

Not all apparently gynandromorphic butterflies are gynandrous: The case of *Polyommatus icarus* and its relatives (Lepidoptera: Lycaenidae)

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ABSTRACT

Beside the more than two thousand normal specimens of *Polyommatus icarus* (Rottemburg, 1775) yielded by rearing experiments, there was one perfectly bilateral dichromatic individual first considered to be gynandrous. On the basis of analysing genitalia traits, wing surface covering scale micromorphology, and the spectral characteristics of the blue colour generated by the cover scales, the gender of the specimen has been identified as female. This exemplar was investigated in comparison with gynandrous specimens from the collections of the Hungarian Natural History Museum exhibiting various degrees of intermixing of blue and brown coloration. Focus stacking microscopy for detailed scale morphology and UV–visible reflectance spectroscopy was used for the characterization of the optical properties. Inspecting literature references and the Lycaenidae collection of the museum, further examples have been found for female bilateral dichromatism in the closely related polyommatine lycaenid species *Lysandra bellargus* (Rottemburg, 1775) and *Lysandra coridon* (Poda, 1761) what suggests that polyommatine female dichromaticity may be displayed by the manner of bilaterality and mosaicism, phenomena hitherto solely connected to gynandromorphy.

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1. Introduction

Lepidoptera is one of the most heterogeneous insect order displaying an astonishing phenotypic diversity. Behind this diversification, there is also an unusual high variance of chromosome numbers (Grimaldi and Engel, 2005; Wright et al., 2024). During the full metamorphosis of the individual insect, sex chromosomes influence molecular cell development (Ma et al., 2014). As a consequence, males and females often have grossly different phenotypes (Traut et al., 2008). However, according to multiple observations, there are individuals with mixed female heterogametic (ZW) and male unigametic (ZZ) chromosomes which reveal this phenomenon externally, as well as internally causing various sexual anomalies (Narita et al., 2010). The occurrence of individuals consisting of phenotypically male and female parts has been

reported repeatedly in arthropods. They have been found in both natural and laboratory (Campos et al., 2022) populations of almost all orders of insects as well as non-insects (Schärer, 2016).

In a recent review, the gynandromorphs and the intersexes were discussed with emphasis on arthropods (Fusco and Minelli, 2023). The diversity of the processes potentially responsible for the production of the morphologically anomalous sexual phenotypes and the complexity of mechanisms involved both in the primary establishment of sex and in the translation of the latter into sexual differentiation of the individual; accordingly, its different body parts make it difficult to precisely identify the causes of the sex anomalies. Beyond phenotypes with well-organized symmetric character displacement, there are morphs with chaotic display of male and female traits. In these cases, the different sexual characters are intermixed resulting in a non-clear-cut appearance, posing difficulty for a positive identification of the real causing effects. When during individual development at the stage of the chromosomes ZW and ZZ segregation and the formation of pools of cells with a different set of sex chromosomes a problem or a chain of

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problems appear that results in an intersexual or gynandrous individual. Hitherto, it is generally considered that the bilateral and mosaic morphs of adult Lepidoptera are gynandrous. Gynandromorphy in butterflies is relatively well documented; the phenomenon already attracted the interest of early (Ochsenheimer, 1816; Rudolphi, 1828; Wiskott, 1895, 1897; Oberthür, 1910), as well as recent workers (Schurian, 1989; Scriber et al., 2009).

Sexually dimorphic Lepidoptera species, like the representatives of the family Lycaenidae, are particularly well suited for the identification of sexual anomalies as the flat wings with sex-dependent scale morphology and colour facilitate the identification and clear localization of the anomalies. Hence the well-developed methodologies for the examination of the genitalia can complement the observed unusual phenotypic characters (Sakamoto et al., 2012). Sexual anomalies are especially conspicuous in Lycaenidae, as strong sexual dimorphism is a typical trait of the family to such an extent, that different sexes of the same biological species were sometimes described as different taxa (Schiffermüller, 1775; Robbins, 1986; Bálint and Wojtusiak, 2003; Bálint and Salazar-Escobar, 2003). Wing pattern and genitalia morphology of bilateral and mosaic lycaenid gynandromorphs or intersexuals were extensively analysed. It was remarked that whilst the dorsally present bilateral gynandromorphic character was not displayed by the ventral wing surface, which did not deviate from the standard, on the other hand, the genitalia displayed sexual anomalies as the structures examined were always aberrant (Cockayne, 1922; Jahner et al., 2015).

Polyommatus icarus is one of the most often recorded Lycaenidae species in Europe, hence it has the English vernacular name Common Blue (Thomas and Lewington, 2010). In the Carpathian Basin, *P. icarus* occurs practically everywhere in mesophilous open habitats producing one to four annual generations, depending on the microclimate of the site. Although it has never been the favourite species of collectors, there are more than 6000 specimens of *P. icarus* in the Lepidoptera Collection of the Hungarian Natural History Museum, collected between 1800 and 2020 in localities from the Pacific coast via the entire Eurasian landmass to the British Isles. In this voluminous material, there are only three gynandromorphic specimens revealing their extreme rarity (Fig. 1).

P. icarus turned to be a good model species for various experimental works in biophotonics we carried out in the last decade (Piszter et al., 2011, 2016, 2022; Bálint et al., 2012). We demonstrated how to breed it in laboratory conditions (Piszter et al., 2022, 2023), and during our experimental works, we recorded the hatching of more than 2000 individuals in three years, none of

them were gynandromorphic. However, one of our breeding experiments in 2019 yielded a perfect bilateral specimen, but a female: one side with typical brown, and at the other side with typical blue wing surface (Fig. 1D). According to our best knowledge, hitherto such peculiar, bilateral dimorphic female phenotype for *P. icarus* was never recognized or documented by the scientific literature.

