

Linking phylogeographic history and contemporary dispersal dynamics: Multiple glacial refugia and restricted but effective present-day gene flow shaped the genetic structure of an endemic newt from the Pyrenees

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Abstract

Historical factors (colonization scenarios, demographic oscillations) and contemporary processes (population connectivity, current population size) largely contribute at shaping species' present-day genetic diversity and structure. In this study, we use a combination of mitochondrial and nuclear DNA markers to disentangle the role of Quaternary climatic oscillations and present-day gene flow dynamics in determining the genetic composition of the newt *Calotriton asper*, endemic to the Pyrenees. Mitochondrial DNA did not show a clear phylogeographic pattern and presented low levels of variation. In contrast, microsatellites revealed five major genetic lineages with admixture patterns at their boundaries. Approximate Bayesian computation analyses and linear models indicated that the five lineages likely underwent separate evolutionary histories and can be tracked back to distinct glacial refugia. Lineage differentiation started around the Last Glacial Maximum at three focal areas (western, central and eastern Pyrenees) and extended through the end of the Last Glacial Period in the central Pyrenees. Our data revealed no evidence of recent gene flow between lineages, whereas borders likely represent zones of secondary contact following expansion from multiple refugia. Within lineages, dispersal distances were relatively short, although long-

distance dispersal (up to 52 km) was achieved by few individuals. This work highlights the importance of integrating past evolutionary processes and present-day gene flow dynamics, together with multilocus approaches, to gain insights on what shaped the current genetic attributes of amphibians living in montane habitats.

Vegetation encroachment drives changes in the composition of butterfly assemblages and species loss in Mediterranean ecosystems

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Abstract

Land abandonment and loss of grazing have been amongst the primary drivers of landscape change in the Mediterranean basin in recent decades. As a consequence, forest cover has greatly expanded in detriment of semi-natural grasslands and areas of cultivation and pasture mosaics. Although predictably important, the impact that this phenomenon has on biodiversity has remained largely unexplored, partly because of lack of appropriate data. Here we make use of an extensive citizen science program, the Catalan Butterfly Monitoring Scheme, to quantify the response of butterfly assemblages to vegetation encroachment in NE Spain. We first adapted an index to describe the preference of 147 butterfly species for open or closed habitats, and found a strong association of most species for open habitats. We then developed a community index to record changes in 54 long-term monitored sites (10 years or more), where plant communities were also periodically monitored. Butterfly assemblages have undergone changes towards species preferring closed habitats in 72% of the studied sites, in parallel to a process of vegetation encroachment in the region. Community changes were linked to population trends, and could be locally predicted by the interaction of the preference of butterfly species for open or closed habitats and the magnitude of vegetation encroachment at each site. These changes were accompanied by frequent extinction events (4.53% of the studied populations), that were highly biased towards species preferring open habitats. Our study confirms and quantifies the threat that vegetation encroachment imposes on biodiversity in this highly diverse region.

Modelling surface thermal conditions of Pyrenean high mountain lakes for spatial and climate change projections

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5th course of PhD thesis

In the current context of existing climate change, it is of paramount importance to have a precise knowledge about the thermal conditions of water bodies in high mountain ranges, such as the Pyrenees, and to know which variables can be used to model and predict thermal variables in water bodies, as temperature is fundamental in biologic and ecological processes. We describe the regional and interannual variability in Pyrenean lakes and ponds and we developed mixed models to identify the key environmental variables to model different water temperatures: mean and maximum summer temperature, accumulated degree-days, diel temperature ranges and oscillation (defined as three days lapse difference in maximum temperatures). We used nine years of data from 59 temperature series of Pyrenean lakes and ponds covering most of the mountain range. We ran the models to predict actual and future temperatures under different warming scenarios for 2267 water bodies of the Pyrenees. For that, we used two scenarios of the Global Climate Models: The RCP2.6 with a reduction in greenhouse effects, and RCP8.5, the one with the highest emissions. We have found that altitude was the main explicative variable for accumulated degree-days, mean and maximum temperature, whereas lake area had the most important effect on diel temperature range and temperature oscillation, with a lesser effect of altitude. Altitude, lake area, catchment morphology and solar radiation together were more explanatory than interannual variability in air temperature. Accumulated degree-days are expected to increase more in smaller lakes and ponds and at lower altitudes, whereas maximum temperatures are expected to increase at higher altitude. Predicted mean summer water temperatures in Pyrenean water bodies can increase 2.0 °C from the present mean 11.3 °C by 2090.

Metallothioneins, a genetic response against metal toxicity in polluted marine environments.

