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Craniodental variation among Macaques (*Macaca*), nonhuman primates

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Abstract

Background: In terms of structure and function, the skull is one of the most complicated organs in the body. It is also one of the most important parts in terms of developmental and evolutionary origins. This complexity makes it difficult to obtain evolutionary assessments if, as is usually the case with fossils, only part of the skull is available. For this reason this study involves a set of comparisons whereby the smallest functional units are studied first, and these built up, through a triple-nested hierarchical design, into more complex anatomical regions and eventually into the skull-as-a-whole. This design has been applied to macaques (*Macaca*) in order to reveal patterns of variation at the different levels. The profiles of such variation have been obtained both within and between species. This has lead to a search for the skull parts that have undergone similar selection pressures during evolution and comparable development patterns in both ontogeny and phylogeny.

Results: Morphometric analysis (Principal Components) was used to obtain these profiles of species and sex separations based on 77 cranial variables from 11 species of macaques. The results showed that 7 functional units could be aggregated into three functionally reasonable anatomical regions on the basis of similarities in profiles. These were: the masticatory apparatus containing mandible, lower teeth and upper teeth, the face as a whole combining maxilla (actually lower face) and upper face, and the cranium as a whole involving cranium and calvaria. Twenty-six variables were finally selected for analyzing the morphology of the whole skull. This last showed an overall profile similar to that revealed in the masticatory apparatus but also contained additional information pertaining to individual species and species-groups separations.

Conclusions: The study provides a model for carrying out analysis of species separations and sex variation simultaneously. Through this design it seems possible to see cranio-dental elements that may result from similar developmental processes, have similar functional adaptations, and show an appropriately integrated structure morphologically. This study also implies that the biological information drawn from part of skull alone, e.g. as in studies of incomplete fossils may provide misleading information.

Background

The skull, unlike most postcranial bones, has a number of different functional units and anatomical regions. It is the most complicated organ in the body both morphologically and functionally. It results from variations in growth and fusion of a number of separate and independent centers of ossification and chondrification. This complexity is, in part, a problem arising from two familiar tissues, cartilage and bone, differentiating in a rather more complex pattern than observed elsewhere in the vertebrate body. In addition, the factors controlling skeletal differentiation and morphogenesis in the skull are necessarily different from those operating elsewhere, for example, in the formation of vertebral column, limb girdle or limb itself [1]. Furthermore, at the level of cell differentiation, it is not simply a matter of endochondral bone and primary hyaline cartilage, but also of other types of skeletal tissues found almost exclusively in the head, principally membrane bone and secondary cartilage [2].

The development of the skull is as complex as its functional integration. For instance, the cranium is strongly influenced by the development of the face in relation to the branchial arches, and by the development of the calvaria in relation to the brain. During the whole of life these changes never cease, though they occur at larger and smaller rates at different times [[3,4]: pp 423–443). These complexities and variations do not just exist between species – resulting in interspecific variation, but also within species between the sexes – intraspecific variation. They are usually considered to accompany changes in phylogeny between species, and growth within species. It is now appreciated that when they are considered simultaneously, these two types of variations in the skull are more complex than expected [5,6]. Moreover, it is likely that, even though the skull is the most complex region of the skeleton, these caveats apply, if only to lesser degree, in other regions of the body.

Interspecific morphometric craniodental variation has been commonly used in order to help clarify controversies in classification, evolution, phylogeny and functional adaptation. Similar analyses, but of intraspecific variation, have been used to reveal differences between the sexes not only in morphology but also in social activities, behavior, ancestral heritage, size and sexual selection [7–9]. These two issues are normally studied separately. Studies integrating these two issues together are quite uncommon [5,6,10]. Yet such analyses, combining these issues, are logical ways to approach complex problems [5,6,11]. Thus profiles of animal group differences, due to differences in variation between species and differences between sexes within each species, can demonstrate how each pattern of variation effects the other, and what is the overall relationship between them.

The main purpose of this study is, thus, to set up a model to analyze craniodental profiles referring to inter- and intra-specific variations with a specially designed method: "the nested procedure". This permits profiles of both animal groups and anatomical variable clusters between functional units, anatomical regions and the whole skull to be compared. This study also illustrates the effects of combining functional units into anatomical regions, and the effect, in turn, of aggregating anatomical regions into the skull as a whole. Such a model has value in many anatomical regions, far beyond the cranio-dental study to which it is applied here.

Macaques are specifically used in this study because they are characterized by great biodiversity and variation. As many as: up to 20 species have been described in this genus by various authors, and they have been placed into as many as four species groups [12–15]. In their geographic variation and natural adaptation, except for humans, no other primate group inhabits a more diverse and extensive range of habitats than the macaques. These range from lowland tropical rain forest (in Indonesia) to high altitude mountains (on the top of the Qinghai-Tibetan Plateau, China), and from dry forest and grassland of northwestern Africa to northerly latitudes of Japan. Thus, the genus *Macaca* provides an ideal model for the study of inter- and intraspecific variation [5,16]. Again, this investigation has value for application, and one that may be applicable to many species that have undergone similar evolutionary processes and biogeographic radiations.

Results

Because, in this study, the variables are all measurements, they naturally all show significant positive contributions to the first PC axis in each analysis. In the second axis, however, only smaller numbers of variables reach significant contributions, and these are mixed positive and negative.

Functional units

Mandible, lower teeth and upper teeth

In the analysis of the first unit, the mandible, (Fig. 1) the first axis mainly arranges species according to size with the larger species more positive (with the minor exception of *M. fuscata*). It is likely therefore that overall size is a predominating factor in this axis (although, of course, other characteristics that happen to be correlated with size will also be included by this statistic – see later). This axis has large and positive eigenvectors for all variables (Table 1).

