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Spatially-dense morphometrics of craniofacial sexual dimorphism in one year-olds

Running Head: Sexual dimorphism in one year-olds

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Abstract

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1 Recent advances in the field of geometric morphometrics allow for powerful statistical
2 hypothesis testing for effects of biological and environmental variables on anatomical shape.
3 This study used partial least-squares regression (PLSR) and the recently developed
4 Bootstrapped Response-Based Imputation Modeling (BRIM) algorithm to test for sexual
5 dimorphism in the craniofacial shape of one year-old humans. We observed a recession of the
6 forehead in boys relative to girls, and differences in the nose, consistent with adult
7 dimorphism. Results also suggest that the degree to which individuals express dimorphic
8 traits is continuous throughout the population. This is also seen in adult dimorphism, but in
9 one-year olds the amount of overlap, between groups is much higher, indicating the strength
10 of dimorphism between sexes is lower. Our results demonstrate early sexual dimorphism that
11 is not attributable to the influx of sex hormones at puberty. This highlights the need to look
12 very early ontogeny for the origins of sexual dimorphism. We suggest future work look at
13 potential mediating effects of this early dimorphism on the later impact of puberty. The subtle
14 shape differences we have detected, may also be applied to the sexing fossilised crania. A
15 common artefact in 3D images of faces of young children is that they often have their mouths
16 open to varying degrees, introducing variability in the data unrelated to anatomy. We
17 describe two PLSR-based methods of correcting this. These methods may facilitate surgical
18 planning and assessment of young children, based on 3D images.

19
20 Keywords: craniofacial, sexual dimorphism, morphometrics, partial least-squares regression,
21 pose, infants

22 Spatially-dense morphometrics of craniofacial sexual dimorphism in one year-olds

23
24 Sexual dimorphism refers to the characteristic morphological differences between males and
25 females. Sexual dimorphism of faces has been confirmed in adults (Tanikawa et al., 2015,
26 Claes et al., 2011, Claes et al., 2014, Hennessy et al., 2005, Velemínská et al., 2012). The
27 developmental, evolutionary and biological bases for sexual dimorphism is of considerable
28 interest to anthropologists and biologists. It is widely supposed dimorphic traits emerge only
29 with the onset of puberty, where increased testosterone triggers a masculinisation of males'
30 faces (Enlow and Hans, 1996, Verdonck et al., 1999). There is evidence for independent

1 effects of both adult hormone levels and those experienced in utero, on adult facial shape
2 (Fink et al., 2005, Schaefer et al., 2005, Penton-Voak and Chen, 2004). It has been proposed
3 that uterine hormone levels organise the underlying ‘architecture’ of the face, which is then
4 activated at puberty (Neave et al., 2003). Such early differences in facial architecture should
5 be detectable very early in life, if sufficiently sensitive methods are employed. Although
6 dimorphism in the size of the neurocranium has been demonstrated in infants and in utero
7 (Joffe et al., 2005, Bulygina et al., 2006) the presence of adult patterns of shape dimorphism
8 has, to the best of our knowledge, only been demonstrated in adolescents, down to
9 approximately age eleven (Koudelová et al., 2015, Ferrario et al., 2003, Bulygina et al.,
10 2006).

11 Many previous studies of sexual dimorphism have employed univariate comparisons of
12 selected inter-landmark measurements (Tanikawa et al., 2015, Kondo et al., 1999, Joffe et al.,
13 2005). However, any fewer than all pairwise distances between all pairs of landmarks, fail to
14 capture the full spatial arrangement and geometrical relationships between landmarks
15 (Dryden and Mardia, 1998). *Geometric morphometrics* (Adams et al., 2004, Mitteroecker and
16 Gunz, 2009, Rohlf and Marcus, 1993), comprises techniques for the analysis of entire
17 landmark configurations. These capture the spatial arrangement between all landmarks by
18 using landmark locations themselves as the fundamental unit of analysis. Previous such
19 studies of children have derived conclusions from visual inspections of the differences
20 between average male and female configurations (Ferrario et al., 2003, Bulygina et al., 2006)
21 and not from statistical hypothesis testing. They have also used a relatively limited
22 representation of the surface defined by anatomical (and some pseudo-anatomical)
23 landmarks. This precludes analysis of the surface where these are not present. **Finally they**
24 **have not controlled for the effect of allometry. Within morphometrics, allometry refers to**
25 **shape changes that accompany changes in size (Klingenberg, 1996). Males’ heads are larger**
26 **than females’ heads, meaning that a general allometric effect may confound the true pattern**
27 **of shape differences attributable to sex (Rosas and Bastir, 2002).**

28 Surface registration algorithms (Hutton et al., 2003a, Claes et al., 2012b, Claes, 2007,
29 Andresen and Nielsen, 2001), allow the automatic mapping of spatially-dense configurations
30 of landmarks from a template shape to every instance in a population. In combination with
31 geometric morphometric techniques, this defines *spatially-dense morphometrics* which can

1 be used to analyse the entire geometry of the surface under study (Claes et al., 2011, Claes et
2 al., 2012c, Claes et al., 2012b, Claes et al., 2014, Claes et al., 2012a, Claes, 2007, Hutton et
3 al., 2003b, Hutton et al., 2003a, Hutton et al., 2001, Hammond et al., 2004, Hammond, 2007,
4 Cox-Brinkman et al., 2007, Andresen et al., 2000, Hennessy et al., 2005).

5 Here we use partial least-squares regression (PLSR; Wold et al., 2001, De Jong, 1993),
6 coupled with a permutation-based framework for significance testing (Kennedy, 1995,
7 Anderson and Legendre, 1999, p. 278) to test for sexual dimorphism in one year-old boys and
8 girls, improving on earlier comparisons of average shapes. We analysed the entirety of the
9 craniofacial complex, whereas previous studies of this age-group have only analysed the face
10 and the anterior neurocranium (Bulygina et al., 2006), or a single measure of head-
11 circumference (Joffe et al., 2005). We also use the Bootstrapped Response-based Imputation
12 **Modeling** (BRIM; Claes et al., 2014)) algorithm to test for sexual dimorphism and determine
13 if dimorphic traits are distributed continuously in the population, **and the degree of overlap in**
14 **morphology of males and females**. Extensions of PLSR were used to correct a problem
15 observed in the sample, of variable mouth openness due to limited co-operation of the young
16 children.

