

Microanatomical Assessment of Nasomaxillary Suture Patency

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ABSTRACT

In addition to acting as a growth site, sutures in the facial skeleton are important for distributing mechanical forces during mastication. In the present study, the extent of fusion of a facial suture is assessed in two samples of adult bushbabies (*Galago moholi* and *Otolemur garnettii*). Microanatomical techniques were used to determine the loci of osseous bridges across the nasomaxillary suture (NMS). Histological sections containing sutures with osseous bridging were rated as “fused.” One of the specimens was studied using micro-computed tomography before paraffin embedding and serial sectioning. At all ages, *O. garnettii* shows more advanced fusion of the NMS than *G. moholi*. The youngest *O. garnettii* shows multiple foci of fusion of the NMS; however, 13% of the posterior most suture is patent. Throughout the NMS of this animal, sutural fusion is isolated to one or two small osseous bridges, typically of woven bone. These bridges are most often on the external (superficial) surface of the suture, but in numerous sections the site of fusion occurs deep to an external notch. In *G. moholi*, the youngest adults studied showed little or no fusion across the NMS. However, the nasal and maxillary bones were indirectly tethered at some levels by other bones that were fused to both nasal and maxillary bones. These results indicate that microanatomical evidence is required to fully assess the extent of fusion of facial sutures. These findings also support previous observations of differing magnitude of suture fusion between the two species. *Anat Rec*, 293:651–657, 2010. © 2010 Wiley-Liss, Inc.

Key words: craniofacial development; facial growth; primate; suture

The extent of fusion of facial sutures has important implications for the study of facial growth and biomechanics (Herring, 2008). Patent sutures serve as passive sites of growth (Pritchard et al., 1956) and also ameliorate strain on bone during mastication (Behrents et al., 1978; Hylander, 1979; Herring et al., 2001; Rafferty et al., 2003). A consideration of facial suture fusion or patency may be crucial to studies that model biomechanics of the facial skeleton (Ross, 2005).

An unfortunate limitation of studying suture fusion in skeletal specimens is that it is difficult to assess whether apparently patent sutures are in fact fused internally (Wang et al., 2006a; Reinholt et al., 2009). Following a study of the more rostrally restricted nasopremaxillary

and premaxillary sutures (Reinholt et al., 2009), the present study assesses the degree of fusion of the nasomaxillary suture (NMS) in adult greater and lesser

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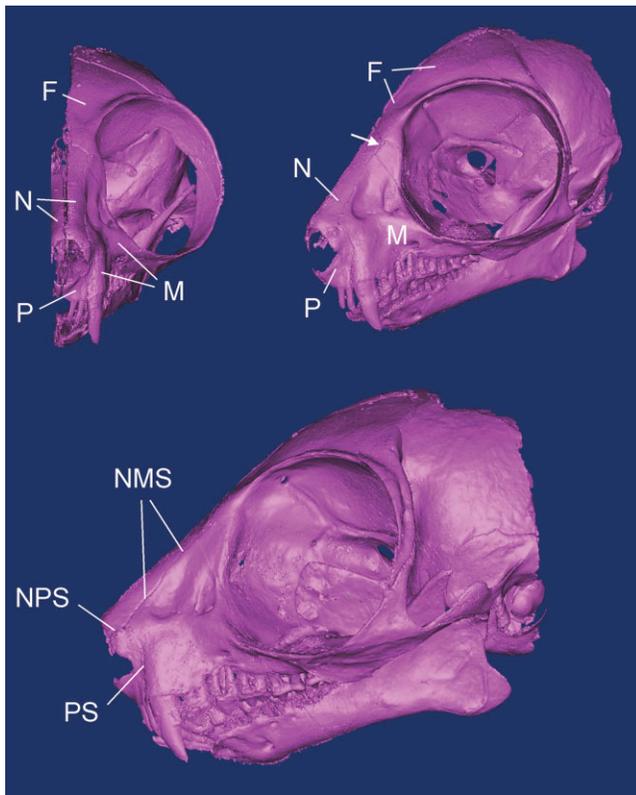


Fig. 1. Micro CT-based reconstruction of a 4-year-old *G. moholi* showing the spatial extent and relationships of the nasomaxillary suture (NMS). Abbreviations: F, frontal; M, maxilla; N, nasal; NPS, nasopremaxillary suture; P, premaxilla; PS, premaxillary suture; arrow, tip of nasal process of frontal bone.

bushbabies. The extent of fusion of the NMS is documented across anteroposterior space.

