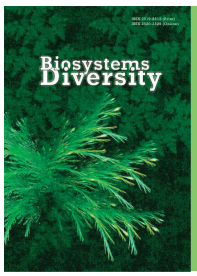

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Evaluation of sexual dimorphism of histochemical activity of phosphatases of the plantar glands of Norway rats (*Rattus norvegicus*)

A. B. Kiladze, N. K. Dzhemukhadze

A. N. Severtsov Institute of Ecology and Evolution, Moscow, Russia

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A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskiy pr., 33, Moscow, 119071, Russia.
Tel.: +7-916-503-44-51.
E-mail: andreykiladze@yandex.ru

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The activity of acid phosphatase, alkaline phosphatase and adenosine triphosphatase in the eccrine and sebaceous glands of the skin of the soles of the paws of male and female Norway rats was studied by histochemical methods. Using the methods of qualimetric analysis, we presented a digitalized form of the enzyme activity, which made it possible to calculate sexual dimorphism indices, reflecting quantitative differences in the enzymatic activity of the skin glands in males and females of Norway rats. For acid phosphatase activity, the sexual dimorphism index was equal to 0.50 in the eccrine glands and equal to 0.33 in the sebaceous glands. For alkaline phosphatase activity, values of sexual dimorphism indices were equal to –0.25 and 0.33 correspondingly, and for adenosine triphosphatase values of sexual dimorphism indices were equal to –0.33 and 0.50. Digital analogues of the histoenzymatic activity of the skin glands were presented as coordinates in three-dimensional space. Using the methods of analytical geometry, we calculated the values of intersexual distances (1.73 for eccrine glands and 1.73 for sebaceous glands), reflecting the cumulative differences in the activity of three types of phosphatases, which can be considered as an integral indicator of sexual dimorphism. Histochemical activity entropy is significant in the eccrine glands. Male entropy value (0.842 bit) was less than female entropy value (0.915 bit), because total actual activity of the males' phosphatases was greater (220% of 300%) than total actual activity of the females' phosphatases (200% of 300%). High entropy level of phosphatase activity was typical for male sebaceous glands (0.998 bit), where the general level of enzymatic activity was significantly reduced (160% of 300%). Because of the highest total actual phosphatase activity of female sebaceous glands (220% of 300%), entropy value was the lowest (0.842 bit). The obtained results show not only sexual dimorphism by histochemical parameters but show different communicational levels of male and female Norway rats, taking into account the important role of the plantar glands as sources of chemical signals determining the character of marking behaviour.

Keywords: skin; glands; histochemistry; qualimetry; analytical geometry; entropy.

Introduction

The skin glands are referred to as the chemocommunication sources, which determine the characteristics of the marking behaviour in mammals (Quay, 1986; Sokolov & Chernova, 2001; Rozhnov, 2011; Vanisova et al., 2016). It is known that trace marking with a secretion of the skin glands is crucial for Norway rats, as it participates in the formation of various behavioural acts (Barnett, 2017), including active investigative behaviour (Dzhemukhadze, 2007a, b). In addition, it is necessary to take into account that Norway rats' plantar skin includes two morphologically distinct groups of glands: eccrine glands and sebaceous glands (Sokolov, 1973; Bell, 1986; Hashimoto et al., 1986; Sokolov & Chernova, 2001; Chernova & Kiladze, 2019). We believe that for a holistic understanding of the nature of the marking behaviour associated with the secretory activity of the glands, it would be necessary to study the histoenzymatic activity of skin glands of both types (Dzhemukhadze, 2007a, b). The enzymes are known to be involved in the intracellular mechanisms of secretion, which indicates a direct link between histochemical phosphatase activity and the functional activity of the skin glands (Dzhemukhadze, 2007a, b). Given that the marking behaviour in animals is activated during the breeding season (Sokolov et al., 1988), the actual task is to identify signs of sexual dimorphism in the histochemical activity of the skin glands in males and females. The study of physiological criteria determining reproductive success is an important practical task related to managing the number of rapidly growing populations of synanthropic rodents (Livingston & Riley, 2003; Khlyap & Warshavsky, 2010; Feng & Himsworth, 2014; Haniza et al., 2015). Norway rats are a real danger as pests of urban infrastructure, agriculture, as well as carriers of various infectious diseases (Sudeikin, 1976; Livingston & Riley, 2003; Feng & Himsworth, 2014). They are capable of migrations, territorial invasions, wide dispersal and movement in space, which requires constant monitoring of the spatial-dynamic parameters of the population of this species (Sudeikin, 1976; Kalinin, 1995; King et al., 2011; Haniza et al., 2015).

