Habitat use and population biology of the Danube Clouded Yellow butterfly *Colias myrmidone* (Lepidoptera: Pieridae) in Romania

István Szentirmai[1*], Attila Mesterházy[2], Ildikó Varga[3], Zoltán Schubert[4], Lehel Csaba Sándor[5], Levente Ábrahám[6] and Ádám Kőrösi[7, 8]

[1]Őrség National Park Directorate, Siskaszer 26/A, Őriszentpéter 9941, Hungary, i.szentirmai@gmail.com

[2]Celldömölk, Hungary, Celldömölk 9500, Hunyadi u. 55., Hungary, amesterhazy@gmail.com

[3]Department of Nature Conservation, Ministry of Rural Development, Hungary, ildiko.varga@vm.gov.hu

[4]Csákánydoroszló 9919, József Attila u. 35., Hungary, sempervivum@index.hu

[5]Tarisznyás Márton Museum, Gheorgheni 535500, Str. Rákóczi Ferenc Nr. 1., Romania, sandorlcs@freemail.hu

[6]Rippl-Rónai Museum, Kaposvár 7400, Fő u. 101., Hungary, dr.levente.abraham@gmail.com

[7]MTA–ELTE–MTM Ecology Research Group, Budapest 1117, Pázmány Péter s. 1/C, Hungary; korozott@gmail.com

[8]Field Station Fabrikschleichach, Biocenter, University of Würzburg, Glasshüttenstr. 5, 96181 Rauhenebrach, Germany

*Corresponding author: i.szentirmai@gmail.com, phone: +36 30 377 5494, fax: +36 94 428 791

Abstract

The Danube Clouded Yellow (*Colias myrmidone*) is one of the most endangered butterflies in Europe. Its distribution range shrank dramatically in the last few decades due to the extinction of populations in Western and Central Europe. Ecological studies were commenced when populations were already at the verge of extinction, thus our knowledge on the population ecology and habitat use of this butterfly is very limited. Here we report the results of a study on habitat preferences, egg distribution and demography of *C. myrmidone* in Romania, perhaps the last remaining stronghold of the species in Central Europe. We found that Danube Clouded Yellow adults occurred mainly in mesophilous grasslands created by forest clearing and then maintained by low intensity grazing allowing bushes and forest-edge vegetation to develop and host *Chamaecytisus* species to grow. Butterflies highly preferred lightly grazed pastures over hay meadows and abandoned grasslands, their density was positively related to host plant density. Egg-laying females preferred habitat patches with relatively high cover of the host plant and tall vegetation. Both apparent survival rate and encounter probability were lower for females than males, and parameter estimations also had much higher errors for females. These indicate that much higher sampling effort is needed to estimate and monitor population parameters for females than for males. Our results provide guidelines for the habitat management and population monitoring of *Colias myrmidone*, thus may significantly contribute to its successful conservation.

Key words: host plant, Chamaecytisus, grassland, grazing, habitat preference, oviposition, demography

Introduction

The Danube Clouded Yellow (*Colias myrmidone*) is one of the most threatened butterfly species in Europe. It was formerly distributed from Germany through Central Europe and Ukraine to the north-west of Kazakhstan, but several populations became extinct from Western and Central Europe by the beginning of the 21st century (Van Swaay et al. 2010; Marhoul and Dolek 2012). Its last populations are small and sparsely scattered in Slovakia, Poland and Romania, whereas their status is unknown in Ukraine, Russia and Kazakhstan. The causes behind its European-wide collapse are poorly known since researchers became interested in the Danube Clouded Yellow only after it had been already at the verge of extinction (Dolek et al. 2005; Freese et al. 2005). Most experts however, attribute its decline to climate change, and to the loss and deterioration of its habitat due to homogenisation and intensification of agricultural management (Dolek et al. 2005; Freese et al. 2005; Konvička et al. 2008; Settele et al. 2008; Settele et al. 2009).

To halt the decline of the Danube Clouded Yellow, one needs to understand its habitat requirements and then apply habitat management suitable for the species. In general, the species inhabits mosaic forest-steppe like landscapes with grasslands, scrublands, scattered trees and very open woodlands (Settele et al. 2009). Lightly grazed and/or patchily mown herb-rich grasslands with scattered trees or bushes are used as breeding sites, where its larval host plants (*Chamaecytisus* spp.) are abundant. Western European studies found that calcareous or dolomitic grasslands on south exposed hillsides, heath-steppes and heath forests, secondary xeric or oligotrophic grasslands and clearings in pine forests are suitable habitats for *C. myrmidone* (Kudrna and Mayer 1990). Egg-laying females prefer sun-exposed sprouts in dense patches of the host plant. Eggs are laid on the tip of fresh shoots that were neither flowering nor yielding (Dolek et al. 2005). This latter result indicates that some kind of management (grazing or mowing) of the habitat is necessary to provide fresh shoots throughout the reproductive period. However, too intensive or uniform mowing may destroy eggs and larvae or remove potential shoots for oviposition and eventually may lead to the collapse of entire populations (Konvička et al. 2008). Although these results provide useful information for the preservation of the Danube Clouded Yellow, there are still many gaps in our knowledge. For example, there have been no detailed studies on its habitat selection, especially in the eastern part of the species' range. There is still no guide how to assess habitat quality for this species, and the spatial structure and dynamics of its populations are also largely unknown, although these are inevitable for the successful conservation of threatened butterflies (Thomas et al. 2001; Örvössy et al. 2013).

The objective of our study was to obtain further data on the habitat requirements of the Danube Clouded Yellow. The study was carried out in Romania, one of the last remaining European strongholds of the species, where still some vivid populations thrive. At regional scale, we were interested in factors determining the distribution of the species, therefore investigated nine potential habitats and related their characteristics to the presence and absence of the species. At the landscape level, we were interested in the suitability of different habitat types within a landscape mosaic, hence we related the density of butterflies to several habitat characteristics. At the habitat level, we were interested in factors influencing females' egg-laying decisions and so we related the frequency of eggs to microhabitat characteristics. Besides, we also aimed to collect information on population sizes and dispersal, therefore conducted mark-recapture studies in some populations.

