



**Myrmecophily of *Maculinea* butterflies in the  
Carpathian Basin (Lepidoptera: Lycaenidae)**

**A *Maculinea* boglárkalepkék mirmekofíliája a Kárpát-  
medencében (Lepidoptera: Lycaenidae)**

PhD Thesis

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Tanúsítom, hogy Tartally András doktorjelölt 2001-2005 között a fent megnevezett Doktori Iskola Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javaslom.

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Dr. Varga Zoltán  
egyetemi tanár





**In memory of my grandparents**



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## **1. Introduction**

### **1.1. Myrmecophily of *Maculinea* butterflies**

Myrmecophily (close association with ants) is a well-known phenomenon in several insect taxa (e.g. HÖLLDOBLER & WILSON 1990). The lycaenid butterflies are particularly interesting in this respect, because the caterpillars of most studied species are associated with ants. Facultative and obligate myrmecophily and the entire range from mutualism to parasitism can be observed among these butterflies (e.g. FIEDLER 1991, 2001, PIERCE & al. 2002).

The genus *Maculinea* is known as the best-known example of obligate parasitism, where after feeding on a host plant for the first three larval instars, the fourth instar caterpillar must complete its development in a host ant nest (e.g. THOMAS & al. 1989; see Chapter 2.2.2, Appendix VI: 1-8).

### **1.2. Why is it important to know the local host ant species?**

*Maculinea* butterflies are often scored as globally endangered (e.g. MUNGUIRA & MARTÍN 1999, VAN SWAAY & WARREN 1999, SETTELE & al. 2002, THOMAS & SETTELE 2004, SETTELE & al. 2005, IUCN 2006; their populations are declining also in the Carpathian Basin: BÁLINT 1991, 1993). Knowledge of the host ant species has been shown to be crucial for the protection of these endangered butterflies (e.g. MUNGUIRA & MARTÍN 1999, SETTELE & al. 2005, IUCN 2006) because the availability of host ants is usually more limiting to *Maculinea* populations than that of the food plants (e.g. ELMES & THOMAS 1992). Furthermore, the identification of the local host ant species can unravel the evolution of this type of parasitic interaction (e.g. ELMES & al. 1998, ALS & al. 2004).

In their seminal work, THOMAS & al. (1989) found that each European *Maculinea* butterfly depends on a single host ant species, at least in a single habitat. Subsequent studies have refined this by demonstrating that host-ant specificity varies between regions and, in addition, multiple host ant use was observed in some

regions (e.g. ELMES & al. 1994, ALS & al. 2002, STANKIEWICZ & SIELEZNIEW 2002, STEINER & al. 2003). Therefore data should be collected over the geographical range of *Maculinea* butterflies.

### **1.3. The aim of this study**

The aim of this study is to give a review (according to Papers I-V) on the host ant use of *Maculinea* populations in the Carpathian Basin. Such knowledge has been missing so far despite a recent book with a detailed chapter on lycaenid butterflies of this region (BÁLINT 1996) and despite the increasing number of local myrmecologists (see e.g. SZABÓ-PATAY 1910, SOMFAI 1959, ANDRÁSFALVY 1961, GALLÉ 1991, FENYŐSINÉ-HARTNER 1994, VÖRÖS 1995, GALLÉ & al. 1998, CSÖSZ 2001, MARKÓ & al. 2006, NAGY (& al.) 2007). Fortunately, the MacMan project on *Maculinea* butterflies (SETTELE & al. 2002, MacMan 2007; see also Acknowledgements, Appendix VI: 21) gave the possibility to fill this hiatus (TARTALLY & CSÖSZ 2004, TARTALLY & VARGA 2005, VÁLYI NAGY & CSÖSZ 2007; Papers I-V).

I supposed that the host ant use of *Maculinea* populations in the Carpathian Basin is more similar to the host ant use found in central than in western Europe (see e.g. SIELEZNIEW & STANKIEWICZ 2002, STANKIEWICZ & SIELEZNIEW 2002, HÖTTINGER & al. 2003, STEINER & al. 2003, BUSZKO & al. 2005, WITEK & al. 2005; vs. THOMAS & al. 1989, ELMES & al. 1998, MUNGUIRA & MARTÍN 1999), and expected that these data would be suitable for testing some formerly published predictions on host ant specificity (see e.g. THOMAS & al. 1989, ELMES & al. 1998, ALS & al. 2002, STEINER & al. 2003, SETTELE & al. 2005).

The mentioning of other taxa connected with the *Maculinea-Myrmica* associations recorded during my surveys might also be interesting because (as far as I know) no data on their host ant use or, what is more, on their occurrence in the Carpathian Basin were available before.

## 2. Materials and Methods

### 2.1. Taxonomy and nomenclature

#### 2.1.1. *Glaucopsyche*, *Maculinea* or *Phengaris*?

Although *Glaucopsyche* SCUDDER, 1872 is often used as generic name also for *Maculinea* VAN EECKE, 1915 species (e.g. SETTELE & al. 1995, Fauna Europaea 2007), I will not use this name for *Maculinea* species because it contradicts with the recent results of phylogenetic analyses (ALS & al. 2004, PECH & al. 2004). According to the formal aspects of priority rule, the generic name *Phengaris* DOHERTY, 1891 should be used (see e.g. FRIC & al. 2007, PECH & al. 2007). However, the International Commission on Zoological Nomenclature (ICZN 2007) has not decided to suppress *Maculinea* by *Phengaris*. Therefore, for simplicity I am following here the most frequently used and established generic name (viz. *Maculinea*).

#### 2.1.2. Separation of Alcon Blues

For a long time Alcon Blues were subdivided traditionally into two species (the Alcon Blue *Maculinea alcon* and the Mountain Alcon Blue *M. rebeli*) based on their different ecological and physiological adaptations, including their life history, use of host plants and host ants (e.g. THOMAS & al. 1989, SCHÖNRÖGGE & al. 2000, SIELEZNIEW & STANKIEWICZ 2007; see also Chapter 4, Paper V). However, recent studies clearly showed that the level of differentiation between these two traditionally subdivided Alcon Blues is lower than expected at the species level (Als & al. 2004, PECH & al. 2004, STEINER & al. 2006, FRIC & al. 2007). BERECZKI & al. (2005, 2006) and PECSENYE & al. (2007) also did not support the differentiation of these two butterflies and questioned the validity of several Alcon Blue forms and subspecies known from and around the Carpathian Basin (for a review: BÁLINT 1996, BERECZKI & al. 2006). Moreover, according to the ICZN (2007), the name “*Maculinea alcon* var. *rebeli* (HIRSCHKE, 1904)” is not available

as valid species name because it refers to an elevational form which was erroneously transferred into the European populations connected with *Gentiana cruciata* initial host plant (KUDRNA & BELICEK 2005). On the other hand, the ecological differentiation of *M. 'rebeli'* from *M. alcon* is important as they were proposed as separate units for conservation because of their different habitats, host plants and host ants (e.g. ELMES & al. 1998, MUNGUIRA & MARTÍN 1999, THOMAS & SETTELE 2004, SETTELE & al. 2005; see also Chapter 2.2.1, 4, Paper V).

According to these, the name *Maculinea rebeli* will be written using single quotes in the followings (as *M. 'rebeli'*) as a “technical name”. *M. 'rebeli'* is used for specimens that developed on *Gentiana cruciata* and *M. alcon* for those that developed on *G. pneumonanthe* initial host plant. However, it should be noted that recent observations 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 & STANKIEWICZ 2004a) and it was found also on some studied sites (see Paper V: Table 1, Appendix III; see also Chapter 2.2.1, 4). According to these, “developed on an initial host plant” practically means in the case of laboratory experiments that the caterpillars were dropped from the plant (Chapter 4.2) but in the case of field experiments it regards to specimens found in patches occupied by the plant (Chapter 4.1).

### 2.1.3. Technical comments on some terms

- “**(*Maculinea*) butterflies**” is used instead of “(*Maculinea*) species” when they include *M. 'rebeli'* because this is not a valid species (see Chapter 2.1.2).
- “**Caterpillar**” is used instead of “(butterfly) larva” to avoid confusion with the larvae of other insects discussed.
- “**Important host**” is used instead of “primary host” because the meaning of “primary host” is often not clear in the available literature and the host ant usage is more complicated in the Carpathian Basin than in western Europe (see Chapters 3, 4). So, defining the “primary host” of a here studied



population would be often bold and I would not like to make more confusion about the meaning of this term. Although “important host” is quite a subjective word, it is usually clear from the results if a host is “important” or not for a population.

- For the clarity of the discussion, “***Maculinea* specimens**” is used instead of “*Maculinea* caterpillars and/or pupae and/or exuvia” found in ant nests.
- To be easy to handle, the **full taxonomic names** are given in Appendix II and not at the first mentioning.
- The names of **four genera start with “M”** (*Maculinea*, *Manica*, *Microdon* and *Myrmica*), the genera of the different species can be checked in Appendix II.

## 2.2. The biology of studied butterflies

### 2.2.1. Host plants

*Maculinea* caterpillars start their life feeding on the developing seeds of specific food plants. *M. teleius* and *M. nausithous* feed on *Sanguisorba officinalis*. *M. arion* uses different *Thymus* species and *Origanum vulgare*. The Alcon Blues develop on different gentian species, while *M. ‘rebeli’* primarily feeds on *Gentiana cruciata*, the ‘classic’ host plants of *M. alcon* are *G. pneumonanthe* and *G. asclepiadea* (e.g. MUNGUIRA & MARTÍN 1999). However, it should be noted that both Alcon Blues use other gentians as well and their host plant specificity overlaps in some regions (see e.g. MUNGUIRA & MARTÍN 1999, KOLEV 2002, SIELEZNIEW & STANKIEWICZ 2004a; see the host plant use of the here studied populations in Chapter 4.1.1, Paper V: Table 1, Appendix III).

### 2.2.2. Myrmecophily

In their last, fourth, larval instar *Maculinea* caterpillars leave the initial host plant and soon must be adopted by a suitable host ant colony (e.g. THOMAS & al. 1989). For social integration into ant nests, the caterpillars mimic the acoustic (DEVRIES & al. 1993) and especially the chemical signals of the host ants (AKINO & al. 1999, ELMES & al. 2002, SCHLICK-STEINER & al. 2004, SCHÖNROGGE & al. 2004).

Ants from only two genera are recorded as hosts of these butterflies. The vast majority of *Maculinea* use hosts from the genus *Myrmica* (e.g. THOMAS & al. 1989, ALS & al. 2002, FIEDLER 2006) but *Maculinea teleius* and especially *M. arionides* have also been recorded using *Aphaenogaster japonica* in Japan (YAMAGUCHI 1988, PIERCE & al. 2002). Consequently, only *Myrmica* species have been recorded as *Maculinea* hosts from Europe (see Appendix V for a review on the important host ants in Europe; see ALS & al. 2004, FIEDLER 2006 and PECH & al. 2007 for a review on the recorded host ant species; host ant specificity of the here studied populations is discussed in Chapters 3, 4 and in Paper I-V).

After entering the ant nests, *Maculinea* caterpillars can exploit the ant colonies in two ways (THOMAS & ELMES 1998). Predacious caterpillars are feeding on the ant brood while cuckoo caterpillars are fed by the worker ants by trophallaxis (but in certain cases the latter ones can also be predators of ant brood; see Chapter 4.2.2, Paper VII). *M. alcon* and *M. 'rebeli'* have cuckoo but *M. arion*, *M. teleius* and *M. nausithous* have predacious caterpillars (THOMAS & ELMES 1998). Though, caterpillars of *M. nausithous* appear to show some cuckoo characters as well (FIEDLER 1990, THOMAS & ELMES 1998, STANKIEWICZ & SIELEZNIEW 2002, THOMAS & SETTELE 2004; see also Chapter 3.2). The feeding type of caterpillars is important for the biology of *Maculinea* populations (THOMAS & ELMES 1998) because predacious caterpillars are subject to scramble competition (i.e. numbers get killed off and just a few big ones survive; THOMAS & WARDLAW 1992) and less likely to survive in high densities than cuckoo caterpillars which suffer from

contest competition (i.e. more but smaller ones survive; THOMAS & al. 1993). According to these significant differences, my results on predacious and cuckoo caterpillars are discussed in two separated chapters (Chapter 3, 4).

### **2.3. Field surveys and determination**

To understand the host ant usage of *Maculinea* butterflies, 1589 *Myrmica* nests were searched in 33 sites in the Carpathian Basin (30 in Hungary and 3 in Transylvania; see Appendix III, IV) between 2000 and 2007. Sites were selected from all *Maculinea* sites known in the two regions that held stable populations. Nests were searched exclusively within a 2 m radius circle around randomly selected host plants, this is the approximate foraging range of workers of the *Myrmica* genus (ELMES & al. 1998). Nests found were carefully opened and checked for *Maculinea* specimens. After excavation the ground and vegetation were restored as close to the original conditions as possible. The number of *Myrmica* nests found varied greatly among the sites, which resulted in unbalanced sample sizes. I restricted searches to reduce disturbance in some of the sites (SETTELE & al. 2005), all of which are of high conservation value and most are 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, Csősz 1999, RADCHENKO & al. 2003; the few questioned samples were revised by S. Csősz; Hungarian Natural History Museum, Budapest). When *Maculinea* specimens were found in a nest, I recorded their number and determined the species using a 20x magnifier lens and an unpublished, earlier version of the key published by ŚLIWIŃSKA & al. (2006).

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

around winter because of starving: ELMES & al. 2004, SCHÖNROGGE & al. 2004, THOMAS & al. 2005; A. Tartally, pers. observ.). However, on some sites investigations were completed not before the flying but before the pupation period since *M. teleius* and *M. nausithous* co-occurred there (see Appendix III for such sites). Pupae of these two butterflies are rather similar (ŚLIWIŃSKA & al. 2006) which can cause the confusion of these two syntopic species in pupal stage. Fortunately the caterpillars of *M. teleius* and *M. nausithous* can be easily distinguished from each other (ŚLIWIŃSKA & al. 2006). The few *M. teleius* and/or *M. nausithous* pupae found on such sites were reared to adulthood (as in Chapter 2.5) for determination or were determined by their allozyme patterns (V. Mester, J. Bereczki & K. Pecsénye, pers. comm.; University of Debrecen).

Some *Maculinea* pupae found were obviously parasitized by ichneumon wasps (Chapter 5.1, Appendix VI: 8, 11). These pupae were moved to the laboratory and were cultured there (as in Chapter 2.5) up to the hatching of the wasps. The adult wasps (*Ichneumon eumerus*: Appendix VI: 10; and *Neotypus melanocephalus*: Appendix VI: 9) were sent to K. Horstmann (Theodor-Boveri-Institut für Biowissenschaften, Würzburg) for determination.

Larvae and pupae of a myrmecophilous hoverfly, *Microdon myrmicae* (Chapter 5.2, Appendix VI: 13, 14), were often found in *Myrmica* nests investigated. Samples of these larvae and pupae were sent to K. Schönrogge (CEH, Wallingford) and S. Bonelli (University of Turin) for determination.

*Myrmica* ants were often covered with a myrmecophilous fungus, *Rickia wasmannii* (Chapter 5.3, Appendix VI: 15). Photos and SEM photos of this fungus were sent to M. Blackwell (Louisiana State University, Baton Rouge) and to X. Espadaler (Universitat Autònoma de Barcelona) for determination.

Voucher samples of the here discussed insects and the fungus are deposited in the Hymenoptera Collection of the Hungarian Natural History Museum (Budapest) and in my collection.

## **2.4. Host ant specificity**

Host ant specificity can be defined in many different ways (THOMAS & al. 2005; see Paper V). In my disquisition the heterogeneity of the number of *Maculinea* specimens between nests of different species was compared using two methods. First, a Fisher exact test was used (by SISA-Tables 2007) to compare the observed number of infected nests with the number expected if they were infected at random. Second, a Chi-square statistic was computed to compare the number of *Maculinea* specimens observed with the number expected, based on the number of nests available. The significance of this was tested by reassigning each nest (and its associated number of *Maculinea* specimens) randomly to one of the *Myrmica* species observed at a site 100 000 times (using the software program MacSamp: NASH 2007), with the constraint that the total number of nests of each species was the same as that observed. 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 is low, except for those cases in which many ant nests have been investigated.

## **2.5. Laboratory experiments**

For several trials (Chapter 4.2), caterpillars of *M.alcon* and *M. 'rebeli'* were adopted by artificial ant colonies (based on WARDLAW 1991, WARDLAW & al. 1998). To drop caterpillars from gentians, shoots of *G. pneumonanthe* with eggs of *M.alcon* and shoots of *G. cruciata* with eggs of *M. 'rebeli'* were collected in the fields (maximum 10 shoots from 10 strong plants per site from conservational reasons) and then cultured in the laboratory. Gentians were kept in glasses of water placed in plastic basins, and could be kept fresh for 2-3 weeks while *Maculinea* caterpillars emerged. Fourth instar caterpillars were collected using a fine brush as they dropped from the flowers in the evenings, and were transferred straight into the foraging arena of an artificial ant nest to be adopted by the ants. Before

introduction, the length of each caterpillar was measured with a ruler. Caterpillars were remeasured one week (for Chapter 4.2.1) or one month (for Chapter 4.2.2) later by putting the ruler onto the glass that covered the artificial nests. This allowed recording the survival and growth of the caterpillars while causing the minimum of disturbance.

The artificial ant colonies were kept in unheated artificial nests (made from clay and glass) joined by silicon tubes to plastic arenas (Appendix VI: 16). These nests were not covered to exclude the light, but were kept in places that never received direct sunlight. The laboratory was not air-conditioned in the summer, but was heated in the colder seasons. The temperature that the ant nests experienced was less variable than under natural conditions, and was often up to 25° C in the warmer periods. A part of the nest area was always kept wet by a cotton wool strand that connected the clay with water. To feed the ants, the arenas of the nests were always provided with a cube of sugar, and various insects (mainly cut-up mealworms, larvae and pupae) as well as granules of a dry diet (given in the Appendix of Paper VI) at a minimum of once a week. The cube of sugar provided continuous food while the dry diet provided the proteins (and maybe essential vitamins and minerals) when there were not enough insects to feed the ant colonies.

When a butterfly caterpillar pupated, it was removed from the ants using a pair of fine forceps and placed in a plastic box with ventilation holes and a moist sponge pad at the bottom. The separation was thought to be important because the ants damage the eclosed butterflies if they are not able to escape from a closed artificial nest and if they are not discovered and separated in time (ELFFERICH 1988).

I tried to do similar experiments with caterpillars of *M. arion*, *M. nausithous* and *M. teleius* but all of these caterpillars died soon in the artificial nests (moreover, even in the artificial colonies of important host ants recorded on the fields). It is difficult to culture predatory caterpillars in the laboratory (THOMAS & WARDLAW 1990; J.C. Wardlaw & J.A. Thomas, pers. comm.).

### 3. The host ant use of predatory *Maculinea* butterflies

#### 3.1. The host ant use of *M. teleius*

(This chapter is based on Paper I)

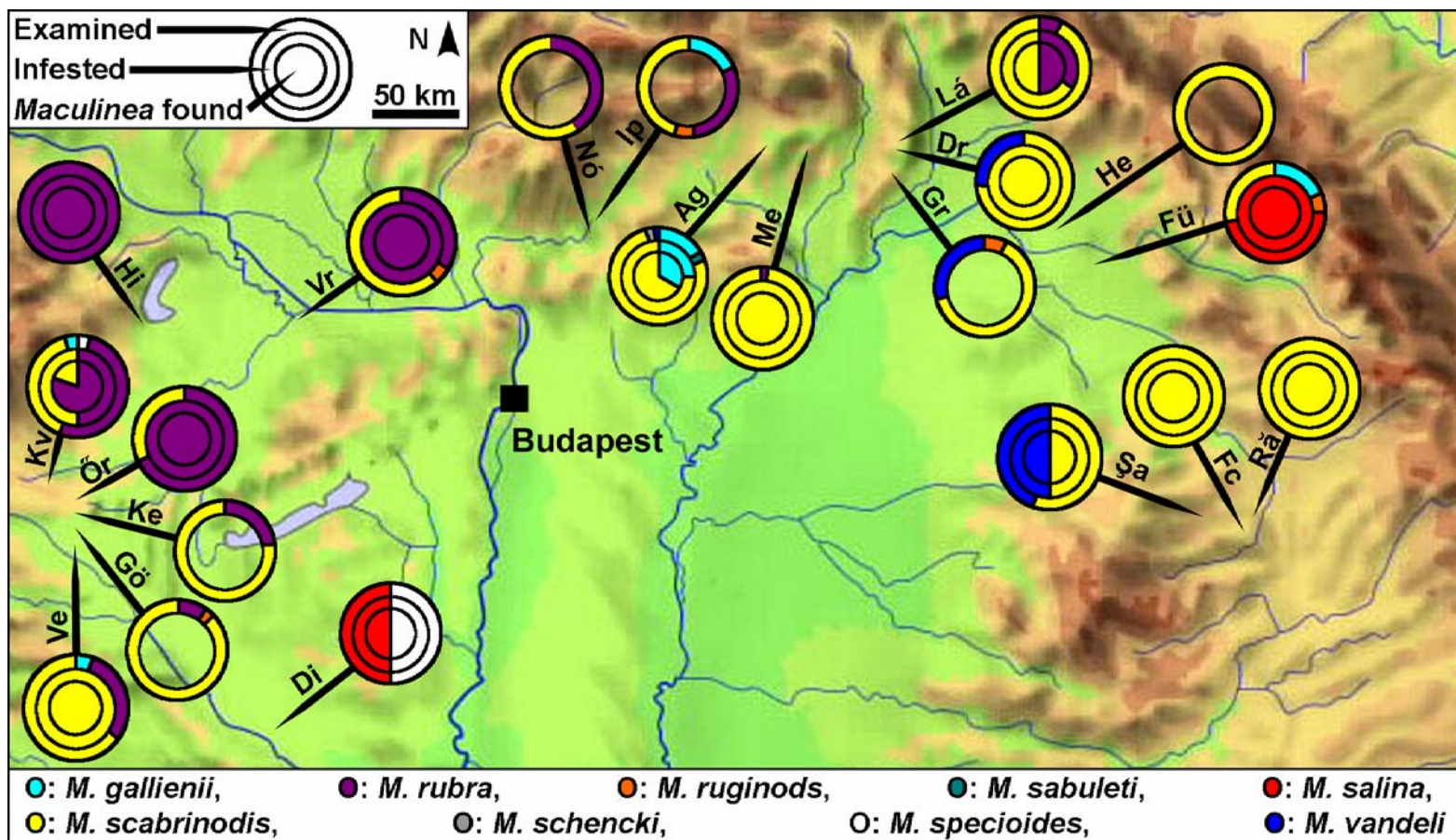
##### 3.1.1. Sites investigated

The host ant use of *M. teleius* was investigated in 20 sites (see Table 1, Fig. 1, Appendix III, IV). Three of these were in Romania Transylvania: Fânațele Clujului (Szénafüvek) Răscruci (Válaszút) and Șardu (Magyarsárd); and 17 in Hungary (Aggtelek, Drahos-rét, Drávaiványi, Fülesd, Gödörháza, Gyertyánkúti-rétek, Hetefejércse, Hidegség, Ipolytarnóc, Kercaszomor, Kétvölgy, Lászlótanya, Meszes, Nógrádszakál, Óriszentpéter, Velemér, Vörös-rét).

##### 3.1.2. Results

A total of 856 nests of nine *Myrmica* species (*M. gallienii*, n = 31; *M. rubra*, n = 92; *M. ruginodis*, n = 7; *M. sabuleti*, n = 2; *M. salina*, n = 16; *M. scabrinodis*, n = 659; *M. schencki*, n = 2; *M. specioides*, n = 2; *M. vandeli*, n = 45) were investigated at the 20 sites (Table 1). In total 114 *M. teleius* specimens were found in 63 nests of six *Myrmica* species (*M. gallienii*, *M. rubra*, *M. salina*, *M. scabrinodis*, *M. specioides* and *M. vandeli*) in 14 sites. Nests of *M. ruginodis*, *M. sabuleti* and *M. schencki* were not found to be infested. Overall, two thirds of the *Myrmica* species examined were used as hosts, and 7.4% of all nests were infested (Table 1).

Five *Myrmica* nests also contained caterpillars of other *Maculinea* species besides *M. teleius*: One *M. rubra* nest also contained caterpillars of *M. nausithous* (Kétvölgy: 8 *M. teleius* + 28 *M. nausithous*; see also Chapter 3.2.1, Paper II: Fig. 5), one *M. scabrinodis* nest likewise contained caterpillars of *M. nausithous* (Răscruci: 1 *M. teleius* + 1 *M. nausithous*, see also Chapter 3.2.2, Paper III) and three *M. scabrinodis* nests contained caterpillars of *M.alcon*, too (Răscruci: 1 *M. teleius* + 1 *M.alcon*, 1 *M. teleius* + 1 *M.alcon*, 1 *M. teleius* + 5 *M.alcon*; see also Chapter 3.2.2, Paper III).



**Fig. 1.** The proportion of nests of different *Myrmica* species examined and infested by *M. teleius* and the proportion of *M. teleius* specimens found in the nests of different *Myrmica* species in the Carpathian Basin (see also Table 1; background map: ZENTAI 1996).



Site (code on Fig 1) <i>p1; p2</i>	<i>Myrmica</i>	No. nests	No. infested	No. nau.	Me. ex.	Me. inf. (range)
Aggtelek (Ag) 0.759; 0.568	<i>M. gallienii</i>	17	2 (11.8 %)	7	0.41	3.5 (1-6)
	<i>M. sabuleti</i>	2				
	<i>M. scabrinodis</i>	78	6 (7.7 %)	15	0.19	2.5 (1-4)
	<i>M. schencki</i>	2				
	<i>M. vandeli</i>	1				
Drahos-rét (Dr) 1.00; 0.602	<i>M. scabrinodis</i>	53	2 (3.8 %)	2	0.04	1.0
	<i>M. vandeli</i>	19				
Drávaiványi (Di)	<i>M. salina</i>	1	1 (100.0 %)	1	1.00	1.0
	<i>M. specioides</i>	1	1 (100.0 %)	1	1.00	1.0
Fánafele Clujului (Fc)	<i>M. scabrinodis</i>	58	1 (1.7 %)	1	0.02	1.0
Fülesd (Fü) 0.410; 0.494	<i>M. gallienii</i>	6				
	<i>M. ruginodis</i>	2				
	<i>M. salina</i>	15	3 (20.0 %)	5	0.33	1.7 (1-3)
	<i>M. scabrinodis</i>	9				
Gödörháza (Gö)	<i>M. rubra</i>	4				
	<i>M. ruginodis</i>	1				
	<i>M. scabrinodis</i>	36				
Gyertyánkúti-rétek (Gr)	<i>M. ruginodis</i>	1				
	<i>M. scabrinodis</i>	9				
	<i>M. vandeli</i>	4				
Hetefejércse (He)	<i>M. scabrinodis</i>	5				
Hidegség (Hi)	<i>M. rubra</i>	8	3 (37.5 %)	17	2.13	5.7 (1-8)
Ipolytarnóc (Ip)	<i>M. gallienii</i>	6				
	<i>M. rubra</i>	10				
	<i>M. ruginodis</i>	2				
	<i>M. scabrinodis</i>	15				
Kercaszomor (Ke)	<i>M. rubra</i>	11				
	<i>M. scabrinodis</i>	37				
Kétvölgy (Kv) 1.00; 1.00	<i>M. gallienii</i>	1				
	<i>M. rubra</i>	14	1 (7.1 %)	8	0.57	8.0
	<i>M. scabrinodis</i>	11	1 (9.1 %)	2	0.18	2.0
	<i>M. specioides</i>	1				
Lászlótanya (Lá) 0.263; 0.075	<i>M. rubra</i>	2	1 (50.0 %)	2	1.00	2.0
	<i>M. scabrinodis</i>	27	2 (7.4 %)	2	0.07	1.0
Meszes (Me) 1.00; 1.00	<i>M. rubra</i>	2				
	<i>M. scabrinodis</i>	200	21 (10.5 %)	31	0.16	1.5 (1-4)
Nógrádszakál (Nó)	<i>M. rubra</i>	7				
	<i>M. scabrinodis</i>	10				
Őriszentpéter (Őr) 1.00; 1.00	<i>M. rubra</i>	21	1 (4.8 %)	1	0.05	1.0
	<i>M. scabrinodis</i>	10				

Site (code on Fig 1) <i>p1; p2</i>	<i>Myrmica</i>	No. nests	No. infested	No. nau.	Me. ex.	Me. inf. (range)
Răscruți (Ră)	<i>M. scabrinodis</i>	49	12 (24.5 %)	14	0.29	1.2 (1-2)
Șardu (Șa) <i>1.00; 1.00</i>	<i>M. scabrinodis</i>	26	1 (3.8 %)	1	0.04	1.0
	<i>M. vandeli</i>	21	1 (4.8 %)	1	0.05	1.0
Velemér (Ve) <i>1.00; 1.00</i>	<i>M. gallienii</i>	1				
	<i>M. rubra</i>	6				
	<i>M. scabrinodis</i>	13	1 (7.7 %)	1	0.08	1.0
Vörös-rét (Vr) <i>0.195; 0.193</i>	<i>M. rubra</i>	7	2 (28.6 %)	2	0.29	1.0
	<i>M. ruginodis</i>	1				
	<i>M. scabrinodis</i>	13				

**Table 1.** The recorded host ant usage of *M. teleius* in the Carpathian Basin (see also Fig. 1, Appendix III, V). No. nests: the number of *Myrmica* nests examined within 2 m from *Sanguisorba officinalis*; No. infested: the number (and percentage) of nests infested with *M. teleius*; No. tel.: the total number of *M. teleius* specimens in nests; Me. ex.: the mean number of *M. teleius* specimens found in examined nests; Me. inf.: the mean number (and range) of *M. teleius* found in infested nests. Two measures of host specificity are given (where the calculation was possible): p1 is the 2-tailed probability from the Fisher exact test of heterogeneity in infection of host ant nests, p2 is the probability from a randomization test of ant nests between species (see Chapter 2.4).

### 3.1.3. Discussion

To our knowledge, this is the first study to focus directly on the host ant use of *M. teleius* in south-eastern Central Europe (although there are some results initially published in papers concentrating on other *Maculinea* species: TARTALLY & CSÖSZ 2004, VÁLYI NAGY & CSÖSZ 2007; Paper II-IV; see also BATÁRY & al. 2007). While the number of infested nests found in a site (Table 1) is usually too small to draw strong conclusions about host ant specificity, there are some general patterns.

*M. teleius* was found with six *Myrmica* species (*M. gallienii*, *M. rubra*, *M. salina*, *M. scabrinodis*, *M. specioides* and *M. vandeli*) therefore this butterfly appears not to have strict host ant specificity in the Carpathian Basin. However, it can be contrasted with the strict host ant specificity (to *M. scabrinodis*) initially found in some French and Polish sites (THOMAS & al. 1989, ELMES & al. 1998).

Later these original results from Poland were supplemented with more data (STANKIEWICZ & SIELEZNIEW 2002, BUSZKO & al. 2005, WITEK & al. 2005) and it is now clear that the host ant use of *M. teleius* is more complex there (see Paper I: Table 1).

If we follow the criteria (see ALS & al. 2004: Supplementary Table 10) that ‘primary hosts’ raise more than 10% of specimens in a population, it could be concluded that all the recorded host ant species would be ‘primary hosts’ in the different sites (Fig. 1, Table 1). Nevertheless, we should be careful with this statement because the number of *M. teleius* specimens recorded in a site was usually too low (more than 10 would be needed) for such a calculation (Table 1). Combining our data (see Paper I), we found only two ant species in the Carpathian Basin which reared more than 10% of *M. teleius* specimens found across the investigated sites (*M. rubra*, 26.3%; *M. scabrinodis*, 60.5%). This is not surprising because these two *Myrmica* species were the most often recorded host ants (see Table 1, Paper I).

There were differences in the mean numbers of *M. teleius* specimens found in the nests of the different *Myrmica* species when the data were combined (see Paper I: Fig. 2), and this number was significantly higher for *M. rubra* than for *M. scabrinodis* when only these two major hosts were examined (see more details in Paper I). Interestingly, *M. rubra* appeared to be more suitable in the western sites while *M. scabrinodis* proved to be more important in the eastern sites (Fig. 1, Table 1). This phenomenon is simple to explain since *M. rubra* is usually rare or missing from the eastern sites (Fig. 1, Table 1). Moreover, this ant usually forms larger colonies than *M. scabrinodis* (SEIFERT 1988) and therefore an average *M. rubra* nest can rear more *Maculinea* caterpillars (e.g. the infested *M. rubra* nest at Kétvölgy contained not only eight *M. teleius* but also 28 *M. nausithous* caterpillars which is an exceptionally high number of predatory *Maculinea* caterpillars within the same nest; see also Chapter 3.2.1, Paper II). Thus, it is not surprising that *M. rubra* was significantly more suitable for *M. teleius* than *M. scabrinodis* (see Paper I).

In Poland, *M. scabrinodis* was regarded as the primary host ant of *M. teleius* for a long time (THOMAS & al. 1989, ELMES & al. 1998) but recent results show that *M. rubra*, if common on a site, is often a significantly more suitable host of *M. teleius* there (STANKIEWICZ & SIELEZNIEW 2002, see also BUSZKO & al. 2005, WITEK & al. 2005). The fact that *M. gallienii* was found to be an important host ant of *M. teleius* on sites in both countries is another similarity between Poland and Hungary (at Kosyń in Poland: STANKIEWICZ & SIELEZNIEW 2002; at Aggtelek in Hungary: Fig. 1, Table 1). However, there were four Hungarian sites where *M. gallienii* was also recorded (Fig. 1, Table) but was not found to be infested.

The situation seems to be different in the case of *M. salina*. This species was recorded in two Hungarian sites and it reared *M. teleius* at both of them (Fig. 1, Table 1). Interestingly, *M. salina* was the only recorded host ant at Fülesd, where nests of *M. scabrinodis* and *M. gallienii* (both of which are hosts in NE-Hungary, Fig. 1, Table 1) were not found to be infested. It would be of peculiar interest to obtain more data from this site to investigate whether there is a similarly strong adaptation of *M. teleius* to *M. salina* as was found to be the case for *M.alcon* on the same site (see Paper IV). One nest of *M. speciooides* (from two nests in two sites) and another of *M. vandeli* (from 45 nests in four sites) were also found to be infested by *M. teleius* (Fig. 1, Table 1). *M. vandeli* has already been recorded as a host of *M. teleius* (one nest with one specimen from France or Poland: THOMAS & al. 1989, ELMES & al. 1998), however, as far as we know, *M. speciooides* and *M. salina* have not been recorded as hosts of *M. teleius* outside Hungary (for a review: ALS & al. 2004: Supplementary Table 10, FIEDLER 2006: Digital Supplementary Material, PECH & al. 2007).

The three *Myrmica* species not infested were among the rarest species in the survey (there were only seven *M. ruginodis*, two *M. sabuleti* and two *M. schencki* nests recorded in total; Table 1), which is consistent with the possibility that this is due to the low level of “sampling” of these species, but this effect may also be of importance for the butterflies themselves, if these *Myrmica* species are consistently rare. This is easily imaginable because there were no significant deviations from

random distribution amongst ant species when the heterogeneity of the number of *M. teleius* specimens in the nests of different *Myrmica* species was compared in the different sites (see Table 1, Paper I).

It requires further investigation to determine if the differences between the host ant use of *M. teleius* on different sites simply reflects host ant availability, whether there is a coevolutionary mosaic of adaptation to local hosts (*sensu* THOMPSON 1999), or some other explanations (see THOMAS & al. 2005; see more details in Chapter 6.1). Moreover, it would be useful to get information on the host ant specificity of *M. teleius* from further western Central European regions (not only from France; THOMAS & al. 1989, ELMES & al. 1998) and so determine if the strength of host ant specificity is generally higher there than it was found in eastern Central Europe (in Poland and Hungary: THOMAS & al. 1989, ELMES & al. 1998, STANKIEWICZ & SIELEZNIEW 2002, BUSZKO & al. 2005, WITEK & al. 2005; Table 1, Paper I) and in Northern Mongolia (WOYCIECHOWSKI & al. 2006).



### 3.2. The host ant use of *M. nausithous*

*M. nausithous* occurs only in two rather separated regions of the Carpathian Basin (in western Hungary and Transylvania; BÁLINT 1996; Appendix III) and the ecological conditions of the known sites on these two regions are quite different (see Appendix III). The results on the host ant use of *M. nausithous* are discussed therefore in two separated subchapters.

#### 3.2.1. Results on western Hungarian *M. nausithous* populations

(This subchapter is based on Paper II)

##### 3.2.1.1. Sites investigated

Seven sites of *M. nausithous* were investigated in western Hungary (Gödörháza, Hidegség, Kercaszomor, Kétvölgy, Óriszentpéter, Velemér and Vörös-rét; see Table 2, Fig. 2, Appendix III, IV). All are marshy meadows with a profusion of the host plant *Sanguisorba officinalis*.

##### 3.2.1.2. Results

A total of 196 nests of five *Myrmica* species (*M. gallienii*, n = 2; *M. rubra*, n = 71; *M. ruginodis*, n = 2; *M. scabrinodis*, n = 120; *M. speciosoides*, n = 1) were found at the seven sites in western Hungary, and 58 overwintered *M. nausithous* caterpillars were found in nine *Myrmica rubra* nests in three of these sites (Kétvölgy, Gödörháza and Hidegség) (Table 2, Fig. 2). The caterpillars found were apparently in good general condition (see Paper II: Fig. 4, 5, Appendix VI: 5).

Caterpillars of *M. teleius* were also found in *M. rubra* nests during our survey (see Chapter 3.1.2), which is not surprising since *M. rubra* is a common host ant of both butterflies (for a review: ALS & al. 2004, FIEDLER 2006). There was a nest infested by the caterpillars of both butterflies (in huge numbers: 28 *M. nausithous* and eight *M. teleius* caterpillars; see Paper II: Fig. 5).

