Genus	Vol. 21(1): 163-168	Wrocław, 30 III 2010

Spectroboard: an instrument for measuring spectral characteristics of butterfly wings – a new tool for taxonomists

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Abstract. The spectroboard, a device developed for measuring spectral properties of the light reflected from butterfly wings, is described and discussed. It allows to set constant illumination conditions necessary for multiple, repetitive measurements which enable to evaluate the spectrum of the light reflected from wings and discriminate the differences between species. Details of its construction and the instruction on how to use the instrument are given and briefly discussed. Spectroboard measurements cause no harm to museum specimens and are safe even for unique types.

Key words: Structural colours, sexual signals, optical properties, repeatable measurements, spectral analysis, taxonomic utility

INTRODUCTION

According to Vane-Wright (1979), optical characteristics of the light reflected from the dorsal surface of butterfly wings are of a great importance for inter- and intra specific communication, especially during sex discrimination by individuals of the same and different species. In our studies on diversity of structural colouration of butterfly wings we accumulated sufficient amount of data supporting that hypothesis.

Measurement of spectral characteristics of the light reflected from the dorsal wing surface of a butterfly wing requires calibrated artificial white light to be used for illumination of the wing. Reflected light is analysed by means of a spectrophotometer. The results of several repetitive measurements are analysed statistically and resulting mean spectral pattern is assigned to a species. The method in itself is easy to use, however, it has to be remembered that in order to maintain the same, stable physical conditions for each consecutive measurement the angle at which the light beam should fall into the wing surface should always be the same. This also applies to the distance between the tip of the illuminating fiber glass above the wing, and a spectral characteristic of calibrated light used for illumination.

The above mentioned conditions can be easily controlled in the "spectroboard", as we call the instrument that permits the analysis of spectral properties of light reflected either from the whole, or from a part of butterfly wing. The advantage of this method is that even the most fragile specimens remain intact after that treatment.

External characters, such as wing colouration pattern, genital structures, etc. traditionally used by taxonomists, can now be supplemented by the spectral data which are characteristic for a species. Therefore, taxonomically important conclusions concerning possible relationships between particular species, or groups of species, can now be taken with better accuracy and confidence.

DESCRIPTION OF THE INSTRUMENT AND ITS OPERATIONAL GUIDE

The instrument described here resembles ordinary wooden spreading board (Winter 2000: 207-210) commonly used by entomologists to fix wings of butterflies in a required position. However, contrary to ordinary spreading boards that are usually built from soft, wooden material, our board was constructed entirely from metal plates (Fig. 1). Both, the left and right plate with flat surfaces horizontal to each other, were covered by smooth plastic sheets. The left part of the board can be moved horizontally, so that the width of the pinning channel between the two parts can be easily adjusted to accommodate the thorax. The board was supplemented with a fiber optic spectrophotometer. In the prototype which we are describing here, Avantes 2048 fiber optic spectrophotometer was used, but any other model can also be chosen.

The both, spectrophotometer and the setting board, make together the main part of a set. To the right side of the board there is a fixed metal plate equipped with a movable metal arm positioned perpendicularly to it. The arm can be moved above the board and fixed in a certain, required height. The illuminating/pick-up fiber glass is mounted within the middle of a copper screw in the arm in such a way that its position on a metal arm can be adjusted and then fixed in a certain position. At the end of the board there is a white pad used for calibration of the spectrophotometer prior to the measurements.

