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QUANTIFICATION OF WING PIGMENTATION AND IDENTIFICATION OF PIGMENTS IN WINGS OF *PALPARES LIBELLULOIDES* (LINNAEUS, 1764) (NEUROPTERA: MYRMELEONTIDAE)

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ABSTRACT

Pigmentation of the wings in Palpares libelluloides was quantified with light absorbance at specific wavelengths. Absorption of yellow parts of the wings in females was higher than in males. In the yellow parts of the wings, the pigments sepiapterin and xanthopterin were identified, whereas in their black spots the melanin was present.

Key words: Neuroptera, Myrmeleontidae, *Palpares libelluloides*, wings, coloration, pigments

QUANTIFICAZIONE DI PIGMENTAZIONE DI ALI ED IDENTIFICAZIONE DI PIGMENTI IN ALI DI *PALPARES LIBELLULOIDES* (LINNAEUS, 1764) (NEUROPTERA: MYRMELEONTIDAE)

SINTESI

La pigmentazione delle ali di Palpares libelluloides è stata quantificata con l'assorbanza della luce a specifiche lunghezze d'onda. L'assorbimento delle parti gialle delle ali è risultato maggiore nelle femmine che nei maschi. Nelle parti gialle delle ali sono stati identificati i pigmenti sepiapterina e xantopterina, mentre nelle macchie nere era presente la melanina.

Parole chiave: Neuroptera, Myrmeleontidae, *Palpares libelluloides*, ali, colorazione, pigmenti

INTRODUCTION

Palpares libelluloides (Linnaeus) occurs as a single species from the tribe Palparini in Europe (Mansell, 1990). It is widely distributed in the Mediterranean reaching Iran and Caucasus (Aspöck *et al.*, 1980). In Slovenia, the species has not yet been found (Devetak, 1996), although it is expected. As predator on insects and spiders, it inhabits grassland areas and scrub communities. Macroscopically, its pale yellow wings with black spots do not substantially differ between males and females and seem to have more mimic than warning appearance. A similar coloration of wings with yellow pigments pteridines (sepiapterin and xanthopterin) and black melanin was reported for *Libelloides macaronius* (Senčič, 2006). The same pigments were detected in the integument of a scorpion fly *Panorpa japonica* (Nakagoshi *et al.*, 1984). Yellow pigments in insects could also be ommochromes, porfirines or compounds originating from food ingested, like carotenoides and flavones (Peters, 1999).

In the present study, the intensity of pigmentation of the wings was measured with absorption of the light. The pigments were extracted from the wings and identified.

MATERIAL AND METHODS

Adult specimens of *Palpares libelluloides* (Linnaeus) were collected in grasslands near the village of Nerezine (44°40' N, 14°24' E) on the island of Lošinj in Croatia

(Fig. 1) and stored at –25°C before use. Intensity of coloration of the wings was measured according to the method described in an earlier paper (Senčič, 2006). Briefly, from each right hind wing (from 10 males and 10 females) about 2 cm long distal part was cut off and clumped between two plates with a 1.9 mm aperture. This holder was inserted on the front of the sample chamber in spectrophotometer (Perkin-Elmer, Lambda 11/Bio). Absorption was measured in parts with intensive and homogenous pigmentation (Fig. 2). The yellow parts were measured at 425 nm wavelength (at the absorption maximum of pteridines (Stark, 1974; Nakagoshi *et al.*, 1984)) and black spots at 650 nm wavelength (as recommended for quantification of melanin (Virador *et al.*, 1999)). The data were statistically analysed with Student's *t* test, the correlation coefficient *r* and *F* test; all tests were performed with the computer package STATISTICA (StatSoft, Inc.).

Identification of melanin was performed by soaking the black part of the wing in the solution of the methylene blue (7 mg l⁻¹) in KCl buffer (0.2 mol l⁻¹), pH = 1 (Lillie, 1954).

