

Conference Programme

6th International Orchid Workshop

Białystok 2018



28 May - 1 june 2018
Białystok, poland





INTRODUCTION

Cypripedium calceolus, Biebrza National Park

HISTORY OF ORCHID MEETINGS IN EUROPE

In 1990, a workshop was organized by Jo Willems in South Limburg, Netherlands, with the aim to bring together scientific information on aspects of orchid biology and discuss future research, management and conservation of orchids. The meeting was very productive, but a regular system of meetings would be useful to keep the connections alive. Thus, after a long gap of 10 years, a regular series of meetings started from the workshop organized by Pavel Kindlmann in České Budějovice, Czech Republic, in 2001. The next meeting was organized by Tiit Kull in 2005 in Haapsalu, Estonia. In 2007 the group met in Tver, Russia, where the workshop was organized by Irina Tatarenko. The most recent one was organized by Giuseppe Pellegrino, which was held in 2013 in Rende, Italy. The lack of space prevents me from naming all other people who helped with the organization of these meetings — the main organizer was never alone. The papers presented in these workshops addressed a wide range of topics in orchid biology, ranging from analysis of problems in orchid taxonomy, via mycorrhizal interactions, seed germination issues, pollination ecology, population dynamics, to management regimes for the conservation of individual species. The meetings were always an excellent opportunity for orchid specialists to meet and exchange ideas. Recently, a group of attendants of these meetings created the European Orchid Conservation Centre with the seat in České Budějovice, Czech Republic, with Pavel Kindlmann as the coordinator. We hope that this will promote orchid research in Europe and facilitate its funding. Any ideas and offers of new activities for this Centre are most welcome.

Pavel Kindlmann

COMMITTEES

Epipactis palustris, Biebrza National Park

ORGANIZING COMMITTEE



DEPARTMENT
OF PLANT ECOLOGY
(from the left side of the photo)

Paweł Mirski
Emilia Brzosko
Ada Wróblewska
Beata Ostrowiecka
Edyta Jermakowicz
Izabela Tałałaj

INSTITUTE OF BIOLOGY^{ww}

Olgierd Aleksandrowicz
Bożena Kozłowska-Szerenos

UNIVERSITY CENTRE
OF NATURE
OF PROF. ANDRZEJ MYRCHA

Wiesław Mikucki
Janusz Kupryjanowicz

PH.D. STUDENTS

Agnieszka Bona
Zofia Korbut
Damian Pogodziński
Aleksandra Mieczkowska



Dactylorhiza incarnata incarnata and *ochroleuca* morphs, Biebrza National Park

SCIENTIFIC COMMITTEE



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naturelle, Paris
France
University of Gdańsk
Poland



Dennis Whigham

The Smithsonian Environmental
Research Center
The North American
Orchid Conservation Center
USA



Neottianthe cucullata, Wigry National Park

CONFERENCE PROGRAMME

Liparis loeselii, Biebrza National Park

MONDAY, 28 MAY

8:00	REGISTRATION University of Białystok, Institute of Biology
10:00	CONFERENCE OPENING
	Welcome words <i>Prof. Robert W. Cibarowski</i> — Rector of the University of Białystok <i>Prof. Beata Godlewska-Żyłkiewicz</i> — Dean of the Faculty of Biology and Chemistry <i>Prof. Emilia Brzosko</i> — Organizer of 6IOW 2018
SESSION 1: BIODIVERSITY, BIOGEOGRAPHY AND CONSERVATION	
10:30	CHAIR: EMILIA BRZOSKO Keynote Speakers Species distribution models and their application in orchid biodiversity research <i>Pavel Kindlmann</i>
11:00	Orchids at their distribution edge in Europe <i>Tiiu Kull</i>
11:30	COFFEE BREAK
12:00	CHAIR: PAVEL KINDLMANN Investigating the factors determining orchid species richness from a macroecological perspective <i>Tsiftsis Spyros</i>
12:20	The influence of ecogeographic isolation on divergence in the South African orchid genus <i>Disa</i> <i>Karl J. Duffy</i>
12:40	Factors associated with the distribution of orchids in the Czech Republic and their comparison from two different regions <i>Zuzana Štípková</i>
13:00	LUNCH
14:00	CHAIR: RICHARD SHEFFERSON How to find determinants of orchid occurrence <i>Iva Traxmandlová</i>
14:20	Factors affecting the dynamics of orchid populations <i>Ekaterina Zheleznaia</i>

14:40	Vegetation types with <i>Ophrys insectifera</i> in Salten, northern Norway, and their importance in conservational contexts <i>Jørn Erik Bjørndalen</i>
15:00	North American Orchid Conservation Center — an update <i>Dennis Whigham</i>
15:20	COFFEE BREAK
SESSION 2: TAXONOMY, PHYLOGENETICS AND EVOLUTION	
15:60	CHAIR: ADA WRÓBLEWSKA Keynote Speaker Have DNA-based analyses lived up to their promise? <i>Richard Bateman</i>
16:20	Phylogeographic implications of the breakdown of mutation-drift equilibrium in the clonal-sexual orchid <i>Cypripedium calceolus</i> <i>Michael F. Fay</i>
16:50	Using a genomic approach, RAD-seq, to unravel the recent evolutionary histories of European orchids <i>Gábor Sramkó</i>
17:10	The taxonomy of <i>Sobralia</i> — a significant but poorly known element of Neotropical orchid flora <i>Przemysław Baranow</i>
17:30	COFFEE BREAK
18:00	The power of movement in orchids, a kinematics study of <i>Porroglossum</i> (Pleurothallidinae) evaluated in the context of their phylogeny reconstructed using NextGen GBS Data <i>Ken Cameron</i>
18:20	Macro- and microevolutionary drivers of allopolyploid evolution in <i>Dactylorhiza</i> (Orchidaceae) <i>Ovidiu Paun</i>
18:40	Molecular phylogeny and taxonomy of the genus <i>Paphiopedilum</i> (Cypripedioideae, Orchidaceae) <i>Marcin Górniak</i>

19:00	Systematic implications of trichomes in problematic species, <i>Polystachya paniculata</i> (Sw.) Rolfe (Orchidaceae) <i>Joanna Mytnik</i>
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TUESDAY, 29 MAY

SESSION 3: MYCORRHIZAL INTERACTIONS AND SEED GERMINATION

8:30	CHAIR: JANA JERSÁKOVÁ Keynote Speakers Mycoheterotrophy and mixotrophy in orchids: an update Marc-André Selosse
9:00	Looking belowground to understand orchid distributions aboveground <i>Hans Jacquemyn</i>
9:30	Is the mycorrhizal fungi distribution limiting the orchid establishment in restored meadows? <i>Hélène Vogt-Schilb</i>
9:50	Partial mycoheterotrophy is more widespread among orchids than previously assumed: A multi-element stable isotope natural abundance approach <i>Gerhard Gebauer</i>
10:10	The mixotrophic <i>Epipactis helleborine</i> does not necessitate a link to C-providing fungi: lessons from pot culture <i>Alzbeta Novotna</i>
10:30	COFFEE BREAK

SESSION 4: POPULATION DYNAMICS AND GENETICS

11:00	CHAIR: TIJU KULL Keynote Speaker Global drivers of orchid demography <i>Richard Shefferson</i>
11:30	Unravelling the population dynamics of orchids as high-fecundity organisms <i>Martha Charitonidou</i>

11:50	Climate-driven variation in demographic responses of <i>Himantoglossum adriaticum</i> at the Northern distribution limit <i>Anna Mária Csergő</i>
12:10	A novelty look at terrestrial orchids through a prism of ecosystem concept <i>Irina Tatarenko</i>
12:30	LUNCH
15:00	DEPARTURE TO SUPRAŚL TOWN
16:00	EXCURSION TO MUSEUM OF ICONS
17:30	GALA DINNER WITH CULTURAL PROGRAMME

WEDNESDAY, 30 MAY

SESSION 5: DECEPTION AND REPRODUCTIVE ECOLOGY

8:30	CHAIR: HANS JACQUEMYN Keynote Speaker The evolution of reproductive isolation in Mediterranean orchids <i>Giovanni Scopece</i>
09:00	Why don't deceptive orchid pollinators go extinct? <i>Amy Martin</i>
09:20	Pollination strategy of a food-deceptive orchid: a community approach <i>Thomas Rupp</i>
09:40	Food or sexual deception? Untangling the pollination strategy of the fly-pollinated orchid <i>Neotinea ustulata</i> (Orchidaceae) <i>Jana Jersáková</i>
10:00	New databases on orchid-pollinator interaction and fruit set in the Euro-Mediterranean region <i>Bertrand Schatz</i>
10:20	Untangling factors underlying distribution of forest orchids <i>Milan Kotlínek</i>
10:40	COFFEE BREAK

11:10	CHAIR: MARC-ANDRÉ SELOSSE On the adaptive role of flower form in sexually deceptive orchids: insights from the Mediterranean genus <i>Ophrys</i> (Orchidinae) <i>Demetra Rakosy</i>
11:30	Parallel adaptation to pollinator attraction in <i>Ophrys</i> <i>Laura Piñeiro Fernández</i>
11:50	Management affects the pollinator abundance but not the reproductive success of butterfly orchids <i>Marilyn Mötelp</i>
12:10	Reproductive ecology of the rare mycoheterotrophic orchid <i>Epipogium aphyllum</i> <i>Julita Minasiewicz</i>
12:30	A case of spontaneous pollinarium reconfiguration within flowers of deceptive orchid <i>Dactylorhiza fuchsii</i> <i>Izabela Tałałaj</i>
12:50	MEETING WITH SPONSORS. VISIT IN THE ANDRZEJ MYRCHA UNIVERSITY CENTRE OF NATURE (GROUP 1)
14:00	LUNCH
15:00	POSTER SESSION. VISIT IN THE ANDRZEJ MYRCHA UNIVERSITY CENTRE OF NATURE (GROUP 2)
16:00	COFFEE BREAK
16:30	PLENARY DISCUSSION. CLOSING WORDS.

THURSDAY, 31 MAY

7:00-21:00	FIELD TRIP TO BIEBRZA NATIONAL PARK AND SUWAŁKI LANDSCAPE PARK
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FRIDAY, 1 JUNE

8:00-18:00	FIELD TRIP TO BIAŁOWIEŻA NATIONAL PARK
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Species distribution models and their application in orchid biodiversity research

Pavel Kindlmann^{1,2}, Zuzana Štípková^{1,2}, Marta Kolanowska^{1,3}, Dušan Romportl^{1,4}

¹Global Change Research Institute, Czech Academy of Science, Czech Republic; ²Institute for Environmental Studies, Czech Republic; ³Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, Poland; ⁴Department of Physical Geography and Geoecology, Charles University, Czech Republic

Species distribution models (SDMs) enable prediction of the occurrence of a species, based on a set of GPS coordinates of known sites and a set of biotic and abiotic characteristics of these sites. The output of these models is a map of the potential distribution of the species, where the likelihood of its occurrence (a number between zero and one) is depicted in the same way as, e.g., altitude in classical maps — usually by different colors. SDMs are especially useful in regions that are not yet fully explored, as they enable one to pinpoint sub-regions of potential occurrence of the species studied. Based on such results, areas with special protection may be declared. SDMs are scarcely used for orchids as orchid habitats are not sufficiently explored in many cases for various reasons (inaccessibility, danger of access) and therefore modelling is the only way to pinpoint places where they could potentially occur but have not been discovered. In addition, many orchid populations are on the verge of extinction and therefore SDMs might help in proposing proper conservation measures for these populations. Finally, objective finding of factors determining their presence may elucidate information about their life history strategies and relative importance of their individual traits. The deficiency of SDM studies on orchids may be because orchidologists may not be aware of the usefulness of these models for their research and may be deterred by their mathematical and statistical complexity when these models are described in the literature. Therefore, a simple and user-friendly guide to these models involving as little mathematics as possible is needed for orchidologists. To address this issue, we show the basic principles of these models and the situations when they are used and problems that may occur in their practical application.

SPEAKER ABSTRACTS

Platanthera bifolia, Biebrza National Park

Orchids at their distribution edge in Europe

Tiiu Kull

Estonian University of Life Sciences, Tartu, Estonia

Many species occurring at their geographical and ecological edges are found growing in small and isolated populations due to the losses of suitable habitats. Small and isolated populations are subject to decline and increased risk of extinction through decreased reproductive performance and offspring fitness. It is caused by increased selfing and mating among closely related individuals thus leading to increased homozygosity and decreased individual fecundity due to inbreeding depression. Orchid species in Europe that have range limits are often more threatened than those with vast distribution area around them. Intense human impact in central Europe has raised the threat level of orchids in these countries but comparatively less at the northern limit of these species. But border area may also be an area in which the hybridization is more intense and thus the abundance of hybrids can be higher, due to the proximity of different forms that inhabit opposite adjacent areas. Furthermore, the populations at the border may have a higher genetic diversity due to the larger amplitude of variation of environmental conditions at the border in comparison to adjacent core areas. The majority of European orchids have Eurasian type of distribution area stretching from the West to the East and sometimes from the Atlantic Ocean to the Pacific. In addition to the studies at the northern and southern distribution border, we should also concentrate on population genetics and ecology of species that are at the eastern or western range border in Europe.

Investigating the factors determining orchid species richness from a macroecological perspective

^{1,2}Spyros Tsiftsis, ^{2,3}Zuzana Štípková, ^{2,3}Pavel Kindlmann

¹Aristotle University of Thessaloniki, Greece; ²Global Change Research Institute, Academy of Sciences of the Czech Republic, Czech Republic; ³Institute for Environmental Studies, Charles University, Czech Republic

Although there are many papers on patterns in plant species richness along the elevational and latitudinal gradients, few refer to orchids and their life history traits. Knowledge of the associations between species richness and environmental gradients are of crucial importance for the conservation and study of the environmental relationships of orchids and may even help to predict the effects of global change on their distribution. We explored the patterns in the distribution of Greek orchids along gradients of elevation, climatic conditions, and latitude. To explore the associations between species richness and the selected predictors, the data was analysed using regression techniques for all orchids, and then for subsets, which were classified depending on the structure of their root system. The predictive power and significance of the predictors was tested using second order polynomial generalized linear models (GLM). Our results demonstrate the different but significant effects of the predictors on the total orchids and their subsets. The GLM analyses revealed that all the variables together accounted for almost 50% or more of the variance in each of the orchid subsets. The maximum elevation was the most significant factor for rhizomatous and intermediate orchids, whereas minimum temperature in the coldest month was highly significant for the tuberous orchids. We reveal that the patterns in the distribution of Greek orchids are associated with a combination of elevation, latitude, and climate. The distributions of rhizomatous and intermediate orchids are mainly associated with the orographic configuration of Greece, whereas the tuberous orchids are widely distributed in the southern, central and north-western areas of Greece, where most of them are mainly delimited by the harsh winter climate.