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The increasing levels of heavy metals derived from human activity are poisoning marine environments, threatening zooplankton and ocean food webs. To protect themselves from the harmful effects of heavy metals, marine animals have several mechanisms, one of which is based on the expression of a set of cysteine-rich proteins called Metallothioneins (MTs). An intensive search of genome and transcriptome databases has prompted us to identify 25 new MTs sequences from 13 species of the Urochordates, exposing a high structural MT variability since that this group of animals has the longest and the smallest metazoan MTs described so far. We have investigated the metal-binding capacity of these MTs and we have demonstrated that they prefer cadmium, rather than other metals such as zinc or copper. This binding preference agrees with the different teratogenic effects that Cd/Zn/Cu-treatments cause on embryonic development of the urochordate *Oikopleura dioica*. To sum up, our work puts an alert on the potential impact that an increase of heavy metals may have on marine organisms affecting their embryonic development and survival, and points to MT genes as molecular biosensors that marine ecologists could use to monitor the genetic stress of natural populations exposed to these toxic compounds.

Biotic and abiotic drivers of microbial community structure in a high iron calcareous-spring.

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Microbial communities differ in their species composition as a result of biotic and abiotic factors. It is expected that extreme environmental conditions may exert a strong filter on the specific microbial community that may be dominated by tolerant species. Fe is an essential element for life but it becomes toxic at concentrations above 1 mg/L. However, several prokaryotes are able to use iron as energy source. We investigated the shift from environmental to biotic drivers of microbial communities along a gradient of chemical stress caused by elevated concentrations of Fe II. A biomonitoring was carried in an Fe spring located in the Llémna watershed (Ter River in Girona, northeast of Spain). Water nutrient content, metal content (in water and biofilm), leaf litter decomposition, fungal biomass, primary production, chlorophyll and biofilm bacterial communities were analysed in five sites downstream of the spring source. This spring is characterized by high conductivity and alkalinity resulting from the dissolution of carbonates and metal-rich ores leading to high concentration of Fe, Sr and B (>1 mg/L), followed by Mn, Ba, Al, Zn and As. Analysis of covariance showed that decomposition rate was significantly greater at site 4, followed by site 3 and significantly lowest at sites 1, 2 and 5 ($p < 0.05$). Moreover, biomass accrual and fungal biomass increased as the chemical conditions improved (high nutrient content, oxygen, pH and low amounts of metals). On the other hand, α -diversity of microbial community followed an opposite pattern, site 1 had the maximum number of amplicon sequence variants (>1000). Analysis of β -diversity of microbial communities performed by Venn diagram, Canonical analysis of principal coordinates (CAP) and cluster analysis showed that site 1 was different from the rest and characterized by *Gallionella*, a Fe-oxidizing bacterium. Therefore, extreme chemical conditions are linked to a unique and very diverse prokaryotic community mainly composed by chemolithoautotrophic bacteria which actually represent the main contribution to the primary production. Moreover, the lowest bacterial diversity and richness reported in the active community growing under non-limiting conditions was attributed to biotic interactions and competitive exclusion rather than to abiotic factors.

Ecological drivers and seasonal change in commercial species distributions of the Northwestern Mediterranean Sea

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Despite multiple studies that focus on marine Mediterranean resources and ecosystems, there is a lack of information considering the importance of seasonality on these ecosystems. This knowledge is key to predict and understand how ecosystems can react to climate change effects and how to improve fishing management. Here, we examined the spatial distribution of eight commercial marine species; including fish, crustaceans and cephalopods, in a highly exploited area of the Northwestern Mediterranean Sea during two different seasons (winter and summer). We hypothesised that the seasonal differences in the water column (with a marked thermocline in summer and absence of it in winter), as well as the spatial heterogeneity of oceanographic conditions, can result on a seasonal variation of species distributions, which may impact ecosystem spatial and functional traits. We employed a Bayesian hierarchical species distribution model with data from two experimental oceanographic surveys conducted during winter and summer of 2013. Our model included seven explanatory variables; depth, type of bottom substrate, water temperature (surface and bottom), sea surface salinity, primary production, and fishing effort as the drivers of species distributions during both seasons. We identified significant drivers in each season and we analysed if they were specific or common to all the studied species. Then, we investigated whether the functional relationships between the predicted and explanatory variables varied from winter to summer. Our results provide solid knowledge about ecosystem response to environmental and anthropogenic drivers, as well as the first systematic quantification of seasonal changes in commercial species distributions in the western Mediterranean Sea. These results could have important management applications and help project regional ecosystem responses to existing or new stressors in the future.

