ESF Exploratory Workshop on

Heterochromatin structure and function: from repetitive DNA sequences to epigenetics

Donja Stubica (Croatia), 20-23 September, 2008

Convenor:
Miroslav Plohl, Department of Molecular Biology, Ruder Boskovic Institute, Zagreb

Co-convenors:
Barbara Mantovani, Dipartimento di Biologia Evoluzionistica Sperimentale, Università degli Studi di Bologna

Fernando Azorin, Department of Molecular and Cellular Biology, Institute of Molecular Biology of Barcelona

John S. (Pat) Heslop Harrison, Department of Biology, University of Leicester
The European Science Foundation (ESF) is an association of 77 Member Organisations devoted to scientific research in 30 European countries. The Mission of ESF is to provide a common platform for its Member Organisations in order to advance European research and to explore new directions for research at the European level. Through its activities, the ESF serves the needs of the European research community in a global context.

The main objectives of ESF for the years 2006-2010 as defined by its current Strategic Plan are to promote Science Strategy and Science Synergy, paving the way for initiatives across disciplinary and geographic boundaries in the European Research Area (ERA).

The Exploratory Workshops scheme is one of the key instruments of the Science Strategy “pillar”. Each year, ESF supports approximately 50 Exploratory Workshops across all scientific domains. The focus of the scheme is on workshops aiming to explore an emerging and/or innovative field of research or research infrastructure, also of interdisciplinary character. Workshops are expected to open up new directions in research or new domains. It is expected that a workshop will conclude with plans for specific follow-up research activities and/or collaborative actions or other specific outputs either within the frame of ESF (e.g. prepare the ground to develop a Forward Look, a Research Networking Programme or a EUROCORES proposal; publication of a Policy Briefing…) or for submission to the EU 7th Framework Programme or to other European or international funding organisations.

European Science Foundation
1 quai Lezay Marnésia
BP 90015
67080 Strasbourg Cedex
France
Fax: +33 (0)3 88 37 05 32
http://www.esf.org

ESF Exploratory Workshops:

Nina Kancewicz-Hoffman
Scientific Coordinator

Valerie Allspach-Kiechel
Senior Administrator
Tel: +33 (0)3 88 76 71 36

Isabelle May
Administrator
Tel: +33 (0)3 88 76 71 46
Email: ew-office@esf.org
http://www.esf.org/workshops
Main Objectives of the Workshop:

Hardly accessible to genome mapping and sequencing projects, heterochromatic genome compartments still represent the least understood parts of eukaryotic genomes that challenge with their ubiquity and diversity and highlight an emerging area of genome research. Centromeres and surrounding heterochromatin are structurally and functionally distinct, epigenetically determined domains of eukaryotic chromosomes, both enriched in repetitive DNA sequences. A rather complex functional network is depicted to exist in these genomic segments, but even general aspects of function-related traits between DNA and protein components are often controversial and poorly understood.

By gathering groups of experts working on different aspects of (peri)centromeric regions, this workshop aims to offer an interactive atmosphere in which heterochromatin and centromere organization, structure and function will be explored. The main objective is to understand roles that repetitive DNA sequences might have in functional organization and evolution of aforementioned regions. In addition, the potential of different non-conventional experimental organisms for the research in the field will be explored. The meeting is structured to enable exchange of knowledge about heterochromatin and centromeres on the DNA and protein level, discussions, and possible integration of new ideas into future plans. The important objective is to establish mutual collaborations, and in particular we hope to establish a research network which will be focused on different aspects of DNA-protein interactions in heterochromatin, with particular emphasis on studies concerning possible functional interactions related to centromere structure and properties.
ESF LESC Exploratory Workshop:
Heterochromatin structure and function: from repetitive DNA sequences to epigenetics
Donja Stubica (Croatia), 20-23 September 2008

PROGRAMME

Saturday 20 September 2008

Morning
13.00-15.00 Lunch

Opening session (Chair: M. Plohl)
16.00-16.30 Opening and foreword
Miroslav Plohl

Presentation of the European Science Foundation (ESF)
Željko Kućan (ESF Standing Committee for Life, Earth and Environmental Sciences)

16.30-17.30 Introduction of each group (5 minutes)
- Pat Heslop-Harrison
- Jiri Macas
- Thomas Schmidt
- Fernando Azorin
- Teresa Palomeque
- Monica Bullejos
- Philippe Castagnone-Sereno
- Gunter Reuter
- Paul Fransz
- Sarit Cohen
- Andrea Luchetti
- Miroslav Plohl

