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RESEARCH ARTICLE

The Skull Shape Analysis of Wistar Albino Rats: A Geometric Morphometric Study

İftar GÜRBÜZ [1*], Yasin DEMİRASLAN[1]

[1]Department of Anatomy, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

ABSTRACT

The aim of the study was to evaluate the skull shape of *Wistar albino* rats using the geometric morphometry method. For this purpose, a total of 52 adult rat skulls, 31 female and 21 male, were evaluated. Skulls were photographed from equal distances in dorsal and ventral directions. 13 and 18 landmarks were marked in dorsal and ventral images, respectively. Percentage variation values of principal components were calculated by performing principal components analysis in the MorphoJ program. Average shape graphs showing the positive and negative boundaries of the first three principal components were obtained. It was defined that scatter plot showing the degree of grouping of male and female rats according to the first and second principal components. The study also examined the allometric effect with regression. Discriminant function analysis was performed to examine gender dimorphism. As a result of the study, it was determined that the most significant shape change was in the zygomatic arc and palate regions according to individuals, while it was also in the neurocranium according to gender. We believe that the study will contribute to anatomy, biology and archeology studies on the shape of the skull.

Keywords: Gender, Geometric Morphometry, Rat, Shape, Skull.

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Wistar Albino Ratların Kafatası Şekil Analizi: Bir Geometrik Morfometrik Çalışma

ÖZ

Çalışmada *Wistar albino* ratlarda kafatası şeklinin geometrik morfometri yöntemi ile değerlendirilmesi amaçlandı. Bu amaçla erişkin 31 dişi, 21 erkek olmak üzere toplam 52 adet rat kafatası değerlendirildi. Kafatasları dorsal ve ventral yönde eşit uzaklıktan fotoğraflandı. Dorsal görüntülerde 13, ventral görüntülerde 18 landmark işaretlendi. MorphoJ programında temel bileşenler analizi yapılarak temel bileşenlerin yüzde varyasyon değerleri hesaplandı ve ilk üç principal componentin pozitif negatif sınırlarını gösteren ortalama şekil grafikleri elde edildi. Erkek ve dişi ratların birinci ve ikinci temel bileşene göre gruplanma derecelerini gösteren dağılım grafiği tanımlandı. Çalışmada ayrıca regresyon ile allometrik etkiye bakıldı. Cinsiyet farkını incelemek için discriminant (ayırma) analizi yapıldı. Çalışma sonucunda wistar rat kafatasında bireyler arasında en belirgin şekilsel değişimin arcus zygomaticus (elmacık kemeri) ve damak bölgesinde olduğu, cinsiyete göre ise neurocranium da olduğu belirlendi. Çalışmanın, kafatasında cinsiyet farklılığı ile ilgili anatomi, biyoloji ve arkeoloji çalışmalarına katkı sağlayacağı kanaatindeyiz.

Anahtar Kelimeler: Cinsiyet, Geometrik Morfometri, Kafatası, Rat, Şekil

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ORCID ID; *İG: 0000-0001-9460-0645, YD: 0000-0003-3612-6142*

*Corresponding author e-mail: iftargurbuz@mehmetakif.edu.tr

INTRODUCTION

The *Wistar albino* rat is an outbred albino rat. This breed was developed at the Wistar Institute in 1906 for use in biological and medical research. It was the first rat to be developed as a model organism at a time when laboratories were primarily using the house mouse (Mus musculus). The Wistar rat is currently one of the most popular rats used in laboratory research. It is characterized by its broad head, long ears, and tail length that is always shorter than its body length (Clause 1998).

One of the structures frequently used in morphometric research on the skeletal system is the skull. The bones that make up the skull are in the flat bones group. Anatomically, the skull is examined in two parts: viscerocranium (facial bones) and neurocranium (cranial bones) (Özcan and Demiraslan 2022). The morphological and morphometrical structure of these bones differs according to species and gender (Çalışır et al. 2023). For this reason, comparative morphometric or geometric morphometric shape analyses on the skull are a real tool in the classification of rats and identification of species (Musser et al. 2005; Odigie et al. 2018).

