The comparative analysis of the cytogenetic and morphometric characteristics of some mammals

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Abstract. 5 species of the *Meriones* genus are known in Azerbaijan. In these species (Libyan Jird (*Meriones libycus* Lichtenstein, 1842)), the studying of the chromosomal homology and intraspecies problems is one of the actual issues. Based on the literature data about the species of Libyan Jird (*Meriones libycus* Lichtenstein, 1842), it turns out that very few researches have been conducted in the physical-geographical region of Jeyranchol - Ajinohur. There is a need to the ecological, morphological and cytogenetic researches of this species in the research area and the researches are carried out by us. At the same time, there is a need of a comparative morphometric researching of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774) species in the research areas, because the comparative morphometric measurements of the species have not been researched in the research areas. In the areas of the Jeyranchol - Ajinohur geographical region, the comparative morphometric measurements of the Common Pipistrelle species are investigating by us. Both areas are located in the semi-desert zone and are characterized by relatively different landscape, vegetation, substrate, altitude and weather conditions (especially precipitation). The Gobustan region is the area with little precipitation and the most wind, and more characterized by the glasswort-wormwood-ephemeral plants. Jeyranchol has wormwood-caperbushes, sparsely shrubby xerophytic vegetation.

Keywords: Chromosome, karyotype, structure, analysis, cell.

1 Introduction

Due to the fundamental change of the ecological conditions, the lifestyle of the rodents and bats, their role in the fields of public farm, health and science has caused great interest of the researchers. The main purpose of our research was to study the cytogenetic and morphological changes caused by environmental factors in the rodents and bats over the years. Both cytogenetic and morphological changes were revealed during the research.

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During the analysis of the comparative mass and body dimensions of the Common Pipistrelle species, it was detected that there is a real difference only in the length of the second ear (tragus). The real differences between the length of the upper tooth level and the length of the face part were detected during the comparative analysis of the cranial dimensions of two populations of Common Pipistrelle.

Therefore, the cytogenetic and morphological studying of the Libyan Jird and Common Pipistrelle populations is one of the main purposes of our research.

2 Materials and methods

2.1 The preparing of the chromosome preparations

The chromosome preparations were prepared from the red bone marrow cells. The modified method of C.E. Ford and J.L. Hamerton [1] was used during the preparation of the preparations. 1-1.5 hours before the slaughter of the animal, 0.04% colchicine solution was injected into its abdominal cavity at the rate of 1 ml per 100 g of the weight. The femur was removed from the killed animal and the bone marrow was washed with a warm solution of 0.56% of KCL by syringe. The cells were kept in 2-3 ml of potassium-chlorine solution at 37 ^{0}C for 5-7 minutes, and then fixed for 30 minutes in a mixture of methyl alcohol and acetic acid in a ratio of 3:1 at +4 ^{0}C. The fixative was changed twice with intermediate resuspension and centrifugation. The preparations were prepared by the method of burning the fixative by dripping it on glass [2].

The differential staining of heterochromatin with the structural structure.

In order to determine the structural heterochromatin, the method of A.T. Sumner [3] was used. 2-6 days after preparation, the preparations are kept in 0.2 N HCl acid solution during 1 hour, then washed with distilled water and put in newly prepared 5% Ba(OH) solution for 5-15 minutes at a temperature of 62 ^{0}C. After that, it is washed several times with distilled water and transferred to Himza dye for 1-1.5 hours in 2SSC buffer. After staining, the preparations were washed with distilled water, dried and put in balsam [4].

2.2 The analysis of the chromosome sets

The preparations were analyzed in the "Amplival" microscope with 100x objective and 10x eyepiece. MF (Automatic microphotography exposure device (objective 100x, eyepiece 10x)). Diploid chromosome number (2n) and total number of chromosome arms (NF) were determined for each form. NF is appointed as the total number of the arms of all the chromosomes, including the sex chromosomes of the female individual. The chromosomes were counted in 30-50 metaphase plates to determine the diploid chromosome number for each animal. The metaphase plates with less scattered chromosomes were selected for the analysis. The metaphases that were too scattered were excluded from the analysis to avoid the counting cells with the missing chromosomes. A kariogram was made for each animal to appoint NF. The metaphase plates of the chromosomes cut in the photos are arranged in pairs according to their size and morphology, and then the chromosomes that are morphologically identical and differ only in size are grouped according to their size couples to their size in kariogram. Chromosome pairs that are morphologically identical and differ only in size are arranged in decreasing order of size in the karyogram. The differential analysis of the colored chromosomes was carried out according to the photographs at the same time. They are paired according to the similarity of the chromosome shape, heterochromatin block and AgNOR distribution. An average of 100 metaphase plates were analyzed for each animal.

