A rare natural melanistic aberration of *Melitaea trivia* (Lepidoptera: Nymphalidae) found during a spring field study in Dobrogea, Romania

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Abstract. During a field study in spring 2022 the goal was to monitor pollinators in underexplored habitats in Eastern Romania, Dobrogea province. Here, also different populations of spring butterflies were observed, including those of the Lesser Spotted Fritillary *Melitaea trivia* (Denis & Schiffermüller, 1975) (Lepidoptera: Nymphalidae). In the Tulceae area, near Babadag, in broadleaved, deciduous woodland steppe with understorey vegetation and clearings, a rare natural melanistic aberration of a *M. trivia* male was found. Pictures of the aberration are shown and compared with upper- and undersides of typical wing patterns, and with similar artificial aberrations produced with injections of sodium tungstate in controlled lab conditions. Such aberrant morph types are discussed in relation to cold shock during the pupation stage and compared to melanistic specimens generated under artificially controlled conditions.

Samenvatting. Tijdens een veldexcursie in het voorjaar van 2022 was het doel om pover onderzochte gebieden gebieden in Oost-Roemenië, provincie Dobrogea, te exploreren. Er werden ook verschillende populaties voorjaarsvlinders waargenomen, waaronder die van de Toortsparelmoervlinder *Melitaea trivia* (Denis & Schiffermüler, 1775) (Lepidoptera: Nymphalidae). In het Tulcea district rond Babadag werd, in een met open loofbos begroeid stuk steppe met onderbegroeiing, een natuurlijke melanistische afwijking van *M. trivia* waargenomen. Foto's van de afwijking worden weergegeven en vergeleken met de typische vormen van de soort, alsook met gelijkaardige aberraties die werden verkregen onder gecontroleerde labo omstandigheden met injecties van natrium tungstaat. Deze afwijkende morphotypes worden besproken in relatie tot een koudeshock proces tijdens het verpoppingstadium en in vergelijking met kunstmatige omstandigheden die dezelfde aberraties teweegbrengen.

Résumé. Lors d'une étude de terrain au printemps 2022, l'objectif était de suivre les pollinisateurs dans des habitats peu explorés dans l'est de la Roumanie, dans la province de Dobrogea. Ici, différentes populations de papillons printaniers ont été observées, y compris celles de *Melitaea trivia* (Denis & Schiffermüller, 1775) (Lepidoptera: Nymphalidae). Dans la région de Tulceae, près de Babadag, dans une steppe boisée de feuillus avec une végétation de sous-bois et des clairières, une rare aberration mélanique naturelle d'un mâle de *M. trivia* a été trouvée. Des photos de l'aberration sont montrées et comparées aux faces supérieures et inférieures de motifs alaires typiques, ainsi qu'à des aberrations artificielles similaires produites par des injections de tungstate de sodium dans des conditions de laboratoire contrôlées. Ces types de morphes aberrants sont discutés en relation avec le processus de choc lié au froid au cours de la nymphose et comparés à des spécimens mélaniques générés dans des conditions artificiellement contrôlées.

Key words: *Melitaea trivia* — Natural aberration — Cold shock — Sodium tungstate — Melanisation process.

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Introduction

During spring 2022, a field study was organized by the Flemish Entomological society workgroup Rhopalocera (VVE WG Dagvlinders), with the goal of monitoring in underexplored areas in Romania, Dobrogea. The focus was on the observation of spring butterflies or spring broods, including those of *Apatura metis* Freyer, 1829, *Euphydryas maturna* (Linnaeus, 1758), *Heteropterus morpheus* (Pallas, 1771), *Parnassius mnemosyne* (Linnaeus, 1758), the rare endemic *Tomares nogelii dobrogensis* (Caradja, 1895), and *Polyommatus* and *Melitaea* species, amongst others. An in depth overview of all observations in the explored habitats including biogeographical distributions will be published in a separate paper (Couckuyt *et al*. 2024, in press). In this paper, we focus on the remarkable field observations made by the two authors on the populations of *Melitaea trivia* (Denis & Schiffermüller, 1775).