The geometric morphometry method is a field of study that investigates and analyzes the variations and changes seen in structural (morphological) features by using landmarks on the surface area that is examined. It allows the comparison and interpretation of structural differences owing to visual graphs obtained with Cartesian coordinates (Slice 2007; Slice et al. 2010; Mitteroecker and Gunz 2009).

Statistically significant differences were determined in the skull related to structural changes due to bone development and function (Reichs and Bass 1998). Research on gender dimorphism in the skull is frequently carried out using the morphometry method (Karaavcı et al. 2024; Gündemir et al. 2023a, 2023b; Dayan et al. 2023; Szara et al. 2022; Gürbüz et al. 2022; Demircioğlu et al. 2021). Researches have previously been carried out in rat skulls with morphometric methods based on the linear measurement method (Olude et al. 2009). However, no study was found that evaluated the structural properties of the skull in *Wistar albino* rats using the geometric morphometric method. In this study, it was aimed to determine the regions where there are structural differences and similarities in the skull of *Wistar albino rats* and to compare them according to the gender factor.

MATERIALS and METHODS

Animals Ethical Approval

The necessary permission for the study was obtained from Burdur Mehmet Akif Ersoy University, Animal Experiments Local Ethics Committee (Date: 29.03.2023, No:996).

Materials

A total of 52 rat (Wistar albino) skulls, 31 female and 21 male, were used in the study. The rats used in the study were chosen from those raised in similar care units and were adult and healthy. Analysis began after the skulls were cleaned of their buttocks by maceration.

Geometric Morphometry

Rat skulls were photographed from equal distances, dorsal and ventral view. In the photographed images, 13 and 18 homologous landmark points were detected from the dorsal direction (Figure 1) and ventral direction (Figure 2), respectively. TPS files of the images were created using the TPSUtil (version 1.79) program (Rohlf 2019). Homologous landmarks detected in TPS Dig2 (Version 2.31) (Rohlf 2018). The program were marked. Thus, the *x* and *y* Cartesian coordinates of the homologous landmarks on the skull were determined. The suitability of landmarks was tested in TPS small (version 1.34) program and their correct placement was proven. In dorsal images the slope and correlation score were determined as 0.99820 and 1.0000 respectively; in ventral images, the slope and correlation score were determined as 0.999895 and 1.0000.

Statistical Analysis

MorphoJ software was used in the study for statistical analysis and evaluation of shape differences (Klingenberg, 2011). In this program, firstly, Generalized Procrustes Analysis (GPA) was performed to eliminate differences in skull placement and position in the photographs (Slice 2007). Thus, the landmarks on images were superimposed, new coordinates were determined and Principal Component Analysis (PCA) was performed. Degrees of interindividual variation were determined as percentage (%) values (Zelditch et al. 2012; Villalobos-Leiva and Benitez 2020). To determine whether shape changes in the skull depending on size, regression and allometric effects were examined. Additionally, Discriminant Function Analysis was performed to determine the differences in skull shape according to gender factors (Zelditch et al. 2012).

