[Summary]

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Plankton-Nets Fields Forever
"During my research I collect planula larvae from gravid Stylophora pistillata colonies with plankton nets which allows me to address developmental biology and immune system maturation questions. When I observe at the plankton nets laying on my colonies, the imagination starts to work... Sometimes I float in an endless field, covered with ghosts, sometimes it feels like a school of the mysterious sea angels coming to say goodbye... Open your mind, in what fantasy would you like to be?"
Keren-Or Amar , Israel Oceanographic and Limnological Research

[éditorial]

Dear friends and colleagues,

Catherine Boyen
Station biologique de Roscoff, France

We are only a few weeks away from the Final Meeting and the Marine Genomics Europe Network of Excellence is drawing to a close—at least in its present form as a contract with the European Commission. It thus time to review the past and to prepare the future and the next few months will be devoted to just that. This one and only, Special Issue Newsletter is part of that process, because to prepare for the future, we must analyse our past actions and identify our strong points and our most significant accomplishments. I can definitely assert that after four years we have truly succeeded in constituting a veritable scientific community. There are countless indicators to demonstrate that the momentum initiated in 2004 was regularly stoked over the past four years. Now, the risk lies in losing all that we have gained through our efforts. This is why we must continue to build a "post-NoE" network together to maintain our cohesiveness and to improve the visibility of our community even more. The challenge in the near future is also to continue to strive so that our community will be considered as a vital player in the elaboration of European and national marine science policies. We have something to contribute to these policies and our voices must be heard. The upcoming publication of the **"The European Flagship in Marine Science for a Sustainable Future"** position paper should help us convince European and national decision-makers of our maturity and of our representativeness. Marine Genomics is clearly a scientific field coming into its own and it is up to all of us to consolidate it and to help it develop in a dynamic way that fosters, for example, wide-reaching integrative systems biology approaches. In its broad sense, Marine Genomics can generate knowledge with important implications for i) understanding the functioning of marine ecosystems in the context of global change; ii) sustainable management of marine resources; iii) the blue biotechnology sector; and iv) a better understanding of marine biodiversity and predicting the impact of climate change on this biodiversity.

We can also be proud of the Education and Training Programme that was implemented by MGE. The acquired experience coupled with the groundwork that was laid in terms of offered courses constitute a solid foundation for a European, or even international, PhD programme. **«The Kolombari declaration»**, the product of a forward planning session that took place in October involving the younger MGE generation and published in this Newsletter, shows that they are ready to take over!

I wish to end this editorial by warmly and sincerely thanking Michèle Barbier, MGE's European Manager who assisted me in the management of the MGE enterprise during these four years. Michèle is now leaving for new professional horizons: she has been hired by the Mediterranean Science Commission (CIESM) in Monaco. I would like to stress that through her enthusiasm and her energy, Michèle Barbier added her own personal touch to MGE and truly contributed to its development, including through artistic channels, which is rather innovative!

See you in Faro, Portugal from 14 to 16 May 2008!

>>> Catherine Boyen
MGE Scientific coordinator

Combined **functional genomic** and **genetic approaches**

in oyster to identify summer mortality-resistant markers

Arnaud Huvet
Sylvie Lapègue
Ifremer France

The oyster *Crassostrea* gigas has global distribution and for the past several years the highest annual production of any freshwater or marine organism. Economic importance of oysters motivates a great deal of biological research, which provides the most immediate scientific rationales for sequencing ESTs and developing genomic tools.

Strong rationales for studying the oyster genome products also come from contrasts to other genomes: membership in the Lophotrochozoa, an understudied branch of the Eukaryotes, high fecundity, with concomitantly high DNA sequence polymorphism. Oysters play also an important, sentinel role, in estuarine and coastal marine habitats, where the majority of humans live, environmental degradation is substantial, and oysters suffer intense mortalities from disease and stress.

Select oysters that are « resistant » or « susceptible » to summer mortalities

The most immediate applications of a wide variety of sequences and genomic tools from *C. gigas* fall under three headings:

- **functional genomics,** in which oysters permit a phylogenetic contrast in studies of genome function, and diversity,
- **comparative genomics,** in which oysters belonging to the Lophotrochozoa help to shed light on mechanisms of evolutionary biology, speciation in the sea and the evolution of sexuality,
- **environmental genomics,** in which oysters are a model for understanding the adaptation through genetic and physiological bases of complex traits (e.g. growth, survival) that are strongly correlated with Darwinian fitness and population responses to environmental change and stresses, such as disease.

Large scale mortalities of *C. gigas* have been reported in all areas of the world where this species is cultivated. Examination of the question of oyster summer mortality in France has suggested that there are complex interactions between the oyster, their environment and opportunistic pathogens. For the oyster, a large genetic basis was shown to exist for observed variation in resistance to summer mortality. This has opened up possibilities of improvement by selection and has in turn allowed us to select oysters that are 'resistant' (R) or 'susceptible' (S) to summer mortalities (Dégremont et al., 2007). As we have demonstrated that the physiological state of the animal plays a major part in this interaction (Huvet et al., 2004; Samain et al., 2007), the selected genetic character is likely to be connected with one or more functions within the oyster that explain the differential survival observed between R and S. >>>

The South Brittany site where oysters were reared

A collaborative work between MGE and Aquafirst

In the framework of the Marine Genomics Europe (MGE) Network of Excellence, a collaborative research of teams shared effort to increase the lack of genomic information in oyster and develop tools of general use, such are cDNA libraries, EST collections and microarray technology. This effort has been joined to the European program "Aquafirst" (coordinator P Prunet, INRA), devoted to genetic and functional genomic approaches for stress and disease resistance marker selection in fishes and oysters, to form a powerful consortium for fish and oyster genomics.

Partners of the "Fish and Shellfish node" of MGE network have produced four cDNA libraries for C. gigas, which have been built by the Max Plant Institute (Berlin) and have resulted in a total of 5712 EST sequences (Tanguy et al., 2008). Multiple normalized libraries were constructed from three tissues (gonad, gills and digestive gland) and are still available for further sequencing in order to increase the EST collections. All these sequences have complemented previous ESTs and mRNA available in Genbank (clones provided by all partners for the microarray) and 8064 ESTs produced by subtraction between R and S oyster lines in the Aquafirst program. All together, these sequences were assembled in a unique database to form 9272 contigs (http://www.sigenae.org/aquafirst/), resulting from the collaborative work of the consortium between MGE and Aquafirst.

Consequently, these two European projects met an agreement to construct a cDNA microarray. PCR amplifications of insert clones and spotting were realized by the MPI (Berlin). The slide contains 9059 unigenes spotted in duplicates. In total, 219 slides were produced and divided into each partner for developing functional genomics studies to reach a common goal: characterize summer mortality-resistant markers and identify the biological mechanism(s) involved in summer survival.

Thus, several experiments were analysed using microarray corresponding to R and S tested in different environments: in the field and in laboratory experiments in response to bacterial challenge, hypoxia or sulfoxia, all presumed to interact in summer mortality (Samain et al., 2007). Part of the hybridizations have been processed at the IFR 140-Ouest Genopole platform (Rennes, France) and are currently under statistical treatment. Correlation coefficients of both technical and biological replicates appeared very high which supports that our data are highly reproducible. First results indicate that among the 9059 unigenes, 438 are differentially expressed between R and S in the field. A second batch of hybridization is running to identify regulation processes of gene networks involved in hypoxia stress in digestive gland of R and S.