In this paper, we pose the following questions for clarifying the physiology of this bilateral female *P. icarus* specimen: (1) what kind of genitalia morphology characterizes the *P. icarus* gynandromorphs, the blue and brown female phenotypes, and the bilateral dimorphic female specimen, (2) what kind of scale micromorphology characterizes the *P. icarus* blue colour generating scales in the mosaic and bilateral gynandromorphs, the blue female phenotype, and the bilateral dimorphic female specimen, and (3) what are the optical characteristics of the blue colour generated by the *P. icarus* gynandromorphs, the blue female, and the bilateral female phenotype? Our results are briefly discussed in the light of genitalia morphology, scale micromorphology, optical characteristics, and historical records with the intent for a better understanding of the appearance of the blue scales on the brown wing surfaces of the females.

2. Materials and methods

2.1. Abbreviations

HNHM = Hungarian Natural History Museum; HUN–REN: Hungarian Research Network, Centre for Energy Research, Institute of Technical Physics and Materials Science.

2.2. Species

The lycaenid butterfly *P. icarus* (Rottemburg, 1775) is a widely distributed species, originally exclusively with a Palearctic range, but most recently introduced to the Nearctic region, too (Rivest and Kharouba, 2021). The habitats are all kinds of mesophilous meadows, larval host plants are various Fabaceae, especially adventive species as *Trifolium*, *Medicago* and *Lotus*.

Classification – Order: Lepidoptera. Superfamily: Papilionoidea. Family: Lycaenidae. Subfamily: Polyommatinae. Tribe: Polyommagini. Subtribe Polyommatina. Genus: *Polyommatus* Latreille, 1804. Type species: *Papilio icarus* Rottemburg, 1775; original designation by Latreille (1804).

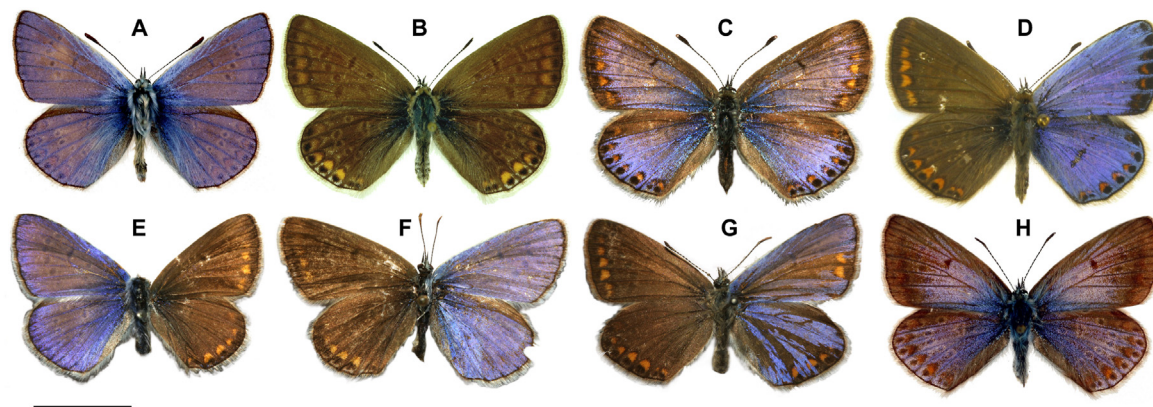


Fig. 1. Various *Polyommatus icarus* specimens (before dissection) representing different phenotypes in dorsal view: male (A), brown female (B), blue female (C), brown-blue bilateral female (D), male-female bilateral gynandromorph (E), female-male gynandromorph (F), mosaic gynandromorph (G), blue female morph variation (H). Scale bar: 1 cm.

2.3. Specimens

More than 6000 specimens of *P. icarus* curated in the HNHM (National Carpathian Basin collection $n \approx 4000$; General collection $n \approx 2000$) were scanned for gynanders or dimorphic female specimens.

For genitalia and scale micromorphology investigations, seven specimens were used, for optical spectroscopy, 25 specimens were measured. All the specimens examined originate from the holdings of the HNHM Lepidoptera collection, they are pinned, set, properly labelled, and curated in the National Collection (Carpathian Basin). Label and other data of the specimens examined are given in Table A.1. Individual butterfly specimens were documented on the digital working stations of the HNHM Lepidoptera collection using an Olympus Camedia C 7070 and an Olympus SZX12 stereomicroscope equipped with an Olympus DP-70 digital camera.

2.4. Genitalia dissections

Dissections were carried out using traditional methods (Higgins, 1975; Winter, 2000). Genitalia were mounted and fixed in the same manner on microscope slides and numbered following the HNHM protocol. Microscope slides were photographed in the digital working station of the HNHM using an Olympus SZX12 stereomicroscope equipped with an Olympus DP-70 digital camera.

2.5. Optical microscopy

Optical imaging of the wing scales was carried out in the HUN–REN using a Nikon Eclipse LV150N (Shinagawa, Tokyo, Japan) microscope in reflected light. The wing scales usually stand at an angle of approximately 15° relative to the wing membrane, so for a better visibility, we used extended depth of focus (EDF) mode to compensate for the narrow depth of field of the microscope objectives.

2.6. Optical spectroscopy

For optical reflectance spectral investigation in the HUN–REN, we used a modular fibre optic spectrophotometer (Avantes AvaSpec-HSC1024x58TEC-EVO, Apeldoorn, Netherlands) and a deuterium–halogen light source (Avantes AvaLight-DH-S-BAL). All spectra were recorded against a diffuse white standard reference (Avantes WS-2). For the measurement of the reflected specular component, we used a normal incidence probe, which combined the incoming illuminating fibres and an outgoing detecting fibre. These measurements were carried out in non-destructive way, using a spectroboard (Bálint et al., 2010). One selected sample per morph was investigated using an integrating sphere (Avantes AvaSphere-30-REFL) setup (Kertész et al., 2021).

3. Results

3.1. Genitalia morphology

Normal polyommata male and female genitalia are symmetric organs as also display expressed by *P. icarus* genitalia (Fig. 2A–B). The blue female phenotype does not show any genitalia peculiarities, nor the bilateral female specimen: both of them have the normal female structures of a pair of papillae anales with long apophysis, an eversible tubular henia with sclerotized posterior capsule (Fig. 2B–D), one of the apomorphies of the tribe (Bálint, 2022).

