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A MORPHOMETRIC STUDY OF SEX DIFFERENCES IN FETAL ILIA

A Thesis

Presented for the

Master of Arts

Degree

The University of Tennessee, Knoxville

Susan Marie Cera Holcomb

December 1992

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DEDICATION

This thesis is dedicated to my family and to my husband, Tim D. Holcomb, for believing in me when I didn't always believe in myself.

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ABSTRACT

Considerable debate has concentrated on whether human fetal skeletal remains exhibit sexual dimorphism. Most attention has focused on the greater sciatic notch of the ilium, since it is a gross morphological characteristic with known sex differences in the adult and is easily seen in fetal skeletal remains. Previous traditional morphometric analyses of the fetal sciatic notch have, however, led to ambiguous results. The purpose of this study is to determine whether differences between the sexes can be discerned when modern morphometric techniques are applied.

Photographs of the ventral side of 133 fetal ilia of known age and sex from the Trotter Collection of Washington University are digitized, and the trace coordinates used for all subsequent analyses. Elliptic Fourier analysis followed by calculation of a discriminant function indicates that there are significant shape differences between male and female fetal ilia (81.8% of females correctly classified, 74.0% of males correctly classified). In order to identify these shape differences, a finite line skeleton is fit to each tracing and used to locate homologous landmarks that encompass the sciatic notch. These landmarks are then used in a Euclidean distance matrix analysis to localize the form differences between the sexes. The comparison of distances shows there are no statistically significant differences between the sexes localized around the greater sciatic notch.

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CHAPTER 1

INTRODUCTION

A problem in human osteology today is the sexing of subadult remains, especially fetal remains. Various differences between human adult remains have been studied over the years, such as sciatic notch width, subpubic angle, ventral arc, and characteristics of the ischio-pubic ramus. Some of these differences have been shown to be fairly accurate indicators of sex (Letterman 1941, Washburn 1948, Phenice 1969). Logically, these aspects of the adult pelvis have been studied in the fetal pelvis to see if differences between the sexes can be detected. Some of these aspects, such as arcs and indices, are difficult to study in fetal skeletal remains since the cartilage between the bones is not intact. Roentgenometric studies of fetuses and neonates have tried to compensate for this problem. Studies using measurements of fetal bones and statistical analysis of these measurements have also been undertaken. Most attention, however, has focused on the width of the greater sciatic notch of the ilium, since this is a gross morphological characteristic with known sex differences in the adult and is easily seen in fetal skeletal remains. The purpose of this thesis is to conduct a morphometric study of fetal ilia of known sex to see if differences between the sexes can be discerned.

This study is relevant to: 1. forensic anthropology and the identification of fetal/infant remains; 2. archaeology and mortuary treatment

of remains in resolving possible sex-biased mortality rates; and 3. a better understanding of the influences of growth and development on sexual dimorphism.

The methodology used here differs from previous studies, such as Boucher (1955, 1957) and Fazekas and Kosa (1978), by offering a more objective way of identifying landmarks on fetal ilia and by controlling for age so that the differences noted can be attributed to sex and not age (Weaver 1980; Hunt 1983, 1990). Additionally, this study analyzes the form of the ilia itself and attempts to identify landmarks based on shape, since gross morphological landmarks are difficult to identify on fetal ilia.

LITERATURE REVIEW

Over the years, various researchers have made attempts to distinguish sex characteristics in fetal ilia. Studies on the sex differences between fetal ilia began as early as 1876 when Fehling noted that male and female pelves could be distinguished as early as the fourth fetal month (cited in Fazekas and Kosa 1978). In 1899, Thomson also stated that sex differences could be seen as early as the fourth fetal month after studying a small sample of apparently only 8 pelves (cited in Reynolds 1945).

Pryor (1923) studied the differences in the time of development of centers of ossification in male and female children. After looking at 554 x-rays of hands of children from 3 months to 14 years of age, Pryor concluded that the bones of the female ossify before the male. He came to the same conclusion for fetuses and newborns after studying 140 x-rays of fetuses (71

male; 69 female) from 10 1/2 weeks to 38 weeks of age and 100 newborns (48 male, 52 female) from a few hours to 10 days old. Pryor notes that the ilium shows ossification in the female in advance of the male. Pryor concluded that "after birth the differences are progressive up to the union of epiphyses with the shaft of the long bones which takes place with the female from 3-4 years in advance of the male" (1923:268).

In 1941, Letterman conducted a comparative study of the greater sciatic notch in adult American whites and blacks. He measured 426 hip bones of cadavers of known age and sex from the Washington University anatomical collection. Letterman noted that the form of the greater sciatic notch is dependent upon the relationship of the hip bone and sacrum. He defined the greatest width of the sciatic notch as the distance between the point on the posterior inferior iliac spine where the lateral margin of the sacrum intersects and the posterior margin of the iliac spine. Sex differences that Letterman noted as statistically significant include: 1. the mean greatest width of the sciatic notch is larger in females than males; 2. the mean greatest height is larger in males than females; and 3. the point where the lines of width and height intersect is shorter in males than females. This illustrates that the greater sciatic notch is deep and narrow in males but wide and shallow in females. Letterman cautions that there is a degree of overlap in the absolute measurement of the width of the sciatic notch of whites. In the races combined, males showed a greater variability than females.

Morton and Hayden (1941) conducted a comparative study of male and female pelves in children. They felt that the factors responsible for variation in the shape of the pelvis were not yet known. Additionally, pelvis shape may be influenced by disease, nutrition, general development, and sex hormones.

Morton and Hayden also noted that sex hormones may be responsible for differences in male and female pelves. They studied x-rays of 59 girls and 16 boys aged 4-15 years old in three views: superior straight, lateral aspect, and subpubic angle. Measurements taken on the x-rays show possible differences are present between males and females before puberty. They concluded that most of the changes in pelvis shape to the adult form take place at puberty and are therefore due to sexual factors, although there may be inherent differences between the sexes before puberty. They noted that there may be other factors that influence pelvis shape which are variable and unidentifiable. Morton and Hayden concluded that the pelves of prepubertal children were indistinguishable with respect to sex, except for possible differences in the position of the greatest transverse diameter of the pelvic inlet. Additionally, they suggested that males and females start out with pelves identical in type and that differences observed between adult male and female pelves do not appear until puberty and are therefore the result of influences of sex hormones.

In a follow-up study, Morton (1942) studied the shape of the female pelvic inlet and the development of sex differences in the bony pelvis. He looked at 27 fetal pelves (10 females, 17 males) from 3 1/2 to 10 months and 143 x-rays (98 females, 45 males) from 3 to 18 years old. In all of the fetal pelves, the pelvic inlet is similar. Additionally, except for a general increase in size, there are no marked changes in the shape of fetal pelves between males and females. Until puberty, Morton noted little differences between males and females. After 11 years of age, girls showed pelvic inlets more broad than long. Morton again concluded that the differences between subadults and adults suggests an important role of sex hormones at puberty.

Reynolds (1945) conducted a study of the bony pelvis in early infancy. As part of the Fels Research Institute Study, serial x-rays were taken of 95 white babies born in southwest central Ohio. The x-rays were taken at birth, 1, 3, 6, 9, and 12 month of age. Several measurements and indices were made, and on all paired measurements the left side was used. Reynolds noted that at birth, ossification is seen in the superior pubic ramus, inferior ramus of the ischium, and much of the ilium. Reynolds concluded, as others had, that the female begins ossification earlier than the male. He also concluded that the pelvis grows fastest between birth and 3 months, so if there are sex differences in the newborn bony pelvis, they are lost during this period of early rapid growth. Reynolds found that two measurements, sagittal inlet breadth and breadth of the sciatic notch, showed differences between males and females. Additionally, boys tended to be larger in measurements which represented the outer structure of the pelvis, while girls tended to be larger in measurements which represented the inner structure of the pelvis, including a relatively larger pelvic inlet.

After noticing differences in the sciatic notch of five fetal skeletons being prepared for forensic study, Boucher (1955) decided to conduct a blind study of sex differences in the sciatic notch of fetal ilia. She collected ilia from 20 stillborn infants and fetuses of known sex aged 6 months to term. Boucher laid the ilia on the dorsal surface (so the ventral side was facing up) and looked with one eye for the point of contraflexure. She defined contraflexure as the "point. . . at which the curvature of the notch changes to that of the contiguous bone edge, and can be seen by eye" (1955:53). Measurements taken included the width between these points, depth of the sciatic notch, and width/depth used as an index for comparison. Boucher concluded that

differences between male and female indices were significant, with the index being larger in females than males. She suggested, therefore, that sex can be determined from the fetal ilium.

In a follow-up study, Boucher (1957) used the same method on American black and white fetuses as she did on British whites (Boucher 1955). The sample size consisted of stillbirths of 96 American blacks and 33 American whites. The depth, width, and sciatic notch index were obtained in the same fashion as in her previous study (Boucher 1955). Boucher found that the sciatic notch index was significantly larger in females than males for British whites and American blacks, but not for American whites. She was unable to explain why there were no significant differences between the sexes in sciatic notch index of American whites.

In 1970, Choi and Trotter conducted a factor and discriminant analysis for 21 measurements of 115 fetal skeletons (28 white males, 21 white females, 31 black males, 25 black females) aged 16-44 weeks from cadavers assigned to Washington University. They argued that since most prior studies had been univariate analyses, the multivariate relationships of weight, length, and allometric growth patterns had been overlooked. The measurements they used included average lengths and weights of four paired bones (femur, tibia, humerus, and radius), average weights of seven other paired or groups of bones (ribs, scapula, ulna, hand, hip, fibula, and foot), and weights of the axial bones (skull, sternum, vertebrae, and sacrum). Age was used as a co-variate. Choi and Trotter postulated that pattern of growth is governed by a few common factors, plus a factor unique to each bone. Factor analysis was used to describe the multivariate characteristics of the skeleton, but it did not establish any differences between races or sexes. Then discriminant analysis

was applied to see if there were any race or sex differences. Discriminant analysis was performed using several ratios of lower limb measurements with age removed. Differences between races were not significant, but differences between the sexes were. Using this procedure, 72% were correctly classified as to sex. Choi and Trotter concluded that "the percentage of correct classification of the sex of the skeleton is lower than comparable percentages obtained for adult skeletons. . . because. . . sexual characteristics are less evident (or less significant) before the age of puberty" (1970:312).

Fazekas and Kosa (1978) conducted an in-depth study of fetal skeletons. They also noted the ilium ossifies as early as the third lunar month and from this point onward, the iliac crest becomes more convex. Since Fazekas and Kosa were unable to discover if Boucher's (1955, 1957) research had been replicated and results confirmed, they decided to test her methodology on their Hungarian sample. The sample Fazekas and Kosa used consisted of 104 skeletons (61 males; 43 females) from 4-10 lunar months of age. They measured the length and depth of the sciatic notch, as did Boucher, but in a different manner, Instead of using two triangular gauges, Fazekas and Kosa modified a gauge calibrated to millimeters by filing off two corners of the gauge so it fit into the sciatic notch. Additionally, Fazekas and Kosa felt the points of contraflexure defined by Boucher were too subjective, so they used fixed anatomical points that are more readily visible on the dorsal surface of the ilium. They defined the length of the notch as:

> equal to the distance between the border of the passage into the articular surface and the tip of the posterior inferior lilac spine. Thus, the greatest protrusion of the posterior inferior iliac spine should be taken as the upper point of the notch, and the passage in the articular surface as the lower limit [Fazekas and Kosa 1978:374].

