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Sexual Dimorphism of the Maxilla in European Hare Using Geometric Morphometric Techniques

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ABSTRACT In this study, 40 European hares (20 male and 20 female) that were obtained from the provinces of Elazığ and Malatya in Turkey, were used. The samples were evaluated by placing 23 landmarks on the ventral of the maxilla. After the landmarks were placed onto the pictures, which were developed by means of Thin Plate Spline (TPS) program, the landmarks were standardized by using Morpheus program. Pairwise test, Principle Component Analysis (PCA) and Discriminant Function Analysis (DFA) were applied to the data. The result of the statistical analysis with geometric morphometric techniques showed no sexual dimorphism (SD) in Europan hares (*Lepus europaeus).*

Keywords: *Lepus europaeus, European hare, Sexual dimorphism, Geometric morphometric techniques, Maxilla*

ÖZ

Avrupa Tavşanlarının Maxillalarında Geometrik Morfometrik Yöntemler Kullanılarak Eşeysel Dimorfizmin Araştırılması*

Bu çalışmada, Türkiye'nin Elazığ ve Malatya illerinden elde edilen 40 adet (20 dişi, 20 erkek) Avrupa tavşanı kullanıldı. Örnekler maxilla'ların ventral'ine 23'er adet landmark konularak değerlendirildi. Thin Plate Spline (TPS) programı ile konulan landmarklar Morpheus programı ile standardize edildi. Verilere pairwise testi, Principle Component Analizi (PCA) ve Discriminant Fonksiyon Analizleri DFA) uygulandı. Geometrik morfometri teknikleri kullanılarak yapılan istatistiksel analiz sonuçları Avrupa tavşanı (*Lepus europaeus)* örneklerinde eşeysel dimorfizm olmadığını göstermiştir.

Anahtar Kelimeler: *Lepus europaeus, Avrupa tavşanı, Eşeysel dimorfizm, Geometrik morfometri yöntemleri, Maxilla*

INTRODUCTION

European hares (*Lepus europaeus* Pallas, 1778) are widespread across the world from Central and South Europe and China to Arctic Circle and desert areas (Demirsoy 1995; Demirsoy 1996; Carleton and Musser 2005; Alves et al. 2008). There are a total of 32 European hare species in the world (Chapman and Flux 2008). Only *Lepus europaeus* of them live in Turkey and it is in superabundant (Demirbaş et al. 2010).

Sexual dimorphism (SD) is when the sexes of a species are distinguishable from each other. Although SD is present in all vertebrate taxa it is not noticeable at first sight. Sexual differentiation starts in the embryonic period and may develop in terms of anatomical and physiological characteristics. SD can occur in terms of body size, body composition, skeletal composition, the brain and nervous systems, weaponry, behaviour, metabolism, pelage and body markings, vocalisation and other organs. SD in the skull can only be seen in animals when male ones go head to head against each other. For example, the skull bones of males are thicker because male animals such as sheep and deer compete for females during mating seasons (McPheron and Chenoweth 2012). The level of SD is affected by environmental factors and therefore SD differs in each population (Bigoni et al. 2010). SD is best considered separately in comparative analyses (Sanger et al. 2013). Sexual dimorphism is very important to understand skeletal structures. Although morphologic studies have some difficulties with quantification, SD characteristics can be researched morphologically and metrically. However, geometric morphometric techniques are more suitable to describe subtle differences in SD structures (Gonzales et al. 2011). Geometric morphometric is a new method and allows for the better assessment of morphologic characteristics (Pretorius et al. 2004).

There are no important differences between male and female European hare except that the males have higher shoulders and are more fearful than the females and that tails of the males straighten up whereas tails of the females are adjoint to the body (Huş 1963). The purpose of this study was to apply geometric morphometric techniques to

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*: This study was summarized from a part of PhD thesis of first author

describe SD in the maxilla of European hare. This study explored SD by means of 23 landmarks placed on the ventral maxilla of European hares obtained from the provinces of Elazığ and Malatya in Turkey.

