show remarkable variation in body size, ranging over two orders of magnitude from the smallest pygmy marmoset (*Cebuella*) to the woolly spider monkey (*Brachyteles*; Hershkovitz 1977; Fleagle 1988). They feed on a variety of animal and plant items and live in virtually all Neotropical forest types (Coimbra-Filho and Mittermeier 1981; Mittermeier et al. 1988). Their phylogenetic relationships are well resolved for all taxa above the genus, and they have had a relatively stable taxonomy at the generic level (Schneider and Rosenberger 1996; Marroig and Cheverud 2001; Schneider et al. 2001). In addition, while existing diversity of species within genera is generally agreed on, phylogenetic relationships among these closely related species are often unknown (Rylands et al. 2000). Besides the diversity of habits and habitats, NWM also display diverse skull morphologies that appear, at least in some cases such as the Pitheciini fruit-seed predators, to present adaptations to their ways of life (Rosenberger et al. 1996). However, studies associating skull morphology with function or diet are still lacking (Kay 1984), and formal tests of evolutionary processes responsible for this diversity are also lacking. Furthermore, the evolutionary basis of morphological variability among species within genera remains unexamined. We specifically test whether or not morphological evolution in the NWM at several taxonomic/phylogenetic levels can be explained by genetic drift.

Methods

The skull sample description, classification, and general methods used to obtain the within-groups V/CV matrices (**W** matrices) are described elsewhere (Marroig and Cheverud 2001). Figure 1 shows the 36 skull landmarks taken on each of the 5,222 NWM skulls using a Polhemus 3Draw digitizer, and table A1 in the online edition of the *American Naturalist* defines these landmarks. Table 1 defines the 39 traits (distances between landmarks) used here along with a classification of those traits according to functional/developmental groups (for details, see Cheverud 1995). Details of the statistical analyses are found in Ackermann and Cheverud (2002). We outline here the general theoretical background for the test of genetic drift as a source of observed interspecific phenotypic diversification. The pattern and magnitude of variation within a population affect variation between diverging species since evolutionary forces rely on intraspecific variation as the fuel for population diversification. If genetic drift is the sole evolutionary force operating, the amount of observed phenotypic divergence between extant population means is expected to be proportional to the initial pattern and amount of variation in the ancestral population. As an analogy to evolution by drift, imagine a diffusion process where the dispersions of the population means in all traits evolving randomly along the available number of dimensions in morphospace are proportional to the initial amount of variation for each trait (variance) and their associations (covariances). Theoretical details might be found elsewhere (Lande 1979, 1980; Lofsvold 1988), but the important point here is to note that drift might be evaluated by a simple comparison of the amount and pattern of variation within and between groups measured in the form of variance/covariance matrices. It is also important to note that the relationship between within- and between-taxon variation does not depend on the structure of the phylogenetic tree within the taxon under consideration; it depends only on the fact that the group is monophyletic.

The possibility of obtaining the observed pattern of morphological differentiation by genetic drift can therefore be evaluated by comparing within- and between-population V/CV matrices (Lande 1979, 1980; Lofsvold 1988; Ackermann and Cheverud 2002). To simplify this com-

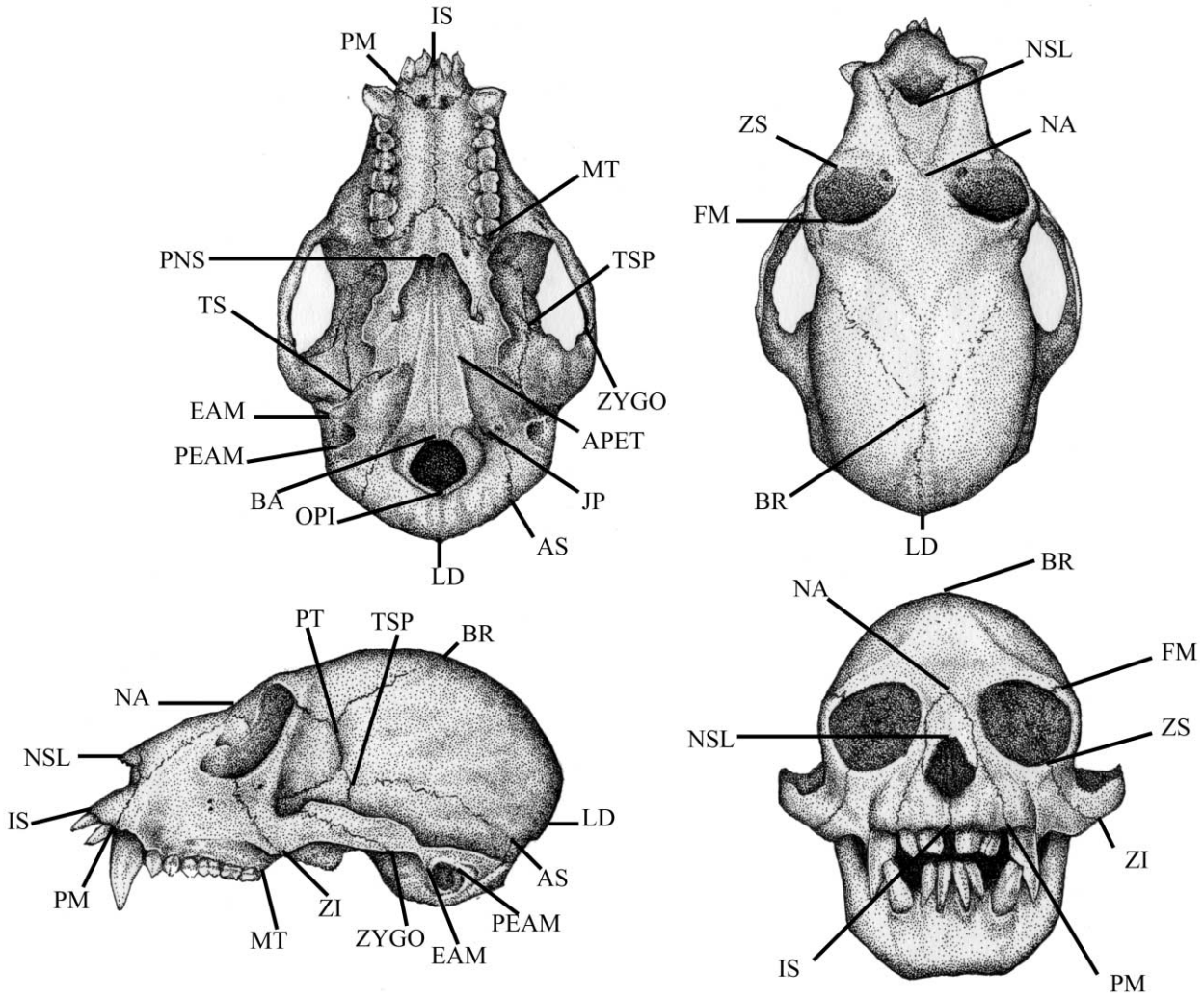

Figure 1: Craniofacial landmarks recorded from New World monkey skulls using three-dimensional digitizer. Refer to table A1 in the online edition of the *American Naturalist* for description of landmarks.

