3rd Symposium on the Biology of Non-Weedy Parasitic Plants

Namur, Belgium, 12-15 September 2013

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The organising committee would like to thank FNRS, the Fund for Scientific Research, for financial support, Brigitte Ferauge for making the badges and Grégory Mahy for organising and guiding the excursion.

General information

The venue
Please respect the house rules: all builings are non-smoking, and noise is not allowed after 22h00 in or near the bedrooms.
The Wi-Fi network of the youth hostel can be used free of charge.
Breakfast is from 7:30 until 9:45.

The excursion on Saturday
We will use the available cars plus some extra vehicles to transport you to several sites with calcareous grasslands and other habitats along the river Meuse. To gain time, we will not lunch in the restaurant, but we have ordered a packed lunch for everyone, which includes sandwiches, a cold salad, a snack, fruit juice and water. You decide when you eat your own lunch, before we leave or during the excursion, but make sure to bring part of it along with you, especially something to drink and maybe a snack or so.

On departure
You should vacate your bedroom before 10:00 on Sunday. We also would like to re-use the badges, so there will be a box where you can leave your badge before leaving, but you can of course keep the piece of paper with your name on it.

Getting to the railway station on Sunday
On Sunday, bus 3 has no service, and bus 4 only goes once an hour, at :03 from “Marronnier” and at :04 from “Parc de la Plante”, with the first bus at 8:03 (see time tables posted at the registration desk and on the website). Make sure to take a bus early enough not to miss your connecting train, otherwise you can share a taxi with other people going to the station. At the railway station, bus 4 halts at the same stop as from which it departs, so you will have to cross the street to get to the train station.

Programme

Thursday 12 September 2013

12:00 ➜ Arrival of participants, registration, poster setup

18.30 ➜ Welcome reception with buffet

Friday 13 September 2013

09.00 **David Watson**
Parasitic plants as drivers of ecological communities: patterns > predictions > processes

10.00 **Andreas Demey**
Impacts of hemiparasitic plants on the vegetation and biogeochemical cycling in two contrasting semi-natural grassland types

10.30–11.00 Coffee break

11.00 **Jakub Těšitel**
Fighting for resources–parasitism, competition and virulence in a hemiparasitic association

11.30 **Petr Blažek**
Response of grassland Rhinanthoid Orobanchaceae to different mowing dates

12.00 **Vojtěch Adamec**
Ecology of early ecotype of *Melampyrum nemorosum*

12.30–13.30 Lunch

14.00 **Jitka Kockova**
Using DNA-barcoding and anatomical methods to reveal host spectra of hemiparasitic plants under natural conditions

14.30 **Pavel Fibich**
Modelling of niches of Central European root-hemiparasitic species

15.00–17.00 **Poster session** with coffee and refreshments

17.00–18.30 walk to the Citadel of Namur

19.00 Dinner

Saturday 14 September 2013

09.00 **Vincent Merckx**
Mycoheterotrophy: plants living on fungi

10.00 **Sidonie Bellot**
The evolutionary retention of plastid genomes in non-photosynthetic plants: A comparative approach centred on the endoparasitic Apodanthaceae

10.30–11.00 Coffee break

11.00 **Gerhard Glatzel**
The evolution of deciduous mistletoes – a hypothesis

11.30 **Milan Štech**
Why is the *Melampyrum nemorosum* group taxonomically difficult?

12.00 **Laurent Natalis**
The role of bumblebee pollinators in hybridization between two *Rhinanthus* species

13.00–18.00 **Excursion** to chalk grasslands

19.00 ➜ Dinner & farewell party

Sunday 15 September 2013

Departure

Abstracts of talks

In chronological order

Parasitic plants as drivers of ecological communities: patterns > predictions > processes

David M. Watson

Institute for Land, Water and Society, Charles Sturt University, Australia

Long regarded as botanical curios or ecological oddities, parasitic plants are routinely excluded from community-level studies impeding our understanding of their interactions and overall contribution to ecosystem function. In this overview of a decade of research, I highlight efforts to fill key knowledge gaps by my research group, focusing on our studies of mistletoes in southern Australia. I begin with pair-wise interactions between mistletoes and their pollinators, seed dispersers and natural enemies, and consider the influence of mistletoes on habitat structure. Building on this existing knowledge, I articulate a series of predictions on the role of mistletoes in forest communities, and summarise a recent large-scale experiment designed to test these predictions. Rather than the presumed direct effects via nutritional resources, marked indirect effects mediated via abundant, enriched litter-fall were apparent, consistent with parallel research on insectivores, arthropods, understorey plants and nutrient dynamics. Rather than specific to mistletoes, I suggest many of these processes are characteristic of parasitic plants generally, acting as facilitators in many ecosystems. I conclude with a series of open questions, identifying persistent gaps in our understanding, suggesting new avenues for ecological research and the potential utility of parasitic plants for large-scale ecosystem restoration.

