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July 10–13, Foz do Iguaçu, Brazil

21st International Chromosome Conference (ICC)

A venue that offers a diversity of scientific approaches to chromosome biology and a diversity of wildlife in Iguaçu National Park

The International Chromosome Conferences (ICC) originated from the Oxford Chromosome Conferences, inaugurated by C.D. Darlington and K.R. Lewis in 1964 and held subsequently in England in 1967 and 1970. The Chromosome Conference grew to an international event with its fourth meeting, held in Jerusalem, Israel in 1972, heralding the beginning of 40 years of technological advances that have expanded our understanding of chromosome biology in model and non-traditional biological systems. Having been hosted in Europe and the United States 16 times since then, this year the ICC will be held across the equator in Foz do Iguaçu, Brazil, on July 10–13, 2016. The event will bring scientists from across the globe to a biannual meeting focused on modern advances in chromosome biology, technology and theory. The Iguaçu National Park, a UNESCO World Heritage Centre, includes the Iguaçu Falls and has been chosen as one of the 'New Natural Seven Wonders of the World'. Home to an amazing diversity of life, including over 2,000 species of vascular plants, exotic mammals such as tapirs, giant anteaters, howler monkeys, ocelots, and jaguars, in addition to hundreds of different bird species and thousands of different insects, the choice of Foz is an excellent analogy for the diverse approaches and systems chromosome biologists explore, and that will be emphasized throughout this conference.

The 2016 ICC program offers seven sessions, beginning with a session on ***Chromosome Structure and Nuclear Architecture***, highlighting the influences and interactions chromosomes have on the three-dimensional space of the nucleus. Session II will focus on ***Specialized Chromosomes***, such as sex chromosomes and B chromosomes, whose structure and behavior are often distinguished from that of autosomal chromosomes. ***Population and Evolutionary Chromosome Biology***, the third session, covers a synthesis of chromosome biology and

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the theoretical approach to understanding the evolution of genomes and species. Sessions IV and V focus on the dynamic nature of chromosomes during cell division, ***Mitosis and Meiosis***, and as influenced by different chromatin states, ***Chromatin and Chromosome Dynamics***. Finally, the conference concludes with two sessions that focus on the technological advances in approaches to studying chromosomes in translational fields, ***Applied Chromosome Biology in Medicine and Agriculture***, and more broadly, ***New Technologies***. Across all of these sessions, talks are presented from invited speakers and those selected from the submitted abstracts, allowing the organizing committee to include new scientists in the program (students, postdocs, and young faculty) as well as late-breaking scientific advances. Poster sessions will promote interactions between established scientists and trainees that will enrich the community and foster mentoring and networking opportunities.

Offering a unique experience in both venue and breadth of chromosome research advances and technologies that span topics in both traditional and emerging model systems, the 21st ICC is an event that promises to synergize an invigorated and fast-paced field. We thank our funding agencies and sponsors, invited speakers, and participants and look forward to an exciting conference!

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Keynote Talk

Using Synthetic Biology to Study Chromosome Structure and Segregation

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The discovery of human centromere proteins using sera from patients with scleroderma-spectrum autoimmune disease led to the modern era of kinetochore studies in which over 100 proteins have now been identified at kinetochores. One insight that came from the use of these antibodies was the discovery that kinetochore assembly is regulated by an epigenetic mechanism. Thus, in stable dicentric chromosomes with 2 copies of the centromeric DNA sequences, only 1 centromere locus nucleates formation of a kinetochore. Our ongoing studies aim at determining the chromatin landscape that promotes kinetochore assembly. In the first part of my talk, I will describe studies using a synthetic human artificial chromosome that enables us to adjust the chromatin composition within the centromeric chromatin. These studies have revealed that heterochromatin is incompatible with kinetochore function. Indeed, nucleating heterochromatin within an active centromere rapidly inactivates that centromere. We have gone on to show that centromere function appears to require ongoing transcription of noncoding sequences. This transcription occurs even though centromeres exist in an environment flanked by 'deep' heterochromatin. Centromeres can apparently function in this environment because they are able to resist normal pathways of transcriptional inactivation by both Polycomb and classic heterochromatin. I conclude that synthetic biology offers a powerful approach to study chromatin function at centromeres. The second part of my talk will introduce PREditOR. Epigenetic regulation in chromatin has the following steps: an *Editor* (writer or eraser) makes or removes a *Mark* on a chromatin protein. A *Reader* either recognizes this mark (or no longer does so if the mark is removed). The binding of the Reader establishes a *Chromatin state* (e.g. euchromatin, heterochromatin, centrochromatin). We refer to this as an E→M→R→C pathway. We have designed an approach called PREditOR (Protein Reading and Editing Of Residues) to dissect and manipulate E→M→R→C pathways. This talk will present data concerning the outcome of using one Reader to target another Reader to the correct location in chromatin, but with the wrong binding dynamics. The results of this analysis reveal that the dynamics of Reader binding in chromatin is a critical factor in determination of the functional chromatin state.

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I. Chromosome Structure and Nuclear Architecture

Invited Talks

I.1

Chromosomal Translocations via Intercentromeric Recombination Underlie Mating Transitions during Fungal Evolution

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Cryptococcus neoformans is a pathogenic basidiomycete fungus present in the environment globally that annually causes more than one million infections, >620,000 deaths, and about one third of all those are HIV/AIDS-associated. *C. gattii* is a related, distinct pathogenic species that is more geographically restricted and causes fewer infections, but often afflicts healthy individuals. While only 2 pathogenic species are currently recognized, as many as 6–9 species populate this monophyletic pathogenic species complex. These pathogenic species are embedded within 2 broader species groups, the *sensu stricto* and *sensu lato* complexes, which are saprobic, insect, or plant/tree associated fungi that are not animal pathogens. The *sensu stricto* complex includes the *Cryptococcus* pathogenic species complex (7 defined species) and just 3 additional species: *C. amylolentus*, *Filobasidiella depauperata*, and *Tsuchiyaea wingfieldii*. The *sensu lato* complex includes *Kwoniella mangrovensis*, *K. europaea*, *K. botswanensis*, *C. heavenensis*, and several other species. How pathogenesis evolved is a central question being addressed via comparative genomics to reveal genes and gene sets unique to pathogens, or lost or gained in particular pathogenic lineages, and higher order changes in genomic architecture. We have discovered extant sexual cycles and defined the mating type loci for several of these species and are contributing to ongoing genome projects with the Broad Institute Fungal Genome Initiative and the Genoscope Dikaryome Project. We found that the genome of the closest nonpathogenic sibling species, *C. amylolentus*, has 14 chromosomes like *C. neoformans* and *C. gattii,* but with extensive translocations and also many intrachromosomal rearrangements, such as inversions and transpositions. We have defined an extant tetrapolar sexual cycle for *C. amylolentus* and found evidence that the *MAT* loci are genetically linked to the large regional repetitive centromeres of their host chromosomes (Chr. 10 and Chr. 11). This finding suggests models in which intercentromeric recombination may have contributed to drive chromosomal translocations and the transition from an ancestral tetrapolar outcrossing saprobic state to the derived pathogenic bipolar configuration. Similar types of intercentromeric chromosomal translocations are apparent by comparing the *C. neoformans* and *C. gattii* genomes, and the *C. amylolentus* and *C. neoformans* genomes, and also *Candida* species, suggesting this may be a general mechanism of genome rearrangement. These findings contribute to a growing appreciation that rather than being

bereft of recombination, gene conversion, and crossing over involving centromeres can occur to impact evolutionary trajectories during mating type locus evolution and speciation.

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I.2

Retroelements and Noncoding RNAs Linked to Centromere Turnover Macropodid Chromosome Evolution

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Two paradoxes of centromere biology have confounded our understanding of the eukaryotic centromere, and consequently of chromosome evolution. The first is that centromere function is highly conserved across eukaryotes, yet centromere-specific proteins that interact with nucleic acids diverge rapidly. The second is that while satellite DNA is found ubiquitously across eukaryotic centromeres, it is considered neither necessary nor sufficient for centromere formation. Major hurdles in understanding centromere evolution lie in the highly repetitive nature of most centromeric DNA and an inability to decouple centromere divergence from species evolution and stochastic processes such as genetic drift and molecular drive. Using comparative cytogenomics, we have identified specific retroelements and RNA sequences that are coincident with active centromere demarcation in a broad range of mammalian species spanning all therian clades, affording the ability to assess the evolution of centromeres in the context of the transcriptional activity of nascent centromeric elements. As an exemplar, the macropodid species complex is typified by rapid chromosome evolution and convergence of karyotypes independent of ancestry. Notably, each of these karyotypic rearrangements involves centromeres, and hybrids between even closely related species possess abnormalities delimited to centromeres. Our ChIP-seq, RIP-seq, RNA-seq, genome assembly, and repeat analysis computational methods have established a testable model of centromere evolution in the context of rapid chromosome and species evolution. Our findings on satellites, retroelements, and noncoding RNA elements will be presented in the context of conflict between nucleic acid binding proteins and centromeric DNA domains, and ultimately chromosome evolution.

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I.3

Structure and Evolution of *Rhynchospora* Vahl (Cyperaceae) Holocentric Chromosomes

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Holocentric chromosomes differ from monocentrics due to the presence of kinetochore activity along most of the chromatid length. This particular centromere organization evolved independently several times among eukaryotes, leading to differences among holocentrics from different organisms. We have shown that holocentrics from the plant genus *Rhynchospora* have centromeres enriched in retrotransposons and satellite DNAs, what had so far not been detected in any holocentromere. Analyses of different *Rhynchospora* species revealed 3 types of satellite DNA organization, associated or not with typical heterochromatin or with holocentromeres. Similar to *Luzula*, *Rhynchospora* species have evolved an inverted meiosis, with segregation of sister chromatids, instead of homologues, in the first meiotic division. A particular centromere organization was, however, observed during meiosis. The structure of *Rhynchospora* holocentrics will be discussed as well as the consequences of holocentricity to karyotype evolution in the genus.

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I.4

Nuclear Morphology and Fertility in Mouse Sperm

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Nuclear organisation – the position chromosomes occupy in the interphase nucleus – has been a topic of interest for many years. The organisation is cell type- and species-specific, and perturbations of chromosome positioning have been linked with disease. One example of this is infertility – infertile men appear to have an altered sperm chromosome arrangement compared to men with no fertility problems. The sperm of mice are exceedingly interesting for nuclear organisation studies; they have a distinctive hook-shaped sperm head, where the acrosome extends from the tip of the hook over the 'hump' of the sperm head. Furthermore, deletions on the Y chromosome affect the shape of the sperm head. How is chromatin packaged within such a shape? Does nuclear morphology alter in mouse models with fertility problems? How are chromosomes positioned, and does this differ between our mouse models? We have developed a general analysis program for assessing nuclear morphology and chromosome territory position within asymmetric nuclei, both pre- and post-FISH. This system has been successfully tested on sperm from multiple species. Using this approach, we have characterised the nuclear morphology of sperm from wild-type mice and mice with a $^{2}/_{3}$ deletion of the Y chromosome, revealing regions of morphological difference between the genotypes. Studying the regions of the nucleus morphologically affected provides insights into the developmental pathways and cytoskeletal components affected by the Yq deletion and helps us link the genes in the deleted region to their phenotypic effects.

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I.5

Variability in Centromere Position in Chromosomes 2 and 4 of Tropical Maize Lines

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Maize chromosome variability has been extensively investigated. The identification of maize somatic and pachytene chromosomes was improved with the development of fluorescence in situ hybridization (FISH) using tandemly repeated satellite sequences as probes. The somatic chromosomes of sister inbred lines derived from a tropical flint corn population [Jac Duro (JD)] and hybrids between them were investigated by FISH-mapping of the180-bp knob repeat, centromeric satellite (CentC), centromeric satellite 4, subtelomeric clone 4-12-1, 5S ribosomal DNA (rDNA), and nucleolus organizer DNA sequences. These markers allowed unequivocal identification of the chromosomes. In the present study, we show that the centromere position of chromosomes 2 and 4 differed from the pattern reported for standard maize lines. The arm ratio value was higher in chromosome 2 (1.44) compared with chromosome 4 (1.30). In pachytene, arm ratios were 1.71 and 1.44, respectively, for chromosomes 2 and 4. These values were significantly different in the JD lines compared with the commonly used line KYS. The pairing behavior at pachytene stage of chromosomes 2 and 4 of a hybrid between a JD line and KYS line was investigated. The homologues were fully synapsed, including the 5S rDNA and CentC sites on chromosome 2, and Cent4 and subtelomeric 4-12-1 sites on chromosome 4. This suggests that homologous chromosomes could pair through differential degrees of chromatin packing in homologous arms differing in size. The arm ratio of the hybrid chromosome 2 was 1.69, thus similar to the JD line. The arm ratio of chromosome 4 was 1.52, intermediate between the parental JD and KYS lines. The results contribute to the current knowledge of maize global diversity and also raise questions concerning the meiotic pairing of homologous chromosomes possibly differing in their amounts of repetitive DNA.

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I.6

Comparative Analysis of Repetitive DNA in the Large, Heteromorphic Chromosome Pair of the Bimodal Species *Eleutherine bulbosa* Miller (Urban)

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Eleutherine bulbosa (Iridaceae, 2n = 12) presents a bimodal karyotype with one chromosome pair 3–4 times larger than the other chromosomes. This large pair has a pericentric inversion comprising 70% of the chromosomes in permanent heterozygosis, resulting in an acrocentric and a metacentric chromosome that show suppressed meiotic recombination in the inverted region. In addition, this large pair is enriched in repetitive DNA sequences and epigenetic marks typical for heterochromatin. In order to understand the possible divergence of repetitive elements in the inverted region, 10 chromosomes of each homologue were microdissected, their DNA was amplified and sequenced by Illumina. The repetitive DNA composition of both homologues was compared using the Galaxy/RepeatExplorer pipeline. Five satellite DNA families (satDNAs), 7 LTR-retrotransposon lineages, LINE sequences, DNA transposons, and ribosomal DNA were identified. The large chromosomes were enriched for satDNAs when compared to the whole genome sequence. The Eb1 satDNA abundance was apparently almost 3 times higher in the metacentric than in the acrocentric chromosome; conversely, 2 novel satellites were higher in the acrocentric than in the metacentric. Among the LTR-retrotransposons, Ty1/Copia Angela, Tork, Ale II, and TAR lineages were obviously more abundant in the metacentric chromosome, while Ty1/Copia Maximus and Ty3/Gypsy Chromovirus were more abundant in the acrocentric. Thus, as consequence of suppressed meiotic recombination, a bimodal karyotype evolved due to the accumulation of repetitive DNA in the large pair of chromosomes.

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I.7

Chromosomal Mapping of Telomeric and Interstitial TTAGG Repeats in Six Species of *Belostoma* (Heteroptera: Belostomatidae)

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The $(TTAGG)_n$ sequence, the ancestral motif of insect telomeres, shows highly conserved chromosomal localization across the phylogenetic tree of insects except those orders in which it was replaced with another motif or an alternative mechanism of telo-

mere elongation. However, in a few species additional interstitial telomeric sequences (ITS) were found. The occurrence of ITS has been associated with karyotype evolution through telomere-telomere fusions. In giant water bugs of the genus *Belostoma*, karyotypes of individual species differ from each other in chromosome number and sex chromosome system. The chromosome number changed during speciation by fragmentation of the single ancestral X chromosome, resulting in a multiple sex chromosome system. Furthermore, several autosome-autosome fusions and a fusion between the sex chromosome pair and the NOR-bearing autosome pair resulted in a reduction of the number of chromosomes and an increase of their size. In this study, we mapped the chromosomal distribution of TTAGG repeats in *Belostoma candidulum* (2n = 12 + XY/XX), *B. dentatum* (2n = 26 + $X_1X_2Y/X_1X_1X_2X_2$), *B. elegans* (2n = 26 + $X_1X_2Y/X_1X_1X_2X_2$), *B. elongatum* (2n = 26 + $X_1X_2Y/X_1X_1X_2X_2$), *B. micantulum* (2n = 14 + XY/XX), and *B. oxyurum* (2n = 6 + XY/XX) by FISH with the insect telomeric probe. Hybridization signals of the probe at the end of chromosomes confirmed the presence of TTAGG repeats in the telomeres of all species examined. In species with reduced chromosome numbers, we observed additional hybridization signals in interstitial positions, indicating the occurrence of ITS. Comparison of the ITS distribution between the closely related species supports the hypothesis that chromosome fusions played a major role in the karyotype evolution during speciation of the genus *Belostoma*.

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I.8

The Genomic Organization of Classical Genomes Based on Shannon Entropy

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The level of organization of DNA sequences has been studied, and a pattern for all organisms is the key point to understand the information contained in it. The Shannon entropy was the first concept concerning entropy using information theory; it is employed to quantify the organizational level of any system. Here, we developed an algorithm to apply the Shannon entropy equation on DNA. It was applied to classical genomes *(Homo sapiens*, *Takifugu rubripes*, *Haemophilus influenza*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, and *Escherichia coli)* to look how long the most organized genomic/chromosomic regions are without overlapping different sequences, as traditionally has been done. The results of the entropy calculations showed a convergence of the entropy of organized regions ($H_{i,i+L}$, where i is the initial position of the segment at the genomic sequence) and the length L of nucleotide segmentation. It is possible to observe the lower entropy values converged to the length of segmentation L ranging from 48 to 1,004 nucleotides. We also identified that the lowest entropy values can be correlated to the genome size using an exponential equation with $R^2 > 0.8$. This result is a paradox since it could be anticipated that larger genomes should have higher entropy values because they have a higher amount of DNA spacers and repetitive DNA, for example, which are more prone to maintain mutations. Now we are working with a hypothesis that includes the selective pressure rate over genomic regions to explain it, and new analyses have been performed.

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I.9

Chromothripsis, Chromoplexy, and Other Chaotic Genomic Rearrangements in Osteosarcoma

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Osteosarcoma (OS) is an aggressive bone tumor with a highly unstable karyotype. A new type of genetic abnormality termed chromothripsis (Greek word, *chromo*: chromosome; *thripsis*: shattering into pieces) was recently described in OS and other cancers. When chromothripsis occurs, a single chromosome is fragmented and reassembled into ten to hundreds of complex genomic rearrangements following a sudden single catastrophic genomic event. The rearrangements associated with chromothripsis are thought to arise because of an atypical repair process, and they are confined to one single or a very few chromosomal regions. Chromothripsis may occur in 2–3% of cancers, but in OS the rate may be much higher at 33%. Chromoplexy (from the Greek *pleko*, which means to weave) is a similarly chaotic class of alteration, marked by adjacent breakpoints derived from separate DNA fusions, which appear to form complex chains of rearrangements. In this study, we performed a summary analysis of 106 OS samples (comprising 11 cell lines and 95 tumors) based on sequencing technology and array-CGH to determine the incidence of the various classes of chaotic genomic rearrangements. We showed that 7 had chromothripsis and a further 17 had chromoplexy/chromothripsis-like rearrangements. Alterations predominantly involved chromosomes 6, 8, 9, 13, and 14. To better understand the clinical significance of chaotic genomic rearrangements in OS, we performed a more detailed analysis of genomic imbalances in 10 OS tumor samples previously published by this laboratory [Hum Mol Genet 18:1962–1975 (2009)]. Chromothripsis-like events (i.e., >25 copy number transitions within 25 Mb) were observed in 3 OS samples (30%), involving the chromosomes 2, 6, 10, 12, 14, and 15. Chromothripsis appears to be an important contributor to OS genomic diversity, and the implications of this new mechanism should be considered when novel treatment strategies are applied to this tumor.

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I.10

Fine Mapping of Late-Replicating Bands Partitions the *Drosophila* Genome into Two Contrasting Types of Domains Whose Properties Are Dependent on Their Size

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We developed a comprehensive approach that combines cytology mapping data of the FlyBase-annotated genes, novel tools for predicting cytogenetic features of chromosomes based on their protein composition [Zhimulev et al., 2014] (reference to be obtained by the author), and data on the replication timing of polytene chromosome bands and obtained the genomic coordinates for all 'black' late-replicating bands of polytene chromosome 2R of *Drosophila melanogaster*. In total, 159 'black' bands were mapped with lengths varying from 15 to 500 kb. The total length was 11.6 Mb for bands and 7.6 Mb for intervals between them. Using published genome-wide datasets for salivary glands and cell cultures, we have shown that the genomic regions corresponding to all mapped 'black' bands share a set of properties earlier described for intercalary heterochromatin regions, namely: (a) Band size-dependent replication delay in polytene chromosomes and in cell cultures; reduced levels of ORC2 binding. (b) Silent chromatin types in different tissues, with short fragments of active chromatin preferentially located at the borders. (c) Enrichment with the SUUR and Lamin proteins, with their distribution profile showing sharp changes at the band borders. (d) Enrichment with genes that have narrow developmental expression patterns and low expression levels, lower gene density, conserved gene order in the *Drosophila* genus, enrichment with male-specific genes. All these properties become more pronounced as the length of the band grows. All the bands, however, differ drastically in these features from intervals between them. The borders of 'black' polytene chromosome bands correspond well to the borders of topological domains. As a conclusion, we have shown that the characteristic pattern of polytene chromosomes compacted 'black' bands alternating with less compact grey bands and interbands reflects a partition of the *Drosophila* genome into 2 global types of physical domains with contrasting properties, with expression depending on domain size manifestation.

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I.11

Tissue-Specific Genome Instability in Synthetic Interspecific Hybrids of *Pennisetum purpureum* and *Pennisetum glaucum*

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Chromosome instability occurs in several interspecific hybrids of plants. We studied the mechanisms underlying the genome instability in hexaploid induced hybrids of *Pennisetum purpureum* (Napier grass) and *P. glaucum* (pearl millet). The hexaploid hybrid produced by chromosome doubling shows genome instability in different tissue types producing micronuclei. The aim of this study is to evaluate the chromosome elimination in different tissues and the ploidy level of hybrids of Napier grass and pearl millet. We verified the genome instability by chromosome counting, estimation of DNA content by flow cytometry, and analysis of centromere activity using a centromere-specific histone H3 (CENH3) antibody, as well as fluorescence in situ hybridization with labeled centromere- and telomere-specific sequences. Comet assay and genomic in situ hybridization using Napier grass and pearl millet genomes as probes were also performed. The results suggest that chromosome elimination is found preferentially in meristematic tissue of hexaploid hybrids, and chromosome elimination is not only caused by centromere dysfunction. In addition, double-strand breaks occur as consequence of the combination of different genomes leading to the formation of chromosome fragments and micronuclei. Chromosomes of both parental genomes are eliminated.

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I.12

Nuclear Repositioning and Loss of Trisomic Chromosome 18 in Human Promyelocytic HL60 Cells Induced by Retinoic Acid

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Vertebrate cell nuclei are complex, highly organized and dynamic structures exhibiting well-defined chromosome territories (CT) surrounded by the interchromatin compartment. CT corresponding to gene-rich chromosomes map preferentially to the nuclear core while gene-poor ones mostly reside in the periphery. The human promyelocytic cell line HL60 exhibits trisomy of chromosome 18, t(5;17)(q35;q21) involving the *RARA/NPM* genes among other translocations, and loss of 1 X chromosome. Treatment of

HL60 cell cultures (6 days) with all-*trans*-retinoic acid (ATRA) induces granulocytic differentiation and nuclear polylobation (HL60-ATRA). We investigated the positioning of trisomic gene-poor CT 18 and gene-rich CT 19 in polylobated HL60-ATRA nuclei compared to their distribution in controls using whole chromosome probes for CT 18 and CT 19 and fluorescence in situ hybridization. The majority of HL60-ATRA polylobated nuclei revealed different relocation patterns of one CT 18, namely (a) segregation to a peripheral lobe; (b) incorporation into a nuclear bud; (c) inclusion into a drumstick-like appendage; (d) elimination inside a micronucleus. Image analysis of HL60-ATRA nuclei revealed a decreased area linked to increased circularity of repositioned CT 18 compared to its 2 homologs and both CT 19. Results obtained in our experimental model suggest that mammalian nuclei may have tailored appropriate tools to count, segregate, oust, and eliminate extra chromosomes.

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I.13

The First Cytogenetic Description in Schizomida, a Micro-Diverse Arachnid Order

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Schizomida is considered a micro-diverse order within Arachnida, including not more than 210 taxonomically described species. This order is subdivided into 2 families, Protoschizomidae with ~15 species endemic to the United States and Mexico and Hubbardiidae that presents a worldwide distribution. The schizomids are small arachnids with a medium size of 5 mm, which live mainly in the leaf litter, but some species also occur in caves. Within the family Hubbardiidae, the genus *Rowlandius* is the most diverse, and in Brazil it is distributed in the Atlantic rainforest and semiarid regions. Cytogenetically, no Schizomida was studied so far. The aim of this work is to describe the karyotype of 2 schizomids from the Brazilian fauna. The sample included 6 females of *Rowlandius ubajara* from Ubajara, CE, and 3 females of *Rowlandius* sp.n. from Floriano, PI. Due to the small size of the individuals, chromosome preparations were obtained from the whole content of the opisthosoma. The slides were stained with 3% Giemsa solution. Mitotic metaphases of the 2 species showed a diploid number of 2n = 22 in *R. ubajara* and 2n = 20 in *Rowlandius* sp.n. In both species, the chromosomes presented predominantly subtelo/acrocentric morphology, with the exception of 1 pair that was biarmed. In *Rowlandius* sp.n. this biarmed pair was easily recognized as the large chromosome of the diploid set. However, in *R. ubajara*, all the chromosomes exhibited a gradual decrease in size. The cytogenetic data obtained here reinforces the idea that karyotype differentiation evolved independently to an increase or decrease of the diploid number in related arachnid orders, such as Schizomida (2n = 20–22), Amblypygi (2n = 66–70), Thelyphonida (2n = 12–49), and Araneae (2n = 7–128). In Schizomida, it is not yet possible to affirm if differentiated sex chromosomes are absent or present, considering that only females presented cells in adequate stage for cytogenetic analyses. Thus, an effort to obtain chromosome preparations of males is indispensable in further studies.

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I.14

The Molecular Mechanisms of Heterochromatin Expansion in Rye Chromosomes

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A prominent and distinctive feature of rye *(Secale cereale)* chromosomes is the presence of massive blocks of subtelomeric heterochromatin, the size of which is correlated with the copy number of tandem arrays. The rapidity with which these regions have formed over the period of speciation remains unexplained. As direct sequencing of such genomic regions is hampered by their repetitive nature, long-range organization, mutual arrangement of monomers within arrays, and molecular features of genomic regions flanking tandem repeats remain poorly explored. We took the advantage of the availability of a subgenomic BAC library created from the short arm of rye chromosome 1R (1RS) to obtain a detailed picture of the organization of tandem repeats and transposable elements (TEs) making up subtelomeric heterochromatin of the chromosome arm. The presence of multiple short monomer arrays, coupled with star-like topology of the monomer phylogenetic trees, indicated a rapid expansion of the tandem arrays. The evolution of subtelomeric heterochromatin appears to have included a significant contribution of illegitimate recombination. Extending this approach to a genome-wide scale by analysis of 454 reads, we found that the composition of TEs in regions flanking the arrays of major tandem repeat families differed markedly from the remaining parts of the genome. Solo-LTRs were strongly enriched, suggestive of a history of active ectopic exchange. The sequence of junctions between TEs and the arrays harbored several short DNA motifs, which could be involved in promoting chromosomal rearrangements. The large amount of DNA sequence data obtained allowed us to conclude that the large blocks of subtelomeric heterochromatin arose from a combined activity of TEs and the expansion of tandem repeats. The expansion was likely based on a highly complex network of recombination mechanisms.

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II. Specialized Chromosomes

Invited Talks

II.1
Origin and Evolution of *Drosophila* Y Chromosomes

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X and Y chromosomes are widely believed to evolve from an ordinary pair of autosomes, the Y chromosome originating through massive gene loss in one of the homologs. However, studies in 12 *Drosophila* species showed that gene gains (instead of gene losses) played the major role in its evolution. Furthermore, most *Drosophila* Y-linked genes have autosomal paralogs, and hence autosome-to-Y transposition must be the main source of *Drosophila* Y-linked genes. Using a recent PacBio assembly of the *D. melanogaster* genome, we showed how these genes were acquired. We identified a new gene *(FDY – flagrante delicto Y)* that originated from a recent duplication of the autosomal gene *vig2* to the Y chromosome. Four contiguous genes were duplicated along with *vig2,* but they became pseudogenes through the accumulation of deletions and transposable element insertions, whereas *FDY* remained functional, acquired testis-specific expression, and now accounts for ~20% of the *vig2*-like mRNA in testis. *FDY* is absent in the closest relatives of *D. melanogaster*, and DNA sequence divergence indicates that the duplication to the Y chromosome occurred ~2 million years ago. Thus, *FDY* provides a snapshot of the early stages of the establishment of a Y-linked gene and demonstrates how the *Drosophila* Y has been accumulating autosomal genes. Contrary to the pattern seen in mammalian sex chromosomes, where most Y-linked genes have X-linked homologs, the *Drosophila* X and Y chromosomes appear to be unrelated.

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II.2
Origin and Regulation of Rye B Chromosomes

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Supernumerary B chromosomes (Bs) are dispensable components of the genome exhibiting non-Mendelian inheritance; they have been widely reported in over several thousand eukaryotes but still remain an evolutionary mystery ever since their first discovery over a century ago. Recent advances in genome analysis significantly improved our knowledge on the origin and composition of Bs. In contrast to the prevalent view that Bs do not harbor genes, recent analyses revealed that Bs of sequenced species are rich in gene-derived sequences. I will summarize the latest findings on supernumerary chromosomes with a special focus on the origin (1), DNA composition (2), gene erosion process (3), and the non-Mendelian accumulation mechanism (4) of rye Bs.

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II.3
The Origin of Multiple Sex Chromosomes in Moths and Butterflies

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The majority of moths and butterflies (Lepidoptera) have a WZ/ZZ (female/male) sex chromosome system, which has evolved from the Z/ZZ system occurring in basal moths and caddisflies (Trichoptera). In quite a large number of species, the WZ pair has been altered by fusion with an autosome pair, resulting in neo-sex chromosomes. The origin of neo-sex chromosomes is best explored in geographical populations of wild silkmoths, *Samia cynthia*, in which gene-based synteny mapping revealed a step-by-step evolution by repeated autosome-sex chromosome fusions. Recent data suggests that neo-sex chromosomes may contribute to the formation of reproductive barriers and play a significant role in adaptive evolution. However, multiple sex chromosomes were found only in a few taxa from different lineages of Lepidoptera, and their evolutionary significance is poorly understood. We examine the origin of multiple sex chromosomes in 2 distant taxa, small ermine moths of the genus *Yponomeuta* and wood white butterflies of the genus *Leptidea*. In the species examined, *Yponomeuta* females show a WZ_1Z_2 trivalent in pachytene oocytes, while *Leptidea* females show a complex species-specific multivalent with 3–4 W chromosomes and 3–6 Z chromosomes. Our research strategy is based on synteny mapping of orthologous genes by BAC-FISH. For this purpose, BAC libraries and transcriptomes of *Y. evonymella* and *L. juvernica* are available. We identify orthologs of sex-linked genes in 2 ways, (i) by search for orthologs of Z-linked genes of the silkworm, *Bombyx mori*, and (ii) by array-CGH, i.e., comparative genomic hybridization of male and female genomic DNAs to microarray slides with oligonucleotide probes. Then the orthologs are used as markers to select BAC clones from the corresponding BAC library. The BAC clones are mapped to chromosomes of particular species of *Yponomeuta* and *Leptidea*, respectively. This approach allows us to identify genomic regions involved in multiple sex chromosomes and determine their origin.

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II.4

Sex-Linked Chromosomal Heterozygosity in the Scorpion *Tityus confluens* Borelli, 1899 (Buthidae)

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Tityus shows holokinetic chromosomes, achiasmatic male meiosis, and absence of heteromorphic sex chromosomes, as all Buthidae. Previous studies of males from a population of *T. confluens* Borelli, 1899 from Reserva Natural Formosa (Argentina) revealed a numerical and structural polymorphism, comprising 4 cytotypes: (A) 2n = 6, II + IV at meiosis I; (B) 2n = 5, V type 1; (C) 2n = 5, V type 2; (D) 2n = 6, VI. In this work, we analyzed the cytogenetic difference between males and females and discuss its possible relation with sex chromosomes. Thirteen males, 6 females, and a litter of 15 embryos (of one of these females) from the same population were studied by C-banding and FISH with a 28S rDNA probe. All females presented 2n = 6 with 2 large and 4 medium-sized chromosomes. rDNA signals were detected at one terminal region of 2 medium chromosomes, and the C-banding pattern allowed us to distinguish 3 chromosome pairs. Within the litter, 8 embryos showed 2n = 6, and 7 embryos presented 2n = 5. Males of cytotype A demonstrated rDNA signals at one terminal region of the bivalent. Cytotypes B and D showed terminal signals at 2 chromosomes of different size, while cytotype C showed signals at one terminal region of a medium chromosome and at an interstitial region of the largest chromosome. The fact that all the analyzed males were structural heterozygotes and all the females were homozygotes could indicate a case of sex-linked chromosomal heterozygosity, the male being the heterogametic sex. We propose that the ancestral karyotype of this species could have had homomorphic sex chromosome pairs XY/XX (male/female), and a fusion between the Y chromosome and an autosome could have occurred. Rearrangements involving the autosomes and the Y chromosome could have given rise to meiotic multivalents in males and bivalents in females. The different karyotypes observed in the litter of embryos support this model.

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II.5

Genomic Analysis of Transposable Elements in *Astyanax correntinus* with Focus on B Chromosome

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Supernumerary (B) chromosomes are additional to the regular complement set (A) and have been found in ~15% of eukaryotic species karyotyped so far. Despite the fact of continual analysis and numerous reports by many researchers, a complete understanding about the origin and specific molecular composition of Bs is limited. A significant feature of these dispensable chromosomes is to accumulate repetitive DNA including transposable elements (TEs), leading to species-specific evolutionary fates. Here, we conducted Illumina next-generation sequencing of the complete genomes of 2 individuals of the fish species *Astyanax correntinus* with (1B) and without (0B) B chromosome to investigate distinct B-related repetitive contents and their relative abundance. Repeats, including TEs, were identified and annotated using RepeatExplorer and RepeatMasker, a collection of software tools for characterization of repetitive elements. This analysis showed a higher percentage of repetitive DNA in 1B as compared to 0B genomes. Clusters with higher read content in 1B than in 0B indicated several transposons such as Gypsy, TC1, Mariner, and Helitron and were ultimately amplified by PCR to confirm their existence using respective specifically designed primers. Furthermore, qPCR and FISH will be performed to validate our results. We expect to explain the possible role of these TEs in the maintenance, composition and evolution of B chromosomes from our preliminary results of repeat annotation analysis in the near future.

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II.6

B Chromosome Can Compensate the Content of Heterochromatin and Methylation in the Genome of *Astyanax scabripinnis* (Teleostei: Characidae)

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5-Methylcytosine (5-mC) is a chemical modification of cytosine-guanine dinucleotide DNA sequences (CpG) in the 5′ position of a guanine residue. It is preferentially located in heterochromatin, although it is also found in structural genes. In mammals, DNA methylation marks the silencing of gene regions. Here, our goal was to investigate the distribution of methylated regions in *Astyanax scabripinnis* by indirect immunofluorescence using a monoclonal antibody specific for 5-mC. The methylation level of total genomic DNA was quantified by enzyme immunoassay (ELISA). 5-mC signals were scattered over the chromosomes of females and males, with preferential accumulation in the B chromosome and further some euchromatic regions. Quantification of heterochromatin was performed using Image-J software, considering the length of heterochromatin in relation to the largest chromosome of the complement. Both the content of heterochromatin (in μm) and the methylation (in %) of the genome showed no significant differences between the sexes or in relation to the presence of B chromosomes (25.8 μm and 13.3% in males with B; 23.9 μm and 10.7% in males without B; 23.3 μm and 13.9% in females with B; 22 μm and 15.3% in females without B). In the B chromosome of *A. scabripinnis* heterochromatin is preferentially located in the pericentromeric regions of the short and long arms of this isochromosome. However, its default replication shows differentially replicating regions suggesting transcriptional activity. Thus, individuals with B chromosomes should have an increase in heterochromatin amount when compared with individuals that do not have them. Nevertheless, the results show that the occurrence of B chromosomes does not influence the total heterochromatin content or increase the amount of methylation in the genome. In conclusion, this evidence suggests that B chromosomes can have a compensatory epigenetic effect of the total content of heterochromatin and methylated regions in *A. scabripinnis*.

