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Cranial growth in normal and low-protein-fed *Saimiri*. An environmental heterochrony

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Abstract

Protein malnutrition has a significant and measurable effect on the rate and timing of growth. Heterochrony is generally viewed as the study of evolutionary changes in the relative rates and timing of growth and development. Although changes in growth commonly result from experimental manipulations of diet, nobody has previously attempted to explain such changes from a heterochronic perspective. We use a heterochronic perspective to compare a group of squirrel monkeys fed a low-protein diet to individuals on a high-protein diet, but, in contrast to previous works, we focus particularly on the effects of environmental and not genetic factors. In the present study, Gould's (1977) and Godfrey and Sutherland's (1996) methodologies for studying heterochrony, as well as geometric morphometrics, are used to compare two groups of *Saimiri sciureus boliviensis*. Two groups of *Saimiri* were constructed on the basis of the protein content in their diets: a high-protein group (HP) (N = 12) and a low-protein group (LP) (N = 12). All individuals are males born in captivity. Two major functional components of the skull, the neurocranium and the face, were analysed. Four minor components were studied in each major component. Comparison of craniofacial ontogeny patterns based on major and minor components suggests that changes in the skull of LP animals can be explained by heterochrony. The skull of LP animals exhibits isomorphism produced by proportioned dwarfism. Our results suggest that heterochrony can be environmentally, rather than exclusively genetically, induced. The study of genetic assimilation (Waddington, 1953, 1956; see Scharloo, 1991; Hallgrimsson et al., 2002) has demonstrated that

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environmentally induced phenotypes often have a genetic basis, and thus parallel changes can be easily induced genetically. It is possible that proportioned dwarfism is far more common than currently appreciated.
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Environmental stresses during development result in transitional growth perturbations. A catch-up process occurs immediately after the perturbation to recuperate normal growth (Prader et al., 1963; Farnum et al., 2003). Farnum et al.'s (2003) studies on cellular processes underlying catch-up suggest that the perturbation is an interruption of "a prepatterned growth program" (2003: 39). The catch-up is manifested in a "sense of size." Individuals suffering the effects of a continual environmental stressing agent, such as malnutrition, may experience dramatic changes in size (Prader et al., 1963; Dammrich, 1991; Loveridge and Noble, 1994). Reichling and German (2000) nevertheless observed little or no difference in size between low-protein and high-protein-fed rats, suggesting that normal adult size may be attained in rats on a low-protein diet via a longer period of growth. Protein malnutrition is effectively known to have a significant and measurable effect on the rate and timing of growth (Malcom, 1979; Pucciarelli, 1981; Golden, 1994). Miller and German (1999) indicated that the significant effect of malnutrition is variation in the growth trajectories rather than the endpoints of growth.

Changes in rate and timing of growth are the focus of heterochronic studies, but the effects of malnutrition have not been assessed from a heterochronic point of view. Heterochrony is the study of evolutionary changes in the relative rates and timing of growth and development (Godfrey and Sutherland, 1995, 1996; Ramirez Rozzi, 2000). Needham (1933) and Gould (1977) identified growth (changes in size), development (changes in shape), and age at sexual or germinal maturation as three "fundamental" aspects of ontogeny. Studies of heterochrony focus on how these three fundamental aspects interact and evolve. Their products (paedomorphosis, isomorphosis, or peramorphosis) are identified only on the basis of comparisons of adult shape. Paedomorphosis is defined as the retention in mature descendants of ancestral juvenile traits, isomorphosis implies acquisition in mature descendants of ancestral adult shape, and peramorphosis is recognized as the acquisition in mature descendants of features that transcend ancestral adult characteristics (Godfrey and Sutherland, 1995).

The pioneering works by Gould (1977) and Alberch et al. (1979) on heterochronic processes and their outcomes were followed by other studies, some of which deliberately or unintentionally altered the meaning of heterochronic terms. Through such alterations, Gould's original search for changes in the relative rates of growth and development became a comparison of the relative changes in two metric traits (e.g., McKinney, 1986; McNamara, 1986; Shea, 1989). Thus, a heterochronic understanding of ontogeny was replaced by an allometric one, which uses, however, the same terminology. Reviews written from differing perspectives have attempted to clarify the meaning of the processes and results of heterochrony (McKinney, 1986; McNamara, 1986, 1988; McKinney and McNamara, 1991; Godfrey and Sutherland, 1995, 1996; Reilly et al., 1997; Ramirez Rozzi, 2000). We believe that 1) heterochrony should not to be confused with allometry; 2) heterochrony can be understood via the analysis of three fundamental aspects of ontogeny, namely size, shape, and time; and 3) Godfrey and Sutherland's (1995, 1996) works stand alone in proposing a useful methodology to test it. Here, we adopt their theoretical framework (Godfrey and Sutherland, 1995; Ramirez Rozzi, 2000).

Although heterochrony was first interpreted as an evolutionary process, it was later observed that

many aspects of intraspecific variation could be explained by heterochrony (i.e., Shea, 1986, 1988, 1989; Berge and Penin, 2000; Berge, 2002; Williams et al., 2002). The comparison between a low-protein-fed group and a high-protein-fed individuals is a study of intraspecific heterochrony, but in contrast to previous works, it has a particular focus on the effect of environmental and not genetic factors.

The first works on heterochrony attempted to explain between-group changes by means of a unique heterochronic process. It is now more widely accepted that different parts of the organism seem to have followed a different heterochronic process, i.e., skull shape in modern humans may be attained by neoteny (Godfrey and Sutherland, 1995), whereas bipedalism may be a peramorphic character (Tardieu, 1998; Berge, 1998, 2002). Heterochronic studies should begin with a consideration of the distinct growth fields affecting each part, perhaps independently. Several factors, such as morphological integration (Olson and Miller, 1958; Marroig and Cheverud, 2001; Bookstein et al., 2003), developmental and functional constraints (Lieberman, 1997; Lieberman et al., 2000; Pucciarelli et al., 2000), as well as different levels of plasticity (Kiliaridis, 1995; Wood and Lieberman, 2001; Giesen et al., 2003), are thought to interact ontogenetically and thus contribute to the expression of adult morphology. As a result of morphological integration, it is expected that functionally and developmentally related characters will be inherited together (Cheverud, 1995; Marroig and Cheverud, 2001; González-José et al., 2004). Environment also plays an important integrative role, since selection favours functionally related traits, which evolve as a single coordinated unit (Cheverud, 1982, 1995). To sum up, studies on heterochrony must analyse both growth and development of potentially independent growth fields.

Functional cranial theory (Klaauw, 1948-1952; Moss and Young, 1960; Moss, 1973) states that craniofacial development needs to be interpreted in terms of changes in functional cranial components (FCC). This theory essentially assumes that skeletal growth may vary in response to mechanical stresses and the demands of the functionally associated soft tissues and cavities, which also grow (e.g., Henderson and Carter, 2002). The FCCs behave relatively independently during growth. They are integrated by a functional matrix (FM) and by a skeletogen unit (SKU) that gives biomechanical support and protection to the FM demands (Moss, 1979). The theoretical conception of functional craniology can be easily integrated into the framework of heterochrony (FCC − growth field).

Several studies have been conducted on the craniofacial anatomy of the genus *Saimiri* (Delattre and Anthony, 1951; Hill, 1960; Thom, 1965; Hershkovitz, 1977; Kaack et al., 1979; Ayres, 1985; Thorington, 1985; Corner and Richtsmeier, 1992; Hartwig, 1995). However, little effort has been made to study the craniofacial anatomy of *Saimiri* from the point of view of functional craniology (Pucciarelli et al., 1990; Dressino, 1991; Dressino and Pucciarelli, 1997). Previous studies on squirrel monkeys growing under controlled conditions have suggested that individuals fed a low-protein diet undergo changes in the neurocranium and face (Pucciarelli et al., 1990; Dressino and Pucciarelli, 1997). Thus, it is possible to compare groups of *Saimiri* fed different amounts of protein to assess whether heterochrony is responsible for their resulting craniofacial differences.

