

Discerning Evolutionary Processes in Patterns of Tamarin (Genus *Saguinus*) Craniofacial Variation

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ABSTRACT Quantitative genetic theory specifies evolutionary expectations for morphological diversification by genetic drift in a monophyletic clade. If genetic drift is responsible for the evolutionary morphological diversification of a clade, patterns of within- and between-taxon morphological variance/covariance should be proportional. We tested for proportionality of within- and between-species craniofacial morphological variation in 12 species of tamarins (genus *Saguinus*). We found that within- and between-taxon morphological variations across the entire genus were not proportional, and hence not likely to be due to genetic drift alone. The primary deviation from proportionality is that size and size-related shape in the cranium is more variable relative to other aspects of cranial morphology than expected under genetic drift, sug-

gesting differential size selection between the two major clades, the small-bodied and large-bodied tamarins. Within each of these major clades, most of the interspecific variation is consistent with the pattern expected under genetic drift, although specific contrasts may indicate the involvement of differential selection. Morphological distances among taxa do not correspond very closely to the phylogeny derived from mtDNA. In particular, *S. oedipus* and *S. geoffroyi* are very distinct morphologically from the rest of the tamarins, although they are phylogenetically the sister clade to a clade containing *S. midas* and *S. bicolor*. Morphological similarity is not a good guide to phylogenetic affinity in the tamarins, especially with regard to deeper nodes in the phylogenetic tree. *Am J Phys Anthropol* 117:260–271, 2002. © 2002 Wiley-Liss, Inc.

Patterns of morphological variation within species play an important role in determining patterns of evolutionary diversification. Among one group of New World monkeys, i.e., the tamarins (family Callitrichidae, genus *Saguinus*; von Hoffmannsegg, 1807), analyses of variation in coat color, body size, and craniofacial morphology have all been applied to the task of understanding relationships among populations (Hershkovitz, 1977; Ferrari, 1993a,b; Moore and Cheverud, 1992; Cheverud, 1995, 1996). This study adds to previous work, exploring morphological divergence across the entire genus *Saguinus*, interpreting this divergence in light of known phylogenetic relatedness and hypothesized biogeographical shifts through evolutionary time, and uncovering the evolutionary processes which may have been responsible for morphological diversification.

The pattern and magnitude of variation within a population is tied to the variation between diverging species; evolutionary forces rely on intraspecific variation as fuel for population diversification. According to Lande (1979, 1980) and Lofsvold (1988), the expected dispersal of average population phenotypes through random genetic drift over generations is a function of genetic variation/covariation, the effective size of the evolving populations, and the time since divergence:

$$\mathbf{B}_t = \mathbf{G}(t/N_e) \quad (1)$$

where \mathbf{B}_t is the dispersion matrix, or between-population variance/covariance matrix, in generation t , \mathbf{G} is the additive genetic variance-covariance matrix of the base population from which the group of species is derived, and N_e is the effective population size of the individual taxa. When working with morphological data drawn from contemporaneous populations, the phenotypic within-group variance/covariance (V/CV) matrix (\mathbf{W}) is often proportional to the additive genetic V/CV (Cheverud, 1988; Roff, 1995, 1996; Koots and Gibson, 1996) and may be substituted for it so that:

$$\mathbf{B} \propto \mathbf{W}(t/N_e) \quad (2)$$

Since t and N_e are constants for any particular comparison, the pattern of between-group phenotypic variation should be proportional to the within-group phenotypic variation ($\mathbf{B} \propto \mathbf{W}$), if the populations

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TABLE 1. Sample sizes and museum allocations¹

	N	AMNH	BMNH	FMNH	NMNH	ORAU	MZUSP
<i>S. bicolor</i>	13	×	×	×			×
<i>S. fuscicollis</i>	289	×	×	×	×	×	×
<i>S. geoffroyi</i>	132	×		×	×		
<i>S. imperator</i>	25	×		×			×
<i>S. inustus</i>	7	×		×			
<i>S. labiatus</i>	26	×	×	×	×		
<i>S. leucopus</i>	27	×	×	×	×		
<i>S. midas</i>	116	×	×	×	×		
<i>S. mystax</i>	72	×	×	×	×		×
<i>S. nigricollis</i>	61	×	×	×	×	×	
<i>S. oedipus</i>	180	×			×	×	
<i>S. tripartitus</i>	12	×	×	×			

¹ AMNH, American Museum of Natural History; BMNH, British Museum of Natural History; FMNH, Field Museum of Natural History; NMNH, National Museum of Natural History; ORAU, Oak Ridge Associated Universities' Marmoset Research Center; MZUSP, Museu de Zoologia da Universidade de Sao Paolo.

have diversified by random evolutionary processes. Similarly, if these patterns of variation are not proportional, one can postulate that other modes of evolutionary phenotypic divergence (such as differential selection) were at work.

Applied to extant tamarins, this hypothesis suggests that if populations (such as subspecies of *S. fuscicollis*) diverged via genetic drift, then the variation pattern between subspecies would be proportional to the morphological variation pattern within them. Under a model of genetic drift, we expect more variable traits to drift further than less variable traits. Conversely, if populations diverged via selective processes, between- and within-group variation patterns may or may not be proportional. Additionally, the deviation of observed divergence from proportionality may provide clues to evolutionary processes responsible for interspecific morphological divergence. We will test hypotheses of evolution by genetic drift in a hierarchical examination of divergence among extant tamarin taxa. Our intent is to provide insight into the processes of organismal evolution within the genus *Saguinus*, by studying the differences between patterns of variation at various levels in phylogeny, and evaluating how these differences may reflect underlying morphological, ecological, or biogeographical shifts over evolutionary time.

MATERIALS AND METHODS

Samples and data

Measurements were obtained from a total of 960 adult tamarin crania. Crania with fused sphenoccipital and sphenoccipital sutures were considered adult. The *Saguinus* specimens were obtained from collections at the American Museum of Natural History (AMNH, New York), the British Museum of Natural History (BMNH, London), the Field Museum of Natural History (FMNH, Chicago), the National Museum of Natural History (NMNH, Washington DC), the University of Tennessee (ORAU, derived from the Marmoset Research Center, Oak Ridge Associated Universities' colony), and the Museu de Zoologia da Universidade de Sao Paolo

TABLE 2. Craniofacial landmarks recorded from tamarin crania using three-dimensional digitizer

Landmark	Description ¹	Position(s)
IS	Intradentale superior, A	Midline
PM	Premaxillary suture at the alveolus, A	Right, left
NSL	Nasale, A	Midline
NA	Nasion, A	Midline
BR	Bregma, AP	Midline
PT	Pterion, AP	Right, left
FM	Fronto-malare, A	Right, left
ZS	Zygomaxillare superior, A	Right, left
ZI	Zygomaxillare inferior, A	Right, left
MT	Maxillary tuberosity, A	Right, left
PNS	Posterior nasal spine, A	Midline
APET	Anterior petrous temporal, A	Midline
BA	Basion, AP	Midline
OPI	Opisthion, AP	Midline
EAM	Anterior external auditory meatus, A	Right, left
PEAM	Posterior external auditory meatus, A	Right, left
ZYGO	Inferior zygo-temporal suture, A	Right, left
TSP	Temporo-spheno-parietal junction, A	Right, left
TS	Temporo-sphenoidal junction at petrous, AP	Right, left
JP	Jugular process, AP	Right, left
LD	Lambda, P	Midline
AS	Asterion, P	Right, left

¹ Designation A (anterior) or P (posterior) after landmark indicates which position(s) the landmark was recorded in. Landmarks are also identified in Figure 1. Adapted from Cheverud (1995).

