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Nectar-plant availability and visitation in the Clouded Apollo butterfly (*Parnassius mnemosyne*)

Summary of PhD Thesis

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Introduction

The relationship between pollinators and flowering plants plays a crucial role in the function of terrestrial ecosystems. The ecological and co-evolutionary interactions in plant-pollinator systems can be studied in many aspects, at very different spatio-temporal scales.

Pollinators visit plants for floral food resources and they pollinate these plants. Floral food resources may be rapidly changing in space and time. Pollinator abundance, diversity, and resource-visit frequency are influenced by the number of floral resource species, quantity, and density of flowers, and the amount and quality of food in flowers. Floral resources are among the strongest factors structuring pollinator communities.

Foraging plays a key role in the life of animals as it fundamentally affects survival and reproductive success, hence pollinators should be able to select among the available resources. A wide range of flowering species with various advertisement cues can be available for insect pollinators, and flower-visiting insects have to choose all the elements covering their dietary needs to acquire the most profitable resources. Foraging behaviour can be different among species, populations, generations, sexes, individuals and developmental phases. It also can change during the lifespan of an individual within a specific phase.

Recently, there has been an increasing interest in pollination studies. However, the development of sampling methods lags behind and are often inappropriate to address specific research questions. Plant-pollinator communities are influenced by many traits of plants, animals, and environmental effects, hence to understand their natural patterns in different spatio-temporal scales, one should use appropriate sampling procedures and analyses. Plant-pollinator interactions can be studied at very different spatio-temporal scales. Many field studies investigated plant-pollinator interactions at a large scale and low resolution, while others applied experiments at high resolution but a small scale. Although many hypotheses can be found on foraging strategies, the long-term, detailed observational datasets at natural conditions are scarce. To understand the ecological relationships between plants and pollinators, we need detailed, long-term field data. These information can also help to plan experiments and conservation management.

Among insect pollinators, some butterflies are suitable model organisms to investigate foraging behaviour, because they can be easily monitored under natural circumstances. Adult butterflies mostly feed on floral nectar, and besides larval food intake, adult nectar-feeding was proved to significantly affect the longevity and reproductive success of several species. Floral species selectivity and the spatio-temporal changes of foraging behaviour are still understudied in butterflies. Studies investigating foraging behaviour in insect pollinators on several spatio-temporal scales are also scarce.

Aims

Measuring floral resource availability for pollinators

In the first part of the thesis, I studied resource availability sampling methods for insect pollinator resource-use. Although understanding complex plant-pollinator relationships requires accurate field data and sampling methods, there are a number of problems and shortcomings in the applied sampling methods. For example, no general methodology for floral resource availability estimates exist. I studied floral resource sampling methods, by investigating a methodological review and a case study. From the studies using floral resource sampling methods, I extracted the specific information on their methodologies. My focus was on how representative were the vegetation samples both spatially and temporally. I also searched for trade-offs between different aspects of sampling investment. In a case study, I compared the quadrat and scanning sampling procedures to estimate floral resources to reveal the difficulties of measuring floral resources and highlight potential methodological biases.

Flower visitation in Clouded Apollo butterflies

In the second part of the thesis, I studied spatio-temporal changes in flower visitation in the Clouded Apollo butterfly (*Parnassius mnemosyne*, Linnaeus, 1758; Lepidoptera: Papilionidae). This species is a suitable model organism to investigate foraging behaviour: adult butterflies of this species spend considerable time on feeding on nectar-plants and it is easy to observe them during feeding and to collect detailed observational data during the individuals' lifespan. However, very little is known on their nectar source use. My aim was to reveal the factors influencing flower visitation in Clouded Apollo butterflies. I studied how the spatio-temporal variation in flower visitation by butterflies was related to the changes in flower abundance, both at population and individual levels. I was interested in which insect-pollinated floral species are available during the flight period, how Clouded Apollo butterflies use them as nectar sources and how floral traits affect flower choice. I investigated inter-annual similarities in floral abundances and in flower visitation. Furthermore, I studied within- and between-year variability in nectar-plant flowering phenology and in visit dynamics, and I investigated how the number of visits related to flower abundance and if this relationship was different among plant species and years. I studied the flower resource-use of individuals over time and the differences between individuals. Finally, I investigated within-habitat resource dependency of spatial distribution in Clouded Apollo butterflies.