Methods

Study species

Danube Clouded Yellow is bivoltine (May-June and July-September) in Europe with a partial third generation in hot summers (Kudrna and Mayer 1990; Settele et al. 2009). Its larval host plants are various species of the genus *Chamaecytisus* (Dolek et al. 2005; Konvička et al. 2008). Females lay eggs on young sprouts of host plants on the upper side of leaves. Caterpillars hibernate either in the litter on the ground (Weidemann 1995) or on the stem of the host plant (Gauckler 1962).

Study sites

Danube Clouded Yellows were studied at nine locations in Romania between 2007 and 2011 (Table 1, Fig. 1). Study sites were selected based on earlier reports on the species' occurrence (Gheorgheni, Cluj-Napoca, Săvădisla, Turda Gorge, Turului Gorge, Piatra Secuiului, Liteni), or if our prior evaluation suggested the sites being potentially suitable habitat for the species (Cheia, Lita). A total area of approximately 50 km^2 was searched for suitable habitats in the Gheorgheni Basin and 250 km^2 in Cluj County. Detailed description of the nine investigated sites is given in the Supplementary Material (S1).

Data collection

Habitat selection of adult butterflies was studied in the habitat mosaic of Gheorgheni site in 2010 (Supplementary Fig. 1). Altogether 21 transects were laid down in a way that they covered most of the potential habitats of the Danube Clouded Yellow from grazed pastures to hay meadows. Transects were 64-495 m long and each of them crossed only one distinct habitat patch of uniform vegetation type and management. Transects were walked with the same velocity every other day between 28 July and 29 August.. Butterflies within 10 metres of both sides of the transect were counted and their sex and behaviour was recorded. For each transect the following variables were also estimated at the beginning of the survey. Variables describing vegetation were *vegetation height*, *host plant density* (estimated on a scale from 0 to 4 (30% cover)) and *bush cover* (estimated on a scale from 0 to 10%). Topographic variables were *slope steepness* (to the nearest 5 degrees) and *direction* (S and E *vs*. N and W facing), while management was characterized with *management type* (grazing *vs.* mowing), *intensity* (from 1: light grazing or occasional mowing to 3: intensive management; judged from vegetation height and the number of grazing animals) and *grazing animal* (sheep, cattle, or both).

Oviposition site selection of females was studied at four sites that were frequently visited by adult butterflies in Gheorgheni between 9 and 15 August 2009. Three 20 × 20 m squares were designated at site D and E, six at site F and two at site G. First, all squares were thoroughly searched for eggs, larvae and pupae of *C. myrmidone*. The species of the plant on which the reproductive stages were found was recorded as well as their position within the plant. Distance of eggs and pupae from ground level was measured by a straight ruler placed perpendicular to the ground. For each square, *host plant cover* (%), *vegetation height* (to the nearest 10 cm), *slope steepness* (to the nearest 5 degrees) and *bush cover* (%) were estimated.

Mark-recapture study was carried out in Gheorgheni site A in 2010, and at Turda Gorge and in Liteni in 2011. In Gheorgheni the study was conducted between 29 July and 28 August covering the entire flight period of the second generation. Sampling was conducted on every second day. At Turda Gorge and Liteni, the study was carried out between 7 and 27 August 2011. Turda Gorge was split into two sites, i.e., site 1 south of the gorge and site 2 north of the gorge. Turda Gorge site 1 and site 2 were visited eight and six times, respectively, and Liteni eight times. During each visit the entire suitable habitat was thoroughly searched for adults by a single person and each individual was caught and marked on the underside of their hindwing using fine-tipped pens with permanent ink (Stabilo S).

Additional data on population biology were collected in Georgheni in 2007 and in Lita in 2011. In Gheorgheni, the study was conducted by three persons between 20 and 23 August in 2007, during which three occupied habitat patches (site A, B, C, see Supplementary S1) were visited two times between 11:00-15:00 hours and adult butterflies were caught and marked. Lita was visited once on 21 August and five individuals were caught and marked.

Statistical analyses

To study habitat selection of adult Danube Clouded Yellows, we calculated their density for each transect (n = 21) by dividing the mean number of observed individuals with transect length. We used the data of sixteen pastures and five hay meadows and we assessed the effects of vegetation, topographic and management related variables on butterfly density. First we tested for correlations among these explanatory variables. We found that hay meadows were significantly different from grazed pastures in all aspects: vegetation was significantly higher on meadows than on pastures (Kruskal-Wallis $\chi^2 = 7.91$, $P = 0.005$), and both host plant density and bush cover were ranked as zero on all meadows. Additionally, meadows were plain so topographic variables were unreasonable. Butterfly density was also significantly lower on meadows (Kruskal-Wallis $\chi^2 = 5.36$, $P = 0.02$), thus we excluded the meadows from further analyses. We also found some correlations among the explanatory variables on pastures: *grazing intensity* significantly influenced *vegetation height* (Kruskal-Wallis $\chi^2 = 6.02$, $P = 0.049$), *slope steepness* was positively correlated with *bush cover* (Spearman's *rho* = 0.692, $P < 0.01$) and marginally non-significantly with *host plant density* (Spearman's *rho* = 0.465, $P = 0.07$), while *host plant density* and *bush cover* were also positively correlated (Spearman's *rho* = 0.594, $P = 0.015$). Based on these results, we constructed six sets of uncorrelated explanatory variables (see Supplementary S2). Due to the small sample size and some heteroscedasticity in our data, we applied conditional inference trees, a non-parametric class of regression trees (Hothorn et al. 2006), with all the six subsets of explanatory variables. Dependent/response variable was density of male and female butterflies (both separately and merged together).

