Published in final edited form as:

Am J Phys Anthropol. 2016 November ; 161(3): 448–455. doi:10.1002/ajpa.23043.

Fetal and neo-natal maxillary ontogeny in extant humans and the utility of prenatal maxillary morphology in predicting ancestral affiliation

Christina L. Nicholas¹

¹Iowa Institute for Oral Health Research, University of Iowa, Iowa City, IA 52242-1322

Abstract

Objectives—The midface of extant *H. sapiens* is known to undergo shape changes through fetal and neo-natal ontogeny; however, little work has been done to quantify these shape changes. Further, while midfacial traits which vary in frequency between populations of extant humans are presumed to develop prenatally, patterns of population-specific variation maxillary shape across ontogeny are not well documented. Only one study of fetal ontogeny which included specific discussion of the midface has taken a 3D geometric morphometric approach, and that study was limited to one population (Japanese). The present research project seeks to augment our understanding of fetal maxillary growth patterns, most especially in terms of intraspecific variation.

Materials and Methods—Three-dimensional coordinate landmark data were collected on the right maxillae of 102 fetal and neo-natal individuals from three groups (Euro-American, African-American, “Mixed Ancestry”).

Results—Shape changes were seen mainly in the lateral wall of the piriform aperture, the anterior nasal spine, and the subnasal alveolar region. The greatest difference across age groups (2nd Trimester, 3rd Trimester, Neonates) was between the second and third trimester. Euro-Americans and African-Americans clustered by population and differences in midfacial morphology related to ancestry could be discerned as early as the second trimester ($p=0.002$), indicating that population variation in maxillary morphology appears very early in ontogeny.

Discussion—The midface is a critical region of the skull for assessing ancestry and these results indicate that maxillary morphology may be useful for estimating ancestry for prenatal individuals as young as the second trimester.

Keywords

Palatal growth; geometric morphometrics; midface

The midfacial morphology of *H. sapiens* varies between populations and this variation is argued to in part reflect climatic adaptations (Churchill et al., 2004; Yokley, 2009; Holton et al., 2013; Evteev et al., 2014). Differences between modern human midfaces and Neandertal

midfaces have also been argued to be due to climatic differences. Inter- and intraspecific differences in midfacial traits have been argued to appear early, often before birth (Shultz 1918, 1920; Mooney and Siegel, 1986a). For example, the populational pattern whereby sub-Saharan African populations have a short or non-existent anterior nasal spine (ANS) while European populations tend to have strongly projecting anterior nasal spines, has been argued to appear as early as the second trimester (Mooney and Siegel, 1986a). A similar pattern of difference in nasal dimensions between populations has been recorded as young as fetal ages (Schultz, 1920).

During prenatal development, the maxilla starts off as two centers of ossification: the premaxilla and the palatal process of the maxilla (also known as the “post-maxilla”). These two centers ultimately fuse at the premaxillary suture which runs across the palatal surface of the maxilla posterior to the maxillary incisors, between the lateral incisors and the canines, and superiorly up, around, the nasal cavity terminating on the frontal process. The timing of this suture fusion differs between individuals, populations, and species, with Europeans showing earlier suture fusion (often in utero) than Africans (typically during the perinatal period through the first two years of postnatal growth) (Mooney and Siegel, 1986b), and Neandertals showing a much later fusion than extant humans (typically well into childhood, sometimes as late as 6-8 years) (Maureille and Bar, 1999).

The palate forms from three centers of ossification: the midline median palatine process, and left and right lateral palatine processes. Anteriorly, the intermaxillary segment forms, which will develop into the premaxilla (housing the anterior maxillary incisors). The palatine processes grow such that both shelves fuse at midline with each other (creating the midpalatal suture), and form a Y-shaped fusion with the premaxilla anteriorly (creating the premaxillary suture); this occurs by roughly 8-10 weeks fetal (Som and Naidich, 2014). Ossification begins anteriorly, in the primary palate (premaxilla) and extends posteriorly (Kim et al., 2004; Warbrick, 1960). Most growth of the face during the prenatal period occurs along the intermaxillary suture (Scott, 1957; Som and Naidich, 2014).

