ABSTRACT: Phengaris ‘rebeli’ Hirschke is a globally threatened obligatorily myrmecophilous and xerothermophilous butterfly. Caterpillars are initially endophytic and feed in flowerheads of Gentiana cruciata L. but they complete their development as social parasites of Myrmica Latr. ants. Relationships with hosts are specific and show geographical variation in Europe. Knowledge on them is vital for effective conservation. In Poland ‘P. rebeli’ was recorded from a dozen or so sites in the SE part of the country and from one locality in the Pieniny Mts. – a low calcareous mountain range on the Polish-Slovak border that constitutes a distinct geobotanical unit with specific climate and diversity of habitats. Present studies aimed to identify host-ants of the butterfly in the Pieniny Mts. A total number of 30 Myrmica colonies were searched in 2008 near larval food plants bearing eggs of ‘P. rebeli’ in the previous season. We observed caterpillars and pupae of the butterfly exclusively in nests of M. schencki Em., i.e. 4 of 4 of them were infested. However, we did not find premature of P. ‘rebeli’ with M. sabuleti Mein., M. rugulosa (Nyl.) and M. rubra (L.) which were also present in the turf. This makes the Pieniny population completely distinct from previously studied populations in SE Poland and similar to those known from Lithuania, France and Spain. It is probably related to the variation in chemical mimicry of P. ‘rebeli’ caterpillars. Our finding contributes to knowledge on the pattern of host-ant use of the butterfly in Europe and is also important for conservation on a local scale.

KEY WORDS: Phengaris ‘rebeli’, Maculinea rebeli, endangered butterfly, ants, social parasitism, myrmecophily, Myrmica schencki, Poland

Phengaris ‘rebeli’ Hirschke, together with other representatives of genus Phengaris Doherty, which, according to Fr ic et al. (2007) is a senior synonym of Maculinea Van Eecke, is one of the most intensively studied butterflies in Europe (Settle et al. 2005). This results from its priority conservation status i.e. vulnerable according to the latest IUCN (2008) red list as well as from its fascinating and complex life history. Initially endophytic caterpillars complete their development as social parasites of ants (Thomas and Settle 2004). Abandonment of extensive management, agricultural improvements and afforestation are reported as the main causes of its decline (van Swaay and Warren 1999). The taxonomic status of P. ‘rebeli’ is also disputed. Recent genetic studies did not find differentiation between it and its sibling P. alcon Den. et Schiff. at species level (Als et al. 2004, Pecsenye et al. 2007). However due to differences in ecology and some developmental features, P. ‘rebeli’ should be treated at least as a distinct conservation unit (Sielezniw and Stankiewicz 2007).
While *P. alcon* is a rather higrophilous butterfly, *P. ‘rebeli’* inhabits xerothermal calcareous grasslands. Adults are on the wing in early summer and females oviposit to the flower heads of *Gentiana cruciata* L. Caterpillars, for the first three instars, *i.e.* 2–3 weeks, are herbivorous, feeding on developing seeds, but at the beginning of the fourth (final) instar they abandon the plants and drop to the ground. If a caterpillar is encountered by a foraging red ant *Myrmica* Latr. worker, it is treated as a lost ant larva and immediately taken to the nest (Elmes et al. 1991a). The initial chemical signature of caterpillars is unspecific and enables adoption by any *Myrmica* worker, but later it comes to closely mimic certain species only. Hence, if the caterpillar gets into the wrong colony its chances for survival will be usually strongly reduced (Akino et al. 1999, Elmes et al. 2004, Schönrogge et al. 2004). In contrast, in the right colony it will be fed with regurgitations and insect prey. As a social parasite the caterpillar gains about 98% of its final body biomass and in the next year (or in two years in a case of biennalism), it finally pupates (Elmes et al. 1991b, Thomas et al. 1998).

During the first studies on host ant specificity of *P. ‘rebeli’*, performed in France and Spain, its prematures were found almost exclusively in *M. schencki* Em. colonies (Thomas et al. 1989, Elmes et al. 1998), with a very small fraction of individuals reared by *M. sabuleti* Mein., *M. scabrinodis* Nyl. and *M. ruginodis* Nyl. Later a *M. schencki* dependent population was discovered only in Lithuania (Stankiewicz et al. 2005c). However, data from Austria, Switzerland, Hungary and Romania indicated a much more complicated pattern of butterfly-ant relationships. Altogether six species were recorded there as hosts *i.e.* *M. sabuleti*, *M. schencki*, *M. scabrinodis*, *M. specioides* Bondr., *M. lonae* Finzi, and *M. sulcinodis* Nyl., and a generally less clear cut pattern of host ant specificity was observed (Steiner et al. 2003, Tartally et al. 2008).