Identification of markers of resistance

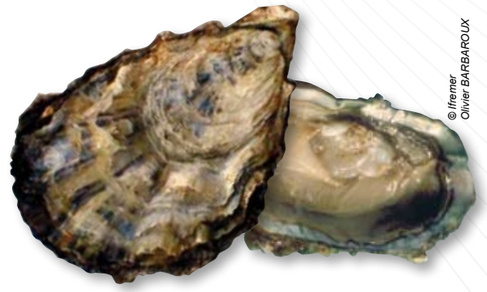
Functional genomics studies allow the characterization of genes that are differentially expressed between R and S progenies. To evaluate these genes as potential candidate markers of resistance to summer mortality, they will be next studied "one by one" in terms of the physiology of these oysters with RNA interference as functional promising tools and also in terms of valuable markers in the search for Quantitative Trait Loci (QTL).

Indeed, genetic markers linked to summer mortality resistance in oyster spat are being sought. This search, using the QTL approach was initiated by the production of F1 'hybrid' families between R and S batches, and then F2 segregating families. The genetic map published by Hubert & Hedgecock (2004) is the basis to be enriched with SNPs (Single Nucleotide Polymorphisms) and new microsatellites detected from EST databases, called "in silico" microsatellites.

The search for SNPs was performed by sequencing some candidate ESTs in the 24 grand-parents (F0) of our families of reference. The first analysis of sequence polymorphism and codon usage bias with a set of 41 nuclear loci revealed a very high level of DNA polymorphism in oysters, in the order of magnitude of the highest levels reported in animals to date *(Figure 1; Sauvage et al., 2007)*. >>>

01
[Scientific highlights]
MGE Newsletter

A total of 290 SNPs were detected, 76 of which being localised in exons and 214 in non-coding regions. Average density of SNPs was estimated to be one SNP every 60 bp in coding regions and one every 40 bp in non-coding regions. Non-synonymous substitutions contributed substantially to the polymorphism observed in coding regions. The non-synonymous to silent diversity ratio was 0.16 on average, which is fairly higher to the ratio reported in other invertebrate species recognised to display large population sizes. Therefore, purifying selection does not appear to be as strong as it could have been expected for a species with a large effective population size. The level of non-synonymous diversity varied greatly from one gene to another, in accordance with varying selective constraints. We also examined codon usage bias and its relationship with DNA polymorphism. A table of optimal codons was deduced from the analysis of an EST dataset, using EST counts as a rough assessment of gene expression. As recently observed in some other taxa, we found a strong and significant negative relationship between codon bias and non-synonymous diversity suggesting correlated selective constraints on synonymous and non-synonymous substitutions. Codon bias as measured by the frequency of optimal codons for expression might therefore provide a useful



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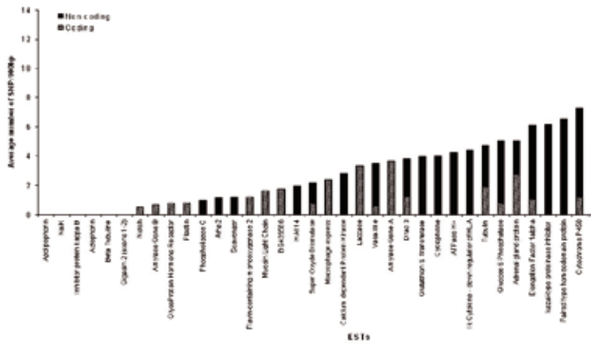
Olivier BARBAROUX

The Pacific cupped oyster Crassostrea gigas (Thunberg, 1793)

indicator of the level of constraint upon proteins in the oyster genome. Complementary studies based on an evolutionary approach of gene polymorphism has been conducted in both *C. gigas* and *C. angulata* with the aim to identify selective effect in functional genes and allowed to identify more SNPs and indels markers (Tanguy et al., unpublished). Furthermore, in silico microsatellites are currently being developed by searching repeat motifs in ESTs from the database. A first set of 18 markers will be soon available (Sauvage et al., unpublished) and other markers are being developed. Microsatellite and SNP markers were then used to construct a genetic map on three F2 families that were also tested for resistance to summer mortality in summer 2006. This experiment combined to the maps will be used to search for QTLs of resistance or susceptibility to summer mortality. ■■■



1

Average level of polymorphism detected in coding and non-coding parts of ESTs.

>>> Bibliography (page 23)

>>> Acknowledgements
The authors are thanking A Canario, F Volckaert, E Zouros and P Prunet, for their help in organising their work respectively within the MGE network (Fish and Shellfish node) and the Aquafirst project. Many thanks to the Sigenae team (http://www.sigenae.org/aquafirst).

Antarctic

Within the MGE remit, there is an element of research on organisms that live in extreme environments. The interest lies in understanding how the organisms manage to survive in what we would consider to be harsh and "unfriendly" conditions, such as excessive heat, salt, lack of water, high UV radiation and cold. My work centres on Antarctic marine life. At the British Antarctic Survey (BAS) we are interested in studying not only how the animals adapt to the cold, but also how they cope with increased sea water temperatures in line with global climate change predictions.

The BAS research directly linked to MGE funding takes the form of two main projects: an MGE funded studentship to Manuela Truebano-Garcia entitled "Stress in marine bivalves" and an MGE collaboration involving a BAS employee, Gavin Burns, being registered as a PhD student at Gothenborg University via a link with the Kristineberg Marine Research Station with his project "Transcriptional regulation of arm regeneration in temperate and Antarctic brittle stars".

Stress in Marine Bivalves

The aim of this project is to compare the heat stress response in the commercially important blue mussel with that of the Antarctic clam. The advantage of using the Antarctic clam is that it is very sensitive to temperature and therefore it is much easier to recognise a response in the expression levels of genes and proteins to heat, also because there is no pollution in the Antarctic, you get clear heat responsive signal without the confounding factors of pollutants or contamination. By identifying the genes and proteins in the Antarctic clam that react to higher temperatures, we hope to use these as biomarkers to monitor heat stress (and the effects of climate change) in the blue mussel. So far Manuela has identified 28 candidate proteins in the blue mussel and 5 proteins in the Antarctic clam that significantly change in response to heat. She is now in the process of identifying these proteins via mass spectrometric analysis and shortly will be able to tell if the same proteins change in both animals and which of these could potentially be used as biomarkers. We are also developing a gene chip, so that we can study in more detail, the expression of the genes that produce these proteins. This PhD studentship is a collaboration between the University of Swansea (UK) for the protein work, the University of the Algarve (Portugal) involved in identifying stress hormones and BAS, where Manuela will look at genes involved in the stress response using a gene chip. This is a real mobility PhD! >>>

Melody Clark
British Antarctic Survey

The main British Antarctic Survey base for biological research is Rothera on the Antarctic Peninsula. It is situated in one of the regions experiencing the most rapid climate change on Earth. Shallow seawater temperatures along the west Antarctic Peninsula have risen in excess of 1°C over the last 50 years. Whilst the IPCC Third Assessment climate model predicts a further 2o°C increase in global seawater temperatures over the next 100 years, albeit with large regional variations and confidence intervals. This does not bode well for the marine life: the marine invertebrates found in the seas around the Peninsula have been shown to be some of the most thermally sensitive creatures on the planet. In warmer conditions, some animals can no longer avoid predation when disturbed as they lose the ability to swim (scallops) or rebury into sediment (clams) with water temperature rises of only 2-3°C.