Most research on variation in prenatal midfacial development has focused on palatal length. Firstly, this measurement can be reliably identified in ultrasound data. Secondly, palatal length is an indicator for normal or anomalous craniofacial growth, and has been argued to potentially be useful in early detection of malformations and syndromes such as cleft palate (Hermann et al., 2015), Marfan syndrome (De Coster et al., 2004), Neurofibromatosis Type 1 (Heervae et al., 2011), and Down syndrome (Alio et al., 2011)).

Qualitative traits, such as anterior nasal spine prominence, have been documented to vary between populations at prenatal ages. Weinberg et al. (2005) investigated a wide range of craniofacial traits to determine which were most useful for estimating ancestry (African-American vs. Euro-American) in fetal samples. They found that occipital squamous shape, ANS prominence, subnasal margin definition, vomer shape, and temporal squama shape were the most accurate for predicting whether an individual was of African or European descent. Three of these five traits are midfacial/nasal, with two of them being maxillary, further emphasizing the utility of the maxilla in determining ancestral affiliation.

Understanding the 3D relationship between midfacial traits through development is an important aspect of understanding growth – and the differences in growth between individuals and populations. To date, only one published study on fetal craniofacial ontogeny has been conducted using modern 3D geometric morphometrics (Morimoto et al., 2008). This study looked at overall craniofacial ontogeny in a sample of Japanese fetal specimens (n=31) using a 3D geometric morphometric approach. They show that the shape and relative position of the midface (i.e., the maxillae and zygomatics) undergo relatively little morphological change prenatally. Based upon their results, Morimoto et al. (2008) suggest that neurocranial changes (in particular the lengthening of the skull antero-posteriorly) are the most noteworthy, which is in agreement with earlier work (Trenouth, 1984).

While Weinberg et al. (2005) have demonstrated that qualitative trait assessment can be used to estimate ancestry starting at prenatal ages, quantitative research on this topic is lacking. To get a more accurate picture of midfacial shape changes through ontogeny, and across populations, three-dimensional geometric morphometric methodologies should be utilized. The present study expands upon the previous literature and looks specifically at differences between African-derived and European-derived populations using 3D coordinate landmark data. Three questions are examined: 1) What are the 3D patterns of prenatal/neonatal maxillary growth? 2) How do they differ between African-Americans and Euro-Americans? And lastly, 3) Can these differences in morphology be used to predict ancestry of individuals from forensics/bioarchaeological contexts?

MATERIALS AND METHODS

Isolated fetal and infant maxillae (n=102) were landmarked using an Immersion Co. Microscribe. Due to the small size of the anatomical structures, only 12 coordinate landmarks were chosen to capture shape variation, focusing largely on the nasal region (Fig. 1). Samples came from the Mall and Lamb Collection (National Museum of Natural History) and the Johns Hopkins Collection (Cleveland Museum of Natural History). The individuals selected represent three ancestry groups, as identified by the researchers who compiled these collections: Euro-American, African-American, and “Mixed Ancestry” (individuals whose museum records indicate both African-American and Euro-American ancestry).

Age at death information was gathered from the museum collections records. Ages ranged from 4 months fetal to 5 months post-natal. Age groups were then created to aggregate similarly-aged individuals, roughly in line with the final two trimesters of pregnancy, with the third group representing neonates: 2nd Trimester: 4-6 months fetal; 3rd Trimester: 7-9 months fetal; Neonates: birth-5 months post-natal (Table 1).

Generalized Procrustes analysis (GPA) was run on the data. A principal components analysis (PCA) was run on the Procrustes superimposed landmark data using MorphoJ (Klingenberg, 2011). The first 6 PCs were found to each represent >5% of the overall variation, and thus were selected for further analysis.

To examine patterns of maxillary growth, age was regressed against the first 6 PCs (derived from the raw GPA data). The sample was then analyzed by Age Group (2nd Trimester, 3rd Trimester, Neonates). These age groupings were compared using canonical variates analysis (CVA). A form space (shape + log centroid size) PCA was also run, to highlight variation across ontogeny (as per Mitteroecker et al., 2005; Bastir and Rosas, 2009).

Prior research has suggested a multi-pronged approach to analyzing allometry in an ontogenetic sample (Ponce de Leon and Zollikofer, 2001). This approach distinguishes between growth (size vs. age), development (shape vs. age), and ontogenetic allometry data (shape vs. size). Thus three regression analyses were run, using the raw age data, centroid size data, and the GPA data.