MATERIALS AND METHODS

NMS were examined in three adult greater bushbabies (*Otolemur garnettii*, 4–20 years of age) and three adult lesser bushbabies (*Galago moholi*, 3.5–5 years of age). The sample and preparation of tissues was described previously (Reinholt et al., 2009). Briefly, portions of the heads were dissected free, decalcified, paraffin embedded, and serially sectioned in the coronal plane at 10–12 μm intervals. Every fifth to tenth slide was stained alternately with either hematoxylin-eosin or Gomori trichrome procedures. Before decalcification and embedding, one 4-year-old *G. moholi* was scanned using a Skyscan 1172 high-resolution micro-CT (micro-computed tomography) scanner (40 kV, slice thickness at 8.85 μm) housed in the laboratory of J.W. Hagadorn, Department of Geology, Amherst College, Amherst, MA. For illustrative purposes, thresholding was used to render three-dimensional images of the *G. moholi* cranium and to identify relationships of the NMS to neighboring bones. Image processing and three-dimensional volume reconstruction were performed with Mimics 12.3 (Materialise, Ann Arbor, MI).

Histological sections of sutures were viewed under a Leica DMLB photomicroscope (Leica Microsystems: Wetzlar, Germany) at 200 \times to 630 \times , using at least every tenth section. After microscopic examination, sutures were rated according to suture fusion or patency. Any osseous bridging constituted a rating of “fused” for a section. The presence or absence of a “notch,” that is, a separation between the nasal and maxillary bones, on the external surface of the suture was also recorded. For each specimen, the number of sections with fused sutures was summed and divided by the total number of sections to calculate the percentage of the suture that was fused. The percentage of sections in which NMS fusion was accompanied by an external notch was also calculated. The unstained sections that followed sections with a fused or patent suture were assumed to be similar (i.e., fused or patent). Thus, the calculated percentages may have some error due to fusion in “patent” regions or small points of patency in “fused” regions.

RESULTS

Three-dimensional rendering of the *G. moholi* cranium reveals that the NMS runs anteroposteriorly along the long axis of the midface, which is moderately elongated in this species (Fig. 1). This reconstruction suggests that the NMS is patent, at least superficially. The suture begins at an intersection with the nasopremaxillary and premaxillary sutures and ends at the nasal process of the frontal bone (Fig. 1). The relationship of the NMS to the premaxillary bone of bushbabies was described in detail elsewhere (Reinholt et al., 2009). Figure 2 illustrates the NMS and its relationship to other cranial bones. Within the approximate anterior 20% of the NMS, the maxillary and nasal bones form a suture that is nearly exclusive of other bones (but see Reinholt et al., 2009, regarding the premaxilla). At the midpoint of the NMS, the ethmoid is intimately related to the deep surface of both the nasal and maxillary bones (Fig. 2). Within the approximate posterior 20% of the NMS, the maxillary and nasal bones are adjacent to a deep portion of the frontal bone, which is continuous with the nasal process shown in Fig. 1.

In *G. moholi*, the extent of fusion of the NMS ranged from 0% to 45%. Figure 3a,b shows the patent suture of a 4-year-old *G. moholi*. However, at the anterior end of the NMS, a deep projection of the maxillary bone fuses to the nasal process of the premaxilla, the latter which is fused to the nasal bone (Fig. 3a,b). The NMS itself of this specimen is entirely unfused (Figs. 2 and 3a,b). In a 5-year-old specimen, intermittent fused regions of the NMS are distributed across the length of the suture, separated from each other by patent portions measuring from 100 μm to 2 mm. In this specimen, fusion is either complete or via small bridges, which occur either deep within the suture or on its external face (Fig. 3c,d). Deep to the NMS, an anterior projection of the ethmoid bone articulates with the nasal and maxillary bones. The ethmoid is fused to either or both of the latter bones. In the 5- and 3.5-year-old specimens, this projection indirectly tethers the nasal and maxillary bones together at some cross-sectional levels (Fig. 3e,f).

