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SHORT COMMUNICATION

Myrmica aloba (Hymenoptera: Formicidae) hosts isolated populations of a hoverfly, a butterfly and an ichneumon species in NE-Portugal

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Abstract Larvae and pupae of the obligately myrmecophilous social parasites *Microdon myrmicae* (Diptera: Syrphidae) and *Maculinea alcon* (Lepidoptera: Lycaenidae) were found using exclusively *Myrmica aloba* (Hymenoptera: Formicidae) host ants in NE-Portugal. *Ichneumon eumerus* (Hymenoptera: Ichneumonidae) was also found developing in *Ma. alcon* pupae in nests of *My. aloba* at the same site. These are the first records of *Mi. myrmicae* and *I. eumerus* for Portugal, and from *My. aloba* nests. Earlier records that *My. aloba* is the only known host ant of *Ma. alcon* in Portugal are confirmed. Further studies on the biology of these isolated peripheral populations are necessary for their well-planned protection.

Keywords Alcon Blue · *Ichneumon eumerus* · *Maculinea alcon* · *Microdon myrmicae* · Social parasitism

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Introduction

Insects developing in ant nests, so-called myrmecophiles, often adapt to the locally available and suitable host ant species. This phenomenon is known for both the socially parasitic *Microdon* Meigen, 1803 (Diptera: Syrphidae) hoverfly and *Maculinea* van Eecke, 1915 (Lepidoptera: Lycaenidae) butterfly larvae (Schönrogge et al. 2002; Thomas et al. 1989).[1] Such local adaptation results in geographical variation in the host ant usage of the same myrmecophilous species (Als et al. 2002; Bonelli et al. 2011; Elmes et al. 1998; Höttinger et al. 2003; Sielezniew and Stankiewicz-Fiedurek 2009; Tartally 2008; Tartally et al. 2008). This variation in specificity is also important for the parasites and parasitoids of myrmecophilous insects. One of the best known examples is the wasp *Ichneumon eumerus* Wesmael, 1857 (Hymenoptera: Ichneumonidae), which specifically seeks out *Mymica* nests infected by *Maculinea* caterpillars, and gains entrance to them by secreting agonistic chemicals that induce in-fighting between worker ants, after which it parasitizes the *Maculinea* larvae (Thomas et al. 2002). The situation is even

[1] Based on formal taxonomic priority, it has been suggested that the generic name *Phengaris* Doherty, 1891 should replace *Maculinea* (see e.g. Fric et al. 2007; Pech et al. 2007). However, the case is still undecided by the International Commission on Zoological Nomenclature (ICZN 2012) and strong arguments for the precedence of *Maculinea* over *Phengaris* have been made (Balletto et al. 2010, and Comments on this Case). Hence we continue to use *Maculinea* as the accepted generic name of these butterflies.

more complex because the *Maculinea* larvae start their life feeding on the developing seeds of specific host plants, and in only in their last (fourth) instar become social parasites (see details in Thomas et al. 1989), which means that not only the suitable host ant, but also the host plant, must be present for the survival of these butterflies and their parasitoids. Because to these stringent requirements, myrmecophilous insects and their parasitoids are rare, are often endangered, and are good indicators of the stability of a community (Munguira and Martín 1999; Schönrogge et al. 2002; van Swaay and Warren 1999). Knowledge of the local host ant species is thus important for the successful protection of such species, as well as for answering interesting evolutionary questions (e.g. Settele and Kühn 2009; Settele et al. 2005).

There are some *Maculinea alcon* [(Denis and Schiffermüller), 1775] populations in the Alvão Natural Park (PNAl) in NE-Portugal, which are isolated from other populations. These populations have been studied only recently (Soares et al. 2012), and subsequently *Myrmica aloba* Forel, 1909 was recorded as the host ant of *Ma. alcon* there (Arnaldo et al. 2011).[2] However a deeper knowledge of the (potential) host ants of *Ma. alcon* in Portugal is essential. Furthermore, there have been no records from Portugal of the presence of the wasp *I. eumerus*, a parasitoid of *Ma. alcon* found elsewhere in Europe (Hinz and Horstmann 2007), nor of the hoverfly *Microdon myrmicae* Schönrogge et al. 2002, which has been recorded with *Myrmica* spp. in several European regions (Bonelli et al. 2011).

Our aim was thus to obtain more data about the host ant specificity of *Ma. alcon* and about the potential presence of *Mi. myrmicae* and *I. eumerus* in this isolated NE-Portuguese region.