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II.7

Populational Variants of B Chromosomes from the Grasshopper *Xyleus discoideus angulatus* (Romaleidae): New Insights about Origin and Evolutionary Dynamics

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B chromosomes, extra elements present in the karyotype of some eukaryote species, have been described in the grasshopper *Xyleus discoideus angulatus*. Although some studies have proposed an autosome as possible origin for the B chromosome in *X. d. angulatus*, little is known about its repetitive DNA composition and evolutionary dynamics. The aim of the present work was to elucidate the evolutionary dynamics of the B chromosome in *X. d. angulatus* by cytogenetic analysis in 27 populations from Pernambuco and Ceará states (Brazil). Therefore, an assessment of B chromosomes was carried out in different populations as well as chromosome measurements and FISH with C_0t-DNA, telomeric and B chromosome sequences in the karyotype of B-carrying individuals. The results showed variations in the prevalence of these elements among populations, in particular a high prevalence was observed in one population from Ceará (Juazeiro do Norte – 2007). Furthermore, some B chromosomes were smaller in certain populations. FISH presented similar patterns for C_0t-DNA probes in all hybridized individuals, whereas telomeric and B-chromosome probes, obtained by microdissection, exhibited variations in their distribution. These results indicate the presence of 3 B chromosome morphotypes in *X. d. angulatus*, evidencing a variation in repetitive DNA composition of these elements throughout evolution. In this species, B chromosomes have an intraspecific origin, having arisen probably from the centromeric region of A chromosomes.

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II.8

Not so Stable after All: Neo-Sex Chromosomes and Speciation in Lepidoptera

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Lepidopteran karyotypes have for long been considered stable, with an ancestral chromosome number of 2n = 62 conserved for at least 140 Myr. Conserved synteny of genes has been reported in several species using *Bombyx mori* (Bombycoidea) as a reference genome, and only few chromosomal rearrangements have been

found. Interestingly, major chromosomal rearrangements of autosomes and sex chromosomes have been recently described in representatives of several (super)families. In the present work, we used different techniques of molecular cytogenetics (GISH, BAC-FISH, TSA-FISH) and molecular biology (qPCR) to describe in detail sex chromosome-autosome fusions in the diverse superfamilies Gelechioidea and Tortricoidea. The gene content of the autosome involved in the fusion may be essential for the spreading and fixation of the neo-sex chromosomes in lepidopteran populations. While the Z chromosome is involved in pre- and post-zygotic isolation, fused autosomes in these superfamilies have been shown to be enriched in larval performance and/or ovary-specific genes. The sex chromosome-autosome fusions can thus be driven by sexual antagonism and/or selection for association of Z-linked reproductive isolation with larval performance. Furthermore, we hypothesize that the amplification of larval performance genes followed by their functional diversification could increase the larval detoxification capacity and contribute to the radiation of lepidopteran clades with neo-Z chromosomes.

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II.9

Novel Unbalanced X-Autosome Translocation in a Woman Affected with Primary Ovarian Failure

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The cause of premature ovarian failure (POF) remains unknown in several cases. Xq chromosomal rearrangements such as deletions or balanced X-autosome translocations (X;A) show 2 critical specific regions: POF1 (Xq26qter) and POF2 (Xq13.3q21.1). Microdeletions at these breakpoints have been characterized in only half of the cases. In the other X;A, 2 mechanisms have been proposed: one is ascribed to the XIST-mediated inactivation to the autosomal translocated region, and the other a position effect variegation due to epigenetic modifications in genes located near the constitutive heterochromatin of the pericentromeric regions. Our purpose is to communicate an apparently balanced translocation between chromosome 1 and the X, but STRs linked to Xq28 loci and the triplet expansion of Fra-X showed 3 alleles, 1 from the father and 2 from the mother. The patient was referred for short stature at the age of 3.1 years. Height was 86 cm (–2 SDS), weight 11 kg (below percentile 3). Infancy showed a delay in growth development and no progressive motor neuropathy. Breast development started at the age of 11 years. At age 14, she presented hypergonadotropic POF. Cytogenetic analyses were performed with CTG, CBG, and RHG banding techniques. Fra-X (Xq27.3) was studied by QF-PCR and in addition several STRs linked to Xq28: DXS8091, DXS8377, DXS1068, DXS8069. The de novo karyotype of the proband was: 46,X,der(X)t(X;1)(qter;q12) meaning that a whole chromosome X was translocated on the heterochromatin 1q12. The STRs linked to Xq27.3 and Xq28 revealed 3 alleles, 2 of maternal and 1 of paternal origin showing a de novo microduplication in the distal portion of Xq. Late-replication studies demonstrated the existence of skewed X inactivation in the derivative X chromosome. This case is a novel X;A translocation found in a woman with POF. The results of the cytogenetic molecular studies support the proposal of the position effect of the translocated X on flanking genes resulting in silencing of gene expression.

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II.10

Cytogenetics of Parasitoid Hymenoptera: Main Features and Implications for Biological Pest Control

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Parasitic wasps are one of the most diverse and taxonomically complicated insect groups. They are haplodiploid organisms, which mainly reproduce by arrhenotokous parthenogenesis. The haploid chromosome number in this group can vary from 3 to 23, with 2 apparent peaks at n = 6 and 11. Chromosomes of parasitoid Hymenoptera are mostly bi-armed; however, the predominance of acrocentrics is characteristic of certain relatively advanced groups. Parasitoid karyotypes harbor 1–6 rDNA clusters per haploid set, but their chromosomes lack the canonical insect telomeric motif, $(TTAGG)_n$. Parasitic wasps can effectively suppress many insect pests, and chromosomal analysis of parasitoids therefore has important implications for biocontrol. Karyotypic studies can be successfully used to detect cryptic species of parasitic wasps. Perhaps the most striking example of this kind is the discovery of a recently described cosmopolitan synanthropic parasitoid of stored-product pests, *Anisopteromalus quinarius* Gokhman & Baur (Pteromalidae). This parasitic wasp was previously confused with an effective biocontrol agent, *A. calandrae* (Howard), but these species have different karyotypes with n = 5 and 7, respectively, as well as alternative life-history strategies. An analogous situation was found in another member of this family with similar biology, *Lariophagus distinguendus* (Förster), in which 2 separate species with n = 5 and 6 were also revealed; 2 consecutive rearrangements are apparently responsible for the transition between karyotypes of these species. Moreover, chromosomal characters can be used to identify adult and immature parasitoid Hymenoptera from natural populations and laboratory stocks, especially within taxonomically complicated groups. In addition, karyotypic analysis of parasitoid Hymenoptera can visualize B chromosomes (up to 6 B chromosomes per diploid set in *Pnigalio gyamiensis* Myartseva & Kurashev (Eulophidae) with 2n = 12). Some of these chromosomes carry specific sex-ratio distorters, which can lead not only to male-biased sex ratios but also to female-biased ones, as in *Aphidius ervi* Haliday (Braconidae) with 2n = 10 + 0–2 B.

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II.11

Sex Chromosome Evolution in Plants – Common and Specific Processes

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In contrast to animals, plant species with separate sexes possessing heteromorphic sex chromosomes are very rare. Phylogenetic analyses show that dioecy in plants originated independently and repeatedly even within individual genera. As our knowledge of the architecture of sex chromosomes in individual plant models increases, the question arises what is the major factor of sex chromosome degeneration? What role did transposable elements play in plant sex chromosome evolution? Is reduced gene expression in the Y/W chromosomes followed by dosage compensation in plants? Much data have been published, but the conclusions are ambiguous. We speculate that although molecular analyses indicate some degree of genetic degeneration of the non-recombining region of the Y chromosome, at least in some plant species, the evolutionary processes forming sex chromosomes in plants may differ from those in animals. We also discuss how genetic and epigenetic processes influence sex expression and sex chromosome degeneration in 2 model dioecious species – *Silene latifolia* and *Rumex acetosa*.

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II.12

A Coverage-Based Study of *ihhb* and 45S rRNA Genes in the B Chromosomes of *Astatotilapia latifasciata* to Highlight Polymorphism Levels

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B chromosomes, also known as supernumerary or accessory chromosomes, are a major source of intraspecific variation and can create polymorphisms in natural populations. By studying Bs, we can gain useful knowledge about the organization, function, and evolution of genomes. Our work is an attempt to investigate genomic duplication events that have acted during the early evolutionary history of B chromosomes in the cichlid fish *Astatotilapia latifasciata*. We have generated high coverage Illumina next-generation sequence data of B– (0B) and B+ (1B and 2B) samples, and the read datasets were aligned against reference genes of the cichlids *Oreochromis aureus* and *Lithochromis rubripinnis*. We have identified a higher number of copies for the Indian Hedgehog b *(ihhb)* gene and the 45S rRNA transcriptional gene-cluster based on differential coverage analysis among B– and B+ samples. Single nucleotide polymorphisms for the corresponding genes were detected in B+ sequencing data. Our results indicate that the *ihhb* gene from the A chromosome accumulated on the B chromosome due to frequent duplication events. The findings also demonstrate that B chromosomes have incorporated the entire 45S rRNA cluster (18S rRNA, internal transcribed spacer 1, 5.8S rRNA, internal transcribed spacer 2, and 28S rRNA) from the A complement set. Furthermore, we expect to understand the role of polymorphism during the evolution of B chromosomes.

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II.13

Repetitive Organization and Differential Methylation Patterns of the MHM Region: Insights from Different Avian Genomes

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The MHM region (male hypermethylated), so far identified at the Z chromosome of Galliformes, is associated with a hypermethylation pattern in males. A hypomethylation pattern, found in females, leads to the synthesis of an ncRNA which is associated with a higher expression of genes that are located near to the MHM region, resulting in a possible local dosage compensation between the sexes. The present study aimed to isolate and characterize this region in Galliformes (chicken, European quail, turkey), Struthioniformes (ostrich), Strigiformes (striped owl, tropical screech-owl, barn owl, burrowing owl), Piciformes (toco toucan), Psittaciformes (hyacinth macaw), and Apodiformes (versicolored emerald, swallow-tailed hummingbird, black jacobin). Adult individuals and 6-day embryos were sexed based on morphological characters and/or by PCR followed by agarose and polyacrylamide gel electrophoresis, SSCP analysis, and automated DNA genotyping. Multiplex PCR for the amplification of MHM segments of *Gallus gallus* led to the characterization of 2 DNA fragments of ~240 bp, localized outside a CpG island, and 750 bp that includes a CpG region. The characterized MHM segments showed a repetitive organization along the chicken genome. These repetitions were arranged into 4 major phylogenetic clusters that reflect their origin and evolution. As observed for chicken, the other studied species also presented 2 different-sized DNA fragments that were associated with the MHM region. A higher identity index (80–97%) was recognized between the chicken MHM region and the obtained nucleotide sequences of European quail, turkey, and ostrich. Enzymatic digestion assays in MHM CpG regions of chicken, quail, and turkey validated a methylation pattern in males and a non-methylation pattern in females. The obtained data evidence that MHM segments are not restricted to Galliformes and demonstrate the potential to use the differential methylation profiles of this region in molecular sexing tests.

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II.14

Genomics of Sex Chromosomes in *Rumex acetosa*

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In contrast to animals, the presence of dioecous species with well-established sex chromosomes is very rare in the plant kingdom. *Rumex acetosa* is a dioecious plant with chromosomal sex determination. Although the evolution of sex chromosomes has been the subject of numerous studies, a global view of sex chromosome structure is still missing in this species. We have flow-sorted and sequenced sex chromosomes and autosomes in *R. acetosa*. We focused on the role of various repetitive elements in the process of Y chromosome evolution. Our data demonstrate that the *R. acetosa* genome was formed by expansion of various repetitive elements with a specific pattern of distribution in the case of sex chromosomes. We show that some tandem repeats and retroelements are ubiquitous in the *R. acetosa* genome but surprisingly absent on the Y or X chromosome. Our work is a combination of multiple approaches: flow-sorting and sequencing of sex chromosomes, assembly and repeat clustering, and FISH analysis. Using these techniques, we were able to quantify the extent of accumulation of individual repeats and their potential role in sex chromosome evolution in *R. acetosa*.

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II.15

Sex Ratio Distortion in a Y Chromosome Deletion Model

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According to Mendel's law of random segregation, both copies of a gene have an equal chance of being inherited. However, there exist instances in which genes act selfishly, exerting 'drive', resulting in that gene being passed on to more than 50% of offspring. When such genes are located on sex chromosomes, a distortion of offspring sex ratio may result. Understanding the biological mechanisms of sex ratio skew and related sperm function aberrations could not only result in benefits for fertility research, but also in the development of methods for large-scale agricultural pre-implantation sex selection. The mouse XY^{RIII}qdel model exhibits a 2/3 deletion of the long arm of the Y chromosome, which has been shown to induce a sustained sex ratio that deviates from wild type by 10% in favour of females. Intracytoplasmic sperm injection has shown the skew to occur as a result of differences in fertilising capacity between individual X- and Y-bearing spermatids. Many spermatid transcripts are shared between sister spermatids as they mature, providing relative functional homogeneity. In addition to the sex ratio skew, sperm morphology is severely altered in the deletion model. To identify the genetic correlates of the phenotype, we have purified round, elongating and mature spermatids using centrifugal elutriation and subjected each population to a series of lysis buffers, separating the cytosolic RNAs from more cytoskeletally-bound, membrane-bound, and nuclear RNAs. Microarray analysis and RNA-sequencing of mRNAs, miRNAs, and lncRNAs from these cellular fractions has yielded a collection of genomic regions which show distinctive expression, and RNA compartmentalisation differences between the 2 models, between and across several sperm maturation stages. These regions contain candidate loci underlying the sex ratio distortion and the morphological defects in the XY^{RIII}qdel sperm.

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II.16

Genome Elimination Caused by a 'Selfish' B Chromosome in the Jewel Wasp *Nasonia vitripennis* Involves a Unique Combination of Altered Histone States

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B chromosomes are supernumerary centric fragments that can induce dramatic alterations to normal cellular processes in order to facilitate B chromosome transmission. One of the most remarkable examples is the complete elimination of the paternally derived genome caused by a B chromosome known as paternal sex ratio (PSR) in the jewel wasp, *Nasonia vitripennis*. Transmitted solely through the sperm to new progeny, PSR 'imprints' the paternal genome so that it becomes lost during the first embryonic mitotic division. Due to the haplo-diploid reproduction of *Nasonia*, this event causes the embryo to develop into a male, the PSR-transmitting sex. A compelling question is how PSR induces paternal genome elimination at the molecular level. Using cytological methodology, we discovered that the paternal genome becomes highly abnormal in its chromatin state immediately preceding elimination. The entire paternal set becomes highly enriched in methylation of histones H3K9, H3K27, and H4K20, 3 marks associated with heterochromatin. In contrast, PSR avoids obtaining these marks despite its close association with paternal chromatin. We identified H3K27me1 as the first abnormal mark to appear, and we propose that this mark leads to the other 2 marks as downstream effects, while all 3 marks collectively contribute to heterochromatinization of the paternal genome. Additionally, we discovered that the paternal genome undergoes proper replication during the first but not the second embryonic S-phase, and that this effect likely results from failure of at least 1 replication-licensing factor, ORC2, to become loaded properly. We speculate that this occlusion of replication factors stems from steric hindrance caused by the heterochromatinized state of the paternal set. We are currently investigating several PSR-expressed transcripts and piRNAs as candidates for mediating the altered state of the paternal chromatin.

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II.17

B Chromosomes in the Cichlid Fish *Astatotilapia latifasciata*: A Landscape Provided by Functional High-Scale Data Analysis

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Supernumerary chromosomes (Bs) are extra elements not homologous to the regular complement and have been described in several eukaryote species. Recent studies have been focused on the structural level, and functional aspects have been poorly explored. *Astatotilapia latifasciata* is endemic to the Lake Victoria system and can harbor 1 or 2 large heterochromatic Bs. Cytogenomic analysis detected thousands of highly fragmented genes in the B of this species. Besides the advances on the genomic content of this element, its functional relevance is still to be elucidated. Considering the importance of Bs, miRNA and mRNA transcriptional profiles of brain, muscle, and gonads of *A. latifasciata* were obtained in order to investigate possible effects linked to B presence or absence. Sixty-four differentially expressed (DE) miRNAs (53 known and 11 novel) were detected between B– and B+ samples. Such data do not suggest an effect of the B itself; instead, B presence appears to act over the A complement genome causing functional modifications in the cell biology. Concerning transcriptomes, a range of transcripts evidenced in the analyzed tissues shows that B chromosomes may be interfering in different pathways. Transcriptome analysis indicates functional implications of Bs based on the identification of several genes related to cell cycle, cell structure maintenance, and immunity response. Various genes targeted by DE miRNAs are also related to cell cycle control. We identified upregulated genes located on the B, inferring its direct interference on cells. On the other hand, most DE genes are located in the regular chromosome complement (autosomes) and are affected by the presence of this extra element, as observed for miRNA data. The changes in the transcriptional profiles involved with cell cycle in B+ samples corroborate that B presence can affect its transmission rate during meiosis, favoring the B maintenance.

D.F. Marques and B.E.A. Fantinatti contributed equally to this work.

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II.18

PcP190 Satellite DNA in Paradoxical Frogs and Its Relationship with Sex Chromosome Differentiation

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Pseudis is very interesting for the study of sex chromosome differentiation because *P. tocantins* is the only species of the genus with highly heteromorphic Z and W chromosomes. The Wq of *P. tocantins* shows a large heterochromatic block that is enriched in the satellite DNA PcP190. Based on an analysis of chromosomal morphology and NOR location, the sex chromosomes of *P. tocantins* are inferred to be homeologous to the homomorphic pair 7 of *P. bolbodactyla*, *P. fusca,* and *P. minuta.* To better investigate these chromosomes, we mapped the PcP190 satellite DNA to the karyotypes of these 3 species and performed a comparative hybridization of female and male genomic DNA (CGH) in the karyotype of *P. bolbodactyla*. In the female karyotypes of *P. bolbodactyla* and *P. fusca*, the PcP190 probe detected only 1 homologue of pair 7, whereas male karyotypes of these species showed no hybridization signal of this probe. PcP190 was located in the pericentromeric region of the short arm of chromosome 7 in *P. bolbodactyla*, while in the karyotypes of *P. tocantins* and *P. fusca*, the PcP190 site was detected at Wq and 7q, respectively. CGH in *P. bolbodactyla* revealed a female-specific region, which is the same region that is labeled with the PcP190 probe. In both male and female karyotypes of *P. minuta*, the PcP190 probe detected the pericentromeric heterochromatin of 7p and the centromeric region of chromosome pair 12. Our results support the homeology between the sex chromosomes of *P. tocantins* and chromosome 7 of *P. bolbodactyla*, *P. fusca,* and *P. minuta*, as well as the hypothesis that pair 7 encompasses the sex chromosomes of *P. bolbodactyla* and *P. fusca*. Our data also suggest the involvement of PcP190 satellite DNA and pericentric inversion in the evolution of these chromosomes.

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II.19

Regulation of the *arid4* Gene by microRNA-205 Is Dependent on Sex and B Chromosome Presence in the Cichlid Fish *Astatotilapia latifasciata*

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B chromosomes (Bs) are extra chromosomal elements present in a considerable number of eukaryote species. Such elements do not follow Mendelian laws of inheritance. Though considered dispensable, studies suggest several effects related to B chromosomes. Such effects can be observed as genetic variability, fitness alteration, and sexual differentiation. The fish *Astatotilapia latifasciata* can carry 0, 1, or 2 Bs in both sexes. Nevertheless, function, maintenance, and behavior of B chromosomes are poorly understood. One possible strategy to investigate the function of Bs is the analysis of miRNA and their target mRNA in B+ and B– cells. In this study, we analyzed the mRNA and miRNA transcripts generated by next-generation sequencing in order to correlate differentially expressed (DE) elements in gonads of specimens with (B+) or without (B–) B chromosomes. We identified DE mRNAs related to transcriptional factors, metabolic processes, structure and transport of molecules, chromatin remodeling, cell cycle, and cell activity. An interesting correlation was observed between dre-miR-205 and its target, *arid4* (AT-rich interactive domain-containing) in gonads. The DE analysis shows miR-205 upregulated and its target gene *arid4* downregulated in B+ males. In B+ females, miR-205 is downregulated and therefore *arid4* is upregu-

lated. The *arid4* gene is a transcriptional factor involved in chromatin remodeling function. Our data support the involvement of the *arid4* gene in chromatin remodeling contributing to the transmission of the B chromosome during female meiosis.

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II.20

The Neo-Y Chromosome of the Cricket *Eneoptera surinamensis* Favored the Burst and Occurrence of the Highest Diversity of Tandem Repeats among Eukaryotes

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Tandem repeats (TRs) constitute a large portion of eukaryote genomes, comprising noncoding sequences repeated hundreds to thousands of times. They are located mostly in heterochromatic regions such as centromere, telomere, intercalary regions, and in addition populate frequently non-recombining sex chromosomes. Here, we examined the genome of the cricket *Eneoptera surinamensis*, with a karyotype composed of 2n = 9, neo-X_1X_2Y and a large genome (5.42 Gb), in order to isolate and characterize the TRs that contributed to sex chromosome differentiation. In this way, Illumina reads were given to RepeatExplorer pipeline to perform graph-based clustering analyses. The analysis of dotplot graphic and PCR confirmed the occurrence of 45 TR families ranging in length from 4 to 517 bp, showing different modular structure and diversity of array forms and accounting for ~14% of the genome. FISH experiments showed that the TRs are located mainly in the pericentromeric regions that correspond to the pericentromeric C-positive bands; additionally some interstitial and distal signals in C-band-negative regions were also seen. Fiber-FISH demonstrated intermingling for some repeats studied. TR enrichment was observed on the neo-sex chromosomes, and an astonishing number of loci on the neo-Y was remarkable for most repeats, besides specific occurrence in this chromosome. qPCR abundance analyses in males versus females showed that the satDNA doses differ significantly between sexes, with males having much higher copies than females for most repeats studied. Interestingly, our results show an intriguing burst of TRs, with occurrence of 45 distinct families in the *E. surinamensis* genome and their enrichment in the highly differentiated neo-X_1X_2Y sex chromosomes with 39 TRs mostly located on the neo-Y. To our knowledge, this is the genome and the neo-Y chromosome with the largest diversity of TRs documented until now among eukaryotes.

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II.21

BncDNA, a B Chromosome-Enriched Sequence Transcribed into a Potentially Noncoding RNA in *Astatotilapia latifasciata*

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B chromosomes have been studied in many species of eukaryotes, including the cichlid fish, *Astatotilapia latifasciata*. However, there are many unanswered questions about the maintenance, inheritance, and functional aspects of these supernumerary chromosomes. The cichlid family has been highlighted as a model for evolutionary studies, including those that focus on mechanisms of chromosome evolution. Individuals of *A. latifasciata* carry up to 2 heterochromatic B isochromosomes that are enriched in repetitive DNA and contain few intact gene sequences. We isolated and characterized a transcriptionally active repeated DNA, called BncDNA (B chromosome noncoding DNA), highly represented across all B chromosomes of *A. latifasciata*. BncDNA transcripts are differentially processed among 6 different tissues, including the production of smaller transcripts, indicating transcriptional variation may be linked to B chromosome presence and sexual phenotype. The transcript lengths and lack of coding characteristics indicate BncRNA might represent a novel long noncoding RNA family (lncRNA). Additionally, the potential for interaction between BncRNA and known miRNAs was computationally predicted, resulting in the identification of possible binding of this sequence in upregulated miRNAs related to the presence of B chromosomes. In conclusion, Bnc is a transcriptionally active repetitive DNA enriched in B chromosomes with potential action in B-involved mechanisms and related regulatory RNAs.

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II.22

Molecular Mapping of Microsatellites and Retrotransposons: Evidence of Evolutionary Mechanisms Involved in the Differentiation of Giant Sex Chromosomes in *Omophoita* (Coleoptera: Alticinae)

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The species of the subtribe Oedionychina (Alticinae) are interesting for evolutionary studies due to the giant sex chromosomes and asynapsis during meiosis. Microsatellites (simple sequence repeats, SSRs) are widely used as molecular markers, showing high levels of polymorphism, and are widespread in the genomes. Furthermore, transposable elements (TE) can influence chromosome rearrangements, originating and moving satellite DNAs. The objective of this study was to elucidate the molecular mechanisms involved in the process of differentiation and evolution of giant sex chromosomes in the genus *Omophoita* by fluorescent in situ hybridization with probes from repetitive DNA. We used SSR probes $(CA)_{15}$, $(CAT)_{10}$, $(GAC)_{10}$, $(CAC)_{10}$, $(GAA)_{10}$, $(GATA)_8$, and $(GA)_{15}$; the probes of TEs were obtained by the C_0t-1 technique and cloning. Chromosome mapping with SSR probes in meiotic cells from 4 species of *Omophoita* showed conserved patterns for autosomes, but diversified patterns in sex chromosomes. The mapping demonstrated differences in the SSR composition in the sex chromosomes. Pericentromeric and distal accumulations in the sex chromosomes were the most common pattern observed. The analysis of autosomes in all species revealed pericentromeric hybridizations in most species. The SSR $(GAA)_{10}$ labeled only 1 bivalent in the pericentromeric region in *O. magniguttis* and *O. personata*. Interesting marks in the sex chromosomes were obtained with $(GATA)_8$ in all species, accumulation in the pericentromeric regions of the X chromosomes and different regions in the chromosome arms. The hybridization with TE clones showed some similarities to the patterns of the SSR markers, indicating that in the evolutionary process of species these elements were present. Considering all results, we can propose differences in the constitution of sex chromosomes of the species studied. Thus, we can infer an evolutionary role of repetitive DNA in the differentiation of chromosomes in the subtribe.

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II.23

Molecular Differentiation Markers for the Analyses of Sex Determination Diversity and Sex Chromosome Evolution in Fish

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Fish show a great variety of sex determination mechanisms, which in the case of genetic sex determination is linked to a similarly high variability of sex chromosome differentiation. Curiously, neither phenomenon follows any obvious phylogenetic pattern. To obtain a better understanding of the biological meaning of the diversity of sex determination and the mechanisms driving sex chromosome evolution, we are attempting to decipher the molecular basis of the primary sex determination mechanisms and the structure and genetic organization of sex chromosomes across a broad diversity of actinopterygian fish. On the one hand, we are analyzing a collection of species that represent major branches of the fish tree of life, and on the other hand, we focus on closely related species within branches of the phylogenetic tree (Esociformes, Danios, Poeciliids). We use high throughput RAD-tag marker mapping in 40 species as well as transcriptomics and long-insert clone sequencing to delineate sex-specific chromosomal regions and to identify candidate sex-determining genes. We find that many species harbor very poorly differentiated sex chromosomes. Our RAD-tag based approach led to the identification of sex-specific markers, allowing the delineation of the extent of recombination suppression, which turned out to be highly variable between species. In several species where genomic resources are already available, sex-specific markers could be used to identify sequence scaffolds that can be assigned to sex chromosomes and some of those even more specifically to regions that are supposed to contain the primary sex-determining gene.

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II.24

Multiple Sex Chromosome System $X_1X_2X_3Y_1Y_2$ in the Brown Howler Monkey *Alouatta guariba clamitans* (Atelidae, Platyrrhini)

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In primates, the most widespread sex chromosome system is XX/XY. In all species of howler monkeys, genus *Alouatta*, multiple sex chromosome systems originated by Y-autosome translocations were observed, but confirmed by meiotic analyses in only 5 of them ($X_1X_1X_2X_2/X_1X_2Y_1Y_2$ in *A. macconelli, A. caraya* and *A. pigra;* $X_1X_1X_2X_2/X_1X_2Y$ in *A. belzebul* and *A. palliata*). In the brown howler monkey *A. guariba clamitans,* diploid numbers ranging from 2n = 45 to 2n = 52, with XX/XY, $X_1X_1X_2X_2/X_1X_2Y$ and $X_1X_1X_2X_2X_3X_3/X_1X_2X_3Y_1Y_2$ sex chromosome systems were described by mitotic studies, but not confirmed by meiotic ones. In this contribution, we sampled 3 males in the wild (in the municipality of Santa María, RS, Brazil) and 1 male and 1 female in captivity at the São Braz breeding center. Peripheral blood samples and testicular biopsies were taken under anesthesia. Two techniques were performed in the latter: spermatocyte microspreads for synaptonemal complexes (SC) and the air-drying technique for the other stages. Immunodetection was performed in spermatocyte microspreads using antibodies anti-SMC3 and anti-CREST. In somatic cells, we observed different diploid numbers for both sexes: 2n = 45, $X_1X_2X_3Y_1Y_2$ and 2n = 46, $X_1X_1X_2X_2X_3X_3$ with 3 metacentric (pairs 9–11), 7 submetacentric (pairs 1–6, 8), and 10 acrocentric chromosome pairs (pairs 12–20, 22). X_1 and X_2 were submetacentric chromosomes, while X_3, Y_1, and Y_2 were acrocentrics. The presence of a sex chromosome pentavalent $X_1X_2X_3Y_1Y_2$ in the males was confirmed by C-banding in metaphase I and immunodetection in prophase I by the clear identification of 5 centromeres. The meiotic behavior was analyzed and compared with the chromosomal descriptions for other howler monkeys.

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II.25

Chromosome Mapping of Microsatellites in Parodontidae (Teleostei, Characiformes): Emphasis on Sex Chromosomes

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Eukaryotic genomes are highly enriched in microsatellites (simple sequence repeats, SSR). These sequences may play a role in DNA replication, recombination, regulation of genic expression, and chromatin organization, making them important for genome studies. Chromosomal mapping of microsatellites provides a huge potential to understand karyotypic differentiation in fishes. In this study, we mapped 16 microsatellite sequences in the genomes of 5 species of Parodontidae *(Apareiodon piracicabae, A. affinis, Apareiodon* sp., *Parodon nasus,* and *P. hilarii)* in order to investigate the accumulation of repetitive DNA in chromosomes and its role in heteromorphic sex chromosome differentiation. The studied species have a diploid chromosome number of 2n = 54, exhibiting absent or homomorphic sex chromosomes *(A. piracicabae)*, proto sex chromosomes *(P. nasus)*, a heteromorphic ZZ/ZW sex chromosome system *(Apareiodon* sp. and *P. hilarii)*, and a heteromorphic ZZ/ZW_1W_2 sex chromosome system *(A. affinis).* The SSR sequences were observed in similar locations on the autosomes of all 5 species. Some of them showed terminal/subterminal signals. In *A. piracicabae*, signals were observed in the subterminal region of the 45S rDNA-bearing chromosome pair. Others revealed marks related to euchromatic regions spread through the genomes. These results suggest that the accumulation of microsatellites is shared in the evolutionary history of Parodontidae. These sequences also act in keeping chromosomal ends and probably have an important role in the genome. Further accumulation was observed in interstitial heterochromatic regions, especially in differentiated sex chromosomes. There was a major accumulation in the W chromosome of *P. hilarii,* corroborating the repetitive DNA invasion proposal acting in genic erosion and heterochromatinization of heteromorphic sex chromosomes. These results enable some inferences about an intense invasion and accumulation of sequences in terminal regions of chromosomes, verifying the role of SSR in the genetic differentiation and evolutionary history of W chromosomes.

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III. Population and Evolutionary Chromosome Biology

Invited Talks

III.1
Anchored Genomes of Australian Vertebrates Provide Insight into Genome Evolution, Disease and Sex Determination

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The advances in genome sequencing technologies are leading to an ever-increasing list of sequenced vertebrate genomes. However, some of the most important questions regarding genome evolution remain unanswered unless these sequence assemblies are anchored to chromosomes. Assigning sequence to chromosomes for 2 sequenced Australian marsupials and an Australian lizard has enabled some important genome evolution questions to be answered, highlighted by several prominent examples. Assigning sequence to tammar wallaby and Tasmanian devil chromosomes has enabled the ancestral marsupial karyotype consisting of 7 pairs of chromosomes to be reconstructed, ending a 40-year-old debate between a 2n = 14 and a 2n = 22 marsupial ancestor. By extending comparisons to eutherian mammals, the karyotype of the therian ancestor could be predicted, providing a glimpse into genome evolution more broadly amongst mammals. In a disease context, comparisons of the arrangement of sequence on Tasmanian devil chromosomes with that of facial tumours found in Tasmanian devils has identified a potential link between chromosomes susceptible to rearrangement during marsupial evolution and rearrangement in tumours from Tasmanian devils, as well as providing some insight into the genomic restructuring events leading to these tumours. Similarly, by comparing the anchored genome of the central bearded dragon with other anchored genomes, such as chicken and anole, and cytogenetic maps for other squamates, it has been possible to determine the evolutionary history of squamate macrochromosomes and gain some understanding of the role microchromosomes have played in squamate genome evolution. The anchoring of sequence to the dragon sex chromosomes has been crucial for the identification of a candidate sex-determining gene in this species. These examples emphasize the importance of chromosome-based genome sequence assemblies for providing a greater understanding of many facets of genome evolution.

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III.2
Chromosomal and Genome Evolution of Land Plants

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The ever increasing number of complete genome sequences accompanied by a series of methodological advances in the fields related to comparative genomics provide unprecedented insights into patterns and mechanisms driving chromosomal evolution in land plants. Paleogenomic data along with improved large-scale phylogenies allowed us to reconstruct a tentative structure of ancestral genomes, infer ancestral chromosome numbers, and to determine the directionality of chromosomal and karyotype evolution. Transcriptome sequencing revealed a wealth of lineage-specific whole-genome duplication events, which had a major impact on plant genome complexity as well as on the mode and tempo of karyotype evolution. Very first genomic insights into understudied groups of green plants such as horsetails, ferns, gymnosperms, *Amborella*, aquatic and carnivorous angiosperms provide us with a unique opportunity to revisit old questions on prevailing patterns and trends of chromosomal and genome evolution across land plants. Similarly, newly available techniques are promising for achieving a better understanding of centromere evolution, interphase chromosome organization, origin of chromosomal rearrangements, and their role in plant speciation. By interlinking these new findings and emerging research fields, I will explore to what extent some of the old questions were solved and what are the newly arising ones.

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III.3
Chromosome Evolution in Terrarana (Amphibia, Anura)

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Using conventional and molecular cytogenetic techniques, the mitotic and meiotic chromosomes of a very large number of species belonging to the neotropical Terrarana frogs are studied in detail. Furthermore, genome sizes of all available species and their mitochondrial DNA sequences are determined. Up to date more than 900 genuine species of Terrarana have been described in the literature. More than 250 of these species were collected during expeditions to Costa Rica, Venezuela, Brazil, Peru, Bolivia, Cuba, Jamaica, Haiti, Dominican Republic, and the Lesser Antilles. The rate of chromosome evolution among Terrarana frog species is so far the highest found for all vertebrates, including mammals. This genus has gone through an explosive radiation and speciation. All types of structural and numerical chromosome mutations have been detected in Terrarana (reciprocal and non-reciprocal translocations, centric fusions and fissions, inversions). Additionally, in most species, there is an exception-

ally high rate of intra-individual somatic and meiotic chromosome instability (complex translocations, fusions, inversions, ring chromosomes, neocentromeres), leading to mosaic chromosome conditions. In several species very unusual sex chromosomes of the XY/XX and ZW/ZZ type, as well as Y-autosomal translocations have been discovered. At least 1 species has mobile 18S + 28S ribosomal RNA genes that move freely between telomeres of the chromosomes, and at least 1 species presents multivalent meiotic chromosomes. Chromosome and genome instability seem to be the leading forces of the conspicuous species diversification (and adaptive radiation) of the Terrarana frogs. In no other extant vertebrate group there is such a high inter-species, inter-individual, and intra-individual chromosome variability as is the case in Terrarana. The compilations and descriptions of all of these autosomal changes, the different sex chromosome systems, and mobile genetic elements have elementary significance for the general understanding of chromosome evolution in vertebrates.

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III.4

Evolutionary Plasticity of Sturgeon Genomes

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Sturgeons are ancient fish from the order Acipenseriformes, comprising 25 extant species inhabiting waters of the Northern Hemisphere. The order takes a basal position within Actinopteri (ray-finned fish minus polypterids). Extant sturgeons are characterized by conserved morphology, slow molecular evolution, late puberty, long life cycle, benthic specialization, high diploid chromosome numbers, various levels of ploidy between species, unclear sex determination systems, and propensity to interspecific hybridization. Recent advances in molecular genetics, genomics, and comparative cytogenetics allowed producing novel data on different aspects of acipenserid biology, including improved phylogenetic reconstructions and deeper understanding of genome structure. Illumina sequencing of the sterlet *(Acipenser ruthenus)* genome coupled with FISH analysis allowed us to characterize the repetitive elements with chromosome-specific localization, facilitating chromosome identification in complex high-chromosomal karyotypes. Microdissection-derived chromosome-specific probes of sterlet revealed extended genomic segments represented in diploid, tetraploid, and 'intermediate' partly degenerated state in the 120-chromosome sterlet karyotype, directly indicating the ongoing diploidization process after the ancient 200 million years old whole-genome duplication event. Comparison between diploid (sterlet) and tetraploid (Siberian sturgeon, *A. baerii*, 2n ≈ 245) karyotypes disclosed that some additional chromosome rearrangements occurred after the second round of polyploidization in the Siberian sturgeon. We hypothesize that a relaxed pachytene check point in sturgeons promotes both propensity to interspecific hybridization and relieved polyploid formation. Due to widespread fertility of sturgeon hybrids with similar ploidy levels, it seems that auto- and allopolyploidization might be important for sympatric speciation in this group.

