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CRANIOFACIAL PHENOTYPIC VARIATION AMONG LABORATORY RAT STRAINS

By

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ABSTRACT

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Variations in craniofacial form are phenotypic traits of an integrated system ultimately designed to insure the survival of the organism. The long-term goal of this line of research is to identify genetic mechanisms and quantify their effect in producing variations in craniofacial form and function. The present study used 4 male and 4 female fully mature adult rats (>6 months old) from eight different strains: Brown-Norway (BN), Sprague-Dawley (SD), Wistar-Kyoto (WKY), Long-Evans (LE), Holtzman (HZ) and Fisher-344 (F344), Buffalo (BUF), and Lewis (LEW). Five strains were inbred (BN, BUF, LEW, F344, WKY) and three strains were outbred (SD, LE, HZ). From these 64 animals: Nineteen craniometric linear measurements were used to characterize the phenotypic craniofacial features relative to size of each strain. Femur length was used as a reference for overall skeletal size. Significant differences existed for all nineteen measurements among the eight strains. In particular, according to the Cranial Index, the Sprague-Dawley strain was characterized as dolichocephalic and the Brown-Norway was

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INTRODUCTION

The success of orthodontic treatment and oral maxillofacial reconstructive surgery largely depends on our knowledge of morphological craniofacial characteristics. The two broad causes of mediators that lead to craniofacial morphology are genes and environment. A better understanding of the relative contributions of these two factors would likely result in more predictable clinical results. Such an understanding has eluded the specialty thus far. Historically, the orthodontic literature has identified a role for genetics in some craniofacial patterns. For example, Rubbrecht (1939) studied the well-known Hapsburg jaw of the Royal German family and demonstrated the presence of mandibular prognathism through many generations. In addition, the primacy of the genetic plan was championed by Brodie (1948), Brash (1929) and MacMillan (1928). These authors supported a view that the form of jawbones was entirely under genetic control and environmental influences including orthodontics could have little effect.

In animal investigations, Nonaka and Nakata (1988a, b) concluded that both additive and non-additive genetic effects play an important role in the craniofacial growth of rats. Rats are considered a favorable model for studies attempting to understand human traits as suggested by Tsolakis (1997). Human craniofacial characteristics may be classified into brachycephalic

(broader cranial widths) and dolichocephalic (longer cranial lengths) with facial patterns that are remarkably similar, i.e., the brachycephalic individual is more likely to have a broad face (euryprosopic) and the dolichocephalic individual is prone to a longer facial length in relation to facial width (leptoprosopic). (Enlow and Hans 1997)

Studies of the craniofacial region may be broadly broken down into three regions; the cranium, the maxilla and the mandible. All three structures are derived from neural crest mesenchyme but follow different pathways of development resulting in two types of bone formation - endochondral and intramembranous. While several studies have examined the developmental aspects of these three facial components in rats (Pucciarelli 1980; Kiliaridis et al. 1996; Navarro 1995; Tsolakis 1997; Behrents and Johnston 1984; Hans et al. 1996) no studies have determined the inter-relation of these three components of the skull of the rat in relation to their genetic make up and the effect of gender and strain on the final craniofacial configuration with respect to shape and size.

A metric evaluation of size differences between various strains may be simply completed using anthropometric or craniometric measures. Alternately, cephalograms may be used in the assessment of size of the cranium with valid results (Park et al. 1999). The use of cephalograms, coupled with currently available sophisticated variance component models allow for shape assessment as well. While it appears a stretch to use x-ray

based human studies to draw genetic conclusions on a human population, because cultural and environmental factors often mimic the genetic factors, laboratory grown, inbred strains overcome this problem. (Valiathan 2001) Genetic variation is reduced by inbreeding and environmental variation is reduced by housing the animals under the same conditions.

The present study evaluated intra-gender, inter-gender and inter-strain morphologic differences among eight different laboratory rat strains. Cephalograms and sophisticated computer programs that facilitate shape assessment were used. We hypothesized that differences in shape and size for head form, the maxilla and the mandible were greater among rat strains than between sexes or within the same sex of the same strain. These differences can be used as phenotypic markers in future studies that will attempt to link genotypic markers with phenotypic traits. Such linkage studies will improve our understanding of the interaction between genes and environment to produce craniofacial form. Because of the law of conservation of genetic material it is likely that discoveries made from rat animal models will have application in humans as well.

LITERATURE REVIEW

The interest in the relationship between genes and craniofacial variation is motivated by clinical observations in orthodontics. The inability of a clinician to predict treatment, which is often times dependent on patient "response", has led to the interesting question. Could the genetic make of the individual be the determining factor? Or perhaps, could portions of the face or specific morphologic features be dominated by genetic control and thereby render themselves immune to mechanical therapies. In 1941, Hughes and Moore mentioned that to correct undesirable morphologic variations produced by heredity and/or the environment, a professional orthodontist should understand the genetic and environmental influences precisely and in detail. However more than thirty years later Smith and Bailit (1977) report that the published research on the genetics of the craniofacial oro-dental region had little influence on the daily practice of orthodontics. They contended that the lack of impact was because the treatment targets and mechanical methods employed failed to capitalize on the genetic differences among patients. They suggested that long-term goals of genetic studies should focus on the detection of major craniofacial genes and on determining their biomechanical action.

The relative contribution of genes and environment to the etiology of malocclusion has been a cause of controversy throughout the twentieth century (Mossey 1999). Genetic programming is clearly predominant during embryonic craniofacial morphogenesis, but the environment is also thought to influence dentofacial morphology. This is particularly true for postnatal facial growth and development. Orthodontic and orthopedic techniques are a specific type of therapeutic environmental influence used in the treatment of malocclusion and other dentofacial deformities. One key to successful treatment is to determine the etiology of the malocclusion. A second key might be to differentiate between the genetic and environmental influences acting on the craniofacial skeleton in that particular individual. Our ability to do either of these is limited by our lack of knowledge. We do not understand genetic mechanisms that control facial growth and we do not fully comprehend how environmental factors modify human craniofacial morphogenesis (Mossey 1999).

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 naive 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."

Further, the current knowledge regarding craniofacial development accepts that all genes are subject to epigenetic signals that dictate spatially and temporally the induction of specific gene. That is, all the information required for growth and development is contained within genes, and hence the subsequent protein-protein interactions as well as gene-environment interactions control the distribution and degree of expression of these genes (Slavkin 1999).

The genetic information of the DNA molecule is based on the sequence of the 4 different bases along the DNA molecule: adenine (A), thymine (T), guanine (G), and cytosine (C), each base on a DNA strand binds by hydrogen bonds to the base on the opposite DNA strand, making up a base pair (Staff 2001). Adenine will bind to thiamine, and guanine only to cytosine. In this way two complementary DNA strands are formed. The information in the DNA is transformed into production of proteins. A gene is a part of the DNA molecule containing the entire necessary base coding for the amino acids that build a particular protein. There about three billion base pairs in the human DNA-molecule and this information is divided into the 46

chromosomes that are found in each body cells. The human DNA molecule in each body cell contains about 100,000 genes. Two thousand base pairs make up an average gene. A single mutation, a wrong letter in the DNA molecule, can result in disease, such as in cystic fibrosis. During transcription, the genetic information from DNA is passed on to the single-stranded mRNA (messenger RNA, ribonucleic acid), which can pass into the cytosol of the cell. At the beginning of each gene there is a base sequence called the gene promotor, which starts the production of the mRNA molecule (transcription of the gene) by binding the enzyme RNA polymerase. The induction or start of gene transcription is a very complicated event where many elements cooperate, such as transcription factors, nuclear receptors, regulatory DNA elements, co-activators, and co-repressors. In the cytoplasm, this genetic information in the mRNA molecule is translated into the formation of proteins (translation) on the ribosome. Three bases on the mRNA molecule attract a transporter molecule and code for one of the twenty possible amino acids the cell uses to build proteins. By determining protein production, genes regulate essential cell functions. Proteins are involved in transporting energy sources in the form of lipoproteins; they are involved in the energy metabolism (hormones are proteins), and in cell receptors etc (Staff 2001). Proteins build, destroy, send and receive signals, and sense changes in their environment. They are the bricks and mortar of cells, the circuitry in our brains, the

defenders against invaders, the chemicals that break down our food and convert it to energy, and the signals that cause us to grow and change.

Before one is able to venture into the field of isolating specific pieces of genetic information, it is of utmost importance to have a starting point. To just begin randomly trying to isolate a specific material is literally like finding a needle in a haystack. One such starting point is to first define some phenotypes that may have a genetic factor that influences them. A phenotype is defined as the detectable outward manifestations of a specific genotype or the form taken by some character or group of characters in a specific individual. A genotype is defined as the specific allelic composition of a cell-either of the entire cell or, more commonly, for a certain gene or a set of genes. By first defining specific phenotypes, it is possible to start isolating the genotype responsible, or partially responsible, for the phenotypic expression.

I. Functional and Positional Cloning

Two contrasting strategies of gene identification can be employed. These strategies are positional cloning or "reverse genetics" and functional cloning or "forward genetics". Positional cloning or "reverse genetics" involves the process of going from gene to phenotype. On the other hand, functional cloning or "forward genetics" starts with the phenotype and proceeds to the gene. Takahashi and his colleagues (1994) suggested "forward and reverse genetic approaches" to achieve the genetic analysis of complex traits in the mouse. Forward genetic approaches begin by

characterizing the phenotype of interest and move towards identifying the genes or gene 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 for any particular phenotype. 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 phenotypic trait and searching for correlations between the phenotypic SDP and the genotypic SDPs of alleles at marker loci (linkage analysis). This part estimates the possibility of any specific genetic loci contributing to the phenotypic 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. Possible protocols for these sets include intercross (F2) or backcross (N2) panels of mice, or congenic strains of mice. Step three determines the importance of the candidate Qualitative Trait Loci (QTLs) and gives more information for accurately mapping their location. The fourth step is to develop/identify target genes. Specifically, to evaluate the contribution of the target genes to the trait of interest experimental animal models such as

knockout, mutant, or congenic strains of mice are usually used for this step in the process (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). Positional cloning was invented by Bender and colleagues, who used it to isolate the bithorax complex in *Drosophila*, and it rapidly, became a routine technique in flies and worms (Lander and Schork 1994).

II. Inbred Rat Strains

Inbreeding is the mating of individuals, which are related to one another by having one or more common ancestors. Brother and sister mating (or its genetic equivalent in terms of other relationships), for twenty or more consecutive generations produces an inbred strain. After twenty generations of full-sib mating the probability that an individual is homozygous at any given locus is at least 0.986, and unexpressed recessive genes are not normally carried within the colony. Inbred strains have a number of properties which make them particularly useful in research. These properties are: isogenicity, homozygosity, phenotypic uniformity, individuality, long-term stability,

identifiability, sensitivity, and international distribution. All individuals within an inbred strain are genetically identical or isogenic. They will be homozygous at all chromosome loci. They are usually more uniform than are nonisogenic stocks. As a consequence of this uniformity, fewer inbred than outbred animals are usually required to achieve a desired level of statistical precision. They will remain genetically constant for long periods of time, since, unlike outbred stocks, they do not respond to selection and do not change as a result of inbreeding. They can be identified by their genetic profile. Finally many of the most common inbred strains are internationally distributed so that work carried out in the United States (USA), Japan, European Union (EU), and elsewhere is often comparable (Festing 1981). Intercrosses occur when two inbred strains are crossed and an F1 generation is produced. F1 hybrid is genetically identical to each other. In contrast to inbred strains, F1 hybrids will be "heterozygous" at all loci at which the parental strains differ. When two F1 hybrids are bred an F2 generation is produced. 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 genetic variation is under the control of experimenter (Festing 1979). The use of inbred strains and Fl hybrids make possible a reduction in the number of experimental animals needed to give conclusive results (Barnett 1975). The genetic identity offers the possibility of

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

III. Strain variations of the Craniofacial Complex in Rats and Mice

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 discriminate 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. The bones examined were the mandible, os coxae (bone of the hip), femur, tibia fibula, scapula and humerus. They found marked variation in the shape of each bone and could successfully discriminate among strains using variability in bone shape. Correct strain classification ranged from 87% for the scapula to 98% for the os coxae (bone of the hip). Successful discrimination between genotypes was obtained with the mandible and three additional bones. The variation in bone shape among genotypes was likely to be under genetic control. Sexual dimorphism was detected in the mandible. Differences in the ages of the animals in the samples might have increased the within-strain variability.

Riesenfeld (1976) in a study of race formation in breeding isolates among different strains of rats reported: the relative nasal length in Wistar rats is significantly greater than in wild rats. The only strain which shows significant degree of brachycephalization is Brown-Norway in both sexes. This change is not due to a shortening of neurocranial length but must be caused by greater cranial width. The relative cranial height is greater in Brown-Norway rats and in male Lewis rats. Brown-Norway rats show a significant broader face than any other strain including wild rats. Riesenfeld states that while this broader face happens to coincide with their exceptional

brachycephalization, it has been shown repeatedly that there is no functional connection between the development of the masticatory musculature and the width of the skull. Thus, this combination seems to be purely genetic (Riesenfeld 1976). Furthermore, Sprague-Dawley male and female rats, Fisher 344 male rats and Brown-Norway male and female rats show a statistically significant lower nasal index than the wild rat. This drop of the nasal index is due to a significant increase in nasal height for the Brown-Norway rats and decrease of nasal width for the Sprague-Dawley rats and female Fisher 344 rats (Riesenfeld 1976). In another publication Riesenfeld (Riesenfeld 1977) concluded that short-faced rats (Sprague-Daley and Buffalo) have markedly shorter lower molars than long-faced rats (Brown-Norway).

IV. Genetic Studies on the Craniofacial Configuration of Laboratory Rats

Rats and mice are commonly used in growth studies, because they have high reproductive rates, mature quickly, and have short life spans. In terms of life span, one year of rats' life is physiologically comparable to thirty human years. Besides, the availability as inbred strains is an important element for investigating complex developmental issues that require detailed genetic analysis. Their larger size often makes rats a better choice than mice to quantify genetic factors in the craniofacial complex (Spence 1940; Nonaka and Nakata 1984). Nonaka and his colleagues reported a series of papers regarding the genetic analyses of the craniofacial complex in the same

sample of rats over several years (Nonaka et al. 1988; Nonaka and Nakata 1984, 1988a, 1988b; Yanagita et al. 1992). Diallel cross among three inbred strains of rats and two inbred closed-colony strains provided the data for their studies. Seventy- five rats of each sex were anesthetized with ether at 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, and 80 days of age. Lateral and ventrodorsal radiographs of the head were taken and a set of eleven cephalograms (one for each time point) was used to investigate craniofacial growth. They conducted the longitudinal study in order to overcome the limitations imposed by using cross-sectional data, i.e. that individual growth patterns are hardly taken into account. The purpose of the study was to clarify longitudinal change and determine the relative contributions of genetic and environmental factors to the phenotypic variation in growth of the craniofacial complex.

In the first part of their study, they examined the lateral view of the craniofacial complex (Nonaka and Nakata 1984). The antagonistic "behavior" between the additive genetic variance and maternal variance through all experimental periods was reported. This means that in the early stage of the growth of rats, the main source of phenotypic variation is attributed to the maternal effect; later, as the maternal effect decreases, the genetic effect becomes significant. In the second part of the study, body weight and overall size of the craniofacial complex (Nonaka et al. 1988) were examined. The inverse behavior between the additive genetic effect and maternal effect was

detected again for body weight and overall size. They added that the maternal effect disappeared earlier in female rats than in male rats and assumed that it may be a result of early maturation in females. The third investigation came from the data of the ventrodorsal radiograph (Nonaka and Nakata 1988a). They found that the contribution of genetic effects increased with age and that environmental variance did not change much throughout the experimental period. They also reported the contribution of the maternal effect on the early stages of craniofacial growth. Then, the role of the genetic component in the craniofacial shape changes was investigated (Nonaka and Nakata 1988b). The authors found that the genotype had a considerable effect on the change of craniofacial shape during growth, while the maternal effect did not have a significant role in the craniofacial shape change during the same period of growth. Yanagita et al. (1992) conducted quantitative genetic analysis of the factor scores from the data using both lateral and ventrodorsal radiographs. Their conclusions were that genetic effects had greater influence on growth changes in craniofacial width and length, while environmental factors had a greater impact on structures of the upper jaw, nasal cavity, and pharynx.

Atchley (1980, 1981, 1983) published another series of papers on the quantitative genetic aspects of variation and covariation in the laboratory rat on the 189th day of age. They dealt with several topics, including the magnitude of heritability of patterns of morphometric size and shape variation,

the patterns of genetic covariation in functional character sets and the dynamics of phenotypic variance and covariance components during ontogeny. All rat strains were originated from a common genetic base (cross of four inbred strains). In the first paper, which investigated live body traits (body weight, tail length and chest circumference), they partitioned the phenotypic variance and covariance and examined the dynamics of these components. They found that the bivariate growth relationships observed between body weight and body circumstances and between body weight and tail length were highly heritable and responded to selection. Systemic change in the narrow sense 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 0.157 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 a 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 (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 mandibular 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 (Atchley 1983).

Most quantitative phenotypes represent complex traits. A complex trait is one 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).

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.

V. Etiology of Strain Variation

Morphological variability in craniofacial dimensions stems from the interplay of a number of genetic and non-genetic factors. There have been a few studies attempting to partition genetic and environmental components of variation within the craniofacial complex.

Quantitative genetics is the part of genetics that study traits with continuous variation. 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 form 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, epistasis, pleiotropy, linkage, and mutation, are taken into account in quantitative genetics.

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 can arise 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. 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 traits 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 phenotype often varies quantitatively across different individuals, as opposed to the classical Mendelian "all-or-none" pattern of expression (Toth and Williams 1998). The genetic study of quantitative traits focuses on their variations. The total phenotypic variation is the sum of the separate components, which include genetic and environmental factors. The genetic variance can be subdivided into additive and non-additive genetic variance. Additive genetic variance is the variance arising from the combination of a number of individual genes. This variance is the main cause of resemblance between relatives, and is the chief determinant of observable genetic similarity. Additive genetic variance is thought to be the response by the population to natural selection. On the other hand, non-additive genetic variance is attributed to the effects of dominance and epistasis effects. In a population, a relatively smaller proportion of the total variation is usually explained by non-additive genetic factors. In the genetic study of rats by Nonaka and Nakata (1984), they observed abnormally large non-additive genetic variance. In addition to the possibility of over-dominance and epistasis, they suggested linkage disequilibrium with heterosis and sampling error as other possible explanations.

Environmental variance includes, by definition, all variation of non-genetic origin. A large variety of factors are known to cause environmental variance. The contribution of each of these factors depends on the characteristics of the organism being studied. Environmental variance is generally considered a source of error that reduces accuracy in genetic studies. Therefore, proper design of experiments with careful environmental management is required to minimize this variance. Nutrition and climate are the most common external factors that are partially under experimental control. Maternal effects are another source of variation. Unfortunately, maternal effect can be difficult to control. Maternal effects can be divided into prenatal and postnatal elements. Prenatal factors are associated with the intrauterine influence of the mother. Postnatal maternal effects are those arising from the influence of the mother on her offspring. Among postnatal maternal influences, those factors related to mothering, such as temperament, maternal instincts, and lactational output are thought to be most important (Falconer 1996; Nonaka and Nakata 1984).

Central to the study of variation is its partitioning into various components. The relative magnitude of these components determines the genetic properties of the population. The partitioning of the variance into its components leads us to evaluate the relative importance of each source of variation, especially the role of heredity versus environment, or nature and nurture (Falconer 1996).

Sewall Wright (1932) was one of the first to attempt a rigorous partitioning of morphometric variability into underlying components. Path analysis was used on a series of skeletal traits to partition multidimensional variability into general, group, and special size factors. General factors are those factors showing systematic influences in all major regions of the body. Group factors are those factors having particular effects on a set of traits and act independently of those affecting other groups of traits. Special factors have effects on single traits.

