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ORIGINAL PAPER

Efficiency of emergency conservation methods: a case study of the alcon blue butterfly (*Phengaris alcon*) and its specific parasitoid

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Abstract

A complicated life history and specific habitat requirements make the Alcon blue butterfly *Phengaris alcon* a very sensitive species to land-use change. Eggs are oviposited on some *Gentiana* and *Gentianella* plants, in whose flowerheads larvae initially feed, after which they continue their development as social parasites in the nests of specific *Myrmica* ants, once they have been adopted by workers. One of the main threats to their existence is mowing at a time when most individuals are still on gentians. In our research, we tested an emergency conservation method on a site of the butterfly subjected to this kind of pressure in the Białowieża Forest (NE Poland). Previous studies showed that the focal population (dependent on *Gentiana pneumonanthe* and *Myrmica scabrinodis*) was characterized by an extremely high density of adults as well as of *Ichneumon eumerus*, i.e. the rare and specific parasitoid of *P. alcon*. In mid-August 2020 we performed an unplanned experiment to save caterpillars present in freshly cut plants. We selected about 1800 shoots bearing the eggs or egg shells of the butterfly. Seven hundred and fifty preadoption larvae were transported to the site and placed close to the nests of host ants located in patches without *G. pneumonanthe*. In the following season we observed that the *P. alcon* infestation rate of experimental colonies was similar to a control patch, and proportions of parasited pupae did not differ, either.

Implications for conservation. Our experiment proved that such actions can be highly effective as emergency conservation methods, and possibly also in (re)introductions of *P. alcon*.

Keywords *Gentiana pneumonanthe* · *Ichneumon eumerus* · Mowing management · *Myrmica scabrinodis* · Wet grassland · *Stenoptilia pneumonanthes*

Introduction

Land use change of agricultural land is currently one of the main causes of declining numbers and local extinction of insects in Europe, and butterflies inhabiting semi-natural grasslands are among the most affected groups. They are the first to disappear in comparison to plants and birds, which makes them both an excellent indicator and an umbrella group in nature conservation (Thomas et al. 2004; Nilsson et al. 2008; Warren et al. 2021; Habel et al. 2022). The charismatic *Phengaris* Doherty (=*Maculinea*) (Lycaenidae) butterflies are particularly sensitive, since they require the co-occurrence of two specific resources. Larvae are initially phytophagous and feed for 2–3 weeks inside the flowerheads of specific plants, but after the third moult they drop to the ground, to be adopted by *Myrmica* ants and complete their development as social parasites. The Alcon blue butterfly *Phengaris alcon* (Den. & Schiff.) is considered the most advanced myrmecophile, since its caterpillars are fed by workers (Thomas and Settele 2004; Tartally et al. 2019b). Moreover, it is the only one of the four European *Phengaris* species which is not listed in the Habitats Directive, although it is threatened similarly to its congenera (Maes et al. 2019; Kajzer-Bonk and Nowicki 2022). Although the species is considered as of Least Concern on the European Red List of butterflies (van Swaay et al. 2010), a more recent analysis suggests that the status should be upgraded to Vulnerable. In as many as 21 of 28 European countries its Red List status is assessed as Vulnerable, Endangered or even Critically Endangered (Maes et al. 2019). Habitats of *P. alcon* suffer from abandonment,

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Fig. 1 A view of the unmown habitat of *Phengaris alcon* on the site in the Białowieża Forest in August **(a)**, a mown part of the site **(b)**, and gentians selected from the hay **(c)**

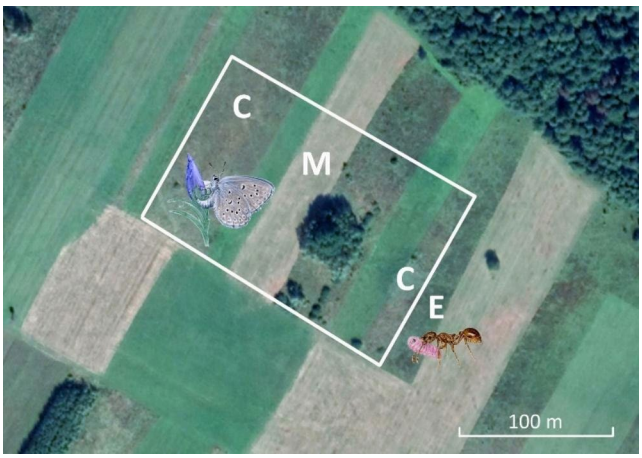

Fig. 2 Orthophotomap of the study of *Phengaris alcon* in the Białowieża Forest showing the mosaic of mowing use. The area covered by Marsh gentians and occupied by the butterfly is marked with a white polygon. A large patch (M) was mown in mid-August 2020, i.e. in the critical period of the life cycle. The adoption experiment was performed at an adjacent patch (E) and control patches are also shown (C).Source of the orthophotomap from 2017: Maxar Technologies (acquired by Google Earth Pro)

leading to slow degradation, usually starting from adverse changes in ant species composition (Tartally et al. 2019a). On the other hand, the effects of intensification tend to be even faster, and a single mowing performed in the critical period of the life cycle may be highly detrimental (Grill et al. 2008).

