

## SPECTRAL ANALYSIS OF THE FUR OF DIFFERENT COLOUR PHASES OF *SCIURUS VULGARIS*: EVIDENCE FOR ADAPTIVE COLOURATION

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**Spectral analysis of the fur of different colour phases of *Sciurus vulgaris*: evidence for adaptive colouration.** — **Yu. Zizda.** — The red squirrel (*Sciurus vulgaris* L.) is one of the most variable mammals by its coat colouration, which allows to distinguish up to 40 subspecies. The distribution of four different subspecies in the region of the Eastern Carpathians, including Poland and Ukraine, based on coat colouration is described in literature sources. The accordance of colour phases to subspecies can be determined only if to place them in a row of individuals having similar colouration. Thus, in this paper, we present the results of research on two forms of the red squirrel having different coat colouration typical for Ukraine (light, or orange-red and dark, or black-brown). The aim of the study was to describe the colour phases of the red squirrel by means of spectral analysis and digital ratios of the fur pigmentation (melanin). In order to achieve this goal, we measured the dependence of the light diffuse reflection on fur samples established with the help of standard chemical methods (using alkali, NaOH in different concentration levels). To conduct the research, fur samples of dark specimens (black, dark brown squirrels) were taken from the Zoological Museum of Uzhhorod National University, while specimens of the light phase (red, orange squirrels) were taken from the Zoological Museum of Ivan Franko Lviv National University. In total, 30 individuals were investigated. According to the rules of light reflection and its differentiation into colours, we expected to obtain distribution curves in the range from 400 to 560–800 nm. In the light forms, the diffusion reflection curve was expected to rise at 560 nm and at 610 nm in the red. With the darkening of the fur, the increase in diffusion reflection should have approached the infrared region of 800 nm. However, according to the obtained results, the reflection spectra for the dark individuals showed much less deviation of the curve's right side than the spectra for most of the red individuals. However, orange samples with the similar deviations of the spectra right side were also observed among the black samples. There was no clear distribution among different colour phases of the squirrel regarding fur colouration intensity. At the same time, it is obvious that different pigments influence the darkness of the squirrel's coat patterns. This fact may indicate the adaptive nature of colour in different colour phases.

**Key words:** squirrel, colour form, spectral analysis, coat coloration.

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### Introduction

Analysis of mammal skulls and fur, which are especially variable by the phenotype and located in collections of zoological museums, helps to resolve issues of species variability, including their colouration. The coat coloration is very important for the adaptation of animals to the particular conditions of their habitats (Ortolani, 1999; Caro, 2005, 2013).

The European red squirrel (*Sciurus vulgaris* L.) is one of the most variable mammals by its fur coloration that allows describing about 40 subspecies (Tonkin, 1983; Lurz et al., 2005). In the Eastern Carpathians and across Ukraine and Poland four subspecies were described based on their fur colouration (Tatarinov, 1956; Zawadzka, 1958; Sidorowicz, 1958, 1971).

The individual colouration polymorphism in the red squirrel is an adaptation and depends on environment conditions of the habitat and the species' adaptation (Wauters et al., 2004; Fratto, Davis, 2011; Zizda, 2016). Conformity of colour phases to subspecies can be determined only if they are placed in one row of individuals with close coloration (Kiris, 1973). Four different phases of the red squirrel can be established in Ukraine: orange, which can be confined to *S. v. vulgaris* or *S. v. varius*, red, which corresponds to *S. v. kessleri*, brown, confined to *S. v. fuscoater*, and black, which was described as *S. v. carpathicus*. In the Ukrainian Carpathians and in the Transcarpathian region, at least three colour phases occur, which are distributed in different altitudinal zones and they usual-

ly co-occur only in urban environments. This allows to conduct a research similar to that by I. Kiris on a smaller but sufficient sample from a smaller study area.

In this paper, we studied two phases of the red squirrel having different coat patterns, typical for Ukraine. There are some differences in the distribution of colour phases in the nature and urban areas thus the description of these phases are rather rough (see Table 1).

Using spectral analysis methods in studies of coloration patterns to investigate adaptability to environmental conditions is known in amphibians and reptiles (Norris, Lowe, 1964). The intensity of fur colouration in different phases of the red squirrel studied by spectral analysis is described by Kiris (1973). The author showed that on the same wave length (a single region of the spectrum) the different colour phases do not produce the same intensity reflection, as it is in nature. At the same time, many questions arise about the methods and results of this experiment. Therefore, it became necessary to conduct a similar study in modern conditions.

