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Host specificity revisited: New data on *Myrmica* host ants of the lycaenid butterfly *Maculinea rebeli*

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Abstract

Larvae of *Maculinea rebeli*, one of the most endangered European butterflies, are obligatory social parasites of *Myrmica* ants. At present, this relationship is thought to be highly specific, with *Myrmica schencki* being regarded as the primary host. Here we present data on six populations from Poland and Austria, including the first record of *Myrmica specioides* as a host, together with published data from other central European countries, which severely questions the inference that *M. schencki* is the exclusive host of *M. rebeli*. Our results indicate that *Myrmica sabuleti* is the most frequently used host ant in central Europe, whereas *M. scabrinodis*, *M. sulcinodis*, *M. specioides* and *M. schencki* are used as secondary hosts. Possible explanations for this highly variable host use include (1) regional differences in semiochemicals, behaviour or social structure of the potential *Myrmica* host species and (2) the existence of different ecological subspecies or cryptic species of *M. rebeli.* Finally, we emphasize the importance of identifying local host ant species prior to further conservation strategies in order to avoid failure of management programs or even damage to populations on the edge of extinction.

Introduction

The Palearctic lycaenid butterfly *Maculinea rebeli* Hirschke, 1904, is one of Europe's most endangered butterflies. Currently it is restricted to 16 countries of central and western Europe (Wynhoff 1998) and its global threat status is ″vulnerable″ (Van Swaay and Warren 1999). In Poland, the national degree of threat is unknown (Van Swaay and Warren 1999). In Austria *M. rebeli* is generally endangered (Huemer et al. 1994), but it is critically endangered in eastern Austria (Schlick-Steiner et al. 2002). All known populations in Poland and eastern Austria are declining (Gornicki, unpublished data, Schlick-Steiner et al. (2002)). The main causes of decline include abandonment of extensive management and intensification of agriculture (Munguira and Martín 1999).

The life cycle of *M. rebeli* is closely associated with *Myrmica* host ants, a relationship which is parasitic and obligatory for the butterfly (Thomas and Elmes 1998; Wynhoff 1998). Eggs are laid on the main host plant *Gentiana cruciata*, on which the larvae feed until the fourth instar. Then the larvae leave the plants by letting themselves drop to the ground, where they depend on adoption by *Myrmica* ants that mistake them for their own brood due to chemical mimicry (Akino et al. 1999). Being fed and cared for by their host and while feeding on the ants' brood, *Maculinea* larvae gain about 98% of their final biomass before they pupate in the ant nest and emerge as adult butterflies after 11 or 23 months (Thomas et

al. 1998; Wardlaw et al. 2000). If the *M. rebeli* larvae are not adopted by a suitable *Myrmica* species, however, they are unlikely to survive until pupation (e.g., Thomas et al. (1989)).

Hence, only records of adult emergences from ant nests, or mature prepupal larvae or pupae inside ant nests, can be regarded as proof of a successful parasite-host interaction (Elmes et al. 1998). To date, the species *Myrmica scabrinodis, M. sabuleti, M. sulcinodis, M. ruginodis* and *M. schencki* have been described as rare secondary hosts (Jutzeler 1989; Thomas et al. 1989; Elmes et al. 1998).

There is also an ongoing discussion on the taxonomic status of *M. rebeli* because of confusion with another closely related taxon, *M. alcon* Denis & Schiffermüller, 1775. In general, however, authors have agreed that two ecologically differing forms can be distinguished: *M. rebeli*, restricted to xerothermous grassland with *Gentiana cruciata*, *Gentianella germanica* or *Gentianella campestris* as host plants, and *M. alcon* exclusively inhabiting moorland and wet grassland with *Gentiana pneumonanthe*, *G. asclepiadea* or *G. germanica* as host plants (Schweizerischer Bund für Naturschutz 1987). In this paper we report that a variety of *Myrmica* host ants are used by *M. rebeli* in Poland and Austria and we explore the potential implications of this finding for future conservation strategies, taking into account all presently available data on the host ants of *M. rebeli*.

