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PHENOTYPIC TRAITS OF THE CRANIOFACIAL SKELETON IN LABORATORY RATS

by

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Submitted in partial fulfillment of the requirements

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PHENOTYPIC TRAITS OF THE CRANIOFACIAL SKELETON IN LABORATORY RATS

Abstract

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This study compared cephalometric measurements from dry skulls and radiographs to assess craniofacial phenotypic traits in rats. The study also examined the distribution of dry skull measurements in the F1 offspring obtained from a Sprague-Dawley male and Brown-Norway female cross.

The dry skulls and radiographs (lateral and ventrodorsal) of eight inbred strains of laboratory rats (four male, four female in each strain, total n = 64) were utilized. Twenty measurements from dried skulls were compared to the corresponding measurements made from radiographs. The results suggested that cephalometric radiographs could be used to characterize strain variation in craniofacial morphometric traits in laboratory rats. This non-invasive method could be useful for longitudinal studies of phenotypic traits in rats.

Dry skull measurements of the F1 hybrids were compared with the measurements from the F0 parents, an age-matched group, and same

gender inbred groups. It was found that measurements could be grouped into three types. The first were measurements that showed large strain variation and gender difference. For this type of measurement, the values for the F1 generation were between the values measured for the parents. Most of the length measurements in the craniofacial complex were in this category. The second type of measurements had less strain variation and larger gender differences. The measurement values of the F1 animals were smaller than those of parents but larger than those of age-matched groups. The width and height measurements commonly belonged to this category. The third type of measurement showed little difference between the F1 generation and parental rats. There was little variation for strain difference or sexual dimorphism for this type of measurement. Tooth measurements were included in this group.

These findings suggested various inheritance patterns of the craniofacial morphometric traits in rats. The study of the patterns in the F1 generation provided the fundamental information needed to investigate mechanisms of inheritance and identify candidate genes for future studies of craniofacial morphogenesis.

DEDICATION

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INTRODUCTION

In the field of craniofacial biology, the subject of genetics is a fundamental topic. Considering that all development is under genetic control, an understanding of genetics is essential for scientific studies of craniofacial growth and development. Orthodontists have studied the human face for over one hundred years. Therefore, the face is a relatively well known part of the human skeleton, and patterns and rates of growth are well characterized (Landauer 1962). However, with our current understanding of genetics the applications to clinical orthodontics are limited.

Despite the fact that those genes composing the child's genome come from his or her parents, we do not have a clear picture of the timing or pattern of future growth of that child. In planning treatment, there is no doubt that orthodontists need a method to predict the long-term as well as short-term growth direction. A better understanding of the genetic basis of facial morphology may be the key to facial growth prediction.

Orthodontists change the morphology of the craniofacial complex by mechanically altering the environment. This raises several important questions. Are there genetic factors that modulate a patient's response to orthodontic or orthopedic forces? Or as mentioned by Arya et al. (1973), is a particular area of the craniofacial complex more heritable than the complex as

a whole? Is one area more heritable than another area? Individual variation in treatment response is a troublesome area in a clinical orthodontics. An improved understanding of the biologic basis for variations in response to similar therapies may prove to be the single most important factor in improving patient care.

LITERATURE REVIEW

I. Why Does Genetics Matter in Orthodontics?

I.1. Orthodontics – Working with Facial Growth

Diagnosis and treatment planning in orthodontics often involves evaluating general physical development of the patient in relation to the amount of attained growth and the amount of potential growth yet to come. Most experienced orthodontists agree that "good" growth leads to "good" treatment and "poor" growth results in "poor" treatment. However, prediction of future growth with and without orthodontic treatment is uncertain. There is no doubt that therapeutic outcome is strongly influenced by growth during treatment (Proffit and Ackerman, 1994).

During the orthodontic decision making process, practitioners often ask the following questions: How much more will my patient grow? When will he or she grow? In what direction will their face grow? Will they follow the facial characteristics of their parents? If so, how much? To date the answers to these questions are best guess estimates based on the similarity in family facial features. It is assumed that the basis of that resemblance is genetic (Hunter 1990).

Further, the current knowledge regarding craniofacial development accepts that all development is under genetic control and that all genes are

subject to epigenetic signals that dictate the time and place that a particular gene is switched on. That is, all the information required for growth and development is contained within genes, and gene-gene interactions as well as gene-environment interactions control the distribution and degree of expression of these genes (Slavkin 1999).

In 1941, Hughes and Moore stated that to correct undesirable variations provided by heredity and environment, the professional orthodontist should have a thorough understanding of the genetic and environmental influences affecting craniofacial development. Interest in the relationship between genes and craniofacial variation is motivated by the clinical needs of orthodontists. After many years of observing various treatment responses, experienced orthodontists have assumed that some portions of the face or particular morphologic features are dominated by genetic control and are difficult to manipulate by mechanical therapies. Therefore, orthodontists are interested in identifying morphologic characteristics that can be altered by treatment, and in understanding the growth control process associated with those structures. An understanding of genetics is essential to that end.

Smith and Bailit (1977) contended that despite a long-standing interest and extensive study of genetic influences on the dentition, the daily practice of clinical orthodontics has not changed. That is, they argued that treatment targets and methods failed to reflect consideration of the genetic differences among patients. Through the critical review of the literature, they pointed out

two main reasons for this lack of improvement. The first was the fact that there are some intrinsic limitations in human genetic research. The second factor was related to the concept of malocclusion and basic assumption and methods used in the orthodontic research. They suggested that long term goals of genetic studies should focus on the detection of major genes and determination of their biochemical action.

While summarizing some of the existing ideas relating to the issue of growth modification, Carlson (1999) emphasized the importance of understanding the concepts of craniofacial growth relevant to the emerging field of genetics. He noted that the attempt to explain the mechanisms of craniofacial growth only by the concepts of "nature-nurture" or "form-function" is based on a false dichotomy rooted in a naïve understanding of genes, gene action, and extrinsic factors. That may reflect ignorance about the actual cell physiology and the degree of plasticity that may be possible within a biologic system. He stated,

> "…. there is nothing in biological development and growth that does not have a genomic basis…. All biological cells and tissues, and thus growth and form of morphological structures and whole complexes, have a degree of plasticity at some stage of ontogeny during which they are capable of being influenced to a variable degree by factors extrinsic to the genome."

I.2. Etiology of Craniofacial Deformity and Malocclusion

Etiologic factors for malocclusion and dentofacial deformity have been one of the main issues in craniofacial research. These factors were classified as specific causes, heredity influences, and environmental influences (Proffit

1993). In spite of the long-term investigation into possible etiologic factors, only small portions of malocclusions have a specific known cause. The majority assumed to be the result of a complex and poorly understood combination of hereditary and environmental influences.

In the early part of the twentieth century, accompanied by the rapid development of Mendelian genetics, the view that malocclusion was primarily the result of inherited dentofacial proportions dominated the literature. In those days, the potential effects of orthodontic treatment were considered limited, and the orthodontist's role was to adapt the dentition to the existing facial features. There was little hope of correcting skeletal problems (Proffit 1993). Brodie (1946) stated that orthodontic treatment couldn't change facial growth. The teeth and the alveolar process were considered as the only area of the face where change could be expected or induced. The biological basis for this theme was the belief that heredity predetermined facial type and form and that tooth position is malleable. Therefore the orthodontists' primary role was to correct a malocclusion by moving teeth into a more harmonious position relative to the facial type and jaw relationship present (Carlson 1999).

The 1970s and 1980s were the era in which the role of environmental influences was emphasized. The failure of heredity to explain most variation in craniofacial deformity led to extreme optimism about growth modification therapy. In the 1990s, a more balanced view seemed to be emerging. Although the precise role of heredity as an etiologic factor has not yet been

defined, the relatively high heritability of craniofacial dimensions and the relatively low heritability of dental components has been established.

The secular change in the incidence of crowding and malocclusion often led us to relate the increased outbreeding to at least part of the increased prevalence of crowding in recent centuries. Changes in environmental factors, such as the increased frequency of chronic mouth breathing, and reduced masticatory stress have also been related to the rapid increase in the prevalence of malocclusion over the past few generations (Proffit 1993).

I.3. Using Family Data for Diagnosis and Treatment Planning

There are several limitations of using population norms in orthodontic diagnosis and treatment planning. Even in the situation where population norms are derived from relatively homogeneous groups of people, mean values give us little information about growth prediction of any one individual drawn from the population (Harris 1975; Harris and Kowalski 1976). Moreover, when we use these population standards in a heterogeneous society such as the "melting pot" structure of the United States, those norms have even more severe limitations. Harris and Kowalski (1976) contended that population norms may lead practitioners to use fixed treatment goals rather than individual goals. They concluded that family data can yield the most dependable information on a patients' growth and development.

Hunter et al. (1970) examined several facial dimensions in family members to determine whether the data from the parents could be a useful predictor of future face size in their offspring. They conducted a cephalometric study of thirty-eight families and found high correlations between parents and progeny. These results support the concept of heredity in facial dimensions. The relationships between fathers and offspring were particularly strong for mandibular dimensions. However, when the regression equations using parental data were applied to predict the adult dimension of their offspring, the clinical value was doubtful. The similar finding, which shows a strong father-offspring relationship especially for the mandibular measurements, was reported by Nakata et al. (1973). They claimed that a better understanding of the relative contributions of genetic and environmental factors should improve the accuracy of predicted facial growth.

Harris (1975) contended that most malocclusions are the direct result of heredity, therefore, the family represents the primary source for predicting the patient's own growth record. Using the family data in the class II, division 1 patient, the most efficient set of predictive measurements was proposed by the step-wise regression. It was shown that when a select set of measurements of a sister is combined with those of the female patient, the predictive value of those measurements is greatly improved. The attempt to study heredity as a predictor of facial growth was also found in the family study by Saunders et al. (1980). Lateral cephalometric radiograph studies in

members of 147 families suggested that the use of a few selected cephalometric measurements of the parents and siblings of a patient might enhance the ability to predict the facial growth characteristics and be useful in clinical practice.

Houston and Brown (1980) presented a different opinion regarding family likeness and growth prediction. They supported the concept of polygenetic control of craniofacial features and argued that this is irrelevant to the facial growth prediction. They stated,

> "…. if family resemblance is to be used for prediction it must be shown that, with growth, a child becomes more like its parents in the relevant features. But this is not the case."

I.4. Development and Growth of the Craniofacial Skeleton

After the primordia of all other structures of the head (brain, cerebral nerves, eyes, otic structure, blood vessels, muscles, etc.) developed, the embryonic development of skull begins by condensation of mesenchyme. In the area of the cranial base and in the pharyngeal arches, the condensed mesenchyme differentiates into cartilage and the cartilaginous primordium, the chondrocranium, develops. Whereas, in the area of calvaria and face, the differentiation of the desmocranium from the mesenchymal tissue begins and the center of intramembranous ossification develops. The chondrocranium in the skull base joins with the nasal capsule and otic cartilage. Once the ossification centers appear, these centers increase both in size and in number and the cartilage is replace by bone. Gradually, the cartilage

precursors disappear and remnants are left between the ossified parts as the thin strips called synchondroses. In the desmocranium, the remaining mesenchymal tissue between the bones forms the suture.

The growth of the chondrocranium in the skull base occurs as a result of growth at the synchondroses, followed by endochondral ossification. By the time the sphenooccipital synchondrosis ossifies, growth of the cranial base is completed. The desmocranium, which forms the calvaria and facial skeleton, grows by continued surface modeling. The addition of bone at periosteal, endosteal, and sutural interfaces results in new bone being added to the pre-existing bone. Each of the bones then increases in size and the sutures become increasingly ossified (Limborgh 1970).

I.5. Craniofacial Growth Theories and Genetics

Debates concerning control mechanisms in craniofacial growth and development were one of the main themes in craniofacial biology during the latter part of the nineteenth century and middle third of the twentieth century. A series of four or five relatively distinct and competing theories of craniofacial growth were developed. Each of these identified one or more primary causal factors that determined craniofacial growth and form (Carlson 1999).

The remodeling theory was the first general theory of craniofacial growth. Early research focused on defining the distinction between appositional and interstitial growth of bone. The findings of experimental research led to understanding and acceptance of the concept that bone does

not grow interstitially. Bone grows by apposition or resorption on the surface. Based on this theoretical foundation, the remodeling theory supposed that the growth of the craniofacial skeleton occurs entirely by selective apposition and resorption of bone at its surface. Sutures and cartilages have little or no major role in craniofacial growth. At this time, rediscovery of Mendel's experiments resulted in significant advances of the mathematical laws of inheritance within the field of genetics. However, the nature of the gene and the mechanisms of gene action during the development were all unknowns (Giblin and Alley 1942; Carlson 1999).

The sutural theory was formulated by Sicher and Weinmann and became widely accepted during the 1940s. In this theory, the proliferative growth of sutural connective tissue and cartilaginous joint tissue was considered as the primary cause for the skull growth and form. According to this view, the growth of the suture and cranial base synchondroses is directly controlled by intrinsic genetic factors just as the growth of the epiphyseal plate of a long bone. The mandible was perceived as a bent long bone and the condylar cartilage was regarded as equivalent to the epiphyseal plates. The suture and the cartilages in the craniofacial skeleton were considered the primary centers of bone growth. The inherited pattern of growth was expressed through these control centers. Therefore, the sutural theory supported the concept that the growth of the face and jaws could not be changed (Brodie 1946). During this period of time, developmental genetics,

(which focuses on how genes govern developmental process), was just beginning and our understanding of the process was limited (Sicher 1947; Limborgh 1970; Carlson 1999).

During the 1950s, the nasal septum theory was suggested by Irish anatomist, James H. Scott (1956). After conducting several experimental studies of cranial sutures, Scott and colleagues concluded that sutures played no determining role in the growth of craniofacial skeleton even though they were major sites of growth (Scott 1956; Moss 1957). Rather, sutures were considered as permissive, secondary, and compensatory sites of bone formation and growth. Sutural growth was assumed to be entirely dependent on extra-sutural influences and modified by local environmental factors. According to the nasal septum theory, intrinsic growth controlling factors are only present in the cartilages found within the cranial base and, in particular, the anterior extension of the chondrocranium, the nasal septal cartilage. Scott stated that nasal septum is most active and important for craniofacial skeletal growth, cranial base synchondroses have a longer-lasting effect on craniofacial growth, and condylar cartilages determine the growth of mandible. He also asserted that the anterior-inferior growth of the nasal septal cartilage brings the maxillary complex downward and forward and the growth of condylar cartilages push the mandible downward and forward. During this period of time, the field of developmental genetics was now addressing some of the essential questions regarding the specific mechanisms that relate to the

mode and timing of gene activity. DNA structure was discovered by Watson and Crick in 1953 and a concept of gene action, the operon theory of Jacob and Monod, was initially described in 1963. These two essential findings, the structural basis of the gene and the concept of the genes as regulatory switches, led directly to the DNA recombinant technology 25-30 years later. And it forms the foundation of research in the mechanism of gene action during development (Scott 1954; Limborgh 1970; Carlson 1999).

Both Scott and Moss initially studied the role of sutures in the craniofacial growth, and both came to the same conclusion, which denied the primary determining role of suture in bone growth. While Scott proposed the nasal septum theory, Moss was influenced by European functional morphologist, van der Klauuw and came up in strong defense of the theory that the growth of the skull is entirely secondary. In the first half of the twentieth century, as the previous three theories had been shown, the issue was where is heredity expressed, under the premise that craniofacial growth is largely inherited, intrinsically regulated, and predetermined. This premise was refused in the functional matrix hypothesis, which was claimed by Moss and his co-workers. The functional matrix hypothesis argues that heredity and genes play no significant determining role in the growth of the craniofacial skeleton and instead, the craniofacial complex grows in direct response to its extrinsic, epigenetic environment. According to the functional matrix hypothesis, the skull is composed of various functional cranial components

and each component is comprised of two elements, a functional matrix and a skeletal unit. The functional matrix includes all the soft tissues and spaces that perform a given function. The skeletal unit applies to the bony structures that support the functional matrix and are permissive for that function. During the 1960s and 1970s, debate about the functional matrix hypothesis, which presented an entirely new way to consider craniofacial growth, was extensive, extremely spirited and often emotional. The opponents criticized the facts that Moss used unfamiliar and obscure terminology and relied on overly simplistic assumptions about function. Despite those critiques, the functional matrix hypothesis had a significant impact on the next forty years of craniofacial research and provided a conceptual framework for the possibility of growth modification (Moss 1957, 1972, 1981, 1997a, b, c, d; Moss and Young 1960; Limborgh 1970; Watson 1982; Carlson 1999).

From the early 1970s through almost the present time, the servosystem theory of craniofacial growth developed by Petrovic, Charlier, and Stutzmann, has been most prominently articulated and debated. Their principal interests were in the development and growth of connective tissue and in the intrinsic and extrinsic hormonal factors that affect cartilage growth. They recognized the dissimilarities of different growth cartilages, primary (e.g., epiphyseal, sphenoccipital synchondrosis, and nasal septal) and secondary (e.g., condylar, coronoid, angular, and those in some craniofacial sutures) cartilages. The growth of primary cartilage appears to be subject to

the intrinsic cell-tissue related properties and the extrinsic systemic endocrine system. In this case, the effect of local biomechanical and functional factors is reduced to modulation of the direction of growth, without effect on the amount of growth. On the other hand, the growth of secondary cartilage is highly adaptive and responsive to both extrinsic systemic factors and local biomechanical and functional factors. In this case, the amount of growth can be modulated by an appropriate orthopedic device. Reduced to its most fundamental principals, the servosystem theory can be summarized as follows. First, under the influence of the cartilaginous cranial base and nasal septum the midface grows downward and forward. Second, proprioceptors within the periodontal regions and temporomandibular joint detect occlusal discrepancy and activate the muscles for mandibular protrusion. Third, activation of mandibular protruding muscles have influences directly on the condylar cartilage and indirectly through the blood supplies to temporomandibular joint, stimulating cartilage to grow. Finally, hormonal factors, both directly and indirectly, influence muscle function and responsiveness of the condylar cartilage. The principal feature of the servosystem theory is its dependence on experimental demonstration of hypotheses and the major significance of this theory is that it provides a guide for future experimentation. In respect to developmental genetics, the importance of the servosystem theory arises from the fact that the basic approach of this theory is based in cell physiology and biology. The

servosystem theory addressed the expression of genes influencing the growth and adaptability of cells and tissues in the craniofacial complex (Petrovic and Stutzmann 1997; Carlson 1999).

I.6. Growth of Craniofacial Skeleton in Rats

Several longitudinal studies investigated skull growth in rats (Spence 1940; Asling and Frank 1963; Moore 1966; Wright et al. 1966; Cleall et al. 1968; Vilmann 1969; Hughes et al. 1978; Vilmann and Moss 1981). Through a longitudinal radiographic exam on a total of fifteen male and ten female albino rats, Moore (1966) found that the cranium gains about 93% of its adult size (fifth month) by the age of one month and finishes growth by the third month. Whereas, the facial skeleton and mandible attain about 75% of their adult size by the first month after birth and, by the end of the fourth month, the face and mandible almost complete their growth by attaining 97% of their fifth-month size. It is shown that the basic patterns of relative growth in the rat skull are similar to those of primates. The same conclusion regarding similarity in relative growth pattern between rats and human was made by Spence in 1940. Spence's study characterized the adult rat skull as a flat cranium and a long snout or upper face. It was shown that the outline of the cranium appeared to have reached its full dimensions by the seventeenth day, while the snout continued to form.

Asling and colleagues published two papers of roentgen cephalometric studies on female and male rat skull development from 27-280 days of age

(Asling and Frank 1963; Wright et al. 1966). In the first report dealing with female rat skulls, it was shown that the neurocranium increased in length but very slightly in width and height. The ratio between viscerocranial and neurocranial length had noticeable change with rapid viscerocranial growth. In the second paper, sex differences in the rat skull were defined. The male rat skulls generally showed more rapid and prolonged growth characteristic of skeletal development especially in facial length and width, demonstrating distinctive sex differences in vicerocranium. The same finding was noticed earlier by Hatai (1907). Vilmann's study (1969) showed significant sex differences in growth of the single bones in the cranial base from twenty-one days of age. This result was explained as an expression of a difference in time of maturation between males and females, comparable to what is found in humans around the adolescence growth spurt. Hughes et al. (1978) measured the cranial index and facial index in addition to the lineal craniofacial measurements. Their study demonstrated that males became significantly larger than females in each of the absolute linear measurements studied, but in cranial and facial indices there were no significant sex differences.

II. Strain Variation in the Craniofacial Morphometric Traits

II.1. Quantitative Traits

Quantitative traits are different from the characteristics of qualitative traits in several aspects. First, quantitative genetics is the study of degree

rather than kind. Second, phenotypes of quantitative traits vary in a continuous way from individual to individual, and do not fall into discrete classes. In contrast, qualitative traits can be placed into distinct phenotypic groups. Third, gene effects on the quantitative difference are thought to be relatively small and variations can also arise from non-genetic events as well. Fourth, gene differences at many loci usually influence quantitative differences and it is impossible to identify the individual genes by their segregation. On the other hand, a gene difference at a single locus causes an easily discernible difference of qualitative traits (Falconer 1996).

The study methods of quantitative genetics are different from those of qualitative genetics in two aspects. In the first place, larger groups of individuals composing many generations are needed. Secondly, the study of quantitative differences demands the measurement, and not just the classification, of the individuals (Falconer 1996).

II.2. Multivariate Nature of the Parameter

Quantitative characters which show continuous variation are different from the characters of qualitative genetics in that the measured traits are not likely to be a sufficiently direct representation of the heritable entities involved (Howells 1953). Most quantitative phenotypes represent complex traits, that is trait in which a one-to-one relationship between genotype and phenotype does not exist (Darvasi 1998). Morphogenesis of highly integrated structures is a multivariate and multidimensional phenomenon. The genetic and non-

genetic interactions during morphogenesis are multivariate in nature, such that pleiotropic gene and environmental factors simultaneously influence large numbers of traits. A major problem in studying quantitative traits is to extricate the various genetic and non-genetic effects, so that major patterns and components can be studied (Atchley et al. 1981; Atchley 1983).

Among those statistical procedures, which were suggested to describe the simultaneous covariation in large suites of traits, the simplest method is principal component analysis. With principal component analysis, the major independent patterns of multivariate covariance are summarized in a small number of vectors and the major patterns of multivariate covariation in the phenotypic, genetic, or environmental correlation matrices can be described (Atchley 1983; Goto et al. 1993).

II.3. Factor Analysis

Factor analysis is a generic term for several methods, which examine possible underlying patterns of relationships among the variables. Principal component analysis is the most common type of these methods. The transformation of a set of observed variables into a new set of composite variables known as the principal components, or eigenvectors is performed in this analysis. Those variables indicating the assessment of similar parameters are grouped together and assigned to factors. Generally, those variables assigned to the same factor have high correlation (Master and Lubinsky 1988).

Factor analysis has been applied on craniofacial measurements in many studies (Landauer 1962; Brown et al. 1965; Wallace and Bader 1967; Nakata et al. 1974; Cleall et al. 1979; Ohtsuki et al. 1982; Master and Lubinsky 1988). It is useful in morphologic study to get a few factors that explain a large amount of the total variation. Yanagita et al. (1992) also used factor analysis in their study and mentioned the advantage of this method, which simplifies the complicated mechanism of the craniofacial morphogenesis by reducing a large set of interrelated variables into a smaller set of independent factors.

II.4. Methods of Obtaining Craniofacial Measurements

An essential condition of quantitative traits is that they should be measurable. The technique of measurement, however, sets a practical limitation on what can be studied (Falconer 1996). Two major methods have often been considered in measuring craniofacial morphometric traits. Roentgenographic cephalometry has been one of those methods used in several studies (Asling and Frank 1963; Hughes et al. 1978; Engstrom et al. 1982; Ohsuki et al. 1982; Nonaka and Nakata 1984, 1988a, 1988b; Nonaka et al. 1998; Kohn 1989; Yanagita et al. 1992). Hughes et al. (1978) mentioned the advantage of using radiographs over other methods such that they are non-destructive and allow repeated observations on the same animals. The longitudinal measurements of variables make it possible to estimate the rates of growth and their associated variance. Spence, who constructed a head

holding device for anesthetized rats while taking radiographs, stated another value of the cephalometric method as follows; radiographs reveal structural details such as suture, sinuses and diploe, which cannot be studied as readily by other methods (Spence 1940). Cleall et al. (1979) regarded roentgen cephalometry as most useful for characterizing the morphology. However, they also concluded that the craniofacial region grows and functions in a three-dimensional manner and there were some limitations in viewing it in only two dimensions.

On the other hand, some studies like those of Byard et al. (1985), Atchely (1983), Atchely et al. (1981) and Landauer (1962) took craniometric measurements of the dry skull, and could overcome some limitations related to radiographs, such as the two dimensional projection of a three dimensional subject, magnification, and x-ray quality.

II.5. Strain variations of Craniofacial Complex in Rats and Mouse

The skeleton of the mouse or rat has been used as a source of strain identification for a long time (Festing 1972, 1974 a, b; Lavelle 1976; Lovell and Festing 1982; Lovell and Johnson 1983). Festing (1972) used multivariate discriminant analysis in the mandible for mouse strain identification. Without using biochemical methods for strain identification, which are costly and laborious, 98% of the mandibles were correctly classified. Moreover, if measurements on other bones had been included, this strain identification method could have been more precise. Festing proposed

this mandible-analysis technique for genetic monitoring of commercially bred laboratory mice (Festing 1974 a, b). Lovell and Festing (1982) used the mandible-analysis method in the rat, which is the second most widely used vertebrate in animal experimentation. They concluded that mandibular shape is also useful for genetic monitoring in rats. Rat mandibles were significantly different between genetically distinct inbred strains. The shape of the mandible could be used for genetically monitoring colonies of rats as well as mice.