Histological examination of every fifth to tenth section reveals that the NMS is mostly fused in a 4-year-old *O. garnettii* and completely fused in a 13-year-old *O.*

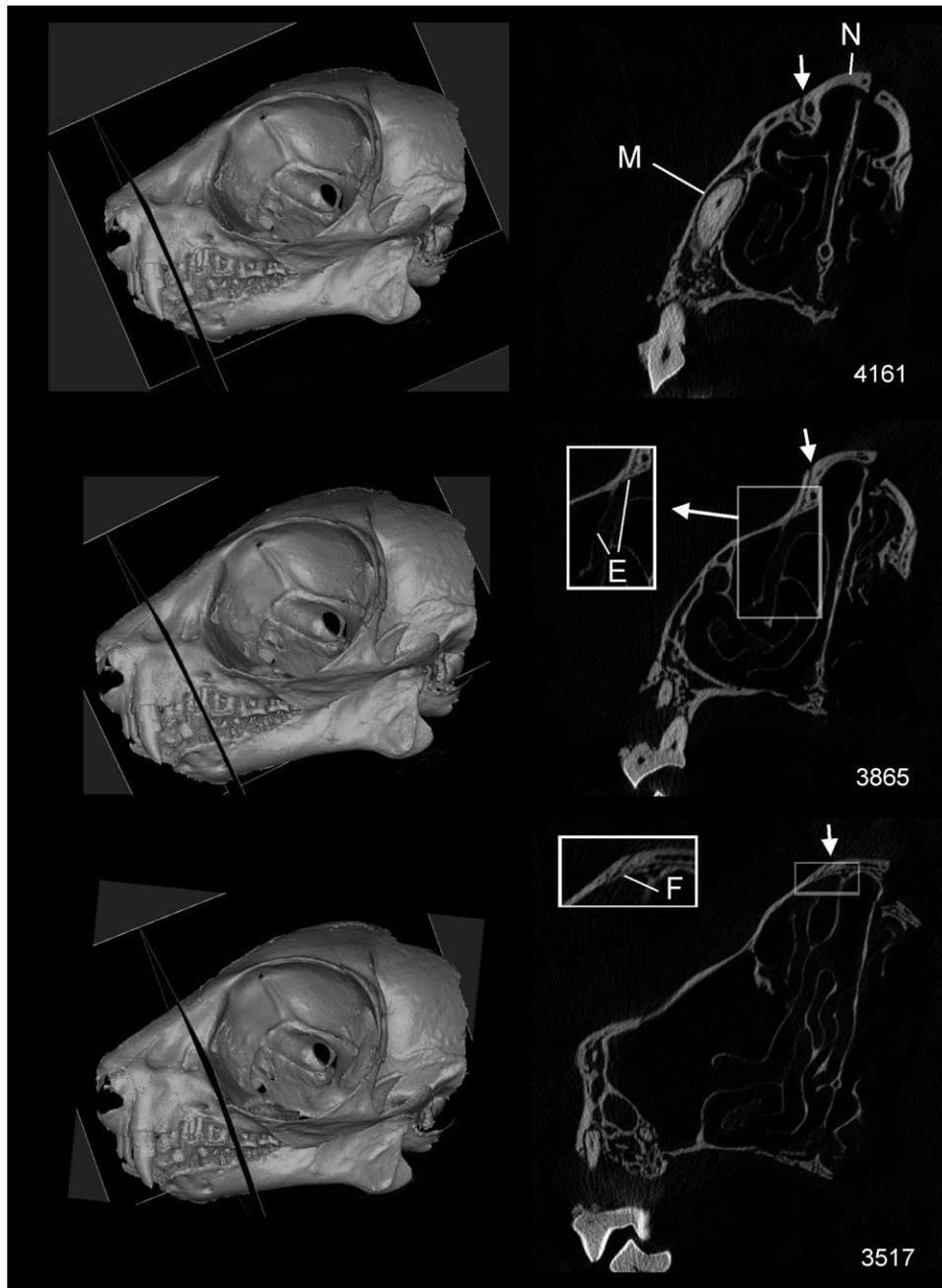


Fig. 2. 4-year-old *G. moholi* showing the NMS and its relationship to other bones at three anteroposterior levels. "Scout" images to the left of each CT slice show approximate anteroposterior level of the slice. Abbreviations: E, ethmoid; F, frontal; M, maxilla; N, nasal. Selected micro-CT slices of this specimen may be viewed as a movie

file at <http://srufaculty.sru.edu/timothy.smith/tds-web-pages/smith-nasal-fossa.htm> or at <http://www.interscience.wiley.com/jpages/1932-8486/suppmat>. This file shows the proximity of internal, anterior processes of the ethmoid bone (arrow) to the NMS.