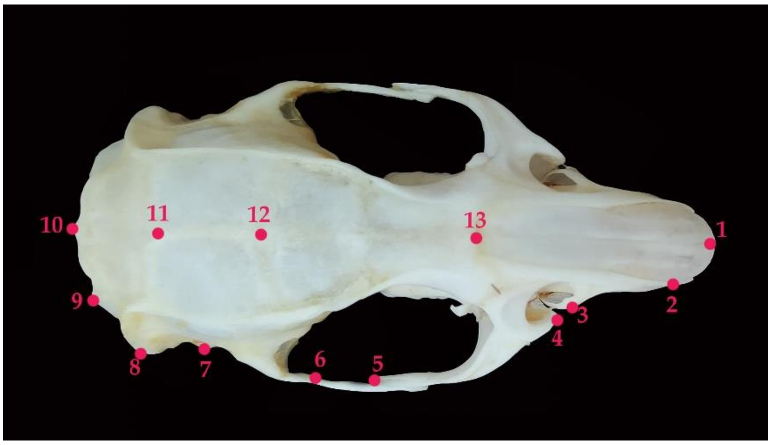

Figure 1. The landmarks on the dorsal view of rat skull

1. Cranial point of incisive bone (os incisivum), 2. Craniolateral point of nasal bone (os nasale), 3. The most lateral point of maxilla, 4. The most cranial point of zygomatic arc (arcus zygomaticus), 5. At the caudal end of maxillar process (processus maxillaris) of zygomatic arc (arcus zygomaticus), 6. At the cranial end of temporal process (processus temporalis) of zygomatic arc, 7. External meatus acusticus (meatus acusticus externus), 8. The lateral point of temporal bone (os temporale), 9. The most caudolateral point of occipital bone (os occipitale), 10. Caudomedial point of occipital bone, 11. The stura between occipitale and parietal bone (os parietale) (Lambda), 12. The sutura between parietal and frontal bone (os frontale) (Bregma), 13. The sutura between frontal and nasal bone.

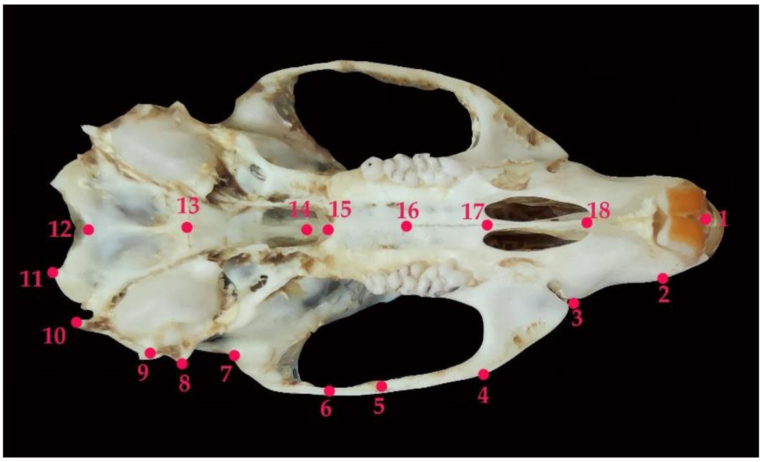

Figure 2. The landmarks on the ventral view of the rat skull

1. Cranial point of incisive bone, **2.** The most craniolateral point of maxilla, **3.** Lateral point of infraorbital foramen (*foramen infraorbitale*), **4.** The most craniolateral point of zygomatic arc, **5.** At the caudal end of maxillar process of zygomatic arc, **6.** At the cranial end point of temporal process of zygomatic arc, **7.** Caudal angle of zygomatic arc, **8.** Cranial point of external meatus acusticus, **9.** Caudal point of external meatus acusticus, **10.** Jugular pocess (*processus jugularis*), **11.** Caudal point of occipital condyle (*condylus occipitalis*), **12.** Ventral point of foramen magnum, **13.** Sutura between occipital (*os occipitale*) and sphenoid (*os sphenoidale*) bone, **14.** Medial point of sutura between sphenoid and vomer bone, **15.** Caudomedial point of palatina (*os palatinum*), **16.** At the medial sutura between palatinum and maxilla, **17.** Craniomedial point of palatine process (*processus palatinus*) of maxilla, **18.** Caudomedial point of incisive bone

RESULTS

In the study, according to the principal component analysis performed in the dorsal direction, a total of 22 principal components were calculated and 32 principal components were calculated in the ventral images. In dorsal oriented images, the first principal component had a degree of variation of 19.431%, and in ventrally oriented images it had a degree of variation of 20.758%. In the dorsal and ventral images, the degree of variation in the first three principal components was determined as a total of 48.923% and 47.052%, respectively. The percentage variation values of the sequential principal components were determined to be close to each other (Table 1, Table 2).