Lavelle (1976) obtained quantitative data on the skulls from three inbred rat strains. Significant differences were detected among the strains and these were attributed to genetic differences. Furthermore, the facial skeleton exhibited greater amount of genetic control than the neurocranium. To detect variation in the mouse skeleton resulting from genetic and environmental causes, Lovell and Johnson (1983) measured a series of six bones from eleven inbred strains and one F1 hybrid. They found marked variation in the shape of each bone and could successfully discriminate between strains using variability in bone shape. Correct strain classification ranged from 87% for the scapula to 98% for the os coxae[1].

III. Where Does Strain Variation Come from?

Morphological variability in craniofacial dimensions stems from the interplay of a number of genetic and non-genetic factors. There have been a

[1] bone of the hip

few studies attempting to partition genetic and environmental components of variation within the craniofacial complex. The review of the literature regarding this subject begins with the study of quantitative genetics.

III.1. Quantitative Genetics

The branch of genetics concerned with continuous variation in traits is called quantitative genetics. Even though there are some differences in the nature of quantitative and qualitative traits, the genes that control the inheritance of quantitative traits are subject to the same laws of transmission and have the same general properties as the genes associated with qualitative traits. Therefore, quantitative genetics can be considered an extension of qualitative Mendelian genetics, and Mendelian principles may be regarded the foundation. Quantitative genetic theories result from analysis of Mendelian inheritance patterns observed in large populations and to the simultaneous segregation of genes at many loci. The effects of other Mendelian genetic properties including dominance[2], epistasis[3], pleiotropy[4], linkage[5], and mutation[6], are taken into account in quantitative genetics.

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[2] those conditions that are expressed in heterozygotes (i.e., individuals with one copy of the mutant gene and one copy of the normal allele)

[3] suppression of the effect of a gene by a nonallelic gene

[4] diverse effects of a single gene or gene pair on several organ systems and functions

[5] co-inheritance of two or more nonallelic genes because their loci are in close proximity on the same chromosome, such that after meiosis they remain associated more often than the 50% expected for unlinked genes

[6] any permanent heritable change in the sequence of genomic DNA

Genetic segregation usually results in discontinuous variation. However, there are two reasons for the continuous variation observed in quantitative variables. First, simultaneous segregation of many genes may influence a single trait. This phenomenon is related to polygenic variation. Second, continuous variation arising from non-genetic causes. Therefore, the difference between qualitative genetics and quantitative genetics may lie in the magnitude of gene effects relative to other sources of variation.

III.2. Polygenic Traits

The early literature attempted to characterize the inheritance of malocclusion by assuming applicability of simple Mendelian genetics and used terms such as dominant, recessive, sex-linked, incomplete penetrance, and expression, partly because they regarded dental traits as discrete or qualitative entities. However, craniofacial variations are now recognized as continuous trait and a simple Mendelian pattern of segregating genes can not been established in family pedigree. In the 1960s, the concept of polygenic inheritance became generally accepted in the study of craniofacial and dental variation; that is, facial development is caused by the action of many genes and environmental effects (Harris 1975; Saunders et al. 1980). When large numbers of genes collectively control the variations in a behavioral or physiological phenotype such as craniofacial morphology, allelic differences at any one locus will probably be associated with small, possibly undetectable, differences in the observed phenotype. For polygenic traits, the

heritability of those phenotypic traits provides evidence for differential gene activity during ontogeny (Atchley et al. 1980).

In the second paper, they studied genetic components of size and shape variation by examining seventeen osteometric characteristics of the rat skull and a long bone from the axial skeleton. The osteometric traits had heritabilities ranging from 0.25 to 0.68, while heritabilities of the three live body traits ranged from 0.12 for body circumference to 0.57 for tail length. They decomposed the phenotypic correlation into a genetic and an environmental correlation and analyzed the genetic correlation structure for those traits. The analyses of genetic correlation showed four genetic patterns of morphogenesis in the rat skull, including a general skull size component, a general skull shape component, a tooth and jaw length component and a palatal and skull width pattern. They also reported a large, genetically determined shape contrast in the rat skull, which involves an inverse relation between skull width and depth versus skull length. Large and complex patterns of genetic correlations in the integrated skeletal system from their data demonstrated the universality of pleiotrophy in morphogenesis (Atchley et al. 1981).

In the third paper reported in 1983, Atchley provided data on the quantitative genetic aspects of variation and covariation for eight mandibular and maxillary traits together with femur length. They showed positive genetic associations between femur length and the length and height of the mandible,

palatal length, and posterior palatal width. Their hypothesis was that because the rat was a long-snouted animal they would find large positive genetic correlations between major length components. Their results supported their hypothesis. There were significant negative genetic relationships between femur length and mandibular tooth row length, and no genetic association between femur length and both maxillary tooth row length and mandibular width. A significant environmental allometric effect was also found in all eight oral traits. They reported the general size effect on the oral structures from their data, which didn't substantiate Limborgh's theory (1970) of more pronounced local factors in craniofacial growth. Regarding the mandible, they concluded that overall mandiblular size is a highly heritable trait. After removal of the overall mandible size effect, the length and width were heritable, while height was not heritable because it was found to be a measure of muscle insertion size (Atchely 1983).

III.6. Genetic Studies on Craniofacial Complex in Human

Historically, there have been two classic ways to determine the influence of heredity on the human craniofacial complex (Proffit, 1993). The first method compares monozygotic with dizygotic twins. And the second method observes similarities and differences in the family members.

It was Galton who first distinguished twin-pairs into identical and fraternal twins and demonstrated the primary differences between two groups (Lundstrom 1984). He published the article of the first scientific analysis of

twins in 1875. Monozygotic twins have identical genotypes, so there is no genetic variance within pairs and all the observed variation can be attributed to the environment or measurement error. On the other hand, dizygotic twins are related as full siblings. But, since they are born at the same time, it can often be assumed that the environmental similarities and dissimilarities between same-sexed fraternal twins are similar to those of identical twins. Galton reasoned that by comparing differences between mono and dizygotic twins, it should be possible to separate out the effect of environment, "nurture", from the effect of heredity or "nature". The proportion of the variability in a certain morphometric trait attributable to heredity can be estimated by comparing identical twins and fraternal twins. That is, a comparison of differences within identical twins (caused by nongenetic factors) with those within fraternal twins (caused by genetic and nongenetic factors) can be carried out after subtracting nongenetic effects from the latter (Lundstrom 1984).

Arya et al. (1973) studied the genetic variability of six mandibular dimensions and three craniofacial dimensions by analyzing the cephalograms of twenty-six monozygotic and sixteen like-sexed pairs of dizygotic twins. They attempted to determine the zygosity using blood grouping, concordance of physical characteristics and other testing methods. The predominant genetic component was found in the variability of all the dimensions measured except the dimension left gonion-right gonion. The question

whether an individual bone (mandible in this study) shows a larger component of genetic variability than is shown by the craniofacial complex was substantiated by the mandibular dimensions demonstrating a greater genetic component. A comparison of the height, length, and width of the mandible indicated that height shows less genetic variability than the others and is therefore more susceptible to environmental influences (such as treatment).

Lundstrom (1984) performed a twin study to investigate heritability in dentofacial measurements. Path analysis was used for forty-two pairs of twins with respect to facial height and facial depth. Several published studies were also summarized, suggesting that the etiology of dentofacial variation and malocclusion might be about 40% genetic. They suggested further study to evaluate the effects of cultural inheritance and family environment. The clinical aspects of genetic studies, including prophylactic possibilities for non-genetic problems and treatment difficulties in genetic anomalies were discussed. The causal factors for non-genetic problems can be grouped into two main categories. The first category includes the factors that can be influenced prophylactically. Examples in this category include nutrition, sucking habits, cultural environment etc. The second factors are inaccessible and cannot be influenced prophylactically. Clinically, it may be important to distinguish between these two factors. The question, "Are genetic anomalies more difficult to treat than non-genetic?", was left unanswered. Even though it is clear that certain genetically induced problems are quite resistant to

treatment, well-documented therapeutic trials are needed to answer the question.

To study cephalometric twin data Kohn (1989) also analyzed the effect of age on trait inheritance. This longitudinal study was expected to provide evidence of differential gene action in the determination of craniofacial morphology, but the results suggested unclear differential gene action.

Twin studies have several limitations. First, it can be difficult to find enough twin pairs to study. In addition, it is sometimes difficult to establish zygosity and to confirm that the same environmental influences existed for both members of twin pairs (Proffit 1993). The second classic way to determine the heritability in the human craniofacial complex is to investigate family members, observing similarities and differences in the parents-offspring pairs and sibling pairs.

Nakata et al. (1974) conducted multivariate analysis of craniofacial measurements in twin and family data. They undertook a rotated factor analysis to explain the variation by a set of composite variables. After computing correlation coefficients on twins, siblings and parent-offspring data for factor scores, they reached a conclusion that each factor could result from the interaction of independent sets of genes with the environment.

Sibling correlations for cranial measurements were examined using lateral cephalograms taken serially (Byard et al. 1984). Generally, sibling correlations gradually increased from age one to adulthood. Most of the

correlations for nine craniofacial measurements investigated in their study were significantly greater than zero. Some of them including sella-nasion, sella-vertex, and basion-nasion were greater than 0.5. Considering 0.5 is the maximum value expected for a completely heritable polygenic trait, this suggested the effect of shared environment among siblings.

While a large number of studies used cephalometric radiographs for craniofacial measurements, Byard et al. (1985) took craniometric measurements of the craniofacial complex on a total of 1,763 individuals in 399 families. Eight principal components were extracted and path analysis was used to analyze family resemblance. Their report showed higher transmissibility for components related to cranial size and facial height than for components of facial breadth and ear dimensions.

Harris and Johnson (1991) reported a longitudinal study regarding the heritability of craniofacial variables on thirty sibship pairs. They distinguished between skeletal components and dental components among the variables and showed a clear dichotomy in the heritability estimates between the two groups of variables. In their study, the skeletal variables had moderate to high heritability increasing with age; on the other hand, the variables representing dental components had low heritability estimate values, decreasing with age. They were cautious in estimating sibling correlations by mentioning the possible co-habitual effects, which means that phenotypic similarities can result from siblings being raised in similar environments.

III.7. Environmental Effects on Craniofacial Complex

Moore (1965) investigated the overall changes in the craniofacial skeleton of rats accompanying reduction in masticatory muscle function following feeding on a soft diet. A 12% reduction in craniofacial bone mass, a 1-2 % decrease in the overall dimensions of the cranial and facial skeleton, and a decrease of 4% in the size of the angular process of the mandible were reported. Another study, which focused on the effect of the altered masticatory muscle function, was reported by Kiliaridis et al. (1985). A soft diet was given to the experimental group of the Sprague-Dawley rats assuming to generate low activity of masticatory function. When rats from the standard diet group and the soft diet group with matched body weight were compared, significant differences in craniofacial morphology were detected. The soft diet group showed a greater anterior facial height (viscerocranial height), a shallower antegonial notch and a decreased gonial process. The authors concluded that the functional activity of the masticatory muscle might influence the general craniofacial growth pattern as well as bone remodeling in local areas.

Pucciarelli's study (1980), which examined the effects of strain, sex, and nutrition on craniofacial differentiation in Wistar and Holtzman rats found that 55% of total cranial variation was due to nutritional factors. The remaining 45% of variation were attributed to biological factors, which could be partitioned into 26% and 19% due to race and sex, respectively. These

results suggested that nutritional factors may influence craniofacial growth to a greater extent than genetic factors. Using male Holtzman rats, Pucciarelli (1981) observed the effect of nutrition on the growth of the functional components of the craniofacial complex. The craniofacial complex was divided into neurocranial, respiratory, and masticatory components. The growth of the masticatory component seemed to be most affected by nutritional status. The respiratory component was less affected followed by the neurocranium.

Engstrom et al. (1982) conducted a cephalometric study to investigate the influence of a low calcium and vitamin D-deficient diet on the growth and form of the skull of the growing rat. The low calcium and vitamin D-deficient rats were compared to normal rats of the same age and body weight. They found the nutrient deficient rats do have reduced growth and altered form of the skull. This finding implied an impaired osteogenesis in growth sites determining the growth and form of the viscerocranium and its relation to the neurocranium in the experimental groups. The finding that the main influence of the deficient diet was on the viscerocranium and the border area between the viscero- and neurocranium was explained by the fact that in the late growing stages of the normal rat, viscerocranial growth predominates.

IV. Detecting Genes that might Control Craniofacial Morphometric Traits

Development in molecular genetic techniques makes it possible to assess DNA variation directly among individuals rather than estimating

genetic influence indirectly via resemblance between relatives (Plomin et al. 1991). Detecting sets of genes that relate to the craniofacial morphometric traits is a key step in discovering the molecular networks that modulate craniofacial morphogenesis (Toth and Williams 1998). Moreover, the potential ability to identify genes and their function provides opportunities for new diagnostic tests and treatments for complex craniofacial abnormalities (Darvasi 1998).

IV.1. Quantitative Trait Loci (QTLs)

Quantitative trait loci (QTLs) are genes or genomic regions that control a large portion of the total phenotypic variance in quantitative traits. In the quantitative traits, the probability distribution of phenotypes across different individuals shows approximately Gaussian distribution. For some quantitative traits, however, a relatively small number of genes may account for much of the variation in expression. The large phenotypic effects associated with these genes can often be seen in probability density plots as modal peaks or S-shaped deflections in the distribution pattern. In these cases, it will often be possible to map a QTL.

QTL mapping depends on two critical factors; the amount of phenotypic variance controlled by the locus and the precision with which the phenotypic trait can be measured. If phenotypic variance is low or if individual gene effect is large, then it may be possible to relate phenotype expression with a distinct number of independent major QTLs. Even if many genes

control a trait, mapping a QTL may nonetheless be practical if one or two of the QTLs account for a disproportionately large portion of the phenotypic variance (Toth and Williams 1998).

The essence of QTL association analysis is simply to correlate allelic variation for a marker with phenotypic variability in the population for a quantitative characteristic (Plomin et al. 1991). To find main genomic regions that control the phenotypic variability and then to detect possible genes located in those regions are general goals of QTL analysis (Toth and Williams 1998).

IV.2. Functional and Positional Cloning

Takahashi and his colleagues (1994) suggested "forward and reverse genetic approaches" to achieve the genetic analysis of complex traits in the mouse. "Forward genetics" or functional cloning starts with the phenotype and proceeds to the gene. On the other hand, "reverse genetics" or positional cloning starts with the gene and proceeds to the phenotype.

Forward genetic approaches begin by characterizing the phenotype of interest and move toward identifying the gene or genes that control the expression of that particular phenotype. A forward genetic strategy can be divided into four sequential steps of assessment. The first step starts with characterizing the phenotypes of various inbred strains of mice and identifying strain variation of any particular phenotypes. The second step has two parts. Part one is to distinguish the phenotypes of an appropriate set of recombinant

inbred (RI) mice. Part two is to assess the association between variation in the phenotype and allelic variation at genetic markers in published data sets. Part two involves determining a strain distribution pattern (SDP) for that trait and searching for correlations between the strain SDP and the SDPs of alleles at marker loci (linkage analysis). This is the part of the second step that finds the possibility of any specific loci contributing to the trait of interest. If there is any evidence, then, a third step will follow. The third step tests whether these genomic loci control phenotypic variance in other RI sets, in intercross (F2) or backcross (N2) panels of mice, or in congenic strains of mice. This determines the importance of candidate QTLs and gives more information for accurate mapping of their location. The fourth step is a gene-targeted approach. To specifically evaluate the contribution of these genes to the trait of interest, knockout, mutant, or congenic strains of mice may be studied (Toth and Williams 1998).

Positional cloning, which was originally referred to as "reverse genetics", is one of the gene identification methods. While functional or expression cloning needs the information about the protein product and/or function of the responsible gene, positional cloning does not need any knowledge of function of the gene to clone it. In the positional cloning, mapping the responsible gene to its correct location on a chromosome is accomplished first and then the function of the gene can be studied (Collins 1992).

IV.3. Intercross and Backcross

For genetic analysis of individual characteristics in experimental animals, different breeding schemes need to be considered. Intercross and backcross breeding schemes are often used in genetic studies. In both schemes, the progenitors are chosen from inbred or outbred strains, and these parental animals are crossed to get the first filial (F1) generation. In the intercross breeding scheme, two F1 individuals (siblings) are crossed to obtain F2 offspring. On the other hand, the backcross breeding scheme yields the first generation (N2) by crossing F1 individuals with one of the progenitors. Next (N3) offspring would be obtained by crossing individuals from the N2 generation to individuals from the parental generation.

IV.4. Recombinant Inbred Strain

Generating recombinant inbred (RI) strains begins with the initial cross of two inbred strains. The F1 population from this initial cross is mated to get an F2 generation. F1 hybrids are heterozygous for all loci. Beginning with the F2 generation, brother-sister mating is carried out for more than twenty generations. This mating procedure develops inbred lines, which are homozygous for all genes. During the F2 and inbreeding process, the original parental genotypes are recombined by crossing over of homologous chromosomes several times per chromosome for each RI strain (Klein 1978; Plomin and McClearn 1993).

RI strains have at least three advantages over segregating (F2 or backcross (BC)) populations (Plomin and McClearn 1993; Belknap 1998). First, RI strains possess all the advantages of any inbred strain, which include direct comparisons of genetic and phenotypic information across time, traits, and laboratories on the same set of readily available and stable genotypes. Second, the fact that RI strains are homozygous at all loci is more informative for QTL mapping than the usually intermediate-scoring of heterozygotes composing only half of the F2 population, or the total absence of one homozygote class in BC populations. Third, in contrast to an F2 or BC population in which each genotype is represented by only one animal, measurements of the same genotype can be replicated on several animals per strain in RI strains. This results in a more accurate assessment of the phenotype associated with each genotype in RI strains.

The major disadvantage of RI strains in QTL mapping studies over an F2 or BC population is the limited number of genotypes available. Since each genotype is represented by a single RI strain, the number of the RI strains limits the number of available genotypes. For example, the most commonly used RI sets in the mouse, BXD RI series, are limited to no more than twenty-six or twenty-seven distinct genotypes. In contrast, F2 or BC populations can have any number of genotypes, since each genotype is represented by a single animal and large number of animals can be generated (Belknap 1998).

IV.5. Inbred Strains and F1 Hybrid

Inbreeding is the mating of individuals, which are related to one another by having one or more common ancestors. Brother and sister mating for twenty or more consecutive generations generate an inbred strain. After twenty generations of full-sib mating the probability that an individual is homozygous[10] at any given locus is at least 0.986, and unexpressed recessive genes are not normally carried within the colony. All individuals within an inbred strain are genetically identical. F1 hybrid, i.e. the first generation cross between two inbred strains is genetically identical to each other. In contrast to inbred strains, F1 hybrids will be heterozygous[11] at all loci at which the parental strains differ. F2 hybrids will also be heterozygous at many loci but will not be genetically identical to each other. The great advantage of using the inbred strains lies in the fundamental property that individuals of the same inbred strain are genetically same, and thus one important variable in any experimental situation is under the control of experimenter (Festing 1979). The use of inbred strain and F1 hybrid make possible a reduction in the number of experimental animals needed to give conclusive results (Barnett 1975). The genetic identity offers the possibility of

[10] an individual possessing a pair of identical alleles at a given locus on a pair of homologous chromosomes

[11] an individual who has two different alleles at a given locus on a pair of homologous chromosome

determining the genotype of the whole strain from examination of a single individual (Festing 1979).

IV.6. Genetic Homologies in Human, Mouse, and Rat

Homologous segments refer to chromosome segments marked by at least one gene that has been mapped in two or more species. This homologous segment is regarded as the basic unit of comparative gene mapping among different species. Conserved synteny is defined as a chromosomal region that has two or more homologous genes, regardless of gene order. Conserved linkages are those segments where both synteny and gene order are conserved (Nadeau 1989).

Since the mid-1980s, thousands of genes have been reported to have genetic homology among different species, such as fruit fly, fish, mouse, rat, human etc. The investigation of genetic homology and the strategy of comparative gene mapping suggest several applications of this knowledge. Genetic homologies would provide substantial support for using animal models to study growth and development (Slavkin 1999). The chromosomal distribution of homologous genes may provide a unique opportunity for understanding many important aspects concerning genome organization and evolution. An important application of comparative mapping in medicine involves predicting the location of disease loci in one species given their locations in another species. This is the way in which one can identify and screen human samples in advance of knowing a gene's identity. The

comparative mapping may also help to explain the genetic basis of a complex disease (Nadeau 1989). QTLs identified in experimental animals, such as mouse and rats, and located in chromosomal regions syntenic to the human, have particular importance as possible candidate chromosomal regions for QTL association studies of human (Plomin and McClearn 1993).

While reviewing the status of rat gene map, Levan et al. (1991) discussed the comparative mapping of rat, mouse, and man. They found that genes on the same chromosomes in one species tend to be syntenic also in another. Ten rat synteny groups were showed to be syntenic in the mouse. Comparison between human and rat gene mappings revealed rat synteny groups have been dispersed to a much greater extent in the human genome than in the murine. This finding was expected based on evolutionary considerations.

Studies of genetic homologies among different species have increased dramatically since the recent introduction of biochemical and molecular methods of characterizing and mapping of genes. Recently, Seldin et al. presented the Davis Human/Mouse/Rat Homology Map in their Internet web site (http://www.ncbi.nlm.nih.gov/Homology). This homology map shows genes in homologous segments of DNA from human and mouse sources. Two hundred and one homology groupings have been discovered so far.

V. Orthodontics and Genetics in the Next Century

Slavkin (1999) outlined two directions for ongoing research lin craniofacial biology. First, toward understanding the molecular basis of growth and development and second, toward utilizing biomimetics for repair and regeneration of craniofacial and skeletal tissues.

A scientific revolution in developmental molecular biology led us to increase our understanding about the mechanisms controlling craniofacial growth and development (Thesleff 1996; Carlson 1999). So far, knowledge regarding gene and development accepts that genes provide all of the information required for growth and development. Using DNA as a template, RNA molecules are synthesized, and the process of synthesizing a protein is directed by the sequence of a specific mRNA. Protein products determine cell traits and changes in cell activity ultimately result in the form of an individual. The sequence and rate of gene expression, on the other hand, is influenced by interactions with other genes as well as with the environment. These regulatory influences can be explained as physical or chemical signals. Those signals are perceived by the receptors on cells, tissues, and organs and then, complex biochemical reactions may be initiated. The understanding of those signals and the biochemical mechanisms will give us the possibility for growth modification in orthodontic treatment (Gelehrter and Collins 1990; Slavkin 1999).

The studies involved in the mutation of individual bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs) suggest that the specific shape of individual bones may be determined by the expression of particular BMPs and GDFs or, by their combined effects. In particular, BMPs appear to play an important role in the regulation of hard tissue development. New possibilities for clinical dentistry may be expected in their therapeutic use as stimulators of new bone and dentin formation in the near future (Thesleff 1995).

By the end of 2003, the Human Genome Project will expect to have a complete and accurate DNA sequence representing the genetic blueprint and evolutionary history of the human species. One of the important values of this new gene map is its utility for identification and isolation of genes that cause human disease or increase the susceptibility to disease (Collins et al. 1998). The twenty-first century will be the time when we attempt to transfer the findings in developmental genetics and craniofacial morphogenesis into the field of orthodontics.

STATEMENT OF THE THESIS

The present study was designed to explore phenotypic variation in facial morphology. An animal model was chosen to allow both inheritance and growth to be manipulated and observed. Methods of measuring craniofacial features were compared and / or critically examined. Radiographs were used to develop a non-destructive assessment method to track heritable traits. There is a need to develop non-destructive assessment methods and to test their ability to track heritable traits. By mating a dolichocephalic-type Sprague-Dawley rat with a brachycephalic-type Brown-Norway rat, the pattern of craniofacial morphometric traits in F1 hybrids was observed. The goal of this research was to provide valuable preliminary data for genetic projects in the Department of Orthodontics at Case Western Reserve University (CWRU).

The present study had two objectives to achieve. The first is to compare invasive and non-invasive methods for obtaining relevant trait variables in the craniofacial complex of rats. Specifically, the direct measurements from the dry skull (craniometrics) and the same measurements from x-ray (cephalometrics) will be compared to determine the relative accuracy of x-ray measurements in characterizing the quantitative traits in the craniofacial complex of rats. If acceptable accuracy can be obtained using radiographs, those measurements will allow future longitudinal

studies. The second aim of the present study is to examine the distribution of measures of interest in the offspring (F1) of a cross between Brown-Norway and Sprague-Dawley strains. The observation of the F1 generation will produce the fundamental information on the phenotype needed to investigate mechanisms of inheritance and identify candidate genes for future studies of craniofacial morphogenesis.

MATERIALS AND METHODS

I. Study Samples

This study included two independent investigations. In part one, dry skulls and radiographs (lateral and ventrodorsal) of eight inbred strains (four males, four females in each strain, total n = 64) of laboratory rats were used. The eight strains were Sprague-Dawley (SD), Brown-Norway (BN), Lewis (L), Fisher-344 (F), Wistar-Kyoto (WK), Long-Evans (LE), Holtzman (H), and Buffalo (B). Dry skulls and radiographs were derived from the previous animal experiments at the department of orthodontics in Case Western Reserve University (Moullas et al. 1999). All sixty-four of the animals were older than six months.

