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Research article

Patterns of host ant use by sympatric populations of *Maculinea alcon* and *M. 'rebeli'* in the Carpathian Basin

A. Tartally[1], D.R. Nash[2,*], S. Lengyel[3] and Z. Varga[1]

[1] *Department of Evolutionary Zoology and Human Biology, University of Debrecen, H-4032, Egyetem tér 1, Debrecen, Hungary, e-mail: tartally@gmail.com, zvarga@tigris.klte.hu*
[2] *Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen, Denmark, e-mail: DRNash@bio.ku.dk*
[3] *Department of Ecology, University of Debrecen, H-4032 Debrecen, Egyetem tér 1, Debrecen, Hungary*
Current address: Department of Zoology, North Carolina State University, David Clark Labs, 100 Brooks Avenue, Raleigh, NC 27695–7617, USA, e-mail: szabolcslengyel@yahoo.com

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Abstract. *Maculinea* butterflies show social parasitism via obligatory myrmecophily as their larvae are adopted and raised to pupation by *Myrmica* ants. Suitable hosts differ for different *Maculinea* species, and host ant specificity can further differ at the population-level. Although early studies suggested single ant species as main hosts for each *Maculinea* species, it has recently become clear that their host ant specificity is more complex. *Maculinea alcon* and *Maculinea 'rebeli'* have variously been separated according to adult and larval morphology, phenology, and their use of different ecosystems, including host plant and host ant species. However, recent genetic evidence has questioned their separation as good species. Here we compare the use of host ants by *M. alcon* and *M. 'rebeli'* at the regional scale in NE-Hungary and Transylvania (Romania), where molecular studies have found no species-level separation between the two forms. We opened 778 nests of *Myrmica* ants and searched for *Maculinea* specimens (larvae, pupae and exuviae) shortly before imago emergence from the nest in seven *M. alcon* sites, six *M. 'rebeli'*-sites and one site where both *M. alcon* and *M. 'rebeli'* are syntopic. In all, *Maculinea* caterpillars were found in the nests of seven different ant species (*M. alcon* was recorded mainly with *Myrmica scabrinodis* and occasionally with *M. salina* and *M. vandeli*; *M. 'rebeli'* used mainly *M. scabrinodis*, *M. sabuleti* and *M. schencki* and occasionally *M. lonae* and *M. specioides*). *Myrmica scabrinodis* was found to be a general host of both *M. alcon* and *M. 'rebeli'*, which is the first record for a common host ant of these two closely related butterflies within the same region. However there were also differences in host ant use patterns between the sites occupied by the two *Maculinea* taxa, which reflect differences in *Myrmica* communities between the two types of habitat. Possible explanations for the similar but not identical host use patterns of *M. alcon* and *M. 'rebeli',* and their relevance for the question of whether they are separate species are discussed.

Keywords: Myrmica, myrmecophily, social parasitism, host specificity.

Introduction

Close association with ants (myrmecophily) is known from numerous insect taxa (e.g., Hölldobler and Wilson, 1990). Most butterflies of the family Lycaenidae have facultative or obligate myrmecophilous larvae, and the outcome of the association ranges from mutualism to parasitism (Fiedler, 1991, 2006; Pierce et al., 2002). Species of the lycaenid genus *Maculinea* van Eecke, 1915 are obligate, socially parasitic myrmecophilous butterflies. The caterpillars start their life feeding on the developing seeds of specific food plants but complete their development during the last larval instar in an ant nest (e.g. Thomas et al., 1989 and references therein). For social integration into ant nests the caterpillars mimic the acoustic (DeVries et al., 1993) and especially the

* Author for correspondence.

chemical signals of the host ants (Akino et al., 1999; Elmes et al., 2002; Schlick-Steiner et al., 2004; Schönrogge et al., 2004).

Because of their joint reliance on specific host plants and host ants, *Maculinea* butterflies are generally rare, and are considered to be globally endangered (Munguira and Martín, 1999; Van Swaay and Warren, 1999; Settele et al., 2002, 2005; Thomas and Settele, 2004; IUCN, 2007). The availability of host ants is often more limiting to *Maculinea* populations than that of the food plants, therefore, the knowledge of host ant use of *Maculinea* populations is critical for their protection (Elmes and Thomas, 1992).

The range and level of host specificity is especially relevant in the Alcon Blues, as they have been conventionally subdivided into the Alcon Blue *Maculinea alcon* ([Denis and Schiffermüller], 1775) and the Mountain Alcon Blue *M. rebeli* (Hirschke, 1904) based on their use of different host ant and host plant species (Thomas et al., 1989). As their common names suggest, the habitats of the two Alcon Blues are also different, with *M. alcon* occurring on boggy meadows, wet heaths and fens, and *M. rebeli* inhabiting nutrient-poor xerothermic and calcareous mountain grasslands (Munguira and Martín, 1999).

Recent studies have, however, shown that the level of differentiation between *M. alcon* and *M. rebeli* is lower than expected at the species level (DNA-based phylogeny: Als et al., 2004; adult morphology and ecology: Pech et al., 2004; larval cuticular compounds and egg morphology: Steiner et al., 2006). Enzyme polymorphism studies by Bereczki et al. (2005, 2006) and Pecsenye et al. (2007) have shown that *M. alcon* and *M. rebeli* populations in the Carpathian Basin exhibit a considerable amount of local genetic structure, but that this differentiation is better explained by geographical distribution than species differentiation or habitat use. Recent observations also suggest that host plant use and habitat characteristics do not conform to the traditional clear-cut differentiation between *M. rebeli* and *M. alcon* in SE-Europe (Kolev, 2002; Sielezniew and Stankiewicz, 2004a).

Their specificity to different host ants has long been thought as one of the main differences between the two Alcon Blues (Thomas et al., 1989). Host ants of the Alcon Blues are known exclusively from the genus *Myrmica* Latreille, 1804 (Als et al., 2004; Settele et al., 2005). Larvae of the Alcon Blues spend 11 or 23 months in the ant nest where they are fed by the ants by trophallaxis ("cuckoo-type" caterpillars, Thomas and Elmes, 1998) and may also prey on ant brood (e.g., Tartally, 2004). In their seminal work, Thomas et al. (1989) found *Myrmica ruginodis* Nylander, 1846 as the main host ant of *M. alcon* in the Netherlands and *M. schencki* Viereck, 1903 for *M. rebeli* in France. Subsequent studies have refined this by showing that the two butterflies use different main host ant species in different parts of their geographical range (Elmes et al., 1994, 1998; Als et al., 2002; Meyer-Hozak, 2002; Sielezniew and Stankiewicz, 2002; Höttinger et al., 2003; Steiner et al., 2003). The main hosts reported in these studies include *M. rubra* (Linnaeus, 1758), *M. ruginodis* and *M. scabrinodis* Nylander, 1846 for *M. alcon*; and *M. schencki* and *M. sabuleti* Meinert, 1860 for *M. rebeli* (see Als et al., 2004 and Settele et al., 2005 for a review of major and minor host ant species). Due to the massive geographical variation in host ants, data are necessary from the entire geographical range of Alcon Blues to understand host specificity and the factors influencing these patterns. Furthermore, efficient conservation of *Maculinea* species and their habitats is impossible without proper knowledge of local host ants (Munguira and Martín, 1999; Settele et al., 2002).

The aim of this study was to compare host ant specificity of *M. alcon* and *M. rebeli* populations in the Carpathians, where they are regionally sympatric. Such a comparison has been lacking so far (see Elmes et al., 1994, 1998; Als et al., 2002; Meyer-Hozak, 2002; Sielezniew and Stankiewicz, 2002; Höttinger et al., 2003; Steiner et al., 2003). We hypothesised that niches of *M. alcon* and *M. rebeli* overlap with regard to their host ant species because they do not show species-level genetic differentiation in this region (Bereczki et al., 2005, 2006; Pecsenye et al., 2007).

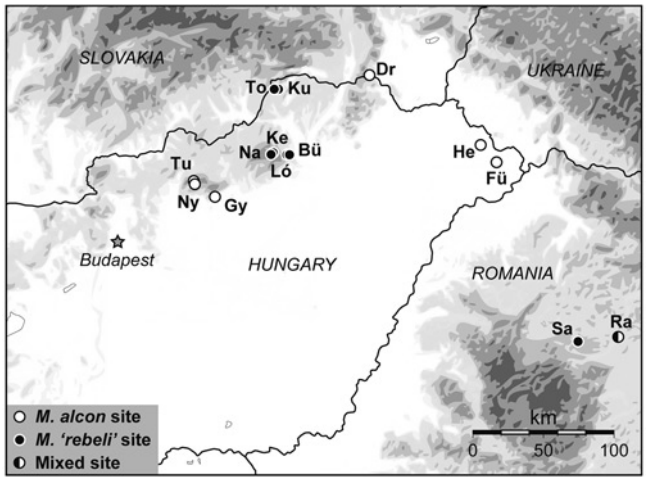

Fig. 1. Map of north-east Hungary and Transylvania, showing the location of the sample sites. Codes for each site are given in Table 1. Shading represents local topology.

