

Comparative histochemical analysis of phosphatases activity of the skin glands in some artiodactyls

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We determined the histochemical activity of phosphatases of sweat and sebaceous glands in the withers of three artiodactyls species — roe deer *Capreolus capreolus* Linnaeus, 1758, musk deer *Moschus moschiferus* Linnaeus, 1758, and *Saiga tatarica* Linnaeus, 1766. Equal values of acid phosphatase activity (60% level of relative activity), alkaline phosphatase (80% level of relative activity), and adenosine triphosphatase (100% level of relative activity) for both the sweat and sebaceous glands of roe deer founded. Only for alkaline phosphatase, a 60% level of relative activity founded in the sweat glands of musk deer, while the remaining parameters of phosphatase activity in both types of skin glands of musk deer showed zero values. Moderate levels of activity of acid phosphatase and alkaline phosphatase (60% level of relative activity), as well as an average level of adenosine triphosphatase activity (80% level of relative activity) are typical for both the sweat and sebaceous glands of saiga. Synchronicity in the parameters of histochemical activity in both the sweat and sebaceous glands revealed, which may be associated with physiological adaptation. Coordinate analysis based on digital analogues of the histoenzymatic activity of the skin glands made it possible to calculate the Euclidean distances between the studied species. In the sweat and sebaceous glands the following individual distances identified: (1) distances between roe deer and musk deer are 5.92 and 7.07; (2) distances between the roe deer and saiga are coincide and equal to 1.41 and 1.41; (3) distances between musk deer and saiga are 5.00 and 5.83. Phenetic analysis, which includes 18 parameters of histoenzymatic activity — three types of phosphatases in the sweat and sebaceous glands of three species of artiodactyls — proximity of the values of roe deer and saiga at an obvious distance of the parameters of musk deer revealed. The Euclidean distances for the three species of artiodactyls were: (1) distance between the roe deer and musk deer is 9.22; (2) distance between roe deer and saiga is 2.00; (3) distance between musk deer and saiga is 7.68.

Keywords: Histochemistry; Phosphatases; Analytical geometry; Cluster analysis; Artiodactyls; Ruminants; Pecora; Roe deer; Musk deer; Saiga

Introduction

Research of the histochemistry of mammalian cutaneous gland enzymes is an urgent biological task, which allows expanding the understanding of the physiology of the glandular apparatus of the skin (Sokolov et al., 1988), the main function of which is to produce a secret that has an important functional value and ensures homeostasis of the animal's body (Sokolov, 1982; Quay, 1986; Sokolov & Chernova, 2001). That is why close attention should be paid to the study of enzyme systems involved in secretion (Saga, Morimoto, 1995; Saga, 2001, 2002; Cui & Schlessinger, 2015), while the determination of histoenzymatic activity will allow us to characterize the physiological parameters of the sweat and sebaceous glands (Dzhemukhadze, 2007), which belong to modular systems of the skin with different morphological structures (Chernova & Kiladze, 2019). The sweat glands located in the thickness of the skin have a tubular structure ending in a glomerulus, and the sebaceous glands adjacent to the hair are characterized by a bunch-like shape (Hashimoto et al., 1986; Bell, 1986; Sokolov & Petrishchev, 1997; Sokolov & Chernova, 2001). It is believed that the secret of sweat and sebaceous glands encodes both reactively changing conditions of the body and, on the contrary, characterizes more stable physiological parameters of an individual (Dzhemukhadze, 2007). It is obvious that the histochemistry and cytochemistry of enzymes requires an extension of the data processing methods used, previously presented in the descriptive-symbolic form (Montagna & Noback, 1947; Tuzuner & Bennett, 2018). Translation of signs indicating the gradation of enzyme activity into digital analogues allows using the methods of qualimetry, statistics and mathematics, which provides not only the possibility of identifying relationships that can be discussed from a biological point of view, but also gives the necessary rigor and evidence of the obtained histochemical results (Dzhemukhadze & Kiladze, 2008; Azgaldov & Kostin, 2011; Lobanov, 2013).