Study sites and methods

Poland

Field studies were carried out at the previously undescribed (*cf.* Buszko (1997)), most NE population of *M. rebeli* in the Przemyskie Foothills, SE Poland, close to the Ukrainian border (Table 1, Figure 1, the exact site name and position are not presented to avoid exploitation by collectors). In late May 2001, *M. rebeli* larvae were searched for in all *Myrmica* nests located in a circular area of 12.5 m^2 around *Gentiana cruciata* plants, as most *Myrmica* species forage at a distance of up to 2 m from the nest (Elmes et al. 1998). Only gentians on which oviposition had been observed in the previous year, were chosen. The numbers of prepupal larvae or pupae of *M. rebeli* in *Myrmica* nests were recorded.

Ants were determined according to Radchenko et al. (1997) and Wardlaw et al. (1998). The identification of *Maculinea* larvae was unequivocal, as *M. rebeli* was the only adult *Maculinea* species present at the site.

Austria

Field studies were carried out in May and June 2001 in five populations of *M. rebeli* in Lower Austria and Burgenland, Austria (Nördliches Weinviertel, Rohrwald, Steinfeld, Rotwald, Günser Gebirge; Table 1, Figure 1). These sites are separated by distances of between 35 and 150 km. At each site, *M. rebeli* larvae were searched for in all *Myrmica* nests located in a circular area of 12.5 m^2 with randomly chosen *G. cruciata* plants in the center. Additional larvae were recorded in nests found by baiting and tracking *Myrmica* ant workers in four further plots of 12.5 m^2 (Elmes and Wardlaw 1982) and by intensively searching near *G. cruciata* stems in the other parts of the sites. Moreover, at each site five modified pitfall traps (Majer (1978); ethanol:glycerin = 5:1, detergent added) were set up 5 cm away from gentian stems for four weeks to complete the list of *Myrmica* species present at each site. Ants and butterfly larvae were determined according to Seifert (1988, 1996) and Schweizerischer Bund für Naturschutz (1987), respectively.

Results

We searched a total of 53 nests of four *Myrmica* species at the six sites in Poland and Austria and found a total number of 75 mature prepupal larvae or pupae of *M. rebeli* in the nests of the following four species: *M. sabuleti* Meinert, 1861, *M. scabrinodis* Nylander, 1846, *M. specioides* Bondroit, 1918, and *M. schencki* Viereck, 1903 (Table 1). Most individuals were discovered in nests of *M. sabuleti* (11 nests, 56 *Maculinea*), while only two infested nests of *M. scabrinodis* (7 *Maculinea*) and one each of *M. specioides* (6 *Maculinea*) and *M. schencki* (6 *Maculinea*) were found.

At the Polish site, *M. sabuleti* and *M. scabrinodis* were simultaneously used as hosts. Seven out of ten *M. sabuleti* nests searched were infested, whereas only 2 out of 15 *M. scabrinodis* nests were infested. *M. schencki* was present (1 nest), but not identified as a host.

In Austria, a total of 223 additional workers of six *Myrmica* species were trapped (*M. specioides*, *M.*

Table 1. Host specificity of *Maculinea rebeli* in Europe (Poland, own findings; Austria: own findings, Switzerland: Jutzeler (1989) and Jutzeler and Agosti (1991); Germany: Meyer-Hozak (2000); France and Spain: Elmes et al. (1998). Only emerging adults, pupae or prepupal larvae, or larvae of nearly prepupal size, 14 months after adoption (Germany) are considered). All *Myrmica* species found at the investigated sites are listed. Numbers of sites and of *Myrmica* nests searched are given. The total number of nests containing at least one fully grown immature stage of *M. rebeli* is given beneath each species; figures in parentheses indicate the total number of individuals observed, if available. A zero indicates nest(s) searched, but no host records, + indicates co-existence at the site. No entry indicates either that the species has not been recorded coexisting (Austria, Germany, France, Poland Spain) or that information is lacking (Switzerland).

