Bologna
15 September 2014
Botanic Garden
Aula B
BiGeA - Department of Biological, Geological and Environmental Sciences
Via Irnerio 42

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biodiversity Conservation:
the plant and pollinator perspectives to enhance ecosystem services

15:00 PRE-CONFERENCE WORKSHOP
Chairman: Prof. Giovanna Aronne (University of Naples Federico II)
Results and good practices of the project Life+ PP-ICON
Dr. Marta Galloni (Dept. BiGeA, University of Bologna)
Dr. Laura Bortolotti (CRA-API, Bologna)
Measuring pollinator performance in Papilionoideae from Argentina
Prof. Angela Etcheverry (Universidad Nacional de Salta, Argentina)
Habitat management for realising multiple agroecosystem services
Prof. Mario Balzan (MCAST, Malta)
SHARP species based approach for sustainable conservation of rare endangered plants
Prof. Giovanna Aronne (University of Naples Federico II)

16:45 DISCUSSION
17:30 Guided tour of the Botanic Garden
Prof. Giovanni Cristofolini (SMA, University of Bologna)

The workshop is open, no registration is required.
Contact person: Marta Galloni, tel. 0512091318 - email: marta.galloni@unibo.it
Welcome to the
International Meeting on Plant Reproduction

Organizing Committee

President: Davide Pettener, Director of Dept. BiGeA, University of Bologna
Vice-president: Marta Galloni, Dept. BiGeA, University of Bologna

Members

Laura Bortolotti, CRA - Unità di Ricerca di Apicoltura e Bachicoltura
Massimo Nepi, Dept. of Life Sciences, University of Siena
Stefano Del Duca, Dept. BiGeA, University of Bologna
Giulio Galla, Dept. DAFNAE, University of Padua
Gianni Barcaccia, Dept. DAFNAE, University of Padua
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Carlo Ferrari, Dept. BiGeA, University of Bologna
Alessandro Chiarucci, Dept. of Life Sciences, University of Siena
Giovanni Cristofolini, Herbarium and Botanic Garden, University of Bologna
International Meeting on Plant Reproduction

Programme

September 16th, 2014

Aula F – Via Belmeloro, 14

8:30 - 9:00 Opening and registration

9:00 - 10:40 1st and 2nd Symposium:
“Reproductive barriers in plants: male-sterility and self-incompatibility”
“Reproductive modes in plants: sexuality vs. apomixes”

Chairs: Stefano Del Duca - Dept. BiGeA, University of Bologna
Giulio Galla - DAFNAE, University of Padua

9:00 Invited lecture: The self-incompatibility fertilization system in Rosaceae. Genetic and agricultural aspects - Martin Goldway (Tel-Hai College, Israel)

9:40 Oral I-1: Cytoskeleton modification plays a critical role during self-incompatibility in pear pollen tube – S. Del Duca, I. Aloisi, C. Faleri, G. Cai

10:00 Oral I-2: Discovery of nuclear male-sterility in leaf chicory: an overview on its genetic determinism and use in breeding programs - A. Ghedina, G. Galla, S. Tiozzo Caenazzo, G. Barcaccia

10:20 Oral II-1: De novo sequencing of the *Hypericum perforatum* L. flower transcriptome to identify genes and miRNAs that are related to plant reproduction sensu lato - G. Galla, M. Volpato, T. Sharbel, G. Barcaccia

10:40 - 11:00 coffee break

11:00-13:00 3rd Symposium

“Nectar: plant interface for interactions with biotic environment”
Chair: Massimo Nepi - DSV, University of Siena

11:00 Invited lecture: Sipping from a poisoned chalice: plant drugs, toxins, and pesticides in nectar and their influence on bee behavior - Geraldine Wright (Institute of Neuroscience, University of Newcastle, UK)

11:40 Invited lecture: Interactions among plants, pollinators and nectar microbial communities: patterns and effects - Clara de Vega (Estación Biológica de Doñana, CSIC, Sevilla, Spain)


12:40 Oral III-2: Monitoring of *Osmia* bees reared with beta-Alanine and gamma-Amino Butyric Acid diets - S. Sagona, M. Nepi, L. Bortolotti, M. Galloni, G. Bogo, A. Felicioli
Dept. BiGeA, Via Selmi 3
13:00 - 15:00  Lunch Break and Poster Session

Aula F – Via Belmeloro, 14
15:00-18:10  4th Symposium
   “Plant mating and animal pollination: a complex natural system”
   Chairs: Marta Galloni - BiGeA, University of Bologna, Laura Bortolotti - CRA-API, Bologna

15:00 Invited lecture: Pollinators and pollination challenge: the Mediterranean case - Theodora Petanidou (University of the Aegean, Mytilene, Greece)

15:40 Invited lecture: Is main sugar composition of floral nectar determined by pollinators in Scrophularia species? – Tomás Rodríguez-Riaño (Universidad de Extremadura, Spain)

16:20 - 16:30  coffee break

16:30 Oral IV-1: Honey bee behaviour flexibility and adaptation: reinforcing specialized associations while handling diverse species of flowers - M. Giovanetti


17:10 Oral IV-3: Reproduction, modularity and important species of Mt. Lesima grasslands (Northern Apennine): a season-wide network analysis - P. Biella, S. Assini, M. Barcella, J. Ollerton

17:30 Oral IV-4: Insect pollination and nectar secretion in protandrous flowers of Geranium macrorrhizum L. - M. Masierowska

17:50 Oral IV-5: Habitat fragmentation effects and ecological determinants of plant reproductive success: the response of an insect-pollinated species - S. Nora, A. Aparicio, R. Albaladejo

18:10  Closure of the meeting
“Reproductive barriers in plants: male-sterility and self-incompatibility”

The self-Incompatibility fertilization system in Rosaceae, genetic and agricultural aspects

M. Goldway, A. Raz, A. Zisovich, R. Nyska, R.A. Stern

Department of Biotechnology, Faculty of Life Sciences, Tel-Hai College, Upper Galilee 12210, Israel e MIGAL, Galilee Scientific Research Institute, P.O. Box 831, Kiryat Shmona 11016, Israel (goldway@migal.org.il)

Rosaceae are incapable of self-fertilization due the RNase mediated Gametophytic Self-Incompatibility (GSI) system. The system is governed by a multi allelic S-locus which holds a pistil specific ribonuclease gene (S-RNase) and pollen specific F-Box genes (SLF/SFB). The SLF/SFBs are components of the ubiquitinating SCF complex that poly-ubiquitinats selected proteins leading to their degradation by the 26S proteasome. Due to the self-incompatibility, Rosaceae orchards (pears, apples, plums, apricots etc.) contain at least two cultivars that pollinate each other. Since the S-locus is heteroallelic, the S-RNase and the SLF/SFB genes serve as markers for cultivar genotyping. Applying these markers, we found that semi-compatibility between cultivars reduced fruit set and could explain cases of low yields in regions with suboptimal conditions for pollination and fertilization. Following this notion, all cultivars are S-genotyped and, to assure better yields in suboptimal regions, orchards are designed considering full-compatibility between adjacent cultivars.