17.30-17.50 Coffee break

17.50-18.30 Satellite DNAs, chromosomes and heterochromatin
Pat Heslop-Harrison (University of Leicester, UK)

18.30-19.10 Evolution of satellite DNA sequences: stability versus rapid changes
Miroslav Plohl (Ruder Boskovic Institute, Zagreb, HR)

20.00 Dinner

Sunday 21st September 2008

Session 1: DNA aspects of heterochromatin (Chair: S. Cohen, N. Meštrović)

09.00-09.25 Satellite DNAs in non-canonical reproducing systems
Andrea Luchetti (Università degli Studi di Bologna, IT)

09.25-09.50 Extrachromosomal circular DNA and the plasticity of tandem repeats in eukaryotes
Sarit Cohen (Tel-Aviv University, IL)

09.50-10.15 Extrachromosomal circular DNA: Recombination by-product or a key intermediate in evolution of plant satellite repeats?
Alice Navratilova (Institute of Plant Molecular Biology, Cezke Budejovice, CZ)
### Session 1: Heterochromatin structure and function: from repetitive DNA sequences to epigenetics

**ESF LESC Exploratory Workshop:**

**Heterochromatin structure and function: from repetitive DNA sequences to epigenetics**

**Donja Stubica (Croatia), 20-23 September 2008**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.15-10.40</td>
<td>Satellite DNAs in closely related species: the case of Tribolium beetles</td>
<td>Brankica Mravinac</td>
<td>Ruder Boskovic Institute, HR</td>
</tr>
<tr>
<td>10.40-11.00</td>
<td>Coffee break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.00-11.25</td>
<td>Satellite DNAs in Chrysomelidae (Coleoptera)</td>
<td>Pedro Lorite</td>
<td>Universidad de Jaen, ES</td>
</tr>
<tr>
<td>11.25-11.50</td>
<td>Satellite DNA organization and evolution in neotropical cactophilic Drosophila</td>
<td>Gustavo Kuhn</td>
<td>University of Leicester, UK</td>
</tr>
<tr>
<td>11.50-12.15</td>
<td>Constitutive heterochromatin blocks in giant sex chromosomes</td>
<td>Juan Marchal</td>
<td>Universidade de Jaen, ES</td>
</tr>
<tr>
<td>12.15-13.00</td>
<td>Round table: DNA aspects of heterochromatin (Chair: S. Cohen, N. Meštrović)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.00-15.00</td>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.00-15.40</td>
<td>Epigenetic processes controlling heterochromatin differentiation in Drosophila and Arabidopsis</td>
<td>Gunter Reuter</td>
<td>Martin-Luther University Halle-Wittenberg, DE</td>
</tr>
<tr>
<td>15.40-16.20</td>
<td>Centromere identity and function</td>
<td>Fernando Azorín</td>
<td>Institute of Molecular Biology of Barcelona, ES</td>
</tr>
<tr>
<td>16.20-16.50</td>
<td>Genetic and epigenetic consequences of a paracentric inversion in Arabidopsis thaliana</td>
<td>Paul Fransz</td>
<td>University of Amsterdam, NL</td>
</tr>
<tr>
<td>16.50-17.10</td>
<td>Coffee break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.10-17.40</td>
<td>Heterochromatin decondensation in Arabidopsis triggered by light stress</td>
<td>Paul Fransz</td>
<td>University of Amsterdam, NL</td>
</tr>
<tr>
<td>17.40-18.10</td>
<td>The structural organization of major DNA sequences at Beta centromeres</td>
<td>Thomas Schmidt</td>
<td>Dresden University of Technology, DE</td>
</tr>
<tr>
<td>18.10-19.00</td>
<td>Round table: Protein data and epigenetics (Chair: F. Azorin, G. Reuter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.00-21.00</td>
<td>Dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.00-22.00</td>
<td>Round table: Defining a basis for European-scale joint research project I (Chair: Pat Heslop-Harrison, M. Plohl)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Monday 22nd September 2008