To investigate oviposition site selection Spearman rank correlation tests were used to relate number of eggs to the cover of host plant, vegetation height, slope steepness and bush cover. These analyses were based on data collected in Gheorgheni in 2009.

All statistical analyses were performed using R 3.0.0 statistical software (R Development Core Team 2012).

Sufficient data for detailed analyses of population demography was collected at Gheorgheni site A in 2010 and at Turda Gorge site 2 in 2011. Though in the latter year several other sites were sampled, the number of butterflies marked and/or recaptured on those sites was too low (Table 2). All analyses were performed using program MARK 6.2 (White and Burnham 1999). First, a fully time-dependent Cormack-Jolly-Seber (CJS) model was fitted to the data in which the two parameters (apparent survival rate φ and encounter probabilty p) were sex-dependent as well. Then we tested the goodness-of-fit (GOF) of this model to our data by estimating the $\hat{c}$ value which quantifies the amount of overdispersion. The $\hat{c}$ equals to 1 in case of perfect fit, but as a working rule of thumb, provided $\hat{c} < 3$, we can relatively safely state that the model fits well (Lebreton et al. 1992). Then we performed a backward model selection based on AIC values using $\hat{c}$ adjustment to account for the lack of fit in the initial model. When no single model proved to outperform the others (*i.e.* there was not a single highly supported model) then we applied model averaging to yield more robust parameter estimations (Burnham and Anderson 2002). Finally, we fitted a Jolly-Seber (JS) model to the dataset and performed model selection and parameter estimation as described above. We used the so called POPAN parameterisation of the JS model which has two additional parameters: probability of entry to the population from the superpopulation *pent*, and size of the superpopulation *N* (Schwarz and Arnason 1996).

Results

Habitat selection

Danube Clouded Yellow was found at four out of nine surveyed locations (Table 1). Sites occupied by the Danube Clouded Yellow were hill slopes at an altitude between 520 and 950 metres, with variable exposure and slope steepness between 15% and 60%. Unoccupied locations did not seem to differ from occupied ones regarding the above mentioned parameters. Vegetation of inhabited sites was typically mesophilous grassland dominated by *Brachipodium* and *Agrostis* species, whereas unoccupied sites were dominated by xeric or xero-mesophilous grasslands dominated by *Stipa* species. Habitats of the Danube Clouded Yellow were managed by light grazing, whereas the species was absent from intensively grazed or unmanaged grasslands. The abundance of the host plants did not differ between occupied and unoccupied locations.

In the habitat mosaic of Gheorgheni, Danube Clouded Yellow adults primarily used grazed pastures, whereas hay meadows were seldom visited (see Methods). Density of males and females were not correlated (Spearman's *rho* = 0.408, *P* = 0.117). Conditional inference trees suggested that *host plant density* had significant effect on butterfly density: Fig. 2 shows that butterfly density was significantly higher on those seven pastures where host plant density was not zero. When data of the two sexes were analysed separately, we found that female density was also highly significantly affected by host plant density (Supplementary Fig. 2). If we used subsets of predictors without host plant density, we found that slope steepness and bush cover also had significant effects: butterflies preferred steeper slopes with higher bush cover (Supplementary Figs. 3, 4). However, no effect of any environmental variable on male density could be revealed.

Oviposition site selection

In Gheorgheni, eggs were exclusively laid on *Chamaecytisus triflorus* (n = 34), whereas 43.3% of pupae (n = 30) were found on other plant species including *Origanum vulgare* and *Calamagrostis villosa.* 91.2% of eggs were laid on the upper surface of the leaves, and in 97.1% of the cases only a single egg was laid per plant. Eggs were laid 21.5 ± 2.7 cm above ground level, while pupae were found 42.7 ± 3.4 cm above ground level.

Oviposition site selection was related to the density of host plant and vegetation height, as the number of eggs per unit area increased with these variables (Spearman rank correlation, host plant density: Spearman's *rho* = 0.714, *n* = 14, *P* = 0.004; vegetation height: Spearman's *rho* = 0.652, *n* = 14, *P* = 0.011; Fig. 3). In contrast, egg numbers were related to neither slope steepness (Spearman's *rho* = 0.187, *n* = 14, *P* = 0.523), nor bush cover (Spearman's *rho* = -0.086, *n* = 14, *P* = 0.769).

Population biology

The numbers of marked and recaptured butterflies in each site are given in Table 2. Recapture rate was invariably much lower for females in all study populations. The bootstrap GOF test suggested that the fully time-dependent CJS model fitted well to both datasets since $\hat{c}$ values were close to one. For the Gheorgheni 2010 dataset, apparent survival rate φ was higher for males and showed a decreasing trend in both sexes during the flight season (Supplementary Table 1). Average residency of males ranged between 2.37 and 6.13 days, while that of females was between 1.64 and 3.14 days. Encounter probability *p* was remarkably higher for males and also showed a declining trend for both sexes throughout the flight season. Probability of entry *pent* was equal for the sexes and showed a temporal pattern (Supplementary Fig. 5). Peaks of *pent* indicate high rate of birth and/or immigration between two consecutive sampling occasions. Population size of females (N = 536, SE = 80.1, 95% CI: 411–731) was estimated almost two times higher than that of males (N = 285, SE = 14.4, 95% CI: 262–319), although the 95% CI was much wider for females. For the Turda Gorge 2 dataset, survival rate was considerably higher for males, while encounter probability was slightly higher for females. However, the 95% CI's of both parameters were much wider for females (Table 3). Average residency of males (5.2 days) was also much higher than that of females (2.3 days). In the best JS model, survival rate and encounter probability were time-constant and equal for sexes, *pent* was time-dependent and equal for sexes (Table 3). Population size of males (N = 67, SE = 13.4, 95% CI: 51–108) was estimated much higher than that of females (N = 13, SE = 3.8, 95% CI: 9–27).

