Hungarian University of Agricultural and Life Sciences

Assessment of honey bee and wild bee communities and investigation of competition between them in a high nature value landscape with traditional cultivation

DOI: 10.54598/003450

PhD thesis booklet

Demeter Imre

Gödöllő
2023

The doctoral school

Name: Doctoral School of Biological Sciences

Discipline: Biological sciences

Head of school: Dr. Nagy Zoltán
Head of department, DSc
Hungarian University of Agriculture and Life Sciences
Institute of Agronomy,
Department of Plant Physiology and Plant Ecology

Supervisor 1: Dr. Sárospataki Miklós
Associate professor, PhD
Hungarian University of Agriculture and Life Sciences
Institute for Wildlife Management and Nature Conservation
Department of Zoology and Ecology

Supervisor 2: Dr. Balog Adalbert
Associate professor, Vice rector, PhD
Sapientia Hungarian University of Transylvania
Department of Horticulture

……………………………………… ….……………………

Approval of the head of school Approval of the supervisor

BACKGROUND AND OBJECTIVES

Pollinators provide key services to both crop and wild plants, but they are also sensitive to agricultural intensification and the loss or conversion of high nature value habitats. Maintaining healthy pollinator communities is critical for food security. By some estimates, pollinating insects provide a global service to food production worth nearly $210 billion. In addition, with more than 87.5% of flowering plant species worldwide benefiting from animal pollination, conserving pollinators is also essential to the conservation of wider biodiversity.

The most important group of pollinators are bees, which visit and pollinate more than 90% of the world's 107 leading crops. More than 20,000 bee species have been described worldwide, of which nearly 50 species are bred and about 12 species are commonly used for plant pollination, such as the western honey bee (*Apis mellifera*), the eastern honey bee (*Apis cerana*), some bumble bee species, stingless bees, and solitary bees. Pollination by bees has many benefits for humans, such as providing a reliable and diverse supply of seeds and fruit, maintaining wild plant populations that underpin biodiversity and ecosystem functioning, producing honey and other bee products, and supporting cultural values. It is also a proven fact that the presence of wild bees in crop production is important even when the presence of honey bees is high. Indeed, wild bee communities often prove to be more efficient pollinators than honey bees, and interactions between species can increase pollination efficiency. Diverse bee communities provide a high quality and stable supply of pollination services, but recent intensification of agriculture has greatly reduced the diversity and numbers of wild bee communities.

In pre-industrial agriculture, high nature value grasslands provided the nutrient base for production by providing fodder for animals, which in turn provided the natural fertiliser needed for crop production. These natural grasslands are among the most species-rich ecosystems in the world, as they are mainly hay meadows and pastures with spontaneously established vegetation, i.e., not intensively cultivated and not fertilised. In these areas, mild anthropogenic disturbance (usually in the form of optimum grazing or mowing) is essential, or at least very important, for maintaining habitats. In addition to maintaining plant species richness, high nature value grasslands are essential habitats for pollinators, as high plant diversity helps to provide pollinators with a continuous resource throughout the season.

Pollinators can also feed on crops, but only for a brief period of the season. For the rest of the year, they rely on the surrounding high nature value habitats for vital life functions such as feeding, sheltering, nesting, breeding, and wintering sites. In intensively cultivated agricultural landscapes, only small fragments of such high nature value habitats remain, typically as linear elements such as field boundaries and roadsides. However, such small areas of uncultivated habitat fragments often contain impoverished fauna compared to larger grassland areas. It is important to understand the role of local and regional factors in controlling insect species richness and abundance in these habitats if viable pollinator populations are to be maintained in farmland. The survival of viable pollinator populations on farmland is highly dependent on the preservation of high nature value habitats in agricultural landscapes, which are otherwise subject to repeated anthropogenic impacts.

One of the main drivers of the decline of wild bees is linked to changes in land use, loss of natural and high nature value areas, loss of nesting and feeding sites and loss of main flower sources. The fragmentation of high nature value areas and their intensive cultivation leads to the fragmentation of habitats, and changes in land use result in poor, homogeneous landscape structures that can affect the size and permeability of the remaining habitat patches. All this can reduce the gene flow between pollinating populations in the short term, but in the long term it can have an impact on the persistence of the population. The ever-increasing use of agents for plant protection weakens the bees' immune response, making them more susceptible to parasites and diseases. The number and diversity of wild bees has decreased significantly in recent years, as these species find it difficult to adapt to harmful environmental effects.