In agreement with Boucher's (1955) findings, Fazekas and Kosa also found significant differences between the sexes with the index of sciatic notch length/sciatic notch depth. They concluded that sex differences can only be determined from measurements of the sciatic notch and not the ilium as a whole. Fazekas and Kosa stated that using their measurements and indices, the sex of a fetus could be established with certainty in 44.2-59% of the cases, but this number rises to 70-80% "if the cases not absolutely characteristic of sex are added" (1978:383). They cautioned that in 10-15% of their cases, measurements of the sciatic notch were the same for males and females.

In 1980, Weaver evaluated fetal and infant ilia for seven traits, six metric and one nonmetric. Weaver noted that the onset of major sexual differentiation begins with the appearance of fetal testosterone by the 10th week, with a peak around the 15th week. After this point, testosterone levels decrease and remain relatively low until puberty. Since sexual differentiation in fetal males is a direct result of prenatal testosterone levels, characteristics diagnostic of sex should be visible in the fetus, at least after the 15th week, and remain stable or possibly decrease as growth may mask those characteristics. Using a sample of 153 fetal and infant skeletons of known age, sex, and race from the collections of the Smithsonian Institution, Weaver measured the following traits: sciatic notch width, sciatic notch depth, ilium anterior length, ilium posterior length, iliac height, and iliac width. Three indices were calculated from these measurements. One nonmetric trait, auricular surface elevation, was also analyzed. Weaver defined auricular surface elevation:

> if the sacro-iliac surface was elevated from the ilium along its entire length and along both the anterior and posterior edges of the sacro-iliac surface, the auricular surface was considered elevated and was so scored [1980:192].

Weaver noted that complete elevation as he defined it was not very common in adult females. While none of the metric traits showed any significant sex differences, auricular surface elevation proved to be 91% accurate for fetal males and males six months of age. Weaver concluded that sex determination of fetal and infant skeletal remains will probably be based on criteria similar to that used for adult skeletons.

Hunt (1983) studied age changes in shape and morphology of subadult ilia. As part of this study and one other (Hunt 1990), he indirectly tested Weaver's auricular surface elevation as an indicator of sex. Hunt used a sample of Arikara subadult ilia of unknown age and sex to statistically analyze growth and development changes in bone as well as investigating possible sex differences. Eight measurements, maximum iliac length, maximum iliac height, pre-auricular portion, post-auricular portion, auricular height, sciatic notch width, sciatic notch depth, iliac crest curvature, and two nonmetric traits, auricular surface elevation and pre-auricular spine, were analyzed. For the nonmetric traits, there was a positive relationship between elevated auricular surface and absence of a pre-auricular spine as well as non-elevated auricular surface and presence of a pre-auricular spine. Hunt noted this was an age-related feature because as femur length increases, an elevated auricular surface is noted with the absence of a pre-auricular spine, then a leveling of the elevation occurs, and then a non-elevated auricular surface with the presence of a pre-auricular spine is noted. In 1990, Hunt noted that the distribution of elevated versus non-elevated auricular surface

is so unbalanced that it cannot reflect sexual variation. Hunt concluded that the morphology of the auricular surface in fetal ilia is related to growth, not sex.

In his study of sex determination of fetal and neonate skeletons, Shutkowski noted that there is probably not a "lack of sex-differentiated structures, but a deficiency of criteria with effective diagnostic values" (1987:347). Shutkowski used the raw data of hip and thigh bone measurements from Fazekas and Kosa (1978). Out of the seven indices Shutkowski used, five were of value in discriminating sex. In all of these indices the sciatic notch contributes at least one dimension, indicating the value of this structure. Classification according to sex was certain 63.2-71.1% of the time. Shutkowski noted the sex of more than 60% of the ilia used could be discerned with one indicator only: sciatic notch depth/width. Of all the functions, the one with the best discriminatory capacity was sciatic notch width/femur length with approximately 70% accuracy for both sexes. For all the other functions, certainty of classification varied, with females usually better classified than males.

In summary, a review of studies of fetal ilia since the late 1800s show various results. Some researchers stated that sex differences were visible by the fourth fetal month, while others felt any sex differences in the hip bones resulted from the influence of sex hormones at puberty. It was noted by Weaver (1980) that there are hormones active during fetal development which also influence sex. It is worthwhile to understand the activity of hormones that influence sex fetally because if hormones influence pelvic girdle shape at puberty, they may also have effects during fetal development.

CHAPTER 2

HORMONAL INFLUENCES ON FETAL SEXUAL DIFFERENTIATION

Researchers argue that hormonal influence is important in sexual dimorphism at adolescence; however, hormones also have important influences at other times, even embryologically. Some research indicates there is sexual dimorphism in embryos as early as the 16th day. Hunt (1966) notes that at this time, Barr bodies first become visible in diploid female cells. After this, Barr bodies are present at least part of the time in proliferating female cells and permanently in postmitotic ones such as neurons. As for males, Hunt suggests the possibility that the Y chromosome is most active in embryonic life and at that time initiates a masculine pattern not only in reproductive organs, but in general retardation of development which continues until the end of adolescence in many organs of the body. Until the 7th week, the sexes are indistinguishable except for Barr bodies in female cells. The gonad is bipotential and consists of an outer cortex and an inner medulla before it starts to differentiate towards its specific sex. According to Moore (1977), the Y chromosome has a strong testis-determining effect on the medulla of the bipotential gonad. In its presence, primary sex chords differentiate into seminiferous tubules; in its absence an ovary is formed. Consequently, in the presence of XY, the medulla differentiates in the male and the cortex regresses, while the opposite occurs in the female.

This primary differentiation may depend directly on the sex chromosomes in the gonad itself. Other reproductive organs develop in response to the presence or absence of a hormonal stimulus. Fetal testes produce androgens somewhat like testosterone of the adult. In the presence of this substance, adjacent ducts and external genitalia become masculine. In the absence of this androgen, adjacent ducts and external genitalia become feminine. Human male sexual differentiation is completed by the 16th week; human female sexual differentiation is completed by the 20th week (Hunt 1966).

Hunt (1966) also noted that retardation of development of ossification in males may begin at about the same time that the testis begins to differentiate from a bipotential gonad (approximately 7 weeks). By 20 weeks, the male is some two weeks retarded; by 40 weeks, the male is delayed by 4 weeks relative to the female.

Acheson (1966) notes that there are different components that influence the rate and pattern of skeletal maturation. Two of these are sex associated and hormonal. Sex associated factors include the increased maturation rate of females. This increased rate of development originates fetally and is accentuated around the 9th-10th year. Sex differences in patterns of ossification are clearly seen in the hip joint and pelvis, where relative to the os coxae, the femur matures more rapidly in girls. The time of onset of ossification is systematically more variable in males than females. Those sex differences concerned with the overall rate of maturation probably operate through the endocrine glands, but pattern differences may originate from genetic differences in the skeletal tissue of the two sexes.

The sexes also differ in response of their maturation to the environment. In response to environmentally induced slowing of maturation, the female is more stable than the male in skeletal maturation. If factors contributing to retardation of growth are eliminated, there is a greater increase in growth in males during a compensatory period.

Secretions of the endocrine glands are also important in the rate and pattern of skeletal maturation. Glandular roles have been deduced to some extent from clinical abnormalities and animal experiments. For example, a normally functioning thyroid gland is essential for normal skeletal maturation. There is little evidence that indicates that the growth hormone of the pituitary gland plays any significant role in the control of skeletal maturation.

Sex hormones, however, are of considerable importance. According to Acheson (1966), the epiphyses of the male eunuch are unfused, but growth continues in the growth cartilage plates. In the absence of gonadal secretions, epiphyseal closure remains incomplete or does not occur at all. Epiphyseal closure can, however, be induced therapeutically by steroid hormones appropriate to that sex. It seems that osteogenesis in skeletal cartilage is initiated and maintained by thyroid hormones until puberty. The thyroid hormone cannot complete the process and full skeletal maturity is gained only through the increasing influence of sex hormones secreted by normal glands. There is a close temporal relationship in both sexes between the time of maximum acceleration of skeletal maturation and of growth. This maximum acceleration for both processes occurs earlier in the female than the male. It appears that ". . . gonadal endocrine activity appropriate to (each) sex seems to be important" in rates of maturation (Acheson 1966:495).

Anderson (1966) argues that sexual differentiation of the fetus is not controlled by sex determining genes, but by unknown "inductor" substances. These substances, secreted by the fetal testis, influence development of the genital ducts. Animal experiments have shown that a lack of gonads at a critical time will result in female development. If gonads are removed or destroyed early in fetal life, the genitalia will be feminized. However, administration of androgens may influence the development of masculine genital ducts.

In summary, there are hormones active during fetal life that affect primary sex characteristics. Human sexual differentiation in soft tissue is completed by the 20th week in utero. These soft tissue changes are influenced by fetal hormones. Therefore, it is possible that these hormonal differences are also influencing sexual dimorphism in fetal ilia.

CHAPTER 3

NEW MORPHOMETRIC APPROACHES TO CONTINUOUS FORMS

While landmarks may be relatively simple to define on the adult innominate, the fetal ilium is a smooth continuous form with few obvious landmarks. Consequently, while it may make sense to measure sciatic notch width in an adult, it does not in a fetus. A problem common to most previous studies of fetal ilia is the use of inappropriate morphometric methods. For example, Boucher (1955, 1957) argues for using her method of measuring the sciatic notch to determine sex, but it is subjective and does not work on all populations, such as American whites. Fazekas and Kosa (1978) state they are testing Boucher's method, yet they use the opposite side of the ilia, different landmarks, and a different measuring tool. In Weaver's 1980 study, a possible nonmetric trait for sexing fetal ilia was observed, but according to Hunt (1983, 1990), it is an age and not sex-related trait. Additionally, indices, used by Shutkowski (1987), Boucher (1955, 1957), and Fazekas and Kosa (1978), are linear measurements which are largely size dependent while the shape between the points is ignored. While an index can be a valuable indicator of proportions, it does not necessarily measure shape (Lestrel 1974). In an attempt to avoid these problems, this study uses newer morphometric methods, elliptic Fourier analysis and medial axis transformation, that are appropriate for smooth forms.

