Chromosome Res (2014) 22:393–437
DOI 10.1007/s10577-014-9435-7

21st International Colloquium on Animal Cytogenetics and Gene Mapping

(21st ICACGM), June 7–10, 2014, Ischia, Naples, Italy

Published online: 23 July 2014

ABSTRACTS

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Plenary Session

L1

Molecular cytogenetics in veterinary diagnosis and research

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Fifty years ago Ingemar Gustavsson made the first observation of a chromosome abnormality in a farm animal. The common rob1/29 translocation in cattle has been associated with reduced fertility, prompting efforts at eradication. Since then many other chromosome abnormalities have been identified in domestic species, including sex chromosome abnormalities in race horses, and these have been discussed at many meetings of the ICACGM. Interest in diagnostic veterinary cytogenetics has grown alongside research into comparative genomics and karyotype evolution of farm animals. The current place of molecular cytogenetics in both diagnosis and research in this field is discussed here in several demonstration projects, including artificial insemination, the fertility of mules and infertility in farm and companion animals due to sex chromosome disorders. Chromosome-specific painting probes, and especially 7-colour FISH probes, have been valuable additions to classical techniques in the resolution of problems associated with high diploid numbers and difficult to distinguish acrocentrics in animal cytogenetics.

L2

Chromosomes, genome analysis and a transforming landscape of applications in the twenty-first century

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Chromosome analysis has been the center-point for nuclear genome analysis for a long time—perhaps over a century. While initially it provided a peek into the structure and organization of the chromosome, it later led to the discovery of chromosome abnormalities and their impact on phenotypes. Also, it allowed increased understanding of the potential causes for various diseases. However, since the advent of a range of gene mapping and genome analysis techniques beginning early 1990s, time and again it has been suggested that the scope and utility of chromosome analysis will decline and fade into oblivion. Understandably, the "Golden Era" of chromosome analysis may be over, however, some basic aspects of analysis coupled with molecular techniques are indispensable and

irreplaceable, and are therefore in demand even today. Nevertheless, the transition from simple karyotype analysis to banding, study of chromosome abnormalities, application of molecular techniques including in situ hybridization (FISH), chromosome painting, Zoo-FISH, multi-color FISH, etc., bear the hallmarks of how the field has progressed and diversified, and how increasing details have enhanced our understanding of the structure, organization and interaction of chromosomes during interphase, metaphase or prometaphase in various tissue types and developmental stages. The field of chromosome studies has vastly broadened from mere studying normal and abnormal karyotypes and chromosome rearrangements to studying various diseases including cancer, and their impact on variation in phenotypes. Chromosome evolution, speciation, ancestral karyotypes/chromosomes, and visible/molecular imprints of rearrangements represent the creation and expansion of yet another whole new challenging/exciting field. Overall, while the focus is shifting from studying 'chromosome under the microscope' to the analysis of 'message and/or material it carries' and its impact on phenotypic variation, production (in domesticated animals), health and diseases, more defining moments are yet to come, as is the case with any field of research. The challenge, however, would be: how will we be able to exploit all this to the advantage of mankind and animals while maintaining harmony with nature?

L3

Genome resequencing identifies loss of function variants underlying fertility, inbreeding depression and heterosis

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Traditional selection practices are based on selection to improve the additive genetic merit of economically relevant traits. However, much of the US commercial beef industry capitalizes on non-additive genetic merit through heterosis which is achieved by crossbreeding. While the molecular basis for heterosis and its obverse, inbreeding depression, are not well understood the existence of large numbers of loss of function (LOF) alleles within breeds may explain both phenomena. The explanation postulates the existence of overlapping sets of LOF alleles between breeds, with the extent of the intersection set being determined by the genetic distance between breeds. If these LOF alleles are phenotypically detrimental (i.e., they are functionally responsible for QTL within breeds), inbreeding within each breed will increase the proportion of individuals that are homozygous for LOF alleles at each locus and phenotype will be depressed. When breeds are crossed, heterozygosity at loci with LOF alleles will increase and will be maximized when the breeds carry LOF alleles at completely distinct loci (the intersection set is null). Under this model we would expect to see heterosis maximized for crosses between genetically distant breeds where evolution would be expected to result in a small overlap in the number of loci sharing LOF alleles (distinct LOF mutations within the same gene). This is an intriguing and testable model which suggests an approach to simultaneously identify QTL which have large non-additive effects within breeds and which contribute to heterosis in breed crosses. We first sequenced the genomes of 11 US registered Angus bulls to a 30× depth of coverage and found 1,720 putative heterozygous LOF alleles in 1,477 genes suggesting that there are likely to be a very large number of LOF alleles at varying allele frequencies within livestock populations. Of these, 1,053 genes were annotated and 176 (16.7 %) were identified in the mouse genome informatics database as possessing a lethal phenotype in homozygous knockout mice suggesting that these loci are causal variants for fertility QTL in Angus. We are currently in the process of resequencing the genomes of 160 bulls from 9 numerically important beef breeds to identify LOF alleles and other variants predicted to be damaging to protein function. From the set of detected mutations, ~50,000 will be designed onto a custom genotyping assay which we shall use to genotype 10,000 animals to estimate allele frequencies and identify lethals as mutations for which no homozygotes are observed when expected based on sample size. These loci can be used to generate molecular breeding values for fertility and mate selection strategies can be used to improve fertility within beef cattle based on knowledge of genotype at these loci. By genotyping phenotyped populations from different breeds and crossbreds used in GWAS we shall be able to test