### Session 3: Model organisms and genome projects (Chair: T. Schmidt, J. Macas)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.00-09.40</td>
<td>Genome projects and heterochromatin, nematodes and diffuse centromeres</td>
<td>Philippe Castagnone-Sereno (INRA/UNSA/CNRS, FR)</td>
</tr>
<tr>
<td>09.40-10.05</td>
<td>Satellite DNAs and insect models – <em>Tribolium</em></td>
<td>Nevenka Meštrović (Ruder Boskovic Institute, HR)</td>
</tr>
<tr>
<td>10.05-10.30</td>
<td>Repetitive DNAs in bivalve molluscs</td>
<td>Andrea Luchetti (Università degli Studi di Bologna, IT)</td>
</tr>
<tr>
<td>10.30-10.55</td>
<td>Higher order structure of a major centromeric satellite of sugar beet</td>
<td>Gerhard Menzel (Dresden University of Technology, DE)</td>
</tr>
<tr>
<td>10.55-11.15</td>
<td>Coffee break</td>
<td></td>
</tr>
<tr>
<td>11.15-11.40</td>
<td>High throughput genome sequencing as a new tool for global characterization of satellite DNA</td>
<td>Jiri Macas (Institute of Plant Molecular Biology, Ceske Budejovice, CZ)</td>
</tr>
<tr>
<td>11.40-12.05</td>
<td>Satellite DNAs and haplo-diploid insect models</td>
<td>Teresa Palomeque (Universidad de Jaen, ES)</td>
</tr>
<tr>
<td>12.05-12.30</td>
<td>Pericentromeric satellite DNA in a rodent family</td>
<td>Monica Bullejos (Universidad de Jaen, ES)</td>
</tr>
<tr>
<td>12.30-13.05</td>
<td>Round table: Model organisms and genome projects (Chair: T. Schmidt, J. Macas)</td>
<td></td>
</tr>
<tr>
<td>13.05-15.30</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>15.30-17.00</td>
<td>Round table: defining a basis for European-scale joint research project II (Chair: Pat Heslop-Harrison, M. Plohl)</td>
<td></td>
</tr>
<tr>
<td>17.00-17.30</td>
<td>Coffee break</td>
<td></td>
</tr>
<tr>
<td>17.30-19.00</td>
<td>Conclusions and preparation of joint report (Chair: M. Plohl, F. Azorin)</td>
<td></td>
</tr>
<tr>
<td>20.00</td>
<td>Conference Dinner and informal discussions</td>
<td></td>
</tr>
</tbody>
</table>

## Tuesday 23rd September 2008

Morning  
*Departure*
Satellite DNAs, chromosomes and heterochromatin

P. Heslop-Harrison; University of Leicester, Leicester, United Kingdom

Many methods of study of DNA over three decades - ranging from gradient centrifugation, reannealing or denaturation curves and gel separation of restriction digests, to sequence analysis - show the abundance of various forms of satellite DNA in the genomes of all plants and animals. But the variation in nature of these sequences is enormous: examples range from monotonic repeats of single nucleotides, dinucleotides, to 10,000bp motifs; localization at 1 or 100,000 chromosomal locations; specificity to one species to wide distribution over wide taxonomic breadth; presence on one chromosome or on all chromosomes; specificity to contrasting domains or arm classes in chromosomes. Few of these features can yet be related to function, selection, evolution or control, but during this meeting I hope that the comparative approach involving a range of different repeat types, techniques and organisms will allow us to develop new models and paradigms for understanding satellite DNA. Information and references, and soon my presentation, are available from www.molecyt.com.

Evolution of satellite DNA sequences: stability versus rapid changes

M. Plohl; Ruđer Bošković Institute, Zagreb, Croatia

Genomic composition, copy number and/or nucleotide sequence of satellite DNAs is usually a taxon-specific feature. The library model explains the occurrence of these differences by amplification-contraction of satellite families preexisting in a set common for a group of organisms. Despite rapid alterations in copy numbers, nucleotide sequences of some studied satellite DNAs can remain unchanged during extremely long evolutionary periods. Dual character of satellite DNAs, ability for long-time sequence conservation, and at the same time proneness to change their genomic composition can be a general feature of tandem repeats, what might be advantageous in some genomic regions such as in and around centromeres. A model of satellite DNA life cycle will be presented.
Satellite DNA dynamics in non canonical reproductive systems
B. Mantovani, A. Luchetti; Università degli Studi di Bologna, Bologna, Italy

The majority of animals reproduce through gonochorism in an at least presumed panmictic situation. Genetic studies therefore mainly apply to this canonical reproductive strategy. But, leaving aside true asexual reproduction, quite different sexual situations occur in the Kingdom Animalia, from unisexuality to bisexual hermaphroditism, androdioecy and hybridogenesis. A further deviation from a canonical mating system is given by eusocial organisms, where gonochorism takes place, but panmissy is prevented. Obviously, all these different strategies are expected to have a deep impact on nuclear genome dynamics. We will discuss data on satellite DNA variability and evolution obtained in two Hexapoda models, i.e. in the stick insect genus Bacillus (where gonochorism together with unisexuality - either natural or linked to hybridization events - occur) and in the eusocial termite genus Reticulitermes.