Fourier methods "are of interest when there are few (if any) homologous landmarks on a structure or when the outline shape itself is of interest rather than its relationship to various landmarks" (Rohlf 1990:167). Fourier analysis fits a curve to a form that allows separation of the total form into orthogonal components of size and shape (Lestrel 1974:140). Lestrel (1974) notes that any complex two-dimensional shape can be broken down into simpler components, which, when added, re-describe the shape. He defines Fourier analysis as a numerical procedure of a summation of an increasing number of terms in a series until a reasonably good fit to the original form is found. A Fourier series contains both sine and cosine terms. Sine denotes asymmetry of the form while cosine denotes symmetry. The sum of the sine and cosine terms in the series are called harmonics. Kuhl and Giardina (1982) illustrated that there are elliptic properties to Fourier coefficients and these properties can be used to normalize a Fourier representation so that it is invariant to size, rotation, and starting point of the contour, but loses no shape information. Fourier analysis using these normalizations is referred to as elliptic Fourier analysis. In a study comparing Fourier methods, Rohlf and Archie (1984) found elliptic Fourier most generally useful. They note that one of the most important factors of elliptic Fourier analysis is that overall shape is being dealt with, not changes in distances between points. Ferson et al. (1985) observe that although elliptic Fourier analysis is sensitive to the complexity in closed curves, it can discern subtle differences among nearly oval shapes. They also argue that Fourier coefficients can be used as variables in multivariate analyses. Care must be taken in the interpretation of results from such an analysis since

biological interpretation cannot be attached to the individual Fourier coefficients.

Bookstein et al. (1982) consider landmarks to be more biologically valuable than an overall shape analysis. To obtain landmark information based on shape, Bookstein (1979) developed an algorithm to find the line skeleton, or symmetric axis, of a shape. This algorithm is based on symmetric axis work by Blum (1973) and Blum and Nagel (1978). In describing a shape using the symmetric axis, a collection of discs are fit inside the outline, tangent to at least two points on the outline, and the loci of the centers of the discs are located. The set of these centers constitute the symmetric axis or medial axis. Straney (1990) describes the symmetric axis as a continuous and branching curve that lies in the middle of a smooth, continuous, closed form. He notes that the importance of Bookstein's (1979) line skeleton algorithm lies in the branch points it constructs in landmark free forms. Medial axis offers two types of landmark substitutes: end points of the axis and triple points where branches off of the main axis occur (Bookstein 1991). However, to be useful, Straney (1990) notes that homology must be established between points in order for comparisons to be made. He does not mean homology as for biologically real entities, but instead as an operational procedure for making comparisons valid. Oxnard (1973a, 1973b) used a comparison study of modern human, chimpanzee, pygmy, and Australopithecine innominates to illustrate medial axis transformation.

Once a line skeleton is obtained, the landmarks denoted by end points and triple points must be analyzed. Lele (1991) and Lele and Richtsmeier (1991) present an argument and a method for using Euclidean distances to compare landmark data in different shapes. Lele argues that Euclidean

distance matrix analysis is valuable for the comparison of forms because it uses "the only real information one has. . . the relative positions of landmarks, or equivalently the distances between them" (1991:415).

Lacking a consensus method to determine sex of fetal ilia and few collections to develop and/or test methods upon, modern morphometric analysis presents an intriguing potential solution to sexing fetal ilia. This approach could show differences in overall shape and if there are overall shape differences, identify landmarks around the sciatic notch to see if the differences are localized here. Given a large enough sample of fetal ilia, such a morphometric study could be of value. The purpose of this study is to conduct such a morphometric analysis on a large sample of fetal ilia to determine if there are any differences in shape between males and females.

CHAPTER 4

MATERIALS AND METHODS

Photographs of 133 fetal ilia of known age and sex from the Trotter Collection of Washington University were used. The photos are of the ventral side of the left ilium, except for 3 which are right ilia (see Figures 1 and 2). There are 55 females, 73 males, and 5 unknowns between the ages of 16-58 weeks post menstrual, with an average age of 30 weeks.

The photos were digitized using the software SigmaScan by Jandel Corporation with a pen and GTCO Corporation Type 5A translucent digitizing tablet connected to an IBM PC-XT. Straney (1990) recommended digitizing one outline at several different point densities because line skeletons can be sensitive to digitization. After following this suggestion, points on the outline were recorded by the computer on a .25 centimeter grid since this density produced the most stable line skeletons. The scale of the photos was 1:2.6 centimeters. All outlines were scaled to actual size (1:1). The x,y coordinates from the digitized plots were then loaded into an elliptic Fourier analysis program developed by F. James Rohlf and Scott Ferson (1990 Michigan Morphometrics Workshop) to conduct an overall shape analysis. Elliptic Fourier analysis distorts an ellipse until a good fit to the original curve is obtained (see Figure 3). The outlines were normalized for rotation, starting point, and size (Figures 4 and 5 are outlines of Figures 1 and 2). Normalizing for rotation places the principal axis of the first ellipse

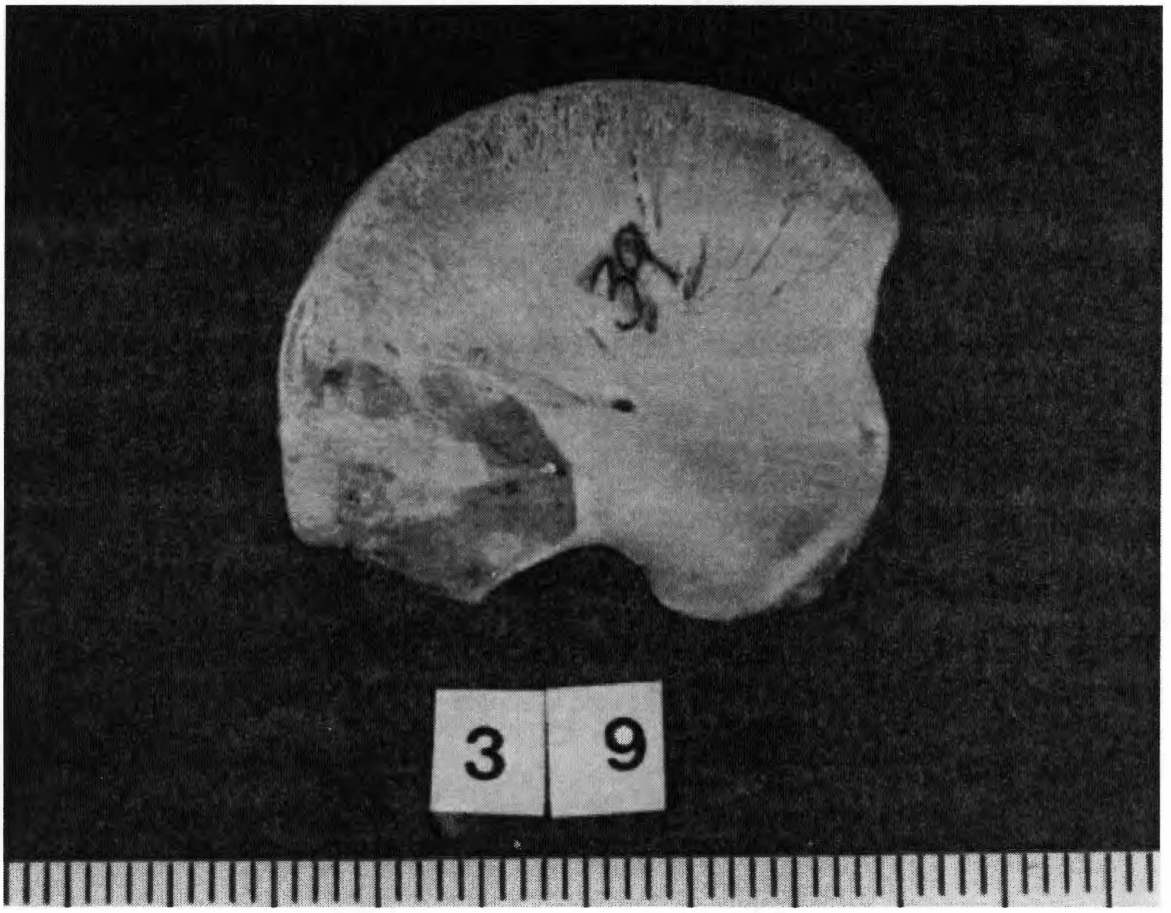

Figure 1. Photograph of left ilium of 26 week old white male.

Figure 2. Photograph of left ilium of 26 week old black female.

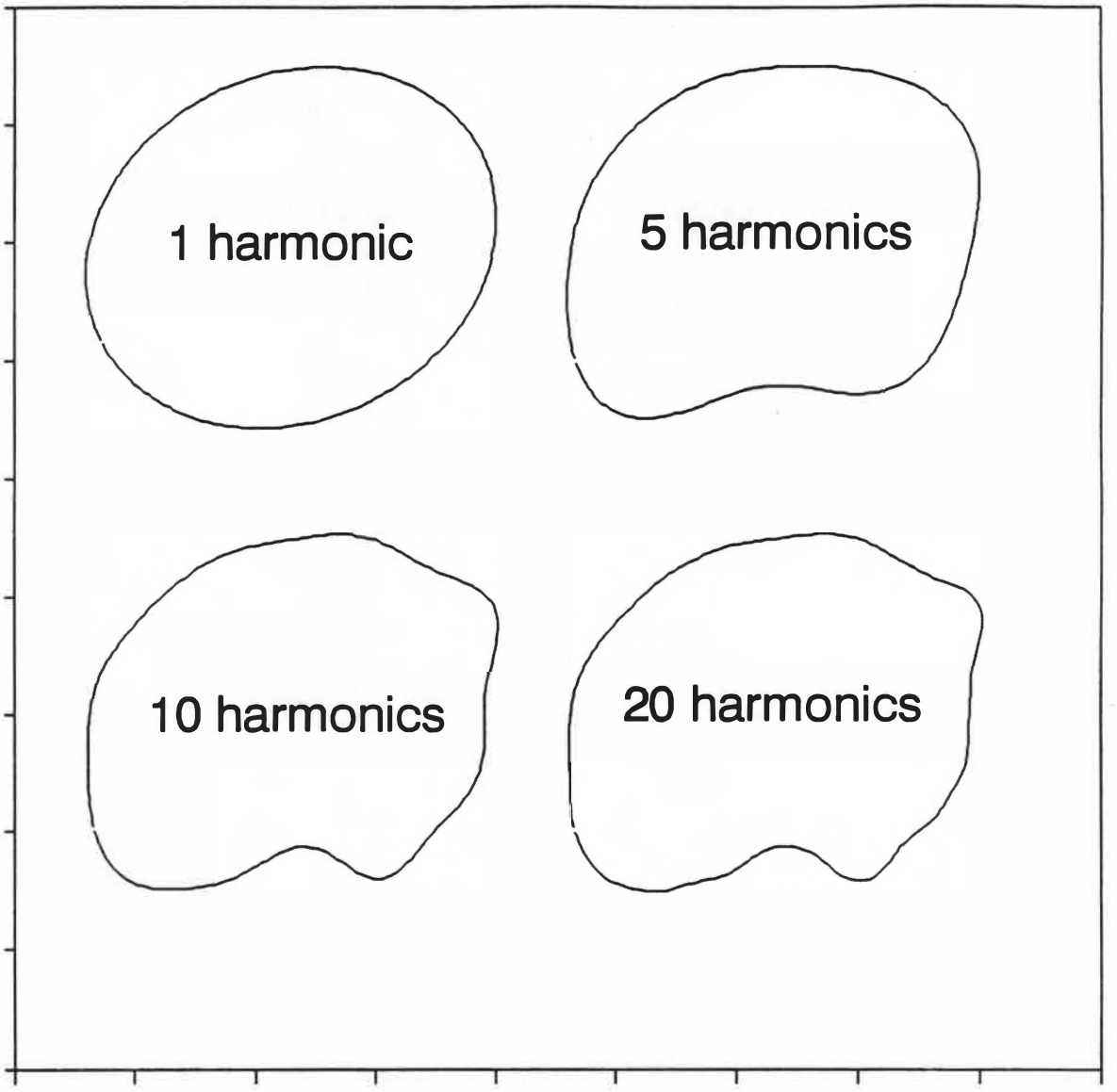

Figure 3. Ellipses at different harmonics. Each tick mark = 1 cm.