Materials and methods

We searched for *Myrmica* nests within 2 m (the approximate foraging zone of *Myrmica* workers, see Elmes et al. 1998) of plants of *Gentiana pneumonanthe* L. (the host plant of *Ma. alcon*) in NE-Portugal in four marshy mountain meadows (see Fig. 1 of Online Resource) where *Ma. alcon* has been recorded, between the 2nd and 8th of July 2010: (1) Three patches near the village of Lamas de Olo (local site name: Libania; 41°22′N, 7°48′W, 1029 m a.s.l., 0.613 ha, population size in 2009 was 3680 and the sex ratio M/F was 1.3: Soares 2009; for more details of this site and population see Arnaldo et al. 2011; Rodrigues et al. 2010a, b; Soares et al. 2012); (2) three patches near the village of Plana dos Mijaceiros (41°22′N, 7°48′W, 974 m a.s.l., 1.179 ha, 50 captured adults in 2008 with a M/F sex ratio of 1.6: Soares 2009);(3) one patch near the village of Pioledo (41°23′N, 7°49′W, 895 m a.s.l., 0.064 ha, unknown population size); and (4) one patch at Moinho (41°22′N, 7°47′W, 1,000 m a.s.l., 0.309 ha, unknown population size) were investigated.

Myrmica nests found around *G. pneumonanthe* were carefully opened and searched for fully-grown *Ma. alcon* and *Mi. myrmicae* larvae and pupae, and the nests were restored after the examination. The number of prepupal *Ma. alcon* larvae and pupae were noted, and the infection of *I. eumerus* in *Ma. alcon* pupae was detected visually (it is clearly visible at this stage: see Fig. 2 of Online Resource). Infected *Ma. alcon* pupae were collected and kept in the dark under humid conditions at room temperature until the imago enclosed. The adult wasps were sent to K. Horstmann (Universität Würzburg) for confirmation of their identification. The recorded *Mi. myrmicae* larvae and pupae were placed into 75 % ethanol and sent to S. Bonelli (University of Turin) for confirmation of our determination. To identify the *Myrmica* species, five to ten workers were collected from each nest and preserved in 75 % ethanol, and were determined in the laboratory (det. AT according to Seifert 1988; Radchenko and Elmes 2010). Host ant specificity of *Ma. alcon* and *Mi. myrmicae* and *I. eumerus* was calculated in two ways (see details in Tartally et al. 2008): First, the 2-tailed probability from a Fisher exact test of heterogeneity in infection of host ant nests (as implemented at http://www.quantitativeskills.com/sisa/), and secondly, the probability from a randomization test of ant nests between species (Nash 2013). The current results were combined with our earlier data (Arnaldo et al. 2011) for these analyses.

Results

In addition to our earlier records from Libania (Arnaldo et al. 2011), we found a total of 46 new *My. aloba* nests and four new *My. ruginodis* Nylander, 1846 nests in the four sites investigated (Table 1). Altogether 99 larvae and pupae of *Ma. alcon* were newly found from six infested *My. aloba* nests, but only at Libania. Together with our earlier records (Arnaldo et al. 2011), *My. aloba* was recorded on all the four investigated sites while *My. ruginodis* was found only on three of them. A total of 85 nests of the two *Myrmica* species were opened (*My. aloba*, n = 77; and *My. ruginodis*, n = 8), and 139 *Ma. alcon* specimens were found in 18 *My. aloba* nests, while none of the *My. ruginodis* nests were infected. At Libania five *I. eumerus* were recorded from *Ma. alcon* pupae found in three *My. aloba* nests. At the same site and at Plana dos Mijaceiros, a total of nine *Mi. myrmicae* larvae and pupae were found in five *My. aloba* nests. No nests infested with both *Ma. alcon* and *My. myrmicae* were

[2] As names of three genera (*Maculinea*, *Microdon* and *Myrmica*) mentioned frequently in this paper start with "M", these are abbreviated with the first two characters for the easier readability.

Table 1 Summary of our earlier (Arnaldo et al. 2011) and current results (see "Results") about the number of *Myrmica* nests found and their recorded use by *Ma. alcon*, *I. eumerus* (recorded from *Ma. alcon* pupae) and *Mi. myrmicae* on four sites in NE-Portugal

Site	No. *Myrmica* nests	Infested with *Ma. alcon*	No. *Ma. alcon* (range *P*1, *P*2)	Parasitized with *I. eumerus*	No. *I. eumerus* (range *P*1, *P*2)	Infested with *Mi. myrmicae*	No. *Mi. myrmicae* (range *P*1, *P*2)
Plana dos Mijaceiros	*aloba* 19					3	5
	ruginodis 3						(1–3, 1.00, 0.76)
Moinho	*aloba* 3						
	ruginodis 1						
Pioledo	*aloba* 7						
Libania	*aloba* 51	20	139	3	5	2	4
	ruginodis 4		(2–32, 0.28, 0.35)		(1–3, 1.00, 1.00)		(1–3, 1.00)

Two measures of host specificity are given: *P*1 is the 2-tailed probability from the Fisher exact test of heterogeneity in infection of host ant nests and *P*2 is the probability from a randomization test of ant nests between species (see "Materials and methods")

recorded, although the expected number of co-infections if the two parasites were distributed randomly (0.78) was small, and our results do not differ significantly from this prediction (Fisher exact test, $P = 0.513$).

