AGGREGATON OF RAT CRANIOFACIAL PHENOTYPIC TRAITS IN A BROWN NORWAY/SPRAGUE-DAWLEY CROSS

By

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Abstract

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Previous studies have shown that the Brown Norway strain (BN) is brachycephalic while the Sprague-Dawley (SD) strain is dolichocephalic. In order to determine which morphometric measures reflect an inherited trait, F2 rats originating from a BN/SD intercross were studied. Thirty-six female and forty-one male F2 generation rats were compared for twenty-one direct linear measurements from dried skulls and femur. Seven mandibular, six maxillary and seven cranial measurements and the length of the femur bone were collected. Mean, variance, and standard deviation was calculated for each measure after appropriate adjustment for age, sex, and litter size. A stepwise regression was used to identify variables for statistical significance. The estimates of variance in the F1 and F2 generations were used to compute broad sense heritability values for the twenty-one phenotypes. Eight variables had high heritability values which indicated they were heritable in this cross; bicoronoid width (29%), bicondylar width (24%), maxillary teeth row length (44%), cranial height (25%), parietal bone length (37%), frontal bone length (45%), occipital bone length (34%), and cranial width (23%). The majority of heritable

measures were directly (5/7) or indirectly (2/7) related to the size and shape of the cranium. Hence, it was conclude that differences in cranial morphology were most likely genetic whereas, morphologic variation in extracranial structures were influenced to a greater degree by the functional demands of breathing and mastication. This rat model might prove valuable in identifying genes that predispose to differences in crainial morphology.

DEDICATION

This thesis is dedicated to my wife, Lisa and our son, Jack. Thank you for your love, guidance and understanding. The two of you have helped me to achieve goals that would never have been possible without your support.

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INTRODUCTION

Being just a few years away from the completion of the human genome project, places clinicians at the door to the genetic frontier. The role of the orthodontist will be expanded in the near future. Orthodontists will be expected to have a grasp on what processes influence the craniofacial complex. The affects of environment and genetics on craniofacial morphology demand attention and have a direct influence on what the orthodontist attempts to modify and control. Having the ability to determine the heritability of a trait might shed some light on predicting facial morphology. Knowing the extent to which genetics controls a trait could modify a treatment plan, or at least help the clinician to direct treatment in such a way as to increase the chance of creating a successful and stable treatment result.

If a trait is determined to possess high genetic control, it may be less amenable to a large treatment change that would be stable over time. A trait primarily under environmental control might be altered in such a way to accomplish a larger, more stable result. Without first knowing the amount of genetic or environmental control, the clinician could be directing a treatment strategy destined to fall short of a desired result.

Investigators need a starting point when attempting to analyze a genetic blueprint for one or more traits. By first finding predictable phenotypic markers with certain heritability values, there should be some key markers that unlock the genetic

background. Animal crosses play an important role in experiments designed with a goal of calculating heretability. It is important to remember that, even though a trait may have a high heritability factor, this does not mean that a trait is unchangeable by the environment.

LITERATURE REVIEW

Approaches for Studying Genetics

Gregor Mendel in 1865 was the first to give a scientific explanation for the inheritance of discrete traits. He used the word "factors" to describe the influence of an unobservable process that was responsible for physical traits in individuals, and that single members of these pairs were transmitted unaltered from parent to offspring (Suzuki et al. 1986). Mendel's work can be summarized into two principles; the principle of segregation of alleles, and the principle of independent assortment. Mendelian segregation relies on the fact that random mating results in an equilibrium distribution of genotypes after only one generation. When two members of a gene pair segregate into gametes, one-half of the gametes carry one member of the pair while the other one-half of the gametes carry the other portion of the pair. During gamete formation, alleles segregate in such a way that each gamete is equally likely to receive either member of the pair. The principle of independent assortment states that one gene is independent of the segregation of alleles of another gene during gamete formation. Genes specifying distinct traits are passed through the gametes independently of one another.

Mendel also showed that when two loci (genes) are on the same chromosome they are said to be linked and they do not segregate independently. If they are very close together, then likely, they are tightly linked and will go together during meiosis.

If they are far apart on the same chromosome, then they may appear to sort independently, as if they are on separate chromosomes. The basis of genetic mapping relies on this dependence of chromosome position (Duke 2000).

The Punnett square is a useful tool to help illustrate possible genotypic and phenotypic results from breeding. In 1902, Sutton and Boveri correlated Mendel's work with the behavior of chromosomes during meiosis. Substitution of the word "chromosome" for Mendel's term "factor" explained how segregation and independent assortment actually describe the movement of chromosomes in meiosis (Griffiths et al. 1993).

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 piece of genetic material is literally like finding a needle in a haystack. There are twenty-three different chromosomes in each human cell from which to locate one gene. The human genome possesses approximately three billion base pairs. An average chromosome contains about one hundred and twenty million base pairs. Two thousand base pairs make up an average gene. A mutation capable of causing a disease may be as small as one base pair (Duke, chpt. 1, 2000).

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.

Two contrasting strategies of gene identification can be employed. These strategies are positional and functional cloning. Functional cloning depends upon application of biological information, while positional cloning is initiated by mapping the responsible gene to its correct location on a chromosome. The process of gene identification by map position was previously referred to as reverse genetics. It is now clear that the process of gene identification by mapping is not reverse at all. The analysis of function of a gene by creating multiple mutations and assessing the effects is the correct definition for the term reverse genetics. Functional cloning is limited to disorders for which biological information about the basic genetic defect exists. Functional cloning depends on the availability of information about the protein product or the function of the responsible gene. Mapping will then follow cloning. Positional cloning has an opposite flow of operation. Mapping becomes essential and function is only determined after the gene is located. When using positional cloning in animal studies, one must first begin with the collection of pedigrees in which the responsible gene segregates. Pedigrees are studied with multiple polymorphic markers until evidence for linkage is identified (Collins 1992).

Positional cloning involves the process of going from gene to phenotype. It is a procedure that begins with a cloned segment of DNA or a protein sequence, having unknown function, and uses this sequence to introduce programmed mutations into

the genome in order to investigate function. Functional cloning is the process of going from phenotype to gene. Positional cloning has taken on the approach of producing mutations by replacing genes of choice (Takahashi et al. 1994).

Nine definitions provided by Takahashi et al. (1994) are imperative for understanding current gene research. First, an inbred strain is a set of animals that is produced by at least twenty consecutive generations of sister-brother or parent-offspring matings that can be traced to a single ancestral pair. Animals of an inbred strain are nearly fully homozygous, providing a defined and consistent genotype for analysis. Second, a congenic strain is a strain that differs from another in defined regions of one or more genetic loci and that is produced by at least ten successive backcrosses or intercrosses to the control strain. This gives the ability to make a double congenic. These animals can be used to evaluate a mutation or allelic variant against a particular inbred strain background. Third, a recombinant inbred strain is a set of strains originating from the second generation of intercrossing of two inbred strains, then inbreeding different lines at least twenty generations. Each recombinant inbred strain has a different, but reproducible, recombined sampling of the genomes of the inbred strains of origin. Fourth, a genetic map is a map based on the segregation of genes or DNA markers in a cross. This type of map allows for the ability to find the position of a gene causing the trait being followed in families. Units of genetic distance are based on recombination rates-that is, morgans or centimorgans. Fifth, a physical map is a map based on the chromosomal position of physical markers in units of physical distance [base pairs or kilobases]. This type of

map is actually biochemical. Sixth, restriction fragment length polymorphs are DNA fragments of different lengths generated by cleaving with a specific endonuclease; they can be used for genetic or physical mapping, particularly in interspecific crosses. Seventh, positional cloning is the process of gene identification, using map position as a starting point. Once the chromosomal region containing the gene is identified, DNA from that region is cloned and the gene is identified from the clones. Eighth, the candidate gene approach involves testing of cloned genes that map in the region of a mutant for involvement in the phenotype associated with a mutation. Ninth, quantitative trait loci are the set of genes that together govern the quantity of a characteristic not completely determined by any one gene acting alone.

Genes affecting facial growth are likely to be polygenic. Thus, there are a large number of genes and each has a small effect on the phenotype. There are many factors affecting an organism's phenotype, and each genotype does not produce a single phenotype. There are an unspecified large number of independently segregating loci contributing in a simple additive fashion to the phenotype. having a norm of reaction that covers a wide phenotypic range. If a phenotype is said to be multifactorial, it is implied that the phenotype is determined by both genetic and environmental factors, frequently without specifying the number of genetic loci involved. Many segregating loci can have alleles that make a difference to the observed phenotype. This demonstrates the need for quantitative genetics in order to study the genetics of continuously varying traits (Griffiths et al. 1993).

It is possible to study quantitative traits with animal models. When purebred

lines show phenotypic traits with nonoverlapping distributions, it is possible to form crosses between the two parental lines and among the resulting progeny lines. Crossing the purebred lines may produce offspring that have an intermediate phenotype, although all three groups may still have a large variance of phenotypic expression. Even though the phenotypic means of the parental lines and the offspring differ significantly, their variances will be quite similar. The variance can be attributed to nongenetic causes because all three groups are genetically homogeneous, since parental lines are homozygous at all loci effecting the specific phenotype and all offspring are heterozygous. When the progeny are selfed, the observed variance among their offspring will be much larger than the two prior generations. By using this increased variance in the third generation, along with the difference in phenotypic means of the two parental lines, it is possible to estimate the number of independent loci influencing a quantitative trait (Suzuki et al. 1986).

Complex or multifactorial traits often do not follow Mendelian dominant and recessive phenotypic distribution patterns. Similar phenotypes can be due to the result of different genotypes. Interactions with other genes, chance effects, or environmental factors can be the cause for these different phenotypes. Different genotypes may result in the same phenotype. It is often impossible to find a genetic marker that shows perfect cosegregation with a complex trait. Individuals with incomplete penetrance will inherit a predisposing allele and yet might not manifest the phenotype. In a phenocopy situation, an individual may not possess a predisposing allele yet might have the phenotype as a result of environmental or

random causes. The penetrance function, specifying the probability of phenotype for each genotype, can also depend on nongenetic factors such as age, sex, environment and other genes. Lander and Schork (1994) outlined the methods available for genetic dissection of complex traits. These methods fall into four categories: linkage analysis, allele-sharing methods, association studies in human populations, and genetic analysis of large crosses in model organisms. Linkage analysis is used to explain the inheritance pattern of phenotypes and genotypes observed in a pedigree. It involves describing the association of genes on a chromosome. Linkage is a measure of frequency of recombination between two markers, which is related to the distance between the markers. An ideal quantitative trait for investigation would be one that does not vary as a consequence of the segregation of alleles at large numbers of loci, but rather as a consequence of segregation of very few loci with major effects (Griffiths et al. 1993). This is the method of choice for studying simple Mendelian traits, but this technique does not work for complex traits. It is hard to find a model that explains the inheritance pattern. Allele-sharing methods test whether affected relatives inherit a region identical-by-descent more often than expected under random Mendalian segregation. Affected sib pair analysis is a well-known special case, in which the presence of a trait-causing gene is revealed by more than the expected fifty percent identical-by-descent allele sharing. The method is more robust for genetic complications than linkage analysis but can be less powerful than a correctly specified linkage model. Haseman and Elston applied this method to studying quantitative traits. The approach is based on the understanding that phenotypic

similarity between two relatives should be correlated with the number of alleles shared at a trait-causing locus. Association studies follow the understanding that allele "A" at a gene of interest is said to be associated with the trait if it occurs with a significantly higher frequency among affected individuals compared with control individuals. This type of study is not concerned with familial inheritance patterns. It is a type of case-control study based on a comparison of unrelated affected and unaffected individuals from a population. Experimental crosses, using rats and mice, make it possible to map polygenic traits with the problem of genetic heterogeneity disappearing because this process allows the study of hundreds of meioses from a single set of parents. A large complex genetic interaction can be probed. Experimental crosses are powerful tools due to their ability to dissect quantitative traits into discrete genetic factors. Quantitative trait locus (QTL) mapping has become possible due to the construction of dense genetic linkage maps for the mouse and the rat. Interval mapping uses phenotypic and genotypic information to estimate the specific genetic code and the most likely quantitative trait locus at every point in the genome. Experimental crosses also facilitate analysis of discrete traits with complex genetic etiology. Experimental geneticists are able to study the physiological effects of individual polygenic factors by creating congenic strains that are different only in the region of a single locus, once an initial mapping is complete. Linkage analysis reveals those trait-causing genes that are different between the two parental strains (Griffiths et al. 1993).

Szpirer et al. (2000) localized fifty-four rat genes and identified new synteny

groups conserved in the human and the rat. The process of localization was completed in order to improve the rat gene map and comparative mapping with humans and mice. Anchor units were generated and four new synteny groups were found to be conserved in humans and rats. These new locations can be useful for the identification of genes involved in the control of some quantitative traits.

Plomin et al. (1991) reported the use of recombinant inbred strains to detect quantitative trait loci associated with behavior. Quantitative trait loci association analysis was completed in order to correlate allelic variation for a marker with phenotypic variability in the population for a quantitative characteristic. In this case, quantitative trait loci refers to identifiable multigenic influences on phenotypic expression. Primarily, recombinant inbred strains were developed to detect major gene effects and to establish linkage for traits that show bimodal recombinant inbred strain distribution patterns (Bailey, 1971). Taylor (1989) reported that there were at least twenty-four established and incipient recombinant inbred series of mice. He also listed one hundred and eighty-five markers that showed one hundred and sixty-three strain distribution patterns distributed across all but four of the twenty mouse chromosomes. The number of available strain distribution patterns has expanded by the use of restriction fragment length polymorphism markers. Recombinant inbred data are important for detecting quantitative trait loci. Quantitative trait loci association analysis can be used to correlate allelic variation for a marker with phenotypic variability in the population for a quantitative characteristic.

Recombinant inbred strain averages can also be employed to investigate associations between quantitative traits and markers (Plomin et al, 1991).

Thoday (1961) developed the basic approach of identifying associations between genetic markers and quantitatively varying traits. A biometrical approach calls for an analysis of variance. It is important to investigate which chromosomes are effective by test-crossing with a stock in which each chromosome of the haploid set is marked. The parental classes will have distinct means, but since they are homogenous they will posses low variances. The recombination classes will be heterozygous with means being between the parental classes, but with relatively high variances.

Plomin (1993) called for the study of F1 strains derived from crosses among existing recombinant inbred strains. He stated five advantages of using this process. First, many F1 strains can be generated by crossing existing recombinant inbred series. Second, there is no need to type for genetic markers in the recombinant inbred F1 strains because their genotypes are known from the recombinant inbred parents. Third, other investigators can reconstitute F1 crosses for comparability across studies. Forth, analysis of F1 crosses provides a replication of the recombinant quantitative trait loci analysis. Fifth, since half of the F1 strains derived from crosses between recombinant strains are expected to be homozygous, it is possible to investigate additive, dominance and heterozygosity effects. Using the same number of recombinant inbred strains has advantages over using recombinant inbred F1 cross

strains, but the larger sample size in F1 studies compensates for the modest genotypic correlation among the F1 strains.

Bailey (1971) described the process of generating recombinant inbred strains created by inbreeding an F2 population acquired from the F1 cross between two progenitor-inbred strains. The F1 generation will be a hybrid; that is, heterozygous for all genes for which the inbred progenitor strains are different. This also means that all F1 animals will be similar in their heterogeneity. When the F1 generation is mated, the F2 progeny will segregate all genes for which the original inbred strains were different. Half of the F2 generation will be heterozygous and half will be homozygous. The homozygous subjects will be split with half in one state and half in another state. Having the progenitors differ in allelic state for a gene implies that half of the derived recombinant strains would be homozygous for one allele and half homozygous for the other allele. Non-overlapping strain distribution patterns imply a single-gene effect; conversely a continuous distribution of recombinant inbred strains suggests multigenic inheritance.

Klein (1978) explained a way to analyze major gene effects using recombinant inbred strains and related congenic lines. It should be realize that, in developing inbred strains, it is possible to establish loss of fitness. This decreases survivability, with this loss attributed to the fixation of a lethal or deleterious recessive allele, or allelic combination. It is possible to test a major gene hypothesis, establish linkage or pleiotropy by the use of recombinant inbred strains and congenic lines developed from the same progenitor strains. A comparison of the mean value

for all recombinant inbred strains, with the mean of their progenitors, yields a test for epistasis. An epistatic gene allele of one gene eliminates expression of the alternative phenotype of another gene, and inserts its own phenotype instead. Heterogeneity of variance among recombinant inbred strains and their progenitors would be indicative of genotype by environmental interaction. Having a limited number of strain distribution patterns relative to the proportion of polymorphic loci in the genetic background of these strains, requires additional evidence from other genetic materials to prove that an observed variation is due to the action of a single gene.

Plomin and McClean (1993) described eight strengths of using a recombinant inbred quantitative trait loci approach in genetics. First, linkages can be detected when the distribution of recombinant inbred means is continuous rather than bimodal. The approach does away with the need to dichotomize recombinant strains into groups resembling one or the other progenitor strain as was traditionally done. Second, the approach can detect quantitative trait loci associations for a phenotype for which progenitor strains do not differ. Third, progenitor-inbred strains may have similar average phenotypes produced by totally different sets of alleles. Forth, it is possible to compare individuals across time and across studies because recombinant strains are inbred. This allows for all markers previously typed for the recombinant inbred series to become available in the search for quantitative trait loci once a recombinant series has been characterized for a particular trait. Fifth, multivariant analyses of genetic correlations and their quantitative trait loci underpinnings can be conducted across studies that employ the same recombinant inbred series. Sixth, it

can identify major-gene effects if they exist. Seventh, the approach can be applied to any quantitative trait regardless of the level of biobehavioral analysis. Plomin and McClearn also made reference to genetic analysis of inbred strains estimating heritability by comparing variances between inbred strains and F2 individuals. The variance among F2 individuals related to the genetic plus the environmental total phenotypic variance. The nongenetic variance was assessed by the variance within inbred strains. The total variance minus the nongenetic variance gave a measure of the genetic variance. The proportion of the total variance due to genetic variance gave an assessment of the heritability.

In a Master's thesis by Y.C. Park (1977) it was stated that the most effective ways of decreasing the variance of a gene number estimate were an increase in the number of generations in a primary selection program, an increase in the number of generations in the two-way selection program, and an increase in the population size. The effect of heritability on sampling variance is important when the gene number is large. Park also pointed out that when heritability of a quantitative character was low, and the gene number was large, many generations of primary selection were required to obtain or estimate the gene number with reasonable precision.

Genetic correlation measures the extent of plieotropic effects of polygenes in pairs of characters or the closeness of linkage between sets of loci influencing the traits and held in allelic disequilibrium. Genetic correlation limits the rate and set the direction of multivariate evolution. Estimating heritability from inbred strain comparisons involves rearing a set of strains, being sure to avoid associating

environmental differences with strain differences. In order to provide an unbiased estimate of gene-imposed differences affecting the characters, it is necessary to keep the sample size large and to choose the strains without regard to their values for the characters to be analyzed. The component of variance among inbred strains, under intense inbreeding, should provide two times the additive genetic variance influencing a trait when compared to a randomly mating population. Standard analysis of variance procedures can be employed to partition the measured characters within and between strains (Hegmann and Possidente 1980).