Age was regressed on the coordinate landmark data with population as a subgrouping in order to test whether differences in ontogenetic scaling might be a confounding factor analyses examining variation between populations. To test for allometry in our sample, a regression of shape was run on centroid size. This test revealed evidence for allometry ($p=0.001$), and thus additional analyses investigating population variation were run using the regression residuals from the allometry test, to control for the effects of allometry when attempting to identify group-level variation.

The data was subdivided by both Age Group (2nd Trimester, 3rd Trimester, Neonates) and Ancestry (African-American, Euro-American) to investigate trends in maxillary ontogeny across development in both populations. For each sub-dataset (Africans 2nd Trimester, Europeans 2nd Trimester, etc.) a GPA and PCA were run and the consensus configuration was used to examine average patterns of morphological shape for each stage of development. Additionally, a separate GPA was run on each Age Group, unaggregated by ancestry. A multivariate analysis of variance (MANOVA) was run on the shape data using Ancestry as a grouping variable to ascertain whether patterns of difference between Euro-Americans and African-Americans could be detected at each stage of growth.

To examine patterns of variation across grouping variables, CVA was run to investigate patterns of shape variation between populations and age groups. A discriminant function analysis (DFA) was run exclusively on the African-American and Euro-American data to determine whether ancestral population could be predicted based upon maxillary shape. A DFA is homologous to a CVA, and CVA can even be considered a multivariate extension of DFA that is used for >2 grouping variables (Timm, 2002). These methods allow the examination of which patterns of shape variation drive differences between groups, in contrast to a PCA which highlights factors which differentiate individuals in the sample. The results of the DFA are validated via leave-one-out cross-validation, a Jackknifing method.

RESULTS

The test for allometry indicated that allometric variation contributed to 9.8% of the overall variation ($p=0.001$). Size related shape variation is intrinsic to ontogenetic variation, and thus analyses related to age were conducted on the raw GPA data. However, to ensure that any signals of population variation were not distorted by the effects of allometry, these tests

were run using both the raw GPA data and the regression residuals. All results showed very similar results across both sets of tests. To take a more conservative approach, the results presented below for the population-specific analyses are based upon the regression residuals.

Pre- and perinatal growth of the maxilla

The raw, unaggregated age data was regressed against PCs 1-6 and was found to be correlated with PC1 ($p=0.025$), PC2 ($p<0.001$), and was approaching significance on PC6 ($p=0.057$). Principal component 1 (Fig. 2) depicts relative maxillary height (in supero-inferior dimensions), nasal height, alveolar breadth, and the relative location of zygo-orbitale (the positive end of the range of variation shows a relatively more antero-medially positioned zygo-orbitale). Principal component 2 describes nasal aperture shape (more triangular to more oval), ANS prominence, palatal curvature, and the height and breadth of the alveolus. To compare overall shape across the latter two trimesters of development and early postnatal growth, individuals were aggregated by age (4-6 months fetal, 7-9 months fetal, birth-5 months post-natal). Morphological changes throughout prenatal development are extensive enough that the landmark data was sufficient to reasonably accurately differentiate between the second and third trimesters. The first 3 PCs showed statistically significant differences across the age groupings ($p=0.0016$; $p<0.001$; $p=0.015$). The results of a CVA indicate that the key distinguishing features across maxillary ontogeny are changes in the A-P height of the alveolar region and to the shape of the anterior nasal aperture (Figure 3). When the three age groups were compared, the Procrustes distances were smallest between Third Trimester and Neonatal, but all three groups were statistically significantly different from each other (Table 2).

As described above, ontogenetic allometry (shape vs. size) was found in the sample ($p<0.001$). As centroid size increases, the nasal aperture becomes taller and the alveolus becomes taller and narrower. Growth (size vs. age) and development (shape vs. age) were also investigated (Figs. 4 and 5). When centroid size was regressed on the raw age data, a moderately strong pattern between size and age was observed (% predicted = 42.85%; $p<0.001$). The percentage of shape variation predicted by age was much lower (5.23%), but also statistically significant ($p<0.001$). In contrast to the linear relationship between shape and size, both growth (size vs. age) and development (shape vs. age) show a non-linear relationship, with the greatest amount of change being seen earlier in development. In both cases, around the early post-natal period, the rate of change levels off somewhat.