Untangling factors underlying distribution of forest orchids

Milan Kotlínek¹, Tamara Těšitelová¹, Jiří Košnar¹, Pavel Fibich¹, Petr Koutecký¹, Lucie Hemrová², Zuzana Münzbergová^{2,3}, Jana Jersáková¹

¹Faculty of Science, University of South Bohemia, Czech Republic; ²Institute of Botany, The Czech Academy of Sciences, Czech Republic; ³Faculty of Science, Charles University, Czech Republic

Factors driving species distribution patterns is a key topic in ecology. In view of dispersal and habitat limitation concepts, orchids are considered limited by seed dispersal but highly habitat specific. In contrast to this assumption, many theoretically suitable sites have stayed unoccupied. Using four forest orchids differing in fungal symbionts and demands for limestone substrate, we studied drivers of their distribution in a fragmented landscape. We combined analyses of seed dispersal and population genetic structure with seed sowing in occupied and unoccupied habitats and with an analysis of mycorrhizal fungi in soil, adults, seedlings, and ectomycorrhizal tips from surrounding trees to the untangle influence of diverse environmental predictors. While 95% of seeds were trapped less than six meter from the mother plants, gene flow study showed effective long distance transport. Germination success of all studied species was influenced by habitat (forest type and soil pH), together with presence/absence of suitable mycorrhizal fungi on the site, and the highest germination rate occurring at the occupied sites. Although seed germination also occurred at unoccupied sites, dispersal limitation seems to play a smaller role in orchid distribution than habitat limitation.

The influence of ecogeographic isolation on divergence in the South African orchid genus *Disa*

Karl J. Duffy¹, Ethan Newman², Steven D. Johnson²

¹Department of Biology, University of Naples, Italy; ²School of Life Sciences, University of KwaZulu-Natal, South Africa

Ecogeographic isolation, the geographic isolation that arises from ecological differences between taxa, represents a major pre-mating barrier and may be seen as a driving force in the divergence of plants. However, the extent of its influence on plant divergence over large spatial scales is unclear. We tested this by quantifying ecogeographic isolation between 24 well resolved sister taxa (12 taxon pairs) belonging to the charismatic orchid genus *Disa* in South Africa. Environmental niche models show that these taxa varied substantially in their overlap niche. As a result, there is an overall low ecogeographic isolation, with *Disa* taxa overlapping in 50% of their ranges on average with their closest relatives. However, ecogeographic isolation was often highly asymmetric between species pairs as the geographical range of one taxon often is in complete sympatry with their sister taxon, while the sister taxon has a highly allopatric range. This high level of sympatry may indicate that divergence may have occurred in the absence of large-scale geographical barriers. However, we found that the proportion of both current range and niche sympatry increased with increasing time since taxon divergence, indicating that divergence occurs mainly in allopatry in *Disa*. These results reveal that ecogeographic isolation may be a primary driver of diversification in these orchids. In addition, other mechanisms such as pollinator mediated selection may account for the current stable sympatry observed within *Disa*. These results emphasise that quantifying the patterns of ecogeographical isolation can greatly improve our understanding of mechanisms underlying orchid divergence.

Factors associated with the distribution of orchids in the Czech Republic and their comparison from two different regions

Zuzana Štípková^{1,2}, Dušan Romportl^{1,3}, Veronika Černocká², Kristina Kosánová², Pavel Kindlmann^{1,2}

¹Global Change Research Institute, Czech Academy of Science, Czech Republic; ²Institute for Environmental Studies, Charles University, Czech Republic; ³Department of Physical Geography and Geoecology, Charles University, Czech Republic

Orchids are an endangered plant group therefore questions about their conservation are lively discussed; however, not all factors affecting their survival and distribution are known. The purpose of this study was to determine and compare the environmental and climatic factors influencing the existence of certain orchid species in their localities among two different model regions — South Bohemia (more or less flat land in lower altitudes) and Jeseníky mountains (mountain range around 1000 m a.s.l.) in the Czech Republic. Our data were analysed using the MaxEnt program, which produces species distribution models and allows for the prediction of potential occurrences of orchids in yet unknown localities. This program also determines the factors affecting species presence. This is important in order to better protect orchids because only by knowing these factors, we can find new localities and can improve management plans. We studied three orchid species growing in South Bohemia as well as in Jeseníky mountains: *Dactylorhiza majalis*, *Dactylorhiza fuchsii* and *Platanthera bifolia*. The main factors affecting their occurrence in both regions were the consolidated layer of ecosystems, habitat heterogeneity and cover of arable land. However, if we compare, for example, preferred type of habitat of each species in a particular region, the results are a bit different for each of the studied regions. Also, a single factor which is important for particular species, can affect its distribution to a different extent in Jeseníky mountains compared to South Bohemia. We also determined area where new sites are most likely to be discovered and illustrated them in the maps of both regions. This approach can help in finding new localities of orchids as well as aid in the understanding of which factors affect the occurrence of these endangered orchid species in both regions.

How to find determinants of orchid occurrence

Iva Traxmandlová^{1,4}, Kristina Kosánová², Zuzana Štípková^{1,2}, Dušan Romportl^{1,3}, Pavel Kindlmann^{1,2}

¹Global Change Research Institute, Czech Academy of Science, Czech Republic; ²Institute for Environmental Studies, Charles University, Czech Republic; ³Department of Physical Geography and Geoecology, Charles University, Czech Republic; ⁴Center of Biology, Geosciences and Environments, University of West Bohemia, Czech Republic

Orchids are an endangered plant group therefore questions about their conservation are lively discussed; however, not all factors affecting their survival and distribution are known. The purpose of this study was to determine the environmental factors influencing the existence of certain orchid species in their localities -South Bohemia. Our data was analyzed using the MaxEnt program, which produces species distribution models and allows for predicting potential occurrence of orchids in unknown localities. This program also determines the environmental factors affecting species presence. This is important in order to better protect orchids because only by knowing these factors, we can find new localities and can improve management plans. We studied two orchid species growing in South Bohemia, Czech Republic: *Cephalanthera damasonium* and *Epipactis atrorubens*. The main factors affecting their occurrence were the consolidated layer of ecosystems, habitat heterogeneity, proportional cover of arable land in the vicinity of the site and vertical heterogeneity. We determined areas where new sites are most likely to be discovered and displayed them in the maps of the area. This approach can help us find new localities of orchids and can help us understand the environmental factors that influence the occurrence of these endangered orchid species.

Factors affecting the dynamics of orchid populations

Ekaterina Zheleznaia

Peoples' Friendship University, State Biological Museum, Russia

A number of factors affect the dynamics of orchid populations including internal (life forms, phytocoenoses and population strategies, external weather, succession, and anthropogenic activities). I will discuss the dynamics of orchid populations in both the European and Asian parts of Russia in terms of changes in vegetation in forests and meadows, zoogenic mosaic of the vegetation, impacts of fires, windfalls, typhoons and anthropogenic activities. Population dynamics of the following species were studied: in Eurasian range - *Dactylorhiza incarnata*, *Epipactis palustris*, *Cypripedium calceolus*, *Cypripedium macranthum*; in predominantly the Asian range: *Cypripedium ventricosum* and *Cephalanthera longibracteata*. Fluctuations were characteristic for *C. calceolus* and *E. palustris* mature populations. In *C. calceolus* populations it manifests in the periodicity of the waves of renewal and the plants' transition to secondary dormancy, while in *E. palustris* populations — only in the latter. Asynchronism of the long-term dynamics of ontogenetic spectra and the number of populations in relation to each other ensures the stability of the entire territorial complex of the *C. calceolus* population. Dynamics of the successive type is a characteristic for young and regressive populations of *E. palustris* and *D. incarnata*. When environmental conditions change, habitats of *D. incarnata* degrade faster than those of *E. palustris*. This is due to a narrower ecological amplitude, as well as illumination, soil moisture and exclusively seed reproduction. *E. palustris* can reproduce both in seed and in a vegetative way. In the European part of Russia and the Russian Far East, seed reproduction of *D. incarnata*, *E. palustris*, *C. calceolus* and *C. longibracteata* often occur in wild boars' diggings. Showing features of ruderal strategy, forest populations expand and increase in density due to fires and windthrows resulting in better illumination conditions. Orchids may exist for a long period in low numbers, in low vitality and even move into secondary dormancy under unfavorable conditions during the next stages of succession. The species having narrow ecological tolerance like *D. incarnata* may disappear during the overgrowing of meadows in the absence of grazing ungulates and haymaking. Special management practices are required in nature reserves by imitating natural processes to establish the forest and meadow mosaic pattern.

Vegetation types with *Ophrys insectifera* in Salten, northern Norway, and their importance in conservational contexts

Jørn Erik Bjørndalen

Jens Bjelkesgate 16A, N-0562 Oslo, Norway

Ophrys insectifera has some of its richest populations in Norway in the district of Salten, just north of the Arctic Circle in northern Norway, an area otherwise known for its high diversity of plants and rich vegetation types that are confined to marble substrate and to sea shell deposits along the coast. The species occurs in the basiphilous pine forests as well as in a variety of calcareous open vegetation types such as e.g. rocks, dry meadows, screes, sand dunes, and sea shell sites. All of the habitat types are generally species-rich, including many orchids and other rare species. They have a high value in conservational contexts. An outline of species composition, appearance in the landscape and notes of ecological features of the vegetation types based on synedrial plots with *O. insectifera*. Most of the localities with *O. insectifera* in the region are protected, mainly as natural reserves. However, further conservation efforts are needed, including management in some of the localities.

North American Orchid Conservation Center — an update

Dennis Whigham, John O'Neill, Melissa McCormick, Julianne McGuinness

Smithsonian Environmental Research Center, USA

The North American Orchid Conservation Center (NAOCC), established by the Smithsonian Institution and the U.S. Botanic Garden, has the goal of assuring the long-term survival of native orchids in the U.S. and Canada. NAOCC has grown from just a few initial collaborators to a network of more than 50 allied individuals and institutions across the US and Canada. The network is comprised of regional groups based in one or more states or provinces, each including at least one nationally-recognized botanic garden. Our approach is to develop an ecologically-based conservation model that includes citizen science volunteers, educators and scientists engaged in three essential endeavors: 1. Conservation of orchid and fungal genetic diversity in seed and fungal banks, 2. development of protocols for propagation and restoration of native orchids, 3. education and outreach to increase botanical literacy using orchids as model organisms. Progress to date includes development creation of regional seed and fungal banks, and protocols which establish best practice methods for collecting samples and procuring collections with approval from landowners. NAOCC has also developed educational materials, (*Go Orchids* website, *orchid-gami* paper punch-out models) and started our first outreach effort in which middle-school students in Maryland, Washington DC, and Florida conduct parallel orchid experiments in classrooms. This discussion will review these programs and other NAOCC activities including efforts to secure sustainable funding to ensure the survival and growth of NAOCC's orchid conservation work.

Have DNA-based analyses lived up to their promise?

Richard M. Bateman

c/o Royal Botanic Garden Kew, Richmond, U.K.

Within a particular group of orchids, species — the fundamental unit of currency in any biological study — remain authoritarian, typological (and thus hypothetical) constructs rather than self-delimiting biological realities until they have been studied through a modern monographic approach that samples widely populations and individual organisms for all relevant taxa. Although some historical information can be extracted from the genome, molecular typology is only marginally more satisfactory than morphological typology. The optimal approach to taxonomic delimitation is integration of genotypic and phenotypic data for the same set of individuals, thereby allowing various forms of statistical delimitation of species and genera by identifying genuine discontinuities. Recently developed DNA-based methods collectively termed next-generation sequencing (NGS) have somewhat strengthened molecular phylogenetic knowledge, but truly effective species delimitation must await the transfer of highly accurate DNA-sequencing technologies from the laboratory to the field, so that all orchid enthusiasts can not only benefit from but also, more importantly, contribute to central repositories molecular and associated morphological data. Thus far, DNA analyses have indeed lived up to their promises — but only in cases where those promises were comparatively unambitious.

Phylogeographic implications of the breakdown of mutation-drift equilibrium in the clonal-sexual orchid *Cypripedium calceolus*

Michael F. Fay¹, Samuel Pironon¹, Ekaterina Zheleznyaya², Michele Sanchez¹, Zoltan Balázs³, Dorina Podar³, Timothy Wilkinson¹, Anne Jäkäläniemi⁴, Tiit Kull⁵, Henry Väre⁶, Roberta Gargiulo¹

¹Royal Botanic Gardens, Kew, UK; ²Peoples' Friendship University of Russia, Russia; ³Babeş-Bolyai University, Romania; ⁴University of Oulu, Finland; ⁵Estonian University of Life Sciences, Estonia; ⁶Finnish Museum of Natural History

We assessed genetic variation and reconstructed demographic history for Eurasian populations of *Cypripedium calceolus*. Samples (>900, 56 locations) were genotyped at 11 nuclear microsatellite loci; plastid sequences were obtained for a subset of these. Genetic diversity analyses revealed high heterozygosity and allele diversity, with no geographical trend, and population differentiation was more compatible with a neutral model of isolation by colonization. Three genetic clusters were identified with extant gene pools from glacial refugia in the Mediterranean, central Europe and eastern Asia. Siberian populations possess different plastid haplotypes, supporting an early divergence for the Asian gene pool. Demographic results based on genetic data are compatible with an admixture event explaining differentiation in Estonia and Romania, and they are consistent with past climatic dynamics inferred through species distribution modelling. The results support a central role of clonal reproduction in shaping genetic diversity of *C. calceolus* and affecting spatial patterns of differentiation, with a small contribution from long-distance dispersal. Genetic drift may play a minor role in the establishment of long-term genetic variation, whereas the dynamics of the clonal system are responsible for the persistence of ancestral variation and stability during glacial periods and post-glacial expansion.

Using a genomic approach, RAD-seq, to unravel the recent evolutionary histories of European orchids

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Genomic methods have revolutionised our understanding of phylogeny and phylogeography of European orchid groups, where classical molecular phylogenetic methods were unable to gain a profound insight. In recent years, we have prepared several RAD-seq libraries for the taxonomically controversial terrestrial orchid genera *Epipactis* (rich in autogamous species), *Dactylorhiza* (rich in allopolyploid species) and *Ophrys* (pseudocopulatory, but less rich in species than often supposed). We gained useful experience when working with these (and other) plants in the laboratory. In this presentation, we will draw the attention of potential users of these powerful genomic methods to some of the pitfalls and caveats that we uncovered during our studies of these orchid groups. We will also offer practical advice on some methodological aspects of RAD-seq that are specific to orchids. As our genomic results have provided new insights into the evolutionary history of these interesting orchids, we can confidently advocate the use of genomic methods such as RAD-seq to other orchid groups, particularly those suspected of undergoing a recent evolutionary radiation.