The bilateral gynandromorph specimens display similar characteristics in genitalia morphology as observed on the wings of the

specimens. These bilateralities are homologous with the wings. Accordingly, where the left side is a male, the genitalia organ corresponds with showing a complete male genitalia structure on the left side, but the right side is a female in its apical part as there is a complete structure of papillae anales with posterior apophysis and a sclerotized formation what seems to be the spermathecal (Fig. 2E). Where the left side is a female, one of the papillae anales with the posterior apophysis appears on the left side with a sclerotized henia (Fig. 2F). The genitalia of the mosaic gynandromorphs display mixed sexual traits and a deformation of great extent, where the male sclerotized parts (valva, tegumen, labis and falx) show more obvious deformations, while the tubular female structures are less recognizable (Fig. 2G).

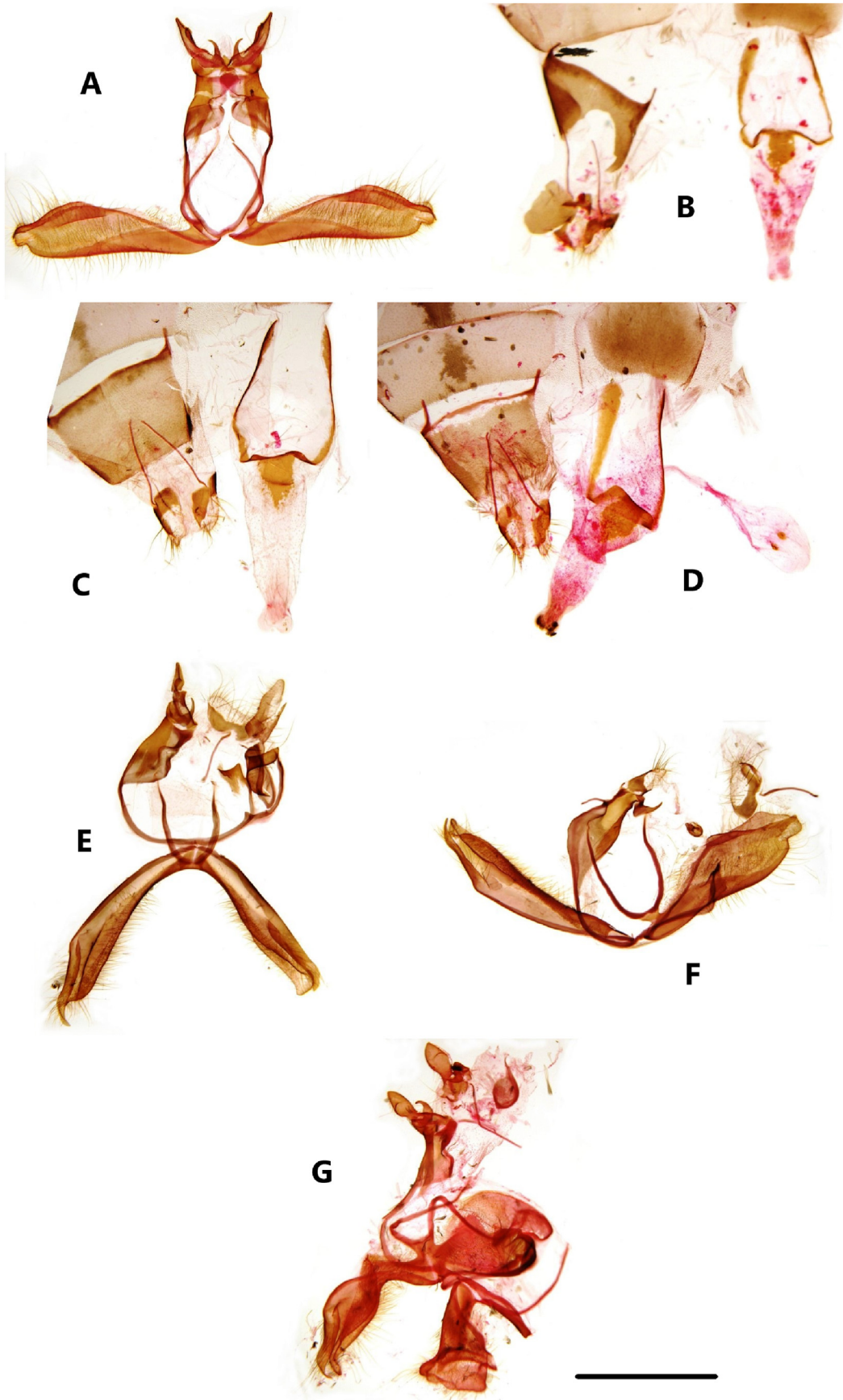
3.2. Scale micromorphology

We documented previously that the sexual dimorphism in the cover scale micromorphology of polyommata lycaenids is distinctive. The male blue cover scales have typically rounded apices and contain colour generating nanoarchitectures (Fig. 3A), whilst female scale apices are dentate and are saturated with brown melanin pigments (Fig. 3B) (Vértesy et al., 2004, 2006). The blue female morphs that have been produced in the laboratory by the prolonged cooling of the freshly formed pupae have cover scales containing photonic nanoarchitectures (Piszter et al., 2019). We confirm here these findings, as in all the specimens examined from this respect, we detected these characteristics: blue coloured cover scales (Fig. 3C–E, G, I). However, in male individuals the colour generating cover scales are always associated with androconia (Fig. 3A), which are placed between the scale rows. This characteristic remains in mosaic and bilateral gynandromorph specimens where the male (blue) parts also possess androconia (Fig. 3D, E, G). Moreover, this trait is kept even between those scale rows which are composed of micromorphologically male and female scales as the androconia are also present there (Fig. 3D). Female wing surfaces are without androconia and covered by scales with dentate apices (Fig. 3B, F, H, J). The dentated end of the scales is retained to a lesser extent even in cases when these scales generate structural colour (Fig. 3C and I). In these cases, the scale ending is a transition between the dentated ending of the female scales (Fig. 3B, F, H, J) and the rounded ending of the male scales (Fig. 3A): the scale ending is undulated (Fig. 3C and I). The blue and brown wing surfaces of the bilateral female specimen are female in all respect: the shape of the cover scale apex is slightly dentated (undulated), and there are no androconia between the rows (Fig. 3I and J).

