Differences in adult phenology, demography, mobility and distribution in two syntopic ecotypes of *Maculinea alcon* (*cruciata* vs. *pneumonanthe*) (Lepidoptera: Lycaenidae) from Transilvania (Romania)

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Summary: We present a Mark-Release-Recapture study performed on the populations of two *Maculinea alcon* ecotypes in a Natura 2000 site from Transylvania, Romania. The Natura 2000 site harbours cultural landscapes with highly biodiverse semi-natural grasslands, among which several meso-hygrophilous meadows represent the only areas with 4 syntopically occurring European *Maculinea* butterfly species and two syntopically occurring ecotypes of *M. alcon*. Previous studies have shown that the two *M. alcon* ecotypes use different host plants and host ants; however our study is the first to focus on adult butterfly population ecology and distribution. In the case of *M. alcon*, conservation of the species has to consider the ecological needs of both ecotypes in order to be meaningful. The unique syntopical occurrence of both ecotypes makes population ecology studies in this area especially important for providing information for conservation management.

Key words: population ecology, mark-release-recapture, *Maculinea alcon* ecotypes, syntopic populations.

Introduction

Traditionally managed grasslands in temperate Europe harbour a high diversity of plants and animals that are specifically adapted to these types of habitats (Johst *et al.* 2006). The abandonment or intensification of the land-use practices in grasslands, their fragmentation and isolation has a dramatic effect on species directly depending on these habitats (Settele & Henle 2003). The species with most specialised life cycles have experienced the most serious decline, because of their need for specific combinations of biotic and abiotic conditions (Habel *et al.* 2007).

Butterflies of the genus *Maculinea* van Eecke, 1915 (synonymised with *Phengaris* Doherty, 1891 by Fric *et al*. 2007) are examples of myrmecophilous species with a complex life-cycle, adapted to traditional cultural landscapes that are threatened at European level (Elmes & Thomas 1992, Simcox *et al.* 2005). After a short period feeding monophagously on their host plant, fourth instar larvae of *Maculinea* caterpillars fall to the ground, where they are adopted by their host ant species from genus *Myrmica* Latreille, 1804 (Thomas *et al*. 1998, Akino *et al*. 1999, Elmes *et al*. 2001). The caterpillars possess a range of adaptations that enable them to enter and exploit host ant colonies (Thomas & Wardlaw 1992, Akino *et al.* 1999, Barbero *et al.* 2009) preying either on ant grubs ("predatory" *Maculinea*) or being fed by trophallaxis ("cuckoo" *Maculinea*) (Malicky 1968, Elmes *et al*. 1991, Thomas & Wardlaw 1992). Because they depend on specific host plants (Asteridae and Rosidae families) and host ants of the genus *Myrmica*, *Maculinea* butterflies can be very sensitive indicators of (butterfly) diversity and habitat degradation. They are considered umbrella species and their conservation benefits many other threatened species (Thomas *et al.* 2005, Maes & Van Dyck 2005, Skórka *et al.* 2007, Anton *et al.* 2007).

Presently most *Maculinea* populations are small and isolated and occupy fragmented habitats, strongly affected by human activities, mainly because of the changes in agriculture over the past decades and the abandonment of traditional land-use (Whynhoff 2001, Thomas & Settele 2004, Schmitt & Rákosy 2007, Van Swaay *et al*. 2012).

Maculinea populations have been reported to be declining in the Carpathian basin and Romania (Bálint 1991, 1993), although, in the latter region, the precarious economical situation largely maintained traditional land-use systems with positive effects on large blue's habitats (Rákosy & Vodă 2008, Vodă *et al*. 2010, Timuş *et al*. 2011). Thus in Romania we can still find most of the European *Maculinea* taxa and in several cases even occurring syntopically: *Maculinea arion* Linnaeus 1758, *M. teleius* Bergsträsser 1779, *M. nausithous kijevensis* Sheljuzhko 1928 and *M. alcon* with two ecotypes (Vodă *et al*. 2010, Rákosy *et al.* 2010, Timuş *et al*. 2011, Hollós *et al.* 2012). In Europe, this is the only area currently identified where the two forms of *M. alcon* co-occur: in the

Natura 2000 site "Dealurile Clujului Est", Romania (Tartally *et al.* 2008, Timuş *et al.* 2011, Czekes *et al.* 2013).

There are 5 known European *Maculinea* species, some of them with several more or less clearly defined subspecies or ecotypes. Controversial taxonomical changes pose problems especially between *rebeli* and *alcon,* and widespread confusion of *alcon* ecotypes with taxa *xerophila* or *rebeli* has been identified in the literature (e.g. Tolman & Lewington 1998).

Because of these taxonomical issues the taxa *M. alcon* and *M. rebeli* are not mentioned on the IUCN 2013 Red list of threatened species.