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Posters and Short Talks

III.5

Cytogenetic Comparison of *Tityus uruguayensis* and a Closely Related Undescribed Species from Eastern Argentina (Buthidae: Scorpiones)

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Tityus uruguayensis Borelli, 1901 is the only described species of the *bolivianus* group from lowlands of eastern South America; however, we have recently discovered another yet undescribed species from the same area (*Tityus* sp. 1). *T. uruguayensis* is widely distributed in Uruguay and southern Brazil, while in Argentina the only known population is from an area around building ruins in El Palmar National Park (EPNP) (Entre Ríos). *Tityus* sp. 1 is endemic to Paraje Tres Cerros (Corrientes), an ancient isolated low altitude hilly area of subtropical eastern Argentina. We cytogenetically compared 9 males of *T. uruguayensis* from EPNP and 3 males of *Tityus* sp. 1. Giemsa staining, C-banding, and FISH with 28S rDNA and $(TTAGG)_n$ telomere probes were performed. Both species presented holokinetic chromosomes and achiasmatic male meiosis. *T. uruguayensis* showed a diploid chromosome number of 2n = 31 and 3 different cytotypes: 12II + VII at meiosis I (7 males), 10II + VII + IV type 1, and 10II + VII + IV type 2. All the males presented the same heptavalent. *Tityus* sp. 1 showed 2 cytotypes: 2n = 32, 16II at meiosis I (2 males) and 2n = 31, 14II + III (1 male). rDNA clusters were detected at 1 terminal region of 2 pairs in *T. uruguayensis* and in 1 pair in *Tityus* sp. 1. Both species showed small C-bands at the terminal regions of almost all chromosomes and conspicuous C-bands that colocalized with rDNA sites. In *Tityus* sp. 1, the largest chromosome of the trivalent showed 1 interstitial C-band, which could indicate that it arose by chromosome fusion. Telomeric signals were exclusively detected at the terminal regions of all chromosomes in both species. The chromosome numbers found in both species are unusually high in the genus and in the family. The presence of 2 NOR pairs in *T. uruguayensis* is remarkable since most of the studied Buthidae species show only 1 NOR pair, despite the variation in chromosome number.

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III.6

Evolution of Karyotype and Sex Chromosomes in Two Families of Haplogyne Spiders, Filistatidae and Plectreuridae

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Spiders belong to one of the most diverse animal orders. One of the 3 primary clades of spiders are araneomorphs, which are divided into 2 groups, haplogynes and entelegynes. Haplogyne cytogenetics is not satisfactorily understood. Several families of haplogynes exhibit an unusual X_1X_2Y sex chromosome system, which generally comprises 2 large metacentric X chromosomes and 1 metacentric Y microchromosome. These chromosomes display a specific achiasmatic mode of end-to-end pairing during male meiosis. In our study, we revealed an X_1X_2Y system in the family Plectreuridae. Furthermore, we analyzed the karyotype evolution of plectreurids as well as Filistatidae, another family with an X_1X_2Y system. We also determined the pattern of nucleolus organizer regions (NORs) by means of fluorescence in situ hybridization for selected representatives of these groups. Male diploid numbers and sex chromosome systems were as follows: Filistatidae: *Afrofilistata* sp. 23 (X_1X_2Y), *Filistatinella* sp. 24 (X_1X_20), *Kukulcania* aff. *hibernalis* 25 (X_1X_2Y), *Pritha nana* 17 (X0); Plectreuridae: *Plectreurys* sp. A 22 ($X_1X_2Y_1Y_2$), *Plectreurys* sp. B 21 (X_1X_2Y), *Plectreurys* sp. C 20 (X_1X_20), *Plectreurys* sp. D 21 (X_1X_2Y). Karyotypes of both families were predominated by metacentric chromosomes and exhibited a low number of NORs. In prithine filistatids the X_1X_2Y system has gradually evolved into the X0 system. The X_1X_20 system of *Filistatinella* sp. arose by loss of the Y chromosome; a subsequent fusion of the X chromosomes produced the X0 system that was observed in *P. nana*. The X_1X_2Y system was also transformed into the X_1X_20 system in some plectreurids. The $X_1X_2Y_1Y_2$ system of *Plectreurys* sp. A most likely arose by duplication of the original Y chromosome. Our results indicate that the X_1X_2Y system is more plastic from an evolutionary point of view than previously thought. Furthermore, our data can be useful to reconstruct the evolution of karyotypes, sex chromosomes, and NORs in haplogynes.

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III.7

Insights into the Dynamics of the rDNA Clusters during the Chromosomal Evolution of Four Heptapterid Species (Pisces, Siluriformes)

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Heptapteridae are characterized by intra- and interspecific variation in chromosome macrostructure, which indicates the existence of cryptic species in some genera, such as *Imparfinis* and *Pimelodella*. This study presents the karyotypes of *Heptapterus* sp. (2n = 52, 32m + 14sm + 6st), *Imparfinis schubarti* (2n = 58, 28m + 28sm + 2st), *Pimelodella avanhandavae* (2n = 46, 20m + 20sm + 6st), and *Rhamdia quelen* (2n = 58, 36m + 16sm + 6st) and classical and molecular cytogenetic markers, providing new insights into the genetics of this poorly-known family. *P. avanhandavae, R. quelen,* and *I. schubarti* presented simple Ag-NORs, which coincided with CMA_3 bands, while *Heptapterus* sp. had multiple Ag-NORs, all confirmed by 18S rDNA FISH. A single chromosome pair with 5S rDNA sites in pericentromeric position was observed in *Heptapterus* sp., *I. schubarti,* and *R. quelen*, and 2 pairs in *P. avanhandavae*. In *R. quelen*, synteny was found between the 5S and 18S rDNA sites which are adjacent, a rare condition in this family, shared by *I. minutus* and *I. mirini*. Blocks of heterochromatin were located in pericentromeric, interstitial, and telomeric regions. While 2n = 58 is considered the plesiomorphic condition, as observed in *Imparfinis* and *Rhamdia,* this condition is not conserved in *Pimelodella* (2n = 46–58) and *Heptapterus* (2n = 52–58). Simple NORs in terminal positions are common; however, *Imparfinis* and *Cetopsorhamdia* present simple NORs in interstitial positions, which is an important marker in *Imparfinis*. Additionally, the multiple NORs found in some *Heptapterus* species, and in *Rhamdia branneri* and *R. voulezi*, may represent an evolutionarily derived condition. One pair carrying the 5S rDNA sites in terminal position is common in this family, although in *Pimelodella* and *Rhamdia*, multiple sites were detected. The cytogenetic data of the present study are consistent with the molecular phylogenies proposed for the Heptapteridae, which indicate a close relationship between *Pimelodella* + *Rhamdia*, as well as between *Imparfinis* + *Heptapterus*, even though these 2 genera present apomorphies in relation to the other members of the family.

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III.8

Structure and Function: The Role of 5S rDNA in Chromosome Evolution of *Ancistrus* sp.

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Chromosome rearrangements are known as an important mechanism that leads Neotropical freshwater fishes to diversification. Among the in tandem repetitive sequences, the rDNA family has an intense genic activity and organizes chromosome sites with less condensed chromatin. In *Ancistrus* (an armored catfish genus), centric fusion is the main chromosome rearrangement which leads to a reduction in diploid number from 54 to 36 chromosomes among the species. In addition, innumerous *Ancistrus* species possess a size/number variation of 5S rDNAs. We report here a characterization of 5S rDNA sequences, concerning function and localization, in order to evaluate the role of these chromosome sites in the origin of centric fusions and diploid number reductions in *Ancistrus* sp. The PCR amplification with 5S rDNA primers resulted in 200-bp and 300-bp fragments. The DNA sequencing analysis of the 2 variants revealed identity with 5S rDNA of different Ostariophysi orders. The 203-bp sequence shows 98% identity with 5S from *Salmo trutta*, *Solea solea*, and *Xiphias gladius*. The 317-bp sequence shows 89% identity with 5S from *Brycon cephalus*, 88% with *Cyprinus carpio*, and 92% with *Leporinus octofasciatus*, named by us, *pseudo5S*. The 2D structure and free energy analysis also resulted in different structures of the rDNA sequences. The 5S rRNA sequence resulted in a straight conformation folding of loops, with a symmetric distribution of unpaired nucleotides and $\Delta G_o = -58.48$, while the *pseudo5S* showed asymmetric loops and a visibly unstable structure, mostly in II, III and IV domains, with $\Delta G_o = -52.89$. In situ localization evidenced 5S rDNA sites, *pseudo5S* sites and syntenic sites. In addition, the centromeric *pseudo5S* site colocalized with an interstitial telomeric site in a large metacentric chromosome pair. In this way, the *pseudo5S* instability could be the main factor concerning loss of function and accumulation in chromosomal rearrangement hotspots.

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III.9

Refinement of the Ancestral Carnivore Karyotype Based on Comparative Chromosome Painting of Pinnipeds (Pinnipedia, Carnivora)

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Karyotype evolution in Carnivora is thoroughly studied by classical and molecular cytogenetics and supplemented by reconstructions of the ancestral carnivore karyotype (ACK). However, chromosome painting was done only for 1 pinniped, the common seal *(Phoca vitulina)*. We report on the construction of a comparative chromosome map for species from all 3 pinniped families: the monotypic walrus (*Odobenus rosmarus*, Odobenidae), 2 Otariidae species: the Steller sea lion *(Eumetopias jubatus)* and the New Zealand sea lion *(Phocarctos hookeri)*, and 2 Phocinae species: the Baikal seal *(Pusa sibirica)* and the bearded seal *(Erignathus barbatus)* using a combination of human (HSA), domesticated dog (*Canis familiaris*, CFA), and stone marten (*Martes foina*, MFO) whole-chromosome painting probes. Employing highly informative dog painting probes, we have identified rearrangements specific for different pinniped lineages. Human and dog autosome painting probes delineated 32 and 68 conservative autosome segments in the genomes of the studied species. Our data show that pinniped karyotype evolution was characterized by few tandem fusions, seemingly absent inversions and a slow rate of genome rearrangements (less than 1 rearrangement per 10 million years). This indicates a very high level of chromosome conservation that manifests in highly similar G-banding patterns of chromosomes. Molecular cytogenetic results favor the hypothesis of a monophyletic origin of pinnipeds. Chromosome painting in Pinnipedia and comparison with non-pinniped carnivore karyotypes provide strong support for a refined structure of ACK with 2n = 38. A plausible order of dog chromosome syntenic segments on ancestral chromosomes was specified by the mapping of dog painting probes in pinniped species.

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III.10

Evolutionary History of Alpha Satellite DNA in Cercopithecini

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Alpha satellite DNA is the major nucleotide element of primate centromeres. This superfamily is composed of tandemly repeated sequences of about 170 bp in length which have diversified to some extent and distribute in different families of alpha satellite sequences. The limited amount of information available in non-human primates is a restriction to the understanding of alpha satellite evolutionary dynamics, which calls for the integration of this element into comparative studies. Chromosomal evolution in the Cercopithecini tribe would be mainly due to non-centromeric chromosomal fissions associated with evolutionary new centromere emergences, making these Old World monkeys a promising model to collect information regarding alpha satellite diversity and dynamics. We carried out targeted high-throughput sequencing of alpha satellite monomers and dimers from the *Cercopithecus solatus* and *C. pogonias* genomes, 2 species that belong to different primary lineages within the Cercopithecini tribe and diverged from each other about 7 million years ago. Computational approaches were used to analyze the collected sequences and infer the existence of alpha satellite families in the studied genomes, as well as their organization with respect to each other. Our analysis provided evidence for the existence of 4 alpha satellite families shared by both species and 2 supplementary families in the *C. pogonias* genome. Fluorescence in situ hybridizations using highly discriminant oligonucleotide probes allowed visualizing these families on *C. solatus* and *C. pogonias* chromosomes and highlighted distinct distribution patterns that both comfort and question alpha satellite DNA evolutionary models. Furthermore, our results suggest special alpha satellite diversity on Cercopithecini evolutionary new centromeres. Finally, the extension of our fluorescence in situ hybridization experiments to other Cercopithecini species allowed us to propose an evolutionary scenario for alpha satellite DNA in the Cercopithecini tribe, wherein alpha satellite evolutionary dynamics appears intimately connected to chromosome rearrangement dynamics.

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III.11

Recurrent Origin and Different Lineages of Ploidy in *Solanum elaeagnifolium*

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Polyploidy has played a major role in the evolution of many eukaryotes. Up to now, most polyploid species studied, both plants and animals, have been recurrently formed from different progenitor populations. *Solanum elaeagnifolium* (Solanaceae) is a weedy species which invades several areas around the world, North America being its probable center of origin. It exhibits a euploid series (2x, 4x, and 6x) for populations growing spontaneously in Argentina, while only diploids were found in the rest of the world. In Argentina, the distribution pattern of *S. elaeagnifolium* ploidy is probably a response to environmental conditions, although historical factors have not been considered to date. Phylogeographic studies could make a significant contribution interpreting the origin and timing of polyploidy. Here, we question if polyploidy has multiple origins from diploid populations in *S. elaeagnifolium*. Chromosome counts and sequencing of 2 chloroplast noncoding regions were carried out in individuals from 18 populations of the species along the entire range of Argentina and from other countries. The haplotype network configuration resulted in 2 main lineages that were not geographically structured. One group comprises all hexaploid populations studied and some tetraploid samples apparently derived from hexaploids. The other group includes the diploid populations (embracing those from the probable center of origin) and its derivate tetraploids. According to these results we concluded that in *S. elaeagnifolium*, tetraploids have been formed recurrently (from diploid or hexaploid progenitors), although it is noticeable that hexaploids have had a different and older origin, considering the high number of mutational steps found between the 2 lineages.

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III.12

Using FISH and GISH Techniques for Recombination Analysis on BC_1 Interspecific Hybrids Involving *Passiflora sublanceolata*

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Detailed karyotype analysis of first generation (F_1) and backcrossed (BC_1) interspecific hybrids and their genitors through classical and molecular cytogenetic methods suggests great homology between the species *Passiflora sublanceolata* and *P. foetida*, with cytological important events in the origin of hybrids. Karyotype characterization was performed in 16 BC_1 plants and their genitors by

conventional staining, fluorescence in situ hybridization with probes for 5S and 45S rDNA and telomeric probes. Genomic identification of hybrids was performed by genomic in situ hybridization (GISH). The diploid chromosome number (2n = 22) was verified in the genitor species *P. sublanceolata* and *P. foetida*. However, variations were observed in chromosome numbers (2n = 20, 21, 22, and 30). Variation in the number of 45S and 5S rDNA sites occurred primarily in BC_1 plants. Telomeric signals were restricted to telomeric regions. GISH made it possible to distinguish genomes from the genitor species, genome-S and genome-F, in F_1 and BC_1 plants, thereby revealing differences in the origin of recombinant chromosomes present in BC_1 plants. Varying numbers of recombinant chromosomes, from 1 to 8, were identified in BC_1 genotypes. Differences in the number and nature of recombinant chromosomes in BC_1 hybrids suggest great homology between the genitor genomes, but differences in meiotic recombination potential in F_1 plants. However, numerical chromosome modifications, such as chromosome elimination and polyploidization, which probably resulted in meiotic errors in genitors, are hypothesized as common events when establishing artificial hybrids involving both genitor taxa.

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III.13

5S Ribosomal Gene Family in the Moray Eel *Gymnothorax unicolor* (Anguilliformes: Muraenidae)

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Moray eels are very common species, mainly in tropical and subtropical waters; nevertheless, they are very little studied. While the monophyly of the family is well-supported by phylogenetic studies, the species taxonomy is still controversial and some genera appear to be non-monophyletic. From a cytogenetic point of view, only 20 of ~200 species have been studied, and for most of them few karyological data are available. Beside a rather conserved diploid number of 42, morays display a greatly diversified karyotype structure and a large amount of heterochromatin. Chromosomal rearrangements as well as chromosome homologies have been reported in several species, resulting in divergent karyotype structures. To further investigate these cytogenetic features, the localization and distribution of specific sequences by FISH are essential for species with no genomic data, but, to date, this molecular cytogenetic technique has been employed in very few moray species with promising results. The karyotype of the Mediterranean brown moray eel *Gymnothorax unicolor* (Delaroche, 1809) was previously investigated and compared with the other Mediterranean species *Muraena helena* and with the congeneric species *G. tile*, by the use of classical techniques that pointed out many chromosomal banding similarities, as well as the occurrence of pericentric inversions and changes in heterochromatin amount. Furthermore, in this species FISH has been employed for the localization of centromeric, telomeric, and 45S ribosomal sequences. In order to extend the study of ribosomal genes in this species, in the present study we carried out the isolation, molecular characterization, and chromosomal mapping of the 5S sequences. These ribosomal genes have been interstitially localized in the acrocentric chromosome pair 10, different from the major ribosomal gene-bearing pair. Within the Muraenidae family, the minor ribosomal gene family has been recently mapped in other 5 species, showing differences in the chromosomal distribution. *G. unicolor* is the only studied species with only one 5S cluster, but a similar location on an acrocentric chromosome has been found in almost all other species. Furthermore, a comparative analysis of the distribution of the 2 ribosomal gene families among morays was performed. These results, even if preliminary, provide novel karyological information for comparative genomic investigations and could also help to understand the cytotaxonomy of this family.

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III.14

Chromosomal Mapping of the Repetitive U2 snRNA Gene in Six Species Belonging to the Neotropical Genus *Leptodactylus* (Anura, Leptodactylidae)

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Small nuclear RNA (snRNA) is a class of RNA molecules involved in the processing of pre-mRNA in the nucleus or even participating in different regulatory cell processes, as well as maintaining telomeres. The snRNAs are always associated with a set of specific proteins in complexes referred as small nuclear ribonucleoproteins (snRNPs). Spliceosomal U RNAs are the most common snRNA components of these complexes. The repetitive sequences of U snDNAs (mainly U1 and U2) have been cytogenetically mapped in several species of Arthropoda, fishes, and mammals, whereas their distribution remains unknown in amphibians. Thus, we present the results of FISH-mapping of the U2 snDNA repetitive sequences in the karyotypes of 6 species of *Leptodactylus* (2n = 22, except for *Leptodactylus* sp. 2, with 2n = 20) collected in Brazil, aiming to reveal their distribution patterns. The used probe, amplified and digoxigenin-labeled by PCR, hybridized to the distal short arm of the metacentric pair 6 in *L. fuscus* (in the *Leptodactylus fuscus* group), *L. latrans* (in the *Leptodactylus latrans* group), *L. petersii*, *Leptodactylus* sp.1, and *Leptodactylus* sp. 2 (in the *Leptodactylus melanonotus* group). A change in this pattern was observed in *L. labyrinthicus* (in the *L. pentadactylus* group) from 2 localities in São Paulo state. A specimen from Lençóis Paulista showed hybridization signals at proximal 10p regions in addition to the distal 6p regions, while *L. labyrinthicus* from Rio Claro exhibited only hybridization signals in the chromosomes 10. The same localization of U2 gene sequences in most of the species analyzed here suggests a relatively conserved pattern, as observed for other studied animals, and an homeomorphy of the chromosome 6 among these species of *Leptodactylus*, including *Leptodactylus* sp. 2, in which at least 1 fusion event is considered to have occurred during its karyotype evolution.

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III.15

Karyotype Diversity of a Curious Fauna of *Astyanax* (Teleostei, Characidae) from Middle Iguaçu River, Paraná, Brazil

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The genus *Astyanax* is highly speciose and widely distributed in the neotropical region. However, information about isolated species, in particular in hydrographic basins, is still scarce. The main goal of this work is to characterize the karyotype of *A. altiparanae*, *A. bifasciatus*, *A. dissimilis*, *A. minor*, *A. ribeirae*, and *A. serratus* from the middle Iguaçu River region, Paraná, Brazil. Cytogenetic and molecular genetic techniques were used. All studied species revealed a conserved diploid number of 50 chromosomes, with different karyotype formulae. The C-banding technique evidenced distinct heterochromatin patterns among species, sometimes matching with the nucleolus organizer regions (Ag-NORs), 18S and 5S rDNA. Simple NORs in *A. altiparanae*, *A. bifasciatus*, and *A. dissimilis*, and multiple NORs in the other species were observed, confirmed by FISH with an 18S rDNA probe in almost all situations. The 5S rDNA sites were revealed in chromosomal interstitial segments, simple in *A. altiparanae*, *A. minor*, and *A. serratus,* and multiple in *A. bifasciatus*, *A. dissimilis*, and *A. ribeirae*. The differences in the karyotype macrostructure observed among species are probably due to non-Robertsonian rearrangements. The analysis of the partial cytochrome c oxidase sequence (subunit I) and genetic distance verified with Kimura 2-parameter revealed enough genetic divergence to distinguish and identify the species, except in *A. bifasciatus* and *A. minor*. The *COI* gene used as a barcode was not resolutive in these species, possibly reflecting a recent speciation process. *A. ribeirae,* endemic to the Ribeira do Iguape River basin, was found for the first time at the Iguaçu basin. This evidence reinforces the fauna-sharing hypothesis among rivers on the crystalline plateau and coastal regions in the south of Brazil. In this study, the first cytogenetic and molecular genetic data were shown for *Astyanax* from the middle Iguaçu River region.

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III.16

Karyotype Diversity in Neotropical Fish: How Far Is Reality Reflected?

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Fish cytogenetics shows a great advance in relation to the knowledge of the number and karyotype formula of countless species. In the neotropical region, progress was very fast. In the order Siluriformes, some families contain certain species, which were quite well studied, for example, *Pimelodus maculatus* (Pimelodidae) and *Rhamdia quelen* (Heptapteridae). What was brought to our attention is the wide variety of karyotype formulae described in these 2 species, that is, populations of the same species within a basin and often in the same river. *P. maculatus* was cytogenetically described by Toledo and Ferrari in 1976 (references to be obtained by the author) having 2n = 56, but so far, with the cytogenetic data published, it shows at least 5 different karyotype formulae. *R. quelen* already presents 2n = 58 and at least 6 karyotype formulae are published, considering these differences actually exist. It is known that karyotype differences originate due to polymorphisms or as a result of the speciation process, but it cannot be expected that these are so frequent. What could be the reason for such diversity? Are there concerns about the chromosomal classification or the incorrect identification of the species? Often, if not exclusively, Levan et al. [1964] is quoted, unaware that this study proposes only the use of capital letters for 2 types, metacentric (M) and telocentric (T), and that at no time the type 'acrocentric' is described. In conclusion, it is possible that the karyotype differences between populations of the same species or among related species are overrated and that it requires a more appropriate chromosomal classification and caution. Therefore, it would be necessary to set up specific discussion groups in order to achieve a standardized way to report on karyotype data in several fish groups.

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III.17

Basic Chromosome Number Implication in the Relationship between Two Series of *Turnera* (Passifloraceae, Turneroideae) with Floral Nectariferous Pockets

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The genus *Turnera* is structured in 11 series: *Annulares, Anomalae, Capitatae, Conciliatae, Leiocarpae, Microphyllae, Papilliferae, Salicifoliae, Sessilifoliae, Stenodictyae,* and *Turnera*, with 2 subseries: *Turnera* and *Umbilicatae*. The flowers of the series *Anomalae* and *Turnera* are the only ones that have nectariferous pockets. In this genus, 3 basic chromosome numbers were found: series

Leiocarpae, Microphyllae, Salicifoliae, and *Stenodictyae* have x = 7; series *Papilliferae* has x = 13, and series *Turnera* has x = 5. In the first molecular analysis of the genus, *T. cearensis* (from the series *Anomalae*) clustered with *T. calyptrocarpa* of the series *Microphyllae* (x = 7). However, according to a cladistic analysis based on morphological characters and basic chromosome numbers, the series *Turnera* and *Anomalae* could be sisters. Hence, the cytogenetic analysis of *T. cearensis* would clarify the position of the series *Anomalae*. For the mitotic analysis, we stained roots with the Feulgen technique, and for the meiotic analysis, we stained anthers from fresh and fixed buds with acetic orcein. In mitosis, we found the chromosome number 2n = 30, and in meiosis, we found 16 cells with 15 II, one cell with 13 II + 1 IV, and 74 cells with 12 II + 1 VI, all of them with the basic number x = 5. The discovery of x = 5 in the series *Anomalae* is crucial information in order to find the actual position of this series in the genus. The evidence suggests that basic chromosome number and floral morphology could be linked, and this is a strong argument in favor of the merger of the series *Anomalae* and *Turnera*. Experimental crosses between *T. cearensis*, *T. calyptrocarpa,* and species of the series *Turnera* would help to clarify this situation.

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III.18

Genome Size, B Chromosomes and Karyotype Parameter Variations of Andean Argentinian Maize Populations *(Zea mays* ssp. *mays)*

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In the present study, the variation in genome size, number and frequency of B chromosomes (Bs) and karyotype parameters was analyzed in 10 populations belonging to 4 maize landraces (Orgullo Cuarentón, Pisingallo, Amarillo Grande, and Garrapata) from the Andean region of the northwest of Argentina. These maize populations were collected in an altitudinal gradient from 910 to 3,900 m.a.s.l. The goal of the present study is to analyze the relationship between these cytogenetic parameters with the altitude of cultivation. Moreover, by studying different karyotype parameters, each population is characterized cytogenetically. The 2C value varied significantly among the populations studied, between 4.5 and 6.2 pg. The average number of Bs, from 0 to 2.7, and the frequencies of Bs, from 0 to 100%, also varied significantly among populations. Variations in different karyotype parameters, such as total chromosome length, intra- and interchromosomal asymmetry indexes, and the percentage of knob heterochromatin were detected. In particular, the heterochromatic knobs showed variations in number, size, and position in the karyotype. Furthermore, FISH experiments were performed to reveal the sequence composition of each knob. The results allowed us to construct a representative idiogram for each population studied, revealing the value of karyotype parameters in the cytogenetic characterization of native maize populations. The correlations found show that Bs and the percentage of heterochromatin are sources of the genome size variation. Besides, the correlations found among the altitude of cultivation with Bs, percentage of heterochromatin, and DNA content suggest that the prevailing environmental features of each altitude of cultivation may also modulate the nucleotype of each maize population from northwestern Argentina.

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III.19

Repetitive DNA and Comparative Cytogenetic Analysis between *Crenicichla saxatilis* and Two Populations of *Crenicichla johanna* (Cichlidae) from Amazon

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Cichlids are one of the most diverse groups of fresh water fish, with 1,300 species, and a geographic distribution covering Africa, South Asia, and North, Central and South America. *Crenicichla* is the largest genus of the South American cichlids with 86 species. Cytogenetic studies are still relatively rare in this genus, although it is important to understand evolutionary genomic changes. Repetitive DNA sequences were mapped in *C. saxatilis* (Bragança, Pará, Brazil), and 2 populations of *C. johanna* (Abaetetuba-AB and Cametá-CA, Pará, Brazil). Chromosome preparations were analyzed by C-banding and FISH with probes of multigene families, telomeric sequences and transposable elements *(Mariner* and *Rex1)*. Significant differences at intra- and interspecific levels were observed. The 2 species analyzed showed the same 2n = 48 and NF = 56, but the arrangement of repetitive DNA sequences presented many differences. *C. saxatilis* presented 18S rDNA and *Mariner* marked at the secondary constriction in the short arm of chromosome pair 1; *Rex1* was dispersed in the karyotype, and interstitial telomeric sequences (ITS) in the centromeric region of pair 1 were observed. In *C. johanna*, 18S rDNA is located in 6 chromosomes for AB population and for CA population in the short arm of chromosome pair 1, and in the terminal region of an acrocentric chromosome. *Mariner* and *Rex1* are both dispersed in AB and CA samples, and 5S rDNA hybridizes at the interstitial region of an acrocentric pair. Despite the difference in location, both have the 18S rDNA and telomeric sequences colocalized. Fiber-FISH revealed that these sequences are intercalated. These differences may be associated with the reproductive isolation of these *C. johanna* populations, leading them to the process of speciation. These cytogenetic variations show that DNA organization is very

dynamic, and the repetitive DNA study becomes important for its role in the structural and functional genome organization.

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III.20

Optimized Probe Pooling and Consecutive Rounds of Multicolor FISH Allowed the Chromosome Identification in the Carnivorous Plant *Genlisea margaretae*

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The genus *Genlisea* A. St.-Hil. of the carnivorous Lentibulariaceae family possesses unique lobster-pot traps (corkscrew-like bundles of root-like subterraneous and chlorophyll-free leaves) to attract and entrap prokaryotes and small eukaryotes. More than 29 species of the genus are phylogenetically classified into 2 subgenera, *Tayloria* and *Genlisea*. The latter is further divided into 3 sections: *Africanae*, *Recurvatae*, and *Genlisea*. The entire genus is characterized by severe genome size differences (25-fold) and chromosome number variations (from 2n = 16 to 2n = 102) and thus, a promising model for genome and karyotype evolution studies. *G. margaretae* (184 Mbp/1C), belonging to the section *Recurvatae* of the subgenus *Genlisea*, has 19 pairs of tiny chromosomes. To establish a marker set that allows the individualization of all chromosomes, we applied an approach that combines an optimized probe pooling with consecutive rounds of multicolor fluorescence in situ hybridization (mcFISH) using bacterial artificial chromosomes (BACs) selected for repeat-free inserts. In total, 51 BACs were assigned to 18 chromosome pairs. They provide a tool for future assignment of genomic sequence contigs to distinct chromosomes as well as for the identification of homeologous chromosome regions (and potentially of chromosome rearrangements, in case more than one BAC label a chromosome pair) in other species of the Lentibulariaceae family.

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III.21

Chromosome Painting in Neotropical Long-Tailed Psittacidae (Aves, Psittaciformes): Phylogeny and Proposal of a Putative Ancestral Karyotype for Tribe Arini

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Most neotropical Psittacidae show a 2n = 70 and a dichotomy in the chromosome patterns. Long-tailed species have biarmed macrochromosomes, while short-tailed ones have telo/acrocentric macrochromosomes. However, the use of chromosome painting with chicken and white hawk probes has demonstrated that the karyotype evolution in Psittacidae included a high number of inter/intrachromosomal rearrangements. Hence, in order to infer about the phylogeny of long-tailed species as well as to propose a putative ancestral karyotype for this group, we analyzed the homology map of *Pyrrhura frontalis* (PFR), comparing it to other previously analyzed long-tailed species. Chromosome preparations were obtained from fibroblast cultures from skin biopsy of a female kept at Parque Zoológico do Rio Grande do Sul. Chromosomes were analyzed by conventional staining and FISH, using whole chromosome paints of *G. gallus* (GGA) and *L. albicollis* (LAL). Conventional staining showed a karyotype with 2n = 70, with biarmed macrochromosomes. The comparison of the results with the putative avian ancestral karyotype (PAK) showed the following correspondence: PAK1 (GGA1) = PFR1q and PFR4, PAK2 (GGA2) = PFR2, PAK3 (GGA3) = PFR3, PAK4 (GGA4q) = PFR1p, PAK5 (GGA5) = PFR5q, PAK6 (GGA6) = PFR6q, PAK7 (GGA7) = PFR6p, PAK8 (GGA8) = PFR7, PAK9 (GGA9) = PFR8, PAK10 (GGA10) = PFR9, and PAK11 (GGA4p) = PFR10. Fusion PAK6/PAK7 (PFR6) showed a paracentric inversion. LAL probes confirmed these results. The results indicate that PFR retained a more basal karyotype when compared to *Anodorhynchus hyacinthinus, Ara macao*, and *Ara chloropterus* because these 3 species show the fusion PAK8/PAK9, not observed in PFR. Hence, we suggest the ancestral karyotype for long-tailed species presented the fusions PAK1q/PAK4 and PAK6/PAK7, and additionally, a pericentric inversion in PAK6/PAK7, while fusion PAK8/PAK9 would have appeared in the common ancestor of *A. hyacinthinus, A. macao*, and *A. chloropterus.*

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III.22

Chromosome Discrimination in Diploid *Fragaria vesca* and *F. iinumae* by BAC-FISH Technique and Its Application to Octoploid Strawberry *F.* × *ananassa*

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The species in the genus *Fragaria* diversified into various ploidy levels, i.e., diploid to decaploid. Although genomic relationships among these species have been proposed, clear results have not been obtained. Also, karyotypes of these species have not yet been determined because of the small sizes of their chromosomes. Here, we present karyotypes of 2 diploid species, *F. vesca* and *F. iinumae*, which are thought to be the ancestors of the present cultivated octoploid strawberry *F.* × *ananassa*, by means of FISH analysis. FISH and GISH analyses were also applied to *F.* × *ananassa*. Slide preparations were made using root tip cells at metaphase and prometaphase stages of the 2 diploid species. Further, 1 or 2 BAC clones were selected from each of the linkage groups of *F. vesca* and used as probes for FISH. From FISH analysis, 7 metaphase chromosomes of the 2 species could be clearly discriminated by the signals of the BAC clones and showed the same distribution patterns of the signals on the chromosomes of the 2 species. Also, application of FISH to prometaphase chromosomes clarified relationships between the karyotypes and the linkage groups of the diploid *Fragaria* species. Application of FISH to *F.* × *ananassa* suggested chromosome rearrangements due to discrepancies of signal numbers from the expected ones. GISH analysis applied to *F.* × *ananassa* with the probes of genomic DNAs of the 2 diploid species showed a wide range of rearrangements among the chromosomes of the octoploid genome.

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III.23

Origin of Neopolyploids by Sexual Polyploidization in *Turnera sidoides* L. (Passifloraceae, Turneroideae)

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Turnera sidoides (x = 7) autopolyploid complex is a suitable model to analyze neopolyploid evolution in natural populations. Here, we analyze natural diploid populations of this species aiming to (1) estimate the frequency of neopolyploids and analyze their mechanism of origin, (2) analyze the spatial distribution of cytotypes and 2n gametes producers at a microscale, (3) estimate the viability and ploidy level of offspring of intercytotype matings, (4) analyze triploids cytogenetically, and (5) estimate the expected rates of neopolyploid formation. The finding of 3.5% triploid embryos contrasts with the rarity of triploid plants detected in natural diploid populations. The 2n gametes involved in neopolyploid formation were mostly of paternal origin. A spatial segregation of cytotypes was detected, although mixed 2x–3x patches were found. In such patches, 2n eggs and/or 2n pollen producers were detected. Triploids produce some n and 2n viable gametes and diploid, triploid, and tetraploid progeny in experimental crosses. The expected neopolyploid formation rates involving 2n pollen were 0.034 (triploids) and 0.00033 (tetraploids), and 0.025 (triploids) and 0.00025 (tetraploids) involving 2n eggs. The rate of neotetraploid formation by bilateral polyploidization was 0.00027. These results suggest that unilateral polyploidization would constitute an alternative mechanism of neopolyploids origin in *T. sidoides*. The rates of neopolyploid formation estimated are consistent with the expected rates in cross-pollinated autopolyploid species. The continuous formation of neopolyploids resulted from successive backcrosses between 2n gametes producers, and their progeny would favor the establishment and persistence of neopolyploids in diploid populations of *T. sidoides*. New generations of polyploids (triploids and tetraploids) would also originate by crossings between triploids or by backcrosses with diploid progenitors that produce 2n gametes. These facts together with the capacity of *T. sidoides* to multiply by rhizomes would enhance the likelihood that a low frequency of neopolyploids can be originated and maintained in natural diploid populations.

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III.24

Cytogenetic Characterization of a Sexual Population of the Brazilian Scorpion *Tityus serrulatus* (Buthidae)

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Tityus serrulatus is a Brazilian endemic scorpion of medical importance, whose wide distribution and occurrence in urban areas was attributed mainly to the parthenogenetic mode of reproduction. Until recently, only all-female *T. serrulatus* populations were known; male populations were found to be restricted to 6 areas of natural environment in the semiarid Brazilian region. *T. serrulatus* belongs to the family Buthidae, which is cytogenetically characterized by the presence of holocentric chromosomes and high levels of chromosomal variability. Only 2 parthenogenetic populations of *T. serrulatus* from an urban environment were chromosomally investigated, showing a diploid number of 2n = 12. The aim of this study is to cytogenetically characterize the males of *T. serrulatus*, regarding the diploid number, chromosome behavior during meiosis, and the location of 28S rDNA. The sample included 4 males from Januaria and 3 from Itaobim, Minas Gerais. Chromosome preparations were obtained from gonads of adult individuals. The slides were stained with 3% Giemsa solution and submitted to FISH. Mitotic metaphase cells of the 7 specimens showed the diploid number of 2n = 12, with 8 chromosomes gradually varying in size, from large to medium, and 4 small-sized chromosomes. Postpachytene cells exhibited 6 bivalents with

chromosomes arranged in a parallel disposition without evidence of chiasmata. All nuclei in metaphase II presented n = 6. FISH technique revealed 28S rDNA localized in the terminal region of one bivalent. The diploid number observed here in males of *T. serrulatus* is similar to that previously registered for 2 parthenogenetic populations. The presence of the 28S rDNA in one bivalent differed from the pattern observed in parthenogenetic females, in which only one chromosome had the major ribosomal sites. Therefore, it is necessary to investigate if this difference is related to the mode of reproduction found in different populations.

