Morphological evolution through integration: quantitative analysis of cranio-mandibular covariance structures in extant hominids

Dissertation

zur Erlangung des Grades eines Doktors der Naturwissenschaften

der Geowissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen

vorgelegt von

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aus Neu Delhi (Indien)
2010

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Dedication

To the two most important people in my life: my mother and sister,

… and to the memory of my father and grandfather.

"I mean by this expression [correlated variation] that the whole organization is so tied together during its growth and development, that when slight variations in any one part occur, and are accumulated through natural selection, other parts become modified. This is a very important subject, most imperfectly understood, and no doubt wholly different classes of facts may be here easily confounded together." (Charles Darwin, "*The Origin of Species*", Chapter V – Laws of variation and correlated variation)

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Thesis summary

The primate skull is a functionally integrated and complex structure. The skull is commonly divided into different functional units, such as the bones and muscles that are involved in mastication, bones of the face, and the bones that house the brain. However, each of the functional units must also function within the skull as an integrated whole. This integration or covariation is reflected in structures varying with change in other structures. Understanding the evolution of integrated or covariance structures provides important insight into the underlying mechanisms that generate phenotypic variability and variation. The main goal of this thesis is to investigate covariance in the cranio-mandibular form of *Pongo*, *Gorilla*, *Pan* and *Homo* using quantitative methods such as landmark-based 3D geometric morphometrics. This thesis comprises three individual studies that address questions related to covariance-generating processes such as: morphological integration, allometry, canalisation and developmental stability. The three studies collectively provide important insight into the underlying mechanisms that generate phenotypic variability and variation in closely related hominid taxa.

Phenotypic variability is of particular interest to biological anthropologists for several reasons one being that majority of the questions addressed in primate evolution centre around morphological variation. The primate cranium is an important source of information for biological anthropologists because it preserves better in the fossil record than most other skeletal components. Due to the lack of large fossil samples, closely related extant hominids have long been used as analogues to better understand phenotypic changes related to developmental and functional adaptations in fossil hominids.

The first manuscript is a study of the patterns of morphological integration between the face, basicranium and cranial vault in adult humans, chimpanzees, bonobos, gorillas and oranugtans. Regions of the mammalian cranium differ in their developmental origin and functional demands. Accordingly, we sub-divide the

cranium into three functional components: (a) facial skeleton, including the zygomatic processes, nasal, lacrimal and maxillary bones (b) cranial vault, consisting of the frontal and parietal bones and (c) basicranium, comprising the non-squamous parts of the temporal and occipital bones. We choose to call these modules "functionally" derived because they are loosely based on Moss' "functional matrix hypothesis" (Moss and Young, 1960); however, they are primarily distinguished based on differential growth patterns. Patterns of integration can help understand the structural relationship between morphological units, providing important insight into how phenotypes can evolve or how they may be constrained. The main goal of this study is to evaluate whether integration patterns vary across these closely related hominid taxa. Results show that even though taxa exhibit species-specific variation, particularly in interactions between the basicranium and other cranial regions, the overall pattern in which cranial regions integrate in these hominids is largely similar. This suggests that the *direction* of integrated shape change is similar and that cranial integration is highly conserved in extant hominids and possibly other primate taxa.

The second manuscript is a study of two covariance-generating processes: canalisation (among-individual variation) and developmental stability (within-individual variation) in the extant hominid cranium. Canalisation and developmental stability refer to concepts that buffer developmental processes from external and internal perturbations in organisms, constraining their evolution along particular pathways. To generate covariance among structures, developmental processes have to affect elements of the cranium in the same way or not at all. Experimental studies on mice and fly wings have revealed that processes such as canalisation and developmental stability contribute to maintaining covariance between structures, and consequently influence an organism's phenotypic variability. This study evaluates, for the first time, whether canalisation and developmental stability affect covariance structures similarly within and across adult hominids and whether these processes are conserved among these taxa. My results show remarkably high correlations between species covariance structures in aspects of canalisation and developmental stability. The main implication of these

results is that covariance structures and the developmental processes maintaining covariance structures are highly conserved across extant hominids. However, covariance structures in the cranium have a complex and integrated relationship to the underlying developmental interactions, making it problematic to pinpoint the precise influence of processes that maintain covariance structures in the hominid cranium.

The third study is on mandibular ontogeny and integration. In this study, I examine patterns of integrated ontogenetic shape change and growth trajectories in both sub-adult and adult humans, bonobos and chimpanzees. We propose that ontogenetic shape differences in the mandible are influenced not only by diverging ontogenetic trajectories among taxa, but also by differing patterns of developmental integration in the corpus and ramus elements. According to the "functional matrix hypothesis" (Moss and Young, 1960; Moss, 1973) different parts of the mandible have semi-independent growth centres. Genetic and morphometric research on mouse mandibles and on some primate mandibles support this claim by showing that the mammalian mandible can be largely sub-divided into two distinct embryonic units, the corpus and the ramus. The main conclusions that can be drawn from this study are that chimpanzees, bonobos and humans have divergent ontogenetic trajectories – a result that has been found with respect of cranial developmental trajectories as well, and that species-specific differences, even between bonobos and chimpanzees, emerge early in ontogeny, as is also noted during cranial ontogeny. Furthermore, my results also demonstrate that the corpus and ramus units of the mandible are semi-independent and do not share the same developmental pathway. The latter provides support for the "functional matrix hypothesis" and serves as an additional explanation for divergent patterns of shape change in closely related hominid taxa. Above all, these results emphasise the need for further research into the integrative nature not only of the primate mandible, but also between aspects of the cranium and mandible.

The overall implications of this thesis are that covariance structures are highly conserved in the extant hominid skull. The evolutionarily conserved nature of covariance structures can be largely attributed to shared developmental

processes and possibly constraints. While all three studies show obvious differences between species, these differences do not alter the covariance structure – that is, the evolutionary direction of integrated shape change is similar among extant and possibly extinct hominids. This further implies that extant analogues can be used to approximate covariance structures for extinct taxa.

Zusammenfassung

Der Primatenschädel ist eine funktional eingebettete und komplexe Struktur. Gewöhnlich wird der Schädel in verschiedene funktionale Einheiten unterteilt, wie Kauapparat, Gesichtsknochen und Hirnschädel. Jede dieser funktionalen Einheiten muss jedoch auch als Teil des gesamten Schädels funktional integriert sein. Diese Integration oder Kovarianz spiegelt sich in Strukturänderungen wieder, die auf der Änderung anderer Strukturen basiert. Das Verständnis der Evolution von integrierten oder Kovarianz-Strukturen bietet wichtige Einblicke in die Mechanismen, die phänotypischer Variabilität und Variation zugrundeliegen. Unterschiedliche Stufen der Assoziation von verschiedenen Strukturen schaffen phänotypische Variabilität, die das Variationspotential einer Struktur oder eines Organismus darstellt. Phänotypische Variabilität ist für die biologische Anthropologie von besonderem Interesse. Dabei bildet die morphologische Variation den wohl bedeutendsten Bestandteil der Forschung in der Primatenevolution. Das Kranium der Primaten ist eine wichtige Informationsquelle für biologische Anthropologen, da die fossile Überlieferung besser ist als bei anderen Teilen des Skeletts. Aufgrund des Mangels an großen fossilen Serien werden nah verwandte, überlebende Hominiden seit langem als Analogien benutzt, um phänotypische Veränderungen zu verstehen, die mit entwicklungsbedingten und funktionalen Anpassungen in fossilen Hominiden zusammenhängen.

Das Hauptziel dieser Arbeit ist die Erforschung der Kovarianz in der cranio-mandibularen Form von Pongo, Gorilla, Pan und Homo, unter Verwendung quantitativer Methoden wie der Landmark-gestützten, geometrischen 3D-Morphometrie. Diese Arbeit umfasst drei individuelle Studien, die sich mit Fragen in Bezug auf Kovarianz-schaffende Prozesse beschäftigen: morphologische Integration, Allometrie, Kanalisierung und Entwicklungsstabilität. Indem die Evolution von integrierten und Kovarianz-Strukturen untersucht wird, bieten die drei Studien

zusammen einen wichtigen Einblick in die zugrundeliegenden Mechansimen der phänotypischen Variabilität und Variation in nah verwandten hominiden Taxa.

Das erste Manuskript ist eine Studie der Muster morphologischer Integration von Gesicht, Basikranium und Kalotte bei erwachsenen Menschen, Schimpansen, Bonobos, Gorillas und Orang-Utans. Die Regionen des Säugetier-Kraniums unterscheiden sich in ihrer entwicklungsgeschichtlichen Herkunft und ihren funktionalen Ansprüchen. Dementsprechend unterteilen wir das Kranium in drei funktionale Komponenten: (a) Gesichtsskelett, inklusive Zygomatikum, Nasale, Lacrimale und Maxilla, (b) Kalotte, bestehend aus Frontale und Parietale und (c) Basikranium, bestehend aus den nicht-squamosen Teilen des Temporale und Occipitale.

Wir haben uns entschieden, diese Module als „funktional" abgeleitet zu bezeichnen, weil sie eine Anlehnung an Moss' „functional matrix hypothesis" (Moss and Young, 1960) darstellen; dennoch sind sie hauptsächlich anhand differentieller Wachstumsmuster abgegrenzt. Integrationsmuster können helfen, die strukturelle Beziehung von morphologischen Einheiten zu verstehen, und liefern so wichtige Hinweise, wie Phänotypen sich entwickeln oder welchen Einschränkungen sie unterliegen. Das Hauptziel dieser Arbeit ist es, zu evaluieren ob Integrationsmuster über nah verwandte hominide Taxa variieren. Die Ergebnisse legen nahe, dass sich trotz einer Spezies-eigenen Variation, insbesondere bei Wechselwirkungen zwischen dem Basikranium und anderen kranialen Regionen, die generellen Muster nach denen kranialen Regionen integrieren, stark ähneln. Dies legt nahe, dass die Richtung des integrierten Gestaltwandels ähnlich ist, was wiederum impliziert, dass die kraniale Integration in lebenden Hominiden und möglicherweise anderen Primaten evolutionär stark konserviert ist.

Das zweite Manuskript ist eine Studie zweier Kovarianz-schaffender Prozesse, wie Kanalisierung und Entwicklungsstabilität im Kranium lebender Hominiden. Kanalisierung und Entwicklungsstabilität beziehen sich auf Konzepte, die Entwicklungsprozesse von externen und internen Perturbationen puffern, und deren Evolution in bestimmte Richtungen lenken. Um Kovarianz zwischen Strukturen zu schaffen, können Entwicklungsprozesse die Elemente des Kraniums

in gleicher Weise oder gar nicht beeinflussen. Experimentelle Studien an Mäusen und Flügeln von Fliegen haben gezeigt, dass Prozesse wie Kanalisierung und Entwicklungsstabilität dazu beitragen, die Kovarianz von Strukturen zu bewahren und so die phänotypische Variabilität eines Organismus beeinflussen. Trotz der enormen Variation im Kranium lebender Hominiden, wird angenommen, dass die Kovarianz-Strukturen unter Primaten relativ stabil sind. Diese Studie evaluiert diese Annahmen, indem erstmals untersucht wird, ob Kanalisierung und Entwicklungsstabilität sowohl inner- wie zwischenartlich die Muster der Kovarianz-Strukturen bei Hominiden in ähnlicher Weise beeinflussen. Meine Resultate zeigen außergewöhnlich hohe Korrelationen bei Kovarianz-Strukturen zwischen den Arten, was nahelegt, dass gewisse Aspekte der Entwicklungsprozesse in lebenden Hominiden stark evolutionär konserviert sind.

Meine Resultate implizieren außerdem, dass Kovarianz-Strukturen im Kranium eine komplexe und integrierte Beziehung zu den zugrundeliegenden entwicklungsbezogenen Interaktionen besitzen, und es deshalb problematisch ist, den genauen Einfluss zu identifizieren, den jene Prozesse ausüben die die Kovarianz im hominiden Kranium aufrechterhalten .

Die dritte Studie beschäftigt sich mit Ontogenese und Integration der Mandibula. In dieser Studie untersuche ich Muster des integrierten, ontogenetischen Gestaltwandels und Wachstumskurven in sub-adulten und adulten Menschen, Bonobos und Schimpansen. Wir zeigen, dass ontogenitsche Gestaltunterschiede der Mandibula nicht nur von divergierenden Wachstumskurven verschiedener Taxa beeinflusst werden, sondern auch von Unterschieden in den Mustern der entwicklungsbezogenen Integration von Ramus und Corpus. Folgt man der „functional matrix hypothesis“ (Moss and Young, 1960; Moss, 1973), haben verschiedene Teile der Mandibula halb-unhabhängige Wachtumszentren. Genetische und morphometrische Forschungen an Mäuse-Mandibulen und einigen Primaten stützen diese Behauptung indem sie zeigen, dass die Mandibula von Säugetieren im Embryo grob in zwei Einheiten unterteilt werden kann, Ramus und Corpus.

Die wichtigsten Ergebnisse dieser Studie sind, dass Schimpansen, Bonobos und Menschen divergierende ontogenetische Wachstumskurven haben - ein Ergebnis, das in Hinblick auf kraniale Entwicklungskurven gefunden wurde. Dies bedeutet, dass artspezifische Unterschiede der Mandibula, sogar zwischen Schimpansen und Bonobos, schon früh in der Ontogenese entstehen, wie auch in der kranialen Ontogenese. Darüberhinaus zeigen meine Ergebnisse, dass Corpus und Ramus als halb-unabhängige Einheiten nicht den gleichen Entwicklungen unterliegen. Dies stützt die „functional matrix hypothesis“ und dient als zusätzliche Erklärung für divergierende Muster des Gestaltwandels in nah verwandten hominiden Taxa. Insbesondere wird durch diese Ergebnisse gezeigt, dass weitere Forschungen notwendig sind, nicht nur an der integrativen Natur der Primaten-Mandibula, sondern auch zwischen Teilen des Kraniums und der Mandibula.

Die übergeordneten Implikationen dieser Arbei sind, dass Kovarianz-Strukturen im Schädel lebender Hominiden stark evolutionär konserviert sind. Die evolutionär konservierte Natur der Kovarianz-Strukturen können größtenteils den gemeinsamen Entwicklungsprozessen zugeschrieben werden. Während alle drei Studien offensichtliche Unterschiede zwischen den Arten aufzeigen, ändern diese Unterschiede nicht die Kovarianz-Struktur - das heißt, die Evolutionsrichtung des integrierten Gestaltwandels ist ähnlich zwischen lebenden, und möglicherweise auch ausgestorbenen Hominiden. Dies impliziert weiterhin, dass über Analogien von lebenden Taxa auf Kovarianz-Strukturen ausgestorbener Arten geschlossen werden darf.

PREFACE

This doctoral thesis is the first comprehensive study of morphological integration in the cranio-mandibular form of extant hominids. The main research question asked here is: Is integration or covariation in the extant hominid skulls similar or different? And whether covariance structures evolve or are conserved across closely related hominids? This thesis is based on three studies, which collectively investigate covariance-generating factors such as morphological integration, allometry, canalisation and developmental stability in extant hominid skulls. These covariance-generating processes directly impact the phenotype by either facilitating or constraining morphological and genetic variability, in turn providing insight into how morphology evolves over time. Data for this thesis comprised of anatomical 3D landmarks taken on 812 crania and 295 mandibles of sub-adult and adult *Pongo*, *Gorilla*, *Pan* and *Homo*. Procrustes based geometric morphometric techniques were used to process and analyse these data. Overall results suggest that despite showing species-specific patterns of morphological changes, the covariance structures in the cranio-mandibular form of extant hominids is highly conserved. These results shed light on aspects of morphological evolution in hominids and possibly all primates that are conserved over a macroevolutionary time scale.

List of tables and figures

Chapter 1: General introduction

INTRODUCTION

Evolution of the mammalian cranium is informed by several complex developmental, genetic and environmental interactions. Differing levels of association among these interactions generates phenotypic variability, which is the potential of a structure or organism to vary (Wagner and Altenberg 1996). Phenotypic variability is of particular interest to biological anthropologists for several reasons one being that majority of the questions addressed in primate evolution centre around morphological variation. The primate cranium is an important source of information for evolutionary anthropologists because it preserves better in the fossil record than most other skeletal components. Due to the lack of large fossil samples, closely related extant hominids have long been used as analogues to better understand phenotypic changes related to developmental and functional adaptations in fossil taxa.

The primate skull is a developmentally integrated and complex structure. Understanding the evolution of integrated or covariance structures provides important insight into the underlying mechanisms that generate phenotypic variability and variation. The main goal of this PhD is on the evolution of covariance structures and covariation patterns in extant hominids, namely *Homo*, *Pan*, *Gorilla* and *Pongo*. This will be achieved by investigating morphological integration, ontogeny, canalisation and developmental stability in the primate skull form (Hallgrímsson et al. 2002). Morphological integration, canalisation and developmental stability directly impact the phenotype by influencing the direction of phenotypic and genetic variability and variation (Hallgrímsson et al. 2002).

Morphological integration in the hominid skull

Coordinated variation among different parts of an organism is referred to as morphological integration (Olson and Miller 1958). More specifically, Olson & Miller (1958) proposed that functionally and developmentally associated traits/characters will be highly correlated and as a result have a higher potential to co-evolve. In their model, morphological traits were treated as inter-related "numerical sets" and integration was defined and quantified as covariation between traits. This concept is similar to one of "correlation Pleiades" furthered by Berg (Terentjev 1931; Berg 1960). Correlation Pleiades addresses the degree of correlation between quantitative traits, in a way similar to examining the magnitude of integration in different parts of an organism. While the former is mainly concerned with relative independence between different morphological regions, the latter also includes the pattern of covariation/correlation between contiguous structures.

The cranium is a complexly integrated structure, comprising several different semi-independent units characterised by differential skeletal growth patterns, muscle activity and bony spaces in which brain and pharynx grow (Moss and Young 1960). The degree of relatedness between and within phenotypic elements varies with varying levels of developmental and functional interactions, subsequently giving rise to semi-distinct components or modules. Modules are semi-independent units that are more tightly integrated within themselves than they are with other contiguous units. Modular elements of a phenotype can evolve independently to some extent without significantly influencing the organization of an organism as a whole (Klingenberg 2008). Over an evolutionary time period features that are developmentally and/or functionally associated are co-inherited and co-evolve. Patterns of integration can offer important insight into the modular nature of phenotypic units, providing a framework to study an organism's evolutionary potential to vary.

More and more studies are now adopting an integrative approach to better understand the evolution and development of the primate skull form. There is some

debate as to whether primates follow a common pattern of integration in the cranium. Some researchers have suggested that homologous cranial regions, in particular those outlined in the functional matrix hypothesis (Moss and Young 1960; Moss 1962; 1968) covary across the primate clade, indicating a shared pattern of integration among all hominoids (Cheverud 1982; 1988; 1995; Ackermann and Cheverud 2004); however, not all studies support this claim (Ackermann and Cheverud 2000; Marroig and Cheverud 2001; Polanski and Franciscus 2006). Ackermann and Cheverud's (2000) work on tamarin crania revealed that *Saguinus geoffroyi* and *Saguinus oedipus* diverged in their variance/covariance structure from other tamarin taxa. Even though the phylogenetic relationship between the Geoffroy's and cotton-top tamarins is not unequivocally resolved, Ackermann and Cheverud's results indicated a common pattern of co-variation, possibly reflecting close phylogenetic relatedness between the two groups and morphological distinction from other tamarins. Marroig and Cheverud's (2001) work on 16 genera of New World monkeys provides insight into whether integration patterns evolve. Although their overall results pointed to a somewhat shared pattern of integration among Platyrrhini, there were subtle differences in inter-specific covariance structures suggesting that morphological integration patterns tend to vary across macroevolutionary time scale. In particular, the taxa showed large disparity in the strength of correlation between neurocranial and facial elements.

Integrative features of the neurocranium and face, especially in studies on modern human cranial integration (Lieberman et al. 2000a; Lieberman 2000b; Bastir 2005; Bastir et al. 2008) has been a popular subjective of investigation. Lieberman (2000a; 2000b) highlighted the role of the basicranium in generating overall integration in the primate cranium, but also suggested that the base and the face were semi-independent from each other. Bastir and Rosas (2005; 2008) used Enlow's (Enlow et al. 1982; 1990) counter-part model to examine the hierarchical nature of integration between the human face and basicranium. Their results implied that the basicranium was not an integrated whole and that the lateral elements covaried more strongly with the mandible than with the midline of the cranial base.

At an inter-specific level, Polanski and Fransicus (2006) and Mitteroecker and Bookstein (2008) compared aspects of the neurocranium and face in chimpanzees, gorillas and modern humans; however, their results were at odds with each other. While the former study suggested a different pattern of integration among these groups, the latter found them to be largely similar. Another point of contention was the lack of association between the face and neurocranium; Polanski and Fransicus (2006) found the modern human cranium to be highly modular, whereas Mitteroecker and Bookstein (2008) suggested strong correlation between the two cranial components. However, it has been suggested that conflicting results could be a consequence of different methodological approaches (Mitteroecker and Bookstein 2007).

Other studies examined cranial integration in extinct and extant hominids using a multi-module approach (Ackermann 2002; Ackermann and Cheverud 2004; Ackermann 2005). Ackermann (2002) found that although integration patterns between different parts of the face showed species-specific differences, the zygomatic region played a key role in generating overall integration in the face of both African apes and modern humans. Bookstein et al. (2003) investigated the evolutionary and ontogenetic integration in the cranial vault, base and face among archaic and modern humans. They concluded that patterns of integration were largely similar during ontogeny, but different in aspects of evolutionary integration primarily due to phylogenetic differences in the basicranium.

Developmental integration: A relatively unexplored area of research is developmental integration, particularly in the mandible. To better understand how morphology co-evolves, it is first essential to understand how morphological variability (and variation) is generated through growth and development. An effective way of investigating this is from an integrative perspective. The developmental aspect of integration was first and most extensively explored in mouse mandibles. Extensive research on murine mandibles has contributed greatly to our general understanding of mandibular biology. Quantitative genetics and developmental biology have shown that the alveolar (tooth bearing corpus) and

ramus are two key regions of variation in the mandible. Studies on mandibular patterns of integration are few and primarily on aspects of the mouse mandible (Leamy 1993; Klingenberg 2003a; Klingenberg et al. 2004) with the exception of Bastir et al. (2005), but they included aspects of the cranium, with the primary objective of determining the degree of morphological integration between the cranium and mandible.

Thus far no work has been done exclusively on morphological integration in the mandible of extinct and/or extant hominids. Along with the cranium, this thesis also explores aspects of integration in the hominid mandible focusing on ontogenetic patterns of shape change.

Canalisation and developmental stability

Canalisation and developmental stability refer to concepts that buffer developmental processes from internal and external perturbations in organisms. More explicitly, canalisation refers to a buffering mechanism against external influences such as environmental and some genetic perturbations arising in the developmental system. Developmental stability is the absence of developmental noise and refers to the buffering against developmental perturbations within individuals. Both processes in a sense result in reducing phenotypic variation in populations and individuals, respectively. The difference between canalisation and developmental stability is the latter: while canalisation is a buffering mechanism against external perturbations among individuals, developmental stability is the buffering process within individuals.

The definition of canalization was first made explicit through the work of Waddington (1942; 1957). According to Waddington (1942; 1957) certain developmental mechanisms work as a buffer against external perturbations, achieving predetermined developmental endpoints. Schmalhausen (1949) also addressed the concept of canalisation, but from the perspective of stabilising selection. Accordingly, mechanisms that resist environmental perturbations simultaneously respond adaptively to the environment. Quantitative genetic models have been widely used to better understand the effects of canalisation on phenotypic evolution (Scharloo 1991; Hall 1999; Hallgrı´msson 2002; Hallgrímsson et al. 2006). The majority of these models also support that canalisation can evolve through stabilising selection (Schmalhausen 1949; Wagner et al. 1997). Only a few studies have explored canalisation at the phenotypic level in primate evolution (Livshits et al. 1998; Reddy 1999; Tardieu 1999; Tague 2002; Hallgrímsson et al. 2006; Willmore et al. 2006).

Conversely, several studies have been conducted on developmental stability at the morphological level in biological anthropology (Jantz and Webb 1980; Corruccini and Potter 1981; Hallgrímsson 1999; Willmore et al. 2005; Willmore et

al. 2006). The most common method of measuring developmental stability or instability is by calculating the differences between sides of symmetrical organisms. Sides of bilateral organisms share a genome and develop under the same environmental conditions. The differences, although small, estimate perturbations/variation in the developmental programme. The majority of these studies have focused on aspects of fluctuating asymmetry because that is one of the ways of measuring developmental stability in the phenotype (Auffray et al. 1996; Auffray et al. 1999a; Debat et al. 2000).

Unlike canalisation, developmental stability has been widely researched in biological anthropology, but mainly in the context of stress and sexual selection (Kohn and Bennet 1986; Kieser et al. 1986a; Manning and Chamberlain 1993; Møller et al. 1995; Wilson and Manning 1995; MØller and Swaddle 1997; Kieser et al. 1997). In recent times, several studies have used genetic models based on mice crania and fly wings to explore the relationship between canalisation and developmental stability at the morphological level (Klingenberg and McIntyre 1998; Klingenberg et al. 1998; Auffray et al. 1999a; Debat et al. 2000; Klingenberg 2003a; Willmore et al. 2005; Debat et al. 2006; Debat et al. 2008; Debat et al. 2009). These studies mainly focused on the covariance structures generated from fluctuating asymmetry components and phenotypic variation, i.e comparing covariance of within fluctuating asymmetry (FA) and among individual variation. A similar study was conducted on macaque crania (Willmore et al. 2005), and showed significant correlations between covariance structures generated by FA and among-individual variation, suggesting that underlying developmental properties responsible for developmental stability and canalisation were similar. However, this claim is contentious and no clear resolution has been reached on the relationship between canalisation and developmental stability. More research in this area on the morphological and genetic level is much needed.

"Functional matrix hypothesis": cranium and mandible

The "functional matrix hypothesis" or the functional matrices approach to craniology (Moss and Young 1960; Moss 1962; 1968; Moss and Salentinjn 1969(b)) has been extensively used throughout this thesis. According to the "functional matrix hypothesis" the skull can be subdivided into components based on functional/mechanical demands, influencing bone growth through the function of soft tissues and cavities within which skeletal components develop.

Moss (Moss 1969) described two types of functional matrices: periosteal and capsular. Periosteal matrices are concerned with the effects of muscle interactions on growth and development of skeletal components. Capsular matrices are concerned with the indirect effects on growth and development caused by soft tissue organs, brain, orbits and pharyngeal cavities. Accordingly, he divided the skull into two major components or modules: the orofacial component, consisting of bones surrounding the nasal, oral and pharyngeal capsules and the neurocranial components composed of skeletal units such as the cranial vault and basicranium that encase the brain. The base of the skull or the chondrocranium is formed by endochondral bone, while the other bones of the cranium are formed by intramembraneous ossification. The facial skeleton ossifies mainly from neural crest precursors and the calvarium (vault) is formed by both maraxial mesoderm and neural crest.

Moss further proposed that elements of one functional unit would covary more strongly with each other than with elements of any other units. This is evident through experimental and morphological studies (Chernoff and Magwene 1999). In human evolution, a commonly considered determinant of cranial shape among hominids is brain growth (Lieberman et al. 2000a; Lieberman 2000b; Lieberman et al. 2002; Bastir and Rosas 2005; Bastir et al. 2008; Bastir and Rosas 2009). The fusion of the cranial vault and base bones form the complete brain case, and shape and size of the cranial vault and basicranium are mainly affected by the growth of the brain; however, different aspects of brain growth and development affect these

regions differently (Moss 1969; Mooney et al. 2002). The development of the neurocranium is primarily determined by the formation of a capsular membrane that surrounds the brain (Mooney et al. 2002). Sutures of the cranial vault bones consist of fibrous tissue, which allow alternations in size and shape of the bones as the cranium reaches adulthood. The basicranium forms the cartilaginous platform on which the brain rests and is considered the most conserved part of the cranium, being least susceptible to epigenetic forces. Most drastic changes in the facial complex have occurred in humans. Lieberman et al (2000a) attributed the tucking of the face under the neurocranium to increase in brain size. In addition, the face is influenced by the growth of the basicranium, masticatory apparatus and the sensory organs (Moss and Young 1960). Thus, even though brain growth has a fairly global effect on the cranium, the local variances created by tissue growth and muscle activity are bound to result in varying levels of intra and inter individual variation.

The mammalian mandible is similarly decoupled into functional matrices as is the cranium. According to the "functional matrix hypothesis" different parts of the mandible have semi-independent growth centres (Moss 1960; Moss 1968 ; Moss 1973). Genetic and morphometric research on mouse mandibles (Leamy 1984; Atchley et al. 1985; Atchley and Hall 1991; Atchley 1993; Leamy 1993; Klingenberg 2003a) and some on primate mandibles (Johnson et al. 1976; Daegling 1996; Willmore et al. 2009a) support this claim by showing that the mammalian mandible can be largely sub-divided into two distinct embryonic units, the corpus and the ramus (Atchley et al. 1985; Atchley and Hall 1991; Atchley 1993). Daegling's (1996) on African ape mandibles found that growth of the corpus was mainly influenced by the developing dentition, whereas the ramus was modulated by the activity of the masticatory muscles. His results showed that because parts of the mandible are semi-independent, both functionally and structurally, no single pattern of development can account for overall morphological variability and variation among different taxa. Moss's original hypothesis mainly relied on epigenetic mechanisms as explanations for morphogenesis. He later revised this hypothesis (Moss 1997(a); 1997(b); 1997(c); 1997(d)) to account for both genomic (intrinsic causes) and epigenetic or extrinsic/proximate processes.

Research goal and objectives

This PhD is a thorough investigation of the potential factors that contribute to the evolution of covariance and covariation patterns in the cranio-mandibular form of extant hominids. This directly provides insight into how phenotypes evolve over time. Furthermore, the thesis employs cutting edge methods of 3D geometric morphometrics to provide a framework to test models from evolutionary developmental biology and physical anthropology. Using models from evolutionary developmental biology can greatly inform anthropological research and help to better understand processes that influence the evolution of the primate skull form. The thesis is divided into three studies that collectively provide important insight into the underlying mechanisms that generate phenotypic variability and variation in extant hominid taxa. Following are the specific objectives for each of the studies:

Morphological integration in the hominid cranium: 1) Quantify morphological integration patterns across extant hominids:

a) Conduct pair-wise comparisons between regions of the cranium: facial skeleton, basicranium and cranial vault
b) Examine *species-specific* patterns and direction of cranial shape covariation patterns among extant hominids.
c) Evaluate whether patterns of cranial integration have a potential to evolve or whether they are constant across closely related taxa.

Canalisation and developmental stability in the hominid cranium: 1) Quantify and assess the effects of different developmental mechanisms on covariance structures in the cranium:

a) Examine whether processes responsible for generating within- and among-individual covariance are distinct within species

b) Examine whether covariance of fluctuating asymmetry (FA), that is direct developmental interactions and among-individual variation in different regions of the cranium is distinct across extant hominids
c) Assess whether covariance of FA is more conserved than individual variation.

Ontogeny and integration in the hominid mandible: 1) Quantify ontogenetic patterns of mandibular shape variation/covariation in *Pan* and *Homo:*

a) Examine ontogenetic shape changes in the mandible of humans, bonobos and chimpanzees to identify at what age stages species-specific features emerge and what they are.
b) Test the pattern and direction of mandibular ontogenetic trajectories in these hominids.
c) Compare ontogenetic shape change in the corpus and ramus separately. Specifically, we are interested in testing assumptions on mandibular growth and development as posited by the "functional matrix hypothesis"
d) Compare the results to findings of previous studies on cranial ontogenetic trajectories.

The data used in this thesis was collected at different museums. Chapter 2 provides a detailed description of the data collected during the course of this PhD. Chapter 2 also provides a general overview of the methods used to analyse these data. Technical details about the statistical analyses are provided in the manuscripts in the results section (Chapter 3 a – c).

Chapter 3 (a –c) provides the results of this thesis as three studies. Chapter 3(a) is the manuscript on morphological integration in extant hominid crania. This manuscript explores the patterns of integration across adult individuals of *Homo*, *Pan*, *Gorilla* and *Pongo*. The main research question addressed in this study is whether patterns of integration among closely related hominids are constant or

evolvable. Integration is quantified as the covariation between functional cranial components (Olson and Miller 1958; Chernoff and Magwene 1999). Three-dimensional morphometrics was used to analyse these data.

Chapter 3(b) is the second manuscript on developmental aspects of covariance matrices. The main question addressed here is, what factors influence evolution of covariance structures in hominid crania. In order to do so, this study compares covariance within and among individual interactions in order to understand the effects of developmental interaction in generating covariance structures in the hominid cranium. This study also explores the relationship between canalisation and developmental stability. Canalisation was estimated by among individual interactions and developmental stability by covariance of fluctuating asymmetry. This study also used 3D morphometrics to analyse the data.

Chapter 3(c) is the third manuscript on ontogeny of the *Pan* and *Homo* mandibles. The main question addressed in this study is at what age stage do species-specific patterns of variation/covariation emerge early in ontogeny and whether *Pan* and *Homo* follow a similar ontogenetic trajectory in the anterior and posterior aspects of the mandible. This study employed the methods of 3D geometric morphometrics to analyse the data.

Chapter 4 is a general outline of future projects - three additional studies - that emerge from this thesis. All the data and partial analyses for these studies have been collected and conducted, respectively.

Chapter 2: General materials and methods

MATERIALS

The total number of specimens measured during the course of this thesis is summarised in Tables 1 and 2. Specific details about the samples used in the different studies are provided in the results chapter. This section is to provide a general overview of the data collection process and the entire dataset.