garnettii. The NMS of a 20-year-old *O. garnettii* also appears to be completely fused but the termination of the NMS is uncertain due to the complete fusion of the NMS and more posterior bones. The NMS of the 4-year-old *O. garnettii* shows multiple foci of fusion of the NMS. Throughout the anterior three-fourths of the suture, scattered patent regions separate these fused segments. The 13% of the posterior most suture is

entirely patent, based on examination of every fifth stained section.

NMS fusion in the 4-year-old *O. garnettii* is very complex. In coronal cross-sections, fusion is isolated to one or two small osseous bridges (Fig. 4a,b). Some cross-sections show dense cords of collagenous tissue bridging the suture, which may or may not have ossified tissue within it. In no single section is the NMS

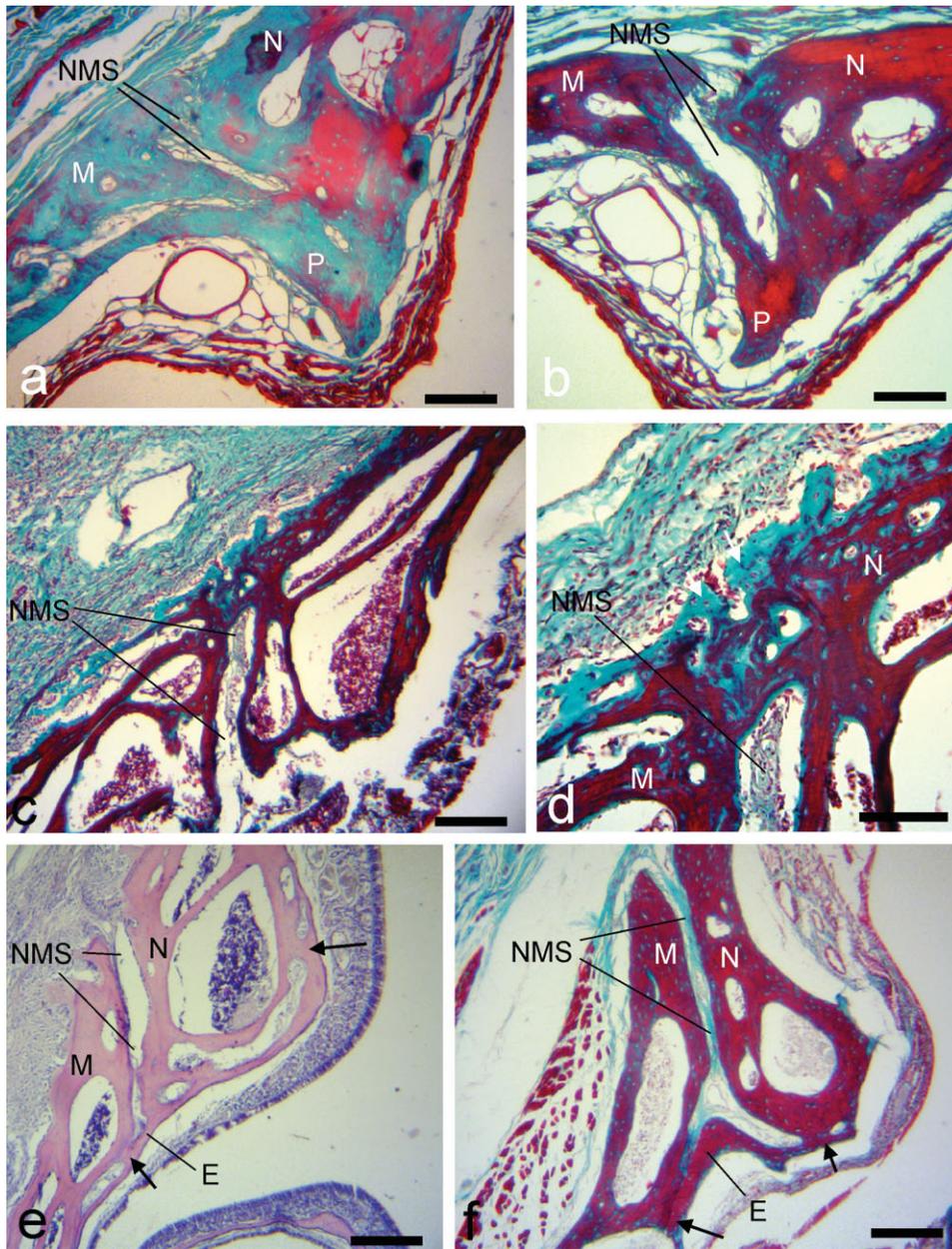


Fig. 3. The nasomaxillary suture (NMS) in *G. moholi*: (a,b) 4 years old; (c–e) 5 years old; (f) 3.5 years old. Note the maxilla is fused to the premaxilla (a), and the latter is fused to the nasal bone (a,b). (c,d) External fusion of the NMS, with primary bone at the surface (white arrows). The ethmoid is fused (black arrows, e,f) to both the nasal and

maxillary bones in some parts of the suture, although the NMS is patent. Stains: (a–d,f) Gomori trichrome stain; (e) hematoxylin and eosin. Abbreviations: E, ethmoid; M, maxilla; N, nasal. Scale bars: (a–c,e,f) 200 μ m; (d) 100 μ m.

entirely obliterated, from superficial to deep extent. Osseous bridges are most often on the external (superficial) surface of the suture (Fig. 4a), but the site of fusion occurs deep to an external notch in numerous sections (Fig. 4b). In the 13-year-old *O. garnettii*, remnants of the NMS can be seen throughout most of its length (Fig. 4c,d), although the suture is often difficult to locate posteriorly due to the great extent of fusion. At numerous intervals, deep notches occur on the external surface of the fused NMS (Fig. 4c). The NMS

of the 20-year-old specimen is nearly obliterated throughout its length.

DISCUSSION

The microanatomical progression of facial and calvarial suture closure has been studied in terms of both normal (Persson et al., 1978) and pathological progression (Mooney et al., 1996). Microanatomical studies of facial sutures are relatively rare. The progression of