When a form space (shape + log centroid size) PCA was conducted, the first 3 PCs each represented >5% of the total variance. Individuals in the youngest age group (1st Trimester) and those in the oldest (Neonates) fell at opposite ends of the range of variation along PC1. The scatterplot below (Fig. 6) is labeled by both Ancestry and Age Group. The clear age trend is easy to discern, while there is no noticeable pattern differentiating the Ancestry groups.

Patterns of variation were investigated further by subdividing the data by both ancestry and age group (Fig. 7). At the 2nd Trimester, both Africans and Europeans show a relatively shorter nasal height, shorter palate, and broader anterior alveolar region. Through development, the frontal process of the maxilla becomes more anteriorly oriented, the nasal

aperture becomes taller, and the alveolar region becomes both taller and relatively narrower. The positioning of subspinale becomes more superior with each subsequent growth stage.

Differences between the African-American and Euro-American samples manifest early, with the 2nd Trimester showing greater anterior alveolar prognathism in African-Americans, and greater anterior nasal spine prominence in Euro-Americans. Throughout ontogeny, differences in subnasal alveolar prognathism magnify, while differences in anterior nasal spine prominence appear more static.

Population variation in fetal/neonatal midfacial morphology

The PCA run on the regression residuals yielded 36 principal components, the first 6 of which each represented >5% of the total variation. Of these 6 PCs, only PC1 ($p=0.0000067$) and PC3 ($p=0.0249$) showed statistically significant differences between the three populations (European-American, African-American, Mixed Ancestry). PC1 described nasal aperture shape (more triangular or more oval), height of the frontal process of the maxilla, and palatal curvature. PC3 describes ANS prominence, alveolar prognathism, and the relative height of the frontal process (Fig. 8).

The CVA indicated that patterns of shape variation between the three groups can be identified ($p<0.001$; Table 3; Fig. 9). Canonical variate 1 describes alveolar prognathism, relative nasal height and depth of palatal curvature; CV1 also clearly separates the two major ancestry groups (African-American and Euro-American). Canonical variate 2 describes the curvature and height of the lateral wall of the piriform aperture (more oval or more triangular) along with a degree of ANS prominence. Due to the fact that all of the Mixed Ancestry sample is in the 3rd Trimester age group, CV2 may depict age-related morphological differences.

When a DFA was run on just the African-American and Euro-American data, shape differences were found to be statistically significant between Euro-Americans and African-American ($p=0.0029$, Procrustes distance $p<0.0001$), and Euro-Americans and Mixed Ancestry individuals ($p=0.0001$, Procrustes distance $p=0.016$). The percentage of each group correctly classified by the DFA was relatively high (African-Americans: 78.7% correct; Euro-Americans: 85.2% correct), and even though the percentages are lower for the cross-validation (African-Americans: 66% correct; Euro-Americans: 62% correct), both are still above 50% (Table 4).

These population-level differences can be discerned as early as the second trimester. When MANOVAs were run on the GPA data for each Age Group individually (i.e., the data was subdivided by Age Group and a separate GPA sub-dataset) using ancestry as the grouping variable, it was found that there were statistically significant differences between the populations (2nd Trimester: $p=0.002$; 3rd Trimester: $p=0.041$; Neonates: $p=0.0152$).

DISCUSSION

When growth was examined, via a regression of centroid size on age in months postnatal, a clear developmental trend can be seen. It also appears that the steepest rate of change occurs

in the youngest ages in the sample, with an apparent leveling out of size in the early postnatal period. A similar trend of a larger magnitude of change in the prenatal period can also be seen in the relationship between shape and age (i.e., development). In contrast, the relationship between size and shape (allometry) is predictably much more linear. When the first two PCs of the form space PCA were examined, it could be seen that there is more shape variability in the first age group (Second Trimester) than the following to age groups (Third Trimester and Neonatal). The third age group (Neonatal) is also completely overlapping in form space with the Second Trimester age group. Taken together, this indicates that the main maxillary growth spurt appears to occur sometime between the second and third trimester.

Prior research on prenatal and neonatal craniofacial ontogeny (Morimoto et al., 2008; Bastir and Rosas 2009) has indicated that allometric growth of the cranium is not one, continuous pattern across all of development. Morimoto et al. (2008) found that prenatal growth of the neurocranium showed the opposite pattern than that seen postnatally (e.g., cranial length increases outstripped cranial breadth increases during fetal growth). Bastir and Rosas (2009) document differential integration between the units of the basicranium across different periods of prenatal and postnatal growth. The results of the present study similarly indicate variation in the timing of growth. Additional research is needed to compare patterns of prenatal and postnatal maxillary growth and development.