				Potential host Myrmica species								
Country	name of site	sites	nests searched	*sabuleti*	*sulcinodis*	*scabrinodis*	*schencki*	*specioides*	*ruginodis*	*rubra*	*vandeli*	*lobicornis*
Poland	Przemyskie Foothills (300 m a.s.l.)	1	26	7(39)		2(7)	0					
	Nördliches Weinviertel (250 m a.s.l.)	1	4	1(4)		+	1(6)		+	+		
	Rohrwald (370 m a.s.l)	1	6	1(1)			0					
Austria	Steinfeld (330 m a.s.l)	1	3	0			+	1(6)				
	Günser Gebirge (420 m a.s.l.)	1	1	1(7)								
	Rotwald (720 m a.s.l.)	1	13	1(5)								
Switzerland		1	7		4							
Germany		1	8	4(17)			0					
France, Spain		10	321	1(6)	+	1(4)	71(299)		1(3)	+	+	+

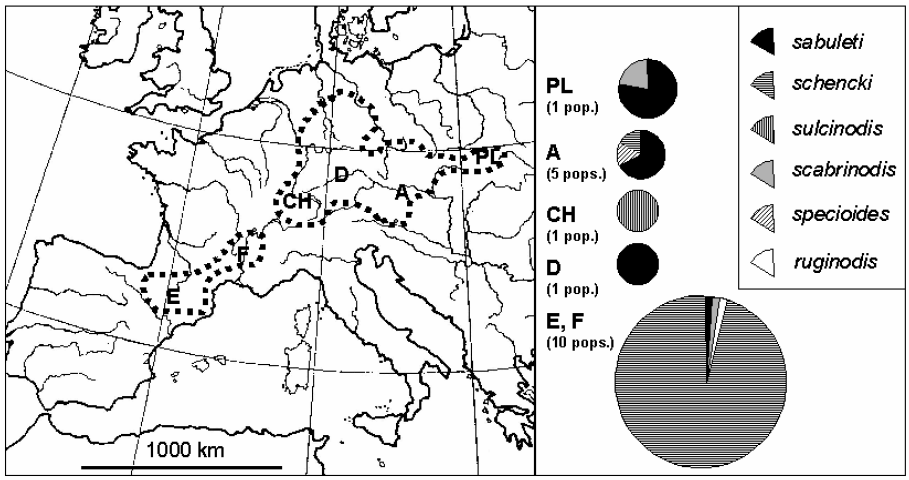

Figure 1. Distribution of *M. rebeli* in Europe (based on post-1980 records in Wynhoff (1998)). Populations with known *Myrmica* host ant species are indicated (PL, Poland: own findings; A, Austria: own findings; CH, Switzerland: Jutzeler (1989) and Jutzeler and Agosti (1991); D, Germany: Meyer-Hozak (2000); F, France and E, Spain: Elmes et al. (1998)). Proportion of use of different *Myrmica* species as host ants (right): The area of each circle is proportional to the total number of *Myrmica* nests out of which adult emergences were observed, or in which pupae, prepupal larvae or larvae of nearly prepupal size, 14 months after adoption (D), were found (PL: 9; A: 6; CH: 4; D: 4; E,F: 74). The number of populations investigated is given in parentheses.

scabrinodis, *M. sabuleti*, *M. rubra* Linnaeus, 1758, *M. ruginodis* Nylander, 1846, *M. schencki*, Table 1). At two sites (Rotwald, Günser Gebirge), *M. sabuleti* was the only *Myrmica* species present and thus the only host ant recorded. In the Nördliches Weinviertel, *M. sabuleti* and *M. schencki* were used as hosts simultaneously (one nest each). In the Rohrwald, *M. sabuleti* was found to be the only host, although *M. schencki* (1 nest) was found to be present near the gentians. In the Steinfeld, *M. specioides* was the only host, although *M. sabuleti* and *M. schencki* were present at the site.

