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Geo-Morphometric Approaches to the Study of Sexual Size Dimorphism in Murid Rodents

Kiran Afshan,[1] Syed Shakeel Raza Rizvi,[2] Ali Muhammad[1] and Mazhar Qayyum[1*]
[1]*Department of Zoology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi-46300, Pakistan*
[2]*Pakistan Museum of Natural History, Pakistan Science Foundation, Islamabad*

Abstract.- Sexual size dimorphism (SSD) based on the presence of external genitalia has extensively been documented in mammals, which however, poses impediments in the identification of the sex of specimens based on morphometric traits. Recent advances in geometric morphometric (GM) and multivariate analyses can assist to differentiate sexual dimorphism in animals. The present study was based on morphometric measurements taken from adult Norway rats. The results showed that the magnitude of SSD with regards to the shape and size differs among the sexes. Males were consistently larger in all morphometric measurements than females but considerable overlap between the sexes resulted in no single measurement being a useful discriminator of sex. A principal components analysis on a correlation matrix of nine morphometric measurements indicated that the first principal component was a good 'body size' indicator explaining 49% of the variance in the original matrix. The new characters ratios tail length/body length (BL), BL/body weight (BW), length of front foot/width of front foot and length of hind foot/width of hind foot were introduced, which increases the discrimination tendency up to 60%. The results of the study accentuate the need to incorporate GM methods in rodent taxonomy and in the identification of specific features rendering sexual dimorphism in murid rodents.

Keywords: *Rattus norvegicus*, sexual size dimorphism, geometric morphometrics, Rodents

INTRODUCTION

In tropical and subtropical countries, the two most important murid rodent species namely *Rattus norvegicus* (Norway rat) and *Rattus rattus* (house rat) have been documented as pests for agricultural crops (Roberts, 1977; Aplin *et al.*, 2003). In many rodents taxa, determining the sex of individuals presents no challenge because of the marked differences between the sexes through the development of external genitalia and secondary sex traits. However, the study of sexual dimorphism has fascinated the biologists and zoologists ever since Darwin (1859, 1874). Sexual size dimorphism (SSD), the variations in body size and body shape between the two sex groups is commonly documented for a great number of organisms, from invertebrates to vertebrates, including mammals (Clutton-Brock and Harvey, 1983; LaBarbara, 1989; Short and Balaban, 1994; Fairbairn, 1997). Previously several studies were based on traditional morphometric measurements to assess the sexual dimorphism in otters and bats (Wiig, 1986: Lynch and Hayden, 1995; Lynch *et al.*, 1996b). Recently the application of multivariate approaches (Burnaby, 1966: Rohlf and Bookstein, 1987; Bookstein, 1991) have permitted these studies to assess shape dimorphism.

The preliminary studies showed that geometric morphometric (GM) techniques offer powerful tools to evaluate morphological variations in animals (Bookstein, 1991, 1996; Rohlf and Marcus, 1993; Marcus and Corti, 1996). The aim of present study was to investigate the use of GM approaches in SSD studies, to identify the unique contributions of these new tools in identification of SSD in Norway rats. The basic objective in present study was to investigate the discriminatory power from a range of morphometric characters taken from two sex groups.

MATERIALS AND METHODS

Capturing and grouping of brown rats

In this study thirty six female and twenty four male rats were captured from warm-temperate areas (Rawalpindi and Islamabad) Pakistan by using metal traps with food bait and brought to the laboratory. They were identified through the morphometric

* Corresponding author name: mazhar_dr@yahoo.com
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measurements and coloration pattern. They were darkly brown in coloration, belonging to most common and widely distributed rat species *R. norvegicus*. The sexing was done based on the inspection of external genitalia. The ages were determined as per criteria laid down by Delattre and Le Louarn (1981). They categorized adult rats (≥130 g) and juvenile rats (≤100 g).

Geometric morphometry

Morphometric measurements were taken only from adult male (n=17) and female (n=17) rats. All measurements were taken by using a flexible tape measure in millimetre (mm) and body weight in grams by using a triple beam balance (MB-2610). The following measurements were recorded: tail length(TL), body length(BL), body width (BW), ear length(EL), length of hind limb(LHL), length of fore limb(LFH), length of front foot(LFF), length of hind foot(LHF), width of front foot(WFF), width of hind foot(WHF), width of neck(WN) and weight(W). The ratios were also calculated as TL/BL, BL/BW, LFF/WFF and LHF/WHF.