Impacts of hemiparasitic plants on the vegetation and biogeochemical cycling in two contrasting semi-natural grassland types

Andreas Demey[1], Ameloot E.[2], Staelens J.[1,3], De Schrijver A.[1], Boeckx P.[3], Hermy M.[2] & Verheyen K.[1]

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Hemiparasitic plants from the family Orobanchaceae play a key role in the conservation of species-rich semi-natural grasslands. They can alter plant community composition and diversity by both parasitism and litter effects. The drainage of resources by the hemiparasite suppresses preferred host species to the benefit of non-host species. On the other hand, hemiparasitic litter inputs enhance nutrient cycling with indirect positive effects on both host and non-host. When parasitism effects dominate, decreased host and total biomass are expected to result in an increased diversity of the non-host community. In contrast, when litter effects compensate for the biomass loss due to parasitism, minimal to no changes in diversity, minor changes in host biomass and an increase in total biomass are expected. The relative importance of the litter pathway is expected to increase with decreasing nutrient status of the ecosystem. Here we report on litter and net community effects of two native hemiparasitic plant species growing in vegetation types with a contrasting nutrient status: *Rhinanthus angustifolius* C.C. Gmel. favoring mesotrophic grasslands (3 sites) and *Pedicularis sylvatica* L. growing in oligotrophic heath-grasslands (3 sites). We first linked hemiparasitic litter nutrient returns to the net effect on biomass production; second, we assessed the net effect of both hemiparasites on species abundances and seedling recruitment; third, we studied the impact of hemiparasitic and non-parasitic litter on gross nitrogen (N) transformations in the soil; and forth, we traced N uptake from hemiparasitic litter by the vegetation.

Fighting for resources–parasitism, competition and virulence in a hemiparasitic association

Jakub Těšitel[1], Tamara Těšitelová[1], James P. Fisher[2], Jan Lepš[1] & Duncan D. Cameron[2]

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Hemiparasitism represents a unique life form on the edge of autotrophy and heterotrophy. Thus, the hemiparasitic interaction is rather complex involving both parasitism and competition. We tested the effect of differential abundance of water and mineral nutrients on the hemiparasitic interaction in a factorial mesocosm experiment with *Rhinanthus alectorolophus* attached to maize and wheat. This approach allowed disentangling the roles of the key resources involved in the hemiparasitic association and separating individual components of the hemiparasitic association.
Mineral nutrients were identified as the main subject of parasitism. Hemiparasite virulence is caused directly by the loss of mineral nutrients to the parasite if this resource is limiting. Water acts as a medium in which the resources are transported. Abundance of mineral nutrients releases the system from a limitation by this resource. In this situation, light becomes limiting. *Rhinanthus* is a generally weak competitor for light compared to the hosts. However, if water is deficient, the hemiparasite ability to capture host xylem stream strongly increases its virulence. The damage inflicted to the host then reduces its competitive strength. Thus, a single mechanism, the ability to extract water from host xylem, is responsible for both resource acquisition and virulence, levels of which vary depending on environmental conditions. Organic carbon uptake from the host presents a significant contribution to hemiparasite carbon budget, especially if its photosynthesis is limited by nutrient deficiency or competition. From the host's point of view, loss of organic carbon to parasitism does not cause a major harm.

Response of grassland Rhinanthoid Orobanchaceae to different mowing dates

Petr Blažek[1], Lepš J.[1], Těšitel J.[1] & Fajmon K.[2]

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Rhinanthoid Orobanchaceae growing in temperate grasslands were negatively affected with agricultural intensification. As these species are annuals, one of the aspects that could cause their decline is the shift in mowing date. We carried out two manipulative field experiments aiming to find out the effect of various mowing dates on hemiparasites' performance.

In the first experiment, we clipped plants of *Rhinanthus minor* and *R. alectorolophus* in situ from mid May to mid July. There is a gap between the dates until when is *Rhinanthus* able to regenerate and since when it has ripe fruits. This period was about 2 weeks long for *R. minor* and at least 8 weeks long for *R. alectorolophus*, and it matches with the usual mowing time in productive meadows. This period can be shortened by making hay instead of silage.