Methods

Measuring floral resource availability for pollinators

For a methodological review, we extracted information on floral resource measuring methodologies from 158 selected studies, investigating insect pollinators in temperate grasslands. We searched papers by a combination of key terms in scientific literature databases. We present descriptive statistics for the extracted variables. We studied if publication dates influenced how representative a study was with respect to the spatio-temporal scale of vegetation samples. We also searched for trade-offs between different aspects of sampling investment, because these were constrained by sampling effort.

In a case study, we compared two methods for sampling floral resources in a single meadow. We recorded species lists of insect-pollinated plants with abundance categories (hereafter scanning methods) and we counted the flowering shoots in quadrats, to estimate floral resources. These methods were compared with respect to the number of species detected, estimates on floral resource abundance, and temporal changes in flowering.

Flower visitation in Clouded Apollo butterflies

We studied flower visitation in Clouded Apollo butterflies from 2009 to 2013 at Leány-kúti rét, and in 2014 at Hegyesd in the Visegrádi-hegység, Hungary, Central Europe. We made field observations on flower visitation of individually marked Clouded Apollo butterflies. We recorded identities, the sex of individuals, time, location and the visited nectar-plant species. We measured the abundance of insect-pollinated flowering plants. We extracted information on flower traits from databases and we measured nectar amounts and sugar concentration. We estimated the spatial distribution of resources (larval host-plant, habitat openness and nectar-plant). Our aims were to reveal natural patterns in flower visitation and our observational field data were primarily suitable for descriptive analyses, rather than statistical hypothesis testing.

Results

Measuring floral resource availability for pollinators

By reviewing pollination studies, we found large methodological differences, e.g. the applied sampling units and count variables varied considerably. Vegetation sampling procedures, such as the spatial and temporal distribution of the sampling units in the study sites were not clearly described or the reasons why a given method had been used remained unexplained in many studies. Sampling units were mostly quadrats or transects. Sampling covered a small proportion (median: 0.69%) of the study sites, with long intervals (median: 30 days), and most studies lasted only one year. The most often used count variables were indirect proxies of floral resources. Positive trends between the representativeness of methodology and the date (years) of publications were not found. However, we found negative relationships in some of the different aspects of sampling, e.g. the proportion of the study sites covered with sampling decreased with increasing site area.

In the case study, the scanning method found more potential nectar-plant species and found the species earlier than quadrat sampling. Quadrats found abundant species with higher odds than the scarce. Flower abundances were correlated between the two methods, although with a remarkable scatter. Flower abundance changed 6% per day compared to the flowering peak.

Flower visitation in Clouded Apollo butterflies

Clouded Apollo butterflies visited 35 nectar plants from the 71 species available. Few nectar plants were frequently visited, many were scarcely visited, and no visits were observed on several abundant species. Plant species differed in their flower traits (e.g. flower colour and type) and in flower abundances. Clouded Apollo butterflies visited frequently the abundant, purple, blue or red coloured flowers with stalk, disk or flag structure.

Although we found inter-annual correlation in flower abundance and flower visitation for all the plant species, flower abundance and visit ratio remarkably varied among years and within flight periods in the seven most frequently visited plant species. The number of visits increased with flower abundance in the seven most frequently visited plant species.

By analysing flower visitation patterns in more details, we found that individuals were considerably different in their resource-use (i.e. individual specialisation). This variation was partly explained by the temporal variation in floral resource availability together with the occurrence of individual butterflies within the flight period. Furthermore, we found temporal changes in lifetime individual resource-use, indicating that butterflies can adjust foraging to the varying resource availability.

Spatial occurrence of butterflies increased with nectar-plant density and habitat openness, although it was not directly influenced by larval host-plant quantity. Spatial occurrence changed over time and it was explained by the temporal changes in nectar-plant spatial distribution.

