



EUROPEAN
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EUROCORES Programme

EuroEEFG

Ecological and Evolutionary Functional Genomics

EUROCORES Programme

European Collaborative Research

The unprecedented scope of genomic information allows ecologists and evolutionary biologists to gain new perspectives on the genes that matter in the environment. This includes insights into the role of genes and their regulation in such varied processes as stress responses (e.g., climate change, temperature, parasites, pathogens, herbivores and contaminants) and the mapping between genotype and phenotype (in, e.g., development, life histories, adaptive population divergence, ecotypic differentiation and speciation).

At the molecular evolutionary level, comparative genomics across species is uncovering the architectural aspects of the genome that influence important evolutionary processes such as gene duplication, horizontal gene transfer, adaptive variation for *cis* and *trans* effects upon transcriptional regulation and, ultimately, speciation. Similarly, genomics tools applied to experimental evolution of key species in the laboratory allow many of these same questions, as well as questions related to short-term adaptive and neutral evolution, to be addressed under highly controlled conditions.

At the other end of the spectrum, field ecologists need to be able to identify and track cells and organisms, e.g., using phylochips and functional gene chips, to assess evolutionary change in ecosystems. Transcriptomics and metagenomics are revolutionising community genetics of microorganisms and our overall understanding of multitrophic organisation of entire communities and, in the foreseeable future, ecosystems. In short, a great deal of integrative state-of-the-art research is waiting as genomics tools come on line.

In its timeframe from 2010 to 2013, the EUROCORES Programme 'Ecological and Evolutionary Functional Genomics' (EuroEEFG) provides the necessary framework and funding for top-quality European research and allows research groups to enter into high-profile collaborations. This, in turn, will help to envelop a large section of ecology and evolution research leading to a wider comparative analysis of pattern and process and ultimately to a more successful scientifically-based management of ecological resources. Multidisciplinary research platforms will enable the necessary coordinated approach to be generated. European scientists are currently at the forefront of this line of investigation and are well placed to explore and exploit this new research field of ecological functional genomics.

Collaborative Research Projects (CRPs)

Methanotrophic Diversity and Gene Expression as a Controlling Factor of Global Methane Consumption (MECOMECON)

(DFG, NWO, RCN, SNF)

Microbes are the catalysts and drivers of ecosystems on Earth. Despite their importance, environmental microbial communities are not on the biodiversity conservation agenda. Nothing is known regarding the vulnerability and resilience of microbial communities, a situation exacerbated by the inherent problem of assigning microbial activity to species identity. This knowledge is currently lacking in global conservation efforts but is urgently needed considering the challenges that global ecosystems are facing. The MECOMECON project proposes methane-oxidising bacteria (MOB) as a model group of microbes to assess the hypothesis that microbial diversity is linked to ecosystem functioning. MOB catalyse a vital ecosystem service (mitigation of the greenhouse gas methane) necessitating investigations of the impact of climate change and anthropogenic disturbance on resilience and vulnerability of these communities. In MECOMECON, diversity of MOB is linked to consumption of methane using diversity profiling techniques (microarray, 454 pyrosequencing) in a range of key pristine and cultivated soils and sediments in Europe covering important methane sources. Classical macro-ecological experiments (gradients, artificial communities, removal experiments, time series after disturbance) will be carried out using the MOB communities of these habitats in order to assess resilience and vulnerability of MOB communities. The parameters obtained (active vs inactive diversity, abundance, methane consumption kinetics) in the set of habitats and disturbances will be implemented in a process model incorporating microbial traits in order to predict response to disturbance from community composition. The latter is of importance for policy or management

guidelines concerning questions related to greenhouse gas emissions or consumption in natural and managed ecosystems. The European Research Consortium synergises by bringing together knowledge on the process and the bacteria, expertise on experimental design and molecular diversity assessment methodology, metagenomic data handling. The habitats covered warrant the value of this work for Europe-wide impact.