## Materials and methods

To study different colour phases of the squirrel we used individuals from the Ukrainian Carpathians and Transcarpathia. Thirty specimens were studied, including dark forms (black, dark brown) from the Zoological Museum of Uzhhorod University (ZMUZH) and light forms (red and orange) from the Zoological Museum of Ivan Franko University of Lviv (ZMD) (acronyms after Zago-rodniuk, Shydlovsky, 2014).

Common methods of chemical analysis, particularly the dissolution of crushed fur in NaOH were used to isolate pigments. Spectral analysis was performed in the visible part of the spectrum. The dependence of diffuse light reflection from the fur was measured. The research consisted of two steps: 1) the reflection spectra were removed from the dry fur; 2) melanin was isolated from the analysed samples in step 1 and then spectra were analysed again.

The accuracy of measurements was checked several times. A spectrometer with a reflection nozzle was used to capture the spectra. An Al<sub>2</sub>O<sub>3</sub> sample was used as standard, as it gave 100 % of full reflection over a wide range. The fur of squirrels was measured in relation to the reflection spectrum of the standard sample (100 %).

## Results and discussion

The spectral dependence of diffuse light reflection on fur (30 squirrels) is presented in Fig. 1. According to the rules of light reflection and its differentiation to colours that can be seen in the spectrum, all light coloured squirrels were expected to have low diffusion reflectance in the spectral range of 400 to 560–580 nm. In orange forms, the start of diffusion reflection lifting should have occurred at 560 nm. With the darkening of the fur, the enhancement of diffusion reflection was expected to shift into the infrared region (up to 800 nm).

Thus, curve line growing for red samples at 610 nm on the graph was expected.

Spectral dependence of diffuse light reflection from the fur is presented in Fig. 1: black lines indicate the dark phase and red lines note the light phase. The X-axis shows the wavelength from 400 to 800 nm (400–480 are blue in the spectrum; 490–540 nm are green in the spectrum; 540–590 nm are yellow-orange; 600–800 are red). The Y-axis is the light reflection intensity. The graph shows the jumps of light reflection curves at 540–590 nm.

Table 1. Two colour phases of the squirrel analysed in this research

Таблиця 1. Дві кольорові форми вивірки, яких було досліджено у цій роботі

Colour phase	Variants of coloration	Corresponding subspecies and their distribution
Dark	black, dark brown	<i>S. v. carpathicus</i> (black) and <i>S. v. fuscoater</i> (dark brown). Both are distributed in the mountains.
Light	red, orange	<i>S. v. kessleri</i> (red) and <i>S. v. vulgaris</i> or <i>S. v. varius</i> (orange). Red forms are distributed in piedmont areas, while orange forms occur on plains.

The thin blue line in the graph shows the standard sample's reflectance spectrum. The results indicated that even dark squirrels have a similar type of line and there is no clear difference between different colour phases. For a convenient further analysis, the graphs of the dependence of the colour intensity on the degree of light permeability of each colour phase were shifted along the Y-axis to the level of one (Fig. 2).

The dark phase is represented by black lines (see Fig. 2). The study showed that dark squirrels have a much smaller right side curve deviation than the most of the light squirrels. Among lines of dark squirrels are lines of light specimens too with a similar deviation of the right part of the spectrum. In addition, some light squirrels showed a higher level of deviation of the right side of the spectrum than that in dark specimens.

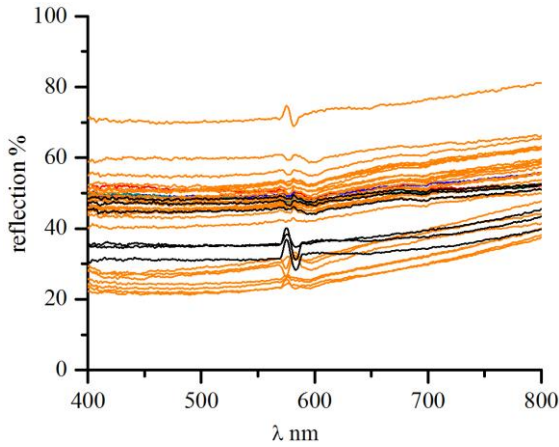


Fig. 1. Spectral dependence of diffuse light reflection on fur of two colour phases of the squirrel: black lines indicate the dark phase and red lines indicate the light phase. X-axis — wavelength; Y-axis — light reflection intensity.