In this study we aim to analyse ecological differences and similarities between the two ecotypes of *M. alcon*: *M. a. 'cruciata'* and *M. a. 'pneumonanthe',* occurring syntopically but with distinct host plant species, *Gentiana cruicata* and *G. pneumonanthe*, in the unique Natura 2000 site from Transylvania (Romania). But in advance, we need to clarify the fact that under the name of *M. alcon 'cruciata'* we do not refer to *M. rebeli, M. alcon xerophila* or *M. rebeli xerophila*. As described by Hirschke (1904), *M. rebeli* is an exclusive alpine species that does not use *Gentiana cruciata* as host plant because this gentian is not found in the type population distribution area (Habeler 2008).

In order to insure the long term survival of these butterflies, it is essential to understand the mechanisms that shape *Maculinea* population dynamics and to apply this knowledge in specific conservation programs (Nowicki *et al.* 2005a). Thus, we applied the mark-release-recapture (MRR) method to study the adult populations of the two *Maculinea alcon* ecotypes with the aim of gathering knowledge of importance for their conservation. The only studies previously conducted on the two ecotypes in the same region analyse host ant specificity (Tartally *et al.* 2008) and oviposition behaviour (Czekes *et al.* 2013). Our study is the first to apply MRR to gather population ecology data about phenology, demography, mobility and distributions in the two syntopically occuring ecotypes of *M. alcon*.

Materials and methods

Study site and species

Our research was carried out in the Natura 2000 site "Dealurile Clujului Est" (Eastern Hills of Cluj), on a northern exposed meso-hygrophilous meadow of *ca* 40 ha named Fânațul Domnesc (located between the villages of Răscruci and Luna de Jos 46.92N, 23.73E, 410-460m a.s.l., Cluj county, Transylvania, Romania).

The study area is characterized by a mosaic of two main vegetation types: *Molinion caeruleae* Koch 1926 in boggy depressions and *Cirsio-Brachypodion* Hadac & Klika in Klika & Hadac 1944 in semi-dry patches. Characteristic species for the first alliance are *Molinia caerulea*, *Sanguisorba officinalis*, *Gentiana pneumonanthe*, *Ranunculus polyanthemos*, *Carex tomentosa*, *Juncus conglomeratus*, *Serratula tinctoria*. For the second alliance, the dominant and frequent species are: *Brachypodium pinnatum*, *Bromus erectus*, *Asperula cynanchica*, *Carex humilis*, *Polygala major*, *Cirsium pannonicum*, *Centaurea scabiosa*, *Ranunculus polyanthemos*, *Prunella grandiflora*, *Gentiana cruciata*, *Veronica teucrium*, *Trifolium pannonicum*, *T. ochroleucon* and others (Paulini *et al.* 2011). The pasture was abandoned progressively after 1990 and in 2011 was partially mown (3% of the entire study area) (Paulini personal communication). In the period 2009-2012 the study area was partially and intensively grazed by sheep.

In Fânațul Domnesc three *Maculinea* species cohabit syntopically*: M. alcon* (with two ecotypes)*, M. teleius* and *M. nausithous kijevensis* (Timuş *et al*. 2011, Czekes *et al.* 2013). The females of one of the *M. alcon* ecotypes oviposit on *Gentiana cruciata,* the other on *Gentiana pneumonanthe*. Since the taxonomy of *Maculinea* butterflies that oviposit on *G. cruciata* is not clear (Steiner *et al.* 2006, Habeler 2008), we prefer to call the two ecotypes according to their host plants, thus: the form using *G. cruciata* is referred to as *M. a. 'cruciata'*, while the form utilising *G. pneumonanthe* as *M. a. 'pneumonanthe'*.

The two gentians used by *M. alcon* ecotypes as host plants, co-occur in some patches, but *G. pneumonanthe* has higher abundance in wet depression with *Molinia caerulea*, while *G. cruciata* is more abundant in semi-dry areas.

For *M. a. 'pneumonanthe'* there is strong evidence that the host ant species is *Myrmica scabrinodis* (Tartally *et al.* 2008, Czekes *et al.* 2013). The *M. a. 'cruciata'* caterpillars were found (*G. cruciata* patches) in *Myrmica schencki* and *M. sabuleti* nests (Tartally *et al.* 2008).

Sampling method

The two syntopic populations of *M. alcon* ecotypes from Fânațul Domnesc were studied by mark-release-recapture (MRR) method. The MRR method was applied in 2010 on *M. a. 'pneumonanthe'* population and in 2011 both on an *M. a. 'cruciata'* and an *M. a. 'pneumonanthe'* populations. Qualitative observations on the flight period of the two ecotypes were registered in the years 2009-2011.