Material and methods

1. Separation of Alcon Blues

Maculinea rebeli was initially described as a form of *M. alcon*, based on differences in habitat use (Hirschke, 1905), and there are morphological differences between the type specimen and typical *M. alcon* (Kudrna and Belicek, 2005). Subsequently, since the discovery of differences in host plant and host ant use, it has become common

Table 1. Characteristics of the study sites (see Fig. 1 for geographic locations). Sites where *Gentiana pneumonanthe* is the main host plant are considered to be *Maculinea alcon* sites, while those dominated by *G. cruciata* are *M. 'rebeli'* sites.

Site	Code	Region	Latitude longitude elevation	Main (additional) host plant	Syntopic *Maculinea* populations	Vegetation	Estimated population size
Bükkszentkereszt	Bü	Bükk mountains	48°04' N 20°38' E 563 m	*Gentiana cruciata*	—	Abandoned mountain hayfield	< 500
Drahos-rét	Dr	Zemplén mountains	48°34' N 21°26' E 742 m	*G. pneumonanthe*	*M. teleius* *M. arion*	Tall-grass marshy meadow	< 500
Fülesd	Fü	Szatmár-Bereg lowlands	48°01' N 22°38' E 111 m	*G. pneumonanthe*	*M. teleius*	Tall-grass marshy meadow	> 1000
Gyilkos-rét	Gy	Mátra mountains	47°48' N 19°58' E 352 m	*G. pneumonanthe*	—	Tall-sedge marshy meadow	> 1000
Hetefejércse	He	Szatmár-Bereg lowlands	48°08' N 22°29' E 108 m	*G. pneumonanthe*	*M. teleius*	Tall-grass marshy meadow	< 500
Kecskeláb-rét	Ke	Bükk mountains	48°05' N; 20°31' E 751 m	*G. cruciata*	—	Abandoned mountain hayfield	< 500
Kuriszlán	Ku	Aggtelek Karst	48°29' N; 20°34' E 333 m	*G. cruciata*	—	Semi-dry sward mesic hayfield	> 1000
Lófő-tisztás	Ló	Bükk mountains	48°04' N; 20°39' E 656 m	*G. cruciata* (*Gentianella austriaca*)	—	Abandoned mountain hayfield	500–1000
Nagy-mező	Na	Bükk mountains	48°04' N; 20°30' E 783 m	*G. cruciata* (*G. pneumonanthe*)	—	Mountain meadow	500–1000
Nyikom-rét	Ny	Mátra mountains	47°54' N; 19°46' E 680 m	*G. pneumonanthe*	—	Tall-grass marshy meadow	< 500
Răscruci	Ra	Câmpia Transilvaniei	46°54' N; 23°47' E 485 m	*G. cruciata* *G. pneumonanthe*	*M. teleius* *M. nausithous*	Extensively grazed tall-grass, meadow steppe (*G. cruciata*) with small marshy depressions (*G. pneumonanthe*)	500–1000
Şardu	Sa	Podişul Someşan	46°52' N; 23°24' E 480 m	*G. pneumonanthe*	*M. teleius*	Tall-grass, marshy meadow	< 500
Tugár-rét	Tu	Mátra mountains	47°54' N; 19°49' E 586 m	*G. pneumonanthe*	—	Tall-grass marshy meadow	< 500
Tohonya-hát	To	Aggtelek Karst	48°29' N; 20°32' E 268 m	*G. cruciata*	—	Oldfield semi-dry sward	> 1000

practise to refer to *M. rebeli* as a separate species, although the basis for this is unclear (Kudrna and Belicek, 2005). More recent studies, however, have found no morphological or genetic differences between *M. alcon* and *M. rebeli* (Als et al., 2004; Pech et al., 2004; Bereczki et al., 2005, 2006; Steiner et al., 2006; Pecsenye et al., 2007). In an attempt to clarify the relationship between host ant use, habitat and host plant use, here we separate the studied populations by using *M. alcon* for populations in wet meadows where caterpillars develop on *Gentiana pneumonanthe*, and *M. 'rebeli'* for those in xerothermic or nutrient-poor mountain grasslands where caterpillars develop on *G. cruciata* host plants. There were three sites (see Table 1) where this separation could not work clearly, because more than one host plant species was used by the populations there (there were eggs on all the syntopic host plant species and caterpillars dropped from all of them in the laboratory, Tartally and Varga, pers. obs.). In these cases *M. alcon* refers to such full-grown caterpillars, pupae and exuviae that were found in patches occupied by *G. pneumonanthe*, while *M. 'rebeli'* refers to those found in patches occupied by *G. cruciata.*

2. Field methods

We studied ant colonies in 12 sites in NE-Hungary and in two sites in Transylvania (Romania) between 2000 and 2007 (Fig. 1, Table 1). Sites were selected from all *Maculinea* sites known in the two regions that held stable populations of either of the Alcon Blues. There were six sites where only *G. pneumonanthe* and four where only *G. cruciata* were found as host plants. At two sites (Nagy-mező and Lófő-tisztás), a few individuals of alternative host plants occurred, but no potential host *Myrmica* spp. nests could be found in their vicinity. However, in Răscruci there was a mosaic structure of semi-dry and boggy patches where *G. cruciata* occurred on the semi-dry and *G. pneumonanthe* on the boggy patches. In this site we chose one semi-dry and an adjacent boggy patch where the border between them was clear (i.e. there was a ca. 10 m wide zone between them without any gentians; these two patches are referred to as Răscruci 'wet' and Răscruci 'dry'). These records of host plants mean that host specificity of *M. alcon* only was studied at seven sites (Drahos-rét, Fülesd, Gyilkos-rét, Hetefejércse, Nyikom-rét, Şardu and Tugár-rét), that of *M. 'rebeli'* at six sites (Bükkszentkereszt, Kecskeláb-rét, Kuriszlán, Lófő-tisztás, Nagy-mező and Tohonya-hát) and that of both butterflies at one site (Răscruci) (see Table 1).

Myrmica nests were searched exclusively within 2 m around randomly selected *Gentiana* host plants, which is the approximate foraging zone of workers of the *Myrmica* genus (Elmes et al., 1998). Nests found were carefully opened and searched for full-grown larvae, pupae and exuviae of *Maculinea* (hereafter referred to as '*Maculinea* specimens'). After excavation, the ground and vegetation were restored to as close to the original conditions as possible. The number of *Myrmica* nests found varied greatly between sites, which resulted in rather unbalanced sample sizes (see below), since we attempted to keep search effort constant across sites. We also restricted searches to reduce disturbance of some sites (Settele et al., 2005), all of which are of high conservation value and protected by law. Five to ten workers were collected from each ant nest and were preserved in ethanol for identification in the laboratory (using Seifert, 1988; Radchenko et al., 2003). When *Maculinea* specimens were found in a nest, we recorded their number and determined the species using a 20x magnifier lens. Larvae and pupae of the Alcon Blues can be easily separated from those of the other European *Maculinea* species (Śliwińska et al., 2006). *M. alcon* and *M. 'rebeli'* have typical 'cuckoo characters', whereas the other European *Maculinea* species have some 'predatory characters' (Thomas and Wardlaw, 1990; Elmes et al., 1991). Data included in this study are from ant nests parasitized exclusively by either *M. alcon* or *M. 'rebeli'*. Voucher samples are deposited in the Hymenoptera Collection of the Hungarian Natural History Museum (Budapest) and in the first author's collection.

Searches were made not earlier than four weeks before the flying period of *Maculinea* at each site. This period is the most appropriate to evaluate which ant colonies reared *Maculinea* larvae to adulthood. Search periods earlier in the life cycle (e.g., spring or previous autumn) are less satisfactory because ant colonies adopting young fourth-instar larvae may later kill them (Thomas et al., 2005).