The taxonomy of *Sobralia* — a significant but poorly known element of Neotropical orchid flora

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Sobralia Ruiz & Pav. genus was established in 1794. Since then, over 240 taxa have been found in Central and South America and were described within the genus which makes it a significant element of orchid flora in Neotropics. Representatives of *Sobralia* are usually tall, erect, terrestrial plants with terminal inflorescence producing a single flower successively. The flowers are large, delicate, and vary in color — white, yellow, pink, violet or purple. The ephemeral flowers of *Sobralia* are often damaged in herbarium material, thus it is difficult to obtain the data necessary for the taxonomic revision of the group. The morphological study is possible only with large amounts of specimens. Until now, few attempts of the infrageneric classification have been made based on the position of the inflorescence and flowers structure. However, taking into account the progress of the methods used in taxonomy and the increase of *Sobralia* species number, the genus requires a modern revision. The phylogenetic study of *Sobralia*, confirmed the polyphyletic character of the genus. *Sobralia* also appears to be highly heteromorphic especially considering the position and architecture of inflorescence, lip form, and the type of its outgrowths, length, and form of stamens of column. The infrageneric relationship of *Sobralia* species was revealed by analyses of three DNA markers (nuclear ITS and *xdh*, and plastid *matK*). The nominal section of the genus appeared to be more related with *Elleanthus* and other genera of Sobralieae than with the remaining species of *Sobralia*. The group differs from other representatives of the genus by the position and architecture of inflorescence, smaller floral bracts, and the morphology of the flowers. The investigations gave backgrounds to elevation the section to a generic rank. The proposed changes make *Sobralia* a monophyletic genus; however, further studies are required. The infrageneric classification should be verified on the basis of both morphological and molecular study results.

The power of movement in orchids, a kinematics study of *Porroglossum* (Pleurothallidinae) evaluated in the context of their phylogeny reconstructed using NextGen GBS Data

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Darwin was fascinated by the power of movement in plants, and if he had known about the genus *Porroglossum*, he almost certainly would have considered these small Neotropical plants to be among the most remarkable of all orchids. We say this because physical stimulation of the flower's labellum causes it to actively snap inward thrusting pollinators against the column in a floral trap. We reconstructed the evolutionary history of *Porroglossum* using a combination of nuclear (*XDH*), ribosomal (*nrITS*), and plastid (*matK*, *ycf1*, *rpl32-trnL*) markers, but interspecific relationships were not fully resolved. Additionally, a majority of the species were characterized by relatively short branch lengths indicating a recent origin for the genus. To compensate, we turned to NextGen genotyping by sequencing (GBS) for phylogenetic inference, and generated thousands of SNPs from the raw sequence data. Support values for the relationships among clades increased substantially (mostly to 100% bootstrap support) and the tree was fully resolved. For our kinematics study we recorded high-speed videos (60fps) of the active, floral snap-traps of nearly all *Porroglossum* species, and used Tracker to calculate the average time to snap-trap closure for each species as well as the average velocity and acceleration of the snap-trap for each species. Furthermore, we implemented statistical methods in R to map the aforementioned continuous traits for each species onto the GBS phylogeny to calculate phylogenetic signal. By doing so, we documented strong signal using both Pagel's and Blomberg's *K* statistic indicating that closely related species resemble each other more than expected by chance in relation to time, velocity, and acceleration. A pattern of correlation with elevation also emerged. Our study, therefore, considers the physiology, structure, pollination biology, and 'behavior' of these remarkable orchids through the lens of their ecology and evolution history.

Macro- and microevolutionary drivers of allopolyploid evolution in *Dactylorhiza* (Orchidaceae)

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Whole genome doubling (WGD) is a central force shaping plant evolution. Early-generation allopolyploids need to quickly accommodate divergent genomes into one nucleus by adjusting organization and function, with ecological consequences. The orchid genus *Dactylorhiza*, with its diverse array of polyploid lineages that are often ecologically distinct, constitutes an excellent system to investigate allopolyploid evolution. With over 5,000 SNPs derived from RADseq, and different phylogenetic and coalescent methods, we documented the origins of more than ten independent *Dactylorhiza* allopolyploids. We bring examples of frequent gene flow in contact zones between related polyploids of different ages. This process enriches the genetic pool of involved lineages, but the patterns observed point to some genomic regions resilient to admixture. This in turn, suggests a strong divergent selection required in order to maintain the observed phenotypic divergence. We further exemplify with RNAseq and smRNAseq the molecular basis of phenotypic and ecological divergence between two of these independent, sibling allopolyploids (*D. majalis* and *D. traunsteineri*). Based on Ks estimates, their diploid parental species have diverged 10 MYA; today their transcriptomes are highly divergent, indicating a major transcriptomic shock associated with the origin of allopolyploids. Today, significant expression differences between these allopolyploids affect several ecologically-relevant genes. For example, genes in the photosynthesis metabolic pathway have been significantly unregulated in *D. traunsteineri*, which has adapted to northern environments. Alternative dominance is confirmed by differential homoeolog expression in each of the two polyploids and does not support a recently formulated hypothesis of a generally dominant genome that is retained over different WGD events. Finally, we conclude that the major transcriptomic divergence observed among the diploid parents became reconciled in different ways in the sibling *Dactylorhiza* polyploids, as a result of an interplay between stochastic genomic alterations and distinct selection pressures specific to their respective environments.

Molecular phylogeny and taxonomy of the genus *Paphiopedilum* (Cypripedioideae, Orchidaceae)

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The division of the genus *Paphiopedilum* into subgenera and sections is based mainly on floral morphology and the types of leaves. The plastid DNA analyses strongly supports the infrageneric approach proposed by Cribb (1998). There are three independent evolutionary lineages, corresponding to the subgenera *Parvisepalum*, *Brachypetalum* and *Paphiopedilum*. However, the relationships between sections in the subgenus *Paphiopedilum* are not clear. Until this study, none of the four low copy nuclear markers analysed by Guo et al. (2015) and one by Górniak et al. (2014) showed topology congruent with plastid and morphological data. Our analyses of nuclear genes support morphological analyses and confirm the existence of two evolutionary lineages within subgenus *Paphiopedilum*: the sections *Barbata* and *Paphiopedilum* and the multiflowered sections (i.e. *Cochlopetalum*, *Pardalopetalum*, *Coryopedilum*). We also proved the existence of a third, monotypic lineage containing *P. canhii*. Based on the molecular data, we propose a new infrageneric classification of the genus *Paphiopedilum*.

Systematic implications of trichomes in problematic species, *Polystachya paniculata* (Sw.) Rolfe (Orchidaceae)

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The pantropical genus *Polystachya* Hook. is the largest genus of the subtribe Polystachyinae Schltr. (Orchidaceae) with over 200 species classified within thirteen sections. One of the most problematic species from the taxonomic point of view is the African epiphytic orchid *Polystachya paniculata* (Sw.) Rolfe, being a unique species among other Polystachyinae, based on its bright orange flowers with red markings on the lip. Some authors consider it to be a member of *Polystachya* sect. *Caulescentes* Kraenzl.; however, the section is characterized by clustered, reed-like stems without prominent pseudobulbs, whereas *Polystachya paniculata* forms distinct cylindrical or lanceolate pseudobulbs. Other authors classify the species within the nominal section of the genus *Polystachya* Hook. The trees topology from the Bayesian and parsimony analysis of combined data shows *Polystachya paniculata* in these analyses as neither a part of the clade representing the section *Polystachya* nor the section *Caulescentes* which form an isolated clade sister to other clades. The results of the recent micromorphological analyses of the labellar trichomes show that *Polystachya paniculata* differs from all other representatives of the sections *Polystachya* and *Caulescentes* in that moniliform hairs are lacking, clavate, cylindrical in 1- to 2-celled trichomes, and are scattered throughout the proximal part of the labellum.

Mycoheterotrophy and mixotrophy in orchids: an update

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A particularity of orchids is their mycoheterotrophic germination, where reserve-less seeds develop into a heterotrophic seedling thanks to the colonization and carbon provided by a symbiotic fungus. In most cases the seedling later forms green leaves and the fungus generally belonging to the polyphyletic 'rhizoctonia' aggregate, then turns into a purely mycorrhizal fungus, which colonizes roots only. At this adult stage, most green orchids are believed to become autotrophic and to reward the fungus with their own photosynthetic carbon, as in most other mycorrhizal associations. However, some species rely on mycoheterotrophy at adulthood and lost photosynthesis. This evolution of non-green species occurred ca. 50 times independently in the orchid family. It was more recently realized that some green orchids, phylogenetically related to mycoheterotrophic species, although photosynthetic, are partially mycoheterotrophic, with a strategy called mixotrophy. In the later species, difference in isotopic abundance (¹³C) between fungal and photosynthetic carbon and the examination of albinos (rare achlorophyllous variants that survive *in natura*, thanks to full mycoheterotrophy) were instrumental in the elucidation of mixotrophy. Mycoheterotrophic and mixotrophic species rely on the symbiotic shifts from the usual rhizoctonia partners to taxonomically and ecologically different taxa, which are either saprotrophic (in the tropics mainly) or mycorrhizal on surrounding trees. Moreover, mixotrophy is viewed as an evolutionary step toward mycoheterotrophy. More recently, isotopic particularities found in most green orchids that are putatively considered autotrophic have raised the possibility that they are mixotrophic as well. Evidence exists that they receive carbon from their fungal partners although a budget of the plant-fungal exchange remains elusive thus questioning the exact definition of the concept of 'mixotrophy'. We discuss this issue in an evolutionary perspective and also address the limits of isotopic approaches, in order to suggest further research on mixotrophy in the orchid family.

Looking belowground to understand orchid distributions aboveground

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The aboveground distribution and abundance of orchid populations depends on a suite of biological and ecological factors, including seed production and dispersal, the availability and belowground distribution of suitable mycorrhizal fungi, and local environmental conditions appropriate for seed germination and growth to a seedling. Disentangling the factors that determine a successful orchid establishment, represents a major challenge and requires a combination of seed germination experiments, molecular techniques, and assessments of environmental conditions. In this talk, I will present results of studies that have investigated the relative role of mycorrhizal fungi in determining the aboveground distribution of orchids. Identification of fungi from large-scale surveys of mycorrhizal associations in a range of orchid species has shown that mycorrhizal fungi may be widespread and can occur in a range of habitats. Seed introduction experiments have revealed that seed germination does not necessarily differ between occupied and unoccupied habitat patches thus suggesting that the availability of fungal associates is not necessarily the limiting factor determining the large-scale distribution of orchid species. Nonetheless, seed germination within sites appears to depend on both biotic and abiotic conditions. Recent molecular studies have shown that fungal abundance declines rapidly with the distance from the adult host plants. Likewise, the decline of fungal abundance around the adult plants coincided with the decline in the neighborhood density of recruits around adults, suggesting that fungal abundance and orchid population dynamics are to some extent related to each other. Interestingly, coexisting orchids appear to have largely distinct mycorrhizal communities and show strong spatial segregation, suggesting that mycorrhizal fungi are important drivers of niche partitioning and contribute to orchid coexistence. Overall, these results indicate that the distribution and abundance of fungal associates in the soil may have a strong impact on the aboveground distribution of its partner within local habitat patches.

Is the mycorrhizal fungi distribution limiting the orchid establishment in restored meadows?

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Natural grasslands in Central Europe have suffered a dramatical biodiversity decline due to the overexploitation, land abandonment, or conversion to arable land. For orchids, the restoration of their populations is particularly challenging due to their dependence to the mycorrhizal fungi for germination, and our poor knowledge on the distribution and diversity of their associated fungi. Here, we investigated how the abiotic (soil conditions, age of restored grasslands) and biotic factors (diversity of mycorrhizal fungi) affect germination of orchids in restored grasslands. We focused on six rhizoctonia-associated orchids in 29 grasslands that had been restored 3 to 28 years ago and also in 31 natural grasslands in the White Carpathians Mountains (Czech Republic). We investigated i) in situ orchid germination (using the seed baiting technique) and ii) the diversity of mycorrhizal fungi (using next generation sequencing), associated with adult orchid roots and soils, in restored and natural grasslands. We found that among the studied orchid species, *Gymnadenia conopsea* and *Neottia ovata* exhibited both the highest fungal diversity in adult plants and germination success rate in restored grasslands. No significant relationship was found between germination success and age of restored grassland. Thus, diversity of orchid mycorrhizal fungi in restored habitats might be one of the major factors limiting the establishment of some orchid species. The slow restoration of orchid species due to the absence of appropriate fungi could be potentially overcome by the targeted introduction of fungi and substrate amelioration of restored sites.

Partial mycoheterotrophy is more widespread among orchids than previously assumed: A multi-element stable isotope natural abundance approach

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Based on their isotopic positioning between autotrophic non-orchids and fully mycoheterotrophic orchids a couple of green-leaved orchids have been elucidated during the last decade as gaining simultaneously carbon from two different sources: own photosynthesis and fungal supply. This mode of mixotrophic carbon gain is called partial mycoheterotrophy. All of these ^{13}C - (and ^{15}N -) enriched partially mycoheterotrophic orchids are mycorrhizal, with fungi simultaneously forming ectomycorrhizas with forest trees. While the majority of green orchids worldwide are mycorrhizal with fungi of the phylogenetic heterogeneous assemblage of rhizoctonias. Recent work has shown that carbon and nitrogen isotope abundance is not suited to unequivocally identify partially mycoheterotrophic species among rhizoctonia-mycorrhizal green orchids. Here we provide an essentially new approach to identify species with partially mycoheterotrophic nutrition among rhizoctonia-mycorrhizal orchids. In addition to carbon and nitrogen stable isotope abundance, we analysed hydrogen isotope abundance in dry tissues of a fully MH orchid. It was also investigated in green orchids associated either with ectomycorrhizal fungi or with rhizoctonias, in autotrophic reference plants and in sporocarps of ectomycorrhizal and saprotrophic fungi growing together in the understorey of a dark beech forest and within a broad range of orchids and autotrophic reference plant from sunny alpine meadows. It turned out that almost all orchids were significantly enriched in heavy hydrogen isotopes regardless of whether they were enriched in heavy carbon isotopes or not. Thus, partial mycoheterotrophy appears to be much more widely distributed among green orchids than previously assumed. Identical oxygen stable isotope abundance in orchids and reference plants allowed us ruling out transpiration as responsible for the differences between orchids and reference plants in hydrogen isotope abundances.