Despite environmental threats and economic difficulties affecting the sustainability of beekeeping, the number of honey bee colonies (*Apis mellifera L.)* worldwide has increased by 45% in the last half century. As a result of intensive agricultural land use, agricultural areas are less suitable for sustainable honey production, so professional beekeepers regularly migrate large beehives to high nature value areas, either to exploit the flourishing resources, or to avoid the dangers of agricultural chemicals, or to avoid periodic food. While honey bee species (*Apis spp.)* very rarely behave aggressively towards other bees, their advanced social systems, large colony sizes and high hive densities often raise concerns about potential resource competition with other pollinators. This is especially true in areas where the western honey bee has been introduced.

Since both honey bees and wild bees feed on nectar and pollen, it is not a new concern that there might be competition for food sources between honey bees in large numbers and wild bees. Interspecific competition can occur when species utilize the same limited resources, and this type of competitive effect is common in plant and animal communities. In natural populations, competition between species over evolutionary time has led to niche differentiation, sometimes observed as character displacement, which minimizes niche overlap between species and thus the potential for competition. At the same time, the intensive use of *A. mellifera* by humans and its explosive population growth (also managed by humans) do not allow this type of niche segregation processes to take place, and thus the competitive effects on wild bee communities can have serious negative consequences in the long term.

In recent decades, new farming systems and techniques have been developed to mitigate the above-mentioned impacts through organic farming and agri-environmental protection systems (AES). At the same time, compared to the above-mentioned pollination crisis, there have been few studies examining the structure and abundance of wild bee communities. This is especially true in Eastern Europe, where thanks to traditional farming, high nature value grasslands have extremely rich wild bee communities. In the future, the thorough study and knowledge of these areas can help in the restoration of other Western European areas, where species-rich habitats like this have almost completely disappeared due to intensive agriculture.

Objectives

Numerous studies of developed countries with intensive agriculture draw our attention to the continuous decrease in the diversity of wild bees. In contrast, few studies examine extremely rich, high nature value, traditionally managed grasslands. Such is the case in many areas of Transylvania, where, despite the rich wild bee fauna, very few surveys have been conducted in the recent period. For this purpose, we began to investigate the diversity and number of wild bees, as well as the competition between honey bees and wild bees in three high nature value areas in Transylvania (Romania). Here we still found habitats where the anthropogenic impact, the number and density of apiaries are low, but at the same time the species richness of the vegetation on the mowing fields is extremely high.

The aim of our study was to:

- assess the wild bee communities living in the examined high nature value areas.
- map rare and faunistically interesting species.
- compare the bee communities of the three areas to have an understanding how different human presence affects these communities.
- demonstrate the effect of honey bees on wild bees, considering the distance from the hives.
- examine whether the abundance and diversity change differently depending on the distance from honey bee hives, if small and large bees are examined separately.

MATERIALS AND METHODS

Description of the study areas

Our research was carried out in 2018 and 2019 in Central Romania (South-eastern Transylvania), in high nature value areas, where the diversity and number of wild bees is expected to be high due to the significant plant species richness, but honey bees (*Apis mellifera*) have at most wild populations present, since artificially maintained apiaries do not occur, or occur only sporadically. In the investigated region, in the counties of Hargita and Kovászna, traditional, extensive farming is still taking place. In the three selected sampling areas, the average altitude above sea level is 530-630 m. The study areas, being located relatively far from the villages, have quite well-preserved high nature value habitat complexes consisting of a mosaic of grasslands and forest-shrub patches. These sites are located relatively close, but they are well separated from each other by the wooded, rocky hills. The lawns are mainly used as mowing grounds, the size of the plots usually does not exceed one hectare. Mowing the mosaic lawn patches takes place at various times, thereby providing a continuous source of food for the pollinators. The investigated mowing fields and lawn patches are part of a valley in all three sampling areas. Although all three valleys are high nature value habitats, different anthropogenic effects can be observed. Some differences can be observed in the diversity and abundance of plants at the three locations, but the plant species composition shows significant overlaps.

One of the characteristics of traditional farming in this region is that more intensive farming is typical only in areas closer to villages. Specifically, this means that land use that negatively affects diversity, such as extensive fertilization or mowing more than twice a year occurs only in areas close to villages The hay harvested from these more intensively used areas in the vicinity of the settlements is largely sufficient to feed the livestock. Choosing the time of mowing and diversifying it at the habitat level is particularly important from the perspective of preserving the biota, since plants with a flowering period after the time of mowing cannot reproduce sexually, so these species do not offer food for bees. Areas further away from the settlement are usually mowed only once a year, which means that the diversity of flowering plants is much higher in these areas due to extensive land use.

Additional reasons for the greater anthropogenic impact of areas closer to the settlement may be poor infrastructure (areas closer to villages are easier to access); protection of areas (areas closer to villages can be more easily protected against damage caused by wildlife); and traditional farming (currently this is less common, but until the beginning of the 21st century, many of the farmers still used horse-drawn carts for harvesting).

