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Veronica longifolia L. as an important initial larval food plant of Scarce Fritillary Euphydryas maturna (LINNAEUS, 1758) (Lepidoptera, Nymphalidae): the ecological uniqueness of populations from the Natura 2000 area "Dolina Biebrzy" (Biebrza Valley) in NE Poland

MARCIN SIELEZNIEW $^{1*}$ , IZABELA DZIEKAŃSKA $^{2}$ 

<sup>1</sup>Department of Invertebrate Zoology, Institute of Biology, University of Białystok, Ciołkowskiego 1J, PL-15-245 Białystok, Poland, e-mail: marcins@uwb.edu.pl <sup>2</sup>Association for Butterfly Conservation (TOM), Kartaginy 1/98, PL-02-762 Warszawa, Poland, e-mail: idziekańska@gmail.com

ABSTRACT. The Euro-Siberian Scarce Fritillary *Euphydryas maturna* is considered a vulnerable species in the European Union and is listed in Annexes II and IV of the Habitats Directive. The butterfly shows a complex pattern of larval plant use throughout its range. In central Europe females oviposit on some trees and bushes, especially *Fraxinus* spp., on which hatched larvae spend their pre-diapause phase of development feeding gregariously in conspicuous webs. However, some herbal plants are also reported in the north and east. During a study performed in the Natura 2000 area "Dolina Biebrzy" (Biebrza Valley) in NE Poland we recorded populations showing a unique mixture of ecological characteristics. Both *Fraxinus excelsior* and *Veronica longifolia* were used as larval food plants before hibernation, and some local populations seemed to be completely dependent on the latter plant. Moreover, in the spring, at one site, we observed larvae feeding on *Salix rosmarinifolia* – the first host record for this plant species. The importance of our findings for conservation, as well as for the monitoring of the butterfly, is discussed.

**KEY WORDS**: Biebrza, *Euphydryas maturna*, Habitats Directive, host plant, oviposition, Poland, *Salix rosmarinifolia*, *Veronica longifolia*.

<sup>\*</sup> Corresponding author: marcins@uwb.edu.pl

#### INTRODUCTION

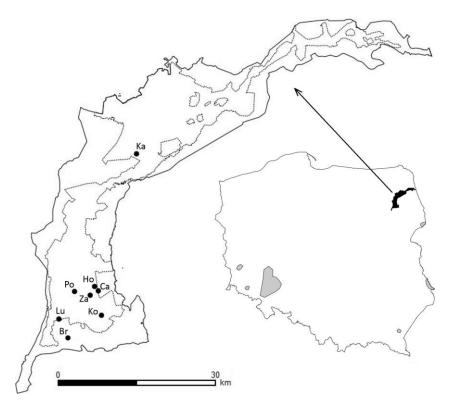
Detailed knowledge of host plant relationships is vital for the effective conservation of endangered phytophagous insects, including many butterflies. Some of them show complex patterns of larval food use throughout their ranges. A good example of such a species is the Scarce Fritillary *Euphydryas* (*Hypodryas*) maturna (LINNAEUS, 1758), which is recorded at scattered sites in the Western Palaearctic from the Alps and northern Balkans to southern Fennoscandia, and from France across central and eastern Europe, southern Siberia to the Altai Mts. and Yakutia (TUZOV et al. 2000, GORBUNOV & KOSTERIN 2007, RÁKOSY et al. 2012, NIEMINEN 2015). The butterfly is univoltine and imagoes are on the wing between May and July depending on latitude, altitude and biotope. Females lay their eggs in batches on the underside of the leaves of host plants. After hatching, larvae feed gregariously within a communal web. In the fourth instar they leave the plants to overwinter close to the ground. In the spring the caterpillars disperse, by their sixth (final) instar they live solitarily, and finally they pupate low in the vegetation, usually on plant stems.

Generally *E. maturna* can be considered a polyphagous species, eating plants belonging to different orders and families, e.g. Oleaceae, Orobanchaceae, Plantaginaceae, Salicaceae, Adoxaceae and Caprifoliaceae. Almost all of them possess secondary compound seco-iridoids and/or iridoid-glycosides, which are sequestered by caterpillars for their own defence, and are also used later for the protection of pupae and imagoes (WAHLBERG et al. 2001, KUUSSAARI et al. 2004, DOLEK et al. 2013). Although the range of host plants used in pre- and post-diapause phases of development is quite wide, in most of Europe females oviposit predominantly on ashes (*Fraxinus* L.) but very occasionally on shrubs, e.g. *Viburnum opulus* L. In the spring caterpillars feed on seedlings or young shoots of the initial food plants, and also on some herbs (CIZEK & KONVICKA 2005, FREESE et al. 2006, RÁKOSY et al. 2012, DOLEK et al., 2013). An important exception is Finland, where eggs are laid mainly on *Melampyrum pratense* L. and *M. sylvaticum* L., which are fed on after hibernation (WAHLBERG 1998, NIEMINEN 2015). Numerous plant species are reported from Russia (from both its European and Asian areas), but without reference to detailed studies (TUZOV et al. 2000, GORBUNOV & KOSTERIN 2007).