Discussion

Measuring floral resource availability for pollinators

Most of the reviewed studies did not carefully design resource availability sampling relative to the complexity of plant-pollinator interactions. Some pollinator studies investigated flower availability superficially, whereas some studies even neglected it completely. We highlighted the advantages and drawbacks of different methods, and recommend a guideline to design sampling more adequately, e.g. to increase coverage and the frequency of sampling floral resources.

We recommend that both quantity and quality, as well as the spatio-temporal distribution of resources should be monitored when sampling floral resources. Sampling methods should be better adapted to the aim of the study and to the complexity of the studied system (spatial heterogeneity, seasonality, number of pollinator species, etc.). To investigate floral resource abundance, the focal pollinators' temporal phenology and foraging range in a specific area should be known. We recommend using shorter sampling intervals and higher coverage than used in most of the reviewed studies.

Although the ultimate solution to estimate floral resource amounts would be directly measuring nectar and pollen, it is not feasible in many cases. Characterising flowers with such direct measures, and collecting larger samples on flower abundance could be a reasonably good compromise, especially when the variability in nectar and pollen amounts were also taken into account. If direct measures on nectar or pollen amounts are not feasible, visual floral units from the pollinators perspective could be the appropriate count variable. Combining different methods that are adequate to provide data with either high spatio-temporal resolution or coverage, can be a reasonable approach.

Remote sensing technologies can help flower resource sampling in the near future. However, such technologies have a low spatial resolution for floral resource sampling (e.g. are unable to detect cryptic plants). Therefore, we think that traditional sampling methods should be further investigated to find efficient, widely usable methods to provide a sound methodological basis for understanding plant-pollinator interactions. We conclude upon both the methodological review and the case study that further field work on optimising sampling techniques is mandatory.

Flower visitation in Clouded Apollo butterflies

Investigating flower visitation in the Clouded Apollo butterfly at different temporal scales and organizational levels offered a detailed insight into the foraging behaviour of a protected butterfly species. We showed that the Clouded Apollo butterflies chose among nectar-plant species, they visited a few nectar plants frequently, many scarcely, and did not visit several abundant species. The selection between nectar plant species based on flower abundance, flower colour and flower type. Flower traits may not be independent of each other and selection is probably influenced by several factors simultaneously. In order to understand the innate colour preferences and learning abilities of butterflies, lab experiments would be required.

Visitation rates of Clouded Apollo butterflies, as well the availability of their resources changed among and within years. The spatio-temporal changes in flower abundances are important factors in flower visitation resulting in a variation in population, among year, and individual diet, as well as in occurrences within a habitat. However, the visited floral species in a given place and time may not be necessarily optimal resources, but can be the best in a range of the available poor resources. We suggest that occasional visitation on a wide range, even suboptimal nectar species might be “sampling behaviour”, allowing adjustments in plastic foraging behaviour when floral rewards change. The difference in flower visitation among years, sites and individuals can be explained by the species’ plasticity in nectar source preferences rather than strong specialisation for a few given plant species in different butterfly populations separated by space and time.

The population level pattern can be understood only by revealing individual level flower visitation. We conclude that dynamic flower visitation changes in the population was the result of the individuals' diet change over their life and the temporal variation in individual occurrences of the butterfly population. We propose that foraging plasticity can be beneficial even for short-living insect pollinators in rapidly changing environments.

The behaviour and spatial occurrence of Clouded Apollo butterflies may be influenced by the spatial distribution of their main resources. Within one habitat, the spatial occurrence of butterflies were explained by the spatial distribution of the most frequently visited nectar-plant and habitat openness, although larval host-plant distribution and climatic effects may explain the Clouded Apollos’ occurrence at a larger scale.