We therefore selected three sampling areas in this region. Vargyas-szoros (46.2034539, 25.5344264) is a nature reserve, located farthest from human settlements, characterized by mowing fields and patches of forest. The area of Homoródalmás (46.2394164, 25.5322366) is located at a medium distance from human settlements and is characterized by mowing fields, pastures, and patches of forest. The Erdőfüle area (46.1731241,25.6236372) is the closest to human settlements, it is characterized by mowing fields, patches of forest and little arable land. In the following, we will refer to the three research areas with the following abbreviations, "V" Vargyas, "A" Homoródalmás and "F" Erdőfüle.

Sampling of bees along a transect

At all three research sites, we took samples four times a year (1 time in May, 2 times in June, 1 time in July) in two consecutive years (2018-2019). In the sampling area close to the Erdőfüle settlement, a beekeeper with his hives was already present before the start of the study, while in the other two areas, our own hives were placed in the two years of the study, at the end of May, after the first field survey.

In each test area, 3 sampling points were designated at a distance of 250, 500, and 1500 m from the placed beehives in 3 repetitions, that is, 9 sampling points belonged to one sample area. Species-rich mowing meadows were selected as the sampling locations, and two people performed the sampling at the 9 sampling points in a randomly set order. At each sampling point, two people at the same time carried out the collection for 20 minutes, continuously walking along a 200 m transect (a pre-designated straight route). At the sampling points, sampling was done in random order, thus avoiding that sampling always takes place at the same time of day on a given transect. One observer was constant both years (the author conducted the surveys), the other observer changed. During sampling, we caught all observed individuals with a butterfly net and preserved them in 70% alcohol. The exceptions were the individuals that we could precisely identify locally (honey bees and some bumble bee species), we did not catch them, we just recorded them. The individuals preserved in alcohol were defined to species level by expert taxonomist Zsolt Józan.

Sampling of Hymenopterans with trap nests

In 2018 and 2019, we placed four trap nests on each of the areas connected to all nine sampling locations. All trap nests were uniquely coded with reference to locations and placed 250, 500 and 1500 m from the hives. The trap nests were made of PVC pipe with a diameter of 12 cm and a length of 23 cm. The tubes were filled with pieces of common reed stems (*Phragmites australis*), which were cut to a length of about 22 cm in such a way that each reed fibre contained a node. We placed the reeds tightly next to each other in the tubes so that they would not fall out. The pipes were placed on trees or shrubs 1-2 m above the ground surface. The reed nests were collected at the end of August 2018 and 2019 and stored outdoors in a shady place. In January 2019 and 2020, the nests were placed in a refrigerator and stored at 4-7°C. In both years, we started collecting data from reed fibres in January. To do this, we cut open each reed, and if we found a nest in it, we recorded it with the unique code of the trap nest and referring to a serial number. Thus, each nest received a unique identification code. The following parameters were recorded in each colonized reed fibre:

- the diameter of the reed.
- the number of occupied brood cells in the nest – empty cells were also counted, but not used in further analyses.
- type of nesting material
- colour of larvae or cocoons (if present)
- the type of food accumulated in the brood cell to feed the offspring, whether it was a nectar-pollen mixture or paralyzed arthropods (usually spiders).

In addition to these parameters, we also counted all the reeds per trap nests. Based on the results, we were able to identify seven groups of nest types. From the 2018 nests, we took several samples (at least two) from each of the seven groups, which we raised at room temperature. In 2019, however, we reared individuals at a much higher rate (10-20 per nest type). After these samples had developed, at least two samples were collected from each nest sample and stored in 70% ethanol. The specimens obtained in this way were determined by a taxonomist, who found the following genera*: Ancistrocerus*, *Auplopus*, *Dipogon*, *Hylaeus*, *Megachile*, *Osmia*, *Symmorphus*, and *Trypoxylon*. Except for the two genera *Ancistrocerus* and *Symmorphus* of the subfamily *Eumeninae* (pot wasps), which could not be distinguished based on the type of nest, each genus was assigned to one nest type. Therefore, based on this information, we distinguished three taxa of solitary bees and four taxa of predatory wasps and determined the corresponding genus name. In both years, if we found spider prey in the nest, they were collected, placed in 70% ethanol, and marked with the unique code of the nest. Spiders were then taxonomically defined at species level where possible (but at least at family level).

Statistical analysis

We divided the collected wild bee species into two groups, bumble bees and other bees (the latter are called small bees hereafter), as they can show significant differences in terms of social behaviour and home range size. Bees with a larger body size can collect food from significantly greater distances than smaller ones. Since the feeding/flying distance and related body size can play a significant role for the purpose of our study, we only used the division according to body size when analysing the results, we did not use the otherwise usual groupings based on food specificity or social behaviour.

