

**XVI Annual Meeting of the Spanish
Group for Floral Ecology**

EcoFlor2019

<https://ecoflor2019.weebly.com/>

March 14th-16th 2019

Granada



EcoFlor2019



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Organizing committee:

Mohamed Abdelaziz, Juan Lorite, José M. Gómez, A. Jesús Muñoz-Pajares & Francisco Perfectti

Workshops organization and instructors:

- *From individual to collective creativity in Science* by Isabel Reche & Francisco Perfectti
- *Integral Projection Models (IPMs): a trait-based approach to investigate mechanisms of population responses to environmental change* by Maria Paniw & Irene Martín

Round table organization:

Marcos Méndez, Ana María Martín González, Jeff Ollerton & María Clara Castellanos

Cover photo credit:

Francisco Perfectti

wednesday 13th

16:00 **WORKSHOPS**

From individual to collective creativity in Science MEDIA ROOM

Integral Projection Models (IPMs): a trait-based approach to investigate mechanisms of population responses to environmental change ROOM A04

thursday 14th

9:00 - 9:45 **PLENARY TALK**

Prof. **Gonzalo Nieto-Feliner**. Real Jardín Botánico de Madrid. Spain. *The role of hybridization in evolution. Hybrid speciation, adaptive introgression and no man's land in between?*

9:45 – 11:00 **ORAL PRESENTATIONS**

11:00 – 12:00 **COFFEE BREAK & POSTER SESSION**

12:00 – 13:15 **ORAL PRESENTATIONS**

13:15 – 15:15 **LUNCH BREAK**

15:15 – 16:30 **ORAL PRESENTATIONS**

16:30 – 17:30 **COFFEE BREAK & POSTER SESSION**

17:30 – 18:00 **ORAL PRESENTATIONS**

18:00 – 19:00 **ROUND TABLE** *Needs for synthesis and opportunities of large scale collaboration in floral biology and pollination research. MEDIATORS:* M. Méndez, A. M. Martín González, J. Ollerton, M. C. Castellanos

19:30 **BAR CONVERSATION AND DISCUSSION**

friday 15th

9:00 - 9:45 **PLENARY TALK**

Dr. **María Clara Castellanos**. University of Sussex. United Kingdom. *Selection and heritability in the wild: understanding floral trait stasis and evolvability*

9:45 – 11:00 **ORAL PRESENTATIONS**

11:00 – 12:00 **COFFEE BREAK & POSTER SESSION**

12:00 – 13:15 **ORAL PRESENTATIONS**

13:15 – 15:15 **LUNCH BREAK**

15:15 – 16:30 **ORAL PRESENTATIONS**

16:30 – 17:30 **COFFEE BREAK & POSTER SESSION**

17:30 – 18:00 **ORAL PRESENTATIONS**

18:00 – 18:30 **NEXT ECOFLOR 2020**

19:30 **BAR CONVERSATION AND DISCUSSION**

saturday 16th

Field trip to **Natural Area of Maro-Cerro Gordo Cliffs** and **Sierra de Lujar and the Contraviesa**. Meeting at Faculty of Sciences at 8:00.

thursday 14th – talks & posters

- 8:45 – 9:00 Welcome and introduction
- 9:00 – 9:45 The role of hybridization in evolution: hybrid speciation, adaptive introgression and no man's land in between? **G. Nieto-Feliner**
- 9:45 – 10:00 Sunbird surprise: A test of the predictive power of the syndrome concept **E. Quintero**, E. Genzoni, N. Mann, C. Nuttman, B. Anderson.
- 10:00 – 10:15 Experimental insights to unravel adaptive evolutionary patterns in plants: the tyranny of phenology **X. Picó**
- 10:15 – 10:30 Phenotypic selection on flower colour and other floral traits in *Silene littorea* **N. L. Rodríguez-Castañeda**, E. Narbona, P. L. Ortiz, M. Arista, M. L. Buide
- 10:30 – 10:45 Can pollinators assess floral rewards through the use of visual cues? **D. Pareja Bonilla**, P. Fernández Díaz, M. Arista Palmero, A. M. Escudero Lirio, P. L. Ortiz Ballesteros.
- 10:45 – 11:00 Virtual lack of prey-pollinator conflict in the carnivorous subshrub *Drosophyllum lusitanicum*: how is it achieved? **F. Ojeda**, C. Carrera
- 11:00 – 12:00 **COFFEE BREAK & POSTER SESSION**
- 12:00 – 12:15 Size matters — for plants and insects as well **H. Miranda**, M. Jarne, A. González, J. Olesen, M. B. Garcia.
- 12:15 – 12:30 Impact of alien rats and honeybees on the reproductive success of an ornithophilous endemic scrambling plant in the Canarian thermophilous forest
J. Jaca, N. Rodríguez, M. Nogales, A. Traveset
- 12:30 – 12:45 Effects of agriculture intensification on the diversity, composition and interaction structure of wild plant-pollinator communities **C. Gómez-Martínez**, J. Cursach, J. Rita, M. A. González-Estévez, C. Cardona, M. Capó, A. Lázaro
- 12:45 – 13:00 Non-native mammals as seed-dispersal rescuers of the Mediterranean dwarf palm *Chamaerops humilis* L. in Mallorca (Balearic Islands) **R. Muñoz-Gallego**, J. M. Fedriani, A. Traveset
- 13:00 – 13:15 Kin recognition and sociability in one-flower vetch (*Vicia articulata*) **I. Martín Brull***, N. García Díaz, F. Ocaña Calahorro, R. Rubio de Casas.
- 13:15 – 15:15 **LUNCH BREAK**
- 15:15 – 15:30 Pollen and stamen mimicry – the most speciose mimicry system of the world **K. Lunau**

- 15:30 – 15:45 What is the true colour of snake's head? Spectral analysis of floral colours in the genus *Fritillaria* **K. Roguz**, S. Köthe, K. Lunau, A. Roguz, M. Zych
- 15:45 – 16:00 An unstable habitat and a narrow-endemic plant: the pollination biology and genetic identity of *Linaria tonzigii* **P. Biella**, G. Federici, A. Akter, N. Tommasi, A. Galimberti, J. Jersáková, A. J. Muñoz-Pajares, F. Mangili, L. Mangili
- 16:00 – 16:15 Why so different? The diversity and factors affecting nectar composition in populations of a rare plant *Polemonium caeruleum* L. in Poland **J. Ryniewicz**, K. Roguz, M. Skłodowski, A. Bajguz, M. Zych
- 16:15 – 16:30 Heterostyly and zygomorphy as complementary mechanisms promoting outcrossing in *Oplonia nannophylla* (Acanthaceae) C. Reaño Jimenez, **E. Olivieri**, M. Faife-Cabrera
- 16:30 – 17:30 **COFFEE BREAK & POSTER SESSION**
- 17:30 – 17:45 Mating networks in a generalist plant species **J. Valverde**, J. M. Gómez, F. Perfectti
- 17:45 – 18:00 The influence of insularity on pollination networks in Australasia **W. E. Drabble**, B. Dalsgaard, A. M. Martín González
- 18:00 – 19:00 **ROUND TABLE** Needs for synthesis and opportunities of large scale collaboration in floral biology and pollination research. M. Méndez, A. M. Martín González, J. Ollerton, M. C. Castellanos

friday 15th – talks & posters

- 9:00 – 9:45 Selection and heritability in the wild: understanding floral trait stasis and evolvability **M. C. Castellanos**
- 9:45 – 10:00 Before and after flowering: patterns of flower, ovule and seed formation in an annual hemiparasitic plant, *Rhinanthus major* (Orobanchaceae) A. Saulnier, **R. A. Wesselingh**
- 10:00 – 10:15 Spatio-temporal variation in pollen limitation in a boreal pine forests in North-Eastern Poland **M. Skłodowski***, A. Szaciłło, M. Zych
- 10:15 – 10:30 The function of precision in heterostyly: an approach using 3D printed flowers **V. Ferrero Vaquero**
- 10:30 – 10:45 Niche divergence and limits to expansion in the high polyploid *Dianthus broteri* complex **J. López-Jurado**, E. Mateos-Naranjo, F. Balao.
- 10:45 – 11:00 Cytogenetic diversity in the diploid-polyploid complex *Linum suffruticosum* s.l. **A. Afonso**, S. Castro, J. Arroyo, E. Olmedo-Vicente, J. Loureiro
- 11:00 – 12:00 **COFFEE BREAK & POSTER SESSION**

- 12:00 – 12:15 Mapping Spanish bee distribution **I. Bartomeus**
- 12:15 – 12:30 Bumblebee learning: epigenetics perspective (EpiBee) **E. E. Montiel**
- 12:30 – 12:45 Tracking pollen transfer with quantum dots – pollination effectiveness of *Heriades truncorum* (Megachilidae) **S. Konzmann**, M. Kluth, D. Karadana, K. Lunau
- 12:45 – 13:00 Earth observation satellites and fossil pollen records as remote sensors of vegetation **F. Alba-Sánchez**, D. Alcaraz-Segura, J. A. López-Sáez, D. Abel-Schaad, G. Vargas, A. González-Hernández
- 13:00 – 13:15 A step-by-step guide for geometric morphometrics of floral symmetry **Y. Savriama**
- 13:15 – 15:15 **LUNCH BREAK**
- 15:15 – 15:30 Spatio-temporal stability of island endemic plant-pollinator interactions: *Erysimum scoparium* on Tenerife **J. Ollerton**
- 15:30 – 15:45 Community-wide assessment of pollination outcomes reveals negative frequency-dependence coupled with interspecific facilitation **P. J. Bergamo**, N. S. Streher, A. Traveset, M. Wolowski, M. Sazima
- 15:45 – 16:00 Pollination of a catnail by elephant-shrews – a unique case of floral specialisation **P. Wester**
- 16:00 – 16:15 Plant-pollinator interactions in a boreal pine forest in North-Eastern Poland **A. Szaciłło**, M. Skłodowski, M. Zych
- 16:15 – 17:30 **COFFEE BREAK & POSTER SESSION**
- 17:30 – 17:45 Dioecy on Canary Islands and sex-ratio in Laurel forest trees **M. Vidal-Rodríguez**, R. Torices, J. M. Fernández-Palacios
- 17:45 – 18:00 Elucidating the role of Macaronesian pigeons on the laurel forest reproduction and dynamic **J. Romero**, M. Nogales
- 18:00 – 18:30 Next EcoFlor 2020

saturday 16th – field trip

8:00 – 19:00 aprox. Meeting at Faculty of Sciences for field trip.

workshops

From individual to collective creativity in Science

Isabel Reche^{*,1}, Francisco Perfectti^{§,2}

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In this workshop, which integrates an oral presentation, videos and group activities, we talk about the need to increase our appreciation of creativity as a driver of science. We begin by learning about the key characteristics of the creative process, reviewing various ways in which creativity occurs, and showing the barriers that prevent us from developing our own creative potential. We present various ways to promote individual and group creativity. Finally, we present the role of groups in fostering creativity and the importance of collective intelligence.

Integral Projection Models (IPMs): a trait-based approach to investigate mechanisms of population responses to environmental change

Maria Paniw^{*,1}, Irene Martín^{§,2}

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Global environmental change affects natural populations through complex pathways. Such pathways include interacting ecological (demographic responses to the environment) and evolutionary (responses in phenotypic and genetic trait distributions to the environment) processes. A flexible, holistic population-modelling framework is therefore required to assess the relative contribution of these processes to population persistence. Integral Projection Models (IPMs), which relate genetic and phenotypic traits to demographic and population-level processes, offer a powerful approach to investigate the numerous mechanisms driving population responses to environmental change. This course will teach basic applications of IPMs, with a special focus on evolutionary dynamics. By the end of the course, attendees will be able to (i) construct IPMs (with data provided by the instructors) and (ii) include trait heritability into IPMs. The course is targeted to all participants of ECOFLOR 2019 with a strong interest in demography.

The role of hybridization in evolution: hybrid speciation, adaptive introgression and no man's land in between?

Gonzalo Nieto-Feliner

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Natural hybridization is considered to affect 25% of plant species and 10% of animal species and the importance of its occurrence in both groups is nowadays beyond doubt. However, improving those frequency estimations appears to be a feasible endeavor compared to that of understanding the evolutionary role and consequences of hybridization and introgression. The importance of natural hybridization stems from its connection to fundamental evolutionary processes such as the development of reproductive barriers between divergent populations, the introduction of adaptive variation through introgression, the formation of new species through homoploid hybrid speciation or allopolyploidy, or the genetic swamping of a restricted population by a congeneric species through repeated backcrossing. The specific focus of this talk is the creative contribution of natural hybridization to evolution by generating new genetic variability open to selection that may ultimately lead to creating new diversity. I will give an overview of the challenges that lie ahead in our understanding of this issue. For instance, the contrast between the wealth of hybrid lineages unveiled by molecular phylogenetic studies and the small number of cases in which we understand well the role played by hybridization in generating and maintaining those hybrid lineages is an intriguing question. There are several possible underlying causes and genomic approaches have dissected the architecture of a number of hybridized genomes, providing clues on the processes that drive their fate. But we lack an extended time perspective that could allow us predicting the evolution of hybridized genomes and we also ignore to what extent we will find general patterns. These uncertainties fuel debates, such as whether hybridization does contribute to generate diversity at all. According to a position mainly grounded on theoretical studies it does not, since the effects of hybridization may be transient and its signatures recent or neutral. The alternative view, mostly embraced by empiricists, considers that the multiplicity of scenarios and intervening factors in which hybridization occurs, corresponding to disparate temporal and spatial contexts, are not easily accommodated into available theoretical frameworks, and thus recommends interpreting case studies with a lighter load of theoretical preconceptions. I will illustrate the diversity of scenarios with my own work on several plant groups.