Extrachromosomal circular DNA and the plasticity of tandem repeats in eukaryotes
S. Cohen, D. Segal; Tel-Aviv University, Tel-Aviv, Israel

An intriguing aspect of the plasticity of the eukaryotic genome is the formation of extrachromosomal circular DNA (eccDNA). We developed a 2-dimensional gel electrophoresis to characterize eccDNA, and using it we detected eccDNA in Xenopus, Drosophila, rodents, plants and humans. eccDNA contains multimers of chromosomal tandem repeats, coding as well as non-coding sequences, including ribosomal genes. The formation of eccDNA does not require chromosomal DNA replication, may be enhanced by DNA damaging agents and likely involves intra-chromosomal homologous recombination and looping-out. Furthermore, rolling circle intermediates of eccDNA occur in Drosophila and in human cells. These phenomena have implications on the plasticity of chromosomal tandem repeats, including expansion, contraction and homogenization.
Extrachromosomal circular DNA: Recombination by-product or a key intermediate in evolution of plant satellite repeats?

A. Navratilova; Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic

Results of the study focused on detection and characterization of extrachromosomal circular DNA (eccDNA) molecules derived from plant satellites will be presented. EccDNA molecules corresponding to nine different families and three subfamilies of satellite repeats were surveyed in ten species from various genera of higher plants using two-dimensional agarose gel electrophoresis followed by Southern blotting. The results demonstrate that satellite repeat-derived eccDNA is common in plant genomes and thus it can be seriously considered as a potential intermediate in processes driving satellite repeat evolution.

Satellite DNAs in closely related species: the case of *Tribolium* beetles

B. Mravinac; Ruder Bošković Institute, Zagreb, Croatia

The sibling species *Tribolium audax* and *T. madens* are so similar in appearance that for a long time they were classified as a single species. Regarding satellite DNA profiles, their genomes have in common two highly repetitive families with repeating units based on a ~110 bp building element. Analyzed junction fragments between satellite families point to intensive rearrangements of DNA sequences in heterochromatic regions, implying the potential origin of novel satellite (sub)families at the array borders. Comparison of highly repetitive DNAs in the two sibling species suggests an evolutionary scenario where a basic building element changes its sequence as well as higher-order architecture, and concurrently generates different repetitive families within each species. Along with geographic isolation, diverged satellite profiles might facilitate the speciation process.
Satellite DNAs in Chrysomelidae (Coleoptera)

P. Lorite Martinez, T. Palomeque Messia; Facultad de Ciencias Experimentales Universidad de Jaen, Jaen, Spain

Chrysomelidae (leaf beetles) is the second largest family of phytophagous beetles within the order Coleoptera, nevertheless the satellite DNA (satDNA) has been analyzed only in four species. The results show great heterogeneity of the satDNA among species in relation to organization and complexity. In two species there is a single satDNA family composed by homogeneous tandem repeats of a simple unit. In another species, the monomers are organized in three types of repeats; monomers and defined higher-order repeats in the form of dimers or even trimers. The three types of repeats are intermixed in the heterochromatic regions. In the last species there are two unrelated satDNA families. Although both satDNAs are located in pericentromeric regions they are not intermixed and when both families are present in the same chromosome, they are located in different chromosome arms.

Satellite DNA organization and evolution in cactus-breeding *Drosophila* species

G. Kuhn; University of Leicester, Leicester, United Kingdom

The *Drosophila buzzatii* cluster is a monophyletic group comprising seven closely-related neotropical cactus-breeding species. Several repetitive DNAs (including both tandem and dispersed repeats) have been studied in this group. In this talk, I will focus on two satellite DNAs (pBuM and DBC-150). A complex pattern of variation across species was found concerning abundance, chromosomal distribution, long-range organization, homogenization rate, nature and age of homogenized repeats. The data have some similarities but also important contrasts with satellites in the *D. melanogaster* subgroup, which is considered as a paradigm for studies on *Drosophila* satDNA organization and evolution. Details on www.drosophila.molcyt.com.
Pericentromeric satellite DNA in a rodent family