### 3.2.1.3. Discussion

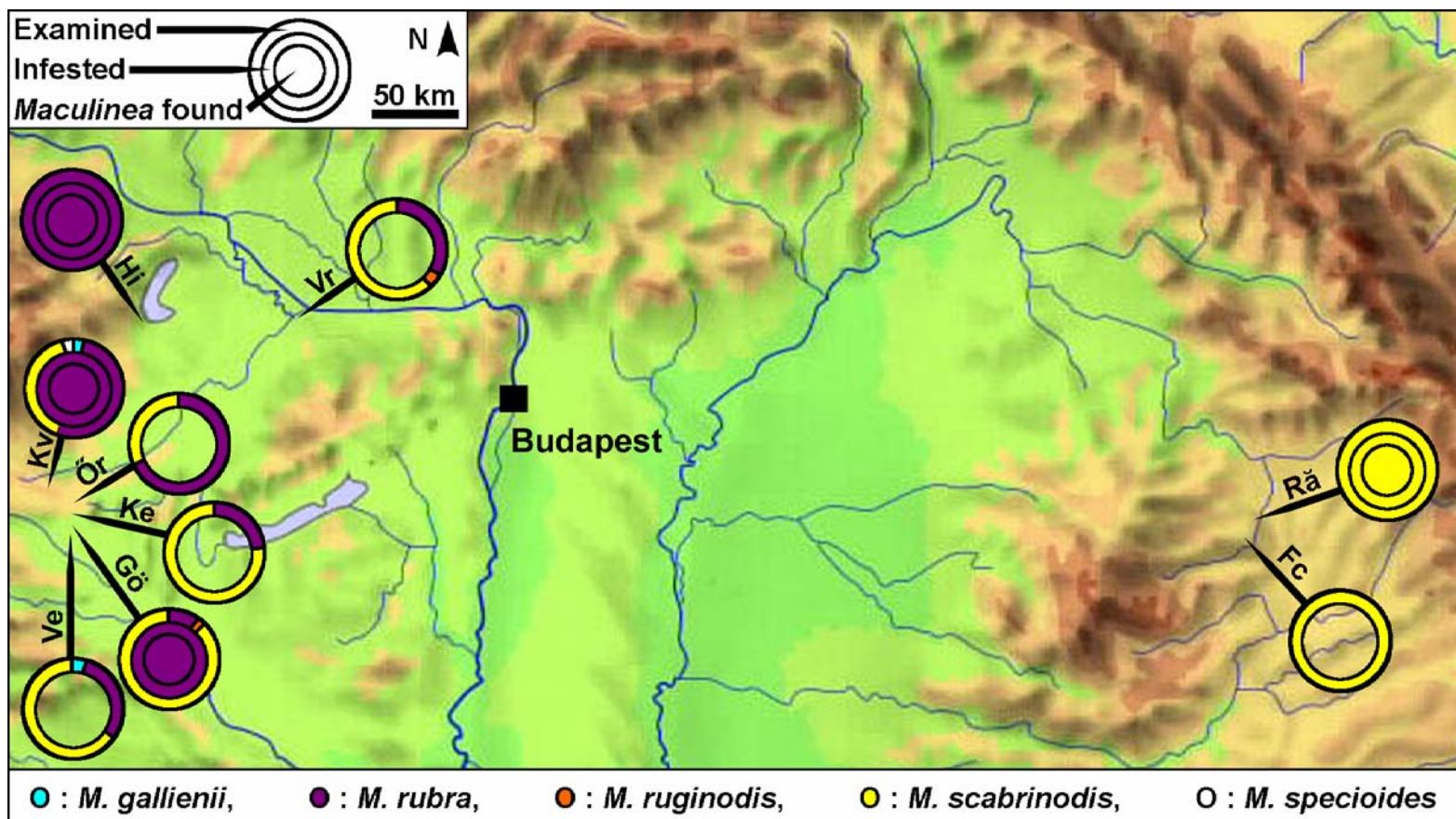
According to these results, we consider that *M. rubra* is likely to be the main host of *M. nausithous* in the Hungarian sites investigated as in most of the previously investigated sites elsewhere (THOMAS & al. 1989, ELMES & al. 1998, KORB 1998, STANKIEWICZ & SIELEZNIEW 2002; M. Witek, pers. comm.; Appendix V). However, it is important to note that our fieldwork in the Őrség region was done in late May but the western Hungarian *M. nausithous* populations start to fly in mid or late July depending on site and year (Z. Varga, pers. observ.). In spite of the relatively early sampling, the nests of the general host *M. rubra* repeatedly contained overwintered *M. nausithous* caterpillars at Gödörháza and Kétvölgy but other *Myrmica* species did not (Table 2, Fig. 2). Similarly, although only one *M. nausithous* caterpillar was found at the Fertő region at Hidegség, this caterpillar was in the prepupal stage and living in a *M. rubra* nest. No *Myrmica* species other than *M. rubra* were found there during our work (Table 2, Fig. 2). These facts reflect the suitability of *M. rubra* as the host for *M. nausithous* at Hidegség. Accordingly, our results definitely support the earlier suggestions that *M. rubra* is the main host of *M. nausithous*, at least in most of this species' western range. The fact that one of the infested *M. rubra* nests contained 28 overwintered *M. nausithous* caterpillars (and eight *M. teleius* caterpillars, see Chapter 3.1.2, Paper II: Fig. 5) also confirms this statement since it is a huge number of parasitizing butterfly caterpillars for a predatory *Maculinea* species (THOMAS & ELMES 1998). This huge number of overwintered caterpillars within one nest appears to support the idea that the caterpillars of *M. nausithous* are possibly intermediate between the cuckoo and the predatory life forms of caterpillars (FIEDLER 1990, THOMAS & ELMES 1998, STANKIEWICZ & SIELEZNIEW 2002, THOMAS & SETTELE 2004). However, the question of the potential for cuckoo behaviour of *M. nausithous* caterpillars still needs thorough investigation in the laboratory. Another explanation of this result could be that these 28 *M. nausithous* caterpillars were found in a large nest of a polydomous *M. rubra* colony (A. Tartally, pers. observ.) which may have had the capacity to rear several caterpillars.



Moreover, because of the relatively early sampling there is no evidence that all of these 28 *M. nausithous* caterpillars were able to finish their development.

*Myrmica scabrinodis* has also been recorded as a host of *M. nausithous* in Spain and Transylvania (see MUNGUIRA & MARTÍN 1999; Chapter 3.2.2). Hosts of *M. nausithous* other than *M. rubra* and *M. scabrinodis* have not been recorded anywhere (see ALS & al. 2004: Supplementary Table 10). During our work none of the 120 *M. scabrinodis* colonies that were searched in western Hungary contained caterpillars of *M. nausithous* in contrast to the host nests of *M. rubra* (Table 2). The number of *Myrmica gallienii*, *M. ruginodis* and *M. specioides* nests that were examined was too small to establish their suitability for being a host of *M. nausithous* in western Hungary. However, we suppose that they cannot serve as important *M. nausithous* hosts in the study sites since their nests were found only in small numbers there. Moreover, several other *Myrmica* species were also formerly recorded from both of the regions investigated in western Hungary – from the Őrség region: *M. sabuleti*, *M. salina* and *M. schencki*; from the Fertő region: *M. microrubra*, *M. gallienii*, *M. sabuleti*, *M. salina*, *M. schencki*, *M. scabrinodis*, and *M. specioides*; according to CSÖSZ & al. (2002) – but we did not find any specimens of these during the surveys. Knowledge of *Myrmica* species distribution and further investigations on these potential *Maculinea* hosts are crucial to studies in the Őrség region because four species of *Maculinea* (*M.alcon*, *M. nausithous*, *M. teleius*, and *M. arion*) co-exist within this region, and in some cases within the same locality (BÁLINT 1996, A. Ambrus pers. comm., Z. Varga pers. observ.; see Appendix III).

It would be desirable to collect more data about the host specificity of *M. nausithous* in western Hungary, because our results suggest that *M. rubra* frequently occurs in *S. officinalis* sites there unlike in eastern Hungary (see Fig. 1). It also appears that this ant species occurs in eastern Hungary in the adjacent marshy forests of the *S. officinalis* sites rather than on the meadows (A. Tartally, pers. observ.). An explanation for the absence of *M. nausithous* from eastern



**Fig. 2.** The proportion of nests of different *Myrmica* species examined and infested by *M. nausithous* and the proportion of *M. nausithous* specimens found in the nests of different *Myrmica* species in the Carpathian Basin (see also Table 2; background map: ZENTAI 1996).

Site (code on Fig 2) <i>p1; p2</i>	<i>Myrmica</i>	No. nests	No. infested	No. nau.	Me. ex.	Me. inf. (range)
<b>western Hungary:</b>						
Gödörháza (Gö) <i>&lt; 0.001; &lt; 0.001</i>	<i>M. rubra</i>	4	4 (100.0 %)	14	3.50	3.5 (2-6)
	<i>M. ruginodis</i>	1				
	<i>M. scabrinodis</i>	36				
Hidegség (Hi)	<i>M. rubra</i>	8	1 (12.5 %)	1	0.13	1.0
Kercaszomor (Ke)	<i>M. rubra</i>	11				
	<i>M. scabrinodis</i>	37				
Kétvölgy (Kv) <i>0.355; 0.233</i>	<i>M. gallienii</i>	1				
	<i>M. rubra</i>	14	4 (28.6 %)	43	3.07	10.8 (3-28)
	<i>M. scabrinodis</i>	11				
	<i>M. specioides</i>	1				
Őriszentpéter (Őr)	<i>M. rubra</i>	21				
	<i>M. scabrinodis</i>	10				
Velemér (Ve)	<i>M. gallienii</i>	1				
	<i>M. rubra</i>	6				
	<i>M. scabrinodis</i>	13				
Vörös-rét (Vr)	<i>M. rubra</i>	7				
	<i>M. ruginodis</i>	1				
	<i>M. scabrinodis</i>	13				
<b>Transylvania:</b>						
Fânațele Clujului (Fc)	<i>M. scabrinodis</i>	58				
Răscruți (Ră)	<i>M. scabrinodis</i>	49	2 (4.1 %)	2	0.04	1.0

**Table 2.** The recorded host ant usage of *M. nausithous* in the Carpathian Basin (see also Fig. 2, Appendix III, V). No. nests: the number of *Myrmica* nests examined within 2 m from *Sanguisorba officinalis*; No. infested: the number (and percentage) of nests infested with *M. nausithous*; No. nau.: the total number of *M. nausithous* specimens in nests; Me. ex.: the mean number of *M. nausithous* specimens found in examined nests; Me. inf.: the mean number (and range) of *M. nausithous* found in infested nests. Two measures of host specificity are given (where the calculation was possible): p1 is the 2-tailed probability from the Fisher exact test of heterogeneity in infection of host ant nests, p2 is the probability from a randomization test of ant nests between species (see Chapter 2.4).

Hungary could be that its host ant is usually not present in the *S. officinalis* sites there (see Fig. 1). However, to answer these questions a better knowledge of the host specificity of *M. nausithous* and a thorough knowledge of the distribution of the host ant species are necessary (see also Chapter 3.2.2).

### 3.2.2. Results on isolated Transylvanian populations

(This subchapter is based on Paper III)

#### 3.2.2.1. Sites investigated

Only two *M. nausithous* sites are known from Transylvania. Both of these are in the Câmpia Transilvaniei (Mezőség) region, near Cluj-Napoca (Kolozsvár): one at Răscruți (Válaszút), another at Fânațele Clujului (Szénafüvek) (see Table 2, Fig. 2, Appendix III, IV; see also RÁKOSY & LÁSZLÓFFY 1997). Both sites are semi-dry meadows with steppe character, with sporadic small boggy depressions (see Paper III: Fig. 2). *S. officinalis*, the host plant, occurs in a mosaic in these small depressions, creating potential metapopulational networks of *M. nausithous* subpopulations (e.g. HANSKI 1999). Both known sites were investigated in this study, but it should be noted that the Câmpia Transilvaniei region is rather poorly studied, so that occurrence of other, as yet undiscovered, *M. nausithous* sites in the area is likely.

#### 3.2.2.2. Results

A total of 107 *Myrmica* nests were found at the two sites (58 at Fânațele Clujului and 49 at Răscruți), and went over for *Maculinea* caterpillars. All 107 nests proved to be *M. scabrinodis*. Two nests from Răscruți were infested by *M. nausithous*, both of them containing only a single *M. nausithous* caterpillar (Table 2, Fig. 2).

Caterpillars of *M. alcon* and *M. teleius* were also found in *M. scabrinodis* nests during our survey (see Chapter 3.1.2, 4.1.2; Paper I, V), which is not surprising since *M. scabrinodis* is a common host ant of both butterflies (for a review: ELMES & al. 1998, ALS & al. 2004, FIEDLER 2006). One of the two nests infested by *M. nausithous* also contained a *M. teleius* caterpillar (see also Chapter 3.1.2).

### 3.2.2.3. Discussion

To our knowledge, this is the first study to provide data on the host ant use of *M. nausithous* in Transylvania. The use of *M. scabrinodis* as a host ant by *M. nausithous* is, on the one hand, not surprising, since this was the only *Myrmica* ant species found in the vicinity of the initial larval host plant; while on the other hand being highly unusual, as this butterfly is found almost exclusively in nests of *Myrmica rubra* in other parts of its range (THOMAS & al. 1989, ELMES & al. 1998, KORB 1998, STANKIEWICZ & SIELEZNIEW 2002; M. Witek, pers. comm.; Table 2, Fig. 2, Chapter 3.2.1, Appendix V). Although *Myrmica rubra* occurs in Transylvania, where it is connected with damp forested habitats in the eastern part of the Carpathian Basin, this ant is not known from the sites investigated here, despite extensive surveys by local myrmecologists (B. Markó, pers. comm.). Other *Myrmica* species (*M. hellenica*, *M. sabuleti*, *M. schencki* and *M. specioides*) have been recorded from the drier patches (MARKÓ 1998, MARKÓ & CSÖSZ 2001; B. Markó, pers. comm.; see Table 4: Răscruci ‘dry’), but only *M. scabrinodis* is known from the boggy depressions where *M. nausithous* can lay eggs on *S. officinalis*. THOMAS & al. (2005) provide some warnings and guidelines about recording host ant use in *Maculinea* butterflies, and although the sample of infested nests that we found was small, we believe that the comprehensive survey that we made of the *Myrmica* fauna on the investigated sites means that these records represent genuine specialization.

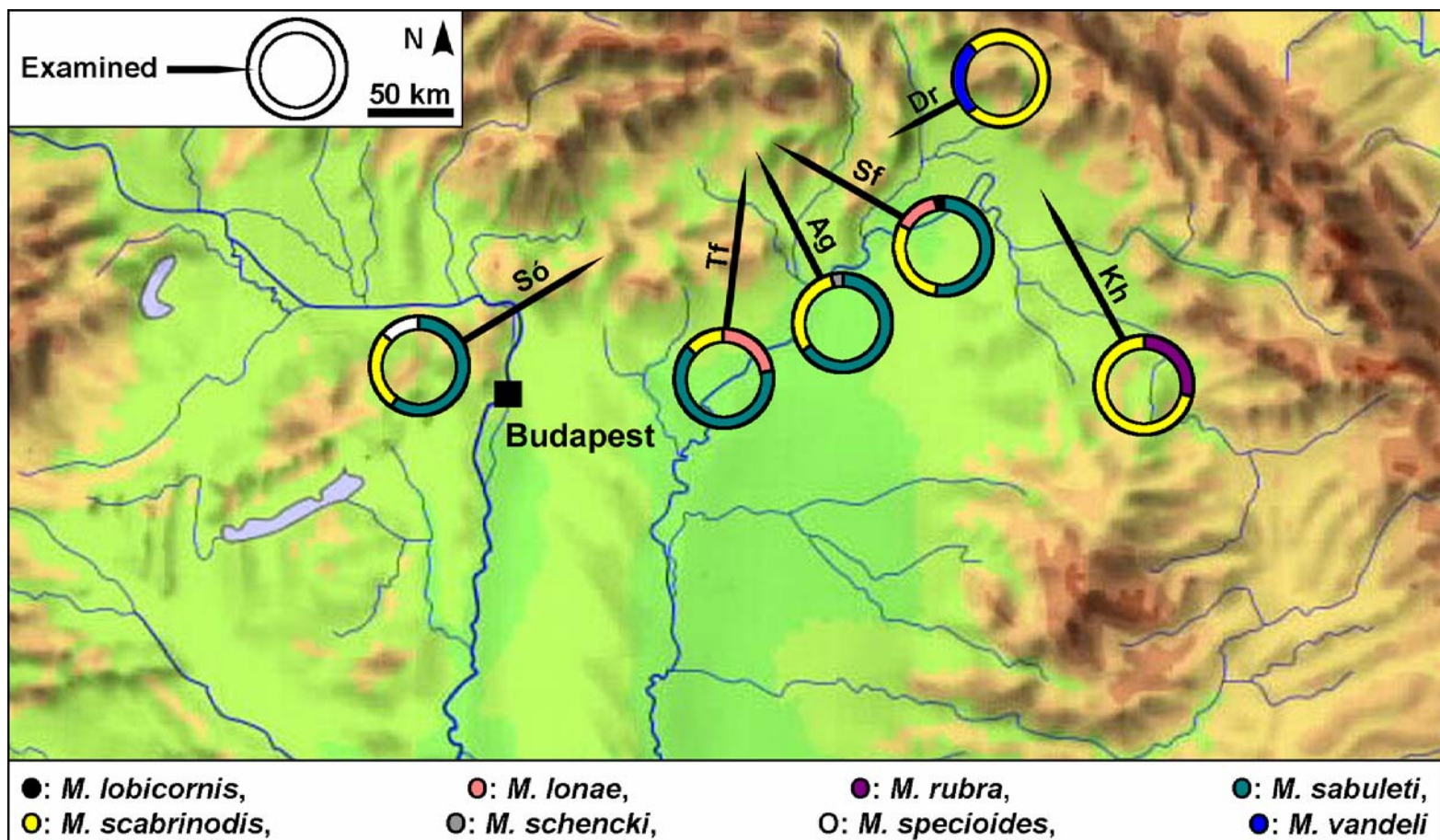
The rate of parasitism of *M. scabrinodis* nests that we found was low (1.9 % of nests investigated overall, 4.1% of nests at Răscruci), which is an order of magnitude lower than parasitism rates previously recorded for *M. nausithous* (STANKIEWICZ & SIELEZNIEW 2002; Chapter 3.2.1; Mean parasitism rate of other studies = 44.9 %; GLM with Binomial Errors:  $\chi^2 = 56.79$ , d.f. = 3,  $p < 0.0001$ ). If the *M. nausithous* populations on these sites persist as a local metapopulation, then high variance in parasitism rates between sub-populations might be expected, so the significance of the low parasitism rate awaits further investigation.

Our records are not the first of *M. nausithous* exploiting *M. scabrinodis*, since MUNGUIRA & MARTÍN (1999) reported this ant as a *M. nausithous* host from Spain. However, apart from this single record, this widespread *Myrmica* species has not been recorded as a host of *M. nausithous* on the other European sites studied (although *M. scabrinodis* is often common on those sites), where *M. rubra* is used exclusively (THOMAS & al. 1989, ELMES & al. 1998, KORB 1998, STANKIEWICZ & SIELEZNIEW 2002; Chapter 3.2.1; see Paper III: Fig. 1, Appendix V). Interestingly *M. nausithous* occurs only in western parts of Hungary where *M. rubra* is common on *S. officinalis* sites, but this butterfly does not occur in central and eastern parts of Hungary where *M. rubra* is rare or missing from such sites. However, *M. scabrinodis* is common in most of the Hungarian *S. officinalis* sites investigated (see Fig. 1, Table 1, Appendix III). Hence, it is an open question why the eastern Hungarian *S. officinalis* sites are not colonised by *M. nausithous* from Transylvania that uses *M. scabrinodis* (see also Chapter 3.2.1). One reason could be the high mountains of Muntii Apuseni that inhibit spread to eastern Hungary for the isolated Transylvanian *M. nausithous* populations as a barrier. Another possible explanation is that *M. teleius* and *M. alcon* populations are in competition with *M. nausithous* in eastern Hungary through their common use of *M. scabrinodis* (see Chapter 3.1, 4.1). Interestingly *M. nausithous* does not occur at Şardu (see Appendix III, IV) where a potential *M. nausithous* site is known near to the Câmpia Transilvaniei region, with high densities of *S. officinalis* and *M. scabrinodis* (see Fig. 1, 4, Table 1, 4). This site is, however, used by *M. teleius* and *M. alcon* (both butterflies exploit *M. scabrinodis* and *M. vandeli* for host ant; Chapter 3.1, 4.1), and appears more similar to the central and western European *M. nausithous* sites (with bushy forest edges; see Appendix VI: 20) than the sites investigated in the Câmpia Transilvaniei region (which are meadows with some isolated bushes; see Paper III: Fig. 2).

### 3.3. The potential host ants of *M. arion*

The host ant specificity of *M. arion* was investigated on six sites (Aggtelek, Drahos-rét, Kaszonyi-hegy, Sósartyán, Szilicei-fennsík and Teresztenyei-fennsík; see Fig. 3, Table 3, Appendix III, IV) and 184 nests of eight *Myrmica* species (*M. lobicornis*, n = 1; *M. linae*, n = 11; *M. rubra*, n = 6; *M. sabuleti*, n = 65; *M. scabrinodis*, n = 91; *M. schencki*, n = 1; *M. specioides*, n = 3; *M. vandeli*, n = 19) were found on these (Fig. 3, Table 3). However, no infested nests were found during the investigations and the eight *Myrmica* species found can only be treated as recorded potential host ant species. It appears that investigating the host ant specificity of *M. arion* is the most difficult among the European *Maculinea* butterflies (see also SIELEZNIEW & al. 2005). *M. sabuleti* was the commonest *Myrmica* species in most sites (Fig. 3, Table 3) and this is the main host ant of *M. arion* in western Europe (THOMAS & al. 1989, ELMES & al. 1998; see Appendix V). This ant was not recorded from the Drahos-rét and the Kaszonyi-hegy but *M. scabrinodis*, the additional host ant in western Europe (THOMAS & al. 1989, ELMES & al. 1998), was relatively common there (Fig. 3, Table 3). The third known host ant of *M. arion* is *M. lobicornis* (Poland, one pupa: SIELEZNIEW & al. 2003, SIELEZNIEW & al. 2005) and one nest of this ant was also found during the investigations (at Szilicei-fennsík; Fig. 3, Table 3). Other host ant species of *M. arion* than *M. sabuleti*, *M. scabrinodis* or *M. lobicornis* have not been recorded anywhere else (for a review: ALS & al. 2004: Supplementary Table 10, FIEDLER 2006: Digital Supplementary Material).





**Fig. 3.** The proportion of nests of different *Myrmica* species examined in *M. arion* sites in the Carpathian Basin (see also Table 3; background map: ZENTAI 1996).



Site (code on Fig 3)	Flying period	Host plant	<i>Myrmica</i>	No. nests
Aggtelek (Ag)	M07-M08	Tpu, Ovu	<i>M. sabuleti</i>	17
			<i>M. scabrinodis</i>	8
			<i>M. schencki</i>	1
Drahos-rét (Dr)	L7	Tpu	<i>M. scabrinodis</i>	53
			<i>M. vandeli</i>	19
Kaszonyi-hegy (Kh)	M07-M08	Ovu	<i>M. rubra</i>	6
			<i>M. scabrinodis</i>	15
Sóshartyán (Só)	M05-M06	Tma, Tpa	<i>M. sabuleti</i>	13
			<i>M. scabrinodis</i>	6
			<i>M. specioides</i>	3
Szilicei-fennsík (Sf)	M05-L06	Tma, Tpa	<i>M. lobicornis</i>	1
			<i>M. lonae</i>	6
			<i>M. sabuleti</i>	21
			<i>M. scabrinodis</i>	12
Teresztenyei-fennsík (Tf)	M05-L06, L07	Tma, Tpa, Ovu	<i>M. lonae</i>	5
			<i>M. sabuleti</i>	14
			<i>M. scabrinodis</i>	3

**Table 3.** The host plants and flying periods of the investigated *M. arion* populations (according to Z. Varga, pers. comm.; L: late, M: mid, Ovu: *Origanum vulgare*, Tma: *Thymus marschallianus*, Tpa: *T. pannonicus*, Tpu: *T. pulegioides*) and the number of *Myrmica* nests (No. nests), as potential host ant colonies, examined within 2 m from the host plants (see also Fig. 3, Appendix III, V).



## 4. The host ant use of cuckoo *Maculinea* butterflies

### 4.1. Field records

(This chapter is based on Paper IV-V)

#### 4.1.1. Sites investigated

The range and level of host specificity is especially relevant in the case of Alcon Blues, as they have been conventionally subdivided into the Alcon Blue *Maculinea alcon* and the Mountain Alcon Blue *M. 'rebeli'* based on their use of different habitats, initial host plant and host ant species. 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 (see Chapter 2.1.2 and Paper V for more details).

According to these facts, the comparison of host ant specificity of *M. alcon* and *M. 'rebeli'* populations in the Carpathian Basin was thought to be especially important, where they are regionally sympatric. Such a comparison has been lacking so far (see ELMES & al. 1994, 1998, ALS & al. 2002, MEYER-HOZAK 2002, SIELEZNIEW & STANKIEWICZ 2002, HÖTTINGER & al. 2003, STEINER & al. 2003).

We studied ant colonies in 15 sites in Hungary and in two sites in Transylvania between 2000 and 2007 (Fig. 4, Table 4, Appendix III, IV). There were 10 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* was studied in 10 sites (Drahos-rét, Fülesd,

Gyertyánkúti-rétek, Gyilkos-rét, Hetefejércse, Kercaszomor, Kétvölgy, Nyikom-rét, Şardu and Tugár-rét), that of *M. 'rebeli'* in six sites (Bükkszentkereszt, Kecskeláb-rét, Kuriszlán, Lófő-tisztás, Nagy-mező and Tohonya-hát) and that of both butterflies on one site (Răscruci) (see Fig. 4, Table 4, Appendix III, IV).

#### 4.1.2. Results

A total of 867 nests of 11 *Myrmica* species (*M. gallienii*, n = 13; *M. lobicornis*, n = 2; *M. lonae*, n = 15; *M. rubra*, n = 25; *M. ruginodis*, n = 16; *M. sabuleti*, n = 85; *M. salina*, n = 45; *M. scabrinodis*, n = 566; *M. schencki*, n = 43; *M. specioides*, n = 12; *M. vandeli*, n = 45) were found in the 17 sites (Fig. 4, Table 4). *M.alcon* or *M. 'rebeli'* specimens were found in 10 % (or 88 nests) of these *Myrmica* ant nests (n = 867) (Table 4). A total of 558 *M.alcon* and 194 *M. 'rebeli'* specimens were found in the ant nests studied (Table 4). *M. scabrinodis*, present in all sites studied, was the only ant species which was used by both Alcon Blues, and thus can be considered as 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 (Fig. 4, Table 4). The rate of parasitism of *M. scabrinodis* nests was significantly higher at *M.alcon* sites than at *M. 'rebeli'* sites (see Paper V).

At five sites, *M. scabrinodis* was the only host ant of *M.alcon* (three *M. scabrinodis* nests at Răscruci contained caterpillars of *M. teleius* too: 1 *M. teleius* + 1 *M.alcon*, 1 *M. teleius* + 1 *M.alcon*, 1 *M. teleius* + 5 *M.alcon* caterpillars; see also Chapter 3.1, 3.2.2). However, *M. vandeli* nests held more *M.alcon* specimens than *M. scabrinodis* nests did in Drahos-rét and Şardu, and *M. salina* nests held more *M.alcon* than *M. scabrinodis* nests in Fülesd (Fig. 4, Table 4). 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 4, Table 4).

When host specificity between species was examined at each site in terms of the distribution of infected and uninfected colonies, only one of the *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 (see Paper V).

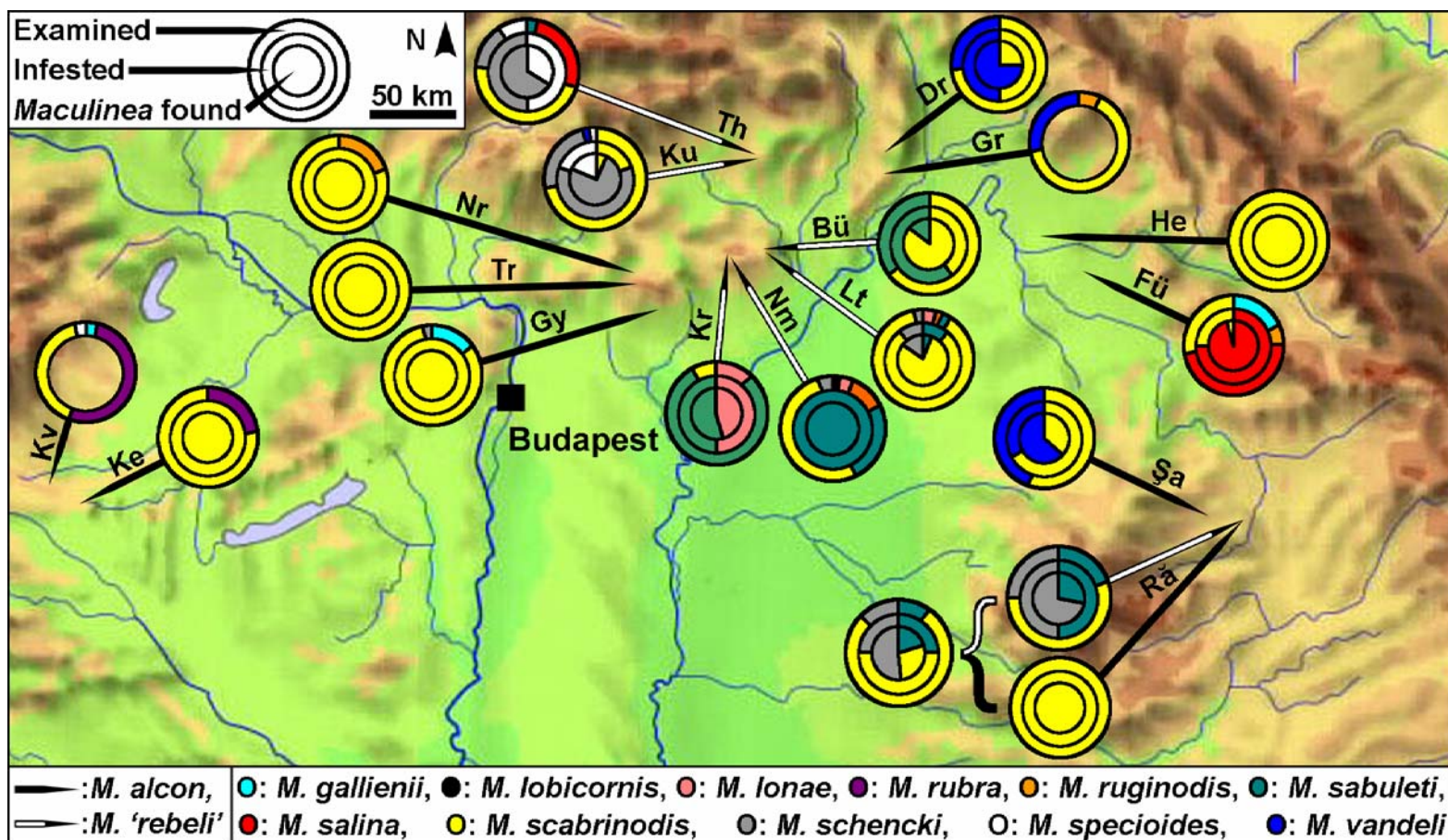
Combining the data for the *M.alcon* and *M. 'rebeli'* areas of Răscruți (Table 4) decreased the significance of the heterogeneity in host use based on the presence or absence of *Maculinea* specimens in nests (as specimens were found in *M. scabrinodis* nests in the 'wet' area of the site), but increased the significance of the heterogeneity in host ant use when numbers of specimens per nest were taken into account (see Paper V for more details). This latter effect is probably primarily due to the increase in power associated with an increased sample size.

More analyses are given in Paper V but these do not contain data from Gyertyánkúti-rétek, Kercaszomor and Kétvölgy.

#### 4.1.3. Discussion

To our knowledge, this is the first study to provide data on the host ant specificity of regionally sympatric populations of *M.alcon* and *M. 'rebeli'* in regions where close genetic similarity of these two butterflies was found (BERECZKI & al. 2005, 2006, PECSENYE & al. 2007; see also Paper V: Fig. 6). 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'*.

Our results show that *M. scabrinodis* is the most important host ant of *M.alcon* in NE-Hungary and Transylvania (Table 4, Fig. 4), which is also the case in central and western Hungary, E-Austria, W-Ukraine, France, Spain and Poland



**Fig. 4.** The proportion of nests of different *Myrmica* species examined and infested by *M. alcon* and/or *M. 'rebeli'* and the proportion of *M. alcon* and/or *M. 'rebeli'* specimens found in the nests of different *Myrmica* species in the Carpathian Basin (see also Table 4; background map: ZENTAI 1996).

Site (code on Fig 4) <i>p1; p2</i>	<i>Myrmica</i>	No. Nests	No. infested	No. a/r	Me. ex.	Me. inf. (range)
<b><i>M. alcon</i>:</b>						
Drahos-rét (Dr)	<i>M. scabrinodis</i>	53	3 (5.7 %)	3	0.06	1.0
0.184; 0.046	<i>M. vandeli</i>	19	3 (15.8 %)	9	0.47	3.0 (1-5)
Fülesd (Fü)	<i>M. gallienii</i>	6				
0.274; 0.239	<i>M. ruginodis</i>	2				
	<i>M. salina</i>	15	6 (40.0 %)	137	9.13	22.8 (3-61)
	<i>M. scabrinodis</i>	9	2 (22.2 %)	3	0.33	1.5 (1-2)
Gyertyánkúti-rétek (Gr)	<i>M. ruginodis</i>	1				
	<i>M. scabrinodis</i>	9				
	<i>M. vandeli</i>	4				
Gyilkos-rét (Gy)	<i>M. gallienii</i>	6				
< 0.001; 0.153	<i>M. scabrinodis</i>	32	23 (71.9 %)	315	9.84	13.7 (1-68)
	<i>M. schencki</i>	1				
Hetefejércse (He)	<i>M. scabrinodis</i>	5	3 (60.0 %)	35	7.00	11.7 (6-20)
Kercaszomor (Ke)	<i>M. rubra</i>	11				
0.561; 0.561	<i>M. scabrinodis</i>	37	4 (10.8 %)	11	0.30	2.8 (1-7)
Kétvölgy (Kv)	<i>M. gallienii</i>	1				
	<i>M. rubra</i>	14				
	<i>M. scabrinodis</i>	11				
	<i>M. specioides</i>	1				
Nyikom-rét (Nr)	<i>M. ruginodis</i>	1				
1.00; 1.00	<i>M. scabrinodis</i>	4	2 (50.0 %)	7	1.75	3.5 (3-4)
Răscruci 'wet' (Ră)	<i>M. scabrinodis</i>	20	4 (20.0 %)	10	0.50	2.5 (1-5)
Şardu (Şa)	<i>M. scabrinodis</i>	26	2 (7.7 %)	4	0.15	2.0 (2-2)
1.00; 0.888	<i>M. vandeli</i>	21	1 (4.8 %)	7	0.33	7.0
Tugár-rét (Tr)	<i>M. scabrinodis</i>	6	4 (66.7 %)	17	2.83	4.3 (1-7)
<b><i>M. 'rebeli'</i>:</b>						
Bükkszentkereszt (Bü)	<i>M. scabrinodis</i>	55	2 (3.6 %)	24	0.44	12.0 (5-19)
0.340; 0.769	<i>M. sabuleti</i>	30	3 (10.0 %)	4	0.13	1.3 (1-2)
Kecskeláb-rét (Kr)	<i>M. lonae</i>	3	1 (33.3 %)	5	1.67	5.0
0.353; 0.353	<i>M. sabuleti</i>	21	1 (4.8 %)	6	0.29	6.0
	<i>M. scabrinodis</i>	2				
Kuriszlán (Kr)	<i>M. scabrinodis</i>	39	1 (2.6 %)	1	0.03	1.0
0.015; 0.045	<i>M. schencki</i>	13	3 (23.1 %)	12	0.92	4.0 (3-5)
	<i>M. specioides</i>	1	1 (100.0 %)	3	3.00	3.0
	<i>M. vandeli</i>	1				

Site (code on Fig 4) <i>p1; p2</i>	<i>Myrmica</i>	No. Nests	No. infested	No. a/r	Me. ex.	Me. inf. (range)
Lófő-tisztás (Lt) <i>0.151; 0.768</i>	<i>M. lonae</i>	8				
	<i>M. ruginodis</i>	2				
	<i>M. sabuleti</i>	5	1 (20.0 %)	5	1.00	5.0
	<i>M. scabrinodis</i>	149	7 (4.7 %)	82	0.55	11.7 (1-33)
	<i>M. schencki</i>	4	1 (25.0 %)	15	3.75	15.0
Nagy-mező (Nm) <i>0.340; 0.325</i>	<i>M. lobicornis</i>	2				
	<i>M. lonae</i>	4				
	<i>M. ruginodis</i>	10				
	<i>M. sabuleti</i>	22	2 (9.1 %)	6	0.27	3.0 (2-4)
	<i>M. scabrinodis</i>	47				
	<i>M. schencki</i>	4				
Răscruți 'dry' (Ră) <i>0.035; 0.142</i>	<i>M. sabuleti</i>	4	2 (50.0 %)	7		
	<i>M. scabrinodis</i>	11				
	<i>M. schencki</i>	5	2 (40.0 %)	18		
Tohonya-hát (Th) <i>0.007; 0.069</i>	<i>M. sabuleti</i>	3				
	<i>M. salina</i>	30				
	<i>M. scabrinodis</i>	51				
	<i>M. schencki</i>	16	2 (12.5 %)	4	0.25	2.0 (2-2)
	<i>M. specioides</i>	10	2 (20.0 %)	2	0.20	1.0
<b>Both:</b>						
Răscruți (Ră) <i>0.090; 0.067</i>	<i>M. sabuleti</i>	4	2 (50.0 %)	7	1.75	3.5 (2-5)
	<i>M. scabrinodis</i>	31	4 (12.9 %)	10	0.32	2.5 (1-5)
	<i>M. schencki</i>	5	2 (40.0 %)	18	3.60	9.0 (3-15)

**Table 4.** The recorded host ant usage of *M. alcon* and/or *M. 'rebeli'* in the Carpathian Basin (see also Fig. 4, Appendix III, V). No. nests: the number of *Myrmica* nests examined within 2 m from *Sanguisorba officinalis*; No. infested: the number (and percentage) of nests infested with *M. alcon* and/or *M. 'rebeli'*; No. a/r.: the total number of *M. alcon* and/or *M. 'rebeli'* specimens in nests; Me. ex.: the mean number of *M. alcon* and/or *M. 'rebeli'* specimens found in examined nests; Me. inf.: the mean number (and range) of *M. alcon* and/or *M. 'rebeli'* found in infested nests. Two measures of host specificity are given (where the calculation was possible): p1 is the 2-tailed probability from the Fisher exact test of heterogeneity in infection of host ant nests, p2 is the probability from a randomization test of ant nests between species (see Chapter 2.4).

(ELMES & al., 1994, 1998, SIELEZNIEW & STANKIEWICZ 2002, HÖTTINGER & al. 2003, SIELEZNIEW & STANKIEWICZ 2004b, TARTALLY & CSÖSZ 2004, VÁLYI NAGY & CSÖSZ 2007; M. Witek, pers. comm.; Table 4, Fig. 4). However, *M. vandeli* and *M. salina* were used rather than *M. scabrinodis* when these two species



were common at a site (Table 4, Fig. 4). *M. vandeli* has previously been reported as a host of *M. alcon* from Poland (SIELEZNIEW & STANKIEWICZ 2004b, STANKIEWICZ & SIELEZNIEW 2005), but *M. salina* is known as a host of *M. alcon* exclusively from Fülesd. Interestingly *M. teleius* was also found only with *M. salina* at this site and was not found with its usual host *M. scabrinodis* there (see Chapter 3.1). The parasitism of *M. salina* rather than *M. scabrinodis* in Fülesd may result from a former bottleneck because this site was used as a rice field in the early 1950s. This may have resulted in a recent local adaptation towards using the more salt-tolerant ant species in a secondarily salinised habitat (see Paper IV).

