

***Myrmica sabuleti* (Hymenoptera: Formicidae) not necessary for the survival of the population of *Phengaris (Maculinea) arion* (Lepidoptera: Lycaenidae) in eastern Poland: Lower host-ant specificity or evidence for geographical variation of an endangered social parasite?**

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Abstract. Larvae and pupae of the obligatorily myrmecophilous *Phengaris (Maculinea) arion* were found for the first time in nests of *Myrmica rugulosa*, *M. hellenica* and *M. schencki* at a site in eastern Poland. *M. sabuleti*, commonly considered as a major host of the butterfly and whose abundance is vital for the survival of *P. arion* populations, was shown to be very rare and not used. Estimated mean density of *Myrmica* nests was very low, which suggests that relatively vast areas are necessary to support a viable population of *P. arion* in similar sandy habitats. This may explain the decline and rarity of the butterfly in the whole region, even though its relationships with ants seem not to be very specific there.

INTRODUCTION

Caterpillars of the Large Blue *Phengaris (Maculinea) arion* (Linnaeus, 1758) (Lycaenidae) complete their development as social parasites of *Myrmica* ants. After the third (final) moult they leave the larval food plant (*Thymus* spp. or *Origanum vulgare*, depending on locality) and are then transported by *Myrmica* workers to their nests where they prey upon the host brood. These relationships are considered not only as obligatory for the butterfly but also as specific i.e. a single species of *Myrmica* is much more tolerant of caterpillars than other species. However, detailed studies on *P. arion* have in fact only been carried out at four sites in Britain, France and Sweden (Thomas et al., 1989; Elmes et al., 1998). They showed that over ninety percent of butterflies emerged from nests of *M. sabuleti* Meinert, 1861 and of the other *Myrmica* species present, only *M. scabrinodis* Nylander, 1846 was successfully exploited as a minor host. A significant difference in the survival rate of *P. arion* larvae among ant species was the most important biological factor in the butterfly's decline in Britain. The more thermophilous *M. sabuleti* was replaced by the less demanding *M. scabrinodis* when vegetation increased in height at abandoned sites (Thomas, 1980). Nevertheless secondary host nests may play an important role for population persistence in seasons with extreme events e.g. droughts (Thomas et al., 2005). Additionally Thomas & Wardlaw (1990) found that colony structure might affect survival, i.e. mortality of *P. arion* caterpillars in *M. sabuleti* nests is higher when queen ants are present.

The complex life history of the Large Blue has triggered enormous scientific and conservational interest in the whole genus *Phengaris* Doherty, 1891 (Thomas & Settele, 2004; Settele et al., 2005), which, according to Fric et al. (2007), is the senior synonym of *Maculinea* Van Eecke, 1915. However, the host-ant relationships of *P. arion* on a larger geographical scale have remained, surprisingly, the least known of all European *Phengaris* taxa (Pech et al., 2007). The first pupa of *P. arion* in Central and Eastern Europe was found in a *M. lobicornis* nest in Poland (Sielezniew et al., 2003). This anecdotal record indicated the importance of further studies in this region in order to obtain a better understanding of the ecological complexity of this species, which is endangered in many European countries (van Swaay & Warren, 1999). Here we present further data from Poland, which for the first time indisputably prove that at least locally the butterfly does not depend on the "classic" host, *M. sabuleti*.

MATERIAL AND METHODS

Our studies were carried out near Tykocin (53°13'N/22°45'E, about 105 m a.s.l.) in the Valley of the Narew River, Podlaskie Voivodship, North-Eastern Poland. The whole site encompasses about 8 ha of open sandy land covered sparsely and almost exclusively by *Juniperus communis* bushes and cut through by a secondary road planted with *Populus balsamifera* trees. Borders are generally clear i.e. pine forest, arable land, wetter places (covered with *Vaccinium vitis-idaea* and *Calluna vulgaris*) and a small river separating the rest from intensive pasture.