The morphological measurements of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774) species were written on the basis of the personal materials and materials stored in the Laboratory of Terrestrial Vertebrates of the Institute of Zoology, collected in different seasons of 1980-2019 from the territories of the Jeyranchol-Ajinohur and Gobustan-Absheron physical-geographic regions.

Somato-craniometry was performed according to the known scheme [5]. For this purpose, a 10-year-old individual was researched. The morphometric calculation of the materials from the different populations of the bats was performed by the variation-statistical method. The degree of accuracy of the differences is based on the known method accordingly. For individuals of both populations, 4 exterior features, mass and 8 craniological indicators were taken [6]. According to this method, if $P<0.05$, the difference between the populations is considered real [7].

3 Results and discussion

Libyan Jird (*Meriones libycus* Lichtenstein, 1823). The ecological and morphological researches of this species have been carried out by us for a long time in the research area. During our comparative researches in the research area, it was detected that there are differences between the morphological and ecological characteristics of these animals [8, 9]. The presence of such differences indicates the need to study the chromosomal homology and intraspecies problems in these species. Therefore, the studying of the chromosome structure of Meriones in Azerbaijan is one of the actual issues and is being investigated by us.

On the basis of the materials obtained by us, the karyotype of the Libyan Jird was studied comparatively in the terrestrial vertebrate laboratory of the Institute of Zoology from Jeyranchol-Ajinohur and Gobustan-Absheron physical-geographical areas [10]. The materials were placed in the collection of the Institute of Zoology after the chromosomes were obtained. In the karyotype of the individuals of this species in all the researched areas, the number of the diploid chromosomes is $2n = 44$, the number of the arms of the autosomal chromosomes is 84, and the total number of the arms is 86. In karyotype, 15 pairs of double-armed chromosomes with meta- and submetacentric structure, 6 pairs of autosomal chromosomes with subtelocentric and acrocentric structure are detected. The karyotype of this species can be divided into three groups. In the first group, as in the other species, the first pair of chromosomes differs in size from the other chromosomes in the same group and is big relatively. The other chromosomes in the first group are in decreasing order of size. The first pair of chromosomes of the subtelocentrics in the second group, in contrast to the karyotype of the other species, forms a row that decreases in size from the chromosomes of its group. The chromosomes of the third group consist mainly of subtelocentric chromosomes with small shoulders. All the chromosomes differ little from each other in terms of size and form a series of decreasing sizes. In contrast to Gobustan and Absheron populations, the intercalary heterochromatin is not detected in the chromosomes of group 1 of Jeyranchol population of Libyan Jird. The heterochromatin is visible clearly in the form of blocks only in the centromeric part of the chromosomes. The 4th and 6th pairs of the second chromosomes of the Jeyranchol population karyotype (and the 12th and 14th chromosomes in the general karyotype) are similar to the heterochromatin composition of the corresponding chromosomes contained in the karyotype of the Gobustan population individuals. But the Gobustan and Jeyranchol populations differ from the other population, i.e. the Absheron population, due to the heterochromatin composition of these chromosomes. The 13th pair chromosome has heterochromatin lines which characteristic for all three populations, that is, it is heteromorphic. So Gobustan and Jeyranchol populations are closer to each other according to the heterochromatin composition. And the

Absheron population is relatively far from them. Our researches are continued.

As can be seen from table 1, it was detected that there is only a real difference in the length of the second ear (tragus) during the comparative mass and body size analysis of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774) species. No real differences were detected in mass and other body measurements (Table 1).

Table 1. The variation in mass (g) and body measurements (mm) of two populations of Common Pipistrelle (♀ – female).