Investigating the GSI mechanism in both Prunus (plum, apricot) and Pyrus (apple, pear, loquat) suggests that even though both subfamilies belong to the Rosaceae their GSI systems differs. It appears that in Pyrus the GSI resembles the “collaborative non-self-recognition” model found in Solanaceae yet, in Prunus there are findings indicating that the system does not correspond with this model. For example, in Pyrus self-compatibility (SC) is due to mutations in the S-RNase gene but not in SFB/SLF genes whereas in Prunus SC usually occurs due to mutations in the SFB gene.

We are using apricot as our research system and propose a model for Prunus which is different than the “collaborative non-self-recognition”. The two main dissimilarities are: 1) in self-pollination, SFB and S-RNase of the same haplotype interact, leading to the protection of the S-RNase from ubiquitination (rather than leading to S-RNase ubiquitination) allowing it to stay active and prevent self-fertilization, and 2) in non-self-pollination, a universal interaction between an F-Box gene and the foreign S-RNase leads to its ubiquitination and degradation allowing cross fertilization.
“Nectar: plant interface for interactions with biotic environment”

Sipping from a poisoned chalice: plant drugs, toxins, and pesticides in nectar and their influence on bee behaviour

G. Wright

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Floral nectar is the main reason most pollinators visit flowers. It is an energy drink for hard-working pollinators: its composition is mainly carbohydrates and amino acids that can be rapidly made into fuel. Nectar may also contain other substances including amino acids, toxins, and even compounds with pharmacological activity. Many pollinators like foraging bees learn to associate floral traits with reward in order to best exploit nectar while it is present. Here, I will discuss how nectar-toxins such as caffeine and commonly used pesticides interact with the honeybee’s ability to learn and remember floral scent. I will discuss the ecological implications of the use of toxins by plants to manipulate pollinators.
Interactions among plants, pollinators and nectar microbial communities: patterns and effects

C. De Vega
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Floral nectar is a complex fluid that plays a decisive role in the establishment of most plant-pollinator mutualisms. However, nectar is not used exclusively by pollinators. Its composition makes it a favourable environment for the growth of microorganisms, and more specifically, yeast and bacteria that are vectored from flower to flower by floral visitors. In this presentation I will review the current understanding of the importance of nectar microbial communities, and present our most recent findings on interactions among plants, pollinators, and nectar microbes. We have observed that yeasts and bacteria are widespread in the nectar of many plant species irrespective of continent or habitat type. Some yeasts and bacteria species are particularly well adapted to flourish in this especially harsh environment of high sugar concentrations and antimicrobial compounds, where they can reach high densities. These microbes actively consume nectar and change its chemical composition, reducing the chemical control of the plant over this key floral trait. Their metabolic activity may also increase flower temperature. I will discuss recent results suggesting that through their effects on nectar physicochemical characteristics, yeasts and bacteria can affect the foraging behaviour of pollinators and ultimately influence both quantitative aspects (pollination success, seed production...) and qualitative aspects (inbreeding levels and genetic diversity of seeds...) of sexual reproduction of plants. Finally, I will propose a new role for nectar, as a reservoir of unexplored microbial biodiversity, and reveal the importance of plant-pollinator interactions in structuring nectar microbial communities.
“Plant mating and animal pollination: a complex natural system”

Pollinators and pollination challenge: the Mediterranean case
T. Petanidou
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Pollination is a key ecosystem service that is essential for the conservation of wildlife and ecosystem functioning (thus, an invaluable resource), and the world’s agricultural production and economy (thus, a valuable resource). On a global scale, pollination services are provided by populations of managed and wild animal pollinators, the majority of which are insects. Bees are the most important pollinator group with an estimated 25,000 species. The Mediterranean Basin, with >2500 species is a hotspot for and a “centre” of bee diversity.

Worldwide pollinators are declining today. Likely causes of the decline are habitat fragmentation and loss, excessive land use and land use change, pesticide use, various pathogens and parasites, climate change: Several of the aforementioned causes lead likely to the Colony Collapse Disorder that has been reported widely. Thus, the maintenance of pollinator diversity has been an increasing global concern and it is considered a daunting threat to the planet, having similar far-reaching impacts, as climate change, chemical waste deposit and biological invasions.

The talk will summarise our knowledge on the pollinators of the Mediterranean: their diversity, the services they provide to natural systems and crops, and the threats they face particularly in this part of the world (biological invasions, wild fires, grazing, excessive bee-keeping, mobile telecommunication antennas, climate change). I will highlight the necessity of creating (i) infrastructure and facilities to support further pollination studies in the Mediterranean (e.g. Mediterranean bee/pollinator collections and taxonomic keys), (ii) human capital and expertise for bee/pollinator taxonomy, and (iii) public awareness vis-à-vis the Red Data list of the Mediterranean pollinators to be followed by the formation of pollinator-friendly management guidelines to be applied in the Basin.

This research is co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework – NSRF), Research Funding Program: Thales – Investing in knowledge society through the European Social Fund.
Is main sugar composition of floral nectar determined by pollinators in *Scrophularia* species?

T. Rodríguez-Riaño, A. Ortega-Olivecnia, L. López, J.L. Pérez-Bote, M.L. Navarro-Pérez

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Nectar traits in angiosperms have been explained by its relationship with pollination syndromes or as due to phylogenetic constrains. However, nectar trait information remains scarce for many plant groups. This work focuses on three groups of *Scrophularia* species, with different flower sizes and principal pollinator groups, to find out whether pollinator type determine the nectar sugar composition or it reflects taxonomic affinities. The sugar composition was similar in all species, having high-sucrose nectar, except for the Macaronesian *Scrophularia calliantha*, which was the only species with balanced nectar. This balanced nectar could be associated with the high interaction rates observed between this species and passerine birds. The nectar sugar composition (high in sucrose) was unrelated to the principal pollinator group, and could instead be considered a conservative taxonomic trait.
Sexual reproduction of flowering plants depends on delivery of the male gamete to the ovule, which occurs through a polarized apical growth of the pollen grain, giving rise to the pollen tube. This process known as pollen germination is characterized by the continuous rebuilding of the cell wall and apical migration of the cytoplasm sustained by cytoskeleton re-organization.

The cytoskeleton of pollen tubes is a network of single and polymer-forming proteins that are involved in many aspects of pollen germination and growth, from the asymmetrical distribution of membrane-bounded organelles to the deposition of cell wall material.