The second axis mostly arranges the groups according to sex, with males more positive. The distances between the sexes vary quite markedly. Two species (*M. mulatta* and *M. fuscata*) display a slightly different directional relationship between the sexes. The contributions of variables to the
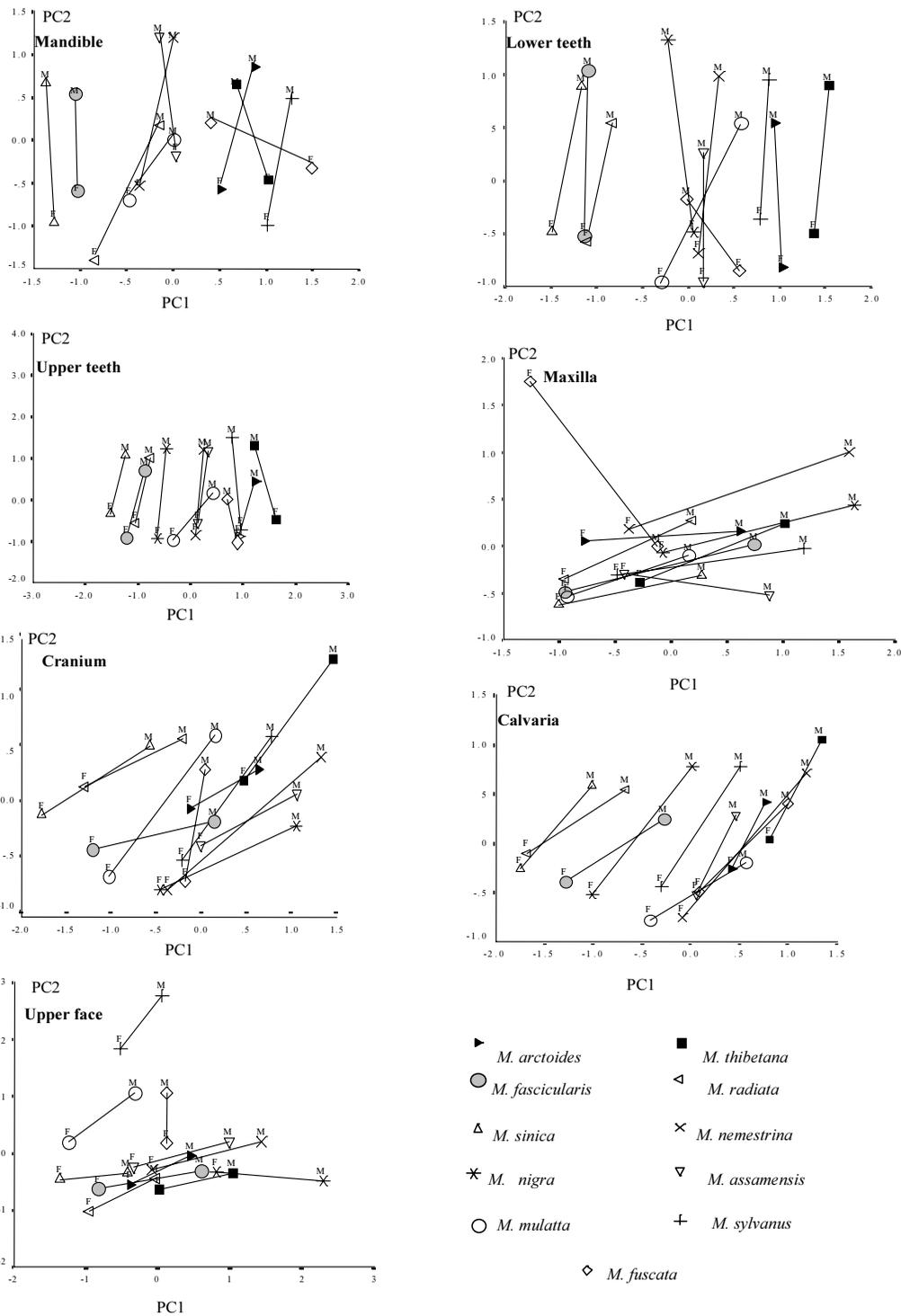
Figure 1
Comparative profiles composed of inter- and intraspecific variations revealed in the first two axes of PCA based on the seven functional units. M: Male; F: Female. Three different profiles are found: 1) mandible, lower teeth and upper teeth in which interspecific variation is revealed along the first axis, intraspecific variation is, however, along the second axis; 2) lower and upper face whose structure is almost opposite to that illustrated in previous one, and 3) calvaria and cranium in which inter- and intraspecific variations are divided orthogonally by the two axes.

Table 1: Percentage of total variation, eigenvalues and eigenvectors for the first two PCA axes of functional units in macaques. The eigenvectors reaching significant contribution level are in bold [*].