the breed specificity of these mutations and their contributions to heterosis and inbreeding.

Keywords: Genome resequencing, Loss of function, Embryonic lethal, Homozygosity

Clinical Cytogenetics and Reproduction

(dedicated to Ingemar Gustavsson)

L4

50 years of studies on bovine 1/29 Robertsonian translocation—from Giemsa staining to genomic analysis

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Fifty years ago, on August 29 of 1964, in NATURE journal a short note entitled "*Chromosome abnormality in three cases of lymphatic leukaemia cattle*" (203: 990, 1964), by Ingemar Gustavsson and Gunar Rockborn, was published. Five years later, in 1969 an extended monograph entitled "*Cytogenetics, distribution and phenotypic effects of translocation in Swedish cattle*" was presented by Ingemar Gustavsson in the HEREDITAS journal (63: 69–169, 1969). These two publications were devoted to the identification of the Robertsonian translocation (centric fusion) between the largest (no 1) and the smallest (no 29) bovine chromosomes. It will be not an overstatement to state that this discovery have launched an era of livestock cytogenetics. Soon after this discovery, due to the deleterious effect on fertility eradication programs against carriers of the translocation have been launched in many countries. In consequence the incidence of this mutation significantly decreased especially in some beef breeds. Interestingly, this mutation has been very rarely diagnosed in dairy cattle breeds, while in beef and local breeds its incidence was much higher and the frequency of the carriers reached up to 40–50 % in some breeds. The 1/29 Robertsonian translocation identified worldwide in dozens of breeds, was extensively studied with the use of different techniques. Among them it is important to mention the following ones: chromosome bandings, fluorescent in situ hybridization (including development of molecular probes dedicated to the detection of this translocation), visualization of synaptonemal complexes at pachytene substage of meiotic prophase I, analysis of meiotic segregation by cytogenetic evaluation of early embryos produced in vivo or in vitro. Recently, genomic analyses (arrayCGH, DNA methylation) were also applied to study the organization of the 1/29 translocated chromosome. The discovery of 1/29 translocation resulted in identification of other centric fusions in cattle, involving almost all autosomes. It should be also mentioned that due to the fact that the detection of the centric fusion is technically easy to carry out this type of mutation has also been diagnosed in a variety of domestic animal species (e.g. sheep, goat, pig, dog, arctic fox etc.), but in some of them was not reported (e.g. horse).

L5

Clinical genetics of sexual development

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In embryos of mammals sex determination occurs since fertilization. When eggs, containing the single X chromosome, are fertilized by sperm carrying X or Y chromosome, produce female or male zygotes respectively. The process that leads to the development of a male phenotype is activated by a gene localized on Y chromosome: SRY gene. First, SRY triggers a cascade of molecular events, known as SOX9 pathway, that induce the undifferentiated urogenital tract to differentiate into testis. Successively, the hormones produced by specific cells of the testis lead to the development of the entire male phenotype of the embryo. On the Contrary in the absence of SRY gene, another molecular pathway, known as β-catenin pathway, is activated and induces the development of the ovaries and, consequently, a female phenotype of the embryo. This process is actually quite complex and flimsy as a minimal alteration or in the structure of the molecules involved or in the activation process leads to the onset of disorders in sexual development (DSDs) of an individual more or less severe. In humans, for example, the incidence of DSDs ranges from 1 in 250 live births for hypospadia to 1 in 10,000 cases of individuals with XX sex reversal (XXSR) Since the discovery in 1990 of the SRY gene

that determines the male gender, many other genes, responsible for inducing DSDs, were identified. It's useful to specify how the cytogenetic analysis has often represented a valid methodology for the identification of these genes. Some animal species are excellent models for the identification and study of these genes since the sexual diseases that show are entirely similar to those present in the human species but characterized by a higher frequency due to the lower rate of abortion against them. In this lesson we will present the current knowledge on the subject and on the genes recognized as responsible for sexual disorders. We will also discuss how animals can be a valuable tool to deepen this knowledge that has yet unexplored aspects.