An intriguing aspect of plant reproductive biology is how pollen–pistil interactions occur and who are the actors of this cross talk, involved in regulation of pollen tube growth inside the pistil.

In the self-incompatibility response, changes to both actin filaments and microtubules are likely to be triggered by specific proteins, resulting in either the de-polymerization of cytoskeleton filaments, or the formation of aberrant structures. Transglutaminases are enzymes that catalyze the post-translational conjugation of polyamines to different protein targets among which the cytoskeleton ones as well as the cross-linking among protein substrates giving rise to protein aggregates. The binding of cytosolic TGase to actin filaments was shown to be Ca2+ dependent.

In the self-pollinated style of Abbè Fétel (A x A, incompatible system), the activity of TGase increased when the pollen tube stopped its growth inside the style leading to the formation of high molecular mass cross-linked products, including aggregates of tubulin and actin, as it is also shown by the in vitro post-translational modification of actin and tubulin catalyzed by purified pollen TGase. On the contrary in Abbè Fétel styles pollinated with Williams pollen (A x W, compatible system), TGase activity decreased during pollen germination.

Immunolocalization of TGase showed that the enzyme was present in the growing region of pollen tubes in the cytoplasm and in membranes; moreover, the enzyme colocalizes with cell wall markers; TGase is likely to be secreted by a mechanism involving both membrane dynamics and the cytoskeleton. Since actin filaments are perturbed during the self-incompatibility response, it is likely that the distribution and the activity of extracellular TGase is also affected, leading to the arrest of pollen tube growth. This enzyme was expressed during pollen germination inside the style to a similar extent in both systems (A x A and A x W) showing that it is only the activity to have been stimulated by some factor dependent on SI.

According to the reported data, we can hypothesize that TGase-modified cytoskeleton proteins may be important for the rapid rearrangement and translocation of the cytoskeleton elements, essential during pollen tube elongation occurring in pollinated styles.
Oral I-2: Discovery of nuclear male-sterility in leaf chicory: an overview on its genetic determinism and use in breeding programs

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The present research deals with the discovery and genetic analysis of male sterile mutants of leaf chicory (Cichorium intybus, 2n=2x=18). Distinct spontaneous male sterile mutants, which to the best of our knowledge are the first ones ever discovered and described in leaf chicory (Cichorium intybus subsp. intybus var. foliosum L.), were characterized in great details for the developmental pathway of microsporogenesis and gametogenesis, and the inheritance pattern of the gene, here named Cims-1, underlying the male-sterility trait. Overall data clearly support a nuclear origin and a monogenic control of recessive type for the male-sterility trait in each of the leaf chicory mutants. Moreover, the fine mapping of the mutant locus was attempted by molecular markers using F2 and BC1 populations segregating for male-sterility. The gene responsible for male-sterility was found tightly linked to a microsatellite of the TC/GA type whose full sequence was recently deposited in the NCBI databases under the accession no. JF748831. A molecular diagnostic assay was then developed to be profitably adopted as a tool of marker-assisted breeding and exploited for an early screening of male-sterile plants within segregating progenies stemmed from back-crosses, with a genotyping error lower than 4%. Additional sequence-target site marker loci residing in the linkage group carrying the Cims-1 locus were developed to increase the accuracy of the marker-assisted selection for male-sterility in breeding parental inbred lines. Four new hybrid varieties of radicchio “Rosso di Chioggia” with different earliness, spanning from 70 to 150 days from transplanting (i.e. T&T 4010, T&T 5010, T&T 4070 and T&T 5070), were bred since 2011 by crossing male sterile partially inbred clones, used as seed parents, with wild type highly inbred lines, used as pollen donors. The parental lines to be used in large-scale pair-wise crosses were selected on the basis of their specific combining ability (SCA) assessed by means of molecular marker analysis (i.e. genetic distances) and agronomic progeny tests (i.e. field performances). The discovery of non-engineered male-sterility in leaf chicory will open new frontiers for maximizing crop productivity in this important cultivated vegetable species through the breeding of F1 hybrid varieties. Introgression of the male sterility trait, initially discovered in “Rosso di Chioggia”, is currently in progress in other biotypes such as “Rosso di Treviso Precoce”, “Rosso di Verona” and “Pain de Sucre”.
Oral II-1: De novo sequencing of the *Hypericum perforatum* L. flower transcriptome to identify genes and miRNAs that are related to plant reproduction sensu lato

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St. John’s wort (*Hypericum perforatum* L.) is a medicinal plant considered to be an attractive model system for the study of the aposporos type of apomixis. Here we sequenced and annotated the *H. perforatum* flower transcriptome to provide critical knowledge toward a better understanding of the genetic control of aposporic and sexual reproduction. We used Roche 454 technology to sequence the transcriptome of single flower parts collected from both apomictic and sexual genotypes, which enabled us to investigate the coding and non-coding RNA fractions of the flower transcriptome and identify RNAs that are likely exclusive to single flower organs or reproductive strategies. Computational investigations were based on 1.5 mln sequences, which were globally assembled to originate 33.860 contigs with an average length of 1.002 bp. The protein-coding sequences of the flower RNAs were annotated using Gene Ontology and Plant Ontology terms in order to identify major biological processes and molecular functions involved in flower development and plant reproduction. Starting from this dataset, we were able to recover and annotate a large number of transcripts related to meiosis, gametophyte/gamete formation, and embryogenesis, as well as genes that are exclusively or preferentially expressed in sexual or apomictic libraries. Computational investigations allowed the identification and characterization of a number of mature miRNAs along with their precursors and potential targets, which were characterized in great details to investigate their regulatory role in biological processes occurring in the flower. As a result, we could demonstrate that *H. perforatum* flowers shares numerous highly conserved miRNAs and that these miRNAs potentially target dozens of genes with a wide range of molecular functions, including response to stress, flower development and plant reproduction. Finally, quantitative PCR analyses and in situ hybridization assays proved the differential expression of six transcripts putatively involved in related to meiosis and gametophyte/gamete formation in apomictic and sexual pistils. Annotation of all identified flower transcripts as well as their qualitative and quantitative expression data will be presented and critically discussed as they prove a far better understanding of molecular bases of flower development and gamete formation in sexual and apomictic *H. perforatum*. 
“Nectar: plant interface for interactions with biotic environment”

Oral III-1: Non-protein amino acids in nectar: are they tools to modulate insect behaviour and increase plant reproductive output?