The British Antarctic Survey base: Rothera

Arm regeneration in temperate and Antarctic brittle stars

The process of regeneration is of great interest to the medical profession: how do cells and tissues regenerate? What genes are involved? How can we activate “normal” cells to produce new growth, particularly in a certain form, rather than just a ball of random cells? Star fish are some of the animals known to regularly regenerate a new arm. For them it is a way of life, a method of avoiding predators. If a fish nips hold of an arm, the star fish loses that part of the arm, escapes and grows a new one. Brittle stars are a type of star fish. Although there are a number of genes that are known to be involved in regeneration, the numbers are limited. Gavin aims with this PhD project to make a gene chip so that he can study thousands of genes at once and identify which ones play a critical role in regeneration. Firstly, however, you have to know how common it is for your particular type of starfish to regenerate its arms. Gavin is looking at two brittle stars, *Ophiura albida* (Swedish) and *Ophionotus victoriae* (Antarctic). Extensive field surveys in Gullmarsfjord (Sweden) and Ryder Bay (Antarctica) prove that the answer in both cases, is very common. However re-growth of arms in the Antarctic brittle star is very slow, it can take over three years to fully develop. If you want to look at the different stages of regeneration, then such slow growth can be a problem when designing experiments (as can the distance from the field site), so Gavin is concentrating at the moment on the Swedish brittle star, which regenerates its arms within weeks. Gavin has only just started, but this is looking to be a very exciting connection between BAS and the Kristineberg Marine Research Station, which would not have happened without Marine Genomics Europe. ■■■

Marine Virus Genomics

If you put all the 10^{31} viruses in the global ocean end to end they would stretch as far as 200 million light years, about 60 galaxies away (Suttle, 2007)! With an average of 10 million viruses in a teaspoon of seawater, they are the most abundant biological particles in the ocean with a weight equivalent to about 1 million blue whales.

Viruses are ‘lubricants’ of the Earth system ‘engine room’ and can be thought of as catalysts for global biogeochemical cycling by transforming planktonic cells to dissolved material; viruses essentially act as biological transformers that accelerate the lysis of (predominantly) bacteria and phytoplankton (Wilhelm & Suttle, 1999). The morphological, biological and genetic diversity of viruses in the ocean is enormous and this is exemplified by the recent explosion of virus sequences obtained from environmental shotgun sequencing and genome sequencing projects (Breitbart & Rohwer, 2005, DeLong et al., 2006, Wilson et al., 2005a, Yooseph et al., 2007). It is important to make sense of such diversity particularly in the context of global environmental change; the next big challenge for oceanic biogeochemistry is to use this genetic diversity to help understand the complexities of ecosystem functioning (Zak et al., 2006).

E. huxleyi: the most numerous coccolithophore in our oceans

Of course a sensible starting point is to investigate manageable components of the oceanic microbial ecosystem, ideally those that are quantitatively significant with regard to both global biogeochemical cycling and climate interactions. Also, perhaps obviously, if post-genomic tools are to be developed and employed it is important that good sequence information is available for virus and ideally host. The globally important marine microalga *Emiliania huxleyi* and its co-occurring viruses, the *Coccolithiviridae* (Allen et al., 2006c, Schroeder et al., 2002) fit these criteria.

1

True colour satellite image of a high reflectance E. huxleyi bloom in the English Channel, south of Plymouth, UK on the 30 July 1999. Water samples taken from this bloom contained up to 1 million E. huxleyi-specific viruses per millilitre. The SeaWiFS image was processed by the Plymouth Marine Laboratory Remote Sensing Group (RSG)

E. huxleyi is the most numerous coccolithophore in our oceans and satellite observations often show massive mesoscale blooms *(Figure 1)*. Until recently the mechanismsof *E. huxleyi* bloom disintegration were poorly understood, but it is now accepted that viruses are intrinsically linked to these sudden crashes *(Figure 2)* (Schroeder et al., 2003, Wilson et al., 2002). Characterization of these viruses revealed that they are large double-stranded DNA viruses with genomes approximately 410 kbp and belong to a new virus genus termed *Coccolithovirus*. (Schroeder et al., 2002, Wilson et al., 2005b).

The largest algal virus genome sequenced to date

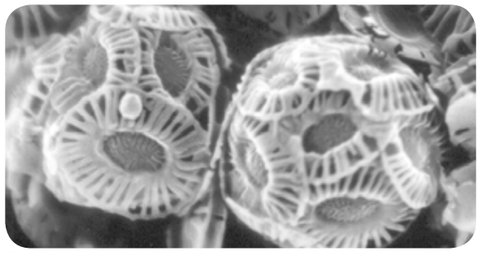
The genome of coccolithovirus type-species EhV-86 was sequenced and revealed a circular genome with a length of 407,339 bp making it the largest algal virus sequenced to date (Wilson et al., 2005a). Some of the homologs in EhV-86 encode a range of unexpected, ecologically relevant genes never previously observed in a virus. Most notably, there are at least 6 genes involved in sphingolipid biosynthesis. Sphingolipids are membrane lipids present in all eukaryotes (and some prokaryotes) and also play a key role in a number of important biological processes, particularly in signal transduction pathways (Futerman & Hannun, 2004). Though to date, we do not know the function of this virus-encoded pathway during infection of *E. huxleyi*. Arguably the most frustrating aspect of the coccolithovirus genome sequence was that just over 400 (86%) of the 473 genes have no database homologs. This presents a huge challenge to determine the function of these virus ‘ORFans’. >>>

>>> Bibliography (page 23)

Willie Wilson
Bigelow Laboratory for Ocean Sciences (USA) & Plymouth Marine Laboratory (UK)
Mike Allen
Plymouth Marine Laboratory (UK) British Antarctic Survey

The coccolithovirus microarray used for multiple purposes

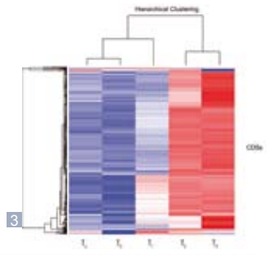
With the help of MGE funds Dr Wilson and his Plymouth based team developed a coccolithovirus microarray to help take this challenge head on. The first generation microarray was developed by spotting oligonucleotides designed against each of the coding sequences (CDSs) from virus genome on to the array (along with a wide range of control oligonucleotides). The *coccolithovirus* microarray has been used for a range of purposes (reviewed by Allen and Wilson (2006)). Initially, the microarray was employed in the annotation of the EhV-86 genome (Wilson et al., 2005a). We used functional information from preliminary expression results to determine correct reading frames for disputed CDSs, in addition, it was used to help to identify new and unannotated CDSs. This was the first time a microarray had been used for these purposes. The primary use of the microarray was to assign virus transcripts into kinetic classes *(Figure 3)* with the distinct aim of helping to determine the function of coordinately expressed genes with no database homologs (Allen et al., 2006b). An opportunistic use of the microarray was to use it as a tool for genome diversity analysis (Allen et al., 2007). Very simply the array was used as a hybridization tool to determine presence or absence (or high divergence) of genes in genomes of related coccolithoviruses. Rather than focusing on a single gene, the microarray allowed us to determine a diversity index based on whole genomes without the need to sequence these genomes. It allowed us to build a picture of the core coccolithovirus genes (eg. Allen et al. 2006c) and identify variable and absent genes between coccolithovirus genomes.