(MZUSP, Brazil), and include the following species: *Saguinus bicolor*, *S. fuscicollis*, *S. geoffroyi*, *S. imperator*, *S. inustus*, *S. labiatus*, *S. leucopus*, *S. midas*, *S. mystax*, *S. nigricollis*, *S. oedipus*, and *S. tripartitus* (see Table 1).

Three-dimensional coordinates were recorded for 36 landmarks using a Polhemus 3Space digitizer (Table 2, Fig. 1). Each specimen was digitized twice to minimize measurement error (see Cheverud, 1995); the average of repeated measures was used for further analyses. A set of 39 linear measurements, averaged between left and right sides and chosen to describe cranial morphology without excessive redundancy, was calculated from the coordinate values (Table 3).

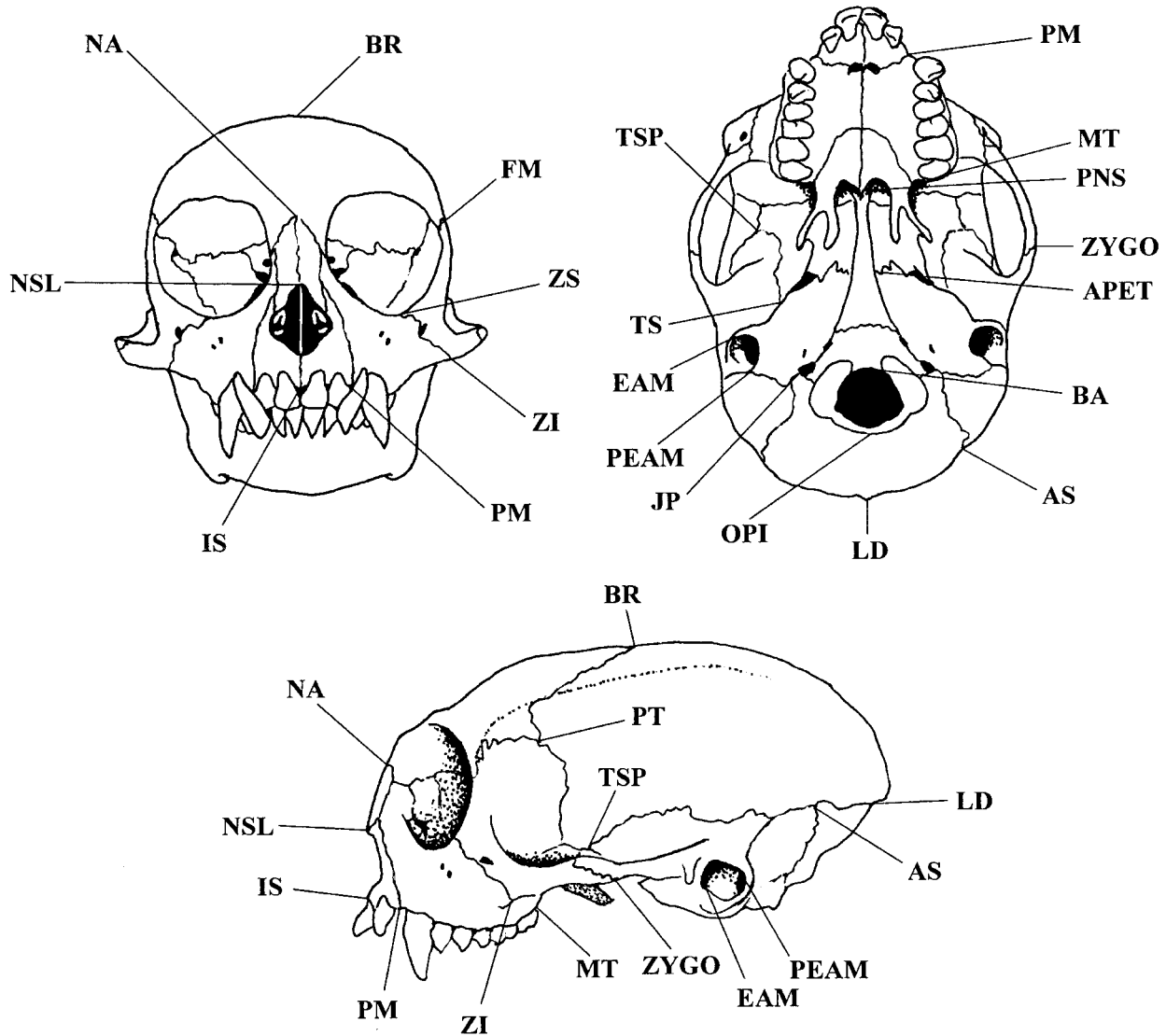


Fig. 1. Tamarin craniofacial landmarks. Landmark abbreviations are spelled out in Table 2.

TABLE 3. Thirty-nine linear craniofacial measurements calculated from the landmarks in Table 2¹

IS-PM	BR-APET	MT-PNS
IS-NSL	PT-FM	PNS-APET
IS-PNS	PT-APET	APET-BA
PM-ZS	PT-BA	APET-TS
PM-ZI	PT-EAM	BA-EAM
PM-MT	PT-ZYGO	EAM-ZYGO
NSL-NA	PT-TSP	ZYGO-TSP
NSL-ZS	FM-ZS	LD-AS
NSL-ZI	FM-MT	BR-LD
NA-BR	ZS-ZI	OPI-LD
NA-FM	ZI-MT	PT-AS
NA-PNS	ZI-ZYGO	JP-AS
BR-PT	ZI-TSP	BA-OPI

¹ Landmark acronyms are defined in Table 2 and Figure 1.