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 & al. 1989, ELMES & al. 1998, MEYER-HOZAK 2002, STEINER & al. 2003, STANKIEWICZ & al. 2005, Vályi Nagy & Csösz 2007). *M. 'rebeli'* specimens were mostly found in nests of *M. sabuleti*, *M. schencki* and *M. scabrinodis* (Fig. 4, Table 4). *M. sabuleti* is known as the main host ant for *M. 'rebeli'* from Poland, E-Westphalia (Germany) and E-Austria (MEYER-HOZAK 2002, STEINER & al. 2003, STANKIEWICZ & SIELEZNIEW 2005). *M. schencki* has also been recorded as the main host of *M. 'rebeli'* from France, Spain and Lithuania (THOMAS & al. 1989, ELMES & al. 1998, STANKIEWICZ & 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 Bükkzentkereszt and Lófő-tisztás; Fig. 4, Table 4). However, this ant species is known only as a secondary host of *M. 'rebeli'* from Poland and France (THOMAS & al. 1989, ELMES & al. 1998, STEINER & al. 2003). *M. specioides* and *M. lonae* are additional hosts of *M. 'rebeli'* in Hungary (Fig. 4, Table 4). *M. specioides* is also known as an additional host from E-Austria (STEINER & al. 2003) but our study is the first to record *M. lonae* as a host of any *Maculinea* species (see ALS & al. 2004: Supplementary Table 10).

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

species accumulation curves from the two types of habitat (see Paper V: 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 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 (see Paper V: Table 1), so that our 'sampling effort' reflected accurately the situation faced by the butterflies.

It has recently been suggested that *Maculinea* butterflies do not show any host specificity (PECH & al. 2007), however, we have clearly heterogeneous use of host ant species in several of the *M. alcon* and *M. 'rebeli'* populations examined (Fig 4, Table 4, see more details and more statistical proofs in Paper V).

Our analysis of microsatellite markers from a small number of individuals collected from areas of the site at Răscruci where *G. pneumonanthe* and *G. cruciata* are used as host plants suggests that there is very little gene flow between these two patches (see Paper V). The allelic diversity of the microsatellites used is high, so that every individual tested had a unique combination of alleles, which also means that the estimated allele frequencies for local populations must be regarded as unreliable in such a small sample. Nevertheless, the clustering of individuals according to host plant availability using population assignment analysis supports the genetic isolation of *M. alcon* and *M. 'rebeli'* in this area. Whether this genetic isolation represents a species boundary, or whether it arises from differences in phenology within a single species, enforced by the differences in flowering phenology of the two host plants, remains an open question. Such differences in phenology clearly exist at Răscruci, where eggs are laid earlier on *G. cruciata* than on *G. pneumonanthe* (which reflects the earlier development of *G. cruciata* flower buds). Comparing the genetic differentiation that we found in Paper V with that found for *M. alcon* in Southern Scandinavia (LOMBORG & al. 2005, NASH & al. 2008; D.R. Nash and M.A. Fürst, pers. comm.) shows that a similar level of genetic differentiation can be found between populations within this species over distances

of a few hundred kilometres ( $F_{ST} = 0.182$ ) as was found in Paper V at most over hundreds of metres. Examining the alleles present at Răscruți (see Paper V) shows that 67% of all alleles were private alleles (i.e. alleles found only within either the *M.alcon* or *M. 'rebeli'* samples), but measured differentiation based on allele length (RST) did not differ from the measured value of  $F_{ST}$ , suggesting that the difference between these groups may arise from genetic drift rather than mutation (HARDY & al. 2003), which would be less likely if *M.alcon* and *M. 'rebeli'* were completely genetically isolated.

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* (and probably *M. salina* too; see Fig. 4, Table 4, 5) is a mutual host for both *M.alcon* and *M. 'rebeli'* in north-eastern Hungary, as it is in Poland (SIELEZNIEW & STANKIEWICZ 2002, STEINER & al. 2003, SIELEZNIEW & STANKIEWICZ 2004b, STANKIEWICZ & 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 (see Paper V). 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 & 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 & 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: Appendix III). 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 & al. 1989) is absent in more central populations. Enzyme polymorphism studies by BERECHKI & al. (2005, 2006) and PECSENYE & 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 BERECHKI & al. 2006). These studies were based on allopatric populations of the two Alcon blues, and are not supported by our preliminary genetic data (Paper V) from a single sympatric population, although the cause of the significant genetic differentiation we find between the two forms remains to be determined.

## 4.2. Laboratory experiments

### 4.2.1. The accelerated development of *M. 'rebeli'* caterpillars

(This subchapter is based on Paper VI)

#### 4.2.1.1. Introduction

It has long been known that *Maculinea* butterflies have an annual life cycle, with caterpillars living for about 10-11 months in *Myrmica* ant nests (THOMAS & al. 1989, THOMAS & WARDLAW 1992, THOMAS & ELMES 1993, THOMAS & al. 1993, THOMAS 1995, WARDLAW & al. 2000, THOMAS & ELMES 2001). More recently, it has been shown that some caterpillars live for an additional year in the ant nests, for a total of about 22-23 months (THOMAS & al. 1998, SCHÖNRÖGGE & al. 2000, ELMES & al. 2001, ALS & al. 2002). While rearing *M. 'rebeli'* caterpillars in the laboratory, an unexpectedly accelerated development was observed, which resulted in pupation as soon as a month after adoption.

#### 4.2.1.2. Materials studied

Between 2000 and 2003, 93 *Maculinea alcon* caterpillars from three sites (Hungary: Fülesd, Gyilkos-rét, Tugár-rét) and 261 *M. 'rebeli'* caterpillars from four locations (Austria: Hochschwab; Hungary: Nagy-mező, Bükkzentkereszt, Tohonya-hát) were introduced (see Chapter 2.5) to 103 artificial laboratory colonies of *Myrmica*. The *Myrmica* colonies were usually collected from the same sites as the gentians. Each colony contained at minimum one queen and 100 workers. The following *Myrmica* species were used: *M. gallienii* (7 cultures), *M. lobicornis* (1 c.), *M. lonae* (1 c.), *M. rubra* (9 c.), *M. ruginodis* (8 c.), *M. rugulosa* (1 c.), *M. sabuleti* (9 c.), *M. salina* (3 c.), *M. scabrinodis* (35 c.), *M. schencki* (8 c.), *M. speciosoides* (3 c.) and *M. vandeli* (1 c.).

#### 4.2.1.3. Results

Several caterpillars died during the period of adoption and the next few days. After this critical period their mortality was lower and the *M. 'rebeli'* caterpillars usually grew very quickly. They were about 3 mm long on introduction, and usually they had grown to about 15 mm a month later. However, seven of them pupated after about a month in different ant nests. These were associated with three *Myrmica* species and came from each of the four *M. 'rebeli'* populations studied (Table 5). Such a quick development during the first months in *M.alcon* caterpillars was never observed in my experiments. The *M.alcon* caterpillars were also about 3 mm long on introduction, but they had grown only to ca. 5 mm a month later and remained about this size in the winter. Two male butterflies from the seven pupae emerged. One of them eclosed 32, the other 47 days after adoption as freshly moulted fourth instar caterpillars (Table 5). These specimens were smaller than average (the forewing length of the one from Bükkszentkereszt was 15 mm and the one from Nagy-mező 15.5 mm), but similarly small specimens often occur under natural conditions. The fast-developing specimens did not show any other obvious differences compared with field-grown specimens (see Appendix VI: 18). The other five pupae rotted or dried out under the unnatural air humidity of the laboratory.

#### 4.2.1.4. Discussion

The fast development of *M. 'rebeli'* under laboratory conditions might be caused by (1) the warmer temperatures in comparison to natural conditions (WARDLAW 1991, WARDLAW & al. 1998), and/or (2) the artificial *Myrmica* nests being more exposed to light than under natural conditions (the more abundant light could influence the larval development of lycaenid butterflies; see e.g. HØEGH-GULDBERG 1968), and/or (3) the more balanced diet the *Myrmica* colonies received.

Locality	Host ant	Adoption	Pupation	Eclosion
Nagy-mező	<i>M. sabuleti</i>	27.07.2002	15.08.2002	28.08.2002
Nagy-mező	<i>M. scabrinodis</i>	27.07.2002	28.08.2002	died
Bükkszentkereszt	<i>M. scabrinodis</i>	11.07.2002	13.08.2002	27.08.2002
Bükkszentkereszt	<i>M. scabrinodis</i>	11.07.2002	13.08.2002	died
Tohonya-hát	<i>M. salina</i>	11.07.2002	15.08.2002	died
Tohonya-hát	<i>M. scabrinodis</i>	11.07.2002	10.08.2002	died
Hochschwab (Austria)	<i>M. sabuleti</i>	14.07.2003	16.08.2003	died

**Table 5.** The *Maculinea* ‘*rebeli*’ caterpillars that pupated in about a month in the laboratory.

G.W. Elmes & J.A. Thomas (pers. comm.) recorded similarly short times of development for *M. ‘rebeli’* from the Pyrenees and the Southern Alps under unnaturally warm conditions and with abundant food. Hence, an accelerated development is known from several populations and is not a unique phenomenon. These results support the plasticity of the developmental time of *M. ‘rebeli’* as the caterpillars develop during one or two years in nature (THOMAS & al. 1998, SCHÖNROGGE & al. 2000, ELMES & al. 2001) or have a conspicuous accelerated development within one year under favourable conditions. In addition, based on my own observations, there is no indication of a two-year development of *M. ‘rebeli’* in Hungary since I have never found semi-developed *M. ‘rebeli’* caterpillars in *Myrmica* nests during the flying period. On the other hand, some semi-developed *M. alcon* caterpillars were observed in various Hungarian sites during the flying period. According to Z. Varga (pers. comm.), ‘dwarf’ adults of *M. ‘rebeli’* regularly appear in several Hungarian populations at the end of the flying period (end of June to mid-July, depending on year and elevation). However, in the laboratory, the two dwarf specimens eclosed in late August. This suggests that undernourished *M. ‘rebeli’* caterpillars may also fully develop within one year under natural conditions and a partly bivoltine life cycle in nature seems to be unlikely. It is known that the growth of the one-year *M. ‘rebeli’* caterpillars tend to accelerate immediately after adoption, stops during winter (meaning that they go into diapause in nature), and resumes in the spring just before pupation (THOMAS & al. 1998). However, based on my laboratory observations the development of *M. ‘rebeli’* caterpillars can be continuous (without diapause) under favourable

conditions, contrary to that of *M. alcon*. These differences were also observed when I reared *M. alcon* and *M. 'rebeli'* caterpillars under the same laboratory conditions but in *Manica rubida* colonies (Chapter 4.2.2).

Further studies are still necessary to investigate the temporal dynamics of the development within *M. 'rebeli'* and *M. alcon* populations and to understand the ecological circumstances influencing these dynamics (see e.g. SIELEZNIEW & STANKIEWICZ 2007).

#### 4.2.1.5. Potential host ants recorded

These laboratory observations were only performed to give some ideas for further experiments but were not aimed as a thorough investigation. It is worth mentioning that during the field experiments some *Myrmica* species were found to rear *M. alcon* and *M. 'rebeli'* caterpillars similarly successful as the host ants (Chapter 4.1). *M. alcon* caterpillars grew similarly well in the colonies of *M. gallienii* and *M. speciosides* for weeks as they did in *M. scabrinodis* and *M. salina* colonies (the latter two are suitable hosts according to field records; Chapter 4.1; see Appendix VI: 17). However, the mentioned artificial colonies and the caterpillars died of an infection during the winter. So, it cannot be stated that these *M. alcon* caterpillars would be able to finish their development at the artificial *M. gallienii* and *M. speciosides* nests. Moreover, it should also be noted that *Myrmica* nests kept in captivity are generally more tolerant of any species of *Maculinea* parasite in their midst than wild nests are, subject to the stress and deprivations of competition in the field (ELMES & al. 2004, SCHÖNROGGE & al. 2004). In the case of *M. 'rebeli'*, two potential host ant species were also found which were not recorded during the field surveys (Chapter 4.1). Six out of ten introduced *M. 'rebeli'* caterpillars pupated successfully in a *M. lobicornis* nest (which is a rare ant in Hungary; see e.g. Appendix III); and one out of three *M. 'rebeli'* caterpillars pupated successfully in a *M. salina* nest (it was pupated in accelerated development: Table 5, Appendix VI: 19); so, probably *M. salina* is a common host



ant of *M. alcon* and *M. 'rebeli'* in the Carpathian Basin, similarly to *M. scabrinodis*, see Fig. 4, Table 4). Of course, it would be desirable to confirm these results also by field data (ELMES & al. 2004, SCHÖNROGGE & al. 2004). In contrast with the former two *Myrmica* species, *M. sabuleti* and *M. scabrinodis* have been recorded as host ants of *M. 'rebeli'* in the here studied regions (see Chapter 4.1 for a review) and it is not surprising therefore that the latter two ant species proved to be suitable hosts also in the laboratory (what is more, accelerated development was observed in their artificial colonies: Table 5).

#### **4.2.2. Is *Manica rubida* a potential host ant of the Alcon Blues?**

(This subchapter is based on Paper VII)

##### **4.2.2.1. Introduction**

*Manica rubida* is quite closely related to ants in the genus *Myrmica* (e.g. ASTRUC & al. 2004), and was classified as a member of this genus by FOREL (1915). This ant occurs in mountains at altitudes of 500-2000 m in Central Europe, parts of Southern Europe, the Caucasus, Asia Minor and the Crimea (CZECHOWSKI & al. 2002). All five European *Maculinea* butterflies occur in some of these regions. Moreover, *M. arion*, *M. alcon* and *M. 'rebeli'* populations are known from the same altitudes (e.g. WYNHOFF 1998). The co-occurrence of *M. rubida*, *M. 'rebeli'* and host gentians with eggs was observed at the same site near Lacul Roșu (Romania: Hargita County; S. Csősz, pers. comm.).

Based on this distribution, the question arose as to whether *M. rubida* could potentially be a host ant of *Maculinea* butterflies. This ant has never been observed in association with any myrmecophilous lycaenid so far (PIERCE & al. 2002, FIEDLER 2006). Hence I tested the ability of laboratory colonies of this ant to adopt and raise caterpillars of *M. 'rebeli'* and *M. alcon*.

#### 4.2.2.2. Materials studied

In the summer of 2003, I introduced 21 caterpillars of *M. alcon* from Mátraszentimre (Hungary: Heves County) and 23 caterpillars of *M. 'rebeli'* from Bükk-szentkereszt (Hungary: Borsod-Abaúj-Zemplén County) into five artificial colonies of *M. rubida* (Table 6). It should be noted that all the caterpillars were from mountains where *M. rubida* has not been recorded (SOMFAI 1959; A. Tartally, pers. observ.).

The *M. rubida* colonies were collected from Sovata (Romania: Mureş County; Maros megye: Szováta) in March 2003. Colonies 1-4 (Table 6) contained one queen, brood, and at least 150 workers, while Colony 5 contained one queen, brood, and only 50 workers. This last colony was used primarily for taking a video record of the interaction, because smaller caterpillars in a dense colony are not easily visible. Colony 5 was videotaped on 5 August 2003 when a freshly adopted *M. alcon* caterpillar and two *M. 'rebeli'* caterpillars (introduced one week earlier) were present. Discrimination between adopted *M. alcon* and *M. 'rebeli'* caterpillars based on size was not difficult because caterpillars of *M. 'rebeli'* grow much quicker than those of *M. alcon* in the Hungarian populations in late summer (see Chapter 4.2.1). Similar phenomena are also known from other countries (e.g. SCHÖNROGGE & al. 2000, SIELEZNIEW & STANKIEWICZ 2007).

The caterpillars of both species were introduced to the foraging arena of the same colony together at the same time. Colonies were then checked for surviving caterpillars once per week (Table 6).

#### 4.2.2.3. Results

After the introduction the *M. rubida* workers carried each caterpillar from the foraging arena to the nest within an hour. Several caterpillars of both *M. alcon* and *M. 'rebeli'* survived in the nests (see Appendix VI: 19) for a number of weeks, up to one and a half months (Table 6). The remains of dead caterpillars were often found in the arena among the rubbish.

Both *M.alcon* and *M. 'rebeli'* caterpillars were about 3 mm long on introduction, and a month later the former had grown to about 5 mm while the latter had grown to about 10 mm. The workers antennated, licked and carried the caterpillars within the nest (see Paper VII: Fig. 1; see also the Digital Supplementary Material of this article, MPEG files showing extracts of the videotape, at <http://www.oegef.at/>). I observed caterpillars eating ant brood, but it was not clear if the caterpillars were also fed directly by the worker ants.

Date (2003)	Colony 1 <i>M. 'rebeli'</i>	Colony 2 <i>M. 'rebeli'</i>	Colony 3 <i>M.alcon</i>	Colony 4 <i>M.alcon</i>	C o l o n y 5	
	<i>M. 'rebeli'</i>	<i>M.alcon</i>	<i>M.alcon</i>	<i>M.alcon</i>	<i>M. 'rebeli'</i>	<i>M.alcon</i>
15 July	10*	10*	-	-	-	-
22 July	4	9	-	-	-	-
29 July	2	8	-	-	3*	-
05 August	2	5	10*	10*	2	1*
12 August	1	4	10	7	2	1
19 August	0	3	7	6	2	1
26 August	0	1	6	3	1	1
02 September	0	0	4	3	1	0
09 September	0	0	1	1	0	0
16 September	0	0	1	0	0	0
23 September	0	0	0	0	0	0

**Table 6.** The number of introduced (\*) and surviving *M.alcon* and *M. 'rebeli'* caterpillars in *M. rubida* colonies from week to week.

#### 4.2.2.4. Discussion

My results show that laboratory *Manica rubida* colonies readily adopted *Maculinea* caterpillars, some of which survived for up to six weeks, during which they grew considerably. This contrasts with the field observations of THOMAS & al. (1989) on another ant of the subfamily Myrmicinae, *Tetramorium caespitum*. They noted that "... several young *M. rebeli* larvae were seen being taken by *Tetramorium caespitum* L. soon after leaving their foodplant, but these were presumably taken as food by this voracious predator, for no trace of them was found in the nests soon afterwards." (THOMAS & al. 1989: 453). Similarly, ELFFERICH (1988) did not observe adoption of *M.alcon* caterpillars by *T. caespitum* nor by *Lasius flavus*, *L. niger*, *Formica fusca* and *F. sanguinea* under artificial conditions. The caterpillars were often carried or tapped by the workers

after the introduction but each of them was dead the next day or was carried out of the nest. However, he observed successful adoption and rearing by *Myrmica ruginodis* and this was the only ant species which licked the caterpillars. ELFFERICH (1988) considered this behaviour as obligatory for the survival of caterpillars. Similarly, in my experiments the caterpillars not only survived and grew but also were licked by the workers of *M. rubida* (see Paper VII: Fig. 1B). These phenomena show the potential suitability of *M. rubida* as a host for *Maculinea*. It is therefore possible that caterpillars of some *Maculinea* populations could fully develop in *M. rubida* nests at high altitudes.

It is important to emphasize that we cannot predict the host ant species used by a particular population of *Maculinea* butterflies using the data recorded from other populations. Different populations of these butterflies have evolved to use different hosts in different parts of their geographical ranges (ELMES & al. 1994, ALS & al. 2002; Appendix V). Moreover, within the islands of Japan *M. teleius* has been recorded as using hosts from two different ant genera: a *Myrmica* species (*M. ruginodis*) and an *Aphaenogaster* species (*A. japonica*) (YAMAGUCHI 1988).

*M. rubida* is remarkably open in its social structure, even allowing for heterospecific colonies (with *Formica selysi*) to be established in the laboratory (ERRARD & JALLON 1987). This could potentially enhance the suitability of this ant as a host of myrmecophilous insects. Additionally MALICKY (1969) reported experimental observations that *Manica* ants show standard, non-aggressive (but rather unspecific) tending behaviour to a range of (unspecified) lycaenid species. It remains a question whether this potential of *M. rubida* as a host of *Maculinea* butterflies – demonstrated in the laboratory – can be realized in the fields. As far as I know there are no records of *Maculinea* specimens from nests of *M. rubida* in the wild, but this may also reflect lack of search effort. On the other hand it has also been shown that a *Myrmica* species that does not act as a host of *Maculinea* from a particular population in the wild can be a good host in the laboratory when well-fed and not subject to stress (ELMES & al. 2004, SCHÖNROGGE & al. 2004).

It has been shown that the primary means of gaining entry to and surviving in *Myrmica* host ant nests is mimicry, whether it be of acoustic signals (DEVRIES & al. 1993) or of brood or colony odours (AKINO & al. 1999, ELMES & al. 2002, SCHLICK-STEINER & al. 2004, SCHÖNROGGE & al. 2004, NASH & al. 2008). My results suggest that the signals of caterpillars matched the template of *Manica rubida* sufficiently, well enough to induce adoption and to some degree acceptance in the nest – which is not surprising considering the close phylogenetic proximity of the genera *Manica* and *Myrmica* (ASTRUC & al. 2004). However, the caterpillars in this study were from sites where *Manica rubida* did not occur. Whether better mimicry of *M. rubida* can evolve in areas where it is found on *Maculinea* sites remains to be seen. In any case it would be interesting to compare the acoustical and chemical signatures of *M. rubida* with those of *Myrmica* species and to *Maculinea* caterpillars from *M. rubida* sites. In addition, it would be interesting to further test whether caterpillars of *M.alcon* and *M. 'rebeli'* in *M. rubida* colonies get all their nutrition by eating the ant brood (which is not an unfamiliar food of "cuckoo-feeder" caterpillars; e.g. ELFFERICH 1988), or whether they can also be fed by trophallaxis by the worker ants as they are in *Myrmica* host nests (Chapter 2.2.2).



## 5. Other recorded taxa connected with the *Maculinea*-*Myrmica* relationship

### 5.1. Parasitoids

Not only *Maculinea* butterflies but also their parasitoid ichneumon wasps are of high interest to evolutionary and conservation ecology because of their extreme adaptations to the myrmecophilous life-style of *Maculinea* and because all are rare and globally endangered (HOCHBERG & al. 1996, MUNGUIRA & MARTÍN 1999, IUCN 2006). *Neotypus* females oviposit on young *Maculinea* caterpillars on the larval foodplant (see Appendix VI: 9) while *Ichneumon* females penetrate *Myrmica* nests to seek *Maculinea* caterpillars (THOMAS & ELMES 1993; see Appendix VI: 10). These parasitoids both emerge from host pupae inside ant colonies and are presumed to have similar specialisations to those described for *I. eumerus* (THOMAS & al. 2002) to escape unharmed from ant nests.

Two parasitoid species (*Neotypus melanocephalus* and *Ichneumon eumerus*) were found during the work on *Maculinea* host ant specificity. However, it should be noted that pupae of *M. arion* and *M. nausithous* were not found and therefore no data on their parasitism were available. Both butterflies are parasitized in western Europe by *Neotypus* species (*N. pusillus* was bred from *M. nausithous* and another *Neotypus* species from *M. arion*: THOMAS & ELMES 1993; J.A. Thomas, J.C. Wardlaw & M.G. Fitton, pers. comm.). According to these, it is easily imaginable that parasitoids of *M. arion* and *M. nausithous* could be found in the Carpathian Basin with further investigation.

#### 5.1.1.1. *Neotypus melanocephalus*

(This subchapter is based on Paper VIII)

The first parasitized *Maculinea* pupa found in the Carpathian Basin was collected at Meszes in a marshy meadow occupied by *Sanguisorba officinalis* (see Appendix III, IV). No other species and no other foodplant of *Maculinea* occur at

this site, except *M. teleius* and *S. officinalis* (Z. Varga, pers. comm.). Moreover, *M. nausithous*, the only other *Maculinea* species that uses *S. officinalis*, is not known from NE-Hungary (BÁLINT 1996). Based on this evidence, this pupa was identified as *M. teleius*. Some hours after collection, a wasp hatched from this pupa. It proved to be *Neotypus melanocephalus* (like Appendix VI: 9).

No more pupae parasitized by *N. melanocephalus* were found while investigating *Myrmica* nests. However, there were two *N. melanocephalus* females collected ovipositing on *S. officinalis* also at Meszes and another flying at Drahos-rét (see Appendix III, IV).

#### 5.1.1.2. *Ichneumon eumerus*

In total 61 *Maculinea* pupae parasitized by *I. eumerus* were found during the research (see Appendix VI: 8, 10-12). Such pupae of *M. teleius* were found in *M. scabrinodis* nests at Aggtelek (1 parasitized pupa) and Meszes (6); and parasitized *M.alcon* pupae were found in *M. scabrinodis* nests at Gyilkos-rét (24), and in a *M. vandeli* nest at Šardu (3); of *M. 'rebeli'* were found in *M. scabrinodis* nests at Lófő-tisztás (8), in *M. schencki* nests at Lófő-tisztás (4) and Răscruci (2), and in *M. sabuleti* nests at Bükkszentkereszt (1), Lófő-tisztás (5), Nagy-mező (5) and Răscruci (2) (see also Appendix III).

Hence the presence of *I. eumerus* in *Maculinea* pupae appears as a good indication that a given ant species is a suitable host ant of *Maculinea* butterflies (THOMAS & al. 2002, SIELEZNIEW & STANKIEWICZ 2004b), the records on *I. eumerus* seem to support the host ant specificity results found in the Carpathian Basin (viz. that *M. scabrinodis* is a suitable host ant of *M. teleius*; *M. scabrinodis* and *M. vandeli* are suitable hosts of *M.alcon*; *M. sabuleti*, *M. scabrinodis* and *M. schencki* are suitable hosts of *M. 'rebeli'*; see also Chapter 3.1, 4.1).

In spite of the fact that the *N. melanocephalus* specimen found at Meszes (Chapter 5.1.1.1) was the first wasp recorded to parasitize *M. teleius* (J.A. Thomas, pers. comm.; see Paper VIII), *I. eumerus* was also recorded parasitizing *M. teleius*,



what is more, at the very same site (Meszes; Appendix III). Furthermore, *I. fulvicornis* has also been recently recorded parasitizing *M. teleius* (HINZ & HORSTMANN 2007). At the same time, only *I. eumerus* has been recorded parasitizing *M. alcon* and *M. 'rebeli'* (THOMAS & ELMES 1993, MUNGUIRA & MARTÍN 1999, SIELEZNIEW & STANKIEWICZ 2004b, HINZ & HORSTMANN 2007; J.A. Thomas, M.G. Fitton & H. Hilpert, pers. comm.).

## 5.2. *Microdon myrmicae*

Similarly to *Maculinea* caterpillars, larvae of *Microdon* hoverflies are also social parasites of ant colonies (HÖLLDOBLER & WILSON 1990) and can be extremely host specific (ELMES & al. 1999). The recently described (SCHÖNROGGE & al. 2002) *Microdon myrmicae* (see Appendix VI: 13, 14) parasitizes nests of *Myrmica* species and therefore might be in competition with *Maculinea* caterpillars (SCHÖNROGGE & al. 2002, BONELLI & al. 2005, THOMAS & al. 2005).

While working on *Maculinea* host ant specificity, the presence of *M. myrmicae* was recorded in two sites in Transylvania (larvae and pupae were found at Răscruți and Șardu) and in 12 sites in Hungary (larvae and pupae were found at Aggtelek, Drahos-rét, Fülesd, Gyilkos-rét, Ipolytarnóc, Kercaszomor, Kétvölgy, Lófő-tisztás, Meszes, Nógrádszakál, Vörös-rét; and there was a flying adult at Velemér) (see Appendix III). *M. scabrinodis* was parasitized in the most (11; both in Hungary and Transylvania) sites but *M. gallienii* (4) and *M. rubra* (2) were also found to be infested (see Appendix III). Moreover, there was an undetermined *Microdon* larva, very similar to that of *M. myrmicae*, found in a *M. sabuleti* nest at the Slovakian Hačava (in a *M. 'rebeli'* site not included in this thesis; unfortunately this larva became rotten and the identification was not possible in the lab; A. Tartally, unpubl. data). *M. sabuleti* and *M. rubra* have not been recorded as host ants of *M. myrmicae* before (known hosts were: *M. scabrinodis*, *M. gallienii* and probably *M. tulinae*; for a review: BONELLI & al. 2005).

Having a specific life cycle, *Microdon myrmicae* is thought to be rare and endangered (SCHÖNROGGE & al. 2002). Interestingly this hoverfly often lives sympatrically with *Maculinea* butterflies and often infests the same *Myrmica* species, or what is more the same nests as *Maculinea* caterpillars (BONELLI & al. 2005; Appendix III; A. Tartally, pers. observ.). The competition of the valuable butterflies and this recently described and thus understudied hoverfly would be worth studying in a more detailed manner.

### **5.3. *Rickia wasmannii***

(This chapter is based on Paper IX)

*Rickia wasmannii* (Ascomycetes: Laboulbeniales; Appendix VI: 15) obligately exploits ants (for a review on Laboulbeniales: BLACKWELL 1994, WEIR & BLACKWELL 2005, 2007; and for one especially on myrmecophilous species: HÖLLDOBLER & WILSON 1990, HERRAIZ & ESPADALER 2007). The ants appear to be neutral to the presence of this fungus on their cuticles (HÖLLDOBLER & WILSON 1990; A. Tartally, pers. observ.). *Myrmica* species are reported to be the usual hosts of *R. wasmannii* (ESPADALER & SANTAMARIA 2003, HERRAIZ & ESPADALER 2007). To the best of our knowledge, this fungus has not been reported previously from the Carpathian Basin.

The occurrence of *R. wasmannii* was checked on 6505 *Myrmica* specimens from 669 colonies collected at the sites involved in this thesis (see Appendix III, IV) between 2001 and 2007, and the density of the fungus on the different parts of the body of infected *Myrmica* specimens was estimated.

The fungus was present on 459 infected ant specimens in 73 colonies at nine Hungarian (Drahos-rét, Gödörháza, Gyilkos-rét, Lászlótanya, Meszes, Nógrádszakál, Nyikom-rét, Tohonya-hát and Tugár-rét) and three Transylvanian (Fânațele Clujului, Răscruci and Șardu) sites (see Appendix III). Although 11 *Myrmica* species (*M. gallienii*, *M. lobicornis*, *M. lonae*, *M. rubra*, *M. ruginodis*, *M. sabuleti*, *M. salina*, *M. scabrinodis*, *M. schencki*, *M. specioides* and *M. vandeli*)

were involved in our work, only four of them (*M. salina*, *M. scabrinodis*, *M. specioides* and *M. vandeli*) were found to be infected (see Appendix III). *M. scabrinodis* was the most common host, and *M. salina* was most heavily infected. The fungus was present on workers (see Paper IX: Fig. 2-4) and dealate (old) queens, but not on males, alate (young) queens and larvae. However, the numbers of males, alate queens and larvae examined were small.

Our results indicate that it is quite probable that *R. wasmannii* could be found at several other sites in the Carpathian Basin with a more intensive survey.



## 6. Conclusions and perspectives

### 6.1. Evolutionary and ecological aspects

My results confirm the recent records from other parts of central Europe that the host ant use of *Maculinea* butterflies is more complex there than it was shown by the seminal works from western Europe (see e.g. SIELEZNIEW & STANKIEWICZ 2002, STANKIEWICZ & SIELEZNIEW 2002, HÖTTINGER & al. 2003, STEINER & al. 2003, BUSZKO & al. 2005, WITEK & al. 2005; vs. THOMAS & al. 1989, ELMES & al. 1998, MUNGUIRA & MARTÍN 1999).

The multiple host ant use of *M.alcon*, *M. 'rebeli'* and *M. teleius* found in this study raises several ecological and evolutionary questions. At least four scenarios can explain multiple host use in the case of *Maculinea* butterflies (THOMAS & al. 2005):

First, *conditions may be benign enough that Myrmica species normally not used as hosts can tolerate Maculinea caterpillars in their nests*. The recent changes in some of the sites studied (e.g. abandonment of grazing in Bükkszentkereszt and Lófő-tisztás, salinisation in Fülesd) 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 caterpillars may be adapted to use different host species*. Under these circumstances a host-ant-related genetic substructure could also be expected within populations. Enzyme polymorphism studies (BERECZKI & al. 2005, 2006, PECSENYE & al. 2007), however, have failed to find such substructuring of *M.alcon*, *M. 'rebeli'* and *M. teleius* populations in the Carpathian Basin, and while the preliminary investigation of microsatellite markers for the Răscruți population in Transylvania does show strong differentiation between *M.alcon* and *M. 'rebeli'* within this site, there is no differentiation between individuals using *M. sabuleti* and *M. schencki* nests (see Paper V: Fig. 6).

Third, *mixed-host populations may occupy habitats in areas that are on biogeographical boundaries between single-host M. alcon, M. 'rebeli' and M. teleius areas*. Under this scenario, either genetic differentiation between the subpopulations using different hosts, or a hybrid population structure with an excess of heterozygotes could also be expected. The genetic data from Răscruți do show evidence for differentiation between *M. alcon* and *M. 'rebeli'* individuals using different host plant species, but not between *M. 'rebeli'* individuals using different host ant species, and there is no evidence of heterozygote excess (see Paper V).

Finally, *mixed-host Maculinea populations may show phenotypic adaptations to more than one host, allowing true multiple host usage*. Recent chemical studies suggest that *M. 'rebeli'* caterpillars may show adaptation to different hosts by synthesising cuticular hydrocarbons specific to both of the local host ant species (SCHLICK-STEINER & 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 & al. 2008).

The complexity of host use patterns can represent a geographical mosaic of coevolution between *Maculinea* butterflies 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 example, for both *M. alcon* and *M. 'rebeli'* sites a pattern of higher level infestation of *Myrmica scabrinodis* nests was found when this host was locally common, and lower levels of infestation when it was rare (Paper V). This is exactly the pattern expected if there is local coadaptation with this host, and it 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 & al. 2008).

Different selection forces may operate in central and peripheral populations. In addition, the drift may act as the dominant force forming the rapid genetic

differentiation in 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 & PULLINS 2004, SCHMIDT & HUGHES 2006). The host ant specificity of *Maculinea* butterflies could also show such variation because the genus *Maculinea* is thought to have evolved in continental East Asia (SIBATANI & al. 1994) and European, particularly western European, populations can be considered peripheral. It would be useful therefore to get more knowledge about the host ant use of *Maculinea* butterflies in Asia because the scarce available data (YAMAGUCHI 1988, WOYCIECHOWSKI & al. 2006) suggest that there is little host ant specificity there (see also PECH & al. 2007). Most of the *M. alcon*, *M. 'rebeli'* and *M. teleius* 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 & al. 1989, ELMES & al. 1998, STANKIEWICZ & al. 2005). Data from this study and other recent studies (STANKIEWICZ & SIELEZNIEW 2002, STEINER & al. 2003, SIELEZNIEW & STANKIEWICZ 2004b, BUSZKO & al. 2005, STANKIEWICZ & SIELEZNIEW 2005, WITEK & al. 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 & al. 2002). It is interesting to note, however, that the most common host ant species in this study, *Myrmica scabrinodis*, is common in north-west Europe, but has never been recorded as a host of *M. alcon* in that area (ELMES & al. 1994, ALS & al. 2002).

My results on *M. nausithous* are also of interest from an evolutionary point of view because ALS & al. (2004) have observed two genetic forms in *M. nausithous*: they found that a specimen from Slovakia strongly diverged from the Polish and the Central-Russian specimens (see ALS & al. 2004: Fig. 2). Potentially, these different forms of *M. nausithous* could use different host-ant species. As far as I know, there are no data on the host-ant specificity of *M. nausithous* from Slovakia, but there are

such data from three neighbouring countries: *Myrmica rubra* was the only recorded host of *M. nausithous* in Hungary, Poland and Ukraine (THOMAS & al. 1989, ELMES & al. 1998, STANKIEWICZ & SIELEZNIEW 2002; M. Witek, pers. comm.; Chapter 3.2.1, see Paper III: Fig. 1). ALS & al. (2004) studied some *M. nausithous* specimens from Slovakia and Poland but they did not examine any from Hungary or Transylvania. Accordingly, it would be worth studying the host-ant specificity of *M. nausithous* in Slovakia and comparing *M. nausithous* specimens from Hungary, Transylvania and Ukraine genetically with Slovakian and Polish ones. It would be desirable to do similar experiments on the southern European fringe populations of *M. nausithous* in Slovenia (see WYNHOFF 1998) and Bulgaria (KOLEV 2002). Furthermore, it would be especially important to involve the isolated Transylvanian populations of *M. nausithous* in the genetic analyses because those populations conspicuously use *M. scabrinodis* but not *M. rubra*, that is used by other populations studied in the nearby regions (discussed above; see Chapter 3.2).

According to my data, the hypothesis (THOMAS & ELMES 1998) could not be confirmed that the host selection of the cuckoo *Maculinea* species is more restricted than of the predators (e.g. the predatory *M. nausithous* was rather host ant specific than the cuckoo *M. 'rebeli'*; see Chapter 3.2, 4.1).

The use of the same host ant species by more myrmecophilous insects and fungus (see Appendix III: e.g. *Myrmica scabrinodis* was infested by *Maculineaalcon*, *M. nausithous*, *M. teleius*, *Microdon myrmicae* and *Rickia wasmannii* at Răscruçi) raises several questions about competition which would be worth studying in more details. Besides, the fact that *M.alcon*, *M. teleius* and *M. nausithous* often co-occur and have common host ants (and even commonly parasitized nests, see Chapter 3.1, 3.2, 4.1, Appendix III) in Hungary and Transylvania, contrast with results from western Europe, where each *Maculinea* species depends on a single, and different *Myrmica* species (THOMAS & al. 1989, ELMES & al. 1998).



## 6.2. Conservation biology

My data on the differences in host specificity between nearby populations are compatible with those of other studies (ALS & al. 2002) and draw attention to the importance of host specificity studies on the local scale. These 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* butterflies from local extinction (see ÁRNYAS & al. 2006 for an example for the successful management of a Hungarian *M. 'rebeli'* site and SETTELE & al. 2005 for several chapters on the management of other *Maculinea* sites in Europe; see also WYNHOFF 2001).

*M. nausithous* was found to be the most restricted geographically and also the most host ant specific *Maculinea* butterfly in the Carpathian Basin (though we do not have data on the host ant specificity of *M. arion* there) which means *M. nausithous* could be the most sensitive *Maculinea* butterfly to the change of *Myrmica* composition there (it was exclusively found with *M. rubra* in western Hungary but with *M. scabrinodis* in Transylvania; see Chapter 3.2). I suggest that – to be successful – the management of *S. officinalis* meadows for western Hungarian *M. nausithous* should include leaving a mosaic of scrub fragments and natural forest edges according to the ecological requirements of the general host ant *M. rubra* (see ELMES & al. 1998; Appendix VI: 20). However, *M. scabrinodis* using *M. nausithous* populations represent a specific life form in Transylvania that needs further investigation and careful protection. The acuteness of this task is underlined by the low density of these populations (see Chapter 3.2.2).

