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PLENARY LECTURE

THE ROLE OF STRUCTURAL GENOMIC VARIANTS ACCURATELY IDENTIFIED FROM WHOLE GENOME SEQUENCES

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As more whole genomes become sequenced, the challenge remains to accurately identify structural genomic variants and to deduce the role of these genetic variants in human pathology and genome evolution. In whole-genome sequences of different tumors, somatic insertions of transposable elements (and other types of structural variants) highlight their potential impact in tumorigenesis. In non-human primate genomes, structural genomic variants can dramatically alter the architecture of specific species' genomes. Moreover, transcriptome analyses across nonhuman primates and humans demonstrates significant effects of species-specific gene duplications on gene expression. Indeed, certain inter-species gene duplications coincide with species-specific gain of expression in a new tissue, implicating these duplications in function acquisition

CHROMOSOME STRUCTURE AND FUNCTION

LINKING CHROMOSOME STRUCTURE TO THE ORGANISATION OF S PHASE IN HUMAN CELLS

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Eukaryotic DNA synthesis is regulated with exquisite precision so that genomes are replicated exactly once before mitosis and cell division occurs. The size and structure of mammalian genomes requires that the initiation of DNA synthesis is activated at about 50,000 sites – the average inter-replicon spacing is ~120kbp - in order to complete synthesis within an S phase that is typically 9-10 hours in length. During the process of DNA synthesis, different regions of the genome are replicated at different but broadly predictable times. Such patterns of replication timing, which define a temporal program during S phase progression, are seen to alter during cell differentiation and correlate with establishing different classes of chromatin on DNA that is replicated at different times of S phase.
In understanding this process, significant progress has been made in recent years using population based studies and 'omics' technologies – e.g., advanced sequencing and chromatin immuno-precipitation. However, as the selection of origins for activation at different times of S phase is known to be significantly probabilistic in nature in mammals we have adopted a suite of single cell approaches to compliment the population studies. By analysing individual chromosomes, within living cells, and replicon organisation on spread DNA fibres we have shown that a major mechanism regulating transitions in the S phase timing program involves the sequential activation of replication domains based on their genetic continuity. Our analysis shows that replicons are generally activated in small clusters, which represent the higher order units of DNA synthesis – replicons are typically ~120kbp in somatic human cells and most clusters contain 500-1000kbp of DNA. These ~1Mbp regions of DNA also correlate with major units of higher order chromosome organisation, which can be visualised as DNA foci within individual chromosome territories and seen as stable entities using genome-wide analysis of chromatin interactions. To expand these experimental studies, we have also used *in silico* approaches to explore how a complex composite of genomic features links chromosome structure, at a range of scales, to fundamental principles of nuclear function - such as DNA sequence, chromatin epistates and the location of different protein molecules bound to DNA. Together these studies show how a complex composite of interactions defines both chromosome structure and the efficacy of genome duplication in mammalian cells.

CELL-LINEAGE-SPECIFIC CONSEQUENCES OF CONDENSIN II-DEFICIENCY ON PLOIDY AND HAEMATOPOIETIC DEVELOPMENT

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Chromosomal Instability (CIN) and aneuploidy are common features in most human tumours, but their prevalence during normal development is less clear. We profiled ploidy during normal development of mouse B, T and erythroid cells, and uncovered marked variability in the frequency of cells with abnormal DNA content both within and between lineages. Frequencies of aneuploidy were elevated during stages of development characterised by rapid cellular proliferation but decreased at subsequent stages, suggesting that aneuploid cells experience a competitive disadvantage. To assess the consequences of elevated CIN during development, we have used mice carrying germline hypomorphic mutations in Caph2, a component of the chromosome restructuring complex condensin II. Homozygous mutants were viable, fertile, and of normal size, but accumulated cells with abnormal DNA content in several cell lineages. The effects on haematopoietic development were remarkably tissue-specific: B cell and erythroid lineages were largely unaffected, whereas $CD4^+CD8^+$ thymic T cells were reduced in number by two orders of magnitude. This developmental block could not be attributed to major abnormalities in transcription or interphase chromosome structure. Instead, thymocytes showed defects in both proliferation and differentiation arising from failed cytokinesis, and a build up of tetraploid, non-cycling cells. Concomitant deletion of p53 restored thymic cellularity, but led to the rapid development of aneuploid thymic lymphomas. P53 mutations had no detectable effect on the development of Caph2-deficient B cells or erythrocytes. These data provide a systematic analysis of ploidy across a cellular differentiation hierarchy, and demonstrate that thymocytes are uniquely vulnerable to condensin II deficiency. We uncover a stage-specific p53 checkpoint that protects from CIN-induced thymic lymphoma at the expense of thymic T-cell output.

GENOMIC COMPOSITION AND TURNOVER OF SATELLITE DNA REPEATS

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Eukaryotic genomes are to a large extent structured by repetitive sequences and remodeled by their evolution. Among them, satellite DNAs, non-coding DNA sequences repeated in tandem, are principal DNA components of heterochromatic chromosomal regions but can also appear as single units or as short arrays interspersed in euchromatic portions of the genome. Each genome can harbor many satellite DNAs, different in sequence and their origin, copy number and genomic distribution. Rapidly achieved dramatic interspecies differences in genomic content of satellite DNAs are, according to the library model, driven by expansions and contractions within a set of sequences common for a group of species. This phenomenon does not necessarily include dramatic alterations in satellite DNA sequences, which can remain well-conserved even during long evolutionary periods. Ability of satellite DNAs to persist in sequence and change rapidly in copy number may guarantee long-term stability of interactions involved in some basic functions of heterochromatic chromosomal segments, such as in and around centromeres, where rapid adjustments of DNA-protein interactions might be crucial for chromosomal integrity and function. The idea that mechanisms related to transposition may determine extensive satellite DNA array rearrangements is supported by sequence similarity shared between some transposable elements and satellite DNAs. For example, tandem repeats can be found as constitutive modules within a class of non-autonomous mobile element MITE (miniature inverted repeat transposable element), and related tandem repeats can appear in satellite DNA arrays. These features indicate that two distinct classes of repetitive sequences, satellite DNAs and mobile elements, are intertwined in a complex network of interrelated sequences and processes that eventually determine repetitive setup of the genome.

NONCODING RNA AND CENTROMERE STABILITY

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The centromeres of most multicellular eukarya consist of highly repetitive DNA composed of large arrays of simple satellites and transposable elements, rendering them intractable to traditional sequencing technologies and assembly algorithms. The demonstrated lack of conservation of centromeric sequences, even among closely related species, suggests that the genomic component of eukaryotic centromeres is relatively rapidly evolving, while function is preserved by conserved epigenetic components. Mounting evidence supports the hypothesis that an RNA component is a crucial part of the epigenetic cascade responsible for centromere, and thus chromosome, stability. Our work in mammalian models spanning all three mammalian infraclasses has identified centromere specific RNA sequences that are processed into a specific class of small RNAs, larger than previously described small RNA classes. We have developed a toolkit that includes bioinformatic, genomics and functional assays to both fully characterize this class of small RNAs and define the role these small RNAs play in centromere stability. Combining our evolutionary studies in a marsupial system and functional assays in an engineered human cell culture system, we have determined that this new class of small RNAs directly affects loading of newly synthesized CENP-A and is a critical component of the centromere assembly cascade.

NEOCENTROMERE AND CENTROMERE EVOLUTION

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In recent years we have used large panels of BAC clones to track the evolutionary history of chromosomes in primates. This approach has disclosed an unprecedented phenomenon: the "centromere repositioning", that is the movement of the centromere along the chromosome without marker order variation. Repositioned centromeres are relatively frequent. In macaque, for instance, 9 out of 21 centromeres are evolutionarily new; in donkey at least 5 such neocentromeres originated after its divergence from zebra (less than 1 million years). A related phenomenon (clinical neocentromeres) has been reported in human clinical cases. Clinical neocentromeres are analphoid centromeres that emerge in ectopic chromosomal regions. Usually they stabilize supernumerary acentric chromosomes which have detrimental phenotypic consequences. Studies on the evolution of the chromosomes where clustering of neocentromeres were reported (3q, 13q, and 15q for instance) disclosed distinct, intriguing relationships between human clinical neocentromeres and evolutionary neocentromeres. Additionally, examples are now available of centromere repositioning events in humans, disclosed by chance because they do not result in phenotypic abnormalities. In 1976 Seuanez et al. described, in the oragutan population, an inversion of chromosome 9 (human 12) polymorphic in the population. Our studies demonstrated that the inversion was actually a further example of a centromere repositioning event.

ARCHITECTURE AND FUNCTIONAL DYNAMICS OF SATELLITE-LESS CENTROMERES IN THE EQUID MODEL SYSTEM

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Centromeres in vertebrates are typically associated with extended arrays of satellite DNA repeats and human clinical neocentromeres seemed to represent the only exception to this rule. The repetitive nature of normal mammalian centromeres hampered a detailed molecular dissection of their function.
We discovered that the centromere of horse chromosome 11 is completely devoid of satellite DNA [1]. This perfectly functional centromere is present in all horses and has been stably inherited over millions of years. We then showed that, in the genus *Equus* (horses, asses and zebras), centromeric function and satellite DNA are often uncoupled [2]. Several centromeres in this genus are similar to that of horse chromosome 11, consisting of unique sequences without detectable satellite DNA. The non-repetitive nature of these centromeres and the availability of the complete sequence of the horse genome make them an ideal system to analyse centromere architecture and plasticity at the molecular level. Recent results on the functional organization of equid satellite-less centromeres will be presented.

[1] Wade et al, Science 2009, 326: 865-867.
[2] Piras et al, PLoS Genet 2010, 6: e1000845.

SIZING THE GENETIC AND PHYSICAL CENTROMERE OF BARLEY CHROMOSOMES AS A PREREQUISITE OF UNDERSTANDING GENOME EVOLUTION IN THE GENUS *Hordeum*

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Genome characterization of agricultural important species such as barley (*Hordeum vulgare*), requires knowledge of the correlation between the genetic and physical maps. Genetic maps are based on recombination values and often show different positions of molecular markers in comparison to physical maps, particularly in the centromere that is generally poor in meiotic recombinations.
In order to improve the resolution of the physical map of barley in the centromeric and pericentromeric regions we use fluorescence *in situ* hybridization (FISH) to order 100 repeat-free single-copy sequences that were genetically assigned to a 10 cM bin around the centromere of 3H of *Hordeum vulgare*. Single-copy probes have been generated by the Kmasker, a tool for *in silico* prediction of single-copy sequences for the large genome species.Twenty one out of twenty two contig-specific single-copy probes, with an average length of 7 kb, marked about 54% of chromosome 3H. Furthermore, the established order of these probes on barley chromosome 3H will be used to test their order in related *Hordeum* species.
Physical or cytological mapping of predefined sequences emerged as a feasible strategy to correct the discrepancy between physical and genetic centromere of barley chromosomes.

CHROMOSOME OBSERVATION BY SCANNING ELECTRON MICROSCOPY USING IONIC LIQUIDS

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The continuous and comprehensive studies on chromosome higher-order structure are necessary to answer the enigma of how metaphase chromosome is constructed. The utilization of electron microscopy to visualize chromosome higher-order structure is somehow limited because of the time-consuming and multiple preparation method required for biological sample observation. Besides the requirement of drying and dehydration which could affect the biological structure, the necessity to coat biological sample by metal or carbon would also cover the real structure of the samples. The development of a new method enabling observation of biological samples without dehydration and metal/carbon coating would be a powerful tool for the biological microscopy. Ionic liquids are a class of ionic solvents that possess advantageous properties of current interest in a variety of interdisciplinary areas of science. By using ionic liquids, biological samples need not be dehydrated or metal-coated. In this study, we have investigated chromosome higher-order structure by using ionic liquids by electron microscopy and evaluated the factors that affect visualization of chromosome higher-order structure.

As a results pre-warmed, well-mixed, and low concentration (0.5~1.0%) ionic liquids provide well-contrasted images, especially when using the more hydrophilic and the higher purity one (1-butyl-3-methylimidazolium tetrafluoroborate). Image contrast and resolution are enhanced by the combination of ionic liquid and platinum blue staining, the use of an Indium Tin Oxide membrane, osmium tetraoxide-coated coverslip, or aluminum foil as substrate, and the adjustment of electron acceleration voltage.

We conclude that the ionic liquid method is effective for the visualization of chromosome higher-order structure by scanning electron microscopy even without dehydration or metal coating.

ASURA (PHB2) INTERACTS WITH SCC1 THROUGH CHROMATIN

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PHB2 (ASURA) is a highly conserved protein. ASURA localizes at multiple cellular compartments, including the cell membrane, mitochondria, and nucleus, providing diverse cellular functions. In our previous work elucidating chromosome higher order structure, we identified ASURA in isolated human metaphase chromosomes (Uchiyama et al., 2005). Further analysis revealed that ASURA knockdown causes precocious sister chromatid separation at prometaphase, marked by loss of centromeric Scc1 without affecting Sgo1 localization (Takata et al., 2007). Nevertheless, the mechanism by which ASURA protects sister chromatid cohesion remains unclear

Present co-immunoprecipitation analysis reveales ASURA interacts with cohesin subunit Scc1 *in vivo*. We show that ASURA associates with chromatin in a similar manner as Scc1 throughout the cell cycle. Furthermore our observation using the Fucci (Fluoroscent ubiquitination-based cell cycle indicator) system indicates that ASURA is important for cohesin maintenance at early mitosis and exhibited different phenotype compared to Sororin (Cohesin establisment protein). We have also identified that the conserved PHB domain is responsible for chromatin targeting of ASURA. Our results suggest that the regulation of sister chromatid cohesion is mediated by ASURA binding to chromatin, where ASURA might be involved in cohesin protection through ASURA-Scc1 interactions.

We have demonstrated that ASURA interacts with cohesin subunit Scc1. We have further showed that ASURA specifically binds to hromatin when Scc1 is associated with chromatin. Our Fucci analysis indicates that ASURA is required for cohesin maintenance at mitosis but not at interphase. Taken together, we propose that ASURA specifically binds to chromatin to directly protect cohesin dissociation from the prophase pathway until mitosis by a mechanism independent from that of Sgo1.

FLIM AS A TECHNIQUE FOR PROBING THE STRUCTURE OF HUMAN METAPHASE CHROMOSOMES

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The higher order structure of chromosomes remains unclear today. Staining increases the contrast of chromosomes under different imaging techniques. Differential staining along the length of a chromosome results in a characteristic striped appearance called "banding". The characteristic banding pattern of each chromosome allows the identification of the chromosomes and provides an insight on the chromosomal substructures wherein heterochromatin and euchromatin can be identified. Traditionally, the contrast between the bands is based on the intensity of the stain. In this study, the sensitivity of a fluorochrome's lifetime to the change of chromosome environments associated with banding was explored using fluorescence lifetime imaging (FLIM). Human metaphase chromosomes fixed in methanol:acetic acid were prepared on glass slides and were stained with various concentrations of DAPI (4',6-diamidino-2-diphenylindole). Multiphoton FLIM was then used to measure the DAPI lifetime.

Variations in the DAPI lifetime along the length of the chromosomes were observed. Two components of the lifetime were observed at high DAPI concentration, which can be attributed to two different binding modes of DAPI: minor groove binding at AT-rich regions and external binding. The lifetime patterns for the chromosomes were obtained and the same pattern was obtained for the same chromosome. It was observed that these patterns correspond to R-banding pattern. This means that the regions where the fraction of shorter lifetime component is higher and the lifetime values of both the short and long components are higher correspond to the GC-rich regions of the chromosome. This suggests that at the GC-rich regions: 1) there is less protection of DAPI from solvent quenching, and 2) there is less compaction of chromatin and thus, self-quenching is less likely.

The results obtained signify that FLIM can be used as a tool to study chromatin compaction in chromosomes.

QUALITATIVE AND QUANTITATIVE B CHROMOSOME GENOTYPING IN THE CICHLID FISH *Astatotilapia Latifasciata*

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B chromosomes, also known as accessory, extra or supernumerary chromosomes, are additional chromosomal elements found in a high diversity of eukaryote groups including fungi groups, plants and animals. These chromosomes are very diverse in size, behavior, composition and also mechanisms of emergence. Despite thousands of reports describing the distribution of supernumeraries, a comprehensive theory for the origin, maintenance and evolution of B chromosomes has not emerged, and new studies are necessary. In order to analyze the B chromosomes of the cichlid fish *Astatotilapia latifasciata* (that possess 0, 1 or 2B chromosomes) we develop PCR- and qPCR-based markers for this chromosome using 454 sequencing data of microdissected B and Illumina whole genome sequencing data generated for B- and B+ animals.
The sequencing results for the microdissected B chromosome resulted in 125,601 reads (~0.8X coverage of the B chromosome). Such data were aligned to other cichlid species and regions with B-variant nucleotides were used to design specific primers to the B chromosome DNA. The primers were designed in the way to have two amplified fragments for B+ samples, and only the control fragment for the B- samples. The PCR maker only detected the B presence/absence. The Illumina data comprised in a total of 401,017,570 reads for the B- samples and 306,823,512 reads for the B+ samples. Comparing the two Illumina datasets, genomic regions with higher coverage in the B+ genome were selected to design primers to be used in qPCR reactions in order to have the gene-dose-ratio value for 0, 1 and 2B animals. The qPCR markers clearly discriminated 0B, 1B and 2B samples. The DNA markers identified were confirmed by FISH-mapping.
The analyzes of B chromosomes genomic content based in the next-generation approach represents a very helpful strategy to obtain markers to detect the B chromosome presence/absence and dosage in the sample of interest. Furthermore, the qPCR also gives information in the copy number of a specific DNA fragment observed in the B.

DISTRIBUTION OF HISTONE H4K5AC IN PLANTS WITH DISTINCT PATTERNS OF PROPHASE CHROMOSOME CONDENSATION AND DIFFERENT GENOME SIZES

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Euchromatic regions are typically hyperacetylated in relation to heterochromatic ones, but less is known about the difference in acetylation intensity between early and late condensed prophase chromatin. In general, species with high DNA amount display uniformly condensed prophase chromosomes whereas those with low DNA amount exhibit an early condensing chromatin (including C-bands) and a late condensing one. Here, we investigated the distribution of H4K5ac in 14 plant species with different genome sizes and prophase chromosome condensation patterns. Chromosomes acetylation level was immunodetected with Upstate primary antibody against H4K5ac and FITC-conjugated secondary antibody. Giemsa and CMA/DAPI staining were used to observe the prophase condensation and heterochromatin patterns, respectively, and flow cytometry was used to estimate the genome size.

We found out that species with small genome size (2C <3 pg) and low average amount of DNA per chromosome (<0.5 pg) (*Bixa orellana, Indigofera hirsuta, Phaseolus lunatus, P. vulgaris, Solanum lycopersicum, Eleutherine bulbosa, Costus spiralis, Emilia sonchifolia* and *Poncirus trifoliata*) displayed late condensed chromatin highly enriched in H4K5ac and early condensing chromatin poorly acetylated. Species with large genomes (1C> 6 pg) and high DNA amount per chromosome (> 1 pg) (*Allium cepa*, *Callisia repens* and *Nothoscordum pulchellum*), had prophase chromosomes uniformly enriched in H4K5ac. Exceptions to these trends were the two species of this sample with holokinetic chromosomes, *Rhynchospora pubera* (2n = 10; 2C = 3.29 pg) and *R. tenuis* (2n = 4; 2C = 0.78 pg), which displayed small genomes but their chromosome condensation and acetylation pattern were similar to those of species with large genomes. Heterochromatic blocks, detected as brilliant CMA or DAPI regions, were always hypoacetylated whereas euchromatic regions could be hypo or hyperacetylated, depending on their condensation timing during prophase.

These results indicate that in species with unevenly condensed prophase chromosomes (usually with small genome size) hypoacetylation is characteristic of all early condensed chromatin, including the heterochromatin, whereas hyperacetylation marks the late condensed chromatin. Differently, species with uniformly condensed prophase chromosomes (those with large chromosomes or holokinetic ones) are

uniformly acetylated, with labeling intensity slightly lower than the late condensed euchromatin of the formers. The uniform labeling is possibly due to the presence of small fractions of hyperacetylated chromatin interspersed with hypoacetylated ones throughout the chromosome. The meaning of the exception found in *Rhynchospora* species is not clear but it may have to do with the holokinetic nature of their chromosomes.

CLASSIFICATION AND ANNOTATION OF LARGE TANDEM REPEATS IN ASSEMBLED GENOMES

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The tandemly repeated DNA forms a significant portion of eukaryotic genomes. The large tandem repeats including satellite DNA are the main component of centromeric and pericentromeric regions that are mostly unassembled. The incomplete characterization of large tandem repeats and satellite DNA limits experimental studies. Here, I present an approach to classification and annotation of large tandem repeats with genome assemblies and unassembled reads.

A non-redundant set of tandem repeats found with TRF in assembled contigs is classified into following types: microsatellites, perfect minisatellites, minisatellites, tandem repeats related to dispersed elements, and large tandem repeats including satellite DNA. I suggest two approaches to the distance computation between arrays: one based on a pairwise sequence alignment and another based on a number of common k-mers between two arrays. The constructed graph of array similarity based on these distances allows defining tandem repeats families and subfamilies for large tandem repeats. The array coverage by unassembled reads provides the information about the assembly quality and its estimated copy number in genome. The annotation includes following steps: prediction of position in the reference genome, copy number estimation, checking of presence of known DNA motifs (e.g. CENP-B box, G-quadruplex), prediction of chromosome position, and annotation with available epigenetic datasets. The k-mer representation of satellite DNA provides a simple approach to the construction of family specific or array specific labeled oligonucleotide probes for the following hybridization *in situ*.

The suggested approach significantly reduces the complexity of satellite DNA analysis workflow from assembled contigs to hybridization probes. Annotated large tandem repeats and satellite DNA could be an important resource for further characterization and overall understanding of chromosome and genome organization and structure.

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NUCLEOSOME-NUCLEOSOME STACKING: A BASIC ELEMENT OF CHROMOSOME STRUCTURE

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Folding of the genomic DNA existing in eukaryotes as linear arrays of regular DNA – histone complexes, nucleosomes, into compact but dynamic chromosomes is driven by energetically favourable interactions between nucleosome core particles (NCPs) and is inherently related to regulation of DNA replication, transcription and repair. However, little is known about molecular structure of the NCP-NCP contacts as well as about the nature and scale of forces involved. Major component of chromatin, DNA, is highly negatively charged polymer and as a consequence chromatin folding is sensitive both to ionic environment and to sequence and modifications of positively charged histone N-termini (tails).
Combining experimental and modelling approaches, we study nucleosome-nucleosome interaction in both general polyelectrolyte and specific structural context. Using novel synthetic organic chemistry/molecular biology methods, folding and intermolecular association of the NCPs and chromatin arrays are studied in dependence of ionic conditions and presence of posttranslational modifications in the histone tails. Also, computational modelling is used to investigate NCP-NCP interaction taking into account atomistic details of the nucleosome structure and adequately describing strong electrostatic forces acting in chromatin. Most experimental data is well described by electrostatic model. However, NCP-NCP close contact (stacking) shows enhanced sensitivity to presence of the H4 histone tail and to acetylation of lysine 16 in this histone (H4K16Ac) which is beyond electrostatics. Surprisingly, we have experimentally found that in addition to the strong effect of H4K16Ac, chromatin displays similar unfolding behaviour in the presence of major cation of cell cytoplasm, K+, while very similar Na+ ions promote chromatin compaction.
Based on our experimental and modelling data as well as on the analysis of the NCP crystal structures, we propose a novel structural model for exceptional influence of H4 K16 acetylation on chromatin compaction. Also we explain molecular mechanism for different effect of K+ and Na+ on the nucleosome stacking.

SPECIFIC CENTROMERE STRUCTURE OF EQUATIONALLY DIVIDING CHROMOSOMES IN THE FIRST MEIOSIS OF WHEAT/RYE AMPHIHAPLOIDS

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In wheat/rye amphihaploids most chromosomes are univalent and their orientation at metaphase I initiates different pathways of meiosis. The meiotic-like pathway includes a combination of the reductional and the equational + reductional steps at AI followed by the second division. The mitotic-like pathway involves equational division of univalents at AI and the absence of the second division. To detect mechanisms underlying the process of sister chromatid separation at AI in mitotic-like division meiocytes, we used centromere labeling as a tool to study the centromere structure of equationally and reductionally dividing chromosomes. It is known that there are differences in the structure of meiotic and mitotic chromosomes. However, is the unknown is the pattern of centromere structure the same in the first and second cases?
The centromere labeling with pACT6-09 probe was compared between univalent chromosomes in amphihaploids and chromosomes in normal rye meiosis (diplotene, diakinesis, MI, MII) and in mitotic metaphase. In amphihaploids MI with equational division, rye MII and rye mitotic metaphase, all studied centromeres had hybridization site looked as thin band with diffuse structure positioned across chromosomes. For comparison, in amphihaploids MI with reductional division, rye MI, the centromeres had single dense hybridization site, and in all cases positioned toward the outside of the univalent or bivalent chromosomes in MI.
The current results suggested a structural difference between centromeres with monopolar and bipolar orientation, so equationally dividing univalents have "mitotic" centromere. Observed centromeres structural difference may reflect their functional difference. Thus, we have additional evidence of mitotic division in amphihaploid meiosis including the key mitotic features such as complete lack of pairing, separation of all chromosomes into sister chromatids, their poleward movement, and absence of the second division.

THE MORE THE MERRIER: RECENT HYBRIDIZATION AND POLYPLOIDY IN *Cardamine*

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Inter-species hybridization and polyploidy are playing a crucial role in speciation of spermatophytes. Nevertheless, surprisingly little is known about the subgenome stability and interplay within hybrid and allopolyploid genomes. *Cardamine schulzii* is one of the classical examples of recent allopolyploidy (<150 yrs). To investigate modes of reported hybridization and assumed polyploidization in *Cardamine* species at Urnerboden, Switzerland, comparative karyotype analysis on a population level was - for the first time - performed by combination of genomic *in situ* hybridization and comparative chromosome painting (GISH/CCP). Parental chromosome complements were identified by GISH, whereas chromosome rearrangements were characterized by CCP.
Urbanska and Landolt [1, 2] reported that hybridization between diploid species *Cardamine rivularis* (2n = 16, RR) and *C. amara* (2n = 16, AA) resulted in the origin of the triploid hybrid *C. ×insueta* (2n = 3x = 24, RRA) was followed by polyploidization towards the alloautohexaploid species *C. schulzii* (2n = 6x = 48, RRRRAA). We have reconstructed cytogenetic maps of many individuals of the parental, triploid and bona fide hexaploid taxa. CCP analysis showed that the parental species share six ancestral chromosomes and differ by chromosome rearrangements on two chromosomes. In the triploid semi-fertile hybrid *C. xinsueta*, parental genomes of two diploid species remained structurally stable during c. 100 years since its origin. Despite an extensive population screening, we did not find a single autoallohexaploid plant of *C. schulzii* with 48 chromosomes. Instead, all plants from the *locus classicus* possesed 46 chromosomes and three individuals had 38 chromosomes. We gained compelling evidence that the alleged recent polyploid *C. schulzii* is not a hexaploid derivative of *C. xinsueta*. Instead, at least two hybridization events involving *C. xinsueta* and the hypotetraploid *C. pratensis* (PPPP, 2n = 4x-2 = 30) resulted in the origin of tri-genomic hypopentaploid (2n = 5x-2 = 38, PPRRA) and hypohexaploid (2n = 6x-2 = 46, PPPPRA). These data show that the semi-fertile triploid hybrid can promote a merger of three different genomes and demonstrate how important is to

revise the routinely repeated textbook examples using modern techniques.
This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189).

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B CHROMOSOMES OF RYE ARE HIGHLY CONSERVED AND ACCOMPANIED THE DEVELOPMENT OF EARLY AGRICULTURE

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Supernumerary B chromosomes (Bs) represent a specific type of selfish genetic elements. As Bs are dispensable for normal growth, it is expected to observe B polymorphisms among populations. To address whether Bs maintained in geographically distinct populations of cultivated and weedy rye are polymorphic, we analyzed the distribution patterns and the transcriptional activity of different B-located repeats.
B chromosomes in cultivated and weedy rye have maintained similar molecular structure at the level of subspecies. The high conservation degree of the nondisjunction control region regarding its transcription activity, histone composition and replication underlines the functional importance of this chromosome region for the maintenance of Bs. The conserved chromosome structure suggests a monophyletic origin of the rye B. As Bs were found in different countries, it is likely that Bs were frequently present in the seed material used in early agriculture.
The surprisingly conserved chromosome structure suggests that although the rye Bs experienced fast evolution including multiple rearrangements at the early evolutionary stages, this process has slowed significantly and may have even ceased during its recent evolution.

B CHROMOSOMES ARE DERIVED FROM MANY AUTOSOMES IN THE CICHLID FISH, *Astatotilapia Latifasciata*

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Approximately 15% of eukaryotes contain supernumerary B chromosomes that frequently represent as much as 5% of the genome. Despite thousands of reports describing the distribution of supernumeraries, a comprehensive theory for the origin, maintenance and evolution of B chromosomes has not emerged. Here we applied next generation sequencing to sequence microdissected B chromosomes, and the complete genomes of individual cichlid fish (*Astatotilapia latifasciata*) with and without B chromosomes, to identify DNA sequences on the B. B sequences were also analyzed through qPCR and *in situ* hybridization mapping.

We found that the B chromosome contains thousands of sequences duplicated from essentially every autosomal chromosome and share sequences with the Bs of other African Lake Victoria species. Most of the B sequences correspond to non-identified highly repetitive sequences, transposable elements, and relics of genes. Although most genes are fragmented on the B chromosome, few genes have a high level of integrity, such kinesin-like protein (KIF11), spindle and kinetochore-associated protein 1 (Ska1) and tubulin beta-5 chain (Tubb5).

We propose a model in which the B chromosome originated early in the evolutionary history of Lake Victoria cichlids from a small fragment of one autosome. DNA sequences originating from several autosomes, including protein-coding genes and transposable elements, subsequently inserted into this proto-B. Intact genes on the B involved with spindle dynamics and kinetochore attachment may play a role in driving the transmission of the B chromosome. The genomic approaches employed here broaden the study of karyotypes and chromosomes in ways not possible using classical, or even molecular, cytogenetics alone, creating new opportunities for understanding the function and evolution of sequences on B chromosomes.

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CONSERVED DNA MOTIFS, INCLUDING THE CENP-B BOX-LIKE, ARE INVOLVED IN SATELLITE DNA ARRAY REARRANGEMENTS

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Satellite DNAs (satDNAs), despite rapid evolution that continuously remodel the genomic landscape, occupy functionally essential centromeric regions. Difficult to be explored due to their repetitive nature and divergence, satDNAs are still hardly accessible frontiers of eukaryotic genomes and knowledge concerning functional significance of satellite DNAs is rather limited.

In this work, we provide a comprehensive analysis of six satDNAs in the library of recently separated root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*. We disclosed two different conserved regions common for analyzed satDNAs. One appeared to be highly similar to the CENP-B box of human alpha satDNA, which emerged, in sequence alignment, as a conserved segment common for six divergent satDNAs shared by closely related genomes. Observed results emphasize it as the most prominent example of the CENP-B box-like motif out of mammals. The proposed feature of the CENP-B box-like motif is to act as a promoter in the hypothesized cut-and-paste transposition-related mechanism. This observation could represent a novel role of the CENP-B box, in addition to the known function in centromere protein binding. We propose that the second conserved sequence motif detected in explored satDNAs is involved in illegitimate recombination. In parallel to alpha satDNAs, we found organization of satDNA arrays in nematodes comparable to that found in human and primates, in the form of simple and complex higher order repeats (HORs). In contrast to human satDNA organization, characterized by phylogenetically distinct HOR and monomeric forms, organizational patterns observed in nematodes are consistent with frequent and continuous shuffling of sequences between HORs and monomeric arrays.

Our results suggest the role of conserved domains in mechanisms that cause rapid shuffling of sequences among divergent satDNAs, on the level of short-segment tracts. In context of satDNA evolution, our finding provides, for the first time, an experimentally verified link between conserved domains and satDNA rearrangement events.

TTAGG MEGA-TELOMERES IN *Bacillus* STICK INSECTS (INSECTA: PHASMIDA)

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The species of the genus *Bacillus*, with their complex genealogy and different reproductive strategies, represent a good model group to study karyotype evolution as well as chromosome structure itself. In order to reveal the composition of *Bacillus* telomeres, we analyzed the facultative partenogenetic species *Bacillus rossius* (with gonochoric and all-female populations) and the obligatory unisexual species *B. atticus* and *B. whitei*. By Southern blot hybridization we evidenced the presence of the insect canonical telomeric TTAGG motif in the genomes of all tested species. Bal31 DNA end-degradation assay proved that TTAGG repeats constitute the terminal regions of *Bacillus* chromosomes. In addition to the chromosome end positions, Bal31 trimming approach also disclosed distally located TTAGG arrays in *B. rossius* and *B. whitei*. The chromosomal distribution of TTAGG repeats was analyzed by fluorescence *in situ* hybridization (FISH), which authenticated the position of TTAGG sequence at the end of all chromosomes in the three species. Interestingly, FISH analysis showed that the telomeres of certain chromosomes of *B. rossius* and *B. whitei* are composed of extremely long TTAGG arrays, providing the first evidence of mega-telomeres not only in insects, but also in invertebrates.
As the species *B. whitei* is the interspecific hybrid between *B. rossius* and *B. grandii grandii*, TTAGG mega-telomeres might represent a chromosomal trait inherited through chromosome complement assembly. In our ongoing studies, we are addressing the evolutionary origin of *Bacillus* mega-telomeres, their persistence in different species and populations, and the mechanisms of their maintenance.
This work has been supported by grants from the Ministry of Science, Education and Sports of the Republic Croatia and Canziani funds.

MECHANISM OF HOW TELOMERIC PROTEIN TRF2 BINDS TO DNA AND CONTRIBUTES TO CHROMOSOME END STABILITY

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The eukaryotic genome integrity and stability is balanced by nucleoprotein complexes, telomeres. Telomeres form caps at the very ends of linear chromosomes and ensure protection of chromosomal DNA against unwanted repairs and genome shortening by cellular machinery during cell division. Any disturbance in integrity of telomeres causes chromosomal instabilities leading to unfavorable chromosomal rearrangements or even to carcinogenesis. Specific telomeric protein complex, shelterin hides the 3'end of DNA in telomeric loop and thus protects naked chromosome end from DNA repair machinery. Shelterin serves as a negative regulator of telomerase, an enzyme that adds telomeric DNA to chromosomal ends to ensure complete genome replication. Protein TRF2 (Telomeric Repeat-binding Factor 2) plays a central role within shelterin. TRF2 recognizes and binds to double-stranded telomeric DNA and folds DNA into telomeric loops. The sequence-specific binding of TRF2 to telomeric DNA is mediated by its C-terminal Myb domain. Sequence of TRF2 contains also positively charged N-terminus domain. It has been proposed that this domain is involved in TRF2 binding to telomeric dsDNA as well.

The effect of the domain on DNA binding was described by comparison of the DNA binding affinity of the truncated protein variant of TRF2 lacking the N-terminal domain with the binding affinity of the full length protein. The studies were completed by interaction measurement of the isolated basic domain with telomeric DNA. In our studies we used an effective combination of fluorescence anisotropy and isothermal titration calorimetry techniques.

These studies provided the first detailed quantitative thermodynamic description of the contribution of the basic domain to the DNA affinity of human TRF2 protein. The obtained findings will be used to further understand which of interactions of shelterin proteins are essential for the regulation of telomere length and thus maintenance of the whole genome stability.

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CENTROMERE STRUCTURE IN THE PEA (*Pisum Sativum*) AND RELATED FABEAE SPECIES IN THE CONTEXT OF CenH3 EVOLUTION

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The centromere is a functional chromosome domain that is essential for faithful chromosome segregation during cell division and that can be reliably identified by the presence of the centromere-specific histone H3 variant CenH3. In most diploid plant genomes, CenH3 is encoded by a single copy gene. A chromosome region containing CenH3 protein constitutes a single compact domain within a morphologically distinct primary constriction. This region usually spans up to a few Mbp composed mainly of centromere-specific satellite DNA which is common to all chromosomes of a given species. Previously we found that the centromeres in the pea (*Pisum sativum*) differ from the other species in several aspects. The pea chromosomes exhibit remarkably long primary constrictions containing 3-5 functional centromere domains defined by the presence of CenH3. All of these domains possess two variants of CenH3, which are designated as CenH3-1 and CenH3-2 and share only 72 % identity. We estimated that the length of chromosome segments delimited by two outermost domains varies between 69 and 107 Mbp. Sequencing of chromatin immunoprecipitated DNA revealed that CenH3-containing domains are almost entirely composed of repetitive DNA sequences belonging to 13 distinct families of satellite DNA and one family of centromeric retrotransposons.