The Lesser Spotted Fritillary *M. trivia* is found in southern Europe, from Spain, to places in Italy, becoming more widespread and common in south-east Europe from Slovenia, Romania to Greece. The species is double-brooded with flying peaks in spring (April/May) and again in June to August. A similar-looking species is the Spotted Fritillary *Melitaea didyma* (Esper, 1778), with which it flies together in south-eastern countries such as Romania; however the latter is less common compared to *M. trivia*. Generally, *M. trivia* is more heavily marked than *M. didyma*. The typical habitats are hot, dry, grassy and flowery places amongst light woodland and scrub up to the tree line (Tolman & Lewington 1997; Tshikolovets 2011). During the field study such habitats were visited and we report here on the observation of an extreme aberration found in a spring population of *M. trivia*.

Results

On 31.v.2022, the two authors were exploring together an old pontico-mediterranean steppe overgrown with small trees situated near Babadag, in the Tulcea district. Passing through this biotope with understorey vegetation of grasses and perennials such as *Knautia macedonica* Griseb., *Verbascum* Schrad and *Astragalus ponticus* Pall. (Fig. 1a), a population of *M. trivia* was found.

Fig. 1. Study site explored near Babadag (Romania, Dobrogea, Tulcea district); a, habitat of *Melitaea trivia*, with feeding and nectaring plants; b, copula of *M. trivia* in its biotope on 31.v.2022; c, several *M. trivia* nectaring on *Knautia macedonica.* © L. Parmentier.

Fresh females and males were observed, some still in copula (Fig. 1b), and especially nectaring on *Knautia macedonica* Griseb. (Caprifoliaceae) (Fig. 1c). Shortly after the second author noticed a very dark specimen, the first author recognized it as an extreme natural aberration of *M. trivia*, remembering other melanistic aberrations of *Melitaea* species seen in collections. The aberration, together with some typical forms were taken for further investigation. The male was set and depicted with upper- and underside in Fig. 2a, b. The upper sides of both forewing and hindwing show a drastic reduction in the orange coloration and typical dotted wing patterns. Instead, for the upperside, a continuous black streak to the submedian wing zone is seen; the remaining two apical spots are fully black. The underside of the wings also show a very melanistic pattern, especially with the subapical dots on the forewing smeared as black lines from the marginal to the median zones; and hind wings with big marginal black dots touching the submarginal zone which is transformed into orange dots surrounded with black. In addition, on the underside of the hindwings, the creamy yellow ground tone of the basal to median zone is transformed into a fully orange-black pattern. For reference, a typical male and female from the same population is shown in Fig. 3a, d with upper- and undersides of both sexes. Here it is seen that the upper side black border is small, solid and uniform, usually touching the postdiscal arches. The underside hindwing marginal spots are approximately triangular rather than rounded. The definitive feature for *M. trivia* is the presence of a small vein that closes the cell in the hindwing.

Fig. 2. Extreme dark melanistic aberration of *M. trivia* (male) collected at a site near Babadag (Romania, Dobrogea, Tulcea district) on 31.v.2022; **a,** upper side; **b,** underside, leg. Philippe Van de Velde, coll. Laurian Parmentier. © L. Parmentier.