The multiple host ant use of *M. teleius*, *M.alcon* and *M. 'rebeli'* found in the Carpathian Basin could suggest that these butterflies can easily adapt to some new host ant species when circumstances (e.g. because of human disturbance) change. However, we should be careful with such interpretations based on the currently limited amount of data available, and need consider that usually not all the

available *Myrmica* species were found to be infested by these vulnerable butterflies. Moreover, it should be mentioned that *M. alcon* and *M. 'rebeli'* (the closely related Alcon Blues; see Chapter 2.1.2 for a review) are different from conservational aspects (e.g. THOMAS & SETTELE 2004, SIELEZNIEW & STANKIEWICZ 2007) and therefore they require various treatments because not only their habitats, host ants and host plants can be different within the same region (see Chapter 2.1.2 for a review) but also their phenology (because of the phenology of their different host gentians: SIELEZNIEW & STANKIEWICZ 2007). These features practically can result in difficulties in the reintroduction programmes when *M. alcon* is introduced to *M. 'rebeli'* habitats or vice versa (SIELEZNIEW & STANKIEWICZ 2007).

My results on rare and understudied species connected to the *Maculinea-Myrmica* relationship (*Ichneumon eumerus*, *Microdon myrmicae*, *Neotypus melanocephalus* and *Rickia wasmannii*) clearly support the earlier statements that *Maculinea* butterflies are suitable umbrella species for valuable sites (see e.g. MUNGUIRA & MARTÍN 1999, SETTELE & al. 2005). Furthermore, this variety of valuable species proves the relevance of the study sites (Appendix III, IV) for further investigations and for protection.

## Summary

Larvae of *Maculinea* (Lepidoptera: Lycaenidae) butterflies are obligate parasites of *Myrmica* (Hymenoptera: Formicidae) ant colonies. Knowledge of the host ant species has been shown to be crucial for the protection of these butterflies. Furthermore, the identification of the local host ant species can unravel the evolution of this type of parasitic interaction. Host-ant specificity may vary between regions; therefore data should be collected over the geographical range of these butterflies.

To understand the host ant usage, 1589 *Myrmica* nests were searched in 33 sites in the Carpathian Basin (30 in Hungary and 3 in Transylvania) between 2000 and 2007; and for some experiments, caterpillars of *Maculinea alcon* and *M. 'rebeli'* were adopted and cultured by artificial *Myrmica* colonies.

*Maculinea teleius* was recorded with six *Myrmica* species. *M. rubra* and *M. scabrinodis* were the most frequently used host ants. *M. rubra* appeared to be more suitable in the western while *M. scabrinodis* proved to be more important in the eastern sites. *M. salina* (first record) and *M. gallienii* were only locally important hosts on a few sites. *M. specioides* (first record) and *M. vandeli* were parasitized in only one case.

*Maculinea nausithous* was recorded exclusively with *Myrmica rubra* in western Hungary but exclusively with *M. scabrinodis* in Transylvania.

Although no parasitized nests by *Maculinea arion* were found, there were eight *Myrmica* species (*M. lobicornis*, *M. lonae*, *M. rubra*, *M. sabuleti*, *M. scabrinodis*, *M. schencki*, *M. specioides* and *M. vandeli*) known as potential host ants from *M. arion* sites.

*Maculinea alcon* was recorded with three *Myrmica* species. *M. scabrinodis* was the general host. *M. salina* (first record) and *M. vandeli* were found only on a few sites where these species were used rather than *M. scabrinodis*.

*Maculinea 'rebeli'* was recorded with five *Myrmica* species. *M. schencki*, *M. sabuleti* and *M. scabrinodis* were the most important hosts. *M. lonae* (first record) and *M. specioides* were also used occasionally.

The laboratory observations confirmed the field results about the host ants of *Maculineaalcon* and *M. 'rebeli'*. Furthermore the potential host ants of *M.alcon* were augmented with *Myrmica gallienii*, *M. specioides* and *Manica rubida* (a closely related ant to *Myrmica* which has not been recorded as *Maculinea* host on the fields). The potential host ants of *Maculinea 'rebeli'* were completed with *Myrmica lobicornis*, *M. salina* and *Manica rubida*. Of course, it would be desirable to confirm these results also by field data.

Seven *Maculinea 'rebeli'* caterpillars pupated after only about a month from the adoption in artificial *Myrmica sabuleti*, *M. salina* and *M. scabrinodis* nests and two of them successfully eclosed. As far as I know, such an accelerated development has not been published before.

*Neotypus melanocephalus* (Hymenoptera: Ichneumonidae) parasitized *Maculinea teleius* (first record).

*Ichneumon eumerus* (Hymenoptera: Ichneumonidae) parasitized *Maculinea teleius* (first record), *M.alcon* and *M. 'rebeli'* at more sites.

Larvae and pupae of *Microdon myrmicae* (Diptera: Syrphidae) were often found in *Myrmica scabrinodis* and sometimes in *M. rubra* (first record) and *M. gallienii* nests.

*Rickia wasmannii* (Ascomycetes: Laboulbeniales) were often found covering *Myrmica scabrinodis* and sometimes *M. salina*, *M. specioides* and *M. vandeli* specimens.

My results confirm that the host ant use of *Maculinea* butterflies is more complex in central Europe than it was shown by the seminal works from western Europe.

*Myrmica scabrinodis* (and probably *M. salina* too, according to the laboratory experiments) can be considered a mutual host of both *Maculineaalcon* and *M. 'rebeli'* in the Carpathian Basin, which is the first record for a common host ant species of these two closely related Alcon Blue butterflies within the same region. This is in harmony with the recent literature which found overlapping variations between these two butterflies.

The host use of *Maculinea nausithous* is also of interest from an evolutionary point of view because the isolated Transylvanian populations used other host ant species (*Myrmica scabrinodis* exclusively) than other central European populations (*Myrmica rubra* exclusively).

According to my data, the earlier hypothesis could not be confirmed that the host selection of the cuckoo *Maculinea* species is more restricted than of the predators.

The use of the same host ant species by more myrmecophilous insects and fungus raises several questions about competition which would be worth studying in more details.

My data on the differences in host specificity between nearby populations are compatible with those of other studies and draw attention to the importance of host specificity studies on the local scale.

*Maculinea nausithous* was found to be the most host ant specific *Maculinea* butterfly in the Carpathian-Basin which means *M. nausithous* could be the most sensitive *Maculinea* butterfly to the change of *Myrmica* composition there.

My results on rare and understudied species connected to the *Maculinea*-*Myrmica* relationship (*Ichneumon eumerus*, *Microdon myrmicae*, *Neotypus melanocephalus* and *Rickia wasmannii*) clearly support the earlier statements that *Maculinea* butterflies are suitable umbrella species for valuable sites.



## Összefoglalás

A *Maculinea* boglárkalepkék hernyói obligát módon *Myrmica* (Hymenoptera: Formicidae) hangyák fészkeiben fejlődnek (VI. függelék 3-8). Konzervációbiológiai szempontból fontos, hogy az egyes hangyagazda fajokat megismerjük. Ezen kívül a helyi hangyagazdák ismerete segíthet tisztázni bizonyos, a lepkék és a hangyák közötti kapcsolat evolúcióját érintő kérdéseket. Mivel a hangyagazda fajok regionálisan különbözhetnek (V. függelék), ezért e lepkék hangyagazda fajait lokális szinten kell megismernünk.

Terepi vizsgálataim során összesen 1589 *Myrmica* fészket vizsgáltam meg 33 kárpát-medencei élőhelyen (harminc élőhelyen Magyarországon és három élőhelyen Erdélyben; lásd: III., IV. függelék) 2000 és 2007 között; és laboratóriumi vizsgálataim során *Myrmica* kolóniákkal adoptáltattam és neveltetem *Maculineaalcon* és *M. 'rebeli'* hernyókat (VI. függelék: 16).

*Maculinea teleius* példányokat hat hangyafajnál találtam (1. ábra, 1. táblázat), melyek közül a *Myrmica rubra* és a *M. scabrinodis* fészkei voltak a leggyakrabban fertőzöttek. Míg azonban a nyugati élőhelyeken a *M. rubra*, addig a keleti élőhelyeken a *M. scabrinodis* volt a fontosabb gazdafaj. Csak néhány élőhelyen, helyi szinten volt fontos gazda a *M. salina* (első adat) és a *M. gallienii*, találtam továbbá egy-egy fertőzött *M. specioides* (első adat) és *M. vandeli* fészket is.

Míg a *Maculinea nausithous* a Dunántúlról kizárólag *Myrmica rubra*, addig Erdélyből csak *M. scabrinodis* fészkekből került elő (2. ábra, 2. táblázat).

Összesen nyolc *Myrmica* faj (*M. lobicornis*, *M. lonae*, *M. rubra*, *M. sabuleti*, *M. scabrinodis*, *M. schencki*, *M. specioides* és *M. vandeli*) került elő a *Maculinea arion* élőhelyeiről (3. ábra, 3. táblázat), mint lehetséges hangyagazda, azonban e lepke példányait egy fészekben sem találtam meg.

A *Maculineaalcon* összesen három *Myrmica* fajnál fordult elő (4. ábra, 4. táblázat), amelyek közül a *M. scabrinodis* volt a leggyakoribb gazdafaj. Ugyanakkor viszont a *M. salina* (első adat) és a *M. vandeli* fészkei csak néhány élőhelyen voltak fertőzöttek, de ott nagyobb arányban, mint a *M. scabrinodis* fészkek.

*Maculinea* 'rebeli' példányokat összesen öt *Myrmica* faj fészkeiben találtam (4. ábra, 4. táblázat), melyek közül a *M. schencki*, a *M. sabuleti* és a *M. scabrinodis* volt a három legfontosabb gazdafaj. Kis számban fertőzöttek voltak a *M. lonae* (első adat) és a *M. specioides* fészkei is.

A laboratóriumi megfigyeléseim megerősítették a *Maculineaalcon* és a *M. 'rebeli'* hangyagazda használatával kapcsolatos fenti terepi tapasztalataimat, de valószínűsítik azt is, hogy a *Myrmica gallienii*, a *M. specioides* és a *Manica rubida* (a *Myrmica* fajok közeli rokona, amelyről terepen még nem bizonyosodott be, hogy *Maculinea* gazdafaj lehetne) is a *M.alcon* gazdafajai lehetnek. Hasonló módon a *Maculinea 'rebeli'* lehetséges gazdafajának bizonyult a *Myrmica lobicornis*, a *M. salina* és a *Manica rubida* (6. táblázat) is. Fontosnak tartom azonban a jövőben e laboratóriumi eredményeket terepi adatokkal is alátámasztani.

Körülbelül egy hónappal az adoptáció után hét *Maculinea 'rebeli'* hernyó bebábozódott a laboratóriumi *Myrmica sabuleti*, *M. salina* és *M. scabrinodis* fészkekben és ebből két példány sikerrel lepkévé is fejlődött (5. táblázat). Tudomásom szerint a *Maculinea* boglárkalepkék ilyen felgyorsult fejlődését az irodalomban elsőként publikáltam.

Előkerült egy *Neotypus melanocephalus* (Hymenoptera: Ichneumonidae) imágó (III. függelék, VI. függelék: 9) egy *Maculinea teleius* bábból (első adat).

Az *Ichneumon eumerus* (Hymenoptera: Ichneumonidae) több élőhelyen is és gyakran parazitálta a *Maculinea teleius* (első adat), a *M.alcon* és a *M. 'rebeli'* bábjaikat (III. függelék, VI. függelék: 8, 10-12).

Sokszor találtam *Myrmica scabrinodis* és néha *M. rubra* (első adat) és *M. gallienii* fészkekben *Microdon myrmicae* (Diptera: Syrphidae) lárvákat és bábokat (III. függelék, VI. függelék: 13, 14).

Gyakran találtam olyan *Myrmica scabrinodis*, és ritkábban olyan *M. salina*, *M. specioides* és *M. vandeli* egyedeket, amelyeket megfertőzött a *Rickia wasmannii* (Ascomycetes: Laboulbeniales; III. függelék, VI. függelék: 15).



Az eredményeim megerősítik azt, hogy a *Maculinea* boglárkalepkék hangyagazda használata sokkal komplexebb Európa középső részein, mint ahogy azt a kontinens nyugati régióiból ismerték.

A *Myrmica scabrinodis* (és a laboratóriumi vizsgálataim alapján valószínű a *M. salina* is) a *Maculinea alcon* és a *M. 'rebeli'* közös gazdájának bizonyult a Kárpát-medencében. E megállapításaim összhangban vannak azokkal az újabb irodalmi adatokkal, amelyek szerint e két lepke genetikai variabilitása átfedő, és hogy filogenetikailag sem különülnek el egymástól.

Érdekesnek bizonyult evolúciós szempontból a *Maculinea nausithous* hangyagazda használata is, mivel elszigetelt erdélyi populációi más gazdafajt használnak (kizárólag *Myrmica scabrinodis*-t), mint a többi közép-európai populációja (kizárólag *Myrmica rubra*-t).

Adataim nem támasztják alá azt a korábbi hipotézist, mely szerint a kakukk *Maculinea* fajok gazdahasználata korlátozottabb, mint a predátoroké.

A kárpát-medencei populációk hangyagazda használatával kapcsolatos adataim rávilágítanak annak fontosságára, hogy a hangyagazda specificitást helyi szinten is vizsgálni kell.

A Kárpát-medencében földrajzilag a legbehatároltabb, és egyúttal a leginkább hangyagazda-specifikus lepkének a *Maculinea nausithous* bizonyult (habár nincsenek ismereteim a *M. arion* hangyagazdájáról), ami azt jelenti, hogy itt ez a *Maculinea* faj reagálhat a legérzékenyebben a *Myrmica* közösség változásaira (lásd: VI. függelék: 20).

A *Maculinea-Myrmica* kapcsolathoz kötődő ritka és hiányosan kutatott fajokra (*Ichneumon eumerus*, *Microdon myrmicae*, *Neotypus melanocephalus* és *Rickia wasmannii*) vonatkozó adataim egyértelműen alátámasztják azokat az irodalmi megállapításokat, melyek szerint a *Maculinea* boglárkalepkék az értékes élőhelyeik ernyőfajainak tekinthetők.



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## List of publications

### This thesis is based on the following papers

(referred in the text by their roman numerals; see Appendix I)

#### Peer reviewed articles in English

- I. TARTALLY, A. & VARGA, Z. 2008: Host ant use of *Maculinea teleius* in the Carpathian-basin (Lepidoptera: Lycaenidae) – Acta Zoologica Academiae Scientiarum Hungaricae 54 (in press) [IF: 0.511]
- II. TARTALLY, A. & VARGA, Z. 2005: *Myrmica rubra* (Hymenoptera: Formicidae): the first data on host-ant specificity of *Maculinea nausithous* (Lepidoptera: Lycaenidae) in Hungary. – Myrmecologische Nachrichten 7: 55-59.
- III. TARTALLY, A., RÁKOSY, L., VIZAUER, T.-C., GOIA, M. & VARGA, Z. 2008: *Maculinea nausithous* exploits *Myrmica scabrinodis* in Transylvania: unusual host ant species of a myrmecophilous butterfly in an isolated region (Lepidoptera: Lycaenidae; Hymenoptera: Formicidae). – Sociobiology 51 (in press) [IF: 0.590]
- IV. TARTALLY, A. 2005: *Myrmica salina* (Hymenoptera: Formicidae) as a host of *Maculinea alcon* (Lepidoptera: Lycaenidae). – Sociobiology 46: 39-43. [IF: 0.590]
- V. TARTALLY, A., NASH, D.R., LENGYEL, SZ., FÜRST, M.A. & VARGA, Z.: Patterns of host ant use by sympatric populations of *Maculinea alcon* and *M. 'rebeli'* in the Carpathian Basin. – Insectes Sociaux (under review) [IF: 1.618]
- VI. TARTALLY, A. 2005: Accelerated development of *Maculinea rebeli* larvae under artificial conditions (Lycaenidae). – Nota Lepidopterologica 27 (2004): 303-308.
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- VIII. TARTALLY, A. 2005: *Neotypus melanocephalus* (Hymenoptera: Ichneumonidae): the first record of a parasitoid wasp attacking *Maculinea teleius* (Lycaenidae). – Nota Lepidopterologica 28: 65-67.

#### Other articles

- IX. TARTALLY, A., SZÜCS, B. & EBSEN, J.R. 2007: The first records of *Rickia wasmannii* CAVARA, 1899, a myrmecophilous fungus, and its *Myrmica* LATREILLE, 1804 host ants in Hungary and Romania (Ascomycetes: Laboulbeniales; Hymenoptera: Formicidae). – Myrmecological News 10: 123. (published lecture Abstract)

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ÁRNYAS, E., BERECKZI, J., TÓTH, A., VARGA, K., PECSENYE, K., TARTALLY, A., KÖVICS, GY., KARSA, D. & VARGA, Z.: Oviposition preferences of *Maculinea alcon* (Lepidoptera: Lycaenidae) influenced by food-plant specialized aphids (*Aphis gentianae*) and fungal infection by *Puccinia gentianae*. – Ecological Entomology (under review) [IF: 1.960]

### Other articles

TARTALLY, A. & CSÖSZ, S. 2004: Adatok a *Maculinea* boglárkalepkék (Lepidoptera: Lycaenidae) kárpát-medencei hangyagazdairól. – Természetvédelmi Közlemények 11: 309-317.

### Book chapters

BONELLI, S., WORGAN, A.D.P., EVERETT, S., NAPPER, E., ELMES, G.W., STANKIEWICZ, A.M., SIELEZNIEW, M., WARDLAW, J.C., CANTARINO, S., TARTALLY, A., BALLETO, E. & SCHÖNROGGE, K. 2005: Host specificity in *Microdon myrmicae*, a sympatric social parasite to the *Maculinea* in moist grassland ecosystems. In: SETTELE, J., KÜHN, E. & THOMAS, J.A. (Eds.) Studies on the Ecology and Conservation of Butterflies in Europe Vol. 2: Species Ecology along a European Gradient: *Maculinea* Butterflies as a Model. – PENSOFT Publishers, Sofia-Moscow, pp. 69-71.

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## Lectures

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- LENGYEL, SZ., SZITTA, E., BÍRÓ, M. & TARTALLY, A. 2005: Experimental adoption of *Maculinea* caterpillars by *Myrmica scabrinodis* ants: behavioural aspects. – MacMan Conference, Laufen
- LENGYEL, SZ., TARTALLY, A. & SZITTA, E. 2004: Jósolható-e a *Maculinea* boglárkalepke-hernyók *Myrmica scabrinodis* hangyák általi befogadásának sikeressége a viselkedés alapján? – 9. Magyar Etológiai Konferencia, Göd, Abstract p. unnumbered
- SÍPOS, J.V., HORVÁTH, R., NAGY, A., RÁCZ I., TARTALLY, A. & VARGA, Z. 2005: *Maculinea alcon* and *Maculinea rebeli*: not two ESU's but two different units for conservation. – MacMan Conference, Laufen
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### Posters

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- ESPADALER, X., TARTALLY, A., SCHULTZ, R., SEIFERT, B. & NAGY, CS. 2007: Regional trends and local expansion rate in the invasive garden ant, *Lasius neglectus* (Hymenoptera, Formicidae): the status of the year 2005. – Insectes Sociaux 54: 293-301. [IF: 1.618]
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# APPENDIX





**Appendix I:** The following papers were used for this thesis

Paper I.

TARTALLY, A. & VARGA, Z. 2008: Host ant use of *Maculinea teleius* in the Carpathian-basin (Lepidoptera: Lycaenidae) – Acta Zoologica Academiae Scientiarum Hungaricae 54 (in press)



1 HOST ANT USE OF *MACULINEA TELEIUS* IN THE CARPATHIAN BASIN  
2 (LEPIDOPTERA: LYCAENIDAE)

3

4 TARTALLY, A. and VARGA, Z.

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8

9 **Key words:** Hungary, *Maculinea teleius*, *Myrmica*, social parasitism, Transylvania

10

11 **Running head:** Host ants of *Maculinea teleius*

12

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20

## 21 ABSTRACT

22

23 Host ant use of *Maculinea teleius* was investigated in 17 Hungarian and three  
24 Transylvanian (Romania) sites by opening *Myrmica* ant nests. A total of 856 nests of  
25 nine *Myrmica* species (*M. gallienii*, *M. rubra*, *M. ruginodis*, *M. sabuleti*, *M. salina*, *M.*  
26 *scabrinodis*, *M. schencki*, *M. specioides* and *M. vandeli*) were found and nests of six  
27 species (*M. gallienii*, *M. rubra*, *M. salina*, *M. scabrinodis*, *M. specioides* and *M.*  
28 *vandeli*) contained 114 *M. teleius* specimens in total. *M. rubra* and *M. scabrinodis* were  
29 the most frequently used host ants. *M. rubra* appeared to be more suitable in the western  
30 while *M. scabrinodis* proved to be more important in the eastern sites. *M. gallienii* and  
31 *M. salina* were only locally important hosts on a few sites. *M. specioides* and *M. vandeli*  
32 were parasitized only once. Five *Myrmica* nests also contained larvae of other  
33 *Maculinea* species. These results show a less restricted host ant use of *M. teleius* in the  
34 central part of the Carpathian Basin than records from France. Our results correspond  
35 with the host ant use data recorded from Poland.

36

37

## 38 INTRODUCTION

39

40 Larvae of the Scarce Large Blue butterfly, *Maculinea teleius* (BERGSTRÄSSER, 1779),  
41 have a socially parasitic life-cycle. They start their development by feeding on seeds in  
42 the flower heads of the host plant *Sanguisorba officinalis* L. A few weeks later, in their  
43 last, fourth instar, they descend to the ground. This is one of the most critical periods of  
44 their life-cycle when they need to be adopted by a suitable host ant colony. It requires  
45 not only a tolerant host ant colony but also a nest which contains enough brood because,

46 after leaving the host plant, larvae of *M. teleius* are predators of the ant brood (e.g.  
47 THOMAS *et al.* 1989, WYNHOFF 2001, THOMAS & SETTELE 2004). Different *Myrmica*  
48 LATREILLE, 1804 species and *Aphaenogaster japonica* (Hymenoptera: Formicidae) have  
49 been reported as host ants of *M. teleius* from different regions (Table 1 and references  
50 therein). It is important therefore to identify the local host ant species of this vulnerable  
51 butterfly for its conservation (MUNGUIRA & MARTÍN 1999, SETTELE *et al.* 2005, IUCN  
52 2006), and also because it can help shed light on the evolution of this type of parasitic  
53 interaction (ELMES *et al.* 1998, ALS *et al.* 2004).

54

55 Our aim was to investigate the host ant use of *M. teleius* in the central part of the  
56 Carpathian Basin. The current paper presents primarily unpublished data,  
57 complementary to our initially reported results (TARTALLY & CSÖSZ 2004, TARTALLY  
58 2005a, b, TARTALLY & VARGA 2005a, b, TARTALLY *et al.* in press).

59

60

## 61 MATERIALS AND METHODS

62

63 The host ant use of *M. teleius* was investigated in 20 sites (Fig. 1, Table 2). Three of  
64 these were in Transylvania, Romania (Fânațele Clujului: 23°37' N, 46°51' E; Răscruci:  
65 46°54' N, 23°47' E; and Șardu: 46°52' N, 23°24' E) and 17 in Hungary (Aggtelek: 48°28'  
66 N, 20°30' E; Drahos-rét: 48°34' N, 21°26' E; Drávaiványi: 45°50' N, 17°49' E; Fülesd:  
67 48°01' N, 22°38' E; Gödörháza: 46°45' N, 16°21' E; Gyertyánkúti-rétek: 48°29' N, 21°22'  
68 E; Hetefejércse: 48°08' N, 22°29' E; Hidegség: 47°23' N, 16°27' E; Ipolytarnóc: 48°14'  
69 N, 19°37' E; Kercaszomor: 46°46' N, 16°18' E; Kétyvölgy: 46°53' N, 16°12' E;  
70 Lászlótanya: 46°33' N, 16°12' E; Meszes: 48°27' N, 20°47' E; Nógrádszakál: 48°12' N,

19°32' E; Óriszentpéter: 46°51' N, 16°12' E; Velemér: 46°44' N, 16°21' E; Vörös-rét:  
47°46' N, 17°42' E). To obtain data on host ant use, *Myrmica* nests were carefully  
opened (usually without full excavation, to minimize disturbance) and the number of *M.*  
*teleius* larvae, pupae or exuvia (= 'specimens' in the following) present was recorded.  
Nests exclusively within 2m of *S. officinalis* host plants were chosen as this is the  
approximate foraging zone of *Myrmica* workers, and nests farther from the host plants  
are unlikely to adopt *Maculinea* larvae (ELMES *et al.* 1998). Investigations were usually  
completed in the early flying period (late July – early August) between 2000 and 2007.  
However, we did the investigation about one month before the flying period on sites  
(see TARTALLY & VARGA 2005a, TARTALLY *et al.* in press) where *M. teleius* and *M.*  
*nausithous* (BERGSTRÄSSER, 1779) co-occurred, because pupae of these two butterflies  
are rather similar (but pupae of *M. alcon* ([DENIS & SCHIFFERMÜLLER], 1775 are  
different from them; see ŚLIWIŃSKA *et al.* 2006). By this method we did not find pupae,  
only prepupal larvae, and the identification of *Maculinea* larvae is straightforward  
(ŚLIWIŃSKA *et al.* 2006). These larvae had obviously spent the winter in their host nests,  
surviving a critical period of their lives (ELMES *et al.* 2004). The number and species of  
*Maculinea* larvae found was noted after determination using a 20x hand lens in the  
field. Five to ten workers were collected from each *Myrmica* nest opened, and were  
preserved in 67 % ethanol for identification in the laboratory (using keys in Seifert  
1988).

91

To quantify the host ant specificity, the heterogeneity of the number of *M. teleius*  
specimens between nests of different species was compared using two methods. First, a  
Fisher exact test was used to compare the observed number of infected nests with the  
number expected if they were infected at random. Second, a Chi-squared statistic was

96 computed comparing the number of *M. teleius* specimens observed with the number  
97 expected based on the number of nests available. The significance of this was tested by  
98 reassigning each nest (and its associated number of *M. teleius* specimens) randomly to  
99 one of the *Myrmica* species observed at a site 100000 times (using the software program  
100 MacSamp: NASH 2007), with the constraint that the total number of nests of each  
101 species was the same as that observed. This gives a measure of the host specificity of  
102 the *Maculinea* at a site based on the observed distribution of *Maculinea* between nests,  
103 but the power of this test to detect heterogeneity in the distribution between ant species  
104 is low except for those cases in which many ant nests have been investigated.

105

106 The overall distribution of *M. teleius* specimens within nests was compared between  
107 species using the ‘MASS’ package (version 7.2-36) of the software package ‘R’  
108 (version 2.5.1; <http://www.R-project.org>) to perform tests that are the equivalent of  
109 General Linear Models with negative binomial errors.

110

111

## 112 RESULTS

113

114 A total of 856 nests of nine *Myrmica* species (*M. gallienii*, n = 31; *M. rubra*, n = 92; *M.*  
115 *ruginodis*, n = 7; *M. sabuleti*, n = 2; *M. salina*, n = 16; *M. scabrinodis*, n = 659; *M.*  
116 *schencki*, n = 2; *M. specioides*, n = 2; *M. vandeli*, n = 45) were investigated within 2 m  
117 from the *S. officinalis* host plants at the 20 sites (Table 2). In total 114 *M. teleius*  
118 specimens were found in 63 nests of six *Myrmica* species (*M. gallienii*, *M. rubra*, *M.*  
119 *salina*, *M. scabrinodis*, *M. specioides* and *M. vandeli*) in 14 sites. Nests of *M. ruginodis*,  
120 *M. sabuleti* and *M. schencki* were not found to be infested. Overall, two thirds of the

121 *Myrmica* species examined were used as hosts, and 7.4% of all nests were infested  
 122 (Table 2).

123

124 Five *Myrmica* nests also contained larvae of other *Maculinea* species besides *M. teleius*:  
 125 One *M. rubra* nest also contained larvae of *M. nausithous* (Kétyölgy: 8 *M. teleius* + 28  
 126 *M. nausithous*; TARTALLY & VARGA 2005a), one *M. scabrinodis* nest likewise contained  
 127 larvae of *M. nausithous* (Răscruci: 1 *M. teleius* + 1 *M. nausithous*, TARTALLY *et al.* in  
 128 press) and three *M. scabrinodis* nests contained larvae of *M. alcon*, too (Răscruci: 1 *M.*  
 129 *teleius* + 1 *M. alcon*, 1 *M. teleius* + 1 *M. alcon*, 1 *M. teleius* + 5 *M. alcon*; A. Tartally,  
 130 unpublished data). It should be noted that other *M. nausithous* and *M. alcon* specimens  
 131 were also found during our surveys in *M. teleius* sites (TARTALLY & CSÖSZ 2004,  
 132 TARTALLY 2005a, b, TARTALLY & VARGA 2005a, b, TARTALLY *et al.* in press; A.  
 133 Tartally, unpublished data).

134

135 The distribution of *M. teleius* specimens found in nests of different *Myrmica* species  
 136 was well described by a negative binomial distribution (GLM with negative binomial  
 137 errors and log-link; dispersion parameter  $k = 0.194$ : Goodness of fit test; Pearson chi-sq  
 138  $= 628.3$ , d.f. = 822,  $p = 0.999$ ), and differed significantly between sites (change in  
 139 deviance = 84.56, d.f. = 19,  $p < 0.001$ ), but not between host species (change in deviance  
 140  $= 11.81$ , d.f. = 8,  $p = 0.160$ ; Fig. 2). When the analysis was repeated with only the two  
 141 most frequently found and infested *Myrmica* species, it became clear that *M. rubra* was  
 142 significantly more suitable for *M. teleius* than *M. scabrinodis* ( $k = 0.196$ : between sites;  
 143 change in deviance = 77.45, d.f. = 18,  $p < 0.001$ : between ant species; change in  
 144 deviance = 4.37, d.f. = 1,  $p = 0.036$ ).

145



146 No population showed significant heterogeneity in host ant use, although *Lászlótanya*  
147 approached significance when the heterogeneity of the number of *M. teleius* specimens  
148 in the nests of different *Myrmica* species was compared (Table 2).

149

150

## 151 DISCUSSION

152

153 To our knowledge, this is the first study to focus directly on the host ant use of *M.*  
154 *teleius* in south-eastern Central Europe (although there are some results initially  
155 published in papers concentrating on other *Maculinea* species: TARTALLY & CSŐSZ  
156 2004, TARTALLY 2005a, b, TARTALLY & VARGA 2005a, b, VÁLYI NAGY & CSŐSZ 2007,  
157 TARTALLY *et al.* in press; see also BATÁRY *et al.* 2007). While the number of infested  
158 nests found in a site (Table 2) is usually too small to draw strong conclusions about host  
159 ant specificity, there are some general patterns.

160

161 *M. teleius* was found with six *Myrmica* species (*M. gallienii*, *M. rubra*, *M. salina*, *M.*  
162 *scabrinodis*, *M. speciosoides* and *M. vandeli*) therefore this butterfly appears not to have  
163 strict host ant specificity in the Carpathian Basin. However, it can be contrasted with the  
164 strict host ant specificity (to *M. scabrinodis*) initially found in some French and Polish  
165 sites (THOMAS *et al.* 1989, ELMES *et al.* 1998). Later these original results from Poland  
166 were supplemented with more data (STANKIEWICZ & SIELEZNIEW 2002, BUSZKO *et al.*  
167 2005, WITEK *et al.* 2005) and it is now clear that the host ant use of *M. teleius* is more  
168 complex there (Table 1).

169

170 If we follow the criteria (see ALS *et al.* 2004: supplementary table 10) that ‘primary  
171 hosts’ raise more than 10% of specimens in a population, it could be concluded that all  
172 the recorded host ant species would be ‘primary hosts’ in the different sites (Fig. 1,  
173 Table 2). Nevertheless, we should be careful with this statement because the number of  
174 *M. teleius* specimens recorded in a site was usually too low (more than 10 would be  
175 needed) for such a calculation (Table 2). Combining our data (see Results), we found  
176 only two ant species in the Carpathian Basin which reared more than 10% of *M. teleius*  
177 specimens found across the investigated sites (*M. rubra*, 26.3%; *M. scabrinodis*,  
178 60.5%). This is not surprising because these two *Myrmica* species were the most often  
179 recorded host ants (see Results and Table 2).

180

181 There were differences in the mean numbers of *M. teleius* specimens found in the nests  
182 of the different *Myrmica* species when the data were combined (Fig. 2), and this number  
183 was significantly higher for *M. rubra* than for *M. scabrinodis* when only these two  
184 major hosts were examined. Interestingly, *M. rubra* appeared to be more suitable in the  
185 western sites while *M. scabrinodis* proved to be more important in the eastern sites (Fig.  
186 1, Table 2). This phenomenon is simple to explain since *M. rubra* is usually rare or  
187 missing from the eastern sites (Table 2). Moreover, this ant usually forms larger  
188 colonies than *M. scabrinodis* (SEIFERT 1988) and therefore an average *M. rubra* nest can  
189 rear more *Maculinea* larvae (e.g. the infested *M. rubra* nest at Kétvölgy contained not  
190 only eight *M. teleius* but also 28 *M. nausithous* larvae which is an exceptionally high  
191 number of predatory *Maculinea* larvae within the same nest; see TARTALLY & VARGA  
192 2005a). Thus, it is not surprising that *M. rubra* was significantly more suitable for *M.*  
193 *teleius* than *M. scabrinodis* (see Results, Fig. 2).

194

195 In Poland, *M. scabrinodis* was regarded as the primary host ant of *M. teleius* for a long  
 196 time (THOMAS *et al.* 1989, ELMES *et al.* 1998) but recent results show that *M. rubra*, if  
 197 common on a site, is often a significantly more suitable host of *M. teleius* there  
 198 (STANKIEWICZ & SIELEZNIEW 2002, see also BUSZKO *et al.* 2005, WITEK *et al.* 2005).  
 199 The fact that *M. gallienii* was found to be an important host ant of *M. teleius* on sites in  
 200 both countries is another similarity between Poland and Hungary (at Kosyń in Poland:  
 201 STANKIEWICZ & SIELEZNIEW 2002; at Aggtelek in Hungary: Fig. 1, Table 2). However,  
 202 there were four Hungarian sites where *M. gallienii* was also recorded (Table 2) but was  
 203 not found to be infested.  
 204  
 205 The situation seems to be different in the case of *M. salina*. This species was recorded  
 206 in two Hungarian sites and it reared *M. teleius* at both of them (Fig. 1, Table 2).  
 207 Interestingly, *M. salina* was the only recorded host ant at Fülesd, where nests of *M.*  
 208 *scabrinodis* and *M. gallienii* (both of which are hosts in NE-Hungary, Fig. 1, Table 2)  
 209 were not found to be infested. It would be interesting to obtain more data from this site  
 210 to investigate whether there is a similarly strong adaptation of *M. teleius* to *M. salina* as  
 211 was found to be the case for *M. alcon* on the same site (see TARTALLY 2005a). One nest  
 212 of *M. specioides* (from two nests in two sites) and another of *M. vandeli* (from 45 nests  
 213 in four sites) were also found to be infested by *M. teleius* (Fig. 1, Table 2). *M. vandeli*  
 214 has already been recorded as a host of *M. teleius* (one nest with one specimen from  
 215 France or Poland; THOMAS *et al.* 1989, ELMES *et al.* 1998), however, as far as we know,  
 216 *M. specioides* and *M. salina* have not been recorded as hosts of *M. teleius* outside  
 217 Hungary (Table 1 and references therein).  
 218

219 The three *Myrmica* species not infested were among the rarest species in the survey  
220 (there were only seven *M. ruginodis*, two *M. sabuleti* and two *M. schencki* nests  
221 recorded in total; Table 2), which is consistent with the possibility that this is due to the  
222 low level of “sampling” of these species, but this effect may also be of importance for  
223 the butterflies themselves, if these *Myrmica* species are consistently rare. This is easily  
224 imaginable because there were no significant deviations from random distribution  
225 amongst ant species when the heterogeneity of the number of *M. teleius* specimens in  
226 the nests of different *Myrmica* species was compared in the different sites (Table 2).

227

228 The other two *Maculinea* species (*M.alcon* and *M. nausithous*) inhabiting marshy  
229 meadows often co-occur and have common host ants (and even commonly parasitized  
230 nests, see Results) with *M. teleius* in Hungary and Transylvania. For example, *M.*  
231 *teleius* and *M.alcon* commonly use *M. salina* at Fülesd, *M. vandeli* at Șardu and *M.*  
232 *scabrinodis* at Drahos-rét, Răscruci and Șardu (TARTALLY *et al.* in press, TARTALLY  
233 2005a, A. Tartally, unpublished data, Fig. 1, Table 2); *M. teleius* and *M. nausithous*  
234 commonly use *M. scabrinodis* at Răscruci and *M. rubra* at Hidegség and Kétvölgy  
235 (TARTALLY *et al.* in press, TARTALLY & VARGA 2005a). These contrast with results  
236 from western-Europe, where each *Maculinea* species depends on a single, and different,  
237 *Myrmica* species (THOMAS *et al.* 1989, ELMES *et al.* 1998).

238

239 It requires further investigation to determine if the differences between the host ant use  
240 of *M. teleius* on different sites simply reflects host ant availability, whether there is a  
241 coevolutionary mosaic of adaptation to local hosts (sensu THOMPSON 1999), or some  
242 other explanations (see THOMAS *et al.* 2005). Moreover, it would be useful to get  
243 information on the host ant specificity of *M. teleius* from further western Central

244 European regions (not only from France; THOMAS *et al.* 1989, ELMES *et al.* 1998) and  
245 so determine if the strength of host ant specificity is generally higher there than it was  
246 found in eastern Central Europe (in Poland and Hungary; THOMAS *et al.* 1989, ELMES *et*  
247 *al.* 1998, STANKIEWICZ & SIELEZNIEW 2002, BUSZKO *et al.* 2005, WITEK *et al.* 2005,  
248 this paper). It would be particularly interesting to test whether the number of host ant  
249 species of *M. teleius* declines towards the western parts of Europe, because the genus  
250 *Maculinea* is thought to have evolved in continental East Asia (SIBATANI *et al.* 1994)  
251 and European populations therefore can be considered peripheral. Similar phenomena  
252 are well known in the host plant specificity of polyphagous butterflies (e.g. DE LATTIN  
253 1967, MARTIN & PULLINS 2004, SCHMIDT & HUGHES 2006). Moreover, to answer this  
254 problem, it would be useful to get more knowledge about the host ant use of *M. teleius*  
255 in Asia because the scarce available data (Table 1 and references therein) suggest that  
256 there is little host ant specificity there.