In order to find whether the large size and multiple domain structure are common also to other species closely related to the pea, we investigated other members of the tribe Fabeae including *Lens culinaris*, 7 species of *Lathyrus* and 8 species of *Vicia*. While orthologs of both CenH3 variants were identified in *Lathyrus* spp., only CenH3-2 orthologs were found in *Lens* and *Vicia* spp.. Phylogenetic analysis revealed that the duplication of the ancestral CenH3 preceded the split of Fabeae species about 16 - 23 million years ago, indicating that the CenH3-1 variant was lost in the lineage leading to *Lens* and *Vicia* species. Centromere sizes differed considerably among the species. The smallest being in *Lens* and *Vicia* species and largest in *Pisum* and *Lathyrus* species.

We demonstrated that centromere size and complexity can be more varied than previously thought. We showed that Fabeae species with the two CenH3 variants have apparently larger centromeres than those with CenH3-2 only. However, further investigation is needed to find if there is a causal link between CenH3 and centromere size or if it is just a coincidence.

SEQUENTIAL HYBRIDIZATION OF SIX SPECIES-SPECIFIC PAINTING PROBES TO MAKE THE FIRST MULTI COLOR FISH IN RIVER BUFFALO (*Bubalus bubalis* 2N=50)

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In recent years, the progress of molecular cytogenetic led to an evolution of the fluorescence *in situ* hybridization (FISH) methods. Multiplex-FISH (M-FISH), spectral karyotyping (SKY), and combined binary ratio labeling FISH (COBRA-FISH) allow the simultaneous visualization of chromosomes in different colors. Their application in human clinical cytogenetic makes plainer the identification of chromosomal abnormalities. Conversely, in animal cytogenetic the use of these methods is still very limited. In this work we propose the first river buffalo multi-color FISH by the production of a small specific chromosome painting probe library.
Six specific river buffalo autosomal painting probes were prepared through conventional microdissection and DOP-PCR. Probes were labeled with digoxigenin-11- and biotin-16-dUTP in a second DOP-PCR. Three sequentially rounds of FISH were achieved for the same slides. Each round was realized using two probes simultaneously hybridized on the mitosis. Biotin-labeled probes were revealed by FITC-avidin, whereas digoxigenin-labeled probes were revealed by antidig-rhodamine. Slides were counterstained with DAPI in antifade. Digital images were captured in gray-scale and pseudo-colored by the computer.
Chromosome microdissection and DOP-PCR were used to produce six species-specific probes, painting 3 out of 5 sub-metacentric river buffalo chromosomes (BBU 1p, 1q, 3p, 3q, 4p and 4q). The simultaneous hybridization of these probes allowed to develop the first multi-color FISH in this species. Nowadays, the lack of chromosome-specific probes commercially available for individual animal species represents a limiting factor for cytogenetic investigations in farm animals. Therefore, the creation of a such collection of probes opens further opportunity for clinical cytogenetic applications also in river buffalo. Acknowledgements: This study was supported by CISIA-VARIGEAV project, National Research Council (CNR) of Italy

PHYSICAL ORGANIZATION AND CENP BINDING ABILITY OF HORSE CENTROMERIC SATELLITE DNA FAMILIES

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Centromeres, cytologically appearing as visible primary constrictions in metaphase chromosomes, are essential for the proper segregation of sister chromatids during cell division. They are the sites of kinetochore assembly and spindle fibre attachment and consist of protein-DNA complexes, in which the DNA component is typically characterized by the presence of extended arrays of tandem repeats (called satellite DNA). However, centromere DNA array length is highly variable, both among homologous and heterologous centromeres, moreover, centromeric DNA sequences are rapidly evolving among species and within chromosomes of the same species. We isolated, from the horse genome, three different centromeric satellite DNA families (37cen, 2PI and 137sat). Here we report on the physical organization of these DNA sequences, as studied by three colour FISH on mechanically stretched metaphase chromosomes and high resolution two colour FISH on "combed" chromatin fibres. Further, the interaction between centromeric satellites and the centromeric antigens CENP-A and CENP-B has been investigated by ChIP-seq and immuno-FISH on extended chromatin fibers.
The relations among the three centromeric satellite DNA families, their relative abundance and the extension of the single arrays have been estimated. The overall organization of the different classes of centromeric horse satellite DNA appears to be a mosaic where the three DNA families display a strictly intermingled association of sequence blocks widely variable in size, the 37cen satellite being the most abundant and the most widely distributed. More than 95% of the DNA fragments, immunoprecipitated using an anti-CENP-A antibody, are represented by the 37cen satellite DNA sequence, morever, immuno-FISH results indicate that only the 37cen sequence contains CENP-A and CENP-B binding domains.
The organizational pattern of horse centromeric satellite DNA sequences confirms that the horse genome is undergoing a high rate of interchromosomal exchange. The analysis of satellite DNA-protein interactions suggests that, in the horse, the 37cen satellite plays a role similar to that of alpha satellite in man, the other satellite DNA sequences being accessory DNA elements, presumably contributing to the organization of pericentromeric chromatin, by interacting with 37cen. This work was funded by Ministero dell'Istruzione dell'Università e della Ricerca, Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale (PRIN).

IDENTIFICATION OF GENES WITH AN INTEREST IN AQUACULTURE IN THE SPECIES *Solea senegalensis* (KAUP 1858) AND DEVELOPMENT OF A PRELIMINARY GENETIC MAP

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The Senegalese sole (*Solea senegalensis*) is a species of great commercial importance, and is a priority species for diversification of aquaculture. The introduction of the cultivation of this species is not definitively established, mainly due to problems of captive breeding. The high mortality during metamorphosis, susceptibility to diseases and low reproductive rate, remain the main problems farmers are facing this species. Following these deficiencies arose the need for research to improve production in the Senegalese sole. The field of genomics has high potential for discovering the molecular basis of biological processes of interest in aquaculture, such as reproduction, disease resistance or growth rates among others.
For the study of the genes of interest a BAC library consisting of 76 384-well plates was used. The BAC chosen (CopyControlTM pCC1BACtm Vector, Epicentre) has two origins of replication, a chloramphenicol resistance gene, some primer binding sites for sequencing the ends of BAC and target recognition restriction enzymes. This library was screened by PCR to pick up genes of interest using specific primers. BAC DNA extraction is performed with the "BACMAX DNA purification kit" (BMAX044, Epicentre). Extracted BACs were labeled by PCR, DOP-PCR or Nick Translation with fluorochromes for the physical location of the genes within the chromosome using the technique of fluorescence *in situ* hybridization (FISH). FISH simple, double and multiple used one, two or three or more different fluorochromes, respectively ligands for specific labeling of DNA excluding the contrast (Liehr et al. 2004) and to check for co-locations. In this sense, to address issues surrounding the culture of this species from a molecular point of view, we are applying massive sequencing techniques (NGS) *de novo* to a BAC library of *S. senegalensis*, using Roche 454 technology. So far, a total of 169.04 Mb in 28 BAC clones containing anonymous and other genes of interest for reproduction, metamorphosis immune system, among others have been isolated and sequenced. On the other hand, we are conducting a preliminary genetic mapping karyotype of S. senegalensis techniques using fluorescence *in Situ* hybridization multiple (M-FISH) using the BAC library clones as hybridization probes.

We have isolated a total of 32 genes related to the immune system, sex determination, metamorphosis, some genes expression and anonymous. Some of the multiple hybridizations performed show co-locations, most often, not genes belonging to the same group. This can be positive in terms of the selection of favorable or unfavorable quantitative traits without affecting genes with similar functions to control other features or properties of interest hold, as might be the case for genes related to the immune system. Conducted hybridizations were used to perform an ideogram or preliminary genetic map of the species to enable the recognition of each chromosome pair. The sequencing and bioinformatic study has provided BACs sequences anchored to chromosomes and allow detailed study of sequences and determination of sintenias related species.

The results presented are a major advance in the understanding of the molecular and genomic organization of Senegalese sole, and they represent a starting point for further studies applied to aquaculture, especially at the level of expression. Also, the preliminary study genetic map is a source of information that can be used in a complementary manner with physical maps and / or linkage.

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NEW EMBRYONIC STEM CELL LINES DERIVED FROM 2N=26 ROBERTSONIAN MICE

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A drawback for the clinical use of pluripotent stem cells (induced pluripotent stem cell, iPSCs, or embryonic stem cells, ESCs) is the difficulty to preserve their euploid karyotype. The mechanisms behind chromosome instability have been mainly studied in cancer cells but remain very poorly known in ESCs. They include dysfunction in the spindle assembly checkpoints, defects in microtubule attachment to the kinetochore and in sister chromatid cohesion and the presence of supernumerary centrosomes, but up to date only the latter has been investigated in ESCs. Model cell lines with a low number of chromosomes would greatly facilitate these studies, particularly when aiming at understanding the interactions between kinetochores and microtubules or sister chromatids. So far, all mouse ESCs (mESCs) lines were derived from laboratory strains with 2n=40 chromosomes; however, although neglected, wild mouse populations own a strikingly variegated karyotype with a chromosome number from 2n=40, 38, 36.... to 2n=22. This reduction is due to centromeric fusions (Robertsonian translocations, Rb) of telocentrics to form metacentrics. With the aim of deriving new Rb mESCs, 2n=26 females were superovulated, oocytes inseminated in vitro with sperm of 2n=26 males and zygotes cultured up to blastocyst. Methods. Blastocysts were singly plated and cultured up to 96h in complete medium on a STO feeder-layer. Cells were routinely passaged mechanically. Chromosomes were prepared according to standard procedures. Rb mESCs were differentiated using the hanging drop protocol to verify their capacity to form embryoid bodies (EBs).
Fifteen Rb mESC lines were derived from 31 blastocysts. Cells grow in round shape colonies and express pluripotency marker genes. Forteen lines formed EBs with cells expressing markers of the three germ layers. Four lines out of 15 showed a 100% tetraploid chromosome complement since early passages, whereas the remaining displayed more than 70-80% of 2n=26 metaphases. Two of the Rb-mESCs lines have been selected to test their capacity to form teratomas in immunocompromised mice and to contribute, when cells are transferred into a blastocyst, to form a new individual.
Our work aims at the production of novel model mESC lines with a reduced chromosome number that will be made available to the scientific community.

PHYSICAL MAP OF THE MAJOR rDNA CLUSTERS AND TELOMERIC SEQUENCES IN THE KARYOTYPES OF FOUR SPECIES OF *Phyllomedusa* (AMPHIBIA, ANURA)

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Intrachromosomal telomeric sequences (ITS) have been found in the karyotypes of a range of species and may represent residual evidence of chromosomal rearrangements. In the present study, the chromosomal distribution of telomere sequences and major rDNA clusters were mapped in four species of Neotropical tree frogs of the genus *Phyllomedusa* (*P. vaillantti, P. tarsius, P. distincta*, and *P. bahiana*), in an attempt to identify possible remnants of chromosomal rearrangement. The number (2n=26) and morphology of the chromosomes are conserved in this genus, except in one intriguing group of species of the *P. tarsius* clade, which present two pairs of telocentric chromosomes.
The chromosomes of the study species were analyzed using FISH techniques with the major rDNA HM123 probe and the telomeric PNAS probe (CCCTAA)3 as well as differential staining with C-banding, Ag-NOR, and the fluorochromes DAPI and Mitramycin (MM). All the clusters of rDNA obtained by the FISH approach were also detected by Ag-NOR, and permitted the differentiation of the species (pair 1 in *P. vaillantii* and *P. tarsius*; pairs 1 and 10 in *P. distincta* and pair 9 and in one homologue of pair 8 in *P. bahiana*). The telomeric probe hybridized at the terminal position of all the chromosomes in all the species analyzed, and was also detected in the centromeric region of pairs 4 and 6 in P. vaillantti and *P. bahiana*, pair 8 in *P. distincta*, and pair 11 in *P. bahiana* and *P. distincta*. A strong signal was detected in the short arm of pair 13 in *P. vaillantti* and in the terminal position in both arms of all the chromosomes of *P. tarsius*. Notably, the distribution pattern of the ITS was associated with the heterochromatin blocks identified in previous studies by C-banding and fluorochrome staining.
It seems likely, however, that the interstitial telomeric repeats do not correspond to phylogenetically informative events of chromosomal rearrangement in the *P. tarsius* clade. The telomeric repeats in the centromere regions may be an important component of the heterochromatin. Interspecific and intraspecific variations in Ag-NORs have been documented in *Phyllomedusa*, and may either reflect the rapid rate of evolution of this character in this genus or represent a polymorphic ancestral trait.
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EVOLUTIONARY DISTRIBUTION AND MEIOTIC BEHAVIOUR OF HOLOKINETIC CHROMOSOMES IN SPIDERS

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All spiders studied so far show monocentric chromosomes except for the haplogyne araneomorph families Dysderidae and Segestriidae. These groups exhibit holokinetic chromosome structure. Holokinetic chromosomes have arisen several times in Eukaryota. They do not contain a centromere and show a specific behaviour during mitosis and meiosis. To elucidate the distribution of holokinetic chromosomes in spiders, we have studied cytogenetics of the superfamily Dysderoidea (families Dysderidae, Oonopidae, Orsolobidae, and Segestriidae) and its sister clade, the family Tetrablemmidae. Cytogenetics of oonopids and orsolobids has been unknown so far. Chromosome preparations were obtained from testes by a spreading technique.
All species examined exhibited low diploid numbers and the X0 system. Male diploid numbers were as follows: Dysderidae: *Dysderocrates storkani* 21. Oonopidae: *Gamasomorpha lutzi* and *Oonops ebenecus* 7. Orsolobidae: *Afrilobus* sp. nov. 5. Segestriidae: *Ariadna* sp. (Namibia) 7. Tetrablemmidae: *Monoblemma muchmorei* 23. Tetrablemmid chromosomes were monocentric, whereas all Dysderoidea showed holokinetic chromosomes. Therefore, the holokinetic chromosome structure is a synapomorphy of this superfamily. Holokinetic chromosomes of Dysderoidea segregated in parallel to the equatorial plane at mitosis. In contrast, they showed telokinetic activity during meiosis. The X chromosome of Dysderoidea was usually the same length (*Ariadna*, oonopids) or somewhat shorter (*Afrilobus*) than autosomes except for the enormous X chromosome of *Dysderocrates*. The X chromosome of studied dysderoids displayed a standard meiotic segregation; its chromatids segregated during the second meiotic division. Following pachytene, spermatocytes of tetrablemmids and dysderoids entered a long diffuse stage, which was marked by a considerable autosome despiralisation. Therefore, chiasmata were visible after this stage only. Our data suggest that male diffuse stage is a synamomorphy of haplogyne spiders.
This study was supported by projects of the Grant Agency of the Charles University (GAUK-830413, SVV-2013-267205).

X-RAY IMAGING OF CHROMOSOMES

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There is a gap in our knowledge of the structure of chromosomes due to the resolution of the available imaging methods. Crystallography provides atomic resolution images up to the size of the nucleosome, consisting of histone proteins wrapped by two turns of double-stranded DNA, forming a cylindrical structure 11 nm in diameter and 6 nm tall. Optical methods, notably fluorescence microscopy, can resolve entire chromosomes down to about 200 nm. Imaging using short wavelength probes such as X-rays and electrons can fill that gap in our structural knowledge between crystallography and optical imaging.
X-ray based imaging has been carried out using synchrotron radiation from Diamond Light Source, Advanced Photon Source and Elettra. The methods include Coherent Diffraction Imaging (CDI), X-ray ptychography, Scanning Transmission X-ray Microscopy (STXM) and Fresnel CDI. Some of these are still under development and require specially designed setups. Because of the high degree of structural order in metaphase human chromosomes, air dried and critical-point dried samples are expected to be representative of the biologically relevant state. Glutaraldehyde cross-linking allows the samples to be fixed while still preserving order down to the tens of nanometre length scale. DNA-specific stains, such as Pt blue, are used to dye the sample to further enhance the contrast. Electron imaging using epoxy-embedded samples with a Serial Block-Face Scanning Electron Microscope (SPFSEM) was performed as well.
Images have been obtained using all methods, but not yet with the resolution expected from the diffraction patterns obtained so far. Three dimensional images have so far only been available by SBFSEM but these require significant image processing before they can be interpreted. Excellent X-ray images have been obtained for inorganic test objects, used to debug the methods and instrumentation. Our latest results will be presented at the conference.

SEX-RELATED GENOMIC SEQUENCES IN TWO CARTILAGINOUS FISH

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Sex determination and differentiation are key events in the development of either the testis or ovary in fish. Sex determination mechanisms include environmental and genetic regulation. Research on sex determination systems and their related genes has been implemented in the teleost species, but the mechanism of sex determination in cartilaginous fish remains largely unknown. In Chondrichthyan fish, the sex chromosomes are known in only a few species. This study reports the data on molecular studies and chromosome localization of SRY-like specific sequences useful to discriminate between various chromosome pairs in the common torpedo, *Torpedo torpedo* (Torpediniformes, 2n = 66; FN = 78), and in the scyliorhinid coral catshark, *Atelomycterus marmoratus* (Carcharhiniformes, 2n = 76; FN = 128), species that do not have morphologically distinct sex chromosomes.
As concerns chromosomal localization of the amplification products, in both species the results obtained showed the presence of hybridization signals on two pairs of chromosomes in the male, one pair of subtelocentric and one pair of acrocentric elements. In the female, FISH showed hybridization signals only on the pair of acrocentric chromosomes. Similar results as in the female were obtained by hybridizing a probe derived from male DNA to female chromosomes and a probe derived from female DNA to male chromosomes. Sequencing products of the female 400 bp band in the two species differed greatly, whereas the male 400 bp bands showed 93% identity and alignment with the human spermatogenesis related genes SPATA 16, SPATA 18 and UTY.
T. torpedo and *A. marmoratus* are phylogenetically very distant. If the sequences are indeed part of the genes involved in the process of spermatogenesis in these species, an evolutionary pressure that led to their conservation in the cartilaginous fish was probably exerted. Moreover, the sequences might also be involved in the mechanism of sex chromosome differentiation.

ORGANIZATION OF TELOMERES IN THE MAMMALIAN GERM LINE: THE ROLE OF NON-CODING TELOMERIC RNA (TERRA) AND TELOMERASE

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Telomeres are ribonucleoprotein complexes capping chromosome ends that maintain genome stability. During spermatogenesis chromosomes undergo many chromatin and nuclear distribution rearrangements, since it comprises two meiotic divisions followed by gametes differentiation. Telomeres are essential to preserve chromosome integrity along these processes, and so for the proper progression of the gametogenic events. Moreover, the telomeric repeat-containing RNA (TERRA), an integral element of the telomeric complex, has been recently related to the regulation of both telomeres structure and telomerase activity. In this context, here we have studied the role of TERRA and telomerase in maintaining telomere homeostasis during mouse spermatogenesis.
TERRA molecules are present as discrete foci at telomeres of mouse spermatocytes during pairing, synapsis and recombination processes between homologous chromosomes. In the same way, telomerase mostly localizes at germ cells telomeres during all the spermatogenesis, while free telomerase co-localizes with free TERRA molecules.
Our results suggest that TERRA may contribute to the preservation of the telomeric integrity during spermatogenesis, both by taking part of the telomeric complex and by binding free telomerase molecules.

COMPARATIVE ANALYSIS OF HUMAN COMMON FRAGILE SITE FRA6E AND ITS MURINE ORTHOLOGUE

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Common fragile sites (CFS) are large unstable sequences of the human genome, which can be involved in cancer chromosomal rearrangements. It is intriguing that in spite of the intrinsic fragility of human CFS, they appear strongly conserved in mammals. By a comparative analysis of human/mouse genomes, the predicted murine fragile regions were identified [1, 2]; however, for the majority of them a validation by molecular cytogenetics is lacking. It has been recently proved that a combination of sequence features and of epigenetic factors account for the expression of fragility at CFS, and this implies that CFS maps derived from classical studies on peripheral blood lymphocytes are not exhaustive; on the contrary, cell-specific CFS, expressing differential fragility, may be expected. Furthermore, while the direct occurrence of breakages at CFS has been defined in cell culture and under replication stress, the possible instability of the same sequences under physiological conditions is emerging [3]. FRA6E is one of the most active CFS of the human genome, it harbours the PARK2 gene, which is involved in a form of autosomal recessive juvenile parkinsonism and it is considered a tumour suppressor gene [4]. Sequence homology and synteny conservation between human and murine regions are high.
We used MEF cell lines and primary cultures of mouse splenocytes, stimulated by Concanavalin A, to define the activity of the predicted murine fragile site after aphidicolin (APH) exposure, and the possible differential response of different cell types [5]. A panel of genomic clones has been used in FISH analyses to define the frequency of APH-induced breakages and the boundaries of the predicted fragile site at chromosome 17A1. Molecular combing has been used for a fine description of the region. Our preliminary results suggest that in the region under investigation an active fragile site is present, with a remarkable differential organization of the core of fragility in human and mouse segments.
CFS represent an important component of the human genome; detailed knowledge of the murine orthologues of human CFS may provide models to define the molecular basis for intrinsic fragility of these sequences, and to understand the evolutionary implications, with a special emphasis to the different cells types and developmental stages involved in sequence instability.

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ANALYSIS OF CHROMOSOMES AT EARLY STAGE OF THE FIRST MEIOSIS DIVISION OF THE SABLE, *Martes Zibellina*

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For the first time we presented the data on the meiosis first stage and described SC karyotype of the sable *Martes zibellina*. There are date of many mammals of SC karyotypes at present (Moses, 1977; Kolomietc et al., 1986; Safronova et al., 2009). Electron microscopic analysis of synaptonemal complexes (SC) formed in the prophase of the first meiotic division, is very informative to investigate the structure of chromosomes
SC-karyotype *M. zibellina* consists of 18 SC autosomal bivalents and sex bivalent. The autosomal bivalents gradually decreasing in length, length axis of the X chromosome is comparable in length to the SC bivalent number 8 (early pachytene) or 6 (average pachytene) the axis of the Y chromosome is 3 times smaller in length than the axis X chromosome karyotype. Famous *M. zibellina* includes 15 double-armed autosomes, medium to fine, and 3 pairs of small two arms autosomes (Orlov, Maligin, 1969; Graphodatsky, Radjabli, 1987). Chromosome X - metacentrics, comparable in size to the autosomes number 5; Y-chromosome - the smallest acrocentrics. The ratio of the lengths of the SC autosomes are consistent with the relative lengths of mitotic chromosomes. However, the length of SC sex chromosomes differ from the lengths of these elements in meiosis. Length axis X chromosome somewhat smaller in mitosis and Y - chromosome length slightly exceeds the length Y of mitotic chromosomes. The axes of sex chromosomes *M. zibellina* synapsis, like most mammals, synaptic length of the X and Y chromosomes in the early stages - the middle of most of the pachytene chromosome Y. In *Mustela vison*, closest to *M. zibellina* kind of studied representatives of the Carnivora, length of the sex chromosome synapsis during meiosis varies greatly, and the late pachytene stage was practically the entire length of the Y chromosome.
Meiosis was studied in ten adult males (2 years) cell population. Preparations spread synaptonemal complexes (SC) in the early stages of meiotic division I (zygotene-pachytene) prepared by (Dresser, Moses, 1977) and painted IC 50% solution of silver nitrate. Synapsis of the sex chromosomes does not differ from that observed in most mammals, including carnivores. The small discrepancy between the relative lengths of the sex chromosomes obtained in the study of the SC and in the study of mitotic karyotype of *M. zibellina*, probably reflect the structural features of the sex chromosomes of the species.

GENOMIC COMPARISON OF THE AKODONTINE RODENTS *Akodon cursor, A. montensis* AND *Necromys lasiurus* (CRICETIDAE: RODENTIA)

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The karyotypically diverse tribe akodontine presents a high level of chromosomal polymorphisms due to Robertsonian rearrangements and pericentric inversions. In this work, we compared the GTG- and CBG-banded karyotypes of three species of this group: *Akodon cursor* (2n=14, FN=19), *A. montensis* (2n=24; FN=42), and *Necromys lasiurus* (2n=34; FN=34). In addition, we performed interspecific genomic comparisons using fluorescent *in-situ* hybridization with total genomic DNA of each species as probes (GISH).
Each probe was hybridized to chromosome preparations of the same species (control) and of the other two species. Suppression experiments were performed with a mix of probe:suppressor (1:100). Comparative analysis after GTG-banding showed almost complete homeologies among the three karyotypes. They differed from each other by centric fusions and fissions and pericentric inversions. CBG-banding revealed the presence of small centromeric heterochromatic regions in most chromosomes of the three species. In the control GISH intraspecific experiments, each probe hybridized to the whole extension of all the chromosomes with more intense labeling in the constitutive heterochromatin. Interspecific GISH experiments between *A. cursor* and *A. montensis* resulted in hybridization of all euchromatic regions but only part of the heterochromatin. Interspecific hybridizations between *N. lasiurus* and the two *Akodon* species resulted in labeling of all euchromatic regions.
Our results point to an extreme conservation between the genomes of the species analyzed and suggest that the differences are restricted to their heterochromatic regions to varying degrees. Further studies will be focused on the genetic bases of the heterochromatin differentiation observed among the three analyzed species.
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REMARKABLE WIDESPREAD INTERSTITIAL TELOMERIC SEQUENCES IN EYELID-LESS MICROTEIIDS: SPECIES-SPECIFIC DISTRIBUTION ON AUTOSOMES AND SEX CHROMOSOMES (SQUAMATA, GYMNOPHTHALMIDAE)

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The Gymnophthalmini is a monophyletic assemblage of nine South American lizard genera – *Calyptommatus, Gymnophthalmus, Micrablepharus, Nothobachia, Procellosaurinus, Psilophthalmus, Scriptosaura, Tretioscincus* and *Vanzosaura* - characterized mainly by lack of eyelids, progressive limb reduction and body elongation associated with sandy environments and fossoriality. They exhibit highly rearranged karyotypes and the largest range in diploid numbers described for microteiids, from 2n=40 with 16M+24m to 2n=62-64 with chromosomes gradually decreasing in size. This diversity is due to the occurrence of species-specific karyotypes, mostly based on comparative analyses of banded chromosomes, supernumeraries and XX:XY sex determination system in *N. ablephara* and in *Micrablepharus* spp., multiple $X_1X_1X_2X_2$:X_1X_2Y sex chromosomes system in *Calyptommatus* spp. and unisexuality in *Gymnophthalmus*. The highest chromosome numbers occurs in the most derived *Nothobachia* (2n=62-64) and *Calyptommatus* (2n=57-58), while the remaining genera present lower diploid numbers, suggesting that chromosomal rearrangements have included major events of chromosome fission. Also, chromosome fusion, pericentric inversion as well as amplification/deletion of heterochromatin and shifts in Ag-NOR are considered during speciation of these South American microteiids.

We present herein the first comparative cytogenetic analyses on 14 species, representing all genera of the Gymnophthalmini plus outgroups, based on telomeric FISH and CBG-banding patterns. In addition to the ordinary telomeric signals, we evidenced profuse ITS variable in number, size, and positioning within the genomes, some of them with facultative co-occurrence with C-heterochromatin in autosomes of all species and conspicuous in X and Y chromosomes in *Micrablepharus*, demonstrating even intrageneric variability.

Associated with other chromosomal markers such as localization of Ag-NORs and distribution of constitutive heterochromatin, the observed patterns of ITS highlight extensive differentiation in this radiation. Further investigations on the origin and maintenance of these frequent and widespread sequences, as well as a more refined characterization including additional repetitive sequences will yield information to the better understanding of the mechanisms of chromosomal evolution in this group of South American microteiids.

3D ELECTRON MICROSCOPY FOR INVESTIGATING THE STRUCTURE OF HUMAN MITOTIC CHROMOSOMES

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The study of human chromosomes has been important as they carry the repository of all genetic material, which is passed from one generation to the next during mitosis. Currently there is a big gap in the knowledge of the structure of these complex organelles, mostly in the range from about 10nm to 250nm. Below this range, traditional X-ray crystallography has provided detailed atomic structure of the nucleosome (6nm), while above this range, visible light microscopy takes over (250nm). At low magnification, scanning electron microscopy (SEM) images of human chromosomes are recognisable. But at higher magnification, SEM images have shown residual nucleoplasm, which partially or completely conceals the chromosomes and prevents high-resolution analysis of chromosome ultrastructure.
We have expanded our study to gain 3D information on chromosome structure using a new method known as serial block face scanning electron microscopy (SBFSEM) technique. Sample is embedded into resin, slices are taken using an ultramicrotome mounted inside the SEM. The block surface is imaged. After each section, the resin block is raised by 20 nm. This automated system can acquire many thousands of images in perfect alignment [1]. We wish to share our progress in suitable sample preparation for 3D imaging of human chromosomes. Chromosome samples have been optimised where methanol acetic acid samples show structural details where as other chromosome preparation methods do not. Contrast agents such as platinum dyes have also being selected and tested. 20 nm slices have been obtained using a diamond knife of a whole human metaphase chromosome embedded in resin. The 20 nm slices have been aligned to demonstrate the FIRST EVER high-resolution 3D human metaphase chromosome reconstruction. This work is part of our BBSRC diamond fellowship ere we are using same chromosome samples and stains to obtain 3D chromosome structure images by diffraction setup which is under development at Diamond light source synchrotron.
2D SEM does not give chromosome structure detail as the surface is concealed with nucleoplasm Sample prep, staining and embedding protocols have been optimised 3D SBFSEM is a new technique and is automated which can take 20 nm slices which are reconstructed The first ever high resolution 3D human chromosome reconstruction is demonstrated.

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STRUCTURE-FUNCTION RELATIONSHIPS DURING TRANSGENIC TELOMERASE EXPRESSION IN *Arabidopsis*

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Telomerase is a protein essential for maintenance of telomere lenght, chromosome stability and totipotency of plant cells. Although a number of intensive studies, the principles of telomerase regulation have not been well understood. Therefore, we investigated the subcellular localization and function of the full-length and truncated forms of the catalytic subunit of *Arabidopsis* telomerase, AtTERT, *in planta*. We investigated stable telomerase expression in *Arabidopsis* wild type and mutant plants and/or transient expression in *Arabidopsis* protoplasts and/or *Nicotiana benthamiana* leaves. We also analysed the ability of distinct domains to reconstitute telomerase activity and to maintain telomeres in parallel

We detected nuclear localization signals in multiple sites in AtTERT as all the studied individual domains of the AtTERT were targeted to the nucleus and/or the nucleolus regardless transient or stable expression. Although we were able to detect the introduced genomic or cDNA AtTERT transgene expression at transcript and protein levels, we did not identified transformants able to fully complement the lack of telomerase functions in tert −/− mutants.

We can conclude that an interactive cross-talk between regulatory elements located downstream and/or upstream of the start codon, and/or some post-translational modifications might play a crucial role in the proper function and effective expression of the AtTERT. Our data suggest that telomerase regulation in plants is more complex compared to the reported mammalian model systems.

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SEX CHROMOSOMES

WEIRD ANIMAL GENOMES, SEX AND THE EVOLUTION OF NEW SEX DETERMINING SYSTEMS

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In humans and other mammals with XX females and XY males, the Y bears a gene (*SRY*) that induces testis differentiation in the embryo and switches on hormones that masculinize it. The human X has more than 1500 genes, but the tiny Y is a genetic wasteland and bears only 45 protein-coding genes, most active only in testis. To discover how human sex chromosomes got to be so weird, we compared the chromosomes, genes and DNA in distantly related mammals and even birds and reptiles (with completely different sex determining systems). Kangaroo sex chromosomes reveal the original mammal sex chromosomes, while the bizarre platypus sex chromosomes (more related to those of birds) tell us that human sex chromosomes and the *SRY* gene are relatively young. The human X and Y evolved from an ordinary chromosome pair as the Y degraded progressively. If Y degradation continues at this rate, it will disappear in just 5 million years. If humans don't become extinct, new sex determining genes and chromosomes must evolve, maybe leading to the evolution of new hominid species.
Where will our new sex genes and chromosomes come from? Whereas mammals (and birds) have rather rigid systems, other vertebrates (particularly reptiles) show great variation in sex determining systems, and we can find many examples of switches in sex determining systems. Using a model of dosage-dependent and temperature dependent sex determination, we can readily understand switches between temperature-dependent and chromosome-dependent sex determination, and even between XY and WZ systems. We also see many ways in which genes or gene copies (often of the same genes which seem to be particularly good at this role) have taken on a sex determining function, and defined new sex chromosome systems.

VERTEBRATE SEX DETERMINATION: HOW DOES IT FIT TOGETHER?

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The sex determining network shows a remarkable plasticity in different orders of the vertebrate lineage. One would expect a well conserved system because of the similarity in sexual reproduction and the importance of proper sex determination in the survival of the species. Evolutionary forces must be significant to actually promote different modes of sex determination. A first division can be made between environmental and genetic sex determination, but even within these two modes different systems of sex determination can be recognised. The implication is that the underlying arrangement of genes with respect to timing of gene activation, mechanism of gene regulation, and dose of gene product varies in different vertebrate orders. The challenge of current research is first to recognise and comprehend the different sex gene networks and second to use comparative genomics to investigate the degree of the plasticity in the different vertebrate orders, find common themes and define ancestral sex determining systems. Model organisms for this research are species of fish, amphibians, reptiles, and mammals (monotremes, marsupials and placentals). Current knowledge and unknowns on sex determination in these species will be discussed.

EVOLUTION OF SEX DETERMINATION MECHANISMS AND SEX CHROMOSOMES IN FISH

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Fish show the widest variety of sex determination mechanisms of all vertebrates, but the evolution and biological meaning of this diversity is barely understood. In several species the primary sex-determining gene has been identified recently, allowing to study the processes that create new sex determination mechanisms and sex chromosomes. The evolution of monogenic sex determination is intimately linked to the formation of sex chromosomes. The small sizes of the mammalian and *Drosophila* Y-chromosomes and of the chicken W perfectly illustrate a prominent aspect of their evolutionary history. Due to the absence of meiotic recombination most of their genetic content, except for some sex specific genes, degenerated and was lost. Thus, the general picture emerged of the ever-shrinking heterogametic sex chromosomes that finally even fade to total disappearance.

A closer look at the evolutionary younger sex chromosomes of fish, amphibians and reptiles shows that this perception is not complete. In these groups – contrary to birds and mammals – sex determination mechanisms show a high turnover and new sex chromosomes appear again and again. Interestingly, a majority of these Y and W chromosomes are considerably larger than their corresponding X and Z counterparts, sometimes more than four times in length, and being the largest chromosome of the whole complement.

We conclude that in the initial phase of sex chromosome evolution Y and W sex chromosomes increase in size, and that size diminishing may be only a late attribute in the evolutionary history. For the emergence of new genetic sex determination mechanisms it becomes clear that besides the classical mode of allelic diversification at one locus of a pair of proto-sex chromosomes, gene duplications of genes that act downstream in the sex determination regulatory network or even genes with totally unrelated functions can create new master male-determining genes.

EVOLUTION OF SEX CHROMOSOMES IN MOTHS AND BUTTERFLIES

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Moths and butterflies (Lepidoptera) represent the largest group of organisms with female heterogamety and holokinetic chromosomes. Although a WZ/ZZ (female/male) sex chromosome system predominates, 'primitive moths' and caddis-flies (a sister order Trichoptera) lack the W chromosome and have a Z/ZZ system. The W chromosome arose later in the evolution of Lepidoptera. In some species the W chromosome was lost, resulting in a derived Z/ZZ system. In some other species, neo-sex chromosomes and multiple sex chromosomes originated by fusion of the ancestral W and/or Z chromosomes with autosomes. In this report, I summarize our knowledge on molecular differentiation of the lepidopteran W and Z chromosomes. I also present our recent results on tracking the evolutionary history of sex chromosomes in selected species of Lepidoptera.

