

***Maculinea alcon* exploits *Myrmica aloba* in Portugal: unusual host ant species of a myrmecophilous butterfly in a peripheral region**

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Abstract Larvae of the obligate myrmecophilous social parasite *Maculinea alcon* (Lepidoptera: Lycaenidae) were found exclusively using *Myrmica aloba* (Hymenoptera: Formicidae) ant hosts in NE-Portugal. This is the first record of the host ant usage of any *Maculinea* species in Portugal, and of any *Maculinea* using *M. aloba* nests. These results on such peripheral European populations confirm that knowledge of the local host ant species is

crucial for the successful protection of these endangered butterflies, and vital for examining the evolution of such interactions.

Keywords Alcon Blue · Biogeography · Conservation · Endangered population · Host-specificity · Iberian Peninsula

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Larvae of *Maculinea*¹ *alcon* ([Denis & Schiffermüller], 1775) (Lepidoptera: Lycaenidae) start their life feeding on the developing seeds of gentian plants and in their last (fourth) instar they become social parasites of *Myrmica* Latreille, 1804 (Hymenoptera: Formicidae) ant colonies (see details in Thomas et al. 1989). The host ant species often vary in different regions (Elmes et al. 1998; Als et al. 2002; Höttiger et al. 2003; Tartally et al. 2008; Sielezniek and Stankiewicz-Fiedurek 2009). Knowledge of the local host ant species is important for the successful protection of these endangered butterflies and for answering evolutionary questions (e.g. Settele et al. 2005; Settele and Kühn 2009). As far as we are aware, no data on the host ant specificity of the Portuguese *M. alcon* populations has been published, and our aim was to fill this gap.

Myrmica nests were searched within 2 m (the approximate foraging zone of *Myrmica* workers, see Elmes et al. 1998) around *Gentiana pneumonanthe* L. host plants in three patches of a marshy mountain meadow with a lot of

¹ According to the formal aspects of priority rule, the generic name *Phengaris* Doherty, 1891 would be used (see e.g. Fric et al. 2007, Pech et al. 2007). However, the International Commission on Zoological Nomenclature (ICZN) has not decided to suppress *Maculinea* by *Phengaris* (ICZN 2010) and the precedence of *Maculinea* over the other name is proposed (Balletto et al. 2010).

Erica tetralix L. plants in NE-Portugal. The meadow is near the village of Lamas de Olo (41°22'N, 7°48'W, 1,029 m a.s.l.) in the Alvão Natural Park (PNAL) and is part of a Site of Community Importance (SCI) for the Mediterranean biogeographical region, listed in 2006 by the European Commission (2006/613/EC of 19 July 2006). The population size of *M. alcon* was determined there as between 1,000 and 2,000 in 2009 (see more details about the site in Soares et al. 2010). The research was carried out in late June-early July, just before the flying period of the butterfly, in 2002 and 2007–2009. *Myrmica* nests found were carefully opened and searched for fully-grown larvae and pupae (“specimens” in the following) of *M. alcon*. After excavation, the ground and vegetation were restored to as close to their original condition as possible. We tried to find a compromise between getting enough data and not disturbing the small site of this endangered butterfly, so we spread the collection of data over several years. Five to ten workers were collected from each ant nest and preserved in 75% ethanol for identification in the laboratory (det. 2002: G.W. Elmes, 2007–2009: AT and SC according to Seifert 1988). Host ant specificity was calculated in two ways (see details in Tartally et al. 2008): p1 is the 2-tailed probability from a Fisher exact test of heterogeneity in infection of host ant nests (as implemented at <http://www.quantitative-skills.com/sisa/>), and p2 is the probability from a randomization test of ant nests between species (using Nash 2009).