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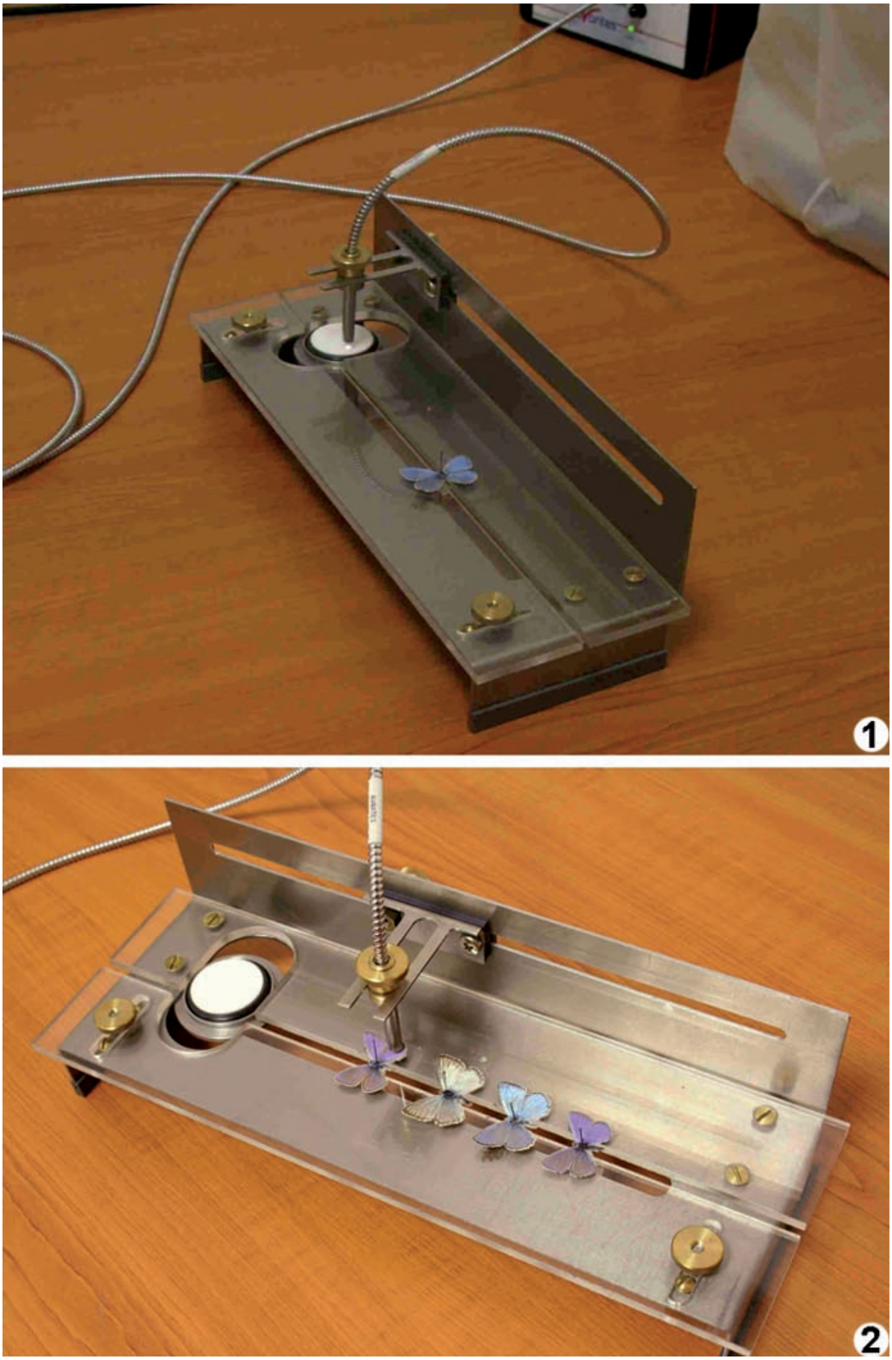
1. The spectroboard seen at the moment of light calibration before the start of spectral measurements (scales: left plastic wing board length = 32 cm; left wing board width = 5 cm; the width of spectroboard metallic base = 10 cm); 2. The spectroboard installed for measuring spectral properties of dorsal surface of wing under normal light incidence. Four different species of *Polyommatus*: *P. thersites*, *P. coridon*, *P. dapnis* and *P. icarus* are ready for measurements. On this photograph the illuminating/pick-up fiber of the spectrophotometer is shown during measuring the light reflected from *P. thersites* hindwing dorsal surface (scales as in Fig. 1)