A total of 169 bilateral and mid-line landmarks and 15 bilateral and mid-line curves (Figure 1 and Table 3) were collected on all modern human crania (Table 2). Three human populations were included in this thesis. The largest sample is from the Lisbon or Luis Lopes collection, which consists of 19th and 20th century populations from Lisbon. The Lisbon collection comprises approximately 1,632 known age and sex individuals exhumed from three cemeteries in and around the greater Lisbon area (Cardoso 2006). The Lisbon collection is housed at the Bocage or Natural History Museum in Lisbon and is an invaluable source of data for physical and forensic anthropologists and paleopathologists because it provides reliable tempo-geographical, calendar ages and sex information. The second human population included here is from the Point Hope Alaskan Ipiutak, Alaska, housed at the American Museum of Natural History (NYC). The population spans from about 100 B.C. to 500 A.D. The age and sex information was obtained from available archaeological artefacts wherever possible and also estimated from the skeletal remains. The third population was taken from the Khoi San collection curated at the Department of Anthropology, University of Vienna. Rudolf Pöch, the founder of the Department for Anthropology Vienna collected dozens of Khoi San individuals' skeletons from the Kalahari Desert during his expeditions to South Africa. When available, the sex determination of the Khoi San skulls was taken from Pöch's fieldnotes (Pacher 1961; Gunz 2006) who determined sex on the cranium based on Martin (Martin 1914; Gunz 2006) and on the available postcranial analysis from Schultz (1924).

On the great ape sample, a total of 157 bilateral and mid-line landmarks and 15 bilateral and mid-line curves were taken on the entire cranium. All great ape

specimens are wild shot individuals housed at different museums (Table 1). The entire gorilla sample was obtained from the Royal Museum of Central Africa (RMCA), Tervuren. All *Pongo* individuals were measured at Zoologische Staatssammlung, Munich. An extensive sample of *Pan troglodytes* was included to address additional questions on chimpanzee taxonomy; the three different sub-species came from four different museums (Table 2). All individuals of *Pan t. verus* measured at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig are of known calendar age and sex. All *Pan paniscus* specimens were measured at the RMCA, which is the only museum in the world that has a collection of bonobos. All measurable bonobo crania were digitised during the course of data collection at the RMCA.

In addition, mandibular data of 55 3D landmarks and 5 curves (Table 3 and Figure 3 and 4) were also measured for all taxa. Additional points were taken on modern human crania to capture human-specific features, which have been previously described qualitatively (Strait 2001). All the 3D landmarks and curves (Table 3) were carefully chosen based on biological homology and repeatability across all included taxa (Harvati et al. ; Braeuer 1988; White and Folkens 2000; Nicholson and Harvati 2006; Singh and Harvati 2007; Singh et al. 2007).

All specimens were measured by a G2X MicroScribe digitiser, which has a measurement accuracy of 0.23mm and is an optimal way of building 3-D datasets. Digitizing of all crania was done in two orientations, dorsal and ventral, in order to register all landmarks as efficiently as possible. While measuring, the specimens were mounted on modelling clay to keep them stationary. In order to match the digitised landmarks from the two separate sessions, we took four "orientation points" in the two views. To ensure matching the two sets of landmarks accurately, these points were taken in exactly the same place each time. DVLR (dorsal-ventral-left-right fitting) program was used to combine the two sets of landmarks to make one individual. More information about this software can be found on the "NYCEP morphometrics group" homepage.

Specimen ages were determined through dental developmental stages and osteometric signatures such as suture closures (White and Folkens 2000; Hall

2005). Three age categories were included: juveniles, adolescents and adults (Table 2). The juveniles were further subdivided into "Juv1" and "Juv2" groups depending on crown completion and eruption of permanent M2. As mentioned previously, calendar ages were available for most of the modern human specimens from the Luis Lopes collection and *P.t. verus* from the MPI-EVA collection.

Types of landmarks

Bookstein (Bookstein 1991) described three different types of landmarks: Type I, Type II and Type III. Type I landmarks include points that occur at the intersection of sutures (eg. asterion, lambda, pterion) and centres of small "inclusions" such as the vertebrate eye or the openings for nerves and blood vessels in the bones of the skull (Bookstein 1991). Type 1 landmarks are easiest to locate across different taxa, particularly mammalian taxa, and tend to be biologically homologous. Type II landmarks are "fuzzy/mathematical" points that are homologous based on geometric similarity. They are considered geometrically homologous because they are always located in the same place, for example, points on maximum curvature (bulges on the bone) and innermost points on concavities. Such landmarks are defined case by case and can often be difficult to locate across wide genera of taxa. Type III landmarks are defined as the "anterior or posterior most" point of a structure. These landmarks are somewhat similar to Type II landmarks, but the former tend to refer to traits on a larger geometric scale.

Outline or curve data is another type of "landmarks set" and provides additional information on shape. Curves are particularly useful to quantify smooth and/or rounded surfaces that do not have too many well-defined landmarks, such as the mammalian cranial vault or the enamel-dentine junction in teeth. Such data provide information about shape that cannot be captured by standard landmarks.

METHODS

This section contains a general introduction to geometric morphometric methods. More detailed and technical explanations of specific methods used in the different studies are provided in the results chapter.

Traditional morphometrics

The discipline of morphometrics is concerned with quantifying and analysing biological form. Studying different aspects of morphological variation is a central concept in most biological sciences. Morphometrics is an integral part of biology in that it provides a rigorous and objective means of quantifying biological variation. Morphometric methods are fast becoming an important methodological tool in physical anthropology, evolutionary and developmental biology, ecology and orthodontics. There are several reasons for applying these techniques in different biological disciplines:

1) Morphometrics allows for more objective examination of biological variation than qualitative descriptions, which makes it easy to test and reproduce results. (Slice 2007).
2) Small-scale variation among morphological structures can be better detected through quantitative methods. Such differences are often times biologically meaningful; thus, having methods that can reliably capture them is important in biology.
3) Morphometrics also provides a tool to examine biological objects with complex and abstract body plans.
4) Quantitative data can be used to test important theories in evolutionary developmental biology, anthropology, ecology, clinical studies such as orthodontics and genetics, to name a few.
5) Finally, morphometrics has been particularly useful in quantifying and analysing fragmentary fossils in physical anthropology (Gunz 2006).

Morphometrics as a discipline has been around for several centuries. Many of the principles underlying this approach can be traced back to biometry. The founding fathers of biometry such as Adolphe Quetelet (1797-1874), Karl Pearson (1857-1936) and Ronald Fisher (1890-1962), to name a few, developed bio-statistical methods to quantify and analyse biological forms; the initial statistical analyses were univariate in nature, meaning with single variables. Later developments in the field of bio-statistics led to the incorporation of analyses including many variables, i.e. multivariate analyses; this type of analysis also included osteometric measurements as variables (Bookstein 1996; Marcus 1996).

A slight shift in these analytical tools, that is, those of traditional morphometrics, was seen in the eighties with the introduction of geometric morphometrics (GM) (Rohlf 1996). The foundation of GM is based on procedures that include Cartesian coordinates of anatomical points (2D or 3D) rather than linear distances and angles previously used in traditional morphometrics. However, perhaps the most important aspect of GM is that it allows a global analysis of biological shape, preserving all the geometric properties of biological structures (Marcus 1996; Zelditch et al. 2004).

The basic tenets of GM were set at the beginning of the 20th century. D'Arcy Thompson, in this seminal book "On growth and form" he stated that morphological shape changes could be represented through "transformation grids", which were based on a Cartesian model. However, Albrecht Duehrer's (Bookstein 1991; Bookstein 1996) work on shape transformations of the human head easily stands as one of the first examples of using such grids to study body proportions. These ideas were put into a modern synthesis by Bookstein (Bookstein 1991) and Kendall (1984), providing a new approach to morphometrics.

Geometric morphometrics

In geometric morphometrics, form is defined as size and shape of an object. The "form" of objects is measured by Cartesian coordinates of (anatomical) points or landmarks based on homology and repeatability across different objects or specimens. The landmark configurations of specimens are then transferred into a "morphospace" or point clouds, preserving the geometric properties of each specimen. A morphospace is a scatter plot of points where each point represents a specific form and every form in this space corresponds to a point. The distances between points represent the degree of similarity between the included forms. That is, the point configurations in the morphospace provide information about the degree of morphological similarity among the forms they represent. Morphospaces are multidimensional spaces. The information related to the differences and similarities among forms provided by the morphospace can be analysed through standard multivariate statistics.

Size and shape

GM methods are primarily concerned with the concept of size and shape and an important advantage of GM is that it can analyse size and shape separately (Bookstein 1991). There have been various different measures for size, such as body mass, length-width measurements, area and volume. GM provides a specific measure for size called the centroid size obtained from the landmarks configurations of each specimen included in the sample. Centroid size is independent from any other variables when landmarks are evenly distributed around their respective means (Slice 2007).

Shape refers to the arrangement of an object relative to its size and different components that constitute that object. Technically, shape is defined as the geometric information that remains when factors such as size, location and rotation are removed. The latter forms the crux of generalised Procrustes analysis, which is described in detail in the section below.

Superimposition techniques

To transfer specimen landmark configurations into a morphospace, different mathematical superimposition techniques such as two-point registration (Bookstein 1991; Bookstein 1996), general resistant fit superimposition (Rohlf 1999) and generalised Procrustes analysis (GPA) have been proposed; the most popular and commonly used among these is GPA (Rohlf and Corti 2000; Slice 2007). In this thesis too we employ GPA as our primary method of shape analysis.

Procrustes is a figure in ancient Greek mythology who mutilated his victims by either stretching or cutting off his victim's body parts to best "fit" them to his bed. The function of a Procrustes superimposition is similar in that according to this procedure shape information is extracted from specimen landmark configurations by scaling, translating and rotating the configurations to achieve a best fit. Scaling the landmark configuration involves removing information that is related to size, that is, all size-related effects. The second step, translating, is when the centroids of the landmark configurations are shifted to coordinates (0,0,0). In the third step, the configurations are rotated around the centroid using least-squares estimates to achieve a best fit. In this three-step procedure, scaling and translating of the configurations can happen interchangeably, but rotation of the configurations can only take place after the first two procedures are completed. The scaled, translated and rotated coordinates are called Procrustes or shape coordinates and can be used in subsequent statistical analysis. In addition, this procedure yields a measure for size, called centroid size (CS). CS is the sum of squared distances of all landmarks from their mean and is also a measure of how much the landmarks disperse from their centroid; the farther the dispersion of landmarks, the bigger the CS. The most significant difference between other superimposition procedures and GPA is that in the latter all landmarks are given an equal role.

The procedure used to convert the landmark configurations into "shape space" is called the Euclidean similarity transformation ((Dryden and Mardia 1998; Zelditch et al. 2004). This shape space is commonly known as "Kendall's shape space" (Kendall 1984) because Kendall developed and proposed the statistical

theory of shape that is now used in GM. Kendall's "shape-space" is a non-linear space that provides a geometric setting for analyzing the Procrustes distance among a set of landmarks. The shapes are represented by the landmarks, which are essentially isotropic distribution of points in this shape space (O'Higgins 2000). The landmark configurations go through several different multidimensional "morphospaces", each with unique statistical properties. Dyden and Mardia (1998) explained the transformation of the original landmarks into shape space as represented by a *p*k* matrix, where *p* is the number of landmarks and *k* the number of dimensions (2D or 3D). The matrix contains as many rows as landmarks and as many columns as dimensions. Translation positions the *k* coordinates and moves the "fixed" specimen landmark configurations into pre-form space, which has *pk-k* dimensions. Rotating the landmarks reduces the dimensions (*k[k-1]/2)* and allows the specimens to enter a form-space, which still contains size. By removing scale/size, the dimension of size is lost and specimens then exist in a *pk-k-k(k-1)/2-1*, which is shape space. The latter is a multidimensional shape-space in which the landmarks lie (Rohlf 1996). The distance between two points in this space is called Procrustes distance. According to Bookstein (1996) Procrustes distance is the only statistically viable shape distance for landmark data.

While Procrutes superimposition is the most commonly used method in GM studies, there are other methods such as thin-plate spline deformation methods (Bookstein 1991; Bookstein 1996) and Eucliedean Distance Matrix (Richtsmeier and Lele 1993).

Thin-plate spline: shape deformation

The difference between coordinates obtained after a Procrustes superimposition and the thin-plate spline superimposition is that the latter can be directly used in statistical tests without adjusting for degrees of freedom (the number of independent measurements in a dataset) in the data. The thin-plate

spline coordinates are generally referred to as partial warp scores. The partial warps represent the position of each specimen in shape space and can be used to examine small-scale shape changes (Rohlf 1999). The results obtained from using partial warps and Procrustes coordinates is the same as long as the degrees of freedom for the Procrustes coordinates are adjusted for correctly.

The primary purpose of thin-plate spline deformation grids is to visualise shape changes. That is, such deformation grids can be used to interpolate between landmarks, taking into account all the displacements between landmarks relative to each other. In short, this method serves as a convenient tool for visualising shape changes and obtaining shape variables with correct degrees of freedom.

Euclidean Distance Matrix Analysis

EDMA is the other school of superimposition methods that is almost as widely used as Procrustes based techniques. EDMA too uses landmark coordinates as raw data, but rather than keep them in coordinate form, the data are converted into Euclidean inter-landmark distances between all possible pairs of landmarks (Richtsmeier and Lele 1993). However, the matrix of distances is not concerned with translating, reflecting and rotating landmark configurations. Mean shapes are obtained by standardising the mean forms of each specimen by a scaling factor or the geometric mean. The scaled distances can be used to explore shape changes among different specimens. The differences/similarities in shapes can be tested statistically by applying a Monte Carlo parametric bootstrap procedure (Lele and Cole III 1996).

Multivariate statistics

GM is a powerful and efficient way of analysing biological data. The usefulness of these methods is due to the fact that it provides a means to statistically test hypothesis about size and shape of structures, making the study of overall morphological variation objective and reliable. The most commonly used statistical methods involve multivariate statistics. Depending on the questions and data at hand, there are several different statistical approaches used in GM studies. This section will give a general overview of the different methods available and also a brief background on the methods employed in this thesis. However, relevant application and technical details regarding specific methods can be found in the results section.

Ordination methods

Two widely used ordination methods and the ones that will be discussed here at principal components analysis (PCA) and canonical variates analysis (CVA). These methods are exploratory techniques and their primary use is to describe variations between individuals and/or groups included in a dataset; they cannot be used to test hypotheses.

PCA is an effective technique, which is used to examine overall variation in a given sample. PCA is based on calculations of an eigenvalue decomposition of a covariance matrix and is essentially an eigenvector-based multivariate technique. This method transforms the original set of variables entered into the analysis into principal components (PCs). The PCs are not correlated to each other and account for the maximum amount of variation in the sample; in most cases, the first few PCs explain most of the variance in the dataset. The presentation of results in a PCA is also simplified because it can be done through plots and figures. In GM, a PCA can be conducted directly on Procrustes shape coordinates, which provides a description of shape variation in a dataset without the effects of isometric size, i.e. centroid size. A PCA can also be performed in "form-space" which is when centroid

size is re-introduced into the analysis as a variable and allows exploration of size related shape differences, for example allometric changes in a dataset. Many studies using geometric data (Procrustes coordinates) also refer to PCA as "relative warps analysis" (RWA).

The second ordination method widely used is canonical variates analysis (CVA). The major difference between PCA and CVA is that while the former explores overall variation within a dataset, the latter is mainly concerned with differences among groups rather than among individuals. In several ways PCA and CVA are similar. Both methods construct a new coordinate system, in this case, the component of a CVA are called canonical variates (CVs) and are eigenvector-based techniques. But the main information described by the CVs is related to group mean differences in the dataset rather than individuals. Moreover, for effective interpretation of CVs, each group in the sample has to share a particular trait that is discontinuous in nature; for example, species or sex.

Multivariate regression analysis

A regression analysis focuses on the relationship between a dependent and independent variable; in this case, the dependent variable will generally be shape because in GM the focus is mainly on shape changes. When applying this theory to shape, we employ multivariate regressions because shape is multidimensional. The most common regression analysis is shape on size, which is predominantly used in allometric studies, particularly with ontogenetic data. To test for significant differences between size and shape, permutation tests can be performed (Good 2000). Regression analyses can also be run to test the differences between group means and angles among taxa and/or populations and age categories (Zelditch et al. 2004).

2-block Partial-least squares

2B-Block Partial-least squares (2B-PLS) is a method for exploring the pattern of co-variation between two blocks of variables. This technique is particularly useful in examining morphological integration (Rohlf and Corti 2000; Mitteroecker and Bookstein 2007). But this method can also be used to analyse the relationship between shape and other variables such as age, climatic variables, time or behaviours. The partial least squares approach has been extensively used in social sciences (Bookstein 1990) and is only recently being applied to biological datasets (Bookstein et al. 2003; Klingenberg 2003a; Gunz and Harvati 2007; Mitteroecker and Bookstein 2007; Bastir et al. 2008; Mitteroecker and Bookstein 2008). Two-block partial least squares analysis emerges from a group of statistical techniques that is based on a singular value decomposition of the between-block covariance matrix (Mardia et al. 1979; Rohlf and Corti 2000; Mitteroecker and Bookstein 2007). The blocks of variables are chosen *a priori* to the analysis and therefore this method cannot be utilised to *find* blocks of variables. A 2B-PLS differs from a multivariate regression analysis in that it does not assume variables to be either dependent or independent; it treats them both symmetrically. Accordingly, it finds pairs of axes - one axis per block - which account for the maximum amount of co-variation between the two sets of variables examined. Moreover, the axis in one block is only correlated to the corresponding axis in the other block, making it easy to examine co-variation patterns one pair of PLS axes at a time (Mitteroecker and Bookstein 2007). Because PLS techniques use a mathematical calculation called the singular-value decomposition, the vectors/axes of PLS are referred to as singular values. In some studies on shape co-variation patterns, 2B-PLS axes are referred to as singular warps (Bookstein et al. 2003; Mitteroecker and Bookstein 2007).

Software packages

In recent years a number of new 2D and 3D software packages have been introduced to conduct GM analyses. These are particularly useful for running complicated statistical analyses if researchers lack computer-programming skills.

Moreover, these packages facilitate visualisation of plots and figures, which are integral for interpreting results. The Stony Brook University morphometrics website (http://life.bio.sunysb.edu/morph/index.html) contains a useful list of software packages for various statistical analyses and visualisation tools. Another good resource for morphometrics programmes is the NYCEP website (http://www.nycep.org/nmg/programs.html). The programmes listed on the NYCEP website are mainly for data formatting and structuring. Among the most commonly used morphometrics softwares are: Thin-plate spline series, MorphoJ, Morphologika, Morpheus, Integrated morphometrics package and Edgewarp. All these programmes are free and downloadable from their respective websites. Moreover, advanced tool kits such as AMIRA and Viewbox are also useful for visualising datasets that deal with surfaces of landmarks and CT data.

Table 1: Total number of crania measured

Species	No. of individuals	Source
Pan t. troglodytes		Naturkundemuseum, Berlin
Adults		
Female	22	
Male	33	
Total adults	55	
Adolescents		
Female	0	
Male	0	
Juveniles		
Female	2	
Male	2	
Unknown	11	
Total juveniles	15	
Grand total	70	
Pan t. verus		MPI-EVA, Leipzig & Peabody Museum, Boston
Adults		
Female	29	
Male	28	
Unknown	3	
Total adults	60	
Adolescents		
Female	1	
Male	2	
Total adolescents	3	
Juveniles		
Female	1	
Male	1	
Unknown	3	
Total juveniles	5	
Grand total	68	
Pan t. schweinfurthii		RMCA, Tervuren
Adults		
Female	5	
Male	14	
Unknown	34	
Total adults	53	
Adolescents		
Female	4	

Male	4	
Unknown	8	
Total adolescents	16	
Juveniles		
Female	6	
Male	2	
Unknown	8	
Total juveniles	16	
Grand total	85	
Pan paniscus		RMCA, Tervuren
Adults		
Female	25	
Male	23	
Unknown	11	
Total	59	
Adolescents		
Female	2	
Male	3	
Unknown	5	
Total	10	
Juveniles		
Female	8	
Male	2	
Unknown	15	
Total	25	
Grand total	94	
Gorilla beringei graueri		Naturkundemuseum, Berlin RMCA, Tervuren
Adults		
Female	27	
Male	28	
Total adults	55	
Adolescents		
Female	2	
Male	1	
Total adolescents	3	
Juveniles		
Female	5	
Male	7	
Unknown	11	
Total juveniles	23	
Grand total	81	

Pongo pygmaeus pygmaeus		Zoologische Staatssammlung, Muenchen
Adults		
Female	50	
Male	34	
Total adults	84	
Adolescents		
Female	5	
Male	1	
Total adolescents	6	
Juveniles		
Female	7	
Male	7	
Total juveniles	14	
Grand total	104	
Homo sapiens		
Luis Lopes		MNHN, Lisbon
Adults		
Female	49	
Male	96	
Unknown	3	
Total adults	148	
Adolescents		
Female	10	
Male	7	
Unknown	1	
Total adolescents	18	
Juveniles		
Female	8	
Male	5	
Unknown	2	
Total juveniles	15	
Grand total	181	
Point Hope		AMNH, New York
Adults		
Female	42	
Male	32	
Unknown	12	
Total adults	86	
Adolescents		
Female	4	
Male	0	
Unknown	1	

Total adolescents	5	
Juveniles		
Female	3	
Male	0	
Unknown	12	
Total juveniles	15	
Grand total	106	
Khoisan		University of Vienna, Vienna
Adults		
Female	8	
Male	10	
Unknown	1	
Total adults	19	
Adolescents		
Female	0	
Male	3	
Total adolescents	3	
Juvenile		
Female	1	
Male	0	
Total juveniles	1	
Total	23	
Grand total	310	
Total number individuals measured	812	

Table 2: Total number of mandibles measured

Species	No. of individuals	Source
Pan t. troglodytes		Naturkundemuseum, Berlin
Adults		AMNH, New York City
Female	11	
Male	9	
Unknown	34	
Total adults	54	
Adolescents		
Female	2	
Male	2	
Unknown	2	
Total adolescents	6	
Juveniles		
Female	3	
Male	3	
Unknown	3	
Total juveniles	9	
Grand total	69	
		MPI-EVA, Leipzig & Peabody Museum, Boston
Pan t. verus		
Adults		
Female	1	
Male	4	
Total adults	5	
Grand total	5	
		RMCA, Tervuren and AMNH, New York City
Pan t. schweinfurthii		
Adults		
Female	4	
Male	20	
Unknown	13	
Total adults	37	
Adolescents		
Female	2	
Male	2	
Unknown	2	
Total adolescents	6	
Juveniles		
Female	3	
Male	2	

Unknown	5	
Total juveniles	10	
Grand total	53	
		RMCA, Tervuren
Pan paniscus		
Adults		
Female	15	
Male	16	
Unknown	10	
Total adults	41	
Adolescents		
Female	2	
Male	2	
Unknown	7	
Total adolescents	11	
Juveniles		
Female	2	
Male	2	
Unknown	8	
Total juveniles	12	
Grand total	23	
Pongo		Zoologische Staatssammlung, Muenchen
Adults		
Female	5	
Male	4	
Unknown	9	
Total adults	18	
Adolescents		
Female	0	
Male	1	
Total adolescents	1	
Juveniles		
Female	4	
Male	7	
Total juveniles	11	
Grand total	30	
Homo sapiens		
Luis Lopes		MNHN, Lisbon
Adults		
Female	8	
Male	26	
Unknown	19	
Total adults	53	

Adolescents	6	
Female	6	
Male	4	
Unknown	16	
Total adolescents		
Juveniles	3	
Female	2	
Male	7	
Unknown	12	
Total juveniles	81	
Grand total		
		AMNH, New York
Point Hope		
Adults	3	
Female	2	
Male	11	
Unknown	16	
Total adults		
Adolescents	5	
Unknown	5	
Total adolescents		
Juveniles	8	
Unknown	8	
Total juveniles	29	
Grand total		University of Vienna, Vienna
Khoisan		
Adults	2	
Female	6	
Male	1	
Unknown	9	
Total adults		
Adolescents	0	
Female	1	
Male	1	
Total adolescents	10	
Grand total		
Total number of mandibles measured	295	

Table 3: Definition of landmarks

Cranial landmarks Midline (Ventral points)	Definitions
1. Inion	Point at which the superior nuchal lines merge in the midline. Located below the external occipital protuberance, but above the tuberculum linearum. (Hublin, 1978; Braeur, 1988).
2. Lambda	The apex of the occipital bone at its junction with the parietals, in the midline – where the lambdoidal and sagittal sutures meet; there is often an intercalary or apical bone at the site, in which case lambda is to be found by extending the general curving course of each half of the lambdoid suture to their intersections with the midline (or halfway b/t these intersections) (Howells, 1973; Braeur, 1988).
3. Opisthion	Midline point at the posterior margin of the foramen magnum – taken on rim of the foramen or the lower edge of the margin of the foramen (Howells, 1973; White & Folkens, 1991).
4. Basion	On the anterior border of the foramen magnum (opposite opisthion), in the midline, at the position pointed to by the apex of the triangular surface at the base of either condyle, i.e., the average position from the crests bordering this area. Generally taken between hypobasion and the endobasion (Howells, 1973; Braeuer, 1988).
5. Mid-point on basio-spehno synchrondrosis	Median sagittal point taken on basio-spehno synchorodrosis, but on the occipital bone. In apes this is difficult to see, but locate remnants of the suture and take it on the basilar part of the occipital bone, just on the margin of the suture.
6. Mid-point between inion and opisthion	Instrumentally determined point taken on the mid-sagittal of the nuchal plane between inion and opisthion – occipital crest region (as defined by Lahr).
7. Opisthocranion	Instrumentally determined point at the rear of the cranium; defined as the midline ectocranial point at the farthest chord length from glabella (White & Folkens, 1991). This point occasionally falls on the external occipital protuberance (Braeuer, 1988).
8. Hormion	Median sagittal point on the base of the vomer bone, point lies between the two vomeris alae – taken on the sphenoid bone, directly below the posterior midline of the vomer bone (Braeuer, 1988; Baab, personal communication).
9. Staphylion	Point on the interpalatal suture where a line drawn between the deepest parts of the notches (free edges) at the rear of the palate crosses the midline (White & Folkens, 1991).
10. Midline anterior palate	Anterior most point on the midline of transverse palatine bone – always take point in the middle even if sutures do not

	line up (Harvati, 2003).
11. Incisive foramen	Midpoint on posterior end of foramen.
Bilateral points	
12. Asterion	The common meeting point of the temporal, parietal, and occipital bones, on either side; if the meeting point is occupied by a wormian bone (os astericum), extend the lambdoid suture onto its surface, and then extend the other two sutures (temporo-parietal, temporo-occipital) to the first line, finding asterion as the point midway between the intersections if these do not coincide; use only the part of the last two sutures (ca. 1cm) which is nearest the point, in finding these directions. If the lambdoid (or other) suture is complex or composed of wormian bones, trace a pencil line along the center of the area covered by the complexity, as well as can be done, to find the main axis of the suture (Howells, 1973).
13. Occipital condyle post.	Posterior apex (midpoint) on occipital condyle – taken on the condyle.
14. Occipital condyle ant	Anterior apex (midpoint) on occipital condyle – taken on the condyle.
15. Lateral points on basilar bone	Lateral most points on the basilar bone – taken on occipital bone
16. Jugular foramen Post.	Most poterio-lateral point on the foramen – taken on the suture, but on the occipital end – if gap present, take lateral most point on occipital bone, where the suture would have been.
17. Jugular foramen Ant	Taken on the anterior most point of the foramen, on the occipital bone.
18. Hypoglossal canal	Medial point on the anterior margin of the opening of the canal – close to the condyles.
19. Superior nuchal line	Beginning of nuchal line, close to asterior – trace where the outline of the line begins (Harvati, 2003).
20. Inferior nuchal line	Beginning of the nuchal line, close to anterior end of the occipito-mastoid suture (Harvati, 2003).
21. Parietal notch/ Entomion	Point taken where on the posterosuperior border of the temporal where the squamosal and pareitomastoid sutures meet (Braeuer, 1988).
22. Mastoidale	Most inferior point on the mastoid process (Braeuer, 1988).
23. Crest tip	Most inferior point on the juxtamastoid eminence – but generally taken in the middle of the crest (Harvati, 2003).
24. Anterior point on articular eminence	Most anterior point on the articular surface of the articular eminence – close to the spehnosquamosal suture (Lockwood, 2002).
25. Entoglenoid process	Most inferior point on the entoglenoid process – taken on the temporal bone (Harvati, 2003).
26. Inferior point on articular surface	Most inferior point on the medial margin of the articular surface of the articular eminence – close to the entoglenoid process, but point taken on the margin of the articular

	surface (Lockwood, 2002).
27. Point on articular tubercle	Lateral point - deepest point - on the lateral margin of the articular surface of the articular tubercle (Harvati, 2003).
28. Center point on articular eminence	Instrumentally determined point on the center of the articular eminence – between the medial and lateral points on the articular eminence (Lockwood, 2002).
29. Mandibular fossa	Deepest point in the fossa – following Lockwood et al (2002), if there was no deepest point, like in the apes, the center of the fossa is taken (Lockwood, 2002).
30. Postglenoid process	Most inferior point on the postglenoid process – could be below mid-point in mandibular fossa (Lockwood, 2002).
31. Lateral squamotympanic fossa	Intersection of squamotympanic fissure with lateral edge of mandibular fossa – taken as being between the lateral edge of the meatus and the postglenoid process (Baab, personal communication).
32. Apex of the petrous part of temporal bone	Taken on the temporal bone (Lockwood, 2002).
33. Foramen Ovale	Lateral point on the foramen (Lockwood, 2002).
34. Foramen spinosum	Point taken on posterior-lateral margin of spinosum.
35. Posterolateral point on carotid canal	Taken on the posterolateral margin of the canal.
36. Stylomastoid process	Lateral point taken on the vagina of the process, even when process is absent (Lockwood, 2002).
37. Stylomastoid foramen	Postero-lateral point on the margin of the foramen – where the bone would have been.
38. Tympanomastoid fissure	Point on lateral border of fissure (Baab, personal communication).
39. Porus of EAM	Most inferior point on the external acoustic porus. (Lockwood, 2002).
40. Tympanic plate	Most inferolateral point on the tympanic element of the temporal bone (Lockwood, 2002).
41. Lateral vaginal plate/lateral petrotympanic crest	Lateral origin of petrotympanic crest (Harvati, 2003).
42. Medial vaginal plate/Medial petrotympanic crest	Most medial point of petrotympanic crest at level of carotid canal (Harvati, 2003).
43. Point on supraglenoid surface	Point of inflection where the braincase curves laterally into the supraglenoid gutter, in coronal plane of mandibular fossa (Lockwood, 2002).
44. Point on lateral zygomatic process	Point on the lateral margin of the zygomatic process of the temporal bone at the position of the postglenoid process (Lockwood, 2002).
45. Auriculare	Point vertically above the center of the external auditory porus at the root of the zygomatic process, a few millimeters above porion (White & Folkens, 1991).
46. Radiculare	Point taken in front of auriculare, at the root where the zygomatic arch joins the squmaous of the temporal bone – the deepest point at the junction of the arch with the squamous.