257

258 The multiple host ant use of *M. teleius* found in Hungary and Transylvania could  
259 suggest that this butterfly can easily adapt to some new host ant species when  
260 circumstances (e.g. from human disturbance) change. However, we should be careful  
261 with such interpretations based on the currently limited amount of data available.

262

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Recorded host ants	Hungary (8)	Japan (1)	Mongolia (7)	Romania (8)	Poland (4, 5, 6)	Poland & France (2, 3)
<i>Aphaenogaster japonica</i> FOREL, 1911		+				
<i>Myrmica angulinodis</i> RUZSKY, 1905			+			
<i>M. forcipata</i> KARAVAIEV, 1931			+			
<i>M. gallienii</i> BONDROIT, 1919	+				+	
<i>M. kamtschatica</i> KUPYANSKAYA, 1986			+			
<i>M. kurokii</i> FOREL, 1907						
<i>M. lobicornis</i> NYLANDER, 1846						
<i>M. rubra</i> (LINNAEUS, 1758)	+				+	+
<i>M. ruginodis</i> NYLANDER, 1846		+			+	
<i>M. rugulosa</i> NYLANDER, 1846					+	
<i>M. sabuleti</i> MEINERT, 1860						+
<i>M. salina</i> RUZSKY, 1905	+					
<i>M. scabrinodis</i> NYLANDER, 1846	+			+	+	+
<i>M. specioides</i> BONDROIT, 1918	+					
<i>M. vandeli</i> BONDROIT, 1920	+			+		+

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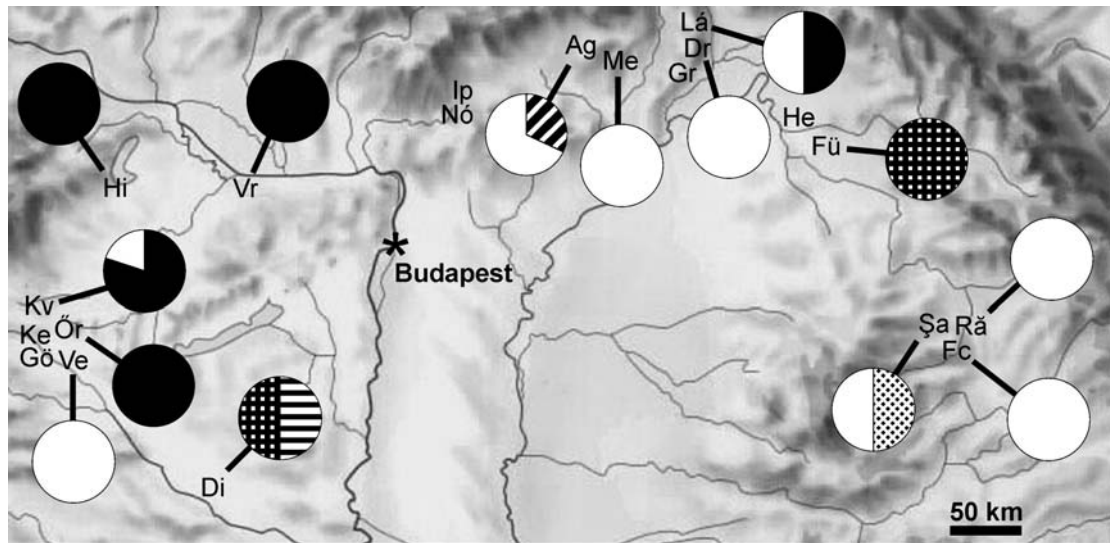
383 **Table 1.** Recorded host ants of *M. teleius* from the most intensively studied regions384 (references in parentheses: 1: Yamaguchi 1988, 2: Thomas *et al.* 1989, 3: Elmes *et al.*385 1998, 4: Stankiewicz & Sielezniew 2002, 5: Buszko *et al.* 2005, 6: Witek *et al.* 2005, 7:386 Woyciechowski *et al.* 2006, 8: this paper; for a more detailed review: Als *et al.* 2004:387 Supplementary table 10, Fiedler 2006: Digital supplementary material, Pech *et al.* 2007:

388 Table 1, 5).

Site (code on Fig. 1)	<i>Myrmica</i>	Sample size	Infested with <i>M. teleius</i>	Total number of <i>M. teleius</i>	Mean in examined nests	Mean in infested nests (range)	P1	P2
Aggtelek (Ag)	<i>M. gallienii</i>	17	2 (11.8 %)	7	0.41	3.5 (1-6)	0.759	0.568
	<i>M. sabuleti</i>	2						
	<i>M. scabrinodis</i>	78	6 (7.7 %)	15	0.19	2.5 (1-4)		
	<i>M. schencki</i>	2						
	<i>M. vandeli</i>	1						
Drahos-rét (Dr)	<i>M. scabrinodis</i>	53	2 (3.8 %)	2	0.04	1	1.00	0.602
	<i>M. vandeli</i>	19						
Drávaiványi (Di)	<i>M. salina</i>	1	1 (100.0 %)	1	1.00	1	•	•
	<i>M. specioides</i>	1	1 (100.0 %)	1	1.00	1		
Fânațele Clujului (Fc)	<i>M. scabrinodis</i>	58	1 (1.7 %)	1	0.02	1	•	•
Fülesd (Fü)	<i>M. gallienii</i>	6					0.410	0.494
	<i>M. ruginodis</i>	2						
	<i>M. salina</i>	15	3 (20.0 %)	5	0.33	1.7 (1-3)		
	<i>M. scabrinodis</i>	9						
Gödörháza (Gö)	<i>M. rubra</i>	4						
	<i>M. ruginodis</i>	1						
	<i>M. scabrinodis</i>	36						
Gyertyánkúti-rétek (Gr)	<i>M. ruginodis</i>	1						
	<i>M. scabrinodis</i>	9						
	<i>M. vandeli</i>	4						
Hetefejércse (He)	<i>M. scabrinodis</i>	5						
Hidegség (Hi)	<i>M. rubra</i>	8	3 (37.5 %)	17	2.13	5.7 (1-8)	•	•
Ipolytarnóc (Ip)	<i>M. gallienii</i>	6						
	<i>M. rubra</i>	10						
	<i>M. ruginodis</i>	2						
	<i>M. scabrinodis</i>	15						
Kercaszomor (Ke)	<i>M. rubra</i>	11						
	<i>M. scabrinodis</i>	37						
Kétvölgy (Kv)	<i>M. gallienii</i>	1					1.00	1.00
	<i>M. rubra</i>	14	1 (7.1 %)	8	0.57	8		
	<i>M. scabrinodis</i>	11	1 (9.1 %)	2	0.18	2		
	<i>M. specioides</i>	1						
Lászlótanya (Lá)	<i>M. rubra</i>	2	1 (50.0 %)	2	1.00	2	0.263	0.075
	<i>M. scabrinodis</i>	27	2 (7.4 %)	2	0.07	1		
Meszes (Me)	<i>M. rubra</i>	2					1.00	1.00
	<i>M. scabrinodis</i>	200	21 (10.5 %)	31	0.16	1.5 (1-4)		
Nógrádszakál (Nó)	<i>M. rubra</i>	7						
	<i>M. scabrinodis</i>	10						
Őriszentpéter (Őr)	<i>M. rubra</i>	21	1 (4.8 %)	1	0.05	1	1.00	1.00
	<i>M. scabrinodis</i>	10						
Răscruți (Ră)	<i>M. scabrinodis</i>	49	12 (24.5 %)	14	0.29	1.2 (1-2)	•	•
Șardu (Șa)	<i>M. scabrinodis</i>	26	1 (3.8 %)	1	0.04	1	1.00	1.00
	<i>M. vandeli</i>	21	1 (4.8 %)	1	0.05	1		
Velemér (Ve)	<i>M. gallienii</i>	1					1.00	1.00
	<i>M. rubra</i>	6						
	<i>M. scabrinodis</i>	13	1 (7.7 %)	1	0.08	1		
Vörös-rét (Vr)	<i>M. rubra</i>	7	2 (28.6 %)	2	0.29	1	0.195	0.1998
	<i>M. ruginodis</i>	1						
	<i>M. scabrinodis</i>	13						

390 **Table 2.** The number of *Myrmica* nests found and their recorded use by *M. teleius* in the  
391 investigated sites (see also Fig. 1); Two measures of hosts specificity are given: P1 is  
392 the 2-tailed probability from the Fisher exact test of heterogeneity in infection of host  
393 ant nests. P2 is the probability from a randomization test of ant nests between species  
394 (see Materials and Methods); •: test can not be calculated.

395



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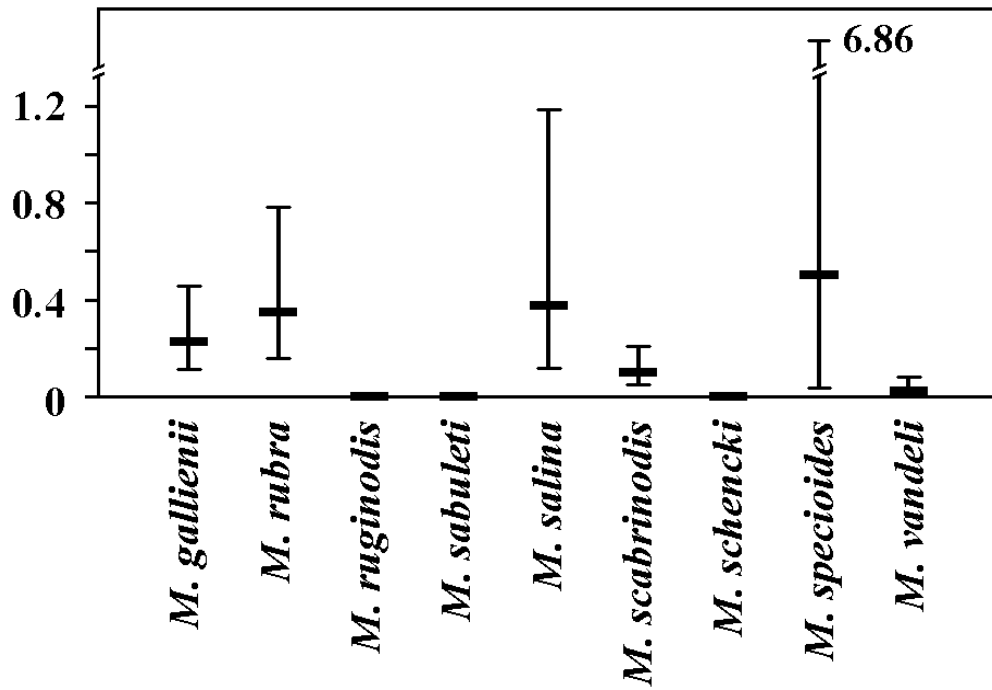
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▨: *M. gallienii*, ■: *M. rubra*, ▩: *M. salina*, □: *M. scabrinodis*, ▤: *M. speciosus*, ▦: *M. vandeli*

**Fig. 1.** The investigated sites and the proportion of *M. teleius* specimens found in nests of different *Myrmica* species (see Table 2 for more details and for a key to site codes)



400

401

402 **Fig. 2.** The mean number ( $\pm$  SE) of *M. teleiuss* specimens found in nests of the different  
 403 *Myrmica* ant species combining all data. Standard errors are based on a General Linear  
 404 Model with negative binomial errors ( $k = 0.194$ ) and log-link, and so are asymmetrical.





## Paper II.

TARTALLY, A. & VARGA, Z. 2005: *Myrmica rubra* (Hymenoptera: Formicidae): the first data on host-ant specificity of *Maculinea nausithous* (Lepidoptera: Lycaenidae) in Hungary. – Myrmecologische Nachrichten 7: 55-59.



## ***Myrmica rubra* (Hymenoptera: Formicidae): the first data on host-ant specificity of *Maculinea nausithous* (Lepidoptera: Lycaenidae) in Hungary**

András TARTALLY & Zoltán VARGA

### **Abstract**

In total, 58 overwintered *Maculinea nausithous* (BERGSTRÄSSER, 1779) larvae were found in nests of *Myrmica rubra* (LINNAEUS, 1758) from three sites in West Hungary. These results confirm that *M. rubra* is, in general, the host ant of *M. nausithous*. Twenty-eight larvae of *M. nausithous* and eight larvae of *M. teleius* (BERGSTRÄSSER, 1779) were found in a single *M. rubra* nest, which is a huge density for predacious butterfly species.

**Key words:** *Maculinea nausithous*, *Myrmica rubra*, host-ant specificity, Hungary

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### **Introduction**

Larvae of *Maculinea* VAN EECKE, 1915 (Lepidoptera: Lycaenidae) are obligate parasites of *Myrmica* LATREILLE, 1804 (Hymenoptera: Formicidae) colonies in Europe for most of their lives. After developing on an initial host plant, the last (fourth) instar caterpillars must be adopted by a suitable host ant colony to survive. They spend the majority of their lives within the ant nest and also pupate there (e.g., THOMAS & al. 1989). Knowledge of the host-ant species is crucial for the protection of these endangered butterflies (e.g., ELMES & al. 1998, MUNGUIRA & MARTIN 1999). Host-ant specificity may vary between regions as in the case of *Maculinea alcon* (DENIS & SCHIFFERMÜLLER, 1775) where it has been shown that different populations have evolved using different hosts in different parts of their geographical ranges (e.g., ELMES & al. 1994, 1998, ALS & al. 2002, TARTALLY 2005). Therefore data should be collected over the geographical range of a butterfly species' distribution. Data on the host-ant specificity of *Maculinea alcon*, *M. rebeli* (HIRSCHKE, 1904) and *M. teleius* (BERGSTRÄSSER, 1779) have been gathered from Hungary (TARTALLY & CSÖSZ 2004, TARTALLY 2005), but equivalent data for *M. arion* (LINNAEUS, 1758) and *M. nausithous* (BERGSTRÄSSER, 1779) are yet to be presented. The first records from Hungary on the host-ant specificity of *M. nausithous* (the Dusky Large Blue butterfly; Fig. 1) are given here.

### **Material and Methods**

*Maculinea nausithous* occurs only in the western part of Hungary (BALINT 1996). Two sites of the Órség region (at Kétvölgy, Fig. 2: 46° 53' N, 16° 12' E, on 26 May 2004; and at Gödörháza: 46° 45' N, 16° 21' E, on 25 May 2004) and one site of the Fertő region (at Hidegség: 47° 23' N, 16° 27' E, on 12 July 2005) were investigated in West Hungary (see Tab. 1, Fig. 3). Although the two sites of the Órség region were investigated relatively early in the

year (see Results and Discussion), our surveys were done a long time after the caterpillars had overwintered. During the winter, when ant colonies are starving, non-host colonies kill caterpillars more frequently than colonies of the host species (ELMES & al. 2004, SCHÖNROGGE & al. 2004). Moreover the caterpillars were larger during these investigations than at adoption (see Figs. 4 and 5). Thus, we believe that these surveys were carried out after the most critical periods of the caterpillars' life cycle.

All three studied sites are marshy meadows with a profusion of *Sanguisorba officinalis* L., which is the initial host plant species of *M. nausithous* (e.g., THOMAS 1984, WYNHOFF 2001). *Myrmica* nests within two metres of *S. officinalis* plants were opened carefully to check the presence of *M. nausithous* larvae in the three sites. Altogether 76 nests of five *Myrmica* species (determination according to SEIFERT 1988) were investigated (Tab. 1). The determination of *Maculinea* caterpillars (according to E. Śliwińska & M. Woyciechowski, pers. comm.) was confirmed by the allozyme patterns of some specimens (V. Mester, K. Pecsenye & J. Bereczki, pers. comm.). Reference samples are stored in the first author's collection and in the Hymenoptera Collection of the Hungarian Natural History Museum in Budapest.

Host specificity index ( $F$ ) was calculated following THOMAS & ELMES (1998) to compare the strength of the host specificity in the studied Hungarian populations with published data for other populations of *M. nausithous* (see also ALS & al. 2002).

### **Results and Discussion**

During our surveys 58 overwintered *M. nausithous* larvae were found in total, all of them in *Myrmica rubra* nests (Tab. 1, Fig. 4) in the three investigated sites ( $F = \infty$ ). These caterpillars were apparently in good general condi-

tion (see Figs. 4, 5). According to these results, we consider that *M. rubra* is likely to be the main host of *M. nausithous* in the Hungarian sites investigated here as in most of the previously investigated sites elsewhere (ELMES & al. 1998, KORB 1998, THOMAS & al. 1989, STANKIEWICZ & SIELEZNIEW 2002). However, it is important to note here that our fieldwork in the Őrség region was done in late May but the Hungarian *M. nausithous* populations start to fly in mid or late July depending on site and year (Z. Varga, pers. observ.). In spite of the relatively early sampling, the nests of the general host *M. rubra* repeatedly contained overwintered *M. nausithous* larvae at Gödörháza and Kétvölgy but other *Myrmica* species did not (Tab. 1). Similarly, although only one *M. nausithous* larva was found at the Fertő region at Hidegség, this larva was also found living in a *M. rubra* nest. No *Myrmica* species other than *M. rubra* were found there during our work (Tab. 1) and this larva was in the prepupal stage. These facts reflect the suitability of *M. rubra* as the host for *M. nausithous* at Hidegség. Thus, our results definitely support the earlier suggestions that *M. rubra* is the main host of *M. nausithous*, at least in most of this species' western range. The fact that one of the infected *M. rubra* nests contained 28 overwintered *M. nausithous* larvae (Tab. 1, Fig. 5) also confirms this statement since it is a huge number of parasitizing butterfly larvae for a predatory *Maculinea* species (THOMAS & ELMES 1998). This huge number of overwintered larvae within one nest appears to support the idea that the larvae of *M. nausithous* are possibly intermediate between the cuckoo and the predatory life forms of caterpillars (FIEDLER 1990, THOMAS & ELMES 1998, STANKIEWICZ & SIELEZNIEW 2002, THOMAS & SETTELE 2004). However, the question of the potential for cuckoo behaviour of *M. nausithous* larvae still needs thorough investigation in the laboratory. Another explanation of this result could be that these 28 *M. nausithous* caterpillars were found in a large nest of a polydomous *M. rubra* colony (A. Tartally, pers. observ.) which may have had the capacity to rear several caterpillars. Moreover, according to the relatively early sampling there is no evidence that all of these 28 *M. nausithous* larvae were able to finish their development. What we do know (see THOMAS & WARDLAW 1992, THOMAS & al. 1993) is that predacious caterpillars are subject to scramble competition (i.e., numbers get killed off and just a few big ones survive) and less likely to survive in high densities than cuckoo species which suffer from contest competition (i.e., more but smaller caterpillars survive). The *M. rubra* nest, which contained 28 *M. nausithous* larvae, was also infected by eight *M. teleius* larvae at the time of the investigation (Tab. 1, Fig. 5). Previously *M. rubra* has not been recorded as a host of *M. teleius* in Hungary (TARTALLY & CSÖSZ 2004, TARTALLY 2005) but it is mentioned as the main host of *M. teleius* in some Polish sites (STANKIEWICZ & SIELEZNIEW 2002). The most widespread host for *M. teleius* recorded in Europe is *M. scabrinodis* (THOMAS & al. 1989, ELMES & al. 1998, STANKIEWICZ & SIELEZNIEW 2002, TARTALLY & CSÖSZ 2004) and overwintered larvae of *M. teleius* were also found in nests of *M. scabrinodis* in the Őrség region (A. Tartally, unpubl. data).

*Myrmica scabrinodis* has also been recorded as a host of *M. nausithous* in Spain (see MUNGUIRA & MARTIN 1999). Hosts of *M. nausithous* other than *M. rubra* and



Fig. 1: A marked female of *M. nausithous* on a *Sanguisorba officinalis* flowerhead at Hidegség (photo by A. Ambrus).



Fig. 2: The site of *Maculinea nausithous* at Kétvölgy. Red lines sign the narrow zone where the nests of *M. rubra* occurred (photo by Z. Varga at the investigation).

*M. scabrinodis* have not been recorded anywhere (see ALS & al. 2004: supplementary tab. 10). During our work none of the 47 *M. scabrinodis* colonies that were searched contained larvae of *M. nausithous* in contrast to the host nests of *M. rubra* (Tab. 1). The number of *Myrmica gallienii* BONDROIT, 1920, *M. ruginodis* NYLANDER, 1846 and *M. speciosus* BONDROIT, 1918 nests that were examined was too small to establish their suitability for being a host of *M. nausithous* in Hungary. However, we suppose that they cannot serve as important *M. nausithous* hosts in the study sites since their nests were found only in small numbers there. Moreover, several other *Myrmica* species were also formerly recorded from both of the regions investigated – from the Őrség region: *M. sabuleti* MEINERT, 1861, *M. salina* RUZSKY, 1905, and *M. schencki* VIERECK, 1903; from the Fertő region: *M. microrubra* SEIFERT, 1993, *M. gallienii*, *M. sabuleti*, *M. salina*, *M. schencki*, *M. scabrinodis*,

Tab. 1: The number of *Myrmica* nests examined at the Hungarian sites (see Fig. 3) and the detailed results. The host specificity index ( $F$ ) was calculated following THOMAS & ELMES (1998). There was one nest (\*) which contained 28 *M. nausithous* and eight *M. teleius* larvae in total (see Fig. 5).

Site	<i>Myrmica</i> species	Sample size	Colonies with <i>M. nausithous</i>	Number of <i>M. nausithous</i> larvae in the infected nests	$F$
Kétvölgy	<i>M. rubra</i>	14	4	3, 6, 6, 28* ( $\Sigma$ 43)	$\infty$
	<i>M. scabrinodis</i>	11	0	0	
	<i>M. specioides</i>	1	0	0	
	<i>M. gallienii</i>	1	0	0	
Gödörháza	<i>M. rubra</i>	4	4	2, 2, 4, 6 ( $\Sigma$ 14)	$\infty$
	<i>M. ruginodis</i>	1	0	0	
	<i>M. scabrinodis</i>	36	0	0	
Hidegség	<i>M. rubra</i>	8	1	1 ( $\Sigma$ 1)	( $\infty$ )

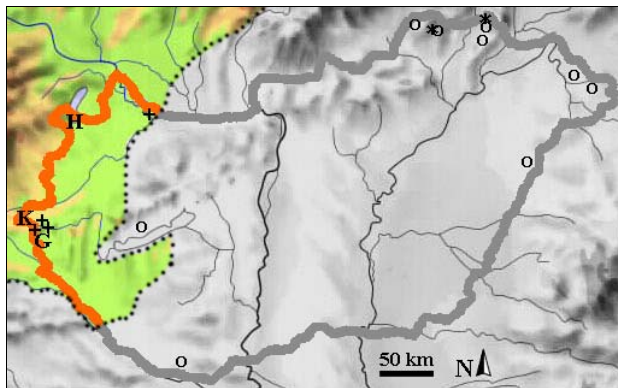


Fig. 3: The sites where *Maculinea nausithous* caterpillars were found in *Myrmica rubra* nests in Hungary (G: Gödörháza, K: Kétvölgy, H: Hidegség; see Tab. 1). Coloured area: the distribution of *M. nausithous* in Hungary and surrounds (according to BÁLINT 1996). *S. officinalis* - *M. teleius* sites (o, +, \*) where: neither *M. rubra* nor *M. nausithous* are known (o); *M. rubra* is known but *M. nausithous* is not (\*); both *M. rubra* and *M. nausithous* are known but there are no data on the host-ant specificity of the butterfly (+) (A. Tartally, pers. observ.).

and *M. specioides*; according to CSÖSZ & al. (2002) – but we did not find any specimens of these during the surveys. Knowledge of *Myrmica* species distribution and further investigations on these potential *Maculinea* hosts are crucial to studies in the Őrség region because four species of *Maculinea* (*M. alcon*, *M. nausithous*, *M. teleius*, and *M. arion*) co-exist within this region, and in some cases within the same locality (BÁLINT 1996, A. Ambrus pers. comm., Z. Varga pers. observ.).

These results are also of interest from a phylogenetic point of view because ALS & al. (2004) have observed two genetic forms in *M. nausithous*: they found that a specimen from Slovakia strongly diverged from the Polish and the Central-Russian specimens (see ALS & al. 2004: Fig. 2 and Supplementary Tab. 1). Potentially, these different forms of *M. nausithous* could use different host-ant species as has been found in *M. alcon* and *M. rebeli* (e.g.,



Fig. 4: An overwintered *Maculinea nausithous* caterpillar in a *Myrmica rubra* nest at Gödörháza (photo by P. Kozma at the investigation).

THOMAS & al. 1989); these two butterflies have been shown to be less different genetically than the two genetic forms of *M. nausithous* (ALS & al. 2004). As far as we know, there are no data on the host-ant specificity of *M. nausithous* from Slovakia, but there are such data from two neighbouring countries: *Myrmica rubra* was the only recorded host of *M. nausithous* both in Poland and Hungary (THOMAS & al. 1989, ELMES & al. 1998, STANKIEWICZ & SIELEZNIEW 2002; Tab. 1). ALS & al. (2004) stud-





Fig. 5: These 28 *Maculinea nausithous* and eight *M. teleius* caterpillars were found together in one *Myrmica rubra* nest (see Tab. 1). There is also a *M. rubra* worker on this photo to enable estimation of the size of the caterpillars (photo by P. Kozma at the investigation).

ied some *M. nausithous* specimens from Slovakia and Poland but they did not examine any from Hungary. Accordingly, it would be worth studying the host-ant specificity of *M. nausithous* in Slovakia and comparing *M. nausithous* specimens from Hungary genetically with Slovakian and Polish ones. It would be desirable to do similar experiments on the European southern fringe populations of *M. nausithous* in Slovenia (see WYNHOFF 1998), Bulgaria (KOLEV 2002) and especially the isolated and acutely endangered populations in Transylvania (Romania; BÁLINT 1996, RÁKOSY & LÁSZLÓFFY 1997, T. Cs. Vizauer pers. comm., A. Tartally, pers. observ.).

It would also be desirable to collect more data about the host specificity of *M. nausithous* in Hungary, because our unpublished results suggest that *M. rubra* frequently occurs in *S. officinalis* sites in West Hungary but not in East Hungary (see Fig. 3). It also appears that in East Hungary this ant species occurs in the adjacent marshy forests of the *S. officinalis* sites rather than on the meadows. An explanation for the absence of *M. nausithous* from East Hungary (see Fig. 3) could be that its host ant is usually not living in the *S. officinalis* sites there. However, to answer these questions a better knowledge of the host specificity of *M. nausithous* and a thorough knowledge of the distribution of the host ant species are necessary.

We suggest that – to be successful – management of *S. officinalis* meadows for Hungarian *M. nausithous* should include leaving a mosaic of scrub fragments and natural forest edges according to the ecological requirements of *M. rubra* (see ELMES & al. 1998; Fig. 2).

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#### Zusammenfassung

Insgesamt 58 überwinterte Raupen von *Maculinea nausithous* (BERGSTRÄSSER, 1779) wurden in Nestern von *Myrmica rubra* (LINNAEUS, 1758) an drei Stellen in Westungarn gefunden. Unsere Ergebnisse bestätigen, dass *M. rubra*, im allgemeinen, als Wirtsameise von *M. nausithous* fungiert. In einem *M. rubra* Nest fanden wir 28 Larven von *M. nausithous* und acht Larven von *M. teleius* (BERGSTRÄSSER, 1779) – eine für räuberische Schmetterlinge enorme Zahl von Larven.

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### Paper III.

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*Maculinea nausithous* exploits *Myrmica scabrinodis* in Transylvania: unusual host  
ant species of a myrmecophilous butterfly in an isolated region (Lepidoptera:  
Lycaenidae; Hymenoptera: Formicidae). – Sociobiology 51 (in press)



***Maculinea nausithous* Exploits *Myrmica scabrinodis* in  
Transylvania: Unusual Host Ant Species of a Myrmecophilous  
Butterfly in an Isolated Region (Lepidoptera: Lycaenidae;  
Hymenoptera: Formicidae)**

by

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Zoltán Varga<sup>1</sup>

## ABSTRACT

Isolated populations of the myrmecophilous Dusky Large Blue butterfly (*Maculinea nausithous*) occur in Transylvania (Romania). The hitherto unknown host ant specificity of these populations was investigated at two sites, where *Myrmica scabrinodis* was the only potential host ant found. A total of 107 *M. scabrinodis* nests were opened in early summer to check for the presence of *M. nausithous* larvae, and two of them contained overwintered larvae. Our observations suggest that, like the habitat, the host ant of these isolated populations essentially differs from other central European *M. nausithous* populations studied, which use exclusively *Myrmica rubra*.

Keywords: host specificity, local host, *Maculinea nausithous*, myrmecophily, *Myrmica scabrinodis*, Transylvania

## INTRODUCTION

Larvae of *Maculinea nausithous* (Bergsträsser) are obligate social parasites of *Myrmica* Latreille ant nests, after developing on *Sanguisorba officinalis* L. host plant (e.g. Thomas *et al.* 1989). The identification of the local host ant species is not only crucial for the conservation of this vulnerable butterfly (Munguira & Martín 1999, Settele *et al.* 2005, IUCN 2006), but also because it can help shed light on the evolution of this type of parasitic interaction (Elmes *et al.* 1998, Als *et al.* 2004). *M. nausithous* almost exclusively exploits

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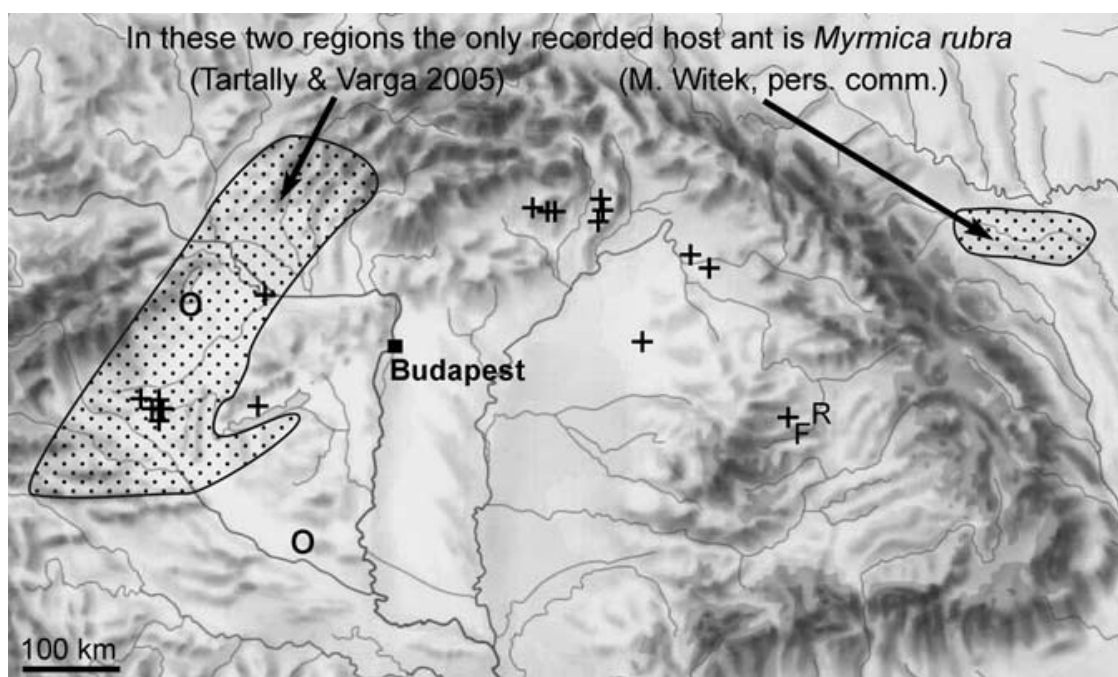


Fig. 1. The distribution and host ant use of *Maculinea nausithous* in and around the Carpathian-Basin. F: Fânațele Clujului, R: Răscruci (the sites investigated in this study), +: myrmecologically investigated (by A.T.) *Sanguisorba officinalis*–*Maculinea teleius* sites where *M. scabrinodis* was recorded, o: such sites where *M. scabrinodis* was not recorded (data from Bálint 1996, Wynhoff 1998, recent and unpublished data; see also Tartally & Varga 2005: Fig. 3).

*Myrmica rubra* (Linnaeus) nests in Europe (Thomas *et al.* 1989, Elmes *et al.* 1998, Korb 1998, Stankiewicz & Sielezniew 2002, Als *et al.* 2004, Tartally & Varga 2005; Fig. 1). However, *Maculinea* host ant specificity may vary between regions (e.g. Elmes *et al.* 1998), and *M. nausithous* has some rather isolated populations in Transylvania (Romania) (Rákossy & Lászlóffy 1997; Fig. 1) that differ somewhat in habitat from other *M. nausithous* sites. The aim of this study was therefore to investigate host ant use in these isolated populations.

## MATERIALS AND METHODS

Only two *M. nausithous* sites are known from Transylvania (Fig. 1). Both of them are in the Câmpia Transilvaniei region, near Cluj-Napoca: one at Răscruci (N46°54', E 23°47' 485 m a.s.l.; exact localities are not given to avoid exploitation), another at Fânațele Clujului (N46°51', E23°37'; 540 m; more details of this site are given by Rákossy & Lászlóffy 1997). Both sites are semi-dry meadows with steppe character, with sporadic small boggy depressions (Fig. 2). *S. officinalis*, the host plant, occurs in a mosaic in these small



Fig. 2: The site at Răscruci where *Maculinea nausithous* larvae were found in *Myrmica scabrinodis* nests (photo by L. Rákósy; compare with Tartally & Varga 2005: Fig. 2, where *M. nausithous* was found with *Myrmica rubra*).

depressions, creating potential metapopulation networks of *M. nausithous* subpopulations (e.g. Hanski 1999). Both known sites were investigated in this study, but it should be noted that the Câmpia Transilvaniei region is rather poorly studied, so that occurrence of other, as yet undiscovered, *M. nausithous* sites in the area is likely.

To obtain data on host specificity, *Myrmica* nests within 2 m of *S. officinalis* host plants were carefully opened (usually without full excavation, to minimize disturbance) on both sites, and the presence or absence of *M. nausithous* larvae was recorded. Nests within 2m of host plants were chosen as this is the approximate foraging zone of *Myrmica* workers, and nests further from the host plants are unlikely to adopt *Maculinea* larvae (Elmes *et al.* 1998). The investigations were from late May to early July 2002 and 2007, so that all the recorded larvae had spent the winter in their host nests, surviving one of the most critical periods for the butterfly (Elmes *et al.* 2004). Investigations were completed before the pupation period in mid July, since *M. teleius* (Bergsträsser) and *M.alcon* (Denis & Schiffermüller) also develop in the boggy depressions

(and *M. arion* (Linnaeus) in the adjacent drier patches at Fânațele Clujului), and pupae of *M. teleius* and *M. nausithous* are rather similar (Śliwińska *et al.* 2006) which could result in the confusion of these two syntopic species. However, the identification of *Maculinea* larvae is straightforward (Śliwińska *et al.* 2006). The number and species of *Maculinea* larvae found was noted after determination using a 20x hand lens in the field. Five to ten workers were collected from each *Myrmica* nest opened, and were preserved in 67 % ethanol for identification in the laboratory (using keys in Seifert 1988).

## RESULTS

A total of 107 *Myrmica* nests were found within 2 m from the *S. officinalis* host plants at the two sites (58 at Fânațele Clujului and 49 at Răscruci), and checked for *Maculinea* larvae. All 107 nests proved to be *M. scabrinodis* Nylander. Two nests from Răscruci were infested by *M. nausithous*, both of them containing only a single *M. nausithous* larva. Larvae of *M. alcon* and *M. teleius* were also found in *M. scabrinodis* nests during our survey (A. Tartally, unpublished data), which is not surprising since *M. scabrinodis* is a common host ant of both butterflies (for a review: Elmes *et al.* 1998, Als *et al.* 2004). One of the two nests infested by *M. nausithous* also contained a *M. teleius* larva.

## DISCUSSION

To our knowledge, this is the first study to provide data on the host ant use of *M. nausithous* in Transylvania. The use of *M. scabrinodis* as a host ant by *M. nausithous* is, on the one hand, not surprising, since this was the only *Myrmica* ant species found in the vicinity of the initial larval host plant, while on the other hand being highly unusual, as this butterfly is found almost exclusively in nests of *Myrmica rubra* in other parts of its range (Thomas *et al.* 1989, Elmes *et al.* 1998, Korb 1998, Stankiewicz & Sielezniew 2002, Tartally & Varga 2005 and M. Witek, pers. comm.). Although *Myrmica rubra* occurs in Transylvania, where it is connected with damp forested habitats in the eastern part of the Carpathian-Basin, this ant is not known from the sites investigated here, despite extensive surveys by local myrmecologists (B. Markó, pers. comm.). Other *Myrmica* species (*M. hellenica* Finzi, *M. sabuleti* Meinert, *M. schencki* Viereck, and *M. specioides* Bondroit) have been recorded

from the drier patches (Markó 1998, Markó & Csősz 2001; B. Markó, pers. comm.; A. Tartally, pers. observ.), but only *M. scabrinodis* is known from the boggy depressions where *M. nausithous* can lay eggs on *S. officinalis*. Thomas *et al.* (2005) provide some warnings and guidelines about recording host ant use in *Maculinea* butterflies, and although the sample of infested nests that we found was small, we believe that the comprehensive survey that we made of the *Myrmica* fauna on the investigated sites means that these records represent genuine specialization.

The rate of parasitism of *M. scabrinodis* nests that we found was low (1.9% of nests investigated overall, 4.1% of nests at Răscruci), which is an order of magnitude lower than parasitism rates previously recorded for *M. nausithous* (Stankiewicz & Sielezniew 2002, Tartally & Varga 2005, A. Tartally, unpublished data; Mean parasitism rate of other studies = 44.9%; GLM with Binomial Errors:  $c^2 = 56.79$ , d.f. = 3,  $p < 0.0001$ ). If the *M. nausithous* populations on these sites persist as a local metapopulation, then high variance in parasitism rates between sub-populations might be expected, so the significance of the low parasitism rate awaits further investigation.

