Tooth Morphology as a Basis for Distinguishing Monozygotic and Dizygotic Twins

ANDERS LUNDSTRÖM

Department of Orthodontics, Royal School of Dentistry, Stockholm, Sweden

THE PRESENT INVESTIGATION is based upon two series of casts, one of 70 twin pairs of like sex collected at the Dental School of the University of Michigan in Ann Arbor, and the other of 54 like sexed pairs at the Dental School of Columbia University in New York. The first series was diagnosed as to zygosity by Sutton, Vandenberg and Clark at the Institute of Human Biology in Ann Arbor and the second by Osborne and De George of the Sloan Kettering Institute of Cancer Research in New York. The casts were made by Dr. J. Alderisio, former Assistant Professor of Orthodontics at the Dental School, University of Michigan, and by Dr. S. Horowitz, Assistant Professor of Orthodontics at the Dental School at Columbia University.

Previous studies

Although there are a number of publications dealing with the genetic control of tooth morphology (Weitz, 1924; Reif, 1928; Korkhaus, 1930, 1939; Kraus, 1951, 1957; Ludwig, 1957) there seems to be only one investigation of the application of genetic variability in tooth form in distinguishing between monozygotic and dizygotic twins (Kraus, Wise and Frei, 1959). Eleven out of 17 earlier described traits for the lower first premolars (Kraus & Furr, 1953) were recorded in four out of six sets of triplets. Concordance and discordance were determined and the frequency of concordance in the possible pairing was used as an indication of zygosity. For all four sets the diagnoses from tooth morphology were identical with the diagnoses on the basis of blood types.

PRESENT INVESTIGATION

Zygosity diagnosis

The zygosity diagnosis of the Ann Arbor material was performed in the following way (Sutton, Vandenberg and Clark, 1962). The twin pairs were first assigned to two groups on the basis of concordance or discordance with respect to blood groups ABO, MN, Rh, Kell and Duffy and the secretor factor. Discordance for one or more of the antisera used (anti-A, absorbed anti-A, anti-B, anti-M, anti-N, anti-C, anti-D, anti-E, anti-c, anti-e, anti-K, and anti-Fy^a) was regarded a sufficient but not a necessary condition for dizygosity. It was estimated that roughly 10 per cent of the concordant twin pairs were dizygous, and several supplementary observations were therefore made to detect

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the dizygous concordant twins, namely, of physical appearance, finger-prints, eye color, visual inspection of head and hand radiographs, and dental casts. A particularly careful examination was made of eye color and iris pattern, which were inspected on Kodachrome transparencies by three observers independently. The concordant twins that all three observers agreed could be distinguished on the basis of the iris patterns were considered to be most probably dizygous.

The use of such a classification for determining the extent to which tooth morphology can be applied in distinguishing monozygous and dizygous pairs might involve some risk of circular reasoning, as an inspection of the dental casts was included in the series of observations conducted for diagnosing dizygous twins. However, the casts obviously played a minor role in the diagnosis and, furthermore, tooth morphology was not referred to specifically in this connection. It is therefore improbable that appreciable bias has been introduced in the present investigation.

Osborne and De George (1959) also based their diagnoses chiefly upon serologic evidence. The blood of the twins was tested for A_1, A_2, B, O; M, N, S; C, D, E, c of the Rh series; Kell, Duffy, and P. A difference in any one of these blood factors was taken as proof that a pair was dizygotic. When, in a supplementary study, five pairs of unlike sex were found to agree as regards all the blood factors tested, it became apparent that it would be necessary to introduce additional diagnostic criteria. For the series studied the following characteristics were found to be of particular value: head hair color, eye color, eye detail pattern, tongue rolling, P.T.C. taste reaction, ear lobe form, chin form, and mid-phalangeal hair. The twin pairs were assigned to three groups: (1) definitely similar, (2) definitely dissimilar or (3) questionably similar in respect of each of these eight characteristics. All twin pairs of like sex for which there was agreement as to blood factors were then separated and classified as *similar, dissimilar* or *similar (?)*. A twin pair that was recorded as definitely similar for all eight criteria was classed as *similar*. A pair that was recorded as definitely dissimilar in two or more of the eight criteria, or as dissimilar in one and questionable in three or more was classed as *dissimilar*. All others were classed as *similar (?)*. Of the 96 pairs of like sex comprising the series, 10 were classified as *dissimilar* and 10 as *similar (?)*. The former were listed as dizygotic and the latter as monozygotic. A separate study of dermatoglyphic patterns and facial photographs did not disclose any cause for changing the previous classification. According to Osborne and De George any tendency to error would be toward including closely similar dizygotic pairs in the monozygotic group; for this reason they call their procedure "a proved dizygotic method."