17 Method

18 *Sample*

19 **To test for sexual dimorphism in an homogenous one year-old population** 473 infants, were
20 imaged at the age of one ($M=363.47$, $SD=8.83$ days old). One child's sex was not recorded,
21 and was excluded from the analyses of sexual dimorphism, although they were included in
22 the sample used to assess the mouth closing techniques. The final sample with known sex
23 contained 248 boys and 224 girls. These were a subsample of the Asking Questions about
24 Alcohol (AQUA; Muggli et al., 2014) cohort. The mothers were a cross-section of a low-risk
25 pregnancy population recruited during their first visit to an ante-natal clinic. Data about
26 lifestyle and biological factors that may affect craniofacial shape were collected, with a focus
27 on maternal alcohol consumption. Analyses were conducted to determine if the effect of sex
28 reported here was independent of these factors (see supplementary material, section 2). In all
29 included cases parent reported ethnicity was either "white" or of European extraction.

1 *Image acquisition and pre-processing*

2 **The outer surface of the facial soft-tissue was encoded as a three-dimensional image.** In a 3D
3 image, faces are represented as a “mesh”. This comprises a cloud of points (vertices) in three-
4 dimensions and a triangulation of neighbouring vertices that defines the outer surface of the
5 face.

6 Images were collected at the Royal Children’s Hospital, Melbourne, Australia using the
7 3dMD 7-pod system (3dMD corporation Atlanta GA). This captures a full 360^o image of the
8 head including the face and cranium. Images were taken with a tight-fitting stocking placed
9 over the cranial vault, so as to capture its shape, unobscured by hair, which was pressed very
10 tightly against the scalp.

11 **In order to establish anatomical correspondence across the sample of images,** spatially-dense
12 configurations of quasi-landmarks were automatically indicated onto each 3D image via a
13 non-rigid registration (see Snyders et al., 2014 for technical details, see Claes et al., 2012b for
14 a non-technical description). **This is essentially a spatially-dense, automatic extension of the**
15 **process of manually indicating anatomical landmarks onto a subject or specimen.** Quasi-
16 landmarks are defined as the vertices of a template mesh (e.g. an average one-year old face)
17 Each point may or may not have a well-defined biological meaning (hence quasi-) but
18 together they provide a representation of the entire surface.

19 **Although differing patterns of asymmetry in males and females may be of interest (Claes et**
20 **al., 2012c), in this work we concentrate on the symmetric component of shape variation.**
21 Asymmetry was removed by superimposing a reflected copy of the face onto the original and
22 taking the mean of the two faces as a symmetrical version of that face (Claes et al., 2011).

23 *Generalised Procrustes Analysis (GPA)*

24 **In order to isolate differences in the scans due to anatomical variability, from those**
25 **differences due to the location, rotation and size of the images, all scans were co-aligned to**
26 **the sample mean via Generalised Procrustes Analysis (GPA; Gower, 1975).** GPA iteratively
27 estimates the mean (the *Procrustes mean*) configuration of the sample, to which all faces are

1 subsequently aligned. Standard GPA is sensitive to gross, localised form differences such as
 2 the “Pinocchio” effect (Zelditch et al., 2004), and so robust Procrustes superimposition was
 3 used (Claes et al., 2012a). To remove differences in size, all faces were scaled to the mean
 4 size of all faces in the sample. Although it is more usual to scale all faces to unit centroid size
 5 (see below), the mean size of the sample was used to keep the regression coefficients in mm
 6 units, as the magnitude of shape differences may be of interest to clinicians. It was
 7 empirically verified that this had no effect on the statistical inferences drawn here (data not
 8 shown). As a measure of size we use centroid size:

$$\sqrt{\sum_{i=1}^k d_i^2}$$

9 where d_i is the distance of the i_{th} quasi-landmark from the centroid (mean) of the landmark
 10 configuration.

11 The co-ordinates of the Procrustes mean configuration were subtracted from each face to
 12 define the *Procrustes residuals*. The Procrustes residuals of the entire sample (\mathbf{P}) can be
 13 represented as a matrix:

$$\mathbf{P} = \begin{bmatrix} 1_{x_1}, 1_{y_1}, 1_{z_1} & \cdots & 1_{x_k}, 1_{y_k}, 1_{z_k} \\ \vdots & \ddots & \vdots \\ j_{x_1}, j_{y_1}, j_{z_1} & \cdots & j_{x_k}, j_{y_k}, j_{z_k} \end{bmatrix}$$

14 the elements of \mathbf{P} code the deviation of each point from the Procrustes mean configuration.
 15 Each row contains the k quasi-landmarks which together define the shape of a particular face.
 16 There are j number of faces. The geometric interpretation of this matrix is as a point-cloud in
 17 $k \times 3$ dimensional space, with the Procrustes mean at the centre. Each face is a single point
 18 within this space.

19 *Partial least-squares regression (PLSR)*

20 **PLSR was used to characterise the effects of sex and size on shape.** PLSR was accomplished
 21 by the SIMPLS method (De Jong, 1993). PLSR models the association between one or more
 22 predictor variables and one or more response variables. In this case the matrix of Procrustes

1 residuals (defining facial shape) constituted the matrix of response variables. Procrustes
2 residuals are highly correlated, and there are more Procrustes residuals than observations in
3 our sample. In such situations PLSR is preferable to ordinary least-squares regression (Wold
4 et al., 1984, Shrimpton et al., 2014).