MATERIALS and METHODS

The samples were collected between 2007 and 2008 from the provinces of Elazığ and Malatya, which are located in the east of Turkey. The samples were selected from animals that had died from natural causes. The samples which were 20 male and 20 female European hare were examined in this study. The locations from which the samples were collected can be seen in Figure 1.

Figure 1. Geographical distribution map of the analysed samples of *Lepus europaeus*

The samples were embalmed using the method described by Mursaloğlu (1965). The maxilla of the samples were equipped to technique of maceration according to the method described by Taşbaş and Tecirlioglu (1965). After the maceration the genders were marked on the maxilla. The specimens were photographed with a FinePix s7000 digital camera. In total 23 morphological characters were measured. Thin Plate Spline (TPS), Morpheus, Past and SPSS programs were used for the geometric morphometric techniques.

The landmarks and TPS program were used according to Bookstein (1989) and Rohlf (2002) and the landmarks used are shown in Fig 2. The photographs were developed into TPS files using Tps Util 1.44 to Rohlf (2005). The landmarks defined are as follows:

1. Rostral tip of alveoli of incisive
2. Rostral tip of palatine fissura (incisive foramen)
3. Interface of palatine fissuras with recess in medial
4. Caudal tip of palatine fissura (incisive foramen)
5. The widest point of palatine fissura (incisive foramen)
6. Anterior extremity of the toothrow
7. Vanishing point of rostrum and zygomatic arch in the ventral
8. Most anterior point of the orbit
9. Caudal end of the hindmost molar tooth
10. Pterygoid process
11. Rostral tip of the orbit
12. Styloglosal process of the tympanic bullae
13. Occipito-caudale
14. Opistocranion
15. Caudal tip of the foramen magnum (Opisthion)
16. Caudal end of the medial edge of the occipital condyle (top point of the foramen magnum)
17. Rostral end of the occipital foramen in the midline (basion)
18. Foramen ovale
19. Widest top point of the sphenoid bone
20. The rostral interface point of the sphenoid bone on the medial line
21. Basisphenoid foramen
22. Rostral end of the curvature of the palatine bone (Lamina horizontalis ossispalatini)
23. Palatal fenestrae