In the second experiment, we mowed permanent plots with *Rhinanthus major* (= *R. angustifolius*) and *Melampyrum nemorosum* in three dates in June and July. Whereas there was no clear pattern in population change for *Rhinanthus*, *Melampyrum* was obviously suppressed by mowing in June and thrived after July mowing. Despite that *Rhinanthus* probably also did not produce seeds when mown in June, its seeds from the surroundings were able to reach a central plot over a 2m buffer zone, compared to seeds of *Melampyrum*.

We conclude that not only the increased biomass itself, but also the timing of harvesting and way of fodder conservation are key factors affecting hemiparasites population dynamics.

Ecology of early ecotype of *Melampyrum nemorosum*

Vojtěch Adamec, Tamara Těšitelová, Jitka Kocková & Jakub Těšitel

Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic

Melampyrum nemorosum contains two distinct ecotypes. The late ecotype is common ecotone and broad leaved forests species in Central Europe. It flowers in second half of June and in July. The early ecotype flowers one month earlier. It is considered criticaly endangered. In central Europe, it occurs only on mown and species rich meadows located in White Carpathians where it has several known localities.
We were interested in distribution limitations of early ecotype in the lanscape. Sowing experiment was established to determine, if the limited occurence is caused by poor dispersal or habitat limitation. Effect of hemiparasite on community was assessed after four years of monitoring. We also studied host spectra of both ecotypes. This was done by DNA-barcoding of attacked host-roots combined with anatomical sections of samples. For questioning if the oaks and bushes can facilitate growth of early ecotype, early ecotype populations were mapped and the bivariate O-ring functions were computed.
Sowing experiment showed unsignificant effect of early ecotype on its hosts. It grew on all sown-sites, even after four ears, so the reason of limited occurrence is poor seed dispersal. Low virulence of early ecotype in combination with poor seed dispersal and annual life cycle serves most probably as mechanism how to persist on site. Host spectra between ecotypes differs strongly. Early ecotype attacks herb meadow species whereas the late ecotype parasitises mainly woody forest and bush species. It reflected also in results of O-ring functions. These showed mostly repulsion of early ecotype from woody vegetation.

Using DNA-barcoding and anatomical methods to reveal host spectra of hemiparasitic plants under natural conditions

Jitka Kockova & Tesitel J.

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Host identification of root-parasitic plants under natural conditions has always been a challenging task requiring dissection of parasite's root system and tracing the host root to the shoot. Revealing the host range and possible preferences has been especially difficult in rather generalist hemiparasitic species. This study aimed to test the effectiveness of a DNA barcoding-based approach to reveal the host associations.
Root systems of three root hemiparasites *Rhinanthus minor*, *R. major* and *Melampyrum nemorosum* were examined for haustorial connections. Samples of host roots were separated and subjected to DNA extraction and genotypification by sequencing the *trnL* intron of cpDNA. Anatomical structure of the haustoria was furthermore checked for functionality of the connection using semi-thin sections of the haustoria samples (standard sample preparation for TEM).
The amplification and sequencing yielded relatively high success rate (c. 70 %) of obtaining quality sequences. Most samples were classified to species and all to the level of genera. The three hemiparasitic species differed in their respective host spectra; *Rhinanthus minor* seemed to prefer grasses to forbs, whereas *R. major* and *Melampyrum nemorosum* preferred forbs. Anatomical structure of some haustoria showed that the connection to certain forbs (e. g. *Plantago lanceolata*) was non-functional due to host defense reaction. Furthermore, both mutual parasitism and cannibalism of the three species were also observed.
The DNA-barcoding approach based on the *trnL* intron proved an effective tool for revealing host associations of root-parasitic plants. Use of this readily available method promises a major advancement in the biology of root-parasitic plants.

Modelling of niches of Central European root-hemiparasitic species

Pavel Fibich[1], Těšitel J.[1], de Bello F.[1,2], Blažek P.[1], Chytrý M.[3] & Lepš J. [1,2]

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Root-hemiparasites are considered an important functional group of plants due to their ability to affect competitive relationships in plant communities and alter nutrient flows. On the basis of these effects, hemiparasite can enhance community diversity and stability. Although several field and modelling studies identified e.g. the response of hemiparasite populations on site productivity, projections of hemiparasite occurrence still heavily rely on expert knowledge of ecological behaviour of individual hemiparasitic species.