Juan A. Marchal Ortega, A. Sanchez Baca; Facultad de Ciencias Experimentales Universidad de Jaen, Jaen, Spain

The satellite DNA Msat-160 has a monomer unit of 160 bp, it is repeated in tandem and is AT rich. Several studies demonstrated that this satellite was present in the genome of 12 *Microtus* species, but it is absent in another 8 species of this genus and in several arvicoline species belonging to 8 different genera. For this reason it was considered specific for the genus *Microtus*. However, we have recently analyzed this satellite DNA in the genome of species belonging to three other genera of arvicoline rodents: genus *Chionomys*, *Arvicola* and *Pitymys*. The characteristic location of this satellite DNA is maintained among the species analysed, being located mainly in the pericentromeric heterochromatin and in the sex chromosomes heterochromatin. Despite this fact, the autosomal and X chromosome distribution of Msat-160 follows a species specific pattern.

Epigenetic processes controlling heterochromatin differentiation in *Drosophila* and *Arabidopsis*

T. Rudolph, S. Phalke, S. Lein, M.Walther, K. Heidrich, H. Baisch, O. Nickel, I. Hofmann, A. Fischer, K. Irmler, S. Meinelt, D. Jain, G. Reuter; Martin Luther University Halle-Witenberg, Halle (Saale), Germany

In *Drosophila* heterochromatin is established at the end of cleavage in syncytial blastoderm when nuclei show an apico-basal Rabl conformation. In this process histone H3K4 demethylases and histone H3K9 deacetylases play a pivotal role. Clonal analysis shows that the early-determined epigenetic state is afterwards stably maintained during development. An alternative pathway controls retrotransposon silencing. In *Drosophila* DNMT2 dependent DNA methylation during early embryogenesis initiates retrotransposon silencing, which is maintained consecutively by SUV4-20 dependent H4K20 trimethylation.

For genetic dissection of molecular processes controlling heterochromatic gene silencing in *Arabidopsis* we developed a new test system for transcriptional gene silencing (TGS), which is based on T-DNA transgenes containing tandem repeats of the *Luciferease* reporter gene. Altogether 31 TGS suppressor mutations defining 18 different genes were isolated after EMS
mutagenesis. Genetic and molecular analysis revealed a sequence of interdependent molecular processes. Initially H3K9 and symmetric DNA methylation are established followed by asymmetric DNA methylation and processes controlling higher order chromatin structure at the transgenes. Several of the newly identified functions represent heterochromatin-associated proteins and control organisation of chromocenter heterochromatin in *Arabidopsis*. The differences between molecular processes controlling heterochromatic gene silencing in animal and plant systems will be discussed.

**Centromere identity and function**

F. Azorin; Institut of Molecular Biology of Barcelona, CSIC, and Institut for Research in Biomedicine (IRB), Barcelona, Spain.

Centromere identity is regulated epigenetically. The centromere is determined by the formation of a specialized chromatin structure containing the centromere-specific histone H3 variant, cenH3. In this contribution, I will revise the present knowledge on the epigenetics mechanisms that regulate centromere structure and function. In particular, I will address the molecular mechanism(s) accounting for the specific deposition of cenH3 at centromeres and, specifically, the contribution of regulated proteolysis. Here, I will also report on the identification of the molecular determinants linking cenH3 to recruitment of kinetochore proteins.

**Genetic and epigenetic consequences of a paracentric inversion in *Arabidopsis thaliana***

P. Fransz; University of Amsterdam, Amsterdam, Netherlands

Chromosomal rearrangements can affect recombination and gene expression in the neighbouring regions especially when heterochromatin segments are involved. We show that the heterochromatic knob (hk4S) in the short arm of chromosome 4 is the result of a paracentric inversion in the accessions Columbia (Col) and Wassileskija (Ws), covering more than 1200 kb of heterochromatic and euchromatic segments. We have fine-mapped the
borders of the inversion using a combination of pachytene-, DNA fiber- and interphase-FISH and examined meiotic recombination frequencies and epigenetic features in the rearranged area and flanking regions. The extremely low sequence variation in the inversion fragment between Col and Ws, compared to flanking regions suggest that outcrossing rather than mutation events is responsible for the overall polymorphism between the two ecotypes. The epigenetic data indicate that chromatin structure and gene expression profile are hardly affected by the paracentric inversion.