In central Europe, *E. maturna* almost exclusively inhabits woodland areas with ashes present in the stand. The butterfly requires mosaics of forests, clearings and low intensity managed flower-rich meadows. Its habitats are negatively affected by, among other factors, abandonment and changes in woodland management leading to canopy closure and the disappearance of food plants (FREESE et al. 2006). As a consequence, *E. maturna* is considered a vulnerable species in Europe: it is included in Annexes II and IV of the Habitats Directive as well as in Appendix II of the Bern Convention. In some countries it

has become extinct or is critically endangered (VAN SWAAY & WARREN 1999, ELIASSON & SHAW 2003, KONVIČKA et al. 2005, VAN SWAAY et al. 2010b).

Poland is still an important regional stronghold for *E. maturna*, which has recently been recorded in nearly fifty 10 km grid squares in that country (MALKIEWICZ 2012). However, its distribution range is highly disjunctive, encompassing the SW part of the country (mainly Lower Silesia) and some areas in the east (the Biebrza Valley, the Białowieża Forest, the Bug Valley in the Lublin Upland and one isolated site in the Podkarpacie region) (Fig. 1). In Lower Silesia the butterfly inhabits mainly riparian mixed forests of *Quercus robur* L., *Ulmus* L. and *Fraxinus excelsior* L., as well as oak-hornbeam forests. Females oviposit there on *F. excelsior* and only *V. opulus* is sometimes used as an alternative (MALKIEWICZ 2012).



**Fig. 1.** Location of study sites of *Euphydryas maturna* at the Natura 2000 site "Dolina Biebrzy" (Biebrza Valley) in NE Poland and other distribution sites of this butterfly in Poland (marked in grey; modified from MALKIEWCZ 2012): Br – Brzeziny A-D, Ca – the Tsar's Road (Carska Droga), Ho – Honczarowska Causeway (Grobla Honczarowska), Lu – Łuczane, Ko – Kopcie, Ka – Kapice, Po – Pogorzały, Za – Zabłudnik. The dotted line shows the boundary of the Biebrza National Park.

However, little is known about the host-plant relationships of the populations of *E. maturna* inhabiting eastern Poland. In this contribution we describe observations of initial larval food plants carried out in the Natura 2000 area "Dolina Biebrzy" (Biebrza Valley), which has proved to be unusual for Europe and therefore important also from the conservational point of view.

#### MATERIAL AND METHODS

The Habitats Directive Area "Dolina Biebrzy" (Biebrza Valley) is situated in NE Poland and covers an area of 1 210.03 km<sup>2</sup>. It includes the entire area of the Biebrza National Park (BNP), the largest national park in Poland (592.23 km<sup>2</sup>), which was founded in 1993 primarily to protect one of the most valuable wetland complexes in central Europe. Its variety of biotopes (including mineral islands surrounded by fens) generates a high richness of butterflies. More than 100 species have been recorded there, i.e. two-thirds of the species known from Poland, including four listed in Annex II of the Habitats Directive (FRACKIEL 1999, FRACKIEL & SIELEZNIEW 2009 and unpublished). These species, i.e. Lycaena helle (DEN. et SCHIFF.), L. dispar (HAW.), Euphydryas maturna and Coenonympha oedippus (FABR.), were the objects of an inventory performed in 2012 and 2013 in order to prepare a conservation plan for a Natura 2000 site. Among other things, some local populations of E. maturna were found in the lower and middle basins of the Biebrza Valley, as well as in the N2000 area situated to the south of the BNP in the Narew Valley (Fig. 1). In late July/August 2012 and 2013 we visited the sites to look for larval webs. Additionally, we had come across batches of eggs on larval food plants and ovipositing females earlier in the season. At one site we also observed caterpillars feeding in the spring after diapause.