COMMENTS:

Sunbird surprise: A test of the predictive power of the syndrome concept

Elena Quintero^{*,1}, Eléonore Genzoni², Nigel Mann³, Clive Nuttman⁴, Bruce Anderson⁵.

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Floral syndromes are thought to be the product of convergent evolution, where the floral characters of unrelated species have evolved similar forms in response to shared pollinators. A contentious corollary is that floral form should be predictive of pollinators. In the forests of Uganda, we came upon the inflorescences of *Thonningia sanguinea*, a parasitic plant which previous literature suggested was part of a brood site pollination mutualism with flies (Calliphoridae and Muscidae). The general phenotype of the inflorescence suggested pollination by vertebrates, and the phenotypic similarity with several species of rodent pollinated *Protea* suggested to us that terrestrial mammals may be important pollinators. Pollinator observations and quantifications of pollen loads demonstrated that *T. sanguinea* is not visited by mammals, and that sunbirds are likely the most effective pollinators. The fact that the syndrome concept drove us to question the published literature on fly pollination demonstrates the usefulness of the concept. However, due to several phenotypic traits which did not conform to the classic sunbird pollination syndrome, sunbird visitation came as a surprise. While the syndrome concept is very useful, pollinator predictions based on syndrome traits should always be treated as working hypotheses.

COMMENTS:

Experimental insights to unravel adaptive evolutionary patterns in plants: the tyranny of phenology

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Plants adapt to varying environments by adjusting a myriad of non-independent phenotypic traits. Although the process is highly inefficient (e.g. massive mortality rates per generation, costly structures constrained genetically), plants eventually find their way to succeed. Our understanding of how adaptive evolution works is quite limited and too often conditioned by pre-existing ideas, interests and particularities of the study system. In this contribution, I will present unpublished results from experiments conducted in natural settings to shed light into the nature of adaptive evolution in the annual plant *Arabidopsis thaliana*. In particular, I am interested in highlighting the hierarchy of traits underlying adaptive evolution. Based on the evidence gathered during the last few years, I will pose the hypothesis that adaptive evolution in plants is chiefly determined by phenology, in particular by adjusting flowering time to varying environmental conditions, and that the rest of traits would simply be subordinated to phenology. Additional support to this hypothesis will be provided from the gene-trait-environment relationships revealed by the combination of genomics and spatial statistics. The take-home message of this contribution is that any evolutionary study in plants, whatever its nature and scope, should take phenology and its environmental cues into account to better understand the evolutionary relevance of any other study trait.

COMMENTS:

Phenotypic selection on flower colour and other floral traits in *Silene littorea*

Nancy L. Rodríguez-Castañeda^{*,1}, Eduardo Narbona², Pedro L. Ortiz¹, Montserrat Arista¹, M. Luisa Buide².

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Variation in flower colour among individuals within the same population is known as floral colour polymorphism (FCP). In this study, we investigated factors involved in FCP maintenance in *Silene littorea*, an usually pink-flowered plant with white-flowered individuals in some populations. It was carried out in two wild polymorphic populations in Galicia, with contrasted proportions of white-flowered plants (20% Barra, 80% Melide). To test if there are different selective pressures in the two studied populations, we explored whether phenotypic selection (PS) acts on different floral traits (colour, display size and morphology) by calculating differentials (S) and selection gradients (β). We used path analysis to assess how floral traits, seed predation and fruit infestation affect female fitness. Overall, plants from Barra have flowers with significantly higher corolla area, and significantly higher values in all fitness measures. This population presented low herbivory levels in infestation/fruit (Curculionidae infestation). In both populations, plants with a higher flower production showed a higher herbivory rate. In Barra, S was significant and positive for calix colour, daily floral display and total flower number, but β was only significant for total flower number, showing a direct effect of this trait in selection. In Melide, S have negative significant values for corolla colour and positive for daily floral display and total flower number, but again, β was only significant for total flower number. Therefore, the different proportion of white-flowered individuals in Melide seem to be a consequence of indirect PS on flower colour mediated by direct selection on flower number.

COMMENTS:

Can pollinators assess floral rewards through the use of visual cues?

Daniel Pareja Bonilla*, Pilar Fernández Díaz, Montserrat Arista Palmero, Antonio Marcial Escudero Lirio, Pedro Luis Ortiz Ballesteros.

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Pollinators use floral designs as advertisement, therefore imposing a selection pressure on animal-pollinated-plants, whose fitness depends upon pollinator visitation. The high variability in floral traits such as colour, scent, size and shape has been usually interpreted as result of this selection. The association between floral traits and rewards has been traditionally interpreted through the concept of “pollination syndromes”. That implies that pollinators would discriminate among plants that offer different rewards by the means of floral signals. Historically, pollination syndromes have been interpreted through human perception of floral cues, but if the goal of certain floral traits is to attract pollinators, then we should expect them to be tuned for pollinator senses. The aim of this study is to test the possible association between flower colour and reward offer at both community and species levels in the Mediterranean. Given that bees are the most important pollinators in this area, we used Chittka’s colour hexagon to obtain standardized perceptual colour parameters from flower colour spectra. At community scale, we quantified flower size and colour, and pollen and nectar production from 134 entomophilous plant species from Mediterranean communities. At species level, we analyzed the intraspecific association among colour and rewards in 34 species in order to assess whether bees can use colour to choose among flowers with different reward quantities when foraging from a specific species. We corrected the analysis including phylogenetic distances among species as covariate. At community scale, flower size was the most important visual cue showing strong, significant and positive correlation with both rewards and also with green contrast. In addition, pollen volume showed association with the flower green-contrast and the sugar mass with the flower hue. Intraspecific variability in flower colour and rewards was found in all the studied species. Only in four species significant associations between pollen volume and colour parameters (hue, chroma, UV chroma and background contrast) were found. Sugar mass showed significant associations with colour parameters (hue, UV chroma, brightness, and green and background contrasts) in seven species. Results indicate that flower size is the most important cue for attraction to pollinators, which can link size and rewards. Moreover, the association between green contrast and size suggests that these traits are important for long distance attraction.

COMMENTS:

Virtual lack of prey-pollinator conflict in the carnivorous subshrub *Drosophyllum lusitanicum*: how is it achieved?

Fernando Ojeda^{*,1}, Ceferino Carrera².

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Charles Darwin was the first to demonstrate that carnivorous plants do assimilate nutrients from captured prey. Regarding *Drosophyllum lusitanicum*, he suggested that it must be “in some manner strongly attractive” to insects. That efficient attraction has been classically attributed to a noticeable rancid-sweet, luring odor. Since this species, like most carnivorous plants, has entomophilous flowers that also attract insects for the purpose of pollination, a pollinator–prey conflict might arise. Most carnivorous species overcome a conflict by a marked spatial separation between leaf-traps and flowers. In *Drosophyllum*, although the spatial separation between leaves and flowers is small, a virtual lack of prey-pollinator conflict has been reported. Here, we explored the hypothesis that a conspicuous difference in attractive scents between leaf-traps and flowers might determine the strong segregation between pollinator and prey insect guilds in this species. To do so, we used direct thermal desorption techniques to study the volatile organic compounds (VOCs) emitted by its leaves and flowers. We detected two families of VOCs, one related to insect attraction and other related to plant defence (e.g. antimicrobial function). However, almost identical VOC profiles were found in both leaves and flowers. Therefore, odor discrimination does not seem to explain satisfactorily the virtual lack of pollinator-prey conflict in *Drosophyllum*.

COMMENTS:

Size matters — for plants and insects as well

Hector Miranda^{*,1}, Maria Jarne¹, Adela González², Jens Olesen³, Maria B Garcia¹.

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Biotic interactions are the glue of biodiversity, and therefore a factor of paramount importance for the persistence of species. Such vital connections among organisms, however, are systematically overlooked when assessing their current conservation status and threats. Threatened plants, for example, are considered vulnerable due to their reduced extension, occupancy or low abundance, while their dependence of biotic interactions, or their role as suppliers of food or microhabitats for animal visitors, has been long-forgotten. In this study we focused on the association between “plant population size” and “diversity of plant-animal interactions”, in order to explore to what extent biotic interactions can be lost when populations become smaller due to fragmentation or density decline. After selecting some case studies of Pyrenean plants listed in regional or European catalogues, we recorded for two years all kinds of aerial interactions in a small and three similar sized fragments inside a nearby large population. Taxonomical and functional diversity were calculated and compared, as well as β -diversity. Some vulnerable plants resulted to sustain an enormous diverse array of insects, spiders or snails. Although there were differences among plant species in the way taxonomical and functional diversity structured among the 4 units sampled, small populations tended to show lower values. That means that the decline or reduction of an endangered plant entails a reduction in functional diversity, most probably simultaneously affecting the population dynamics and persistence of both the plant and many other organisms interacting with it.

COMMENTS:

Impact of alien rats and honeybees on the reproductive success of an ornithophilous endemic scrambling plant in the Canarian thermophilous forest

Julia Jaca^{*,1}, Noemí Rodríguez², Manuel Nogales², Anna Traveset¹.

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Islands harbor a considerable portion of global biodiversity and endemic biota and at the same time are the recipients of the largest proportional numbers of alien invaders. Such invaders may jeopardize the performance of native species, through either their direct or indirect effects. In this study, we investigated the reproductive ecology of the endemic scrambling perennial herb *Canarina canariensis* in remnants of the former thermosclerophyllous woodland of Tenerife (Canary Islands), assessing how two widespread alien invasive species, the honeybee (*Apis mellifera*) and the black rat (*Rattus rattus*), affect its reproductive success. The honeybee visits its flowers whereas the black rat consumes both its flowers and fruits. We compared the pollination effectiveness of different animal guilds (vertebrates vs insects) by means of selective enclosures and determined the level of floral herbivory. Three bird species (*Phylloscopus canariensis*, *Cyanistes teneriffae* and *Sylvia melanocephala*), a lizard (*Gallotia galloti*) and two insects (*A. mellifera* and the butterfly *Gonepteryx cleobule*) were the main flower visitors. *Phylloscopus canariensis* was the most frequent visitor in the early flowering season whereas *A. mellifera* predominated in the flowers at the mid and late flowering. Birds increased fruit set, whilst lizards and insects had a negligible effect. Rats consumed about 10% of the flowers and reduced fruit set to one third. Besides contributing little to plant reproduction, *A. mellifera* might interfere with bird pollination by depleting flowers of nectar. We conclude that both alien species can threaten *C. canariensis* reproduction and hence population sustainability in the thermosclerophyllous vegetation. Honeybees, in particular, may become especially detrimental if beekeeping keeps expanding, or if they become active earlier due to global warming.

COMMENTS:

Effects of agriculture intensification on the diversity, composition and interaction structure of wild plant-pollinator communities

Carmelo Gómez-Martínez^{*,1}, J. Cursach², J. Rita², M. A. González-Estévez¹, C. Cardona³, M. Capó², A. Lázaro¹.

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Agriculture intensification results in extreme habitat loss and landscape homogeneity, which in turn may affect the diversity and composition of local communities and cause an overall biotic homogenization across the landscapes. Besides, the structure of species interactions, and the functional role of species in pollination networks may change notably along the disturbance gradient. However, the effects that agriculture intensification has on biodiversity and the structure of pollination interactions in managed landscapes are still unclear. In this study, we aim to assess whether agricultural intensification in the landscape affects: (I) the diversity of plants and flower visitors, (II) community composition, (III) biotic homogenization at the landscape level and, (IV) the structure of pollination networks. For that, we selected 20 wild *Olea europaea* communities across Mallorca Island, representing a gradient of agriculture intensification in the landscape (estimated as landscape heterogeneity and percentage of natural and agricultural habitats in 1-km buffer zone around the communities). Within each study community, we marked one hectare to carry out vegetation censuses. In these same areas, we used hand-nets to capture flower visitors on wild flowers by walking along three permanent 100 x 2 m transects for one hour (5 replicates per site from April to July 2018). By means of GLMM, we assess variations in plant and insect diversity along the agriculture intensification gradient. We use multivariate and analyses and network analyses to evaluate the changes in the composition of communities and in the structure of pollination interaction, respectively. We hypothesise that along the gradual loss of natural habitats, it would be a decrease in species diversity and an increase in biotic homogenization at the landscape level. In addition, we expect species diet reshuffling and a reduction in network size and robustness as agriculture intensification increases, with potential negative effects on the pollination service provided in these managed landscapes.