Extraction and analysis of the yellow pigments were performed from 6 fore and 6 hind wings. The wings were separated in yellow (93.8 mg) and black parts (9.0 mg) in two mortars. After grinding with 42 mg of quartz sand for 5 min, the extraction was performed with 600 and 300 µl of 0.4% sucrose, respectively, in a dim light. After 10 min of centrifugation at 1000 g, the reextraction of each pellet was performed with 250 µl of 0.4% sucrose. The absorption spectra of pooled supernatants



Fig. 1: A pasture near Nerezine (Lošinj), July 1992. (Photo: D. Devetak)
Sl. 1: Pašnik blizu Nerezin (Lošinj), julij 1992. (Foto D. Devetak)

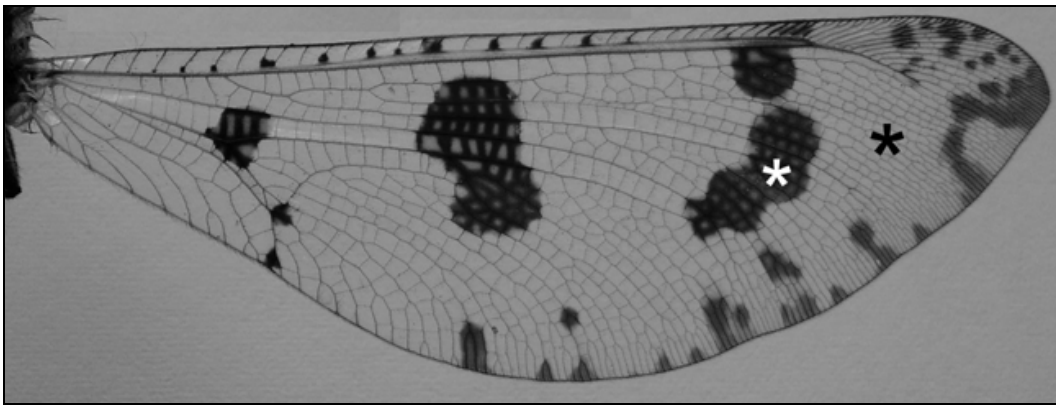


Fig. 2: Hind wing of ant-lion *Palpares libelluloides*. Black and white asterisks indicate the points where absorption was measured. Length of the hind wing is 55 mm.

Sl. 2: Zadnje krilo volkca vrste *Palpares libelluloides*. Črna in bela zvezdica kažeta mesti, kjer je bila merjena absorpcija. Dolžina zadnjega krila je 55 mm.

were scanned at 350–700 nm. The freeze-dried supernatants (0.5 mg of each) were dissolved in 3 μ l of deaerated water and applied (3 \times 0.5 μ l) on aluminium sheet with Silica gel 60 F₂₅₄ (6 \times 20 cm; Merck, Germany). The marker (0.1 mg in 1 μ l) was sepiapterin (Schircks, Switzerland). The paper chromatography (with paper MN214, Macherey-Düren, Germany) was performed under the same conditions, except that a double amount of samples was applied.

RESULTS

Both in males and females the yellow parts of the wings absorbed the light at 425 nm wavelength less intensively than black parts at 650 nm (Tab. 1), but absorption of the yellow parts in females was significantly higher than in males, $F_{1,18} = 4.43$ at $p = 0.049$. In absorption of the black parts of the wings, there was no difference between males and females, $F_{1,18} = 0.13$ at $p = 0.726$. The ratio "absorption of the yellow part of the wing at 425 nm / absorption of the black part of the wing at 650 nm" varied individually (data not shown). No correlation between these two parameters was obtained in males ($r = 0.29$, $p = 0.412$) nor in females ($r = 0.27$, $p = 0.449$).

In the wings' black parts, the presence of the black pigment melanin was confirmed. After reaction of the dark parts of the cut wings with methylene blue at pH 1, a dark green coloured margin appeared.

From the yellow as well as black parts of the wings, yellow extracts with the absorption maximum at 420–425 nm (characteristic for pteridines) were obtained. Absorptions of light at 425 nm wavelength of the first extracts were 0.557 and 0.221, and 0.428 and 0.172, of

the second extracts. According to the calculations from this data, the concentration of the yellow pigments is 8.3 times higher in black spots than in the yellow parts of the wings.

Tab. 1: Intensity of pigmentation of the wings of *P. libelluloides*, expressed as absorption of the light. Yellow parts were measured at 425 nm and black parts at 650 nm. Values are means \pm standard deviation, $N = 10$, * = $p < 0.05$.