The relative importance of heredity in determining phenotypic characteristics is termed the "heritability" of the trait. Heritability can be further subdivided into broad and narrow sense heritabilities. When the total genotypic contribution to phenotypic variation is considered, this is called heritability in the broad sense. This total genotypic variance can be further partitioned into additive variance (contributions from individual alleles), dominant variance (contributions from pairs of homologous alleles at a locus), and epistatic variance (contributions from combinations of non-homologous loci). The narrow sense heritability calculates the contribution of additive genetic variance to the phenotypic variation and expresses the extent to which phenotypes are determined by the genes transmitted from the parents (Falconer 1996; Atchley 1983).

VI. The future in Orthodontics

Slavkin (1999) states that the future of the research in craniofacial biology is two-fold: 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) -- members of the TGF-beta super family of secreted signaling molecules -- 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).

The Human Genome Project is completed and an accurate DNA sequence representing the genetic blueprint and evolutionary history of the human species is available. 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. Among the findings from the Human Genome Project, the most important ones are: People have only 26,000 to 40,000 genes. Inherited genetic mutations arise about twice as often in men than women. Only 1% to 1.5% of human DNA carries instructions for making proteins, compared to the 3% to 5% the researchers had expected. Along the stretches of DNA, genes tend to occur in clusters, like cities separated by vast stretches of countryside.

Some 200 human genes apparently arose from genes that were somehow inserted into humanity's early vertebrate ancestors by bacteria (Associated Press).

VII. 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).

STATEMENT OF THE THESIS

The present study was designed to evaluate the phenotypic variation in craniofacial morphology. Specifically, the rat animal model was used so genetic and environmental factors could be controlled and assessed. Direct measurements from the dry skulls (craniometry) and x-rays (cephalometry) were used to characterize the quantitative traits in the craniofacial complex of rats. In particular, size and shape of the cranium, maxilla, and mandible were measured and compared for intra-strain and inter-strain variation using the Procrustes Algorithm.

We hypothesize that shape and size differences in the (i) cranium (ii) maxilla and (iii) mandible are greater among animals of different rat strains than among animals within the same strain. In other words, the hypothesis can be simply stated. The inter-strain variation will be significantly greater than the intra-strain variation. The null hypothesis then becomes the following. Interstrain variation in rat craniofacial morphology is similar to intrastrain variation.

The present study has 3 specific aims:

> Specific aim 1. Create the database for craniofacial configuration among eight rat strains.
>
> Specific aim 2. Determine variance in <u>size</u> of the cranium, maxilla and mandible among eight rat strains (inter-strain), and

evaluate the modified Cranial Index, using craniometric measurements.

Specific aim 3. Determine variance in shape of the cranium, maxilla and mandible among eight rat strains (inter-strain), and between genders of the same strain (intra-strain, i.e. inter-gender), and within the same gender (intra-strain, i.e. intra-gender). Measurements will be collected using cephalometrics and shape variation will be separated from size variation using the Procrustes Algorithm.

The goal of this research was to provide valuable preliminary data for genetic projects in the Department of Orthodontics at Case Western Reserve University to investigate mechanisms of inheritance and identify candidate genes for the morphogenesis of the craniofacial complex.

MATERIALS AND METHODS

I. Study Samples

Eight strains of laboratory rats were used in the present study. Five strains were inbred strains and three were outbred strains. The eight strains (n=4 males, 4 females in each strain) were Sprague-Dawley (SD), Brown-Norway (BN), Lewis (LEW), Fisher-344 (F344), Wistar-Kyoto (WKY), Long-Evans (LE), Holtzman (HZ), and Buffalo (BUF). The inbred strains were BN, BUF, LEW, F344 and WKY. Their characteristics and generational age are presented in the Table 1. The SD, LE and HZ were the outbred strains. Since the rat skeleton is not fully mature until 6 months of age, we set our criteria for an adult rat to be defined as greater than 6 months old. In order to assure that the environmental factors, including housing, food, and animal care were the same among strains, retired breeders or excess animals were obtained from a single animal vendor (Harlan, Indianapolis, IN). All animals were housed at Room K-27, Wade Park Unit, Veterans Affairs Medical Center, Cleveland, OH, and were provided with water and food *ad libitum* for at least 3 weeks before sacrifice. Animals were under the direct observation of the veterinary staff and were in good health. A total of sixty four adult animals, which were between six and fourteen months old at the time of sacrifice, were studied (Moullas et al. 1999). SD and BN were chosen

because they exhibit the largest differences in breathing patterns and both of them are normotensive (Strohl et al. 1997). The BN strain is sold as having undergone at least 11 generations of brother-sister mating (F11 and Barrier 207). This results in animals that are basically genetic twins. The BN's color is brown non-agouti. Body size is medium and reproduction is very poor. They are not very docile (Riesenfeld 1976; Festing and Staats 1973). The SD breathes slower and deeper when compared to the BN. The BUF is albino, has a low incidence of dental caries, and grows various tumors in older animals. The BUF strain is sold as having undergone at least four generations of brother-sister mating (F4). Body size and reproductive capacity are medium (Riesenfeld 1976). The WKY strain is sold as having undergone at least twenty generations of brother-sister mating (F20 and Barrier 205). The WKY is albino. The WKY rat is widely used in Europe, whereas, the SD is more popular in the United States. The LEW rat was selected because it exhibits different physiological characteristics during sleep. The LEW strain is sold as having undergone at least 45 generations of brother-sister mating (F45 and Barrier 202B). It is docile and high fertility (Festing and Staats 1973). The F 344 is albino but carry non-agouti black or hooded types. They grow various induced and transplanted tumors. They are of small body size but weight gains are above average (Harrington 1981). They have excellent reproductive capacity (Riesenfeld 1976). The F344 strain is sold as having undergone at least 44 generations of brother-sister

mating (F44 and Barrier 202A). It is chosen because of its docility (Harrington 1981). It has been widely used to study processes associated with aging. The LE, HZ, BUF are variations of albino rats and have all been used previously for cephalometric studies.

TABLE 1.--The Five Inbred Strains and Their Generational Age.

Strain	Coat color	RT1 Haplotype	Generational Age
BN/RIjHsd	non-agouti brown	n	F11, Barrier 207
BUF/SlmRIjHsd	Albino	b	F4
F344/NHsd	Albino	lvl	F44-F45, Barrier 202A
LEW/SsNHsd	Albino	l	F45-F47, Barrier 202B
WKY/NHsd	Albino	l	F20, Barrier 205

Prior to any experimentation, the protocol for this study was submitted and approved by the Institutional Animal Care and Use Committee of Case Western Reserve University.

Animals were individually identified and assigned an identification number. At that time, animals were euthanized according to the 1986 AVMA (American Vetenery Medical Association) with carbon dioxide inhalation for period of five minutes. Immediately following sacrifice, anthropomorphic data (body length-nose to anus-, body weight) were taken. Body mass index (Lee Index) was calculated by dividing the cube root of body weight in grams by body length in millimeters (Moullas et al. 1999).

The cephalometric x-rays (lateral and dorsoventral) were taken using a modified cephalostat (Figure 1) designed by Palmer (1978) to insure

reproducible object-film and source-film distances and the Broadbent-Bolton cephalometer (Series 1K550, Bolton Fund, Cleveland, Ohio) and standard occlusal film (Kodak, Df46, Rochester, NY) at 70KV, 20mA with exposure time of 3 seconds. After the x-rays were taken, the rats were defleshed according to standard protocols. Briefly, the skin was removed from the head, and right hind leg, the femur removed, and the fleshy skull and femur with musculature intact were placed in the same bag and were frozen at the – 20 C until ready for use. The frozen skulls and the right femurs were transferred to 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 (Moullas et al., 1999). Figures 2 – 6 show the dry skulls and mandibles.

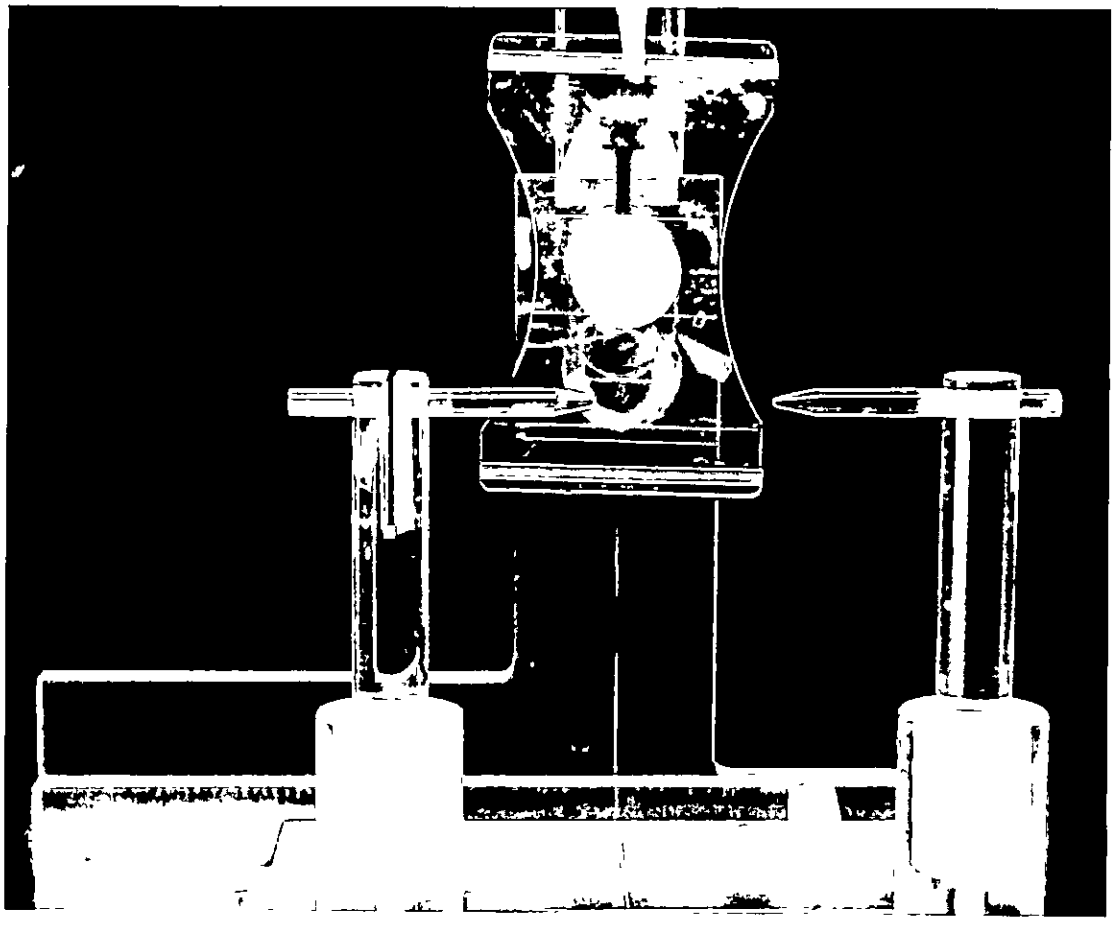


Figure 1.The modified cephalostat designed by Palmer.

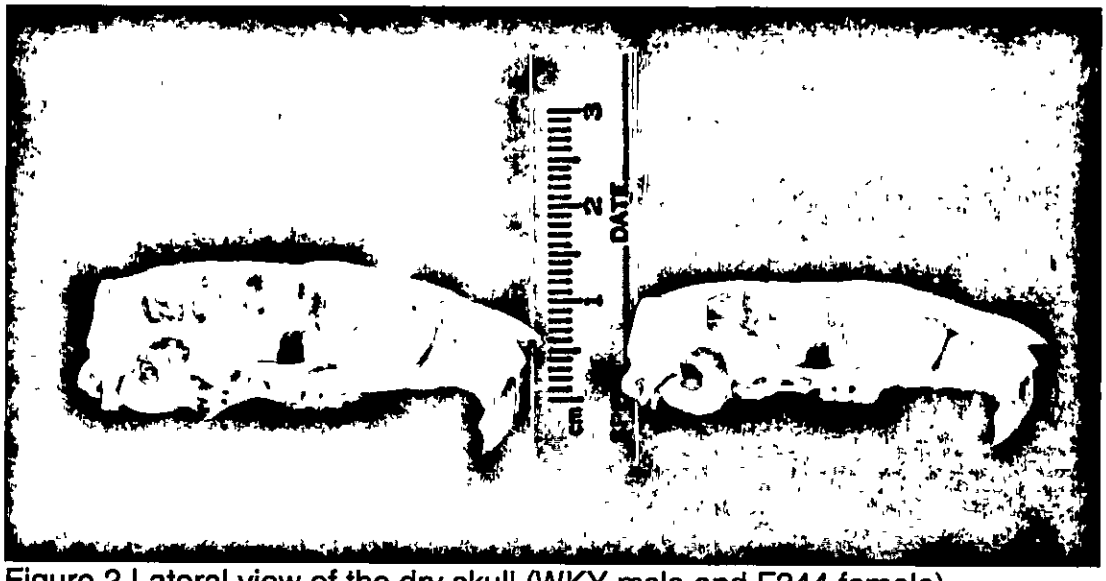


Figure 2.Lateral view of the dry skull (WKY male and F344 female).

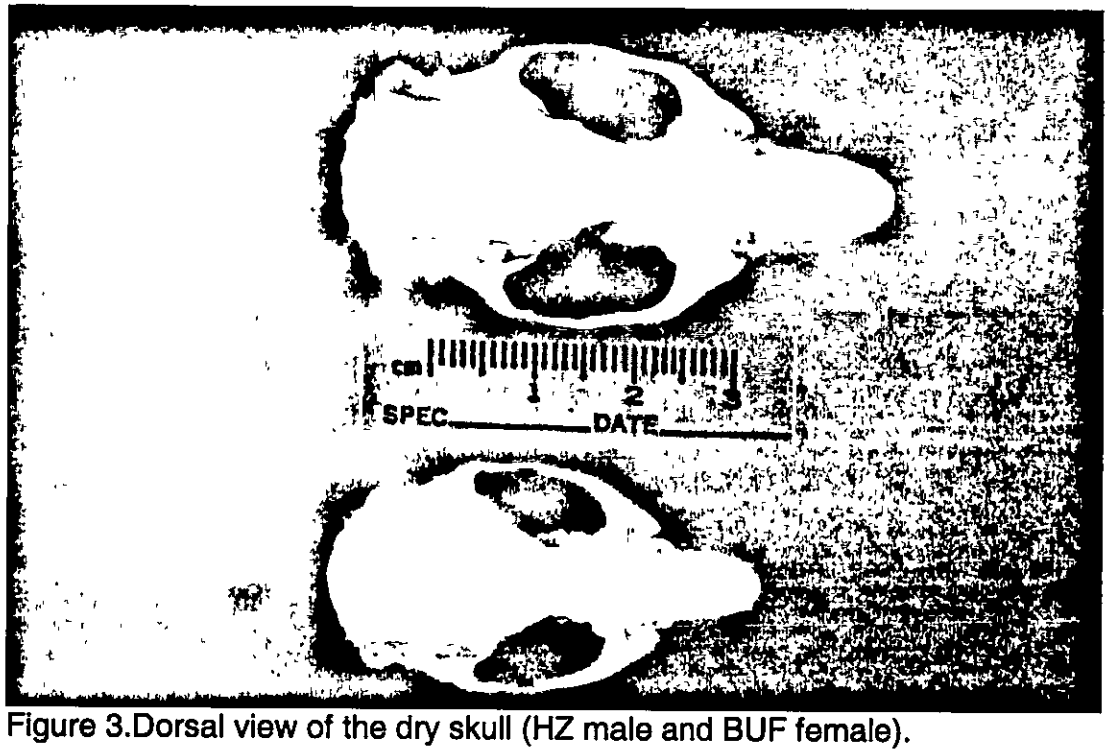


Figure 3.Dorsal view of the dry skull (HZ male and BUF female).



Figure 4.Ventral view of the dry skull (HZ male and WKY female).

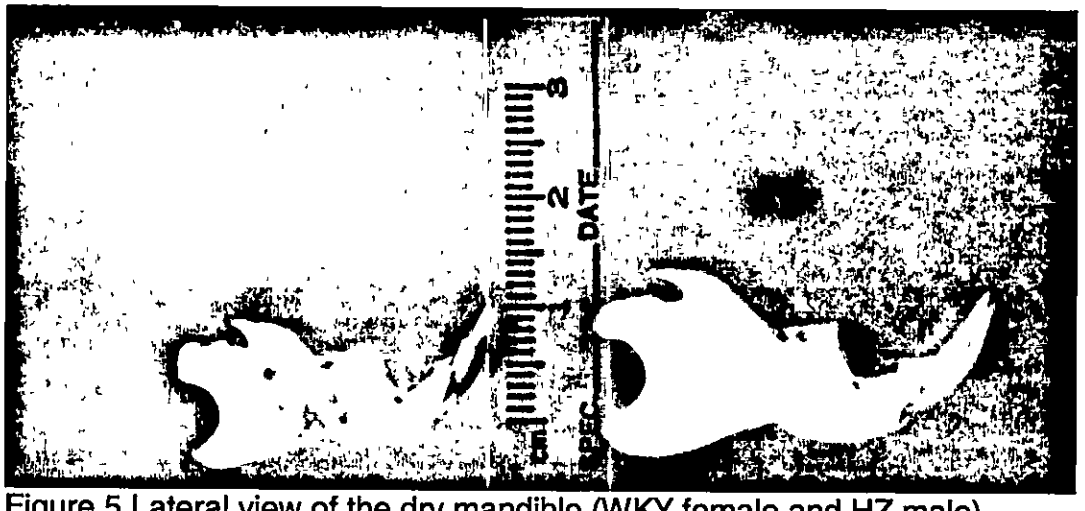


Figure 5.Lateral view of the dry mandible (WKY female and HZ male).

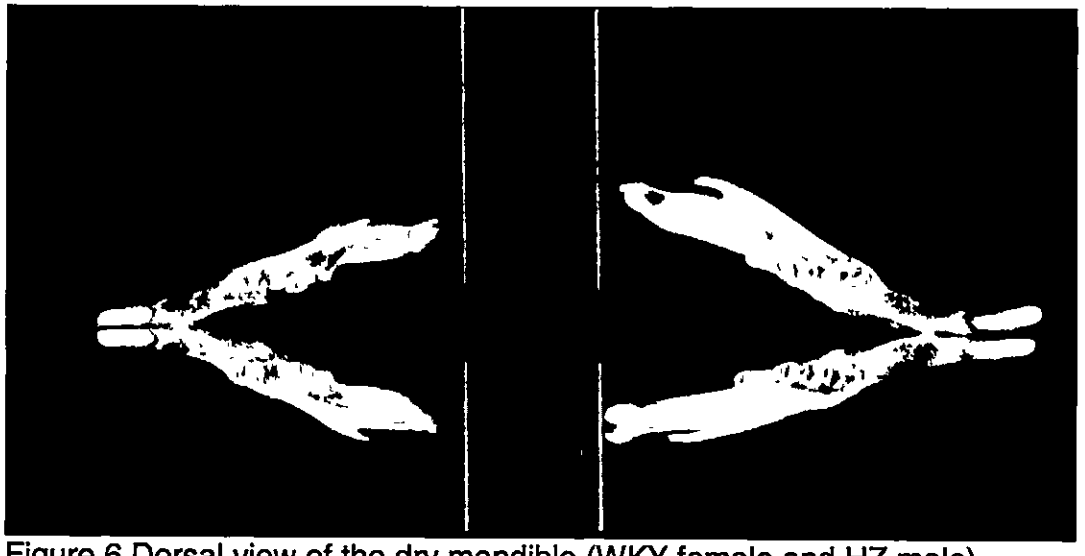


Figure 6.Dorsal view of the dry mandible (WKY female and HZ male).

II. Anatomic Landmarks

Nineteen linear measurements were utilized to characterize the craniofacial features. Descriptions of each measurement along with its construction are found in Table 3 and are illustrated in Figure 8. The following anatomic landmarks were used for the twenty-one linear morphologic traits. The anatomical landmarks are listed in Table 2 and illustrated in Figure 7.

TABLE 2.--The Anatomical Landmarks Used for the Craniometric Linear Measurements.