Across the continent, *P. alcon* forms several ecotypes, adapted to different species of *Gentiana/Gentianella* plants and/or *Myrmica* ants (Bereczki et al. 2018; Tartally et al. 2019b). In the case of Central European hygrophilous populations, caterpillars depend on Marsh gentian (*Gentiana pneumonanthe* L.) and almost exclusively on *M. scabrinodis* Nyl. (Tartally et al. 2019b). In 2016 one such site characterized by a high abundance of Marsh gentian (Fig. 1a) was discovered in the Białowieża Forest, a high value nature area in NE Poland. It turned out that it possessed some unique features, namely the highest density of adults (790 individuals/ha) of all populations of the ecotype studied so far (Dziekańska et al. 2020). Subsequently, the presence of a specific and rare parasitoid was detected. *Ichneumon* cf. *eumerus* Wesmael which infests caterpillars when they are already in ant nests, using allomones (Thomas et al. 2002), turned out to be, as an imago, approximately three times as numerous as its host (Dziekańska et al. 2020).

Due to land ownership structure, the site located near the village of Budy (N52°44'10", E23°44'7"; 150 m above sea level) was a mosaic of variously used meadow stripes (Fig. 2), which probably enabled the population to remain in good condition despite the lack of targeted protection. Not all treatments were beneficial for the butterfly, and more specifically mowing in late June (i.e. shortly before the butterfly emergence which usually happened in mid-July) prevented females from laying eggs on the host plants. However, in 2020 we found that a large part of the site (ca. 0.22 ha) was mown at critical time, i.e. the end of the flight period, when most of the *P. alcon* population was on *G. pneumonanthe* (Fig. 1b). The vast majority of the caterpillars present in drying plants did not have the chance to reach the fourth (final) instar when they left the gentians to be adopted by *Myrmica* workers. In this unexpected situation, we spontaneously decided to perform an unplanned experiment aimed to give some caterpillars the chance to continue their development, and to evaluate whether such actions can be effective. Taking into account that larvae of *P. alcon* obtained from cut plants are easily adopted by ants in artificial conditions (see e.g. Sielezniew and Stankiewicz 2007), we assumed that it would be even more possible in natural conditions.

Methods

On 19–20 August 2020 (i.e. 1–3 days after mowing) we selected gentians bearing eggs or egg shells of *P. alcon* from the hay (Fig. 1c). Such plants are easy to find, since oviposition occurs mostly on flower buds (Van Dyck and Regniers 2010), and egg shells remain attached after caterpillars hatch

Fig. 3 Bouquets with gentians selected from the hay **(a)**, early-reared 4th instar larvae of *P. alcon***(b)**, and adoption of a larva by a worker of *Myrmica scabrinodis* observed in the field **(c)**

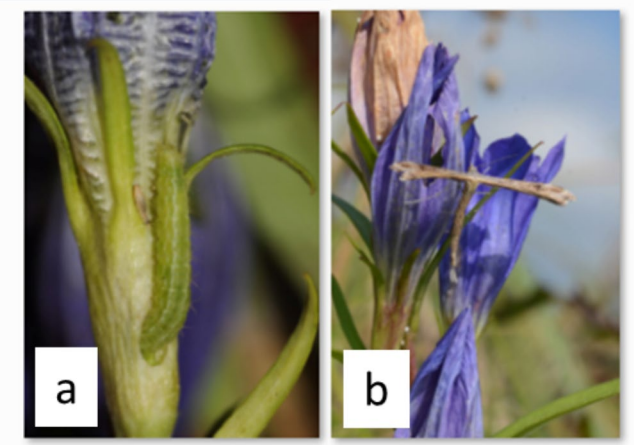

Fig. 4 A larva of *Stenoptilia pneumonanthes* found on a gentian selected from the hay **(a)**, and an adult reared and released at the *P. alcon* site **(b)**

through the egg base (Thomas et al. 1991). A total of about 1800 shoots, 15–40 cm long, were divided into bouquets and placed in containers with water. To reduce drying, most of the leaves, especially the lower ones, were cut off. Then the bouquets were placed in bowls lined with paper towels (Fig. 3a) and kept in close to natural conditions (on the terrace of a country house). The bowls were inspected twice a day (in the morning and evening) to look for caterpillars leaving the plants. Preadoption larvae (Fig. 3b) were placed in small plastic containers with a small damp piece of paper towel. Then they were immediately put into a refrigerator and kept at 6–8 °C. A total number of about 950 4th instar caterpillars were obtained between August 21 and September 9. An unexpected side effect was the detection of plume moth *Stenoptilia pneumonanthes* (Büttner) (Lepidoptera, Pterophoridae), whose larvae pupated on the paper towels, or were found directly on plants. Numerous adults emerged in early September (Fig. 4).

