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A Discriminant Function Analysis of Deciduous Teeth to Determine Sex

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ABSTRACT: Studies of deciduous teeth have concluded that crown size differences in these teeth between males and females are not reliable sex discriminators, in contrast to such differences in permanent teeth. This study measured the mesiodistal and faciolingual crown diameters of all deciduous teeth, as well as those of the permanent first molars, of 162 children from the Burlington Orthodontic Growth Study, conducted earlier in Burlington, Ontario, Canada. All 40 deciduous tooth diameters (20 mesiolingual and 20 faciolingual) were significantly different between the sexes, as were the permanent tooth diameters. Using three to five measurements of deciduous teeth, discriminant analyses of several samplings of these children produced discriminant functions in which 76 to 90% of the holdout samples were correctly classified by sex. Combinations of deciduous and permanent measurements were used to classify 83 to 85% of the holdout samples correctly. When compared with published data on other sample populations, the Burlington group is the most dimorphic for deciduous teeth and is within the range of permanent tooth dimorphisms of other populations. The level of classification accuracy, when using discriminant analysis of the deciduous teeth, can approach the accuracy levels of analysis using the permanent teeth.

KEYWORDS: odontology, dentition, human identification, sex determination

While a variety of metric and morphological measurement methods have been developed to determine the sex of adult skeletons, there is no consensus on a method for determining the sex of subadult (preadolescent) remains. The sex of subadult humans can be inferred by comparing the dental development with the postcranial development in the same individual, since males mature more slowly skeletally [1]. However, the accuracy levels attained using this method have been questioned [2], and the method itself is difficult to apply to incomplete skeletal remains. Choi and Trotter [3] used long bone weight and length ratios to produce a sex classification accuracy of 72% for fetal skeletons, but the use of bone weights makes this method inapplicable for exhumed bones. One recent promising method [4] has used the iliac auricular surface elevation to sex fetal and infant skeletons, achieving a classification accuracy of 43 to 75% for females and 73 to 92% for males. This method, however, relies on a single, nonmetric trait.

The fact that significant sexual dimorphism occurs in the permanent dentition [5-9] suggests that there might be significant sexual dimorphism in the deciduous teeth. While it is recommended for forensic anthropology that determination of the sex of adult skeletons by using the teeth should only be done as a verification for other methods [10],

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it has also been said that "for children . . . the deciduous teeth represent the only factor useful for sex diagnosis" [11].

Several studies have determined that a small but significant dimorphism does exist in the deciduous dentition, but these studies do not attempt to apply classificatory procedures for separating the sexes [12-15]. Only one previous study has employed discriminant function analysis of deciduous tooth measurements to classify a sample by sex. Black [16] measured the mesiodistal and faciolingual diameters of the right deciduous dentition from casts of 133 white American children, 64 females and 69 males. Based on his observation that only 5 of the 20 measurements were significantly different by sex, he concluded that the deciduous dentition displays much less sexual dimorphism than the permanent dentition of a related adult sample. He also concluded that the discriminant functions calculated from the diameters of deciduous teeth were much less accurate for sex classification than were discriminant functions derived from the permanent tooth diameters of the same children [17]. Black correctly classified by sex 64 to 68% of the deciduous sample from which he had originally derived the functions.

The purposes of the present study were to determine the following:

(a) whether the mesiodistal and faciolingual dimensions of both the right and left deciduous crowns of a specific population sample would display significant sexual dimorphism and the extent of the dimorphism;

(b) whether the groups of deciduous variables, derived from the discriminant function analysis of those diameters, would classify by sex a second holdout sample with an accuracy of 75% or greater; and

(c) whether the addition of the permanent first molar measurements would have an effect upon the classification accuracy of the discriminant function.

Materials and Methods

Dental measurements were taken from 162 dental casts, 80 female and 82 male, of children aged 3 to 4 years. An additional 84 casts, 45 female and 39 male, were drawn from that same group of children at 16 years of age, in order to obtain the permanent first molar measurements. The casts were selected from a sample of 1380 children involved in the Burlington Orthodontic Growth Study, a longitudinal growth study conducted from 1952 to 1972 in Burlington, Ontario, Canada. The Burlington study, whose children comprised 85 to 90% of the population of children in Burlington at that time, is considered to contain a sample representative of the majority population of children in Ontario, described as being mainly Caucasian and Anglo-Saxon [18].

