Sociobiology
An international journal on social insects

SHORT NOTE

First Data on the Host Ant Usage of Large Blue from the Carpathian Basin

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Article History

Edited by
Jean C. Santos, UFU, Brazil
Received 10 October 2016
Initial acceptance 07 December 2016
Final acceptance 16 January 2017
Publication date 29 May 2017

Keywords
Social parasitism, *Maculinea arion*, Phengaris, *Myrmica specioides*, *Myrmica scabrinodis*, Hungary.

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Abstract
The protected *Maculinea arion* is an obligate myrmecophilous butterfly (Lepidoptera, Lycaenidae). Fourth instar larvae and pupae develop in *Myrmica* (Hymenoptera: Formicidae) ant nests. Host ant specificity varies geographically, and knowledge of the local host ant species is important to understand the biogeography and evolution of this species, and vital for its conservation. Here we report the first data on the host ant usage of *M. arion* in the Carpathian Basin, one prepupal caterpillar from a *Myrmica specioides* and one pupa from a *M. scabrinodis* nest. *Myrmica specioides* is a new host ant species of *M. arion*. It is important to collect further data on the host ant usage of *M. arion*, despite the difficulties of data collection.

Maculinea van Eecke (1915) (Lepidoptera: Lycaenidae) species are endangered and protected in Europe (Gimenez Dixon, 1996; Munguira & Martín, 1999; Settele et al., 2005). They have an extraordinary life cycle, where larvae start their development on specific host plants. After feeding on the developing seeds of these plants, the fourth instar caterpillars then complete their development in *Myrmica* Latreille (1804) (Hymenoptera: Formicidae) ant nests (Thomas et al., 1989). Different *Maculinea* populations are adopted to different *Myrmica* species (Als et al., 2002; Tartally, 2008), which means that their protection can only be successfully managed with knowledge of the local host ant species (Munguira & Martín, 1999; Settele et al., 2005). Furthermore, such knowledge is interesting both biogeographically and evolutionarily (Nash et al., 2008).

The Large Blue butterfly, *Maculinea arion* (Linnaeus, 1758), is a well-studied *Maculinea* species, especially from its conservation aspect (Thomas, 1995). However, our knowledge about its host ant specificity is lower than is the case for the other European species (Settele et al., 2005; Hayes, 2015), because it is very difficult to find *M. arion* caterpillars in *Myrmica* nests (Sielezniew et al., 2010a). Hence, every observation of its host ant usage is important, and especially from the areas where *M. arion* exists in two phenological forms ('spring' and 'summer' types, based on their flight periods, see Bereczki et al. 2011, 2014, 2015 for more details). The Carpathian Basin is such a region, where data have not been available about the host ant specificity of *M. arion*.

To get such data, *Myrmica* nests were carefully opened and the presence of *M. arion* caterpillars was checked at 12 *M. arion* sites (Table 1 of Supplementary File) since the spring of 2000. After excavation the ground and vegetation were restored as close to the original conditions as possible. Only *Myrmica* nests found ~2 m within around the initial host plants (*Thymus* spp. and *Origanum vulgare*: Munguira

& Martín, 1999) of the butterfly were checked, as this is the approximate foraging range of *Myrmica* workers (Elmes et al., 1998). Excavations were carried out just before, or at the beginning of, the usual flying periods of the different populations. Search periods earlier in the life cycle are less suitable because ant colonies adopting young fourth-instar caterpillars may later kill them (typically around winter because of starving: Elmes et al., 2004). Five to ten workers were collected from each *Myrmica* nest and preserved in ethanol for identification in the laboratory (according to: Seifert, 1988; Radchenko & Elmes, 2010).

Altogether 289 nests of nine *Myrmica* species [*M. lobicornis* Nylander, 1846; *M. lonae* Finzi, 1926; *M. rubra* (Linnaeus, 1758); *M. ruginodis* Nylander, 1846; *M. sabuleti* Meinert, 1861; *M. scabrinodis* Nylander, 1846; *M. schencki* Viereck, 1903; *M. specioides* Bondroit, 1918; *M. vandeli* Bondroit, 1920] were opened and only two (i.e. less than 1%) were infected with *M. arion*, at two different sites. One spring arion pupa was recorded in a *M. scabrinodis* nest, and a summer arion prepupa with *Myrmica specioides* (see Table 1 and Fig 1 of Supplementary File). The identification of phenological forms was carried out based on collection date (see Table 1 of Supplementary File for details). The specific identification of these pre-adult stages was confirmed using the COI genetic barcoding gene (see Table 1 of Supplementary File for the accession numbers).

Myrmica scabrinodis has already been recorded as a host of *M. arion* from Western Europe, although just in a few cases (Thomas et al., 1989; Elmes et al., 1998). However, as far as we know, this is the first record of *M. specioides* as a host ant of *M. arion.*

The known host ant species of *M. arion* are: (I) *Myrmica sulcinodis* Nylander (1846) and *M. lonae* from Italy (Sielezniew et al., 2010b; Casacci et al., 2011); (II) *M. hellenica* Finzi (1926); *M. lobicornis*; *M. lonae*; *M. rugulosa* Nylander (1849); *M. sabuleti*; *M. scabrinodis* and *M. schencki* from Poland (Sielezniew & Stankiewicz, 2008; Sielezniew et al., 2010b; Sielezniew et al., 2010c; Sielezniew et al., 2010a) (III); *M. scabrinodis* and a *M. specioides* from the Carpathian Basin (this paper); and (IV) *M. sabuleti*, as the well-proved main host, from Western Europe (England, France and Sweden: Thomas et al., 1989; Elmes et al., 1998; Nielsen, 2012) where *M. arion* is also recorded from *M. scabrinodis* and *M. lonae* nests, but only in a few cases.

According to these observations, it seems that *M. arion* shows less host ant specificity in Central than in Western European regions (similarly to the other European *Maculinea* species: Tartally, 2008). However, this does not exclude local adaptations to some host ant species in Central Europe, such as the *M. schencki* using Polish populations (Sielezniew et al., 2010c). This phenomenon could be explained by the geographic mosaic of coevolution between the butterflies and their host ants (Nash et al., 2008).

Because *M. arion* can sometimes be found with *Myrmica* species, which are not suitable to maintain populations (Thomas, 1980), we should be cautious not to place too much emphasis on single host ant records. On the other hand, it is very important to publish all host ant records, including single observations, because the greater the available data about host ant usage, the greater is our understanding of the biology and conservation potential of this endangered butterfly. At the same time, it is important to emphasize that finding *Myrmica* nests infected with *M. arion* is extremely difficult, and requires dedicated and systematic surveys (Sielezniew et al., 2010a). Therefore, building up detailed knowledge about the host ant usage of this butterfly across its range would need a much more intensive research involving numerous competent people.

Acknowledgements

To: DR Nash and two anonymous reviewers (revising), WP Pfliegler (Supplementary File: Fig 1b), "MacMan" RTD project (EVK2-CT-2001-00126), Hungarian Scientific Research Fund (OTKA K109223 and K84071), 'InDifferAnt' Marie Curie Intra European Fellowship (AT), 'AntLab' Marie Curie Career Integration Grant (AT), János Bolyai Scholarship of the Hungarian Academy of Sciences (AT and JB).

Supplementary material

http://dx.doi.org/10.13102/sociobiology.v64i1.1206.s1572

http://periodicos.uefs.br/index.php/sociobiology/rt/suppFileMetadata/1206/0/1572

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