## **RESULTS**

A total of 99 larval webs of *Euphydryas maturna* (38 and 61 on *Veronica longifolia* and *Fraxinus excelsior* respectively) were observed at eight sites in the study area. In one locality only *V. longifolia* was used as the initial food plant, while at four others both *V. longifolia* and *F. excelsior* supported local populations. Finally, in three localities *F. excelsior* was the exclusive host plant (Table 1).

**Table 2.** Study sites of *Euphydryas maturna* in the "Dolina Biebrzy" (Biebrza Valley) Natura 2000 site with information on the plant species used by pre-diapause larvae.

Site name, its location and coordinates	Biotope description	Estimated area	Observation dates	Food plants present (number of larval
				webs found)
Brzeziny (Narew Valley south of the Biebrza NP); N53°14', E22°31'	Complex of five habitat patches (overgrowing litter meadows, thickets, edges of small deciduous forests, woodlots) separated mainly by hay meadows and spaced 0.3-1.9 km apart.	2.0 ha	21 July 2012 21 August 2012 20 July 2013	Veronica longifolia (4) Fraxinus excelsior (2)
<b>Luczane</b> (the lower Biebrza basin); N53°16°, E22°29°	Two mineral islands (separated by a distance of 100 m) surrounded by fens and covered by well-lit deciduous forest, thickets and grasslands	8.0 ha	22 July 2013	Veronica longifolia (20)
Pogorzały (the lower Biebrza basin); N53°18', E22°32'	A mineral island covered by deciduous forest, thickets and grasslands. <i>E. maturna</i> was mostly observed close to dirt roads, along a distance of nearly 3 km.	5.0 ha	21 August 2012	Veronica longifolia (5) Fraxinus excelsior (9)
Zabludnik (the lower Biebrza basin); N53°18', E22°35'	A mineral island covered by well-lit deciduous forest and thickets	4.5 ha	19 August 2012	Veronica longifolia (3) Fraxinus excelsior (25)
Kopciewo (the lower Biebrza basin); N53°16', E22°37'	Small, mostly overgrown clearings in a deciduous forest.	0.4 ha	21 August 2012	Fraxinus excelsior (1)

Table 2. Continued.

Site name, its location and coordinates	Biotope description	Estimated area	Observation dates	Food plants present (number of larval webs found)
Honczarowska Causeway (Grobla Honczarowska) (the lower Biebrza basin); N53°19°, E22°35°	By the side of a dirt road (causeway) with some ash trees running from east to west.	1.0 ha	21 August 2012	Fraxinus excelsior (6)
The Tsar's Road (Carska Droga) (the lower Biebrza basin); N53°18°, E22°36°	By the side of a tarred road with a few ash trees.	0.1 ha	19 August 2012	Fraxinus excelsior (4)
Kapice (the central Biebrza basin); N53°32', E22°44'	Clearings and roadside verges on mineral islands (Kozikowo and Chmielnikowo) covered by deciduous forest.	4.0 ha	23 August 2012	Veronica longifolia (6) Fraxinus excelsior (14)

A female ovipositing on V. longifolia (Fig. 2a) was first observed on 12 June 2012 on the verge of a dirt road near the village of Brzeziny in the Narew Valley, south of the BNP. This record drew our attention to the potential use of this plant there and elsewhere on the N2000 site. During subsequent visits to the site we monitored larval development (Fig. 2bf), and six further larval webs were found on the southern edge of a deciduous forest located about 100 m away on a slight elevation surrounded by hay meadows. Interestingly, we never discovered batches of eggs or larval webs on a large ash growing just 150 m away. In the same complex of hay meadows, small forests, woodlots and thickets we also found four other local populations of E. maturna. The most important patch included overgrowing litter meadows and the edge of a young pine forest with an admixture of some young ashes with a total area of 0.8 ha. Almost all the larval webs were recorded on V. longifolia; only one observation related to a small, 2 m high F. excelsior tree. We noticed that adjacent meadows were mown during the flight period of the butterfly, so some larval webs may have been destroyed. At this site in early May 2014 we also observed, postdiapause, dozens of final instar larvae feeding mostly on leaves of Salix rosmarinifolia L. (accompanied by numerous larvae of Lymantria dispar L. Single caterpillars were also recorded on V. longifolia (Fig. 2g), Populus tremula L. and V. opulus.

Two other local populations near Brzeziny were supported exclusively by ashes, on which batches of eggs or larval webs were found on trees growing at the edge of a small forest and in woodlots. In the fifth habitat patch, however, only a single larval web was recorded on *V. longifolia*.