Plant-pollinator systems are highly vulnerable to human impacts and little is know about the ecological requirements of many insect species. The Clouded Apollo butterfly is endangered and protected under the Bern Convention. Besides its monophagous larval diet, diet selectivity in the adult phase contributes to its vulnerable status. Hence, extreme fluctuations in factors influencing food resources such as weather can be a potential risk for Clouded Apollo butterflies, since they primarily use only a small number of food resources. In contrast, complementary resources combined with plastic foraging behaviour that we found in Clouded Apollo butterflies may mitigate the effect of these processes, thus the lack of a few resources may not threaten the entire plant-pollinator system or the viability of a single population. Our small-scale study on the spatial distribution of Clouded Apollo butterflies support that their optimal habitats consist of small open patches rich in nectar sources and close to rich larval host-plant patches. Large open areas lacking nectar-plants may serve as ecological traps that attract butterflies without the necessary resources. Furthermore, afforestation of suitable habitats may involve local extinction.

In Hungary, the colline and mountain meadows are highly valuable habitats for a variety of plant and insect species, including the Clouded Apollo. The habitat of this butterfly species, mosaic forest-meadow edges supporting heterogeneous and divers flowering plant communities can be maintained today only by human management. We suggest that a well-established conservation management needs detailed information on the ecological requirements of the focus species. For this, in the future, locally adapted small-scale forest and meadow management should be applied, then regularly monitored by a combination of appropriate sampling methods, hence more detailed nature conservation recommendations could be concluded from its results.

Our results at different spatio-temporal scales indicate that Clouded Apollo butterflies are sequential specialists, i.e. short-term specialists (i.e. individuals mostly visit the actually most rewarding resource), and long-term generalists (i.e. butterflies are able to switch among plant species according to resource profitability). Selection favours plasticity in a rapidly changing environment. Fine-grained environmental variation should be particularly high for long-lived species encountering multiple environments over their lifetime, thus behaviour should necessarily be flexible. We suggest that this applies also for relatively short-lived species such as the Clouded Apollo, because environmental variation is fast enough to alter food availability within an individual's lifetime. Hence, probably lot of pollinators are sequential specialists, and we may find similar result in other species, if we had similar detailed datasets. Ultimately, the relative pace of environmental change compared to individual lifespan may be a key factor in resource-use plasticity.

New scientific results

1. We reviewed the floral resource sampling methods frequently used in pollination studies. We revealed that vegetation sampling was presented in many studies insufficiently. We found trade-offs between different aspects of sampling investment.

2. We compared two sampling procedures to estimate floral resources. We showed that the two methods and the two sampling persons differed in finding species, estimating flower abundance and flowering phenologies. We recommend a guideline for more appropriate sampling designs, e.g. the need to increase sampling coverage and frequency.

3. Clouded Apollo butterflies choose among nectar plant species. Selection was mainly explained by flower abundance, flower colour and flower type.

4. We showed that flower abundance and visit ratio varied among years and within flight periods. The number of visits increased with flower abundance in the most frequently visited plant species.

5. We found differences among individuals in their resource-use. This variation was partly explained by changing floral resource availability over time, together with individual butterflies occurring in different time windows during the flight period. We found that the individuals' resource-use changed during their lifetimes. Clouded Apollo butterflies are sequential specialists, i.e. short-term specialists (individuals are specialists at narrow time windows) and long-term generalists at the levels of the individual during its lifetime, the population as well as the species, i.e. change flexibly among resources.

6. Spatial occurrence of Clouded Apollo butterflies within one habitat were explained by the spatial distribution of the most frequently visited nectar-plant and habitat openness. Butterfly spatial occurrence changed over time, and this can be explained by the temporal changes in nectar-plant spatial distribution.

Own scientific publications related to the topic of the present thesis

Full text papers in peer-reviewed journals with impact factor

Szigeti, V., Kőrösi, Á., Harnos, A., Nagy, J., Kis, J.: **Comparing two methods for estimating floral resource availability for insect pollinators in semi-natural habitats**, Annales de la Société entomologique de France (N.S.), 52. 289–299, 2016a.

Szigeti V., Kőrösi Á., Harnos A., Nagy J., Kis J.: **Measuring floral resource availability for insect pollinators in temperate grasslands – a review**, Ecological Entomology, 41. 231-240, 2016b.

