NOTE

Eur. J. Entomol. **101**: 693–696, 2004
ISSN 1210-5759

Simultaneous exploitation of *Myrmica vandeli* and *M. scabrinodis* (Hymenoptera: Formicidae) colonies by the endangered myrmecophilous butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae)

MARCIN SIELEZNIEW[1] and ANNA M. STANKIEWICZ[2]

[1]Department of Applied Entomology, SGGW – Warsaw Agriculture University, Nowoursynowska 166, PL-02787 Warszawa, Poland; e-mail: sielezniew@sggw.waw.pl

[2]Laboratory of Social and Myrmecophilous Insects, Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, PL-00679 Warszawa, Poland; e-mail: ams@miiz.waw.pl

Key words. *Maculinea alcon*, host-ant specificity, *Myrmica scabrinodis*, *Myrmica vandeli*, *Ichneumon*, myrmecophily, endangered species, Poland

Abstract. Host-ant specificity of *Maculinea alcon*, an endangered obligatory myrmecophilous lycaenid butterfly, was studied at two sites in southern Poland (Świętokrzyskie Mts near Kielce). *M. alcon* larvae and pupae were found in nests of both *Myrmica vandeli* and *M. scabrinodis*, which is the typical host in Poland and Southern Europe. To our knowledge this is the first record of *M. vandeli* as a host of *M. alcon*. Our results show that *M. scabrinodis* and *M. vandeli* are about equally suitable as hosts. We hypothesize that both host ant species are closely related and have similar chemical profiles, and that the *M. alcon* "*scabrinodis-vandeli*" populations we studied belong to the *M. alcon* "*scabrinodis*" race. More than half of the *M. alcon* pupae, both from *M. vandeli* and *M. scabrinodis* nests, were parasitized by a single wasp species of the genus *Ichneumon*, which also suggests that the cuticular chemistry of the two ant species is similar.

INTRODUCTION

The Alcon Blue, *Maculinea alcon* (Denis & Schiffermüller, 1775), is a representative of the Palaearctic genus *Maculinea* van Eecke, 1915, which includes about seven obligatory myrmecophilous species (Lepidoptera: Lycaenidae). All of these are initially herbivorous as caterpillars, feeding on specific host plants, but spend their last (fourth) instar in *Myrmica* Latreille, 1804 ant colonies as social parasites (Thomas, 1995). Because of their extraordinary life history and sensitivity to environmental change, *Maculinea* butterflies are locally and globally endangered (van Swaay & Warren 1999; Munguira & Martin, 1999).

M. alcon inhabits hay meadows, wet heaths and fens. Females lay eggs on *Gentiana pneumonanthe* L. and some other gentians (Munguira & Martin, 1999). After the third moult caterpillars leave the plants and fall to the ground where, if lucky, they are encountered by foraging *Myrmica* ants, which carry them to their nests. Adopted caterpillars mimic ant chemicals (Elmes et al., 2002) and spend 10 months underground being fed like the ant larvae by worker ants. Among the other four European species of the genus *Maculinea*, only *M. rebeli* Hirschke, 1904 has the same "cuckoo" habit of parasitism, and the remaining species exploit *Myrmica* colonies by eating the ant brood (Thomas, 1995).

Butterfly-ant relationships in the genus *Maculinea* are highly specific. In western Europe *M. alcon* uses three *Myrmica* species along a north-south gradient. *M. scabrinodis* Nylander, 1846 is recorded as the host-ant in France, Spain and southern Netherlands, *M. ruginodis* Nylander, 1846 in northern Netherlands and Denmark, *M. rubra* (L., 1758) in Denmark and Sweden (Elmes et al., 1994; Als et al., 2002). However, Eastern European populations of *M. alcon* have hardly been studied so that the distribution and general habitat requirements of *M. alcon* in Eastern Europe is incomplete. During our first survey in Poland we identified *M. scabrinodis* as the host-ant of *M. alcon*. We suggested that this ant species is the most important host throughout the country but stressed the need for further studies (Sielezniew & Stankiewicz, 2002). This paper presents additional findings on the ecology and host ant use by *M. alcon* and reports an hitherto unknown host ant.