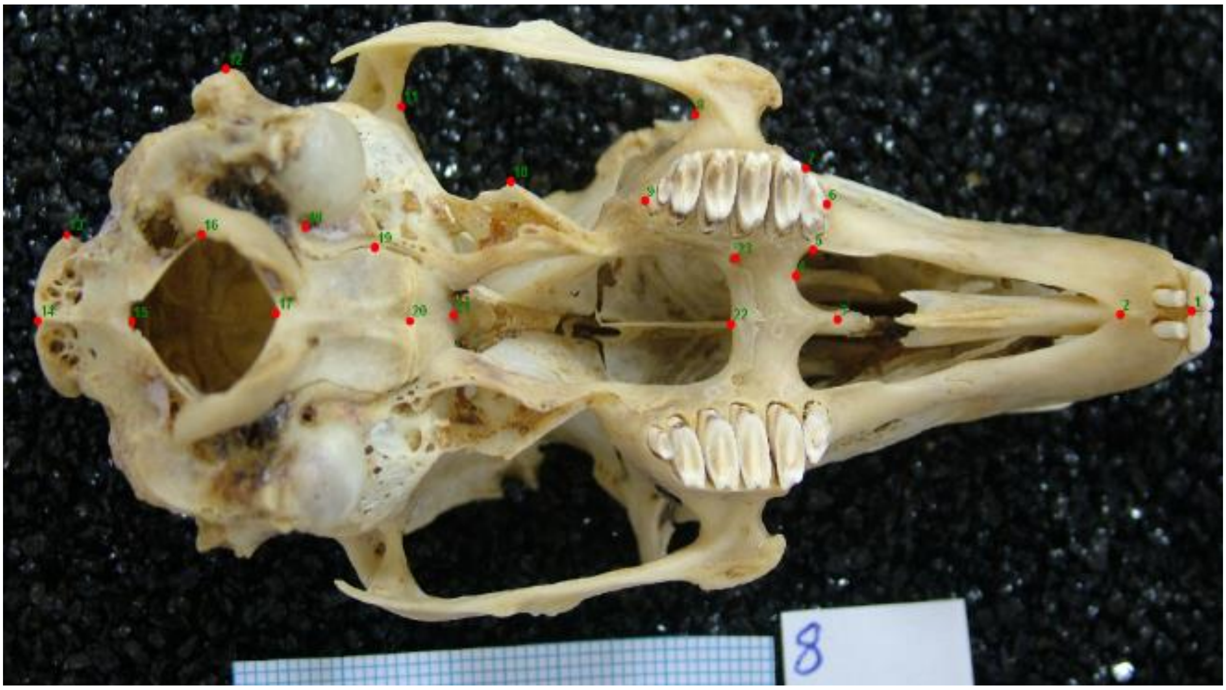

Figure 2. Landmarks placed on the ventral side of the maxilla

The landmarks were placed on the ventral side of the maxilla using TpsDig 2.14 to TPS files. The marking landmarks attended to Elbroch (2006). The landmarks were tested for correctitude using TPS small 1.20. In these tests the correlation value was taken between tangent space (y coordinate) and procrustes space (x coordinate). The correlation value was 1 and the slope value close to 1. Shapes can be defined in a configuration of landmark points after the differences due to location, scale and orientation are removed with landmark-based analyses (Bookstein 1991; Bookstein 1996).

The effects of location, scaling and orientation were typically removed using Generalized Procrustes Superimposition (GPA) (Rohlf 1999; Slice 2001) with respect to a common reference form. For correlation between morphometric characters, Principle Component Analyses were carried out using 2.0 version of the Past program. The differences inside each gender were minimised to be seen the differences between genders were applied Discriminant Function Analyses (DFA) using the SPSS 17.0. Consequently, SD tested between European hare groups from the provinces of Elazig and Malatya in Turkey.

RESULTS and DISCUSSION

Characteristics of the businesses

In the present study the Past program was used to test the differences between the maxilla of male and female European hares. Thus, the samples were grouped according to their sex in the mdt file in Morpheus program after TPS. Consequently, it was produced as an excel file. The first group consisted of females and the second group consisted of males and the number of samples in each group was 20. Multivariate Principal Component Analyses were applied to both groups.

As a result, the European hare male and female individuals were compared using Multivariate Principal Component Analyses in the past program. In these analyses, which explored SD in the same species groups no difference was seen in terms of maxilla namely SD(Fig 3-5).

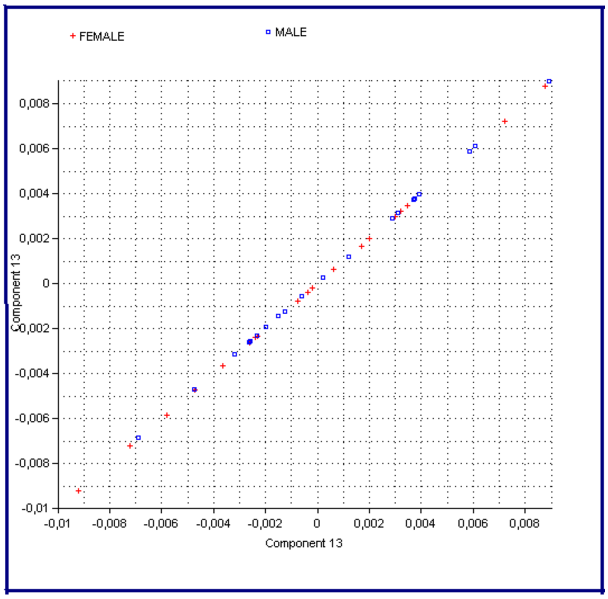

Figure 3. The graphics of Principal Component Analyses for SD in *Lepus europaeus* (a)