2 *Virus (approx. 190 nm diameter) attached to a coccolith from an E. huxleyi cell.*

> ***« The advent of the genomic era has accelerated our understanding of genome function beyond all expectations »***

A second-generation microarray has now been developed that contains a much greater coverage over 2 coccolithovirus genomes (Allen et al., 2006a) and crucially, oligonucleotides for over 2000 host *E. huxleyi* ESTs. Some of the ESTs were generated from libraries constructed at different stages of an EhV infection ***(a joint MGE initiative between PML and AWI)***. Preliminary results from this study revealed that transcripts from some host photosynthesis-related genes were no-longer detectable 6h into infection, suggesting that virus propagation in *E. huxleyi* influences regulation of photosynthesis. (Kegel et al., 2007). Oligonucleotides for these (plus many other) host genes have been spotted on a second-generation virus-host microarray. This will allow us to look at the response of the host to virus infection at the genome level. The construction of the coccolithovirus microarray has rapidly increased the understanding of the virus and its infection strategy, yet clearly there is still much to learn. Future work will, without doubt, build on the techniques described here to further our knowledge of this amazing virus and even other algal viruses. Indeed, the advent of the genomic era has accelerated our understanding of genome function beyond all expectations. Microarrays are a powerful tool, but are typically used in well studied model biological systems in which complex transcriptional cascades are mapped with ever increasing precision. However, as we show here, microarrays can prove to be just as powerful and useful a tool when dealing with less studied, non-model systems. ■■■



3 *Temporal expression analysis of EhV-86 genes during the first 4 hours of infection of host E. huxleyi. The figure shows bi-clustering of coding sequence (CDS) and sample expression profiles in combination with heat map visualization. Each column represents the averaged log2 expression data for three independent samples for an infection time point. Each row in the heat map represents one CDS color-coded for expression level from low (blue) to medium (white) to high (red). Branches on the top and side of the heat map show the identified clusters in the sample, i.e., samples with the most-similar expression profiles across all genes and genes with similar expression profiles across all samples. As expected, results for uninfected (Tu) samples and samples at 0 h post-infection (T0) are very closely related. The later time points T2 and T4 are also closely related. T1 is the most interesting, showing a cluster of CDSs which go from "off" in the Tu and T0 samples to "on."; intriguingly CDSs in this cluster all contain a novel upstream putative promoter, not present anywhere else in the genome (Allen et al., 2006b).*

>>> Bibliography (page 23-24)

New technics on technology platform

The biological research achieved, in the last years, great result thanks especially to the huge progress of the molecular genetics. New technologies allow, in fact, to identify, to isolate and to characterize genes more easily that in past and therefore to understand the functions of proteins and complex metabolic pathway in a more straight way.

In the beginning the genetic studies developed following a "direct approach" finding a gene starting from his product, the protein, whose study furnishes the information to identify the corresponding gene. This strategy, also fundamental, has been not so useful, because of the few information available fof a lot of proteins. It is more currently used an "inverse approach" (the so-called inverse genetics): a gene is identified without any knowledge of its product but starting from other considerations, for example its homology with important genes studied in model-organism, simpler than the mammals. From these considerations it emerges that the possibility to study different model-organism and to develop appropriate techniques represents an absolute necessity for the progression of researches in the field of the molecular genetics.

Projects based on Molecular Biology at the Stazione Zoologica "A. Dohrn" of Naples, are supported by the Molecular Biology Service (SBM), coordinated by Dr. Elio Biffali (Senior technologist), that offers, with the collaboration of Dr. M. Borra (Technologist) and F. Campili, E. Mauriello, R.Pannone and R. Sepe (Technical staff), to all researchers of the Institute different facilities. The Molecular Biology Service,thank's to the various services offered, was present as «Technology Platform», in the Network of Excellence Marine Genomics Europe (MGE).

• Basic Activities

preparation of media and plate for bacteriology and media for animal cell culture, supply of restriction and modifying enzymes, stocking up of chemicals and disposables, preparation of competent cells for bacterial transformation etc. Besides the Service handles the provisioning of normal and modified synthetic oligonucleotides.

• Sequencing

Sequences produced are ≃ 650 bases long in average with more than 97.5% accuracy, starting from plasmids, PCR fragments, phages. The potentiality of the system endowed by the Service is more than 1000 sequences per day and it can support high productivity projects too. The Service at the moment produces more than 15 000 sequences per year.

• Fragment analysis (microsatellites) and SNiPs

About 6 000 samples analysed per year, mainly for genetic population studies.

• Real Time PCR

The use of such new technology is in rapid increase. The Service supports the laboratories of the Institute in the planning of the experimental strategies and, moreover, it provides the automated preparation of the reactions in plate for high productivity experiments, the run of the samples and, if required, the analysis of the data.

• Lab. Automation and High throughput projects

The Service is equipped with a robotic system able to realize high productivity projects for the research activities that needs, in brief times, the analysis of an high number of samples. The Service is able to accomplish to different demands: genomic DNA extractions, mini-preparations of plasmidic DNA, sequencing and PCR reactions setup and cleanup, fragments analysis reaction setup, qPCR reactions setup, samples quantitation and normalization, replica plates, cherry picking and gridding etc. >>>

Marco Borra,
Stazione Zoologica «Anton Dohrn» (Napoli Italy)

Moreover the Service offers technical-scientific consultation, training, technical and market survey, comparative tests of new products and protocols and often is involved in scientific and technical-scientific collaborations with laboratories of other Institutes and in partnership with companies operating in the Molecular Biology compartment.
The Service have also an active role concerning the feasibility study and/or the realization of innovative technological assay applied to the model-systems and to the needs of the research laboratories. An example of such activity are the protocols developed within the *Marine Genomics Network of Excellence and published* on the Newsletter and on the *MGE* Web pages (bibliography).
Great attention is given to the training activity, carried out at different levels, receiving students or researchers that need to be introduced to the Molecular Biology techniques or that needs to develop and to optimize protocols to use in their own laboratory. Equally the SBM acts as pilot lab for the development of new methods. Training and development of protocols on-demand has been a substantial part of the SBM activity within the *Marine Genomics Network of Excellence*.