Landmarks in the Mandible	
Ap	The most inferior-posterior point of the angular process
Id	Infradentale. The most superior point on the alveolar process between the lower incisors
Co r, l	The most superior point of the condyle
C r, l	The most superior point of the coronoid process
Lm	The most superior point of the mandibular alveolar process on the first lower molar
Landmarks in the Maxilla	
Ul	Supradentale. The most inferior point on the alveolar process between the upper incisors
Pns	The most posterior point of the palatal process on the palatal bones on the midline
Um	The most inferior point of the maxillary alveolar process on the first upper molar
Mum r, l	Mesiobuccal cusp of the upper first molar
Dum r, l	Distobuccal cusp of the upper last molar
Mx r, l	The most anterior point on the maxillary bone at the infra-orbital foramen
Landmarks in the skull	
Nf	The most anterior point of the frontonasal suture on the midline
O	The most posterior point on the external occipital protuberance
N	The most anterior point (tip) of the nasal bone
Brg	Bregma
Lbd	Lambda
Zg r. l	The most lateral point on the zygomatico-maxillary suture
So	The most inferior (ventral) point on the spenoccipital synchondrosis
P	The most superior point of the parietal bone perpendicular to spenoccipital synchondrosis.
Landmarks in the Cranial Base	
So	The most inferior (ventral) point on the spenoccipital synchondrosis
Ba	The most anterior point on the margin of the foramen magnum

TABLE 3.--Craniometric Linear Measurements and Its Construction.

Measurements In the Mandible		
A1	**Ap-Id**	Mandibular body length
A2	**Ap-Lm**	Mandibular body length
A3	**Ap-Co**	Mandibular ramus height
A4	**Ap-C**	Mandibular ramus height.
A5	**Co r-Co l**	Bicondylar width
A6	**C r- C l**	Bicoronoid width
Measurements In the Maxilla		
B1	**Ul-Um**	Premaxilla length
B2	**Ul-Pns**	Overall palatal length
B3	**Mum r-Mum l**	Anterior palatal width
B4	**Dum r-Dum l**	Posterior palatal width
B5	**Mx r-Mx l**	Maxillary width
B6	**Mum r-Dum r**	Right maxillary tooth row length
B7	**Mum l-Dum l**	Left maxillary tooth row length
Measurements In the Skull		
C1	**So-P**	Skull height
C2	**Nf-O**	Skull length
C3	**Lbd-Brg**	Parietal bone length
C4	**Zg r-Zg l**	Skull width
C5	**N-Nf**	Nasal bone length
C6	**Ba-So**	Cranial base

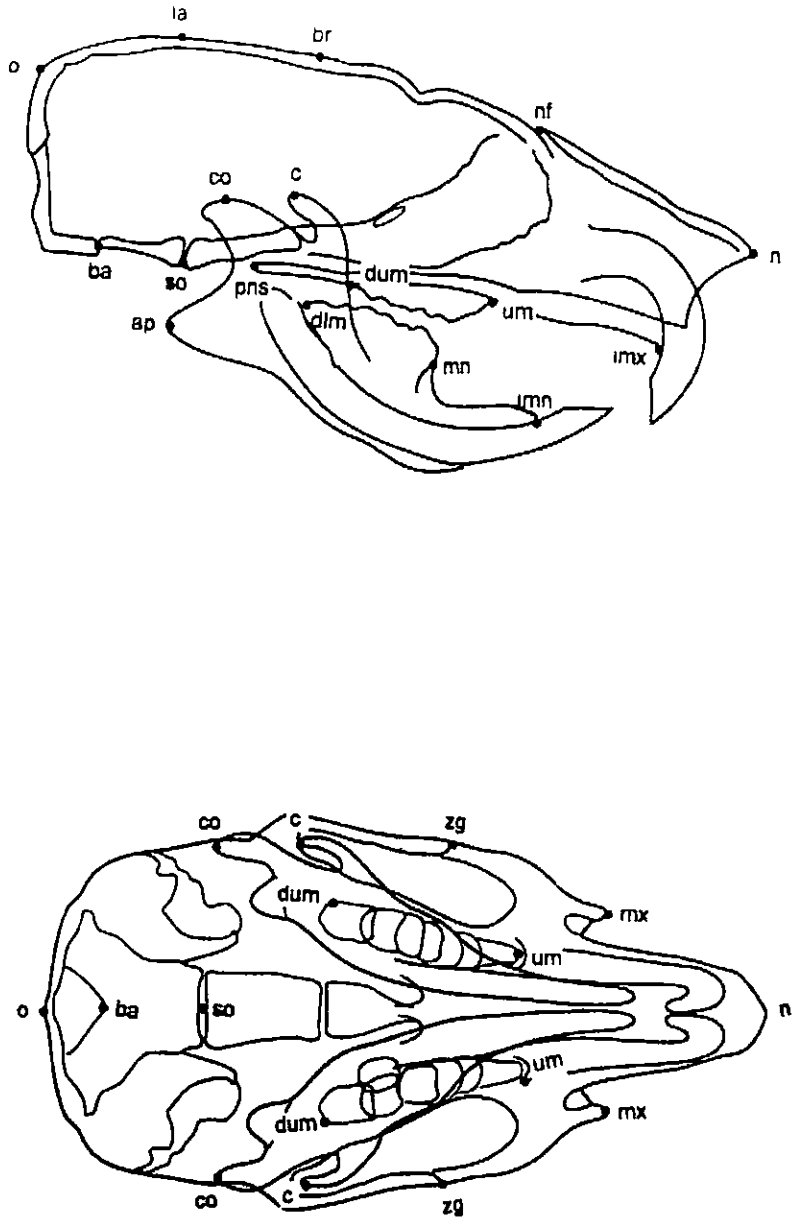


Figure 7.Anatomical landmarks in lateral view (top) and in dorsoventral view (bottom) used for the craniometric linear measurements. See Table 2 for landmark abbreviation definitions.

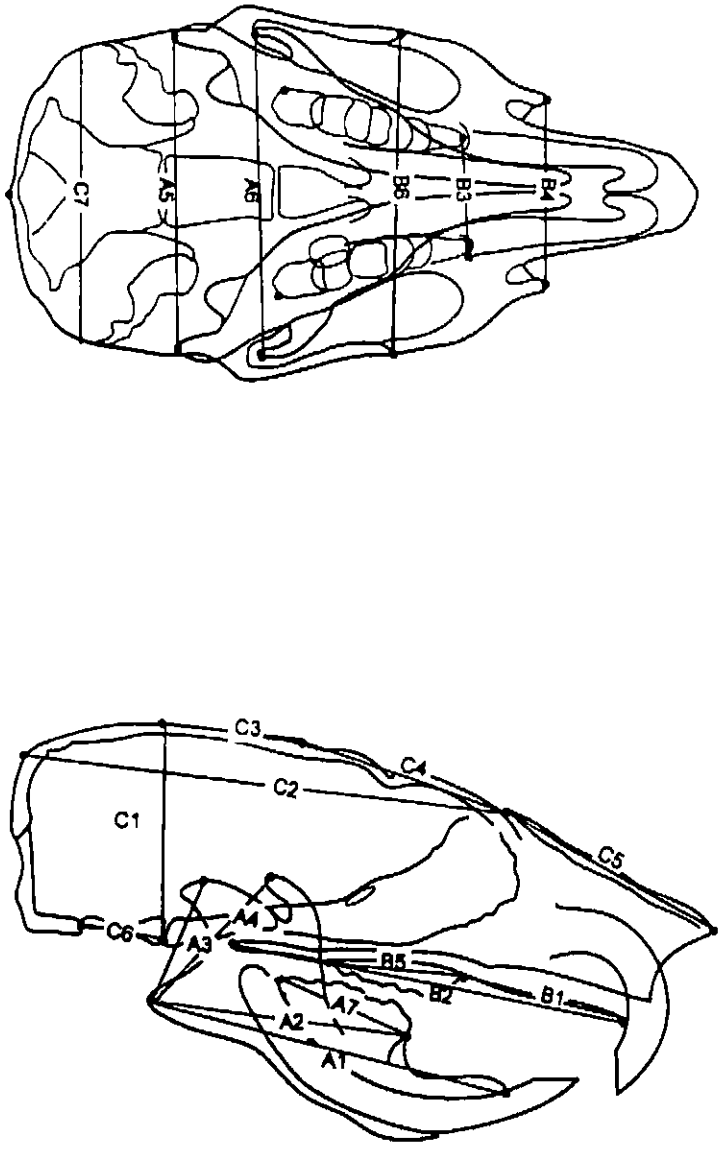


Figure 8.Craniometric linear measurements in lateral view (top) and in dorsoventral view (bottom). See Table 3 for linear measurement construction.

III. Procrustes Landmarks

Seventeen cephalometric landmarks at the lateral view, Figure 9 and 11, (11 landmarks at the nasomaxillary complex and the cranium and 6 landmarks at the mandible,) and fourteen cephalometric landmarks at the dorsoventral view, Figure 10 and 12, (10 landmarks at the nasomaxillary complex and the cranium and 4 landmarks at the mandible) were used as a constellation to capture total craniofacial phenotypic variation. The cephalometric landmarks in lateral and dorsal-ventral x-rays for the cranium and mandible are listed in Table 4-7 respectively. Figures 9-12 are shown the lateral and dorsal-ventral x-rays with the landmarks for the cranium and the mandible respectively.

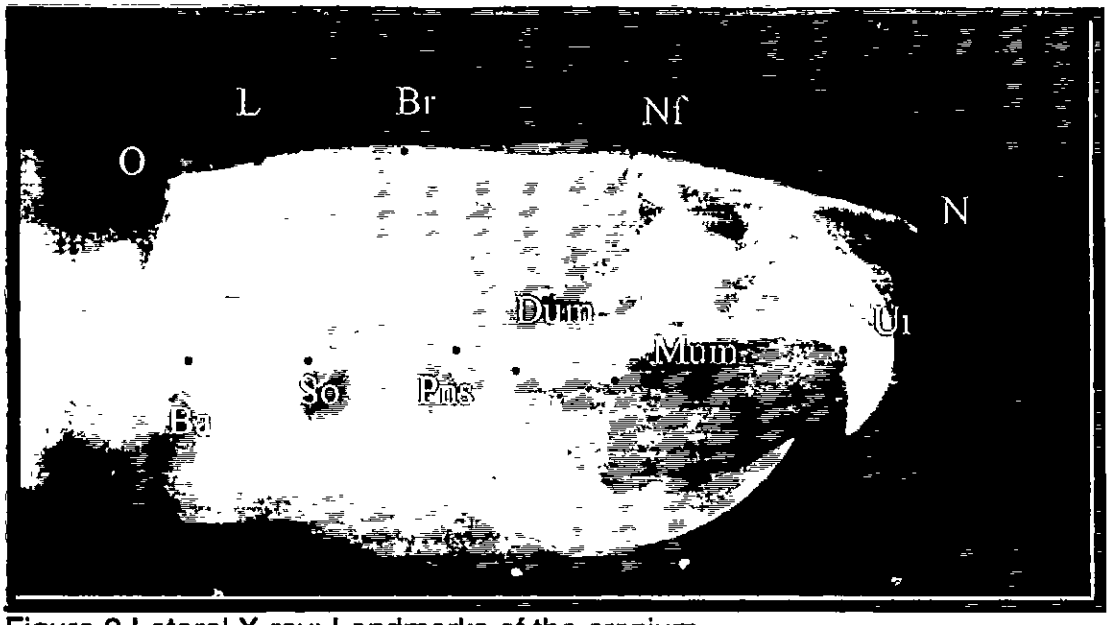


Figure 9.Lateral X-ray: Landmarks of the cranium.

TABLE 4.--The Landmarks Used for the Procrustes Shape Analysis in the Lateral X-ray for the Cranium.

Lateral X-ray: Cranium	
N	The most anterior point (tip) of the nasal bone
Nf	The most anterior point of the frontonasal suture on the midline
Br	Bregma. The junction of the parietal bone and frontal bone on the midline
L	Lambda. The junction of the parietal bone and interparietal bone on the midline
O	The most posterior point on the external occipital protuberance
Ba	The most anterior point on the margin of the foramen magnum
So	The most inferior (ventral) point on the spenoccipital synchondrosis
Pns	The most posterior point of the palatal process on the palatal bones on the midline
Dum	Distobuccal cusp of the upper last molar
Mum	Mesiobuccal cusp of the upper first molar
Ul	Supradentale. The most inferior point on the alveolar process between the upper incisors

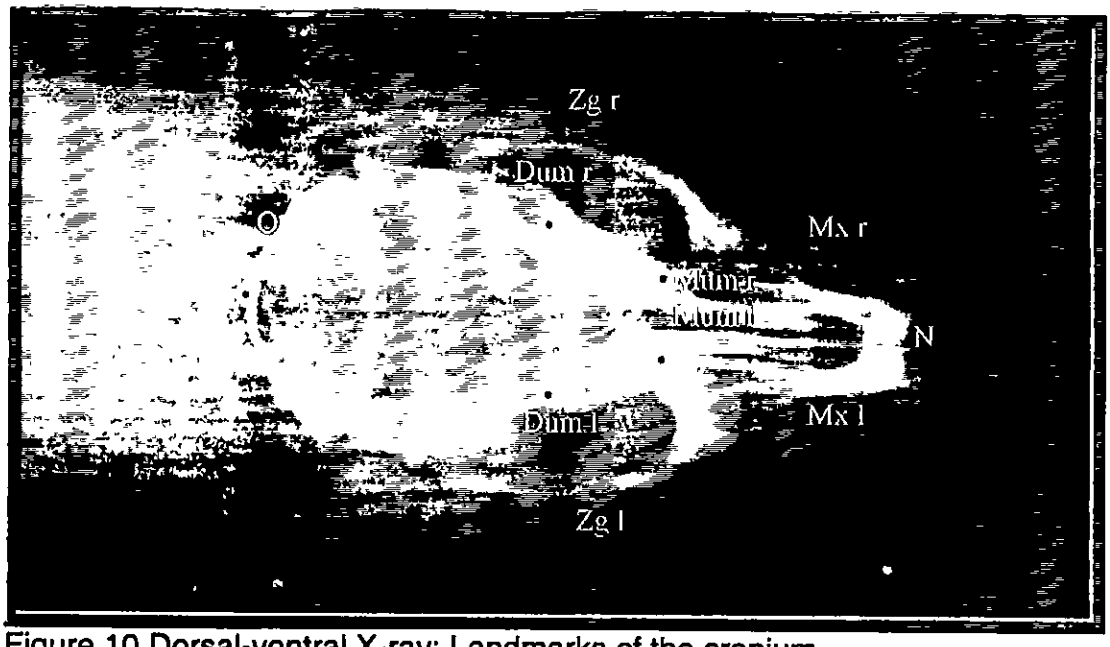


Figure 10.Dorsal-ventral X-ray: Landmarks of the cranium.

TABLE 5.--The Landmarks Used for the Procrustes Shape Analysis in the Dorsal-ventral X-ray for the Cranium.

Dorsal-Ventral X-ray: Cranium	
N	The most anterior point (tip) of the nasal bone
Mx r	The most anterior point on the right maxillary process of the maxilla
Mx l	The most anterior point on the left maxillary process of the maxilla
Zg r	The most lateral point on the right zygomatic process
Zg l	The most lateral point on the left zygomatic process
O	The most posterior point on the occipital protuberance
Dum r	Distobuccal cusp of the upper right last molar
Dum l	Distobuccal cusp of the upper left last molar
Mum r	Mesiobuccal cusp of the upper right first molar
Mum l	Mesiobuccal cusp of the upper left first molar

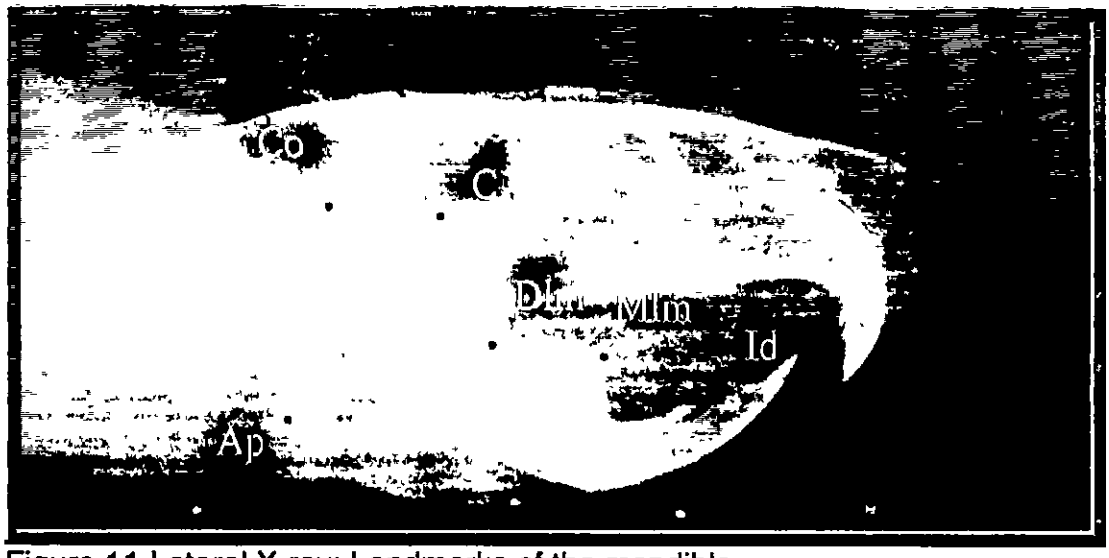


Figure 11.Lateral X-ray: Landmarks of the mandible.

TABLE 6.--The Landmarks Used for the Procrustes Shape Analysis in the Lateral X-ray for the Mandible.

Lateral X-ray: Mandible	
Id	Infradentale. The most superior point on the alveolar process between the lower incisors
Mlm	The most superior point of the mandibular alveolar process on the mesial of the first lower molar
Dlm	The most superior point of the mandibular alveolar process on the distal of the terminal lower molar
C	The most superior point of the coronoid process
Co	The most superior point of the condyle
Ap	The most inferior-posterior point of the angular process



Figure 12.Dorsal-ventral X-ray: Landmarks of the mandible.

TABLE 7.--The Landmarks Used for the Procrustes Shape Analysis in the Dorsal-ventral X-ray for the Mandible.

Dorsal-ventral X-ray: Mandible	
Co r	The most superior point of the Right condyle
Co l	The most superior point of the Left condyle
C r	The most superior point of the Right coronoid process
C l	The most superior point of the Left coronoid process

IV. Data Collection

Craniofacial measurements were made on the dry skulls (bony structures) using a micro-caliper (Digimatic, Mitutoyo Co, Tokyo, Japan) and recorded to the nearest 0.05 mm. Nineteen linear measurements were utilized to characterize the craniofacial features. To increase the precision, all measurements were obtained twice by the same investigator (AM) and averaged. All measurements were entered into a Microsoft™ Excel© spreadsheet for compilation of statistics. Means and standard deviations were calculated.

The cephalograms (lateral and dorsoventral) were scanned at 300 dpi (12 pixels/millimeter or 0.1 millimeter/pixel). The Baumrind method (Baumrind and Frantz 1971a, b) of registering four corner fiducials was used on all radiographs. A template designed by Moullas, Figure 13, at Case Western Reserve was used to punch four fiducials in each of the standard occlusal films. The fiducials provide objective landmarks for comparison of multiple operators' digitization of the same radiograph. Seventeen cephalometric landmarks in the lateral view (six landmarks at the mandible, eleven landmarks at the nasomaxillary complex and the cranium) and fourteen cephalometric landmarks in the dorsoventral view (four landmarks at the mandible and ten landmarks at the nasomaxillary complex and the cranium) were used to capture the craniofacial phenotypic variation. The x and y coordinates were obtained using IDL™ 5.4 (Interactive Data Language,

Research Systems, Inc. – A Kodak Company, Boulder, CO) software. The IDL™ 5.4 software routines were modified by Krishna Subramanyan to determine the average (Procrustes Grand Mean) of all the landmarks found for each group (strain). The averages were then compared to each other, using the Procrustes algorithm for visual data in the form of Cartesian plots.