Quantitative genetic theory is based primarily on estimating variance and covariance and less on mean. Lovell and Johnson (1983) conducted a study on the quantitative genetic variation in the skeleton of the mouse involving variation between inbred strains. Six bones from eleven inbred strains and one F1 hybrid mice were measured. The bones examined were the mandible, os coxae, femur, tibia-fibula, scapula and humerus. Considerable variation in the shape of each bone was found and successful discrimination between the strains was obtained. Historically, the mandible has been used for the identification of mouse strains. Successful discrimination between genotypes was obtained with the mandible and three additional bones while two other bones provided moderate separation of genotypes. The variation in bone shape among genotypes was likely to be under genetic control. The degree of sexual dimorphism varied among bones, and a sex difference was detected in the mandible. The sex difference in the shapes of bones was larger in some genotypes than others with variation in shape also found within the strains.

Differences in the ages of the animals in the samples might have increased the within-strain variability. The authors described a simple technique that is capable of detecting considerable variation in the mouse skeleton, much of which presumably has a strong genetic basis

The systematic genetic dissection of quantitative traits using complete restriction fragment length polymorphism linkage maps is a complex and rewarding endeavor when done correctly. Any of the individual polynucleotide sequences produced by digestion of DNA with a restriction endonuclease becomes a restriction fragment. Restriction fragment length polymorphism mapping is a technique in which DNA restriction fragment length polymorphisms are used as a reference loci for identification and isolation in relation to known genes, or other restriction fragment length polymorphism loci. Polymorphism is an ancient Greek term meaning many forms which, in this case, is a coexistence of two or more phenotypes attributable to the alleles of one gene. Restriction enzymes make sequence specific cuts that produce a heterogeneous population of fragments with identical ends. Markers for DNA can be produced by the use of restriction enzyme target sites. Successive digestion by different restriction enzymes produces fragments that can be separated electrophoretically on polyacrylamide gel with the resulting banding creating a map. At the molecular level, the phenotypes are the restriction fragments of varying size. Hybridization to a particular probe should reveal the coexistence of two or more restriction fragment patterns, which are referred to as restriction fragment length polymorphism. The process involves taking probes and using them

in Southern hybridizations against restriction-enzyme-digested DNA preparations from a sample (Griffith 1993).

Having complete restriction fragment length polymorphism linkage maps help make it possible to dissect quantitative traits into discrete genetic factors. Genetic dissection of a quantitative trait will only be successful when some of the quantitative trait loci segregating in the cross have large phenotypic effects. Studying single genetic markers one-at-a-time has three drawbacks. First, the phenotypic effects of quantitative trait loci are systematically underestimated. Second, the genetic locations of quantitative trait loci are not well resolved because distant linkage cannot be distinguished from small phenotypic effects. Third, the number of progeny required for detecting quantitative trait loci is larger than necessary. A reduction of seven fold in the number of progeny to be genotyped can be achieved by doing interval mapping and selective genotyping. This involves growing a large population, but only genotyping those individuals that have phenotypes that deviate substantially from the mean. The ability to map quantitative trait loci underlying a quantitative trait depends on the magnitude of phenotypic effect. Many more progeny will be needed to detect genotypes that have small effects on phenotypes. Using intense artificial selection, for and against a trait, to create two strains from a large outbred population followed by inbreeding will produce the ideal situation. Unselected strains possessing extreme phenotypic differences are also a good starting point. Progeny with phenotypes more than one standard deviation from the mean comprise about thirty-three percent of the total population but contribute about eighty-one

percent of the total linkage information. Progeny with phenotypes more than two standard deviations from the mean comprise about twenty-eight percent of the total linkage information. True phenotypic outliers may represent artifacts once one goes beyond the five percent tails of the distribution (Lander and Botstein 1988).

Wallace and Bader (1967) determined that when a number of continuous variables were measured on a relatively large number of individuals, it was highly desirable to treat all variables simultaneously and reduce the large number of correlations and complex interactions to a few effects and simple interactions. They conducted a factor analysis of morphometric traits in the house mouse. Correlation coefficients were among the several measurements, or variables, that comprised the basic data of the factor analysis. These coefficients were effects and the aim was to reduce them to a few causes or "factors".

Factorial analytic technique was used to determine the sets of factors and discover their causes. Variables in a factor analysis are not assigned independent or dependent status, and no prior assumptions are made concerning the functional relationships among the variables. Common factors and unique factors are the two major types of existing factors. Common factors include general factors present in all variables and group factors that are in more than one variable, but not in all. Unique factors are solely associated with an individual variable and represent that portion of a variable not ascribable to its correlation with other variables in the set. The factor loading or coefficient represents the degree of association, or correlation of the variable, with the factor. The whole variance of the trait is divided into common and

unique factors. By using a factor analysis one is able to allow a more general picture of the forces, or fields of influence, at work to emerge more clearly than could be obtained from studying the individual correlation coefficients.

Genetic Influences on the Craniofacial Growth and Development of the Rat

A basic understanding of the rat calvaria is essential when trying to uncover phenotypic variability of the rat skull. Moss thoroughly studied the growth of the calvaria in the rat and explained its osseous morphology, determining that the growth of the skull vault of the rat was not a haphazard process, but that it followed a definite ontogenetic sequence (Moss 1954). The growth of the neuro-cranium is considered complete at thirty-four days of age with the growth of the visceral cranium continuing long past this time. The parietal and posterior portion of the frontal bones achieve near adult dimension within thirty-four days of age, with the anterior portions of the frontal bones and other bones of the visceral complex exhibiting continued growth past thirty-four days of age. The neuro-cranium is basically complete in its growth by thirty-four days of age. The visceral cranium and the neuro-cranium develop at different rates. Moss determined that the growth of the rat neuro-cranium was not accomplished by means of an interstitial expansive force originating within the soft tissues of the suture, but rather the periosteal osteogenic tissues, ectocranial as well as endocranial, appeared to be the primary growth sites. Osteogenesis occured on all surfaces of the rat calvaria under sixty days of age, and the shape of the calvarial bones was not predetermined by the location of the sutures.

Two areas of importance influence the end result of growth and development.

They are genetics and environment. Nonaka et al. (1988a) reported on the genetic and environmental factors in the longitudinal growth of rats pertaining to body weight and overall craniofacial size. They realized that the genetic and environmental components had a variable effect according to different ages of the rats. The work was based on one hundred and fifty rats with eleven cephalograms taken of each rat at intervals from ten days to eighty days. Forty-two landmarks were recorded on each cephalogram, and a mathematical model was used to include additive genetic, non-additive genetic, maternal, and environmental effects. It was concluded that the additive genetic component increased regularly from day ten to day eighty as growth proceeded. Non-additive genetic variance was attributed to dominance and epistasis and it contributed very little to the total variance. The genetic effect clearly played an important role in the growth of rats with age. The maternal variance was the maximum, and accounted for a large proportion at day ten and then declined rapidly by days forty to fifty and remained low or was not significantly different from zero. The maternal component was greater in males than in females, possibly due to the fact that females reach the adult size faster than males. Thus it appeared that the genetic effect became larger with age of the rat and the maternal effects diminished. Maternal effects were defined as prenatal and postnatal factors. The postnatal factors relate to the mothering ability such as temperment, maternal instincts, and lactational output.

Nonaka and Nakata (1988b) also reported on the genetic and environmental factors in the longitudinal growth of rat ventrodorsal size on one hundred and fifty

rats. It was also determined that the genetic component of variance significantly increased until day eighty. Inversely, the maternal component of variance showed a large value during the early stage of postnatal growth and decreased to a very small amount by day eighty. Eleven ventrodorsal cephalograms were taken on each organism, and eighty-eight landmarks were defined.

Nonaka and Nakata (1988c) described the genetic and environmental factors in the longitudinal growth of rats pertaining to craniofacial shape change. The investigation used one hundred and fifty rats from five different strains. Eleven lateral cephalograms were taken on each rat over an eighty-day period. Forty-one landmarks were locate and compared. The sample was divided into five groups consisting of fifteen possible pairs within the litter, ninety pairs representing rats from different strain pairs, one hundred and eighty maternal half-sibs, one hundred and eighty paternal half-sib pairs, and thirty pairs representing the same rats. Using a common environment for inbred strains, one can assume that the differences within strains are environmental in nature. The differences between strains should be due to the genetic differences of the individual strains. In contrast, the differences between maternal and paternal half-sibs are assumed to be caused by maternal effects common to maternal half-sibs. The significance of the maternal effect was tested by calculating the ratio of maternal half-sib variation verses paternal half-sib variation. The significance of the genetic effect was tested by calculating the ratio of between-strain pair variation verses within-strain pair variation. The dissimilarity of individuals from the same inbred strain is influenced only by environmental effects if

the specimens share the same genotype. Though the genetic effect was not always significant, it does contribute significantly to the shape of the craniofacial complex. A considerable genetic effect was found to act on the change of the craniofacial shape during growth. Information obtained from the maternal and paternal half-sib groups seems to suggest that the maternal effect does not act on the change of the craniofacial shape during growth. The present study will therefore use adult rats in order to have a more genetic rather than environmental maternal effect on the size and shape of craniofacial morphology.

Size and shape differences will lead to variability to phenotypes. Atchley and Rutledge (1980) reported on the genetic components of size and shape of the laboratory rat dealing with the dynamics of components of phenotypic variability and covariability during ontogeny. The size and shape of an organism can be envisioned as being controlled metric characters whose phenotypic expression often changes considerably during ontogeny and evolution. Ontogenetic body traits in six genetic strains of laboratory rats were examined. All lines originated from a common genetic base (cross of four inbred strains). Three live body measurements consisting of body weight, tail length, and chest circumference were recorded ten times beginning at week two and continuing until week twenty-five when net growth was minimal. It was emphasized that rearing progeny in a crossfostering design permits the use of the covariance of full-sibs reared apart for estimating genetic parameters. In the absence of dominance and epistasis, each crossfostering pair forms a two by two factorial and the variance between genetic mothers has the genetic expectation of half of the

additive genetic variance (covariance). This absence will disregard a situation in which the differential phenotypic expression of genotypes at one locus depends upon the genotype at another locus. The heritability relationship estimates between body weight and body circumference and between body weight and tail length indicated that these bivariant growth relationships are highly heritable and would respond to selection. Heritability of these traits may be providing evidence of differential gene activity during ontogeny.

Atchley et al. (1981) took an even deeper look into multivariate covariance patterns in the rat and mouse skull with regard to the genetic components of size and shape. They stated that even with genetic data, comparison of the magnitude of estimated genetic correlation coefficients was often not meaningful since single genetic correlation coefficients were a function of gene frequencies, selection regimes, and evolutionary histories. As a result, comparisons potentially can vary considerably between genetic strains or populations of the same species, and multivariate patterns of correlations may be more stable. Genetic correlations among morphometric traits reflect associations arising from pleiotropy and linkage. Pliotropic genes have more than one distinct phenotypic effect, and linkage is transitory and in randomly mating populations will have little long-term evolutionary consequences for polygenic traits. Rats do have a large number of linkage groups. Atchley et al. stated that palatal width traits made a strong contribution to genetic covariance while other skull width characteristics contributed much less. The final analysis of genetic correlation structure indicated that there are four well-defined

genetic patterns of morphogenesis in the skull of the rat. They are a general skull size component, a general skull shape component, a tooth and jaw length component, and a palatal and skull width component. A genetically determined shape contrast in the rat skull involving an inverse relation between skull width and depth versus length was also reported by Atchley.

Two years later Atchley (1983) reported on eight mandibular and maxillary traits as well as the femur in regards to quantitative genetic aspects of variation and covariation. Femur length had a positive genetic association with mandibular height and length, palatal width, and posterior palatal width. A significant negative genetic relationship between femur length and mandibular tooth row length was reported. There also appeared to be no genetic association between femur length and both maxillary tooth row length and mandibular width. The overall size of the mandible was concluded to be a highly heritable trait. The length and width of the mandible were found to be heritable. In contrast, the mandibular height is not heritable due to the fact that was found to be a measure of muscle insertion. This leads to the impression that most of the dimensions of the length and width of the craniofacial complex of the rat are affected by mostly genetic guidance.

A growth study has more strength if it can be performed over a period of time and not rely solely on cross-sectional data. Cleall et al. (1971) performed a longitudinal study to determine the growth of the craniofacial complex in the rat. The goal of the study was to quantify the growth of bone at specific sites and provide a baseline for future studies investigating the influence of alterations in the local

environment on the growth of bone. The investigators studied changes in the rat craniofacial complex from the post-weaning period to early adulthood, with specific reference to quantitative and qualitative assessment of bone growth in the coronal plane. Using a coordinate analysis technique, they also studied the relative position of selected sites of measurement at the beginning and end of the experimental period. Male Long-Evans rats were used in the study. Thirty-six rats were analyzed to describe growth of the cranial vault and the nasomaxillary complex, and forty-two rats were used to describe growth of the mandible. The study used a sequence of bone marking agents to determine growth. The following is a summary of conclusions made from this study. Remodeling was instrumental in the mandible. Area relocation was crucial to the retromolar region that allowed the original angular process to become incorporated into the elongating body of the mandible. Differential growth was important in the morphogenesis of the inferior border of the mandible. Deepening of the antegonial notch was the result of a greater rate of apposition at its periphery than at its center. This differed from humans who have the notch deepen by a process of resorption directly in the area. The cranial vault enlarged in the lateral and vertical dimensions by appositional growth on both the intracranial and extracranial surfaces. Compensation for the increase in intracranial volume was made at the sutures and by differential growth at the centers and edges of the bone that reduced the curvature. Growth in depth requires alterations involving intracranial bone resorption at specific sites. The cranium and nasal cavity were found to have different patterns of bone growth. The nasal cavity had appositional

growth on the outer surfaces with compensating bone resorption occurring on the intranasal surfaces. Sutures were most active and vary greatly in the amount of growth and also in the relative amount of growth demonstrated by the bones at each side of the suture. The oral region more closely resembled the cranial region since appositional growth occured both on the inferior surface of the palate and on the inner aspects of the body and much of the ramus of the mandible. Growth in width was due to the mid-palatal suture and possibly the symphyseal connective tissue. A striking dissimilarity between rats and humans is an area of symphyseal connective tissue connecting the two sides of the mandible, while the human mandible is a single continuous bone.

Yanagita et al. (1992) conducted a genetic study of craniofacial growth in male rats by using factor scores. Craniofacial growth of F1 male offspring, produced by a complete diallele cross of five strains of rats, was determined with the use of cephalometry. Dorsoventral and lateral cephalograms were taken of five strains of inbred rats. Sixty-four landmarks were determined for the dorsoventral cephalogram, while twenty-six landmarks were developed for the lateral cephalogram, and diet, temperature, and humidity were standardized for all the organisms. By using factor analysis the experimenters were able to reduce a large set of interrelated variables into a smaller set of independent factors, thereby simplifying the craniofacial morphogenesis. By excluding the inbred strains of offspring that were of the same genotype as the parental line in the genetic analysis of the F1 population, which was produced from the complete diallele cross mating of five strains of rats, the portion of

the non-additive genetic variance became comparable to that of the additive genetic variance. This was essential since inbred strains of rats might be more sensitive to a minor environmental change compared with the F1 hybrid strains of rats, resulting in the existence of a large nonadditive genetic variance attributed to the dominance effect. The important conclusions reached were that the growth changes in the widths and lengths of the craniofacial complexes in male rats were more strongly impacted upon by genetic effects. Conversely, growth changes of the upper jaw, nasal cavity, and pharyngeal part were more strongly impacted upon by environmental effects associated with the functions of mastication and respiration.

McAlarney et al. (1992) used anatomical macroelements in order to study the craniofacial growth of the rat. Their method used boundary data to interpolate internal growth values. Longitudinal landmark coordinates from midsagittal radiographic tracings of twenty-two female albino rat skulls of various ages were averaged. The neural skull and the facial skull were analyzed with the facial skull subdivided into olfactory and respiratory regions. Maximum elongation ratios were calculated for approximately four hundred interior points. Macroelement and finite element interpolants differ in the size and shape of allowable anatomical elements. A macroelement representation of an anatomic area may be subdivided into finite elements. By dividing the craniofacial components into functional units, the authors were able to describe the growth of bones comprising elemental boundaries and the direct comparison of anatomical macroelement results with data on the growth of biological structures within each macroelement. Significant neural growth changes

occurred at seven to fourteen days and consisted of the cerebellum undergoing a more rapid growth than other portions of the brain, while the olfactory lobes underwent minimal growth In the forty to sixty day range, large differences in relative growth of parts of the brain do not occur. There was a decrease in the angle between the occipital and basisphenoid bones due to growth of structures external to and below the neural skull. In the one hundred twenty to one hundred fifty-day range there was a large elongation about bregmabasion due to the straightening of the cranial vault. After the growth of the skull was almost complete, there was a rise in the cranial vault related to the growth of the cervical-cranial muscles, which keep the head erect.

Pertaining to the facial skull of the rat, there is ongoing altering in size while shape remains constant. Maximum growth in the olfactory skull occurs about the nasion and fronto-ethmoidal junction and about the posterior nasal spine. The elongation correlates with the large growth of the ethmoid as compared to other areas of the olfactory skull, and this is related to the transmission of incisive masticatory forces.

The function of surrounding tissue structures may play a role in craniofacial growth and development. Sugaware et al. (1999) devised a way to study the craniofacial growth in the whole rat head transplant as a means of determining how a non-functional rat head grows and develops. Ten female Lewis rats were used. The whole heads of the rats were transplanted to the bodies of isohistogenic rats by means of microvascular anastomosis. The transplanted heads had neither scars nor moving soft tissue that could modify growth around facial bones. Since a series of different

vital stains were used, differential growth could be analyzed. This process helped to overcome some of the drawbacks of doing a cross-sectional study. Ten-day-old rats were used as the donors, while eight-week-old rats were used as the recipients. Lateral cephalograms were taken at ten, twenty, thirty, and forty days after transplantation. An advantage of this technique was that the transplanted heads could "survive" without such soft-tissue functions as mastication, breathing, and eye movement. There was no marked blood flow reduction to the craniofacial skeleton. A Possible drawback was that the transplanted heads were under the influence of adult level hormones, such as growth hormone and thyroid hormone. In addition, the change of the balancing position of the transplanted head resulted in a different effect of gravity on the head. Their results found that the craniofacial complex, consisting of the skeleton and soft tissue, grew in the absence of typical organ function and the direction and amount of craniofacial bone growth was basically self-regulated. Craniofacial bone growth appeared to be pre-programmed genetically. None of the nasomaxillary linear measurements showed any significant difference between graft and control animals. Nasomaxillary measures included facial height, facial length, and maxillary length. Respiration was regarded to play only a minor role in the developing nasomaxillary complex. It was concluded that the cranial content was unlikely to be a major determinant of neurocranial growth and that this growth was under tight genetic control. In summary the study supported the hypothesis that cranialfacial growth occurs in the absence of muscle function and brain growth.

Other studies have been conducted to try and find a correlation between

function and growth in laboratory rats. Katsaros et al. (1994) conducted a morphometric study in the anterior facial skeleton of the growing rat to test the functional masticatory influence on sutural growth. Twenty male albino Sprague-Dawley rats were used; ten receiving a normal hard diet while the other ten received a ground-up soft version diet. The experiment rats were twenty-eight days of age at the start of the study which continued for twenty-eight days. The findings suggested that by changing the diet consistency there was a reduction in masticatory force that altered morphology of the plane facial sutures. Rats fed a soft diet had sutural bony surfaces that were closer to each other, especially in the middle aspect of the snout area. The lower functional demand brought on by the softer diet applied a lower tension force on the bony elements of the upper viscerocranial complex. The hard diet sutural separation was explained as a result of increased tension in the periosteal membrane within the suture due to a higher masticatory function. The authors believed that the dimensions and morphology of the facial sutures were significantly affected by reduced masticatory function.