Table 1. Principal component analysis on dorsal view of rat skulls

PC	Eigenvalues	%Variance	PC	Eigenvalues	%Variance
1	0.00016825	19.431	12	0.00001836	2.121
2	0.00014692	16.969	13	0.00001617	1.868
3	0.00010843	12.523	14	0.00001298	1.499
4	0.00007522	8.688	15	0.00001022	1.180
5	0.00007383	8.527	16	0.00000746	0.861
6	0.00005130	5.925	17	0.00000541	0.624
7	0.00004249	4.908	18	0.00000472	0.545
8	0.00003932	4.541	19	0.00000355	0.409
9	0.00002922	3.374	20	0.00000250	0.288
10	0.00002586	2.987	21	0.00000202	0.233
11	0.00001995	2.304	22	0.00000168	0.194

Table 2. Principal component analysis on ventral view of rat skulls

PC	Eigenvalues	%Variance	PC	Eigenvalues	%Variance
1	0.00018902	20.758	17	0.00001031	1.132
2	0.00014718	16.163	18	0.00000961	1.055
3	0.00009225	10.131	19	0.00000843	0.926
4	0.00008292	9.107	20	0.00000481	0.528
5	0.00006442	7.075	21	0.00000444	0.487
6	0.00005182	5.691	22	0.00000368	0.404
7	0.00004343	4.769	23	0.00000294	0.323
8	0.00003264	3.585	24	0.00000286	0.314
9	0.00002875	3.157	25	0.00000211	0.232
10	0.00002711	2.977	26	0.00000189	0.207
11	0.00002236	2.455	27	0.00000158	0.174
12	0.00002096	2.302	28	0.00000135	0.149
13	0.00001465	1.608	29	0.00000097	0.107
14	0.00001424	1.564	30	0.00000049	0.053
15	0.00001209	1.328	31	0.00000045	0.049
16	0.00001044	1.147	32	0.00000040	0.044

As a result of principal component analysis, PC1, PC2 and PC3 graphics were presented in Figure 3. It was observed that the most change was at Landmark 5, 6, 7, 12, and 13 levels in dorsal images and at Landmark 1, 2, 3, 4, 5, 6, and 7 levels in ventral images. It was determined that the greatest change in ventral and dorsal images was at the arcus zygomaticus level. However, it was determined that the change was mostly on neurocranium in dorsal images, but also mostly on viscerocranium in ventral images. The change in positive-negative boundaries was greater in ventral images than in dorsal images.

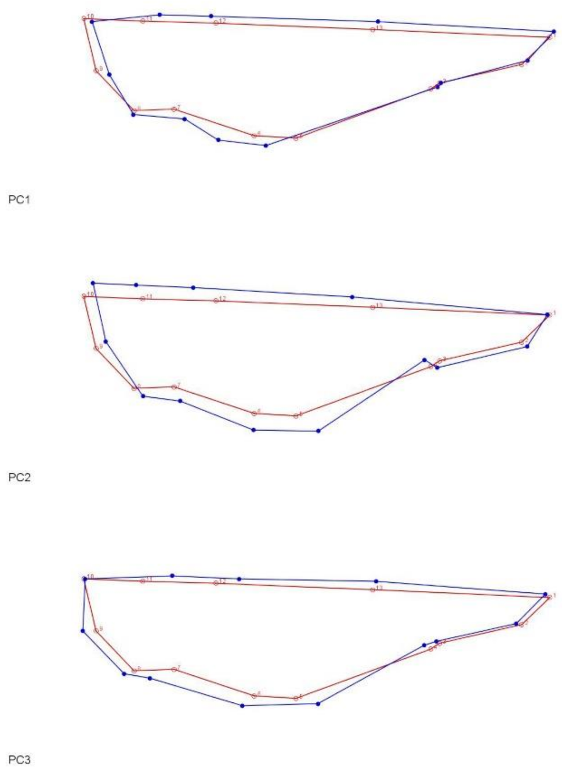

Figure 3. Shape changes in dorsal images of the skull relative to PC1, PC2 and PC3 (Blue outlines represent the average shape configuration, while red outlines indicate shape changes associated with the positive ends of the PC axes)