47. Porion	Uppermost point on the margin of the external auditory meatus.
48. Infratemporale	Intersection of the infratemporal crest and spehnosquamosal suture (Braeuer, 1988).
49. Stenion	Most medial point on the spheno-squamosal suture - close to foramen spinosum (Braeuer, 1988).
50. Pterygoid canal	Point on sphenoid bone, at the apex of the petrous bone, where there is a notch on the sphenoid bone – below the petrygoid canal (Harvati, 2003).
51. Zygomatic root alveolar	Point where malar root arises from the maxilla, projected onto buccal alveolar surface (Harvati, 2003).
52. Zygomatico-temporal suture – inferior	Anteroinferior point of zygomaticomaxillary suture, in antero-lateral view (Harvati, 2003).
53. Zygomatico-temporal suture – superior	Anterosuperior point of zygomaticomaxillary suture (Harvati, 2003).
54. Frontomalare posterior	Point on the frontozygomatic suture – other side of frontomalare orbitale – temporal end of zygomatic bone. Point on external cranial vault where frontal, sphenoid and malar bones join.
55. Frontosphenomalare	Midpoint on suture between palatine and sphenoid bones (Harvati, 2003).
56. Spheno-palatine suture	Point of contact – on the alveolar bone – between the incisors. *Where the alveolar bone is missing or presence of periodontal disease, this point is estimated a little. But if the alveolar has completely atrophied, point is not taken. (VALID FOR ALL DENTAL POINTS).
57. I1-I2 inter-alveolar septum	
58. I2-canine contact	Point of contact between 2nd incisor and canine – taken on the alveolar bone.
59. Canine-P3 septum	Point of contact between Canine-P3 – on alveolar bone.
60. P3-P4 septum	Point of contact between P3-P4 – on alveolar bone.
61. P4-M1 septum	Point of contact between P4-M1 – on the alveolar bone.
62. M1-M2 septum	Point of contact between M1-M2 – on the alveolar bone.
63. Distal M3	Midpoint on distal margin of M3 – point taken on alveolar bone.
64. Ectomolare	Disto-Buccal side on alveolar margin of M3 (Braeuer, 1988).
65. Endomolare	Take it on mid-point on lingual side of M2, on the alveolar margin instead of tooth (Braeuer, 1988).
Midline (dorsal view)	
1. Bregma	Posterior border of the frontal bone in the median plane; the general course of the suture as a whole should be lightly drawn with a pencil, and the bregma established on this; the sutures may meet with rounded external edges, resulting in a cleft or depression at their junction. Bregma is then to be established "in the air," i.e., it its correct position but at the level of the general surface of the bone (Howells, 1973).
2. Metopion	Visually determined point on the median sagittal between

	the frontal peaks on the frontal bone – midpoint on horizonatal line drawn from the two frontal peaks, perpendicular to the medial sagittal line. Take point at the cross-section of the horizontal line and sagittal line. In apes this will be very difficult to determine, but found generally between post-toral sulcus and bregma (Braeuer, 1988).
3. Post-toral sulcus	Minima of concavity on midline post-toral frontal squama – above glabella. (Harvati, 2003).
4. Glabella	Point on median sagittal plane, between the supercillary arches. It serves as most protruding forward point at the head in ear-eye level). (Most anterior midline point on the frontal bone) (Braeuer, 1988; White & Folkens, 1991). Sometimes the arches merge into glabella. In apes, this point is taken between the "arches", which form a torus.
5. Nasion	Intersection of the fronto-nasal suture and the median plane; if there is irregularity near the midline, rectify the general curve of the fronto-nasal suture with a pencil so as to find the correct level for nasion (Howells, 1973).
6. Rhinion	Midline point at the inferior free end of the internasal suture (White & Folkens, 1991).
7. Nasiospinale	Thin projection of bone on the midline at the inferior margin of the nasal aperture- but not taken on projection, because that is often broken off, taken as the mid-point on the inferior margin of the nasal aperture – where the nasal spine intersects the margin – apes do not have an anterior nasal spine – this point is homologous across taxa.
8. Prosthion	Median sagittal (antero-inferior) most point on premaxilla – taken on the most prominent/forward projecting portion of premaxilla.
9. Alveolare	(Infradentale superius) – midline point at the inferior tip of the bony septum between the upper central incisors – below prosthion.
10. Incision	Point on the occlusal surface between the central incisors.
Bilateral points	
11. Mid-orbit torus superior	Point on superior aspect of supraorbital torus, directly above mid-torus inferior (Harvati, personal communication).
12. Mid-orbit torus inferior	Point on inferior margin of supraobrital torus roughly at the middle of the orbit (Harvati, personal communication).
13. Dacryon	Point where the lacrimomaxillary suture meets the frontal bone (White & Folkens, 1991).
14. Frontomalare Orbitale	Point where the frontozygomatic suture crosses the inner orbital rim (White & Folkens, 1991).
15. Orbitale	The lowest point on the orbital margin (White & Folkens, 1991) Most inferior point on the zygomaticomaxillary suture

	(White & Folkens, 1991).
16. Zygomaxillare	Located below the inferior orbital rim on the facial surface – superior-lateral point.
17. Infraorbital foramen	Instrumentally determined as the most lateral point on the margin of the nasal aperture (White & Folkens, 1991).
18. Alare	The point in the depth of the notch between the temporal and frontal processes of the zygomatic (Brauer, 1988; White & Folkens, 1991).
19. Jugale	Point where the coronal suture crosses the temporal line (White & Folkens, 1991).
20. Stephanion	Anterior pterion - Pterion is a region, rather than a point, where frontal, temporal, parietal, and sphenoid meet on the side of the vault; the sutural contact pattern is this area is highly variable – this point is taken at the fore end of the suture (sphenoparietalis juncture) (Braeuer, 1988; Harvati, personal communication).
21. Spehnion	The intersection of the most anterior surface of the lateral border of the orbit and a line bisecting the orbit along its long axis (Howells, 1973).
22. Ectoconchion	Instrumentally determined as the most prominent lateral point on the surface of the zygomatic arch (White & Folkens, 1991).
23. Zygion	To be taken on the margin of the rim of the canal.
24. Mid-point on superior margin of optic canal	To be taken on the superior margin of the canal – on the bone.
25. Mid-point on superior margin of superior orbital fissure.	Point on the coronal suture where the breadth of the frontal bone is greatest look – below stephanion (Brauer, 1988).
26. Coronale	The point where the temporal line reaches its most anteromedial position on the frontal bone (White & Folkens, 1991).
27. Frontotemporale	Instrumentally determined midpoint between bregma and asterion.
28. Midpoint parietal	
Curves/Lines	Definition
Midsagittal profiles:	
1. Glabella-Bregma	From glabella to bregma – take it in the center even if Bregma is not centered.
2. Bregma-Lambda	From bregma, along sagittal suture (to be taken in a straight line - not following the 'zig-zag' of the suture).
3. Lambda-Inion	From lambda to inion – to be taken in a straight line, passing over opisthocranion.
4. Inion-Opisthion	From inion to opisthion – to be taken in a straight line and over occipital crest (if crest is present).
5. Basion-Hormion	From basion to hormion, along the middle of the basilar bone.
6. Nasion-Glabella	From nasion to glabella.

7. Prothion-nasiospinale	From prosthion to nasiospinale.
Oblique lines:	
8. Oblique parietal	From bregma to asterion through midparietal point – to pass through the instrumentally determined midpoint between bregma and asterion – to be taken bilaterally (Harvati, personal communication). In apes, bregma is generally located anterior to the sagittal crest, but asterion is sometimes on the nuchal crest.
9. Oblique parietal	From anterior pterion to lambda – to pass through the center of the parietals – to be taken bilaterally. If pterion is X shaped rather than H shaped, take pterion center of X formation. *would help to first define midpoints between landmarks before taking the oblique lines. Sometimes mid-point between bregma-asterion is the same for pterion-lambda.
Other bilateral lines:	
10. Biauriculare	From auriculare to apex – to be taken bilaterally (Howells, 1973)
11. Mastoid outline (1)	Outline mastoid from porion to the mastoidiale (Harvati)
12. Mastoid outline (2)	From mastoidale-outlining digastric notch from mastoidiale to Stylomast. Foramen. * A freely projecting mastoid process is absent in apes, especially chimpanzees and orangutans. Mastoid bone in apes has a "roughened" and externally rounded surface. Mastoidale is often missing in apes. And the digastric fossa is often represented by a very shallow groove.
13. Occipito-mastoid suture	From post-lat jugular for. to asterion (Harvati).
14. Squamosal suture	From asterion to entoglenoid process *(suture near it).*
15. Glenoid rim	From deepest lateral glenoid anteriorly around glenoid fossa and back (Harvati).
16. Inf. Zygomatic bridge posterior	From deepest lateral glenoid to zygotemp suture inferior (Harvati). From zygotemp suture inf. to the alveolar margin f the zygomatic root (Harvati).
17. Inf. Zygomatic bridge anterior	From auriculare to Zygomaticotemp. suture superior (Harvati).
18. Sup. Zygomatic bridge posterior	From Zygomatico-temp. suture superior to frontozygomatic suture (Harvati).
19. Sup. Zygomatic bridge anterior	From zygorbitale to inferior most point on zygomaxillare suture.
20. Zygomatico facial suture	Outline of superior nuchal line (starting close to asterion) – to be taken bilaterally.
21. Superior nuchal lines	Asterion to lambda, following the suture in a straight line – to be taken bilaterally.
22. Lambdoid suture	From bregma to left anterior pterion – to be taken bilaterally (Harvati).

23. Coronal suture	From Frontomalare Posterior right to Frontomalare posterior left (Harvati).
24. Supraorbital torus	Outline the condyles from anterior apex point along lateral margin to posterior apex point along medial margin to anterior point.
25. Occipital condyles	From opisthion to basion (right to left), along rim.
26. Foramen magnum	
Mandibular landmarks	
1. Gonion	The outer point on either side of the lower jaw at which the jawbone angles upward.
2. Posterior ramus	Mid-point on posterior border of the ramus
3. Condyle	Mid-point on condyle
4. Condyle medial	Middle on medial side of condyle
5. Condyle lateral	Middle on lateral side of condyle
6. Condyle posterior	Middle point on the posterior side of the condyle
7. Root of sigmoid process	Point on root of sigmoid process
8. Sigmoid notch	Deepest point on the sigmoid notch
9. Coronoid	Highest anterior point on coronoid
10. Superior anterior ramus	Superior most point on the anterior ramus
11. Inferior anterior ramus	Inferior most point at the intersection of the ramus and corpus
12. Mandibular foramen anterior	Anterior most point on mandibular foramen
13. Mandibular foramen posterior	Posterior most point on the mandibular foramen
14. M_3	Mid-point on the posterior margin of M_3
15. M_1 lingual side (middle)	Mid-point on the lingual margin of M_1
16. M_1 buccal side (middle)	Mid-point on buccal side of M_1
17. Pre-molar alveolar septum	Point on alveolar margin of pre-molar-M_1 alveolar septum
18. Canine-P_3 alveolar septum	Point on alveolar margin of canine-P_3
19. I_2-Canine alveolar septum	Point on I_2-Canine alveolar septum
20. Mental foramen	Posterior margin of mental foramen
21. Mental foramen alveolar border (above)	Point on alveolar margin above mental foramen
22. Mental inferior border (below)	Point on alveolar margin below mental foramen
23. Incision	Between the incisors
24. Gnathion	The most inferior point of the mandible in the midline
25. Infradentale	The apex of the septum between the mandibular central incisors

26. Mandibular orale 27. Superior mental spine 28. Inferior mental spine	Point between and behind the first two incisors
Curves	
Condyle to coronoid	Posterior border of medial foramen Inferior point on inferior spine
Right posterior condyle-gonion	Root of sigmoid root along the sigmoid notch from condyle or coronoid
Coronoid-ramus root	Posterior condyle point until gonion From coronoid to superior point on anterior ramus, where the ascendening ramus meets the body.
Condyle outline	Medial-lateral post –lateral medial anterior
Symphysis	Gnathion to infradentale

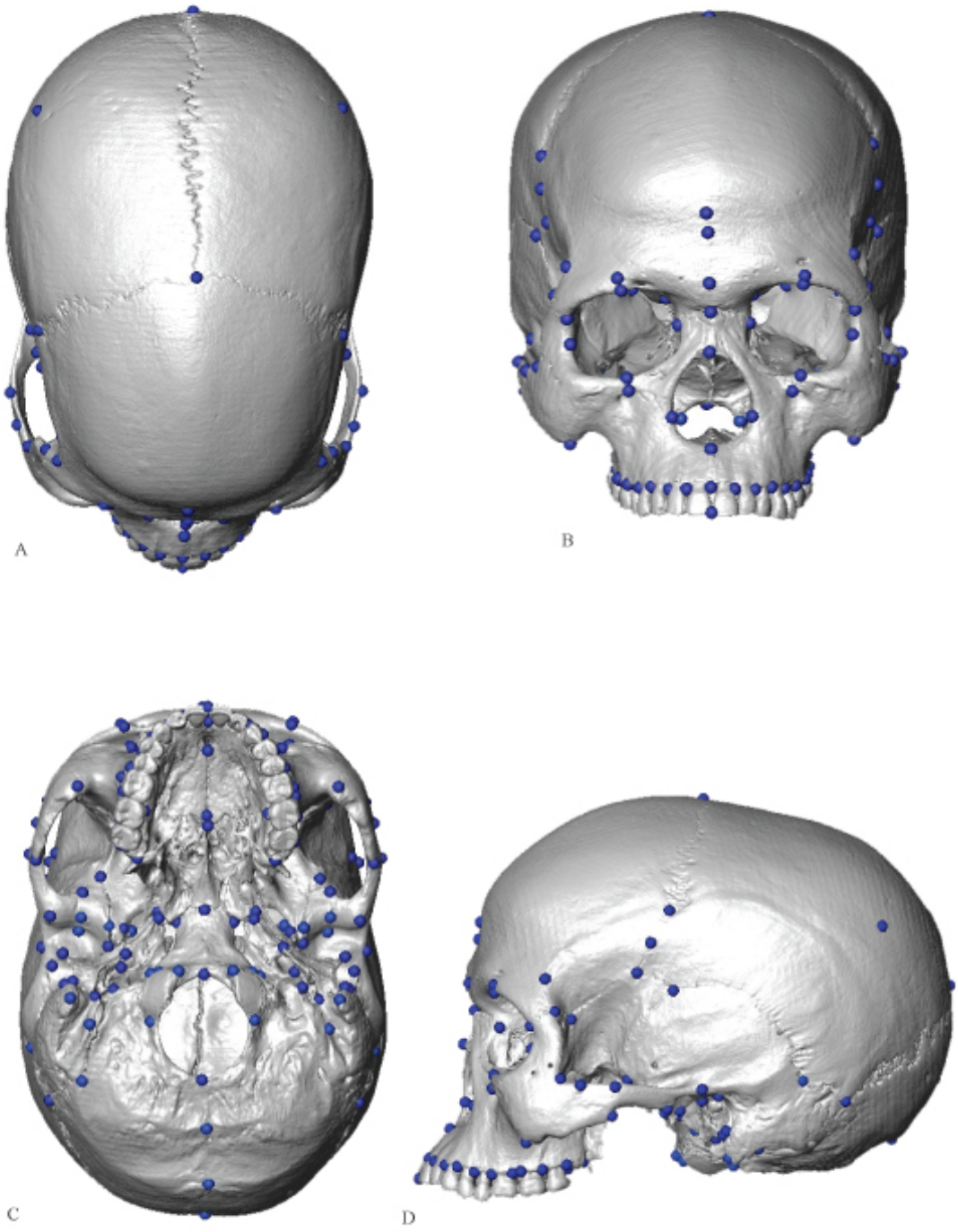

Figure 1: Cranial landmarks corresponding to Table 3

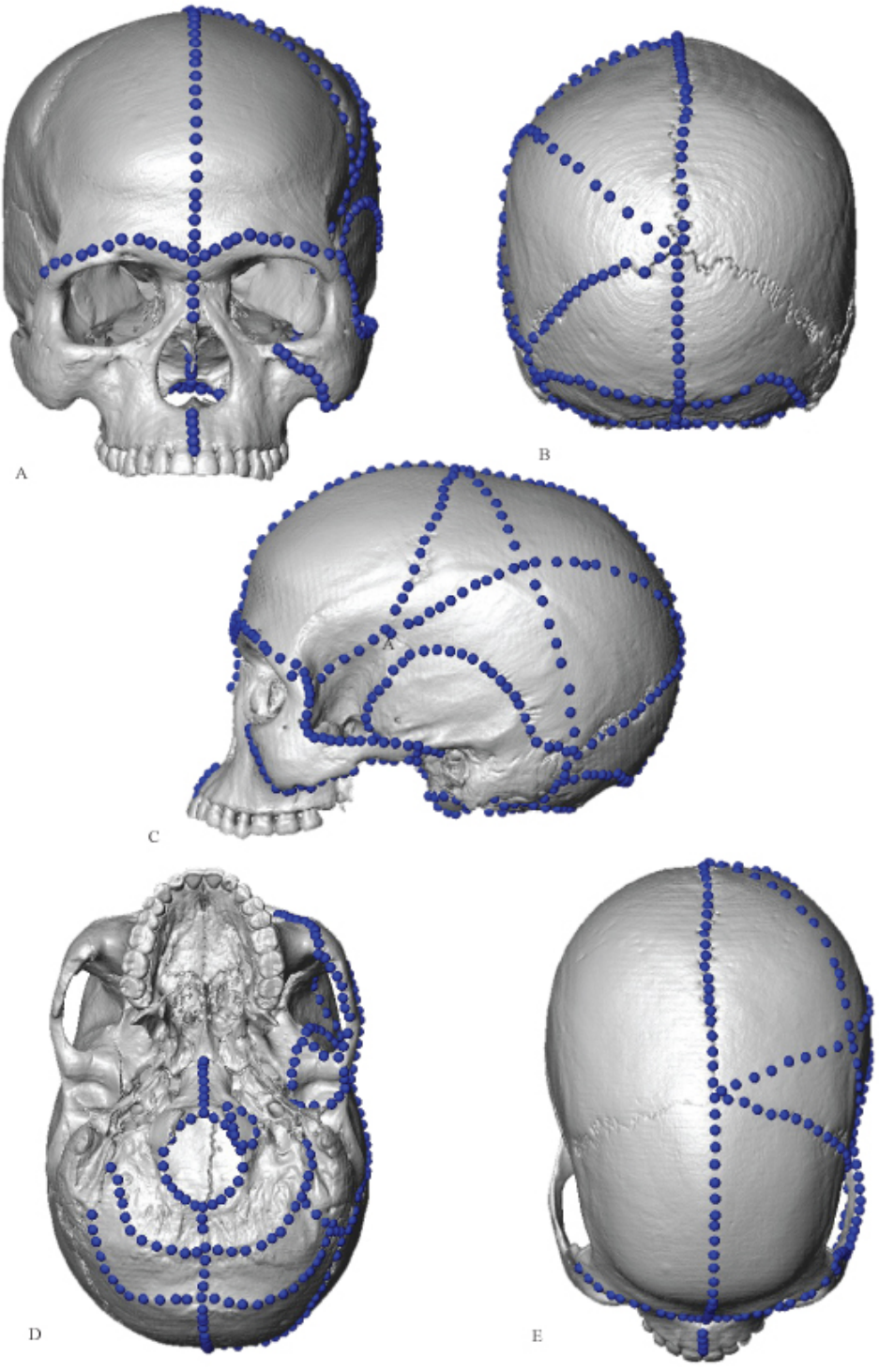

Figure 2: Cranial curves corresponding to Table 3

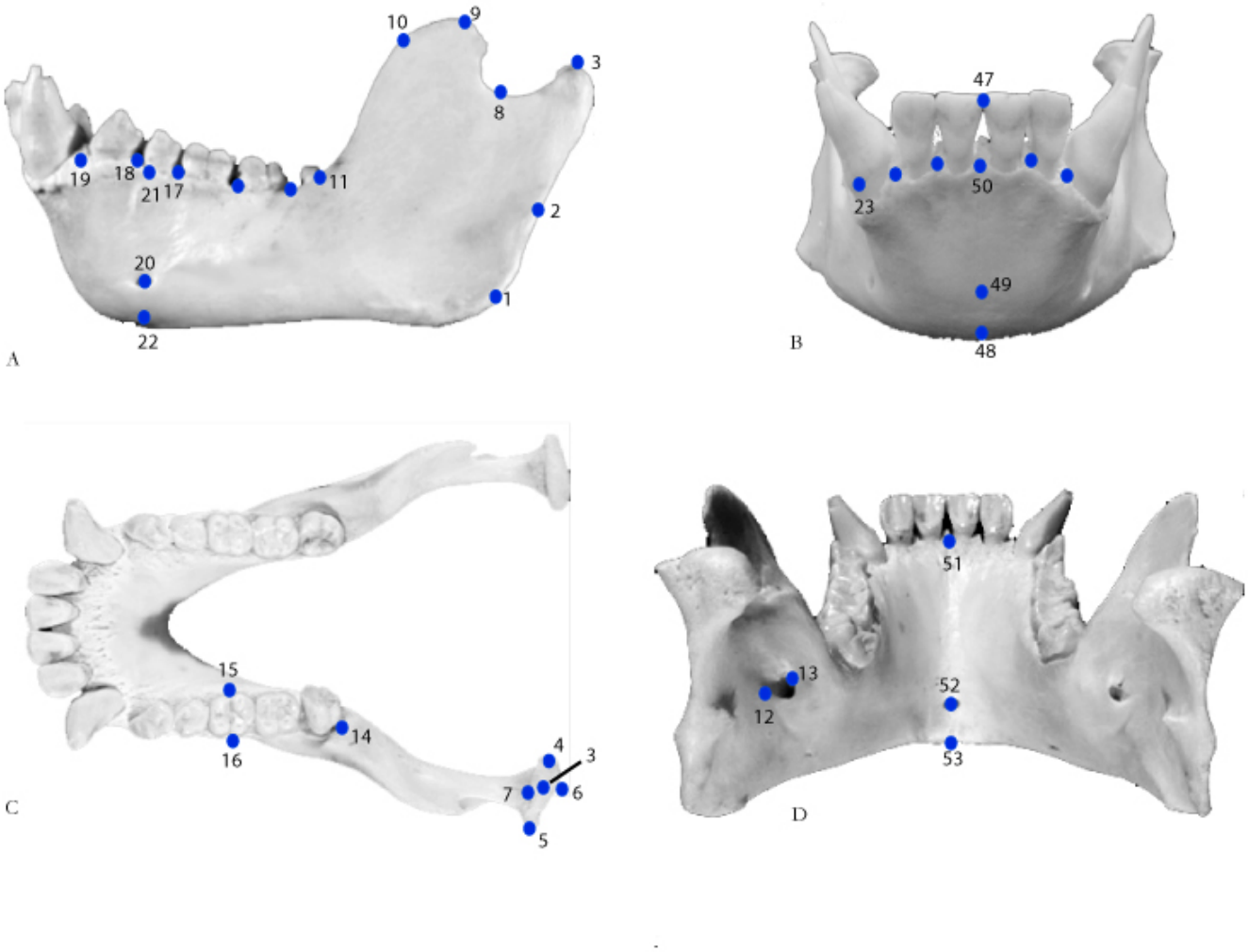

Figure 3: Mandibular landmarks corresponding to Table 3

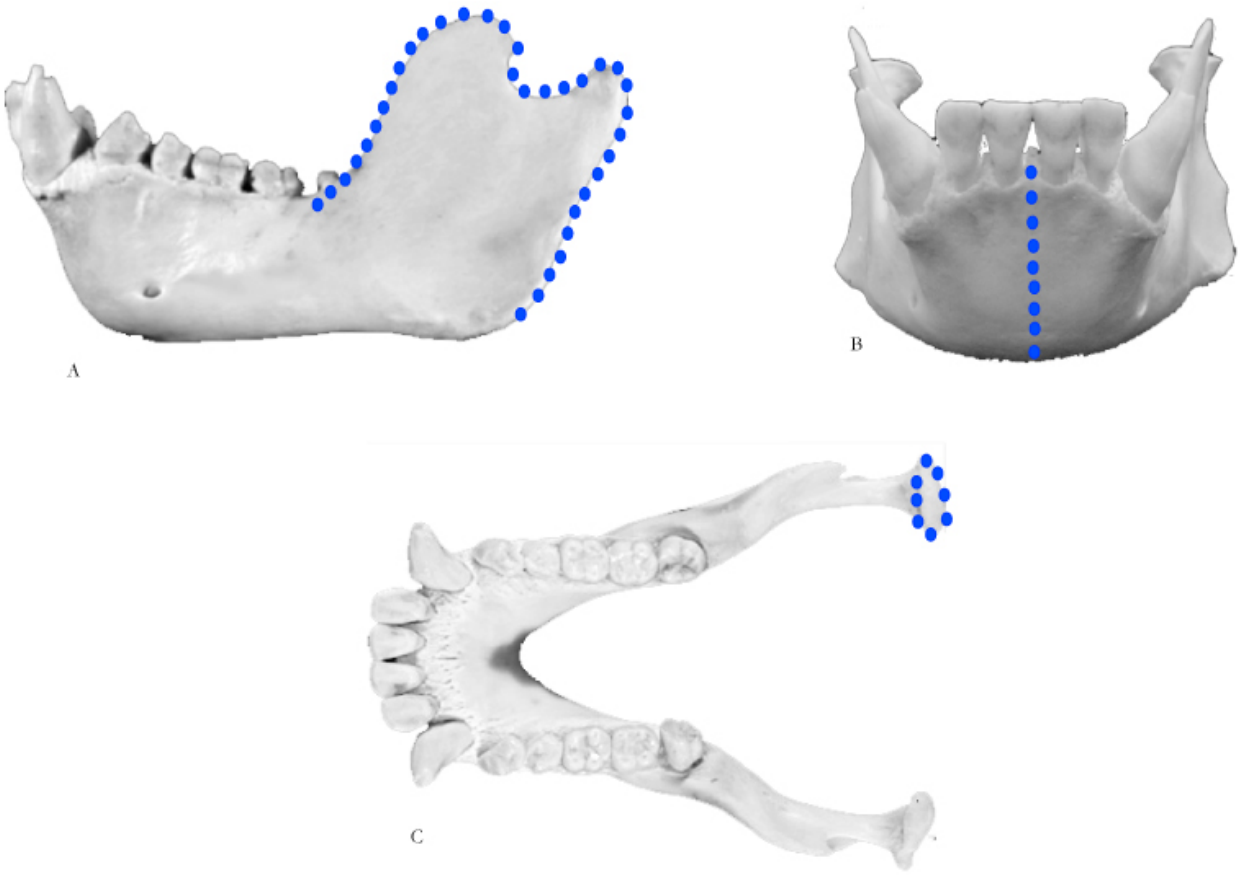

Figure 4: Mandibular curves corresponding to Table 3

Chapter 3: Results

Results

Manuscript 1: Cranial integration in Homo, Pan, Gorilla *and* Pongo

(In review in *Journal of Human Evolution*)

Abstract

Morphological integration refers to coordinated variation among traits that are closely related in development and/or function. Patterns of integration can offer important insight into the structural relationship between phenotypic units, providing a framework to address questions on morphological variability and variation. The latter is particularly important in biological anthropology because majority of the questions addressed in human evolution centre around morphological variation. Integrative features of the primate cranium have recently become a popular subject of study. However, an important question about the extent to which patterns of morphological integration differs among *Homo, Pan, Gorilla* and *Pongo* still remains unanswered. To address this question, we conducted a Procrustes based geometric morphometrics study to quantify and analyse species-specific shape covariation patterns across extant hominids. We did this by collecting fifty-four 3-D landmarks on the entire cranium of 407 adult individuals. We then subdivided the cranial landmarks corresponding to functionally related regions as outlined in the "functional matrix hypothesis". Sub-dividing the cranium in this manner allowed us to test hypotheses based on functional integration. Our results suggest that morphological integration patterns in the hominid cranium are complex, depicting both inter-specific similarities and differences, but the overall trend leans more towards a shared common pattern. The main implication of these results is that patterns of integration among these hominids are highly conserved, despite showing certain species-specific trends.

This is an important finding because it further implies that the general direction of evolutionary shape change is similar in extant and possibly extinct hominids.

Introduction

Phenotypic integration can impact the direction and rate of evolutionary change by either facilitating adaptation or acting as a constraint on morphological evolution. Since the seminal work of Olson and Miller the concept of morphological integration has been widely applied in studies of phenotypic evolution (Cheverud 1982; 1988; Zelditch and Carmichael 1989; Pigliucci et al. 1991; Couly et al. 1993; Cheverud 1995; Ackermann and Cheverud 2000; Daegling and Jungers 2000; Klingenberg 2000; Marroig and Cheverud 2001; Strait 2001; Ackermann 2002; Hallgrímsson et al. 2002; Klingenberg 2003a; Ackermann and Cheverud 2004; Ackermann 2005; Young and Hallgrímsson 2005; Goswami 2006; Polanski and Franciscus 2006; Gunz and Harvati 2007; Ackermann 2009; de Oliveira et al. 2009; Martinez-Abadias et al. 2009). Morphological integration refers to coordinated variation among parts of an organism that are functionally and/or developmentally related (Olson and Miller 1958). This concept is similar to one of "correlation Pleiades" furthered by Berg (Terentjev 1931; Berg 1960). Correlation Pleiades addresses the degree of correlation between quantitative traits, similar to examining the magnitude of integration among different parts of an organism.

The concept of morphological integration has been widely researched in evolutionary and developmental biology, but James Cheverud (1982; 1988, 1995) was the first to actively apply it in studies on primate evolution, mainly the primate cranium. The general consensus among most studies on cranial integration in primates is that developmentally and/or functionally related traits are highly correlated and tend to co-vary in a similar pattern across most primates – supporting the initial hypothesis posited by Olson and Miller (Cheverud 1989; 1995{Ackermann, 2005; Ackermann and Cheverud 2000; Ackermann and Krovitz 2002; Ackermann and Cheverud 2004). However, some studies on the facial skeleton and neurocranium show some ape species to exhibit differing patterns of integration between functionally and/or developmentally related features (Ackermann and Krovitz 2002). Integrative aspects of the basicranium have also been an important subject of investigation, particularly among modern humans.

Lieberman *et al.* (2000a, b) emphasised the role of the basicranium in generating overall integration in the primate cranium, but also suggested that the cranial base and face are semi-independent of each other.

While a number of these studies have explored different aspects of cranial integration in primates, majority of them focused on the common patterns of integration across species. Moreover, the question - what is the pattern of cranial integration in primates and is that pattern constant across all taxa needs to be further addressed. Here, we depart from previous research by investigating species-specific patterns of integration in extant hominids: *Pongo, Gorilla, Pan* and *Homo.* The main aim of this study is to quantify and analysis integration patterns between different cranial regions. Integration is reflected as the covariation within and between structures. The purpose of examining patterns of integration is to evaluate whether they have a tendency to evolve or are conserved across extant hominids. However, we are primarily concerned with the evolution of cranial shape integration patterns rather than the relative contribution of biological factors that generate trait co-variation. In doing so, our study will reassess previous findings on cranial integration patterns in living hominids. Our objectives are as follows: 1) to use hypotheses based on functional considerations to provide a comparative framework for our study. The context of function here is that of the "functional matrix hypothesis" (Moss 1962; 1968; 1969; 1997(a)); 2) to examine *taxa-specific* patterns of cranial shape covariation among all extant hominids; 3) finally, to address whether covariation patterns evolve and whether some aspects of such patterns are more conserved than others.

Cranial modules

Regions of the mammalian cranium differ in their developmental origin and functional demands. Accordingly, we sub-divided the cranium into three functional components: (a) facial skeleton, including the zygomatic processes, nasal, lacrimal and maxillary bones (b) cranial vault, consisting of the frontal and parietal bones and (c) basicranium, comprising the non-squamous parts of the temporal and occipital bones. We choose to call these modules "functionally" derived because

they are loosely based on Moss' functional matrix hypothesis (Moss and Young 1960; Moss 1968; 1997(a); 1997(b); 1997(c)); however, they are primarily distinguished based on differential growth patterns.

The facial skeleton follows a somatic growth pattern, whereas the neurocranium follows a neural growth pattern (Moore and Lvelle 1974); typically, the face grows well into adulthood making it more susceptible to environmental stimuli (Collard and Wood 2001; Bastir and Rosas 2004a). Consequently, differential growth patterns and muscular/functional demands make the face semi-independent of the neurocranium. Neurocranial components can be largely divided into the basicranium and vault. Growth in the cranial vault is through intramembranous ossification and the basicranium grows from endochondral ossification. The basicranium is additionally influenced by somatic growth factors largely attributed to hormones affecting the corresponding cartilage growth (Bogin 1988; Hall 2005).

Materials and methods

The total sample comprises dried crania of 407 adult individuals of *Pan, Gorilla, Homo* and *Pongo* (Table 1); the sex ratio was nearly equal in all groups. Only specimens with fully erupted dentition and fully fused sphenooccipital synchondrosis were considered as adults and included in the study. Fifty-four 3D landmarks were measured on the entire cranium (Figure 1). Landmarks were chosen based on anatomical correspondence and repeatability across the taxa. For individuals with prominent mid-sagittal and nuchal crests, landmarks such as bregma and lambda were taken on top of the crests and two additional points were taken on the neurocranium on either side of the landmarks; the latter two points and landmarks taken on top of the crests were later averaged and the averaged points were included in the analysis. All individuals with missing and/or mislabelled landmarks were excluded from the analysis. An assessment of intra-observer error is provided in Table 2.

Measurements were taken by a G2X MicroScribe (Immersion Corporation, San Jose, CA), which has a measurement accuracy of 0.23mm. Digitizing was done in

two orientations, dorsal and ventral, in order to register all the landmarks as efficiently as possible. While measuring, the specimens were mounted on modelling clay to keep them stationary. In order to match the digitised landmarks from the two separate sessions, we took four "orientation points" in the two views. To ensure matching the two sets of landmarks accurately, these points were taken in exactly the same place each time. DVLR (dorsal-ventral-left-right fitting) program was used to combine the two sets of landmarks to make one individual. More information about this software can be found on the "NYCEP morphometrics group" homepage.

Geometric morphometrics

We used Procrustes based geometric morphometric methods to analyse our data. Landmark configurations of each specimen were superimposed using the generalized Procrustes superimposition (GPA) method (Rohlf and Slice 1990; Bookstein 1996; Rohlf 1999). GPA is used as a standard procedure in most recent geometric morphometric studies on shape analysis. The procedure involves extracting shape coordinates by translating, scaling and rotating the landmark configurations; hence, removing all information unrelated to shape. A size measure, commonly known as centroid size, and related to the dispersion of landmarks around their baricenter, is obtained for each specimen (Dryden and Mardia 1998).

In the present study, as part of the Procrustes superimposition, object symmetry was calculated by reflecting and relabeling each set of paired landmarks (Klingenberg and McIntyre 1998; Klingenberg et al. 2001). Object symmetry is a form of bilateral symmetry in which a structure is inherently symmetrical (Klingenberg et al. 2002). A biological form such as the mammalian cranium is a good example of object symmetry. The object symmetry analysis was conducted in *MorphoJ* (Klingenberg 2008a), which as part of the Procrustes fit yields separate components for symmetry and asymmetry. Subsequent multivariate statistical analyses were done on the symmetric shape components, which are calculated through reflected relabeling of the paired landmarks.