In the class Mammalia system, the order Artiodactyla occupies a significant place, many of which have a certain hunting, commercial and economic significance, while some species are under national and even international protection because of their vulnerable status (Price & Gittleman, 2007). We have studied the skin cover in the withers of such species as roe deer, musk deer, and saiga, while it is believed that the world population of musk deer (230 thousand individuals) and saiga (165 thousand individuals) tends to decrease, which allowed the International Union for Conservation of Nature and Natural Resources (IUCN Red List) to give quite alarming statuses for these species — Vulnerable A2d+3d+4d and Critically Endangered A2acd, respectively (Siberian Musk Deer..., 2012; Nyambayar et al., 2015; IUCN SSC..., 2018; Vaughan, 2019). The main products of hunting for musk deer are the musk deer jet, obtained from the preputial gland and used in the perfume industry (Sokolov et al., 1987; Sokolov & Chernova, 2001; Prikhod'ko, 2003; Yi et al., 2020), while the saiga have the main value in the horns used in traditional

Chinese medicine (Vaughan, 2019; Doughty et al., 2019). Against this background, the global roe deer population is quite large (15 million individuals), which allows for quite active hunting and commercial activities (Lovari et al., 2016), however, in this case, environmental management of the population of these artiodactyls is also required (Marcon et al., 2019), even with the current favorable status (Least Concern). Hunting for roe deer is carried out because of the meat fraction, skins that go to suede production, as well as trophy horns (Spiers, 1973; Weiner, 1973; Kuznetsov, 2005; Khludeev & Gordienko, 2008).

The purpose of this work is to research the histoenzymatic activity of the sweat and sebaceous glands of three representatives of the order Artiodactyla, belonging to the suborder Ruminantia and the infraorder Pecora, however, and the studied species are representatives of three different families, namely: (i) family Cervidae includes the roe deer *Capreolus capreolus* Linnaeus, 1758, (ii) family Moschidae includes the musk deer *Moschus moschiferus* Linnaeus, 1758, and (iii) family Bovidae includes the saiga *Saiga tatarica* Linnaeus, 1766 (Pavlinov & Khlyap, 2012).

On the one hand, this circumstance in a certain way will show the species variability of histoenzymatic activity, and on the other hand, will allow to compare the obtained data with each other due to the taxonomic affinity of species using a qualimetric scale (Azgaldov et al., 2015; Rozhkov, 2018), a three-dimensional coordinate system (Aljohani, 2016), and also cluster analysis methods (Gower, 1967), which are based on the phenetic parameters of enzyme activity of the skin glands. Previously, these methods we tested on the example of rodents (Kiladze & Dzhemukhadze, 2019, 2020) which makes them universal for the analysis of various groups of animals.

Materials and Methods

Following species of artiodactyls selected as the object of the research (harvest wildlife locations are indicated in brackets): (1) roe deer *C. capreolus* (Belarus), (2) musk deer *M. moschiferus* (Eastern Siberia, Russia), and (3) saiga *S. tatarica* (Kazakhstan), belonging to the same sex (males), age (adult mature individuals) and hunting season (November-December). For histochemical analysis, sweat and sebaceous glands of the skin from the withers researched. Three skin samples (1×1 cm) obtained from each individual in 10% neutral formalin solution for up to 24 hours in the cold fixed. The thickness of the frozen "floating" slides was 15 μm (Pears, 1960; Barca & Anderson, 1963). Histochemical reactions to acid phosphatase (ACP), alkaline phosphatase (ALP), and adenosine triphosphatase (ATPase) performed according to Gomori and Burstone (Gomori, 1952; Burstone, 1962). The microscopic examination of the histological slides carried out using a Carl Zeiss microscope (manufacturer VEB Carl Zeiss Jena, Germany).

Digital data processing involves the creation of a qualimetric scale of enzyme activity. In this regard, the ranking of the activity of skin gland enzymes carried out with a sign, digital and relative representation of the results, namely: – / 0 points / 0% — traces or absence of activity; – (+) / 1 point / 20% — indistinct activity; + (–) / 2 points / 40% — low activity; + / points / 60% — moderate activity; ++ / 4 points / 80% — average activity; +++ / 5 points / 100% — high activity (Kiladze & Dzhemukhadze, 2019, 2020). The relative characteristic of enzyme activity is defined as the ratio of actual activity to maximum activity, with the result expressed as a percentage (Kiladze & Dzhemukhadze, 2013).

