Variation in the developmental and morphological interaction between the nasal septum and facial skeleton

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Abstract

While the nasal septum exerts a morphogenetic influence on the facial skeleton, there is evidence that this relationship is highly variable. To better appreciate the precise role of the septum, it is important understand the variable interaction between the septum and surrounding skeleton during ontogeny. Here we analyzed nasal septal and facial skeletal postnatal phenotypic variation using cross-sectional samples of C3H/HeJ and C57BL/6J mice. Initial observations indicated between-strain variation in the magnitude of septal deviation, suggesting differences in septal and facial skeletal interaction. We examined whether variation in septal deviation is due to ontogenetic differences in septal size, or whether variation in facial skeletal growth imposes spatial constraints on the septum. Using microCT we quantified septal size and deviation, and collected coordinate landmark data, which we analyzed using geometric morphometrics. C3H/HeJ mice were significantly more deviated than C57BL/6J during development. We found no differences in septal size between the two strains. However, while both strains exhibited an ontogenetic increase in snout length, C3H/HeJ mice exhibited a non-allometric reduction in nasal bone length. This appears to be influenced by between-strain variation in the spatial relationship between the nasal septum and nasofrontal suture. Unlike C57BL/6J mice, the C3H/HeJ nasal septum is positioned anterior to the nasofrontal suture potentially limiting an early direct influence of septal growth (e.g., through interstitial expansion) on sutural growth. Ultimately, our results underscore that while the septum is a key facial growth center, its precise influence on facial growth can vary even in narrow morphological and taxonomic ranges.

Keywords: Sutures, microCT, cranial base, septal deviation
Introduction

According to the nasal septal traction model, the nasal septal cartilage is a key midfacial growth center that has a significant morphogenetic influence on the ontogeny of the facial skeleton (Scott, 1953; Latham, 1970; Catala and Johnston, 1980; Copray, 1986; Wealthall and Herring, 2006; Holton et al., 2011). Through a combination of interstitial cellular division, extracellular matrix production, and endochondral ossification along the septoethmoidal junction (Scott, 1953; Baume, 1961; Catala and Johnston, 1980; Copray, 1986; Wealthall and Herring, 2006), the expanding septum exerts a tissue-separating force, transmitted via ligamentous attachment, (Latham, 1970; Gange and Johnston, 1974; Siegel et al., 1985, 1990; Mooney and Siegel, 1986, 1991), to the sutures of the facial skeleton. This mechanical force induces an osteogenic response along the bony fronts of the sutures (c.f., Opperman, 2002; Mao, 2006) resulting in increased anterior facial growth.

Experimental support for the nasal septal traction model has largely come in the form of studies that impede normal growth of the nasal septum. Surgical alteration of the nasal septum and septal-premaxillary complex (e.g., septal extirpation, resection of septopremaxillary ligament, etc.) typically leads to deficient anterior growth of the snout resulting in reduced nasal bone, maxillary, and premaxillary length (Wexler & Sarnat, 1961; Sarnat & Wexler, 1966, 1967; Ohyama, 1969; Riesenfeld, 1970; Latham et al. 1975; Friede & Morgan, 1976; Friede, 1978; Wada et al. 1980; Rhys-Evans & Brain, 1981; Squier et al. 1985; Siegel et al., 1990, 1992). However, despite the influence of the nasal septum on the anterior growth of the facial skeleton as indicated by experimental studies, there is sufficient evidence to suggest that this developmental relationship is highly variable. Unlike long-snouted mammals such as rabbits, surgical resection of the nasal septum in shorter faced mammals (i.e., cats, ferrets, and
chimpanzees) does not result in reduced anterior facial skeletal growth (e.g., Freng, 1981; Cupero et al., 2001; Siegel and Sadler, 1981).

The differential influence of the septum on the dynamics of anterior facial skeletal growth is further evident across broad mammalian comparisons. In mammalian taxa that are characterized by reduced anterior-posterior facial dimensions, there is a greater discordance between the growth of the nasal septum and the surrounding facial skeleton. Comparative studies indicate that while longer snouted mammals have a very low frequency of nasal septal deviation (~0.0%), the frequency of deviation increases considerably in mammals characterized by shorter midfaces (Gray, 1978; Takahashi 1987). For example, within primates Takahashi (1987) documented that among long-snouted strepsirrhines, approximately 100% are characterized by straight nasal septa. The frequency decreases among shorter-faced anthropoid primates, with only 40% of gorillas, 59% of chimpanzees, and 3% of humans lacking septal deviation. As such, reduced facial length in some taxa imposes spatial constraints on the expanding septum resulting in deviated nasal septal growth.