Diversity indices (Shannon, Simpson) and diversity profiles were calculated for both groups separately and for the entire wild bee community. The endangered status of the species was determined based on the European Red List. To characterize the diversity of wild bee communities, in addition to species and individual numbers, we also compared the species composition and dominance relationships of the areas. Dominant and subdominant species were those whose dominance, relative to the total material collected, was above 1% and 0.5%, respectively. To compare bee communities, we calculated pairwise Jaccard similarity indices between areas.

The number and abundance of species were compared between the individual areas depending on the years and the distances from the beehives. The distribution conditions of the data were checked with the Shapiro–

Wilkinson test. Since the samples did not show a normal distribution, Mann-Whitney and then Tukey pairwise comparisons were performed on them. The density of honey bees, large bees and small bees was compared using the same method between sites at all distances (250, 500 and 1500 m). The diversity profiles of each distance were plotted, and the diversity pair between years was analysed with a T-test.

The Kruskal-Wallis test was used to compare the abundance of wild bees in the periods before the placement of the hives (sampling period 1) and after the placement of the hives separately, for all distances (250, 500 and 1500 m). Comparisons were made with PAST version 4.02.

The effect of the density of honey bees on small and large bees was investigated by area and year depending on the distance from the hive using the MANOVA test. The interactions were compared using the chi-square (χ^2) test, considering the differences between the covariance matrices, and then standardizing them with the mean squared error of approximation. The initial comparison was made between the density of honey bees and the density of small and large bees for each distance separately. Due to the small number of endangered species, the comparisons were made without statistical analyses, the differences were only shown according to the species and number of individuals. Statistical analyses were performed in R version 3.0.1 (R CORE TEAM, 2012). Canonical correspondence analyses were used to examine the effect of years and sites on species composition of wild bee communities. In these cases, sites and years were used as components, and the number of species as variables. Species composition was also analysed based on site and distance from hives. Analyses were performed in PAST version 4.02.

RESULTS

Survey of wild bee communities, 2018

During the 2018 collection period, we detected a total of 1,882 individuals of 129 wild bee species in the three areas. The collected material contained 12 bumble bee species (1049 individuals) and 117 other wild bee species (833 individuals). According to the European Red List of the IUCN, one species is EN Endangered, 11 species are NT Near Threatened, and 24 species are DD Data Deficient, the other species are LC Least Concern.

The number of species and individuals was the highest in area V, and the number of unique species (species found only in the given area) was also the highest here.

The diversity profiles of the areas are remarkably similar, they intersect, so we cannot talk about real differences in diversity. Only in the case of the diversity profiles calculated for bumble bees, the curves do not cross, but here too they run very close to each other.

The number of species overlapping between pairs of areas (which occurred exclusively in the given two areas) was almost the same in areas A–F and A–V, while significantly fewer common species were found in areas F–V. The values of the Jaccard similarity indices also showed that the similarity between the F–V areas was the smallest, while the A area was not significantly different from either the V or the F areas.

In the nest traps placed in 2018 and processed in 2019, we discovered 1,070 nests in 5,442 reeds. The utilization of the nests ranged between 13-30% on average, which can be said to be average compared to similar research (20%). The number of nests built by small bees (126 nests) was relatively low compared to nests built by solitary wasps (944 nests). The most nests were built by *Hylaeus* species (81), followed by *Osmia* (23) and the fewest nests

(22) by *Megachile* species. Most of the nests were inhabited by solitary wasps, and even within them *Trypoxylon* was the dominant species (575). Species belonging to the subdominant *Dipogon* genus built 191 nests.

Survey of wild bee communities, 2019

During the 2019 collection period, we detected a total of 1,724 individuals of 87 wild bee species in the three areas. The collected material contained 11 bumble bee species (1338 individuals) and 77 other wild bee species (386 individuals). According to the European Red List of the IUCN, 6 species are NT Near Threatened, and 18 species are DD Data Deficient, the other species are LC Least Concern. In 2019, the number of species was the highest in area F, and the number of unique species (species found only in this area) was also the highest here. At the same time, as in the previous year, the number of individuals was also the highest in area V.

In 2019, the diversity profiles of the areas are also very similar, they intersect, so we cannot talk about big differences in diversity now either. Some differences can be observed in the diversity profiles calculated for wild bees and bumble bees. When studying the wild bees together, the F area turns out to be more diverse compared to the other two areas. In the case of bumble bees, the diversity of area A was lower compared to the other two areas.

