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Genetic Covariance Structure of Incisor Crown Size in Twins

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Abstract. Previous studies of tooth size in twins and their families have suggested a high degree of genetic control, although there have been difficulties separating the various genetic and environmental effects. A genetic analysis of variation in crown size of the permanent incisors of South Australian twins was carried out, with structural equation modeling used to determine the relative contributions of genetic and environmental factors. Maximum mesiodistal crown dimensions of maxillary and mandibular permanent incisors were recorded from dental models of 298 pairs of twins, including 149 monozygous (MZ) and 149 dizygous (DZ) pairs. The analysis revealed that: (i) an adequate fit required additive genetic and unique environmental components; (ii) augmenting the model with non-additive genetic variation did not lead to a significant improvement in fit; (iii) there was evidence of shared environmental influences in the upper central incisors of males; (iv) the additive genetic component constituted a general factor loading on all eight teeth, with group factors loading on antimeric pairs of teeth; (v) unique environmental effects were mostly variable-specific; (vi) most factor loadings on antimeric tooth pairs could be constrained to be equal, indicating a symmetry of genetic and environmental influences between left and right sides; and (vii) estimated heritability of the incisor mesiodistal dimensions varied from 0.81 to 0.91.

Key words: tooth size, twins, genetics, environment.

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Introduction

Tooth size in human populations has been the subject of numerous investigations to determine, among other aspects, the patterns of variability of different teeth, associations within and between the dental arches, and the relative degrees of influence of genetic and environmental factors. The findings have been reviewed recently by Kieser (1990) and Lauweryns *et al.* (1993). An examination of within-pair differences in mesiodistal and buccolingual crown dimensions in 75 pairs of twins provided evidence for (i) strong genetic control of individual crown dimensions, (ii) the existence of independent genes or groups of genes contributing to variability in mesiodistal and buccolingual dimensions, and (iii) independent genetic determination of maxillary and mandibular teeth (Potter *et al.*, 1976). In fact, most evidence points to mesiodistal and buccolingual crown dimensions being to the largest extent genetically determined (*e.g.*, Alvesalo and Tigerstedt, 1974; Garn, 1977). Although morphogenetic field theory (Butler, 1939; Dahlberg, 1945) implies that there should be distinct patterns of heritability within each tooth class (incisor, premolar, and molar), results of previous investigations in twins and siblings have been inconsistent (*e.g.*, Lundström, 1948; Alvesalo and Tigerstedt, 1974; Mizoguchi, 1977).

Unfortunately, most previous studies of the dentition do not provide estimates of the role of common or family environment, maternal effects, interaction between genes (epistasis), or genotype-environment interaction, and so the estimates of heritability obtained probably represent the upper limit to the true values. Modern approaches to estimating heritability involve structural equation modeling, which allows hypotheses regarding the relative contributions of genetic and environmental influences to the variation within, and covariation between, variables to be tested (Jöreskog, 1973; Martin and Eaves, 1977; Heath *et al.*, 1989; Neale and Cardon, 1992). Briefly, linear structural equation models are fitted to raw data or summary covariance or correlation matrices by maximum likelihood

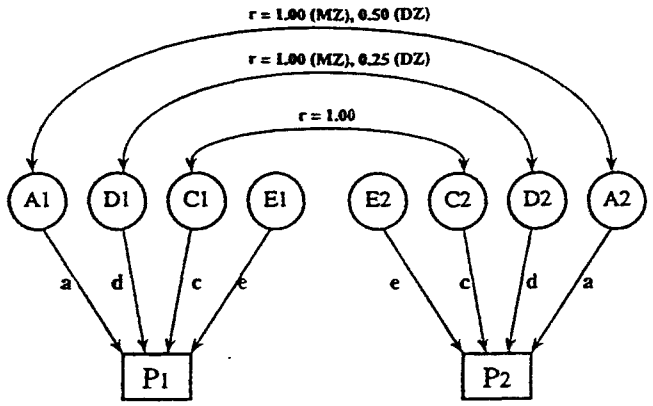

Figure 1. Univariate path diagram showing four potential influences affecting the phenotypes of MZ and DZ twin pairs. P1 and P2 represent the phenotypes of the first and second twin pair members, respectively. The latent factors A, D, C, and E denote the additive genetic variation, non-additive genetic variation, common environmental variation, and unique environmental variation, respectively, for each twin. The double-headed arrows indicate the correlations (r) between latent factors in co-twins. The path coefficients, a, d, c, and e, indicate the relative importance of each of the contributing influences, A, D, C, and E (from Neale and Cardon, 1992).

or other methods. Models may incorporate additive (A) and non-additive (D) genetic variation, and shared (C) and random (E) environmental effects, or a subset of these parameters. Along with efficient parameter estimates, the method provides a test of goodness-of-fit to the data. In one such investigation of Pima Indian families, it was estimated that 35% of the variance in the lateral incisor mesiodistal dimension was due to genetic and environmental transmissible (shared) factors, considerably less than that proposed in earlier studies (Potter *et al.*, 1983).

The aim of this investigation was to analyze the covariance structure of the mesiodistal dimensions of permanent incisor crowns in 298 pairs of South Australian twins, to quantify the relative contributions of genetic and environmental factors to each incisor, and to test whether the size of each tooth was determined independently. Heritabilities were also calculated and examined to see if they supported the predictions of Butler's field theory with respect to the central and lateral incisors.