COMMENTS:

Non-native mammals as seed-dispersal rescuers of the Mediterranean dwarf palm *Chamaerops humilis* L. in Mallorca (Balearic Islands)

Raquel Muñoz-Gallego^{*,1,2,4}, José M. Fedriani^{2,3}, Anna Traveset⁴.

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Megafauna extinctions often lead to the disruption of plant-animal interactions, such as the seed-disperser mutualisms, which might entail severe consequences for plant populations and entire communities. This is especially patent in anachronistic plant species whose legitimate dispersers have gone extinct. Interestingly, their contemporary persistence might be possible thanks to surrogate dispersers or seed dispersal “rescuers”. We know very little on how these relevant functional replacements are contributing to the performance of present-day plant-frugivore networks. The dwarf palm *Chamaerops humilis* L. is a Mediterranean endemism with fleshy fruits and typically dispersed by mammals. Despite its ecological importance and wide distribution in some of the Mediterranean islands, no information exists about its seed dispersal on these depauperated-fauna systems. In this study, we aim at identifying and quantifying the relative importance of introduced frugivores on the island of Mallorca (Balearic Islands), where no native terrestrial mammals exist. Specifically, we assess for the first time the seed dispersal effectiveness (SDE) for *C. humilis* on islands; we evaluate the quantitative component by fecal and regurgitation sampling surveys, and the qualitative component by means of seed germination experiments and seedling growth measures. Introduced goats (*Capra hircus* L.) and pine martens (*Martes martes* L.) were the local mammal fruit consumers of *C. humilis* identified in our study sites. Results suggest that goats are much more important quantitatively than pine martens, due to the high number of fruits handled in each foraging bout and their extremely high abundance on the island. On the contrary, pine marten-ingested seeds showed the highest final seedling emergence success and seedling growth, thus its qualitative contribution on *C. humilis* seed dispersal is higher than that of goats. Overall, SDE was almost 9-fold higher for goats than for pine martens. We conclude that these two non-native mammal species are effective seed dispersal “rescuers” of *C. humilis* in this and probably other Mediterranean islands, where humans led to the extinction of its native seed dispersers, as it was the case of the goat-like *Myotragus balearicus* in Mallorca and Menorca. Other aspects, currently in development in a doctoral thesis, such as the joint impact of the palm borer moth *Paysandisia archon* (Cestniidae) and dense herbivore populations, goats, on the interactions between the endemic Mediterranean dwarf palm and subsequent animal associates (pollinators, seed dispersers, seed predators, and herbivores) will also be discussed in this talk.

COMMENTS:

Kin recognition and sociability in one-flower vetch (*Vicia articulata*)

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Feralization can be defined as the reverse process of domestication. Ferals are wild organisms descended from ancestors and selected to develop and reproduce under human-controlled conditions. That is the case of several legume species that had great importance in European agriculture but now survive only in wild or naturalized populations. One-flower vetch was the third most cultivated crop in Spain in the early 20th century, even though nowadays its agricultural use has all but disappeared. However, the plant can be found as part of Mediterranean herbaceous communities. Hence, *V. articulata* is a suitable candidate to study the evolutionary consequences of feralization. Crops are required to grow in dense populations, often surrounded by close kin. Therefore, it has been hypothesized that domestication and crop selection entail indirect selection for sociability and kin recognition. This might be especially relevant for legumes such as one-flower vetch, which grows in small and scattered populations under natural conditions. Here, we present a study of kin recognition and sociability in this plant, and whether they have been influenced by domestication and/or feralization. For this purpose, we selected one-flower vetch seeds from six different “families” (three maternal plants of each accession), including three wild and three crop accessions. Once the seeds were germinated in a growth chamber, they were sown in a common garden under reduced fertilization (i. e., limited resources). The experimental design consisted of the two domestication levels and three social environments: isolated individuals, individuals in dense populations, and in the latter case, surrounded by either relatives or non-relatives. Each treatment had nine replicates ($n = 162$ pots, 486 plants). Based on our experimental data, plants grown surrounded by non-relatives were more aggressive. This aggressiveness was correlated with higher biomass production and thus increased biodiversity effects, which might be beneficial in fodder crops. Plants grown with relatives were significantly more erect, smaller and with shorter internodes. This trait combination resulted in lower absolute fitness, although these plants were more productive in terms of fitness by vegetative biomass. This major fitness by biomass could be translated into a higher reproductive allocation, which would be favoured in grain crops. These results are indicative of strong kin recognition in *V. articulata*, more marked in domesticated accessions. We conclude that the social environment is important in determining plant fitness and performance. Moreover, the effects of domestication on sociability traits are complex and should be investigated taking into account both the plant’s natural history and agronomic use.

COMMENTS:

Pollen and stamen mimicry – the most speciose mimicry system of the world

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Pollen of flowering plants primarily had a UV-absorbing yellow colour caused by fungicidal and bactericidal protective flavonoid pigments that also shield against ultraviolet light. The UV-absorbing yellow colour was thus the first colour cue of flowers even before the onset animal pollination. Pollen eating and pollen collecting flower visitors are known to innately respond to visual cues of pollen and stamens. Many structures of melittophilous flowers mimic the UV-absorbing yellow colour of pollen and stamens to exploit the innate preferences of bees and hoverflies. Mimicry systems have models, mimics and signal receivers as protagonists. The evaluation of the flora of the Alps shows that 70% flowering plants possess yellow and UV-absorbing pollen grains. More than 30% of the 4328 flowering plants of the Alps display one kind of pollen and stamen mimicry, such as yellow and UV-absorbing floral guides, staminodes, filamental hairs, and styles. Pollen eating and collecting animals such as bees and hoverflies are considered as signal receivers and add up to several hundred species. Pollen and stamen mimicry is a complex phenomenon including true mimicry, mimicry without model, sensory exploitation and signal standardization. True stamen mimicry occurs in diclinic species in which the styles of the pistillate flowers mimic the stamens of the staminate flowers. Stamen mimicry without model comprises the phenomenon that for many stamen mimics no specific model can be identified. Stamen mimics exploit innate responses of bees and hoverflies towards this signal: Bees make their first physical contact with flowers often at the lower edge unless they are directed towards real pollen or pollen mimicking floral structures; Hoverflies of the genus *Eristalis* innately extend their proboscis towards UV-absorbing yellow colour of pollen and pollen mimicking structures. This innate preference of bees and hoverflies for pollen and pollen mimicking structures is still present in experienced individuals. In flowering plants exhibiting floral polymorphism such as dicliny and heterostyly, or different flowering phases such as dichogamy, the stamens are often cryptically coloured and stamen mimics replace its signaling function such that flower visitors cannot use the presence, length, and filling resp. of stamens as an indicator of reward. In many heterantherous flowers one stamen morph takes the signaling function. There is evidence that pollen and stamen mimicry is a worldwide phenomenon and thus constitutes the by far most speciose mimicry system.

COMMENTS:

What is the true colour of snake's head? Spectral analysis of floral colours in the genus *Fritillaria*

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Numerous studies showed that flower color is under strong selection by pollinators. Unfortunately, despite its importance in plant-pollinator interaction, there are few analyses of color properties of flowers in larger plant clades, especially from temperate regions. In order to fill in this gap, we studied floral color properties in the temperate genus *Fritillaria* (Liliaceae). We chose fritillaries because the genus comprises over 120 closely related species exhibiting considerable variation in pollination biology and ecology. In our study we analyzed spectral reflectance and assembled false colour pictures for 32 members of this genus. The results of our study indicate, that in case of fritillaries flower colour may be determined by the phylogenetic constraints to some extent, however, several *Fritillaria* examples indicate, that when the ecological constellation changes, there is a potential for changing floral characters including flower colour. For example, red or orange flower colour of *F. eduardii*, *F. imperialis*, *F. recurva* and *F. gentneri* is not common among closely related species, but indicates adaptation to attraction of birds, which in case of the mentioned species are the new pollinators.

COMMENTS:

An unstable habitat and a narrow-endemic plant: the pollination biology and genetic identity of *Linaria tonzigii*

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In this study, several aspects of the natural history of *Linaria tonzigii*, an endangered narrow endemic plant of the central Alps, were investigated. This plant inhabits an area of less than 10 km², it occurs above the treeline in steep gravel accumulations (scree) and it has a high cultural value because it is the symbol of the local botanical association. Nevertheless, the information on the biology of the species was previously very limited. We have investigated the pollination biology of this species by studying its autocompatibility and autogamy in wild populations. Furthermore, we have looked at the nectar production activity, sugar concentration, nectar robbery and pollinator activity. We have also sequenced 4 regions of the DNA, we have compared the genetic identity of the plant with related species occurring in the area and we have also reconstructed the phylogeny of the genus in order to place the species in a wider evolutionary history. Altogether, this research highlighted aspects of the biology of the species which were ignored before. Knowing the biology of endangered species could help understanding how to conserve them and it is a priority when they occur in highly dynamic and unstable habitats.

COMMENTS:

Why so different? The diversity and factors affecting nectar composition in populations of a rare plant *Polemonium caeruleum* L. in Poland

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Polemonium caeruleum is a red-listed, boreal plant species with SW limit range in Poland. Flowers of *P. caeruleum* are hermaphrodite, dichogamous and protandrous, visited by a broad spectrum of insects. Under the influence of several factors (mainly agricultural treatments) number of individuals of this plant in some Polish populations decreases. We chose 15 populations of *P. caeruleum* to our study. Experiments conducted in years 2014 to 2018 showed that populations of this plant in Poland differ in terms of seed production and frequency of insect visits (and their taxonomic composition). We speculated that nectar composition may be one of the factors involved in shaping those features. In 2018 we collected nectar samples from 15 population of *P. caeruleum* distributed across Poland. Sugars and amino acids (AAs) content was determined using high-performance liquid chromatography (HPLC). We found that nectar composition, regarding both sugars and AAs, is highly variable across populations. In contrast to previous studies, our findings demonstrate that the most common AAs and sugars in nectar of *P. caeruleum* were, respectively, glutamine and glutamic acid, and sucrose or fructose. Additionally, we have also specified some basic habitat parameters: habitat fertility (nitrogen, potassium and phosphorus content) and soil properties for each of the 15 populations involved in the study that could explain the recorded differences. The project was supported financially by the Polish National Science Centre grant no. 2014/15/B/NZ8/00249 (to MZ).

COMMENTS:

Heterostyly and zygomorphy as complementary mechanisms promoting outcrossing in *Oplonia nannophylla* (Acanthaceae)

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Oplonia nannophylla is a Cuban endemic species that presents an atypical reproductive system, because its flowers are heterostylous and zygomorphic. It has been suggested that heterostyly would be useless in zygomorphic flowers, since they are already adapted for cross-pollination. Therefore, it is interesting the existence of two different mechanisms to favor this phenomenon. Hence, we determine the possible effect of zygomorphy and heterostyly on the reproductive success of this species. For this purpose, floral biology was characterized, analyzed the reproductive system and described the ecology of the pollination. To determine the degree of zygomorphism, geometric morphometry techniques were used. A new index is proposed, which allows to determine the reciprocity degree of pistils and stamens of each individual, with respect to the rest of the individuals of the opposite morph in the population. No differences were found in the morphometric variables and in the pollen production among the morphs, so there is no polymorphism in the ancillary characters. In addition, it lacks a system of heteromorphic incompatibility, which could represent an adaptive advantage when pollinators are unpredictable. The small floral display and the low nectar production could be the cause of the low rates of visits. There was no statistically significant relationship of the reciprocity degree and zygomorphy with the reproductive success, which could indicate a complementary role of those mechanisms to ensure outcrossing since the low visitation rates of pollinators and the self-compatibility of the species in the studied localities.

COMMENTS:

Mating networks in a generalist plant species

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Contemporary pollen flow in animal-pollinated plants results from the foraging movements of pollinators. These are influenced by pollinator's preferences on different aspects of individual plants and consequently, non-random matings may arise. The determination of these patterns is therefore necessary to understand the importance of plant-pollinator interactions on the evolution of plant populations. In a population of the generalist plant *Erysimum mediohispanicum* (Brassicaceae) we characterized the phenotype and microenvironment of the plants flowering in each of two consecutive years. For each year we analyzed the mating patterns occurring among these plants, as well as the patterns of pollinator visitation and foraging movements. We found that matings mainly occurred among nearby plants presenting synchronous flowering times. Additionally, plants growing in microenvironments with similar light availability mate more frequently. As a consequence of these patterns, the resulting mating network showed that plants tend to mate with the most promiscuous ones and in clusters. This structure fitted the predictions of the foraging movements of pollinators. Our study links individual-based plant networks with contemporary pollen flow and demonstrates that in a generalist plant species the interactions with pollinators, and thus pollen flow, can be structured.