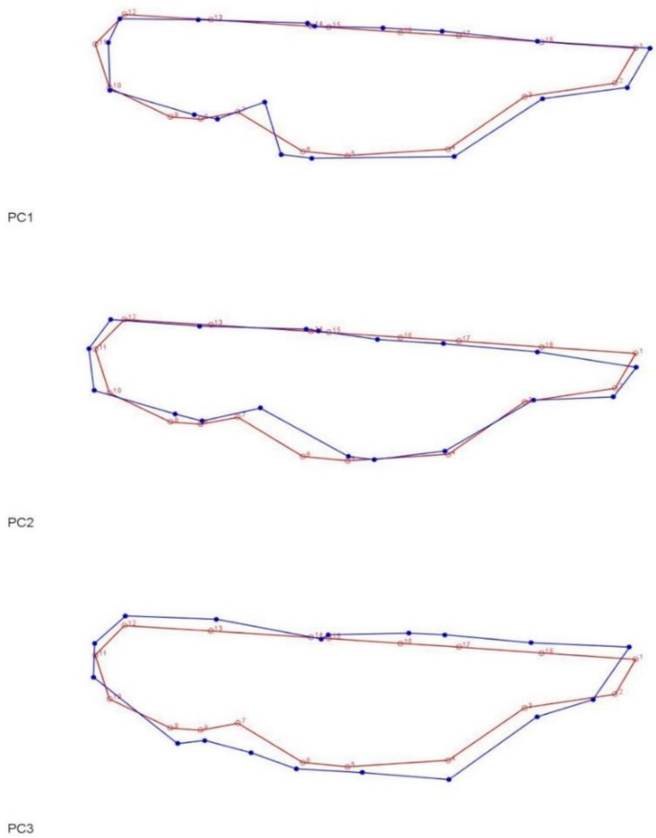

Figure 4. Shape changes in ventral images of the skull according to PC1, PC2 and PC3 (Blue outlines represent the average shape configuration, while red outlines indicate shape changes associated with the positive ends of the PC axes)

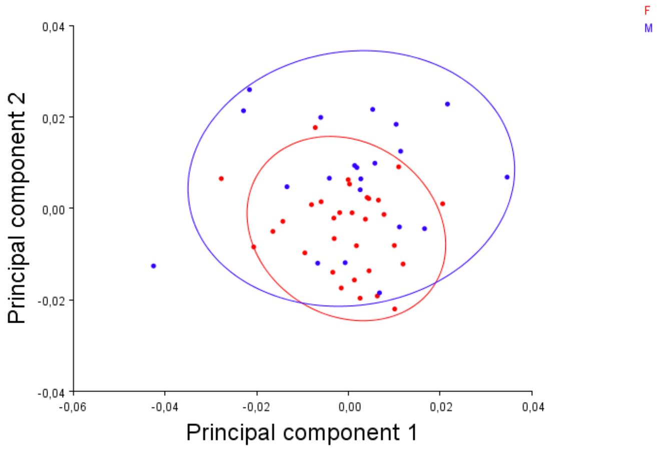

Figure 5. Scatter plot on the principal component axis 1 and principal component axis 2 for sexes on the dorsal images (Blue: male, Red: female).

In Figure 5 and Figure 6, distribution graphs of the skulls in dorsal and ventral images were presented. Scatter plots showed the degree to which skulls were grouped by gender. Accordingly, it was seen that the male and female skulls were not clearly separated from each other.