Materials and methods

Study population and measurement methods

Alginate dental impressions were obtained from 82 female MZ and 67 male MZ twin pairs, 48 female DZ and 44 male DZ pairs, and 57 opposite-sexed DZ pairs. The twins ranged in age from 6 to 62 years, although 90% were between 10 and 25 years old, and the mean age was 16.5 years. Corrections for age were not considered necessary, since the final sizes of dental crowns are determined before emergence of the teeth into the oral cavity, and since measurements were precluded where there was any evidence of attrition affecting the dimension. Zygosities were confirmed by examination of the blood antigens ABO, Rh, MNS, Jk, and Fy, as well as serum enzyme polymorphisms ACP, AK1, ESD, GLO, GPT, PGD, PGM1, and PGP, and protein polymorphisms GC, HP, Pi, and C3. The probability of dizygosity, given concordance for all systems, was less than 1%. Facial photographs and fingerprints provided confirmatory evidence of zygosity status. Data collection methods were approved by the Committee on the Ethics of Human Experimentation, University of Adelaide (Approval No. H/07/84), and all participants were informed volunteers.

Using stone models prepared from the impressions, we measured the maximum mesiodistal and buccolingual crown diameters (following Moorrees *et al.*, 1957) from all emerged and sufficiently intact permanent teeth, except the third molars. The measuring equipment was comprised of sharpened Mitutoyo digital vernier calipers, connected through a multiplexer unit to an Apple IIC microcomputer. To estimate the reliability of the measurement procedure, two investigators measured 50 models independently. Although 56 dimensions were measured, the results of genetic analyses for only the mesiodistal dimensions of the eight permanent incisors are reported here.

Statistical methods

Mean values and standard deviations were calculated for male and female twins for each variable. Student's *t* tests were performed for comparison of mean values between sexes, zygosity groups, and first- and second-born twins. Male and female variances were compared by the variance ratio (F) test. Pearson correlation coefficients were calculated between all pairs of teeth, and between twins for each tooth.

Before proceeding with modeling of covariance structure, we explored the data to test for any genotype-by-environment (GxE) interaction, and to determine the likelihood of detecting any non-additive genetic variation that may have existed. The presence of GxE interactions may be indicated by significant regression of MZ pair variances on MZ pair means (Jinks and Fulker, 1970). In the absence of GxE interaction, directional dominance is indicated by significant regression of DZ pair variances on DZ pair sums, or by significant coefficients of skewness evident in DZ twins only (Martin *et al.*, 1978). The probability of detecting dominance by fitting models to twin data is generally low, even when there are complete dominance and high heritability, unless it has a strong directional component (Martin *et al.*, 1982). As a test for GxE interactions and directional dominance in our data, the absolute pair difference, which is proportional to the square root of the intrapair variance, was regressed onto pair sum, and onto the square of the pair sum. In case the relationship was not linear, square and logarithmic (log) transformations of the data were also tested for significant regression. Coefficients of skewness were calculated and compared between MZ and DZ twin pairs.

Structural modeling was then implemented, with the program Mx (Neale, 1994) used to account for genetic and environmental covariation between the incisor crown dimensions. Implicit in our model-fitting procedure were all the usual assumptions of the twin method—that mating is random, that trait-related shared environmental influences on MZ and DZ twins are equal, and that there is no GxE interaction or gene-environment covariation (Jinks and Fulker, 1970). Fig. 1 shows four of the influences which can be modeled (A, C, D,

Table 1. Percent measurements present, and percent substituted from each source

Tooth[a]	U1	U2	L1	L2
Present	96.6	93.8	98.6	98.0
Other Side	1.9	1.6	1.1	1.3
Other Twin	1.3	2.0	0.2	0.6
Mean	0.2	2.6	0.2	0.2
Total	3.4	6.2	1.4	2.0

[a] U = upper, L = lower, 1 = central incisor, 2 = lateral incisor.

and E) for a pair of twins. The correlations of A1 with A2 and D1 with D2 are fixed according to genetic theory. Additive genetic variation causes DZ correlations to be about half the MZ correlations. Dominance tends to decrease the DZ correlation to below half the MZ value, and common environment increases it above half the MZ value. By definition, unique environmental influences are uncorrelated, and shared environmental influences are perfectly correlated. Since fitting models with four parameters to data from a classical twin study (MZ and DZ twins reared together) results in an underidentified model, subsets of three or fewer parameters are chosen. The choice is made simpler by negative confounding of genetic dominance with common environmental influences (Grayson, 1989; Hewitt, 1989), so that a twin model may not contain both D and C.

Variable-length files of raw data were set up as described in Neale (1994) and utilized directly for the univariate analyses. For the multivariate analyses, the input data took the form of variance-covariance matrices, generated for each of the five twin sex-zygosity groups, by means of the preprocessor, PRELIS (Jöreskog and Sörbom, 1986). List-wise deletion of twin pairs with one or more missing values, necessary to produce positive-definite variance-covariance matrices, would have resulted in the loss of up to 26% of the data. Since the proportion of the total data set missing was reasonably small, ranging from 1.4% for the lower lateral incisor to 6.2% for the upper lateral incisor (Table 1), an imputation procedure was applied following a substitution hierarchy. Values were substituted from the antimeric tooth when present. If the antimere was absent, the value from the co-twin was used in same-sexed twin pairs. Where this was also missing, or the twins were opposite-sexed, the sex-specific mean was used. To reduce the impact of the imputations, we removed twin pairs with fewer than 60 of the 112 values present (56 values in each twin), leaving 78 MZ female, 61 MZ male, 44 DZ female, 41 DZ male, and 48 DZ male-female twin pairs. The age range for the 272 twin pairs was consequently reduced to between nine and 46 years.

We began by analyzing each variable separately, fitting a path coefficient model with unique environmental influences only (E model). Where this failed, the model was extended to include additive genetic variation (AE model), or shared environmental variation (CE model). Finally, ACE and ADE models were fitted, where D (non-additive genetic variation) incorporates both dominance and epistatic interaction variance, which cannot be separated when only MZ and DZ twins are used (Mather, 1974). Path coefficients (a, d, c, e) were estimated, and χ^2 values for goodness-of-fit of the models were calculated.