The growth study structure allowed control of such variables as nutritional and health status, genetic relatedness, population background, age, and sex. During the time of the study, the average household income for Burlington was well above the national average; the children were well situated financially and geographically for achieving optimal health and nutritional levels [18]. The case selection eliminated any possible cases of genetic disorder or serious illness.

The cast selection was rigorous. Casts which were of poor quality, broken, or chipped; casts which contained damaged, excessively crowded, or morphologically abnormal teeth; and casts which exhibited attrition, caries, or restorations were eliminated from this sample.

The measurements were made on all 20 deciduous teeth, on both the right and left sides, and on the four permanent first molars using needlepoint Helios dial calipers reading to 0.05 mm. All measurements were taken by one observer (C. D.). The mesiodistal crown diameter was measured according to the method described by Moorrees [19]. In this case, the diameter refers to the distance between the *contact points*, which are determined by the "ideal" anatomical relationship between the tooth position and

the curve of the dental arch, as opposed to the *maximum* crown diameter. The mesiodistal crown diameter may be difficult to take from evulsed teeth, although for the anterior teeth the two measurements are usually one and the same [20]. In the cheek teeth it should be possible to identify contact facets to take the mesiodistal crown diameter. The faciolingual crown diameter was measured according to the methods described by Townsend [21], Margetts and Brown [14], and Hillson [20], parallel to the mesiodistal crown diameter.

Statistical Procedures

The data were first assessed for normality using the Kolmogorov-Smirnov one-sample test [22]. In addition to the descriptive statistics, we also examined significant sex differences for each measurement using the *t*-test for independent samples, as well as the coefficient of variation to assess and compare the variability of tooth size for each tooth type. The percentage of sexual dimorphism was calculated according to the method of Garn et al. [6]

$$\left(\frac{\text{Male } \bar{X}}{\text{Female } \bar{X}} - 1.0 \right) \times 100$$

To determine if combinations of certain crown diameters would prove effective in classifying by sex a sample of unknown sex, the multivariate statistical technique, discriminant function analysis, was employed. In discriminant analysis, however, the cases used in developing a discriminant function will be classified with a greater accuracy by that same function than will cases from a related group of unknown sex [23,24]. Consequently, more meaningful assessments of the predictability or classification accuracy of discriminant functions will be derived if those functions are applied to *holdout samples*. Classificatory accuracy of the resulting discriminant function is cross-validated by applying it to a *holdout* sample, a separate set of cases from the study sample for which the sex is deliberately entered as unknown. The classification accuracy should be at least 25% greater than that achieved by chance [25]. For this study, the criterion for accuracy was set at 50% better than chance, or a 75% classification accuracy, given the level of accuracy achieved with the permanent dentition in other studies.

The original study cases were split into four different groups (Table 1), each containing a holdout sample and one to two analysis samples of varying sizes. The selection of four groups resulted from our desire to find out whether the level of classification accuracy achieved initially with one group could be repeated using slightly different combinations of cases in the holdout group and whether the same cluster of variables would be included in the resulting discriminant functions. Calculations of Box's *M* test [22] detected equality of group covariance matrices and, indirectly, deviations from multivariate normal distributions.

Initially, direct and stepwise discriminant analyses were performed on the entire sample. Subsequently, stepwise and direct discriminant analyses were run using Groups A through D with their appropriate holdout samples, which were not included in the production of the discriminant functions for these groups.

The Intraobserver Error Study

Intraobserver error, or replicability of measurement, was assessed from 40 randomly selected casts, 20 female and 20 male, remeasured five weeks after the completion of the original data gathering, and involved a total of 1600 measurement scores. A difference of 0.10 mm was taken as the normal unit of permissible measurement difference between

TABLE 1—Structure of the sample groups used for discriminant function analysis.