In this regard, the aim of our work was to study the histoenzymatic activity of phosphatases of the eccrine and sebaceous glands of the soles of the paws of male and female Norway rats. The methods used include not only classical methods of histoenzymology (Montagna & Noback, 1947; Hashimoto & Ogawa, 1963; Schofield et al., 1977), but also modern mathematical approaches that allow calculating sexual dimorphism indices (Lovich & Gibbons, 1992; Taniguchi et al., 2017; Kiladze & Dzhemukhadze, 2018) based on semi-quantitative parameters (Kiladze & Dzhemukhadze, 2013; Azgaldov et al., 2015) of phosphatase activity, as well as identifying the intersex distance in the enzyme activity, presenting it in the form of coordinates in three-dimensional space (Aljohani, 2016). In addition, we calculated entropy (Martín & Rey, 2000) for appraisal of instabilityof histoenzymatic activity of cutaneous glands, which are resources of chemical signals (Kiyokawa et al., 2004).

Material and methods

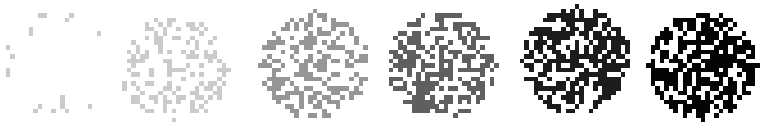
As an object of study, the eccrine and sebaceous glands of the skin of the soles of the paws of 10 adult male and 6 adult female Norway rats (*Rattus norvegicus* Berkenhout, 1769) were studied. The wild Norway rats were captured in various parts of Moscow City and Moscow region. All institutional and national ethical guidelines for care and use

of laboratory animals were followed. Plantar skin samples were fixed in 10% cold neutral formalin for up to 24 hours. The thickness of the frozen "floating" sections was 15 μm. Histochemical reactions to acid phosphatase (ACP), alkaline phosphatase (ALP) and adenosine triphosphatase (ATPase) were carried out according to Gomori and Burstone (Gomori, 1952; Burstone, 1962) in modifications by Dzhemukhadze (2007a, b), developed specifically for skin glands. Every histochemical reaction was controlled in a traditional way using incubation media without substrates (Pears, 1960; Barca & Anderson, 1963; Dzhemukhadze, 2007a, b). In the given scale of enzyme activity, qualimetry methods (Azgaldov et al., 2015) associated with the presentation of digital analogues (Table 1) corresponding to the degree of histoenzymatic activity of the skin glands were used (Kiladze & Dzhemukhadze, 2013).

Sexual dimorphism indices were determined by the following algorithm: (1) if the parameter of females (F) dominates over the parameter of males (M), then the ratio (F/M) − 1 was used; (2) if the parameter of males (M) exceeds the parameter of females (F), then another calculation was applied, namely: − (M/F) + 1 (Lovich & Gibbons, 1992; Taniguchi et al., 2017).