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III.25

Cytogeography of *Psidium cattleianum* Sabine (Myrtaceae)

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Psidium cattleianum (cattley guava) is a fruit plant native to South America. It presents a broad distribution, occurring in the Atlantic forest, from Bahia to Rio Grande do Sul, in Brazil, and in Uruguay. The species has 2 distinct morphotypes, one producing yellow fruits and the other red fruits. Chromosome numbers of 2n = 44, 55, 66, 77, and 88 have been previously reported. We aimed to investigate the distribution of chromosome numbers in populations of *P. cattleianum* along the Atlantic forest. The aim was to collect 5 trees out of 12 different populations. We collected 88 individuals with fruits and determined the chromosome number in 43 individuals until now. We found 9 cytotypes: 2n = 33, 44, 55, 66, 77, 88, 99, 110, and 132; 2n = 77 was the most common; 2n = 33, 99, 110, and 132 are reported here for the first time for *P. cattleianum*, and 2 = 132 is the highest number ever reported in the genus *Psidium*. We found populations exclusively with the yellow morphotype and populations with both morphotypes, but never with the red morphotype only. In addition, we noted that the yellow morphotype tended to present a broader distribution and a larger range of cytotypes relative to the red morphotype. Presently, we cannot associate the color of fruits with the determined cytotype. After CMA/DAPI banding and FISH, we observed that the number of bands increases with the growing level of ploidy. We believe that the high number of cytotypes in the species may be associated with its wide distribution in Brazil.

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III.26

Karyotype and Genome Evolution of Crucifers (Brassicaceae)

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The Brassicaceae family comprises 320 genera and over 3,600 species classified into some 50 bona fide monophyletic tribes. The feasibility of comparative cytogenetic analyses and the available whole genome sequences for a dozen of crucifer species (e.g., *Arabidopsis lyrata*, *Camelina sativa*, *Brassica rapa*, *B. oleracea, Schrenkiella parvula*) allowed us to reconstruct ancestral genomes and infer prevalent trends in genome evolution in the Brassicaceae. A working hypothesis of an ancestral crucifer karyotype (ACK) with 8 chromosomes (n = 8) and 22 genomic blocks was repeatedly corroborated for the crucifer crown group. During the diversification of the mustard family, ACK evolved following 4 basic evolutionary trajectories: (i) genomic stasis without major chromosomal reshuffling (e.g., *A. lyrata*, *Capsella rubella*), (ii) genome reshuffling but stasis of the ancestral chromosome number (e.g., *Arabis alpina*, *Cardamine*), (iii) descending dysploidy (n = 5–7) associated with genome shuffling (e.g., *A. thaliana*, *Neslia paniculata*), and (iv) whole genome duplication/triplication followed by genome diploidization including descending dysploidy (up to n = 4). Detailed analyses of crucifer genomes helped to (re)discover major mechanisms underlying chromosomal and karyotype evolution in the Brassicaceae; these mechanisms include inversions, reciprocal chromosome translocations, centromere invactivation/loss, or centromere repositioning.

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III.27

Multiple Patterns of Genome Evolution in the Brassicaceae: A Lesson from the Polyploid-Rich Genus *Cardamine*

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Cardamine (bittercress) is one of the largest Brassicaceae genera (200 spp.). The genus exhibits a large karyological diversity (2n = 16 to ~256). Due to the feasibility of comparative chromosome painting and genomic in situ hybridization in Brassicaceae, we documented both recurrent and deviating patterns of genome evolution in *Cardamine* polyploids. (i) The North American *C. cordifolia* with a triploid-like chromosome number (2n = 24) is a diploidized tetraploid. The ancestral tetraploid chromosome number (2n = 32) was reduced to a triploid-like number through 4 terminal chromosome translocations ('chromosome fusions'). The *C. cordifolia* genome provides valuable insights into the mechanisms of post-polyploidy rediploidization in plants. (ii) In Europe, some tetraploid (2n = 32) populations of *C. pratensis* are on the way to decrease their chromosome number by 'chromosome

fusions' (2n = 30 and 28). On the contrary, some diploid (2n = 16) populations of *C. pratensis* contain hyperdiploid plants with 1–4 additional chromosomes (2n = 17, 18, 19, and 20). (iii) We elucidated independent origins of several European and Asian tetra- and octoploid (2n = 64) species of the *C. flexuosa* complex through hybridization events involving 3 diploid progenitor species. (iv) In *Cardamine*, hybridization and polyploidization is ongoing. We reconstructed the origin of the triploid hybrid *C.* × *insueta* (2n = 24, RRA) by hybridization between *C. amara* (2n = 16, AA) and *C. rivularis* (2n = 16, RR) ~100 years ago. Hybridization involving *C.* × *insueta* and the hypotetraploid *C. pratensis* (2n = 30, PPPP) resulted in the origin of the hypohexaploid *C. schulzii* (2n = 46, PPPPRA). This shows how a semifertile triploid hybrid can promote the origin of trigenomic allopolyploids.

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III.28

Polyploidy-Driven Diversification and Diploidization: The Origin of the Australian and New Zealand Crucifers

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We previously showed that 3 endemic Australian crucifer species (tribe Microlepidieae, Brassicaceae) have undergone a whole genome duplication (AUS-WGD) followed by a diversification and species-specific diploidization, generating some of the lowest chromosome numbers (n = 4–7) known for the crucifers [Mandáková et al., 2010, Plant Cell]. The New Zealand genus *Pachycladon* has undergone either the AUS-WGD or an independent WGD followed by less extensive genome reshuffling towards n = 10 genomes [Mandáková et al., 2010, BMC Evol Biol]. To further elucidate the origin and fate of Australian/New Zealand mesopolyploid genomes, we analyzed 12 species (9 genera) with variable chromosome numbers (n = 4, 5, 6, and 7) by comparative chromosome painting and gene sequencing. We concluded that all members of the Microlepidieae tribe originated through the AUS-WGD. This allopolyploid WGD most likely spurred the diversification of the group on the Australian continent. Cytogenetic signatures and multigene *(CHS*, *PHYA*, *ndhF)* phylogenies suggest that the ancestral allopolyploid genome had most likely 30 chromosomes (n = 15) and was formed through an inter-tribal hybridization (tribe Crucihimalayeae Smelowskieae/Descurainieae), dated to ~10.6 MYA, followed by a long-distance dispersal from Asia to Australia. The AUS-WGD was followed by independent and massive genome rediploidizations towards the extant diversity of quasi-diploid genomes (n = 4–7) of the Australian Microlepidieae taxa. The New Zealand genus *Pachycladon* was formed by an independent WGD event.

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III.29

Distribution of Repetitive DNA Sequences in Seven Species of the Plant Family Solanaceae Juss

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The Solanaceae family is considered one of the largest botanical families among angiosperms, comprising around 2,500 species and 100 genera. The major Solanaceae diversity is found in neotropical regions. Some of these families' genera have been cytogenetically studied and have presented uniformity in some karyotype features, such as chromosome number and morphology, but a great diversity in relation to repetitive DNA. The study of repetitive DNA is of great importance for taxonomy and evolution since it allows a greater understanding of genome organization and the paths that led to the diversification of species or groups phylogenetically related. In this study, 7 species were sampled, *Brunfelsia uniflora* D. Don, *Cestrum laevigatum* Schltdl, *C. mariquitense* Kunth, *Solanum acerifolium* Sendt., *S. inodorum* Vell., *S. sanctaecatharinae* Dunal, and *S. pseudocapsicum* L. Information about chromosome number, repetitive DNA sequences rich in AT and CG, and 5S and 18S rDNA were obtained. Fluorescence banding (CMA/DAPI) and FISH were used for repetitive DNA mapping. Two species of the *Cestrum* L. genus present 2n = 24, *B. uniflora* 2n = 22, and the *Solanum* species 2n = 24. Fluorescence banding showed distinct patterns of heterochromatin distribution among some species ($CMA^+/DAPI^-$, $CMA^+/DAPI^0$, $CMA^0/DAPI^+$), with number variation (3–28) and different positions (terminal, interstitial position, and NOR associated). Differentiation was also observed in the distribution of the rDNA sequences between 3 genera. It was possible to identify 1 pair of 18S rDNA sites in *S. acerifolium*, *S. sanctaecatharinae*, and *B. uniflora*; 2 pairs in *C. laevigatum* and *C. mariquitense*, and 18 pairs in *S. inodorum*. All species showed 1 chromosome pair with a 5S rDNA site, except *S. acerifolium* and *C. mariquitense* (2 pairs). Finally, the differences in the distribution of heterochromatin and rDNA may be useful in the identification of 3 genera and shed light on the evolutionary trends in Solanaceae.

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III.30

AflaSAT-1, a Satellite DNA in the Grasshopper *Abracris flavolineata*: Sequence and Chromosomal Evolution in A and B Chromosomes

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Satellite DNAs (satDNA) are sequences repeated a hundred to thousand times and are tandemly arrayed. They are enriched mainly in heterochromatin located in centromeres and telomeres, or in intercalary regions, and moreover, they can spread out in specific chromosomes, such as B chromosomes. Here, we isolated the first satDNA in the grasshopper *Abracris flavolineata*, named AflaSAT-1, by restriction enzyme digestion. The sequence was characterized combining cytogenetic, molecular and genomic approaches, aiming to understand the structural organization, evolution in A and B chromosomes, and the possible functionality of this sequence. The AflaSAT-1 is a satDNA shared with other grasshopper species, but our data suggests that it is exclusively enriched in the genome of *A. flavolineata*. The AflaSAT-1 monomers recovered from individuals belonging to 6 populations and from the microdissected B chromosomes (μB-DNA) presented almost no variation between populations, but 4 exclusive mutations in μB-DNA were noticed, reflecting the common higher mutational rate in B chromosomes. The analysis using the sequenced genome revealed distinct organization for the satDNA with occurrence of clusters containing only the AflaSAT-1 or containing the AflaSAT-1 associated with other satDNA, named AflaSAT-2, but AflaSAT-2 was never found alone. Regarding the chromosomes, in the population from Rio Claro/SP, the AflaSAT-1 and AflaSAT-2 were interlinked with each other forming large blocks in the centromeres of almost all chromosomes, except in the smallest element (pair 11). In the B chromosome, only a small centromeric signal was noticed. At the level of population, the chromosomal distribution for AflaSAT-1 showed slight variations, such as absence of some marks, additional clusters, and heteromorphism. These variations were reflected in AflaSAT-1 copy number estimated by qPCR, suggesting a dynamic expansion or elimination of this satDNA. Finally, the cDNA analysis revealed constitutive transcription signals for AflaSAT-1 in distinct tissues of adults, and in distinct life cycle phases in individuals harboring B chromosomes.

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III.31

Evolutionary Chromosome Trends in Spermacoceae s.s. Tribe (Rubiaceae)

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Few groups among the great diversity of species were studied using chromosomal data associated with plant systematics. 'The cytogenetic renaissance' has happened with the development of new methods that combine phylogenetic and cytogenetic approaches. The *Spermacoce* clade is a group of the Spermacoceae tribe (Rubiaceae), and chromosomal data are very limited. For a few species, counts and some chromosomal characteristics are available, but such data were never related to the group phylogeny. This study aimed to investigate the karyotype evolution in the light of the systematic of the Spermacoceae s.s. tribe [sensu Karehed et al., 2008] (reference to be obtained by the author) for the purpose of a better understanding of the processes involved in their diversification. The chromosomal number of the Spermacoceae s.s.tribe was consulted in studies, published articles, and online platforms. For a total of 48 species, we recorded the diploid number and determined the basic number and ploidy level. Each of these characters was plotted as categorical data in a phylogenetic tree proposed earlier under disorderly maximum parsimony. We applied the reconstruction ancestral character analysis using the Mesquite v.3.04 program. The Spermacoceae s.s. tribe has a great variability in chromosomal numbers (2n = 14, 28, 30, 42, 56, 64, and 84) with most genera presenting x = 14 as a basic number. *Ernodea* and *Crusea* are the only genera that presented x = 7 but are in distinct clades. The monospecific genus *Hydrophylax* exhibited the basic number x = 28. We observed many polyploidy events that are important mechanisms for the evolution of the tribe process.

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III.32

Karyotype Diversification in *Rineloricaria* Species (Actinopterygii: Siluriformes: Loricariidae)

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Cytogenetic studies in fish of the *Rineloricaria* genus have already shown a high variation in diploid number (2n). The present study assessed the species *Rineloricaria latirostris* (Laranjinha River – Cinzas Basin and Barra Grande River – Ivaí Basin) and *R. pentamaculata* (Barra Grande River) aiming to describe the karyotype organization of its members and understand the mechanisms that lead to the variation of chromosome numbers. *R. pentamaculata* karyomorph B (2n = 54) occurs in sympatry and syntopy with karyomorph A (2n = 56) in the Barra Grande River, without any evidence of hybrid formation. The analysis of chromosome mark-

ers by FISH showed a small variation of 5S rDNA sites and the occurrence of interstitial telomeric sites (ITS) traces in a metacentric (M) chromosome pair of karyomorph B, indicating a Robertsonian fusion and a numeric reduction to 2n = 54. Two populations of *R. latirostris* (Laranjinha and Barra Grande Rivers) had 2n = 46 and the same karyotype organization. Even keeping the diploid number, the analysis of rDNAs and $(TTAGGG)_n$ sequences using FISH mapping indicated that Robertsonian fusion events occurred in the evolutionary history of this species. In populations of *R. latirostris*, the occurrence of 1 large M chromosome pair carrying 18S rDNA and 1 large M chromosome pair carrying 5S rDNA colocated with $(TTAGGG)_n$ sequences, in addition to a third large M chromosome pair carrying ITS vestiges, all with centromeric location, may indicate fusions between subtelocentric or acrocentric chromosome pairs. These results support that numerous fusion events occurred in the karyotype differentiation within this species. Therefore, in this study, we assessed the telomeric instability, chromosomal breaks, and rearrangements due to ITS vestiges detection, in addition to the probable role of rDNAs in chromosome fusions in karyotype diversification of this group.

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III.33

A Centromeric Repeat of *Solanum chomatophilum* and Its Dynamics in Natural Populations

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The centromere is an essential chromosome locus since it is responsible for organizing the kinetochore, a proteinaceous structure where the spindle fibers attach to promote chromosome segregation. A variant of histone H3, named CenH3, is found in centromeres in all eukaryotes studied so far and is considered as a mark for centromeric function. Centromeres usually contain repetitive DNA and retrotransposons, which often diverge rapidly and can differ among closely related species. Centromeric DNA of *Solanum chomatophilum* (2n = 2x = 24, genome P, the closest to genome A of potato – *S. tuberosum*) was isolated by ChIP (chromatin immunoprecipitation) and sequenced using Illumina platform. Centromeric repeats were identified using RepeatExplorer pipeline. A highly repetitive DNA element, Sc83, was identified, cloned, and sequenced. FISH mapping of Sc83 was performed in *S. chomatophilum* and several other diploid *Solanum* species, including *S. tuberosum* group *Phureja* (DM1–3516R44, genome A), *S. verrucosum* (genome A), *S. jamesii* (genome B), *S. palustre* (genome E), and *S. lycopersicum* (genome T). Repeat Sc83 is exclusively present in the centromeres of chromosome 3 of *S. chomatophilum*. However, Sc83 was detected in a nonidentified chromosome in some plants. Intraspecific variation of this repeat was examined in 80 different plants; 55 contained only the signals on chromosome 3, and 25 contained the third signal. Sc83 was also consistently found in 8 and 6 chromosomes in *S. verrucosum* and *S. palustre*, respectively, but was not detected in the remaining species. These results show a recent origin of the Sc83 repeat and a highly dynamic evolution of the centromeres in *Solanum* species.

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III.34

Cytogenetic Analysis of the Genome in *Brachiaria* (Poaceae) Species and Its Interspecific Hybrids

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The grass genus *Brachiaria* (Poaceae) comprises species showing different ploidy levels and reproduction modes. Despite the great economic and agronomic importance of forage species used in genetic breeding, studies on genomic constitution and relationship within the genus are limited. Accordingly, molecular cytogenetic analyses can help us understand genomes and their differentiation and the species' phylogenetic relationships. The objective of this study was to investigate the genomic relationships among *B. ruziziensis*, *B. decumbens*, *B. brizantha*, and interspecific hybrids by means of GISH, FISH, and meiosis, and nuclear genome quantification by flow cytometry. The hybrid 1863 *(B. ruziziensis* × *B. brizantha)* presented 2n = 36, 3.24 pg of nuclear DNA, and 4 and 7 sites of 45S and 5S rDNA, respectively. The hybrid Mulato II [(*B. ruziziensis* × *B. decumbens*) × *B. brizantha*)] presented 2n = 36, 4 and 7 sites of 45S and 5S rDNA, respectively, and an increase in DNA content (3.83 pg) compared to the average content of the parents (3.43 pg). The hybrid 963 *(B. ruziziensis* × *B. decumbens)* showed 2n = 38 in all metaphases, exceeding 2 chromosomes compared to the expected number and an increase of 0.29 pg (9%) of DNA, considering the average DNA content of the parents (3.33 pg) and the presence of 5 sites of 45S rDNA and 7 sites of 5S rDNA. GISH, using total nuclear DNA, performed in *B. decumbens*, *B. brizantha*, and *B. ruziziensis* showed variations in the hybridized genome percentage and in the chromosome region of this hybridization. Chromosomal rearrangements were observed as well as differences in the contribution of parent genomes in the constitution of the hybrids. GISH results associated to chromosomal pairing in meiosis of the interspecific hybrids confirmed that parental species are closely related, and they share similarities between chromosomes/genomes, which were considered homeologous.

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III.35

Interspecific Chromosome Painting in *Aotus* sp. (Primates: Cebidae)

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Cytogenetic data have been very useful in the identification and establishment of phylogenetic relationships in Platyrrhini, especially in taxa with high chromosome variability, such as *Aotus* (owl monkeys), which have diploid numbers ranging from 2n = 46 to 58. Fusions/fissions, pericentric inversions, and Y/autosome translocations have been described as the main rearrangements in this genus. We analyzed a pair of *Aotus* sp. captured in the Amazon and kept at the Fundação Zoobotânica de Belo Horizonte, Brazil. Chromosome preparations were obtained from fibroblast cultures and analyzed after GTG- and CBG-banding, and in situ hybridization with telomeric and human chromosome-specific (HSA) probes. The specimens had 2n = 49 (male) and 50 (female) and FN = 68. Chromosome painting allowed us to identify 11 conserved HSA chromosomes (HSA 6, 9, 12, 13, 17, 18, 19, 20, 21, 22, and the X chromosome). HSA 4, 8, 10, 11, and 16 labeled 2 chromosome pairs of *Aotus* sp., whereas HSA 1, 2, 3, 5, 7, and 15 were split into 3 or more segments. The following associations were found: HSA 1/3, 1/16, 2/7, 2/20, 3/21, 4/15, 5/7, 5/15 (twice), 7/11, 8/18, 10/11, 10/22, 15/14/15/14, and 16/22. Our analysis showed that the male has a translocation Y/16 and that *A*. sp. has a karyotype different from the other 3 species of *Aotus* analyzed by chromosome painting with human probes (*A. nancymaae*, *A. griseimembra*, and an unidentified species). A comparison between the painted karyotypes of *Aotus* and the putative ancestral Platyrrhini karyotype (APK) allowed us to propose an ancestral *Aotus* complement with 2n = 52.

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III.36

Cytogenetic Data on Agro-Predatory Ant Species *Megalomyrmex incisus* and Its Host *Mycetophylax conformis* (Myrmicinae: Formicidae)

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Karyotype description is an important tool for taxonomy and the understanding of the evolution of organisms. Ants are among the evolutionarily most successful groups of animals, occurring virtually in all environments. Comprising ~14,000 described species, the family Formicidae has cytogenetic data available for only 750 species. In this study, we carried out the first karyotype description of the new agro-predatory *Megalomyrmex incisus,* and evaluated the chromosome counts of its host *Mycetophylax conformis* from another population kilometers apart from those studied previously. Colonies from both species were sampled from seashores of Ilhéus, Bahia state and transferred to the laboratory. Metaphase spreads were prepared from the cerebral ganglia of post-defecant larvae. Slides were examined and pictures of the best metaphases were taken under Olympus BX50 light microscopy. We classified the chromosomes following the nomenclature proposed by Levan et al. [1964] (reference to be obtained by the author), which is based on centromere position: acrocentric (A), subtelocentric (ST), submetacentric (SM), and metacentric (M). The chromosome number observed for *M. incisus* was 2n = 50. The karyotype of this species consists of 45 metacentric and 5 submetacentric pairs. Thus, the karyotype formula found for the diploid set would be 2K = 90M + 10SM and a diploid number of the arms 2AN = 100. Yet, the host *M. conformis* has a diploid number of 30 chromosomes. The karyotype consists of 16 metacentric pairs and 4 submetacentric pairs, which represents a karyotype formula 2K = 22M + 8SM and a diploid number of the arms 2AN = 60. *M. incisus* showed a chromosome number within the range already known for the Myrmicinae subfamily, but such a high chromosome number has a low frequency across species. *M. conformis* showed the same chromosome number and morphology of previously studied populations suggesting karyotype stability.

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III.37

Phylogeny, Genome Size Evolution and Surprisingly Diverse Chromosome Numbers in the (Sub)Tropical Genus *Globba* (Zingiberaceae)

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Nuclear DNA content (i.e., absolute genome size) usually varies among species and genera, and similarly, chromosome numbers can be very variable. There is still limited knowledge of genome size evolution; in particular, studies focused on tropical plant groups are extremely rare. Moreover, polyploidy in tropics is rather surprising because polyploidization preferably arises in extreme (e.g., cold) conditions. The genus *Globba* (~100 species) from the economically important family Zingiberaceae is widely distributed in the Indochinese floristic region. There are 3 subgen-

era and 7 sections recognized according to the current infrageneric classification. *Globba* is a polyploid complex having a surprisingly high diversity of chromosome numbers. The main aim of our study was to get a better insight into chromosomal and genome evolution of *Globba*. We sequenced nuclear (ITS) and chloroplast (matK) DNA regions of more than 100 *Globba* accessions. The dataset was supplemented by 50 GenBank accessions to cover ~70 species. The previous infrageneric classification was largely confirmed, but an additional section needs to be recognized around *G. nisbetiana*. Using flow cytometry, we estimated the DNA content (2C) of more than 100 accessions. Genome size varied more than 4-fold, ranging from 1.1 pg in *G. nuda* to 4.4 pg in *G. marantina*. Species from the evergreen section *Sempervirens* tend to have the highest genome sizes. Eight different chromosome numbers were found (2n = 20, 22, 24, 28, 32, 34, 48, and ~96), and their distribution generally followed the phylogeny. Putative polyploidy was observed in several sections. Taxa with 2n = 20, 22, and 24 have surprisingly large genome sizes, considerably larger than those with 2n = 32 and similar to those with 2n = 48.

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III.38

Cryptic Diversity among Populations of *Ancistrus* (Siluriformes, Loricariidae) Revealed by Cytogenetic Analyses and DNA Barcoding: Evidence of New Species in the Basin of the Paraná River in Brazil

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Ancistrus is characterized by ample variation in chromosome numbers and structure, and a high level of cryptic diversity. In the Paraná River basin, only 1 species, *Ancistrus cirrhosus*, has been described from Misiones (Argentina). By combining data on mitochondrial DNA *(COI)* and chromosomal markers from different *Ancistrus* populations in the tributaries of the Paraná and specimens from Misiones, we detected 4 distinct evolutionary lineages in this basin. All the specimens exhibited 2n = 50 but had distinct karyotype formulae. The populations from Córrego 19 and the Keller River showed 12m+18sm+12st+8a (females) and 11m+18sm+13st+8a (males), consistent with an XX/XY system. The X chromosome (metacentric) of the populations varies in quantity and distribution of heterochromatin blocks and could not be distinguished from the Y chromosome (subtelocentric) by C-banding. Specimens collected in the Mourão River were 12m+18sm+12st+8a, with an additional B microchromosome. These 3 populations were included in a single clade in the molecular phylogeny, indicating that they represent a single species, despite the cytogenetic differentiation represented by the heteromorphic sex chromosomes, which may have originated very recently in these populations. The population of the São Francisco Verdadeiro River (14m+16sm+14st+6a) was assigned to a second clade, together with Arroio Iguaçu, while the specimens from Ocoí and São Francisco Falso Rivers (10m+18sm+16st+6a) were assigned to a third clade. Lastly, the population from San Juan (Argentina), which corresponds to *A. cirrhosus*, corresponds to a distinct clade from the other populations of the Paraná basin. Clusters of 18S rDNA in 6 populations of *Ancistrus* were observed in a single chromosome pair but at different positions, in some cases, in synteny with the 5S rDNA sites. Multiple 5S sites were observed in 3 populations. Overall, the cytogenetic data indicate the existence of cryptic diversity and support the hypothesis that at least 4 *Ancistrus* species may coexist in the Paraná basin.

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III.39

Cytological Characterization of the *Guarani's* Maize Landraces of Northeastern Argentina: Variations in the Genome Size and the Cytological Markers

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To contribute to the knowledge of the cytogenetic diversity of the *Guarani's* maize landraces, we studied 20 populations from northeastern Argentina. In indigenous settings from the subtropical forests of the Misiones Province, up to 15 *Guarani's* maize landraces were found. They showed remarkable phenotypic differences and high genetic diversity despite growing in a small and restricted ecogeographical area without significant differences in altitude, climatic, or biological conditions. We performed DAPI staining and FISH using the following probes: the knob (180 bp and TR-1), rDNA (18S and 5S), and centromeric (CentC and CMR) repetitive sequences. We also evaluated the variability of the genome size by flow cytometry. In *Zea,* knobs have been observed in 34 distinct cytological locations, varying in number, size, and sequence compositions. Previous studies reported that the differences in the DNA content were related to the variations in the number and size of the knobs. However, the variation of other repetitive sequences could not be discarded. The *Guarani's* populations showed knobs, with different frequencies, in almost all chromosome arms (except 6S). The number (8–22), percentage (~5–18%), and sequence compositions of the knobs showed variability among populations. Moreover, variation in the percentages of CentC and CMR sequences was detected. Karyotype parameters and FISH allowed the characterization of each population. The 2C value presented interpopulational (4.6–6.29 pg) and intrapopulational (1.08- to 1.63-fold) variation. This study is a contribution to better understand and conserve the maize landraces, which is important due to the increasing threats to the gene pool of native maize.

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III.40

Multidirectional Chromosome Painting with Whole Chromosome Probes from *Burhinus oedicnemus* (Charadriiformes) in the Genome of *Glyphorynchus spirurus* (Passeriformes, Furnariidae) Reveal Multiple Fissions during Karyotype Evolution of Birds

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The Wedge-billed Woodcreeper (*Glyphorynchus spirurus*, Passeriformes, Furnariidae) has a wide distribution ranging from Central America, west to the Andes, throughout central Amazonia, and south along the Atlantic coast of Brazil. *G. spirurus* represents a polytypic species with a least 13 subspecies, most endemic or associated with particular areas of endemism. These characteristics make *G. spirurus* an ideal model organism to investigate the influence of chromosomal barriers on population structure. In order to contribute to our understanding of the chromosomal evolution of Passeriformes, we analyzed the karyotype of 2 specimens of *G. spirurus paraensis* (GSP) collected in Tapajós endemism area by G-banding and FISH, including chromosome painting with probes from *Burhinus oedicnemus* (BOE, Charadriiformes). Here, for the first time, we describe cytogenetic data of GSP from the Amazon Region, Brazil. The specimens analyzed presented 2n = 80 and FN = 84, typical of Passeriformes. A gross comparison showed that the 2 karyotypes have no morphological differences. This karyotype has 3 pairs of subtelocentric, 8 pairs of acrocentric macrochromosomes, and 56 microchromosomes (MCS). The Z and W chromosomes are acrocentric. The BOE probe hybridization resulted in 32 fluorescent signals, and the Z probe showed cross-hybridization with the W chromosome and 2 MCS. Comparative analysis using G-banding and chromosome painting showed that the karyotype complement of GSP experienced multiple fissions when compared to the BOE karyotype, corroborating the fission-fusion model of evolution of the MCS. Only 2 macrochromosomes (BOE 3 and 7) and 4 MCS (BOE 17–20) have been preserved in the GSP karyotype. Cross-hybridization suggests a compound chromosomal sex determination in BOE, where the sequences would be homologous to W and MCS in the GSP, or hybridized in these regions by high similarity of repetitive DNA sequences.

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III.41

Genomic Organization of Transposable Elements *Rex1* and *Rex3* in Species of Tribe Ancistrini (Siluriformes, Locariidae)

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The family Loricariidae is the second largest of neotropical fishes (order Siluriformes). Its tribe Ancistrini has 217 species and 29 genera. Cytogenetically, this tribe shows the largest karyotype variation, with species having 2n = 34 to 2n = 54, with diverse systems of sex chromosomes, chromosomal and rDNA polymorphism. Considering that studies about this tribe and about transposable elements (TEs) are rare and limited to few species and fish groups, the aim of this study was to analyze the composition and chromosomal localization of TEs of the referred tribe. TEs (*Rex1* with 500 bp and *Rex3* with 400 bp) were amplified in 8 species (*Ancistrus claro*, *A. cuiabae*, *A.* cf. *dubius*, *Lasiancistrus* cf. *schomburgkii*, *Ancistrus* sp. 08, *Ancistrus* sp. 12, *A. tombado*, *Ancistrus* sp.) from hydrographic basins of Paraguay and Amazonia. FISH revealed that *Rex1* and *Rex3* showed a dispersed distribution in all chromosomes of the analyzed species. Conspicuous blocks were observed in the telomeric regions of all samples, in heterochromatic regions and in 2 pairs of sex chromosomes. In *Ancistrus* sp. 08, *Rex1* and *Rex3* showed a differential hybridization in the long arm of a submetacentric chromosome pair in both male and female. In *A.* cf. *dubius*, *Rex1* hybridized to all chromosomes, except in the nucleolus organizer region, while *Rex3* preferentially hybridized to telomeric regions. In *Ancistrus* sp., *Rex1* primarily hybridized to 1 large acrocentric chromosome, while *Rex3* was dispersed along the chromosomes. This is the first report of TEs in the tribe Ancistrini, bringing new information about the distribution of repetitive sequences in the genome of these species and about the organization and evolutionary dynamics of these TEs.

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III.42

Chromosome Homologies of Anteaters in Argentina (*Myrmecophaga tridactyla* and *Tamandua tetradactyla*, Myrmecophagidae, Xenarthra)

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The Myrmecophagidae family is a clade of xenarthran mammals with 2 existent genera and 3 living species endemically distributed in the Neotropics. The karyological relationships of the genera of this family are poorly understood. The karyotype and cytotypes of 39 individuals (20 males and 19 females) of *Myrmecophaga tridactyla* (*Mt*, 2n = 60 and NF = 110) and 29 individuals (17 males and 12 females) of *Tamandua tetradactyla* (*Tt*, 2n =

54 and NF = 108) were studied throughout their geographical distribution in Argentina. Peripheral blood lymphocytes were cultured to obtain mitotic metaphases and G-, C-, and NOR-banding along with telomeric FISH were carried out. Spermatocyte microspreads were used to analyze synaptonemal complexes (SC) from testicular biopsies (n = 2).The G-banding pattern and chromosome size showed a full homology for most pairs, and this was confirmed by SC. Pairs 2, 6, and 7 of *Tt* were the result of the centromeric fusion of pairs 9-20, 14-17, and 24-26 of *Mt*, respectively, and the FISH analysis in *Tt* revealed telomeric signals at the centromeric regions in these chromosomes, supporting this hypothesis. The differences in the number of chromosome arms between *Mt* and *Tt* may be explained by pericentric inversion mechanisms. The analysis of SC confirmed equal size morphology of the sex chromosomes between species; both sex chromosomes were submetacentric with an X chromosome larger than the Y. These results provide a scenario for karyotype evolution in which *Tt* may have evolved from an ancestral karyotype, actually represented by *Mt,* by 3 chromosome fusions and constitute a contribution to the intra- and interspecific phylogenetic relationships in Myrmecophagidae and the superorder Xenarthra.

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III.43

Integrating Morphological, Cytogenetic and Molecular Markers for Investigation of Taxonomic Synonymy in *Astyanax* (Teleostei, Characidae)

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Taxonomy of cryptic species is an obstacle to biological knowledge. The genus *Astyanax* is composed of enigmatic fishes in relation to its taxonomy and a high amount of species, many of them in the process of being described. *A. serratus* is considered endemic to the Iguaçu River, and *A. laticeps* is found in the coastal basins from Uruguay to the Brazilian southeast. The aim of this study was to verify whether *A. serratus* and *A. laticeps* constitute a single species or present enough variability to be distinguished as cryptic species. Integrated analyses of morphology, cytogenetics and molecular genetics were performed. In both species, there is evidence of a pre-jaw with 2 series of teeth, bony hooks on the anal and pelvic fins of males, and a humeral spot with a narrow anteroventral extension. Cytogenetic techniques revealed the same results concerning the diploid number (2n = 50), karyotype formula (4m+24sm+6st+16a), and the fundamental number (FN = 84). The heterochromatin distribution was heterogeneous in both species, with some intrapopulation variations. The nucleolus organizer regions (Ag-NORs) were multiple and in most cases confirmed by FISH using 18S rDNA probes, matching the heterochromatic sites. However, 18S rDNA is found in different chromosome pairs in each species, while the 5S rDNA was located in chromosome pair 19 in both species. The interspecific variations verified by the techniques used are common in *Astyanax* and possibly derived from non-Robertsonian events. The partial sequence analysis of the *COI* gene revealed an interspecific genetic divergence of 0.2%, which is less than the considered threshold of divergence of fish species (2%). These different levels of analysis suggest that *A. serratus* and *A. laticeps* may represent synonymous species. Thus, the geographical distribution of *A. laticeps* may be extended to the Iguaçu River basin, explained by the fauna-sharing hypothesis, caused by tectonic activities of the crystalline shield.

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III.44

Cytogenetic Analysis in Teiidae (Squamata, Lacertilia) Species from the Brazilian Semiarid Region

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Cytogenetic studies in the Teiidae family (Squamata, Lacertilia) have shown that karyotype data are important tools in phylogenetic and systematic studies within this group. In this study, we describe the karyotypes of 2 Teiidae species from the semiarid region of Brazil: *Ameivula ocellifera* and *Salvator merianae*. The karyotypes were analyzed by C and Ag-NOR banding, and in *S. merianae*, we also applied FISH with 18S rDNA, telomeric and microsatellite sequences as probes. In both sexes of *A. ocellifera*, we found identical karyotypes, with 50 telocentric/subtelocentric elements (24 macrochromosomes and 26 microchromosomes). Constitutive heterochromatin was observed in the pericentromeric and telomeric regions of most macrochromosomes. A single NOR was observed in the terminal region of the long arm of chromosome pair 5. These data do not show divergences regarding individuals from other regions of the country discarding any doubt about the taxonomic status of the analyzed individuals, presently denominated *Ameivula* cf. *ocellifera*. Concerning *S. merianae*, we found 2n = 38, identical in both sexes, of which 5 pairs corresponded to biarmed macrochromosomes, and 14 pairs were microchromosomes. Discrete heterochromatic segments were found in the centromeric region of most chromosomes. Clusters of 18/28S rDNA were located in the terminal portion of a single metacentric chromosome pair, corresponding to pair 2. Telomeric probes produced the characteristic signals in the terminal region of chromosomes. In addition, we detected pericentromeric signals in some biarmed chromosomes of *S. merianae*, suggesting the occurrence of fusions during the karyotype diversification of this species. Mi-

crosatellites produced signals uniformly distributed in all chromosomes, in addition to more intense ones in specific telomeric and pericentromeric regions in some chromosome pairs. The comparison with other studies indicates that despite the wide distribution of the *S. merianae* species, the macrostructure organization of the karyotype remains unchanged, showing stability in the diploid number and chromosome morphology of the group.

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III.45

Karyotype and DNA Content Patterns of the Genus *Arachis* in a Phylogenetic Context

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Arachis is a South American genus that includes 82 autogamous and geocarpic species. Most species are diploids with x = 10, 4 are diploids with x = 9, and 5 are tetraploids with x = 10. Based on morphology and cross-compatibility, these species were arranged in 9 sections and different genomes. Classical and molecular cytogenetics revealed large karyotype variability among species and has also been very important for the infrageneric arrangement of the genus. The objective of this research was to make inferences about the direction of changes in different chromosome markers and DNA content during the evolution of the genus. For this purpose, new cytogenetic (FISH with 45S and 5S rDNA and heterochromatic markers) and DNA content (flow cytometry estimated 2C values) data, together with those available, were analyzed in a phylogenetic context based on (available and new data) ITS sequences. This analysis revealed that in the evolutionary history of the genus: (1) there is a general tendency of a genome size (Cx) increase, although independent reductions occurred more than once, (2) reduction of the basic chromosome number (from x = 10 to x = 9) occurred twice, (3) polyploidization based on x = 10 occurred more than once, (4) 'A chromosomes' originated only once, (5) different patterns of DAPI/CMA heterochromatic bands were observed in 2 (out of 3) of the main clades, and (6) the number of 5S rDNA loci is generally 1 pair (exceptions are found in different clades), while the number of 45S rDNA is more variable (1–5 pairs). Most of the chromosome characters analyzed according to the ITS phylogenetic hypothesis for genus *Arachis* did not show a unidirectional evolutionary history.