3. Host ant specificity

Host specificity can be defined in many different ways (Thomas et al., 2005). Here we use the term 'host specificity' to refer to the ability of *Maculinea* butterflies to develop within the nests of particular host ant species, but to have reduced or no development in the nests of others. Ideally, to quantify specificity, the number of *Maculinea* larvae discovered by each species of ant should be known, as well as the number that survive and develop to adulthood in the ants' nests (Thomas et al., 2005). Directly measuring the numbers of caterpillars that are discovered by a particular ant species in the field was not feasible in this study, due to limited access to field sites and the practicalities of collecting data over a large area, so here we have assumed that the proportion of nests of the different *Myrmica* species found within 2 m of randomly chosen *Gentiana* plants is equal to the proportion of caterpillars of Alcon Blues that are discovered by the different ant species (see Discussion). Specificity is then assessed by comparing the distribution of *Maculinea* specimens within ant nests in the early summer with the distribution expected if they were randomly discovered by the observed distribution of host ants.

Even within this framework, however, specificity can be measured in different ways. The main factor that needs to be taken into account is that within a potential host ant species, ant nests vary considerably in their susceptibility to parasitism by *Maculinea* larvae, so that the typical pattern of infestation is of many uninfested nests, several with moderate levels of infestation, and a few nests with high levels of infestation. Such patterns of infestation are typical for macroparasites where hosts vary in susceptibility, and often follow a negative binomial distribution (Anderson and May, 1978). The consequence of this is that if a limited number of nests of a particular ant species is sampled in an area, the probability of finding only uninfested nests is much higher than if the parasites were more evenly distributed.

Host ant specificity at each site was therefore quantified in four separate ways. 1) Heterogeneity in the number of infested and uninfested nests of *Myrmica* species on each site was tested by using an extended version of the Fisher exact test, generalized to more than two compared samples (Carr, 1980, as implemented at http://www.quantitativeskills.com/sisa/). This allows the relative proportion of infested nests to be compared with the number of nests that are available, but takes no account of the number of *Maculinea* specimens that have developed within each nest. 2) The heterogeneity of the number of *Maculinea* specimens between nests of different species was compared using a Chi-squared statistic, the significance of which was tested by reassigning each *Maculinea* specimen randomly to one of the nests at a site regardless of species and calculating the same chi-squared statistic 100000 times. This gives a measure of the host specificity of the *Maculinea* at a site if there was no overdispersion of individuals between nests. 3) The number of *Maculinea* specimens within the nests of each *Myrmica* species found was compared using a general linear model with overdispersed negative binomial errors, as implemented in the 'aod' package (version 1.1–22) for the statistical software 'R' (version 2.5.1; http://www.r-project.org/). This is a powerful way of testing quantitative differences between the number of *Maculinea* raised by different host ant species when the data are highly over-dispersed, but assumes a particular form of overdispersion (the negative binomial distribution), and may not be appropriate for small samples. 4) The heterogeneity of the number of *Maculinea* specimens between nests of different species was compared using a Chi-squared statistic, the significance of which was tested by reassigning each nest (and its associated number of *Maculinea* specimens) randomly to one of the *Myrmica* species observed at a site, with the constraint that the total number of nests of each species was the same as that observed, and calculating the same chi-squared statistic 100000 times. This gives a measure of the host specificity of the *Maculinea* at a site based on the observed distribution of *Maculinea* between nests, but the power of this test to detect heterogeneity in the distribution between ant species will be low except for those cases in which many ant nests have been investigated.

The number of *Myrmica* species found at each site was compared between *M. alcon* and *M. 'rebeli'* sites using a General Linear Model with Poisson errors, as implemented in JMP 7.0. The total number of *Myrmica* nests examined at each site, plus the interaction of between this and the type of site were included in the analysis as covariates to examine any effect of 'sampling effort' on the diversity of the ant community at each site, and the model simplified by backward elimination of insignificant terms. The number of host ant species found at each site was examined in the same way. The composition of the *Myrmica* community was compared between *M. alcon* and *M. 'rebeli'* sites using ANOSIM, as implemented in the software package PAST 1.79 (Hammer et al., 2001).

Table 2. The number of ant nests found within 2 m of host plants (*Gentiana* spp.) at each site, their infection with *M. alcon* or *M. 'rebeli'*, and statistical tests of host ant specificity at each site: P1 = probability from Fisher exact test. χ^2 = value of the chi-squared statistic of heterogeneity in host ant use. P2 = probability associated with the χ^2 statistic based on 100000 randomizations of *Maculinea* specimens between nests. k = dispersion parameter of the negative binomial distribution fitted to the number of *Maculinea* found in nests at each site. Δd = change in deviance in a General Linear Model with negative binomial errors due to differences in infestation between host ant species at each site. P3 = probability that such a change in deviance would arise by chance. P4 = probability associated with the χ^2 statistic based on 100000 randomizations of host ant nests between species. Significant p-values are marked in bold. See text for details of these tests.

Maculinea	Site	*Myrmica*	Number of nests	Number with *Maculinea*	*P1*	Number of *Maculinea*	χ^2	*P2*	k	Δd	*P3*	*P4*
M. alcon	Drahos-rét	*M. scabrinodis*	53	3	0.184	3	14.6	< **0.001**	0.140	5.41	**0.020**	**0.046**
		M. vandeli	19	3		9						
	Fülesd	*M. scabrinodis*	9	2	0.274	3	146.23	< **0.001**	0.143	13.2	**0.004**	0.239
		M. gallienii	6	0		0						
		M. ruginodis	2	0		0						
		M. salina	15	6		137						
	Gyilkos-rét	*M. scabrinodis*	32	23	< **0.001**	315	70.65	< **0.001**	0.407	17.8	< **0.001**	0.153
		M. gallienii	6	0		0						
		M. schencki	1	0		0						
	Hetefejércse	*M. scabrinodis*	5	3	—	35		—	—	—	—	—
	Nyikom-rét	*M. scabrinodis*	4	2	1.000	7	1.75	0.359	0.735	1.67	0.196	1.000
		M. ruginodis	1	0		0						
	Răscruci 'wet'	*M. scabrinodis*	20	4	—	10		—	—	—	—	—
	Şardu	*M. scabrinodis*	26	2	1.000	4	1.6	0.236	0.033	0.199	0.656	0.888
		M. vandeli	21	1		7						
	Tugár-rét	*M. scabrinodis*	6	4	—	17		—	—	—	—	—
M. 'rebeli'	Bükkszentkereszt	*M. scabrinodis*	55	2	0.340	24	5.41	**0.027**	0.023	0.498	0.481	0.769
		M. sabuleti	30	3		4						
	Kecskeláb-rét	*M. scabrinodis*	2	0	0.353	0	12.74	**0.009**	0.039	0.825	0.662	0.353
		M. lonae	3	1		5						
		M. sabuleti	21	1		6						
	Kuriszlán	*M. scabrinodis*	39	1	**0.015**	1	51.84	< **0.001**	0.227	15.7	**0.001**	**0.045**
		M. schencki	13	3		12						
		M. specioides	1	1		3						
		M. vandeli	1	0		0						
	Lófő-tisztás	*M. scabrinodis*	149	7	0.151	82	10.95	**0.037**	0.016	1.67	0.796	0.768
		M. lonae	8	0		0						
		M. ruginodis	2	0		0						
		M. sabuleti	5	1		5						
		M. schencki	4	1		15						
	Nagy-mező	*M. scabrinodis*	47	0	0.340	0	18.27	**0.017**	0.054	16.1	**0.024**	0.325
		M. lobicornis	2	0		0						
		M. lonae	4	0		0						
		M. ruginodis	10	0		0						
		M. sabuleti	22	2		6						
		M. schencki	4	0		0						

Table 2 *(Continued)*

Maculinea	Site	*Myrmica*	Number of nests	Number with *Maculinea*	*P1*	Number of *Maculinea*	χ^2	*P2*	*k*	Δd	*P3*	*P4*
M. 'rebeli' *(Continued)*	Răscruci 'dry'	*M. scabrinodis*	11	0	**0.035**	0	36.64	< **0.001**	0.274	21.3	0.077	0.142
		M. schencki	5	2		18						
		M. sabuleti	4	2		7						
	Tohonya-hát	*M. scabrinodis*	51	0	**0.007**	0	19.43	**0.012**	0.309	14.7	**0.005**	0.069
		M. sabuleti	3	0		0						
		M. salina	30	0		0						
		M. schencki	16	2		4						
		M. specioides	10	2		2						
Both	Răscruci	As above			0.090		56.74	< **0.001**	0.163	5.71	0.058	0.067