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Floral and extrafloral angiosperms’ nectar is undoubtedly recognized as a valuable energetic alimentary resource for a large variety of animals. Through nectar consumption insects, small mammals, birds, marsupials and reptiles establish more or less specialized interactions with plants. But according to recent studies, nectar mediates interactions that are much more complex than simply alimentary relations. From the more abundant nectar compounds, i.e. sugars and amino acids mainly responsible for its high alimentary value, attention of scientists is moving towards compounds that are not directly connected with its alimentary importance. Among these, secondary compounds appear to have a special role in regulating interaction with other organisms. Although very few is known about their ecological roles in nectar, recently it was proved that secondary compounds may affect the nectar feeders’ behaviour interacting with their neurobiology. We addressed a special focus on one class of secondary compounds: the non-protein amino acids. Their presence in floral nectar has been reported since long time but their ecological function has not been investigated. Data from several phylogenetically unrelated species indicate that they may represent a consistent part of the total amino acid content of floral nectar (25-45%) and generally the more abundant ones are γ-amino butyric acid (GABA) and β-alanine. The study of nectar chemistry in a restricted taxonomic context (i.e. the tribe Lithospermeae of the Boragniaceae family) revealed that GABA concentration is particularly high in species with specialist bee- and bumblebee-pollination whilst β-alanine increases in species pollinated by flies and passerine birds. This result suggested to test the effect of an artificial diet consisting of 20% sucrose solutions enriched with GABA and β-alanine on some species of Apoidea. Results show that some species of insects increase their mobility, flying activity and survival time when fed with the artificial diet and particularly with the solution enriched with GABA. Although we used high concentration of the non-protein amino acid (20-fold higher than the concentration occurring in nectar), these results push us to further consider the role of GABA in increasing the mobility of insects between flowers and their foraging activity and thus their pollination performances.
Oral III-2: Monitoring of *Osmia* bees reared with beta-Alanine and gamma-Amino Butyric Acid diets

S. Sagona 1, M. Nepi 2, L. Bortolotti 3, M. Galloni 4, G. Bogo 3,4, A. Felicioli 1

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4) Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy

*Osmia* bees (Hymenoptera: Megachilidae) are pollinating insects that are becoming more widely used in agriculture. They are solitary and univoltine bees, and have a strong sexual dimorphism. These insects have the maximum of fly and foraging activities in the early spring and are active even in adverse weather conditions and very low temperatures (5-8°C), that are forbidding for the *Apis mellifera*. The agricultural interest in using *Osmia* bee as pollinator derives from these characteristics. In this investigation the two non-protein amino acids beta-Alanine and gamma-Amino Butyric Acid (GABA) are used as nectar compounds to observe the *Osmia* bees behaviour. Two species of *Osmia*, *O. cornuta* (Latreille, 1805) and *O. bicornis* (Linnaeus, 1758), were reared in plexiglass tubes as boxes, closed with a metallic net. Each rearing tube had three 0.8 ml Eppendorf plastic vials containing the diet inserted in an artificial flower, 7 plastic test tubes and some pieces of soft white paper. Three diets were tested, each on 5 female *Osmia* bees: control diet (sucrose 20% w/v), sugar syrup with beta-Alanine 46 mM and sugar syrup with GABA 15 mM (amino acids concentration 20 times higher than in natural nectar). Five parameters were observed randomly for 5 minutes each time for all the life span of the reared bees: 1) how many bees were localized on the metallic nets, 2) on the artificial flower, 3) under the paper, 4) how many remained in the test tubes and 5) how many flew. One trial for *O. cornuta* and two for *O. bicornis* were carried out. Results showed a significant survival rate increase for the *Osmia* bees reared with the GABA diet compared to the other diets. Concerning the control diet, more *Osmia* bees stayed in the test tubes and under the paper, while *Osmia* bees from the rearing box with a GABA diet had a significant increase of the parameters of motility (flight and localization on the net). This investigation was mainly focused on the identification of a proper methodology and of valid parameters to study the effect of nectar compounds on the solitary bees behavior. Although further investigations could be made to perfect this type of ethological studies, results showed that the observed parameters and the methodology used for *Osmia* bees rearing seem to be an effective method.
“Plant mating and animal pollination: a complex natural system”

Oral IV-1: Honey bee behavioural flexibility and adaptation: reinforcing specialized associations while handling diverse species of flowers

M. Giovanetti
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Pollination is recognised as a key mutualism between plants and animals that strongly influenced the evolution of angiosperms. Among animal pollinators, bees played a major evolutionary role (Cardinal & Danforth 2013). Today, facing a “pollination crisis” and an alarming decline in bee population worldwide, evidence of important gaps in the understanding of the bee-flower relationship come to light. One of this gap applies to the new mutualisms that alien plant species establish with native pollinators. The focus is often on the reproductive success of the alien species, and pollinators are usually investigated in the context of visitation rates on alien and native flowers, flower constancy, pollen transfer. Beyond these questions, certainly important and of immediate consequences, an evolutionary point of view should consider the behavioural bases favoring the adoption of an alien flower as food source. To what extent are bees learning how to exploit a new source and does it imply a pollination service that may help the spread of aliens?

Due to its worldwide distribution, the honey bee (*Apis mellifera*) contribution to the spread of alien plant species may turn out to be especially important. Key factors for a successful interaction are the way individual foraging matches with the characteristics of the new food sources and how far bee ability can go in recognising and selecting alien flowers. Such data may convey important information on how robust the newly established relationship is, and finally help in defining the evolutionary pathway that drove/drives the extraordinary success of angiosperms-insect pollinator development. Short and long term learning in bees is associated to temporal and spatial characteristics of food sources (Menzel 1985). Data on honey bee foraging behaviour on alien species, from the handling to the selection of individual flowers, highlighting links with flower morphological characteristics and resource content and presentation (pollen and/or nectar), will be presented and discussed in relation to the importance of floral display, on the one hand, and behavioural flexibility, on the other, finally shaping the ecology and evolution of bee–flower interactions.

References:
Menzel R 1985. Experimental behavioral ecology 31, 55-74
Oral IV-2: Temporal variation and efficiency of the pollinator community of the rare *Dictamnus albus*

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The abundance and composition of pollinator communities may vary in space and time due to several natural factors, with consequences on the efficiency of pollination and on plant fitness; on the other hand, flowering phenology and abundance may act on the preferences and behaviour of an insect community. We present the results of a 4-year study performed in a natural population of *Dictamnus albus* within the LIFE+ Project “Plant-Pollinator Integrated CONservation approach: a demonstrative proposal” (LIFE09/NAT/IT000212, http://www.pp-icon.eu).