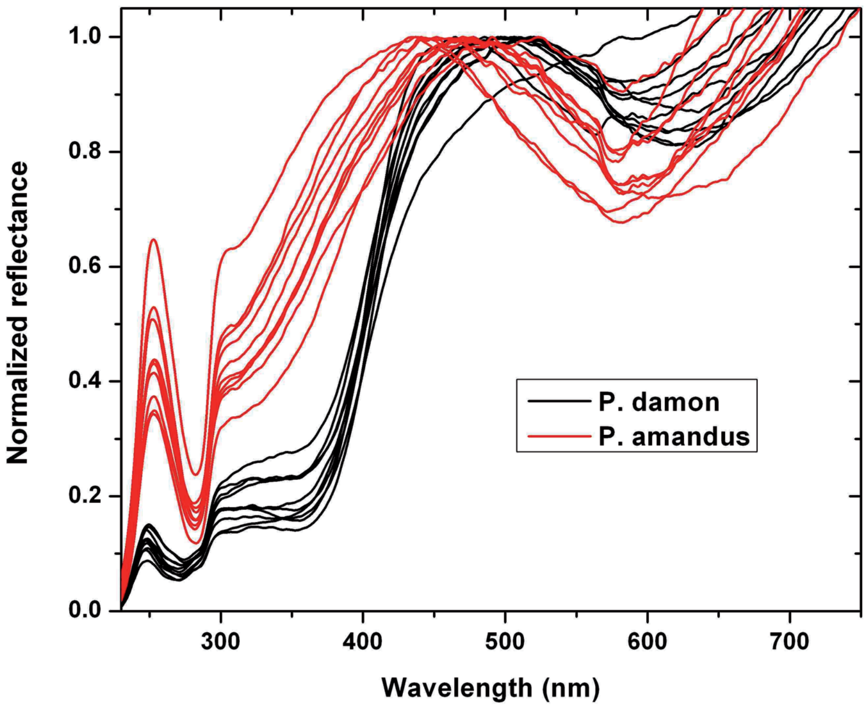


1, 2. Explanation - see facing page

As it was mentioned above, the system allows to obtain spectral characteristics of specimens already mounted on pins, so it is especially suitable for taking data from unique and rare specimens stored in research collections. Because of obvious reasons the data labels on the pin below the specimen have to be temporarily removed before it is placed inside the pinning grove between the two metal plates. Depending on the sector of the wing to be studied the position of the fiber optic cable should be adjusted accordingly. If a series of specimens is to be measured they have to be arranged in line and the position of the spectrophotometer above them should be properly fixed (Fig. 2). For each measurement a protocol has to be made including specimen label data, its name and sex.

DISCUSSION

In earlier methods, measurements of spectral characteristic of light reflected from a butterfly wing could be performed only on wing detached from an insect body. Therefore, in the past, scientists investigating structural colouration patterns in butterflies had limited number of taxa available for studies. This was due to the fact that many tropical species were represented in museum collections only by few specimens, or



3. Reflectance spectra graphs of *Polyommatus damon* (Denis & Schiffermüller, 1775) and *Polyommatus amandus* (Denis & Schiffermüller, 1775) measured in the area of the discal cell apex, composed from ten male individuals of each species

even single types. Detaching the wings from those specimens was not permitted so spectral data for many species are still unknown.

The spectroboard equipment proposed by us makes it possible to uncover the spectral patterns of all specimens, including types, so that their morphological characters can be supplemented with spectral characters.

With this new method, it is now possible to discriminate hitherto unrecognized, or undescribed species (Bálint, Kertész & Wojtusiak 2006) and use species specific spectral characters to support hypotheses on possible evolutionary relationships between various groups of butterflies, especially those belonging to the families displaying structural colours. The method may also help to study the degree of variation of spectral patterns within a population of a species in relation to its geographical distribution and selection of particular types of habitat.

It has to be remembered that the light reflected in normal incident illumination is only a part of the spectrum responsible for optical characteristic of butterfly wings. Therefore, in our research we also carried out extensive measurements of various lycaenid butterflies using different, goniometric techniques (Kertész *et al*. 2006, Biró *et al.* 2007). In these experiments it was necessary to detach wings from specimens and cut them into small pieces. The results confirmed that, if measurements are done by means of spectroboard and are carefully performed in a preset, constant illumination condition, the discrimination between species specific reflection patterns is possible with a high degree of accuracy. It also applies to the species that exhibit so called, iridescent colouration. For example, spectral patterns obtained for ten specimens of lycaenids belonging to two different *Polyommatus* species were sufficiently distinctive to permit their discrimination (Fig. 3).

Results of our research also indicate that the intraspecific variations of the spectral reflection patterns are very slight but the species specific pattern was always well defined. Sometimes it may happen, that one of the measured specimens of the population can show much higher variation than all the other. In such a case, closer examination usually reveals that the specimen was probably mishandled during relaxation and mounting and the observed difference probably resulted from the changes of the nano structure of its scales.

The results of our work on spectral analyses support the hypothesis of Vane-Wright (1979) mentioned at the beginning of the introduction that the light reflection pattern is characteristic for a species (Bálint *et al*. 2007, 2008*a* and 2008*b*). There are some indications that butterfly optics may be important not only for communication between sexes, but also may influence the efficiency of camouflage (Bálint *et al*. 2009) and mimicry (Bálint & Biró 2009). Butterfly wing optics therefore, opens a promising field of research, and we hope that the spectroboard measurement method developed by us will be useful for taxonomists and ethologists.

Acknowledgements. The work in Hungary was supported by OTKA-NKTH research grant No 67793.

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