Results of genomic *in situ* hybridization (GISH), comparative genomic hybridization (CGH), and FISH with W-chromosome painting probes along with available sequence information suggest that the W chromosomes are almost entirely composed of repetitive sequences and often exhibit considerable sequence uniformity. Moreover, they evolve rapidly even between closely related species. On the contrary, the gene rich Z chromosomes are highly conserved among Lepidoptera as demonstrated by synteny mapping of Z-linked genes between distant species using FISH with probes derived from bacterial artificial chromosome (BAC) clones. Despite the high conservation of Z chromosomes, Lepidoptera exhibit a relatively high incidence of neo-sex chromosomes. For example, geographical populations of wild silkmoths, *Samia cynthia* ssp., show a step-by-step evolution of the neo-sex chromosomes by repeated autosome–sex chromosome fusions. In moths of the family Tortricidae, the neo-Z chromosome arose by a fusion between an ancestral Z and an autosome enriched in genes involved in detoxification of plant secondary metabolites.

The high conservation of Z chromosomes across the phylogenetic tree is consistent with the remarkable stability of lepidopteran genomes. However, frequent fusions of sex chromosomes with autosomes in different lineages of Lepidoptera phylogeny point to the possible role of neo-sex chromosomes in speciation. In addition, we believe that the neo-Z chromosome in tortricids represents an evolutionary key innovation that increased their adaptive potential in plant-herbivores interactions and thus contributed to their radiation.

X_1X_2Y SYSTEM OF HAPLOGYNE SPIDERS AND ITS EVOLUTION

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The spider infraorder Araneomorphae is composed of two lineages, Entelegynae and Haplogynae. Both of them show a variety of sex chromosome systems. The rare X_1X_2Y system has been found in six haplogyne families. It is usually composed of two large metacentric X chromosomes and a metacentric Y microchromosome. These chromosomes display an achiasmatic end-to-end pairing by the ends of both arms at male meiosis. The conservative structure and specific meiotic pairing of chromosomes of the X_1X_2Y system suggest that families with this system form a monophyletic lineage. The present study is aimed at evolution of the X_1X_2Y system. Chromosome preparations were obtained from testes by a spreading technique.

The diploid numbers and sex chromosome systems were as follows: Filistatidae: *Kukulcania aff. hibernalis* 25 (X_1X_2Y). Pholcidae: *Holocnemus pluchei* 27 (X0), *Hoplopholcus forskali* 28 (X_1X_20), *Modisimus elongatus* 17 (X0), *Pholcus opilionoides* 25 (X_1X_2Y). Sicariidae: *Sicarius terrosus* 21 (X_1X_2Y). Sex chromosomes showed mostly a metacentric morphology. Chromosomes of both the X_1X_2Y and X_1X_20 system exhibited an end-to-end pairing in male germ, namely from premeiotic interphase to metaphase I. The X chromosomes continued association at anaphase I.

The X_1X_2Y system of haplogyne spiders exhibits a conservative structure except for the family Pholcidae. In the genus *Pholcus* (Pholcinae), one metacentric X chromosome was transformed into a submetacentric one, most probably by a pericentric inversion. The X_1X_2Y system of the subfamily Holocneminae has evolved gradually into the X0 system: the X_1X_20 system of *Hoplopholcus* has arisen by loss of the Y chromosome; subsequent fusion of the X chromosomes has produced the X0 system (Holocnemus). Which evolutionary transformation has produced the X0 system of *Modisimus* (Modisiminae) remains unclear. The considerable diversity of sex chromosomes in Pholcidae can be used to reconstruct the phylogeny of this family.

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YET ANOTHER CASE OF NEO-SEX CHROMOSOMES IN "MICROMOTHS": GELECHIOIDEA

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The Gelechioidea is a mega-diverse superfamily of Lepidoptera that includes 18000 described species. Despite the fact that this group includes many species of economic importance, it is unarguably one of the least studied large lepidopteran superfamilies. This also applies to our knowledge of the genome architecture in Gelechioidea. To date, only 33 species of the families Xylorictidae (1 sp.), Elachistidae (7 sp.), Oecophoridae (1 sp.), Coleophoridae (10 sp.) and Gelechiidae (14 sp.) were examined cytogenetically. The haploid chromosome numbers of the majority of the species range from 28 to 31. An interesting fact is that 26 out of the 33 species have a strikingly large pair of chromosomes. In order to determine the nature of this chromosomal pair, we examined chromosomes of two species of Gelechiidae, the tomato leafminer *Tuta absoluta* (n=29) and the Angoumois grain moth *Sitotroga cerealella* (n=30), using genomic *in situ* hybridization (GISH) and comparative genomic hybridization (CGH).

In female pachytene complements of both species, GISH and CGH identified the longest bivalent by strong binding of the female-derived genomic DNA probe to one member of this bivalent, the W chromosome. We have demonstrated, therefore, that the largest pair of chromosomes represents the W and Z sex chromosomes.

The unusual size of the W and Z together with the reduced chromosome number observed in vast majority of the species, when compared with the ancestral karyotype of n=31, suggest that these are in fact neo-sex chromosomes. Thus, as leaf-rollers of the superfamily Tortricoidea, moths of the superfamily Gelechioidea likely represent yet another example of the neo-sex chromosome system that arose by fusion of an ancestral WZ pair with a pair of autosomes. This fusion might have occurred early in the radiation of Gelechioidea. The relevance of this rearrangement in the radiation of this group is yet to be elucidated.

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SEQUENCING AND *DE NOVO* ASSEMBLY OF THE EQUINE MSY

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The Y chromosome plays a central role in male fertility, sex determination, and evolution of cross species reproductive barriers. Studying the structure, organization and gene content of the chromosome in horse is therefore of great interest for understanding both stallion reproductive biology and genome evolution of equids. The male specific region of the Y (MSY) is evolved from dynamic evolutionary processes that led to many novel lineage-specific genes, multicopy gene families, segmental duplications, palindromes and directional repeated sequences. Because of the challenges faced when sequencing genomic/chromosomal regions with such features, only 3 MSY assemblies have been published to date (i.e., human, chimp, and macaque). The horse genome was sequenced in a mare and lacks the Y chromosome data. In order to finish an important portion of the horse genome sequence, we developed a high-quality assembly of the euchromatic portion of the equine MSY.

We initially constructed a physically ordered high-density BAC contig map using a combination of metaphase-, interphase- and fiber-FISH mapping, sequence tagged site (STS) content analysis, chromosome walking, and direct Sanger sequencing of selected BACs. This map covers ~12 Mb, with 197 BACs ordered into 5 contigs, of which one comprises mainly multicopy sequences. The map provides roughly 5-fold coverage of the euchromatic portion of the MSY. We selected a minimum tiling path consisting of 54 BACs that we divided into 12 pools, barcoded, and pyrosequenced on a 454 titanium instrument yielding 26X coverage for the predominantly single-copy region and 135X coverage for the multi-copy region. In order to extend the length of contigs, resolve assembly of repeats containing regions, duplications, and build supercontigs, we generated additional 5X coverage 454 sequence data using 8-kb insert matepair libraries. Multi-copy BACs were also individually sequenced on the Illumina MiSeq using 2X150 PE runs and HiSeq using 2X100 PE runs (300X and 5000X fold coverage, respectively) to obtain a comprehensive catalog of all sequences present, estimate their copy number, and assist in their assembly. Finally, we generated single-molecule sequences of the pooled minimum tiling path BACs on a PacBio RS yielding reads of up to 12,000 bp with 40X coverage to build supercontigs. This approach has yielded a nearly complete assembly of the euchromatic portion of the MSY. Currently, the mean N50 of the contigs is 38,815 with the maximum length of 161,813 bp and scaffolds up to 2.2 Mb. The final assembly incorporates 454, Illumina, and PacBio data.

Our equine MSY assembly fills a major gap in the equine genome sequence and enables novel studies examining the contribution of Y genes and transcripts to male sexual development, reproductive biology, and the role they play in stallion fertility. Our study also provides new insight into the evolutionary processes governing the evolution of the Y chromosome because it is among the few MSYs sequenced and assembled to date.

INDEPENDENT SEX CHROMOSOME EVOLUTION IN LOWER VERTEBRATES: A MOLECULAR CYTOGENETIC OVERVIEW IN THE ERYTHRINIDAE FISH FAMILY

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The Erythrinidae fish family is an excellent model for analyzing the evolution of sex chromosomes. Different stages of sex chromosome differentiation, from homomorphic to highly differentiated ones can be found among the species of this family. However, what would be the ancestral status of the sex chromosomes among the Erythrinidae species that present homomorphic karyotypes between the sexes? The presence of heteromorphic sex chromosomes, although showing different evolutionary stages, would be present in the Erythrinidae family as a whole, or would be a particular attribute of only some of its species? Three distinct sex chromosomes were microdissected and used for the preparation of probes for chromosome painting procedures. Besides, the cytogenetic mapping of several classes of repetitive DNAs was also used in order to highlight the evolutionary relationships of the sex chromosomes among different erythrinid species and genera.

It was demonstrated that the sex chromosomes can follow distinct evolutionary pathways inside this family. Reciprocal hybridizations with whole sex chromosome probes revealed that different autosomal pairs have evolved as the sex pair, even among closely related species. In addition, different putative proto-XY pairs were found in some species/karyomorphs lacking heteromorphic sex chromosomes, indicating that the sex chromosomes may follow distinct evolutionary paths even among closely related species.

It was demonstrated that sex chromosomes can emerge independently and following distinct patterns of differentiation, even in closely related species as well as in the same type of sex chromosome system, suggesting that the sex chromosome turnover may play an important role in speciation processes.

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MEIOTIC SEX CHROMOSOME INACTIVATION IN LEPIDOPTERA: NEW DATA

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Inactivation of the heterologous sex chromosomes during meiotic prophase I was observed in various species with both the XY (e.g., mouse, opossum, locust, and fruitfly) and WZ (e.g., chicken) systems. In both systems, meiotic sex chromosome inactivation (MSCI) has some similar but also some different features. MSCI is always associated with epigenetic modifications of chromatin; however, organisms differ in the timing and type of chromatin changes. Regardless some dissimilarities, there is accumulating evidence that MSCI is universal among organisms with heteromorphic sex chromosomes. So far, the vast majority of data on MSCI comes from organisms with male heterogamety (XY system). Moths and butterflies (Lepidoptera) represent the largest group of organisms with female heterogamety (i.e., with WZ/ZZ and derived sex chromosome systems) and are thus good candidates for next research on MSCI.

In this work, we examined patterns of several histone modifications by immunolabelling in female meiotic cells of three different lepidopteran species, the flour moth (*Ephestia kuehniella*), the silkworm (*Bombyx mori*), and the codling moth (*Cydia pomonella*). In all species, histone modifications associated with transcriptional activity showed no differences between the sex chromosomes and autosomes. Although markers of transcriptionally inactive heterochromatin (di- and tri-methylated histone H3 lysine K9) were more or less accumulated on the W chromosome, the pattern of these modifications on the Z chromosome was comparable with autosomes.

The lack of immunolabelling with both studied heterochromatin markers together with the high level of other tested histone modifications on the Z chromosome during pachytene in all three species examined suggests the absence of MSCI in Lepidoptera. Moreover, a high level of histone H4 acetylation and a relatively low level of heterochromatin marker H3K9me3 on the W chromosome indicate transcriptional activity also of this 'genetically inert' sex chromosome during meiosis.

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INSIGHTS INTO SEX CHROMOSOME EVOLUTIONARY RELATIONSHIPS IN SQUAMATE REPTILES: THE CASE OF *Anolis* (REPTILIA, IGUANIDAE)

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In Iguania, both temperature-dependant sex determination and genetic sex determination (GSD), with an XY system, have been reported. Species with XY sex chromosome systems, including multiple X chromosomes, are present among anoles. *Anolis carolinensis* is known to be a GSD species, and the occurrence of an XY system in this species has recently been hypothesized and an X specific region characterised. The aim of this study was to determine the evolutionary relationships between those sex chromosomes within the genus *Anolis* that show certain morphological variability (XY as macro- or microchromosomes; X_1X_2Y systems).

In this study*, A. carolinensis* and *A. sagrei* chromosomes were flow sorted and painting probes produced. PCR-assisted mapping on sorted chromosomes allowed the identification of the X specific region in microchromosomes of *A. carolinensis* and in the macro X-chromosome of *A. sagrei*. The paints from *A. carolinensis* sex chromosomes were used in experiments of chromosome painting on eight species of *Anolis* (*A. allisoni, A. bartschi, A. cybotes, A. equestris, A. pogus, A. sagrei, A. sabanus, A. valencienni*) representing the phylogeny of this genus. The results show that in the species with a derivative complement (2n= 28-30) and heteromorphic sex chromosomes (either XY or X_1X_2Y), the A. carolinensis paints always marked the Xs and the Y. In the 4 species with a complement similar to that of *A. carolinensis* (2n = 36; 12 macro- and 24 microchromosomes), the *A. carolinensis* paints always marked three chromosomes in males and four in females. This asymmetry could be the result of Y divergence that would hamper the hybridisation of the Y-probe.

In conclusion, the data demonstrate that surprisingly, despite the morphological variability, the *Anolis* sex chromosomes are united by sharing a sex chromosome specific region. It will be of interest to search for sex determining genes in this region and extend the characterization of this region to other squamate species. This research was supported by PRIN2009/20093HYH97 grant from MIUR (Italy) to VCB.

TOAD GRASSHOPPERS (PAMPFAGIDAE, ORTHOPTERA) AS NEW MODEL OF SEX CHROMOSOME EVOLUTION

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Most of the grasshopper species have X0 sex determination system (White, 1973; Hewitt, 1979). However NeoX NeoY sex determination system occurs as result of centric fusion of autosome and X chromosome. Usually this translocation is observed in distinct taxa (Bidau et al., 2011) but in Pamphagidae family many species have NeoX NeoY sex determination system. Karyotype of XX/X0 species in this group consists from 19 acrocentric autosomes and acrocentric X chromosome while NeoX/NeoY species have 18 acrocentric autosomes, submetacentric NeoX chromosome and acrocentric NeoY chromosome of variable length In some of Pamphagidae species NeoY chromosome is from fully homologous to long arm of NeoX chromosome in other it is small chromosome with large pericentric C-positive block (Bugrov, Warchalowska-Sliwa, 1997; Bugrov, Grozeva, 1998). Presence of NeoY chromosome on different stages of size reduction makes Pamphagidae family perspective model of sex chromosome evolution.

In this research DNA probes were generated by microdissection of sex chromosomes of different species. FISH of NeoY DNA probe with chromosomes of same species revealed strong signal in pericentric C-positive block on small NeoY chromosome and weak signal in other C-positive and euchromatic regions. Distal part of NeoX chromosome of showed homology to NeoY DNA probe. Cross-hybridization of obtained probes showed no homology with C-positive blocks on NeoY chromosomes of close related species. Computer analysis of cross-hybridization images revealed homology between autosomal arm of NeoX chromosome and NeoY of close related species.

We suggest that reduction of NeoY chromosome size in Pamphagidae was due to deletion of euchromatic part of NeoY chromosome accompanied by DNA amplification which leaded to formation of pericentric C-positive block. No hybridization signal in pericentric C-positive regions of autosomes and NeoX chromosome suggests that repeats in NeoY chromosomes evolved independently from other C-positive regions. Cross-hybridization image analysis data suggests that most likely NeoX NeoY chromosomes in Pamphaginae subfamily derived from fusion of same chromosome elements but further evolution of repetitive sequences in C-positive blocks of NeoY chromosome was independent. This work was supported by grant from RFBR № 12-04-01233-a, and grant from OPTEC LLC

THE IMMUNOFLUORESCENT STUDY OF SEX CHROMOSOME SYNAPSIS AND RECOMBINATION IN MALE GUPPIES (*Poecilia Reticulata*)

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Whether it is known how the sex chromosome pairing difficulties are overcome in mammalian male meiosis, the ways of solving this problem in evolutionary young sex chromosome systems of many lower vertebrates remain poorly studied. The study of sex chromosome meiotic behavior is an important tool that may throw light on their structure and function. For this study we have chosen the guppy, which has an XY sex determination system. Guppy acrocentric sex chromosomes were previously shown to consist of a proximal homologous segment and distal heteromorphic segment. They are differentiated enough to be recognized in the meiotic cell, but their meiotic behavior was studied only briefly. To visualize the meiotic chromosomes and recombination points we used the antibodies for synaptonemal complex lateral element protein (SYCP3) and the protein of mature recombination nodules (MLH1). Centromeres and sex bivalents were identified by the modified DAPI staining technique.
We found that pairing between X and Y starts later than between autosomes, and the longer Y-chromosome shortens and thickens until reaching the length equal to that of the X-chromosome. It was suggested that the pairing is initiated at the proximal homologous segment, and late adjustment occurs at distal heteromorphic segment. Our results indicate that the synapsis progresses in opposite direction, i.e. the non-homologous pairing of the heteromorphic segment precedes the homologous pairing of the proximal segment. The recombination nodules at the sex bivalent are located mostly near its distal end, as the nodules at the autosomal bivalents. It was thought that the distal part of guppy Y-chromosome is chromosome-specific and does not recombine. According to our results, the non-recombining part is sub-terminal, thus the guppy Y-chromosome has two homologous regions separated by the non-homologous one.
Thus, we found that in male guppy sex chromosomes synapse at non-homologous region prior to homologous region and recombine at distal segment. The discovered patterns are similar to that of autosomes, indicating lesser degree of differentiation that was suggested.

HIGH HETEROMORPHISM BETWEEN HETEROCHROMATIN OF CHROMOSOMES Z AND W OF *Pseudis tocantins* (ANURA, HYLIDAE)

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Pseudis tocantins is the only species of its genus to show heteromorphic sex chromosomes. Consequently, this group is interesting for the study of sex chromosome differentiation. On its long arm (q), chromosome Z of *P. tocantins* bears an interstitial NOR, which is adjacent to a pericentromeric heterochromatic band. Wq is larger than Zq due to a larger heterochromatic band, which is adjacent to a pericentromeric NOR. Although paracentric inversion and heterochromatin amplification could have occurred during the differentiation of these chromosomes, little is known about the heterochromatic blocks that differ in size between them. Methods To better characterise these sex chromosomes, we employed Comparative Genome Hybridisation (CGH) and chromosome painting with probes generated from microdissected Z chromosomes. Using PCR, we also isolated, from both female genomic DNA and microdissected Z chromosomes, a number of DNA sequences that belong to a satellite DNA family known as PcP190EcoRI, originally described for the anuran *Physalaemus cuvieri* and possibly derived from 5S rDNA. The PcP190EcoRI and 5S rDNA sequences were mapped on the *P. tocantins* karyotype.
CGH identified a Wq-specific region, coincident with its heterochromatic band. The probe generated from the microdissected Z chromosomes did not enable the detection of the entire chromosome but did reveal a region in the heterochromatic segment of Zq. In contrast, this probe produced no hybridisation signal in the W chromosome. The PcP190EcoRI sequences isolated from female genomic DNA differed from those isolated from the microdissected Z chromosomes, with only an approximately 71% nucleotide similarity between them. Curiously, when used as probes in the FISH experiments, the PcP190EcoRI variant isolated from female genomic DNA exclusively revealed the heterochromatic block of Wq and produced no hybridisation signal in chromosome Z. A 5S rDNA sequence of 760 bp was mapped distally in 5q, and its nucleotide sequence differed greatly from that of PcP190EcoRI.
CGH and FISH with probes obtained from microdissected Z chromosomes showed that the heterochromatic blocks of the long arms of chromosomes Z and W differ in not only size but also composition. Chromosomal mapping on Wq of the PcP190EcoRI variant isolated from

female genomic DNA suggested that, at least with respect to the number of repeats, this sequence may have played a role in the differentiation of the sex chromosomes of *P. tocantins*.
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NEW INSIGHTS ON THE SEX CHROMOSOMES OF *Physalaemus rphippifer* (ANURA, LEPTODACTYLIDAE)

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Cytogenetic analyses of Brazilian specimens of *Physalaemus ephippifer* have revealed interpopulational variations that can be used to distinguish among three karyotypes according to the NOR-bearing chromosome pair 8. Although in certain populations (such as that from Viruá, in Roraima State), the short arm (p) of chromosome 8 bears an NOR, which is adjacent to a C-band, in other populations (such as that from Alenquer, in Pará State), an additional NOR, which is located close to the telomere, is also present in the long arm (q) of chromosome 8. A third karyotype is observed in the population from Belém municipality, in Pará State, in which pair 8 was recognized as the sex chromosome pair. The chromosome W observed in this population differs from chromosome Z by the presence of an additional segment in the short arm, composed of a distal NOR and an adjacent terminal C-band. Zq and Wq bear a distal NOR, and no difference between these chromosome arms has been reported to date. A better characterization of these chromosomes Z and W, as well as chromosome 8 of the other populations, is important to investigate their common origin and the evolutionary differentiation of the sex chromosomes Z and W.
Methods A probe (Zqter probe) for the segment located between the NOR and the telomere of the Zq of males from Belém was generated from 22 microdissected segments using the GenomePlex Single Cell WGA Kit (Sigma-Aldrich). The digoxigenin-labeled probe was hybridized to the karyotypes of specimens from Belém, Viruá and Alenquer. Results In the female karyotype of *P. ephippifer* from Belém, the Zqter probe detected the segment between the NOR and the telomere of Zq, but no probe signal was observed in chromosome W. In metaphases from males from Viruá and Alenquer, the Zqter probe detected the heterochromatic region adjacent to the pericentromeric NOR in 8p, and no signal was observed in 8q.
The lack of a Zqter probe signal in Wq suggests that Zq and Wq are dissimilar, offering a contrary finding from what could be inferred from classical cytogenetics. Interestingly, the results obtained for the karyotypes found in Viruá and Alenquer suggest that the 8p (and not 8q) of these karyotypes are most likely homeologous to Zq of the karyotype found in Belém, though the 8q found in Alenquer bears a distal NOR similarly to Zq. This finding is in agreement with the detection of a satellite DNA present in the NORs of Zq, Wq and Wp in the NOR of 8p but not in the NOR of 8q of the specimens from Alenquer.

CONSERVED SEX DETERMINATION ACROSS ADAPTIVELY RADIATED *Anolis* LIZARDS

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Although recent molecular-cytogenetic studies of the rate of chromosomal rearrangement suggested large chromosomal conservatism in squamate reptiles, wide variability in sex determining systems is documented in this group. However, studies testing homology of sex chromosomes across and within major squamate lineages are still infrequent. The aim of this study is to perform comparative analysis of homology of sex chromosomes across *Anolis* lizards, the highly diversified clade, which went through the extensive adaptive radiation.
Using qRT-PCR, we demonstrated X-linkage of five genes, known to be X-linked in *Anolis carolinensis*, in other 17 species representing most of the phylogenetic diversity of the genus *Anolis*. All tested X-linked genes proved to have twice as high doses in female in comparison to male genomic DNA relatively to the dose of a house-keeping gene EF1a and several tested autosomal loci linked to the chromosome 6 in *A. carolinensis*. We demonstrated that this X-linkage is shared in all tested species of anoles as well as in *Petrosaurus thalassinus* and two species of the genus *Sceloporus* (Phrynosomatidae) serving as outgroups.
Based on phylogenetic distribution of species included into our project, we can conclude that presence of sex chromosomes homologous with sex chromosomes of *A. carolinensis* was the ancestral state in the genus *Anolis* and that this state is still widely conserved across Anolis radiation. The sex linkage of tested genes in the outgroups also suggests that the homology of sex chromosomes might be common characteristic and likely ancestral situation in all iguanas (Pleurodonta).
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AN INHERITED X CHROMOSOME ABERRATION IN THREE RELATED MARES

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Fertility problems in female horses are often associated with monosomy or trisomy of the X chromosome. In addition, XY sex-reversal has been shown to be a frequent cause for lack of fertility in mares. A mare that was mated several times to different stallions but never got pregnant was presented to our clinic. Her mother had a similar history of fertility problems. We could prepare chromosomes from the patient\'s mother, the patient and a half-sister of the patient.
Chromosome banding by the CBG-technique revealed the lack of the heterochromatic band (Xq21) on one of the X chromosomes. Using fluorescence *in situ* hybridization (FISH) with an equine Y chromosome painting probe, which also hybridizes to the heterochromatic band on the long arm of the X chromosome, showed weaker hybridization signals on this X chromosome. The same result was observed in all three mares ananlyzed.
The deletion might influence the function of genes located in this region. DIAPH2 is a good candidate gene because it may play a role in the development and correct function of ovaries.

ABERRANT 'SEX CHROMATIN' FORMED BY B CHROMOSOME IN THE MEDITERRANEAN FLOUR MOTH, *Ephestia kuehniella* (LEPIDOPTERA)

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Most lepidopteran species have a WZ/ZZ sex chromosome system. In female interphase nuclei, the W chromosome forms a heterochromatin body, the so-called W-chromatin or sex-chromatin. The sex-chromatin can be easily seen in highly polyploid cells of Malpighian tubules or silk glands, where it consists of multiple copies of the W chromosome. In females of *Ephestia kuehniella*, most somatic tissues show only one sex-chromatin body per cell. The male cells display no sex-chromatin.
Recently, we observed aberrant 'sex-chromatin' bodies in some individuals of the *E. kuehniella* wild-type strain WT-C02. Their morphology was similar to sex-chromatin, but the bodies were smaller, occurred in both sexes, and their number varied between individuals. Preparations of mitotic and meiotic chromosomes from the bodies-carrying larvae revealed the presence of one to several heterochromatic supernumerary elements, i.e. B chromosomes. To find out the origin of the B chromosomes, we performed fluorescent *in situ* hybridization (FISH) with two types of painting probes prepared by laser microdissection, a W-chromatin-derived probe and a B-probe derived from the heterochromatin bodies. The W-probe painted the whole W chromosome but did not hybridize to B chromosomes. The B-probe painted all B chromosomes, demonstrating thus their common origin, but surprisingly did not identify any other chromosome or chromosomal segment. We also performed FISH with an 18S rDNA probe and a (TTAGG)n telomeric probe. None of these probes hybridized to B chromosomes. The absence of telomeric repeats was verified by Southern hybridization with the same result. Nevertheless, positive results of Southern hybridization of the B-probe to genomic DNAs extracted from B-free individuals support the intraspecific origin of the B chromosomes.
Despite the great similarity, heterochromatin bodies observed in interphase nuclei of the WT-C02 individuals of *E. kuehniella* have nothing in common with the W-chromatin. We have demonstrated that these bodies are derived from copies of a single B chromosome. This B chromosome does not contain major rDNA. Its ends are not protected by TTAGG telomeric repeats, and yet the B persists for many generations in the WT-C02 strain without any obvious changes. Although some results indicate that the B chromosome could arise from the genome of *E. kuehniella*, its true origin remains to be elucidated.

PLANT SEX CHROMOSOMES: STRUCTURE, FUNCTION, AND EVOLUTION

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Heteromorphic sex chromosomes have evolved only in few species of dioecious plants. More generally, dioecy is not so common in plants as in animals. In the XX/XY animal systems the Y chromosome is largely degenerated due to loss of recombination with the X chromosome. Some recent data show that the Y chromosome in plants displays some indications of degeneration: accumulation of retroelements, microsatellites, and other repeats.

We analysed the nuclear genome of two model dioecious plants – white campion (*Silene latifolia*) and sorrel (*Rumex acetosa*). These genomes were low-pass 454-sequenced from male and female samples and the data obtained were analysed and compared. Several retroelements and other repeats revealed were used as probes for FISH mapping. We also performed the GISH experiments to estimate phylogenetic relationship among related plant species. To analyze sexual dimorphism in *S. latifolia*, RT-PCR technique was used. While the mass sequencing data show a close similarity of sexes in *S. latifolia*, there are big changes in male genome contrary to female genome in the other model dioecious plant *R. acetosa*. Together with heterochromatic character of the sorrel Ys it seems that the sex chromosomes in this species are much evolutionary older than in other dioecious plants. We also performed a phylogenetic analysis of sex determination in *Silene* species. Dioecious species occur in the genus *Silene* at least in two distinct sections – *Elisanthe* and *Otites*. In the *Elisanthe* section the pair of heteromorphic sex chromosomes comes from the same pair of autosomes. Our AFLP mapping analysis has shown that it is not true for a representative of the *Otites* section – *S. colpophylla*. Its pair of sex chromosomes is homomorphic and originated from another pair of autosomes. Moreover, our data indicate that in *S. otites* there is a ZW/ZZ (F/M) sex determination system with heterogametic females.

Highly polymorphic sex determination systems in the genus *Silene* show that evolution of dioecy is at the beginning and tries to evolve in different ways. We have also searched available databases whether there exist genes that are differently expressed in male and female plants early during vegetative stage of development. Using RT-PCR approach out of 60 genes tested we have found that a majority of them is ubiquitinously expressed, but one gene was specifically expressed only in female leaves and two other genes were expressed exclusively in male leaves. This is the first molecular evidence of early pre-flowering sexual dimorphism in angiosperms.

NUCLEAR ORGANIZATION

AND DYNAMICS

LONG-RANGE, DIRECTED MOVEMENT OF HSP70 TRANSGENES TOWARDS NUCLEAR SPECKLES ACCOMPANIED BY CHROMATIN STRETCHING AFTER TRANSCRIPTIONAL ACTIVATION

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Association of gene loci with specific nuclear compartments increasingly is proposed as a possible mechanism for regulating gene expression. However, nearly nothing is known about how gene loci become associated with different nuclear compartments after changes in gene activity. Previously we showed that tethering acidic activators to a peripheral chromosome site led to an apparently active movement of the tagged chromosome site away from the nuclear periphery. However, the physiological relevance of these results was not clear given the highly artificial nature of this system. Using live cell imaging, we are now directly visualizing the dynamics of Hsp70 transgene arrays, tagged with the Lac operator/repressor system, relative to nuclear speckles, tagged with a eGFP-SON fusion protein, after Hsp70 transcriptional activation. We are also visualizing Hsp70 transcriptional activity using the MS2 RNA labeling system. Examining a plasmid Hsp70 transgene array, we observed increased interphase movements of transgenes after heat shock, with net movements from ~0.5 – 6 μm after heat shock in ~50% of cells in which the transgene array before heat shock was not already near a nuclear speckle. Using mCherry-CENPA tagged centromeres as fiducial marks, we verified that these observations represented true, long-range chromosome movements and were not the result of nuclear rotation or shape changes. Average transgene velocities during periods of long-range movements ranged from 1-2 μm/min. Long-range movements were typically directional, moving towards pre-existing nuclear speckles, and frequently accompanied by visible transgene stretching parallel to the direction of movement, suggesting an active, force generating mechanism. Experiments now in progress are aimed at temporally correlating transcriptional activity with speckle association.

CONTROL OF CHROMOSOME DUPLICATION IN MAMMALS

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Half a century ago the elucidation of the DNA structure was quickly followed by the visualization of replicons in DNA fibers. These very high-resolution analyses were followed by a painstaking effort to catalog the regulatory and enzymatic machinery required for the precise and complete duplication of cellular DNA. Translation of these molecular findings onto the context of intact cells have been hindered by the inherent cellular complexicity and the inability to resolve individual structural and functional replication units by conventional labeling/imaging technology.

To connect 1D DNA replication information with whole cell 3D data over the entire S-phase in mammalian cells, we combined super-resolution 3D structured illumination microscopy and quantitative time-lapse analysis of S-phase dynamics with DNA replication fiber and genome size analyses.

We found that the subnuclear replication structures can be optically resolved down to single replicons during all S-phase stages, which sets aside the conventional view of replication foci as complex entities corresponding to clustered replicons. Further, our data suggest that S-phase dynamics is primarily dictated by chromatin folding and synthetic replisome complexes assemble on template DNA. Accordingly, individual replicons within the chromatin context and not replicon clusters represent the basic unit of DNA replication. Combining experimental data and theoretical modeling we developed a minimal comprehensive model for DNA replication in mammalian cells that can reproduce the observed temporal progression of genome replication and its spatial dynamics in cells.

NON-CODING RNA POLYMERASE II TRANSCRIPTS ARE REQUIRED FOR THE STRUCTURAL AND FUNCTIONAL INTEGRITY OF THE NUCLEOLUS

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The synthesis of rRNAs by RNA polymerase I in the nucleolus is a tightly regulated process that links active transcription of a subfraction of rDNA genes to cell proliferation. By high-resolution confocal fluorescence microscopy imaging, we observed that inhibition of RNA polymerase II (RNAP II) transcription in mammalian cells led to the disassembly of nucleoli into sub-structures. This structural defect correlated with a strong reduction of rRNA production, revealing a linkage of intact nucleolus structure with its rRNA synthesis function. By microinjecting purified nucleoli-associated RNAs we could demonstrate that these RNAs were able to partially rescue nucleoli disassembly in cells treated with RNAP II inhibitors. Thus, purified nucleoli contain RNAP II transcripts that are essential for maintaining a functional nucleolus structure. To identify the nature of these transcripts we conducted a deep sequencing analysis in the presence/absence of RNAP II inhibitors. The RNAP II-dependent nucleolar transcripts identified in this manner were then knocked-down to evaluate their structural function in the nucleoli. With this approach, we identified specific non-coding RNA sequences, the expression of which was required for both the compact structure of the nucleolus as well as for rRNA synthesis. Our results reveal a previously unknown link between RNAP II and RNAP I activity, and provide a mechanism that could couple the regulation of rRNA production to cues from the cell environment. Furthermore, the inhibition of rRNA synthesis by knockdown of non-coding RNAs might represent a novel approach to target proliferating cells in anti-cancer therapies.

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VISUALIZING GENE CONVERSION AND MEIOTIC RECOMBINATION IN *Arabidopsis*

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Chromosomes are amazingly dynamic molecules that undergo intricate conformational changes and movements during each phase of the cell cycle. Among the most significant dynamic changes they experience is the process of recombination (crossovers – CO, and gene conversions – GCs) during meiosis. In most organisms COs are required to ensure proper chromosome segregation, and together with GCs serve to provide allelic diversity among gametes and progeny. Errors in these processes can have catastrophic consequences ranging from infertility, genome instability, cancer and aneuploidy. Previously we developed a novel visual assay system that enables us to detect and quantify recombination events directly in the gametes of the model plant *Arabidopsis thaliana*. Our assay, called the Fluorescent Tagged Line (or FTL) system, uses transgenes encoding fluorescent marker proteins regulated by a pollen-specific post-meiotic promoter inserted across the genome of *qrt1-2*/*qrt1-2* plants. Here we will describe the use of the FTL system to provide a genome-wide characterization of gene conversion events, and to uncover novel genes involved in meiotic recombination. We will also describe improvements to the FTL system that enable high throughput flow cytometry-based analysis of COs, CO interference and GC events.

CELL CYCLE CONTROL OF CHROMOSOME SEGREGATION

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The error-free segregation of duplicated chromosomes during cell division is critical for the development and health of all organisms. In humans, extra or missing copies of chromosomes (aneuploidies) are common causes of genetic disorders and birth defects, while chromosomal instability (defined as chromosomal aberrations that change over time) is typical of most cancers. Chromosomal instability is generally correlated with increased malignancy and likely to favor not only tumor progression but also the emergence of resistance to anti-cancer therapy. Many chromosome aberrations in tumor cells are thought to result from deregulation of the molecular machinery that controls chromosome segregation during M phase. This stage of the cell cycle is governed largely by posttranslational mechanisms, notably protein phosphorylation and ubiquitin-dependent proteolysis. Central to chromosome segregation is the mitotic spindle, a highly dynamic microtubule-based structure that captures chromosomes through centromere-associated kinetochores. In turn, the bipolarity of the mitotic spindle critically depends on the correct number of centrosomes; these function as microtubule organizing centers throughout cell cycle progression and need to be duplicated exactly once in every cell cycle.

To understand the regulatory circuits and multi-protein complexes that govern chromosome segregation and the centrosome duplication cycle, we combine cell and molecular biology with proteomics approaches and advanced microscopy to analyze key components of human kinetochores and centrosomes, as well as the spindle apparatus. In my talk, I will summarize my laboratory's recent work on the regulation of kinetochore-microtubule interactions by mitotic protein kinases, with a particular focus on the tripartite Ska complex, the KMN network and the role of Aurora B in the correction of attachment errors.