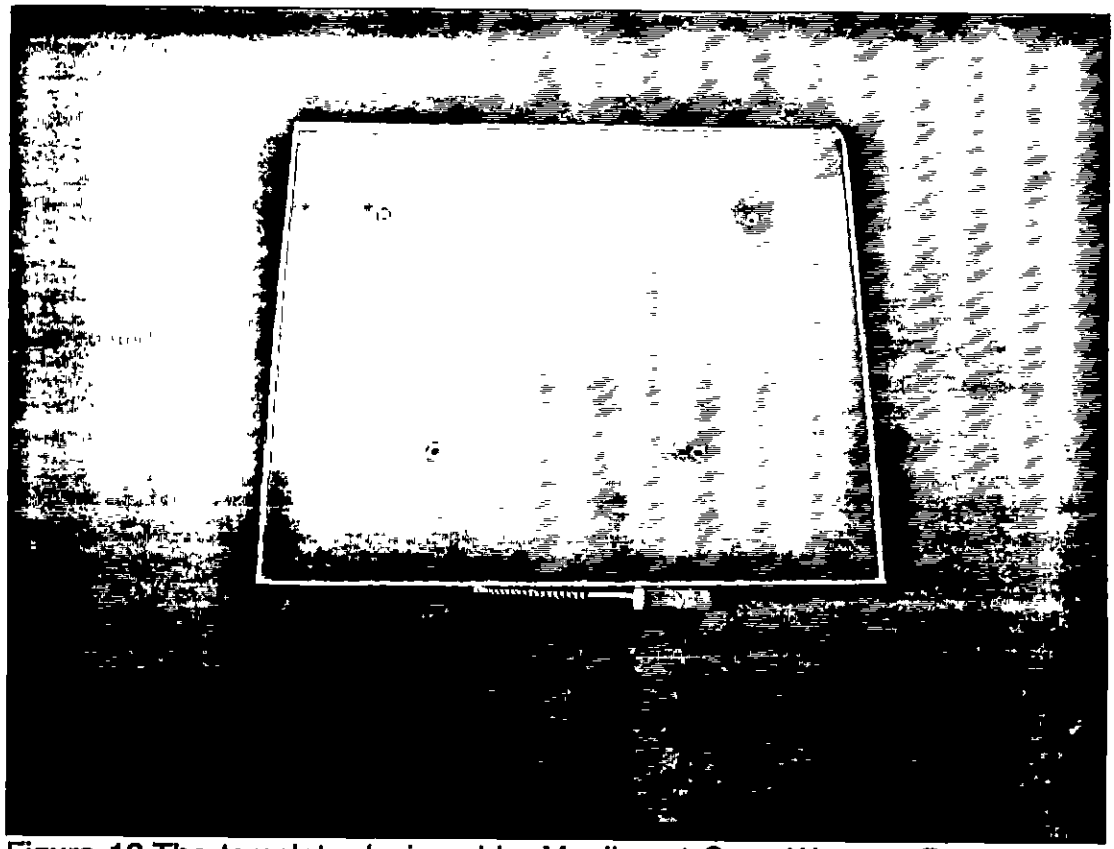


Figure 13.The template designed by Moullas at Case Western Reserve was used to punch four fiducials in each of the standard occlusal films.

In order to establish intra-operator reliability, the author identified the landmarks in 25% of the total sample (16 rats from two different strains) three separate times (T1, T2, T3). A method called thin plate spline based superimposition, proposed by F. Bookstein (1989) is used to superimpose the fiducials of each film over one another. The fiducial markers provide the stable configuration framework upon which each landmark configuration is superimposed. The method uses both linear and non-linear components of the warps, unlike the Procrustes fit, which uses only the linear components. The standard deviation was measured for the three data sets of measurements. The intra-observer reliability testing reports the measuring consistency of one person on different occasions (Kamoen 2001).

V. Data Analysis

V.1. Determining the Variance in Linear Measures

To determine the variance in linear measures of the cranium, maxilla and mandible among 8 rat strains (inter-strain), we used nineteen direct dry skull craniometric measurements. We calculated the means and standard deviations for each measurement from the dry skull for all sixty-four animals. Visual comparisons of craniometric means for nineteen measurements were made in bar charts. The modified Cranial Index (width x100/length) by Moullas was calculated by dividing the cranial width (right zygomatico-maxillary suture to left zygomatico-maxillary suture) to the cranial length (frontonasal suture to external occipital protuberance).

V.2. Determine the Variance in Shape

To determine the variance in shape of the nasomaxillary complex and mandible among 8 rat strains (inter-strain) and between the gender (inter-gender, or intra-strain) in each strain, we digitized a constellation of 31 points on the cephalograms (lateral and dorsal-ventral) and utilized the Procrustes Algorithm to compare the shape of the constellations.

A customized software program written in Interactive Data Language (IDL) was used. (IDL Software, Research Systems Inc. (Kodak subsidiary), Boulder, CO). IDL supports vector operations and rich sets of mathematical and statistical routines. Krishna Subramanyan PhD provided the user interface, image file reading capabilities, landmark identification files and math functions to support the landmark configuration superimposition and constellation averaging.

Procrustes fit is a least square fitting process in order to minimize the distances between homologous landmarks preserving the shape and factoring out size variation. Shape is preserved through affine transformation process involving rotation, scaling and translation. i.e., each configuration is moved (translated) in to a unit space (scaling). The unit space is a Cartesian coordinate system having the x and y coordinates spanning -1 to 1. Later, the configurations are rotated with respect a target configuration, by minimizing the distance between homologous landmarks. A best rotation is computed

through a series of math functions such as matrix multiplications, and singular value decomposition.

The averaging procedure uses a two step procedure. In the first step, the first configuration is assumed to be the target configuration. The rest of the configurations are fitted to the target using the Procrustes algorithm. Next, an average of homologous landmarks was computed via simple summing and division by the number of configurations. In the second step, the average computed in the first step is assigned as the target configuration. All the original landmark configurations are fitted to the average configuration using the same Procrustes fitting process. Finally, a new average landmark configuration is computed. This becomes the average landmark configuration.

Procrustes Distance (PD) is the Euclidean distance between homologous landmarks of the average configuration and the original configuration. PD is computed by taking square root of sum of squares of x and y differences. The square root of the sum of all the homologous distances provides the unique distance characterizing differences between the average and source configuration. Similar comparisons can be also computed between any two configurations. The differences between the configurations are the result of shape differences between the landmark sets, differences due to size are partialled out during the scaling process.

VI. Procrustes Method

- Create a Procrustes Grand Mean for males and females in each group (strain).
- Compare each individual male or female to his or her respective Procrustes Grand Mean (intra-gender variation).
- Compare the male Procrustes Grand Mean versus female Procrustes Grand Mean within each group (inter-gender or intra-strain variation).
- Create a Procrustes Grand Mean for each group (8 strains).
- Compare each group's Procrustes Grand Mean to each other (inter- strain variation).

The Procrustes algorithm is a mathematical calculation that allows shape comparison of two or three-dimensional objects. The Procrustes analysis is based on the following two assumptions: 1) Landmarks are homologous. This means that the landmarks are created from the same biological processes in the separate individuals, being compared, and 2) The number of landmarks was sufficient to capture the shape of the biological region of interest.

The superimposition separates size variation from shape variation (Palomo 1997).

The Procrustes algorithm has the following advantages: 1) Size is treated as a separate variable and does not overdetemine the results, 2) No landmark is seen as primary or stationary with this superimposition technique, and 3) The results are presentable in diagrammatic form that looks like the original data rather than a column of numbers (Palomo 1997).

A Procrustes Grand Mean is not just an average of the x, y coordinate values among all the rats in the group (strain). The Procrustes grand Mean removes size variance by scaling each subsequent subject to the first subject in the group. The normalization of the coordinate space makes the Procrustes grand Mean a convenient method of describing shape differences. The shape difference is measured in metric units (e.g., millimeters). The IDL™ 5.4 software (Research Inc. Boulder CO) was applied to the landmark coordinates of all sixty-four rats for all eight groups to produce eight Procrustes Grand Means. Next, the within group variance was calculated by comparing the eight Procrustes Grand Means to each of the rats within their respective groups. The goal of comparing individual rats to the Procrustes Grand Mean and creating Procrustes distances between the rats and the Procrustes Grand Mean was to determine the within strain standard deviation. The IDL™ 5.4 software fitted the landmarks of each rat to the Procrustes Grand Mean through translation, rotation, and scaling. In other words, the configurations are superimposed and mean shape (the Procrustes mean) was calculated. The displacement of each landmark from the Procrustes mean (the Procrustes residuals) was calculated and these were the input to statistical analysis. The toolbox IDL™ 5.4 was also used to graphically display the landmarks of each of the eight individual rats within a group about the Procrustes Grand Mean.

The between group differences were investigated only after the Procrustes distances between the eight Grand Means were calculated. A Procrustes superimposition of the Procrustes Grand Means to each other was also made. The IDL™ 5.4 software was used to calculate Procrustes distances between two grand means in the same manner in which Procrustes distances were calculated between a single individual rat and a Procrustes Grand mean. One of the two Procrustes Grand Means in each comparison was translated, rotated, and scaled to the other Procrustes Grand Mean for each of the eight Grand Mean comparisons.

The Procrustes distances between two Grand means were normalized for the calculation of the percentage of overall shape difference at any one landmark. Normalization of each landmark distance required division by the overall Procrustes distance total for each particular Grand Mean-to-Mean comparison. The pairwise Procrustes distances between Procrustes Grand Mean generated by IDL™ 5.4 provided the amount of shape difference, but not the direction of shape difference. A plot of the Procrustes superimposition of each pair of Procrustes grand means used different labels for each group. These plots showed the direction of shape difference between the two groups.

RESULTS

The results will be presented in two parts. The first section includes the direct linear measurements from the dry skulls (craniometry) and deals with the variance in linear dimensions of the craniofacial complex among the eight rat strains. The second section includes the cephalometric data. This section deals with the variance in shape of the craniofacial complex among the eight rat strains. The cephalometric data have been analyzed using two different methods. The first method employed Procrustes Superimposition and quantified the shape difference in millimeters (mm) that existed among the rats of the same gender (intra-gender variation), between genders of the same strain (inter-gender variation) and finally among the different strains (inter-strain variation). The second method involved normalization of the data and calculation of the percentage each landmark contributed to the overall shape difference. The landmarks were separated into two craniofacial regions: craniomaxillary and mandible.

Anthropomorphic data (body length-nose to anus-, body weight) are presented in Table 8. Body mass index (Lee Index) was calculated by dividing the cube root of body weight in gram by body length in mm.

TABLE 8.--Means and Standard Deviations of Body Length (mm), Body Weight (g), and Lee Index.

	BN	SD	HZ	WKY
B.L (cm)	22.44±1 74	25.19±1.16	26 42±1 80	23 87±1 38
B.W (gr)	309.50±90.67	432 50±82 60	520.12±120 88	351.25±83 92
Lee Index	0.2984	0 2990	0.3021	0.2929

	BUF	LEW	F344	LE
B.L (cm)	25 21±1 89	24.19±1 57	23 49±2 04	24.87±2.03
B.W (gr)	451 37±102 56	385.25±84 29	339.75±106.19	465.00±118.45
Lee Index	0.3022	0.2987	0 2934	0 3090

I. Craniometric Linear Measurements

Craniofacial measurements were made on the dry skulls (bony structures) using a micro-caliper (Digimatic, Mitutoyo Co, Tokyo, Japan) and recorded to the nearest 0.05 mm. Nineteen linear measurements were utilized to characterize the craniofacial features. To increase the precision, all measurements were obtained twice by the same investigator (AM) and averaged. All measurements were entered into a Microsoft™ Excel© (Redmond, Washington) spreadsheet for later statistical analysis. Means and standard deviations were calculated and are presented in Tables 9-11. Visual comparisons can be made from the Figures 14-16. The comparison of the modified Cranial Index among the eight rat strains is presented at the Figure 17.

Mandible

Overall, in terms of mandibular length: 1) gonion-infradentale, significant differences were as follows H* > BN, F, WK* and B > F, WK* 2) gonion-alveolar process, significant differences were as follows H* > SD, WK, F*.

In terms of mandibular height: 1) gonion-condyloid process, significant differences were as follows LE*, H, BN > F, WK, L* 2) gonion-coronoid process, significant differences were as follows BN*, H > L, F, WK*.

In terms of mandibular width: 1) bicondylar width, significant differences were as follows SD*, H, LE > L, WK, B* and SD*, H > F and SD* > BN 2) bicoronoid width, significant differences were as follows H*, SD > B, L* and LE, WK > L*.

Maxilla

Overall, in terms of maxillary length: 1) premaxilla length, significant differences were as follows H*, LE, SD, B > BN, WK* 2) overall maxillary (palatal) length, significant differences were as follows H*, SD, LE > WK, L, BN* 3) maxillary teeth row length (right, left), significant differences were as follows H* > SD, B, L, F* and LE > L, F* and L, B, SD, BN > F*.

In terms of maxillary width: 1) anterior palatal width H*, L > F, WK, SD* and H* > B, BN and LE > WK, SD* 2) posterior palatal width H*, BN, B, L, LE > WK, F* and H* > BN, B, L, LE, SD 3) maxilla width H*, SD > L, F* and H* > WK.

Skull

Overall, in terms of skull length: 1) skull length, significant differences were as follows H*, LE, SD, B > L, BN* and H* > WK, F 2) parietal bone length, significant differences were as follows B* > BN* 3) nasal bone length, significant differences were as follows H*, LE > BN, L, WK*.

In terms of skull height, significant differences were as follows WK*, H, LE > F*. Even the WK is one of the smallest rat, it exhibits the highest skull along with attrition of the upper molars. These characteristics are subject of further investigations.

In terms of skull width, significant differences were as follows H* > WK, BN, L, F* and LE > F*.

In terms of cranial base, significant differences were as follows H* > BN, WK* and SD > WK*.

In terms of the Cranial Index, at the two extremes of the spectrum lied the inbred BN with 88.6 and the outbred SD with 76.9. The Sprague-Dawley (SD) would be characterized as dolichocephalic (Cranial Index: 76.9), and the Brown-Norway (BN) would be characterized as brachycephalic (Cranial Index: 88.6).

Finally, the femur length was not significantly different among the rat strains.

TABLE 9.--Means and Standard Deviations for the Mandibular Measurements.

	Ap-Id	Ap-Lm	Ap-Co	Ap-C	Co r-Co l	C r-C l
SD	26.7±1 2	19.4±0.6	12.3±1.05	15.3±0.7	17.8±1 01	19.01±1
BN	26 1±1.9	20.2±1 6	12 8±0.6	16.1±0 9	16.4±0.6	17 6±0.8
LEW	26.6±1.1	20 07±0.8	10.8±0.5	14.4±0.6	15 5±0.5	16.7±0.6
F344	25.4±1.6	19.03±1 1	11 2±0.6	13.9±0 6	15.6±0.6	17 7±0 7
WKY	25 2±1.02	19 3±0.9	11.2±0.5	13.7±0.6	15.2±0.3	18.7±1.3
LE	27.8±2.3	20.8±1 7	12.9±1 2	15.8±1 2	16.6±0.7	18.9±1 3
HZ	28 9±1.7	21.6±0.9	12.8±0 7	15.9±0.6	16.7±0.3	19.2±1.3
BUF	28.3±1 3	20.7±1.03	11 5±1.03	15.1±1	15.1±0 3	17 2±0.5

TABLE 10.--Means and Standard Deviations for the Maxillary Measurements.

	Ul-Um	Ul-Pns	Mum r-Mum l	Dum r-Dum l	Mx r-Mx l	Mum r-Dum r	Mum l-Dum l
SD	14.2±0.8	24.2±0.8	5.7±0.4	6.9±0.2	12.1±0.7	6 6±0.07	6 6±0 1
BN	12.3±0.6	22.05±1 01	6.2±0 5	7.2±0.3	11 3±0.5	6.7±0.1	6.7±0.1
LEW	12.6±0.6	22.1±0.8	6.9±0 1	7.1±0.2	11.01±0.6	6.5±0.1	6.6±0.1
F344	13 2±0.8	22.4±1.2	6.25±0.3	6.5±0.2	9 8±0.6	6.2±0.2	6.3±0 1
WKY	12.2±0.6	22.1±0 8	6.03±0.4	6 5±0.3	11 1±0.3		
LE	14 2±1.2	24.1±1.6	6.7±0.3	7 1±0.4	11.6±1.08	6.8±0.2	6.8±0.2
HZ	14.8±0.9	25.2±1 1	7 2±0 3	7.7±0.2	12.4±0.5	6.9±0.1	6 9±0.09
BUF	14.1±1.7	23.9±1.4	6.5±0.2	7.2±0.1	11.5±0.5	6 6±0.1	6 6±0.1

TABLE 11.--Means and Standard Deviations for the Skull Measurements.

	So-P	Nf-O	Lbd-Brg	Zg r-Zg l	N-Nf	Ba-So
SD	12.8±0.7	31.05±1.3	7.5±0.5	23.9±0.6	18.9±1.3	7.9±0.4
BN	12.8±0.8	26.4±1.2	5.8±0.5	23.4±1.3	17.4±0.6	7.02±0.5
LEW	13.1±0.2	28.08±1.1	6.8±0.2	23.08±1.1	17.4±0.7	7.2±0.4
F344	12.03±0.5	28.6±1.6	7.3±0.4	22.1±1.4	18.2±1.2	7.3±0.6
WKY	13.8±0.6	28.7±0.8	7.2±0.3	23.5±1.3	17.07±0.8	6 9±0.4
LE	13.3±0.9	31 1±2.1	7 2±0.7	24.6±1.7	19 8±1.4	7.4±0.8
HZ	13.7±0 5	32.2±1.4	7 5±0.7	25.8±1.2	19.9±1.2	7.9±0.5
BUF	13 07±0.4	30.8±1.8	8.2±0.6	24.1±1.1	18.7±1.1	7 6±0.4

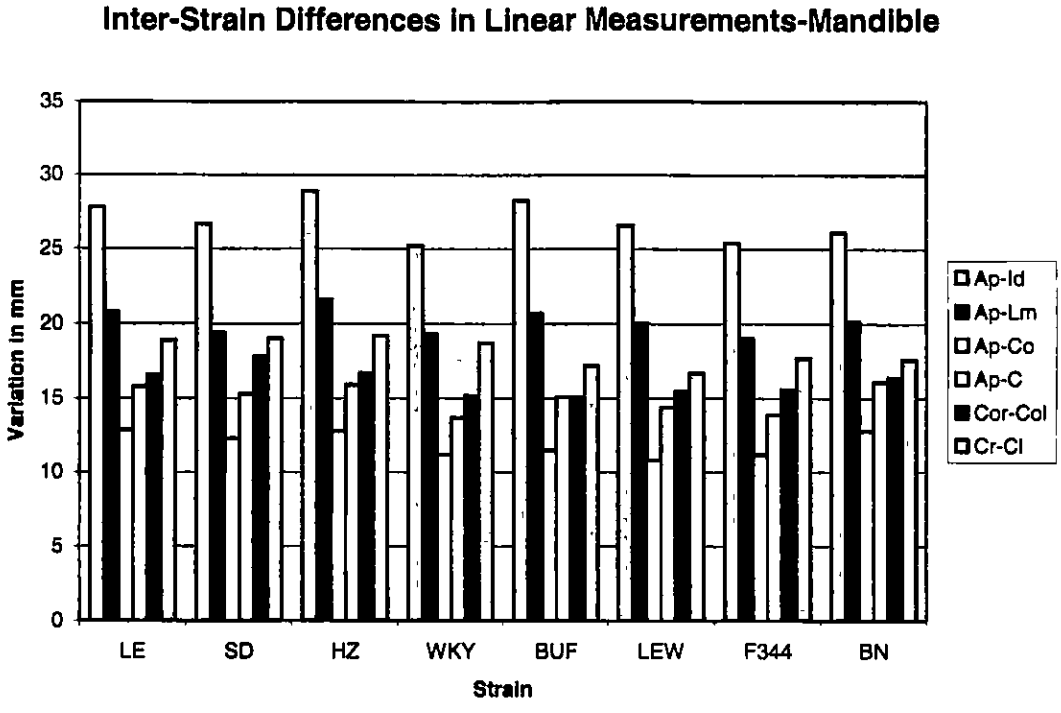


Figure 14.Visual comparisons of craniometric means for mandibular measurements.