parison, **W** matrices are reduced to their principal components (PCs). The PCs of the within-population V/CV matrix are ordered by their level of variance (eigenvalues) and are uncorrelated with one another so that on the scale of the principal components, the **W** matrix is a simple diagonal matrix with no covariances among components. Principal component scores are calculated for each population by multiplying trait means by the standardized within-population PC loadings. The between-population variance for each principal component can then be calculated as the variance among population mean PC scores.

If diversification occurred through genetic drift, the between-population variances for within-population principal component scores should be proportional to the within-population variances given by the eigenvalues. On a logarithmic scale, we can write the relationship of between- and within-population variances as a linear regression with

$$\ln B_i = \ln\left(\frac{t}{N_e}\right) + \beta \ln (W_i), \tag{1}$$

where B_i is the between-population variance and W_i is the within-population variance for the ith eigenvector, t is the time in generations, and N_e is the effective population size (Ackermann and Cheverud 2002). If differentiation was produced by genetic drift, we expect a regression slope (β) of 1.0 for the regression of between- on within-population variance. A significant deviation from a slope of 1.0 in-

Table 1: First seven principal components extracted from the pooled-within-genera (**W**) variance/covariance matrix

Trait	Functional/developmental group	1	2	3	4	5	6	7
ISPM	Oral	.06	.01	.05	.00	.02	.00	.04
ISNSL	Nasal	.13	.02	**.14**	**−.14**	.02	−.06	−.01
ISPNS	Oral, nasal	.22	.04	**.19**	.05	**.18**	.00	**.32**
PMZS	Oral	.15	.02	**.16**	.00	.09	−.09	.08
PMZI	Oral	.17	.03	**.15**	**.19**	**.19**	**−.29**	.07
PMMT	Oral	.12	.03	.09	.09	.08	−.03	**.11**
NSLNA	Nasal	.08	.00	.04	.07	**.17**	.02	.07
NSLZS	Nasal	.12	.01	.09	.04	.09	−.06	**.16**
NSLZI	Oral, nasal	.21	.03	**.16**	**.16**	**.19**	**−.23**	**.10**
NABR	Cranial vault	.21	**.54**	−.11	−.01	**−.37**	−.07	.05
NAFM	Orbit	.12	.01	.06	.01	.00	.01	.09
NAPNS	Nasal	.17	.02	.09	.06	**.12**	.02	**.13**
BRPT	Cranial vault	.12	**.40**	−.11	**.23**	**−.36**	−.03	**.11**
BRAPET	Cranial vault	.12	**.13**	−.01	**.24**	**−.23**	**.21**	**−.16**
PTFM	Orbit	.03	**.13**	**.36**	−.10	−.02	.05	**−.11**
PTAPET	Cranial vault	.20	**−.14**	**−.29**	.05	.01	−.01	−.04
PTBA	Cranial vault	.32	**−.14**	**−.24**	.04	.00	−.01	.00
PTEAM	Cranial vault	.26	**−.15**	**−.26**	−.02	−.02	−.07	−.04
PTZYGO	Zygomatic	.28	**−.18**	**−.22**	**−.21**	.13	.07	−.01
PTTSP	Cranial vault, zygomatic	.16	**−.18**	**−.38**	−.01	.07	−.04	−.04
FMZS	Orbit	.06	.00	−.01	−.09	−.04	.06	−.10
FMMT	Zygomatic	.22	.02	**.13**	−.08	.03	.03	−.10
ZSZI	Oral	.11	.02	.08	.14	**.14**	**−.15**	.02
ZIMT	Oral	.12	.02	.09	.01	.03	−.07	−.08
ZIZYGO	Zygomatic	.19	.00	.06	**−.39**	−.10	**.50**	**.32**
ZITSP	Zygomatic	.21	.01	**.18**	**−.32**	−.03	**.22**	**−.19**
MTPNS	Oral	.09	.01	.05	−.03	.00	.00	.02
PNSAPET	Cranial base	.15	.01	**.11**	**−.17**	−.09	−.07	**−.37**
APETBA	Cranial base	.13	.00	.07	−.02	.02	.02	.05
APETTS	Cranial base	.05	.01	.01	.03	−.01	.01	.06
BAEAM	Cranial base	.12	.02	.04	.03	−.01	.04	.07
EAMZYGO	Zygomatic	.14	.02	**.14**	−.05	−.04	**−.28**	**−.56**
ZYGOTSP	Zygomatic	.16	.00	**.13**	**−.17**	.04	.02	−.08
LDAS	Cranial vault	.05	**.10**	.00	**.26**	.13	**.28**	−.08
BRLD	Cranial vault	.07	**−.59**	**.36**	**.37**	**−.50**	**.14**	.02
OPILD	Cranial vault	.07	**.15**	−.05	**.40**	**.35**	**.52**	**−.33**
PTAS	Cranial vault	.28	**−.10**	**−.15**	.10	**−.21**	−.10	.03
JPAS	Cranial base	.09	.01	.04	.09	.01	.07	.01
BAOPI	Cranial base	.01	.01	.00	.01	−.08	−.03	.06
Eigenvalue		20.35	8.15	7.36	3.75	3.30	3.12	2.43
% of the variance		29.58	11.85	10.70	5.45	4.80	4.54	3.54

Note: The classification of the 39 distances between landmarks in functional/developmental groups is also presented. Boldface indicates more extreme loadings values.

dicates a pattern not likely to have been produced by genetic drift. Regression slopes above 1.0 indicate that one or more of the first few PCs (most often the first PC) are more variable, relative to the other PCs, than expected under genetic drift. This could occur through diversifying selection for the highly variable PC or by stabilizing selection on the later PCs but not on the highly variable dimension. Slopes significantly <1 occur when species are relatively highly divergent along minor PCs. Again, this can occur through strong diversifying selection along these dimensions or stabilizing selection on the remaining PCs. The ratio of time and effective population size (t/N_e) is the regression constant and does not alter the expectation of 1.0 for the regression slope. Under a strictly neutral

evolutionary model, increasing divergence time will also increase the dispersion among groups and consequently the regression constant, leaving the expectation for the regression slope unchanged.

