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Metric Analyses of the Teeth and Faces of South Australian Twins

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Abstract. Procedures are described for the acquisition and analysis of data in a study of the dental and facial characteristics of South Australian twins. Comparisons of the mesiodistal diameters of maxillary incisors in MZ and DZ twins revealed heterogeneity of total variances and evidence of inequality of mean values for some dimensions between MZ and DZ twins. Previous estimates of heritabilities for tooth size, relying on classical assumptions in twin research, may be exaggerated. A preliminary analysis of facial shape was undertaken using a procedure for shape matching based on a least squares fit of homologous coordinates. There was evidence of mirror-imaging in some MZ twin pairs and differences in facial asymmetry between male and female DZ twins. Future extensions of the study using methods for three-dimensional shape analysis are described.

Key words: Tooth size, Facial morphology, Twins, Craniofacial genetics, Asymmetry, Mirror-imaging, Shape analysis

INTRODUCTION

Most twin studies of dentofacial morphology, based on the classical method of comparing variance within monozygous (MZ) and dizygous (DZ) twin pairs, have indicated a strong genetic component to observed variation [13,15,16]. However, traditional approaches assume that environmental effects are similar for MZ and DZ twins and that results can be directly extrapolated to the general population. These assumptions have been challenged, among others, by Christian and coworkers who developed alternative models to analyse twin data [4-7]. Recent applications of these methods to dental data have disclosed sources of heterogeneity between zygosities, together with evidence of higher environmental covariance for some traits in MZ compared with DZ twins. When these factors are taken into account, estimates of heritability, particularly for occlusal variables, have been considerably reduced [8,18,22]. Findings such as these are very relevant in increasing our understanding of the causes and treatment of those variations in the arrangement of teeth and jaw position that we classify as malocclusions. In time, rational methods for preventing these dentofacial anomalies may stem from such studies.

Metric studies of the teeth and faces in twins also provide an opportunity to assess the magnitude and nature of morphological asymmetries, including the phenomenon of mirror-imaging. They may also provide a better insight into the nature of the twinning process itself. Boklage [1] has provocatively stated that "MZ twins, DZ twins, and singletons are not the same kind of people when the question is about the way their heads are built". Our study aims to test this hypothesis, among others, by providing reliable estimates of genetic variability for both dental and facial variables, and also by quantifying the magnitude and location of asymmetries in these structures.

This paper gives a brief description of the type of records being collected and some of the methods developed to acquire, display and analyse data in both two- and three-dimensional formats. Findings of a preliminary genetic analysis of dental crown size in twins using the methods of Christian [7] are given, together with the results of an initial comparison of facial shape in twins, using a method of least squares matching of homologous data points described by Sneath [26] and elaborated by Siegel [24].

SUBJECTS AND METHODS

Records and observational data for over 140 pairs of South Australian twins, mainly teenagers, have been collected. Most of the subjects enrolled in the continuing study are listed in the National Health and Medical Research Council of Australia Twin Registry which is maintained in Melbourne. The main records of dentofacial morphology consist of dental casts and stereo photographs of the face. In addition, intra- and extraoral monophotographs are obtained, together with palm- and fingerprints for collaborative research in dermatologlyphics. Medical histories, including birthweight and length, are recorded and also information relating to laterality. Blood samples are obtained for determination of zygosities.

A detailed description of the facial stereophotogrammetric methods which we employ has been provided elsewhere [2]. The equipment consists of matching left and right Hasselblad motor-driven cameras mounted on a rigid machined frame, following the specifications and photographic technique of Savara et al [20]. Quantification of the stereophotographs on a Wild Analytical Stereoplotter at the South Australian Department of Lands provides a set of x, y and z coordinates which represent the surface morphology of the face in three dimensions (3D). Processing of the 3D coordinates by computer enables facial contourgrams to be generated in any orientation or more elaborate displays to be produced by applying software packages developed mainly for application in computer-assisted design and manufacturing [28]. Figures 1 and 2 provide examples of images that can be generated by computer.