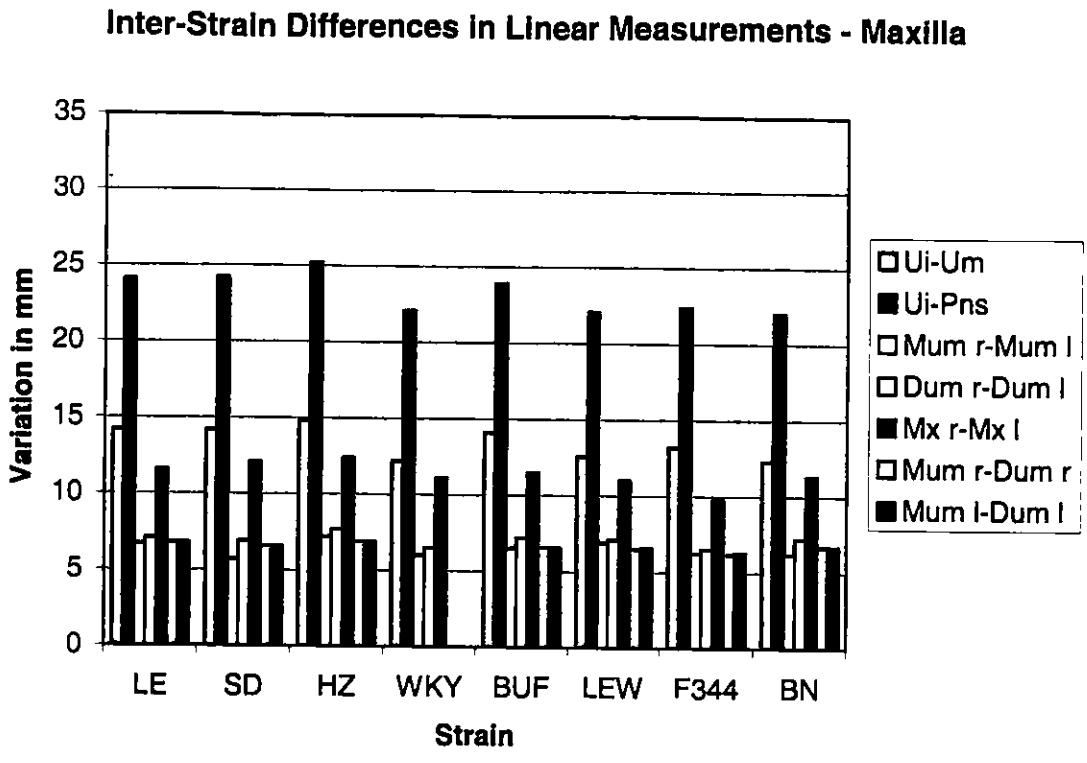


Figure 15.Visual comparisons of craniometric means for maxillary measurements.

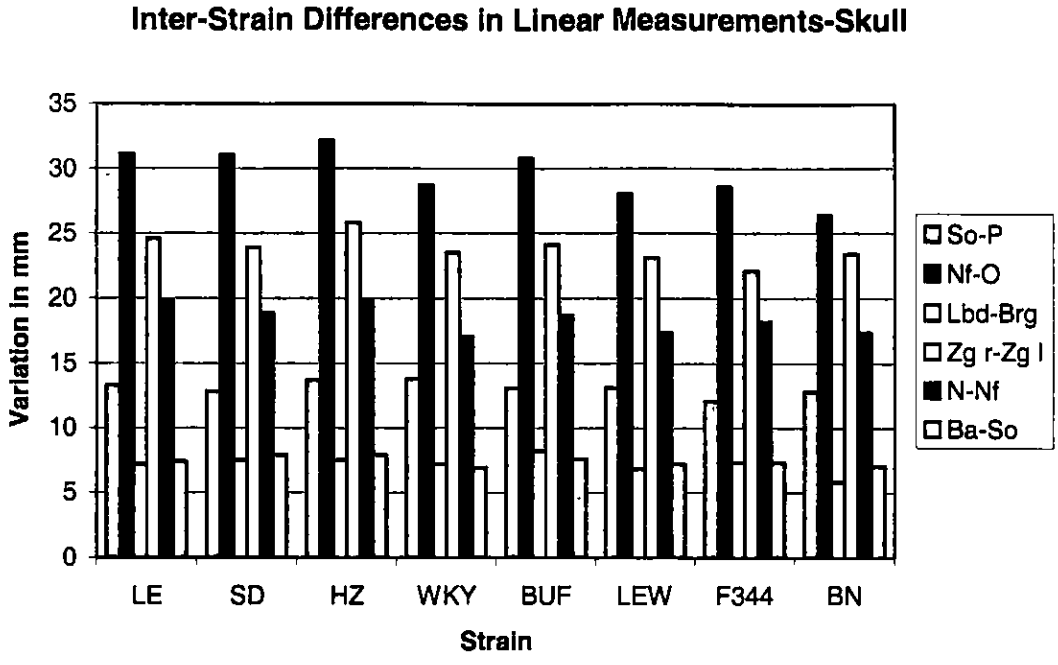


Figure 16.Visual comparisons of craniometric means for skull measurements.

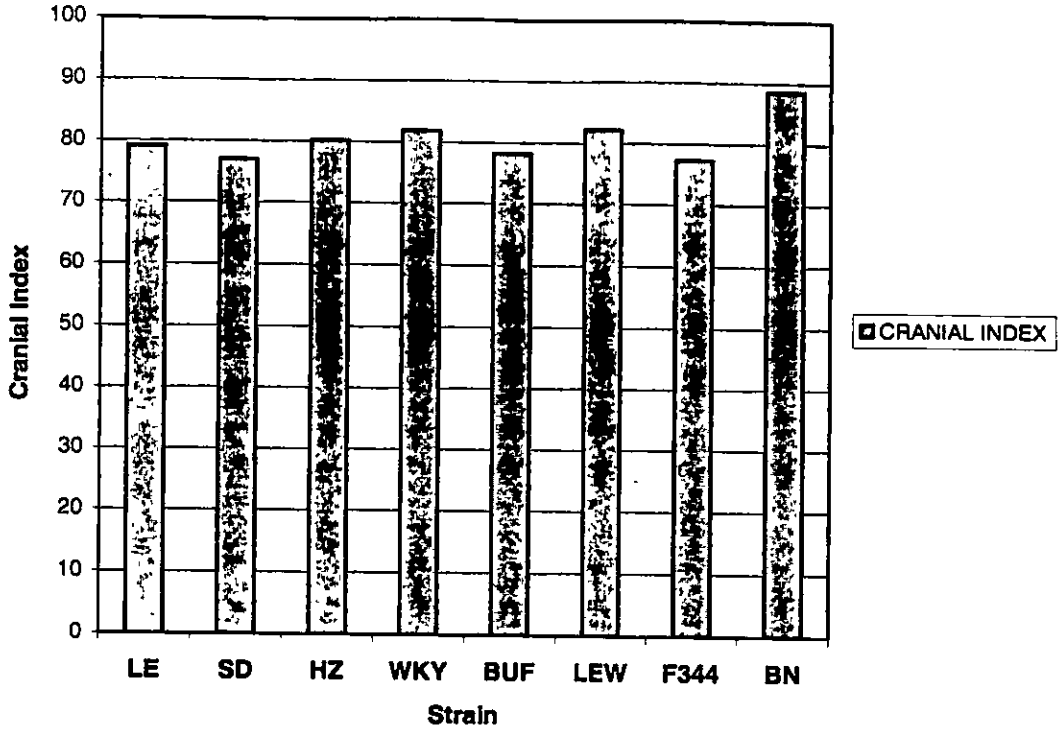


Figure 17.Comparison of the Cranial Index among the eight rat strains.

II. Procrustes Analysis

II.a. Intra-gender, Intra-strain Variation

The means and standard deviations of shape differences in mm for cranium, mandible and total shape difference within each strain for males and females separately are presented in Tables 12 and 13 respectively, and for visual comparison in Figures 18 and 19. All the males in the inbred strains (BN, WKY, BUF, LEW, F344) present the smallest intra-gender shape variation compared to the males in the outbred strains (SD, HZ) which exhibit the highest male intra-gender shape variation, 18.7 mm and 15.1 mm

respectively. The exception is the LE strain that has one of the smallest male intra-gender shape variation 3.6 mm. The same pattern was observed for the females.

TABLE 12.--Mean and Standard Deviation of Shape Differences for Males.

Intra-Gender MALES	LE	SD	HZ	WKY	BUF	LEW	F344	BN
CRANIUM (21 LM)	2.7±0.2	14.5±4.1	10.1±1.8	8.1±1.5	4.8±4.6	4.7±1	2.8±2.6	7.1±1.1
MANDIBLE (10 LM)	0.9±0.1	4.2±1.5	5.1±0.7	2.5±0.3	1 4±1.5	1.5±0.1	0.8±1	3 7±0.1
TOTAL VARIATION (31 LM)	3.6±0.3	18.7±5.6	15.1±2.5	10.6±1.8	6.2±6.2	6.2±1.1	3.6±3.6	10.7±2.1

All values are in millimeters.

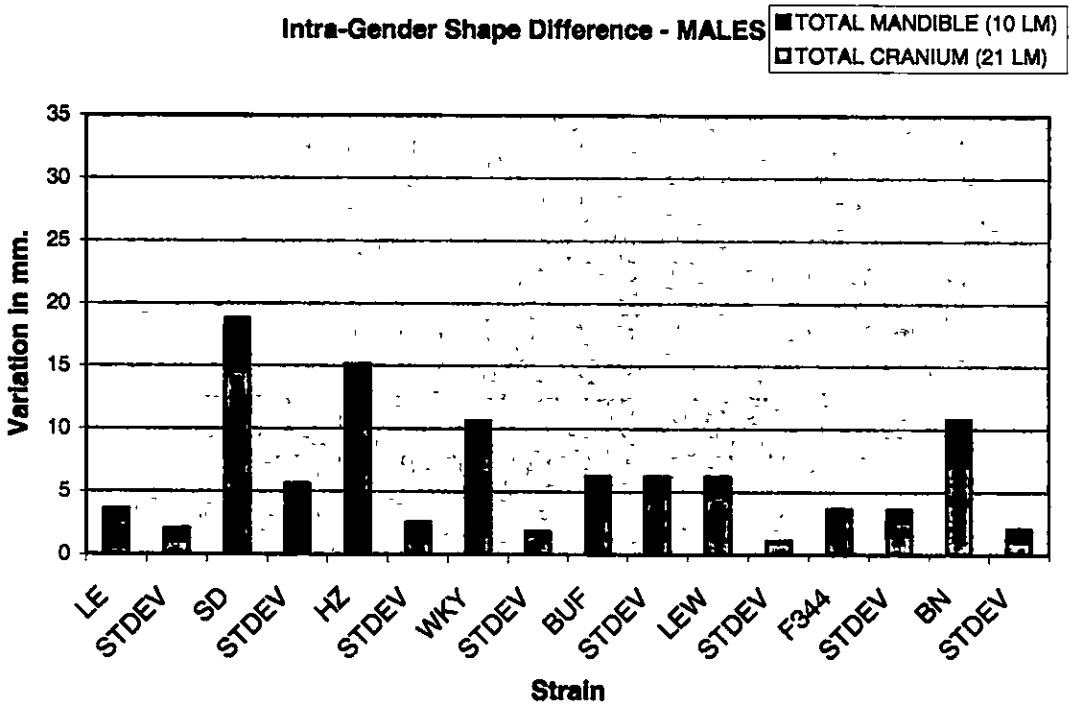


Figure 18.Visual comparisons of mean and standard deviation of shape differences for males.

TABLE 13.--Mean and Standard Deviation of Shape Differences for Females.

Intra-Gender FEMALES	LE	SD	HZ	WKY	BUF	LEW	F344	BN
CRANIUM (21 LM)	3.2±0.4	10.6±3.8	11.7±3.7	9.8±3.2	6.5±4.7	5.6±1.2	3.4±3.1	8.9±1.7
MANDIBLE (10 LM)	1.3±0.2	2.4±0 7	4.1±1.9	3.7±0.4	2.1±2.3	2.2±0.2	1.2±1.4	5.4±3.2
TOTAL VARIATION (31 LM)	4.5±0.6	13.3±4.5	15.8±5.6	13.5±3.6	8 7±7.02	7.8±1.4	4.6±4.5	14.3±4.9

All values are in millimeters.

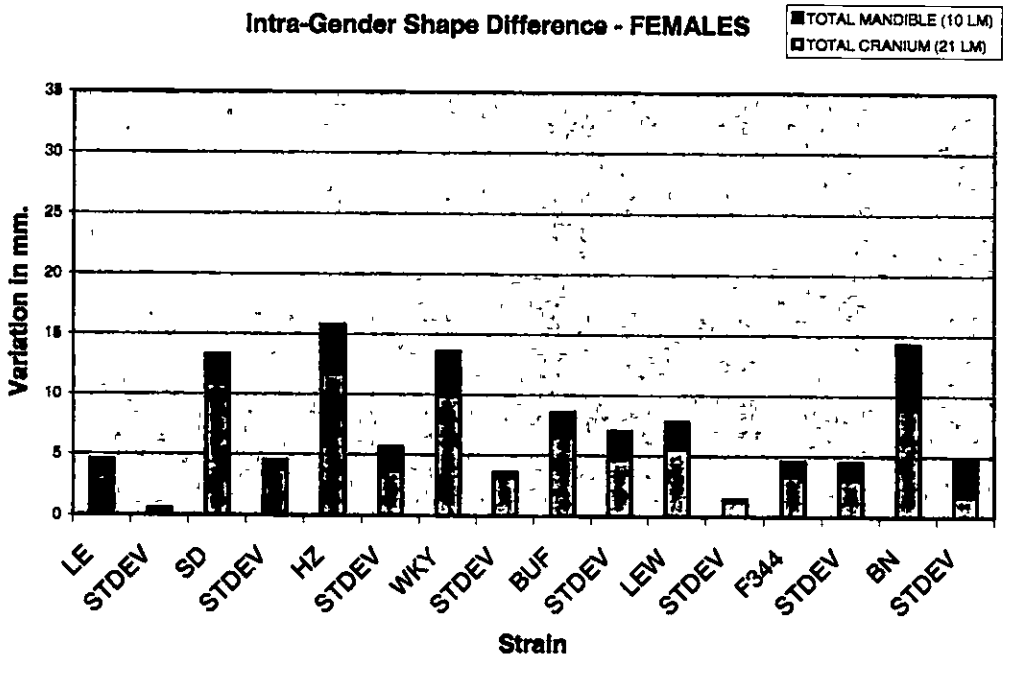


Figure 19.Visual comparisons of mean and standard deviation of shape differences for females.

II.b. Inter-gender, Intra-strain Variation

The means of shape difference in mm for cranium, mandible and total shape difference in mm between the males and females in each strain are presented in Table 14 and for visual comparison at the Figure 20. The largest shape difference between the females and males exists within the Buffalo strain (total: 26.7 mm, cranium: 20.1 mm, mandible: 6.6 mm), whereas the smallest sexual dimorphism exists within the Wistar-Kyoto strain (total: 11. 9, cranium: 10.35, mandible: 1.55 mm). The four inbred strains BN, WKY, LEW, and F344 have the smallest shape differences between the males and females, whereas the outbred strains (SD, HZ, LE) have the largest shape differences. The exception to this pattern was observed for the inbred BUF strain which exhibited the largest inter-gender shape variation.

TABLE 14.--Mean of Shape Differences Between Males and Females within Each Strain.

Inter-Gender M vs. F.	LE	SD	HZ	WKY	BUF	LEW	F344	BN
CRANIUM (21 LM)	14	15.35	12.12	10.35	20.1	11.71	12.19	9.12
MANDIBLE (10 LM)	7.14	2.59	6.04	1.55	6.62	4.15	2.55	6.12
TOTAL DIFFERENCE (31 LM)	21.1	17.9	18.2	11.9	26.7	15.9	14.7	15.2

All values are in millimeters.

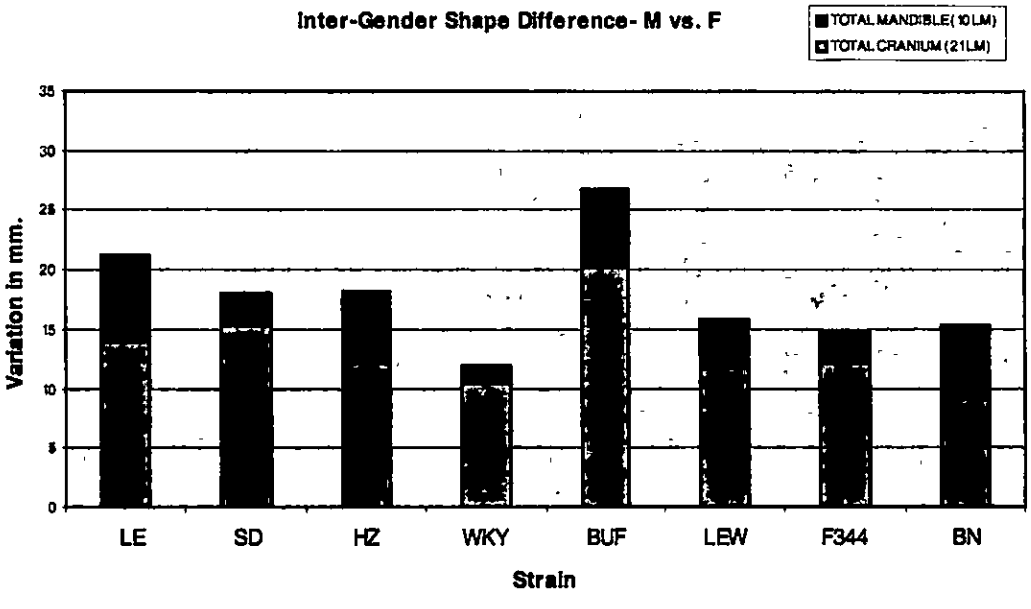


Figure 20.Visual comparisons of mean of shape differences between males and females within each strain.

TABLE 15.--Percentage Contribution to the Overall Inter-Gender Shape Difference at any One Landmark.

MALES vs. FEMALES								
	%LE	%SD	%HZ	%WKY	%BUF	%LEW	%F344	%BN
CRANIUM & NASOMAXILLA								
Lateral X-ray								
Tip Nasal Bone	3.84	0.87	3.21	3.48	1.17	1.40	2.71	0.82
Nasion	4.28	2.32	5.08	2.86	3.27	2.59	2.57	3.45
Bregma	3.66	1.68	3.28	4.60	1.93	1.75	2.81	4.45
Lamda	2.13	0.54	5.08	4.24	2.75	0.39	3.24	0.75
Occipital	1.42	0.70	1.93	2.82	2.77	0.65	2.03	3.38
Basion	2.90	2.10	4.96	2.02	1.56	1.83	5.01	1.29
Sphynoccipital Synhondrosis	3.19	1.09	2.18	6.49	0.29	1.23	4.60	2.60
PNS	2.66	1.12	1.28	0.06	2.71	1.57	2.48	1.93
Distal Terminal Molar	4.75	1.63	0.42	1.15	2.13	0.60	1.43	3.69
Mesial First Molar	1.34	1.69	1.09	0.69	2.00	1.99	2.33	1.13
Upper Incisor & Premaxilla	8.06	0.64	0.65	0.73	0.67	1.29	0.82	0.63
Dorsal X-ray								
Tip Nasal Bone	1.55	2.43	1.45	0.79	3.32	2.34	1.11	5.49
R Maxillary Process	4.20	7.61	4.16	1.06	4.66	1.24	0.79	3.15
L Maxillary Process	1.28	6.63	4.46	1.92	4.00	3.63	2.95	3.84
R Zygoma	2.48	3.59	2.10	11.95	5.31	2.45	1.04	3.83
L Zygoma	1.77	4.57	3.86	9.33	4.61	7.23	6.11	5.75
Occipital	3.68	9.84	2.04	3.46	5.18	4.35	2.69	4.38
R Distal Terminal Molar	6.93	8.79	8.03	5.56	7.18	4.05	1.83	3.79
L Distal Terminal Molar	1.71	9.82	6.31	6.82	8.73	16.24	18.45	3.07
R Mesial First Molar	0.55	9.11	1.93	10.10	5.10	13.80	12.08	1.57
L Mesial First Molar	4.03	8.55	3.85	6.13	5.69	3.09	5.86	1.82
MANDIBLE								
Lateral X-ray								
Mandibular Incisor	2	0.38	0.53	1.56	1.07	1.14	1.07	1.57
Mand first Molar	0.98	1.32	1.15	0.84	2.36	2.96	2.58	3.94
Mand last Molar	4.25	1.42	4.54	3.90	1.42	3.70	2.45	2.37
Coronoid	4.47	3.09	6.10	2.26	0.94	5.41	0.77	9.40
Condyle	4.10	0.84	4.09	1.92	0.29	1.87	2.69	0.57
Gonion	2.83	1.27	1.92	1.09	0.98	5.12	2.35	6.88
Dorsal X-ray								
R Coronoid	3.31	2.58	4.22	0.40	5.25	0.92	1.11	4.36
L Coronoid	4.91	0.88	3.60	0.29	3.66	1.77	1.57	3.04
R Condyle	2.68	2.43	4.04	0.41	4.85	1.23	1.16	4.96
L Condyle	4.34	0.21	3.40	0.26	3.80	2.01	1.62	3.73

II.c. Inter-strain Variation

The means of shape difference in mm for cranium, mandible and total shape among all the eight laboratory rat strains are presented at the Table 16 and for visual comparison at the Figure 21.