Even within humans there is evidence that nasal septal deviation is associated with reduced facial dimensions (Freng et al., 1988; Mays, 2012; Holton et al., 2012). However, the size of the nasal septum appears to be a significant contributing factor to the magnitude of deviation as well. Comparisons of septal deviation in European- and African-derived samples reveal that significantly greater magnitudes of deviation in European-derived subjects are associated with nasal septa that are approximately 35% larger than in African-derived subjects (Holton et al., 2012). Collectively, the results of experimental and comparative studies highlight the complex and highly variable relationship between the nasal septum and other aspects of the facial skeleton.
To more fully appreciate the morphogenetic influence of nasal septal growth on facial skeletal form, it is necessary to develop a better understanding of the variable interaction between the nasal septum and surrounding skeleton during ontogeny. This is key not only to understanding the influence of the nasal septum across a wide range of mammalian variation, but also to understanding the precise influence of the nasal septum on human facial form (e.g., Holton et al., 2012; Hartman et al., in press). In particular, this is critically important when extrapolating the results of experimental animal studies to human facial growth dynamics (e.g., Siegel, 1976; Siegel and Mooney, 1990) especially with regard to clinical procedures involving surgical intervention affecting the nasal septum in growing children, such as septoplasty and cleft palate repair (Farrior and Connolly, 1970; Béjar et al., 1996; Hall and Precious, 2013; Lawrence, 2012).

In the present study, we use microCT data to characterize postnatal phenotypic variation in the nasal septum and facial skeleton using cross-sectional samples in two commonly used mouse strains, C3H/HeJ and C57BL/6J, that we have observed to exhibit significant variation in the magnitude of nasal septal deviation (Fig. 1). The degree of variation in nasal septal deviation in these strains of mice suggests that even within narrow ranges of taxonomic and morphologic variation (e.g., intra-species comparisons) there may be considerable differences in the developmental and morphological relationship between the nasal septum and facial skeleton. Using data from mice ranging from 3-15 weeks of age, we investigated whether there is, in fact, variation in the developmental relationship between the nasal septum and facial skeleton during growth. We also investigated the degree to which potential differences may be due to ontogenetic variation in nasal septal size, as in human population comparisons (e.g., Holton et
al., 2012) versus potential spatial constraints imposed by the surrounding facial skeleton, as in broader mammalian comparisons (Gray, 1978; Takahashi 1987).

To accomplish this, we first quantified nasal septal deviation to determine the magnitude of difference between C3H/HeJ and C57BL/6J mice. We further examined whether differences in the magnitude of nasal septal deviation are established early in ontogeny, or whether septal deviation increases with development. Next, we examined whether differences in nasal septal deviation between the two strains are associated with variation in the growth of the nasal septum or variation in the midline space available for the growing septum. In other words, is septal deviation in C3H/HeJ mice due to increased septal growth, or does the size of the snout impose spatial constraints on the growing septum. Finally, we examined the morphological relationship between nasal septal growth and ontogenetic changes in facial skeletal shape. This included an examination of shape variation to determine whether there are between-strain morphological differences in the relationship between the septum and facial skeleton.

Materials and Methods

We examined the growth and development of the nasal septum and facial skeleton using cross-sectional samples of C3H/HeJ and C57BL/6J mice at 3-, 9-, and 15-weeks of age. Sample sizes are found in Table 1. To control for the potential mitigating effects of sexual dimorphism, only male mice were analyzed. The University of Iowa Animal Use and Care Committee approved all study protocols (#4041015).

To quantify differences in nasal septal deviation between C3H/HeJ and C57BL/6J mice we followed the methods used in Holton et al. (2012) and Hartman et al. (in press). First, we
manually segmented the nasal septum as a volume (mm$^3$) that included the nasal septal cartilage, perpendicular plate of the ethmoid, and vomer from the surrounding anatomy (Fig. 2). Next, we segmented a non-deviated midline volume (mm$^3$) following the space directly between the superior and interior attachment sites of the nasal septum as shown in the bottom of Fig. 2d. This second volume served as a non-deviated model of the nasal septum and represents the minimum amount of space available for the nasal septum in the midline nasal cavity (i.e., a measure of nasal cavity size). Individual nasal septal deviation values were calculated as a percentage of nasal septal volume relative to the volume of the non-deviated model [(nasal septal volume/non-deviated volume) x 100]. Thus, a value of 100% indicates the absence of nasal septal deviation (i.e., nasal septal volume is equal to the non-deviated modeled volume), while a percentage >100% is indicative of septal deviation (i.e., nasal septal volume is larger than the non-deviated modeled volume).