<http://www.mecomecon.org>

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Molecular Architecture of Environmental Adaptation in Natural Populations of the Nematode *Caenorhabditis elegans* (NEMADAPT)

(DFG, FWO, NWO)

Almost all species adapt their life-history or behaviour in the face of continuous environmental challenges. Two distinct adaptive responses can be favoured under these conditions: a phenotypic plastic response and an evolutionary response. In case of a phenotypic plastic response, selection favoured the ability of individuals to adapt by adjusting metabolic processes or specific behaviours. Adaptation is thus determined at the transcriptomic or proteomic level. In case of an evolutionary response, selection acts on the genetic composition of the population as a consequence of differential mortality and reproductive success. Adaptation is thus manifested at the DNA level. In both responses, the options for fitness maximisation are constrained by trade-offs such as increasing stress resistance at the cost of offspring number. But so far it is unknown 1) which genes are the target of natural selection and how they contribute to phenotypic variation, and 2) how trade-offs are regulated in natural populations under different environmental regimes.

We will address these questions by studying natural populations of *C. elegans*. It is a cosmopolitan nematode displaying high local genetic diversity, accessible to molecular high-throughput screens and allows for associations between genotype and phenotype to pinpoint the *loci* responsible for phenotypic variation.

By taking advantage of the model *C. elegans* we are one of the first initiatives to combine phenotypic screens, association mapping and RNAi-mediated gene validation and unravel the molecular architecture of adaptation in natural populations.

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Molecular and Ecological Analysis of *Arabis Alpina* Perennial Life-History Traits along Environmental Gradients (*A. alpina* perennality)

(DFG, NWO, VR)

Flowering plants exhibit annual or perennial life strategies. The life cycle of annual plants is completed within one year, during which they germinate from seed, grow vegetatively, flower, produce seeds and senesce. In contrast, perennials can live for many years, and after flowering often revert to vegetative growth so that the life cycle is divided into periods of vegetative development and flowering. The overall aim of this project is to evaluate whether variation in perennality-related traits is important in the adaptation to different environments and to shed light on the genetic basis of variation in this trait, using *Arabis alpina* as a model species. We have recently shown that the *A. alpina* PEP1 gene, an orthologue of the *Arabidopsis* gene FLC, encodes a repressor that prevents flowering until the plant has been exposed to vernalisation, and after vernalisation restricts the duration of flowering. The purpose of this collaborative project is to gain insight into how the perennial life strategy of *A. alpina* varies along environmental gradients and to test whether this is associated with allelic variation at the PEP1 gene. We therefore aim to assess the natural phenotypic variation of life history and perennality-related traits throughout the native European distribution range, encompassing various different environments, and to link it to the natural allelic variation of PEP1 in order to evaluate its relevance for seasonal flowering, duration of flowering and iteroparous life history. Furthermore, we aim to evaluate the adaptive significance of both the phenotypic and PEP1 sequence variation. To reach these aims the strategy encompasses three major approaches: 1) phenotypic scoring of indigenous populations; 2) molecular analysis of PEP1; and 3) reciprocal transplantation experiments. The CRP will involve three laboratories and one associated partner. The geographical distribution of the partners reflects the native European range of the target species.

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How to Live in a Mosaic of Stressors – an ecological genomics approach on the water flea *Daphnia* (STRESSFLEA)

(AKA, DFG, GAËR, SNF)

STRESSFLEA develops and uses genomics tools to unravel patterns and mechanisms of adaptation to anthropogenic and natural stressors in natural populations, using the water flea *Daphnia magna* as a model system. *Daphnia* is a key model organism in ecology, evolutionary biology and ecotoxicology, and is rapidly developing to become a leading model invertebrate in ecological genomics. *Daphnia* has clear assets as an ecogenomic model because of its ecological importance and life cycle features (short generation time, clonal lineages, layered dormant egg banks). STRESSFLEA aims to 1) obtain insight into the genomic underpinning of genetic adaptation to specific stressors (predation, parasitism, habitat unpredictability); 2) identify gene function by linking gene expression to trait values; 3) obtain insight into the genomics of adaptation to multiple stressors; and 4) reconstruct evolutionary processes over an extended time axis through the use of genomic markers and candidate genes in layered egg banks.

