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INTENSITY OF WING PIGMENTATION AND IDENTIFICATION OF PIGMENTS IN WINGS OF OWL-FLY *LIBELLOIDES MACARONIUS* (SCOPOLI, 1763) (NEUROPTERA: ASCALAPHIDAE)

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Abstract – Coloration intensity of wings from owl-fly (*Libelloides macaronius*) was checked with absorption of the light; at 425 nm of the yellow parts of the wings and at 650 nm of the black parts. The difference between males and females was not statistically significant. In black parts of wings the black pigment melanin was identified. In both parts (yellow and black) the yellow pigment sepiapterin and one unidentified yellow pigment were present. They were extracted with 0.4% sucrose and analysed on paper and thin–layer chromatography on Silica gel.

KEY WORDS: Neuroptera, Ascalaphidae, *Libelloides macaronius*, wings, coloration, pigments, sepiapterin, melanin

Izvleček – INTENZITETA PIGMENTIRANOSTI KRIL IN IDENTIFIKACIJA PIGMENTOV V KRILIH METULJČNICE *LIBELLOIDES MACARONIUS* (SCOPOLI, 1763) (NEUROPTERA: ASCALAPHIDAE)

Intenziteto obarvanosti kril metuljčnice (*Libelloides macaronius*) smo določali z absorpcijo svetlobe pri valovni dolžini 425 nm za rumene dele kril in pri valovni dolžini 650 nm za črne dele kril. Razlike med samci in samicami niso bile statistično pomembne. V črnih delih kril smo dokazali črni pigment melanin. V obeh delih (rumenih in črnih) je bil prisoten rumen pigment sepiapterin in še en neidentificiran rumen pigment. Ekstrahirali smo ju z 0.4% saharozo in analizirali s papirno in tankoplastno kromatografijo na silikagelu.

KLJUČNE BESEDE: Neuroptera, Ascalaphidae, *Libelloides macaronius*, krila, obarvanost, pigmenti, sepiapterin, melanin

Introduction

Libelloides macaronius (Scopoli) inhabits Central, Eastern and South Europe and Palaearctic part of Asia (Aspöck et al., 2001). In Slovenia the species is widely distributed near the Adriatic coast in the SW, but occurs also in certain warmer places all over the country (Devetak et al., 2002). Its yellow wings with black spots have a warning coloration. The differences in colour intensities between males and females are not substantial when we observe them with naked eye. A similar coloration with yellow pteridine (sepiapterin) and black melanin was reported in the integument of scorpion fly *Panorpa japonica* (Nakagoshi et al., 1984). Yellow pigments of insect origin could also be ommochromes and porfirines or from food accepted carotenoides and flavones (Peters, 1999). Our aim was to asses a potential difference in intensity of coloration of the male and female wings, and extraction and identification of the pigments.

Material and methods

Specimens of *Libelloides macaronius* (Scopoli) were collected in grasslands near the village Nerezine (44° 40' N, 14° 24' E) on the island Lošinj in Croatia and stored at –25°C before use. Intensity of coloration of the wings was measured with absorption of the light in situ after slightly modified method of Stark (1974). Each right rear wing (from 30 males and 30 females) was cut off, clumped between two plates with a 1.9 mm aperture and this holder was inserted on the front of the sample chamber in spectrophotometer (Perkin-Elmer, Lamda Bio). Absorption was measured in parts with intensive and homogenous pigmentation (Fig. 1). The yellow parts were measured at the wavelength 425 nm (at the absorption maximum of pteridines (Nakagoshi et al., 1984; Stark, 1974)) and black spots at the wavelength 650 nm (recommended for quantification of melanin (Virador et al., 1999)). Student's "t" test was used to calculate the statistical significance between results for males and females.

Melanin was identified by soaking the black part of wing in the solution of the methylene blue (7 mg/L) in KCl – buffer (0.2 mol/L) pH 1 (Lilie, 1954).