The aim of the present study is to assess whether heterochrony can account for intraspecific differences due to variation in environmental factors that are experimentally induced. In order to do this, a) craniofacial ontogeny patterns of the male squirrel monkey were characterised on the basis of its SKUs; b) the effect of a low-protein diet on craniofacial adult individuals was established; and c) appropriate methodologies to tackle heterochrony (Godfrey and Sutherland, 1995, 1996; Ramirez Rozzi, 2000), as well as geometric morphometrics, were applied to compare low-protein-fed and high-protein-fed *Saimiri*.

Material and methods

The sample

Twenty-four weanling squirrel monkey males (*Saimiri sciureus boliviensis*), born in captivity at

the CAPRIM (Argentine Primate Center), were used to carry out this study. They were assigned to two groups: 1) high-protein-content group (HP): twelve animals fed ad libitum a 20% protein-content diet; 2) low-protein-content group (LP): twelve individuals fed ad libitum a 10% protein-content diet. Both diets were prepared daily in our laboratory (Table 1) (for details see Pucciarelli et al., 1990, 2000; Dressino and Pucciarelli, 1997; Console et al., 2001a, b). The HP individuals were sacrificed at 9 and 24 months of age, whereas LP specimens were sacrificed between 15 and 26 months of age. Table 2 gives the age in months at which each individual was sacrificed. Craniofacial data were collected directly from the sacrificed individuals.

Age estimation

The eruption of M3 is taken as the beginning of the adult stage in Primates (Smith, 1989; Smith et al., 1994). In *Saimiri sciureus*, M3 erupts between the 15th month (lower molar) and the 19th month (upper molar) (Galliari and Colillas, 1985). Since the youngest LP individual died at 15 months, all undernourished animals can be considered adults. In others words, following Reilly et al.'s (1997) suggestions, we assume that adult craniofacial shape is the shape at the time of M3 eruption. However, to confirm the inclusion of all LP animals in the adult stage, independent-sample t-tests of differences between individuals who died at 15–19 months and those sacrificed at 24 months old were conducted. The t-tests and geometric morphometric analysis (see below) showed that there are no differences between them. Therefore, all LP individuals were considered to have already attained their adult size and shape. Volumetric and morphometric indices were employed to compare, by means of independent-sample t-tests, LP animals with adult HP specimens in order to establish the nutritional effect of the low-protein diet on craniofacial size and shape.

Table 1
Composition of high-protein (HP, 20%) and low-protein (LP, 10%) diets

Component	High-protein diet (g)	Low-protein diet (g)
Soybean meal	28.0	9.9
Wheat meal	14.7	8.0
Glucose	0.0	6.7
Skimmed milk	10.6	4.9
Wheat bran	5.6	5.6
Saccharose	3.5	3.5
Rice meal	3.3	6.6
Corn starch	3.0	21.4
Margarine	4.2	6.7
Egg	7.0	3.2
Vitamin mixture	1.5	1.5
Salt mixture	1.5	1.5
Water	17.1	20.5
Total	100.0	100.0

Table 2
Sex, group, and age in months at which each individual was sacrificed

Sex	Group	Age (months)
M	Control	9
M	Control	9
M	Control	9
M	Control	9
M	Control	9
M	Control	9
M	Control	24
M	Control	24
M	Control	24
M	Control	24
M	Control	24
M	Control	24
M	Undernourished	15
M	Undernourished	16
M	Undernourished	16
M	Undernourished	16
M	Undernourished	17
M	Undernourished	18
M	Undernourished	19
M	Undernourished	19
M	Undernourished	20
M	Undernourished	24
M	Undernourished	24
M	Undernourished	26

Craniofunctional analysis

Using functional cranial theory, postulated by van der Klaauw for mammals (Klaauw, 1948-1952) and by Moss and Young (1960) for humans, the skull was divided into two major SKUs: the neurocranium and the face. Each major SKU was divided into four minor SKUs (or components). The neurocranium comprised the anterior, middle,

posterior, and the otic components. The face included the optic, respiratory, masticatory, and the alveolar components. Skulls were oriented in the Frankfurt plane. Raw data consisted of length (L), width (W), and height (H) of the major components (neurocranium and face), as well as those of minor components. Further details on measurements are described in Table 3. Cranial

Table 3
Length, width, and height of the major and minor components. Landmarks used in the geometric morphometric analyses are marked with asterisks

Component		Code	Measurement
Major components			
Neurocranium	Length	NL	Nasion*-opisthocranion*
	Width	NW	Eurion-eurion
	Height	NH	Basion*-vertex
Face	Length	FL	Inner prosthion*-vomerobasilar*
	Width	FW	Zygion-zygion
	Height	FH	Nasion*-External prosthion*
Minor components			
Anteroneural	Length	ANL	Glabella*-bregma*
	Width	ANW	Pterion-pterion
	Height	ANH	Bregma*-vomerobasilar*
Midneural	Length	MNL	Bregma*-lambda*
	Width	MNW	Same as NW
	Height	MNH	Basion*-bregma*
Posteroneural	Length	PNL	Opistion*-opisthocranion*
	Width	PNW	Asterion-asterion
	Height	PNH	Lambda*-opistion*
Otic	Length	OTL	Posterior-inferior limit of tympanic bone - midpoint of sagital limit of petrous bone
	Width	OTW	Most posterior* to most anterior* external points of the auditive meatus
	Height	OTH	Porion*-inferior and external point of the auditive meatus*
Optic	Length	OL	Dacrion-intersfenoidal foramen
	Width	OW	Dacrion-ectoconquium
	Height	OH	Midpoint of supraorbitary – midpoint of infraorbitary
Respiratory	Length	RL	Nasospinale*-posterior nasal spine*
	Width	RW	Alare* left-Alare right*
	Height	RH	Nasion*-Nasospinale
Masticatory	Length	ML	Lower border zygomatic synchondrosis- posterior border of the glenoid cavity
	Width	MW	Anterior sulcus of the sphenotemporal crest- lower point of the zygotemporal synchondrosis*
	Height	MH	Lower border of the zygotemporal synchondrosis*- upper temporal line at the coronal intersection*
Alveolar	Length	AL	External Prosthion*-posterior alveolar border*
	Width	AW	From left to right second-third molars width
	Height	AH	Palatal depth at midsagital/second-third molars width

Table 4
Volumetric (VI) and morphometric (MI) indices

Symbol	Formula	Description
Major components		
NVI	$NVI = \sqrt[3]{NL*NW*NH}$	neurocranial volumetric index
FVI	$FVI = \sqrt[3]{FL*FW*FH}$	facial volumetric index
NMI	NMI = 100*NVI/(NVI + FVI)	neurocranial morphometric index
Minor components		
ANVI	$ANVI = \sqrt[3]{ANL*ANW*ANH}$	anteroneural volumetric index
MNVI	$MNVI = \sqrt[3]{MNL*MNW*MNH}$	midneural volumetric index
PNVI	$PNVI = \sqrt[3]{PNL*PNW*PNH}$	posteroneural volumetric index
OTVI	$OTVI = \sqrt[3]{OTL*OTW*OTH}$	otic volumetric index
OVI	$OVI = \sqrt[3]{OL*OW*OH}$	optic volumetric index
RVI	$RVI = \sqrt[3]{RL*RW*RH}$	respiratory volumetric index
MVI	$MVI = \sqrt[3]{ML*MW*MH}$	masticatory volumetric index
AVI	$AVI = \sqrt[3]{AL*AW*AH}$	alveolar volumetric index
ANMI	ANMI = 100*ANVI/(ANVI + MNVI + PNVI + OTVI)	anteroneural morphometric index
MNMI	MNMI = 100*MNVI/(ANVI + MNVI + PNVI + OTVI)	midneural morphometric index
PNMI	PNMI = 100*PNVI/(ANVI + MNVI + PNVI + OTVI)	posteroneural morphometric index
OTMI	OTMI = 100*OTVI/(ANVI + MNVI + PNVI + OTVI)	otic morphometric index
OMI	OMI = 100*OVI/(OVI + RVI + MVI + AVI)	optic morphometric index
RMI	RMI = 100*RVI/(OVI + RVI + MVI + AVI)	respiratory morphometric index
MMI	MMI = 100*MVI/(OVI + RVI + MVI + AVI)	masticatory morphometric index
AMI	AMI = 100*AVI/(OVI + RVI + MVI + AVI)	alveolar morphometric index

landmarks were recorded using a Microscribe 3-DX digitizer and InScribe-32 software (Immersion Corp., San Jose, CA). Length, width, and height were obtained from the same view in order to avoid errors in integrating these within a common system of landmarks. Volumetric and morphometric indices were obtained according to the formulae shown in Table 4. The volumetric index is the geometric mean and corresponds to the size of the component (Jungers et al., 1995). Volumetric indices were obtained for the two major components. The morphometric index is the relative contribution of a component. The morphometric index of the neurocranium is the relative contribution of the neurocranium to skull size; the morphometric indices of minor components correspond to their relative contribution to the size of major components. Since shape can be defined as a ratio, i.e., the relative size of a part to the whole (Gould, 1966, 1977), the morphometric index gives an estimation of shape. The morphometric index for the neurocranium corresponds to skull shape. Morphometric indices for the four minor components of the neurocranium define the shape of the neurocranium. In a similar way, the shape of the face is given by the morphometric indices of the four minor components. Because morphometric indices were calculated independently for the four minor components of the face and for the four components of the neurocranium (Table 4), their sums for each major component, by definition, equal 100.