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III.46

Geographical Patterns of Morphological, Cytotype and cpDNA Haplotype Variation of *Turnera sidoides* L. Complex (Passifloraceae, Turneroideae): Evolutionary and Biogeographical Implications

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Turnera sidoides (x = 7) is an excellent model to study the action of evolutionary processes in the Chaco Domain. This complex of perennial, rhizomatous herbs ranges across southern Bolivia, Paraguay and Brazil, Uruguay and Argentina, reaching 39°S. Five subspecies and 7 morphotypes were recognized in the complex. Additionally, it shows diploid to autooctoploid cytotypes. Here, we analyze the geographical distribution of morphological and genetic (cytotype and cpDNA haplotypes) variation to interpret the current patterns of diversification of the complex. Our survey revealed 2 main centers of species diversification. Most taxa have diploid and polyploid cytotypes. Within the taxa, both exclusive and shared cpDNA haplotypes were found in diploid and polyploid populations. The main concentration of diploids is located in the northwestern center. Their frequency regularly decreases along a north-south gradient up to 30°S, and from higher to lower altitudes. In the eastern center, a few isolated diploid populations of 4 different subspecies were detected. Tetraploids are by far the most widespread and occupy almost the entire species range, whereas the frequency of higher ploidy levels increases to the western and eastern species boundaries, in regions with the wettest and driest regimes, respectively. *T. sidoides* diversification would occur at 2.11 MYBP. The finding of the ancestral haplotype in diploids and polyploids occurring at the higher regions, together with the results of ancestral area reconstruction suggest that during Pleistocene geomorphological and climatic changes, the highlands along the Peripampasic arc and the adjacent lowlands represented putative centers of diversification of the complex. Our comprehensive analysis further yields evidence of the dispersal pathways of the *T. sidoides* taxa that led to the present species boundaries by means of polyploids. The Peripampasic arc would provide suitable conditions for diploids to survive and differentiate in allopatry and the main rivers as dispersal pathways toward the Chaco-Pampean plain.

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III.47

Chromosomal Rearrangements in Phyllostomid Bats and Their Contribution to Diversification and Adaptation

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The neotropical bat family Phyllostomidae is a remarkable assemblage to study the role of chromosomal changes in adaptation and generation of biodiversity in mammals. Herein, the chromosomal homologies of representatives of 9 phyllostomid subfamilies were investigated using the technique of chromosome painting. Using 3 outgroups and the set of chromosome probes of *Macrotus californicus*, we were able to infer the ancestral karyotype for the family and trace chromosomal rearrangements throughout their evolutionary history. The minimal number of chromosome rearrangements required to form the ancestral karyotypes of 9 of the 11 subfamilies of Phyllostomidae was determined, and the rates of chromosomal evolution in all lineages were calculated using a dated molecular phylogeny as reference for time of divergence and relationship between taxa. Rates of chromosomal evolution were not homogeneous across phyllostomid lineages and one of the highest rates of fixation of chromosomal rearrangements was found for a Phyllostominae species, *Tonatia saurophila*. We discuss the trends of breakage and reshuffling of chromosomal blocks in the karyotype evolution of phyllostomids, reevaluate the concept of karyotype megaevolution and propose that chromosomal rearrangements were one of the promoters of the creation of the high diversity in this group of bats. Finally, the transposable element annotation of 2 phyllostomid whole-sequenced genomes *(M. californicus* and *Desmodus rotundus)* and mapping of LINE1 sequences provided insights on the factors that have influenced differential rates of chromosomal evolution in the group.

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III.48

Identification of Transposable Elements in *Scinax centralis* (Anura; Hylidae) Genome: Searching for New Cytogenetic Markers

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The anuran genus *Scinax* is composed of 115 species, although little is known about their cytogenetic features. Repetitive sequences, such as transposable elements (TEs), compose a great part of the genome and have been isolated to increase the knowledge of chromosome structures and chromosome evolution. This study aimed to detect and characterize TEs from genomic sequences of *S. centralis,* an endemic Brazilian Cerrado species, as cytogenetic markers for future studies. We used data from next-generation sequencing, platform Illumina MiSeq. Nextera library was prepared using a DNA sample obtained from 1 specimen collected at Silvânia, GO. Library quality and quantity was tested by Agilent Bioanalyzer and by QT-PCR; finally, it was paired-end sequenced using MiSeq Reagent v3. The initial quality control of the DNA sequences was performed on FastQC software. We used Trimmomatic to remove sequences with low-quality bases (Phred Q<30). A de novo assembly was obtained using dipSPAdes. This draft genome assembly was used to identify repetitive sequences using RepeatMasker, having *Xenopus (Silurana) tropicalis* as a database. In order to isolate the TEs, primers were designed using Primer3. The sequences were assembled in 4,890 scaffolds with N50 = 394. TEs comprised 11.38% of the analyzed sequences, corresponding to 225,237 bp. LTR retrotransposons (Copia, Gypsy, DIRS, ERV1, and Pao) were the most abundant retroelements followed by LINEs (CR1, L1, L2, Penelope, and Rex-Babar) and SINEs. DNA transposons were also found, including the superfamilies hAT, Kolobok-T2, Maverick, PiggyBac, Tc1-Mariner, and Tc2-Mariner. In the genome of *S. centralis*, L1 elements were the most abundant ones. We designed 17 primer pairs to isolate Tc1-Mariner, Tc2-Mariner, and Rex-Babar. Those elements were already characterized for some anurans and detected in specific chromosomes in frogs and also in fishes. This study represents the first step towards a genomic and cytogenetic characterization of *S. centralis*.

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III.49

Localization of Aphidicolin-Induced Fragile Sites in Brown Brocket Deer (*Mazama gouazoubira*, Artiodactyla; Cervidae)

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Mazama gouazoubira is a deer that shows chromosomal polymorphism due to B chromosomes and Robertsonian translocations because of fragile sites (FS) that generate chromosomal instability and rearrangements. This finding could be related to chromosomal differentiation in the *Mazama* genus. Thus, we evaluated the presence and localization of FS in *M. gouazoubira* by FS inductions in 6 animals using aphidicolin (0.1 and 0.2 μM) in fibroblast cultures. Conventional staining was performed to examine the mitotic index (MI) and FS (gaps and breaks), while only the preparations treated with aphidicolin were subjected to G-banding for the localization of the FS. MI showed no cytotoxicity with the concentrations of aphidicolin used. Out of 1,200 analyzed metaphases by conventional staining, FS were observed in 47.75%. A total of 1,673 FS were detected. The FS generated chromatid gaps (35.98%), chromosomal gaps (29.94%), chromatid breaks (16.14%), and chromosomal breaks (17.94%). Six hundred metaphases were G-banded identifying 214 FS located in the X chromosome (20.56%), and in autosomes pairs 5 (18.23%), 14 (15.89%),

4 (12.15%), 1 (9.82%), 34 (7%), 16 (5.61%), 6 (3.27%), 8 (2.34%), 17 (1.87%), 10 (1.4%), 18 (1.4%), and 13 (0.47%). These results allowed us to build an FS map of *M. gouazoubira*, which may be a useful tool in inferring the FS related to the breakpoints that generate intraspecific chromosomal polymorphism and the chromosomal rearrangements responsible for the karyotype evolution and the process of speciation that occurred between the species of the *Mazama* genus.

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III.50

Chromosome Evolution in Brazilian Scorpions of the Genus *Rhopalurus* and Related Genera (Scorpiones: Buthidae)

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Rhopalurus is the third most diverse genus from the Brazilian scorpion fauna, with 9 species taxonomically described. Cytogenetically, only 2 species were examined, which showed the diploid number of 2n = 28. The aim of this work is to understand the processes related to chromosome differentiation in the Brazilian species *Rhopalurus, Physoctonus*, and *Troglorhopalurus*. The chromosomes were conventionally stained using 3% Giemsa solution, silver-impregnated to detect the nucleolus organizer regions (NORs), and submitted to FISH to localize the 28S rDNA and $(TTAGGG)_n$ telomeric sites. The analysis of mitotic and meiotic cells in all species revealed holocentric chromosomes and the following diploid numbers: 2n = 28 in *R. agamemnon, R. pintoi*, and *R. rochai*; 2n = 26 in *R. debilis, Rhopalurus* sp.n.1, and *Rhopalurus* sp.n.2; 2n = 25 in *R. guanambiensis*; 2n = 22 in *R. crassicauda*; 2n = 20 in *T. translucidus*, and 2n = 20–22 in *R. lacrau.* These results reveal that a diploid number higher than 2n = 20 is a common feature among the species of this group, differing from other Brazilian Buthidae genera, such as *Ananteris*, *Isometrus*, and *Tityus*. Prophase I spermatocytes exhibited the synaptic and achiasmatic chromosome behavior and formation of multivalent associations in *R. crassicauda, R. guanambiensis, R. pintoi*, and *Rhopalurus* sp.n.2. These multivalent associations probably were a consequence of reciprocal translocations or fission/fusion chromosomal rearrangements. In prophase I cells of 1 female of *T. translucidus,* we verified bivalents with similar behavior to that observed in males of scorpions, indicating the absence of genetic recombination in meiosis. Despite the intraspecific variation of the diploid number, most species analyzed in this study presented a conserved pattern of 2 NORs and 28S rDNA cistrons, localized in the terminal region of the chromosomes. The presence of $(TTAGGG)_n$ telomeric repeats was observed in the 10 species studied, occurring only in terminal regions of the chromosomes, including the specimens carrying the heterozygous chromosome rearrangements.

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III.51

Exploring the Satellite DNAs from Squirrel Monkeys (*Saimiri* genus)

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New World monkeys (NWM; Platyrrhini) represent a diverse group of neotropical primates with several important model species used in medical, genomics, and evolutionary studies. As for most eukaryotes, the repetitive fraction of NWM genomes is mostly unexplored. Herein, we took advantage of the genome sequence of the squirrel monkey *Saimiri boliviensis* to explore the satellite DNAs (satDNAs) of this genus. After clustering *S. boliviensis*' sequencing reads by similarity, we identified 2 abundant satDNAs. The first is homologous to the alpha satDNA known to be part of centromeres in primates. In the majority of NWMs, the monomer sequence is ~340 bp, a duplication-derivative of the 170-bp sequence found in Old World primates (OWPs). PCR and genomic analysis confirmed the 340-bp size in *S. boliviensis*, *S. vanzolinii*, and *S. sciureus*. Based on genome analysis, we estimated that the alpha satDNA represents nearly 1% of the *S. boliviensis* genome. FISH in *S. vanzolinii* confirmed the centromeric location of the alpha satDNA in all chromosomes, whereas in *S. sciureus* the repeat appears to be absent in chromosome 6. Furthermore, signal intensity varies among chromosomes in both species. This could reflect the presence of chromosome-specific variants as described for the 170-bp repeat in OWPs. In addition to the alpha satDNA, we identified a large satDNA with monomers of ~1,500 bp that comprises 2.2% of the genome and displays similarity with the CapA satDNA first described in *Cebus apella*. Chromosome location of both satDNAs will be analyzed in other species of *Saimiri* and may provide valuable insights into chromosome evolution and help to clarify the still uncertain *Saimiri* taxonomy.

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III.52

Cytological Markers in the Karyotype of *Tropidurus hispidus* (Spix, 1825) (Squamata,Tropiduridae) Analyzed by Conventional and Molecular Cytogenetic Techniques

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Studies involving chromosome markers in natural populations provide valuable information about the evolutionary processes and structural changes that drove the karyotype evolution of the species. In this study, we applied different cytogenetic markers to describe the karyotype of *Tropidurus hispidus* (Squamata, Tropiduridae) from the Brazilian semiarid region. This species comprises the most representative lizard belonging to the neotropical family Tropiduridae. We examined this species using conventional (Giemsa-staining, Ag-impregnation, C-banding) and molecular (chromosomal mapping of ribosomal, telomeric, and microsatellite repeats) cytogenetic approaches. *T. hispidus* showed a diploid number of 2n = 36, composed of 6 pairs of biarmed chromosomes and 12 pairs of microchromosomes. Conspicuous heterochromatic blocks were evidenced in both telomeric and pericentromeric regions of 6 pairs of macrochromosomes. The NORs were located in the terminal region of metacentric pair 2. This region was coincident with hybridization signals using the 18S rDNA probe. Positive hybridization signals with telomeric probes showed signals in telomeric regions of all chromosomes, as well as in the pericentromeric portion of 4 metacentric and 4 microchromosome pairs, indicating vestiges of chromosome rearrangements in the history of the group. Microsatellite sequences $(CA)_n$, $(CAA)_n$, $(CAC)_n$, $(GA)_n$, $(GAA)_n$, and $(GAG)_n$ showed distinct distribution patterns: while $(CA)_n$, $(CAC)_n$, and $(GAG)_n$ produced scattered signals along the chromosomes, specific bright signals were produced by $(GA)_n$, $(CAA)_n$, and $(GAA)_n$ sequences. Studies with species belonging to the Tropiduridae family have shown a conserved diploid number and few differences regarding the presence of secondary constrictions and the number of NORs. The chromosome macrostructure of *T. hispidus* remains unchanged and the individuals analyzed showed similarities with previous cytogenetic studies conducted within the group.

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III.53

Evolutional Inference of *Omophoita communis* (Coleoptera, Alticinae): Cytogenetic, Morphological and Molecular Differentiation

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The genus *Omophoita* is the most cytogenetically studied among the Ordionychina subtribe. The analyzed individuals have a uniform karyotype of 2n = 22 and a few variations of the diploid numbers and different sex chromosome systems. In the Alticinae subfamily, there are identification problems in several genera and taxonomic issues, such as the Alticinae/Galerucinae monophyly. The aim of this study was to analyze 2 *Omophoita communis* populations using conventional cytogenetic, morphological, and molecular analyses. The specimens were sampled in Ponta Grossa, Paraná and in Nonoai, Rio Grande do Sul. The cytogenetic data showed a difference in diploid number and chromosome morphology, demonstrating a large variation in population. The individuals were separated into 2 cytotypes. Cytotype I showed a karyotype of 2n = 22, a karyotype formula 14m+4sm+4A, and sex chromosomes with extremely large size and asynaptic in meiotic cells. Cytotype II showed 2n = 12, a karyotype formula 7m+4sm+1A with Xy chromosomes. These are large when compared to other Coleoptera species, although smaller than cytotype I. This variation was seen for the first time in the *Omophoita* genus. The comparative analysis of chromosomes showed that the mean size of cytotype II chromosomes is 2.5× cytotype I; thereby, we can infer a derivation process based on chromosomal rearrangement by fusion. Furthermore, the aedeagus morphology has shown that the genitals differ in their morphology, indicating a possible differentiation pattern of the 2 species. Phylogenetic reconstruction using the cytochrome oxidase I mitochondrial gene also shows that cytotypes differ in their sequences. Concerning the cytotypes, there are 2 cryptic species; one possible explanation could be the similar phenotypic characteristics as the occurrence of mimicry.

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III.54

Karyotype Differentiation in Species of the *Omophoita* Genus (Coleoptera, Chrysomelidae, Alticinae): 5S rDNA Mapping and Dispersion

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Most of the Oedionychina species that have been cytogenetically analyzed have a uniform karyotype of 2n = 22 = 20+X+y with giant and asynaptic sex chromosomes. The analysis of nucleolus organizer regions (NORs) in Coleoptera has been performed mainly by silver impregnation, and they are mostly associated with an autosomal pair. However, mapping of this gene by FISH has been performed in only a few species of Coleoptera. The aim of this study was to cytogenetically characterize 4 species of *Omophoita* by mapping ribosomal genes in an attempt to establish the karyotype and chromosomal differentiation strategies among the species. Amplification of the 5S rDNA generated a fragment of ~94 bp and showed 84–89% of similarity with the 5S rDNA of other species of insects. Chromosomal mapping of 5S rDNA showed the presence of 1 autosomal pair in *Omophoita octoguttata* and *O. personata*. Regarding *O. magniguttis*, it was possible to observe 2 pairs carrying the 5S cistron, and in *O. sexnotata*, all the autosomes had this cistron. This pattern of dispersion was described for the first time in Coleoptera. Thus, each chromosome was microdissected separately and each one was amplified with the whole genome amplification kit (WGA4 – Sigma). The 5S fragments obtained from each chromosome of *O. sexnotata* were sequenced, resulting in similar sequences of 5S rRNA, EnSpm transposable element, retropseudogene of 5S, and microsatellite. The sequence analysis using the dot plot method indicates the presence of repetitive DNA. The performance of double FISH and fiber FISH showed the colocalization of these clusters, and that the clusters were interspaced with a variable amount of classes of rDNA.

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III.55

Mitotic Cytogenetic Analysis of *Zosis geniculata* (Araneae, Deinopoidea, Uloboridae)

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The family Uloboridae comprises 18 genera and 279 species distributed worldwide with the greatest diversity in tropical and subtropical regions. Literature review has shown that only 8 Uloboridae species belonging to 3 genera were subject to cytogenetic analysis. These data revealed that cytogenetically analyzed Uloboridae species possess diploid numbers ranging from 2n = 10 to 2n = 22, basically acrotelocentric or telocentric ones, and the occurrence of male sex determination chromosome systems of types XO, X_1X_2, and $X_1X_2X_3$. The aim of this study is to report the first mitotic cytogenetic analysis of *Zosis geniculata*. Male and female specimens as well as egg cases were collected in several states of Brazil (Alagoas, Bahia, Espírito Santo, Minas Gerais, Pernambuco, Rio de Janeiro, São Paulo, and Sergipe). Gonads or embryos were submitted to colchicine treatment, hypotonic solution, and Carnoy's I fixative. Pieces of gonads or whole embryos were macerated in glacial acetic acid (45%) on slides, spread over the slide surface and finally dried on a hot plate (50–55°C). Chromosome preparations were routinely stained using Giemsa. All analyzed spermatogonial, oogonial, and embryo metaphase cells of *Z. geniculata* showed female and male diploid numbers of chromosomes, respectively, with 2n = 9 and 2 = 10, which suggest the occurrence of a XO sex chromosome determination system. The chromosome size of the autosomal set shows little difference, and all 8 chromosomes are metacentric. The X chromosome exhibits a submetacentric morphology and slight difference in size, when compared with those of the autosomal set. Based on the available literature data on chromosome morphology, only Uloboridae species with a diploid number ranging from 2n = 17 to 2n = 22 have an acrotelocentric or telocentric chromosome morphology, suggesting that the reduction of the diploid chromosome number in *Z. geniculata* probably occurred due to centric fusions.

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III.56

Cytological Studies in Species and Interspecific Hybrids of *Passiflora* Native to Argentina

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Passiflora has more than 500 species distributed worldwide. There are 19 natives from Argentina belonging to 4 subgenera with 3 basic numbers: *Passiflora* (11 species) with x = 9, *Tacsonioides* (1) with x = 9, *Decaloba* (5) with x = 6, and *Dysosmia* (2) with x = 10. The aim of this project is to perform a cytological study of Argentinian species of *Passiflora* contributing to a breeding program to obtain ornamental cold tolerant phenotypes. Interspecific crossings were carried out among 9 species. The meiotic behavior of 8 hybrids among *P. alata*, *P. caerulea*, *P. amethystina*, and *P. elegans* showed variable chromosome pairing with evidence of cryptic structural hybridity. *P. amethystina* and *P. caerulea* were the species showing the highest fertility in their hybrids. The genome size of 14 species and 37 hybrids was estimated by flow cytometry and

tested by ANOVA. In the species analyzed, the C value varied from 0.54 to 2.52 pg, and significant differences were detected among the species. The hybrids showed gains and losses of DNA as a result of the genomic shock from interspecific hybridization. The karyotypes of 7 species were analyzed using DAPI/CMA banding and FISH with 18S rDNA sequence as a probe. Hybridization signals colocated with $DAPI^-/CMA^+$ regions. The number of rDNA sites varied from 1 pair in species with x = 6, to 3 pairs in *P. amethystina* and *P. caerulea* with x = 9. The cytogeography of Argentinian species was modeled with Maxent software, analyzing 10,309 collection sites of herbarium specimens worldwide. Species with x = 6 and x = 9 were located only in America, while the species with x = 10 were also found in Africa and Oceania. The progress of this work will contribute to elucidate the several hypotheses about the basal number of the genus, the mechanisms involved in chromosomal evolution, and the relationships among the species of *Passiflora*.

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IV. Mitosis and Meiosis

Invited Talks

IV.1
Dynamic Interaction of Meiotic Telomeres with the Nuclear Envelope

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During meiotic prophase I, homologous chromosomes undergo synapsis and recombination. The nuclear envelope plays a crucial role during these processes. Early at prophase, telomeres attach to the nuclear envelope and actively move at the plane of the inner nuclear membrane. These movements are essential for proper synapsis and recombination. Attachment and movement of telomeres require a reorganization of the meiotic nuclear envelope. According to current models, in most species telomere movement involves microtubules in the cytoplasm. The forces generated there are transduced into the nucleus via the LINC complexes of the nuclear envelope. Despite of important progress in the past, little is known about the structure of meiotic telomere anchoring sites. This is due to the complexity of the structure that can hardly be resolved by light microscopy or standard electron microscopy. To overcome these limitations, we are investigating the organization of mouse meiotic telomeres by super-resolution imaging and electron microscope tomography. Novel structural and quantitative data will be presented and discussed in detail.

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IV.2
Maize Abnormal Chromosome 10: Distribution, Molecular Makeup, and the 'Neocentromere Gene'

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The maize abnormal chromosome 10 (Ab10) haplotype is a classic meiotic drive system that activates neocentromeres at heterochromatic regions called knobs, preferentially segregating them to female progeny. While this phenomenon and others like it are called meiotic drive, Ab10 remains the only example where meiotic chromosome behavior is directly affected. Here, we identify a family of kinesin-14A genes on Ab10, called the Kinesin driver *(Kindr)* complex, that are necessary for this phenotype. At least 9 *Kindr* genes are clustered in a ~1-Mb region of the distal tip of the chromosome. We show that 4 mutants of meiotic drive have defects in the structure or expression of the *Kindr* gene family and lack neocentromere activity at the major knob repeat. Two mutants are gene-family-wide epialleles that involve changes in *Kindr* DNA methylation primarily in the CHG context, in one case abolishing *Kindr* expression and the other reducing it 10-fold. Sequence comparisons show that KINDR evolved from a minus-end directed kinesin that regulates spindle pole formation; however, it is truncated and modified at the cargo-binding domain. We speculate that KINDR binds to chromatin at its N-terminal end and to microtubules by its C-terminal motor domain, thereby driving knobs to spindle poles and skewing meiotic transmission.

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IV.3
Control of Mitotic Chromosome Segregation by Cooperating Protein Kinases

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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. Essential for chromosome segregation is the spindle assembly checkpoint (SAC), a surveillance mechanism that monitors the attachment of all mitotic chromosomes to the microtubule-based spindle apparatus. High up in the hierarchy of SAC signaling is the protein kinase monopolar spindle 1 (Mps1), which controls the recruitment of several evolu-

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tionarily conserved SAC components to unattached or misaligned kinetochores. Recently, we and others have discovered that the consensus phosphorylation motif of Mps1 is very similar to that of polo-like kinase 1 (Plk1). This surprising observation raised the tantalizing possibility that human Plk1 cooperates with Mps1 in SAC signaling. Our recent work indicates that this is indeed the case [von Schubert et al., 2015, Cell Reports 12:66–78]. Specifically, we demonstrate that cooperation between Plk1 and Mps1 involves the phosphorylation of at least 2 kinetochore-associated common substrates. As a result, Plk1 activity enhances Mps1 catalytic activity as well as the recruitment of SAC components to kinetochores. We conclude that Plk1 strengthens the robustness of SAC establishment at the onset of mitosis and supports SAC maintenance during prolonged mitotic arrest.

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IV.4

Epigenetic Regulation on Centromere Propagation and Establishment in *Caenorhabditis elegans*

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The centromere is a specialized chromosomal domain that directs chromosome segregation. Centromeric DNA sequences and sizes vary among organisms, but all active centromeres contain a histone H3 variant, CENP-A, forming a foundation for kinetochore assembly. To epigenetically maintain centromere identity through cell cycles, CENP-A diluted during DNA replication is replenished. In *Caenorhabditis elegans*, licensing factor M18BP1^{KNL-2} is known to recruit CENP-A^{HCP-3} to holocentromeres. We show that RbAp46/48^{LIN-53}, a conserved histone chaperone, is also required for CENP-A^{HCP-3} localization. Indeed, RbAp46/48^{LIN-53} and CENP-A^{HCP-3} localizations are interdependent. RbAp46/48^{LIN-53} localizes to the centromere during metaphase in a CENP-A^{HCP-3}- and M18BP1^{KNL-2}-dependent manner, suggesting CENP-A^{HCP-3} loading may occur before anaphase. RbAp46/48^{LIN-53} does not function at the centromere by histone acetylation, H3K27 trimethylation, or its known chromatin-modifying complexes. RbAp46/48^{LIN-53} may function independently to escort CENP-A^{HCP-3} for holocentromere assembly but is dispensable for other kinetochore protein recruitment. Nonetheless, depletion of RbAp46/48^{LIN-53} leads to anaphase bridges and chromosome missegregation. This study unravels the holocentromere assembly hierarchy and its conservation with monocentromeres. The centromere position is usually maintained through cell cycles and generations. However, neocentromeres can form at ectopic locations in cancer cells or after chromosomal rearrangements. De novo centromeres can also form in artificial chromosomes (ACs) at low frequency, following the introduction of centromeric DNA into human cells. In *C. elegans*, DNA injected into the germline form ACs, which establish de novo centromeres more rapidly and frequently than human ACs. We have developed a real-time, in vivo model in *C. elegans* to monitor AC segregation and study the mechanism of centromere establishment. We found that heterochromatin hinders centromere formation. In consistence, open chromatin marks H3K9ac and H4ac are enriched on new ACs. Tethering the histone deacetylase HDA-1 to ACs specifically reduced AC histone acetylations and segregation frequency, indicating that histone acetylations are necessary for efficient de novo centromere establishment.

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Posters and Short Talks

IV.5

Meiotic Chromosomes in *Astyanax xavante* (Characiformes: Characidae)

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Astyanax xavante is an endemic fish of the Avoadeira Stream, located in Parque Estadual da Serra Azul (PESA), middle Araguaia River basin. The species shows 2n = 50 and NF = 92, with an 8m+16sm+18st+8a formula. Multiple nucleolus organizer regions (NORs) were detected by silver staining and hybridization of 18S rDNA, while the 5S rDNA probe only showed a signal in chromosome 6. The heterochromatin distribution is mostly telomeric, with exception of pair 22, which has a large heterochromatic block and is not found in other *Astyanax* species. There is no information about meiotic chromosomal behavior in this species. So, this study aimed to evaluate the meiotic pairing in *A. xavante*. For this purpose, male testes were processed to obtain meiotic chromosomal suspensions, and the kidneys were used to confirm the diploid number by mitotic analysis. Conventional Giemsa staining, C-banding, and silver staining were performed. We observed cells in different stages: leptotene/zygotene, pachytene, metaphase I and II, interphase nuclei, and spermatogonial metaphases. A large heterochromatic block was observed in most of the phases, and probably this corresponds to chromosome pair 22, which has a large block besides telomeric heterochromatin. The number of Ag-NOR sites varied according to the analyzed phase. The cells in spermatogonial stage showed 50 chromosomes. In pachytene and metaphase I, cells were found with 25 bivalents, while metaphase II showed 25 chromosomes, confirming the chromosome number of the species.

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IV.6

BUB1 and SURVIVIN Proteins Are Not Degraded after a Prolonged Mitosis in HCT116 Cells and Accumulate in the Nucleus

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Spindle poisons activate the spindle assembly checkpoint and prevent mitotic exit until cells die or override the arrest. BCL2 proteins have been associated to regulation of cell death after a prolonged mitosis, but less is known about the cells that survive the arrest. In a normal mitosis, proteins such as CYCLIN B, SECURIN, BUB1, and SURVIVIN are degraded in order to allow mitotic exit and keep them at low levels in the next interphase. In contrast, exit from a prolonged mitosis depends only on degradation of CYCLIN B, and it is not known if other proteins decrease or remain at high levels. Here, we analyzed levels and localization of the mitotic proteins BUB1 and SURVIVIN in cells that escaped from a paclitaxel-mediated prolonged mitosis. We compared cells with a short arrest (HCT116) with cells that spent more time in mitosis (HT29) after paclitaxel treatment. BUB1 and SURVIVIN are not degraded and keep a nuclear localization in HCT116 cells after a mitotic arrest. Besides, BUB1 was observed in nuclear foci with no colocalization with centromere proteins. In contrast, levels of BUB1 and SURVIVIN in HT29 cells decrease during the arrest and were not present in cells that reached the next interphase. We found a morphological heterogeneity in HCT116 cells that escaped from the arrest. Time-lapse imaging showed that this heterogeneity was due to the way cells exit mitosis in a cytokinesis-like mechanism. Thus, our results show that BUB1 and SURVIVIN can be maintained at high levels after a mitotic arrest, which may promote resistance to cell death. Besides, exit from mitosis and organization of cytokinesis-like mechanism may underlie morphological heterogeneity.

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IV.7

Study of the Meiotic Behavior of the Highly Polymorphic Karyotypes of a Population of *Rineloricaria* aff. *langei* (Siluriformes, Loricariidae) from the Basin of the Iguaçu River in Paraná, Brazil

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Considerable numerical and/or structural polymorphisms were found in *Rineloricaria* species, such as *R. latirostris*, *R. lima*, and *R. lanceolate*, which indicate the occurrence of Robertsonian fusions. A similar situation has been found in specimens of *Rineloricaria* aff. *langei*, collected from the Iguaçu River, located in União da Vitória (Paraná, Brazil), in which karyotypes showed 2n = 64–68, with different formulae, resulting in a total of 9 distinct karyotypes. In specimens with 2n = 68 (6m+4st+58a), 2n = 67 (3m+6st+58a; 2m+7st+58a), and 2n = 66 (3m+5st+58a; 2m+6st+58a), the number of acrocentric chromosomes was maintained, even when the diploid number was reduced. However, in karyotypes with 2n = 65 (6m+6st+53a; 6m+3st+54a; 4m+6st+55a) and 2n = 64 (3m+6st+55a), the number of acrocentric chromosomes varied. This suggests a reduction in the diploid number due to Robertsonian fusions as well as other types of rearrangement such as inversions and translocations, which also contribute to the polymorphisms in this species. FISH with a telomeric DNA probe revealed the presence of interstitial telomeric sites (ITS) in 4 karyotypes. All the karyotypes shared the same nucleolus organizer pair (first pair of subtelocentric chromosomes), as indicated by FISH using the 18S rDNA probe. The 5S rDNA sites are distributed in 2 chromosome pairs in most of the karyotypes, in varying positions. Meiotic analyses in individuals with 65 and 67 chromosomes showed bivalent (in most cases) and univalent chromosomes, and in 1 case, a possible multivalent chromosome. An atypical configuration was observed in the gonads of an individual with 2n = 67 (2m+7st+58a) during metaphase I, where an association was observed between 2 nonhomologous chromosomes, a small acrocentric and a subtelocentric with subterminal chiasmata. This partial homology provides evidence of chromosomal rearrangements, which contributed to the observed polymorphisms. The karyotype variants observed in the present study may have been derived from cross-mating between members of this population, reflecting the complex karyotype evolution of this species.

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IV.8

Structural Variations of the Unpaired Axes from Sex Chromosomes in Different Mammalian Species

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The X and Y chromosomes behave in a special way during the first meiotic prophase in mammalian spermatocytes. While the autosomes fully synapse by the formation of synaptonemal complexes, the heteromorphic X and Y chromosomes synapse only partially and form the XY body [Solari, 1974] (reference to be obtained by the author). In mammals, the synaptonemal complex is a highly conserved protein structure and is essential for synapsis, maintenance of integrity of the chromosome cores, and meiotic recombination. The aim of the present study is to analyze the structural and functional variations of the chromosomal axes of the XY pair in mammals. Testicular tissues from adult males of the different species of mammals (*Cavia porcellus, Galea musteloides, Felis catus, Canis familiaris,* among others) were analyzed by optical/electron microscopy and immunofluorescence of meiotic proteins involved in synapsis (SYCP3, SYCP1, and SYCE3), sister-chromatid cohesion (SMC3), meiotic recombination (MLH1), and chromatin silencing (BRCA1, γ-H2AX, 3meH3K27, and RNA pol2). The comparison of the X and Y chromosome axes of the mentioned mammals shows strikingly large, species-specific differentiations, which consist of thickenings, splittings, branchings, pearl necklace-like formations and loops. The unpaired axes of the XY pair undergo stepwise changes along pachytene substages. The transcriptional silencing and chromatin remodeling of the XY body are the basic and conserved mechanisms among mammals; however, variations in the structural components of the synaptonemal complex and unpaired axes were observed in a significant variety of mammals, from eutherians to metatherians. A deeper analysis of these variations and their functional aspects may give new prospects for research on the molecular structure of the components of the synaptonemal complex.

G. De Luca and M.F. Fernández contributed equally to this work.

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IV.9

Divergent Crossover Patterns among Galloanserae in spite of Conserved Karyoptype Structure

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It is known that the pattern of crossover (CO) distribution is affected by karyotypic features such as chromosome size, centromere position, and heterochromatic content. Birds are comparatively less variable than mammals in this regard, so it could be predicted that their recombination landscapes will be less variable as well. In order to test this prediction, we investigated the CO rates and distribution in 5 species of domestic Galloanserae that show conserved karyotype structure and genome sizes. To this end, we used immunolocalization of the mismatch-repair protein MLH1 that labels CO sites along the synaptonemal complexes during pachytene. We found that the total genetic maps are in the range of 2,655 to 3,105 cM, and in most cases, MLH1 foci on macrobivalents show a multimodal profile along chromosome arms. A departure from this general feature was found in the helmeted guinea fowl *(Numida meleagris)*, a species that diverged from the chicken about 30 MYA. In this species, the genetic map spans only 2,220 cM and MLH1 foci are strongly localized towards opposite ends of the bivalents. Furthermore, the global CO rate in the guinea fowl (1.8 cM/Mb) is very similar to that of certain passerines, but lower compared to other species of Galliformes investigated so far. The present data suggest that broad-scale recombination patterns can be significantly modified in the absence of drastic karyotype rearrangements, and that, in contrast to mammals, CO rates might not show a strong phylogenetic signal in birds.

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IV.10

Loss of the Line-Like Holocentromere Structure during Inverted Meiosis in a Holocentric Plant

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The centromeres are the regions responsible for the correct segregation of chromosomes during mitosis and meiosis. Holocentric chromosomes, which are characterized by multiple centromere units along each chromatid, have particular adaptations to ensure regular disjunction during meiosis. Here, we show that holocentromeres undergo a differential reorganization between mitosis and meiosis in the Cyperaceae *Rhynchospora pubera* by in situ detection of CENH3, CENP-C, and tubulin as well as centromeric repeats. In contrast to mitotic chromosomes, which show a line-like holocentromere within a longitudinal groove, during meiotic metaphase I, multiple clusters of centromere units (cluster-holocentromeres) in interaction with spindle fibers accumulate along the poleward surface of equatorially dividing bivalents. During metaphase II onset, cluster-centromeres are visualized as single or double clusters in the mid-region of each chromatid. In contrast to meiosis I, a differential orientation of chromatids and spindle interaction with cluster-centromeres was observed, resulting in an inverse order of chromatid segregation events (inverted meiosis). A line-like holocentromere organization is restored after meiosis at first pollen mitosis. Our findings demonstrate the plasticity of centromeres and provide the characterization of a newly identified dynamic centromere organization during meiosis among holocentric organisms.