O1

Analysis of male infertility: a case study in pigs

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Infertility is a significant problem in humans, affecting up to 15 % of couples. Male (co-)factors, leading mostly to spermatogenesis failure, are involved in almost 50 % of the cases. Male infertility is also of major interest in farm-animal populations. On the one hand, a reduction in male fertility can be responsible for major economic losses at the farm level. Otherwise, due to the importance of the male pathway in the creation and dissemination of genetic progress, male infertility can lead to a strong reduction of the efficiency of genetic selection programs. Origins of infertility are still unknown in more than 90 % of the cases in Human but they may be genetic or environmental causes. We recently developed a research program aiming at deciphering the putative genetic mechanism explaining the bad semen quality parameters observed in boars routinely controlled before reproduction, thanks to cytogenetic, array-CGH and array-painting analyses.

Preliminary results will be presented with a particular attention for an oligo-astheno-terato-spermic boar carrying an asymmetric reciprocal translocation involving chromosomes SSC1 and SSC14. CNVs research, meiotic pairing, recombination and segregation analyses, as well as breakpoints characterization have been carried out and the corresponding results will be presented.

O2

Identification of Chromosomal Translocations in Pigs using FISH with Subtelomeric Probes and the development of a novel screening tool for their application

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Reciprocal chromosome translocations have established to affect fertility in pigs leading to reduced litter sizes and hypoprolificacy. With an increasing emphasis in the commercial pig breeding industry on using a small population of boars for artificial insemination, the potential economic costs of using hypoprolific boars are significant. At present screening for translocations is only performed by karyotyping which, while technically straightforward, requires animal specific expertise for karyotype analysis, which can be unattractive to the industry. The use of subtelomeric probes and fluorescence in situ hybridisation (FISH) eliminates the need for this level of expertise whilst also offering greater accuracy and the ability to identify cryptic translocations. At present, however a universal FISH based screening test for porcine translocations has yet to be developed.

Probes were designed that map to the subtelomeric regions of each chromosome arm to enable detection using FISH. BACs were identified from the subtelomeric region of the p-arm and q-arm of each porcine chromosome and directly labelled with Texas Red or FITC (p-arm and q-arm respectively) prior to

fluorescence microscopy and image capturing using SmartCapture 3 software (Digital Scientific UK).

Clear signals were obtained from each subtelomeric probe. These were tested on normal animals and animals that exhibit translocations, providing preliminary evidence that this technique is a valid tool for the identification of translocations that affect fertility in pigs.

When combined with a tool originally developed for humans to enable the simultaneous detection of all porcine chromosomes on one slide (Multiprobe™ Device), the speed and cost of chromosomal analysis for translocations that affect fertility will be greatly improved, therefore offering significant benefits to animal genetic research and the animal breeding industry.

O3

The incidence of translocations in young breeding boars in Canada

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The objective of the project was to carry out the first systematic screening program for chromosomal abnormalities in young breeding boars in Canada. To date, a total 300 young boars from 4 different breeds (Duroc, Landrace, Pietrain and Yorkshire) were karyotyped by G-banding. Four previously unreported reciprocal translocation including rcp(1;5), rcp(3;4), rcp(8;13) and rcp(7;15) and one previously reported Robertsonian translocation rob(13;17) were found. Consequently, the frequency of chromosome abnormalities in this study was 1.67 %. By extending the sampling to other members of the pedigree, it was determined that rcp(3;4) and rob(13;17) were inherited from their dams and rcp(8;13) was a "de novo" event. Comparing with the herd average, average litter size of rcp(3;4), rcp(8;13) and rcp(7;15) translocation carrier boars was noted to be reduced (24 %, 24 % and 38 %, respectively) while for carriers of rob(13;17), it was only slightly reduced (9 %). Interestingly, for rcp(3;4), the overall reduction in litter sizes for female carriers was substantially lower (only 4 %) compared to male carriers (24 %). Chromosome analysis of live offspring from 2 full litters of carrier boars showed a 20 and 40 % transmission rate to progeny for rcp(7;15) and rob(13;17), respectively. More studies need to be carried out to further investigate the effects of these translocations. (Research support was obtained from NSERC, Agriculture and Agri-Food Canada, and the Canada Research Chairs program).