Digital analogues of the histochemical activity of the sweat and sebaceous glands of roe deer, musk deer and saiga presented in the form of coordinates in three-dimensional space, where the activity of ACP plotted on the x-axis, ALP plotted on the y-axis, ATPase plotted on the z-axis (Kiladze & Dzhemukhadze, 2019, 2020). Three-dimensional graphs created using the program "CPM 3D Plotter", available at <https://technology.cpm.org/general/3dgraph/>.

Euclidean distances for the histochemical parameters of sweat and sebaceous glands determined between roe deer (A) and musk deer (B), roe deer (A) and saiga (B), and musk deer (A) and saiga (B), while the distances (d) determined as follows way:

$$d = \sqrt{(B_{ACP} - A_{ACP})^2 + (B_{ALP} - A_{ALP})^2 + (B_{ATPase} - A_{ATPase})^2}$$

The automated calculation of distances (d) performed using the site: <http://www.mathsolution.ru/math-task/lp-dist-2points>.

For the phenetic constructions, the initial data were digital analogs of the activity of three types of phosphatases for two types of glands from three types of artiodactyls, that is, 18 histochemical parameters that were subjected to clustering by Ward's method using the computer program "STATISTICA 10" (StatSoft, USA).

Results

Given the environmental relevance of the research objects, as well as their significant commercial potential, great importance is given to the research of general biological patterns of artiodactyls, which can include features of the functioning of the skin glands, the physiological aspects of which are largely related to enzyme systems.

Histoenzymatic analysis of the sweat and sebaceous glands of the skin of the withers of roe deer, musk deer, and saiga suggests the identification of gradations of activity of the three types of phosphatases, shown both in sign form and in the form of digital analogues (Table 1), determined by the qualimetric scale.

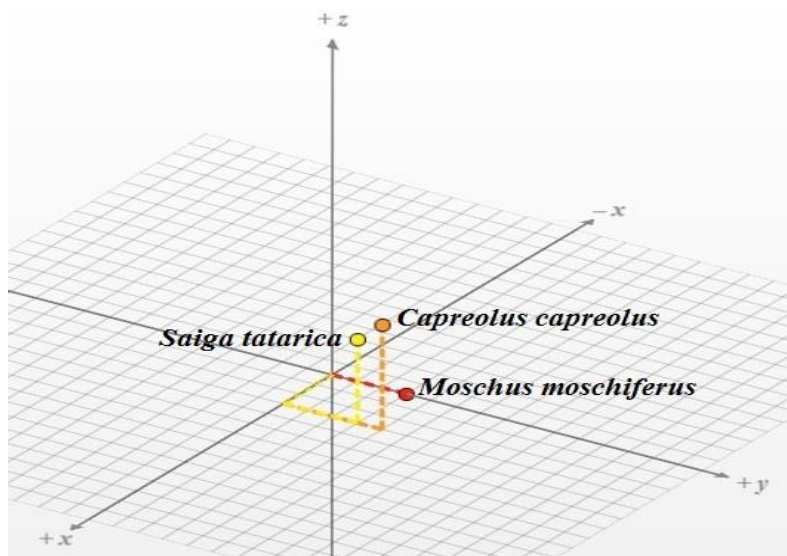
Table 1. Histoenzymatic activity of phosphatases of cutaneous glands in the withers of the studied artiodactyls.

Type of phosphatase	Roe deer <i>C. capreolus</i>		Musk deer <i>M. moschiferus</i>		Saiga <i>S. tatarica</i>	
	Sweat glands	Sebaceous glands	Sweat glands	Sebaceous glands	Sweat glands	Sebaceous glands
Acid phosphatase, points	+ / 3	+ / 3	– / 0	– / 0	+ / 3	+ / 3
Alkaline phosphatase, points	++ / 4	++ / 4	+ / 3	– / 0	+ / 3	+ / 3
Adenosine triphosphatase, points	+++ / 5	+++ / 5	– / 0	– / 0	++ / 4	++ / 4