The mixotrophic *Epipactis helleborine* does not necessitate a link to C-providing fungi: lessons from pot culture

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Epipactis helleborine (L.) Crantz is a mixotrophic orchid that recovers carbon both from its mycorrhizal fungi and its own photosynthesis. The fungi mycorrhizal in *Epipactis helleborine* mostly belongs to *Tuber* as well as other ascomycetes and Sebacinaceae, which are all ectomycorrhizal on surrounding trees. These trees are the ultimate C source of the whole consortium. The fungal organic matter is enriched in ^{13}C due to a fractionation against ^{12}C at the tree/fungus ectomycorrhizal interface, and the gain of C by *E. helleborine* from its mycorrhizal fungi thus entails a ^{13}C enrichment of its tissues as compared to these of surrounding C3 autotrophic plants. Due to this dependence on fungal networks with surrounding trees, *E. helleborine* is believed, as other mixotrophic plants (e.g. *Epipactis* or *Cephalanthera* spp.), not to be transplantable. Yet, there are (very) few reports of successful transplantations and of pot cultures of *E. helleborine*. One author (J.D.) established pot cultures of *E. helleborine* (from the subspecies *minor* and *moratoria*) issuing from different natural German populations that survived over 4 years. On the fourth year, isotopic investigations showed no ^{13}C enrichment of leaves as compared to these of C3 autotrophic weeds growing in pots, supporting an autotrophic growth. To further understand and characterize the life mode of these plants, we identified root fungi by barcoding using next-generation sequencing. We report that the ratio of rhizoctonias (usual orchid mycorrhizal fungi belonging to Ceratobasidiaceae, Tulasnellales and Serendipitaceae, which provide few or no C to orchids) to ectomycorrhizal fungi (that massively provide C to mixotrophic orchids, provided they access a tree) is, as expected, very unusual. *E. helleborine* can therefore survive in an autotrophic way, similarly to its ancestors. Our results show the extreme plasticity mixotrophy can occur in some species and have important consequences for orchid conservation.

Global drivers of orchid demography

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Data from nearly 300 populations of over 100 species of plants were used to evaluate the demographic characteristics of orchids, particularly relative to other plants that are capable of vegetative dormancy (i.e. the phenomenon in which herbaceous perennial plants remain belowground for ≥ 1 year). Most species displaying dormancy exhibit related life history costs, especially associated with sprouting. Mean annual proportions of dormant plants are higher in shorter-lived species and decrease from holomycotrophs to mixotrophs to autotrophs. Maximum duration of dormancy differs between species and between populations within species, and neither it, nor mean proportions of dormant plants are similar in closely-related species, suggesting lability in the evolution of dormancy. Maximum duration of dormancy is greater in species exhibiting sprouting costs, and it varies with latitude. Loss of foliage in one year is a common driver of high future dormancy. Throughout the history of the land plants, including the common ancestors of the ferns and flowering plants, dormancy appears to have evolved repeatedly, probably reflecting different genetic contexts across phylogeny. However, the evolutionary history of dormancy suggests a common origin in orchids. This analysis reveals an urgent need for more long-term demographic studies to examine the role of temporally-varying factors, including weather and herbivory, on sprouting behaviour in plants, and in orchids in particular.

Unravelling the population dynamics of orchids as high-fecundity organisms

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Orchids belong to the Type-III strategists group which are characterized by very large fecundity and high levels of mortality at early stages. Although factors associated with their reproduction and population size are known to affect their survival, little research exists on the orchid population dynamics. In the present study, we present findings of a model on orchid population dynamics. We have created a dynamic population model which was run under various scenarios of fecundity, mortality coefficient, environmental variability, initial population size, and exploring the dependence of population variability on these factors, as well as the potential extinction risk. Population size is affected by an interplay between environmental variability and fecundity, causing changes within and between generations, as well as increasing the extinction risk. The population variability is thus observed to be dependent on both these variables. Population variability increases proportionally with environmental variance and also with fecundity at small values. This increase with fecundity slows down for large fecundity values. As expected, the probability of extinction increases as the populations get smaller, and as environmental variability gets larger. However, extinction risk increases with higher fecundity, even in populations with many individuals. Further studies in this area will help us understand more about the population ecology of orchid species and how to confront the associated conservation challenges.

Climate-driven variation in demographic responses of *Himantoglossum adriaticum* at the Northern distribution limit

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Understanding the effects of climate constraints on population vital rates, is key to predicting population dynamics and persistence probability of plant species. Estimating with accuracy which vital rates are most responsive to climate conditions and the integrated effects of vital rate variation on long-term population dynamics requires detailed demographic observations with high temporal resolution; however, such data is not available for most plant species. In this study we used demographic data collected over 22 years for a population of *Himantoglossum adriaticum* H. Bauman in Keszthely, Hungary, to quantify the effect of climate constraints on demographic vital rates and long-term population persistence at the species' Northern geographic range limit. We expected that in this species with a Mediterranean core area of distribution, the northern population is limited by the interaction between summer and winter temperature and summer and winter precipitation. We fitted vital rate models for mature and young plants separately. Plant size increased the probability of survival and growth in both young and mature plants, while in mature plants it decreased the probability of dormancy and it increased the reproductive effort. Summer and winter climate conditions did not have a consistent effect on all vital rates across mature and young plants. High winter temperature and precipitation combined, increased the survival probability and growth, while high summer temperature and precipitation combined increased the flowering probability and number of flowers of mature plants. High summer precipitation decreased the survival and growth, and high winter precipitation decreased the dormancy probability of young plants. Our results suggest that climate limits population performance through different demographic pathways and via interacting effects of different climate components. Occasional favourable seasons might have a boosting effect on different vital rates by enhancing the overall long-term population viability in populations situated at species' geographic range limits.

A novelty look at terrestrial orchids through a prism of the ecosystem concept

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Terrestrial orchids are involved in a wide range of ecosystems throughout the world, despite the general regard of this plant family as highly specialized. The ecosystem concept is designed to assess a flow of matter and energy linking living organisms and their abiotic environment into a system. Orchids themselves can be recognized as small ecosystems directing carbon flow from photosynthesis and from symbiotic fungi, utilizing phosphorus with and without help of mycorrhiza, depositing carbohydrates and proteins, and releasing them with nectar and pollen. Flows of energy and matter vary greatly among different orchid species. Plant morphology captures those variations and gives an opportunity to assess them without instrumental help. A concept of Growth Habit explains the relationship between a plant body and its environment. This talk will explore the links between two concepts, orchid species representing different types of ecosystems and growth habits.

The evolution of reproductive isolation in Mediterranean orchids

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Food and sexually deceptive orchids are characterized by different levels of pollinator specificity. Whereas food-deceptive orchids show weak pollinator isolation, sexually deceptive species display a highly specialized pollination. Comparative analyses show that specialized orchids mostly rely on few pre-mating reproductive barriers and have very little postmating isolation. In contrast, orchids with weak pollinator isolation, such as food-deceptive orchids, show strong evidence for multiple, postmating reproductive barriers. The role of pre-mating barriers in this latter group has been often underestimated. However, a recent study showed that phenological and microhabitat isolation has strongly contributed to reproductive isolation in sympatric diploid and tetraploid populations of a food-deceptive species thus suggesting that these barriers can evolve in sympatric populations to avoid or limit the formation of triploids. Sympatric populations of food-deceptive species can represent sites where selection against hybridization may strengthen prezygotic barriers between related species by improving the fitness of individuals that are less likely to hybridize and thus promoting divergence in traits involved in pre-mating barriers. Nonetheless, when several hybridizing species co-exist, their interaction is more complex because diverging traits in a species pair can overlap with those of other co-occurring species. We tested this by investigating pre- and postmating prezygotic isolation among closely related food-deceptive Mediterranean orchids. We found that the investigated species were separated by strong prezygotic barriers, but in a mosaic pattern showing large differences depending on the crossing combination and direction. We also found that the combination of phenological and pollinator isolation, i.e. two barriers that affect the likelihood of inter-specific pollen exchange, was correlated with post-mating prezygotic isolation, with this latter being stronger between sympatric individuals. These findings suggest that different prezygotic mechanisms can be reinforced by selection and when multiple closely related species co-exist, prezygotic isolation can produce a mosaic pattern depending on species ecological interactions.

Why don't deceptive orchid pollinators go extinct?

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Co-evolutionary mutualisms, such as pollination, provide reciprocal fitness benefits - but what happens when one species imposes a greater cost on the others? The sexually deceptive Australian and New Zealand Tongue Orchids, *Cryptostylis* spp., are extreme deceivers, causing their wasp pollinator, *Lissopimpla excelsa*, to ejaculate and waste limited sperm. Yet these orchids achieve outstanding pollination rates (up to 90%), but why don't their exploited pollinators go extinct? We propose a novel mechanism explaining the persistence of costly co-evolutionary relationships where costs are buffered by both population-level counter-adaptations and a new concept, species-level 'resilience' traits. To explore this, we integrate field ecology and mathematical models. Our field experiments indicate counter adaptations by male *Lissopimpla excelsa* wasp populations at sites with orchids. These male wasps spend less time mating with orchids and are less likely to waste sperm resulting in more sperm and longer antennae than populations at sites without orchids. Our mathematical models support our 'resilience' hypothesis. Haplodiploidy is a surprisingly common trait across nearly all insects fooled by sexually deceptive orchids. Unmated Haplodiploid pollinator females deprived of sperm by orchids can still produce offspring, albeit all sons. These overabundant sons could further enhance orchid pollination rates and fitness, while providing enough males and sperm to maintain the wasp population, thus providing a 'resilience' trait. Our model found that when exposed to the same level of exploitation, haplodiploid orchid pollinators will persist over an evolutionary time while diploid pollinators can go extinct within 300 generations.

Pollination strategy of a food-deceptive orchid: a community approach

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The phenomenon of mimicry in plants is frequently found in orchids. *Traunsteinera globosa* is a food-deceptive orchid assumed to mimic a guild of plants with locally differing model species. Its major pollinators are widely distributed, generalist syrphid, and empidid flies. In previous studies, putative model plants were mainly selected by visual similarities to the orchid which was judged by the human eye and quantitative analysis of visual and olfactory floral cues restricted to these species. Similarly, the pollinator spectrum was only investigated for *T. globosa* and these models. It remains unknown whether these orchids share pollinators also with other co-flowering plants and if so, how similar their floral traits are to the orchid and the putative models. We analysed visual and olfactory flower cues of an alpine plant community containing *T. globosa*, observed the pollinator network, recorded the orchid's fruit set, tested the importance of scent for pollinator behaviour in an experiment, and identified physiologically active scent compounds. Our results show that in addition to the putative models, another eight co-flowering plants shared pollinators with the orchid. Six of them were fly-blue as the orchid and two were fly-purple. All co-visited plants shared a number of physiologically active scent compounds with the orchid and several of them were not available in the putative model plants but the overall scent blends differed among the species. The behavioural experiment showed that pollinating flies use the orchid's scent as an attractive cue. These results point to a more generalist pollination strategy of *T. globosa*, involving not only the putative models, but also other co-flowering plants.

Food or sexual deception? Untangling the pollination strategy of the fly-pollinated orchid *Neotinea ustulata* (Orchidaceae)

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European deceptive orchid species are pollinated predominantly by Hymenopterans who are searching for food or sexual partners. One of the exceptions is *Neotinea ustulata*, which seems to be adapted to pollination by tachinid flies; however, some flower-visiting beetles were reported as well. Likewise, food deception may also occur as its flowers are nectarless. To untangle the pollination strategy of the species, which possess two phenologically distant varieties (i.e. *N. ustulata* var. *ustulata* and var. *aestivalis*), we recorded pollinators in various parts of Europe, and evaluated important visual and olfactory cues for the pollinator attraction. We found that the early flowering variety is pollinated by a broader spectrum of pollinators, including flies and bees, while the later flowering variety is pollinated solely by male tachinid flies. The very dark top of an inflorescence is highly contrasting with the white flowers, its colour is indistinguishable in a fly-vision model from the fly abdomen colour and it serves as an optical signal for fly landing. Our chemical analysis of floral extracts showed presence of diverse alkanes and alkenes, such as isomers of Tri-, Penta-, Hepta- and Nonacosene. Several of those compounds were also found in cuticular extracts of tachinid flies and electroantennography showed that they are electrophysiologically active. The compound (Z)-11-Tricosene, which was dominant in the flower extracts and tachinid females, is rarely found in plants and its presence in flowers addresses the occurrence of chemical mimicry. Our results suggest that *N. ustulata* evolved a complex pollination system, which combines both food and sexual deception for pollination.

New databases on orchid-pollinator interaction and fruit set in the Euro-Mediterranean region

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Biotic interactions have played a major role in the generation of biodiversity in both the plant and animal kingdoms. One of the major challenges in the study of plant-pollinator interactions is to determine what factors underlie similarity in pollination niches between plants, a question that is relevant both to ecology and evolutionary biology. In addition, the relative contribution of plant-pollinator interactions and other environmental factors in determining plant reproductive success is important both to predict the evolutionary outcome of pollination interactions and to set up appropriate conservation strategies. In this context, we present here two new databases concerning Euro-Mediterranean orchids: 1) one on orchid-pollinator interactions (1278 interactions between 243 orchid and 773 pollinator species) and 2) one on the fruit set of these orchids (171 orchid species from 1750 populations). By analysing the first database using a network approach, we show that specialization in orchid-pollinator interactions depends on orchids' pollination strategy, but also on their geographical range. We further demonstrate that the number and identity of insect families with which orchids interact depend on their pollination strategy and phylogeny, while the number and identity of insect species with which they interact depend on their spatio-temporal distribution. This suggests that if there are consistent associations between some pollination strategies or phylogenetic groups and some insect families, at a finer scale, orchid-pollinator interactions may be more opportunistic than previously thought. By analysing the second database using generalized linear mixed models, we show that orchid fruit set varies among pollination strategies and may be influenced by floral display traits, while ecological preferences of orchid species seem to have no impact on their reproductive success. These two databases on orchid-pollinator interactions and fruit set will allow comparative analysis on reproductive ecology of Euro-Mediterranean orchids and provide relevant information for population dynamics and conservation studies.

On the adaptive role of flower form in sexually deceptive orchids: insights from the Mediterranean genus *Ophrys* (Orchidinae)

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Sexually deceptive orchids have evolved into a complex combination of floral traits in order to lure male insects into attempting to copulate with their flowers. While flower scent has been shown to play the primary role in mediating mostly species specific relationship between these orchids and their pollinators, little is known about the adaptive role of flower form. Flower form could reinforce the role of scent by ensuring both short distance pollinator attraction and the mechanical fit between flowers and their pollinators. Pollinator mediated selection could thus play a crucial role in shaping the immense diversity of flower forms in sexually deceptive orchids. Alternatively, the commonness of cryptic species and high variation in floral form may be indicative of relaxed selection. Here we present the results of the first study investigating the adaptive role of flower form in the Mediterranean genus *Ophrys*. By integrating pollinator choice experiments with traditional and three-dimensional geometric morphometrics, we address not only the functional importance of flower form, but also provide a new perspective into the evolution of cryptic *Ophrys* species. Our results show that in *Ophrys* pollinator, mediated selection acts by increasing the mechanical fit between the orchids and their pollinators. However, selection pressures varied between components with which pollinators mechanically interact and those on which pollinators move more freely. Varying selection pressures on mechanically-active and —inactive form components were reflected in a strongly modular flower structure, which probably facilitates a flexible response of different flower form components to pollinator shifts. The functional dichotomy between mechanically-active and —inactive components may also provide an explanation for the prevalence of cryptic species in the genus *Ophrys*. We suggest that taking the functional differences between flower form components into account can prove to be valuable for elucidating the evolution and diversification of the flower form in sexually deceptive orchids.