O4

Mix of two chromosomal aberrations in a newborn calf 2n=60,XX, t(11;25)(q11;q14-21)

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A newborn calf of the Agerolese breed underwent cytogenetic investigation because presented hyperflexion forelimbs, red eyes and inability to stand up. Anamnesis revealed the mother, phenotypically normal, was carrier of a t(11;25)(q11,q14-21). The newborn died after a few weeks and no internal alterations were found by veterinarian after the post mortem examination. The mother presented, after a cytogenetic investigation, a reciprocal translocation between chromosome 11 and 25 and the presence of two ders: der11 and der25, for the position of corresponding centromere. On the other hand, the veal revealed a different chromosomal aberration in comparison to her mother. In fact, after R-banded karyotype, the calf showed both chromosomes 25, one chromosome 11 and one der (der25). FISH analysis was performed with the same BAC clones used to detect the translocation in the mother: BAC142G06 mapped on the proximal region of both BTA25 and der25; BAC513H08 mapped to BTA 25q22dist; BAC533C11 mapped to the proximal region of BTA11 and der25. Finally, we confirmed both the localization of the breakpoints on band q11 (centromere) of chromosome 11 and q14-21 of chromosome 25, and the loss of the der11. In this way, it is showed a different cytogenetic aberration in the veal: a partial trisomy of chromosome 25 and a partial monosomy of chromosome 11. We have

been studying a correlation between this aberration and some gene involved comparing it with corresponding human clinical cases.

Acknowledgements. This study was supported by the Rural Development Plan under the Project "PSR, Misura 214, e2 of Campania, Razze Autoctone a Rischio di Estinzione della Regione Campania (RARECa)".

O5

A new case of 64,XX sex reversal syndrome in a Spanish purebred horse

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Abnormal morphologies in horses are related in most of the cases to atypical chromosomal complements. An higher percentage of these horses are characterized as animals with unspecified sex or with disorders in the sexual development (DSD). We studied the case of a 3 years old Spanish Purebred horse submitted to karyotiping due to its abnormal sexual morphology. On physical examination, the animal showed an abnormal foreskin, two prominent nipples, a tight scrotum and retained testicles. His sexual behaviour and libido were normal for a male horse. The animal was castrated at 2 years of age and the pathological study showed no abnormal tissue. The animal was karyotyped using conventional citogenetic techniques (chromosome counting and C-banding) and in situ fluorescent hybridization with two specific WCPP for ECAX and ECAY chromosomes showing a female 64,XX chromosomal complement. The presence of three different genes linked to sex chromosomal in the blood DNA of the horse was studied using PCR. Resulting in a positive amplification for ZFX and AMX and no amplification of the SRY, ZFY and AMY genes. Four different microsatellite makers linked to the ECAX chromosome were analysed to determine the number of chromosomal copies. The genotyping of LEX 026, TKY38, TKY270 and LEX003 showed two different alleles in each marker, denoting the presence of at least two different ECAX chromosomes. All the molecular studies were repeated using DNA obtained from hair follicles to discard the presence of a blood chimerism case. The results were identical than those obtained previously. Based on our results we diagnosed the horse as a 64,XX SRY negative DSD carrying a male-like genitalia. To our knowledge, this is the second time that this kind of abnormality was reported in the Andalusian horses and the first time showing this kind of morphological abnormalities. At the present time, the cause of this abnormal sexual development remains certainly unknown. However, the most accepted theory is the occurrence of an androgen exposure during sexual development of the embryo leading to the masculinization of the female foetus. Finally, we suggest the use of genetic and cytogenetic diagnostic tools in the veterinary practice as a valuable tool to determine the origin of reproductive failures among horses.