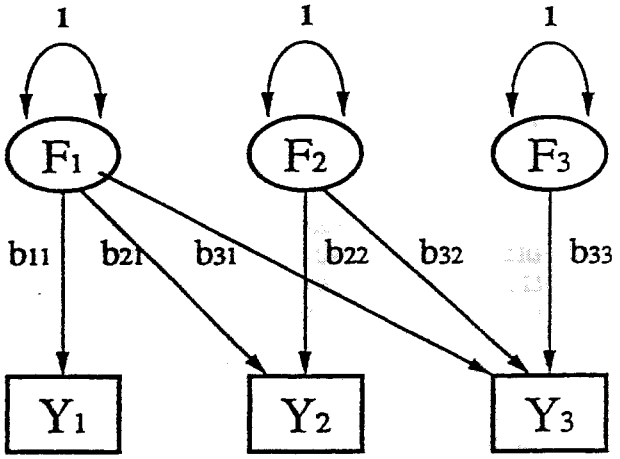

Figure 2. Path diagram depicting a Cholesky decomposition model with three measured variables (Y1 to Y3) being explained by three latent factors (F1 to F3). The double-headed arrows indicate the variance of the latent factors (from Neale and Cardon, 1992).

Akaike's Information Criterion (AIC = χ^2 minus two times the degrees of freedom) was used to indicate the parsimony of each model (Akaike, 1987). The smaller or more negative the AIC, the better the parsimony and fit of a model. The general approach is that of accepting a more complex model only when a simpler one has failed. In addition, comparisons of the χ^2 and AIC values between complex and simpler models may indicate significance of the various components. Various hypotheses can be tested by setting different combinations of paths to zero, and examining the difference between the resulting goodness-of-fit χ^2 and AIC values. We estimated heritability (h^2) from the ratio of genetic variation to total phenotypic variation, using the parameter estimates from the model with the best fit.

The most parsimonious model for each tooth was applied to four subsets of the data base, namely, female same-sexed twin pairs, male same-sexed twin pairs, all four same-sexed twin groups, and the five sex-zygosity groups. We evaluated heterogeneity of causes of variation between the sexes by adding the χ^2 values for the fits of the model to male and female groups separately, and then subtracting this sum from the χ^2 generated by fitting the model jointly to the four groups.

The multivariate analysis was conducted for each gender separately and was comprised of three main steps, each of which utilized the best model from the previous step. In the first stage, Cholesky decomposition models were applied to all eight variables. These models estimate all possible paths of covariation in an attempt to account for as much variation as possible, having as many factors as there are variables and as many loadings as there are observed correlations. The path diagram of a Cholesky decomposition of three variables (Y1, Y2, Y3) into three factors (F1, F2, F3) is shown in Fig. 2. The first factor (F1) loads on all the variables, the second (F2) loads on all but the first variable, the third (F3) loads on all but the first and second variables, and so on (Neale and Cardon, 1992). The Cholesky model is a unique factorization of the covariance structure. It therefore provides a limiting test of how well any model with A, E, C, or D factors will fit. Simpler models will

Table 2. Descriptive statistics for the mesiodistal dimension (mm) of permanent incisors in each sex

	Males[c]			Females		
Tooth[a]	N[b]	Mean	SD	N	Mean	SD
UR2	237	6.87	0.56	269	6.62	0.53
UR1	240	8.82	0.55	288	8.52	0.56
UL1	241	8.82	0.56	282	8.53	0.54
UL2	238	6.87	0.59	277	6.61	0.57
LR2	248	6.05	0.41	289	5.84	0.41
LR1	246	5.44	0.37	286	5.26	0.38
LL1	249	5.44	0.35	285	5.26	0.37
LL2	246	6.05	0.41	289	5.83	0.40

[a] U = upper, L = lower, R = right, L = left, 1 = central incisor, 2 = lateral incisor.
[b] N = sample size; SD = standard deviation.
[c] Male and female distributions significantly different ($p < 0.001$) for all variables.

Table 3. Correlation coefficients (x100) of incisor mesiodistal dimensions for each sex

	Females (N = 278 to 308)							
	UR2	UR1	UL1	UL2	LR2	LR1	LL1	LL2
UR2	—	66	62	**90**[a]	54	52	52	54
UR1	56	—	94	61	69	71	74	70
UL1	55	91	—	62	69	70	72	71
UL2	88	56	56	—	51	49	49	53
LR2	53	66	67	53	—	73	75	88
LR1	46	63	63	46	71	—	89	73
LL1	45	60	61	45	65	88	—	73
LL2	46	62	64	49	85	68	63	—

Males (N = 254 to 273)

[a] Values in bold type represent correlations between antimeric tooth pairs.
All values are significant ($p < 0.001$). N values vary due to different numbers of missing values.

display a worse fit than this by the χ^2 criterion, but are preferred if more parsimonious (as estimated by AIC) or more appropriate on theoretical grounds. The first model again consisted of an E matrix alone, followed by AE, CE, ACE, and ADE models.

In the second stage, principles of parsimony and biological theory were used to test models involving combinations of factors loading on all eight incisors ("general" factors) and factors loading on one or more pairs of incisors ("group" factors). Once a favorable genetic model was determined, the third step involved the same approach to elucidate the structure of the individual environmental covariation. Finally, as in the univariate analysis, χ^2 tests of heterogeneity between male and female data were applied to the most parsimonious models, and estimates of heritability were obtained.

Results

Measurement reliability

The mean squared differences between the sets of measurements obtained by the two investigators were small, with no value exceeding 0.01 mm. The technical error of measurement, or Dahlberg statistic (Dahlberg, 1940), averaged 0.06 mm, with a range of from 0.04 to 0.07 mm. The reliability of the measurement technique was estimated as the ratio of true to observed variance, where the true variance was calculated as the observed minus the error variance. For our test-retest data, the estimated (inter-observer) reliability of measuring dental casts ranged from 0.96 to 0.99, with an average value of 0.98.