In Gheorgheni in 2007, we detected two dispersal events: a male moved from site B to A, and another male from site C to A. No dispersal event was detected among the rest of the sites in Romania.

Discussion

As pointed out by the European Action Plan of the Danube Clouded Yellow (Marhoul and Dolek 2012), gaps in our knowledge are important threat for the species. The present study made an attempt to fill these gaps related to three main aspects of the species' ecology. By mapping populations in Romania we extend knowledge on the distribution of this highly threatened butterfly and draw attention to a formerly unknown populations. By studying habitat use and oviposition behaviour, we contribute to the understanding of habitat requirements and necessary management of the species' habitat. Our data on population biology may provide a starting point to detailed studies of metapopulations and population viability, urged by the action plan as well.

Habitat selection

We found three populations of Danube Clouded Yellow in Cluj County and one in Gheorgheni in Romania. The population in Lita was formerly unknown, and none of them in Cluj County was recorded during a survey by Buttefly Conservation (UK) European Interest Group in 2009 (Marhoul and Dolek 2012). Based on our data, these populations can serve as targets for future conservation actions.

Our investigations of nine potential habitats revealed that the Danube Clouded Yellow inhabits secondary grasslands on variably exposed hill slopes, where its larval host plant, *Chamaecytisus triflorus* is present. Interestingly, none of the two sites with *Chamaecytisus albus* was occupied, although this is a potential host plant species as well. Occupied sites were, with a single exception (Turda Gorge), in place of former spruce or beech forests and their vegetation was some kind of mesophilous grassland, whereas unoccupied ones were often in place of oak forests and were covered by xeric or xero-mesophilous grasslands. These results suggest that Danube Clouded Yellow prefers habitats with relatively humid microclimate. All of the occupied sites were partially covered by bushes surrounded by well-developed forest-edge vegetation, and they were lightly grazed by cattle or sheep, whereas intensively grazed or unmanaged sites were either unoccupied, or population density was extremely low as in Turda Gorge. These results indicate that suitable habitats of the Danube Clouded Yellow are mesophilous grasslands created by forest clearing and then maintained by such low intensity grazing that allows bushes and forest-edge vegetation around them to develop and *Chamaecytisus* species (especially *C. triflorus*) to grow. Our results provide further evidence for the guidelines given by Van Swaay et al. (2012) for the habitat management of *C. myrmidone*. Although our results indicate that intensive grazing affects host plant density negatively, other evidences show that some kind of disturbance of the habitat is necessary for two reasons. First, disturbance (grazing, mowing or burning) halts natural succession and maintains secondary grasslands and forest-edges (Godwin 1929;

Gibson and Brown 1992). Second, removing old branches of the host plant initiates resprouting and provides fresh twigs suitable for egg laying (Dolek et al. 2005). Our results provide further evidence for the statement of the European Action Plan (Marhoul and Dolek 2012), that both intensification of agriculture and abandonment of pastures pose threat to the Danube Clouded Yellow.

Our results thus support previous studies showing that Danube Clouded Yellow inhabits heterogeneous habitats with a mosaic of grasslands and scrublands (Kudrna and Mayer 1990; Dolek et al. 2005; Freese et al. 2005; Settele et al. 2009). Bushes are important habitat features since they support the surrounding forest edge vegetation, which is essential for *Chamaecytisus* species (Zieliński 1975; Skalická 1986; Cristofolini 1991). The preference of the species for mesophilous habitats is in line with studies in the White Carpathians, Czech Republic (Konvička et al. 2008). This result is, however, in contrast with studies in the western part of the species' range, where it prefers xeric grasslands of sun-exposed calcareous hill sides (Freese et al. 2005). We argue that this difference may be explained by climatic differences, since xeric habitats of the more humid Germany may provide similar conditions as mesophilous habitats of the dryer Central-Eastern European countries.

Within the habitat complex of Gheorgheni, the Danube Clouded Yellow clearly preferred grazed hill slopes against mown meadows. This was especially true for females, which hardly ever visited hay meadows. The difference between sexes may be due to the fact that females are bond to pastures rich in host plant to lay eggs, but males might be less sedentary and can use meadows for nectaring. Alternatively, females' detectability in hay meadows might be even lower than in pastures (Tabashnik 1980).

Within pastures, habitat patches with relatively higher host plant cover were preferred. These patches were on relatively steeper slopes with higher bush cover. We suspect that management intensity is the factor behind these relationships, because grazing pressure is lower on less accessible steep slopes and therefore both bushes and the host plant can thrive here. This idea is however, not supported by the lack of statistically significant relationship between butterfly density and grazing intensity. The controversy may be due to the method of estimating grazing intensity. Our estimation was based on the number of grazing animals observed during the study, which is both a very rough estimation and represents intensity only during the short study period. Vegetation structure, however, may be related more closely to past than to present management, since the cover of forest-edge vegetation and host plant is the result of a long management history (Peterken and Game 1984). Moreover, a larger sample size and finer measurement of environmental variables would allow us to make more robust inferences.

For egg-laying, females preferred habitat patches with relatively high cover of the host plant and tall vegetation. Similar results were obtained in Germany, where egg-laying events concentrated in patches with more than 30 shoots of the host plant (Romstöck-Völkl et al. 1999). Vegetation height may indicate grazing intensity again and predict the survival chances of eggs and larvae. Although earlier studies on a North-American *Colias* sp. did not find differences in oviposition preferences of different generations (e.g. Tabashnik et al. 1981), we note, that oviposition behaviour may still differ between generations, so our results on the second generation may not be valid for the first or the third generation (Šlancarová et al. 2013).