5
6 Geometrically PLSR defines the vector through the point-cloud that best captures the
7 covariance between predictor/s (e.g. sex) and facial shape. The effects of sex and centroid
8 size on shape were separated, by including both as predictors in the regression model. This
9 forces each regression path to model different variability, thus isolating their independent
10 effects on shape. The vector of elementwise products of size and shape was also included as a
11 predictor to model the interaction between the two variables.

12
13 The permutation based significance testing protocol described in Claes et al (2014), was used
14 to determine the significance, both of the overall effect of each variable on facial morphology
15 and of the effect on each individual quasi-landmark. All significance tests were based on
16 1000 permutations.

17 *Bootstrapped response-based imputation modeling (BRIM)*

18 The algorithm BRIM (Claes et al., 2014) was also used to characterise the effects of sex on
19 shape, and to determine if dimorphic features are distributed continuously in the population.

20 The core principle in BRIM, with reference to the current problem, is that the categories
21 “Male” and “Female” are imprecise; it is likely that they do not capture the full range of
22 maleness/femaleness. Some people have very masculine or feminine faces, and some appear
23 more androgynous. BRIM iteratively extracts the regression vector (using PLSR) that best
24 characterises the effect of the predictor on the response variables and derives a RIP
25 (Response-based Imputed Predictor) value for each face, which indicates that face’s position
26 along this regression vector. Thus, the dichotomous variable (male/female) is transformed
27 into a continuous variable that represents each face’s masculinity-femininity. The algorithm
28 consists of two nested loops, which are described in supplementary material (section 1) and
29 elsewhere (Claes et al., 2014). This imputation can be ‘conditioned’ by including other
30 variables in the model. We conditioned this analysis with centroid size and the interaction

1 term (described earlier), to ensure the regression path and RIPs were independent of these
2 effects.

3

4 The hypothesis test for BRIM is an ANOVA test of mean difference in the RIP variables,
5 with sex as the independent variable. This tests for a difference in the groups' position along
6 the regression vector. Significance is determined using an empirically estimated F-
7 distribution (see supplementary material section 1). The regression vector extracted can be
8 visualised by regressing Procrustes residuals onto shape and using the methods of
9 visualisation described below.

10

11 *Correction of mouth openness*

12 **Non-anatomical variation in the images, due to mouth openness**, was removed by defining a
13 variable to represent the degree of mouth openness for each face. As a preliminary step all
14 faces were rated by the first author (HM) on a **continuous** scale from 0 **through to** 3,
15 indicating how open the mouth was.

16 Partial least-squares regression defines the direction in the point-cloud that captures the
17 covariance between the variable and the shape. When more than one variable is included as a
18 predictor it defines a direction for each variable. Each direction will be orthogonal to each
19 other and thus statistically independent; two regression vectors cannot account for the same
20 variability. Including a variable that will account for the irrelevant variability, such as ratings
21 of mouth openness, is one method of removing the irrelevant variability. It follows that the
22 residuals of the model with mouth openness as a predictor constitute corrected versions of
23 each face. This corrected face could be explicitly constructed by adding its corresponding
24 residuals to the Procrustes mean configuration (which was subtracted during GPA).

25 A limitation of the above method is that the accuracy of the correction of an individual face is
26 determined entirely by the rating it was initially given and the overall consistency of the
27 ratings across the sample. The accuracy of the correction can be controlled by allowing an
28 operator to manually adjust the degree of the correction. The regression coefficients, are a
29 vector of weights on each Procrustes residuals (the vector of *ms* below). These weights

1 constitute a transformation that can be applied to the face, to manually open or close the
 2 mouth. The degree to which the mouth is opened or closed is determined by adjusting t , while
 3 observing its effect on the facial image. Specifically a transformed version of a particular face
 4 is calculated by:

$$5 \quad \begin{bmatrix} f'_{x_1} \\ f'_{y_1} \\ f'_{z_1} \\ \vdots \\ f'_{x_k} \\ f'_{y_k} \\ f'_{z_k} \end{bmatrix} = \begin{bmatrix} f_{x_1} \\ f_{y_1} \\ f_{z_1} \\ \vdots \\ f_{x_k} \\ f_{y_k} \\ f_{z_k} \end{bmatrix} + t * \begin{bmatrix} m_{x_1} \\ m_{y_1} \\ m_{z_1} \\ \vdots \\ m_{x_k} \\ m_{y_k} \\ m_{z_k} \end{bmatrix}$$

6 where the vector of f s contains the original landmark locations of the face, and f' is the
 7 corrected locations. A primitive graphical user interface was built which enabled the
 8 operator (HM) to interactively adjust t , using a slider, while visualising the results. t was
 9 adjusted until the mouth was judged closed and then the corrected image was saved.

10

11 *Visualising regression effects.*

12 The effects of variables on face shape can be illustrated using **false-colour** maps. These are
 13 the Procrustes mean shape colour-indexed according to some value of interest. Regression
 14 coefficients define a set of weights on each quasi-landmark. Together they define how much
 15 and the direction in which the landmark is displaced per unit of the predictor. Imagine an
 16 arrow at each landmark. The values of interest represented in the heat maps are: the “Effect”
 17 (magnitude) i.e. the length of the arrow at each point, or the magnitude of the effect in the
 18 lateral, vertical and depth direction; the proportion of the variance at each quasi-landmark
 19 explained (partial R^2) by the predictor; and the significance of the effect at each quasi-
 20 landmark.

21 Another way to visualise the effect is to apply the transformation to the Procrustes mean face
 22 as described above for correcting the scans of individual faces, to create a *morph* or *shape*
 23 *transformation*. It is most sensible to construct morphs within the actual range of the

1 predictor, but extrapolating beyond this can help to elucidate a small effect, even if it results
2 in morphs that are anatomically implausible.