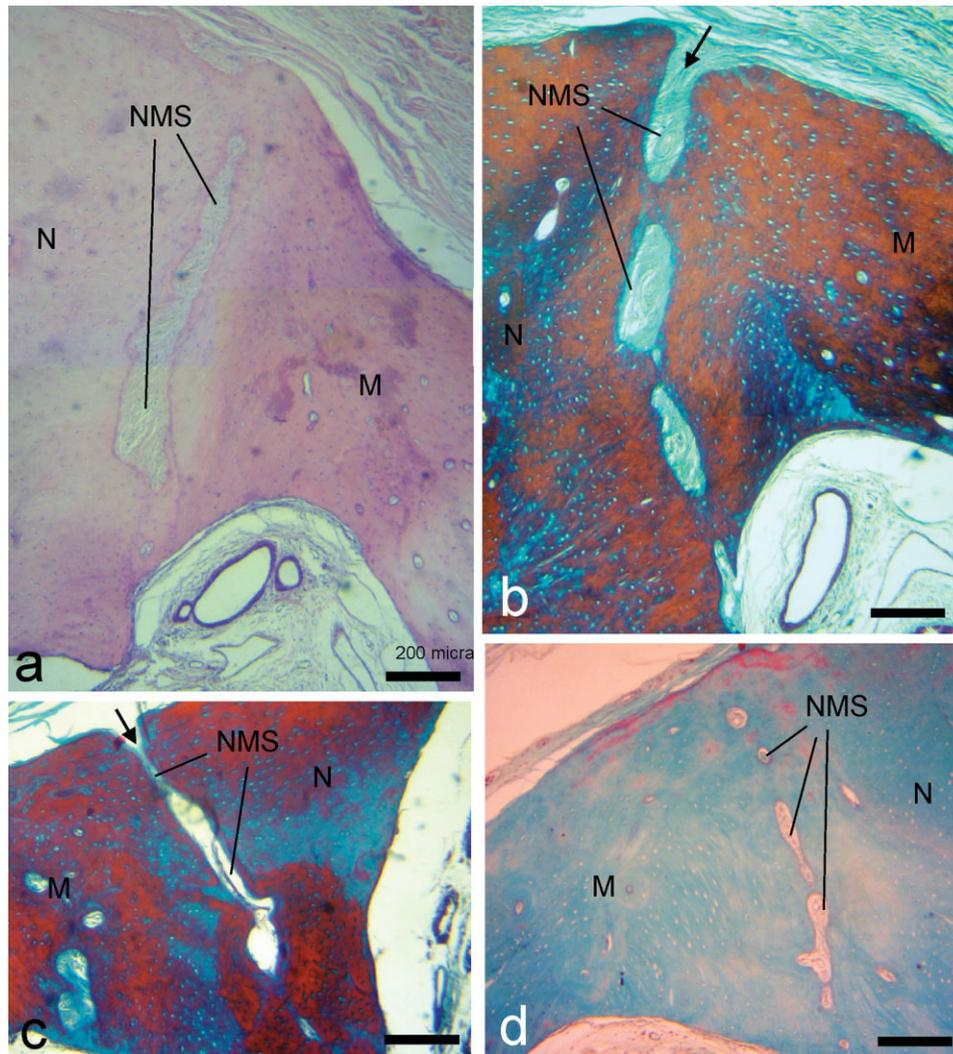


Fig. 4. The nasomaxillary suture (NMS) in *O. garnettii*. Traces of the NMS can be seen in younger (4 years old, **a,b**) and older (13 years old, **c,d**) greater bushbabies. External notches (arrows) sometimes occur superficial to fusion sites. Basophilic cement lines, which may

demarkate the original contour of the NMS, are visible on the margins of the osseous bridges in (a). Stains: (a) hematoxylin and eosin; (b–d), Gomori trichrome stain. Abbreviations: M, maxilla; N, nasal. Scale bars: 200 μ m.

synostosis of the midpalatal suture was previously studied in humans and rabbits (Persson et al., 1978; Korbmayer et al., 2007), and the premaxillary and nasopremaxillary sutures were studied in bushbabies (Reinholt et al., 2009).

Microanatomical observations of the NMS of bushbabies suggest a similar progression of synostosis as described previously for normal facial and calvarial sutures. The series of *Galago* specimens examined here suggests that synostosis initially occurs via limited isolated bridges, similar to the “spicules” described by Persson et al. (1978), in midfrontal and midpalatal sutures. In *Galago* and *Otolemur* specimens with more advanced synostosis, the previous margins of the suture are still seen. Even portions of sutures that show continuous bridging (i.e., all examined sections in a series show synostosis) frequently still show partial separation of the nasal and maxillary bones (Figs. 3c and 4c). More extensive bridg-

ing initially leaves trace “remnants” of the suture, and cement lines border the bridge sites (Fig. 4a,b). Also observed are collagenous connections between sutural fronts, which may be sites of initial ossification, as described by Persson et al. (1978), in rabbits and humans.