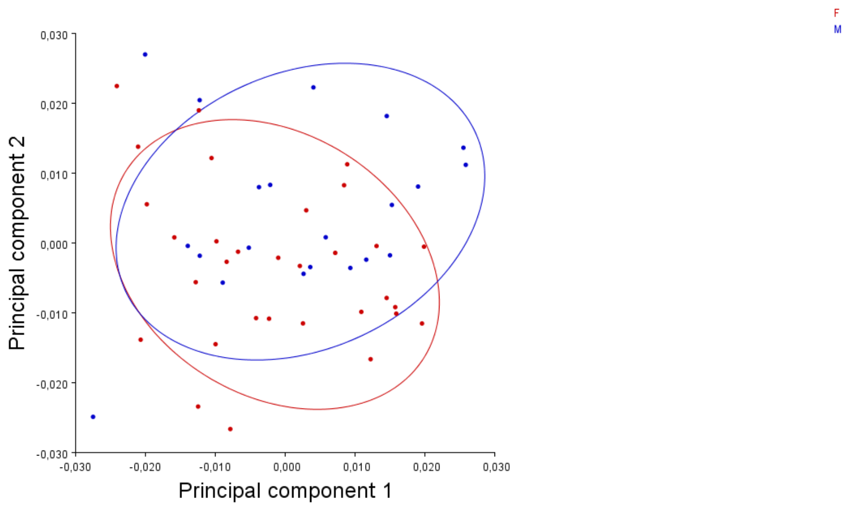

Figure 6. Scatter plot on the principal component axis1 and principal component axis 2 for sexes on the ventral images (Blue: male, Red: female).

Before discriminant function analysis, the allometric effect was examined by regression to evaluate whether the size effected on the shape. The values are presented in the Table 3. Accordingly, the effect of size on shape was statistically significant ($p<0.05$). This effect was removed from the data set with multivariate analysis (Residuels/prediction from regression) and then the analysis continued.

Table 3. Regression analysis for sexual dimorphism comparison in centroid size, in both ventral and dorsal views of rat skulls.

Skull view	Group (n)	%Predicted	p value
Dorsal	Males (21), females (31)	5.2372	0.0033
Ventral	Males (21), females (31)	3.9063	0.0289

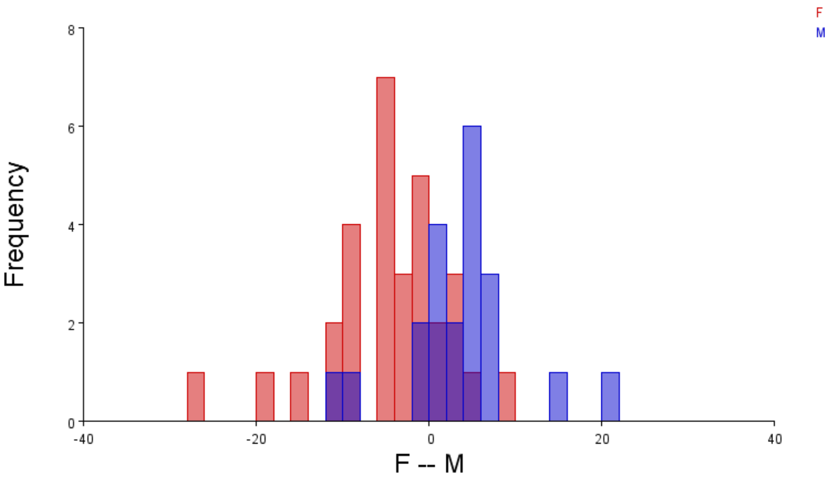

Figure 7. Cross validation score of discriminant function analysis on the dorsal view (Red: Female rat skull, Blue male rat skull).