STRESSFLEA brings together the key European research groups developing genomic resources for studying responses to stressors in natural *Daphnia* populations (supplemented with a pivotal research group from the USA), and combines transcriptome analysis, targeted genome scans, QTL analysis and gene mapping, microarrays, proteomics and methylome analysis to identify candidate *loci* and associated SNP markers, analyse gene function, and apply this knowledge to reconstruct evolutionary dynamics in a palaeogenomics approach and analyse the dynamics and signature of local adaptation. The legacy of STRESSFLEA will include firm insight into the genomic underpinning of local adaptation to single and multiple stressors, insight into the dynamics of micro-evolutionary adaptation over extended timescales, a strongly elaborated genomics and transcriptomics toolbox for ecological

genomics using the model system *Daphnia*, and a firmly established network of European research groups that collaborate on ecological and functional genomics of natural populations, using *Daphnia* as a model.

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Ecological and Evolutionary Plant Epigenetics (EpiCol)

(DFG, FWF, NWO, SNF)

Genetic differences as the basis for selection and evolution in all organisms are well documented, while the role of epigenetic diversity is more hypothesised than experimentally proven. The essential insights into epigenetic mechanisms gained in plant science make it seem obvious to address the questions 1) whether and how information outside of the DNA sequence can influence phenotype and fitness, and 2) whether environmental factors can change epigenetic features and generate epialleles.

The originally proposed CRP was developed by five European experts in epigenetic, ecological and ecogenomic research. They will join forces, using their established methodology, to deliver proof of concept for the role of epigenetics in plant adaptation to environmental change. Work with the inbreeding species *Arabidopsis thaliana*, with a plethora of genetic, epigenetic and genomic information and available in numerous accessions from different habitats, will be complemented by experiments with *Scabiosa columbaria*, an outbreeding species for which consequences of habitat fragmentation and inbreeding depression are well documented. Well-characterised plant material will be analysed for epigenetic diversity, subjected to controlled environmental conditions and studied in subsequent generations. Epigenetic analysis will comprise DNA methylation and chromatin modifications on individual sequences as well as whole genomes.

The project is expected to result in a better understanding of the extent, significance and generality of epigenetic variation as an additional mechanism for plant adaptation. In the light of significant environmental changes in the near future, it might help increase the weight of European research in this field.

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Deep-sea Primary Production: (meta)genomic view on ecosystem functioning (DEEP_C)

(CNR, DFG, NWO)

The deep ocean covers two-thirds of our planet and teems with microbial life. Understanding the roles of deep-sea microbial communities is therefore essential for understanding global biogeochemical cycling, which in turn is pivotal to all other forms of life. The breakthrough discovery that marine bathypelagic realms are significant zones of autotrophic carbon dioxide fixation, i.e. areas of dark ocean primary production, is perhaps the most exciting application of modern molecular approaches in the field of deep-sea microbiology. Considering that the deep-sea environment represents the biggest ecosystem on our planet, it is surprising that the contribution of bathypelagic chemolithoautotrophic production to the microbial food web and to global carbon cycling in general has not been studied yet. After all, it represents a major carbon dioxide sink in the System Earth.

Recently, members of Marine Group I of *Thaumarchaeota* have been identified to be potentially involved in deep-sea chemolithotrophic production of organic carbon. Several independent studies have estimated the global archaeal dissolved inorganic carbon (DIC) fixation rate of $4\text{--}8 \times 10^{11} \text{ kg C yr}^{-1}$. This newly fixed carbon represents a substrate for a largely unknown deep-sea food web including microbial and metazoan members. Besides autotrophy, marine *Thaumarchaeota*, which are very abundant in the deep sea, are likely to play a pivotal role in the ocean's nitrogen cycle because they perform the first step in the nitrification process, namely the conversion of ammonia to nitrite. Thus, it seems that these creatures are capable of growing using ammonia as their energy source and carbon dioxide as their carbon source.