The direct utility of these results for biomechanical interpretations are uncertain and possibly quite limited. In addition to the small available sample size, details concerning the diet of the captive animals used in this study are unknown. Therefore, speculation concerning how the material properties of the foods they ate affected NMS fusion is not possible. Microanatomical differences (e.g., temporomandibular joint morphology) have been described among different species of captive primates that consumed similar diets (Burrows and Smith, 2007). Such differences presumably are attributable to different phylogenetic histories, although this

has not been explicitly examined. Likewise, this study cannot distinguish functional and phylogenetic signals because it investigates only two taxa and cannot control for these factors (Garland and Adolph, 1994). Nevertheless, our results suggest these issues may be readily addressed using existing osteological material from a broader taxonomic sample.

Some interpretation of the progression of synostosis among different facial sutures can be made by comparison to previous findings based on other sutures (Reinholt et al., 2009). The results of the present study suggest that the NMS exhibits less extensive fusion than the nasopremaxillary suture in *G. moholi* and possibly in *O. garnettii* (interpretation of the latter can only be based on the extent of fusion of the youngest specimen). Comparison with the extent of fusion of the premaxillary suture is more difficult. In *G. moholi*, the premaxillary suture was previously reported to be patent in two female specimens and partially fused in two male specimens, and no relationship to age was apparent (Reinholt et al., 2009). In contrast, observations on the present sample suggest fusion of the NMS is most likely an age-related process.