The between-population V/CV of the within-population eigenstructure could also deviate from proportionality if the principal component scores of the diverging populations are correlated with one another. By definition, the within-population principal components are uncorrelated, and if genetic drift causes population diversification, we expect the mean between-population principal component scores to remain uncorrelated. However, correlations among between-population PC scores could arise by correlated selection (coselection) on these dimensions. The between-population V/CV matrix (**B**) expected under diversifying directional selection is

$$\mathbf{B} = \mathbf{GCG}, \tag{2}$$

where **G** is the genetic V/CV matrix and **C** is the V/CV matrix among selection gradients for the traits (Felsenstein 1988). Within-population PCs are, by definition, uncorrelated so that **G** is a diagonal matrix. Thus, any correlations evident in **B** must arise from **C** (selective covariance in Felsenstein 1988), correlations between traits' selection gradients (coselection from now on). This indicates a tendency within a clade for certain morphological features to be coselected.

We test for significant correlations among the first several principal component scores for each comparison involving four or more taxa. As a general rule, we correlate $n - 1$ number of PCs, with n being the number of taxa in the analyses. We apply two criteria in deciding whether or not significant correlation among PC scores exists. First, we inspect univariate probabilities, and second, we look at the Bartlett χ^2 test probabilities available in SYSTAT 10. The latter tests a global hypothesis concerning the significance of correlation in the matrix. However, because of the small samples of taxa used here and potential nonnormality of the between-species means, we used it here only as a guide to possible significant correlations in the matrix. Whenever significant probabilities were found by the Bartlett χ^2 test and for at least one pair of PC scores, we rejected the null hypothesis of genetic drift. Since several proportionality tests are considered at each level of the taxonomic hierarchy, tests at the same level will be corrected for multiple comparisons using the Bonferroni criterion. Of course, the power to detect deviations from a slope of 1 for the regression of between- on within-taxon V/CV matrices or nonzero between-population correlations among within-population V/CV matrix principal components depends critically on the number of taxa included in the comparison. This, in turn, is controlled by the outcome of evolutionary diversification processes.

Results

The first seven PCs of the pooled-within-genus V/CV matrix are provided in table 1. Together, these PCs account for 70.4% of the total within-genus variation. The first PC is an allometry vector representing variation in cranial size and associated shape. The second PC is basically a within–cranial vault factor and contrasts traits within this functional/developmental group. Specifically, PC2 represents a factor where an enlarged frontal bone is associated with a smaller posterior vault, both in length and height, with landmarks bregma being dislocated posteriorly and pterion (PT) inferiorly. The third PC contrasts oral/zygomatic with cranial vault traits being essentially a masticatory factor involving also a repositioning of the landmark PT in the cranial vault. The fourth PC contrasts zygomatic with cranial vault traits, with smaller zygomatic group distances associated with larger cranial vault distances and vice versa. The fifth PC contrasts oral/nasal elements again with cranial vault traits. The sixth PC contrasts a few oral traits involving landmark zygomaxillare inferior with zygomatic and vault traits. The seventh PC contrasts oral/nasal traits with cranial vault and base and might be interpreted as a face versus neurocranium factor. Interestingly, these seven PCs obtained from the pooled-within-genus V/CV matrix are nearly identical to those obtained from the pooled **W** matrices at the subfamily and among-families levels (vector correlations all higher than 0.98). Thus, the interpretation of these factors given above also applies to those levels. We also calculate vector correlations between these seven PCs and those obtained separately from genus-specific **W** matrices. The same basic pattern of factors is found, especially for the first 2 PCs, even though sometimes the PCs are extracted in different orders. For example, PC6 of the *Cebus* **W** matrix had a vector correlation of 0.88 with PC5 of the among-genus matrix, while the reverse correlation between PC5 (*Cebus*) and PC6 (among genera) is 0.95. Table 2 presents the PC correlations for each genus according to their homology with PCs in table 1. Some genera deviate substantially in the less variable of these factors, particularly *Saguinus* and *Saimiri*. We will focus on these first seven PCs (table 1) and interpret nonhomologous PCs as necessary

The combined results of both drift tests (regression and correlation) are depicted on the known relationships among Platyrrhine genera in figure 2 (Schneider 2000; Marroig and Cheverud 2001). A representative cranium is displayed for each of the groups. Species analyzed within each genus may be found in table A2 in the online edition of the *American Naturalist*. Genetic drift is accepted as the