The number of overlapping species between pairs of areas (occurring only in the given two areas) was the highest in areas A–F, slightly lower in areas F–V, while significantly fewer common species were found in areas A–V. Jaccard similarity index values showed that the highest similarity was between areas F–A, while area V showed slightly lower similarity with both areas F and A

In the trap nests placed in 2019 and processed in 2020, we discovered 1,073 nests out of 3,715 reeds. The utilization of the nests ranged between 13-

54% on average. In the trap nests placed in 2019, we found 5 bee species (*Hylaeus difformis, Hylaeus annulatus, Megachile centuncularis, Osmia caerulescens, Osmia leaiana*), of which the first 2 species were not caught in 2018 and 2019 using sweep net method. Based on these, we can say that we detected a total of 159 wild bee species in our study areas during the two years using the two sampling methods.

Community structure parameters based on the aggregated data of the two years

During the collection period of 2018 and 2019, a total of 3,606 individuals of 157 wild bee species were observed in the three areas. The collected material contained 13 bumble bee species (2387 individuals) and 144 other wild bee species (1219 individuals). According to the European Red List of the IUCN, one species is EN Endangered, 12 species are NT Near Threatened, and 35 species are DD Data Deficient, the other species are LC Least Concern.

Summing up the data of the two consecutive years, the number of species was the highest in area F, and the number of unique species (species found only in this area) was also the highest here. Both Shannon and Simpson indices showed very little variation between areas in both years.

The diversity profiles of the areas are also very similar and intersect, so we cannot speak of major differences in diversity even based on the aggregated data of the two years. Only in the diversity profiles that consider all wild bee species, some deviations can be observed, according to which the F area is more diverse than the other two areas.

The number of species overlapping between pairs of areas (occurring only in the given two areas) was almost the same in the F–V and A–V areas, while significantly more common species were found in the F–A areas. The values of the Jaccard similarity indices showed that the similarity between areas F–A was the lowest, while area V was almost identical to areas F and A.

Between the two years, we experienced a significant species exchange. The number of species detected in both years was 36 in area F, 30 in area A, 31 in area V, and a total of 59 species in the three areas. From 2018 to 2019, 43 species were below the detection threshold from areas F and A, 51 species from area V and 70 species in total from the three areas. At the same time, from 2018 to 2019, we detected 29 new species in area F, 18 species in area A, 12 species in area V and 28 species in all three areas.

Competition between wild bees and honey bees

During the two recording years, we observed a total of 158 species and 13,164 individuals, of which 72% (9,542) were *Apis mellifera*. The dominant bumble bee species were *Bombus humilis* (889 individuals), *Bombus terrestris* (874 individuals), *Bombus pascuorum* (225 individuals), *Bombus hortorum* (174 individuals), *Bombus ruderarius* (79 individuals) and *Bombus sylvarum* (67 individuals), and the dominant small-bodied bees include *Andrena flavipes* (152 individuals), *Andrena ovatula* (81 individuals), *Lasioglossum calceatum* (67 individuals), *Eucera nigrescens* (55 individuals), *Eucera longicornis* (53 individuals) and *Halictus tumulorum* (44 individuals).

In 2018, we observed a significant difference in abundance between the first sampling period (before hive placement) and the other sampling periods (after hive placement) at 500 m and 1500 m from the hives in area A and at 250 m from the hives in area V. There is also a difference in abundance at 250 m between the third sampling period and the first and second sampling periods in sampling area F, and at 1500 m between the fourth sampling period and the first and second sampling periods. The same differences can also be detected in the diversity profiles and diversity indices. At the same time, we cannot draw clear conclusions about the competition based on these results, rather these results show the seasonal changes of the communities.

A significant difference between the years can be detected if the species richness of small and large bees is examined together at different distances from the hives. From 2018 to 2019, we experienced a significant decrease in the number of species at sites A and F at a distance of 500 and 1500 m, respectively. In area V, however, there was a significant decrease in the number of species in all three distances (this does not mean that the given species disappeared from the area, but it did not occur in detectable quantities).

Looking at the numbers of individuals, we also found significant differences between years in several areas. From 2018 to 2019, the abundance decreased significantly in area A at a distance of 1,500 m, in area V at a distance of 500 m, and in area F at a distance of 1,500 m. At the same time, abundance increased in area A at a distance of 250 m, and in area F at a distance of 250 m and 500 m.

The changes in wild bee populations within the same years, taking into account the distance from the beehives, were the following separately in each location:

Differences in the number of species depending on the increasing distance from the beehives, broken down by years:

2018: minor increase in area V, no change in areas A and F.

2019: no change in area V, small decrease in area A, strong decrease in area F.