MATERIAL AND METHODS

Studies were conducted at two *M. alcon* sites in southern Poland near Kielce, Świętokrzyskie Mts. The Mt. Otrosz site (50°49'N/20°41'E; 350 m a.s.l.) is a high, isolated, wet meadow surrounded by mixed forest. Less than one hundred *G. pneumonanthe* plants were found on a small (approx 0.5 ha) part on this meadow, which was covered mainly with vegetation that can be classified as *Molinion*. In some places *G. pneumonanthe* co-occurred also with *Calluna vulgaris* (L.), while in others it grew among small *Pinus sylvestris* L. trees or *Frangula alnus* Mill. bushes. The Jasiów site (51°01'N/20°39'E; 350 m a.s.l.) is generally very similar to the previous site but in area is twelve times bigger (about 6 ha) and consists of three connected meadows. Host plants were present almost everywhere and their distribution was fairly even.

Both sites were investigated just before the *M. alcon* flight period at the beginning of July 2003, with one-day between the surveys at Otrosz and Jasiów. Areas within 1 m of a few dozen *G. pneumonanthe* plants on every site were searched for nests of *Myrmica* ants. Some colonies were easy to find because of well-developed solaria, while others were less visible and hidden in tufts of grass or in *Sphagnum* moss. All nests were very carefully opened and checked for the presence of *M. alcon*. Larvae and pupae were counted and recorded using a digital video camera, which allowed later measurement of length by comparison with a standard frame. Every pupa was also carefully examined against the light for the possible presence of a parasitoid pupa. After inspection, pupae and larvae were immediately returned to colonies. Some parasitized pupae were taken to the laboratory,

TABLE 1. The number of *Myrmica* nests within 1 m of *G. pneumonanthe* at two *M. alcon* sites in S-Poland and the number of the nests parasitized by *M. alcon*.

Site	*Myrmica* ant species	Sample size	% of all *Myrmica* nests	Number of nests with *M. alcon*	Total number of *M. alcon*	% of nests with *M. alcon*	Mean number of *M. alcon* per infested nest
Otrosz	*M. scabrinodis*	19	63	5	19	26	3.8
	M. vandeli	11	37	7	24	64	3.4
Jasiów	*M. scabrinodis*	32	62	16	47	50	2.9
	M. vandeli	13	25	3	13	23	4.3
	M. ruginodis	7	13	0			
Both sites	*M. scabrinodis*	51	62	21	66	41	3.1
	M. vandeli	24	29	10	37	42	3.7
	M. ruginodis	7	9	0			

where they were kept until the adult wasps emerged. Samples of 10–20 workers from each nest were collected. Nests were subsequently covered and the surrounding vegetation restored to minimize the impact of our investigation. On both sites the average density of *Myrmica* nests was estimated, using 20 squares (1 m^2 each) positioned at random. Identification of ants was made according to Czechowski et al. (2002) and Radchenko et al. (2003).

The significance of the differences in frequency of *M. alcon* in the nests of different *Myrmica* species and the proportion of parasitized and unparasitized pupae in the nests of the two ant species were tested using Fisher's exact tests. Differences in numbers of alcon individuals per nest were tested using Kruskal-Wallis tests and differences in pupal size using Wilcoxon tests.

RESULTS

Three *Myrmica* ant species were recorded at both sites within foraging range of *G. pneumonanthe* plants (Table 1). A total of 82 *Myrmica* colonies was sampled. *M. scabrinodis* (62%), *M. vandeli* (25%) and *M. ruginodis* (13%) were present at Jasiów while only *M. scabrinodis* (63%) and *M. vandeli* Bondroit, 1919 (37%) were found at Otrosz. At both sites there were 0.5–2 nests per 1 m^2. The only other ants found were *Lasius flavus* (F., 1782) and *Formica cunicularia* Latreille, 1798, but they were very rare compared to *Myrmica*.

M. alcon caterpillars developed successfully in both *M. scabrinodis* and *M. vandeli* colonies (Table 1), but not *M. ruginodis* colonies. At Jasiów a greater proportion of the nests of *M. scabrinodis* (50%) were parasitized, whereas at Otrosz *M. vandeli* (64%) was the most common host. Combining the data for both sites, 41% of the *M. scabrinodis* colonies had *M. alcon* and 42% of those of *M. vandeli*. A Fisher's exact test reveal no difference between these percentages (p = 1.0).

The general distribution of *M. alcon* individuals in the nests of both *Myrmica* species is given in Fig. 1. *M. alcon* in *M. vandeli* nests were more advanced in development than those in *M. scabrinodis* nests. In 10 nests of *M. vandeli* there were 36 pupae and 1 prepupa of *M. alcon*, whereas in 21 *M. scabrinodis* nests there were: 35 pupae, 12 prepupae and 19 larvae of various size (3.5–13 mm) (Fig. 2). The mean number of caterpillars/pupae per parasitized *M. scabrinodis* nest was 3.8 at Otrosz (min 1, max 7) and 2.9 at Jasiów (1, 7) and for *M. vandeli* nests it was 3.4 (1, 7) and 4.3 (1, 10), respectively. A Kruskal-Wallis test did not reveal significant differences between sites or ant species ($H = 0.35$, $p = 0.95$). Pupae from *M. vandeli* nests were significantly bigger than those from *M. scabrinodis* nests ($W = 798$, $p < 0.05$) (Table 2).