Estimates of variance

Measures were taken to prevent the inclusion of subspecific and sexual variation in estimates of within-group variation. A MANOVA indicated sig-

nificant differences among the subspecies of *S. fuscicollis*, between the subspecies of *S. midas*, and between the wild and colony (Oakridge)-derived groups of *S. oedipus*. Therefore, these populations were treated as distinct groups, along with the other species of tamarins. For all of the species (excluding *S. oedipus*) there were few sex differences (19/425 comparisons) which were significantly different at $P = 0.05$, only two of which were significantly different at $P = 0.01$. Additionally, these differences were not consistently larger in one sex. Therefore, corrections for sex differences in these species were not considered necessary. Within *S. oedipus*, a MANOVA indicated significant multivariate differences for the wild group, with more than half of the variables significantly different between sexes (sex difference is more than 0.1 standard deviation units); these differences were corrected by adding the difference between male and female means to each female (a correction to only four females). The colo-

ny-raised group of *S. oedipus* (hereafter called Oakoed) had no significant differences by sex.

Phenotypic within-population variance/covariance matrices for these sex-corrected 39 craniofacial variables were obtained using the residual covariance matrix from a MANOVA with the 39 traits as dependent variables, and taxonomic affiliation (either specific or subspecific groups as described above) as the independent variable, thus pooling the covariances across the taxonomic units of interest (Lofsvold, 1986, 1988; Turelli, 1988). Phylogenetic relationships within the genus *Saguinus* (see Cropp et al., 1999) are used to circumscribe these populations for analysis, i.e., subspecies within a single species, closely related species within a single lineage, and closely related lineages within the entire genus. Based on evidence from mitochondrial DNA, Cropp et al. (1999) divide the genus *Saguinus* into large-bodied and small-bodied clades. The small-bodied clade contains three species: *S. fuscicollis*, *S. tripartitus*, and *S. nigricollis*. The phylogenetic organization of this small-bodied clade places *S. f. fuscus* closer to *S. nigricollis* than to the other *S. fuscicollis* subspecies or to *S. tripartitus*. Cropp et al (1999) suggest that elevating *S. f. fuscus* to the species *S. fuscus* would maintain monophyly in *S. fuscicollis*. For the purpose of this paper, the phylogenetic divisions of Cropp et al. (1999) were adopted. All tamarins are first analyzed as a whole (23 populations, 960 individuals). Next, the small-bodied clade is analyzed as a whole (12 populations, 362 individuals), and in two groups: the Fuscicollis group (*S. fuscicollis* subspecies + *S. tripartitus*; 10 populations, 288 individuals) and the Nigricollis group (*S. nigricollis* + *S. fuscicollis fuscus*; 2 populations, 74 individuals). The large-bodied tamarin clade contains the remaining tamarin species and is analyzed separately (11 populations, 598 individuals). Again following the phylogenetic distinctions of Cropp et al. (1999), the large-bodied clade divides into two groups: the central large-bodied (CLB) tamarins (containing *S. mystax*, *S. labiatus*, *S. imperator*, and *S. inustus*; 4 populations, 130 individuals), and the northern large-bodied (NLB) tamarins (7 populations, 468 individuals). Within the CLB group, the Labiatus/Imperator group (containing *S. labiatus* and *S. imperator*; 2 populations, 51 individuals) is also analyzed. The NLB tamarins are also subdivided into two subgroups: the Oedipus group (*S. oedipus*, Oakoed, *S. geoffroyi*, and *S. leucopus*; 4 populations, 339 individuals), and the Midas/Bicolor group (*S. midas* subspecies, and *S. bicolor*; 3 populations, 129 individuals), and within the Midas/Bicolor group, the Midas group (containing *S. midas* subspecies; 2 populations, 116 individuals) is analyzed separately. To sum, 11 analyses are performed, representing lineages of tamarins at different positions and levels in the *Saguinus* hierarchy (Fig. 2).

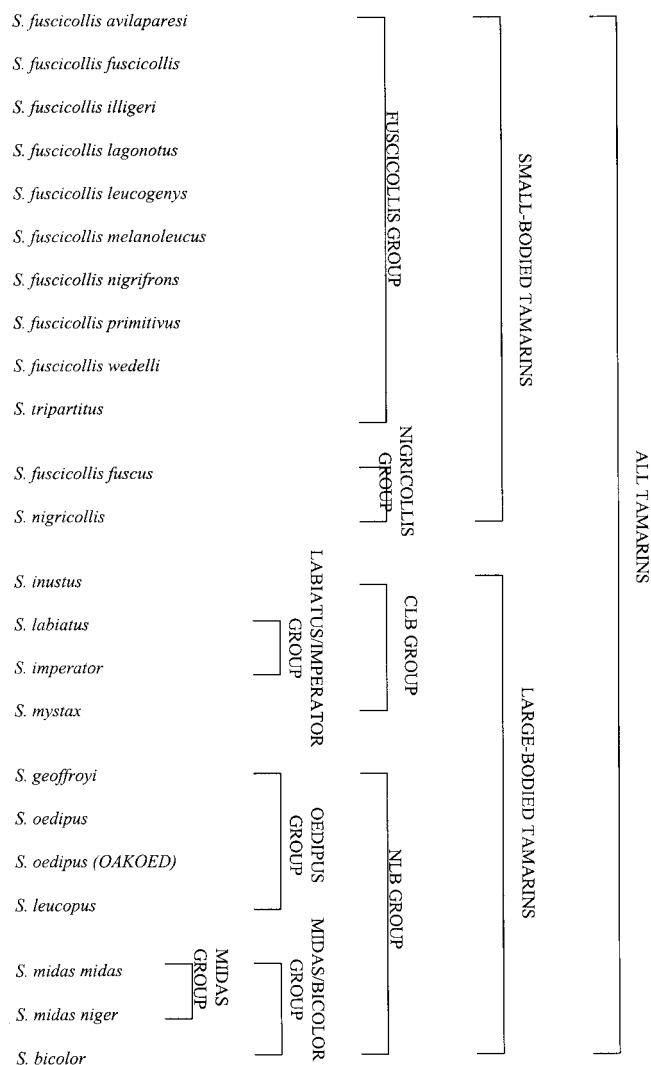


Fig. 2. *Saguinus* hierarchy, adapted from Cropp et al. (1999), and used for constructing analyses.

Determining the role of genetic drift

The possibility of obtaining the observed pattern of interspecific morphological differentiation by genetic drift can be evaluated by comparing within- and between-population V/CV matrices, as described above in Equations 1 and 2. To simplify this comparison, we first obtain a mathematically simple form of the within-population V/CV matrix, its principal components. The principal components of the within-population V/CV matrix are ordered by their level of variance and are uncorrelated with one another, so that on the scale of principal components, the within-population V/CV matrix is a simple diagonal matrix with no covariances among components. Principal component scores can be calculated for each population mean (**Y**) by multiplying the n by k matrix of population means (**X**) by the k by k matrix of standardized eigenvectors (**E**)

$$Y = XE \tag{3}$$

where n is the number of populations, k is the number of traits, and each eigenvector is standardized to a length of 1.00 (sum of squared elements equals 1). The between-population variance for each principal component can then be calculated as the variance among population mean principal component 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, Equation 1 can be written as a linear regression with

$$\ln B_i = \ln(t/N_e) + \beta \ln(W_i) \quad (4)$$

where B_i is the between-population variance and W_i is the within-population variance for the i th eigenvector. If differentiation was produced by genetic drift, we expect a regression slope (β) of 1.00 for the regression of between- on within-population variance. A significant deviation from a slope of 1.00 indicates a pattern not likely to have been produced by genetic drift.