Given the normality of the distribution frequencies, the volumetric and morphometric indices were used to document changes in size and shape from juvenile (9 months old) to adult (24 months old) HP animals (independent-samples t-tests). We could thus characterise the craniofacial ontogeny of squirrel monkeys.

Geometric morphometric analysis

As an alternative to the pure craniofunctional analysis, size and shape changes were studied following geometric morphometric methods—a useful approach for quantitative characterization, analysis, and comparison of biological forms (Bookstein, 1991; Rohlf, 1993; Marcus et al., 1996; Dryden and Mardia, 1998; Lele and Richtsmeier, 2001). Our raw data consisted of 21 three-dimensional landmarks registered in lateral view

(Table 3). Each individual is described by a landmark configuration. Once the landmark configurations were collected for each specimen, a series of algorithms were applied. The first step involved the superimposition of all landmark configurations via translation and rotation using the Procrustes method (Goodall, 1991). From the superimposed configurations, a consensus mean configuration was obtained and used as a reference. All configurations were scaled according to a linear size measure known as the centroid size (the square root of the sum of squared distances from each landmark to the specimen's centroid). To analyse shape, centroid size was set equal to 1. Residuals from the consensus configuration were modelled with the thin-plate spline interpolating function (Bookstein, 1991). Parameters of the fitted function represent the new set of variables defining a new matrix, namely the weight matrix. Each row of the weight matrix represents an individual and each column represents the new variables. Due to the properties of the thin-plate spline function (Bookstein, 1991), the new variables can be analysed using traditional multivariate techniques and shape change or shape differences can be visualised as deformation grid splines. The weight matrix was used in a relative warp analysis, which is the analogue of a principal component analysis for this kind of data (Rohlf, 1993), and which decomposes shape variability into statistically independent factors. Relative warps are statistically independent factors of shape variation that account for the largest, second largest, and successively smaller proportions of the total sample variance in shape.

Finally, landmark coordinate data were studied using a coordinate-system-free approach: Euclidean distance matrix analysis, or EDMA (Lele and Richtsmeier, 1995, 2001; Richtsmeier et al., 2002). EDMA is a coordinate-system invariant method (Lele and Richtsmeier, 2001) for comparing form, shape, or growth differences between two samples. It uses landmark coordinates as raw data and describes a three-dimensional object by the matrix of Euclidean distances between all possible unique landmark pairs (Ackermann and Krovitz, 2002). This matrix of distances is called the form matrix (FM). The form matrix [or FM (A) for object A] is an equivalent representation of the landmark coordinate data that is invariant to the nuisance parameters of translation, rotation, and reflection (Lele and Richtsmeier, 2001). Shape matrices, including all the possible interlandmark distances within each shape, were obtained after standardizing the mean form matrices by a scaling factor (the geometric mean). Interlandmark differences in size (scaling factor) and shape were used to explore skull size and shape changes (Lele and Richtsmeier, 1995, 2001; Richtsmeier et al., 2002). Lele and Cole (1996) described a procedure for testing the significance of differences in shape and size based on the computation of the z-statistic. Under the null hypothesis of equality of shapes, this value should be close to zero. A parametric bootstrap (Monte Carlo) procedure is usually used to calculate the $100(1-\alpha)\%$ confidence interval. If this interval contains the value zero, the null hypothesis is not rejected. Lele and Cole (1996) suggested using $\alpha = 0.1$. As a simple extension, one can also test whether or not the "size" measures of two populations are different. If one is interested in whether or not the scaling measure (C) differs between populations, one can also test whether or not the 90% Monte Carlo confidence interval for the quantity C1−C2 includes zero. This enables the researcher to decompose a significant difference in "form" into tests of differences in shape and scale (Lele and Cole, 1996).

Heterochronic tests

The first step in the analysis of heterochrony was to observe whether LP animals showed the same relationship of size to shape as HP individuals (i.e., size/shape association or dissociation). We used Gould's (1977) metric for testing the dissociation of size and shape. In a plot of logarithmically transformed trait data and size scales, LP adult shape (i.e., the trait/size ratio) was compared to that of HP adults to ascertain whether the LP adult ratio is identical to that of larger, smaller, or similarly sized HP specimens (Fig. 1). Since age is known for all individuals, Gould's (1977) clocks were also employed to detect heterochrony. Gould's method is useful in assessing modifications in size and the shape of single traits (measured on the y-axis).

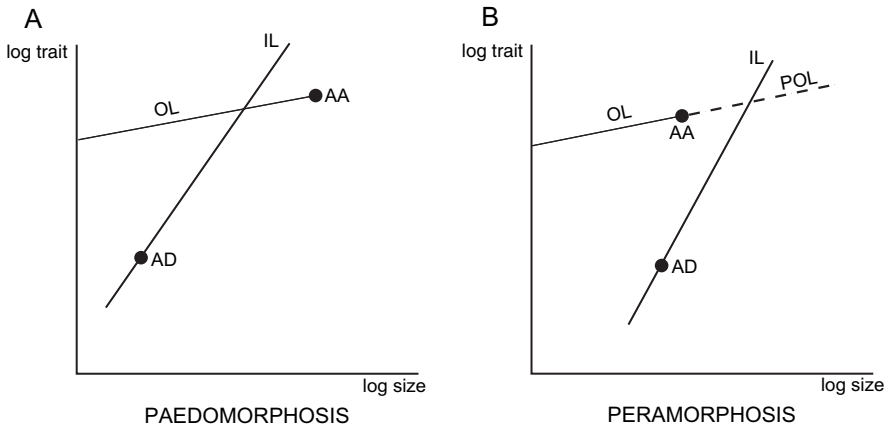

Fig. 1. Gould's (1977) metric for dissociation allows one to ascertain whether adult descendant shape (AD), in our case, that of adult LP animals, matches the shape of a larger, smaller, or similarly sized adult ancestor (AA), in this study, adult HP animals. One need only ask where the line of isometry (IL) of the adult descendant (AD) crosses the ancestral line of ontogeny (OL). In our study, the ontogeny line of HP animals was the line connecting the HP juvenile and adult shapes. In A, the descendant adult isometry line crosses the juvenile portion of the ancestral ontogeny line; thus, the descendant is paedomorphic. In B, the isometry line crosses the projection of the ontogeny line (POL); the adult descendant's shape matches what the ancestor would have acquired had its ontogeny not stopped at AA. In this case, the descendant is peramorphic. A descendant would be isomorphic if the isometry line passes through AA.

However, the interpretation may become ambiguous when more than two traits contribute to size variation because traits must be analysed independently (each with its own metric of dissociation and clock). Gould's methods were therefore applied only to the analysis of the major components (neurocranium and face). For this analysis, "size" was measured by the neurocranial (NVI) and the facial (FVI) volumetric indices, and "shape" by the neural-cranial morphometric index (NMI) (Table 4).