We propose that cooperation between the tripartite Ska complex and the KMN-network, a well-characterized microtubule-binding module, is essential for stable end-on attachment of microtubules to chromosomal kinetochores and hence for the error-free segregation of chromosomes during cell division.

MEIOTIC CHROMOSOME PAIRING IN *Sordaria macrospora*. INTERPLAY BETWEEN CHROMOSOME STRUCTURE AND RECOMBINATION

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During meiosis dynamic changes in chromosomes disposition within the nucleus include homolog juxtaposition that culminates in formation of synaptonemal complex (SC) at pachytene, formation and elimination of the bouquet configuration, and disassembly of SC accompanied by global chromosomes reorganization and completely decondensed chromatin, the so-called "diffuse stage".
After DNA replication meiotic prophase is devoted to chiasmata formation that, in conjunction with cohesion between sister chromatids, provide the linkages required for correct orientation on the spindle and accurate segregation of homologous chromosomes at the first meiotic division.
Chiasmata are cytological manifestations of DNA crossovers (COs), recombination events between homologous chromosomes. Meiotic recombination begins via programmed DSBs and local biochemical changes at DNA level occur in close temporal and structural coordination with changes in chromosome structure.
The filamentous ascomycete *Sordaria macrospora* is a particularly favorable organism to analyze interrelations between chromosomes structure and recombination as stages of alignment of homologous chromosomes occur in a clean progression, with all chromosomes aligned and chromosomal axes completely formed immediately after DNA replication and thus before formation of the SCs.
Basic events of meiosis have been examined in *Sordaria* studying proteins necessary for recombination and formation of chromosome structure. These include the DSB transesterase Spo11, the RecA homolog Rad51, DNA-helicases, proteins specifically involved in crossing-overs formation, and proteins components of the chromosomal axes. The emerging results confirm the intricate connections between recombination and chromosome structure. Topologically regular alignment of homologous chromosomes is strictly related to regular recombination events and different molecules operate at different stages, even independently of their enzymatic role in recombination, to promote regular progression of meiosis.
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DYNAMIC AND STOCHASTIC EPIGENETIC STATE SWITCHING STUDIED IN ENGINEERED MAMMALIAN CELL SYSTEMS

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Chromatin-mediated epigenetic gene regulation is crucial for cellular identity in higher eukaryotes. The epigenetic state plays an important role in directing gene expression variability, achieving long-lasting, unperturbed expression states. We study the molecular mechanisms of dynamic and stochastic epigenetic network behavior in different cells in a clonal population. Such dynamic nature could explain the lack of conclusive understanding of epigenetic regulation since most observations assume cells are in phase and are based on cell-population averages.

We use engineered mammalian cell systems allowing to modulate the (epi)genetic state at a single gene-cassette integrated at predefined regions of the genome and measure input-output relationships of (epi)genetic regulation in multiple cells in a population. We developed techniques allowing systematic, quantitative measurements of changes in transcriptional activity as function of the epigenetic state in single living mammalian cells with high-precision microscopy combined with the development of stochastic modeling-technology. In addition, we determine the statistics of cellular and nuclear RNA concentrations in single cells using single molecule-RNA FISH in combination with nuclear/cytoplasmic volume measurements.

We show that modulation of the epigenetic state of an integrated gene-cassette through targeting of epigenetic regulatory proteins such as Heterochromatin protein 1 (HP1) and Methyl-CpG binding protein 2 (MeCP2) enables the induction of epigenetic state-switching.

Simulation of our stochastic nucleosomal model demonstrates that histone-modification patterns can be established at minute-scale. Strong synergism between sliding and weak recruitment properties of histone-modifying enzymes can induce very stable epigenetic states even in the absence of chromatin looping, whereas chromatin looping introduces epigenetic state-switching between two opposite histone-modification states. Currently, we are experimentally testing such robust dynamic state-switching.

Our approach of stochastic modeling and quantitative sampling in designed mammalian cell systems is proof-of-principle to understand

complex epigenetic regulatory behavior and opens an unexplored field of research with great potential for medicine.

GENOMIC ORGANIZATION OF REPETITIVE DNAS IN THE ASIAN SEABASS (*Lates calcarifer*) GENOME AND THEIR CHROMOSOMAL LOCALIZATION

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As part of the genome project on the Asian seabass, we are generating an inventory of repeat elements of the genome. We identified and characterized several classes of repetitive DNAs through nucleotide sequencing and chromosome mapping.
The karyotype of the Asian seabass showed diploid number of 2n=24 and the karyotype formula of 1M+1SM +11ST+11A (FN=37). In addition to the 24 chromosomes, the karyotype indicated the presence of extra microchromosomes, suggesting the occurrence of B-chromosomes. All chromosome spreads contained various numbers of GC-rich B-chromosomes, whereas AT-rich microchromosomes were detected in 50% of spreads. We characterized a few repeat elements corresponding to tandem and dispersed class. Satellite-like tandem repeats shared a similarity with satellite DNA of other species and mapped on the chromosomes. Satellite DNA were clustered in the pericentromeric (periCEN) heterochromatin of all chromosomes, but some of them were accumulated in pritelomeric (priTel) and periCEN regions of the several pairs of the chromosomes. Telomere signals were associated only with ends of all chromosomes. The other group of tandem repeats was the rRNA multigene family.
FISH signal for 5S rDNA was located on chromosome 6, whereas that for 18S rDNA was found on the chromosomes 3 and 14 in the periCEN region. We sequenced two pairs of BAC clones that were pulled out from a library with 5S and 18S rRNA-derive primers. Their assembly revealed the genomic organization of the periCEN regions. Among retransposon elements, the Rex group is the most common in the different fishes species. Using specific primers, we cloned Rex1 and mapped on the periCEN and priTel areas of all chromosomes. Sequence analysis of the regions containing rRNA conserved units have shown that unit of rRNA located between different dispersed repetitive elements, including Rex3 and Tc1/mariner complex.
Several classes of repetitive DNA are identified in the Asian seabass genome. All of these have transcripts found in our Asian seabass transcriptome assembly. B-chromosomes of the Asian seabass are organised by a heterochromatin different from that were identified in the periCEN regions of the somatic chromosomes. The analysis of various repetitive elements confirmed that repetitive sequences form specific

patterns of compartmentalization on the individual chromosomes. Synteny analysis of the regions containing rRNA with those of other fish species showed the presence of conserved features.

TRANSCRIPTIONAL ACTIVITY OF TRANSPOSABLE ELEMENTS IN COELACANTH

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Coelacanths are considered to be the most basal extant relatives of the terrestrial vertebrates occupying a pivotal position in the evolution. Moreover these "living fossils" show a seemingly conservative morphology especially from the Triassic onwards, a morphological stasis suggesting a slow evolutionary rate was recently confirmed by the genome sequencing of the African coelacanth *Latimeria chalumnae* (Amemiya et al., 2013). Since bradytelic genome evolution might indicate a decrease of transposable element activity, the transcription of genome repeats was evaluated through next generation techniques RNA-sequencing analyses. Transcriptomic data of *L. chalumnae* muscle and of Indonesian congener *L. menadoensis* liver and testis were compared.

The mapping of sequencing reads to the repeat library used to annotate the African coelacanth genome highlighted a significant percentage of transposable element transcriptional activity in both species. Major contributors are Long Interspersed Nuclear Element (LINE) retrotransposons, especially from the CR1 family; some particular elements such as LF-SINE and L2 LINE sequences seem to be more expressed than other elements. Data showed differences between tissues: this variability often depended on the presence of a transposable element insertion into the UTRs of tissue-specific genes highly expressed. This mechanism is particularly important in the gonads, where the high expression level of a L2 LINE element might represent the beginning of a new transpositional burst in successive generations. This analysis allowed also to identify a new *Latimeria*-specific family of Short Interspersed Nuclear Elements called CoeG-SINEs highly repeated in the genome, undergoing a possible transcriptional silencing.

Overall, our results represent a fascinating snapshot of genome dynamicity showing the transcriptional activity of transposable elements in a genome considered as frozen in time.

REPETITIVE DNA FAMILIES FROM B-CHROMOSOMES ARE COMMON AMONG DIFFERENT SPECIES OF *Cestrum* (SOLANACEAE)

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Some plant groups present a large variability in the repetitive DNA accumulation and distribution. *Cestrum* (Solanaceae) is one of these groups. Although their species possesses stability in the diploid number ($2n = 2x = 16$) and chromosome morphology, there are a large diversity in the types and distribution of heterochromatin. These ones can be organized in: i) C-Giemsa$^+$ bands, ii) CMA$^+$ bands associated or not with 45S or 5S rDNA, (iii) DAPI$^+$ bands associated or not with CSRs (cold-sensitive regions), and (iv) CMA$^+$/DAPI$^+$ bands. B-chromosomes have been described in four species of *Cestrum* (*C. parqui, C. parqui* × *C. aurantiacum, C. intermedium* and *C. strigilatum*). For all of them, some repetitive families occurring in the Bs were identified, such as AT-rich SSR, 45S and 5S rDNA, C-Giemsa and C-CMA/DAPI bands and *Ty*3-*gypsy*-like retroelements. In this study, the B-chromosome of *C. strigilatum* was microdissected and amplified using random primers. The aim was to produce a micro-library for probes obtaining, sequencing and FISH. The results showed that although all the B-chromosome was hybridized by the probe, it was evident that the long arm exhibited a greater accumulation of signal. Besides, various hybridization signals were detected adjacent to DAPI bands in the A-chromosomes. Were still found hybridization sites located at pericentromeric region of the pair 8, adjacent the 5S rDNA. These data indicated that A and B-chromosomes share similar sequences. When we did the FISH with B-chromosome probe of *C. strigilatum* against the chromosomes of *C. mariquitense*, several signals adjacent to heterochromatin were detected. These data showed that the accumulation and dispersion of repetitive DNA families are dynamic events in the genomes of *Cestrum*. Although of significant contribution of repetitive DNAs for the intra- and interspecific karyotype differentiation in species of this genus, there are some repetitive families that still are shared by different species. These data can also indicate that the initial proposal for independent origin of Bs in *Cestrum* can be changed.

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NEW DATA FROM *Danio rerio* CONFIRMED V-SINEs AS POTENTIAL miRNA TARGETS

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Short interspersed nuclear elements (SINEs) are members of class 1 transposable elements (retrotransposons), that are present in most eukaryotic organisms. SINEs are chimeras of transcribed RNA genes and simple repeats that proliferate, via reverse transcription, by using the enzymatic machinery of autonomous elements. Particularly, the V-SINEs superfamily includes a central conserved domain preceded by a 5'-end tRNA-related region and followed by a potentially recombinogenic (TG)(n) tract, with a 3' tail derived from the 3' untranslated region (UTR) of the corresponding LINE. We studied 40 chromosome location potentially embedding V-SINE regions in *D. rerio*, available in ENSEMBL data base, together with three *D. rerio* validated V-SINE sequences, from NCBI.
The canonical G+C/A+T rate was calculated and resulted of 0.5417, suggesting a relative low stability of the V-SINE regions. Two different cladograms were then realized, by using both experimental V-SINE consensus and the three confirmed V-SINEs, by using the Kimura's distance. The distance plot for each of the three validated sequence versus each of the predicted one was realized. After having select the predicted V-SINEs sequences more clusterized with the validated ones, we tested them as potential targets of microRNAs (miRNAs), a small evolutionarily conserved non-coding RNAs that seem to post-transcriptionally regulate the expression of thousands of genes in a broad range of organisms, in both normal physiological and disease contexts, usually interacting with specific nucleotide motifs in the 3' UTR of a target mRNA, in a one-to many relatio. In our work, an *in silico* hybridization was performed by applying RNAhybrid program (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/). The Mean Free Energy (MFE) of miRNA:V-SINEs was estimated with the maximum Microcosm target score. The screening was carried out by using three different miRNAs: dre-let7a.1, dre-mir2193 and dre-mir193b, all retrieved from Mirbase (www.mirbase.org). Dre let-7a.1 is one of more conserved member of let-7 family, while conversely dre-mir2193 is specific for *D. rerio* species and dre-mir193b seems to be prone to interact with V-SINEs. Moreover, two recognized miRNA target 3' UTR gene regions, Zgc 162180 and Crsp 7 were compared with the data set.
The results obtained allowed to hypothesize that V-SINE gene associated can be involved in post-trascriptional regulation by short non-coding RNAs and represent a potential target of action of different miRNAs.

DINOFLAGELLATE RIBOSOMAL GENES ORGANIZATION AND CHROMOSOMAL STRUCTURE

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Dinoflagellates are unicellular eukaryotic algae best known by their capacity to reach very high cell densities in coastal areas causing so-called "red tides". These red tides may can cause severe anoxia and thus have severe negative effects on the ecosystem. Additionally, they can also result in serious human health consequences, as many dinoflagellate species produce several types of potent toxins that may accumulate in the food chain. Numerous studies have focused on the ecological factors triggering red tides, but very little is known about the cytological mechanisms involved. Information on dinoflagellate chromosome structure and organization during the cell cycle is scarce. The main reason for this lack of knowledge is probably the difficulty in studying dinoflagellate genomes: they contain huge amounts of DNA per cell, which can be up to 70 times higher than in human cells.

It had previously been suggested that in dinoflagellates chromosomes are both permanently condensed through the cell cycle and morphologically identical. In contrast, our studies revealed that dinoflagellate chromosomes show different levels of condensation during the cellular cycle. At the maximum condensation level (metaphases) different chromosome morphologies are visible. Also, we found inter-species differences: whereas *A. minutum* shows big amounts of ribosomal genes clustered on non-differentiated chromosomes, *A. catenella* appears to have specialized chromosomes on which the genes are located. The genus *Karenia* and *Karlodinium* show more typical eukaryotic patterns for ribosomal gene location, with *Karenia brevis* and *Karenia selliformis* having apparently double chromosomal number than *Karlodinium mikimotoi* and *Karlodinium veneficum*.

Several assumptions about dinoflagellates chromosomes need to be revised. Additionally, we show that NOR patterns are species-specific and in groups containing huge amounts of DNA show novel eukaryotic distribution.

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A MORE RELAXED NUCLEAR ORGANISATION OBSERVED IN PHA STIMULATED HUMAN LYMPHOCYTES: A 2D AND 3D EVALUATION

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Chromosomes occupy distinct positions in the interphase nucleus termed chromosome territories (CTs). This position seems to underlie a functional significance (i.e. gene regulation). Nuclear architecture is dependent on cell type, cell cycle etc. The aim of this study was to investigate whether differences in organisation of all 24 human chromosomes in lymphocytes can be observed in presence and absence of phytohaemagglutinin (PHA) utilizing both 2D and 3D approaches. Peripheral blood was obtained from a healthy male and female subject and cultured using standard protocols with and without PHA. Individuals were karyotyped to exclude chromosomal abnormalities. Fluorescence *in situ* hybridisation for all 24 human chromosomes was utilized. A custom algorithm evaluated chromosome position in 2D based on fluorescence signal intensity. Chi-squared was used to measure statistical significance. 3D data was collected using wide-field microscopy and rendering and assessment of the distance of the CT to the nearest nuclear edge was performed using Imaris software.
2D analysis of CT position in the female and male sample without PHA stimulation revealed 21/23 and 21/24 territories exhibited nonrandom organisation compared to 16/23 and 14/24 in PHA stimulated cells respectively. Five CTs (3, 7, 9, 11, 13) in the female and 7 CTs in the male (4, 5, 7, 9, 11, 18, Y) switched from nonrandom (no PHA) to a more random (plus PHA) position. Preliminary data from 8 CTs in 3D shows a movement of more peripherally located CTs (no PHA) towards more central positions (13, 18 ,20) compared to PHA stimulated cells. More centrally located CTs in PHA stimulated cells also exhibited a preferentially more interior location compared to un-stimulated cells. In addition, a considerable increase (approximately 2-3 fold) in cell and CT volume was observed in PHA stimulated cells.
This study provides evidence of a more defined organisation in cells lacking PHA compared to activated cells. In addition, nuclear and CT enlargement was observed in activated cells, this finding may be attributed to the activation of cells leading to a higher degree of chromatin decondensation. The less defined nuclear organization in PHA activated cells could be linked with an adaptation of CTs to the transcriptional activation profile occurring by PHA and the need for certain chromosomes to be flexible in order to establish "active" chromosome neighborhoods.

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HLXB9 GENE EXPRESSION, AND NUCLEAR LOCATION DURING IN VITRO DIFFERENTIATION OF NEUROBLASTOMA CELLS

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The homeobox gene HLXB9 is constitutively expressed in young children with Acute Myeloid Leukemia (AML) by virtue of the chromosomal translocation t(7;12). Moreover, HLXB9 was identified as a locus involved in the autosomal dominant Currarino Syndrome, also known as Hereditary Sacral Agenesis (HAS) syndrome. The HLXB9 gene is also involved in the development of pancreatic beta cell and motor neuronal (MN) cells with an essential role in motor neuronal differentiation. In mice lacking Hlxb9 function, there is an aberrant specification of MN identity associated with defects in the migration of MNs and in the projection of motor axons.
Here we analysed the correlation between nuclear location of HLXB9 and its expression level during in vitro differentiation of a neuroblastoma cell line. We analysed the in vitro differentiation from replicative to differentiated cells after induction with retinoic acids, and we evaluated, at time 0, 6 and 12 days after start of differentiation, the nuclear localization of HLXB9 obtained by 2D analysis of fluorescence *in situ* hybridization using a probe containing the entire gene. The expression levels of the HLXB9 gene during cell differentiation was done by real time quantitative PCR. We observed during differentiation a significant change in the nuclear position of the HLXB9 gene, that moved from a peripheral location in the replicative cells, to a more internal position during differentiation. This result reflects the different expression level of the gene as obtained by real time PCR.
These findings lead to consider HLXB9 not only as a marker of development and consolidation of motor neuron, but more generally, as a marker of the early stages of neuronal differentiation, also involving a chromatin remodelling pathway, in addition to known markers that are found in completely differentiated neurons as GAP-43, NF, MAP2, TAU.

CELL-CYCLE DYNAMICS OF PERICENTROMERIC HETEROCHROMATIN IN HUMAN CELLS

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The interphase nucleus is a heterogeneous and compartmentalized organelle. Nuclear organization contributes to the regulation of major cellular functions: replication, repair and transcription of the genome. This organization is dynamic and varies during cell-cycle and differentiation. The underlying mechanisms are still a matter of extensive investigation. Several experimental studies, mostly conducted in mouse, have shown that the centromeric regions (CR), that are composed of tandemly repeated DNA sequences, have a specific organization in the interphase nucleus. CR could play a major role in shaping the spatial organization of the genome by interacting with stable nuclear structures, but also with each other, forming structures called chromocenters. Few studies have been conducted on human CR, possibly due to the complexity of the underlying DNA sequences.
We have developed new experimental approaches in order to accurately and systematically describe the organization of CR in human cells, taking into account progression through the cell-cycle. LNA-modified probes are used for the specific detection of various centromeric repeats with a single nucleotide resolution. The results of immuno-FISH experiments are analyzed using TANGO (Ollion et al. Bioinformatics 2013, http://biophysique.mnhn.fr/tango/), a generic and open-source framework we developed for high-throughput analysis of 3D fluorescence images dedicated to the study of nuclear organization. By studying the position of specific centromeres with respect to the nuclear periphery and nucleoli, we have shown that CR display chromosome-specific behaviors that are highly dynamic during the cell-cycle. All studied CR showed an increased nucleolar association frequency in cycling cells compared to quiescent cells. We also have shown that human CR associate with each other forming an heterochromatic compartment similar to mouse chromocenters.
Our experimental approach combined with high-troughput analysis allowed us to define observables describing the spatial organization of CR through the cell-cycle. This will allow us to characterize its modifications in response to the inhibition of candidate genes in order to understand the underlying mechanisms.

CHROMATIN MODIFICATIONS AND REPLICATION DYNAMICS OF HETEROCHROMATIN FROM VOLE RODENTS

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Some vole species are a remarkable model for heterochromatin investigation in mammals. They have a genome enriched in heterochromatin that arranges at pericentromeric regions and, notably, in large blocks coupled to the sex chromosomes. The last ones are an identifiable landmark during the cell cycle due to their prominent condensation easily visualized at DAPI staining. While DNA content has been extensively analyzed little is known about the epigenetic modifications controlling structure and dynamic of heterochromatin in voles. In order to better understand how it functions and is organized within the nucleus, with especial focus on the sex heterochromatin, we have performed a detailed characterization by immunofluorescence of several epigenetic marks in cells from two species, *Microtus cabreare* and *Microtus agrestis*. Furthermore, we have assessed the replication dynamics of sex heterochromatin by a combination of procedures including pulses labeling analyses with modified nucleotides, immunostaining and live cell time lapse microscopy using fluorescent tagged proteins.
We have first analyzed the pattern of three hallmarks of constitutive heterochromatin: H3K9me3, Heterochromatic Protein 1 (HP1) and cytosine methylation. In interphase cells from both species heterochromatic blocks are enriched in H3K9me3 but lack HP1 and present reduced levels of methylation at cytosine residues. Histone modifications defining facultative heterochromatin, H3K27me3 and macroH2A, are also observed but do not overlap with H3K9me3 signals in female cells. As expected, heterochromatic blocks are depleted on typical euchromatin marks H3K4me2 and H4K8ac. Our assays have characterized the temporal order of replication, divided into several consecutive patterns based on labeling with the replication factor PCNA, and clearly defined the replication timing of sex heterochromatin to mid-late S phase. During mid S-phase large replication structures colocalized with H3K27me3 and macroH2A signals, while H3K9me3 stained-heterochromatin shows replication labeling subsequently at late S-phase.
Heterochromatic blocks from the sex chromosomes of voles display a distinctive pattern of epigenetic marks during interphase as they lack two main features of constitutive heterochromatin, HP1 protein and DNA methylation. Accordingly both marks seem not necessary to their correct organization and package within interphase nucleus. This unusual type of

heterochromatin however follows the dogma of replication during S phase, occurring at mid-late S-phase.

THE *Arabidopsis* CONDENSIN CAP-D SUBUNITS ARE REQUIRED FOR CORRECT CHROMATIN ORGANIZATION, GROWTH AND FERTILITY

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In plants as in other eukaryotes, the SMC (Structural Maintenance of Chromosome) protein complexes cohesin, condensin and SMC5/6 are essential for sister chromatid cohesion, chromosome condensation, DNA repair and recombination. The presence of paralogous genes for various components of the different SMC complexes allowed the diversification of their biological functions during the evolution of higher plants.
In *Arabidopsis thaliana* we identified two candidate genes (Cap-D2 and Cap-D3) expressing conserved proteins associated with condensin. *In silico* analyses suggest that both genes are controlled by factors acting in a cell cycle-dependent manner. Cap-D2 is essential because homozygous T-DNA insertion mutants were not viable. The heterozygous mutant showed wild-type growth habit but reduced fertility. For Cap-D3 we selected two homozygous mutants expressing truncated transcripts that are obviously not fully functional. Both mutants show reduced pollen fertility and seed set (one of them also reduced plant vigour), a lower chromatin density and frequent (peri)centromere association in interphase nuclei. Sister chromatid cohesion was impaired compared to wild-type in cap-D3 but not cap-D2 mutants. At high resolution (Structured Illumination Microscopy) we found for both cap-D mutants no alteration of the chromatin substructure. The presence of CAP-D3 and of the cohesin subunit SMC3, both forming reticulate structures in 2C-16C nuclei, suggests that cohesin and condensin complexes are important for interphase chromatin architecture in differentiated nuclei.
The results suggest that CAP-D proteins are required for fertility, growth, chromatin organization, sister chromatid cohesion and in a process preventing the association of centromeric repeats.

ORGANIZATION OF TELOMERES WITHIN THE HUMAN SPERM NUCLEUS: AN ESSENTIAL REQUIREMENT FOR NORMAL FERTILIZATION AND EMBRYOGENESIS?

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In a wide variety of cell types there is a distinct evolutionarily conserved higher order chromatin organisation within the interphase nucleus. We hypothesize that chromatin organisation within the sperm nucleus may be crucial for fertilization and normal embryogenesis. Previously, we have demonstrated organisation of 6 whole chromosomes within the sperm nucleus. The purpose of this study was to evaluate whether we can observe specific localisation of smaller discrete regions of the genome, specifically the telomeres. Nine normozoospermic males were recruited. Telomere organisation for chromosomes 18, 19, 21, 22 and X within spermatozoa was performed utilizing fluorescence in-situ hybridisation. Telomere organisation was assessed via two means: radial (interior-peripheral) and polar (head-tail). A total of 9,000 sperm nuclei were examined (100 cells/chromosome/subject/analysis). The Chi-squared goodness-of-fit test was performed to determine nonrandom organisation of telomeres.

All telomeres tested exhibited nonrandom organisation for both radial and polar analysis ($p<0.05$), with the exception of the telomeres for chromosome 18 (which displayed a random organisation in one subject). A distinct hierarchy of telomere position was identified both radially and longitudinally. Our findings indicate a preferential radial nuclear organisation of telomeres with respect to the nuclear interior to the periphery (22, X, 19, 21 and 18 respectively) and longitudinally from the sperm head to the tail (19, X, 22, 21 & 18 respectively).

Preferential longitudinal and polar organisation was observed for all investigated telomeres with the exception of chromosome 18 in one subject. Paternal chromosomes are hypothesised to be withdrawn from the nucleus in a sequential order allowing gradual exposure of chromosomes to the ooplasm. This gradual decondensation and remodeling of specific regions could contribute to the differential gene activation patterns within the early embryo. This study provides evidence of nonrandom organisation of human telomeres within the sperm nuclei. This hierarchical organisation of chromatin suggests that those telomeric regions occupying the apical head region and the nuclear periphery are more likely to be exposed and activated first. We hypothesise that alterations within this organisation may be associated with infertility and abnormal embryogenesis. Support: NM NIH/NIGMS R25 GM061347

ALTERED CHROMATIN PACKAGING IN MALE FACTOR INFERTILITY

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Within the nucleus, chromatin is packaged in a highly organised manner. Chromosomes are not randomly distributed, but rather occupy distinct chromosome territories. This strict order of nuclear organisation participates in the regulation of expression patterns and therefore deviation results in a variety of complications such as cancer, laminopathies and infertility. In the human sperm cell chromosomes adopt a 'chromocentric' model of nuclear organisation, where centromeres form a 'chromocenter' and telomeres cluster around the nuclear periphery. Such organisation in the sperm cell is essential for correct fertilisation events and early embryogenesis. Using Fluorescence *in situ* Hybridisation (FISH) experiments and Chromomycin A3 (CMA3) staining, we have studied telomere localisation and protamine deficiency in sperm nuclei from oligoasthenozoospermic (OAT) males compared to fertile control males.
Results show altered telomere staining patterns and increased positive CMA3 staining in OAT males compared to fertile control donors.
From these experiments we can conclude that chromatin packaging is altered in males with severely compromised semen parameters. These data provide insights into the complex nature of male factor infertility and reiterate the importance of highly specialised nuclear organisation in sperm fertilisation potential.
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EPIGENETICS AND GENE EXPRESSION

EFFECTS OF VPA AND TSA TREATMENT ON HEPG2 CELLS UNDER NORMOGLYCEMIC AND HYPERGLYCEMIC CONDITIONS

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Valproic acid (VPA) and trichostatin A (TSA) are known histone deacetylase (HDAC) inhibitors, whose epigenetic modulation is strategic for the development of new therapeutic approaches. Recent reports suggest HDAC inhibition as a possible treatment for diabetes mellitus, preventing destruction of pancreatic beta cells and ameliorating the organism hyperglycemic state.
Under normoglycemic conditions, a reduction on chromatin packing states concomitant with an increase in nuclear area and perimeter were observed on VPA- and TSA-treated cells. Results suggestive of increased H3K9ac and H3K4me2 levels and decreased H3K9me2 levels were also promoted by VPA and TSA. However, under hyperglycemic conditions, sometimes a decrease in nuclear area and perimeter was observed, but no chromatin remodeling was detected, following treatments. Furthermore, in comparison to untreated controls, the H3K9ac level was unchanged or decreased, while the H3K9me2 level increased. Comparison between normoglycemic and hyperglycemic controls revealed chromatin decondensation accompanied by higher H3K9ac and lower H3K9me2 abundance in the latter.
These results indicate that, under normoglycemic conditions, inhibition of HDAC in HepG2 cells induces chromatin remodeling and alteration in the histone methylation state. Nonetheless, under hyperglycemic conditions in which the epigenetic characteristics here studied are already altered in a similar way as those provided by treatments with VPA and TSA in normoglycemic controls, HDAC inhibition does not seem to affect them or, in some cases, these characteristics are even becoming similar to a control situation.

SISTER CHROMATID EXCHANGE (SCE) IN TWO ENDANGERED PIG BREEDS RAISED IN SOUTHERN-ITALY

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Chromosome stability ensures that genetic information is correctly transmitted during the DNA replication and cell proliferation. Several studies report a variable rate of genome stability according to the livestock species analysed. Sister chromatid exchange (SCE) test has been largely applied to test chromosome stability in animal cells naturally exposed to environmental mutagens present in the food chain or during *in vitro* cell exposure. SCE-mean number may vary among and between species depending by species, breed and environmental conditions.

In this study, representative groups of animals of two endangered pig breeds (Casertana and Siciliana black) were analyzed by using SCE-test to check their chromosome stability under their normal breeding and environmental conditions. 28 pigs, 16 males and 12 females from Casertana breed (from 3 different farms), and 18 pigs, 8 males and 10 females, from Nero Siciliano breed (from two farms) were studied. 5-Bromodeoxyuridine was added to cell cultures 26 hours before harvesting. Colcemid (0.1 µg/10 ml) was added 1.5 h before harvesting. SCE mean numbers were 6,54± 2,86 and, 6,56± 2,9 in Casertana and Siciliana pig breeds, respectively. No statistical differences were found between the two breeds, neither between males or females within the same breed.

The data obtained in the two pig breeds reveals that SCE-mean value in pig is one of the lowest compared to other livestock species studied so far supporting the hypothesis that pig genome is more stable than that of other species, probably depending also for the young age of animal breeding of this species, compared to that of other domestic species.

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EVOLUTION AND FUNCTION OF NON-SMC SUBUNITS OF SMC5/6 COMPLEX

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Structural Maintenance of Chromosomes (SMC) proteins are highly conserved from bacteria to humans and play fundamental roles in chromosome dynamics, gene regulation and DNA repair. SMC1/3 (cohesin) complex is involved in sister chromatid cohesion, SMC2/4 (condensin) contributes to chromosome condensation and SMC5/6 complex is involved in several DNA repair pathways. SMC5/6 heterodimer associates with four non-SMC elements (Nse).. The human Nse3 ortholog MAGEG1, founding member of MAGE (Melanoma Antigen Gene) protein family, interacts with both NSE1 (RING-finger containing protein) and NSE4 subunits. The NSE1-MAGEG1-NSE4 subcomplex bridges the head domains of Smc5-Smc6 heterodimer.

Using yeast two-hybrid and co-immunoprecipitation experiments we have analyzed the ability of human MAGE proteins to form NSE1-MAGE-NSE4 subcomplexes. Most of MAGE proteins retained the ability to bind members of NSE4 family (NSE4a, NSE4b, EID1, EID2 and EID2b) suggesting a tight evolutionary relationship between these families but binding with NSE1 (RING-finger) protein has diverged. Using mutagenesis we have identified a conserved hydrophobic surface within the C-terminal domain of Nse3/MAGE that interacts with Nse4 orthologs that binds to the above Nse3/MAGE surface. Using luciferase assay in HEK293 cells we have shown that different MAGE-EID pairs can affect transcription.

In our work we have determined interacting domains of Nse3/MAGEG1 and Nse4/EID proteins. The conservation of interacting surface suggests their tight co-evolution. Functional analysis showed involvement of both protein families in transcription regulation and uncovered one possible function of enigmatic MAGE proteins.

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EPIGENETIC MODIFICATIONS OF PLANT CENTROMERIC CHROMATIN INVESTIGATED USING META-POLYCENTRIC CHROMOSOMES OF *Pisum sativum*

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Pea (*Pisum sativum* L., 2n=14) chromosomes are exceptional in the structure of their centromeric regions which appear to be intermediates between monocentric and polycentric types. The so-called meta-polycentric chromosomes show extended primary constrictions which contain 3-5 separate clusters of the centromeric histone variant CenH3. Large pericentromeric regions and multiple CenH3 loci make pea chromosomes suitable for analyzing the distribution of various epigenetic modifications that are supposed to play an important role in the determination of (peri)centromeric chromatin.
Chromosome suspensions obtained from synchronized, formaldehyde-fixed root tip meristems were attached to slides by centrifugation and subjected to immunodetection of various epigenetic modifications of histones H2A and H3 using specific antibodies. These were combined with pea CenH3 antibodies to visualize the position of functional centromere domains. Chromosomes were examined by fluorescence wide-field or Structured Illumination Microscopy (SIM) to achieve an optical resolution of approximately100 nm. Results While there was no specific epigenetic profile detected for the core centromeric (CenH3-containing) chromatin, several histone methylation and phosphorylation marks clearly distinguish pericentromeric chromatin from that of chromosome arms.
Because of its distinct centromere organization and size, pea chromosomes are ideal models for visualizing histone modifications across primary constrictions of mitotic chromosomes. The observed distinct epigenetic profile of the pericentromere is indicative of its important role in centromere determination and maintenance.

CONTRIBUTION OF HISTONE HYPOACETYLATION AND HYPERMETHYLATION TO THE MAINTENANCE OF THE CONDENSED STATE OF THE CHROMOCENTER HETEROCHROMATIN OF *Triatoma infestans*, VECTOR OF THE CHAGAS' DISEASE

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Triatoma infestans is characterized by several particular cell biology characteristics, including presence of holokinetic chromosomes and development of unusual meiotic process, somatic polyploidy, nuclear fusion, deep resistance to starvation, and conspicuous heterochromatin bodies (chromocenters) where DNA methylation has not been detected. Whether or not histone modifications have a role on the condensed state of these bodies has not yet been studied. Histone epigenetic marks in chromocenters and euchromatin as well as chromatin remodeling induced by valproic acid (VPA) and sodium butyrate (NaBt), inhibitors of histone deacetylases (HDACis), were investigated here as a contribution to the knowledge of the chromatin structure of this insect.

Modifications in histones H3 and H4 were compared between the heterochromatin and the euchromatin of fully-nourished nymphs of *T. infestans* by immunocytochemistry. The effect of various VPA and NaBt concentrations on chromocenter condensation was studied morphologically and microspectrophotometrically. The insect survival and molting rates in nymphs was accompanied 155 days after their treatment with the HDACis. Acetylated H3K9, H4K8 and H4K16 residues and mono- and dimethylated H3K9 and H4K20 residues were mostly observed in the euchromatin. Trimethylated H3K9 signals were deeper in the chromocenters. Decondensation of the chromocenter chromatin occurred under specific VPA and NaBt treatments. The insect survival and molting rates were slightly reduced following treatment with 1.0 mM VPA.

Based on histone epigenetic marks, chromatin remodeling induced by VPA and NaBt, and no previous indication of DNA methylation in the chromocenters, histone hypoacetylation and hypermethylation are suggested as significantly contributing to the maintenance of the condensed state of the chromocenter heterochromatin in *T. infestans*.

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LOSS OF SPECIFIC REPETITIVE SEQUENCES IN *Arabidopsis thaliana* FAS X RAD51B MUTANT PLANTS

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The Chromatin Assembly Factor 1 (CAF1) is one of the most important chromatin remodelers. This histone H3/H4 chaperone is involved in replication-dependent nucleosome assembly as well as in DNA replication and repair like nucleotide excision, homologous recombination or non-homologous end joining. CAF1 complex is very well conserved, it is composed of three subunits, in *Arabidopsis thaliana* are called as FASCIATA 1 (FAS1), FASCIATA 2 (FAS2) and MULTICOPY SUPPRESSOR OF IRA1 (MSI1). Our previous data showed that deletion of FAS1 or FAS2 gene leads to a severe and specific loss of two kinds of tandem repeats in A. thaliana – 45S ribosomal DNA units and telomeres.