In the second part of the study, four month old F1 offspring were obtained from a Sprague-Dawley and Brown-Norway cross courtesy of Drs. Strohl and Thomas (Veterans Affairs Medical Center, Cleveland, OH.). These two strains were selected because of their different craniofacial morphology. A previous study showed that the craniofacial dimensions of the Sprague-Dawley strains were characterized as dolichocephalic head form and those of the Brown-Norway strains had more brachycephalic characteristics (Moullas et al. 1999). Animals from Sprague-Dawley and Brown-Norway colonies, obtained from a single vendor (Harlan, Indianapolis, IN), were bred to

produce a F1 offspring. Prior to breeding, the rats received a subcutaneous implanted identification marker, which can be read for both animal ID and body temperature. The marker was placed by injection under Halothane anesthesia. Three females were placed with one male to produce F1 progeny. In the present study, the cross between Sprague-Dawley male (F0) and Brown-Norway female (F0) was used. All animals were housed at Room K-27, Wade Park Unit, Veterans Affairs Medical Center, Cleveland, OH. Animals were caged with appropriate bedding and with water and food *ad libitum*. The rats were sacrificed with carbon dioxide inhalation, and anthropometric data (body length (nose to anus) and body weight) were measured. The flesh skulls and right femurs were obtained and placed into an aquarium housing flesh-eating beetles maintained at the Cleveland Museum of Natural History, Cleveland, OH. This process successfully removed all soft tissue from the bony skeleton without damaging the fragile bony structures.

II. Anatomic Landmarks

The following anatomic landmarks were used to study the phenotypic traits of craniofacial skeleton. These points are illustrated on the diagram in Figure 1.

II.1. Mandible

ap The most inferior and posterior point on the outer surface of the angular process

imn The junction of the inner surface of the right and left mandibular incisors and the shaft of the mandible

mn The average of the right and left intersections of the mandibular alveolar process and the anterior surface of the mandibular first molars

co The most posterior and superior point on the curvature of the averaged outline of the left and right condyles

c The most posterior and superior point on the curvature of the averaged outline of the left and right coronoid processes

dlm The average of the right and left intersections of the mandibular alveolar process and the posterior surface of the mandibular third molars

II.2. Maxilla

um The intersection of the hard palate and the anterior surface of the averaged maxillary right and left first molars

imx The junction of the inner surface of the right and left mandibular incisors and the premaxilla

pns The most posterior point on the palatal process of the palatine bone

mx The most anterior point on the maxillary process of the maxilla

dum The average of the right and left intersections of the maxillary alveolar process and the posterior surface of the maxillary third molars

zg The most lateral point on the zygomatic process

II.3. Cranium

ba The most anterior point on the margin of the foramen magnum

so The outer point of the middle of the sphenoccipital synchondrosis (at the midline)

o The most posterior point on the external occipital protuberance

nf The junction of the nasal bone and the frontal bone on the midline

la The junction of the parietal bone and interparietal bone on the midline

br The junction of the parietal bone and frontal bone on the midline

n The most anterior point on the nasal bone

III. Data Collection

Linear dimensions were taken with a micro-caliper (Digimatic, Mitutoyo Co, Tokyo, Japan) and recorded to the nearest 0.05 mm. Twenty craniofacial variables (Tables 1-3, Figure 2) were measured from the dry skulls and radiographs for the first study. In the second study, only the dry skull measurements were taken. In the dry skull, the bilateral measurements were measured individually and then averaged before they were recorded. In the radiograph, the middle point was taken from the bilateral landmarks. Cephalometric measurements were recorded directly from the radiographs without tracing films. To increase the precision, all variables were measured twice by the same investigator and averaged. All measurements were entered into a Microsoft™ Excel© spreadsheet for compilation of statistics. Means and standard deviations were calculated.

IV. Data Analysis

IV.1. Craniometry vs. Cephalometry

The purpose of the first part of the study was to determine the relative accuracy of the non-invasive method in characterizing the quantitative craniofacial traits by comparing the craniometric measurements with cephalometric measurements in rats. The analysis of twenty craniofacial measurements obtained from sixty-four dry skulls and 125 lateral and ventrodorsal radiographs (three lateral radiographs were missing) proceeded

in three steps. Step one involved calculating means and standard deviations for each measurement from the dry skull and radiograph for all sixty-four animals. Visual comparisons of craniometric and cephalometric means for twenty measurements were made in bar charts. Step two involved calculating the magnification of each radiograph. Means and standard deviations for magnification were calculated for each variable. Step three involved repeating step one in each of eight strains and assessing the difference between strain variations revealed by craniometry and those shown by cephalometry. Using t-tests the values for each strain were ranked for each measurement, and the differences in the order of the rank between craniometry and cephalometry were examined.

IV.2. Distribution of Variables in F1 offspring

The aim of the second part of the study was to observe the distributions of the same measurements in F1 offspring. The analysis of twenty measurements obtained from twenty-three F1 offsprings (n = 11 male, n = 12 female), three F0 parent generation (two Sprague-Dawley males and one Brown-Norway female), six month old Sprague-Dawley males (SDM 6, n = 4) and Brown-Norway females (BNF 6, n = 4), and four month old Sprague-Dawley males (SDM 4, n = 4) and Brown-Norway females (BNF 4, n = 4) proceeded in two steps. Step one involved calculating means and standard deviations for all continuous variables. Step two involved visual comparison of means and standard deviations of different groups of animals using bar

charts. The animal groups are parent and offspring group, inbred strains of SDM and BNF of different age groups, and age-matched SDM, F1 male (XSM), F1 female (XSF), and BNF group.

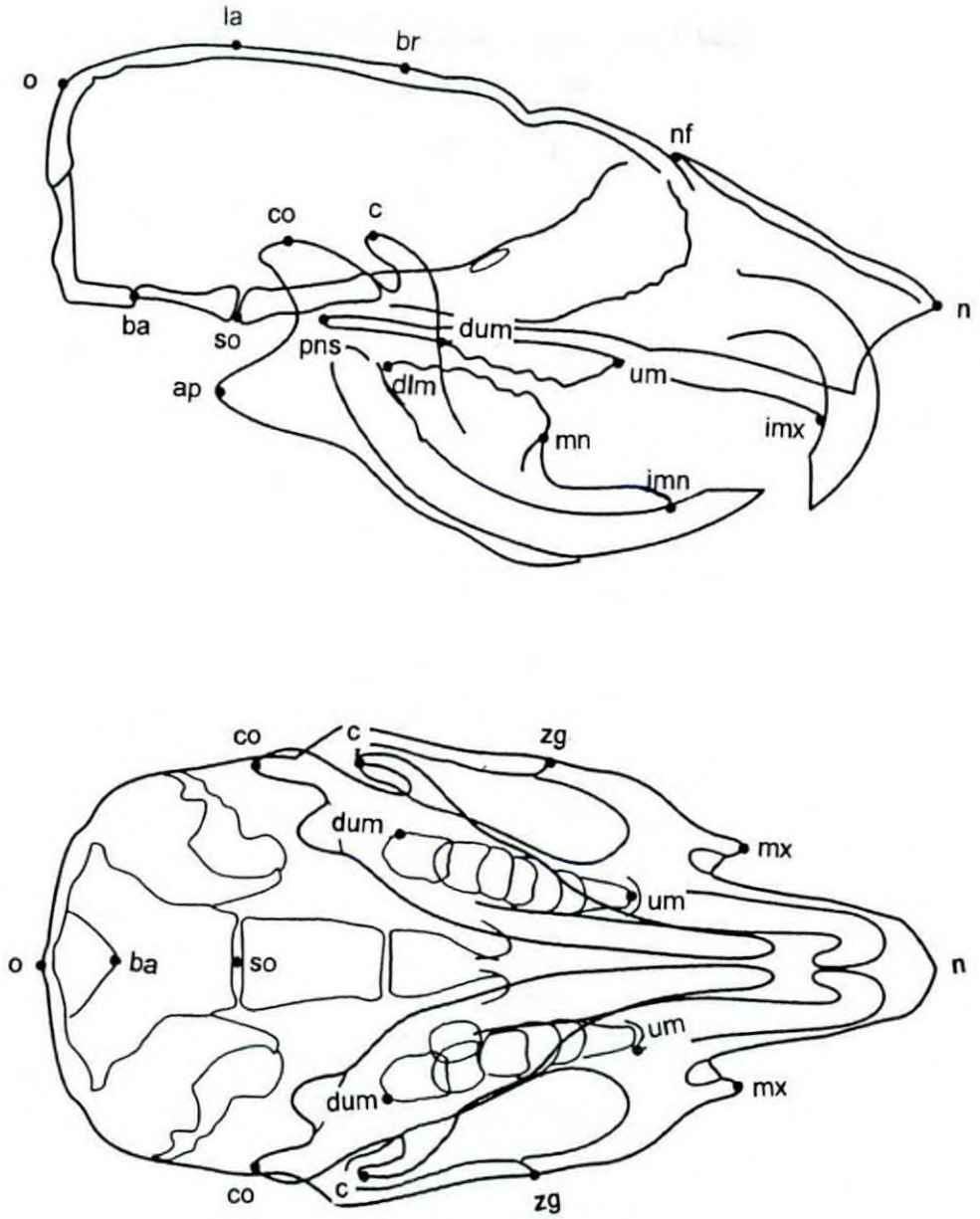


Figure 1. Anatomic landmarks in lateral view (above) and ventrodorsal view (below)
Definition of landmark abbreviations is found on pages 52-54.

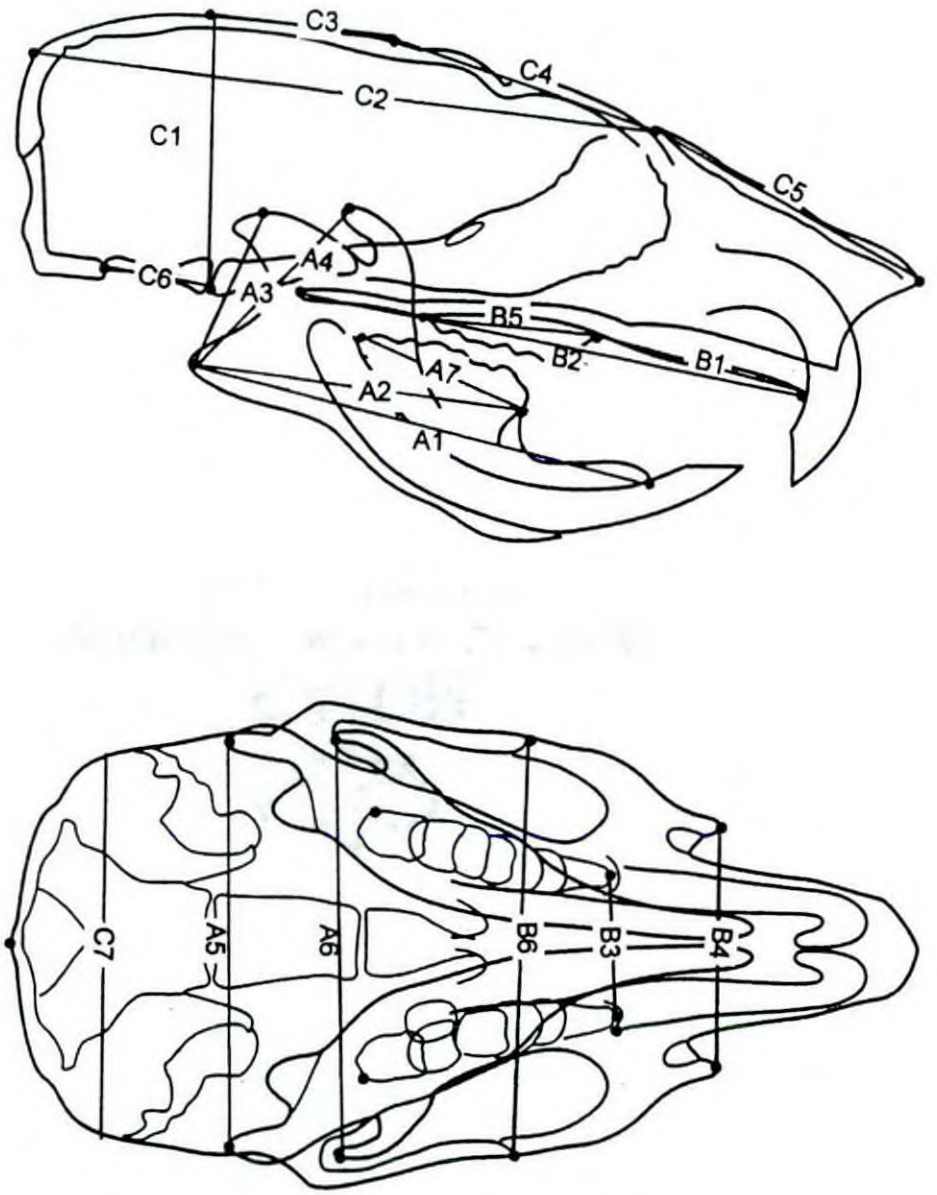


Figure 2. Variables measured in lateral view (above) and ventrodorsal view (below)

Table 1.--Variables used to measure phenotypic traits in mandible

Variables	Landmarks employed	Measures
A1	ap-imn	Mandibular body length in overall
A2	ap-mn	Mandibular body length excluding diastema
A3	ap-co	Mandibular ramus height (condylar process)
A4	ap-c	Mandibular ramus height (coronoid process)
A5	co-co	Bicondylar width
A6	c-c	Bicoronoidal width
A7	dim-mn	Mandibular teeth row length

Table 2.--Variables used to measure phenotypic traits in maxilla

Variable	Landmarks employed	Measures
B1	imx-um	Length of premaxilla
B2	imx-pns	Overall palatal length
B3	um-um	Palatal width
B4	mx-mx	Maxillary width
B5	dum-um	Maxillary teeth row length
B6	zg-zg	Width of zygomatic arch

Table 3.--Variables used to measure phenotypic traits in cranium

Variables	Landmarks employed	Measures
C1	so-la	Cranial height
C2	o-nf	Overall cranial length
C3	la-br	Parietal bone length
C4	br-nf	Frontal bone length
C5	nf-n	Nasal bone length
C6	ba-so	Occipital bone length
C7	Maximal width at occipital bone	Cranial width

RESULTS

The results will be presented in two sections. The first section will deal with the comparison of the direct measurements from the dry skulls and the same measurements from radiographs. The second section will deal with the distributions of measures of interest in an F1 cross between Brown-Norway and Sprague-Dawley strains.

I. Comparison between Craniometry and Cephalometry

Figures 3-5 show means and standard deviations (S.D.) for craniometric and cephalometric measurements in all sixty-four animals. The standard deviation of each measurement was quite similar between craniometry and cephalometry. In general, there was an observed difference in S.D.; the cephalometric S.D. was larger than the S.D. of craniometry. A notable exception to this pattern was bicondylar width (A6 (c – c)).

Tables 4-6 show the mean and S.D. of enlargement in radiograph for twenty measurements. The measurements were divided into the measurements from lateral radiographs and those from ventrodorsal radiographs. The measures of enlargement on the radiographs were not consistent. The range of magnification was 3–12%. The cranial width measurement (C7) showed the smallest mean magnification (3%) and the

measurement of parietal bone length (C3) had the greatest mean magnification (12%). When the measurements from lateral radiograph and those from ventrodorsal radiograph were compared, no consistent pattern was found.

Table 4.--Enlargement in cephalometric measurements of mandible

Mandible	Lateral					Ventrodorsal	
	A1	A2	A3	A4	A7	A5	A6
Mean	4%	6%	6%	4%	4%	9%	8%
S.D.	0.02	0.02	0.02	0.02	0.02	0.04	0.03

Table 5.--Enlargement in cephalometric measurements of maxilla

Maxilla	lateral			Ventrodorsal		
	B1	B2	B5	B3	B4	B6
Mean	4%	5%	5%	11%	5%	4%
S.D.	0.02	0.02	0.03	0.06	0.03	0.01

Table 6.--Enlargement in cephalometric measurements of cranium

Cranium	lateral					ventrodorsal	
	C1	C2	C3	C4	C5	C6	C7
Mean	7%	4%	12%	5%	4%	8%	3%
S.D.	0.02	0.02	0.05	0.04	0.04	0.04	0.01

Tables 8-13 provide means and S.D. of eight strains in all measurements, comparing craniometric and cephalometric measurements. Table 8 lists mandibular measurements in males. If the differences in the order of rank between craniometry and cephalometry were found, there was no significant strain variation of measurements in almost all cases. However, there were four exceptions. In A3 (ap – co) craniometric measurement, F was

larger than WK. However, cephalometric measurement failed to detect that difference. In A5 (co – co) craniometric measurement, LE was larger than F, and WK was larger than B. Those differences were not found in cephalometric measurements. On the other hand, WK was larger than F in A2 cephalometric measurement, which was not seen in the craniometric measurement.

Table 9 has mandibular measurements in females. As a whole, variations in order of the rank were detected in all measurements. However, statistical significance was found in A2 (ap – imn), A4 (ap – c), and A5 (co – co). For A2 measurement, BN was larger than L, but the difference was not found in the cephalometric measurement. For A4 measurement, cephalometric measurement failed to detect the strain variation between SD and LE, WK and F, and L and WK. For A5 measurement, craniometry caught the strain variation between SD and H, but cephalometry could not find it.

Table 10 presents maxillary measurements in males. Major variation was seen in palatine bone width (B3) measurement. For B3 (um – um) measurement, craniometry revealed the strain differences between L and WK, L and B, and B and WK, but cephalometry did not show the differences in those strains. Instead, cephalometric measurement of B3 reported the difference between BN and WK, which did not exist in craniometric measurement.

Table 11 shows maxillary measurements in females. As seen in males, major variation was found in B3 measurement. Cephalometric measurement failed to detect strain variation between L and F, and L and LE. Instead, the differences between BN and F, LE and SD, and BN and SD were only found for the cephalometric measurements.

Tables 12 and 13 have cranial measurements in males and females respectively. There was minor variation in the order of the ranks, but a statistically significant difference was found for only one measurement, C6 in female. For C6, the cephalometric measurement showed a difference between BN and F, which was not found for the craniometric measurement.

II. Distributions of Measurements in F1 animals

II.1. Description of the Animal Sample in the Second study

The results of the twenty measurements for eleven F1 male (XSM) and twelve F1 female (XSF) animals were compared with different age groups of inbred Sprague-Dawley males and Brown-Norway females. The Sprague-Dawley male groups included ten month old F0 parent generation (n = 2), six month old animals (n = 4) from the cephalometric study of strain variation, and 4 month old animals obtained from the vendor. The Brown-Norway female groups included the nine month old F0 parent generation (n = 1), six month old animals (n = 4) from the cephalometric study, and four month old animals (n = 4) obtained from the vendor. F0 generation animals were included in this study to compare the characteristics of parents and offspring.

Six-month-old animals were used to increase the number of adult animals in the sample. Previous rat studies (Spence 1940; Asling and Frank 1963; Moore 1966; Wright et al. 1966) showed that facial growth was almost completed by the end of the fourth month so, five-month-old rats were considered adult animals. The four-month-old animals were used because they were closest in age to the F1 animals among the available data in this study. Age, length, and weight characteristics pertaining to these animals are presented in table 7. For six-month-old animals, length and weight data were not recorded in the previous study (Moullas 1999).

In males, there was no statistically significant difference in weight or length between 109 day old SD (SDM 4) and 129.9 day old XSM animals. On the other hand, the 123.9 day old XSF animals were heavier and longer than 150 day old BN (BNF 4) or the 285 day old BNF founder. The 320-day-old SDM founder was heavier and longer than any of the SDM 4 or the XSM animals.

Table 7.--Age, weight and length characteristics of samples

Breed/Sex	Age (d)		Weight (g)		Length (cm)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
SDM F0 (n = 2)	320.0	0	497.5	13.4	25.5	0.7
SDM 4 (n = 4)	109.0	0	437.3	21.1	23.5	0.4
XSM (n = 11)	129.9	5.9	419.6	22.2	23.9	0.2
XSF (n = 12)	123.9	6.1	244.3	10.0	20.9	0.2
BNF 4 (n = 4)	150.0	0	175.0	20.8	19.9	0.6
BNF F0 (n = 1)	285.0	0	237.0	0	20.5	0

The second part of this study will be reported in three parts. First, the measurements between F1 hybrid animals with the F0 parents will be compared. Second, the measurements for the three different age groups of inbred SDM and BNF will be compared. And third, the age-matched groups (i.e. SDM 4, XSM, XSF, and BNF 4) will be compared.

II.2. Comparison between F0 and F1

Mandible

Figure 6 presents the descriptive craniometric measurements of mandible for F0 (SDM F0, BNF F0) and F1 (XSM, XSF) animals. Statistically significant differences between XSM and XSF were found in all mandibular measurements except in bicondylar width (A5 co-co) and mandibular teeth row length (A7 dlm-mn). XSM measurements were smaller than SDM F0 in almost all measurements except A2 (ap – mn) and A7 (dlm – mn), and XSF measurements were smaller than BNF F0 in almost all except A6 (c – c) and A7 (dlm – mn).

Mandibular body length was measured in two ways. A1 (ap-imn) measured overall body length and A2 (ap-mn) excluded the space between the incisors and first molars from A1. In A1 measurements, F1 hybrids were shorter than F0 parents in both sexes. On the other hand, A2 (ap – mn) measurement was larger in XSM than SDM F0.

Mandibular ramus height was measured in two ways. A3 (ap-co) measured the distance between angular process and condylar process and

A4 (ap-c) measured the distance between angular process and coronoid process. For both A3 and A4 measurement, the same pattern was found, which were smaller measurements in the F1 hybrid than the F0 parents.

Mandibular width was also measured in two ways. A5 (co-co) measured the distance between right and left condylar processes, and A6 (c-c) measured the distance between right and left coronoid processes. For A5 measurement, BNF F0 was even wider than XSM, and for A6 measurement, XSF and BNF F0 had similar values.

For mandibular teeth row length, F1 hybrids showed a slightly higher measurement in both sexes

Maxilla

Figure 7 presents the descriptive craniometric measurements for the maxilla in F0 and F1 animals. Statistically significant differences between XSM and XSF were found in all maxillary measurements.

Maxillary length was measured in two ways. B1 (um - imx) measured premaxillary length and B2 (pns – imx) measurement included premaxilla, maxilla and palatine bone length. For both B1 and B2 measurements, the characteristic pattern was that F1 values were usually between the F0 male and F0 female values.

Three different width measurements were taken in the nasomaxillary complex. B3 (um-um) measured palatine bone width, B4 (mx-mx) measured maxillary width, and B6 (zg-zg) determined zygomatic arch width. For palatine

bone width, XSF was slightly narrower than the other three groups. For both maxillary width and zygomatic arch width, the pattern that F1 was smaller than F0 in animals of the same sex was observed.

For maxillary teeth row length (B5 (dum – um)), there was no difference between male groups. Female groups had a little shorter teeth row length than male groups.

Cranium

Figure 8 presents the descriptive craniometric measurements of cranium for F0 and F1 animals. Statistically significant differences between XSM and XSF were found in all cranial measurements.

For cranial height (C1), male groups demonstrated similar values, but in the female groups XSF was shorter than female parent (F0).

In the cranium, four different length measurements were examined. C2 (o-nf) measured overall cranial length, C3 (la-br) measured parietal bone length, C4 (br-nf) measured frontal bone length, and C6 (ba-so) measured occipital bone length. In all four cranial length measurements, the same pattern of distribution was found. The F1 measurements were between those for F0 animals and males were longer than females. C5 (nf-n) measured nasal bone length, however, it was included in the cranium measurements due to the position of landmarks. For C5 (nf – n), female groups showed similar measurement values.

For cranial width measurement (C7), F1 was smaller than F0 in both sexes.

II.3. Comparison among different age groups

As a second step, three different age groups of inbred animals were compared by gender. The groups were Sprague-Dawley male (SDM) F0 (10 month), SDM 6 (6 month), and SDM 4 (4 month) in males. And there were Brown-Norway female (BNF) F0 (9 month), BNF 6 (6 month), and BNF 4 (4 month) in female groups.

Mandible

Figure 9 demonstrates means and standard deviations for mandibular measurements in the male groups. Overall mandibular body length (A1), ramus height (A3, A4), and bicoronoid process width (A6) increased slightly with age. Another length measurement, A2, did not show any difference among groups. Bicondylar width (A5) was similar in SDM 6 and SDM4, but increased in SDM F0. Mandibular teeth row length was similar in all three groups.

Figure 10 illustrates the distribution of mandibular measurements in female groups. A1, A2, A3, A4, and A5 measurements were increased continuously after four months old. BNF 6 had the largest value in A6 (c – c) measurement. There was no difference in teeth row length.

Maxilla

Figure 11 shows means and standard deviations for maxillary measurements in males. Palatine bone width (B3) and maxillary teeth row length (B5) were almost same among three groups. Overall maxillary length (B2) and zygomatic arch width (B6) were increased continuously. Increases in premaxillary bone length (B1) continued after four months. Maxillary width also increased for the SDM F0 group.

Figure 12 shows descriptive maxillary measurements in female groups. Except for teeth row length (B5), all measurements continued to increase after four months. There was no difference in B5 (dum – um) measurements.

Cranium

Figure 13 presents cranial measurements for the male groups. Cranial height (C1) and cranial length (C2) did not change much after six months. C3, C4, C5, and C6, all of which include sutural landmarks, were rather decreased in SDM F0. Cranial width (C7) slightly increased.

Figure 14 displays means and standard deviations for cranial measurements in the three female groups. C1, C2, C5 and C7 measurements gradually increased in older groups. In C3 and C4 measurement, BNF 6 values were smaller than other groups. C6 measurements were rather constant.