« Training and development of protocols on-demand has been a substantial part of the SBM activity »

In conclusion the experience matured during the years underlines that the centralization of complexes technological processes and the management of the necessary equipments; of the provisioning of the reagents for the molecular biology and of the inherent know-how is advantageous for the whole Institute both from the economic point of view that in terms of productivity. ■■■

Molecular Biology Service

overview

SEQUENCING
ABI 3730 (48)
More than 1 000

FRAGMENT ANALYSIS
Beckman : CEQ2000XL
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HTS ROBOTIC SYSTEM
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Quantitati
Re-
Normalizati
Glycerol
PC
Sequencing
Purificati
Dye

And Technical-scientific advice and training
New strategies and technologies Pilot Lab
Scientific collaborations
Institution of new peculiar services

REAL TIME PCR
Semi-automated

BASIC
Supplyin
Competent cells
Enzymes
Oligos
Chemicals
Disposable
And more

>>> Bibliography (page 23-24)

New approaches for functional genomics of marine microorganisms

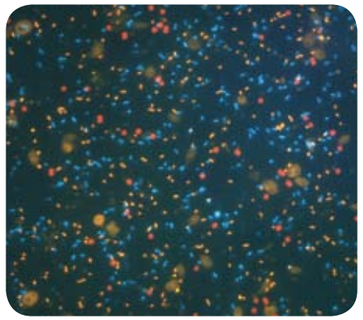
Berlin, June 21-24, 2007

Wolfgang Hess
University Freiburg (Germany)
Richard Reinhardt
Max Planck Institute for Molecular Genetics (Germany)
Chris Bowler
Ecole Normale Supérieure (France)

With financial support from Marine Genomics Europe, the Exploratory Workshop "New approaches for functional genomics of marine microorganisms" was held in Berlin, Germany, from June 22-23.

Background to this meeting was the fact that considerable funds have recently been invested both in Europe as well as in the U.S. into metagenomics and to sequence genomes of laboratory isolates which are representative for ecologically important marine organisms. As a consequence, the nearly exponential increase in new sequence information from total genomes as well as from environmental samples challenges the traditional methodologies of functional genomics-based approaches for functional interpretation.

Therefore New and efficient tools are required for the large scale experimental as well as computational analysis of gene functions. These approaches include newly developed algorithms and databases for the efficient prediction of gene functions as well as novel experimental solutions, e.g., high-speed cell identification and sorting by flow cytometry, RNAi-based gene characterization, new applications of microarrays, variations of large scale mutagenesis systems, high throughput mass spectrometry, etc.



légende à venir

The objective of the two day Exploratory Workshop was to present and discuss innovative approaches and emerging technologies for functional genomics in marine microorganisms, including prokaryotes, unicellular microalgae and picoeukaryotes. The two overarching topics for the two days were « Life in the Ocean » for day One and « Emerging technologies – novel approaches » for day Two. With our program we aimed at an open exchange of new ideas and a discussion of the power of these emerging tools in order to spread their application and foster their integration into existing research objectives. We restricted the number of participants to not more than 20 in order to keep this Exploratory Workshop as informative and intensive as possible. The workshop finished with a general discussion about how the field can move forward over the next years. ■■■

The Exploratory Workshop was held at the Harnack-House in Berlin, which is an old villa of historical importance located within the city and close to the Max-Planck-Institute for Molecular Genetics.

The 18 participants came from seven different countries and, except three local participants, all stayed together directly in the Harnack-House guest house (for all names and contact details, see detailed list at the end of this document). The participants had all been selected based on their previous experience with genome analyses as well as their specific knowledge on molecular details of particular biological processes like e.g. technical problems of genome analysis, computational biology, structural biology, photosynthesis, nitrogen metabolism, environmental signal processing, or stress responses within the frame of ecologically meaningful parameters.
This Exploratory Workshop was a joint activity of the microbial and algal nodes of the EU-funded Network of Excellence in Marine Genomics and was considered as a great success by all participants.

>>> Bibliography (page 24)
>>> List of participants (page 25)

The Bielefeld Bioinformatics platform

Data Management and Data Integration in the "-omics" Sciences

V. Mittard Runte
A. Goesmann
Center for Biotechnology (CeBiTec), Bielefeld University, (Germany)

Over the last years, modern high-throughput techniques in genome and post-genome research have also entered the marine sciences. Today, massively parallel DNA sequencing or hybridization approaches allow to identify not only the gene repertoire but also the gene regulatory networks of an organism. The huge amounts of data acquired from such experiments can only be handled with intensive bioinformatics support that has to provide an adequate infrastructure for storing and analyzing these data. Thus, bioinformatics has to deliver efficient data analysis algorithms, user friendly tools and software applications, as well as extensive hardware infrastructure for answering such questions.

The Bioinformatics Resource Facility (BRF) located in Germany at Bielefeld University provides extensive hardware and software resources to a large interdisciplinary spectrum of users at the CeBiTec [1] but also within various national and international projects such as the EU FP6 network of excellence Marine Genomics Europe [2].

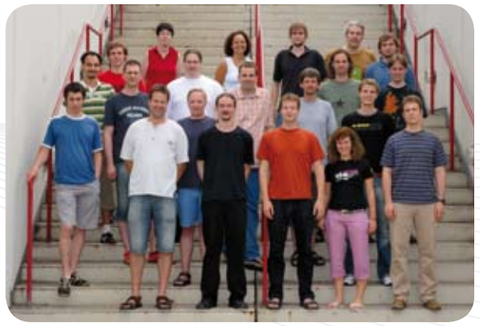
The group focuses on data management for genome and post-genome research projects that require new software solutions for systematic data acquisition, secure data storage of structured information, and high-throughput data analysis. All these features enable the users to browse through a hierarchy of data ranging from raw experimental data to highly structured complexes such as EST analyses, complete genome annotations or results of microarray data analyses.

Within the bioinformatics platform, a bioinformatics portal *(Figure 1)* has been created for the Marine Genomics Europe (MGE) community that provides a central access point for all data sets and various software tools.

High-throughput sequence analysis and genome annotation

While the web-based genome annotation system called GenDB [3] was successfully used for the automatic and manual annotation of a dozen microbial genomes, the sequence analysis and management system called SAMS [4] is currently applied for detailed analysis of large sequence sets (e.g. ESTs or pyro- sequencing reads). GenDB (current version 2.4) is available as open source under the GNU public license (GPL). The GenDB annotation engine will automatically identify, classify and annotate genes using a large collection of software tools. It also offers user interfaces that allow expert annotation with large, geo-graphically dispersed teams of experts. Genes to be annotated can be categorized by functional class or gene location using some ontologies or functional classification schemes such as GO, TIGR roles, or COG. In addition to its use as a production genome annotation system, it can be employed as a flexible framework for the large-scale evaluation of different annotation strategies.