In the lower Biebrza basin the study involved six sites. Three of these were mineral islands surrounded by fens. At the Łuczane site there were no ashes and larval webs were found exclusively on *V. longifolia*. The plants occupied generally grew in sheltered spots to the south and east of trees or shrubs. At the other two sites (Pogorzały and Zabłudnik) larval webs were recorded on both *V. longifolia* and *F. excelsior*. At the former we noticed that patches with numerous imagines (observed at the peak of the flight period) were not necessarily those where preimaginal instars would later be present. Moreover, ashes with egg batches/larval webs were recorded exclusively in the northern part of this mineral island, while in the southern part *V. longifolia* was used since ashes were absent altogether. *Viburnum opulus* was also present in a patch frequently visited by butterflies nectaring on *Scabiosa* L. flowers but no eggs/larval webs were found there. At both Pogorzały and Zabłudnik *V. longifolia* plants with larval webs grew in sheltered sunny spots (usually with a southerly exposure) close to trees and shrubs. Larval webs on ashes were also mostly exposed to the south and were situated 1-8 m above ground. The height of the ash trees used varied between 1 and 20 m.



**Fig. 2.** *Veronica longifolia* as a host plant of *Euphydryas maturna*: **a**) a female ovipositing on a leaf (24 June 2012); **b**) two egg batches with developing larvae inside (21 June 2013); **c**) a plant with larval webs and **d**) caterpillars in the web (21 July 2012); **e**) and **f**) larvae feeding in the prewintering phase (21 July 2012); **g**) a larva feeding in the spring on a shoot of *V. longifolia* (4 May 2014).

At the three remaining sites in the lower Biebrza basin, *F. excelsior* was only recorded as an initial larval food plant of *E. maturna* during observations performed in 2012. Along the Honczarowska Causeway (Grobla Honczarowska) larval webs were observed 2-6 m above the ground and the occupied trees, 4-10 m in height, grew exclusively to the north of the causeway. Along the Tsar's Road (Carska Droga) larval webs were situated 2-2.5 m above the ground (S and SW exposure) on two young ashes (5 and 8 m in height) growing close to the tarred road. Then at Kopciewo, one larval web was found on an ash (10 m tall) at a height of 3 m. However in the previous season, at the same site, egg batches were recorded on two ashes as well on a *V. opulus* bush. Unfortunately, it was not possible to find out whether the larvae had managed to complete their pre-diapause development, as a *V. opulus* shoot with eggs had probably been eaten by an elk. Additionally, in mid May 2012, a larva was observed feeding on *Melampyrum nemorosum* L. This plant grew abundantly in the immediate vicinity of almost all the occupied ash trees at all the study sites.

Finally, at Kapice, the only locality of *E. maturna* in the middle Biebrza basin, both *F. excelsior* and *V. longifolia* were used. It is worth noting that a female ovipositing on *V. longifolia* was observed in the neighbourhood of a large ash where batches of eggs had already been laid. Occupied *V. longifolia* plants were exposed to the south or east. Almost all possible exposures were recorded as far as larval webs found on *F. excelsior* were concerned. The height of the ash trees used varied between 1.5 and 15 m and larval webs were recorded 1-2 m above the ground. In shady spots near narrow dirt roads *E. maturna* was recorded exclusively in preimaginal stages on ashes, i.e. those spots were probably visited exclusively by ovipositing females. On the other hand no larval webs were observed on a meadow with an abundance of *V. longifolia*, and adults generally avoided this area. However, the habitat there was different, i.e. it was wetter with abundant *Polygonum bistorta* L., for example, and its specific herbivore lycaenid butterfly *Lycaena helle*. Generally, the local distribution ranges of *L. helle* and *E. maturna* did not overlap.

#### DISCUSSION

The simultaneous use of *Fraxinus excelsior* and *Veronica longifolia* observed on the Biebrza Valley (Dolina Biebrzy) site N2000 is unique. Ash is the most common food plant of pre-diapause larvae of *Euphydryas maturna* in central Europe (including SW Poland), but until now there have been no records concerning *V. longifolia* from this part of the range (MALKIEWICZ 2012, DOLEK et al. 2013). In contrast, females from the Finnish populations refuse to use *F. excelsior* and oviposit mainly on *Melampyrum* L. There are also single records of *V. longifolia* and *Viburnum opulus* from Finland (WAHLBERG 1998,

NIEMINEN 2015). In this Scandinavian context it is therefore worthy of note that ELIASSON & SHAW (2003) failed to detect oviposition on *V. longifolia* in Sweden. The only other data about the use of *V. longifolia* come from Russia, both its European (Komi Republic) and Asian parts (Krasnoyarsk area, Omsk area, Novosibirsk Province) (GORBUNOV & KOSTERIN 2007). From the Komi Republic *Plantago lanceolata L., Viola arvensis* MURR. and *V. canina* L. have also been reported (GORBUNOV & KOSTERIN 2007).