Our aim is to model species niches of Central European hemiparasites based on the plant community composition data available in the Czech National Phytosociological Database. We identified vegetation units within which individual hemiparasites occur. Further, we adopted a Beals index-based approach to refine the niche shape resulting in a classification of relevés into three groups: i. hemiparasite present, ii. hemiparasite is absent but the species composition of the relevé suggests that it is part of its "dark diversity", iii. hemiparasite is absent and is not included in the site species pool. The first two groups of relevés are considered as to be within the species niche of the hemiparasite. For each species, we visualized its niche and environmental gradients within it using NMDS. Ellenberg indicator values were projected in the ordination space in order to provide further interpretation and comparison between individual hemiparasitic species. Finally, we used a functional traits-based approach to locate the species beta-niche on trait gradients across the whole vegetation.

Mycoheterotrophy: plants living on fungi

Vincent S.F.T. Merckx

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In rainforests plants compete intensively for light. To escape this competition several plant lineages have evolved a remarkable mode of life known as ‘mycoheterotrophy’. Mycoheterotrophic plants obtain carbon from mycorrhizal or saprotrophic fungi, and thus they are not directly parasitic on other plants. Full mycoheterotrophy evolved over 40 times independently in land plants and occurs in nearly all major lineages of land plants. There are over 500 fully achlorophyllous mycoheterotrophic plant species and most occur only in tropical rainforests. In parallel with parasitic plants, mycoheterotrophic plants showcase some of the most extreme adaptations observed in plants and therefore it is no surprise that they have attracted the attention of botanists for centuries. However, their rarity and cryptic nature have challenged their study and consequently many questions about the biology of these enigmatic plants have been proven difficult to answer. As a result of increased research efforts, helped by technological and methodological advancements, our understanding of mycoheterotrophy has considerably broadened in recent years. For many mycoheterotrophic lineages we now have data on their phylogenetic affinities and fungal associates that provide unique insights into their distribution, ecology, and evolution. Yet it remains challenging to unravel the processes that have driven some plant lineages to switch to a diet of fungi.

The evolutionary retention of plastid genomes in non-photosynthetic plants: A comparative approach centred on the endoparasitic Apodanthaceae

Sidonie Bellot & Susanne S. Renner

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Since their acquisition via endosymbiosis around a billion years ago, the plastid genomes of all photosynthetic plants have lost numerous genes due to transfer to the nuclear genome. Indeed, every single plastid gene has been transferred to the nucleus in some lineage, rejecting early ideas about a "minimal" conserved plastome. This process of nuclear transfer is fundamentally different from the loss of plastid genes in parasitic and mycoheterotrophic plants, which are under relaxed selection for the maintenance of photosynthesis. Surprisingly, all 17 non-photosynthetic species of angiosperms with fully sequenced plastomes (*Cuscuta*, Funk et al., BMC Plant Biology, 7: 45, 2007; Orobanchaceae, Wicke et al., in review; Orchidaceae, Delannoy et al., Mol. Biol. Evol. 28: 2077-2086, 2011; Logacheva et al., Genome Biol. Evol. 3: 1296-1303, 2011; Barrett and Davis, Am. J. Bot. 99: 1513-1523, 2012) retain varying combinations of plastid genes, apparently always including possibly functional *clpP*, *accD*, *ycf1* and *ycf2*. What could explain such plastome conservation in heterotrophic angiosperms? Is it insufficient time for complete genome loss, a DNA function required even in non-photosynthetic plants, or some structural role independent of plastid genomes' protein/RNA-coding functions? To distinguish between these possibilities, we are testing whether the age of a non-photosynthetic lineage correlates with the extent of pseudogenization of its plastome. Our tests are based on the comparison of the 5 published non-photosynthetic lineages (*Cuscuta*, *Orobanchaceae*, *Neottia*, *Rhizanthella*, *Corallorhiza*) plus two new plastomes of Apodanthaceae for which we have Illumina and 454 data. If lineage age is unrelated to the pseudogenization of plastid genes, this would argue that the retention of plastid genomes is selectively advantageous even in parasitic angiosperms.