Parallel adaptation to pollinator attraction in *Ophrys*

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Sexually deceptive orchids of the genus *Ophrys* use a combination of flower traits, such as coloration, shape, and scent to attract pollinators. Pollination is achieved by successfully deceiving male insects into pseudo-copulate with the flower. *Ophrys* represents a system characterized by a high degree of specificity to pollinator. This plays an important role in reproductive isolation when acting as a strong pre-zygotic barrier between species. Within the genus, parallel species radiations have occurred, whereby distantly related species have evolved adaptations to the same pollinators. Here, we focus on two pair of species from distantly related *Ophrys* lineages that have adapted to attract the same solitary bees. Understanding the similarities in genes and selected traits that lead to pollinator attraction in these parallel cases can be enlightening into the adaptation and speciation processes. A combination of genomic (transcriptome annotation, RNAseq, population genetics), and phenotypic (pigment and floral volatile extraction, geometric morphometric) analysis in a comparative context is used to determine the genetic architecture of pollinator attractive traits and the degree of parallelism within the genus.

Management affects the pollinator abundance but not the reproductive success of butterfly orchids

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In this study we examined the factors affecting the reproductive success (fruit set) of *Platanthera bifolia* and *P. chlorantha* and their pollinators in agricultural landscape and woodlands. In the years 2014–2016 we observed and caught moths during the flowering period of *Platanthera* species (late June–mid July) in four pure *P. bifolia*, five pure *P. chlorantha* and nine mixed populations under management or no management in Estonia. We determined the pollinator species richness, pollinator abundance, fruit set, and visibility of *Platanthera* plants in each population. We found that pollinator assemblages of *P. bifolia* and *P. chlorantha* did not differ between the managed and unmanaged sites. Pollinator abundance had an effect on the fruit set of *P. bifolia* but not on *P. chlorantha*. Presence or absence of management, visibility of plants and pollinator species richness did not affect the fruit set of either plant species. Pollinator abundance was significantly higher in unmanaged populations of both plant species but pollinator species richness did not differ between managed and unmanaged populations of either plant species. Based on our findings, the recommendations for management of semi-natural grasslands consist of promoting larger landscape diversity for the benefit of moth abundance by leaving unmanaged patches in different parts of a grassland among different years. It needs to have rotational or post-fruitlet management at the end of August for higher reproductive success of orchids.

Reproductive ecology of the rare mycoheterotrophic orchid *Epipogium aphyllum*

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Epipogium aphyllum is a non-green orchid that obtains carbon exclusively from its mycorrhizal fungi. It represents a highly specialised ecological interaction between plants and fungi called mycoheterotrophy. It is hypothesised that the narrow specialisation upon mycorrhizal association, typical for most MH plants, may also be a strong determinant upon their reproductive biology. *E. aphyllum* associates mostly with *Inocybe* spp. Such taxa would be expected to reveal generalization in the pollination system and/or autogamous self-pollination, since the specialisation on two traits is believed to be evolutionary unstable. Reproductive ecology of mycoheterotrophic plants, however, fundamental in understanding their evolution, remains empirically understudied. This also applies to ghost orchids for which our knowledge of reproductive biology is based on scarce and anecdotal reports. To fill this gap in knowledge, we studied pollination ecology, breeding system, flowering phenology and reproductive efforts in three populations of *Epipogium aphyllum* for three consecutive years. Field studies were also supported by population genetic analyses based on developed microsatellite markers as well as anatomical, embryological, and chemical studies. Our results confirm neither generalised pollination syndrome nor autogamous pollination in *Epipogium aphyllum*. This species is outcrossing and is being pollinated by *Bombus* species. Its flowers emit various chemical attractant and offer nectar as a reward, a cooperative interaction that has been debated for a long time. The very short lifetime of flowering shoots (12-17 days) contrasts with the long and varied anthesis time at population level (up to 21 weeks). The later features explain the difficulties in assessing the population's size or even confirming the presence of the plant in a locality (as recognized by its English name). Reproductive effort differed significantly between the populations. Its connection with mycorrhizal fungi and environmental variables will be discussed.

A case of spontaneous pollinarium reconfiguration within flowers of deceptive orchid *Dactylorhiza fuchsii*

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Orchids are strongly pollinator limited and represent many adaptations to self-pollination. The time when selfing takes place during floral lifespan is crucial for evolutionary consequence as it may guarantee a reproductive assurance (prior and delayed selfing) or competes with cross pollination during anthesis. In the case of spontaneous autogamy, many reports have indicated that it does not fall into strict classes of floral lifespan and is instead continuously distributed. In orchids facultatively autogamous it is usually noticed at the end of anthesis. A few reports referring *Dactylorhiza* species indicated accidents of delayed self-pollination due to caudicle bending movement, but others have stated that this genus is strictly allogamous. We have found the mechanism, time and intensity of spontaneous autogamy in *Dactylorhiza fuchsii*. Self-pollination was going through pollinarium twisting, to the side and downwards, which was different than caudicle banding forwards on the body of the pollinator. Pollinarium reconfiguration (*pr*) within the flower may finish at various moment of the movement, but fruits were set more frequently when at least one pollinium adheres to the stigma receptive surface. *Pr* started during anthesis and finished at the end. We recorded various stages of *pr* movement on a half of analysed, fresh opened flowers. At the end of anthesis it was recorded on at least one flower per inflorescence on 60-80% of analysed individuals per population. Autogamous fruits were rare. We discussed different aspects of *pr* appearance, reproductive results (fruit and seed set) and evolutionary consequences.

Factors influencing the distribution of *Epipactis* species in three regions of Hungary

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Significant taxonomic changes occurred in the last few decades in the helleborines (*Epipactis* spp.). Floristic data and knowledge about the distribution of the majority of taxa are incomplete. The aim of our study is to map the distribution of helleborines in Hungary in three regions (Keszthely Hills, West-Bakony Hills, Zala Hills) and to determine their ecological preferences. Between 2014 and 2017 localities of 3911 individuals of 15 *Epipactis* taxa were recorded. Using geographical (elevation, aspect, slope), soil (soil type, bedrock) and forestry (species composition, age, canopy and shrub layer coverage) data, we investigated the habitat preference of helleborines using correspondence analysis and cluster analysis. The three examined regions are significantly different in their species compositions. Almost 50% of the recorded plants were *E. purpurata* in Zala Hills, *E. nordeniorum* in the West-Bakony Hills and *E. helleborine* in the Keszthely Hills. Keszthely Hills was the single region, where every one of the 15 recorded taxa occurred. Only *E. nordeniorum* had considerable populations in all three regions. There are well separated groups of species. Some taxa occur on weathered sediments and loess in beech forests with high canopy closure (*E. leptochila*, *E. nordeniorum*, *E. albensis*, *E. tallosii*), while others prefer dolomite or sand bedrock and open mixed forests (*E. helleborine*, *E. microphylla*, *E. voethii*, *E. neglecta*, *E. peitzii*, *E. muelleri*, *E. atrorubens*). *E. leptochila*, *E. peitzii* and *E. neglecta*, all belonging to the *E. leptochila* complex, were well distinguished by their bedrock, soil type, and forest type preferences that confirmed their taxonomic discrimination. The species composition of forests and soil factors proved to be the most influential factors on occurrences. Our aim is to use these results to predict where certain *Epipactis* species are more likely to occur so that we can better evaluate the potential natural value of vegetation units.

POSTER ABSTRACTS

Dactylorhiza viridis, Biebrza National Park

Low copy nuclear gene (*Xdh*) sequences reveal at least five independent evolutions of mycoheterotrophy in tribe Neottieae

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Tribe Neottieae comprises of six genera, *Aphyllorchis*, *Cephalanthera*, *Epipactis*, *Limodorum*, *Neottia*, and *Palmorchis*, with about 240 species in the temperate to subtropical Northern Hemisphere. This group represents an ideal model for the synthesis of the evolution process from autotrophs to mycoheterotrophs, as diverse nutritional modes exist in this group. To analyze the process associated with the evolution of nutritional modes, it is crucial to establish a robust phylogenetic framework of the tribe. In this study, we performed sequencing of the nuclear xanthine dehydrogenase gene (*Xdh*) together with nuclear internal transcribed spacer (ITS) of ribosomal DNA and the plastid maturase K (*matK*) regions using 41 taxa across all the genera in the tribe, and analyzed phylogenetic relationship using Maximum Likelihood (ML) and Bayesian inference methods. All six genera were confirmed as monophyletic. After the divergence of *Palmorchis*, the second diverged *Neottia* became a sister group to the remaining four genera. Among these four genera, *Aphyllorchis* and *Limodorum*, as well as *Cephalanthera* and *Epipactis*, made clades and further formed sister groups to each other. Evolution of mycoheterotrophy likely occurred at least five times independently, viz., a clade in *Neottia*, a clade comprising *Aphyllorchis* and *Limodorum*, *Cephalanthera humilis* and *C. exigua* clade, *C. subaphylla* and *C. austiniae*. Our results also suggest that fungal partner shifts from rhizotonia to ectomycorrhizal fungi happened before the evolution of mycoheterotrophy.

Monophyly or paraphyly — The taxonomy of *Elleanthus* (Orchidaceae)

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In 1827 Carl Bořivoj Presl described genus *Elleanthus* using two species *E. linifolius* C. Presl and *E. lancifolius* C. Presl. The *E. lancifolius* was later designated by Britton and Wilson (1924) as generitype. The genus comprises nearly 150 taxa which are found from Mexico and throughout Central America, along with the Greater and Lesser Antilles to the tropical and subtropical parts of South America. Most representatives of *Elleanthus* are caespitose plants with simple or branched stems. Leaves are sessile on a sheath tightly or loosely embracing a stem, plicate or conduplicate. Leaf blade is elliptic to lanceolate, rigid, delicate, coriaceous or thin and sometimes the leaf can be long and linear like grass. The inflorescence is several- or many-flowered, always terminal, dense and capitate, or lax and elongate, straight or hanging. Occasionally the rachis is fractiflex which is an important diagnostic feature. The flowers are usually bright coloured, i.e. red, orange, purple or violet are less frequently yellow, white, or greenish. The sepals are similar in size and shape and they are usually lanceolate, elliptic or ovate-lanceolate. In some species, dorsal sepals are characterized by keels on their outer surface running along the mid-vein. The petals are oblong-elliptic to lanceolate and sometimes ligulate. Lip distinctly varies in size and shape as well as in the presence of a pair of ovoid to oblong calli located in a saccate or ventricose base. Hitherto the phylogenetic study of *Elleanthus* and other related genera showed the monophyletic character of this genus. However, the strong polymorphism of *Elleanthus* in generative as well as vegetative parts, is the reason for many systematic ambiguities in reference to the genus. Depending on the taxonomical perspective, it is treated as a monophyletic group or it can be divided into smaller genera. The results of our study include the review of herbarium material, specimens preserved in liquid, and living plants in the field as well as molecular analyses.

Molecular phylogeny of Cranichideae with emphasis on Prescottiinae (Orchidaceae) inferred from Xdh gene analysis

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Prescottiinae is a subtribe of terrestrial orchids with exclusively Neotropical distribution. Previous molecular studies have revealed that Prescottiinae belong to the paraphyletic group and genera in subtribe form two highly supported clades, *Stenoptera*-clade (*Aa*, *Myrosmodes*, *Gomphichis*, *Altensteinia*, *Porphyrostachys*, *Stenoptera*), and *Prescottia*-clade (*Prescottia*, *Galeoglossum*, *Pseudocranichis*). However, topologies of phylogenetic trees presented in various studies are not congruent. Relationships between Prescottiinae, Cranichidinae s.s. and Spiranthininae seem to be unresolved because of relatively low support for parts of clades. Nevertheless, Prescottiinae are usually lumped into broad concept of Cranichidinae. Previous phylogenetic analyses were based on ITS and various plastid DNA sequences. In the present study, the phylogeny of the group is inferred from the low-copy nuclear gene *Xdh*. The obtained results confirm the paraphyletic character of Prescottiinae which has been reported from previous works. Phylogenetic trees topologies are congruent in parsimony and Bayesian analyzes. However, the relationship among clades are unresolved by parsimony, and only Bayesian tree can provide a strong support for the resolution (Spiranthinae—(*Prescottia* clade—(*Stenoptera* clade—Cranichidinae))). The obtained results are discussed using morphology of the group in relation to the classification of the subtribe Prescottiinae among Cranichideae and compared with previously published studies.

A molecular timescale for the subtribe Polystachyinae Schltr. (Orchidaceae) based on nuclear ITS and plastid data

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Representatives of Polystachyinae (Orchidaceae) are found in southern North America and in both tropical and subtropical South America and Africa, Madagascar (and the neighbouring islands) and south-eastern tropical Asia and New Guinea. There are 10 genera and more than 200 species recognized within the group. The largest genus — *Polystachya* is divided into 13 section and contains 196 species. The subtribe is well characterized by presence of pseudobulbs, conduplicate, distichous leaves, and a paniculate or racemose inflorescence which arises from the top of the pseudobulbs and non-resupinate flowers (that possess a prominent mentum and an uppermost lip). Although a number of papers concerning morphology as well as phylogeny of Polystachyinae have been presented in the last years, the taxonomic relationships within the studied group are still not fully resolved. Molecular clock analysis were used to estimate the diversification time of presented evolutionary lineages within Polystachyinae as well as the possibility of hybridization events between them. Analyses were carried on representatives of all genera recognized within the group using nuclear ITS region as well as combined plastid data. The analyses were performed using BEAST 1.8.4 software. Calibration point for subtribe Polystachyinae was selected according to Givnish et al. (2015) and was set as 27.34 My ago. Divergence times for representatives of studied groups were estimated with a log normal relaxed molecular clock using the Yule model of speciation. The results were discussed according to the known geographical distribution of particular genera as well as their morphological affinity.

How can genome changes contribute to orchid hyperdiversity?

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Whole-genome changes and chromosome rearrangements are supposed to play an important role in plant diversification but little is known about their contribution to the extraordinary diversity observed in some lineages. The exceptionally rich subtribe Pleurothallidinae, comprising of more than 5000 morphologically highly diverse species in 20 to 100 genera native to tropical America, represents an ideal model system that can deepen our knowledge of orchid genome structure and its evolution in association with diversity. Therefore, we employed Hyb-Seq sequencing, flow cytometry, and karyology to get insight into phylogenetic relationships within Pleurothallidinae, nuclear genome sizes and endoreplication patterns, and chromosome numbers and rearrangements, respectively. Our preliminary phylogeny mostly supports the current taxonomic division. Nuclear genome size (1C-value) ranges from 0.22 to 5.41 pg and chromosome numbers span from 12 to more than 60; however, no direct link between both traits were found. Approximately one half of the investigated species exhibited progressively partial endoreplication, a unique phenomenon ascribe to orchids, and the minimum proportion of replicated genome is about 19%. Our results indicate that the hyperdiverse morphological variation in Pleurothallidinae might stem from their extremely diverse genomes and a high diversification rate.