Godfrey and Sutherland (1996) suggested a "multivariate perspective" method for analysing heterochrony. This method has two advantages: 1) many traits can be analysed altogether, and 2) the comparison enables researchers to assess whether groups follow similar developmental pathways. Sizes of each trait, total size (corresponding to the sum of trait sizes), and shape (i.e., the relative sizes of traits) are represented by bars (Fig. 2). Changes in shape through ontogeny are given by modifications of the distribution of ratios from one growth class to another. Two groups show similar developmental pathways if their trait-ratio distributions change in the same manner through ontogeny, regardless of the correspondence (or lack of correspondence) of trait-ratio distributions at comparable ages or sizes (Fig. 2). In the present work, this multivariate perspective was employed to complete the analysis of heterochrony. Confidence about the occurrence of heterochrony should only be manifested if different methods reveal the same patterns (Godfrey and Sutherland, 1995, 1996; Ramirez Rozzi, 2000). We used StatView software to conduct these statistical analyses.

Heterochronic processes were also studied using landmark coordinate data by means of two geometric morphometric methods: relative warp analysis and EDMA. Relative warps capture independent aspects of shape variation that can be plotted as age-related patterns of morphological change. The combined observation of the first relative warps, explaining pure shape changes, and a shape-independent representation of size, the centroid size, provides an alternative method to test heterochronic hypotheses (for a previous application of this analysis, see Ponce de León and Zollikofer, 2001). To evaluate heterochronic processes using EDMA, we tested the statistical significance of shape difference matrices among the three groups, as well as differences in the scaling factor (size). Due to its explicit dissociation of size and shape, this method provides yet another useful tool for studying heterochrony.

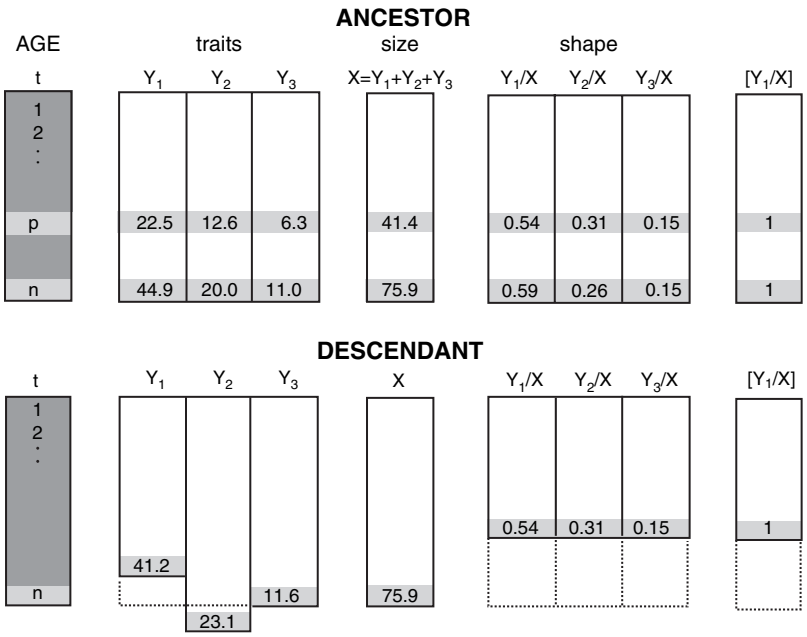

Fig. 2. Multivariate perspective methodology (after Godfrey and Sutherland, 1996: 32). Age, trait size, total size, and shape are represented by vectors or bars that record, in a fashion similar to Gould's clock, how the "descendant" differs from its ancestor. Total size is the sum of the sizes of individual traits at any point along the ontogenetic trajectory. The shape vectors show each trait's proportion of total size. The sum of the ratios is 1. In the case presented here, the adult descendant's shape is the ratio distribution 0.54, 0.31, and 0.15. It is similar to the juvenile ancestor's shape. Size in the adult descendant is the same as that of the adult ancestor, signalling dissociation of size and shape. Because descendant adult shape is similar to ancestral juvenile shape, the descendant is paedomorphic, and because size and shape are dissociated, paedomorphosis was obtained through neoteny. This method allows researchers to analyse several traits simultaneously and to test whether ontogenies have followed similar developmental pathways.

Table 5
Volumetric (VI) and morphometric (MI) index values for juvenile and adult HP *Saimiri*

	Juvenile			Adult			t-test
	X	SD	SE	X	SD	SE	
Size							
NVI	39.5	0.781	0.319	41.21	0.887	0.362	**
FVI	23.24	0.809	0.330	26.49	0.595	0.243	**
Shape							
NMI	62.96	1.024	0.418	60.87	0.461	0.188	**
ANMI	33.87	0.563	0.230	34.02	0.387	0.158	
MNMI	36.18	0.573	0.234	36.75	0.288	0.118	
PNMI	21.07	0.743	0.303	20.34	0.490	0.200	
OTMI	8.88	0.253	0.103	8.90	0.432	0.176	
OMI	35.10	0.933	0.381	31.02	0.912	0.372	**
RMI	26.95	1.136	0.464	25.84	0.445	0.182	
MMI	21.07	1.837	0.750	22.99	0.523	0.214	*
AMI	16.88	3.435	1.402	20.14	0.937	0.383	*

* = $P < 0.05$, ** = $P < 0.01$.

Results

General morphological patterns

Table 5 shows volumetric and morphometric index values for juvenile and adult HP animals, as well as tests of the significance of their differences. Size (volumetric indices, VI) and shape (morphometric indices, MI) changed from juvenile to adult *Saimiri*. The size of the neurocranium (NVI) and the face (FVI) increased, while the relative size of the neurocranium (NMI) decreased significantly, suggesting that the face grew more than the neurocranium (Fig. 3). It is useful to compare these results with those obtained by the geometric morphometric methods (Fig. 4). Visualisation of the results of a thin-plate spline analysis also demonstrates a reduction in the relative size of the whole neurocranium (Fig. 4a). In particular, lambda, opisthocranion, and bregma exhibit a relative shift forward in the adult specimens, thus indicating a reduction of the relative size of the vault.

Despite the change in neurocranial size, there was no change in the relative sizes of minor components (ANMI, MNMI, PNMI, OTMI), which suggests that neurocranial shape remained constant during growth. In the face, the optic component (OMI) changed significantly from the juvenile to the adult stages in *Saimiri*, contributing less to the latter than the former age stage. The masticatory (MMI) and the alveolar (AMI) morphometric indices were, in contrast, proportionally higher in adults than in juveniles; thus, facial shape did change during growth. This result is also evident in the infero-posterior displacement of landmarks situated on the zygomatic arch, the upward displacement of the upper temporal line at the coronal intersection, and the anterior expansion of prosthion and nasospinale (Fig. 4a, b). The respiratory morphometric index (RMI) was the only facial component whose relative size did not change during growth.

Adult LP animals are smaller than adult HP specimens; the FVI and the NVI were significantly reduced by the dietary stress (Table 6). The NMI was not significantly altered, indicating that the relative size of the neurocranium in the skull is not affected by dietary stress. The relative sizes of the neurocranial minor components showed slightly significant (MNMI) or insignificant (ANMI, PNMI, OTMI) differences between HP and LP animals. Figure 4c similarly shows that adult LP and HP animals differ between little in the ontogenetic displacement of neurocranial and facial landmarks. This suggests that adult neurocranial shape is quite similar in both groups. Facial shape, however, did differ between LP and HP animals. The LP animals were characterised by a relatively larger optic (OMI) component and a relatively smaller masticatory (MMI) component than in HP specimens (Table 6).

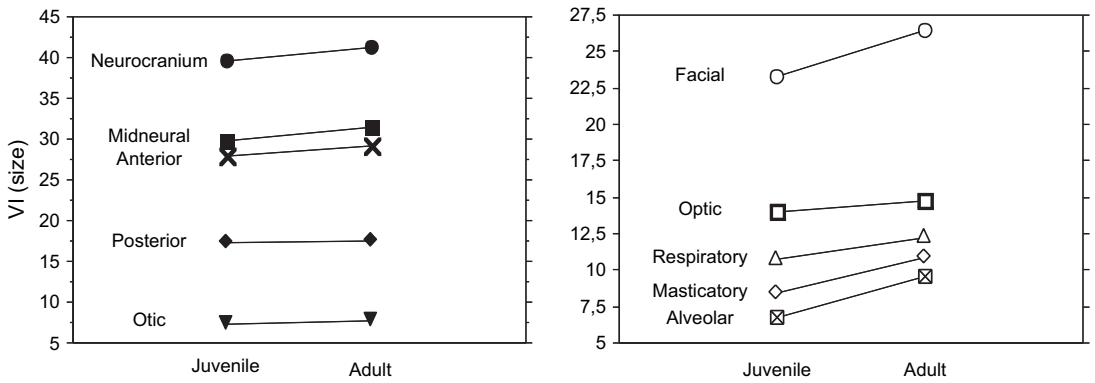

Fig. 3. Changes in size (VI: volumetric indices) from juvenile to adult in *Saimiri*. Change in size (growth) is more important in the face than in the neurocranium. All minor components of the face grow from the juvenile to the adult stage of ontogeny, but in the neurocranium, only the anterior and midneural components grow significantly.