3 **Results**

4 *Mouth Correction*

5 Figure 1 displays false-colour maps of the regression of Procrustes residuals onto mouth
6 openness. Figure 2 illustrates the estimated transformation with morphs constructed within
7 the actual range of mouth openness ratings ($t=0$, $t=3$) and extrapolated beyond it ($t=-3$, $t=6$).
8 The estimated transformation is a good approximation of the action of opening or closing the
9 mouth. The variability present in the sample before and after each correction was investigated
10 using Principal Components Analysis (see supplementary material section 3). Results
11 indicated that the variability due to variation in mouth openness was removed by both
12 corrections.

13 The sexual dimorphism analysis was performed on data corrected using each method of
14 mouth correction and without any correction (see supplementary material section 4). No
15 difference was observed in the outcome of the analysis between the methods. In particular
16 there was no difference in the dimorphism in the lower face where the mouth openness would
17 have been expected to have more effect. For simplicity the analyses on the uncorrected
18 sample are reported below.

19 *Sexual Dimorphism of Size and Shape*

20 The global (centroid) size of males' ($M=21120.70$, $SD=501.16$) heads were significantly
21 larger than females' ($M=20624.26$, $SD=479.08$) heads ($F=119.90$, $p<.001$, $\eta^2=.203$), see
22 Figure 3.

23 [Insert Figure 3 about here]

24
25 **There was no interaction between the effects of size and shape ($p=.449$) indicating that the**
26 **effects of sex and size on shape can be considered independently.** Overall the effect of sex
27 was significant ($partial R^2 = .008$, $p=.002$). The strongest difference in the facial appearance

1 is around the forehead, where sex accounts for up to 4% of the total variance, but there are
 2 also differences in the vault shape (see Figure 4 and Figure 5). The forehead in females
 3 protrudes by approximately 1 mm relative to males, and as a consequence the naso-frontal
 4 angle is more acute. The nose is narrower and smaller in females than in males. Female
 5 cheeks are more prominent in the area lateral to the nasolabial crease. The effect around the
 6 ears indicates that females' lower earlobes tend to point inwards whereas the males' earlobes
 7 point more outwards.

8
 9 The effect of size was also significant (partial $R^2 = .008$, $p < .001$). The effect is concentrated
 10 in the nasal region and on the upper lip and consists primarily of a superior and anterior
 11 displacements of these landmarks as centroid size increases. The upper portion of the ears
 12 appear to point out more in larger children. Not surprisingly, faces that are larger, also appear
 13 to have greater general fat deposition (see Figure 4 and Figure 6).

14 [Insert Figure 4 and Figure 5 about here]

15 *BRIM*

16 The distribution and means of RIP values are shown in Figure 7 and the extracted regression
 17 vector is shown in Figure 8. The females ($M=1.51$, $SD=0.09$) tend to have higher RIP values
 18 than males ($M=1.43$, $SD=0.09$; $F=72.03$, $p < .001$, $\eta^2=.133$). This indicates that males and
 19 females differ in their mean position along the extracted regression path; higher values
 20 correspond to increased femininity of the face. There is also overlap between the male and
 21 female groups. This indicates that there is a continuum of masculinity-femininity in one year-
 22 olds. Similar effects, to those observed in the PLSR analysis, are evident around the cranial
 23 vault and nose. The effect on the ears disappeared, the effect on the cheeks is reduced.

24
 25 [Insert Figure 6, Figure 7 and Figure 8 about here]

26
 27 In order to provide a more comprehensive picture of the overlap between males and females,
 28 we plotted centroid size against RIP scores (Figure 9). This represents the difference between
 29 the groups in a size-and-shape space, where the multivariate shape is compressed into a
 30 single dimension.

31 [Insert Figure 9 about here]

Discussion

This study investigated sexual dimorphism in the craniofacial morphology of a sample of one-year old humans. We used partial least-squares regression and BRIM, coupled with a spatially-dense representation of the soft-tissue anatomy, to test for statistically significant differences in the shape of the entire craniofacial complex. We also included centroid size in the regression, allowing us to separate the effects of size and sex on shape.

We observed that global size of the face and cranial vault was larger in males than females. Partial least-squares regression showed an effect of sex the shape of the forehead, with the girls' foreheads protruding more forwards than the boys'. Females' noses were also smaller and narrower than males. This effect on the forehead and nose may signal a relatively smaller basicranium in females. A smaller basicranium could make both the forehead relatively more bulbous as well as narrowing the maxilla and nasal passages. There was also an effect on the cheeks, possibly corresponding to greater fat deposition on the cheeks in girls. Interestingly also the lower ear-lobes of girls have a tendency to point inwards more than those of boys.

BRIM extracted a similar, though not identical vector of sexual dimorphism in the sample, identifying the differences in the forehead and nose as those that best differentiated the groups. A recession of the forehead in males, relative to females is a classic dimorphic feature, which has been identified consistently in adults (Hennessy et al., 2005, Claes et al., 2014, Rosas and Bastir, 2002). The presence of this trait has been previously only been documented in adolescents as young as 11 (Ferrario et al., 2003) and 12 (Koudelová et al., 2015). In adults the forehead slopes backwards in both males and females, but is more recessed for males. In one year-olds the forehead generally protrudes forwards in both sexes, but is relatively less protruding in males. To the best of our knowledge this is the first study to document shape dimorphism in this adult direction in such a young sample. Curiously Bulygina et al. (2006), in a longitudinal study of lateral radiographs, observed the opposite dimorphism in younger children (0.5 through to 8 years), that is the male forehead protruded more than the female, with the trend reverting into the clearly adult pattern at puberty (their Figure 7). Their sample size was very small (14 for each sex) and did not apply significance tests to their analysis of shape, and so sampling error in that study may explain the different results.

1 The differences in the shape of the nose is also quite consistent with differences observed in
2 adult soft-tissue studies (Claes et al., 2014, Hennessy et al., 2005). Koudelová et al. (2015)
3 observed differences in the nose emerging at 14 years (their Figure 2), but not earlier. Such
4 differences were not investigated by Bulygina's (2006) study of hard tissue landmarks and
5 were not observed by Ferrario (2003), possibly due to the sparse landmark configurations
6 used to represent the shape of the face.