Integrative taxonomy of the section *Pseudophrys* (*Ophrys*, Orchidaceae): making use of both genetic and phenotypic data

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Accurate species delimitation is a prerequisite for most researches about biodiversity and its management. Integrative taxonomy has been advocated for a long time, yet tools allowing true integration of genetic and phenotypic data have been developed quite recently and applied to very few models. In particular, these tools have never been applied to orchids, despite many discussions about species delimitations in this family. In this study, we investigated species boundaries within a group of twelve *Pseudophrys* taxa by analyzing genetic, morphometric and chemical data in a Bayesian framework. We found that these twelve taxa were merged into four species when only genetic data were used, while most formally described species were recognized as such when only phenotypic data were used. The result of the IBPP analysis performed on both genetic, morphometric and chemical data supports the proposal to merge *Ophrys bilunulata* and *O. marmorata* on the one hand, and *O. funerea* and *O. zonata* on the other hand. We are convinced that this integrative taxonomic approach holds great promise to conduct taxonomic revisions in other orchid groups.

Genetic diversity and population dynamic patterns of *Malaxis monophyllos* in Europe and its conservation implications

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Malaxis monophyllos is a rare orchid with a fragmented boreal-montane distribution in Europe and is associated with both natural swampy and anthropogenic habitats. In this study, we investigated genetic and demographic structures of populations throughout the whole European geographic range. The weak phylogeographic structure and low population differentiation ($F_{ST}=0.074$) with high admixture within populations of *M. monophyllos* were observed. These results serve as evidence of multidirectional gene flow between European populations that presumably took place in the past, when its distribution range was more continuous and the populations were less isolated. Our results revealed the relatively low genetic diversity of *M. monophyllos*, due to effect of the small population sizes and inbreeding, as the driving forces operating within the European part of its range. The statistically highest values of genetic diversity were found in populations from the boreal region, while populations from the mountainous regions were characterized by a reduced level of genetic diversity compared to boreal ones. The demographic data, in turn, have illustrated the highly dynamic spatial and temporal structure of its populations over a short-term observation span (5-6 years), with almost complete exchange of individuals in populations. This ephemeral character of populations characterized by a low genetic differentiation may correspond to a metapopulation dynamic model for this species in Europe. It may be important in cases of rare ephemeral plants. In this case target the conservation efforts on improving generative reproduction and conserving all suitable habitats for these species, not only with existing populations.

Endohyphal bacteria communities within the orchid-fungus network

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Orchid species interact with different organisms to exploit the resources necessary for their growth. Amongst them, the association with mycorrhizal fungi has been shown to improve orchid species nutrient uptake during germination and subsequent growth. This mutualistic association allowed orchid species to develop diverse resource acquisition strategies through different functional traits of their mycorrhizal fungi. Consequently, different orchid species can coexist in a given habitat without competing for the same resources. It has been recently suggested that other microorganisms such as endohyphal bacteria (e.g. bacteria living inside fungal cells) could be involved in orchid-fungus interactions. They could be involved in production of growth factors that might stimulate fungal germination, mycelia growth, and indirectly impact the host-plant life strategy. Some studies have shown the important role of endohyphal bacteria in phosphate acquisition and transport into arbuscular mycorrhizal symbiosis; however, little is known about the interaction between the orchid's mycorrhizal fungi and their endobacteria. In this study we analysed the presence and identity of endohyphal bacteria among 65 samples of fungal DNA cultures from 10 different orchid species using the high-throughput sequencing (NGS) method, with the use of universal bacterial primers 515FB/806RB. Through this sequencing method, the diversity and the community composition of endohyphal bacteria was analysed, and the specificity between fungi-bacteria interaction was examined, as well as the phylogenetic relation between all the different network's partners.

Symbiotic fungi associated with a leafless epiphytic orchid, *Taeniophyllum glandulosum*

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The leafless epiphyte is one of the most intriguing life styles in orchids in which the roots are sole photosynthetic organs. Although such orchids are likely to be more dependent on mycorrhizal fungus compare to those with well developed green leaves, study of mycorrhizal association in leafless epiphyte is still limited. Among the approximately 290 species of 7 genera in leafless epiphytic orchid, *Taeniophyllum* is the largest genus and comprises of approximately 180 species. *Taeniophyllum glandulosum* is widely distributed in Japan, Himalaya, and Malaysia. In this study, mycorrhizal fungi of *T. glandulosum* were molecularly identified using a total of 45 mature plants and 3 seedlings from 9 sites. Seedlings were collected by the in-situ seed baiting method. DNA was extracted from the root samples or directly from fungal coils and nrDNA ITS regions which were amplified with three primer combinations: ITS1F/ITS4, ITS1F/ITS4B, ITS1OF/ITS4OF. For low quality sequences, PCR was re-performed using taxon-specific primer LR21-Cer. The results of molecular identification showed that most of the sequences obtained from the root samples belonged to *Ceratobasidium* and were grouped into 3 operational taxonomic units (OTUs) defined by 97% sequence identity. OTU1 accounted for 80% of all *Ceratobasidium* sequences, suggesting that *T. glandulosum* is mainly associated with this fungal group. To investigate the effect of host tree species on mycorrhizal association, mycorrhizal fungi from 5 host tree species were compared; however, there was no correlation between tree species and fungal partner.

Improvement of seed germination of Orchideaceae in disturbed habitats

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Restored habitats are in their development towards high species diversity often limited by seed availability. Orchids, producing large number of easily dispersed seeds, may however be limited by the presence of suitable mycorrhizal fungi on which their germination fully depends on. Sowing orchid seeds together with their fungi could be a practical way to introduce them to restored habitats. To test this idea, we sowed seeds of *Orchis mascula*, *Platanthera bifolia*, *Anacamptis pyramidalis* and *Gymnadenia conopsea* at nine meadows restored on arable land in White Carpathians Mountains using the seed packet technique. The seeds were sown alone or together with fungal isolates which were proved to be efficient during *in vitro* germination. After one year of sowing, we collected the seed packets and evaluated the seed germination progress by visual assessment of protocorms which were complemented by molecular analysis of fungi in protocorms as well as in the surrounding soil. The poster presents preliminary results on amelioration of orchid germination in situ.

In vitro asymbiotic germination of eleven orchid species of Greece

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Asymbiotic seed germination of orchids is a complex process and most of these technical difficulties are due to the requirement for a plant-fungus symbiotic relationship under field conditions. More than 100 Orchidaceae species grow natively in Greece and Mature seeds of 11 orchid species (belonging in 7 genera) were collected in 3 localities (Mt. Hymettus, Mt. Poikilo and Mt. Falakro): *Anacamptis collina*, *Dactylorhiza sambucina*, *Gymnadenia conopsea*, *Neotinea lactea*, *N. maculata*, *N. tridentata*, *Ophrys mammosa*, *O. mycensis*, *O. helenae*, *Serapias bergonii*, *Spiranthes spiralis*. Germination tests included a variety of pre-treatments and culture media. Seeds were pre-treated with either calcium hypochlorite and Tween solution or bleach for various time durations depending on the species. The three *Ophrys* species were, additionally, cold-stratified for 3 days at 4 °C prior to the pre-treatment. Germination experiments were performed using 5 culture media (Knudson, Malmgren, Orchimax, Murashige & Skoog, modified Murashige & Skoog), at 20 °C in the dark. Several species were also tested for germination in petri dishes, filled only with water, without any pre-treatment, at laboratory conditions in darkness. Seeds of *O. mammosa*, *N. maculata* and *D. sambucina* showed the highest germination percentages while the latter two species were further tested in darkness and in light, with or without pre-treatment in a range of various temperatures.

How can nutrients affect orchid-fungus mycorrhizal symbiosis?

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Orchid distribution in nature is usually highly fragmented, which is often explained by specific interactions of orchids with other organisms. Mycorrhizal fungi are likely to play a role in orchid distribution since the young developmental stages of orchids are completely mycoheterotrophic. Nutrients can affect distribution of many plant species directly, as it was reported in orchids. However, it is unclear whether there can be also an indirect effect of nutrients on orchid distribution mediated by mycorrhizal fungi. To test this assumption, we performed symbiotic *in vitro* cultures of *Dactylorhiza majalis* with two *Ceratobasidium* and two *Tulasnella* isolates. We used two-compartment Petri dishes to apply nutrients to both organisms or only to the fungus. Three compounds were tested separately or in combination: nitrate, ammonium, and phosphate. Seed germination rate and protocorm size were measured. Our results show that each nutrient affect mycorrhizal symbiosis differently. The ammonium was detrimental to the symbiosis generally. Fungi were growing intensively but the orchid was not. However, mild concentrations of nitrates were able to stimulate growth of protocorms associated with *Ceratobasidium*. Low concentrations of phosphates stimulated growth of protocorms were associated mainly with *Tulasnella*. Surprisingly, when adding nitrates only to fungus it did not stimulate protocorm growth. Our results show that nutrients can affect orchid mycorrhizal association through both a direct effect on orchids as well as an indirect effect mediated by the fungus.

Emergence, growth and demographic characteristics of seedlings in *Himantoglossum adriaticum*

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The endangerment of many orchid species has brought interest in the study of orchid biology. The recruitment events can have a strong effect on population persistence, especially when plants propagate exclusively by sexual reproduction. The Adriatic Lizard orchid (*Himantoglossum adriaticum*) is a Natura 2000 and a CITES species. Permanent plots (26) were set and 93 plant individuals were marked between 1999-2009 to study the recruitment dynamics in a *H. adriaticum* in Keszthely Hills (Hungary). Seedlings emerged in large numbers in the 3rd year after the adult plants had flowered. The emergence of seedlings was continuous during the vegetation period and their survival depended on environmental factors. Most seedlings emerged during autumn but hot and dry autumn, as well as cold winters, were unsuitable for seedling emergence. Seedling emergence was linked to the status of the parent plant: when the parent plant was dormant, the seedlings also remained in the soil. The status of the parent plant in the previous years did not affect the number of emerged seedlings. A large proportion of the seedlings (94%) had one leaf at the time of emergence and the width of the leaf varied between 0.1-1 cm. Undamaged seedlings reached their maximum leaf area during the following spring, while damaged or unable to compensate individuals were excluded. Seedling mortality rate was size-dependent, and it decreased as the size of the seedlings increased. While for the seedlings survival rather than emergence was critical, in juveniles progressing to more mature stages was a difficult transition. The results suggest that the recruitment dynamics affect strongly the persistence of the studied population. Therefore, we suggest that monitoring for conservation should be tailored to protect the recruitment events in this species.

Long-term studies of orchid populations – mysteries remain

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In this poster, we present long-term population data which demonstrate that many questions remain in the quest for a complete understanding of the ecology of orchid populations. The majority of these questions point to incomplete knowledge about the early life history stages of orchids: seed germination, protocorm dynamics, and dormancy, as well as the abundance and distribution of mycorrhizal fungi. We have monitored populations of three native orchids on Smithsonian property for decades and have also conducted population-focused research on a federally-threatened orchid species at several locations in Virginia and West Virginia. The species that we have monitored are: *Aplectrum hyemale*, *Corallorhiza odontorhiza*, *Galearis spectabilis*, and *Isotria medeoloides*. These species represent different life history strategies and temporal patterns have differed, even in the same forest. Populations of *A. hyemale* have long-lived individuals but new plants appear regularly and are often as large as the plants that have been present and monitored for years. After a long period of relative stability, a population of *C. odontorhiza* has declined markedly for unknown reasons. Plots with *G. spectabilis* have been dynamic and new individuals appear yearly, even though there is no evidence that seeds have successfully been produced in or near any of the monitored plots. *I. medeoloides* populations are small, and ongoing monitoring shows that individuals can remain dormant for at least 9 years. The research will identify what additional information is still needed to assure the survival of these and other orchids as the North American Orchid Conservation Center continues to develop and expand its efforts.

Differential phenotypic selection on floral odours in three orchid taxa growing in distinct environments

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How variations in the selection exerted by pollinators on floral traits can contribute to taxonomic and floral diversification is an important question in evolutionary biology. Variations in floral traits between conspecific populations are often interpreted as local adaptations to geographically structured pollinator communities, but only a handful of studies have pointed out the very process that may have generated such a pattern, by quantifying the selection exerted on these traits in the field. In addition, few of these studies have focused on floral odours, despite the well-known implication of olfactory signals in pollinator attraction. Pollinators could select for the most scented individuals, but they could also drive chemical divergence between conspecific populations, as different insect guilds may select for distinct blends of floral compounds. In this study, we focused on three closely related orchid taxa, *Anacamptis coriophora*, *A. fragrans* and *A. coriophora* var. *martrinii*, which grow in different environments and are pollinated by distinct insect guilds. We first investigated differentiation in terms of floral odours between these three taxa, and we then quantified the selection exerted on these odours in seven populations. We found that the three studied taxa were characterized by distinct floral scents, with two dominant compounds per taxon, one common to these three taxa and one taxon-specific. In addition, we found a significant selection on relative amounts of 1,4-Dimethoxybenzene in two *A. coriophora* populations and of *p*-Anisaldehyde in one *A. fragrans* population. Our study is one of the first to document variations in the selection exerted on floral odours between closely related orchid taxa. Our results suggest that pollinator-mediated selection may have contributed to chemical divergence between *A. coriophora* and *A. fragrans*.

Catasetum ochraceum (Orchidaceae) population structure and pollination

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Catasetum ochraceum is a terrestrial orchid with a disrupted distribution in both Colombia and Brazil. A population was followed during a year at La Vorage, a Colombian site, where observation of pollination was performed. It is known that *Catasetum* spp. are pollinated by male euglossinae bees. Bee traps with low chemical attractors for male euglossinae bees with 1-8 cineole and methyl salicylate were used to collect bees carrying *Catasetum* pollinarium. 50 orchids were recorded, measured, and mapped for follow-up observations. From those 50 orchids, 29 were juveniles and had no evidence of inflorescence production, while 21 were adults. During the observation non marked plants flowered and 41 plants survive through the year. Also 18 bees from 8 species were collected. The bee species recorded included the following species: *Eulema nigrita*; *Exaerete smaragdina*; *Euglossa despecta*, *E. ignita*, *E. modestior*, *E. tridentata*, *E. deceptrix* and *E. liopoda*. From those, *E. deceptrix* and *E. liopoda* carried pollinarium from *C. ochraceum*. Those observation suggested that the population has the potential for growth and that there are pollinators in the area that can make the population viable.