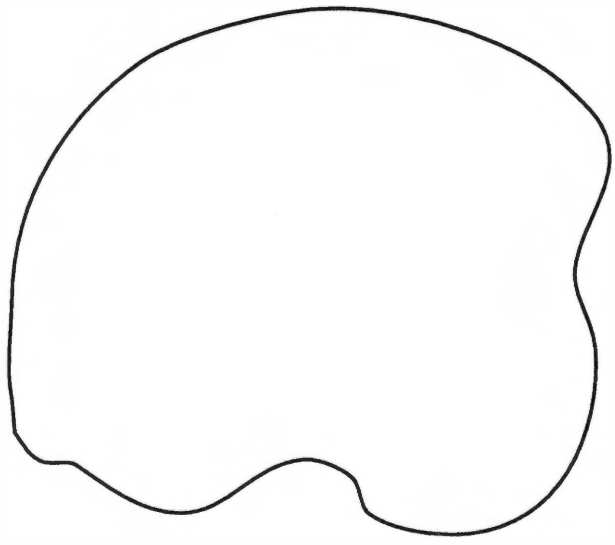

Figure 4. Outline of # 39.

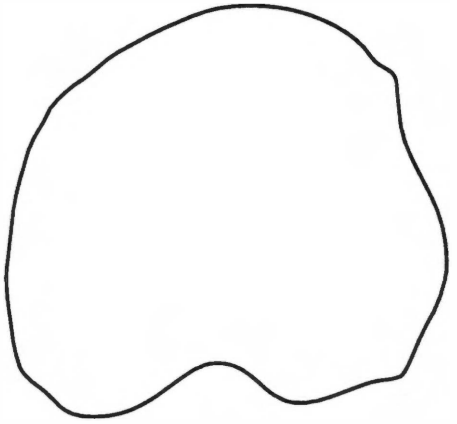

Figure 5. Outline of # 15.

horizontally. The starting point of an outline is irrelevant if it is normalized. Size normalization makes the length of all outlines equal to 2(pi). Seventeen harmonics were used for all outlines. Some outlines had a better fit at twenty harmonics, but others had a best fit at seventeen. To maintain consistency, all outlines were analyzed at seventeen harmonics. Four coefficients are recorded for each harmonic. Normalizations cause degeneration of the first three coefficients, so for 17 harmonics there are 4(17)-3 coefficients. The first eight Fourier coefficients were chosen arbitrarily for use in statistical analyses.

A multiple analysis of variance (MANOVA), or discriminant function, was run on 128 ilia (5 were discarded because sex was unknown) using the first 8 Fourier coefficients, ignoring size influences. In the statistical analyses, size is a proxy for developmental age. Also, a multiple analysis of covariance (MANCOVA) was run, controlling for size. This was done in order to ensure that it was shape differences between males and females and not size differences between younger (smaller) and older (larger) individuals that were being analyzed.

The Fourier outlines were loaded into a line skeleton program developed by Donald O. Straney and Robert D. Kriegel called LineSkel (1990 Michigan Morphometrics Workshop). The Fourier outlines were used in the line skeleton analysis since they were smoother and easier to run with this program. Straney noted that the line skeleton is "sensitive to minor digitizing errors, producing branches caused by dimples or pimples in the outline shape" (1990:192). Smoothing the outline can prevent such occurrences. The line skeleton of each ilium was calculated (see Figures 6 and 7). Line skeletons were similar between ilia. Landmarks forming a triangle around

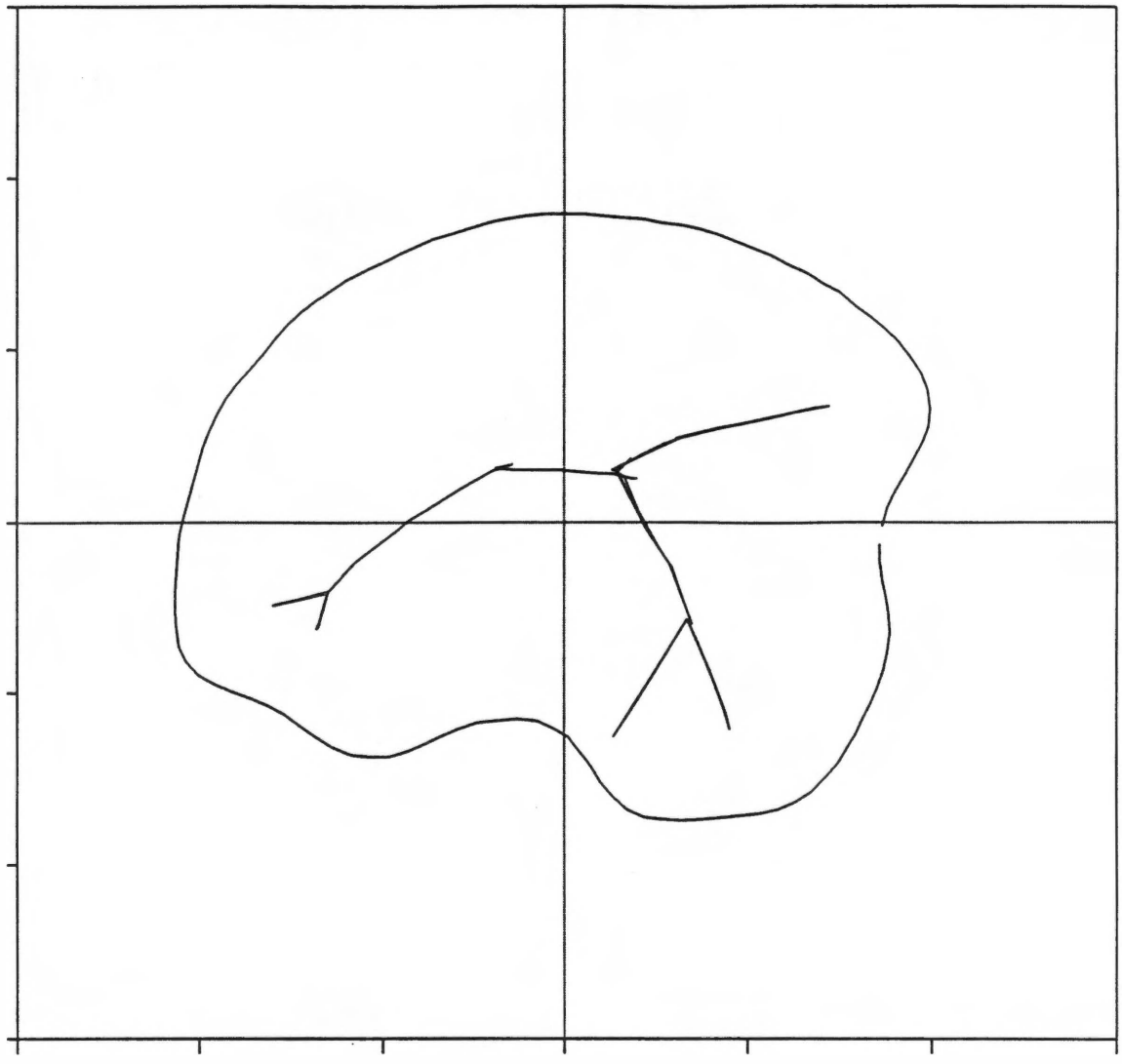

Figure 6. Line skeleton of #39. Each tick mark = .5cm.

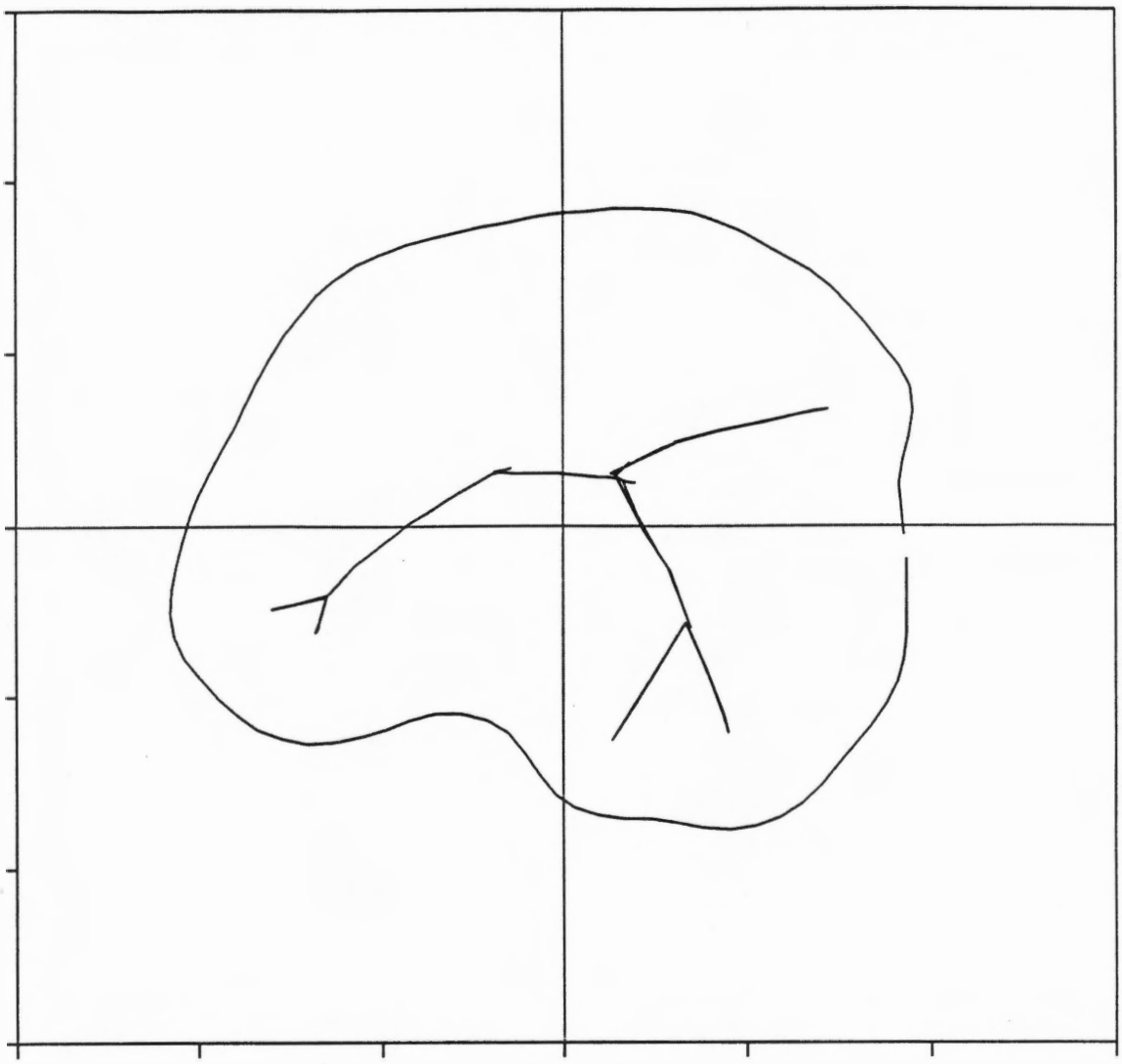

Figure 7. Line skeleton of #15. Each tick mark = .5cm.