Ulgen et al. (1997) also studied the influence of masticatory hypofunction on craniofacial growth and development in rats. The results contradicted those reported by previous studies, and showed that there was no significant effect of masticatory hypofunction on the cranial growth and development in growing rats. Masticatory hypofunction did affect the growth and development of the maxillofacial skeleton, with twenty-one control rats were compared to fourteen experimental rats. The rats were sacrificed at ninety days of age and direct anthropometric cranial, maxillary and

mandibular measurements were made. Differences in the growth and development of the hypofunction group lead to changes in the vertical direction. The regions where statistically significant changes were found and attributed to masticatory hypofunction were as follows: total skull length, total anterior face height, lower anterior face height, ramus mandibular height and corpus mandibular height. Premaxillary length, and maxillary width were reduced, but foramen incisivum was increased. No significant differences were found between thirty days and ninety days for mean weight of the normal and hypofunction groups, with this lack of a weight difference being unique when compared to the results of those found by Katsaros et al. (1994). The lack of a difference in weights showed that there was no nutritional deficiency to create a disparity in the growth and development of the rats. Perhaps rats showing weight differences in other studies proves there was also a nutritional deficiency along with the difference in masticatory function. The combined effects could lead to differences in craniofacial growth and development.

One huge environmental effect placed on organisms are the very beings that gave them life. Itula et al. (1983) helped define the maternal influence on growth of laboratory rats. Maternal influence concerns those factors affecting the expression of phenotypic traits in offspring that are influenced by the mother, in addition to, and apart from, the direct influence of transmitted genes. The effect on the offspring is an environmental one. Two thousand and eighty-nine rats were utilized to estimate maternal influence on growth from weaning (twenty-one days) to sixteen weeks of age. Sprague-Dawley rats represented one of the strains analyzed. Estimates of

hadditive genetic effects were found to be negative at weaning and increased to positive intermediate values during post-weaning growth. The effects of maternal influence due to additive genetic effects were important at weaning and diminished at later stages of growth. There was an antagonism between maternal environment and genes affecting the offspring's growth

Inbreeding is very helpful for obtaining experimental samples, but the process does have some drawbacks. White (1972) uncovered some inbreeding effects upon growth and maternal ability in laboratory mice. A reciprocal cross-fostering technique was used to test the effects of inbreeding. Postnatal maternal performance was linearly depressed by increasing the level of inbreeding. When the more inbred suckled dams were compared to the more outbred suckled dams, it was discovered that at twelve and twenty days of age the inbred suckled young were smaller. By increasing the levels of inbreeding in the litter, the birth weight and weight at twelve, twenty-one, forty-two, and fifty-six days was depressed significantly. In the first part of the study, increased inbreeding had a linear depression of weight on both males and females. The second part of the study, inbreeding to had a curvilinear depressing weight relationship for female rats and a linear depressing weight relationship for males. The curvilinear effect on females was concluded to be due to the lack of depression until inbreeding reached levels of fifty to sixty percent, after which significant inbreeding depression developed. In comparing post-weaning traits, males were found to be significantly larger than females in all stages of growth. The differences in inbreeding effects among the sexes were slightly different, and the final

results were basically similar.

Inbreeding has been shown to have an effect on variation. Robertson (1951) reported evidence that inbreeding may cause a decline in phenotypic variation and in some cases it is known to have increased variation above the original value after several generations of brother-sister matings. There are four possible reasons for this occurrence. First, the variation due to recessive genes at low frequencies will increase with inbreeding. Second, natural selection for heterozygotes may be opposing the trend towards homozygosis produced by inbreeding. Third, the greater part of the variation is environmental in origin and therefore will not be affected by inbreeding. The changes in genetic variance may be undetectable against the background of the environmental variance. Forth, the inbred lines may differ form the random-bred stock in their response to environmental changes.

Craniofacial Growth: The Importance of Genetics and Function

The question of the impact of genetics verses environment on craniofacial growth and development is of great interest to orthodontic practitioners. Brodie contended that the development of the skull, face and dentition is based on an inheritable growth pattern. The genetic factors involved acted separately on different components of the craniofacial complex, but the identity of these components and their specific mode of genetic control remains largely unknown (Brodie, 1941). This craniofacial pattern is set early in life and will persist through time. Many studies have tried to piece together the puzzle of control mechanisms for the final form of the human face and skull. Moss (1969) described the theory of the functional matrix in

detail. In Moss' view, the periosteal and capsular matrices play primary morphogenetic roles in skeletal tissue growth. Cranial growth is due to the combination of both periosteal and capsular matrix activities. Spatial translation and changes in form allow growth to occur. Capsular matrices are regarded as volumes enclosed and protected by both the neurocranial and the orofacial capsules. Muscles and teeth help construct the periosteal matrices. The neural mass is what makes up the capsular matrix in the neural skull. The functioning space of the oronasopharyngeal cavity is the matrix in the facial skull. The net effect of the periosteal matrix is to alter the size and shape of their respective skeletal units. This occurs by means of the processes of osseous deposition and resorption or by cartilaginous or fibrous tissue multiplication. The capsular matrices alter the volume of the capsules within which the functional cranial components are embedded. This causes a passive translation of these cranial components in space. Under this theory the processes of functional matrices give the skull and face their final size and shape. There are three kinds of craniofacial matrixes; skeletal (osseous, cartilaginous, and tendinous), tissue (glands, teeth, organs, supporting tissue, vessels, and nerves), and spatial (nasal, oral, and pharyngeal cavities). Many functions are served by the craniofacial complex. An environmental need leads to a functional change that ends in structural adaptation. This is one way that evolutionary selection of specific craniofacial size and shape may be described by using the functional matrix theory.

Moss (1997) expanded on the interaction of the functional matrix and genomic ideas to help explain their roles in growth and development. Should one

believe that every biologic structure is of specific size, shape, and position because of a built-in genetic code has specified the instructions for such end products? This would imply that there is nothing that will make a phenotypic change possible because the genotype has ingrained within its self a predestined map for expression. Moss suggested that epigenetic stimuli, created by operations of related functional matrices and their skeletal unit adaptive responses, created the new information sequentially, as ontogenesis proceeds. Ontogenesis exhibits developmental cascades, with multiple branching points where decisions are made between alternate developmental pathways. Some epigenetic stimuli are believed to be responsible for the decisions and not some predetermined genetically encoded information.

McNamara (1980) stated that, when applying the idea of function and structural changes to patients, it is important to understand that functional adaptations occur rapidly, but are gradually followed by structural adaptations. He believed there was an intimate relationship between the functional and structural components of the craniofacial region. Even when there was an existing malocclusion, this condition reflected a state of balance regardless of the nature of the individual components. The only way to achieve a stable result of treatment was to have the resultant skeletal and dentoalveolar configuration exist in harmony with the associated musculature and other soft tissue. Theoretically, McNamara also felt there should be no need to retain mechanically a structural relationship which has been achieved concomitant with the elimination of compensatory muscle function.

Can the craniofacial and the facial skeletons be broken down into separate

areas of study? Lavelle (1978) published a study that stated the craniofacial and facial skeletons closely parallel one another and are similar to that of the cranial base. The final data described the degree of association between one region of the skull and another. It would be a mistake to regard the facial skeleton in isolation relative to the craniofacial skeleton as a whole or the cranial base. The brain case, facial skeleton, and various occlusal categories have a complex interaction. The location and orientation of the facial skeleton is partially dependent upon the growth and development of the brain and its extensions. This is because the facial skeleton is located within the cranial base flexure. A brachycephalic skull form has a closed cranial base flexure. This leads to the nasomaxillary complex being located in a more posterior and superior location than a dolichocephalic nasomaxillary complex. The mandible is then located more forward and upward in brachycephalics. This points out a complex issue. A genotype may allow for the phenotypic expression of an area, but that same area is also dependent on the size, shape, and position of other structures near by. One must separate out the intrinsic issues for an area from forces acting from some other locations that have their own genotypes and influences.

Hiernaux (1970) reported on the influences of environment and genetics upon growth. Heredity and environment both play a role on growth and the adult body form, and human gene pools tend to come to a dynamic equilibrium with the environment, namely climate. By comparing genetically similar groups, born and reared in different environments, it is possible to show the influence of environment on phenotypic expression of the growth potential. It has been stated that the period of

maximum sensitivity to irreversible deleterious environmental effects on growth is around birth, with the female being more resistant to such effects. Realizing that the environment might be more responsible for traits having a large phenotypic range, certain traits may be more affected by environmental constraints. A trait that has little phenotypic variation, when subjected to a wide range of environmental dissimilarities, may be a trait under mostly genetic control.

It is essential to understand whether or not there is transmission of craniofacial dimensions. Devor (1987) conducted an analysis of eleven craniofacial dimensions in four different human populations with the four samples coming from published literature on nuclear family correlations. Age and sex were removed from the raw data by regression. Analysis of the data indicated that human craniofacial dimensions exhibited relatively constant transmissibility at an intermediate level in the heritability value range of 0.45% - 0.60%. Most twin studies show levels of heritability slightly above 0.60%. In this range one can assume that genetic factors are at least twice as important an nongenetic factors in determining the observed variation of the craniofacial traits. By using twins in studies it may be possible that some residual twin resemblance may be operating that might not be genetic. The role of genetics in determining the range of craniofacial dimensions was reported as being large. The author cautioned on using soft tissue traits, being aware of strong gene/environment interactions, while complex gene/gene interactions may have an important role as well.

Krogman (1973) compiled an appraisal of facial growth and development.

He stated that man has inherited a complex of craniofacial bones and tissues hundreds of millions of years old. This complex was at the cephalic end of primitive fish. The base of the brain-box was cartilage and gave basic support to the cephalic complex. This cartilage became bone and formed the cranial base and some facial midline structures. About two hundred million years ago, a Devonian fish changed food habits and burrowed into the mud, and the facial skin became bony hard for the purpose of protection. The current human craniofacial complex manifests its ancient heritage with cartilaginous bone (cranial base and nasal septum) and membranous bone (cranial vault, most of the facial skeleton). The bones preformed in cartilage came before those derived from the skin. Through human embryology it is possible to trace parts of the face to the ectodermal neural crest cells originating from neural folds at the margins of the neural plate. Most craniofacial trait complexes involve multifactorial inheritance patterns. Krogman stated that the degree of overbite, overjet and interincisal angle may be familial. The growth of the facial skeleton and the linear growth of the long bones share an orderly pattern.

Determining the heritability of attained growth in the human face was examined by Hunter et al. (1976). In order to avoid data being obscured by analyzing growing children, only families with both parents and two or more children twenty-one years or older were included in the study. The adult offspring were compared with both parents using lateral cephalograms. The statistical relationships were strongest between father and offspring and were consistently highest between the same measurements in parents and offspring. Mandibular dimensions showed

particular strong relationships between fathers and offspring while the statistical relationships between mothers and offspring were lower. Only the relationship of facial height between mother and their offspring was similar to the father-offspring relationship. This study used cross-generation relationships of attained growth. Regression equations using parental facial dimensions were of questionable clinical value for prediction of the adult dimensions in offspring, but the authors felt their data supported the hypothesis that facial dimensions were largely inherited.

Other studies have shown that familial patterns do exist in the human craniofacial complex. Harris et al. (1973) attempted to describe genetic factors in the shape of the craniofacial complex. Almost all of the dento-facial characteristics of interest to the orthodontist are polygenic and continuously variable. It was noted that relationships between localized areas might show hereditary resemblances more consistently than those involving larger and more wide spread areas since different genes might affect different parts of the skull. Their study compared the frequency of concordance of small bony contours between related and nonrelated individuals. If the two individuals of a pair showed concordance for a contour, then it was assumed that the individuals shared more common genes for the bony area than if the contours were not superimposable. In every subgroup and for each of the contours considered, the proportion of concordant measurements were always higher for sibs than for the non-related controls. Even though all the contours showed some familial aggregation, the strength of the aggregation was not the same for all the contours studied. It was discovered that the shape of the palate had more functional

mechanisms exerting control rather than purely genetic factors. The posterior border of the ramus also showed a weak familial pattern. This type of research indicates an existence of familial similarities in certain components of the craniofacial complex.

Nakata et al. (1973) attempted to describe the craniofacial complex by a few meaningful factors and to detect genetic variability of these factor scores by the covariance of related subjects. Thirty-three cephalometric variables and height were measured on six hundred and thirty individuals from one hundred and fifty-seven families. It was found that most of the variation could be explained by nine independent factors. They were anterior lower face height, mandibular depth, anterior cranial inclination, posterior cranial inclination, anterior maxillary height, posterior cranial base, mandibular ramus height, maxillary depth and anterior cranial length. The authors reported highest heritability for a lower facial height factor derived from radiographic measurement of twin and parent-offspring pairs. It was suggested that it might be more appropriate to examine the relevant variables as multivariant factors rather than to treat them as independent traits. The findings suggested that dominance effects exist for some factors but not for others. For some traits both genetic and environmental factors influenced the same morphogenic pathway. The authors also suggested that each factor, measured by a linear combination of a set of variables, could have resulted from the interaction of independent sets of genes with environment.

Family resemblance for components of craniofacial size and shape was investigated by Byard et al. (1985). The study consisted of eight principal

components extracted from thirty anthropometric measurements of the craniofacial complex. One thousand seven hundred and sixty-three individuals from three hundred and ninety-nine families were investigated. The eight components studied were cranial size, craniofacial breadth, nasal height, ocular breadth, lower facial breadth, mouth height, upper facial height, and ear dimensions. It could not be determined by this study if the genetic or common environmental factors influencing variation for each component were themselves independent. The null hypothesis of no assortative mating was rejected for the nasal height component. Other hypotheses that were rejected included no common sibling environmental effect for cranial size, craniofacial breadth, and nasal height component. Finally, the hypothesis that transmission of determinates from mother and father were equal was rejected for components corresponding to craniofacial breadth and upper facial height. This finding indicated than autosomal polygenic inheritance was not an independent acting mechanism, thus implicating some effect of familial environment. Transmissibility was shown to be higher for components related to cranial size and facial height than for those related to facial breadth or ear dimensions. Byard et al. (1984) also stated that sibling correlations were generally lowest during the first year of life and highest as adulthood approached. Most correlations for cranial measurements of siblings were significantly greater than zero and exceeded 0.5, the maximum value expected for a completely heritable polygenic trait. The data produced values in excess of what was expected for genetic similarity, thus confirming that a common sibling environment contributed to sibling resemblance for craniofacial dimensions in the

sample. The cranial dimensions sella-nasion, sella-vertex, and basion-nasion had the highest correlations averaged across all ages.

Many human faces show much similarity, yet there also exists variability expressed as size and shape differences in adult craniofacial complexes. Similar familiar patterns of facial form and development are abundant in the general population. Major morphologic types of faces behave differently in terms of growth and treatment responses. Cleall et al. (1979) conducted a study on craniofacial morphology using a component analysis technique. Six hundred and twenty-two subjects were studied at age eight years through adulthood. Thirty-three landmarks on lateral cephalograms were used to obtain fifty-six linear and angular variables relating to both hard and soft tissue structures. A series of six major factors were determined; 1) vertical facial components, 2) anteroposterior aspects of facial morphology, 3) midfacial and dental protrusion, 4) relationship of the mandible and the dentition to the profile, 5) horizontal base-line relationships, and 6) maxillary incisor relationships. Factors one, two and three demonstrated specific age changes while factors one and two showed statistically reliable changes resulting from treatment and/or growth. This type of study not only helps to define the morphology of the craniofacial complex, and highlights areas amenable to stable treatment related changes.

A study by Arya et al. (1973) stressed the genetic variability of craniofacial dimensions. Twenty-six pairs of monozygotic and sixteen lide-sexed pairs of dizygotic twins were investigated using lateral cephalograms. The genetic variability

of six mandibular dimensions and three craniofacial dimensions were examined. Mandibular dimensions demonstrated a greater component of genetic variability than that demonstrated by craniofacial dimensions. The genetic component was predominant in the variability of all the dimensions measured except the dimension of left gonion-right gonion in which the environmental component of the variability was predominant. The length and the width dimensions of the mandible appeared to have a greater component of genetic variability than did the height dimensions. This indicated that the height dimension of the mandible was more susceptible to such environmental influences as therapeutic procedures. Once again a practitioner has scientific evidence from which to base treatment planning protocol for effective correction of specific problem areas.

Malocclusion is a result of environment and genetics. Clinicians view the phenotypic expression of a mixture of the environment and genetic interactions. Local environmental forces such as thumb sucking, tongue thrust, premature loss of teeth from to caries or trauma, and facial injuries can result in a malocclusion. Most malocclusion, however, is the direct result of heredity. Angle's class II molar relationship (mandibular dentition in a retruded relation to the maxillary dentition), Angle's class III molar relationship (with the prognathic mandible), and dental crowding (tooth size-jaw size discrepancy) are representations of malocclusion that have hereditability, or a familial basis. Modern clinicians should evaluate familiar patterns for all patients in order to obtain a better understanding of the presenting condition, its origin and treatment.

MATERIALS AND METHODS

The present study was preceded by two investigations. Moullas et al., in 1999, analyzed radiographs (lateral and ventrodorsal) and dry skulls of eight different rat strains. The eight inbred strains (four males and four females in each strain, total sample of sixty-four) were Sprague-Dawley, Brown Norway, Lewis, Fisher-344, Wistar-Kyoto, Long-Evans, Holtxman, and Buffalo. All strains were obtained from the same vendor to ensure that environmental conditions would be the same. For example, Brown Norway strains were certified as having undergone at least twenty generations of brother-sister mating. This resulted in animals that were basically genetic twins. All of the specimens in the sample were older than six months. The experimentation was conducted at the Case Western Reserve University Department of Orthodontics. In particular, two strains were selected for study because they showed facial types that are opposites. The Sprague-Dawley strain is characterized as dolichocephalec, while the Brown Norway strain is brachycephalic. Strain variation appears to be large enough to study the variance of selected traits into genotypic and non-genotypic components.

In 1999, Park et al. analyzed four month old F1 offspring obtained from a Sprague-Dawley and Brown Norway cross courtesy of Drs. Kingman Strohl and Agnes Thomas (Veterans Affairs Medical Center, Cleveland, Ohio). The Sprague-Dawley strain was characterized as having a dolichocephalic head form. It possessed

a long, skinny face and skull. The Brown Norway strain was characterized as having a brachycephalic head form. It possessed a short, wide face and skull. (Moullas et al. 1999). The two strains were selected due to their opposite craniofacial morphology. The Sprague-Dawley and Brown Norway rat strains were obtained from a single vendor (Harlan, Indianapolis, Indiana). The two strains were used as the F0 founder generation. They were crossed to produce an F1 generation. An F1 generation is defined as the first filial generation produced by crossing two parental lines. Direct anatomical skull measurements of the results of hybrids were compared to the F0 parents, and three phenotypic patterns were reported. The first showed large strain variation and gender differences, while the values for the F1 generation were between the values measured for the parents. The second pattern had less strain variation and larger gender differences. The craniofacial measurements of the F1 animals were smaller than the parents. Sexual dimorphism was present in this category. The third phenotypic group showed little difference between the two generations of rats. Length measurements on the craniofacial complex fell under the first pattern, while width and height fell under the second pattern. Tooth measurements were found in the third group. The findings suggested various inheritance patterns for these craniofacial morphometric traits in rats with the F1 hybrid generation providing valuable information on how phenotypes might be related to genotypes. Important genetic information can be dissected from analysis of an F2 generation because individual genotypes will vary. The F2 generation is defined as the second filial generation produced by selfing or intercrossing the F1 generation.