COMMENTS:

The influence of insularity on pollination networks in Australasia

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In certain tropical and subtropical areas, birds contribute significantly to the pollination native flora. In order to understand how birds interact with plants, these communities have been converted into ecological networks of interactions between plants and pollinators. Whilst there have been extensive efforts to describe the characteristics of the pollination networks of Neotropical pollinator birds (i.e. hummingbirds), Australasian pollinator birds (i.e. honeyeaters) are relatively understudied. It is important to understand how species interact with plants within these communities, and potential macroecological differences on these communities. In this study we examine the differences in network structure between plant-honeyeater communities on mainland Australia and the surrounding islands. We hypothesise that island communities will be more generalised than their mainland counterparts, as has been shown in hummingbird networks. Nevertheless, as honeyeater networks are significantly more generalised than hummingbird networks, we explore whether the observed mainland-island pattern still holds among honeyeater-plant interactions, and potential relationships with other macroecological features and species traits.

COMMENTS:

Selection and heritability in the wild: understanding floral trait stasis and evolvability

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Floral trait variation at the macroevolutionary level is often believed to be the result of adaptations to pollinators. Yet studies measuring pollinator-mediated natural selection on floral traits frequently find little evidence for strong evolutionary change taking place in wild populations. A possible reason for this 'paradox' is the prevalence of periods of stasis, when pollinator-mediated selection on flowers is relaxed under stable conditions, followed by more unstable periods where pollinator changes provide innovative selection. I will present the results of two studies that examine floral trait evolution under opposite pollination situations. The Mediterranean gorse (*Ulex parviflorus*) has a stable pollination environment dominated by a single pollinator, *Apis mellifera*. The common foxglove, bumblebee-pollinated *Digitalis purpurea*, is native to Europe but introduced and naturalised in many areas of the world, including neotropical mountains where hummingbirds are added to the visitor spectrum. In wild populations of both systems we study the pollination ecology and measure natural selection along with marker-based heritability to explore the role of pollinators in current and potential evolution of flowers.

COMMENTS:

Before and after flowering: patterns of flower, ovule and seed formation in an annual hemiparasitic plant, *Rhinanthus major* (Orobanchaceae)

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Plants in the genus *Rhinanthus* are annual hemiparasites which can use a large range of grassland species (mainly grasses and legumes) as hosts. The vernal ecotype, which occurs in hay meadows, has a relatively short life cycle, as seed maturation should be completed before mowing. Plant biomass in *R. major* is highly dependent on attachment to a good host and local conspecific density, and it can vary strongly among plants. Biomass determines the amount of resources available for flower and ovule formation before anthesis and for formation of the relatively heavy (2–3 mg) seeds after flowering. Visitation rates to *R. major* are high, so pollen limitation is unlikely to play a role in seed set patterns. We studied patterns of flower bud abortion, ovule numbers and seed production in naturally developing plants and in plants in which flowers at 6 out of 8 nodes in the inflorescence had been removed just before opening, to reduce resource competition. Small plants had a higher rate of flower bud abortion overall, and within inflorescences the highest rates of flower bud abortion were found on the first (basal) and last node of the inflorescence. Ovule production did not vary with flower position within the inflorescence, but a higher plant biomass increased the number of ovules produced per flower. In control plants, seed set per flower decreased from the bottom to the top of the inflorescence. In the treatments with a reduced flower number, seed production increased in the remaining flowers. Each capsule has an upper and a lower locule, and the ovules are arranged horizontally along the two placenta in each locule. An upper locule contains on average 8.1 (two rows of 4) ovules, with slightly more ovules in the lower locule (8.6), with rarely 6 ovules per row. Seed set in the control plants was around 0.4, while this was 0.5–0.65 in the treated plants. Unexpectedly, seed set was highest in the central and distal ovules: in both locules, the first (basal) ovule set seed only half as often as the second ovule. Treated plants increased seed set at all ovule positions. We discuss the possible explanations for these patterns.

COMMENTS:

Spatio-temporal variation in pollen limitation in a boreal pine forests in North-Eastern Poland

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Boreal pine forest showing specific ecological conditions for reproduction of zoogamous plants of forest floor form a natural vegetation in NE Poland. However, little is known on the extent and variation of the level of reproductive success in local plant populations. According to recent opinions pollen limitation, i.e. a reduction in plant seed set due to inadequate quantity or quality of available pollen, may be an important driver of the evolution of plant reproductive strategies. Therefore, over three years we studied pollen limitation in several populations of four native (*Campanula persicifolia* L., *Hepatica nobilis* Mill., *Hypericum perforatum* L. and *Rubus idaeus* L.) and one invasive (*Impatiens parviflora* DC.) zoogamous plant species commonly growing in pine forests in Wigry National Park. For all studied species we confirmed significant levels of pollen limitation index (PLI) which seems to be caused by low pollen transfer in the environment. This, in turn, may suggest rather low activity of pollinators. High values of PLI for invasive *I. parviflora* shows that this species can increase seed set in response to supplemental pollination.

COMMENTS:

The function of precision in heterostyly: an approach using 3D printed flowers

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Heterostyly is a floral polymorphism hypothesized to improve cross-pollination and to reduce sexual interference and gamete wastage. In distylous populations some plants present the flowers with stigmas above the anther whorl, ie. long-styled morph, whereas others have the anthers below the stigma, ie. short-styled morph. Distylous populations are characterized by high reciprocity and precision between sexual whorls of the two morphs; however, in recent years several studies have described other polymorphisms that differ from distyly in the degree of reciprocity and precision between morphs. The *Disassortative Pollen Flow Hypothesis* postulates that reciprocity in distylous flowers enhances legitimate pollination (ie, pollen flow between flowers of different morphs) in comparison to illegitimate pollination (pollen flow among flowers of the same morph). However, the functional significance of precision is still unknown. In this study we used 3D printed flowers to estimate flow rates in three types of floral polymorphisms. We found both reciprocity and precision affect pollen flow between morphs and besides, there are differences in this effect depending on the type of polymorphism.

COMMENTS:

Niche divergence and limits to expansion in the high polyploid *Dianthus broteri* complex

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Niche evolution in plant polyploids remains controversial and evidence for alternative patterns has been reported. Using the autopolyploid *Dianthus broteri* complex (2x, 4x, 6x and 12x) as a model, we aimed to integrate three scenarios, competitive exclusion, recurrent origins of cytotypes and niche filling, into a single framework of polyploid niche evolution. We hypothesized that high polyploids would tend to evolve towards extreme niches when low ploidy cytotypes have nearly filled the niche space. We used several ecoinformatics and phylogenetic comparative analyses to quantify differences in the ecological niche of each cytotype and to evaluate alternative models of niche evolution. Each cytotype in this complex occupied a distinct ecological niche. The distributions were mainly constrained by soil characteristics, temperature and drought stress imposed by the Mediterranean climate. Tetraploids had the highest niche breadth and overlap due to their multiple origins, while the higher ploidy cytotypes were found in different, restricted, non-overlapping niches. Niche evolution analyses suggested a scenario with one niche optimum for each ploidy, including the two independent tetraploid lineages. Our results suggest that the fate of nascent polyploids could not be predicted without accounting for phylogenetic relatedness, recurrent origins or the niche occupied by ancestors.

COMMENTS:

Cytogenetic diversity in the diploid-polyploid complex *Linum suffruticosum* s.l.

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Polyploidy is widespread in angiosperms and has long been considered as an important mechanism of speciation. Genome duplications bear broad-scale consequences on gene expression and developmental processes, potentially leading to immediate morphological, reproductive, physiological and ecological shifts. *Linum suffruticosum* s.l. is composed by perennial plants with dimorphic populations presenting a heteromorphic self-incompatibility system. Nevertheless, this complex has a problematic taxonomy, with several varieties, subspecies and/or species, being described by distinct authors. Additionally, according with preliminary data, *Linum suffruticosum* s.l. is also cytogenetically diverse in east Spain. Still, information for most of the distribution range of the group remains largely unavailable. The objective of this study was to determine the cytogenetic variation for the entire range of *Linum suffruticosum* s.l. For that, we investigated 152 populations from Iberian Peninsula, south-east France, north-west Italy and Morocco. A high cytogenetic diversity was found in the complex with five major cytogenetic entities being detected, diploids, tetraploids, hexaploids, octoploids and decaploids. Such cytogenetic diversity was only observed in the Iberian Peninsula and Morocco, with the rest of the distribution range surveyed being homogeneously diploid. Each genome size category corresponded to specific chromosome numbers, however a high heterogeneity in genome size was observed within ploidy levels. Most of the populations were composed by only one cytotype, but a few mixed-ploidy populations were observed. The high cytogenetic diversity detected in the field might suggest that unreduced gamete formation and hybridization events seem frequent in this polyploid complex. The importance of assessing cytogenetic diversity and understanding cytotype distribution patterns will be discussed in the context of *Linum suffruticosum* s.l. Study funded by a CONFLISEX research grant (Spanish MINECO CGL2013-45037-P) and by the Portuguese Foundation for Science and Technology (grant SFRH/BD/108451/2015 and project IF/01267/2013).

COMMENTS:

Mapping Spanish bee distribution

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Spain is a hot spot of bee diversity, but surprisingly, we still lack a clear picture of when and where the more of 1000 bee species occur. Integrating data from general repositories, scientists and naturalists, we can provide a comprehensive map of bee diversity in Spain. By using such extended dataset, we can track how bee diversity has changed during the last 50 years, at least for some well-sampled regions. For example, we show that since 1970, local bee diversity has decreased in average by 33% near Valladolid. By providing an open database of bee occurrences (<http://www.abejassilvestres.es/>) we aim not only to stimulate new research, but also to make accessible this information to the general public.

COMMENTS:

Bumblebee learning: epigenetics perspective (EpiBee)

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The bumblebee *Bombus terrestris* has an important role as pollinators in the natural environment, and also in agricultural production. Thus, know as they learn to recognize flowers that forage, and as they are innovating in their access to the pollen and nectar, would be of huge interest for biology and agriculture. The process of learning and memory storage has been extensively studied in mammals, finding changes in gene expression associated with these phenomena. During recent decades, however, it has begun to glimpse the importance of the epigenetic control of gene expression. DNA methylation is the epigenetic mechanism more studied, associated with the repression of genes, is a necessary element in the formation of memory. Currently, thanks to recent massive sequencing techniques, it is possible to know the methylation state of nucleotide of a whole genome (sequencing of bisulfite, BS-seq) and establishing the methylation profile (methylome). In bees, the methylome of queens and workers has differential markings, and these changes increase with the experiences that individuals accumulate, i.e. with learning. Recently, other epigenetic mechanism has shown its importance, the hydroxymethylation of DNA, which unlike the methylation is associated with the activation of gene expression. In both mice and bees, the inhibition of the enzyme that catalyzes its production (Tet1) facilitates the consolidation of memory and making difficult memory loss. In bumblebees, changes between reproductive and non-reproductive workers methylomes have been analyzed, but still a thorough analysis of the hydroxymethylation of DNA has not been done. In fact, very recently a new method has been released that allows to know the hydroxymethylation state of nucleotides with BS-seq sequencing. These two epigenetic actors, DNA methylation and hydroxymethylation, seem to be in a delicate balance that regulates gene expression, and that in brain it is responsible for the behavior modulation. More studies are needed to understand how these two epigenetic mechanisms are coordinated and complement each other. In the EpiBee project, we intend to do just that, to understand how patterns of methylation and hydroxymethylation change in two modes of learning: visual learning and the learning of object manipulation.

COMMENTS:

Tracking pollen transfer with quantum dots – pollination effectiveness of *Heriades truncorum* (Megachilidae)

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Oligolectic bees are morphologically and behaviourally adapted to effectively collect pollen on their host plants. They often transport pollen loosely in their scopa, a brush-like structure on their abdominal venter or long hairs on their hind legs. The megachilid bee *Heriades truncorum* is a specialist bee foraging on plants of the family Asteraceae and collecting pollen by tapping its abdomen on the pollen-presenting florets which places pollen grains directly in the ventral scopa. Considering that on one hand, this is an adaptation to effectively deprive the plants of their pollen, but on the other hand, scopal pollen is still viable and might be transferred to receptive stigmas during abdominal drumming, we tested the bees' pollination effectiveness. We used quantum dots as pollen labels to track pollen transfer by female *H. truncorum* collecting pollen on inflorescences of *Inula ensifolia* and *Pulicaria dysenterica*. As expected, marked pollen was mostly accumulated in the scopa and on the hind legs that regularly compact pollen into the scopa. Analysing visited inflorescences showed that only few (< 0.2) pollen grains are transferred from the last inflorescence visited before to a receptive stigma. Including pollen from the same inflorescence and pollen from all previously visited inflorescences, on average $20.63 (\pm 24.83 \text{ SD})$ pollen grains are deposited on each receptive stigma during one visit. The ratio of germinated pollen tubes is significantly lower for inflorescences visited once by *H. truncorum* compared to open, freely accessible inflorescences, which suggests that the bees possibly mainly deposit pollen from the same inflorescence on receptive stigmas of these self-incompatible plants. In conclusion, a single visit by *H. truncorum* does not grant the plant high reproductive success, but the bees' abundance and flower constancy might reduce this disadvantage.