Table 2: Results of regression and correlation drift tests

Taxa	B	95% CI Lower	95% CI Upper	PCs	N	Bartlett	df	P	r	P	Average $\lvert r\rvert$	PCs
Among families	**1.30**	**1.02**	**1.59**	**1, 4, 6**	4	5.4	3	.142	**.949**	**.050**	.922	**1–3**
Within families:												
Atelidae	1.05	.80	1.30	4, 6	**4**	**11.1**	**3**	**.011**	.854	.146	.698	…
Pithecidae	**1.24**	**1.02**	**1.47**	**1, 2, 6**	**4**	**10.6**	**3**	**.014**	−.901	.099	.778	…
Cebidae	**1.26**	**1.07**	**1.45**	**1, 7**	**8**	**56.7**	**21**	**<.001**	**.946**	**<.001**	.711	**1–(2, 3, 4, 5, 6); 2–(3, 4, 5, 6); 3–(4, 6); 4–(5)**
Among subfamilies	**1.34**	**1.18**	**1.50**	**1, 4, 7**	**7**	**56.2**	**15**	**<.001**	**.885**	**.008**	.644	**1–(2, 3, 4); 2–(4, 5); 5–(4, 6)**
Among genera	**1.30**	**1.14**	**1.47**	**1, 4, 5, 7**	**16**	**284.4**	**105**	**<.001**	**.946**	**<.001**	.512	**1–(2, 3, 4, 5, 6, 7); 2–(3, 4, 5, 7); 3–(4, 7); 4–(5, 6, 7); 5–(6, 7)**
Within genera:												
Callitrhix	1.00	.90	1.10	2, 7	**16**	**194.6**	**105**	**<.001**	**.887**	**<.001**	.362	**1–2; 3–(6, 9)**
Amazon clade	**.83**	**.74**	**.93**	**2**	**11**	**72.8**	**45**	**.005**	**.751**	**.008**	.339	**1–9; 3–4**
Atlantic Forest clade	1.04	.87	1.22	2, 3, 5, 6	**5**	**56.4**	**10**	**<.001**	**.927**	**.023**	.464	**1–2**
Saguinus	1.04	.94	1.15	1, 4, 5, 7	**31**	**867.6**	**435**	**<.001**	**.726**	**<.001**	.255	**1–(2, 4); 2–(4); 4–(7)**
Large-bodied clade	.93	.82	1.03	4, 6	**17**	**199.3**	**120**	**<.001**	**−.686**	**.002**	.307	**3–5**
Small-bodied clade	.98	.87	1.08	4, 6	**14**	**142.9**	**78**	**<.001**	**−.815**	**<.001**	.346	**5–7**
Ateles	.96	.81	1.11	5	**4**	**3.3**	**6**	**<.001**	.926	.074	.621	…
Cebus[a]	.97	.84	1.10	5, 6	*10*	*58.8*	*36*	*.010*	**.857**	**.002**	.390	**4–9**
Chiropotes[a]	.91	.77	1.05	3, 4, 6	*4*	*9.2*	*3*	*.024*	**.983**	**.017**	.849	**1–3**
Callicebus	1.07	.95	1.19	1, 7	**13**	**116.1**	**66**	**<.001**	**.867**	**<.001**	.435	**1–(4, 6); 6–(4, 7)**
Alouatta	1.09	.92	1.26	1	6	12.8	10	.237	**−.921**	**.009**	.514	**2–4**
Saimiri	.99	.89	1.10	…	**10**	**61.7**	**36**	**.005**	**−.836**	**.003**	.358	**2–3**
Pithecia	.91	.76	1.06	2, 4, 7	7	18.1	15	.257	.739	.058	.423	…

Note: For the regression test, the regression coefficients along with the 95% confidence interval (CI) are shown as well as the principal components (PCs) that deviate significantly from the regression line. For the correlation test, the number of taxa included (N), the value found in the Bartlett test along with associated degrees of freedom (df), and probability (P) are shown. In addition, the highest individual Pearson's correlation value (r) found in the correlation of PCs and associated probability (P) are shown. The average value of all correlation coefficients found in modulus (average $\lvert r\rvert$) and PCs significantly correlated at 5% are also shown. Regression coefficients significantly different from 1.0 are shown in bold. Significant Bartlett statistics are also shown in bold as well as the highest individual correlation coefficient in each matrix of PCs scores.

[a] Diversification within these genera fail to reach the Bonferroni correction for multiple tests in the Bartlett multivariate significance (in italic) but might represent true deviation from the null model, given the stringency of the Bonferroni criteria as well as the highly individual values of Pearson's correlation (r) and associated P.

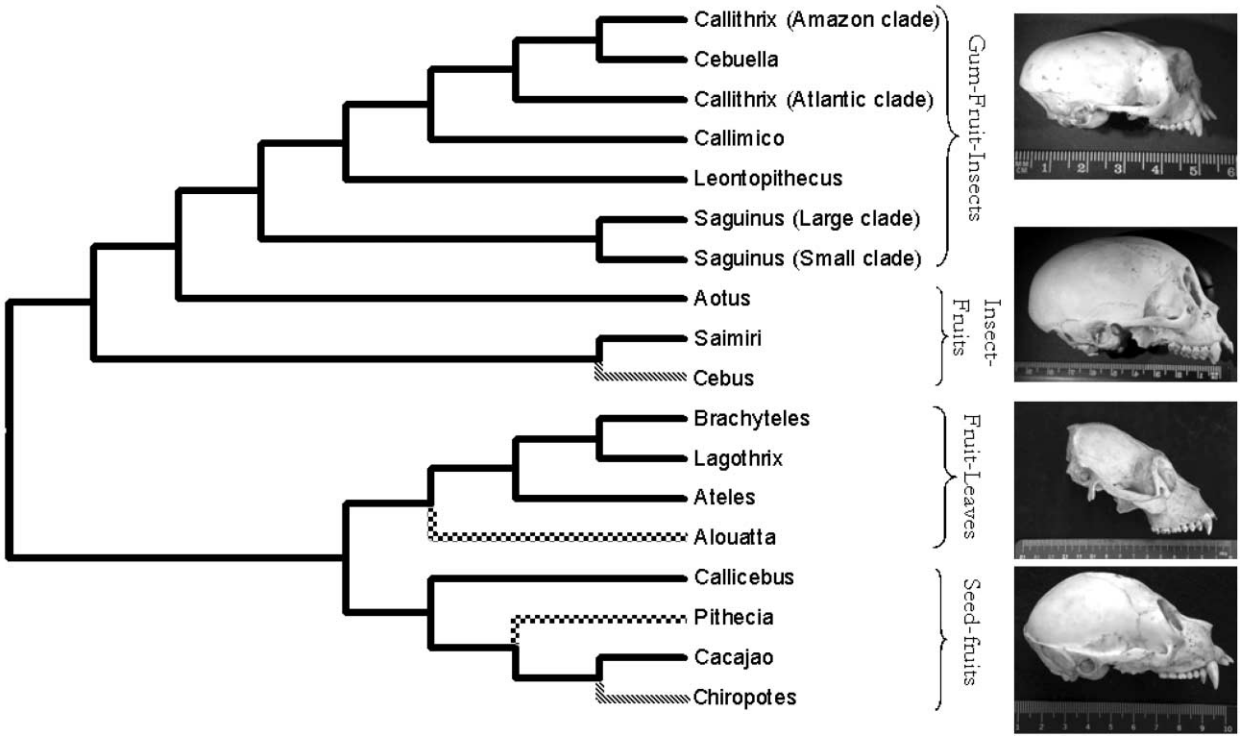

Figure 2: Phylogenetic tree depicting the best-known evolutionary relationships among Platyrrhines. Phylogenetic relationships among species within several genera were not available, and those relationships that were considered as unresolved polytomies are not shown (species analyzed within each genus might be found in table A2 in the online edition of the *American Naturalist*). Checkered lines indicate phenotypic diversification consistent with the null hypothesis of genetic drift, while solid lines depict phenotypic diversification by selection. Diagonal lines represent the two genera with diversification of their species consistent with natural selection at a nominal level of 5%, while one of them may represent a false positive. For simplicity, the 26 species and subspecies of the genus *Saguinus* are not shown, and details of their phenotypic evolution may be found in Ackermann and Cheverud (2002).