We compared the magnitude of nasal septal deviation between C3H/HeJ and C57BL/6J mice by testing for significant differences in nasal septal deviation values within each age group (i.e., 3-, 9-, and 15-weeks). We then examined whether variation in deviation between the two strains was due to an increase in the size of the nasal septum or a decrease in the available space for the nasal septum by testing for significant differences in nasal septal and non-deviated modeled volumes. Given that our nasal septal variables were not normally distributed, all comparisons were made using non-parametric Mann-Whitney U-tests.

To further assess developmental variation in nasal septal deviation, we examined the ontogenetic allometry of the volume of the nasal septum relative to a) the non-deviated modeled volume and b) overall craniofacial size measured as the centroid size of a series of two-dimensional midsagittal coordinate landmarks (Fig. 3). Specifically, we tested whether nasal
septal volume in C3H/HeJ mice scales with greater positive allometry relative to non-deviated modeled volume and craniofacial centroid size when compared to C57BL/6J mice. That is, do C3H/H3J mice exhibit a disproportionate increase in nasal septal volume relative to available measures of craniofacial size during ontogeny? Allometric slopes were calculated using reduced major axis regression of log-transformed variables.

Finally, we examined the relationship between the nasal septum and two-dimensional midsagittal craniofacial shape using the landmarks in Fig. 3. Landmark configurations were scaled and aligned using Procrustes superimposition techniques. We then used multivariate regression analysis to examine allometric changes in facial shape (dependent variables) relative to nasal septal volume (independent variable) across the combined C3H/HeJ and C57BL/6J samples. Next, we extracted residual shape information from the multivariate regression (i.e., shape variation that is independent of the ontogenetic increase in nasal septal volume). Using the residual shape data, we conducted a principal components analysis to examine whether there are key non-allometric shape differences (i.e., differences established early in ontogeny) that account for variation in nasal septal deviation between the two strains. All geometric morphometric analyses were conducted using MorphoJ (Klingenberg, 2008-2010).

**Results**

The C3H/HeJ mice were characterized by a significantly greater level of nasal septal deviation at 3-, 9-, and 15-weeks of age when compared to the C57BL/6J mice (Table 1; Fig 4a). While on average the C57BL/6J sample exhibited mean septal deviation values of ≈100% across all three age groups (indicating a general absence of septal deviation), the level of septal
deviation in the C3H/HeJ mice increased with age from 107.42% in the 3-week age group to 115.45% in the 15-week age group. The increasing level of septal deviation is evident in microCT images of representative mice from the 3-, 9-, and 15-week groups (Fig 5). In contrast to the C57BL/6J mice, which maintain a straight nasal septum throughout ontogeny, the nasal septum in C3H/HeJ mice becomes increasingly curved and buckled, especially in the anterior region of the septal cartilage.

With regard to the size of the nasal septum, septal volume was, on average larger in the C57BL/6J when compared to the C3H/HeJ mice, particularly at age 15-weeks (6.88 mm$^3$ and 5.73 mm$^3$ respectively). Nevertheless, there were no significant differences between the two strains of mice for any of the age groups (Table 1; Fig 4b). There was, however, a sizable difference in the available midline space for the nasal septum as evidenced by differences in the size of the non-deviated modeled volumes (Table 1; Fig 4c). In all three age groups, the available space was reduced in the C3H/HeJ mice, and was significantly different in the 9-week and 15-week age groups.

The discrepancy in growth between the nasal septum and the amount of midline space available for the septum is further evident in the allometric scaling of nasal septal volume and the volume of the non-deviated model (Fig. 6a, Table 2). For a given non-deviated modeled volume, the C3H/HeJ mice exhibited a larger nasal septal volume across the entire size range when compared to the C57BL/6J mice. Moreover, the results of our RMA regression analysis indicate that the nasal septal volume in C3H/HeJ mice scaled with positive allometry (slope=1.13, 95% CI=1.02-1.24). Thus, as the available space for the nasal septum increases, nasal septal volume becomes disproportionately larger in this strain. This is in contrast to nasal septal volume in the
C57BL/6J mice, which scaled isometrically with respect to the non-deviated modeled septal volume (slope=0.99, 95% CI=0.96-1.02).