7 The distributions for RIP values of each sex overlap in adults (Claes et al., 2014, their Figure
8 4B) as we have reported here for one year-olds. This indicates that the features identified are
9 distributed continuously within each sample i.e. there is a continuum of
10 masculinity/femininity in facial shape within one year-olds, as there is in adults. The
11 distributions for one year-olds overlap much more than in adults, suggesting that more one
12 year-olds than adults occupy an androgynous middle region of the continuum. Comparison of
13 the distributions of RIP values of sex for different ages may add an interesting new
14 dimension to the study of the ontogeny of sexual dimorphism.

15 The observed effect of sex was independent of the effect of size on shape. There was also a
16 significant effect of size, or *allometry*, which was independent of the effect of sex. Our
17 allometric effect consisted primarily of anterior and posterior displacement of the landmarks
18 around the nose and upper lip as centroid size increases. Furthermore the upper portion of the
19 ears point out and there appears to be greater general fat deposition as centroid size increases.
20 No strong claims can be made regarding whether this effect is consistent with adults, as the
21 effects are on regions with deep soft-tissue. The only similar analyses of adults have been
22 performed on skulls (Rosas and Bastir, 2002), or have not separated the effects of allometry
23 and sex making comparison of these findings difficult.

24 It is generally assumed that an increase in testosterone around puberty triggers a
25 masculinisation of male faces, leading to dimorphism between the sexes (Enlow and Hans,
26 1996, Verdonck et al., 1999). It is well-established that serum testosterone is related to facial
27 masculinity in adults (Penton-Voak and Chen, 2004, Schaefer et al., 2005). Our results
28 demonstrate that some dimorphic traits are present in a subtler form at one year-old, long
29 prior to this pubertal increase in serum testosterone. This may represent the effect of the sex-
30 hormone levels in the uterine environment on the early configuration of the face,

1 hypothesised by Neave (2003). The difference may also be attributable to inherited sex-
2 linked facial characteristics, and not directly to uterine hormone levels. In either case these
3 differences should be present in newborns. Future work should make use of the powerful
4 methods employed here to also test for shape differences in newborns, and explicitly
5 investigate the effect of the uterine hormone environment.

6 This work quantifies an early sexual dimorphism that is not attributable to the influx of sex
7 hormones at puberty. It may be of interest in future to investigate the relationship between
8 this early dimorphism and the later impact of puberty. For example, how dimorphic a face is
9 prior to puberty may or may not mediate the magnitude or direction of the changes that occur
10 at puberty (e.g. faces that are already masculine may experience a more profound
11 masculinisation at puberty).

12 Different developmental mechanisms may lead to sexual dimorphism (Shea, 1986). Studies
13 of hominids, including humans, support the notion that adult sexual dimorphism is in part due
14 to ontogenetic scaling (that is that adult females resemble more juvenile males) and in part
15 due to divergence in growth trajectories (Schaefer et al., 2004, Cobb and O'Higgins, 2007,
16 O'Higgins and Collard, 2002). The ontogenetic scaling component of adult sexual
17 dimorphism can be explained by differences in the time taken to mature, with males
18 continuing to grow along the same trajectory for a longer time, or by differences in the rate of
19 maturation, with males progressing faster along a common growth trajectory, for a similar
20 length of time. Shea (1983) terms these mechanisms time and rate hypermorphosis,
21 respectively. It is possible that different mechanisms operate differently at different times in
22 ontogeny. For example Cobb and O'Higgins (2007) argued that, in apes, sexual dimorphism
23 prior to puberty was a product of ontogenetic scaling, but subsequent sexual dimorphism was
24 produced by a deviation of male and female growth trajectories. Within our sample of a
25 single juvenile ontogenetic stage, it is not possible to determine the developmental
26 mechanisms that produced the dimorphism, but it does demonstrate the need for future work
27 to look very early in ontogeny, to develop a full understanding of the development of
28 dimorphic characteristics.

29 In anthropology a common problem is determining the sex of fossilised crania (Franklin et
30 al., 2005, Deshmukh and Devershi, 2006, Abdel Fatah et al., 2014). This is particularly

1 difficult for pre-adolescent remains (Molleson et al., 1998). Our results demonstrate that there
2 are shape differences in the crania that distinguish the sexes very early in ontogeny. Shape
3 may be more useful than size for sexing juveniles where the age, and therefore expected size
4 of the crania is unknown.

5 Cranial sexing is usually achieved using either a univariate or multivariate approach. The
6 univariate approach treats a particular trait (e.g. bizygomatic breadth) as a binary classifier.
7 That is, an appropriate threshold is determined, and observations on one side are considered
8 male and those on the other are considered female. A multivariate approach defines a
9 *discriminant function* into which values on multiple traits can be used to determine the sex of
10 an observation (see Deshmukh and Devershi, 2006 for a comparison of the two approaches).
11 The former is limited in that any particular trait is unlikely to serve as an optimal classifier.
12 The latter is limited in that a decision must be made regarding which traits are important and
13 which are not. Furthermore, the common method of linear discriminant-function analysis is
14 sensitive to the number of traits included in the model and collinearity between traits. A
15 PLSR-based approach may, therefore, be desirable.

16 A PLS regression vector through shape-space constitutes a single dimension that captures the
17 variability in all traits (landmarks) that is most strongly associated with the predictor (sex).
18 RIPs, being projections onto this vector, can be interpreted as a univariate ‘compression’ of
19 this multivariate variability. We suggest that RIPs can be used as a univariate binary
20 classifier, keeping the simplicity of the univariate approach, whilst capturing the important
21 multivariate shape information. We report classification accuracy using RIPs and describe a
22 method of building and assessing a classification model in supplementary material (section
23 5). On the current data the model performs above chance. We suggest that future work can
24 investigate this issue more thoroughly using 3D hard-tissue representations derived from 3D
25 images of skeletons, or CT of living subjects, and examine classification accuracy on
26 juveniles of different ages.