Difference in abundance according to increasing distance from beehives, divided into years:

2018: growth is observed in all areas (sturdy growth in area A and minor growth in areas V and F).

2019: no change in area V, a decrease can be observed in areas A and F

The changes in wild bee populations between the two recording years, taking into account the distance from beehives in each location separately, were as follows:

Differences in the number of species depending on the increasing distance from the beehives between years:

The area A: 250 m no change; 500 m decrease; 1500 m decrease.

The area V: 250 m no change; 500 m decrease; 1500 m decrease.

The area F: 250 m no change; 500 m decrease; 1500m decrease.

Difference in abundance according to increasing distance from beehives between years:

The area A: 250 m decrease; 500 m no change; 1500 m decrease.

The area V: 250 m no change; 500 m decrease; 1500 m no change.

Area F: 250 m increase; 500 m no change; 1500 m decrease.

There was a notable change in species richness and species composition, as well as in abundance from one year to the next. The percentage of species replacements in the sampling areas was as follows: 67% in area A, 66% in area V and 63% in area F. Depending on the distance from the hives, we observed a significant decrease in diversity from one year to the next at all distances, except for site F, 250 m from the hives.

In 2018, the species richness of small bees increased significantly in area V as the distance from the hives increased, while no similar increase was observed in areas A and F (MANOVA and Chi square test). At the same time, in 2019, the species richness of small bees decreased as the distance from the hive increased, i.e., the trend was opposite for all three areas. Furthermore, in 2019, the number of small bee species in the study areas was significantly lower compared to 2018.

In 2018, we experienced a significant increase in the number of small bees in areas A and V as the distance from the hive increased, but we did not experience a similar increase in area F. At the same time, in 2019, the number of small bees decreased as the distance from the hives increased, i.e., the trend was opposite for all three areas. Furthermore, the number of small bees in the study areas was significantly lower in 2019 compared to 2018.

Examining bumble bee species separately, the differences are not as spectacular as in the case of small bees. In 2018, the number of bumble bees in areas A and F increased as the distance from the hive increased, while in area V the number of bumble bees decreased, i.e., the trend was the opposite. In 2019, the number of bumble bees increased significantly in area F as the distance from the hive increased, there was no meaningful change in area V, and the number of bumble bees decreased significantly in area A.

Overall, the data show that honey bees can generally have a negative effect on both small-bodied and bumble bees, as well as endangered species, but if we consider years and distances from hives as explanatory variables and species group as response variables in the analyses, then there is a significantly negative effect can only be detected on small bees.

Canonical correspondence analyses again showed a large difference in species composition between the years when areas were considered as a grouping factor.

DISCUSSIONS

The importance of wild bee communities in high nature value habitats

Our investigation brought important and interesting results from a faunistic point of view. In recent faunistic works on bumble bees in Romania, no faunistic data from the region of Hargita and Kovászna counties were found. At the same time, the information obtained on the species on the European Red List is particularly important, since new data emerging on both endangered and data-deficient species can be irreplaceable from a nature conservation point of view. In 2018 and 2019, 5 of the 12 bumble bee species classified as NT were found only in 1 specimen each, but the occurrence of the other 7 NT species turned out to be more frequent, and there were even some that were dominant (*Andrena ovatula*, 81 individuals) or subdominant (*Andrena hattorfiana*, 19 individuals) species in our study.

Based on the faunistic evaluation of the data from our field recordings, we can say that compared to the literature data, the species richness of our sampling areas was remarkably high. In 2018 and 2019, we collected a total of 24 *Megachilidae*, 39 *Andrenidae*, 23 *Anthophoridae* and 13 *Apidae* species, and the total number of species was 157. We collected about 22% of the 726 wild bee species registered in Romania and 33% of the 40 Bombus species in our study areas.

Examining the consequences of the different anthropogenic effects between the sample areas, we found that the comparison of species and individual numbers, as well as diversity values and profiles, did not show large or consistent differences between the sample areas. Based on this, it can be said that the small difference between the anthropogenic impacts on the areas was not enough to cause significant differences in the number of species and diversity. However, the differences in anthropogenic effects, although not strong, can be detected based on the similarities/differences in the species composition. Based on the results of the first year, the two areas with the most different anthropogenic influence (V–F) showed the least similarity in species composition, even though they were the closest to each other. At the same time, the 2019 data differ significantly from the 2018 data. Thus, it can be seen from the two-year data that the A-F area shows the least similarity in the species composition. Our studies indicate that the extensive use of the investigated areas enables the development of diverse, species-rich bee communities, even at our sampling site closest to human settlements. Monitoring the bee communities of these and similar meadows can provide useful data for the preservation of these grasslands of high natural value.