The present study compared an F2 sample within its generation as well as between generations to the F1 and F0 generations. Seventy-seven total F2 dry skulls and right femurs were obtained in a manner identical to the projects of Moullas and Park. The F0 generation ages ranged from eight to twelve months old. Four months to fourteen and a half months was the age range in the F1 generation. All of the F2 generation rats were four months old. The F1 generation responsible for creating the F2 generation consisted of five male rats and five female rats. The F0 founding generation responsible for rearing the F2 pedigree consisted of two Sprague-Dawley males and four Brown Norway females. The present investigator measured the dry skulls and femurs of the F0 and F1 generations, and measured the same dimensions in the F2 generation to eliminate any possible inter-observer error. Other specimens existed for the F0 and F1 generation that were not direct ancestors in this specific pedigree. They were analyzed and used for a larger between generation comparisons.

A subcutaneous implanted identification marker was placed in each animal prior to breeding. It was used for animal identification as well for monitoring of body temperature. The marker was placed by injection under halothane anesthesia. All animals were housed at Room K-27, Wade Park Unit, Veterans Affairs Medical Center, Cleveland, Ohio. The caged animals received appropriate bedding with food and water *ad libitum*. A method of carbon dioixide inhalation was used at the time of animal sacrifice. Anthropometric data (body length, measured from nose to anus, and body weight) were recorded at the time of specimen sacrifice.

The flesh skulls and right femurs were dissected and placed into an aquarium

housing flesh-eating beetles maintained by Dr. Timothy Matson at the Cleveland Museum of Natural History, Cleveland, Ohio. This process allowed for the successful removal of all the soft tissue from the bony skeleton without damaging the fragile bony structures. The specimens received a final soak in ammonia followed by a twenty-four hour drying period. Before each parent generation was sacrificed they were bred to ensure offspring for future specified studies.

A microcaliper (Digimatic, Mitutoyo Co., Tokyo, Japan) was used to measure twenty craniofacial linear areas on each skull. The same linear dimensions were performed in the F1 and F0 generations. Seven measurements were performed on the mandible. The maxilla was defined by six linear measurements. The cranium was analyzed by seven measurements. The femur bone was measured in length. The following anatomic landmarks were used to study the phenotypic traits of the craniofacial skeleton. These landmark points are illustrated on the diagrams in Figures 1 and 2.

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

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

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

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

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

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

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

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

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

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

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

zg The most lateral point on the zgomatic process

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

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

o The most posterior point on the external occipital protuberance

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

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

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

n The most anterior point on the nasal bone

Based on the locations of the above mentioned landmarks, linear measurements were obtained. Seven linear variables defined the mandible, six were constructed for the maxilla and the cranium was defined by seven linear

measurements. Figure 3 depicts the variables measured in a ventrodorsal view of a rat skull, while Figure 4 displays the variables measured in a lateral view of a rat skull (see appendix).

The mean, standard error and standard deviation were calculated for each of the F0, F1 and F2 generations. All values were determined in unadjusted and adjusted forms. Excel and SPSS statistical processing programs were employed to determine the means, standard errors and standard deviations for all twenty-one variables. Variances were calculated for the F1 and F2 rats. The standard deviation was squared in order to get the variance for each trait. Heritability values were determined for each of the twenty-one variables, unadjusted and adjusted, in the F2 generation. In order to calculate heritability estimates the following formula was used:

$$\text{Heritability} = \frac{\text{Variance(F2 generation)-Variance(F1 generation)}}{\text{Variance(F2 generation)}}$$

The variance in the F2 generation was due to genetic and environmental determinates. The variance in the F1 generation was due to environmental causes. The previous formula represented genetic and environmental influence in the F2 generation minus the environmental influence in the F1 generation all divided by the genetic and environmental influence of the F2 generation. When the variance in the F2 generation became larger than the variance in the F1 generation a positive heritability estimate was achieved.

A heritability value of plus one implied that a trait was estimated to be under total genetic control. A value closer to zero was interpreted as a trait regarded to be under very little genetic control and more influenced by the environment. The

twenty-one variables were also analyzed by adjusting for age, sex and litter size. This was done to exclude confounding factors due to differences in age, sex and litter size between and within the different rat strains. In order to get adjusted values it was necessary to create syntax programs using SPSS and applying them to the raw data. Figure 5 displays the SPSS syntax file program written in order to create residual values. Figure 6 displays the SPSS syntax file program used for generating unadjusted and adjusted standard deviations, means and standard errors. This approach provided the necessary adjusted standard deviations required to calculate the adjusted heritability values.

In order to assure reliability of the measurement technique, ten rat skulls and femurs were measured for a second time. A table of random digits was employed to select the ten specimen numbers. SPSS statistical package was employed to generate a reliability analysis. Table 5 displays the reliability results for each variable. The following formula was used to calculate reliability:

$$\frac{MSb - MSw \times 100}{MSb + Msw} = \text{reliability}$$

where MSb equals Mean Square between and MSw equals Mean Square within.

RESULTS

The purpose of this study was to determine which craniofacial characteristics in a cross between Brown Norway and Sprague-Dawley rat strains were heritable. The two strains possessed opposing craniofacial morphologic characteristics. Each of the twenty-one variables were measured in the parental generation Brown Norway and Sprague-Dawley rats, and in the F1 and F2 rat generations. The raw data can be found in Tables 13-24 (see appendix).

The variables were adjusted for age, sex and litter size. These three adjustment factors were considered independent variables and accounted for a percentage of the variation in each measurement. The adjustment factors were considered independent variables and under the control of the present investigator. The twenty-one variables measured in this project were considered dependent variables. Differences in age, sex and litter size led to large variations in the F1 generation. A stepwise regression analysis was used to try and explain variation in the twenty-one variables. This regression first put into the model the single independent variable that accounted for most of the variability, and then successively inserted the variable that explained most of the remaining (residual) variability. Predetermined significance levels were used to terminate the procedure if one, or all, of the independent variables failed to explain the variability in the twenty-one dependent variables. The final result was a prediction model and not necessarily a

model for the causation of variability in the dependent variable This was similar to that of Elston (1994).

What actually was determined was broad sense heritability, which measured the extent to which a phenotype was determined by genotype. Heritability values were computed for each of the twenty-one variables. Table 4 breaks down the heritability values into unadjusted and adjusted results. Heritability values of "one" represented variables with little to no variation due to environment, while heritability values of "zero" represented variables totally due to environment. Ten of the twenty-one variables had heritability values in the positive range, in either the unadjusted or adjusted forms. Two variables were mandibular measurements, and two variables were maxillary measurements. The remaining six variables were cranial measurements. However not all ten variables possessed a high enough positive heritability estimate to be considered heritable.

Variable A5 measured bicondylar width and had values ranging form 0.24 adjusted to 0.38 unadjusted. Variable A6 measured bicoronoid width. Its heritability value was about 0.28 in both forms. Variable B2 measured overall palatal length and was negative in the unadjusted state and only 0.003 in the adjusted form. This was not positive enough to be classified in the heritable grouping. Maxillary tooth row length, represented by variable B5, ranged from 0.272 unadjusted to 0.436 adjusted. Cranial height, depicted by C1, was negative in the unadjusted form and 0.245 in the adjusted form. C2, which measured overall cranial length, was another variable that was negative in one state and to small to be considered heritable in the other state.

The C3 variable was unique because in the unadjusted form it was 0.369 and had no adjusted value. This variable measured parietal bone length. Variable C4 was a measure of frontal bone length. Its heritablity range went from 0.183 unadjusted to 0.449 adjusted. Variable C6 was a measure of occipital bone length. The heritability ranged from 0.228 unadjusted to 0.342 adjusted. The variable C7 was a measure of cranial width. It had heritability values range from 0.087 unadjusted to 0.229 adjsuted.

In the unadjusted form variable A5, which measured bicondylar width had the highest unadjusted heritability value (0.379) of all twenty-one variables. Variable C4 which measured frontal bone length, had the highest adjusted heritability number (0.449). Three variables (B2, C1, and C2) had heritability values that were negative before adjustment, positive following adjustment. C1 went from a negative value of 0.05 all the way to a positive 0.245.

In order to ensure that measurement of these traits were reliable; a second measurement of ten randomly picked specimens was conducted. The results are found in Table 5. All but three of the twenty-one variables had reliability values above 0.90. Of the eight variables considered to be heritable, only one fell below 0.90; C6, measuring occipital bone length, had a value of 0.88. Ensuring repeatability of the measurements reduced the phenotypic variance due to random environmental variation. This increased the accuracy of the heritability measures because measurement error was determined not to be adding to the variance.

Table 6 lists each variable, what it measured, the adjusted value along with

Soon Jung Park's classifications. No pattern between Park's groupings and the heritability values could be concluded.

Each of the twenty-one variables were adjusted for age, sex and litter size. This was done by a stepwise procedure using an SPSS statistical syntax program. The adjusted values were found using a linear regression model. This method allowed for an incorporation of factors to obtain the best fit. Table 7 indicates how each variable fit with the factors to be used in adjusting the data. A1, B1, B2, B6, C5 and C7 were adjusted for age, sex and litter size. C2, C6 and femur were adjusted for sex and litter size. A3, A4, A7, B4 and C1 were adjusted for age and sex. A2, A5, A6, B3, B5 and C4 were adjusted only for sex. C3 could not be adjusted by any of the three factors.

Tables 8 through 12 show the unadjusted and adjusted measured means plus and minus their standard errors for the three generations for each of the twenty-one variables. In order to create a normal distribution, some outlier data was omitted intentionally from this data. When necessary individual rat variable results, or in some cases, total rat measurements were omitted. This explains the reason why some of the sample numbers do not add up to the total raw data numbers. The F0 generation was divided into two strains chosen for their opposite craniofacial forms. One parent generation (P1SD) was labeled to represent the F0 Sprague-Dawley strain. The second parent generation (P1BN) represented the F0 Brown Norway generation.

Figures 7 through 9 show the unadjusted means for all twenty-one variables. Figures 10 through 12 show the adjusted means for the twenty-one variables. All

generations, including F0 divided between the two parent strains, are represented. Theoretically, if generations were bred to infinity, then the means of the ensuing generations would approach the mid-point between the means of the parent F0 generations. Displaying the means of the generations, showing the F0 generation broken down into P1 and P2, allowed for the detection of heterosis. Heterosis occurred when the F1 offspring means were outside the mean range of the two parent strains. The unadjusted mandibular variables A1, A2, A6, and A7 were above the parent mean range. Variables A3 and A4 fell below the parent range. For the adjusted mandibular variables, only A7 was above the parent range, while A3 and A4 fell below the parent range. B6 was the only maxillary unadjusted mean above the parent range, and B4 was below the parent range. Adjusted maxillary variable B4 scored above the range while no variables fell below the range. C7 was the only unadjusted cranial variable outside the mean range of the parent generation. It scored above the range and no other mean, adjusted or unadjsuted, fell outside the parent mean range for cranial measurements.

Tables 13 through 24 display the raw data gathered in this study. Rat ID numbers were used to identify the specimens. All generations were represented. F0 was represented with a zero, F1 with a number one and F2 with a number two in the generation column. Litter size described how many siblings the identified rat possessed; for example, a litter size number of eight defined seven males or females plus the rat in question. Males were labeled with a number one and females with a number two. The age in months was also defined.

DISCUSSION

The Brown Norway strain has craniofacial dimensions considered to be brachycephalic. This is in contrast with the Sprague-Dawley having a dolichocephalic craniofacial complex (Moullas et al., 1999). It is possible to study quantitative traits with animal models. When purebred lines show phenotypic traits with non-overlapping distributions, it is possible to form crosses between parental lines and among the resulting progeny. Crossing the purebred lines may produce offspring that have an intermediate phenotype, although all three groups may still have a large variance of phenotypic expression. Even though the phenotypic means of the parental lines and the offspring differ significantly, their variances will be similar. The variance can be attributed to nongenetic causes because all three groups are genetically homogeneous, since parental lines are homozygous at all loci effecting the specific phenotype and all offspring are heterozygous. When the progeny individuals are selfed the observed variance among their offspring will be much larger than the two prior generation's variance. By using this increased variance in the third generation, along with the difference in phenotypic means of the two parental lines, it is possible to estimate the number of independent loci influencing a quantitative trait (Suzuki, el al., 1986).

The variance in the F2 generation was due to genetic and environmental determinates. The variance in the F1 generation was due mainly to environmental

reasons. The previous heritability formula represented genetic and environmental influence in the F2 generation minus the environmental influence in the F1 generation all divided by the genetic and environmental influence of the F2 generation. When the variance in the F2 generation became larger than the variance in the F1 generation a positive heritability estimate was achieved.

Several rat craniofacial skeleton growth studies have reported that skull growth is completed, or at least nearly completed, by the end of the fourth month (Spence 1940; Asling and Frank 1963; Moore 1966; Wright et al. 1966). This is why the youngest rats in this study were no less than four months old. The sample size was one hundred and fifty-one adult rats. Rats were chosen for this project because they have high reproductive rates, mature quickly and have short life spans. Thirty-five human years is equal to about one year for a rat when comparing physiology. Rats also lend themselves well to creating inbreed strains. Rats were chosen over mice due to the need for a larger size in order to quantify genetic factors in the craniofacial complex (Nonanka and Nakata 1984)

This study found similar results to previous studies that showed the nasomaxillary complex morphology is largely due to environment and not genetics. Previous investigators (Atchley 1983; Arya 1973) have reported that the length and width dimensions of the rat mandible are highly heritable traits, while height was not heritable and found to be a measure of muscle insertion. This study failed to show a correlation in mandibular length measurements, although it did find genetic effects in the width dimension. Bicondylar width and bicoronoidal width were both found to

have good heritability values. A possible reason for this study not finding mandibular length to be heritable could be the selection of landmarks and variables employed. Smaller linear units might have shown a positive heritability correlation. The landmark imn, defining the anterior limit of variable A1, which measured mandibular body length, received the lowest reliability value of all the landmarks used in this study. Previous findings of little genetic control on mandibular height were supported by this study. Yanagita et al. (1992) concluded that changes in width and length of the craniofacial complex were strongly influenced by genetic effects. Of the eight variables found by the present investigator to have high heritability values, four were length measures, three were width measures and one (cranial height) was a measurement of height. Harris and Johnson (1991) reported that dental conponents have low heritability. This is a case, the present study found some conflicting results. Maxillary tooth row length was found to have good heritability values in both the unadjusted and adjusted forms.

The majority of the measures were directly (5/7) or indirectly (2/7) related to the size and shape of the cranium. The extracranial structures may be under more functional demands of mastication and breathing. The posterior morphology of the mandible is related to the size and shape of the cranium in order to function correctly. The genetic control resulting on the size and shape of the cranium may also exert its effect on the posterior mandibular width dimension. This may contribute to the heritability values for the mandibular variables bicondylar and bicoronoid width.

One should appreciate that this study was conducted between two rat strains

considered to have opposite craniofacial forms. The strains were considered to have cephalic measures on the opposite ends of the spectrum. Although this might have been true for some variables, it was not true for others. Had different strains been analyzed, different variables may have scored high heritability values compared to the present findings.

CONCLUSIONS

This study of cross between Brown Norway and Sprague-Dawley rats, determined that some craniofacial characteristics showed heritability of craniofacial morphology. Eight of twenty-one variables analyzed were found to demonstrate high heritability values. Bicondylar width and bicoronoid width were two mandibular traits considered to have high heritability values. Maxillary tooth row length was the only maxillary variable to show heritability. The cranium had five variables found to demonstrate heritability: cranial height, parietal bone length, frontal bone length, occipital bone length and cranial width.

Of the three dimensions of space, only one height variable, total cranial height, indicated heritability. Three width variables: bicondylar width, bicoronoid width and cranial width revealed high heritability estimations. The four length variables that showed high heritability values were: maxillary tooth row length, parietal bone length, frontal bone length and occipital bone length. This study found that the majority of the cranial measurements possessed high heritability values, excluding those measurements that described the nasal complex, which might have been influenced more by the environmentally controlled functional airway rather than genetic elements of control. The majority of heritable measures were directly (5/7) or indirectly (2/7) related to the size and shape of the cranium. Hence, the differences in cranial morphology suggest a genetic cause, whereas, morphologic variation in

extracranial structures appear be influenced to a greater degree by the functional demands of breathing and mastication

All individuals within an inbred strain are genetically identical. In constrat, the F1 animals were identical and heterozygous for parental genotype. F2 hybrids were also be heterozygous, but not homozygous with each other. This study found eight phenotypic traits might serve a future gene-targeted approach in detecting genes responsible for predicted heritable traits. This study took the first steps of characterizing the phenotypes of two inbred rat strains and identifying strain variations of particular phenotypes.