Population biology

Our estimations on population sizes suggest that the Gheorgheni population is sufficiently large, while the population at Turda Gorge site 2 is quite small. At the latter site, the sampling period covered only two weeks which does not allow us to estimate the total population size, moreover, the number of sampling occasions (n = 6) over the two weeks proved to be too low to collect sufficient data on females. Population density may extremely differ between populations. While we observed more than 150 individuals per hectare in Gheorgheni, less than two individuals were recorded per hectare in Turda Gorge. These extreme differences may be explained by differences in the quality of the two habitats.

Our results in Gheorgheni proved that there was dispersal between nearby subpopulations. Unfortunately the study was too short to estimate how frequent these dispersal events were and how they relate to the distance between subpopulations. We did not observe any dispersal event between populations in Cluj County probably due to large interpatch distances (13 km on average) and very low population densities. Relatively low apparent survival rates and short residency suggest that *C. myrmidone* live in open populations in our study sites where the precise delineation of the boundaries of habitat patches used by the butterfly is difficult. Presumably, this species needs an extensive, mosaic area as a habitat that provides all inevitable resources for completing its life cycle (Marhoul and Dolek 2012).

In our detailed analyses, we estimated lower apparent survival rate and encounter probability for females, although the high uncertainty in parameter estimations for females allows us only to suspect that there is a significant difference between sexes in *C. myrmidone.* The large error of parameter estimations in females has serious implications for conservation and monitoring of the species, because it suggests that precise estimations on population parameters of females require relatively high sampling effort, at least in the habitats involved in the present study. In their seminal work on North-American montane *Colias* species, Watt et al. (1977) also found shorter residence for females that they attributed to their higher mortality. Though we found no evidence on higher female emigration, that could explain their lower apparent survival, Watt et al. (1979) demonstrated that females' dispersal propensity was higher in the second brood of *Colias philodice eriphyle*. Females' lower encounter probability is also supported by earlier studies on *Colias* spp., for example in *Colias philodice eriphyle* (Tabashnik 1980). Kingsolver (1983) pointed out that males of montane *Colias* species in North America spend more time in flight and make flights of longer duration than females. Thus females might be less detectable which can explain their lower catchability. We suppose that similar sexual differences in flight behaviour may occur in *C. myrmidone* as well, although detailed observations on individual behaviour would be inevitable to test these hypotheses. Finally, we note that in our study we investigated the second generation of *C. myrmidone*, which might differ from the first generation in terms of mobility and population density (e.g. Ide 2002; Karlsson and Johansson 2008).

Conservation recommendations

Our results indicate that one of the most viable populations of the Danube Clouded Yellow may exist in Gheorgheni. Since the area is not protected, the preservation of the population could be assured by designation of the area to the Natura 2000 network and maintain favourable extensive grazing management. In the contrary, the population at Turda Gorge is extremely small and thus may be prone to extinction. Here therefore decreasing grazing pressure to improve habitat quality would be necessary. Based on our results on habitat use we recommend that existing habitats of the Danube Clouded Yellow should be managed with extensive grazing, meaning not more than a few animals per hectare. The removal of bushes should be avoided since these are important for the development of forest-edge vegetation.

Acknowledgements

We are grateful to the Őrség National Park Directorate for its support for the field work in 2007. Field work in 2009 was supported by a Zöld Forrás grant (K-38-08-00149A) of the Ministry of Environment and Water. We thank to Csaba Tibor Vizauer for his help in the field work in 2010. We are grateful to two anonymous Reviewers for their useful comments on an earlier version of this manuscript.

References

Burnham KP, Anderson DR (2002) Model selection and multimodel inference (2nd ed.) Springer, New York, pp. 496

Cristofolini, G (1991) Taxonomic Revision of Cytisus Desf. Sect. Tubocytisus DC. (Fabaceae) – Webbia 45(2): 187–219

Dolek M, Freese A, Geyer A, Stetter H (2005) The decline of *Colias myrmidone* at the western edge of its range and notes on its habitat requirements. Biologia 60: 607–610

Freese A, Dolek M, Geyer A, Stetter H (2005) Biology, distribution, and extinction of Colias myrmidone (Lepidoptera, Pieridae) in Bavaria and its situation in other European countries. J Res Lep 38: 51-58

Gauckler K (1962) Regensburger Sandbiene, Regensburger Heufalter und Regensburger Geißklee in ihrem süddeutschen Lebensraum. Denkschr Regensburg Bot Ges NF 19: 26-34

Gibson CWD, Brown, VK (1992) Grazing and Vegetation Change: Deflected or Modified Succession? J Appl Ecol 29: 120-131

Godwin H (1929) The subclimax and deflected succession. J Ecol 17: 144-147

Hothorn T, Hornik K, Zeileis, A (2006). Unbiased Recursive Partitioning: A Conditional Inference Framework. J Comput Graph Stat 15: 651-674

Ide J-Y (2002) Mating behaviour and light conditions cause seasonal changes in the dispersal pattern of the satyrine butterfly *Lethe diana*. Ecol Entomol 27: 33-40

Karlsson B, Johansson A (2008) Seasonal polyphenism and developmental trade-offs between flight ability and egg laying in a pierid butterfly. Proc R Soc B 275: 2131-2136

Kingsolver JG (1983) Ecological significance of flight activity in *Colias* butterflies: implications for reproductive strategy and population structure. Ecology 64: 546-551

Konvička M, Beneš J, Čížek O, Kopeček F, Konvička O, Vítaz L (2008) How too much care kills species: Grassland reserves, agri-environmental schemes and extinction of *Colias myrmidone* butterfly from its former stronghold. J Insect Cons 12: 519-525

Kudrna O. & Mayer L. 1990: Grundlagen zu einem Artenhilfsprogramm für *Colias myrmidone* (Esper, 1780) in Bayern. Oedippus 1: 1-46