Szigeti, V., Kőrösi, Á., Harnos, A., Kis, J. **Temporal changes in floral resource availability and flower visitation in a butterfly**, Arthropod-Plant. Inte., *in press*.

Full text papers in peer-reviewed journals without impact factor

Szigeti V., Harnos A., Kőrösi Á., Bella M., Kis J.: **Kis Apolló-lepkék (*Parnassius mnemosyne*) élőhelyhasználata nektárforrásuk és lárvális tápnövényük függvényében [Habitat use, larval host-plant and nectar-plant distribution in the Clouded Apollo butterfly *Parnassius mnemosyne*]**, Természetvédelmi Közlemények, 21. 311-320, 2015.

Conference abstracts

Oral presentations

Vajna F., **Szigeti V.**, Kis J. **Virágmélység és pödörnyelvhossz kapcsolata a kis Apolló-lepkénél [Relationships between proboscis length of the Clouded Apollo butterfly and flower depth of its nectar sources]**, II. Országos Lepkésztalálkozó [2nd National Butterfly Meeting], Szögliget, 2016.

Kis J., **Szigeti V.**, Kőrösi Á., Harnos A., Vajna F., Sáfrán N., Gór Á. **Kedv, erények, lepkék: viselkedésbiológiai és ökológiai kutatások kis-Apolló lepkéknél (*Parnassius mnemosyne*) [Mood, virtue, butterflies: behavioural and ecological studies in the Clouded Apollo (*Parnassius mnemosyne*)]**, II. Országos Lepkésztalálkozó [2nd National Butterfly Meeting], Szögliget, 2016.

Vajna F., **Szigeti V.**, Kis J.: **Testméretfüggő táplálékválasztás kis Apolló-lepkéknél [Body-size dependent foraging strategies in Clouded Apollo butterflies]**, 6. Szünzoológiai Szimpózium [6th Universal Zoology Symposium], Budapest, 2016.

Vajna F., **Szigeti V.**, Kis J. **Kis Apolló-lepkék (*Parnassius mnemosyne*) pödörnyelvhosszának kapcsolata nektárforrásaik pártacsőhosszával [Relationships between proboscis length of the Clouded Apollo butterfly (*Parnassius mnemosyne*) and the flower depth of its nectar-plants]**, Magyar Etológiai Társaság XVII. Konferenciája [17th Annual Conference of the Hungarian Ethological Society], Dobogókő, 2015.

Szigeti V., Kőrösi Á., Harnos A., Nagy J., Kis J. **Nektárnövény választás a Kis Apolló-lepkénél (*Parnassius mnemosyne*) [Nectar-plant choice in the Clouded Apollo butterfly (*Parnassius mnemosyne*)]**, A Magyar Etológiai Társaság XV. Kongresszusa Kivonat- és programfüzet [Abstract of 15th Annual Conference of the Hungarian Ethological Society], 35, 2013.

Szigeti V., Danka C., Nagy J., Kőrösi Á., Kis J. **Nektárnövény fogyasztás és kínálat a kis Apolló-lepkénél *Parnassius mnemosyne* [Nectar-plant availability and visitation in the Clouded Apollo butterfly *Parnassius mnemosyne*]**, 9. Magyar Ökológus Kongresszus Keszthely, Programfüzet előadások és poszterek összefoglalói [Abstract of 9th Hungarian Congress of Ecology, Keszthely], 98, 2012.

Poster presentations

Szigeti V., Kőrösi Á., Harnos A., Kis J. **Individual specialization in flower visitation in the Clouded Apollo butterfly (*Parnassius mnemosyne*)**, 3. Student Conference on Conservation Science, Hungary, Tihany, 2017

Gór Á., **Szigeti V.**, Kis J. **Kis Apolló-lepkék (*Parnassius mnemosyne*) táplálkozási stratégiái [Foraging strategies in the Clouded Apollo butterfly, *Parnassius mnemosyne*]**, Magyar Etológiai Társaság XVIII. Kongresszusa [18th Annual Conference of the Hungarian Ethological Society], 2016.