The between-population V/CV of the simplified within-population V/CV matrix could also deviate from proportionality if the principal component scores of the populations were 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 also remain uncorrelated. We will test for significant correlations among the first several principal component scores for each comparison involving three or more taxa. Of course, the power to detect deviations from a slope of one or nonzero between population correlations depends critically on the number of taxa included in the comparison.

Other methods for comparing V/CV matrices are also available. Proportionality can be tested using Flury's common principal component approach that tests for matrix similarity in a hierarchical fashion (Flury, 1987; Cowley and Atchley, 1992; Phillips and Arnold, 1999). We chose not to use this approach because with large samples, as included in many of the analyses reported here, even biologically trivial deviations from the model are statistically significant and the method does not provide an appropriate association metric with which to judge the degree of similarity (Ackerman and Cheverud, 2000; Marroig and Cheverud, 2001; Cheverud and Marroig, unpublished results). V/CV matrix similarity can also be measured using a random skewers approach, in which the relative response of V/CV matrices to random selection vectors is measured (Cheverud, 1996; Marroig and Cheverud, 2001; Ackermann, 2002). The results of such an analysis are very similar to those presented here. We chose to use the relative variance of within-taxon V/CV principal components for the present analysis because the simplified form of the within-taxon V/CV matrix led

to straightforward expectations under a model of genetic drift that could be easily tested with standard statistics. A simulation of this method under conditions of evolution by genetic drift also confirmed the statistical properties of the test, in that when evolution occurred in a manner consistent with genetic drift, the tests used here rejected drift 5% of the time at the nominal 5% significance level.

Sample sizes for these 11 comparisons vary greatly, from 51–960, and also vary considerably in the number of taxa compared. The consequences of varying sample size for the analyses carried out here are complex, with effects on the estimation of both the within- and between-taxon V/CV matrices. In all but two comparisons, the within-taxon V/CV matrices are calculated from over 100 individuals and thus should be fairly robust estimates. However, several comparisons are made with only 2–4 taxa. The reduced number of taxa results in less power to test for between- and within-V/CV matrix similarity. This reduced power is evident in the relatively low R^2 values and consequent large standard errors of the slope of the between- on within-taxon variances for the within-taxon principal components. It also results in a lack of power to test for between-taxon correlations among the within-taxon principal components. Taxon means are reasonably well-estimated relative to the magnitude of between-taxon morphological differences, making this a relatively minor source of error.

Because the first PC generally represents size and size-related shape change and can have an important effect on the regression of between- on within-population variances (Jolicœur, 1963; Reyment et al., 1984), the first principal component vector in each analysis is also examined in order to describe regions of positive and negative allometry. Each PC1 is converted into an allometry vector by dividing it by $1/\sqrt{39}$ (the expected loading if the variation were equally distributed between the 39 traits, with isometry equal to a loading of 1). Allometry vectors are tested for significance against isometry, using a standard t -test, and those variables that are significantly above or below 1 are interpreted as positively or negatively allometric, respectively.

Comparisons with independently derived results

To evaluate the morphological, phylogenetic, and biogeographical meaning of the results, they are interpreted in the context of estimates of similarity that are independently derived. First, multivariate discriminant function analysis (DFA) (Klecka, 1980) is performed on the 39 variables grouped by 12 different taxa (*S. bicolor*, *S. fuscicollis*, *S. geoffroyi*, *S. imperator*, *S. inustus*, *S. labiatus*, *S. leucopus*, *S. midas*, *S. mystax*, *S. nigricollis*, *S. oedipus* (excluding the colony-raised individuals), and *S. tripartitus*), corrected for sex differences (see above). The canonical scores of the group means are used to calculate the morphological distances (D^2) between the species, with the relative magnitude of morpho-

TABLE 4. Results of regression of between-group on within-group variance for within-group principal components and between-group correlations among within group principal components as a test for genetic drift¹

Group	Consistent with drift	Slope	95% confidence	R ²	Correlation probability
All tamarins	No	1.12	1.02 <> 1.22	0.94	0.0001
Small-bodied tamarins	No	0.95	0.80 <> 1.09	0.82	0.0063
Fuscicollis group	Yes	0.92	0.78 <> 1.06	0.82	0.0540
Nigricollis group	No	0.48	0.08 <> 0.87	0.14	
Large-bodied tamarins	Yes	1.03	0.90 <> 1.16	0.87	0.9020
Central large-bodied group	No	0.79	0.60 <> 0.97	0.67	
Labiatus/Imperator	No	0.35	-0.02 <> 0.72	0.09	
Northern large-bodied group	Yes	0.99	0.87 <> 1.11	0.88	0.3310
Midas group	No	0.77	0.55 <> 0.99	0.57	
Midas/Niger	Yes	1.08	0.73 <> 1.43	0.51	
Oedipus group	Yes	1.16	0.92 <> 1.41	0.72	

¹ Includes whether result is consistent with a model of genetic drift (Yes/No), the regression slope, its confidence interval, associated R² value, and the probability of no correlation among the first several principal components. All regressions are significantly greater than zero at <0.001.

logical distance (D²) representing the degree of morphological distinction (Klecka, 1980). A cluster diagram is generated from these distances, utilizing the average linkage method, UPGMA, as recommended by Sneath and Sokal (1973).

The second independently derived measure of similarity is the phylogenetic tree of Cropp et al. (1999) and the biogeographical dispersal hypotheses drawn from their analysis. Cropp et al. (1999) generated their phylogenetic tree by manually aligning homologous sites of cytochrome b and D-loop mtDNA sequences and analyzing them with phylogenetic analysis using parsimony (PAUP). The D-loop contained sufficient variation for phylogenetic resolution of species and subspecies among the tamarins because it is a highly variable region of mtDNA. The outgroup reference taxa consisted of *Callimico*, *Callithrix*, *Cebuella*, and *Leontopithecus*, and the most parsimonious phylogenetic reconstruction for the genus *Saguinus* was obtained using the heuristic search option (for further details, refer to Cropp et al., 1999). While the mtDNA data represent only one locus inherited solely through the female line, these data comprise the only comprehensive molecular data set available from which to derive a phylogenetic hypothesis for the tamarins. The third measure of interspecific similarity is based on judgments by Hershkovitz (1977) about relationships within the genus. These judgments are based on coat color variation, a particular hypothesized orthogenetic pattern of coat color mutation, and biogeography. The representation of relationships by Hershkovitz (1977) is serial rather than cladistic, but can be recast in a cladistic form consistent with his statement of relationships. Patterns of morphological divergence will be compared with patterns of evolutionary relationship given by mtDNA (Cropp et al., 1999) and the hypothesis of Hershkovitz (1977; see also Cropp et al., 1999), using Mantel's test (Dow and Cheverud, 1985). Mantel's test provides the probability that two matrices have a given level of similarity under a null hypothesis of no structural similarity.