Production state and dynamics of seagrass meadows in Andalusia over the last centuries and millennia

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Abstract

Posidonia oceanica is a long-living marine phanerogam that buries a significant part of the belowground production forming an organic bioconstruction known as mat. Mats have been proven to be reliable archives of long-term environmental change. With the aim of understanding seagrass community production dynamics over the long-term, mat cores retrieved along the Andalusian coast (South Spain) were analysed using Fourier Transform Infrared (IR) Spectroscopy, which is a form of vibrational spectroscopy in which vibrations arise from inorganic and organic components in the soil. The structure of minerals and organic compounds determine their IR spectra and much literature exists on spectra of reference components and their diagnostic peaks. After processing seagrass soils spectra and selecting common diagnostic peaks, these were analysed with Principal Component Analysis. Four main IR signals were identified: algal (aliphatic organic matter) vs seagrass production (aromatic organic matter); mineralogical composition of inorganic terrestrial fluxes (carbonatic vs siliciclastic material); changes in the seagrass production (polysaccharides), probably affected by post-depositional degradation of the organic matter; and biogenic silica production. Meadows with higher organic matter content, and probably a highest production, are those with higher signal of aromatics and polysaccharides. We also

observed that the more productive a seagrass meadow is, the more cyclical its dynamics.

How redesigning organic vegetable cropping systems by managing agroecological service crops affects the agroecosystem functioning across Europe?

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Understanding the ecological implications as well as agronomic benefits provided by a specific cropping design, might give farmers, advisors and policy makers, a wide picture of the benefits and disadvantages of the cropping designs evaluated. The introduction of agroecological services crops (ASC) in the crop rotation is identified as one of the most widely adopted strategies for improving the environmental performance of the cropping systems. In organic vegetable productions, ASC are usually terminated prior the cash crop transplantation to avoid competition. In this context, the adoption of no-till practices for ASC management also can help to redesign the cropping system to provide agronomic and environmental benefits. Nonetheless, in Europe, no-till practices in organic vegetable systems are very limited in commercial productions, and the scarce available scientific literature has been carried out in Italy.

In order to fill this knowledge gap, we gathered data from eleven organic vegetable trials over two years located across a wide range of pedo-climatic conditions across Europe. The support for a common effect of the cropping redesign across trials was tested by means of a meta-analytic approach based on a weighted version of the Stouffer's method. Redesigning the cropping system by ASC incorporation and adopting no-till management resulted in an enhancement of the ground beetles and rove beetles activity density, an increase of the potential recycling energy within the system and a better weed control at early stages of crop growth in different vegetable systems, soils and climatic conditions. Conversely, an important reduction of cash crop yield and quality, potential pest biological control by spiders, and energetic efficiency of the production was observed across trials. Therefore, the scientific evidences observed in our studies across Europe provides valuable information for farmers, advisors and policy makers to better understand and balance the cropping designs evaluated. Further research is required to evaluate whether the yield gap might be reduced by specific breeding programmes for organic no-till. Moreover, studies adopting a gross margin approach are required to analyse whether the no-till cropping design associated costs compensates the reduction of cash crop yield and quality.

Structuring and adaptation at a turtle pace: population genomics of the loggerhead turtle (*Caretta caretta*)

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In the light of the biodiversity crisis reported by the global assessment report on biodiversity and ecosystem services, the assessment of marine biodiversity is one of scientific research priorities. The loggerhead turtle is one of the best oceanic migrators, distributed in all tropical and warm-temperate areas and is the most abundant sea turtle in the Mediterranean Sea. Conservation measures in the eastern Mediterranean, where most loggerhead turtle rookeries are found, are crucial to support and monitor nesting populations. However, population structuring of Mediterranean rookeries is not yet fully resolved and previous studies did not consider the role of local adaptation. We used genomic data obtained by 2b-RAD libraries on 202 individuals of 9 loggerhead turtle populations in the Mediterranean (~25 samples per population). Using ~80000 markers we detected deep signals of structuring by using discriminant analysis of principal components (DAPC) and principal coordinate analysis (PCoA). We analysed regional missing loci and recover them to refine the analysis of population structuring. We also analysed outlier loci to assess the potential importance of local adaptation among populations despite the high migratory capabilities of the species. Finally we gathered environmental variables to test its potential role in regional adaptation. As a summary, the application of genomic approaches to non-model organisms can unveil novel patterns and drivers for population structuring that should be incorporated in management and conservation strategies.