Ultimately, we intend to compare 3D dental and facial contours within and between twin pairs. However, this initial report describes more conventional analyses of tooth

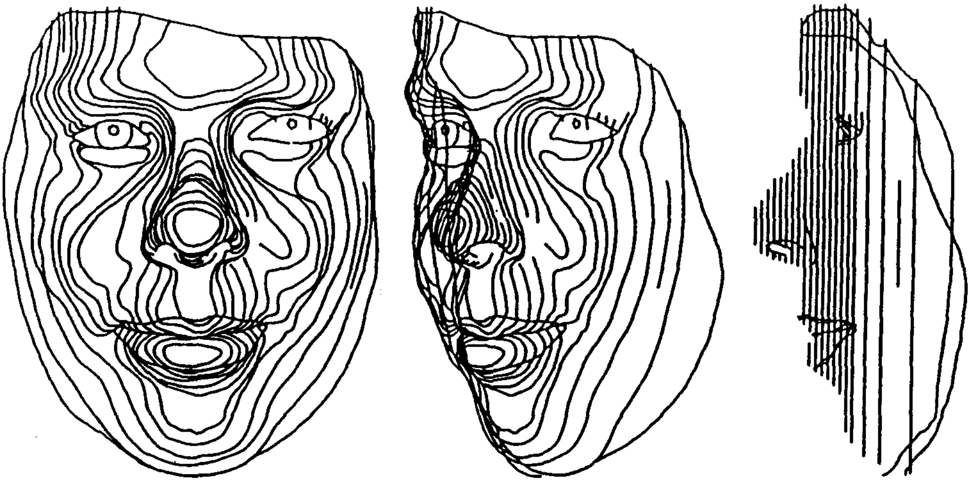

Fig. 1. Facial contourgrams of a subject from the twin study generated by computer in three orientations.

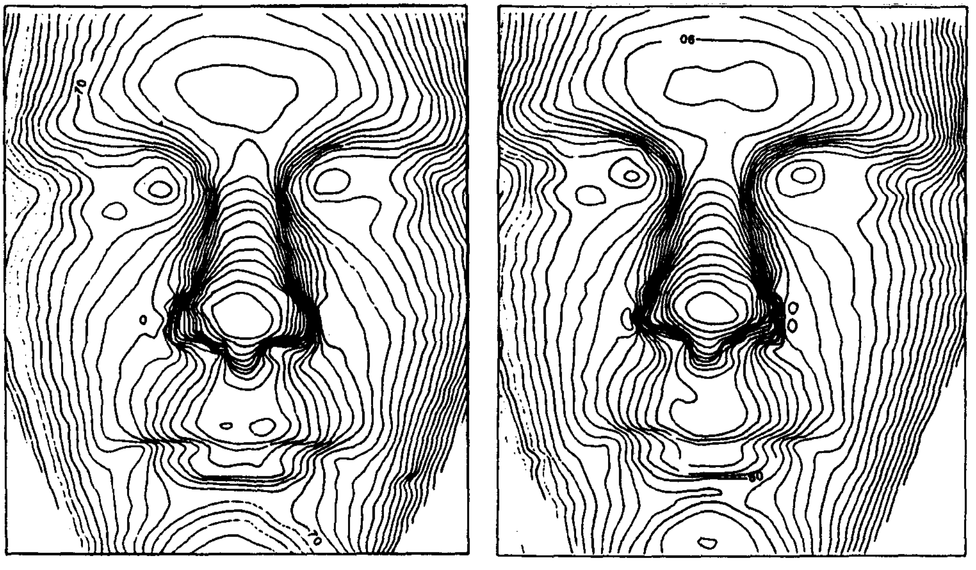

Fig. 2. Contourgrams of MZ twin boys generated by profiling on a stereoplotter.

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size and facial morphology limited to two-dimensional data obtained by conventional photogrammetric techniques.