Breeding system and pollination mechanism of *Dichaea pendula* (Aubl.) Cogn. (Orchidaceae)

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The floral biology, breeding system, and pollination mechanism of *Dichaea pendula* were studied in the Andean Colombian Forest at 2200 m.a.s.l. The *D. pendula* reproductive system was assessed using five controlled pollination experiments in the field: i) non-manipulated unbagged flowers, ii) non-manipulated bagged flowers, iii) hand self-pollination, iv) hand cross-pollination, and v) emasculation. To study the pollination process, a total of 280 observations hours were performed in the field between 8:00 to 16:00 h during several days of flowering seasons. *Dichaea pendula* presented two flowering seasons per year: March-May and October-November. At the first period the orchid produced 5-148 flowers per plant and at the second season, it produced 2-106 flowers per plant. The breeding system treatments showed that the studied population is self-incompatible and pollinator dependent as only fruits were formed with the hand cross-pollination treatment (ca. 70%) and with non-manipulated unbagged flowers treatment, where limited natural pollination events occurred (ca. 5%). The major pollinator activity on flowers was recorded between 10:00 and 12:00 h. These visits were carried out by male euglossine bees especially when direct sunlight reached the flowers. The pollinator of *D. pendula* was determined as *Euglossa nigropilosa* and the pollination mechanism consists of the odour-collecting behaviour of male bees which are attracted to the floral aromatic compounds of flowers. Early analyses suggest that floral scent of *D. pendula* is due primarily to methoxylated phenolic compounds. To the pollinaria adheres to the clipeo of the bee, it must spend between 60 and 80 seconds while collecting the fragrance of the flower and transferring it from its front legs to the hind legs. Among fragrance-rewarding orchids, *Dichaea* is one of the least studied groups in terms of pollination ecology. Thus, this study is a contribution to the knowledge of the biology of this Neotropical genus.

Beauty attracts beauty: a case of *Masdevallia* pollination (Orchidaceae: Pleurothallidinae) by fruit flies (Diptera: Drosophilidae)

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Orchids are known as one of the most advanced groups of plants in the context of adaptation to different forms of zoogamy. The pollination by flies (myophily), with all its variations, is the second most popular pollination pathway in the Orchidaceae, with pollination by species of about 20 families of Diptera. *Masdevallia* Ruiz. & Pav. is probably one of the most recognized members of the Pleurothallidinae Lindl. The genus comprises of about 350 species distributed from southern Mexico to southern Brazil, with the highest diversity being found in the Andes of Ecuador and Colombia, Peru, and Bolivia. During our studies on orchid biology carried out in Estación Experimental de Orquídeas de la Familia Archila in Cobán (Guatemala), we have observed pollination of two species of genus *Masdevallia*: *M. floribunda* and *M. tuerckheimii* by fruit flies (Diptera: Drosophilidae). These two orchid species were found on the trunks of numerous trees located across the area of the Station. All of them were located in a rather shady area. Both *Masdevallia* species are spread naturally across the Station. The flowering period ranges from June to February which coincide with the rainy season. The flies visited flowers regularly from the early morning until the sun dusk. Further studies led us to the identification of the pollinator as a representative of the genus *Zygothrica* Wiedemann. This species-rich genus, widely distributed in the Neotropical and Indopacific regions, is defined by a prominent and sharp facial carina, a medially cleft oral margin, and a sclerotized proboscis with an acute joint between the mentum and submentum. Adult males have their heads distinctly broadened (hypercephaly), much wider than the thorax - the body proportions being the most conspicuous character for *Zygothrica*. As further research revealed, this was the first report of a *Zygothrica* species being effective pollinators of *Masdevallia floribunda* and *M. tuerckheimii*.

Morphological, histological, and ultrastructural features of flowers of *Lepanthes calodictyon* (Orchidaceae, Pleurothallidinae)

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Orchids represent a variety of adaptations which enhance the effectiveness of pollination. One of the mechanisms is pseudocopulation which is widely found in Pleurothallidinae. The pseudocopulation success is an effect of simultaneous presence of features such as three dimensional structure of the flower, vivid contrastive colouration, and emission of fragrances. Here we present results of our studies on *Lepanthes calodictyon*. Histochemistry analyses, fluorescence, scanning, and transmission electron microscopy were used. The research revealed single cellular papillae on the petals' and labellar's surfaces. Histochemistry analyses showed the large amount of lipids concentrated inside the papillae. The cells were protein rich and starchless. Cytoskeleton of the cells were well developed. In electron microscopy the dense cytoplasm with numerous endoplasmic reticulum, mitochondria and plastoglobuli are noticeable. Our results overlap with other research findings made on flowers of Orchidaceae which have led to the conclusion about granulocrine secretion and osmophore activity on the petals' and labellar surfaces.

Floral anatomy and ultrastructure in *Dactylorhiza incarnata/maculata* complex

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Four *Dactylorhiza* species within the *Dactylorhiza incarnata/maculata* complex were examined to identify differences in floral traits, using micromorphological, histochemical, and ultrastructural studies on spur and labellum structure. Flowers were collected at anthesis from four monospecific populations in Gdańsk Pomerania, northern Poland. The *Dactylorhiza* flowers revealed secretory activity of the epidermis of the spur mouth (possible osmophores) and of the inner epidermis of spurs (possible nectar traces) in *D. incarnata*, *D. maculata* subsp. *fuchsii* and *D. majalis* subsp. *majalis*. The secretion was absent on papillae in *D. maculata* subsp. *maculata*. However, the presence of slightly developed micro-channels, vesicles in periplasmic space, and plastoglobuli in plastids may be responsible for the production of a meagre amount of fragrance or nectar. On the other hand, the presence of papillae and trichomes within spurs and their striated texture may function as tactile stimuli for pollinators, expecting the presence of nectar, as it was observed in other food-deceptive orchid species.

Pollinators and visitors of *Dactylorhiza majalis* in the NE Poland

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The Orchidaceae represents diversity of pollination mechanisms. About one-third of orchid species are deceptive. The most common mechanism is generalized food deception (GFD) occurring in 38 genera which exploit the innate food foraging behaviour of pollinators. One of orchid genera representing GFD is *Dactylorhiza* where the majority of taxa are nectarless and deceive their pollinators. In early flowering *Dactylorhiza majalis* bees and beetles have been reported as main flower pollinators, although in different parts of geographical range pollinators assemblage are different. To indicate pollinators and flower visitors of *D. majalis* in the north-eastern Poland, orchid inflorescences were video-recorded. A total of 27 insects belonging to 18 species were found. Dipterans were the most abundant arthropods (ca. 50%, mostly hoverflies), followed by Hymenopterans (33.3%), Coleopterans (7.4%), and Lepidopteran species (11.1 %). Among all recorded insects only five *Apis mellifera* individuals were observed to carrying pollinia. Visits lasted from 27 s to 56 s on inflorescence and during single bout honeybees visited two-five flowers on inflorescence, in different populations. Similarly to previous published studies, we confirmed only melittophily syndrome (pollination by honeybees) in *D. majalis*.

JAKIE ZDJĘCIE?



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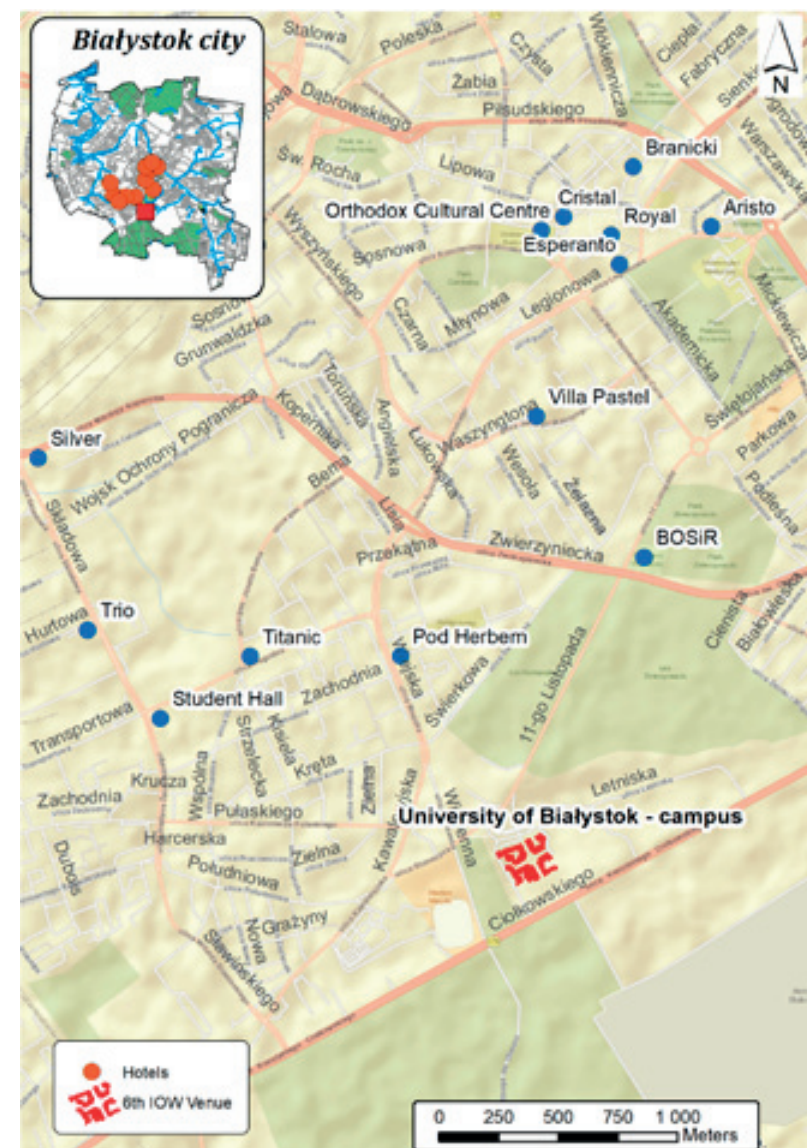


Dactylorhiza fuchsii, Wigry National Park



VENUE

Epipactis atrorubens, Wigry National Park



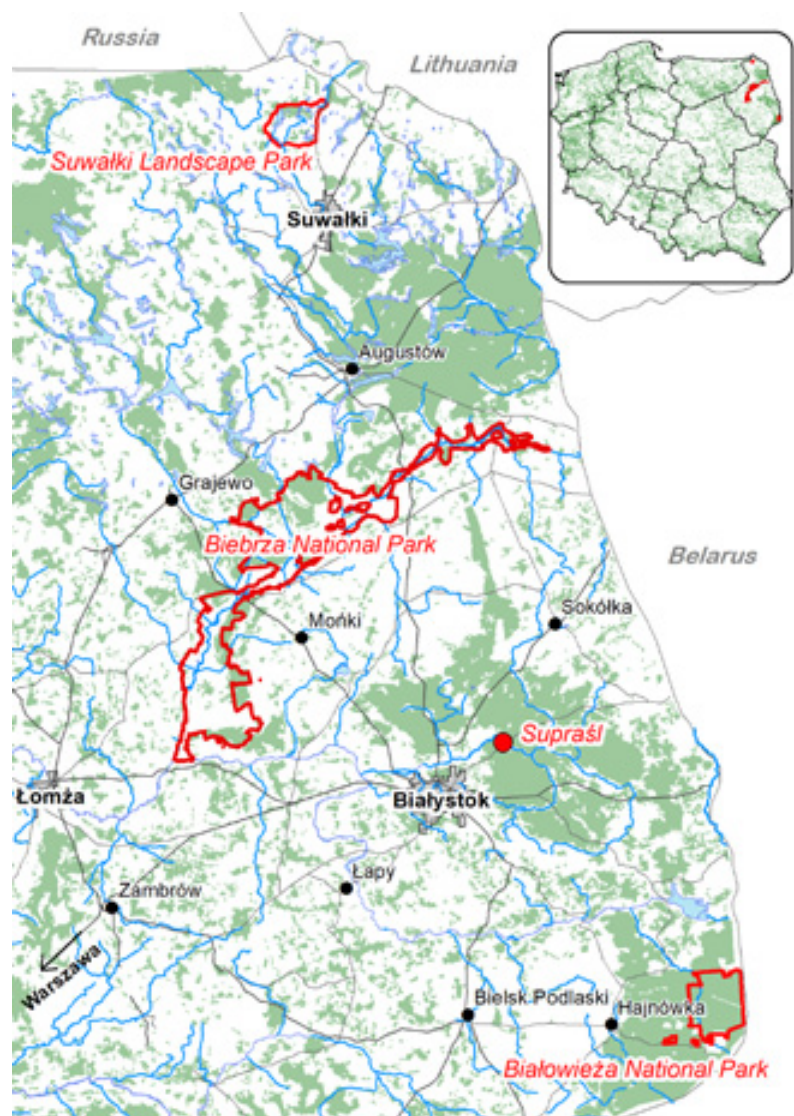
Białystok, with a population of approximately 300 thousand inhabitants, is the largest city in north-eastern Poland. It forms an administrative, scientific, economic and cultural hub for this part of Poland. It is a multi-cultural town, which has a significant number of Belarusians, Russians, Ukrainians and Tartars in addition to Poles. There are three large universities and a few smaller ones, which comprises of approximately 40,000 students. Białystok is known as the capital of "The Green Lungs of Poland," a region that is located in the most nature-valuable part of the country and is surrounded by large forest, river valleys, and culturally-rich farmland landscape. The city is also known for Ludwik Zamenhof, who created the international language Esperanto. There are two theatres and an opera house in the city. In the summer a number of large music festivals take place outdoors. The most recognizable building in Białystok is the Branicki Palace and its surrounding park. Białystok's space is well organized and is easily accessible via private or public transportation and on foot or by bicycle. The Guardian newspaper in 2014, reported Białystok as the most satisfactory city for its residents compared to other Polish cities.

The University of Białystok was established in 1997 as a branch of Warsaw University after 29 years of its existence. Today the University is one of the largest and strongest academic centres in north-eastern Poland. The University consists of nine faculties, including one located abroad in Vilnius. Classes and lectures are delivered by approximately 850 academic teachers (nearly 200 are independent research scholars). The University educates about 13 thousand students in 31 fields. PhD degree studies are carried out within five University units, including the Faculty of Biology and Chemistry. The University is constantly developing. Four departments are already located in a newly built academic campus. A modern Nature Centre established for the popularization of earth sciences is also located at the campus, while field studies and education are facilitated by the field station in Biebrza National Park. Special studies in the field of chemistry are supported by a modern Bio-Nano Techno Centre. All students can develop their artistic interests at the University Culture Centre, which has a chamber event hall and rooms for workshops, conferences, and meetings.



