

# Relationship between the butterfly *Phengaris rebeli* and its larval host plant *Gentiana cruciata* in Lithuanian population

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Relationship between the endangered herbaceous plant *Gentiana cruciata* and its specialist herbivore, the endangered butterfly Alcon Blue, *Phengaris rebeli*, was studied in Lithuanian population which is the northernmost one over the whole known species range in Europe. The size of *P. rebeli* population was estimated basing on eggshell counts on the larval host plant. Oviposition behaviour of *P. rebeli* was investigated during summer season of 2011 on a grassland area in Neris Regional Park, Lithuania. Potential host plants were checked and eggshells of *P. rebeli* were counted. A total of 619 eggshells were counted on 248 out of 536 *G. cruciata* stems checked. Selective choice of both host plants and site for oviposition on host plant was revealed. Approximately 89% eggs were laid on the organs of the uppermost whorl. Plants selected by females for oviposition almost always had a luxuriant growth and most of them were higher than the surrounding vegetation.

**Key words:** Alcon Blue, Cross Gentian, egg deposition, Neris Regional Park

## INTRODUCTION

Butterflies of the Palearctic genus *Phengaris* Doherty, which according to Fric et al. (2007) is a senior synonym of *Maculinea* Van Eecke, are among the most intensively studied insect species in Europe (Settele et al., 2005). This is mainly because of its conservation status and complicated and fascinating life history. Females of *Phengaris* lay their eggs on a specific foodplant and approximately three weeks, later larvae hatch from eggs and feed on the green seeds or flowers of the host plant (Stankiewicz et al., 2005; Arnyas et al., 2006). On reaching the fourth instar, the larvae drop down to the ground and wait for foraging *Myrmica* ants, which take them to their own nests (Witek et al., 2008). In the nests of ants *Phengaris* caterpillars become social parasites, spending 11 or 23 months underground to complete their development (Thomas, Settele, 2004; Tartally et al., 2008).

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Both population of *Phengaris* butterflies' larval food plants and that and of their host ants are very sensitive to changes in land use. In general, the habitat of *Phengaris* butterflies depends on grassland managed to a great extent. Therefore, any change in land use, including either intensification, or contrariwise, activity abandonment, can be highly destructive (Rutkowski et al., 2009).

The lycaenid butterfly *Phengaris rebeli* Hirsche, 1904 is one of Europe's most endangered butterfly species (Steiner et al., 2003). The life cycle of this species is very complex, as the larvae depend both on host plant *Gentiana cruciata* (during the first three larval instars) and on *Myrmica* ants (larvae complete development as social parasites of *Myrmica* ants) (Sielezniew, Dziekanska, 2009). The flight period of *P. rebeli* butterflies is between the second week of June and the third week of July (Arnyas et al., 2009). The butterfly prefers dry grasslands and lays its eggs mainly on *G. cruciata* (Kery et al., 2001).

*Gentiana cruciata* is a hemicytrophite scapose plant of small size, reaching on average 20–40 cm in height. It is widespread in most parts of Europe (except Portugal, Great Britain and Scandinavia) and in Western Asia. *G. cruciata* grows on dry calcareous soil on forest edges, bushy slopes, pastures, grasslands, on sunny slopes and dry meadows (Grubičič et al., 1995). In July–August this herb typically produces 1–10 stems, each with 10–20 dark-blue flowers. On average, single fruit contains about 100 seeds (Dolek et al., 1998; Kery et al., 2001).

Lithuanian populations of *P. rebeli* belong to the northernmost one in the whole known species range in Europe and are situated about 500 km away from the nearest population in Eastern Poland (Stankiewicz et al., 2005; Sielezniew et al., 2012). There were known nine local populations of *P. rebeli* in five localities of Lithuania in 2007 (Švitra, 2007). Recently there were revealed several localities where extra local populations of *P. rebeli* occur in Lithuania (Bačianskas, 2009; Gliwa, Šeškauskaitė, 2009; Švitra et al., 2011).

Although *G. cruciata* is widespread over the territory of Neris Regional Park, there are recorded only four more or less isolated populations of *P. rebeli*, which occur throughout the upper valley of the river Dūkšta (localities Bradeliškės I, Bradeliškės II and Rusėnai) and throughout the valley of the river Neris (locality Bielazariškės) (Švitra, 2007). Both *G. cruciata* and *P. rebeli* were included into the list of endangered and protected species of Lithuania.