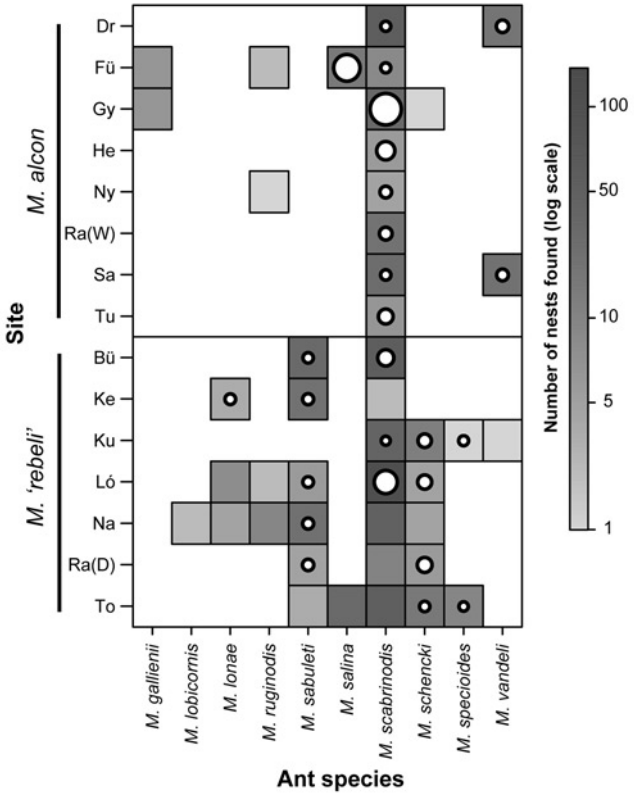

Fig. 2. Matrix showing the distribution of different *Myrmica* species (columns) between the sample sites (rows). A shaded box represents the presence of that species of *Myrmica*, with the depth of shading representing the number of nests found. Those species that were found to be infected with *Maculinea* at each site are marked with a circle, the diameter of which is proportional to the number of *Maculinea* specimens found. Codes for each site are given in Table 1.

Results

1. Ant species present at the sites

A total of 778 *Myrmica* ant nests were found within 2 m of the host *Gentiana* spp. plants in the 14 sites (Fig. 2; Table 2). Only *M. scabrinodis* was present at all sites, and overall it was by far the most frequent ant species (69 % of all ant colonies found). Nine other ant species were also present in the areas searched (Fig. 2; Table 2), with *M. sabuleti* and *M. schencki* present on six sites and *M. ruginodis* present on four. There was a greater number of *Myrmica* species on *M. 'rebeli'* sites (mean ± SE: 4.0 ± 0.53) than on *M. alcon* sites (2.0 ± 0.38; Fig. 3; Likelihood-Ratio $\chi^2 = 5.11$, $p = 0.024$), but the number of *Myrmica* species could equally well be explained by the total number of *Myrmica* nests discovered within 2 m of *Gentiana* plants at each site (Fig. 3a; L-R $\chi^2 = 4.91$, $p = 0.027$), which was higher for *M. 'rebeli'* sites than *M. alcon* sites, so this difference may simply reflect 'sampling effort' (see discussion). When the number of host species recorded at each site was examined, however (Fig. 3b), there was no significant relationship between this and type of site (*M. 'rebeli'* vs *M. alcon* sites; L-R $\chi^2 = 1.26$, $p = 0.260$) or total number of *Myrmica* nests (L-R $\chi^2 = 1.27$, $p = 0.259$).

When the community of ants found at each site was examined, on the other hand, there were clear differences between *M. alcon* and *M. 'rebeli'* sites (ANOSIM; $p = 0.009$). This reflects the absence of *M. lobicornis*, *M. lonae*, *M. sabuleti* and *M. specioides* from *M. alcon* sites, and the absence of *M. gallienii* from *M. 'rebeli'* sites (Fig. 2; Table 2). When only host ant species were considered, the two types of site were even more distinct (ANOSIM, $p = 0.0005$).

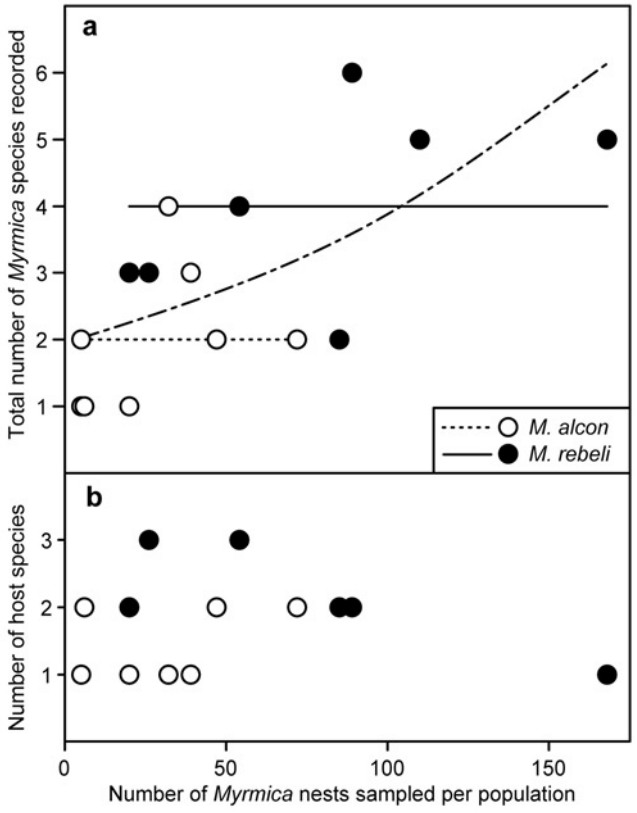

Fig. 3. a) Relationship between the total number of *Myrmica* nests found within 2 m of gentian plants at each site, and the number of *Myrmica* species represented by those nests. The number of *Myrmica* species is equally well described by the means for the different types of site (horizontal lines), or by the total number of *Myrmica* nests independent of type of site (curved line: fitted regression line from GLM with Poisson errors). **b**) Relationship between the total number of *Myrmica* nests found within 2 m of gentian plants and the number of *Myrmica* species found to host *Maculinea* specimens at each site.

2. Frequency of Maculinea *parasitism*

Maculinea caterpillars, pupae or exuviae were found in 84 (or 11 %) of the *Myrmica* ant nests found (n = 778) (Table 2). A total of 741 *Maculinea* specimens were found in the ant nests studied (Table 2). The mean ± S.D. number of *Maculinea* per nest was 0.95 ± 4.94 and ranged between 0 and 68 caterpillars per ant nest (n = 778). Ant nests in *M. alcon* sites held significantly more *Maculinea* specimens (2.42 ± 8.32 *Maculinea* per nest) than did ant nests in *M. 'rebeli'* sites (0.35 ± 2.22; Median test, S = 4.67, p < 0.001), and when all ant nests on a site were considered, parasitism levels were higher on *M. alcon* sites (23.4 %) than on *M. 'rebeli'* sites (5.6 %; GLM with Binomial errors: Likelihood-Ratio $\chi^2 = 9.20$, p = 0.0024). There was no apparent size dimorphism of Alcon blue caterpillars in the examined nests, as has been found elsewhere (Thomas et al., 1998), but two particularly small *M. alcon* caterpillars were found at Gyilkos-rét in one *M. scabrinodis* nest with 68 caterpillars, probably as a result of contest competition between the larvae for the colony resources (Thomas et al., 1993). The absence of larval size dimorphism is consistent with earlier observations of *M. 'rebeli'* in this area (Tartally, 2005a).

3. Specificity of host ant use based on presence or absence of infections

M. scabrinodis, present in all sites studied, was the only ant species which was used by both Alcon Blues, and thus can be considered a general host ant for *M. alcon* and *M. 'rebeli'* in north-eastern Hungary, although no infested nests were found on *M. 'rebeli'* sites in Transylvania (Table 2). The rate of parasitism of *M. scabrinodis* nests was significantly higher at *M. alcon* sites than at *M. 'rebeli'* sites (Fig. 4; GLM with binomial errors; between types of site, L-R $\chi^2 = 36.6$, $p < 0.0001$), reflecting the overall levels of parasitism at the two types of site. However, it also increased with the degree to which *M. scabrinodis* dominated the local ant community (L-R $\chi^2 = 12.48$, p = 0.0004) in the same manner for both types of site (type of site × proportion of *M. scabrinodis*; L-R $\chi^2 = 0.34$, p = 0.558).