Our records are not the first of *M. nausithous* exploiting *M. scabrinodis*, since Munguira & Martín (1999) report this ant as a *M. nausithous* host from Spain. However, apart from this one record, this widespread *Myrmica* species has not been recorded as a host of *M. nausithous* on the other European sites studied (although *M. scabrinodis* is often common on those sites), where *M. rubra* is used exclusively (Thomas *et al.* 1989, Elmes *et al.* 1998, Korb 1998, Stankiewicz & Sielezniew 2002, Tartally & Varga 2005; see Fig. 1). Interestingly *M. nausithous* occurs only in western parts of Hungary where *M. rubra* is common on *S. officinalis* sites, but this butterfly does not occur in central and eastern parts of Hungary where *M. rubra* is rare or missing from such sites. However, *M. scabrinodis* is common in most of the Hungarian *S. officinalis* sites investigated (Fig. 1). Hence, it is an open question as to why the eastern Hungarian *S. officinalis* sites are not colonised from Transylvania by *M. scabrinodis* using *M. nausithous*. One reason could be that the high mountains of Muntii Apuseni are barriers for the isolated Transylvanian *M. nausithous* populations that inhibit spread to eastern Hungary. Another possible explanation is that *M. teleius* and *M. alcon* populations are in competition with *M. nausithous* in eastern Hungary through their common use of *M. scabrinodis*.

as their primary host ant (Tartally & Csősz 2004, Tartally & Varga 2005; A. Tartally, unpublished data). Interestingly *M. nausithous* does not occur at Șardu (in a hilly region at the western border of the Transylvanian-Basin; N46°52', E23°24'; 480 m; the easternmost "+" on Fig. 1) where a potential *M. nausithous* site is known near to the Câmpia Transilvaniei region, with high densities of *S. officinalis* and *M. scabrinodis* (A. Tartally, unpublished data). This site is, however, used by *M. teleius* and *M.alcon* (both butterflies exploit *M. scabrinodis* and *M. vandeli* Bondroit for host ant; A. Tartally, unpublished data), and appears more similar to the central and western European *M. nausithous* sites (with bushy forest edges; see: Tartally & Varga 2005: Fig. 2) than the sites investigated in the Câmpia Transilvaniei region (which are meadows with some isolated bushes; Fig. 2). All these facts suggest that the Transylvanian *M. nausithous* populations represent a specific life form that needs further investigation and protection. The acuteness of this task is underlined by the low density of these populations. Moreover, phylogeographic studies of these populations would be of major interest, since Als *et al.* (2004) found considerable genetic diversity within European *M. nausithous* samples, suggesting potentially cryptic species.

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## Paper IV.

TARTALLY, A. 2005: *Myrmica salina* (Hymenoptera: Formicidae) as a host of *Maculineaalcon* (Lepidoptera: Lycaenidae). – Sociobiology 46: 39-43.



# ***Myrmica salina* (Hymenoptera: Formicidae) as a Host of *Maculineaalcon* (Lepidoptera: Lycaenidae)**

by

András Tartally

## **ABSTRACT**

Numerous overwintered *Maculineaalcon* (Denis & Schiffermüller 1775) larvae and a single pupa were found in nests of *Myrmica salina* Ruzsky 1905 in the Szatmár lowland, NE Hungary. This ant species appears to be the primary host of *M.alcon* at this site. *M. salina* has previously been recorded as a host of *Maculinea teleius* (Bergsträsser 1779) at this, and one other site. Nearby, other local populations of *M.alcon* use *Myrmica scabrinodis* Nylander 1846 as a host, so it appears that this particular *M.alcon* population has become locally adapted to use *M. salina* as a host ant because of the secondary salinization of this site.

**Keywords:** *Myrmica salina*, *Maculineaalcon*, host specificity, myrmecophily, new host species, local adaptation, Hungary.

## **INTRODUCTION**

Larvae of *Maculinea* van Eecke 1915 are obligate parasites of *Myrmica* Latreille 1804 colonies during most of their development. The host ant species has been shown to be crucial for the protection of these endangered butterflies (see e.g. Elmes *et al.* 1998). *Myrmica rubra* (Linnaeus 1758), *M. ruginodis* Nylander 1846 and *M. scabrinodis* Nylander 1846 are the known hosts of *Maculineaalcon* (Denis & Schiffermüller 1775) in western Europe, where different populations of this butterfly have evolved to use these three hosts in varied proportions (Thomas *et al.* 1989; Elmes *et al.* 1994, 1998; Als *et al.* 2002). In contrast, only *M. scabrinodis* was considered a host of *M.alcon* in central Europe (Sielezniew & Stankiewicz 2002, Höttinger *et al.* 2003, Tartally & Csősz 2004). Sielezniew & Stankiewicz (2004) recently found two localities in Poland where both *M. scabrinodis* and *M. vandeli* Bondroit 1920 are the hosts of *M.alcon*, increasing the global number of known *Myrmica* host species (see: Als *et al.* 2004: Supplementary Table 10) to four.

## METHODS

During 2002 and 2004 I repeatedly searched a known *M. alcon* site in a slightly salinized marshy meadow near the village of Fülesd (Szatmár lowland, NE Hungary; N 48.05°, E 22.75°) for *Myrmica* nests. Each nest (2 *M. gallienii* Bondroit 1919, 1 *M. ruginodis*, 9 *M. salina* Ruzsky 1905 and 4 *M. scabrinodis* nests; determination according to Seifert 1988) situated within 2 m of the initial host plant *Gentiana pneumonanthe* L. was investigated (see: Als *et al.* 2002) in a 20 x 50 m area. Further nests were not opened in order to minimize the disturbance to this small, protected and endangered site.

## RESULTS

On 7 May 2004 two *M. salina* nests were found to contain 61 and 5 *M. alcon* larvae respectively. The nests were marked and the site was revisited on 29 June 2004 (the flying of *M. alcon* started three weeks later, Varga personal communication). At this time, the ants had vacated these disturbed nests, presumably taking the *M. alcon* larvae with them. However, a single *M. alcon* pupa and 39 prepupal larvae (which is a huge number; see: Elmes *et al.* 1994, Thomas & Elmes 1998, Als *et al.* 2002, Höttinger *et al.* 2003, Tartally & Csősz 2004) were found in another *M. salina* nest, about 25 m from the nest which contained 61 *M. alcon* larvae in May. An additional three prepupal *M. alcon* larvae were also found in another *M. salina* nest. No *M. alcon* larvae or pupae were found in the nests of other *Myrmica* species on this site. Voucher samples (revised by Csősz) are stored in the authors collection and in the Hymenoptera Collection of the Hungarian Natural History Museum/Budapest.

## DISCUSSION

These results do not exclude the possibility that *M. alcon* may use other species of *Myrmica* as hosts at Fülesd because the number of *M. scabrinodis*, *M. gallienii* and *M. ruginodis* nests examined was small. However, they do show that *M. salina* is an important host ant of *M. alcon* at this site, as had already been suggested by adoption experiments (Tartally & Csősz 2004, Tartally unpublished work). Presumably, *M. scabrinodis* was the only host ant of *M. alcon* from Hungary recorded in the published literature (Tartally & Csősz 2004) and *M. salina* has never previously been implicated as a host of *M. alcon* in Hungary (Tartally unpublished work) or in Transylvania, W. Romania (Csősz personal communication). Moreover, prepupal *M. alcon* larvae were observed exclusively in *M. scabrinodis* nests at a near lying

lowland site in NE Hungary (about 32 km from Fülesd, at Hetefejércse, Bereg lowland; N 48.15, E 22.45; Tartally unpublished work). These observations suggest that *M. alcon* has locally adapted to use *M. salina* as its primary host ant at Fülesd. This is likely to be a relatively recent phenomenon, since one part of the site at Fülesd was used as a rice field during the 1950s, which may have resulted in the secondary salinization of the soil. *M. salina* is characteristic for habitats with a particularly high salinity (Seifert 1988).

Sielezniew & Stankiewicz (2004) hypothesized that *M. vandeli* (probably a temporary social parasite of *M. scabrinodis* nests, Elmes *et al.* 2003) has a similar chemical profile to *M. scabrinodis*, allowing *M. alcon* larvae to easily develop in the nests of *M. vandeli* at two Polish sites. It would also be interesting to compare the chemical signatures of *M. salina* with those of *M. scabrinodis* and *M. alcon* larvae from Fülesd.

Nests of *M. salina* from the Fülesd site and at another Hungarian site were found to have *Maculinea teleius* (Bergsträsser 1779) larvae (Tartally & Csősz 2005). These were the first records for *M. salina* as a host ant of any *Maculinea* species (see: Als *et al.* 2004: Supplementary Table 10, but see: Tartally & Csősz 2004). My observations in 2004, of a single caterpillar being found with *M. salina* on 7 May and another on 29 June, give additional support for the use of *M. salina* by *M. teleius* at Fülesd. I have never found larvae of *M. teleius* and *M. alcon* together in the same *M. salina* nest.

Under laboratory conditions a *Maculinea rebeli* (Hirschke 1904) larva from Hungary has also been raised to pupation in a *M. salina* colony (Tartally 2004). This is not surprising considering the overall genetic similarity of *M. alcon* and *M. rebeli* (Als *et al.* 2004, Bereczki *et al.* 2005), although these butterflies often have different host ant species in the same region (see: Thomas *et al.* 1989; Elmes *et al.* 1994, 1998; Als *et al.* 2002; Sielezniew & Stankiewicz 2002; Höttinger *et al.* 2003; Steiner *et al.* 2003; Sielezniew & Stankiewicz 2004; Tartally & Csősz 2004). This larva pupated only 35 days after adoption, but this laboratory result has not been confirmed by field records yet (Tartally 2005).

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Paper V.

TARTALLY, A., NASH, D.R., LENGYEL, SZ., FÜRST, M.A. & VARGA, Z.: Patterns of host ant use by sympatric populations of *Maculineaalcon* and *M. 'rebeli'* in the Carpathian Basin. – Insectes Sociaux (under review)



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

**Running head:** Host ants of sympatric Alcon Blues in the Carpathian Basin

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

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**Summary.** *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. *Maculineaalcon* and *Maculinearibli* have traditionally been separated according to their use of different host plant and host ant species, but recent genetic evidence has questioned their separation as good species. Here we compare the use of host ants by *M.alcon* and *M.rebili* at the regional scale in NE-Hungary and Transylvania, 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.rebili*-sites and one site where both *M.alcon* and *M.rebili* are sympatric. *Myrmica scabrinodis* was found to be a general host of both *M.alcon* and *M.rebili*, 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* species, which may simply reflect differences in *Myrmica* communities between the two types of habitat. A pilot comparison of the segregation of a small number of *Maculinea* from the sympatric site based on nine variable microsatellite markers suggests that there is no clear genetic differentiation between *M.alcon* and *M.rebili* individuals. Possible explanations for the similar but different host use patterns of *M.alcon* and *M.rebili* and their relevance for the question of whether they are separate species are discussed.

## 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; Pierce et al., 2002; Fiedler, 2006). 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 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; Thomas and Settele, 2004; Settele et al., 2005; IUCN, 2006). 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).

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

83

84 Their specificity to different host ants has long been thought as one of the main differences between  
85 the two Alcon Blues (Thomas et al., 1989). Host ants of the Alcon Blues are known exclusively  
86 from the genus *Myrmica* Latreille, 1804 (Als et al., 2004; Settele et al., 2005). Larvae of the Alcon  
87 Blues spend 11 or 23 months in the ant nest where they are fed by the ants by trophallaxis  
88 (“cuckoo-type” caterpillars, Thomas and Elmes, 1998) and may also prey on ant brood (e.g.,  
89 Tartally, 2004). In their seminal work, Thomas et al. (1989) found *Myrmica ruginodis* Nylander,  
90 1846 as the main host ant of *M.alcon* in the Netherlands and *M.schencki* Viereck, 1903 for *M.*  
91 *rebeli* in France. Subsequent studies have refined this by showing that the two butterflies use  
92 different main host ant species in different parts of their geographical range (Elmes et al., 1994,  
93 1998; Als et al., 2002; Meyer-Hozak, 2002; Sielezniew and Stankiewicz, 2002; Höttinger et al.,  
94 2003; Steiner et al., 2003). The main hosts reported in these studies include *M.rubra* (Linnaeus,  
95 1758), *M.ruginodis* and *M.scabrinodis* Nylander, 1846 for *M.alcon*; and *M.schencki* and *M.*  
96 *sabuleti* Meinert, 1860 for *M.rebeli* (see Als et al., 2004 and Settele et al., 2005 for a review of  
97 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; Pecsénye et al. 2007).

## **Material and methods**

### *1. Separation of Alcon Blues*

*Maculinea rebeli* was initially described as a subspecies of *M.alcon*, based on differences in habitat use (Hirschke, 1904). In recent years, since the discovery of differences in host plant and host ant use, it has become common 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; Pecsénye 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 humid 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. observ.). 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 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 in seven sites (Drahos-rét, Fülesd, Gyilkos-rét, Hetefejércse, Nyikom-rét, Șardu and Tugár-rét), that of *M. rebeli* only in six sites (Bükkszentkereszt, Kecskeláb-rét, Kuriszlán, Lófő-tisztás, Nagy-mező and Tohonya-hát) and that of both butterflies on one site (Răscruci) (see Table 1).

Approx position of  
figure 1 and 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 exuvia 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 among 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). Estimating the numbers of caterpillars that are discovered by a particular ant species in the field is not normally practicable, so here we have assumed that the proportion of nests of the different *Myrmica* species found within 2m of randomly chosen *Gentiana* plants is equal to the proportion of caterpillars of Alcon Blues that are discovered by the different ant species. 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 are 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 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 overdispersed, 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 ant community was compared between *M. alcon* and *M. ‘rebeli’* sites by using the software package EstimateS 8.0 (Colwell, 2006) to compute sample-size corrected pair wise similarity indices for the ant communities between every pair of sites, and then by comparing the values of these indices between sites where different host plants were present with those where the same host plant was present. A range of different indices were used, but all gave essentially the same result, so the index presented here is the Chao abundance based sample-size corrected version of Sørensen’s index (Chao et al., 2005).

#### 4. Genetic analysis

For the site at Răscruci, where *M.alcon* and *M.rebeli* populations were sympatric (patches with *G.pneumonanthe* and *G.cruciata* were separated by less than 20 m), some preliminary population genetic analysis was carried out on 8 *M.alcon* caterpillars collected from *M.scabrinodis* nests and on 10 *M. 'rebeli'* caterpillars, 5 of which were collected from *M.schencki* nests and 5 from *M.sabuleti* nests. Only small samples were taken at this site to minimize damage to the population, which has only recently been discovered, and the size and extent of which is not yet known. Genetic differentiation between these two groups was compared by examining variation among alleles at nine microsatellite loci (Macu 20, Macu 26, Macu 28, Macu 29, Macu 30, Macu 31, Macu 40, Macu 44, Macu 45). The number of individuals compared was small, so this must be regarded as a preliminary analysis. Methods for DNA extraction were the same as those presented by Zeisset et al. (2005). PCRs (20 µl) contained ca. 25 ng template DNA, 20 pmol of each primer, 80 µmol of each dNTP, 1× GeneAmp PCR buffer II (10 mM Tris-HCl pH 8.3, 50 mM KCl; Applied Biosystems) in 1.5 mM MgCl<sub>2</sub> Solution and 0.4 U AmpliTaqGold polymerase (Applied Biosystems). Thermal cycling was carried out in a Hybaid PCR Express thermocycler and consisted of: 15 min at 95 °C, then 30 cycles of 30 sec at annealing temperature, 30 sec at 72 °C and 30 sec at 95 °C, followed by 1 min at annealing temperature and a final extension of 30 min at 72 °C. Allelic lengths were determined on an ABI 3130xl sequencer using 5' fluorescent labelled forward primers. Microsatellite genotypes were obtained using GeneMapper 4.0 (Applied Biosystems).

Differentiation between *M.alcon* and *M. 'rebeli'* samples was tested by calculation of Wright's  $F_{ST}$  and comparison on this value with zero based on 10000 permutations using the software package SPAGeDi version 1.2g (Hardy and Vekemans, 2002), which was also used to test for mutation vs drift in microsatellite allele size by comparing Slatkin's  $R_{ST}$  with Wright's  $F_{ST}$  (Hardy et al. 2003). The population assignment packages STRUCTURE 2.2 (Pritchard et al., 2000) and BAPS 5.0

(Corander and Marttinen, 2006) were used to examine possible population substructure within the individuals tested, and to compare this with their origin from *M. alcon* or *M. 'rebeli'* areas. STRUCTURE was run using the no admixture model with independent allele frequencies, and a burn-in time of 100000 generations and 500000 MCMR replicates. Values of k between 1 and 18 were explored, each replicated a minimum of five times, and that giving the highest posterior probability chosen. Clustering of individuals was performed in BAPS, giving 18 as the maximum number of groups.

## 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 diversity of *Myrmica* species on *M. 'rebeli'* sites (Shannon-Weiner index  $\pm$  SE =  $1.25 \pm 0.18$ ) than *M. alcon* sites ( $0.63 \pm 0.22$ ;  $F_{1,13} = 4.72$ ,  $p = 0.049$ ). More *Myrmica* nests were also discovered within 2m of *Gentiana* plants on *M. 'rebeli'* sites (mean  $\pm$  SE;  $78.9 \pm 19.5$ ) than on *M. alcon* sites ( $28.3 \pm 8.5$ ;  $F_{1,13} = 6.22$ ,  $p = 0.027$ ), so this difference could simply reflect 'sampling effort'. To control for this, the relationship between number of ant nests examined at a site and the number of potential host species found was examined using EstimateS 8.0 (Colwell, 2006) to produce species accumulation curves for the two types of site. This showed that over the range of nest numbers found on *M. alcon* sites (5-69 nests) the number of ant species found fell within the 95% confidence interval of the number found on *M. 'rebeli'* sites (Fig. 3), suggesting that the difference in number of ant species may well reflect the difference in 'sampling effort' between the two types of site.

Approx. position of table 2  
and figure 2

Approx. position of Figure 3

280

281 However, when the community of ants found at each site was examined, there were clear  
282 differences in the species composition, with ant communities being more similar within the *M.*  
283 *alcon* and *M. 'rebeli'* groups of sites than between them (Fig. 4). This reflects the absence of *M.*  
284 *lobicornis*, *M. lona*, *M. sabuleti* and *M. speciosus* from *M. alcon* sites, and the absence of *M.*  
285 *gallienii* from *M. 'rebeli'* sites (Fig. 2).

Approx position of Figure 4

286

## 287 2. Frequency of *Maculinea parasitism*

288 *Maculinea* caterpillars, pupae or exuviae were found in 84 (or 11%) of the *Myrmica* ant nests found  
289 ( $n = 778$ ) (Table 2). A total of 741 *Maculinea* specimens were found in the ant nests studied (Table  
290 2). The mean  $\pm$  S.D. number of *Maculinea* per nest was  $0.95 \pm 4.94$  and ranged between 0 and 68  
291 caterpillars per ant nest ( $n = 778$ ). Ant nests in *M. alcon* sites held significantly more *Maculinea*  
292 specimens ( $2.42 \pm 8.32$  *Maculinea* per nest) than did ant nests in *M. 'rebeli'* sites ( $0.35 \pm 2.22$ ;  
293 Median test,  $S = 4.67$ ,  $p < 0.001$ ).

294

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

296 *M. scabrinodis*, present in all sites studied, was the only ant species which was used by both Alcon  
297 Blues, and thus can be considered a general host ant for *M. alcon* and *M. 'rebeli'* in north-eastern  
298 Hungary, although no infested nests were found on *M. 'rebeli'* sites in Transylvania (Table 2). The  
299 rate of parasitism of *M. scabrinodis* nests was significantly higher at *M. alcon* sites than at *M.*  
300 *'rebeli'* sites (Fig. 5; GLM with binomial errors; between types of site, Likelihood-Ratio  $\chi^2 = 36.6$ ,  
301 d.f. = 1,  $p < 0.0001$ ), and at both sites it increased with the degree to which *M. scabrinodis*  
302 dominated the local ant community (L-R  $\chi^2 = 12.48$ , d.f. = 1,  $p = 0.0004$ ) in the same manner for  
303 both types of site (type of site  $\times$  proportion of *M. scabrinodis*; L-R  $\chi^2 = 0.34$ ,  $p = 0.558$ ).

304

Approx. position of  
Figure 5



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%) out 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. followed a Poisson distribution) (Table 2).

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

#### 5. Genetic differentiation between Răscruci *M.alcon* and *M. 'rebeli'*

Although sample sizes were small (8 individuals for the *M.alcon* area and 10 individuals from the *M. 'rebeli'* area), there was considerable variation at all the nine loci used, with the number of alleles per locus among the 18 individuals ranging from 4 to 15. Every individual had a unique combination of alleles. There was highly significant genetic differentiation between *M.alcon* and *M. rebeli* samples ( $F_{ST} = 0.112$ ,  $p < 0.0001$ ), but none between the *M. rebeli* using *M. schencki* and those using *M. sabuleti* ( $F_{ST} = -0.005$ ,  $p = 0.953$ ). Genetic differentiation between *M.alcon* and *M. rebeli* samples based on microsatellite allele length ( $R_{ST}$ ) was 0.183, and did not differ significantly from  $F_{ST}$  based on 10000 permutations of alleles ( $p = 0.220$ ; Hardy et al., 2003). Analysis of the multilocus genotypes of the 18 individuals using the population assignment programs STRUCTURE and BAPS also clustered the individuals according to the host plant area. STRUCTURE suggested that the individuals should be divided into 2 groups, and these groups

corresponded perfectly with the samples collected from the *M.alcon* and *M. 'rebeli'* areas (Fig 6). BAPS produced nine clusters of individuals, which also separated the individuals from *M.alcon* and *M. 'rebeli'* areas, but not the *M. 'rebeli'* from *M. sabuleti* and *M. schecki* nests (Fig. 6). When BAPS was forced to divide the samples into two groups, these also corresponded to the *M.alcon* and *M. rebeli* samples, except for sample *M. scabrinodis* 87-2 which was assigned to the *M. 'rebeli'* group (but only with a probability of 0.67, compared with  $> 0.8$  for all other group assignments).

Approx. position of Fig. 6
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## 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 regions where close genetic similarity of the two butterflies was found (Bereczki et al., 2005, 2006; Pecsénye et al., 2007; see also Fig. 6). 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'*.

Our results show that *M. scabrinodis* is the most important host ant of *M.alcon* in NE-Hungary and Transylvania, (Table 2; Fig. 1, 4), which is also the case in central and western 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. 1, 4). *M. vandeli* has previously been reported as a host of *M.alcon* from Poland (Sielezniew and Stankiewicz, 2004b; Stankiewicz and Sielezniew, 2005), but *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, 2005).

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. 1; 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. 1). 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. 1). *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 species accumulation curves 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 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 *Maculinea* butterflies do not show any host specificity (Pech et al., 2007), however, we have clearly heterogeneous use of host ant species in several of the populations examined (Table 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  $\nu$  P3; 0.695, P1  $\nu$  P4; 0.837, P3  $\nu$  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.

Our analysis of microsatellite markers from a small number of individuals collected from areas of the site at Răscruți where *G. pneumonanthe* and *G. cruciata* are used as host plants suggests that there is very little gene flow between these two areas. The allelic diversity of the microsatellites used is high, so that every individual tested had a unique combination of alleles, which also means that the estimates of allele frequencies for local populations must be regarded as unreliable in such a small sample. Nevertheless, the clustering of individuals according to host plant availability using population assignment analysis supports the genetic isolation of *M. alcon* and *M. 'rebeli'* in this area. Whether this genetic isolation represents a species boundary, or whether it arises from differences in phenology within a single species, enforced by the differences in flowering phenology of the two host plants, remains an open question. Such differences in phenology clearly exist at Răscruți, where eggs are laid earlier on *G. cruciata* than on *G. pneumonanthe* (which

reflects the earlier development of *G. cruciata* flower buds). Comparing the genetic differentiation that we found in this study with that found for *M.alcon* in Southern Scandinavia (Lomborg et al. 2005; Nash et al., submitted; Nash and Fürst, unpublished data) shows that a similar level of genetic differentiation can be found between populations within this species over distances of a few hundred kilometres ( $F_{ST} = 0.182$ ) as was found here at most over hundreds of metres. Examining the alleles present at Răscrući shows that 67% of all alleles were private alleles (i.e. alleles found only within either the *M.alcon* or *M. 'rebeli'* samples), but differentiation measured based on allele length (RST) did not differ from the measured value of  $F_{ST}$ , suggesting that the difference between these groups may arise from genetic drift rather than mutation (Hardy et al., 2003), which would be less likely if *M.alcon* and *M. 'rebeli'* were completely genetically isolated.

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; Steiner et al., 2003; Sielezniew and Stankiewicz, 2004b; 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 absent in more central populations. Enzyme polymorphism studies by Bereczki et al. (2005, 2006) and Pecsénye 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). These studies were based on allopatric populations of the two Alcon blues, and are not supported by our preliminary genetic data from a single sympatric population, although the cause of the significant genetic differentiation we find between the two forms remains to be determined.

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, 2005) 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; Pecsénye et al., 2007), however, have failed to

find such substructuring of *M. alcon* and *M. 'rebeli'* populations in NE-Hungary, and while our preliminary investigation of microsatellite markers for the Răscruci population in Transylvania does show strong differentiation between *M. alcon* and *M. 'rebeli'* within this site, there is no differentiation between individuals using *M. sabuleti* and *M. schencki* nests. 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. Our genetic data does show evidence for differentiation between individuals using different host plant species, but not between *M. 'rebeli'* individuals using different host ant species, and there is no evidence of heterozygote excess. 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., submitted).

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., submitted).



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. 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 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.

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

**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.  $\chi^2$  = value of the chi-squared statistic of heterogeneity in host ant use. P2 = probability associated with the  $\chi^2$  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.  $Dd$  = change in deviance in a General Linear Mode 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  $\chi^2$  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.

<i>Maculinea</i>	Site	<i>Myrmica</i>	Number of nests	Number with <i>Maculinea</i>	P1	Number of <i>Maculinea</i>	$\chi^2$	P2	$k$	$Dd$	P3	P4
<i>M. alcon</i>	Drahos-rét	<i>M. scabrinodis</i>	53	3	0.184	3	14.6	<b>&lt; 0.001</b>	0.140	5.41	<b>0.020</b>	<b>0.046</b>
		<i>M. vandeli</i>	19	3		9						
	Fülesd	<i>M. scabrinodis</i>	9	2	0.274	3	146.23	<b>&lt; 0.001</b>	0.143	13.2	<b>0.004</b>	0.239
		<i>M. gallienii</i>	6	0		0						
		<i>M. ruginodis</i>	2	0		0						
		<i>M. salina</i>	15	6		137						
	Gyilkos-rét	<i>M. scabrinodis</i>	32	23	<b>&lt; 0.001</b>	315	70.65	<b>&lt; 0.001</b>	0.407	17.8	<b>&lt; 0.001</b>	0.153
		<i>M. gallienii</i>	6	0		0						
		<i>M. schencki</i>	1	0		0						
	Hetefejércse	<i>M. scabrinodis</i>	5	3	—	35		—	—	—	—	—
	Nyikom-rét	<i>M. scabrinodis</i>	4	2	1.000	7	1.75	0.359	0.735	1.67	0.196	1.000
		<i>M. ruginodis</i>	1	0		0						
	Răscruci 'wet'	<i>M. scabrinodis</i>	20	4	—	10		—	—	—	—	—
	Șardu	<i>M. scabrinodis</i>	26	2	1.000	4	1.6	0.236	0.033	0.199	0.656	0.888
		<i>M. vandeli</i>	21	1		7						
	Tugár-rét	<i>M. scabrinodis</i>	6	4	—	17		—	—	—	—	—
<i>M. 'rebeli'</i>	Bükkszentkereszt	<i>M. scabrinodis</i>	55	2	0.340	24	5.41	<b>0.027</b>	0.023	0.498	0.481	0.769
		<i>M. sabuleti</i>	30	3		4						
	Kecskeláb-rét	<i>M. scabrinodis</i>	2	0	0.353	0	12.74	<b>0.009</b>	0.039	0.825	0.662	0.353
		<i>M. lonae</i>	3	1		5						
		<i>M. sabuleti</i>	21	1		6						
	Kuriszlán	<i>M. scabrinodis</i>	39	1	<b>0.015</b>	1	51.84	<b>&lt; 0.001</b>	0.227	15.7	<b>0.001</b>	<b>0.045</b>
		<i>M. schencki</i>	13	3		12						
		<i>M. speciosoides</i>	1	1		3						
		<i>M. vandeli</i>	1	0		0						
	Lófő-tisztás	<i>M. scabrinodis</i>	149	7	0.151	82	10.95	<b>0.037</b>	0.016	1.67	0.796	0.768
		<i>M. lonae</i>	8	0		0						
		<i>M. ruginodis</i>	2	0		0						
		<i>M. sabuleti</i>	5	1		5						
		<i>M. schencki</i>	4	1		15						
	Nagy-mező	<i>M. scabrinodis</i>	47	0	0.340	0	18.27	<b>0.017</b>	0.054	16.1	<b>0.024</b>	0.325
		<i>M. lobicornis</i>	2	0		0						
		<i>M. lonae</i>	4	0		0						
		<i>M. ruginodis</i>	10	0		0						
		<i>M. sabuleti</i>	22	2		6						
		<i>M. schencki</i>	4	0		0						
	Răscruci 'dry'	<i>M. scabrinodis</i>	11	0	<b>0.035</b>	0	36.64	<b>&lt; 0.001</b>	0.274	21.3	0.077	0.142
		<i>M. schencki</i>	5	2		18						
		<i>M. sabuleti</i>	4	2		7						
	Tohonya-hát	<i>M. scabrinodis</i>	51	0	<b>0.007</b>	0	19.43	<b>0.012</b>	0.309	14.7	<b>0.005</b>	0.069
		<i>M. sabuleti</i>	3	0		0						
		<i>M. salina</i>	30	0		0						
		<i>M. schencki</i>	16	2		4						
		<i>M. speciosoides</i>	10	2		2						
Both	Răscruci	As above			0.090		56.74	<b>&lt; 0.001</b>	0.163	5.71	0.058	0.067

## FIGURE LEGENDS

**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.

**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.

**Fig. 3.** Species accumulation curves based on drawing random samples of *Myrmica* nests from the pool of those found on *M. alcon* (dotted lines) and *M. 'rebeli'* (solid lines) sites. The thick, central line for each type of site represent the mean number of *Myrmica* species resampled, and the thinner, outer lines the 95% confidence limits around this mean. The region over which sampling took place on *M. alcon* sites in the field (i.e. between 5 and 69 nests per site) is shaded.

**Fig. 4.** Comparison of the pairwise similarity in ant communities between sites that hosted only *M. alcon*, only *M. 'rebeli'* and between sites that differed in the *Maculinea* 'species' that they hosted. The figure shows mean values  $\pm 1$  SE for Chao's abundance-based and sample-size corrected extension of Sørensen's diversity index (Chao et al., 2005) for each pair of sites. Overall, there was a significant difference between the three comparisons ( $F_{2,102} = 6.29$ ,  $P = 0.003$ ). Means that do not differ based on post-hoc Tukey tests are marked with the same letter.

**Fig. 5.** 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.

**Fig. 6.** Results of population assignment analysis of 14 individuals collected at Răscruci based on nine variable microsatellite loci. Each individual is represented by a single horizontal bar. Individuals are grouped according to the nine clusters identified by BAPS, and the genetic distance between these clusters is shown as a neighbour-joining tree to the right. Analysis with STRUCTURE optimally divided the individuals into two groups. The length of the black segment of each bar represents the probability that that individual belongs to group 1, while the hatched segment is the probability of membership of group 2. The code for each individual represents the species of ant and nest in which it was found. If several individuals were found in the same nest, they received consecutive numerical suffixes. *M. 'rebeli'* specimens are marked in bold type.

Figure 1

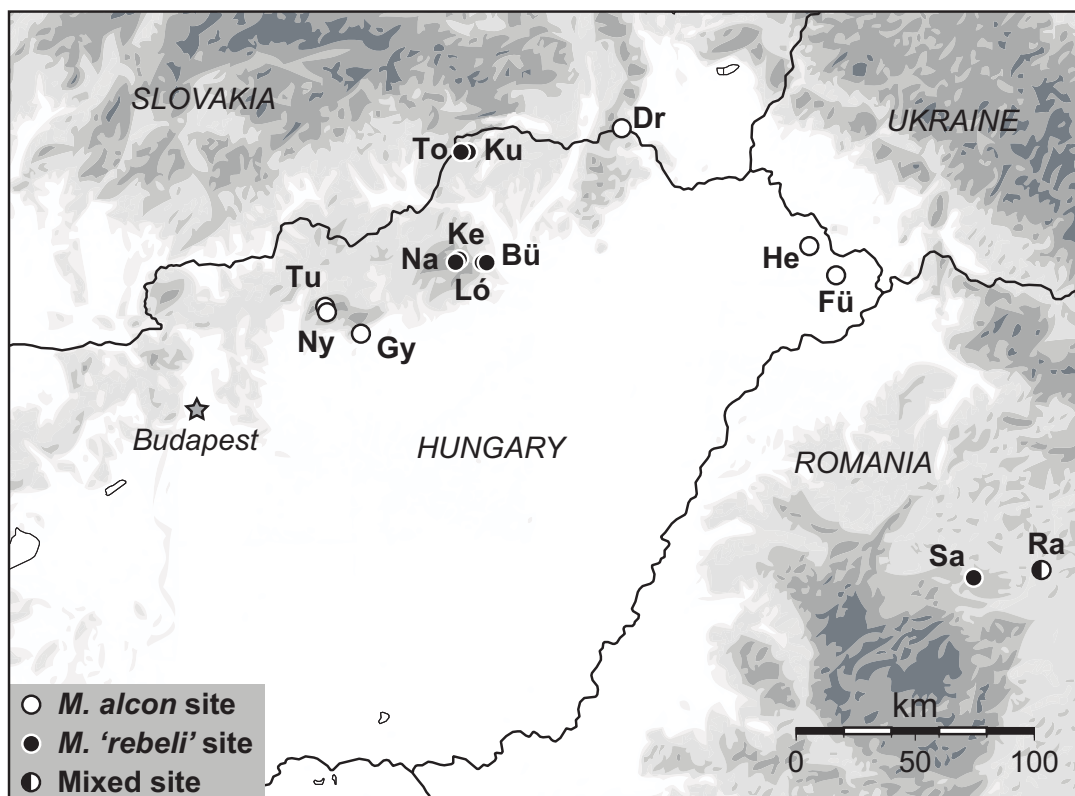


Figure 2

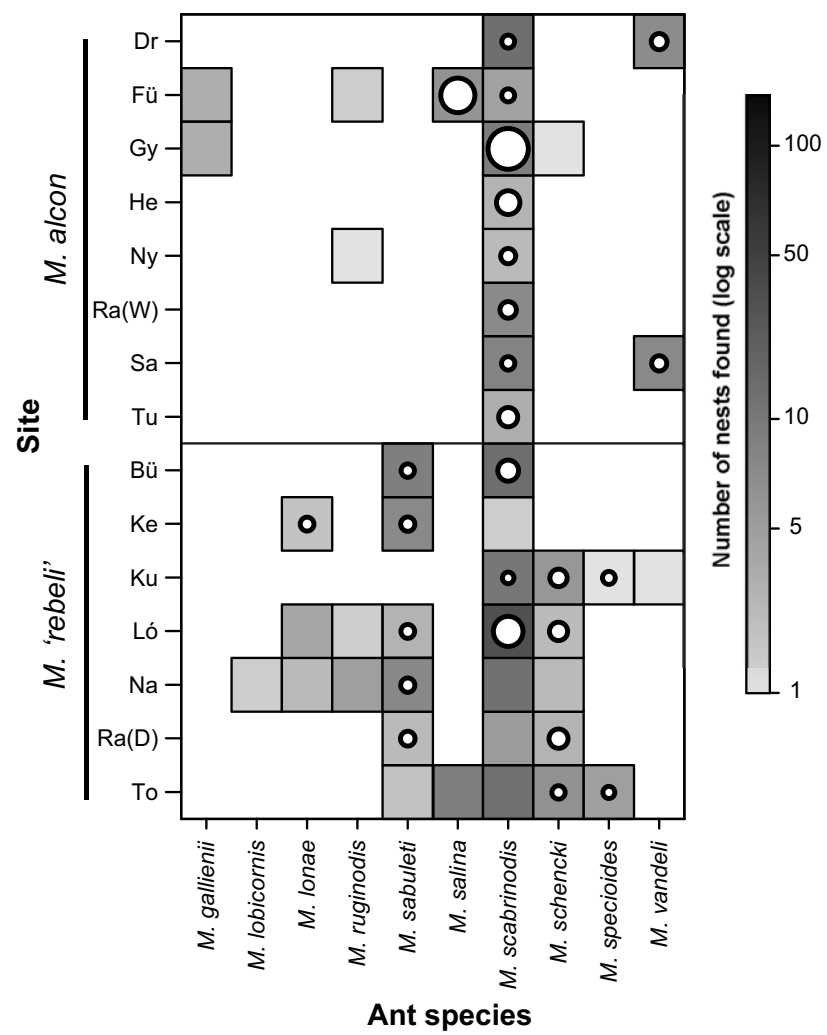


Figure 3

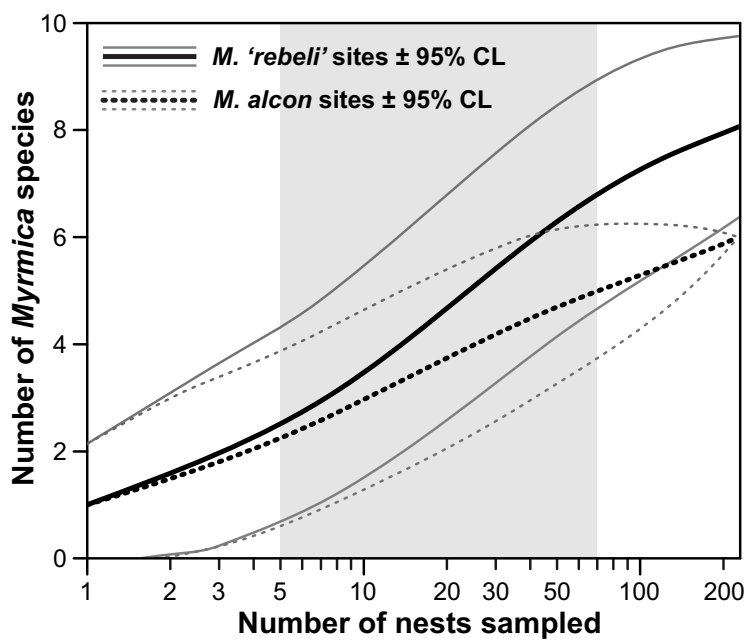




Figure 4

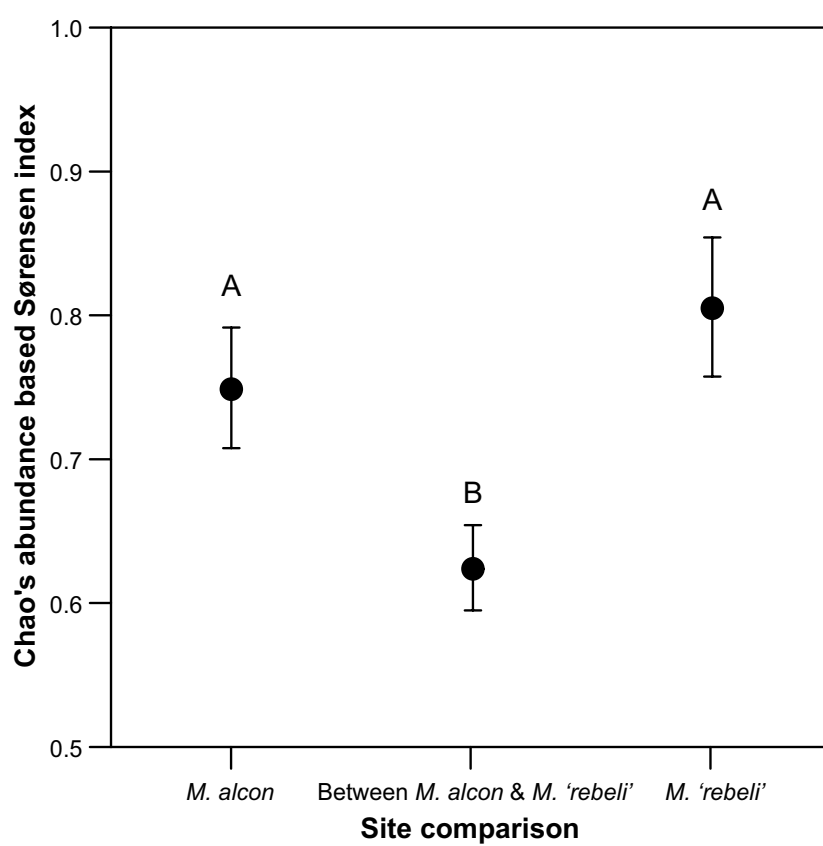


Figure 5

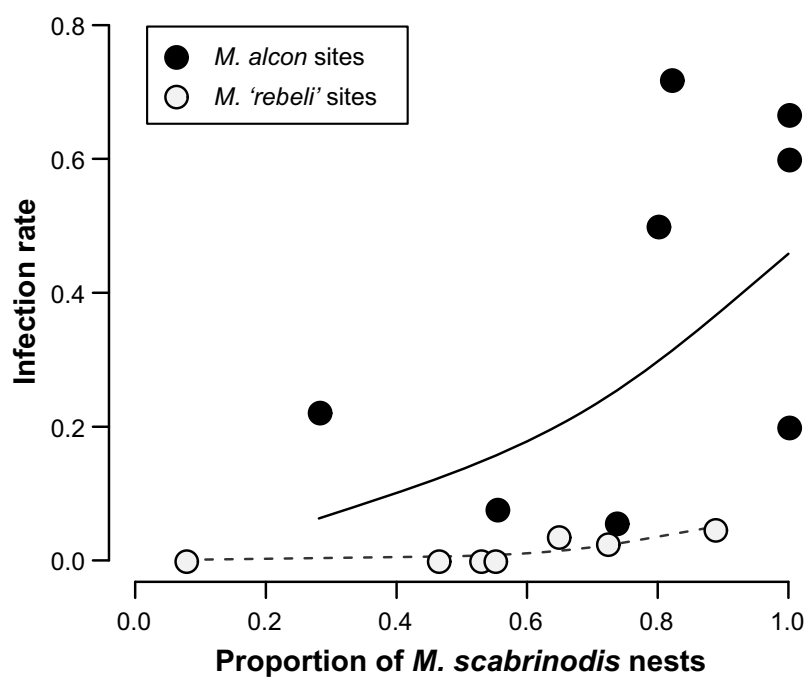
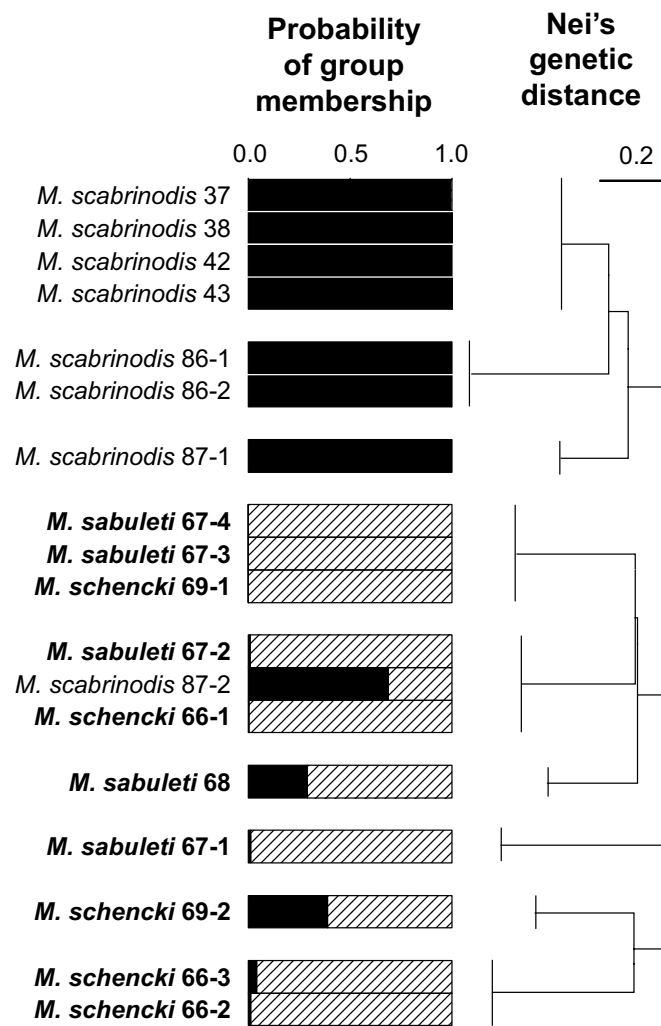


Figure 6





## Paper VI.

TARTALLY, A. 2005: Accelerated development of *Maculinea rebeli* larvae under artificial conditions (Lycaenidae). – *Nota Lepidopterologica* 27 (2004): 303-308.