RESULTS

Variation in size/shape vectors

All regressions of logged between-group variation against logged within-group variation are significantly greater than zero, with $P < 0.001$; results of these regressions are shown in Table 4. Of the 11 hierarchical levels analyzed, 5 have slopes significantly different from one: all tamarins, Nigricollis group, central large-bodied group, Labiatus/Imperator group, and Midas/Bicolor group. Divergence within these groups is therefore unlikely to be due to genetic drift alone. Several other groups display patterns of between-group variance consistent with that expected by genetic drift, including the small-bodied tamarins and their Fuscicollis group, the large-bodied tamarins and their northern group, and the Midas/Niger and Oedipus subgroupings within the northern large-bodied Tamarins.

It is possible to characterize the reasons for deviations from a slope of 1.00 by inspection of the regression residuals. For example, a negative deviation of the PC1 variance (below the regression line) would indicate that for PC1, the variation between populations is less than expected, given the variation within populations. Since the first principal component represents size (and corresponding allometric shape), this would indicate that the groups are less different from each other in size than expected. Following this line of reasoning, the analysis of all tamarins displays relatively too much variation between groups on the first principal component. Size and allometrically related shape are too variable among species to have been produced by drift alone. Furthermore, the slope is no longer significantly different from 1.0 when PC1 is removed from the analysis. In the Nigricollis group, the central large-bodied group, and the Midas/Bicolor group, several minor PCs have far too much between-group variance relative to other PCs for drift to be responsible for divergence. Finally, the Labiatus/Imperator group has far too little variance be-

tween groups in PC1 for differentiation to have occurred by genetic drift.

We also tested for significant between-group correlations among the within-group principal components, since the drift model predicts no correlation among them. Significant correlations were found for the all tamarin and small-bodied tamarin comparisons, indicating a deviation from expectations under genetic drift. For the all tamarin comparison, PC1 was significantly correlated with PC4 and PC5, while PC1 and PC2 were correlated between groups in the small-bodied tamarin comparison. No significant correlations were found for the *Fuscicollis* group, the large-bodied tamarins, or the northern large-bodied tamarins. Other comparisons contained too few species for this test.

To summarize, the overall pattern in the genus *Saguinus* is one where variation between the populations in the first few principal components, and especially the first, is more than would be expected under genetic drift, given the pattern of variation within species. Furthermore, there are significant correlations among the first five principal components. This indicates that divergent size selection occurred during the period of tamarin diversification, but it is unlikely that this selection was on size alone because of the correlations among principal components. Within the small-bodied tamarins, between- and within-group variation is largely proportional, but there is a strong significant correlation between the first two principal components, again indicating divergent selection among these taxa. The other sets of taxa that diverge from the pattern expected by drift are the *Nigricollis* group, the central large-bodied group, the *Labiatus/Imperator* group, and the *Midas/Bicolor* group. These groups contain small sets of taxa, ranging in size from 2–4. In all cases, minor within-group PCs are much more variable between taxa than would be expected under genetic drift, suggesting divergent selection. The divergence among the *Fuscicollis* group taxa, the large-bodied tamarins, northern large-bodied tamarins, *Midas* tamarins, and *Oedipus* group taxa are consistent with genetic drift.

Patterns of allometry

With minor deviations (see below), members of the genus *Saguinus* share the same allometry vector. The common tamarin allometry pattern is positively allometric for calvarium height and palate length, and negatively allometric for midface and palate width, basicranium width and length, and zygomatic arch flare. These patterns generally indicate positive growth in the cranial vault at the expense of relatively reduced of oral, orbital, zygomatic, and basicranial regions.

The first principal component often has undue influence on a regression line because it has a disproportionately large variance. Because of this, the regressions of the five groups with deviance from a slope of one, or with outliers, were repeated without

the first principal component. If this variable is driving the deviation, then it is important to look at the properties of the first vector (the allometry vector) to better understand these size differences. Only 2 of these 5 groups have different between- and within-group variation patterns due to the impact of differential size selection. For the all tamarin group, the deviation from drift is largely due to size, with more size variation between the groups than expected. For the *Labiatus/Imperator* group, the deviation from drift was also due to size, although in this case, size differences between groups were not as variable as expected. Additionally, this test shows that in the *CLB* group, the deviation from drift was *not* allometric (not size-related). The allometry vectors of the large- and small-bodied clades, of the all tamarin group, and of the *Labiatus/Imperator* group are shown in Table 5 to illustrate the differences between them. Note that the allometry vector of all tamarins is actually not all that different from the allometry vector of either the small-bodied or large-bodied tamarins; the pattern of variation within these groups is similar, despite the fact that the amount of variation in size between the constituents of the all tamarin group is too large.

Comparative analyses

Results of the discriminant function analysis are shown in Table 6 and Figure 3. The cophenetic correlation coefficient (Sneath and Sokal, 1973) between the cluster diagram distances and the raw distances is 0.94, indicating that the cluster diagram is a good representation of the data. The cluster analysis grouped the members the small-bodied clade of Cropp et al. (1999) in a single cluster. The members of the *Midas* group, *S. midas* and *S. bicolor*, are also grouped together. These two clusters are then linked with *S. labiatus*, *S. imperator*, *S. mystax*, and *S. inustus* at higher levels. At the other extreme, two members of the *Oedipus* group, *S. geoffroyi* and *S. oedipus*, are quite distinct from all other tamarins. The third *Oedipus* group member, *S. leucopus*, is not included in this cluster but is linked to the remaining tamarins just prior to inclusion of the *S. oedipus*-*S. geoffroyi* cluster.