Descriptive statistics

The mean values and standard deviations for each crown dimension in males and females are listed in Table 2. Student's *t* tests revealed significant differences in mean values between the sexes for all eight incisors ($p < 0.001$), with males having larger teeth than females. Variance ratio tests revealed no significant differences in variances between the sexes ($p > 0.05$). There was no evidence of a relationship between mean and variance from either variance ratio tests or regression analysis of pair variances on pair sums. Further *t* tests revealed no significant differences in average incisor crown size between first- and second-born twins or between the zygosities in either sex ($p > 0.05$).

Correlation coefficients for pairs of tooth dimensions within each sex are given in Table 3. All correlations were significant ($p < 0.001$). The strongest correlations were between antimeric teeth, with values ranging from 0.85 to 0.94. Table 4 displays the correlation coefficients between co-twins for each of the sex/zygosity groups. Values for MZ twins ranged from 0.79 to 0.90 ($p < 0.001$), those for DZ same-sexed twins from 0.33 to 0.76 ($p < 0.05$), and those for DZ opposite-sexed twins from 0.10 to 0.43 (most $p < 0.05$). The standard errors of the between-twin correlations for the DZ twin groups (from 0.11 to 0.15) were approximately double those for the MZ twins (from 0.05 to 0.07).

Testing for genotype by environment interaction and directional dominance

For the raw data, regressions of absolute pair difference on pair sum and pair sum squared did not suggest GxE interaction or directional dominance. Only two of the 40 regressions (eight teeth by five twin groups) of absolute pair difference on pair sum were significant ($p < 0.05$), these being for the lower left lateral incisor in MZ males and the lower left central incisor in DZ females. The same variables provided significant results for the regression on pair sum squared, as did the lower right lateral incisor in MZ males. The square and log transformations of the data did not improve linearity. Above all, there was no evidence of stronger relationship between these variables in DZ compared with MZ twins. These tests provide little evidence of dominance in the data.

Univariate analyses

A model with only a unique environmental factor (E) was rejected ($p < 0.001$) for all groups and all variables. The AE model was adequate for all variables except for the upper right central incisor in the "Male" group and in the "All" group. Table 5a shows the squared standardized parameter estimates for the AE models fitted to each incisor crown

Table 6. Goodness-of-fit statistics for the multivariate analysis in female and male twins

			Females			Males		
No.	Model	df[a]	χ^2	Prob	AIC[b]	χ^2	Prob	AIC
	STEP 1: Cholesky models							
1	ACE	164	236.74	< 0.001	-91.26	244.22	< 0.001	-83.78
2	ADE	164	236.65	< 0.001	-91.35	256.69	< 0.001	-71.31
3	AE	200	243.86	0.019	-156.14	265.94	0.001	-134.06
4	CE	200	360.71	< 0.001	-39.29	346.89	< 0.001	-53.11
5	E	236	644.34	< 0.001	172.34	647.98	< 0.001	175.98
	STEP 2: Model 3, vary A							
6	Cholesky, 5 factors	206	243.86	0.036	-168.14	271.11	0.002	-140.89
7	Cholesky, 4 factors	210	245.65	0.046	-174.35	279.15	0.001	-140.85
8	General, Upper, Lower Lateral, Central	212	244.63	0.062	-179.37	276.19	0.002	-147.81
9	General + 4 group factors[c]	220	252.43	0.066	-187.57	290.00	0.001	-150.00
10	General factor only	228	445.76	< 0.001	-10.24	459.10	< 0.001	3.10
11	Group factors only	228	435.64	< 0.001	-20.36	463.06	< 0.001	7.06
12	- With symmetry in:							
13	- General factor	224	254.20	0.081	-193.80	311.46	< 0.001	-136.54
14	- Group factors	224	257.11	0.064	-190.89	299.15	< 0.001	-148.85
15	- All	228	258.96	0.078	-197.04	317.32	< 0.001	-138.68
	STEP 3: Model 15, vary E							
16	General + 8 specific factors[d]	248	327.27	0.001	-168.73	343.74	< 0.001	-152.26
17	- Specific factors only	256	400.90	< 0.001	-111.10	367.28	< 0.001	-144.72
18	- Model 16, with symmetry	256	338.34	< 0.001	-173.66	351.99	< 0.001	-160.01

[a] df = degrees of freedom.
[b] Akaike's Information Criterion (AIC) = χ^2 - 2df.
[c] Group factors represent the four antimeric tooth pairs.
[d] Specific factors represent each of the eight incisors. The best model in each step is highlighted in bold.

constraints were applied to the additive genetic factors (Model 15). The model for male data was improved by symmetry constraints on all but the general genetic factor, which was better left unconstrained (Model 14). The χ^2 value increased by 18.2 (4 df; $p < 0.001$) when the general genetic factor was constrained (Model 15). The third step further investigated the environmental covariation, using the best-fitting model for the additive genetic covariation (Model 15). The factor loadings from the Cholesky decomposition of the unique environmental covariation are listed in Tables 7a and 7b. The largest factor loadings were on the diagonal of the matrix, indicating that the effects of environment on each tooth separately were greater than those on any groupings of the teeth. The second highest loadings involved antimeric teeth. Exploration of the unique environmental covariation thus began with models comprising a general factor affecting all eight incisors and eight specific factors, one for each tooth. When these submodels were applied for the unique environmental covariation, the shift from Cholesky decomposition to one general and eight specific factors improved the fit for the males, but worsened it for the females, with a difference in χ^2 of 68.3 (20 df; $p < 0.001$). As with the additive genetic variation, the differences in χ^2 values between models indicated that the environmental covariation contained both group and specific factors. Comparison of models 16 and 17 yielded $\chi^2 = 73.6$ for females and $\chi^2 = 23.5$ for males (8 df; $p < 0.01$). The model with a general factor alone was so unlikely, it resulted in a nonsensical χ^2 and probability level. Symmetry constraints on all factors improved the fit for both sexes (Model 18). The path diagram for this model is depicted in Fig. 3. The factor loadings of additive genetic and individual environmental components are summarized in Fig. 4. Heritability estimates (h^2) ranged from 0.81 to 0.91 in females, and from 0.84 to 0.89 in males (Fig. 5).