the sciatic notch were recorded for analysis in a Euclidean distance program comparing averages of form matrices developed by Lyle W. Konigsberg in FORTRAN based on Lele and Richtsmeier (1991).

As a test to see how different this matrix is from a matrix of constants, Lele and Richtsmeier (1991) developed the *T* statistic. A form matrix of the average distances between points is obtained for males and females. Ratios of the average male to average female distance are calculated. A ratio of the maximum/minimum is developed from the preceding two steps; this is the *T* statistic. *T* is a useful test statistic because the calculation of null distribution is uncomplicated since it is invariant under scaling yet it is sensitive to changes in shape. Fifty-two ilia were used in this analysis (28 females and 24 males aged 22-43 weeks) because although the line skeletons were fairly similar among the ilia, not all had homologous points around the sciatic notch. Sixty-six were thrown out because the point went to the posterior inferior spine instead of around the notch (see Figure 8). Six were discarded because the line skeleton went to the anterior inferior spine (see Figure 9). Three were rejected because the line skeletons went from the anterior inferior spine to the posterior inferior spine with no branches around the sciatic notch (see Figure 10). Four were not usable line skeletons (see Figure 11), and 2 were dropped from the distance analysis because sex was unknown. Distances between points of the triangles were also compared and an angle of the triangle opposite the sciatic notch was calculated (see Figure 12).

The distance analyses were bootstrapped 2499 times. Bootstrapping is a statistical procedure used if the distribution of a sample is unknown or non-normal. The bootstrap replaces any distributional assumption by using random resampling from the observed data distribution. In the case of Lele

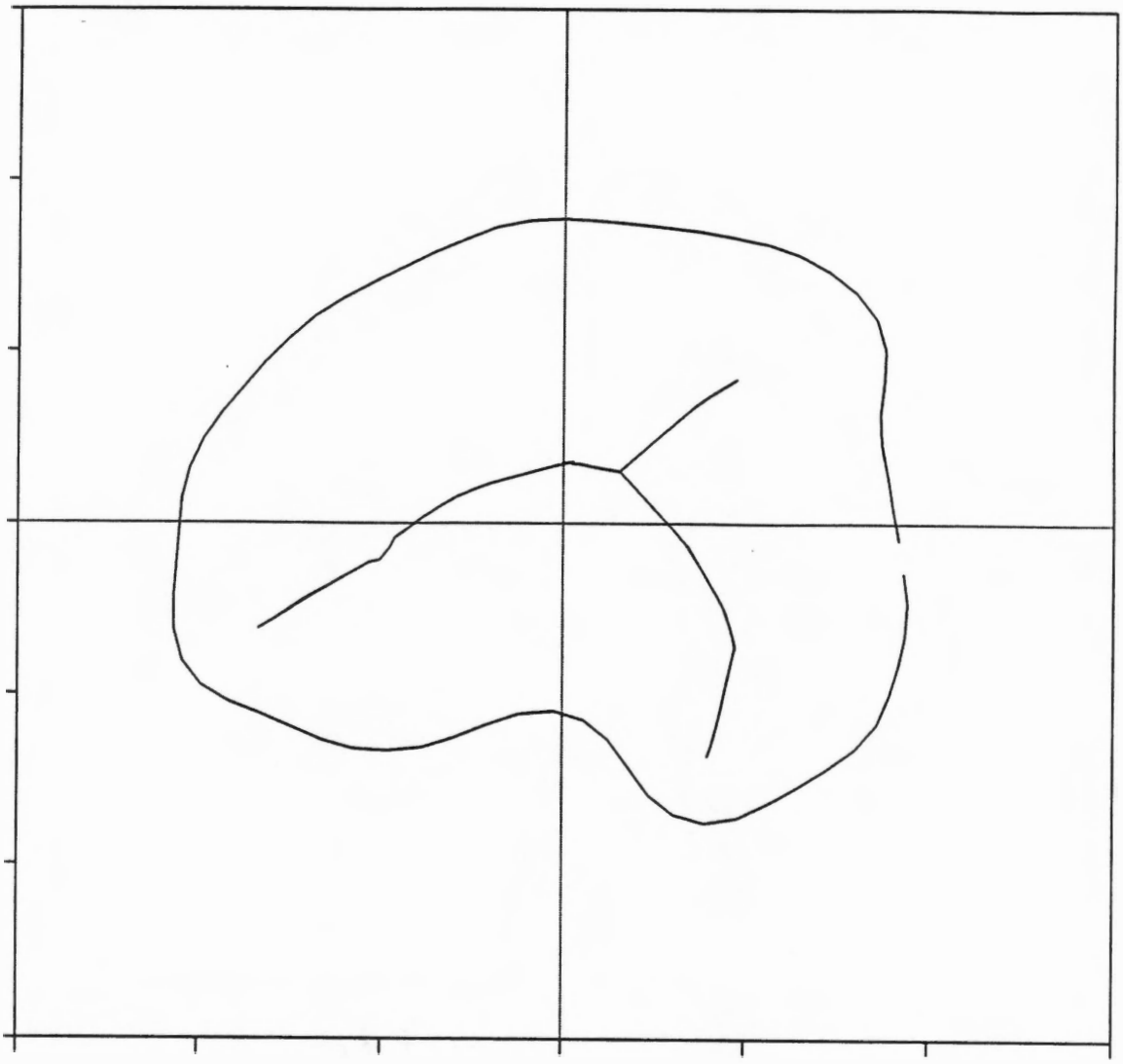

Figure 8. Line skeleton of #5. Each tick mark = .5cm.

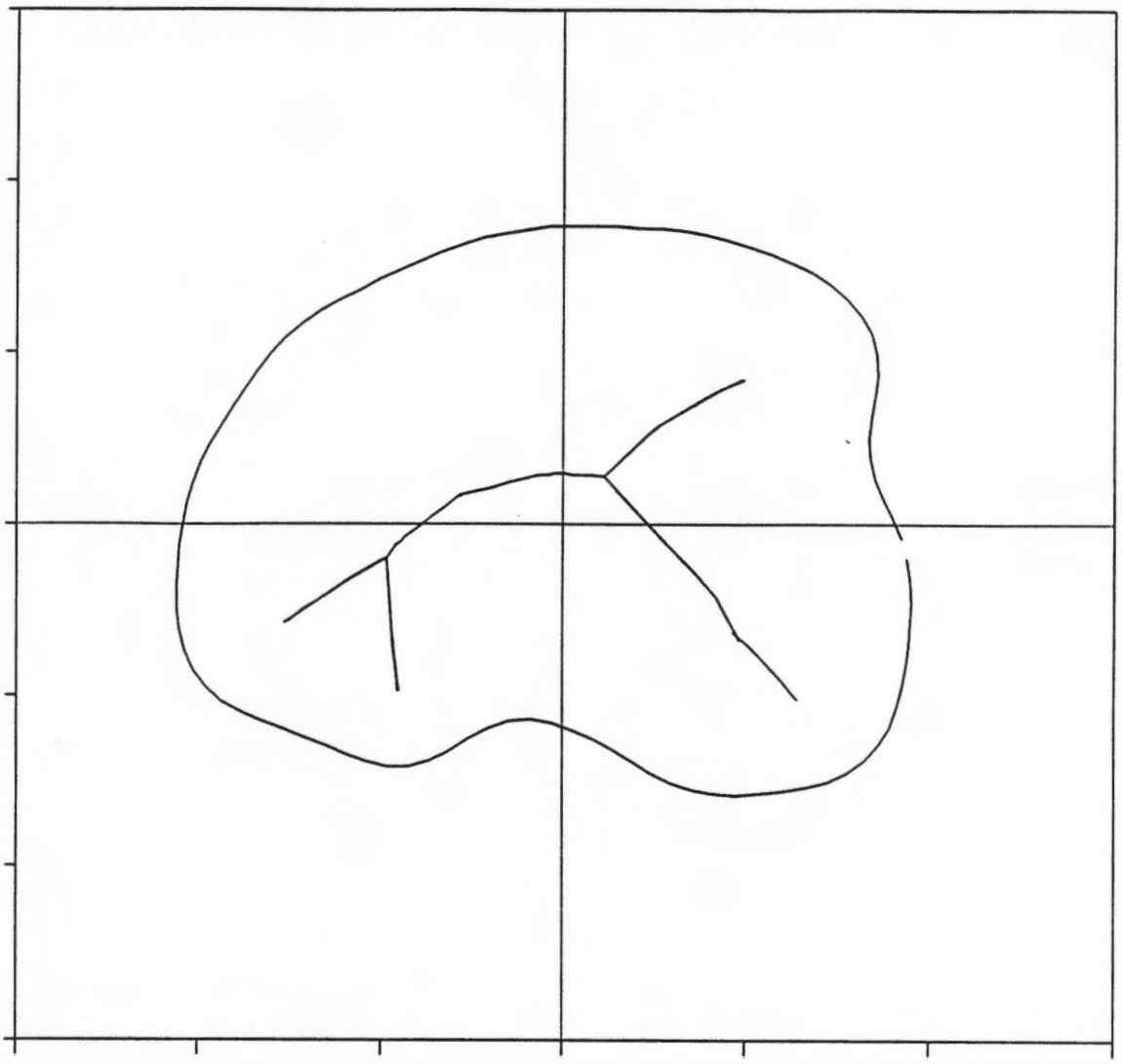

Figure 9. Line skeleton of #31B. Each tick mark = .5cm.