Heterochromatin decondensation in *Arabidopsis* triggered by light stress

P. Fransz; University of Amsterdam, Amsterdam, Netherlands

Our goal is to understand the structure, formation and function of heterochromatin in *Arabidopsis thaliana*. Heterochromatin in this model system is confined to 6-10 discrete chromocenters, which display most heterochromatin characteristics. We observed dramatic changes in chromocenter condensation under several biotic and abiotic conditions. For example, a major reduction (75%) in heterochromatin compaction occurred, when the light intensity decreased to 10%. Strikingly, decondensation of heterochromatin appeared reversible, since raising the light intensity to original levels restored normal values of the heterochromatin fraction. The extent of chromatin reduction appeared dependent on the ecotype and the light regime. Using mutant and QTL analysis we discovered photoreceptors (CRY2, PHYB) and a chromatin modifier (HDA6) to be involved in light-dependent chromatin compaction.

The long-range organization of *Beta* centromeres

T. Schmidt; Dresden University of Technology, Dresden, Germany

The DNA composition of plant centromeres is variable, however, their structural sequence organization follows similar rules. We analyzed the long-range organization of the centromeric DNA of *Beta vulgaris* mutants which carry a monosomic chromosome fragment derived from *Beta procumbens* or *Beta patellaris*. These chromosome fragments resemble
minichromosomes with centromere activity and provide an experimental system for the molecular analysis of individual plant centromeres. Comparative physical mapping of the fragment centromeres included BAC analyses and FISH on pachytene chromosomes and fibre-FISH. Minichromosome centromeres have a complex structure and consist of repetitive sequences including different satellite DNAs and centromere-specific Ty3-gypsy retrotransposons. In particular, the Ty3-gypsy retrotransposon Beetle1 is highly amplified and a major component of the centromere.

Structure and dynamics of the root-knot nematode genome, from satellite DNA to whole-genome sequence.

P. Castagnone-Sereno; Interactions Biotiques et Santé Végétale INRA/UNSA/CNRS, Sophia Antipolis, France

Root-knot nematodes (Meloidogyne spp.) are polyphagous crop pests of major worldwide importance, exhibiting extreme diversity in terms of reproductive mode (from amphimixis to mitotic parthenogenesis) and chromosomal complement (diploid and polyploid forms). Our research interest focuses on the structure and dynamics of their genome (with emphasis on satellite DNA sequences) in relation to i) the cytogenetic diversity displayed within this genus and ii) the high adaptive ability of these organisms. The very recent achievement of the sequencing and annotation of the M. incognita genome will no doubt help elucidating fundamental aspects of the biology and evolution of this ubiquitous parasite.

Satellite DNAs and insect models – Tribolium

N. Meštrović; Ruder Bošković Institute, Zagreb, Croatia

The genome of the red flour beetle Tribolium castaneum was recently sequenced and draft assembly that represent about 70 % of the genome was produced. In silico analysis of the abundance and distribution of repetitive DNA revealed that approximately 30% of the assembled genome is composed of repetitive DNA (Wang et al, Genome Biology 2008, 9:R61). In assembled genome satellite DNAs build only 2.5%. This results is in contrast with
Experimental data revealed that highly abundant (peri)centromeric 360 bp satellite makes up to 17% of the genome. It has been proposed that “unknown” part of the genome represents regions of highly repetitive DNA that could not be assembled. Understanding the organization and features of this kind of sequences can help in efforts to gain a complete picture of genome structure. I will present our preliminary in silico and experimental analysis of new satellite DNAs identified from reptiles that failed to assemble into scaffolds. In addition, a comprehensive analysis of 360 bp satellite DNA described previously will be presented in the light of new data.

**Long-term conservation of a satellite DNA library in the Class Bivalvia**

**A. Luchetti; Università degli Studi di Bologna, Bologna, Italy**

The “library” hypothesis explains why related taxa could share a number of repetitive DNA sequences that may have been species-specifically amplified, but the evolutionary dynamic of satellite DNA “libraries” are still debated and poorly understood. A promising animal model is given by the class Bivalvia. The occurrence of a transposon-related, transcribed satellite DNA family across the Class indicates an exceptional antiquity of these tandem repeats (~540 Myr). Monomers are mostly unchanged between species, apart some group of repeats and sequences from Pterioida and Myoida that are monophyletic.