II.4. Age-matched Comparison: Four-month-old F1 Hybrids to Four-month-old Inbred Strains

Mandible

Figure 15 has descriptive mandibular measurements for SDM 4, F1 male (XSM), F1 female (XSF), and BNF 4 rats. Female XSF animals were larger than BNF 4 in all mandibular measurements. In A1 (ap – imn) and A5 (co – co) measurements, F1 value were between SDM 4 and BNF 4 value. In A2 (ap – mn), A3 (ap – co), A4 (ap – c) and A6 (c – c) measurements, the biggest values were found in XSM group. Teeth row length was similar in all groups of animals and showed small standard deviation.

Maxilla

Figure 16 demonstrates the distribution of maxillary measurements. The two maxillary length measurements (B1 and B2) revealed the pattern in which F1 measurements were between SDM 4 and BNF 4. In three width measurements (B3, B4, and B6), XSM was bigger than SDM and XSF was also bigger than BNF. BNF 4 had the shortest teeth row length. XSF were bigger than BNF 4 in all maxillary measurements.

Cranium

Figure 17 shows the means and standard deviations for cranial measurements in four groups. XSF had larger measurements than BNF 4 in all cranial measurements. Four length measurements in cranium (C2, C3, C5, and C6) from F1 hybrids existed between SDM 4 and BNF 4 measurements.

C1, C4, and C7 measurements of XSM were bigger than SDM4 measurements.

Finally, the means and standard deviation of all measurements in all eight groups of animals are shown in Tables 14-17.

DISCUSSION

I. Craniometry vs. Cephalometry

In studying the quantitative traits, one of the essential natures of those traits is that they should be measurable. The technique of measurement and the accuracy of methods, therefore, become important issues in the study of quantitative traits (Falconer 1996). Two methods, craniometry and cephalometry, have been used in characterizing craniofacial skeletal morphology. The objective of the first part of the present study was to investigate the relative accuracy of cephalometric measurements in identifying strain variations of craniofacial morphology in laboratory rats. The previous study at the Department of Orthodontics, Case Western Reserve University, demonstrated that dry skull measurements of eight different strains of rats could detect the phenotypic variations in craniofacial morphometric traits (Moullas et al. 1999). The question in the present study was whether the same measurements taken from radiograph could reveal the same strain variation of inbred rat strains observed in the dry skull measurements. The answer of this question will be a sound basis for determining whether or not we can use cephalometrics in the ongoing genetic study of craniofacial growth.

The measured values of skull characteristics have two sources of variation, which are biological variation and measurement errors (Engstrom et al. 1982; Mausner and Kramer 1985). As seen in the previous study, there is detectable biological variation, strain variation in this case, in the craniofacial skeleton of different strains. So, if the cephalometric measurement failed to detect the strain variation, the measurement errors in radiographs may be so large that they override the strain variation.

In dealing with the measurement errors, random error and systemic error are considered (Mausner and Kramer 1985). Random error is measurement variation mainly due to chance. It affects the reliability of measurement. To compare the difference of the reliability of the measurements in craniometry and cephalometry, all variables were measured twice and the difference between the first measurement and the second measurement was calculated. The means and standard deviation of the absolute number of the difference were computed. Table 17 demonstrates between measurement differences in craniometry and cephalometry. There existed discrepancies in the mean of between measurement differences (Table 17). Systemic error often has a recognizable source and affects validity of the measurement. If systematic error inherent in cephalometry were large, cephalometric measurements would not be suitable for the study.

The fact that the calculated enlargement of measurement (Table 1,2, and 3) did not show any consistent pattern might be explained as follows;

first, the divergence of x-ray beam from the x-ray source may cause the variation of magnification depending on the location of landmarks. Second, though the distance from the midsagittal or midfrontal plane to the x-ray source was fixed, the distance between the film and the x-ray source was varied depending on the animal size. Third, the variation may reflect the measurement error, especially the difficulty of locating the radiographic landmarks accurately.

Among the mandibular measurements, those measurements related to the condyle (A3 (ap – co), A5 (co – co)) revealed common erroneous variables. In the lateral radiograph, the condyle is superimposed by the ear rods and other structures in the cranium. So, the accuracy in finding 'co' point was decreased in the lateral radiograph. This is also seen in the human cephalogram. On the other hand, the fact that the standard deviation of A5 in radiographic enlargement showed larger value may be explained in the different way. Bicondylar or bicoronoid width in the radiograph was taken when the mandible was connected to the cranium. So, other structures were filled in the space between both processes. In the dry skull, the mandible was disconnected from the cranium and the distance between the condylar or coronoid processes seemed to be varied depending on the degree of shrinkage of bones. In both craniometric and cephalometric measurements, A1 and A2 demonstrated wide range of between measurement difference. This may be due to the vague definition of 'ap' point.

For the maxillary measurements, B3 (um – um) showed major variation in defining strain variations. The obscure definition of this point made it hard to pinpoint this landmark. This was reflected in the large mean and standard deviation of measurement enlargement in radiograph. B4 (mx – mx) measurement also had a vague definition and the large range of between measurement differences in both methods might be the evidence of it. The landmark 'pns' is the end point of thin palatal process of palatine bone. Defining this point in the lateral radiograph was not easy and this was reflected in the broad range of between measurement differences in the cephalometric measurement.

For the measurements in the cranium, those landmarks from the sutures seemed to involve inaccuracy in the cephalometric measurement. This was contrary to the statement by Spence (1940), that described one of the values of cephalometric method as revealing structural details such as sutures. When the sutures, running on the cranial surface obliquely, projected to the lateral view, the measurements from those landmarks included possible systemic errors. A larger mean enlargement and standard deviation of cephalometric measurement in C3 (la – br) and large range of between measurement differences might display the possibility of the error. However, there was no significant difference in defining strain variations between craniometry and cephalometry.

When we consider the timing of growth as an important factor in an orthodontic treatment planning procedure, the cephalometric technique suggests a potential to investigate the strain variation of the growth spurt and growth rate. Changes of growth rate in the F1 generation can also be investigated by a longitudinal study using cephalometric technique.

In studying craniofacial growth of rats, the cephalometric technique has been considered as a valuable method by several authors (Spence 1940; Asling and Frank 1963; Hughes et al. 1978; Engstrom et al. 1982). On the other hand, methodological error related to the cephalometrics forced this method to be considered as an unreliable method (Cleall et al. 1968). The results shown in this study suggested that even though there were slight variations in the cephalometric measurements, those variations had little influence on defining strain variation of craniofacial morphometric traits in laboratory rats. The cephalometric radiographic technique seems to be of value in studies of quantitative traits of rat skulls, especially because of the potential for the longitudinal growth study. The variation from the methodological error was found to be smaller than strain variations. The cephalometric radiographic technique, used to study the morphometric traits in craniofacial skeleton of the rats, was found to be suited for the purpose of detailed descriptions.

II. Distributions of Measurements in F1 Animals

II.1. Increase of Craniofacial Measurement in Aging

Several longitudinal studies of skull growth of rats (Spence 1940; Asling and Frank 1963; Moore 1966; Wright et al.1966) reported that by the end of the fourth month the growth of the craniofacial skeleton in rats is almost completed. Contrary to previous studies, the present investigation showed the increase of skull size after six months old of age. So, instead of comparing F1 animals with parent animals, age matched four months old inbred animals were used for study. Though small sample size prevents us from making a conclusive statement, the fact that all animals in this study were inbred and F1 hybrid offers a preliminary information.

II.2. Distributions of Measurements in F1 Animals

As stated by Festing (1979), the properties of individual F1 hybrid may not be predictable from the characteristics of the parental strains. The purpose of the second part of this study was to observe the distributions of measurements in F1 hybrids. This observation was expected to provide fundamental information for ongoing study. The comparison between age matched group (XSM and SDM 4, XSF and BNF 4) was major focus of the present study.

The distribution of age, weight, and length characteristics (Table 1) demonstrated a little different trend in weight and length. For weight, the

mean values of XSM and XSF were between those of SDM 4 and BNF 4. For length, XSM mean was almost same with SDM 4 mean and XSF mean was larger that BNF 4. This might reflect that the overall length component in F1 hybrid expressed earlier than in the inbred strains.

Several authors (Hatai 1907; Wright et al. 1966; Vilmann 1969) observed the gender differences in the rat skull. The present study detected this property in the F1 hybrids.

Both mandibular teeth row length (A7) and maxillary teeth row length (B5) did not have any between group difference. Individual differences in the group were also very small. This might suggest that dental component is fixed between these strains. This may relate to the finding of Harris and Johnson (1991), who showed dental components had low heritability. In the mandibular length measurement, the trend shown in the A1 (ap - imn) measurement disappeared. This might reflect that A2 (ap – mn) measurement contained the variation of teeth component, diastema between incisors and first molars.

As was mentioned in the previous section, the measurements of bicondylar width and bicoronoid process width in dry skull have some potential of error, due to shrinkage after removing soft tissue component between two processes. The larger range of standard deviation may reflect that possibility.

The rat study by Atchley (1983) and human study by Arya et al. (1973) both indicated that among mandibular length, width and height dimensions, the mandibular height is more susceptible to the environment and is less heritable. The present investigation did not reveal a different trend in the distribution of measurements among these three dimensions. Instead, mandibular height measurements (A3 and A4) demonstrated less variation within group animals.

Among female groups, all measurements from XSF were larger than BNF4. XSF group was larger than BNF6 group in most of the dimensions except ramus height (A3 and A4), mandibular width (A5 and A6), palatine bone width (B3), and cranial height (C1). The finding that in all three-height measurements of the present study older groups (BNF6 and BNF0) demonstrated bigger value might reflect the fact that for the three planes of space in the craniofacial complex, growth in the height dimension is completed last (Proffit 1993).

While explaining phenotypic uniformity in inbred strain, Festing (1979) mentioned that F1 hybrids tended to be more uniform than inbred strains. Inbreeding may result in an increased sensitivity to environmental influences and this may lead to an increased phenotypic variation compared to F1 hybrid. Alternatively, the rats may not be bred to complete homozygosity. This phenomenon was demonstrated in some variables such as mandibular body length (A1), bicondylar width (A5), premaxillary bone length (B1), maxillary

length (B2), cranial height (C1), frontal bone length (C4), and nasal bone length (C5).

II.3. Patterns in the Measurement Distributions of Inbred and F1 Hybrid Animals

Certain patterns of measurement distributions were found in the comparison of inbred parent animals and F1 hybrid animals. To analyze the patterns, three factors of variation need to be considered. Those factors are strain variations, which are variations in Sprague-Dawley and Brown-Norway strains, age variation, which means difference from four-month-old F1 animals and fully-grown parents, and gender differences. The comparisons in Figures 6-8, which compared parent animals and F1 hybrid animals, included all three factors. For example, the distribution of B2 measurement in Figure 7 showed the values for F1 animals are between the values for F0 animals. The variables in this distribution pattern comparison included the characteristics of full-grown Sprague-Dawley male, full-grown Brown-Norway female, growing F1 hybrid male, and growing F1 hybrid female. Therefore, the comparison in Figures 6-8 contained all three factors, strain, age, and gender.

The age-matched comparison in Figures 15-17 excluded the age factor. The distribution of measurement in Figure 15-17 included the variation of strain and gender.

To exclude the gender factor, different sets of comparison were made (Figures 18-23). Six-month-old inbred Sprague-Dawley and Brown-Norway

male and female data were available from the previous cephalometric study. The comparison of Sprague-Dawley male (SDM 6), male F1 (XSM), and Brown-Norway male (BNM 6) or the comparison of Sprague-Dawley female (SDF 6), female F1 (XSF), and Brown-Norway female (BNF 6) ruled out the gender factor and that comparison included strain and age factors (Figures 18, 20, and 22).

By examining these factors, three unique categories of distribution patterns were found in F1 measurement distribution. The first type is the pattern that the measurements of F1 animals are between parents' measurements. For this type, the distribution in the age-matched comparison and the same gender comparison also showed the same pattern. In the same gender comparison, F1 values were between the same gender Brown-Norway and Sprague-Dawley. The variables which belonged to this category were A1 (ap – imn), B1 (um – imx), B2 (pns – imx), C2 (o – nf), C3 (la – br), C4 (br – nf), C5 (nf – n), and C6 (ba – so). All length measurements except A2 (ap – mn) were in this group. The strain variation of these variables were larger in inbred Brown-Norway and Sprague-Dawley animals. There was large sexual dimorphism in these groups of measurements.

The second type of distribution was the pattern that the F1 values were smaller than parents' value in both sexes but F1 values were bigger than age-matched inbred strains (SDM 4 and BNF 4). The strain variation in the same gender comparison was small and F1 values were smaller than both strain

values in the same gender comparison (Figures 18-23). Sexual dimorphism was present in the measurements of this category. This type included A3 (ap – co), A4 (ap – c), A5 (co – co), A6 (c – c), C1 (height), and C7 (width). The sub-type of this pattern was seen in B4 (mx – mx) and B6 (zg – zg). These two measurements showed the same pattern as the main pattern in the parent-offspring comparison and age-matched comparison. In the same gender comparison with the two inbred strains, however, female F1 measurements were between SDF 6 and BNF 6, and male F1 measurements were larger than SDM 6 and BNM 6. This second group contained the width measurements in mandible, maxilla, and cranium.

The last pattern was the type that did not show any difference among group measurements. These measurements didn't show any strain variation in the same gender comparison and didn't show any sexual dimorphism, either. Two teeth row measurements (A7 and B5) belonged to this category and palatine bone width (B3) fell into this pattern.

In conclusion, the findings in the present study suggested various inheritance patterns of the craniofacial morphometric traits in rats. The study of the patterns in the F1 generation provided the basic information for future study.

III. Future Study

The present study is a starting point of an ongoing project of genetic study at the Department of Orthodontics in Case Western Reserve University.

The first step has been to characterize the phenotypes of various inbred strains of rats and identify strain variation of phenotypes. The next step will be to inspect the phenotypic traits in F2 animals. Assessing the association between variation in the phenotype and allelic variation at genetic markers will be the following step to move toward identifying genes.

Study of the traits in F2 animals will make it possible to conduct a quantitative genetic analysis in which the importance of genetic and non-genetic components of variance is evaluated. Phenotypic variations detected in the F1 generation are ascribed mainly to environmental factors, since all individuals in F1 generation are genetically identical. Phenotypic variations of F2 generation will be partitioned into genetic and environmental factors. Therefore, genetic variance can be estimated by subtracting phenotypic variations in F1 generation from those in F2 generation. By calculating the contribution of genetic variance to the phenotypic variation, the heritability of certain traits will be estimated. This information is very useful in determining the target of orthodontic and orthopedic treatment. For maximizing the treatment effect and the stability of treatment result, the traits, which are less heritable and more susceptible to environment, need to be defined in clinical orthodontics.

Slavkin (1999) mentioned that genetic homologies provided substantial support for using animal models to study growth and development in human. The comparative mapping of rat, mouse, and human have been reported by

several authors (Nadeau 1989; Levan et al. 1991; Seldin 1999). Thus, findings from this rat genetic study will provide fundamental information in human genetics.

SUMMARY AND CONCLUSIONS

There were two objectives in the present study. The first purpose was to decide relative accuracy of the cephalometric measurements in the study of craniofacial morphometric traits in rats. And the second aim was to examine the distribution of measurements in the F1 rats obtained from a Sprague-Dawley and Brown-Norway cross.

For the first purpose, twenty measurements were taken from the rat skulls in eight different strains and the same measurements were taken from the lateral and ventrodorsal radiographs of the same rats. The results shown in this study suggested that even though there were slight variations in the cephalometric measurements, those variations had little influence on defining strain variation of craniofacial morphometric traits in laboratory rats. The cephalometric radiographic technique seems to be of value in studies of quantitative traits of rat skeleton, especially because of its potential for longitudinal assessment.

For the second purpose, comparisons were made in parent-offspring group, age-matched inbred-F1 group and same gender inbred-F1 group. Three different types of measurement groups were found. The first group of measurements had large strain variation and gender difference. In this type

of measurements, F1 values were between the values for parents. Most of the length measurements belonged to this category. The second pattern of measurements showed small strain variation in the same sex comparison and the F1 values were smaller than the same sex inbred strains. Sexual dimorphism was present in this category. The measurements of F1 animals were smaller than those of parents but larger than those of age-matched animals. The width and height measurements were included in this group. The third pattern of measurement distribution showed little difference of measurements in F1 animals and parents animals. There was little strain variation and sexual dimorphism in this type of measurements. Teeth measurements fell into this category.

The findings in the present study suggest various inheritance patterns of the quantitative morphometric traits in the craniofacial complex in rats. The observation of the F1 generation provides the fundamental information needed to investigate mechanisms of inheritance and identify candidate genes for future studies of craniofacial morphogenesis.

Table 8.--Means and S.D. of mandibular measurements in 32 male rats

	A1 (ap - imn)				A2 (ap - mn)				A3 (ap - co)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNM	26.80	0.60	27.86	0.70	20.43	0.18	21.82	0.54	13.76	0.43	14.37	0.55
SDM	28.15	0.28	28.92	0.27	20.03	0.41	20.82	0.34	13.99	0.51	14.75	0.28
LM	26.44	0.98	27.75	0.99	19.68	0.44	20.86	0.69	12.13	0.35	12.87	0.29
FM	26.08	0.39	27.09	0.27	18.96	0.46	20.10	0.43	13.23	0.21	13.84	0.22
WKM	25.86	0.26	27.56	0.27	19.01	0.14	21.07	0.12	12.55	0.14	13.92	0.00
LEM	28.88	0.35	30.78	0.57	21.01	0.44	23.28	0.53	14.95	0.32	15.43	0.38
HM	29.78	0.79	31.20	0.39	21.46	0.54	23.08	0.26	14.31	0.42	15.22	0.11
BM	27.96	0.57	28.97	0.75	20.45	0.74	21.53	0.77	13.51	0.50	14.30	0.44

	A4 (ap - c)				A5 (co - co)				A6 (c - c)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNM	16.24	0.46	16.59	0.52	18.64	0.37	19.47	0.48	18.48	0.52	19.80	0.59
SDM	15.93	0.23	16.26	0.36	17.54	0.42	19.34	0.72	18.57	0.63	20.20	0.40
LM	14.21	0.33	14.80	0.37	17.65	0.41	19.32	0.09	17.42	0.49	18.96	0.14
FM	14.73	0.21	15.26	0.30	17.05	0.18	19.44	0.31	18.23	0.32	20.33	0.46
WKM	14.06	0.16	15.21	0.27	17.29	0.13	19.08	0.46	19.51	0.26	20.62	0.11
LEM	16.72	0.32	18.09	0.39	18.33	0.55	19.42	0.85	20.19	0.66	21.18	1.00
HM	16.37	0.29	17.17	0.46	18.84	0.12	20.50	0.21	20.63	0.52	21.98	0.48
BM	15.50	0.25	16.04	0.39	16.83	0.24	19.19	0.70	17.68	0.36	19.38	0.49

	A7 (dlm - mn)			
	CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.
BNM	7.47	0.16	7.78	0.23
SDM	7.29	0.16	7.51	0.16
LM	7.59	0.10	7.77	0.13
FM	7.29	0.08	7.48	0.06
WKM	7.65	0.08	8.03	0.17
LEM	7.94	0.23	8.39	0.20
HM	7.87	0.12	8.17	0.14
BM	7.53	0.12	7.75	0.21

Table 9.--Means and S.D. of mandibular measurements in 32 female rats

	A1 (ap - imn)				A2 (ap - mn)				A3 (ap - co)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNF	24.47	0.16	25.16	0.35	18.49	0.08	19.54	0.16	12.57	0.30	13.03	0.25
SDF	26.54	0.30	26.88	0.40	18.72	0.45	19.37	0.34	12.40	0.25	13.34	0.23
LF	24.85	0.24	26.25	0.21	18.20	0.17	19.75	0.35	11.27	0.25	12.07	0.31
FF	23.85	0.16	24.78	0.48	17.31	0.39	18.52	0.61	11.70	0.13	12.41	0.47
WKF	24.01	0.36	25.11	0.17	17.55	0.32	18.68	0.50	11.58	0.14	12.45	0.25
LEF	26.27	0.70	27.16	0.65	19.15	0.62	20.36	0.48	12.47	0.34	13.36	0.38
HF	27.62	0.46	28.36	0.36	20.26	0.44	21.43	0.57	12.72	0.17	13.42	0.34
BF	26.19	0.73	26.92	1.01	18.98	0.72	20.05	0.70	12.45	0.92	13.05	0.70

	A4 (ap - c)				A5 (co - co)				A6 (c - c)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNF	14.66	0.35	14.92	0.42	17.81	0.38	18.46	0.18	17.73	0.34	18.69	0.27
SDF	14.67	0.14	14.80	0.07	17.97	0.63	19.40	0.13	18.04	0.90	19.52	0.25
LF	13.20	0.07	13.84	0.13	16.82	0.26	18.47	0.62	16.26	0.28	17.86	0.37
FF	13.34	0.08	13.87	0.26	16.52	1.04	17.97	0.36	17.02	0.92	18.78	0.36
WKF	13.02	0.05	14.03	0.32	16.48	0.18	17.76	0.30	17.68	0.55	18.66	0.46
LEF	14.24	0.16	14.96	0.28	17.38	0.39	18.89	0.58	18.10	0.58	19.51	0.53
HF	15.11	0.20	15.71	0.41	17.87	0.22	20.16	0.34	18.22	0.58	20.23	0.55
BF	14.36	0.58	15.07	0.55	16.73	0.75	18.65	0.62	16.98	0.75	18.49	0.52

	A7 (dlm - mn)			
	CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.
BNF	7.39	0.09	7.61	0.08
SDF	7.33	0.15	7.47	0.16
LF	7.61	0.37	7.78	0.20
FF	7.24	0.10	7.52	0.08
WKF	7.28	0.03	7.55	0.13
LEF	7.79	0.27	8.12	0.36
HF	7.95	0.23	8.17	0.15
BF	7.47	0.21	7.88	0.30

Table 10.--Means and S.D. of maxillary measurements in 32 male rats

	B1 (imx - um)				B2 (imx - pns)				B3 (um - um)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNM	12.54	0.44	12.97	0.43	22.80	0.77	23.65	0.67	6.79	0.03	7.10	0.28
SDM	14.82	0.32	15.44	0.34	25.11	0.55	26.26	0.42	6.68	0.26	7.37	0.43
LM	13.22	0.39	13.47	0.38	22.90	0.52	23.97	0.51	7.26	0.09	7.68	0.23
FM	13.65	0.21	14.25	0.23	23.09	0.57	24.32	0.21	6.75	0.14	7.49	0.28
WKM	12.79	0.27	13.75	0.15	22.72	0.25	24.17	0.21	6.69	0.10	7.93	0.39
LEM	15.05	0.47	15.68	0.48	25.37	0.50	26.47	0.81	7.17	0.14	8.16	0.32
HM	15.35	0.30	16.02	0.45	25.92	0.69	27.61	0.59	7.56	0.16	8.38	0.20
BM	14.48	0.57	15.06	0.67	24.96	0.97	26.19	1.07	6.88	0.04	7.89	0.35

	B4 (mx - mx)				B5 (dum - um)				B6 (zg - zg)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNM	11.70	0.41	12.38	0.57	7.64	0.22	7.97	0.20	25.54	0.78	26.22	0.71
SDM	12.21	1.30	12.58	1.52	7.83	0.16	8.17	0.17	25.54	0.31	26.58	0.46
LM	11.44	0.46	11.98	0.44	7.56	0.09	8.00	0.11	24.42	0.65	25.29	0.57
FM	10.18	0.22	10.76	0.09	7.40	0.07	7.94	0.18	23.98	0.25	24.94	0.25
WKM	11.38	0.25	11.75	0.31	8.27	0.25	8.56	0.07	25.07	0.41	26.11	0.30
LEM	12.59	0.49	13.15	0.25	8.09	0.14	8.70	0.26	26.95	0.73	27.58	0.96
HM	13.25	0.29	13.82	0.22	8.21	0.20	8.81	0.21	27.42	0.39	28.51	0.24
BM	11.95	0.55	12.77	0.48	7.76	0.28	8.24	0.25	25.58	0.32	26.56	0.56

Table 11.--Means and S.D. of maxillary measurements in 32 female rats

	B1 (imx - um)				B2 (imx - pns)				B3 (um - um)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNF	11.77	0.14	12.11	0.17	21.18	0.27	21.86	0.37	6.55	0.13	6.93	0.05
SDF	13.40	0.34	14.00	0.34	23.45	0.37	24.56	0.37	6.49	0.18	7.25	0.23
LF	12.16	0.13	12.62	0.13	21.20	0.65	22.38	0.15	7.20	0.19	7.63	0.09
FF	12.40	0.15	12.88	0.31	20.92	0.71	22.52	0.20	6.36	0.08	7.49	0.20
WKF	11.62	0.14	12.50	0.22	20.92	0.46	22.38	0.43	6.37	0.25	7.33	0.59
LEF	13.10	0.28	13.63	0.32	22.37	0.62	23.89	0.56	6.70	0.19	7.67	0.14
HF	14.21	0.25	14.74	0.32	24.16	0.71	25.48	0.44	7.31	0.25	8.23	0.12
BF	12.92	0.46	13.41	0.44	22.86	0.56	23.98	0.35	6.73	0.26	7.35	0.33