Number of Comparable Tooth Pairs

An important factor in a study of tooth morphology is the number of teeth in the dentition that are available for examination of tooth form. Teeth may be non-available because of non-eruption or extraction, hypodontia, severely defective crowns from caries, enamel hypoplasia, or trauma.

In the present study teeth with, for instance, central or single proximal

fillings were usually found to be acceptable. Where only one tooth of a homologous pair was defective it could still be included in the material. A bilateral comparison is, however, of greater value since it is not uncommon to find small deviations in tooth shape between the left and right sides.

The number of teeth available for co-twin comparison on one or both sides in the Ann Arbor and New York series were on an average 12.4 and 10.3, respectively. This difference is probably due, at least in part, to the fact that the latter series was composed entirely of adult twins (over 18 years) whereas the former included many younger twins with fewer lost or badly decayed teeth. The Ann Arbor series contained only 7 pairs out of 70 having less than 8 teeth comparable against 14 such pairs out of 54 in the New York material.

It is not only the number of available teeth that is of importance but also the types of teeth on which the comparison is based. Teeth with a relatively large variability are of greater value than those with small individual differences. The upper incisors and the lower premolars more often have distinct traits than the lower incisors and canines. The molars and upper premolars seem to assume an intermediate position in this respect. The distribution of the different teeth with respect to their availability to concordance and discordance determinations is shown in tables 1 and 2. In both series the four incisors are best represented (in 93-99 per cent of the Ann Arbor series and in 77-94 per cent of the New York series), whereas the third molars were the least often available (about 3 and 15-17 per cent).

METHOD AND RESULTS

The study was performed by the blind technique. The examiner was unaware of the diagnosis reached on the basis of serologic and general anthropology. The pairs were presented at random so that the observer had no clue as to whether a particular pair of casts belonged to a monozygotic or dizygotic

TABLE 1. INDIVIDUAL TEETH AVAILABLE FOR INTRA-PAIR COMPARISON IN THE ANN ARBOR SERIES (70 TWIN PAIRS)

Intra-pair comparison possible	I_1	I_2	C	P_1	P_2	M_1	M_2	M_3
				Upper Jaw				
Bilaterally	56	58	60	56	41	46	41	—
Unilaterally	9	9	7	12	17	11	15	2
Total	65	67	67	68	58	57	56	2
Percentage of total number of twin pairs (70)	92.9	95.7	95.7	97.1	82.9	81.4	80.0	2.9
				Lower Jaw				
Bilaterally	61	59	59	64	46	32	38	1
Unilaterally	5	9	10	5	13	14	14	1
Total	66	68	69	69	59	46	52	2
Percentage of total number of twin pairs (70)	94.3	97.1	98.6	98.6	84.3	65.7	74.3	2.9

twin pair. The casts of the Ann Arbor series were randomized with the help of a table of random numbers. The New York series was collected in such a way that the casts could be expected to be in a random order, and no special measures were taken to ensure randomization. This proved to be not quite satisfactory, since the material contained considerably more monozygotic than dizygotic pairs (34:20), a fact that came to light during the examination and, being unexpected, had a puzzling effect on the examiner. A presentation of the material according to random numbers would probably have been better, although it would then have been necessary to use some dizygotic pairs twice.

To start with, a preliminary examination of the Ann Arbor series was made by three observers. All were orthodontists and had a knowledge of genetics. Observers 1 and 3 had had previous experience of twin investigations on teeth and jaws. Observer 3 (the author) had undertaken a parallel study to the present one on a similar series, for which, however, no serologic examinations had been performed to confirm the diagnoses; he was therefore better trained than observers 1 and 2. The casts of the twin pairs were compared by each observer independently of the other two, and a diagnosis was made on the basis of the shape and, to some extent, the size of the teeth (for differences evident to the unaided eye). The results of these comparisons are shown in table 3.