At four sites, *M. scabrinodis* was the only host ant of *M. alcon.* However, *M. vandeli* nests held more *M. alcon* specimens than did *M. scabrinodis* nests in Drahos-rét and Şardu, and *M. salina* nests held more *M. alcon* than *M. scabrinodis* nests in Fülesd (Fig. 2; Table 2). Nests of five *Myrmica* species (*M. lonae, M. sabuleti, M. scabrinodis, M. schencki* and *M. specioides*) were parasitized by *M. 'rebeli'*, of which *M. lonae* and *M. specioides* were rare hosts (Fig. 2; Table 2).

When host specificity was examined at each site in terms of the distribution of infected and uninfected colonies between species, only one of the 5 *M. alcon* sites, Gyilkos-rét, showed evidence of host ant nests being used in a proportion that differed from their availability, with *M. scabrinodis* being infected more often than expected. For *M. 'rebeli'* sites, three out of six showed heterogeneity in host use, with all three sites having fewer infected nests of *M. scabrinodis* than expected (Fig. 2; Table 2).

4. Specificity and frequency distribution of Maculinea *in host nests*

The numbers of *Maculinea* specimens in *Myrmica* nests was highly clumped, with 694 nests (89 % of all nests examined) being uninfected, and with more than 50 % of *Maculinea* specimens being found in just 11 nests (1.4 %). Not surprisingly, therefore, we found significant heterogeneity in host ant use at 10 out of 12 sites where more than one *Myrmica* species was present when the distribution was tested against that expected if *Maculinea* specimens were assigned to nests at random (i.e. following a Poisson distribution) (Table 2).

The distribution of number of *Maculinea* specimens within host nests at each site was well described by a

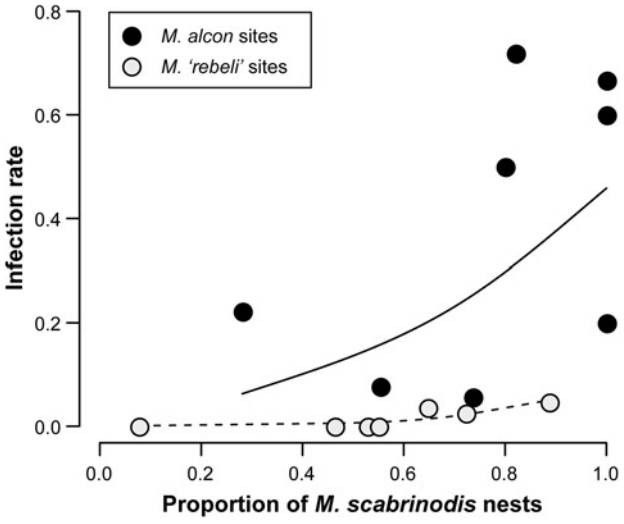

Fig. 4. Relationship between the proportion of *Myrmica* nests at each site that were *M. scabrinodis* and the infection rate of those nests (i.e. the proportion of *M. scabrinodis* nests that were infected with *Maculinea*). Lines are fitted logistic regression lines.

negative binomial distribution (Goodness of fit test, all $p > 0.75$), with dispersion parameter k ranging from 0.016 to 0.375 (Table 2). Testing whether there were differences in the number of *Maculinea* specimens found infecting different host ant species, assuming a negative binomial distribution between nests, showed significant differences in host ant use at three *M. alcon* sites and three *M. 'rebeli'* sites, with the Răscruci 'dry' site being on the border of significance (Table 2).

When nests were randomly reassigned between species, only two sites showed significant heterogeneity in host use, the *M. alcon* site Drahos-rét, and the *M. 'rebeli'* site Kuriszlán, although that for Tohonya-hát was on the borders of significance.

Combining the data for the *M. alcon* and *M. 'rebeli'* areas of Răscruci (Table 2) decreased the significance of the heterogeneity in host use based on the presence or absence of *Maculinea* specimens in nests (as specimens were found in *M. scabrinodis* nests in the 'wet' area of the site), but increased the significance of the heterogeneity in host ant use when numbers of specimens per nest were taken into account. This latter effect is probably primarily due to the increase in power associated with an increased sample size.

Discussion

To our knowledge, this is the first study to provide data on host ant specificity of regionally sympatric populations of *M. alcon* and *M. 'rebeli'* in an area where close genetic similarity of the two butterflies was found (Bereczki et al., 2005, 2006; Pecsenye et al., 2007). Both *M. alcon* and *M. 'rebeli'* use more host ant species in north-eastern Hungary and Transylvania than elsewhere in their studied range (see details below). Ten *Myrmica* species were recorded in the two types of Alcon Blue habitats and only three of them (*M. ruginodis*, *M. lobicornis* and *M. gallienii*) were not exploited by either *M. alcon* or *M. 'rebeli'*.

There are many potential pitfalls in examining the host ant use of *Maculinea* butterflies, since there are several possible reasons for finding *Maculinea* caterpillars in nests of non-host species (Thomas et al., 2005). By sampling in early summer, we ensured that the *Maculinea* caterpillars and pupae we found must have survived the winter in a *Myrmica* colony, which is unlikely to have experienced the benign conditions that can lead to the toleration of caterpillars by non-host species in the laboratory (Thomas et al., 2005). However, it is possible that the *Myrmica* colony that raised the *Maculinea* caterpillars is not the same colony in which they are found in early summer, as *Myrmica* colonies frequently emigrate, and take-over of abandoned nests (perhaps with resident *Maculinea* caterpillars) by other *Myrmica* species is a distinct possibility (Thomas et al., 2005). On the other hand, laboratory studies suggest that the Alcon blues are so tightly integrated into their host colony structure (Thomas and Elmes, 1998), that they are among the first individuals to be moved when nest emigration takes place (Thomas et al., 1998), so this problem is less likely to arise than with the predatory *Maculinea* species. Our assumption that *Maculinea* caterpillars are retrieved by the different ant species in proportion to the number of nests of those ant species that we found close to food plants may be a larger potential source of inaccuracy in our host specificity data. Although it would theoretically pay female *Maculinea* to lay their eggs on host plants close to nests of the local host ant species, the field evidence so far suggests that *Maculinea* butterflies do not bias their egg distribution in this manner (see Musche et al., 2006 for a recent review). Clumping of foodplants, or eggs on foodplants (Clarke et al., 1998) could also potentially cause biases in our measurement of host specificity, but if these clumps are distributed randomly with respect to ant species distributions, then our measurements based on the distribution of number of caterpillars between nests will take such clumping into account, but this inevitably adds extra noise to the sampling procedure.

Our results show that *M. scabrinodis* is the most important host ant of *M. alcon* in north-east Hungary and Transylvania, (Table 2; Fig. 2, 4), which is also the case in central and W-Hungary, E-Austria, W-Ukraine, France, Spain and Poland (Elmes et al., 1994, 1998; Höttinger et al., 2003; Tartally and Csősz, 2004; Sielezniew and Stankiewicz, 2002, 2004b; Vályi Nagy and Csősz, 2007; M. Witek, pers. comm.; A. Tartally, unpubl. data). However, *M. vandeli* and *M. salina* were used rather than *M. scabrinodis* when these two species were common at a site (Table 2; Fig. 2, 4). *M. vandeli* has previously been reported as a host of *M. alcon* from Poland (Sielezniew and Stankiewicz, 2004b; Stankiewicz and Sielezniew, 2005). Since this species is thought to be a

temporary social parasite of *Myrmica scabrinodis* (Elmes et al., 2003), its use as a host at sites where it is common could potentially reflect take-over of already parasitized *M. scabrinodis* nests (Thomas et al., 2005), or parasitism of established colonies. In either case, adaptations by *M. alcon* to parasitism of *M. scabrinodis* may also facilitate parasitism of *M. vandeli*, which may itself mimic the recognition cues of its temporary host. *M. salina* is known as a host of *M. alcon* exclusively from Fülesd. Parasitism of *M. salina* rather than *M. scabrinodis* in Fülesd may result from a recent local adaptation towards using the more salt-tolerant of the two ant species in a habitat where secondary salinisation started in the early 1950s (Tartally, 2005b).