Mandible

	I	2
Eigenvalue	11.66	1.13
Percentage	72.9	7.0
Cum. Per	72.9	79.9
Eigenvectors		
LBCB	**.821**	**.468**
LBMB	**.913**	.006
LIAW	**.584**	**.697**
CONDYLL	**.674**	-.084
CONDYLW	**.870**	-.200
CONM1	**.959**	-.127
CONM3	**.907**	-.149
MAM1	**.794**	-.372
MAT	**.789**	-.139
MANDLAL	**.950**	.033
MANDH	**.921**	-.090
MANDSYM	**.910**	.207
BICONDY	**.944**	-.171
MANCORW1	**.835**	-.155
SYMWID	**.900**	.045
LTRLALV	**.793**	.265

Upper teeth

	I	2
Eigenvalue	10.33	64.60
Percentage	64.6	9.8
Cum. Per	64.6	74.4
Eigenvectors		
UI1MDL	**.534**	**.521**
UI2MDL	**.714**	.082
UCMDL	**.658**	**.532**
UP3MDL	**.707**	.030
UP4MDL	**.875**	-.163
UM1MDL	**.874**	-.181
UM2MDL	**.897**	-.249
UM3MDL	**.890**	-.235
UI1BLL	**.621**	.571
UI2BLL	**.809**	.246
UCBLL	**.706**	.449
UP3BLL	**.874**	-.011
UP4BLL	**.876**	-.097
UM1BLL	**.885**	-.294
UM2BLL	**.905**	-.292
UM3BLL	**.897**	-.252

Upper face

	I	2
Eigenvalue	6.02	.67
Percentage	75.2	8.3
Cum. Per	75.2	83.6
Eigenvectors		
BIORBW	**.855**	.236
INTORBW	**.770**	.576
PIRH	**.903**	-.008
PIRW	**.760**	-.419
UFACEH	**.886**	-.249
INFRMAL	**.938**	.105
BIZYGW	**.939**	-.053
MUZL	**.869**	-.170

Calvaria

	I	2
Eigenvalue	3.98	.88
Percentage	56.9	12.6
Cum. per	56.9	69.4
Eigenvectors		
CALVL	.889	-.177
BPORW	.626	-.189
MIDPARW	.845	.003
POSTORB	.631	-.252
OCCH	.673	-.318
FORMAGL	.630	.526
FORMAGW	.611	.609

Lower teeth

	I	2
Eigenvalue	9.87	1.81
Percentage	61.7	11.3
Cum. Per	61.7	73.0
Eigenvectors		
LI1MDL	**.437**	**.616**
LI2MDL	**.635**	**.346**
LCMDL	**.657**	**.379**
LP3MDL	**.690**	**.473**
LP4MDL	**.870**	-.094
LM1MDL	**.862**	-.147
LM2MDL	**.896**	-.292
LM3MDL	**.885**	**-.313**
LI1BLL	**.618**	**.341**
LI2BLL	**.782**	.165
LCBLL	**.641**	**.457**
LP3BLL	**.877**	.099
LP4BLL	**.892**	-.194
LM1BLL	**.861**	**-.338**
LM2BLL	**.893**	**-.334**
LM3BLL	**.882**	**-.310**

Maxilla

	I	2
Eigenvalue	4.72	.60
Percentage	78.6	10.0
Cum. Per	78.6	88.6
Eigenvectors		
MUZL	**.943**	-.083
PALLENG	**.657**	-.106
PALWID	**.896**	-.157
UBCB	**.922**	-.037
UBMB	**.893**	-.158
UIAW	**.680**	**.729**

Cranium

	I	2
Eigenvalue	6.41	.76
Percentage	80.1	9.6
Cum. per	80.1	89.1
Eigenvectors		
BCRANL	**.954**	.017
CRANL	**.964**	-.044
CRANW	**.935**	.096
BIYGW	**.956**	-.050
POSTORB	**.576**	**.774**
OCCH	**.736**	**-.369**
ANTBASI	**.976**	-.098

[*]: Definitions of the variables are listed in the appendix.

second axis are variable, including both positive and negative trends. The anterior breadth measurements of the jaw (mandibular bicanine breadth-LBCB and mandibular bimolar breadth – LBMB) and moment arm of the masseter-MAM1) show greatest influence on this axis.

Figure 1 also confirms that the variation profile for both first and second axes described for the mandible also exists within two of the other units (the lower teeth and the upper teeth). For the teeth, the principal variables contributing to the second axis are mainly measurement related to the placement of the incisors (LI1MDL, LI2MDL,

Table 2: Percentage of the total variation, eigenvalues and eigenvectors in the first two principal components of the three anatomic regions. The eigenvectors reaching significant contribution level are in bold.

Masticatory apparatus

	1	2
Eigenvalue	16.02	2.25
Percentage	61.6	8.7
Cum. Per	61.6	70.3
Eigenvectors		
LBCB	**.824**	**.321**
LBMB	**.903**	-.206
LIAW	**.681**	**.382**
CONMI	**.891**	-.070
MAMI	**.724**	**-.305**
MANDLALV	**.906**	.055
MANDH	**.881**	-.034
BICONDYL	**.897**	-.175
UI1MDL	**.614**	**.446**
UI2MDL	**.731**	-.081
UCMDL	**.764**	**.417**
UP4MDL	**.836**	**-.305**
UM1MDL	**.848**	-.271
UI1BLL	**.630**	**.417**
UI2BLL	**.801**	.043
UCBLL	**.761**	.313
UP4BBLL	**.842**	-.232
UM1BLL	**.846**	**-.386**
LI1MDL	**.491**	**.559**
LI2MDL	**.622**	.165
LCMDL	**.688**	.279
LP4MDL	**.849**	-.191
LM1MDL	**.838**	-.199
LCBLL	**.677**	**.383**
LP4BLL	**.872**	-.200
LM1BLL	**.816**	**-.354**

Face as a whole

	1	2
Eigenvalue	7.63	.95
Percentage	69.4	8.6
Cum.	69.4	78.0
Eigenvectors		
MUZL	**.928**	.152
BIORBW	**.804**	**-.386**
INTORBW	**.698**	**-.447**
PALLENG	**.944**	.155
PALWID	**.963**	.175
UBCB	**.884**	.233
UBMB	**.911**	-.009
UIAW	**.586**	**.606**
PIRH	**.875**	-.244
PIRW	**.723**	-.205
UFACEH	**.873**	-.009