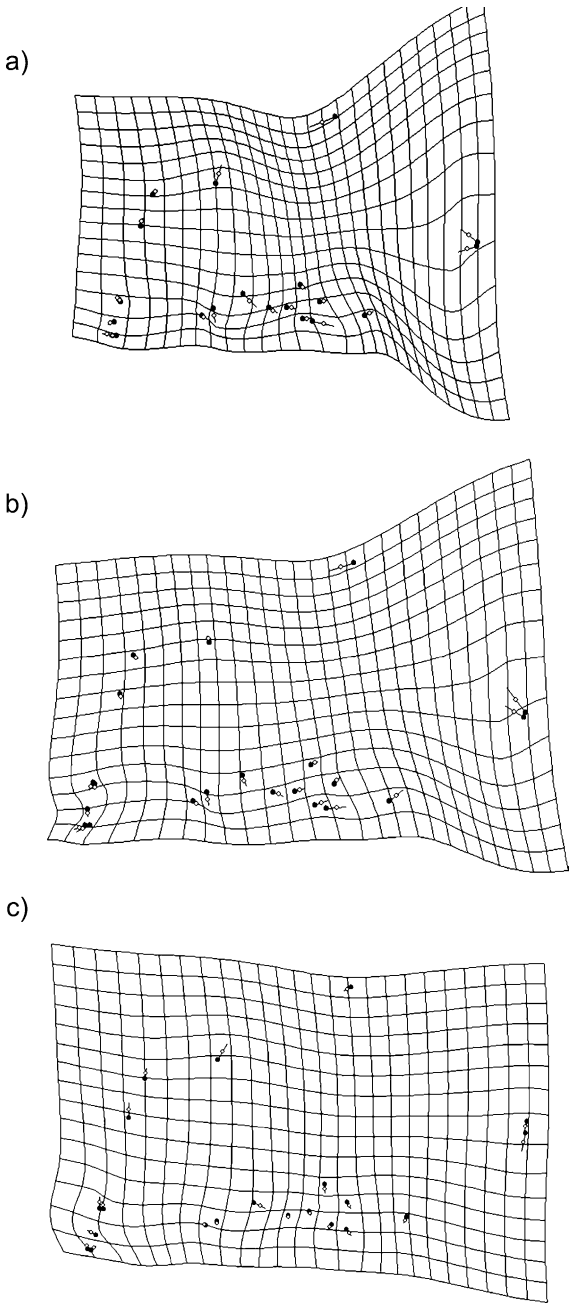

Fig. 4. Thin-plate spline analyses based on Procrustes superimposition of a) juvenile HP (reference) and adult HP (target); b) juvenile HP (reference) and adult LP (target); and c) adult LP (reference) and adult HP (target). Solid circles: reference landmarks; open circles, target landmarks. Vectors depict the direction and magnitude of the change from the reference to the target. To facilitate visualisation of shape changes, all splines were magnified two times.

An alternative comparison of the three groups is provided by the analysis of relative warps and centroid sizes (Fig. 5). These results corroborate the craniofunctional analysis: both LP and HP adults present near zero or positive values along relative warp 1, thus demonstrating a common shape for both adult samples. This result is also evident in the thin-plate spline analysis, which suggests small shape differences between LP and HP adult specimens (Fig. 4c) and similar ontogenetic changes from the juvenile HP condition to either adult LP individuals (Fig. 4b) or adult HP individuals (Fig. 4a). In terms of size, HP animals are larger than are LP individuals, which tend to overlap with HP juveniles (Fig. 5). Positive values of the relative warp 1 mainly reflect the differences explained above, that is, a relative decrease in the neurocranial size of the adults. Remaining relative warps are not significantly correlated with size, nor do they discriminate the three groups. They thus provide no information of utility to studying heterochrony.

Heterochrony

Metric of dissociation and clocks

In a bivariate graph (Fig. 6), log neurocranial size (NVI) is plotted against the logged size of the entire skull (ES, i.e., the sum the two major volumetric indices, NVI + FVI). Both ES and neurocranium size differ in juvenile vs. adult HP individuals. Plotting these points allows us to depict Gould's (1977) line of ontogeny for juvenile and adult HP *Saimiri*. Gould used "lines of isometry" to depict individuals who differ in size but not shape from individuals at various points along the line of ontogeny. In the case of *Saimiri*, both ES and neurocranial size are smaller in LP adults than in adult HP specimens. However, the skull shape (or relative size of the neurocranium) of the former matches that of adult HP individuals. Note that Gould's line of isometry (IL) passing through the mean (ACP) for adult LP animals crosses the HP line of ontogeny at exactly the point corresponding to the adult HP value (ratio = 0.882). This indicates that the shape of the skulls of LP adults resembles that of HP adult *Saimiri*, despite their differences in size (Fig. 6).

Table 6
Volumetric (VI) and morphometric (MI) index values for *Saimiri* fed a 20% protein-content diet (HP) and a 10% protein-content diet (LP)

	HP			LP			t-test
	X	SD	SE	X	SD	SE	
Size							
NVI	41.21	0.887	0.362	39.04	1.087	0.314	**
FVI	26.49	0.595	0.243	24.67	0.658	0.190	**
Shape							
NMI	60.87	0.461	0.188	61.28	0.672	0.194	
ANMI	34.02	0.387	0.158	34.66	0.794	0.229	
MNMI	36.75	0.288	0.118	36.11	0.586	0.169	*
PNMI	20.34	0.490	0.200	20.27	0.767	0.221	
OTMI	8.90	0.432	0.176	9	0.376	0.108	
OMI	31.02	0.912	0.372	33.57	0.942	0.272	**
RMI	25.84	0.445	0.182	25.66	0.931	0.269	
MMI	22.99	0.523	0.214	21.68	1.005	0.290	**
AMI	20.14	0.937	0.383	19.09	1.360	0.393	

* = P < = 0.05, ** = P < 0.01.

Size and shape are thus dissociated. Gould's clock for NVI shows the same phenomenon (Fig. 7): the age bar (grey) is the same in HP and LP animals because both samples comprised 24 month-old specimens. The shape arrow for LP animals points to the shape of HP adults since both have the same ratio (0.882). The size arrow of LP adults (1.804) is directed between those of juvenile (1.797) and adult (1.831) HP animals. Size and shape are dissociated. The LP animals attain the same shape at the same age as do HP specimens, but at a smaller size. Gould's metric for size/shape