Group	Analysis Sample Type	Total, <i>N</i>	Number Used for Analysis	Number Used for Classification
A	deciduous	141	136	138
	deciduous and permanent	63	63	63
	holdout	21	...	21
B	deciduous	122	119	121
	holdout	40	...	40
C	deciduous	141	138	140
	deciduous and permanent	63	63	63
	holdout	21	...	21
D	deciduous	120	117	117
	deciduous and permanent	42	42	42
	holdout	42	...	42

an original and a repeat measurement [20]. The significance of any error was evaluated using Dahlberg's method for determining the standard deviation of a single determination [14,21], the direct difference method [26], and Sandler's *A*-statistic [26,27].

Results

Univariate Analysis

The Kolmogorov-Smirnov test showed that all the diameters of both deciduous and permanent teeth were normally distributed by sex. The means for male subjects were consistently greater than those for females for the diameters of the 40 deciduous teeth and those of the 8 permanent teeth, although there was considerable overlap in the ranges (Fig. 1). The *t*-tests revealed significant differences between males and females for all of the 40 deciduous diameters at the 5% level and for 25 of the diameters at the 0.1% significance level. Overall, the maxillary deciduous dentition displayed more significant differences between the sexes, especially faciolingually. However, the highest individual differences occurred mesiodistally, in the mandibular canines, and then faciolingually, in the maxillary right central and lateral incisors and the maxillary left second deciduous molar (Fig. 2). The eight permanent diameters were all significantly different between the sexes at the 5% level or less.

The tooth dimensions of deciduous teeth were more variable in the female for all teeth except the canines, as expressed by the coefficient of variation. For both sexes, the faciolingual dimensions were more variable than the mesiodistal dimensions. In both sexes, the lateral incisors were more variable than the central incisors in the maxilla, while the central incisors were more variable than the laterals in the mandible. For both sexes, the second deciduous molars were less variable than the first deciduous molars in both the maxilla and the mandible. In general, the second deciduous molar was the least variable tooth for either sex in either jaw, although in the female maxilla the mesiodistal diameter of the canine varied less than that of the second deciduous molar.

The calculation of the percentage of sexual dimorphism did not reveal any systematic pattern when the diameters were ranked. The dimorphism percentage ranged from 1.91 to 6.44%. The individual diameters displaying the highest dimorphism percentage are shown in Fig. 2. When the right and left sides were averaged, dimorphism was greater

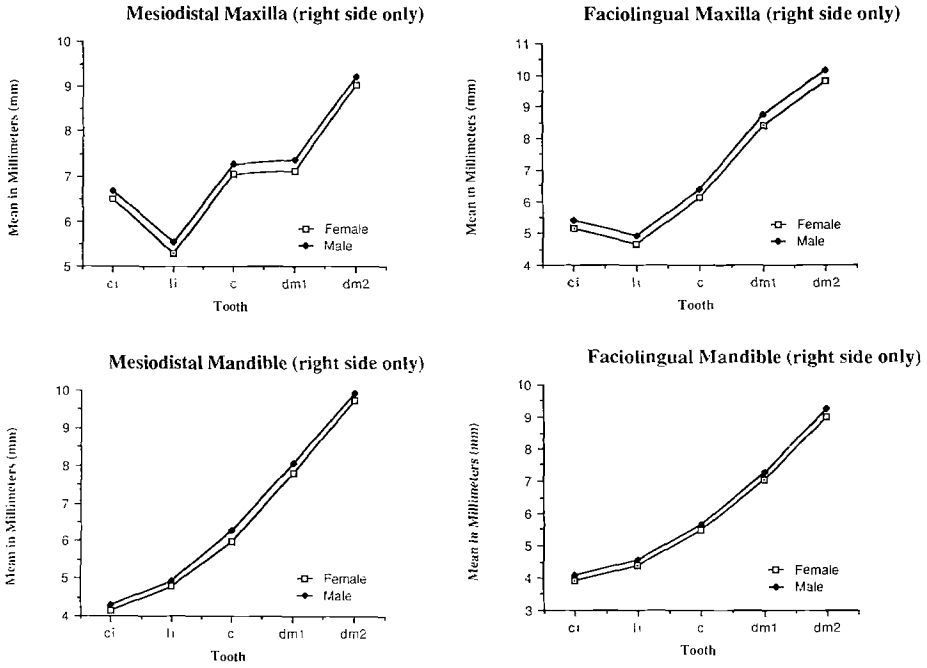


FIG. 1—Comparison of male and female means for the mesiodistal and faciolingual deciduous crown diameters. The right side is rounded to one decimal place.