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IV.11

High Variability of Multivalent Associations in Holocentric Chromosomes of *Tityus (Archaeotityus)* Scorpions

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The subgenus *Tityus (Archaeotityus)* comprises sedentary scorpions that live in the upper layers of the leaf litter. Five species *(Tityus clathratus*, *T. maranhensis*, *T. paraguayensis*, *T. pusillus*, and *T. silvestris)* were examined regarding the diploid number, chromosome behavior during meiosis, and repetitive DNA sequences. Intraspecific variation in the diploid number was observed in 2 species, and the presence of multivalent associations formed by a variable number of elements was visualized within and among populations of 4 species. Spermatogonial metaphase cells showed: 2n = 16 in *T. paraguayensis* and *T. silvestris*, 2n = 20 in *T. maranhensis*, and 2n = 19 and 2n = 20 in *T. clathratus* and *T. pusillus*. Early pachytene cells of *T. clathratus* revealed unsynapsed chromosomal segments, which were continuous with completely paired regions. Total synapsis occurred in late pachytene cells. In postpachytene nuclei, a high variability in the number of bivalents and/or elements involved in multivalent associations was verified in *T. clathratus*, which showed 11 distinct meiotic configurations, and *T. pusillus* that exhibited polyploid cells. Metaphase II nuclei indicated that all chromosomes possessed regular disjunction and balanced segregation. Metaphase cells submitted to silver impregnation exhibited 2 NORs localized in terminal/subterminal chromosome regions. FISH with a 28S rDNA probe in meiotic cells revealed clusters in the terminal region of 1 bivalent-like element in *T. maranhensis*, *T. paraguayensis*, and *T. pusillus* and in 2 chromosomes involved in multivalent associations in *T. silvestris*. FISH with a $(TTAGGG)_n$ probe exhibited typical telomeric signals in most of the elements or at least in one chromosome end. The variability of the diploid number such as observed in *T. clathratus* and *T. pusillus* was known only for *T. bahiensis*. Additionally, multivalent associations and the presence of polyploidy in meiosis I seem to be a common feature in *Archaeotityus* species.

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IV.12

Variability in the Amount of Homoeologous Pairing among F1 Hybrids Depends on the Genotype Constitution of the Parental Species

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Genes involved in the exclusive pairing of homologous chromosomes have been described in several polyploid species, but little is known about the activity of these genes in diploids (which have only 1 dose of each homoeologous genome). Analysis of the meiotic behavior of *Glandularia* species and natural and artificial hybrids and polyploids suggests that in allopolyploids, where homoeologous genomes are present in 2 doses, regulator genes prevent homoeologous pairing. The different meiotic phenotypes in diploid F1 hybrids between *G. pulchella* and *G. incisa* strongly suggest that this pairing regulator gene possesses an incomplete penetrance when homoeologous genomes are in only 1 dose. Moreover, the meiotic analysis of natural and artificial F1 hybrids suggests that the genetic constitution of parental species influences the activity of pairing regulator genes and is mainly responsible for the variability of the amount of homoeologous pairing observed in diploid hybrids. We found evidences of spatial separation of parental genomes in the hybrids, suggesting that PRG genes would affect the distance between homologous and/or homoeologous chromosomes, and that this distance would depend on the genotype constitution of parental species. Based on phylogenetic, cytological and morphological studies, we suggest that the Ph-like genes of *Glandularia* would have originated in SA diploid species. The cytogenetic characteristics of this genus make it an important model to analyze and explore the activity of pairing regulator genes at different ploidy levels in greater depth.

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IV.13

Chromosome Bearing rDNA Loci Involved in Meiotic Chromosome Abnormalities

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Lathyrus macrostachys (2n = 14) is a South American endemic species. An initial screening of some populations of this species showed many chromosome irregularities during meiosis in spite of the reported high stability of its karyotype. Chromosomes located outside of the metaphase plate, bridges and multiple spindles are among the most frequent irregularities observed that led to the formation of aneuploid microspores. On this basis, a population study of the meiotic behavior was conducted and FISH with 45S and 5S rDNA was used in pollen mother cells (PMC) in order to shed light on the mechanisms that cause the irregularities and the chromosomes involved in aberrant meiotic behavior. The study was conducted in 3 populations from NE Argentina. The results obtained here revealed that those chromosomes bearing either 45S or 5S rDNA loci were involved in the chromosome abnormalities observed in higher frequency than expected by chance. The 45S rDNA loci were mainly involved in the formation of bridges, while the 5S rDNA loci were associated to the chromosomes located outside of the metaphase plate, in first and second divisions. These chromosomes were further correlated with the occurrence of multiple spindles in the second division. Silver staining revealed that all additional microcells observed in tetrad stage and 88.19% of micro pollen grains had nucleoli. All the data confirm that the chromosomes with rDNA loci are involved in the generation of aneuploid pollen grains. The association between the meiotic bridges and SAT chromosomes may be related to the fact that in many instances the fibrillar center of nucleoli is formed by the rDNA genes of 4 chromatids that may remain entangled as the first division progresses, giving rise to bridges. However, to explain the relationship between the chromosomes bearing the 5S rDNA loci and the observed chromosomes located outside of the metaphase plate, more research is needed.

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IV.14

Immunodetection of the Synaptonemal Complex in Achiasmate Meiosis of Scorpions

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The synaptonemal complex (SC) is a meiosis-specific proteinaceous structure evolutionarily conserved in a wide variety of taxa. It is related to specific events, such as homologous pairing, synapsis, recombination, and chromosome disjunction. The formation of SC is essential for genetic recombination; however, its presence does not indicate the occurrence of crossing over. In scorpions, all species cytogenetically investigated up to now seem to have an achiasmatic mode of meiosis in males but with the formation of SC. For the first time, we studied the SC in scorpions using immunostaining. The analyses were accomplished in 3 Brazilian scorpion species, 1 with monocentric chromosomes *(Bothriurus rochai)* and 2 with holocentric chromosomes *(Ananteris balzanii* and *Tityus strandi)*. Microspead spermatocytes were subject to immunodetection using a commercial antibody against the protein SMC3, which recognizes a cohesin component along vertebrate SCs, and detected with a secondary antibody conjugated to Alexa Fluor-488 fluorochrome. In pachytene cells of the 3 scorpion species, the immunostaining showed well-preserved SCs, which allowed the identification of their entire length. These characteristics of the SC were similar between species with monocentric and holocentric chromosomes. Additionally, some nuclei of *A. balzanii* presented single filaments non-synapsed axes in terminal and/or interstitial regions of 1 or 2 SCs. These results reveal that the commercial antibody against SMC3 from vertebrates can also be useful to detect this cohesin component in scorpions, including chromosome regions with atypical synaptic behavior. In all species, the SCs were maintained until the postpachytene substage of meiosis. In species with achiasmate meiosis, the presence of SC in late prophase I cells seems to be important to maintain the connection between homologous chromosomes, ensuring balanced chromosome segregation.

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IV.15

Synapsis and Recombination in Grey Goose *(Anser anser)*

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Birds are characterized by extremely high recombination rates compared to mammals and a very conservative karyotype (consisting of several macrochromosomes and a large number of small microchromosomes), which makes them an excellent model for studying evolutionary factors affecting the recombination rate. Comparative analysis of recombination in bird species with similar karyotypes and different habitats could help to reveal the adaptive component of the recombination rate variation. However, recombination has been studied only in a few bird species. Furthermore, sex chromosomes are significantly different between bird taxa allowing us to study the steps in their evolution. We analyzed recombination and sex chromosome behavior in oocytes of the grey goose *(Anser anser)* using immunolocalization of the synaptonemal complex (SC) protein SYCP3, mismatch repair protein MLH1 (marking recombination sites), and centromere proteins. We found that most recombination characteristics exceeded the highest parameters observed in chicken. The mean number of MLH1 foci was 73.6 ± 7.8 foci per cell, the length of the genetic map was 3,680 cM, and the approximate recombination rate was 2.9 cM/Mb. However, recombination density per unit of SC length was

lower due to significantly longer SCs (0.24 MLH1 foci/μm). It also results in a high proportion of microchromosomes that had more than one MLH1 focus. We built recombination maps for 5 macrochromosomes and revealed a low level of chromosomal and centromeric interference, indicating that a large number of microchromosomes is not the only cause of the high recombination rate. We analyzed recombination and pairing features of the ZW sex-bivalent and found that synapsis started from the short arms of Z and W chromosomes after pairing of autosomes. One MLH1 signal is usually observed at mid/late pachytene in the region of synapsis initiation that holds less than 9% of the short arm of the Z chromosome. It is consistent with the finding that inversion in the short arm of the W chromosome is located next to the pseudoautosomal region of Anseriformes [Zhou et al., 2014] (reference to be obtained by the author). Nonetheless, sex chromosomes synapsed along the entire length of the W chromosome at mid-pachytene and then equalized through shortening of the Z and stretching of the W chromosome at late-pachytene. A similar process of synaptic adjustment has been observed in sex chromosomes of neognathae birds studied so far.

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IV.16

Determining the Missegregation Rates of Individual Human Chromosomes

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Recurrent aneuploidies are associated with cancer and ageing in humans; however, mechanisms underlying these are unclear. Human chromosomes vary greatly in size, gene density, heterochromatin content and structure among other properties, and therefore may behave differently to one another under cellular stresses affecting chromosome replication and segregation. However, it is not fully understood whether some chromosomes are more prone to missegregation, and whether this varies depending on the specific mechanism causing aneuploidy. To address these important questions, we are performing the first comprehensive examination of chromosome missegregation rates of individual human chromosomes under different cellular stresses. To do this, we have employed FISH imaging of centromeres, coupled to high-throughput analysis using the ImageStream$^{®X}$ cytometer, allowing us to score missegregation events in tens of thousands of cells and generate highly accurate rates of chromosome gain or loss for each chromosome. Findings are then validated using traditional microscopy and centromeric FISH assays. Using this approach, we have identified striking differences in rates of chromosome gain and loss between individual chromosomes. For example, under conditions where improper kinetochore-microtubule attachments are promoted (nocodazole washout treatment), we observe high rates of chromosome 18 loss, and X chromosome gain. Chromosome 18 is also lost at high rates following chromosome missegregation induced by defects in DNA replication in the preceding cell cycle. This suggests a general instability of this chromosome and possibly explains the frequent loss of chromosome 18q in colorectal cancer. The X chromosome is also lost at high rates following DNA replication stress-induced chromosome missegregation. This result is striking since the loss of sex chromosomes is associated with ageing in humans, suggesting that DNA replication stress may contribute to sex chromosome aneuploidy that is observed in ageing cells.

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IV.17

Transcriptome Analysis Reveals New Features of Meiotic Gene Expression and Meiotic Sex Chromosome Inactivation in Mouse

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Gene expression analysis of meiotic stages is hindered by the high number of different cell types coexisting inside the testis. Moreover, the characterization of short, poorly represented cell stages such as initial meiotic prophase ones (leptotene and zygotene) has remained elusive, despite their crucial importance for understanding the fundamentals of meiosis. We have developed a flow cytometry-based approach for obtaining highly pure stage-specific spermatogenic cell populations, including early meiotic prophase. This methodology was combined with next-generation sequencing, enabling the analysis of meiotic and postmeiotic gene expression signatures in mouse with unprecedented reliability. Interestingly, we found that an important proportion of the meiotic program is already on at early meiotic prophase, including most genes for meiotic recombination and segregation. Moreover, a considerable number of those genes have a marked expression peak at lepto-zygotene stages, their transcripts being present for a very short time lapse and decreasing before the pachytene stage. Our results also afford new insights concerning meiotic sex chromosome inactivation (MSCI). On one hand, for the first time we have found a cluster of X-linked genes in mouse that escape MSCI, being upregulated in pachytene; among them, many code for sperm-related proteins. Besides, we observed that transcriptional reactivation of the X chromosome after meiosis is not uniform along the chromosome, but concentrated on one-half, thus suggesting undisclosed mechanisms of epigenetic regulation.

R. Rodríguez-Casuriaga and I. da Cruz contributed equally to this work.

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V. Chromatin and Chromosome Dynamics

Invited Talks

V.1
Pericentromeric Satellites: Implications for Chromosome Evolution and Misregulation in Disease

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Nearly half of the human genome consists of noncoding repetitive DNA elements, including tandem satellite repeats in large blocks at the pericentromeric regions of chromosomes and intergenic repetitive elements. While both repeat types were long thought to remain mostly silent, recent evidence indicates that repeats can be expressed, but the extent and regulation of their expression or their potential function(s) remain to be elucidated. The location of satellite sequences within pericentromeric regions is largely conserved; this is in contrast to the apparent lack of sequence conservation among even closely related species. Due to their critical location within regions vital for cell division, it is expected that tight regulation of pericentromeric satellite sequences is essential for both epigenetic and genetic stability. We have developed tools to examine the effect of pericentromeric satellite expression on cell division and the distribution of key regulatory proteins. Our data suggests induced expression of pericentromeric satellite RNA is tightly linked to both epigenetic instability and aberrant cell division. Further, using a comparative genomics approach, we examine the evolutionary conservation of a subset of satellite sequences found to be particularly prone to misregulation in cancer.

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V.2
PI3Kδ Inhibitors Increase Genomic Instability by Upregulating AID Expression

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Activation-induced cytidine deaminase (AID) is a B cell-specific enzyme that initiates class switch recombination and somatic hypermutation of immunoglobulin genes, essential mechanisms to generate different classes of antibody and antibody diversity for the antigens. AID expression is tightly controlled in B cells to limit its genotoxic effects. PI3Kδ acts downstream of the B-cell receptor to suppress AID expression. Potent oral PI3K inhibitors such as the p110δ inhibitors idelalisib or duvelisib are used for the treatment of different types of lymphoma. We investigated whether inhibition of PI3K could result in increased AID expression and, consequently, genomic instability. By applying a genome-wide translocation sequencing technique (high-throughput genomic translocation sequencing approach, HTGTS), we studied chromosomal translocation formation and aberrant mutations in activated primary mouse B cells under treatment with idelalisib and duvelisib to show that these inhibitors significantly increased both AID protein and mRNA levels compared to controls. Remarkably, in B cells treated with idelalisib or duvelisib, we identified a significantly higher number of translocations in known AID off-target genes as well as novel hotspots of translocations. Unbiased genome-wide analysis of translocation revealed that AID targets localized in the TSS region and predominantly grouped within super-enhancers and regulatory clusters. HTGTS analysis on AID-deficient B cells showed that these translocations were dependent on AID. Hence, our data demonstrate that in normal B cells PI3Kδ inhibitors increase genomic instability by an AID-dependent mechanism. Finally, we observed that both idelalisib and duvelisib increased AID expression in human lymphoma cell lines (MCL and CLL). We adapted the HTGTS technique to human cells by introducing DSBs in the human c-*myc* locus by CRISPR/Cas9 technology. Through this approach, we demonstrated that also in human lymphoma B cells, treatment with idelalisib or duvelisib increased the formation of translocations to AID off targets. In conclusion, we showed that idelalisib or duvelisib increase genomic instability in normal and neoplastic B cells by enhancing AID expression. Since several B cell malignancies imply treatment with these drugs for years, these effects of PI3Kδ inhibitors on the genomic stability of B cells should be carefully taken into account for therapeutic outcomes and clinical protocol design.

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V.3
Molecular Architecture of Vertebrate Centromeres and Kinetochores

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The kinetochore is formed on centromeric DNA as an important interface with microtubules from the mitotic spindle for correct chromosome segregation during mitosis. The kinetochore is specified on centromeric chromatin by sequence-independent epigenetic mechanisms in vertebrates, and a centromere-specific histone H3 variant, CENP-A, is an important epigenetic marker for the kinetochore specification. Rarely, centromere repositioning results in neocentromere formation at ectopic sites in a chromosome. (Neo)centromeres should have specific chromatin features to be a functional centromere. However, the mechanisms governing how and where neocentromeres form are largely unknown. Thus, we established a chromosome-engineering system in chicken DT40 cells that allowed us to efficiently isolate neocentromere-containing chromosomes and examined specific chromatin features of neocentromeres. We would like to introduce our recent progress in the analysis of centromeric chromatin for kinetochore assembly. Following the establishment of centromeric chromatin, various

proteins are assembled on centromeres to form the functional kinetochore. To examine the assembly pathway of the kinetochore, we localized various kinetochore proteins in the non-centromere locus to create 'artificial kinetochore', following depletion of an endogenous centromere. Based on this experiment, we are able to test whether the artificial kinetochore is functional. Combining these results with gene-knockout experiments, we propose a model for kinetochore assembly, which will be presented.

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V.4

The Golden Nuggets Buried in the Open Chromatin in Plant Genomes

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Plant genome research has entered the post-genome sequencing era. Genes can be readily identified because of the powerful next-generation sequencing tools. However, transcription in eukaryotes is regulated by orchestrated binding of regulatory proteins to various types of *cis*-regulatory DNA elements (CREs), mostly promoters and enhancers. Identification and annotation of large amounts of noncoding but functional CREs have become a new frontier in plant genome research. Genomic regions containing active CREs are known to be hypersensitive to DNase I digestion because these regions are free of nucleosomes or undergo dynamic nucleosome displacements. These regions are called DNase I hypersensitive sites (DHS) and can be identified using recently developed genomics techniques. Our lab has developed techniques for DHS identification in plants. We have demonstrated various applications of DHS datasets in plant genomics and epigenomics research, most important for revealing footprints derived from binding of regulatory proteins and identifying enhancers located in intron and intergenic regions.

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V.5

Chromatin Dynamics in *Arabidopsis* during Heat Stress and Differentiation

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Chromosomal DNA in eukaryotes is organized into chromatin in a dynamic manner which affects all processes that operate on DNA. The basic subunit of chromatin, the nucleosome, consists of a core of 8 highly conserved histone proteins, for which several variants occur and that can carry specific post-translational modifications. Nucleosome position and composition are important elements of epigenetic information. While DNA sequences are copied faithfully during replication, the arrangement, composition, and modification of the associated nucleosomes can vary during growth or between tissues and individual cells. These dynamic changes affect gene expression patterns and contribute to differentiation and development. Nucleosome occupancy and modifications, however, have also been shown to vary in response to environmental stimuli. Previous work from our lab revealed that extended heat stress can cause substantial rearrangement of heterochromatin, dissociation of nucleosomes from DNA, and expression changes of many genes in the model plant *Arabidopsis thaliana*. Although most of these changes are transient, they have the potential to result in lasting effects in some cells. Therefore, we study the process of nucleosome dis- and re-association during and after heat stress, the role of histone chaperones, and concomitant gene expression changes. We also follow the process of heterochromatin decondensation and changes in nuclear architecture upon heat stress and during differentiation by live-imaging and quantifying changes with semi-automated image analysis techniques.

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Posters and Short Talks

V.6

Cytogenetic Study in a Group of Brazilian Infertile Men with Severe Oligozoospermia or Non-Obstructive Azoospermia Attending an Infertility Service

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Male infertility affects about 7% of the world population and is considered a multifactorial syndrome including a wide spectrum of disorders. More than half of the cases are idiopathic, genetic factors explain 10–15% of the etiology of severe male infertility, and of these 8% are due to chromosomal abnormalities. Investigations of the production of unbalanced gametes in balanced inversion carriers have been made to a much lesser extent than in translocation carriers. Nevertheless, a handful of studies have reported ranges of unbalanced sperm of 1–54%. In addition to other well-described etiologies, genetic causes of male infertility are now more commonly diagnosed. Despite this, the molecular and genetic factors underlying the cause of infertility remain largely undiscovered. Thus, individuals with abnormal semen analysis should at first be evaluated by classical cytogenetics. The chromosomal profile of 30 men with idiopathic infertility (oligozoospermia or azoospermia) treated at the Human Reproduction Labora-

tory of the Clinical Hospital (LabRep – HC), Federal University of Goiás (UFG) was evaluated by CTG-banding, interview with the individual, and chart review data collection. The results of the cytogenetic analysis of oligozoospermic (18/30) and azoospermic (12/30) men, aged between 24 and 59 years, revealed aneuploidy of sex chromosomes in 3 azoospermic cases (47,XXY, 2 cases and 47,XXY[9]/46,XY[2], 1 case). Also, an inversion of chromosome 9 in 2 cases (one oligospermic and one azoospermic with 47,XXY) was observed. Genetic tests help to identify men with azoospermia or oligozoospermia that may benefit from reproductive techniques. Furthermore, our samples will be submitted to a screening for microdeletions in the azoospermia factor (AZF) region of the Y chromosome, since these are also a major cause of male infertility.

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V.7

Asymmetrical Distribution of Single-Copy FISH Signals Revealed Differences in the Chromatin Packaging of Sister Chromatids at Mitosis

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The cell cycle-regulated process of chromosome condensation is a prerequisite for the accurate segregation of sister chromatids. To study whether the mitotic chromatin packaging of sister chromatids is identical, single-copy FISH was performed on metaphase chromosomes of barley. Simultaneous detection of differentially labelled ~4-kb long single-copy probes revealed that 95% of analyzed chromosomes showed an asymmetric signal distribution between sister chromatids. Hence, although mitotic sister chromatids encode the same genetic information, the packing of DNA at the chromosomal level differs to a certain degree. To correlate the cytogenetic and the genetic map of barley, the chromosomal positions of 9 single copy probes assigned to the short arm (ranging from 24.67 to 43.91 cM) and 3 and 4 probes assigned to the long arm (ranging from 62.11 to 68.98 and 115.74 to 137.25 cM, respectively) of chromosome 3H were determined. The probes, all of them corresponding to highly recombining regions, resulted in FISH signals at distal regions of the chromosome. Thus, in contrast to the recombination-poor region around the centromere, which comprises more than half of chromosome 3H, long genetic distances in distal regions translate into short physical distances. The order of sequences along the chromosome can only be resolved for probes which are more than 21 Mb apart from each other. Furthermore, we were using the probes of the short arm together with 18 additional single-copy probes to 'paint' the entire arm of chromosome 3H. This is a first step towards successful chromosome painting in plant species with large genomes.

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V.8

Complex Tandem Repeats in the Euchromatin of *Drosophila virilis*

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Simple tandem repeats (TRs) are a common feature of the euchromatin in eukaryote genomes and have been studied in detail in several species. Conversely, complex TRs residing in the euchromatin are much less investigated. Herein, we have characterized a recently identified abundant family of complex ~172-bp TRs in the genome of *Drosophila virilis*. These repeats occupy about 0.85% of the genome (~20,000 copies) and can be found in arrays with up to 14 kb. The analysis of over 4,000 TRs from the *D. virilis* genome assembly evidenced that these TRs are enriched in some chromosomes (3, 4, and 5) and nearly absent in the other elements (2, 6, and the X). FISH of these TRs to the polytene chromosomes confirmed and extended the computational analysis, evidencing the same chromosome enrichment but also an accumulation of the TRs towards the distal portions of all chromosomes and their paucity from centric and pericentric heterochromatin. The average pairwise distance for TRs within the same array is 7.6% and for TRs from different arrays 20.8%, indicating a concerted mode of evolution. We found the 172-bp TRs very close to annotated genes in *D. virilis* (205 and 229 hits in the up- and downstream 5-kb gene flanking regions, respectively) and within introns (161 hits). We also found that small RNAs matching the 172-bp TRs are predominant in embryos and gonads of *D. virilis*, with 27-fold enrichment in gonads over adult carcasses and 18-fold enrichment in embryos over carcasses. Altogether, our results point to a role for the 172-bp TRs as regulatory elements in the *D. virilis* genome. These TRs could belong to a regulatory network either by recruiting specific transcription factors or by altering local chromatin states and thus influencing gene expression.

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V.9

Glucose Level of Culture Medium Affects the Response of HepG2 Cells Chromatin to Histone Deacetylase Inhibitors

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Histone deacetylase inhibitors (HDACi), including valproic acid (VPA) and trichostatin A (TSA), are known to promote chromatin remodeling in several cell models. Liver is the key organ that releases glucose into the circulation under fasting conditions. An increase in hepatic glucose production due to insulin resistance or insulin deficiency is the central event in the development and progression of diabetes. It is thus of interest to investigate whether the glucose level of the culture medium, which is a fundamental pa-

rameter for cell life, would affect the action of VPA and TSA on liver cells. The effect of VPA and TSA treatments on HepG2 cells cultivated under normoglycemic (LG) or hyperglycemic (HG) conditions was investigated to assess changes in the chromatin and epigenetic landscape. Under LG conditions, VPA and TSA treatments promoted global chromatin decondensation, a conclusion that was obtained by image analysis parameters and identification of H3K9ac and H3K9me2 abundance. Moreover, a shift of H3K9ac nuclear localization to the periphery of the nucleus concomitant with disruption of HP1-α from pericentromeric heterochromatin and G1 cell cycle arrest was observed, which might be related to heterochromatin disruption and the spatio-temporal replication properties of different chromatin domains. Regarding the results obtained after HG exposure, some evidences indicated that hyperglycemia per se promotes overall decondensation of the chromatin with loss of repressive histone modifications and increase of activating histone marks. Interestingly, VPA and TSA treatment under HG conditions did not intensify these results. Therefore, chromatin alterations promoted by these drugs under HG in HepG2 cells may be more a function of the different nuclear domains and genes regulated than of global remodeling. These data suggest that the long-held views regarding HDAC inhibition may be subject to more flexibility than previously imagined.

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V.10

Engineering of Large Tandem-Repeated Arrays of Nucleosome Positioning Sequences in Yeast to Study Determinants of Nucleosome Spacing in vivo

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Eukaryotic genomes are packaged into chromatin. The basic repeating unit of chromatin is a nucleosome, which consists of a histone octamer wrapped around 147 bp of DNA. Nucleosomes are arranged into regularly spaced arrays, with the length of the linker varying among cell types and organisms. Nucleosome organization is critical for gene regulation. Determinants of nucleosome organization include chromatin remodelers, competition with DNA-binding proteins, and DNA sequence preferences. It remains difficult to estimate the relative importance of each of these factors in living cells. Tandem-repeated DNA sequences represent a particular case of sequence organization in the genome, which have been documented as supporting particular chromatin organizations. In order to study the effect of tandem DNA repeats on nucleosome organization in living cells, we developed a strategy to assemble tandemly repeated chromatin arrays of nucleosome positioning sequences in the budding yeast genome. This method allowed us to assemble up to 10 kb of nucleosome positioning repeats in the chromosome XIII of *Saccharomyces cerevisiae*. We engineered synthetic arrays of varying length, resulting in 167, 197, and 237-bp long repeats. Nucleosome occupancies on these artificial arrays are analyzed by quantitative PCR on mononucleosomal fragments isolated by MNase digests of yeast chromatin. Our results on nucleosome positioning patterns in vivo on these synthetic arrays will be presented. Our system should give valuable information on the ability of a repeated DNA sequence to influence chromosomal organization in wild-type yeast cells. A precise positioning of the nucleosome by the repeated array in living cells would strongly support the role of the underlying DNA sequence in nucleosome organization and pave the way for specific chromatin engineering in living cells.

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V.11

Determining the Role of Centromeric Noncoding RNA Transcription in Chromosome Segregation in the Budding Yeast *Saccharomyces cerevisiae*

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The centromere is crucial for ensuring accurate chromosome segregation. Yet, centromeric DNA sequences are not conserved among organisms, and centromeres can be regulated epigenetically. Centromeric transcription is a common epigenetic factor identified in many eukaryotes, and increasing evidences indicate that centromeric transcription and its corresponding noncoding RNA (cenRNA) may control the activity of the centromere. Centromeric transcription in different organisms is cell cycle-dependent, but the exact timing is not conserved. The budding yeast *Saccharomyces cerevisiae* centromere is thought to be DNA sequence-dependent. Nonetheless, a recent study showed that transcriptional activity at the budding yeast centromere is important for centromere function. Our preliminary result shows that the budding yeast centromere is transcribed during S phase, coincident with centromeric protein A (CENP-A) centromeric loading time, similar to the situations in human cells and fission yeast. We propose that the conserved function of cenRNA is to promote CENP-A loading. The regulation of centromeric transcription remains elusive in many eukaryotes. In budding yeast, we identified 2 proteins that repress centromeric transcription, a centromere-binding factor Cbf1 and a histone H2A variant Htz1. *cbf1Δ* and *htz1Δ* have increased cenRNA levels and elevated centromeric plasmid loss rates. Cbf1 and Htz1 bind to the centromere and centromere-proximal region, respectively, and regulate centromeric transcription at both the DNA and nucleosome levels. We hypothesize that Cbf1 and Htz1 are released from the centromere during S phase to induce centromeric transcription. We will further investigate the role of centromeric transcription and cenRNA in centromere function and its regulation. This study will clarify the role of centromere transcription as a conserved epigenetic mechanism across eukaryotic centromeres in maintaining chromosome stability.

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V.12

Investigation of Histone Post-Translational Modifications in Adrenocortical Tumor Cells Stimulated with Fibroblast Growth Factor 2

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Despite being a growth factor, FGF-2 has anti-proliferative and tumor suppressive functions in some contexts. In the Ras-driven mouse tumor cell line Y1 it promotes G0/G1 transition but delays S phase and permanently blocks cells in G2/M, leading to senescence. In order to investigate if FGF-2 induces differential histone post-translational modifications related to these effects, histone extracts from Y1 cells of different time points after FGF-2 stimulation were used for Western blotting (WB) assays. No important alterations were detected for H4K5ac, H4K8ac, and H4K12ac. However, H4K16ac levels were diminished after FGF-2 treatment as seen by WB and FACS. In addition, H4K20me1 peaks after 30 min of FGF-2 stimulation. H4K16ac and H4K20me1 are involved in DNA damage and repair, so we started to investigate if FGF-2 induces DNA damage by H2AX-γ labelling and COMET assays. The first indicates that FGF-2 induces DNA damage after 1 h and, in agreement, the second shows that FGF-2 induces 3-times more DNA damage than serum. We are investigating the levels of writers and erases of H4K16ac/K20me1 (KAT8, SIRT1/2, SET8 and PHF8) by WB and qPCR. Moreover, we intend to knock down the expression of these enzymes through shRNA. We believe that our results will help to clarify the role of chromatin in these unexpected effects of FGF-2 in a Ras-driven cell line.

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V.13

Cytogenetic Differentiation Data among *Characidium* Populations (Actinopterygii: Crenuchidae)

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Characidium has 56 valid species distributed throughout the Neotropical region. It has a diploid number of 2n = 50 chromosomes and may present a ZZ/ZW sex chromosome system which makes it a model for studies of sex chromosome differentiation. Samples of 2 populations of *Characidium (C.* cf. *pterostictum* and *C. gomesi)* from the Chapecó River, Uruguai basin and São João River, Upper Paraná River basin, Brazil were subjected to conventional Giemsa staining and C-banding technique. Probes of 18S rDNA, 5S rDNA, and the W chromosome (isolated from *C. gomesi*) were also applied to chromosomal localization by FISH. *C. gomesi* possesses heterochromatic blocks in the pericentromeric region of all chromosomes and the W chromosome is entirely heterochromatic. The W-specific probe was in situ localized on the W subtelocentric chromosome. The 5S rDNA sites were located in metacentric pair 5, while the 18S rDNA sites were located in metacentric pair 1 and in the submetacentric pair 17. *C.* cf. *pterostictum* presented heterochromatin in the pericentromeric region of most of the chromosomes and in the interstitial region of the long arm of the sex chromosomes. The W-specific probe from *C. gomesi* was located on the W metacentric chromosome of *C.* cf. *pterostictum*. 5S rDNA sites were identified in metacentric pair 1 and 18S rDNA sites in submetacentric pair 18. Different to other *C. pterostictum* populations, these specimens do not have rDNA sites on the sex chromosomes. These cytogenetic data differentiate the population of *C.* cf. *pterostictum* (Chapecó River) from all other populations of *C. pterostictum* analyzed up to this moment, demonstrating populational differentiation in the absence of gene flow. These results show cytogenetic differentiation among *Characidium* populations, indicating the occurrence of new species.

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V.14

Differential Hypomethylation of the Repetitive *Tol*2-Rich Region in Species of the Genus *Bodianus* (Perciformes, Labridae)

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Perciformes represents the largest order of vertebrates exhibiting exceptional models for the comprehension of the genetic structure and karyotype evolution in marine species. Its representatives reveal both examples of extreme conservatism and chromosomal diversification. Special attention has been given to the organization and structure of fish chromosomes, primarily with respect to the characterization and mapping of heterochromatic regions. However, the cytological characterization of epigenetic modifications is still unknown for the majority of this group. The Labridae family has been characterized by the presence of a peculiar chromosomal region in the short arm of the second subtelocentric chromosome pair, here named BOD. This region, which occurs in species of the *Bodianus* genus – *B. insularis, B. pulchellus,* and *B. rufus* – is exceptionally decondensed, argentophilic, GC-neutral, heterochromatic and, contrary to the classical secondary constrictions, it does not show signals of hybridization with 18S rDNA probes. In this sense, aiming at the characterization of the BOD

region of the metaphasic chromosomes of *B. insularis* and *B. pulchellus*, the methylation pattern, the distribution of sequences (TTAGGG)n, the retrotransposon *Tol*2 and the 18S and 5S rDNA were analyzed with FISH. The immunolocalization of 5-methylcytosine (5mC) revealed hypermethylated chromosomal regions, dispersed in the whole extension of the chromosomes of both species, while the BOD regions showed a hypomethylated pattern. The hypomethylation of the BOD region is associated with the precise collocation of the *Tol*2 elements, suggesting an active participation of these elements in the regulatory epigenetic process. These evidences provide an interesting perspective of the differential methylatory action during the cell cycle, as well as the possible role of the *Tol*2 elements in functional processes of genomes of fishes.

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V.15

Characterization of *Arabidopsis* CAP-D2 and CAP-D3 Condensin Subunits

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The condensin complex is conserved in all eukaryotes. Yeast has 1 condensin complex, while higher eukaryotes have 2 of them (condensin I and II). In *Arabidopsis thaliana,* both condensin I and II are conserved, but little is known about their function. To better characterize plant condensins, we focus on the *A. thaliana* subunits CAP-D2 (condensin I) and CAP-D3 (condensin II) to elucidate their participation in the condensin complex formation, their distribution during the cell cycle, and their function. Pull-down experiments confirmed that CAP-D2 and CAP-D3 interact with the other putative subunits of the condensin I and II complexes, respectively. To address the colocalization in interphase nuclei, antibodies against different condensin subunits were used and super-resolution microscopy was applied. The tested subunits localized within the euchromatin, but they were absent from the heterochromatin and the nucleolus. Condensins are best known for their role in shaping mitotic and meiotic chromosomes, but they also organize interphase chromatin as described for *Drosophila* and vertebrates. Previous experiments showed that in *A. thaliana* the lack of CAP-D3 leads to decondensed chromosome territories and centromere association during interphase. To study the function of CAP-D3 in more detail, further repetitive DNA sequences were analyzed by FISH. Thereby it was found that CAP-D3 also affects the arrangement of 45S rDNA, but not that of 5S rDNA.

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V.16

What Do We Know about the Heterochromatin of *Cebus* (Primates, Platyrrhini)? An In-Depth Genomic Revision

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Heterochromatin has been proposed as a putative factor involved in karyological diversification among vertebrates and in mechanisms leading to speciation. Our work in *Cebus*, one of the most complex groups within platyrrhine primates, highlightened a peculiar genomic feature: the presence of conspicuous regions of repeated sequences distributed throughout the karyotype. This trait refers not only to most of the chromosome pairs with heterochromatic blocks but also to the composition of them. Here, we analyze the heterochromatin implication in *Cebus* genome dynamics by studying different genomic parameters and components. We addressed a molecular cytogenetic approach of more than 250 individuals from 7 of 12 recognized species. *Cebus spp.* showed a high degree of homology with each other. The proportion of heterochromatin in their karyotypes correlates with genome size, from smallest to largest: *C. xanthosternos*, *C. nigritus, C. cay, C. libidinosus, C. apella, C. olivaceus,* and *C. albifrons*. FISH applying our own specific heterochromatic probe of *C. cay* in different *Cebus* and other Platyrrhini species allowed proposing that this heterochromatin is genus-specific with taxonomic diagnostic value. C-band heteromorphism and polymorphism frequencies were not randomly distributed. Chromosome pairs 6, 17, and 19 were the most variable for *C. cay* and *C. nigritus*. DAPI-CMA3 patterns were similar among *Cebus* species, where C-blocks showed conspicuous heterochromatic CMA3 bands, corresponding to regions of DNA with a GC-rich composition. CGH analyses showed that interspecific divergence is mostly related to differences in Y chromosome composition. For *C. nigritus* and *C. cay* we found differences in the proportion of DNA between different genomic regions, with *C. cay* having more repetitive DNA and *C. nigritus* more coding DNA. Our results showed that heterochromatin has an evident role in *Cebus* genome dynamics and support the hypothesis that larger genomes with GC-rich heterochromatic DNA are more stable and protected.