	B4 (mx - mx)				B5 (dum - um)				B6 (zg - zg)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNF	10.91	0.40	11.37	0.36	7.67	0.13	7.92	0.17	23.00	0.34	23.79	0.32
SDF	12.02	0.23	12.44	0.12	7.75	0.13	8.07	0.10	23.91	0.13	24.78	0.21
LF	10.50	0.24	10.92	0.21	7.42	0.20	7.87	0.10	22.63	0.32	23.52	0.26
FF	9.15	0.04	9.90	0.12	7.19	0.18	7.43	0.34	21.31	0.32	22.31	0.31
WKF	10.86	0.30	11.24	0.22	8.21	0.42	8.33	0.65	22.70	0.60	23.72	0.45
LEF	10.56	0.38	11.56	0.19	7.68	0.18	8.15	0.32	23.58	0.56	24.66	0.44
HF	12.17	0.54	12.84	0.41	8.09	0.12	8.55	0.21	25.09	0.54	26.10	0.58
BF	11.54	0.35	12.14	0.28	7.57	0.17	8.07	0.24	23.54	0.64	24.57	0.58

Table 12.--Means and S.D. of cranial measurements in 32 male rats

	C1 (height)				C2 (length)				C3 (la - br)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNM	13.75	0.31	14.43	0.15	27.78	0.32	28.35	0.47	6.62	0.33	7.48	0.56
SDM	13.42	0.27	14.11	0.29	32.49	0.56	33.79	0.52	8.68	0.26	9.18	0.46
LM	13.55	0.25	14.27	0.20	29.07	0.62	30.34	0.94	7.71	0.31	8.67	0.17
FM	12.33	0.22	13.32	0.19	30.26	0.44	31.66	0.39	8.71	0.43	10.02	0.31
WKM	14.27	0.20	15.12	0.01	29.71	0.49	32.04	1.04	8.18	0.35	8.86	0.57
LEM	14.08	0.35	15.12	0.43	33.01	0.48	34.21	0.62	8.26	0.72	9.04	0.50
HM	14.05	0.28	15.06	0.31	33.21	1.39	34.28	1.49	8.63	0.99	9.30	0.94
BM	13.45	0.19	14.46	0.37	32.72	0.57	34.16	0.64	9.48	0.49	10.48	0.51

	C4 (br - nf)				C5 (nf - n)				C6 (ba - so)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNM	14.07	0.26	14.89	0.34	18.20	0.67	19.07	0.86	7.50	0.14	8.03	0.13
SDM	16.16	0.32	17.16	0.62	20.17	0.48	21.09	0.61	8.34	0.22	8.74	0.24
LM	14.14	0.47	14.90	0.73	18.24	0.25	18.78	0.32	7.64	0.22	8.21	0.06
FM	14.26	0.39	14.94	0.44	19.43	0.26	20.02	0.73	7.87	0.06	8.29	0.21
WKM	14.33	0.17	16.16	0.01	18.05	0.47	18.56	0.33	7.39	0.30	7.95	0.15
LEM	16.46	0.49	17.34	0.64	21.34	0.25	22.77	0.71	8.12	0.44	8.84	0.15
HM	16.55	1.03	16.79	0.94	21.07	0.34	22.54	0.74	8.72	0.16	9.39	0.05
BM	15.58	0.44	16.57	0.34	19.87	0.31	20.27	0.28	8.21	0.07	8.65	0.13

	C7 (width)			
	CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.
BNM	19.37	0.52	19.99	0.57
SDM	19.39	0.33	19.81	0.18
LM	18.97	0.19	19.45	0.29
FM	18.84	0.15	19.73	0.20
WKM	19.51	0.12	20.10	0.24
LEM	21.03	0.66	21.74	0.47
HM	20.55	0.56	21.25	0.56
BM	19.56	0.46	20.27	0.47

Table 13.--Means and S.D. of cranial measurements in 32 female rats

	C1 (height)				C2 (length)				C3 (la - br)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNF	12.41	0.15	13.10	0.10	26.40	0.38	26.98	0.21	6.53	0.36	7.56	0.36
SDF	12.39	0.09	12.98	0.06	30.48	0.84	31.68	0.95	8.79	0.36	9.75	0.28
LF	12.69	0.11	13.61	0.15	27.58	0.54	28.40	0.40	7.38	0.29	8.48	0.25
FF	11.54	0.15	12.68	0.11	27.62	0.55	28.87	0.54	7.87	0.20	8.83	0.42
WKF	13.48	0.18	14.38	0.13	28.48	0.53	29.74	1.04	7.78	0.22	8.45	0.34
LEF	12.58	0.35	13.55	0.27	29.32	0.70	30.35	0.84	7.71	0.52	9.00	0.73
HF	13.07	0.22	13.82	0.14	31.50	1.01	32.78	0.60	8.40	0.30	9.19	0.38
BF	12.55	0.45	13.34	0.38	29.16	0.78	30.44	0.88	8.64	0.29	9.64	0.44

	C4 (br - nf)				C5 (nf - n)				C6 (ba - so)			
	CRANIO		CEPHALO		CRANIO		CEPHALO		CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
BNF	13.27	0.30	13.73	0.17	16.99	0.23	17.50	0.23	6.57	0.18	7.14	0.23
SDF	14.81	0.36	15.28	0.32	18.16	0.65	19.07	0.68	7.66	0.15	7.95	0.12
LF	13.33	0.19	13.56	0.15	17.16	0.12	17.99	0.38	7.04	0.08	7.93	0.16
FF	13.07	0.48	13.97	0.46	17.15	0.27	17.58	0.69	6.81	0.20	7.57	0.13
WKF	13.55	0.19	14.36	0.73	16.44	0.31	17.07	0.40	6.62	0.09	7.31	0.15
LEF	14.67	0.33	14.97	0.44	18.66	0.61	19.21	0.54	6.91	0.18	7.55	0.18
HF	15.85	0.37	16.76	1.20	19.27	0.77	20.21	1.47	7.65	0.18	8.55	0.11
BF	13.86	0.46	14.60	0.39	18.13	0.56	18.73	0.65	7.46	0.31	8.08	0.11

	C7 (width)			
	CRANIO		CEPHALO	
	MEAN	S.D.	MEAN	S.D.
BNF	17.57	0.09	18.02	0.14
SDF	17.99	0.17	18.47	0.24
LF	17.95	0.10	18.42	0.13
FF	17.45	0.06	17.94	0.22
WKF	17.68	0.22	18.28	0.47
LEF	18.59	0.05	19.16	0.20
HF	19.23	0.55	19.77	0.47
BF	18.66	0.40	19.31	0.39

Table 14.—Means and S.D. of mandibular measurements in SDM F0, SDM 6, SDM 4, XSM, XSF, BNF F0, BNF 6 and BNF 4

	A1 ap-imn		A2 ap-mn		A3 ap-co		A4 ap-c		A5 co-co		A6 c-c		A7 dlm-mn	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
SDM F0	28.51	0.83	20.33	0.54	14.00	0.01	16.31	0.37	18.50	0.41	18.96	0.46	7.43	0.16
SDM 6	28.15	0.28	20.03	0.41	13.99	0.51	15.93	0.23	17.54	0.42	18.57	0.63	7.29	0.16
SDM 4	27.92	0.59	20.11	0.37	13.12	0.12	14.61	0.20	17.69	0.85	17.56	0.46	7.67	0.06
XSM	27.58	0.32	20.53	0.39	13.15	0.25	15.59	0.19	17.52	0.73	18.31	0.67	7.67	0.09
XSF	25.46	0.51	18.75	0.34	11.94	0.28	14.03	0.30	17.09	0.58	17.09	0.51	7.56	0.14
BNF F0	25.63		19.03		13.08		14.92		17.88		17.04		7.31	
BNF 6	24.47	0.16	18.49	0.08	12.57	0.30	14.66	0.35	17.81	0.38	17.73	0.34	7.39	0.09
BNF 4	23.82	0.63	17.91	0.25	11.48	0.16	13.57	0.35	16.84	0.64	16.66	0.67	7.27	0.09

Table 15.—Means and S.D. of maxillary measurements in SDM F0, SDM 6, SDM 4, XSM, XSF, BNF F0, BNF 6 and BNF 4

	B1 imx-um		B2 imx-pns		B3 um-um		B4 mx-mx		B5 dum-um		B6 zg-zg	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
SDM F0	14.68	0.66	25.58	0.71	6.78	0.12	13.10	0.17	8.07	0.05	25.98	0.02
SDM 6	14.82	0.32	25.11	0.55	6.68	0.26	12.21	1.30	7.83	0.16	25.54	0.31
SDM 4	13.56	0.28	24.20	0.51	6.68	0.15	12.25	0.19	7.95	0.17	24.61	0.22
XSM	13.51	0.24	23.86	0.30	6.80	0.13	12.37	0.21	8.02	0.12	25.66	0.36
XSF	12.53	0.44	22.20	0.40	6.40	0.16	11.23	0.24	7.80	0.11	23.33	0.44
BNF F0	11.88		21.43		6.74		11.59		7.58		23.76	
BNF 6	11.77	0.14	21.18	0.27	6.55	0.13	10.91	0.40	7.67	0.13	23.00	0.34
BNF 4	10.94	0.47	20.02	0.51	6.03	0.11	9.86	0.26	7.44	0.15	21.36	0.46

Table 16.—Means and S.D. of cranial measurements in SDM F0, SDM 6, SDM 4, XSM, XSF, BNF F0, BNF 6 and BNF 4

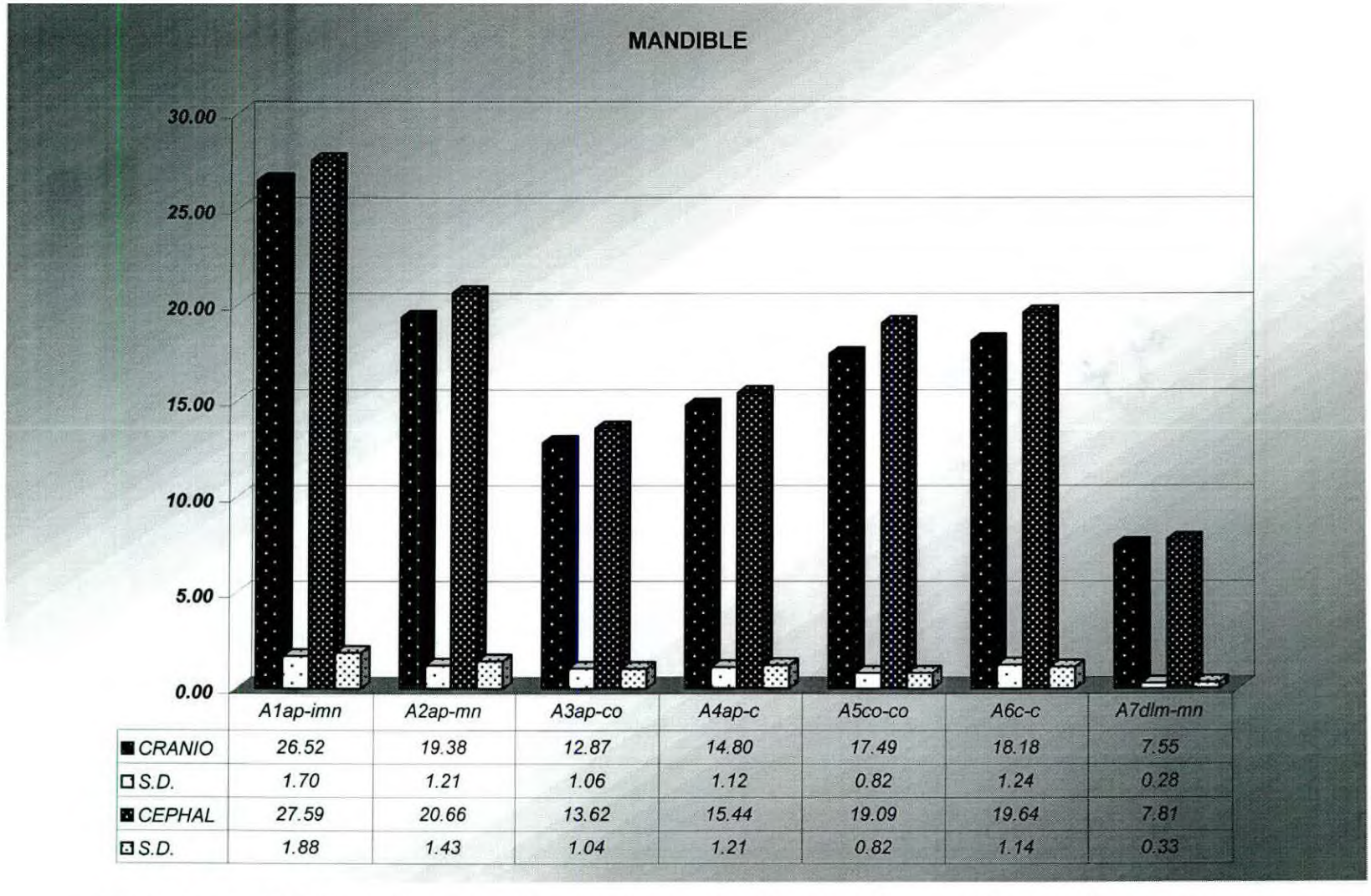
	C1 height		C2 length		C3 la-br		C4 br-nf		C5 nf-n		C6 ba-so		C7 width	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
SDM F0	13.52	0.24	32.18	0.07	8.62	0.08	15.91	0.05	19.40	1.20	8.13	0.22	19.59	0.22
SDM 6	13.42	0.27	32.49	0.56	8.68	0.26	16.16	0.32	20.17	0.48	8.34	0.22	19.39	0.33
SDM 4	12.68	0.30	30.99	0.51	9.07	0.30	15.33	0.42	18.76	0.36	7.49	0.19	18.82	0.19
XSM	13.44	0.21	30.26	0.33	8.01	0.30	15.38	0.34	18.71	0.35	7.48	0.11	19.05	0.19
XSF	12.29	0.13	28.18	0.58	7.45	0.32	14.32	0.37	17.16	0.30	6.82	0.15	17.81	0.23
BNF F0	12.99		26.73		7.30		13.82		17.18		6.53		18.30	
BNF 6	12.41	0.15	26.40	0.38	6.53	0.36	13.27	0.30	16.99	0.23	6.57	0.18	17.57	0.09
BNF 4	11.89	0.33	25.68	0.43	6.80	0.09	13.41	0.77	15.39	1.08	6.31	0.14	16.78	0.36

Table 17.--Between measurement difference in Craniometry and Cephalometry

	|M2 – M1|[1] Craniometry		|M2 – M1| Cephalometry		
	Mean	S.D.	Mean	S.D.	
A1 ap-imn	0.52	0.35	0.41	0.36	
A2 ap-mn	0.40	0.32	0.50	0.37	
A3 ap-co	0.11	0.09	0.36	0.31	**
A4 ap-c	0.30	0.25	0.30	0.28	
A5 co-co	0.10	0.09	0.27	0.23	**
A6 c-c	0.13	0.10	0.31	0.27	**
A7 dim-mn	0.14	0.12	0.22	0.20	**
B1 um-imx	0.13	0.11	0.16	0.13	
B2 pns-imx	0.14	0.12	0.29	0.34	**
B3 um-um	0.20	0.16	0.32	0.28	**
B4 mx-mx	0.31	0.22	0.38	0.30	
B5dum-um	0.12	0.11	0.26	0.21	**
B6 zg-zg	0.09	0.08	0.22	0.18	**
C1 height	0.15	0.14	0.25	0.21	**
C2 length	0.29	0.24	0.26	0.25	
C3 la-br	0.53	0.28	0.36	0.38	**
C4 br-nf	0.26	0.24	0.30	0.44	
C5 nf-n	0.13	0.14	0.31	0.32	**
C6 ba-so	0.12	0.17	0.37	0.27	**
C7 width	0.09	0.09	0.19	0.17	**

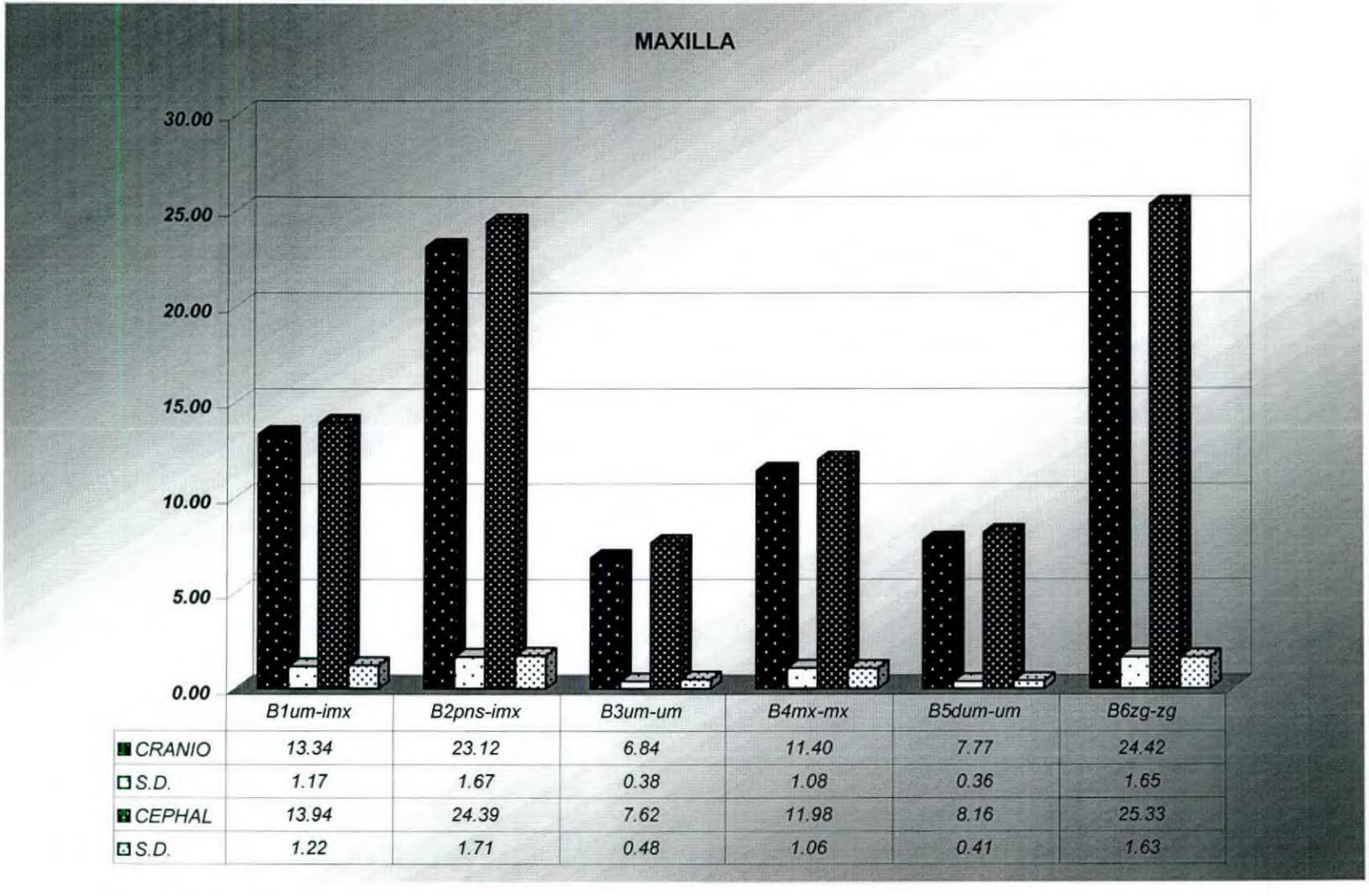
[1]absolute number of difference between 2nd measurement and 1st measurement

** $p < 0.01$



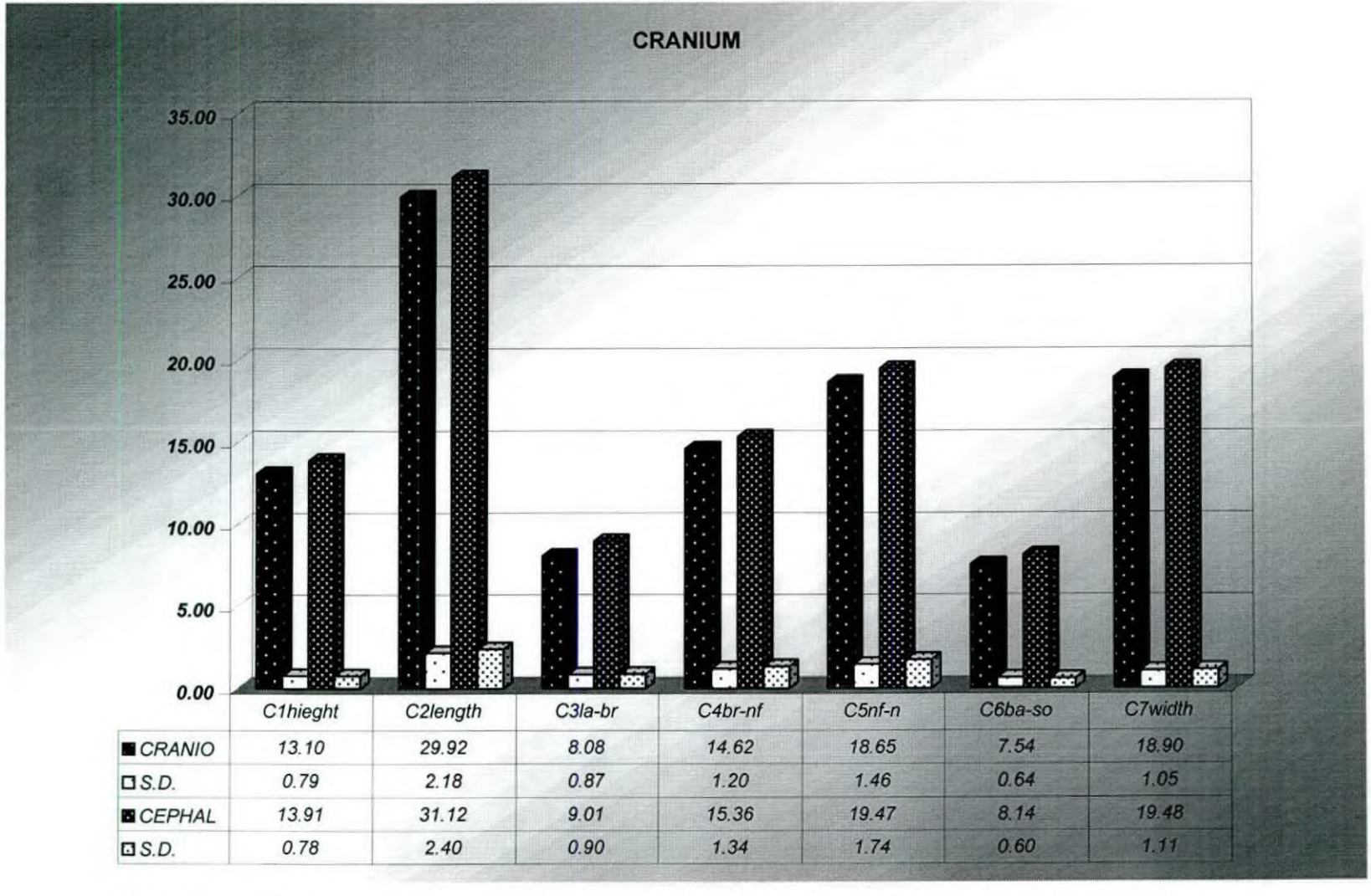
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
CRANIO	26.52	19.38	12.87	14.80	17.49	18.18	7.55
S.D.	1.70	1.21	1.06	1.12	0.82	1.24	0.28
CEPHAL	27.59	20.66	13.62	15.44	19.09	19.64	7.81
S.D.	1.88	1.43	1.04	1.21	0.82	1.14	0.33

Figure 3. Means and S.D. of mandibular measurements from dry skulls (CRANIO) and radiographs (CEPHAL) in 64 rats



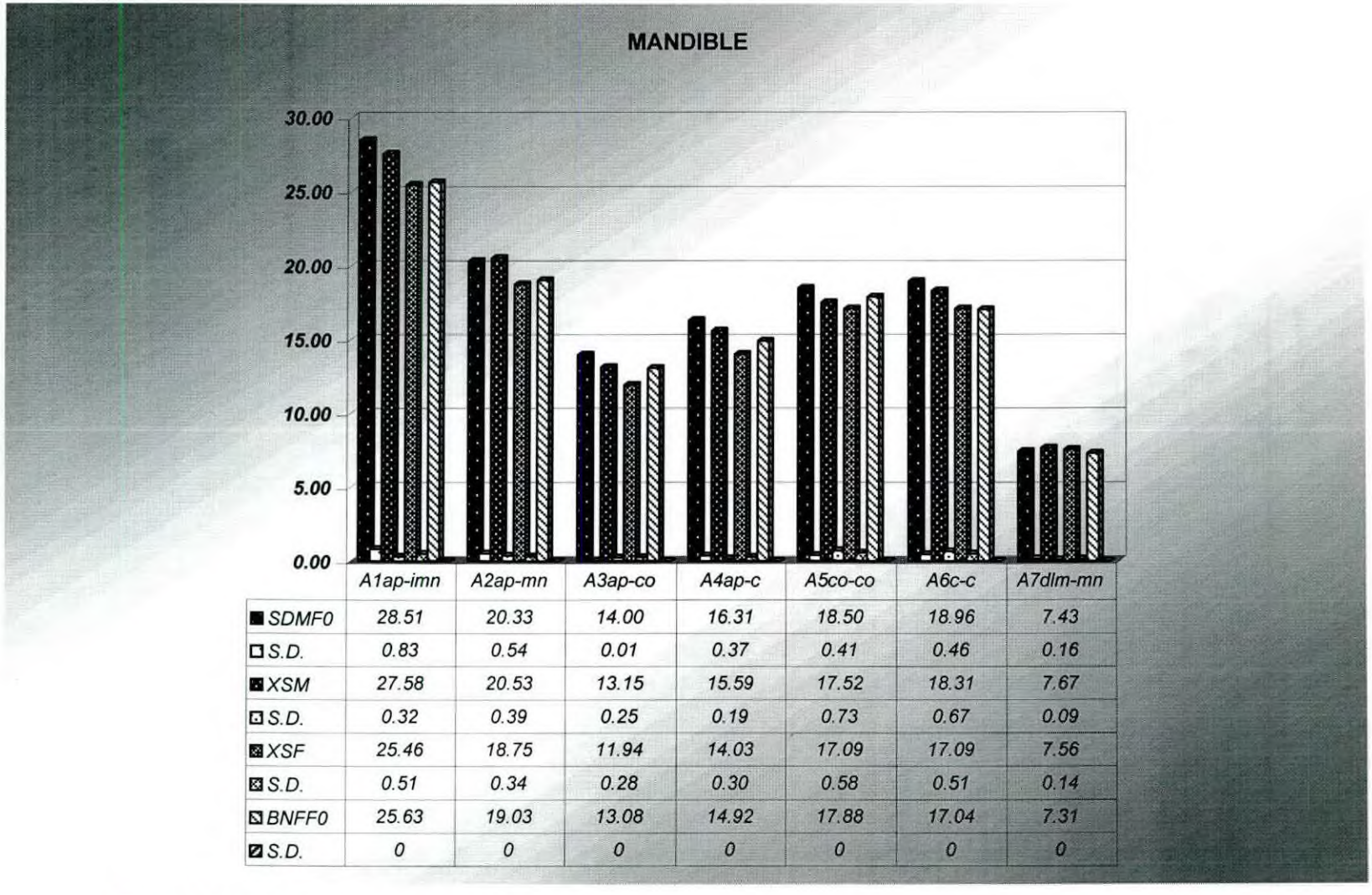
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
CRANIO	13.34	23.12	6.84	11.40	7.77	24.42
S.D.	1.17	1.67	0.38	1.08	0.36	1.65
CEPHAL	13.94	24.39	7.62	11.98	8.16	25.33
S.D.	1.22	1.71	0.48	1.06	0.41	1.63