Model 18 was used to test the data for heterogeneity of fit between sexes. This model produced a goodness-of-fit of $\chi^2 = 338.3$ for females, $\chi^2 = 352.0$ for males (256 df; $p < 0.001$), and $\chi^2 = 724.1$ (528 df; $p < 0.01$) when fitted to all four same-sexed matrices. Subtracting the sum of the χ^2 values for the sexes considered separately from the third (joint) fit gave a heterogeneity Chi-square of $\chi^2 = 33.8$ (16 df; $p < 0.01$), indicating significant heterogeneity between female and male twins.

Discussion

Descriptive statistics

The only significant difference in the distributions of male and female data involved the means, with males having larger incisors than females. The correlation analysis provided evidence of common environmental influences on the upper central incisors in males, since DZ correlations were almost as high as those for MZ twins. In fact, the DZ correlations were greater than half the MZ correlations for all eight variables in the males, especially for all central incisors and for the lower central incisors in the females.

Table 4. Between-twin correlation coefficients (x100) for incisor mesiodistal dimensions, within each of the five sex/zygosity groups

Twin Group[a]	N (pairs)[d]	UR2	UR1	UL1	UL2	LR2	LR1	LL1	LL2
MZ Twins									
FF	72-81	87 (06)[b]	90 (05)	89 (05)	85 (06)	84 (06)	79 (07)	87 (06)	82 (07)
MM	63-67	87 (06)	82 (07)	86 (07)	89 (06)	84 (07)	86 (06)	87 (06)	85 (07)
DZ Twins									
FF	42-46	45 (14)	40 (14)	41 (14)	41 (14)	39 (14)	52 (13)	50 (13)	33 (14)
MM	37-43	54 (14)	76 (11)	61 (13)	47 (15)	45 (14)	60 (13)	56 (13)	50 (14)
MF	45-55	10 (15)[c]	33 (13)	29 (13)	25 (15)[c]	43 (13)	42 (13)	22 (14)[c]	39 (13)

[a] F = female, M = male. All other abbreviations as in Table 2.
[b] Numbers in parentheses represent standard errors associated with the correlation coefficients.
[c] Correlation not significantly different from zero ($p > 0.05$).
[d] N values vary due to different numbers of missing values.

dimension separately, within each of the data subsets. Equivalent parameter estimates for the ACE models fitted to the upper central incisors are given in Table 5b. The ACE model displayed a significantly better fit than the AE model for the upper right central incisor of males, with a difference between the two χ^2 values of 7.94 [1 degree of freedom (df); $p < 0.005$]. Low probabilities (from 0.05 to 0.10) were also obtained for the upper left central incisor, AE and ACE models, in the males. Significant heterogeneity in fit of the ACE model occurred between males and females for the upper right central incisor ($p < 0.05$), while there was no significant difference in fit for the upper left central incisor ($p > 0.95$).

Table 5a. Squared standardized parameter estimates (x100) for univariate AE models applied to incisor mesiodistal dimensions

Tooth	Data Subset	a^{2}[a]	e^2	χ^{2}[b]
UR2	Females	86	14	7.10
	Males	85	15	3.82
	Same Sex	85	15	12.59
	All	86	14	25.97
UR1	Females	90	10	3.24
	Males	84	16	19.44[c]
	Same Sex	87	13	25.15[d]
	All	88	12	33.65[e]
UL1	Females	89	11	2.28
	Males	89	11	12.44[d]
	Same Sex	89	11	15.57
	All	90	10	21.35
UL2	Females	85	15	2.16
	Males	88	12	6.92
	Same Sex	87	13	10.14
	All	87	13	17.80
LR2	Females	82	18	9.22
	Males	84	16	6.63
	Same Sex	83	17	16.28
	All	83	17	19.62
LR1	Females	78	22	4.64
	Males	86	14	3.37
	Same Sex	82	18	12.64
	All	82	18	19.94
LL1	Females	85	15	4.30
	Males	89	11	2.08
	Same Sex	86	14	10.23
	All	88	12	21.63
LL2	Females	82	18	4.91
	Males	86	14	3.26
	Same Sex	84	16	8.76
	All	85	15	13.57

[a] a^2 = additive genetic variation, e^2 = unique environmental variation.
[b] Degrees of freedom for χ^2 are 7 for females and males, 16 for "Same-Sex", and 21 for "All" twins for the AE model.
[c] $p < 0.01$; [d] $0.05 < p < 0.10$; [e] $p < 0.05$.