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V.17

Chromosomal Mapping of Microsatellites $(GATA)_n$ and Transposable Element Mariner Sequences in *Characidium* (Characiformes: Crenuchidae)

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The *Characidium* (Characiformes) may present a ZZ/ZW sex chromosome system, varying in the degree of differentiation. These heteromorphic sex chromosomes are absent in some species, while in others they are found with different heterochromatinization and morphology patterns, some presenting 45S rDNA sites. The heteromorphic sex chromosome differentiation is based on recombination supression, chromosomal rearrangements, heterochromatinization, and accumulation of repetitive DNA. In this study, we mapped the microsatellite $(GATA)_n$ and the transposable element Mariner in the genomes of *C. gomesi, C. heirmostigmata,* and *C. zebra*, highlighting the location of these repeats and their possible role in the evolution of sex chromosomes. The probe for $(GATA)_n$ sequences showed hybridization signals on all autosomes, especially on the terminal region. In *C. gomesi* the same probe showed hybridization signals only on the short arm of the W chromosome. The probe for Mariner sequences interestingly revealed the same pattern distribution as the $(GATA)_n$ sequences, even in the W chromosomes of *C. gomesi* and in the W pericentric region of *C. heirmostigmata*. The $(GATA)_n$ repeat is considered a motif for GATA-binding proteins related to decondensation of the genic regions, where there are genes related to the oocyte development, especially in heterochromatic regions of heteromorphic sex chromosomes. That the GATA motif was detected only in the short arm of the W chromosome of *C. gomesi* may indicate the existence of coding genes in this region. The absence of these repeats in the major portion of the W chromosomes may represent the lack of coding genes. The transposable element Mariner presented a distribution pattern similar to the $(GATA)_n$ sequence. Transposable elements are able to shape both genic regions and even the entire genome. In the *Characidium* karyotypes, the distribution of $(GATA)_n$ sequence and the transposable element Mariner is not involved with W chromosome gene erosion and differentiation.

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V.18

Ionizing Irradiation Does Not Promote Chromosomal Breakages in the Fragile Sites 45S rDNA of the *Lolium multiflorum*

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In *Lolium*, 45S rDNA sites have shown high decondensation in metaphase chromosomes, constituting regions slightly stained with DAPI. These regions are denominated fragile sites (FSs) that have been widely studied in humans. FSs might be prone to form chromosomal fragments and rearrangements, unless repair mechanisms such as homologous recombination (HR) or non-homologous end joining (NEJH) play a role at the DNA break-site. Thus, this study aimed at investigating if such sites are hotspots for the occurrence of breakages induced by X-ray in *Lolium multiflorum*. *Hordeum vulgare* was used as a comparative model. Seedlings of both species were irradiated with 50Gy X-ray and evaluated 1 day following the irradiation and thereafter at 7-day intervals for a 28-day period using FISH with 45S rDNA and telomere probes. *H. vulgare* did not survive after a few days of irradiation due to the increased rate of chromosomal abnormalities. Chromosomes of *L. multiflorum* exhibited abnormalities such as deletions, fusions, translocations, and chromosomal fragments with and without 45S rDNA sequences, yet over the 28-day trial, it had a decrease in the rate of chromosomal damage. Despite being considered to be FSs, the 45S rDNA sites of *L. multiflorum* are not hotspots to chromosomal breakages after the induction with X-ray. The lack of fragments with 45S rDNA can be explained by the absence of double-strand DNA breaks (DSBs) in these sequences, not being necessarily the genome sites where the irradiation interacts or due to DSBs in 45S rDNA that were more efficiently repaired by mechanisms like HR or NEJH compared to other chromosomal regions where the breakages have also occurred.

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V.19

Chromosomal Replication Dynamics and DNA Microarrays Suggest Gene Activity in B Chromosomes in *Astyanax scabripinnis* (Teleostei, Characidae)

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B chromosomes are genetic components found in various taxonomic groups. No evidence of phenotypic effects related to the occurrence of B chromosomes was found in fish so far, including the model *Astyanax scabripinnis*. This study used 35 specimens of *A.* aff. *scabripinnis* from the stream of Lavrinha Farm, Paraíba do Sul watershed, Brazil. In the fish population individuals with 2n = 50 and 2n = 51 chromosomes were found, with one metacentric B isochromosome confirmed by the whole chromosome painting (WCP) probe which was obtained by DOP-PCR amplification of a microdissected B chromosome. The goal of this work was to obtain high-resolution patterns of chromosomal bands, to gain data on the location, nature, and variability of the chromatin of the B chromosome, and to elucidate possible transcriptional regions. The recognition of initial replication bands, potentially transcriptional targets, and evidence of an allusive organization to isocores present in mammalian chromosomes was possible with the use of in vivo 5BrdU. In addition, DNA microarray assay was performed with cDNA obtained from gonad tissue of adult males and females with and without B chromosomes through blades with total genome of zebrafish. The integrity of total RNA samples was verified by microfluidic electrophoresis, with RIN >9. The occurrence of an RNA band of about 180 bp only in females and overexpressed with B chromosomes is noteworthy. The results of cross-hybridization on DNA chip allowed the identification of differentially expressed genes among the sexes and among females with and without a B chromosome. These evidences suggest that the B chromosome has a role in the transcription of genes in zebrafish females, with a possible heterotic role.

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V.20

A Holistic View on Evolution and Stability of Genomes

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The nuclear genetic information of eukaryotic organisms is binned in an organism-specific number of linear chromosomes. The number of genes in eukaryotes varies from ~5,000 to >100,000, the number of chromosome pairs from 1 to >100, and the nuclear DNA content by more than 2,400-fold in flowering plants. The apparently uncorrelated ratio of genetic complexity and nuclear DNA content is called 'C-value paradox' [Thomas, 1971] or more recently 'C-value enigma' [Gregory, 2001] (references to be obtained by the author). The reason why DNA content, chromosome numbers, and gene content vary independently of each other is still a matter of speculation. The same is true for the questions of whether there is a general tendency for increase or decrease of genome size, whether genome size has an adaptive value and, if so, what that value is. It is also not clear what the evolutionary importance of distinct mechanisms is which mediate chromosome number alteration. The main sources of genome structure variability are sexuality, allopolyploid whole genome duplication (WGD), and DNA double-strand break (DSB) mis-repair. Based on literature and own data, we discuss strategies for the evolution of genome size and karyotype structure in the context of genome stability. We hypothesize 3 strategies for genome evolution: (i) genome shrinking caused by deletion-biased DSB repair and counteracted by WGD which is associated with subsequent dysploid chromosome number reduction; (ii) genome expansion mainly due to DSB repair biased against deletions, but favoring insertions including retroelement dispersion; (iii) genome stasis due to balanced insertion/deletion ratios during DSB repair yielding relatively constant genome sizes and rather stabile karyotypes. Stochastic mutations and/or selection pressure may cause strategy switches.

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V.21

Chromosome Characterization and Mapping of Transposable Elements in *Proechimys* (Rodentia: Echimiydae)

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Rodents of the genus *Proechimys* (Echimyidae family) are characterized by a complex taxonomy due to their morphological variation and wide karyotype variation due to chromosome rearrangements. We characterized the karyotypes of 2 individuals of *Proechimys* sp. (2n = 28, FN = 48) from Corumbá (MS) and 3 females and 2 males of *Proechimys* sp. (2n = 14–16) from São José do Xingu (MT) and Alto Paraíso (RO) in the Amazon region of Brazil. Our analyses included GTG- and CBG-banding patterns, Ag-NOR and FISH with telomeric and rDNA sequences. We also investigated the relationship between repetitive sequences (LINE-1 and SINE-B1) and karyotype evolution. The observed variation in the diploid number (2n = 14–16) resulted from a fusion/fission involving chromosomes of pair 1, and a Robertsonian rearrangement involving the X chromosome and an autosome, leading to a sex

chromosome system XX/XY_1Y_2. The transposable elements LINE-1 and SINE-B1 showed a dispersed distribution in the chromosomes of both species. Interestingly, LINE-1 sequences also hybridized in the pericentromeric regions of most autosomes and the X chromosome, whereas SINE-B1 labeled the pericentromeric regions of pair 1 and the X chromosome of the specimens with 2n = 14–16. The distributions of LINE-1 and SINE-B1 suggest that these repetitive sequences may be related to the chromosome rearrangements of the analyzed species and with the different cytotypes found in *Proechimys*.

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V.22

Effects of Epigenetic Modulator Drugs on DNA Methylation of HeLa Cells

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Valproic acid (VPA) is an important epigenetic drug that works as a histone deacetylase (HDAC) inhibitor, inducing histone hyperacetylation and chromatin unpackaging in several cell types. Currently, DNA demethylation is also being considered part of the VPA action, as shown in HEK293 cells and rat brain cells. This process may occur in a replication-independent active way, possibly with TET proteins participation and 5-hydroxymethylcytosine (5hmC) formation. Recent studies have also detected a 5hmC increase in different cell types after treatment with the well-established DNA methyltransferase (DNMT) inhibitor 5-aza-2′-deoxycytidine (5-aza-CdR), which is known to promote DNA demethylation in a cell cycle-dependent passive way. In the present work, the process of DNA demethylation following VPA and 5-aza-CdR treatment was studied in HeLa cells. The cells were treated with 1.0 mM VPA for 4 h and then cultivated in the absence of the drug for 24 and 48 h or treated with 5.0 μM 5-Aza-CdR for 28 h. Immunoassays for 5-methylcytosine (5mC) and 5hmC were performed to evaluate changes in signals related to total DNA methylation and hydroxymethylation. Quantitative PCR assays were used to investigate gene expression levels of *TET1* and *DNMT1*. Lower fluorescence intensity for methylated DNA in contrast to higher fluorescence intensity for hydroxymethylated DNA was observed in VPA- and 5-aza-CdR-treated cells compared to untreated controls, which could be related to an active demethylation induction by both drugs. The *DNMT1* mRNA levels were not affected by VPA or 5-aza-CdR treatment. *TET1* gene expression decreased after VPA exposure, indicating that other enzymes associated with active demethylation may be involved in this process. Interestingly, 5-aza-CdR treatment increased *TET1* expression, suggesting that the agents analyzed here may promote active demethylation by different pathways. Taken together, these results help to elucidate the multiple effects performed by these drugs on epigenetic markers.

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V.23

The Role of the SMC5/6 Complex in *Arabidopsis thaliana*

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Structural maintenance of chromosome (SMC) complexes are members of a large ATPase protein family. Representatives of SMCs are cohesins which play a role in sister chromatid cohesion, condensins which are essential for chromosome condensation, and finally the SMC5/6 complex. The SMC5/6 complex has been mainly described as a DNA repair complex, but there is also evidence that it has an influence on chromosome stability, dynamics, and DNA replication. The depletion of SMC5/6 subunits leads to sister chromatid cohesion defects in yeast, chicken, and human cells. Nevertheless, the role of this complex is still unclear in plants. Therefore, we analyzed the SMC5/6 subunits NSE1, NSE3, NSE4a, NSE4b, and SMC5 of *Arabidopsis thaliana* in more detail. By investigating T-DNA insertion mutants, we found NSE1, NSE4a, and NSE4b to be required for fertility, NSE1 and SMC5 for plant development, and NSE4a for normal mitosis and meiosis. NSE3- and NSE4a-35S::EYFP fusion proteins indicated their distribution within euchromatin but the absence from heterochromatin and nucleoli. A similar signal distribution was present after employing polyclonal antibodies against NSE3, SMC4a, and SMC5 of *A. thaliana*.

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VI. Applied Chromosome Biology in Medicine and Agriculture

Invited Talks

VI.1

Germplasm Enhancement and Chromosome Remodeling in Wheat-Wide Hybridization

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Plant breeding may lead to a narrowing of genetic diversity of cultivated crops, thereby affecting sustained selection gains in crop improvement. Germplasm enhancement is an important aspect of wheat genetics and breeding. *Thinopyrum elongatum* and *Th. intermedium*, the wild relatives of wheat, have been suggested as a potentially novel source of resistance to several major wheat diseases including Fusarium Head Blight (FHB). A series of wheat (cv. Chinese Spring, CS)-*Th. elongatum* addition, substitution and ditelosomic lines were assessed for resistance to FHB. The results

indicated that the lines containing chromosome 7E of *Th. elongatum* gave a high level of resistance to FHB; the infection did not spread beyond the inoculated floret. Furthermore, it was determined that the novel resistance gene(s) of 7E was located on the short arm (7ES) based on a difference in FHB resistance between the two 7E ditelosomic lines. *Th. ponticum*, *Th. intermedium*, and tetraploid *Th. elongatum* contained useful and potential genes for wheat improvement. Amphiploids and partial amphiploids were released from the hybrids between wheat and *Thinopyrum*, and their genome chromosomal constitution was revealed by using GISH and multicolor GISH. New amphidiploids were obtained between wheat and rye, new 1B/1R translocation lines have been released and their centromere structure and functional analysis revealed that commercial wheat varieties contained fusion centromere in this translocated chromosome.

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VI.2

Human Artificial Chromosomes and TAR Cloning Technology for Genome Studies and Biomedicine

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Transformation-associated recombination (TAR) cloning allows selective isolation of full-length genes and genomic loci as large circular yeast artificial chromosomes (YACs) in yeast. The method has a broad application for structural and functional genomics, long-range haplotyping, characterization of chromosomal rearrangements, and evolutionary studies. Also, the benefit of combining the TAR gene cloning technology with the HAC (human artificial chromosome) gene delivery system for gene expression studies will be discussed. HAC-based vectors offer a promising system for delivery and expression of full-length human genes. HACs avoid the limited cloning capacity, lack of copy number control and insertional mutagenesis due to integration into host chromosomes that plague viral vectors. Recently, we engineered the HAC with a single loxP gene adapter site and a defined structure and demonstrated its utility for delivery of several full-length genes and correction of genetic deficiencies in human cells. We also showed that phenotypes arising from stable gene expression can be reversed when cells are 'cured' of this HAC by inactivating its kinetochore in proliferating cell populations, a feature that provides a control for phenotypic changes attributed to expression of HAC-encoded genes, thereby aiding in proper interpretation of gene function studies. Also, we demonstrated that HAC-bearing ES cells were indistinguishable from their wild-type counterparts: they retained self-renewal potential and full capacity for multilineage differentiation during mouse development, whereas the HAC itself was mitotically and transcriptionally stable during this process. The HAC vectors have a great potential for gene function studies, gene therapy, regenerative medicine, screening of anticancer drugs and biotechnology.

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VI.3

Applications of Cytogenomic Characterization of Chromosomal Rearrangements to Establish a More Precise Phenotype-Genotype Correlation

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Structural or numerical chromosomal aberrations are detected in 0.6% of newborns and are responsible for variable phenotypic findings which include congenital anomalies, developmental delay, intellectual disability, and infertility. Analysis by classical cytogenetics remains the main tool for diagnosis of chromosomal abnormalities. However, its resolution is limited, leading to difficulties in planning treatment as well as unknown risks for the families due to undefined diagnosis. Microarray-based comparative genomic hybridization (aCGH) allows the analysis of the whole human genome, providing precise information of the copy number of the DNA sequences involved in each genomic region and contributing to the identification of abnormal regions defined by classical cytogenetics. We have evaluated 4 patients with abnormal karyotypes characterized by the presence of additional material (n = 2) and marker chromosomes (n = 2) without a definitive syndromic diagnosis. Cytogenomic studies were conducted using the 2×400K platform (Agilent®), and the results were analyzed by Nexus 7.0 software. Molecular cytogenetic techniques such as FISH and mBand were applied for confirmation of chromosomal breakpoints and to validate the aCGH results. It was possible to provide molecular characterization of Jacobsen syndrome in one patient with a ring chromosome 11, defining the characteristic phenotype of deletion of region 11q24.2q25; to relate characterization of Pallister-Killian syndrome related to the gain in the region 12p11.21p13.31; to diagnose trisomy of the short arm of chromosome 8 by gains in 8p12, 8p11.22p11.23, 8p22, and 8p23.2p23.3 regions; and to provide a phenotypic correlation in one patient with cat eye syndrome, characterized by the gain of 22q11.1. A combination of cytogenetic and cytogenomic analysis in patients with structural chromosome aberrations further elucidated their diagnosis and established a more accurate correlation between karyotype, phenotype, and genotype.

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VI.4

Genomic Architecture Facilitating Interstitial *PTEN* Deletions in Prostate Cancer

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The high frequency of *PTEN* tumor suppressor gene deletions at 10q23.3 and the recurrent activating genomic fusion rearrangements of *ERG* with the *TMPRSS2* gene because of a deletion at 21q22 in prostate cancer draw attention to the importance of somatic intrachromosomal deletions in this neoplasia. Deletion of *PTEN* at 10q23.3 occurs in 30% of human prostate cancers and is associated with aggressive metastatic potential, poor prognosis, and androgen-independent disease. This high frequency of recurrent *PTEN* deletions in prostate cancer suggests that there may be unusual genomic features close to this locus that facilitate intrachromosomal deletions in the DNA flanking the *PTEN* genomic region. To explore possible mechanisms for deletions in the *PTEN* region, a meta-analysis of copy number variation (CNV) in 662 published human genome array datasets was conducted. The most common loss was 8p (NKX3.1). The CNV distribution in other genomic subgroups was characterized by losses at 2q, 3p, 5q, 6q, 13q, 16q, 17p, 18q, and *PTEN* (10q23.3), and acquisition of 21q22 deletions associated with the *TMPRSS2-ERG* fusion rearrangement. Parallel analysis of advanced and primary tumors in the cohort indicated that genomic deletions of *PTEN* and the *TMPRSS2-ERG* gene fusion were enriched in advanced disease. The overall percentage of CNVs was significantly higher when *PTEN* was deleted, suggesting that tumors with this deletion were characterized by intrinsic chromosomal instability. Mapping of the CNVs on chromosome 10 leading to *PTEN* interstitial deletions showed that the common breakpoint region was between *BMPR1A* and *PTEN*. Furthermore, this breakpoint interval coincides with a repeat-rich region of 400 kB containing segmental duplications, which comprise at least 13 homologous inverted repeat sequences. Together, these data suggest that a strong selective growth advantage for loss of *PTEN* and subsequent upregulation of PI3K/AKT, combined with the close proximity of *PTEN* to a large unstable segment of repeated DNA, could lead to recurrent interstitial deletions of the *PTEN* gene in prostate cancer.

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VI.5

Chromosome Therapy: Correction of Large Chromosomal Aberrations via Ring Chromosomes in Induced Pluripotent Stem Cells (iPSCs)

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Approximately 1 in 500 newborn infants are born with chromosomal abnormalities that include trisomies, translocations, large deletions, and duplications. There is currently no therapeutic approach for correcting such chromosomal aberrations in vivo or in vitro. Recently, we attempted to produce induced pluripotent stem cell (iPSC) models from patients that contained ring chromosomes: one with a ring chromosome 17 (r17) and 2 patients with different ring chromosomes 13 (r13). Surprisingly, while all 3 of the lines were reprogrammed to iPSCs efficiently, the ring chromosomes were eliminated and replaced by a duplicated normal copy of chromosome 17 in the r17 line and normal copies of chromosome 13 in the r13 lines [Bershteyn et al., 2014] (reference to be obtained by the author). This finding suggested a potential therapeutic strategy to correct large-scale chromosomal aberrations. We hypothesize that a chromosome with a large aberration could be corrected by producing a ring chromosome from the aberrant chromosome in iPSCs, which would then be eliminated and replaced by a normal chromosome. We are testing this hypothesis by attempting to induce ring formation in patients with large deletions of chromosome 17 via a Cre/loxP approach. If successful, we will have a generalizable system of 'chromosome therapy' for the correction of large chromosomal aberrations by the induction of ring chromosomes through genome editing followed by loss of the ring and duplication of the normal chromosome.

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Posters and Short Talks

VI.6

Duplication 16q11.2q21: A Region Associated with Sexual Dimorphism

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Partial trisomy 16q is a rare chromosomal rearrangement in live-born infants. Affected patients carrying a partial trisomy 16q have a wide clinical spectrum. We present clinical and cytogenomic data from a male patient with a de novo 16q11.2q21 in tandem duplication. The patient was born at 41 weeks gestation after an uncomplicated pregnancy. He presents bladder exstrophy with ambiguous genitalia, epispadia, bilateral inguinal hernia, cardiac malformations, and dysmorphic features. Cytogenomic analysis was performed by GTG-banding, chromosomal microarray, and FISH which revealed

the final result as 46,XY.arr 16q11.2q21(46,441,545–62,089,933)×3 dn. The in silico analysis showed that in this trisomic region there are 2 candidate loci: (1) a metallothionein *(MT)* gene cluster at 16q13, and (2) a testis, prostate and placenta expressed gene *(TEPP)* at 16q21. Since glucocorticoid hormones regulate the expression of metallothionein genes as well as the increase in glucocorticoid receptors which has been associated to sexual dimorphism, it is possible that urogenital abnormalities observed in our patient may be due to the increase in glucocorticoids response elements. In this way, we suggested for the first time that 16q13 and 16q21 could be critical regions to the development of sexual diseases.

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VI.7

Genomic and Chromosome Characterization of 'Kettu' Triticale by Cytogenetic and Molecular Techniques

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Triticale (wheat × rye) is an excellent resource as animal food. Kettu is a triticale obtained at the Universidad Nacional de Río Cuarto (UNRC, Argentina). This hybrid has a complex origin because it has undergone repeated backcrosses. As a result, there are doubts about the current chromosome composition. Kettu has been introduced and used in Argentina as part of the UNRC program for germoplasm development of tricepiro, a trihybrid product between triticales × trigopiros. The aim of this work was to determine the genome and chromosome composition of Kettu triticale through molecular and cytogenetic techniques. In situ hybridization with total genomic DNA from rye as a probe (GISH) allows detecting the presence of the R genome, while the use of a highly repeated DNA sequence of *Secale cereale* (pSc119.2) allowed the identification of R chromosome of rye and the A and B chromosomes of wheat. To determine the presence of D genome chromosomes we used specific microsatellites. The results obtained indicated that Kettu is a hexaploid triticale (2n = 6x = 42 chromosomes). After performing FISH and GISH studies, we detected that this hybrid contains all 14 rye chromosomes and all 14 chromosomes of the B genome of wheat. The remaining 14 chromosomes would correspond to the A and D genomes of wheat. The use of specific microsatellites for each chromosome of the D genome allows determining the presence of 5, 6, and 7 chromosomes of this genome. These results confirm that the simultaneous use of molecular markers and cytogenetic techniques are a useful tool for breeding programs that involve the use of this hybrid and allow knowing the evolution and stabilization of tricepiros that contain a Kettu triticale as one of the parents.

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VI.8

Cytogenetic Characterization of Sex Chromosomes in the Y-Short *Anastrepha fraterculus* Strain

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The South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae), has a XX/XY sex determination system. Information about tephritid species reported the presence of genes encoding male-determining factors linked to the Y chromosome, although no information is available for the species we study. Cytological analysis performed in the *A. fraterculus* strain maintained in our laboratory enabled the identification of 2 of the 6 reported morphological variants of Y chromosomes (Y_5 and Y_6). An *A. fraterculus* strain carrying Y_5 (shorter and more frequently found) was purified and analyzed. We performed the characterization of the Y heterochromatin by DAPI/CMA and C-bands and compared our results to the *A. fraterculus* reference karyotype (which includes a short and submetacentric Y chromosome with its constitutive heterochromatin distributed in almost the entire chromosome and has AT- and GC-rich regions). The Y-short *A. fraterculus* population retains the same heterochromatin distribution of the original population as was expected. The constitutive heterochromatin extends throughout almost the entire Y chromosome showing AT- and GC-rich regions. Furthermore, we identified an AT-rich heterochromatin in both termini of the X chromosome. Further analysis by FISH co-localized the ribosomal genes in the constitutive heterochromatin of the Y chromosome, whereas the ribosomal gene cluster on the X chromosome is localized at the terminal position on the long arm. This cytological characterization provides new insights into further exploring *A. fraterculus* genetics towards the identification of male-determining genes in this pest species and the selection of suitable strains for the development of environmental-friendly control strategies.

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VI.9

Point Mutation of *CENH3* (CENP-A) Impairs Loading to Centromeres and Induces Haploid Plants

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Centromere-specific histone H3 variant CENH3 (CENP-A) is essential for establishing functional kinetochores in many organisms. Any error in transcription, translation, modification, or in-

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corporation can affect the ability to assemble intact CENH3 chromatin and can cause centromere inactivation. Here, we show that a single point amino acid exchange in the centromere target domain of CENH3 leads to reduced CENH3 loading to centromeres in barley, sugar beet, and *Arabidopsis thaliana*. We generate cenh3 L130F-complementation plants in *A. thaliana* cenh3-null mutants. Crosses between cenh3 L130F-complemented plants and wild-type *A. thaliana* plants generate wild-type haploid offspring. On the other hand, cenh3 L130F-complementated plants do not generate haploid offspring after selfing. Those results suggest that only a competitive situation during early embryogenesis generates uniparental chromosome elimination in *A. thaliana*. The high degree of evolutionary conservation of the identified mutation site offers promising opportunities for application in a wide range of crop species in which haploid technology is of interest.

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VI.10

FISH Confirmation of a Reciprocal Translocation in Chestnut

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The American chestnut (*Castanea dentata*, 2n = 2x = 24), once known as the 'King of the Appalachian Forest', has been nearly decimated by chestnut blight caused by a fungal pathogen *(Cryphonectria parasitica)* that was accidentally imported from Asia in the late 1800s on nursery stock of Chinese chestnut (*C. mollisima*), which is resistant to the chestnut blight disease. Efforts are being made to transfer blight resistance gene(s) from Chinese chestnut into American chestnut using backcross breeding and genetic engineering. Interspecies crosses often unveil structural differences such as translocations, inversions, and/or insertions/deletions of chromosomal segments. Kubisiak et al. [1997] (reference to be obtained by the author) first developed a genetic map of chestnut using an inter-specific F2 family where the formation of 2 of the 12 genetic linkage groups were not resolved but formed a single complex group. A later discussion of these results raised a possibility of the presence of a reciprocal translocation involving linkage groups (LGs) B and E. In an earlier meiocyte analysis of an F1 tree we confirmed the presence of reciprocal translocation, but FISH of mitotic chromosome spreads indicated that LGs B and E were not involved (unpublished). More recent genetic mapping data (unpublished) has indicated that *C. mollissima* 'Vanuxem' is heterozygous for a reciprocal translocation involving LG_H and LG_L. Presently, FISH is being performed in mitotic (prophase and metaphase) and meiotic (pachytene) chromosome spreads of 'Vanuxem' using LG-specific BAC clones to explore this apparent H/L translocation and to study the structural details of this genome. Our preliminary FISH data for BACs from either LG show hybridization signals on 2 homologous chromosome pairs (mitotic metaphase chromosome spread), which is an indication of structural rearrangements that have had occurred between these 2 specific chromosomes. We also observed 4 of the 6 BACs from LG_L (all labeled with digoxigenin hapten and detected with green fluorochrome), selected across its entire length (2.7, 16.0, 22.5, 31.0, 35.6, and 47.0 cM), hybridized to about 40% of the pachytene chromosome, while the other 2 BACs hybridized towards the distal 10% of the opposite arm of the same chromosome, leaving about 50% of the chromosomes with no BAC-FISH signal. In addition, 1 of 7 BACs from LG_H (all labeled with biotin hapten and detected with spectrum orange fluorochrome), selected across its entire length (1.3, 6.3, 14.9, 17.5, 36.0, 46.0, and 57.9 cM), hybridized close to one of the LG_L BACs (positioned a third from one end of the chromosome or positioned fourth from the other end), leaving about 50% of the chromosome with no BAC-FISH signal. This undetected portion of the LG_L chromosome could be a region of a translocated segment from the LG_H chromosome. Additional research is needed to confirm this hypothesis of reciprocal translocation in chestnut.

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VI.11

Karyotype-Phenotype-Genotype Correlation in a 4p;12q Rearrangement

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Structural chromosomal rearrangements are potentially associated with the development of genetic disorders due to disruption, inactivation, or gene dosage alterations. We report the genomic characterization of structural chromosomal rearrangements involving the 4p and 12q regions in 2 cousins (P1 and P2), using a combination of classical cytogenetics, FISH, and array-CGH. Both patients shared the same chromosomal abnormality, global developmental delay, and craniofacial dysmorphims but had distinct phenotypic findings. P2 was more severely impaired and showed a severe mental disability, seizures, midface hypoplasia, microtia and deafness, which was absent in P1. Our analysis demonstrated a 4p16 deletion and trisomy 12qter for both patients

[46,XX,der(4)t(4;12)(p16;q24.3)]. Array-CGH (2×400K Platform, Agilent®) analysis showed a small difference in size between the losses and gains in the rearranged region. P1 was 4p16.3(73,629–2,780,849)×1;12q24.31q24.33(121,446,691–133,851,895)×3 and P2 was 4p16.3(69,881–2,780,849)×1;12q24.31q24.33(121,458,011–133,851,895)×3. The 12q24.31q24.33 duplication was 11.3 kb larger in P1, comprising 139 genes and 138 genes for P2. The 4p16.3 loss was 3.7 kb larger in P2, involving 53 genes for both patients. The exclusively duplicated region in P1 contained *C12orf43*, a cholesterol regulator associated with coronary diseases. The deleted region of chromosome 4 comprised the Wolf-Hirschhorn syndrome critical region (OMIM#194190) *WHSC1*, *WHSC2,* and *LETM1*. *WHSC1* and *WHSC2* losses are associated with intellectual disability, development delay, and craniofacial dysmorphisms, similar to those described in both patients. *LETM1* gene loss is correlated with seizures, but only P2 presented seizures, suggesting that other mechanisms may be associated with this finding. Our results are in agreement with the gene dosage effects described in the literature, indicating that monosomies have more severe impacts on patients than trisomies. This study has provided a precise definition of the breakpoints at 4p16.3 and 12q24.31q24.33, as well as the parental origin of the rearrangement for improved genetic counseling.

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VI.12

Cytogenetically Visible Copy Number Variations (CG-CNVs) in Banding and Molecular Cytogenetics of Human: About Heteromorphisms and Euchromatic Variants

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Copy number variations (CNVs) having no (obvious) clinical effects have been rediscovered as major part of the human genome in 2004. However, for every cytogeneticist, microscopically visible harmless CNVs (CG-CNVs) are well known since decades. Harmless CG-CNVs can be present as heterochromatic or even as euchromatic variants in clinically healthy persons. Here, I provide a review on what is known today on the still too little studied harmless human CG-CNVs, point out which can be mixed up with clinically relevant pathological CG-CNVs, and shortly discuss that the artificial separation of euchromatic submicroscopic CNVs (MG-CNVs) and euchromatic CG-CNVs is no longer timely. Overall, neither so-called harmless heterochromatic nor so-called harmless euchromatic CG-CNVs are considered enough in evaluation of routine cytogenetic analysis and reporting. This holds especially true when bearing in mind the so-called 2-hit model suggesting that combination of per se harmless CNVs may lead to clinical aberrations if they are present together in one patient.

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VI.13

Acute Myeloid Leukemia with Translocation t(6;8)(q25;q22) and Loss of the Y Chromosome

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Acute myeloid leukemia (AML) is a heterogeneous disease characterized by an excessive accumulation of immature myeloid blasts in the bone marrow preventing normal hematopoiesis. Molecular and cytogenetic abnormalities in AML consist of mutations in genes that affect normal cell proliferation and development. One of the most frequent nonrandom karyotypic abnormality associated with AML is the translocation t(8;21)(q22;q22), especially in subtype M2 according to the French-American-British (FAB) classification. The t(8;21)(q22;q22) involves the genes *RUNX1* (*AML1*) and *RUNX1T1* (*ETO*) at 8q22 and 21q22, respectively. We report a case of a 9-year-old male patient with AML M2 whose cytogenetic analysis in bone marrow revealed 2 clones. One abnormal clone with the karyotype 45,X,–Y,t(6;8)(q25;q22) and another normal 46,XY clone in a 3:1 rate. Immunophenotypic study showed an immature population positive for CD19 and CD56 markers. The patient started induction of chemotherapy without good response, after approximately 5 months he was dead. Cases with t(6;8)(q25;q22) have been reported by Berger [1988] in a patient with AML M2 and by Okasuka [2001] in a patient with extramedullary T lymphoid blast crisis Philadelphia-positive chronic myeloid leukemia after allogeneic bone marrow transplantation representing an additional translocation (references to be obtained by the author). A number of cases with t(8;21) could present other abnormalities such as loss of a sex chromosome. Variants of t(8;21)(q22;q22) have also been reported with participation of a third chromosome. The case presented here could be a variant of t(8;21) involving chromosome 6. Further studies such as FISH are needed to confirm this assumption. It is known that t(8;21)(q22;q22) confers a good prognosis but the prognosis of t(6;8)(q25;q22) still remains unclear.

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VI.14

Comparative Cytogenetics among *Vigna aconitifolia* (Jacq.) Maréchal, *V. unguiculata* (L.) Walp. and *Phaseolus vulgaris* L. Using BAC-FISH

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Vigna aconitifolia (Jacq.) Maréchal, commonly called mat bean or moth bean, is a drought-resistant legume, native from India, Pakistan, and Myanmar, that was domesticated in the arid and

semi-arid regions of desert plains of western India. In the present study, a comparative cytogenetic analysis among *V. aconitifolia*, *V. unguiculata* (L.) Walp., and *Phaseolus vulgaris* L. genomes was performed using BAC-FISH of BACs belonging to *P. vulgaris* and sequences of 45S and 5S rDNA on mitotic chromosomes of *V. aconitifolia* (*Va*, 2n = 22). Sequences were previously mapped in *P. vulgaris (Pv)* and *V. unguiculata (Vu)* chromosomes, and in this work, data were compared, contributing to the macrosynteny analysis between both genera. Twenty-five clones from *P. vulgaris* 'BAT93' BAC library, corresponding to 11 linkage groups, were hybridized in situ on *V. aconitifolia* chromosomes. Sixteen showed single sites, 3 showed duplicated sites, and 6 BACs showed no signal. Breaks of synteny were observed between *V. aconitifolia* and *P. vulgaris*, such as translocations (involving BACs from *Pv*2, *Pv*3 and *Pv*9 and BACs from *Pv*1 and *Pv*8) and duplications (BAC from *Pv*11 and BACs from *Pv*2). Two translocation events were also indicated between *V. aconitifolia* and *V. unguiculata*, the first involving *Vu*8-*Vu*3 in relation to *Va*3, and the second involving *Vu*3 in relation to *Va*3 and a second unknown *Va* chromosome. A collinear break was also observed between *V. aconitifolia* and *V. unguiculata*: 2 BACs from *Pv*4 hybridized on opposite arms of *Va*11 as previously reported for *Pv*4 but on the same arm of *Vu*11, indicating a pericentric inversion for chromosome 11 between both *Vigna* species. These results allowed a comparative cytogenetic map construction and demonstrated several macrosynteny and collinearity breaks, collaborating to the understanding of the structure and genome organization and karyotype evolution in both genera from the Phaseoloid clade.

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VI.15

Transcription of Pericentromeric Major Satellite DNA in Lung Cancer

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Tandemly repeated (TR) noncoding DNA of pericentromeric and centromeric regions of chromosomes is the base of constitutive heterochromatin. These sequences can be decondensed and transcriptionally active. The decondensation and transcription of TR are shown both in physiological (cell cycle, development, differentiation) and pathological (tumorigenesis, cell stress) conditions. However, not much is known on how satellite DNA is transcribed during carcinogenesis and whether its transcription is associated with any specific cell type of a heterogeneous tumor mass and could be used as a predictive diagnostic marker. In this work, we used a mouse model of K-ras^{G12D}-induced lung tumorigenesis to investigate the transcription of the pericentromeric major satellite (MaSat) in lung tumor development. This K-ras-induced tumor model is suitable to analyze tumor lesions and stroma, called tumor microenvironment, from the same lung. Firstly, by qPCR and FISH analysis we assessed the level of MaSat transcription in tumor lesions and in tumor microenvironment and normal non-tumor mouse lung was used as a control. In most cases, cells isolated from the tumor microenvironment showed higher MaSat transcription than tumor lesions as shown by qPCR. However, transcription of MaSat in tumor lesions was consistently reduced compared to normal lung. To understand the mechanisms of up-regulation of MaSat DNA in the lung tumor microenvironment, we focused on cancer-associated fibroblasts which are major cell components in the tumor stroma and undergo re-modelling during tumor progression such as activation by tumor-secreted factors to create a niche for beneficial tumor cell growth. We found that transcription of MaSat DNA was upregulated in response to treatment with TGFb to activate fibroblasts into a cancer-associated subtype. Our results suggest that transcription of mouse MaSat is upregulated in lung fibroblasts during cancer-associated remodelling and could be a source of cells with transcriptionally active satellite DNA in a lung tumor microenvironment.