The most significant implication of our findings has a potentially critical, though indirect, bearing on our understanding of suture biomechanics. *In vivo* and *in vitro* studies demonstrate that patent craniofacial sutures serve to redistribute forces in adjacent cortical bone (Behrents et al., 1978; Hylander, 1979; Herring and Mucci, 1991; Herring and Teng, 2000; Herring et al., 2001). Specifically, while closed sutures exhibit patterns of strain that are similar to those seen in adjacent cortical bone, patent sutures exhibit higher strain indicating that they serve to dampen strain (Wang et al., 2008). Based on this role of sutures in mitigating bone strain, Ross (2005) suggested that it may be important to incorporate sutures into finite element (FE) models that are used to assess global variation in stress and strain. Only a few comparative finite element studies of the skull have incorporated sutures, and it is not yet clear whether the strain-mitigating effects of sutures are limited to adjacent bone alone or whether they affect larger regions of the skull. For example, Kupczik et al. (2007) showed that the patency or fusion of the zygomatic-temporal suture in an FE model of a macaque not only affected bone strain magnitudes by a factor of two but also influenced fairly local patterns of strain distribution. In contrast, a study of the effects of sutural fusion in an FE model of a lizard skull demonstrated again that sutures alleviated local strain, but that they also had significant impacts on strain levels in areas far removed from the sutures (Moazen et al., 2009). Many more studies are needed before we can make broad statements about the impact of sutures on craniofacial biomechanics. However, it appears that they are important in at least some vertebrates and are certainly likely to impact the mechanical performance of the skulls of mammals during their ontogeny.

As observed for the premaxillary and nasopremaxillary sutures (Reinholt et al., 2009), the NMS exhibits external notches or grooves in some areas when they are, in fact, fused on their internal aspects. This raises a concern, as previously articulated by Reinholt et al., (2009), that such sutures may be rated as patent if observed grossly on skeletonized specimens. This is

clearly also of concern for interpretation of sutural growth. Although it has been suggested that foci of suture fusion may “reopen,” at least transiently (Persson et al., 1978), once a suture is even partially fused, its function as a growth site is thought to be terminated (Herring, 1974). Therefore, undetected sites of fusion present a potential challenge for studies of both growth and suture mechanics.

A similar issue raised by this study is the articulation of more than two bones at sutural fronts. Processes of the premaxillary and ethmoid bones deep to the NMS fuse with and indirectly join the nasal and maxillary bones together at certain locations along the NMS. To our knowledge, this phenomenon has not entered discussions of suture biomechanics. Yet, a suture with such indirect “tethering” at the deep aspect is presumably less mobile as a patent suture without such connections.

The important effects of age, sex, heredity, and phylogeny on sutural fusion and patency in primates are relatively well-studied based on external evidence (e.g., Wang et al., 2006a,b; Cray et al., 2008). This study strongly indicates the need for a broad survey of suture microanatomy to inform interpretations of primate craniofacial growth and biomechanics. Interpretations of the sample studied herein are limited due to small sample size. Nonetheless, this sample hints that age and sex may be critical variables relating to early events in the complex process of sutural fusion, that is, the manner in which fusion occurs throughout its internal to external limits. Thus, microanatomical examination of larger samples of primates of known chronological or skeletal age would clarify fusion patterns relating to age and sex. The observation of indirect tethering of facial bones suggests a possible mechanism for phylogenetic patterning of facial organization, specifically regarding sutural interfaces, that may affect facial biomechanics. The ethmoid complex, in particular, articulates with intramembranous bones in a variable pattern across primates, notably within the orbit (Cartmill, 1978). These external patterns in the organization of facial bones suggest that sutural articulations may be phylogenetically variable at deeper levels as well. Thus, the incidence of indirect “tethering” of externally visible sutures by deeper articulations with other bones may vary among lineages of primates.

Micro-CT has emerged as a viable tool to address the issues of externally undetectable loci of suture fusion as well as indirect tethering of facial bones. There are some limitations to studying sutural fusion patterns with micro-CT, owing to resolution and sectioning plane details, (Reinholt et al., 2009). However, because it is a noninvasive method (Recinos et al., 2004; Stadler et al., 2006), micro-CT may be preferable to direct histological sectioning, and therefore ideal for a broader study using relatively rare skeletal samples such as primates.

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