## Accelerated development of *Maculinea rebeli* larvae under artificial conditions (Lycaenidae)

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**Abstract.** In the years 2000 to 2003, 93 *Maculineaalcon* ([Denis & Schiffermüller], 1775) larvae from three localities in Hungary and 261 *M. rebeli* (Hirschke, 1904) larvae from three localities in Hungary and from one locality in Austria were introduced into 103 *Myrmica* colonies in the laboratory. Seven specimens of *M. rebeli* pupated after only about a month in artificial *Myrmica scabrinodis* Nylander, 1846, *My. sabuleti* Meinert, 1860 and *My. salina* Ruzsky, 1905 nests. This phenomenon was found in each of the four studied populations. Two pupae successfully eclosed, one 32 and the other 47 days after adoption. The other five pupae died. These results confirm observations that the developmental time of *M. rebeli* larvae can be plastic. A similarly accelerated development of *M.alcon* larvae was never observed. The accelerated development of *M. rebeli* larvae might be attributed to (1) the higher temperatures in the laboratory as compared with natural conditions, and/or to (2) the artificial *Myrmica* nests which were more exposed to light than under natural conditions, and/or to (3) the balanced artificial diet that the *Myrmica* colonies received.

**Key words.** *Maculinea*, *Myrmica*, myrmecophily, host ant, ant diet, accelerated development.

### Introduction

Larvae of *Maculinea* van Eecke, 1915 are obligate parasites of *Myrmica* Latreille, 1804 (Hymenoptera: Formicidae) colonies for most of their life. It has long been known that the butterflies have an annual life cycle, with larvae living for about 10–11 months in *Myrmica* ant nests (Thomas 1995; Thomas & Elmes 1993, 2001; Thomas & Wardlaw 1992; Thomas et al. 1989, 1993; Wardlaw et al. 2000). More recently, it has been shown that some larvae live for an additional year in the ant nests, for a total of about 22–23 months (Als et al. 2001; Elmes et al. 2001; Schönrogge et al. 2000; Thomas et al. 1998). While rearing *Maculinea rebeli* (Hirschke, 1904) larvae in the laboratory, an unexpectedly accelerated development was observed, with pupation as soon as a month after adoption.

Although genetic (Als et al. 2004; Bereczki et al. in press) and morphological (Pech et al. 2004) differentiation between the traditionally separated species *M.alcon* ([Denis & Schiffermüller], 1775) and *M. rebeli* is rather low, the two taxa have different physiological and ecological adaptations (see e.g. Schönrogge et al. 2000; Thomas et al. 1989). I use '*M. rebeli*' for populations which develop on *Gentiana cruciata* and '*M.alcon*' for those which develop on *G. pneumonanthe*. However, the host plant affinities of these two taxa need to be re-investigated for their significance for identification purposes (see e.g. Kolev 2002; Munguira & Martin 1999; Sielezniew & Stankiewicz 2004).

### Material and Methods

Between 2000 and 2003, 93 *Maculineaalcon* and 261 *M. rebeli* larvae were reared in 103 artificial laboratory colonies of *Myrmica*. For this purpose, plants of

*G. pneumonanthe* with eggs of *M. alcon* from three localities (Hungary: Fülesd, Gyöngyös, Mátraszentimre) and plants of *G. cruciata* with eggs of *M. rebeli* from four localities (Austria: Hochschwab; Hungary: Bükk-plateau, Bükkszentkereszt, Jósvalő) were collected. In the laboratory the gentians were kept in glasses of water placed in plastic basins, and could be kept fresh for 2–3 weeks while the *Maculinea* larvae emerged. Fourth instar larvae were collected using a fine brush as they dropped from the flowers in the evenings, and were transferred straight into the foraging arena of an artificial *Myrmica* nest to be adopted by the ants. Before introduction, the length of each caterpillar was measured with a ruler. Caterpillars were remeasured after one month by putting the ruler to the glass that covered the artificial nests.

The *Myrmica* colonies usually were collected from the same sites as the gentians. Each colony contained at minimum one queen and 100 workers. They were kept in Debrecen (Hungary) in unheated nests (made from clay and glass) joined by silicon tubes to plastic arenas. These nests were not covered to exclude the light, but were kept in places that never received direct sunlight. The laboratory was not air-conditioned in the summer, but was heated in the colder seasons. The temperature that the *Myrmica* nests experienced was less variable than under natural conditions, and was often up to 25° C in the warmer periods. A part of the nest area was always kept wet by a cotton wool strand that connected the clay with water. To feed the ants, the arenas of the nests were always provided with a cube of sugar, and various insects (mainly cut-up mealworms, larvae and pupae) as well as granules of a dry diet at a minimum of once a week (see appendix). The cube of sugar provided continuous food while the dry diet provided the proteins (and maybe essential vitamins and minerals) when there were not enough insects to feed the ant colonies.

The following *Myrmica* species (identified by Tartally & Csősz) were used: *My. lonae* Finzi, 1926 (1 culture); *My. vandeli* Bondroit, 1920 (1 c.); *My. rugulosa* Nylander, 1849 (1 c.); *My. salina* Ruzsky, 1905 (3 c.); *My. speciosides* Bondroit, 1918 (3 c.); *My. gallienii* Bondroit, 1919 (7 c.); *My. schencki* Viereck, 1903 (8 c.); *My. ruginodis* Nylander, 1846 (8 c.); *My. rubra* (Linnaeus, 1758) (9 c.); *My. sabuleti* Meinert, 1860 (9 c.) and *My. scabrinodis* Nylander, 1846 (35 c.).

When a butterfly larva pupated, it was removed from the ants using a pair of fine forceps and placed in a plastic box with ventilation holes and a moist sponge pad at the bottom. This was thought to be important because the ants damage the eclosed butterflies if they are not able to escape from a closed artificial nest and if they are not discovered and separated in time (Elfferich 1988). Voucher samples of ants, dead pupae, exuviae, and butterflies are stored in the author's collection.

## Results

Several larvae died during the period of adoption and the next few days. After this critical period their mortality was lower and the *M. rebeli* larvae usually grew very quickly. They were about 3 mm long on introduction, and usually they had grown to about 15 mm a month later. However, seven of them pupated after about a month in different ant nests. These were associated with three *Myrmica* species and came from



**Tab. 1.** The *Maculinea rebeli* larvae that pupated in about a month in the laboratory.

Locality	Host	Date of adoption	Date of pupation	Date of eclosion
Hungary / Bükk-plateau	<i>My. sabuleti</i>	27.07.2002	15.08.2002	28.08.2002
Hungary / Bükk-plateau	<i>My. scabrinodis</i>	27.07.2002	28.08.2002	died
Hungary / Bükkzentkereszt	<i>My. scabrinodis</i>	11.07.2002	13.08.2002	27.08.2002
Hungary / Bükkzentkereszt	<i>My. scabrinodis</i>	11.07.2002	13.08.2002	died
Hungary / Jósvalő	<i>My. salina</i>	11.07.2002	15.08.2002	died
Hungary / Jósvalő	<i>My. scabrinodis</i>	11.07.2002	10.08.2002	died
Austria / Hochschwab	<i>My. sabuleti</i>	14.07.2003	16.08.2003	died

each of the four *M. rebeli* populations studied (Tab. 1). Such a quick development in *M. alcon* larvae was never observed in my experiments during the first months. The *M. alcon* larvae also were about 3 mm long on introduction, but they had grown only to ca. 5 mm a month later and remained about this size in the winter. Two male butterflies from the seven pupae emerged. One of them eclosed 32, the other 47 days after adoption as freshly moulted fourth instar larvae (Tab. 1). These specimens were smaller than average (the forewing length of the one from Bükkzentkereszt was 15 mm and the one from Bükk-plateau 15.5 mm), but similarly small specimens often occur under natural conditions. The fast-developing specimens did not show any other obvious differences compared with field-grown specimens. The other five pupae became rotten or dried out under the unnatural air humidity of the laboratory.

## Discussion

The fast-pupating larvae were reared by three different species of *Myrmica*: *My. scabrinodis*, *My. sabuleti*, and *My. salina*. According to field observations, the former two are suitable host ants for *M. rebeli* in Hungary (Tartally & Csősz 2004) and *My. sabuleti* is also suitable in Eastern-Austria (Steiner et al. 2003). However, there are no records of *My. salina* as a host of *M. rebeli* yet (Als et al. 2004; Tartally & Csősz 2004). It is important to note that in well-fed laboratory nests the survival of adopted larvae is usually better than in nature (Elmes et al. 2004; Schönrogge et al. 2004).

The fast development of *M. rebeli* under laboratory conditions might be caused by (1) the warmer temperatures in comparison to natural conditions (Wardlaw 1991; Wardlaw et al. 1998), and/or (2) the artificial *Myrmica* nests being more exposed to light than under natural conditions (the more abundant light could influence the larval development of lycaenid butterflies; see e.g. Høegh-Guldberg 1968), and/or (3) the more balanced diet the *Myrmica* colonies received – thus, my diet seems to be suitable for *Myrmica* colonies as supplementary food.

Elmes & Thomas (pers. comm.) recorded similarly short times of development for *M. rebeli* from the Pyrenees and the Southern Alps under unnaturally warm conditions and with abundant food. Hence, an accelerated development is known from several populations and is not a unique phenomenon. These results support the plasticity of the developmental time of *M. rebeli* as the larvae develop during one or two years in nature (Elmes et al. 2001; Schönrogge et al. 2000; Thomas et al. 1998) or have a conspicuous accelerated development within one year under favourable conditions. In addition,

based on my own observations, there is no indication of a two-year development of *M. rebeli* in Hungary since I have never found semi-developed *M. rebeli* larvae in *Myrmica* nests during the flying period. On the other hand, some semi-developed *M.alcon* larvae were observed in various Hungarian sites during the flying period. According to Varga (pers. comm.), ‘dwarf’ adults of *M. rebeli* regularly appear in several Hungarian populations at the end of the flying period (end of June to mid-July, depending on year and elevation). However, in the laboratory, the two dwarf specimens eclosed in late August. This suggests that undernourished *M. rebeli* larvae may also fully develop within one year under natural conditions and a partly bivoltine life cycle in nature seems to be unlikely. It is known that the growth of the one-year *M. rebeli* larvae tend to be fast immediately after adoption, stops during winter (meaning that they go into diapause in nature), and resumes in the spring just before pupation (Thomas et al. 1998). However, according to my laboratory observations the development of *M. rebeli* larvae can be continuous (without diapause) under favourable conditions, contrary to that of *M.alcon*. These differences were also observed when I reared *M.alcon* and *M. rebeli* larvae under the same laboratory conditions but in *Manica rubida* (Latreille, 1802) colonies (Tartally 2004). Further studies are still necessary to investigate the temporal dynamics of the development within *M. rebeli* and *M.alcon* populations and to understand the ecological circumstances influencing these dynamics.

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## Appendix

### The recipe of the dry diet

**I n g r e d i e n t s .**            100 cm<sup>3</sup> (=14–15 g) freeze-dried fish <sup>1</sup>  
   1 level tablespoon of flour  
   1 pinch of sea salt  
   1 vitamin pill <sup>2</sup>  
   1 egg

<sup>1</sup> You can buy it in well-equipped pet shops as food for cats and turtles (I used a Hungarian product: Bio-Lio). If you cannot find freeze-dried fish you can dry some lean pieces of cooked fish or chicken in the sun or under an infra-red lamp.

<sup>2</sup> Choose the type that contains the daily portion of multiple vitamins, essential minerals, and salts for an adult (I used Supradyn).

**P r e p a r a t i o n .** Grind the fish into powder and mix it with the flour, the salt, and the pulverised pill. Beat the egg slightly and add a little to the dry components. You need to get a hard paste in order to be able to form a ball, then leave it to desiccate for about half an hour. When its consistency is suitable, grate it with a cheese grater onto a sheet of paper. You will get various sizes of granules. Spread the granules on the paper and leave them to dry for about a day. The dried granules keep their quality (= the ants like them) for about half a year at room temperature in a dark and ventilated place.

I have planned this diet to culture *Myrmica* colonies because it is essential for the *Myrmica* colonies to be fed with protein and sugar (Wardlaw et al. 1998). However, the Bhatkar diet (Bhatkar & Whitcomb 1970) – which is presumably the most popular artificial ant diet (see e.g. Hölldobler & Wilson 1990) – contains very little protein (Buschinger & Pfeifer 1988).

## Paper VII.

TARTALLY, A. 2004: Is *Manica rubida* (Hymenoptera: Formicidae) a potential host of the *Maculineaalcon* (Lepidoptera: Lycaenidae) group? – Myrmecologische Nachrichten 6: 23-27.



## Is *Manica rubida* (Hymenoptera: Formicidae) a potential host of the *Maculinea alcon* (Lepidoptera: Lycaenidae) group?

TARTALLY András

### Abstract

The caterpillars of *Maculinea* butterflies are obligate parasites of nests of *Myrmica* (or in certain cases *Aphaenogaster*) ants during most of their development. *Manica rubida* (LATREILLE, 1802) is closely related to ants of the genus *Myrmica*, and can occur on *Maculinea* sites. Laboratory colonies of *M. rubida* were therefore tested for their ability to raise caterpillars of *Maculinea rebeli* (HIRSCHKE, 1904) and *M. alcon* (DENIS & SCHIFFERMÜLLER, 1775). After introduction into the foraging arenas of these colonies, all caterpillars were taken into the nest by worker ants, where they were often carried and licked. Several caterpillars of both butterflies survived and increased in size for a number of weeks, up to one and a half months. These results suggest that *M. rubida* could potentially act as a host for *Maculinea* caterpillars, although whether any local populations have evolved to specialise on this potential host remains to be demonstrated in the fields.

**Key words:** *Manica*, *Maculinea*, host specificity, myrmecophily

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### Introduction

Myrmecophily is a well-known phenomenon among several insect taxa (HÖLLDOBLER & WILSON 1990). The lycaenid butterflies are particularly interesting in this respect, because the larvae of most species studied are associated with ants. Facultative and obligate myrmecophily and the entire range from mutualism to parasitism can be observed among these butterflies (FIEDLER 1991, PIERCE & al. 2002). The best-known example of obligate parasitism is the genus *Maculinea*, where after feeding on a host plant for the first three larval instars, the fourth instar larva must complete its development in a host ant nest. Knowledge of the host ant species has been shown to be crucial for the protection of these endangered butterflies (e.g., ELMES & THOMAS 1992). Ants from only two genera are recorded as hosts of these butterflies: The vast majority of *Maculinea* use hosts from the genus *Myrmica* (e.g., THOMAS & al. 1989, ALS & al. 2002, STEINER & al. 2003) but *Maculinea teleius* (BERGSTRÄSSER, 1779) and especially *M. arionides* (STAUDINGER, 1887) have also been recorded as using *Aphaenogaster japonica* FOREL, 1911 in Japan (YAMAGUCHI 1988, PIERCE & al. 2002).

*Manica rubida* (LATREILLE, 1802) is quite closely related to ants in the genus *Myrmica* (e.g., ASTRUC & al. 2004), and was classified as a member of this genus by FOREL (1915). This ant occurs in mountains at altitudes of 500 - 2000 m in Asia Minor, the Caucasus, Central Europe, the Crimea and parts of Southern Europe (CZECHOWSKI & al. 2002). All five

European species of *Maculinea* occur in some of these regions. Moreover, *M. arion* (LINNAEUS, 1758), *M. alcon* (DENIS & SCHIFFERMÜLLER, 1775) and *M. rebeli* (HIRSCHKE, 1904) populations are known from the same altitudes (e.g., WYNHOFF 1998). The co-occurrence of *Manica rubida*, *M. rebeli* and host gentities with eggs was observed at the same site near Lacul Roșu (Romania: Harghita County; S. Csősz, pers. comm.).

Based on this distribution, the question arose as to whether *Manica rubida* could potentially be a host ant of *Maculinea* butterflies. This ant has never been observed in association with any myrmecophilous lycaenid so far (FIEDLER 2001). Hence I tested the ability of laboratory colonies of this ant to adopt and raise caterpillars of *M. rebeli* and *M. alcon*.

### Material and methods

Although the level of genetic (ALS & al. 2004; J. Bereczki, K. Pecsénye & Z.S. Varga, pers. comm.) and morphologic (PECH & al. 2004) differentiation between the traditional species *M. alcon* and *M. rebeli* is rather low, I differentiate "*M. rebeli*" from *M. alcon* here mostly for physiological and ecological reasons, and because sympatric populations usually have different host ant species in the same region. In Western Europe, *M. rebeli* primarily uses *Myrmica schencki* VIERECK, 1903 (THOMAS & al. 1989) but *M. alcon* mainly uses *Myrmica scabrinodis* NYLANDER, 1846, *M. rubra* (LINNAEUS, 1758) or *M.*



Tab. 1: The number of introduced (\*) and surviving *Maculineaalcon* and *M. rebeli* caterpillars in *Manica rubida* colonies from week to week.

Date (2003)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	
	<i>M. rebeli</i>	<i>M. rebeli</i>	<i>M.alcon</i>	<i>M.alcon</i>	<i>M. rebeli</i>	<i>M.alcon</i>
15 July	10*	10*	-	-	-	-
22 July	4	9	-	-	-	-
29 July	2	8	-	-	3*	-
05 August	2	5	10*	10*	2	1*
12 August	1	4	10	7	2	1
19 August	0	3	7	6	2	1
26 August	0	1	6	3	1	1
02 September	0	0	4	3	1	0
09 September	0	0	1	1	0	0
16 September	0	0	1	0	0	0
23 September	0	0	0	0	0	0

*ruginodis* NYLANDER, 1846 (THOMAS & al. 1989, ELMES & al. 1994, ALS & al. 2002) host ants. However, in Central Europe *M.alcon* is almost always found in colonies of *M. scabrinodis* (SIELEZNIEW & STANKIEWICZ 2002, HÖTTINGER & al. 2003, TARTALLY & CSÖSZ in press), and *M. rebeli* is mostly found in nests of *Myrmica sabuleti* MEINERT, 1861, *M. schencki* and *M. scabrinodis* (STEINER & al. 2003, TARTALLY & CSÖSZ in press).

In the summer of 2003, I introduced 21 caterpillars of *M.alcon* from Mátraszentimre (Hungary: Heves County) and 23 caterpillars of *M. rebeli* from Bükk-szentkereszt (Hungary: Borsod-Abaúj-Zemplén County) into five artificial colonies of *Manica rubida* (Tab. 1). It should be noted that all the caterpillars were from mountains where *M. rubida* has not been recorded (SOMFAI 1959; pers. observ.). The same methods were used in the collection and introduction of the caterpillars and in the keeping of the *M. rubida* colonies as had previously been used for culturing *Maculineaalcon* and *M. rebeli* caterpillars in artificial *Myrmica* colonies (TARTALLY in press).

The *Manica rubida* colonies were collected from Sovata (Rumania: Mureş County) in March of 2003. Colonies 1 - 4 (Tab. 1) contained one queen, brood, and at least 150 workers, while Colony 5 contained one queen, brood, and only 50 workers. This last colony was used primarily for making a video record of the interaction, because smaller caterpillars in a dense colony are not easily visible. Colony 5 was videotaped on 5 August 2003 when a freshly adopted *M.alcon* caterpillar and two *M. rebeli* caterpillars (introduced one week before) were present. Discrimination between the two *Maculinea* species after adoption based on size was not difficult because *M. rebeli* caterpillars grow much quicker than those of *M.alcon* in the Hungarian populations in late

summer (TARTALLY in press and unpublished data). Similar phenomena are also known from other countries (e.g., SCHÖNROGGE & al. 2000).

The caterpillars of each butterfly species were introduced to the foraging arena of the same colony together at the same time. Colonies were then checked for surviving caterpillars once per week (Tab. 1). Before introduction, the length of each caterpillar was measured with a ruler. Caterpillars were remeasured after one month by putting the ruler to the glass which covered the artificial nests. This allowed the survival and growth of the caterpillars to be recorded while causing the minimum of disturbance.

## Results

After introduction the *Manica rubida* workers carried each caterpillar from the foraging arena to the nest within one hour. Several caterpillars of both *M.alcon* and *M. rebeli* survived in the nests for a number of weeks, up to one and a half months (Tab. 1). The remains of dead caterpillars were often found in the arena among the rubbish.

Both *M.alcon* and *M. rebeli* caterpillars were about 3 mm long on introduction, and a month later the former had grown to about 5 mm while the latter had grown to about 10 mm. The workers antennated, licked and carried the caterpillars within the nest (Fig. 1; see the MPEG files showing extracts of the videotape at <http://www.zool.klte.hu/macman> or <http://www.oegef.at/>). I observed caterpillars eating ant brood, but it was not clear if the caterpillars were also fed directly by the worker ants.

## Discussion

My results show that laboratory *Manica rubida* colonies readily adopted *Maculinea* caterpillars, some of which survived for up to six weeks, during which they



grew considerably. This contrasts with the field observations of THOMAS & al. (1989) on another ant of the subfamily Myrmicinae, *Tetramorium caespitum* (LINNAEUS, 1758). They noted that "... several young *M. rebeli* larvae were seen being taken by *Tetramorium caespitum* L. soon after leaving their food-plant, but these were presumably taken as food by this voracious predator, for no trace of them was found in the nests soon afterwards." (THOMAS & al. 1989: 453). Similarly, ELFFERICH (1988) did not observe adoption of *M. alcon* caterpillars by *T. caespitum* nor by *Lasius flavus* (FABRICIUS, 1781), *L. niger* (LINNAEUS, 1758), *Formica fusca* LINNAEUS, 1758 and *F. sanguinea* LATREILLE, 1798 under artificial conditions. The caterpillars were often carried or tapped by the workers after the introduction but each of them was dead the next day or was carried out of the nest. However, he observed successful adoption and rearing by *Myrmica ruginodis* and this was the only ant species which licked the caterpillars. ELFFERICH (1988) considered this behaviour as obligatory for the survival of caterpillars. Similarly, in my experiments the caterpillars not only survived and grew but also were licked by the workers of *Manica rubida* (Fig. 1B). These phenomena show the potential suitability of *M. rubida* as a host for *Maculinea*. It is therefore possible that caterpillars of some *Maculinea* populations could fully develop in *M. rubida* nests at high altitudes.

It is important to emphasize that we cannot predict the host ant species used by a particular population of *Maculinea* butterflies using the data recorded from other populations. Different populations of these butterflies have evolved to use different hosts in different parts of their geographical ranges (ELMES & al. 1994, ALS & al. 2002). Moreover, within the islands of Japan *M. teleius* has been recorded as using hosts from two different ant genera: a *Myrmica* species (*M. ruginodis*) and an *Aphaenogaster* species (*A. japonica*) (YAMAGUCHI 1988).

*Manica rubida* is remarkably open in its social structure, even allowing for heterospecific colonies (with *Formica selysi* BONDROIT, 1918) to be established in the laboratory (ERRARD & JALLON 1987). This could potentially increase the suitability of this ant as a host of myrmecophilous insects. Additionally MALICKY (1969) reported experimental observations that *Manica* ants show standard, non-aggressive (but rather unspecific) tending behaviour to a range of (unspecified) lycaenid species. Whether the potential of *M. rubida* as a host of *Maculinea* butterflies that I have shown in the laboratory is realized in the field remains to be demonstrated. As far as I know there are no records of *Maculinea* pupae, caterpillars or exuvia from nests of *M. rubida* in the wild, but this may also reflect lack of search effort. On the other hand it has also been shown that a *Myrmica* species that does not act as a host to *Maculinea* from a particular population in the wild can be a good

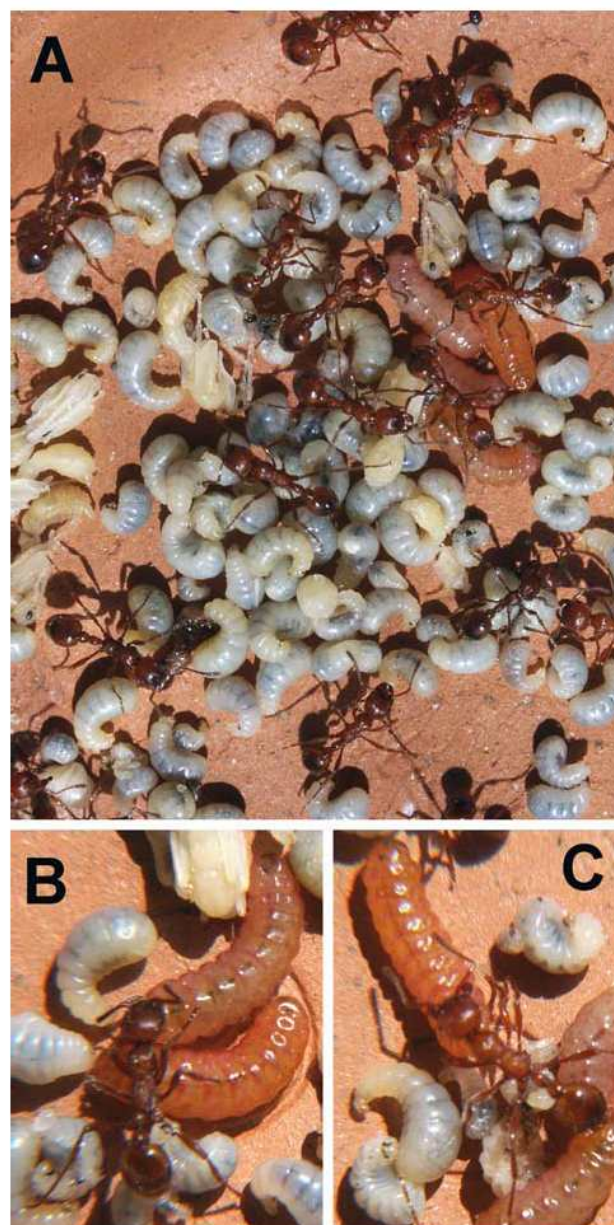


Fig. 1: These well-fed *Maculinea rebeli* caterpillars were not only at peace among the *Manica rubida* brood (A) but the workers often licked (B) and carried (C) them (photos by Péter Kozma of Colony 2 on 12 August 2003).

host in the laboratory when well-fed and not subject to stress (ELMES & al. 2004, SCHÖNROGGE & al. 2004).

It has been shown that the primary means of gaining entry to and surviving in *Myrmica* host ant nests is mimicry, whether it be of acoustic signals (DEVRIES & al. 1993) or of brood or colony odours (AKINO & al. 1999, ELMES & al. 2002, SCHLICK-STEINER & al. 2004, SCHÖNROGGE & al. 2004). My results suggest that the signals of caterpillars matched the template of *Manica rubida* sufficiently, well enough to induce adoption and to some degree acceptance in the nest – which is not surprising considering the close phylogenetic proximity of the genera



*Manica* and *Myrmica* (ASTRUC & al. 2004). However, the caterpillars in this study were from sites where the *Manica rubida* did not occur. Whether better mimicry of *M. rubida* can evolve in areas where it is found on *Maculinea* sites remains to be seen. In any case it would be interesting to compare the acoustical and chemical signatures of *M. rubida* with those of *Myrmica* species and to *Maculinea* caterpillars from *M. rubida* sites. In addition, it would be interesting to further test whether caterpillars of *M. alcon* and *M. rebeli* in *M. rubida* colonies get all their nutrition by eating the ant brood (which is not an unfamiliar food of "cuckoo-feeder" caterpillars; e.g., ELFFERICH 1988), or whether they can also be fed by trophallaxis by the worker ants as they are in *Myrmica* host nests.

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### Zusammenfassung

Die Larven von *Maculinea*-Bläulingen leben den Großteil ihrer Entwicklung als obligate Parasiten in Nestern von *Myrmica*-Ameisen (oder in Einzelfällen von *Aphaenogaster*). *Manica rubida* (LATREILLE, 1802) ist der Gattung *Myrmica* nahe verwandt und kommt unter anderem auch in Lebensräumen von *Maculinea* vor. Ich testete daher im Laborversuch, ob *M. rubida* Larven von *Maculinea rebeli* (HIRSCHKE, 1904) und *M. alcon* (DENIS & SCHIFFERMÜLLER, 1775) großziehen kann. Nach dem Einsetzen der *Maculinea*-Larven in die Arenen der Laborkolonien wurden alle Larven von Ameisenarbeiterinnen ins Nest gebracht, wo sie häufig umgelagert und beleckt wurden. Einige Larven beider Schmetterlingsarten überlebten und wuchsen einige Wochen, maximal einhalb Monate lang. Diese Ergebnisse legen nahe, dass *M. rubida* ein potentieller Wirt von *Maculinea*-Larven sein könnte. Ob sich tatsächlich lokale *Maculinea*-Populationen auf diesen potentiellen Wirten spezialisiert haben, muss allerdings im Freiland überprüft werden.

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## Paper VIII.

TARTALLY, A. 2005: *Neotypus melanocephalus* (Hymenoptera: Ichneumonidae): the first record of a parasitoid wasp attacking *Maculinea teleius* (Lycaenidae). –  
Nota Lepidopterologica 28: 65-67.



## ***Neotypus melanocephalus* (Hymenoptera: Ichneumonidae): the first record of a parasitoid wasp attacking *Maculinea teleius* (Lycaenidae)**

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**Abstract.** A *Maculinea teleius* (Bergsträsser, 1779) pupa was found near Meszes (NE-Hungary) in a *Myrmica scabrinodis* Nylander, 1846 nest. Some hours later emerged a wasp that proved (det. K. Horstmann) to be *Neotypus melanocephalus* Gmelin, 1790 (Ichneumonidae). The wasp with the exuvium and specimens of the host ant are deposited in the Hymenoptera Collection of the Hungarian Natural History Museum (25.vii.2002; Meszes; leg. A. Tartally). It would be desirable to obtain more *Neotypus* specimens from *M. teleius* pupae to test if the wasp really is *N. melanocephalus*, or a form of *N. pusillus* Gregor, 1940, or even a new cryptic species of *Neotypus*.

**Key words.** *Neotypus*, *Maculinea*, *Myrmica*, Ichneumonidae, Lycaenidae, parasitoid, Hungary.

The larvae and pupae of four of the five European species of *Maculinea* van Eecke, 1915 butterflies are known hosts of parasitoids from the Ichneumonidae family. The two cuckoo *Maculinea* species for which the caterpillars are fed by worker ants (Thomas & Elmes 1998) have *Ichneumon* sp. parasitoids. *Ichneumon eumerus* Wesmael, 1857 was recorded as a parasitoid of *M. rebeli* (Hirschke, 1904) and the same or a sibling *Ichneumon* species attacks *M. alcon* ([Denis & Schiffermüller], 1775) as well (Thomas & Elmes 1993; Munguira & Martin 1999; Sielezniew & Stankiewicz 2004; Thomas, Fitton & Hilpert, pers. comm.). Two of the three *Maculinea* species, of which the caterpillars are predators of ant broods (Thomas & Elmes 1998), have *Neotypus* parasitoids: *N. pusillus* Gregor, 1940 was bred from *M. nausithous* (Bergsträsser, 1779) (Thomas & Elmes 1993) and another *Neotypus* sp. from *M. arion* (Linnaeus, 1758) (Thomas, Wardlaw & Fitton, pers. comm.). So far as known, each of these parasitoids is host-specific to a single *Maculinea* species, but until now no parasitoid wasp of the predatory *M. teleius* (Bergsträsser, 1779) was known.

*Maculinea* species and their parasitoids are of high interest to evolutionary and conservation ecology because of their extreme adaptations to a myrmecophilous life-style and because all are rare and globally endangered (IUCN 2004; Hochberg et al. 1996; Munguira & Martin 1999). The larvae of these butterflies feed briefly on specific foodplants before being adopted by *Myrmica* ants (Hymenoptera: Formicidae) in which colonies they live as social parasites for 11–23 months (Thomas & Elmes 1998). *Neotypus pusillus* oviposits on young *M. nausithous* larvae on the larval foodplant while *Ichneumon* spp., perhaps in response to the different population structure found in cuckoo species, penetrate *Myrmica* nests to seek *Maculinea* larvae (Thomas & Elmes 1993). These parasitoids both emerge from host pupae inside ant colonies and are presumed to have similar specialisations to those described for *I. eumerus* (Thomas et al. 2002) to escape unharmed from nests.

Between 2000 and 2003, hundreds of *Myrmica* nests at eight sites were examined for caterpillars, pupae, and exuvia of *M. teleius* to measure host specificity and to investigate whether parasitoids of this butterfly occur in the Carpathian Basin, Hungary.

Twenty-four caterpillars, one exuvium, and eight pupae of *M. teleius* were found at five sites. One of the pupae contained a parasitoid. This was collected on 25 July 2002 near the village of Meszes (NE-Hungary; Borsod-Abaúj-Zemplén County) in a marshy meadow with a profusion of *Sanguisorba officinalis* (I intentionally do not give the exact location as a precaution against collectors; only two more pupae were found there but these were not parasitized). The pupal cases of *M. teleius* and *M. nausithous* are hard to distinguish, but this pupa was found in a *Myrmica scabrinodis* Nylander, 1846 nest within one meter from a *S. officinalis* plant. *My. scabrinodis* is the main host ant of *M. teleius* in Europe (Thomas et al. 1989; Stankiewicz & Sielezniew 2002; Tartally & Csősz 2004) and no other species of *Maculinea* nor any other foodplant of *Maculinea*, occur at this site (Varga, pers. comm.). Moreover, *M. nausithous*, the only other *Maculinea* species that uses *S. officinalis*, is not known from NE-Hungary (Bálint 1996). Based on this evidence, this pupa was identified as *M. teleius*. Some hours after collection, a wasp hatched from this pupa. The wasp with the exuvium and specimens of the host ant (*My. scabrinodis*) were placed into a small vial with 75% ethanol. The full sample is deposited in the Hymenoptera Collection of the Hungarian Natural History Museum (25.vii.2002; Meszes; Tartally leg.). The wasp was sent to Dr. Klaus Horstmann (Theodor-Boveri-Institut für Biowissenschaften, Würzburg) for determination; it proved to be *Neotypus melanocephalus* Gmelin, 1790, a species that had not previously been recorded as a parasitoid of any *Maculinea* host (Thomas, pers. comm.).