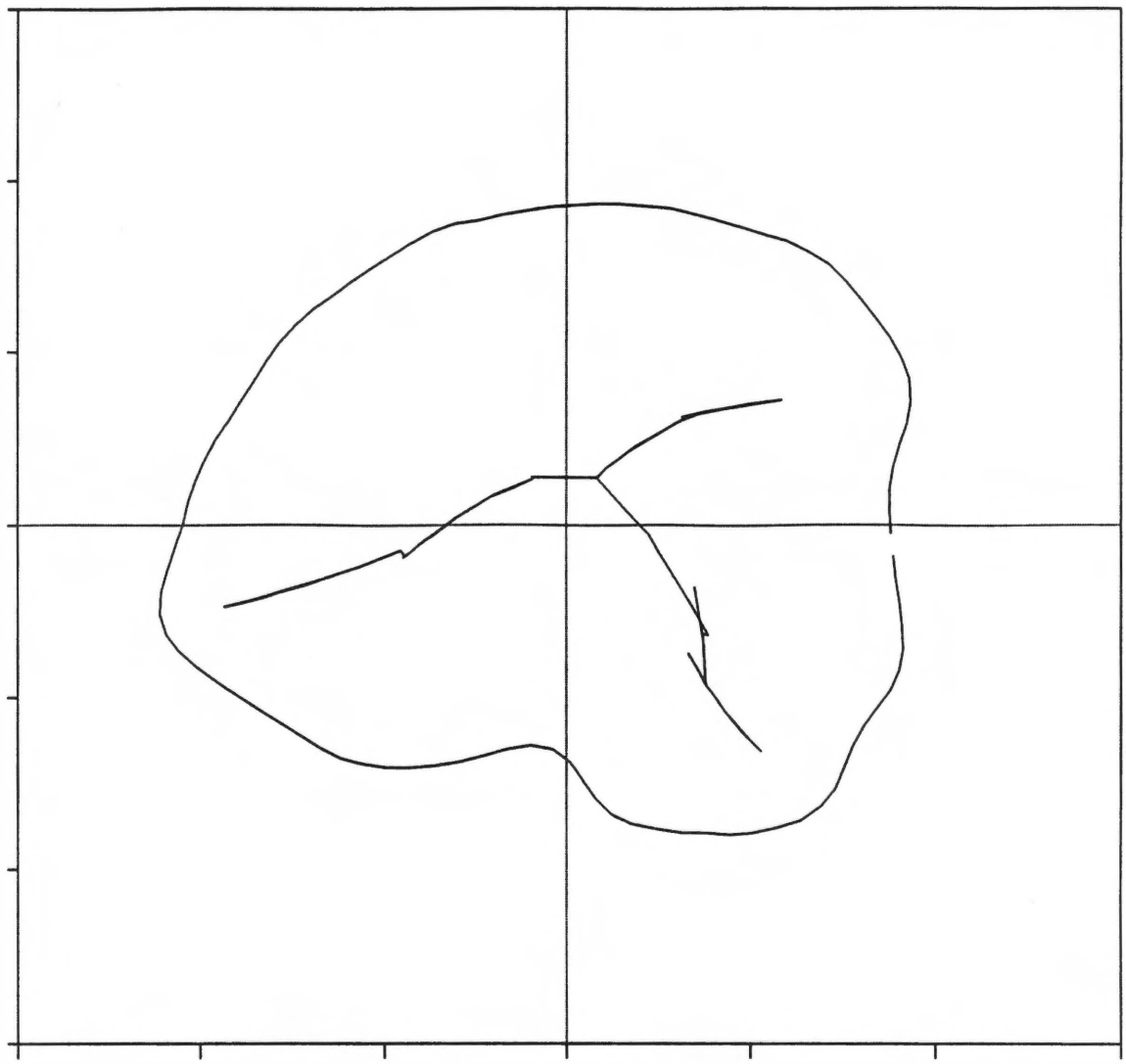

Figure 10. Line skeleton of #27A. Each tick mark = .5cm.

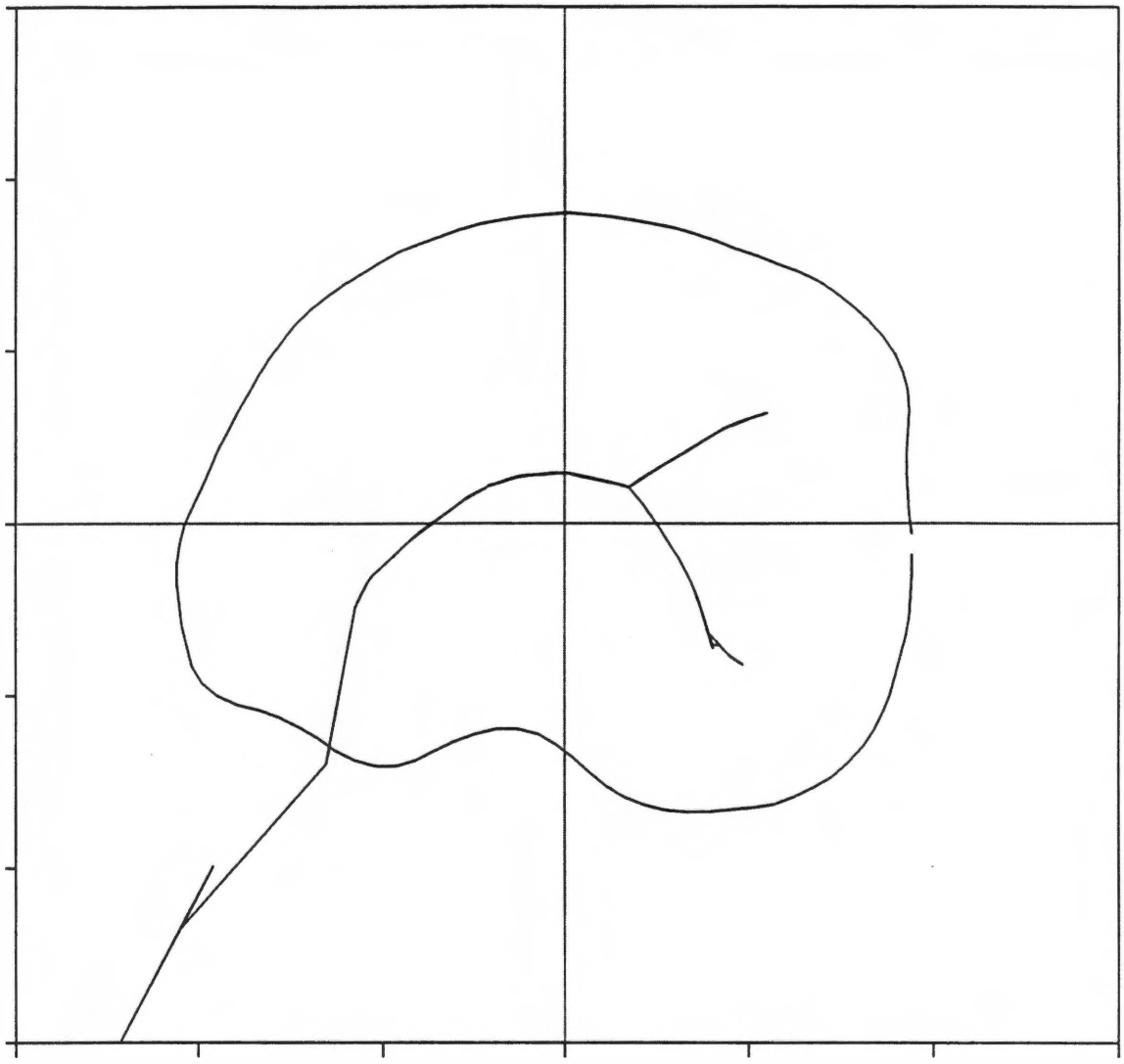

Figure 11. Line skeleton of #30A. Each tick mark = .5cm.

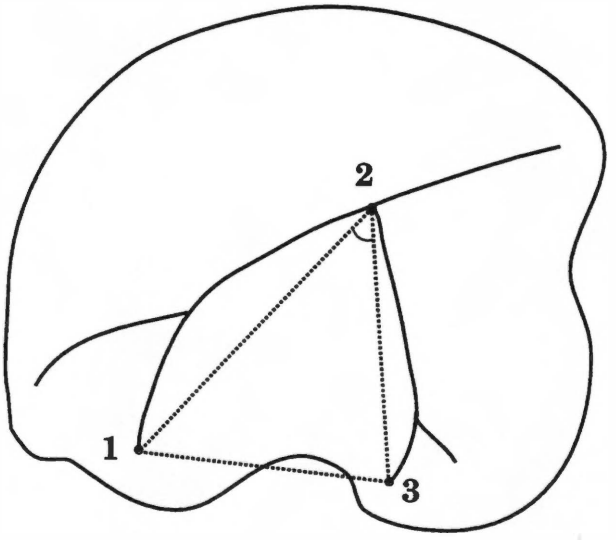

Figure 12. Schematic drawing of triangle and angle measured.

and Richtsmeier's distance statistics, where the distribution is certainly not normal, the bootstrap is an appropriate statistical testing procedure. Bootstrapping estimates the amount of variability of the population from which the current sample is drawn. Samples of size 52 are selected with replacement at random and tested; these are the bootstrapped samples (Diaconis and Efron 1983). Significance was then tested from these results.

CHAPTER 5

RESULTS

The results from the elliptic Fourier analysis are as follows. The mean shapes for males and females are presented in Figure 13. The forms for the smallest and largest males and females are presented in Figure 14, while Figure 15 depicts the overall smallest and largest shapes (see Figure 15). The MANOVA procedure, ignoring size, showed significant differences in overall shape between the sexes with a p-value of .003. Females were correctly classified in 81.8% of the cases, while males were correctly classified in 74.0% of the cases, with an overall correct classification of 77.3% (see Figure 16). The discriminant coefficients and constant are given in Table 5.1. The sectioning point is zero.

Since shape differences between the sexes was noted, the MANCOVA procedure, controlling for size, was performed to verify these are differences in shape between males and females and not differences between young and old. MANCOVA also showed significant differences between sexes with a p-value of .004. Correct classification for both sexes is no different than the correct classification given for the MANOVA procedure. Therefore, there are overall shape differences in fetal ilia between males and females and size is not a confounding factor. Since these procedures showed significant differences between sexes, a triangulation of points around the sciatic notch was studied to see if the differences were localized here.