APPENDIX

TABLE 1.—Mandibular variables A1-A7, defining landmarks and anatomic structures measured

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

TABLE 2.-- Maxillary variables B1-B6, defining landmarks and anatomic structures measured

Variables	Landmarks employed	Measures
B1	imx-um	Premaxilla length
B2	imx-pns	Overall palatal length
B3	um-um	Palatal width
B4	mx-mx	Maxillary width
B5	dum-um	maxillary tooth row length
B6	zg-zg	Width of zygomatic arch

TABLE 3.-- Cranial variables C1-C7, defining landmarks and anatomic structures measured

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

TABLE 4--Unadjusted and Adjusted Heritability Values

Mandible			Maxilla		
Var	Unadj	Adj	Var	Unadj	Adj
A1	-0.3	-1.11	B1	-0.62	-0.65
A2	-0.37	-0.21	B2	-0.28	0.003
A3	-0.33	-0.32	B3	-0.16	-0.15
A4	-0.87	-0.78	B4	-0.48	-0.42
A5	0.379	0.243	B5	0.272	0.436
A6	0.279	0.285	B6	-0.85	-1.1
A7	-0.13	-0.05			
Cranium			Femur Length		
Var	Unadj	Adj	Var	Unadj	Adj
C1	-0.05	0.245	FEM	-0.41	-0.73
C2	-0.17	0.069			
C3	0.369	****			
C4	0.183	0.449			
C5	-0.21	-0.79			
C6	0.228	0.342			
C7	0.087	0.229			

TABLE 5.--Reliability Values for: Mandibular(A1-A7), Maxillary(B1-B6) Cranium(C1-C7), and Femur Measurements

	A1	A2	A3	A4	A5	A6	A7
Rel	0.66	0.99	0.91	0.89	0.9	0.95	0.93
	B1	B2	B3	B4	B5	B6	
Rel	0.96	0.98	0.92	0.99	0.96	0.99	
	C1	C2	C3	C4	C5	C6	C7
Rel	0.98	0.97	0.94	0.99	0.98	0.88	0.99
	Femur						
Rel	0.99						

TABLE 6--Variable, adjusted heritability values and Soon Jung Park's Groupings

Variable	Area Measured	Heritability	Park Group
A1	Mand Body Length	-1.108	I
A2	Mand Body Length W/O Diastema	-0.207	None
A3	Mand Ramus Height (Condyle)	-0.322	II
A4	Mand Ramus Height (Coronoid)	-0.783	II
A5	**Bicondylar Width**	**0.243**	II
A6	**Bicoronoidal Width**	**0.285**	II
A7	Mand Teeth Row Length	-0.052	III
B1	Length of Premaxilla	0.655	I
B2	Overall Palatal Length	0.003	I
B3	Palatal Width	-0.155	III
B4	Maxillary Width	-0.423	II
B5	**Maxillary Teeth Row Length**	**0.436**	III
B6	Width of Zygomatic Arch	-1.097	II
C1	**Cranial Height**	**0.245**	II
C2	**Overall Cranial Length**	**0.07**	I
C3	Parietal Bone Length	No Value	I
C4	**Frontal Bone Length**	**0.449**	I
C5	Nasal Bone Length	-0.785	I
C6	**Occipital Bone Length**	**0.342**	I
C7	**Cranial Width**	**0.229**	II
Femur	Length of Femur Bone	-0.727	None

TABLE 7—Adjustments selected for each variable

	Adjustment		
Var	Age	Sex	Litter
A1	yes	yes	yes
A2	no	yes	no
A3	yes	yes	no
A4	yes	yes	no
A5	no	yes	no
A6	no	yes	no
A7	yes	yes	no
B1	yes	yes	yes
B2	yes	yes	yes
B3	no	yes	no
B4	yes	yes	no
B5	no	yes	no
B6	yes	yes	yes
C1	yes	yes	no
C2	no	yes	yes
C3	no	no	no
C4	no	yes	no
C5	yes	yes	yes
C6	no	yes	yes
C7	yes	yes	yes
Fem	no	yes	yes

TABLE 8.--Unadjusted and adjusted means
± standard errors, all three generations: variables A1-A5

Var	Gen	Stat	N	Mean	± S.E.
A1	P1 SD	Unadj	6	27.09364	0.371381
		Adj	6	27.58966	0.320572
	P2 BN	Unadj	5	25.9425	0.352323
		Adj	5	26.51942	0.544258
	F1	Unadj	45	27.36667	0.187043
		Adj	45	27.11667	0.131904
	F2	Unadj	75	26.73933	0.1272
		Adj	75	26.8851	0.093808
A2	P1 SD	Unadj	11	19.50545	0.286757
		Adj	11	20.24149	0.208093
	P2 BN	Unadj	8	19.77	0.312381
		Adj	8	19.85103	0.216293
	F1	Unadj	45	20.54378	0.166722
		Adj	45	20.22547	0.099396
	F2	Unadj	75	20.02787	0.110475
		Adj	76	20.2162	0.076195
A3	P1 SD	Unadj	10	12.007	0.254855
		Adj	10	11.36319	0.100649
	P2 BN	Unadj	7	12.17286	0.249129
		Adj	7	11.36276	0.10319
	F1	Unadj	45	11.14867	0.100622
		Adj	45	11.05938	0.070406
	F2	Unadj	76	10.8464	0.033647
		Adj	76	10.8464	0.033647
A4	P1 SD	Unadj	10	14.98273	0.261305
		Adj	10	14.97811	0.168727
	P2 BN	Unadj	8	15.26	0.204599
		Adj	8	14.88786	0.166888
	F1	Unadj	45	14.76422	0.143526
		Adj	45	14.65552	0.098251
	F2	Unadj	76	14.31368	0.080658
		Adj	76	14.41847	0.057756
A5	P1 SD	Unadj	11	15.27091	0.563701
		Adj	11	15.684	0.05875
	P2 BN	Unadj	8	16.31875	0.522362
		Adj	8	15.57376	0.061064
	F1	Unadj	40	15.413	0.09111
		Adj	40	15.67948	0.028062
	F2	Unadj	75	15.8968	0.084424
		Adj	75	15.67686	0.021512

TABLE 9.--Unadjusted and adjusted means ± standard errors, all three generation: variables A6-B3

Var	Gen	Stat	N	Mean	± S.E.
A6	P1 SD	Unadj	11	16.74273	0.418639
		Adj	11	17.09255	0.126449
	P2 BN	Unadj	8	17.0025	0.277275
		Adj	8	16.85528	0.131431
	F1	Unadj	41	17.21951	0.115264
		Adj	41	17.08281	0.060398
	F2	Unadj	76	17.05987	0.099687
		Adj	76	17.07718	0.0463
A7	P1 SD	Unadj	10	7.223	0.084709
		Adj	10	7.255173	0.012882
	P2 BN	Unadj	8	7.16125	0.078112
		Adj	8	7.210875	0.015772
	F1	Unadj	45	7.344667	0.029166
		Adj	45	7.318851	0.010432
	F2	Unadj	75	7.359467	0.021219
		Adj	75	7.358096	0.003838
B1	P1 SD	Unadj	11	14.01545	0.170685
		Adj	6	13.37408	0.1663
	P2 BN	Unadj	5	12.1725	0.19778
		Adj	5	12.81839	0.329515
	F1	Unadj	45	13.166	0.104857
		Adj	45	13.00832	0.077066
	F2	Unadj	76	12.68289	0.063464
		Adj	76	12.80736	0.049797
B2	P1 SD	Unadj	6	24.056	0.272051
		Adj	6	23.26073	0.24782
	P2 BN	Unadj	5	21.61625	0.27326
		Adj	5	22.38333	0.494174
	F1	Unadj	45	22.95556	0.134693
		Adj	45	22.78408	0.109995
	F2	Unadj	76	22.38579	0.091737
		Adj	76	22.52494	0.074066
B3	P1 SD	Unadj	11	6.4	0.104403
		Adj	11	6.334671	0.036035
	P2 BN	Unadj	8	6.43375	0.087545
		Adj	8	6.267055	0.037455
	F1	Unadj	45	6.354222	0.042174
		Adj	45	6.331897	0.017212
	F2	Unadj	76	6.313816	0.030104
		Adj	76	6.330291	0.013195

TABLE 10.--Unadjusted and adjusted means
± standard errors, all three generations: variables B4-C2

Var	Gen	Stat	N	Mean	± S.E.
B4	P1 SD	Unadj	10	12.13545	0.14962
		Adj	10	11.73213	0.112212
	P2 BN	Unadj	8	11.7675	0.134028
		Adj	8	11.62494	0.109516
	F1	Unadj	45	11.64689	0.099527
		Adj	45	11.619	0.056836
	F2	Unadj	76	11.47526	0.06291
		Adj	76	11.52668	0.038789
B5	P1 SD	Unadj	10	7.859	0.078308
		Adj	10	7.659323	0.023242
	P2 BN	Unadj	8	7.501875	0.07363
		Adj	8	7.615712	0.024158
	F1	Unadj	45	7.693556	0.026601
		Adj	45	7.657534	0.011102
	F2	Unadj	75	7.637867	0.024151
		Adj	75	7.656498	0.00851
B6	P1 SD	Unadj	6	24.002	0.426809
		Adj	6	24.58422	0.323139
	P2 BN	Unadj	5	23.8175	0.337918
		Adj	5	23.64099	0.522185
	F1	Unadj	45	24.33956	0.209227
		Adj	45	23.96689	0.141327
	F2	Unadj	76	23.43776	0.118292
		Adj	76	23.65526	0.094824
C1	P1 SD	Unadj	10	12.42636	0.206059
		Adj	10	12.72365	0.122842
	P2 BN	Unadj	8	12.85	0.170378
		Adj	8	12.58154	0.119778
	F1	Unadj	44	12.74364	0.080702
		Adj	44	12.6541	0.05885
	F2	Unadj	76	12.54487	0.059943
		Adj	76	12.58808	0.042543
C2	P1 SD	Unadj	7	30.46556	0.479221
		Adj	7	29.61961	0.221617
	P2 BN	Unadj	5	27.55125	0.337414
		Adj	5	28.45924	0.544546
	F1	Unadj	44	29.61545	0.154789
		Adj	44	29.31202	0.101962
	F2	Unadj	76	28.99816	0.109098
		Adj	76	29.1785	0.07512

TABLE 11--Unadjusted and adjusted means
± standard errors, all three generations: variables C3-C7

Var	Gen	Stat	N	Mean	± S.E.
C3	P1 SD	Unadj	10	7.61	0.267885
		Adj		****	****
	P2 BN	Unadj	8	6.60875	0.128222
		Adj		****	****
	F1	Unadj	44	7.345909	0.070677
		Adj		****	****
	F2	Unadj	75	7.3704	0.06813
		Adj		****	****
C4	P1 SD	Unadj	11	14.70909	0.264728
		Adj	11	14.45169	0.131954
	P2 BN	Unadj	8	14.05125	0.301209
		Adj	8	14.20409	0.137153
	F1	Unadj	44	14.64682	0.099967
		Adj	44	14.44153	0.063027
	F2	Unadj	75	14.32907	0.08471
		Adj	75	14.43565	0.048316
C5	P1 SD	Unadj	6	18.41818	0.258889
		Adj	6	18.10687	0.237445
	P2 BN	Unadj	5	16.99875	0.205838
		Adj	5	17.34962	0.42592
	F1	Unadj	44	17.99227	0.139015
		Adj	44	17.64129	0.105296
	F2	Unadj	75	17.19893	0.096875
		Adj	75	17.39677	0.070323
C6	P1 SD	Unadj	7	7.54	0.136834
		Adj	7	7.226744	0.077465
	P2 BN	Unadj	5	6.7975	0.155446
		Adj	5	6.802321	0.20382
	F1	Unadj	44	7.186818	0.052705
		Adj	44	7.116108	0.036079
	F2	Unadj	75	6.99	0.045937
		Adj	75	7.065083	0.026477
C7	P1 SD	Unadj	6	18.25636	0.24844
		Adj	6	18.43687	0.179559
	P2 BN	Unadj	5	18.165	0.212039
		Adj	5	17.89579	0.286979
	F1	Unadj	44	18.36114	0.092197
		Adj	44	18.1287	0.076134
	F2	Unadj	76	17.84671	0.073402
		Adj	76	17.97693	0.05251

TABLE 12--Unadjusted and adjusted means
± standard errors, all three generations: femur variable

Var	Gen	Stat	N	Mean	± S.E.
FEM	P1 SD	Unadj	7	32.66182	0.609072
		Adj	7	31.51475	0.445678
	P2 BN	Unadj	5	29.04429	0.706409
		Adj	5	27.87238	2.073908
	F1	Unadj	45	31.07511	0.304413
		Adj	45	30.67995	0.2374
	F2	Unadj	76	29.71947	0.197028
		Adj	76	30.10802	0.167251

TABLE 13.--Raw data

RAT ID#	GEN F:	litter size	Sex 1m2f	AGE mths	A1	A2	A3	A4	A5
204	0		1	9	28.37	20.69	13.56	15.76	18.3
243	0	2	1	10 1/2	27.59	19.79	XXX	16.58	18.29
283	0	5	1		25.58	18.23	11.05	13.94	13.94
382	0	5	1	8	28	19.78	11.98	15.48	14.31
384	0	5	1	8	28.13	20.4	11.96	15.06	13.45
534	0	5	1	11	29.19	20.81	12.81	16.05	15.3
209	0		2	9	26.16	18.73	12.65	14.54	15.52
212	0		2	9	25.90	18.15	12.11	14.13	17.35
386	0		2	9	25.74	18.64	11.28	14.33	13.76
530	0	2	2	10	26.33	19.43	11.42	14.49	13.64
532	0	2	2	10	27.04	19.91	11.25	14.45	14.12
221	0		1	9	26.43	19.92	XXX	15.69	18.3
235	0	6	1	12	28.1	21.62	12.46	16.4	14.7
218	0		2	9	24.78	18.82	12.75	14.70	17.86
239	0	6	2	11 1/2	25.68	19.82	12	15.52	14.64
253	0	6	2	12	26.06	20.22	11.83	14.99	15.49
254	0	6	2	12	25.4	19.5	11.08	14.74	15.99
255	0		2	9	25.63	19.03	13.08	14.92	17.86
256	0	6	2	12	25.46	19.23	12.01	15.12	15.71
298	1	2	1	11	29.85	22.85	12.43	16.43	16.01
300	1	2	1	11	29.01	21.55	12	16.06	15.73
304	1	1	1	5	29.02	21.47	11.47	15.79	14.61
306	1	2	1	10	29.8	22.78	12.9	16.74	15.93
317	1	7	1	4 1/2	28.2	21.34	11.36	15.5	15.81
318	1	7	1	4 1/2	27.65	20.87	11.32	15.27	15.73
320	1	7	1	4 1/2	27.97	20.91	10.92	15.03	15.82
331	1	10	1	4 1/2	28.03	21.37	11.17	14.82	15.37
332	1	10	1	4 1/2	28.36	21.37	11.38	14.88	14.8
333	1	10	1	4 1/2	29.14	21.88	12.03	15.75	15.23
334	1	10	1	4 1/2	28.04	21.02	11.66	15.82	15.38
335	1	10	1	4 1/2	28.04	21.31	11.60	15.33	15.97
336	1	10	1	4 1/2	27.97	21.19	10.96	14.96	XXX
351	1	3	1	14 1/2	26.25	19.42	10.53	13.88	16.03
352	1	3	1	14 1/2	26.7	19.81	11	14.32	15.78
353	1	3	1	14 1/2	26.61	19.83	10.33	14.22	15.37
365	1	3	1	4	29.41	22.21	13.02	17.09	15.81
366	1	3	1	4	27.42	20.4	11.58	15.57	15.88

TABLE 14.—Raw data

RAT ID#	A6	A7	B1	B2	B3	B4	B5	B6	C1
204	18.6	7.25	14.93	XXX	6.85	12.89	8.01	XXX	13.18
243	18.65	7.56	14.22	25.07	6.85	12.87	8.15	26.02	13.44
283	15.02	XXX	13.43	22.93	5.99	12.17	7.42	22.06	11.71
382	16.25	7.07	14.43	24.35	6.26	11.97	7.77	24.24	12.67
384	15.64	7.27	14	24.59	5.92	12.41	XXX	24.42	12.62
534	17.01	6.86	14.7	25.67	6.16	12.54	7.83	26	13.42
209	17.94	7.54	13.57	23.64	6.41	12.08	8.21	24.28	12.26
212	18.17	7.53	13.16	23.87	6.66	11.82	8.07	24.23	11.84
386	15.08	6.81	13.54	23.21	6.31	11.58	7.77	22.42	11.73
530	15.74	7.13	13.8	23.42	6.83	11.75	7.72	22.77	11.7
532	16.07	7.21	14.39	23.81	6.16	11.41	7.64	23.58	12.12
221	18.17	7.43	12.38	22.47	6.6	12.19	7.83	25.03	13.62
235	16.08	7.13	13.38	22.97	6.57	12.39	7.44	25.35	13.5
218	17.94	7.44	11.96	21.6	6.61	11.35	7.63	23.31	12.36
239	16.1	6.88	12.12	21.43	6.36	11.56	7.635	23.29	12.75
253	17.24	7.13	11.82	21.39	6.4	12	7.24	24.13	12.66
254	17.03	6.87	11.53	20.48	6.2	11.64	7.22	22.9	12.47
255	17.04	7.31	11.88	21.43	6.74	11.59	7.58	23.76	12.99
256	16.42	7.1	12.31	21.16	5.99	11.42	7.44	22.77	12.45
298	18.87	7.38	14.1	24.55	6.78	12.39	7.66	27.51	13.42
300	17.66	7.37	14.1	24.14	6.53	12.03	7.68	25.95	13.22
304	16.73	7.43	13.69	23.95	6.48	12.18	7.76	25.27	12.97
306	18.16	7.29	14.24	24.29	6.84	12.45	7.7	27.24	13.78
317	17.46	7.25	13.85	23.65	6.36	12.24	7.62	24.83	13.18
318	17.29	7.23	13.95	23.49	6.34	11.51	7.73	24.51	13.03
320	17.14	7	14.15	23.69	5.77	11.36	7.57	24.65	12.88
331	17.71	7.24	13.1	23.23	6.6	12.2	7.73	25.22	12.96
332	17.31	7.45	13.56	23.65	6.65	12.83	7.63	25.27	13.48
333	17.51	7.62	13.19	23.5	6.51	12.69	8.03	26.04	13.46
334	17.81	7.68	13.72	24.1	6.59	12.17	7.99	25.89	13.5
335	17.67	7.70	13.33	23.48	6.46	12.5	7.60	25.45	13.09
336	XXX	7.19	13.4	23.57	6.61	11.88	7.70	24.99	13.09
351	17.03	7.31	13.44	23.47	6.13	11.6	7.72	22.8	12.51
352	17.01	7.35	13.1	22.25	6.26	10.84	7.89	23.22	12.49
353	16.4	7.41	12.84	22.63	6.3	10.74	7.87	23.16	12.46
365	17.95	7.44	14.77	24.47	6.66	12.1	7.8	26.92	13.56
366	17.63	7.01	13.55	23.35	6.47	11.34	7.43	24.25	12.62

TABLE 15.—Raw data

RAT ID#	C2	C3	C4	C5	C6	C7	FEMUR
204	32.47	8.49	16.36	19.58	8.04	19.78	34.1
243	32.19		15.75	18.95	7.92	19.44	35.29
283	27.83	6.76	13.32	17.9	6.57	17.2	30.89
382	30.26	8.6	14.03	18.55	7.75	18.26	34.13
384	30.32	7.74	14.81	18.79	7.83	18.27	33.94
534	31.32	5.95	15.37	19.99	8.06	18.81	35.5
209	30.88	8.35	15.02	18.22	7.66	18.5	30.94
212	29.92	8.07	14.55	18.04	7.37	17.66	29.57
386	XXX	7.58	13.96	17.11	7.32	17.45	30.75
530	29.49	7.62	14.13	17.64	7.3	17.64	32.05
532	29.83	6.94	14.5	17.83	7.12	17.81	32.12
221	28.85	6.76	14.82	17.53	7.18	19.13	31.24
235	29.09	6.07	15.62	17.99	7.61	18.91	31.99
218	27.23	6.35	14.38	16.85	6.86	17.82	27.81
239	27	6.7	13.45	16.28	6.76	17.78	28.02
253	27.9	6.61	13.94	17.16	6.81	18.26	29.27
254	26.85	6.69	13.12	16.42	6.27	17.61	27.44
255	26.73	7.3	13.82	17.18	6.53	18.3	27.54
256	26.76	6.39	13.26	16.58	6.36	17.51	XXX
298	31.64	6.71	16.1	19.97	7.91	19.18	35.35
300	30.63	7.24	15.48	19.34	7.71	18.84	34.05
304	30.75	7.39	15.33	18.88	7.57	19.18	33.33
306	31.06	7.76	15.45	20.07	7.53	19.67	35.45
317	30.4	7.98	14.83	18.57	7.47	18.74	31.95
318	30.02	6.49	15.11	18.16	7.23	18.61	31.62
320	29.98	7.82	14.53	18.28	7.3	18.33	31.95
331	30.06	7.42	15.04	18.86	7.31	18.57	32.25
332	31.04	8.12	15.51	18.41	7.45	18.68	32.89
333	31.02	7.77	15.62	18.37	7.73	18.79	33.18
334	30.3	6.87	15.42	18.77	7.44	19.17	32.94
335	29.74	7.94	15.07	18.85	7.53	19.15	33.17
336	30.56	8.07	15.47	18.88	7.36	18.52	32.69
351	28.23	7.16	13.53	17.15	6.72	17.83	28.89
352	28.39	7.79	13.6	17.33	6.89	18.2	28.99
353	28.91	7.09	14.7	16.79	6.81	18.34	28.94
365	30.7	7.6	15.12	19.85	7.43	19.59	35.35
366	29.69	7.06	14.59	17.08	7.19	18.33	31.44