27 This sample was collected as part of a larger study on the effects of alcohol intake during
28 pregnancy (Muggli et al., 2014). This study recruited mothers during normal, low-risk
29 pregnancy to monitor the effects of typical levels of alcohol intake on a range of outcomes
30 including face shape. As such the sample represents a typical cross-section of one year-olds

1 and the effect of alcohol consumption was expected to be unrelated to the effect of sex. To
2 confirm this, the analysis was repeated on a subsample of mothers who did not drink at all
3 while pregnant (see supplementary material). The effect on the forehead was present,
4 although the effects on other regions were not statistically significant. This is most likely due
5 to the substantially reduced sample size and statistical power in that analysis. We cannot
6 exclude the possibility that the effects on other regions are specific to mothers who drank
7 some alcohol during pregnancy.

8 Instantaneous image capture by 3D stereo-photogrammetry reduces the requirement for
9 prolonged co-operation that often hampers the collection of anthropometric measurements in
10 children, such as those used in the planning and assessment of craniofacial surgery. Even
11 with this a residual problem remains in images of young children that a neutral facial pose is
12 difficult to ensure. This can greatly affect the accuracy of such measurements (Hermann et
13 al., 2016). Stereo-photogrammetry, coupled with a mouth-correction protocol may therefore
14 greatly facilitate the collection of such measurements on this otherwise difficult population.

15 We suggest correcting mouth openness by including it as a covariate in a PLSR model, or
16 using PLSR to define a transformation that can be applied manually to the face. Inspection of
17 the residual variability after treating the data with either approach (supplementary material
18 section 3), suggests that both successfully remove the variability due to mouth openness, and
19 both are probably quite adequate for a PLSR based analysis. For applications where the
20 accuracy of the correction at the level of an individual is important, such as anthropometry
21 for surgical planning, manual correction is likely preferable.

22 To the best of our knowledge, this is the first study to demonstrate the presence of sexual
23 dimorphism, consistent with adult patterns, in a sample of one year-olds. **We quantify a
24 sexual dimorphism that is not attributable to the influx of sex-hormones at puberty. This may
25 represent the effect of uterine hormone levels or inherited sex-linked facial characteristics.
26 We highlight the need to look very early in ontogeny for the beginnings of sexual
27 dimorphism and suggest future studies investigate potential mediating effects of early
28 dimorphism on the impact of puberty. Furthermore, the study illustrates the power of
29 spatially dense morphometrics to detect subtle differences in the shape of anatomical
30 structures, which may be applied to the problem of sexing fossilised crania. We also**

1 illustrate the application of PLSR-based techniques to correcting non-standard pose in 3D
2 images, which may facilitate surgical planning and assessment from 3D images of this age
3 group.

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16 classification models.

17 *Author Contributions*

18 H.M. conducted the analyses reported here, developed the manual mouth-closing procedure,
19 assembled the first draft of the manuscript and incorporated the suggestions of co-authors.
20 T.P. offered extensive suggestions for revision of the manuscript. I.S. conducted preliminary
21 analyses on the data and first identified the presence of sexual dimorphism. P.C. oversaw the
22 implementation and application of the analyses, and provided extensive comments on the
23 manuscript. JH and EM oversaw the collection of the images in the AQUA study and
24 contributed to manuscript content.

25 *Conflict of Interest*

26 The authors declare they have no conflicts of interest.

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Figure Legends

Figure 1. The regression of Procrustes residuals onto ratings of mouth openness. Shows the effect magnitude (effect) and the effect decomposed into the lateral, vertical and depth directions. In “lateral”, red indicates (viewers) right-directed displacement, blue indicates left-directed displacement. In “vertical” red indicates superior displacement, blue indicates inferior displacement. In “depth” blue indicates posterior displacement and red indicates anterior displacement. The units of the colour-bars for these images are mm. Partial R^2 indicates the amount of variability in the location of each quasi-landmark that is explained by mouth openness. $p < .05$ indicates the areas where partial R^2 was significant.

Figure 2. The transformation estimated by the regression onto mouth openness ratings. Morphs were constructed by varying the value of t . 0 and 3 represent the limits of the variation that is actually present in the population. -3 and 6 are exaggerations of the effect.

Figure 3. Sex differences in head size. Shows the distributions (left) and means (right) of head centroid size of males and females. Error bars indicate the standard error of the mean.

Figure 4. The regression of Procrustes residuals onto sex and centroid size, including an interaction in the model. Shows the overall magnitude of the predicted vertex displacements (Effect) and the magnitude of the predicted displacements in the lateral, vertical and depth directions, from males to females. They are colour indexed to mm displacement, per unit of the predictor. The proportion of variance that the predictor variable predicts at each individual vertex is shown in Partial R^2 and the significance of the effect on each landmark (yellow regions are significant at $\alpha = .05$).

Figure 5. Effect of sex on face shape. The centre two columns show the typical male ($t=1$) and typical female ($t=2$) faces predicted by the model. The outer columns show the difference between these two typical faces magnified 5 times ($t=-4$, $t=7$).