One may therefore conclude that the Biebrza Valley populations exhibit a mixture of the ecological characteristics represented by central and eastern European populations. We also hypothesise that the use of *V. longifolia* is probably more common in E Poland, especially since a single web was found at a site in the Bug Valley in the Lublin Upland, where no ashes grow (Sielezniew unpublished). Interestingly, WAHLBERG (2001) suggests that an ancestor of the *Euphydryas* group probably specialised on plants from the Plantaginaceae and Caprifoliaceae: the use of *V. longifolia*, belonging to the former family, could be an indication of a primary feature.

The *Euphydryas* group has the broadest host-plant family spectrum among the Melitaeini, and there are many examples of complex patterns of host plant use and unexpected switches among its representatives (WAHLBERG et al. 2001, KUUSSAARI et al. 2004). Actual preferences are often not easy to determine, however. For instance, in the case of *E. aurinia* (ROTT.) it was found under experimental conditions that *Succisa pratensis* MOENCH (i.e. the most commonly used larval food plant throughout Europe) is preferred even by females originating from sites where it is absent (SINGER et al. 2002). In contrast, our observations of *E. maturna* suggest that both plants are equally attractive to the Biebrza Valley populations, i.e. *V. longifolia* is used not only when there are few ash trees in the neighbourhood. However, one has to bear in mind that the pattern of host plant use is influenced, amongst other factors, by spatial variation in both host plant abundance and quality (KUUSSAARI et al. 2000).

Oviposition on *V. longifolia* has important implications for conservation, since eggs or larval webs present on herbs are more prone to destruction due to agricultural use compared to those on trees or shrubs. When meadows are mown twice a year the first mowing is usually in June. This may therefore negatively affect developing eggs/early hatched larvae, or at least prevent oviposition on cut *V. longifolia* plants and reduce access to nectar resources. Late mowing is probably less detrimental, although its real impact on larvae that have already dropped to the ground requires assessment. Moreover, it would be advisable and beneficial to mark all plants with visible eggs/larval webs and exclude these patches from management and/or to leave uncut strips of vegetation along forest edges or hedgerows. We hope that our recommendations, consistent with those specified by VAN SWAAY et al. (2010a) and included in the conservation plan for the "Dolina Biebrzy" Natura 2000 site, will be implemented.

The use of alternative plants could be beneficial in view of ash dieback – a fungal disease causing extensive damage to ash trees across Europe (PRZYBYŁ 2002, MCKINNEY et al. 2011). Symptoms indicative of this disease, i.e. crown dieback, were already observed in the BNP during this study. *Veronica longifolia* is therefore an important potential alternative initial food plant for *E. maturna*. However, it is worth mentioning that although the plant is a perennial, it is also somewhat ephemeral, and may be seriously affected by the intensive feeding of caterpillars, especially in the post-diapause phase. Therefore, resources of *V. longifolia* should be sufficiently abundant to prevent over-exploitation.

Little is known about the larval food plants of *E. maturna* in the spring. However, observations from the Brzeziny site are consistent with data from elsewhere in Europe, suggesting that the spectrum of species used is wider than in the pre-wintering phase. Interestingly, *Salix rosmarinifolia* was recorded in our studies as a host plant of *E. maturna* for the first time. Knowledge relating to local populations developing on ash trees in the pre-wintering period remains inadequate. The typical presence of *Melampyrum nemorosum* near ashes selected for oviposition and our single record from Kopciewo, as well as data from Sweden (ELIASSON & SHAW 2003), may indicate the importance of this plant. Finally, it is also very likely that *V. longifolia* is eaten in the spring by larvae initially developing on ashes.

The knowledge acquired regarding host plant use is important not only in the context of conservation but also in the monitoring of this butterfly. The relative estimate of population numbers is based, among other factors, on counts of webs in summer, and habitat quality is evaluated by the accessibility of trees (*F. excelsior*) and bushes (*V. opulus*) and their exposure to the sun (MALKIEWICZ 2012). There is no doubt that a modified methodology should be developed for populations depending largely on *V. longifolia*.

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