COMMENTS:

Earth observation satellites and fossil pollen records as remote sensors of vegetation

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In a world facing rapid environmental changes, exploring the expected effects of habitat changes on the structure and function of biodiversity is a critical issue to guide climate change policy, conservation and management actions. Understanding how vegetation structure and function responded to past climate changes will help to improve our future biodiversity conservation efforts. In this sense, the fossil pollen record has proven to be a pertinent proxy for exploring the interactions between past climates and species dynamics. Our goal is to understand the extent to which taxa assemblages comprise relationships between vegetation functioning and structure and climate. We hypothesize that based on the relationship between pollen records and current vegetation functioning, we can reconstruct past vegetation structure and functioning. The study area covers the Western Mediterranean. First, we extracted raw pollen data from EPD and EMPD using EPD-r package. The raw pollen data were classified into plant functional types (PFTs) according to life form, phenology, leaf shape, climatic tolerance, etc. Then, the functional categories were used to find functional similarities in the fossil-pollen samples using Analog Technique (MAT). For vegetation structure we used CORINE Land Cover. Descriptions of vegetation functioning and identifications of Ecosystem Functional Types (EFTs) were based on MODIS (Moderate Resolution Imaging Spectroradiometer) satellite images of the Enhanced Vegetation Index, albedo and land surface temperature. From the original raw pollen, we used 77 taxa, including trees, shrubs, and herbs, that were assigned to 22 PFTs. These selected pollen taxa were optimized to be more sensitive for detecting changes in vegetation composition throughout the Western Mediterranean. MAT analysis allowed us to draw a clear picture of vegetation structure and composition by incorporating a greater number of taxa into our analysis and by blending many taxa from disparate regions. We characterized ecosystem functioning in terms of annual amounts (annual mean), seasonality (annual standard deviation) and phenology (date of the maximum) of carbon dynamics, radiative balance and sensible heat. Based on these descriptors, we identified EFTs that were then compared to the pollen-derived PFTs. Knowing how ecosystem functions responded to past environmental changes can serve as a sentinel to anticipate expected effects of current, and ongoing, climate change on biodiversity and ecosystem functions and services. The successful combination of pollen data and satellite-based vegetation descriptions is a powerful tool, enabling the extrapolation of vegetation dynamics beyond the limited domain of direct observations.

COMMENTS:

A step-by-step guide for geometric morphometrics of floral symmetry

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I am presenting a step-by-step guide for the morphological analysis of corolla and the decomposition of corolla shape variation into its symmetric and asymmetric components. The shape and symmetric organisation of corolla are key traits in the developmental and evolutionary biology of flowering plants. The various spatial layout of petals can exhibit bilateral symmetry, rotational symmetry or more complex combination of symmetry types. Here, I describe a general landmark-based geometric morphometric framework for the full statistical shape analysis of corolla and exemplify its use with four fully worked out case studies including tissue treatment, imaging, landmark data collection, file formatting, and statistical analyses: (i) bilateral symmetry (*Fedia graciliflora*), (ii) two perpendicular axes of bilateral symmetry (*Erysimum mediohispanicum*), (iii) rotational symmetry (*Vinca minor*), and (iv) combined bilateral and rotational symmetry (*Trillium undulatum*). The necessary tools for such analyses are not implemented in standard morphometric software and they are therefore provided here as functions running in the R environment. Principal Component Analysis is used to separate symmetric and asymmetric components of variation, respectively, quantifying variation among and within individuals. For bilaterally symmetric flowers, only one component of left–right asymmetric variation is extracted, while flowers with more complex symmetric layout have components of asymmetric variation associated with each symmetry operator implied (e.g., left–right asymmetry and adaxial–abaxial asymmetry). Fundamental information on the genetic, developmental, and environmental determinants of shape variation can be inferred from this decomposition (e.g., directional asymmetry, fluctuating asymmetry) and further exploited to document patterns of canalization, developmental stability, developmental modularity and morphological integration. Even if symmetry and asymmetry are not the primary interest of a study on corolla shape variation, statistical and anatomical arguments support the use of the framework advocated. This didactic protocol will help both morphometricians and non-morphometricians to further understand the role of symmetry in the development, variation and adaptive evolution of flowers.

COMMENTS:

Spatio-temporal stability of island endemic plant-pollinator interactions: *Erysimum scoparium* on Tenerife

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Endemic oceanic island plant-pollinator relationships have generated particular interest because there is a theoretical mismatch in the expectations of the outcomes of dispersal and establishment of biotically pollinated plants to islands. The ancestors of endemic plants should, in theory, be more likely to establish on an island if they are generalists capable being pollinated by a wide range of taxa. At the same time, island pollinator faunas tend to be of rather low diversity and abundance, and plant reproduction is often pollinator limited, making pollination niche segregation more likely via specialisation on a single pollinator or functional group of pollinators. The relative importance of specialist versus generalist strategies for biotic pollination are therefore unclear for island plants. At the moment, however, too few detailed studies of oceanic island plant-pollinator interactions have been conducted to properly assess the generality of any patterns and further case studies are required, particularly over a longer time scales and across multiple populations to assess spatiotemporal variation in the interactions between plants and their pollinators. Since 2003 I have been studying the pollination ecology of a Canary Island endemic plant species, the Canary Wallflower *Erysimum scoparium* (Brassicaceae), on Tenerife. *E. scoparium* employs a flower colour change strategy to manipulate its principal pollinators, which comprise a guild of medium sized bees, dominated by an endemic solitary bee *Anthophora alluaudi* (Apidae). The interaction between *E. scoparium* and *A. alluaudi* is stable over that time period and in multiple populations over an altitudinal range of almost 1000 m (range = 1300 to 2250 m.a.s.l.). The period of study includes one of the driest winters recorded on Tenerife and I discuss the effect of changes in community composition on the ecology of this endemic plant-pollinator interaction, and the reproductive success of the plant.

COMMENTS:

Community-wide assessment of pollination outcomes reveals negative frequency-dependence coupled with interspecific facilitation

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Pollination is frequently thought to be under positive conspecific frequency-dependence, and thus, would destabilize plant coexistence via *Allee effects*. Interspecific competition or facilitation for pollination may enhance or diminish such effects, respectively. We assessed several pollination variables (visitation rate, pollen receipt and pollen tube growth) of 67 species in a pollinator-depauperated tropical plant community, expecting negative frequency-dependence and interspecific facilitation due to the low pollinator availability. We related them to three flower availability variables: community-level conspecific frequency (number of flowers of the species in the community), floral display (number of flowers of the focal plant) and neighbor-level heterospecific density (number of flowers of heterospecific neighbors that shared pollinators with the focal plant). All variables showed negative conspecific frequency-dependence, although weaker for pollen tubes. Thus, intraspecific competition in pollination is a possible mechanism stabilizing interspecific competition. Moreover, interspecific facilitation in pollen receipt prevailed, which is expected in pollinator-depauperated communities. The prevalence of facilitation diminished for pollen tubes, since some interactions turned neutral. We identified for the first time plant traits that modulate the prevalence of competition vs. facilitation: specialization (if pollinated by one or several groups of pollinators), floral display and pollination system (if pollinated by bees, flies or hummingbirds). The combination of negative conspecific frequency-dependence and facilitation fosters coexistence, suggesting pollination as a niche axis maintaining diversity.

COMMENTS:

Pollination of a catnail by elephant-shrews – a unique case of floral specialisation

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Plant pollination by small non-flying mammals (therophily) is a rare phenomenon. In South Africa, therophilous plant species investigated so far are pollinated by mice (Rodentia) and sometimes secondarily by non-related elephant-shrews (Macroscelidea, Afrotheria). The South African endemic, holoparasitic *Hyobanche atropurpurea* (Black catnail, Orobanchaceae) has long-tubed and black flowers. Its flowers are most frequently visited by long-tongued elephant-shrews (*Elephantulus edwardii*, Cape rock elephant-shrews) and to a lesser extent by short-tongued mice (mainly *Acomys subspinosus*, Cape Spiny mice). Based on the close mechanical fit between the long-tubed flowers and the elephant-shrews' snout and tongue morphology, *H. atropurpurea* is hypothesised to be adapted to elephant-shrews as pollinators. Elephant-shrews carry much more pollen than mice in the fur around their snouts and in their scats. Extensive observations, video monitoring and pollen transfer experiments showed that elephant-shrews contact the plants' reproductive organs during almost every flower visit whereas flower visiting mice rarely make contact with the stamens and stigma. Compared to mice, the elephant-shrews transferred pollen more often and in significantly larger numbers to the stigma of the flowers. Moreover, flowers were often damaged or eaten by mice, but never by elephant-shrews. These results suggest that *H. atropurpurea* is specialised for pollination by elephant-shrews.

COMMENTS:

Plant-pollinator interactions in a boreal pine forest in North-Eastern Poland

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Interactions between flowering plants and animal pollinators are one of crucial ecosystem functions. Pollen vectors are necessary for most of the plant species in order to be pollinated and produce maximum number of seeds. Moreover, plant reproductive success is one of the crucial factors influencing the proper functioning of ecosystems, and the presence of both specialist and generalist pollinators is necessary to achieve proper pollination. Recently, the network approach has been widely used to map plant pollinator interactions. This technique, however, was mostly applied in Europe for semi-natural ecosystems, whereas natural communities have been seldom studied. In our project we focused on boreal pine forests in Wigry National Park (NE Poland). Over two years we captured floral visitors using a transect approach and mapped local pollination networks. The paper discusses the characteristics of the studied networks and reports the results of the experiment where we tested the impact of managed honeybee on local interactions, introducing *A. mellifera* colonies to our experimental sites.

COMMENTS:

Dioecy on Canary Islands and sex-ratio in Laurel forest trees

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Dioecy, or separate male and female individuals, is the most extreme sexual specialization in plants. Only ~4-6% of flowering plant species are dioecious, however its incidence is much higher on island ecosystems (e.g. 21% of the New Caledonia's flora is dioecious). The origin of this sexual system on insular ecosystems can be an ancestral condition (the species was already dioecious when arrived) or a derived trait (dioecy evolved in-situ). Although dioecy has received considerable attention on many oceanic islands, it is surprising the lack of knowledge from Macaronesian archipelagos. A preliminary study suggests that a 75% of the dioecious species in Canary Islands might be originally dioecious. Specifically, the laurel forest at the Canary Islands stands out with a high level of paleoendemisms and thus it could also harbour the highest number of dioecious species. To assess differences in dioecy presence and its origin at the different island ecosystems, we reviewed sexual system of all the 705 native flowering plant species in the Canaries. In the whole archipelago, 12% of the genera had at least one species displaying some degree of sexual dimorphism (dioecy, gyno- and androdioecy, subdioecy, trioecy and polygamy. Most of the dioecious species had woody habit (93%), presence of inconspicuous flowers (70%) and fleshy fruits (60%). Finally, the laurel forest showed the highest incidence of dioecy (11.5% of its species presented sexual dimorphism). Additionally, we surveyed sex ratios of natural and reforested populations of *Morella faya* (a dominant dioecious species in the laurel forest). Although there was a trend toward male-biased sex ratios, most of the populations did not show any significant departure from 1:1. Besides, several populations showed a significant departure from a random males and females distribution. These preliminary data provide the first steps to understand the reproductive ecology of key species of these degraded forests and it will help to design future studies on other species of the laurel forest.

COMMENTS:

Elucidating the role of Macaronesian pigeons on the laurel forest reproduction and dynamic

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Traditionally, the three Macaronesian endemic pigeons have been considered as key seed dispersers due to their status as largest frugivorous in their relative simple island communities. However, recent evidences based on faeces analysis suggest that this assumption overestimated the ecological contribution of these birds in the laurel forest. But faecal dispersion is not the only way to seed dispersal, and pigeons often regurgitate the larger seeds. In this contribution we try to elucidate the role of Macaronesian endemic pigeons by three different methods: 1) doing a comprehensive literature review, 2) performing feeding trials in captive conditions, and 3) understanding the ecological signals in the laurel forest between different scenarios/islands. We found that the ecological role of Macaronesian pigeons are situated in a complex position between seed dispersers and seed predators depending on the plant species with which interact. Moreover, ecological and evolutive signals suggest potential impacts on laurel forest diversity and dynamic if these bird populations decline or disappear.

COMMENTS:

poster abstracts

Plant-pollinator interactions: Comparison between native and invasive terrestrial plant species in the Balearic Islands

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Invasive plant species are often regarded as potential competitors of native species in the receptive communities due to their greater capacity for colonization and expansion, but little is known about whether invasive can compete for pollination services with native plants. In the present study, we hypothesized that the showy flowers of the invasive species compete with native species, which shares the same habitat and flowering time, influencing pollinator visitation. To test this hypothesis, a field work was carried out on native and invasive species coexisting in the same habitat. We focused on 5 pairs, 2 pairs inhabiting sandy coastal sides and 3 pairs inhabiting torrents in Mallorca from May to September 2018. Our preliminary analysis shows that the showy flowers of the invasive species does not play a major role in the competition between the studied pairs. Other factors are contributing in this competition, such as the location of the plant within the vegetative community, the abundance of flower buds per individual, the flowering period and the climatic changes per season as well.