O6

Disorders of Sexual Development (DSD) in dogs with ambiguous external genitalia—survey of 30 cases

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The genetic background of disorders of sexual development (DSD) may be related with chromosomal aberrations, gene mutations or may have a multifactorial etiology. During the last 10 years we have analyzed 30 dogs subjected to cytogenetic (Giemsa staining and whole sex chromosome painting) and molecular analyses (detection of the *SRY* and *ZFY* genes) due to the abnormalities of their external genitalia. In most cases (21) the enlarged clitoris with a bone was observed in phenotypic females with 78,XX chromosome set and lack of the Y-derived genes (*SRY* and *ZFY*). The consequent characteristics were observed in the following breeds: Bernese Mountain Dog (1), Cocker Spaniel (4), German Shepherd (2), Miniature Pincher (1),

American Staffordshire Terriers (4), Yorkshire Terrier (1), Tibetan Terrier (1), French Bulldog (2), Kerry Blue Terrier (1), Leonberger (1), Mops (2) and Beagle (1). In ten cases, out of the mentioned above, the gonadectomy was performed and the histological analysis revealed the presence of testes or ovotestes. Moreover, eight DSD cases, representing Moscow Watchdog, Yorkshire Terrier (2), Scottish Terrier, Dachshund, Syberian Husky and mongrel dogs (2), with a male karyotype (78,XY) and presence of the *SRY* gene were described. The following abnormalities of external genitalia were observed in these dogs: hypospadias, rudimentary penis, abnormal prepuce or female phenotype with enlarged clitoris. In addition, one case with leukocyte chimerism (78,XX/78,XY) was diagnosed in Shih Tzu dog with a rudimentary penis and prepuce located in a position typical of a male. We conclude, that cytogenetic evaluation of DSD animals is a crucial step toward precise classification of the observed sexual disorders. Our study showed that the most frequently diagnosed DSD in dogs is testicular/ovotesticular DSD (78,XX; *SRY*-negative), while chromosome abnormalities are very rare. Thus further studies focused on molecular etiology of canine DSD are very important.

(*supported by National Science Center in Poland—grant 2012/05/B/NZ9/00907*).

P1

Cytogenetic study of a reciprocal translocation (1;6)(q17;p11) in a subfertile boar

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The presence of chromosome abnormalities in domestic pig is well known for its negative impact on the reproductive parameters and the viability of any offspring produced. For this reason it is recommended to perform a cytogenetic screening in young boars before entering services at AI centres. In the Department of Animal Reproduction belonging to INIA (Spain), cytogenetic analysis of pig populations is realised routinely with the aim of identifying possible carriers of chromosome abnormalities. Boars producing a decrease in litter size are also studied as urgent cases. A cytogenetic analysis of a boar (synthetic line from Large-White with a small percentage of Pietrain), known to produce smaller litter size when its semen was used for AI, was made. Data on litter size parameters was not provided from the AI centre. The boar karyotype was determined by analysis of cultured peripheral blood lymphocytes. Chromosome banding was done following GTL-banding and C-banding techniques. A minimum of 20 metaphases were analysed and placed in karyotypes according to the International Standard for domestic pig. It was verified that this boar had a reciprocal translocation between chromosome 1 and chromosome 6 rcp(1;6)(q17;p11). Among the over 160 different reciprocal translocations described in pigs, at the moment there are five affecting both chromosomes but in different breakpoints. In our case, the q-arm of chromosome 1 (SSC1) appeared shortened, with the absent distal fragment found at the tip of the p-arm of chromosome 6 (SSC6). Subsequently to this finding we were able to study the offspring of this boar. Within 16 animals tested, 6 had the same chromosomal alteration (2 females and 4 males) and 14 of them were found to have C-band polymorphism in the acrocentric chromosome 17(SSC17). This last abnormality was not found in the boar subject to this study, being C-band polymorphism frequently found in our laboratory when animal are analysed.

P2

Identification of a pericentric inversion in chromosome 4 in a boar from artificial insemination center

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An inversion is a structural chromosomal disorder that can be explained as a rearrangement in which a segment is being taken out of a chromosome, turned through 180° and inserted back in its original position. The inversion is considered paracentric if the breakpoints are in the same arm of a chromosome, and pericentric if both breakpoints are in different arms on either side of the centromere.

This report describes a new pericentric inversion of chromosome 4 in a boar with poor reproductive

performance considering it belongs to a hyperprolific synthetic breed. The breakpoints described are (p15;q24). Inversions have been described for only six porcine chromosomes, namely SSC1, 2, 4, 6, 8 and 9. Apparently, the inversions have a minimum impact in carriers because they don't involve loss or gain of DNA. However, they can perturb spermatogenesis and lead to the production of unbalanced gametes through the formation of an inversion loop. These recombinant gametes would be responsible for stillbirth or congenital abnormalities.