source of divergence in those cases where both tests failed to reject the null hypothesis. Because the phylogenetic relationships among species within most genera are unknown, species within genera were tested as in unresolved polytomies. As noted above, this does not affect the interpretation of tests for **W** matrix and **B** matrix proportionality, although it would affect the value of the constant. For marmosets (*Callithrix*), the relationships are those obtained from Tagliaro et al. (1997) and van Roosmalen et al. (2000). For tamarins (*Saguinus*), the relationship between the large and small clades was obtained from Cropp et al. (1999).

Both regression and correlation approaches indicate that morphological evolution above the level of species within genera cannot be explained by genetic drift alone and that natural selection was involved in the diversification of cranial morphology among higher taxa (table 2). Figure 3 shows a typical regression line of **B** on **W** variances and associated 95% confidence limits. Notice that PC1, PC4, and PC5 are above the line, indicating that these PCs deviate significantly from expectations based on drift, being more variable that expected. Conversely, PC7 is below the line, indicating that this factor carries less variation than expected. Basically, the same result is obtained from the regression at the among-subfamilies level, with PC1, PC4, and PC5 above the line and PC7 below it. Figure 4 shows a plot of correlations between PC1 and PC4 at the among-genera level and for PC2 and PC4 at the subfamily level. The pattern of correlations among the first seven PCs is basically the same at the among-genera and among-subfamilies levels (table 2). The correlation of PC1 and PC4 among genera suggests that allometric size along with cranial vault and zygomatic traits were coselected during evolutionary diversification of Neotropical primate genera. In addition, allometric size is correlated with all remaining PCs except PC6. Principal component 6 shows only a moderate correlation with PC4 and PC5. Moreover, except for a low correlation between PC3 and PC5, all remaining correlations among the first seven PCs are significant ($P < .05$), indicating coselection among these factors. Prin-

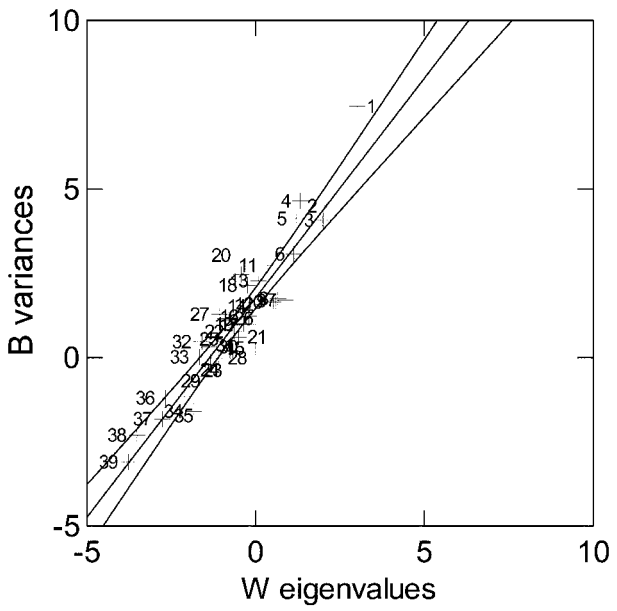

Figure 3: Regression of **B** variances on **W** eigenvalues and associated 95% confidence limits at the among-genus level.

cipal components 5 and 7 are positively correlated with each other but are negatively correlated with all remaining PCs. All these correlations indicate coselection of traits independent of any within-taxon association.

Morphological evolution among species within each of the nine genera with sufficient species and specimens per species to be analyzed shows a mix of divergence by natural selection and genetic drift (fig. 2; table 2). The null hypothesis of genetic drift could not be rejected for any genus by the regression test. The only exception was the Amazon clade within the marmosets (*Callithrix*), which has a regression coefficient significantly <1.0. However, five of the nine genera (*Callithrix*, *Saguinus*, *Ateles*, *Callicebus*, and *Saimiri* as well as both clades within the marmosets and tamarins) showed significant correlation among PC scores after Bonferroni correction (table 2; fig. 5). Two additional genera, *Cebus* and *Chiropotes*, showed significant correlations among PCs at the nominal .05 level but failed to reach significance after Bonferroni correction for multiple comparisons ($P = .05/9$). Out of the nine within-genus comparisons, we would expect at most only one false positive test for correlation among PCs, so it is likely that at least one of these genera also displays divergence through differential selection. If we consider a sequential Bonferroni threshold (Rice 1989), *Cebus* may be included in the significant results because its probability is .010, and that is the same as the sequential Bonferroni threshold ($.05/5 = .01$) result. For two genera, *Alouatta* and *Pithecia*, genetic drift is a sufficient explanation for morphological diversification (fig. 2; table 2). These genera contained six and seven taxa, respectively, and so were not especially lacking in diversity or power to detect deviations from drift-based expectations. However, it is worth noting that PC2 and PC4 present a high and significant negative correlation, indicating that selection might also be acting in howlers (*Alouatta*) despite the nonsignificant multivariate Bartlett test. Thus, genetic drift is sufficient to explain diversification in cranial morphology in some NWM genera.