The aims of this study were: (1) to evaluate relative density of *Phengaris rebeli* host plant *Gentiana cruciata* using the method of clumps and (2) to reveal some ecological relationships between *G. cruciata* and *P. rebeli* checking distribution of *P. rebeli* eggshells on host plant.

## MATERIALS AND METHODS

Locality Bradeliškės I (54°49'34"N, 24°57'09"E) is one of those where the most abundant populations of *Phengaris rebeli* occur in Neris Regional Park (Švitra, 2007). Bradeliškės I is located in the Dūkšta river valley near village Bradeliškės, in the Dūkštos environs, Vilnius administrative district, Lithuania. Clay based gentle dry slopes exposed to south, southeast and east with meso-thermophile

grasslands, covered by tall grass communities are characteristic for the habitat.

The size of *Gentiana cruciata* population was assessed by counting the number of flowering gentian plants. In the grassland representative squares were selected from the four patches in July 2011 and, in addition to the recording of the eggshells, characteristics of the *G. cruciata* plants and location of eggshells were recorded, namely: the number and height of the stalks; the number of flower-buds per stalk; the number of eggshells on the different whorls of the stems; the number on flowers and buds on a single stalk; the number of eggshells on adaxial and abaxial surfaces of *G. cruciata* leaves. The data collected were used to analyze possible preference of *Phengaris rebeli* females for the site choice for oviposition (Meyer-Hozak, 2000; Arnyas et al., 2006). This study was carried out immediately after the end of adult flight period.

## RESULTS AND DISCUSSION

In Bradeliškės I locality there were found growing four small patches of *G. cruciata* plants. In this locality all the patches were situated on open slopes of the southern, south-eastern or south-western exposure and community of tall grass covered the slopes. Vegetation cover was mostly 100%, and only in a few cases it was slightly less dense (covered 75–95%). A total of 238 clumps of *G. cruciata* were collected in this area. The total number of stems was 536 with average 2.25 stems per clump. Of those, 248 stems contained 619 eggshells of *P. rebeli*. Clumps containing 3 or 4 stems were most frequent (47%). The percentage of clumps with 1–2 stems was also high (25.9%), while only a few clumps collected contained more than 8 stems (Table 1). Most of the

Table 1. Distribution of *Gentiana cruciata* stems and eggshells among the clumps with different number of stems

No. of <i>G. cruciata</i> stems / clumps	Stems	Eggshells	Eggshells / stem
1–2	139	105	0.76
3–4	252	266	1.06
5–6	104	148	1.42
7–8	24	51	2.12
9–10	17	49	2.88
Total	536	619	1.15

stems in this locality were intact and fertile. Significantly more eggshells were found namely on intact fertile stems.

Microclimatic conditions play an important role for butterfly oviposition. As all gentians grew in sunny area, theoretically they were equally available for egg deposition (Küer, Fartmann, 2005). However, only 40% of the stems contained eggshells. Females preferred plants growing in a certain density, i. e. those collected in clumps with 3–4 stems. Oviposited *G. cruciata* plants (containing eggshells) were generally higher and had more flowers than those containing no eggshells. Most stems chosen by females for oviposition were the highest ones among available (Table 2).

Analysis of egg distribution on the whorls of each *G. cruciata* stem revealed the following features. Females laid 89% of their eggs on organs of the uppermost whorl (Table 3). When the distribution of eggs in the organs of different whorls was compared, the adaxial surface of the plant leaves was revealed as clearly the most preferred oviposition site (60%). Hardly any eggshell (0.01%) was found on the stalks of a stem.

Basing on the number of eggshells counted and entire area checked, it is possible to predict the size of the local butterfly population. At the beginning of August 2011, gentians in the area checked contained 619 eggshells of *P. rebeli*. Therefore, based

on the scheme suggested by Meyer-Hozek (2000), one could estimate population size at Bradeliškės I locality as approximately equal to 15–20 adult *P. rebeli* butterflies.