FIELD TRIPS

Herminium monorchis, Rospuda Valley



Malaxis monophyllos, Rospuda Valley

BIEBRZA NATIONAL PARK

Cephalanthera rubra, Biebrza National Park



Biebrza National Park is the largest wetland complex in Poland, protected by the Ramsar Convention and is also the country's largest national park (59 233 ha). It was established in 1993 to protect vast wetlands of Biebrza river valley. The valley itself was formed during the central Polish and Baltic glaciations 10 – 30 thousand years ago. Since then a peat layer has developed, that locally reaches up to 10 m thickness. Although this area was partly drained by the canals that were built even as far as 19th century, the wetlands are still well preserved and governed by natural hydrological dynamics. In the early spring, the river forms a great stop-over site for huge numbers of migrating birds. Habitats in Biebrza National Park differ from various peat bogs and marshy forests to sandy dunes, therefore the overall biodiversity is very high. Vegetation comprises of 70 natural and semi-natural plant communities, particularly well-represented by sedge, sedge-moss, reed communities and mineral islands. Vascular plants count for over 900 species in the area of the national park and over 1000 species in the whole valley. Fauna of Biebrza Marshes is known for the country's largest population of elk and for over 270 birds species including 180 species breeding with Europe's rarest warbler and eagle species (Aquatic Warbler, Greater Spotted Eagle). There is also great diversity threshold of invertebrates (over 750 species of moths and butterflies, 500 species of beetles and 450 spider species). Many of the valuable habitats are connected to extensive agriculture and they need human effort to be sustained in the current shape. Therefore, Biebrza National Park is an area that needs a lot of attention of conservationists' and scientists' Those specialists can find agreeable conditions to study in the Gugny Field Station of the Institute of Biology, University of Białystok.

ORCHIDS OF THE BIEBRZA NATIONAL PARK: *Cephalanthera rubra*, *Coeloglossum viride*, *Corallorhiza trifida*, *Cypripedium calceolus*, *Dactylorhiza fuchsii*, *Dactylorhiza incarnata*, *Dactylorhiza maculata*, *Dactylorhiza majalis*, *Dactylorhiza ochroleuca*, *Epipactis atrorubens*, *Epipactis helleborine*, *Epipactis palustris*, *Goodyera repens*, *Gymnadenia conopsea*, *Liparis loeselii*, *Neottia ovata*, *Neottia nidus-avis*, *Orchis palustris*, *Orchis coriophora*, *Platanthera bifolia*.

www.biebrza.org.pl

Living on an island — long-term dynamics of *Cypripedium calceolus* populations in the Biebrza National Park (NE Poland)

Emilia Brzosko, Edyta Jermakowicz, Paweł Mirski, Beata Ostrowiecka, Izabela Tałałaj, Ada Wróblewska

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From the conservation point of view, knowledge about population dynamics is of key importance in the prediction of rare species' population. It is also important to evaluate the extinction probability and plan conservation strategies. Since endangered species are represented by isolated populations, their dynamics depend mainly on the population demographic processes. During 28-years of studies, we observed the spatiotemporal variability of three *C. calceolus* populations and determined factors enabling their long-term persistence under conditions of environmental isolation on mineral islands in the Biebrza valley. Three populations represented distinct types of dynamics and different level of vegetative spread and generative reproduction. Generally, inter-annual fluctuations of demographic parameters were observed in all populations, although there is a decreasing trend for many of them in the last years. Especially important is the decrease of flowering parameters. Our results document the low level of fruiting in *C. calceolus* populations and low visitation rate (3.5% pollinaria removal in the biggest population), which confirms pollinator deficiency. Therefore, we can conclude that pollinator limitation is a crucial factor causing low reproductive success in *C. calceolus* populations in the Biebrza valley. The main factors influencing demography of *C. calceolus* populations on mineral islands in the Biebrza National Park are the character of vegetation and its changes due to natural processes (succession), human activity (cutting of trees) and climatic changes (lower precipitations disturbed hydrology). Natural succession provides the expansion of trees and shrubs and increases shading. Human activities produce open areas which are quickly dominated by expansive grasses and sedges that eliminate other flowering plants (including orchids), which are vulnerable to competitors. Changes in the character of vegetation on mineral islands restrict both sexual reproduction and the vegetative growth of orchid individuals, and may threaten population persistence.

Rare orchids translocation between mineral islands in Biebrza Valley (north-eastern Poland): effectiveness and recipient site selection

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Long-term studies of environmentally isolated orchid population in the Biebrza Valley revealed that some species (for example *Cypripedium calceolus* and *Cephalanthera rubra*) decrease population size and lower reproductive parameters (flowering and/or fruiting). In order to maintain these species and increase connectivity between existing populations we carried out translocation experiment. Thirty soil monoliths with vegetative orchid plants were dug out after flowering and were transplanted to three different sites on new mineral islands (half in 2012 and half in 2013). Recipient sites were chosen according to similarity of soil moisture, pH and phytosociological characteristics to donor sites among 68 potential recipient sites on unoccupied mineral islands. After translocation, we monitored the number of shoots for 4-5 years and retrospectively conducted principal component analysis (PCA) in order to compare the similarity of donor sites and chosen recipient sites. Four years after transplantation, we found new *C. calceolus* populations in good condition, but *C. rubra* transplants emerged only in the first and second season. An increase in shoot number was observed in the new *C. calceolus* populations, especially in the one found most similar to the donor ones (according to PCA analysis). Moreover, fruiting was observed in two out of three newly established *C. calceolus* populations.

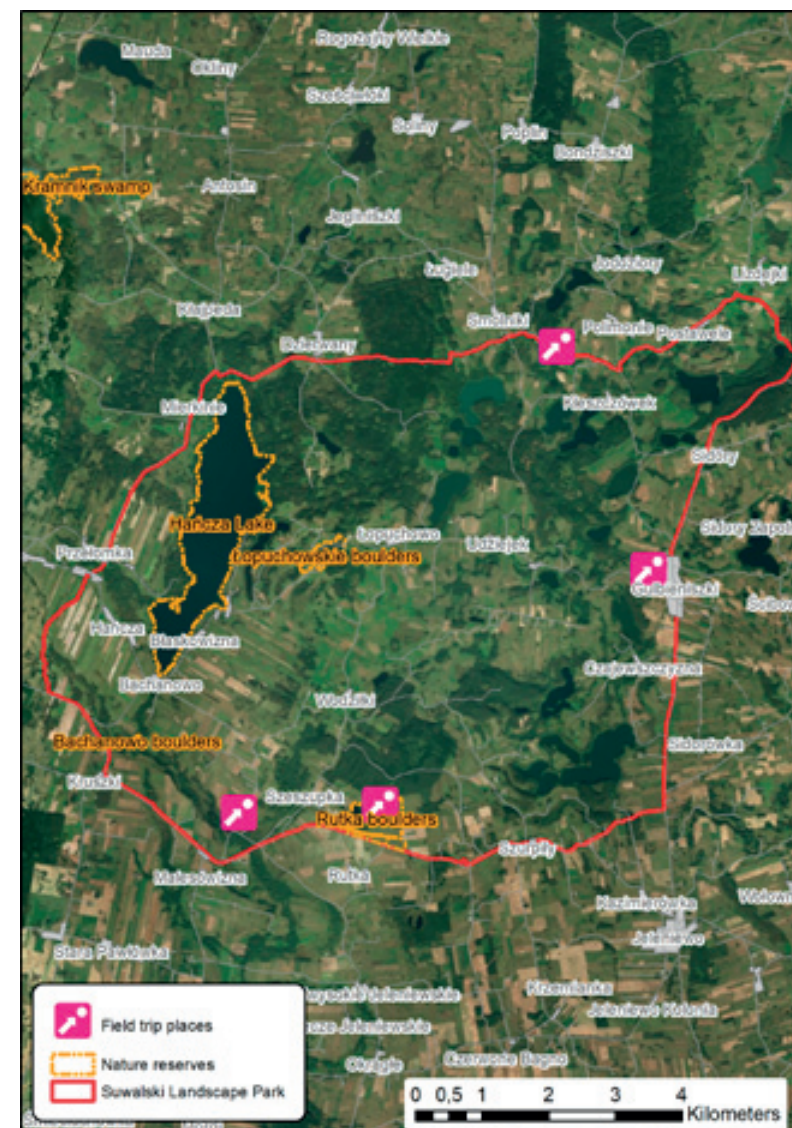


Corallorhiza trifida, Rospuda Valley

**SUWAŁKI
LANDSCAPE PARK**

Dactylorhiza baltica, Suwałki Landscape Park

Dactylorhiza baltica, Suwałki Landscape Park



Suwałki Landscape Park is located in the coldest, north-eastern corner of Poland, near the Lithuanian border. It is the oldest Polish landscape park, founded in 1976 to protect the diverse postglacial landscape. It covers 6,284 ha, including 26 lakes and two river valleys (Szeszupa and Czarna Hańcza). One of those lakes — Hańcza, is the deepest lake in Poland (108 m). The main value of the Park is its varied, charming landscape forms. It includes moraines, kame mounds and eskers that are shaped by the melting ice masses during last glaciation. Among them there are scattered groups of boulders and depressions filled with lakes or wetlands. A mosaic of various habitats creates conditions for rich flora with numerous representatives of various geographical plant elements: boreal, southern and montane. There are over 700 species of herbaceous plants, among which several postglacial relicts are considered most valuable. In terms of fauna, the most distinctive groups are fish, molluscs and crustaceans inhabiting deep, clean, and well-oxygenated waters of the country's deepest lake Hańcza. Suwałki Landscape Park was also a key refuge site for European beaver, decades ago, when its population was on the edge of survival.

ORCHIDS OF THE SUWAŁKI LANDSCAPE PARK: *Corallorhiza trifida*, *Dactylorhiza baltica*, *Dactylorhiza fuchsii*, *Dactylorhiza incarnata*, *Dactylorhiza maculata*, *Dactylorhiza majalis*, *Dactylorhiza viridis*, *Epipactis atrorubens*, *Epipactis palustris*, *Goodyera repens*, *Hammarbya paludosa*, *Liparis loeselii*, *Malaxis monophyllos*, *Neottia nidus — avis*, *Neottia ovata*, *Orchis coriophora*, *Orchis mascula*, *Platanthera bifolia*, *Platanthera chlorantha*.

Epipactis helleborine, Biebrza National Park



BIAŁOWIEŻA NATIONAL PARK

?????



Out of the vast forests which used to cover Central Europe centuries ago, only few parts remain with a condition similar to primeval. One is **Białowieża National Park**, often considered a model forest in ecological studies. It is one of the oldest Polish national parks and the country's only natural area listed as a UNESCO World Heritage Site. The unique, natural and mature wood stands have been protected since 1921 as a nature reserve and since 1932, a national park. Białowieża Primeval Forest is divided in two parts by the Polish - Belarusian border. One third of the Polish part is protected as a national park. It covers 10,500 ha, out of which over 6,000 is under strict protection. This is where natural processes are main conservation goal and human activity is strictly limited. The forest is entirely dependent on natural factors, which makes it very diverse, especially on meso- and micro-scale. Multigenerational forest comprises of centuries-old trees as well as younger generations emerging in the natural gaps. Whole forest is rich in dead wood, including very old and large oak trunks. Altogether, it forms great conditions for late succession mature-forest and saproxylic species. Białowieża National Park is the home for a great variety of organisms: 809 vascular plants, over 3 thousands cryptogams and fungi, almost 200 moss and 283 lichen species. Fauna is also impressively diverse: over 8000 invertebrates, 120 breeding birds and 52 mammal species. The latter group is best represented by the iconic European bison — the symbol of great conservation success. It was restored from the last three captive individuals of the lowland population and there is currently about 500 individuals in the Białowieża Forest and few hundred in other Polish lowland forests.

ORCHIDS OF THE BIAŁOWIEŻA NATIONAL PARK: *Cephalanthera rubra*, *Dactylorhiza fuchsii*, *Dactylorhiza incarnata*, *Dactylorhiza maculata*, *Dactylorhiza majalis*, *Epipactis atrorubens*, *Epipactis helleborine*, *Epipactis palustris*, *Goodyera repens*, *Neottia cordata*, *Neottia nidus-avis*, *Neottia ovata*, *Platanthera bifolia*, *Platanthera chlorantha*.

www.biebrza.org.pl

FIELD PRESENTATION

Białowieża Forest - the relic of naturalness

Bogdan Jaroszewicz

Białowieża Geobotanical Station, University of Warsaw, Poland

USEFUL PHRASES

Gymnadenia conopsea, Biebrza National Park

IN ENGLISH	IN POLISH	PHONETICS
good morning	dzień dobry	[dzeń dobri]
good evening	dobry wieczór	[dobri vyetʃur]
good morning	dzień dobry	[dzeń dobri]
good night	dobranoc	[dobranots]
goodbye	do widzenia	[do veedzeńa]
hello, hi	cześć	[tʃeʃtʃ]
please here you are	proszę	[proʃe]
thank you	dziękuję	[dzenkuye]
I'm sorry. Excuse me	przepraszam	[pʃeprafam]
My name is ...	Nazywam się...	[nazivam sɕ ...]
I got lost	man say: zgubiłem się woman say: zgubiłam się	[zgubeewem sɕ] [zgubeewam sɕ]
I don't understand	Nie rozumiem	[ńe rozumyem]
Where is it?	Gdzie to jest?	[gdze to yest]
I'm looking for ...	Szukam ...	[ʃukam]
Where is the police station ?	Gdzie jest komenda policji?	[gdze yest komenda poleetsyee]
Please call the police/ /the ambulance	Proszę wezwać policję/ /pogotowie ratunkowe	[proʃe vezvatɕ poleetsyee/ /pogotovye ratunkove]
Where is ... street ?	Gdzie jest ulica ...?	[gdze yest uleetsa ...]
Where's the toilet?	Gdzie jest toaleta?	[gdze yest toaleta]
Where is the hotel ...?	Gdzie jest hotel ... ?	[gdze yest hotel]
How can I get there?	Czym tam dojadę?	[tʃim tam doyehatɕ]
Please call a taxi.	Proszę zawołać tak- sówkę	[proʃe zavovatɕ taksufke]
I'm staying in hotel.	Mieszkam w hotelu	[myeʃkam fhotelu]

How much is an overnight stay?	Ile kosztuje nocleg?	[eele koʃtuye notslek]
Please wake me up at	Proszę obudzić mnie o godzinie	[proʃe mńe obudzeetɕ o godzeeńe]
Where is the railway station?	Gdzie jest dworzec kolejowy?	[gdze yest dvoʒets koleyovi?]
Where is the information office?	Gdzie jest informacja?	[gdze yest eenformatsya]



Neottia ovata, Biebrza National Park

As organizers of the 6th International Orchid Workshop (2018), we would like to express our sincere gratitude to the following institutions, organizations, societies and companies for their generous support.



Rector of the University
of Białystok



FACULTY OF BIOLOGY AND CHEMISTRY
UNIVERSITY OF BIAŁYSTOK



Ministerstwo Środowiska
Przyrody i Klimatu
i Energetyki



European Orchid Conservation Center



Orchid Specialist Group



WNIOSKI BIOLOGICZNE ORGANIZACJI



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Anacamptis coriophora, Narew Valley