Discussion

Our results point to an adaptive diversification of cranial morphology among the higher taxa of Platyrrhini. Also, species in most genera apparently diversified in skull morphology by natural selection. The first principal component (allometric size) was above the 95% confidence interval (CI) in all regressions above the within-genera level (except for Atelidae, that was not significantly different from 1.0). Moreover, PC4 is also above the 95% CI and PC7 below the 95% CI in all regressions, while PC6 was below the 95% CI at the among-families level and above it in Pithecidae. These results indicate that there is more variation in allometric size (PC1) and in a factor contrasting zygomatic versus cranial vault traits (PC4) than expected under genetic drift and less than the expected amount of variation in PC7. It is important to keep in mind that our comparisons were phylogenetically structured in that monophyletic groups were examined. However, the degree of relationship or time since origin of the species within a genus may vary among New World monkeys. However, this should not affect the expectations for PC variances and correlations within any specific monophyletic group. Interestingly, removing allometric size (PC1) from the regression analyses results in nonsignificant deviation of the regression coefficients from the null expectation at the among-families level and within the Pithecidae and Cebidae families. This indicates that especially strong divergent selection on size alone was sufficient to produce a significant departure from null drift model at those levels.

All regression tests at the level of species within genera failed to reject the null hypothesis of genetic drift, except for the Amazonian marmoset clade (*Callithrix*), which shows less between-taxon variation than expected given within-taxon variability for the first seven PCs. This means that the level of variation was proportional within and between species, which is as expected under a drift model for most comparisons among species within genera. Traits (PCs) that are relatively highly variable within species are also relatively divergent between species. However, there

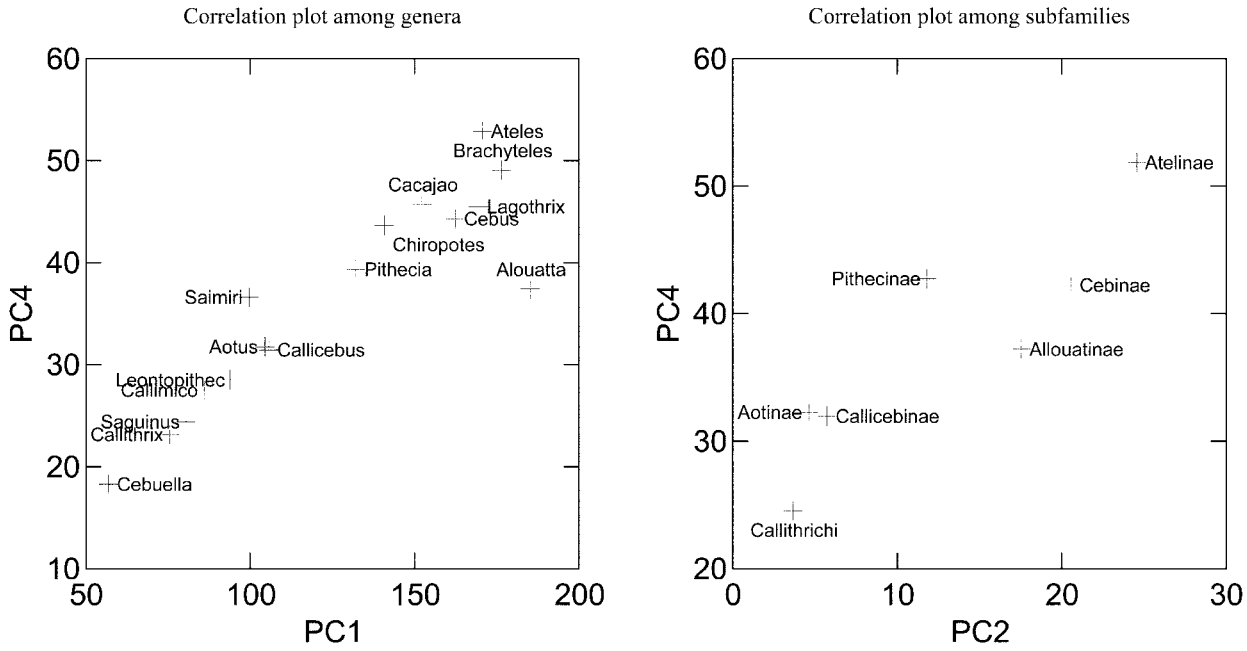

Figure 4: Plot of correlation between PC1 and PC4 at the among-genera level and for PC2 and PC4 at the subfamily level

is significant correlation among PC scores in several of the nine genera tested. This indicates that the highly correlated PCs were coselected during the divergence of species within the genus. Principal components may be coselected when they interact with each other in performing some common function or because of correlated environmental factors separately producing selection on various PCs. The correlated environmental factors may or may not represent functional correlations at the ecological level. For example, one general pattern apparent at all levels (table 2) is the correlation between allometric size and several other PCs. This indicates that these independent morphological dimensions were coselected during the evolutionary diversification of NWM. Perhaps the ecological motor powering diversification was diet based and resulted in overall size changes to adapt NWM to new and specialized dietary (Fleagle 1988) adaptive zones. As a result, not only were size and associated allometries (as defined and measured by PC1) selected, but also other factors involved in mastication (PC3, PC6), the relative size of the face versus the neurocranium (PC4, PC5, PC7), and aspects of cranial vault morphology (PC2) were coselected together with PC1. There is no implication of a common genetic architecture (genetic correlation) among these coselected PCs because they are independent of one another and genes affecting allometric size may be entirely different from those affecting the remaining variation (besides the portion due to allometry) in facial and neurocranial morphology. Instead, correlations of these factors are brought about by the correlation of their selection pressures (**C**). Moreover, correlations among some PCs may reflect cooperation of these factors in performing some function. Size changes might require concomitant morphological adjustments beyond those brought about by allometry to maintain masticatory function, for example, and result in the coselection of PC1 and PC3.