We documented a similar scaling relationship with regard to craniofacial centroid size (Fig 6b, Table 2). Nasal septal volume in C3H/HeJ mice scaled with greater positive allometry (slope=0.81, 95% CI=0.59-1.03) compared to the C57BL/6J mice (slope=0.68, 95% CI=0.56-0.81). Thus, as craniofacial centroid size increases during ontogeny the C3H/HeJ mice exhibit a disproportionate increase in nasal septal volume we compared to the C57BL/6J mice. It is also of note that $R^2$ value for the C3H/HeJ mice ($R^2=0.81$) is lower than the value for the C57BL/6J mice ($R^2=0.90$). This suggests that nasal septal volume is not as strongly correlated with craniofacial size in the C3H/HeJ sample.

The results of our multivariate regression analysis demonstrate that during ontogeny there is a significant relationship between nasal septal volume and midsagittal craniofacial shape across the combined C3H/HeJ and C57BL/6J sample ($P<0.001$) with septal volume explaining 21% of shape variation. As illustrated in Fig. 7, an ontogenetic increase in nasal septal volume is associated with an elongation of the facial skeleton. This is evident both in the relative increase in nasal bone length (landmarks 1 & 3), as well as the relative increase in premaxillary length (landmarks 2 & 4). Facial elongation is further evident by the relative posterior displacement of the midline anterior cranial base (landmarks 6 & 7), which also represents the caudal surface of the nasal septum (i.e., the posterior-most aspect of the perpendicular plate of the ethmoid). The pattern of facial elongation is associated with a relative reduction in the height of the rostrum as evidenced by the inferior displacement of the nasal bones at the frontonasal suture (landmark 3).

Our principal components analysis of allometry-corrected shape variables (i.e., residuals from our multivariate regression analysis) reveals that there are distinct morphological
differences in craniofacial shape between C3H/HeJ and C57BL/6J mice. There was clear separation between the two strains along PC1 (Fig. 8), which accounted for 67% of non-allometric variation. Along the major axis of variation the C3H/HeJ mice (negative PC scores) were characterized by relative reduction in the length of the nasal bones (landmarks 1 & 3) relative to the C57BL/6J mice (positive PC scores). Moreover, there were considerable shape differences along the junction of the midline anterior cranial base (landmarks 6 & 7) with C3H/HeJ mice exhibiting a less retroflexed (i.e., flatter) cranial base relative to C57BL/6J mice. As a result, the caudal surface of the midline anterior cranial base (i.e., the posterior aspect of the perpendicular plate of the ethmoid) in C3H/HeJ mice is displaced anteriorly relative to the nasofrontal suture (Fig. 9). In contrast, the nasofrontal suture is positioned posterior to the anterior cranial base in C57BL/6J mice.

Discussion

The nasal septum is a key facial growth center that has a significant influence on the ontogenetic development of anterior-posterior dimensions of the facial skeleton (e.g., Scott, 1953; Wexler & Sarnat, 1961; Latham, 1970; Catala and Johnston, 1980; Copray, 1986; Siegel et al., 1990, 1992; Wealthall and Herring, 2006; Holton et al., 2011). Despite the importance of the nasal septum in contributing to normal facial growth processes, there is considerable variation in the morphological and developmental relationship between the septum and surrounding facial skeleton across mammalian taxa. In the present study we highlight the variable nature of this relationship using two strains of mice that exhibit previously undescribed variation in the magnitude of nasal septal deviation.
In our cross-sectional comparison we found that C3H/HeJ mice exhibited a greater degree of septal deviation during ontogeny when compared to C57BL/6J mice. A significant difference in deviation between the two strains was present by three-weeks of age and the difference increased in magnitude through age 15-weeks. Unlike human population variation in nasal septal deviation, which can be influenced by variation in the absolute size of the nasal septum (Holton et al., 2012), there were no significant differences in septal volume between the two strains across all three age groups. Instead, as evidenced by the significantly smaller non-deviated modeled volumes in C3H/HeJ mice, nasal septal deviation was associated with a significant reduction in the midline nasal cavity space available for the expanding septum. Thus, while facial skeletal and nasal septal growth was isomorphic in C57BL/6J mice, there was a disruption of the normal growth process between the nasal septum and surrounding facial skeleton in C3H/HeJ mice that resulted in a high magnitude of septal deviation.