Identification of yellow pigments was performed from 6 rear wings. The wings were separated in yellow parts (2.8 mg) and black parts (3.5 mg) into two mortals. After grinding with 42 mg of quartz sand for 5 min, the extraction was performed with 300 µL of 0.4% sucrose in a dim light. After 10 min of centrifugation at 1000 g, the extraction was repeated. The absorption spectra of pooled supernatants were scanned at 350 – 700 nm. The freeze-dried supernatants were dissolved in 10 µL of deaerated water and applied (3 x 1 µL) on aluminium sheet with Silica gel 60 F_{254} (7.5 x 20cm; Merck, Germany) for thin-layer chromatography. On last starting point

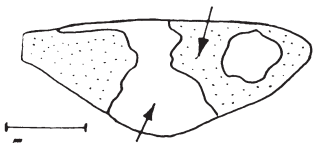

Fig. 1: Rear wing of *Libelloides macaronius*. Arrows indicate the points where absorption was measured.

the 1μL of solution containing 0.1 mg of pure sepiapterin (Schircks, Switzerland) was applied. The solvent was n-propanol – 1% ammonium hydroxyde (2:1, V/V) (Hama et al., 1965). Developing was performed in dark. The chromatogram was dried with warm air and checked at day light and under fluorescence at excitation wavelength 365 nm in fluorescence analysis cabinet (Spectroline CM-10, USA). The paper chromatography (with paper MN214, Macherey-Düren, Germany) was performed at the same conditions.

Results

The yellow and black parts of wings absorb light of specific wavelengths quite differently, but differences between males and females at equal parts of wings were not statistically significant (Table 1).

Table 1: Absorption of yellow and black parts of the wings of *Libelloides macaronius* at wavelengths 425 and 650 nm, respectively. Values are mean ± S. D., N = 30, * = p< 0.05.

	Absorption of the yellow parts of wings at 425 nm	Absorption of the black parts of wings at 650 nm
Males	2.860 ± 0.182	1.426 ± 0.176
Females	2.830 ± 0.192	1.450 ± 0.135

The presence of melanin was confirmed with methylene blue at pH 1 as a dark green coloured margins at the dark spots of the cut wings.

Extraction of the yellow and black parts of wings resulted as yellow extracts with the absorption maximum at 420 – 425 nm, characteristic for the presence of pteridines. The first extract from yellow parts had at 425 nm absorption 0.958 and from the black parts 0.426. Absorption of the second extracts were 0.631 and 0.230, respectively.

The thin-layer chromatography revealed the presence of two yellow spots (at least two yellow pigments) in both extracts. The first spot with Rf 0.33 was not identified, while the second with Rf 0.56 the length of migration corresponded to the migration of pure sepiapterin (Figure 2). Paper chromatography (Figure 3) confirmed these results. The yellow spot with Rf 0.27 was not identified and yellow spot with Rf 0.44 was at the same position as pure sepiapterin.

Discussion

Sexual dimorphism of *Libelloides macaronius* is not marked, although the wings of females were found to be slightly, but statistically significant larger than in the males (Devetak et al., 2002). After our absorption data the differences in intensity of coloration of wings were not statistically significant. Moderate low absorption of the black spots is consequence of distribution of the black pigments only near the veins