Table 1

The degree of histoenzymatic activity in the cell presented in various forms (Kiladze & Dzhemukhadze, 2013)

The visual characteristic of the activity reflecting the proportion of sediment						
Grey scale reflecting the degree of activity						
Verbal characteristic of activity	traces or lack of activity	indistinct activity	low activity	moderate activity	medium activity	high activity
Standard activity designation (SAD) in the form of characters	–	–(+)	+(–)	+	++	+++
Qualimetric activity designation (QAD), points	0	1	2	3	4	5
Relative activity (K_{Enzyme}), %	0/5×100 = 0	1/5×100 = 20	2/5×100 = 40	3/5×100 = 60	4/5×100 = 80	5/5×100 = 100

For the first time, using the methods of analytical geometry (Coolidge, 1948; Aljohani, 2016), the histochemical activity of the skin glands of male (A) and female (B) Norway rats was presented as coordinates of three-dimensional space, that is, A (ACP, ALP, ATPase) and B (ACP, ALP, ATPase). The distance (d), equivalent to the length of the segment AB, shows the distance between the histochemical activity of the skin glands of different-sex individuals of Norway rats, that is,

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

Three-dimensional graphics were built using the CPM 3D Plotter program hosted on the site https://technology.cpm.org/general/3dgraph.

Informational theory of Shannon (Shannon, 1948; Martin & Rey, 2000) provides for the following entropy (H) calculation formula:

$$H = -\sum_{i=1}^{n} P_i \log_2 P_i,$$

where P_i – possibility (frequency) ith variant; n – value number, which can be taken by the system.

Results

At the first stage, we changed over characters of histoenzymatic activity to digital analogue forms, and we also calculated sexual dimorphism indices (Table 2). The ACP activity did not reach maximum values in the eccrine and sebaceous glands, but in the latter it was higher. The low ACP activity in the eccrine glands was established in males, while females demonstrated a moderate activity of this enzyme, which led to the positive value of the index of sexual dimorphism. The sebaceous glands of males were characterized by moderate activity of ACP and the medium activity of this enzyme was typical for females. In this regard, the value of the index of sexual dimorphism also had a positive value.

The activity of ALP had different values. However, it was slightly higher in the eccrine glands compared to the sebaceous glands. High activity of ALP in the eccrine glands of males and medium activity of this enzyme in females was established. For this type of gland, a negative index of sexual dimorphism was recorded. The sebaceous glands of males were characterized by moderate activity of ALP and the medium activity of this enzyme was shown in females. The sexual dimorphism index was positive.

ATPase activity in both types of glands did not reach maximum values. However it was higher in the eccrine glands than in the sebaceous glands. The eccrine glands were characterized by medium ATPase activity in males and moderate activity value of this enzyme was revealed in females. In this regard, the index of sexual dimorphism was negative. The sebaceous glands were characterized by a low value of ATPase activity for males and moderate activity of this enzyme was demonstrated in females. The sexual dimorphism index was positive.

At the next stage, the histoenzymatic activity of the plantar glands of Norway rats was presented as coordinates, resulting from not only analytical calculations of the intersexual distances (Table 3), but also showing them in three-dimensional space (Fig. 1).

Table 2

Histoenzymatic activity of the skin glands of the soles of the paws of male and female Norway rats (*Rattus norvegicus*)

Skin glands	SAD in the form of characters		QAD, points / %		Sexual dimorphism index
	males	females	males	females	
Acid phosphatase:					
eccrine	+(–)	+	2 / 40	3 / 60	0.50
sebaceous	+	++	3 / 60	4 / 80	0.33
Alkaline phosphatase:					
eccrine	+++	++	5 / 100	4 / 80	−0.25
sebaceous	+	++	3 / 60	4 / 80	0.33
Adenosine triphosphatase:					
eccrine	++	+	4 / 80	3 / 60	−0.33
sebaceous	+(–)	+	2 / 40	3 / 60	0.50

Table 3

The distance between the histochemical activity of the skin glands of the soles of the paws of male and female Norway rats (*Rattus norvegicus*)

Skin glands	Coordinates of males – A (ACP, ALP, ATPase)	Coordinates of females – B (ACP, ALP, ATPase)	Intersexual distance equivalent to the length of the segment AB
Eccrine	A (2, 5, 4)	B (3, 4, 3)	1.73
Sebaceous	A (3, 3, 2)	B (4, 4, 3)	1.73

Despite the visual and quantitative differences in the relevant coordinates, reflecting the activities of ACP, ALP and ATPase, the calculated intersexual distance showed equal values for both the eccrine and sebaceous glands of the plantar skin of the Norway rats.