Reduced midline nasal cavity space in C3H/HeJ mice was due, at least in part, to a relative reduction in the anterior growth of the nasal bones when compared to C57BL/6J mice. The results of our multivariate regression of nasal septal volume and facial shape indicate that during development, the growth of the nasal septum in both C3H/HeJ and C57BL/6J mice was correlated with an increase in the anterior-posterior dimensions of the palate and nasal bones. This pattern is consistent with the nasal septal traction model of facial growth (e.g., Scott, 1953; Latham, 1970; Catala and Johnston, 1980; Copray, 1986; Wealhall and Herring, 2006; Holton et al., 2011). However, in spite of the increase in anterior facial skeletal growth, C3H/HeJ mice were characterized by a disproportionate reduction in nasal bone length when compared to C57BL/6J mice. This was evident in the results of our principal components analysis of allometry-corrected shape data (i.e., size-independent shape variation) indicating that this pattern
is present by at least the third week of postnatal growth. While nasal bone length increased during ontogeny in C3H/HeJ mice, the pattern of increasing septal deviation indicates that the anterior growth of the nasal septum outpaces the anterior growth of the nasal bones. As such, deviation in C3H/HeJ mice is a manifestation of developmental constraints imposed by the surrounding facial skeleton.

Variation in the morphological relationship between the nasal septum and anterior facial dimensions in C3H/HeJ and C57BL/6J mice is similar to that documented by Gray (1978) and Takahashi (1987) who found an inverse correlation between facial length and nasal septal deviation across a broad range of mammalian taxa. Similarly, experimentally reduced facial growth via fixation of the circummaxillary sutures, which results in a reduction in the anterior-posterior dimensions of the facial skeleton, leads to increased nasal septal deviation and altered nasal septal/facial skeletal relationships (Rönning and Kantomaa, 1985; Holton et al., 2011). Given that we did not experimentally alter craniofacial growth in our sample of C3H/HeJ mice, the presence of reduced nasal bone growth coupled with septal deviation suggests that even within the same species, there is considerable variation in the developmental relationship between the nasal septum and the anterior growth of the facial skeleton.

Reduced anterior-posterior facial dimensions in other mouse models have been attributed to altered development of the nasal septum and intrinsic variation in nasofrontal sutural growth (Lozanoff et al., 1994; Ma and Lozanoff, 1996; McBratney et al., 2003; Neslon and Williams, 2004; Fogelgren et al., 2008). While we are unable to directly link reduced nasal bone growth to a specific dynamic in the present morphometric study, it is likely that variation in nasal bone length in our sample can be attributed, at least in part, to between-strain differences in the spatial relationship between the nasal septum and nasofrontal suture. The midline anterior cranial base,
which also serves as the posterior border of the perpendicular plate of the ethmoid, was less retroflexed (i.e., flatter) in C3H/HeJ mice when compared to C57BL/6J mice. This has the effect of positioning the nasal septum anterior to the nasofrontal suture in C3H/HeJ mice. This is in contrast to C57BL/6J mice in which the nasal septum spans the ventral surface of the suture.

The nasal septum exerts a measurable tissue-separating force along the nasofrontal suture during ontogeny (Al Dayeh et al., 2013). Force transmission is largely the result of ligamentous attachment from the expanding nasal septum to the surrounding facial bones. Experimental resection of the septopremaxillary ligament (Latham, 1970; Gange and Johnston, 1974; Siegel et al., 1985, 1990), as well as ligamentous attachment spanning from the septum to the rostral tip of the nasal bones (Long, 1985) results in a reduction anterior facial growth in a wide range of animal models, including rodents. It is the transmission of force, via ligamentous attachment at the rostral edge of the expanding septum that likely accounts for the ontogenetic increase in nasal bone length evident in both C3H/HeJ and C57BL/6J strains. However, the anterior displacement of the nasal septum relative to the nasofrontal suture, coupled with reduced nasal bone length in C3H/HeJ mice, suggests that the nasal septum may have less of a morphogenetic influence on nasofrontal suture growth when compared to C57BL/6J mice.

During postnatal development, nasal septal expansion is driven by high rates of chondrocyte proliferation (Searls, 1977, 1979; Vetter et al., 1984a,b; Copray, 1986; Wealthall and Herring 2006; Al Dayeh, et al., 2013). This includes the cartilage along the ventral surface of the nasofrontal suture prior to the ossification of the perpendicular plate of the ethmoid, which occurs approximately 10 days postnatal in mice (Wealthall and Herring, 2006). Thus, the influence of the nasal septum on early postnatal nasal bone growth is potentially derived from a combination of indirect septal forces transmitted by ligamentous attachment, as well as a more
direct influence from interstitial cartilage expansion in the region of the suture. Due to the anterior displacement of the nasal septum relative to the nasofrontal suture in C3H/HeJ mice, it is possible that early interstitial cartilage growth (to the degree that it influences osteogenic activity in the nasofrontal suture) has less of an influence on the anterior growth of the nasal bones, thus resulting in an early developmental deficiency in nasal bone length. Given that the discrepancy in nasal bone length between C3H/HeJ and C57BL/6J mice is non-allometric in nature, and therefore independent of growth-driven morphological variation (at least from 3-15 weeks), suggests that these differences are established early in development. The influence of variation in the spatial relationship between the nasal septum and nasofrontal suture, as well as the potential influence of early postnatal chondrocyte proliferation on nasofrontal sutural growth, however, remains to be directly tested.