The effect of honey bees on wild bee communities in species-rich high nature value grasslands

A number of studies investigating the competition between wild bees and honey bees report negative effects of honey bees. In our studies, the effect is not clear when looking at the entire wild bee community. In the first year of the study, we observed much lower numbers of species and individuals near the hives in almost all cases. This negative trend was more pronounced in the two areas where honey bees were placed only in the study year (areas A and V). No similar trend was observed in the second year. The negative effects of honey bees may pose a wider risk to bee communities where honey bees are not native. European wild bee communities are probably more tolerant of honey bee invasion, so it is conceivable that they were able to compensate for the negative impact of honey bees placed in the area from one year to the next at the community level. In areas where honey bees are native, competition does not always occur, as niche overlap is insufficient for effective competition for a food source, or resource scarcity does not occur to a degree that results in detectable competition.

Considering the species richness of large and small bees separately, a significant decrease in the number of species can only be detected for small bees, not for large bees. Honey bees tend to have a more significant impact on generalist bees than on oligolectic bees. In the control area we examined (area F), where 60 families of honey bees have been regularly resettled during the season for years, the number of bumble bees was 70% lower in 2018 and 48% lower in 2019 compared to the other two study areas. The honey bees that have been present in area F for a long time and in larger numbers were therefore most likely able to exert a greater effect on bumble bees, reducing their number and causing bumble bees to avoid areas where the number of honey bees is high, and thus the lack of a food source or interference is also significant. In addition to the directly perceptible competitive effects, competition with honey bees can also have effects that, through the reduction of body size and the resulting change in fecundity, only have an actual effect on the bumble bee community in the long term, over several years. This means, based on the data of our own investigations, the cautious conclusion can be drawn that the direct effects of the mass appearance of honey bees on bumble bees in such species-rich habitats are not immediately noticeable, but in the longer term they would probably be detectable, especially if the weight gain of the bumble bee colonies and the body sizes of the individuals could also be recorded.

The species exchange and decrease in the number of small bees may be the result of competition, but confirmation of this would require further research. Bee foraging distance is related to body size. The maximum movement range of solitary wild bees, which are smaller than honey bees, is between 150 and 600 m. In contrast, honey bees can travel several kilometres. In the case of small bees, even if their numbers do not decrease near apiaries, low nectar and pollen levels can have a negative effect. The body size of the adult bee is directly determined by the amount of pollen and nectar consumed by the larva. Smaller offspring are more likely to die during development and hibernation. Smaller individuals are less likely to find a nesting place. Low levels of food sources can increase the number of parasites in the nest, as females spend more time foraging and therefore leave nests unattended for longer periods of time. Furthermore, smaller bees require less energy to fly and maintain nesting sites. In addition, small bees require less pollen and nectar to raise their offspring. In areas with large apiaries, the amount of pollen and nectar may be sufficient for small bees, but not for large species. In this case, they are forced to look for sufficient food somewhere further away, or to broaden their food spectrum to other plants, and thus the structure of the wild bee community near the hives may also change.

In April and May 2018, it was significantly warmer and there was less rainfall than in the same period in 2019, which could have significantly increased the competition effect due to scarcity of floral resources.

Based on these, it can be seen that the effects of competition are not always effective in the short term, often the consequences only appear after a longer period. Based on what was described above, it is conceivable that this species and population decline are the result of competition with honey bees. Based on the literature described above, it is possible that this species and population decline is a consequence of the honey bee competition and the unusual weather synergy. At the same time, further, preferably longer-term research is needed to clearly state that all of this is due to the competition effect.

Studies that examined competition as a function of honey bee density (e.g., by distance from hives) found that competition was strongest near honey bees (usually within 800 m). Minimal or no effect was observed with increasing distance, suggesting that the effect of honeybees may be local.

Among the results of our study, we could not show a significant effect on the number of individuals or the number of species depending on the distance. The degree of competition, and thus its direct effects, may depend on the availability of resources, such as nectar quantity and quality (sugar content). Significant impacts can occur where resources are scarce, for example in homogeneous, intensively used landscapes. At the same time, competition may have insignificant effects when abundant resources are available or heterogeneous landscapes are examined. In addition to the heterogeneity of the landscape, the distance dependence of the effect of beekeeping can also vary seasonally depending on the number of flowering plants in the area. Our study areas are characterized by great heterogeneity, and due to extensive pasture and mowing, the naturalness of the areas and the richness of plant species are quite high. It is probably due to this that the direct, short-term effects of the competition, manifested in a decrease in the number of individuals and species, are not noticeable in our results. At the same time, it is important to know that the mass presence of honey bees and the competition with them may not only have short-term effects on solitary wild bees. Henry and Rodet found an average reduction in body length of 12% for wild bees located within 0.65 km of honey bee hives, and average weight of wild bees was 33% lower.