To clarify the underlying mechanism of this phenomenon we combined the FAS knock out with knock out of candidate genes participating in DNA repair. As FAS mutants were reported to have increased levels of HR and we have previously shown that the rDNA loss and telomere shortening are independent of meiosis, AtRad51B, a paralogue of the Rad51 recombinase, was chosen for this purpose. The level of 45S rDNA and potential changes in expression of other genes participating in HR pathway were explored by qPCR. The telomere lengths were determined by Terminal Restriction Fragment (TRF) analysis and sensitivity of plants to agents causing dsDNA breaks and replication fork stalling was tested. Sites of DNA damage response were visualized by γH2AX and levels of general chromosome rearrangement were studied by counting aberrant anaphases.

The telomere dynamic and impact of this knocked out proteins were observed in four consecutive generations of fas1-/- rad51b-/- and fas2-/- rad51b-/- mutants.

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ANALYSIS OF ITS2 VARIATION AT GENOMIC AND EXPRESSION LEVELS BY MEANS OF 454 SEQUENCING REVEALS TWO HAPLOTYPES BEING SPECIFIC TO B CHROMOSOMES

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Eyprepocnemis plorans is a grasshopper species bearing different supernumerary (B) chromosomes, which harbor variable amounts of ribosomal DNA (rDNA). Although these Bs are heterochromatic and presumably genetically inert, their rDNA has shown activity in some individuals from several Spanish populations as deduced from cytological (nucleolus formation by B) and molecular (detection of B-transcripts) evidences. Here we analyze by 454 Next Generation Sequencing (NGS) the DNA sequence of the ITS2 rDNA in genomic DNA (gDNA) and complementary DNA (cDNA) from 18 *E. plorans* different males from Torrox population: B-lacking, B-carrying with inactive rDNA and B-carrying with active rDNA. We included six nucleotide labels in the primers in order to run the amplicons in 1/8 of a 454 plate.

This yielded a total of 129,349 ITS2 sequences whose analysis revealed the existence of 11 different haplotypes. We quantitatively analyzed the five most prevalent (H1, H2, H3, H4 and H5) and three other less common haplotypes (H6, H7 and H8), constituting 99.84% of the sequence collection. H1, H2 and H8 appeared with similar frequency in the gDNA and cDNA, but H3, H4, H6 and H7 were underrepresented in the cDNA -suggesting underexpression-, and H5 was more frequent in the cDNA than in the gDNA -indicating overexpression-. Remarkably, the frequency of the H4 and H7 haplotypes in gDNA showed significant positive correlation with B chromosome numbers, and were absent in 0B males, thus suggesting that both are B specific. In fact, H4 and H7 are unique in showing the adenine insertion on which previous molecular detection of B-specific transcripts was based. Analysis of B-carrying males showed underexpression of the H4 and H7 haplotypes, matching the fact that only half of them had B-rDNA expression.

Taken together, our results show that the rDNA contained in B chromosomes is highly repressed, with expression levels below average even in males carrying active B chromosomes.

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CHROMOSOME AND GENOME EVOLUTION

THE COMPARTMENTALIZED GENOMES OF VERTEBRATES: A UNIFIED VIEW AND A LINK WITH GENE EXPRESSION

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The first hint at a genome compartmentalization came from the earliest chromosome banding investigations of Caspersson. An approach at the DNA level demonstrated the compositional compartmentalization of vertebrate genomes.
Compositional genomics is a strategy based on base composition and short-sequence frequencies of DNA molecules or sequences. Since short-sequences (*e.g.* trinucleotides) determine the fine structure of DNA and the DNA-protein interactions, compositional genomics is based on genome structure and function.
The compositional genomic strategy showed that the human genome, a typical mammalian genome, is compartmentalized, like the genomes of other vertebrates (and eukaryotes in general), in an ordered mosaic of isochores. These are long (>300 kb), compositionally fairly homogeneous DNA stretches that belong to a small number of families characterized by different GC levels and different frequencies of short sequences. In turn, GC-poor and GC-rich families belong to two genome spaces, a large, gene-poor, closed chromatin genome desert which replicates late in the cell cycle and a small gene-rich, open chromatin genome core which replicates early. The isochores from the two compartments are clustered into GC-rich and GC-poor macroisochores that are correlated with chromosomal bands and with chromatin structures in the interphase nucleus.
Correlations hold between the base composition of protein-coding sequences and that of contiguous, inter- and intra-genic, non-coding sequences, as well as between the base composition of isochore families and structural/functional genome properties, such as gene density and gene expression. All the compositional correlations represent a set of comprehensive rules that amount to a genomic code.
The two major conclusions are: 1) The relative compositional homogeneity of isochores and the compositional correlations are explained by the observation that they are essential requirements for the expression of protein-coding sequences. This indicates that the regulation of gene expression, which is harbored in the non-coding sequences, is the major determinant of genome structure. 2) The co-variation of all the genome properties tested with GC levels of isochore families (and with each other) imply that the human genome may be visualized as a co-varion. This leads to a unified view of the compartmentalized genomes of vertebrates and other eukaryotes.

THE AVIAN GENOME AND EVOLUTIONARY DYNAMICS. THE ROLE OF HSBS AND EBRS

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Every genome needs a good map (Lewin at al 2009) however modern "next generation" sequencing (NGS) technologies struggle fully to assemble whole genomes *de-novo*. This is disappointing as mapping information on a chromosomal basis brings the opportunity to address a number of fundamental biological questions with regard to genome evolution. Modern molecular genomic research generally applies neo-Darwinian principles to focus on the role of individual genes but the role of whole chromosomes and homologous synteny blocks (HSBs) is often disregarded. This is despite the ubiquity of HSBs and the clear phenotypic consequences and evolutionary implications of chromosome rearrangement. In mammals, there are sufficient fully assembled reference genomes to make assembly of new NGS genomes possible by comparative analysis. For bird species however only three such reference genomes are published (chicken, zebra finch and turkey) and complete chromosomal assembly needs to be achieved by other means. Classical approaches involving karyotyping and fluorescence *in situ* hybridisation (FISH) have provided much needed information about the mechanisms of chromosome evolution in birds. My lab has generated and collated a body of data on the comparative genomics of birds as generated by zoo-FISH. Zoo-FISH is however limited in its level of detail at a genic level; NGS genome assemblies however tend to result in a number of scaffolds (the greater the depth of sequence, the larger the scaffold but rarely extending to the length of a whole chromosome). The combination of karyotyping, zoo-FISH, BAC mapping by FISH and bioinformatics has the potential to assemble genomes to a chromosomal level and thus accurately identify HSBs and Evlutionary Breakpont Regions (EBRs). HSBs and EBRs can be displayed interactively using the "Evolution Highway" browser (Larkin and colleagues, Royal Vet College) and allow us to address fundamental questions pertaining to genome evolution in non-mammalian and mammalian vertebrates. These hypotheses concern the randomness (or otherwise) of HSBs and EBRs, that EBRs coincide with recombination hotspots and that transposable element density is increased in EBRs. Gene ontology studies allow us to generate further hypotheses pertaining to the reasons for the prevalence of HSBs and EBRs in the context of evolution. Comparative cytogenomics in birds and other animals is therefore moving from a largely descriptive science to one in which genome function and organisation is correlated.

THE EVOLUTION OF MAMMALIAN CHROMOSOMES: HYPOTHESES FROM SYNTENIC ASSOCIATIONS

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Cytogeneticists have always had interest in reconstructing ancestral karyotypes. Early workers discussed ancestral karyotypes in terms of diploid and fundamental number. Then ancestral karyotypes were proposed by comparing banded chromosomes. Molecular cytogenetic was a step ahead because homology was established not on morphology, but on DNA content. Chromosome painting used parsimony, commonality and outgroups to determine the syntenies, associations and landmarks present in ancestral genomes. Unfortunately, chromosome painting was unsuccessful between placental mammals and marsupials or monotremes, or birds, or reptiles, even if it works well enough within each of these groups to postulate ancestral karyotypes for each.

An alternative to molecular cytogenetics is to compare sequence assemblies. A virtual map can be made to electronically paint and compare chromosomal homologies between various vertebrate groups. Here we compare the syntenic associations present in placental mammals with those of other vertebrates for which genome assembly data are available including opossum, tammar wallaby, platypus, chicken, and green anole lizard.

It is long been appreciated that the platypus genome has a notable similarity with birds and reptiles. Here we show that this similarity extends to the common syntenic associations between platypus, birds and lizard. Surprisingly, there is also by far a greater similarity in association structure between opossum/tammar with birds and lizards than with placental mammals. Previously, there was more interest in features, which were held in common between marsupials and placentals, but we suggest that it is equally important and perhaps even more informative to look at the ancestral vertebrate associations that are conserved in marsupials and montremes, but lost in placentals.

The loss of the majority of ancient vertebrate associations helps us better appreciate the magnitude of what happened at the origin of placentals. It does not seem to be an exaggeration to suggest that placentals have had a virtual genomic revolution at the association level. We caution that this is a preliminary analysis valuable for speculation and pointing to aspects of genome architecture that might merit further attention.

CHROMOSOMAL ABNORMALITIES IN IVP PRE-IMPLANTATION BOVINE EMBRYOS: AN UP-DATE

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The overall efficiency of the *In Vitro* Embryo Production (IVEP) industry is strongly hampered by the low rate of blastocyst production which, on the average, doesn't exceed 30 %. Chromosomal abnormalities (C.A.) in the developing embryos are thought to be one of the main responsible for such a failure. The present work updates the actual knowledge on the incidence of chromosomal abnormalities in *in vitro* as well as in *in vivo* produced bovine (*Bos taurus L., 2n=60,XY*) embryos by reviewing nearly 30 major cytogenetic papers published since 1968.
C.A.(haploidy+aneuploidy+polyploidy+mixoploidy) have been found to depend not only upon the *in vitro* or *in vivo* conditions but also upon the day of collection, morphological quality, rate of cleavage and development. Under the *in vitro* systems, 1,650 (day 2-3) and 613 (day 7-8) embryos were analyzed, respectively. From day 2-3 to day 7-8, C.A. significantly ($P<0.01$) increase (19.5 *vs* 42.5%); more precisely, haploidy and polyploidy tend to decrease (from 3.0 to 1.9 and from 11.0 to 7.7 %, respectively), mixoploidy tends to increase (from 2.8 to 32.5%) while aneuploidy remains basically unchanged. Under the *in vivo* systems, 161 (day 7-8) and 159 (day 12-18) embryos were analyzed, respectively. From day 7-8 to day 12-18, C.A. significantly ($P<0.01$) increase (19.2 *vs* 43.3%); more precisely, while haploidy , aneuploidy and polyploidy tend to 'zero', mixoploidy dramatically increases (from 13.7 to 42.7%). C.A. were found to be significantly ($P<0.01$) higher in day 7-8 *in vitro* than in day 7-8 *in vivo* embryos (45.2 *vs* 19.2%, respectively). Mixoploidy was the major abnormality found in day 7-8 *in vitro* as well as in day 7-8 *in vivo* embryos (32.5 and 13.7%, respectively). However, when the *in vivo* embryos were analyzed at 12-18 days, the proportion of mixoploidy dramatically increased up to 42.7%. Higher incidences of C.A. were found : (a) in low-quality embryos compared to the good-quality ones, (b) in the late-cleaving embryos compared to the early cleaving and (c) in embryos showing the lowest rate of development. Since mixoploidy concerns cells which are preferentially allocated in the trophectoderm (TE), future cytogenetic analysis should focus on discriminating between TE and inner cell mass (ICM). The importance of ICM derived 'stem cells' for the biomedical industry is also stressed.
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CLINICAL CYTOGENETICS IN HORSES: CASES IN SPANISH PURE BREED (PRE)

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A more common than though percentage of reproductive alterations and unexplained infertilities observed in horses are related to chromosomal abnormalities mainly associated to the sexual pair of chromosomes. However, most of them remain undiagnosed due to the difficulty in this kind of analysis and the lack of interest and knowledge among breeders and veterinarians in these cases of abnormalities. Since a few years ago the laboratory of Animal Cytogenetic of the University of Cordoba is working with the Spanish Purebred (PRE) Breeders Association using a diagnose strategy combining, microsatellite markers and conventional and molecular cytogenetic techniques. All individuals showing abnormal or uncertain results in the Short Tandem Repeats (STR) paternity test, phenotypic abnormalities or unexplained infertility were submitted to a complementary microsatellites panel specifically linked to sexual pair and to cytogenetic analysis. Using this strategy, we detected in the last year nine equines presenting chromosomal abnormalities, including a 64, XX; a sex reversal 64,XX; a 63,X0/64,XY; two cases of 63,X0/64,XY (one mosaicism and one chimerism) and three cases of 64,XY sex reversal. It is interesting that some of these animals didn't show any morphologic or physiologic abnormality in preliminary analyses. However, their chromosome complements were detected as abnormal after the evaluation of the routinely STR markers used as filiations test. In the last year, using a new set of STR markers associated to the sexual chromosomes (5 markers in the ECAX and 4 markers in the ECAY), we improved our detection threshold of sex related chromosomal abnormalities.

The use of a diagnostic approach combining routine parentage QF-PCR based STR screening tests with classical or molecular cytogenetic analysis could be a interesting procedure that allows early detection of chromosomal abnormalities in horses saving time, efforts and breeders' resources.

POLYPLOID GENOME EVOLUTION IN CROP PLANTS

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Polyploidy and whole genome duplication are wide spread in most eukaryotic species, but recent events are found more frequently in plant than mammalian species. Recent advances in deep and whole-genome sequencing of many diverse plant genomes have revealed ancient events occurring hundreds of millions of years ago with strong signatures still remaining in the genomes. Others, in both wild plants and crops, are detected by chromosomal analysis and may have occurred evolutionarily recently, or even in a single generation.

Our group uses fluorescent *in situ* hybridization (FISH) with repetitive and total genomic DNA to elucidate chromosome organisation during evolution, hybrid formation and polyploidization. We study both mitosis and meiosis and combine these cytological analysis with molecular marker and sequencing approaches.

I will discuss the significance of the three rounds of whole genome duplication in the ancestry of banana (*Musa*), occurring between 115 and 65 million years ago, and the modern polyploid events giving triploid and tetraploid cultivars. These events will be compared with those in the lineage leading to hexaploid wheat, and the chromosomal organisation in the triploid *Crocus sativus*, Saffron.

Whole genome duplications enable species separation, genome mutation can occur without loosing functional proteins, and multiple alleles of each gene can be present. However, genome interactions and regulation of meiosis are complicated and despite some advantages, polyploidy may be an evolutionary dead-end.

We thank many visitors and students to our lab and the funding they have received to collaboarate with us. For further information and references see our website at www.molecularcytogenetics.com (www.molcyt.com).

LINKING GENOME SEQUENCE WITH KARYOTYPIC INFORMATION: KARYOTYPE DEFINITION, COMPARATIVE GENOMICS AND FULL GENOME SEQUENCE OF THE GYR FALCON (*Falco rusticolus*)

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Karyotype is essentially a low-resolution map of the genome of any eukaryotic species and thus any whole genome sequence should, ultimately, be accompanied by anchoring to the relevant chromosomes. As sequencing technologies improve, the main advantage of them is their speed and drastically reduced cost; compared to traditional (e.g. Sanger) approaches. A major disadvantage however is that the shorter read lengths make de-novo assembly of hitherto un-described species much more difficult and thus chromosomal information in terms of a basic karyotype is crucial. Also essential is comparative genomics with well-described species (e.g. chicken) to anchor the genome assembly to a known reference. There are about 30 species of falcon in the world and many have huge cultural importance for hunting and sport, especially in the Arabian Peninsula. Among these, Peregrine and gyrfalcon are the most important both appear as endangered on the CITES list. Despite this, the genome of all *Falco* species has remained poorly described in terms of classical cytogenetics comparative genomics and genome sequencing. In this study Gyrfalcon chromosomes have been prepared to produce the first Karyotype and standard ideogram. Chicken chromosome paints have been applied on Gyrfalcon chromosomes to establish inter-specific syntenies between the two species and a whole genome sequencing effort has been initiated.
High quality chromosome preparations were produced that generated distinct banding patterns usind DAPI and propidium Iodide. A karyotype scheme of Gyrfalcon was successfully produced and Zoo FISH was successful using chicken paints for chromosomes 1-9, Z and some microchromosomes displayed a number of fissions and fusions compared to the avian ancestor. Whole genome sequencing produced a coverage of 11x and allowed comparative studies.
The results of this study and comparison to other studies (Nishida et al. 2008) suggested that *Falco rusticolus* (Gyrfalcon) and *Falco tinnunculus* (the common kestrel) have highly rearranged, but similar karyotypes and both represent a common *Falco* ancestor. Future work will focus on linking the genome sequence to the karyotype.

CYTOGENETIC ANALYSIS OF *Anurogryllus* sp. (ENSIFERA, GRYLLIDAE) FROM VIÇOSA, MINAS GERAIS, BRAZIL

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Cytogenetic analysis of *Anurogryllus sp*. collected in Rio Claro (São Paulo, Brazil) evinced intrapopulational karyotype polymorphism originated by Robertsonian fusions from a basic macrochromosomal structure with male diploid number 2n=22+X0, in which all autosomal chromosomes are acro-telocentric and two of them, the ones of the 5th pair, bear nucleolar organizer regions of different sizes. The X chromosome is the largest element of the karyotype and the only metacentric one. Adult males and females, as well as embryos, have karyotypes with homozygous or heterozygous conditions due to presence of one up to six metacentric chromosomes. Silver impregnation of nucleolar organizer regions (AgNORs) showed that transposition of rDNA cistrons also occurs in addition to events of whole arm fusion, increasing the number of chromosome bearing nucleolar activity. Female karyotypes and their offspring embryos confirmed that this population is formed by a species complex with high karyotypic plasticity. The aim of this work was to perform the Chromosome preparations were obtained using embryos, midgut caecum and ovary cells. Embryo mitotic chromosomes were subjected to the AgNOR method or fluorescent *in situ* hybridization (FISH) for detection of rDNA cistrons. Synaptonemal complex (SC) analysis were performed using nymph testicular cells.cytogenetic analysis of Anurogryllus sp. collected in Viçosa (Minas Gerais, Brazil), located 550km away from Rio Claro.
All adults and embryos exhibited male or female diploid numbers of 23(22+X0) or 24(22+XX) chromosomes, respectively. The autosomal set is formed by acro-telocentric chromosomes which slightly decrease in size; the X chromosome is metacentric and the largest of one in the karyotype. FISH analysis evidenciated that the short arms of the 5th autosomal pair bear a variable number of rDNA cistrons. The autosomal bivalents exhibited regular SC formation and the sex chromosome was easily identified in pachytene spreads because of its single axis.
The cytogenetic description of *Anurogryllus sp*. collected in Viçosa matches the basic macrochromosomal organization observed in the previously analysed population from Rio Claro. The variable number of rDNA cistrons bearing the autosomal 5th pair can be explained due to transposition events occurring at the short arm sites, which probably do not synapse but can associate during prophase I

VARIATION IN CHROMOSOMAL RDNA CLUSTERS EXPLAINED BY THE MOLECULAR SIGNATURE OF THE DEMOGRAPHIC PROCESS AND HISTORICAL POPULATION EXPANSION IN *Phyllomedusa hypochondrialis* (ANURA, HYLIDAE)

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This study investigated micro-evolutionary patterns of genomic change based on a comparison of the dynamics of the nucleolar organizer region (NOR) and molecular data on *Phyllomedusa hypochondrialis*, a Neotropical tree frog from the Amazon recently recorded in the Brazilian Cerrado savanna. The diversity of NOR was examined by the Ag-NOR method and FISH approach using the 28SrDNA probe on 166 specimens from 16 populations in the Brazilian Amazon and Cerrado, with samples for molecular analyses being collected at 27 sites. Two datasets were constructed using mitochondrial (ND2 and 16S gene) and nuclear (Rhod and SIA) DNA, used separately in each analysis. A Bayesian population-level phylogeny was reconstructed in MrBayes. Genetic signatures of past demographic processes were identified by the mismatch distribution of pairwise differences in mtDNA. Molecular diversity was quantified using F-statistics (FST) and hierarchical AMOVA. Haplotype networks were constructed in SplitsTree.

The results show NOR diversity among *P. hypochondrialis* populations, with NOR-bearing chromosomes in three major pairs (4, 7 and 8). All NOR sites were detected in pericentromeric regions, a common NOR rearrangement hotspot. Two major clades were recognized: one with the haplotypes from the Amazon rainforest and one with the haplotypes from the open Cerrado habitats. The Amazonian clade had NOR in pair 8, whereas the Cerrado clade presented NOR in pairs 7 and 4. Intra-populational NOR variation was detected in the population from Barreiras (Cerrado clade), Bahia state, with additional NOR sites in one homologue of pairs 3 or 5. Analyses of historical demography indicate significant population expansion within the Cerrado clade. Considerable regional structuring was detected by FST and gene flow among populations was low

As chromosome morphology is very conserved among populations, NOR variation indicates inter-chromosomal transposition in the *P. hypochondrialis* genome, although these rearrangements are not detected cytogenetically. The mismatch distribution indicates a historical population expansion followed by new reproductive niches, which fixed a NOR-bearing chromosome in each subgroup. The molecular signature indicated rapid population in the Cerrado clade of central Brazil,

corroborated by the NOR variability among populations. This scenario indicates high transposition rate in NORs due to the concerted evolution of rRNA genes. We conclude that the dynamics of NOR sequences in the genome has contributed to evolutionary changes in the architecture of *P. hypochondrialis* karyotypes.

RECOMBINATION PLASTICITY IN A WILD MICE ROBERTSONIAN POPULATION

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Understanding the mechanisms underlying speciation has been of special interest in biology. Traditionally it has been considered that chromosomal reorganizations would play a role in the speciation process by, either underdominant fitness effects or by the suppression of recombination in heterokaryotypes. With the aim to better understand the impact, if any, of chromosomal rearrangements in genetic recombination we have analysed the meiotic dynamic in the Barcelona Robertsonian (Rb) polymorphism zone, a wild house mouse population with a wide range of diploid numbers (from 2n=22 to 2n=40) due to the presence of Rb fusions.

Analyses revealed the presence of a high heterogeneity in meiotic recombination events between Rb mice. Moreover, a significant decrease in the number of crossovers per cell was observed in Rb specimens when compared to standard ones. We also observed an alteration of crossovers distribution along the synaptonemal complex towards the telomere in Rb chromosomes compared with acrocentric ones.

Results suggest that the presence of chromosomal rearrangements would reduce the overall recombination rate affecting different meiotic parameters and alter the crossovers distribution in rearranged chromosomes. We hypothesize that the recombination suppression observed in Rb mice could be one of the causes of decreased gene flow observed in this zone of Rb polymorphism.

ON THE ORIGIN OF TETRAPLOID *Hordeum marinum ssp. gussoneanum*

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Hordeum marinum Huds., which possesses the Hordeum Xa-genome, is a complex species that usually is recognized as having two subspecies: *H. marinum* (2n=2x=14) and *H. marinum gussoneanum* (Parl.) Thell. (2n=2x=14, 2n=2x=28). Their relationships, the rank of the taxa, and the origin of the polyploidy forms remain points of debate. The earliest hypothesis supported by a number of cytogenetic studies that analysed C-banded karyotypes and examined chromosome pairing in meiosis indicates that the tetraploids seems to be of autoploid origin. However, allopolyploidy has been strongly supported by molecular phylogenetic analyses using single nuclear markers. Nevertheless, additional cytogenetic analyses are needed to reveal the true genomic constitution of the different *H. marinum* taxa and cytotypes. To understand the genomic constitution of *H. marinum* and infer the parents of the tetraploid form, in the present work we analyse the karyotypes of several *H. marinum* accessions, representing all taxa and cytoypes, applying FISH to reveal the distribution of several DNA sequences (tandemly repetitive Triticeae DNA sequences and SSRs)

Specific chromosomal markers characterise and distinguish the genomes of all taxa and cytotypes. Two subgenomes are detected in the tetraploids. One of these has the same chromosome complement as diploid gussoneanum; the second subgenome, although similar to the chromosome complement of diploid *H. marinum sensu lato*, appear to have no counterpart in the *H. marinum marinum* accessions here analyse. The tetraploid forms of *H. marinum gussoneanum* therefore appear to have come about through a cross between a diploid *H. marinum gussoneanum* progenitor and a second, related - but unidentified - diploid ancestor.

In conclusion, the present results reveal the genome structure of the different *H. marinum* taxa and demonstrate that allopolyploids, which should deserve taxonomic recognition, are segmental in nature with two close related homeologous genomes.

CHROMOSOMAL STUDIES IN *Coscoroba coscoroba* (AVES, ANSERIFORMES) REINFORCE THE *Coscoroba-Cereopsis* CLADE

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Coscoroba swan (*Coscoroba coscoroba*), endemic from southern South America, is traditionally considered a link between tribes or an early branch from the common ancestor leading to true geese and swans. Recently, an interesting association between coscoroba swan and Cape Barren goose (*Cereopsis novaehollandiae*) as sister-groups has been proposed. *C. novaeholladiae* has the most divergent karyotype among Anseriformes, with 2n=92. There are no descriptions of the karyotype of *C. coscoroba*. Methods: Cell cultures were obtained from feather pulp of a female of *Coscoroba coscoroba*. Chromosomes were obtained by standard arrest with colcemid (Gibco), hypotonic treatment with 0.075 M KCl, and cell fixation in methanol/acetic acid (3:1). Initial analyses included conventionally stained metaphases (Giemsa 5% in 0.07 M phosphate buffer, pH6.8). G-banding with trypsin was performed according to Seabright (1971). We used whole chromosome probes of *Gallus gallus* (pairs 1-10 and Z). *In situ* hybridization experiments followed standard protocols as described earlier.
We found a 2n=98 in *Coscoroba coscoroba*, the highest diploid number among Anseriformes so far. Pairs 1 and 2 are biarmed, while the other macrochromosomes are telocentric chromosomes, including the sex chromosomes (figure 1a). The G-banding pattern of the first nine pairs and sex chromosomes are shown in figure 1b. Whole chromosome probes of *Gallus gallus* each hybridized onto one pair of chromosomes, except for probes corresponding to pair 4 of *G. gallus*, which hybridized onto two different chromosome pairs.
C. coscoroba has the highest diploid number for an Anseriforme so far, with 2n=98. *G. gallus* pairs 1-10 were conserved. Hence, the high diploid number found in *C. coscoroba* is a consequence of microchromosome fissions, because macrochromosomes have conserved syntenies, as revealed by FISH using whole chromosome probes. The present study reinforces the phylogenetic proximity of *Coscoroba coscoroba* to *Cereopsis novaehollandiae*, due to their similar diploid numbers.

PECULIARITIES OF OOGENESIS IN HYBRIDOGENETIC EUROPEAN WATER FROG COMPLEX (*Pelophylax esculentus* COMPLEX)

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Hybridogenesis represents one of known clonal ways by which interspecies hybrids can be reproduced. During hybridogenesis, gametogenesis proceeds with deviations, when one of the parental genomes can be eliminated while the other one can be endoreduplicated. Among natural interspecies hybrids, *Pelophylax esculentus* as a hybrid between P. ridibundus (RR genome, n=13) and *P. lessonae* (LL genome, n=13) serves as an appropriate model for studying of hybridogenesis. To study the features of female gametogenesis associated with hybridogenesis we analyzed the karyotypes transmitted in growing oocytes of di- and triploid *P. esculentus*.

We characterized oocyte karyotypes of 9 diploid (RL) and 14 triploid (11 frogs with RRL genotype and 3 – with LLR genotype) hybrid females with genome composition being determined by DNA flow cytometry. Genomes transmitted in oocytes of hybrid frogs were identified according to differences between sets of lampbrush chromosomes. We found that 9 triploid RRL hybrids produced oocytes with 13 bivalents similar to P. ridibundus chromosomes while oocytes of triploid RLL frogs contained 13 bivalents identical to *P. lessonae* chromosomes. Thus, in germ cells of the majority of triploids, genome represented in one copy was eliminated, while two remaining genomes formed 13 bivalents. Most diploid hybrids produced oocytes with 13 bivalents, 26 bivalents and 26 univalents similar to *P. ridibundus* chromosomes. To form such oocytes, genome of *P. lessonae* was eliminated in germ cells of diploid hybrids while genome of *P. ridibundus* was endoreduplicated ones or even two times. In oocytes of two triploid RRL frogs we observed 39 bi- and univalents where 26 bi- or univalents identical to *P. ridibundus* chromosomes and 13 bi- or univalents corresponded to *P. lessonae* chromosomes. Three diploid hybrids produced oocytes with 26 bivalents and 26 univalents, where either 13 bi- or univalents corresponded to chromosomes of each parental species. To form 39 bivalents in oocytes of triploid frogs and 26 bivalents with chromosomes of both parental species in oocytes of diploid frogs, whole genome endoreduplication should have been occurred. Neither endoreduplication nor elimination occurred during oogenesis to form oocytes with 39 univalents in triploid frogs and 26 univalents with chromosomes of both parental species in diploid hybrids.

The results obtained allow to evaluate presence of endoreduplication and elimination during gametogenesis in *P. esculentus* females.

TWO *Genlisea* SPECIES WITH AN EIGHTEEN-FOLD GENOME SIZE DIFFERENCE – A CYTOGENETIC CHARACTERIZATION

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Flowering plants vary more than 2,300- fold in their genome size from 63.6 Mbp in *Genlisea aurea* [1] to 150.000 Mbp in *Paris japonica* [2]. The monophyletic carnivorous genus *Genlisea* (Lentibulariaceae), comprising at least 29 species, is characterized by a striking plasticity of genome size (25-fold). This is the largest range found within a genus so far. This feature makes *Genlisea* an interesting subject to study mechanisms of genome and karyotype evolution. However, cytogenetic data were largely lacking so far.
For *G. nigrocaulis* (86 Mbp) with one of the smallest plant genomes and the 18-fold larger genome of *G. hispidula* we determined the chromosome numbers and investigated the chromosomal constitution regarding heterochromatin and the sub-nuclear distribution of DNA and histone methylation marks. The proportion and chromosomal distribution of rDNA and telomere sequences as well as of diverse retrotransposons and newly identified tandem repeats including putative centromeric sequences were analyzed by fluorescent *in situ* hybridization.
The obtained data not only provide for the first time an overview on cytological characteristics of *G. nigrocaulis* and *G. hispidula* but also allow in combination with ongoing genomic approaches to elucidate mechanisms responsible for shrinking and expanding of genomes within the genus.

[1] Greilhuber et al, Plant Biol 2006, 8: 770-777.
[2] Pellicer et al, Bot J Linn Soc 2010, 164: 10-15.

COMPARATIVE ANALYSIS OF SEQUENCE COMPOSITION AND STRUCTURE OF CENTROMERES BETWEEN GARDEN PEA (*Pisum sativum*) AND ITS WILD RELATIVE *P. fulvum*

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An unusual organization of functional centromeres has recently been described for pea (*Pisum sativum*) mitotic chromosomes, consisting of 3-5 distinct CenH3 domains embedded within extended primary constrictions. Contrary to most species studied so far, pea centromeres are not composed of a single type of satellite repeat, but there are thirteen families of satellite repeats associated with CenH3 domains, differing in their monomer length and sequence composition. There is no family present on all chromosomes and individual CenH3 domains on a single chromosome often contain different repeats.
Since similar, multi-domain structure was also detected in centromeres of the related species *P. fulvum* using CenH3 antibody, a comparative analysis of these two species was performed in order to investigate how they differ with respect to associated satellite repeats. Repetitive sequences were identified using similarity-based clustering of genomic NGS reads and repeats associated with CenH3 domains were revealed using ChIP-seq. Although overall repeat composition of the two species is very similar, the spectrum of satellites associated with centromeric chromatin is partially different. Work is in progress to identify homeologous chromosomes by investigating *P. sativum* x *P. fulvum* hybrids in order to follow evolutionary changes in centromeres of individual chromosomes.
This approach has a great potential to reveal evolutionary dynamics of peculiar pea centromeres and provide insight into the role of satellite sequences in organization and evolution of centromeres in plants.
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CNV MAP OF THE JAPANESE QUAIL GENOME

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Chicken and Japanese quail are evolutionary close avian species, representatives of Galliformes order, Phasianidae family. They have very similar karyotypes (2n=78) with a high degree of conservation suggesting the absence of interchromosomal rearrangements. In contrast, numerous intrachromosomal rearrangements have accompanied their karyotype evolution. Accumulation of repetitive sequences has probably led to the increased size of quail genome (1.41pg in Japanese quail vs. 1.25 pg in chicken). Copy number changes (gain or loss of DNA) could reflect genome evolutionary plasticity. CNV is a form of structural variations displaying copy number differences of DNA stretches larger than 1 kb.

Comparative genomic hybridization on chicken CGH 385K whole-genome tiling arrays (Roche NimbleGen) was performed to reveal a global variation in Japanese quail genome. Using this method of molecular cytogenetics we have evaluated unbalanced copy number changes (gains/losses) in quail DNA (the test) relative to Red Jungle Fowl DNA (the reference). In total, 183 copy number variants (CNVs) were detected in two quail individuals. Overlapping CNVs form 87 discrete copy number variable regions (CNVRs), of which 59 are losses and 28 gains. Some CNVRs completely or partially overlap with 289 annotated genes and are enriched with four transcription factor binding sites. On the whole, CNV regions are detectable in most chicken chromosome assemblies (except for 15, 17, 19, 32) and cover 9.41 Mb.

It is interesting that Japanese quail and chicken being diverged 35 million years have much more CNVs if compared to phylogenetically distant turkey, duck and zebra finch (Volker et al 2010). To date, four avian interspecific CNV maps have been successfully built including our study. Remarkably, genomes of Japanese quail and chicken reveal much more CNVs if compared to phylogenetically distant ones of turkey, duck and zebra finch. But CNVs are of a comparable size in the four species. A majority of CNVs are lineage-specific CNV-prone regions unique to Japanese quail. At the same time, one fifth of all quail CNV regions are present in duck, turkey and zebra finch. Some of shared CNV regions contain known chromosomal breakpoints and several genes. Altogether, available data on avian CNV comparative maps and finding of common CNV regions in five bird species support the CNV hotspot hypothesis (Skinner, Griffin 2012).

COMPARATIVE GENOMICS BETWEEN PIG, CATTLE AND HUMAN: ESTABLISHING PRECISE CHROMOSOMAL SYNTENIES USING GENALYZER AND FISH

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The availability of genome sequence information for an organism allows genomic analysis from a chromosomal perspective ("chromonomics"). Significant progress in human, pig and cattle genomics has been made in recent years, allowing the determination of evolutionary breakpoint regions (EBRs), intrachromosomal rearrangements and homologous synteny blocks (HSBs) by *in silico* analysis; for example the pig genome is now it's tenth iteration. While it is possible to determine the precise location of EBRs by zoo-FISH using BACs, such studies are time-consuming and arduous. With the opportunity to access free online sequence information as well as the availability of sequence analysis tools, both HSBs and EBRs can be mapped precisely, with FISH used only for confirmation. Here the available sequence information for human, pig and cattle was successfully compared and contrasted by the use of the program GenAlyzer. Selected pig/human syntenies were confirmed independently by FISH using BACs designed to map to the EBRs.
In total 115 evolutionary breakpoint regions were identified between pig and human, 184 between pig and cattle and 149 between human and cattle and selected pig/human syntenies were independently confirmed by FISH.
Data generated has provided us with reasonable confidence that GenAlyzer data represents an accurate indicator of the real biological comparisons between the species in this study.
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CHROMOSOME PAINTING IN BARLEY – A NEW MILESTONE IN CYTOGENETICS OF CEREALS

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Chromosome painting describes fluorescence *in situ* hybridization with chromosome-specific, composite probes to label selected chromosomes in metaphase and interphase. This procedure developed into a major tool in clinical and research molecular cytogenetics. It has been successfully applied in many animals and contributed to the identification of structural chromosome changes that accompanied their evolution and speciation. The attempts to utilize chromosome painting with composite chromosome probes in plants failed, mainly due to presence of dispersed repeats in painting probes.

Here we describe a novel approach suitable for chromosome painting in plants with large genomes that are composed mainly of repetitive DNA sequences. We used barley chromosome 1H as a model. The method relies on the ability to prepare chromosome painting probes composed mainly from low-copy coding sequences. DNA for the probe preparation was isolated using gene capture from DNA of flow-sorted chromosome 1H. Illumina sequencing confirmed significant enrichment of the captured DNA for sequences specific for 1H. The use of highly specific probe in combination with a large amount of blocking DNA resulted in specific labeling of barley chromosome 1H when applied to barley mitotic metaphase spreads.