We found *M. aloba* Forel, 1909 nests in all three patches investigated. Only one other *Myrmica* species, *M. ruginodis* Nylander, 1846 was found, and only in two patches. A total of 30 nests of the two *Myrmica* species were opened (*M. aloba*, $n = 26$; and *M. ruginodis*, $n = 4$), and 40 *M. alcon* specimens were found in 12 *M. aloba* nests. Thus, 46% of *M. aloba* nests were infested, with a mean number of *M. alcon* specimens in all nests of 1.54, and 3.33 in only the infested nests (range: 2–7). Although only *M. aloba* nests were infected, the small number of *M. ruginodis* nests found meant that we could not rule out this species as a potential host, and that measures of local host specificity were non-significant ($p_1 = 0.130$, $p_2 = 0.190$). The number of specimens per *Myrmica* nest was higher than one, and thus similar to other populations in Europe (Als et al. 2002; Tartally et al. 2008; Witek et al. 2008).

To the best of our knowledge, these are the first records of the host ant usage of any *Maculinea* species in Portugal, and of any *Maculinea* from *M. aloba* nests (for a review see Fiedler 2006; Pech et al. 2007). *M. aloba* was found as host in all three patches and in all four summers. Thus, this ant species appears to be the most important host of this butterfly in this locality. This is supported by the fact that only this *Myrmica* species was found in two other nearby *M. alcon* sites, although only five uninfested nests were

found in total within 2 m of *G. pneumonanthe* (at PNAL near to Lamas de Olo: 41°21'N, 7°47'W, 1,000 m a.s.l., two nests; and 41°22'N, 7°48'W, 980 m a.s.l., three nests). However, care should be taken when generalising from the known host ant of a population to the host ant usage within a region (see e.g. Als et al. 2002; Tartally et al. 2008), therefore the host ant usage on the latter sites requires more investigation.

The distribution of *M. aloba* is restricted to the Iberian Peninsula and some nearby islands in Europe (Seifert 1988), typically on salt marshes with a high water table and an altitude of up to 1,400 m, and it is therefore not surprising that this ant has not been recorded as a *Maculinea* host from other regions. However, Elmes et al. (1998) report *M. aloba* from some Spanish *M. alcon* sites, but they found only *Myrmica scabrinodis* Nylander, 1846 as the host of *M. alcon* there. *Myrmica ruginodis*, the other recorded potential host ant, is known as an important *M. alcon* host in the Netherlands and Denmark, but not in other parts of Europe, despite its occurrence on some *M. alcon* sites there (Elmes et al. 1998; Als et al. 2002; Höttlinger et al. 2003; Tartally et al. 2008; Witek et al. 2008; Sielezniew and Stankiewicz-Fiedurek 2009). Whether *M. ruginodis* is used by the Portuguese *M. alcon* populations is still an open question. Some other *Myrmica* species are also known as hosts of *M. alcon* in different parts of Europe: *M. scabrinodis* in Austria, France, the Netherlands, Hungary, Spain, Poland, Ukraine and Transylvania (Romania); *M. vandeli* Bondroit, 1920 in Hungary, Poland and Transylvania; *M. rubra* (Linnaeus, 1758) in Denmark, the Netherlands and Sweden and *M. salina* Ruzsky, 1905 in Hungary (see references above). All of these records are for the ‘classical form’ of *M. alcon* occurring on boggy meadows, wet heaths and fens. The host ant usage of the form inhabiting nutrient-poor xerothermic and calcareous mountain grasslands, usually called *M. ‘rebeli’* (Hirschke 1904), is more complex (see details in Tartally et al. 2008).

Our results confirm that peripheral populations often adapt to special circumstances (e.g. Bernays and Graham 1988; Martin and Pullin 2004), in this case to a host ant that is unknown from other regions. According to these and to earlier results on different host ant species in different regions (see above), the host ant usage at the local scale needs to be considered for all European populations in the design and implementation of conservation management aimed to protect threatened *Maculinea* van Eecke, 1915 butterflies from local extinction, and especially in the case of peripheral populations. Future research could concentrate on clarifying the effect of the grazing regime on the host ants and plants at the Alcon Blue sites at Lamas de Olo, because according to our opinion it can be critical for the conservation of this butterfly there.

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