Tooth Size

Christian and colleagues have emphasised the need to test several assumptions commonly made in traditional genetic analyses. Firstly, a modified t-test is used to assess differences in mean values between the twin groups for the traits under consideration. Significant differences would indicate biological differences associated with the twinning process [6,7]. Secondly, an F-test is performed to detect the presence of any heterogeneity of total variances between twin groups which would indicate that environmental factors may differ between MZ and DZ twins [4]. If variance inequality is noted, the arithmetic mean of the between-pair and within-pair mean squares is used as an unbiased estimate of genetic variance [4]. Thirdly, Christian et al [5] have pointed out that genetic variance estimates will be biased if there is evidence of unequal environmental covariances in MZ and DZ twins. An F-test is used to compare the within-pair and between-pair mean squares of DZ twins. If this ratio fails to exceed the value of one by an appreciable amount, then evidence for genetic variance is probably present only in MZ twins and it is therefore unlikely that a significant proportion of the total variance is genetic.

In a sample of 90 twin pairs, 49 MZ and 41 DZ pairs, measurements of the maximum mesiodistal crown diameters of maxillary central and lateral incisors were obtained from dental casts. Recordings were made to an accuracy of 0.10 mm using a modified dial caliper and following the definition of Moorrees et al [14]. Teeth which were not fully erupted or which showed evidence of attrition or trauma were excluded. Measurements were not attempted where caries, restorations, calculus or plaque obscured a dimension. Duplicate measurements performed on 20 subjects showed that experimental errors were small as assessed by the standard deviations of a single determination which ranged from 0.10 to 0.13 mm.

Descriptive statistics for tooth size, including means and variances were determined initially for males and females separately. Estimates of genetic variance, intraclass correlation coefficients and heritability estimates were then calculated according to the methods of Christian [7].

Facial Morphology

To quantify facial asymmetry and compare the facial morphology of twins, we have developed approaches which do not rely on matching measurements derived from an arbitrary mid-facial axis as has been proposed previously [3,11,21,29].

We have applied two related methods for shape comparisons, each based on the principles of least squares matching of sets of homologous points. The first used the well-known algorithm of Sneath [26] in which all points are treated equally in minimizing the discrepancies between shapes. The second procedure, described by Siegel [24] and Siegel and Benson [25], uses a repeated median approach to identify and quantify regions of maximum congruence between the shapes. In our preliminary applications dealing with a small set of facial coordinates the minimization procedures led to almost identical results for both methods. The results of the Sneath procedure are presented in this paper.

We have simplified our representation of facial morphology by defining 12 key reference points identified on standardised photographs [Fig. 3]. Replicability trials within

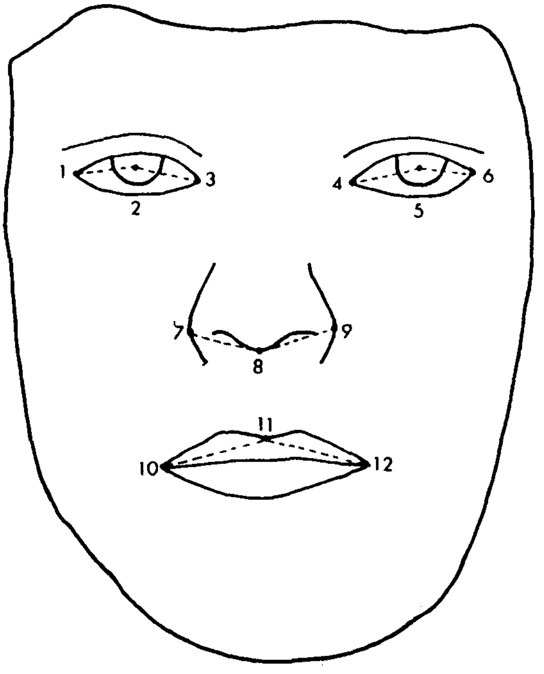

Fig. 3. Reference points used in the analysis of facial asymmetry.