Lebreton J-D, Burnham KP, Clobert J, Anderson DR (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. Ecol Monogr 62: 67-118

Marhoul P, Dolek M (2012) Action Plan for the Conservation of the Danube Clouded Yellow *Colias myrmidone* in the European Union. European Commission

Örvössy N, Kőrösi Á, Batáry P, Vozár Á, Peregovits L (2013) Potential metapopulation structure and the effects of habitat quality on population size of the endangered False Ringlet butterfly. J Insect Cons 17: 537-547

Peterken GP, Game M (1984) Historical factors affecting the number and distribution of vascular plant species in the woodlands of central Lincolnshire. J Ecol 72: 155-182

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Romstöck-Völkl M, Völkl W, Leibl F (1999) Die Situation von *Colias myrmidone* Esper (Lepidoptera, Pieridae) im Raum Regensburg. Schriftenreihe des Bayerischen Landesamtes für Umweltschutz 150: 219-225

Schwarz CJ, Arnason AN (1996) A general methodology for the analysis of open-model capture recapture experiments. Biometrics 52: 860-873

Settele J, Kudrna O, Harpke A, Kühn I, van Swaay C, Verovnik R, Warren M, Wiemers M, Hanspach J, Hickler T, Kühn E, van Halder I, Veling K, Vliegenthart A, Wynhoff I, Schweiger O (2008) Climatic Risk Atlas of European Butterflies. Pensoft, Sofia-Moscow, 712 pp

Settele J, Dover J, Dolek M, Konvička M (2009): Butterflies of European ecosystems: impact of land use and options for conservation management. In: Settele J, Shreeve TG, Konvička M, Van Dyck H (Eds), Ecology of Butterflies in Europe. Cambridge University Press

Skalická A (1986) Chamaecytisus triflorus (Lam.) in der Tschechoslowakei. Preslia 58: 21-27

Šlancarová A, Bednářová B, Beneš J, Konvička M (2013) How life history affects threat status: Requirements of two Onobrychis-feeding lycaenid butterflies, *Polyommatus damon* and *Polyommatus thersites*, in the Czech Republic. Biologia 67: 1175-1185

Tabashnik BE (1980) Population structure of pierid butterflies. III. Pest populations of *Colias philodice eriphyle*. Oecologia 47: 175-183

Tabashnik BE, Wheelock H, Rainbolt JD, Watt WB (1981) Individual variation in oviposition preference in the butterfly, *Colias eurytheme*. Oecologia 50: 225-230.

Thomas JA, Bourn NAD, Clerke RT, Stewart KE, Simcox DJ, Pearman GS, Curtis R, Goodger B (2001) The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. Proc Roy Soc B 268: 1791-1796

Van Swaay C, Cuttelod A, Collins S, Maes D, López Munguira M, Šašić M, Settele J, Verovnik R, Verstrael T, Warren M, Wiemers M, Wynhoff I. (2010) European Red List of Butterflies. IUCN and Butterfly Conservation Europe. Luxembourg: Publications Office of the European Union

Van Swaay CAM, Collins S, Dušej G, Maes D, López Munguira M, Rákosy L, Ryrholm N, Šašić M, Settele J, Thomas JA, Verovnik R, Verstrael T, Warren M, Wiemers M, Wynhoff I (2012) Dos and Don’ts for butterflies of the Habitats Directive of the European Union. Nature Conservation 1: 73–153

Watt WD, Chew FS, Snyder LRG, Watt AG, Rothschild DE (1977) Population structure of pierid butterflies. I. Numbers and movements of some montane *Colias* species. Oecologia 27: 1-22

Watt WD, Han D, Tabashnik BE (1979) Population structure of pierid butterflies. II. A “native” population of *Colias philodice eriphyle* in Colorado. Oecologia 44: 44-52

Weidemann HJ (1995) Tagfalter beobachten, bestimmen. Naturbuch Verlag, 659 pp

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study 46: S120-S139

Zieliński J (1975) Rodzaj Cytisus L. s.l. w Polsce. Arbor Kórnickie 20: 47-111

.

Table 1: General description of study sites investigated in 2007-2011.

Study site	Area (ha)	Altitude (m)	Exposure	Steepness (%)	Bush cover (%)	Management	Host plant cover (%)	Danube Clouded Yellow
Gheorgheni	250	930-950	variable	40-60	5-30	light grazing	10-30	present
Liteni	8	800-900	W	35-45	40	light grazing	5-10	present
Lita	10	550-600	N	15-20	10	light grazing	5-10	present
Turda Gorge	100	520-750	N	20-30	40	intensive grazing	< 5	present
Cluj-Napoca	10	650-700	N	10-20	5	intensive grazing	< 5	absent
Săvădisla	5	520-540	S-SW	40-45	20-40	unmanaged	10-20	absent
Turului Gorge	200	400-500	variable	40-55	20	unmanaged	< 5	absent
Cheia	20	400-500	SE	50	60	unmanaged	5-10	absent
Piatra Secuiului	200	500-900	W-SW	30-40	30	light grazing	10-20	absent

Table 2 Number of marked and recaptured individuals in MRR studies.

Site	Year	Number of visits	Number of marked		Number of recaptured	
			males	females	males	females
Gheorgheni	2007	2	29	33	3	4
Gheorgheni	2010	19	209	178	95	29
Liteni	2011	8	65	26	5	1
Lita	2011	1	4	1	--	--
Turda Gorge 1*	2011	8	33		4	
Turda Gorge 2	2011	6	40	8	12	1

*Due to the loss of data the sex of some individuals was missing

Table 3 Parameter estimations of CJS and JS models for the Turda Gorge site 2 dataset from 2011. In case of the CJS model the unconditional SE is reported.