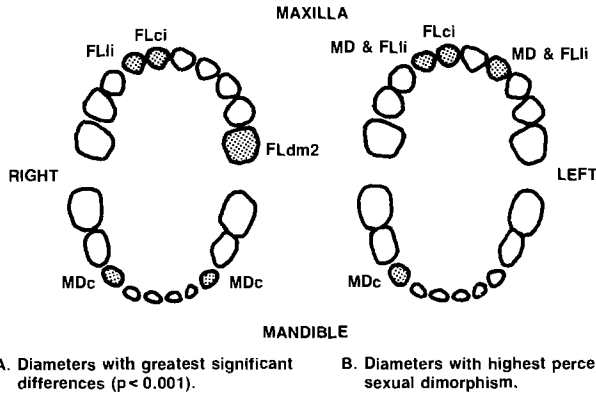


FIG. 2—Deciduous crown diameters displaying the greatest significant differences and highest percentage of sexual dimorphism.

in the maxillary deciduous dentition than in the mandibular, and greater faciolingually than mesiodistally (Fig. 3).

The average dimorphism percentage in the permanent first molar was 4.11% in the maxilla mesiodistally and 5.62% faciolingually. In the mandible the average dimorphism percentage in the permanent first molar was 5.86% mesiodistally (1.96% in mandibular tooth dm2 mesiodistally) and 2.70% faciolingually (2.67% in mandibular tooth dm2 faciolingually).

Multivariate Analysis

Initially, in the discriminant analyses of the four groups (Groups A through D), stepwise analyses were run on the *analysis samples* (the holdout samples were kept separate) of each group to determine the total classificatory variables selected by the statistical calculations as the most effective discriminators of sex. The total variables chosen by the analyses were as follows: Group A, 14 variables; Group B, 17 variables; Group C, 17 variables; and Group D, 12 variables. Next, for the classification of the holdout samples, direct analyses were run with the first five variables from the totals of the stepwise analyses for Groups A and B and with the first four variables for Groups C and D (Fig. 4). Subsequently, the variables were further subdivided into maxillary and mandibular sets and the holdout samples were again classified.

Table 2 summarizes the direct discriminant analyses results for all groups with non-significant Box's *M* test values. Table 3 summarizes the resulting discriminant function equations. Using from three to five deciduous diameter variables for the discriminant analyses, four discriminant functions were derived which yielded a degree of classification accuracy of greater than 75%. A combination of four maxillary variables and one mandibular variable was needed to achieve an accuracy level of 80% with the Group B holdout sample (*N* = 40, originally the error study sample) and of 90% with the Group A holdout sample (*N* = 21). The four maxillary variables alone, with the Group A holdout sample, and the three maxillary variables alone, with the Group D holdout sample (*N* = 42), both produced a classification accuracy of 76%.

When discriminant analyses were run including the permanent first molar dimensions, preliminary stepwise analyses chose three permanent molar diameters, the faciolingual diameter of the right maxillary first molar and the faciolingual and mesiodistal diameters of the left mandibular first molar, as the most effective classification variables. Direct discriminant analyses run with Group C, using the four deciduous diameters originally