TABLE 16.--Raw data

RAT ID#	GEN F:	litter size	Sex 1m2f	AGE mths	A1	A2	A3	A4	A5
367	1	3	1	4	27.66	20.6	11.51	15.22	15.4
XSM 1	1	2	1	4 1/2	28.76	22.17	11.25	15.24	XXX
XSM 2	1	2	1	4 1/2	28.59	21.89	11.25	15.19	13.65
XSM 3	1	2	1	4	27.70	20.88	11.27	14.71	15.13
XSM 4	1	8	1	4	28.08	21.68	11.60	15.43	XXX
XSM 5	1	3	1	4	28.61	21.75	11.56	15.07	XXX
299	1	8	2	11	28.01	20.9	10.87	15.19	14.96
301	1	2	2	10	27.72	20.89	11.16	15.11	15.8
303	1	8	2	12 1/2	27.54	20.98	11.6	15.44	16.02
305	1	8	2	9	27.1	20.02	11.48	14.74	14.87
307	1	8	2	12	27.63	20.9	11.75	15.45	16.23
319	1	7	2	4	26.44	19.54	10.77	14.1	15.66
321	1	7	2	4	26.39	19.59	10.52	14.05	15.06
322	1	7	2	4	26.58	19.68	10.49	14.36	16.26
323	1	7	2	4	26.29	19.9	10.66	14.33	14.98
337	1	10	2	4 1/2	26.07	19.53	10.44	13.74	15.03
338	1	10	2	4 1/2	26.29	19.67	10.20	13.57	15.64
339	1	10	2	4 1/2	25.45	19.48	11.03	13.81	15.44
340	1	10	2	4 1/2	26.53	19.57	10.65	14.09	15.18
354	1	3	2	4	25.81	19.01	10.12	13.18	15.78
355	1	3	2	4	25.16	18.85	9.99	12.97	15.25
356	1	3	2	4	26.04	19.50	10.83	13.74	15.42
357	1	3	2	4	25.05	18.24	10.65	13.44	15.23
XSF 1	1	8	2	4	26.61	19.87	10.51	13.53	XXX
XSF 2	1	8	2	4	26.00	19.01	10.68	13.78	14.49
XSF 3	1	8	2	4	26.35	19.79	10.34	13.77	13.96
XSF 4	1	3	2	4	25.57	19.00	10.85	13.68	15.82
405	2	6	1	4	28.38	20.97	11.14	15.49	16.62
406	2	6	1	4	29.61	22.36	11.45	16.02	16.77
496	2	12	1	4	27.89	20.97	10.35	14.49	15.59
497	2	12	1	4	28.42	21.18	11.07	14.79	16.14
498	2	12	1	4	27.78	20.9	11.24	15.2	16.33
499	2	12	1	4	27.9	20.89	10.4	14.62	16.17
504	2	12	1	4	27.07	20.09	10.37	14.58	15.23
505	2	12	1	4	27.47	20.85	10.55	14.74	16.89
506	2	12	1	4	27.44	20.69	10.94	14.5	16.49
508	2	11	1	4	26.12	19.21	10.67	14.17	15.2
509	2	11	1	4	27.32	20.27	11.74	14.74	15.74
510	2	11	1	4	27.56	21.1	11	15.19	16.42

TABLE 17.--Raw data

RAT ID#	A6	A7	B1	B2	B3	B4	B5	B6	C1
367	17.15	7.21	13.9	23.41	5.93	11.25	7.79	24.36	12.98
XSM 1	XXX	7.72	13.35	24	6.62	12.16	8.15	26.04	12.99
XSM 2	16.93	7.43	13.62	23.33	6.36	12.2	8.13	25.98	13.26
XSM 3	18.15	7.60	12.88	23.12	6.41	12.76	7.67	25.02	13.35
XSM 4	19.76	7.61	13.28	23.68	7.05	12.4	7.95	25.19	13.37
XSM 5	XXX	7.81	13.59	23.84	6.69	12.61	7.81	25.65	13.2
299	17.05	7.26	13.19	22.91	6.36	11.9	7.59	24.8	12.63
301	16.99	7.27	13.11	22.8	6.46	11.85	7.56	24.44	12.8
303	16.95	7.28	13.25	23.28	6.84	12.2	7.59	24.74	13.11
305	16.51	7.14	13.15	23.18	6.43	12.12	7.54	24.66	12.66
307	18.19	7.21	12.82	22.62	6.4	11.61	7.48	24.81	12.7
319	17.13	7.15	13.48	22.54	6.07	10.98	7.37	22.32	11.72
321	16.6	7.35	13.26	22.3	6.09	10.71	7.57	22.62	12.23
322	16.85	7.27	13.32	22.56	5.91	10.54	7.69	23.24	12.25
323	16.54	7.3	13.34	22.34	5.93	10.37	7.69	22.86	12.15
337	17.13	7.37	12.18	21.84	6.22	11.29	7.60	23.19	12.12
338	17.21	7.53	12.33	22.04	6.31	11.19	7.68	23.03	12.33
339	16.71	7.34	12.66	21.98	6.09	11.16	7.59	23.19	12.01
340	16.34	7.15	12.2	21.68	6.18	11.21	7.68	23.11	12.23
354	17.14	7.38	12.47	22.15	6.29	11.01	7.62	23.26	12.15
355	16.46	6.88	11.94	20.87	5.91	10.91	7.40	22.33	12.04
356	17.05	7.19	12.03	21.69	6.13	11.15	7.93	22.87	12.18
357	16.98	7.20	11.75	21.41	6.04	10.85	7.77	22.31	XXX
XSF 1	XXX	7.30	12.9	22.32	6.18	11.28	7.58	23	12.2
XSF 2	16.24	7.21	12.53	21.87	6.37	11.15	7.40	23.07	12.22
XSF 3	15.6	7.50	12.12	22.06	6.3	11.27	7.56	23.32	12.03
XSF 4	17	7.50	11.74	21.72	6.03	10.93	7.71	22.7	12.11
405	17.57	7.34	13.6	23.66	6.48	12.15	7.66	24.35	13.26
406	18.25	7.59	13.69	23.67	6.94	12.61	7.88	25.33	13.57
496	16.21	7.36	13.33	23.42	6.28	12.1	7.82	24.25	12.7
497	17.63	7.43	13.12	23.34	6.73	11.99	7.96	25.07	12.57
498	17.84	7.06	13.21	22.98	6.5	11.83	XXX	24.87	13.51
499	17.37	6.99	13.4	23.6	6.2	12.04	7.36	24.92	13.16
504	16.32	7.29	12.77	22.68	6.15	11.24	7.95	23.81	12.59
505	17.35	7.45	13.02	22.88	6.68	11.25	7.56	24.44	12.72
506	17.68	7.39	13.19	23.61	6.62	11.86	7.62	24.19	12.87
508	17.31	7.3	13.06	22.23	6.09	11.41	7.21	23.3	12
509	16.66	7.35	13.17	23.05	6.45	11.56	7.66	23.48	12.86
510	16.77	7.46	13.06	23.23	6.29	11.21	7.78	24.48	13.01

TABLE 18.--Raw data

RAT ID#	C2	C3	C4	C5	C6	C7	FEMUR
367	30.1	6.55	15	18.02	7.24	18.61	31.64
XSM 1	30.73	7.46	14.93	18.84	7.56	19.2	32.69
XSM 2	30.63	7.54	15.03	18.86	7.56	18.88	33.13
XSM 3	30.86	7.81	15.16	17.35	7.48	18.81	31.54
XSM 4	30.55	7.6	15.58	18.46	7.51	19.13	32.02
XSM 5	30.32	7.5	15.11	18.66	7.38	19.01	32.47
299	30.09	7.98	14.65	17.99	7.08	18.12	30.95
301	29.66	7.23	14.55	18.19	7.37	18.23	31.15
303	29.83	8.3	14.21	18.05	7.41	18.29	31.02
305	29.59	7.54	14.17	18.36	6.87	18.09	31.06
307	29.13	6.91	14.59	18.09	7.15	18.13	31.17
319	28.89	7.08	14.51	17.28	6.55	17.67	28.88
321	28.28	7.26	14.14	17.01	6.96	17.83	29.05
322	28.69	7.13	14.22	17.26	6.83	17.91	29.58
323	28.7	7.4	13.97	16.95	6.63	17.56	29.38
337	28.92	7.38	14.25	17.23	6.86	17.61	29.33
338	29.07	7.24	14.56	17.37	6.92	17.53	29.31
339	28.69	7.31	14.25	17.19	6.78	17.73	29.03
340	28.49	7.31	13.49	17.14	6.95	17.73	28.83
354	28.6	7.15	13.56	17.64	6.84	17.72	29
355	27.4	7.3	13.67	16.13	6.56	17.33	27.77
356	28	7.12	14.06	17.06	7	17.85	28.6
357							
XSF 1	28.92	6.82	14.26	17.17	7.55	17.89	30.01
XSF 2	28.96	7.19	13.91	17.16	6.96	17.86	29.72
XSF 3	28.68	5.99	14.45	17.64	6.84	17.8	29.13
XSF 4	28.18	6.84	13.68	16.95	6.8	17.65	28.84
405	29.77	6.81	14.55	18.26	6.55	19.02	32.17
406	29.71	8.7	14.47	19	7.98	19.28	33.3
496	30.47	8.95	13.76	18.41	7.47	18.29	31.45
497	30.24	7.7	14.69	17.88	7.12	19.05	30.8
498	29.81	7.46	14.45	17.5	7.28	18.88	31.23
499	31.21	7.31	15.4	17.61	7.31	18.71	31.79
504	30.25	7.85	14.68	17.36	7.42	18.34	31.53
505	29.89	7.04	14.93	17.77	7.16	18.65	31.66
506	28.63	7.14	13.66	18.23	7.29	18.16	31.25
508	29.21	7.42	14.71	17.45	6.69	17.66	29.48
509	29.18	7.57	15.02	17.67	7.05	18.2	30.9
510	29.04	6.86	14.37	17.96	7.31	18.53	30.78

TABLEe 19.--Raw data

RAT ID#	GEN F:	litter size	Sex 1m2f	AGE mths	A1	A2	A3	A4	A5
511	2	11	1	4	25.68	19.21	11.66	14.63	14.82
524	2	11	1	4	26.67	19.84	9.71	13.37	16.66
525	2	11	1	4	27.13	19.79	10.58	14.07	15.59
526	2	11	1	4	27.58	20.6	10.47	14.14	15.79
527	2	11	1	4	26.38	20.17	11.22	14.9	15.02
528	2	11	1	4	26.88	19.96	11.47	14.2	16.67
528	2	11	1	4	26.88	19.96	11.47	14.2	16.67
529	2	11	1	4	27.08	20.68	11.15	14.75	17.83
549	2	7	1	4	27.2	20.17	11.68	14.95	15.98
550	2	7	1	4	27.1	20.52	11.35	15.53	15.93
551	2	7	1	4	28.69	21.77	10.88	15.21	17.14
552	2	7	1	4	27.02	20.17	11.17	14.93	15.4
553	2	7	1	4	27.65	20.7	12.35	15.32	XXX
554	2	7	1	4	XXX	XXX	11.55	15.08	16.27
557	2	6	1	4	26.87	20.19	10.33	14.23	16.92
558	2	6	1	4	28.83	21.71	11.18	15.74	16.91
559	2	6	1	4	27.33	20.29	10.6	14.73	16.84
560	2	6	1	4	27.75	18.95	10.14	13.68	14.15
561	2	6	1	4	27.03	20.48	12.16	15.21	16.59
562	2	6	1	4	27.86	21.07	10.97	14.61	16.36
568	2	9	1	4	28.66	22.2	10.68	15.22	16.58
569	2	9	1	4	27.65	20.69	11.64	15.08	16.15
573	2	11	1	4	28.09	22.07	10.55	15.05	15.14
574	2	11	1	4	27.12	20.74	10.52	14.53	15.95
575	2	11	1	4	28.26	21.7	11.3	14.6	15.73
576	2	11	1	4	25.98	19.67	10.61	13.79	15.35
577	2	11	1	4	26.98	19.89	10.92	14.36	16.34
578	2	5	1	4	28.12	21.79	11.13	15.33	16.92
589	2	6	1	4	26.32	19.82	10.21	13.54	16.52
407	2	6	2	4	25.87	19.47	10.49	14.31	16.58
408	2	6	2	4	28.29	21.67	10.97	15.07	16.46
409	2	6	2	4	25.3	19.24	10.58	13.47	15.87
500	2	12	2	4	25.55	18.9	9.96	13.42	15.17
501	2	12	2	4	25.46	18.56	9.68	12.73	14.41
502	2	12	2	4	25.41	18.82	10.44	13.65	14.69
503	2	12	2	4	25.64	19.07	10.86	14.08	14.91
507	2	12	2	4	25.66	19.19	9.98	13.52	16.57
512	2	11	2	4	25.69	19.28	10.16	13.42	15.18
513	2	11	2	4	25.69	19.26	10.85	14.17	15.93
514	2	11	2	4	24.42	18.38	10.68	13.37	15.44

TABLE 20.--Raw data

RAT ID#	A6	A7	B1	B2	B3	B4	B5	B6	C1
511	16.38	XXX	12.44	21.95	6.25	11.49	8.18	23.07	12.64
524	18.53	6.97	13.09	23.23	6.27	12.02	7.41	23.21	13.22
525	17.66	7.24	12.43	22.35	6.35	11.31	7.74	23.21	12.9
526	17.56	7.45	12.72	22.96	5.95	12.08	7.64	23.88	12.89
527	16.81	7.49	12.72	22.84	6.62	11.67	7.94	24.35	12.97
528	18.05	7.2	13.34	23.45	6.04	11.08	7.66	23.59	12.46
529	19.89	7.5	13.17	22.49	6.28	11.91	7.9	24.14	13.01
549	17.06	7.52	13.11	22.81	6.81	11.48	7.59	22.4	12.26
550	16.45	7.33	13.21	22.96	6.2	11.74	7.63	23.81	13.13
551	18.11	7.45	13.18	23.47	6.41	12.22	7.8	25.01	13.54
552	16.64	7.47	12.91	22.43	6.4	11.55	7.51	24.05	12.54
553	19.53	7.55	13.12	23.06	6.8	12.59	7.31	24.08	13.31
554	16.63	7.56	13.33	23.06	6.68	12.11	8.14	25.05	12.42
557	18.09	7.23	13.18	22.39	6.26	11.92	7.73	23.85	12.83
558	17.87	7.33	13.66	23.72	6.48	11.98	7.69	25.16	13.17
559	18.15	7.6	13.33	22.93	6.2	11.52	7.87	24.14	12.61
560	15.71	7.43	11.98	20.98	6	11.05	7.55	22.37	11.94
561	18.24	7.39	13.13	23.16	6.88	11.82	7.86	24.44	12.67
562	18.37	7.58	13.19	23.32	6.75	12.37	7.9	25.52	13.01
568	18.74	7.28	13.52	23.45	6.88	12.31	7.6	25.25	13.67
569	17.47	7.24	13.47	23.35	6.55	12.36	7.67	24.58	13.46
573	16.74	7.28	13.54	23.56	6.74	12.28	7.52	24.71	12.98
574	17.66	7.48	12.43	22.38	6.16	11.85	7.39	23.83	12.39
575	17.24	7.28	12.68	22.91	6.57	11.37	7.66	24.28	13.02
576	16.69	7.46	12.09	21.5	5.8	11.5	7.59	23.57	12.19
577	17.12	7.14	13.06	22.18	6.52	11.25	7.67	24.13	12.76
578	17.72	7.38	13.17	23.28	6.28	11.54	7.71	24.68	13.78
589	17.17	7.38	12.14	21.61	6.57	10.75	7.55	23.33	12.14
407	16.89	7.19	11.96	21.16	6.16	10.39	7.6	22.89	12.47
408	17.08	7.22	13.37	23.39	6.47	11.75	7.36	24.83	13.5
409	17.39	7.42	12.73	21.92	6.15	10.53	7.39	22.37	12.02
500	15.85	7.27	12.07	21.4	6.28	11.2	7.67	22.67	11.84
501	16.29	7.28	12.52	21.9	5.91	10.96	7.55	22.04	12
502	15.22	7.19	12.11	21.66	6.08	10.59	7.28	22.01	11.87
503	16.23	7.14	12.32	21.83	6	11.16	7.58	22.27	12.3
507	17.13	7.17	11.9	21.63	6.29	10.95	7.53	23.01	12.23
512	16.6	7.2	12.55	21.86	6.02	10.57	7.4	22.25	12.41
513	17.17	7.42	11.66	21.1	6.12	10.95	7.79	22.79	12.3
514	17.3	7.03	12.16	21.72	6.07	10.88	7.33	22.02	11.92

TABLE 21.—Raw data

RAT ID#	C2	C3	C4	C5	C6	C7	FEMUR
511	28.37	7.69	14.52	17.39	7.29	17.87	31.18
524	28.52	6.65	14.37	17.06	6.82	17.78	31.33
525	29.81	7.02	14.59	17.24	7.22	18.29	30.68
526	28.99	7.42	14.07	17.61	7.6	18.38	31.36
527	29.15	7.45	14.82	17.32	7.11	18.03	31.5
528	29.72	8.11	14.17	17.68	6.95	17.96	30.91
529	29.71	8.19	14.57	16.95	7.12	18.36	31.22
549	28.88	7.97	14.66	18.16	6.84	17.66	29.81
550	29.96	6.73	15.28	18.12	7.55	19.06	30.96
551	30.53	6.94	15.14	18.33	7.82	18.45	32.58
552	29.4	7.96	17.57	17.4	6.87	18.12	30.12
553	29.82	8.43	14.8	17.74	7.26	18.4	30.56
554	30.36	XXX	14.55	17.97	7.46	18.52	31.99
557	29.51	6.99	14.68	17.86	7.04	17.91	31.54
558	30.55	7.73	14.71	18.51	7.75	19.08	32.15
559	30.02	8.01	14.82	17.92	7.36	17.89	30.98
560	27.72	6.21	13.81	16.04	6.68	16.92	28.08
561	29.52	7.76	15.24	17.85	7.03	18.15	31.66
562	29.61	7.76	14.95	18.4	7.42	18.98	31.53
568	30.08	6.15	15.05	17.78	7.48	18.31	31.28
569	29.25	7.05	14.72	17.88	7.21	18.37	31.14
573	30.56	7.92	15.22	18.39	6.01	18.24	31.21
574	29.41	6.94	14.37	18.26	7.32	18.24	30.26
575	29.73	6.72	15.29	18.07	7.22	17.43	31.67
576	27.86	6.62	13.51	17.47	XXX	17.73	29.11
577	28.94	6.84	14.45	17.71	6.91	18.04	27.66
578	29.39	7.1	14.1	18.19	7.68	18.5	32.53
589	27.99	6.9	13.84	16.82	6.63	17.28	28.22
407	28.73	6.91	13.69	16.65	7.1	17.84	27.93
408	29.63	7.48	14.73	17.79	7.51	18.67	31.24
409	27.39	6.73	13.65	16.53	6.21	16.97	28.01
500	27.71	7.19	13.83	16.33	6.31	17.37	27.67
501	28.3	7.55	13.69	16.65	6.65	17.3	28.32
502	28.53	7.22	14.45	16.67	6.42	17.24	27.48
503	28.75	7.05	13.9	16	6.65	16.99	28.27
507	28.28	7.52	13.36	16.33	6.79	17.74	28.71
512	28.2	6.36	14.01	16.6	6.69	16.97	28.52
513	30.17	7.6	16	14.3	7	17.48	27.04
514	28.28	8.2	13.93	16.36	6.34	17.09	30.17