and between recorders and using data from photographs of the same individuals taken on separate occasions indicated that the landmarks can be located with an acceptable degree of accuracy. Landmarks were located on tracings made on enlarged projections of the facial photographs and then digitised. Coordinate sets representing the key facial features were stored on computer for 41 pairs of twins; 10 MZ male pairs, 11 MZ female pairs, 10 DZ male pairs and 10 DZ female pairs. The coordinates were brought to natural size according to a metric scale included in each photograph. Coordinates representing the mirror-images of each face were generated using a mathematical transformation of the coordinates, which essentially "flipped over" the image around its y axis and resequenced the points to create its own mirror. The following comparisons of facial shapes were then made: between twin pairs, using the original coordinates to quantify similarity in facial appearance between each twin pair; between the original coordinates of each twin and that twins's mirror image, to assess individual facial asymmetry; and between the original coordinates of a twin and the mirror-image coordinates of the cotwin, to assess mirror-imaging between twin pairs. The degree of similarity between shapes was indicated by the root mean square (RMS) of the residual distances between homologous points remaining after shape 2 had been brought to maximum agreement with shape 1 by a scalar, linear and angular transform according to Sneath [26].

RESULTS

Table 1 provides a summary of the descriptive statistics derived for tooth size in male and female twins separately. Although the sample sizes were small when the sexes were considered separately, they are of similar magnitude to those reported in other studies of tooth size in twins [23]. Of considerable interest were the findings of an inequality of mean values between male MZ and DZ twins for the maxillary right central incisor dimension,

Table 1 - Mean Values and Total Variances for Mesiodistal Tooth Size in South Australian Twins

Tooth	Males				Females			
	MZ(22)		DZ(12)		MZ(30)		DZ(12)	
	$\overline{X}$	s^2	$\overline{X}$	s^2	$\overline{X}$	s^2	$\overline{X}$	s^2
Left I2	6.8	0.46	6.9	1.22*	6.6	0.59	6.6	0.53
Left I1	8.8	0.53	9.2	0.77	8.5	0.83*	8.5	0.35
Right I1	8.8	0.57	9.2*	0.56	8.4	0.74*	8.5	0.31
Right I2	6.9	0.58	7.0	0.78	6.6	0.51	6.6	0.39

* Significantly greater at $p < 0.05$ when MZ and DZ values compared.
Number of teeth measured indicated in parenthesis.

together with heterogeneity of total variances between male MZ and DZ twins for the maxillary left lateral incisor, and both central incisors in female MZ and DZ twins. Whilst we would stress the greatest caution in attempting to place biological implications on these results, they emphasise that the basic assumptions of the classical twin approach were invalidated in this instance in four of a possible eight comparisons.

Table 2 gives estimates of genetic variance and heritability estimates following Kang et al [12] for our twin sample, with data for males and females combined. Where significant differences in mean values were noted between the sexes for tooth size, a correction factor was applied to the female data which equated to the mean difference. Values of intraclass correlation coefficients in MZ and DZ groups are also given in Table 3.

Table 2 - Estimates of Genetic Variance (G) and Heritability (h^2) in Tooth Size for 49 MZ and 41 DZ pairs

Tooth	G	h^2
Left I2	0.03^a	0.21
Left I1	0.10^b	0.31
Right I1	0.13^b	0.41
Right I2	0.12^c	0.42

[a] Nonsignificant; between-component estimate used as DZ total variance significantly greater than MZ.
[b] Significant difference between MZ and DZ means.
[c] $p < 0.01$; genetic variance and h^2 statistically significant.

Table 3 - Values of Intraclass Correlation Coefficients in 49 MZ and 41 DZ Twins for Tooth Size

Tooth	Correlation	
	MZ	DZ
Left I2	0.83	0.47
Left I1	0.76	0.35
Right I1	0.83	0.34
Right I2	0.78	0.40

Only the estimate of genetic variance for the maxillary right lateral incisor was found to be significant, with an associated heritability estimate of 0.42. The inequality of mean values between MZ and DZ twins for central incisors invalidated further genetic analysis, whilst the between-component estimate of genetic variance for the maxillary left lateral incisor, applied because of variance heterogeneity, failed to reach significance.

The values of intraclass correlations, which have often been used in the past to provide heritability estimates, were on average approximately 0.80 for MZ twins and 0.40 for DZ twins. For example, the *Holzinger heritability coefficient*, $(r_{MZ} - r_{DZ})/(1 - r_{DZ})$ or the *path analysis estimate*, $2(r_{MZ} - r_{DZ})$, described among others by Kang et al [12] would both seemingly have led to a considerable overestimation of genetic variance in this example.