Observers 1 and 3 both proved to be very reliable in diagnosing the dizygotic pairs (with 3 and 0 of the 32 pairs given as monozygotic) but less reliable in judging the monozygotic pairs (8 and 5, respectively, given as dizygotic). Observer 2 made 8 mistakes for the dizygotic against 2 for the monozygotic. It is conceivable that these discrepancies are manifestations of a systematic difference in judgement, with a tendency for observers 1 and 3 to regard borderline cases as monozygotic and observer 2 to regard them as dizygotic.

Observer 3 afterwards performed a more detailed comparison of the twin

TABLE 2. INDIVIDUAL TEETH AVAILABLE FOR INTRA-PAIR COMPARISON IN THE NEW YORK SERIES (52 TWIN PAIRS FOR THE UPPER AND 53 PAIRS FOR THE LOWER JAWS)

Intra-pair comparison possible	I_1	I_2	C	P_1	P_2	M_1	M_2	M_3
				Upper jaw				
Bilaterally	42	37	38	29	15	25	28	3
Unilaterally	3	10	7	13	15	12	13	5
Total	45	47	45	42	30	37	41	8
Percentage of total number of twin pairs (52)	86.5	90.4	86.5	80.8	57.7	71.2	78.8	15.4
				Lower jaw				
Bilaterally	38	40	49	39	21	17	24	4
Unilaterally	3	5	1	11	18	9	9	5
Total	41	45	50	50	39	26	33	9
Percentage of total number of twin pairs (53)	77.3	84.9	94.4	94.4	73.6	49.1	62.3	17.0

casts. Comparisons were made tooth for tooth and the presence of concordance (K), minor (D1) or major discordance (D2) were noted. If there was uncertainty associated with a particular decision, this was indicated by placing a question mark after the notation. When such a record had been made for all the comparable teeth of a twin pair a total evaluation was made and the twin pair in question was denoted as monozygotic (MZ), probably monozygotic (MZ?), probably dizygotic (DZ?) or dizygotic (DZ).

The following types of discordance between homologuous teeth were recorded as major:

1. Differences in the number of cusps.
2. Marked differences of fissure-arrangements.
3. Marked form-differences of the crown, *e.g.*, a short and/or broad crown for one tooth and a long and/or narrow for the other.
4. Marked differences in palatal surfaces of upper incisors or canines with, *e.g.*, a rather smooth surface for one tooth and two or more strong enamel ridges for the other.

Drawings were made in order to describe major differences. Minor discordance was recorded for tooth-pairs with less obvious differences, such as more or less curved facial, or lingual surfaces, more round or more angulated occlusal surfaces, more or less pointed or otherwise differently formed cusps.

Examples of the type of differences upon which the tooth-morphology diagnoses were based are given in Fig. 1 and 2.

The results of the classification are given in table 3, line 3b. There is close agreement between the results of the preliminary and detailed examinations. In seven cases the classification was changed: two monozygotic pairs that had first been assigned to DZ? were transferred to MZ; two monozygotic pairs were changed from MZ to MZ?, and one monozygotic pair from MZ? to MZ; one monozygotic pair was changed from DZ to DZ? and one dizygotic pair from DZ? to DZ. Five of the seven changes were thus in the "right" direction and two "wrong." It is not unlikely that in a few cases the recollection of the comparison between the results of the three observers may have had an influence on the detailed evaluation.