44 of the 71 *M. alcon* pupae (62%) were parasitized by ichneumon wasps. The adults reared from pupae were identified as belonging to the genus *Ichneumon* L., 1758 and closely resembled *I. eumerus* Wesmael, which is recorded as a parasitoid of *M. rebeli*. The proportion of parasitized pupae was similar in *M. scabrinodis* (57%) and *M. vandeli* (67%) nests (Fisher's exact test, p = 0.47). Pupae parasitized by the *Ichneumon* were smaller than unparasitized ones but the difference was not significant ($W = 705$, $p = 0.09$) (Table 2).

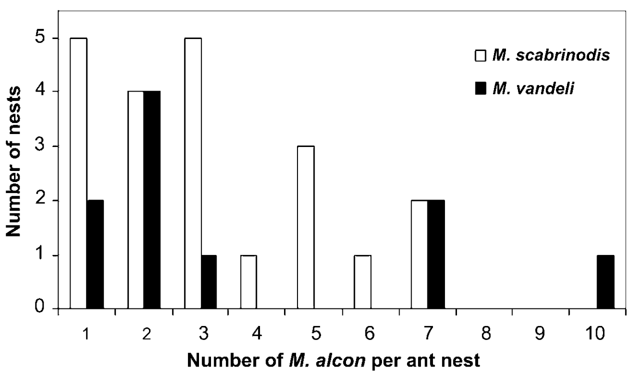

Fig. 1. Frequency distribution of *M. alcon* caterpillars/pupae in the nests of two *Myrmica*.

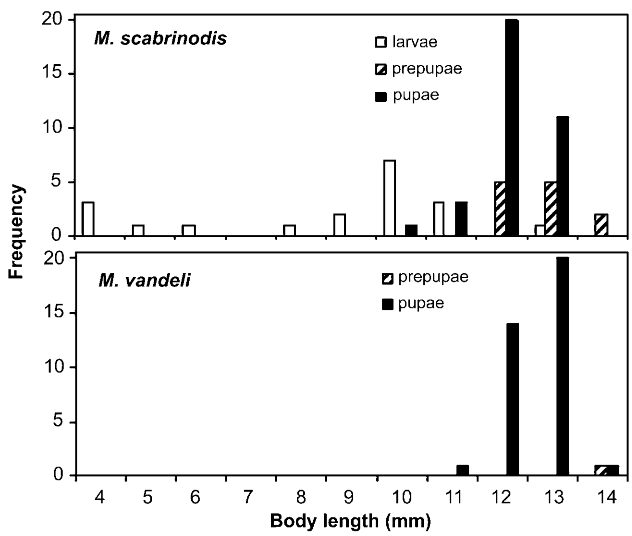

Fig. 2. Frequency distribution of the sizes (in mm) of *M. alcon* caterpillars and pupae in the nest of two *Myrmica* species species.

TABLE 2. Comparison of the number and the body length of parasitized and unparasitized pupae of *M. alcon* found in nests of two ant species.

M. alcon pupae	parasitized by *Ichneumon* sp.		unparasitized		Total	
Myrmica species	N	Mean ± SD (mm)	N	Mean ± SD (mm)	N	Mean ± SD (mm)
M. scabrinodis	20	11.6 ± 0.6	15	11.8 ± 0.6	35	11.7 ± 0.6*
M. vandeli	24	12.0 ± 0.4	12	12.2 ± 0.8	36	12.0 ± 0.5*
Total	44	11.8 ± 0.5	27	12.0 ± 0.7	71	11.9 ± 0.6

* significantly different ($W = 705$, $p < 0.05$).