For the analysis of EST libraries, the SAMS pipeline includes the processing of the raw sequence data (e.g. base calling, quality and vector clipping), the processing of ESTs using different tools (e.g. BLAST), and also the clustering and assembly of the sequences. Finally, the system provides a web based visualization of the results. New features like SteN – the statistical electronic Northern tool - (a SAMS integrated application to scan libraries of an EST-project for differentially expressed genes), or pie chart graphics for viewing the distribution of TCs according to their functional classification such as KEGG, COG, and GO were added with the latest SAMS releases (current version 2.0) to help the users in their analysis. New tools such as HAMAP, PRIAM and PatScan search for protein sequences have also been integrated to enhance support for functional genomics. >>>



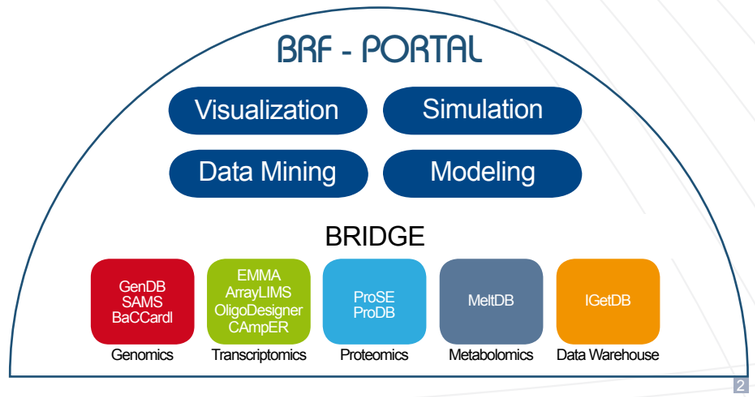
Group photo of the Bioinformatics Resource Facility (BRF).

Transcriptome and proteome data analysis

In the field of transcriptomics, the EMMA2 (current version 2.4) software is provided as an open source and MAGE compliant software platform for the evaluation of data derived by genome-wide transcriptomic studies [5]. It provides automated pipelines for data processing, allowing an automated or manual analysis of expression profiles. It also provides automated tools to perform data normalization, statistical test methods for differentially expressed genes and clustering the measured data. Furthermore several visualizations of data and extracted information are provided. Detailed experimental setups and protocols as well as all raw data sets are stored in a separate LIMS component called ArrayLIMS. ArrayLIMS is a Microarray Laboratory Information Management system developed in close cooperation with biologists that are carrying out microarray experiments for many years. It provides a permanent and consistent storage of the microarray experiment data as well as a fast information retrieval, making the data rapidly available. The stored data is standardized, consisting of the hybridization steps (e.g. RNA production), production of the hybridization targets or the hybridization itself. It is also possible to store images of the hybridized and scanned slides as well as the corresponding data files.

Overview page of the MGE bioinformatics portal. 1

Data access across different components is mediated via the BRIDGE architecture *(Figure 2)*, a domain spanning query software [6], allowing users for example to be able to view gene expression data projected onto a KEGG metabolic pathway which is cross-linked with all available sequence annotations for the corresponding enzyme. ■■■



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The BRF platform currently comprises software for high-throughput sequence analysis (SAMS) and genome annotation (GenDB), transcriptome (ArrayLIMS, and EMMA) and proteome (ProDB and ProSE) data analysis. A new software module for metabolome data analysis (MeltDB) and a Data Warehouse (IgetDB) for efficient queries on large data sets are also being developed as well as a new tool (CampER) for automatic analysis, annotation and storage of real-time PCR experiments performed with different real-time PCR systems such as Lightcycler® and the Opticon®. All components are linked via the BRIDGE integration layer providing an interface for further data mining, modeling, simulation, and visualization.

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Workshop on marine genomics:
an ocean of techniques
Crete, Greece, October 8-11, 2007

As part of the MGE Training & Education Programme a workshop for junior scientists was organized in Crete (Greece) the 8th to 11th of October, 2007. The workshop was financially supported by the T & E budget as well as the MGE Exploratory Workshop fund.

Ulrika Hjelm
Kristineberg Marine Station (Sweden)

The event took place at the Orthodox Academy of Crete in the village of Kolombari with support from the University of Crete and the Hellenic Centre for Marine Research. The aim was to provide PhD students and post docs working in the field of marine genomics an arena for discussions of scientific and non-scientific subjects. The workshop was set up with four different sessions; three with scientific focus and one session dealing with career issues.

- **Genome analysis**
- **Functional analysis**
- **Image analysis**
- **Career development**

The research oriented themes were covered by invited speakers and by talks selected from abstracts. The starting point for these sessions was the techniques and how they are used for studying different organisms and answering a wide range of biological questions. The career development session included talks and discussions on different aspects of career development at a European level; within academia as well as within the private sector.

One of the evenings the participants were split up in sub-groups for roundtable discussions on different issues of « genomics today & in the future ». The discussions were lively and continued the days after in a smaller working group which put together a written statement reflecting the views of the larger group (The declaration of Kolombari, see page 17).

The career session was divided into two parts, the first one starting with a talk by Dr Sohail Luka from the European Commission (DG research - Universities and Researchers) on “Facilitating Researchers’ Career Development through Mobility” followed by speakers covering different post docs fellowships (Marie Curie, HFSP and EMBO); giving evidence about their experience on moving from academy to industry as well as from academy to starting up a company; and outlining the Two Body Problem and other gender issues in career development. A nice and friendly poster session followed and moved slowly into a Cretan evening with traditional food and dance. The next day the second part of the career session was set up as an informal chat-with-the-Prof session where the participants sat down in smaller groups and over a cup of coffee talked with a senior scientist about any career issues they had an interest in.

The workshop was considered a great success by all involved. A warm and relaxed atmosphere was created generating a nice integration between the members of the group of junior marine genomic scientists as well as with the more senior researchers. ■■■

The workshop participants at the terrace of the Orthodox Academy of Crete

>>> List of participants (page 25)

The declaration of Kolombari
MGE PhD and post-doctoral fellows’ opinions on marine genomics

40 of Europe’s young scientists working in the field of marine genomics met in Crete (Greece) in October 2007. The future of marine genomics in general and of the MGE Network in particular was discussed during this meeting which generated the following statements from these representatives for the new generation of marine researchers.

MGE PhD and post-doctoral fellows through
Alexander Alsen, Pierre-Olivier de Franco, Marina Panova, Andres Ritter, Jennifer Rock, Paola Squarzoni and Manuela Truebano

Gender

- We support the 40-60% gender balance consideration for speakers, teachers and scientific steering committee when organising a course or conference. This balance brings diversity and equality into the scientific community, without neglecting the importance of scientific quality. In this respect we also support gender equality in any scientific project leadership.

Technological Platforms

- We wish to receive more information on MGE (?) Technological Platforms by the organization of workshops or courses at the different Platforms with visits to the facilities included.
- We encourage key hubs expertise laboratories (in particular areas) to host short stays to learn techniques. Funding should be available to encourage this scheme.
- Universities and government agencies should facilitate research work/out put by sharing infrastructure, which should enhance scientific collaborations.

Education

- It would be of great interest for us to extend the idea introduced during this workshop of meeting senior scientists in small, informal gatherings to discuss career issues. We would like to make this a common feature in other marine genomics courses, conferences or meetings. A follow-up career development session that could be organised at a future workshop should provide exercises on CV writing for industry versus academia application and advice on how to “market “ourselves best.
- We would like a continuation and extension of the MGE Training & Education scheme with practical, short training courses (and summer courses?). We support the idea that the knowledge acquired during these courses should be spread within host institutions on the return from the course.
- We greatly appreciate the possibility of being part of both universities and governmental institutions so different by nature and yet so close in their goals. In relation to this, we give great importance to the implementation of an international post-graduate programme that would allow mobility and provide experiences not accessed otherwise. We would like to emphasize and continue the effort made to structure the marine genomics community through workpackage organisation (tasks and data to be share).