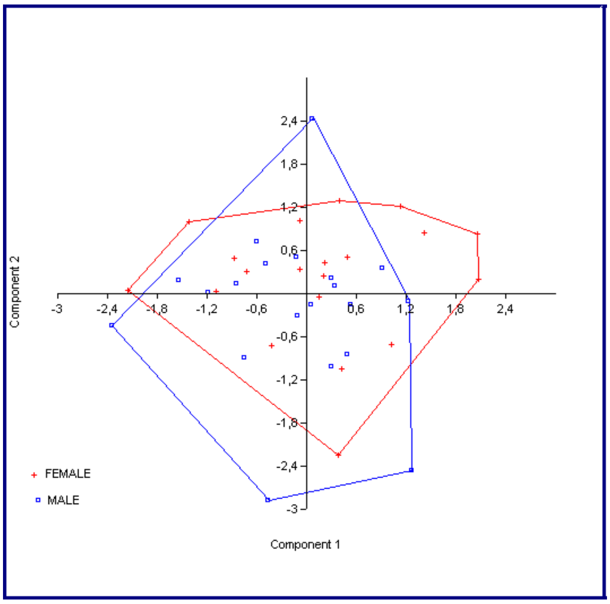

Figure 4. The graphics of Principal Component Analyses for SD in *Lepus europaeus* (b)

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Pair-wise test summary...
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pTest options [possible]...

    nSamples: 999
       alpha:     0.050

Data file: C:\DOCUME~1\XP\DESKTOP\LEPUS-~1\LEPUS-~1.TPS
     nObj: 43
     nDim: 2
     nPts: 23

   Effect: Group
   Groups: FEMALE n=20
             MALE n=20
             g3:3 n=3

       alpha:    0.050
     nPairs: 3
alpha_prime:     0.017

             FEMALE       MALE       g3:3
         ---------------------------------
  FEMALE|         -
    MALE|    0.004*          -
    g3:3|    0.779       0.911          -
```

Figure 5. The result of the pairwise test in Morpheus program for SD of *Lepus europaeus*

SD in head size and shape has been documented in many animals (Herrel et al. 2018). However, this dimorphism shows diversity. For example, for the Serbian red fox (*Vulpes vulpes*), the male cranium is about 5% larger than that of the females (Jojic et al. 2017). Plateau zokor (*Eospalax baileyi*) has SD in body and skull measures. Su et al. (2018), found positive results in favour of male individuals. Lapoint et al. (2017) showed that the skull of male stoats are longer than those of females.

Cardini and Tongiorgi (2003) investigated SD on the premolar tooth wear and the size and shape of the mandible of the yellow-bellied marmot. According to their results, SD was moderate depending on sexual maturity and social role of the adult male and females. Mandibular shape modification was related to the different behavioural ecology of immature and adult marmots. Corti et al. (2001) researched SD between groups of the genus *Proechimys.* However, they found no variation in the ventral and dorsal of the maxilla. Vallejo et al. (2017) examined the cranial morphology of *Megadontomys* (Rodentia: Cricetidae). Researchers showed that for mandibles, neither size nor shape was significantly different between sexes ($p>0.05$). For both skull and mandible, the interaction between sex and species was not significant ($p > 0.05$).

Given the scientific research conducted on the European hare, Bo Frylestam (1980) confirmed that juvenile European hares showed no clear tendency in SD in terms of body weight. Sobocinska-Janeszek (1976) inquired SD in the 34 different skeletal bones of the *Lepus europaeus* but they only found sexual differences in pelvis and os sacrum bones and couldn't confirm any sexual discrimination in the maxilla of the European hare. Segovia et al. (2006) could not observe SD in the vomeronasal systems of rabbits. Similarly, in this study SD wasn't found in European hare samples collected from the provinces of Elazig and Malatya in Turkey.

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