Figure 4. Means and S.D. of maxillary measurements from dry skulls (CRANIO) and radiographs (CEPHALO) in 64 rats



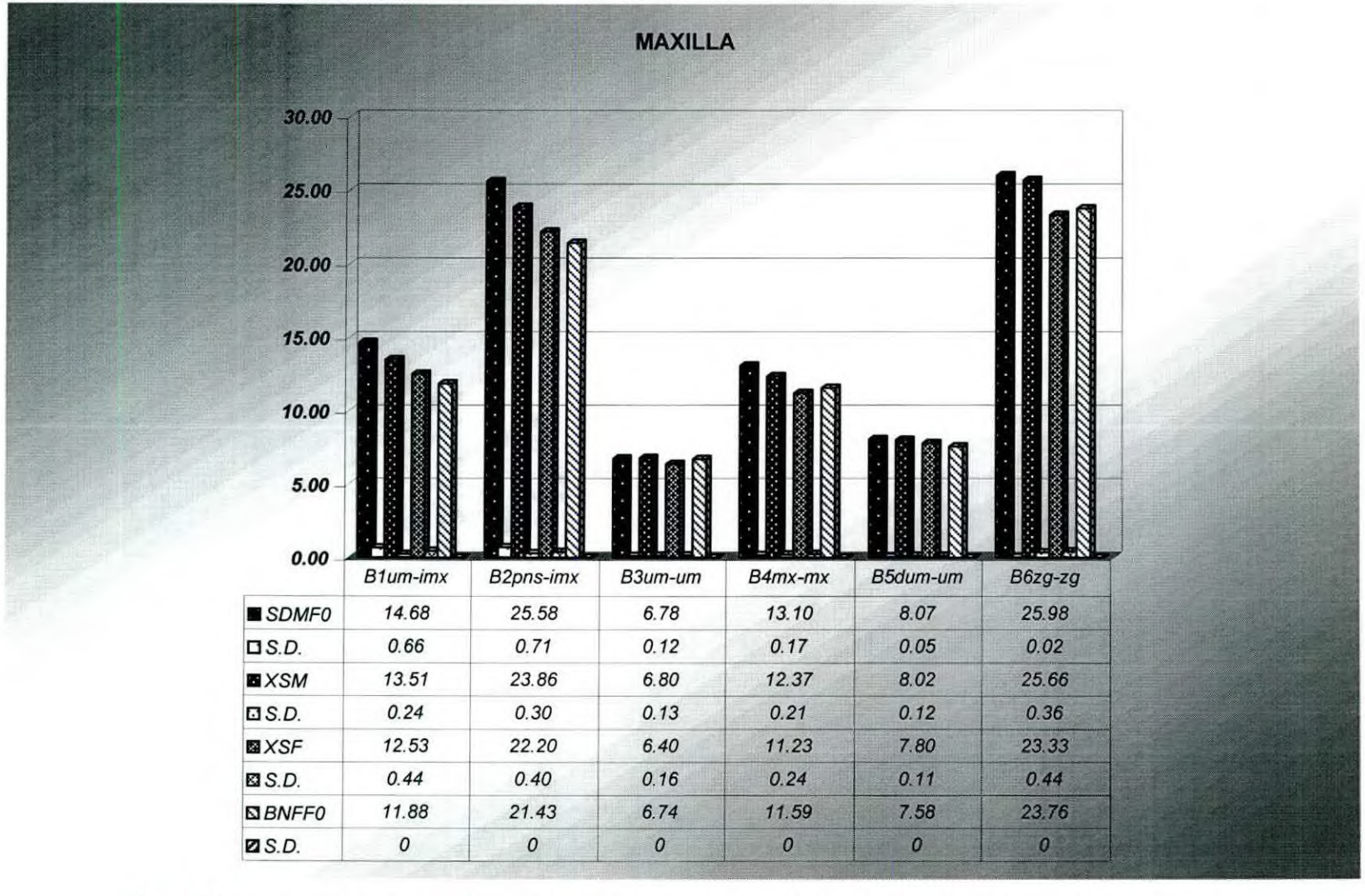
	C1hieght	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
CRANIO	13.10	29.92	8.08	14.62	18.65	7.54	18.90
S.D.	0.79	2.18	0.87	1.20	1.46	0.64	1.05
CEPHAL	13.91	31.12	9.01	15.36	19.47	8.14	19.48
S.D.	0.78	2.40	0.90	1.34	1.74	0.60	1.11

Figure 5. Means and S.D. of cranial measurements from dry skulls (CRANIO) and radiographs (CEPHAL) in 64 rats



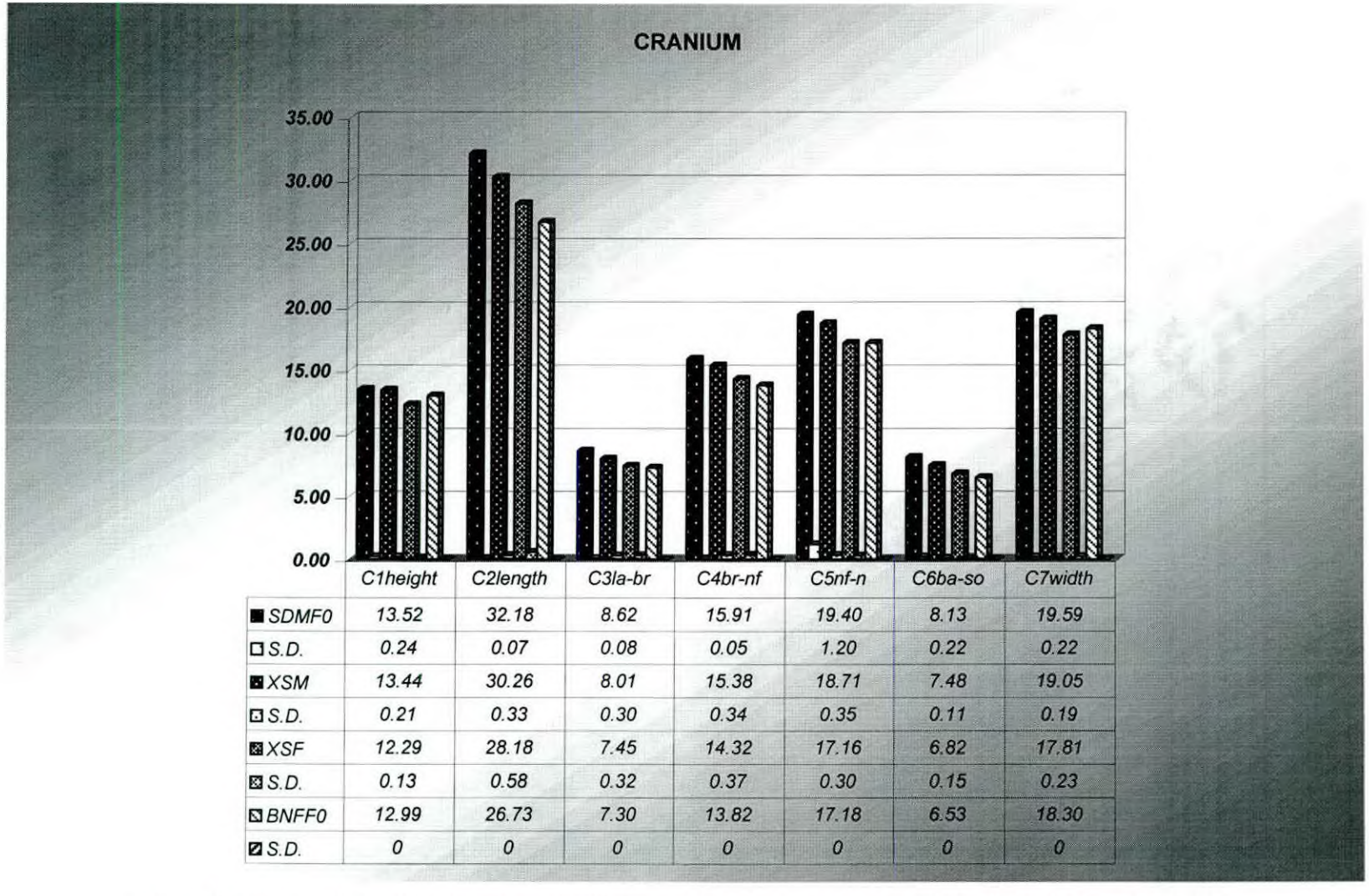
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
SDMF0	28.51	20.33	14.00	16.31	18.50	18.96	7.43
S.D.	0.83	0.54	0.01	0.37	0.41	0.46	0.16
XSM	27.58	20.53	13.15	15.59	17.52	18.31	7.67
S.D.	0.32	0.39	0.25	0.19	0.73	0.67	0.09
XSF	25.46	18.75	11.94	14.03	17.09	17.09	7.56
S.D.	0.51	0.34	0.28	0.30	0.58	0.51	0.14
BNFF0	25.63	19.03	13.08	14.92	17.88	17.04	7.31
S.D.	0	0	0	0	0	0	0

Figure 6. Means and standard deviations (S.D.) of mandibular measurements in SDM F0, XSM, XSF, and BNF F0



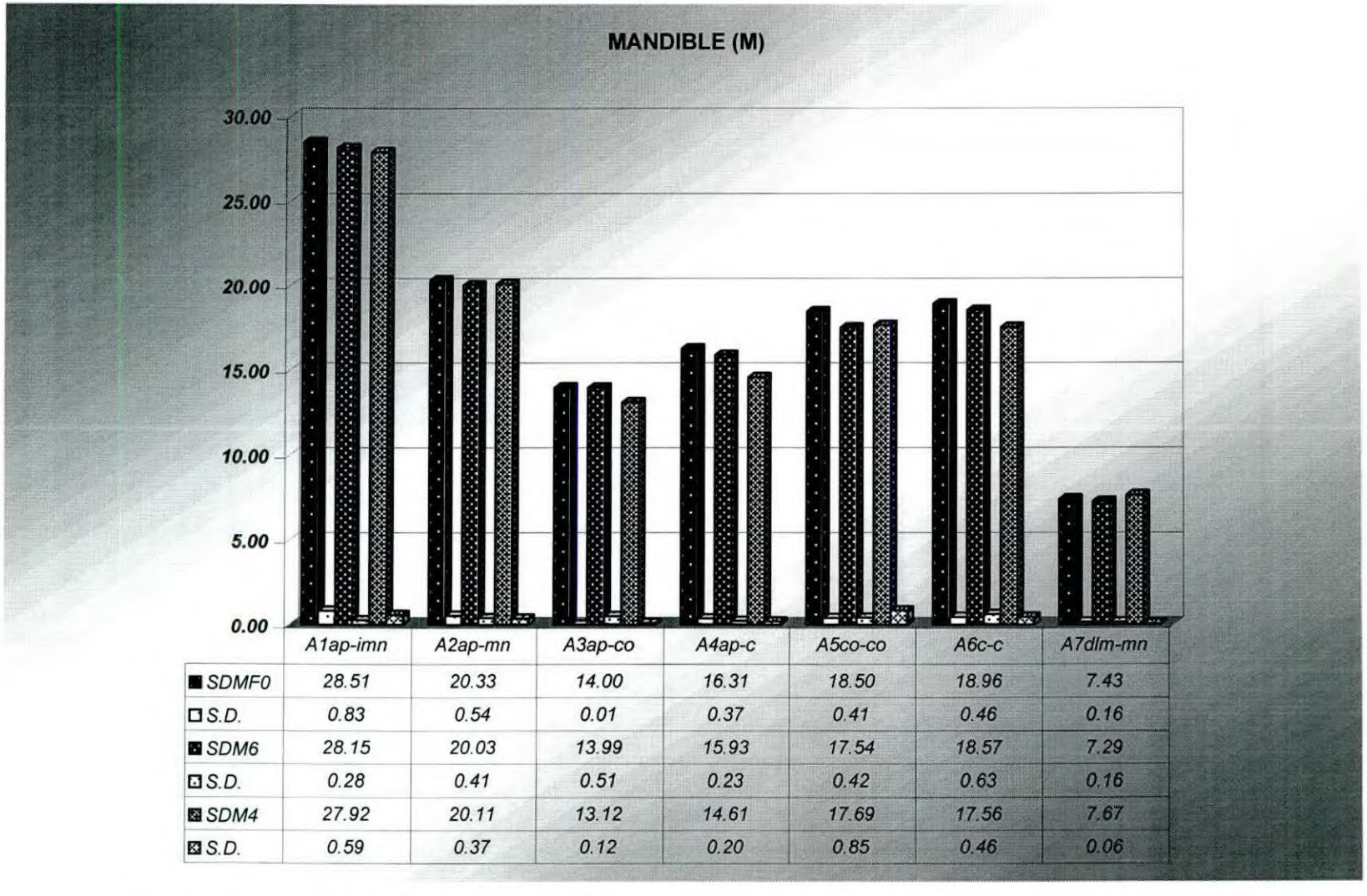
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
SDMF0	14.68	25.58	6.78	13.10	8.07	25.98
S.D.	0.66	0.71	0.12	0.17	0.05	0.02
XSM	13.51	23.86	6.80	12.37	8.02	25.66
S.D.	0.24	0.30	0.13	0.21	0.12	0.36
XSF	12.53	22.20	6.40	11.23	7.80	23.33
S.D.	0.44	0.40	0.16	0.24	0.11	0.44
BNFF0	11.88	21.43	6.74	11.59	7.58	23.76
S.D.	0	0	0	0	0	0

Figure 7. Means and standard deviations (S.D.) of maxillary measurements in SDM F0, XSM, XSF, and BNF F0.



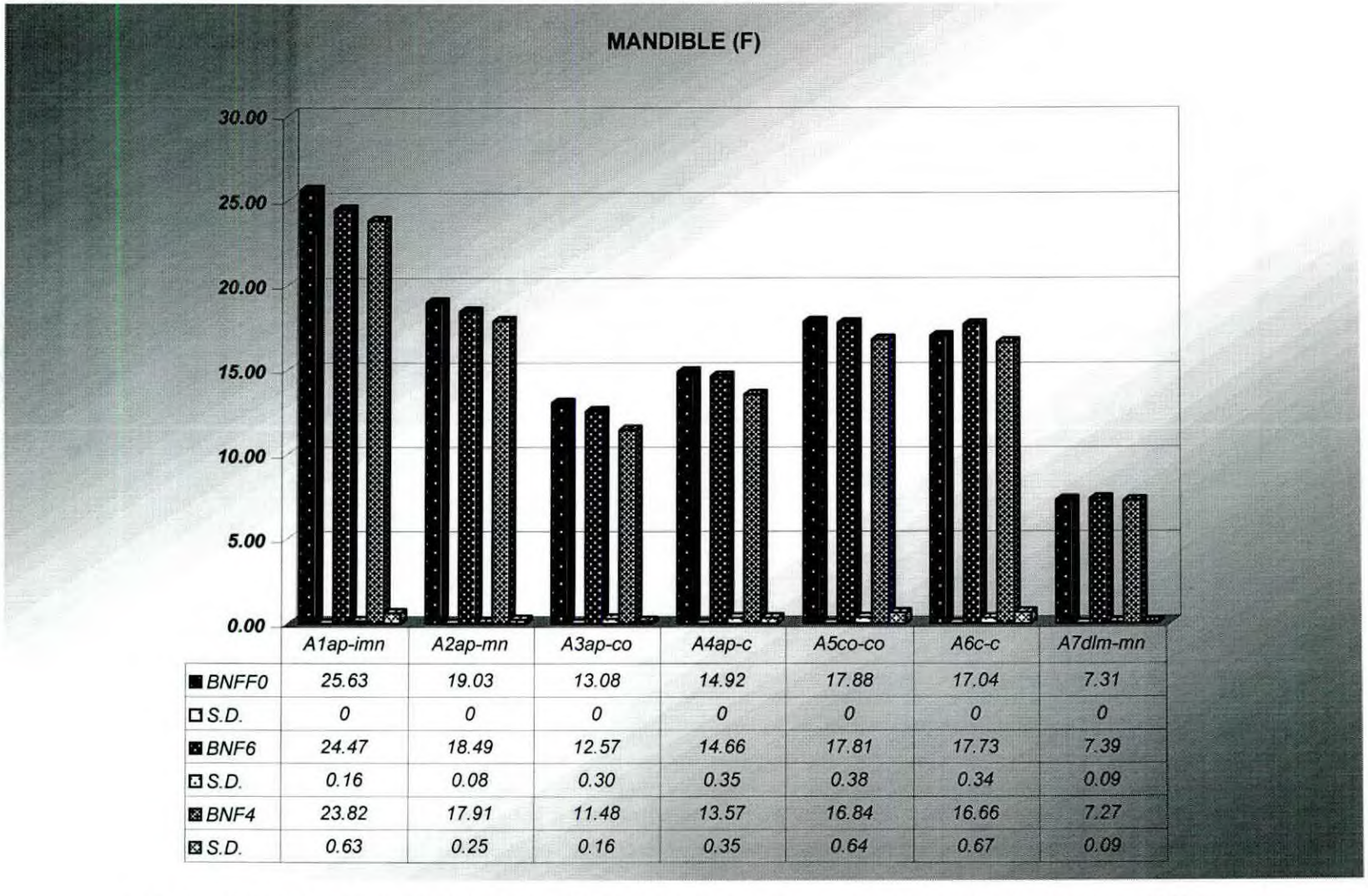
	C1height	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
SDMF0	13.52	32.18	8.62	15.91	19.40	8.13	19.59
S.D.	0.24	0.07	0.08	0.05	1.20	0.22	0.22
XSM	13.44	30.26	8.01	15.38	18.71	7.48	19.05
S.D.	0.21	0.33	0.30	0.34	0.35	0.11	0.19
XSF	12.29	28.18	7.45	14.32	17.16	6.82	17.81
S.D.	0.13	0.58	0.32	0.37	0.30	0.15	0.23
BNFF0	12.99	26.73	7.30	13.82	17.18	6.53	18.30
S.D.	0	0	0	0	0	0	0

Figure 8. Means and standard deviations (S.D.) of cranial measurements in SDM F0, XSM, XSF, and BNF F0.



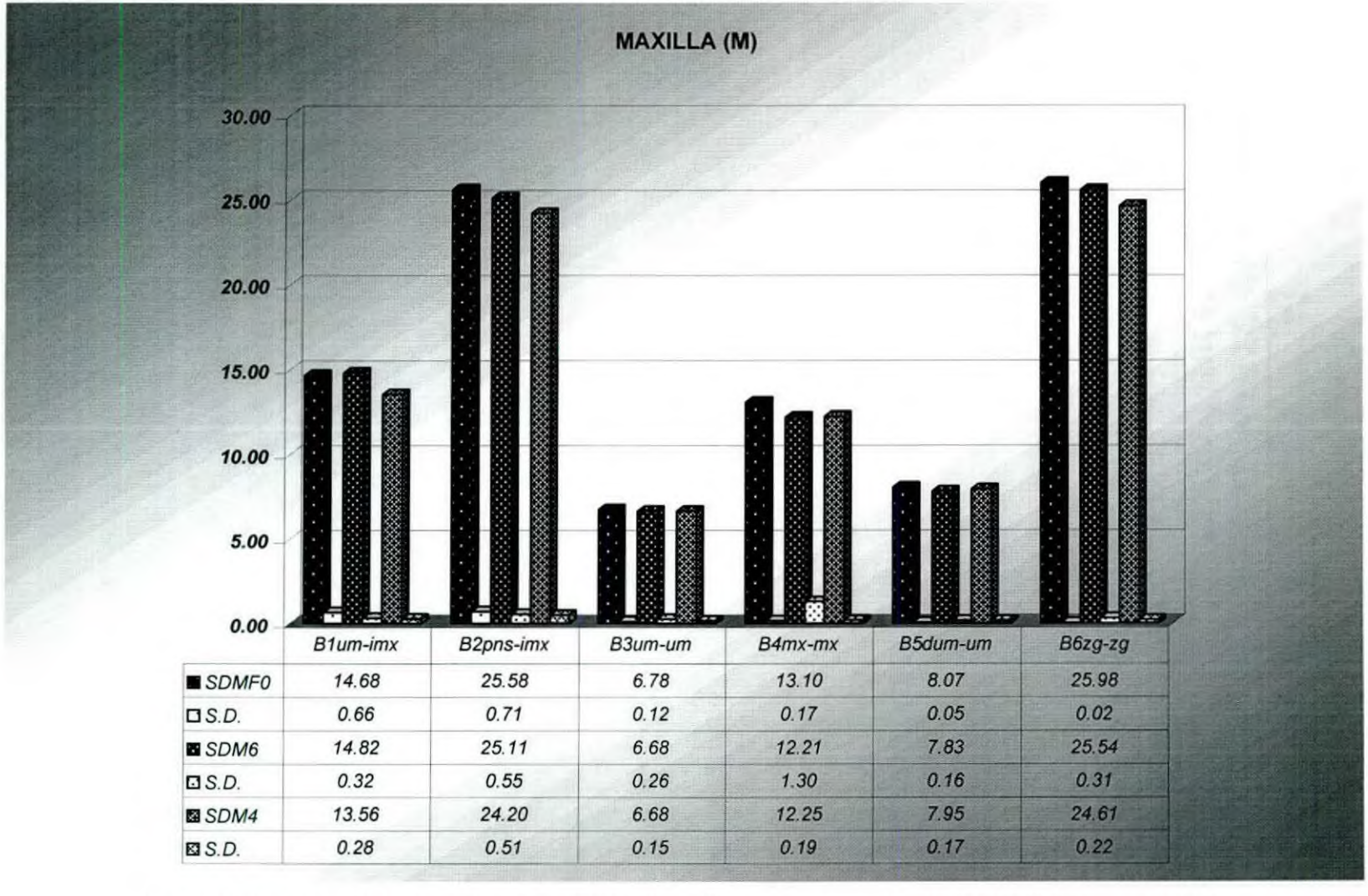
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
SDMF0	28.51	20.33	14.00	16.31	18.50	18.96	7.43
S.D.	0.83	0.54	0.01	0.37	0.41	0.46	0.16
SDM6	28.15	20.03	13.99	15.93	17.54	18.57	7.29
S.D.	0.28	0.41	0.51	0.23	0.42	0.63	0.16
SDM4	27.92	20.11	13.12	14.61	17.69	17.56	7.67
S.D.	0.59	0.37	0.12	0.20	0.85	0.46	0.06

Figure 9. Means and standard deviations (S.D.) of mandibular measurements in SDM F0, SDM 6, and SDM 4