Table 4 summarizes the results of a replicability study of the facial photographic method. When the repeated photographs, obtained on separate occasions a week apart, were matched by the least-squares method using a scalar factor to minimize any error arising from difference in the positioning of the photographic scale between examinations, the RMS residuals ranged from 0.7 mm to 1.9 mm, averaging 1.19 mm. The residuals

Table 4 - Comparison of Facial Shapes Determined from Photographs of 9 Subjects Obtained on Two Occasions[1]

Subject	Distance Measure	
	No scalar adjustment	Scalar adjustment
1 F	1.13	0.99
2 M	2.24	1.34
3 F	1.72	1.23
4 M	1.53	1.31
5 M	0.94	0.93
6 M	1.21	0.73
7 M	2.33	1.09
8 M	3.16	1.24
9 M	5.85	1.85
Average	2.23	1.19
SD	1.53	0.32

[1]Comparisons carried out by least squares matching [26] with and without scalar adjustment for size differences between 1st and 2nd determinations.

were higher when no scalar adjustment was used. These results indicated a low level of experimental error resulting from variations in head posture between the two examinations or from difficulties in locating and digitizing the landmarks. While the error residual averaged only 1 mm per point, this value could be expected to improve with the refinement of procedures for 3D data acquisition when mathematical compensation for variations in head posture can be effected.

The comparisons of facial morphology in MZ and DZ twin pairs are given in Table 5.

Table 5 - Facial Shape Comparisons in MZ and DZ Pairs[1]

	Males			Females			Total		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
MZ pairs	10	2.01*	0.61	10	2.05	0.64	20	2.03	0.61
DZ pairs	10	3.19*†	0.97	11	2.36†	0.64	21	2.75	0.90

1 Comparisons between the two twins of each pair were carried out by the method of least squares matching [26] with scalar adjustment for size differences between the faces. Values shown are RMS residuals.

* Mean RMS values for DZ and MZ males differ significantly ($p < 0.01$).

† Mean RMS values for DZ males and DZ females differ significantly ($p < 0.05$).

The root mean square (RMS) of the residuals between homologous points after shape-fitting has been taken as a measure of similarity between shapes. As expected, there was a high degree of concordance between the faces of the MZ twin pairs, the RMS residuals averaging 2.05 mm and 2.01 mm for girls and boys respectively. These values did not differ significantly from each other but each significantly exceeded the average RMS residual error of 1.19 mm shown in Table 4 ($p < 0.01$). The least squares matching between the faces of the DZ twins resulted in average RMS residuals, 2.36 mm for girls and 3.19 mm for boys, that were greater than the RMS values for MZ twins, significantly so in the boys ($p < 0.01$). The average RMS for DZ boys was significantly greater than that for DZ girls ($p < 0.05$) indicating a trend for DZ twin girls to resemble each other more closely than DZ boys, a result that we intend to investigate further with a larger sample.

Table 6 gives the results of matching facial images in the male MZ twin sample. There was no general trend for marked mirror-imaging or symmetry differences between Twin A and B based on average values (none of the comparisons yielded significant differences). However, average values tend to mask individual variability and in fact, when individual twin pairs were compared, several examples of apparent mirror-imaging were noted.

For example, twin pairs 14, 43, 45, 50 and 52 all showed evidence of mirror-imaging in the facial landmarks as they provided lower RMS values for the match between one twin and the mirror-image of the other than they did between original faces. Twins 45A, 60A, 43B and 56B gave low RMS scores when their facial images were matched with their own mirror-images. This indicates a high degree of bilateral symmetry in facial morphology of these individuals.