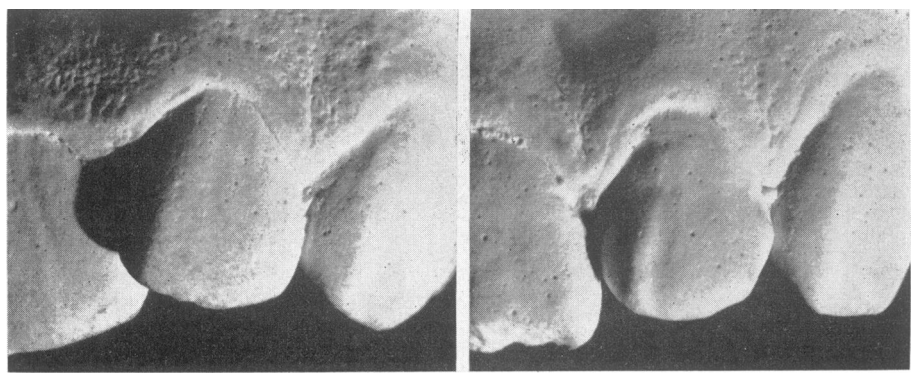

FIG. 1. Upper left lateral incisors from twin pair no. 6, Ann Arbor. According to blood groups and general somatic evaluation this pair was monozygotic. Differences in tooth morphology led to the tooth morphology diagnosis: dizygotic (?).

The diagnosis of the New York pairs was performed by the same detailed procedure as before, but only by observer 3. The results (table 4) are in close agreement with those of the Ann Arbor material. Complete identity was obtained with the original diagnosis for 19 out of 20 pairs that were originally

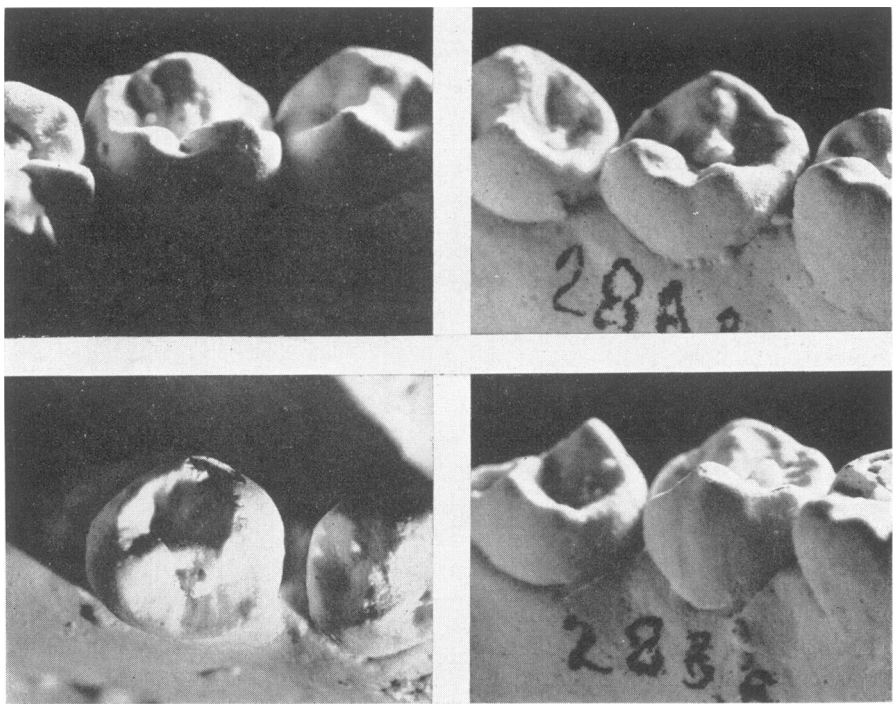

FIG. 2. Lower second premolars from twin pair no. 28, Ann Arbor. According to blood groups and general somatic evaluation this pair was monozygotic. Differences in tooth morphology, especially the fact that the lower second premolars, of one twin had two lingual cusps and those of the other had only one such cusp on both sides, led to the tooth morphology diagnosis: dizygotic.

TABLE 3. CORRESPONDENCE BETWEEN ORIGINAL AND TOOTH MORPHOLOGY DIAGNOSES OF ZYGOSITY, ANN ARBOR SERIES (3 OBSERVERS)

		38 monozygous pairs (original diagnosis)			
	Tooth-morphology diagnosis	MZ	MZ?	DZ?	DZ
Observer	1	16	14	3	5
	2	33	3	2	0
	3a*	28	5	3	2
	3b	29	6	2	1
		32 dizygous pairs (original diagnosis)			
	Tooth-morphology diagnosis	DZ	DZ?	MZ?	MZ
Observer	1	27	2	3	0
	2	19	5	5	3
	3a*	31	1	0	0
	3b	32	0	0	0

*For observer 3 the second set of values (3b) reports a more detailed examination.

classed as dizygotic (diagnosis definite for 17 pairs and with reservation for two). The dizygotic pair assessed as monozygotic was recorded as MZ?. This pair was serologically identical, but differed as to iris pattern, PTC tasting, ear lobe and mid-phalangeal hair, and was diagnosed as DZ? by Osborne and De George. Out of the 34 pairs assessed originally as monozygotic, 31 were diagnosed as monozygotic (17 definite and 14 with reservation) and three as dizygotic (two definite and one with reservation). Out of these three pairs one pair was denoted as MZ? by Osborne and De George due to differences in tongue rolling and PTC tasting.