**Higher-order structure of a major sugar beet (Beta vulgaris) centromeric satellite**

**G. Menzel; Dresden University of Technology, Dresden, Germany**

Structural differences of the monomer of a major sugar beet (Beta vulgaris) centromeric satellite are caused by size variations as well as an interspersion of a non-homologous repetitive motif. Both repeats form higher-order satellite units, which are exclusively present in B. vulgaris and closely related wild beets. Fluorescent in situ hybridization (FISH) on metaphase and interphase chromosomes of these species reveals different degrees of centromer-specific amplification of the higher order units on a subset of chromosomes, thus indicating the diversification of centromeric satellites during species radiation.
High throughput genome sequencing as a new tool for global characterization of satellite DNA

J. Macas; Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic

The lecture will focus on utilization of highly parallel sequencing data for identification and comprehensive characterization of satellite DNA families in complex eukaryotic genomes. It will provide an overview of advantages of the second generation sequencing technologies over conventional, cloning-based repeat sequencing and analysis, and demonstrate their efficiency for satellite repeat characterization in several higher plant genomes.

Satellite DNAs and haplo-diploid insect models

T. Palomeque Messia, P. Lorite Martinez; Facultad de Ciencias Experimentales Universidad de Jaen, Jaen, Spain

In the haplo-diploid insects, unfertilized eggs develop as haploid males, and fertilized eggs develop as diploids females. The Hymenopteran ants, whose satellite DNA (satDNA) is been studied by our group, are haplo-diploids. It has been suggested that the processes of molecular evolution of the satDNA would be altered by the special constrain imposed by the haplo-diploid system. The satDNA of ants shows a relative lack of homogenization and fixation which could be due to the haplodiploidy. In this system, the mutation rate in haploid males could counteract the efectiveness of the genome-turnover mechanism. The existence of a motif similar to CENP-B box and the existence of transposable elements inserted into the satDNA are also features of satDNA of ants. Finally, the transcription of satDNA appears to be a general phenomenon in Hymenoptera, although the role of the transcripts is unknown.
Constitutive heterochromatin blocks in giant sex chromosomes

M. Bullejos Martin; Facultad de Ciencias Experimentales Universidad de Jaen, Jaen, Spain

The genus *Microtus* is a very interesting mammalian group owing to the characteristic sex chromosomes described in some of them. In fact, four species (*M. cabrerae, M. agrestis, M. chrotorrhinus* and *M. rossiaemeridionalis*) have extremely large X and Y chromosomes, known as “giant”, due to the presence of enlarged blocks of constitutive heterochromatin. Thus, the X chromosome of *M. agrestis*, the longest found in mammals, comprises 20% of the genome, whereas the X chromosome of *M. cabrerae* represents nearly 15% of the genome. Several repeated sequences, mainly satellite DNAs, middle repeated sequences of complex organization or genetic mobile elements, have been described in the heterochromatic blocks of the giant sex chromosomes of this group. These sequences show a high number of interspecific variations among sex chromosomes, suggesting high complexity, in composition, distribution and organization, and an independent origin for the sex heterochromatin in each *Microtus* species.
List of Invited Participants

**Convenor:**
1. Miroslav PLOHL  
   Department of Molecular Biology  
   Rudor Boskovic Institute  
   Bijenicka cesta 54  
   10002 Zagreb  
   Croatia  
   plohl@irb.hr

**Co-Convenors:**
2. Fernando AZORIN  
   Department of Molecular and Cellular Biology  
   Institute of Molecular Biology of Barcelona  
   CSIC  
   c/Josep Samitier, 1-5  
   08028 Barcelona  
   Spain  
   fammbmc@ibmb.csic.es

3. John Seymour (Pat) HESLOPHARRISON  
   Department of Biology  
   University of Leicester  
   University Road  
   Leicester LE1 7RH  
   United Kingdom  
   phh4@le.ac.uk

4. Barbara MANTOVANI  
   Dipartimento di Biologia Evoluzionistica Sperimentale  
   Università degli Studi di Bologna  
   Via Selmi 3  
   40126 Bologna  
   Italy  
   barbara.mantovani@unibo.it

**ESF Representative:**
5. Željko KUČAN  
   Department of Chemistry  
   Faculty of Science  
   University of Zagreb  
   Horvatovac 102 a  
   1000 Zagreb  
   Croatia  
   zkuican@chem.pmf.hr

**Participants:**
6. Eva ŠATOVIĆ  
   Department of Molecular Biology  
   Rudor Boskovic Institute  
   Bijenicka 54  
   10002 Zagreb  
   Croatia  
   esatovic@irb.hr