For the MRR study, butterflies were captured every second or third day (with exceptions caused by unfavourable weather) between 9:00 A.M. and 7:00 P.M. Altogether 18 field trips were conducted in both years between the 11 June – 9 August in 2010 and 2011. In the case of each capture/recapture, gender and behaviour (rapid flight, search flight, sitting on host plant, sitting on food plants, oviposition, mating) of the captured individual, capture time and coordinates of the exact location were recorded. Coordinates were recorded with a GPS device Garmin GPSmap 60 CSx.

In order to map the host plants of the two ecotypes: *Gentiana cruciata* and *Gentiana pneumonanthe*, a grid of squares of 50x50 m was established that covered the whole area of Fânațul Domnesc. Each square of the grid was investigated and all individuals of the two plants were marked with a GPS device (Garmin GPSmap 60 CSx) in June and July both years 2011 and 2012.

Statistical analyses

MRR data from the years 2010 and 2011 was analyzed to estimate the current population size, flight distance and average lifespan of adults of *M. a. 'pneumonanthe'* and *M. a. 'cruciata'* ecotypes. Data was analysed separately for each ecotype with the Cormack-Jolly-Seber type constrained models (Schwarz & Arnason 1996, Schwarz & Seber 1999) using the program MARK 6.0 package (Cooch & White 2010). The performances of the models were assessed with the Akaike Information Criterion corrected for small sample size (AICc) (Akaike 1973, Hurvich & Tsai 1989). As recommended by Burnham & Anderson (2001), after running predefined models in program Mark, we selected the model with the lowest ΔAICc and the smallest number of parameters.

Individual life span was estimated using the formula $\hat{e}=(1-\varphi)^{-1}-0.5$ ($\hat{e}$ is the individual life span, φ is the survival probability) (Nowicki *et al*. 2005b). The parameters resulted from analysing the data with the program Mark 6.0, survival and capture probability, were used to estimate daily number of individuals in each capture occasion (i.e. days of capture) and recruitment of new individuals into the population and the total number of individuals in the two populations (of the two ecotypes).

We compared the number of marked individuals, daily population estimates and flight distances in both years between the two ecotypes with a Mann-Whitney U-test (with the program Past 2.09, Hammer *et al.* 2001), because data did not follow a normal distribution.

Flight distances were calculated separately for males and females within season as the distances between consecutive recaptures, and daily flight distances from same day recaptures for both sexes and ecotypes using program ArcMAP 9.2. Comparisons between flight distances of different ecotypes and sexes were also computed with a Mann-Whitney U-test (with the program StatView 5.0), because datasets were not normally distributed. A Chi squared goodness of fit test was applied (using the site http://www.quantpsy.org/chisq/chisq.htm) on the seasonal and daily flight distances to check if the sample is evenly distributed on three and four, respectively, distance classes (0-100 m, 101-200 m, 200-300 m, over 300 m). For *M. a. 'cruciata'* daily flight distances we could not perform this test because sample size was too small.

In order to compare the spatial distributions of the *G. cruciata* and *G. pneumonanthe* plants and of the adults of *M. a. 'cruciata'* and *M. a. 'pneumonanthe'* we overlaid a grid of 20x20 m over the map of Fânațul Domnesc with the help of the program Quantum GIS 2.2.0 - Valmiera. Of these we randomly selected 4 times 25 squares and counted the number of plant and butterfly individuals of each ecotype. Correlation between the distribution of host plants and adult butterflies of the two ecotypes was tested using Spearman Rank Correlation with the program Past 2.09 (Hammer *et al.* 2001).

Results

Phenology and population parameters

M. a. 'cruciata' flew for approximately two weeks, between the 14th and 26th of June in 2011. Previous observations from the years 2009 and 2010 showed