We observed insect behaviour during flower visits, recording the collected rewards, the contacts with receptive stigmas, the movements along the raceme and among plants, and also noting flower abundance and phenological stage. The pollinating ability of each visitor taxon was estimated as an index of pollination performance based on the frequency of visits, fidelity to the dittany (i.e., the percentage of *Dictamnus* pollen on the total pollen load) and pollination efficiency (i.e., the percentage of contacts with receptive stigmas). Our results indicate a slight variation in the composition of visitor guilds among years, and a considerable ecological specialization of the plant-pollinator system: within the spectrum of visitors, only medium-big sized bees (*Habropoda tarsata*, *Xylocopa* spp., *Bombus* spp.) play a significant role as *D. albus* pollinators. Megachilids (*Megachile, Osmia*) show an intermediate efficiency, partly due to their smaller body size; however their high frequency of visit contributes to their role as pollinators. On the contrary, small halictid bees only act as pollen or nectar thieves. These results were helpful for the implementation of concrete conservation measures addressed to pollinator safeguard and habitat restoration, such as the setting of specific nesting sites for solitary bees or bumblebee colonies, as well as the selection of nectariferous flowering species for habitat management. Fruit and seed set of control and pollen-augmented flowers highlighted a variation in pollen/pollinator limitation among years, which may be due to Allee effect (i.e., quantitative limitation) or to high inbreeding between close relatives (i.e., qualitative limitation).

This research has been performed within the Life+ Project PP-ICON (http://pp-icon.eu) co-financed by the LIFE Programme of the European Union (LIFE09/NAT/IT000212)
Oral IV-3: Reproduction, modularity and important species of Mt. Lesima grasslands (Northern Apennine): a season-wide network analysis

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In our work, we investigated the structure of plant-pollinator interactions, the modules, the key taxa of two montane grasslands sited on Mt. Lesima (at about 1650 m a.s.l., Northern Apennine, Pavia Province), which were previously analyzed according to the phytosociological method: *Brachypodium rupestre* and *Festuca laevigata* community and *Laserpitium siler* and *Sesleria pichiana* community (*Bromion erecti, Brometalia erecti, Festuco-Brometea*). To record insects foraging on flowers, a sampling scheme based on three plots of 2.5x2.5 m was established in each plant community, sampled weekly for 20 minutes, twice a day. Seasonal groups of plants that flower together (pheno-cluster) were identified treating the number of per-species inflorescences as variables of reproductive effort. On such seasonal units, we performed a network analysis of plant-pollinator interactions.

*Laserpitium siler* and *Sesleria pichiana* community resulted more complex and richer than *Brachypodium rupestre* and *Festuca laevigata* community. Despite the differences in floristic composition, proportions of functional pollinator groups were similar. Moreover the module architecture (detected by means of the weighted Newman’s modularity measure) was constant along the season: cores of a high number of insect species visiting few plant species flanked by plants visited by large Apidae, completed by species interactions not structured into proper modules.

Regarding the plant-pollinator networks, low levels of realized links (connectance) were detected, suggesting a probable bias towards specialization in plants. Nevertheless, network metrics changed over the season: as the season progressed to the final stages, both plants and insects became more generalists (H2’), possibly due to an increase in the pollinator/plant ratio. Nestedness values (NODF) were generally low. Thus, the nested structures are based on few species as pivot clusters, probably as result of altitude effect on insects. Two types of pivot species (that links different modules) were detected. The first type is characterized by high values of number of links, of weighted betweenness and of sharing partner and their presence provides a cohesive structure. In the second type, other plants with high link number, considerable WB value but low d’ one play an important role in biodiversity maintenance because they are visited by insects that otherwise would not forage on the grasslands.
Oral IV-4: Insect pollination and nectar secretion in protandrous flowers of *Geranium macrorrhizum* L.

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The genus *Geranium* L. contains species characterized by different ways of flowers development, ranging from homogamy to various degree of dichogamy. Moreover, several trends in pollination mechanism with gradual transition between automatical self-pollination and cross-pollination by insects have been observed. To full reproductive success, zoophilous *Geranium* flowers must attract their pollinators by offering nectar and/or pollen as a reward.

In the present study, the insect pollination in *Geranium macrorrhizum* L. as well as nectar secretion and accessibility were investigated. Two pollinating treatments: open pollination and geitonogamous/autogamous pollination with exclusion of insects were carried out. An assemblage of insects visiting *Geranium* flowers was monitored. Moreover, a visiting rate as well as size and localization of pollen load transported on an insect body were studied. Nectar secretion was examined across male and female floral phases. Localization of nectaries and accessibility of nectar were determined.

*Geranium macrorrhizum* exhibits an incomplete protandry with an overlap in the presentation of the pollen and stigma. This species produced fully developed fruits and seeds via autogamy, geitonogamy and xenogamy. The fruit set decreased by 63%, after geitonogamous/autogamous pollination without insects but seed number/flower did not differ between treatments.

Flowers facing horizontally during anthesis, are slightly zygomorphic with upwardly curved generative organs. Nectar secreted by 5 discoid glands is concealed in ~10 mm deep calyx. The greatest average values of the nectar amount, nectar concentration as well as nectar-sugar quantity occurred in the female-stage flowers when nectar becomes the major reward available in flowers. Nectar collecting insects must touch anthers or pistil with their abdominal site, which results in sternotribic deposition of pollen.

Key pollinators of *G. macrorrhizum* were honey bees (94.8%) and bumblebees (3.13%) with the mean number of visits per flower-min-1 = 0.24 and 0.05. The number of pollen grains transported on bees body ranged from 16 to 253 and *Geranium* pollen comprised only 19.12% of all pollen grains found, on average. Overall, *G. macrorrhizum* exhibited characters associated with bee pollination.
Oral IV-5: Habitat fragmentation effects and ecological determinants of plant reproductive success: the response of an insect-pollinated species

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Habitat fragmentation has become a major concern in ecology and conservation biology. Many landscapes that were once continuous now exist only as scattered small fragments with different degrees of connectivity and under the influence of edge effects. Thus, habitat fragmentation influence important ecological factors responsible of plant reproductive success, such as conspecific neighbourhood density or flowering synchrony. Further, animal-pollinated species are particularly susceptible to habitat alteration indirectly by changes in the local pollinators behaviour, composition and abundance.

In this study, we assess the influence of habitat fragmentation on the pollination biology and reproductive success of the insect-pollinated Mediterranean shrub *Myrtus communis* L. By analysing individual plants and populations in a gradient of fragmentation and through structural equation modelling (SEM) we investigate the interactions between multiple ecological and landscape factors and the plant reproductive success (fruit set, seed set and number of seeds per fruit). Further, we conducted censuses of pollinators in each population as a main driver to explain differences in plant reproductive output. Overall, our study stresses how the spatial architecture of the landscape at different levels and plant-pollinator interactions shape species reaction to habitat fragmentation, thus highlighting the interest of multi-scale approaches.
POSTER SESSION

“Reproductive barriers in plants: male-sterility and self-incompatibility”

Analysis of genetic stability within and genetic diversity between male-sterile and male-fertile inbred lines of fennel (Foeniculum vulgare Mill.) for breeding new F1 hybrid cultivars