Starting with the youngest age group (2nd Trimester: 4-6 months fetal), differences can be seen between the Euro-Americans and African-Americans ($p=0.002$). The data in this study is cross-sectional, thus rate of change cannot be observed. However, the overall pattern of morphological change is very similar between the two populations, with differences in ANS prominence appearing early (in agreement with Mooney and Siegel, 1986) and difference in subnasal alveolar prognathism appearing later. This data would appear to suggest that when attempting to assess ancestry by traditional visual scoring methods, subnasal alveolar prognathism may not be useful until perinatal/neonatal ages.

Anterior nasal spine prominence is visually scored based upon the relationship between the anterior-most tip of the anterior nasal spine and the point of greatest inflection along the alveolar clivus. This trait has been shown to exist along a continuum which at one end shows a morphology that has essentially no prominence/projection, and at the other extreme can show several millimeters of projection (Franciscus, 1995; Weinberg et al., 2005). African-derived populations show a higher frequency of less projecting nasal spines, while European-derived populations tend to have greater ANS prominence, thus this trait is commonly included in ancestry estimation of unidentified crania. Interestingly, while ANS prominence is described by PC3, CV1 from the CVA by ancestral group (which differentiated between African-Americans and Euro-Americans) did not show pronounced variation in ANS prominence. This suggests that other aspects of maxillary shape, perhaps including more complex shape variation that is difficult to qualitatively assess via visual observation of specimens, may be important in estimating ancestry (at least for the populations included in this analysis).

The pattern of morphological variation that seems to differ the most among the populations was subnasal alveolar prognathism. It is worth noting that Weinberg et al. (2005) did not find a statistical association between ancestry and a visual coding of alveolar prognathism. This discrepancy may arise from various potential factors. Firstly, Weinberg and colleagues' primary sample was the Mall and Lamb Collection at the NMNH with alveolar prognathism scored for 51 individuals (n=27 African-Americans, n=24 Euro-Americans). The sample for the current study is comprised of individuals from the Mall and Lamb sample (n=31) together with individuals from the John Hopkins Collection at the CMNH (n=71), and is both larger in overall size and in the number of African-American individuals included. It may therefore be that the Weinberg et al. (2005) sample may not have been large enough to detect a pattern of variation in this trait, or that the two collections are not equally representative of the entire pattern of population variation in midfacial morphology. Another possibility is that subnasal alveolar prognathism, in and of itself, is not as important to distinguishing between populations as is an overall pattern of shape variation which includes variation in alveolar prognathism. The results presented here do concur with early reports in the literature that differences in subnasal alveolar prognathism could be detected prenatally (e.g., Hauschild, 1937).

The maxilla is one of the most useful single bones in the cranium for estimating ancestry. This holds true across *H. sapiens* and genus *Homo* more broadly, due to climate-related variation in nasal morphology coupled with dentofacial variation such as facial prognathism. Recent work indicates that this midfacial variation arises through differential deposition and resorption of bone across taxa, which can be seen in early childhood (Lacruz et al., 2015). An interesting avenue for future research might compare bone depositional patterns at the fetal/neonatal period of development, examining whether differences in subnasal alveolar prognathism in modern humans arise from differential depositional patterns. The current study confirms the results of work by Mooney and Siegel (1986) and Weinberg et al. (2005) that showed population variation in maxillary traits developing, in some cases, prenatally. While not all maxillary traits that vary by population follow this pattern (such as the internal nasal floor, see Nicholas and Franciscus, 2014), there is sufficient shape variation at these young ages for it to be easily quantified using a relatively restricted set of coordinate landmarks. Geometric morphometric methods' ability to quantify continuous variation, along with variation that may be more difficult to parse due to the physically small size of fetal material, may help to increase accuracy when trying to estimate ancestry for fetal or neo-natal individuals. Future work in this area should compare qualitative assessments with a quantitative approach.