Figure 6. Effect of head centroid size on head shape. This shows the face predicted by the regression model with centroid size equal to ± 6 standard deviations

1 *Figure 7 Imputed masculinity-femininity. This shows the distribution (left) and mean (right) of the RIP values*
2 *for males and females. Error bars indicate the standard error of the mean.*

3

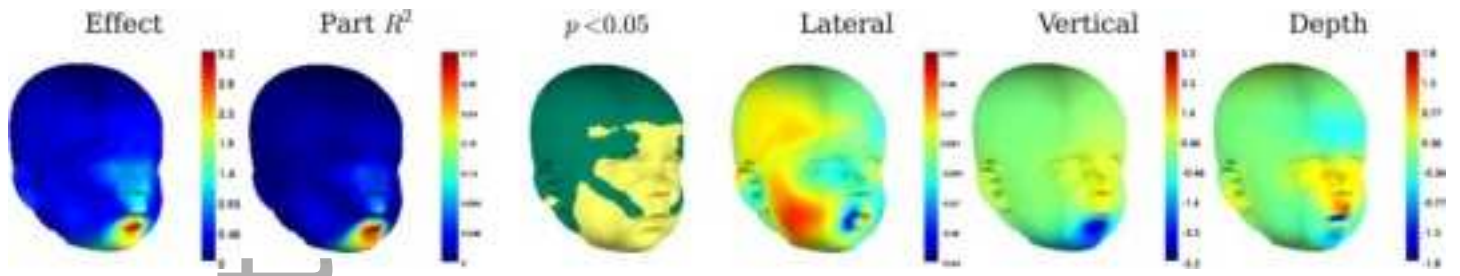
4 *Figure 8 Regression of Procrustes residuals onto RIP variables. This shows the regression path best*
5 *characterising the difference between males and females estimated by the BRIM algorithm.*

6

7 *Figure 9. RIP scores and centroid size for males and females. 95% confidence ellipses for males (solid line) and females*
8 *(dotted line) are also shown.*

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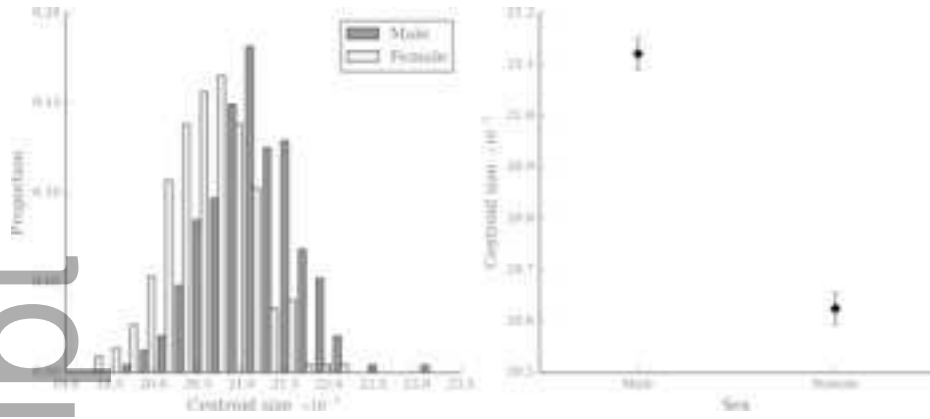
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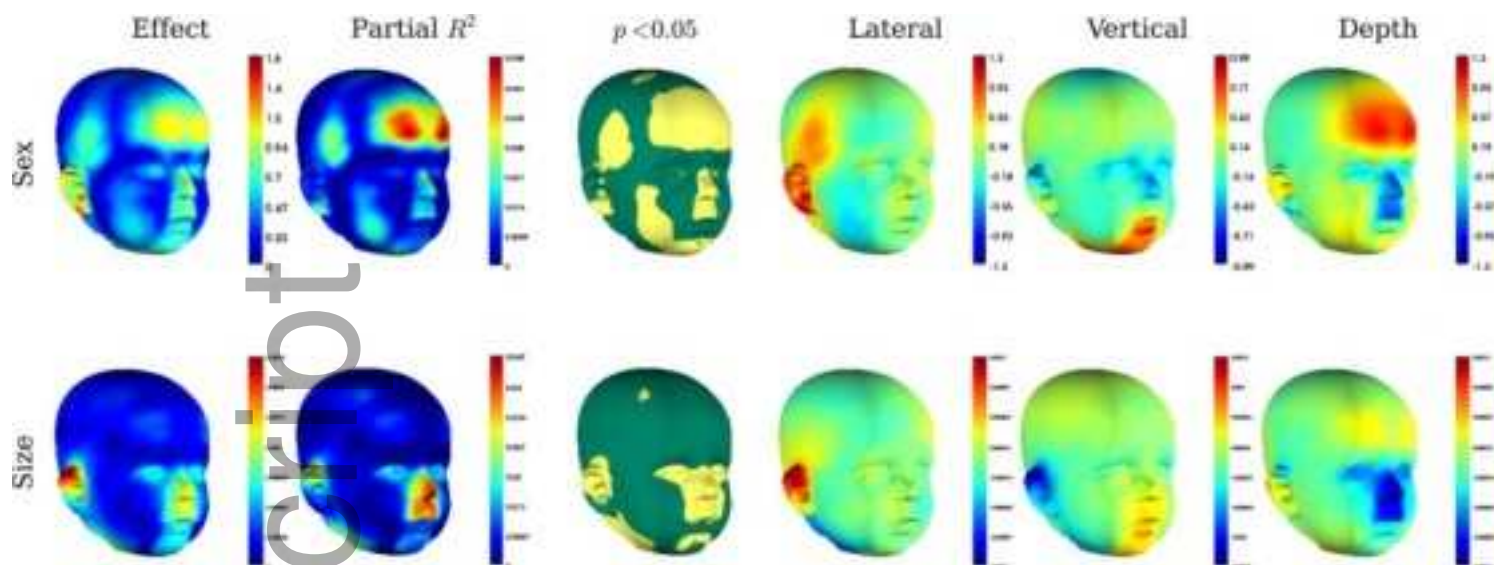
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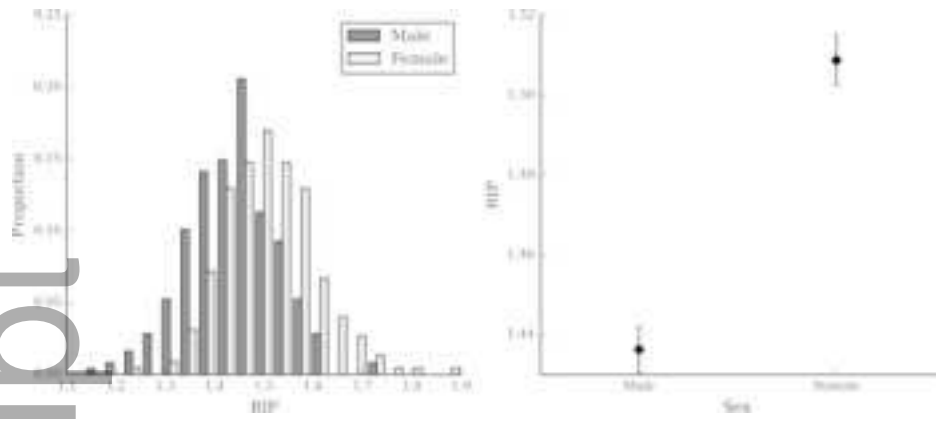
-6 SD