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Fennel (Foeniculum vulgare Mill.) is well known worldwide as an aromatic, culinary and medicinal herb. Bulb fennel is also a relevant crop in the Mediterranean basin, and particularly in Italy, is as emerging crop in other countries, such as the USA. Despite its agronomic and pharmaceutical interests, researchers face the almost complete lack of biological data for this species, being nucleotide sequences and molecular markers available very scanty. In this research we describe a new and robust AFLP-based technology and its exploitation to assess the genetic stability and diversity existing within and between inbred lines, respectively. In our estimates, this method allowed the amplification and visualization of an average of 190 amplicons per primer combination (EcoRI+3/Msel+3 and PstI+2/Msel+3, equivalent to restriction sites and selective bases). The reproducibility of DNA fingerprints was computed on a number replicated experiments and it appears to be higher than 97%. Validation of the method was conducted using 240 plants that belong to inbred lines, including male steriles, maintainers and pollen donors. Here we also describe the discovery, cloning and analysis of microsatellite regions and the development of the first set of SSR markers in fennel. It is worth mentioning that the most discriminant microsatellites, characterized by high polymorphism information content, revealed regular and adjacent tri-nucleotide repeats, such as (CAA)₆(TAA)₅ and (AAG)₅(TGA)₃, but also imperfect and longer motifs, such as tetra- or penta-nucleotide repeats (GenBank, submission ID 1735181). On the whole, this research describes the development and assessment of molecular markers suitable for fingerprinting and genotyping accessions of fennel, including cytoplasmic male-sterile mutants, that will have great applied utility for marker-assisted breeding programs aimed at the characterization and selection of parental inbred lines, and the constitution of new commercial F1 hybrids in this species.
Self-incompatibility in olive: cytological observations support sporophytic-like features and molecular findings reveal a non-conventional S-locus in *Olea europaea* L.

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Self-incompatibility is one of the most important barriers adopted by many flowering plants to prevent inbreeding, thus maintaining diversity within species. The elucidation of this reproductive constraint is crucial in olive (*Olea europaea* L.) because it may help to improve breeding strategies and orchard management. The available literature reports that a gametophytic self-incompatibility (GSI) system might exist in olive, even though neither cytological nor molecular data were provided. A molecular approach failed to find S-RNase (S-locus ribonuclease) and SLF (S-locus F-box containing protein), the genes responsible for GSI. Here we present data from cyto-histological observations and bio-molecular experiments, supporting the presence in olive of a sporophytic self-incompatibility (SSI) system. The main genes known to play a crucial role in SSI are SRK (S-locus receptor kinase) and SLG (S-locus glycoprotein), the female determinants, and SCR (S-locus cysteine rich protein), the male determinant. On the whole, we demonstrated that olive is a self-incompatible species and the rejection of self-pollen occurs at the stigma level, suggesting a sporophytic-type mechanism similar to that occurring in *Brassica* species. Furthermore, we cloned candidate genes for female and male determinants as orthologous of the genes that control the SI system in the Brassicaceae family, showing a tissue-specific expression in pistils and anthers, respectively, and we also documented a differential expression pattern of SI genes in self-compatible and self-incompatible cultivars. Nevertheless, both genetic results (i.e. independent segregation of OeSRK and OeSCR-like genes) and molecular evidences (i.e. no interaction between OeSRK and OeSCR-like proteins) support a non-conventional S locus in *Olea europaea*. In conclusion, on the basis of cytological observations we hypothesized that olive is a self-incompatible species displaying sporophytic-like features and then demonstrated that the molecular pathway leading to the recognition and rejection of self-pollen is dissimilar to the one adopted by Brassica species. Our genetic and molecular findings are robust and suggest a new and yet unknown mechanism controlling self-incompatibility in olive.
Seed germination and conservation of endemic species from the Central Apennines

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Seed germination is a critical stage for the establishment of plants as its success is determinant for plant species propagation (Rajjou et al., 2012; Annu. Rev. Plant Biol. 63:507-33). Each species has specific requirements for seed germination (Schütz and Milberg, 1997; Oikos 78: 420-428) which involves particular features of seed and environmental factors. Several germination traits have been claimed to be specific to high-altitude species (i.e. rapid onset of germination after snowmelt and high seed viability). Endemic species are a significant feature of high mountain Mediterranean climate which are one of the most threatened systems in Europe. Many efforts should be addressed to improve the conservation strategies for Mediterranean mountain species considering that the survival of endemic and threatened species is based on different and complementary conservation approaches and techniques. The definition of germination protocols, in particular for species characterized by small populations and for which data are missing, could be an important step in this direction. The objective of this research was to analyze seed germination capability of two endemic species of the Central Apennines included in the Regional Red List: Aquilegia magellensis and Campanula fragilis subsp. cavolini. Freshly-matured seeds of the considered species were collected from small wild populations growing on Mount Majella (Italy). Debeaujon and Koornneef (2000; Plant Physiol. 122: 415-424) show the role of gibberellins in promoting seed germination. Exogenous application of gibberelic acid (GA3) overcomes seed dormancy in several species. Accordingly, the following treatments were carried out for the considered seed types: control treatment (0 ppm GA3 treatment); 250 ppm GA3 treatment; 500 ppm GA3 treatment; cold-wet stratification treatment. The results show significant differences in germination rate between the species. The treatment with 500 ppm GA3 has a significant effect on A. magellensis germination rate. The cold-wet stratification treatment increases germination in A. magellensis and decreases it in C. fragilis. The observed differences in seed dormancy may be also related to the seed coat thickness. On the whole our results highlight the importance of the selected treatments to favor germination capability and may be used for a protocol in reinforcement projects of the wild populations as a means of reducing the extinction risk.
Verbascum tzar-borisii (Scrophulariaceae) is one of the rarest species not only in the Bulgarian flora but also in the world. It is represented by about 1000 individuals in two small localities in Southeastern Bulgaria – close to villages Chernevo and Ravna, Varna district. The taxon is included in the Bulgarian Biodiversity Act and in the Red Book of Bulgaria as ‘Critically Endangered’. The recent studies show its relatively low germination rate in situ, that reaches about 30% in laboratory conditions. In 2014 an Action Plan for the Conservation of V. tzar-borisii was developed and approved by the National Biodiversity Council at MOEW. One of the provided actions for improving the population state of the species is the increasing of the population size. In this relation the aim of the current work is to study the biology and to establish a scientific basis for in vitro cultivation of V. tzar-borisii. The biology of the species was investigated carefully. Seeds of the species were disinfected with NaClO and in vitro germinated on basal MS medium. The process was successfully stimulated by soaking seeds into 0.35% solution of GA3 primary to cultivation: 85% germinated during the first week versus 29% of the control. In contrast, GA3 supplemented in the medium (1 mg/l) strongly inhibited germination; after 6 weeks seeds were transferred on GA3 free medium but only 14% of them germinated. Seedlings developed normal leaves and roots, than plantlets were potted in soil mixture and 79% of them were successfully ex vitro adapted in growth chamber with gradual decrease of air humidity for 4 weeks. All of them survived under greenhouse conditions. Experiments on in vitro rapid multiplication are in progress using media containing different plant growth regulators. This research received support from the project №2570/13.06.2013. „Field investigations for distribution and populations’ size of selected fungi and plants in Bulgaria” financed by the Bulgarian Executive Environment Agency.
“Nectar: plant interface for interactions with biotic environment”