Measurement error: Repeat measurements were taken for 200 specimens, out of which 48 were re-digitized two years apart - at the beginning and end of data collection - to further assess measurement accuracy. Measurement error was quantified using the Procrustes ANOVA method outlined in Klingenberg and McIntyre (1998). This method is analogous to the two-factor ANOVA model developed by Palmer and Strobeck (1986; Palmer 1994), which estimates measurement error relative to the signal of asymmetry in biological datasets. The Procrustes ANOVA involves a four-step procedure: quantification of among individual shape variation in the dataset; calculating the effects of directional asymmetry; accounting for fluctuating asymmetry, which is done by calculating each side x specimen interaction; and finally, quantifying variability among replicates, which is the residual and a value for measurement error in the dataset. Procrustes ANOVA yields large degrees of freedom, more so than in a regular ANOVA. For the purposes of this study, the Procrustes ANOVA was mainly employed to look at measurement error and was performed separately for each taxon in the sample.

Analysis of covariation: We use the 2-block partial-least squares (2B-PLS) approach to examine phenotypic integration between developmentally and functionally defined modules. The partial least squares approach has been extensively used in social sciences (Bookstein 1990) and is only recently being applied to anthropological datasets, to name a few(Bookstein et al. 2003; Gunz and Harvati 2007; Mitteroecker and Bookstein 2007; Bastir et al. 2008; Mitteroecker and Bookstein 2008). Two-block partial least squares analysis emerges from a group of statistical techniques that is based on a singular value decomposition of the between-block covariance matrix (Mardia et al. 1979; Rohlf and Corti 2000; Mitteroecker and Bookstein 2007). Accordingly, it finds pairs of axes - one axis per block per dimension - which account for the maximum amount of covariation between the two sets of variables examined. Moreover, the axis in one block is only correlated to the corresponding axis in the other block, making it easy to examine covariance patterns one pair of PLS axes at a time (Rohlf and Corti 2000; Bookstein et al. 2003; Klingenberg 2003b; Mitteroecker and Bookstein 2007).

In our study, we first sub-divided the landmarks into the respective blocks as outlined in the "functional matrix hypothesis" and performed separate Procrustes superimpositions for each block (face, basicranium and cranial vault) of landmarks. We then sub-divided the taxon Procrustes shape coordinates of the three blocks; no new Procruestes fits were performed at this stage. Subsequently, to investigate taxa-specific patterns of integration between the blocks, we performed separate pooled within-group 2B-PLS analyses for each species: the groups here being sexes, populations and sub-species within each taxon. Pooled within-group analysis subtracts the mean differences between sexes, populations and sub-species within each taxon. Because investigating sexual dimorphism is not one of the objectives of this study we chose to pool the sexes to reduce for differences between males and females, particularly in gorillas and orangutans.

To further test whether the direction of integrated shape change, i.e. integration patterns, is similar across these hominids, we computed and tested the angles between the PLS vectors for each taxa for each pair-wise comparison of blocks (eg. face vs. basicranium) via a bootstrap test (Efron and Tibshirani 1994). The null hypothesis in the bootstrap test was that the PLS vectors have the same directions in the respective species' shape spaces. The resampling is done from the PLS scores, species by species separately. This allows for all aspects in the test to remain species-specific, particularly the singular values.

Shape changes were visualised on surfaces generated in AMIRA (Mercury Computer Systems Inc.). This was done by warping the PLS vectors of the respective species onto the grand mean shape of the face, basicranium and cranial vault. The original surface used was that of a modern human cranium. Rather than using the species mean shapes, we used the grand mean shape to avoid the influence of mean differences between species when interpreting shape co-variation patterns. Using a common mean does not show the extreme changes in the taxa, but it eliminates the problem of mean differences between species. Only the landmarks listed in Figure 1 were used. All analyses were conducted in *MorphoJ* (Klingenberg 2008a) and programming software R version 2.6.2 (R development core team 2008).

Results

Measurement error: We calculated measurement error for each group separately using the Procrustes ANOVA method. Results indicate that measurement error was negligible in all groups (Table 2).

Functional components

Here, we focus on the first two dimensions of shape change because over 60% of the total covariance in the dataset (of all respective taxa) is explained by these vectors.

Associated shape changes in the face and basicranium: PLS 1 shows similarity between chimpanzees and bonobos in the pattern of associated shape change. Covarying features include narrowing of the face associated with a prognathic premaxilla and broadening associated with an orthognathic premaxilla (Figure 2). Changes in the basicranium are more pronounced in bonobos, but both taxa show slight broadening of the cranial base with a narrowing of the face. Results of the bootstrap test (supplementary section: Table 4) show that angles computed between chimpanzee and bonobo PLS vectors are not significantly different ($p > 0.1$). However, both chimpanzees and bonobos are significantly different ($p < 0.01$) from gorillas along PLS 1 and 2, respectively. Gorillas are distinct in their pattern of shape change in the basicranium, which is mainly seen in the widening of the posterior cranial base associated with minimal shape changes in the face. However, gorillas are only significantly different ($p < 0.03$) from orangutans along PLS 1. Human have a uniform pattern of covariation, where a relatively elongated basicranium covaries with a relatively elongated face and slightly progranthic maxillary region, and a relatively wide basicranium with a wide and relatively orthognathic face. However, humans are only significantly different ($p < 0.05$) from bonobos and orangutans along PLS 1. Orangutans show a relatively elongated and narrow basicranium covarying with a wide face and retracted maxillary region, and a relatively wide and short basicranium covarying with an elongated face and prognathic maxillary region. Orangutans are significantly different ($p < 0.04$) from

humans and gorillas along PLS 1. Only bonobos were significantly different ($p = 0.01$) from gorillas along PLS 2. Overall, while the angles between the species-PLS vectors are not 0° or parallel and some do suggest significant differences between species, majority of the pair-wise comparisons do not show strong support for species-specific direction of integrated shape change. Therefore, the null hypothesis of parallel trajectories cannot be fully rejected in the face vs. the basicranium analysis.

Basicranium vs. cranial vault: PLS I captures subtle changes in covariation patterns among the taxa. Bonobos show a short and slightly broad basicranium covarying with a robust and narrow cranial vault and a narrow basicranium covarying with a globular and posteriorly wide cranial vault (Figure 3). Bonobos are only significantly different ($p < 0.01$) from orangutans along PLS 2 (supplementary section: Table 4). Chimpanzees show a short and broad cranial base covarying with a markedly globular cranial vault and a slightly narrow base with a robust cranial vault. Chimpanzees are not significantly different ($p > 0.05$) from any taxa along PLS 1 and 2. Gorillas show similar associated shape changes to chimpanzees with a broad cranial base covarying with a posteriorly broad and robust cranial vault and narrow cranial base with an overall narrow cranial vault. Gorillas are also not significantly different ($p > 0.05$) from any other taxa along PLS 1 and 2. Humans show little change in the overall shape of the cranial vault, but shape changes along PLS 1 shows a relatively wide basicranium associated with a posteriorly wide cranial vault and a slightly narrow basicranium associated with a narrow and robust cranial vault. However, humans are only significantly different ($p = 0.03$) from chimpanzees along PLS 2. Orangutans show the most marked shape changes, with a relatively short cranial base covaring with a posteriorly broad and robust cranial vault and a narrow and elongated cranial base covarying with an overall narrow cranial vault. Orangutans are most similar to chimpanzees in their pattern of integration between the base and vault. Bootstrap results show that orangutans are significantly different ($p < 0.01$) from bonobos, gorillas and humans along PLS 1. Overall, results show that the basicranium vs. cranial vault analysis are similar to

that of the face vs. bascranium results in that majority of the pair-wise comparisons between species PLS vectors are not significantly different from each other. Given that majority of the pairwise comparisons between PLS vectors is not significant, the null hypothesis of sameness of PLS vectors cannot be fully rejected.

Face vs. cranial vault: All taxa show similarities along PLS 1 in their associated shape changes in the cranial vault and face (Figure 4). The general pattern of integration suggests that a narrow face covaries with a relatively narrow face and wide face with a relatively wide cranial vault. This integrated pattern of shape change is most marked among humans; the other apes tend to show a robust and slightly posteriorly expanded cranial vault covarying with a relatively narrow face and a more globular cranial vault covarying with a broad face. With the exception of bonpbos being significantly different ($p < 0.01$) orangutans along PLS 2, the other taxa angles computed between species PLS vectors are small with non-significant results along both PLS 1 and 2 (supplementary section: Table 4).

Discussion

Changes in developmental and/or functional processes such as single mutations, developmental buffering mechanisms and brain growth can influence morphological integration or covariation within and among morphological traits/units (Hallgrimsson et al. 2007a; Hallgrimsson et al. 2007b). Given the different genetic, developmental and environmental factors that influence human and nonhuman ape biology, it seems unlikely that extant hominids share the same pattern of covariation in the cranium. We tested this assumption in a genera wide extant hominid sample and found that despite showing certain species-specific differences, cranial covariation patterns on the whole are similar among these hominids. This suggests that cranial integration patterns are in fact conserved among humans, bonobos, chimpanzees, gorillas and oranugtans. While similar conclusions were previously drawn regarding cranial integration in African apes and modern humans, no previous studies had been conducted on large samples of all great apes and modern humans.

Cranial integration

An important difference between the present study and previous research on cranial integration in extant hominids is that here we focus on species-specific integration patterns among three major functional regions of the cranium. The functional regions are defined based on Melvin Moss' "functional matrix hypothesis" (Moss and Young 1960; Moss 1962). The "functional matrix hypothesis" posits that these three regions have differential growth patterns depending on the respective interaction between the bone, surrounding soft tissue and organ growth, particularly the brain. Integrated shape changes suggest that taxa are most similar in their pattern of integration between the face and cranial vault and most divergent in the face and basicranium. In the face and cranial vault comparison, the main similarities in integrated shape space are related to an elongated face covarying with a robust cranial vault, and a globular cranial vault covarying with a wide face. However, certain features, for example cranial vault cresting and expansion of the posterior vault are more pronounced in great apes than in modern humans. The angles computed between the species PLS-vectors range from 45˚ - 75˚ and are not significantly different from each other along PLS 1. The only significant difference found between taxa was between bonobos and gorillas along PLS 2. The relatively large angles are expected in such high-dimensional spaces and to show any significant differences the angles would have to be close to 90˚ and/or larger. The apparent similarities in shape change particularly between the African and Asian apes suggest that certain interactions between cranial regions are conserved across these taxa.

The face and basicranium, and cranial vault and basicranium distinguish taxa better than the cranial vault and face. The basicranium has been found to have the strongest phylogenetic signal among the different regions of the skull (Lieberman et al. 2000; Lieberman et al. 2000a; Lieberman et al. 2004). The basicranium is also regarded as key in integrating the cranium as a whole. The differences found in integrated shape features between taxa could be an indication of phylogenetic

divergence in those features among these taxa (Lieberman et al. 2002; Lieberman et al. 2004; Cardini and Elton 2008). However, the latter does not imply that these features can be used to reconstruct relationships among hominids in phylogenetic analyses. The significant differences found between taxa are not consistent with the phylogenetic relationships derived from molecular data. However, results from both the 2B-PLS analysis and bootstrap test do show oranugtans to be the most significantly different from the other taxa, especially humans, in their integrated shape changes.

Morphological differences between the Asian apes and other taxa for the most part can be attributed to the structural relationship and positioning of the splanchnocranium on the neurocranium (Shea 1985). Even though we did not directly examine the relative positioning of the cranial structures, the overall shape and interaction of the blocks are influenced by their relative placement, affecting the way in which the two regions are integrated among the ape taxa. Structural and positional association between traits no doubt influences the evolution of integration patterns and warrants additional attention when examining association between different morphological elements.

Our findings also set humans apart from the other taxa in aspects of integrated shape space and this is corroborated by previous studies on cranial integration (Ackermann and Krovitz 2002; Lieberman et al. 2002; Bastir and Rosas 2004; Polanski and Franciscus 2006; Mitteroecker and Bookstein 2008). A study on 28 different modern human populations conducted by Gonzales-Josè *et al.* (2004) found humans to have a stable variance/covariance structure. Due to a limited modern human sample we were unable to conduct a thorough examination of intra-specific patterns of integration, but the shape covariation seen here corroborates previous findings on modern human covariance patterns. A narrow face co-varying with a narrow basicranium and cranial vault, in contrast to a short and broad face co-varying with similarly dimensioned basicranium and vault seems to be a more consistent finding in modern humans than in the other apes.

The differences seen among the taxa should not be overemphasised, particularly those of the bootstrap tests. As mentioned previously, low and high

significant values are difficult to interpret in this context because of the high dimensional space in which the angels between species PLS vectors are computed. Given that the angles have to be very high to count as significant, the reasonable conclusion drawn here from these results is that the null hypothesis of similar patterns of integration and direction of shape change cannot be fully rejected. Moreover, Ackermann (2002; 2005) pointed out that differences among species could be influenced by sample structure and size. In her own study she found that small and/or unevenly distributed samples, such as combining sub-species may potentially bias the results. In our study, only the chimpanzee sample had three different sub-species. We accounted for possible sub-species, population and sex differences by conducting pooled-within group analyses for each species. This subtracts possible mean differences that arise from sex, population and/or subspecies differences within populations, thus facilitating near equal comparisons between taxa.

Evolvability and constraint

Integration studies are key to understanding whether the underlying developmental and functional interactions are conserved or evolvable across a macroevolutionary time scale. One of the main objectives of examining patterns of cranial integration is to evaluate whether these patterns have a tendency to evolve or whether they are conserved across extant hominids. As stated above, our overall results suggest that despite showing species-specific differences, cranial integration patterns are conserved in these hominids and possibly all primates. This does not strictly imply that these patterns are constant and invariable, but that while there is a degree of variability that manifests as species-specific differences, it does not alter the overall covariance structure of these taxa. So the question that arises here is what maintains covariation patterns?

While we did not specifically investigate the relative contribution of biological mechanisms that influence covariance in the hominid cranium, some studies have proposed that covariance structures are maintained by a network of complex developmental processes. Any changes in the underlying developmental

interactions can alter the patterning of covariance within and between structures. According to the palimpsest model proposed by Hallgrimsson et al. (Hallgrímsson et al. 2002; Hallgrimsson et al. 2007a; Hallgrimsson et al. 2007b) covariance structures are representations of various "layers" of developmental and genetic interactions. These interactions influence different aspects of covariance structures differently, resulting in varying levels of integration among structures. "Tinkering" or variation in any of the underlying interactions changes the covariance structure. In light to Hallgrimsson's (2007a) model because our results suggest a conserved pattern of integration among extant hominids, it suggests that the covariance-generating processes and variation in those processes are similar across these taxa. That is, the variation or possible changes in the covariance structures are similar in magnitude.

Differences and similarities of integration patterns seen among human and non-human apes could be attributed to a combination of selection pressures and underlying genetic adaptations (Gagneux and Varki 2001). For example, the constancy of covariance patterns in humans, as shown by Gonzalez-Jose *et al*. (2004) could be a result of strong stabilizing selection acting to conserve common developmental and/or functional properties within human populations to the exclusion of nonhuman apes. Stabilizing selection reduces variability within a population, but favours the average or common traits in a population, conserving aspects of development and/or function across evolutionary time.

Conclusion

Four main conclusions can be drawn from this study. First, the interaction between the face and cranial vault captures maximum similarity in pattern of integration than pair-wise comparisons that include the basicranium. Second, orangutans are consistently significantly different from humans in pair-wise comparisons between the basicranium and face and basicranium and cranial vault. Third, covariation patterns among these taxa are by and large similar, suggesting that these patterns are conserved across extant hominids.

Acknowledgements

We thank the following curators for allowing us access to the various collections: Dr. Emmanuel Gilissen and Wim Wendelen of the Royal Museum of Central Africa, Tervuren; Diana Carvalho of Museu Nacional de História Natural, Lisbon; Ian Tattersall and Gisselle Garcia of the American Museum of Natural History, New York; Dr. Hendrik Turni and Dr. Frieder Mayer of the Naturkunde Museum, Berlin; Dr. Katrin Schaefer of the University of Vienna and Dr. Richard Kraft of the Zoologische Staatssammlung, Munich. We are grateful to Dr. Philipp Mitteroecker, Dr. Philipp Gunz and Dr. Fred Bookstein for extensive discussion and comments. This work was supported by the Marie-Curie "EVAN" training network (MRTN-CT-019564) and the Max Planck Society.

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Table 1: Extant hominids included in this study

Species	No. of individuals	Source
Pan t.troglodytes	42	Naturkundemuseum, Berlin
Pan t. verus	35	MPI-EVA, Leipzig & Peabody Museum, Boston
Pan t. schweinfurthii	45	Royal Museum of Central Africa, Tervuren
Pan pansicus	36	Royal Museum of Central Africa, Tervuren
Gorilla beringei	49	Royal Museum of Central Africa, Tervuren
Pongo pygmaeus	68	Zoologische Staatssammlung, Munich
Homo sapiens	132	
Luis Lopez	69	Natural History Museum, Lisbon
Point Hope	48	American Museum of Natural History, New York
Khoisan	15	University of Vienna, Vienna
Total	**407**	

Table 2: Measurement error tests: separate Procrustes ANOVA tests for the taxa included in this study. Individual effects represent overall variation in the dataset; Side is the measure for directional asymmetry; Individual * side is the measurement for fluctuating asymmetry; Error 1 is the measurement error calculated from the variation among repeat measurements. Sum of squares (SS) and mean squares (MS).

Gorillas: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.17612759	5.77846E-05	3048	8.95	<.0001
Side	0.00181909	1.58182E-05	115	2.45	<.0001
Ind * Side	0.01782854	6.4596E-06	2760	33.24	<.0001
Eror 1	0.0010818	1.944E-07	5566		

Bonobos: Shape, Procrustes ANOVA:

Effect(param.)	SS	MS	df	F	P
Individual	0.15301585	0.000022733	6731	7.46	<.0001
Side	0.00269028	2.33937E-05	115	7.68	<.0001
Ind * Side	0.01857773	0.000003048	6095	9.18	<.0001
Error 1	0.00016078	3.322E-07	484		

Humans: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.08007854	2.74148E-05	2921	5.92	<.0001
Side	0.00221287	1.92423E-05	115	4.16	<.0001
Ind * Side	0.01224441	4.6293E-06	2645	24.56	<.0001
Error 1	0.00018247	1.885E-07	968		

Orangutans: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.329559	6.19006E-05	5324	11.58	<.0001
Side	0.00381311	3.49826E-05	109	6.55	<.0001
Ind * Side	0.02562663	5.3433E-06	4796	9.48	<.0001
Error 1	0.00583472	5.637E-07	10350		

Chimpanzees: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.10109703	2.48762E-05	4064	7.62	<.0001
Side	0.00194026	1.68718E-05	115	5.17	<.0001
Ind * Side	0.0120169	3.2655E-06	3680	14.1	<.0001
Error 1	0.00005604	2.316E-07	242		

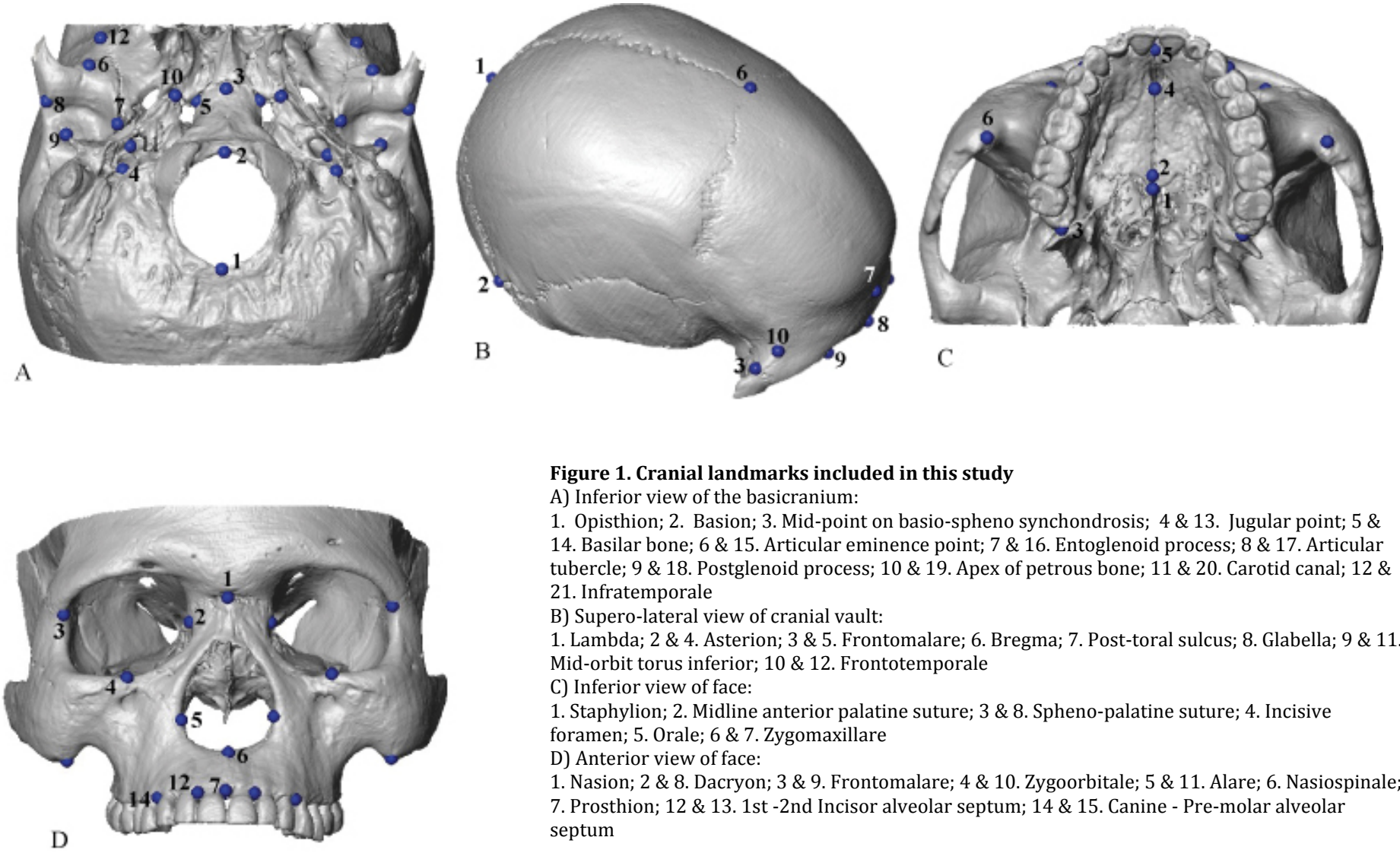

Figure 1. Cranial landmarks included in this study
A) Inferior view of the basicranium:
1. Opisthion; 2. Basion; 3. Mid-point on basio-spheno synchondrosis; 4 & 13. Jugular point; 5 & 14. Basilar bone; 6 & 15. Articular eminence point; 7 & 16. Entoglenoid process; 8 & 17. Articular tubercle; 9 & 18. Postglenoid process; 10 & 19. Apex of petrous bone; 11 & 20. Carotid canal; 12 & 21. Infratemporale
B) Supero-lateral view of cranial vault:
1. Lambda; 2 & 4. Asterion; 3 & 5. Frontomalare; 6. Bregma; 7. Post-toral sulcus; 8. Glabella; 9 & 11. Mid-orbit torus inferior; 10 & 12. Frontotemporale
C) Inferior view of face:
1. Staphylion; 2. Midline anterior palatine suture; 3 & 8. Spheno-palatine suture; 4. Incisive foramen; 5. Orale; 6 & 7. Zygomaxillare
D) Anterior view of face:
1. Nasion; 2 & 8. Dacryon; 3 & 9. Frontomalare; 4 & 10. Zygoorbitale; 5 & 11. Alare; 6. Nasiospinale; 7. Prosthion; 12 & 13. 1st -2nd Incisor alveolar septum; 14 & 15. Canine - Pre-molar alveolar septum

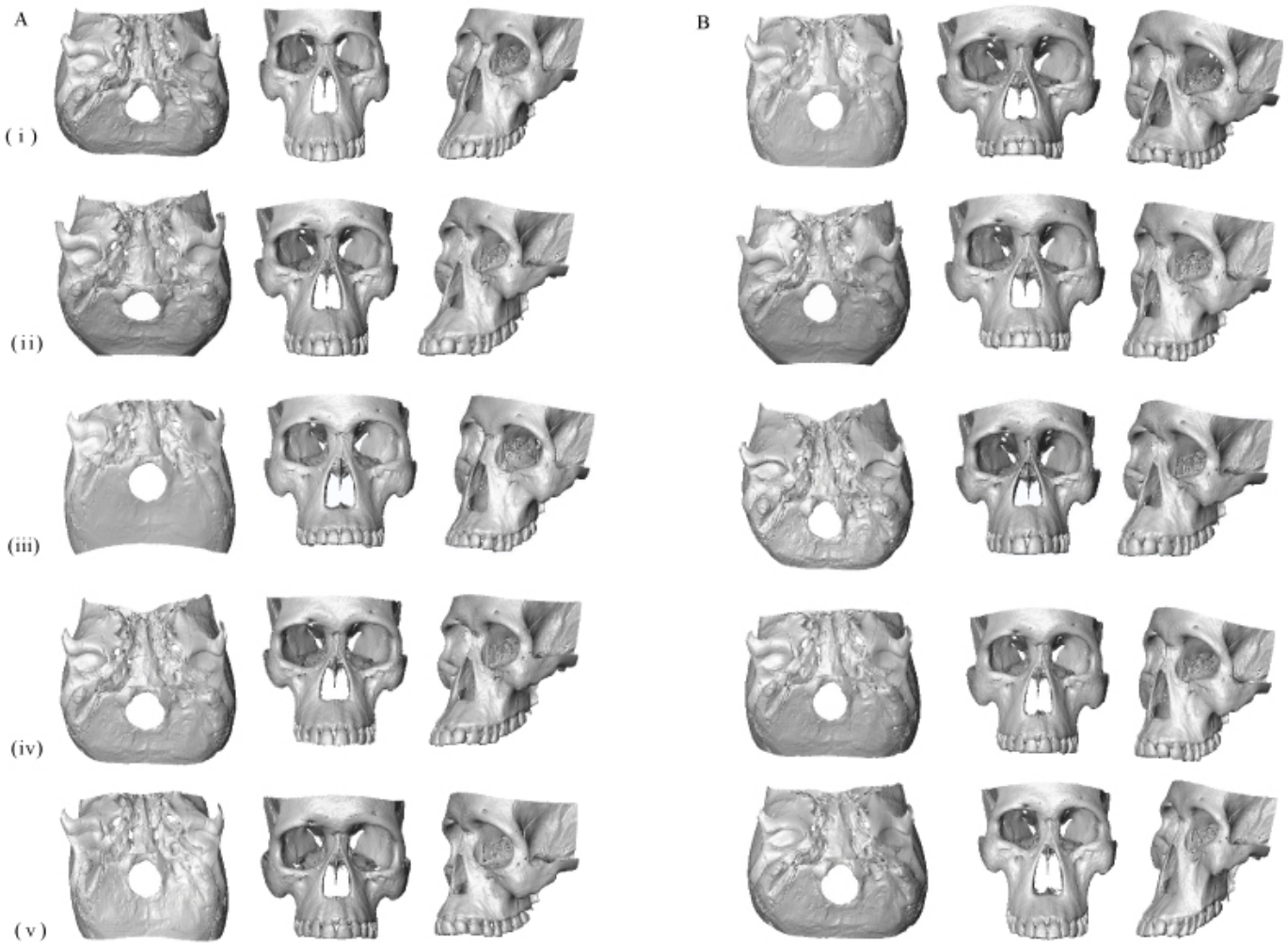

Figure 2: 2B-PLS analysis of the face vs. basicranium: A) Lower scores; B) Higher scores. Showing species-specific shape changes along PLS 1 of the anterior and lateral face and inferior basicranium. (i) Bonobos; (ii) Chimpanzees; (iii) Gorillas; (iv) Humans; (v) Oranugtans

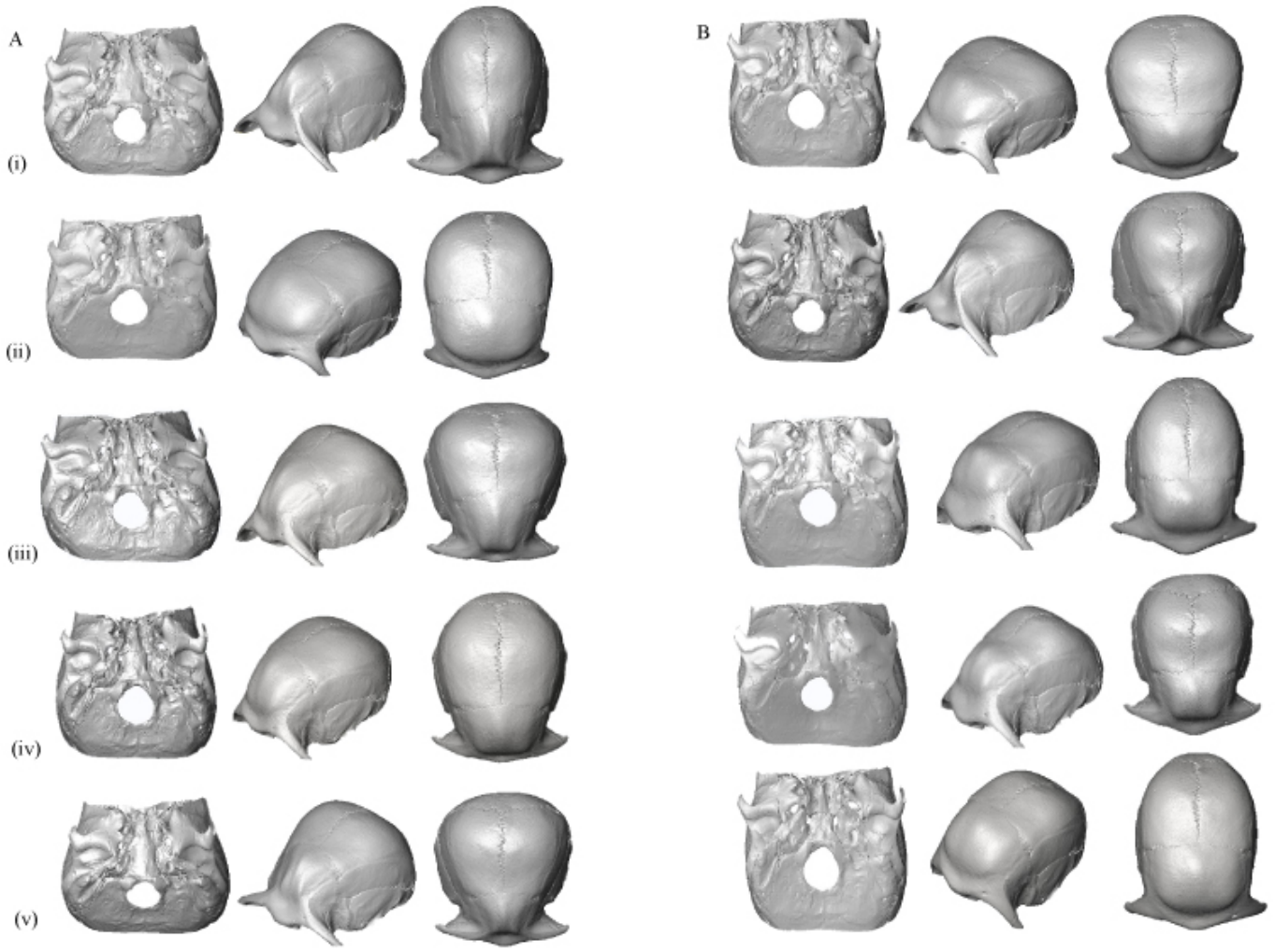

Figure 3: 2B-PLS analysis of the basicranium vs. cranial vault: A) Lower scores; B) Higher scores. Showing species-specific shape changes along PLS 1 of the anterior and lateral face and inferior basicranium. (i) Bonobos; (ii) Chimpanzees; (iii) Gorillas; (iv) Humans; (v) Oranugtans