COMMENTS:

A long travel across the ocean. The case of *Astydamia latifolia* in Canary Islands: dispersal and colonization

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Understanding the distribution of species has aroused scientific interest since ancient times. In this study, we intend to hypothesize a possible dispersal mechanism that allows us to understand the current distribution of *Astydamia latifolia* in the Canary Islands. The study of the fruits by scanning electron microscopy allowed to verify the existence of an air space around the seed, the presence of two chambers, one in the apical part and another in the basal part of the fruit, and the presence of waxes. With these results, flotation analysis were made, obtaining an average buoyancy (T50) of 7.5 days and a maximum buoyancy (T100) of 20 days, results that confirmed that the diaspores requires at least 7 days to be able to cross the distance that separates the Canary Islands of the northwest African coast and the distance between the different islands. Two tests were performed to analyze the embryonic viability and thus verify how it varied in the presence of salt. The result of the germination tests was that the viability decreased with the presence of salt (81.65%) with respect to the presence of distilled water (86.8%), we obtained the same result with the tetrazolium test, greater viability in distilled water (82.5%) than in salt water (50.3%). Based on these results, *Astydamia latifolia* is considered a thalassochorus species. In addition to these dispersal analyzes, a pilot study was carried out in which the genetic variability of the plastidial DNA was analyzed, obtaining as more variable regions *psal-accD*, *rpl32F-trnL* (UAG) and *psbD-trnT* (GGU), with these regions and individuals sampled made haplotype networks where the relationships between the haplotypes found and their distribution in the islands could be observed, being able to infer the number of colonizations that occurred, but always in a preliminary way.

COMMENTS:

Bee and floral traits affect the characteristics of the vibrations experienced by flowers during buzz-pollination

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During buzz pollination, bees use their indirect flight muscles to produce vibrations that are transmitted to the flowers and result in pollen release. Although buzz pollination has been known for >100 years, we are still in the early stages of understanding how bee and floral characteristics affect the production and transmission of floral vibrations. Here we analysed floral vibrations produced by four closely related bumblebee taxa (*Bombus spp.*) on two buzz-pollinated plants species (*Solanum spp.*). We measured floral vibrations transmitted to the flower to establish the extent to which the mechanical properties of floral vibrations depend on bee and plant characteristics. By comparing four bee taxa visiting the same plant species, we found that peak acceleration (PA), root mean-squared acceleration (RMS) and frequency varies between bee taxa, but that neither bee size (intertegular distance) or flower biomass (dry weight) affect PA, RMS or frequency. A comparison of floral vibrations of two bee taxa visiting flowers of two plant species, showed that, while bee species affects PA, RMS and frequency, plant species affects acceleration (PA and RMS) but not frequency. When accounting for differences in the transmission of vibrations across the two types of flowers, using a species-specific 'coupling factor', we found that RMS acceleration and peak displacement does not differ between plant species. This suggests that bees produce the same initial acceleration in different plants but that transmission of these vibrations through the flower is affected by floral characteristics.

COMMENTS:

NAM genes are involved in the control of the fusion and number of stamens in Papaveraceae Juss.

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Papaveraceae show very different configurations of androecium. Papaveroideae androecium has numerous free stamens, while in Fumarioideae it is formed by six stamens arranged into two bundles of three fused stamens. We conducted a comparative functional study by Virus-Induced Gene Silencing (VIGS) of the NO APICAL MERISTEM (NAM) genes, belonging to the NAC transcription factors in *Eschscholzia californica* (Papaveroideae) and *Cysticapnos vesicaria* (Fumarioideae). 47% of the analyzed flowers in *C. vesicaria* showed unfused or partially unfused stamens, and often stamens were fused to gynoecium, and gynoecium malformation were observed. This suggests a function of NAM genes in stamen fusion of *C. vesicaria*. In contrast, stamen number but not fusion was affected when the two NAM paralogs were silenced in *E. californica* indicating functional divergence of NAM genes in Papaveroideae and Fumarioideae. In addition, we will present our latest results on phylogeny, expression, and function of NAM genes in *C. vesicaria* and *E. californica* to understand their role in stamen organization.

COMMENTS:

EcoPRAT, reproductive ecology of the threatened orchid *Anacamptis robusta*: habitat alterations, mutualisms and hybridizations

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Anacamptis robusta, also known as meadow orchid, is a threatened orchid in the Balearic Islands. Despite being widely distributed in North Africa, its unique population in the rest of the Mediterranean Basin is located at S'Albufera de Mallorca Natural Park. Its pollinators and their interaction are still unknown. In the densest patch of the population (Son Bosc's area) it coexists with *Anacamptis fragrans* and produces intermediate hybrids (*Anacamptis x albuferensis*). Furthermore, the population of Son Bosc is high perturbed. The attempts of construction has negatively affected the pollinators network as reported by Traveset et al. (2018). In this project, I aim to study the effect of the perturbation to the ecology of this species, its mutualisms with *A. fragrans* and the fertility of *A. x albuferensis*. For these purposes, I will (1) compare fitness traits between perturbed and conserved areas, (2) use differential GPS to evaluate the effects of the interaction with *A. fragrans* and (3) test the fertility of the hybrid through hand-pollination treatments. I present the first experimental approaches of the project expected to be executed during spring 2019, in the context of the research fellowships for young researchers promoted by AEET.

COMMENTS:

B chromosome accumulation in *Dipcadi serotinum* (L.) Medik

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The plant species *Dipcadi serotinum* L. Medik ($2n = 8$) is characterized by a non-bimodal asymmetric karyotype, with three heterobrachial chromosomal pairs of increasing size, and a smaller hyperheterobrachial one (Ruiz Rejón et al., 1978). This karyotype could be the result of a chromosomal reduction from an initial polyploid condition ($2n = 12$). The presence of a variable number of accessory chromosomes (B-chromosomes) is also common in different populations of this species. These B-chromosomes can be considered as a by-product of the evolution of the standard karyotypes (Camacho et al., 2000). In this work, the frequency of B-chromosomes in *D. serotinum* and its relation to the fitness have been analyzed. Out of 50 individuals studied from La Cabrera, Madrid, 64% showed the presence of B-chromosomes, ranging from 0 B (36%) to 7 Bs (4%). This high value of B-chromosome indicates that this species might have a certain degree of tolerance to the presence of these extra chromosomes. For the same population, we have also shown that pollen viability decreases in individuals carrying B-chromosomes. Thirty-seven years ago, Ruiz Rejón et al. (1981) found almost one third of the current frequency of chromosomes B (24.24%) in the same population. The high number of chromosomes Bs detected today may be the result of a process of accumulation generated by the meiotic impulse (Jones 1991), which occurs in the parasitic or egoistic model (Shaw & Hewitt 1990), where equilibrium is the result of increase in the number of its frequency and the detrimental effects on the fitness of the hosts (Camacho et al, 2000).

COMMENTS:

Reproductive barriers and mating systems in *Erysimum incanum* species complex

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The causes and consequences of speciation have been the focus of researchers since the first studies on evolution. The causes of speciation lie on the interaction between ecological and genetic mechanisms, being the creation of reproductive barriers by means of natural selection (ecological speciation) a basic question. One of the most recurrent consequences of speciation in plants is the evolutionary transitions between different mating systems. In the present work we explore the reproductive barriers between the different species in the *Erysimum incanum* species complex. The complex is composed by three subspecies of *E. incanum*, *E. wilczekianum* and *E. repandum*. These species are distributed through the Western Mediterranean basin and they mainly differ in flower size, suggesting the existence of important dissimilarities in mating systems. We studied the mating systems of these species carrying out over 5000 hand-pollinated crosses in more than 200 different plants. On the same plants, three different treatments were done: selfing crosses, outcrossing, and hybrid crosses. More than 500 controlled crosses were made to estimate the post-zygotic reproductive barriers between them. We found strong but asymmetric barriers to gene flow between these species depending on their estimated mating system. We also found important differences in the mating system presented by them and in the mechanisms promoting the success of their reproductive strategies.

COMMENTS:

Temporal stability of a high mountain hybrid zone: the case of a hybrids between *Erysimum mediohispanicum* and *E. nevadense* (BRASSICACEAE)

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Hybrid zones are particularly interesting to shed light on evolutionary mechanisms driving adaptation and speciation, as well as to study the interaction between divergent genomes and to understand the mode and rate at which reproductive isolation is built. In this work we studied a secondary contact zone between *Erysimum mediohispanicum* and *E. nevadense*. From the *E. mediohispanicum* population, the lowland distributed species, to *E. nevadense*, the high mountain species, we phenotyped 570 individuals in two reproductive sessions separated by 10 years (270 plants in 2007 and 300 plants in 2017). We quantified trait related to the plant size, traits related to the flower size and traits related to the relative distribution of the reproductive organs into the flowers. We also sampled during over 30 hours the plant-pollinator interactions in this transect, quantifying and characterizing the pollination assemblages. We found significant differences between the species in traits related to the plant size and flower size. Some traits showed a natural gradient from *E. mediohispanicum* to *E. nevadense*, for instance: plant height or flowering stalk diameter. While some other traits showed significant *hybrid vigour*: number of flowers and number of flowering stalk. The pollinators visiting *E. mediohispanicum* plants were mainly bees and bee flies, while *E. nevadense* plants were ants. In the hybrid zone the pollinator assemblage was composed by a combination of pollinators found in *E. mediohispanicum* and *E. nevadense* populations. It has been very interesting to find the same patterns of phenotypic differentiation and pollinator assemblages characterizing this contact zone after 10 years since the first measure. Our work suggests a high level of the hybrid zone resulting from the secondary contact between *E. mediohispanicum* and *E. nevadense* in Sierra Nevada National Park.

COMMENTS:

Not a matter of size or spines alone – collectability of echinate pollen grains by *Bombus terrestris*

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Bee-pollinated plants face a dilemma in that bees both passively transport pollen grains among conspecific flowers and actively collect pollen to feed their larvae. Therefore, mechanisms that reduce pollen collection by bees have evolved in melittophilous plants. Spines on large pollen grains of the Hollyhock, *Alcea rosea*, have previously been described to reduce the collectability of pollen grains by corbiculate bees. We analysed the influence of the pollen grain properties diameter, spine length, and spine density on the collectability of echinate pollen by buff-tailed bumble bees (*Bombus terrestris*). Bumble bees individually foraging on one species of a variety of plants were either clearly able or unable to collect pollen from a plant, but neither pollen grain size, spine length, nor spine density prove to be an absolute disqualifier for pollen collectability. As pollen morphology is no suitable indicator of pollen collectability, we tested pollen adhesion to bumble bee hairs for several plant species and found that collectable pollen grains adhere significantly less strongly to bees' bodies than non-collectable pollen. Consequently, it is probably the composition of the pollenkitt surrounding the grains that determines pollen collectability by corbiculate bees. When pollen foragers were offered plants with non-collectable pollen, they did not shift to nectar collection but seized visiting flowers, while nectar foragers performed regular foraging bouts on these plants. Pollen that is uncollectable for corbiculate bees limits pollen depletion by generalist bumble bees and probably also honey bees while maintaining them as pollinators, which is especially advantageous when specialists are scarce or absent.

COMMENTS:

Plastome assembly, annotation and comparative phylogenomics in *Linum bienne* and *L. usitatissimum*

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Since its domestication in the Near East ca. 10.000 years ago, the cultivated linseed (*Linum usitatissimum* L., Linaceae) has become a globally important crop, with a yearly production of 2.794 Ktonnes of linseed oil and 781 Ktonnes of linen fiber (UN-FAO Figures for 2017). Because of its economic interest, the identification of genetic and genomic resources is of enormous importance for crop improvement. However, cultivated varieties include moderate genetic diversity (Uysal et al. 2002; Genet. Resour. Crop. Ev. 57:1109), and breeding efforts were limited to few germplasm lines (Zapata et al. 2004; J. World Prehist. 18:283). The limited genetic variation in *L. usitatissimum* has constrained the generation of new cultivars, making introgression from wild progenitors important to produce varieties better adapted to a changing climate and to support a growing population. While substantial efforts have contributed to understand genomic variation in *L. usitatissimum*, little is known about its closest relative, *L. bienne*. This is surprising since *L. bienne* appears to play an important role for the improvement of linseed. For example, the adaptation of cultivated linseed to north-eastern European latitudes required genetic transfers from its closest relative *L. bienne*, capturing genes involved in flowering initiation (Gutaker et al. 2019; Sci. Rep. 9: 976). Our study aimed to understand the genetic diversity and structure of the closest wild relative of linseed based on comparative phylogenomics using fully assembled plastomes.

COMMENTS:

Who are mistletoes visitors and pollinators?