The evolution of deciduous mistletoes–a hypothesis

Gerhard Glatzel[1], Richter H.[2], Lin R.[3], Lee S.[4], Devkota M.[5] & Amico G.[6]

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The vast majority of mistletoe species is evergreen. A few deciduous mistletoe species occur at the northern and southern fringes of the geographic range of mistletoes. Deciduous *Loranthus* species can be found in eastern Central Europe, Italy and the Balkans as well as in East-Asia. In Europe the range of the deciduous *Loranthus europaeus* overlaps with the range of the evergreen *Viscum album* which parasitizes a fairly large number of different tree species but very rarely oaks, while *L. europaeus* parasitizes mainly on oaks. In East-Asia several deciduous *Loranthus* species are described with *Quercus* sp. being their preferred hosts. Overlapping in range, the evergreen *V. album coloratum* parasitizes a similar spectrum of hosts as *V. album* in Europe but is also common on oaks. Evergreen *Loranthus* species can be found in the warm temperate and subtropical forests of the southern Himalayas and southern China. Assuming that deciduous *Loranthus* species evolved from evergreen subtropical species, we hypothesize that differences in the morphology and anatomy of the haustoria and wood anatomy of their hosts to be responsible. *Loranthus* has a single, wedge shaped haustorium with direct vessel to vessel connections to large vessels of the host. *Viscum* has a complex haustorium with many cortical strands and secondary sinkers, well suited for water uptake from narrow vessels of diffuse porous trees which do not freeze in the winter. When the large vessels of ring-porous oaks freeze they are embolized and cannot supply *Loranthus* with water.

Why is the *Melampyrum nemorosum* group taxonomically difficult?

Milan Štech[1], Chlumský J.[1], Daneck H.[2], Fér T.[2], Herbstová M.[1], Koutecký P.[1], Košnar Jan[1], Košnar Jiří[1], Trávníček P.[3] & Suda J.[2, 3]

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Many annual hemiparasitic genera like *Melampyrum*, *Euphrasia*, *Rhinanthus*, *Odontites* are characterized by very complex pattern of morphological variability. We have tried to find evolutionary processes that have generated the amazing variation in *Melampyrum nemorosum* group, which is the most taxonomically difficult group of the genus *Melampyrum*, using modern biosystematic tools (DNA flow cytometry, isozyme analysis, sequencing of cp-DNA and nr-DNA, and AFLP analysis). Ancient inter-specific hybridization is suggested as one of the evolutionary processes that have generated the amazing variation in this group. Rapid speciation rate and adaptation to environmental condition are supported by differentiation of genome size at the intraspecific level as like as genetic differentiation among populations. Our ongoing study of the group has allowed many long-standing taxonomic controversies to be resolved. Our understanding of the intraspecific genetic variation was reshaped, as was the taxa circumscription, their distribution, and phylogenetic relationships.

The role of bumblebee pollinators in hybridization between two *Rhinanthus* species

Laurent C. Natalis & Renate A. Wesselingh

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To shed light on the role played by pollinators in the diversification of angiosperms, focus is needed on how floral isolation varies locally in the early stages of plant divergence. We studied a hybridizing Orobanchaceae pair (*Rhinanthus minor* and *R. angustifolius*) with strong similarities in flower morphology. The two species shared the same suite of pollinator species (bumblebees), but *R. angustifolius* is more attractive because of its higher rewards levels. We examined how ethological isolation changes locally in relation to relative *Rhinanthus* frequencies, spatial configurations, and pollinator assemblages. Interestingly, no relationship was found between floral isolation and the local pollinator assemblage, but species frequency and spatial arrangement strongly influenced bumblebee behaviour, and thus potentially hybrid formation. When both species were in equal proportions, bumblebees preferred *R. angustifolius*, while at unbalanced frequencies, the more abundant species was preferred, although this was less pronounced when the less rewarding *R. minor* predominated. Some bees foraged on both *Rhinanthus*, thus allowing interspecific pollen transfers. Although *Rhinanthus* has been cited as a case of mechanical isolation resulting from interactions between bee behaviour and differences in stigma and anther placement, pollen placement on bees was similar with both *Rhinanthus*, and cross-specific stigmatic pollen deposition was equal in both directions, thus we found no support for efficient mechanical reproductive isolation. We also measured bumblebee visitation rates to hybrids relative to their parents in populations of each parental species. Bumblebees treated hybrids almost as equal to the background species and more often rejected the non-resident *Rhinanthus*. Bumblebees treated hybrids almost as equal to the background species. In a *R. angustifolius* background, bumblebees visited hybrids more often than *R. minor*, while visitation rates to the hybrids were equal to both parental species on a *R. minor* background.