Cranium and calvaria

	1	2
Eigenvalue	7.65	1.07
Percentage	58.9	8.2
Cum. per	59.9	67.1
Eigenvectors		
CALVL	**.944**	.020
BPORW	**.935**	-.001
MIDPARW	**.809**	.265
INTFRMAL	**.931**	-.063
POSTORB	**.575**	.126
TEMFOSL	**.888**	-.077
TEMFOSW	**.861**	-.137
ZYGH	**.743**	-.259
OCCH	**.664**	-.232
FORMAGL	**.557**	**.456**
FORMAGW	**.513**	**.642**
GLENOL	**.446**	**-.451**
GLENOW	**.860**	-.113

Whole skull

	1	2
Eigenvalue	15.12	2.85
Percentage	56.0	10.6
Cum. per	56.0	66.6
Eigenvectors		
LBCB	**.869**	.223
LIAW	**.689**	**.532**
CONMI	**.931**	-.188
MAMI	**.763**	-.276
UI1MDL	**.570**	**.659**
UCMDL	**.820**	.176
UI1BLL	**.634**	**.459**
UM1BLL	**.771**	-.239
LI1MDL	**.438**	**.773**
LI2MDL	**.606**	**.406**
LCBLL	**.758**	.123
LM1BLL	**.774**	-.189
MUZL	**.921**	.056
BIORBW	**.810**	-.281
INTORBW	**.708**	-.208
PALWID	**.860**	-.058
UBCB	**.897**	.172
UIAW	**.826**	**.387**
PIRH	**.848**	-.264
CALVL	**.893**	-.294
MIDPARW	**.731**	**-.396**
INFRMAL	**.917**	-.188
POSTORB	**.653**	-.089
OCCH	**.595**	-.163
FORMAGW	**.441**	-.285
GLENOL	**.546**	-.081

LI1BLL, UI1MDL and UI1BLL), canines (LCMDL, LCBLL, UCMDL and UCBLL) and the breadths of the lower molars (LM1BL, LM2BLL and LM3BLL) (Table 1).

Maxilla and Upper Face
Though it might be expected that the maxilla (bearing the upper teeth) would be like the mandible (bearing the lower teeth) in fact careful inspection of the maxillary variables shows that they are primarily measures of the lower face, not of the tooth-bearing alveolus. It is therefore of major interest that the separations differ from those in the first three units. Sex separations are mainly along the first axis and species separations along the second (Fig. 1).

Again, of course, the variables contributing significantly to the first axis are all large and positive, and those to the second axis fewer and both negative and positive (Table 1). It is the fact that these separations are reversed as compared with the first three analyses that implies that reading the first axis as simply size and the second as something else is inappropriate – see discussion.

The upper face resembles the maxilla in that, for most species, the sex separations (males more positive) are mainly along the first axis, and the inter-specific variation (bigger species more positive) along the second axis (Fig. 1). Two variables, interorbital width (INTORBW) and width of the piriform aperture (PIRW) make significant contributions positively and negatively, respectively, to the second axis.

Calvaria and Cranium

These two regions also provide very similar profiles (Fig. 1) but, in contrast with the first two, a third pattern of relationships – one in which the separations between species and between sexes are along diagonals between the first two axes. The separations between the sexes (intraspecific variation) are diagonal from lower left to upper right. The interspecific variation is diagonal in the opposite way, from upper left to lower right. The first axis, as before, contains large and positive contributions from each variable but these do not separate either of the types of variation. The second axis contains contributions from occipital height (OCCH), postorbital constriction (POSTORB) and the length and breadth of the foramen magnum (FORMAGL and FORMAGW) (Table 1). These contribute therefore to both types of group separation.

Anatomical Regions

Results for the anatomical regions entirely parallel those for the constituent functional units.

Masticatory apparatus

For the masticatory apparatus twenty-six of the forty-eight variables from units of mandible, upper teeth and lower teeth were chosen (Table 2). The selection is based on including representation of the three different units and on keeping the variables that have been shown to make important contributions to the analyses in the previous analyses. These three units which individually display similar profiles, show, when aggregated into an anatomical region: the masticatory apparatus, the same overall profile: that is – separations of species according to overall size (largest species more positive) on the first axis, and sexes (with males more positive) on the second (Fig. 2).

The first two axes account for 70.3% of the total variation. In PC2, LBCB, LIAW, LI1MDL, UCMDL, UI1BLL and UCMDL are positive and are associated with the anterior teeth and canines (Table 2). Four variables, MAM1, UP4MDL, UM1BLL and LM1BLL, have significant, but slightly lower negative eigenvectors in this axis. The latter three values are associated with the sizes of the posterior teeth.

Face as a whole

In the analysis of the aggregation of these two units: maxilla and upper face, into the face as a whole, almost all variables are used, only two variables, interfrontomalare breadth (INFRMAL) and bizygomatic breadth (BIZYGW), being omitted. The species are separated, in the same way as in the constituent units, according to size (largest species being positive) in the second axis, and sexes (with larger males more positive) in the first.

The first two axes account for 78.0% of the total variation (Table 2). Maxillary incisor alveolar breadth (UIAW) shows the greatest positive eigenvector, and it is contrasted with biorbital breadth (BIORBW) and interorbital breadth (INTORBW) which make the greatest negative contributions to the second axis (Table 2).