Model	sex	parameter	estimation	SE	CI low	CI up
CJS	male	Phi	0.875	0.070	0.665	0.961
	female	Phi	0.740	0.201	0.269	0.957
	male	p	0.335	0.136	0.132	0.625
	female	p	0.358	0.285	0.047	0.863
JS	both	Phi	0.826	0.053	0.698	0.907
	both	p	0.414	0.123	0.207	0.655
	both	pent	0.186	0.080	0.076	0.391
	both	pent	0.261	0.097	0.116	0.487
	both	pent	0.251	0.104	0.103	0.497
	both	pent	0.241	0.097	0.101	0.473
	both	pent	0.000	0.000	0.000	0.000
	male	N	66.599	13.416	50.462	107.626
	female	N	12.910	3.809	9.279	26.849

Figure legends

Fig. 1: Overview of the location of study sites in Romania.

Fig. 2: Conditional inference tree: density of Danube clouded yellows was significantly affected by host plant density (*P* = 0.005). Butterfly density was significantly higher on pastures where host plant was present (n = 7).

Fig. 3: Number of Danube clouded yellow eggs in relation to (a) larval host plant density and (b) vegetation height.

Fig. 1

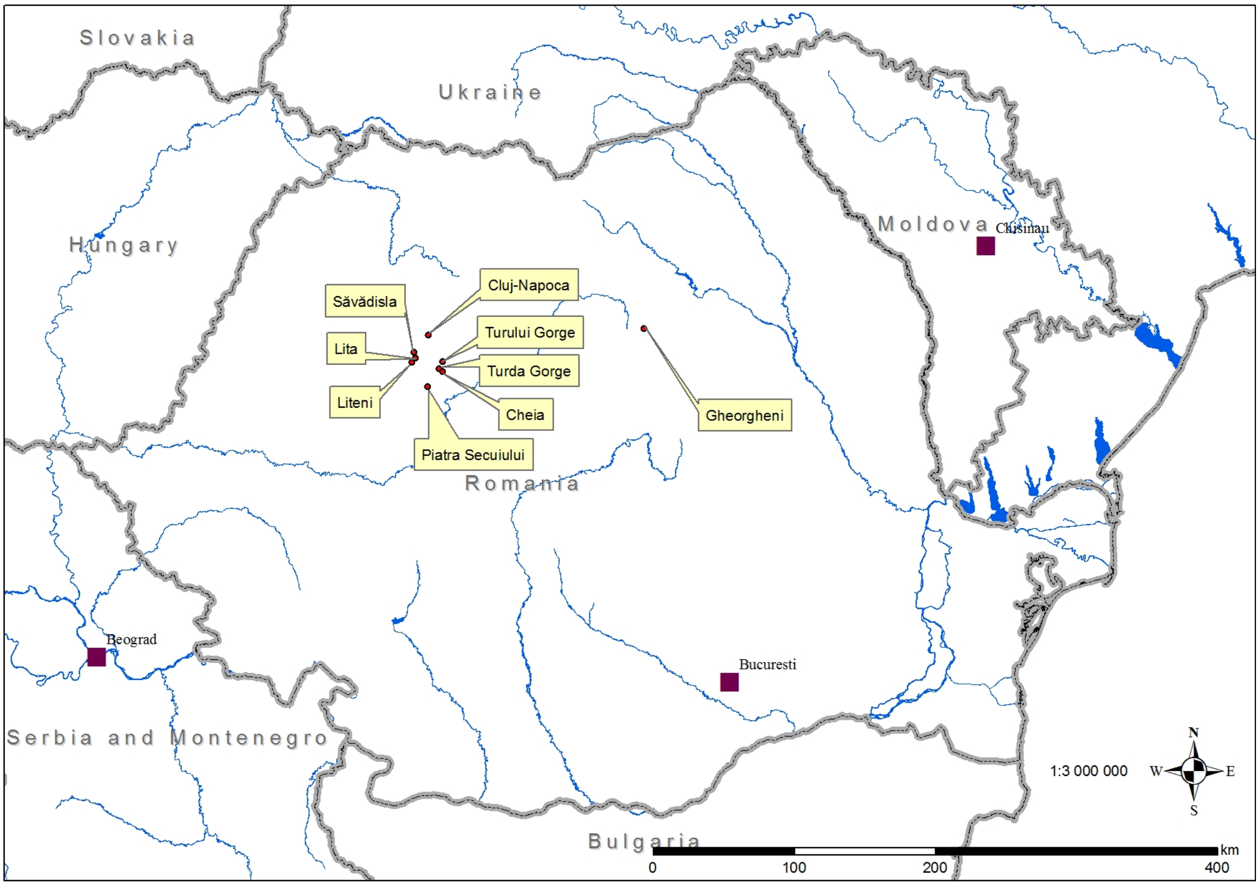

Slovakia
Ukraine
Hungary
Moldova
Chisinau
Cluj-Napoca
Săvădisla
Lita
Turului Gorge
Turda Gorge
Liteni
Cheia
Piatra Secuiului
Gheorgheni
Romania
Beograd
Bucuresti
Serbia and Montenegro
Bulgaria
1:3 000 000
N
W
E
S
0
100
200
400
km