Poster abstracts

In alphabetical order

Plant density and seed production of *Rhinanthus minor* under long-term Ca, N, P and K fertiliser application in the Rengen Grassland Experiment (Germany)

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Rhinanthus minor L. is a summer annual and facultative hemiparasitic plant common in low-productivity grasslands. Survival of this species is strictly dependent on sufficient seed production in each vegetative season. We aimed to evaluate the effect of different fertiliser treatments on plant density per m^2, seed production per individual plant and seed production per m^2. All data were collected in unfertilised control, Ca, CaN, CaNP, CaNPKCl and $CaNPK_2SO_4$ treatments of the Rengen Grassland Experiment in June 2010. Plant density per m²ranged from 5 to 745 in CaNPKCl and control treatments, seed production per individual plant from 24.9 to 65 seeds in control and $CaNPK_2SO_4$ treatments, and total seed production per m^2 from 195 to 18142 seeds in CaNPKCl and control treatments, respectively. High density of small plants in low productivity control contrasted highly with low density of tall plants in fully fertilised treatments. We concluded that for sufficient seed production per plot, high plant density of small *R. minor* plants is more relevant than low density of tall plants with high number of seeds.

Phylogeny of the genus *Rhinanthus* (*Orobanchaceae*)

Vinciane Mossion & Renate A. Wesselingh

Biodiversity Research Centre, Earth & Life Institute, Louvain University, Louvain-la-Neuve, Belgium

The genus *Rhinanthus* consists of around 25 species, each with several seasonal ecotypes, which have often been described as subspecies. Since the taxonomy of the species in the genus is based solely on morphological characters, and recent genetic research has shown unexpected patterns within two of the species already, the time has come to construct a molecular phylogeny for the whole genus based on multiple DNA sequences and multiple accessions. A new PhD project will start soon to perform this task, combined with a morphological description of the resulting taxonomical units.

Germination and growth of *Rhinanthus minor* populations on west-east gradient in Europe

Vilem Pavlu[1,2], Pavlu L.[1], Tesitel J.[3], Gaisler J.[2] & Hejcman M.[1]

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[3] *University of South Bohemia, Faculty of Science*

Rhinanthus minor (Scrophulariaceae) is annual hemiparasites occurring in grasslands across Europe. Despite the continental range of occurrence, there are only few studies comparing biology of multiple populations. We set up an experiment to study germination, growth and effect on host *R. minor* populations across the east-west gradient in Europe (Scotland, Wales, Germany, Czech Republic, Estonia). The germination dynamics was studied in climatic chamber in the dark at 4°C. Here, we also assessed the effect of sodium hypochlorite treatment, a widely used surface-sterilization procedure, in addition to the comparison between individual populations. The dynamics of *R. minor* growth and the effect on the host was studied in a mesocosm experiment with either diploid or tetraploid variety *Lolium perenne* (ryegrass) as host species and under glasshouse or outdoor conditions.
The average germination of all population was about 60 %. The fastest germination was revealed in Central European populations (Germany, Czech Republic) whereas the slowest one was recorded in Scotland. The treatment of seeds by sodium hypochlorite increased germination by 10%. The total ryegrass biomass production was higher in tetraploid than diploid variety and also the stimulation of *R. minor* growth was greater with tetraploid than diploid ryegrass hosts. A negative linear relationship between the total ryegrass and *R. minor* biomass was revealed under the greenhouse conditions, whereas under outdoor conditions the relationship was not so straightforward. Growth dynamics and effects on hosts were significantly different among the *R. minor* populations, and similarly we revealed the importance of genotypic variation within a single host species (*L. perenne*).

Seed morphology of species of the genus *Pedicularis* L. (*Orobanchaceae* Vent.) of the Eastern European flora.

Olena Peregrym

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Pedicularis L. is complicated genus of the angiosperms, that represented by the hemiparasitic herbaceous plants. This genus includes 21 species in the Eastern European flora, which belong to three subgenera and five sections (Ivaninova, 1981).

Our aim was to describe seed morphology of the *Pedicularis* species from the Eastern Europe, to assess taxonomic significance of seed morphology, and to hypothesize the possibilities of its applicability for taxonomic reconstructions.

Seed morphology of 20 species of *Pedicularis* was studied by light and scanning electron microscopy, a terminology followed by Artyushenko (1990) and Stern (1992). The size, shape and ornamentation of seeds surface were analyzed. We delimited primary and secondary structures of the testa according to Barthlott. The contour, shape, features of periclinal and anticlinal walls of the cells were included in the primary structure. The secondary structure is defined morphological features of a cuticle.