Cranium as a whole

Finally, the two cranial units, both involved in portions of the skull to do with the brain and both demonstrating similar profiles to each other in this study, are aggregated as the cranium as a whole including all of the variables of the overall cranium. In this case, and in contrast to the prior aggregations, the resulting analysis provides a different result in that it is not the same as the individual sub-units. The first axis (Fig. 2) mainly separates intraspecific variation (sex differences). This differs from that for each constituent unit where this variation is diagonal across two axes. The second axis mainly distinguishes interspecific variation (species size differences) and again this is different from each of the component units where this variation is diagonal across two axes. However, the two sets remain orthogonal as in the individual unit analyses. The picture is actually rather similar to that of the component units but has been rotated so that the sex and species variations are orthogonal and linked with each axis, instead of being orthogonal but at about 45° to each axis.

The first two axes account for 67.1% of the total variation (Table 2). The length and breadth of the foramen magnum (FORMAGL and FORMAGW) show the greatest positive, while glenoid length (GLENOL) makes the greatest negative, contribution to PC2.

The Whole Skull

The final aggregation is achieved by analysis of twenty-seven variables representing the whole skull selected to cover overall morphology (Table 2). As in the last section, these variables were selected from those making significant contributions in the first two axes, whilst considering the balance between regions. As in all other analyses, the first PC1 eigenvectors are positive; the remaining axes include both positive and negative eigenvectors.

The first axis reveals species size relationships (interspecific variation, Fig. 2). Sex differences (intraspecific variation) lie in the second axis. The total variation accounted by the first two axes is 66.6% (Table 2). The variables
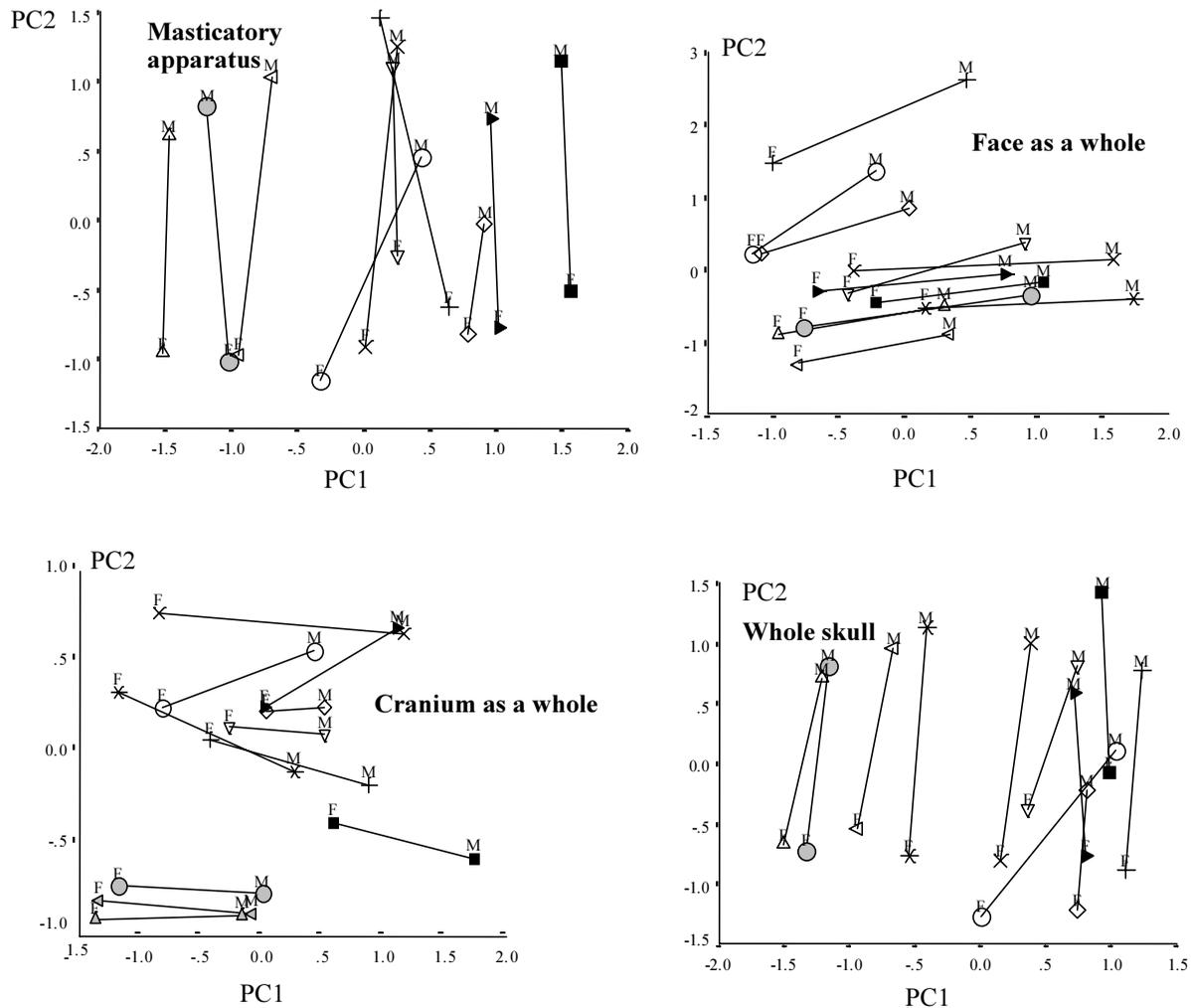
Figure 2
Comparative profiles based on the three anatomic regions. Three patterns of profiles are detected: 1) that expressed by the masticatory apparatus and the whole skull in which inter-and intraspecific variations are exposed along the first and second axes, separately; 2) that illustrated by the face as a whole. Its structure is opposite to that shown in the previous pattern, and 3) that demonstrated by the cranium as a whole. Its pattern is opposite to that expressed by the first one, and also different from pattern 2 in terms of separation among species and arrangement along the second axis.

showing the highest eigenvectors in PC2 are all positive. They are incisor alveolar breadth (LIAW), UI1MDL, UI1BLL, LI1MDL, LI2MDL and UIAW, which, as found previously, are associated with the anterior dental dimensions. However, one variable, midparietal breadth (MID-PARW), is a significant negative eigenvector.