In Poland *P. ‘rebeli’* was recorded for the first time in the Pieniny Mts in 1987 (Buszko 1997, Felger pers. comm.). Later a dozen or so populations were discovered in eight 10 km UTM squares in south-eastern Poland *i.e.* in the Przemyskie Foothills, Bieszczady Mts. and Beskid Niski Mts. (Stankiewicz et al. 2005b, Warecki and Sielezniew 2008, Michalczuk and Warecki unpublished) (Fig. 1). Interestingly, in Poland, field studies as well as laboratory experiments (captive rearing) concerning the largest known population of *P. ‘rebeli’* from the south-eastern part of the country (vicinity of the town of Przemyśl, 49°46′N, 22°46′E) proved indisputably that *M. schencki* is not used there at all and *M. sabuleti* and *M. scabrinodis* are suitable hosts (Steiner et al. 2003, Stankiewicz et al. 2005b, Sielezniew and Stankiewicz 2007). The importance of *M. sabuleti* was also revealed in other studies at another site in the SE of Poland (near locality Komańcza 49°21′N, 22°03′E), where additionally *M. rugulosa* Nyl. was also observed as a rare secondary host (Stankiewicz et al. 2005a). However, *M. schencki* colonies were not infested again. Based on those findings some general conclusions about the host-ant specificity of *P. ‘rebeli’* in Poland were even drawn (Sielezniew and Stankiewicz 2006). Knowledge about host ants is vital for the conservation of *Phengaris* butterflies because particular *Myrmica* species show different preferences as regards exposure, height and vegetation structure as well as management practices (Elmes et al. 1998).

The population of *P. ‘rebeli’* from the Pieniny Mts, has never been studied in detail. The Pieniny Mts. are a distinct low (up to 1050 m a.s.l.) limestone mountain range 35 km long and 6 km wide within the Western Carpathian region situated at the borderland

Table 1. The number of *Myrmica* nests within 2 m of *Gentiana cruciata* and the number of the nests parasitized by *P. ‘rebeli’*.

<table>
<thead>
<tr>
<th><em>Myrmica</em> species</th>
<th>Number of nests</th>
<th>Number of nests with <em>P. ‘rebeli’</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. sabuleti</em></td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td><em>M. schencki</em></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><em>M. rugulosa</em></td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td><em>M. rubra</em></td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>
Host ant specificity of *P. rebeli* in Pieniny Mts. of Poland and Slovakia. Pieniny Mts. constitute a separate geobotanical with specific mild climate and diversity of habitats. Polish part of the Pieniny belongs to the best studied areas in the country in terms of flora and fauna. Some plants (including two endemic species) and some animals were not found elsewhere in Poland (Razowski 2000). As far as butterflies are concerned the Pieniny are famous of a recovery programme of a *Parnassius apollo* population (Adamski and Witkowski 2007).

*P. rebeli* has always received less attention and therefore until now it has not been clear why the butterfly is relatively rare, in spite of an abundance of the larval food plant. We found out that *G. cruciata* is widespread and quite common in the Pieniny National Park and its surroundings but *P. rebeli* is much more local. The presence of the butterfly is quite easy to detect, due to eggshells which remain on host plants many weeks after the end of the flight period. In 2005 only about two thousand eggs were counted (Stankiewicz et al. 2005b), which indicates that no more than a few dozen imagines were on the wing, taking into consideration data on female fecundity from the mark-release-recapture studies of Meyer-Hozak (2000).

However egg load is very uneven and the area where oviposition occurs varies between seasons. For our research we chose an area where the density of plants with eggs was highest in 2006 and 2007 (Fig. 2a). It encompassed about 1 ha of mainly south exposed slopes near the locality named Sromowce Niżne (49°24′N, 20°24′E; 500–550 m a.s.l.), sparsely covered with bushes (mainly *Prunus spinosa* L.), and more or less intensively grazed by sheep. Other rare xerothermophilous butterfly species occurring sympatrically were: *Colias alfacarensis* Ribbe, *Satyrium acaciae* (Fabr.), *Phengaris arion* (L.) and *Iphiclides podalirius* (L.).

In late July 2007, 37 patches with plants numerously loaded with eggs (at least 5 per shoot) were marked with GPS and small plastic markings were also placed in the turf. At the end of May and in early June 2008 we searched turf in a radius of up to 2 m of all marked plants which is approximately the foraging zone of *Myrmica* workers, according to Elmes et al. (1998). At every patch first we investigated carefully one randomly chosen 1 m square to evaluate the density of ant colonies and then we looked for ants also outside the square but still within the patch.

A total number of 30 colonies (16 within the squares) of *Myrmica* ants was found. The most common species was *M. sabuleti* (14 nests) and the other three species recorded there were: *M. rugulosa* (7), *M. rubra* (L.) (5) and *M. schencki* (4). The mean density of *Myrmica* nests near gentians was estimated at 0.43 m⁻². Identifications were made in the laboratory according to Czechowski et al. (2002). Voucher samples of about 5 specimens from every colony are deposited in the first author’s collection.