The altered spatial relationship between the anterior cranial base/perpendicular plate of the ethmoid and the nasofrontal suture documented in the present study suggests that cranial base morphology (e.g., variation in the degree of retroflexion), relative to other cranial structures, has significant consequences for variation in anterior-posterior facial dimensions in C3H/HeJ and C57BL/6J mice. Indeed, the influence of cranial base morphology on the dimensions of the facial skeleton is well established during ontogenetic development as well as across a wide range of morphological variation in mammals (de Beer, 1937; Weidenreich, 1941; Enlow, 1990 Lieberman et al., 2000a,b; Bastir and Rosas, 2006; Bastir et al., 2006). While we are unable to establish the underlying causal determinants of variation in the orientation of the anterior cranial base, the degree of midline cranial base flexion is significantly influenced by neural development, including relative brain size (Ross and Ravosa, 1993; Strait, 1999; Lieberman et al., 2000a, 2000b, 2008; McCarthy, 2001; Ross et al., 2004; Bastir et al., 2010). As such,
variation in cranial base angle, and the resulting effects on the spatial relationship between the anterior cranial base and the nasofrontal suture, is potentially associated with variation in neural growth patterns. Lieberman et al. (2008) examined the relationship between brain size and cranial base angle in a variety of mouse strains including C3H/HeJ and C57BL/6J mice. Their results, however, did not seem to indicate an appreciable difference in encephalization between the two strains. However, in spite of differences in relative brain size values across a wide range of morphological variation in multiple strains of mice, encephalization only accounts for approximately 8% of the variation in cranial base angle (Lieberman et al., 2008). Therefore, it is possible that other factors, such as potential allometric variation in brain shape, may account for the differences in anterior cranial base morphology observed in the our analysis. A detailed three-dimensional analysis of cranial base and neural development in C3H/HeJ and C57BL/6J mice would help elucidate how the interaction between these structures (e.g., potential variation in transverse neural dimensions; Fig. 5) effects of the development of the anterior cranial base and the spatial position of the nasal septum.

In spite of the reduction in nasal bone length in C3H/HeJ mice, we did not identify any meaningful differences in palate length between the two strains of mice. We previously documented that reduced anterior facial growth via circummaxillary suture fixation in a pig model, coupled with a normal ontogenetic increase in nasal septal length, resulted in a compensatory increase in the length of the premaxillary region (Holton et al., 2011). In the present study, however, C3H/HeJ mice, exhibiting both reduced nasal bone growth, and a normal increase in nasal septal size, relative to C57BL/6J mice, did not exhibit a meaningful difference in the relative length of the premaxillae. The reason for the inconsistency in the results is unclear. It is possible, given that we focused solely on two-dimensional morphological variation
in the midsagittal plane, that compensatory growth occurred in other regions of the premaxillae that were not included in the present analysis.

Conclusions

Historically there has been debate regarding the role of the nasal septum in normal facial growth and development (e.g., Moss, 1964; Moss and Salentijn, 1969). However, while it is generally accepted that the nasal septum exerts an influence on facial skeletal growth (e.g., Scott, 1953; Baume, 1961; Catala and Johnston, 1980; Copray, 1986; Wealthall and Herring, 2006), this influence appears to be highly variable within and between mammalian taxa. While there are likely multiple underlying causal determinants for the variable influence of the nasal septum, the results our comparative morphometric analysis suggest that the spatial relationship between the nasal septum and key facial growth sites can a) reduce the total amount of anterior nasal bone growth, and b) restrict the amount of space available for the nasal septum resulting in deviated septal growth. Importantly, our results document that the developmental and morphological relationship between the nasal septum and surrounding facial skeleton is highly variable even within a narrow range of morphological and taxonomic variation.

Acknowledgements

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References


Figure Legends

Fig. 1. Comparison of C57BL/6J and C3H/HeJ mice. (a) Three-dimensional microCT reconstruction of mice in lateral view. (b) Cross-section through the snout in the region of the red dashed line in (a) illustrating variation in nasal septal deviation.