3.3. Spectral characteristics

The reflectance of the wings was measured using integrating sphere light collection on one specimen per morph (Fig. 4A), while all blue specimens were investigated by non-destructive normal-incidence spectroscopy (Bálint et al., 2010) to prepare the peak wavelength statistics (Fig. 4B).

The male reflectance spectrum shows all the characteristics we know from our previous results regarding spectral characteristics of *P. icarus* (Piszter et al., 2011, 2016, 2023; Bálint et al., 2012): the main reflectance peak is around 390 nm, and there is a small peak at 255 nm (Fig. 4A). The reflectance of the blue female morph from the HNHM collection shares the characteristics of the male spectrum, only the peak wavelength is slightly redshifted to 400 nm, similarly to what we have experienced before (Piszter et al., 2019; Kertész et al., 2017). This also can be seen in Fig. 4B, where the statistics of the peak wavelengths measured in normal-incidence were analysed in form of box plots. The structural colour of the



gynandromorphs does not differ in any essential character from the male and the blue female spectra. The bilateral female specimen fits also into this pattern, with the notable difference that the wings were more absorbing in the 600–800 nm wavelength range (Fig. 4A), suggesting higher melanin content of the ground scales. This may be a direct consequence of the fact that the bilateral female was the most recent specimen and was also bred in captivity, therefore it was preserved in perfect condition compared to the older, wild-caught museum specimens (Kertész et al., 2019).

4. Discussion

4.1. Genitalia morphology and wing coloration

Our findings correspond with some previous results: male and female genitalia morphologic characteristics reflect the main sexual traits of the specimen's wing (Kuznetsov, 1926; Jahner et al., 2015). Therefore, specimens showing bilateral appearance on the basis of wing surface colouration, also show this peculiarity in their genitalia. The examined *P. icarus* bilateral gynandromorphic specimens display the same characteristics exhibited by the wings in their genitalia morphology, too. The genitalia bilaterality is homologous with the wings, accordingly when the right side is a perfect male this is mirrored also by the genitalia organ, but the left side is a composition of both sexes, and vice versa. However, it has been recorded in Lycaenidae that superficially bilateral gynandromorph specimens may also have compact male genitalia (Shuey and Peacock, 1985). Our bilateral female *P. icarus* specimen has a normal female genitalia organ what confirms that the specimen is indeed a female, and not gynandromorph. This agrees with the findings of Cockayne (1922), who stated that all “intersexual” *Plebejus argus* (Linnaeus, 1761) specimens with blue scalings without androconia have female genitalia, but in spite of this, he considered them as “intersexual” specimens.

4.2. Spectral characteristics

We demonstrated earlier using a large sample that the male dorsal wing surface colour of *P. icarus* is species-specific and it shows very little variation in a wide geographic and temporal range (Kertész et al., 2019). The spectra of the blue *P. icarus* females resulted from the cooling experiment (Piszter et al., 2019; Kertész et al., 2017) showed a similar blue colour as that of the males resulted from the same experiment, but the variability of the females was larger than the males'. The spectral characteristics of the gynandromorphic specimens in Fig. 4 are similar in all details in the spectral range of the structural coloration (approximately 300–550 nm) with the typical spectra of blue *P. icarus*. The bilateral female fits also in this picture, as the displayed blue colour does not have any characteristic difference from conspecifics, only the higher amplitude of the blue peak is present.

4.3. Lycaenid female bilaterality and mosaic dichromatism

We are of the opinion that our peculiar bilateral *P. icarus* specimen is not gynandromorph, but a rare female aberrant individual. Although Cockayne (1922) tackled the phenomenon, his summary was not conclusive, as he considered all his mosaic or bilateral specimens with blue colour but without androconia being “intersexual”. However, we suspect that amongst specimens considered

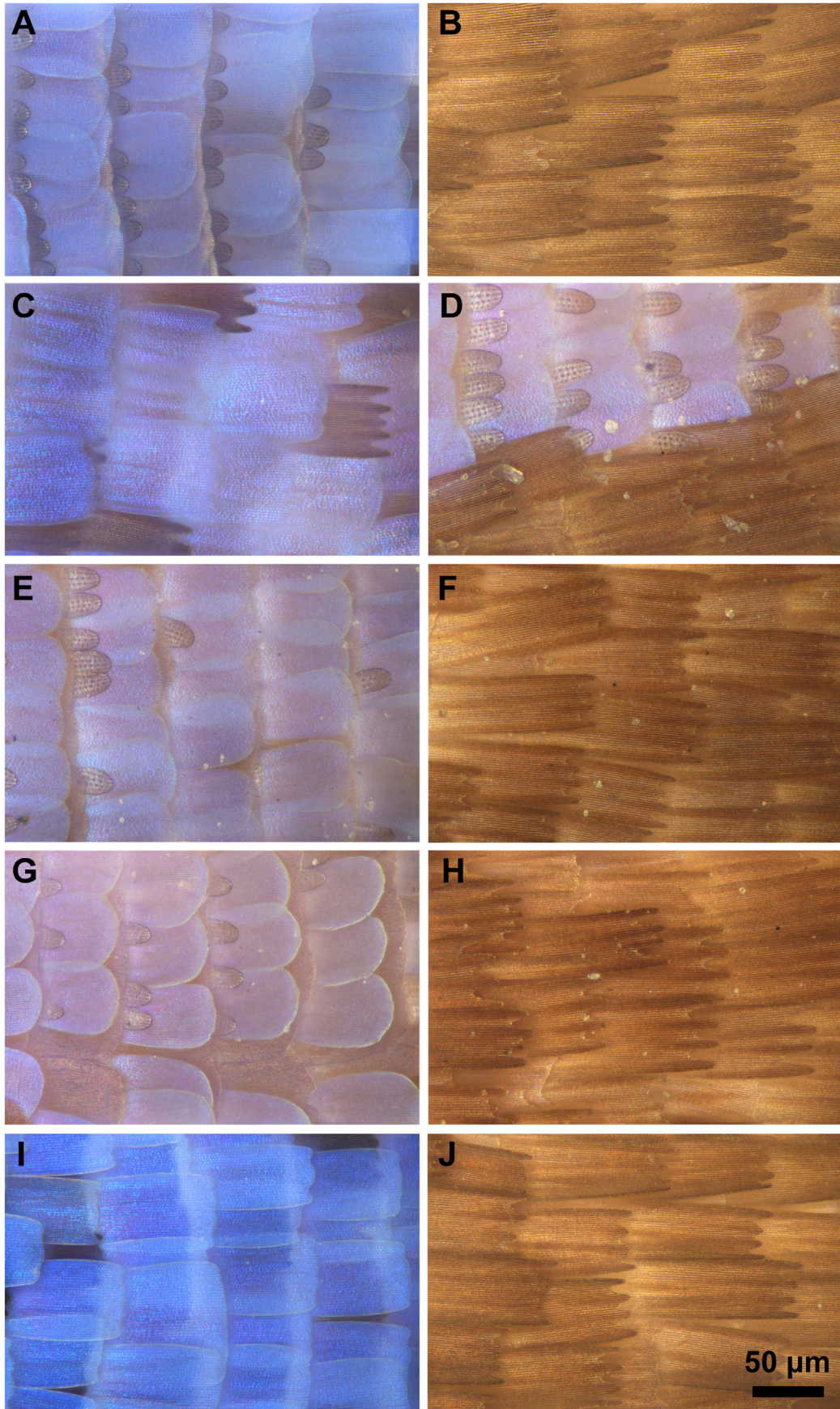
hitherto bilateral or mosaic gynandromorph, there are female specimens. This unclarified issue also turned up when we inspected the literature. The following three references appeared as supporting evidence for this scenario.