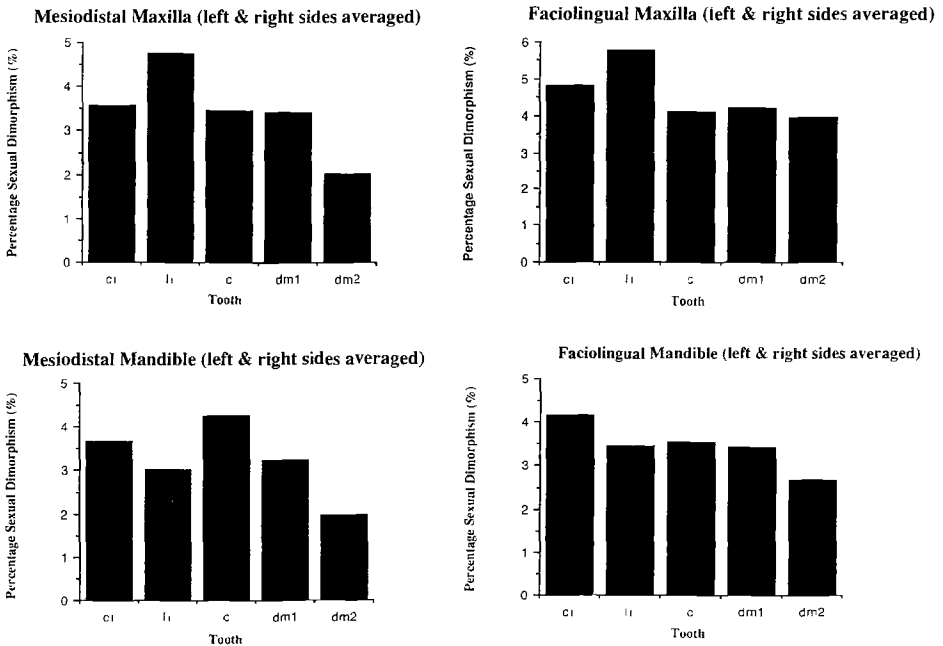


FIG. 3—Percentage of sexual dimorphism in the deciduous dentition.

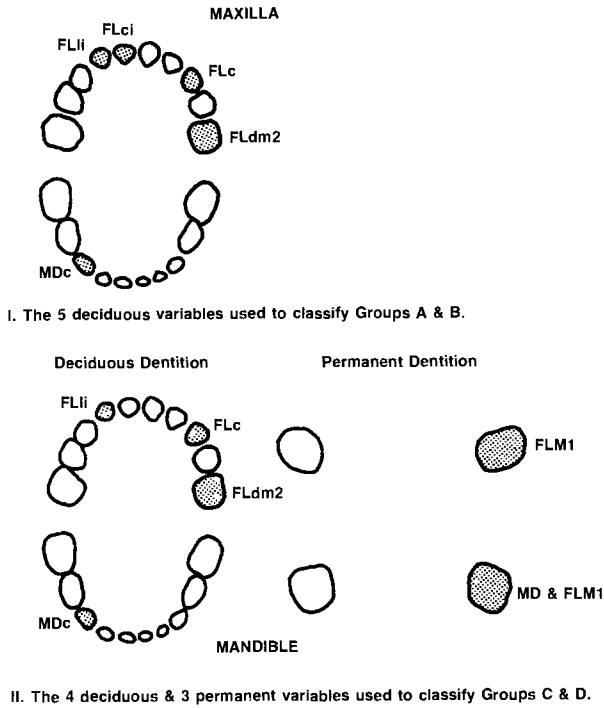


FIG. 4—The classificatory variables used in the direct discriminant analysis.

chosen by stepwise analysis and the three permanent molar variables, produced a classification accuracy of 85.7% with the holdout samples (Table 2).

Discussion

The fact that the Burlington data proved to be normally distributed for males and for females justified the subsequent tests of significance. Significant sexual dimorphism occurs in all tooth types, with all male means being greater than female means. The sexual dimorphism percentage expressed in the Burlington data (1.91 to 6.44%) is small compared with many skeletal variables but is comparable to that determined for the permanent teeth of various populations [6,8,9]. The teeth displaying the greatest sexual dimorphism are the maxillary lateral incisors, the faciolingual right maxillary central incisor, and the mesiodistal right mandibular canine.

Previous studies of human deciduous teeth have concluded that the expression of sexual dimorphism is less in the deciduous dentition than in the permanent dentition [12,13,16]. On the other hand, male means for tooth crown diameters are generally greater than female means in both the deciduous and permanent teeth, particularly for the mandibular canines [6,8,28-30]. Moss [29] maintains that the greater male canine crown diameters result from differences in enamel thicknesses due to the longer period of amelogenesis in the male. Completion of tooth crown calcification occurs earlier in the female than in the male for both the deciduous and permanent teeth [31-33]. Female deciduous crowns are actually larger than male crowns prenatally, but continued enamel deposition occurs postnatally in males [34].