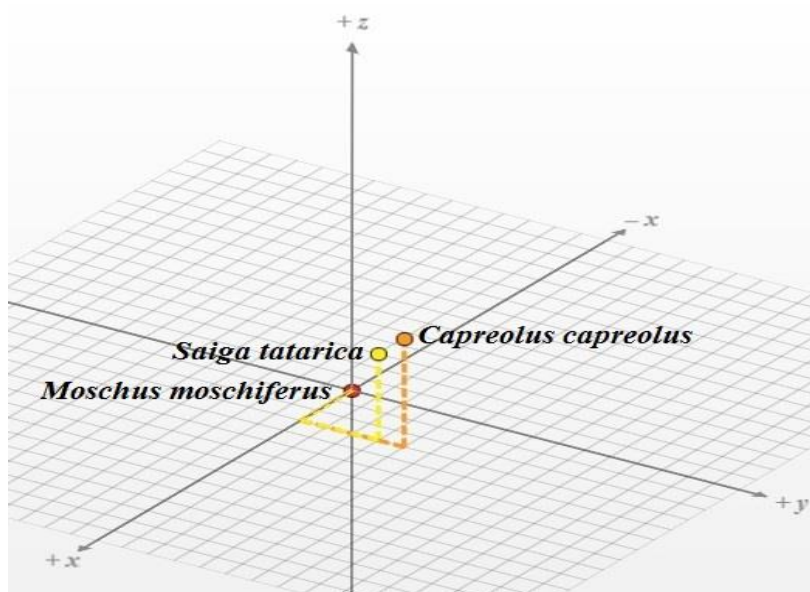
Analyzing the above results, we can indicate that the same activity levels of ACP, ALP, and ATPase of roe deer for both sweat and sebaceous glands founded. For ACP, a 60% level of the relative manifestation of the enzyme is characteristic, for ALP — 80%, for

ATPase —100%. Musk deer is characterized by a minimal level of activity for all types of phosphatases in both sweat and sebaceous glands. The results obtained indicate only the presence of traces or the absence of an enzyme in the cells of the skin glands. A moderate level of activity was detected only for ALP in the sweat glands, which suggests a 60% relative character. Moderate level of activity only for ALP in the sweat glands detected, which suggests a 60% relative nature of the histoenzymatic manifestations on the preparations. Moderate and average levels of ACP, ALP and ATPase are typical for the sweat and sebaceous glands of saiga, while the maximum level of enzyme expression in the preparations not detected. If ACP and ALP have a 60% relative value, then for ATPase it reaches 80% level.

Taking into account the key importance of phosphatases in the process of secretion, we modeled three-dimensional phosphatase spaces, where the values of the activity of ACP, ALP, and ATPase founded as coordinates, both in the sweat (Figure 1a) and in the sebaceous (Figure 1b) glands of roe deer, musk deer and saiga. Interpretation of the enzymes activity in the form of coordinates made it possible to calculate the Euclidean distance between the studied animal species, thereby showing the degree of proximity of the phosphatase activity for two types of glands of the skin (Table 2).



(a)



(b)

Figure 1. The activity of ACP (+x), ALP (+y), and ATPase (+z) of the sweat (a) and sebaceous (b) glands of the skin of the roe deer *C. capreolus*, musk deer *M. moschiferus*, and saiga *S. tatarica* in three-dimensional space.

Table 2. Interspecific distances of histoenzymatic activity of phosphatases of sweat and sebaceous glands in the withers area of the studied artiodactyls.

Pairwise comparison of species	Sweat glands	Sebaceous glands
Roe deer – Musk deer	d = 5.92	d = 7.07
Roe deer – Saiga	d = 1.41	d = 1.41
Musk deer – Saiga	d = 5.00	d = 5.83

Individual values of the activity distances of the three types of phosphatases indicate that both the sweat and sebaceous glands of the musk deer parameters are sharply different from the values of the activity of roe deer and saiga. The maximum distance is

typical for sweat and sebaceous glands between the roe deer and musk deer, and the minimum distance that coincides for both types of glands is registered between roe deer and saiga. The distance values between musk deer and saiga are intermediate. In support of the found regularities, a cluster analysis of the data performed, which can be considered as an assessment of the phenetic similarity of the phosphatase activity of three artiodactyl species (Figure 2).