DISCUSSION

A factor of importance in evaluation of the results is the reliability of the original diagnoses. As has already been mentioned, it is easier to establish dizygosity than monozygosity, since evidence for the former is provided by serologic discordance or marked discordance in external features. For this reason, the close agreement obtained in the dizygotic series between the original diagnosis and that made on the basis of tooth morphology would perhaps be expected.

To establish monozygosity is more difficult, however, since a few of the pairs classed as concordant by serologic analysis are in fact dizygotic. By using other anthropologic features it is certainly possible to distinguish most of the pairs of serologically concordant dizygotic twins, but it cannot be ruled out that occasional dizygotic pairs are so closely similar that they cannot be distinguished from monozygotic pairs displaying relatively marked non-genetic difference.

Thus, in cases where dizygosity is indicated on the basis of tooth morphology but monozygosity on the basis of serology and general anthropology, it is not necessarily the latter diagnosis that is the correct one.

It might be mentioned that all six serologically concordant Ann Arbor pairs that were originally designated as DZ were also recorded as DZ on the basis of tooth morphology. For four of these the original diagnosis was DZ and for two DZ?. In the New York series there were also four pairs of the same type (all DZ?). The diagnosis on the basis of tooth morphology was DZ for three of these and MZ? for one.

It is interesting to observe that the proportion of pairs for which the original diagnosis and that based on tooth morphology were not in agreement

TABLE 4. CORRESPONDANCE BETWEEN ORIGINAL AND TOOTH MORPHOLOGY DIAGNOSES OF ZYGOSITY, NEW YORK SERIES

	34 monozygous pairs (original diagnosis)			
Tooth-morphology diagnosis	MZ	MZ?	DZ?	DZ
Observer 3	17	14	1	2
	20 dizygous pairs			
Tooth-morphology diagnosis	DZ	DZ?	MZ?	MZ
Observer 3	17	2	1	0

was slightly higher for the New York than the Ann Arbor series (7.4 against 4.3 per cent, respectively). The percentage of pairs classed as MZ? and DZ? was also higher for the New York material (33.3 per cent) than for the Ann Arbor material (11.4 per cent). These differences are probably due, at least in some degree, to the greater difficulty of performing a zygosity diagnosis on the basis of tooth morphology in older people. This is also reflected in the number of teeth available for comparison in the two series. Another possible