Discussion

These new data confirm our earlier records (Arnaldo et al. 2011) that *My. aloba* is an easily available and suitable host ant of *Ma. alcon* in NE-Portugal. *My. ruginodis* was also recorded there, but in very low numbers, and not from all the studied sites. It is well accepted that *My. aloba* is a sister species of *My. scabrinodis* Nylander, 1846 (Radchenko and Elmes 2010; Seifert 1988), which is the primary host ant of *Ma. alcon* in Central and Western Europe (e.g. in Spain and France: Elmes et al. 1998), and therefore it is not surprising that *My. aloba* is a good host ant of *Ma. alcon* in the Libania site, as we have not recorded *My. scabrinodis* on any of the investigated sites (despite that it is a sympatric with of *My. aloba* in much of the Iberian Penninsula: Radchenko and Elmes 2010; Seifert 1988). Given the apparent absence of other suitable hosts, it is likely that *Ma. alcon* also uses *My. aloba* as host ant at Pioledo, Moinho and Plana dos Mijaceiros, although this needs further investigation. As *My. ruginodis* is known as a host ant of *Ma. alcon* only from NE-Europe (Als et al. 2002; Elmes et al. 1998) and seems to be a relatively rare potential host ant species on the investigated sites (Table 1), we think this ant unlikely to be a host of *Ma. alcon* in NE-Portugal, but we cannot rule it out. This question also needs further investigation.

To the best of our knowledge, these are the first records of *I. eumerus* from Portugal (for a review see Hinz and Horstmann 2007). As *Ma. alcon* (or any *Maculinea* species) has been recorded from *My. aloba* nests only from our study sites (see above), these should also be the first records of *I. eumerus* from the nests of this ant species. The nearest known *I. eumerus* populations to this region are in Spain and France (Hinz and Horstmann 2007, see Fig. 1 of Online Resource), and the Portuguese populations seem to be rather isolated from these. As *I. eumerus* infects only *Maculinea* spp. caterpillars (Hinz and Horstmann 2007), the possibly refugial presence of this parasitoid in NE-Portugal raises the question of whether the host *Maculinea* species were more widely distributed in the Iberian Peninsula in the cool temperature phases of the last glacial period. Genetic surveys could help to answer this question.

Both the records of *Mi. myrmicae* from Portugal and from *My. aloba* nests are new to science (for a review see Bonelli et al. 2011), and give reasons for further thorough investigations. The presence of this hoverfly on the North-Eastern part of the Iberian Peninsula suggests a wider distribution of this species in Western-Europe than previously thought, as the nearest records of *Mi. myrmicae* to NE-Portugal are from NW-Italy, W-Switzerland, SW-Germany, The Netherlands, S-England and Ireland, but there are none from Spain, France or NW-Africa (Bonelli et al. 2011, see Fig. 1 of Online Resource). The usage of *My. aloba* as a host by *Mi. myrmicae* on two of our investigated sites can probably be explained in the same way as its use by *Ma. alcon* (see above); i.e. the dominant potential host ant on our sites was *My. aloba,* which is a sister species of *My. scabrinodis* (Radchenko and Elmes 2010; Seifert 1988) and the latter is the general host ant of *Mi. myrmicae* in other parts of Europe (Bonelli et al. 2011). Despite the fact that *My. ruginodis* is known from Hungarian, Polish and Ukrainian *Mi. myrmicae* sites, this ant has never been recorded as a host of *Mi. myrmicae* (Bonelli et al. 2011). It is intriguing that we found no nests of *My. aloba* simultaneously parasitized by *Ma. alcon* and *Mi. myrmicae*, but our sample sizes are too small to draw any firm conclusions from this.

Portugal, on the western part of the Iberian Peninsula, is a rather isolated region of Europe, the isolation of which is increased by the dispersal barrier offered by the Pyrenees.

It is well known that peripheral populations often adapt to special circumstances (e.g. Bernays and Graham 1988; Martin and Pullin 2004), and here we found such isolated (and possibly refugial) populations of three endangered insect species on the same sites in nests of a single ant species that is endemic to the Iberian Peninsula and some nearby islands (Radchenko and Elmes 2010; Seifert 1988). Based on the local co-occurrence of *Ma. alcon*, *I. eumerus* and *Mi. myrmicae* populations in a peripheral region, we suggest well-planned protection of these sites in Portugal. We also suggest careful but thorough surveys of similar Portuguese sites to get more knowledge about the distribution of these three species. The biology of the recorded populations and of their host plant(s) and host ant(s) should also be intensely but carefully surveyed for their successful conservation. The small sites investigated here appear to be largely isolated from each other (based on capture-recapture studies of *Ma. alcon*: Soares 2009), which suggests that they are likely to be sensitive to disturbance, and their successful protection should be a priority.

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