The morphological distance matrix can also be compared to various phylogenetic hypotheses for the tamarins. Using Mantel's test, morphological distance is not significantly similar (matrix $r = 0.19$, $P = 0.0758$) to distances based on the mtDNA-derived phylogenetic tree given by Cropp et al. (1999). However, morphological distances are significantly similar ($r = 0.61$, $P < 0.0001$) to phylogenetic tree distances, based on the discussion by Hershkovitz (1977) of tamarin relationships (see Cropp et al., 1999). Using a three-way Mantel's test (Dow and Cheverud, 1985), morphological distances are significantly more similar ($r = 0.40$, $P = 0.0143$) to relationships based on Hershkovitz (1977) than they are to relationships based on the mtDNA phylogeny (Cropp et al., 1999). A major distinction between the

TABLE 5. Allometry vectors of within-group covariance matrices¹

Trait	All tamarins	Small-bodied clade	Large-bodied clade	Labiatus/Imperator
IS-PM	0.54***	0.47***	0.58***	-0.37***
IS-NA	0.57***	0.64**	0.53***	-0.38*
IS-PNS	1.81***	1.62***	1.91***	0.13
PM-ZS	0.71***	0.41***	0.84*	0.08**
PM-ZI	1.14*	1.00	1.19**	0.71
PM-MT	1.12*	0.82**	1.28***	0.51
NSL-NA	0.14***	0.11***	0.12***	1.03
NSL-ZS	0.96	0.77*	1.05	-0.09***
NSL-ZI	1.51***	1.55***	1.45***	1.01
NA-BR	1.44***	0.92	1.69***	0.94
NA-FM	0.99	0.68***	1.18*	-0.04***
NA-PNS	1.45***	1.46***	1.41***	0.85
BR-PT	0.97	0.34***	1.28**	1.78*
BR-APET	0.48***	0.56**	0.35***	1.91*
PT-FM	0.12***	0.12***	0.09***	-0.47**
PT-APET	1.44***	1.30**	1.48***	1.45
PT-BA	2.07***	2.03***	2.04***	1.78**
PT-EAM	1.54***	1.72***	1.38**	2.15**
PT-ZYGO	1.42***	1.72***	1.21	1.46
PT-TSP	0.88	1.06	0.77*	1.14
FM-ZS	0.22***	0.39***	0.15***	0.16**
FM-MT	0.91	0.97	0.85*	-0.45**
ZS-ZI	0.75***	0.85	0.67***	0.91
ZI-MT	0.31***	0.23***	0.31***	-0.64**
ZI-ZYGO	0.80**	0.85	0.75**	0.14
ZI-TSP	0.24***	0.64***	-0.03***	0.40
MT-PNS	0.69***	0.67**	0.70***	0.72
PNS-APET	0.16***	0.01***	0.17***	0.30
APET-BA	0.63***	0.73**	0.56***	-0.04**
APET-TS	0.37***	0.38***	0.37***	-0.25**
BA-EAM	1.16**	1.20*	1.14*	0.37
EAM-ZYGO	0.07***	0.06***	0.04***	0.18*
ZYGO-TSP	0.59***	0.81*	0.41***	0.01**
LD-AS	0.94	0.59***	1.13	0.03*
BR-LD	0.81	1.53**	0.34**	1.58
OPI-LD	0.95	0.82	1.02	1.04
PT-AS	1.69***	1.99***	1.43**	2.46**
JP-AS	0.79***	0.76**	0.79**	1.33
BA-OPI	0.17***	0.24***	0.12***	0.13*

¹ Standardized first principal component for each species was converted (divided by (1/√39)) to identify regions where variables diverge from isometry. Values greater or less than 1.0 are positively or negatively allometric, respectively. Asterisks signify significant deviation from isometry. Results with highest levels of significance are considered biologically important.

* $P < 0.05$.
 ** $P < 0.01$.
 *** $P < 0.001$.

TABLE 6. Mahalanobis distance D^2 , based on discriminant function analysis between tamarins

	<i>S. bicolor</i>	<i>S. fuscicollis</i>	<i>S. geoffroyi</i>	<i>S. imperator</i>	<i>S. inustus</i>	<i>S. labiatus</i>	<i>S. leucopus</i>	<i>S. midas</i>	<i>S. mystax</i>	<i>S. nigricollis</i>	<i>S. oedipus</i>	<i>S. tripartitus</i>
<i>S. bicolor</i>	0											
<i>S. fuscicollis</i>	31	0										
<i>S. geoffroyi</i>	41	63	0									
<i>S. imperator</i>	33	31	47	0								
<i>S. inustus</i>	34	40	55	39	0							
<i>S. labiatus</i>	32	18	60	30	37	0						
<i>S. leucopus</i>	32	47	49	42	51	35	0					
<i>S. midas</i>	18	25	61	27	37	25	41	0				
<i>S. mystax</i>	31	30	54	25	34	25	43	31	0			
<i>S. nigricollis</i>	29	9	58	29	40	12	37	21	29	0		
<i>S. oedipus</i>	56	64	25	68	73	71	69	63	72	59	0	
<i>S. tripartitus</i>	33	10	58	31	46	25	54	35	30	15	67	0

mtDNA-based phylogeny and the pattern of morphological distances is the extreme morphology of *S. oedipus* and *S. geoffroyi* contrasted with their deeply embedded position in the phylogeny.

DISCUSSION

Determining the relationship between variation and evolution is fundamental to our understanding

of the processes of evolution. In this study, patterns of variation within and between populations were compared in order to identify the evolutionary processes involved in the morphological diversification of tamarins. This was done by studying the patterns of within- and between-taxon variation at various levels of the tamarin phylogenetic hierarchy. Recall that evolutionary theory indicates a proportional