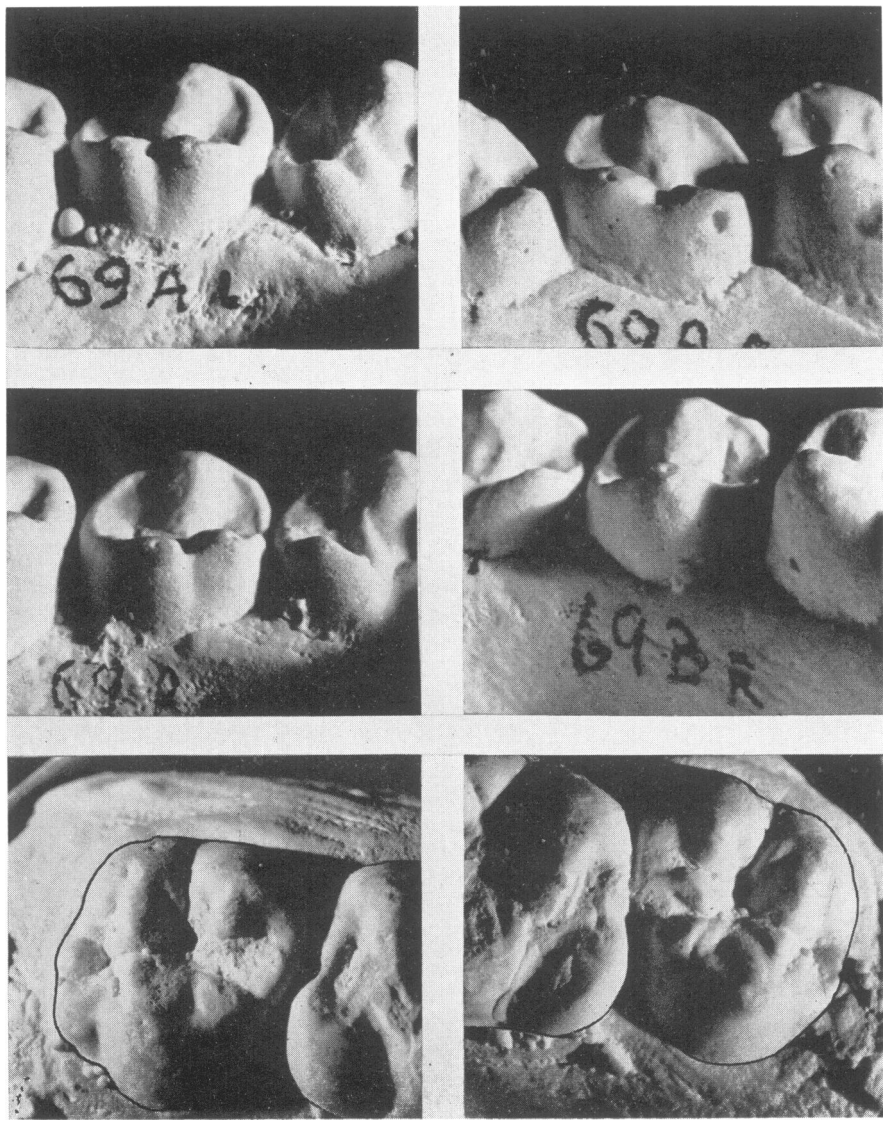

FIG. 3. Lower second premolars from pair no. 69 and upper second molars from one twin of pair no. 65, Ann Arbor. The B-twin of the monozygotic pair no. 69 had two lingual cusps on one side and one lingual cusp on the other side. The A-twin of the dizygotic pair no. 65 shows similar asymmetry of the upper second molars.

explanation of the differences is the unusually large proportion of monozygotic pairs in the New York series. The observer was, as already noted, puzzled thereby and might for this reason have tended to place question marks after his monozygotic diagnoses.

SUMMARY

The results of the study on tooth morphology show that on the whole there was a close agreement with the diagnoses of zygosity made on the basis of general anthropology (including serologic determinations). For 117 out of the 124 pairs studied (94.4 per cent) the same final diagnoses were obtained. The discrepancies demonstrated cannot be proved to be due to incorrect diagnosis on the basis of tooth morphology nor can this possibility be ruled out. In six pairs out of seven the difference consisted of a diagnosis of dizygosity by tooth morphology where monozygosity was diagnosed on grounds of general anthropology (two pairs DZ? and four pairs DZ). In only one pair was a dental morphologic diagnosis of monozygosity (MZ?) made where the anthropologic factors indicated dizygosity (DZ?). This pair was serologically identical, but differed as to iris pattern, PTC tasting, ear lobe and mid-phalangeal hair.

The presence of a difference in the number of cusps on the left and right sides, whereby, for instance, the lower second premolar had one lingual cusp on one side and two such cusps on the other (Fig. 3), indicates a variation in the expressivity of the genes, even in the case of a character determined so strongly by heredity as tooth morphology. This means that the different shape of a particular tooth of two twins of a pair cannot be regarded as proof of dizygosity, even if the discrepancies are bilateral (Fig. 2). If other teeth are strikingly similar in form, monozygosity is probably the correct diagnosis. If the present study had been based on this principle two Ann Arbor pairs would probably have been diagnosed as monozygotic, as was indicated by the general anthropology.

As a general conclusion it would seem that for twin pairs for which a large enough number of permanent teeth are available for comparison of tooth morphology, a fairly reliable judgment can be made on whether there is monozygosity or dizygosity by an observer having a good knowledge of the variation in tooth morphology.

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