Multivariate analyses

The results of the first stage of the multivariate analysis (Table 6) were consistent with those of the univariate analyses, in that the AE model displayed the best fit for each gender (Model 3). In the second stage, one of the best-fitting models for the additive genetic covariation (Model 9) was comprised of a general factor and four group factors, one each for the antimeric pairs of incisors. The general genetic factor alone was demonstrated to be insufficient, with the differences in χ^2 values between Models 9 and 10 being 193.3 for females and 169.1 for males (8 df; $p < 0.001$). The group factors for antimeric tooth pairs were also insufficient on their own, with the differences in χ^2 values between Models 9 and 11 being 183.2 for females and 173.1 for males (8 df; $p < 0.001$). For the females, the fit improved when symmetry

Table 5b. Squared standardized parameter estimates (x100) for univariate ACE models applied to mesiodistal dimensions of the upper central incisors

Tooth	Data Subset	a^2	c^{2}[a]	e^2	χ^{2}[b]
UR1	Females	90	0	10	3.24
	Males	28	56	16	11.50[c]
	Same Sex	65	22	13	23.16[c]
	All	88	0	12	33.65[d]
UL1	Females	89	0	11	2.28
	Males	65	24	11	11.12[c]
	Same Sex	80	9	11	15.23
	All	90	0	10	21.35

[a] c^2 = common environmental variation.
[b] Degrees of freedom for χ^2 are 6 for Females and Males, 15 for "Same-Sex", and 20 for "All" twins for the ACE model.
[c] $0.05 < p < 0.10$; [d] $p < 0.05$.

Beyond this, the correlations suggested an influence of unique environment—since MZ correlations were less than one—and additive genetic factors where DZ correlations were approximately half the MZ values. The difference in standard errors of the correlations between MZ and DZ twins is partly due to the smaller sample sizes of DZ compared with MZ twins, and partly indicative of genetic influence on tooth size.

Genetic and environmental components

The main finding from both uni- and multivariate model-fitting was that variation in incisor crown size was explicable mostly by additive genetic and individual environmental variance, with no need for non-additive genetic or shared environmental variance. This supports the previous finding for the upper lateral incisor in Pima Indians (Potter *et al.*, 1983), in which most of the genetic variation was additive, with unique environmental effects as well. An expected artifact of the imputation procedure used for the multivariate analyses was the spurious occurrence of common environment, caused by the substitution of sex-specific means or values from the co-twin. Since common environment was not a significant factor in these analyses, the imputation procedure did not appear to have any significant impact on our findings.

Our analyses revealed a general genetic factor (gene or group of genes) which influenced all of the incisors, in contrast to the evidence of independent genetic determination of maxillary and mandibular teeth advocated by Potter *et al.* (1976). However, the finding is consistent with studies of individuals with chromosomal abnormalities (*e.g.*, Alvesalo *et al.*, 1991), implying that human sex chromosomes influence the thickness of dental crowns, and also with recent molecular genetic investigations (Lau *et al.*, 1989; Nakahori *et al.*, 1991; Salido *et al.*, 1992) showing that genes on the human sex chromosomes influence enamel formation. It has been hypothesized further that sequence differences between the genes on X and Y chromosomes contribute to the observed sexual dimorphism in tooth size (Lau *et al.*, 1990; Fincham *et al.*, 1991). In addition to a general genetic factor, there were additive genetic influences on antimeric pairs of teeth, and unique environmental factors operating on each tooth, and on the eight incisors as a group. The slight reduction in goodness-of-fit to the female data which occurred when the model was changed from a Cholesky decomposition to one general and eight specific unique environmental factors was not considered biologically significant, but more a reflection of the power of the twin study to detect environmental influences, especially since the factor loadings displayed a pattern very similar to those in the males (Tables 7a and 7b).

The only exception to the finding of additive genetic and individual environmental influences in our data involved the upper right central incisor in male twins, for which there was evidence of a common environmental effect. This is consistent with the relative magnitudes of the correlation coefficients in male DZ and MZ twins. The significant heterogeneity between females and males for this tooth suggests that genetic and environmental factors differed significantly between the sexes. On re-checking the data for outliers, we noted a pair of male DZ twins with unusually small teeth. When they were excluded from the analyses, the correlations decreased slightly, but the univariate and multivariate analyses and the χ^2 test for heterogeneity in the multivariate analyses remained unchanged. It is therefore unlikely that outliers were responsible for the apparent effect of shared environment.

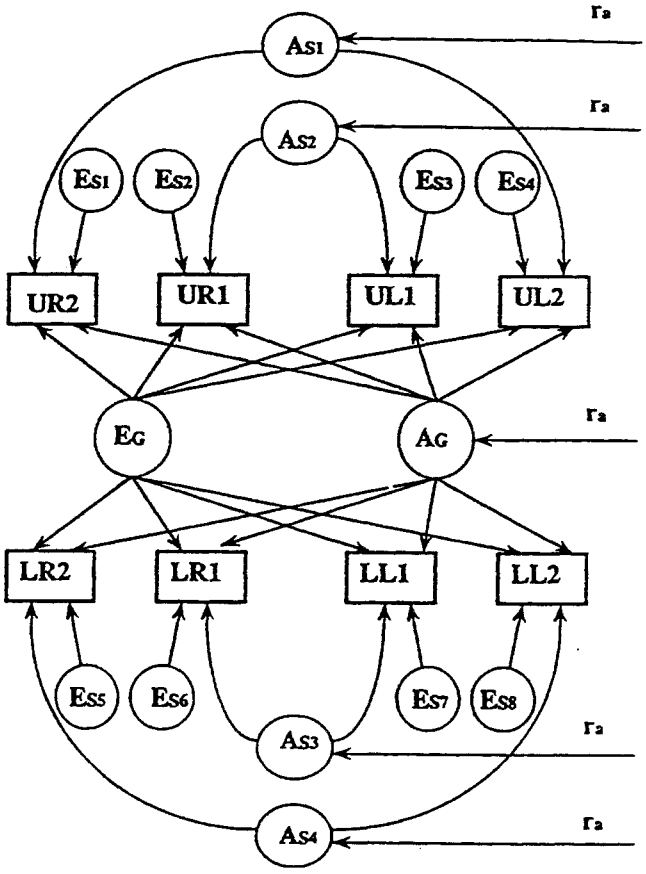

Figure 3. Path diagram for the best-fitting multivariate model (Model 18), illustrating the hypothesized covariance structure of the mesiodistal dimension for the eight incisor crowns in one twin. A_G and $A_{S(1-4)}$ denote the general and group additive genetic factors, respectively, for twin 1, while E_G and $E_{S(1-8)}$ denote general and specific unique environmental factors in twin 1. The entire diagram should be duplicated for twin 2, with the double-headed arrows indicating correlations (r_a = 1.0 for MZ, 0.5 for DZ twins) between the twin members.