Fig. 2. Three dimensional rendering of segmented nasal septum (blue) and non-deviated septal model (green) in a C3H/HeJ mouse. (a) Rendering of mouse cranium (dorsal aspect cropped) with nasal septum and non-deviated model in situ. (b) Isolated nasal septum and non-deviated model. The discrepancy between the actual deviated septum and the modeled non-deviated septum is illustrated in (c), which overlaps the reconstructed nasal septum and the reconstructed non-deviated model. Septal deviation was calculated as [(nasal septal volume/non-deviated...
model volume x 100] following Holton et al. (2012) and Hartman et al (in press). (d) Coronal CT sections of a deviated C3H/HeJ mouse. The nasal septum (blue) was segmented by tracing the septum in the coronal plane from the anterior to posterior septal borders. The nondeviated model (green) was segmented by following the superior and inferior borders of the nasal septum.

Fig. 3. Two-dimensional midsagittal landmarks used to assess craniofacial morphology.

1= rhinion; 2= prosthion; 3= nasion; 4= maxillary-premaxillary suture; 5= posterior nasal spine; 6= anterior cranial base; 7= sphenoid-ethmoidal synchondrosis; 8= mid-sphenoidal synchondrosis; 9= sphenoid-occipital synchondrosis; 10= basion.

Fig. 4. Comparison of (a) nasal septal deviation, (b) nasal septal volume, and (c) modeled volume in C57BL/6J (black) and C3H/HeJ (white) mice. *Significant difference (P<0.05).

Fig. 5. Transverse microCT images of representative C57BL/6J (top row) and C3H/HeJ (bottom row) at 3-, 9-, and 15-weeks of age. The nasal septa (dashed lines) of C57BL/6J mice remain straight during ontogeny, while the nasal septa of C3H/HeJ mice become increasingly deviated.

Fig. 6. Allometric scaling of the nasal septum. Scatter plots of septal volume and modeled septal volume (a) and septal volume and facial centroid size (b) with reduced major axis regression lines for C3H/HeJ (open circles, dashed line) and C57BL/6J (black circles, solid line) mice.

Fig. 7. Variation in craniofacial shape that is correlated with an ontogenetic increase in nasal septal volume across C57BL/6J and C3H/HeJ mice. Facial shape associated with a smaller nasal
septal volume (i.e., at 3-weeks of age) is pictured on the left, while facial shape associated with a larger nasal septum (i.e., 15-weeks of age) is pictured on the right. The black wireframe models represent size-correlated shape variation, while the gray wireframes represent the mean shape.

Fig. 8. Non-allometric shape variation along PC1 (67%). Variation along the PC1 axis distinguishes between C3H/HeJ mice (open circles) and C57BL/6J mice (black circles). Wireframe models illustrated shape variation between C3H/HeJ (left) and C57BL/6J (right) mice. The black wireframe models represent shape variation along PC1, while the gray wireframes represent the mean shape.

Fig. 9. Two-dimensional midsagittal and three-dimensional microCT images illustrating variation in the spatial relationship between the anterior cranial base and the nasofrontal suture in C3H/HeJ (a & c) and C57BL/6J (b & d) mice. As a component of non-allometric shape variation between the two strains, the anterior cranial base is positioned anterior to the nasofrontal suture in C3H/HeJ mice, while the anterior cranial base is positioned posterior to the nasofrontal suture in C57BL/6J mice.
Table 1. Descriptive statistics for nasal septal variables. Significant differences in bold.

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<th>Age</th>
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<tr>
<td>Septal deviation (%)</td>
<td>101.74 (1.60)</td>
<td>107.42 (2.50)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Septal volume (mm$^3$)</td>
<td>3.66 (0.44)</td>
<td>3.62 (0.15)</td>
<td>0.84</td>
</tr>
<tr>
<td>Non-deviated model (mm$^3$)</td>
<td>3.60 (0.39)</td>
<td>3.38 (0.19)</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>9-weeks of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septal deviation (%)</td>
<td>101.46 (1.57)</td>
<td>113.58 (3.59)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Septal volume (mm$^3$)</td>
<td>5.63 (0.28)</td>
<td>5.47 (0.51)</td>
<td>0.92</td>
</tr>
<tr>
<td>Non-deviated model (mm$^3$)</td>
<td>5.55 (0.31)</td>
<td>4.83 (0.45)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>15-weeks of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septal deviation (%)</td>
<td>100.52 (0.58)</td>
<td>115.45 (2.09)</td>
<td>0.02</td>
</tr>
<tr>
<td>Septal volume (mm$^3$)</td>
<td>6.88 (0.22)</td>
<td>5.73 (0.22)</td>
<td>0.06</td>
</tr>
<tr>
<td>Non-deviated model (mm$^3$)</td>
<td>6.21 (0.45)</td>
<td>4.70 (0.36)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table 2. RMA regression parameters for nasal septal volume relative to modeled volume and craniofacial centroid size.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Modeled Volume</th>
<th>Craniofacial Centroid Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>95% CI</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>1.13</td>
<td>1.02-1.24</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>0.99</td>
<td>0.96-1.02</td>
</tr>
</tbody>
</table>
Fig. 1. Comparison of C57BL/6J and C3H/HeJ mice. (a) Three-dimensional microCT reconstruction of mice in lateral view. (b) Cross-section through the snout in the region of the red dashed line in (a) illustrating variation in nasal septal deviation.