Adoption experiments were carried out on patches where the larvae of *P. alcon* could not get to the ant colonies naturally, i.e. (i) at least 5 m from the nearest gentians, therefore clearly out of the foraging zone of *Myrmica* ants, estimated at 2 m (Elmes et al. 1998), or (ii) where the host plants were present but, due to June mowing, no eggs could be laid on them. After locating an ant nest, workers were identified with a magnifying glass to select *M. scabarinodis* colonies. Having detailed knowledge on local *Myrmica* species composition, this was relatively easy due to some characteristic features associated with antennae (Czechowski et al. 2012). Nests where there seemed to be few workers were rejected.

Adoptions were performed in the evenings, i.e. at the peak of *Myrmica* activity (Elmes et al. 1991). A cohort of 15 larvae was placed in close proximity to the nests. At the beginning of the experiment we monitored 10 cases and found that all larvae were picked up by workers within half an hour, every time (Fig. 3c). Each of the *M. scabrinodis* colonies near which the larvae were placed for adoption was marked with GPS and a metal tag inserted about 10 cm from the nest, usually located in a tuft of grass. Seven hundred and fifty caterpillars were put up for adoption near 50 *M. scabrinodis* colonies, away from gentians, in a patch unmown in that season located approximately 120–150 m from the patch in which the gentians were collected. The remaining caterpillars (ca. 200 individuals) were placed next to the *M. scabrinodis* nests in a closer patch (ca. 70–100 m away) with host plants without *P. alcon* eggs (gentians were not available for oviposition due to mowing in July). The latter part of the experiment could not be completed since we noticed that our markings, clearly visible in the low turf, had been destroyed by someone. So we were forced to limit our activities to patches with higher vegetation.

The next year (2021), we evaluated the results of our experiment. Between July 10 and 15, i.e. in the period in which finding grown larvae and pupae was to be expected,

we attempted to find the marked nests. This turned out to be difficult due to the lush vegetation, higher than in the previous year, which was apparently related to the wetter season (this was indicated by data acquired from the Białowieża weather station located approximately 8 km south-east of the study site and run by the Polish Institute of Meteorology and Water Management - National Research Institute (IMGW-PIB). To increase our chances a metal detector was used, but even with its help 23 of the 50 nests were not found. Trampling by bisons visiting this meadow could be a factor, since we noticed their feces, and moreover some tags were ripped off and bent. All the nests found were very carefully opened with a knife and examined for the presence of *P. alcon*. It is known that full-grown larvae are carried by workers to upper chambers during the day and that pupation takes place there as well (Als et al. 2002), therefore to minimize our impact we examined the upper chambers without nest excavation. Nests in which we could not find anything were searched more thoroughly. After the procedure we covered the nests and the surrounding vegetation was restored as exactly as possible.

As a control we carried out a similar investigation in patches with gentians which bore eggs in the previous season. The online Social Science Statistics calculator was used for comparisons between the two groups.

Results

In the patch where we placed caterpillars for natural adoption we managed to find 27 nests (only *M. scabrinodis*) and 14 of them (52%) were infested by *P. alcon*. A total number of 30 butterfly prematures (25 pupae and five larvae) of *P. alcon* were recorded (Fig. 5). The number of individuals per nest varied from one to four (1.9 on average). Then, at least 14 pupae (47% of all individuals) were parasitised by *I. eumerus*. All caterpillars and healthy-looking pupae were left in the colonies, and we collected only some parasite pupae for further experiments.

In the control patch we opened 27 *M. scabrinodis* colonies, and *P. alcon* was found in 13 of them (47%), so this proportion did not differ from the experimental group (X^2 test, $p = 0.79$). Variation in the number of individuals (total 46, including 41 pupae and five larvae) was higher (1–10), as well as average number per nest (3.8), but the difference was not significant compared to the experiment results (Mann-Whitney test, $p = 0.10$). At least 29 pupae (63%) were parasitised and the infestation rate did not differ significantly from the experiment, either (X^2 test, $p = 0.16$).