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VI.16

Case Reports with t(8;14)(q11;q32) in Mixed-Phenotype Acute Leukemia and Acute Lymphoblastic Leukemia

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The translocation t(8;14)(q11;q32) is a non-common abnormality found in acute lymphoblastic leukemia (ALL) that affects the genes *CEBPD* (8q11) and *IGH* (14q32). The most frequent abnormalities associated with t(8;14)(q11;q32) are the gain of chromosome X, trisomy 21 as an acquired abnormality, and t(9;22)(q34;q11). We report 2 cases of female patients with acute leukemia and t(8;14)(q11;q32). The first one is a 6-year-old patient with mixed-phenotype acute leukemia (MPAL) with the karyotype 46,XX,t(8;14)(q11;q32),del(13)(q14q32)[16]/46,XX[4] in bone marrow, negative for fusion genes in ALL *(E2A/PBX1, MLL/AF4, BCR/ABL p190, BCR/ABL p210, TEL/AML1)*, negative for fusion genes in acute myeloid leukemia (AML) *(AML1/ETO, CBFB/MYH11)*, and negative for common mutations in AML *(FLT3, NPM1)* as detected by PCR. Immunophenotype study was positive for lymphoid lineage markers: CD45, CD19, CD79a, CD34, CD38, CD10, CD66c, CD81, CD123, CD9, nuTdT, CD24, HLA-DR, but weakly positive for cytoplasmatic myeloperoxidase (cyMPO). The second case is a 21-year-old patient with ALL type B, with the karyotype 47,XX,+X,t(8;14)(q11;q32)[8]/46,XX[12], negative for the fusion genes in ALL detected by PCR. Immunophenotype study was positive for the markers: CD45, CD19, CD79a, CD34, CD66c, CD10, CD15, CD20, CD38, CD81, CD123, CD9, and NG2. To date and according to the Mitelman database, 68 cases of ALL with t(8;14)(q11;32) have been reported. Our patients are receiving chemotherapy; the first case began 5 months ago and the second case 2 weeks ago. We are still following them to evaluate the prognosis of this abnormality.

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VI.17

β-Catenin Targeting Cytokinesis for Ovarian Cancer Metastasis Control

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Metastasis remains the major cause (>90%) of cancer deaths. Despite aggressive treatment, the rates of cancer patient survival in the past several decades have not effectively improved. Thus, alternative conceptual and practical approaches are required. It is now increasingly clear that the tumor microenvironment, rather than tumor cells per se, is the key determining factor for tumor progression. However, previous studies have focused mainly on tumor cells, the microenvironment remains poorly understood, especially cells of the immune system, which are the most important regulators of metastasis in the microenvironment that can affect tumor behavior. Recently, we have developed a new isogenic ovarian cancer model with different metastatic properties to unravel key factors mediating metastasis. HM was unique in its ability to metastasize consistently to the peritoneum, mimicking the major dissemination route of human ovarian cancer. In contrast, NM failed to form detectable metastases although it was equally tumorigenic. Interestingly, in our pilot studies, we showed for the first time that HM, but not NM tumor cells, have a selective advantage in activating M1 macrophages to tumor-associated macrophages, which supported tumor cell growth. Interestingly, this interaction also significantly increased the ploidy level in a significant fraction of HM. β-catenin, whose expression was activated in HM, but not NM, plays a key role in this process. These findings provide important new mechanistic insights in tumor metastasis with implications for novel treatments.

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VII. New Technologies

Invited Talks

VII.1

Fully Automated FISH Analysis for B-Cell Chronic Lymphocytic Leukemia: A Faster Alternative to Manual Double Scoring

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Automated imaging can assist in the scoring of common FISH tests associated with B-cell chronic lymphocytic leukemia. We have demonstrated a concordance of results between CytoVision GSL-120 and manually scored slides of 100% for the panel of Kreatech FISH probes. The talk aims to highlight the importance of slide preparation and quality control when implementing automated imaging in the FISH lab, and also presents workflow and efficiency gains offered by implementation of the Leica CytoVision GSL-120 scanning system when used in conjunction with Kreatech FISH probes. Leica Biosystems continues to develop complete FISH solutions with our novel repeat free Leica Biosystems Kreatech FISH probes and our world leading CytoVision imaging platform.

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VII.2

Chromosome-Centric Approaches to Plant Genomics

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The analysis of the nuclear genome in plants has been facilitated by new DNA sequencing technologies, which excel in high throughput. Nevertheless, the production of high quality reference genome assemblies in species with large and polyploid genomes remains challenging due to DNA sequence redundancy. To overcome these difficulties, our team has developed an approach, which consists in dissecting nuclear genomes to their functional units – chromosomes. This is achieved by isolating intact mitotic chromosomes from synchronized root tip meristems and their sorting by flow cytometry. DNA of flow-sorted chromosomes is intact and suitable for all applications of molecular biology and genomics. This complexity reduction approach is suitable for de novo genome assembly and facilitates validation of whole genome shotgun assemblies. Sequencing particular chromosomes, such as B chromosomes and sex chromosomes, enables the analysis of their structure and origin. Other attractive applications of chromosome genomics include identification of chromosomes with integrated transgenes, characterization of alien chromatin in alien introgression lines, and development of molecular markers from chromosomes of interest. As re-sequencing of large genomes remains expensive, chromosome sorting offers a targeted and cost-effective approach to sequence only the chromosome of interest, if needed isolated from higher number of individuals. In some species, chromosomes cannot be discriminated from each other due to similarity in size. A solution is to sort single copies of chromosomes and amplify their DNA. Although this procedure does not provide high molecular weight DNA, it delivers DNA from a particular chromosome even if it cannot be resolved from other chromosomes. The number of uses of flow-sorted chromosomes in plant genomics keeps growing, and the approach can be used in any species from which a liquid suspension of intact mitotic chromosomes can be prepared.

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VII.3

Peeling the Onion – The Epigenetic DNA Modification Layers of Chromatin

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The chromatin structures are very complicated complexes of genomic DNA, many types of RNAs, and a myriad of proteins. All of these components are synchronized to interpret the meaning of DNA that lays the blueprint of all lives. It is well know that epigenetic machineries are essential elements for gene control and regulation. Besides histones and other regulatory proteins, DNA methylation and hydroxymethylation have been shown to be essential in gene regulation of all high organisms. We examined the DNA methylation and hydroxymethylation effects on the overall DNA structure and its relation to the double helix stability. Additionally, using high resolution melt methods, we performed thermodynamic stability analysis of synthetic DNAs comprised entirely of cytosine, 5-methylcytosine (5-mC), 5-hydroxymethylcytosine (5-hmC), and 5-glucosylmethylcytosine (5-gmC) at all cytosine positions. Structurally, the presence of 5-mC in DNA serves to increase the thermodynamic stability of the double helix, but interestingly, the presence of 5-hmC in DNA decreased the thermodynamic stability. This is consistent with the general notion that DNA methylation tightens the helix, making it more refractory to proteins and transcriptional repression, while hydroxymethylation may serve the opposite function, to open the helix and make it more receptive to ancillary factors involved in transcription and/or elements of transcriptional activation or repression. Therefore, the epigenetic modifications of DNA contribute to additional dynamic layers of chromatin structure.

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VII.4

Hidden Features of Satellite DNA Revealed by Novel Sequencing and Bioinformatic Approaches

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Satellite repeats are among the major constituents of complex eukaryotic genomes, yet they are usually only poorly characterized even in extensively studied species. This is mainly due to their genomic organization into long arrays of nearly identical repeated units which represent a serious obstacle to satellite DNA assembly and further investigation using conventional approaches. This problem becomes even more evident when dealing with genome shotgun sequencing data provided by currently used next-generation sequencing platforms. In this talk, I will give an overview of emerging approaches which can be used to overcome the limitations of assembly-based satDNA investigation. Novel bioinformatic pipelines will be discussed that allow assembly-free identification and characterization of satellite repeats from short next-generation sequencing reads, based on a combination of graph-based read clustering and *k*-mer analysis. Examples will be provided demonstrating efficiency of these approaches, and their ability to reveal a wide range of satellite repeats including those with extremely long monomers. In addition, I will present data obtained from several plant species using the latest generation of single molecule sequencer, the Oxford Nanopore MinION, which can produce ultra-long reads up to 100 kb in length. Such reads can be employed to directly reveal internal structures of long arrays of satellite DNA, thus circumventing error-prone assembly of these regions from the short reads.

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VII.5

Introduction to Enza Zaden

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Enza Zaden's head office is in the Dutch town of Enkhuizen. The company was established by Jacob Mazereeuw in 1938. Enza Zaden is active worldwide with about 1,700 employees of more than 40 nationalities. The Dutch region in which Enza Zaden's head office is located is also known as 'Seed Valley'. That's the part of the Netherlands between the towns of Enkhuizen and Warmenhuizen in the province of Noord-Holland. Dozens of companies specializing in breeding, production and sale of high-quality seeds and propagating material can be found here. All those companies have joined forces in the Seed Valley foundation with the aim of strengthening their cooperation and anchoring the companies and the sector in the province of Noord-Holland. **Vitalis:** In the past decade the popularity of organic vegetables has grown tremendously all over the world, causing a substantial increase in the demand for strong, organically grown varieties of top quality. Our subsidiary Vitalis Biologische Zaden (Organic Seeds) is a completely organic vegetable breeding company catering to professional organic growers. Vitalis supplies a wide range of organic vegetable seeds of distinctive quality to customers all over the world. The organically certified seeds are produced and processed using exclusively organic methods. They consequently meet the specific requirements and wishes of both organic consumers and organic growers. **KeyGene:** KeyGene is constantly searching for new ways of making our breeding processes faster and more efficient. Enza Zaden is one of the founding fathers of KeyGene, one of the largest biotechnological companies in the world specializing in vegetable genetics. The company performs pre-competitive research to enable the participating breeding companies to make their breeding programs more effective and more efficient. Besides searching for new markers for more genes, KeyGene also develops software with which analytical methods and breeding programs can be more easily recorded.

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VII.6

Detection of Partial Deletions of the Y Chromosome AZFc Region in Infertile Men in a Group of Brazilians Attending an Infertility Service Using the Multiplex Ligation-Dependent Probe Amplification

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Microdeletions of the Y chromosome are the second most frequent genetic cause of spermatogenetic failure in infertile men, occurring in 15%. Thirty-two samples of men with azoospermia (13/28) or severe oligozoospermia (15/28) with idiopathic infertility who attended the Human Reproduction Laboratory of the University Hospital (LabRep-HC), Federal University of Goiás (UFG) were analyzed. The samples were collected and characterized with the SALSA MLPA probemix P306-A1 to detect deletion/duplication of one or more DNA sequences of AZF. The kit contains 53 probes, 14 probes of the AZFa region, 16 probes of the AZFb region, 13 probes of the AZFc region, 2 probes of the *DPY19L2* gene (located on chromosome 12), and 9 probes of controls (autosomal regions). We used the Coffalyser software from NET (MRC-Holland) to evaluate the MLPA quality and data. We observed that 85.7% (24/28) of patients have standard amplification of the probes within the normal range and 14.2% (4/28) of patients (3 azoospermia patients and 1 oligospermia patients) showed amplification alterations. The oligospermic patient presented 7 duplication probes (CDY2A_2, BPY2_4, BPY2_5, DAZ2_2, DAZ2_3, RBMY2CP, CDY1B). One azoospermic patient presented 3 duplication probes (KDMS_4, BPY2_2, PPP1R12BP), the second 11 deletion probes (CDY2A_3, BPY2_1, BPY2_2, BPY2_3, BPY2_4, BPY2_5, DAZ2_1, DAZ2_2, DAZ2_3, RBMY2CP, CDY1B), and the third 8 deletion probes (CDY2A_2, BPY2_4, BPY2_5, DAZ2_1, DAZ2_2, DAZ2_3, RBMY2CP, CDY1B). These probes are located in the AZFc region. The AZFb and AZFc regions are responsible for initiating the process of spermatogenesis, and the AZFc region is responsible for termination or maturation. The partial duplication of the AZFc region is associated with male infertility. Changes in the AZFa region were not detected in any patient. This study confirmed that male infertility in azoospermia or severe oligozoospermia cases with an unknown cause is associated with a microdeletion of the Y chromosome. Array-CGH studies will be done to confirm these changes.

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VII.7

DNA Crystals Exhibit a Twisted-Grain-Boundary-Columnar (TGBC) Texture

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Columnar and lateral order arrangements have been reported for non-smectic DNA mesophases. Simultaneous presence of helical and columnar textures has also been considered for DNA molecules. Although these textures are typical of recently described twisted-grain-boundary-columnar (TGBC) liquid crystals, it has not as yet been considered in DNA studies. With the object of finding out whether DNA can exhibit a TGBC texture, 6 mg/ml highly polymerized thymus DNA (Sigma-Aldrich) in saline solution was analyzed in the present work using advanced polarized light microscopy, the elected methodology for liquid crystal studies. A volume of 10 µl drops of this solution was dripped on special glass tension-free slides for differential interference contrast-polarized light microscopy. Pre-liquid crystals and crystals were observed to develop from the edge surface to the center of the DNA drops obeying a drying gradient. The DNA crystals were studied rotating the microscope stage; in all relative positions acquired by these crystals, a birefringence phenomenon could be detected. The most characteristic crystal morphology was observed when the long axis of the crystals was oriented parallel to the analyzer's azimuth, coinciding with the drop's edge surface, which was perpendicular to such an azimuth. Under these conditions, a twisting texture was exhibited by the columns, whereas an intertwisting fibrous texture occurred between the columns. A 3D overlap of these structures was also observed. These results allowed concluding, for the first time, that DNA crystals have TGBC characteristics probably generated from TGBC liquid crystals' mothers. These properties were the consequence from self-assembly, self-organization laws that are mandatory during formation of supramolecular structures, including that of the DNA. The capacity of DNA to lead to the production of highly supramolecular constructs with TGBC properties probably generates a stereoarrangement that plays a part in the expression of the gene machinery.

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VII.8

Investigating the Effect of CRISPR-dCas9-Based Binding of the Centromeric Histone H3 Variant CENH3 to Non-Centromeric Loci

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Plant artificial minichromosomes were proposed as promising tools in improving crop production and plant breeding by enabling the stacking and linked transfer of multiple genes required for complex traits. The construction of minichromosomes is

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thought to be achieved by either a 'top-down' or 'bottom-up' approach, the first focusing on reducing a chromosome down in size to its essential constituents and the latter aiming to assemble these constituents de novo and introduce them into plant cells. Proof-of-concept for the 'top-down' approach was achieved by telomere-mediated chromosomal truncation in *Arabidopsis*, maize, barley, and rice. Genome engineering has made significant progress since the introduction of the CIRSPR-Cas9 system to eukaryotes. Apart from precisely introducing mutations, a catalytically dead version of Cas9 (dCas9) fused to transcription modifiers was used to impact on gene expression patterns in a number of organisms including plants. Here, we present a novel approach of linking a centromeric protein to dCas9. We fused dCas9 with the centromeric histone H3 variant CENH3 in order to tether CENH3 to non-centromeric loci in *A. thaliana*. First, we are exploring the feasibility of this approach by simultaneously visualizing CENH3 localization and dCas9 target regions. Second, we are hypothesizing that the initial binding of CENH3 might attract other members of the plant kinetochore complex. Therefore, we are investigating whether targeted binding of CENH3 to non-centromeric loci might lead to de novo centromere formation. Since de novo centromere formation initiates a condition under which a chromosome carries 2 centromeres, we are focusing on the occurrence of anaphase bridges.

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VII.9

Quantitative Morphometric Analysis as a Tool for Investigating the Evolution of Sperm Morphology and Nuclear Organisation in Mouse

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Every species has a characteristic sperm shape, and alterations in sperm morphology have been associated with fertility defects in multiple species including mouse, rat, pig, cattle, and human. Sperm morphological abnormalities can be due to inherited genetic factors, disease, exposure to environmental toxins such as xenoestrogens and anti-androgens, or DNA damage by factors such as ionizing radiation. Accurate assessment of sperm morphology is therefore of use when characterizing novel phenotypes in transgenic mouse models and has also been applied in toxicological studies as an indication of the genotoxicity of a given test compound. Historically, sperm morphological analysis has generally been carried out by manual scoring, which is laborious, requires significant amounts of training, and suffers from a lack of consistency between observers. We have therefore designed a novel image analysis package for detailed analysis and comparison of nuclear shapes both within and between populations. We show that this can readily distinguish the shapes of sperm from different laboratory mouse strains and (in reciprocal F1 hybrid crosses) demonstrate autosomal and Y-linked inheritance for aspects of sperm head shape. Within the nucleus of both somatic cells and sperm, chromosomes are known to occupy specific sub-nuclear 'addresses' known as chromosome territories. Previous work has shown that this level of organization may be conserved between species, and in particular that in mammalian sperm the sex chromosomes appear to occupy a privileged location underneath the acrosome. To date chromosome positioning has only been assessed in morphologically simple, symmetrical sperm such as the ovoid spatulate sperm found in humans, pigs, and cattle and the linear fibrillar sperm found in birds. We show by FISH in combination with sperm morphology analysis that the location of chromosome territories is conserved across a range of mouse species, and in particular that the X and Y chromosomes occupy the same sub-acrosomal region as in other mammalian species.

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VII.10

Invadolysin: From 'Ugly Chromosomes' to Serum Protease

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We have utilized *Drosophila* as a model system to identify novel and conserved genes essential for progression through the cell cycle and normal cellular physiology. From our analyses of mutations that gave rise to abnormally condensed chromosomes, we identified invadolysin – a zinc-metalloprotease that we have shown to link cell division and cell migration in *D. melanogaster* [McHugh et al., 2004]. Invadolysin localizes to lipid droplets in mammalian cell lines, and *D. invadolysin* mutants have a decreased triglyceride:protein ratio [Cobbe et al., 2009]. Invadolysin is the first metalloprotease localized to lipid droplets, and physiological functions remain at this point speculative. Invadolysin additionally interacts with mitochondrial ATP synthase subunits [Di Cara et al., 2013] and plays a role in angiogenesis [Vass and Heck, 2013]. As many proteases function in catalytic pathways, it is intriguing that the first genetic interactor of invadolysin is a ubiquitin protease (nonstop) – targeting histone H2B, and thereby linking to the chromosome defects we observed initially [Gururaja Rao et al., 2015] (references to be obtained by the author). Interestingly, mono-ubiquitinated H2B was elevated in both *invadolysin* and *nonstop* mutants but localized surprisingly to the cytoplasm. As core histones (along with many other proteins) are stored in lipid droplets, we speculate that lipid droplets may play an as yet unidentified role in chromatin dynamics. We have recently discovered that a secreted form of invadolysin is present in vertebrate serum and invertebrate hemolymph. As the gene is essential for life, we hypothesize that the secreted form of invadolysin may be playing a crucial role to normal physiology. We plan to address whether this novel form of invadolysin serves as a potential biomarker for any human disease states.

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VII.11

Characterization of Translocation by Chromosome Sequencing on Flow-Sorted Chromosomes: Robust Methods for Identification of Genomic Breakpoint Junctions

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Chromosome translocation is a key feature in chromosome abnormalities and can lead to the formation of a fusion gene. Although it is identified by cytogenetic analysis based on banding patterns or chromosome painting, it is hard to characterize the breakpoint at the sequence level. Chromosome sorting by flow cytometry shows flow karyotypes and enables us to generate chromosome painting probes. Abnormal chromosomes are often found to form weak peaks in the flow karyotypes, allowing distinguishing them from normal alleles. In this study, we sorted derivative chromosomes in a human tumor cell line, Ishikawa 3-H-12, and a dog cell line, MDCK, to characterize their genomes. Approximately 2,000 chromosomes of t(9;14) from the Ishikawa cell line and t(27;X) from MDCK were amplified by a WGA kit used for preparation of the genomic DNA fragment library. Chromosome-specific sequencing was performed by the Ion PGM sequencer. The breakpoint junction in der(9) was identified at 9p24.3 and 14q13.1, with the formation of a fusion gene. Sequence analysis of coding regions around 14q13.1 based on the Ion Ampliseq technology showed the differences of SNP frequencies between the upstream and downstream regions of the breakpoint junction. The genomic breakpoint junctions unique to each cell line can be precisely determined through chromosome sequencing.

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VII.12

Analysis of a Noncoding Region of the *SIX3* Gene in Patients with Holoprosencephaly

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Holoprosencephaly (HPE) is characterized by a defect of the middle line of the embryonic forebrain, when a segmentation failure of the previous neural tube occurs. Mutations in the *SHH*, *ZIC2*, *SIX3* genes were detected and are related to 33% of cases, but few studies about the noncoding region are available. Mutations in the gene *SIX3* are present in 1.3% of HPE cases in humans and are associated with a complex phenotype, varying from a single central incisor to ciclopy. A total of 44 individuals with HPE, registered at the database of the Rehabilitation Craniofacial Anomalies Hospital, USP, Bauru, were analyzed by next-generation sequencing in the laboratory of the National Human Genome Research – National Institutes of Health, Bethesda, Md., USA. The Illumina Platform HiSeq2000 was used and the analysis made in paired-end reads of 100 bp to analyze noncoding elements of the *SIX3* gene in 15 male and 29 female samples with heterogeneous phenotypes of HPE. All patients have some type of cleft, hypotelorism, and microcephaly. We found 28 variants, 27 SNPs and 1 indel. Twenty alterations have already been described, while 8 other dbSNP have not been described; 24 are located in the intergenic region, 2 in the 3′ UTR, and 1 upstream of the gene. Although the mapping of complex disease genes is difficult, an increase in the number of susceptibility genes has been identified as a result of the availability of the complete genomic sequence, dense marker maps, and high yield genotyping platforms. However, in many cases, the true susceptibility variant(s) remain unknown and extremely difficult to identify. The identification which of those millions of variants is functional is important for health and research, and bioinformatics methods are required to assess the probability of functionality based on extensive experimental data.

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VII.13

Upgrading Molecular Cytogenetics to Study Reproduction and Reproductive Isolation in Mammals, Birds, and Dinosaurs

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The past 10–15 years have seen a revolution in the field of genomics, first with the human genome project, followed by those of key model and agricultural species (chicken, pig, cattle, sheep) and, most recently, ~60 de novo avian genome assemblies. The ultimate aim of a genome assembly is to create a contiguous unbroken length of sequence from p- to q-terminus to facilitate studies of gene mapping, trait linkage, phylogenomics, and gross genomic organization/change. Chromosome rearrangements are biologically relevant both in the context of reduction in reproductive capability of individual animals and in the establishment in reproductive isolation as species evolve and diverge. Moreover, a karyotype effectively represents a low-resolution map of the genome of any species. In investigating all these aspects, FISH remains the tool of choice, and this study describes a step change in its use thus: (1) Isolation of sub-telomeric sequences from the pig and cattle

genome assemblies to develop a device for the screening of both overt and subtle chromosome rearrangements. This device worked successfully and was the basis for the development of a routine screening test now used in the pig (and potentially in the future the cattle) breeding industries. The work also facilitated an assay of the integrity of the respective genome assemblies, revealing serious errors in sub-telomeric builds of pig chromosomes. Numerous translocations were detected, most notably a 5:6 cryptic translocation that would not have been detected by classical means. (2) Isolation of evolutionarily conserved sequences from the chicken and zebra finch genome builds to develop similar probes and devices designed to assay for comparative genomics and genome evolution in any avian species. This device worked on the chromosomes of all species attempted and successfully detected chromosomal rearrangements. The hypothesis that certain groups were under constant change was accepted for Psittaciformes species but not Falconiformes. (3) Use of the technology developed in 1 and 2 to complete scaffold-based genome assemblies in several key avian species recently sequenced. We have nearly completed the genome assemblies of peregrine falcon, pigeon, budgerigar, and ostrich genomes at the full chromosomal level and the information was uploaded to Evolution Highway. (4) Use of bioinformatic tools to re-create the overall genome structure (karyotype) of both Saurian and Avian ancestors, then retrace the gross evolutionary changes that have occurred down the dinosaur (and various avian) lineages. Gene ontology analysis of homologous synteny blocks and evolutionary breakpoint regions revealed enrichment for genes involved in chromosome rearrangement (consistent with the formation of the signature fragmented karyotype of birds (and probably dinosaurs), and body size, consistent with the overall gross reduction in size as dinosaurs evolved into birds. Taken together, these results represent significant novel insights into gross genomic organization and rearrangements in extant and extinct terrestrial vertebrates. It has the added benefit of developing both physical and online tools for future use in academic studies and for feeding a growing global population.

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VII.14

Domestication and Repetitive DNA Genome Fraction in *Capsicum chinense*

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The chili pepper *Capsicum chinense* belongs to the Solanaceae family. It is a diploid, self-pollinating crop and is closely related to potato, tomato, eggplant, tobacco, and petunia. It is 1 of the 5 domesticated chili peppers with several commercial varieties. The species is native to South America with the center of diversity in the Amazon biome. Many authors have questioned the species status of *C. chinense,* perhaps because it is the least known of the 5 domesticated taxa with respect to the center of origin and probable progenitors. To gain a better understanding of *C. chinense* evolution and domestication, we used a next-generation low-coverage sequencing of the cultivated pepper *Habanero* (*C. chinense* Jacq.) and the wild *C. chinense* and a graph-based clustering approach for the repeat sequence characterization as implemented in the RepeatExplorer pipeline. In total, we identified that more than 60% of the genome of both the cultivated and wild *C. chinense* was represented by repetitive sequences. Both class I and II transposable elements were present. The predominant type of transposable element was the long terminal repeat (LTR) retrotransposons accounting for 31.25% of the genome. Most of the LTRs were *Gypsy* elements. An accumulation of members of the *Caulimoviridae* pararetrovirus family was also observed. A large number of *Caulimoviridae* elements have previously been detected in *C. annum* and other Solanaceae species and might have had a role in the expansion of the pepper genome in both heterochromatic and euchromatic regions. No differences in type or percentage of repetitive sequences were observed between the cultivated and wild forms. This fact suggests that the domestication process in this species did not affect this genome fraction which seems quite conserved.

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VII.15

Comparative Male and Female Characterization and Expression of the *dmrt1* Gene of *Apareiodon* sp. (Characiformes, Parodontidae)

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The DMRT (doublesex and mab-3 related transcription factor) gene family is widely conserved from invertebrates to humans. Vertebrate *Dmrt1* gene expression occurs predominantly in testis and is a strong candidate for male sex-determining gene studies. In this respect, the understanding of the function of *Dmrt1* in sex determination is important for understanding the cascade of sex differentiation. Structural characterization of the *dmrt1* locus and protein prediction of *Apareiodon* sp. was conducted by bioinformatic analyses of male and female genomes sequenced by Illumina HiSeq and PCR amplification of cDNA using specific primers. Expression of *dmrt1* of 8 adult male and female *Apareiodon* sp. (ZZ/ZW) was quantified by qRT-PCR. We found 5 exons of *dmrt1* which contain 887 bp for male and female. The protein has 2 domains, the DM DNA-binding domain and doublesex mab3-related transcription factor 1. Promoter prediction of ~6,000 bp upstream of the gene revealed 8 similar/equal regions between male and female. However, 8 regions were different, characterized by 6 additional regions (insertions) in female and 2 additional regions (insertions) in male. SSA (Signal Search Analyses Server) software detected a TATA box, initiator and GC-box promoters in male, and initiator and GC-box promoters in female. Several relicts of transposable elements were found in the promoter region, HatN45_DR (54 bp) was present in the male GC-box, Mariner-1 SSA (150 bp) in the female GC-box, L2–5_GA (162 bp) in male

and female initiator, and ENSPM-7_DR (56 bp) in the male TATA box, indicating molecular exaptation of these sequences. *dmrt1* expression was significantly ($p < 0.05$) higher in male gonads compared to the female gonads. Thus, structural differences of the *dmrt1* region can indicate differential expression between the sexes, suggesting an important role for this gene in spermatogenesis. *dmrt1* chromosomal mapping and in-depth molecular analysis of the promoter are very important for more concrete conclusions about this gene.

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VII.16

Super-Resolution Microscopy – Applications in Plant Cytogenetics

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Most of the present knowledge about chromatin and protein organization and their dynamics in chromosomes and cell nuclei is based on molecular methods as well as on cytological investigations. While electron microscopy allows identifying nuclear substructures till a resolution of ~1 nm, the resolution of fluorescence microscopy is limited due to the diffraction limit of light. However, the advantage of that technique is the possibility to identify and co-localize differently labelled structures. The recently developed super-resolution microscopy techniques, such as Structured Illumination Microscopy (SIM) and Photoactivated Localization Microscopy (PALM), allow analyzing structures beyond the diffraction limit of light at a resolution of ~120 nm and of ~20 nm, respectively. Here, I demonstrate how super-resolution microscopy has been used in plant cytogenetics for better understanding the organization and function of chromosomes and cell nuclei. Our research focuses especially on analyzing the centromeres of mono- and holocentric species, the organization of interphase chromatin, the distribution and quantification of structural proteins (e.g. SMC complex components, such as that from cohesin, condensin ,and SMC5/6), and on enzymes (e.g. RNA polymerase II and interacting factors). In addition, the super-resolution techniques will be critically evaluated.

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VII.17

Cytogenetic Analysis as Additional Tool in Identifying Initial Development Stages of Fish in Tributaries of the Paranapanema River (Brazil)

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The cytogenetic data on neotropical fish in their vast majority were obtained from adult animals in which the most appropriate organ or tissue to get the best quality for chromosome preparations could be chosen. In the case of this study, which includes an innovative approach in Brazil, it was necessary to adapt the methods to be applied to initial stages of fish development (larvae and juveniles). The objective of the present study was to cytogenetically analyze samples of fish in early stages of development of the tributaries of the Paranapanema River to characterize the main areas of maintenance of the fish fauna. This type of work provides a basis for future environmental actions, conservation of biodiversity, preservation, and recovery of affected areas. Chromosome preparations were obtained according to Bolla [1987], Baski and Means [1988], and the short term culture by Fenocchio et al. [1991], with several modifications (references to be obtained by the author). In this study 31 taxonomic entities belonging to 4 orders (Characiformes, Siluriformes, Perciformes, and Gymnotiformes) were cytogenetically identified. In many cases, especially in those species that most often appeared in the samples, it was possible to establish a positive correlation between morphological identification and chromosomal characteristics found.

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VII.18

Fourier Transform-Infrared (FT-IR) Microspectroscopy of Chromatin DNA from Valproic Acid-Treated HeLa Cells

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Valproic acid (VPA), a well-known histone deacetylase inhibitor, has been recently demonstrated to also affect the DNA methylation status in several cell types, including HeLa cells. Changes in DNA methylation levels that are induced in the liver cells of diabetic mice reflect on their Fourier transform-infrared (FT-IR) spectral characteristics [Vidal et al., 2014] (reference to be obtained by the author). In the present study, our goal was to investigate whether the DNA demethylation process previously demonstrated in VPA-treated HeLa cells by immunofluorescence could also reflect on DNA FT-IR spectral profiles. DNA was extracted from HeLa cells treated with 1 or 20 mM VPA for 4 h and examined on gold-recovered slides. The spectra were obtained using an Il-

luminat IR II™ microspectroscope connected to an Olympus microscope equipped with an all-reflecting objective, and Grams/AI 8.0 software. Although apparently no difference was introduced by VPA treatments in the IR spectral window concerning with $-CH_3$ stretching vibrations, absorbances due to $-CH_3$ bending vibrations characterized at 1,375 cm^{-1} decreased in the DNA spectra from VPA-treated cells. The same occurred for a ratio here proposed to compare absorbances obtained at 1,375 cm^{-1} and at the frequency assigned to the contribution of overall cytosines. Present findings are in agreement with changes in 5-methyl-cytosine abundance detected for the DNA of VPA-treated HeLa cells.

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VII.19

Methodological Strategy to W Chromosome Characterization of *Apareiodon* sp. (Characiformes, Parodontidae)

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Parodontidae presented species with different sex chromosome systems: proto-sex chromosome, ZZ/ZW, and ZZ/ZW_1W_2. There are also species with an absence of heteromorphic sex chromosomes. Structural and genetically distinct Z and W chromosomes have evolved independently in many groups of eukaryotes. The first step in the evolution of sex chromosomes is recombination restriction between a pair of proto-sex chromosomes. Alleles that are advantageous in males but disadvantageous in females can lead to genetic and chromosomal differentiation. In our study we sequenced male (ZZ) and female (ZW) genomes of *Apareiodon* sp. and microdissected W chromosomes of *Parodon hilarii* to identify DNA sequences specific for sex chromosomes. Genomic DNA was sequenced by Illumina HiSeq and W by Illumina MiSeq. We obtained about 17-fold coverage of short-reads for male, 25-fold coverage of short-reads for female, and <1-fold coverage of short-reads for the W chromosome. The genomic short-reads were assembled using the Velvet software. In order to obtain better quality of W reads, they were aligned to the *Apareiodon* sp. reference female genome using paired-end mode of Bowtie2 with the –sensitive option. Then the W reads that align with the genome were assembled, and results demonstrated small contigs of the chromosome. NCBI-blast-2.2.18+ and Samtools software was used to obtain contigs of W or Z chromosomes from the female genome, using W contigs as query. Repeat Explorer software identified a similar percentage of retrotransposons and DNA transposons in the W contigs. BLAST2GO was applied for gene identification. This workflow allowed finding some linked genes in the sex chromosomes. Further alignments of male and female genomes will be also performed to identify chromosome-specific contigs. This study is promising for understanding the sexual evolution and why genes originally present on both proto-Z and proto-W chromosomes can be lost in the heterochromatic W chromosome of Parodontidae.

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Art and Chromosome Science

YouNome: Your Personalized Genome in 25 Self-Portraits

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We present a unique science-art collaboration designed to engage, educate, and inspire the general public about 'personalized genomics'. Keith Robinson (the artist) has produced 25 self-portraits, each representing the 24 human chromosomes plus mitochondrial DNA by altering his self-image to facilitate genetic understanding and reference art history, popular culture, and effects on the viewer. The collection (accompanied by explanatory notes outlining the rationale for representing each chromosome in the chosen manner) is relevant both to specialists and the general public. To increase public engagement, in collaboration with digital artists, we have combined computer algorithms using face alteration software that will take a full-face image of any person and output 25 self-portraits in the same styles as the original artwork. This creates a 'personalized genome' for anyone interested in one. From scientists' perspective the project explores, who (even among professionals) knows what genomic information looks like? What does the output of a genome sequencer mean to the average person? How can (personalized) genomics be presented to an audience in an informative, memorable, and challenging way? How can we raise awareness of the concept of personalized genomics and what it means to society? From an artist's perspective, throughout history artists have created self-portraits, whether for vanity, self-scrutiny, or cultural commentary, (e.g. Rembrandt, Van Gogh, Cindy Sherman), and public response to the artist's vision of themselves differs to that of other portraits. Why is this? Is it the artist's or the viewer's self-analysis? What makes up and informs 'the self?' If the altering of self-image is informed by genomics what then is the relationship between self and genome? What do we see when a self-portrait is informed with contemporary genomic understanding? Do we see the art in its own right or simply an engaging way to convey a scientific concept? Many authors (e.g. Matt Ridley's 'Genome') have attempted to classify or anthropomorphize human chromosomes by giving each a 'character'. For some this is straightforward, e.g. an extra chromosome 21 is diagnostic of Down syndrome, recognizable by characteristic facial features; presence of a Y chromosome is sufficient and necessary to impart the male gender. For others, it is necessary to rely on genes that reside on it that might impart a disease or notable physical feature e.g. albinism on chromosome 15. Other examples include chromosome 2 that arose during human evolution from a fusion of 2 smaller great ape chromosomes. The subject is thus depicted as an 'ape-man' in a Rembrandtesque style and palette to give the por-

trait a traditional, early feel. Chromosome 10 represents gene-environment interactions: the *CYP17* gene on chromosome 10 makes cortisol, an excess of which can cause stress. The portrait is thus a stressed looking face painted under stress with frenetic mark-making and anxious jolting colors influenced by the style of Vincent Van Gogh. To the best of our knowledge, this collection is unique in that it will be the first time an artist's view of themselves has been informed in such a way and the first time that genomics has been represented in such a way.

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Comic Books: Integrating Chromosome Biology Data and Art in Scientific Divulgation

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Comic books represent an integration of writing and fine arts that are usually focused on a general public. Since their format is attractive and simplified, comics can be used to disseminate scientific knowledge outside the scientific community and facilitate learning of curricula in different disciplines. Indeed, there is a lot of registers of the use of these materials aiming to disseminate science mainly among students. Here, we present 2 comic-based materials that were performed by undergrad and graduate students and by university professors in order to address scientific information for the ordinary society. The first material, 'Ci&Cia: o que é essa tal ciência?' ('Ci&Cia: what is this thing called science?'), intended to make clear to the readers that there is a systematic and logical approach to discovering how things work and which are the basis of the scientific method. This material reports the story of a fellow that finds the answer to this question throughout daily situations like cooking food at home. The characters of this story were named in honor to Brazilian scientists that brought significant contributions to the society. This comic book was distributed to students of elementary schools of Botucatu city (São Paulo State, Brazil), and questionnaires were used before and after the reading of the material, in order to evaluate its efficiency on clarifying its main topics (what is science and scientific method). The qualitative and quantitative data analyses of the performed evaluations will be used to direct future book editions and also to propose school activities that could be performed during science classes using this comic book as a supporting educational material. The second book is composed of a set of cartoons that address topics as cell, DNA, chromatin and chromosomes, issues that are covered by the fundamental and high school curricular contents and also in undergraduate classes. These comic strips have an attractive language and could be used to clarify some concepts and mechanisms associated with cell biology. The described playful materials, due to their ability to motivate readers, represent consistent instruments to transpose scientific background from universities to the general public and can serve as an intermediate step to support disciplinary learning concepts. Free download files of the publications are available at UNESP Virtual Museum School (http://www.ibb.unesp.br/#!/museu-escola/).

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