Given the relatively small number of taxa included in some of the comparisons, lack of power may be responsible for failures to reject the null hypothesis of genetic drift. Small numbers of taxa in a unit result in higher standard errors for the slopes in the regression test and require higher correlations among PCs to reach statistical significance. This does not seem to be an especially important factor in the regression tests because none of the within-genus slopes deviate from 1.0 by more than 0.10 units and even the narrowest 95% CI for a generic slope is >0.20. In general, this is also not likely to be responsible for failure to reject the null hypothesis with the PC correlation test, although the failure of *Chiropotes* to reach the Bonferroni-based critical probability threshold may be due to the fact that there are only four taxa in this genus. It is difficult to increase the power to detect deviations from drift in a systematic study because this can only be accomplished by recognizing more taxa per genus. The recognition of subspecies in *Saguinus* leads to the large sample of taxa analyzed here. Recognition of subtaxa or distinct popu-

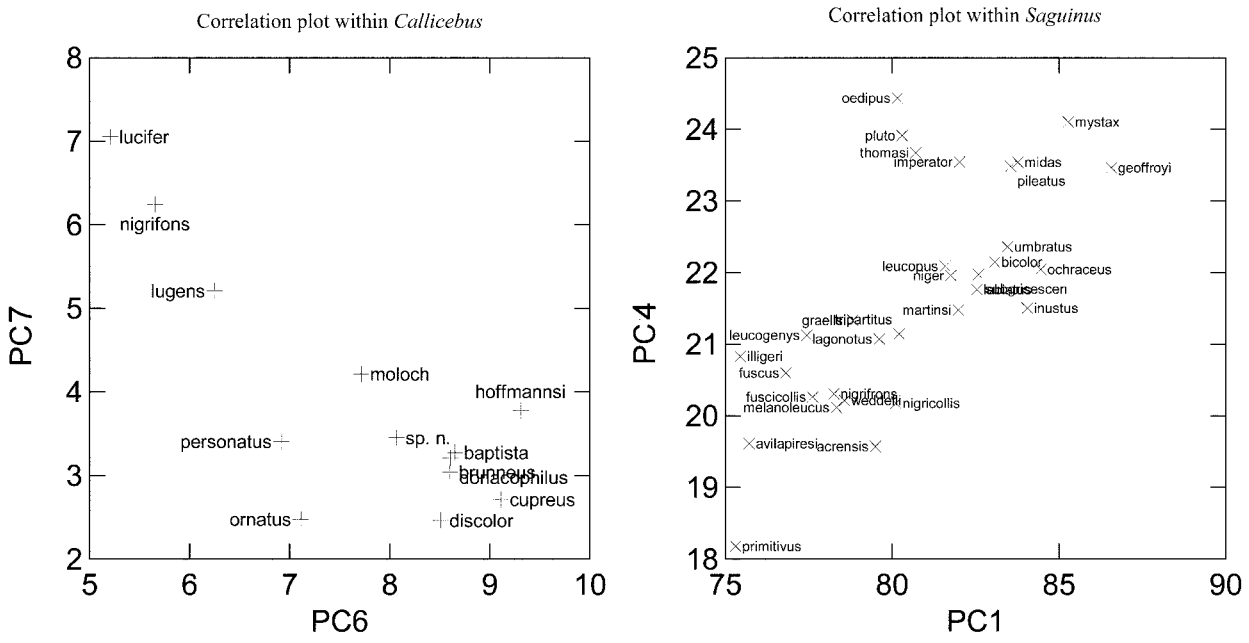

Figure 5: Plot of correlation between PC1 and PC4 for species within *Saguinus* and PC6 and PC7 for species within *Callicebus*

lations within some species could be used to increase the sample of taxa per genus. However, taxonomic sampling is also limited by a paucity of specimens. In this study, sample coverage was as complete as museum collections allowed. However, some species were not included because too few specimens are available for study.

Morphological changes associated with speciation are usually thought of as adaptive, and processes producing taxonomic diversity and phenotypic diversity are considered strongly linked. Speciation either occurs through adaptive diversification (Endler 1977) or is permissive of it. Our results suggest that this linkage often occurs in the NWM, but it is not necessarily as universal as supposed. Morphological evolution among species may, in some instances, be nonadaptive. Our failure to reject the null hypothesis of genetic drift as the source of diversification among *Alouatta* and *Pithecia* species and the failure of *Cebus* and *Chiropotes* species to reach Bonferroni-adjusted significance raise the possibility that neutral evolution of a complex structure like the mammalian skull occurs with an appreciable frequency at the interspecific level. While the Bonferroni correction might be too stringent in casting doubt on otherwise significant results, our results indicate that diversification within one and perhaps two or three genera is consistent with genetic drift. However, natural selection cannot be ruled out as an explanation for diversification even within these genera. While this alternative to the null hypothesis of genetic drift might seem odd at first, the argument is quite simple. Whenever natural selection operates uniformly on all dimensions in the morphological space during diversification, a proportional increase of the between-population variability in relation to within-population variability will result, mimicking the pattern expected under drift. For simplicity, this might be envisaged in terms of the **W** matrix PCs. If selection (or differences in selection between clades in our case) is uniform (all elements or coefficients similar), then a uniform selection gradient times the nonuniform variances will result in a response to selection proportional to the **W** matrix variances. One particular situation where this might happen is selection operating through a common developmental system that imposes common patterns of variation and covariation among traits despite changes in allelic frequencies in the genetic system (Marroig and Cheverud 2001).

Our results can be interpreted within a Simpsonian view of nature based on adaptive zones (Simpson 1953). He argues that bursts of evolution and diversification result from the invasion of new adaptive zones that he defined as a set of similar ecological niches distinct from those occupied by other life forms. Broad adaptive zones might then be further subdivided into narrower zones, these again into subzones, and so on down to narrowest bands equivalent to populations (Simpson 1953). Adaptive zones therefore involve the use of resources (food and space) as well as resistance to predation, parasitism, and competition

occurring within the adaptive zone (Van Valen 1971). Major clades in the Platyrrhini are clearly associated (fig. 2) with broad dietary types (Marroig and Cheverud 2001) such as the gum-insect-fruit type, the insect-fruit type, the seed-fruit type, and the leaf-fruit type, which in turn are also associated with size differences, from the smallest gum-eating marmosets to the largest ateline fruit and leaf eaters (Fleagle 1988). Here, we have shown that the cranial diversification of these clades is not consistent with random differentiation by genetic drift. Perhaps invasion of diet-defined adaptive zones and further subdivision within them was one key unleashing adaptive evolution in the Neotropical primates. Indeed, since we found that intergeneric and higher-level differences were inconsistent with diversification through genetic drift, it could be that the separate genera are recognized as such, in part, because their ancestral species diverged adaptively into different feeding niches. Thus, variation among species within genera in which adaptive divergence has occurred may be the seeds of future generic level differences. However, cranial diversification within some genera appears consistent with random differences produced through genetic drift. This is not to say that speciation was driven by drift but only that morphological evolution in a certain suite of cranial characters is consistent with genetic drift. Unfortunately, there is no detailed information about the amount of variation in diet among species within NWM genera, so correlations of dietary and morphological diversification within genera are not possible at this time. In the future, it will be interesting to evaluate whether or not species in those genera whose cranial diversification is consistent with genetic drift are also less variable in their diets than species within genera that diversified by natural selection.