Discussion

M. specioides had so far not been found to rear any *Maculinea* species (Wardlaw et al. 1998) and is therefore a new host record. Other data on the host use of *M. rebeli* from central Europe are available only from one site in Switzerland and one site in Germany (Jutzeler 1989; Jutzeler and Agosti 1991; Meyer-Hozak 2000), (Table 1, Figure 1). Overall, *M. sabuleti* is most frequently used (6 of 8 sites, 15 of 24 infested nests), while at the other sites *M. sulcinodis* and *M. specioides* were found to be the only hosts. In central Europe, eleven *Myrmica* species occur in potential *M. rebeli* habitats (excluding socially parasitic species like *M. microrubra* Seifert, 1993, three of the *Myrmica species* being rare (*M. salina*, Ruzsky 1905, *M. lobicornis*, Nylander 1846, *M. lonae*, Finzi 1926, *cf.* Seifert (1996)). Despite the limited number of observations, only three of the remaining eight species (*M. rubra, M. ruginodis, M. rugulosa* Nylander, 1849) have not been reported as hosts of *M. rebeli* in central Europe. In western Europe, data are available from ten sites in the southern French Alps and Spanish Pyrenees (Elmes et al. 1998): *M. rebeli* larvae have been found in nests of four *Myrmica* species (Table 1), nearly always with *M. schencki* (71 of 74 infested *Myrmica* nests). Although *M. schencki* is a rare species in these habitats, and only adopts one third of the larval population, larval mortality of *Maculinea rebeli* is 29 times higher in the nests of other *Myrmica* species, 95% of full grown *Maculinea* larvae are produced in nests of *Myrmica schencki* (Thomas and Elmes 1998).

While earlier authors postulated low host specificity for *Maculinea* species in general (e.g., Malicky (1969)), today the relationship is thought to be highly specific. Based on field observations in Spain and France, on laboratory experiments and on chemical analyses, *M. schencki* is regarded as the primary host of *M. rebeli* (e.g., Thomas et al. (1989) and Hochberg et al. (1992), Clarke et al. (1998), Elmes et al. (1998), Akino et al. (1999)). Field observations from central Europe, however, question the assumption that *M. schencki* is the primary host of *Maculinea rebeli* in

general. Only in France and Spain can a single host species (*M. schencki*) be regarded as significantly preferred (data for Poland, the Austrian populations together, and France/Spain separately subjected to χ^2-tests). On the other hand, there are significant differences between the host-use in the different regions (Fisher's Exact Test, $P < 0.05$). Thus, the new findings indicate regional differences of this ant-butterfly relationship as known for *Maculinea alcon* (Elmes et al. 1994; Als et al. 2001) and considered likely for *Maculinea teleius* (Stettmer et al. (2001), Stankiewicz and Sielezniew, 2002). Possible explanations, alone or in combination, include: (1) Regional differences of semiochemicals (*cf.* Nowbahari et al. (1990)) and consecutive differences in adaptation of the chemical camouflage of *M. rebeli* larvae, or regional differences of behaviour, e.g., level of aggressiveness, or of social structure (e.g., level of polygyny, *cf.* Seppä and Walin (1996)) of the potential *Myrmica* host species, which lead to different patterns of host use in the same butterfly-species. (2) Different ecological subspecies, or even cryptic species, of *M. rebeli* may exist (*cf.* Elmes et al. (1994)). This could mean that, in contrast to a western European taxon that almost exclusively uses *M. schencki* as a host, in central Europe another taxon with different host use occurs. On the other hand, it is also possible that host specificity is simply lower in central Europe.

Evidence in favour of one or the other of these hypotheses is lacking at present. Thus, detailed field observations on *M. rebeli* populations in the other parts of its range are needed, especially as only few authors have so far collected large larvae and pupae in nests. Such surveys will take time, which is problematic because the danger of local extinction continues to increase (e.g., Schlick-Steiner et al. (2002)). We should take warning from the conservation-history of *M. arion* in Great Britain – the butterfly went extinct due to lack of understanding of its ecological requirements (Thomas 1999). Our study shows that in the meantime any conservation strategy should be based on the assumption that *M. rebeli* may well have other primary hosts than *M. schencki*. Priority must thus be given to investigating the distribution of local *Myrmica* host ants (due to the precarious situation of *M. rebeli*, with utmost caution!) in all viable populations of *M. rebeli*, instead of only considering *M. schencki*, as has been done in the recent past (*cf.* Kockelke et al. (1994) and Settele et al. (1995)).

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