63x32mm (300 x 300 DPI)
Fig. 2. Three dimensional rendering of segmented nasal septum (blue) and non-deviated septal model (green) in a C3H/HeJ mouse. (a) Rendering of mouse cranium (dorsal aspect cropped) with nasal septum and non-deviated model in situ. (b) Isolated nasal septum and non-deviated model. The discrepancy between the actual deviated septum and the modeled non-deviated septum is illustrated in (c), which overlaps the reconstructed nasal septum and the reconstructed non-deviated model. Septal deviation was calculated as \[(\text{nasal septal volume/\text{non-deviated model volume}}) \times 100\] following Holton et al. (2012) and Hartman et al (in press). (d) Coronal CT sections of a deviated C3H/HeJ mouse. The nasal septum (blue) was segmented by tracing the septum in the coronal plane from the anterior to posterior septal borders. The nondeviated model (green) was segmented by following the superior and inferior borders of the nasal septum.

38x14mm (300 x 300 DPI)
Fig. 3. Two-dimensional midsagittal landmarks used to assess craniofacial morphology. 1=rhinion; 2=prosthion; 3=nasion; 4=maxillary-premaxillary suture; 5=posterior nasal spine; 6=anterior cranial base; 7=spheno-ethmoidal synchondrosis; 8=mid-sphenoidal synchondrosis; 9=spheno-occipital synchondrosis; 10=basion.
59x27mm (300 x 300 DPI)
Fig. 4. Comparison of (a) nasal septal deviation, (b) nasal septal volume, and (c) modeled volume in C57BL/6J (black) and C3H/HeJ (white) mice. *Significant difference (P<0.05).
Fig. 5. Transverse microCT images of representative C57BL/6J (top row) and C3H/HeJ (bottom row) at 3-, 9-, and 15-weeks of age. The nasal septa (dashed lines) of C57BL/6J mice remain straight during ontogeny, while the nasal septa of C3H/HeJ mice become increasingly deviated.
Fig. 6. Allometric scaling of the nasal septum. Scatter plots of septal volume and modeled septal volume (a) and septal volume and facial centroid size (b) with reduced major axis regression lines for C3H/HeJ (open circles, dashed line) and C57BL/6J (black circles, solid line) mice.
Fig. 7. Variation in craniofacial shape that is correlated with an ontogenetic increase in nasal septal volume across C57BL/6J and C3H/HeJ mice. Facial shape associated with a smaller nasal septal volume (i.e., at 3-weeks of age) is pictured on the left, while facial shape associated with a larger nasal septum (i.e., 15-weeks of age) is pictured on the right. The black wireframe models represent size-correlated shape variation, while the gray wireframes represent the mean shape.
Fig. 8. Non-allometric shape variation along PC1 (67%). Variation along the PC1 axis distinguishes between C3H/HeJ mice (open circles) and C57BL/6J mice (black circles). Wireframe models illustrated shape variation between C3H/HeJ (left) and C57BL/6J (right) mice. The black wireframe models represent shape variation along PC1, while the gray wireframes represent the mean shape.
Fig. 9. Two-dimensional mid-sagittal and three-dimensional microCT images illustrating variation in the spatial relationship between the anterior cranial base and the nasofrontal suture in C3H/HeJ (a & c) and C57BL/6J (b & d) mice. As a component of non-allometric shape variation between the two strains, the anterior cranial base is positioned anterior to the nasofrontal suture in C3H/HeJ mice, while the anterior cranial base is positioned posterior to the nasofrontal suture in C57BL/6J mice.

138x75mm (300 x 300 DPI)