One potential source of shared environmental contribution to tooth size is the hormonal composition of the uterine environment. In humans, males have larger teeth on average than females. If androgens contribute to increased tooth size and are able to diffuse from one twin to the other, then we might predict an increased similarity in dental dimensions of male DZ twins. This would be reflected in statistical analyses as a common environmental effect in males. In other mammals, testosterone diffuses between fetuses through the amniotic membranes (Fels and Bosch, 1971), or *via* the maternal circulation (Meisel and Ward, 1981). Indirect evidence for hormonal exchange between human twins arises from a preliminary study of opposite-sexed twins, in which we noted a trend toward larger teeth in females with twin

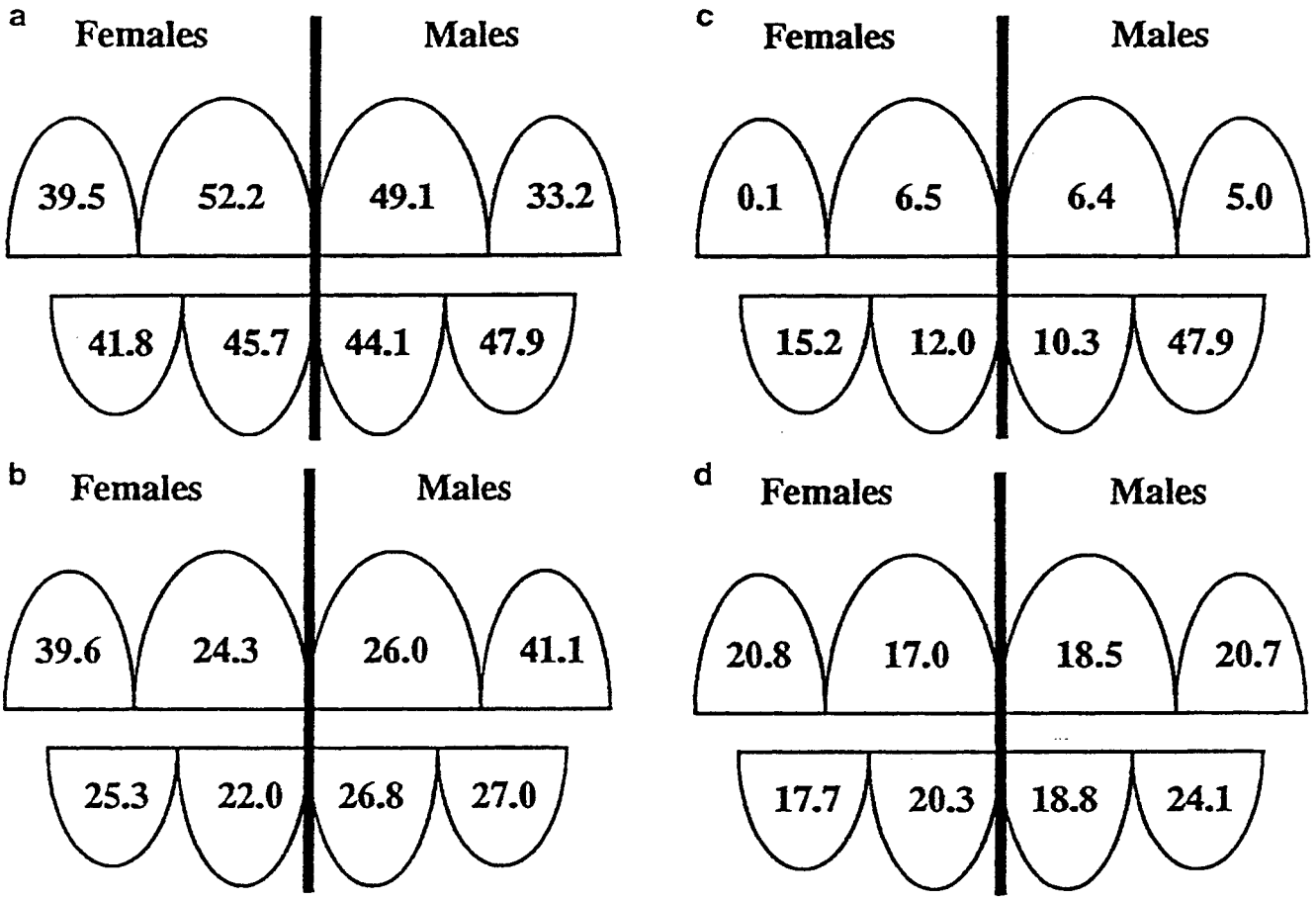

Figure 4. Standardized factor loadings (x100) from Model 18 in females (left) and males (right). Only one side of the dentition is displayed, since loadings are identical on each side. (a) Loadings for the general additive genetic factor. (b) Loadings for the additive genetic factor for antimeric pairs of teeth. (c) Loadings for the general unique environmental factor. (d) Loadings for the unique environmental factor for individual teeth.

brothers, than in females with twin sisters (Dempsey *et al.*, 1994), indicating a possible masculinizing effect on females. However, if this type of sibling interaction does occur, one would expect to find decreased variance among male DZ twins relative to male MZ twins (Neale and Cardon, 1992). No such decrease in variance was found in our data.