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European mistletoe (*Viscum album* subsp. *austriacum*) is a parasitic dioecious plant whose pollination biology is barely known. In this study, we recorded the potential and real floral mistletoe visitors, and conducted an exclusion experiment to determine the importance of insects, wind and apomixis as determinants of fruit set. The exclusion experiment revealed that mistletoe requires insect visits to produce fruits. The effect of wind was negligible, while no fruit was developed by apomixis. The potential floral visitors of mistletoe were identified by aerial pitfalls under mistletoes. The main species found were the hemipteran specialist *Cacopsylla visci* (49.9%) and dipterans (SubO. Nematocera, 20%; and Sub.O Brachycera, 16.8%). In line with this, the real visitor community, identified by diurnal direct census, were quite similar (*Cacopsylla visci*, 58.9%; SubO. Nematocera, 6%; and Sub.O Brachycera, 7.2%), with an increase of ants (6.5%), *Apis mellifera* (11%) and other bees as *Lasioglossum* sp., *Andrena* sp., *Ceratinia cucurbitina* and *Bombus terrestris* (1.5%). In contrast, the guild of floral visitors is composed of hymenopterans and dipterans, being different between plant sexes. Female flowers received 1187 out of 1370 flower-pollinator interactions (87%), mainly by bees (*Apis mellifera*, 76.5%; *Lasioglossum* sp., 3%), while male flowers only received 13% of visits, but were visited by a more diverse guild (O. Diptera: Fam. Calliphoridae, 35.5% and Fam. Simuliidae, 18.6%; *Apis mellifera*, 32.4%; and Fam. Formicidae, 10.4%). Due to the low number of male flowers visits, the study was complemented with nocturnal censuses by light traps. The 41% of the captured moths carried mistletoe pollen on its abdomen, revealing that at least male flowers were visited during the night by *Panolis flammea* (17.3%), *Caradrina noctivaga/flavirena* (15.4%), *Rheumaptera andalusica* (9.6%), *Spudaeae ruticilla* (9.6%) and *Valeria jaspidea* (9.6%), and thus can potentially act as nocturnal mistletoe pollinators. Overall, the study discards wind and apomixis as relevant, while shows the importance of insects as pollinators for mistletoe. Diurnal visits were carried out by *Apis mellifera*, dipterans and ants. Female and male mistletoes show a clear mismatch between male and female visitors, both in number and identity. Roughly half of captured moths carried mistletoe pollen, suggesting that nocturnal pollination could play an important role on mistletoe fruit set.

COMMENTS:

Floral filters excluding bees

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Floral filters are mechanisms that limit access to flowers and rewards for distinct flower visitors. Here we focus on floral filters that exclude bees from flower visits. Mechanical floral filters act by means of the morphological fit between flowers and bees or by the force and technique bees need to handle flowers e.g. with masked entrances or keels enclosing the reproductive organs. The study case Bladder Senna (*Colutea arborescens*) demonstrated that honey bees and bumble bees are unable to handle the flowers. Large *Xylocopa* bees can open the keel as well as small *Megachile* bees which anchor their mandibles at two ridges on the standard to lever the keel. Thereby, they are able to exert more force than larger honey bees and bumble bees. Many bees are excluded by specific floral rewards and secondary attractants. It is known that flowers producing perfume as the only floral reward are visited exclusively by perfume-collecting male orchid bees, resin-producing flowers only by female bees using floral resins for nest construction, and oil-producing flowers only by oil-collecting bees. The admixing of bitter tasting substances or toxins also limits consumption of nectar and pollen by bees and might be interpreted as a physiological floral filter. Some flowers prevent pollen collection by bees to benefit pollination effectivity. Orchids attach incollectable pollinaria on the bees' body for pollen transfer. Some species of the families Malvaceae, Convolvulaceae, Cucurbitaceae, and Asteraceae produce spiny pollen grains that cannot be groomed into the bees' corbiculae. Consequently, corbiculate bees only visit these flowers for nectaring. Also, buzz-pollinated flowers exclude bees from pollen collection that are unable to sonicate the flowers. Sensory exclusion plays a dominant role in ornithophilous flowers. Most bird-pollinated flowers are red and UV-absorbing, whereas the rare red bee-pollinated flowers reflect ultraviolet light. While for birds both colours are attractive, for red-blind bees the bird-pollinated flowers appear black and thus inconspicuous. White bird-pollinated flowers exclude bees by reflecting ultraviolet light and thus reducing the attractiveness for bees. Yellow bird-pollinated flowers absorb ultraviolet light and do not display an ultraviolet bull's eye which seemingly increases the handling time of bees. Many manipulations of bees by flowers are less strict and do not fully exclude bees but rather manipulate the bees' handling of flowers in order to deposit pollen grains on so-called safe sites which bees are less capable of grooming.

COMMENTS:

Is intra-plant variation in nectar production an energy saving strategy? An experimental test

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Nectar is one of the main floral rewards offered by plants to pollinators and can be energetically expensive. Increased investment in nectar production (quantity or quality) may result in higher rates of pollinator visitation, but also incurs on increased risks of geitonogamy and higher costs of reproduction. Intra-plant variation in reward production has been visualized as a geitonogamy reducing strategy, but its role as an energy saving mechanism has rarely been considered. Here, we test if a lower investment in nectar production coupled with increased intra-plant variation, functions as a deceit mechanism allowing plants to save resources and still maintain pollinator service. To this end, we set a series of experiments with artificial plants in which we controlled the amount and intra-plant variation in nectar investment. We designed a 2 x 2 factorial experiment with two levels of nectar quality (36 and 32% of sugar concentration) and two levels of intra-plant variation ($\sigma^2 = 0$ and $\sigma^2 \approx 50$). Each experimental setting was exposed to a captive colony of bumblebees (*Bombus impatiens*) and the number of visits to each experimental plant was registered. Our results indicate that plants without variation in nectar production among their flowers, had 35.8% fewer visits compared to variable plants irrespectively of the amount invested in nectar production. Accordingly, variable plants may invest up to 12.5% less sugar than constant plants and still obtain higher rates of pollinator visitation. Overall, our results suggest that intra-plant variation in reward production may constitute a resource saving strategy releasing resources that could be used on other plant functions (defense, growth, reproduction).

COMMENTS:

Pollinator mediated evolution of floral traits in *Digitalis purpurea* after range expansion

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Evolution of novel floral form can occur when plants experience a change in their pollinators, like for example when they extend their range into novel environments. Understanding how variation in the pollinator community visiting a plant and exerting natural selection on its floral traits can help us understand floral evolution and is also crucial for predicting how plants will respond to environmental change. This study investigates floral variation and patterns of selection in the biennial herb *Digitalis purpurea* by comparing native populations in the UK with naturalised populations in tropical mountains in Colombia and Costa Rica, with respect to potential differences in the pollinator assemblage present in the native and non-native range. The tropical populations were established at least 200 years ago. Pollinator censuses in 11 populations and tests for the pollinating ability of each species indicate differences in the pollinator assemblage between continents, including visitation by hummingbirds and a high incidence of nectar robbing in the new range. In parallel, we found variation in floral morphology, particularly in the size of the proximal portion of the corolla tube, where a constriction restricts access to nectar to visitors with long mouth parts. We found consistent directional selection for larger proximal corolla tubes in the naturalised non-native populations, while no selection on corollas was present in two native European localities. We discuss potential explanations for these patterns in the light of adaptation to new pollinator environments.

COMMENTS:

Phenotypic and reproductive strategy variation associated to ploidy level in *Erysimum incanum*

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Ploidy level variation in plants is frequent and many times this variation has a significant effect on plant traits, as for example plant phenotype. The variation on the plant phenotype can also modify the mating strategy by modifying the relative inversion on male and female gametes and/or by modifying the interaction with pollinators. In the present study we used over 400 plants of *E. incanum* representing the three different cytotypes in this species which inhabits Iberian Peninsula and North Africa. We analyzed the phenotype differentiation by measuring different traits related to the plant size, flower size and reproductive organ distribution in the flower. We also explored the investment of each plant on reproduction by measuring the total production of pollen grains, ovules and the efficiency in self-pollen deposition. Finally, we explored the associations between phenotypic traits and reproduction investments in each of the ploidy levels. We found significant differences between the cytotypes in the flower size and the production of pollen and ovules. We did not find any difference in the efficiency of self-pollen deposition. But we found that traits related to flower size can be associated to the male reproductive investment. Our result suggest that some *E. incanum* cytotypes could be changing their mating system associated to changes in the phenotype because of the ploidy variation.

COMMENTS:

Phenological responses to climate change in communities of plants species with contrasting functional strategies

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Understanding how plant species will respond to the new environmental conditions predicted by global change models is nowadays a crucial research aim in ecology. Variation in plant reproductive phenology is an important plant feature to evaluate species' responses to climate change as it has direct implications for community dynamics and ecosystem functioning. However, to what extent coexisting plant species are sensitive to different climate scenarios in order to maintain fitness homeostasis is a question that remains largely untested. In this study, we carried out an experiment under controlled conditions using five annual plant species co-occurring in Mediterranean grasslands of oak-Savannahs (Dehesas) that spanned a wide range of different functional strategies. Seedlings of these species were transplanted into mesocosms filled with a mixed substrate of sterile sand-loam and peat. Each mesocosms was considered as a community of 18 individuals in which its spatial design allows us to target specific individuals with varying identity and number of competitors. This design was done to decompose the direct effect of environmental conditions and the indirect effect of neighbors (including both intra and interspecific competition) on plant performance. A total of 144 communities were equally distributed into two glasshouse units subjected to different temperature ranges, using a randomized block design with four climatic treatments: a 'control' treatment (simulating current averaged meteorological conditions in Mediterranean savanna-like systems), a 'dry' treatment (with a reduction of 33% in watering, anticipating future predictions of climate change models), a 'warming' treatment (with an increase of 3-4°C), and a 'dry+warming' treatment (to evaluate the interactive effects of both sources of abiotic stress). This study will significantly contribute to advance our understanding on how species' responses to different climate change scenarios will modify community composition, and target phenological traits as key indicators of species performance and community dynamics.

COMMENTS:

INAPERTURATE POLLEN1 is involved in the formation of pollen apertures in *Eschscholzia californica*

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The apertures of the pollen grain play a significant role in pollen survival and successful reproduction. The main diversity of the angiosperm pollen grain is associated with variations in the apertural pattern (the shape, number and position of the apertures on the surface of the pollen grain). Nevertheless, little is known about the genetic determinism of the apertural system. *INAPERTURATE POLLEN1* (INP1) gene is involved in the formation and, location of the aperture areas in the *Arabidopsis thaliana* pollen grains. We conducted a molecular analysis and a functional study of INP1 in the basal eudicot *Eschscholzia californica* (Papaveraceae, Ranunculales). We characterize two variants of INP1 differing in 4 amino acid residues. The phylogenetic analysis indicates that both variants do not cross specific limits. INP1 is expressed in all organs of the plant, although the highest levels of expression occur in the stamens. During the phases of pollen development, INP1 begins its expression before the appearance of the pollen stem cells and the expression is maximum in the tetrad phase, from when the levels decrease sharply to maintain minimum levels of expression until the mature pollen phase. These levels of expression correspond to the protein levels detected during pollen ontogeny, suggesting the absence of posttranscriptional regulation of the gene. INP1 silencing resulted in inaperturate pollen grains, what validates the function of the gene in *E. californica* and suggests that the function of INP1 is conserved in basal Eudicot lineages.

COMMENTS:

PipeLogeny: An automated pipeline for phylogenetic reconstruction

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Here we introduce PipeLogeny, a software package to integrate and automatize the different steps of the phylogenetic reconstruction under the R environment. Based on a combination of existing and newly developed functions, PipeLogeny allows the user to create and update local sequence databases, download accessions from GenBank, review stop codons in an alignment, concatenate different markers, and perform and visualize phylogenetic trees. In addition, our software automatically produce inputs for selecting the best partitioning scheme and evolutionary model using Partition Finder. PipeLogeny also calls mafft or muscle to perform multiple alignments with or without a reference, Gblocks to delete poorly aligned sites, and aliview to visualize alignments. PipeLogeny is ideal to conduct phylogenetic projects as it considerably reduce time required to convert aligned or unaligned sequences into complete inputs for the most popular software for phylogenetic reconstruction (such as MrBayes and RaxML). The package is still under development, and the first stable version will aim to also include a Graphic User Interface and tools to facilitate the GenBank submission process. The beta version of PipeLogeny, together with the manual, data examples, and vignettes, are available in <https://sites.google.com/site/pipelogeny/>.