The distribution of *P. rebeli* eggshells on *G. cruciata* was not equal among stems. The intact fertile stems were the most attractive to females. The number of whorls with flowers / flower buds and the prominence of the stems from the surrounding vegetation were known as two important factors affecting oviposition (Arnyas et al., 2006).

The flowering period of *G. cruciata* lasts at least twice as long as the flight period of *P. rebeli*. Females, especially late flying ones, also lay their eggs on flowers in bloom, as the number of eggshells increases in a cumulative manner, together with a shift of the phenological stage of stems (Arnyas et al., 2009).

It was known that *P. rebeli* prefers luxuriant specimens of its larval host plant *G. cruciata* that are easy to reach (Dolek et al., 1998; Meyer-Hozek, 2000). Visual attraction is an important factor when searching for suitable host plant (Dolek et al., 1998). Stems that grow higher than the surrounding vegetation are less shaded and offer better microclimatic conditions for a quick development of eggs and caterpillars (Küer, Fartmann, 2005).

The same trends in female egg laying behaviour were characteristic for *P. rebeli* butterflies

Table 2. Height of stems, number of flower-buds and occurrence of eggshells on *Gentiana cruciata*

Height of stems (cm)	<10	10–20	20–30	30–35	35–40	Total
Number of stems	87	89	138	142	80	536
Number of eggshells	2	29	65	270	253	619
Number of eggshells per stem	0,02	0,33	0,47	1,90	3,16	1,15
Number of flower-buds	13	317	1 070	1 229	890	3 519
Number of eggshells per flower-buds	0,15	0,09	0,06	0,22	0,28	0,18

Table 3. The number of eggshells on different parts of the first, second, third and fourth whorls of *Gentiana cruciata* stem

Whorl, No.	I (upper)	II	III	IV	Total
Flower	160	10	6	3	179
Stalk	3	2	1	0	6
Upper surface of the leaf	332	28	9	2	371
Underleaf surface	56	5	1	1	63
Total	551	45	17	6	619

of Lithuanian population, present in the patch located in the most northernmost direction and existing quite isolated from the main area of the whole species range in Europe.

*G. cruciata* is a hemicryptophyte plant and reproduces vegetatively giving rise to clumps of stems made up of various numbers of fertile and sterile stems (Arnyas et al., 2006). Thus, population can survive the loss of a large proportion of seeds if the site is suitable for vegetative growth.

A few ways are suggested to assure regeneration of *G. cruciata* populations. WallisDeVries (2004) suggested that small-scale sod cutting as the best means to promote existing gentian populations. This way gaps are created in which the seeds can germinate and the young seedlings can grow protected. The extensive grazing is also appropriate as management for *P. rebeli* habitats containing *G. cruciata* as host plant.

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#### LIETUVOS POPULIACIJOS GENCIJONINIO MELSVIO (*PHENGARIS REBELI*) IR JO MITYBINIO AUGALO *GENTIANA CRUCIATA* EKOLOGINIAI RYŠIAI

##### *S a n t r a u k a*

Tirti ryšiai tarp dviejų nykstančių rūšių – melsvojo gencijono (*Gentiana cruciata*) ir šiuo augalu lervos stadijoje mintančio gencijoninio melsvio (*Phengaris rebeli*) – populiacijų. Populiaciniai parametrai vertinti pagal ant mitybinio augalo padėtus gencijoninio melsvio kiaušinėlius. Tyrimai atlikti 2011 m. vasarą Neries regioninio parko teritorijoje (Vilniaus rajonas). Suskaičiuoti ir įvertinti visi pasirinktoje teritorijoje augantys gencijonai ir ant augalo padėti gencijoninio melsvio kiaušinėliai. Ant 248 iš 536 augančių melsvojo gencijono stiebų rasta 619 drugio kiaušinėlių. Nustatyta, kad 89 % visų kiaušinėlių padėti įvairiose augalo viršutinio menturio vietose, dažniau viršutinėje lapo pusėje nei apatinėje. Gencijonai, ant kurių rasti drugio kiaušinėliai, pasižymi vešlumu ir augumu, todėl vizualiai ryškiai išsiskiria pievos augalijoje.

**Raktažodžiai:** gencijoninis melsvys, melsvasis gencijonas, kiaušinėlių dėjimas, Neries regioninis parkas