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Table 6 - Facial Asymmetry and Mirror Imaging in Male MZ Twins[1]

	Between Twins	With own mirror Twin A	With own mirror Twin B	With opposite mirror
T12	2.00	3.78	3.13	3.44
T14	2.38	2.34	2.40	1.25
T28	1.03	1.27	1.41	1.59
T40	1.42	2.01	1.74	1.97
T43	1.81	1.22	0.97	1.69
T45	1.52	1.06	2.02	1.40
T50	2.93	2.05	2.35	2.34
T52	2.85	2.77	2.29	1.72
T56	1.95	1.30	0.95	1.94
T60	2.21	0.86	1.62	2.41
Mean	2.01	1.87	1.89	1.98
SD	0.61	0.91	0.69	0.63

[1] Comparisons carried out by method of least squares matching [26] with scalar adjustment for size differences between images.
Values shown are RMS residuals.

Figure 4 shows the faces of MZ twin boys, T14A and T14B, who display asymmetry and some mirror-imaging of the key facial features. Analyses of these faces are summarized in Figure 5 showing the closest comparison was between T14A and the mirror-image of T14B.

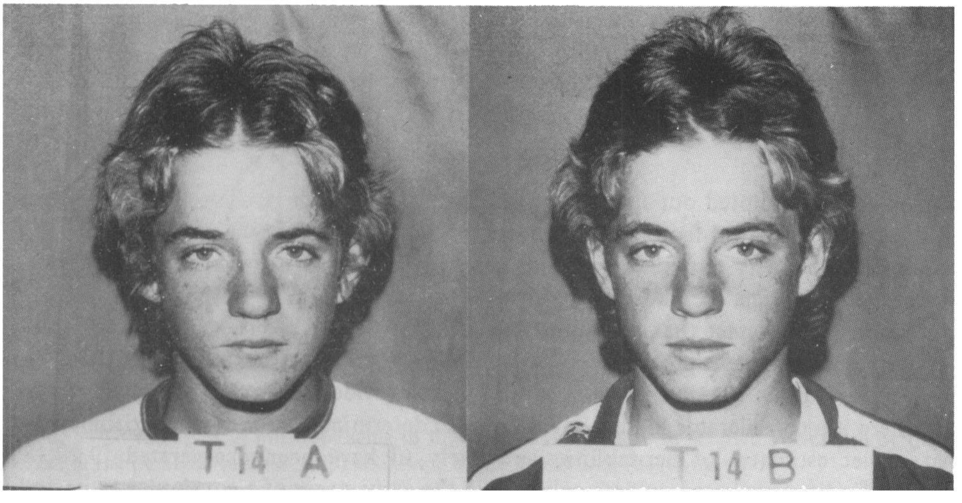

Fig. 4. MZ twin boys, T14A (left) and T14B (right), showing differences in facial asymmetry and some mirror-imaging.

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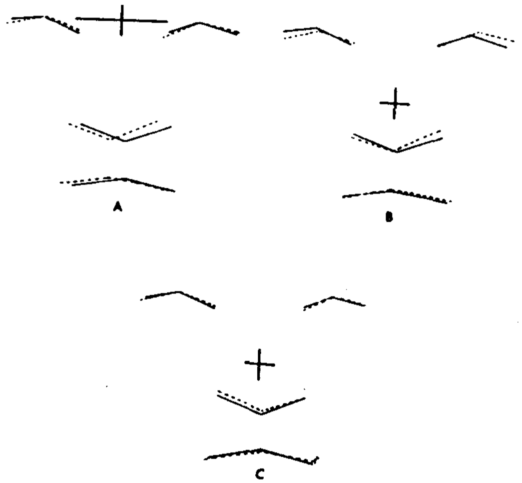

Fig. 5. Comparison of 12 facial reference points in MZ twin boys showing: A, T14A and T14B superimposed on the mid-pupil line to highlight the asymmetry of mid- and lower-faces; B, T14B superimposed on T14A according to least squares fit around the common centre of gravity; C, Mirror-image of T14B superimposed on T14A according to least squares fit around the common centre of gravity.

It is of interest to compare the dentitions of two pairs of MZ twins (Figure 6), where further evidence of mirror-imaging is present in the location of the Carabelli tubercle on the maxillary first molar and in the other twins the shape of the dental arches.

Table 7 gives results of facial matching in the femal MZ twin sample. Again, no general trend for mirror-imaging was noted based on comparisons of mean values, howewer some individual twin pairs did show evidence of mirror-imaging, for example T3, T29 and T51.