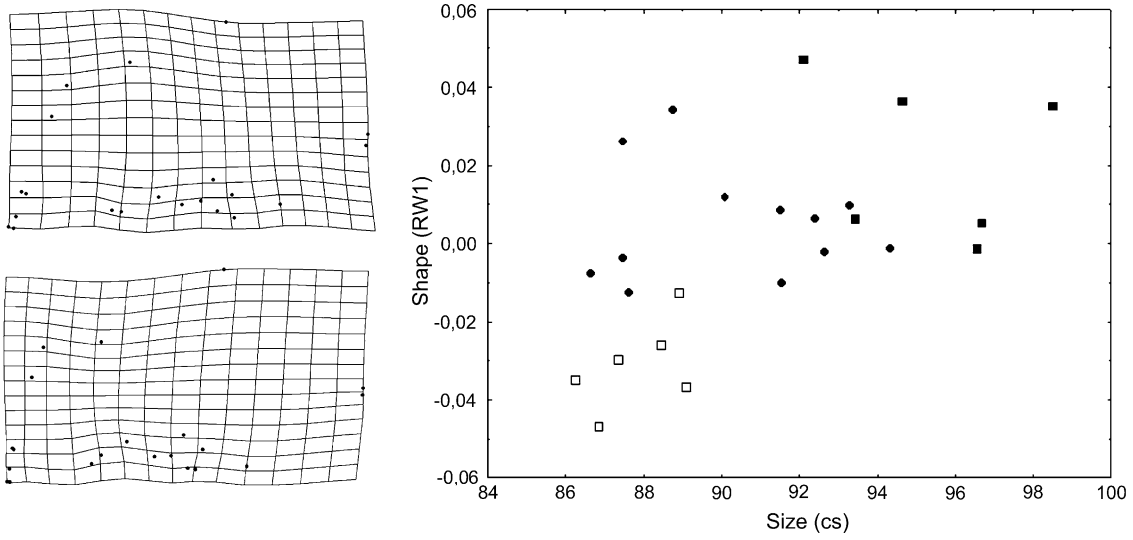

Fig. 5. Scatterplot of size (centroid size) against shape (relative warp 1). Relative warp 1 accounts for 29.35% of variation. Solid squares: adult HP; open squares: juvenile HP; solid circles: adult LP. Shape changes (departures from the consensus) associated with positive and negative values of the relative warp 1 are presented as splines on the left side of the graph.

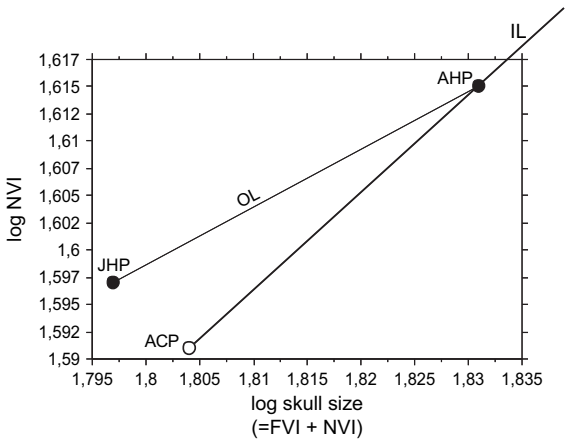

Fig. 6. Gould's metric of size/shape dissociation. The logged size of the skull—i.e., the sum of the face volumetric index (FVI) and the neurocranium volumetric index (NVI)—is plotted against logged neurocranium size (NVI). Solid points represent the ratio of neurocranium size to skull size in juvenile (JHP) and adult (AHP) HP animals. These points determine the ontogeny line (OL). The isometry line (IL) passing through the ratio corresponding to LP adults (ALP) crosses the OL at the point AHP, indicating that the shape of ALP is the same as that of AHP (ratio = 0.882). ALP and AHP are isomorphic.

dissociation and Gould's clock model both suggest that a low-protein diet can produce heterochronic changes. Since shape has not changed but there was a reduction in size, that heterochronic shift must be interpreted as isomorphosis by dwarfism.

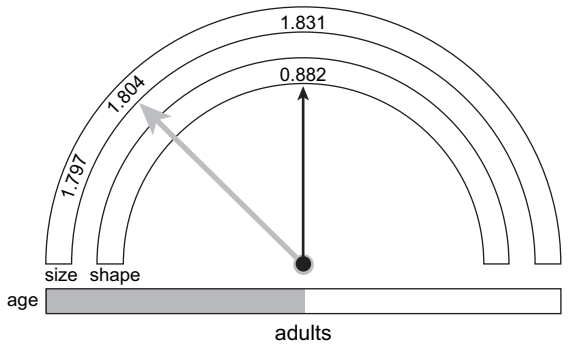

Fig. 7. Gould's clock applied to squirrel monkeys. In this example, because shape is similar in adult HP and adult LP animals but size is smaller in the latter, this clock depicts isomorphosis by dwarfism (see text for further explanation).

Multivariate perspective

The heterochronic process apparently revealed using the methods described above should be tested by an analysis of size (VI) and shape (MI) changes of the minor components using the multivariate perspective method. If heterochrony holds at this finer level of analysis, then it is expected that 1) components that differ between LP and HP adults will be those that differ between juvenile and adult HP animals, and 2) similar directional changes in components will apply across both comparisons. In the present case, if isomorphosis via dwarfism applies across minor components, then we should expect adult LP individuals to differ from HP individuals in having components that are smaller in size but similar in shape. If the similarity in the shape of major components (observed above) is achieved through different ontogenetic pathways (resulting in a different distribution of relative sizes of minor components in LP and HP adults), then heterochrony is not possible. Instead, we would conclude that the similar shapes of major components were obtained coincidentally, with minor components affected by different processes. Alternatively, if the relative size distributions of minor components are similar, heterochrony may be considered plausible.

The multivariate perspective method for the minor neurocranial components (Fig. 8) showed that the minor components increased in size from the juvenile to the adult HP animals. In LP animals, each of the minor components, except in ANVI, was similar to or smaller in size than in juvenile HP individuals. Size in LP specimens, as expected, was also smaller than in juvenile HP *Saimiri*. The relative size of neurocranial minor components did not show significant differences between juvenile and adult HP (see above), suggesting that the shape of the neurocranium remained stable during postnatal growth. If heterochrony has occurred, we would expect the same to apply to our comparison of HP and LP adults. This was confirmed by our experiment, in which the only exception, the midneural component, differed only at the .05 level of significance (Table 6, Fig. 9). Therefore, the neurocranial size was smaller in LP animals than in HP individuals, but the neurocranial shape did not change.

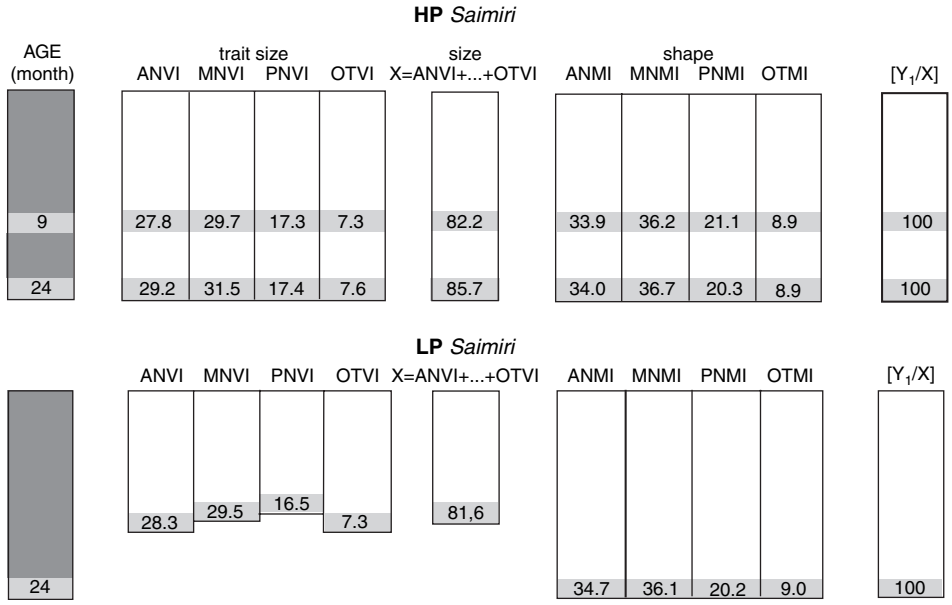

Fig. 8. The multivariate perspective method for minor components of neurocranium of squirrel monkeys. Trait size and total size are smaller in LP animals than in adult HP individuals and even than in juvenile HP, except in ANVI. Shape does not change from juvenile to adult HP animals. LP individuals do not differ in shape from adult HP animals.