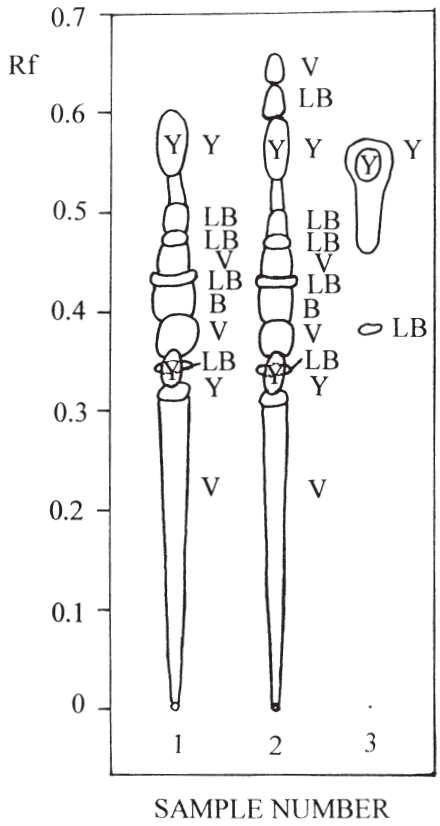

Fig. 2: Thin-layer chromatography of extracts from wings of *Libelloides macaronius*. Samples: 1 – from yellow parts, 2 – from black parts, 3 – pure sepiapterin. Yellow spots, visible at daily light, are indicated with Y in the centre of spot. On right side is indicated the colour of fluorescence at 365 nm: B – blue, LB – light blue, V – violet, Y – yellow.

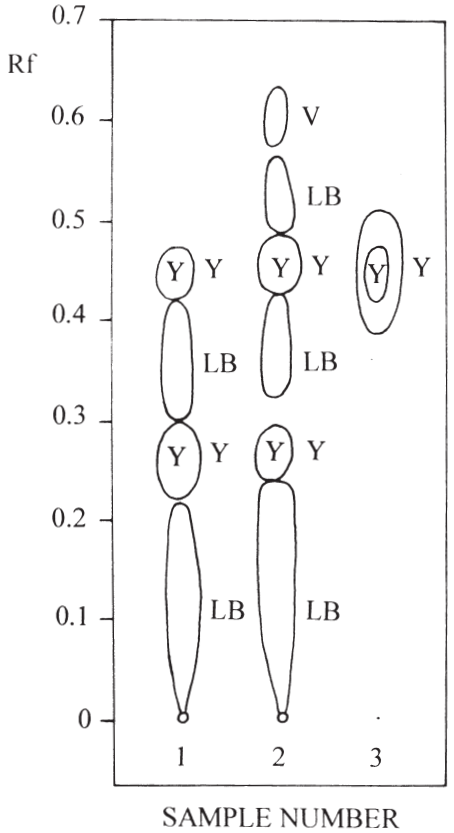

Fig. 3: Paper chromatography of extracts from wings of *Libelloides macaronius*.
Samples: 1 – from yellow parts, 2 – from black parts, 3 – pure sepiapterin. Yellow spots, visible at daily light, are indicated with Y in the centre of spot. On the right side is indicated the colour of fluorescence at 365 nm: LB – light blue, V – violet, Y – yellow.

and fields between them are yellow in the middle (personal observation). The absorption of the yellow pigments in black spots was lower than in yellow spots (data not shown). An alternative method for determination of the colour intensity of wings with potentially lower scattering interference on structures requires a colour camera, which is connected to personal computer (Windig, 1999).

The presence of black pigment melanin in black parts of the wings was identified only with the histochemical method (Lilie, 1954), because the extraction after the

method for determination of melanin in mice melanocytes (Virador et al., 1999), was completely unefficient. After extraction with 0.85 M KOH (Siegrist and Eberle, 1986), the black pigment was only partially solubilized (data not shown).

In contrast to the black pigments, the yellow pigments pteridines are slightly soluble, but in solutions easily oxidizable and photolabile, as reported by Hama et al. (1965), Nakagoshi et al. (1984) and Tomic-Carruthers et al. (2002). In attempt to stabilise them we checked extractions at dim light with 50% ethanol at 100ºC (Nakagoshi et al., 1984), 50% ethanol at room temperature and water solutions of five reducing compounds: 0.1% β-mercaptoethanol in 0.1 M ammonium acetate buffer pH 6.5 and water solutions of 0.05% ascorbic acid, 0.02% potasium metabisulfite, 0.24% rosmarinic acid (Roth, Germany) 9% and 0.4% sucrose. The best results were obtained with sucrose, equal at both concentrations (data not shown). Pigments were also labile during concentrating and freeze-drying in dark could not be substituted with evaporation in dark at room temperature (data not shown). In *Panorpa japonica* the successive extraction of yellow sepiapterin with 50% ethanol at 100ºC and black melanin from integuments was performed (Nakagoshi et al., 1984), but before extraction the yellow and black parts were not separated.