Tab. 1: Intenziteta obarvanosti kril pri volkcu vrste *P. libelluloides*, izražena z absorpcijo svetlobe. Njegovi rumeni deli so bili merjeni pri valovni dolžini 425 nm, črni pa pri 650 nm. Vrednosti so podane kot srednje vrednosti \pm standardni odklon, $N = 10$, * = $p < 0.05$.

Gender	Absorption of the yellow parts of wings at 425 nm	Absorption of the black parts of wings at 650 nm
Males	0.537 \pm 0.143	0.818 \pm 0.119
Females	0.681 \pm 0.136*	0.834 \pm 0.115

Identification of the pigments with thin-layer chromatography revealed the presence of two yellow spots with Rf 0.35 and 0.56 in both extracts (Fig. 3). The first spot was not identified, while the second corresponded to the marker sepiapterin. In both extracts, two yellow pigments were also identified after paper chromatography (Fig. 4). The first pigment with Rf 0.27 was after comparing the Rf values (Hama *et al.*, 1965) identified as xanthopterin, while the second with Rf 0.44 corresponded to the marker sepiapterin.

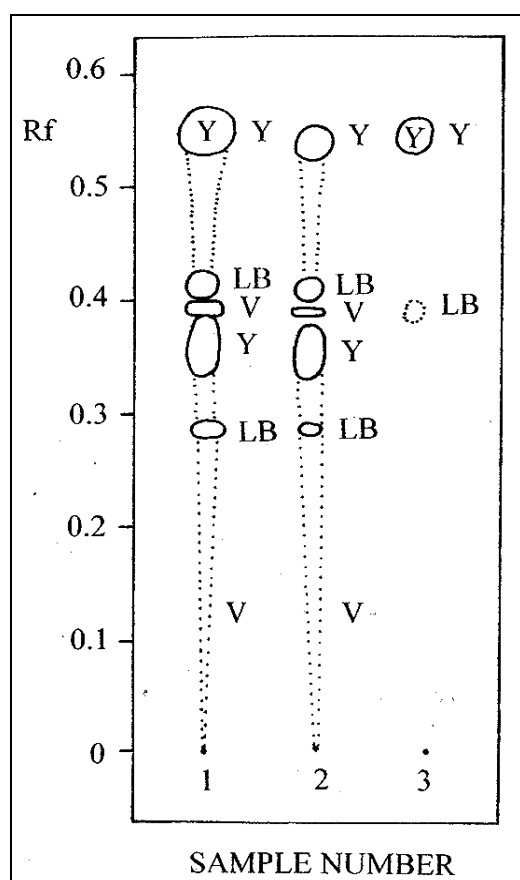


Fig. 3: Thin-layer chromatography of the extracts from the wings of ant-lion *P. libelluloides*. Samples: 1 – from yellow parts, 2 – from black parts, 3 – pure sepiapterin. Yellow spots visible at daily light are indicated with Y in the centre of the spot. On the right side, the colour of fluorescence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

Sl. 3: Tankoplastna kromatografija ekstraktov kril volkca vrste *P. libelluloides*. Vzorci: 1 – od rumenih delov, 2 – od črnih lis, 3 – čisti sepiapterin. Rumene lise, ki so vidne pri dnevni svetlobi, so označene z Y v sredini lise. Na desni strani so označene barve fluorescence pri 365 nm: LB – svetlo modra, V – vijolična, Y – rumena.

DISCUSSION

The pale yellow wings of *Palpares libelluloides* have relatively weak absorption of the light at wavelength specific for yellow pigments. More pronounced dark parts have higher absorption as well. In comparison with the related species *Libelluloides macaronius* with intensive yellow and black coloured wings (Senčič, 2006), the absorptions were at *P. libelluloides* lower by about 5 and 2 times, respectively. The higher pigmentation of the wings of females, which was determined pho-