Entropy of histochemical activity was significant in the eccrine glands. Male entropy value (0.842 bit) was less than female entropy value (0.915 bit), because total actual activity of the males' phosphatases was greater (220% of 300%) than total actual activity of the females' phosphatases (200% of 300%) (Table 4). A high entropy level of phosphatase activity was typical for male sebaceous glands (0.998 bit), where the general level of enzymatic activity significantly decreased (160% of 300%). Because of the highest total actual phosphatase activity of female sebaceous glands (220% of 300%), entropy value was the lowest (0.842 bit) (Table 5).

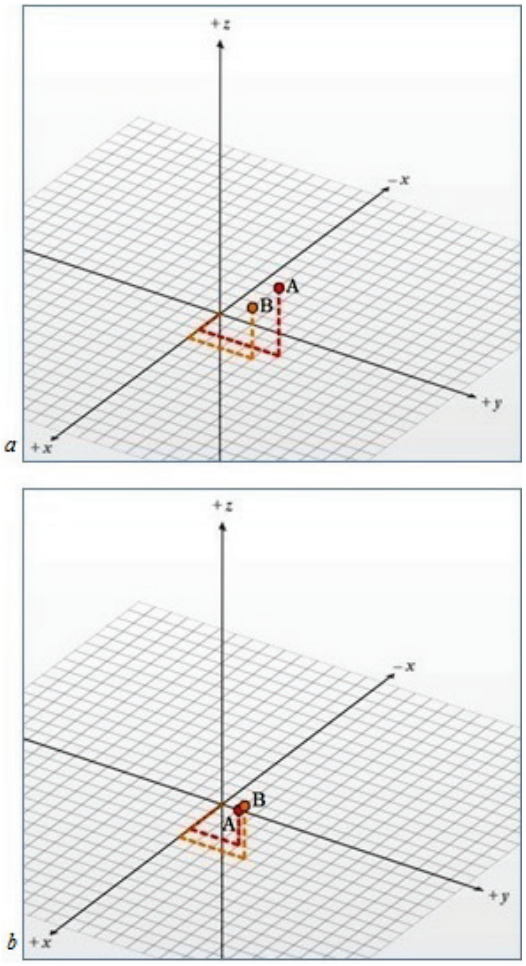


Fig. 1. The spatial ratio of the histoenzymatic activity of the eccrine (*a*) and the sebaceous (*b*) glands of the soles of the paws of male (*A*) and female (*B*) Norway rats (*Rattus norvegicus*): + x − ACP activity; + y − ALP activity; + z − ATPase activity (graphics were built using the site https://technology.cpm.org/general/3dgraph)

Table 4
Entropy analysis of enzyme activity in the eccrine glands of the soles of the paws of male and female Norway rats (*Rattus norvegicus*)

Types of phosphatase	Males			Females		
	SAD	QAD, points	K_{Enzyme}, %	SAD	QAD, points	K_{Enzyme}, %
Acid phosphatase	+(−)	2	40	+	3	60
Alkaline phosphatase	+++	5	100	++	4	80
Adenosine triphosphatase	++	4	80	+	3	60
Total actual activity of phosphatases:						
in percentage ratio (%)	–	–	220	–	–	200
in frequencies (P_1)	–	–	0.73	–	–	0.67
Total unrealized activity of phosphatases:						
in percentage ratio (%)	–	–	80	–	–	100
in frequencies (P_2)	–	–	0.27	–	–	0.33
Maximum activity of the phosphatases:						
in percentage ratio (%)	–	–	300	–	–	300
in frequencies ($P_1 + P_2$)	–	–	1.00	–	–	1.00
Entropy, bit*		0.842			0.915	

Note: P_i – possibility (frequency) i^{th} variant; n – value number, which can be taken by the system; in this example, n = 2 with respect to P_1 and P_2.