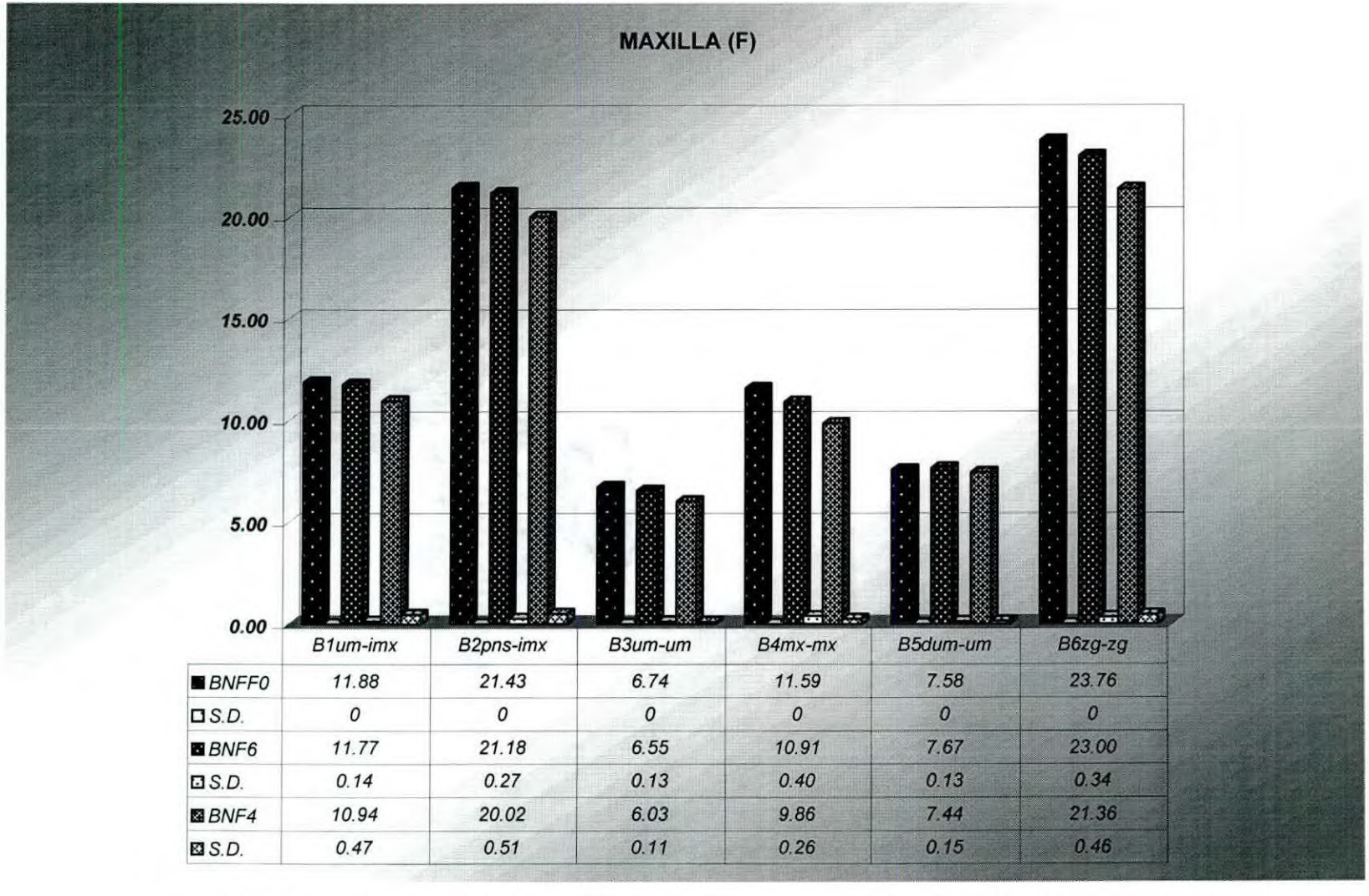
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
BNFF0	25.63	19.03	13.08	14.92	17.88	17.04	7.31
S.D.	0	0	0	0	0	0	0
BNF6	24.47	18.49	12.57	14.66	17.81	17.73	7.39
S.D.	0.16	0.08	0.30	0.35	0.38	0.34	0.09
BNF4	23.82	17.91	11.48	13.57	16.84	16.66	7.27
S.D.	0.63	0.25	0.16	0.35	0.64	0.67	0.09

Figure 10. Means and standard deviations (S.D.) of mandibular measurements in BNF F0, BNF 6, and BNF 4



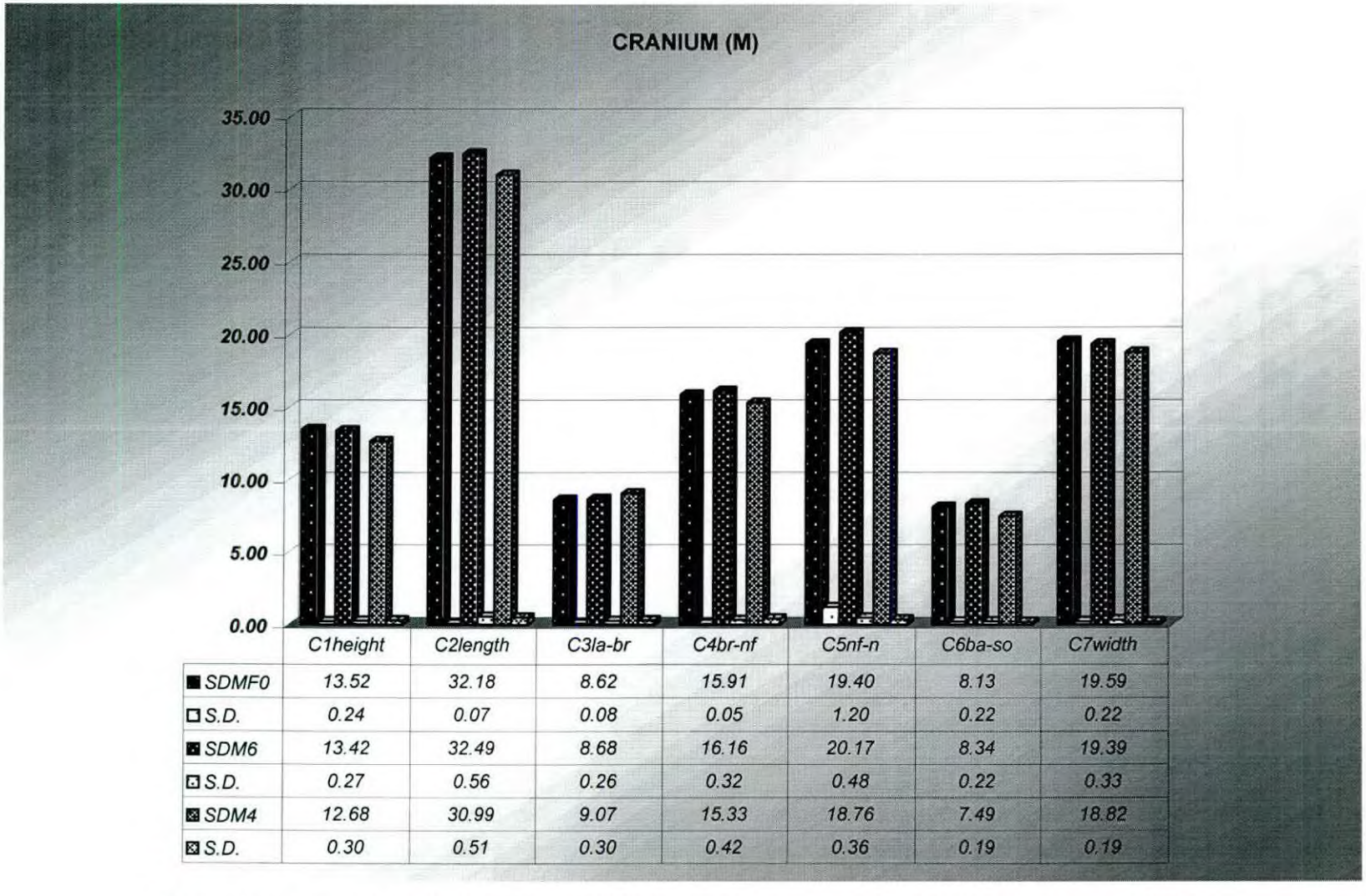
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
SDMF0	14.68	25.58	6.78	13.10	8.07	25.98
S.D.	0.66	0.71	0.12	0.17	0.05	0.02
SDM6	14.82	25.11	6.68	12.21	7.83	25.54
S.D.	0.32	0.55	0.26	1.30	0.16	0.31
SDM4	13.56	24.20	6.68	12.25	7.95	24.61
S.D.	0.28	0.51	0.15	0.19	0.17	0.22

Figure 11. Means and standard deviations (S.D.) of maxillary measurements in SDM F0, SDM 6, and SDM 4



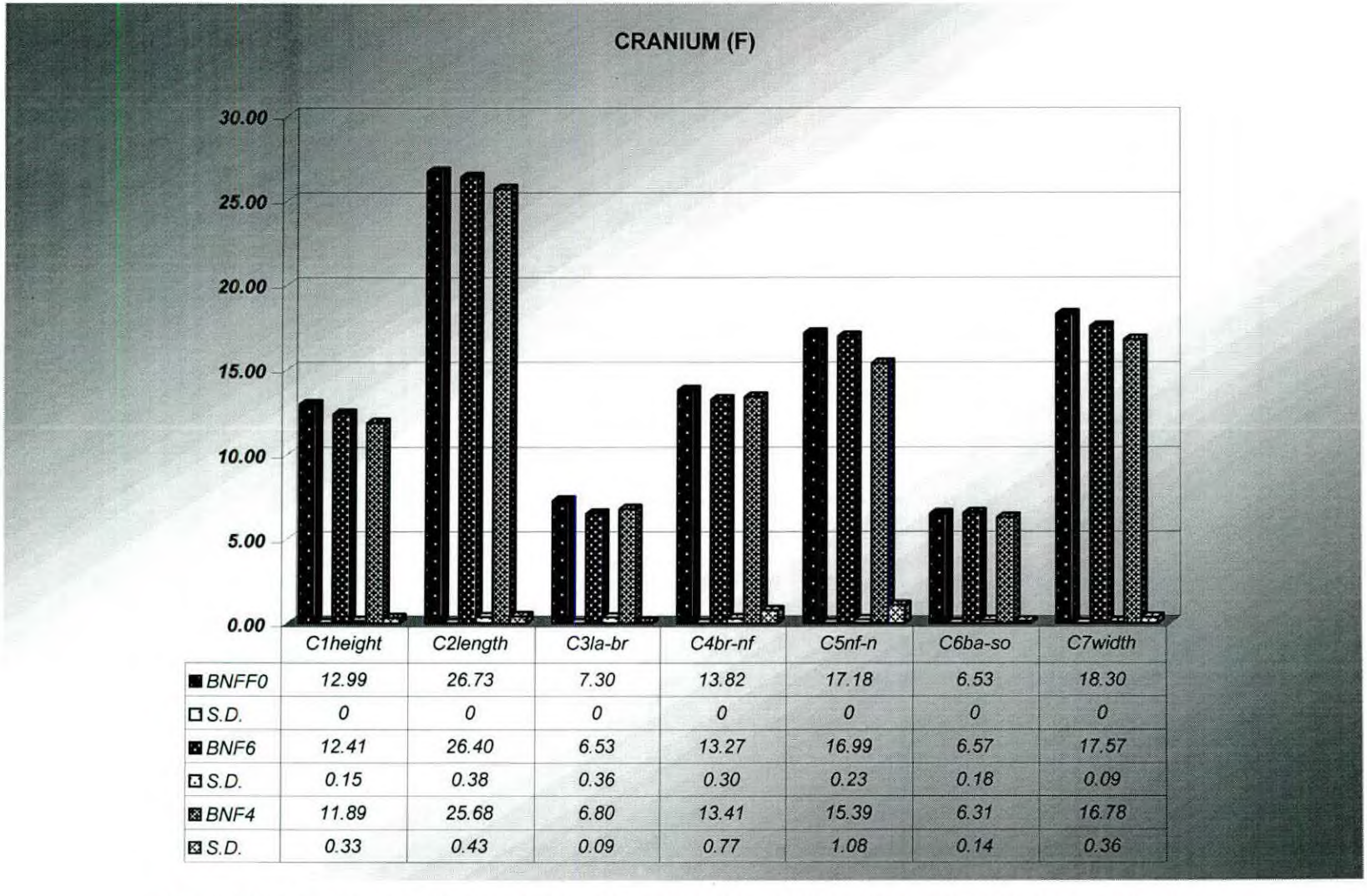
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
BNFF0	11.88	21.43	6.74	11.59	7.58	23.76
S.D.	0	0	0	0	0	0
BNF6	11.77	21.18	6.55	10.91	7.67	23.00
S.D.	0.14	0.27	0.13	0.40	0.13	0.34
BNF4	10.94	20.02	6.03	9.86	7.44	21.36
S.D.	0.47	0.51	0.11	0.26	0.15	0.46

Figure 12. Means and standard deviations (S.D.) of maxillary measurements in BNF F0, BNF 6, and BNF 4



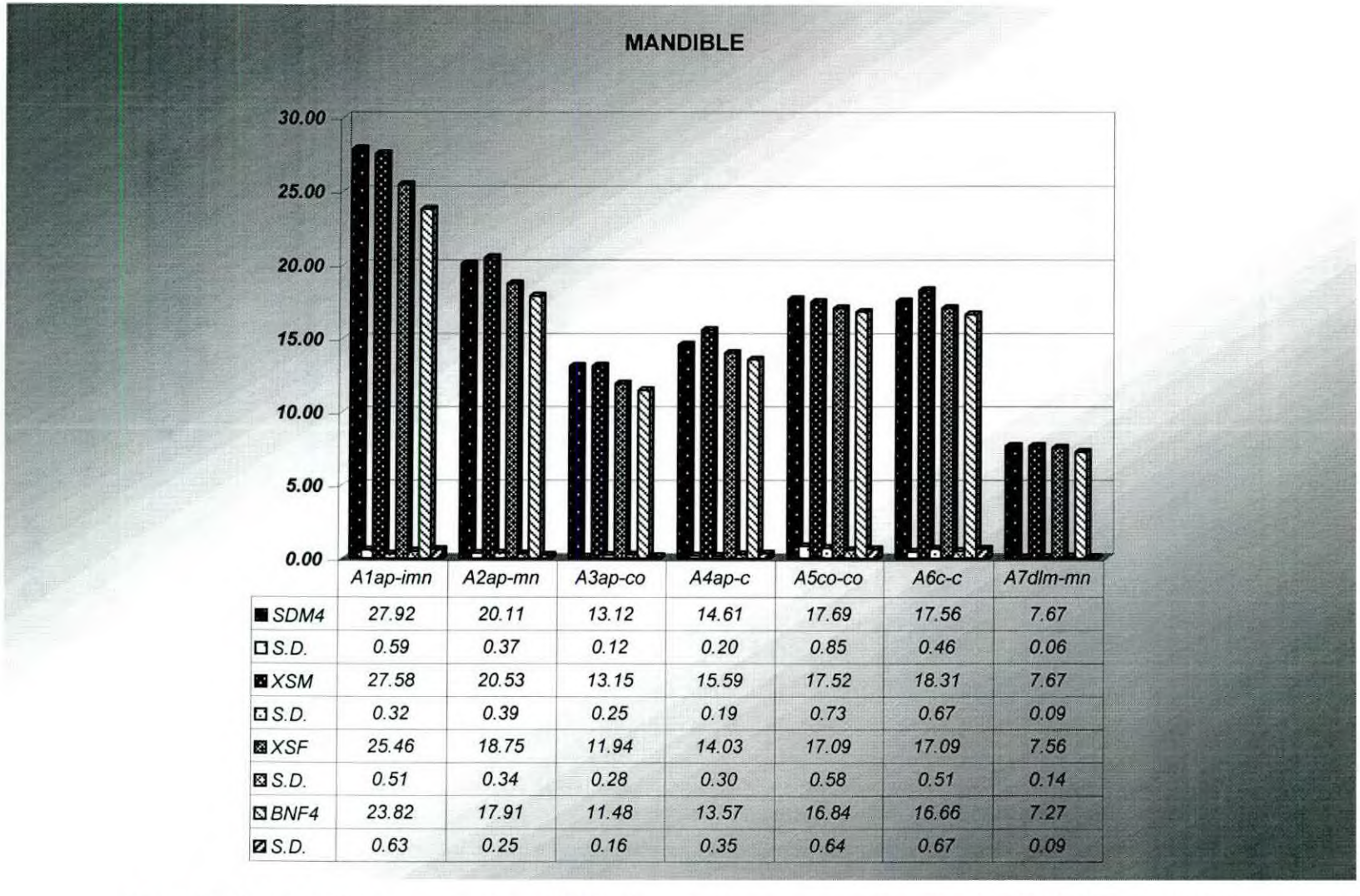
	C1height	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
SDMF0	13.52	32.18	8.62	15.91	19.40	8.13	19.59
S.D.	0.24	0.07	0.08	0.05	1.20	0.22	0.22
SDM6	13.42	32.49	8.68	16.16	20.17	8.34	19.39
S.D.	0.27	0.56	0.26	0.32	0.48	0.22	0.33
SDM4	12.68	30.99	9.07	15.33	18.76	7.49	18.82
S.D.	0.30	0.51	0.30	0.42	0.36	0.19	0.19

Figure 13. Means and standard deviations (S.D.) of cranial measurements in SDM F0, SDM 6, and SDM 4



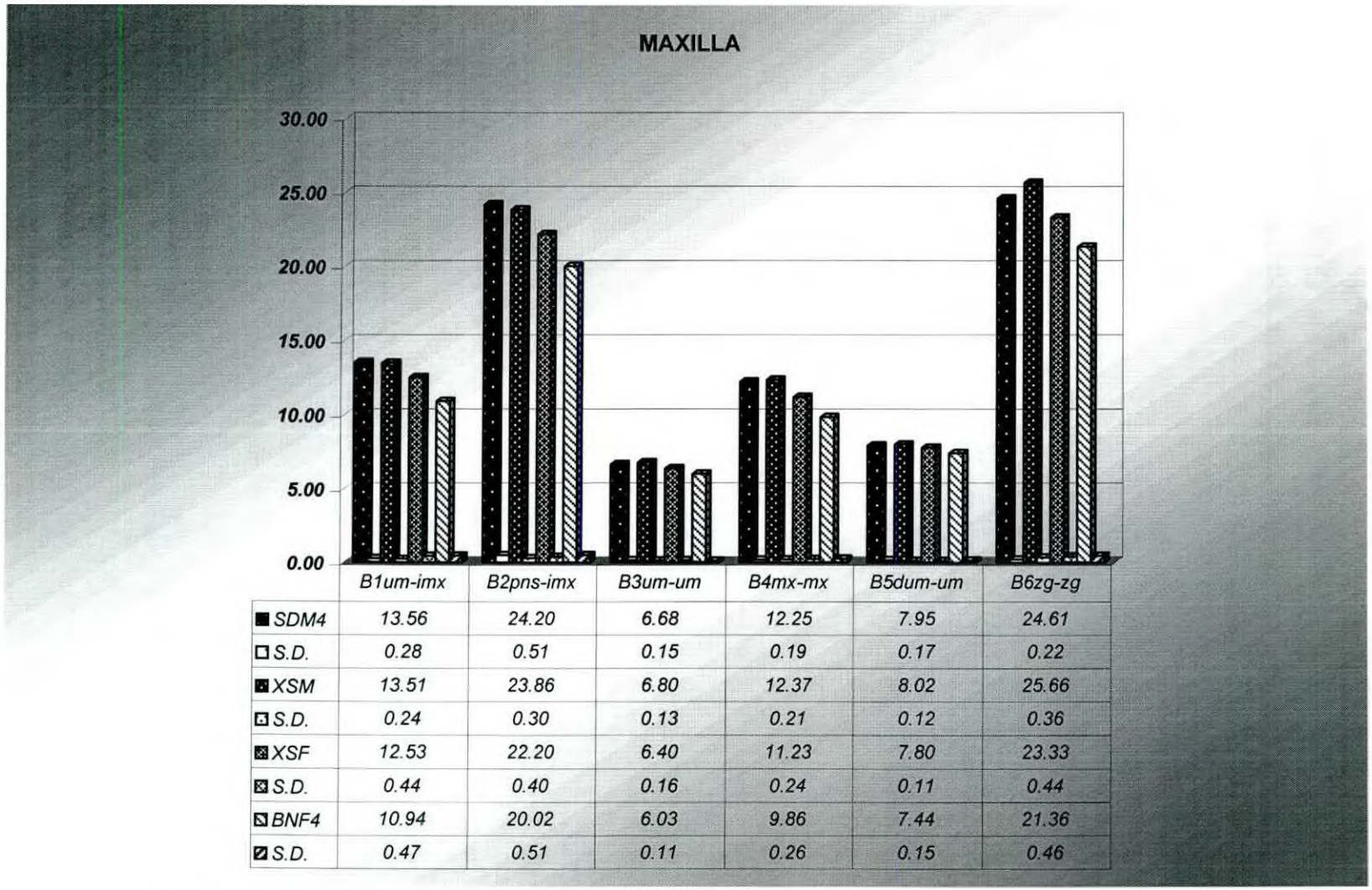
	C1height	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
BNFF0	12.99	26.73	7.30	13.82	17.18	6.53	18.30
S.D.	0	0	0	0	0	0	0
BNF6	12.41	26.40	6.53	13.27	16.99	6.57	17.57
S.D.	0.15	0.38	0.36	0.30	0.23	0.18	0.09
BNF4	11.89	25.68	6.80	13.41	15.39	6.31	16.78
S.D.	0.33	0.43	0.09	0.77	1.08	0.14	0.36

Figure 14. Means and standard deviations (S.D.) of cranial measurements in BNF F0, BNF 6, and BNF 4



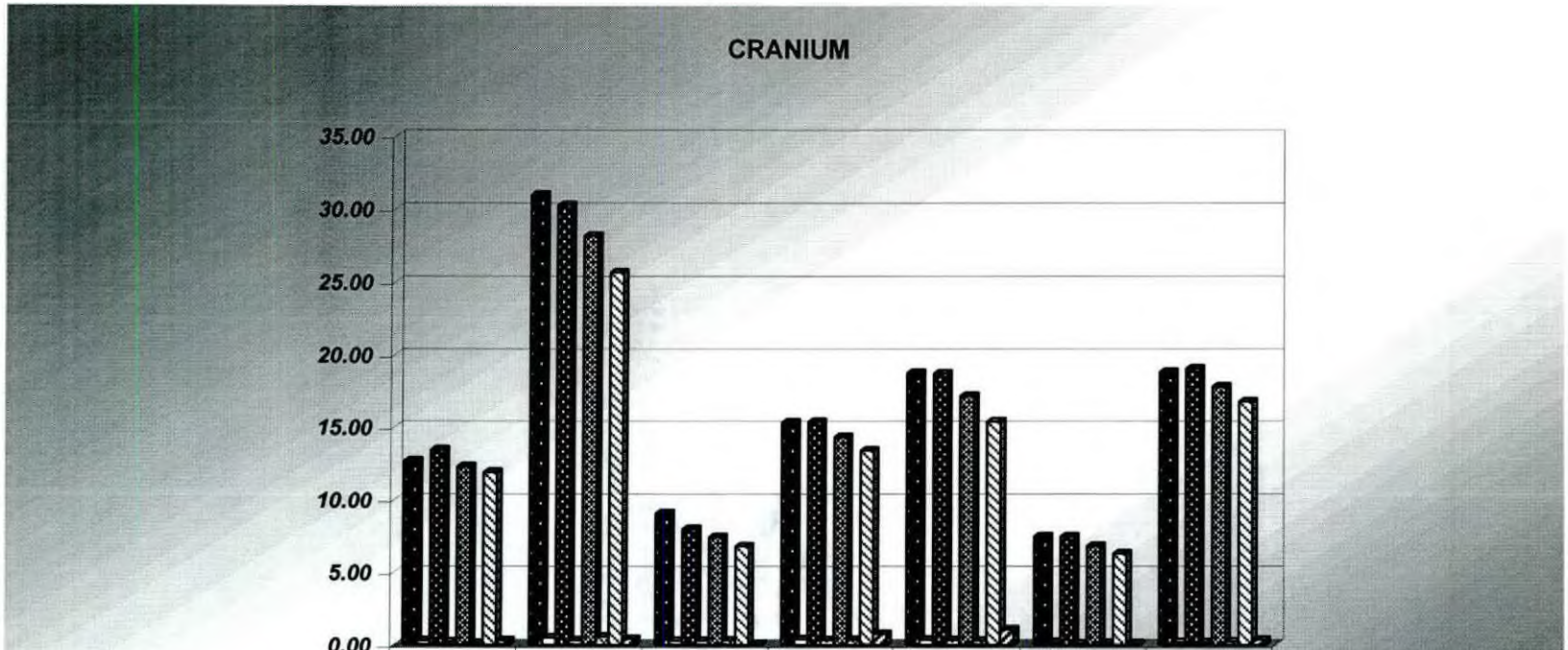
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
SDM4	27.92	20.11	13.12	14.61	17.69	17.56	7.67
S.D.	0.59	0.37	0.12	0.20	0.85	0.46	0.06
XSM	27.58	20.53	13.15	15.59	17.52	18.31	7.67
S.D.	0.32	0.39	0.25	0.19	0.73	0.67	0.09
XSF	25.46	18.75	11.94	14.03	17.09	17.09	7.56
S.D.	0.51	0.34	0.28	0.30	0.58	0.51	0.14
BNF4	23.82	17.91	11.48	13.57	16.84	16.66	7.27
S.D.	0.63	0.25	0.16	0.35	0.64	0.67	0.09

Figure 15. Means and standard deviations (S.D.) of mandibular measurements in SDM 4, XSM, XSF, and BNF 4