The largest inter-strain shape difference among all the rats exists between Brown-Norway strain (total: 19 mm, cranium: 14.8 mm, mandible: 4.2 mm) and the Procrustes Grand Mean for all the rat strains, whereas the smallest inter-strain difference exists at the Buffalo strain (total: 9.9 mm, cranium: 6.6 mm, mandible: 3.3 mm) and at the Sprague-Dawley strain (total: 9.9 mm, cranium: 7.1 mm, mandible: 2.8 mm). As a pattern, the four out of five inbred strains (BN, WKY, LEW, and F344) exhibit larger shape variation and differ the most when compared to the outbred strains (SD, HZ). Exception is the BUF which exhibits the smallest of all shape variation even though it is inbred, as mention earlier. The outbred strains (SD, HZ) present the smallest shape differentiation with exception of the LE which exhibits high shape variation.

The four inbred strains BN, LEW, WKY, and F 344 present a pattern of increasing shape difference while we move from the intra-gender variation to inter-gender and finally to inter-strain variation. In other words, the shape differences are greater among the different rat strains than within the strains (between the genders) or even within the same gender. The males are more similar to the males in the same strain, differ more from the females of the

same strain and as a whole group (strain) they differ considerable larger amount. Specifically, the BN intra-gender total shape difference is for males 10.7 mm and for females 14.3 mm. When we compare the BN males to the BN females (inter-gender) we notice that they differ 15 mm and finally when we compare the BN as a whole group to the other strains (inter-strain) then the total shape difference is 19 mm. The LEW intra-gender total shape difference is for the males 6.2 mm and for the females 7.8 mm. Their inter-gender sexual dimorphism is considerably higher. The LEW males differ from the LEW females 15.9 mm total, and when we compare the LEW strain to the other strains (inter-strain) the total shape difference is 15.5 mm. Similarly, the F 344 presents an intra- gender variation of 3.6 mm and 4.6 mm for F344 males and F344 females respectively. The F344 males differ from the F344 females 14.7 mm. The F344 strain differs 16.9 mm from all the other strains.

At the two extremes of the spectrum lie the inbred BN with 19 mm and the outbred SD with 9.9 mm.

TABLE 16.--Mean of Shape Differences Among all the Rat Strains.

Inter-Strain	LE	SD	HZ	WKY	BUF	LEW	F344	BN
CRANIUM (21 LM)	9.1	7.1	9.1	12.8	6.6	9 4	12.9	14 8
MANDIBLE (10 LM)	7 6	2 8	1 8	5.5	3.3	6 1	4	4.2
TOTAL DIFFERENCE (31 LM)	16.7	9.9	10.9	18 3	9 9	15.5	16.9	19

All values are in millimeters.

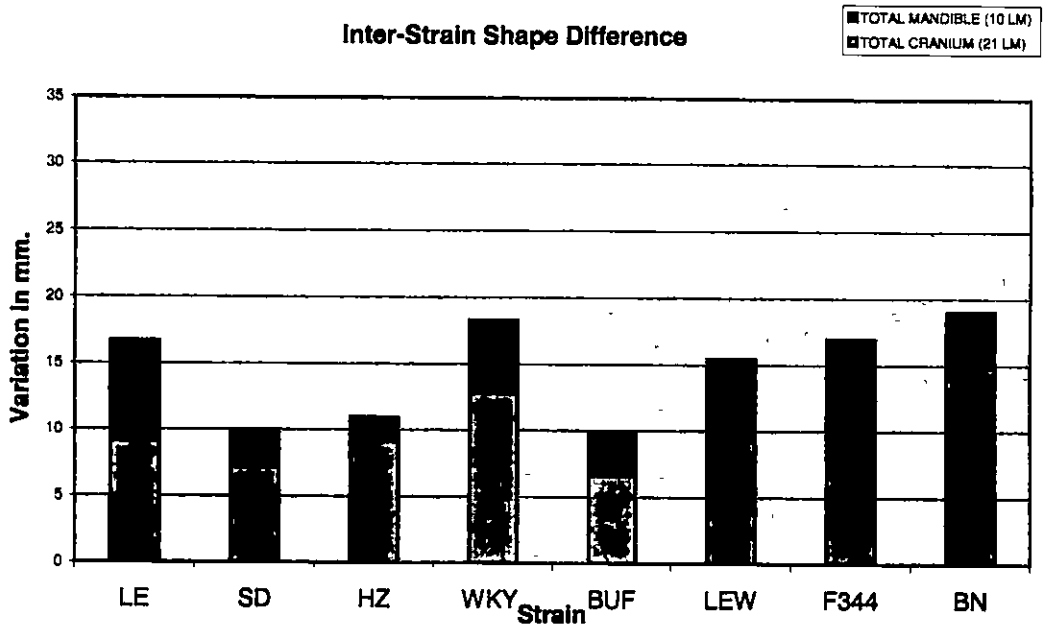


Figure 21.Visual comparisons of mean of shape differences among different strains.

TABLE 17.--Percentage Contribution to the Overall Inter-Strain Shape Difference at any One Landmark.

	%LE	%SD	%HZ	%WKY	%BUF	%LEW	%F344	%BN
CRANUM & NASOMAXILLA								
Lateral X-ray								
Tip Nasal Bone	4.61	3.13	4.74	4.10	0.84	3.66	0.62	3.71
Nasion	3.80	1.59	3.99	4.20	7.95	4.25	2.13	5.60
Bregma	2.72	6.19	5.05	5.21	9.17	1.99	4.86	4.36
Lamda	1.65	6.96	2.52	4.76	3.94	3.17	2.06	2.45
Occipital	3.64	2.01	3.58	0.25	3.13	4.40	2.53	6.27
Basion	1.61	5.15	4.18	4.22	2.50	1.00	2.61	3.11
Sphynoccipital Synhondrosis	2.44	6.74	4.34	6.30	5.34	2.21	5.46	4.76
PNS	2.44	2.34	3.34	3.66	5.47	6.17	2.99	4.39
Distal Terminal Molar	3.81	3.38	3.81	2.59	2.47	4.01	2.12	4.00
Mesial First Molar	3.16	3.54	5.62	0.83	2.75	3.59	1.48	5.48
Upper Incisor & Premaxilla	5.00	3.09	3.97	1.74	2.97	2.29	1.40	2.32
Dorsal X-ray								
Tip Nasal Bone	1.89	1.47	0.43	2.82	0.52	1.85	0.90	2.04
R Maxillary Process	0.69	2.41	2.43	1.47	2.59	1.12	3.95	3.36
L Maxillary Process	2.64	2.19	2.58	0.85	1.05	1.19	4.84	3.09
R Zygoma	1.19	3.35	5.13	6.14	1.29	2.75	2.16	2.64
L Zygoma	3.90	2.62	2.36	6.33	1.84	1.56	3.08	1.46
Occipital	2.42	1.76	5.60	1.83	0.81	0.75	4.21	1.35
R Distal Terminal Molar	1.05	4.13	4.52	3.41	4.49	1.20	5.20	3.09
L Distal Terminal Molar	2.84	2.35	4.50	0.95	2.57	8.38	4.10	4.48
R Mesial First Molar	1.74	0.41	4.15	4.19	3.06	4.63	11.17	6.70
L Mesial First Molar	1.02	6.53	5.92	4.04	1.69	0.45	8.33	3.12
MANDIBLE								
Lateral X-ray								
Mandibular Incisor	1.17	4.78	2.02	0.30	1.14	4.57	0.70	3.54
Mand first Molar	1.67	5.52	2.66	4.28	3.81	2.68	2.84	2.56
Mand last Molar	1.21	1.18	1.70	1.65	1.45	1.84	1.93	2.54
Coronoid	2.86	3.73	2.51	4.65	5.51	5.30	3.40	4.20
Condyle	0.91	1.20	1.66	1.26	4.78	3.23	0.71	1.76
Gonion	1.56	5.41	0.10	2.71	6.29	7.54	2.17	2.98
Dorsal X-ray								
R Coronoid	8.93	2.44	1.44	3.98	4.38	3.57	3.22	1.69
L Coronoid	10.34	1.41	1.22	4.06	1.42	3.69	2.44	0.79
R Condyle	7.56	2.11	1.83	3.66	3.99	3.31	3.40	1.61
L Condyle	9.30	0.80	1.67	3.58	0.44	3.64	2.75	0.63

DISCUSSION

The present study was intended to detect and evaluate craniometric and cephalometric information with regard to the contributions of genetics to the overall craniofacial configuration. We evaluated the intra-gender, inter-gender and inter-strain craniofacial morphologic differences among laboratory rat strains. The present study is the first step of an ongoing genetic study at the Department of Orthodontics in Case Western Reserve University, which is to characterize the craniofacial phenotypes of various rat strains (inbred and outbred) commonly used in research, and identify the craniofacial strain variation. Results indicate that a wide difference in craniofacial characteristics exists within different strains from a single species, the rat. The present data provide a normative database for the adult rat, in which environmental factors including housing, food and water intake, and caretaker handling were the same. Results indicate that even within the confines of a regulated environment, animals given the same food (rat chow) exhibit wide variations in craniofacial features. The significance of the strain effect argues for the notion of genetic predominance over environmental influence in the development of craniofacial characteristics. It would be reasonable to assume, given the standardized and controlled environment all strains were subjected to, that the influence on their respective craniofacial development

would be identical. Studies done in development, with various environmental interventions to affect mandible and maxilla form have shown that food (Pucciarelli 1980), masticatory muscle function (Kiliaridis et al. 1996), dysfunction (Navarro 1995), condylectomy (Tsolakis 1997), influence of the trigeminal nerve (Behrents and Johnston 1984), and effects of antirat nasal septum cartilage antisera (Hans et al. 1996), can be important factors in mandibular and/or maxillary development. However, in the present work, all rats were allowed to develop normally during the developmental period and into adulthood.

Several studies with rats have reported that the craniofacial growth is completed by the end of the fourth month (Spence 1940; Asling and Frank 1963; Moore 1966; Wright et al. 1966). Hence, six month old rats were used. Rats were used over mice due to their larger size. This is very important in order to quantify with better accuracy and precision the craniofacial complex. The measured craniofacial traits have two sources of variation, which the biological variation and the measurement errors (Engstrom et al. 1982; Mausner and Kramer 1985). In effort to study quantitative traits, such as cranium, nasomaxillary complex and mandibular size and shape, the method of assessment, accuracy of recordings and reliability become critical. In dealing with measurement errors, random error and systemic error are considered (Mausner and Kramer 1985). Random error is mainly due to change. It affects the reliability of measurement. The sources of

cephalometric landmark unreliability include: lack of agreement on anatomical landmark definitions, landmark structural radiopacity, unexpected right-left asymmetry, estimated landmarks, operator fatigue (Baumrind and Frantz 1971a, b; Dean et al. 1998). To increase the precision, all craniometric measurements were obtained twice by the same investigator (AM) and averaged. Also, in order to establish intra-operator reliability for the cephalometric landmarks, the author (AM) identified the landmarks in 25% of the total sample (sixteen rats from two different strains) three separate times (T1, T2, and T3). A method called thin plate spline based superimposition, proposed by Fred L. Bookstein was used to superimpose the fiducials of each film over one another. The fiducial markers provide the stable configuration framework upon which each landmark configuration is superimposed. The method uses both linear and non-linear components of the warps, unlike the Procrustes fit, which uses only the linear components. The standard deviation was measured for the three data sets of measurements. The intra-observer reliability testing reports the measuring consistency of one person on different occasions (Kamoen 2001). Systemic error often has a recognizable source and affects validity of the measurement. The sources of cephalometric landmarks inaccuracy include: lack of accurate head and x-ray source registration, digitizing errors, film scanner-based errors, software interface (e.g., poor zoom or mouse tools). Most of these problems were overcome by comparing inter-fiducial distances taken from the film and from

the scanned film at the computer display (Baumrind and Frantz 1971a, b; Dean et al. 1998). The inter-fiducial distance controls enlargement during the scanning process.

I. Inbred vs. Outbred strains

Ia. Intra-gender/Intra-strain

All the males and females of the inbred strains (BN, WKY, BUF, LEW, F344) present small intra-gender shape variation when compared to the males in the outbred strains (SD, HZ) which exhibit a higher male and female intra-gender shape variation. An, exception is the LE that is an outbred strain but has one of the smallest male and female intra-gender shape variation of 3.6 mm and 4.5 mm respectively. These results seem logical because the inbred strains being pure and genetically homogeneous should show that males are phenotypically similar to each other and shouldn't present large craniofacial differentiation from one to other. Results show the same trend for the females. Outbred strains, on the other hand show larger intra-gender/intra-strain differentiation which can be explained by the fact that they are not genetically similar.

Ib. Inter-gender/Intra-strain

Four of the five inbred strains (BN, WKY, LEW, and F344) demonstrate the least shape difference between genders, while the outbred strains (SD, HZ, LE) have the largest shape differences between males and females. An

exception observed among the inbred strains was the BUF; exhibiting the largest inter-gender shape variation.

Ic. Inter-strain

In general, the inbred strains tend to differ in shape more than the outbred strains when compared to the grand mean. The lone exception to this trend was the BUF that was found to be fairly close to the grand mean. The outbred strains that were found to be closer to the grand mean also had one exception the LE. Specific note should be made of the two inbred strains (BN and WK) that presented a high level of craniofacial shape differences from the inbred BUF and the outbred SD.

Darwin at his classical "Origin of the Species" states that all domestic strains are likely derived from a single wild type genotype. In other words, domesticated rats bred in vendors kennels likely come from a common ancestor. He also states that domesticated rats are likely to show variations that exceed those found in wild rats. Perhaps we can interpret the gender variations along these lines; also maybe the universal rat average approaches the wild type. Said another way, wild rats are born, thrive and reproduce, or not, according to Darwin's concept of natural selection; whereas, domesticated rats, laboratory rats are born, thrive and reproduce, or not, based on selective breeding determined by the plans of the investigator.

II. Strain Use for Experimentation

The identification of the genes responsible for a given phenotype, utilizing inbred strains that differ widely in expression for the specific phenotype, is based on two widely held principles of biology: genes cause traits and not vice versa and in a segregating F2 generation where genes that cause a given trait (such as shape of the craniofacial complex) will remain associated with that trait, and other genes will segregate randomly relative to the trait. As a beginning point to dissect the genes and mechanisms responsible for the development of the craniofacial complex, we report wide divergence in the size (modified Cranial Index) and shape (Procrustes Analysis) of the craniofacial complex between the BN or WK and BUF or SD. This deviation provides a basis to begin a genetic analysis of this complex trait in the rat.

III. Linear Measurements vs. Shape Analysis

Using the Procrustes shape analysis the two inbred strains, BN and WK present craniofacial phenotypic differences when compared to the inbred BUF and the outbred SD. Using the modified Cranial Index the inbred BN has the highest Index, followed by the WKY and LEW (more brachycephalic), where the outbred SD has the lowest Index, followed by the inbred BUF and F344 (more dolichocephalic).

CONCLUSIONS

In the present study there were three specific aims. Create a database for craniofacial configuration among eight rat strains, determine variance in size of the cranium, maxilla and mandible among strains (inter-strain), using craniometric measurements and evaluate the Cranial Index, and determine variance in shape of the cranium, maxilla and mandible among strains (inter-strain), and between genders of the same strain (intra-strain, i.e., inter-gender), and within the same gender (intra-strain, i.e., intra-gender). Measurements were collected using cephalometrics and were analyzed using the Procrustes Algorithm.

For the first specific aim, we preserved the dry skulls, mandibles and femurs of the sixty-four animals for future reference. The means and standard deviations of all the craniometric measurements of the fully mature animals were presented. In addition, the Procrustes grand means of the strains were generated and are available for any future study.

For the second specific aim, the results suggest that the Sprague-Dawley strain was characterized by craniofacial dimensions that were decidedly dolichocephalic (the lowest Cranial Index: 76.9). In contrast, the Brown-Norway rats demonstrated more brachycephalic craniofacial features and the highest Cranial Index: 88.6. Overall, the length of the skull was

greatest in the Holtzman rat, and shortest in the Brown-Norway. Mandible length was greatest in Holtzman and shortest in Wistar-Kyoto. Maxilla length was greatest in Holtzman and shortest in Brown-Norway. No differences in femur length were found among the strains.

For the third specific aim, the results shown that all the males and females of the inbred strains (BN, WKY, BUF, LEW, F344) present small intra-gender shape variation when compared to the males in the outbred strains (SD, HZ) which exhibit a higher male and female intra-gender shape variation. An, exception is the LE that is an outbred strain but has one of the smallest male and female intra-gender shape variation. The least inter-gender (i.e. intra-strain) variation was found among four inbred strains BN, WKY, LEW, and F344. Interestingly, the greatest inter-gender variation was found for the BUF strain. Outbred strains (SD, HZ, and LE) have the largest intra-strain differences and the most noticeable sexual dimorphism. Inter-strain variation was largest among the four inbred strains BN, WKY, LEW, F344 although differences were also observed for the outbred strains SD and HZ strains. The BN has the largest inter-strain craniofacial shape variation where the SD has the least inter-strain shape variation. In fact the LE is still showed differences even though it is an outbred strain.

The four out of five inbred strains BN, LEW, WKY, and F 344 present a pattern of increasing shape difference while we move from the intra-gender variation to inter-gender and finally to inter-strain variation. In other words, the

shape differences are greater among the different rat strains than within the strains (between the genders) or even within the same gender. The males are more similar to the males in the same strain, differ more from the females of the same strain and as a whole group (strain) they differ considerable larger amount. Our results for the four inbred strains (BN, WKY, LEW, and F344) support our hypothesis that shape differences in the craniofacial complex are greater among the different rat strains than between genders of the same strain or within the same gender.

The two inbred strains, BN and WK present a high level of craniofacial phenotypic differences when compared to the inbred BUF and the outbred SD. As a beginning point to dissect the genes and mechanisms responsible for the development of the craniofacial complex, we report wide divergence in the size (Cranial Index) and shape (Procrustes Analysis) of the craniofacial complex between the BN or WK and BUF or SD. This deviation provides a basis to begin a genetic analysis of this complex trait in the rat.

The present study completed the first steps of characterizing the craniofacial phenotypic traits of five inbred strains and three outbred strains. Further more we identified strain variation of the particular craniofacial phenotypes. The observations provide the necessary information needed to consider different rat strains for craniofacial research.