Discussion

There are good reasons to discuss the obtained results in relation to the histochemical and mathematical methods used. Histoenzymology allows us to determine activity rates of three types of phosphatases in the plantar glands of Norway rats given not only in standard characters but in digital analogues too (Walker, 2006; Dzhemukhadze & Kiladze, 2008; Kiladze & Dzhemukhadze, 2013).

Using the index of sexual dimorphism made it possible to quantify the nature of differences in the histochemical parameters of the skin glands of male and female Norway rats, while the calculated values of the indices were symmetrical with respect to the zero value. It is believed that such a calculation allows for more correct scaling of the intersexual differences (Lovich & Gibbons, 1992; Taniguchi et al., 2017; Kiladze & Dzhemukhadze, 2018, 2019).

Table 5
Entropy analysis of enzyme activity in the sebaceous glands of the soles of the paws of male and female Norway rats (*Rattus norvegicus*)

Types of phosphatase	Males			Females		
	SAD	QAD, points	K_{Enzyme}, %	SAD	QAD, points	K_{Enzyme}, %
Acid phosphatase	+	3	60	++	4	80
Alkaline phosphatase	+	3	60	++	4	80
Adenosine triphosphatase	+(−)	2	40	+	3	60
Total actual activity of phosphatases:						
in percentage ratio (%)	–	–	160	–	–	220
in frequencies (P_1)	–	–	0.53	–	–	0.73
Total unrealized activity of phosphatases:						
in percentage ratio (%)	–	–	140	–	–	80
in frequencies (P_2)	–	–	0.47	–	–	0.27
Maximum activity of the phosphatases:						
in percentage ratio (%)	–	–	300	–	–	300
in frequencies ($P_1 + P_2$)	–	–	1.00	–	–	1.00
Entropy, bit*		0.998			0.842	

Note: see Table 4.

The intersexual distance can be considered as an integral indicator of sexual dimorphism, combining the differences in the activity of the three types of phosphatases. Equal intersexual distance values indicate the synchronous nature and stable differences in the intracellular mechanisms of secretion in the two types of plantar glands in males and females.

Based on the received entropy values, we can draw the conclusion that the most stable histoenzymatic activity is typical for male eccrine glands and female sebaceous glands, then female eccrine glands follow and finally the least stable histochemical parameters are characteristic of the male sebaerous glands because the total actual activity of phosphatases is minimal in them. In this regard, the communicational state of females (0.879 bit) is more stable in comparison with males (0.920 bit) due to the lower average entropy values. We can draw the conclusion that there is an inverse relationship between total actual activity of phosphatases and entropy value. The higher the histoenzymatic activity level, the lower the entropy value, which reflects the "disorder" measure of enzyme systems (Schmalhausen, 1968). The biological meaning of the received data is quite evident: the secretory activity of cutaneous glands will be higher at higher values of histochemical activity of phosphatases, thus the entropy level will be lower for this process. At different and reduced levels of phosphatase activity, entropy value increases, which is a reflection of the unstable metabolism of cellular systems, one of the physiological manifestations of which is the production of a secretion (Bell, 1986; Hashimoto et al., 1986; Zhang et al., 2018; Diao et al., 2019).

It is advisable to include the above indices of sexual dimorphism, as well as the integral intersex distance and entropy by histochemical parameters of the skin glands, in the system of physiological criteria that affect reproductive parameters (Barnett, 2017) which require constant monitoring in order to control the population dynamics of Norway rats (Davis, 1953; Harper & Bunbury, 2015).

Conclusion

The study of the marking behaviour of Norway rats must be accompanied by an analysis of the functional parameters of the skin glands,

whose secretory activity is directly dependent on the examined histoenzymatic parameters presented in qualimetric form, which made it possible not only to calculate sexual dimorphism indices, but also analytically determine the intersexual distance represented in three-dimensional space. Carrying out entropy analysis allowed us to discover the instability degree of enzyme systems in the cutaneous glands of male and female Norway rats, as well as to show the difference in the communication state of opposite-sex individuals.

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