DISCUSSION

M. vandeli has not previously been recorded as a host of *M. alcon*. This is also the first record of this species being the major host-ant of any *Maculinea* species. It is recorded as a minor host ant of *M. teleius* in France (Elmes et al., 1998). The sites are also among the very few localities where two species of *Myrmica* ants host "cuckoo" *Maculinea* species with similar effectiveness. Thomas & Elmes (1998) suggest that the exploitation of ants by *M. alcon* and *M. rebeli* incurs the cost of increased specificity. Only at our sites and some Danish sites is host-specificity incomplete, and both *M. rubra* and *M. ruginodis* used as hosts. According to Als et al. (2002) there are two possible explanations for this: a lower local specificity of *M. alcon* or the existence of two distinct sub-populations adapted to different hosts. In addition, Elmes et al. (2002) found that hydrocarbon profiles of *M. rubra* and *M. ruginodis* are the most similar among all investigated *Myrmica* species, and assigned *M. alcon* individuals reared by them to a single northern race. Biosynthesis of chemicals by *M. rebeli*, another "cuckoo" *Maculinea* species, which mimicks the host's brood recognition pheromones, is the first step in the integration of adopted caterpillars within a host-ant colony (Akino et al., 1999).

M. vandeli chemicals were not analysed but the simultaneous presence of *M. alcon* in *M. scabrinodis* and *M. vandeli* nests also indicates the close affinity of these two ant species. Both *M. vandeli* and *M. scabrinodis* are placed in the *scabrinodis*-complex of the genus, with *M. vandeli* being a much rarer species, which usually coexists with *M. scabrinodis*. The occurrence of mixed colonies is also reported from Britain and *M. vandeli* is suspected of being a temporary social parasite of *M. scabrinodis* (Radchenko et al., 2003). Hence we suppose that our *scabrinodis-vandeli* populations of Alcon Blue belong to the "*scabrinodis*" race of *M. alcon*.

The incidence of parasitation of *M. alcon* pupae by *Ichneumon* sp. was similar in *M. scabrinodis* and *M. vandeli* colonies. If we assume that the behaviour of this parasite is similar to *I. eumerus*, which locates and parasitizes larvae of *M. rebeli* in *M. schencki* nests using specific allomones (Thomas et al., 2002), then the *M. alcon* parasite must be adapted to penetrate both *M. scabrinodis* and *M. vandeli* colonies. This corroborates the hypothesis that the chemical profiles of both ant species are similar.

M. alcon in *M. vandeli* nests were usually more advanced in development than those in *M. scabrinodis* colonies. The very small larvae observed in the latter host may suggest a growth polymorphism similar to earlier reports for *M. rebeli* (Thomas et al., 1998) and *M. alcon* (Als et al., 2002). We can not exclude the possibility that some larvae in *M. scabrinodis* nests take two years to develop but we suggest that the size difference may reflect differences in nest size and general suitability. *M. vandeli* colonies were usually bigger and had conspicuous solaria, while *M. scabrinodis* colonies were smaller and hidden in turf, especially in *Sphagnum* moss. Perhaps the more populous colonies and higher temperatures in *M. vandeli* nests accelerated the growth of *M. alcon* larvae. The significantly bigger pupae in *M. vandeli* nests suggest better conditions. Thus the presence of small larvae in *M. scabrinodis* nests may result from a deficiency of workers rather than from growth polymorphism. Observations of medium sized larvae in *M. scabrinodis* nests at the end of the flight period (Sielezniew & Stankiewicz, unpubl.) may confirm this. Also we did not observe "slow developers" in our previous studies. Thus the existence of a growth polymorphism in the *scabrinodis* race of *M. alcon* needs confirmation by much larger field samples or by studies using artificial nests.

Two types of *Myrmica* combinations were recorded at *M. alcon* sites prior to the present study. In meadows situated near forests, *M. scabrinodis* co-occurs with *M. rubra* and *M. ruginodis* in various proportions. This species composition is typical of most European sites (Elmes et al., 1994; Sielezniew & Stankiewicz, 2002). However in open fen communities in eastern Poland the grasslands are dominated by *M. gallienii*, with *M. scabrinodis* present in the drier patches of *Molinion* vegetation, where *G. pneumonanthe* occurs (Sielezniew & Stankiewicz, 2002 and unpubl.). Thus there is a third unique *Myrmica* community in a different fraction of the *M. alcon* habitat. A favourable *Myrmica* species composition, with almost all the colonies of potential hosts, is an ideal site for *M. alcon* populations and our present sites probably belong to this category. The presence of specialized parasitoids is another indicator of a high quality *Maculinea* habitat, as they are only able to survive in large stable populations (Munguira & Martin, 1999). Unidentified ichneumon parasitoids of *M. alcon* were only previously recorded from Spain and France (Elmes et al., 1994).