More host species were recorded for *M. 'rebeli'* than for *M. alcon* in NE-Hungary and Transylvania, and this region appears to have a greater diversity of hosts than other parts of Europe (see Thomas et al., 1989; Elmes et al., 1998; Meyer-Hozak, 2002; Steiner et al., 2003; Stankiewicz et al., 2005; Vályi Nagy and Csősz, 2007). *M. 'rebeli'* specimens were mostly found in nests of *M. sabuleti, M. schencki* and *M. scabrinodis* (Fig. 2; Table 2). *M. sabuleti* is known as the main host ant for *M. 'rebeli'* from Poland, E-Westphalia (Germany) and E-Austria (Meyer-Hozak, 2002; Steiner et al., 2003; Stankiewicz and Sielezniew, 2005). *M. schencki* has also been recorded as the main host of *M. 'rebeli'* from France, Spain and Lithuania (Thomas et al., 1989; Elmes et al., 1998; Stankiewicz et al., 2005). Thus, it appears that *M. 'rebeli'* uses *M. schencki* and *M. sabuleti* as the main host in different parts of Europe. *M. scabrinodis* was also an important local host of *M. 'rebeli'* (in Bükkszentkereszt and Lófő-tisztás; Table 2; Fig. 2). However, this ant species is known only as a secondary host of *M. 'rebeli'* from Poland and France (Thomas et al., 1989; Elmes et al., 1998; Steiner et al., 2003). *M. specioides* and *M. lonae* are additional hosts of *M. 'rebeli'* in Hungary (Table 2; Fig. 2). *M. specioides* is also known as an additional host from E-Austria (Steiner et al., 2003) but our study is the first to record *M. lonae* as a host of any *Maculinea* species (Als et al., 2004).

The difference in diversity of host use by *M. alcon* and *M. 'rebeli'* may in part reflect the greater diversity of *Myrmica* found on *M. 'rebeli'* sites. Comparison of the relationship between the number of *Myrmica* nests and the number of *Myrmica* species from the two types of habitat (Fig. 3) suggests that the greater diversity of *Myrmica* species found on *M. 'rebeli'* sites may, in turn, reflect greater 'sampling effort' (i.e. greater number of examined nests) on these sites. The time and effort expended in searching the two types of site was similar, so the difference in the number of nests examined on the two sets of sites primarily arose because the *M. alcon* sites were generally smaller and supported lower *Maculinea* populations than the *M. 'rebeli'* sites (Table 1), so that our 'sampling effort' reflected accurately the situation faced by the butterflies.

It has recently been suggested that there is little support for local host specificity in *Maculinea* butterflies (Pech et al., 2007), and that patterns of apparent host specificity reflect undersampling of alternative hosts. However, we clearly have heterogeneous use of host ant species in several of the populations examined (Table 2; Fig. 2). The test used to examine heterogeneity makes some difference to the result found, but generally there is a similar pattern of specificity across sites as measured in terms of the presence or absence of infection (P1 in table 2), abundance-based models based on the negative binomial distribution (P3 in table 2), and randomization of nests (P4 in table 2). The P-values from each of these tests are highly correlated (Spearman rank correlations: P1 *v* P3; 0.695, P1 *v* P4; 0.837, P3 *v* P4; 0.729). Each method used to assess host specificity has its advantages and disadvantages (see methods), but models based on the negative binomial distribution may provide the best compromise between power and taking into account the clumped distribution of *Maculinea* between nests. The absence of any relationship between the number of ant nests sampled within a site, and the number of host species recorded (Fig. 3b) also confirms that the local specificity we observe is not as a result of undersampling of the local ant fauna.

At the regional scale, our study does, however, support the conclusion of Pech et al. (2007) that the total number of host species recorded for any *Maculinea* species is an increasing function of the number of populations examined. This does not, however, reflect lack of specificity of *Maculinea-Myrmica* interactions, but that the processes underlying the evolution of specificity occur at the local rather than global or regional scale. This is unsurprising, since the availability and density of different potential host species varies widely across the Palaearctic (Czechowski et al., 2002) and regionally (Als et al. 2002; this study).

The complexity of host use patterns can represent a geographical mosaic of coevolution between *M. alcon*/*'rebeli'* and the local host ant species (*sensu* Thompson, 1999). The geographical mosaic model predicts that geographic variation in the strengths and reciprocity of coevolution may lead to differences in host ant use at both the regional and local level. For both *M. alcon* and *M. 'rebeli'* sites we found a pattern of higher levels of infestation of *Myrmica scabrinodis* nests when this host is locally common, and lower levels of infestation when it is rare. This is exactly the pattern expected if there is local coadaptation with this host, and mirrors the pattern found for the interaction between *M. alcon* and *Myrmica rubra* in north-west Europe, where a coevolutionary geographic mosaic of chemical mimicry is thought to exist (Nash et al., 2008).

Different selection forces may operate in central and peripheral populations. For example, polyphagous butterflies are often specialised on a smaller number of host plants in peripheral areas than in central parts of the range, and host plants may be different for different

peripheral populations (e.g. de Lattin, 1967; Martin and Pullins, 2004; Schmidt and Hughes, 2006). The host ant specificity of Alcon Blues could also show such variation because the genus *Maculinea* is thought to have evolved in continental East Asia (Sibatani et al., 1994) and European, particularly western European, populations can be considered peripheral (Pech et al., 2007). Most of the *M. alcon* and *M. 'rebeli'* populations known to use only one main host ant species have been reported from the periphery of their geographical range, i.e. in western and northern Europe (Thomas et al., 1989; Elmes et al., 1998; Stankiewicz et al., 2005). Data from our study and other recent studies (Steiner et al., 2003; Sielezniew and Stankiewicz, 2004b; Stankiewicz and Sielezniew, 2005) show that multiple host ant use may be more frequent in central Europe than in western Europe, although this may also reflect differences in the composition of *Myrmica* communities between these areas (Czechowski et al., 2002). It is interesting to note, however, that the most common host ant species in our study, *Myrmica scabrinodis*, is also common in north-west Europe, but has never been recorded as a host of *M. alcon* in this area (Elmes et al., 1994; Als et al., 2002). More knowledge on the host ants and host plants of Alcon Blues in the Asian (e.g., southern Siberia, Kazakhstan), southern and eastern European (e.g., Balkans) part of their range would therefore be valuable.

The pattern of host ant use may be an important tool in understanding species boundaries in the Alcon Blue complex. Our results show that *M. scabrinodis* is a mutual host for both *M. alcon* and *M. 'rebeli'* in north-eastern Hungary, as it is in Poland (Sielezniew and Stankiewicz, 2002, 2004b; Steiner et al., 2003; Stankiewicz and Sielezniew, 2005). We have also demonstrated that the host ant community associated with the two host plants, *Gentiana pneumonanthe* and *G. cruciata*, is different, so that differences in host ant use by *M. alcon* and *M. 'rebeli'* could potentially be due to host ant availability rather than specialization of these two forms on different host species. Of particular note, here, is the absence of *Myrmica schencki* from all but one of the sites where *G. pneumonanthe* is the initial host plant, but its commonness and use as a major host on *G. cruciata* sites. The one *M. alcon* site where *M. schencki* was found should therefore be a priority for future examinations of host ant use by Alcon blues. Such differences in host ant availability between sites where *G. pneumonanthe* and *G. cruciata* are found are not at all surprising, given the generally different habitats that these two plants occupy, and the different community of *Myrmica* ants that are expected to be associated with these habitats (Elmes et al., 1998). In addition, recent observations suggest that host plant use by Alcon blues is more complex than previously thought (e.g., *M. alcon* uses '*rebeli* host' *Gentiana cruciata* in Poland: Sielezniew and Stankiewicz, 2004a; *M. 'rebeli'* uses '*alcon* host' *G. asclepiadea* in Bulgaria: Kolev, 2002; *M. 'rebeli'* uses '*alcon* host' *G. pneumonanthe* in NE-Hungary at Nagy-mező: Tartally and Varga, pers. obs., Table 1). These findings suggest that both host ant and host plant species may be locally shared by the two Alcon Blues in central Europe. Therefore, the species-level distinction between *M. alcon* and *M. 'rebeli'* based on their use of host ants and host plants at the western margin of their distribution range (e.g. Thomas et al., 1989) is less pronounced in more central populations. Enzyme polymorphism studies by Bereczki et al. (2005, 2006) and Pecsenye et al. (2007) also did not support the separation of *M. alcon* and *M. 'rebeli'* in the Carpathian Basin, and led these authors to question the validity of several Alcon Blue forms and subspecies known from and around the Carpathian Basin (for a review see Bereczki et al., 2006).

On the other hand, *Myrmica scabrinodis*, the one host ant that is exploited by both *M. alcon* and *M. 'rebeli'* is parasitized at different rates in the different types of habitat (Fig. 4). Since this reflects the overall parasitism levels of all *Myrmica* nests at *M. alcon* and *M. 'rebeli'* sites, it may reflect differences in the distribution, abundance or phenology of host plants, but this pattern could also arise because of different levels of specialization of the two forms of *Maculinea* on *M. scabrinodis*. More data on the distribution of host plants and *Maculinea* eggs from these sites is needed to differentiate between these possibilities, but the similar relationship between the levels of exploitation of *Myrmica scabrinodis* and its abundance found in the two types of habitat (Fig. 4) argues in favour of similar levels of specialization.