APPENDIX A

REPORT ON STATUS OF APPROVED ANIMAL STUDIES

Annual Review of IACUC

Case Western Reserve University
Institutional Animal Care and Use Committee
School of Medicine Room WG77
10900 Euclid Avenue Cleveland OH 44106-4912
Phone (216)368-3413 Fax (216)368-3305 veterinarian Reviewers (216) 368-3490

FEB 26 RECD

For IACUC use only
Year (and protocol # if any)
KC

Title of Project: GENETIC DISSECTION OF CRANIOFACIAL MORPHOLOGIC TRAITS

Principal Investigator HANS MARK	Office Phone 368 3249	Email mgh4@po.cwru.edu
Dept ORTHODONTIC	Building/Location code DENTAL SCHOOL	
Approval Date 3/17/97	Funding AACF NIDR	Mail Date 12/13/98	Returned 02/23/98

Items above and below were completed from information contained in the IACUC/ARC database. Please make corrections directly on this form and complete any blank lines.

The person to be given responsibility for the following animal care is accurate. I understand that any animal research activity not described in the approved protocol must obtain the approval of the IACUC.

Signature of Investigator/Author — 2-20-98

Signature of Department Chair or Director

KIM [illegible] Dunn	Robert C Unger	[illegible]
3-24-98	ARC Director/Associate Director	CWRU Attending Veterinarian
Date	3/24/98 Date	4/7/98 Date

Project Status

- [X] active
- [ ] not yet active
- [ ] canceled
- [ ] completed

If animal studies are currently being done
If no animals were studied in the past year
If no animals were studied in the past year and no plans for future studies
If studies are completed and no animals remain

Please note: Protocols cover work over a long year period, thus the exact number of animals approved by the IACUC should reflect that period. If they do not, please adjust the number of animals and provide justification. (See no. 4 page 2 of this document)	Species #1 (2) RATSHO EBOX	Species #2	Species #3	Species #4
Total number of animals approved by the IACUC	40			
Total number of animals available*	40			
How many animals will you need for the coming year?	see note:	40		

If THIS PROJECT IS CANCELLED, COMPLETED OR NOT YET ACTIVE, PLEASE STOP HERE.

*If this number does not agree with your records please contact the IACUC office at 368 3815

note future animals will come from Dr Thomas's genetic project, protocol # 960157

IACUC Annual Review
Principal Investigator HANS SEARK
Protocol Number 97-1101

PLEASE PROVIDE DETAILS OF CHANGES IN (Please type additional sheets if needed If no changes please note)

1 Experimental Design

none

2 Animal Procedures - yes. Euthanasia is done by CO_2 inhalation.

3 Species (Please provide, in lay language, a written narrative that addresses appropriateness of species)

none

4 Number of animals (Please provide justification) increased to 64 rats These include different strains requested by reviewers p includes n this number Animals from multiple species procured from other investigators (esp K Smith) no post mortem specimens - ie Zucker etc neurology etc minimum sizes

5 Method of anesthesia euthanasia analgesia administration of new drugs)

CO_2 inhalation

6 Addition of hazards (carcinogens radioisotopes infectious agents, biologic materials) [Attach copy of approved hazard form]

none

7 Change in location of procedures

none

8 Personnel added/deleted [When adding personnel please attach a Personnel Health and Training form and include a signed statement from each person added that they have read and understand their role in the protocol] Do not list individuals that are already listed on your protocol or that you have already added to your protocol

none

IACUC Annual Review
Principal Investigator HANS MARK
Protocol Number 97-0140

Page 1

*For all studies involving anesthesia and/or having the potential to cause more than momentary pain or distress, please provide as required by the USDA documentation that ALTERNATIVES to the proposed procedures utilizing fewer animals animals lower on the phylogenetic scale or less painful procedures have been sought.

n/a

I have used the following methods and sources to search for such alternatives (Check all that apply)

- [ ] AGRICOLA Database (Nat'l Ag Libr)
- [ ] MEDLINE Database
- [ ] CAB Abstracts Database
- [ ] BIOSIS Database
- [ ] Animal Welfare Info Center (301)344-3704
- [ ] Direct contact with colleagues (must document source and substance of information)
- [ ] Laboratory Animal Science (Journal)
- [ ] Lab Animal (Journal)
- [ ] Lab Animal (UK) Journal)
- [ ] Quick Biblio Series (AGRICOLA)
- [ ] Alternatives to LAB Animals Journal
- [ ] Others (Please specify)
- [ ] Key words use

n/a

[ ] No alternatives to the procedure(s) were identified n/a

[ ] Alternatives were unsuitable because (complete) n/a

Date of most recent search 12/97
Years covered by search 1990 to 1997
Frequency of search 1 / year

Review of the relevant literature as describe in the original protocol is on going to insure that the study remains original and is not unnecessarily duplicated (Please indicate [X] yes [ ] no)

The following is not required for the Annual Review however the ARC requests the information from all investigators

Have you encountered problems with your animal procedures? If yes, please give pertinent details

Not at all We simply procure aged (old) rats, euthanize them with CO_2 and harvest the tissue

Have you encountered problems with the care of your animals?

animals are sacrificed as they arrive at the ARC

no

Do you have suggestions for how the IACUC or the ARC may be more helpful to your research?

The ARC really is quite helpful to this investigation We acknowledge their assistance in the planning of this project, and particularly in their suggestions concerning the defleshing of rats without damage to the bony structures which are the subject of our investigation

APPENDIX B

Rat Strain Variation of Craniofacial Morphologic Traits

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Abstract

Variation in response to treatment is one of the major confounding factors faced by the orthodontic practitioner. Although poor patient compliance is most often cited as the cause of poor response it is likely that at least some of the variation is due to genetic susceptibility to a given therapy. The long-term goal of this line of research is to identify genetic mechanisms and quantify their effect in producing variations in treatment response. To achieve this goal more information is needed on the relationship between dentofacial morphology and genetics. The present study represents a step in that direction. We used 4 male and 4 female fully mature adult rats (>6 months old) from eight different strains. From these 64 animals, 19 linear measurements (6 measures for the mandible, 7 measures for the maxilla, 6 for the skull and cranial base) derived from 21 anatomic landmarks were used to characterize the phenotypic craniofacial features of each strain. Femur length was used as a reference for overall skeletal size. Both cephalometric and direct skeletal measurements were collected and compared. Significant differences existed for all 19 measurements among the 8 strains. In particular, dolichocephalic craniofacial dimensions characterized Sprague-Dawley rats. In contrast, Brown-Norway rats demonstrated more brachycephalic craniofacial features. Overall, the length of the skull was greatest in the Holtzman rat, and shortest in the Brown-Norway. Mandible length was greatest in Holtzman and shortest in Wistar-Kyoto. Maxilla length was greatest in Holtzman and shortest in Brown-Norway. Similar results were found for both cephalometric and direct skeletal measurement techniques. No differences in femur length were found among the strains. These data provide a normative database in fully mature adult rats of both sexes and various strains. The strain variations were large enough to allow future studies to partition the variance of selected traits (size, shape of mandible, maxilla, skull,

and cranial base) into genotypic and non-genotypic (environmental) components.

Introduction

Successful orthodontic treatment depends on at least three major factors. First, the practitioner must be able to recognize normal and abnormal craniofacial morphology. This ability helps the practitioner generate an orthodontic problem list. From the problem list, a logical plan for treatment can be developed. Second, the orthodontist must be knowledgeable with regard to the technical aspects of orthodontic mechanotherapy. This allows the clinician to implement the specific plan to modify dental and skeletal morphology. And third, the orthodontist should have some understanding of the expected physiologic response of this particular patient to a orthodontic and orthopedic force. Although the first two factors are well described in the orthodontic literature, a biological basis for the third factor has been largely ignored. Instead, variations in patient response to treatment have usually been summarily dismissed as a problem with compliance with treatment i.e. the patient who did not respond to bionator therapy simply did not wear the bionator. This simplistic explanation of differences in treatment response completely ignores the possibility of innate variations in susceptibility to treatment. These variations are necessarily related to differences in individual cellular responses to: mechanical stimuli, neurotrophic signals, membrane control, oxygen tension, bioelectric potentials, pH levels, temperature effects, nutrition, as well as enzymatic and hormonal factors. Since individual differences in these factors forms the underlying basis for observed differences in craniofacial morphology, a logical approach to understanding the basis for biologic variation in treatment response would be

to determine if these observed phenotypic morphologic differences can be traced to specific genotypic origins.

The influence of genetics on facial features has been documented. As early as 1939, Rubbrecht studied the well-known Hapsburg jaw of the Royal German family, and suggested that the resemblance of mandibular prognathism through many generations was due to genes[10]. Similarly Brodie, Brash and MacMillan reported that the form of the jawbones was entirely under genetic control and environmental influences had little effect. [11,12,13] Although the "functional appliance" revolution of the last 20 years has challenged the notion that the size and shape of the maxilla and mandible cannot be altered by therapeutic intervention, the degree to which modifications can be made is generally thought to be less that 10% of the overall size of the part. ** In addition, clinicians have long realized that the effectiveness of functional appliances in some patients is extraordinary, whereas in others, the effect seems minimal. To begin to understand this phenomenon the National Institute for Dental and Craniofacial Research funded 5 major randomized clinical trials in orthodontics. Despite this massive effort to acquire more complete information on the effectiveness of various orthodontic modalities much uncertainty still exists. This uncertainty exists because a major confounding factor in randomized clinical trials of orthodontic therapy is that the variation within a given treatment group exceeds the variation observed between the two competing treatment modalities. ***Baumrind Seminars in Orthodontics Evidence Based issue*- Until we have a better understanding of the basis for this observed variability in treatment response it is unlikely that randomized clinical trials will be able to help clinicians decide which appliance is most likely to work for their patient.

While several studies have examined the developmental aspects of facial components, no studies have evaluated the relative contribution of genetics and gender to the formation of the adult craniofacial phenotype. Ultimately we

need to evaluate the genetic relationship of size and shape of the maxilla and mandible using more sophisticated variance component models. In human studies, plane x-rays are widely used by the orthodontic practitioner to estimate and plan craniofacial reconstruction. However, x-ray representations alone, do not factor in variations caused by genetics and cultural background, or behavioral patterns which may have affected craniofacial deformities or other aberrations as seen in patterns of malocclusion. In contrast to human studies animal models have two important advantages. First, animal models allow for controlled mating of inbred populations. Second, animals can be sacrificed to allow the skeletal and occlusal patterns generated by this controlled breeding to be measured directly. In addition it is possible to compare measurements made from x-ray films to those made by direct measurement of the same bony areas. The rat has been used as a successful model in the examination of craniofacial growth and development. Furthermore, Nonaka and Nakata concluded that both additive and non-additive genetics effects play a very important role in the craniofacial growth with age of the rats. Importantly there is a large body of literature on the use of various functional orthodontic appliances to modify the craniofacial skeleton of the growing rat. The present study was designed to evaluate morphologic differences among rat strains. We hypothesize that differences in craniofacial characteristics, head form, shape and size of the maxilla and mandible are greater among rat strains than between sexes or within same sex animals of the same strain.

Materials and Methods

Eight different rat strains were chosen for this study: 1) Sprague-Dawley (SD), 2) Brown-Norway (BN), 3) Lewis (L), 4) Fisher-344 (F), 5) Wistar-Kyoto (WK), 6) Long-Evans (LE), 7) Holtzman (H), 8) Buffalo (B). Four

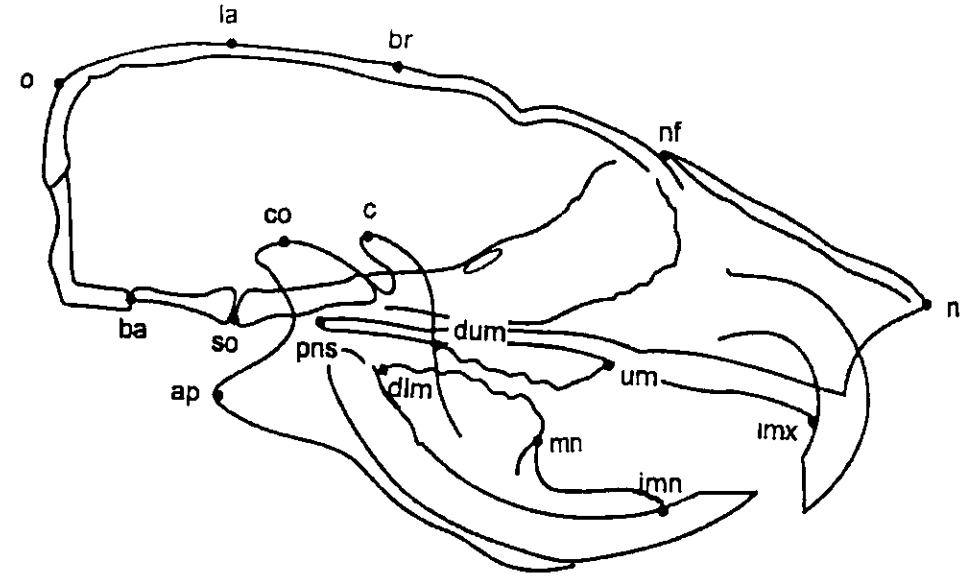
male and four female rats from each strain were used. All animals were at least 6 months of age and were obtained from a single animal vendor (Harlan, Indianapolis, IN). All animals were housed at Case Western Reserve University for at least 3weeks before testing and were provided food and water. Animals were under the direct observation of the veterinary staff and were in good health. A total of 64 adult animals, between 6 and 14 months old at the time of testing were studied. SD and BN were chosen because they exhibit the largest differences in breathing patterns. The WK is the parental strain of SD crosses that make obese and hypertensive rats. The WK rat is widely used in Europe whereas the SD is more popular in the United States for animal studies on orthodontic/orthopedic interventions. The L rat was selected because it exhibits different physiological characteristics during sleep. The F has been widely used to study processes associated with aging. The LE, H, B are variations of albino rats and have all been used previously for cephalometric studies. The Institutional Animal Care and Use Committee of Case Western Reserve University approved all protocols used for this study.

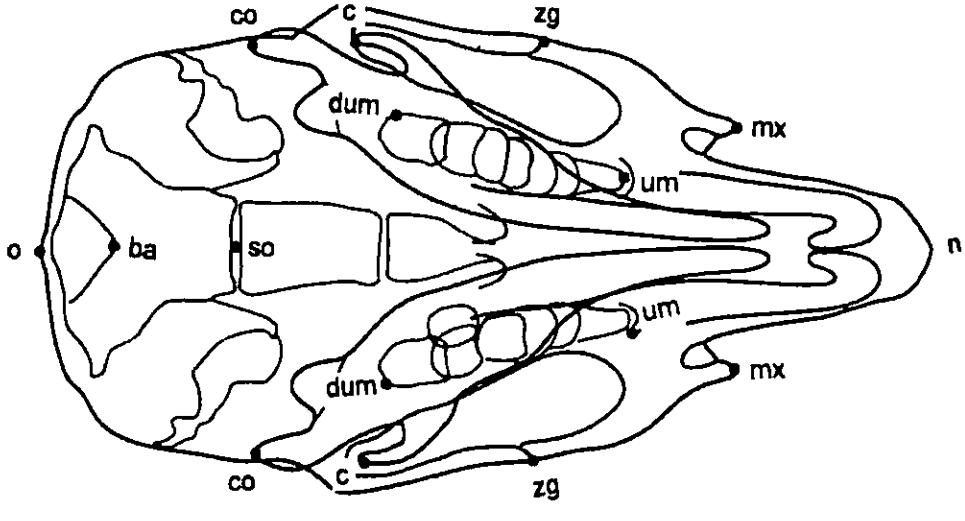
Animals were individually identified and assigned an identification number. At that time, animals were euthanized according to the 1986 AVMA (American Vetenery Medical Association) with carbon dioxide inhalation for period of 5 minutes. Immediately following sacrifice, anthropomorphic data (body length-nose to anus-, body weight) were taken. Body mass index (Lee Index) was calculated by dividing the cube root of body weight in grams by body length in millimeters. All rats were radiographed in the lateral and dorsoventral projection using a modified Bolton-Brush cephalometer as previously described. (Hans et al)Next, rats were defleshed according to standard protocols. Briefly, the skin was removed from the head, and right hind leg, the femur removed, and the fleshy skull and femur with musculature intact were placed in the same bag and were frozen at the –20 C until ready

for use. The fleshy skulls and femur were 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 injury to any of the fragile bony structures. All craniofacial measurements were made on the dry skulls (bony structures) using a micro-caliper (Digimatic, Mitutoyo Co, Tokyo, Japan). The following anatomical landmarks used are listed in Table 1 and illustrated on Figure 1.

Figure 1. See TABLE 1 for Key to Abbreviations

Anatomical landmarks (lateral)

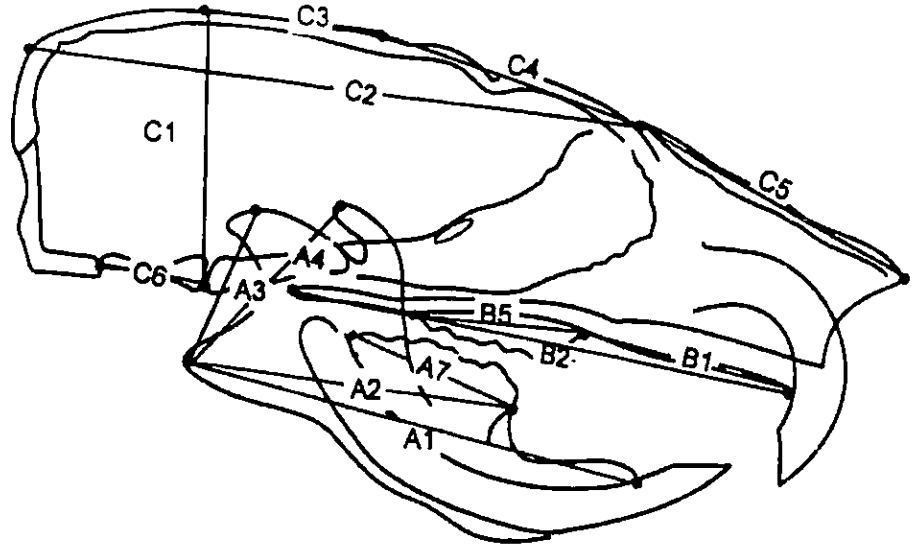




Anatomical landmarks (dorsoventral)

Figure 2- See TABLE 2 for Key to Abbreviations

Anatomical measurements (lateral)



Anatomical measurements (dorsoventral)

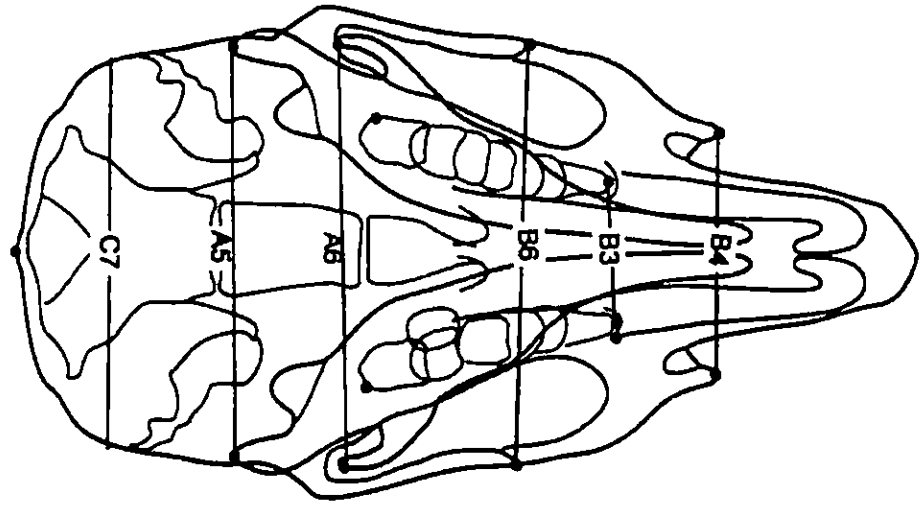


TABLE 1.--Anatomic Landmarks

Ap	The most inferior-posterior point of the angular process
Id	Infradentale. The most superior point on the alveolar process between the lower incisors
Co r, l	The most superior point of the condyle
C r, l	The most superior point of the coronoid process
Lm	The most superior point of the mandibular alveolar process on the first lower molar
Ui	Supradentale. The most inferior point on the alveolar process between the upper incisors (ui)
Pns	The most posterior point of the palatal process on the palatal bones on the midline (pns)
Um	The most inferior point of the maxillary alveolar process on the first upper molar (um)
Mum r, l	Mesiobuccal cusp of the upper first molar (mum)
Dum r, l	Distobuccal cusp of the upper last molar (dum)
Mx r, l	The most anterior point on the maxillary bone at the infra-orbital foramen
Nf	The most anterior point of the frontonasal suture on the midline
O	The most posterior point on the external occipital protuberance
N	The most anterior point (tip) of the nasal bone
Brg	Bregma
Lbd	Lambda
Zg r. l	The most lateral point on the zygomatico-maxillary suture
So	The most inferior (ventral) point on the spenoccipital synchondrosis
P	The most superior point of the parietal bone perpendicular to spenoccipital synchondrosis.
So	The most inferior (ventral) point on the spenoccipital synchondrosis
Ba	The most anterior point on the margin of the foramen magnum

Anatomic Measures

The following twenty-one linear measurements were utilized to characterize the craniofacial features. Descriptions of each measurement along with its construction are found in Table 2 and are illustrated in figure 2.