Statistical analysis

Characters normality was tested using the Q-Q plots and comparison of means made with t-test. The multivariate analysis was applied to estimate morphometric variations among male and female adults of *Rattus norvegicus*. The morphometric variations between them were assessed by using size-free canonical discriminant analysis on covariance of log-transformed measurements. This analysis removes the effect of within-group ontogenetic variations by regressing each character on the first pooled within-group principal component (a multivariate size estimate) (Dos Reis *et al.,* 1990). The male and female adults of *Rattus norvegicus* morphometric analysis were conducted by using BAC v.2 software and the PAD (Permutaciones, Analisis Discriminante) which are module of the CLIC programme (http://www.npl.ird.fr/morphometrics) (Dujardin, 2002; Dujardin and Le Pont, 2004; Dujardin *et al.,* 2010). The results were reflected statistically significant when $p<0.05$. The non-redundant measurements used were TL, BL, BW, EL, LHL, LFL, LH, LP, WH and WP. PC1 could be known as a general indicator of size (Bookstein, 1989), so that the resulting factor maps for adult male and female *Rattus norvegicus* clearly illustrate global size differences in the populations analyzed. The PCA was based on the correlation matrix among nine morphometric measurements and placed nine independent axes through the dataset to explain maximum variance in the dataset (Manly, 1994).

RESULTS

Geometric morphometry

The statistical comparison showed no significant differences in characters among males and females. Adult male and female of *R. norvegicus* were not distinct morphologically. The significant degrees of overlap for each of the sixteen morphometric measurements were determined and although males showed consistently larger morphometric measurements for all measured characters (Table I). No single morphometric measurement could be used to predict the sex of *R. norvegicus* with any reliability. The male and female data was subjected to further analysis.

Body size and shape indicator

The PCA was carried out on the nine morphometric measurements taken from males and females *R. norvegicus*. Principal components were taken from a matrix of correlations among the morphometric measurements (Table II), with the first eigenvector showing an eigenvalue less than one and explaining 49% of the total variation in the original correlation matrix (Table II). Character loadings were all positive on the first principal component (PC1) axis, indicating that PC1 scores are predominantly a measure of *R. norvegicus* body size. The second principal component (PC2) axis is a balanced one, with positive and negative values. In morphometric studies, PC2 is an axis reflecting body shape (Rising and Somers, 1989). Together, the first two eigenvectors explained 66% of the total variation.

A second PCA was performed using the possible determinant characters ratios: TL/BL, BL/BW, LFF/WFF and LHF/WHF. Using these four ratios, the success of correct classification increased to 60%.

Table I.- Means, standard deviations (SD) and ranges of twelve Morphometric measurements of male (n= 17) and female (n=17) of *Rattus norvegicus*.

Morphometry	Female (n=17)		Male (n=17)		p-value
	Mean±SD	Range	Mean±SD	Range	
Tail length (mm)	166.00±29.56	200-100	178.59±13.97	200-150	0.126^{NS}
Body length (mm)	178.65±40.36	230-100	197.65±22.59	250-170	0.103^{NS}
Body width (mm)	57.76±12.32	85-80	65.00±9.84	35-50	0.68^{NS}
Ear length (mm)	17.12±3.71	20-25	18.71±1.76	10-15	0.124^{NS}
Length of hind limb (mm)	48.12±8.71	60-70	55.29±8.00	30-40	0.18^{NS}
Length of fore limb (mm)	40.41±7.22	50-60	49.12±6.90	25-30	0.001^{*}
Length of front foot (mm)	18.82±1.78	20-50	20.12±1.45	15-17	0.027*
Length of hind foot (mm)	36.18±4.85	45	40.12±3.82	30	0.013*
Width of front foot (mm)	11.94±2.33	15-17	11.82±1.91	8-10	0.87^{NS}
Width of hind foot (mm)	17.12±2.83		17.12±2.55	11	1.00^{NS}
Width of neck(mm)	38.24±6.11	50-50	41.56±4.73	30-35	0.09^{NS}
Weight (g)	190.05±98.62	335-392	254.21±63.63	48-158	0.032*
TL/BL	0.96±0.22	1.70-1.06	0.91±0.09	0.78-0.77	0.43^{NS}
BL/BW	3.13±0.52	3.85-3.67	3.08±0.39	1.67-2.43	0.75^{NS}
LFF/WFH	1.63±0.3	2-2.5	1.74±0.31	0.94-1.33	0.27^{NS}
LHF/WHF	2.17±0.46	3.46-3.67	2.4±0.48	1.50-1.84	0.157^{NS} -

P-values are from t-tests between the sexes for each morphometric measurement mean;
^{NS} Not significant; *significant

Table II.- Character loadings on principal component axes (PCI, PCII and PCIII) for a principal component analysis (PCA) extracted from a correlation matrix of nine morphometric measurements taken from 34 *Rattus norvegicus*.