COMMENTS:

A polyploid origin and the reproductive biology of the rare and endemic species *V. aragonensis* appear to be crucial for its survival in southern Iberian mountains

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Climate warming is causing the disappearance of suitable areas for cold-adapted species in southern Iberian mountains. Consequently, some studies have demonstrated that some plants are shifting their ranges towards higher altitudes, while other studies indicated that certain plants have adapted to new environmental conditions. In this context, the maintenance of genetic diversity is crucial since it is associated with the adaptive potential of plant populations. Rare and endemic species are expected to show low levels of genetic variability due to the effect of genetic drift and inbreeding, limited gene flow, and habitat specificity. By contrast, polyploids may compensate such effects through the increase of genomic dosages, thus potentially increasing allelic diversity. This has been associated with ecological adaptations, competitiveness, and better colonization abilities than diploids in harsh high-mountain habitats. *Veronica aragonensis* Stroh is a tetraploid endemic species that shows a narrow distribution range mostly restricted to Pyrenean and pre-Pyrenean mountains of Aragón. Besides, a single, isolated population has been reported from the highest mountain within the Baetic System. This plant has specialized to inhabit calcareous mountain screes mainly located at the top of the mountains. *Veronica aragonensis* is considered a rare species and it is included in regional Red Lists. In this study, nine microsatellite markers were used to assess the genetic diversity, structure and levels of gene flow within and among populations. Our analyses have found no more than two alleles per locus for individual SSR amplifications of *V. aragonensis*. This SSR pattern is only compatible with the species being either a highly selfing autotetraploid or an allopolyploid. The loss of many duplicated genomic regions from one of the parental genomes following allopolyploidization (i.e., biased fractionation) could explain the SSR patterns observed. Our results show a broad range of different expected heterozygosity values and inbreeding coefficients, suggesting that different, but often complementary, reproductive strategies might be modelling the genetic patterns within populations of *V. aragonensis*. Notably, during the last field campaign in the Baetic System, ants were identified as floral visitors in many plants of *V. aragonensis*. The relative importance of wind-pollination, ants or other potential insect-pollinators, as well as vegetative multiplication effects in the genetic patterns of this species should be further investigated.

COMMENTS:

Saplings distribution in Mediterranean Forest communities mediated by Janzen-Connell effect.

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The coexistence between plant species is a concept that has been studied for a long time. The Janzen-Connell effect is one of the main hypothesis that try to explain it. However, most of the studies have been developed in Tropical systems, but a little is known in Mediterranean ones. The existence of this effect in a community promotes high biodiversity; being the recruits located under conspecific canopy more exposed to pathogens and herbivories than recruits of other species. Thus, different species of plants have more chance to recruit under heterospecific adult plants. The aim of this work is to study whether this effect is present in southeastern mixed forests of Oaks and Pines of the Iberian Peninsula. Here, we analysed spatially sapling distributions regarding to conspecific adult locations. This allows detecting density dependent patterns evidencing possible Janzen-Connell effects. Our results showed different patterns depending on the species of adult plant where the species of sapling were under located. Consequently, we established different patterns between adults-saplings that allow knowing whether there is attraction, neutrality or repulsion between each pair of species. In case of a repulsion effect under conspecific canopy, we incorporate specialist fungal pathogen in the point pattern analysis. These analyses showed some evidences that Janzen-Connell could be a present mechanism in some plants of Mediterranean forest communities.

COMMENTS:

Organic management increases the complexity and stability of plant-pollinator networks in olive orchards

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Intensive agriculture threatens the biodiversity and the provision of ecosystem services (ES). The pollination ES is currently a major concern due to its importance for the production of food and the conservation of wild plants. Studies have frequently analyzed the abundance, richness or diversity of solitary bees to study the effects of environmental gradients on this key group of pollinators. It is still unexplored, however, how different managements affect the properties of pollination networks, which can be more informative and insightful than traditional methods. In this work, we used 96 bee nest traps to build bee-pollen mutualistic networks in 18 paired olive orchards with different herb cover management (intensive vs. extensive). We analyzed the bipartite networks at the field scale to study how differences in local management affect the properties of these networks. Specifically, we considered six network metrics: linkage density, interaction diversity, weighted connectance, interaction evenness, robustness and niche overlap. We also run multivariate analyses to explore management impacts on the spatial homogenization of bee-plant communities and their interactions (i.e. β -diversity). Results showed that organic management increased linkage density, interaction diversity and robustness of the networks, conferring more complexity and stability to these systems. Also, it decreased plant niche overlap, unveiling higher functional diversity. No differences were observed for connectance nor interaction evenness. Moreover, organic farms showed a higher β -diversity of plant communities, bee communities and their pairwise interactions than conventional farms. These results highlight the bad effects of unsustainable management on the pollination function beyond the loss of species, through the simplification and homogenization of pollination networks.

COMMENTS:

Influence of repeated DNA in the viability of the pollen grains in three species of the genus *Asphodelus*

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Repeated sequences represent an important fraction of the eukaryotic genomes which tends to accumulate in heterochromatic regions. In this study, we have carried out an analysis of interphasic nucleus of three species of the genus *Asphodelus* (*A. fistulosus* L., *A. cerasiferus* J. Gay, and *A. tenuifolius* Cav.), belonging to the Family Xanthorrhoeaceae (Dumortier), to test if there are significant differences in the amount of heterochromatin in their nuclei. In previous studies, a direct relationship between the amount of heterochromatin and repeated DNA quantity has been established, so here differences in size of heterochromatic blocks were considered as differences in total amount of repeated DNA (Stebbins 1966). *A. tenuifolius* was the less heterochromatic species (then, with less amount of repeated DNA), followed by *A. fistulosus* and *A. cerasiferus*, being the latter the most heterochromatic species (with higher amount of repeated DNA). The influence of the amount of DNA repeated in many phenotypic characters have been described (Watson 1988). In this study, after the analysis of 4500 pollen grains, we found that the amount of repeated DNA in these species of *Asphodelus* correlates negatively with its viability. It is known that a large amount of heterochromatin is adaptive in extreme environments, while a smaller amount accelerates cell division times by shortening the development time. Thus, and considering the life cycle *A. cerasiferus* (perennial) and *A. tenuifolius* (annual), a greater amount of heterochromatin, which increases the resistance of the perennial species in extreme environments, would act as an evolutionary reinforcement to guarantee a greater number of reproductive seasons, thus compensating the loss of fertility. While, on the other hand, the smaller amount of DNA repeated that we found in *A. tenuifolius* would reinforce the fertility and the smaller time of development, acting like beneficial factor in the annual biotype. For its part, *A. fistulosus* can behave as a biennial or triennial plant, which would explain its intermediate position. A molecular analysis revealed that satellite DNA family (CL90 family) is part of the heterochromatin of these species. This repeated DNA was first described in the genome of the plant *Dipcadi serotinum* (L.) Medik. Considering that both the genus *Asphodelus* (Xanthorrhoeaceae), and the genus *Dipcadi* (Hyacinthaceae) belong to Order Asparagales, this family of satellite DNA exhibit a high degree of conservation, something that is not usual in this type of sequences, usually with a rapid rate of change.

COMMENTS:

Biogeography of *Arabidopsis thaliana* relict lineage across the Iberian Peninsula and Morocco

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As a consequence of global climate change, species distribution is expected to be dramatically altered. Hence, the identification of the historical, biotic and abiotic drivers have become one of the main goals for ecology and evolution. Nevertheless, there is much debate concerning whether geographic boundaries are driven by merely geographic and climatic characteristics and/or by biological and historical processes, such as dispersion, post-glacial re-colonization history and human activities. *Arabidopsis thaliana* in the Iberian Peninsula and Morocco represents a useful model system to assess the effects of these evolutionary and ecological processes on the distribution patterns, mostly due to three factors. First, the high-quality genetic resources of the *A. thaliana* population of the Iberian Peninsula, which is also available for the public, made this population one of the wild populations of which the genetics is described in greatest detail. Second, the multiple paleo-botanic records allow us to follow the community changes throughout the last glacial maximum (LGM). Third, many studies reconstructing the historical change of the main forestall species in the Iberian Peninsula are available. Furthermore, little is known about the specific range limits and the environmental characteristics defining the difference between the genetic units of *A. thaliana*. Although previous studies described climatic features as the main explanatory factor, in this study we included satellite images from MODIS (i.e. photosynthetic activity) as representation of the biotic features, hence improving the fit of the predicted probabilities of the *A. thaliana* distribution. In addition, the literature suggests that contemporary and recently introduced populations seem to be associated with altered more disturbed areas and the relict ascension to the more stable forests, although it remains to be explored. We will explore two ways to include the historical stability of the communities where these populations belong to explain the difference between the *A. thaliana* ascension in their distribution patterns. The New tools and resources, such as spatial distribution models (SDMs), MODIS image, and predicted vegetation layers, enable the use of more reliable distribution models accounting for biotic and abiotic factors as well as the historical dynamics of the species. This contribution will shed light on the importance of analyzing relict, contemporary and introduced ranges separately, in order to better understand the historical and present processes shaping the observed *A. thaliana*'s distribution range.

COMMENTS:

Micromorphological traits and phytochemical patterns of endangered *Clinopodium rouyanum* (Lamiaceae)

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Clinopodium rouyanum is an endemic species from the mountains of the island of Majorca (Balearic Islands). A chemical composition analysis of the essential plant oil (obtained by distillation SDE) and volatiles emitted by green parts, flowers, corollas and tube corollas (obtained by SPME and ST methods) and analysed by GC and GC-MS was conducted. Additionally, a study on the morphological types and distribution of trichomes was carried out by electron scanning microscopy. The essential oil and volatiles were characterized by a high concentration of oxygenated monoterpenes (83.3-99.9 %), whose principal compounds were pulegone (73.0-93.9%) and menthone (3.1%-11.8%). Non-glandular tricellular trichomes, peltate trichomes, and capitate trichomes were observed on leaves, calyx, corolla and stems. Volatile components of *C. rouyanum* show a feeding deterrent activity against generalist molluscan herbivore that could represent an effective defence that favours the conservation of this endangered species.

COMMENTS:

Shrub role on the removal of *Quercus* acorns and seedling emergence in Valdelosa (Salamanca)

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Quercus species are very representative of the arboreal stratum of Mediterranean forests and offer a variety of natural resources. They present limitations for their regeneration, as the seedling phase is very sensitive to the environmental conditions. In addition, their acorns are a nutritional resource for the wild and domestic fauna, therefore, they experience a high rate of consumption. The shrubs suppose a good method for the regeneration of plant species, since they increase the survival of the seedlings by reducing water stress and direct solar radiation. In this work we study how the presence of *Cytisus multiflorus* and *Cistus ladanifer* affects the removal of acorns of *Quercus ilex*, *Quercus suber* and *Quercus faginea*, and the emergence of the seedlings of these *Quercineae*. For this purpose, two field experiments are carried out with three different environments: "Escoba", "Abierto" and "Jara"; in order to measure the removal and the emergency. The results obtained indicate that the rate of consumption of acorns is very high in all environments, there being a clear preference for holm oak and gall oak acorns compared to cork oak. The presence or absence of exclusion cages for small herbivores does not reduce the removal, only delaying in time due to the enormous pressure exerted by consumers in the area. The emergency was late and scarce, the "Jara" environment was downright negative. Regarding species, there were no significant differences in the emergence of the seedlings. Finally, the alteration produced by large herbivores such as *Sus scrofa* should be taken into account in case of reforestation plan are carried out in this area.

COMMENTS:

Nectary morphoanatomy and nectar traits of the bromeliad *Billbergia distachia* are strongly related to its single pollinator favoring plant reproduction

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Among the floral attributes present in plants with biotic pollination, nectar has an important role in attracting pollinators. Nectar production dynamics may show variations that can be associated with environmental and ecological factors, as well as with the ultrastructural diversity of the floral nectary. In this context, we recorded nectar production dynamics from a morphofunctional perspective of the hummingbird pollinated bromeliad *Billbergia distachia*. Floral visitors were observed for about 70 hours in two flowering periods (2016/2017). Nectar production pattern were determined through three treatments: standing crop, pattern of nectar secretion (accumulated nectar) and nectar removal effects. The treatments were analysed by analysis of variance using the Bonferroni test for multiple comparisons between means. A correlation test (Pearson's r) was performed to verify the relationship between nectar production and pollinators frequency. Morphoanatomical studies of the nectary were done by light, scanning, and transmission electron microscopy. The nectar samples were processed through gas-liquid chromatography (GLC) to determine the types of sugars and for the detection of the amino acids composition was carried out on the basis of the OPA (ophthaldialdehyde). *Billbergia distachia* flower anthesis lasts during approximately 60 hours and only the scale-throated hermit *Phaethornis eurynome* was observed as pollinator. Nectar production and the period of major pollinator activity were synchronous ($r = 0.72$), with nectar peak in volume and sugar mass in the afternoon period (3 - 4 pm) on both days of anthesis. Nocturnal nectar production dropped significantly on both nights during anthesis. The ultrastructural features of the nectary showed some evidences of nectar reabsorption in flowers during the night and after anthesis, such as an increase in the number of vesicles and plastids with starch grains. It can be inferred that physiological aspects, such as the photosynthetic metabolism CAM, may also be a key factor in the nocturnal pause in nectar production. Thus, both aspects - reabsorption and photosynthesis - may be important to the reproductive strategy of this species and can be associated with the nutrient metabolic process at night, which allows the flowers to resume nectar production in the early hours of the following day. Moreover, considering that *B. distachia* is self incompatible, ensuring visits of its single pollinator is essential for successful reproduction. In this way, nectary morphoanatomy, nectar traits and a saving energy mechanism through nectar reabsorption contribute with a well-established relationship between *B. distachia* and *P. eurynome*.

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