- (1) In a monograph of *Lysandra coridon*, several specimens have been documented as “ordinary gynandromorph variant” and various infrasubspecific names were given to them (Bright and Leeds, 1938). Some of these specimens are certainly mosaic females, as even for the naked eye, the deeper greenish blue colour of the female morph f. *syngrapha* is different than the normal male silvery green colour, probably due to the lack of the “fur” covering the androconia, what strongly influences the reflectivity of the male wing surface generating the “silvery” tone (Fig. 5). In the HNHM, there is a gynandrous specimen with similar characteristics with the sample figured under the number 22 in the original plate of the monograph (see Fig. 5, lowest specimen of the left column, and Fig. 6A–B): the left side in both specimens is female, but forewing in the right side shows structural blue colour, whilst the hindwing of the same side displays mosaic characteristic.
- (2) In a review paper, a bilateral female of *Lysandra bellargus* was referred as, ... mosaic gynandromorph in which male (blue) and female (brown) features are mixed” (Negri and Pellicchia, 2012). However, this specimen is a mosaic dichromatic female, as the blue colour of the *L. bellargus* male is different and males rarely have discal line in the dorsal forewing surface. We could find similar female specimens of *L. bellargus* in the HNHM collection, where certain areas of dorsal wing surfaces show mosaicism (Fig. 6C–D).
- (3) In a paper dealing with gynandromorphism in *Lycaeides*, one of the specimens considered to be a mosaic gynandromorph turned to be having normal female genitalia, therefore the specimen of *Lycaeides melissa* (Edwards, 1873) was not taken into account (Jahner et al., 2015). As it has been reported that specimen has intact female genitalia, we are of the opinion that this is another example of a mosaic dichromatic female specimen.

4.4. Female dichromatism in *Polyommata*

It is well-documented that polyommata lycaenid females beside the pigmented “normal” dark morph have many individual variations which have dorsal wing surfaces with suffusion of blue colour generating scales (Bálint et al., 2023; Tshikolovets, 2011). This resulted almost uncountable names proposed for these variations (Courvoisier, 1914–1921; Bridges, 1994), but there was little experimental effort to unravel the developmental forces behind the female dichromatic morphs. We note that it is a general phenomenon of the subtribe that in populations at the edge of the species' range the “abnormal” blue female phenotype became the dominant one (Benyamini, 2023; Tennent, 1996), and this was correlated also with environmental changes. It was pointed out in our experimental work run on *P. icarus* that the genetic information needed from the production of the photonic nanoarchitectures responsible for the blue coloration of the *P. icarus* males can be activated in female individuals using prolonged cold stress (Piszter et al., 2019; Kertész et al., 2017).

Fig. 2. *Polyommatus icarus* genitalia organs: normal male clasper and valve (aedeagus removed) (A); normal female duct and henia plus papillae anales with posterior apophysis (B); blue female duct and henia plus papillae anales with posterior apophysis (C); bilateral female duct and henia plus papillae anales with posterior apophysis (D); bilateral (left female, right male) gynandromorph (E); bilateral (left female, right male) gynandromorph (F); mosaic gynandromorph (G). Scale bar: 2 mm.



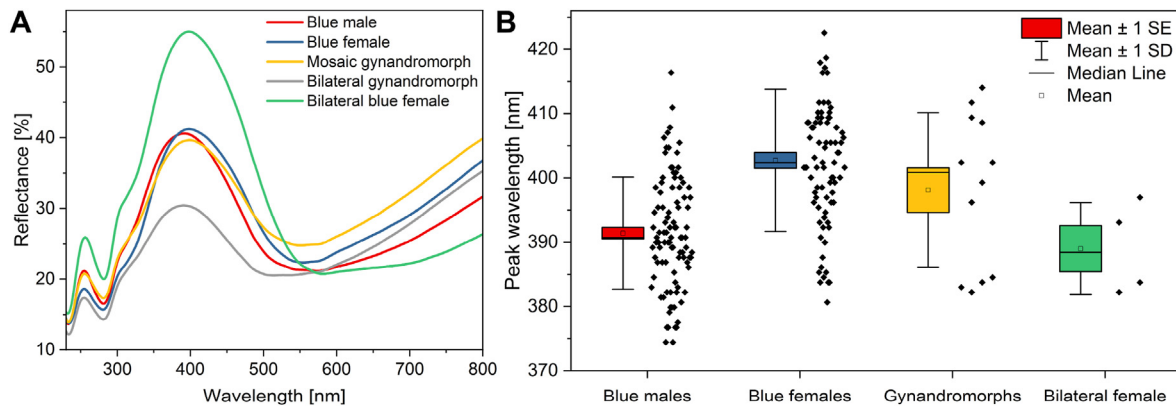


Fig. 4. *Polyommatus icarus* reflectance data of the dorsal blue coloration measured on various specimens: integrating sphere reflectance spectra on different blue morphs (A); box plot statistics of the peak wavelengths measured with a normal-incidence probe on all investigated specimens (B).