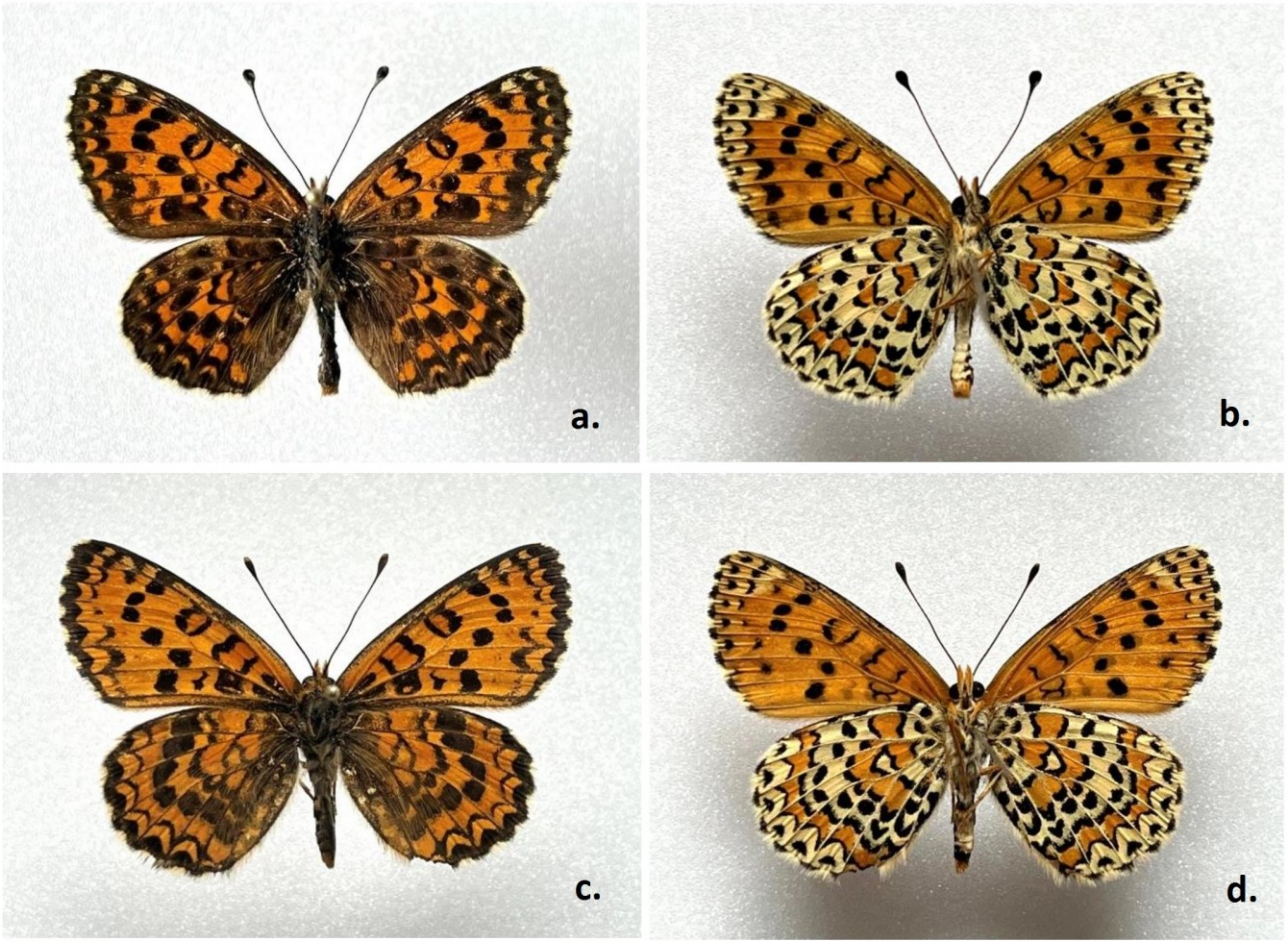

Fig. 3. Normal form of *M. trivia*. male and female collected at the same site near Babadag (Romania, Dobrogea, Tulcea district) on 31.v.2022; a, b, male upper side and underside; c, d, female upper side and underside, leg. & coll. Laurian Parmentier. © L. Parmentier.

Additionally, the first author had contact with colleagues who informed him of such dark aberrations being reproduced under controlled lab circumstances, with injections of sodium tungstate at the beginning of the pupation stage. Via T. Maertens, such artificially made aberrations were obtained and depicted in Fig. 4 for comparison. An artificially produced *M. trivia* female aberration (Fig. 4a, b) is resembling well the aberrant wild male. Other and less pronounced aberrations of a male and female *M. trivia* are also shown (Fig. 4c-f). This artificially produced female was obtained under controlled conditions by microinjections of 1,0 M sodium tungstate in freshly formed pupae (3–5h after pupation) by the Slovakian entomologist Ladislav Misko who is skilled in this technique (pers. comm. T. Maertens).