In human research a relation has been found between inversions and male infertility and abortion. When the inverted segments involve more than 50 % of the chromosome length, the risk of aneusomy in the offspring becomes very important.

In contrast in animal studies researching the meiotic segregation pattern of inversions in pigs, the estimated proportion of recombinant gametes was very low and no correlation was found between size of the inverted fragment and the proportion of the recombinant gametes.

P3

A complex structural reciprocal translocation with three chromosomes involved in a boar

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Chromosome abnormalities in domestic pig are a major cause of reduced reproductive performance. The most common chromosomal rearrangements are reciprocal translocations that involve two chromosomes, leading to reproductive consequences decreasing litter size. In these abnormalities the spermatogenesis produces a high proportion of genetically unbalanced gametes, which are effective at fertilization but result in early embryonic loss of progeny. The present study reports a novel and complex rearrangement of chromosome segments in the domestic pig. A metaphase analysis is done through the GTL banding technique from cultured peripheral blood lymphocytes of boar (Large White) showing low litter size in sows inseminated with seminal doses from the carrier boar. In a total of 39 metaphase cells was found a reciprocal translocation between chromosomes 1, 2 and 17. This translocation affects three chromosomes with four break points. While chromosomes 2 and 17 had a single breakpoint, chromosome 1 had two breakpoints in the same arm (1q11q17). The breakpoint for autosome 2 was in the GTG-positive band 2p16 and the one for autosome 17 was in the GTG-negative band 17q13. The broken distal segment of autosome 1 was an inverted insertion in the short arm of chromosome 2. The broken proximal segment of autosome 1 was attached to chromosome 17. The broken distal segment of autosome 17 was inserted in short arm of chromosome 1. Finally, the distal segment of arm p of chromosome 2 was an inverted insertion in the altered chromosome 17. The two peculiarities of this rare reciprocal translocation were that autosome 1 had two break points, and rearranged acrocentric chromosome 17 had small segments from the three chromosomes involved.

P4

A case of sex chromosome mosaicism 64,XX/65,XXY/66,XXYY in mare

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A cytogenetic study was carried out in 2 year old polish pony mare with abnormal external genitalia, showing a residual vulva and vagina (with the external urethral orifice) in the perineal area under the tail, with what appeared to be an enlarged clitoris or glans penis at the bottom edge. The horse behavior was primarily that of a stallion (jumping mare in heat and vocalization). It was determined that the horse had modest levels of testosterone in its blood (0.49 ng/ml). Rectal and USG examination was not performed due to horse behavior. This mare showed a karyotype in mosaic form 64,XX/65,XXY/66,XXYY. The chromosome aberration was confirmed by fluorescence in situ hybridization with both whole chromosome painting X and Y probes. We analyzed 298 mataphases in total, in which 292 showed 64,XX, four presented 65,XXY and two cells with 66,XXYY karyotype (97.98 %, 1.34 % and 0.68 %

respectively). The DNA analysis with the PCR and PCR/RFLP methods showed an absence of *SRY, AMELY, ZFY, DDX3Y* and *Y3B12* genes as well as 11 STS Y specific markers (*CLY002, CLY003, CLY004, CLY043, CLY044, CLY045, CLY047, CLY049, CLY050, CLY055, CLY059*). These results suggest that the Y chromosome detected in the investigated animal was the result of deletion of euchromatic portion of ECAY that comprised of above-mentioned markers.

P5

The prenatal diagnosis in cat by modern karyotyping techniques

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The purpose of this study is to realize the karyotype of the foetus in female cats from the amniotic liquid using new techniques. The amniotic liquid was sampled by amniocentesis under ultrasound control in aseptic conditions. The incubation of the amniocytes was made at 38.3–38.5 °C, in 5%CO_2, at pH=7.3. The cultivation of the amniocytes was made in two nutritive media, special for amniocytes Ham F10 and Amniochrome. The incubation lasted 18 days with a periodic change of the nutritive medium. After 18 days, the amniocytes were submitted to the hypotonic treatment, fixed and Giemsa stained. For G banding chromosomes the partial denaturation with Tripsine and Giemsa stain were used. The examination was made under a motorized Zeiss Axioscope 2 microscope connected with a camera and a computer with Metasystem Ikaros software for human chromosomes karyotyping, which we adapted for the karyotyping of chromosomes in cats. In G staining, 10 mitosis per slide were examined and 20 mitosis in G banding chromosomes were studied. In the examined mitosis, there were not observed chromosomal aberrations. The use of this technique is very facile compared to the classical methods of karyotyping and the software was easy to use for dogs' chromosomes, so we recommend its use for the study of chromosomes in animals of increased biological value, for prenatal diagnosis.