« We believe that even if we are not able to keep marine genomics funding, it should not stop us from keeping its essence; maintaining bridges and collaborations »

- We would like to be trained on the meaning and relevance of intellectual property.

Science

- We want to maintain and pursue the network MGE as broad as it is with key biological/ ecological questions as common goals for exploring new techniques and methodologies.
- We support the integration of all data generated from whole marine model genome sequencing.
- We want to concentrate more on the data analysis, and welcome any new methodologies and approaches on this.
- We should use genetic tools to investigate ecological questions by focusing on the more biological/ecological relevant organisms.
- We believe that metadata are very important to helping find patterns of repetition and variability but it is also very important to keep in mind the loss of accuracy between experiment and computation. >>>

Communication

- For all the reasons highlighted above we strongly feel that we belong to the marine genomics community - an extension to our everyday based, smaller scientific grounds. We agree with the principles and aims of the MGE organisation and admire the way they have been carried out. **The most valuable tool MGE has given us is the power of communication and collaboration between different laboratories and institutions. In this respect we would like to express our support to international integration, integration of laboratories, of data and most importantly of expertise.**
- We believe that even if we are not able to keep marine genomics funding, it should not stop us from keeping its essence; maintaining bridges and collaborations. This is possible through a small platform with common links as a web site, a newsletter and any internal communication forms which should be maintain with a small administrative structure to animate the network. ■■■

The MGE-GAP awards
two outstanding women in science
Crete, 10 October 2007

Every Network of Excellence has a Gender Action Plan (GAP) and with good reason. There are still significant hurdles to be taken in reaching full representation for women in science. Over the past three years our GAP has supported 17 one-month fellowships for women, mentoring and gender education. We have met with an enthusiastic response from both men and women and look forward to new activities during 2008.

At the end of the second year we decided to offer two awards of €5 000 each for Outstanding Research by Women in Marine Biological Sciences with an Emphasis on Genomic Processes. Criteria for selection included not only excellence in research but also excellence in teaching, service and outreach to young women in science. The senior category was open to nominees with ten or more years of experience following their PhDs; and the junior award to researchers with <10 years of experience. From a field of 18 top candidates, the committee was faced with a challenging but pleasant task. It is with great pleasure that we announce the winners: Dr. Melody S. Clark, British Antarctic Survey, Cambridge (Sr. award) and Dr. Kristin Tessmar-Raible, EMBL-Heidelberg (Jr. award).

Melody completed both her BSc (1983) and PhD (1988) from the University of London in genetics. During her post-doc years, she worked on MHC and calcium regulation genes in *Fugu* as well as skeletal development. Since 2003 she has held the position of Project Leader of the Antarctic Genomics Facility at BAS. She has published >70 papers and five books. She describes her work as “Icebergs and Enzymes”.

Kristen completed her Diploma (2000) from the University of Heidelberg and her PhD (2004) from the University of Marburg (summa cum laude). She is currently an independent group leader in the Developmental Biology Unit where she focuses on functional genomics and development of sensory-neurosecretory, forebrain cells in *Platynereis* and zebra fish. She has focused on vertebrate eye development and the signalling pathways involved in eye induction. She has published 14 papers. She describes her latest work as, “Ancient brains and lunar periodicity in marine worms”.

Rather than going on here, I will let them speak for themselves.

Melody Clark

Melody Clark award for senior category

It is a great honour to receive this prize from MGE. I hope both the junior and senior awards encourage women not only to strive to achieve in science, but to believe in themselves and enjoy science as a career. Studying science is not an easy option, but the rewards are great: no two days are the same and every-so-often you get that fantastic day with the amazing result you’ve been waiting for. I have always been interested in genetics and now have what I consider to be the best molecular biology job in the World: enzymes and icebergs: what a combination!

But how did I get to where I am today? After obtaining my degree and PhD, both in Genetics, from the University of London in the UK, I took a string of short-term post-doctoral positions developing my skills in a range of areas from plant cytogenetics to the high-profile puffer fish genome project. Since August 2003, I have been a Project Leader with the British Antarctic Survey (BAS) and lead their genomics facility. So from having worked on a single animal (literally!) which has been completely sequenced, I now work on a wide range of Antarctic animals on which there is no sequence data what-so-ever, a real challenge, but one I thoroughly enjoy! >>>

Jeanine Olsen
University of Groningen, Netherlands

My group studies how animals adapt to the extreme cold and how they may react in the face of predicted climate change. We use molecular biology as a tool to answer physiological and ecological questions: real "Environmental Genomics". At the moment we are making the first links between gene expression measured in controlled laboratory experiments and their environmental context in wild populations. In the future I see us being able to accurately predict the effect of climate change on not only individual species, but also whole ecosystems from the microbial through to higher predators. Genomics, in close collaboration with other disciplines, will allow us to do this. Integrated, multidisciplinary research is the future and tomorrow's scientists must be able to see the bigger picture if they want to answer the important questions such as how our world will be affected by climate change.

But how many of tomorrow's top scientists will be women? Very few, given today's statistics. There are role models out there, like Professor Susan Greenfield, Head of the Royal Institution, but for many women scientists, she is an exception, their concerns lie closer to home with families, child-care and short-term contracts. Can we change this? I think so. In my opinion, governments should actively encourage work place crèches, as these are essential to minimise the domestic chaos associated with trying to run a full-time career and a family. We must also address the problem of short-term contracts and lack of career structure. We lose some very talented people with our current system, but it particularly affects women with family commitments. I don't have children and its still not been easy and there is a long way to go, I still have goals I want to achieve. Dogged determination has got me a job I love. Hang on in there and go for it Ladies, do not accept second best!

> « *Integrated, multidisciplinary research is the future and tomorrow's scientists must be able to see the bigger picture if they want to answer the important questions* »

Tessmar-Raible award for junior category

"The nervous system has always fascinated me"

I was absolutely thrilled when I learned that I was selected for the Junior Award for "Outstanding Women in Marine Biological Science Prize" of the Marine Genomics Europe Network of Excellence. I am very grateful to this network, not only for this prize, but also because it has already offered me many opportunities for fruitful scientific exchange during my time as a post-doc, helping me to develop my own scientific profile. The nervous system has always fascinated me, in all its facets. As an undergraduate student, I spent almost a year to investigate the nervous system of the nematode, *Caenorhabditis elegans*, resulting in one of the first studies that demonstrated that the nematode nervous system, despite its simplicity, exhibits left-right asymmetry . Then, I spent roughly another year studying the development of the eye in fish and frog, discovering new molecules that play a role in vertebrate eye and forebrain development. My work on the nematode and on the vertebrates allowed me to look in great detail into specific molecular processes involved in nervous system development. But one issue that puzzled me was how these nervous systems relate to one another, and from which origins the vertebrate nervous system arose. In addition, I knew from my zoology lessons about the huge variety of animal life beyond the classical molecular model species. Therefore, I started to work on the nervous system of a marine worm, *Platynereis dumerilii*. As an annelid, >>>

it represents a large animal group not covered by the most common invertebrate model species, which are insects and nematodes. And indeed, things became very interesting, because I cloned a key photosensory molecule from this worm that was known from vertebrates, but was apparently lost from *Drosophila* and *C.elegans*, helping us to re-interpret eye evolution. Meanwhile, I have discovered similar examples for the neurosecretory system Thus, although switching to a non-standard species comes with many technical challenges, these findings convinced me that *Platynereis* is very well suited to reconstruct original functions of the nervous system.