The multiple host ant use found in this study raises several ecological and evolutionary questions. At least four scenarios can explain multiple host use in the *M. alcon*/*'rebeli'* complex (Thomas et al., 2005). First, conditions may be benign enough that *Myrmica* species not normally used as hosts can tolerate *Maculinea* caterpillars in their nests. The recent changes in some of the sites studied (e.g., salinisation in Fülesd, Tartally, 2005b) may be favourable for some *Myrmica* species, reducing interspecific competition for resources and making them more tolerant of *Maculinea* caterpillars. However, this explanation is unlikely to hold for most sites under normal field conditions, and further study is necessary to fully test it. Second, mixed-host *Maculinea* populations may be polymorphic and different larvae may be adapted to use different host species. Under these circumstances we would expect host-ant-related genetic substructure within populations. Enzyme polymorphism studies (Bereczki et al., 2005, 2006; Pecsenye et al., 2007), however, have failed to find such substructuring of *M. alcon* and *M. 'rebeli'* populations in NE-Hungary. Third, mixed-host populations may occupy habitats in areas that are on biogeographical boundaries between single-host *M. alcon* and *M. 'rebeli'* areas. Under this scenario we would also expect either genetic differentiation between the subpopulations using different hosts, or a hybrid population structure with an excess of heterozygotes. Finally, mixed-host *Maculinea* populations may show phenotypic adaptations to more than one host,

allowing true multiple host use. Recent chemical studies suggest that *M. 'rebeli'* larvae may show adaptation to different hosts by synthesising cuticular hydrocarbons specific to both of the local host ant species (Schlick-Steiner et al., 2004), and *M. alcon* may exploit different hosts that share similar chemistry, allowing a geographical mosaic of adaptation depending on local host ant availability (Nash et al., 2008).

Our data on the differences in host specificity between nearby populations are compatible with those of other studies (Als et al., 2002) and draw attention to the importance of host specificity studies on the local scale. Our results support the hypothesis that local adaptations towards using non-primary host ants may increase the diversity of host ant use patterns at the regional scale. The variation in host ant use at the local scale needs to be considered in the design and implementation of conservation management aimed to preserve threatened *Maculinea* spp. from local extinction.

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References

Akino T., Knapp J.J., Thomas J.A. and Elmes G.W. 1999. Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond. B* **266:** 1419 – 1426

Als T.D., Nash D.R. and Boomsma J.J. 2002. Geographical variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. *Ecol. Entomol.* **27:** 403 – 414

Als T.D., Vila R., Kandul N.P., Nash D.R., Yen S.H., Hsu Y.F., Mignault A.A., Boomsma J.J. and Pierce N.E. 2004. The evolution of alternative parasitic life histories in Large Blue butterflies. *Nature* **432:** 386 – 390

Anderson R.M. and May R.M. 1978. Regulation and stability of host-parasite population interactions. *J. Anim. Ecol.* **47:** 219 – 247

Bereczki J., Pecsenye K., Peregovits L. and Varga Z. 2005. Pattern of genetic differentiation in the *Maculinea alcon* species group (Lepidoptera, Lycaenidae) in Central Europe. *J. Zool. Syst. Evol. Res.* **43:** 157 – 165

Bereczki J., Pecsenye K. and Varga Z. 2006. Geographic versus food plant differentiation in populations of *Maculinea alcon* (Lepidoptera: Lycaenidae) in Northern Hungary. *Eur. J. Entomol.* **103:** 725 – 732

Carr W.E. 1980. Fisher's exact test extended to more than two samples of equal size. *Technometrics* **22:** 269 – 270

Clarke R.T., Thomas J.A., Elmes G.W., Wardlaw J.C., Munguira M.L. and Hochberg M.E. 1998. Population modelling of the spatial interactions between *Maculinea rebeli*, their initial foodplant *Gentiana cruciata*, and *Myrmica* ants within a site. *J. Insect Conserv.* **2:** 29 – 37

Czechowski W., Radchenko A. and Czechowska W. 2002. *The Ants (Hymenoptera, Formicidae) of Poland:* Museum and Institute of Zoology PAS, Warszawa. 200 pp

de Lattin G. 1967. *Grundriss der Zoogeographie.* Hochschullehrbücher für Biologie 12. Fischer G. Verlag, Stuttgart. 602 pp

DeVries P.J., Cocroft R.B. and Thomas J.A. 1993. Comparison of acoustical signals in *Maculinea* butterfly caterpillars and their obligate host *Myrmica* ants. *Biol. J. Linn. Soc.* **49:** 229 – 238

Elmes G.W., Akino T., Thomas J.A., Clarke R.T. and Knapp J.J. 2002. Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. *Oecologia* **130:** 525 – 535

Elmes G.W., Radchenko A.G. and Thomas J.A. 2003. First records of *Myrmica vandeli* Bondroit (Hymenoptera, Formicidae) for Britain. *Br. J. Ent. Nat. Hist.* **16:** 145 – 152

Elmes G.W. and Thomas J.A. 1992. Complexity of species conservation in managed habitats: interaction between *Maculinea* butterflies and their ant hosts. *Biodivers. Conserv.* **1:** 155 – 169

Elmes G.W., Thomas J.A., Hammarstedt O., Munguira M.L., Martín J. and Van Der Made J. 1994. Differences in host-ant specificity between Spanish, Dutch and Swedish populations of the endangered butterfly, *Maculinea alcon* (Denis et Schiff.) (Lepidoptera). *Memorab. Zool.* **48:** 55 – 68

Elmes G.W., Thomas J.A. and Wardlaw J.C. 1991. Larvae of *Maculinea rebeli*, a large blue butterfly, and their *Myrmica* hosts: wild adoption and behaviour in ant-nests. *J. Zool. Lond.* **223:** 447 – 460

Elmes G.W., Thomas J.A., Wardlaw J.C., Hochberg M., Clarke R.T. and Simcox D.J. 1998. The ecology of *Myrmica* ants in relation to the conservation of *Maculinea* butterflies. *J. Insect Cons.* **2:** 67 – 78

Fiedler K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). *Bonn. Zool. Monogr.* **31:** 5 – 210

Fiedler K. 2006. Ant-associates of Palearctic lycaenid butterfly larvae (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae) – a review. *Myrmecologische Nachrichten* **9:** 77 – 87

Hammer Ø., Harper D.A.T. and Ryan P.D. 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontol. Electronica* **4:** 1 – 9

Hirschke H. 1905. Eine neue hochalpine Form der *Lycaena alcon* F. *Jber. Wien. ent. Ver.* **15 (1904):** 9 – 11

Hölldobler B. and Wilson E.O. 1990. *The Ants.* Springer-Verlag, Berlin. 732 pp

Höttinger H., Schlick-Steiner B.C. and Steiner F.M. 2003. The Alcon blue *Maculinea alcon* (Lepidoptera: Lycaenidae) in eastern Austria: status and conservation measures. *Ekologia (Bratislava)* **22:** 107 – 118

IUCN 2007. 2007 IUCN Red List of Threatened Species. www.iucnredlist.org, accessed on 11 March 2008

Kolev Z. 2002. The species of *Maculinea* van Eecke, 1915 in Bulgaria: distribution, state of knowledge and conservation status (Lycaenidae). *Nota lepid.* **25:** 177 – 190

Kudrna O. and Belicek J. 2005. On the "Wiener Verzeichnis" its authorship and the butterflies named therein. *Oedippus* **23:** 1 – 32

Martin L.A. and Pullin A.S. 2004. Host-plant specialisation and habitat restriction in an endangered insect, *Lycaena dispar batavus* (Lepidoptera: Lycaenidae) I. Larval feeding and oviposition preferences. *Eur. J. Entomol.* **101:** 51 – 56

Meyer-Hozak C. 2002. Population biology of *Maculinea rebeli* (Lepidoptera: Lycaenidae) on the chalk grassland of Eastern Westphalia (Germany) and implications for conservation. *J. Insect Cons.* **4:** 63 – 72

Munguira M.L. and Martín J. (Eds) 1999. *Action Plan for the Maculinea butterflies in Europe.* Nature and Environment, No 97. Council of Europe Publishing, Strasbourg. 64 pp

Musche M., Anton C., Worgan A. and Settele J. 2006. No experimental evidence for host ant related oviposition in a parasitic butterfly. *J. Insect Behav.* **19:** 631 – 643