The multivariate perspective method for the facial minor components (Fig. 10) showed that the four minor components (OVI, RVI, MVI, AVI) increased in size from the juvenile to the adult HP animals. In LP animals, the size of minor components was intermediate between those of juvenile and adult HP specimens. An exception was the optic component (OVI), which was similar to that of adult HP. Also, total size in LP animals was intermediate between those of juvenile and adult HP specimens. The relative size changed significantly in three out of the four facial minor components from juvenile to adult HP animals. Specifically, the optic component (OMI) decreased, while the masticatory (MMI) and alveolar (AMI) components increased. The respiratory component (RMI) did not change. In LP animals, the relative size of these three minor components showed an intermediate value between those of juvenile and adult HP individuals (Fig. 11). The relative size of the respiratory component (RMI) was not affected by the low-protein diet. Thus, the facial size of the stressed animals was smaller than that in adult HP individuals and the facial shape in LP animals resulted from a developmental arrest. The multivariate perspective analysis of minor cranial components suggests that differences observed between HP and LP animals were due to changes occurring through the same

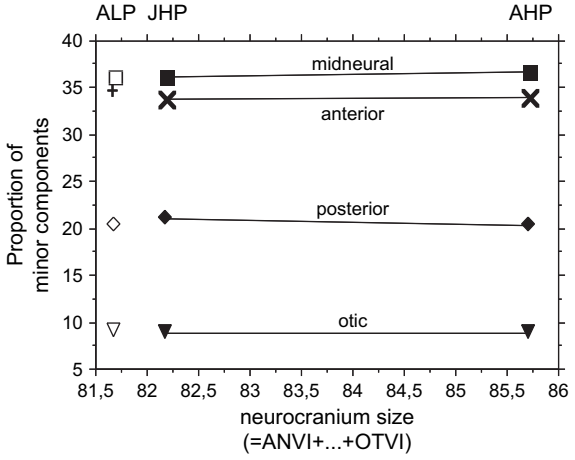

Fig. 9. Neurocranial minor component proportions do not change from juvenile (JHP) to adult (AHP) HP animals. Total size in adult LP individuals (ALP) is smaller than in juvenile HP specimens, but the proportions are not different.

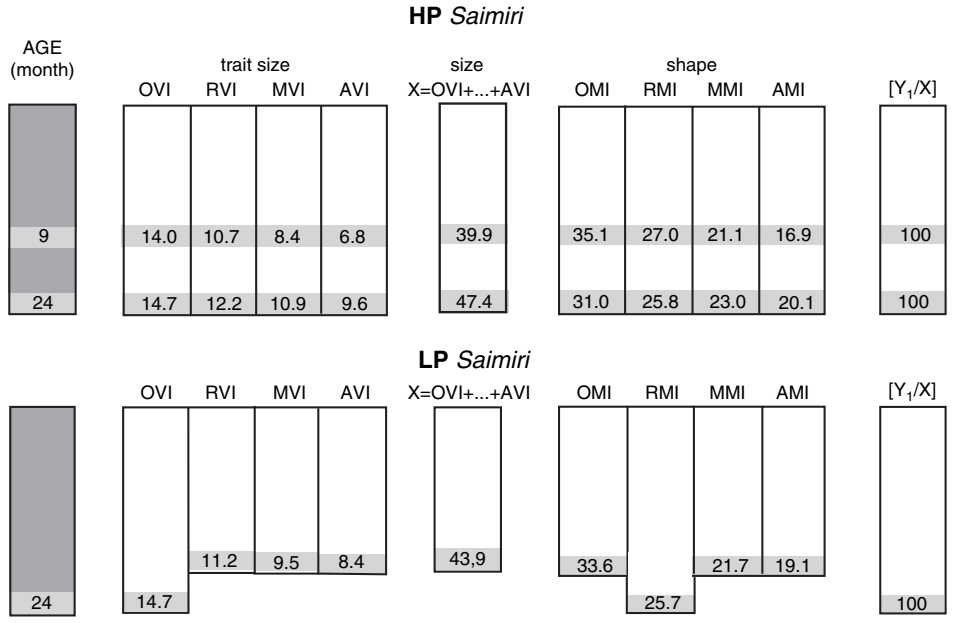

Fig. 10. The multivariate perspective method for minor components of the face (see text for explanation).

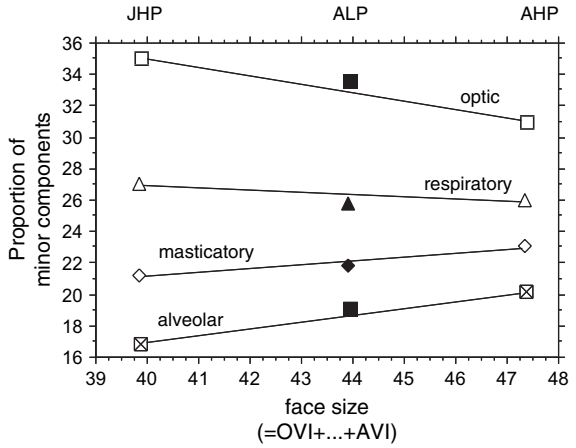

Fig. 11. Changes in the proportions of the minor components of the face. Size in LP animals (ALP) is smaller than in adult HP individuals (AHP) but bigger than in juvenile (JHP) HP animals. The proportions of optic, masticatory and alveolar components change from juvenile to adult HP specimens. In LP, the proportions of these components fall between juvenile and adult HP values. Only the proportion of the respiratory component in LP animals does not follow this pattern; it is close to the adult HP value. It is noteworthy that this minor component does not change from juvenile to adult HP animals.

developmental pathway. This can be interpreted as resulting from heterochrony. The face followed a different process (developmental arrest) to that observed at skull level (isomorphosis via dwarfism) (see Discussion).

Geometric morphometrics

Figure 5 provides a useful tool to test heterochrony since it combines in a simple bivariate plot a representation of pure shape changes (the relative warps) and a shape-independent linear estimation of size (the centroid size). Furthermore, shape changes corresponding to extreme values along the shape axis can be visualised in the form of thin-plate splines. This analysis corroborates the results of the craniofunctional clocks and the dissociation graphics depicted above: LP animals tend to have smaller skulls than adult HP specimens, but the shape observed in LP animals is similar to that of HP *Saimiri*. Geometric morphometrics thus reveals a dissociation of size and shape.

EDMA was used here as an alternative method to study the dissociation of size and shape. Table 7 summarizes differences in shape and size for all the possible group comparisons. Results are congruent

Table 7
Results of the EDMA carried out among the three groups. Size and shape differences are displayed. The scaling variable is the geometric mean of distances. The LP sample was chosen as the bootstrap referent because it is the largest sample. Probability values were obtained following Lele and Cole (1996)

Comparison	Shape Difference (z-statistic)	Size Difference (scaling variable difference)
Adult LP vs. Adult HP	0.0667 (not significant)	−1.26871 ($p < 0.01$)
Adult LP vs. Juvenile HP	−0.14827 ($p < 0.01$)	0.77174 ($p < 0.01$)
Adult HP vs. Juvenile HP	0.21496 ($p < 0.01$)	2.04045 ($p < 0.01$)

with previous tests in the sense that differences between LP and HP adult animals are restricted to size changes, whereas shape in both groups tends not to be significantly different.

In summary, Gould's methods for testing heterochrony (i.e., his metric of size/shape dissociation and his clock model), as well as geometric morphometrics, support the view that changes in diet can produce heterochrony. For *Saimiri* on low-protein diets, a reduction in skull size was not accompanied by a concomitant change in skull shape. The differences in skull size and shape of LP and HP *Saimiri* adults conform to the expectations of the heterochronic process of isomorphosis by dwarfism.

Discussion

Growth, development, and maturation are the three fundamental aspects of ontogeny (Gould, 1977). Growth is defined as changes in size over time, and development as changes in shape over time (Gould, 1977; Godfrey and Sutherland, 1995). Growth and development in *Saimiri*'s craniofacial ontogeny is characterised by 1) an increase in size in major components; 2) a change in skull shape shown by a decrease in the relative size of the neurocranium as a consequence of relatively greater postnatal growth of the face; 3) no change in neurocranial shape, or the relative contribution of minor components in neurocranium; and 4) a change in facial shape, mainly due to changes in the relative sizes of the optic, masticatory, and alveolar components. These aspects of *Saimiri* craniofacial ontogeny agree with those found in previous studies (Pucciarelli et al., 1990; Dressino and Pucciarelli, 1997).