TABLE 22.—Raw data

RAT ID#	GEN F:	litter size	Sex 1m2f	AGE mths	A1	A2	A3	A4	A5
515	2	11	2	4	25.26	18.86	10.14	13.53	16.45
516	2	11	2	4	25.34	18.95	10.14	13.68	14.15
517	2	11	2	4	25.67	19.26	10.15	13.58	15.86
519	2	11	2	4	25.64	19.23	10.01	13.79	15.86
520	2	11	2	4	25.58	19.2	10.85	13.33	15.35
521	2	11	2	4	24.96	18.88	10.56	13.87	16.32
522	2	11	2	4	25.92	19.55	9.63	13.46	14.99
523	2	11	2	4	25.88	19.62	10.66	14.13	15.58
555	2	7	2	4	25.82	18.93	10.92	13.92	15.85
564	2	9	2	4	27.63	20.06	10.32	13.9	15.93
565	2	9	2	4	25.8	18.8	11.65	14.22	15.39
566	2	9	2	4	26.2	19.63	9.94	13.62	16.4
567	2	9	2	4	26.76	20.03	10.19	14.05	15.88
570	2	9	2	4	27.22	19.91	10.76	14.27	15.93
571	2	9	2	4	27.03	19.38	10.83	14.14	16.31
572	2	9	2	4	27.3	20.6	10.43	13.96	14.85
579	2	11	2	4	25.77	19.57	10.47	14.35	15.63
580	2	11	2	4	27.1	19.9	10.91	14.78	15.63
581	2	11	2	4	26.06	19.65	11.31	14.28	15.73
582	2	11	2	4	25.12	19.26	11.44	14.21	16.04
583	2	11	2	4	26.05	19.54	10.51	14.1	14.76
585	2	5	2	4	25.76	19.21	10	13.69	15.51
586	2	5	2	4	26.25	19.99	10.45	14.47	16.77
587	2	5	2	4	26.01	19.24	9.73	13.32	15.35
588	2	5	2	4	25.47	18.68	10.1	12.67	15.39

TABLE 23.--Raw data

RAT ID#	A6	A7	B1	B2	B3	B4	B5	B6	C1
515	17.58	7.08	11.72	21.33	6.3	10.89	7.27	22.9	12.37
516	15.71	7.43	11.98	20.98	6	11.05	7.55	22.37	11.94
517	16.68	7.09	12.31	21.16	5.97	11.44	7.28	21.87	12.08
519	15.74	7.48	12.07	21.42	6.05	10.75	7.66	22.39	11.78
520	17.23	7.33	12.37	22.05	6.2	11.35	7.35	22.51	11.98
521	17.1	7.7	11.82	21.15	6.16	10.98	7.68	22.11	11.98
522	16.05	7.14	12.63	22.03	6.36	11.22	7.47	22.6	12.4
523	16.39	7.33	12.12	21.56	6.13	10.23	7.65	22.35	12.05
555	16.43	7.48	12.48	22.27	6.07	11.11	7.68	22.67	11.98
564	16.35	7.43	12.36	22.31	6.04	11.75	7.68	22.62	12.28
565	16.83	7.13	12.09	22.09	6.24	11.36	7.46	22.22	12.22
566	16.53	7.44	12.08	21.69	6.21	11.15	7.9	23.35	12.29
567	16.68	7.42	13.32	22.94	6.59	11.53	7.8	23.34	12.51
570	17.05	7.61	11.96	21.5	6.39	11.78	7.57	23.51	12.23
571	17.37	7.71	12.32	22.55	6.5	11.17	7.83	23.64	12.2
572	16.55	7.25	12.65	22.33	6.42	12.1	7.52	23.23	12.36
579	15.97	7.36	12.13	21.56	5.9	11.34	7.63	22.14	11.98
580	16.7	7.17	12.02	21.41	6.32	12.16	7.35	23.36	12.7
581	16.83	7.97	12.5	22.24	6.2	11.54	8.17	22.71	12.16
582	16.89	7.25	12.54	22.05	5.97	11.62	7.63	22.28	12.43
583	15.83	7.83	12.15	22.01	6.2	10.57	7.76	22.2	12.1
585	16.4	7.34	12.25	21.73	6.22	11.71	7.57	22.87	12.24
586	17.38	7.41	12.2	22.09	6.34	11.65	7.62	23.05	11.88
587	15.78	7.36	12.44	21.83	6.14	10.78	7.61	22.28	11.91
588	16.11	7.47	12.08	21.34	6.27	10.59	7.8	21.37	11.77

TABLE 24.--Raw data

RAT ID#	C2	C3	C4	C5	C6	C7	FEMUR
515	28.02	7.03	13.4	16.82	6.8	17.46	28.56
516	27.72	6.21	13.81	16.04	6.68	16.92	28.08
517	27.29	6.42	13.92	16.67	6.69	17.41	28.57
519	28.05	7.64	13.76	16.47	6.37	17.35	27.14
520	28.9	8.04	13.67	17.09	6.66	17.18	28.82
521	28.64	7.47	13.61	16.16	6.52	17.25	27.48
522	28.65	7.3	13.79	16.11	6.76	17.48	28.18
523	28.61	7.67	13.87	16.4	6.87	17.3	27.52
555	28.5	7.69	13.87	16.9	6.75	17.32	27.83
564	28.97	8.48	13.44	17.15	7.11	17.08	28.94
565	28.27	7.69	13.8	17.02	7.11	17.19	29.01
566	27.81	7.26	13.54	16.19	6.87	17.6	29.41
567	27.75	7.38	16.18	15.55	6.87	17.31	28.48
570	28.59	6.91	13.85	17.1	6.96	17.89	28.69
571	27.92	7.63	14.12	16.72	7.17	17.43	29.2
572	29.19	7.47	14.1	16.58	6.84	17.5	28.96
579	28.23	7.32	13.51	16.76	6.62	16.47	27.99
580	29.32	7.16	14.38	17.11	7.21	18.2	29.25
581	31.02	7.81	XXX	XXX	7.16	17.56	28.44
582	27.9	8.25	13.93	16.27	6.83	17.48	28.79
583	27.74	7.75	14.02	17.49	7.16	17.47	27.78
585	27.65	6.43	13.3	16.53	6.57	17.19	27.87
586	28.56	7.78	13.33	16.71	6.55	17.77	27.7
587	28.73	7.41	14.16	16.58	6.77	17.05	27.12
588	27.09	6.7	13.52	16.07	6.4	17.11	25.95

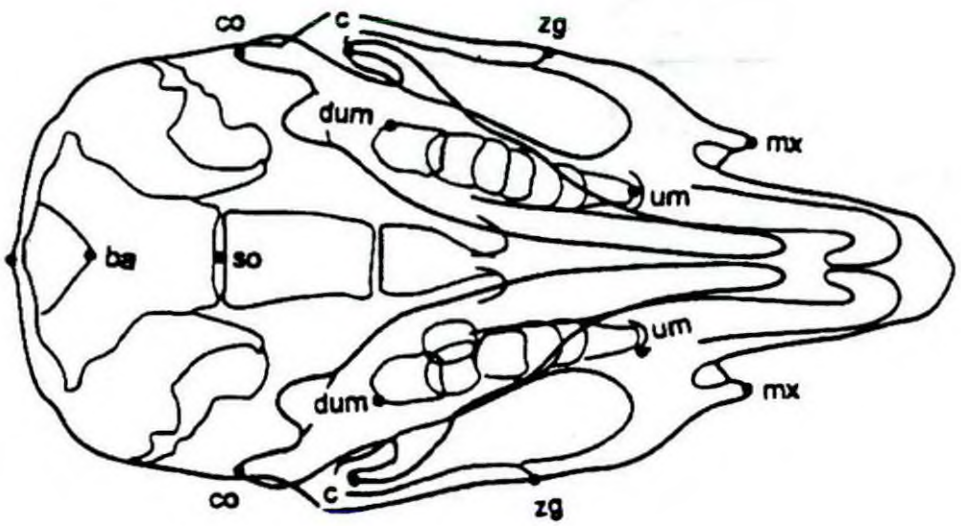


Figure 1. Anatomic landmarks in ventrodorsal view

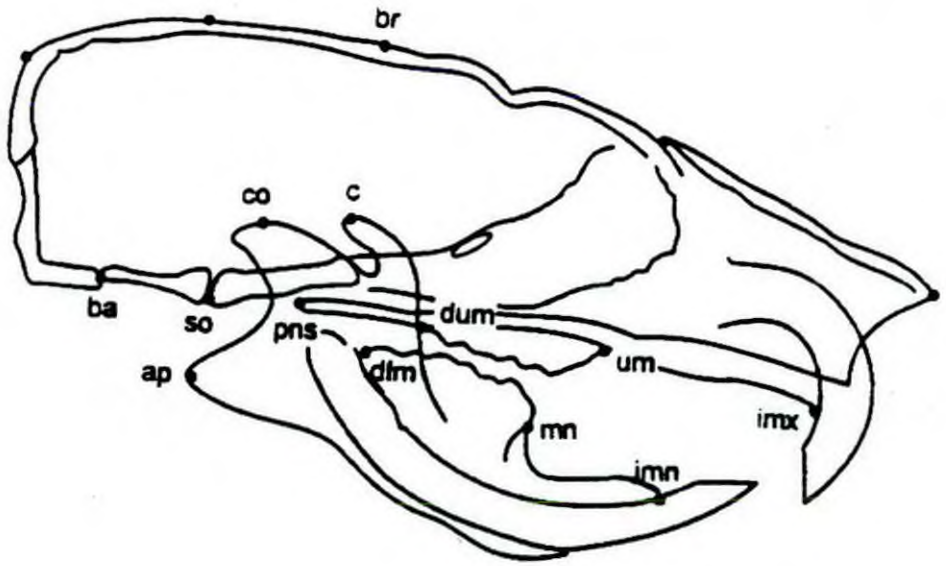


Figure 2. Anatomic landmarks in the lateral view

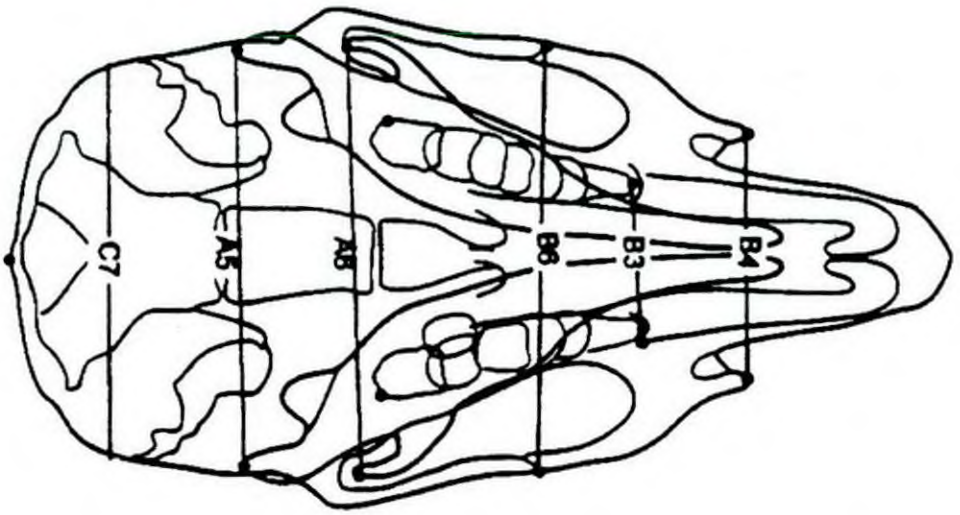


Figure 3. Variables measured in the ventrodorsal view

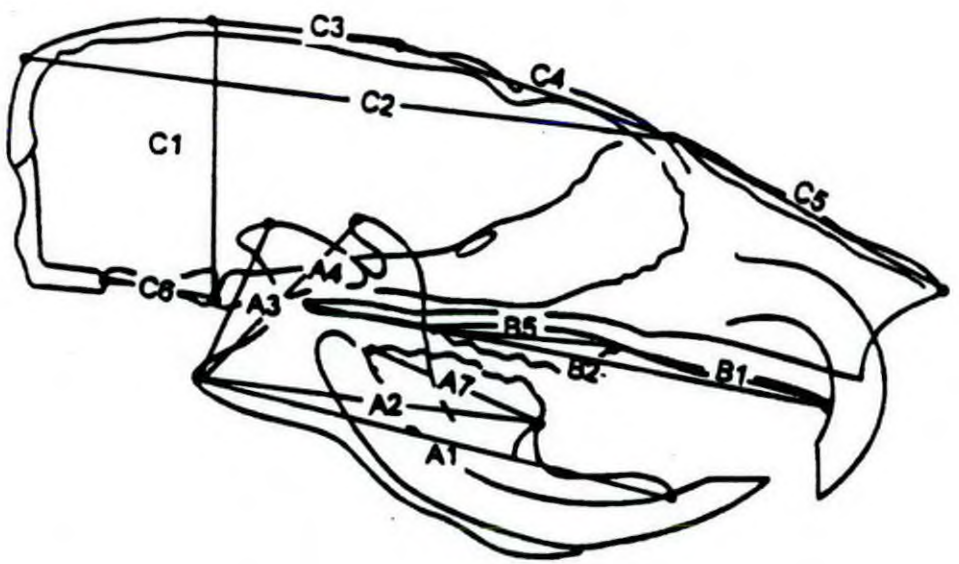


Figure 4. Variables measured in the lateral view

```
REGRESSION
/MISSING LISTWISE
/STATISTICS COEFF OUTS R ANOVA
/CRITERIA=PIN(.05) POUT(.10)
/NOORIGN
/DEPENDENT a1
/METHOD=STEP WISE age sex litter size
/SAVE PRED ZRESID.
```

Figure 5. SPSS syntax file program for generating residual values.

```
ONEWAY
a1 a2 a3 a4 a5 a6 a7 b1 b2 b3 b4 b5 b6 c1 c2 c3 c4 c5 c6 c7 fem BY gen
/STATISTICS DESCRIPTIVES
/MISSING ANALYSIS .
ONEWAY
paasla1 paasla2 paasla3 paasla4 paasla5 paasla6 paasla7 paaslb1 paaslb2
paaslb3 paaslb4 paaslb5 paaslb6 paaslc1 paaslc2 paaslc4 paaslc5 paaslc6
paaslc7
paaslfem BY gen
/STATISTICS DESCRIPTIVES
/MISSING ANALYSIS .
ONEWAY
raasla1 raasla2 raasla3 raasla4 raasla5 raasla6 raasla7 raaslb1 raaslb2 raaslb3
raaslb4 raaslb5 raaslb6 raaslc1 raaslc2 raaslc4 raaslc5 raaslc6 raaslc7 raaslfem
BY gen
/STATISTICS DESCRIPTIVES
/MISSING ANALYSIS .
```

Figure 6. SPSS syntax file program for generating unadjusted and adjusted standard deviations, means and standard errors

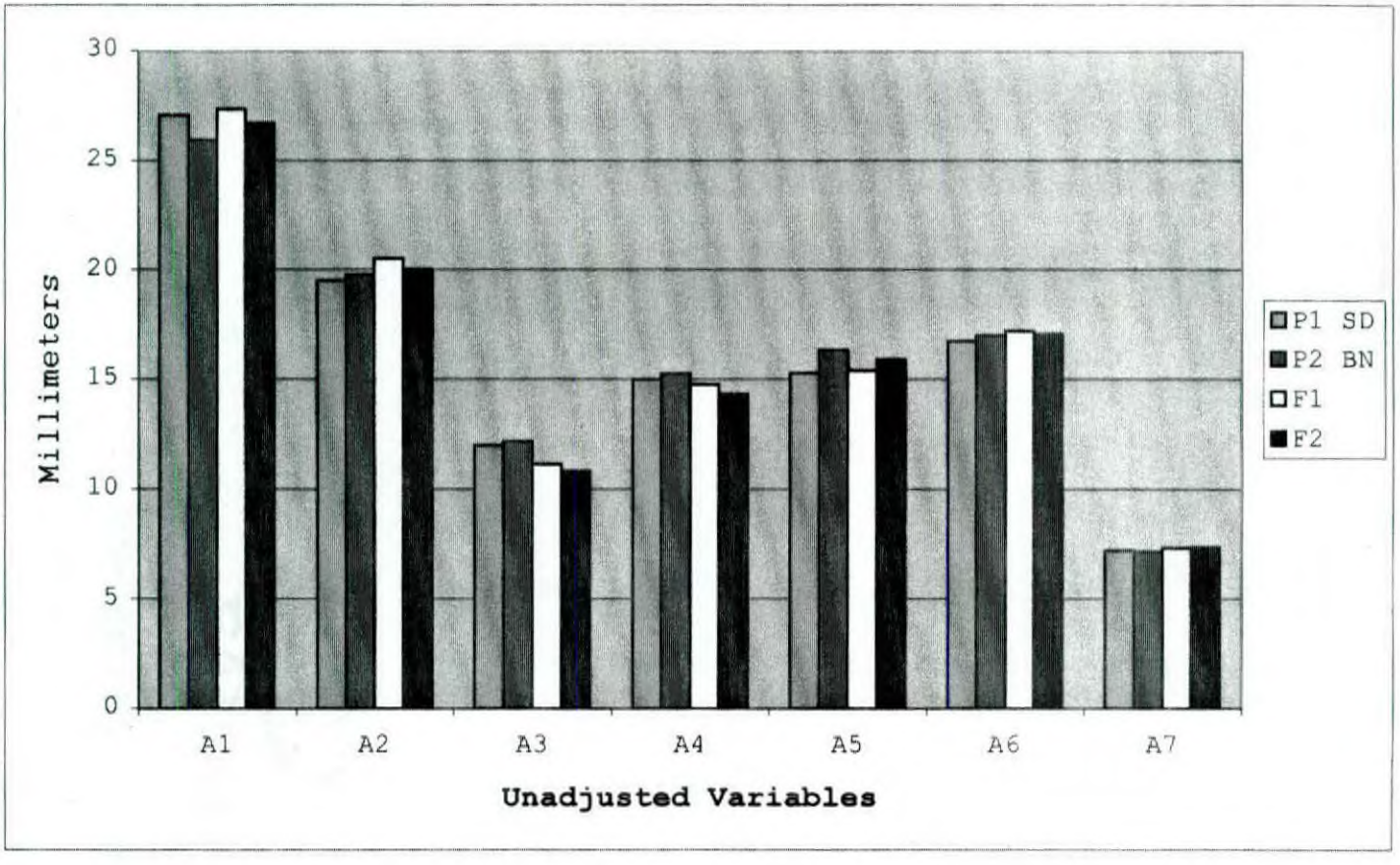


Figure 7. Unadjusted measurement means in millimeters for variables A1-A7. F0 parent generation broken down into Sprague-Dawley (SD) and Brown Norway (BN) strains. F1 and F2 generations also displayed