Discussion

Our research showed that in an emergency, (i) collection of plants bearing eggs, (ii) rearing of caterpillars and (iii) their subsequent release in the vicinity of ant nests can mitigate the adverse effects of bad management. Caterpillars obtained from mown gentian plants can be successfully used for adoption in natural conditions. The adoption rate of caterpillars in the field is unknown, and so is how many of them finally produce adults. Nonetheless, the lack of significant differences in infestation rates between experimental and control patches suggests that our actions closely imitated natural conditions. Very similar values were also obtained in the case of our previous intensive research at this site (Dziekańska et al. 2020).

Hence, we suggest that this could be an excellent method for *P. alcon* reintroduction, enabling immediate verification of whether local hosts are really appropriate, and taking into consideration possible co-evolution leading to local adaptations (Nash et al. 2008). Hence, the source for reintroduction could be caterpillars obtained from host plants located outside the foraging zone of local host ants, so as not to deplete a donor population. Females of *P. alcon* are guided by plant characteristics rather than the presence of specific *Myrmica* species (Fürst and Nash 2010; Wynhoff et al. 2015).

To our knowledge, there have been no attempts to reintroduce *P. alcon* anywhere so far. But here it is worth noting that it was larvae that were used for the successful

Fig. 5 A healthy pupa with a prepupa **(a)**, and a parasited pupa of *P. alcon***(b)**, found in nests of *M. scabrinodis* close to where preadoption larvae were placed in the previous season

reintroduction of *P. arion* in the UK (Thomas et al. 2009). The alternative is to release adult butterflies, as in the reintroduction of two other congenera, i.e. *P. teleius* and *P. nausithous* in the Netherlands (Wynhoff 1998). The latter approach seems to make sense only if donor populations are very large. The short lifespan and sensitivity of *Phengaris* adults could be also problematic taking their transportation and breeding on a destination site into consideration.

From our experiments it is also clear that parasitoid females are not guided by gentians in the precise location of their hosts. Hence further studies are needed to establish whether they are able to distinguish *M. scabrinodis* from other *Myrmica* ants, as reported for *I. eumerus*, attacking the xerothermophilous ecotype of *P. alcon* dependent on *Gentiana cruciata* L. and *Myrmica schencki* Em. (Thomas and Elmes 1993). It is also unknown when parasitation occurs in the case of the hygrophilous form of *P. alcon.* Thomas and Elmes (1993) report that larger caterpillars are preferred, but in contrast to *G. cruciata* populations, *P. alcon* larvae developing on *G. pneumonanthe* grow very little before overwintering (Sielezniew and Stankiewicz 2007). Therefore they possibly become attractive for *I. eumerus* only in June, and wasp females have to survive until then.

Parasitoids are usually not considered as targets for insect conservation, being less charismatic than e.g. their butterfly hosts. However there is no doubt that *I. eumerus* is much rarer than *P. alcon*, and parasitoids are considered as ultimate indicators of *Phengaris* systems (Hochberg et al. 1998). The uniqueness of our site is additionally evidenced by the presence of *S. pneumonanthes*, a species which had never been observed in this part of Poland before (Buszko and Nowacki 2017). This rare micromoth is related to *G. pnemonanthe* and *G. cruciata* (Gelis 1996), and its biology is little studied but it can definitely also be affected by intensification of land use. On the other hand, this species will probably benefit from conservation activities aimed at the Alcon blue butterfly.

Autumn mowing is recommended as a conservation measure for *Phengaris* species on wet meadows (Grill et al. 2008), and early or late extensive grazing could be an alternative (Moschetti et al. 2020). In the case of mowing we suggest leaving some patches unused every year, so as not to accidently harm other vulnerable but less conspicuous inhabitants with little studied requirements, such as parasitoids or micromoths for whom uniform management could be destructive. Spatially varying uncut refuges in wet grasslands foster many other butterfly species of conservation concern and that they have already been integrated in many management schemes (Scherer and Fartmann 2023).

Our case also shows a kind of paradox existing in nature conservation, which applies not only to Poland. We had to legalize our activities, since keeping and transporting a protected species is against the law. While mowing of a meadow leading to high mortality of the same species is perfectly legal. Although our experiment proved that such actions can be highly effective as emergency conservation methods, and possibly also in (re)introductions of *P. alcon* and related species, it would be better to avoid such situations through the proper management of occupied meadows.

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Author contributions I.S. designed the experiment, I.S. and M.S. performed the field studies. I.S. wrote the manuscript with input from M.S. Both co-authors accepted the final version of the manuscript.

Declarations

Competing interests The authors declare no competing interests.

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