Our results are in accord with those of previous studies of postnatal craniofacial growth in *Saimiri* (Corner and Richtsmeier, 1992). Craniofacial growth follows the same pattern observed in mammals where postnatal growth of the face contributes more than growth of the neurocranium in changes observed postnatally (Enlow, 1966; Michejda et al., 1979; Pucciarelli, 1981; Sirianni et al., 1982; Nanda et al., 1987; Oyhenart, 1988; Miller and German, 1999). In fact, the lower postnatal neurocranial growth in mammals results from the development of the brain earlier than any other structure in mammals (i.e., Topinard, 1891; Delattre, 1951; Moss and Young, 1960; Moss, 1973; Michejda, 1975; Sirianni and Swindler, 1979; Sirianni, 1985; Hartwig, 1995). Corner and Richtsmeier (1992) showed that the growth of the neurocranium in *Saimiri* is completed rather early in ontogeny. The absence of neurocranial changes in shape in our study would suggest that brain development was achieved in *Saimiri* by the 9th month of age.

Moss (1973) suggested that facial growth is explained by the volumetric expansion of the oro-naso-pharyngeal cavity. Corner and Richtsmeier (1991) found in *Cebus apella* that the muzzle grows far more than the orbital cavity (which showed the smallest amount of growth). In *Saimiri*, we showed that three out of four minor components increased from the juvenile to the adult HP stage, with growth in the optic one being the least. The explanation for the relatively high post-lactational increase of the masticatory component is linked to the development of the permanent dentition that occurs after the 9th month of age. Clearly, after infancy, the masticatory apparatus follows a common growth pattern in several primate species (Schultz, 1962). The low growth of the optic component is linked to its association with the soft tissues of and surrounding the eye, the eye being an outgrowth of the brain and following a neural

growth pattern (Cheverud, 1995). To sum up, differences in growth rates of the neurocranium and face in *Saimiri* result from a general pattern of morphological integration across mammals responding to the disjunction of neural and somatic growth systems that have different embryological origins (Cheverud, 1995).

Previous studies of malnutrition in *Saimiri* (Pucciarelli et al., 1990; Dressino and Pucciarelli, 1997) suggested that a low-protein diet affects the growth of both major craniofacial components, the face and neurocranium. DeRousseau and Reichs (1987) found that size and shape were affected in *Macaca* from Cayo Santiago when they were fed on a diet of improved quality. In *Saimiri*, skull size was affected by low-protein diet; however, skull shape was not. This indicates that size and shape were dissociated in skull. Malnutrition affected facial size, facial shape, and neurocranial size. Malnutrition 1) slowed facial growth and development as well as neurocranial growth, and 2) affected facial growth more than neurocranial growth, resulting in a smaller skull but with a similarly shaped neurocranium.

Miller and German (1999) observed that malnutrition in rats produces changes mainly in the timing of skull growth but not in adult size (contra Elias and Samonds, 1977; Pucciarelli, 1978, 1981; Yayha and Millward, 1994). The offset of growth is delayed in low-protein-fed animals in order to attain a size similar to that of high-protein-fed individuals; in other words, they found catch-up growth in animals under prolonged environmental stress, although catch-up can take a long time. In contrast, our experience shows that skull size is affected in *Saimiri* on a low-protein diet, but shape is not. It is possible that the contrary observations result from our comparing groups before complete catch-up is expected. Exploring morphological integration, Cheverud (1982, 1995) suggested that environmental integration is strong in functional or developmentally integrated traits when the neurocranium and face are treated as two different units, whereas genetic integration is stronger than environmental and phenotypic integration when the skull is considered as a whole. Cheverud and colleagues (Cheverud and Midkiff, 1992; Cheverud et al., 1992) observed that facial growth may be adjusted in a compensatory manner to altered neurocranial growth in order to maintain the structural integrity of the skull. They suggested that changes in neurocranial morphology can lead to specific complementary changes in facial morphology. The integration between the neurocranium and the face is "a matter of degree" (Cheverud, 1995: 70). Perhaps the different morphologies observed in protein-deprived rats and squirrel monkeys result from different degrees of morphological integration of functionally or developmentally integrated traits in the skull. Genetic integration is apparently stronger in *Saimiri* producing changes in the face and in the neurocranium that preserve their normal adult proportions in individuals subjected to environmental stress. In contrast, among rats, the functionally/developmentally related traits are influenced more by environmental integration than by genetic integration, and environmental changes differentially affect facial and neurocranial growth.

The similar shapes but different sizes of the skulls of HP and LP individuals demonstrate that these groups of *Saimiri* attained their adult morphology via two different growth trajectories. The paths by which similar skull size is achieved in rats on high and low-protein diets were also different (Miller and German, 1999). This indicates that intraspecific variability in development can explain a considerable amount of intraspecific differences in size and/or shape among adults.

Heterochrony sensu Gould (1977) occurs when changes in ontogenetic pathways affect the final shape, resulting in paedomorphosis or peramorphosis. However, Gould also recognized two processes through which descendant adults can preserve ancestral adult shape: gigantism (when the adult descendant is bigger than the adult ancestor) and dwarfism (when the adult descendant is smaller than the adult ancestor). Gigantism and dwarfism are not fundamentally different from other processes that are commonly recognized as heterochronic (Ramirez Rozzi, 2000). They are characterised by a dissociation of size and shape; they can be recognized when comparing the size and shape of ancestors and descendants at given ages. We consider these two processes as heterochronic, despite the fact that

they involve neither paedomorphosis nor peramorphosis. Gigantism and dwarfism are heterochronic processes that result in isomorphosis (Reilly et al., 1997; Godinot, 2000), since adult shape has not changed.

Differences between HP and LP *Saimiri* can be explained by heterochrony. A variety of methods appropriate for testing heterochrony (Gould, 1977; Alberch et al., 1979; Godfrey and Sutherland, 1995, 1996) were applied in this study. They show that LP individuals are proportioned dwarfs in relation to HP specimens. Their similar skull shape but different size is confirmed by geometric morphometrics. Intraspecific heterochrony has been observed in previous studies (i.e., Shea, 1986, 1988, 1989; Berge and Penin, 2000; Berge, 2002; Williams et al., 2002). Environmentally induced intraspecific heterochrony is known in insects and amphibians (see Reilly et al., 1997). The present study has shown that intraspecific heterochrony can also be produced by environmental stimuli in mammals, more specifically, that perturbation in diet can result in proportioned dwarfism. From the perspective of genetic assimilation (Waddington, 1953, 1956; see Scharloo, 1991; Hallgrimsson et al., 2002), the environmentally induced phenotype probably has a genetic basis, and thus parallel changes can be induced genetically. Proportioned dwarfism may be far more common than is currently appreciated.

Functional cranial theory (Klaauw, 1948-1952; Moss and Young, 1960; Moss, 1973) suggests that the skull can be divided into functional cranial components. Each FCC reflects a certain degree of developmental integration. As mentioned before, Cheverud (1982, 1995) suggested that the kind of integration prevailing (environmental or genetic) depends on whether the neurocranium and face are treated as two different units or the skull is considered as a whole. It is similar for heterochrony. Proportioned dwarfism is observed when the skull of LP individuals is compared with the skull of HP specimens. Similar shape in LP and HP skulls is attained by a developmental arrest of the face in LP animals. When the face and neurocranium are considered separately, heterochronic processes affecting these two components are different. The neurocranium of LP individuals conforms to proportioned dwarfism because it has the same shape as but is smaller in size than that of HP animals. In contrast, the analysis of the FCC of the face suggests an arrest (or truncation) of development. Skull growth trajectories are different in LP and HP individuals, whereas face growth trajectories are similar in these two groups. In fact, the heterochronic process observed at a higher level of analysis depends on the interaction of processes at lower levels. In other words, it is an emergent process that does not reproduce at a high level the processes that occur at lower levels. In our case, proportioned dwarfism in the skull of LP *Saimiri* is obtained by proportioned dwarfism in the neurocranium and a paedomorphic face.[1]

In summary, malnutrition produced a change in size but not shape of the skull of LP *Saimiri*. This difference is the result of a heterochronic process; undernourished animals are proportioned dwarfs. Intraspecific heterochrony has been reported previously, but we report here, in contrast to other heterochronic studies of mammals, a case of intraspecific heterochrony produced by an environmental stress.

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[1] It is very likely that if proportioned dwarfism occurs in the face of LP animals, the relationship between the neurocranium and face would change and therefore that LP and HP individuals would not show similar skull shapes.

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