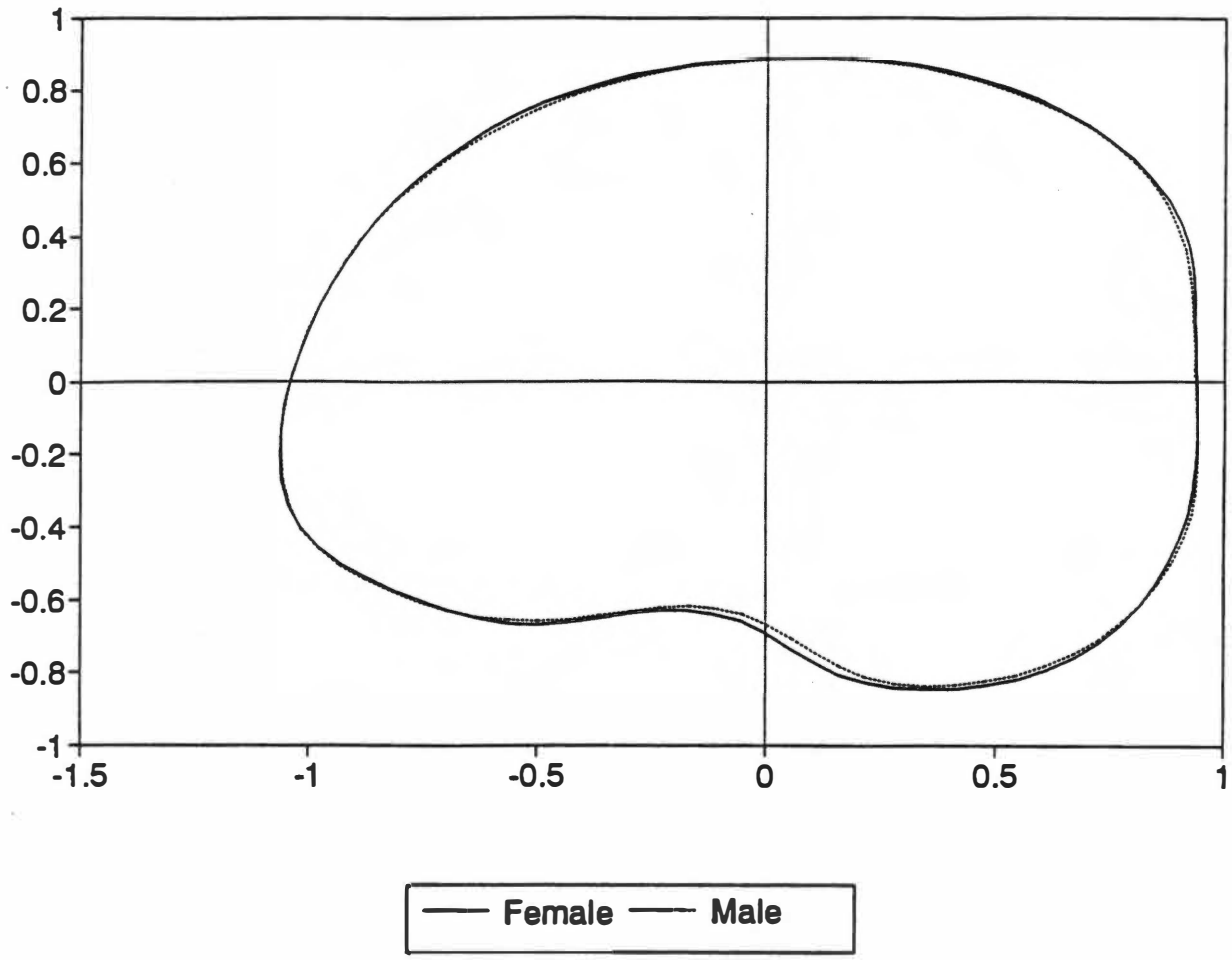

Figure 13. Mean male and female Fourier outlines.

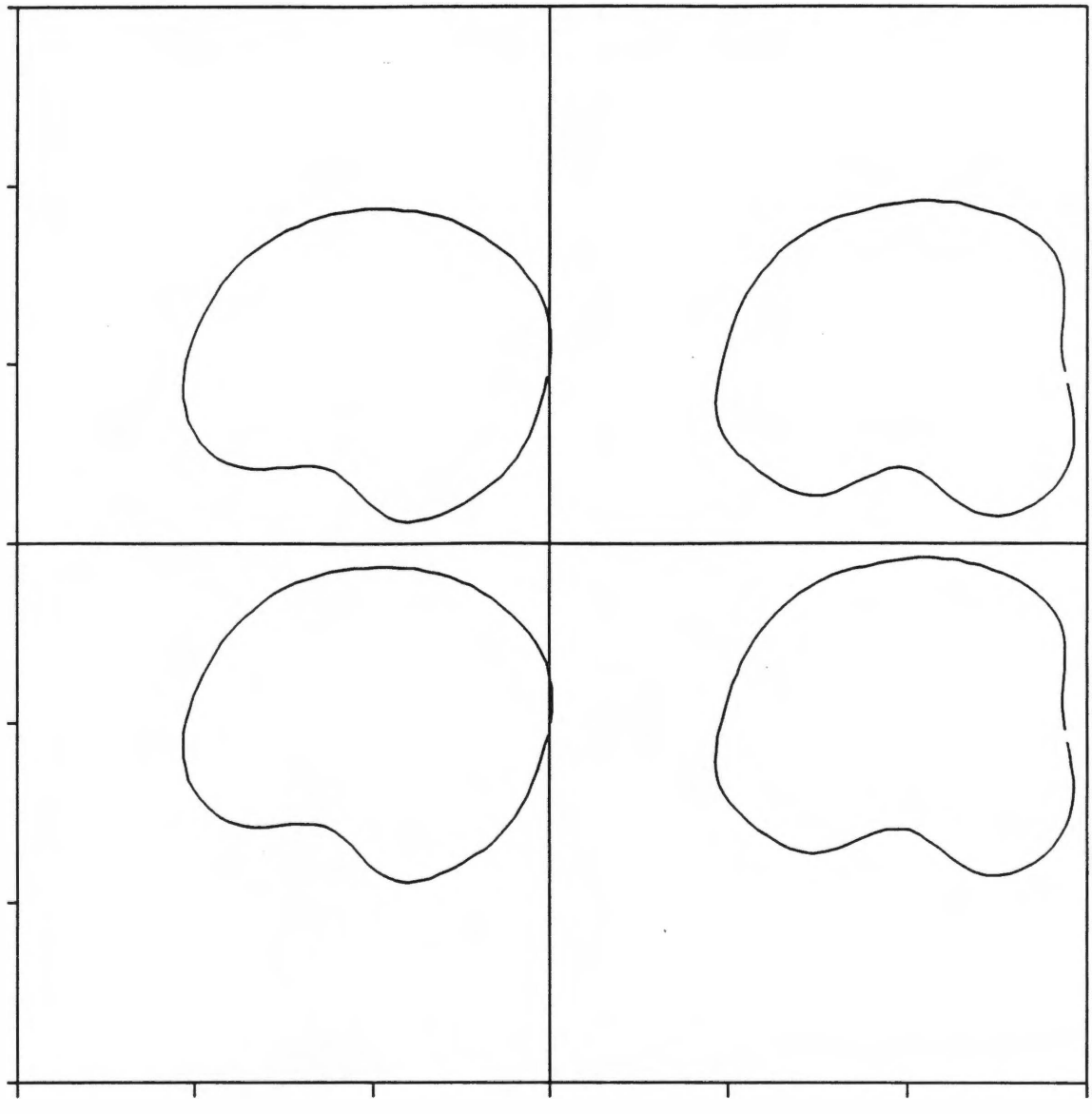

Figure 14. Top row, left to right: smallest male and largest male. Bottom row, left to right: smallest female and largest female. Each tick mark = 1 cm.

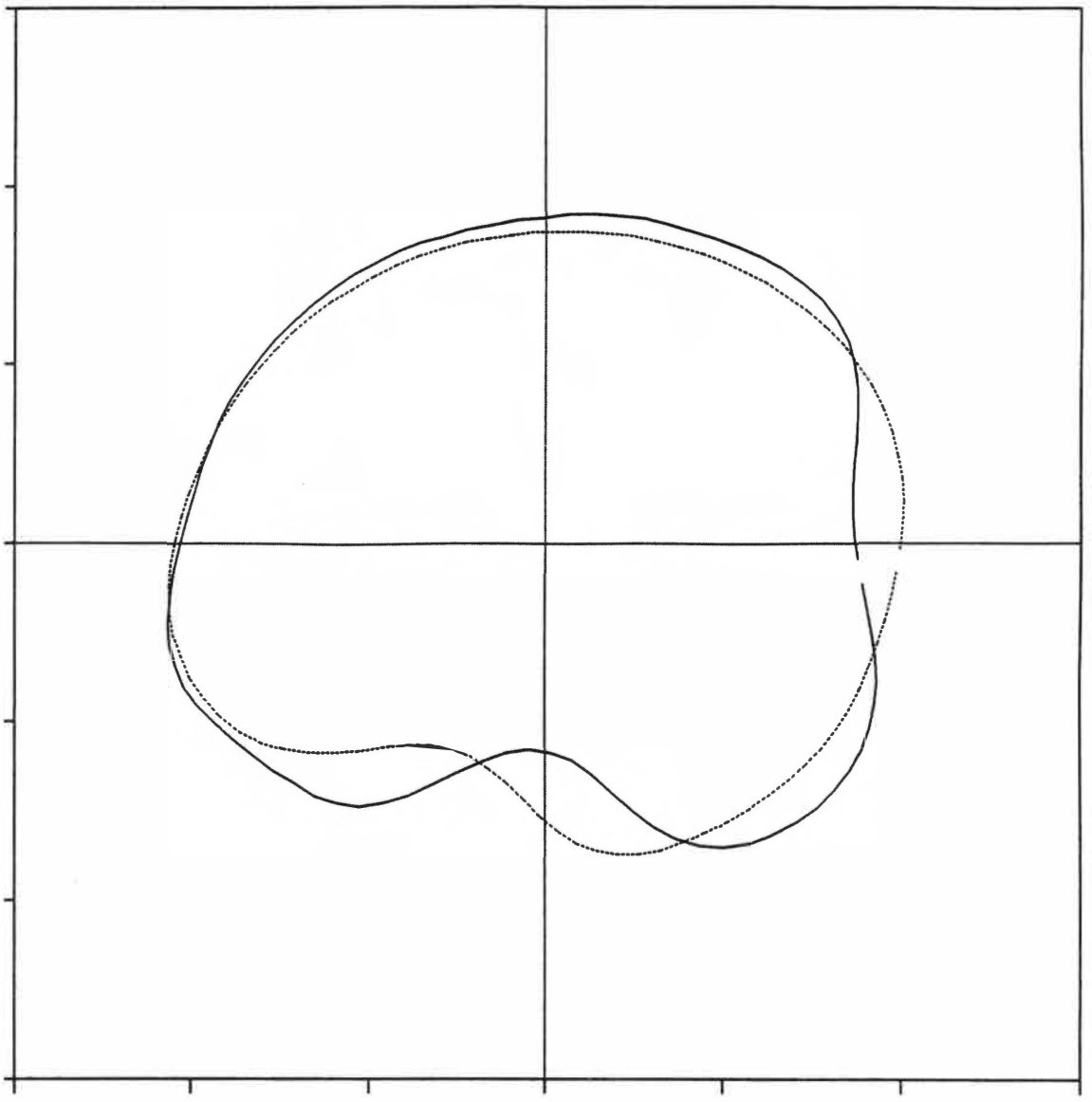

Figure 15. Overall smallest and largest ilia.