Discussion
The results revealed in this study indicate that craniodental variation among species and sexes in these macaques varies in different functional units, anatomical regions,

and the whole skull. The study provides information in relation to: 1) detecting differences between sex separation within species, and species separation among species, 2) detecting the effects of aggregating variables into larger and larger anatomical parts, 3) providing information about the way in which the variables contribute to the sex and species groups and to the anatomical regions at each level of the study and, 4) providing information about overall separations of species.

The seven functional units exhibit three distinctive patterns of separation of the species and sex groups. These distinctive patterns are further emphasised when units are aggregated into anatomical regions. These coincide with the functional adaptations and developmental history relating to these regions. In the final aggregation of the three regions into the whole skull the pattern exhibited by the masticatory apparatus seems to predominate, data about species separations were, as matter of fact, revealed in higher axes [5].

Thus, whatever the analysis, the main variations, inter-species size differences and intra-species sex differences seem mainly to be orthogonal. However, the actual relationship between these two varies from analysis to analysis. In the masticatory apparatus species size differences are expressed along the first axis and sex size differences along the second. In other words, some species with small body size, such as, *M. radiata, M. sinica* and *M. fascicularis,* are allocated on the left side of PC1. Others with large body size, such as, *M. thibetana, M. sylvanus* and *M. arctoides,* are allocated on the right side of PC1. Likewise larger males are allocated on the upper part of the second axis and smaller females occupy the lower side of that axis. In the face as a whole, this is reversed. In the cranial units there is an intermediate relationship, with each being expressed at about an angle of 45c° to the principal axes, though this is further changed in the aggregation into the cranium as a whole. In the analysis of the whole skull there seems to be a return to the picture for the masticatory apparatus. However, that there is a lower total variation in the first two axes (66.6%) than expressed in these axes in functional units and anatomical regions may explain that higher axes, PC3 and PC4, also provided useful additional information [5].

Several points of discussion flow from this summary of the results.

First, there is the question of size
This relates to the biological meaning that is often postulated for first principal component axes. When the raw data are measurements the first principal component contains information about overall size differences among specimens. This is usually said to be confirmed by the pattern of all positive contributions of these variables to this axis. The further assumption is then made that this set of associations is due to size, that it is therefore a size axis, and that, also therefore, the remaining axes are reflecting shape. In this study it is clear that this cannot be the case.

The species and sex differences are almost identical in the various units, regions and the whole skull. Which difference appears in the first axis and which in the second seems to depend solely upon which unit or region is ana-

lysed. Thus, species size differences appear in the first axis for masticatory region and sex size differences in the first axis for whole face region. Presumably, therefore, when species size differences are larger than sex size differences they appear in the first axis with all positive variable contributions and vice versa. The pattern of all positive contributions in the first axis is not because that axis is size alone, but simply because it is the first axis in a study of measurements.

It is likely that the reversals between species and sex differences are only because, in the different regions, there are differences in the degree of each. The patterns of species size and sex size differences are so similar that it cannot be the case that one is **size** and the other **shape,** and that these vary in the different units and regions. It must be the case that the results are due simply to size differences as well as shape in both axes. When species size differences are larger than sex size differences, they appear in the first axis with all positive variable contributions (and this occurred in the masticatory apparatus). When sex size differences are larger than species size differences, the reverse is found (and this occurred in the face as a whole. And the situation is even more complex because in some case, the relationships between species size differences and sex size differences are such that each appears in each axis (in the units of the cranium). In other words, there is more than one kind of size, never mind any shape differences that there may be, and as a result, each axis must contain some size and some shape.

Second, there is the question of anatomical regional differences
The fact that the mandible is very similar to both upper and lower teeth may be related to their unique and common developmental pattern. According to Bjõrk, Solow and Lundstrõm [17–19], the development of the mandible is quite different from other cranial parts and closely related to the development of teeth. Different areas in the mandible, however, have different growth mechanisms differing markedly from each other. For instance, though the body of the mandible is formed by intramembranous ossification, the growth of the alveolar portion bone is linked to dental migration,, but the the lower border of the mandible is not. The growth of the coronoid and angular processes are closely related to the function of masticatory muscles attached to them. However, these various processes are a part of a single bone that has a common set of functions. Most importantly, despite the differences in their growth mechanisms, they follow the same growth pattern.

We might have expected that the maxilla would show a similar pattern. The alveolar process of the maxilla is as closely related to the function and development of the

maxillary teeth as the alveolar portion of the mandible is to the mandibular teeth. And of course, there is a functional relationship between the two sets of teeth. However, the actual variables measured here, though they were labelled as maxilla, do not, in fact, characterise the alveolar portion of the maxilla alone, but rather the entire maxilla, most of which is the lower face. This fits with the previously known information that the development of the entire mandible is more relevant to dental development than of the entire maxilla [20].

A different finding, though still related to development and function, may be operating in the face. The picture for the facial units differ from those for both the masticatory apparatus and the cranium, though there seems to be more overall similarity to the cranium. Is this in part because the function and development of face involve a greater disparity in range of functions and developmental processes than the masticatory apparatus? Thus, in addition to being part of the facial structure, the maxilla also plays an important role in the development and function of mastication [5,21]. Referring to the growth of the maxilla, the only part subservient to erupting teeth is the alveolar process that is closely related to dental development. In contrast, however, most of the maxillary bone involves other factors. Thus, even though disturbance of tooth buds could lead to loss, malformation, or malposition of the teeth causing changes in the maxillary alveolus, the general size and shape of the maxilla are not affected by the changes in the teeth. The maxilla is also a large contributor to the nasal cavities and orbital floor, and therefore its growth is of fundamental importance to facial, nasal and orbital growth patterns [22]. Near its upper extremity, the upper face is influenced by the attached cranium, thus influenced by cranial base growth patterns and the growth of the brain. All this must influence its morphology in a more complex way than is the case for the masticatory apparatus.