Рис. 1. Спектральна залежність дифузійного відбивання світла від хутра вивірки двох кольорових форм: чорні лінії — це темна форма, червоні — світла. Вісь X — довжина хвилі; вісь Y — інтенсивність відбивання світла.

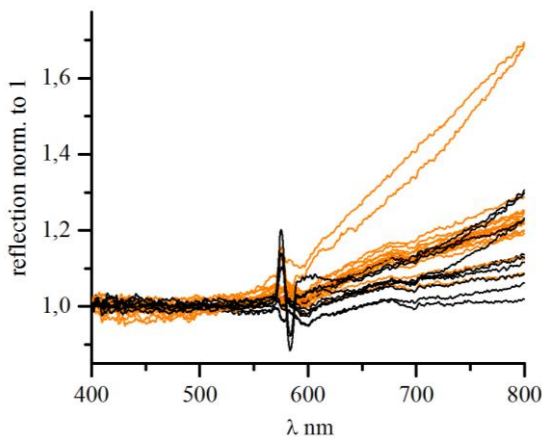


Fig. 2. Spectral dependence of diffuse light reflection on squirrel fur when Y-axis is equated to one. X-axis — wavelength; Y-axis — light reflection intensity.

Рис. 2. Спектральна залежність дифузійного відбивання світла від хутра вивірки приведена до одиниці по осі Y. Вісь X — довжина хвилі; вісь Y — інтенсивність відбивання світла.

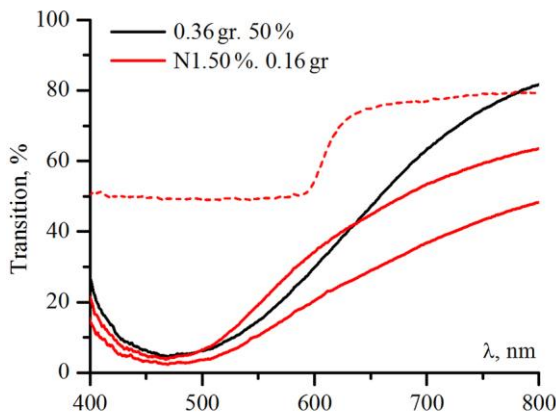


Fig. 3. Spectral dependence of light transition on squirrel fur dissolved in NaOH

Рис. 3. Спектральна залежність пропускання світла від хутра вивірки, розчиненого у NaOH.

Thus, an unambiguous separation of colour phases was not observed. Noteworthy that the amplitude curve of dark squirrels at 580 nm is higher than that of light squirrels on average.

The next step included the analysis of two samples of each colour phase in a 100 % and 50 % NaOH solution. Additionally, the samples were subjected to ultraviolet radiation. Results are presented in Fig. 3. The dashed line shows the reflection of a standard light phase sample. The black line in the figure corresponds to the samples of the dark phase in a 100 % concentration solution. The samples of the light phase (red lines) are close to the spectrum of the dark phase (upper red line — light phase in 100% solution, lower red line — light phase in 50 % solution). In the analysis, we expected a right side spectral line deviation at 600 nm for the light phase. As we can see in Fig. 3, upward shifts in both phases start at 500 nm and gradually increase to 800 nm. The dark phase showed a larger deviation (right part of the line) than the light phase. However, in case of adding other samples to the graph, the black and red lines will overlap.

## Conclusions

Results show that the light and dark colour phases of the European red squirrel cannot be distinguished by spectral analysis of fur pigments and both phases have similar spectra. Therefore, the description of the squirrel's fur coloration in digital terms requires a different approach.

It is obvious that the different colour phases of the European squirrel must be described carefully in terms of colouration intensity (amount, saturation) but not by the type of fur pigments (red or black). If the hypothesis is justified, the shade of the squirrel's fur might logically depend on environmental conditions. The amount or saturation of the fur pigment is important when squirrels need to protect their skin from harmful UV radiation or to use heat more effectively. Thus, our results of spectral analysis allow us to conclude that coat colouration in the European red squirrel is an adaptive ecological feature.

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