Both sites are examples of a very interesting biodiversity complex. *M. alcon* is recorded in Poland in a few localities mainly in the south and east of the country. A severe decline has occurred in the last decades, as in many other European countries (van Swaay & Warren, 1999). Mt. Otrosz and especially Jasiów, because of its large area, are important strongholds for *M. alcon* in Poland. For *M. vandeli* as a host ant these localities are the only known sites in Poland and are at the north-east edge of the species range. Moreover *M. vandeli* occurred at high densities at both sites, and in proportions relative to *M. scabrinodis* comparable only to localities in the French Alps (Elmes & Thomas, 1985). We hope that the presence of this butterfly species, as well as its parasitoid and a rare species of ant will be a strong argument for the conservation of the whole habitat.

ACKNOWLEDGEMENTS. We are very grateful to J. Buszko for access to unpublished data on *M. alcon* distribution in Poland and to M. Gwardian, for the precise site location. We also thank three anonymous referees for valuable and constructive comments on the manuscript. This work was supported by KBN – The State Committee for Scientific Research (grant no. 3-PO4G-026-24).

REFERENCES

AKINO T., KNAPP J.J., THOMAS J.A. & ELMES G.W. 1999: Chemical mimicry and host specificity in the butterfly Maculinea rebeli, a social parasite of Myrmica ant colonies. *Proc. R. Soc. Lond. (B)* **266**: 1419–1426.

ALS T.D., NASH D.R. & BOOMSMA J.J. 2002: Geographical variation in host-ant specificity of the parasitic butterfly Maculinea alcon in Denmark. *Ecol. Entomol.* **27**: 403–414.

CZECHOWSKI W., RADCHENKO A. & CZECHOWSKA W. 2002: *The Ants (Hymenoptera, Formicidae) of Poland.* Museum and Institute of Zoology PAS, Warsaw, 200 + 1 pp.

ELMES G.W. & THOMAS J.A. 1985: Morphometrics as a tool in identification: a case study of a Myrmica from France (Hymenoptera, Formicidae). *Act. Coll. Insectes Soc.* **2**: 97–108.

ELMES G.W., THOMAS J.A., HAMMARSTEDT O., MUNGUIRA M.L., MARTIN J. & VAN DER MADE J.G. 1994: Differences in host-ants specificity between Spanish, Dutch and Swedish populations of the endangered butterfly Maculinea alcon (Denis et Schiff.) (Lepidoptera). *Memorab. Zool*. **48**: 55–68.

ELMES G.W., THOMAS J.A., WARDLAW J.C., HOCHBERG M.E., CLARKE R.T. & SIMCOX D.J. 1998: The ecology of Myrmica ants in relation to the conservation of Maculinea butterflies. *J. Insect Cons*. **2**: 67–78.

ELMES G.W., AKINO T., THOMAS J.A., CLARKE R.T. & KNAPP J.J. 2002: Interspecific differences in cuticular hydrocarbon profiles of Myrmica ants are sufficiently consistent to explain host specificity by Maculinea (large blue) butterflies. *Oecologia* **130**: 525–535.

MUNGUIRA M.L. & MARTIN J. 1999: *Action Plan for the Maculinea Butterflies in Europe.* Nature and Environment, No. 97. Council of Europe Publishing, Strasbourg, 64 pp.

RADCHENKO A., ELMES G.W., CZECHOWSKA W., STANKIEWICZ A., CZECHOWSKI W. & SIELEZNIEW M. 2003: First records of Myrmica vandeli Bondroit and M. tulinae Elmes Radchenko et Aktaç (Hymenoptera: Formicidae) for Poland, with a key for the scabrinodis- and sabuleti-complexes. *Fragm. Faun.* **46**: 47–57.

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

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

THOMAS J.A. 1995: The ecology and conservation of Maculinea arion and other European species of large blue butterfly. In Pullin A.S. (ed.): *Ecology and Conservation of Butterflies*. Chapman & Hall, London. pp. 180–197.

THOMAS J.A., ELMES G.W. & WARDLAW J.C. 1998: Polymorphic growth in larvae of the butterfly Maculinea rebeli, a social parasite of Myrmica ant colonies. *Proc. R. Soc. Lond. (B)* **265**: 1895–1901.

THOMAS J.A., KNAPP J.J., AKINO T., GERTY S., WAKAMURA S., SIMCOX D.J., WARDLAW J.C. & ELMES G.W. 2002: Parasitoid secretions provoke ant warfare. *Nature* **417**: 505–506.

VAN SWAAY C.A.M. & WARREN M. 1999: *Red Data Book of European Butterflies (Rhopalocera).* Nature and Environment, No. 99. Council of Europe Publishing, Strasbourg, 260 pp.

Received January 29, 2004; revised May 24, 2004; accepted July 8, 2004