In the case of the cranium, the situation may be yet more complex still. When the two parts of the cranium are analyzed together, PC1 reflects primarily sex size differences (intra-specific variation) and PC2 species size differences (inter-specific variation). This differs from the analysis of the component units where all relationships are oblique to both axes. It is also the opposite of the masticatory region where PC1 separates species and PC2 sexes, but similar to the face as a whole. Such results may imply that the development of cranium and calvaria, quite different from the masticatory apparatus but similar to the face, is also complex. This may be associated with the dual origin of the calvaria from both neural crest and membrane bone (e.g. Thomson [23]). Therefore, although cranium and calvaria exhibit a similar picture referring to the two types of separate variations, they results imply that they may in-

teract differently in terms of functional, developmental and growth processes.

The results based upon the aggregated but selected variables for the whole skull are more complex again. The overall profile of differences is actually very similar to that found in the masticatory apparatus alone. However, there is a major difference to the pattern in the masticatory apparatus in this ultimate study. That is, a relative lower total variation in the first two axes may imply that, in the analyses of the whole skull, the information content of the main regions must be independent of each other to a great enough degree that information spills over into other axes, such as PC3 and PC4 [5]. This would not have been revealed without doing the studies on units and regions. And it indicates just how misleading it might be to use information from only small skull regions (which is often all that is available for fossils) in attempting to move directly from morphometric analyses to phylogenetic evaluation.

The analyses on the whole skull also imply that in the selection procedure choosing variables from the units is necessary to understand to what extent similar parts with similar functions and similar developmental histories give similar results. The same is true in the anatomical regions in order to see the effect of aggregation of regions into overall skull. In this process of variable reduction, though attempts have been made not to bias the numbers of variables through over- or under-representation of specific skull regions, it is inevitable that the masticatory apparatus is overly represented in a numeric sense. This may actually be not inappropriate because information from teeth (each tooth is its own unit notwithstanding the fact that there are also trends along the tooth row) is under a degree of independent genetic control.

The analyses of the whole skull also provide further confirmatory information that reflects these additional complexities. It has provided a considerable amount of new information that was not easily recognized in the earlier studies: additional information existing in axes 3 and 4 [5]. That is, there are marked sex separations in different directions in some species along PC3: e.g., *M. nigra*, *M. nemestrina*, *M. mulatta* and *M. fascicularis* and in other species, such as *M. mulatta*, *M. assamensis*, *M. arctoides* and *M. sylvanus* along PC4. These separations are not parallel as in the first two axes and this implies that their causation is not due to a single common factor, but some factor or factors different for each species. Likewise in these axes, there are marked distinctions of individual species and these, too, are in various directions rather than being parallel, implying that they are not due to a similar single factor such as overall size. Thus, the species with small body size, such as *M. fascicularis*, *M. radiata* and *M. sinica*, are always

allocated in the negative direction of PC1. Others with large body size, fore instance *M. sylvanus*, *M. thibetana* and *M. arctoides*, are dispersed in the positive direction along the same axis. This type of information might be especially important in understanding the phylogenetic relationships of the species.

A great deal of what is found here seems to relate to functional and developmental factors that are common to macaques (probably common to all anthropoidea). Some parts of the result may refer however, to differences that are restricted to macaques (and possibly other closely related Old World monkeys). Thus that inter-specific variation is larger than intra-specific variation in the masticatory apparatus may be related to the fact that the greatest variation between species found in the muzzle region of this genus [5]. There is mounting evidence that, at least within some groups of primates and at lower taxonomic levels, the morphology in this area is not a useful indicator of phylogeny because of its great variation [24,25]. This may especially apply to a genus like *Macaca* the species of which vary considerably in size. As a result, variation between sexes is expressed in the second axis (even though a large component of this variation is also size). The same may be also true regarding the profile expressed by the whole skull which is similar to that illustrated by the masticatory apparatus. The variation of the whole skull can also reflect variation in species size because body size is tightly related to skull size. For example, the latter is frequently used to substitute the former in the studies in which body size needs to be considered but is not available [6,26,27]. It is also possible, however, that this similarity may be related to fact that a quite number of variables related to masticatory apparatus used in the final analysis.

Third, there is the question of species separations
This study indicates that there are different profiles in different species even within a group as closely related as these species within a single genus such as *Macaca*. This additional information resides in the interactions of information from the separate regions that is produced by the aggregation of parts into the whole skull analysis. It would thus be missed in studies that only include univariate data or even multivariate data if they involved only restricted parts of the skull. On the other hand, remarkable sex and species separations are also found in the higher axes (see especially Pan [5]). Thus, additional information besides that in the first two axes should be considered.