These results open avenues for a wider use of chromosome painting in plants with large genomes and represent the first step towards chromosome painting in interphase nuclei to study their 3D organization and its dynamics.

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THE B CHROMOSOME OF RYE IS A BY-PRODUCT WHOLE GENOME EVOLUTION AND IS RICH IN TRANSCRIPTIONAL ACTIVE PSEUDOGENE-LIKE FRAGMENTS

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Supernumerary B chromosomes (Bs) are optional additions to the basic set of A chromosomes (As), and occur in all eukaryotic groups. They differ from the basic complement in morphology, pairing behaviour, and inheritance, and are not required for the normal growth and development. The current view is that B chromosomes are parasitic elements and comparable to selfish DNA like transposons. Although Bs were described first a century ago, little is known about their origin and molecular make-up. We provide insights into the origin, evolution and drive mechanism of a rye B chromosome.
Bs of rye are rich in gene-derived sequences, which allowed us to trace their origin to fragments of A chromosomes with the largest parts corresponding to rye chromosomes 3R and 7R. Compared to As, Bs were also found to accumulate large amounts of specific repeats and insertions of mitochondria and plastid DNA. About 11% of the B-localized pseudogene-like fragments are transcribed in a tissue-type and genotype-specific manner. In addition, some B-located sequences cause in trans down-regulation of A chromosome-encoded genes.
We propose a comprehensive model of B chromosome evolution, including its origin by recombination of several A chromosomes followed by capturing of additional A-derived and organellar sequences and amplification of B-specific repeats. We conclude that in analogy to the evolution of sex chromosome, rye B evolution is characterized by erosion of its gene content and by accumulation of repetitive DNA. However, in contrast to sex chromosomes, the B is nonessential for the host and therefore requires an accumulation mechanism to counteract purifying selection. usions*

COMPARATIVE GENOMICS AND GENOME EVOLUTION OF AVIAN MICRO-CHROMOSOMES THROUGH THE USE OF NOVEL CHROMOSOME PAINTING TOOLS

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The genome evolution of avian macro-chromosomes has been studied extensively through zoo-FISH however micro-chromosomes have been relatively under-studied due to the lack of probe availability. To date, information is only available for turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchos*), zebra finch (*Taeniopygia guttata*) in comparison to chicken (*Gallus gallus*). Microchromosome pools (named R1-R9) were created by flow sorting and labelled with FITC. Concurrently FISH BAC probes were designed near the ends of each micro-chromosome using the CHORI-261 Chicken BAC library. In order to make chromosomal assignments to each pool, BACs were labelled with Texas red and co-hybridised with the FITC labelled micro paints. zoo-FISH was then performed using the micro paints on to Gyr falcon (*Falco rusticolus*), budgerigar (*Melopsittacus undulatus*), ostrich (*Struthio camelus*) houbara (*Chlamydotis undulate*), goose (*Anser cygnoides*) and duck (*Anas platyrynchos*) chromosomes. Images were captured using fluorescence microscopy and SmartCapture 3 software (Digital Scientific UK).
BAC probes successfully anchored micro-chromosome pools R1-R9 to the chicken genome assembly. Following this classification zoo-FISH of micro-chromosome paints on to various bird species confirmed the conserved nature of micro-chromosomes 11-19 while, for the first time, accurately assigning chromosome fusions in Falconiform and Pssitaciform species.
Micro-chromosome assignment allows more detailed comparison between species. This comparison confirms synteny of micro-chromosomes in most bird species. As predicted fusions were identified in the Gyrfalcon, which possesses an atypical avian karyotype with a diploid chromosome number of only 2n=50, indicating extensive chromosomal fusions from the ancestral avian karyotype.

WHOLE-GENOME TRIPLICATION AND SPECIES RADIATION IN THE SOUTHERN AFRICAN TRIBE HELIOPHILEAE (BRASSICACEAE)

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The unigeneric tribe Heliophileae includes c. 90 *Heliophila* species, all endemic to southern Africa. The tribe is morphologically the most diversified Brassicaceae lineage in every aspect of habit, foliage, flower, and fruit morphology. Despite this diversity, virtually nothing is known about its origin and genome evolution. Here we present the first in-depth information on chromosome numbers, rDNA *in situ* localization, genome structure, and phylogenetic relationship within the Heliophileae.
Chromosome numbers determined in 27 *Heliophila* species range from 2n = 16 to 2n = c. 88, but 2n = 20 and 22 prevail in 77% of species. Chromosome number variation largely follows three major lineages (A, B, and C) resolved in the ITS phylogeny. B-clade species possess mostly 2n = 20, whereas 2n = 22 is the dominating number in C clade. The number and position of 5S and 45S rDNA loci vary between species and cannot be employed as phylogenetically informative characters. Seven species with different chromosome number and from the three ITS clades were analyzed by comparative chromosome painting. In all species analyzed, 90% of painting probes unveiled three homeologous chromosome regions in Heliophila haploid chromosome complements.
These results suggest that all *Heliophila* species, and probably the entire tribe Heliophileae, experienced a whole-genome triplication (WGT) event. We hypothesize that the mesohexaploid ancestor arose through hybridization between genomes resembling the Ancestral Crucifer Karyotype with n = 8. The WGT has been followed by species-specific chromosome rearrangements (diploidization) resulting in descending dysploidy towards extant quasi-diploid genomes. More recent neopolyploidization events are reflected by higher chromosome numbers (2n = 32-88). The WGT might have contributed to diversification and species radiation in the Heliophileae.
This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189).

A TRIBE-SPECIFIC RECIPROCAL TRANSLOCATION CONFIRMS THE MONOPHYLY OF CARDAMINEAE (BRASSICACEAE)

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Tribe Cardamineae (bitter-cress relatives) includes 12 genera and c. 340 species distributed worldwide and growing predominantly in aquatic habitats. Genomes of all Cardamineae species are based on eight chromosomes (x = 8). We used the Ancestral Crucifer Karyotype (ACK; n = 8) consisting of eight ancestral chromosomes and 24 conserved genomic blocks as the basis for the reconstruction of karyotype evolution in eight Cardamineae genera (*Armoracia, Barbarea, Cardamine, Dentaria, Leavenworthia, Nasturtium, Rorippa*, and *Sisymbrella*).

Comparative chromosome painting with *Arabidopsis thaliana* painting probes arranged according to the structure of ACK revealed that the analyzed Cardamineae species share six ancestral chromosomes with ACK (AK1-AK5 and AK7), whereas two chromosomes (AK6 and AK8) were involved in a whole-arm translocation event (chromosomes AK6/8 and AK8/6).

As the two translocation chromosomes were identified in all Cardamineae taxa analyzed, the AK6-AK8 translocation occurred prior to the diversification of the tribe, and thereby corroborates the monophyly of the Cardamineae. Notably, Cardamineae genomes displaying extensive genome collinearity compared to the eight ancestral chromosomes of ACK, exhibit a ramarkable genome stasis over millions of years of their evolution. These data suggest that the intra-tribal diversification in Cardamineae was not caused by or associated with gross chromosomal alterations.

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TRENDS OF CHROMOSOME EVOLUTION IN CRUCIFERS (BRASSICACEAE)

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The medium-sized mustard family comprises 320 genera and over 3,600 species with cosmopolitan distributions. Phylogenetically the family is formed by three major lineages (I to III) and 49 usually monophyletic tribes, while more than 30 genera and 90 species are pending tribal assignment. Chromosome and genome evolution in crucifers was reconstructed by comparative genetic mapping followed by more recent whole-genome sequence assemblies and comparative chromosome painting studies. Due to the steadily increasing number of species with known genome structures, prevalent trends in chromosome and genome evolution in the Brassicaceae are emerging.

Base chromosome number x = 8 is prevailing across Brassicaceae taxa and a genome with eight linkage groups was apparently ancestral to the whole family. Ancestral Crucifer Karyotype (ACK) comprising eight chromosomes (AK1 – AK8) and 24 conserved genomic blocks was inferred as ancestral to lineage I and II. In lineage I tribes, the ACK genome remained conserved in a number of extant species, whereas in other groups the 24 genomic blocks were reshuffled by translocations and inversions during multiple independent dysploidy events (n=8 ---> n=7, 6 and 5). In the ancestry of lineage II, the ACK genome was reshuffled by chromosome number reduction (n = 8 ---> n = 7) toward the Proto-Calepinae Karyotype (PCK) with seven chromosomes. In tribes which experienced recent whole-genome duplication events (mesopolyploidy), the extent and tempo of genome reshuffling toward diploidized genomes exceed that observed in true diploid genomes. Descending dysploidy events in crucifers were mediated by reciprocal translocations accompanied by a centromere loss or through end-to-end translocations and subsequent centromere inactivation. No chromosome fissions (ascending dysploidy) were observed in the genome evolution of Brassicaceae. Several case studies suggest that diversification and speciation in Brassicaceae was not linked to major chromosome rearrangements.

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DECIPHERING THE DIPLOID ANCESTRAL GENOME OF THE MESOHEXAPLOID *Brassica rapa*

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The genus *Brassica* includes several important agricultural and horticultural crops. Their current genome structures were shaped by whole-genome triplication followed by extensive diploidization. The availability of several crucifer genome sequences, especially that of Chinese cabbage (*Brassica rapa*), enables study of the evolution of the mesohexaploid Brassica genomes from their diploid progenitors.
We reconstructed three ancestral subgenomes of *B. rapa* (n = 10) by comparing its whole-genome sequence to ancestral and extant Brassicaceae genomes. All three *B. rapa* paleogenomes apparently consisted of seven chromosomes, similar to the ancestral translocation Proto-Calepineae Karyotype (tPCK; n = 7), which is the evolutionarily younger variant of the Proto-Calepineae Karyotype (n = 7). Based on comparative analysis of genome sequences or linkage maps *of Brassica oleracea, Brassica nigra*, radish (*Raphanus sativus*), and other closely related species, we propose a two-step merging of three tPCK-like genomes to form the hexaploid ancestor of the tribe Brassiceae with 42 chromosomes. Subsequent diversification of the Brassiceae was marked by extensive genome reshuffling and chromosome number reduction mediated by translocation events and followed by loss and/or inactivation of centromeres.
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GENOME EVOLUTION AND THE ORIGIN OF AUSTRALIAN CRUCIFERS

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We showed previously that three endemic Australian crucifer species (Brassicaceae) have undergone a whole-genome duplication (WGD) followed by extensive and species/lineage-specific diploidization [1]. Some of the lowest chromosome numbers (n = 4-7) known for crucifers can be found among the Australian endemics.
The present study aimed to analyze the genome structure in species from different Australian crucifer genera by comparative chromosome painting (CCP). Ancestral Crucifer Karyotype (ACK) with eight chromosomes served as a reference genome for CCP analyses. The structure of two ACK chromosomes was investigated in 12 species belonging to nine genera (*Arabidella, Ballantinia, Blennodia, Cuphonotus, Geococcus, Harmsiodoxa, Microlepidium, Phlegmatospermum,* and *Stenopetalum*) with variable chromosome numbers (n = 4, 5, 6, and 7). As all the inspected genomic blocks were found duplicated and rearranged within the analyzed genomes, we conclude that all the genera experienced presumably the same mesopolyploid WGD event, followed by massive karyotype reshuffling toward diploid-like genomes. Mechanisms of genome diploidization will be dicussed. Asymmetric single-strand conformation polymorphism (SSCP) approach was used to infer the phylogenetic relationships among the Australian crucifers and to identify putative parental genomes of these ancient polyploids. Analyses of paralogous copies of two single-copy genes, phytochrome A (phyA) and LUMINIDEPENDENS (LD), suggest that one of the parental genomes of Australian mesopolyploid genera can be identified within the tribe Smelowskieae which is native in North America and Asia. This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189).
This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189).

[1] Mandáková et al, The Plant Cell 2010, 22: 2277-2290.

WHAT DETERMINES CHROMOSOME BREAKPOINTS IN CRUCIFER GENOMES?

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Genomes of crucifer species (Brassicaceae) show extensive inter-species chromosome collinearity as shown by comparative chromosome painting studies and comparative genomic sequencing. The extant crucifer genomes are descendants of the Ancestral Crucifer Karyotype (ACK) with eight chromosomes (AK1 – AK8) and 24 conserved genomic blocks (A – X). The ancestral proto-genome structurally resembled the sequenced *Arabidopsis lyrata* genome (n = 8). In several crucifer lineages, the ACK genome has been altered by large-scale chromosomal rearrangements reshuffling the 24 genomic blocks on eight or less chromosomes (n = 4 - 8). Here we aimed to analyze what delimits the evolutionary conserved syntenic blocks and whether specific sequence motifs can be associated with the genomic block breakpoints. In multi-species comparison, we compared the arrangement of genomic blocks and chromosome breakpoints sequences among the sequenced genomes of *A. lyrata* (n = 8), *A. thaliana* (n = 5), *Brassica rapa* (n = 10), and *Schrenkiella parvula* (previously *Thellungiella parvula*; n = 7).

The analysis involved two steps: (i) precise localization of chromosome breakpoints along the sequenced genomes, and (ii) sequence analysis of the localized breakpoint regions. Genome-wide inter-species collinearity was detected by SynOrths which identifies syntenic genes shared between Brassicaceae genomes. The genome of *Arabidopsis thaliana* was used as a reference. Nucmer, a part of the MUMmer software, was used to compare breakpoint regions between the analyzed genomes. We also performed *de novo* identification of transposable elements in all four species using the RepeatModeler and RepeatMasker. Randomly chosen genome regions outside the purported breakpoints were analyzed by the same methods.

Our preliminary data suggest that simple repeats and transposable elements are distributed across breakpoint regions and randomly selected genome regions with a comparable probability. In part the difficulty to pinpoint specific breakpoint sequences can be due to imperfect genome assemblies for some of the crucifer species. Several breakpoints coincide with lost ancestral centromere regions (paleocentromeres). Indepth comparative genome analyses are ongoing.

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ISOLATION AND CHARACTERIZATION OF THREE INTERSPERSED REPEATED DNA SEQUENCES IN THE WESTERN WHIP SNAKE (*Hierophis viridiflavus*)

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To date the knowledge of repeated elements in the snake genome is scarce and mostly limited to various satellite and microsatellite sequences, found in species belonging to different taxonomic groups. In a wider study concerning phylogeography of the European whip snake, *Hierophis viridiflavus*, we identified three new repeated sequences. Then, we performed a study aimed at characterizing these sequences, underlining their principal features with particular regard to nucleotide composition, genomic quantification and chromosome localization.
Two of the three repeated sequences differed in length (240 and 360 bp, respectively), but showed a 5' segment of about 110 bp with an identity higher than 90%. Moreover this shared segment has a trait of 85 bp showing an identity of 77% with an interspersed *L1element of Anolis carolinensis*. Both sequences of 240 and 360 bp are organized as interspersed elements, almost exclusively localised on the W sex chromosome, representing about 0,6% of the genome. The third sequence, long about 240 bp, did not show any significant identity with the other two isolated sequences and with any other database deposited sequences. This sequence, too, is an interspersed element, representing about 2% of the genome. Differently from the other two W specific sequences, this third repeated sequence is interspersed on both autosomes and ZW sex chromosomes.
We identified three new interspersed repeated sequences in *H. viridiflavus*. Two of them are W-specific elements related to an L1 element of *A. carolinensis*. The third sequence is an unknown interspersed element, localised on both autosome and ZW sex chromosomes. Comparative genomic and chromosome hybridizations on other related snakes provided useful systematic and phylogentic information.

Oecomys catherinae (CRICETIDAE – SIGMODONTINAE): CHROMOSOMAL CHARACTERIZATION AND COMPARATIVE GENOMIC MAPPING WITH WHOLE CHROMOSOME PROBES OF *Hylaeamys megacephalus*

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The genus *Oecomys* (Rodentia, Sigmodontinae) has 16 frugivore tree-living species. They live in the Amazon rainforest, Atlantic forest, gallery forests, pantanal and cerrado environments in Brazil. The diploid number (2n) ranges from 58 to 86. *O. catherinae* (OCA) is found in the Atlantic forest; two karyotypes were described for this species, both with 2n=60, but with Fundamental Number (FN) 62 and 64. Methods Two males and two females of *Oecomys catherinae* were studied by G- and C-banding, NOR staining, FISH with telomeric and 18S rDNA and chromosome painting with whole chromosome probes of *Hylaeamys megacephalus* (HME). The sample was collected at Carajás, Pará, Brazil (6°07'31"S; 50°04'17"W).
The karyotype of OCA has 2n=62 and FN=62. Constitutive Heterochromatin (CH) is found in the pericentromeric region of all pairs and all of the short arm of the X chromosome. The Y is almost all heterochromatic. No Interstitial Telomeric Sequences (ITSs) were found. The 18S rDNA probe hybridized to the short arm of five acrocentric pairs. The FISH with the 24 whole chromosome probes of HME in OCA demonstrated 37 homologous sequences. Fifteen HME pairs have conserved synteny (HME 2, 3, 4, 7, 8, 11, 12, 15, 18, 20, 21, 24, 25, 26 and X). Five probes (HME [9,10], 14, [16,17], 18 and 23) hybridized to two regions each and four probes (HME 1, 5, 6 and [13,22]) to three regions of OCA. The following HME probes associations were found: HME: 19/23 (OCA 7), 20/13 (OCA 8), 26/11 (OCA 10) 22/21 (OCA 17), 9/14/5 (OCA 3).
This study extends the known geographic distribution of OCA and describes a new karyotype for this species. Comparative genomic mapping with HME probes shows that the genomes of these species are greatly reorganized.

TELOMERIC DNA SEQUENCES AND CHROMOSOMAL EVOLUTION IN SALMONID FISHES

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The common ancestor of the Salmonidae family experienced a whole genome duplication (WGD) event between 25-100 million years ago. The hypothetical karyotype of the first tetraploid ancestor has been proposed to comprise 96 uni-armed chromosomes. To recover disomic segregation, genomes of the extant salmonid fishes have undergone rediploidization process including numerous chromosome fusions and inversions resulted in formation of many different karyotypes in which the diploid numbers vary from 52 to 102 (FN= 72-168). Telomeric DNA sequences observed far from the chromosomal termini might be relics of such karyotypic reorganizations. Thus, in the present study we proposed to analyze chromosomal distribution of the telomeric repeats in six Salmonid species from three families. (TTAGGG)n sequences were also localized on the chromosomes of the Northern pike (*Esox lucius*) (Esociformes) representing the closest non-tetraploid sister group to the Salmoniformes.
Application of FISH with PNA telomere probe and PRINS with (CCTAAA)7 primer revealed telomeric signals at the very ends of all chromosomes in the Atlantic salmon (*Salmo salar*) (2n= 58, FN= 74), brown trout (*Salmo trutta*) (2n= 80, FN= 100), the European huchen (*Hucho hucho*) (2n=82, FN=112), rainbow trout (*Oncorhynchus mykiss*) (2n=59-61, FN= 104), European whitefish (*Coregonus maraena*) (2n= 80+1-6 B, FN=98), the European grayling (*Thymallus thymallus*) (2n=100, FN=167-170) and the Northern pike (2n= 50, FN= 50). Moreover, terminally located telomeric sequences were detected on the supernumerary (B) chromosomes observed in the whitefish karyotype. Additional non-terminal sites of the telomeric fluorescence hybridization spots were observed at the pericentromeric regions of six medium-sized metacentric chromosomes in the European grayling karyotype. In all Salmonid fishes examined here, interchromosomal variation in the intensity of the fluorescence signals after hybridization with the telomeric probe has been detected.
The differences in the telomere hybridization signal intensity observed on the different chromosomes are likely related to the variations in their respective telomere lengths. The lack of the ITS at the putative fusion sites in most of the Salmonid bi-armed chromosomes may suggest p-arm telomeres were lost in the course of the chromosome breakage that preceded chromosome fusions or experienced successive loss and degeneration after interstitial insertions.

ORGANIZATION OF SELECTED REPETITIVE SEQUENCES IN THE *Chenopodium* GENOME

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The genus *Chenopodium*, commonly known as the goosefoot genus, includes about 120 species. Most of the species are herbaceous annuals weeds distributed over large areas in Americas, Asia and Europe. However, this genus includes also several crop species. Most of the crops, like Andean *C. quinoa* or *C. berlandieri* subsp. Nuttalie are polyploids and belong to *Chenopodium* s. str evolutionary lineage (Fuentes-Bazan et al. 2012). Comparative analyses of repetitive sequence organization provides valuable information and are essential for understanding the origin and evolution of these crop plant. Genomic organization and chromosomal localization of two repetitive sequences 12-13P and 18-24J were analyzed in selected *Chenopodium* species (*Chenopodium* s. str lineage). The 18-24J clone were isolated from *C. quinoa* genome and earlier results suggested that the clone could be specific to one parental diploid genome of *C. quinoa* and related species. The second analyzed sequence (12-13P), also isolated from quinoa genome, is clustered in centromeric and pericentromeric regions of its chromosomes.

The Southern blot analysis indicated that the genomic organization of 18-24J clone was very similar in most analysed species while 12-13P clone showed different patterns of hybridization signals for diploid and tetraploid species. After fluorescent *in situ* hybridization disperse hybridisation signals were observed on chromosomes of all analysed species. In most of diploid species all chromosomes in karyotype, showed hybridization signals of similar intensity. Among polyploids the hybridization signals were observed either only on 18 chromosomes of the complement or two groups of chromosomes, one with strong and one with week hybridization signals, were observed depending on the species. Hybridization signals of the second analysed clone 12-13P were observed generally in centromeric and pericentromeric localization in analysed species. In polyploid genomes usually most of the chromosomes of the complement indicate hybridization signals whereas in diploid only few chromosome pairs showed 12-13P hybridization signals.

Comparative analyses of evolution of repetitive sequences are potentially useful for understanding of evolutionary relationships between *Chenopodium* species.

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COMPARATIVE ZOO-FISH ANALYSIS WITH RIVER BUFFALO 1Q PAINTING PROBE REVEALED THE CONSERVATION OF SUCH CHROMOSOME IN ALPACA (*Vicugna pacos*)

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The alpaca (*Vicugna pacos*, 2n=74) belongs to the family Camelidae, order Cetartiodactyla. Karyotypic studies indicated extensive similarities within all members of the family with only slight variations in the amount and distribution of heterochromatin. Recently, molecular cytogenetic investigation led to the establishment of genome-wide comparative maps between camel, cattle, pig and human. Cross-species chromosome painting represents a very useful approach to define homologous DNA conserved segments. In this study we perform a comparative ZOO-FISH with river buffalo (BBU, 2n=50) 1q probe to identify the homologous chromosomes in alpaca.

BBU1q painting probe was prepared through microdissection and DOP-PCR. Briefly, fixed lymphocytes were spread onto a 24x60 mm coverslip, which was treated for GTG-banding. 15 copies of the 1q chromosome were scraped from the coverslip, collected and amplified by DOP-PCR. A second PCR reaction was performed for the labeling of DNA with digoxigenin-11-dUTP. Fluorescent *in situ* hybridization was carried out according to standard protocols. The labeled probe was revealed by antidig-rhodamine. Slides were counterstained with DAPI in antifade.

The painting probe produced by chromosome microdissection and DOP-PCR was tested first on river buffalo metaphases to verify the specific hybridization on the long arm of BBU1q. Then, the same probe was cross-hybridized on alpaca chromosomes (VPA). A strong fluorescent signal was detected on VPA1q. According to the standard karyotype of alpaca and the DAPI banding, the signal covers the entire region of VPA1q1, whereas the region VPA1q2, including the telomere, did not show any signal. This result adds further interesting knowledge to the comparative cytogenetics of the farm animals and their evolution. Further buffalo painting probes will be cross-hybridized on alpaca metaphases to shed light on the comparative map of these two species.

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CHROMOSOMAL REARRANGEMENTS AND HYBRID FERTILITY: A CASE OF THE COMMON SHREW IN EUROPEAN RUSSIA

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Whole-arm chromosomal rearrangements including Robertsonian (Rb) fusions and whole-arm reciprocal translocations (WARTs) have been implicated in speciation, particularly in mammals. To study this possible role of chromosomal rearrangements in reproductive isolation, the common shrew *Sorex araneus* is an interesting model, showing much chromosomal variability (Rb or WART) including subdivision into more than 70 chromosomal races. Hybrids between races are often complex heterozygotes, forming medium-to-long chain or ring configurations at meiosis, due to differing sets of metacentrics in the hybridising races. Such hybrids could be expected to have meiotic abnormalities and consequent low fertility. Meiotic pairing was studied in adult males from the hybrid zone between the Moscow and Neroosa chromosomal races (European Russia). Synaptonemal complexes (SCs) were analysed by electron and immunofluorescent microscopy after application with anti-SYCP3, anti-MLH1, anti-γH2AX and anti-centromere protein antibodies.
Hybrids between these races produce a ring-of-four (RIV) configuration at meiosis I consisting of four metacentrics gm/go/no/mn, together with seven bivalents (af, bc, jl, hi, kr, pq, tu) and the sex trivalent XY1Y2, all as expected from the G-banded karyotype. The RIV showed apparent asynapsis around the centromeres but these regions did not stain with anti-γH2AX antibodies. Only the "true" X arm of the sex trivalent was irregularly thickened and covered with γH2AX as is typical for mammalian males. The distribution of MLH1 foci corresponded to regions of homologous autosomal synapsis.
The analysis of chromosome synapsis and MLH1 signals in hybrid males with RIV meiotic configurations provided evidence of remarkably orderly meiotic process. Meiotic recombination proceeded as expected, and there were no signs of pachytene arrest or shortage of mature, active sperm (Matveevsky et al. 2012, Pavlova 2013). We plan studies of chromosome synapsis in female hybrids from this hybrid zone and investigations of hybrids from other hybrid zones where different types of complex heterozygotes are produced. These studies on the common shrew will help assess the importance of chromosomal rearrangements in speciation in mammals.
The study was supported by RFBR (12-04-31200, 12-04-31425).

CENTROMERIC INSERTION AND TRANSLOCATIONS ARE INVOLVED IN THE DISPLOIDY OF *Phaseolus leptostachyus* BENTH (FABACEAE)

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The genus *Phaseolus* L. belongs to the family Fabaceae and comprises approximately 75 species distributed across America, five of which of economic importance. Most of its species have 22 chromosomes, with the exception of three species of the *leptostachyus* group (*Phaseolus leptostachyus, P. macvaughii*, and *P. micranthus*), which have 2n = 20. Recently, bacterial artificial chromosomes (BACs) were mapped in Phaseolus vulgaris (common bean) by FISH, and comparative cytogenetic maps were established for *P. lunatus* (lima bean) and *P. microcarpus*, a wild, distant-related species. Only a few chromosomal inversions were identified between these genomes, showing an overall conservation of synteny. In the present study, the same chromosomal markers were mapped in *P. leptostachyus*. Additionally, the *Arabidopsis*-like telomeric sequence and the 5S and 45S rDNA were also used as probes in FISH experiments.

All chromosomes showed telomeric sequences at the ends of both arms, with no interstitial site observed. A single 45S rDNA site was observed in the short arm of chromosome 6, which is conserved in all species of the genus analyzed so far. The 5S rDNA site was observed in the proximal region of the short arm of the largest chromosome pair. BACs selected for the same chromosome of *P. vulgaris* hybridized to different chromosomes in *P. leptostachyus*, suggesting multiple translocation events. Paracentric and pericentric inversions were also hypothesized. The combination of BAC probes indicated that a centromeric insertion of chromosome 10 into chromosome 11 was the primary event responsible for the formation of largest pair of *P. leptostachyus*, thereby reducing its chromosome number. In addition, a translocation involving the end of the long arm of chromosome 6 into the end of the long arm of this chromosome pair (10/11) was also observed.

Although previous comparative maps have indicated that structural rearrangements were rare in *Phaselous*, several rearrangements involving various chromosomes seemed to be involved in the reduction from x = 11 to x = 10 in the genus.

EXTENSIVE GENOMIC REORGANIZATION IN *Necromys lasiurus* (MUROIDEA: CRICETIDAE) DEMONSTRATED BY CHROMOSOME PAINTING

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Necromys lasiurus (NLA, Sigmodontinae, Cricetidae) has 2n=34 and FN=34, with extensive chromosomal reorganization, as demonstrated by previous studies with chromosome painting using *Mus musculus* (MMU) whole chromosome probes. Because of the phylogenetic distance between these species, some lacunae occurred, as NLA16 did not hybridize with any probe from MMU. A better analysis can be obtained if probes from a species phylogenetically closer are used. Methods One male *N. lasiurus* was collected in Marabá, Pará State, Brazil (5°21'51"S; 49°07'02"W). Its karyotype was analyzed by G-banding and mapped using the 24 whole chromosomes probes from *Hylaeamys megacephalus* (HME, Sigmodontinae, 2n=54 and FN=62), which is phylogenetically close to NLA. These data were compared with *Cerradomys langguthi* (CLA, Sigmodontinae), and MMU.

All the 24 probes from HME hybridized in NLA (2n=34 and FN=34) showing 39 syntenic blocks. Fifteen probes of HME hybridized in just one pair of NLA, where four correspond to a whole chromosome and 11 are associated with other syntenic groups. Nine probes of HME have two signals in NLA and two have three signals. Thirteen syntenic associations were found in NLA. The karyotype of NLA here described was previously mapped by others with probes of MMU. HME and NLA are highly reorganized when compared with MMU. The HME probes covered all the genome of NLA, including the lacunae found with MMU. Our comparative analysis between NLA and CLA had shown five shared syntenic associations.

NLA has a highly rearranged genome when compared to HME and these species are greatly reorganized in relation to MMU. The shared syntenic associations between NLA and CLA probably are shared also by most of the Sigmodontinae species.

KARYOTYPIC EVOLUTION AND SEX CHROMOSOMES IN ADAPTIVELLY RADIATED GECKOS OF THE GENUS *Paroedura* (SQUAMATA: GEKKOTA)

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Gekkotan lizards represent the highly specious clade of squamate reptiles with extraordinary variability in sex determination. The gecko genus *Paroedura* consists of 17 species endemic to Madagascar and the Comoros Islands, where they went through a significant adaptive radiation. Although these lizards are easily bred in captivity and one species is a model laboratory lizard, karyotypes of only two species of the genus have been described. The aims of our project were to describe karyotypes of both sexes of species representing major phylogenetic lineages of the genus using conventional and molecular-cytogenetic methods, perform phylogenetic analysis of karyotype evolution within the genus and uncover sex chromosomes.

Altogether, we acquired karyotypes of both sexes in nine species of the genus. We observed that six species (*P. masobe, P. karstophila, P. oviceps, P. stumpffi, P. lohatsara, P. picta*) have the diploid chromosome number 2n=36 with all macrochromosomes acrocentric except the third submetacentric pair. Two species (*P. bastardi, P. ibityensis*) have 2n=34 with the first and the third pair of chromosomes submetacentric and all other macrochromosomes acrocentric. *P. gracilis* possesses the diploid chromosome number 2n=38 with all chromosomes acrocentric. We detected the signal in the FISH with the telomeric probe only in the telomeric regions in seven species and intersticial telomeric signals in two species. C-banding in combination with DAPI staining uncovered the presence of homomorphic ZZ/ZW sex chromosomes with heterochromatinized W chromosome in five species.

Phylogenetic distribution of the studied species suggests that the karyotype 2n=36 is ancestral for the genus. Chromosome fission of the third submetacentric pair of ancestral karyotype seems to be responsible for the origin of the derived karyotype in *P. gracilis* and chromosome fusion of two medium sized acrocentric pairs formed the karyotype shared by the sister species *P. bastardi* and *P. ibityensis*. Discovery of the intersticial telomeric signals in two species might be connected with further intra or interchromosomal rearrangement within the clade. Detection of sex chromosomes in five species was enabled by differential staining, Z and W chromosomes are homomorphic, but probably highly differentiated at the sequence level. Discovery of sex chromosomes within the genus *Paroedura* further document variability of sex

determining systems in geckos. Future studies should focus on testing homology of sex chromosomes in *Paroedura* and in other geckos to reveal evolutionary transitions in sex determination in this important clade.

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COMPARATIVE ANALYSIS OF GERBILS: INSIGHTS INTO GENOME CONSERVATION AND RATE OF CHROMOSOME EVOLUTION AMONG SOUTHERN AFRICAN TAXA

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The subfamily Gerbillinae contains 15 genera which are subdivided into three lineages: (i) the basal *Pachyuromys* and *Desmodillus*, (ii) *Tatera, Gerbilliscus* and *Desmodillus* (tribe Taterini) and (iii) *Taterillus, Gerbillus, Sekeetamys, Meriones, Rhombomys* and *Psamommys* (tribe Gerbillini). These suprageneric clades are essentially based on mtDNA sequence data (cyt b and 12S rRNA) with additional support from DNA-DNA hybridization and allozyme data. Comparative chromosomal banding studies among representative taxa suggests that karyotype differences largely attributed to Robertsonian fusions, which are prone to homoplasy. The aim of this study was to conduct a genome wide comparison among gerbils using chromosome painting in search of cytogenetic signatures defining the various lineages and also to re-assess the impact of Robertsonian rearrangements in evolution of gerbils (i.e. to differentiate homoplasy from hemiplasy) using cladogenesis dates from the consensus phylogeny. We have made paint probes from flow-sorted chromosomes of *G. paeba* (2n=36). Using comparative G- and C-banding and chromosome painting, we investigated the karyotypic relationship among *G. paeba*, the North African *Psammomys obesus* (2n=48), West African *Gerbilliscus kempi* (2n=48), and the southern African *Desmodillus auricularis* (2n=50), *G. afra* (2n=44), *G. leucogaster* (2n=40) and *Gerbillurus tytonis* (2n=36).
The molecular cytogenetic data revealed remarkable genome conservation: the six species share a high proportion of conserved chromosomes, and differences are due to 10 Robertsonian (Rb) rearrangements (three autapomorphies, three synapomorphies and four hemiplasies/homoplasies). Our results suggest that chromosome evolution in *Desmodillus* occurred at a rate of ~1.25 rearrangements per million years, and that the rate among *Gerbilliscus* during 8 million years is ~1.25 rearrangements per million years. The recently diverged *Gerbillurus* (*G. tytonis* and *G. paeba*) share an identical karyotype, while *Gerbilliscus kempi, G. afra* and *G. leucogaster* differ by six Rb rearrangements (a rate ~1 rearrangement per million years), suggesting a very slow rate of chromosomal evolution in the southern African gerbils. Our data demonstrates that the rate of chromosome evolution in *Gerbilliscus* is relatively slow in comparison to other gerbil lineages, which suggests evolutionary rate heterogeneity within these rodents. A more

complete Zoo-FISH analysis including more represntatives of the 15 genera comprising the subfamily Gerbillinae (currently underway) should provide further assessment of chromosome evolution among these rodents.

CHROMOSOME EVOLUTION IN CRICETINAE (MYOMORPHA, RODENTIA)

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Subfamily Cricetinae (Myomorpha, Rodentia) is monophyletic group (7 genera, 18 species) of widespread Palaearctic hamsters. Comprehensive comparative chromosome analyses (based on G-banding and chromosome painting) were previously done for representatives of 6 genera. *Cricetulus sokolovi, Allocricetulus curtatus* and *two A. eversmanni* subspecies were included onto that comparative chromosome map based on G-banding data only. We performed here chromosome painting on chromosomes of these species using golden and Chinese hamsters chromosome-specific probes. Moreover, we have integrated these data with the comparative chromosome maps of hamsters published earlier (Romanenko et al., 2007). Our data specify the mode of karyotype evolution and phylogenetic relationships between the cricetin species and genera.

FISH results showed that some chromosome homologies established using G-banding only between *Allocricetulus* species as well as *C. sokolovi* and other Cricetinae were erroneous (Romanenko et al., 2007). Using chromosome painting we have detected species- and subspecies-specific associations of *M. auratus* (MAU) chromosomes and built the comparative chromosome map for *Allocricetulus* species/subspecies. Syntenies MAU13/3/11/7/2, 5/9/14/16/15 and 8/18 are common for all investigated animals and, therefore, probably represent ancestral associations for the genus (Romanenko et al., 2013). Association MAU13/3/11/7/2 containing another segment of MAU7 was also revealed in *C. sokolovi* karyotype. Synteny MAU5/11/14/9/19/17 detected both in *A. curtatus* and *C. sokolivi* karyotypes and is an example of homoplasy. We revised comparative chromosome map for genera *Cricetulus* and *Cricetus* and propose new structure of putative ancestral karyotypes and also suggest new scheme of karyotype evolution in Cricetinae.