The sex chromosomes are known to have a direct effect on tooth size. The Y chromosome influences the timing and rate of body development, producing slower male

TABLE 2—Direct discriminant analysis results.^a

Group	Number of Variables Used				Analysis Sample		Holdout Sample, N	Correctly Classified, %
	Maxilla		Mandible		Sex	N		
	D	P	D	P				
Deciduous Diameters Only								
A	4	...	1	...	M	73	9	100.0
					F	68	12	83.3
					M+F	141	21	90.5
B	4	...	1	...	M	62	20	80.0
					F	60	20	80.0
					M+F	122	40	80.0
D	3	...	1	...	M	63	19	73.7
					F	57	23	73.9
					M+F	120	42	73.8
A	4	M	73	9	77.8
					F	68	12	75.0
					M+F	141	21	76.2
B	4	M	62	20	75.0
					F	60	20	70.0
					M+F	122	40	72.5
D	3	M	63	19	84.2
					F	57	23	69.6
					M+F	120	42	76.2
A	1	...	M	73	9	66.7
					F	68	12	75.0
					M+F	141	21	71.4
B	1	...	M	62	20	65.0
					F	60	20	65.0
					M+F	122	40	65.0
D	1	...	M	63	19	68.4
					F	57	23	73.9
					M+F	120	42	71.4
Deciduous and Permanent Diameters								
C	3	1	1	2	M	29	10	80.0
					F	34	11	90.9
					M+F	63	21	85.7
C	3	1	M	29	10	80.0
					F	34	11	90.9
					M+F	63	21	85.7
C	1	2	M	29	10	80.0
					F	34	11	90.9
					M+F	63	21	85.7

^aAbbreviations: D, deciduous teeth; P, permanent teeth.

TABLE 3—Discriminant function equations.*

Group	Equation
<i>4 Maxillary and 1 Mandibular Variables</i>	
A	1.500 (FL R max li) + 1.091 (FL R max ci) + 0.654 (FL L max dm2) - 1.489 (FL L max c) + 1.640 (MD R mand c) - 20.342
B	1.380 (FL R max li) + 0.896 (FL R max ci) + 0.357 (FL L max dm2) - 1.474 (FL L max c) + 2.266 (MD R mand c) - 19.736
<i>3 Maxillary and 1 Mandibular Variables</i>	
D	1.899 (FL R max li) + 1.174 (FL L max dm2) - 1.750 (FL L max c) + 1.653 (MD R mand c) - 20.138
<i>4 Maxillary Variables</i>	
A	1.625 (FL R max li) + 1.239 (FL R max ci) + 1.135 (FL L max dm2) - 1.141 (FL L max c) - 18.564
B	1.690 (FL R max li) + 0.967 (FL R max ci) + 1.184 (FL L max dm2) - 1.097 (FL L max c) - 18.192
<i>3 Maxillary Variables</i>	
D	2.084 (FL R max li) + 1.688 (FL L max dm2) - 1.353 (FL L max c) - 18.425
<i>1 Mandibular Variable</i>	
A	3.079 (MD R mand c) - 18.861
B	3.051 (MD R mand c) - 18.699
D	3.000 (MD R mand c) - 18.407
<i>4 Deciduous and 3 Permanent Maxillary and Mandibular Variables</i>	
C	0.542 (FL R max li) + 0.279 (FL L max dm2) - 0.723 (FL L max c) + 1.058 (MD R mand c) + 1.837 (FL L max M1) + 0.628 (MD L mand M1) - 1.692 (FL L mand M1) - 17.423
<i>3 Deciduous and 1 Permanent Maxillary Variables</i>	
C	0.574 (FL R max li) + 0.393 (FL L max dm2) - 0.371 (FL L max c) + 1.521 (FL L max M1) - 21.314
<i>1 Deciduous and 2 Permanent Mandibular Variables</i>	
C	2.049 (MD R mand c) + 0.887 (MD L mand M1) - 0.516 (FL L mand M1) - 16.872

*Abbreviations: FL, faciolingual; MD, mesiodistal; L, left; R, right; max, maxillary; mand, mandibular.

maturation [35] and acts both additively and to a greater extent than the X chromosome on tooth size [36]. Females lacking one X chromosome have smaller teeth than do normal females, while females with an extra X chromosome do not show increased tooth size [36].