There are several known *M. nausithous* populations infected by *N. pusillus* that co-occur with *M. teleius* in Europe, but in those studied in the Rhône valley (France), there is strong evidence that *N. pusillus* never parasitizes *M. teleius* (Thomas, pers. comm.). It would be worth checking more widely whether *N. pusillus* uses only *M. nausithous* as a host or whether it can infect *M. teleius* too. This is important because *N. melanocephalus* has, at times, been synonymised with *N. pusillus*; but current studies in the EU ‘MacMan’ programme suggest that *N. melanocephalus* is a cryptic sibling species (Thomas, pers. comm.), a phenomenon well known in parasitoid taxa (Godfray 1994). It would be desirable to obtain more *Neotypus* specimens from *M. teleius* pupae and to test whether this wasp really is *N. melanocephalus*, or a form of *N. pusillus*, or even a new cryptic species of *Neotypus*. If, as I suspect, it is both a good species and specific to *M. teleius*, then, like other parasitoids of *Maculinea*, it will by definition be rarer and more threatened than its host, and its populations will be in greater need of conservation (Hochberg et al. 1996; Thomas et al. 2002).

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## Paper IX.

TARTALLY, A., SZÜCS, B. & EBSSEN, J.R. 2007: The first records of *Rickia wasmannii* CAVARA, 1899, a myrmecophilous fungus, and its *Myrmica* LATREILLE, 1804 host ants in Hungary and Romania (Ascomycetes: Laboulbeniales; Hymenoptera: Formicidae). – Myrmecological News 10: 123 (published lecture Abstract)



## Abstract\*

**The first records of *Rickia wasmannii* CAVARA, 1899, a myrmecophilous fungus, and its *Myrmica* LATREILLE, 1804 host ants in Hungary and Romania (Ascomycetes: Laboulbeniales; Hymenoptera: Formicidae)**

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Myrmecol. News 10: 123

*Rickia wasmannii* CAVARA, 1899 (Ascomycetes: Laboulbeniales) obligately exploits ants (for a review on Laboulbeniales: WEIR & BLACKWELL 2005; and for one especially on myrmecophilous species: HERRAIZ & ESPADALER 2007). The ants appear to be neutral to the presence of this fungus on their cuticles (A. Tartally, pers. obs.). *Myrmica* LATREILLE, 1804 (Hymenoptera: Formicidae) species are reported to be the usual hosts of *R. wasmannii* (HERRAIZ & ESPADALER 2007). To the best of our knowledge, this fungus has not been reported previously from the Carpathian Basin. The occurrence of *R. wasmannii* was checked on 5788 *Myrmica* specimens from 580 colonies collected between 2001 and 2006 at 26 sites in Hungary and three sites in Transylvania, Romania (Fig. 1), and the density of the fungus on the different parts of the body of infected *Myrmica* specimens was estimated. The fungus was present on 353 infected ant specimens in 45 colonies at nine Hungarian and two Transylvanian sites (Fig. 1). Although 11 *Myrmica* species (*M. gallienii* BONDROIT, 1920; *M. lobicornis* NYLANDER, 1846; *M. lonae* FINZI, 1926; *M. rubra* (LINNAEUS, 1758); *M. ruginodis* NYLANDER, 1846; *M. sabuleti* MEINERT, 1861; *M. salina* RUZSKY, 1905; *M. scabrinodis* NYLANDER, 1846; *M. schencki* VIERECK, 1903; *M. specioides* BONDROIT, 1918 and *M. vandeli* BONDROIT, 1920) were involved in our work, only four of them (*M. salina*, *M. scabrinodis*, *M. specioides* and *M. vandeli*) were found to be infected. *M. scabrinodis* was the most common host, and *M. salina* was most heavily infected. The fungus was present on workers (Figs. 2 - 4) and dealate (old) queens, but not on males, alate (young) queens and larvae. However, the numbers of males, alate queens and larvae examined were small. Our results indicate that it is quite probable that *R. wasmannii* could be found at several other sites in the Carpathian Basin with a more intensive survey.

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Figs. 2 - 4: *Rickia wasmannii* on a *Myrmica scabrinodis* worker (photo by J.R. Ebsen, SEM photos by D.R. Nash). ➔

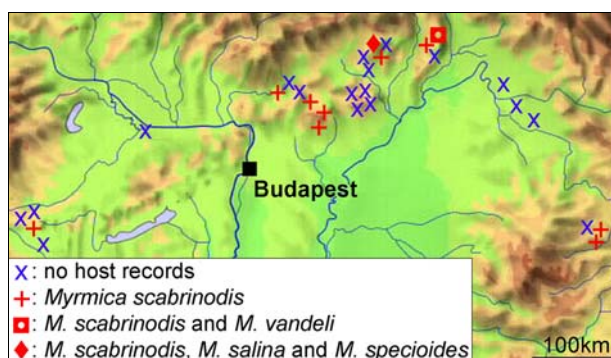
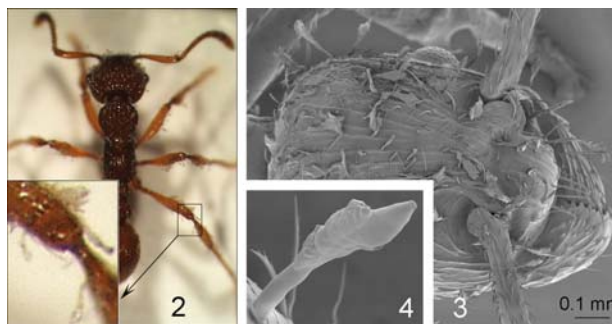


Fig. 1: Investigated sites in the Carpathian Basin and the recorded host ants of *Rickia wasmannii* (map by L. Zentai).

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\* Based on a presentation at the 2<sup>nd</sup> Central European Workshop of Myrmecology at Szeged, Hungary, 17 - 19 May 2007



## **Appendix II: Scientific names of taxa mentioned in the thesis**

(according to: CAVARA 1899, SEIFERT 1988, SIMON 1992, PECH & al. 2004, Fauna Europaea 2007, Hymenoptera Name Server 2007)

### **INSECTS:**

#### ***Aphaenogaster* MAYR, 1853 (Hymenoptera: Formicidae)**

*Aphaenogaster japonica* FOREL, 1911

#### ***Formica* LINNAEUS, 1758 (Hymenoptera: Formicidae)**

*Formica fusca* LINNAEUS, 1758

*Formica sanguinea* LATREILLE, 1798

*Formica selysi* BONDROIT, 1918

#### ***Glaucopsyche* SCUDDER, 1872 (Lepidoptera: Lycaenidae)**

#### ***Ichneumon* LINNAEUS, 1758 (Hymenoptera: Ichneumonidae)**

*Ichneumon eumerus* WESMAEL, 1857

*Ichneumon fulvicornis* GRAVENHORST, 1829

#### ***Lasius* FABRICIUS, 1804 (Hymenoptera: Formicidae)**

*Lasius flavus* (FABRICIUS, 1781)

*Lasius niger* (LINNAEUS, 1758)

#### ***Maculinea* VAN EECKE, 1915 (Lepidoptera: Lycaenidae)**

*Maculineaalcon* ([DENIS & SCHIFFERMÜLLER], 1775)

*Maculinea arion* (LINNAEUS, 1758)

*Maculinea arionides* (STAUDINGER, 1887)

*Maculinea nausithous* (BERGSTRÄSSER, 1779)

*Maculinea rebeli* (HIRSCHKE, 1904)

*Maculinea teleius* (BERGSTRÄSSER, 1779)

#### ***Manica* JURINE, 1807 (Hymenoptera: Formicidae)**

*Manica rubida* (LATREILLE, 1802)

***Microdon* MEIGEN, 1803 (Diptera: Syrphidae)**

*Microdon myrmicae* SCHÖNROGGE, BARR, WARDLAW, NAPPER, GARDNER, BREEN, ELMES & THOMAS 2002

***Myrmica* LATREILLE, 1804 (Hymenoptera: Formicidae)**

*Myrmica aloba* FOREL, 1909

*Myrmica gallienii* BONDROIT, 1920

*Myrmica hellenica* FINZI, 1926

*Myrmica lobicornis* NYLANDER, 1846

*Myrmica lonae* FINZI, 1926

*Myrmica microrubra* SEIFERT, 1993

*Myrmica rubra* (LINNAEUS, 1758)

*Myrmica ruginodis* NYLANDER, 1846

*Myrmica rugulosa* NYLANDER, 1849

*Myrmica sabuleti* MEINERT, 1861

*Myrmica salina* RUZSKY, 1905

*Myrmica scabrinodis* NYLANDER 1846

*Myrmica schencki* VIERECK, 1903

*Myrmica specioides* BONDROIT, 1918

*Myrmica sulcinodis* NYLANDER, 1846

*Myrmica tulinae* ELMES, RADCHENKO & AKTAÇ, 2002

*Myrmica vandeli* BONDROIT, 1920

***Neotypus* FÖRSTER 1869 (Hymenoptera: Ichneumonidae)**

*Neotypus melanocephalus* GMELIN, 1790

*Neotypus pusillus* GREGOR, 1940

***Phengaris* DOHERTY, 1891 (Lepidoptera: Lycaenidae)**

***Tetramorium* MAYR, 1855 (Hymenoptera: Formicidae)**

*Tetramorium caespitum* (LINNAEUS, 1758)

**FUNGUS:**

***Rickia* CAVARA, 1899 (Ascomycetes: Laboulbeniales)**

*Rickia wasmannii* CAVARA, 1899



## PLANTS:

### ***Calluna* SALISB. (Dicotyledonopsida: Ericaceae)**

*Calluna vulgaris* (L.) HULL

### ***Carex* L. (Monocotyledonopsida: Cyperaceae)**

### ***Deschampsia* P.B. (Monocotyledonopsida: Gramineae (Poaceae))**

*Deschampsia caespitosa* (L.) P.B.

### ***Gentiana* L. (Dicotyledonopsida: Gentianaceae)**

*Gentiana asclepiadea* L.

*Gentiana cruciata* L.

*Gentiana pneumonanthe* L.

### ***Gentianella* MÖNCH (Dicotyledonopsida: Gentianaceae)**

*Gentianella austriaca* (A. & J. KERN.) HOLUB

### ***Molinia* SCHRANK (Monocotyledonopsida: Gramineae (Poaceae))**

*Molinia coerulea* MÖNCH agg.

### ***Origanum* L. (Dicotyledonopsida: Labiatae)**

*Origanum vulgare* L.

### ***Pinus* L. (Coniferopsida: Pinaceae)**

*Pinus nigra* ARN.

### ***Salix* L. (Dicotyledonopsida: Salicaceae)**

*Salix rosmarinifolia* (L.) HARTM.

### ***Sanguisorba* L. (Dicotyledonopsida: Rosaceae)**

*Sanguisorba officinalis* L.

### ***Thymus* L. (Dicotyledonopsida: Labiatae)**

*Thymus marschallianus* WILLD.

*Thymus pannonicus* ALL.

*Thymus pulegioides* L.

## Appendix III: Characteristics of the investigated sites

**Key for abbreviated taxon names in Appendix II and IV:** alc: *Maculinea alcon*, alo: *Myrmica aloba*, ari: *Maculinea arion*, gal: *Myrmica gallienii*, Ich: *Ichneumon eumerus*, lob: *Myrmica lobicornis*, lon: *Myrmica lonae*, Mic: *Microdon myrmicae*, Myr: *Myrmica* species recorded from the site, nau: *Maculinea nausithous*, Neo: *Neotypus melanocephalus*, reb: *Maculinea rebeli*, Ric: *Rickia wasmannii*, rub: *Myrmica rubra*, rug: *Myrmica ruginodis*, sab: *Myrmica sabuleti*, sal: *Myrmica salina*, sca: *Myrmica scabrinodis*, sch: *Myrmica schencki*, spe: *Myrmica specioidea*, sul: *Myrmica sulcinodis*, tel: *Maculinea teleius*, tul: *Myrmica tulinae*, van: *Myrmica vandeli*

+: the species occurs with the *Myrmica* species; ?: the species occurs on the site but no data on the host *Myrmica* species; see notes after the table

Site (on Appendix IV)	Vegetation	Myr	alc	reb	tel	nau	ari	Neo	Ich	Mic	Ric	Notes
<b>Aggtelek</b> (Ag) 48°26' N; 20°30' E 340 m a.s.l.	Marshy meadow with <i>Molinia coerulea</i> agg. and <i>Deschampsia caespitosa</i> , dense stands of <i>Sanguisorba officinalis</i> ( <i>M. teleius</i> ); AND <i>Calluno-Genistetum</i> heath with large sprout-colonies of <i>Thymus pulegioides</i> on the clumps of ant nests and with <i>Origanum vulgare</i> at the forest edges ( <i>M. arion</i> )	gal sab sca sch spe van			+		?			+		1
<b>Bükkszentkereszt</b> (Bü) 48°04' N; 20°38' E 563 m a.s.l.	Abandoned mountain hayfield with <i>Gentiana cruciata</i>	sab sca		+	+				+			
<b>Drahos-rét</b> (Dr) 48°34' N; 21°26' E 742 m a.s.l.	Tall-grass, mountain hayfield and marshy meadow with <i>Molinia coerulea</i> agg., dense stands of <i>Sanguisorba officinalis</i> , sprout-colonies of <i>Thymus pulegioides</i> on the clumps of ants nests	sca van	+		+		?	?		+	+	1

Site (on Appendix IV)	Vegetation	Myr	alc	reb	tel	nau	ari	Neo	Ich	Mic	Ric	Notes
<b>Drávaiványi-legelő</b> (Di) 45°50' N; 17°49' E 98 m a.s.l.	Tall-grass, tall-sedge marshy meadow with <i>Sanguisorba officinalis</i>	sal spe			+							
<b>Fânațele Clujului</b> (Fc) Szénafüvek* N23°37'; E46°51' 540 m a.s.l.	Extensively grazed tall-grass meadow steppe with small marshy depressions, tall-forb vegetation with <i>Sanguisorba officinalis</i>	sca	?		+	?	?				+	2
<b>Fülesd</b> (Fü) 48°01' N; 22°38' E 111 m a.s.l.	Tall-grass marshy meadow with <i>Molinia coerulea</i> agg. and <i>Deschmopsia caespitosa</i> and large stands of <i>Sanguisorba officinalis</i> and <i>Gentiana pneumonanthe</i>	gal rug sal sca		+		+				+		
<b>Gödörháza</b> (Gö) 46°45' N; 16°21' E 407 m a.s.l.	Marshy meadow with <i>Molinia coerulea</i> agg. and <i>Carex</i> spp., dense stands of <i>Sanguisorba officinalis</i> and <i>Gentiana pneumonanthe</i> , with low scrubs of <i>Salix rosmarinifolia</i>	rub rug sca			?	+					+	
<b>Gyertyánkúti-rétek</b> (Gr) 48°29' N; 21°22' E 700 m a.s.l.	Extended mosaic of mountain hayfield and marshy meadow ( <i>Molinietum</i> ) with large patches of <i>Sanguisorba officinalis</i> and <i>Gentiana pneumonanthe</i>	rug sca van	?		?							
<b>Gyilkos-rét</b> (Gy) 47°48' N; 19°58' E 352 m a.s.l.	Tall-sedge, marshy meadow with dense stands of <i>Gentiana pneumonanthe</i>	gal sca sch		+					+		+	
<b>Hetefejércse</b> (He) 48°08' N; 22°29' E 108 m a.s.l.	Tall-grass mosaic of lowland hayfield ( <i>Alopecuretum</i> ) and marshy meadow with <i>Molinia coerulea</i> agg., patches of <i>Sanguisorba officinalis</i>	sca	+		?							

Site (on Appendix IV)	Vegetation	Myr	alc	reb	tel	nau	ari	Neo	Ich	Mic	Ric	Notes
<b>Hidegség</b> (Hi) 47°23' N; 16°27' E 117 m a.s.l.	Tall-grass, tall-sedge marshy meadow with <i>Sanguisorba officinalis</i>	rub			+	+						
<b>Ipolytarnóc</b> (Ip) 48°14' N; 19°37' E 168 m a.s.l.	Tall-grass, tall-sedge marshy meadow with <i>Sanguisorba officinalis</i>	gal rub rug sca			?					+		
<b>Kaszonyi-hegy</b> (Kh) 48°15' N; 22°29' E 120 m a.s.l.	Mosaic of tall-grass meadow with <i>Origanum vulgare</i> , hedgerow and tall-forb forest-fringe vegetation, abandoned orchards	rub sca					?					1
<b>Kecskeláb-rét</b> (Kr) 48°05' N; 20°31' E 751 m a.s.l.	Abandoned mountain hayfield and semi-dry meadow with <i>Gentiana cruciata</i>	lon sab sca		+								
<b>Kercaszomor</b> (Ke) 46°46' N; 16°18' E 240 m a.s.l.	Mosaic of tall-grass hayfield, marshy meadow with <i>Sanguisorba officinalis</i> and willow scrubs	rub sca	+		?	?	?			+		2
<b>Kétvölgy</b> (Kv) 46°53' N; 16°12' E 305 m a.s.l.	Mosaic of tall-grass hayfield, marshy meadow with <i>Sanguisorba officinalis</i> and heath with <i>Calluna vulgaris</i>	gal rub sca spe	?		+	+				+		3
<b>Kurisztlan</b> (Ku) 48°29' N; 20°34' E 333 m a.s.l.	Mosaic of semi-dry sward and mesophilic hayfield with <i>Gentiana cruciata</i>	sca sch spe van		+								

Site (on Appendix IV)	Vegetation	Myr	alc	reb	tel	nau	ari	Neo	Ich	Mic	Ric	Notes
<b>Lászlótanya</b> (Lá) 46°33' N; 16°12' E 620 m a.s.l.	Mosaic of tall-grass hayfield, marshy meadow with <i>Sanguisorba officinalis</i>	rub sca van			+						+	
<b>Lófő-tisztás</b> (Lt) 48°04' N; 20°39' E 656 m a.s.l.	Mosaic of abandoned mountain hayfield and semi-dry grassland with <i>Gentiana cruciata</i> and partly with <i>Gentianella austriaca</i>	lon rug sab sca sch		+					+	+		4
<b>Meszes</b> (Me) 48°27' N; 20°47' E 165 m a.s.l.	Tall-grass, tall-sedge marshy meadow with <i>Sanguisorba officinalis</i>	rub sca			+			+	+	+	+	
<b>Nagy-mező</b> (Nm) 48°04' N; 20°30' E 783 m a.s.l.	Mosaic of irregularly grazed mountain meadow and semi-dry grassland with <i>Gentiana cruciata</i> and partly with <i>G. pneumonanthe</i>	lob lon rug sab sca sch		+					+			5
<b>Nógrádszakál</b> (Nó) 48°12' N; 19°32' E 165 m a.s.l.	Tall-grass, tall-sedge marshy meadow with <i>Sanguisorba officinalis</i>	rub sca			?					+	+	
<b>Nyikom-rét</b> (Nr) 47°54' N; 19°46' E 680 m a.s.l.	Tall-grass, marshy meadow with <i>Gentiana pneumonanthe</i>	rug sca	+								+	
<b>Őriszentpéter</b> (Őr) 46°51' N; 16°12' E 230 m a.s.l.	Mosaic of tall-grass hayfield, marshy meadow with <i>Sanguisorba officinalis</i> and willow scrubs	rub sca			+							

Site (on Appendix IV)	Vegetation	Myr	alc	reb	tel	nau	ari	Neo	Ich	Mic	Ric	Notes
<b>Răscruci</b> (Ră) Válaszút* 46°54' N; 23°47' E 485 m a.s.l.	Extensively grazed tall-grass meadow steppe with <i>Gentiana cruciata</i> with small marshy depressions, tall-forb vegetation with <i>Sanguisorba officinalis</i> and <i>G. pneumonanthe</i>	sab sca sch	+	+	+	+			+	+	+	6
<b>Şardu</b> (Şa) Sárd* 46°52' N; 23°24' E 480 m a.s.l.	Tall-grass, tall-sedge marshy meadow with patches of <i>Sanguisorba officinalis</i> and locally dense stands of <i>Gentiana pneumonanthe</i>	sca van	+		+				+	+	+	7
<b>Sóshartyán</b> (Só) 48°04' N; 19°42' E 250 m a.s.l.	Rupicolous grassland with <i>Thymus marschallianus</i> and <i>T. pannonicus</i> on eroded sand-stone with large plantations of black pine ( <i>Pinus nigra</i> )	sab					?					1
<b>Szilicei-fennsík</b> (Sf) 48°30' N; 20°33' E 550 m a.s.l.	Mosaic of rupicolous and steppic grassland with <i>Thymus marschallianus</i> and <i>T. pannonicus</i> and lanuginose oak scrub forest with xerothermic forest-fringe	lob lon sab sca					?					1
<b>Teresztenyei-fennsík</b> (Tf) 48°27' N; 20°37' E 330 m a.s.l.	Mosaic of semi-dry sward and steppic grassland with <i>Thymus marschallianus</i> and <i>T. pannonicus</i> ; patches of lanuginose oak scrub forest and xerothermic forest fringe with <i>Origanum vulgare</i>	sab sca					?					1
<b>Tohonya-hát</b> (Th) 48°29' N; 20°32' E 268 m a.s.l.	Old fallow, regenerated and managed (irregularly grazed and mowed at the end of the vegetation period) semi-dry sward with <i>Gentiana cruciata</i>	sab sal sca sch spe									+	
				+							+	
				+							+	

Site (on Appendix IV)	Vegetation	Myr	alc	reb	tel	nau	ari	Neo	Ich	Mic	Ric	Notes
<b>Tugár-rét</b> (Tr) 47°54' N; 19°49' E 586 m a.s.l.	Tall-grass, marshy meadow with <i>Gentiana pneumonanthe</i>	sca	+								+	
<b>Velemér</b> (Ve) 46°44' N; 16°21' E 198 m a.s.l.	Marshy meadow with <i>Molinia coerulea</i> agg. and <i>Carex</i> spp., dense stand of <i>Sanguisorba officinalis</i>	gal rub sca				?				?		
<b>Vörös-rét</b> (Vr) 47°46' N; 17°42' E 115 m a.s.l.	Tall-grass, tall-sedge marshy meadow with <i>Sanguisorba officinalis</i>	rub rug sca			+	?					+	

\*: The Hungarian names of Transylvanian sites.

1: See notes on host plants and flying period of *M. arion* in Table 3.

2: These two sites (Fânațele Clujului and Kercaszomor) are not included in Chapter 3.3 because only very few *M. arion* specimens were observed there.

3: There was one *M. rubra* nest that contained 28 *M. nausithous* and 8 *M. teleius* larvae in total.

4: *Gentianella austriaca* occurs here in low density and it is used by *M. rebeli* here as an additional host plant among the main host *Gentiana cruciata*.

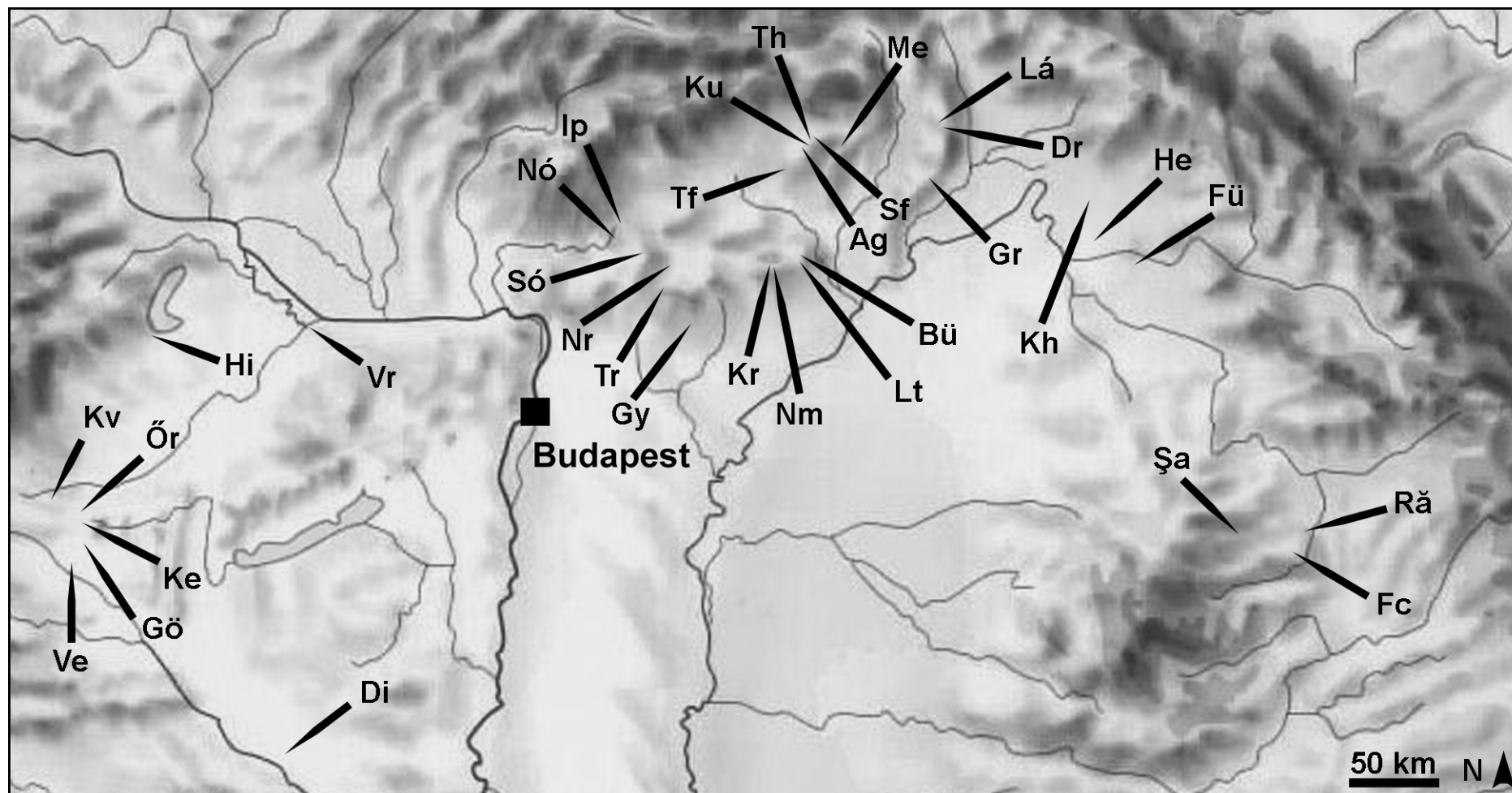
5: *Gentiana pneumonanthe* occurs here in low density and it is used by *M. rebeli* here as an additional host plant among the main host *G. cruciata*.

6: *S. officinalis* and *G. pneumonanthe* were common on the humid and *G. cruciata* on the dry patches. Both gentians were used here by the “*M.alcon-rebeli*” population. *M. sabuleti* and *M. schencki* were found only on the dry patches but *M. scabrinodis* was common on both types of patches. There were four *M. scabrinodis* nests infected by two different *Maculinea* species: 1 *M. teleius* + 1 *M.alcon*, 1 *M. teleius* + 1 *M.alcon*, 1 *M. teleius* + 5 *M.alcon*, 1 *M. teleius* + 1 *M. nausithous* larvae.

7: *I. eumerus* was found only with *M.alcon*.

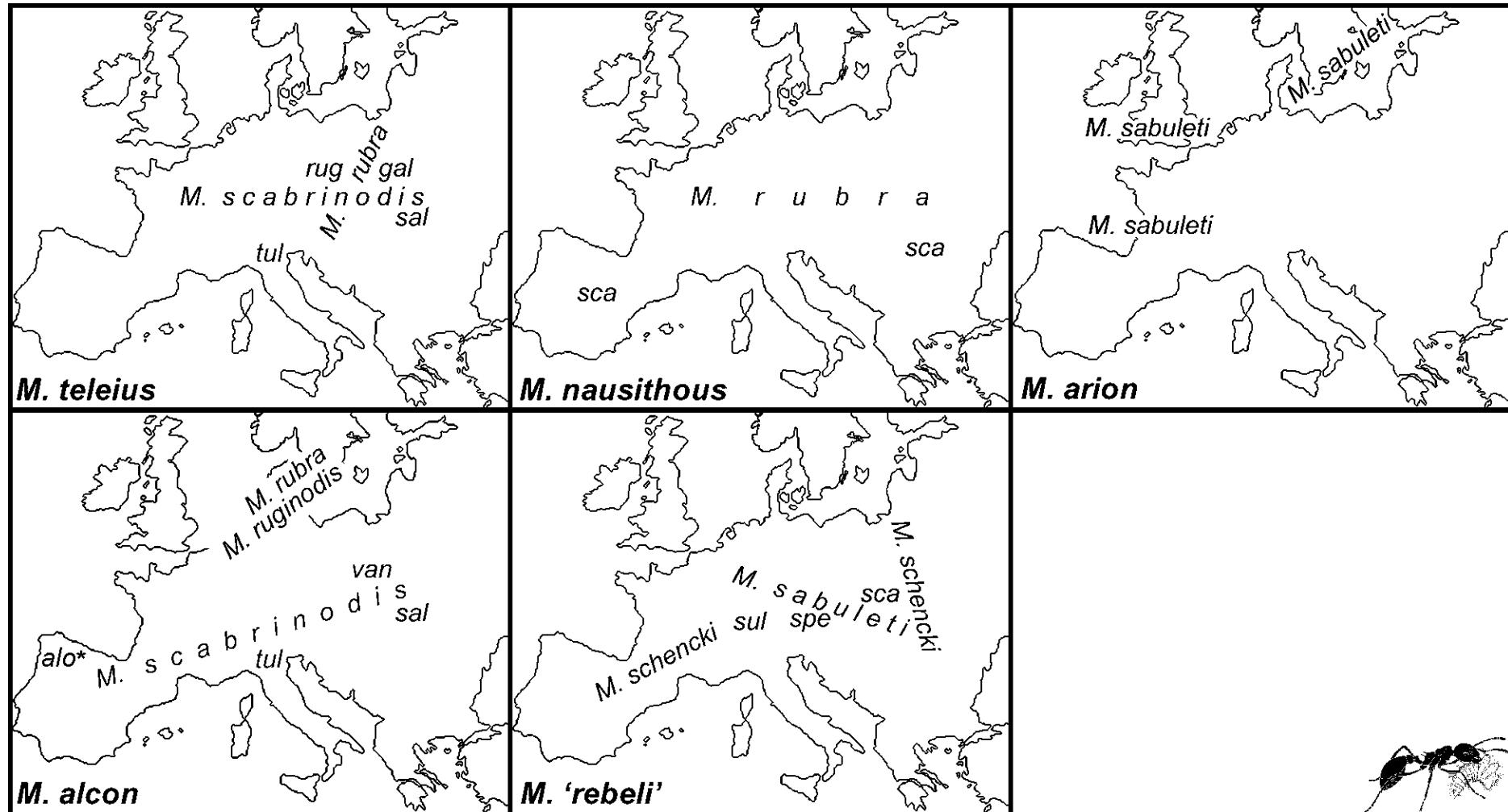
## Appendix IV: Localities of the investigated sites

(the key for the abbreviated site names is in the first column of Appendix III; background map: ZENTAI 1996)





## Appendix V: Known important host ants of *Maculinea* butterflies in Europe



(according to the thesis and the references cited therein, \*: P.S. Arnaldo, A. Tartally & S. Csősz, unpubl. data; see ALS & al. 2004, FIEDLER 2006, PECH & al. 2007 for a review on all the recorded host ant species; abbreviations show locally important host ants, see Appendix III for a key)

## Appendix VI: Photos

**1:** *Maculinea teleius* female (by Zoltán Varga)

**2:** *Maculinea 'rebeli'* eggs on *Gentiana cruciata* (by Péter Kozma)

**3:** A *Myrmica scabrinodis* worker is adopting a young *Maculineaalcon* caterpillar (by Péter Kozma)

**4:** Two *M. scabrinodis* workers are carrying a developed *M.alcon* caterpillar (by József L. Szentpéteri)

**5:** A *Maculinea nausithous* caterpillar in a *Myrmica rubra* nest (by Péter Kozma)

**6:** A *Maculinea teleius* caterpillar in a *M. scabrinodis* nest (by Péter Kozma)

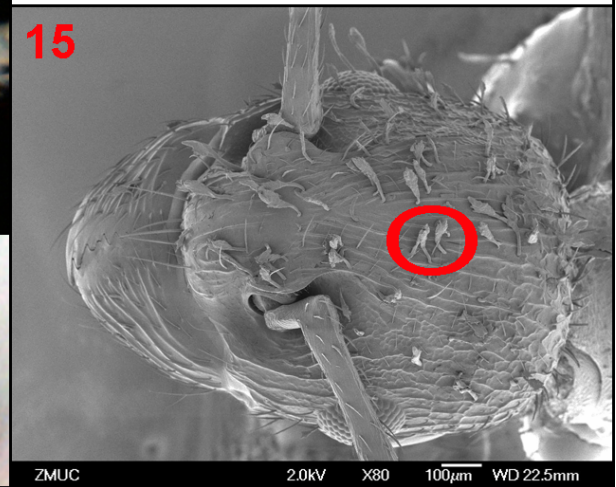
**7:** Four *M. 'rebeli'* caterpillars in a *Myrmica sabuleti* nest (by Péter Kozma)

**8:** Four *M. rebeli* pupae in a *M. sabuleti* nest, arrow indicates a pupa infected by *Ichneumon eumerus* (by Péter Kozma)



- 9:** A *Neotypus melanocephalus* female is stinging *M. teleius* or *M. nausithous* larvae on a *S. officinalis* flowerhead (by Marcin Sielezniew)
- 10:** An *I. eumerus* female is stinging *M.alcon* caterpillars in a *M. scabrinodis* nest (by Marcin Sielezniew)
- 11:** *I. eumerus* is developing in a *M.alcon* pupa in a *M. scabrinodis* nest (by Marcin Sielezniew)
- 12:** An exuvium of a *M.alcon* pupa (found in a *M. scabrinodis* nest) is left by an *I. eumerus* wasp (by Péter Kozma)
- 13:** A *Microdon myrmicae* larva (found in a *M. scabrinodis* nest) (by Péter Kozma)
- 14:** An adult *M. myrmicae* (found in a *M. scabrinodis* nest) is hatching from the pupa (by Péter Kozma)
- 15:** A *M. scabrinodis* worker is covered by *Rickia wasmannii* (SEM photo by David R. Nash)





**16:** The artificial nest which was used for keeping artificial *Myrmica* and *Manica rubida* colonies in the laboratory (this photo was taken on a *M. scabrinodis* colony where two *M. 'rebeli'* caterpillars were developing) (by Péter Kozma)

**17:** Two caterpillars and a pupa of *M. 'rebeli'* which were developed by accelerated development at an artificial *Myrmica salina* colony (by Péter Kozma)

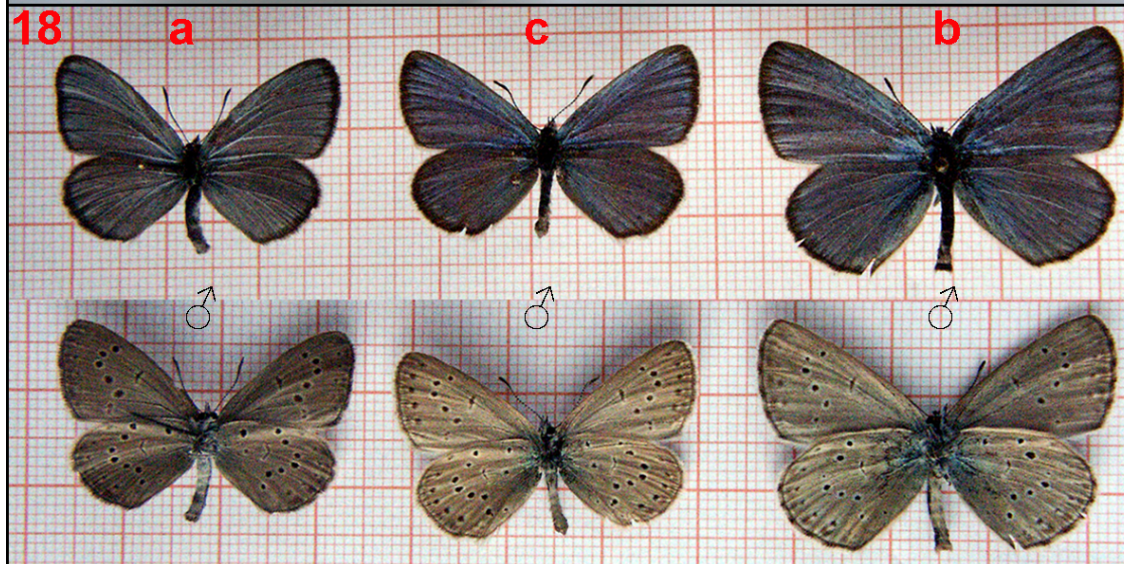
**18:** The *M. 'rebeli'* adult that eclosed 32 days after adoption in the laboratory (a) is smaller than an average size field-raised specimen (b) but similar to a smaller field-raised specimen (c) from the same area (N-Hungary, Nagy-mező) (by Péter Kozma)

**19:** Four well-fed *M. 'rebeli'* caterpillars at peace in an artificial *Manica rubida* colony (by Péter Kozma)

**20:** An example for the specific treatments required by *Maculinea* butterflies: *Myrmica rubra*, the exclusively recorded host ant of *Maculinea nausithous* in western Hungary (see Chapter 3.2.1.), occurred only in the narrow zone at the forest edge indicated by the red lines (by Zoltán Varga)

**21:** The MacMan logo (see SETTELE & al. 2002, MacMan 2007)









**Myrmecophily of *Maculinea* butterflies in the Carpathian Basin**  
**(Lepidoptera: Lycaenidae)**

**A *Maculinea* boglárkalepkék mirmekofiliája a Kárpát-medencében**  
**(Lepidoptera: Lycaenidae)**

Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében  
a Biológia tudományágban

Írta: Tartally András, okleveles biológus (ökológus)

Készült a Debreceni Egyetem Biológia Tudományok Doktori Iskolája  
(Biodiverzitás programja) keretében

Témavezető: Dr. Varga Zoltán

A doktori szigorlati bizottság:

elnök:	Dr. ....	.....
tagok:	Dr. ....	.....
	Dr. ....	.....

A doktori szigorlat időpontja: 200... . . . .

Az értekezés bírálói:

Dr. ....	.....
Dr. ....	.....
Dr. ....	.....

A bírálóbizottság:

elnök:	Dr. ....	.....
tagok:	Dr. ....	.....
	Dr. ....	.....
	Dr. ....	.....
	Dr. ....	.....

Az értekezés védésének időpontja: 200... . . . .