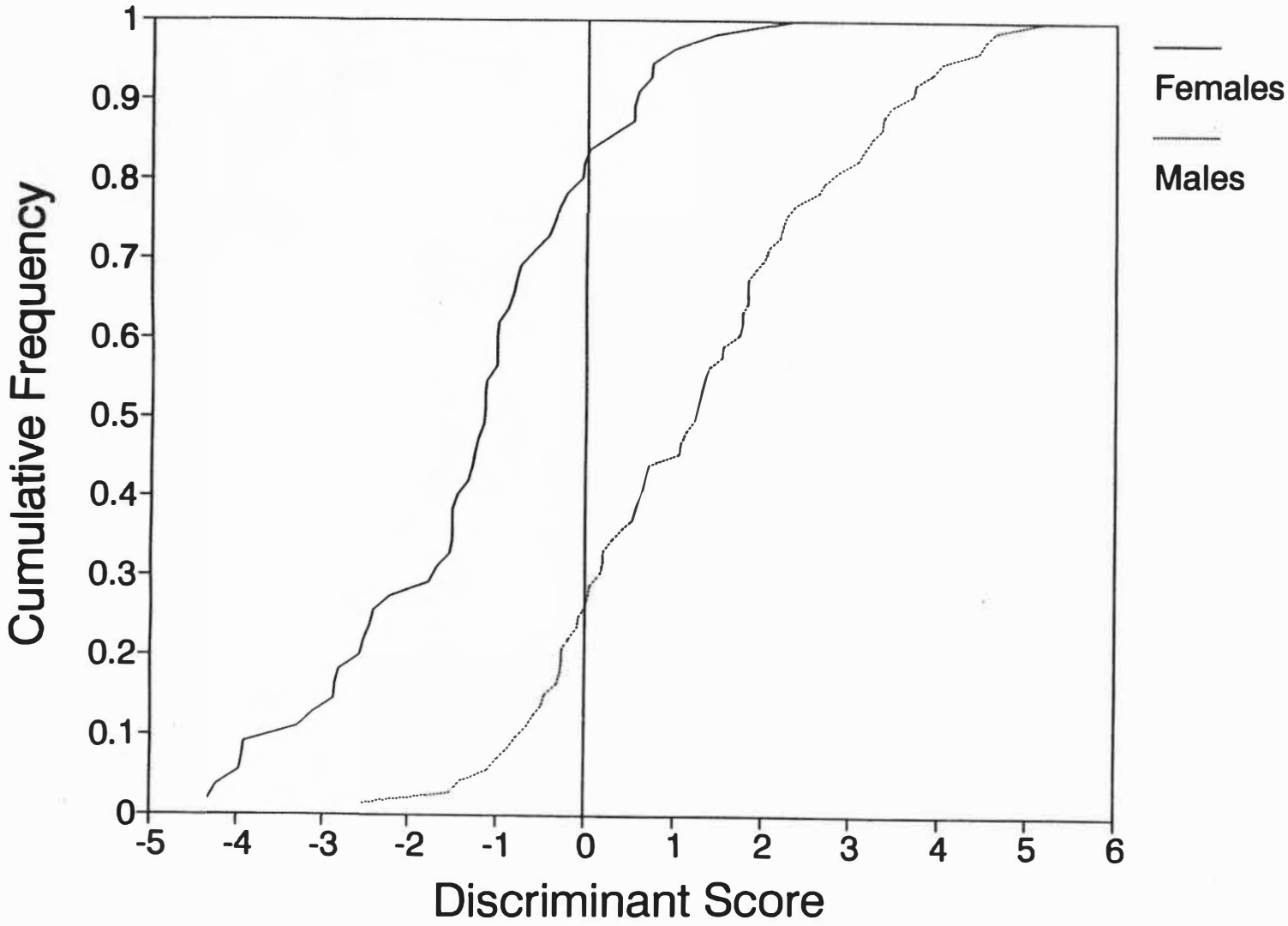

Figure 16. Graph of correct classification.

Table 5.1. Discriminant values from MANOVA procedure.

Fourier Coefficient	Harmonic	Discriminant Coefficient
1	1d	-71.82
2	2a	77.53
3	2b	204.56
4	2c	65.30
5	2d	58.18
6	3a	-424.58
7	3b	17.05
8	3c	-191.84
9	3d	126.96
10	4a	72.70
11	4b	-633.04
12	4c	-95.78
13	4d	-151.89
14	5a	579.00
15	5b	-124.65
16	5c	-162.44
17	5d	339.55
18	6a	-100.16
19	6b	800.16
20	6c	-522.49
21	6d	-228.56
22	7a	-314.06
23	7b	52.10
24	7c	-50.66
25	7d	-439.99
26	8a	-257.36
27	8b	-379.39
28	8c	317.56
29	8d	-68.06
Constant		-66.44

The comparison of distances between average triangles using the *T* statistic showed no statistical significance between the sexes (p=.32). The expected *T* statistic is 1 if there are no shape differences between males and females. The observed *T* statistic was 1.072. Distances between points of the triangle also showed no significant differences between males and females (see Table 5.2).

Table 5.2. Distances between points of triangles.

Points	Average female distance	Average male distance	p-value
1 to 2	.859cm	.834cm	.496
1 to 3	.842cm	.877cm	.484
2 to 3	.809cm	.834cm	.497

In the calculation of the angle opposite the sciatic notch, the average angle for females was smaller, 60.5 degrees, than the average angle for males, 63.4 degrees. The 95% confidence intervals in angle for females is 55-65 degrees and 60-66 degrees for males.

CHAPTER 6

DISCUSSION AND CONCLUSION

DISCUSSION

Results from the elliptic Fourier analysis seem promising in that they are analyzing overall shape differences between males and females and that these differences are significant. Correct classification (77% overall) is slightly better in this study than in Choi and Trotter (1970), Fazekas and Kosa (1978), and Shutkowski (1987) who obtained correct classification of 72%, 44-60%, and 70% respectively. (It must be noted that Shutkowski's data was "jackknifed" in order to obtain an unbiased classification). Ferson et al. argue that "it is possible and valuable to quantify shape variation sensu stricto, independently of homological information" using Fourier analysis (1985:67). They agree with Bookstein et al. (1982) that individual Fourier coefficients are not biologically meaningful in and of themselves, but "can yield useful information about the overall shape of an organism" (Ferson et al. 1985:67). It must be cautioned, however, that some (e.g. Bookstein et al. 1982) feel the use of Fourier coefficients in biometrics is inadequate. The criticism of Fourier analysis that Bookstein et al. make "is not that systematic differences in form are ignored. . . *but that they are made uninterpretable*" (emphasis in original) (1985:92). Bookstein et al. argue that

"the homology function by which we describe correspondences between outlines is an independent source of biometric information" (1982:86).

Following this argument, homologous landmarks of fetal ilia identified by the line skeleton program were also analyzed for differences between sexes. Over half of the original sample had to be discarded for this part of the analysis since landmarks around the sciatic notch were homologous for only 52 ilia (see Figures 6-11). These ilia ranged in age from 22 to 43 weeks, with an average age of 33 weeks. These ilia are older, on average, than the entire sample, and it may be differences in shape due to age that the line skeleton was detecting around the sciatic notch. However, in some instances it appeared that there should have been a branch segment around the sciatic notch (the outline had an obvious "dip" there), but the end point was instead at the posterior inferior spine (Figure 8). It is possible this occurred because the pull of the shape to the posterior inferior spine may be stronger there for the line skeleton than that around the sciatic notch. It is also possible this is a shortcoming of the line skeleton program. After locating these homologous landmarks as a triangle around the sciatic notch, the average forms of the triangles were compared as were distances between each point of the triangle. Lack of significant differences between males and females in the distance analyses could indicate one of two things, or both: 1. Assuming there are differences between sexes in the shape of fetal ilia (as the Fourier analysis suggests), these differences are not localized around the sciatic notch; 2. The sample size is too small (N=52) for the analysis to be meaningful.

The result of a smaller angle for females than for males is perplexing. Since adult females show wider and shallower sciatic notches (Letterman

1941), and Boucher (1955) noted a similar appearance in fetal ilia, it was expected the measurement of this angle would be larger for females reflecting a wider sciatic notch. Angular measurements may not be valuable in the sense that they are basically a ratio of the sides that make up the angle. The degree of overlap of angles between males and females is not too surprising, considering there was no significant differences in triangles between males and females and the angle is just a ratio of those measurements. It is possible that the results of the angular measurements may be an artifact of the line skeleton. The triple point is in the middle of the shape, far removed from the sciatic notch itself.

CONCLUSION

Analysis of overall shape differences in fetal ilia between males and females by elliptic Fourier analysis indicates significant differences between the sexes. Although it has been argued the results of Fourier methods are not biologically meaningful (Bookstein et al. 1982), Fourier analysis is a valuable morphometric tool for comparing overall shape differences. Taking sample size into account, Euclidean distance matrix analyses of points around the sciatic notch illustrated differences between the sexes could not be localized here.

Gross morphological differences in the greater sciatic notch of adult males and females have led to the study of the possibility of such differences in subadult ilia. Most often it has been argued that no differences will be found since the influence of hormones at puberty influence the shape of adult

ilia. However, there are hormones that are active during fetal development that influence sex and therefore may influence the shape of fetal ilia, as hormones active during puberty may influence adult ilia.

Most studies of sex differences in subadults have focused on the greater sciatic notch, since differences in this region are apparent between adult males and females. Modern morphometric techniques appear to be useful in the study of sex differences of fetal ilia. Such methods are more objective and therefore offer themselves more readily to replication than the methodologies of Boucher (1955, 1957), Fazekas and Kosa (1978), and Weaver (1980). These techniques potentially provide important insights into the analysis of shape and shape differences. However, until more is understood about the influence of sex on growth and development, especially skeletally, the sexing of subadult remains will continue to be problematic.

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VITA

Susan Marie Cera Holcomb was born in Warren, Ohio, on March 9, 1967. She attended elementary schools in Mineral Ridge and Niles, Ohio, and moved to Orlando, Florida, after her freshman year of high school and graduated from Bishop Moore High School in May 1985. She entered the University of Central Florida in the fall of 1985 and received a Bachelor of Arts degree with honors in Anthropology in May of 1989. She then matriculated at the University of Tennessee, Knoxville, in August 1989 and received a Master of Arts degree in Anthropology in December 1992.

While at the University of Tennessee, Susan volunteered her time in the Osteology Laboratory and participated in some contract work with the South Dakota Archeological Resource Center. Susan has been a member of the American Association of Physical Anthropologists since 1990. She is interested in all aspects of physical anthropology, especially human evolution and variation, skeletal biology, and human osteology.

Now that Susan has her masters degree, she's going to Disney World.