Overall, population variation in midfacial morphology in fetal and neonatal individuals was detectable through the analysis of these 12 standard, and commonly preserved, osteological landmarks. Furthermore, as expected, the individuals of the Mixed Ancestry group clustered most closely with the African American group. The relative inaccuracy of the cross-validation analysis in correctly assign shapes to a population may represent the relatively small sample size included in this analysis, especially for the Euro-American sample which was much smaller than the African-American sample.

The use of newer ultrasound technologies have made it increasingly possible to obtain 3D midfacial shape data without relying on dry specimens (e.g., Rotten et al., 2002; Roelfsema et al., 2006; Faure et al., 2007; Chen et al., 2011; De Jong-Pleij et al., 2011; Hermann et al., 2015). Additionally, retrospective use of MRI might be useful in some instances, and has successfully been used to study dental arch measurements (Mailath-Pokomy et al., 2012). The current study relies on data from two of the largest collections of fetal individuals in the United States. New work going forward might investigate the utility of assessing anthropologically-relevant information on fetal growth from ultrasound data.

ACKNOWLEDGEMENTS

Many thanks to the curators who provided access collections: Lyman Jellema (Cleveland Museum of Natural History); and David Hunt (National Museum of Natural History, Smithsonian). Many thanks also to Steven Miller and Nancy Budd for their proofreading and assistance. I am especially appreciative of the very helpful feedback from the two anonymous reviewers and the Associate Editor. This research was funded by the University of Iowa's Department of Anthropology. Support was also provided by NIH T90 DE023520-02.

LITERATURE CITED

- Bastir M, Rosas A. Mosaic evolution of the basicranium in *Homo* and its relation to modular development. *Evol Biol*. 2009; 36:57–70.
- Chen M, Wang HF, Leung TY, Sahota DS, Borenstein M, Nicolaides K, Lao TT-H, Lau TK. Frontomaxillary facial angle at 11 + 0 to 13 +6 weeks in Chinese population. *J Matern Fetal Neonatal Med*. 2011; 24:498–501. [PubMed: 20593975]
- Churchill SE, Shackleford LL, Georgi JN, Black MT. Morphological variation and airflow dynamics in the human nose. *Am J Hum Biol*. 2004; 16:625–638. [PubMed: 15495233]
- De Coster P, De Pauw G, Marten L, De Paepe A. Craniofacial structure in Marfan syndrome: a cephalometric study. *Am J Genet A*. 2004; 131:240–8.
- De Jong-Pleij EA, Ribbert LS, Mantern GT, Tromp E, Bilardos CM. Maxilla-nasion-mandible angle: a new method to assess profile anomalies in pregnancy. *Ultrasound Obstet Gynecol*. 2011; 37:562–9. [PubMed: 20922777]
- Evtsev A, Cardini AL, Morozova I, O'Higgins P. Extreme climate, rather than population history, explains mid-facial morphology in northern Asians. *Am J Phys Anthropol*. 2014; 153:449–462. [PubMed: 24374801]
- Faure JM, Captier G, Baeumler M, Boulot P. Sonographic assessment of normal fetal palate using three-dimensional imaging: a new technique. *Ultrasound Obstet Gynecol*. 2007; 29:159–65. [PubMed: 17252526]
- Franciscus, RG. Ph.D. dissertation. University of New Mexico; Albuquerque: 1995. Later Pleistocene nasofacial variation in Western Europe and African and Modern Human origins.
- Hauschild R. Rassenunterscheide zwischen negriden und europiden primordial-cranien des 3. Fetalmonats. *Ze Morphol Anthropol*. 1937; 36:215–279.
- Heervae E, Peltonen S, Pirttiniemi P, Happonen R-P, Visnapuu V, Peltonen J. Short mandible, maxilla and cranial base are common in patients with neurofibromatosis 1. *Eur J Oral Sci*. 2011; 119:121–7. [PubMed: 21410551]
- Hermann NV, Darvann TA, Sundberg K, Kreiborg S, Joergensen C. Maxillary length in 11- to 26-week-old normal fetuses studied by 3D ultrasound. *Prenatal Diagnosis*. 2015; 35:571–6. [PubMed: 25663194]
- Holton N, Yokley T, Butaric L. The morphological interaction between the nasal cavity and maxillary sinuses in living humans. *Anat Rec*. 2013; 296:414–426.
- Kim CH, Park HW, Kim K, Yoon J-H. Early development of the nose in human embryos: a stereomicroscopic and histologic analysis. *Laryngoscope*. 2004; 114:1791–800. [PubMed: 15454774]

- Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Res.* 2011; 11:353–357.
- Lacruz RS, Bromage TG, O'Higgins P, Arsuaga J-L, Stringer C, Godinho RM, Washaw J, Martinez I, Gracia-Tellez A, Bermudez de Castro JM, Carbonell E. Ontogeny of the maxilla in Neanderthals and their ancestors. *Nature Comm.* 2015; 6:8996.
- Mailath-Pokomy M, Klein K, Worda C, Weber M, Brugger PC, Czerny C, Nemec U, Prayer D. Maxillary dental arch biometry: assessment with fetal MR imaging. *Prenatal Diagnosis.* 2012; 32:530–535. [PubMed: 22511243]
- Maureille B, Bar D. The premaxilla in Neandertal and early modern children: ontogeny and morphology. *J Hum Evol.* 1999; 37:137–152. [PubMed: 10444349]
- Morimoto N, Ogihara N, Katayama K, Shiota H. Three-dimensional ontogenetic shape changes in the human cranium during the fetal period. *J Anat.* 2008; 212:627–635. [PubMed: 18430090]
- Mooney MP, Siegel MI. Racial variation in anterior nasal spine (ANS) formation: testing the traction hypothesis. *Am J Phys Anthropol.* 1986a; 69:242.
- Mooney MP, Siegel MI. Developmental relationship between premaxillary-maxillary suture patency and anterior nasal spine morphology. *Cleft Palate J.* 1986b; 23:101–107. [PubMed: 3457657]
- Nicholas CL, Franciscus RG. The ontogeny of nasal floor shape variation in extant humans. *Am J Phys Anthropol.* 2014; 155:369–378. [PubMed: 25043897]
- Ponce de Leon MS, Zollikofer CPE. Neanderthal cranial ontogeny and its implications for late hominid diversity. *Nature.* 2001; 412:534–538. [PubMed: 11484052]
- Roelfsema NM, Hop WC, Wladimiroff JW. Three-dimensional sonographic determination of normal fetal mandibular and maxillary size during the second half of pregnancy. *Ultrasound Obstet Gynecol.* 2007; 29:159–65. [PubMed: 17252526]
- Rotten D, Levailant JM, Martinez H, Ducou Le Points H, Vicaud E. The fetal mandible: A 2D and 3D sonographic approach to the diagnosis of retrognathia and micrognathia. *Ultrasound Obstet Gynecol.* 2002; 19:122–30. [PubMed: 11876802]
- Schultz AH. Relation of the external nose to the bony nose and nasal cartilages in whites and negroes. *Am J Phys Anthropol.* 1918; 1:329–341.
- Schultz AH. The development of the external nose in Whites and Negroes. *Contrib Embryol.* 1920; 34:175–190.
- Scott JK. The growth in width of the facial skeleton. *Am J Orthod.* 1957; 43:366–71.
- Som PM, Naidich TP. Illustrated review of the embryology and development of the facial region, part 2: Late development of the fetal face and changes in the face from the newborn to adulthood. *Am J Neurodiol.* 2014; 35:10–18.
- Suri S, Tompson BD, Cornfoot L. Cranial base, maxillary and mandibular morphology in Down syndrome. *Angle Orthod.* 2010; 80:861–9. [PubMed: 20578856]
- Timm, NH. *Applied multivariate analysis.* Springer; New York: 2002.
- Trenouth MJ. Shape changes during human fetal craniofacial growth. *J Anat.* 1984; 139:639–651. [PubMed: 6526716]
- Warbrick JG. The early development of the nasal cavity and upper lip in the human embryo. *J Anat.* 1960; 94:351–62. [PubMed: 13842854]
- Weinberg SM, Putz DA, Mooney MP, Siegel MI. Evaluation of non-metric variation in the crania of black and white perinates. *Forensic Sci Int.* 2005; 151:177–185. [PubMed: 15939150]
- Yokley T. Ecogeographic variation in human nasal passages. *Am J Phys Anthropol.* 2009; 138:11–22. [PubMed: 18623075]

Anterior landmarks

Palatal landmarks

Figure 1.