As result of our study we distinguished three types of the ornamentation: ladder-like (*P. labrodorica, P. oederi*), rugose (*P. lapponica, P. resupinata, P. sylvatica*) and reticulate (14 studied species). Reticulate type includes regular reticular (*P. compacta, P. hacquetii, P. hirsuta, P. verticillata*), reticular-colicular (*P. kaufmannii, P. physocalyx, P. sibthorpii*), reticular-foveate (*P. dasystachys, P. palustris, P. exaltata*), reticular-membranous (*P. dasyantha, P. sceptrum-carolinum, P. sudetica*) and reticular-cristate (*P. amoena*) types.

Ornamentations of seed surface are diverse within *Pedicularis* genus. Seed characteristics of the species mentioned may be used like an additional diagnostic feature for their delimitation.

Taxonomic status and phylogeography of the Iberian endemic *Odontites recordonii* Burnat & Barbey (tribe Rhinantheae, Orobanchaceae)

Pinto Carrasco D., Delgado-Sánchez L., Sánchez Agudo J.A., Rico Hernández E. & Martínez Ortega M.M.

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The genus *Odontites* Ludw. comprises ca. 26 hemiparasitic species distributed mainly around Mediterranean. Most of the species are restricted to the W Mediterranean and show limited distribution areas in the Iberian Peninsula and North Africa.
Odontites recordonii Burnat & Barbey is endemic to E and NE Spain (rare and with a scattered distribution in C Spain). Based on morphological characters, the species seems to be closely related to the *Odontites vernus* group.
Amplified fragment length polymosphism (AFLP; 11 species, 295 individuals, most of them corresponding to *O. recordonii*) was used to obtain 852 scorable alleles using 3 primer combinations, in order to try to shed light on the phylogenetic position of *O. recordonii* and to reconstruct the phylogeographic patterns within the species.
Our data confirm a taxonomic structure in three species groups (i.e., *O. pyrenaeus*, *O. purpureus* and *O. vernus* groups), which is in accordance with previous taxonomic treatments. *Odontites recordonii*, *O. vernus* (Bellardi) Dumort. and *O. kaliformis* (Pourr. ex Willd.) Pau are distinct closely related species and hybridization among them was not detected.
Three geographically well structured groups of populations were found within *O. recordonii*, which are not separated by evident biogeographic barriers. An admixture analysis detected some degree of admixture among populations from different areas in a couple of cases. Furthermore one long distance dispersal event was identified.
Last, our data do not support that *O. eliassennenii* Pau (described from NW Spain) can be recognized as a separate species and it is therefore treated as a synonym of *O. recordonii*.

Hybridization in *Rhinanthus*: fitness and introgression

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Rhinanthus minor L. and *R. angustifolius* C.C. Gmel are known to form viable hybrids whenever the two species co-occur. We have shown before that in naturally mixed populations, hybrids close to *R. angustifolius* are usually more abundant than hybrids close to *R. minor*. Bumblebee behaviour favouring pollen transfer between F_1 hybrids and *R. angustifolius* appears to be a good explanation for this pattern (see oral presentation by Laurent Natalis), but we actually know very little about hybrid fitness under natural conditions. This new PhD project aims to quantify hybrid and parental fitness in a range of ecological conditions by sowing seeds of known descent in plots and determine biomass, flower and seed production.
From previous research we know some genetic markers from *R. minor* have a higher probability of appearing in advanced hybrids close to *R. angustifolius*, indicating linkage with a genomic region conferring higher fitness. We want to expand the number of markers studied and construct a genetic map to identify these regions of higher and lower hybrid fitness using the plants studied in the field.

Does elevated stomatal conductance provide a photosynthetic gain benefit to hemiparasitic *Melampyrum pratense* in the forest understory environment with sunflecks?

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Root-hemiparasitic plants from the Orobanchaceae family obtain resources by two distinct ways, their own photosynthesis and by exploiting other species. Although own photosynthesis provides a crucial contribution to carbon and energetic budget of hemiparasites, its role is often neglected while the role of parasitic resource acquisition is exaggerated. Here we asked whether *Melampyrum pratense*, a hemiparasitic herb occurring in deciduous forest understory, displays an effective exploitation of sunflecks due to elevated stomatal conductance, a physiological trait shared by many hemiparasitic species of Orobanchaceae. The principle should lie in the fact that the hemiparasite does not need to open its stomata from the closed state when it is illuminated by a sunfleck. Therefore, it should reach the light saturated rates of photosynthesis in a shorter time than non-parasitic species with normal behaviour of stomata. We examined photosynthetic responses of *Melampyrum pratense* to simulated sunflecks in spring and summer. Co-occuring *Viola riviniana* was chosen as a non-parasitic reference. Light response and induction curves were measured under contrasting conditions in spring and summer, i.e. before and after the oak forest canopy closure. In addition to the physiological measurements, light conditions on the forest floor were recorded every two minutes during both seasons to capture the sunfleck patterns in the understory.