Title

Global warming transforms the functional identity of Mediterranean coralligenous assemblages

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Summary

Climate change threatens tropical and temperate reefs globally. In the Mediterranean Sea, coralligenous assemblages are one of the most diverse and emblematic hard-bottom habitats that are highly threatened by global warming. In recent decades, more than 30 coralligenous species from different phyla were affected by warming-induced mass mortality events (MMEs). Field observations together with aquaria thermotolerance experiments recently unraveled the presence of response diversity among co-occurring species, which could contribute to the maintenance of functionality in spite of compositional changes driven by climate change. However, the actual consequences of shifts in species composition and their relative abundances for the long-term functionality of these habitats still remain largely unknown. In this study, we explored for the first time how two different facets of functional community structure (eg. functional richness as a proxy of functional diversity, as well as functional identity) are responding to global warming. To that end, we applied a trait-based approach to long-term photographic series from different coralligenous habitats impacted by marine heatwaves in different extents over the last two decades. Our results show that in spite of a relative stability of functional richness (the breadth of functions present in the community) in all sites tested, important long-term directional changes in the trajectory of the functional identity across the multidimensional trait space occurred in all affected assemblages. Although the direction of this shift was site-specific, it was mainly driven by a reduction in slow-growing, long-lived and late-reproducing species towards an increase in species with a shorter life span, faster reproduction and growth. Conversely, those habitats that did not suffer mass mortality remained remarkably stable over decadal time scales in spite of the steady warming. Overall, these results indicate that despite the great response diversity present within Mediterranean temperate reefs, marine heatwaves have the potential to trigger important shifts in their functional trajectories. Since these extreme events are expected to be more frequent and intense over the following decades, the functional stability of many coralligenous habitats may be at risk.

Thesis supervisors:

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COLONIZATION – PERSISTENCE TRADEOFFS REVEAL DIFFERENTIAL PERFORMANCE OF OCCASIONAL AND PERSISTENT MEMBERS IN MICROBIAL COMMUNITIES

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Fitness equalising mechanisms such as tradeoffs are recognized as one of the main factors promoting stable coexistence in community ecology, however they have rarely been explored in microbial communities. Although microbial communities are highly diverse, the coexistence of their multiple taxa is largely attributed to niche differences and high dispersal rates, following the motto “everything is everywhere, but the environment selects”. We have used a dynamical stochastic model based on the theory of Island Biogeography to study highly diverse microbial communities over time, in three perturbed or naturally stressed habitats. After establishing its validity and accuracy, we have found for the first time a colonization – persistence tradeoff in natural microbial communities, maintained at different taxonomical levels. This tradeoff was largely driven by occasional taxa, despite persistent taxa also showed it in a perturbed soil community. Dynamical models are crucial for understanding temporal patterns and processes in highly diverse communities. Our work highlights the importance of equalising mechanisms in the maintenance of diversity in microbial communities, especially in stressed or disturbed habitats.

Winter is coming: assessing the role of coastal wetlands for bat conservation during the hibernation period

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Climate Change has already had observable effects on the planet such as rising temperatures, droughts and weather instability, causing deep changes in current ecosystem dynamics. Bats are highly sensitive to these changing conditions, particularly in wetlands, as they have three periods in their life cycle strongly related to environmental temperature and food resources availability: hibernation, migration and breeding season. During the last years, warmer winter days are becoming less exceptional which might prevent bats to hibernate as they cannot keep torpor over 10°C. In the Mediterranean region bat hibernation has generally been understudied. While some authors suggest that wetlands would be able to produce a large concentration of insects, guarantying bats' winter survival, others suggest that bats do not wake up during the hibernation. The aims of this study were: a) evaluate the role of different habitats within coastal wetlands as winter foraging zones and b) scan and assess current knowledge gaps about bats in wetlands and research opportunities for future bat conservation. We acoustically sampled four different habitats (lagoon, rice paddies, urban areas and saltmarshes) during two winters in Ebro's Delta. Passive ultrasound detectors were placed in each habitat for eight weeks. In parallel, a deep systematic review of all available indexed publications about bats in wetlands was carried out. Our preliminary results show a remarkably high bat activity during both winters steadily, being urban areas and wetlands the habitats with highest levels. Roost availability, warmer temperatures around urban areas and insect availability in wetlands might explain these patterns. On the other hand, according to the review, contrasted to other bat studies, research in wetlands has not markedly increased during the last decades. The well-spread usage of traditional sampling methods (i.e mist nets), rather difficult to apply in open areas, versus bioacoustics explains why wetlands are globally underrepresented in scientific studies.

However, as some of the interviewees agreed, due to its crucial role as a feeding hotspot during winter season, wetlands should be especially targeted for future research and conservation management plans.