4.5. *In vivo*

The dynamic signal of the structural colour in flight (Bálint et al., 2009) is composed by the whole wing surface, therefore in the case of bilateral female the blue is 50 % mixed with the pigmental brown colour. Theoretically *in vivo* it may emit similar signal as a female *P. icarus* with 50 % coverage of blue colour (see Fig. 1H), but there is no experimental evidence how this may work. For the case of the closely related *Meleageria daphnis* (Schiffmüller, 1775), it was recorded that mosaic gynandromorphic specimens were found in copula with males, but displayed grossly different behaviour than normal females (Dantchenko et al., 1995). We are of the opinion that these gynandromorphic specimens were not “intersexual”, but they were mosaic females. This is supported by the fact that gynandromorphs of *P. icarus* have genitalia showing mostly male characters, and these cannot fit with ordinary male clasping organs. Although some of them were most probably incapable for oviposition as had been recorded for the bilateral female of *L. melissa* (Jahner et al., 2015) but because the population survives for several decades, some females must be fertile. Indeed, Cockayne (1922) reported “intersexual” *P. argus* females with mosaic gynandromorph appearance were fertilized, they could oviposit, and in one recorded case, caterpillar hatched from an egg. This may occur also for *M. daphnis*. Fertilized dimorphic females may explain the long-term existence of these remarkable populations. For the case of *P. icarus*, we are not aware of any such phenomenon.

4.6. Genetic background

The experimental observation (Piszter et al., 2019; Kertész et al., 2017) that the female descendants of typical brown females can exhibit blue scales after the prolonged cooling of the freshly formed pupae indicates that the genetic information needed for the production of the photonic nanoarchitectures characteristic for the males is present in each scale generating cell of both the males and females. Our observation (Piszter et al., 2019) that the increase of the cooling time on one hand causes the increase of the number of blue scales, on the other hand, the modification of the micron-scale morphology of the blue scales (Fig. 7) indicates that turning from brown to blue of a certain scale is a complex process, which is controlled individually in each scale. At short cooling times (4 weeks, Fig. 7A), the dentated shape of the female scales is

preserved, but the colour generating nanoarchitecture is already present. At longer cooling times (8 weeks, Fig. 7C), even the external morphology of the scales is altered from the dentated shape, they switch to a rounded characteristic, similar to the blue scales of the males (Fig. 3A). The mosaic gynandromorph (Fig. 3D) clearly has blue scales with this typical male morphology and exhibits androconia in the blue region. On the other hand, the scales of the blue female (Fig. 3C) show some traces of the dentated apices, and no androconia are visible. In the case of bilateral gynandromorph (Fig. 3G), again, the blue scales have the typical external morphology of the male scales and androconia are present. In the case of the bilateral female (Fig. 3I), the blue scales show traces of the dentated ending and no androconia are present. Therefore, one may conclude that there are two different ways of turning the normally brown scales of females into blue scales: i) in true gynandromorphs, where male type blue scales are observed and androconia are present; and ii) in females with blue scaling, where the traces of the dentated scale ending can be observed and no androconia are present.

4.7. Outlook

It seems justified to formulate the hypothesis that the two routes of appearance of the blue scales on the wings of the females have different mechanism and different genetic background. In the case of the true gynandromorphs – mosaic, or bilateral – at a certain developmental stage a group of cells continues the division as male cells and all later cells, which are produced after several steps of division will exhibit male character. The most convenient indicator for this route is the presence of the androconia. In the case of the females exhibiting blue scales – locally, or over the whole wing, like in the case of the bilateral female – due to some genetic factor, the inhibition of the formation of blue scales is switched off and some scales or all scales will develop the blue colour-generating nanoarchitecture. The dependence on the duration of the cooling time observed in the case of the freshly formed pupae subjected to prolonged cooling indicates that the factor switching off the formation of the photonic nanoarchitecture is present immediately after pupation and may decay during the prolonged cooling (Piszter et al., 2019; Kertész et al., 2017). In those scales, in which the concentration of the substance silencing the expression of the genes that govern the formation of the photonic

Fig. 3. *Polyommatus icarus* dorsal wing surface optical images: male (A); brown female (B); blue female (C); mosaic gynandromorph blue-brown mixed region (D); mosaic gynandromorph blue (male) region (E); mosaic gynandromorph brown (female) area (F); bilateral gynandromorph blue (male) side (G); bilateral gynandromorph brown (female) side (H); bilateral female blue side (I); bilateral female brown side (J).