DISCUSSION

Proffit [19] has pointed out that only a small percentage of malocclusions are associated with specific etiological factors, such as genetic syndromes, embryological disturbance or trauma. The majority represent variations in normal development where both genetic and environmental effects are likely to be involved. To date, our understanding in this area is limited, despite the fact that it is fundamental to the development of rational preventive and corrective treatment procedures.

Our findings in relation to tooth size in twins provide support for recent suggestions that there is a considerable hidden environmental determinance for dental variability, and that earlier estimates of heritabilities are likely to have been exaggerated [9,17,18].

Our findings are based on data collected in the early stages of a continuing twin study and for this reason they should be regarded as preliminary. However, in spite of small sample sizes, they point to a trend for heterogeneity in variances, and possibly mean values, for dental traits in twin groups. We intend to expand this aspect of our study to in-

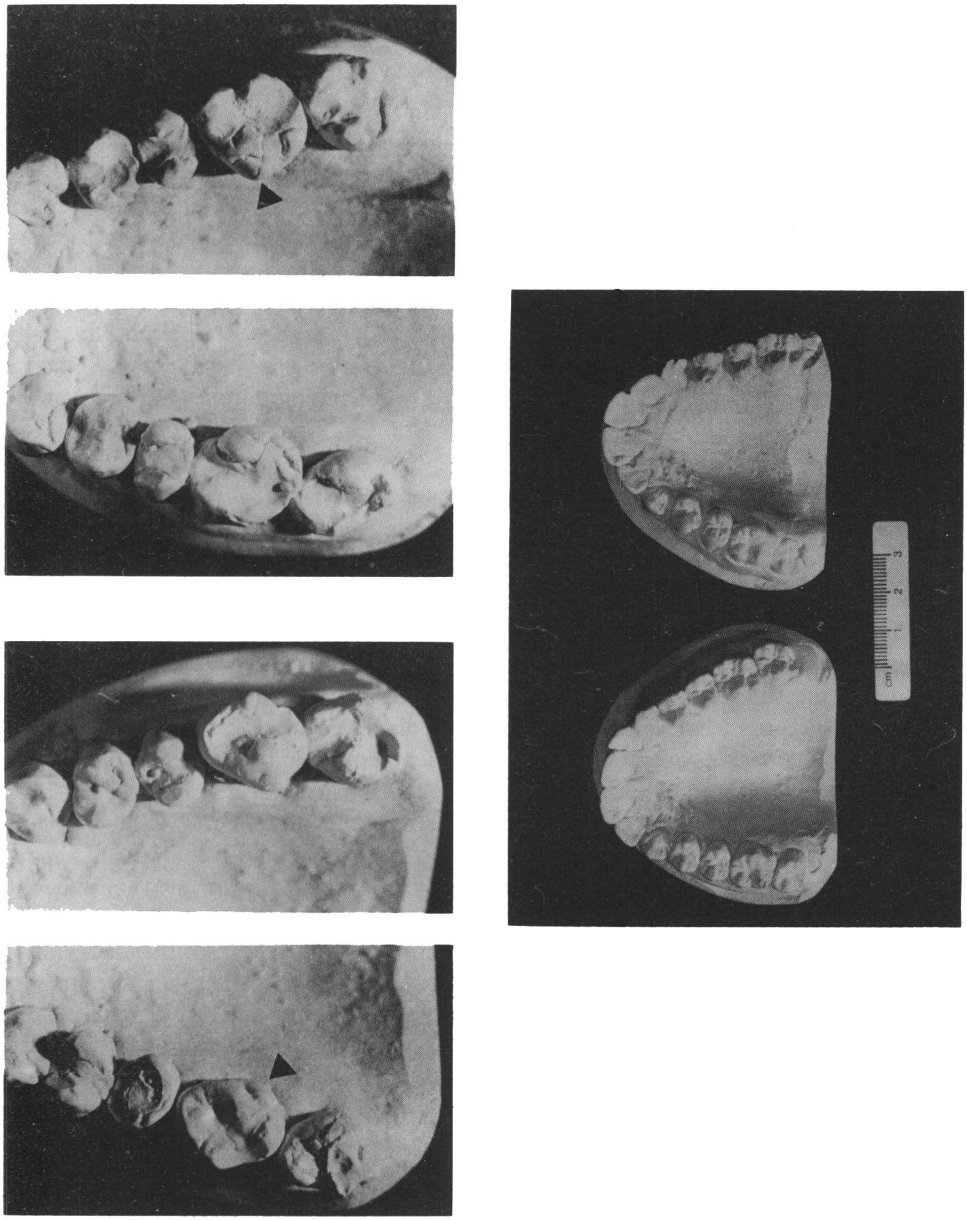

Fig. 6. Two examples of mirror-imaging in the maxillary dentitions of MZ twins displayed by the location of the Carabelli tubercle (top) and shape of the dental arch (bottom).

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Table 7 - Facial Asymmetry and Mirror Imaging in Female MZ Twins[1]

	Between Twins	With own mirror Twin A	With own mirror Twin B	With opposite mirror
T1	1.96	1.74	2.76	2.45
T3	3.06	1.26	2.70	2.65
T6	2.30	1.91	2.90	2.96
T8	1.18	3.23	3.45	3.53
T29	1.90	1.32	2.94	1.79
T37	1.38	0.63	1.55	1.41
T38	1.48	1.09	3.01	1.95
T41	1.75	1.21	1.28	1.89
T46	2.62	1.15	0.76	2.75
T51	2.83	1.57	2.68	2.39
Mean	2.05	1.51	2.40	2.38
SD	0.64	0.70	0.88	0.63

[1] Comparisons carried out by method of least squares matching [26] with scalar adjustment for size differences between images.
Values shown are RMS residuals.

clude dental measurements of all the teeth derived from larger samples. Furthermore, by subdividing the twins according to features such as birth weight and laterality, we hope to clarify the influence of developmental factors on dental morphology.