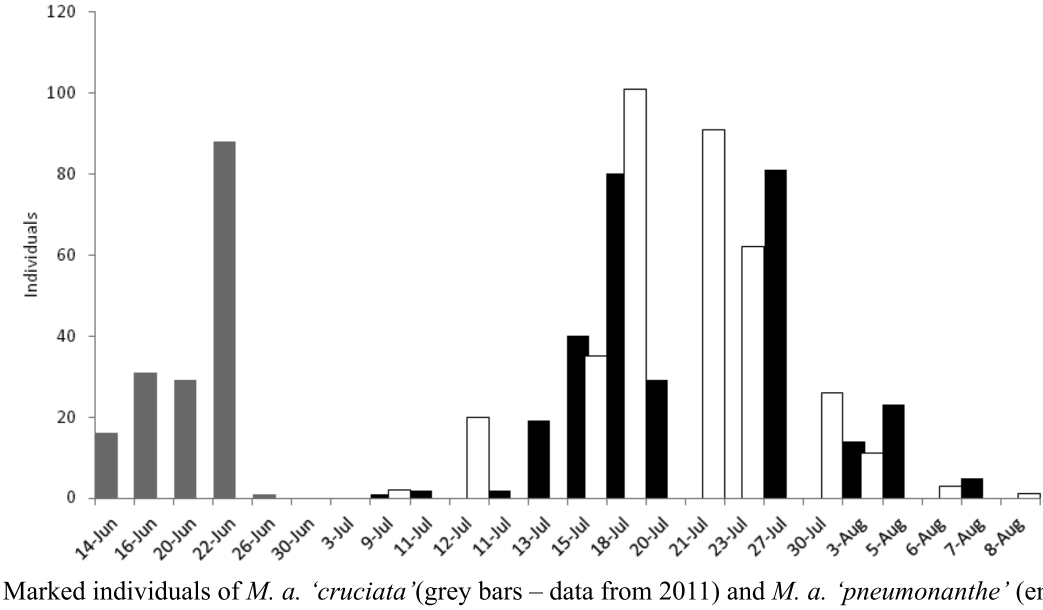

Figure 1. Marked individuals of *M. a. 'cruciata'*(grey bars – data from 2011) and *M. a. 'pneumonanthe'* (empty bars – data from 2010, dark bars – data from 2011) ecotypes in the investigation area Fanatul Domnesc (Cluj County, Romania).

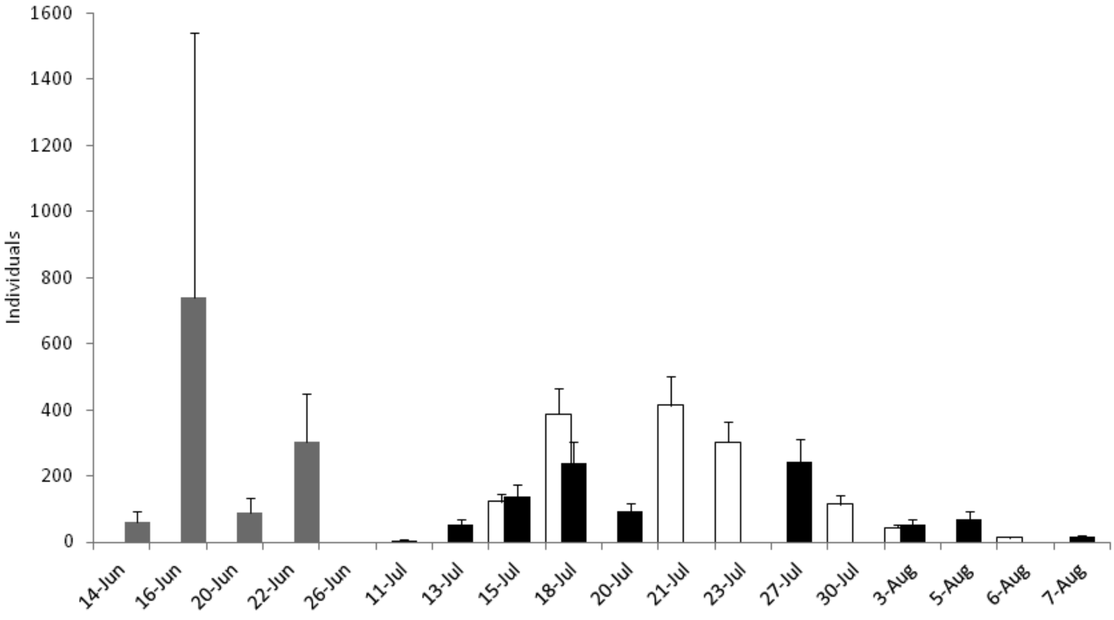

Figure 2. Within-season daily population size estimates (*Ni*). Empty bars –*M. a. 'pneumonanthe'* 2010, dark bars - *M. a. 'pneumonanthe'* 2011 and grey bars – *M. a. 'cruciata'* 2011.

that the flight period of this ecotype extended until the 30th June at the latest. *M. a. 'pneumonanthe'* flew for approximately 4 weeks, between the 11th of July and the 7th of August in 2010 and 2011 (Fig. 1).

In 2010 330 individuals (204, 62% males and 126, 38% females) of *M. a. 'pneumonanthe'* were captured and marked. Of these 40 (12.1%) individuals (29 males and 11 females) were recaptured at least once on a different capture occasion.

In 2011 294 individuals (225, 77% males and 69, 23% females) of *M. a. 'pneumonanthe'*, and 165 individuals (112, 68% males and 53, 32% females) of *M. a. 'cruciata'* were captured and marked. Of these 20 (6.8%) individuals (16 males and 4 females) of *M. a. 'pneumonanthe'*, and 16 (9,7%) individuals (13 males and 3 females) of *M. a. 'cruciata'* were recaptured at least once on a different capture occasion (Table 1). The number of marked individuals did not differ significantly between the two ecotypes (Mann-Whitney U-test, p>0.05).