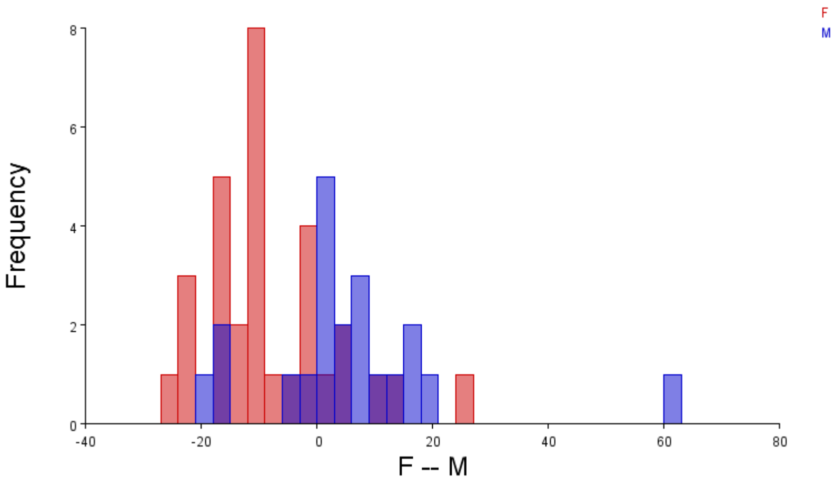

Figure 8. Cross validation score of discriminant function analysis on the ventral view (Red: Female rat skull, Blue male rat skull).

The cross-validation score determined by discriminant function analysis was shown in the graph in Figures 7 and 8. These graphs show the distribution of male and female skulls. According to this analysis, it is seen that 17 skulls from males and 24 skulls from females were grouped correctly in dorsal images, and 16 skulls from males and 25 skulls from females were grouped correctly in ventral images. As a result of dorsal analysis, Procrustes and Mahalanobis distance were determined as 0.0154 ($p<0.0001$) and 3.2274, respectively, and in ventral images, these values were determined as 0.0173 ($p<0.0001$) and 4.4596, respectively. The average shape obtained as a result of discriminant function analysis is shown in Figures 9 and 10. In this figure, the general shape of the skull of male and female rats was similar. However, when looking at the average shape of males and females in dorsal and ventral images, small differences were observed in the neurocranium.

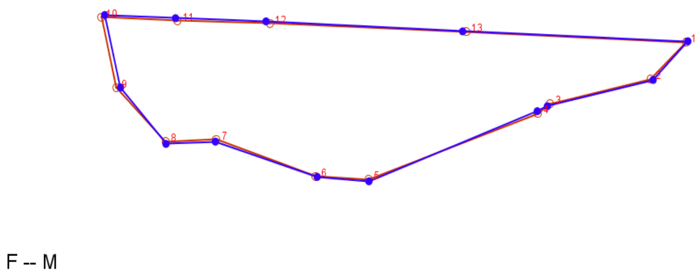

Figure 9. Discriminant function analysis on the dorsal view. (Red: female rat skull, Blue: male rat skull).

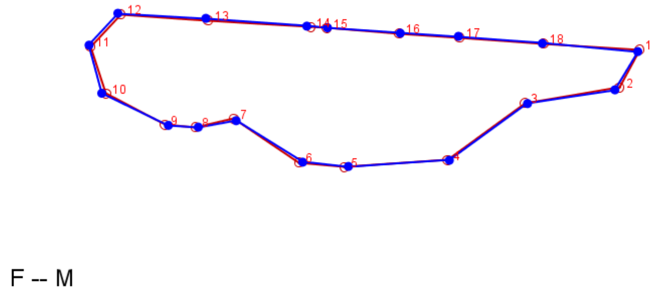

Figure 10. Discriminant function analysis on the ventral view. (Red: female rat skull, Blue: male rat skull)