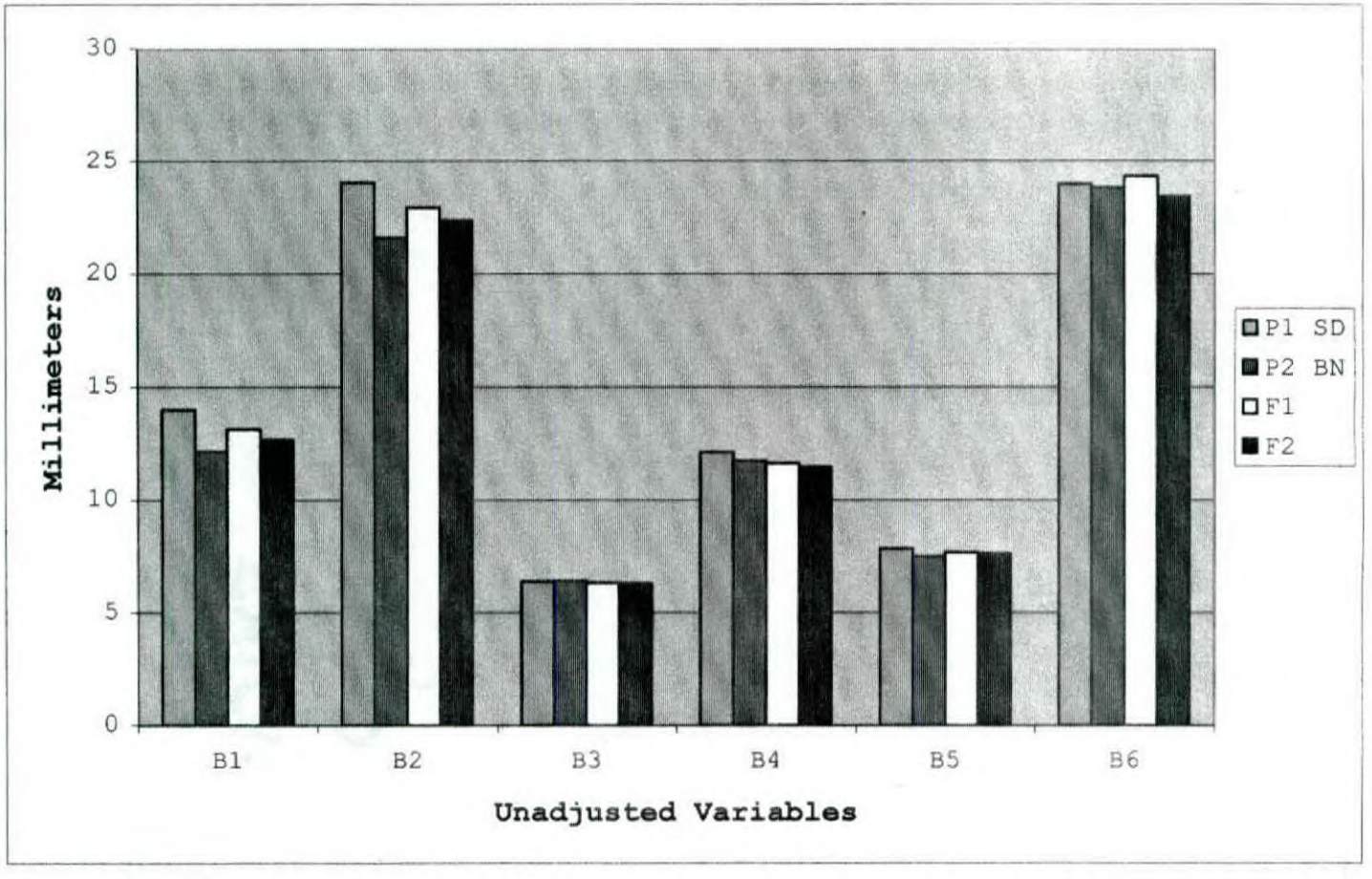


Figure 8. Unadjusted measurement means in millimeters for variables B1-B6. F0 parent generation broken down into Sprague-Dawley (SD) and Brown Norway (BN) strains. F1 and F2 generations also displayed

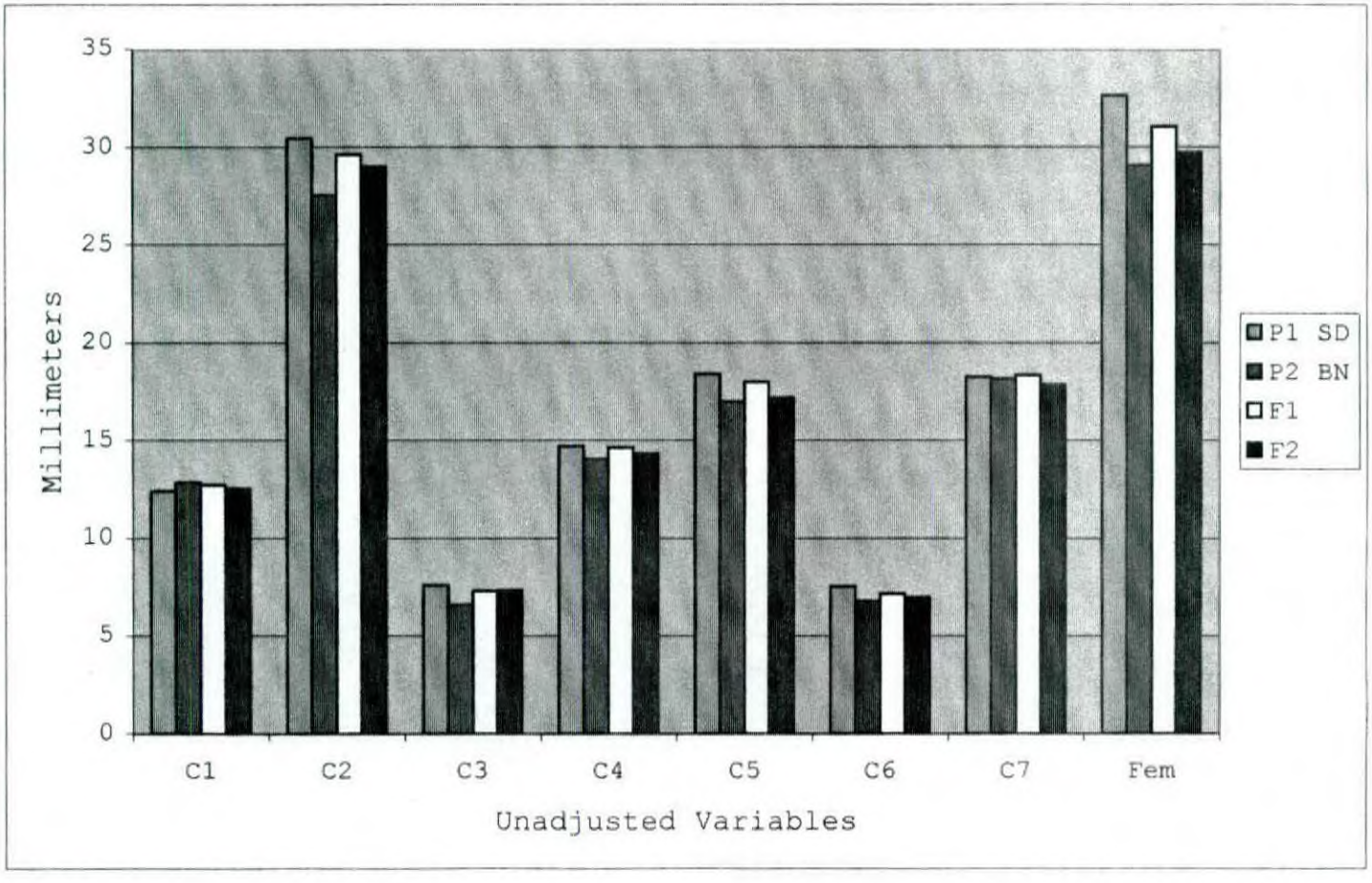


Figure 9. Unadjusted measurement means in millimeters for variables C1-C7 and femur. F0 parent generation broken down into Sprague-Dawley (SD) and Brown Norway (BN) strains. F1 and F2 generations also displayed

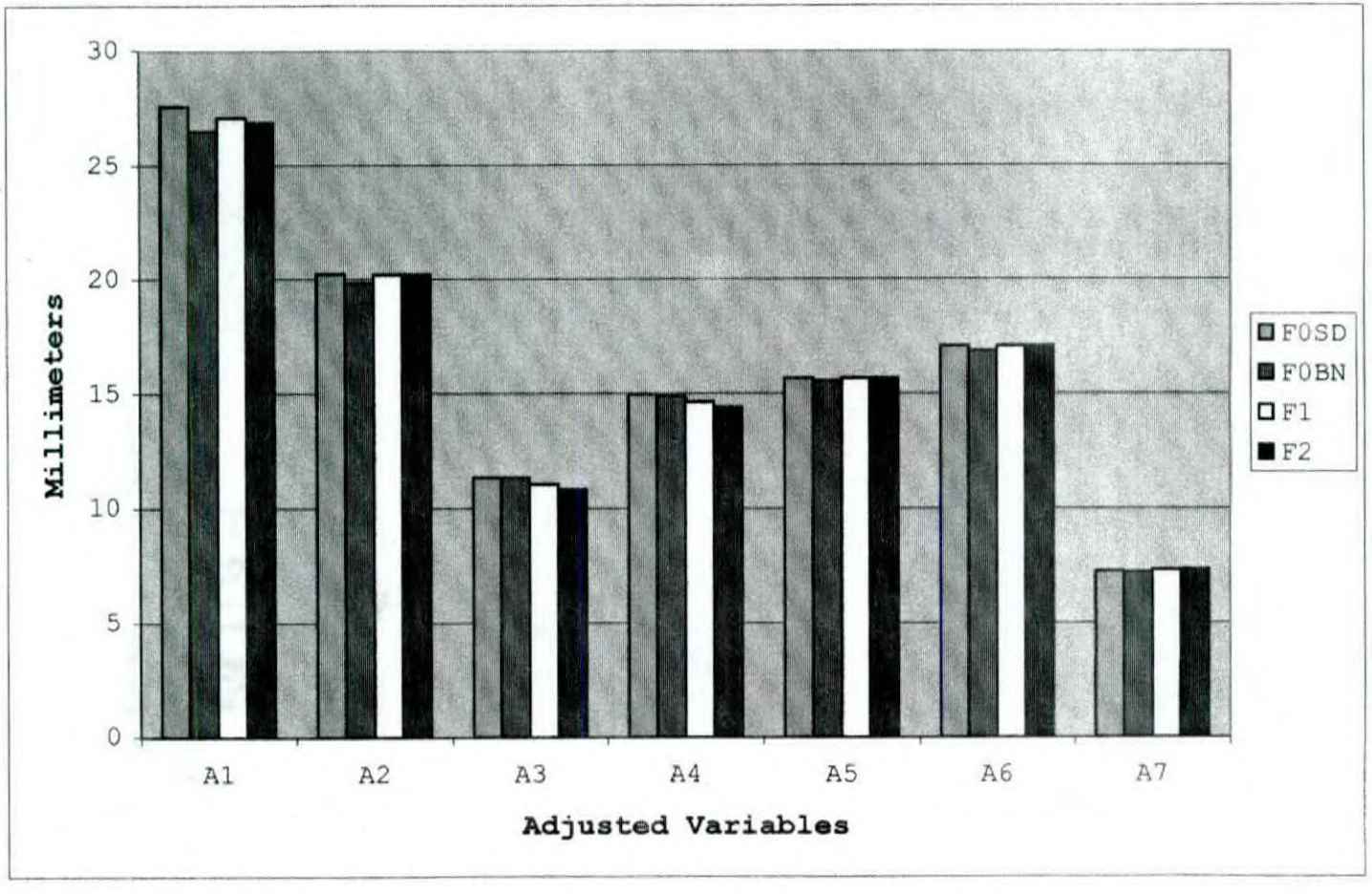


Figure 10. Adjusted measurement means in millimeters for variables A1-A7. F0 parent generation broken down into Sprague-Dawley (SD) and Brown Norway (BN) strains. F1 and F2 generations also displayed

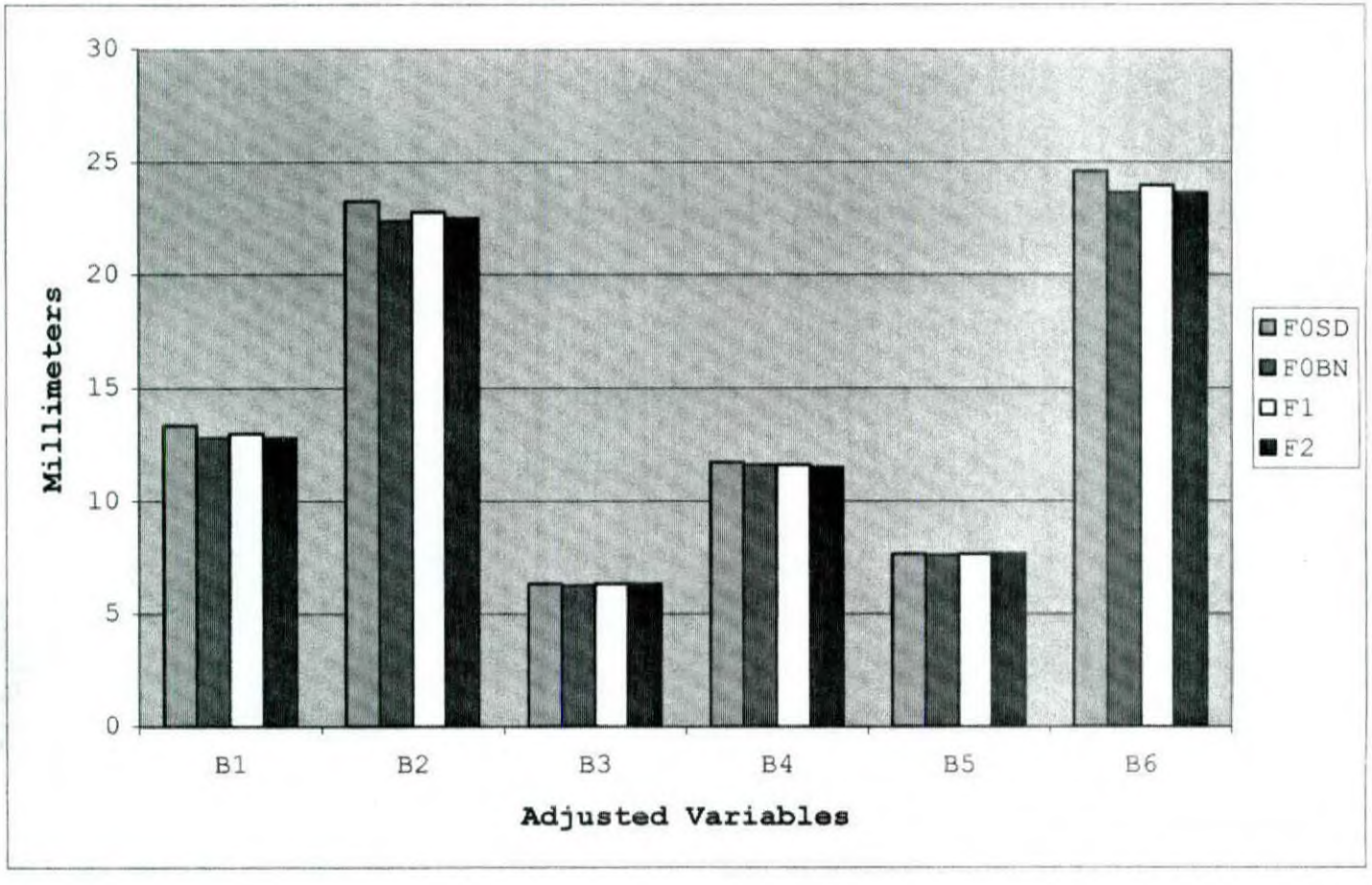


Figure 11. Adjusted measurement means in millimeters for variables B1-B6. F0 parent generation broken down into Sprague-Dawley (SD) and Brown Norway (BN) strains. F1 and F2 generations also displayed

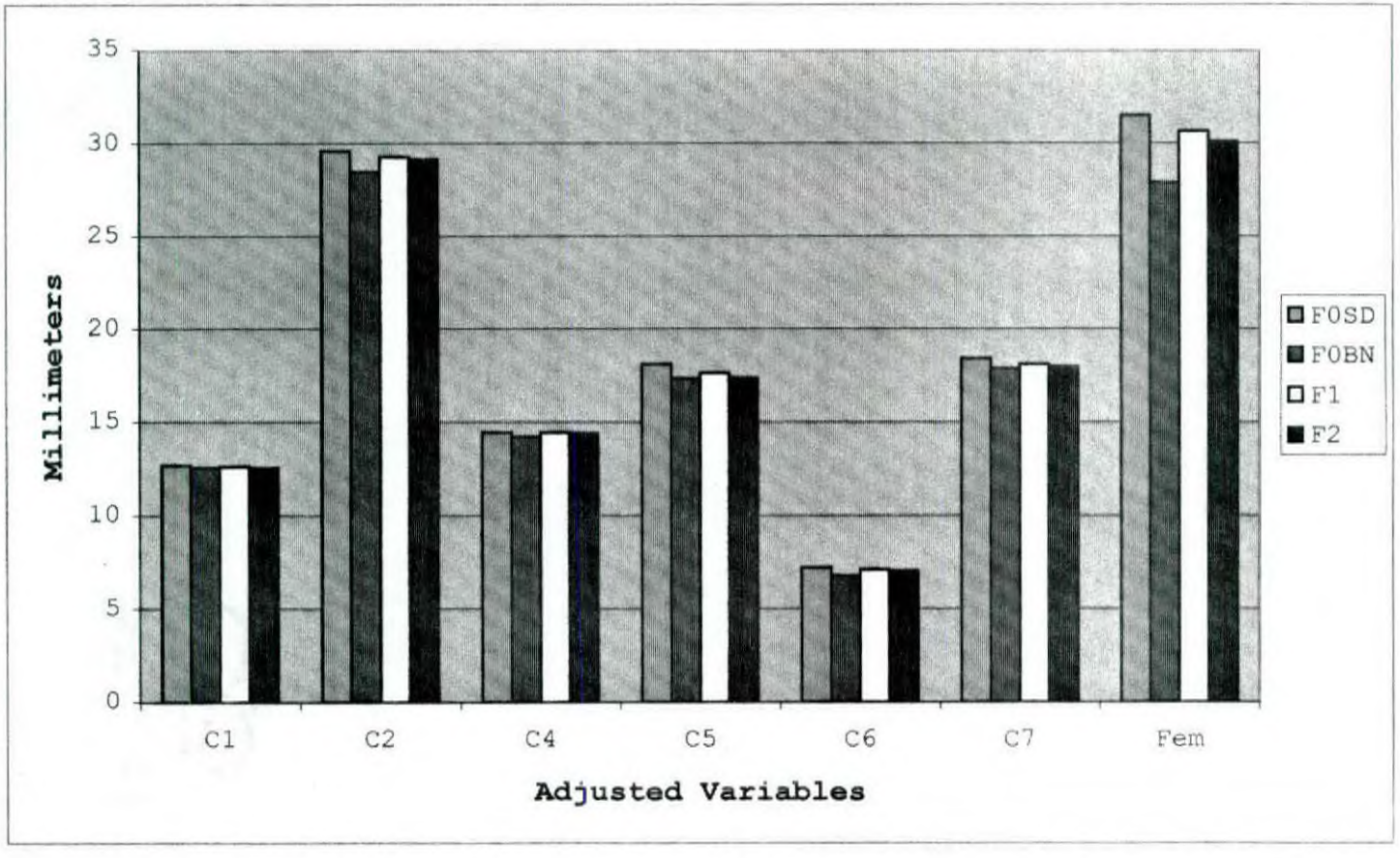


Figure 12. Unadjusted measurement means in millimeters for variables C1-C7 and femur. F0 parent generation broken down into Sprague-Dawley (SD) and Brown Norway (BN) strains. F1 and F2 generations also displayed

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