Body measurements	Genus	Jeyranchol				Gobustan				Degree of difference	
		N	lim	M1	m1	N	lim	M2	m2	T	P
Body mass	♀	5	3.7-4.9	4.38	0.25	5	4.2-5.0	4.64	0.16	0.88	0.41
Body length	♀	5	39.0-43.0	41	0.84	5	37.5-41.0	39.3	0.72	1.54	0.16
Tail length	♀	5	29.5-35.0	31.3	1.14	5	28.0-33.0	30.3	0.96	0.74	0.48
Ear length	♀	5	11.0-12.6	11.72	0.39	5	9.0-13.0	11.3	0.74	0.50	0.63
2nd Ear (tragus) length	♀	5	5.5-6.0	5.9	0.11	5	6.0-6.5	6.4	0.11	3.21	0.014
Wing length	♀	5	31.0-32.0	31.42	0.185	5	30.0-32.0	30.9	0.37	1.26	0.24

As can be seen from the table 2, the real differences between the length of the upper tooth level and the length of the facial part of Common Pipistrelle during a comparative analysis of the cranial dimensions of its two populations were detected.

The exposure of the research areas to different environmental influences and the difference between the vegetation are as the cause to the examples of the real differences in the analysis of the comparative mass and body measurements of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774) species. Both areas are located in the semi-desert zone and are characterized by relatively different landscape, vegetation, substrate, altitude and weather conditions (especially precipitation). The Gobustan region is the area with little precipitation and the most wind, and more characterized by the glasswort-wormwood-ephemeral plants. Jeyranchol has wormwood-caperbushes, sparsely shrubby xerophytic vegetation (Figure 1, 2, 3, 4, 5).

Table 2. The variation in skull measurements (mm) of two populations of Common Pipistrelle (♀ – female).

Skull dimensions	Genus	Jeyranchol				Gobustan				Degree of difference	
		N	Lim	M1	m1	N	Lim	M2	m2	T	P
The total length of the skull	♀	5	11.5-12.1	11.76	0.15	5	11.4-11.9	11.6	0.09	0.91	0.39
Skull width	♀	5	6.0-6.4	6.24	0.075	5	6.0-6.8	6.3	0.15	0.36	0.73
The length of the upper tooth level	♀	5	4.6-5.0	4.76	0.08	5	4.4-4.6	4.48	0.09	2.33	0.05
The length of the lower tooth level	♀	5	4.0-4.5	4.24	0.095	5	4.2-4.4	4.26	0.04	0.19	0.85
The length of the brain part	♀	5	3.9-4.5	4.14	0.115	5	4.0-4.6	4.22	0.11	0.50	0.63
The length of the face part	♀	5	4.7-5.2	5	0.115	5	4.2-4.9	4.54	0.125	2.71	0.03
The length of the drum part	♀	5	2.7-3.3	2.88	0.12	5	2.5-2.9	2.64	0.075	1.70	0.13
The width of the drum part	♀	5	2.0-2.3	2.14	0.05	5	1.7-2.4	2.02	0.14	0.81	0.44

Fig. 1. The determination and appearance of the body dimensions of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774).

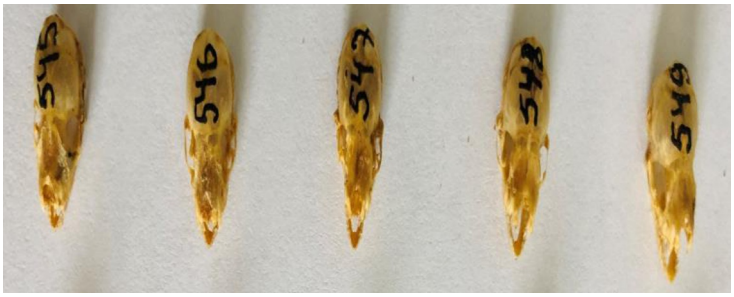

Fig. 2. The top view of the skull bones of the Jeyranchol individuals of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774).

Fig. 3. The bottom view of the skull bones of the Jeyranchol individuals of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774).

Fig. 4. The top view of the skull bones of the Gobustan individuals of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774).

Fig. 5. The bottom view of the skull bones of the Gobustan individuals of the Common Pipistrelle (*Pipistrellus pipistrellus* Schreber, 1774).

4 Conclusions

The study of the Libyan gerbil (Meriones libycus) and the Eurasian pipistrelle (Pipistrellus pipistrellus) in the Jeyranchol-Ajinohur and Gobustan-Absheron regions revealed important ecological, morphological and cytogenetic differences between the populations. In particular, the Libyan gerbil shows differences in chromosome structure, such as differences in heterochromatin composition between populations. These differences indicate the need for further studies on chromosomal homology and intraspecific variation. Similarly, the Eurasian pipistrelle shows differences in cranial dimensions, particularly ear length, while other body measurements remain unchanged. This ongoing study contributes to our understanding of how environmental factors shape the genetic and morphological traits of these species.

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