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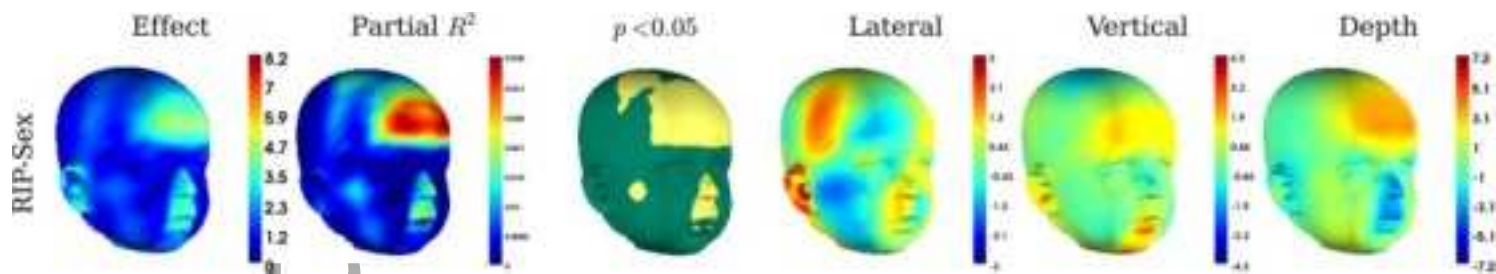
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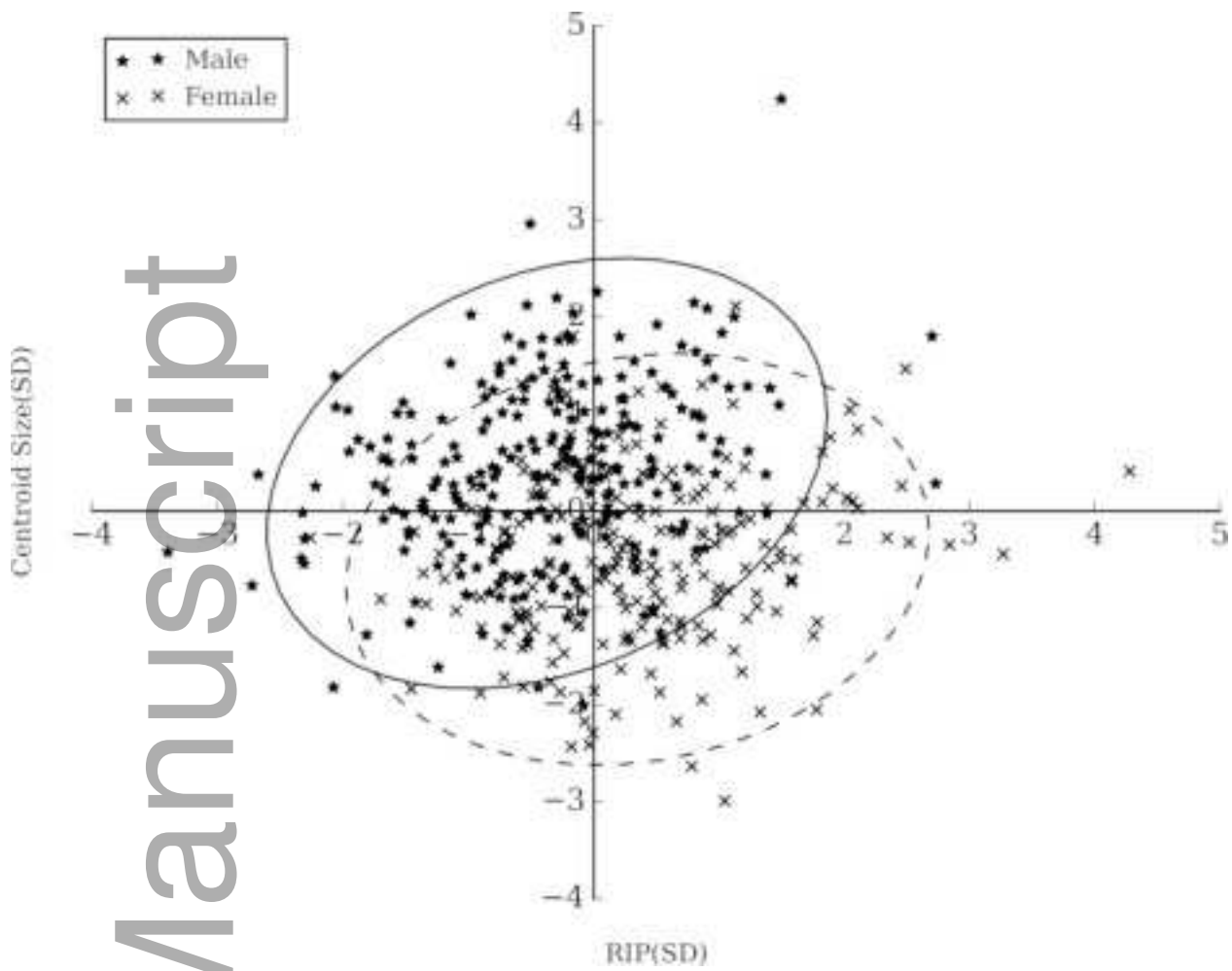


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