Hybridization and genetic variation in *Euphrasia* species

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The genus *Euphrasia* L. belongs to the most taxonomically complex genera in Europe. This fact is caused especially by considerable similarity of the majority of the species, by intraspecific variation, hybridization, which occurs very often in this genus, and phenotypic plasticity. From the taxonomic point of view, *Euphrasia stricta* and *E. nemorosa* are the most critical species in the Czech Republic because of existence of morphologically intermediate populations. It has been assumed that hybridization is an important source of variability, and taxonomy of species has been questioned, but moleculat evidence was lacking.

A hybridization experiment was performed to find out if both species can act as mother plants and whether the fitness of F1 individuals was decreased. The genetic variability and the rate of hybridization and introgression were studied by microsatellites. Results were correlated with morphological characteristics of populations.

Ongoing project on genetic and morphometric variation of rare and common *Euphrasia* species is presented. Comparison of morphometric a genetic variability among plants with assumed diffetent dominant type of reproductive system is planned. Less genetic variability shlould be present in small-flowered species (e.g. *E. nemorosa*). Due to autogamy, they should make more uniform populations than species with large (e.g. *E. officinalis*) and medium-sized (e.g. *E. stricta*) flowers. Genetic variability of these species should be thus concentrated to variability between populations while in species with larger flowers, which are more allogamous, should be higher intrapopulation variability. Also common species should have higher genetic and morphometric variability than rare species.

Interactions between root-hemiparasites and herbivorous insects

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Hemiparasitic plants are known to play an important role in meadow ecosystems regulating competitive relationships and nutrient cycling. Due to their ability to extract mineral nutrients from host xylem, hemiparasites often display higher concentrations of them, namely nitrogen, in their leaf tissues compared to co-occurring plants. This is known to accelerate decomposition of hemiparasite litter. In addition it should increase attractiveness of hemiparasite tissues for herbivores due to a favourable N/C ratio. This theoretical expectation however lacks support from any field observation or experiemental data. In a pilot study on interactions between hemiparasites and herbivores, we aim a) to compare intensity of herbivory between hemiparasites and co-occurring non-parasitic species b) to identify herbivore communities feeding on hemiparasites in various stages of ontogenetic development. The study is conducted on meadows of Čertoryje National Natural Reserve (White Carpathian Mts., Czech Republic) with abundant hemiparasitic *Melampyrum nemorosum* and *Rhinanthus major* (=*angustifolius*). The comparison of the intensity of herbivory between the hemiparasites and other species is based on comparing proportions of leaves damaged by herbivores. Herbivore communities present on hemiparasites were sampled at three sampling intervals (mid-spring, late spring and early summer) to identify herbivore species feeding on young plants, flowering, and fruiting plants.

Looking for Achilles' heel of *Calamagrostis epigejos*. Can we suppress the expansive grass by hemiparasitic *Rhinanthus* species?

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Calamagrostis epigejos is a clonal grass frequently expanding to seminatural grassland communities in Central Europe. This expansion threatens biodiversity due to a massive spread of the grass and its ability to competitively exclude most co-occurring species. *C. epigejos* conserves mineral nutrients in its underground tissues and displays a highly effective resorption of nutrients from the photosynthetic tissues in the end of growing season. As a result, thick layer of slowly decomposing litter is produced preventing establishment of seedlings of other species. In addition, other species do not have access to nutrients captured by *C. epigejos* providing further competitive advantage to the grass. Due to the massive underground resource storage in roots, suppressing *C. epigejos* by standard management practices (mowing etc.) is difficult, laborious and costly.
Hemiparasites could however attach to *C. epigejos* roots and a withdraw the nutrients directly from the storage organ of the host. Still, hemiparasites rarely occur spontaneously in stands of *C. epigejos* due to the suppressive effect of the litter layer. In a series of experiments, we demonstrate that *Rhinanthus* species are able to parasitize *C. epigejos* if the litter layer is removed. The suppressive effect of *Rhinanthus* on *C. epigejos* is tremendous. In particular, *R. alectorolophus* can virtually wipe out *C. epigejos* from infested plant communities. Therefore, we suggest the hemiparasites as a highly effective tool to reverse *C. epigejos* expansion into species-rich grassland communities.

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