DISCUSSION

In studies conducted on the skulls of rodents from different species, it has been reported that the structural differences observed on the cranium may be due to nutritional differences (Samuel et al. 2015). For example, African giant rats have been reported to be fed with household waste and less fibrous foods, and *Thryonomys (T.) swinderianus* species rodents have been reported to be fed with roughage and foods with high fiber content. It has been stated that this situation may cause changes in the anatomical parameters of the skull (Samuel et. al., 2015). Samuel et al. (2015), in a study conducted on the skulls of two species of African rodents, reported that although both rodents had similar tooth and jaw structures, the maxillo-dental structure of *T. swinderianus* was longer than that of *Cricetomys gambianus*. Researchers (Samuel et al. 2015) stated that partial shape changes in the bone structure are observed with strong horizontal movements of the head during chewing, and therefore the chewing movement causes specific changes in the morphological structure of the skull and jaw. In the study, it is thought that the shape difference detected in the zygomatic arc region where the chewing muscles attach, and in the palate region may be due to the chewing movement (biting force). Identification of the characters that constitute cranial variation is important for determining population history, adaptation, and genetic structure (Lieberman et al. 2000; Abdel-Rahman et al. 2009; Herrel et al. 2012). Differences in the shape and size of the skull allow understanding of its development from embryo to adult (Herrel et al. 2012). During the developmental process, the morphological structure of the cranium varies according to age, genetic and environmental factors. One of these sources of phenotypic variation is gender difference (Gannon and Racz 2006). Phenotypic variation based on gender differences is important for animal behavior, ecology, generation mobility, and evolution (Leblanc et al. 2001). Shape differences can be detected according to gender factor with morphometric methods. In a morphometric study conducted with linear measurements on Black-hooded rats (Hughes et al. 1978), it was determined that skull measurements in male rats were significantly bigger than in females, but there was no difference between genders in cranial and facial indices. Çalışır et al. (2023) reported that the craniofacial index value was statistically significant according to the breed factors in male rats. However, Çalışır et al. (2023) stated that there is no difference in cranial and facial indices according to gender. In this study, when compared by

gender using discriminant function analysis, small differences were observed in the neurocranium, while no significant difference was observed in the viscerocranium.
Another structure that affects shape changes in the skull is the mechanical role of cranial sutures (Sharp et al. 2023). In a study on the role of cranial suture's in the rat skull (Sharp et al. 2023), it was reported that cranial sutures in regions such as the temporomandibular joint or maxilla may be more significantly affected than other regions during feeding. However, it has been emphasized that these sutures surrounding the brain (neurocranium) may be important in allowing the brain to expand rather than biting power during growth. In this study, it was determined that the structural variation of the skull between individuals was observed mostly in the part where the chewing muscles attach (zygomatic arc, palate region), while the variation according to gender was found in the neurocranium. While this variation determined between individuals is more related to chewing function, it is thought that the variation determined by gender may be more related to the genetic and developmental processes.

CONCLUSION

As a result, in the inter-individual comparison, the shape changes were evident in the functional areas to which the chewing muscles attach, especially on the zygomatic arc region and the palate region. In this study, we also tried to determine the effect of gender factors on skull shape in rats. In the gender comparison, although male and female skull shapes were observed to be generally similar, minor shape changes were observed on the neurocranium. Accordingly, it was determined that the neurocranium of male rats in dorsal images and female rats in ventral images was slightly narrower. As a result of the evaluation made according to the grouping characteristics, it was determined that 80% of the males in the dorsal images and 80% of the females in the ventral images were grouped correctly. When all the findings of the research were evaluated, it was concluded that despite the differences between the genders, these features were not sufficient to distinguish the two genders from each other.

Conflict of Interest: The authors have no conflicts of interest to report.

Authors' Contributions: İG and YD contributed to the project idea, design, and execution of the study. İG contributed to the acquisition of data. İG analyzed the data. İG drafted and wrote the manuscript. YD reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical Approval: This study was carried out at Burdur Mehmet Akif Ersoy University, Animal Experiments Local Ethics Committee (Date: 29.03.2023, No:996).

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