For *M. a. 'cruciata'* a population of 1073 individuals was estimated in the year 2011 with the model Phi(.) p(t) (i.e. the model showing constant survival probability and different capture probability over time) (Table 2). For *M. a. 'pneumonanthe'* a population of 1277 individuals was estimated for the year 2010 with the model Phi(.) p(g) (i.e. the model showing constant survival probability and different capture probability for males and females) and a population of 1296 individuals for the year 2011 with the model Phi(t) p(g) (i.e. the model with different survival probability in time and different capture probability for males and females).

The survival probability (*Phi*) was 0.85 (±0.09 SE) for *M. a. 'cruciata'*, and 0.71 (±0.04 SE) and an average of 0.54 (±0.34) for *M. a. 'pneumonanthe'* in 2010 and 2011 respectively. The capture probability (*p*) was on average 0.21 (±0.28 SE) for *M. a. 'cruciata'*, and 0.26 (±0.11 SE) and 0.33 (±0.22 SE) for *M. a. 'pneumonanthe'* in 2010 and 2011 respectively. Individual life span was calculated at 6 days for *M. a. 'cruciata'* in 2011 and at 3 and 2 days respectively for *M. a. 'pneumonanthe'* in 2010 and 2011 (Table 2).

Daily population estimates (*Ni*) for each capture occasion did not differ significantly either between years for *M. a. 'pneumonanthe'* and between ecotypes in 2011 (Mann-Whitney U-test: p>0.05) (Fig. 2). With the exception of *M. a. 'pneumonanthe'* males in 2010 (unimodal pattern), for both ecotypes the adult recruitments during the flight period (*Bi*) have well-defined bimodal patterns (Fig. 3). The recruitment is similar between years for *M. a. 'pneumonanthe'* and also between ecotypes in 2011 (Mann-Whitney U-test: p>0.05).

Table 1. Summary of the MRR study on *Maculinea alcon 'pneumonanthe'* (*M.a.'p'*) and *M. alcon 'cruciata'* (*M.a.'c'*) in 2010 and 2011 from Fânațul Domnesc (Cluj County, Romania).

Year	Ecotype	Marked individuals			Recaptured Individuals*			Recapture Ratio (%)
		Males	Females	Total	Males	Females	Total	
2010	*M.a.'p'*	204	126	330	29	11	40	12.12
2011	*M.a.'p'*	225	69	294	16	4	20	6.80
2011	*M.a.'c'*	112	53	165	13	3	16	9.69

*individuals recaptured at least once on a different capture occasion in the same study site

Table 2. Basic parameters of the Cormack-Jolly-Seber model (p average daily capture probability; φ average daily survival rate), population estimates (computed with MARK 6.0 program), sex ratio and individual life span in *Maculinea alcon ‘pneumonanthe’* (*M.a.’p’*) and *M. alcon ‘cruciata’* (*M.a.’c’*) populations, in 2010 and 2011 from Fânațul Domnesc (Cluj County, Romania).

Year	Ecotype	Model	Seasonal population size	Sex ratio (males:females) %	φ	p Males	p Females	Lifespan (days)
2010	*M.a.’p’*	Phi(.) p(g)	1313	52:48	0.712	0.32	0.20	2.98
2011	*M.a.’p’*	Phi(t) p(g)	1297	73:28	0.461	0.36	0.29	2.05
2011	*M.a.’c’*	Phi(.) p(t)	1073	68:32	0.845	0.21	0.21	5.98

Generally, captured individuals and also estimates show a clear male–biased ratio. The sex ratio differed significantly from the expected 1:1 ratio especially in 2011 for both ecotypes (Table 2).

Mobility and distribution

The mean seasonal flight distance (distances between consecutive recapture dates) for *M. alcon ‘cruciata’* was 100 m, and 97 m and 75 m for *M. a. ‘pneumonanthe’* in 2010 and 2011 respectively. The distances from same day recaptures were similar: 121 m for *M. alcon ‘cruciata’* in 2011 and 97 m and 110 m for *M. a. ‘pneumonanthe’* in 2010 and 2011 respectively. We found no significant differences of seasonal and same day flight distances between sexes and between ecotypes (Mann-Whitney U-test p>0.05 in all cases). The maximal seasonal flight distance registered (distance between consecutive recapture dates) was 345 m for a female *M. a. ‘cruciata’*.

Over 95% of seasonal flight distances were less than 200 m in both ecotypes and the Chi squared goodness of fit test showed that the sample was unevenly distributed on distance categories (χ^2=34.9, DF=2, p=3x10^{-8}) with most flight distances in up to 200 m for *M. a. ‘cruciata’* and up to 100 m for *M. a. ‘pneumonanthe’* (Fig. 4). Same day flight distances followed the same unevenly distributed pattern on distance categories with most distances under 200 m for *M. a. ‘pneumonanthe’* (χ^2=63.1, DF=3, p=0).