In general, examining the impact of honey bees on wild bees through diversity and community structure, we saw a drastic decrease from one year to the next (i.e., diversity values varied by 63-67% between years, depending on the location). Recent studies have investigated the potential for honey bee-wild bee competition in areas where *Apis mellifera* is native and where it is not. Although a comparison is not possible due to the different habitats, the conclusion is that large-scale beekeeping generally causes foraging competition between species and reduces the diversity of wild bee species and local populations of wild bees. At the same time, compared to our own results, apart from the decrease in the number of species between years, we could not detect any other clear short-term negative effects on wild bee communities in the immediate vicinity of honey bees. On the one hand, this may be due to the high heterogeneity, naturalness, and plant species richness of the study areas, as well as the quantity and quality of nectar available, which all may reduce the competition effect. On the other hand, it may also be because the honey bee is considered to be native in the investigated areas. It is easy to imagine that the impact on the wild bee community in such areas would only be felt in the long term, over several years. At the same time, the relatively small colony of bees placed for the experiments was probably not enough to exert a significant competitive effect. According to several studies, small apiaries have practically no effect, and can create a competitive situation much less. At the same time, some conservationists advocate a complete ban on beekeeping in nature reserves. It would be necessary to summarize these semi-contradictory positions and results in such a way that a compromise solution could be developed, taking into considering the interests of both nature conservation and farmers (beekeepers). For example, instead of completely banning beekeepers from protected areas, the number of hives that can be placed in a given area could be limited. According to certain studies, e.g., the hive density of 3.1 bee colonies/km^2 and 3.5 bee colonies/km^2 does not yet cause significant changes in wild bee communities.

Applying the appropriate hive density in a given area is extremely difficult, as current apiaries often consist of 100-200 bee colonies, and honeybees can fly up to several kilometres. All of this can be influenced by the density of wild pollinators, as well as the fact that in most cases nature reserves are not homogeneous, and the available flowering plants change from season to season and from year to year. Therefore, it would be important to examine whether the relocation of larger apiaries (100-200 families) has similarly mild consequences. With such tests, it would be possible to determine the approximate amount of honey bee load in nature conservation areas that the wild bee communities living there can still tolerate.

NEW SCIENTIFIC RESULTS

- We conducted a survey of bee fauna and bee community structure in traditional, under extensive land use high nature value areas in the part of Transylvania where bee fauna surveys had not been conducted before. Based on our results, it can be said that the slight difference in anthropogenic effects between the three study areas does not clearly explain the observed differences.
- Based on our results, the traditional, extensive pasture/mowing cultivation used there enables the formation of diverse, species-rich bee communities even in the sampling locations closest to human settlements.
- We showed a significant difference in species richness and number of individuals between the two study years, as well as high values of species exchange.
- The competitive effect between the honey bees placed in the study areas and the wild bee communities living there was not clear, and there were differences between the years.
- A more significant competitive effect was observed only in the case of small bees, which was manifested in the fact that there was a significant decrease in the number of species and individuals from one year to the next.
- The number of bumble bees in area F (Erdőfüle) was lower compared to the other areas, which may be a consequence of the long-term competitive effect.

PUBLICATIONS RELATED TO THE TOPICS OF THE THESIS

Demeter, I., (2018). A háziméhek és a vadméhek közötti kompetíció lehetősége és következményei. *Határhelyzetek XI.: Diszciplínák találkozása – nyelvek és kultúrák érintkezése Budapest, Magyarország: Külgazdasági és Külügyminisztérium* 570 p. pp. 376-393.

Demeter, I., Balog, A., Józan, Z., Sárospataki, M. (2021). Comparison of wild bee communities of three high nature value meadow habitats at Harghita–Covasna Region, Transylvania, Romania. *Acta Zoologica Academiae Scientiarum Hungaricae*, *67*(2), 161-175. https://doi.org/10.17109/AZH.67.2.161.2021 *IF:* 0,923

Lajos, K., **Demeter, I.**, Mák, R., Balog, A., Sárospataki, M. (2021). Preliminary assessment of cavity-nesting Hymenopterans in a low-intensity agricultural landscape in Transylvania. Ecology and Evolution, 11(17), 11903-11914. https://doi.org/10.1002/ece3.7956 *IF:* 2,91

Demeter, I., Balog, A., Sárospataki, M. (2021). Variation of small and large wild bee communities under honey bee pressure in highly divers natural habitats. Frontiers in Ecology and Evolution, 846. https://doi.org/10.3389/fevo.2021.750236 *IF:* 4,171