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Literature Cited

Ackermann, R. G., and J. M. Cheverud. 2002. Discerning evolutionary processes in patterns of tamarin (genus *Saguinus*) craniofacial variation. American Journal of Physical Anthropology 117:260–271.

Arnold, S. 1992. Constraints on phenotypic evolution. American Naturalist 140(suppl.):S85–S107.

Barton, N. H., and M. Turelli. 1989. Evolutionary quantitative genetics: how little do we know? Annual Review of Genetics 23:337–370.

Cheverud, J. M. 1988. A comparison of genetic and phenotypic correlations. Evolution 42:958–968.

———. 1995. Morphological integration in the saddleback tamarin (*Saguinus fuscicollis*) cranium. American Naturalist 145:63–89.

———. 1996. Quantitative genetic analysis of cranial morphology in the cotton-top (*Saguinus oedipus*) and saddle-back (*S. fuscicollis*) tamarins. Journal of Evolutionary Biology 9:5–42.

Clayton, G. A., and A. Robertson. 1955. Mutation and quantitative variation. American Naturalist 89:151–158.

Coimbra-Filho, A. F., and R. A. Mittermeier. 1981. Ecology and behavior of Neotropical primates. Vol. 1. Academia Brasileira de Ciências, Rio de Janeiro.

Cropp, S. J., A. Larson, and J. M. Cheverud. 1999. Historical biogeography of tamarins, genus *Saguinus*: the molecular phylogenetic evidence. American Journal of Physical Anthropology 108:65–89.

Endler, J. A. 1977. Geographic variation, speciation and clines. Princeton University Press, Princeton, N.J.

Felsenstein, J. 1988. Phylogenies and quantitative characters. Annual Review of Ecology and Systematics 19: 445–471.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Fleagle, J. G. 1988. Primate adaptation and evolution. Academic Press, New York.

Hershkovitz, P. 1977. Living New World monkeys (Platyrrhini). University of Chicago Press, Chicago.

Kay, R. F. 1984. On the use of anatomical features to infer foraging behavior in extinct primates. Pages 21–53 *in* J. Cant and P. Rodman, eds. Adaptations for foraging in nonhuman primates. Columbia University Press, New York.

Koots, K. R., and J. P. Gibson. 1996. Realized sampling variances of estimates of genetic parameters and the difference between genetic and phenotypic correlations. Genetics 143:1409–1416.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain : body size allometry. Evolution 33:402–416.

———. 1980. The genetic covariance between characters

maintained by pleiotropic mutations. Genetics 94:203–215.

Lemos, B., G. Marroig, and R. Cerqueira. 2001. Evolutionary rates and stabilizing selection in large-bodied opossums skulls (Didelphimorphia: Didelphidae). Journal of Zoology (London) 255:181–189.

Lofsvold, D. 1988. Quantitative genetics of morphological differentiation in *Peromyscus*. II. Analysis of selection and drift. Evolution 42:54–67.

Lynch, M. 1990. The rate of phenotypic evolution in mammals from the standpoint of the neutral expectation. American Naturalist 136:727–741.

Marroig, G., and J. M. Cheverud. 2001. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology and ontogeny during cranial evolution of New World monkeys. Evolution 55:2576–2600.

Mittermeier, R. A., A. B. Rylands, A. F. Coimbra-Filho, and G. A. B. Fonseca. 1988. Ecology and behavior of Neotropical primates. Vol. 2. World Wildlife Fund, Washington, D.C.

Provine, W. B. 1989. Sewall Wright and evolutionary biology. University of Chicago Press, Chicago.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Roff, D. A. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity 74:481–490.

———. 1997. Evolutionary quantitative genetics. Chapman & Hall, New York.

Rosenberger, A. L., M. A. Norconk, and P. A. Garber. 1996. New perspectives on the Pitheciines. Pages 329–333 *in* M. A. Norconk, A. L. Rosenberger, and P. A. Garber, eds. Adaptive radiations of Neotropical primates. Plenum, New York.

Rylands, A. B., H. Schneider, A. Langguth, R. A. Mittermeier, C. P. Groves, and E. Rodríguez-Luna. 2000. An assessment of the diversity of New World primates. Neotropical Primates 8:61–93.

Schneider, H. 2000. The current status of the New World monkey phylogeny. Anais da Academia Brasileira de Ciencias 72:165–172.

Schneider, H., and A. L. Rosenberger. 1996. Molecules, morphology, and Platyrrhini systematics. Pages 3–19 *in* M. A. Norconk, A. L. Rosenberger, and P. A. Garber, eds. Adaptive radiation of Neotropical primates. Plenum, New York.

Schneider, H., F. C. Canavez, I. Sampaio, M. A. M. Moreira, C. H. Tagliaro, and H. N. Seuanez. 2001. Can molecular data place each Neotropical monkey in its own branch? Chromosoma 109:515–523.

Simpson, G. G. 1953. Major features of evolution. Columbia University Press, New York.

Tagliaro, C. H., M. P. Schneider, H. Schneider, I. C. Sampaio, and M. J. Stanhope. 1997. Marmoset phylogenetics, conservation perspectives, and evolution of the mtDNA control region. Molecular Biology and Evolution 14:674–684.

Turelli, M. 1988. Phenotypic evolution, constant covariances and the maintenance of additive variance. Evolution 43:1342–1347.

Turelli, M., J. H. Gillespie, and R. Lande. 1988. Rate tests for selection on quantitative characters during macroevolution and microevolution. Evolution 42:1085–1089.

van Roosmalen, M. G. M., T. van Roosmalen, R. A. Mittermeier, and A. B. Rylands. 2000. Two new species of marmoset, genus *Callithrix erxleben*, 1777 (Callitrichidae, Primates), from the Tapajós/Madeira interfluvium, South Central Amazonia, Brazil. Neotropical Primates 8:2–18.

Van Valen, L. 1971. Adaptive zones and the orders of mammals. Evolution 25:420–428.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

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