![Fig. 1](image-url)
Fig. 2. *Phengaris rebeli* site in the Pieniny Mts.: (a) general view with distinctive tracks trampled by sheep; (b) patch of habitat where larvae and pupae of the butterfly were found in ant nests; (c) male; (d) female ovi¬-positing on *Gentiana cruciata*; (e) larva, pre-pupae and (f) pupae in *Myrmica schencki* colonies.
All *Myrmica* nests found were carefully opened and examined for the presence of *P. rebeli* pre matures. It turned out that all nests of *M. schencki* hosted larvae or pupae of the butterfly (Fig. 2e, 2f) and none of the other *Myrmica* colonies were infested, and this difference in the rate of parasitism was significant (λ² = 30.0, *P* <0.0001) (Table 1).

We found a total number of 11 pre matures: 6 full grown larvae, 2 pre-pupae and 3 pupae (1, 2, 3 and 5 specimens in individual nests). However, as far as infested colonies are concerned our searching was restricted to surface chambers so as not to disturb them excessively. Therefore, it might be possible that further larvae were present there. After examination *P. rebeli* pre matures were left with their hosts and the ground and vegetation were restored to their original appearance as exactly as possible. Nests where we could not find anything were examined much more thoroughly to exclude the overlooking of caterpillars and pupae.

Our findings are unexpected taking into consideration previous data from Poland. Larvae from the other studies in south-eastern Poland (Przemyśl, 175 km away from the recent study stands) were usually quickly killed by workers, even when introduced to artificial nests of *M. schencki* (Sielezniew and Stankiewicz 2007). Therefore data from the Pieniny Mts. prove that host-ant specificity is reversed with relation to the south-eastern part of the country. According to Elmes et al. (2002) such differences can be explained by the variation in chemical mimicry of caterpillars i.e. in cuticular hydrocarbon profiles. The presently studied population seems to be much more similar to the Lithuanian one in spite of the considerable geographic distance (660 km) between them, compared with the nearest studied site at Komańcza in SE Poland (120 km). It will be very interesting to determine genetic differentiation in those three populations in the future.

The geographic variation in host ants’ use is well documented for sibling *P. alcon* (Elmes et al. 1994, Als et al. 2002). However in Poland all populations probably belong to one race, dependent mainly on *M. scabrinodis* (Stankiewicz et al. 2005b). Our findings concerning *P. rebeli*, however, indicate the importance of further studies.

The area of *P. rebeli* occurrence in the Pieniny Mts. has undoubtedly changed many times over the years. Originally the butterfly probably inhabited less intensively grazed or rarely mown grasslands. *P. rebeli* could abandon overgrown grasslands and colonise newly created suitable habitats which appeared in the mosaics of narrow plots reflecting the complicated structure of land ownership in Poland. Until the 1970s oats and potato crops had been cultivated in the area where the butterfly is now encountered. Borders between the small fields are still visible. After abandonment the partial regeneration of xerothermic vegetation, followed by an invasion of *Myrmica* ants finally created favourable conditions for *P. rebeli*.

Our studies indicate that a deficiency of *M. schencki* nests is at the moment the main factor limiting the population size of *P. rebeli* in the Pieniny Mts. All four colonies of the host ant were found in close proximity in one sheltered patch of the site, with no traces of heavy grazing activity but with relatively short turf (Fig. 2b). However, *M. sabuleti* was recorded mainly in the open and on south exposed slopes with coverage of *Thymus pulegioides* L. and characteristic mounds created by *Lasius flavus* (E) colonies. Next, *M. rubra* preferred the neighbourhood of bushes and *M. rugulosa* nests were observed in various places with short sward. *M. rubra* is the least thermophilous species of all *Myrmica* at the site, and their presence might be a reflection of bush coverage. It was also observed in plain parts of the pasture where *G. cruciata* were numerous (there were very few eggs there). However *M. rugulosa* is quite tolerant to pressure related to disturbance of the ground caused by animals (Czechowski et al. 2002).

The studied site of the butterfly is outside the Pieniny National Park and therefore remains unprotected. Under such circumstances implementation of any conservation plan will be difficult. It is also not clear how to manage perfectly the habitat of *P. rebeli*, but grazing activity should certainly be controlled and reduced (not stopped!). Removal of some hawthorn bushes is also recommended. Simultaneous monitoring of the *P. rebeli* population (by egg counting) and of *Myrmica* ants (species composition and nest density) will be essential to develop optimum management practices.
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REFERENCES


Sielezniew M., Stankiewicz A.M. 2007 – Differences in the development of the closely related myrmecophilous butterflies Maculinea
Host ant specificity of *P. 'rebeli' in Pieniny Mts.*


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