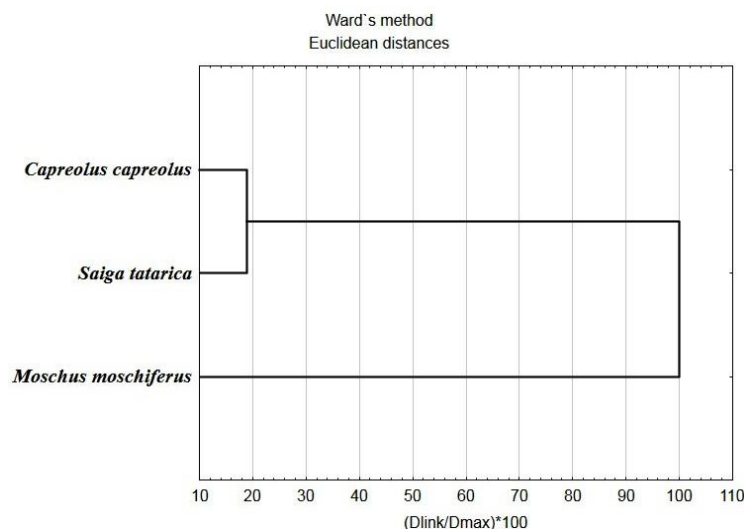


Figure 2. Phenogram of histoenzymatic activity of sweat and sebaceous glands of the skin of the roe deer *C. capreolus*, musk deer *M. moschiferus* and saiga *S. tatarica*.

The given phenogram, as well as the Euclidean distances shown in Table 3, indicate a significant distance between the parameters of the activity of musk deer phosphatases and the activity values of the enzymes of the roe deer and saiga skin glands, which are combined into a common cluster.

Table 3. Interspecific distances of histoenzymatic activity of phosphatases of skin glands in the withers area of the studied artiodactyls.

Euclidean distances	<i>C. capreolus</i>	<i>M. moschiferus</i>	<i>S. tatarica</i>
<i>C. capreolus</i>	0.00	9.22	2.00
<i>M. moschiferus</i>	9.22	0.00	7.68
<i>S. tatarica</i>	2.00	7.68	0.00

Discussion

Turning to the discussion of the results, it is important to indicate that the withers area of the three artiodactyl species can be considered as a model topographic area, characterized by a typical and stable level of functional activity of the skin glands (Sokolov & Petrishchev, 1997). Obviously, the revealed histochemical differences in the three representatives of artiodactyls are determined by species characteristics, as well as lifecycle and habitat (Burbaitė & Csányi, 2009; Wang et al., 2015; Slaght et al., 2019). In addition, attention is drawn to the synchronicity in the histoenzymatic characteristics for the two types of glands, which may indicate a similar intensity of secretion. Perhaps these levels of enzymatic activity in the sweat and sebaceous glands can be explained by the necessary physiological adaptation (Taylor, 1986; Gagnon et al., 2012). Qualimetric data processing allowed us to calculate the relative level of histoenzymatic activity from the maximum possible value, which made it possible to obtain information expressed as a percentage (Kiladze & Dzhemukhadze, 2013). The construction of phosphatase fields and drawing coordinates on them, which are digital analogues of the activity of ACP, ALP, and ATPase revealed a factorial effect of the type of skin glands on the enzymatic activity characteristic of the studied artiodactyl species.

Interpretation of histoenzymatic activity in the form of coordinates allowed using the Euclidean metric to calculate the distance between three species of animals for two types of glands. The results indicated that there are significant distances between the phosphatase activity of artiodactyls, since the values of musk deer are diametrically opposite to the level of activity of roe deer and saiga. These distances confirmed by cluster analysis, indicating similar trends in the phenetic parameters used. If compared the results with the taxonomic hierarchy of infraorder Pecora, we can fix the discrepancy, since the more closely related families are Cervidae, including roe deer, and Moschidae, including musk deer, while they somewhat distanced from Bovidae, which include the saiga (Pavlinov & Khlyap, 2012). It is obvious that the obtained results on the histochemistry of the cutaneous glands of three artiodactyl species should be considered only as an additional criterion for the species.

Conclusion

Thus, the final results can be formulated as the following theses: (1) species-specific parameters of the activity of ACP, ALP, and ATPase in the sweat and sebaceous glands of roe deer, musk deer, and saiga determined; (2) synchronicity of the histochemical activity of sweat and sebaceous glands in the withers area for the studied artiodactyl species revealed, which is possibly due to functional adaptation; (3) shows a significant distance in the histoenzymatic parameters of the activity of the skin glands of musk deer in comparison with roe deer and saiga, which was demonstrated both in coordinate space and using cluster analysis.

We believe that the data obtained using modern methods of qualimetric and biometric processing may be of interest for further comparative biological researches in the field of histochemistry and morphology of the skin glands of other mammalian groups.

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