When comparing the spatial distribution of the adults and host plants for both ecotypes we registered a significant high correlation between *M. a. ‘pneumonanthe’* and *G. pneumonanthe* (r_s=0.67, p=1.7x10^{-10}) and a lower correlation between *M. a. ‘cruciata’* and *G. cruciata* (r_s=0.30, p=0.003). No other correlations were found between distributions of the two plants, between the distributions of the two ecotypes and the distributions of plants and irrespective ecotypes.

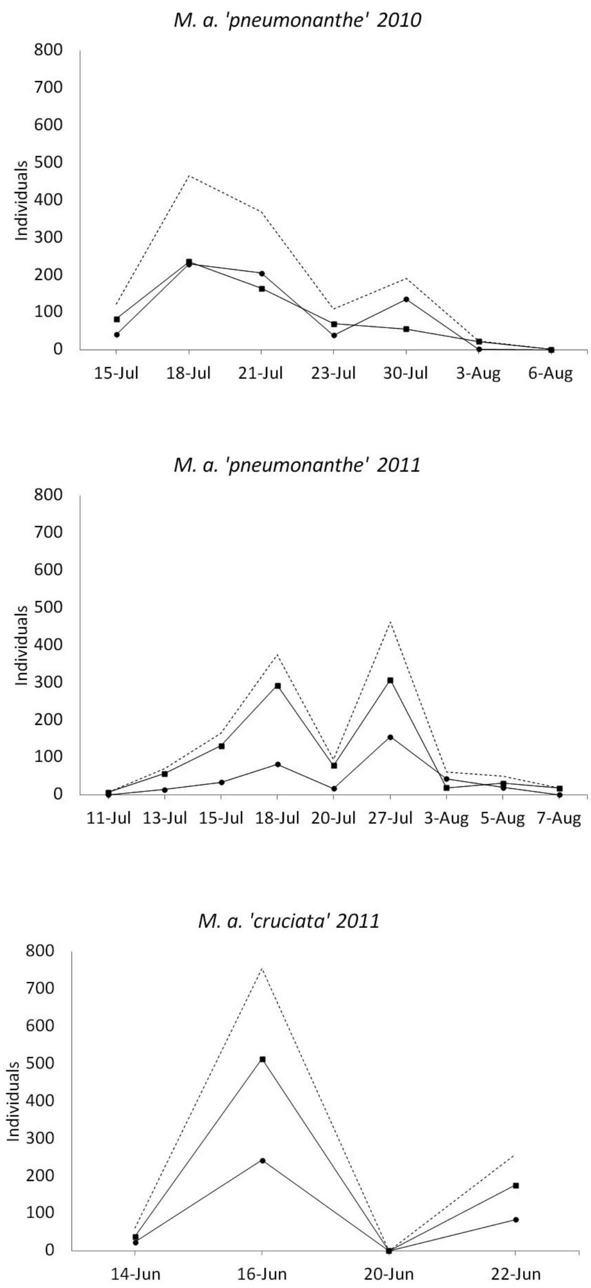

Figure 3. Within – season recruitment (*Bi*) in the populations investigated. *Broken line* represent total recruitment between consecutive capture days, *squares* – males and *circles* – females.

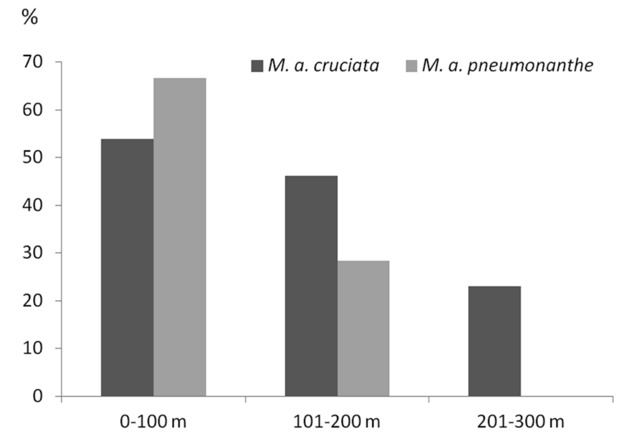

Figure 4. Frequence of flight distance categories of the two ecotypes of *M. alcon* in 2010 and 2011 in Fânațul Domnesc (Cluj County, Romania).

Discussion

Phenology and population parameters

Our phenology results show that the two ecotypes occurring syntopically in Fânațul Domnesc (Cluj County, Romania) are well separated in their flight periods.

Considering our phenological data, the two ecotypes from Transylvania were clearly separated in each year by at least two weeks. Phenological differences are possibly due to the flowering phenology of the two host plants.

The possibility of the two ecotypes being two generations of the same species has to be excluded in this case, as *M. a.* '*cruciata'* ecotype needs on average 11-23 months (Thomas *et al.* 1998, Schönrogge *et al.* 2000, Sielezniew & Stankiewicz 2007, Timuş unpubl. data) and exceptionally (in optimal laboratory conditions) at least 6 weeks (Tartally 2005) for the preadult development. Furthermore, Sielezniew & Stankiewicz (2007) found that only a 3 month period of cooling triggered the completion of development (i.e. an obligatory diapause) in both ecotypes.