Discussion

In this small study we report on an extreme wild melanistic aberration of *M. trivia* in comparison with those produced artificially under controlled circumstances. In butterflies, phenotypic colour pattern modifications can have two causes: modifications induced by temperature shock and those induced via the general stress response (Shimajiri & Otaki 2022). Studies of colour pattern modifications in butterflies induced by temperature shock from evolutionary and developmental viewpoints were pioneered by Shapiro (Shapiro 1981) and Nijhout (Nijhout 1984, 1991). Generally, under colder circumstances a more darkened, melanistic habitus is generated. Such temperature-shock-type modifications appear to be induced via a systemic factor (i.e., a cold shock hormone) (Otaki 1998; Mahdi *et al*. 2010) as well as a local factor (i.e., an extracellular matrix) (Otaki 1998, Dhungel & Otaki 2009). Thus, in the case of our wild aberrant male, the melanistic wing form may be produced during a cold shock for several days just after the formation of the pupa. As the pupation stage takes approximately two to three weeks and there was a cold weather period in the Tulcea area around half of April 2022 (Weatherspark.com), this is a plausible explanation.

In comparison, and besides the *Melitaea* specimens shown in this paper, melanistic aberrations by cold shock have also been reported in other butterfly genera, including *Junonia* (Mahdi *et al*. 2010), *Lycaena* and *Maculinea* (Otaki & Yamamoto 2003), *Pecris* (Nijhout 1984), *Polyommatus* (Kertész *et al.* 2017; Piszter *et al.* 2019), *Nymphalis* (Shapiro 1981), *Papilio* (Umebachi & Osanai 2003; Shimajiri & Otaki 2022), *Vanessa* (Nijhout 1984) and others. While the response to cold shock is obviously quite variable from individual to individual, it should be noted that in such specimens the colour pattern on the homologous wing surfaces of any individual (left and right ventral hindwing, for instance) always showed identical pattern aberrations (Shimajiri & Otaki 2022).

Fig. 4. Extreme melanistic aberrations of *M. trivia* produced via sodium tungstate (1,0 M) injections 3–5h. post pupation; a, b, female upper side and underside, with an extreme dark aberration; c, d, aberrant female upper side and underside; e, f, aberrant male upper side and underside, both produced with a lower amount of sodium tungstate (1,0 M), ex. ovo 05.2013, Slovakia, leg. L. Miško. © L. Parmentier.

About 25 years ago, Otaki (1998) discovered that similar, if not identical, colour pattern modifications can be induced by injections of oxyanions, such as sodium tungstate, into pupae. Indeed, as shown here, it is remarkable that the wild aberrant male under natural conditions is almost identical in aberrant wing pattern to the artificially produced female (Fig. 4a, b) obtained under controlled conditions by microinjections of sodium tungstate. Other modification inducers of wing patterns were also discovered later on, such as acid carboxypeptidase, heparin and other sulphated polysaccharides (Serfas & Carroll 2005; Sourakov 2017). As colour patterns of cold shocked or modification-induced specimens of a given species are often similar to natural colour patterns of wild captured specimen, these treatments have been used for many lepidopteran species to understand developmental physiological aspects of colour pattern formation (Otaki 2008; Kertész *et al.* 2017; Sourakov 2017; Piszter *et al.* 2019). Indeed, wing colour of Lepidoptera is an important trait not only for mating choice, but also for mimicry (escape from predators) (Shimajiri & Otaki 2022) and can be linked with speciation. In conclusion, aberrant specimen as described in this article are interesting to untangle the relationship between wing colour pattern formation, genetics, and environmental circumstances (also stressors). With modern genomic techniques and the upcoming availability of whole genomes of species they can be a tool to better understand the genes involved in these complex processes.

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