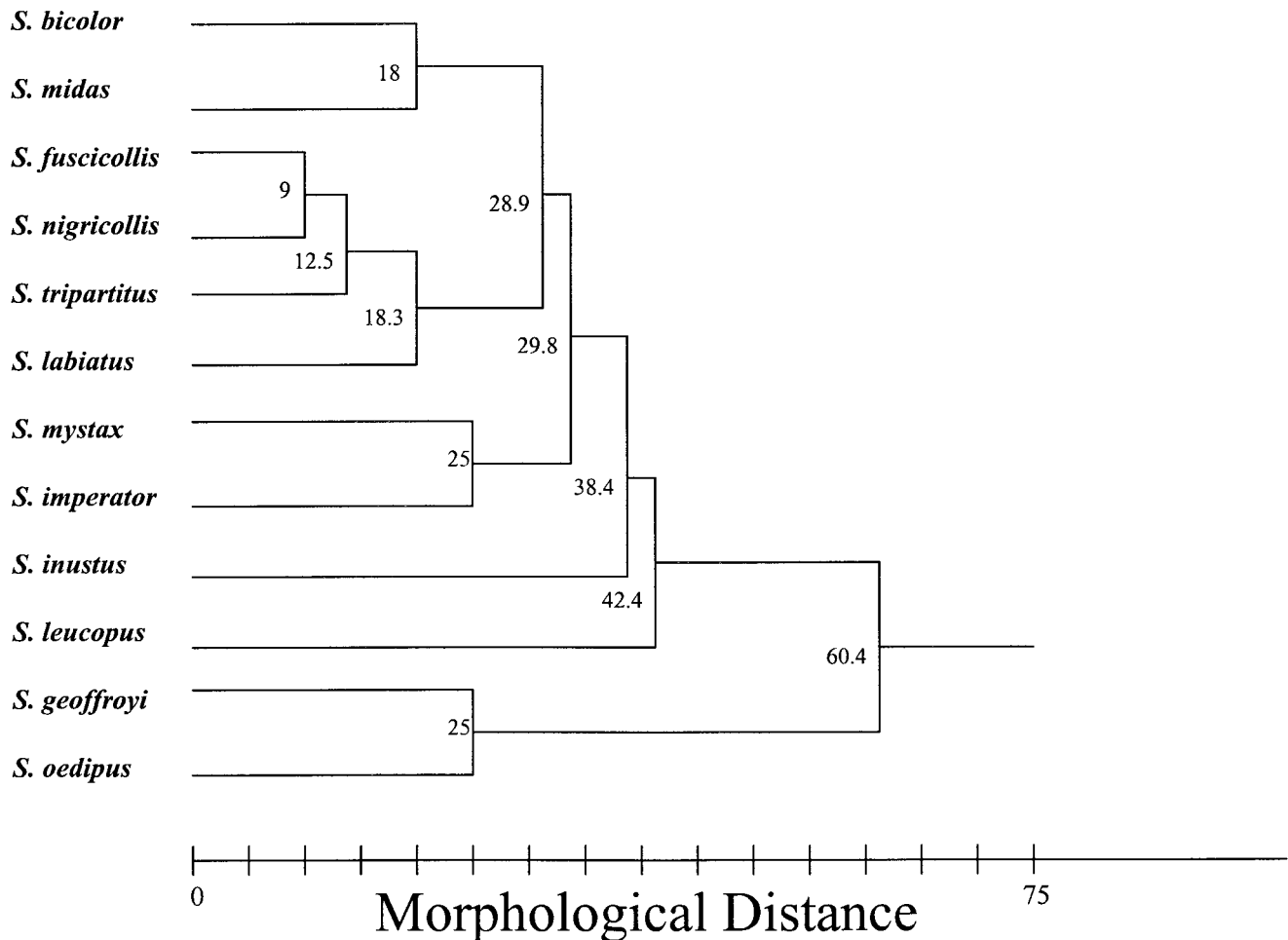


Fig. 3. Morphological relationships among tamarin species, derived from a UPGMA analysis of Mahalanobis D^2 values.

relationship between within-group and between-group phenotypic variation ($B \propto W$) if populations have diversified via random evolutionary processes (Lande, 1979, 1980; Lofsvold, 1988). Similarly, if these patterns of variation are not proportional, other diversifying evolutionary processes likely were at work. We used two indicators of proportionality: the slope of the regression of between-group on within-group variation for the principal components of the within-group V/CV matrix, and the presence of between-group correlations among these principal components.

The overall pattern for the genus *Saguinus* shows that there is too much variation in the first principal component between populations comprising the genus for divergence to have occurred by random processes alone. This indicates that divergent size selection is likely to have been responsible, in part, for the morphological diversification of the two major tamarin clades. This is perhaps not surprising, given that the two major clades have quite distinct body sizes and feeding behaviors (Garber, 1992; Cropp et al., 1999). However, the presence of significant between-taxon correlations between PC1 and PCs 4 and 5 indicates that tamarin diversification

was not due to divergent size selection alone. Rather, in order to induce such correlations, diversifying selection must also have occurred for nonallometric aspects of cranial shape. While divergent size and shape selection is indicated for the tamarins, it is not possible from this analysis alone to determine the absolute selection responsible for this pattern. For example, selection could have been for larger size in the large-bodied clade and smaller size in the small-bodied clade if the common ancestor was of intermediate size. Alternatively, selection could have been for smaller size in both clades, but stronger in the small-bodied clade, if the common ancestor was larger than the extant tamarins. Likewise, we cannot place this selection in time. It could be that selection occurred only during the early diversification of these clades. Alternatively, differences in selection could have occurred at the origins of the clades and continued through today. Garber (1992) argued that selection for smaller size occurred in the small-bodied clade as part of a specialization for use of vertical postures for insect-foraging on large vertical supports. Our results are consistent with this hypothesis.

Genetic drift is also rejected as the sole diversifying force for the small-bodied tamarins because of a significant correlation between the first two principal components. Within the small-bodied tamarins, the divergence of *S. fuscicollis* subspecies (plus *S. tripartitus*) could be a result of random processes, although the differences between *S. nigricollis* and *S. fuscicollis fuscus* in the first few PCs are actually too small, relative to lower PCs, to represent random divergence. This limited between-population variability within the Nigricollis group might represent the effects of common stabilizing selection on size, or divergent selection for nonsize-related shape changes. However, because of the very short time since divergence (Cropp et al., 1999) and the small sample of taxa involved in this comparison ($n = 2$), these results cannot be considered conclusive.

Diversification within the large-bodied tamarins as a whole is consistent with drift. However, the differences between populations of the central large-bodied group in the first few principal components are too small to be explained by random drift, and are not allometric, although they may be the result of similar stabilizing size selection or divergent shape selection within this clade. Within this central group, the differences between *S. labiatus* and *S. imperator* are also not consistent with random processes alone. In this case, size is not as variable between groups as it should be, indicating either divergent selection on shape and/or common stabilizing selection on size. Conversely, the differences between populations within the northern group are consistent with the expectations of genetic drift, although within the northern tamarins, one group (comprised of *S. midas* and *S. bicolor*) diverges from this pattern, and shows a reduced between-group variation for early PCs. Again, this may indicate common stabilizing selection on size or divergent size selection within this Midas/Bicolor group, although again, the inclusion of only a few taxa in this comparison prevents definitive results.

To summarize, divergence of the major tamarin clades indicates too much interspecific size variation to have been produced by genetic drift alone. The general patterns of variation within the small-bodied tamarins and within the large-bodied tamarins suggest that morphological divergence occurred largely through random drift, although lower hierarchical levels within each clade show some evidence for nonrandom divergence. This suggests that subgroups within each clade diverged through a combination of selection and drift processes.