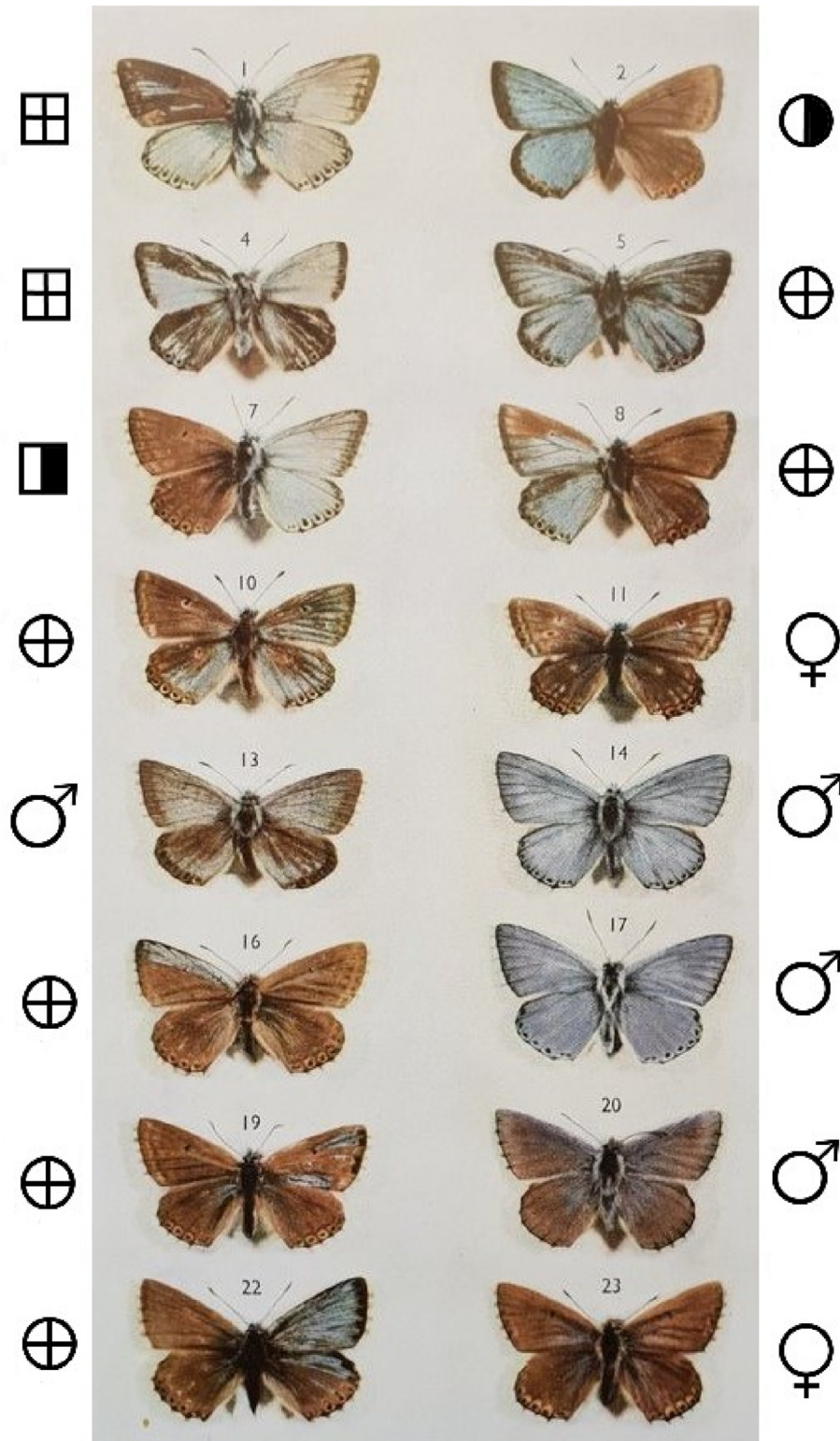


Fig. 5. Documentations of specimens in the monograph of *Lysandra coridon* (Bright and Leeds, 1938) showing various degrees of mosaicism plus full bilaterality, and individual variations. Square with plus sign: mosaic gynanders; square half filled: bilateral gynander; circle with plus sign: female mosaic dichromatic specimen; circle half filled: bilateral dichromatic female specimen, male (♂) and female (♀) signs: symmetric individual variations. Specimens in same magnification, forewing costa length of specimen marked with "1" = 17 mm.



Fig. 6. Aberrant polyommata specimens showing “gynandrous” characteristics (both in HNHM). Mosaic gynandromorph *Lysandra coridon* with left female side, male right forewing and mosaic gynandrous right hindwing (A). These characteristics are mirrored by the ventral wing surfaces (B). Mosaic dichromatic female specimen of *Lysandra bellargus* showing structural colour generating scales in the dorsal wing surface of the left forewing costa (C). As dichromatism is displayed only by dorsal wing surfaces, consequently ventral wing surfaces show normal female characteristics (D). Scale bar: 1 cm.

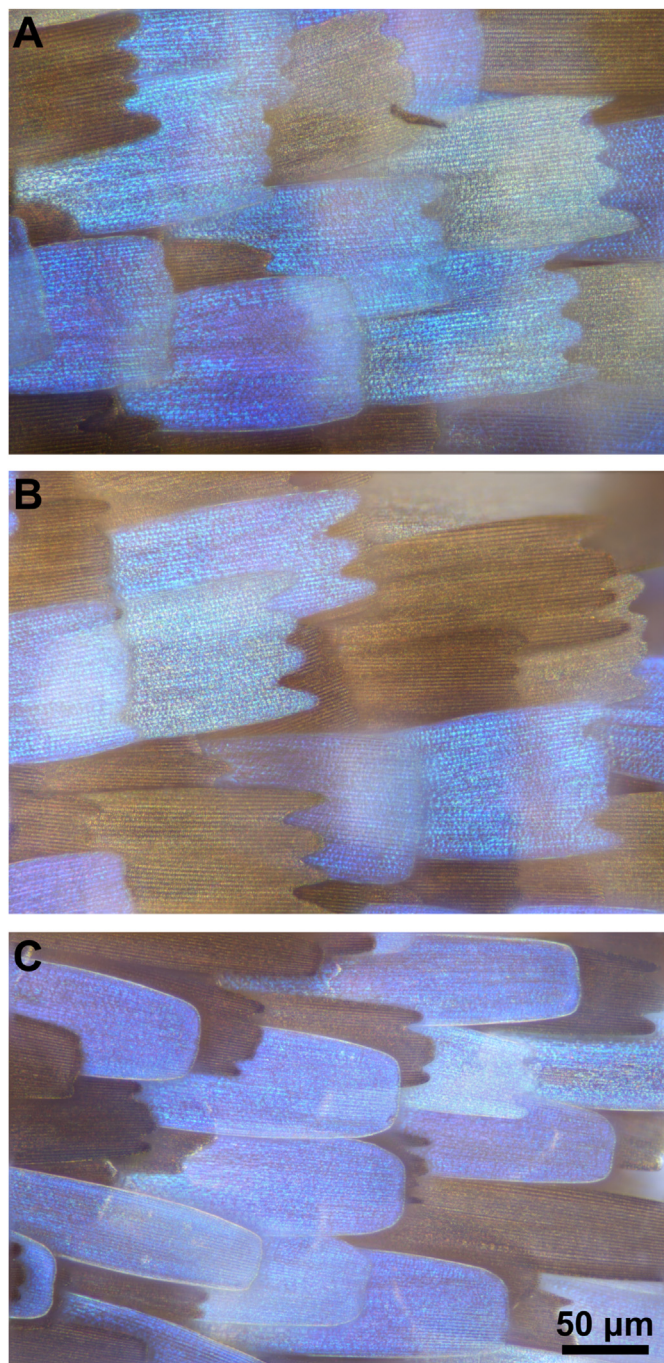


Fig. 7. Optical microscope images of female *Polyommatus icarus* specimens from the cooling experiment in 2019 (Piszter et al., 2019). The pupae were cooled for 4 weeks (A), 6 weeks (B), and 8 weeks (C) prior eclosion which resulted in differently shaped blue scales.