My future work as an independent group leader will try to address the function of ancient sensory-neurosecretory cell types in the brains of both polychaetes and vertebrates Amazingly, many ancient light- and chemoreceptive neurosecretory cell types are located deep inside the vertebrate brain, but their function is basically unknown. For the polychaete, one hypothesis that I have is that at least one of these cell types is involved in lunar reproductive periodicity. I am therefore also investigating the molecular control of lunar reproductive periodicity and its interplay with circadian and seasonal rhythms, a question for which the marine worm I work on is ideally suited.

Following my path through science, I have often wondered why there are so few female group leaders in biology. Especially in Germany, women in leading scientific positions are astonishingly rare. From my discussions with many other women, I feel that our under-representation often results from problems that couples face when they want to combine two careers with one another (and especially with a family). Most commonly, the person to "step back" in these situations is the woman – a role model that has found especially wide acceptance in the (West) German society. Whereas it is encouraging that many organizations, including the DFG or the Robert Bosch Stiftung, have increased their efforts to support women in Science, I feel that it still requires a general (not only German) change of mind at the employing institutions and grant-giving agencies to address the needs of dual career couples, and unleash the energy that is currently wasted in tedious dual job searches, or in situations where partners commute between different cities. There could be individual solutions, such as dedicated partner fellowships, or institutional solutions that award institutes that actively promote / recruit dual career couples. This will not only ease the difficulties dual career couples are currently facing. It will thereby also foster a new generation of future role models.

> « *I feel that it still requires a general change of mind at the employing institutions and grant-giving agencies to address the needs of dual career couples* »

>>> Bibliography (page 24)

08

MGE Final General Assembly

Faro (Portugal), 12-16 May 2008

For further information on how to participate, please visit the website http://ccmar.ualg.pt/actividade_detalhe.htm?i=66 or contact infoccmar@ualg.pt

Organizing committee:
C. Boyen
I. Arnone
H. Moreau
J. Querellou
E. Lubzens
A. Canario

Main topics:

> Scientific sessions highlighting the major advances made throughout i MGE in comparative, genomics, environmental genomics, and functional genomics across the four nodes (EDD, Fish and Shellfish, Algae and Microbes)
> Scientific sessions around the 4 flagships projects: presentation of main scientific results of these projects and on associated topics
> Cross cutting sessions will be organized for Education and Gender issue
> Outcomes from projects run at the Technology platforms and the Bioinformatics centre
> Future collaboration with other consortia working in marine sciences will be stimulated through an open show case exhibition
> Poster session with a special prize.

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>>> Combined functional genomic and genetic approaches in oyster to identity summer mortality-resistant markers (page 04)

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>>> New technics on technology platform (page 11)

High throughput genomic DNA purification, PCR and fragments analysis reaction setup on microalgae using Macherey-Nagel Nucleospin 96 Plant Kit and Beckman Coulter's Biomek® FX* Laboratory Automation Workstation equipped with ORCA® robotic arm;* M. Borra, E. Mauriello, F. Campili, E. Biffali. Marine Genomics Europe (NoE) Technology platform # 22 SZN-Stazione Zoologica "A. Dohrn" Naples, It
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>>> The MGE-GAP awards two outstanding women in science (page 19)

Selected publications

Clark, M.S; Thorne, A.S; Pura*, J; Grubor-Laj*i*, G; Kube, M; Reinhardt, R; Worland, MW.
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>>> Workshop: New approaches for functional genomics of marine microorganisms

Berlin, June 21-24, 2007 (page 13)

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>>> Workshop on marine genomics: an ocean of techniques

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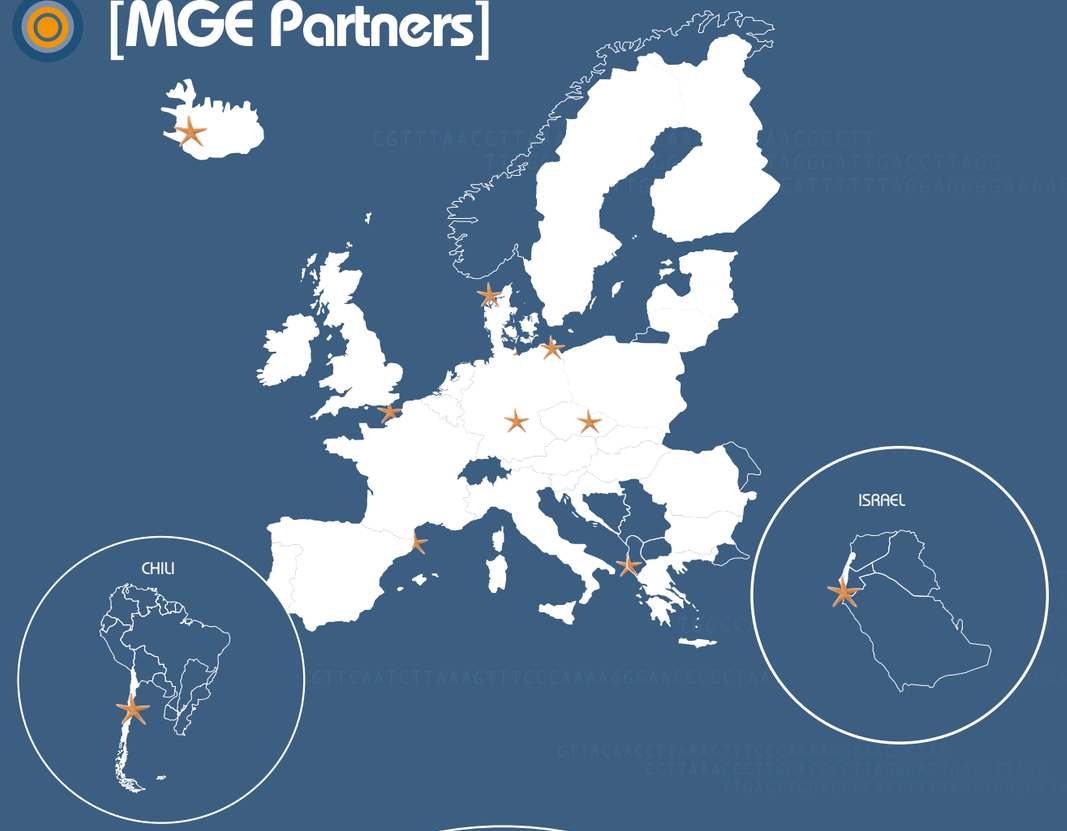

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