Patterns of interspecific craniofacial variation do not match mtDNA-based phylogenetic relationships very closely. Both kinds of variation agree in grouping the small-bodied tamarins together, *S. midas* and *S. bicolor* together, and *S. oedipus* and *S. geoffroyi* together. However, the relationships among these groups and the central large-bodied tamarins are quite different in the different data sets. A primary difference is the placement of the Oedipus

group tamarins as the sister clade to the *S. midas*-*S. bicolor* clade in the mtDNA phylogeny rather than as an extreme outlying group, as in the morphological distance phenogram. The distinctiveness of the Oedipus-group tamarins was also noted in their patterns of within-species morphological variation (Ackermann and Cheverud, 2000). Indeed, patterns of morphological distance are more similar to the phylogenetic relationships derived from Hershkovitz (1977; see Cropp et al., 1999) than those based on mtDNA. However, this aspect of the mtDNA phylogeny is very well-supported, as is the phylogeny as a whole (Cropp et al., 1999), so that we accept the mtDNA-based phylogeny as a generally accurate representation of tamarin phylogenetic relationships. Therefore, in the tamarins, morphological similarity is not necessarily an accurate guide to phylogenetic propinquity. This is consistent with the analysis indicating that some of the morphological diversity in tamarins is due to nonrandom processes.

Cropp et al. (1999) found that molecular phylogenetic evidence is consistent with specific dispersal patterns for tamarins. This dispersal could have occurred "in two major waves from an origin somewhere south of the Amazon" (Cropp et al., 1999, p. 65). One wave (including predecessors of the small-bodied tamarins) moved westward, with a secondary subspecific dispersal back south and eastward. The other wave, which included predecessors of the northern large-bodied tamarins, moved northeast towards the Amazon delta, with a secondary migration west along the northern edge of the continent. This dispersal pattern is pictured in Figure 4. While this dispersion scenario is consistent with the phylogenetic observations, other scenarios, such as the Amazonian Lagoon hypothesis (Marroig and Cerqueira, 1997), may also be consistent with tamarin distributions and phylogeny.

Our analysis indicates that the mechanism driving the initial split between the small-bodied and large-bodied tamarins probably was not drift alone, and may indicate that natural selection was working differently in the two clades, perhaps coupled with ecological factors involved in their initial separation (Garber, 1992). The first divergence of the small-bodied tamarin ancestors from each other seems to have involved processes other than genetic drift because of the significant between-taxon correlation among the first two within-taxon principal components. Drift was likely responsible for the continued divergence of subspecies of *S. fuscicollis* during their secondary southeastwards dispersal. However, common stabilizing selection on size or divergent shape selection may have been involved in the differentiation of *S. nigricollis* and *S. fuscicollis fuscus*. Similarly, the initial divergence of groups within the other major clade (the large-bodied tamarins) may have resulted from random drift processes during the initial northeastern migration and with the continued secondary westwardly divergence of the Oe-

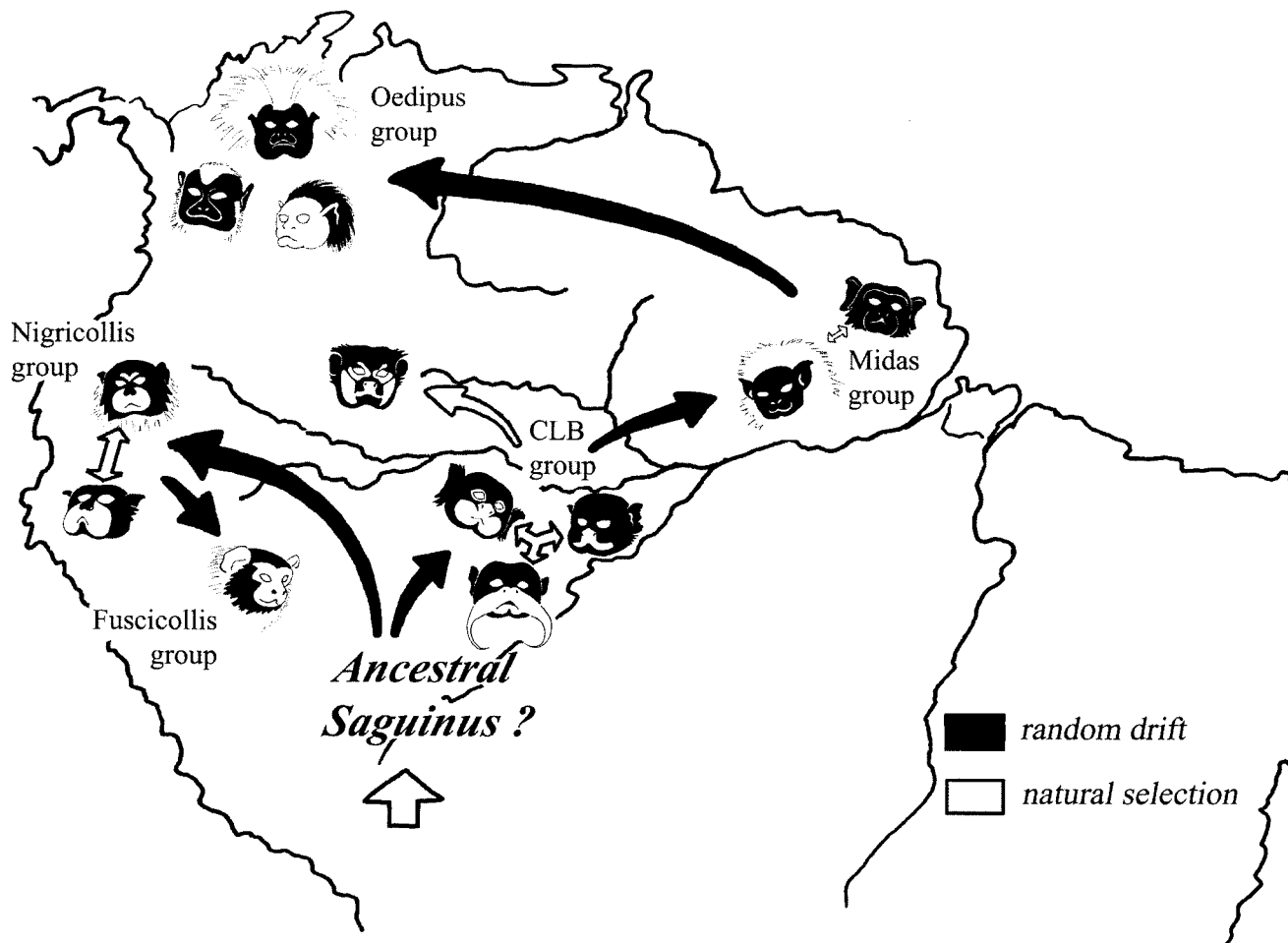


Fig. 4. Map of tamarin dispersal, with possible evolutionary processes directing this dispersal. Arrows directed at a group indicate evolutionary processes likely to be responsible for morphological diversification.

dipus group. The populations within this Oedipus group also diverged through drift. Meanwhile, common stabilizing selection on size or divergent shape selection was involved in the differentiation of the groups (CLB and Midas/Bicolor) that remained in the environments of the initial migration.

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