The main habitat of *P. arion* (Fig. 1) and the study area (about 2 ha) is limited to more sheltered parts of the site and is confined to an area with the larval food plant *Thymus serpyllum* (about 15% coverage of open ground) where imagines of the



Fig. 1. Typical habitat of *Phengaris arion* at the site near Tykocin (NE Poland).

butterfly were regularly encountered. The type of vegetation can be classified as plant communities of All. *Corynephorion canescens* (Ass. *Spergulo vernalis-Corynephorum*). Patches of bare ground are very numerous and often quite extensive. Except for tussocks of grasses (mainly *Corynephorus canescens*) and herbs like *T. serpyllum*, *Helichrysum arenarium*, *Scleranthus perennis*, *Sedum acre* and *Spergula morisonii*, lichens also form a very characteristic component of the turf. The site is part of a local pasture commonwealth, but the land inhabited by *P. arion* is only rarely grazed by cattle because of its low productivity. Fires and local sand digging are much more important disturbances.

The flight period of *P. arion* on the site near Tykocin is typical for the region and lasts up to about six weeks from mid June to late July. The highest numbers of individuals are usually on the wing in early July when at least a dozen or so individuals might be observed daily. The relative abundance of the butterfly as well as the extent of the habitat compared to other sites in the region was one of the most important reasons for the selection of the study area.

Myrmica nests were examined in the first half of June or last days of May to maximize the chances of finding full-grown larvae or pupae (which develop in about three weeks), which is important in determination of host ant specificity of *Phengaris* butterflies (Thomas et al., 1989). Areas of turf were carefully inspected within 2 m of *Thymus* plants in patches where ovipositing females were most frequently observed. Occasionally we used sugar cubes as bait to attract workers and therefore to facilitate localisation of their colonies. The *Myrmica* nests located were excavated and checked for presence/absence of *P. arion* individuals. Voucher samples of 5–10 workers were collected. Studies were performed in 2003–2007 and every year just 1–3 days were dedicated to examination of the nests to minimise the possible negative effects of our activities. Addi-



Fig. 2. Four individuals of *Phengaris arion* found in a single colony of *Myrmica rugulosa* at the site near Tykocin (NE Poland).

tionally, to estimate the average density of *Myrmica* colonies, 270 randomly chosen squares (1 m² each) were surveyed in 2007. We also collected representatives of other ant genera to obtain non quantitative data on general species composition. All ants were identified in the laboratory using Czechowski et al. (2002).

RESULTS

During the five years of this study a total number of 58 colonies (6–16 every year) were excavated. They belonged to five species of *Myrmica* i.e. *M. schencki* Emery 1895, (22 nests), *M. rugulosa* Nylander, 1849 (15), *M. hellenica* Finzi, 1926 (17), *M. sabuleti* (3) and *M. scabrinodis* (1). We found 15 individuals of *P. arion*: 4 pupae, 9 full-grown larvae and 2 medium-sized larvae in chambers up to about 10 cm below ground level (Table 1). The highest number of individuals was recorded in 2007 from a *M. rugulosa* nest where one pupa, one prepupa and two caterpillars were found (Fig. 2.). *M. rugulosa* was also the most often infested *Myrmica* species (27% colonies with *P. arion*) compared to *M. hellenica* (18%) and *M. schencki* (9%). However, Fisher's Test showed no significant differences in these proportions.

The average density of *Myrmica* nests turned out to be very low i.e. about 0.07 per 1 m². Colonies were hidden in the turf, without solaria and therefore very difficult to find. *M. rugulosa* and *M. hellenica* nested often under *Thymus* plants, while entrances to *M. schencki* nests, with

TABLE 1. The number of *Myrmica* nests examined and their recorded use by *P. arion* at the site near Tykocin (NE Poland).

<i>Myrmica</i> ant species	Sample size	Percent of all <i>Myrmica</i> nests	Number of nests with <i>P. arion</i>	Number of <i>P. arion</i> in infected nests (number of pupae and full-grown larvae)	Percent of nests with <i>P. arion</i>
<i>M. rugulosa</i>	15	26	4	8(7): 1(0), 1(1), 2(2), 4(4)	27
<i>M. hellenica</i>	17	29	3	5(4): 1(1), 2(2), 2(1)	18
<i>M. schencki</i>	22	38	2	2(2): 1(1), 1(1)	9
<i>M. sabuleti</i>	3	5	0	0	0
<i>M. scabrinodis</i>	1	2	0	0	0
Total	58	100	9	15(13)	16

characteristic chimneys, were usually in patches of bare ground in much more dense soil close to junipers.