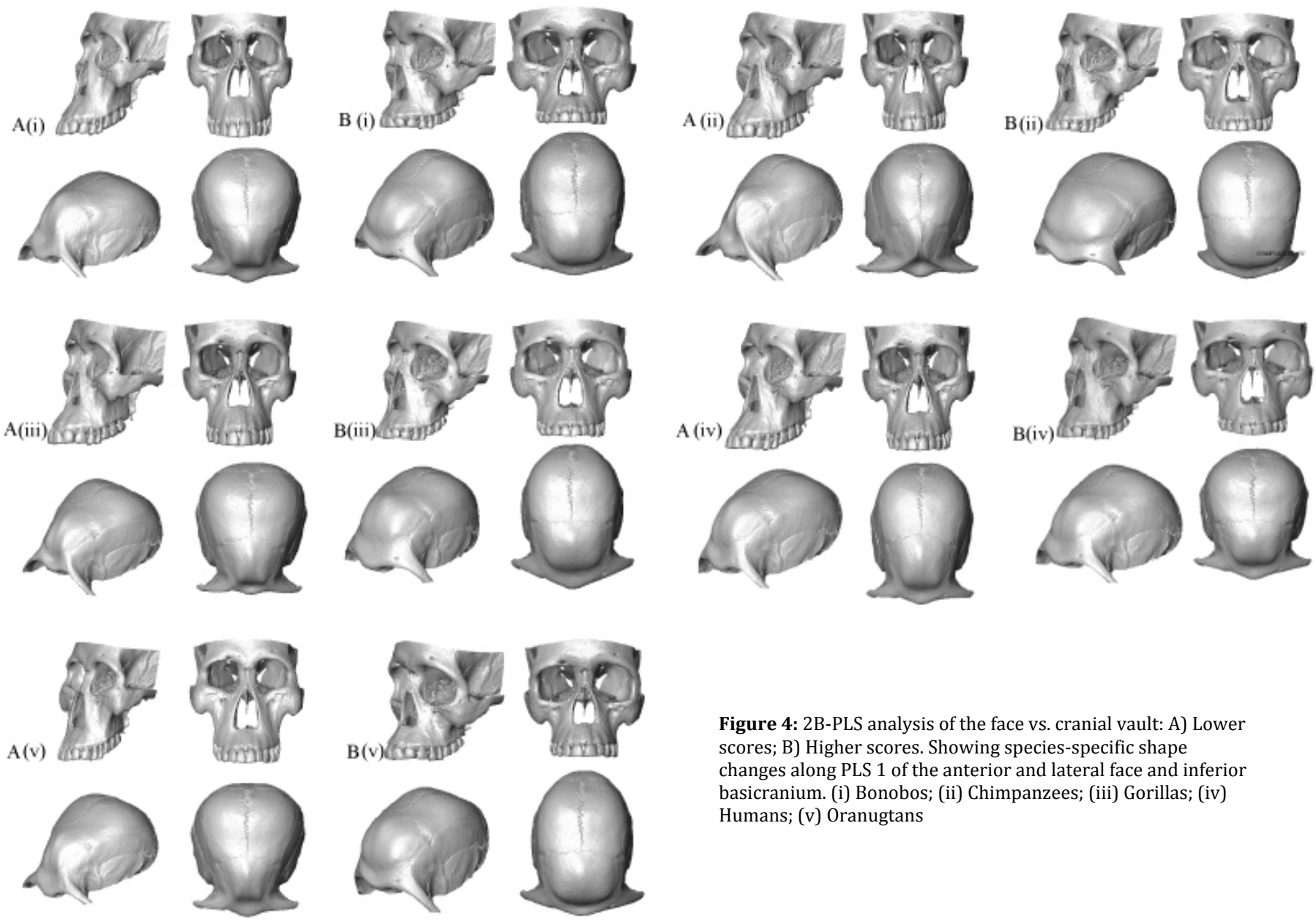

Figure 4: 2B-PLS analysis of the face vs. cranial vault: A) Lower scores; B) Higher scores. Showing species-specific shape changes along PLS 1 of the anterior and lateral face and inferior basicranium. (i) Bonobos; (ii) Chimpanzees; (iii) Gorillas; (iv) Humans; (v) Oranugtans

Appendix

Table 4: Angles computed between species PLS vectors for the face vs. basicranium, basicranium vs. cranial vault and face vs. cranial vault analyses, respectively. The p-values are generated from a bootstrap test of pair-wise comparisons between species angles.

basicranium vs. vault

		bonobo	chimp	gorilla	human
bonobo	pls1	0	79.279069	74.059683	83.162655
p-value		0	0.0643	0.0672	0.0672
bonobo	pls2	0	52.999185	81.933193	85.215627
p-value		0	0.7368	0.1844	0.1192
chimp	pls1	52.768696	0	58.215566	73.429154
p-value		0.1559	0	0.2061	0.1878
chimp	pls2	69.604485	0	79.754069	88.536337
p-value		0.458	0	0.237	0.0348
gorilla	pls1	43.921314	37.73362	0	62.141484
p-value		0.3207	0.4678	0	0.3077
gorilla	pls2	76.179666	86.631259	0	74.393268
p-value		0.3202	0.0838	0	0.3926
human	pls1	44.858619	65.331135	61.046202	0
p-value		0.6089	0.2406	0.3003	0
human	pls2	80.568199	65.92158	67.68763	0
p-value		0.2304	0.5585	0.5331	0
orang	pls1	47.115805	46.408867	41.578108	74.717921
p-value		0.1396	0.1439	0.2687	0.1004
orang	pls2	89.487749	71.427475	52.658834	82.958745

p-value		0.0096	0.3633	0.716	0.1488

face vs. basicranium

		bonobo	chimp	gorilla	human
bonobo	pls1	0	67.209759	69.145257	86.928279
p-value		0	0.3291	0.2605	0.0451
bonobo	pls2	0	85.999581	52.842455	66.944335
p-value		0	0.0906	0.7637	0.566
chimp	pls1	75.45766	0	82.690006	65.503008
p-value		0.2343	0	0.1027	0.3531
chimp	pls2	33.878037	0	80.437884	57.747107
p-value		0.9874	0	0.2056	0.7496
gorilla	pls1	83.242568	89.853969	0	59.1343
p-value		0.0857	0.0021	0	0.2908
gorilla	pls2	89.414499	86.086595	0	68.29637
p-value		0.0135	0.101	0	0.4964
human	pls1	77.779197	77.030323	47.496849	0
p-value		0.1816	0.1748	0.4694	0
human	pls2	83.958472	76.696935	81.90569	0
p-value		0.1616	0.3549	0.2141	0
orang	pls1	60.425788	84.720884	82.811843	83.2752
p-value		0.4059	0.0617	0.0243	0.0317
orang	pls2	75.066673	73.132766	66.586512	85.167821
p-value		0.3467	0.4103	0.5011	0.1209

face vs. vault

		bonobo	chimp	gorilla	human
bonobo	pls1	0	59.95338	73.28334	54.804863
p-value		0	0.1121	0.0814	0.175
bonobo	pls2	0	85.679494	85.430048	68.989248
p-value		0	0.0896	0.0668	0.4366
chimp	pls1	52.768696	0	43.428157	55.227702
p-value		0.1882	0	0.2864	0.0296
chimp	pls2	69.604485	0	79.39597	82.825987
p-value		0.3518	0	0.2122	0.1498
gorilla	pls1	43.921314	37.73362	0	53.382074
p-value		0.4288	0.3051	0	0.1466
gorilla	pls2	76.179666	86.631259	0	77.66473
p-value		0.2064	0.0639	0	0.2183
human	pls1	44.858619	65.331135	61.046202	0
p-value		0.3332	0.0061	0.0581	0
human	pls2	80.568199	65.92158	67.68763	0
p-value		0.1728	0.4475	0.4287	0
orang	pls1	47.115805	46.408867	41.578108	74.717921
p-value		0.2569	0.0153	0.2322	0.0017
orang	pls2	89.487749	71.427475	52.658834	82.958745
p-value		0.0098	0.3579	0.7115	0.1325

Manuscript 2: Evolution of covariance structures in the cranium of Homo, Pan, Gorilla *and* Pongo – *a developmental perspective*

In prep.

Abstract

Covariance structures are maintained by underlying developmental and genetic interactions. Changes in these interactions can alter the covariance within and between morphological regions and traits. Experimental studies on mice and fly wings have revealed that processes such as canalisation and developmental stability play an important role in maintaining covariance within and between structures, and consequently influence an organism's phenotypic variability. This study is the first of its kind to compare the influence of canalisation and developmental stability on cranial covariance structures in extant hominids. The origin and distinction between canalisation and developmental stability has been contested by several studies – the main contention being whether these processes are distinct or whether they have the same functional/genetic origin. Studies have shown support for both distinction and similarity between canalisation and developmental stability. However, those studies have only been conducted on rodents and *Drosophila*. Here, we extend the scope of previous research by comparing the patterning of cranial covariance structures in extant hominids to examine the influence of canalisation and developmental stability, subsequently evaluating whether these two processes are distinct or not in different regions of the hominid cranium. This approach will provide insight into factors that influence overall phenotypic variability and whether these developmental processes are conserved across closely related taxa. We use landmark based 3D geometric morphometric techniques and multivariate statistics to compute and analyse covariance structures within and between taxa. Our results show that canalisation and developmental stability are, to an extent, distinct processes in the hominid cranium, suggesting that they do not share the same functional/genetic origin. We

also found remarkably high correlations between species covariance structures, which suggest that certain aspects of developmental processes are conserved across extant hominids. However, our overall results demonstrate that covariance structures in the cranium have a complex and integrated relationship to the underlying developmental interactions and that it is problematic to pin-point the precise influence of developmental processes that maintain covariance structure in the hominid cranium.

Introduction

The evolution of phenotypic covariance patterns is essential for understanding how microevolutionary processes influence macroevolution (Steppan 1997a). Developmental processes such as canalisation and developmental stability have been named as possible factors that structure and guide the direction of phenotypic change (Waddington 1942; Debat et al. 2000; Hoffmann and Wood 2001; Hallgrímsson et al. 2002; Willmore et al. 2006; Debat et al. 2009). Canalisation is a process that buffers development against environmental and genetic disturbances to maintain phenotypic constancy (Schmalhausen 1949; Waddington 1957) and minimises variation among individuals that is generated from genetic and environmental perturbations at the population level. Developmental stability is similar to canalisation, but it arises from buffering against developmental perturbations generated within individuals. Fluctuating asymmetry (FA) has often been used to assess developmental stability (Auffray et al. 1999a). FA is estimated by the small random differences between the two sides of bilateral structures. Bilateral structures share the same genome and any deviations from bilateral symmetry are indicative of developmental perturbations and the structure's level of developmental stability. FA is an estimation of the level of within-individual variation.

Even though developmental stability and canalisation have been widely studied, the relationship between these processes is still under extensive debate (Klingenberg et al. 1998; Rutherford and Lindquist 1998; Hoffmann and Wood 2001; Hallgrímsson et al. 2002; Re´ale and Ruff 2003; Santos et al. 2005; Willmore et al. 2005; Breuker et al. 2006; Willmore et al. 2006; Debat et al. 2009). Contention mainly arises from the claim that canalisation and developmental stability have distinct developmental origins, and therefore influence the phenotype differently: while the former is thought to suppress variation at the external level the latter is believed to do so internally.

The relationship between canalisation and developmental stability can be investigated by comparing the pattern of covariance of FA and among-individual variation. The focus of this study is on the pattern of covariance structures generated by FA and among-individual variation in different cranial elements of closely related extant hominids, namely *Pongo, Gorilla, Pan* and *Homo.* Specifically, we aim to address: 1) whether processes responsible for generating within- and among-individual covariance are distinct within species; 2) whether covariance of FA, that is direct developmental interactions, and among-individual variation in different regions of the cranium is distinct across extant hominids; 3) whether covariance of FA is more conserved than individual variation. In an evolutionary context, a central question is whether patterns of genetic and phenotypic covariances remain constant among closely related species. According to Steppan (1997a;1997b), during speciation certain microevolutionary processes manifest as macroevolutionary patterns and understanding the macroevolutionary patterns requires prior understanding of the evolution of covariance structures. By investigating covariance structures within and among individuals, we attempt to gain insight into the evolutionary conservation of developmental processes that influence phenotypic covariance patterns in the primate cranium.

To simplify and conduct a thorough investigation of the relationship between FA and phenotypic variation in the cranium, we sub-divided the cranium into the face, basicranium and cranial vault. According to Moss and others (1960a; 1969(b)) the cranium can be divided into regions related to periosteal and capsular matrices, where the former corresponds to skeletal tissue growth and activity, and the latter to the bony compartments for organs such as the brain and pharynx. Moss (Moss and Young 1960b; Moss 1962) further elaborated on the differences between the face, basicraium and cranial vault as being influenced by differential growth patterns and embryonic origin. While the face follows a somatic growth pattern, the neurocranium follows a neural growth pattern (Moore and Lvelle 1974). The neurocranial components can be largely divided into the basicranium and vault. Growth in the cranial vault is through intramembranous ossification and the basicranium grows from endochondral ossification. The basicranium is additionally

influenced by somatic growth factors largely attributed to hormones affecting the corresponding cartilage growth (Bogin 1988; Hall 2005).

The comparative approach used in this study is similar to Debat et al. (2006) study on *Drosophila* wing shape variation on the effects of FA and inter-individual variation on phenotypic variability. In another similar study on mouse crania, Debat et al. (2000) concluded that developmental stability and canalisation are distinct from each other. Their findings are further supported by a number of other studies (Rutherford and Lindquist 1998; Debat et al. 2000; Re´ale and Ruff 2003; Santos et al. 2005; Debat et al. 2006; Rego et al. 2006) that found disparity between developmental stability and canalization. However, other studies on primates and invertebrates (Møller 1990; Livshits and Smouse 1993; Møller et al. 1995; Tardieu 1999; Hallgrímsson et al. 2002; Tague 2002; Willmore et al. 2005) found significant correlations between covariance structures generated by FA and among-individual variation, suggesting that underlying developmental properties responsible for developmental stability and canalisation were similar.

Phenotypic covariance structures among closely related taxa are generated by a combination of several mechanisms, such as developmental interactions, constraints, environmental factors and natural selection. It is difficult to tease apart these different processes and close to impossible without the aid of experiments. A comparative approach examining the influence of developmental processes such as canalisation and developmental stability on patterning of covariance structures directly addresses the question whether covariance structures are as stable as previously proposed or whether they have the potential to evolve among closely related taxa, contributing to phenotyptic variability. Therefore, better understanding the patterning of covariance structures is imperative for understanding the morphological evolution.

Materials and methods

Total sample comprises dried crania of 385 adult individuals of *Pongo*, *Gorilla*, *Pan* and *Homo* (Table 1); the sex ratio was nearly equal in all the groups.

Fifty-four 3D landmarks were measured on the entire cranium using a G2X MicroScribe (Immersion Corporation, San Jose, CA); all measurements were taken by one observer (NS). Landmarks (Figure 1) were chosen based on anatomical correspondence and repeatability across taxa. While measuring, the specimens were mounted on modelling clay to keep them stationary. Only specimens with fully erupted dentition and fully fused spheno-occipital synchondrosis were included in the study. For individuals with prominent mid-sagittal and nuchal crests, landmarks such as bregma and lambda were taken on top of the crests and two additional points were taken on the neurocranium on either side of both landmarks. Because sexual dimorphism is not the subject of interest in this study, the projected points and landmarks taken on top of the crests were later averaged; the averaged point was used in the analyses. All individuals with missing and/or mislabelled landmarks were excluded from the analysis. An assessment of intra-observer error is provided below.

Geometric morphometrics

The application of geometric morphometric techniques to calculate fluctuating asymmetry was first proposed by Bookstein (1991). One of the advantages of these methods is that they incorporate the information of a morphological structure in its entirety (for detailed description of this approach, see Auffray et al. (1996; 1999a), Bookstein (1991), Klingenberg and McIntyre (1998), Rohlf and Marcus (1993) and Smith et al. (1997).

Landmark configurations for each specimen were superimposed using generalized Procrustes superimposition (GPA) (Rohlf and Slice 1990). GPA is widely used in geometric morphometric studies to extract shape information from landmark data. The procedure involves extracting shape coordinates by translating, scaling and rotating the landmark configurations; hence, removing all information unrelated to shape. A size measure (centroid size) which is the sum of squared distances between corresponding landmarks, is obtained for each specimen (Dryden and Mardia 1998).

In the present study, during the Procrustes superimpositition object symmetry was calculated by reflecting and relabeling each set of paired landmarks (Klingenberg and McIntyre 1998; Klingenberg et al. 2001). Object symmetry is a form of bilateral symmetry in which a structure is inherently symmetric; such a structure has a biological midline plane used to render the left and right sides as mirror images of each other (Klingenberg et al. 2002). A biological form such as the mammalian cranium is a good example of object symmetry. The object symmetry analysis was conducted in *MorphoJ* . This software package yields separate components for symmetry and asymmetry. FA analyses were done using the asymmetric components of shape, calculated as the difference between the original landmark configuration and its mirror image. The asymmetric component generated after this procedure accounts for the within- individual variation and the symmetric component, in which all influence of asymmetry has been removed, accounts for among-individual variation.

Measurement error

Estimating measurement error is particularly crucial in studies of fluctuating asymmetry because large measurement errors can inflate the signal of FA. To ensure as much accuracy as possible, replicate measurements were taken for all individuals used in this study; of these 48 were re-digitized two years apart (at the beginning and end of data collection) to gain reliable estimates for FA. Measurement error was quantified using the Procrustes ANOVA method outlined in Klingenberg and McIntyre (1998) and was performed separately for each taxon included in the study. This method is analogous to the two-factor ANOVA model developed by Palmer and Strobeck (1986; Palmer 1994), which estimates measurement error relative to the signal of asymmetry in biological datasets. The Procrustes ANOVA involves a four-step procedure: 1. quantifying among-individual shape variation in the dataset; 2. calculating the effects of directional asymmetry; 3. accounting for fluctuating asymmetry (calculating each side * specimen interaction); and finally, 4. quantifying variability between original and replicate measurements, which estimates measurement error in the dataset. The Procrustes ANOVA yields

larger degrees of freedom, more so than in a regular ANOVA (Klingenberg and McIntyre 1998).

Analyses of covariation

Symmetric and asymmetric components: Landmarks of the face, basicranium and cranial vault were subjected to separate Procrustes fits, yielding symmetric and asymmetric components. As stated above, the symmetric components represent among-individual variation and asymmetric components represent FA in the data. For each taxon the symmetric and asymmetric components were then subdivided from the face, basicranium and cranial vault; separate Procrustes fits were not performed on the taxa at this stage. When object symmetry is calculated, the symmetric and asymmetric components occupy orthogonal subspaces of the same tangent space, making direct comparisons of the respective covariance matrices uninformative (Klingenberg et al. 2002). An alternative procedure is to use only one side of the (paired) landmarks for comparing covariance matrices of the symmetric and asymmetric components. Thus, the latter procedure was employed in this study and all comparisons of covariance matrices was done only for the paired landmarks as outlined in (Klingenberg et al. 2002)

To compare covariance matrices, both inter- and intra-specifically, we computed matrix correlations for the following: 1) intra-specifically between the species symmetric and asymmetric covariance matrices; 2) inter-specifically between the species symmetric covariance matrices; 3) inter-specifically between the species asymmetric covariance matrices; 4) inter-specifically between all possible pairs of species symmetric and asymmetric covariance matrices together (Debat et al. 2006).

When calculating the matrix correlations the diagonal element of the matrices were included to account for both variance and covariance as both contain information about similarity and/or differences of the matrices. The matrix correlations computed between intra- and inter- species covariance matrices were tested with a matrix permutation test against the null hypothesis of complete

dissimilarities between the matrices (Mantel 1967; Cheverud 1989; Klingenberg et al. 2002). The permutation procedure was carried out for the landmarks, keeping the *x* and *y* coordinates of each landmark together (Klingenberg et al. 2002). The matrix correlations were done in *MorphoJ*.

To visualise the relationship of the species covariance matrices we used metric multidimensional scaling (MDS), which uses a matrix of distances. Metric MDS is an effective way to transform a distance matrix into a set of coordinates that account for the maximum amount of information present in the matrix. Distances between each pair of covariance matrices were defined and computed as one minus the matrix correlation (Debat et al. 2006). The output of the distance matrix was then used in the metric MDS analysis. This method is also an effective way to explore and visualise the similarities or dissimilarities in a dataset - in this case that being the distances among the taxa-specific covariance matrices. The distances from the matrix correlations and subsequent metric MDS analyses were calculated using the programming software R.

Results

Measurement error

Main effects except for side, i.e. directional asymmetry, were significant according to the results from the Procrustes ANOVA (Table 2). In addition, error related to digitising was negligible compared to the signal of fluctuating asymmetry component in all taxa (Table 2). Results from the Procrustes ANOVA indicate that measurement error in the dataset is not significant enough to interfere with the following analyses.

Analysis of covariation: intra-species comparison of covariance matrices

Symmetry vs. asymmetry: Matrix correlation comparisons between covariance of FA and among-individual variation show moderate association between the covariance structures of the face, basicranium and cranial vault in all taxa (Table 3). The null hypothesis of total dissimilarity between the matrices is therefore rejected in all

instances. In the face, bonobos, chimpanzees and gorillas show relatively lower correlations than humans and orangutans. Bonobos show the weakest correlation between the covariance matrices in the bascranium. However, in all species correlations between covariance of FA and individual variation in the basicranium are slightly higher than in the face and cranial vault. In the cranial vault, humans show a relatively high association between the covariance structures and chimpanzees a relatively low one; bonobos, oranugtans and gorillas show a moderate association in the cranial vault.

Analysis of covariation: inter-species comparison of covariance matrices

Symmetric components: Matrix correlations for covariance of among-individual comparisons between taxa are fairly high (Table 4), in all cranial regions. Matrix permutation tests against the null hypothesis of complete dissimilarity of taxa covariance matrices is rejected, showing that *Homo*, *Pan*, *Gorilla* and *Pongo* share a strong association between respective covariance structures of the face, basicranium and cranial vault.

Distances between covariance matrices calculated as one minus the matrix correlations are illustrated in the MDS plots in Figure 2. The ordination of covariance matrices shows no consistent pattern or clustering of taxa in the face, cranial base and vault. Covariance of among-individual variation in the face shows chimpanzees and gorillas to cluster closer together, to the exclusion of orangutans, humans and bonobos. Covariance structure of the basicranium shows chimpanzees and orangutans to cluster closer together than to any other taxa. The cranial vault shows orangutans and gorillas to group together; humans are set apart from the other apes.

Asymmetric components: Results for covariance of FA are similar to among-individual interactions in that the matrix correlations are high between taxa covariance matrices among all three cranial components (Table 4). Results from the

permutation test reject the null hypothesis of total dissimilarity between the taxa covariance matrices.

As in the symmetric components, the MDS plots of covariance of FA neither show any consistent patterning nor do they show particular clustering of taxa with respect of the three cranial regions (Figure 2). However, the patterning of FA covariance matrices was different from that of among-individual variation. The covariance of FA in the face shows chimpanzees and humans to cluster closer together to the exclusion of the other taxa. This is different from the patterning of the matrices of among-individual interactions. Covariance of FA in the basicranium shows no particular grouping between taxa, unlike in among-individual variation where chimpanzees and orangutans cluster close together. In the cranial vault chimpanzees and orangutans group closer together in covariance of FA, but orangutans cluster with gorillas in among-individual variation.

Symmetric vs. asymmetric components: Matrix correlation results of the combined analysis of all possible pair-wise covariance matrices of FA and among-individual interactions show large disparity between covariance of FA and among-individual variation in all three cranial regions (Figure 3). In aspects of the face, the taxa distribution is different for covariance of FA and among-individual variation. Orangutans are distinct from the other taxa in their covariance structures of the face; bonobos are slightly set apart from the other taxa in aspects of FA; whereas humans, chimpanzees and gorillas group close together in both covariance of FA and among-individual variation. Patterning and distribution of taxa covariance matrices is only similar in the basicranium analysis. Bonobos are distinct in their covariance matrices from the other taxa, and humans, gorillas and orangutans group close together in aspects of asymmetry rather than symmetry. The cranial vault shows a different distribution and patterning of taxa covariance matrices from that of the face and basicranium. Humans are distinct from the other taxa in their covariance structure of the cranial vault. The other taxa group close together, but differ in their pattern of distribution between symmetric and asymmetric components.

Discussion

In this study we aim to examine and provide a framework for comparing mechanisms responsible for maintaining phenotypic covariance structures over macroevolutionary time. We did this by examining the patterning of cranial covariance structures generated by within- and among-individual variation in *Homo*, *Pan*, *Gorilla* and *Pongo*. By calculating object symmetry and separating symmetric and asymmetric components we were able to examine possible sources of variation that generate covariance in biological structures (Klingenberg 2003b;2005). Based on previous work on extant and extinct hominids we have reason to believe that different cranial components possibly carry different biological signals (Lieberman et al. 2000a; Gunz et al. 2005; Mitteroecker et al. 2005; Harvati and Weaver 2006). Our results suggest that taxa do not follow a consistent pattern of clustering in aspects of among- individual variation and covariance of FA, but instead show different patterns of inter-specific association in covariance structures of the respective cranial regions.

Intraspecific comparisons of within and among individual variation

Although a number of studies have investigated the effects of developmental stability and canalization in different organisms, only few have looked at these processes in primates. A study on macaque crania from Cayo Santiago showed an overlap between environmental, fluctuating asymmetry and among individual phenotypic variation, suggesting that processes that determine developmental stability and canalization are not entirely different (Willmore et al. 2005). While not completely supporting the claim of direct congruence between intra- and inter-individual developmental interactions, they did not reject disparity between these processes either.

Matrix correlation results of the within-species comparisons conducted in the present study show moderate association between covariance of FA and among-individual variation in all taxa. Moderate associations between within- and among-individual variation in the cranium raises the question about how distinct developmental processes are in *Homo*, *Pan*, *Gorilla* and *Pongo*. Humans consistently show higher correlations between within- and among-individual interactions than the other taxa in all three cranial regions. This suggests that processes responsible for developmental stability and canalisation may be less distinct in human crania than the other apes.

Among the face, basicranium and crania vault, the basicranium had the highest correlations between covariance of FA and among-individual variation in all species. The high correlation indicates some congruence between developmental stability and canalisation. Among the three regions the cranial vault showed the lowest correlation between covariance of within- and among-individual interactions even though the null hypothesis of complete dissimilarity was not upheld. This suggests only moderate association between the covariance of FA and among-individual variation in the vault of these hominids.

Several previous studies have addressed similar questions with varying datasets (Pigliucci et al. 1991; Wilkins 1997; Rutherford and Lindquist 1998; Debat et al. 2000; Rutherford 2000; Hallgrímsson et al. 2002; Queitsch et al. 2002; Re´ale and Ruff 2003; Willmore et al. 2005), but no clear conclusion has been reached on the association between the canalisation and developmental stability. Debat et al.'s (2000) study on mouse crania found that the two processes affect morphological traits differently. Debat et al. (2000) among others (Rutherford and Lindquist 1998; Hoffmann and Wood 2001; Re´ale and Ruff 2003) support the initial claim made by Waddington (1957) that canalisation and developmental stability are genetically and functionally independent. But other studies on mice have found strong correlations between FA and among-individual variation in aspects of both skull and post-cranial elements (Clarke 1997; Hallgrímsson et al. 2002; Klingenberg 2003a), suggesting possible congruence between the underlying genetic processes that regulate within- and among- individual developmental interactions. The latter has

been suggested to be particularly true for characters related to locomotion which bear direct relevance for inclusive fitness and for which maintaining bilateral symmetry is crucial (Clarke 1997; Debat et al. 2000). However, a study on limb lengths of crickets failed to find a strong correlation between FA and morphological variation, refuting the assumption that bilateral symmetry is essential for traits related to locomotion (Re´ale and Ruff 2003).

Thus, in light of previous findings our results fall somewhere in between. While the within species comparisons reject the null hypothesis of total dissimilarity between within- and among-individual variation in the cranium, the association between the two covariance structures, as indicated by the matrix correlation, is only moderate. Moreover, the level of association between covariance of FA and among-individual variation was different among the face, basicranium and cranial vault, suggesting a degree of modularity among these regions.

Interspecific comparisons of within and among individual variation

Thus far no study has compared the pattern of covariance of FA and among-individual variation in a genera wide context. Our results from the matrix correlations show that association between taxa covariance structures in symmetry and asymmetry, respectively, is remarkably strong. This suggests that aspects responsible for generating cranial covariance structures in extant hominids are similar and possibly conserved across these taxa. However, this holds true only for separate comparisons of among-individual covariance and covariance of FA.

Despite showing high correlations between species covariance matrices in both the symmetric and asymmetric components of the face, basicranium and cranial vault, the pattern of taxa distribution is dispersed and inconsistent (Figure 2). The patterning of taxa covariance structures of FA and among-individual variation further implies that the association between species covariance structures is different for the three regions; this is true for separate comparisons of covariance of FA and among-individual variation. However, correlations results show bonobos to have the strongest association with chimpanzees (Table 4). The differences in the

patterns of taxa distribution between regions of the cranium in aspects of symmetry and asymmetry further indicate that developmental stability and canalisation in the cranium may have at least partially different developmental origins. However, given the moderately strong correlations found in within-species comparisons (Table 3), the null hypothesis of total dissimilarity can be rejected.

Melvin Moss' (1968) functional matrix hypothesis states that different structures of the cranium have specific mechanical and developmental properties and are responsible for carrying out specific functions. These properties strongly co-vary with, and are influenced by, the spatial arrangement of the bony capsules in which bone grows and by the movement of the surrounding soft tissue. Variation generated by muscle activity and developmental interactions within a region are likely causes that affect developmental interactions among different cranial regions differently. Evidence of differing signals from the three cranial regions also suggests a degree of modularity within these regions that are informed by, and to an extent respond to, different selection pressures. Evaluating the level of modularity and developmental origin of these cranial regions is beyond the scope of this study, but further investigation on this topic will enhance our understanding of cranial evolution.

A different pattern emerges in the second set of analyses, which combines the symmetric and asymmetric components. This was primarily conducted to address the objective whether covariance of FA is more conserved than among-individual variation. Our results suggest that neither covariance of FA nor among-individual variation is more conserved in extant hominids, as indicated by the dispersion of taxa in both clusters of covariance matrices. However, our results also suggest that FA and among-individual variation affect covariance structures differently, indicating that processes that influence covariance structures in the cranium may have different developmental origins. A similar approach adopted in a study on the effects of Hsp90 on the wing shape of *Drosophila melanogaster* by Debat et al. (2006) found covariance of FA to be by far more conserved than inter individual variation. Even though it is not possible to conduct similar studies on primates, it is clear from our results that the relationship between phenotypic

covariance of FA and among individual variation is different in primates than in *Drosophila melanogaster.*

Conclusions

Six main conclusions can be drawn from this study: 1) In the within-species comparisons, humans show the strongest correlations between covariance of FA and among-individual variation in the face, basicranum and cranial vault, possibly suggesting that the human crania is less modulated than the other ape crania; 2) the correlation between within and among individual variation is not homogenous across the three cranial regions, suggesting a degree of modularity within the regions; 3) all taxa show very high correlations among covariance of among-individual variation, suggesting strong association among factors that influence covariance structures in aspects of non-direct developmental interactions – this was consistent for the face, basicranium and cranial vault; 4) between species association in covariance of FA are also high, suggesting similarity across species in aspects of direct developmental interactions that influence covariance structures; 5) Neither covariance of FA nor among-individual variation is more conserved in extant hominid crania; 6) While our overall results reject the notion that developmental stability and canalisation are distinct processes, the association between covariance of FA and among-individual variation is only moderately strong.

Acknowledgements

We thank the following curators for allowing us access to the various collections: Dr. Emmanuel Gilissen and Wim Wendelen of the Royal Museum of Central Africa, Tervuren; Diana Carvalho of Museu Nacional de História Natural, Lisbon; Ian Tattersall and Gisselle Garcia of the American Museum of Natural History, New York; Dr. Hendrik Turni and Dr. Frieder Mayer of the Naturkunde

Museum, Berlin; Dr. Katrin Schaefer of the University of Vienna and Dr. Richard Kraft of the Zoologische Staatssammlung, Munich. We are grateful to Dr. Philipp Mitteroecker and Dr. Fred Bookstein for discussion. This work was supported by the Marie-Curie "EVAN" training network (MRTN-CT-019564) and the Max Planck Society.

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Table 1: Specimens used in this study

Species	No. of Individuals	Source
Pan troglodytes	115	Royal Museum of Central Africa, Tervuren Natural History Museum, Berlin Max Planck Institute for Evolutionary Anthropology, Leipzig
Pan paniscus	34	Royal Museum of Central Africa, Tervuren
Gorilla beringei	43	Royal Museum of Central Africa, Tervuren
Pongo pygmaeus	65	Zoologische Staatssammlung, sektion Säugetiere/mammology, München
Homo sapiens	128	Natural History Museum, Lisbon American Museum of Natural History, New York
Total	385	

Table 2: Measurement error tests: separate Procrustes ANOVA tests for the taxa included in this study. Individual effects represent overall variation in the dataset; Side is the measure for directional asymmetry; Individual * side is the measurement for fluctuating asymmetry; Error 1 is the measurement error calculated from the variation among repeat measurements. Sum of squares (SS) and mean squares (MS).

Gorillas: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.17612759	5.77846E-05	3048	8.95	<.0001
Side	0.00181909	1.58182E-05	115	2.45	<.0001
Ind * Side	0.01782854	6.4596E-06	2760	33.24	<.0001
Eror 1	0.0010818	1.944E-07	5566		

Bonobos: Shape, Procrustes ANOVA:

Effect(param.)	SS	MS	df	F	P
Individual	0.15301585	0.000022733	6731	7.46	<.0001
Side	0.00269028	2.33937E-05	115	7.68	<.0001
Ind * Side	0.01857773	0.000003048	6095	9.18	<.0001
Error 1	0.00016078	3.322E-07	484		

Humans: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.08007854	2.74148E-05	2921	5.92	<.0001
Side	0.00221287	1.92423E-05	115	4.16	<.0001
Ind * Side	0.01224441	4.6293E-06	2645	24.56	<.0001
Error 1	0.00018247	1.885E-07	968		

Orangutans: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.329559	6.19006E-05	5324	11.58	<.0001
Side	0.00381311	3.49826E-05	109	6.55	<.0001
Ind * Side	0.02562663	5.3433E-06	4796	9.48	<.0001
Error 1	0.00583472	5.637E-07	10350		

Chimpanzees: Shape, Procrustes ANOVA:

Effect	SS	MS	df	F	P (param.)
Individual	0.10109703	2.48762E-05	4064	7.62	<.0001
Side	0.00194026	1.68718E-05	115	5.17	<.0001
Ind * Side	0.0120169	3.2655E-06	3680	14.1	<.0001
Error 1	0.00005604	2.316E-07	242		

Table 3: Within-species comparisons of covariance of FA and individual variation

Species	Face: symmetry vs asymmetry	Base: symmetry vs asymmetry	Vault: symmetry vs asymmetry
Bonobo	0.5553607 (p = 0.0209)	0.58104628 (p=0.0005)	0.52280587 (p=0.1672)
Chimpanzee	0.57709582 (p=0.0549)	0.67316591 (p=0.0005)	0.59999104 (p=0.0438)
Gorilla	0.52076287 (p=0.0370)	0.65527158 (p=0.0008)	0.48982042 (p=0.4151)
Human	0.68372665 (p=0.0072)	0.69083101 (p=0.0021)	0.68749768 (p=0.0821)
Orangutan	0.63461384 (p=0.0011)	0.68874522 (p=0.0001)	0.55710518 (p=0.1247)

Table 4. Face: comparisons of between (A) among individual variation and (B) covariance of FA; Base: comparisons of between (C) among individual variation and (D) covariance of FA; Vault: comparisons of between (E) among individual variation and (F) covariance of FA.