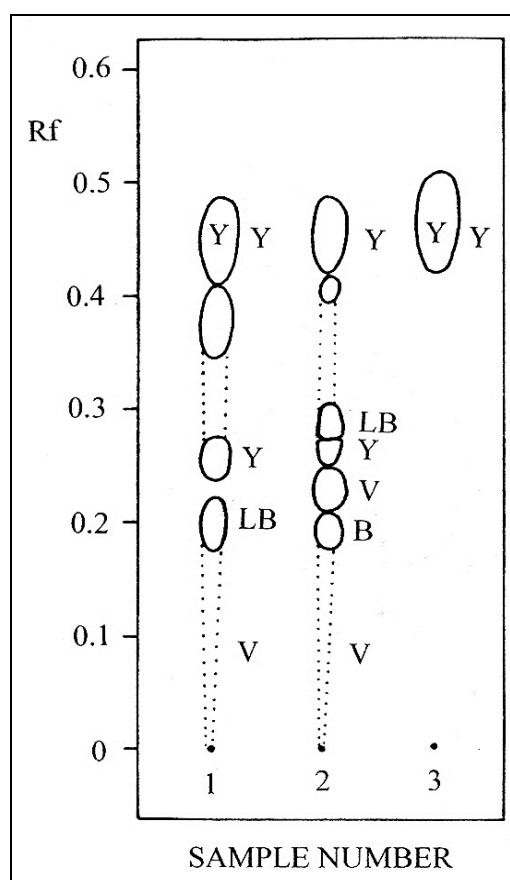


Fig. 4: Paper chromatography of the extracts from wings of the ant-lion *P. libelluloides*. Samples: 1 – from yellow parts, 2 – from black parts, 3 – pure sepiapterin. Yellow spots visible at daily light are indicated with Y in the centre of the spot. On the right side, the colour of fluorescence at 365 nm is indicated: B – blue, LB – light blue, V – violet, Y – yellow.

Sl. 4: Papirna kromatografija ekstraktov kril volkca vrste *P. libelluloides*. Vzorci: 1 – od rumenih delov, 2 – od črnih lis, 3 – čisti sepiapterin. Rumene lise, ki so vidne pri dnevni svetlobi, so označene z Y v sredini lise. Na desni strani so označene barve fluorescence pri 365 nm: B – modra, LB – svetlo modra, V – vijolična, Y – rumena.

tometrically, could not be detected with the naked eye. Extraction of yellow pigments revealed 8.3 times higher concentration of them in black spots than in yellow parts of the wings. Owing to the colour stability during the adult stage (D. Devetak, pers. comm.) this phenomenon is not a protection effect of black pigments against photodegradation of yellow pigments. The pigments (black melanin, yellow sepiapterin and xanthopterin) were the same as in the wings of *L. macaronius* (Senčič, 2006), which is in accordance with their taxonomic relationship. Although the yellow spots on chromatograms were

in the same positions, the differences were obtained in number and pattern of the spots, which have fluorescence in other colours.

The problem of instability of the pigments in solutions remained unsolved. In dried state (freeze-dried extracts and also in wings), they were stable on air and daily light for more months, but in solution the colour visibly disappeared in just a few minutes. For this reason, spontaneous oxidation of some pigments may occur during the two-dimensional thin-layer chromatography, as reported for 7,8-dihydrobiopterine (Tomic-Carruthers *et al.*, 1996). For a more detailed qualitative and quan-

titative analysis, the high pressure liquid chromatography (HPLC) must be used (Tomic-Carruthers *et al.*, 2002), where the sample is not exposed to free oxygen and the time of procedure is short.

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DOLOČANJE INTENZITETE OBARVANOSTI KRIL IN IDENTIFIKACIJA PIGMENTOV V KRILIH VOLKCA VRSTE *PALPARES LIBELLULOIDES* (LINNAEUS, 1764) (NEUROPTERA: MYRMELEONTIDAE)

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POVZETEK

Pri volkcju vrste *Palpares libelluloides* (Linnaeus, 1764), ki ima blede rumena krila s črnimi lisami, je avtor s pomočjo absorpcije svetlobe ugotovil intenziteto obarvanosti kril, s kemijsko reakcijo, papirno in tankoplastno kromatografijo pa identificiral pigmente. Intenziteto obarvanosti rumenih delov kril je meril z absorpcijo svetlobe pri valovni dolžini 425 nm, črnih lis pa pri 650 nm. Absorpcije rumenih delov kril so bile pri samicah višje kot pri samcih. V črnih lisah je dokazal obstoj črnega pigmenta melanina. Iz rumenih delov kot tudi iz črnih lis je ekstrahiriral rumene pigmente. S papirno in tankoplastno kromatografijo na Silikagelu 60 F₂₅₄ je v obeh vzorcih identificiral sepiapterin in ksantopterin.

Ključne besede: Neuroptera, Myrmeleontidae, *Palpares libelluloides*, krila, obarvanost, pigmenti

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