TABLE 2.--Anatomic Measures

A1	**Ap-Id**	Total Mandibular length
A2	**Ap-Lm**	Posterior Mandibular length
A3	**Ap-Co**	Mandibular ramus height
A4	**Ap-C**	Mandibular coronoid height.
A5	**Co r-Co l**	Bicondylar width
A6	**C r- C l**	Bicoronoid width
B1	**Ul-Um**	Premaxilla length
B2	**Ul-Pns**	Overall palatal length
B3	**Mum r-Mum l**	Anterior Maxillary arch width
B4	**Dum r-Dum l**	Posterior Maxillary arch width
B5	**Mx r-Mx l**	Maxillary width
B6	**Mum r-Dum r**	Right maxillary tooth row length
B7	**Mum l-Dum l**	Left maxillary tooth row length
C1	**So-P**	Skull height
C2	**Nf-O**	Skull length
C3	**Lbd-Brg**	Parietal bone length
C4	**Zg r-Zg l**	Facial width
C5	**N-Nf**	Nasal Bone length
C6	**Ba-So**	Cranial Base Length

Statistical Analysis

A one-way analysis of variance (ANOVA) with correction for multiple comparisons was used to detect differences among strains for such variable. The Statistical Package for the Social Sciences (SPSS, Chicago, Illinois) was used for all calculations. Between-strain differences were investigated for those outcome variables for which strain were significant ($P< 0.05$) predictors from the ANOVA; sample means were compared after adjustment for weight, length, and Lee index. To adjust for the effect of multiple comparisons, a Bonferroni correction was employed when comparing means between strains, such that P values were taken to be significant.

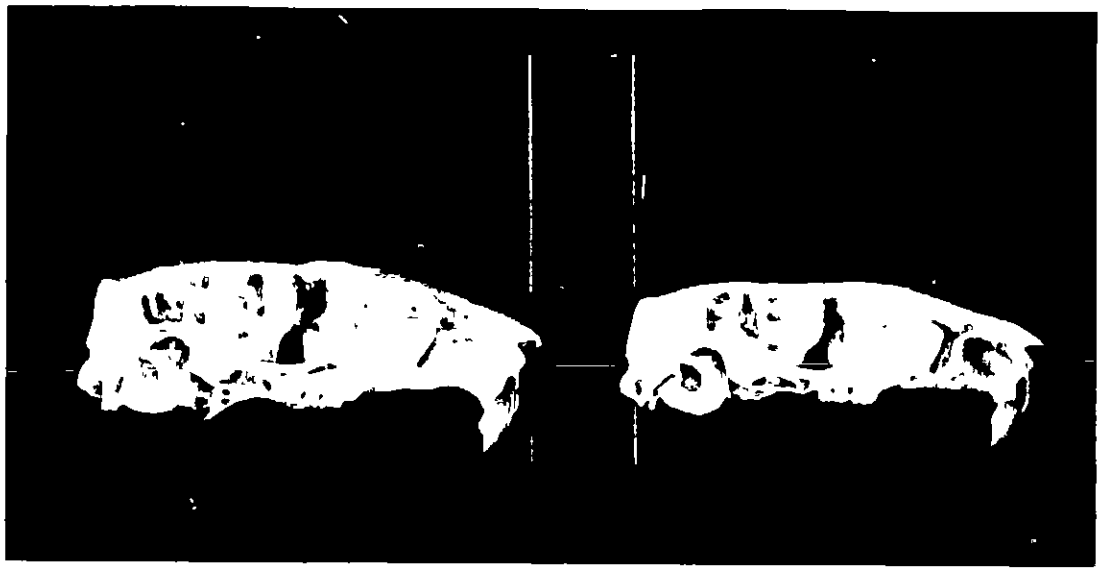


Figure 3.Lateral view of the dry skull (WKY male and F344 female)



Figure 4.Dorsal view of the dry skull (HZ male and BUF female)

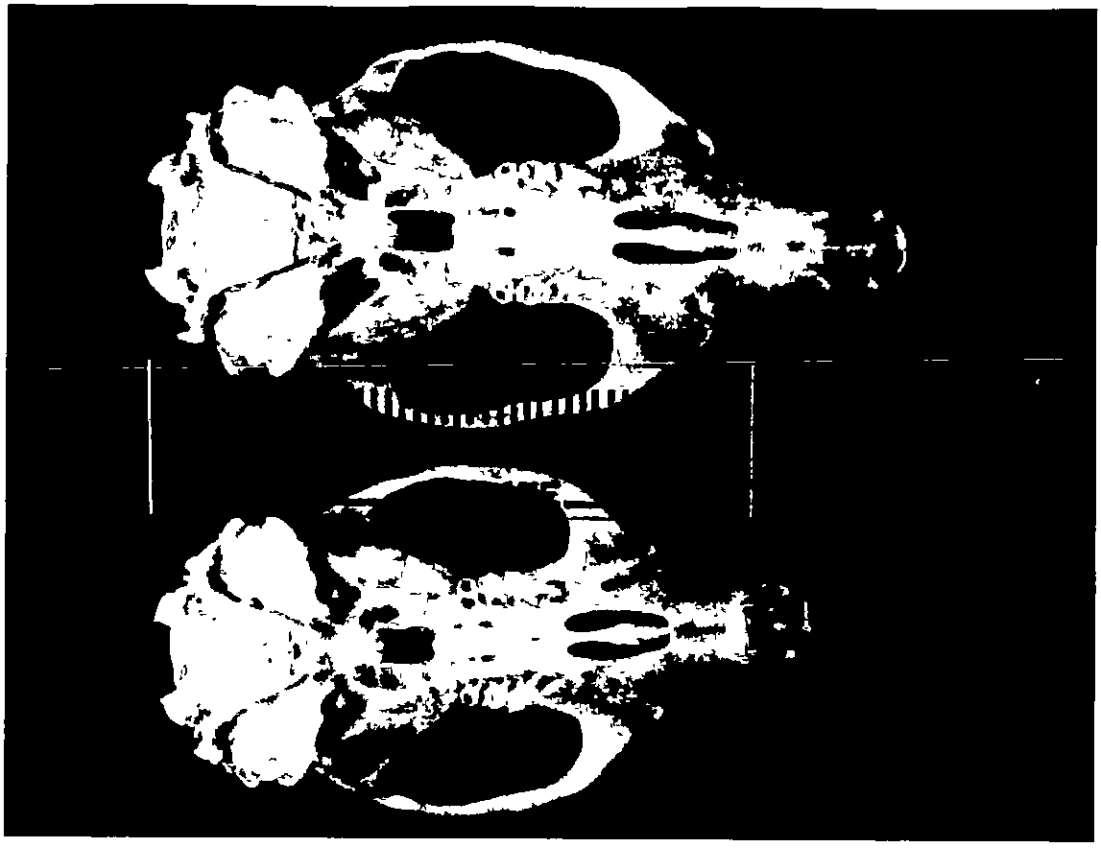


Figure 5.Ventral view of the dry skull (HZ male and WKY female)

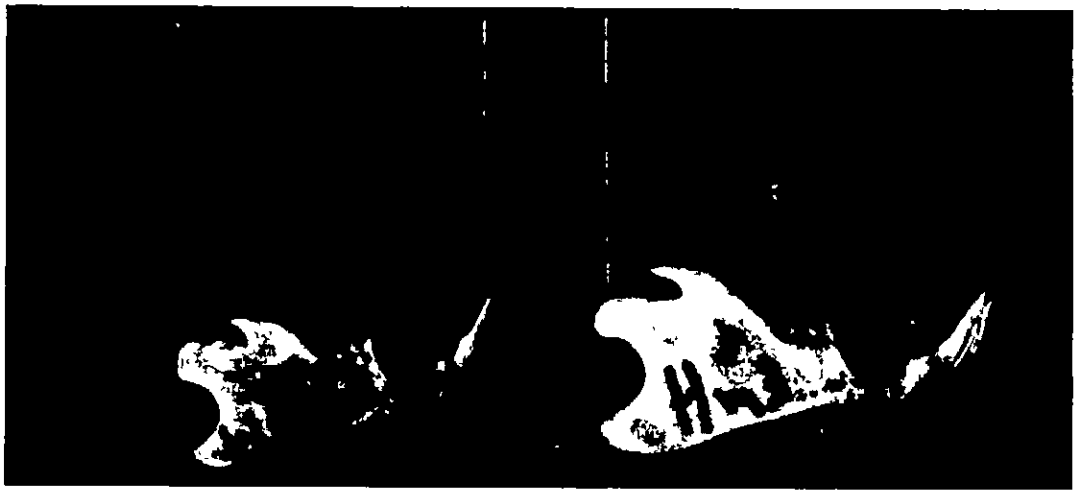


Figure 6.Lateral view of the dry mandible (WKY female and HZ male)

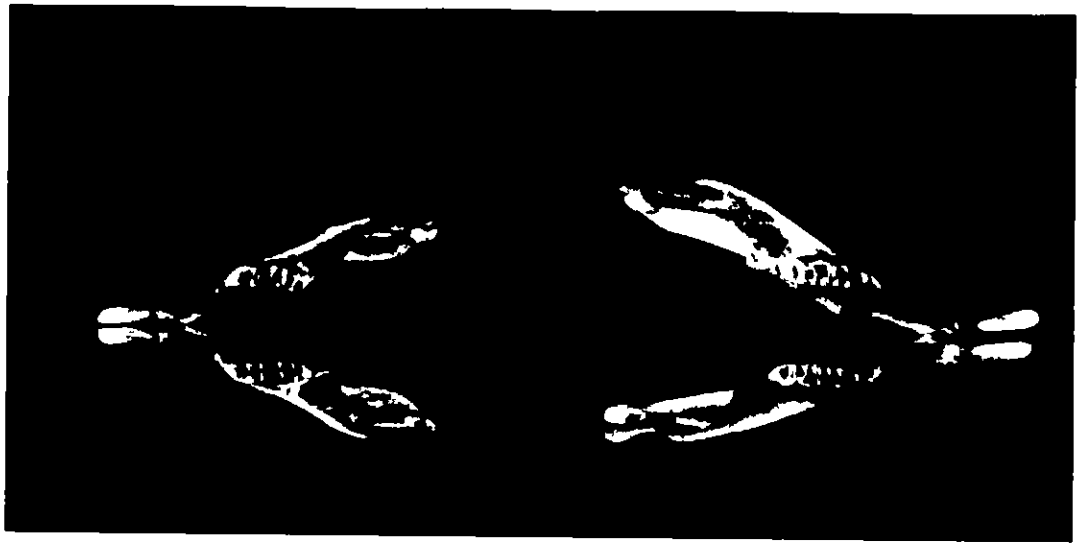


Figure 7.Dorsal view of the dry mandible (WKY female and HZ male)

RESULTS

A summary of the most significant findings will be presented by anatomic group.

Mandible size: TABLE 3 shows the means and the standard deviations for the mandibular measurements. Overall, in terms of mandible length: 1) gonion-infradentale, significant differences were as follows H* > BN, F, WK* and B > F, WK*. 2) Gonion-alveolar process, significant differences were as follows H* > SD, WK, F*. In terms of mandible height: 1) gonion-condyloid process, significant differences were as follows LE*, H, BN > F, WK, L*. 2) Gonion-coronoid process, significant differences were as follows BN*, H > L, F, WK*. In terms of mandible width: 1) bicondylar width, significant differences were as follows SD*, H, LE > L, WK, B* and SD*, H > F and SD* > BN. 2) bicoronoid width, significant differences were as follows H*, SD > B, L* and LE, WK > L*.

Maxilla size: TABLE 4 shows the means and the standard deviations for the maxillary measurements. Overall, in terms of maxilla length: 1) premaxilla length, significant differences were as follows H*, LE, SD, B > BN, WK*. 2) Overall maxilla (palatal) length, significant differences were as follows H*, SD, LE > WK, L, BN*. 3) Maxilla tooth row length (right, left), significant differences were as follows H* > SD, B, L, F* and LE > L, F* and L, B, SD, BN > F*.In terms of maxilla width: 1) anterior palatal width H*, L > F, WK, SD* and H* > B, BN and LE > WK, SD*. 2) Posterior palatal width H*, BN, B, L, LE > WK, F* and H* > BN, B, L, LE, SD.3) Maxilla width H*, SD > L, F* and H* > WK.

Skull size: Figures 3, 4, 5, 6, 7 are photographs of the dry skulls and dry mandibles. It is readily apparent that a wide difference exists among the strains. TABLE 5 shows the means and the standard deviations for the skull measurements. Overall, in terms of skull length: 1) skull length, significant differences were as follows H*, LE, SD, B > L, BN* and H* > WK, F 2) parietal bone length, significant differences were as follows B* > BN* 3) nasal bone length, significant differences were as follows H*, LE > BN, L, WK*.In terms of skull height, significant differences were as follows WK*, H, LE > F*. Even the WK is one of the smallest rat exhibits the highest skull along with attrition of the upper molars. These characteristics are subject of further investigations. In terms of skull width, significant differences were as follows H* > WK, BN, L, F* and LE > F*. In terms of cranial base, significant differences were as follows H* > BN, WK* and SD > WK*.

TABLE 3.--Means and Standard Deviations for the Mandibular Measurements.

	Ap-Id	Ap-Lm	Ap-Co	Ap-C	Co r-Co l	C r-C l
SD	26.7±1.2	19.4±0.6	12 3±1.05	15.3±0.7	17 8±1.01	19.01±1
BN	26.1±1.9	20.2±1 6	12 8±0.6	16.1±0.9	16.4±0.6	17 6±0.8
LEW	26 6±1 1	20.07±0.8	10 8±0.5	14.4±0.6	15.5±0.5	16 7±0 6
F344	25.4±1.6	19.03±1.1	11.2±0.6	13.9±0 6	15.6±0.6	17 7±0.7
WKY	25 2±1.02	19.3±0.9	11.2±0.5	13 7±0.6	15 2±0.3	18 7±1 3
LE	27 8±2.3	20.8±1.7	12.9±1.2	15.8±1.2	16 6±0.7	18 9±1.3
HZ	28.9±1.7	21.6±0.9	12.8±0 7	15.9±0.6	16.7±0.3	19.2±1.3
BUF	28.3±1.3	20.7±1.03	11.5±1 03	15 1±1	15 1±0 3	17 2±0.5

TABLE 4.--Means and Standard Deviations for the Maxillary Measurements.

	Ul-Um	Ul-Pns	Mum r-Mum l	Dum r-Dum l	Mx r-Mx l	Mum r-Dum r	Mum l-Dum l
SD	14.2±0.8	24.2±0.8	5.7±0.4	6.9±0.2	12.1±0.7	6.6±0.07	6.6±0 1
BN	12.3±0.6	22.05±1 01	6.2±0.5	7 2±0.3	11 3±0.5	6.7±0.1	6.7±0.1
LEW	12.6±0.6	22.1±0.8	6.9±0.1	7 1±0.2	11.01±0.6	6.5±0.1	6 6±0.1
F344	13.2±0.6	22.4±1.2	6.25±0.3	6 5±0.2	9 8±0.6	6.2±0.2	6.3±0 1
WKY	12.2±0.6	22.1±0.8	6.03±0.4	6.5±0.3	11.1±0.3		
LE	14.2±1.2	24.1±1.6	6.7±0.3	7.1±0.4	11.6±1.06	6.8±0.2	6.8±0.2
HZ	14.8±0.9	25.2±1.1	7.2±0.3	7.7±0.2	12.4±0.5	6.9±0.1	6.9±0.09
BUF	14.1±1.7	23.9±1.4	6.5±0.2	7 2±0.1	11 5±0.5	6.6±0.1	6.6±0.1

TABLE 5.--Means and Standard Deviations for the Skull Measurements.

	So-P	Nf-O	Lbd-Brg	Zg r-Zg l	N-Nf	Ba-So
SD	12.8±0.7	31.05±1.3	7.5±0.5	23.9±0.6	18.9±1.3	7.9±0.4
BN	12.8±0.8	26.4±1 2	5.8±0.5	23 4±1.3	17.4±0.6	7.02±0.5
LEW	13.1±0.2	28.08±1.1	6.8±0.2	23.08±1.1	17.4±0.7	7.2±0.4
F344	12.03±0.5	28.6±1.6	7.3±0.4	22.1±1.4	18.2±1.2	7.3±0.6
WKY	13.8±0.6	28.7±0.8	7.2±0.3	23.5±1.3	17.07±0.8	6.9±0.4
LE	13.3±0.9	31.1±2.1	7.2±0.7	24.6±1.7	19.8±1.4	7.4±0.8
HZ	13.7±0.5	32.2±1.4	7 5±0 7	25.8±1.2	19.9±1.2	7.9±0.5
BUF	13.07±0.4	30.8±1.8	8.2±0.6	24.1±1.1	18.7±1.1	7.6±0.4

Finally, the femur length was not significantly different among the rat strains.

DISCUSSION

The present study was designed to evaluate strain and gender differences among 8 strains of adult rats. Overall the results indicate that demonstrable differences in craniofacial characteristics exist. The present data provide a normative database for the adult rat, in which environmental factors including housing, food and water intake, and caretaker handling were the same. Our work suggests that even within the confines of a regulated environment, animals given the same food (rat chow) will exhibit measurable differences in craniofacial features. The significance of the strain effect supports the hypothesis that genetic factors influence craniofacial characteristics. In fact the differences in the size and shape of the mandible were larger than the differences reported by Petrovic et. al. in rats treated with a hyperpropulsor compared to controls. This finding is similar to the clinical findings in orthodontically treated patients where the differences found within a given treatment group i.e. headgear versus bionator group were greater than the observed between group differences.ref Studies on rat development, with various types of interventions to affect mandible and maxilla form have shown that such things as food, masticator muscle function/dysfunction, condylectomy, and maxillary restriction have shown that these extrinsic factors can be important factors in mandibular and maxillary development. However, in the present work, all rats were allowed to develop normally over the period of active growth, and well into adulthood. Yet the differences between the strains were greater than those reported for various intervention models. This is an interesting point especially considering that in Europe investigators commonly use the Wistar rat and in the United States

the most common species is the Sprague Dawley. Such species variation may account for some of the observed differences in results observed in these animal studies. In other words, we have always assumed that a rat is a rat, however, just like humans; rat strains demonstrate considerable differences in craniofacial morphology.

Summary and Conclusions

In the future, the nature of healthcare will be dramatically changed by the increase in understanding of the genetic basis for disease susceptibility. If orthodontics is to keep pace with other healthcare professions we must begin to understand the biologic basis for craniofacial variation. Improved understanding of the biology involved will in turn lead to a better understanding of the etiology of malocclusion. The ultimate goal of this line of research is to allow orthodontists to treat the cause rather than the effect of malocclusion. In the future, we expect that orthodontists will be able to tailor their treatment to the individual. The orthodontist of the future might use a functional in susceptible individuals, headgear for others and perhaps orthognathic surgery for orthopedically resistant genotypes. Establishing an animal model with genetically different craniofacial morphologic patterns is a necessary step in this exciting direction.

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