Nash D.R., Als T.D., Maile R., Jones G.R. and Boomsma J.J. 2008. A mosaic of chemical coevolution in a large blue butterfly. *Science* **319:** 88 – 90

Pech P., Fric Z., Konvička M. and Zrav J. 2004. Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae) based on morphological and ecological characters: evolution of parasitic myrmecophily. *Cladistics* **20:** 362 – 375

Pech P., Fric Z. and Konvička M. 2007. Species-Specificity of the *Phengaris* (*Maculinea*) – *Myrmica* Host System: Fact or myth? (Lepidoptera: Lycaenidae; Hymenoptera: Formicidae). *Sociobiology* **50:** 983 – 1003

Pecsenye K., Bereczki J., Tihanyi B., Tóth A., Peregovits L. and Varga Z. 2007. Genetic differentiation among the *Maculinea* species (Lepidoptera: Lycaenidae) in eastern Central Europe. *Biol. J. Linn. Soc.* **91:** 11 – 21

Pierce N.E., Braby M.F., Heath A., Lohman D.J., Mathew J., Rand D.B. and Travassos M.A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol.* **47:** 733 – 771

Radchenko A., Elmes G.W., Czechowska W., Stankiewicz A., Czechowski W. and Sielezniew M. 2003. First records of *Myrmica vandeli* Bondroit and *M. tulinae* Elmes, Radchenko et Aktaç (Hymenoptera: Formicidae) for Poland, with a key for the *scabrinodis*- and *sabuleti*-complexes. *Fragm. Faun.* **46:** 47 – 57

Schlick-Steiner B.C., Steiner F.M., Höttinger H., Nikiforov A., Mistrik R., Schafellner C., Baier P. and Christian E. 2004. A butterfly's chemical key to various ant forts: intersection-odour or aggregate-odour multi-host mimicry? *Naturwissenschaften* **91:** 209 – 214

Schmidt D.J. and Hughes J.M. 2006. Genetic affinities among subspecies of a widespread Australian lycaenid butterfly, *Ogyris amaryllis* (Hewitson). *Austral. J. Zool.* **54:** 429 – 446

Schönrogge K., Wardlaw J.C., Peters A.J., Everett S., Thomas J.A. and Elmes G.W. 2004. Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. *J. Chem. Ecol.* **30:** 91 – 107

Seifert B. 1988. A taxonomic revision of the *Myrmica* species of Europe, Asia Minor and Caucasica (Hymenoptera, Formicidae). *Abh. Ber. Naturkundemus. Görlitz* **62:** 1 – 75

Settele J., Kühn E. and Thomas J.A. (Eds) 2005. *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient:* Maculinea *Butterflies as a Model.* Pensoft, Sofia. 289 pp

Settele J., Thomas J.A., Boomsma J., Kuehn E., Nash D., Anton C., Woyciechowski M. and Varga Z. 2002. MACulinea butterflies of the habitats directive and European red list as indicators and tools for conservation and MANagment (MacMan). *Verhandlungen der Gesellschaft für Ökologie* **32:** 63

Sibatani A., Saigusa T. and Hirowatari T. 1994. The genus *Maculinea* van Eecke, 1915 (Lepidoptera: Lycaenidae) from the East Palaearctic Region. *Tyô to Ga* **44:** 157 – 220

Sielezniew M. and Stankiewicz A. 2002. First data on host-ant specificity of parasitic butterfly *Maculinea alcon* (Den. & Schiff.) (Lepidoptera: Lycaenidae) in Poland and eastern Europe. *Fragm. Faun.* **45:** 123 – 130

Sielezniew M. and Stankiewicz A. 2004a. *Gentiana cruciata* as an additional host plant of *Maculinea alcon* on a site in eastern Poland (Lycaenidae). *Nota lepid.* **27:** 91 – 93

Sielezniew M. and Stankiewicz A.M. 2004b. Simultaneous exploitation of *Myrmica vandeli* and *M. scabrinodis* (Hymenoptera: Formicidae) colonies by the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae). *Eur. J. Entomol.* **101:** 693 – 696

Śliwińska E.B., Nowicki P., Nash D.R., Witek M., Settele J. and Woyciechowski M. 2006. Morphology of caterpillars and pupae of European *Maculinea* species (Lepidoptera: Lycaenidae). *Entomol. Fenn.* **17:** 351 – 358

Stankiewicz A.M. and Sielezniew M. 2005. *Maculinea alcon* and *M. rebeli* in Poland: distribution, habitats, host ant specificity and parasitoids. In: *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient:* Maculinea *Butterflies as a Model* (Settele J., Kühn E. and Thomas J.A., Eds), Pensoft, Sofia. pp 65 – 68

Stankiewicz A.M., Sielezniew M. and Švitra G. 2005. *Myrmica schencki* (Hymenoptera: Formicidae) rears *Maculinea rebeli* (Lepidoptera: Lycaenidae) in Lithuania: new evidence for geographical variation of host-ant specificity of an endangered butterfly. *Myrmecologische Nachrichten* **7:** 51 – 54

Steiner F.M., Schlick-Steiner B.C., Höttinger H., Nikiforov A., Moder K. and Christian E. 2006. *Maculinea alcon* and *M. rebeli* (Insecta: Lepidoptera: Lycaenidae) – one or two alcon blues? Larval cuticular compounds and egg morphology of East Austrian populations. *Ann. Naturhist. Mus. Wien.* **107B:** 165 – 180

Steiner F.M., Sielezniew M., Schlick-Steiner B.C., Höttinger H., Stankiewicz A. and Górnicki A. 2003. Host specificity revisited: New data on *Myrmica* host ants of the Lycaenid butterfly *Maculinea rebeli*. *J. Insect Cons.* **7:** 1 – 6

Tartally A. 2004. Is *Manica rubida* (Hymenoptera: Formicidae) a potential host of the *Maculinea alcon* (Lepidoptera: Lycaenidae) group? *Myrmecologische Nachrichten* **6:** 23 – 27

Tartally A. 2005a. Accelerated development of *Maculinea rebeli* larvae under artificial conditions (Lycaenidae). *Nota lepid.* **27:** 303 – 308

Tartally A. 2005b. *Myrmica salina* (Hymenoptera: Formicidae) as a host of *Maculinea alcon* (Lepidoptera: Lycaenidae). *Sociobiology* **46:** 39 – 43

Tartally A. and Csősz S. 2004. Adatok a magyarországi *Maculinea* fajok (Lepidoptera: Lycaenidae) hangyagazdáiról. [Data on the ant hosts of the *Maculinea* butterflies (Lepidoptera: Lycaenidae) of Hungary.] *Term. véd. Közlem.* **11:** 309 – 317

Thomas J.A. and Elmes G.W. 1998. Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. *Ecol. Entomol.* **23:** 457 – 464

Thomas J.A., Elmes G.W., Schönrogge K., Simcox D.J. and Settele J. 2005. Primary hosts, secondary hosts and 'non-hosts': common confusions in the interpretation of host specificity in *Maculinea* butterflies and other social parasites of ants. In: *Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient:* Maculinea *Butterflies as a Model* (Settele J., Kühn E. and Thomas J.A., Eds), Pensoft, Sofia. pp 99 – 104

Thomas J.A., Elmes G.W. and Wardlaw J.C. 1993. Contest competition among *Maculinea rebeli* butterfly larvae in ant nests. *Ecol. Entomol.* **18:** 73 – 76.

Thomas J.A., Elmes G.W. and Wardlaw J.C. 1998. Polymorphic growth in larvae of the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond. B* **265:** 1895 – 1901

Thomas J.A., Elmes G.W., Wardlaw J.C. and Woyciechowski M. 1989. Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia* **79:** 452 – 457

Thomas J.A. and Settele J. 2004. Butterfly mimics of ants. *Nature* **432:** 283 – 284

Thomas J.A. and Wardlaw J.C. 1990. The effect of queen ants on the survival of *Maculinea arion* larvae in *Myrmica* ant nests. *Oecologia* **85:** 87 – 91

Thompson J.N. 1999. Specific hypotheses on the geographic mosaic of coevolution. *Am. Nat.* **153:** S1 – S14

Van Swaay C.A.M. and Warren M. 1999. *Red Data Book of European Butterflies (Rhopalocera).* Nature and Environment, No. 99. Council of Europe Publishing, Strasbourg. 260 pp

Vályi Nagy M. and Csősz S. 2007. Host ant specificity of the Large Blue butterfly, *Maculinea alcon* (Denis & Schiffermüller, 1775), in the Carpathian Basin (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae). *Myrmecological News* **10:** 124