Fig. 2

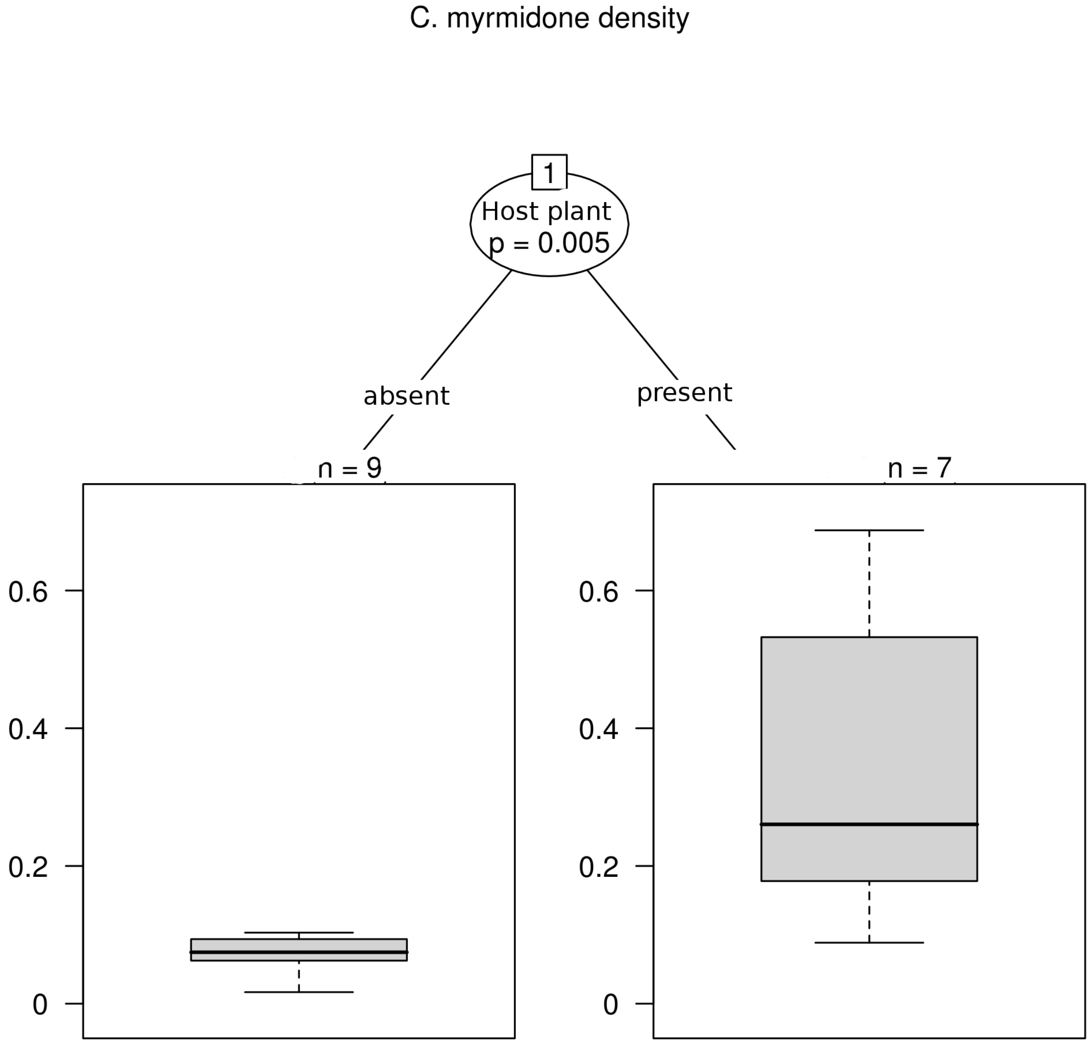

C. myrmidone density
1
Host plant
p = 0.005
absent
present
n = 9
n = 7
0.6
0.4
0.2
0
0.6
0.4
0.2
0

Fig. 3a

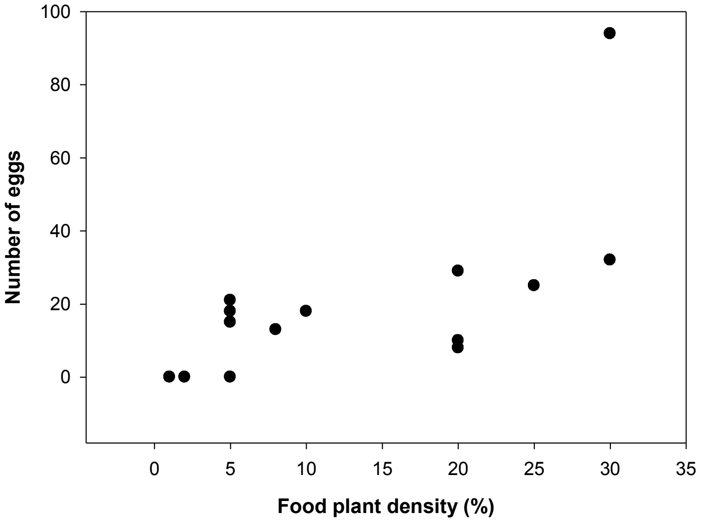
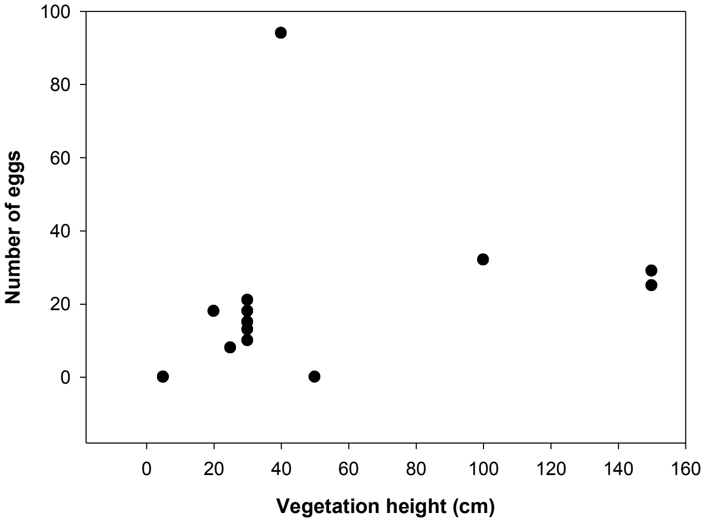

Number of eggs
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Food plant density (%)

Fig. 3b


Number of eggs
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
Vegetation height (cm)