Comparative chromosome painting has enabled analysis of 14 Cricetinae species, making the subfamily the most completely studied by these methods among rodents. In total, chromosome painting demonstrated the high degree of conservation in genome organization of *Allocricetulus*, *Cricetulus* and *Cricetus* hamsters. Robertsonian fusions played a major role in the karyotype evolution of these genera as well as in *Mesocricetus*. However full-scale comparative investigations of the subfamily will be needed to resolve the taxonomic relationships in Cricetinae.

ON HOW THE STUDY OF GENETIC RECOMBINATION IS PROVIDING NEW INSIGHTS INTO MAMMALIAN CHROMOSOMAL EVOLUTION

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Analyzing how mammalian genomes are organized and by which mechanisms genome reshuffling is involved in speciation are fundamental for understanding the dynamics of genome evolution. It has been recently argued that chromosomal rearrangements could reduce gene flow and potentially contribute to speciation by the suppression of recombination. In this context, the aim of this study is to analyze how chromosomal rearrangements are organized and cross-related with recombination hotspots.

Here we address these questions through a multidisciplinary approach, combining computational and experimental methods and by studying the genomes of pivotal mammalian species. We have first established whole-genome comparisons in order to detect homologous syntenic blocks (HSBs) and evolutionary breakpoint regions (EBRs) in both primates and rodent genomes. Subsequently, we analyzed the reorganized regions detected in relation to high-resolution genome-wide maps of recombination rates based on (i) SNPs data, and (ii) meiotic crossovers and double-strand breaks. By this way we provide a highly refined map of the reorganizations and evolutionary breakpoint regions in primate and rodent genomes based on orthologous genes and genome sequence alignments. Our results reveal the existence of a relationship between chromosomal reorganizations and recombination. We detect that rearranged chromosomes presented significantly lower recombination rates than chromosomes that have been maintained collinear during evolution. Importantly, inverted regions had lower recombination rates than collinear and non-inverted regions.

Our results provide new evidences on the effect of genome reshuffling on recombination rates genome-wide, especially for inversions. Such observations have important implications for the effect of recombination in shaping the genomic architecture of organisms and, in particular, how this impacts the speciation process.

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THE CHROMOSOMES OF A FRESHWATER INVADER

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The crustacean *Procambarus clarkii* (Girard, 1852) is a recent freshwater invader introduced for aquaculture into Spain from Louisiana in 1973, and now present in several countries of Europe. From 1989 it is present in Northern and Central Italy and in the next decade it rapidly expanded in Southern Italy and islands. Although its economic and ecological value, the cytogenetics of *P. clarkii*, and in general of decapods, remains poorly studied. This is due to technical constraints in obtaining good chromosomal preparations as well as the features of the chromosome complement made up of a high number (over 100 chromosomes in most of the species) of little sized chromosomes, sometimes presenting supernumerary chromosomes. In Cambaridae (Astacidea),the number of chromosomes varies in a broad range from 2n=102 in *Procambarus digueti* to 2n=254 in *Eupagurus ochotensis*; for *P. clarkii* both 2n= 200 and 2n=188 has been published. We present a cytogenetic characterization of mitotic and meiotic chromosomes of P. clarkii present in the Sardinian freshwaters, by heterochromatin staining techniques (C-, DAPI- and CMA3- bandings) and FISH of the major ribosomal genes and of the telomeric pentameric repeats (TTAGG)n, present in most arthropods.
Chomosomal preparations have been obtained by a direct method from testicular and somatic (hepatopancreas) tissues of ten *P. clarkii* specimens captured in the Southern Sardinian basins. From the counting of mitosis and meiotic metaphase I and II, the chromosome number varied from 184 to 192. At the first meiotic metaphase, all chromosomes were paired, without observable asynaptic chromosomes. Heterochromatin was located in all the centromeres and in paracentromeric regions of several chromosomes. DAPI staining showed large fluorescent blocks in most chromosomes. 45S rDNA FISH produced fluorescent signals on eight mitotic chromosomes and on four meiotic diplotene bivalents. Chromomicin A3 staining localized the GC-rich DNA in correspondance with NORs. The (TTAGG)n probe labelled all telomeres as well as a large interstitial region.
Our results allowed to localize the heterochromatic regions, most of which composed by AT-rich DNA, while the NOR-associated heterochromatin resulted GC-rich. The presence of AT-rich heterochromatin has been found in other Astacidea. FISH localized the nucleolar organizer regions on four chromosome pairs and pointed out the presence of the pentameric telomeric repeats not only at chromosomal ends but in interstitial position too. Our findings give new insights for physical mapping studies in crustacean decapods, till now very scarce, and provide chromosome markers useful for comparative karyologycal analyses in Astacidea.

CHROMOSOMAL VARIABILITY IN BUTHIDAE SCORPIONS REVEALED BY CLASSICAL, MOLECULAR AND ULTRASTRUCTURAL CYTOGENETIC APPROACHES

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The Buthidae scorpions show a high interspecific and intraspecific variability in diploid number, holocentric chromosomes with synaptic and achiasmatic behaviour, and complex chromosome associations during meiosis I. In this work, the mitotic and meiotic chromosomes of 14 buthids (*Ananteris balzanii, Physoctonus debilis, Rhopalurus agamemnon, Rhopalurus rochai, Tityus bahiensis, Tityus confluens, Tityus costatus, Tityus fasciolatus, Tityus maranhensis, Tityus martinpaechi, Tityus paraguayensis, Tityus pusillus, Tityus stigmurus* and *Tityus trivittatus*) were studied using classical, molecular and ultrastructural cytogenetic methods, aiming to establish the mechanisms responsible for diversity of chromosomal number and/or origin of the complex multivalent associations.
In these species, the diploid number ranged from 2n=6 to 2n=28. Multivalent chromosomal associations were encountered in pachytene and postpachytene cells of species of all genera, with exception of *P. debillis* and *T. trivittatus*. Moreover, an intraspecific variability regarding to the presence or absence of chromosome chains and the number of chromosomes involved in multivalent associations was observed in *A. balzanii, R. agamemnon, R. rochai, T. bahiensis, T. maranhensis, T. paraguayensis* and *T. pusillus*. Silver-impregnated cells and nuclei submitted to the fluorescent *in situ* hybridization revealed no variation regarding to the presence of two terminal active nucleolar organizer regions and rDNA sites, respectively. However, the analysis of meitotic cells showed that individuals carrier of same chromosomal configurations during the prophase I can differ in the localization of rDNA sites. Microspread prophase I nuclei of *R. agamemnon, R. rochai, T. bahiensis* and *T. fasciolatus* revealed synaptonemal complex with a tripartite organisation and well-preserved structure until later stages of prophase I, absence of kinetochore plate and recombination nodules. Single and discontinuous axes, gaps and interlocking, which are indicative of the occurrence of heterozygous chromosomal rearrangements and/or heterosynapsis, were also visualised.
All these results permitted us to suggest that: 1) intraspecific variability occurred as consequence of rearrangements of fission/fusion type in *Ananteris* and *Tityus* species, and reciprocal translocation in *Rhopalurus* species; 2) individuals with the same diploid number can differ in the structural organisation of chromosomes. Financial support: FAPESP and CNPq.

A COMPARATIVE STUDY OF MEIOTIC RECOMBINATION IN DOMESTIC CATTLE (*Bos taurus*) AND COMMON ELAND (*Taurotragus oryx*)

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Synapsis of homologous chromosomes and meiotic recombination are prerequisites for completion of the meiotic process and equal distribution of chromosomes into gametes. Meiotic synapsis is mediated by the synaptonemal complex (SC). A method of immunolocalization of MLH1 protein on the major structural template of SC detected by the antibody against protein SCP3 is used for mapping of the physical sites of crossovers on chromosomes. The karyotype of common eland (2n=31/32) differs from the ancestral karyotype of the family Bovidae present in cattle (2n=60, 29 pairs of acrocentric autosomes and X, Y chromosomes) by centric fusions. All autosomes except chromosomes corresponding to chromosomes 13 and 25 of *Bos taurus* are fused into 13 submetacentric chromosomes and autosome 13 is fused with Y-chromosome in common eland. The frequency and distribution of crossovers was compared in pachytene spermatocytes of domestic bulls and common elands by immunofluorescence and FISH methods

A total of 1245 pachytene spermatocytes were analysed in elands and bulls. The mean total numbers of MLH1 foci per cell were 36.2 in elands and 46.4 in bulls. The frequency of recombination in bulls was significantly higher than in elands. The mean total length of all autosomal SCs was 211.7 in elands and 236.9 in bulls. There was a statistically significant correlation between the total length of all autosomal SCs and the total number of MLH1 foci per cell. The length and the number of MLH1 foci of chromosomes 13 and 25 were compared between elands and bulls. The mean length of chromosome 13 and the mean number of MLH1 foci on this chromosome were significantly higher in bulls than in elands, where chromosome 13 is fused to the Y-chromosome.

Fusions are one of the mechanisms of evolution and divergence of the species of the Bovidae. Despite the evolutionary relationship between common eland and cattle the overall frequency of meiotic recombination in common eland (2n=31) is significantly lower than in domestic cattle (2n=60).

CYTOGENETIC AND DNA MARKERS REVEALED GENETIC DELINEATION OF MORPHOLOGICALLY PECULIAR *Cymbidium tigrinum* AND *C. aloifolium* (ORCHIDEACE)

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Cymbidium Sw., or boat orchid, is a myriad orchid with evergreen foliage and arching sprays of delicately colored and waxy flowers comprises of 52 evergreen species. Molecular approaches including single primer amplification reaction (SPAR) method which consist of random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR) and directed amplification of minisatellite DNA regions (DAMD) marker systems for genetic variation analysis at DNA level, physical localization of 45S rRNA gene loci using fluorescent *in situ* hybridization (FISH) as well as phylogenetic assessment of various species and inter-relationships using nuclear ribosomal internal transcribed spacer (ITS) region was carried out in several *Cymbidium* species from north-east India. Molecular data evidently distinguished two taxa viz. *C. tigrinum* and *C. aloifolium* both at molecular cytogenetical as well as DNA levels.

UPGMA based ISSR clustering pattern followed by SPAR methods evidently distinguished the representatives of *C. aloifolium* and *C. tigrinum* with distinct genetic distance supported by high bootstrap values. These two cymbidiums also showed decondensed, dispersed, extended form of *in situ* hybridization signals of 45S rDNA as transcriptionally active signals of fluorescence, where as rest of the species revealed condensed (non-active) signals. Thus, it evidences the existing heteromorphism in size, intensities and their appurtenance. The extended form of hybridization signals, both at interphases and metaphases of the *C. aloifolium* and *C. tigrinum*, reveals the transcriptional activity of ribosomal genes through FISH technique. Sequence data obtained from nrITS region also supports the distinctiveness of both cymbidiums being the base of the trees almost every time.

The genetic delineation and distinctiveness of *C. tigrinum* and *C. aloifolium* might be due to their entirely different habitats as well as their peculiar morphological characteristics. Such genetic uniqueness may be considered as an effort by these species to adapt to the extreme environmental conditions in which they grow and propagate. Such observations provide useful chromosome landmarks and offer valuable evidence about genome evolution, speciation in the genus *Cymbidium* and address conservation concerns for this horticulturally important genus.

DYNAMIC KARYOTYPE EVOLUTION AND MULTIPLE SEX CHROMOSOMES IN WOOD WHITE BUTTERFLIES

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Lepidopteran genomes consist of small-sized holokinetic chromosomes with an ancestral chromosome number of n=31, and a prevalent WZ/ZZ sex chromosome system with female heterogamety. Although the holokinetic nature of lepidopteran chromosomes is supposed to facilitate karyotype evolution mainly due to chromosomal fusions and fissions, recent studies revealed a highly conserved synteny of genes between chromosomes of distantly related taxa and evolutionary stability of karyotypes. The high degree of conservation at the chromosomal level across the phylogenetic tree of Lepidoptera contrasts with exceptional diversity found in some taxa. A typical example is the butterfly genus *Leptidea*, which shows karyotype variability not only between but also within species.

In this work we studied karyotypes of three cryptic *Leptidea* species (*Leptidea juvernica, L. sinapis*, and *L. reali*) by means of standard and molecular cytogenetic techniques. Their chromosomes numbers ranged from 2n=85-91 in *L. juvernica* (Czech population) and 2n=69-73 in *L. sinapis* (Czech population) to 2n=51-55 in *L. reali* (Spain population). Besides inter- and intraspecific variability in the *Leptidea* species, we observed significant differences in chromosome numbers and localization of cytogenetic markers (rDNA and H3 histone genes) between the offspring of individual females. Using FISH with the (TTAGG)n telomeric probe on mitotic and meiotic chromosomes we also demonstrated the presence of multiple chromosomal fusions and other complex rearrangements. The analysis of meiotic chromosomes in pachytene oocytes by genomic *in situ* hybridization (GISH) combined with telomere-FISH revealed multiple sex chromosomes in all three species with the following constitutions: W1W2W3Z1Z2Z3Z4 in *L. juvernica* and *L. reali*, and W1W2W3Z1Z2Z3 in *L. sinapis*.

We confirmed inter- and intraspecific karyotype differences in chromosome number and structure in three closely related wood white butterflies. The karyotype variability is likely due to irregular chromosome segregation of multivalent meiotic configurations. Our results suggest a dynamic karyotype evolution and point to the role of chromosomal rearrangements in speciation of *Leptidea* butterflies. Moreover, our study revealed a curious sex chromosome constitution with 3 W and 3-4 Z chromosomes, which could play an important role in reproductive isolation between *Leptidea* populations.

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PERCENTSAT - A REPETITIVE SEQUENCE IN RODENTIA CONSERVED ACROSS TIME: WHAT'S THE MEANING?

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Initially named "junk DNA" or "selfish genetic material", repetitive sequences are currently considered an essential fraction of eukaryotic genomes, allocating a number of important functions, such as regulation and reorganization of genomes. In this study, we isolated, sequenced and characterized a novel (peri)centromeric repetitive sequence (PERcentSat) in the rodent species *Peromyscus eremicus* (PER). Moreover, we investigated the presence of orthologous PERcentSat in another rodent genome, *Rattus norvegicus* (RNO), and in the primate *Aotus trivirgatus* (ATR).

Physical mapping of PERcentSat on PER chromosomes reveals that this sequence presents a chromosome distribution characteristic of a tandem repeat, sharing the orthologous PERcentSat sequences here isolated (ATR, PER and RNO) similarity values above 90%. Molecular *in silico* analysis of the isolated sequences revealed that its monomeric unit presents a 21 bp unit and an AT content of ~ 52%. The high similarity between the orthologous PERcentSat and two other reported satDNAs, MSAT21 from *Microtus* and the human centromeric satellite HSAT6, suggests that PERcentSat originated from an ancestral satDNA with a repeat unit of 18 bp. Moreover, *in silico* identification of the transcription factor binding sites in PERcentSat: Heat Shock Factor 1 (HSF1) and CRE-Binding protein 1/c-Jun heterodimer (CRE-BP1/c-Jun), suggests that PERcentSat transcription is induced by stress stimuli.

The physical mapping and *in silico* characterization of PERcentSat suggest that this sequence can correspond to a novel satellite sequence, isolated for the first time in the present work. The presence of orthologous PERcentSat with a high level of similarity in a phylogenetically distant species (91.9 million years divergence Rodentia-Primates), suggests a functional meaning. Furthermore, the identification of putative transcription factors reveals that PERcentSat may be involved at the regulation level and this feature seems to have been conserved throughout evolution.

ONE, TWO, MANY: AMPLIFICATION OF HSFY IN SUID SPECIES

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The long arm of the domestic pig (*Sus scrofa*) Y chromosome contains highly repetitive sequences, classically visualised by FISH images of BAC clones painting the entire chromosome arm. However, it has been uncertain whether expressed or indeed functionally important sequences lurk within.

We show here that HSFY has become amplified on pig Yq. We can distinguish 'long' and 'short' variants, which vary mainly by the presence or absence of an artiodactyl-specific SINE in the single intron. We can detect expression of both exons from one or either form in testis, and the second exon in a range of other tissues including brain. Consequently, we looked for HSFY variants in a range of other suid species, attempting to date the amplification. We successfully amplified sequences from individual animals across nine species of suids, from peccaries to warthogs and more closely related *Sus* species.

We found at least two variants of HSFY in all species, predominantly the 'long' and 'short' forms seen in domestic pig. Thus the initial expansion appears to predate the diversification of modern suids. A comparison with cattle, in which HSFY is also amplified, suggests that this is an independent event and raises the possibility of sequences 'prone' to amplification on Y chromosomes.

PRELIMINARY CYTOGENETIC AND MOLECULAR ANALYSES OF *Scorpaena plumeri* FROM VENEZUELA IDENTIFY TWO DISTINCT TAXA

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Scorpaena plumieri is a reef associated subtropical fish occurring in the West Atlantic, from Massachusetts to southern Brazil, and around Ascension and St. Helena islands, in the East Atlantic. External morphology, meristic data and pigmentation do not allow a clear cut distinction of this species from its closely related form, the East Pacific *S. mystes*, currently a valid nominal species, but previously considered as *S. plumieri mystes*. In this study, specimens classified as S. plumieri, collected around Isla Margarita, Venezuela, were cytogenetically analysed and taxonomically identified through mitochondrial molecular markers (COI and 16S rRNA gene sequences).
In the examined specimens two cytotypes (CI and CII) have been identified. CI is characterized by 2n=48, all subtelo/acrocentric chromosomes, and NF=48; CII is characterized by 2n=48, 2 metacentric+46 subtelo/acrocentric chromosomes, and NF=50. The two cytotypes differ also for the constitutive heterochromatin distribution and for the nucleolar organizer regions location. For both molecular markers, sequence analysis yield high genetic distances between the two cytotypes. The phylogenetic tree, obtained retrieving sequences of other Scorpaenidae from GenBank, shows that the CI and CII individuals fall in two distinct and well supported lineages. In addition, the 16S sequences from the CII individuals belong to the same cluster that includes the 16S *S. mystes* sequences.
Thus, both cytogenetic and molecular data identify two distinct entities within the presumptive *S. plumieri* sample from Isla Margarita, possibly disclosing the existence of cryptic species. Additional sampling/karyotyping/sequencing is certainly needed in the area, along with a deeper morphological re-examination of local spotted scorpionfish.

DYNAMICS OF KARYOTYPIC EVOLUTION IN SQUAMATE REPTILES INFERRED FROM COMPARATIVE GENE MAPPING

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Squamate reptiles have large variation not only in the chromosome number (2n = 24 – 46) but also in morphology. They generally exhibit two different types of karyotypes. One is the karyotype with few or no microchromosomes and the other type consists of macro- and microchromosomes. Phylogenetic relationship provides us the possibility that Gekkota and Lacertidae of Scincomorpha with few microchromosomes retain the ancestral state of squamate karyotypes and microchromosomes appeared by fissions of macrochromosomes which occurred in the ancestral karyotype. However, this chromosomal constitution such as Gekkota is not observed in the majority of squamate reptiles with macro- and microchromosomes; therefore, the alternative explanation should be considered that microchromosomes disappeared by fusions between macro- and microchromosomes and/or between microchromosomes in the lineage of geckos and lacertid lizards. Here, comparative analysis of chromosome structures with chicken (*Gallus gallus*) were conducted for four representatives of squamate reptiles: *Leiolepis reevesii rubritaeniata* (LRE, Iguania); *Elaphe quadrivirgata* (EQU, Serpentes), *Varanus salvator macromaculatus* (VSA, Platynota), and *Gekko hokouensis* (GHO, Gekkota) to clarify the process of karyotypic evolution and the origins of microchromosomes in Squamata.
Comparison of genetic linkages between chicken and five squamate reptiles revealed that 13 linkage groups of chicken macrochromosome segments (GGA1p, 1q, 2p, 2q, 3p, 3q, 4q, 5, 6, 7, 8, and Z) were corresponded to LRE, EQU and VSA macrochromosomes, and in some pairs of chromosomes of GHO. All microchromosomes of LRE, EQU and VSA were homologous to chicken microchromosomes, whereas approximately half of chicken microchromosomes were homologous to macrochromosomes in these three species. By contrast, the other four pairs of GHO chromosomes were composed of chromosome segments that are homologous to LRE and/or EQU microchromosomes.
The primitive karyotype of squamate reptiles is considered to be composed of macro- and microchromosomes. Repeated fusions of microchromosomes might have occurred frequently in the lineage of

Gekkota, leading to the disappearance of microchromosomes and the appearance of small-sized macrochromosomes.

AMPLIFICATION OF GENES ON MAMMALIAN B CHROMOSOMES

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B chromosomes are additional to standard karyotypes that may vary in size, number and morphology even between cells of the same individual. Previously it was generally believed that B chromosomes found in some plant, animal and fungi species lacked genes and were thought to be formed of dispensable selfish and parasitic sequences. Recently molecular cytogenetic studies showed the presence of additional copies of essential genes on B chromosomes.

Here we present a method for identification of new genes on B chromosomes of different species, which is based on selection and characterization of B-specific cDNAs. Using this technique we showed the presence of both autosomal and X chromosome specific genes on B chromosomes of the red fox, raccon dog, Siberian roe deer, brown brocket deer and collared lemming.

Discovery of a large autosomal segment in B chromosomes of the different mammalian species and detection of B specific protein-coding genes transcription contradicts the hypothesis of totally silent and heterochromatic nature of B chromosomes. We propose that the origin, evolution and effect on host of B-chromosomal gene copies might be similar to autosomal segmental duplications, which reinforces the view that additional chromosomal elements might play an important role in genome evolution.

MOLECULAR CYTOGENETIC STUDIES ON THE PROCESS OF GENOMIC AND CHROMOSOMAL EVOLUTION IN *Xenopus laevis* AFTER WHOLE-GENOME DUPLICATION AND THE ORIGIN AND EVOLUTION OF SEX CHROMOSOMES IN ANURAN SPECIES

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A draft assembly of the first genome sequence in amphibians was generated in the Western clawed frog (*Xenopus (Silurana) tropicalis,* Pipidae, Anura) (2n = 20). However, there is still no detailed information on genome and chromosome structures for the other anuran species. Comparative gene mapping among many anuran species enables us to understand the process of karyotype evolution in anurans. Here we show the latest data of our research on the process of genomic and chromosomal reorganization in an allotetraploid species, the African clawed frog (*Xenopus laevis*, Pipidae, Anura) (2n = 36), after whole-genome duplication (WGD) and the origins and evolution of sex chromosomes in anurans.

We constructed a high-resolution cytogenetic map of 140 functional genes for *X. tropicalis* using FISH. Then, to examine the process of genomic and chromosomal reorganization in *X. laevis* after WGD, we performed comparative gene mapping for *X. laevis* using 60 cDNA clones of genes that covered entire regions of 10 pairs of *X. tropicalis* chromosomes. Hybridization signals on two pairs of homoeologous chromosomes were detected for 50 of 60 (83%) genes, and genetic linkages were highly conserved between the two species and also between homoeologous chromosome pairs of *X. laevis*. Furthermore, we identified the sex-linked genes of *X. laevis*, the Japanese wrinkled frog (*Rana rugosa*, Ranidae, Anura) (2n = 26) and the Japanese bell-ring frog (*Buergeria buergeri*, Rhacophoridae, Anura) (2n = 26) by comparative gene mapping. Genetic linkages of sex chromosomes were different among *X. laevis, X. tropicalis* and *R. rugosa*. By contrast, in *B. buergeri*, the homology with the *X. tropicalis* sex chromosomes was found for the sex chromosomes and a pair of autosomes.

Comparative chromosome mapping between *X. tropicalis* and *X. laevis* revealed that the loss of duplicated genes and inter- and/or intrachromosomal rearrangements occurred much less frequently in *X. laevis* lineage, suggesting that these events were not essential for diploidization of the allotetraploid genome in *X. laevis* after WGD. Comparative mapping among *X. laevis, X. tropicalis, R. rugosa* and *B. buergeri* suggested the diversity of sex chromosomal origins of anuran species and the possibility that *X. tropicalis* and *B. buergeri* have the same origin of sex chromosomes.

KARYOTYPE DIFFERENTIATION IN BRAZILIAN GRAY BROCKETS (*Mazama gouazoubira* AND *Mazama nemorivaga*; ARTIODACTYLA; CERVIDAE)

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The taxonomic classification of the gray brocket deer has been uncertain, but currently *Mazama gouazoubira* (MGO) and *Mazama nemorivaga* (MNE) are described as distinct species. The karyotype of *M. gouazoubira* (2n=70; FN=70) is similar to that of *M. nemorivaga* (2n=68; FN=70). MGO has 34 autosomal pairs, a large acrocentric X chromosome and occasional B chromosomes, whereas MNE has 33 autosomal pairs, a large number of B chromosomes and a submetacentric X. This study aimed to refine the karyotypic differences between MGO and MNE species.
Lymphocytes and fibroblast from 15 MGO and 8 MNE individuals were cultured and chromosomal preparations were conventionally stained for standard and banded chromosomes (Giemsa, GTG and CBG and Ag-NOR). Fluorescence *in situ* hybridization (FISH) was performed using ribosomal and telomeric probes in both species, and flow sorted chromosome probes from MGO were hybridized to MNE metaphase spreads. All MGO individuals had diploid number (2n) and fundamental number (FN) of 70, and all chromosomes were acrocentric. Compared to this MNE showed three distinct karyotypes: 2n=68/69 FN=70 (standard karyotype, female/male); 2n=67 FN=70; 2n=69 FN=72, with a variation of 2 to 6 B chromosomes and sex chromosomes XX / XY1Y2 due to a X-autosomal tandem fusion . The male variant karyotype with 2n=67 FN=70 + 03 B was different from the standard karyotype due to a submetacentric pair, resulting from a centric fusion between chromosomes 2 and 32. The Ag-NORs were terminally located on the two largest autosomes in both species and ribosomal hybridizations corroborated these results and also labeled B chromosomes of both species. C-banding showed large heterochromatic blocks in all centromeric regions in both species including the B chromosomes. The Y chromosome did not have a distinct heterochromatic centromere. C-banding also visualized an interstitial band located in the middle of MNE

Xq that can potentially be a sign of the X-autosome rearrangement. All MGO chromosomes showed one-to-one correspondence with MNE chromosomes. Interestingly, the B chromosome probe hybridized exclusively to the B chromosomes in both species.
We conclude that the karyotypes of the two species are essentially similar, except for autosomal and the X-autosomal fusions in MNE that cause variation in diploid chromosome number and the sex chromosome system.

INTENSIVE RECOMBINATIONS HAVE LED TO TANDEM REPEAT EXPANSION AND ENLARGEMENT OF RYE SUBTELOMERIC HETEROCHROMATIN

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The rye *Secale cereale* genome is among the largest plant genomes and contains 8.3 × 109 bp, exceeding by approximately 30–40% the genomes of its closest relatives—wheat (diploid) and barley, respectively. A distinctive feature of rye chromosomes is large subtelomeric heterochromatin blocks. Using a BAC library, we studied the sequence composition of subtelomeric heterochromatin of rye chromosome 1 short arm, 1RS, concurrently analyzing a dataset of 454 rye genome reads.
Several multicopy tandemly repeated DNA families are major components of the rye subtelomeric heterochromatin. Some families emerged only after the ancient species *S. silvestre* diverged from a common cereal ancestor. 1RS houses several long arrays of each family. The arrays are of different lengths and display specific patterns of hierarchical arrangement into multimeric blocks, where monomers form various higher-order repeat (HOR) units in the central part of arrays. Analysis of 454 reads has shown that the regions adjacent to tandem repeat arrays mainly (over 90%) contain rearranged copies of various class 1 and 2 mobile element families earlier identified in the barley and wheat genomes. Simple repeats are abundant in the adjacent DNA arrays, exceeding their average content in the rye genome by one order of magnitude. Among the simple repeats, we have identified a 13-bp degenerate motif with two alternating triplets in it. This motif is similar to the binding site for the zinc finger of PRDM9, a protein involved in regulation of mammalian hotspot recombination. Analysis with Dendroscope 3.0 suggested that monomers during the evolution were involved in numerous recombination events, which followed an explosion pattern and led to amplification and emergence of new heterogeneous monomer variants.
Thus, numerous recombinations both within the monomer arrays and in the adjacent DNA regions have led to expansion of tandem repeats and enlargement of heterochromatin regions, thereby contributing to an increase in the *S. cereale* genome.
The work was supported by the Russian Foundation for Basic Research (grant #12-04-00512) and Siberian Branch of the Russian Academy of Sciences (integration project #51).

CHROMOSOMAL ABERRATION AND DISEASE

THE NEW CYTOGENETICS AFTER THE TSUNAMI OF MOLECULAR KARYOTYPE

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The last 10 years have witnessed a profound change in diagnostic cytogenetics. The advent of molecular karyotype through techniques of CGH- and SNP-array allows us to highlight causative genomic imbalances in 15-20% of individuals with intellectual disabilities and / or multiple congenital anomalies compared to only 5% of conventional cytogenetics.
Even in prenatal diagnosis using molecular karyotyping in pregnancies with normal karyotype but abnormal ultrasounds has allowed the identification of causative imbalances in 6%.
Beyond the diagnostic power, the new technologies have also allowed us to identify new types of genomic alterations highlighting, among others, a hitherto unsuspected genomic instability in the early stages of embryonic development.

THE CHROMOSOMAL BASIS OF MALE INFERTILITY

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Infertility in humans is surprisingly common occurring in approximately 15% of the population wishing to start a family. Despite this, the genetic factors underlying the cause of infertility remain largely undiscovered. Additionally, the paternal contribution to infertility and pregnancy loss in humans is often overlooked. Our current understanding of the chromosomal basis of male infertility specifically: chromosomal aneuploidy, structural and numerical karyotype abnormalities and how this impacts the embryo will be reviewed. Chromosomal aneuploidy is the leading cause of pregnancy loss and developmental disabilities in humans and is typically perceived to be predominantly maternal in origin. However, the paternal contribution to aneuploidies involving the sex chromosomes is significant. Evidence to date, suggests infertile men have a significantly increased proportion of aneuploid sperm compared to their fertile counterparts. Furthermore, males with structural and numerical rearrangements are also at an increased risk of producing aneuploid sperm. Since the inception of intracytoplasmic sperm injection (ICSI), concerns have been raised regarding the genetic consequences of utilizing sperm from infertile men. Although few in number, studies to date, demonstrate a lack of selection against chromosomally abnormal sperm. The ultimate answer to this question may lie in combing aneuploidy assessments with qualitative analysis of SNP-based testing data in embryos. This technology can be used to identify parental haplotypes that can be used to detect the parental origin of any chromosomal abnormalities. This will ultimately provide the most accurate assessment of the relationship between sperm aneuploidy and its impact on embryo aneuploidy.

In addition, to better understand the paternal contribution to fertilization and embryogenesis it may not be enough to look at just the number of chromosomes within a sperm. It is well established that chromosomes occupy distinct positions within the interphase nuclei, conferring a potential functional implication to the genome. Therefore it may be crucial to establish whether a specific pattern of chromatin organisation within the sperm cell is required for normal fertilization and embryogenesis. Our current understanding of how chromatin is organized in spermatozoa and the potential implications in fertilization and embryogenesis will also be discussed.

UNIVERSAL APPROACHES FOR DETECTING GENETIC DISEASE IN IVF EMBRYOS: TOWARDS UNDERSTANDING THE CYTOGENETICS OF EARLY HUMAN DEVELOPMENT

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Preimplantation Genetic Diagnosis (PGD) – the diagnosis of genetic disorders in human IVF embryos - is about to enter its 25th year as clinical procedure. From the outset however, it has had been impeded by the time consuming, expensive delay associated with tailoring a bespoke test to each couple before treatment. The further complication of having to ability only to detect either the monogenic disorder in question or the chromosomal complement of the embryo potentially limits its application. Interrogation of single nucleotide polymorphism microarrays (SNP chips) can facilitate high-resolution cytogenetic diagnosis and recently we adapted this technology for monogenic disorder detection also (Karyomapping [1]). By linkage analysis of parental genotypes, an affected sibling and single cells from IVF embryos Karyomapping makes it possible to identify informative loci for each of the four parental haplotypes on each chromosome and map the inheritance pattern of the disease loci on them. In recent months Karyomapping has entered clinical validation, which has resulted in pregnancies and live births. Karyomapping is potentially capable of detecting the spectrum of monogenic and chromosome disorders and thus has the potential for widespread and global application. Moreover it is a tool that can, for the first time, detect the incidence and origin of chromosome abnormalities in early human development. Uniform chromosome abnormalities in human preimplantation embryo at cleavage stages have their origin in meiotic errors in the gametes whereas post-zygotic mitotic errors result in chromosomal mosaicism. While the levels of chromosome abnormality have been described in detail in ongoing pregnancies and live births, studies in preimplantation human embryos are based on limited technologies that do not have the ability to determine the phase and parent of origin. Karyomapping using genome wide SNP analysis can be applied to sperm, oocytes, single blastomeres or whole embryos to determine not only the existence of chromosome errors but also their origin. Evidence suggests that aneuploidy of maternal meiotic origin shows significant differences to those seen in pregnancy, and that post zygotic changes (principally whole chromosome losses) are commonplace. Patterns of nuclear organisation similar to those seen in committed cells can be observed, particularly in aneuploid nuclei.

[1] Handyside et al, J Med Genet 2010, 47: 651-658.

CHROMOSOMAL MICROARRAY ANALYSIS AS A FIRST-LINE TEST IN IN HIGH- AND LOW-RISK PREGNANCIES

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A significant number of important and large prospective clinical trials on chromosome microarray analysis(CMA) for prenatal testing that have been published recently. The combined data from these studies clearly indicate that, compared with conventional karyotyping, the use of CMA in prenatal diagnosis improves substantially the detection rate of pathogenic chromosomal abnormalities. The findings provide evidence for the feasibility of introducing CMA into routine prenatal diagnosis practice, indicating that it is acceptable to apply CMA as a first-line diagnostic test, at least concurrently with conventional karyotyping.
Statements issued by the American College of Obstetrics and Gynecology (ACOG), Canadian College of Medical Geneticists (CCMG) and Italian Society of Human Genetics (SIGU) have recommended that CMA should be offered as an adjunctive tool to selected groups of high risk pregnancies (e.g. those with abnormal ultrasound findings and normal conventional karyotyping results), using the technique as a second-line test only, after standard karyotyping. The main reasoning behind this relates to the fact that CMA performed for indications other than abnormal ultrasound findings would likely be associated with a low positive predictive value, since the vast majority of fetuses tested would be clinically unaffected.
The higher detection rate by CMA compared with conventional karyotyping reported in the recent prospective clinical trials is not confined to cases with abnormal ultrasound findings. The results of the above studies demonstrate the improved diagnostic ability of CMA to detect clinically relevant abnormalities and the utility of bringing CMA into routine prenatal practice as a primary diagnostic tool for a number of other indications.
Furthermore, the findings clearly indicate that offering CMA only as a second-line test in high-risk pregnancies may substantially limit prenatal diagnostic potential, since a significant proportion of copy number variants (CNVs) that can cause serious disability are not detected by traditional karyotyping.
In conclusion, there is a growing body of evidence on the diagnostic superiority of CMA over conventional karyotyping, even in low risk pregnancies. Findings to date provide substantial evidence for the introduction of CMA as a first-line test for all pregnant women undergoing invasive prenatal testing, regardless of risk factors, with the ultimate goal of improved prenatal diagnosis and a lower risk of giving birth to a chromosomal abnormal neonate.

THE USE OF MICROARRAY FOR ANEUPLOIDY SCREENING AND TRANSLOCATIONS IN OOCYTES AND EMBRYOS

GIANAROLI L.

(Abstract not received)

DOES EXPOSURE OF HUMAN LYMPHOCYTES TO GENOTOXIC AGENTS ALTER GENE POSITIONING?