nanoarchitecture, falls below a certain limit, the nanoarchitecture will develop and turn some scales into blue scales. If the concentration falls to even lower values, after the eight weeks cooling, even the external morphology of the scales may be altered.

The scenario outlined above suggests that originally both sexes possessed the structural coloration and the loss of this in the case of the females is an adaptation. The main advantages of such a loss are: *i*) the less conspicuous coloration reduces the predation risk; the brown females are much more difficult to observe against the green background of the vegetation than the blue males; *ii*) the more efficient use of the solar energy (Biró et al., 2003). The silencing of the genes responsible for the formation of the blue colour-generating photonic nanoarchitecture may be achieved by an epigenetic route (Burggren, 2017).

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CRediT authorship contribution statement

Zsolt Bálint: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gergely Katona:** Writing – review & editing, Visualization, Data curation. **Krisztián Kertész:** Writing – review & editing, Visualization, Methodology, Investigation, Data curation. **Gábor Piszter:** Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Balázs Tóth:** Writing – review & editing, Resources, Methodology, Investigation. **László Péter Biró:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

None.

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Appendix

Table A.1

Polyommatus icarus specimens used for the investigations.

Serial number	Phenotype	Locality	Date	Collector	Investigations	Database number
1	normal male	Nógrád vármegye: Nógrádszakál, Rárós	1959.X.15.	Lipthay	spectroscopy	none
2	normal male	Pest vármegye: Tahi	1971.VIII.10.	Szőcs	spectroscopy	none
3	normal male	Pest vármegye: Szentendre	1951.VII.22.	Reskovits	spectroscopy	none
4	normal male	Heves vármegye: Bükk-hegység, Hutarét	1951.VII.1.	Issekutz	spectroscopy	none
5	normal male	Heves vármegye: Bükk-hegység, Telekessy-menedékház	1943.VIII.24.	Reskovits	spectroscopy	none
6	normal male	Heves vármegye: Bükk-hegység, Lök-völgy	1951.IX.7.	Reskovits	spectroscopy	none
7	normal male	Fejér vármegye: Bakonykúti	1996.VII.22.	Rác	spectroscopy	none
8	normal male	Fejér vármegye: Bakonykúti	1996.VII.22.	Rác	spectroscopy	none
9	normal male	Fejér vármegye: Bakonykúti	1996.VII.9.	Rác	spectroscopy	none
10	normal male	Pest vármegye: Nagykovácsi, Nagyszénás	2010.VIII.13.	Bálint	spectroscopy	none
11	normal male	Pest vármegye: Nagykovácsi, Nagyszénás	2010.VIII.13.	Bálint	spectroscopy	none
12	normal male	Pest vármegye: Nagykovácsi, Nagyszénás	2006.VI.17.	Bálint	spectroscopy	none
13	blue female	Budapest: XXII. Kerület, Tétényi-plató	1971.IX.10.	Rác	spectroscopy	33677 HNHM
14	blue female	Budapest: XII. Csillebérc	1948.IX.8.	Neugebauer	spectroscopy	33715 HNHM
15	blue female	Pest vármegye: Pilisszentkereszt, Hosszú-hegy	1973.VIII.26.	Szemerédi	spectroscopy	33738 HNHM
16	blue female	Vas vármegye: Velem	1978.VI.21.	Nyíró	spectroscopy	33674 HNHM
17	blue female	Veszprém vármegye: Balatonfüred, Koloska-völgy	1981.V.31.	Podlussány	spectroscopy	33692 HNHM
18	blue female	Heves vármegye: Eger, Ostorosi erdő	1927.VII.30.	Reskovits	spectroscopy	33759 HNHM
19	blue female	Borsod-Abaúj-Zemplén vármegye: Telkibánya	1971.VI.25.	Rác	spectroscopy	33670 HNHM
20	blue female	Pest vármegye: Nagykőrös	1960.VIII.22.	Balogh	spectroscopy	33685 HNHM
21	blue female	Szlovákia: Rozsnyó	1943.VII.30.	Fabricius	spectroscopy	33741 HNHM
22	blue female	Erdély: Székelykeresztúr	1943.VI.15.	Móczár	spectroscopy	33730 HNHM
23	mosaic gynandromorph	Románia: Bánát, Perjámos M. pr	1936.VIII.22.	Lipthay	spectroscopy/ genitalia	genitalia TB 2337
24	bilateral gynandromorph	Heves vármegye: Bükk-hegység, Hársas tető	1950.V.20.	Reskovits	spectroscopy/ genitalia	genitalia TB 2327
25	bilateral gynandromorph	Borsod-Abaúj-Zemplén vármegye: Jósfa	1976-VII.29.	Szemerédi	spectroscopy/ genitalia	genitalia TB 2329
26	bilateral female	Pest vármegye: Érd	2019. XI.12.	KFKI tenyészet	spectroscopy/ genitalia	genitalia TB 2328
27	blue female	Hajdú-Bihar vármegye: Debrecen-Belváros	1981.VIII.22.	Makranczy	genitalia	genitalia TB 2335
28	normal male	Budapest: Püskösdfürdő	1966.VII.10.	Wettstein	genitalia	genitalia TB 2333
29	normal female	Fejér vármegye: Várpalota-Inota	1968.VI.1.	Nyíró	genitalia	genitalia TB 2334

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