Considering the population parameters, the two ecotypes were similar in population size, survival, capture probability and recruitment. Only individual life span was considerably higher in *M. a. 'cruciata'*, with 6 days, compared to *M. a. 'pneumonanthe'* with only 3 days.

2011 was characterised by lower recapture rates. This should be reflected in larger population size estimates in both ecotypes. However when population parameters of 2011 were compared to those of 2010 for *M. a. pneumonanthe*, no significant differences were found. We have thus used these estimates in order to check for possible differences between population parameters of ecotypes, as the work and analysis method was the same for both.

Both ecotypes showed a skewed sex ratio and a bimodal recruitment in the year 2011.

Árnyas *et al.* (2005) suggested that a deviation from the expected sex ratio can be traced down to the higher recapture rates of males, due to the fact that they are more active and fly higher, searching for less mobile females. Females, on the other hand, tend to fly lower because they are searching for host plants in the undergrowth and may be easily overlooked.

After Nowicki *et al.* (2005a, 2009) the bimodal pattern of adult recruitment during the flight period (*Bi*) indicates the presence of biannual larvae. Biannual larvae were found in all European *Maculinea* species: *M. rebeli* (Thomas *et al.* 1998), *M. alcon, M. arion* (Schönrogge *et al.* 2000), *M. teleius* and *M. nausithous* (Witek *et al.* 2006). Even though our data shows a bimodal recruitment pattern within season for both ecotypes, we found no larval polymorphism in Fânațul Domnesc in the *Myrmica* colonies (Timuş unpublished data). Also, larval development in laboratory experiments with ecotypes from the same area, never indicated polymorphic growth rates (Timuş unpublished data), in contrast to other laboratory studies that identified fast and slow developing larvae (Schönrogge *et al*. 2000, Witek *et al*. 2006).

Mobility and distribution

An increasing number of studies showed that host plant development rather than host-ant presence influences the butterflies' oviposition choice (Thomas *et al.* 1997, Nowicki *et al.* 2005c, Fürst & Nash 2010). In Fânațul Domnesc the distribution of butterflies of both ecotypes showed correlations with the distribution of the respective host plant. *M. a. 'pneumonanthe'* showed a higher density in patches of *G. pneumonanthe* resulting in a higher correlation coefficient between butterfly and plant distribution. *M. a. 'cruciata'* was more dispersed, similarly to the host plant distribution, *G. cruciata*, resulting in a weaker, but still significant, correlation between the two distributions. At the same time, distributions of the two host plants did not overlap and correlations between ecotype and irrespective host plant were not found.

Sielezniew & Stankiewicz (2004) presented the hypothesis that *M. alcon* ([Denis & Schiffermüller]1775) could also use *G. cruciata* apart from *G. pneumonanthe* as an additional host plant, by studying oviposition preferences. But their study could not prove that eggs laid on *G. cruciata* could sustain viable offspring, because only one larva was found in ant nests in the vicinity of *G. cruciata* plants. Also the study of Czekes *et al.* (2013), conducted in the same area of Fânațul Domnesc (Cluj County, Romania), showed clearly separated oviposition behaviour and preference in the two ecotypes for their respective host plant.

Butterflies of both ecotypes proved to be very sedentary. Their limited mobility and distribution overlapped with that of their host plant indicates that they establish home - ranges, not moving far from their emergence site. This phenomenon has been reported also in other recent MRR studies for other *Maculinea* species (Hovestadt & Nowicki 2008, Kőrösi *et al.* 2008, van Langevelde & Wynhoff 2009, Hovestadt *et al.* 2011, Skórka *et al.* 2013). Hovestadt & Nowicki (2008) suggested that keeping close to the place of eclosion is an adaptation of *Maculinea* butterflies to myrmecophily.

The establishing of home-ranges of *Maculinea* butterflies has serious implication in conservation. A low number of emigrants increases the risk of extinction of declining populations, and affects the colonisation process dynamics (Nowicki *et al.* 2005a). Even though on rare occasions *Maculinea* butterflies fly over longer distances and can succeed in colonizing new areas, the probability of such an event is probably lower than the local extinction rate (Nowicki *et al.* 2005a).

Implications for conservation

Meadows that shelter more than 2 *Maculinea* species are very rare. The habitats in the Natura 2000 site 'Dealurile Clujului Est' are probably unique in Europe, because in some northern exposed meso-hygrophilous meadows of this site four of the European *Maculinea* taxons co-habit syntopically: *Maculinea arion*, *M. alcon 'pneumonanthe'*, *M. alcon 'cruciata'* (also on southern exposed sites), *M. teleius* and *M. nausithous kijevensis*. Such places are particularly fragile because of each *Maculinea* taxon's specific ecological requirements which make their conservation a hard task.