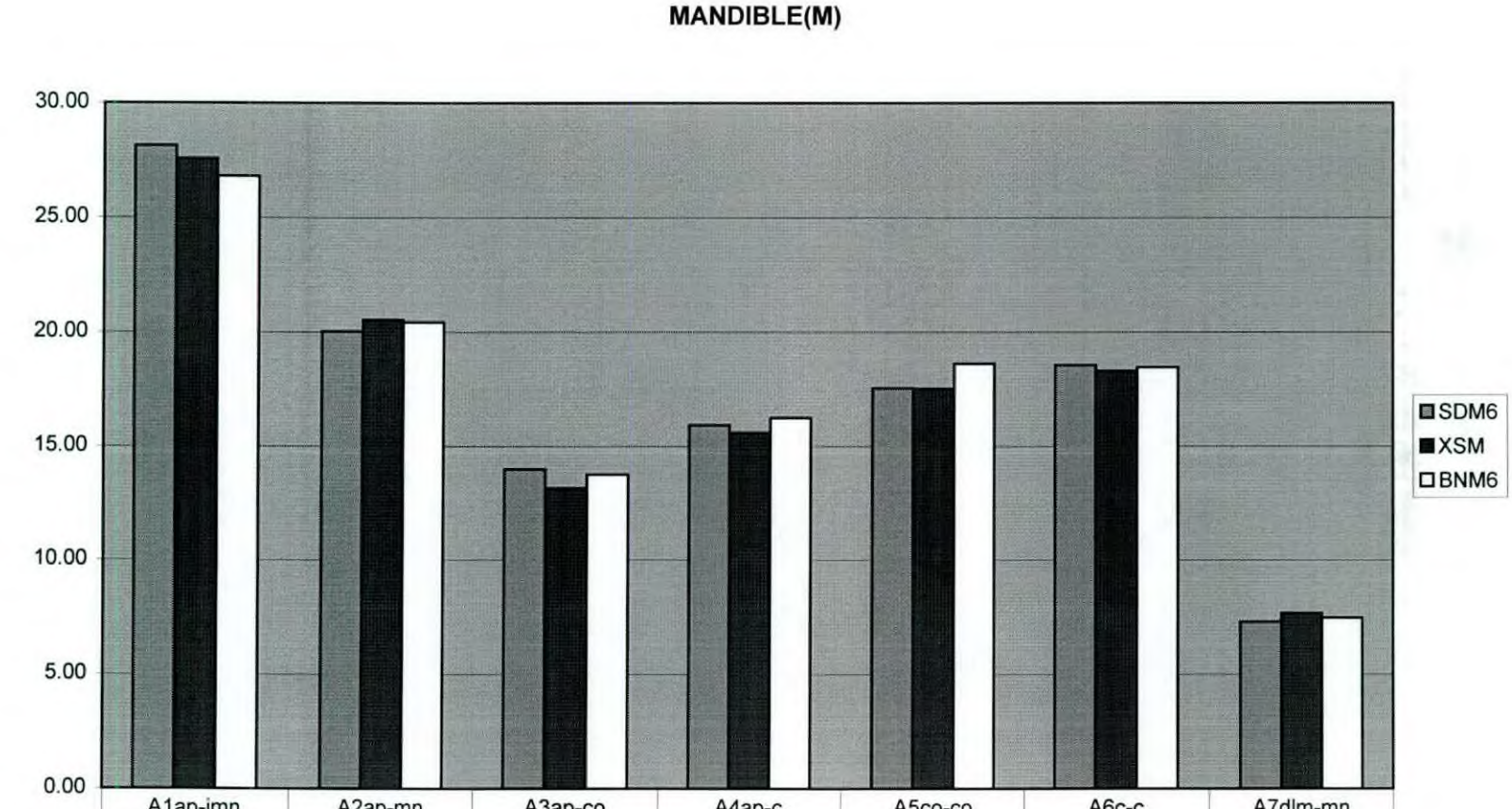
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
SDM4	13.56	24.20	6.68	12.25	7.95	24.61
S.D.	0.28	0.51	0.15	0.19	0.17	0.22
XSM	13.51	23.86	6.80	12.37	8.02	25.66
S.D.	0.24	0.30	0.13	0.21	0.12	0.36
XSF	12.53	22.20	6.40	11.23	7.80	23.33
S.D.	0.44	0.40	0.16	0.24	0.11	0.44
BNF4	10.94	20.02	6.03	9.86	7.44	21.36
S.D.	0.47	0.51	0.11	0.26	0.15	0.46

Figure 16. Means and standard deviations (S.D.) of maxillary measurements in SDM 4, XSM, XSF, and BNF 4



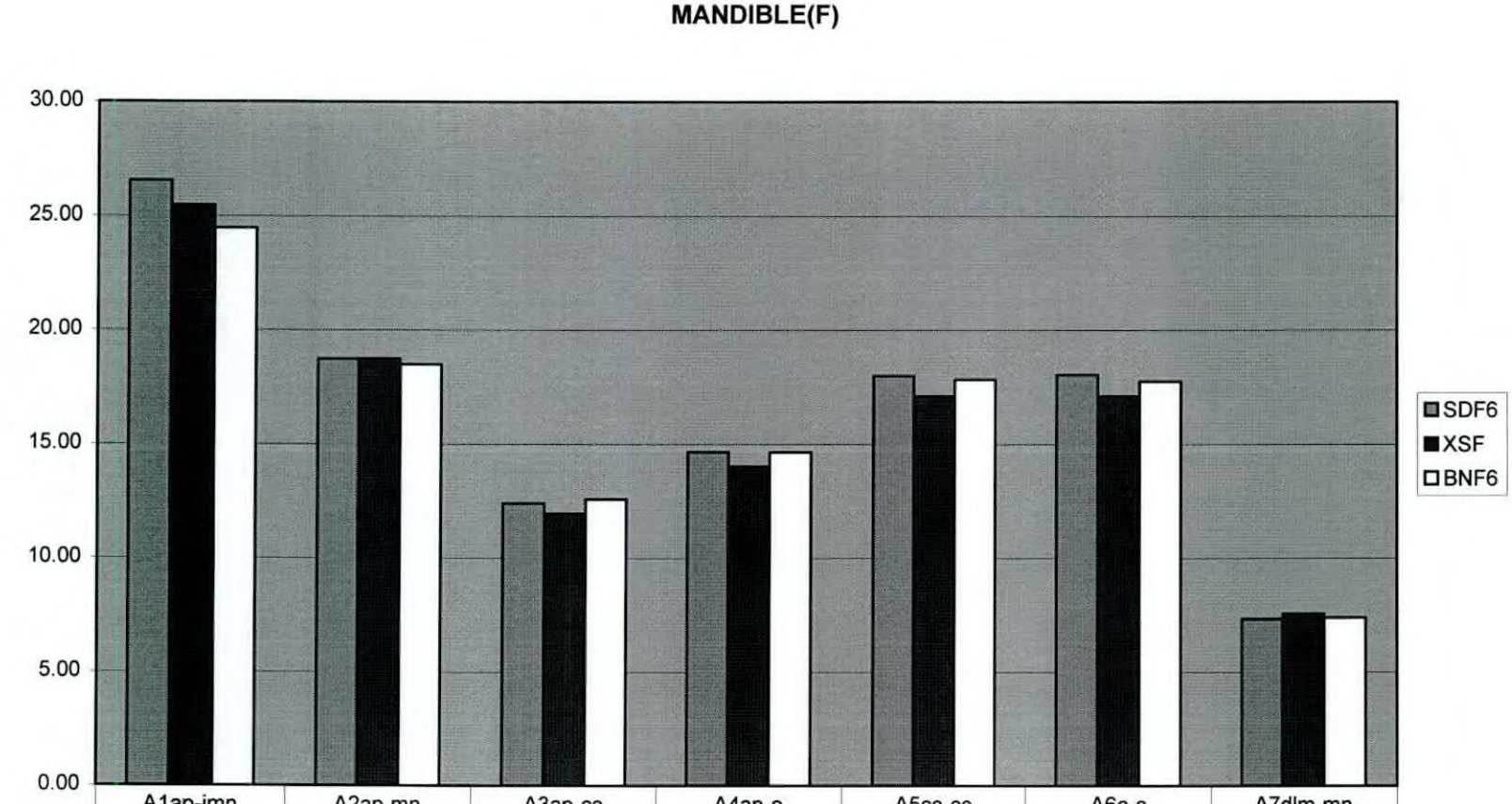
	C1height	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
SDM4	12.68	30.99	9.07	15.33	18.76	7.49	18.82
S.D.	0.30	0.51	0.30	0.42	0.36	0.19	0.19
XSM	13.44	30.26	8.01	15.38	18.71	7.48	19.05
S.D.	0.21	0.33	0.30	0.34	0.35	0.11	0.19
XSF	12.29	28.18	7.45	14.32	17.16	6.82	17.81
S.D.	0.13	0.58	0.32	0.37	0.30	0.15	0.23
BNF4	11.89	25.68	6.80	13.41	15.39	6.31	16.78
S.D.	0.33	0.43	0.09	0.77	1.08	0.14	0.36

Figure 17. Means and standard deviations (S.D.) of cranial measurements in SDM 4, XSM, XSF, and BNF 4



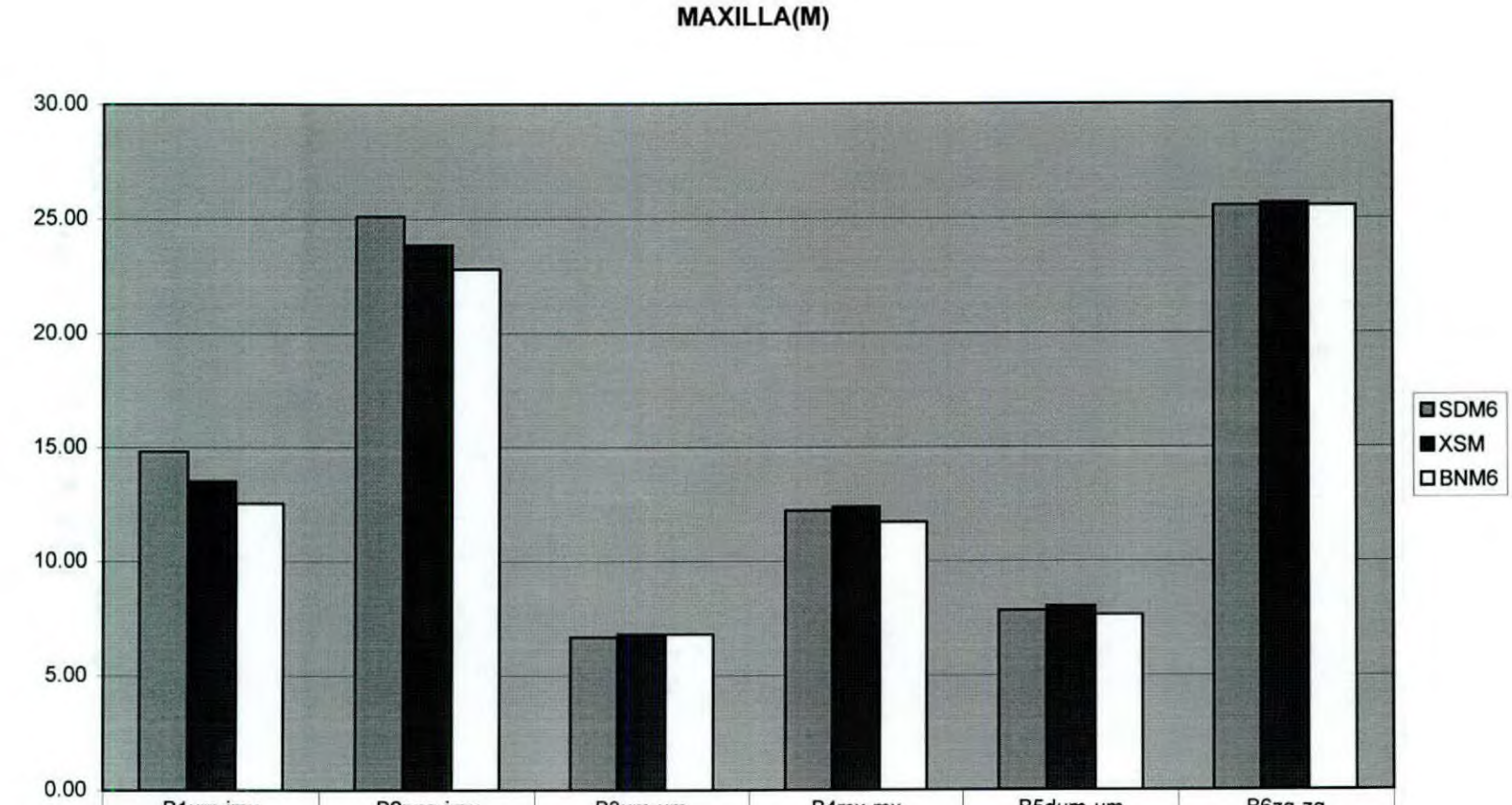
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
SDM6	28.15	20.03	13.99	15.93	17.54	18.57	7.29
XSM	27.58	20.53	13.15	15.59	17.52	18.31	7.67
BNM6	26.80	20.43	13.76	16.24	18.64	18.48	7.47

Figure 18. Means of mandibular measurements in SDM 6, XSM, and BNM 6



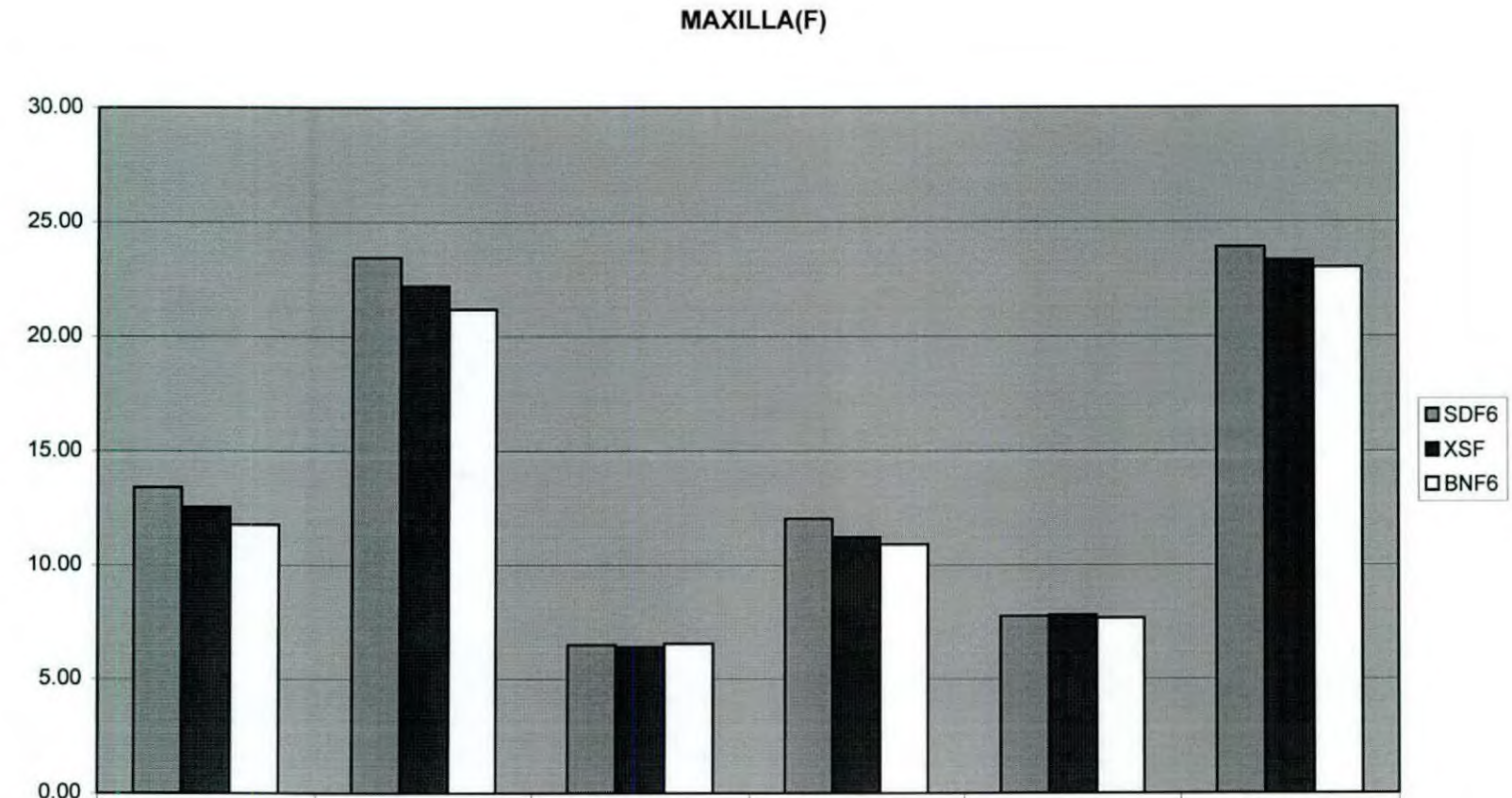
	A1ap-imn	A2ap-mn	A3ap-co	A4ap-c	A5co-co	A6c-c	A7dlm-mn
SDF6	26.54	18.72	12.40	14.67	17.97	18.04	7.33
XSF	25.46	18.75	11.94	14.03	17.09	17.09	7.56
BNF6	24.47	18.49	12.57	14.66	17.81	17.73	7.39

Figure 19. Means of mandibular measurements in SDF 6, XSF, and BNF6



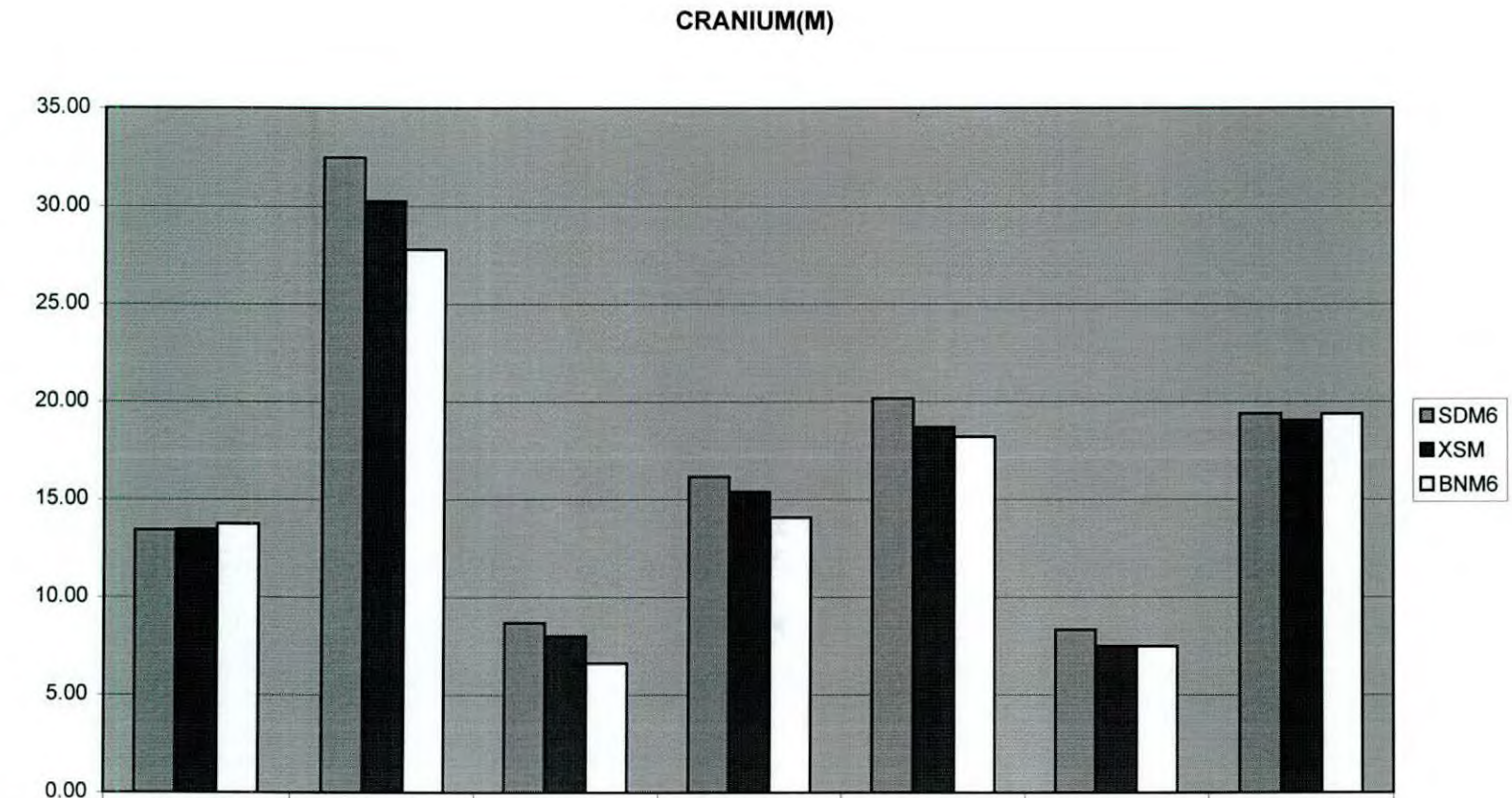
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
SDM6	14.82	25.11	6.68	12.21	7.83	25.54
XSM	13.51	23.86	6.80	12.37	8.02	25.66
BNM6	12.54	22.80	6.79	11.70	7.64	25.54

Figure 20. Means of maxillary measurements in SDM 6, XSM, and BNM 6



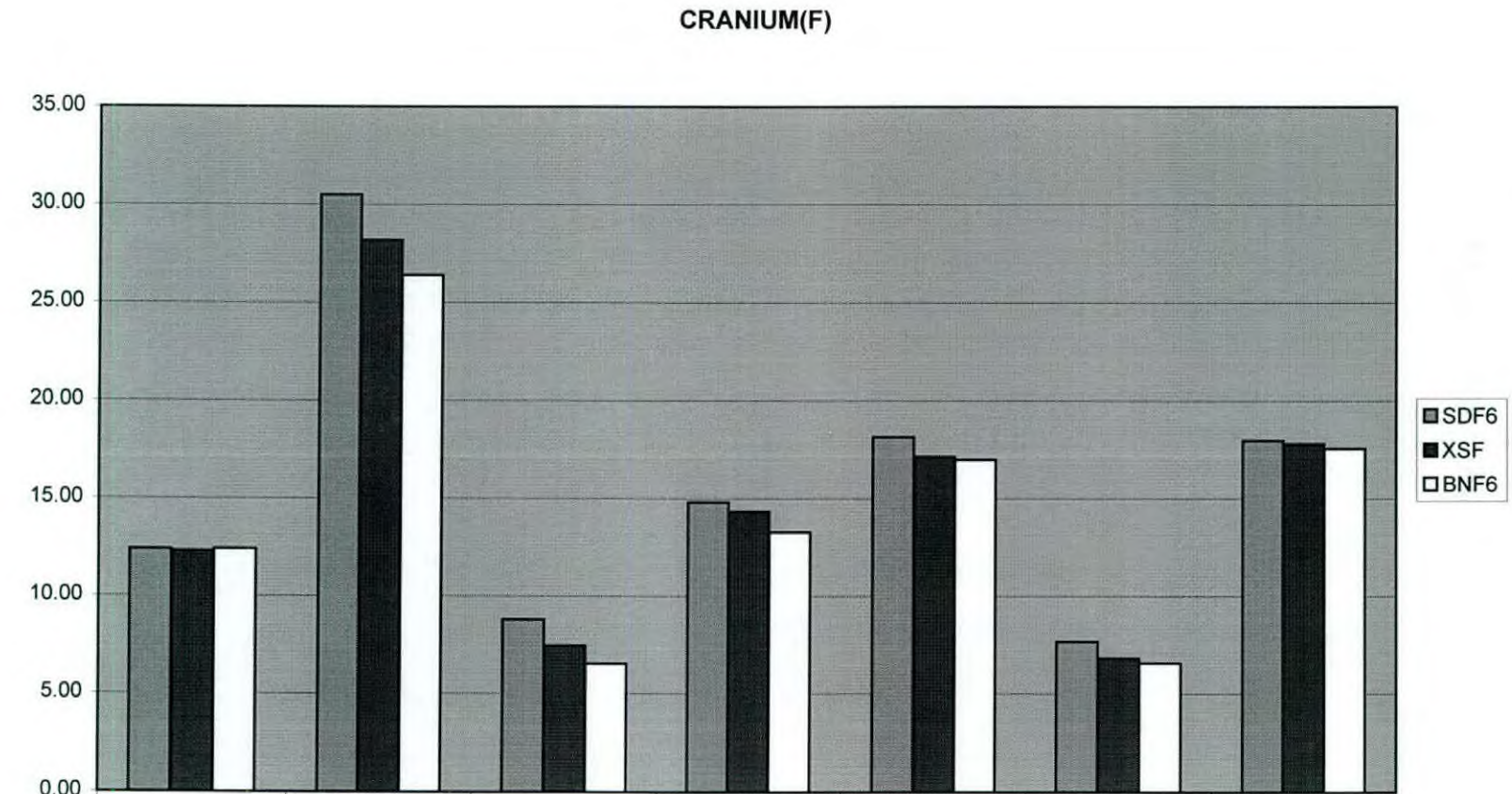
	B1um-imx	B2pns-imx	B3um-um	B4mx-mx	B5dum-um	B6zg-zg
SDF6	13.40	23.45	6.49	12.02	7.75	23.91
XSF	12.53	22.20	6.40	11.23	7.80	23.33
BNF6	11.77	21.18	6.55	10.91	7.67	23.00

Figure 21. Means of maxillary measurements in SDF 6, XSF, and BNF 6



	C1height	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
SDM6	13.42	32.49	8.68	16.16	20.17	8.34	19.39
XSM	13.44	30.26	8.01	15.38	18.71	7.48	19.05
BNM6	13.75	27.78	6.62	14.07	18.20	7.50	19.37

Figure 22. Means of cranial measurements in SDM 6, XSM, and BNM 6



	C1height	C2length	C3la-br	C4br-nf	C5nf-n	C6ba-so	C7width
SDF6	12.39	30.48	8.79	14.81	18.16	7.66	17.99
XSF	12.29	28.18	7.45	14.32	17.16	6.82	17.81
BNF6	12.41	26.40	6.53	13.27	16.99	6.57	17.57

Figure 23. Means of cranial measurements in SDF 6, XSF, and BNF 6

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