Other ants collected at the site were: *Formica cinerea* Mayr, 1853, *F. cunicularia* Latreille, 1798, *F. fusca* Linnaeus, 1758, *F. pratensis* Retzius, 1783, *F. rufibarbis* Fabricius, 1793, *F. truncorum* Fabricius, 1804, *Lasius niger* (Linnaeus, 1758), *L. alienus* (Förster, 1850), *L. flavus* (Fabricius, 1782), *Solenopsis fugax* (Latreille, 1798) and *Tetramorium caespitum* (Linnaeus, 1758).

DISCUSSION

M. rugulosa, *M. hellenica* and *M. schencki* are not recorded as hosts of *P. arion*. All of them are thermophilous ant species preferring generally drier and less grassy habitats compared to *M. sabuleti* and *M. scabrinodis* (Czechowski et al., 2002). *M. rugulosa* and *M. hellenica* are not recorded until now from *P. arion* sites (Thomas et al., 1989; Pauler-Fürste et al., 1996; Elmes et al., 1998; Sielezniew et al., 2003). For *M. hellenica* this is also the first record of participation in any butterfly-ant relationship (Fiedler, 2006). Two other *P. arion* hosts recorded from Tykocin were already observed supporting another xerothermophilous *Phengaris* butterfly – “*P. rebeli*”, which recently was judged not a good species but only a subspecies or race of *M. alcon* (Als et al., 2004; Pecsénye et al., 2007; Fric et al., 2007). *M. schencki* is the main host of “*P. rebeli*” in western Europe (Thomas et al., 1989) and Lithuania (Stankiewicz et al., 2005b) and there is also a single record of a *M. rugulosa* colony infested from Poland (Stankiewicz et al., 2005a).

Studies on the host ant specificity of this butterfly in Poland appear to be by far the most time-consuming and laborious in comparison to studies of all other *Phengaris* taxa in the country (Stankiewicz & Sielezniew, 2002; Steiner et al., 2003; Sielezniew & Stankiewicz, 2004). Our results do not indicate the best host ant of *P. arion* at the site and we also have no information about the survival rate there of caterpillars in nests of particular *Myrmica* species. Moreover caterpillars of *P. arion* may exploit more than one *Myrmica* colony before pupation and even a change of species is possible. Ants often desert their nest after the brood has been eaten but *P. arion* has some ability to persist until the vacated nest is colonized again and fresh grubs become available (Thomas et al., 2005).

However, according to Thomas (1995) a minimum of 51% co-occurrence of *M. sabuleti* and the host plant is necessary for survival of a *P. arion* population (intrinsic growth rate = 1). Hence it is rather improbable that the “classic” host-ant of the butterfly, *M. sabuleti*, supports the *P. arion* population at the investigated site. Nevertheless, it is also impossible to judge the potential importance of *M. sabuleti* for the population as only three nests were found. In Poland this ant prefers warm grasslands and it is rather rare in very sandy places with extensive patches of bare ground. The type of vegetation at the Tykocin site is quite typical for eastern Poland (Sielezniew et al., 2005) and therefore we hypothesise that the presence of *M. sabuleti* is not vital for the survival of

many *P. arion* populations in the region. However at the moment we are not able to state whether this is a result of lower specificity or geographical variation observed e.g. for *P. alcon* and “*P. rebeli*” (Elmes et al., 1994; Steiner et al., 2003; Stankiewicz et al., 2005b). The potential role of *M. rugulosa*, *M. hellenica* and *M. schencki* as hosts of *P. arion* populations studied by Thomas et al. (1989) and Elmes et al. (1998) is unknown because these ant species were not recorded at the study.