(A) *Species*	Bonobo	Chimpanzee	Gorilla	Human	Orangutan
Bonobo	0	0.82 (p<0.0001)	0.77 (p<0.0001)	0.71 (p<0.0001)	0.75 (p<0.0001)
Chimpanzee	0.82 (p<0.0001)	0	0.83 (p<0.0001)	0.76 (p<0.0001)	0.83 (p<0.0001)
Gorilla	0.77 (p<0.0001)	0.83 (p<0.0001)	0	0.76 (p<0.0001)	0.79 (p<0.0001)
Human	0.71 (p<0.0001)	0.76 (p<0.0001)	0.76 (p<0.0001)	0	0.74 (p<0.0001)
Orangutan	0.75 (p<0.0001)	0.83 (p<0.0001)	0.79 (p<0.0001)	0.74 (p<0.0001)	0

(B) *Species*	Bonobo	Chimpanzee	Gorilla	Human	Orangutan
Bonobo	0	0.78 (p<0.0001)	0.75 (p<0.0001)	0.77 (p<0.0001)	0.71 (p<0.0001)
Chimpanzee	0.78 (p<0.0001)	0	0.80 (p<0.0001)	0.89 (p<0.0001)	0.75 (p<0.0001)
Gorilla	0.75 (p<0.0001)	0.80 (p<0.0001)	0	0.77 (p<0.0001)	0.65 (p<0.0001)
Human	0.77 (p<0.0001)	0.89 (p<0.0001)	0.77 (p<0.0001)	0	0.71 (p<0.0001)
Orangutan	0.71 (p<0.0001)	0.75 (p<0.0001)	0.65 (p<0.0001)	0.71 (p<0.0001)	0

(C) *Species*	Bonobo	Chimpanzee	Gorilla	Human	Orangutan
Bonobo	0	0.79 (p<0.0001)	0.64 (p<0.0001)	0.72 (p<0.0001)	0.74 (p<0.0001)
Chimpanzee	0.79 (p<0.0001)	0	0.77 (p<0.0001)	0.79 (p<0.0001)	0.81 (p<0.0001)
Gorilla	0.64 (p<0.0001)	0.77 (p<0.0001)	0	0.69 (p<0.0001)	0.74 (p<0.0001)
Human	0.72 (p<0.0001)	0.79 (p<0.0001)	0.69 (p<0.0001)	0	0.77 (p<0.0001)
Orangutan	0.74 (p<0.0001)	0.81 (p<0.0001)	0.74 (p<0.0001)	0.77 (p<0.0001)	

(D) *Species*	Bonobo	Chimpanzee	Gorilla	Human	Orangutan
Bonobo	0	0.74 (p<0.0001)	0.59 (p<0.0001)	0.61 (p<0.0001)	0.63 (p<0.0001)
Chimpanzee	0.74 (p<0.0001)	0	0.74 (p<0.0001)	0.79 (p<0.0001)	0.80 (p <0.0001)
Gorilla	0.59 (p<0.0001)	0.74 (p<0.0001)	0	0.73 (p<0.0001)	0.78 (p<0.0001)
Human	0.61 (p<0.0001)	0.79 (p<0.0001)	0.73 (p<0.0001)		0.78 (p<0.0001)
Orangutan	0.63 (p<0.0001)	0.80 (p <0.0001)	0.78 (p<0.0001)	0.78 (p<0.0001)	0

(E) *Species*	Bonobo	Chimpanzee	Gorilla	Human	Orangutan
Bonobo	0	0.84 (p<0.0001)	0.70 (p<0.0001)	0.73 (p<0.0001)	0.72 (p<0.0001)
Chimpanzee	0.84 (p<0.0001)	0	0.78 (p<0.0001)	0.64 (p<0.0001)	0.77 (p<0.0001)
Gorilla	0.70 (p<0.0001)	0.78 (p<0.0001)	0	0.71 (p<0.0001)	0.84 (p<0.0001)
Human	0.73 (p<0.0001)	0.64 (p<0.0001)	0.71 (p<0.0001)	0	0.70 (p<0.0001)
Orangutan	0.72 (p<0.0001)	0.77 (p<0.0001)	0.84 (p<0.0001)	0.70 (p<0.0001)	0

(F) *Species*	Bonobo	Chimpanzee	Gorilla	Human	Orangutan
Bonobo	0	0.87 (p<0.0001)	0.69 (p<0.0001)	0.82 (p<0.0001)	0.84 (p<0.0001)
Chimpanzee	0.87 (p<0.0001)	0	0.82 (p<0.0001)	0.83 (p<0.0001)	0.86 (p<0.0001)
Gorilla	0.69 (p<0.0001)	0.82 (p<0.0001)	0	0.69 (p<0.0001)	0.75 (p<0.0001)
Human	0.82 (p<0.0001)	0.83 (p<0.0001)	0.69 (p<0.0001)	0	0.82 (p<0.0001)
Orangutan	0.84 (p<0.0001)	0.86 (p<0.0001)	0.75 (p<0.0001)	0.82 (p<0.0001)	0

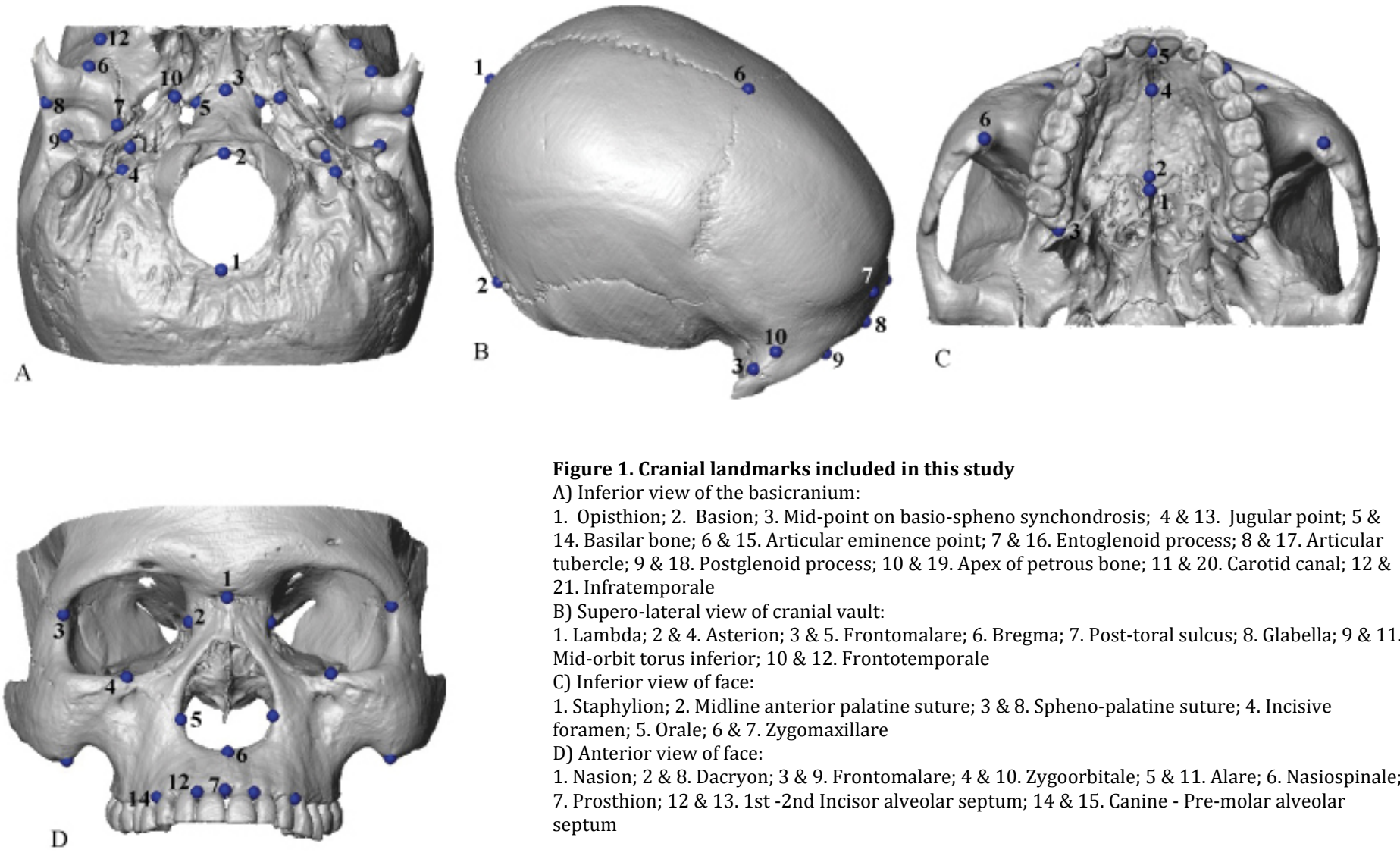

Figure 1. Cranial landmarks included in this study

A) Inferior view of the basicranium:

1. Opisthion; 2. Basion; 3. Mid-point on basio-spheno synchondrosis; 4 & 13. Jugular point; 5 & 14. Basilar bone; 6 & 15. Articular eminence point; 7 & 16. Entoglenoid process; 8 & 17. Articular tubercle; 9 & 18. Postglenoid process; 10 & 19. Apex of petrous bone; 11 & 20. Carotid canal; 12 & 21. Infratemporale

B) Supero-lateral view of cranial vault:

1. Lambda; 2 & 4. Asterion; 3 & 5. Frontomalare; 6. Bregma; 7. Post-toral sulcus; 8. Glabella; 9 & 11. Mid-orbit torus inferior; 10 & 12. Frontotemporale

C) Inferior view of face:

1. Staphylion; 2. Midline anterior palatine suture; 3 & 8. Spheno-palatine suture; 4. Incisive foramen; 5. Orale; 6 & 7. Zygomaxillare

D) Anterior view of face:

1. Nasion; 2 & 8. Dacryon; 3 & 9. Frontomalare; 4 & 10. Zygoorbitale; 5 & 11. Alare; 6. Nasiospinale; 7. Prosthion; 12 & 13. 1st -2nd Incisor alveolar septum; 14 & 15. Canine - Pre-molar alveolar septum

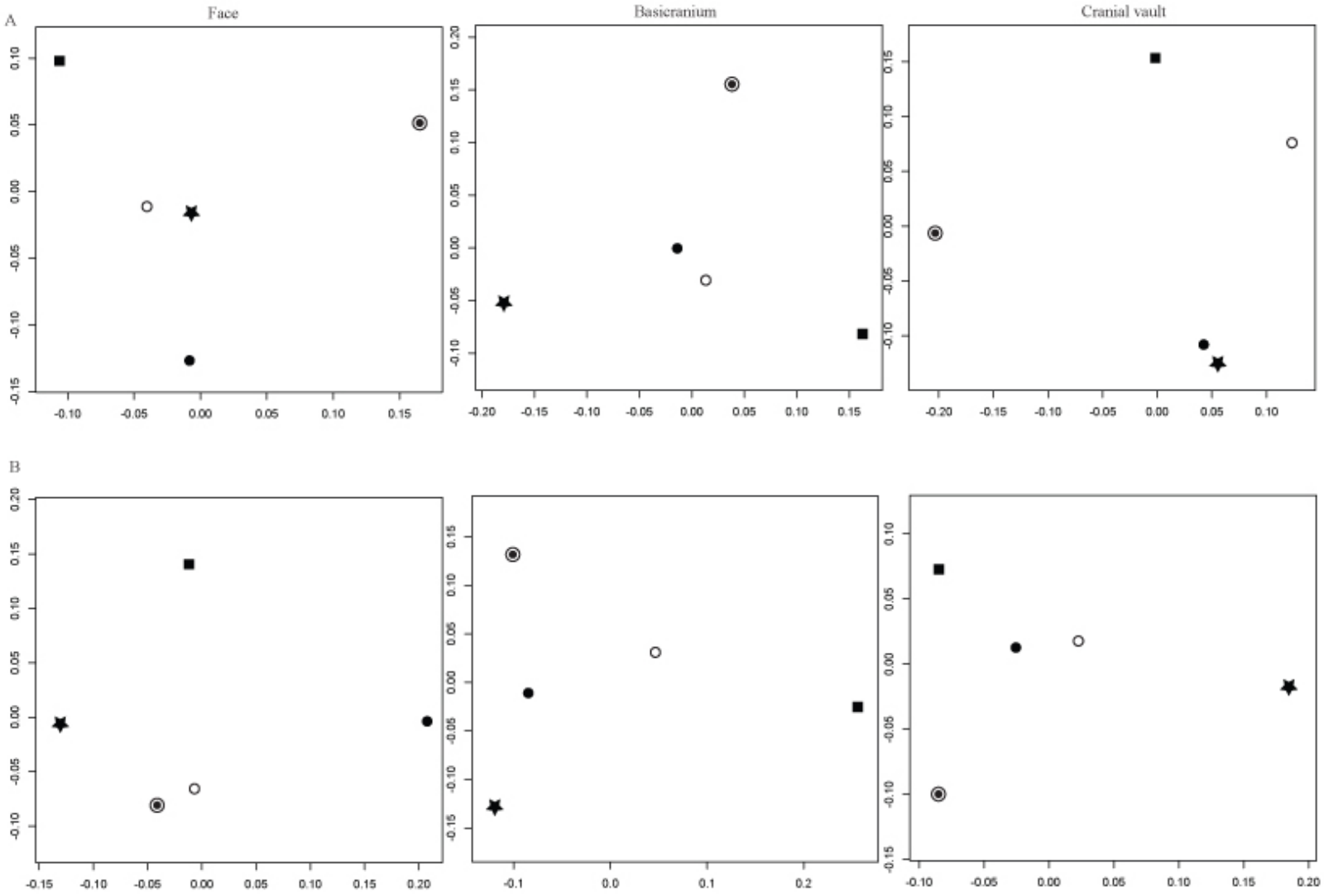

Figure 2: MDS plots of (A) covariance matrices comparisons of among individual variation and (B) covariance of FA
■Bonobo; ○Chimpanzee; ★Gorilla; ◉Human; ●Orangutan

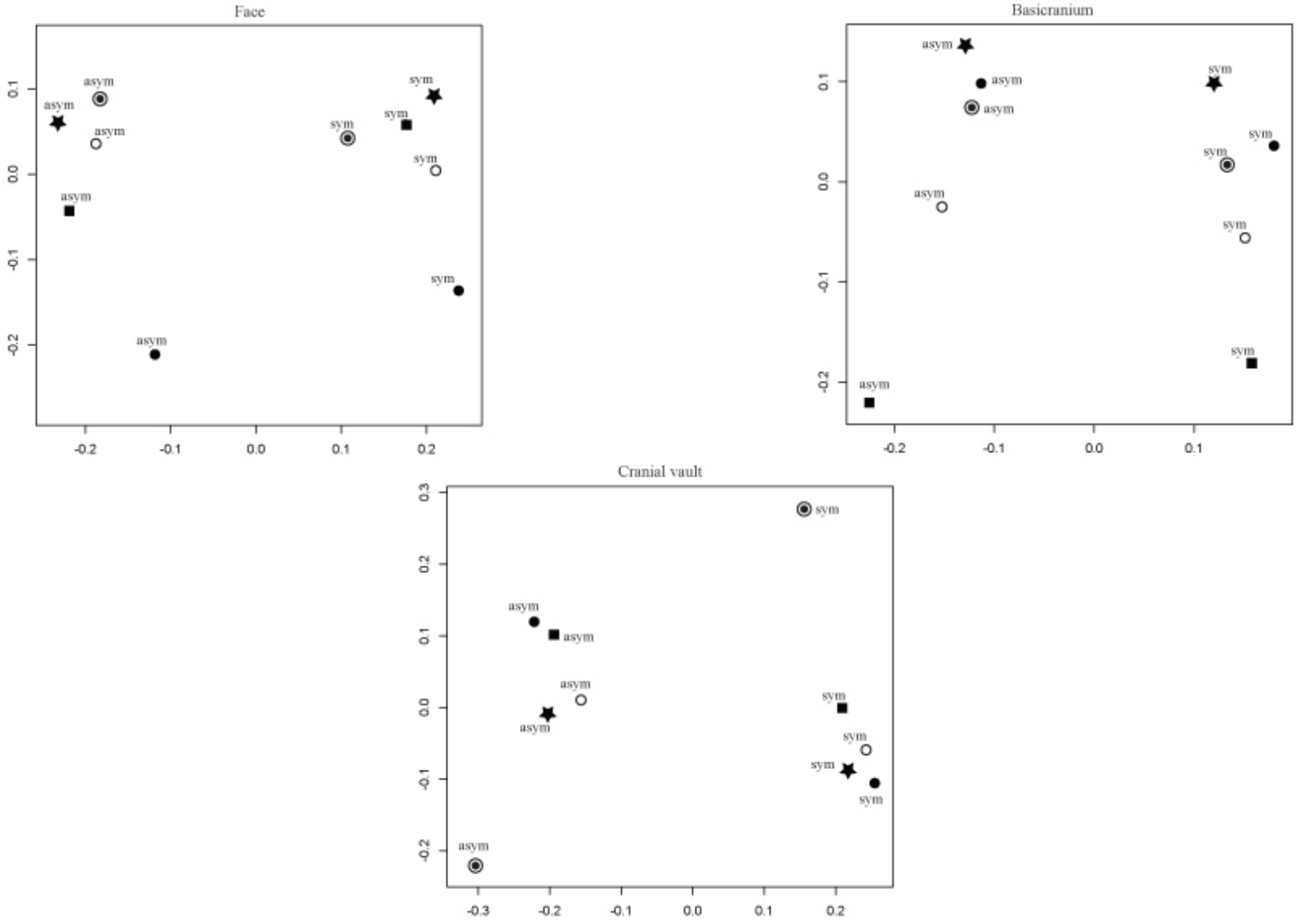

Figure 3: MDS plots of covariance matrices of individual variation and FA.
■Bonobo; ○Chimpanzee; ★Gorilla; ◉Human; ●Orangutan

Manuscript 3: Comparative study of ontogenetic variation in Homo *and* Pan *mandibles*

(Submitted to *American Journal of Physical Anthropology*)

Abstract

Shape variation in extant hominid mandibles has been widely investigated. However, there is still an ongoing debate on whether inter-specific morphological differences can be attributed to the direction and pattern of ontogenetic trajectories. In the present study, we re-examine patterns of ontogenetic shape change and trajectories in 187 sub-adult and adult humans, bonobos and chimpanzees. According to the predictions of the "functional matrix hypothesis", we additionally propose that ontogenetic shape differences in the mandible are influenced not only by diverging ontogenetic trajectories among taxa, but also by differing patterns of ontogenetic shape change in the corpus and ramus. We employ Procrustes based geometric morphometrics to quantify and analyse mandibular form. Thirty 3D landmarks were recorded on the entire mandible; they were analysed both as a whole and separately as corpus and ramus elements. Principal components analyses in shape-space and form space, multivariate regressions as well as taxa mean shape comparisons were used to examine patterns of ontogenetic shape change across chimpanzees, bonobos and humans. Our results suggest that ontogenetic trajectories of shape change in *Pan* and *Homo* are linear, but not parallel. Moreover, shape differences among the taxa are established early in postnatal ontogeny. Separate analyses of the corpus and ramus show that these two regions are semi-independent of each other in their pattern of ontogenetic shape changes. The latter provides support for the "functional matrix hypothesis" and serves as an additional explanation for divergent patterns of shape change in closely related hominid taxa.

Introduction

Humans are distinct from all other living apes in their cranio-mandibular morphology. Species-specific patterns of growth and development largely contribute to this distinction. Studying the ontogeny of morphological structures provides insight into possible evolutionarily conserved and divergent developmental pathways that give rise to taxon-specific traits. One way of examining factors, particularly growth and developmental, which lead to morphological variation is to investigate ontogenetic trajectories among closely related taxa. There has been considerable research on inter-specific differences in the cranio-facial complex of primates (Schultz, 1924; Giles, 1956; Shea, 1983; Richtsmeier and Lele, 1993; Richtsmeier and Walker, 1993; Godfrey and Sutherland, 1994; O'Higgins and Jones, 1998; Bruner and Manzi, 2001; Ponce de Leon and Zollikofer, 2001; Ackermann and Krovitz, 2002; Strand-Vidarsdottir, 2002; Strand Vidarsdottir et al., 2002; Williams et al., 2003; Berge and Penin, 2004; Cobb and O'Higgins, 2004; Mitteroecker et al., 2004a; Zollikofer and Ponce de Leon, 2004; Bastir and Rosas, 2004a; Mitteroecker et al., 2005; Bulygina et al., 2006; Leigh, 2006; O'Higgins et al., 2006; Bastir et al., 2007), but only few have focused on developmental aspects of the mandible (Johnson et al., 1976; Daegling, 1996; Chen et al., 2000; Williams et al., 2002; Willams and Richtsmeier, 2003; Bastir et al., 2007; Bougher and Dean, 2008). Nevertheless, the issue of whether hominids have parallel or divergent cranio-mandibular ontogenetic trajectories is still heavily debated. Here, we conduct a quantitative ontogenetic study of modern human, bonobo and chimpanzee mandibles in order to re-examine shape changes in mandibular morphology and to test hypotheses of divergent vs. parallel developmental trajectories. Our study also includes exploring alternative explanations, such as the "functional matrix hypothesis" (Moss and Young, 1960) to better understand other factors that influence overall mandibular morphology.

Ontogenetic trajectories

Much of the work on cranio-mandibular growth and development in primates has focused on the pattern and direction of ontogenetic change, i.e. ontogenetic trajectories. Most studies on the primate skull can be divided into two camps: one that support divergent ontogenetic trajectories and one that propose parallel developmental trajectories. Earlier studies on cranial development by Schultz (1924) found that the majority of specialised features in humans, other apes and monkeys manifested and diverged among taxa later in the developmental process. This finding was corroborated by relatively recent studies on facial growth (Richtsmeier et al. 1993; Cobb and O'Higgins, 2004; McNulty et al, 2006; Ponce de Leon and Zollikofer, 2006). One study by Richtsmeier et al. (1993) found that facial morphology of macaques, vervet and capuchin monkeys were predominantly influenced by postnatal ontogeny, and not simply extensions of features established prenatally. They concluded that while species-specific features may emerge prenatally, they continue to develop and possibly diverge between taxa later in ontogeny. Cobb and O'Higgins (2004) reached a similar conclusion and further showed that postnatal growth trajectories in the face of extinct and extant hominids were divergent, suggesting different pathways of ontogenetic facial shape change. These findings were corroborated by Mitteroecker et al. (2004a; 2005), who found humans relative to the other apes, to have the most distinct and divergent postnatal morphology and ontogenetic trajectory. Moreover, Mitteroecker et al. (2005) also found bonobos and chimpanzees to have different ontogenetic trajectories in the cranium with respect to the lower and upper face and neurocranium. A slightly different conclusion was reached by McNulty et. al (2006). Although they found African apes, modern humans and the Taung child (*Australopithecus africanus*) to have divergent patterns of cranial development, they showed that diverging trajectories did not greatly impact the adult morphology.

Not all studies on cranial ontogeny show extant hominids to have divergent developmental pathways in the cranium (Shea,1983a,b; Bruner and Manzi, 2001; Ponce de Leo´n and Zollikofer, 2001; Ackermann and Krovitz, 2002; Penin et al.,

2002; Williams et al., 2002; Zollikofer and Ponce de Leo´n, 2004). Classical studies by Shea (1983a,b) have largely attributed cranial differences among African apes to extension and truncation of trajectories, i.e. ontogenetic scaling, rather than divergent developmental pathways. Ackermann and Krovitz's (2002) study on cranio-facial ontogeny of *Gorilla*, *Pan*, *Australopithecus africanus* and *Homo sapiens* directly contradicted the results of Cobb and O'Higgins (2004) and showed extinct and extant hominds to have parallel cranial ontogenetic trajectories, suggesting common growth vectors across the taxa. However, the contradictory results of the latter two studies were attributed to differences in analytical techniques and statistical tests.

Although far less work has been done on mandibular development than the cranium, no resolution has been reached on the pattern of development in the primate mandible. Genera-wide studies on African apes suggest that *Gorilla* and *Pan* do not share common growth vectors in the mandible (Daegling, 1996; Taylor and Groves, 2003). However, in a recent study on *Pan*, Bougher and Dean (2008) found *Pan troglodytes* and *Pan paniscus* to have parallel ontogenetic trajectories, indicating that development in the mandible of, at least, sub-species is similar.

Different conclusions reached by the various studies suggest that cranio-mandibular morphology is complex, highlighting the need for further research. Moreover, contradictory findings could also be a result of analysing different aspects of a structure. The present research not only aims to help improve our understanding of the evolutionary developmental properties of the mandible, but also to provide a framework for addressing potential questions on the integrative aspects of the cranio-mandibular form.

Functional matrix hypothesis

A principal objective of this study is to examine alternative explanations for morphological variation in the mandible, such as that proposed by the "functional matrix hypothesis" for mandibular development (Moss and Young, 1960; Moss, 1973). The "functional matrix hypothesis" states that there exist semi-independent growth centres in different parts of the mandible (Moss, 1973). Genetic and

morphometric research on mouse mandibles (Leamy, 1984; Atchley et al., 1985; Atchley and Hall, 1991; Atchley, 1993; Leamy, 1993; Klingenberg, 2003) and some on primate mandibles (Johnson et al., 1976; Daegling, 1996; Willmore et al., 2009) support this claim by showing that the mammalian mandible can be largely sub-divided into two distinct embryonic units, the corpus and the ramus (Atchley and Hall, 1991). Daegling's (1996) study on African ape mandibles found that growth of the corpus was mainly influenced by the developing dentition, whereas the ramus was modulated by the activity of the masticatory muscles. His results suggest that because parts of the mandible are semi-independent, both functionally and structurally, no single pattern of development can account for the morphological differences among taxa. Here, we extend this research to examine developmental patterns in the corpus and ramus elements of the mandible in *Pan* and *Homo*.

Objectives

The following are the objectives of the present study: 1) examining ontogenetic shape changes in the mandible of humans, bonobos and chimpanzees to identify at what age stage species-specific features emerge and what they are. We predict that species-specific changes will arise early in ontogeny, even between bonobos and chimpanzees, with humans being the most distinct; 2) testing the pattern and direction of mandibular ontogenetic trajectories in these hominids. We predict that ontogenetic trajectories will be linear, but not parallel, at least not between *Pan* and *Homo*, suggesting that the direction of ontogenetic shape change is not conserved in these two hominids; 3) comparing ontogenetic shape change in the corpus and ramus separately. Specifically, we are interested in testing the "functional matrix hypothesis" and we predict that due to functional and embryonic differences, growth in these regions will be different across taxa, further suggesting that overall shape changes in the mandible - particularly inter-specific differences - will be influenced by differential growth patterns; 4) lastly, comparing our results to findings of previous studies on cranial ontogenetic trajectories. We predict that the

pattern of ontogenetic trajectories will differ between the cranium and mandible, suggesting different developmental and functional constraints on cranial and mandibular, morphology.

There are several reasons for conducting work in this area. First, despite there being a number of studies on cranio-facial variation and ontogeny in extant and extinct hominids - both intra and inter-specific - mandibular morphology and ontogeny has received less attention. Second, experimental studies have revealed different functional and developmental centres in the cranium and mandible. Therefore it follows logically that despite the integrative aspects of the cranio-mandibular form, these two elements may differ in the patterns and degrees of variation, suggesting that different developmental constraints operate on the cranium and mandible. Third, studies of this nature provide an important framework for understanding, evaluating and interpreting evolutionary, developmental and functional significance of morphological traits and how they change over time across even closely related species, both extinct and extant.

Materials and methods

Data

This study includes 187 mandibles (Table 1) of modern humans, chimpanzees and bonobos. The ape taxa are wild-shot individuals. The human sample comprises two populations from Portugal (Lisbon) and Alaska (Point Hope). Each specimen is classified according to respective dental development stages (Table 2). The youngest group, "Juv1", consists of individuals with permanent M1 crown exposure. Four humans, one chimpanzee and one bonobo specimen did not have fully erupted permanent M1s, but are included in the "Juv1" stage because no particular shape differences were noted between individuals with only permanent M1 crown exposure and individuals with full M1 eruption. The second category, "Juv2", comprises individuals with permanent M2 crown eruption. The "young adult" group consists of individuals with M3 and permanent canine eruption, but with the basio-spheno synchrondrosis still not fused. Finally, the fourth age

category consisted of adults, with all permanent teeth erupted and basio-spheno synchrondrosis fully fused.

Thirty 3D coordinates were taken on the entire mandible (Figure 1) using a MicroScribe G2X, following and extending on collection protocol described elsewhere (Nicholson and Harvati, 2006); Harvati et al., in press). All specimens were measured by one observer (NS). Intra-observer error was estimated by calculating Procrustes distances between the original and repeat measurements. Individuals with damaged and/or missing landmarks were excluded.

Analytical methods

We used Procrustes based geometric morphometrics in this study (Rohlf and Slice, 1990; Rohlf, 1993; Bookstein, 1996; Rohlf, 1996; Dryden and Mardia, 1998; Rohlf, 1999; Slice, 2007). Generalised Procrustes analysis (GPA) is an effective method, which extracts shape information from 2D-3D landmark data by rotating, scaling and transforming specimen landmark configurations and yields a size measure called centroid size (CS). Centroid size is the sum of squared distances of all landmarks from their mean and is also a measure of how much the landmarks disperse from their centroid; the farther the dispersion of landmarks, the bigger the CS. A principal components analysis (PCA) in shape-space was then conducted on the Procrustes shape coordinates; this was done to explore the overall shape variation in the dataset. Regressions based on PC scores on centroid size were plotted into the PCA graphs to examine and compare shape ontogenetic trajectories of the different taxa. A second analysis of PCA in form-space was conducted and plots were examined in a similar manner as in shape-space. Principal components analysis in form-space uses Procrustes registration, but reintroduces CS into the analysis, including CS as a variable in the PCA (Mitteroecker et al., 2004b). This method has been used in other studies, comparing ontogenetic trajectories in both the cranium and mandible (Mitteroecker et al., 2004a; Bastir et al., 2007). While PCA in shape-space examines ontogenetic shape changes without the effects of isometric

size, (i.e. CS), PCA in form-space addresses all aspects of size related shape changes (Mitteroecker et al., 2004a). Comparing the two analyses can lead to a better understanding of ontogenetic shape and size changes in a biological form. The PCA and regression analyses were done in programming software R (R development core team, 2008).

We analysed the collected mandibular landmarks both as a whole and also as separate "modules". Studies on mouse mandibles show that the mandible consists of two modules – anterior-alveolar region of the corpus and the posterior ramus (Atchley and Hall, 1991; Leamy, 1993; Klingenberg, 2003). We subdivided the landmarks into these two units to examine possible ontogenetic shape differences and trajectories in "modules" that are said to have different embryological origins (Moss, 1973).

In order to examine differences among ontogenetic trajectories, we compared angles between species-specific multivariate regression vectors, computed from within-species regressions of shape (Procrustes coordinates) on log-transformed centroid size (Zelditch et al., 2004); the null hypothesis being that the angle is zero, indicating parallel trajectories among taxa. The approach used here is outlined in Zelditch et al. (2000) and it basically compares the angle between ontogenetic trajectories of two species to the angles between trajectories obtained from a single taxon. The range of angles computed between trajectories within a taxon is calculated using bootstraps (n=2500) and this range is then compared to the angles between-species. If the angles between species exceed the 95% confidence range of the bootstrapped angles computed intra-specifically, then the trajectories are significantly different between taxa. The angles were computed in Integrated Morphometrics Package (Zelditch et al, 2004) and subsequent statistical tests were done in R (R development core team, 2008).

In addition, for comparative purposes we computed mean shapes from the registered Procrustes coordinates of the different age groups across taxa. Human, chimpanzee and bonobo mean shapes were tested by computing the Procrustes distances between mean shapes and running 10,000 rounds of permutation tests to ascertain the statistical significance (*p*-value*)* between the mean shapes. The

Procrustes distances between two mean shapes were tested by randomly rearranging the groups and then re-computing the mean distances each time. The *p* value is the number of these distances that are larger than the original distance divided by the number of permutations (+ 1). Statistical significance was established at $\alpha \leq 0.05$ (Good, 2000). To illustrate the shape changes we constructed wireframe and polygon diagrams in *MorphoJ.* (Klingenberg, 2008a) and *Morphologika* (O'Higgins and Jones, 1998).

To assess for effects of sexual dimorphism in the data, we computed mean shapes for the males and females of the known-sex individuals for each age group in all three taxa. We then calculated Procrustes distances between the mean female and the mean male shapes and tested the differences statistically by 10,000 rounds of permutation tests; significance was established at $\alpha \leq 0.05$ (Good, 2000). All statistical Analyses were done in R programming software (R development core team, 2008).

Results

Measurement Error: The largest Procrustes distance between the repeated specimens and their corresponding originals was 4-5 times smaller than the smallest distance between individuals in the total sample.