In contrast to Stini's [37] assertion that males of a population would exhibit a greater degree of biological variation than would females, the coefficients of variation determined for the Burlington sample show that females are generally more variable than males in

all teeth except the canines. The faciolingual mandible is the most variable, but this dimension does not consistently display the greatest variability in other studies of the deciduous dentition [13-16].

According to the field model of tooth development [38], maxillary lateral incisors vary more than central incisors, mandibular central incisors vary more than lateral ones, and first molars vary more than second molars. The patterns of variability seen in the Burlington data follow the field model except for those for males mesiodistally in the mandibular incisors.

The results of the univariate analyses of the Burlington sample were compared with those published for six other groups, including American Caucasians [16,19], Australian aboriginals [14], Hindu children from western India [15], Swedish children [12], and Icelandic children [13]. With some control for differing measurement methods among the various studies, comparisons were made of the means and sexual dimorphism percentages. The Australian aboriginals exhibited the largest means for males and females, while the Burlington group followed next in overall tooth size. The East Indian group, except for the mesiodistal maxillary canines, was very similar in mean tooth size to the five European-North American groups. Of the two European groups of Scandinavian origin, the Icelandic group had larger measurements, particularly in the posterior dentition. Among the three North American groups, the Burlington group had the largest teeth and the Ohio group the smallest. Because of the differences between these three groups, no one sample could be termed truly representative of modern North American populations of European origin.

The greatest sexual dimorphism percentage in the comparative analysis (Fig. 5) was seen in the Burlington group. The East Indian group was the next most dimorphic, and the Australian aboriginal, American [19], and Swedish groups followed. The Icelandic and Ohio American [16] groups displayed very little sexual dimorphism. Several studies of the permanent teeth of various populations have shown sexual dimorphism to be most strongly expressed in the mesiodistal diameter of the mandibular canines [6,8,9,29]. In the deciduous teeth compared in this study, the greatest dimorphism was displayed in either the incisors or the molars, but there was no pattern characteristic of all the groups.

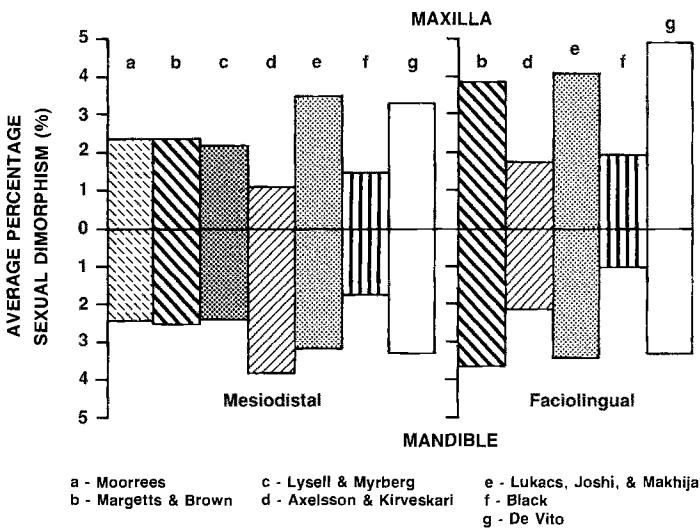


FIG. 5—Comparative data for the average sexual dimorphism percentage.

There was no single pattern in the expression of sexual dimorphism percentage in the deciduous teeth that was specific to any population group. The North American groups, for example, included the most dimorphic group, the Burlington group, and the least dimorphic group, the Ohio group. In addition, the European populations could not be said to be either more dimorphic or less dimorphic than the non-European populations.

Multivariate Analysis

The diameters showing the greatest significant difference in size between males and females or the greatest dimorphism were almost the same as the diameters included in the discriminant functions. These included the faciolingual diameters of the right maxillary central and lateral incisors and of the left maxillary canine and second deciduous molar, and the mesiodistal diameter of the right mandibular canine.