Perhaps *P. arion* is less specific towards the centre of its distribution area and apart from the *M. sabuleti* “race” there is another “continental” one. Data on the other predatory *Phengaris* species, *P. teleius*, suggests a similar pattern. Considered as specific to *M. scabrinodis* in France (Thomas et al., 1989), it turned out to be more of a generalist in Poland, Hungary and Romania, observed altogether with six *Myrmica* species (Stankiewicz & Sielezniew, 2002; Witek et al., 2005; Tartally & Varga, 2008). In Mongolia Woyciechowski et al. (2006) found pupae of *P. teleius* in nests of a further three hosts at sites where overall *Myrmica* species composition was completely different from that in Europe. Authors suggested that *P. teleius* is the least specific parasite among *Phengaris*, which may explain its wide distribution in the Palearctic.

P. arion is not less widespread, and therefore it is very likely that in the east other hosts are also used. *M. sabuleti* was also not suspected to be a host of *P. arion* in Finland, where *M. lonae* instead of *M. sabuleti* was exclusively recorded in the habitat of the butterfly (Kolev, 1998).

Als et al. (2004), considering the results of genetic analyses, suggest the possibility of the existence of further cryptic species among *P. arion* populations. It would be valuable to check whether those differences overlap with variation in host-ant specificity even though molecular studies did not reveal such a pattern for *P. alcon* and *P. “rebeli”*. The ability to simultaneously exploit more than one *Myrmica* species does not preclude the existence of host races. For example caterpillars from Polish populations of *P. alcon* and *P. “rebeli”* in captivity develop successfully both in nests of *M. sabuleti* and *M. scabrinodis*, but with other ants mortality is very high (Sielezniew & Stankiewicz, 2007).

The mean density of *Myrmica* ant nests recorded at Tykocin (700 nests/ha) is very low compared to *P. arion* habitats in Western Europe. On a British site with a breeding habitat considered as suitable but poor there were 6800 nests of the host-ant (*M. sabuleti*) per hectare (Mouquet et al., 2005). In south west Germany the mean nest density of *M. sabuleti* on *P. arion* sites was 2900 nests/ha. According to the modelling of population viability, if that parameter drops to about 500 nests/ha the extinction probability rises to 1 (Pauler-Fürste et al., 1996). At Tykocin the mean density of the most common *Myrmica* ant (*M. schencki*) was estimated at 250 nests/ha and the density of *M. rugulosa* (the most often infested species) at less than 200 nests/ha. Perhaps *P. arion* would

not able to thrive in these relatively unsuitable conditions using a single host.

The low density of host nests in relation to the abundance of the larval food plant also means that many eggs of *P. arion* are laid far from colonies. According to Elmes et al. (1998) *Myrmica* workers only forage over a radius of about 2 m from their nests. Some preliminary observations suggest that this foraging zone is much larger in sandy habitats with extensive patches of bare ground. It may explain the relatively high incidence of caterpillars in *Myrmica* nests at Tykocin (1.7 per infested nest) comparable to data from Britain (Thomas & Wardlaw, 1992).

However even effective exploitation of scarce resources cannot overcome species vulnerability in the case of restricted area. Therefore *P. arion* is a rather rarely encountered species even in habitats that appear suitable, in contrast to other *Phengaris* species in Poland. Our data show that it probably results from low mean density of *Myrmica* ant nests and the lower capacity of individual colonies to support developing butterflies. The recorded mean number of *P. arion* prematures is lower than that for other predacious *Phengaris* species i.e. *P. teleius* and *P. naustithous* studied in Poland (Stankiewicz & Sielezniew, 2002). For *P. alcon* and *P. "rebeli"* (Steiner et al., 2003; Sielezniew & Stankiewicz, 2004) mean numbers per nest are even much higher but this is mainly the result of a more effective "cuckoo" feeding strategy for exploiting of ant colonies (Thomas & Elmes, 1998).

Habitat demands of *P. arion* and its major host ant vary according to regional climate (Thomas et al., 1998). However, until now only the requirements of the 'classic' host *M. sabuleti* were considered. Our data strongly suggest that an understanding of *P. arion* ecology is much more puzzling in Eastern Europe and probably also in other parts of its distribution in the Palaearctic.

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