Sexual dimorphism

Results of the Procrustes distances computed from the mean male and female *shapes* show no significant differences among bonobos, chimpanzees and humans in mandibular shape. This is supported by results from previous studies that found male-female differences mainly in the larger apes: gorillas and orangutans (Chamberlain and Wood, 1985; Taylor, 2002; Taylor and Groves, 2003; Schmittbuhl et al., 2007). Therefore, we pooled the sexes in the following analyses.

Mandibular ontogenetic trajectories in PCA shape-space

The PCA in shape space (Figure 2A) suggests that the ontogenetic trajectories of the taxa are linear. The first two principal components account for 72.32% of the total squared covariance of the sample. *Homo* and *Pan* are clearly separated along PC 1 (63.7%). PC 2 (8.62%) separates the age groups, particularly "Juv1" humans and bonobos from older age groups. One "Juv1" chimpanzee specimen extends the range of variation of the age group into that of "Juv1" bonobos. The permanent M1 crown of this individual has not fully erupted unlike the other specimens in this group and is the youngest individual in the chimpanzee sample; this is the same for an individual in the bonobo "Juv1" age group. The older age groups overlap considerably among the taxa along this axis. Young adult and adult bonobos overlap with "Juv1" and "Juv2" chimpanzee individuals.

Shape changes along PC 1 (Figure 2A) capture the main inter-specific differences between *Pan* and *Homo* mandibles. Modern humans occupy the lower scores on PC 1, which show a parabolic shaped mandible, receding symphyseal region, but marking the presence of a chin and a deep and symmetric sigmoid notch – features characteristic of modern human mandibles. *Pan* occupies the higher scores along PC 1. There is considerable overlap between bonobos and chimpanzees along the first dimension. General mandibular morphology of *Pan* suggests an overall angular and narrow mandible compared to *Homo*. The superior symphyseal region is outwardly projected with clear absence of a chin. The sigmoid notch is shallow and asymmetric relative to humans.

PC 2 (Figure 2A) captures changes mainly among the different age groups. The polygons (Figure 2A) represent ontogenetic changes between humans and *Pan*. "Juv1" humans are clearly separated from the other age groups and show a more parabolically shaped mandible and short ramus relative to the corpus compared to the adult individuals. The symphyseal region marks the presence of a chin even in "Juv1". In *Pan*, there is a slight separation of chimpanzees and bonobos along PC 2. The ontogenetic shape changes are similar to that of humans, but the corpus length relative to the ramus is longer in juvenile *Pan* than in *Homo.*

PC 3 (Figure 2B), which accounts for 6.4% of the total squared covariance in the sample, separates bonobos and chimpanzees. PC 3 also indicates that juvenile bonobos align close to human adults. PC 3 captures differences between chimpanzees and bonobos in aspects of the sigmoid notch, which is more asymmetric in chimpanzees than bonobos, and the symphyseal region, which is more anteriorly projected in the former than in the latter.

PCA in shape-space: anterior and posterior mandibular region

In the anterior-alveolar region, the first two PCs account for 84.8% of the total squared variance in the sample. PC 1 (Figure 3A) accounts for inter-specific differences between *Homo* and *Pan* and PC 2 captures subtle differences between bonobos and common chimpanzees. There is considerable overlap among all the age groups along these two dimensions; this is similar across taxa. The overlap of the age groups in shape-space implies that shape of the anterior mandible region is achieved early in ontogeny. The main distinction between *Homo* and *Pan* in this region is due to the symphyseal outline.

Unlike the anterior mandible, the ramus (Figure 3B) separates the age groups much better, particularly "Juv1" humans along PC 1 (48.5%); "Juv1" bonobos and common chimpanzees also separate from the other age categories. PC 2 (14.6%) captures the differences among common chimpanzees and bonobos better than PC 1, however there is overlap among the younger age groups of chimpanzees and adult bonobos. The main shape differences are in the width of the mandible and aspects of the gonion region.

Shape-space suggests that while there is a clear separation between humans and *Pan* in aspects of the anterior-alveolar region, inter-specific differences are less apparent in the ramus. However, aspects of the ramus better capture ontogenetic shape changes and intra-specific variation than the alveolar region.

Results from the analysis of angles between ontogenetic trajectories within and between-species indicate that angles between humans and bonobos or chimpanzees (Table 3) are higher than the range of angles within-species. Unlike the comparison between *Homo* and *Pan*, statistically significant difference in the

trajectory cannot be established between chimpanzee and bonobo ontogenetic trajectories given the available dataset. However, this does not imply that the angle (Table 3) between the two populations is the same, but rather that we cannot reject a null hypothesis that the difference could be due to limited sampling of a variable population (Frederich and Sheets, 2010) .

Mandibular ontogenetic trajectories in PCA form-space

As seen in the PCA in shape-space, PCA in form-space (Figure 4) also suggests that ontogenetic trajectories between *Pan* and *Homo* are linear. PC 1 accounts for 68% and mainly reflects size and PC 2 accounts for 20% of the total squared variance in the sample. In humans, all age groups show some overlap along PC 1, but less so than in shape-space. A couple "Juv1" humans extend into the range of "Juv2" variation, indicating that some individuals in the younger "Juv1" group despite being larger in size than the other individuals in their group have retained the "Juv1" shape. PC 1 also separates the age groups in *Pan*, but there is considerable overlap between the juvenile common chimpanzees and adult bonobos. PC 2 mainly accounts for differences between *Homo* and *Pan*. Individuals in "Juv2" show considerable overlap with other age groups in shape-space, but separate better in form space; this size difference among age groups is also apparent in *Pan*.

Shape changes in form space are similar to those seen in shape-space, with the exception of some individuals in "Juv1", which are similar in size, but not shape to individuals in "Juv2".

PCA in form-space: anterior and posterior mandibular region

With the inclusion of size as a variable in the PCA (Figure 4A), we see a clearer distinction between the younger and older age groups in aspects of the anterior mandible, along PC 1 (50.7%). PC 2 (35.9%) mainly accounts for inter-specific differences between humans and *Pan* in form space. "Juv1" is clearly separated from the other age groups, but there is considerable overlap among the

other groups across all taxa. This is different from shape-space, where there is overlap among all age categories.

In form-space (Figure 4B) the ramus shows an even better separation of the age groups along PC 1 (72%) than in shape-space. PC 2 (13.4%) captures the differences between humans and *Pan*. As in shape-space, "Juv1" humans are completely separate from the other age groups in form space as well, suggesting that ontogenetic shape changes in the ramus are allometric rather than isometric (as seen in the anterior mandible region).

Mean shape comparisons

Table 4 and Figure 5 show detailed descriptions of all mean shapes. The adult mean forms of humans, common chimpanzees and bonobos are significantly different from each other ($p<0.0001$). "Juv1" humans are significantly different from all age groups within humans and also inter-specifically. "Juv1" chimpanzees are not significantly different ($p=0.0052$) from "Juv2" chimpanzees, "Juv1" bonobos ($p = 0.006$) and "Juv2" humans ($p = 0.008$). "Juv1" bonobos are significantly different ($p<0.0001$) in mean shape from the other age groups intra-specifically. In humans and *Pan* "Juv2-adult" individuals are not significantly different from each other (humans $p=0.3506$; bonobos $p=0.341$; chimpanzees $p=0.8411$), suggesting little difference in mean shapes among these age groups.

Discussion

In the present study our primary aim was to explore different patterns of ontogenetic shape changes in the mandible of two closely related primate taxa: *Pan* and *Homo sapiens*. Our results suggest the following: 1) ontogenetic trajectories in the mandible across these taxa are linear, but not parallel; 2) adult shape of the mandible is achieved early in ontogeny, approximately coinciding with the time of permanent M2 eruption; 3) the anterior and posterior aspects of the mandible follow different patterns of ontogenetic shape change prior to permanent M2-eruption.

Mean mandibular shape comparisons

The few shape changes in mean shape between "Juv2" and adults suggest that the adult mandible shape is established by permanent M2 exposure in both *Pan* and *Homo*. Even though our dataset does not contain infants, the taxon-specific shape differences apparent at "Juv1" suggest that mandibular shape is established earlier in ontogeny. A previous intra-specific study on human mandibles (Chen 2000) found that characteristic shape changes in the mandible take place between 11-15 years of age, coinciding with puberty, rather than younger age groups. These results differ from ours in that we find species-specific features in "Juv1" human individuals ranging from ages 5-10 years. Studies on facial ontogeny have reached similar conclusions: Cobb and O'Higgins (2004) found that ontogenetic shape differences in the face were established early in postnatal development in modern humans as well as other great apes.

Ontogenetic trajectories

With the exception of a few (Mitteroecker et al., 2004a; Mitteroecker et al., 2005; Bulygina et al., 2006; O'Higgins et al., 2006; Bastir and Rosas, 2009), most studies have found ontogenetic trajectories in the cranium to be linear (Richtsmeier and Lele, 1993; Richtsmeier and Walker, 1993; Bruner and Manzi, 2001; Ponce de Leon and Zollikofer, 2001; Zollikofer and Ponce de Leon, 2004). The latter is also true for some studies on ape mandibular growth and development, and is consistent with findings from the present study. Our results show that *Homo* and *Pan* have linear ontogenetic trajectories, which suggest that shape and size changes across the age groups in these taxa are uniform. However, given the lack of very young individuals in our sample this result more specifically indicates that ontogenetic changes in the mandible are stable and alike in these taxa post-permanent M2 eruption. For example, in a study on basicranial development, Bastir and Rosas (2009) found that basicranial growth was modular and non-linear in humans. These results were obtained from radiograph samples of infant humans. They attributed the non-linearity of the growth vectors to modular growth patterns occurring in different regions of the basicranium, implying semi-independence of basicranium

units. Given that species-specific features are found to be established prenatally and that development is modulated in the skull, reflected by non-linear growth vectors, we would have expected to see a similar trend in the mandible had we included individuals with no permanent dentition and/or a broader age range. The latter would be an interesting next step to test hypotheses of developmental modularity in the mandible. We discuss this topic briefly below.

Our results further show that mandibular ontogenetic trajectories in *Pan* and *Homo* are linear and divergent. Studies on cranio-facial growth reached similar conclusions (Lieberman and McCarthy, 1999; Strand Viðarsdo´ttir et al., 2002; Bastir and Rosas, 2004; Cobb and O'Higgins, 2004; Mitteroecker et al., 2004b; Mitteroecker et al., 2005; McNulty et al, 2006; Ponce de Leon and Zollikofer, 2006). Ponce de Leon and Zollikofer (2006) found differences between ontogenetic trajectories not only at the genus level between *Homo* and *Pan*, but also subtle differences between common chimpanzees and bonobos in their pre and postnatal development. McNulty et al.'s (2006) comparisons between developmental trajectories among gorillas, modern humans and common chimpanzees showed that these taxa did not follow a common pattern of development in the cranium. However, pair-wise comparisons between bonobos and common chimpanzees and bonobos and modern humans showed parallel developmental pathways between the taxa. The authors also concluded that the latter result could have been driven by the small sample size of bonobos rather than a developmental signal (Cobb and O'Higgins, 2004; McNulty, 2006). Following that in another study on global and local shape changes in the cranium, Mitteroecker et al. (2005) noted that bonobos and chimpanzees in fact had different growth vectors in the cranium and that shape differences seen between the taxa could not be solely attributed to heterochronic effects. While these studies provide support for divergent trajectories in the cranium, they also corroborate findings from our study that show divergent growth trajectories in the mandible, at least at the genus level.

The stark difference between *Homo* and *Pan*, to an extent, obscures the differences between bonobos and common chimpanzees. Studies by Shea (1983; 1983a; 1985) on ontogeny and allometry suggest that main differences between

common chimpanzees and bonobos are due to "ontogenetic scaling", suggesting that morphological differences in the skull (and in some aspects of the postcrania) are due to extension or truncation of a common growth vector. Even though we did not test for specific differences between common chimpanzees and bonobos, our results show that species-specific shape features in the mandible manifest early in ontogeny in chimpanzees and bonobos (Figures 2 & 3), despite chimpanzees having a slightly longer growth trajectory than bonobos. The former suggests that mandibular morphology of bonobos and common chimpanzees are not simply scaled versions of each other.

In our comparison of angles between taxa ontogenetic trajectories we could not reject the null hypothesis of parallel trajectories (angle= 0^{o}) between common chimpanzees and bonobos. Our results corroborate the findings of Bougher and Dean (2008) in that both taxa of *Pan* show species-specific features early in ontogeny, at infancy according to Bougher and Dean (2008), and parallel developmental trajectories. Moreover, in our results the mandibular variation in shape-space, particularly along PC3 (Figure 2), shows the youngest juvenile groups of bonobos and chimpanzees overlapping slightly with adult modern humans. This supports Schultz (1924) suggestions of sub-adult monkey, ape and human individuals bearing affinity to adult individuals of the respective species, particularly between human and non-human apes.

Furthermore, our analysis of PCA in form-space (Figure 4) suggests that despite overlap in shape-space, particularly among age stages "Juv2", young adults and adults, these groups do not overlap in size. This suggests that after stage "Juv2" growth in the mandible is isometric, which means that growth occurs at the same rate in different parts of the mandible, making shape changes consistent throughout later post-natal development within-species.

Functional matrix in the mandible

An important aspect of this study was to examine whether different regions of the mandible, namely the corpus and ramus, have different ontogenetic patterns of shape change between chimpanzees, bonobos and modern humans (Moss, 1973;

Atchley and Hall, 1991; Atchley, 1993; Hall, 2005). A study on African ape mandibles by Daegling (1996) found that growth in different aspects of the mandible is decoupled to an extent, contributing to species-specific size and shape changes in gorillas and chimpanzees. While the sub-divisions of the mandible in our study are not as many as in Daegling (1996), our results corroborate one aspect of Daegling's findings in showing that patterns of ontogenetic change in the ramus and the corpus are different among bonobos, common chimpanzees and humans. For instance, while the alveolar region in shape-space shows anterior projection in both chimpanzees and bonobos, the shape of the ramus particularly the gonion region is relatively different between the taxa. PCA in form space too suggests that the two regions follow different patterns of ontogenetic shape and size changes in *Pan*.

Within region analyses suggest some similarities between *Pan* and *Homo.* The anterior-alveolar region shows no overlap among the age groups in shape-space in both *Pan* and *Homo*. This implies that shape of the anterior mandible and alveolar region is fixed by permanent M1 eruption (our youngest age group) in all three taxa. However, as expected, while shape of the anterior region is established by "Juv1" stage, size of "Juv1" individuals is distinct from the other age groups as shown in form-space (Figure 4). That is, "Juv1" individuals in *Pan* and *Homo* do not overlap in *size* with the other age groups in their respective species despite overlapping in shape. The lack of overlap in shape, but not in size suggests isometric growth in the alveolar region. In contrast, analyses of the ramus region separately shows "Juv1" individuals of all three taxa to be distinct in both shape and size, suggesting a different developmental pattern in the ramus than the anterior-alveolar region.

These findings can be interpreted in light of the "functional matrix hypothesis" which posits that different regions of the mandible carry different ontogenetic signals. The morphology of the corpus, particularly the anterior-alveolar region, is predominantly driven by the size and shape of the developing dentition and may be more constraint, whereas the ramus maybe less so due to different types of functional demands such as masticatory stress and strain. However, both the corpus and ramus exhibit the same developmental pattern post-M2 eruption, which is consistent with results from the analysis of the mandible as

one unit, suggesting that overall mandibular shape is established by "Juv2" stage in both regions of the mandible in *Pan* and *Homo*.

Conclusions

Five main conclusions can be drawn from this study. First, species-specific features in the mandible are established early in ontogeny, possibly prior to permanent M1 eruption. In particularly, morphological distinctiveness between *Homo* and *Pan* and also to an extent between bonobos and chimpanzees makes the mandible a good candidate for taxanomic evaluation. Second, overall mandibular shape in *Pan* and *Homo* is established by permanent M2 eruption. Third, ontogenetic trajectories between *Homo* and *Pan* are divergent. Direction and pattern of ontogenetic trajectories may in part explain species-specific morphological features that arise during ontogeny. Parallel trajectories across taxa imply that patterns and degrees of shape change influenced by functional/biomechanical and/or developmental processes are conserved among taxa. Divergent ontogenetic trajectories between *Homo* and *Pan* imply that the evolutionary/developmental processes that influence mandibular morphology are different. Fourth, our results closely corroborate findings of studies on cranial ontogeny that suggest different divergent ontogenetic trajectories among hominids, particularly *Pan* and *Homo*. However, our results also suggest that mandibular shape stabilises early in post-natal ontogeny, unlike the cranium that shows continuous ontogenetic variation in shape and size throughout post-natal development as reported by Richtsmeier et al. 1993. Fifth, the hominid mandible consists of semi-independent growth regions and further analysis of integration and modularity are essential to fully understand mandibular ontogeny in primates. A modular approach of compartmentalising structural units, such as examining the corpus and ramus of the mandible, might offer more information on the processes (i.e. morphological integration and modularity) that underlie mandibular form. Even though we did not apply an integrative approach to address inter-specific differences in mandibular morphology, this study demonstrates that different parts of the mandible, such as the corpus and ramus, are semi-independent. Further investigation of the patterns

of integration not just between the corpus and ramus, but among other elements such as the coronoid and condylar processes, will shed light on possible factors that contribute to species-specific morphological change.

Acknowledgements

We thank the following curators for allowing us access to the various collections: Dr. Emmanuel Gilissen and Wim Wendel of the Royal Museum of Central Africa, Tervuren; Diana Carvalho of Museu Nacional de História Natural, Lisbon; Ian Tattersall and Gisselle Garcia of the American Museum of Natural History, New York; Dr. Hendrik Turni and Dr. Frieder Mayer of the Naturkunde Museum, Berlin and Dr. Katrin Schaefer of the University of Vienna. We thank Dr. Kieran McNulty for commenting on the manuscript and greatly improving its content. We are grateful to Dr. Philipp Gunz for assistance with R programming software and Dr. Jean-Jacques Hublin for feedback. This work was supported by the Marie-Curie "EVAN" training network (MRTN-CT-019564) and the Max Planck Society.

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Table 1: Specimens used in this study

Species	No. of Individuals	Source
Pan troglodytes	54	Royal Museum of Central Africa, Tervuren Natural History Museum, Berlin Max Planck Institute for Evolutionary Anthropology, Leipzig
Pan paniscus	56	Royal Museum of Central Africa, Tervuren
Homo sapiens	77	Natural History Museum, Lisbon American Museum of Natural History, New York
Total	187	

Table 2: Description of dental development stages used in this study

Age groups	Dental developmental stages	Humans	Chimpanzees	Bonobos
Juv1	Permanent M1 exposed and/or erupted	14	4	6
Juv2	Permanent M2 erupted	4	9	10
Young adult	M3 exposed and/or erupted, but basio-spheno synchrondrosis still not fused	10	6	6
Adult	Permanent M3 erupted and basio-spheno synchrondrosis fused	49	35	34
Total		77	54	56

Table 3: Angles computed between species ontogenetic trajectories

	Homo sapiens	*Pan paniscus*
Pan paniscus	40.1°	
Pan troglodytes	40.9°	21.4°
Anterior-alveolar region		
	Homo sapiens	*Pan paniscus*
Pan paniscus	60.2°	
Pan troglodytes	57°	20.7°
Ramus		
	Homo sapiens	*Pan paniscus*
Pan paniscus	40.7°	
Pan troglodytes	43.7°	27.6°

Table 4: Description of ontogenetic mean shape differences within and between taxa

Inter-specific mean shape comparisons

Adult	The human mean mandibular shape is markedly more parabolic, with a slightly taller and narrower ramus compared to the corpus, than *Pan*. However, the most distinct feature of humans is the symphyseal outline, which marks the presence of a chin, whereas in *Pan* protuberance of a chin is absent. The sigmoid notch is deeper and more symmetric in humans than *Pan*. Between chimpanzees and bonobos, the former has a deeper and more asymmetric notch than the latter. The gonion region is distinct in humans, and also different between chimpanzees and bonobos. Chimpanzees have a gonion that is more laterally flared than bonobos. The anterior region of the mandible is outwardly projected in *Pan*, particularly in chimpanzees. Chimpanzees have the narrowest (bilaterally) mandible compared to the other two taxa.
Sub-adult	Juvenile humans have the most parabolic shaped mandible, a trait also evident in the adult form. Bonobos have a slightly more parabolic shaped mandible than chimpanzees. Humans also have the shortest overall mandible, with a short corpus relative to the ramus. *Pan* has longer corpora relative to the length of the rami. Even the youngest human individuals show a clear presence of a chin. In *Pan*, even "Juv1" individuals show an anterior projection of the anterior-alveolar region and absence of a chin, but juvenile chimpanzees show slightly more anterior projection of the alveolar than bonobos. The sigmoid notch is deep and symmetric in humans, whereas in *Pan* the deepest point is closer to the coronoid process, particularly in chimpanzees.

Intra-specific mean shape comparisons

Human	The ramus in "Juv1" individuals is relatively shorter than the corpus. The marked parabolic shape of the mandible and a chin is established early in ontogeny. The sigmoid notch is deep, with the deepest point being centrally located along the sigmoid curve. The gonion region is laterally flared in adults compared to the juveniles. Adults show a taller ramus relative to the length of the corpus. There are few shape changes from "Juv2" to adults, suggesting that characteristic features of the human mandible are established early in ontogeny.

Bonobo "Juv1" bonobos have a more parabolic shaped mandible than the older individuals. The symphyseal outline even in the youngest group shows anterior projection of the alveolar region and absence of a chin. In "Juv2", there is an increase in the height of the ramus relative to that of the corpus length and also an overall narrowing of the mandible. Aside from anterior projection of the alveolar region and narrowing of the overall mandible, there are few changes among stages Juv2-adult.

Chimpanzee The general ontogenetic shape changes are similar to humans and bonobos in that juveniles have more parabolic shaped mandibles than adults. Symphyseal outline shows anterior projection of the alveolar region even in juveniles and absence of a chin. The ramus is relatively shorter than the corpus in "Juv1" individuals, but increases in height (relative to the corpus) through ontogeny. The sigmoid notch is deep relative to bonobos, the deepest point being anterior - closer to the coronoid process. However, overall mandibular morphology is achieved after permanent M1 eruption.

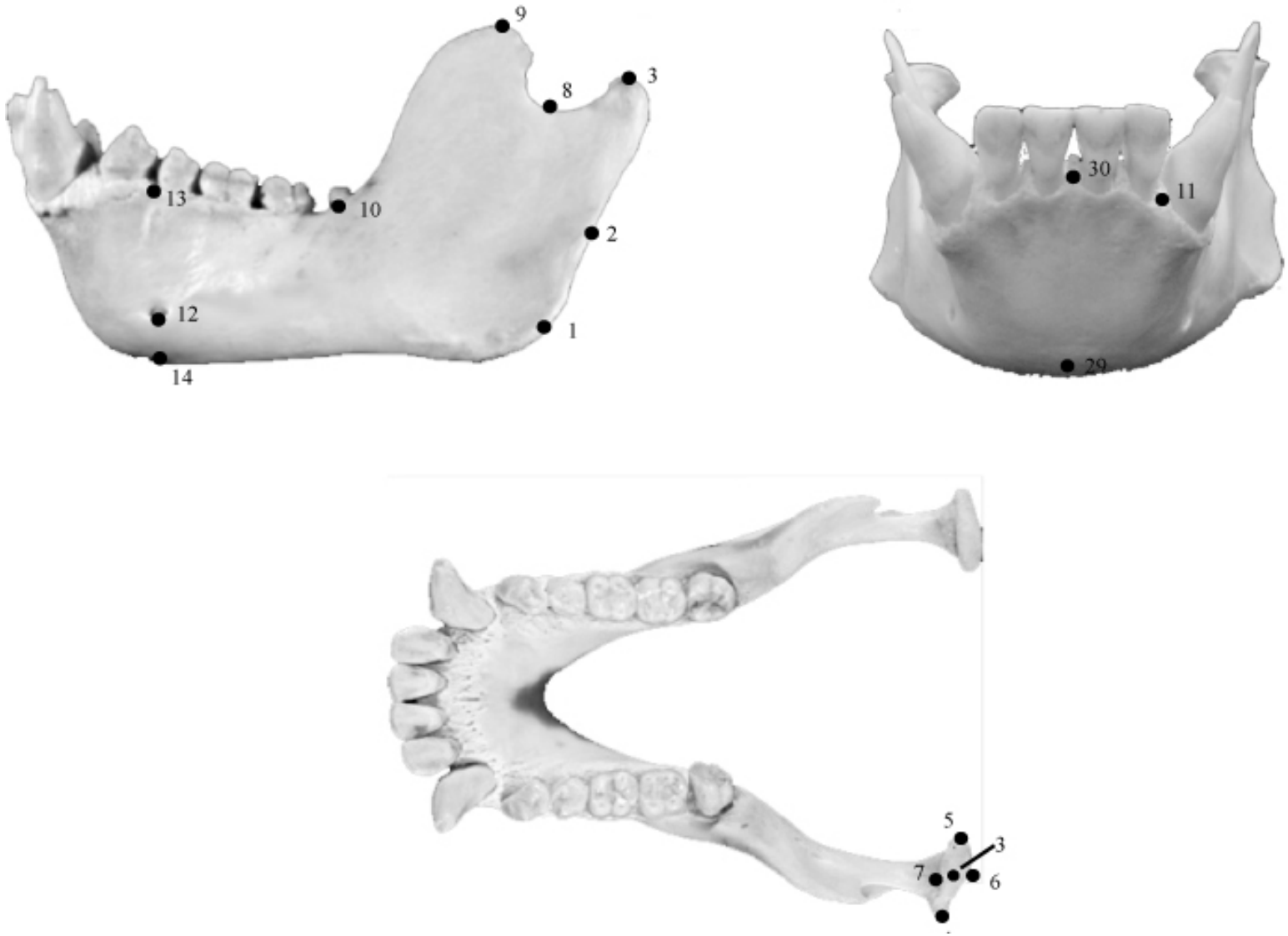

Figure 1. Mandibular landmarks included in this study
1. Right gonion, 2. Right posterior ramus, 3. Right condyle, 4. Right condyle medial, 5. Right condyle lateral, 6. Right Condyle Posterior, 7. Right root sigmoid root, 8. Right deepest point on sigmoid notch, 9. Coronoid process, 10. Right inferior anterior ramus, 11. Right 12-Canine alveolar septum, 12. Right mental foramen, 13. Right alveolar border of ramus, 14. Right inferior border of ramus, 15-28 are landmarks taken on the left side, 29. Gnathion, 30. Infradentale.

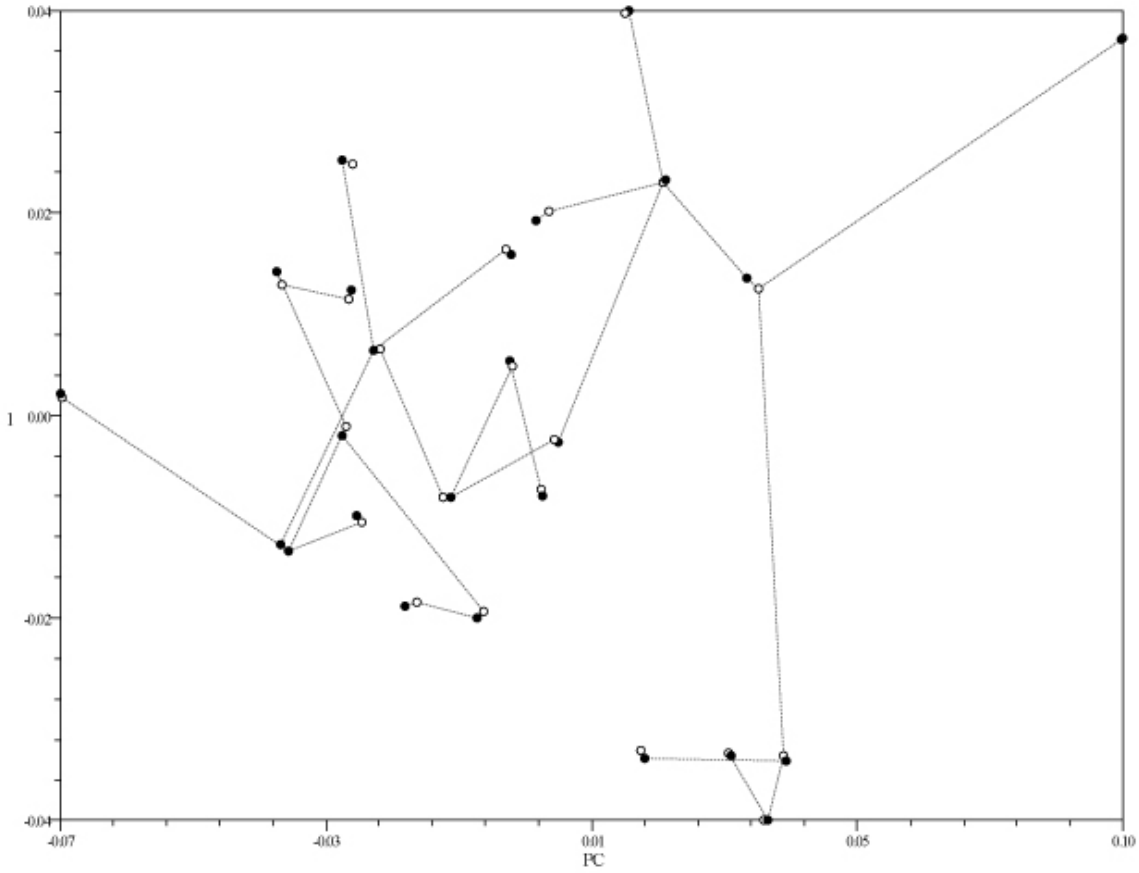

Figure 2: Plot of minimum spanning tree plot showing the Procrustes distances between original and replicate measurements; specimens from all taxa included in this study are represented here. The white dots are the originals and the black dots the replicates.