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Chromatin displays non-random organisation within the nucleus. To date, it has been hitherto poorly investigated whether it is possible to perturb the organisation of specific genes after the induction of DNA damage following exposure to genotoxic agents. Peripheral blood was obtained from 6 healthy subjects. Karyotype analysis was performed in the absence of, and after exposure to genotoxic agents (UVb 320nm-15 minutes and 80mM H2O2-30 minutes). Subsequently, organisation of 12 clinically important genes, located on 12 different chromosomes was analysed by fluorescence *in situ* hybridisation using a custom-made algorithm. This study was approved by the local Institutional Review Board. Random versus non-random organisation and differences in exposed versus controls were assessed by Chi-squared.
A decrease in mitotic index and presence of a variety of chromosomal aberrations was observed compared to controls for both UV and H2O2 samples indicative of DNA damage after genotoxic exposure. Most investigated genes demonstrated a non-random organisation (8/12 - controls and 9/12- for both UV and H2O2). Investigated genes displayed a reproducible hierarchical organisation from the nuclear interior to the nuclear periphery within control samples (CD37; p53; BCR; ABL; IGH; FGFR3; ALK; AML1 PGDFRB; BCL2; hTERC and ETO respectively). This hierarchical organisation remained remarkably consistent in the treated groups compared to controls with only one alteration observed for both UV and H_2O_2. In each case there was a small alteration that resulted in a change in the order of two genes, (BCR and p53 in UV, AML1 and ALK in H_2O_2 samples). Comparisons between control and the two treated samples from the same individual yielded significant differences in the distribution of genes in 9.7% of cases in UV and 19.4% of cases in H_2O_2. Changes affected 9/12 genes but predominantly involved p53 and CD37.
Non-random organisation was observed for most genes in all conditions. Small alterations in the hierarchical ordering of 2 closely located genes were observed in both UV and H_2O_2 treated samples. Significant alterations in the distribution of genes compared to control samples were more frequently observed in the H_2O_2 samples than UV samples but predominantly limited to a handful of tested genes. These findings suggest that there may be as of yet undiscovered crucial requirement for

specific gene positioning that is largely retained even after exposure to genotoxic agents.
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CYTOXICITY AND GENOTOXICITY OF POLYSTYRENE NANOPARTICLES IN BOVINE OVIDUCTAL EPITHELIAL CELLS (BOEC)

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Recently a great interest surrounds the development of nanoparticles (NPs) for potential biomedical applications such as drug delivery, cancer therapy and medical imaging. NP delivery system *in vivo* promises to overcome the epithelial barriers, key obstacle to the administration of drugs, vaccines, plasmid DNA and RNAi material. However, so far the interplay and hazards between nanoscale materials and biological systems have not been fully understood. Highlight of these data we have evaluated the effect of polystyrene nanoparticles (PS-NPs), widely used as model and reference particles, on primary culture of bovine oviductal epithelial cells (BOEC) that form a epithelial barrier of female reproductive tracts involved in the secretion of products essential for gametes and embryos.
BOEC have been cultured with 44 nm fitc-PS-NPs 10ug/ml for 7 days until achievement of cell confluence and treated to study NP influence on the cellular proliferation and chromosomal arrangement, using also confocal microscope, and gene expression by differential display. The confocal analysis has not revealed the NP presence in the nucleus during cell division and the karyotype analysis has not showed stastistically significant chromosomal anomalies compared to control sample. However study of gene expression by differential display has exposed interesting alterations. Particularly, BOEC treated with NPs have showed the activation of expression of a band 100 bp and one of 600 bp. The sequencing has revealed an identity of 99% between the 100 bp band and *Bos taurus* microsatellite DNA clone RP42-69E7, whereas the 600 bp band shared and identity of 87% with *Bos taurus* breed Hereford chromosome 5 genomic scaffold (3517 bp at 5' side: GRAM domain-containing protein 4 6521 bp at 3' side: ceramide kinase (CERK). Microsatellite sequences may occur within promoters and other cis-regulatory regions, playing an important role in modulating of gene expression under stress. The phosphorylation of ceramide (C-1-P) via CERK has been shown to stimulate the production of signaling molecules of cell proliferation. Moreover the interaction of C-1-P with phosphatidylinositol 3-kinase/Akt and Mammalian target of rapamycin indicate that disregulated CERK expression may lead to cancer.

Our results demonstrate that PS-NPs seem do not affect cell division and chromosomal rearrangement, but may interfere with gene expression and progression in cell cycle. Our data represent a preliminary new contribute to understand the complicated interactions and the effects between NPs and biological systems. However other studies are necessary before NP application in biomedical field.

ANEUPLOIDIES DEVELOPMENT IN PREIMPLANTATION HUMAN EMBRYOS

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Genomic rearrangements play a major role in the pathogenesis of human genetic diseases. Preimplantation genetic diagnosis/screening (PGD/PGS) is used to analyze embryos genetically before their transfer into the uterus and to eliminate genetic disorders in offsprings. Studies of human cleavage stage embryos 3 days after fertilization of the oocyte have revealed remarkably high levels of chromosome abnormalities and chromosomal mosaicism than embryos at a later stage of development. We examined chromosome content of human preimplantation embryos at different days post fertilization. Each embryo was first analyzed from a single blastomere at Day 3 and subsequently rebiopsied at Day 5 when partial structural (group B) or numerical (group A) chromosomal aberration was found. In embryos at Day 5 we examined inner cell mass (ICM) and trophectoderm (TE) fractions separately, when it was possible. In the other cases we analyzed cells of embryo altogether. All embryo biopsies were tested for aneuploidies using aCGH.
Most of embryos in group A remained aneuploid and the chromosomal content was absolutely identical at Day 3 and Day 5. By contrast, we observed opposite trend in group B where majority of embryos underwent complete self-correction and were euploid at Day 5. Almost half of previously aneuploid embryos at Day 3 were fully euploid at Day 5 and from genetic point of view suitable for embryotransfer.
Our results confirmed various occurence of chromosomal aneuploidies in different developmental stages of embryos. We also observed that self-correction of partial chromosomal mitotic errors is more effective than reparation of errors affecting whole chromosomes.

MOLECULAR CHARACTERIZATION OF TWO CALYCULIN A - INDUCED HOTSPOTS OF BREAKAGES AT THE HUMAN COMMON FRAGILE SITE FRA11D

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Cancer cells are characterized by their ability to grow and divide at an unregulated, quickened and indefinite rate. Point mutations in tumor suppressor genes and proto-oncogenes, changes in chromosomes copy number and chromosomes structure are various forms of genome instability which constitute a hallmark of cancer cells. Fragile sites are site specific gaps, constrictions or breaks observed on metaphase chromosomes when cells are cultured under partial replication stress conditions. Two parameters are used in their classification: frequency in the population and chemistry of induction. Less than five percent of individuals possess rare fragile sites which are mostly folate stress sensitive. FRAXA, a folate sensitive rare fragile site located at Xq27.3, is responsible for the fragile X syndrome. On the other hand, common fragile sites are found in all individuals and they are induced by low doses of aphidicolin, a partial inhibitor of DNA polymerases alpha, delta and epsilon, or calyculin A, a specific inhibitor of serine/threonine protein phosphatases type 1 and type 2A that leads to a premature chromosome condensation in all phases of the cell cycle (G1, S, G2, and M). Several cancer-specific translocations and deletions containing breakpoints colocalize with common fragile sites. This finding highlights the importance of fragile sites instability in oncogenesis.

In this study, our objective is to isolate at the molecular level the hotspots of breakages at FRA11D, the fragile site cytogenetically mapped to the chromosomal band 11p14. To induce fragile sites, lymphocytes from peripheral blood of three healthy donors were treated by calyculin A at 60 ng/ml. Using Fluorescent *In Situ* Hybridization technique and several Bacterial Artificial Chromosome probes corresponding to our region of interest, we located 2 specific hotspots of breakages at the level of the human genomic clones 1L12 and 283H3.The first clone is localized at the interface between the two chromosomal band 11p14.1 and 11p14.2, a result that confirms previous studies showing that common fragile sites are present at the junction of early replicating and late replicating bands. The second clone covers the mucin 15 gene, a member of a family widely associated with cancer.

This molecular characterization is an essential step to understand the origin of DNA rearrangements observed in this genomic region in several genetic diseases.

GENOMIC ARRAYS IN A POPULATION OF TRANSSEXUALS AND IMPLICATION OF THE GENES ERB, AR, AND CYP19A1 IN THE ETIOLOGY OF TRANSSEXUALISM

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Transsexualism is a gender identity disorder exhibiting a complex and multifactorial etiology, but genetic factors may play an important role. The aim of this study was to analyze the karyotypes from 433 male-to-female transsexuals (MFs) and 273 female-to-male transsexuals (FMs) by CytoScan HD Array, as a new high-resolution tool for detecting genomic imbalances. We also investigated the possible influence of the variable regions of ERβ, AR and CYP19A1 genes on the etiology of transsexualism. Methods: Genomic DNA samples were extracted from peripheral blood using the DNeasy Blood & Tissue Kit from Qiagen. The genome-wide DNA copy number analyses were performed with CytoScan™ HD Array (Affymetrix) in accordance with the manufacturer\'s instructions. The samples were whole genome-amplified, fragmented, hybridized, fluorescently tagged and scanned, according to the manufacturer's protocol. Furthermore, the molecular analysis of variable regions of intron 5 of ERβ, exon 1 of AR, and intron 4 of CYP19A1 genes was carried out in 433 MFs, 273 FMs, 125 control women and 125 control men.
The analysis by high-resolution genome-wide SNP array allowed us to perform an exhaustive analysis of small chromosomal variations but we were unable to find any cytogenetic alteration that could be related to transsexuality. Moreover, with respect to the variability analysis: FMs differed significantly from the control XX group in the mean length of the ERß polymorphism ($p \leq 0.03$). The repeat numbers in ERβ were significantly higher in FMs than in control females ($p \leq 0.042$). For the ERβ gene, the risk of transsexuality was higher (OR: 1.474 [1.012; 17.535]) in the subjects with genotype homozygous for long alleles (LL) than in those with the genotype homozygous for short alleles (SS). MFs differed significantly from the control XY group with respect to the mean length of the CYP19A1 polymorphism ($p \leq 0.003$). The repeat numbers in CYP19A1 were significantly shorter in MFs than in XY controls ($p \leq 0.01$). For the CYP19A1 gene, the risk of transsexuality was higher (OR: 0.4175182 [0.2326678 0.749229]) in the subjects with genotype homozygous for short alleles (SS) than in those with the genotype homozygous for long alleles (LL).
We were unable to find any genomic imbalance that could be related to transsexuality but our data support the finding that the genes ERβ and CYP19A1 are implicated in the risk of transsexuality.

MOUSE OCTOCHROME: FOR CYTOGENETIC CHARACTERISATION OF MOUSE LYR TUMOUR CELL LINE

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The mouse LY-R cell line is an X ray-resistant cell line that is derived from the L5178 Y cell line taken from thymic tumour induced in a DBA/2 mouse by methylcholanthrene. This cell line has been used extensively in studied related to gene expression, gene mutations, telomere length, cell phenotypes, resistant to various radiation and toxic elements; however a complete cytogenetic characterization has not been performed so far. Multicolour fluorescence *in situ* hybridisation (FISH) is well known technique used in cytogenetic mapping and characterisation of cell lines. Here we have carried out an initial characterisation of the LY-R mouse tumour cell line using a newly developed multicolour FISH probe device 'Mouse Octochrome' which allows the detection of the entire mouse karyotype on a single FISH slide. Cells were cultured and chromosome preparations were generated using standard procedures. FISH was performed using Cytocell FISH protocols using Octochrome device and Aquarius Murine liquid probes. To perform multicolour FISH for the entire mouse karyotype on a single slide, we used the Cytocell Mouse Octochrome, which contains 8 squares, seven of which have probes for different chromosome combinations. Each of the seven squares has the probes for 3 different chromosomes labelled in red green and blue colours. Once translocations were observed, results were verified using Cytocell Aquarius Murine FISH probes.

The LY-R cell line had only 2 normal chromosomes (chromosome 3, and 10), the remainder were involved in various abnormalities in a number of distinct clonal populations. So far in this study we have observed a number of numerical and structural abnormalities. These include: aneuploidy; translocations; fragmentations; and chromosome breakages. Aneuploidy was seen in chromosomes 1, 4, 15, 18 and in the sex chromosomes while chromosomes 9,11,12 and 13 were highly fragmented. So far we have detected that 8 different translocations present in the cell line involving chromosome 1 and 7; 2 and 5; 4 and 14; 5 and 9; 6 and 17; 7 and 19; 12 and 13; and 12and 16. Currently this study is on-going in order to further clarify the chromosomal abnormalities present in the cell line.

In conclusion, this study confirms that the LY-R tumour cell line has multiple chromosome aberrations in number of different clonal populations. It is not clear whether these different populations were present in the original thymic tumour from which the cell line was derived or if they have arise from the immortalisation and subsequent sub

culturing process. By further studying the clones and their rearrangements this may become clear. This study also demonstrates that Mouse Octochrome is a reliable technique, which can used to characterise a cell line. Further tests will be performed using locus specific identification probes in order to allow in-depth analysis of the chromosomal translocations present in the cell line.

GENOME WIDE ANALYSIS REVEALS SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH FATNESS AND PUTATIVE NOVEL COPY NUMBER VARIANTS IN THREE PIG BREEDS

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Obesity, excess fat tissue in the body, can underlie a variety of human complaints including heart disease, stroke and cancer. The pig is an excellent model organism for the study of various human disorders, including obesity, as well as being the foremost agricultural species. In order to identify genetic variants associated with fatness, we used a selective genomic approach sampling DNA from animals at the extreme ends of the fat and lean spectrum using estimated breeding values derived from a total population size of over 70,000 animals. DNA from 3 breeds (Sire Line Large White, Duroc and a white Pietrain composite line (Titan)) was used to interrogate the Illumina Porcine SNP60 Genotyping Beadchip in order to identify significant associations in terms of single nucleotide polymorphisms (SNPs) and copy number variants (CNVs).
By sampling animals at each end of the fat/lean EBV spectrum the whole population could be assessed using less than 300 animals, without losing statistical power. Indeed, several significant SNPs (at the 5% genome wide significance level) were discovered, 4 of these linked to genes whose ontologies had previously been correlated with fatness (NTS, FABP6, SST and NR3C2). Quantitative analysis of the data suggested CNV regions containing genes whose ontology suggested fatness related functions (MCHR1, PPARα, SLC5A1 and SLC5A4).
Selective genotyping of EBVs at either end of the phenotypic spectrum proved to be a cost effective means of identifying SNPs and CNVs associated with fatness and with estimated major effects in a large population of animals.
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INTEGRATIVE GENOMIC ANALYSIS OF HUMAN CANCER CELL TYPES BY MFISH, ACGH AND GENE EXPRESSION ANALYSIS

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Cancer cells acquire the hallmarks of malignancy through the accumulation of advantageous mutations over prolonged periods of time. Cancer is a disease of impaired genomic stability. Cancer cell lines have been extensively used as *in vitro* models for cell biological and therapeutical studies, but their chromosomal alterations are not well characterised. Moreover, no correlations were made between chromosome alterations in cancer cell lines with that of gene expression patterns. We characterised human breast and brain cancer cell lines using multi-colour fluorescence *in situ* hybridisation (mFISH), telomere FISH, microarray and array comparative genomic hybridisation (aCGH) techniques.

Breast cancer cell lines (MCF-7, MDA-MB-231, SkBr3, MDA-MB-436, HCC1937) and brain cancer cell lines (A172, KNS60, U251MG(KO), ONS76, MO59K) displayed high levels of both recurrent and non-recurrent chromosome translocations as revealed by mFISH analysis. The gross numerical chromosomal instability and the complex translocations in breast cancer cell lines were much greater than the less aggressive immortal cell lines. In some cancer cell lines, some chromosomes are more frequently targeted for aberrations than others. The mFISH data corroborated the fact that cancer cells have extremely complex genomic aberrations with multiple chromosomal translocations. In an integrated approach using microarray and aCGH analysis, we show that most of the differentially regulated genes in all cell lines were involved in cell cycle and cell death gene clusters. Among the clusters, some cancer cell lines cell line exhibited a relatively different profile compared to the other cancer cell lines.

It may be important to know the basal characteristics of a particular cancer cell line to be used drug discovery studies.

MOSAICISM IN MOUSE EMBRYOS

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Chromosome abnormalities are presented in human and animal embryos and have significant impact on implantation failure, abortion rate or a birth of affected individuals. Besides chromosome abnormalities coming from an oocyte or a sperm, such errors may arise newly in cleavage-stage embryos. This phenomenon leads to a mosaicism, when individual blastomeres bear different chromosome set. To study the occurrence of mosaicism we examined chromosomes of separately collected blastomeres derived from 2-cell mouse embryos.

53 two-cell mouse embryos were disaggregated into individual blastomeres and each was analyzed. In three embryos, only one out of two blastomeres was successfully analyzed, so those embryos were excluded from the study. In another two embryos, an extensive aneuploidy comprising of losses of many chromosomes was detected and not confirmed in second blastomere of these embryos (both were euploid). These two embryos were also excluded on the basis of the probable technical artefact. In the remaining 48 embryos only one embryo was presented with aneuploidy (gain of chromosome 15), however this was not confirmed in a second blastomere (euploid). 28 embryos were males and 20 embryos females (based on concordant finding in both blastomeres).

Examining both cells of the 2-cell mouse embryos, we were able to detect both, meiotic and mitotic errors originating from the first zygote cleavage. According to our results, aneuploidy in mice is very low (2%) and we have also observed that the first zygote cleavage in mice is correct and does not generate chromosome errors that would lead to a mosaicism.

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A NEW WAY TO USE WHOLE-CHROMOSOME PAINTING PROBES (WCPs) IN A CLINICAL CASE

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The analysis of banded karyotypes still remains the best way to evaluate chromosome aberrations that can be later confirmed by FISH analysis with specific markers or whole-chromosome painting probes (WCPs). In this study, we found out a new reciprocal translocation in a phenotypically normal young sheep, from Laticauda breed. This time, we came up with an innovative approach to it, mixing classical cytogenetic to underline the importance to perform banding analysis and, molecular cytogenetic showing a new way to use WCPs get from ders.
Cytogenetic analyses such as RBA-banding and karyotyping were performed to characterize the kind of aberration. Two kind of FISH analyses confirmed it. They included different types of WCPs: two of them get from corresponding cattle chromosomes and another get from the smallest der. Firstly, thanks to RBA-banding, we individuated the chromosomes and their regions involved. Everything was confirmed generating WCPs by chromosome microdissection from cattle chromosomes. Secondly, we come out with the idea to produce the WCPs of the smallest der in order to study its rearrangements on sheep chromosomes. We made up and, hybridized it on both OAR (*Ovis aries*) and BTA (*Bos Taurus*) metaphases. While centromeric signals were detected in the majority of OAR chromosomes due, most probably, to the presence of the entire centromere in the der; 2 sub-centromeric signals on corresponding chromosomes 21 and two distal signals on chromosomes 24 were found out in the BTA metaphases. Finally, we identified the chromosomal aberration as rcp(18q,23q)(q14;q26).
In animal cytogenetic is not very easy get WCPs that work well in Bovids because they are, more often, produced using BTA chromosomes and hybridized in different species. All the probes, used in this study, were self-produced giving new ways of their application and, consequently new results. In this way we underlined both the importance to generate WCPs by microdissection and, a new way to use them. Acknowledgements.
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MITOTIC AND CHROMOSOMAL DEFICIENCIES IN PRIMARY MICROCEPHALY SYNDROME (MCPH)

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Primary Microcephaly (MCPH) is a rare congenital disorder characterized by marked reduction in brain size and mental retardation. MCPH is genetically heterogeneous with eleven loci mapped to date, and ten of the underlying genes identified. While mutations in most of these genes affect cell divisions exclusively in neuroepithelium, two particular MCPH conditions show mitotic alterations extending to other cell types as lymphoblast or fibroblasts. Thus, previous studies defined those alterations as premature chromosome condensation in MCPH1 mutated patient cells and increased missegregation during anaphase in patients affected by a type of MCPH linked to 10q (referred here as MCPH11). Here we provide a novel characterization of the effects of MCPH1 and MCPH11 deficiency on chromosome structure and morphology.
Our analyses revealed that the poor banding resolution observed in routine cytogenetic analyses of MCPH1 patients is due to hypercondensation of the mitotic chromosomes. Moreover, a substantial fraction of those metaphases shows poorly resolved sister chromatids with a twisted appearance. Also, centromere cohesion in chromosomes from MCPH1 patients seems to be reduced as metaphases with premature chromatid separation (PCS) are detected under strong hypotonic conditions. In MCPH11 cells we observed a singular phenotype of encapsulated mitotic cells with chromosomes tightly packed into a layer of membrane occurring again exclusively after strong hypotonic conditions.
We describe a novel series of structural deficiencies during mitosis of MCPH1 and MCPH11 lymphoblast patient cells. Those alterations further characterize the role of both underlying genes in chromosome organization and mitotic division.

IDENTIFICATION OF CHROMOSOMAL TRANSLOCATIONS IN PIGS USING FISH WITH SUBTELOMERIC PROBES AND THE DEVELOPMENT OF A NOVEL SCREENING TOOL FOR THEIR APPLICATION

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Reciprocal chromosome translocations have been established to affect fertility in pigs leading to reduced litter sizes and hypoprolificacy. With an increasing emphasis in the commercial pig breeding industry on using a small population of boars for artificial insemination, the potential economic costs of using hypoprolific boars are significant. At present screening for translocations is only performed by karyotyping, which, while technically straightforward, requires animal specific expertise for karyotype analysis, which can be unattractive to the industry. The use of subtelomeric probes and fluorescence *in situ* hybridisation (FISH) eliminates the need for this level of expertise whilst also offering greater accuracy and the ability to identify cryptic translocations. At present, however a universal FISH based screening test for porcine translocations has yet to be developed. Probes were designed that map to the subtelomeric regions of each chromosome arm to enable detection using FISH. BACs were identified from the subtelomeric region of the p-arm and q-arm of each porcine chromosome and directly labelled with Texas Red or FITC (p-arm and q-arm respectively) prior to fluorescence microscopy and image capturing using SmartCapture 3 software (Digital Scientific UK).
Clear signals were obtained from each subtelomeric probe. These were tested on normal animals and animals that exhibit translocations, providing preliminary evidence that this technique is a valid tool for the identification of translocations that affect fertility in pigs.
When combined with a tool originally developed for humans to enable the simultaneous detection of all porcine chromosomes on one slide (Multiprobe™ Device), the speed and cost of chromosomal analysis for translocations that affect fertility will be greatly improved, therefore offering significant benefits to animal genetic research and the animal breeding industry. Funding: University of Kent, Cytocell

CHARACTERIZATION OF A SMALL SUPERNUMERARY MARKER CHROMOSOME INVDUP (15)

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The small supernumerary marker chromosomes (sSMC) are chromosomal fragments casually rearranged, with a frequency of about 0.044% in the population, reaching up to 0.125% in infertile patients. The most common sSMC derive from chromosome 15 (25-30%) and, among them, we can find most frequently a specific structural rearrangement called isodicentric chromosome (Idic) or sometimes an inverted duplication (Invdup). They often do not contain the critical region (PWACR) for the Prader-Willi/Angelman syndrome and are generally associated with recurrent miscarriages, azoospermia and oligozoospermia. In this study we analyze a sSMC (15) corresponding to the region 15q11.2 inverted and duplicated, using both conventional and molecular cytogenetics, in a man that presents a primary infertility not caused by mutations in the CFTR gene or microdeletions of the Y chromosome.
This invdup(15) shows the occurrence of four NOR regions, and the presence of two hybridization signals for the region D15Z1, which indicates an isodicentric marker. By using FISH techniques we excluded the PWACR region, for the presence of hybridization signals of the probes BAC RP11-11H9 and RP11-720B15 (15q11.2 region) and the absence of the RP11-570N16 (15q12 region) and RP11-408F10 (15q13 region) signals on the marker. The semen analysis shows an oligoastenozoospermia and, in agreement with the literature, a low level of sSMC (15) in the spermatozoa (35%), highlighted by FISH using D15Z1 probe, that could be due to a selection against the marker during spermatogenesis. This would also explain the normal index of fragmentation of the sperm chromatin (DFI = 15%).
In 15q11.2 region POTE B, a gene expressed in the primary spermatocytes, is located. POTE protein induces the apoptosis, and a role of POTE in the maturation of sperm is possible. The replication of this gene could explain the semen alteration, representing a good starting point to understand the mechanisms that relate this sSMC with infertility.

ADVANCES IN IMAGING AND MOLECULAR TECHNOLOGY

NUCLEAR ARCHITECTURE AND FUNCTION STUDIED IN SPACE AND TIME WITH SUPER RESOLUTION FLUORESCENCE MICROSCOPY AND ELECTRON MICROSCOPY

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Super resolution fluorescence microscopy has narrowed the resolution gap to electron microscopy with the added potential of ultrastructural studies of nuclei in living cells. We have used structured illumination microscopy (SIM) [1] to compare the functional organization of nuclei in a variety of cell types and species. Further, we established a protocol for correlative microscopy for sequential studies of individual cells with live cell microscopy, SIM and transmission electron microscopy (TEM) [2]. Correlative microscopy combines the particular strength of each approach and compensates for its specific limitations. New evidence from mouse embryonic stem cells, cells in bovine pre-implantation embryos and human hematopoietic cells supports the chromosome territory – interchromatin compartment (CT-IC) model [3]. Active CTs form a three-dimensonal network of clustered chromatin domains (CDs), characterized by a compact chromatin core enriched in H3K27me3, a marker for transcriptionally silent chromatin, and a less compact periphery, called the perichromatin region (PR) [4, 5]. RNA polymerase II and H3K4me3, a histone modification typical for transcriptionally competent chromatin is enriched in the PR, which lines and also invades a network of interchromatin compartment (IC) channels. This channel network harbors nuclear bodies, pervades all CTs and has direct access to the nuclear pores. The IC channel system and the PR form the active nuclear compartment (aNC), whereas the inactive nuclear compartment (iNC) is represented by the compact chromatin interior of CD-clusters.

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ANALYSIS OF IMAGES OBTAINED BY FISH WITH CHROMOSOME-DERIVED DNA PROBES WITHOUT SUPPRESSION OF REPETITIVE DNA HYBRIDIZATION

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A great amount of interspersed repetitive sequences in chromosomes leads to difficulties in fluorescence *in situ* hybridization (FISH) performed with chromosome-derived DNA probes. This problem can be solved by suppression of repetitive DNA hybridization (Chromosomal *In Situ* Suppression Hybridization) (Lichter et al., 1988). However suppression can't be done on some species. Besides, the use of Cot-1 DNA in CISS-hybridization leads to disappearance of the signals of all repetitive sequences, including chromosome-specific ones. As an alternative to this approach the image processing methods were developed and performed: method (Rens et al., 2006) and VSSIS method (visualization special signal *in silico*) (Bogomolov et al., 2012). Both methods allow us to specify the signal correctly even for species where CISS-hybridization does not show satisfying results or just cannot be conducted. Here we present comparative analysis of results of usual FISH with method of CISS-hybridization and methods of computer image processing. DNA probes of individual chromosomes were made by microdissection following with DOP-PCR amplification (Rubtsov et al., 2000). FISH of microdissection DNA probes obtained from human chromosomes 1, 2, 3, 7, 10, 15, 18 and 19 with human chromosomes was performed according to the standard CISS-hybridization protocol, and without suppression of hybridization of the repetitive sequences (Lichter et al., 1988). For performance evaluation of the approaches the estimate, suggested in paper (Bolzer et al, 1999), was used.

The results obtained showed that the computer methods in general give results similaror better to results obtained with CISS-hybridization. However the case with contrasted SINE and LINE repeats the method of CISS-hybridization shows better results. Also both computer methods were tested on images of CISS-hybridization and FISH with chromosomes other *species*.

Both *in silico* methods can be used to improve results of multicolor FISH as well as CISS-hybridization (especially in case of incomplete suppression). Getting DNA probes from chromosomes with close type of repetitive DNA helps computer methods to efficiently identify specific FISH signal, otherwise difference in content of DNA probes can help to

analyze the distribution of repetitive sequences. Acknowledgements: This study was supported by grant from OPTEC LLC.

FT-IR ASSESSMENT OF CHANGES IN THE DNA METHYLATION STATUS OF LIVER CELLS FROM DIABETIC MICE

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The DNA of the liver cells of non-obese diabetic (NOD) mice under hyperglycemic conditions contains decreased abundance of cytosine methylation. Considering that the Fourier transform-infrared (FT-IR) profiles of DNA in dried samples are differently affected by the DNA base composition, single-stranded form and histone cationic binding [1], it is expected that the methylation status in the DNA could also affect its FT-IR profile. Here, the DNA FT-IR signatures obtained from liver cell nuclei of hyperglycemic and normoglycemic NOD mice of the same age were compared to each other.
A modification of the protocol reported by [2] was used for DNA extraction. Dried DNA samples were examined in an Illuminat IR II™ microspectroscope equipped with ARO objective and Grams software. Comparison of the FT-IR profiles between the hyperglycemic and normoglycemic mice revealed differences in the spectral range of 3600-2800 cm-1, especially when a peak fitting procedure was applied, at 1700-1500 cm-1 and at the spectral fingerprint region. The most elevated peak, especially assigned to DNA base hydrogen bonding, occurred at ~3338 cm-1 for the hyperglycemic mice and at 3369 cm-1 for the normoglycemic mice after a peak estimate method. Interestingly, a peak assigned to vibrations concerned with vas -CH3 and -CH2 groups was more elevated in the DNA from the hyperglycemic mice. However, the area covered by this peak in the less methylated DNA was smaller than that in the DNA from the normoglycemic mice. In addition, when comparing this peak between samples obtained from hyperglycemic and normoglycemic mice, different components resulted from peak fitting. In the spectral regions of PO2- vs and vas stretching, pentose ring vibration and main S-type sugar markers there was increase in vibrational intensities with decreasing DNA methylation.
Changes induced by hyperglycemia in the methylated status of the DNA from mouse liver cells can be assessed through the analysis of their DNA FT-IR profiles, revealing modifications in the vibrational intensities and frequency of several chemical marks including vas -CH3 and -CH2 groups.
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OPTICAL SUPER-RESOLUTION IMAGING OF CHROMOSOMES

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Conventional microscopes allow us to image objects on the 200 nm scale, whilst X-ray crystallography achieves resolutions on the angstrom scale. In between, new super-resolution (SR) microscopes have been used to make images with 20 nm resolution. Essentially, SR methods work by limiting the number of dye molecules that fluoresce at any one time. Among the most popular SR techniques are the so-called reconstruction methods: various chemical systems can be employed to ensure that only a sparse subset of molecules fluoresces. Many thousands of subsets are captured, the fluorophore centre localised and an image reconstructed from the localised points. Surprisingly, SR microscopy has been slow to take hold in chromosome research. There have been few reports of optical SR techniques used to study chromosomes. Two notable works are that of Matsuda et al. with PALM (photoactivated localisation microscopy) on drosophila chromosomes labelled with GFP and Zessin's paper on the dSTORM (direct stochastical optical reconstruction microscopy) imaging of HeLa cell chromatin. The dearth of SR chromosome studies may be due to the lack of suitable labelling methods available; traditional DNA stains do not perform as well as specialised fluorophores in techniques such as dSTORM. It seems necessary, therefore, to find a way of labelling chromosomal DNA with specialised fluorescent probes; several options exist, including probes made using the biopolymer CyDNA.

We have prepared chromosome samples with appropriate dyes for dSTORM using readily available commercial materials and dyes, following protocols that have been reported previously by different groups. We aim to use these samples to allow 2D and 3D images of chromosomes at high resolutions using the new SR microscope available at the Research Complex at Harwell. 3D imaging is achieved by means of optical sectioning using adaptive optics so that axial resolutions of 50 nm are attainable.

Whilst various imaging techniques have elucidated much information about the structure of the chromosome in recent years, new methods are coming to the fore which could reveal vital information about the formation and structure of these complex organelles. Particularly exciting are the non-destructive optical SR methods that have the potential to be used in real-time studies of live cells.

A FLOW MOLECULAR CYTOGENETIC APPROACH TO ANALYZE AND ISOLATE PLANT CHROMOSOMES: FLOW SORTING OF FISH LABELED CHROMOSOMES IN SUSPENSION

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The molecular cytogenetic approach allows karyotype analysis of mitotic cells on slide by using fluorescence *in situ* hybridization (FISH) to detect deletions, translocations and repetitive sequences distribution on chromosomes. The development of flow cytometry (FC) and flow cytogenetics enable to classify and isolate (flow sorting) metaphase chromosomes in suspension according to their DNA content. The sorted chromosome are useful in a broad range of applications reviewed by Dolezel et al. (2012), but in all plants, the possibilities to isolate single chromosomes is restricted to species, or cytogenetic mutants as in the case of wheats, containing chromosome types that differ in size from the standard complement. A reliable method is available now, termed FISHIS ''Fluorescence *In situ* Hybridization In Suspension'' (Giorgi et al. 2013) that combined with FC extends the analytical and preparative power of both molecular and flow cytogenetics giving rise to the new "Flow Molecular Cytogenetics" approach

FISHIS is a wash-less method that relies on readily available fluorescent labeled DNA oligonucleotides probes from simple sequence repeats (e.g. SSR) and employs alkaline DNA denaturation. It effectively generates specific hybridization patterns on chromosomes in suspension allowing a full FC discrimination and flow sorting of chromosomes. The flow molecular cytogenetic analysis of some Triticeae species allowed the ready separation of the A from the B genome chromosome complement of polyploid wheats (*Triticum durum* and *T. aestivum*) and of a number of specific chromosomes from the same species; and the isolation of each chromosome of the wild grain *Dasypyrum villosum* (L.) complement.

FISHIS is a new method that allows to join the high discriminatory capabilities of FISH labeling and the high-throughput of flow cytometry analysis overcoming the need of DNA content differences among chromosomes to flow sort single type chromosomes. In less than 90 mins a specific FISHIS labelling can be performed opening the access to potentially each individual chromosomes of any eukaryotic genome, providing a good chromosome suspension and a suitable probe are both available.

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TYRAMIDE-FISH IS A USEFUL TOOL FOR CYTOGENETIC MAPPING OF GENES IN PLANT SPECIES WITH SMALL AND LARGE CHROMOSOMES

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Direct visualization of short DNA fragments (e.g. genes, ESTs) on compact mitotic chromosomes is very challenging in plant cytogenetics. One of the promising approaches is the application of Tyramide-FISH. However, Tyramide-FISH is not exploited in plant cytogenetics and only few articles have been published using this technique for cytogenetic mapping of genes and RFLP markers. The aim of our study was to optimize a Tyramide-FISH approach for *Allium*, having large chromosomes and *Rosa*, with small chromosomes. In addition integration between physical and genetic maps of species studied and comparative studies were performed.
A direct detection system, using FITC and Cy3 labeled tyramides, was efficient for gene fragment (0.5 - 1.1Kb) localization on *A.cepa* and *A.fistulosum* chromosomes with high signal detection frequency (up to 80%). Application of this direct detection system on rose chromosomes resulted in low signal-to-noise ratio. Therefore, an indirect detection system was optimized for rose chromosomes. By this optimized system, cytogenetic mapping of low-copy genes involved in the abiotic stress response (Phenylalanin Ammonia Lyase (PAL; 1700bp) and Pyrroline-5-carboxylate synthase (P5CS; 1700bp)) and in rose scent production (orcinol O-methyltransferase (OOMT; 1100bp) was successful. In addition, the High Resolution Melting (HRM) technology was used in order to do genetic mapping of these genes and to anchor *R.wichurana* linkage groups to the physical rose chromosomes. Gene fragments (1.1-1.3Kb) of three members of the alliinase gene family and lachrymatory factor synthase (550 bp), involved in the specific onion scent and medical properties production, were used for Tyramide-FISH mapping on the chromosomes of 6 different *Allium* species. It was clear that the evolution of the alliinase genes in *Allium* was accompanied by significant genome organization events including pericentric inversions and duplications. Integration of bulb onion physical and genetic maps was performed using these Tyramide-FISH mapped sequences. Using Tyramide-FISH nuclear-chloroplast insertions (NUPT) of individual chloroplast genes were mapped on the chromosomes of *Allium cepa* and *Allium fistulosum*. The number and the chromosome distribution of NUPT patterns were different between these two species.

Our results clearly show that Tyramide-FISH is a valuable tool for comparative genomics, evolutionary studies and integration of physical and genetic maps.

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