Apart from their suitability for genetic analysis, the teeth provide an excellent model system for studying structural asymmetry by enabling comparisons of size and morphology between antimeric pairs. Using multivariate statistical methods, Boklage [1] has reported significant differences in the distributional relationships of permanent tooth development related to twinship and zygosity: these inequalities may reflect fundamental differences in craniofacial development between these groups. As one of the main limitations in multivariate analyses is small sample sizes, we plan to gather data adequate in amount to test Boklage's hypothesis using 3D representations of surface contours as well as traditional measures of tooth size.

The quantification and comparison of shape has always been an interesting and challenging problem in many fields of biology. Of several methods available we have made use of the Sneath [26] procedure which transforms one shape, represented by a set of 2D coordinates, so that maximum agreement is achieved with a second set of homologous coordinates. The transformation is rigid, involving translation, rotation and, optionally, scalar parameters. Siegel [24] published a programming approach to the Sneath procedure and added his own technique for a robust median resistant fit of two shapes. We have used both the Sneath and the Siegel methods for shape-fitting with as well as without scalar adjustment. Although limited to 2D data sets, the present application demonstrated the value of shape-fitting procedures to quantify biological asymmetry, to reveal evidence of mirror-imaging and to compare facial similarity within MZ and DZ twin pairs.

The application of newer mathematical methods to quantify shape, particularly with

3D visualisation, should lead to a clearer understanding of the role of genetic factors in determining facial variability, and in particular the location and magnitude of asymmetries. Apart from the insights provided by the pioneering work of Nakata [15] and the recent studies of Susanne et al [27] and Hauspie et al [10] who applied univariate and multivariate statistical methods to facial measurements of Belgian twins, understanding of the causes of normal facial variation, particularly asymmetries, is poor. The approaches outlined in this paper will be extended to 3D, providing a more detailed data-base and also reducing the experimental errors in locating facial landmarks or contours which may be increased by minor alterations in head posture.

We believe that the continuing collection and analysis of data from South Australian twins will provide new information on the factors affecting dentofacial variability which will be of considerable relevance clinically. The study may also provide some insight into the nature of the twinning process itself, particularly the way in which twinning influences the development of body symmetry.

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