Using from three to five deciduous diameter variables for the discriminant analyses, four discriminant functions were derived which yielded a degree of classification accuracy greater than 75%. A combination of the four maxillary variables and one mandibular variable was needed to achieve an accuracy level of 80% with the Group B holdout sample and of 90% with the Group A holdout sample. The four maxillary variables alone used with the Group D holdout sample produced a classification accuracy of 76%. The remaining deciduous analyses meeting the criterion correctly classified from 65% to less than 75% of the cases in the holdout samples. When the three permanent molar variables were included with the four deciduous maxillary and mandibular variables, the classification accuracy was 85%.

Black [16] was not as successful in his attempt to use deciduous variables to classify sex, but his measured diameters were not as dimorphic. The success rate achieved in this study is comparable to success rates achieved with permanent dentition measurements when holdout samples are used [24].

The intergroup comparisons raise the question of why the teeth of the Burlington sample seem larger and more dimorphic than those of the samples from the other European groups, particularly the Ohio and Icelandic groups. Human sexual dimorphism is said to be an outcome of a survival strategy, a balancing of the need for a high degree of biological variation within the species with the need for a narrow range of variation in the female, who is physically structured for the support of an infant prenatally and postnatally [37]. Males exhibit more of the extremes in variation than do females and are more affected by extremes in the environment. Theoretically, a population which is well nourished and healthy throughout growth and development would be expected to attain increased or even maximum body size (within the limits of that population's actual potential), including increased tooth size, with males generally exceeding females in overall size and displaying greater variation. An additional expectation would be the expression of a high percentage of sexual dimorphism in the deciduous and permanent teeth.

However, the Burlington sample *could not* be shown to include children who were better nourished or more healthy overall than the children of the Ohio or Icelandic samples, and in the Burlington data females generally varied more than males, except in deciduous canine size. If sexual dimorphism were mainly a function of size, the greatest sexual dimorphism would be expected in the sample of Australian aboriginal children, rather than in the Burlington sample. In addition, although the Icelandic and the Burlington samples both displayed large means (along with the East Indian group), the Icelandic group displayed a lower sexual dimorphism percentage.

Studies of the permanent dentition of various populations demonstrate that a positive correlation between *tooth* size and sexual dimorphism percentage does not exist in humans [6,39]. Garn, Lewis, Swindler, and Kerewsky [6] found that there is only a low significant

correlation between sexual dimorphism in teeth and in *body size*. Frayer and Wolpoff [40] maintain that, from an evolutionary perspective, body size has actually had little impact upon human sexual dimorphism. Therefore, the large tooth size seen in the Burlington group may not be a major factor contributing to the high percentage of sexual dimorphism.

Conclusions

Univariate analysis of the Burlington data reveals significant sexual dimorphism in the 40 deciduous tooth diameters as great as or even greater than that seen in the permanent teeth of several sample populations [6,8,9]. All male means are significantly larger than female means. In a comparison with published studies of deciduous tooth size [12–16,19], the Burlington group proved to be the largest in mean tooth size after the Australian aboriginal group and the most dimorphic. The pattern and degree of sexual dimorphism reported for deciduous crown diameters varies both among and within populations as greatly as that reported for the permanent crown diameters [6]. The differences in mean tooth size and dimorphism among the three North American groups, in this study and others [16,19], show that *no single sample group* can be termed truly representative of a specific population. A positive correlation does not exist between tooth size and sexual dimorphism in the deciduous dentition. Both the Australian sample, which was the largest in tooth size, and the Icelandic sample, which was almost as large in tooth size as the Burlington sample, displayed less sexual dimorphism than did the Burlington sample.

Contrary to the results of a previous North American study [16], discriminant analysis of the Burlington data resulted in sex classification accuracy levels of 75 to 90% of *holdout* samples, levels equal to those seen in analyses of the permanent teeth [17,24,41]. The results obtained with the Burlington data demonstrate that the deciduous teeth, depending upon the group examined, do display significant sexual dimorphism. The discriminant functions derived using deciduous teeth can be as accurate in classifying a sample by sex as those derived from the permanent teeth. The discriminant functions derived through this analysis provide standards for classifying subadult skeletal material by sex, particularly in modern forensic science cases.

Acknowledgments

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