The presence and preservation of vigorous *Maculinea* populations in the area has been fostered over time through a randomized mowing regime of the parcels owned by different farmers. In this system, every year, a part of the parcels which harbour fragments of *Maculinea* populations remain unmown or are mown very late in summer, maintaining in this way the metapopulational structure of the species and having as a result the preservation of the large blues (Timuş *et al.* 2011).

Their reduced mobility, home-range behaviour and distributions closely connected to that of their host plants all lead to the greater susceptibility to isolation of populations through reducing their probability to disperse and colonize new habitats in the two analysed *M. alcon* ecotypes. Thus, habitat fragmentation by land use increases the vulnerability of *M. a.'cruciata'* and *M. a. 'pneumonanthe'*.

The cultural landscapes and mosaics of secondary habitats resulted from differently used meadows in the area Dealurile Clujului, harbouring specific structures, is already threatened by factors such as short- and long-term abandonment of traditional land-use (hand-mowing, extensive grazing etc.), intensification of grazing (especially with sheep), drainage works and local urban development plans (Voda *et al.* 2010, Timuş *et al.* 2011, Paulini *et al.* 2011).

Specifically in the area of Fânațul Domnesc the most threatening activities are abandonment and intensive grazing. The abandonment of mowing and grazing is likely to have a positive short-term impact on *Maculinea* populations (Timuş *et al.* 2011). The positive effect of land use abandonment on butterfly species diversity in general is supported by the studies of Balmer & Erhardt (2000), Cremene et al. (2005), Schmitt & Rákosy (2007), and Rákosy & Schmitt (2011). However, after 4-5 years, this process will most likely go into reverse and cause the decline of biodiversity (Schmitt & Rákosy 2007), affecting the *Maculinea* populations as well. The same, abandonment of heterogeneous mowing of small meadow plots potentially leads to an alteration of the metapopulational structure of *Maculinea* spp (Timuş N. unpubl. data).

The effects of summer grazing on the butterfly populations in abandoned hay meadows depend on the intensity and type of grazing. In general, extensive grazing with cattle can have a positive impact because it prevents or slows down the secondary succession towards shrubs and can therefore contribute to maintaining the butterfly diversity of grasslands. For *M. a. 'cruciata'* and *M. a.'pneumonanthe'* in particular, even an extensive grazing has a negative impact: host plants with eggs can be easily eaten or trampled by sheep (Rus A. unpublished data).

In order to protect the species diversity and the viable metapopulational structures, there is a strong need to develop strategies for maintaining the current mosaic of habitats characterized by different stages of succession (Cremene *et al.* 2005, Skórka *et al.* 2007, Timuş *et al.* 2011), e.g. through more research, support of traditional small-scale farming and active nature conservation management.

Many studies emphasize the importance of maintaining an interconnected network of suitable habitats (Hanski 1999) but also of habitat quality, especially for sedentary species (Thomas *et al.* 2001, Maes & Van Dyck 2005, Habel *et al.* 2007). Conservation of the two *M. alcon* ecotypes should aim at improving habitat quality. Considering their low mobility, the spread of the local populations can be achieved only if high quality habitat is within flight distance (i.e. 200 m). Habitat quality can be improved through implementation of specific land-use measures like extensive mowing once a year, either few weeks before or after the flight period.

Within an interdisciplinary project funded by the Deutsche Bundesstiftung Umwelt (DBU; project number 27559) a pilot agri-environment programme was carried out in 2011 and 2012. This project offered incentives to apply the earliest mowing on the 25th of August and tractor and hand mowing was allowed. The short-term outcomes of this project showed a disadvantage for cattle farmers who were dissatisfied with the lower quality of hay, but on the other hand, most farmers were encouraged by courses and regular support, so that they willingly cooperated in the project (Paulini *et al.* 2012).

Maculinea butterflies were positively affected by the reduction in mowing earlier in the season. Thus, in 2012 a new package of the national agri-environment scheme was introduced for a restricted geographical area, due to the occurrence of the butterflies of the genus *Maculinea:* Agri-environment package 6 "Grasslands important for butterflies esp. *Maculinea* spp". This package aims at maintaining high habitat quality for *Maculinea* butterflies. The payments (240 Euros/ha) can be received for all permanent grasslands and the main requirements are: the earliest mowing date on the 25th of August, mowing only allowed by scythe or small hand mowing machines, grazing with min 0.3 livestock unit/ha (0.3 cows /ha or 1.8 sheep/ha) and max. 0.7 livestock unit/ha (0.7 cow per ha or 4.2 sheep/ha) (Axa II, www.apia.org).

This might be the beginning of long-term education for agri-environment conservation in Romania; however the long-term effects of this measure cannot be estimated yet. The effective implementation of such agri-environment measures often depends on management of local administration and willingness of stakeholders to apply for incentives and practice the recommendations of the measures after NGO support and educational programs are reduced.

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