P6

The 1;29 robertsonian translocation in andalusian indigenous breeds cattle

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The first reports about the incidence of 1;29 robertsonian translocation in indigenous Andalusian cattle breeds appeared in 1984. From that moment this genetic trait was detected in all Spanish cattle breeds. Concerned by its incidence, many breeders associations decided to eliminate all heterozygous carrier animals as reproducers.

In this study, we analyzed 5,345 animals; most of them, (4,163 animals) belongs to the Retinta breed and the rest belongs to others Andalusian indigenous breeds (Lidia, Berrendo en Negro, Berrenda en Colorado, Pajuna, Negra Andaluza and Cárdena). In Retinta, a systematic study began in 1989 showing an initial incidence of 32 %, being today at around 6 % after many years applying the selective breeding policies. By this reason, their field fertility increased from 69 % in1989 to over 80 % in our days. This change is the result of the established policy and whole complete actions inside the Official Selection Scheme established by the Ministry of Agriculture in which the cytogenetic analysis is mandatory for all the animals introduced in the official records.

In the other breeds, all of them endangered, the incidences obtained are 23.3 % in Berrendo en Colorado; 32.6 % in Berrendo en Negro; 15.9 % in Cárdena; 19.4 % in Negra Andaluza and 34.5 % in Pajuna, being the mean of all of them 24.1 %. An interesting exception was detected in Lidia breed (Bullfight) in which we observed 0 % of incidence. In

all of these populations, the Breeders Associations have introduced the cytogenetic analysis to identify the carrier animals avoiding their use as reproducers, obviously if there are enough animals to proceed. In the case in which the effective number of possible sires is very low, the decision to remove carrier animals could be really dangerous. In this case, the animals can be used as breeders but all their offspring is analyzed to identify the non-carrier future reproducers. With these political decisions we expect a significant increase in the fertility of cows as has happened in Retinta breed. In Lidia breed different reasons could explain the non-presence of 1;29 translocation.

P7

The application of a multicolor ZOO-FISH on secondary bovine oocytes showed its potential use for aneuploidy detection

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The female gametes are more susceptible to chromosome segregation errors during meiosis I division and therefore they are the major contributors to the embryo aneuploidies. The evaluation of aneuploidies in bovine oocytes is useful for monitoring the reproductive health of this species and FISH is the main method employed for this purpose. To date only 2–3 chromosomes were simultaneously investigated for aneuploidy detection in cattle oocytes. In this work we propose a multi-color ZOO-FISH by the simultaneous detection of six specific chromosome painting probes on secondary oocytes matured in vitro. Standard procedures were employed for 24 h in vitro oocytes maturation, whereas specific autosomal probes were prepared by microdissection and DOP-PCR using river buffalo mitosis (2n=50). Probes were labelled with spectrum-green and -orange in a second DOP-PCR. Three sequential rounds of FISH were achieved for the same slides. Each round was realized using two probes simultaneously hybridized on MII oocytes with the corresponding first polar bodies (I pb). Slides were counterstained with DAPI in antifade. Digital images were captured in gray-scale and pseudo-colored by the software. Six specific probes, painting 3 out of 5 sub-metacentric river buffalo chromosomes (BBU 1p, 1q, 3p, 3q, 4p and 4q) were sequentially hybridized on BTA secondary oocytes with the corresponding (I pb). The different colors of the probes allowed the identification of six cattle chromosomes (BTA 1, 5, 8, 19, 27 and 28) both on MII and polar bodies evidencing no abnormalities for the investigated cells, but confirming their potential use for aneuploidy detection in bovine oocytes. This result opens further opportunity of investigation for clinical cytogenetic applications also in the other species with difficult CGH karyotype.

This study was supported by CISIA-VARIGEAV project, National Research Council (CNR) of Italy and by RARECA project, PSR-214 of the Campania Region, Italy.