The thin-layer and paper chromatography revealed the presence of two yellow pigments. The pigment with higher mobility was identified as sepiapterin, but pigment with lower mobility was not identified. The absorption spectrum of the extract of the wings with absorption maximum at 420 – 425 nm indicates that this pigment must be very similar to sepiapterin. It may be a product of oxidation and degradation of sepiapterin during aging of the wings and after comparing the Rf values (Hama et al., 1965) it was tentatively proposed to be xanthopterin. This is supported by the identification of sepiapterin only in *Panorpa japonica* one week after emergence (Nakagoshi et al., 1984). For more detailed qualitative and quantitative analysis, the high pressure liquid chromatography (HPLC) must be used, as described Tomic-Carruthers et al. (2002). The two-dimensional thin-layer chromatography is not recommended, because spontaneous oxidation of some pigments during chromatography may occur, as reported for 7,8-dihydrobiopterine (Tomic-Carruthers et al., 1996).

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References

Aspöck H., Hölzel H., Aspöck U., 2001: Kommentierter Katalog der Neuropterida (Insecta: Raphidioptera, Megaloptera, Neuroptera) der Westpaläarktis. *Denisia*, 02: 1-606.

Devetak D., Pirš P., Janžekovič F., 2002: Owl-fly Libelloides macaronius (Scopoli, 1763) in Slovenia and in the northwestern part of Croatia (Neuroptera: Ascalaphidae). *Annales Ser. Hist. Na*t., 12: 219-226.

Hama T., Goto T., TohnokiY., Hiyama Y., 1965: The relation between the pterins and chromathophores in the medaka Oryzias latipes. *Proc. Japan Acad.*, 41: 305-309

Lilie, 1954. In: Bock P., 1989: Romeis Mikroskopische Technik. 403-404 pp. Urban und Schwarzenberg, München.

Nakagoshi M., Masada M., Tsusue M., 1984: The nature of the seasonal colour dimorphism in the scorpion fly, Panorpa japonica Thunberg. *Insect biochemistry* 14(6): 615-618.

Peters W., 1999: Integument. In: Dettner K., Peters W., :Lehrbuch der Entomologie.1-52 pp. Gustav Fischer, Stuttgart.

Siegrist W., Eberle A. N., 1986*:* In situ melanin assay for MSH using mouse B16 melanoma cells in culture. *Anal. Biochem.,* 159: 191-197.

Stark W. S., 1974: Spectral absorption characteristics of sepiapterin measured in situ and in vivo. *Drosophila information service*, 51: 46-47.

Tomic-Carruthers N., Robacker D. C., Mangan R. L., 1996: Identification and age-dependance of pteridines in the head of adult mexican fruit fly, Anastrepha ludens. *J. Insect. Physiol.*, 42(4): 359-366.

Tomic-Carruthers N., Mangan R., Carruthers R., 2002: Age estimation of Mexican fly (Diptera: Tephritidae) based on accumulation of pterins. *J. Econ. Entomol.,* 95(6): 1319-1325.

Virador V. M., Kobayashi N., Matsunaga J., Hearing V., 1999: A standardized protocol for assesing regulators of pigmentation. *Anal. Biochem.,* 270: 207-219.

Windig J. J., 1999: Trade-offs between melanization, development time and adult size in Inachis io and Araschnia levana (Lepidoptera: Nymphalidae)?. *Heredity,* 82: 57-68.

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