Finally, there is the question of methodology
The methods used in this study may provide a means for identifying primitive and shared-derived characteristics for use in cladistic analysis. Thus, instead of moving directly to decisions as to the type and polarity of characters,

studies of this type may provide biological information that can indicate how a character in cladistics should be coded. For instance, most of the information here implies that the individual variables are so complex that each is an actually a compound of several, perhaps many, characters (resulting from different underlying genetic, developmental and functional mechanisms). It may therefore be inappropriate to make a cladistic assessment of any single variable for cladistics (character) as primitive or derived. This study indicates that each variable is a resultant of many different biological processes and inevitably will reflect a compound of characters of different primitivenesses and derivednesses. Indeed, these results indicate that in making the decision that any given variable (character) is primitive or shared derived etc, the only thing one can be certain of is that both assessments must be wrong. Each variable must reflect several different "characters" each of which may be primitive or shared derived. Disentangling such complexity (if it can be done) will require additional study including additional suites of variables characters (for instance, analysis of all breath measurements as separate from all length measurements, and so on).

Conclusions
The profiles composed of the inter- and intraspecific variations of macaques revealed in this study are complicated. 1) Seven functional units are aggregated into three anatomical regions: the masticatory apparatus, the face as a whole and the cranium as a whole. In turn, the three anatomical regions can be aggregated into the skull as a whole. 2) Interspecific variation is the larger than sexual dimorphism in the masticatory apparatus and the whole skull. The opposite is true in the face as a whole and cranium as a whole. 3) Conclusions based on individual functional units or anatomical regions are limited in exploring biological issues concerned with functional morphology, growth, development, phylogeny and classification. It is obvious that the different parts of skull provide different information and thus analyses of partial anatomies (as may be all that are present in fossils) must be interpreted cautiously. Obviously these findings need to be tested in other primates using other anatomical variables (and especially length and breadth combinations of variables and three-dimensional coordinate variables). Their application under these conditions may permit better use of cladistic analysis.

Materials and Methods
Eleven species of the genus *Macaca* are used (Table 3). The materials are housed in a number of institutes, universities and museums in various parts of the world (see Acknowledgements). All specimens are from adult macaques, as judged by the full eruption of M3s. The sexes of the skulls are taken from available records. Sample size is variable between species: from 16 for *M. nigra* to 40 for

the five species (*M. mulatta*, *M. assamensis*, *M. arctoides*, *M. nemestrina* and *M. fascicularis*).

Table 3: Species and numbers (by sex) used in this study

Species	Males	Female	Total
M. mulatta	20	20	40
M. fuscata	11	11	22
M. sinica	14	8	22
M. assamensis	20	20	40
M. radiata	12	12	24
M. arctoides	20	20	40
M. sylvanus	11	10	21
M. nemestrina	20	20	40
M. nigra	8	8	16
M. fascicularis	20	20	40
M. thibetana	11	8	29
Totals	167	157	324

It is usual, in studies like this, to take equal or sub-equal samples for each species. However, in the case of macaques that show great variations in distribution and sub-speciation, it may be inappropriate to take equal sample sizes from one species to another without taking account the amount of variation and the number of sub-species within the different species. According to Groves [28] some species, such as *M. mulatta* and *M. fascicularis* occupying large geographic distribution ranges, have already formed 6 and 10 geographic subspecies respectively. Others, such as *M. sylvanus* and *M. nigra* are found in very small regions and do not display any subspecies divisions. Thus, in order to keep variation proportional between species, the specimens of the species with a great geographic variation should be selected from different geographic populations; sample size should be larger in them than in those with small geographic diversity. This may be a critical factor in studies of a complex genus like *Macaca*.

Intraspecific variation in this study relates to sexual dimorphism. There is no report at present about variations in sexual dimorphism among population groups or subspecies within a specific species. As a result this study uses sub-equal sample sizes between sexes for each species. Again, though it is likely that the natural adult sex ratio in these species is not one-to-one, but in all probability, fewer males to more females, not enough is known about this factor in the field to use it in the current analyses. Again, this is a factor to which greater attention may need to be paid when materials and appropriate information are available.

Seventy-seven linear dental, mandibular and cranial variables are measured on each specimen using digital calipers accurate to 0.01 mm. Length measurements are made parallel to the median sagittal plane, and breadth (or width) measurements are made in the coronal plane. Mesiodistal length of teeth is measured as the greatest length from the most mesial to the most distal points; buccolingual breath is the greatest breath, from the most buccal to the most lingual points.

The complexity of the skull discussed above required the nested research strategy described above. Thus, the variables are grouped into 7 units – mandible, lower teeth, upper teeth, maxilla, upper face, calvaria and cranium [5].

Principal Components Analysis (PCA) is an important method in studying inter-and intraspecific variation simultaneously. It is especially robust in searching for differences of size, sex, function and phylogeny [16] within and between species groups [6]. It would be interesting to know whether this method is also useful for revealing differences between functional units and anatomical regions of the skull. Thus, seven functional units are each first analyzed by PCA to derive the profile of species and sex separations. The units exhibiting similar profile are then grouped together to form composite, anatomical regions in which sex and species profiles are again studied using PCA. Finally a number of the variables, reduced on the basis of these results, are used in PCA investigations of the whole skull.

The division of the skull into functional units, and their sequential aggregation into anatomical regions and then the skull as a whole is aimed at investigating the ways in which the different parts profile the animals' separations, and how the increasing aggregation of parts influences the overall relationships. Such a design strategy allows determination of how variations, between and within species, vary in different parts of the skull. In particular, they provide insight into interpretations that are often made in relation to the first principal component.

Another purpose of this study is to discover the variables that are the main contributors to the components. The criteria for this selection are based on their influences on the first two axes of the respective previous PCAs, which contain most of the variation. Initially, the variables that exhibit significant contributions, with loadings of more than 0.300, are chosen [5,29]. Because different areas may have different numbers of significant variables, the selection of variables also involves choosing balanced proportions of variables from the different units or regions.

All of the statistical analyses are made using the SPSS program for Windows.

Additional material

Additional File
Appendix
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-2-10-S1.doc]

Acknowledgements

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