However, a direct link between deep ocean dark carbon dioxide fixation and the identity of the organisms or assemblages involved in this process has not been established so far and is thus one of the main goals of the DEEP_C proposal.

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Functional Role and Ecotype Divergence in Freshwater Ultramicrobacteria (FREDI)

(DFG, FWF, GAČR, SNF)

Prokaryotic microorganisms in freshwater habitats are centrally involved in carbon turnover processes, yet individual microbial populations greatly differ in their respective metabolic and ecological features. The pelagic zone of freshwater systems is typically dominated by freely suspended ultramicrobacteria. The proposed project will study the functional differences and similarities of three monophyletic groups of freshwater ultramicrobacteria (*P. necessarius*, actinobacteria from the Ac1 clade and alphaproteobacteria affiliated with the LD12 lineage). It will furthermore investigate the sorting of physiologically and genotypically distinct ecotypes within each group by their specific habitats. Single-cell approaches will be combined with (meta) genomic and transcriptomic analyses both on available isolates and on uncultured ultramicrobacteria in experimental studies and comparative *in situ* investigations in different habitats, and the diversity and expression of functional genes (e.g., bacterial rhodopsins) will be assessed. The different levels of interactions and common activities envisaged in the proposed project will for the first time allow a synoptic view of the functional roles and phylogeographic distribution patterns of freshwater ultramicrobacteria that could not be achieved otherwise. It also offers a chance to European scientists to take a leading role in freshwater microbiology through the concerted development and application of novel functional genomic research tools.

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Microbial Oceanography of ChemolithoAutotrophic Planktonic Communities (MOCA)

(DFG, FWF, VR)

Simulation models predict that the oxygen content of the global ocean will decrease by 25% until the end of the century due to an increased stratification of the oceanic surface waters and a rise in temperature. This loss in oxygen will inevitably lead to an expansion of hypoxic and anoxic areas in the global ocean with major consequences for the oceanic carbon and nitrogen cycling. In this project, we assess the functional diversity of chemolithoautotrophic prokaryotic communities in two contrasting marine environments, the deep-water masses of the North Atlantic along a latitudinal gradient and around the redoxcline in the central Baltic Sea. Both environments have been shown previously to harbour highly active chemolithoautotrophic prokaryotic communities with dark carbon dioxide fixation rates approaching surface water phytoplankton activity. Specific focus is put on the functional diversity of prokaryotes in the carbon and nitrogen cycling in both systems, including the sulphur cycle in the central Baltic. Biogeochemical rate measurements are tightly linked to functional gene analyses using among other approaches metagenomics and metatranscriptomics. Information obtained from these analyses will guide further in-depth studies of geochemically relevant processes in the water column of the two systems. Incubation experiments using stable and radio-isotopes in combination with molecular techniques such as SIP-RNA analyses, single-cell analyses using Raman-FISH, NanoSIMS and MICRO-FISH will allow insights into the dynamics of the functional diversity of chemolithotrophic microbial communities in suboxic and anoxic marine planktonic systems. Field studies will be complemented by laboratory model systems with isolated key players in order to understand the adaptive capacity and performance of chemolithoautotrophs in response to different environmental conditions. The combination of these approaches will provide the base for a

significant advancement in our understanding of planktonic chemolithoautotrophy in the dark ocean.

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Cover picture:

The water flea *Daphnia*,
the central model species in STRESSFLEA

Source: Joachim Mergeay

The European Collaborative Research (EUROCORES) Scheme enables researchers in different European countries to develop collaboration and scientific synergy in areas where international scale and scope are required for top class science in a global context. The scheme provides a flexible framework for national basic research funding and performing organisations to join forces in supporting forefront European research in and across all scientific areas. The national organisations support all aspects including scientific coordination, networking and research funding.

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