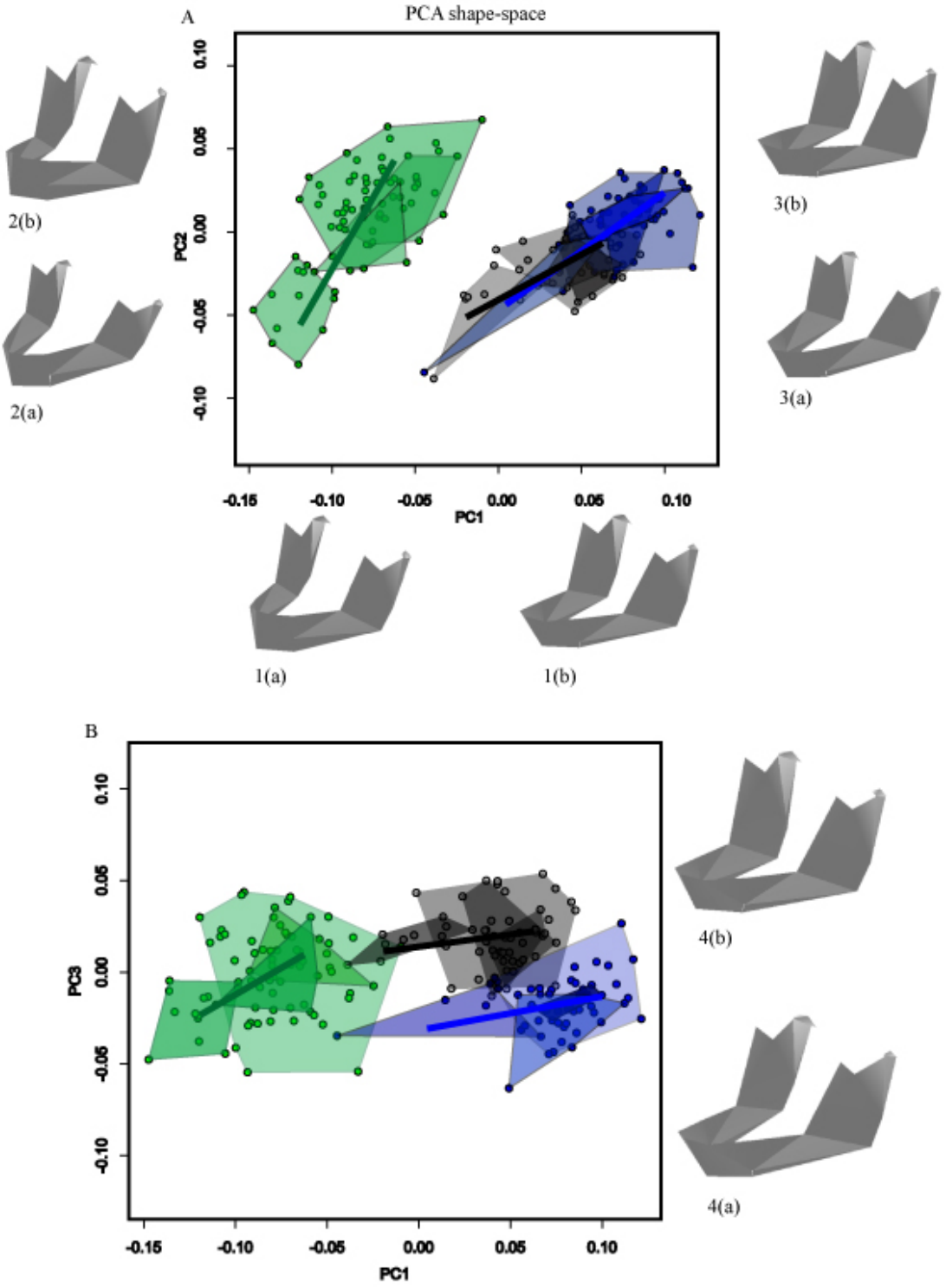

Figure 3. Ontogenetic changes in the mandible in PCA shape-space. A. PC1 vs. PC2: Humans are represented in green, bonobos in black and chimpanzees in blue. Regression vectors are plotted into the graphs. Polygons 1a & b represent overall shape difference between humans and chimpanzees along PC 1. 2a & b represent ontogenetic shape change in humans, particularly between "Juv1" (2a) and the other groups (2b). 3a & b represent ontogenetic shape changes in *Pan* from "Juv1" bonobos

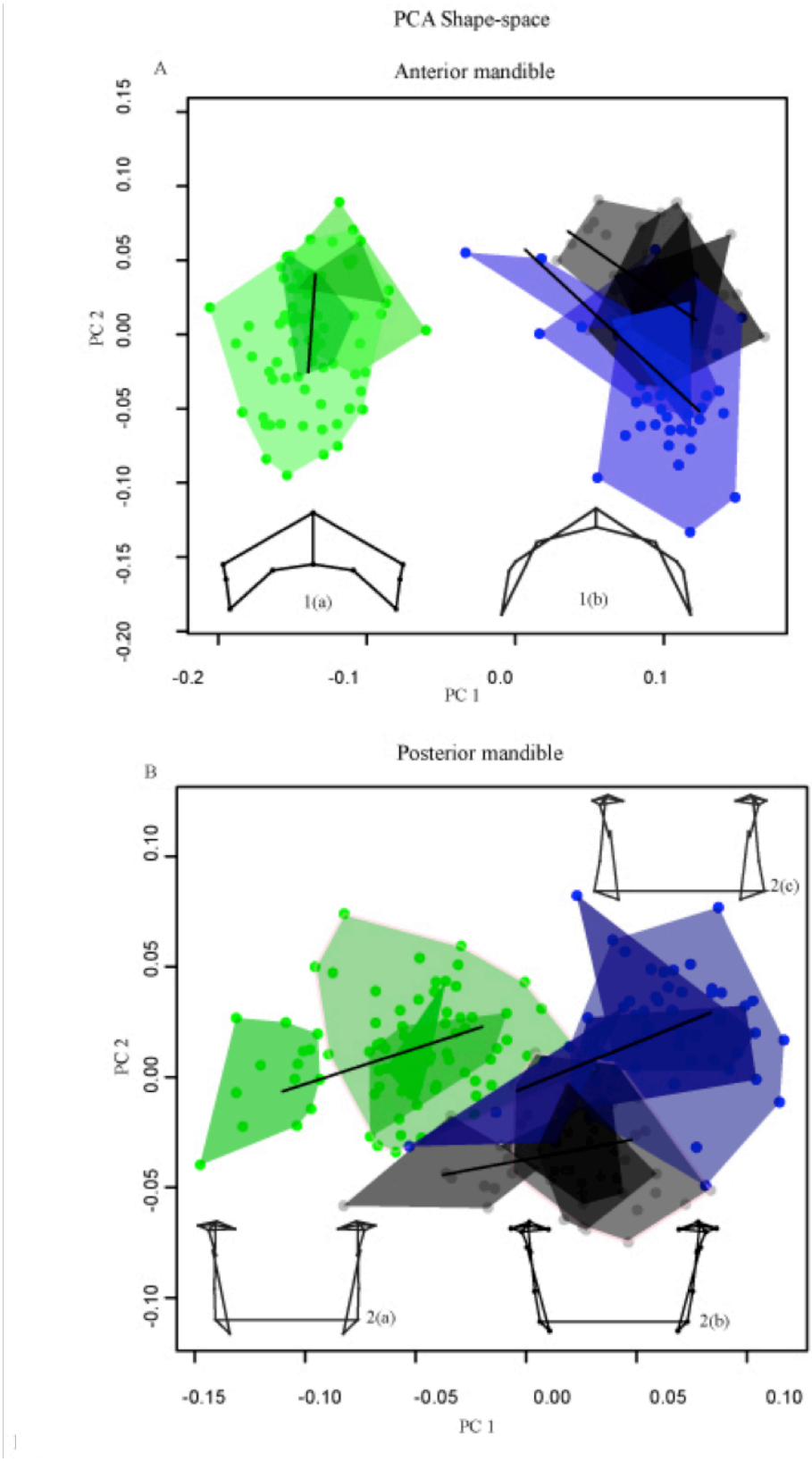

Figure 4. Ontogenetic changes in the anterior-alveolar region and ramus in PCA shape-space: A. PC1 vs. PC2 of the anterior alveolar region: Humans are represented in green, bonobos in black and chimpanzees in blue. Regression vectors are plotted in the graphs. Wireframes 1a & 1b represent shape changes between humans and *Pan* on PC1. B. PC1 vs. PC2 of the ramus: Wireframes 1a, b & c represent shape changes between humans (a) bonobos (b) and chimpanzees (c).

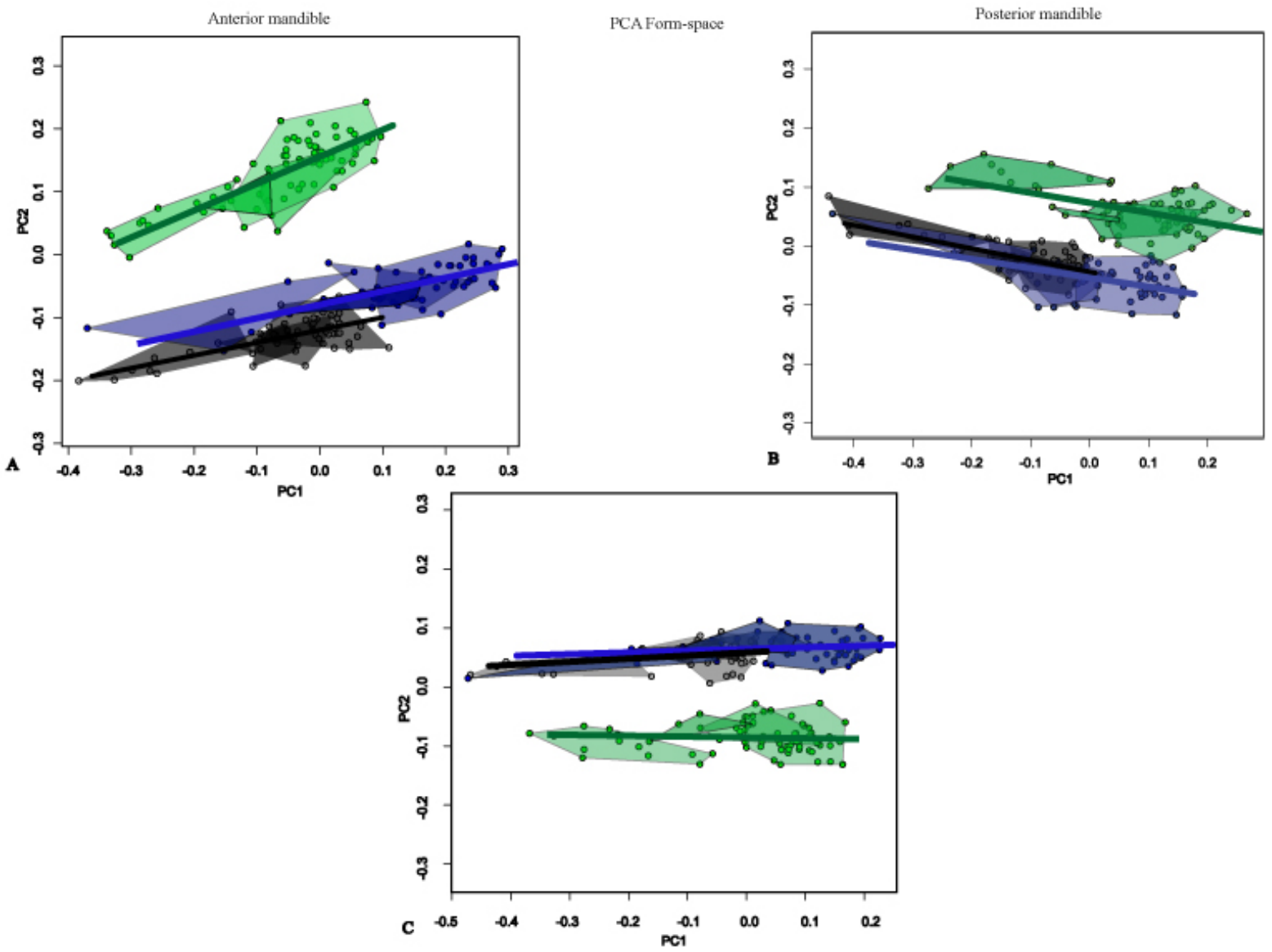

Figure 5. Ontogenetic shape changes in PCA form-space. Humans are represented in green, bonobos in black and chimpanzees in blue. Regression vectors are plotted into the graphs. A. Anterior-alveolar region of the mandible; B. Ramus; C. Complete mandibular form

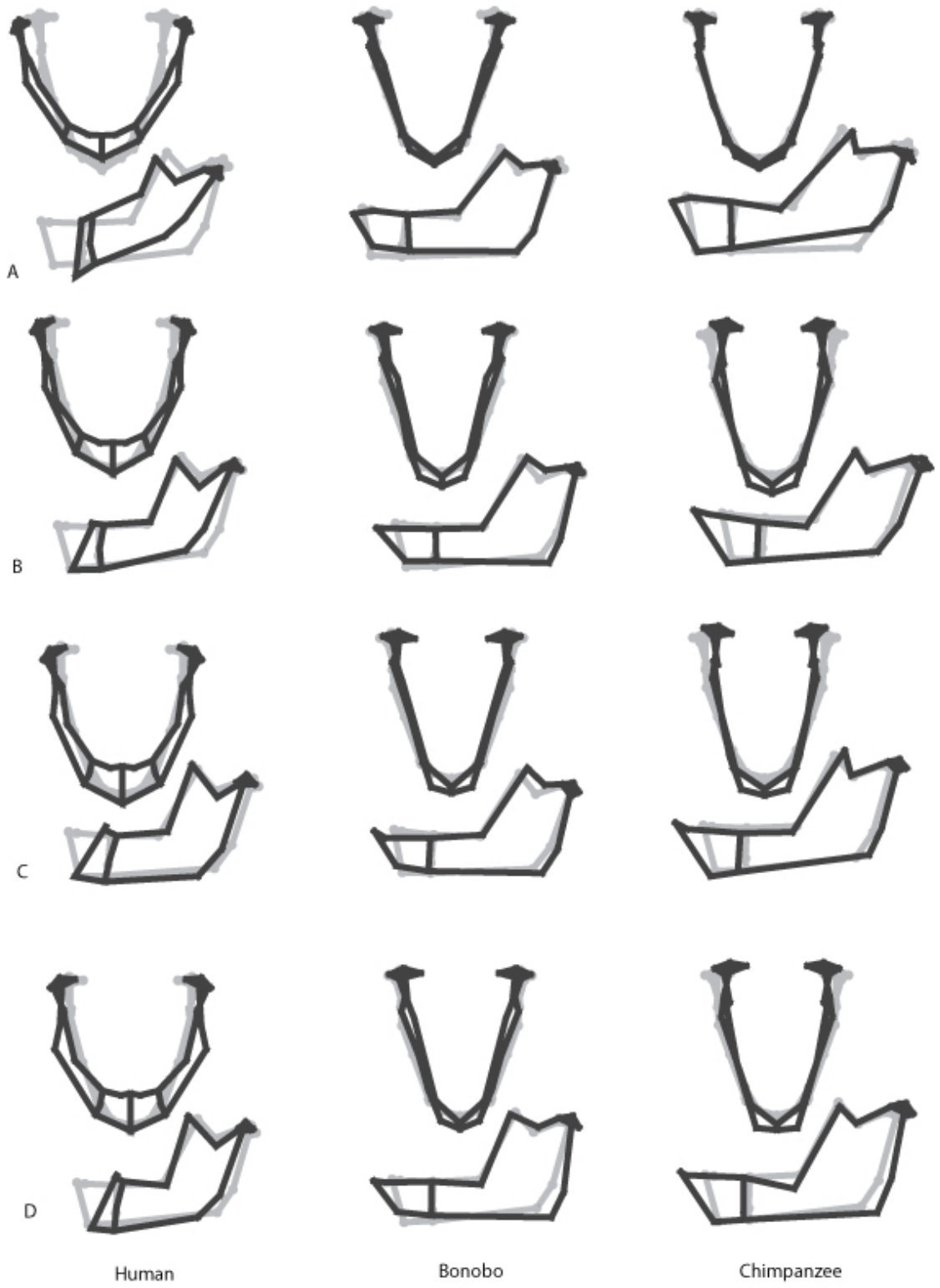

Figure 6. Wireframe diagrams illustrate ontogenetic mean shape changes within and between species. Wireframes in gray represent the grand mean shape of the respective age groups across species and the black represent the species-specific mean shapes. A. "Juv1"; B. "Juv2"; C. "Adolescents; C. "Adults".

Chapter 4: Future Projects

Future projects

Project 1: Patterns of mandibular integration in Pan and Homo

Co-variation patterns

Mandibular integration has been widely researched in mouse mandibles (Leamy 1984; Atchley et al. 1985; Atchley and Hall 1991; Atchley 1993; Leamy 1993; Cheverud et al. 1997; Klingenberg 2003b; Willmore et al. 2009a). Extensive genetic research on murine mandibles has contributed greatly to our general understanding of mandibular integration and development, and allows for further examination of variation/co-variation patterns in a paleoanthropological context. Previous studies have shown that the alveolar (tooth bearing corpus region) and ascending ramus are two key regions of variation in the mandible. Thorough examination of extant hominid patterns of variations, especially in complex structures such as the mandible, will facilitated informed interpretations of the fossil record along with a better understanding of factors that generate morphological variability (evolvability and constraint) and variation.

Objectives

1) Examine the pattern of variation/co-variation between the alveolar-anterior and posterior (ramus) region in *Homo* and *Pan*.
2) Examine the level of modularity between the two regions in *Pan* and *Homo*.

While there have been a number of studies on different aspects of mandibular morphology (Smith 1983; Ravosa 1989; Daegling 1996; Chen et al. 2000; Schmittbuhl et al. 2002; Guy et al. 2008; Miller et al. 2008; Willmore et al. 2009b), no study on primate mandibles has focused exclusively on aspects of co-variation. For instance, co-variation patterns can provide insight into the interaction and relationship between morphological structures. That is, even though aspects of a phenotype, for example the shape of the mandible in common chimpanzees and

bonobos may be similar, two or more components of the mandible may co-vary differently between the taxa. However, this study will not make any assumptions about modularity or strength of co-variation between the corpus and ramus, but rather focus on the pattern of variaton/co-variaiton between the two regions.

Materials

The sample used in this study will comprise 200 adult specimens of common chimpanzees, bonobos and modern humans. Forty 3D landmarks representing the entire mandible were measured by a MicroScribe G2X digitiser. Specimens were mounted on moulding clay to prevent them from moving during digitising. Only specimens with all landmarks present and with minimal damage were included in the analysis.

Methods

Procrustes based geometric morphometric methods will be used to analyse the data. Landmark configurations for each specimen will be superimposed using the generalized Procrustes superimposition (GPA) method (Rohlf and Slice 1990; Rohlf 1999). GPA is used as a standard procedure in most recent geometric morphometric studies on shape analysis. The procedure involves extracting shape coordinates by translating, scaling and rotating the landmark configurations; hence, removing all information unrelated to shape. This method uses the sum of squared distances between corresponding landmarks as a criterion to minimize differences between the specimen landmark configurations. A size measure, commonly known as centroid size, is obtained for each specimen (Dryden and Mardia 1998).

Analyses

To analyse overall variation in adult mandibular morphology of *Pan* and *Homo*, we will conduct a principal components analysis of the Procrustes shape coordinates. To examine morphological integration between the two mandibular components, the landmarks of the anterior and posterior regions will be first sub-divided and subjected to separate GPA. This will be done in order to minimise

possible co-variation between the two regions generated by an overall Procrustes fit. Then, a pooled within-group 2B-PLS (partial least squares) analysis will be conducted to examine the pattern of co-variation between the two regions. A pooled within-group analysis allows the examination of patterns of co-variation for the *same* shape features; it is aimed at estimating possible inter-specific differences between co-variation patterns.

Project 2: Developmental trajectories in different parts of the cranium: a look at developmental modularity

Ontogenetic trajectories and modularity

Several inter-specific studies have been conducted on cranial ontogeny, with the primary aim of comparing overall cranial ontogenetic trajectories between taxa. One specific study by Mitteroecker et al (2005) examined developmental trajectories in the upper and lower face of common chimpanzees and bonobos, concluding that the two taxa followed a different pattern of development in the face. Their study provides an interesting framework to examine questions on ontogeny of different cranial components. According to the predictions of the functional matrix hypothesis (Moss 1997(a); 1997(b); 1997(c)), different regions of the cranium carry different developmental and functional signals, and are therefore, "modular" to an extent. The basic premise of this hypothesis is that different cranial components are influenced by muscle loadings (responsible for bone morphogenesis) and the developing brain, orbits and pharyngeal cavities. The former were referred to as periosteal matrices and the latter as capsular matrices (Moss 1968).

In this study we will examine postnatal ontogenetic trajectories separately for the basicranium, cranial vault and face in *Pongo*, *Gorilla*, *Pan* and *Homo* with the intention of comparing the developmental pattern in the cranium within and between species.

Objectives

1) Within-species: To examine ontogenetic trajectories in the basicranium, cranial vault and face of great apes and modern humans. We will evaluate whether the trajectories of these cranial regions is linear or curvilinear.
2) Between-species: To examine developmental trajectories in the three cranial regions across great apes and modern humans. We will evaluate whether the face, basicranium, cranial vault have parallel or divergent trajectories in extant hominids.

Materials

A total of 500 adult and sub-adult specimens from *Gorilla*, *Pan*, *Homo* and *Pongo* will be used in this study. Landmarks set used will comprise of 60 3D landmarks and 4 curves (semi-landmarks). The data have already been collected as part of this thesis.

Methods

Procrustes (GPA) based geometric morphometrics will be used in this study (please see chapter 2 for details). The statistical methods in this study will comprise: first, principal components analysis to explore overall shape variation in the three cranial regions; second, multivariate regression analysis to compute the ontogenetic trajectories within and between species to compare linearity vs. non-linearity and parallel vs. divergent trajectories; third, pair-wise quantitative measure of the angle between ontogenetic shape trajectories of the different taxa to examine parallel vs. divergent trajectories.

Project 3: An assessment of directional asymmetry in the cranium of Gorilla, Pan and Homo

The proposed project will be the first of its kind to conduct an in depth study of cranial asymmetry and its implications for the evolution and development of human and non-human primate crania. This project will primarily address patterns and degrees of cranial directional asymmetry (DA). DA occurs when there is a consistent bias towards one side of a bilateral body part. The premise is that in optimal conditions development generally produces symmetric forms. In contrast, perturbations caused due to inhospitable genetic and/or environmental conditions during development manifests in deviations from biological symmetry.

Evolutionary context

Asymmetries are observed in both the plant and animal kingdom. Questions on bilateral asymmetries have fascinated evolutionary and developmental biologists since the time of Paul Broca and Marc Dax, particularly in the context of human brain development and handedness. Since the turn of the century, quantification and statistical analysis of asymmetric variation has gained momentum in most biological fields and widely studied in the context of human handedness, language acquisition and laterality of the nervous system and the brain. There have also been studies on the human face, most of which have found a definite asymmetric component in the face. However, very few studies have addressed the evolutionary aspects of asymmetry – how did it evolve in us and is it present in our closest primate relatives?

Ever since the controversial discovery of *Homo floresiensis*, there has been an ongoing debate on whether the Flores fossils represent pathological modern humans or whether they are a distinct species from us. These questions were mainly raised due to the size and asymmetric nature of the fossil cranium. Taphonomic processes resulting in distortion of the specimen definitely are an

important factor in this discussion; however, the diminutive size and cranial bilateral asymmetry are distinct features and not attributed to taphonomy.

Living species are often used as analogues for fossil taxa because studying asymmetry in fossils is problematic because of taphonomic distortion and lack of specimens. A comprehensive study of DA among extant analogues, particularly modern humans, is lacking in evolutionary anthropology. When living taxa are used as analogues for extinct species, an implicit assumption is made that hominids vary in the same way. One aspect of this project will address that claim by comparing patterns of cranial DA across different species and evaluate the *range* (if present) of asymmetric variation in extant hominoids.

Objectives

To investigate cranial asymmetries in humans and closely related hominid species (*Gorilla*, *Pan* and *Homo*) in an adult sample. The main question addressed here will be:

a. Is there a left or right side bias in extant hominoid crania? And is the pattern constant across all taxa included in the study?

Development context

Quantitative studies on the brain have shown differential patterns of growth in terms of size, between the left and right hemispheres of the brain. The bony skull components developing to fit the growing brain should also exhibit some aspects of this asymmetry. We predict that the right side will be larger than the left, more so in bony elements of the neurocranium that encase the brain, than the facial skeleton. A certain amount of natural asymmetry in the skull is always present. However, what degree and pattern of asymmetry is considered "normal"? The two objectives listed above will evaluate the developmental aspects of asymmetric variation, along with estimating the level of asymmetry between pathological and non-pathological individuals. The latter is included in the study because microcephaly is a condition that affects the brain developmentally and has been widely debated in the context of *Homo floresiensis*. This project will provide important insight into the

developmental aspects of asymmetry in the context of both living and extinct hominoid taxa.

Objectives

(a) To examine the level and pattern of DA across an ontogenetic series of extant hominoids. The main question answered here will be:

- Do extant hominoids exhibit different patterns and levels of directional asymmetry at different age stages? And are these patterns similar or different across species.

Materials

The data for this study has already been collected during the course of my PhD, and which will comprise 450 crania of adult and subadult individuals of *Gorilla*, *Pan* and *Homo*. Because our questions are mainly concerned with bilateral asymmetry, all individuals were measured twice in order to ensure accurate estimation of the asymmetric signal in the dataset. Prior to collecting data for my PhD, I conducted a test to assess the effects of systematic measurement error due to handedness. A symmetrical spherical calibration device used to calibrate a surface scanner was measured repeatedly to estimate whether the observer introduced a right-handed bias in the dataset. The measurements were then subjected to a Procrustes ANOVA test (please see results section for detailed explanation) and results showed that the data were free of handedness effects. This is an *indispensible* test for morphometric studies on directional asymmetry and will be conducted again prior to collecting data.

Methods

This project will employ the landmarks-based method of 3D geometric morphometrics to achieve the above-mentioned objectives. A variety of different statistical techniques can be used to analyse the landmark configurations collected in this manner. Here, we will employ a widely accepted method called Generalised Procrustes Analysis (GPA). This method facilitates the analysis of biological shape, and is designed to analyse multiple specimen landmark configurations at a time.

Analyses

This study will use the Procrustes method as proposed by Klingenberg and McIntyre (1998; Klingenberg and McIntyre 1998) for examining asymmetric shape variation in the dataset. Bookstein (1996a) and Auffray *et al.* (1996; 1996) were the first to combine geometric morphometrics in analyses of asymmetry, but their approach was modified by Smith *et al.* (1997) and later by Klingenberg and McIntyre (1998). The method proposed by Klingenberg and McIntyre (1998) is analogous to the two-factor ANOVA model developed by Palmer and Strobeck (1986). The Procrustes ANOVA calculates directional asymmetry in aspects of shape by first scaling the landmarks to unit centroid size (as mentioned above), reflecting one side to its mirror image and then superimposing all specimen landmark configurations. In addition, the Procrustes ANOVA also estimates fluctuating asymmetry and measurement error in the dataset.

The differences in shape and size variation in DA will be tested by the methods outlined in Klingenberg *et al.*(1998), which also account for effects of antisymemtry in the dataset. Briefly, their approach uses *t*-test to test significant differences in size and T^2- test to test significant differences in shape. Shape variation in the sample can be further visualised using multivariate statistical approaches such as principal components analysis (PCA), which is an ordination method and involves the decomposition of the data covariance matrix. Other approaches such a PCA of form space (size and shape) and multivariate regressions will also be conducted to extract maximum information from the dataset. All analyses will be conducted in programming software R and software package *MorphoJ*.

Chapter 5: General conclusions

CONCLUSIONS

The main focus of this thesis was to investigate the evolution and patterning of covariance structures in extant hominid skulls. Covariance structures reflect the organisation of organisms into structures or units that share environmental, genetic, developmental and functional processes. In addition, covariance structures often maintain the conservative or evolvable nature of phenotypes and provide insight into the underlying developmental system. The three individual studies in this thesis focused on morphological integration, canalisation and developmental stability – processes directly responsible for influencing phenotypic evolution. Thus far these concepts have not been comprehensively examined in an anthropological context and little work has been done on a genera wide sample. Therefore, this thesis provides a framework for future studies on primates that aim to address anthropological questions in an evolutionary-developmental biological context.

Morphological integration in the hominid cranium

In recent years, morphological integration in the primate cranium has been a popular subject of investigation in anthropology. One common outcome of studies on primates has been that patterns of cranial integration - that is, the way in which primate crania are integrated, is similar, suggesting that cranial integration is conserved across primates. However, majority of these studies investigated the common aspects of cranial integration. This study was the first of its kinds to examine species-specific patterns of integration across a genera wide sample of all extant hominids. I used cutting edge quantitative and statistical techniques to analyse patterns of integration or covariation across taxa. Results from our study suggest that the overall patterns of integration across *Homo*, *Pan*, *Gorilla* and *Pongo* are similar, but these taxa also exhibit distinct species-specific differences in some aspects of associated shape change between cranial regions; these differences are particularly apparent between human and non-human apes.

So, how can integration studies inform us about the evolution of the primate cranium? These results suggest that cranial integration among extant hominids is conserved, but that these patterns also have the potential to evolve over macroevolutionary time scale. This in turn means that the direction of shape change among even closely related taxa is susceptible to vary, a result that differs from previous studies on cranial integration in primates. The similarities and differences between taxa can be explained in terms of changes in the variance generating developmental processes (Hallgrimsson et al. 2007a; Mitteroecker and Bookstein 2008). For instance, humans tend to be the most distinct among extant hominids and these differences can possibly be explained in terms of how brain growth alters the covariance structures of the face and cranial vault differently than it does in smaller brained hominids (Lieberman et al. 2000a; Lieberman et al. 2002).

So, what does this mean for fossil species? Unfortunately, integration studies are difficult to conduct with fossil specimens because they tend to be few and fragmentary. By using extant analogues we can approximate possible patterns of integration and direction of covarying shape changes in fossil species. In addition, aside from providing insight into the evolutionary direction of shape change, morphological integration also has direct bearing on character assessment in phylogenetic analyses. The nature of states and character complexes is called into question because integration among morphological structures and/or traits questions the reliability of "independent" characters. Accounting for the integrative nature of complex morphological structures, such as the primate skull, is particularly important for studies that determine phylogenetic relationships based on discrete characters.

Covariance structures in the hominid cranium – a developmental perspective

Covariance structures in complex phenotypes such as the primate cranium result from different developmental and environmental factors and thus reflect different interactions at different stages of development (Hallgrimsson et al. 2007a). In this study I investigated the influence of canalisation and developmental stability in different regions of the cranium within and between taxa. Canalisation was

measured as among individual variation and developmental stability as the covariance of fluctuating asymmetry (Klingenberg et al. 2001). With the advent of techniques such as 3D geometric morphometrics shape analyses in a wide range of contexts was made possible and more efficient. This has been particularly true for studies on asymmetry because geometric morphometric techniques preserve the entirety of the biological form.

A study examining the patterning of cranial covariance structures with respect to canalisation and developmental stability has not been conducted before on extant hominids. This work directly addresses and provides insight on how certain underlying developmental processes influence cranial covariance and overall cranial evolution. The most striking finding of this study was that canalisation and developmental stability are distinct processes in the hominid cranium. This suggests that the possible functional and/or genetic origin of these two processes is different. In addition, the high correlations between species symmetric and asymmetric covariance matrices, respectively, further suggest that developmental processes that maintain cranial covariance are similar across extant hominids. However, results from this study also demonstrate that covariance structures in the cranium have a complex and integrated relationship to the underlying developmental interactions and that it is problematic to pin-point the precise influence of developmental processes that maintain covariance structure in the hominid cranium.

Ontogeny and integration the hominid mandible

In the third study we looked at ontogenetic and integrative changes in different aspects of the *Pan* and *Homo* mandible. This study ties in with the previous two in that it addresses yet another important aspect of morphological integration – ontogenetic integration and allometry. Specifically, I set out to compare ontogenetic trajectories and patterns of shape change in the posterior and anterior mandible to test the validity of the "functional matrix hypothesis". The main conclusions that can be drawn from this study are that *Pan* and *Homo* have divergent ontogenetic trajectories – a result that has been found with respect to

cranial developmental trajectories as well. However, this does not imply that the cranium and mandible have similar developmental trajectories. Moreover, species-specific differences, even between bonobos and chimpanzees, emerge early in ontogeny. Our results also demonstrate that the corpus and ramus units of the mandible are semi-independent and do not share the same developmental pathways. Above all, these results emphasise the need for further research into the integrative nature not only of the primate mandible, but also between aspects of the cranium and mandible.

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Acknowledgements

I thank the following curators for allowing me access to the various museum collections: Dr. Emmanuel Gilissen and Wim Wendelen of the Royal Museum of Central Africa, Tervuren; Diana Carvalho of Museu Nacional de História Natural, Lisbon; Ian Tattersall and Gisselle Garcia of the American Museum of Natural History, New York; Dr. Hendrik Turni and Dr. Frieder Mayer of the Naturkunde Museum, Berlin; Dr. Katrin Schaefer of the University of Vienna and Dr. Richard Kraft of the Zoologische Staatssammlung, Munich.

Katerina Harvati my PhD adviser at the University of Tuebingen, for her tremendous support and encouragement throughout my PhD. Katerina also provided me with multiple opportunities to participate in fieldwork, research grants and collaborative projects outside my PhD research, which benefitted and influenced my work greatly. I am grateful to my co-supervisor, Jean-Jacques Hublin of the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) for giving me the opportunity to be a part of a multi-disciplinary Marie-Curie fellowship, the "EVAN" training network, and allowing me to access the facilities at the MPI-EVA. This research would not have been possible without the methodological skills I acquired as a graduate student in the "EVAN" network. I thank my external adviser, Chris Klingenberg of the University of Manchester, who provided invaluable assistance with statistical analyses and constant feedback on my work. His research inspired some of the models I used in my PhD. I will always be thankful to him for taking me on as a student and being a great teacher – not to mention the tremendous patience with which he addressed my incessant questions and confusions!

I cannot thank Philipp Gunz and Philipp Mitteroecker enough for taking the time out to discuss, provide constant feedback and assistance with statistical analyses and computer programming. I learnt a lot from my interactions with them and will always be grateful for their help. Other people who helped me at different stages of my PhD include: Markus Bastir, Katrin Schaefer, Neus Martinez, Paul O'Higgins, Demetrius Halazonetis, Abby Drake, Sam Cobb and Fred Bookstein – I could not have done this without their help and advice.

I am extremely grateful to two people instrumental in encouraging me to do a PhD in the first place: Leslie Aiello and Jon Wagner. If it had not been for Leslie, my M.Sc. dissertation adviser, I would never have approached Katerina and Jean-Jacques with a PhD

project. She has been a great inspiration to me for the last 7 years. Jon Wagner, my undergraduate adviser, encouraged me every step of the way and never faltered to show his support by inviting me to give talks, presentations and graduate school advice to his students. Thanks Jon!

I want to thank all the graduate students and staff of both the "EVAN" training network and department of human evolution (Silke Streiber and Diana Carstens) for making the PhD experience so memorable. Special thanks to Michael Coquerelle (for the endless R discussions and venting), Sascha Senck (for lots of laughs!), Elena Kranioti, Melanie Frelat, Yann Heuze, Valdis Gudmundsdottir, Sahra Talamo (for keeping me motivated), Tsenka Tsanova (for her optimism), Esther Lizano, Sarah Fredline, Katya Guschanski, Johannes Krause (for his gollum impressions!), Simon Neubauer (for lessons in AMIRA), Matthew Skinner, Tracy Kivell (for helpful feedback) and Hannes Napierala (for translating my summary to German – thanks, Hannes!). But most of all to the people who journeyed (literally!) with me through the last four and a half years of ups and downs: Sameer Afsar, Swaminathan, Tamar Barneis, Elke Schwager, Ines Heinz, Ruchira Joshi, Rosemarie Lalor, Carmela Huang, Gauri Malhotra, Ellen Gunnarsdottir (and my little baba, Embla!), Tomislav Maricic, Grit Schubert and Emily Wyman. And a very special thanks to Steve Quatrano for his inspirational words, "you can do ANYTHING you put your mind to!"

I thank my family (Nani, **Amar**, Ravija, Tara, Puja and Karthik) for their love, unwavering support and enthusiasm. In particular, my sister - who taught me persistence and always had faith in my abilities and for giving me the greatest joy ever, my adorable niece Anahi, and my mother- who encouraged me to pursue my goals and be whoever I wanted to be - I could never have done this without them. And finally, my dear friend and teacher, Charles Lockwood, who taught me that, "Natural system - a contradiction in terms. Nature has no system; she has, she is life and its progress from an unknown center toward an unknowable goal. Scientific research is therefore endless, whether one proceeds analytically into minutiae or follows the trail as a whole, in all its breadth and height" (J. W. von Goethe 1823, the essay "Problems"). Thanks, Charles - you will always be missed.

This PhD was funded by Marie-Curie "EVAN" training network (MRTN-CT-019564) and graduate fellowship from Senckenberg Center for Human Evolution and Paleoecology.