Diversity of floral nectar composition in the tribe Lithospermeae (Boraginaceae)

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Nectar is a flower secretion produced by many Angiosperms to recruit and remunerate pollen carriers. The mixture of chemicals contained in this secretion can be shaped by several ecological, physiological or phylogenetic constrains as well as specific preferences of pollinators. Nectar chemistry was reported to be a plastic character that can rapidly changes in response to these constrains, thus elucidating the drivers of nectar chemistry can be important to understand plant diversity and evolution. We studied the diversity of nectar chemistry in the Lithospermeae tribe, the larger one of the Boraginaceae family that group important nectariferous species. Sugar and amino acid profile have been determined by HPLC in 82 species (482 nectar samples) belonging to all the six clades of this tribe. There are clear associations between specific chemical profiles: sucrose-dominant nectar contain a higher percentage of non-protein amino acids (SD profile) than hexose-dominant nectars, and particularly more γ-aminobutyric acid. On the other hand hexose-dominant nectars contain a relatively higher percentage of protein amino acids, and among the non-protein amino acids the more abundant is β-alanine (HD profile). Most of the species in all the clades have SD or sucrose-rich (SR) nectar but there are few species in the clades A (8 species) and C (2 species) that possess an hexose-dominant profile. A clear separation in clade A occur for the Maccaronesian *Echium* species that have an HD nectar and for some of them pollination by bees and birds was reported. In the same clade also the strictly related genus *Lobostemon* shows this dichotomy with *L. fruticosum* having SD nectar whilst *L. momtanum* an HD one. For the latter species pollination by birds is also reported. The genus *Buglossoides* from clade C has a very heterogeneous nectar: *B. purpurocaerulea* and *B. calabra*, perennials growing in arid woods and mainly pollinated by dipterans, have HD nectar; *B. arvensis* and *B. incrassata*, annuals growing in arid pastures, has SR nectar and are pollinated by bees; *B. minima*, living in uncultivated fields, has hexose-rich nectar (HR). Species from all the other clades are in the range of SD-SR profile. Results suggest that a diffused phylogenetic constrain driven nectar chemical composition towards SD profile in Lithospermeae although in some species other factors, such as the habitat and the interactions with pollinators, had a stronger influence in shaping an HD profile.
Amino acids are the main nectar components after sugars. In addition to protein amino acids (among which Proline is the main one) non-protein amino acids (e.g., GABA, β-alanine) are also present and sometimes very abundant, but their contribution to floral attraction to pollinators is not completely clear.

Gentiana lutea subsp. Symphyandra is a perennial and generalist plant; in the studied area, bumble bees are the more important and efficient pollinators. Previous analyses have revealed high concentration β-alanine in G. lutea nectar, and field observations indicated an anomalous behavior in Bombus individuals that collect nectar from this plant, but not in those collecting pollen (Rossi et al., 2014).

To investigate the role of nectar amino acids in plant-pollinator relationships, we analyzed nectar preference under laboratory conditions, using experimental micro colonies of B. terrestris fed with artificially produced nectars. Each trial consisted of the preference analysis (by consumption) of 4 different solutions simulating G. lutea nectar, 3 of them enriched with β-alanine or proline or both amino acids. Nectars were paired in the 6 possible comparisons (dual choice feeding test). Solutions were administered by syringes and consumption was checked at regular intervals (24, 48, 72, and 96 hours). Five trials were performed on B. terrestris workers and two on males.

The same tests were carried out with honeybee foragers (Apis mellifera L.) for comparison, since previous studies (Bertazzini et al., 2010) showed a preference for nectars added with Proline in honey bee workers. The results of the single paired comparisons, analysed by t test, indicate a preference of B. terrestris workers for nectars enriched with β-alanine. Nevertheless, ANOVA test on average individual consumption gave inconsistent results among the 5 different trials, although nectars enriched with β-alanine and with both amino acids are the most consumed.

MANOVA analysis shows that the preference is influenced not only by the presence of amino acids, but also by the colony of origin and the kind of paired choice.

On the contrary, in the two tests performed on B. terrestris males a clear preference for the control solution is observed. Among the enriched solutions, however, also males prefer β-alanine. The results of honey bees tests are under processing.
Is pollinator activity influenced by non-protein nectar aminoacids?

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This study is focused on plant-pollinator relationship in an isolated population of Gentiana lutea subsp. symphyandra. G. lutea L. (Gentianaceae) is a long-lived species that mainly grows on calcareous (sub)-alpine pastures (800–2500 m a.s.l.). Flowering occurs between June and July. The study population is located on the eastern slope of Mount Grande (Northern Apennines; Bologna, Italy), between 1380 and 1460 m a.s.l. This is the only documented disjoint population occurring outside the known distribution range of the subspecies. The main visitors of the plant in this population are bees of the genus Bombus. Observations have been performed during flowering in the last 4 years; in 2011 and 2012 an unusual sluggish behaviour was observed in bumblebees feeding on plant nectar. The abundant nectar of the plant was analysed and it resulted remarkably rich in proline and β-alanine aminoacids; the hypothesis was that this composition could influence feeding choice and insect dynamism, exerting a narcotic effect on pollinators.

In 2013 further investigations were conducted during two successive days and two day intervals (morning and afternoon). Nectar standing crop was recorded sampling nectar from 37 flowers open to insect visitation (12 flowering stems), and nectar production was estimated through samplings from 40 flowers protected by net (20 flowering stems). Furthermore, we analysed the aminoacidic and sugar profiles of nectar collected from i) not yet or just opened flowers with indehiscent anthers and ii) flowers at older anthesic stage with dehiscent anthers.

In the same days and intervals, behavioural observations were performed on the visitors of the genus Bombus, for a total of 9 study hours. Insect movements within and among flowers and inflorescence, collected reward (nectar or pollen), and visitation time were recorded on 155 individuals; insects showing unusual behaviour were captured and identified.