Maxillary Landmarks. (1=superior nasomaxillary suture point; 2=inferior nasomaxillary suture point; 3=ala; 4=inferior-most point on the inferior border of the piriform aperture; 5=anterior nasal spine; 6=subspinale; 7=prosthion; 8=zygo-orbitale; 9=staurion; 10=deepest point of the palate; 11=posterior incisive canal; 12=orale)

Figure 2.

Scatterplot of PC1 and PC2 along with wireframes depicting variation along each PC. It can be seen that there is a shape trend along an age gradient, whereby as individuals grow, they develop shapes more akin to the shape variation at the positive end of variation along PCs 1 and 2. Thus older individuals have taller, narrower nasal apertures and taller, narrower alveolar regions, along with less dramatic anterior nasal spine prominence. The scatterplot is color-coded by Age Group, with 90% confidence ellipses around group means. Wireframes are orientated in lateral view.

Figure 3.

Canonical variates analysis by age group (2nd Trimester: 4-6 months fetal; 3rd Trimester: 7-9 months fetal; Neonates: birth-5 months post-natal). Variation along CV1 differentiates between all three groups, while CV2 seems to primarily describe in palatal shape and nasal spine prominence. Ninety percent confidence ellipses have been placed around the age groups. Wireframes are depicting the extremes of variation along CV1 (bottom) and CV2 (left), and are orientated in lateral view.

Figure 4. Scatterplot of growth (size vs. age). The greatest magnitude of change occurs during the earlier months of development.

Figure 5. Scatterplot of development (shape vs. age). The greatest magnitude of change again appears to occur during the earlier months of development.

Figure 6.

Scatterplot of form space (shape + size) PCA depicting form space PCs 1 and 2. Symbols have been coded by age (color) and ancestry (shape). Wireframes are depicting extremes of variation along PC1 and are oriented in lateral view. In the wireframes, grey circles are the average shape, and the shape variation along the axis is depicted via a wireframe. Variation along form PC1 is primarily age-related variation associated with the development of a taller, narrower nose, a more superiorly oriented frontal process of the maxilla, and a taller, narrower alveolar region.

Figure 7.

Wireframes depicting consensus morphology of each population broken down by Age Group. Wireframes are orientated in lateral view. Mean wireframe shapes from separate GPA analyses of data subdivided by ancestry and age group (i.e., a GPA of all Second Trimester African-Americans) have been superimposed by hand in order to aid in the visual interpretation of the data.

Figure 8.

Scatterplot of PC1 and PC3 coded by ancestry, with 90% confidence ellipses around the means for each ancestry group. Wireframes depict the extremes of variation along PC1 (bottom) and PC3 (left) and are orientated in lateral view. African-Americans and Euro-Americans can be distinguished quite well along PC1, with African-Americans showing wider nasal apertures and more subnasal alveolar prognathism.

Figure 9.

Scatterplot of CVA of the regression residuals, by ancestry, with wireframes depicting the extremes of variation along CV1 and CV2. Wireframes are orientated in lateral view and 90% confidence ellipses have been placed around the three ancestry groups. It can be noted that African-Americans and Mixed Ancestry individuals share the same shape space along CV1.

Table 1

Sample Composition

African-American	Euro-American	Mixed Ancestry
N=61	N=34	N=7
4-6 mo. fetal (2nd Trimester)	7-9 mo. fetal (3rd Trimester)	Birth-5 mo. infant (Neonates)
N=28	N=53	N=21
Afr-Am=18	Afr-Am=35	Afr-Am=8
Euro-Am=10	Euro-Am=11	Euro-Am=13
Mixed Anc=0	Mixed Anc=7	Mixed Anc=0

Table 2

Procrustes distance results (and p-values) from CVA by Age Group

	Second Trimester	Third Trimester
Third Trimester	0.048 (p=0.004)	-
Neonatal	0.071 (p<0.001)	0.04 (p=0.029)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3

Discriminant function and cross validation results for comparisons across ancestry groups.

Af-Am/Eur-Am Discriminant Fx	African-American	Euro-American	Total
African-American	48	13	61
Euro-American	5	29	34
Af-Am/Eur-Am Cross Validation	African-American	Euro-American	Total
African-American	40	21	61
Euro-American	13	21	34

Table 4

Procrustes distance results (and p-values) from CVA by Ancestry

	African-Am	European-Am
African-Am	0.055 (p<0.001)	-
Mixed Ancestry	0.049 (p=0.163)	0.0637 (p=0.019)