7. Monica BULLEJOS MARTIN  
   Departamento de Biologia Experimental,  
   Area de Genetica  
   Facultad de Ciencias Experimentales  
   Universidad de Jaen  
   Campus Las Lagunillas S/N  
   23071 Jaen  
   Spain  
   bullejos@ujaen.es

8. Philippe CASTAGNONE-SERENO  
   Interactions Biotiques et Sante Vegetale  
   INRA/UNSA/CNRS  
   Route des Chappes 400  
   06903 Sophia Antipolis  
   France  
   Philippe.Castagnone@sophia.inra.fr

9. Sarit COHEN KEDAR  
   Molecular Microbiology & Biotechnology Life Sciences  
   Tel-Aviv University  
   Ramat Aviv  
   69978 Tel-Aviv  
   Israel  
   scohen@post.tau.ac.il

10. Constanze FIEGE  
    Cell and Molecular Biology  
    Institute of Botany  
    Dresden University of Technology  
    Zellescher Weg 20b  
    01217 Dresden  
    Germany  
    constanze.fiege@mailbox.tu-dresden.de

11. Paul FRANSZ  
    Swammerdam Institute for Life Sciences  
    University of Amsterdam  
    Kruislaan 318  
    1098 SM Amsterdam  
    Netherlands  
    franz@science.uva.nl
12. Gustavo KUHN
Department of Biology
University of Leicester
University Road
Leicester LE1 7RH
United Kingdom
gcskuhn@rge.fm.rp.usp.br

13. Pedro LORITE MARTINEZ
Departamento de Biologia Experimental, Area de Genetica
Facultad de Ciencias Experimentales
Universidad de Jaen
Campus Las Lagunillas S/N 23071 Jaen
Spain
plorite@ujaen.es

14. Andrea LUCHETTI
Dipartimento di Biologia Evoluzionistica Sperimentale
Università degli Studi di Bologna
Via Selmi 3
40126 Bologna
Italy
andrea.luchetti@unibo.it

15. Jiri MACAS
Biology Centre ASCR
Institute of Plant Molecular Biology
Branisovska 1160/31
37005 Ceske Budejovice
Czech Republic
macas@umbr.cas.cz

16. Nevenka MEŠTROVIĆ;
Department of Molecular Biology
Ruder Boskovic Institute
Bijenicka 54
10002 Zagreb
Croatia
nevenka@irb.hr

17. Gerhard MENZEL
Plant Cell and Molecular Biology
Institute of Botany
Dresden University of Technology
Zellescher Weg 20b
01217 Dresden
Germany
Gerhard.Menzel@tu-dresden.de

18. Valentina MINGAZZINI
Dipartimento di Biologia Evoluzionistica Sperimentale
Università degli Studi di Bologna
Via Selmi 3
40126 Bologna
Italy
vmingazzini@tiscali.it

19. Brankica MRAVINAC
Department of Molecular Biology
Ruder Boskovic Institute
Bijenicka 54
10002 Zagreb
Croatia
brankica@irb.hr

20. Alice NAVRATILOVA
Biology Centre ASCR
Institute of Plant Molecular Biology
Branisovska 1160/31
37005 Ceske Budejovice
Czech Republic
navratil@umbr.cas.cz

21. Teresa PALOMEQUE MESSIA
Departamento de Biologia Experimental, Area de Genetica
Facultad de Ciencias Experimentales
Universidad de Jaen
Campus Las Lagunillas S/N
23071 Jaen
Spain
tpalome@ujaen.es

22. Gunter REUTER
Institute of Biology/Genetics
Martin Luther University Halle-Wittenberg
Weinbergweg 10
06120 Halle (Saale)
Germany
reuter@genetik.uni-halle.de

23. Juan Alberto MARCHAL ORTEGA
Departamento de Biologia Experimental, Area de Genetica
Facultad de Ciencias Experimentales
Universidad de Jaen
Campus Las Lagunillas S/N
23071 Jaen
Spain
jamaor@ujaen.es
24. Thomas SCHMIDT  
Plant Cell and Molecular Biology  
Institute of Botany  
Dresden University of Technology  
Zellescher Weg 20b  
01217 Dresden  
Germany  
Thomas.Schmidt@tu-dresden.de

25. Daniel SEGAL  
Molecular Microbiology & Biotechnology  
Life Sciences  
Tel-Aviv University  
Ramat Aviv  
69978 Tel-Aviv  
Israel  
dsegal@post.tau.ac.il