The results of nectar analysis shows that in 2013 samples amino acids concentration was 100 times lower than in 2011; in parallel, observations on visitors do not reveal a marked abnormal behaviour. This seems to confirm our hypothesis. On the other hand, the few collected Bombus individuals, showing the most sluggish activity, belonged mainly to the Psythirus subgenus, whose adults, especially the males, are known to perform slow movements on flowers. This suggest an alternative explanation to the observed behaviour.
Plant-pollinator interactions in Leguminosae from Argentina

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The study was carried out in Salta Province, Argentina, during five consecutive years (2008–2012). The study area (24.34.53°–25.31.38° S and 65.22.30°–65.39.70° W) extends along the transition zone between Yungas seasonal rain forest and the Chaco dry forest. The studied species belong to five tribes: Dalbergieae, Crotalarieae, Desmodieae, Indigofereae, and Phaseoleae. The predominant color of the flowers was yellow (7 species), followed by purple-violet (5 species), pink-red (5 species) and only one species was pink. The flowering periods occurred between November and May, with an average overlap of 0.80. Larger flowers offered greater amount of both pollen and nectar. Patches with more flowers/area received more visits. Megachilidae species were best pollinators in eight species. Bombus species performed better in three plant species. Conversely, Apis mellifera had the lowest value of performance in six plant species compared with native pollinators. Pollinators removed between 20% and 80% of available pollen but deposited less than 1% of the total removed. Floral constancy was ca. 95%. The average number of pollinators per plant for the entire group was six. We conclude that most bees are polylectic, which would suggest that there is an indirect competition of plants for their pollinators. The divergent floral characters (e.g. size, color, nectar guides, type, quantity and characteristics of the rewards, etc.) and phenological (e.g., flower opening time and duration of anthesis) decrease competition for pollinators among species, while convergent phenological characters (e.g., flowering period) would be a strategy to ensure pollination service. Our results suggest that the studied species are phenotypically specialist, with a complex floral morphology associated with complex mechanisms of pollination. Besides, all species are functionally specialists, i.e., all pollinators were bees belonging to the Superfamily Apoidea (except one wasp species that behaves as an occasional pollinator in Crotalaria pumila). However, some species may be considered ecologically generalist and others could be more specialized, although none is a strict specialist.
Pollen load analysis reveals feeding preference of bee pollinators of *Dictamnus albus*

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*Dictamnus albus* L., commonly known as dittany, is a long-lived perennial herb belonging to the family Rutaceae; its flowers produce abundant pollen and nectar and are visited by several pollinating insects. Observations on insect behaviour while foraging on *D. albus* flowers showed that medium to large bees have the highest percentage of contacts with receptive stigmas, i.e. they show the highest probability of acting as valuable pollinators. However, this can be proved through the analysis of the pollen present on their body. We separately collected the pollen sparse on insect body (head, thorax and abdomen) and the pollen actively collected in specialized structures (corbiculae, scopae), in order to assess the fidelity of each visitor taxon, determined as the mean percentage of specific (*D. albus*) pollen. In addition, we performed a more precise analysis of the pollen load of the main pollinators of *D. albus*: Habropoda tarsata, Bombus spp. and Megachilidae spp.. For these insect taxa we identified pollen grains at the genus or family level, to infer the levels of competition or synergy among co-flowering species, and to obtain additional information on insect feeding habits.

Most visitors showed a high fidelity to *D. albus* in the study area, meaning that it is a good resource of rewards, and the potentially best pollinators of dittany tend to prefer its flowers compared to co-flowering species, indicating a low competition during full blooming. From the insect perspective, different plants may be source of different rewards at a same time. For example, dittany is mainly visited for nectar by most pollinators but other flowering species are visited for pollen or nectar as well.

This study allows us to explore the interactions among species in a complex ecological context and to develop specific conservation actions at both the plant and insect levels.

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Local plant community and bumblebee assemblage in differently managed Mediterranean agroecosystems

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One of the topics relevant to the pollinator-pollinated plant mutualism concerns the question of the extent to which species richness, abundance and composition of plant-pollinator assemblages act in shaping the mutual dependence.

What is the impact of a variation in the abundance of pollinating fauna, induced by external factors (e.g., parasites, human impact), on plant assemblages on a local level? Can a variation in animal composition (e.g., an increase of one pollinating species at the expense of others) directly or indirectly induce significant changes in the abundance of certain plant species?

One of the biggest obstacles to acquiring new knowledge on these issues stems from the scarcity of studies that take population trends into account. To obtain data on these, changes in abundance over time must be monitored by repeating the observations with the same method.

Researchers at the University of Pisa, in the context of the EU-funded project STEP (Status and Trends of European Pollinators) and in collaboration with researchers at the University of Bologna, conducted a study in 2011 using the monitoring method of the transect, which replicated a study conducted 11 years ago as part of a project funded by the Ministry of Agriculture (AMA project).

We present a set of data on the species population trends of the genus *Bombus* Latr. Within this group, while the number of species remains substantially constant over time, the species abundances (i.e., the number of individuals recorded per species) varies considerably from one year to the next. In particular, *Bombus pascuorum* shows an increase in individuals that is about 4 times the average for the number of individuals counted in the overall population of two transects monitored near Bologna in 1998 and 1999.

The species of plants foraged by bumblebees were also recorded, together with their abundance, and differences or similarities in the composition of vegetation are considered.
Contribution of flowering synchrony to species richness in dry meadows

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Facilitative interactions among species may increase the diversity of a community. Such enhancement can be produced through facilitation via shared pollinators among co-flowering entomophilous species. We evaluated the role of flowering synchrony in determining species richness and coverage at the community level in dry meadows.

In particular we tested if 1. there is a non-random flowering pattern at the community level; 2. both the presence and percentage cover of different insect-pollinated species are affected by synchrony.

The study was carried out in the Euganei Hills district (NE Italy). 45 plots (2x2m) were randomly placed over dry meadows and data on species richness and percentage cover were collected. Flowering phenology of all entomophilous species was monitored every 10 days during the growing season (April, 1st to September, 30th 2013).

To estimate flowering time overlap at the community level a mean Co-flowering index (V score; Lepš & Šmilauer, 2003) was performed between all possible pairs of monitored species. The significance of flowering overlap pattern was tested using a null model. For each plot a Co-flowering index was than computed. Spearman’s rank correlation was used to test for a relationship between the V score and the number (and percentage cover) of insect-pollinated species per plot. Species were classified in six groups according to Müeller’s (1881) classification: pollen flowers, flowers with exposed nectar, flowers with partly hidden nectar, flowers with totally hidden nectar, Hymenoptera flowers and Lepidoptera flowers. Only rough groups of pollinators can be assigned to the first four groups, conversely smaller pollinator range characterize Hymenoptera and Lepidoptera flowers.

A non random flowering pattern has been observed at the community level among entomophilous species (p-value<0.001).

In particular, richness and percentage cover of Hymenoptera flowers turned out to be positive related to Co-flowering index (p=0.001 and p=0.02), while no significant correlations were observed for the other groups of species.

In conclusion, our results show that flowering synchrony plays an important role in controlling and enhancing Hymenoptera pollinated species richness in Euganean dry meadows.

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