Morphometric characteristics	Principal component axis		
	PCI	PCII	PCIII
Tail length	**0.781**	**-0.202**	**-0.323**
Body length	0.878	-0.120	0.058
Body width	0.834	-0.391	-0.081
Ear length	0.523	0.163	-0.698
Length of hind limb	0.794	0.344	0.366
Length of fore limb	0.704	0.585	0.300
Length of front foot	0.318	-0.235	-0.093
Length of hind foot	0.518	-0.373	-0.124
Width of front foot	0.127	-0.715	0.520
Eigenvalue	0.3388	0.4895	0.4698
%variance explained	49	17	13

The samples were plotted against PCI and PCII. The *R. norvegicus* variables from adult male and female were significantly (P<0.05) correlated with PC1. The factor map of first two principal components (PC) is shown in Figure 1. The PCI and PCII contributed 49% and 17% size variations respectively. The resulting scatter plot illustrated considerable variations between male and female *Rattus norvegicus* from Pakistan, indicating male rats were larger in size as compared to female rats.

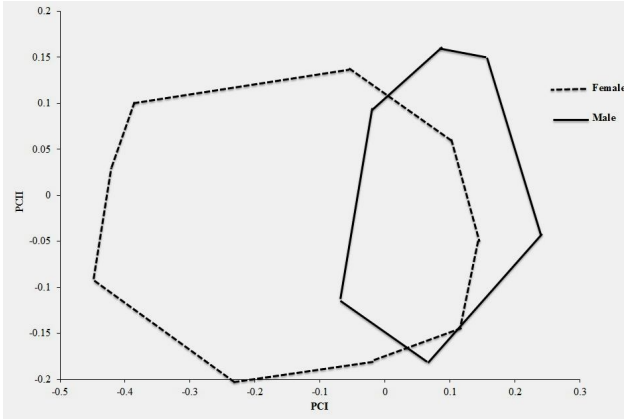

Fig. 1. Factor map corresponding to adult female and male *Rattus norvegicus* specimens. Samples are projected onto the PCI (49%) and PCII (17%). Each group is represented by its perimeter.

DISCUSSION

GM methods are useful in sexual dimorphism not only in visualization of shape variabilties but also in describing the size of mammals. Shape variables can be measured for dimorphism, indicating a sexual shape dimorphism. Many factors involve in shape variabilties between two sex groups these includes, body size variables, behavioral, metabolic and ecological variables (Hood, 2000). Rodent's identification at species level is quite challenging, especially among members of murid rodents due to their ability to undergo major shifts in ecological adaptation with minor morphological changes. In this esteem, GM methods are appropriate in identifying the gaps, discontinuities, discrepancies and overlaps in character shape and size variations (Torres *et al.*, 2010).

In our sample of Norwegian rats, there was significant overlap of single morphometric measurements between the sexes. Thus, none can be used solely to discriminate between the sexes. The PCA of morphometric measurements indicated that PC1 was a good measure of body size (Bookstein, 1989), with all character loadings on PC1 being positive. Based upon the overall separation of individual PC1 scores for male and female rats, we conclude that males are slightly larger than females. The results are in agreement with morphological measurements reported by Zareef *et al.* (2009). The high degree of overlap in these scores, however, suggests that PCA allows sex discrimination with only a moderate degree of reliability. The shape of male and female rats is found significantly different and PCII described the variability between the shapes of two sex groups. In present study the geometric morphometric technique using morphometric approaches was first time used to discriminate sexual size dimorphism in *Rattus norvegicus.*

There are different hypotheses explaining the origin and maintenance of SSD in mammals. The sexual selection is most important for SSD (Clutton-Brock *et al.*, 1977; Leutenegger, 1978; Jungers, 1985; Ford, 1994; Weckerly, 1998), reproductive life history traits, including overall body size as a variable (Leutenegger and Cheverud, 1985; Heske and Ostfeld, 1990; Soderquist, 1995). And the intersexual ecological divergence hypotheses explains divergence in size between the sexes due to their different ecological roles, foraging, behavioral activity patterns, inter and intraspecific competition (Myers,1978; Willig, 1983; Willig and Hollander, 1995; Sullivan and Best, 1997).

CONCLUSIONS

The results of this study showed that GM methods for SSD combined with novel approaches in analysis of multiple multivariate data sets can be used as a supplement to the traditionally collected data that are used in rodent taxonomy and in determining specific features that differentiate the dimorphic sexes. This is particularly important especially in few species of rodents which possess uniquely diagnostic features, making it difficult to recognize closely related species. The following new characters ratios: TL/BL, BL/BW, LFF/WFF and LHF/WHF were introduced to increase the discrimination tendency. The application of GM methods could be useful to determine species boundaries as some rodents are highly polymorphic and display wide range of morphological variations within and between populations of a single species. Thus, population differentiation studies have been suggested, such attempts could be useful for accurate characterization and identification of species.

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