The lack of evidence for non-additive genetic variance in our data does not necessarily mean that it does not exist. Since the preliminary regression analyses of pair sums on pair differences showed no evidence of directional dominance, there was less *a priori* chance of detecting non-additive genetic variation. Additionally, if common environmental influences do exist, they will inflate additive genetic variation and deflate non-additive genetic variation (Martin *et al.*, 1978). Thus, the apparent absence of both non-additive genetic variation and shared environmental variation may be due to having insufficient power to detect them, a difficulty which further sampling may resolve. However, if these contributing factors did exist in our data, they are likely to have been small by comparison with the contributions of additive genetic and unique environmental factors. It is interesting to note that dominance was not a necessary component of the model developed by Potter *et al.* (1983), although statistical power was also a problem in their study.

The advantages of the modeling techniques applied in this analysis include greater ability to separate genetic from non-genetic effects, additive from non-additive genetic variations, and individual from familial environment. It was also possible to test the data for genotype-environment interactions, and to analyze the eight variables simultaneously. Most earlier studies applied heritability estimation procedures to univariate data. The estimates incorporated a number of inseparable genetic and environmental variance components, and/or assumed no genetic interaction (epistasis) or genotype-environment interaction (Bulmer, 1970; Smith, 1974; Mizoguchi, 1977).

Symmetry

The multivariate study indicated that all of the factors for both additive genetic and individual environmental variation operated similarly on antimeric pairs of teeth. The conclusion that antimeric teeth shared the same genetic determinants is consistent with other accounts (*e.g.*, Potter *et al.*, 1976). In a study of dental crown traits in Mexican Indians and Afro-Belizeans, Baume and Crawford (1980) concluded that common genetic factors were likely to influence characters on both sides of the dental arch equally. Asymmetry was proposed to occur through local

Table 7a. Estimated factor loadings for the Cholesky decomposition of unique environmental variance in females

Females	UR2	UR1	UL1	UL2	LR2	LR1	LL1	LL2
UR2	**0.1837**[a]							
UR1	***0.0608***[a]	**0.1703**						
UL1	0.0071	***0.0866***	**0.1553**					
UL2	***0.0698***	0.0242	0.0113	**0.2068**				
LR2	-0.0104	0.0384	0.0391	-0.0032	**0.1585**			
LR1	-0.0046	0.0031	0.0097	0.0134	0.0458	**0.1530**		
LL1	-0.0080	0.0225	-0.0074	-0.0026	***0.0528***	0.0364	**0.1215**	
LL2	-0.0264	0.0164	0.0181	-0.0046	***0.0781***	0.0098	0.0063	**0.1482**

[a] Factor loadings in bold are > 0.1; those in bold italics lie between 0.05 and 0.1.

Table 7b. Estimated factor loadings for the Cholesky decomposition of unique environmental variance in males

Males	UR2	UR1	UL1	UL2	LR2	LR1	LL1	LL2
UR2	**0.2183**							
UR1	-0.0395	**0.1828**						
UL1	-0.0157	***0.0546***	**0.1725**					
UL2	***0.0713***	0.0015	0.0080	**0.1869**				
LR2	0.0064	0.0111	-0.0171	-0.0104	**0.1620**			
LR1	0.0190	0.0072	***-0.0527***	0.0110	0.0142	**0.1216**		
LL1	0.0192	0.0056	-0.0122	-0.0026	0.0095	***0.0531***	**0.1001**	
LL2	0.0077	***0.0514***	0.0291	0.0092	0.0361	0.0059	-0.0021	**0.1407**

environmental conditions within the jaw or by more general intra-uterine developmental effects. Our model for unique environmental factors, like that of Potter *et al.* (1976), comprised independent environmental influences on right and left sides, although constraining antimeric loadings to be equal for unique environmental factors improved the fit of the model, indicating that the influences tended to have the same degree of impact on both sides.

Heritability

Our estimates of heritability, averaging 86%, were reasonably high when compared with estimates of 35% (Potter *et al.*, 1983), 54% (Alvesalo and Tigerstedt, 1974), 60% (Goose, 1971), 64% (Townsend and Brown, 1978a,b), and up to 72% (Rebich and Markovic, 1976) in other studies of human tooth size. They were more compatible with Garn's (1977) estimate of up to 90%. The variation in estimates of heritability among these studies reflects the different statistical approaches used, and probably also the different populations from which samples were drawn, since there may have been greater environmental effects within some than others. As predicted in Butler's field theory, the heritability estimates were slightly higher for both upper and lower central incisors than for the lateral incisors, although the differences were very small (3% for upper and 1% for lower incisors). Expansion of our analyses to the rest of the dentition may shed more light on the theory, but at this stage there is little evidence of differential heritability with position in the incisors.

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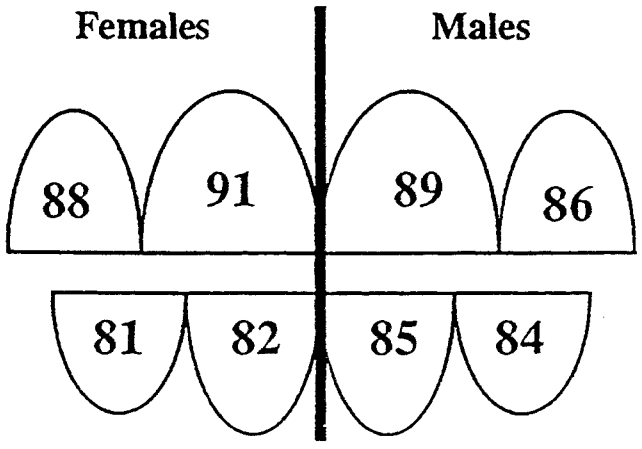

Figure 5. Estimated heritabilities (x100) for the mesiodistal dimension of the permanent incisors in males and females.

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