Szigeti V., Kőrösi Á., Harnos A., Kis J. **Kis Apolló-lepkék egyedszintű élőhely használata [Individual-level habitat-use in Clouded Apollo butterflies]**, II. Országos Lepkésztalálkozó [2nd National Butterfly Meeting], Szögliget, 2016.

Kis J., **Szigeti V.**, Kőrösi Á., Harnos A. **Flower visit patterns in the Clouded Apollo butterfly *Parnassius mnemosyne***, Future 4 Butterflies in Europe, International Symposium, Wageningen, 2016.

Kőrösi Á., **Szigeti V.**, Harnos A., Kis J. **Habitat use of Clouded Apollo butterflies is primarily related to their nectar sources**, Future 4 Butterflies in Europe, International Symposium, Wageningen, 2016.

Szigeti V., Kőrösi Á., Harnos A., Kis J. **Egyedszintű táplálkozási mintázatok a kis Apolló-lepkéknél (*Parnassius mnemosyne*) [Individual foraging patterns in the Clouded Apollo butterfly (*Parnassius mnemosyne*)]**, 6. Szünzoológiai Szimpózium [6th Universal Zoology Symposium], Budapest, 2016.

Szigeti V., Kőrösi Á., Harnos A., Kis J. **Egyedszintű táplálkozási mintázatok a kis Apolló-lepkéknél (*Parnassius mnemosyne*) [Individual foraging patterns in the Clouded Apollo butterfly (*Parnassius mnemosyne*)]**, Magyar Etológiai Társaság XVII. Konferenciája [17th Annual Conference of the Hungarian Ethological Society], Dobogókő, 2015.

Vajna F., **Szigeti V.**, Kis J. **Virágmélység és pödörnyelvhossz kapcsolata a kis Apolló-lepkénél (*Parnassius mnemosyne*) [Relationships between flower depth and proboscis length in the Clouded Apollo butterfly (*Parnassius mnemosyne*)]**, 10. Magyar Ökológus Kongresszus, Veszprém, Absztrakt-kötet [Abstract of 10th Hungarian Congress of Ecology, Veszprém], 157, 2015.

Szigeti V., Kőrösi Á., Harnos A., Nagy J., Kis J. **Kis Apolló-lepkék (*Parnassius mnemosyne*) táplálkozási mintázata [Foraging patterns in the Clouded Apollo butterfly (*Parnassius mnemosyne*)]**, IX. Magyar Természetvédelmi Biológiai Konferencia Absztrakt-kötet [Abstracts of IX. Hungarian Conservation Biology Conference], 119, 2014.

Szigeti V., Kőrösi Á., Harnos A., Bella M., Kis J. **Kis Apolló-lepkék élőhelyhasználata lárvális tápnövényük és nektárforrásuk függvényében [Habitat use, larval host-plant and nectar-plant distribution in the Clouded Apollo butterfly]**, IX. Magyar Természetvédelmi Biológiai Konferencia Absztrakt-kötet [Abstracts of IX. Hungarian Conservation Biology Conference], 118-119, 2014.

Szigeti V., Kőrösi Á., Harnos A., Nagy J., Kis J. **Nectar-plant flowering and consumption in the Clouded Apollo butterfly (*Parnassius mnemosyne*)**, Behaviour 2013, The Sage Newcastle-Gateshead UK, Abstract Book, 156-157, 2013.

Szigeti V., Danka C., Nagy J., Kőrösi Á., Kis J. **Nectar-plant choice and flowering dynamics in the Clouded Apollo butterfly (*Parnassius mnemosyne*) in a small colline meadow – a pilot study**, 2nd Global congress on plant reproductive biology, Pécs, Conference Proceedings, 65, 2012.

Szigeti V., Danka C., Nagy J., Kőrösi Á., Kis J. **Potential and used nectar plant resources and their flowering and consumption dynamics in the Clouded Apollo butterfly *Parnassius mnemosyne* in a small colline meadow – a pilot study**, Kitaibela, 143, 2012.