P8

A cytogenetic investigation on a suspected pseudo-hermaphroditism clinical case in "Rhoen-sheep"

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A lamb of the German "Rhoen-sheep" breed was born spontaneously with deformities of genital organs (pseudovagina with labioscrotal beadings) and urethra after normal twin pregnancy. Its twin brother was healthy and showed no deformities. Classical and molecular cytogenetic investigations were carried out to study possible karyotype defects responsible for the abnormal phenotype. Peripheral blood sample cultures were performed to get both normal and BrdU-treated cultures, the latter to obtain R-banded preparations. Normal cultures were used to perform CBA-banding and FISH-technique. The analysis of the C-banding proved the correct position of the centromeres, whereas the RBA-banding pattern showed karyologically normal arrangement (2n=54,XY). A FISH analysis was carried out to evaluate the eventual level of XX/XY mosaicism

by using specie-specific painting probes for sex chromosomes. One hundred metaphases were scored and all showed normal XY chromosomal arrangement. No metaphases with two X chromosomes were detected. The observed phenotype and the lack of cytogenetic defects led to state that this clinical case might represent a suspected condition of male pseudo-hermaphroditism. In humans, this condition is related to the androgen insensitivity syndrome (AIS). Further investigation is therefore necessary to identify at molecular level the causes of this abnormal phenotype.

P9

Cytogenetic survey in autochthonous endangered animal breeds reared in Campania region (Southern Italy): an up-date

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In the Rural Development Plan RDP 2007–2013, Misura 214, e2, project RARECa of Campania (Southern-Italy), three different Institutions (CNR, University of Naples and IZSM) are involved to study, characterize and valorise some autochthonous endangered animal breeds raised in Campania Region. In this report an up-data on the cytogenetic analyses we performed in horse (Napoletano, Salernitano and Persano breeds), cattle (Agerolese breed), pig (Casertana breed), sheep (Laticauda and Bagnolese breeds) and goat (Cilentana breed) are reported. Up to now, upon 63 Agerolese cattle four females (6.3 %) were found to be carriers of: (a) rob(1;29) (2 animals), (b) rcp(11;25) and (c) a case of partial monosomy and trisomy (2n= 60,XX,t(11;25)(q11;q14-21). All examined horses (34 animals) from Napoletana (14) and Salernitana (20) breeds showed normal karyotypes. Concerning the Laticauda sheep (46 animals), two females were found to be carriers of two new reciprocal translocations while Bagnolese sheep breed (32 animals) and Cilentana goat breed (12 animals) showed normal karyotypes. Furthermore, in Casertana pig breed 52 animals were examined and resulted with normal karyotype.

Acknowledgements. This study was supported by the Rural Development Plan under the Project (PSR, Misura 214, e2 of Campania region, "Razze Autoctone a Rischio di Estinzione della Regione Campania—RARECa").

P10

Chromosomal abnormalities in secondary bovine oocytes matured in vitro up to 48 h

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Preliminary experiments carried out in our laboratory indicated that—by fertilizing oocytes matured in vitro for 24–32 and 48 h—the resulting blastocyst rates decreased from 22.4 % (24 h), to 14.0 % (32 h) to '0' % (48 h). The aim of this study was investigate upon the variation in the incidence of chromosomal abnormalities occurring in bovine oocytes matured in vitro for prolonged periods of time, i.e. from 24 to 32 to 48 h. Abattoir-derived oocytes were matured in vitro using standard procedures, for 24–32 and 48 h. After incubation, the COCs were treated with Ialuronidase (3 mg/ml) to eliminate the cumulus cells, swelled in hypotonic (KCl, 0.075 M) for 5–10 min, fixed individually on microscope slides with Carnoy fixative, air dried and stained with 5 % Giemsa. Conventional karyotypes were prepared from 50 matured oocytes for each time of maturation, providing the following results: chromosomal abnormalities, including unreduced diploid metaphases, hypo-haploidy and hypo-diploidy, increased from 12 % at 24 h, to 20 % at 32 h, to 36 % at 48 h. The Chi-square test (with Yates corrections) showed significant differences ($P>0.01$) in the rate of

chromosome abnormalities from 24 to 48 h of maturation as well as from 32 to 48 h, whereas the differences between 24 and 32 h were not significant. These results confirm previous data and provide further evidence that bovine oocytes to be used in IVF programs should not be matured in vitro longer than 24–32 h.

This work was supported by the RARECA project, PSR, Misure 214 e2 of the Campania Region (Italy).

P11

Four cases of testicular disorder of sexual development (DSD) in cats (38,XY; *SRY*-positive)

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Disorders of sexual development (DSD) are poorly recognized in cats. In the present study we show four male cats with abnormal external genitalia. In case 1 an abnormal orifice of the urethra located close to the anus and rudimentary penis with numerous horny spines were observed. In case 2 an underdeveloped penis with rudimentary spines without scrotum was present. Case 3 had a blind ended vulva (1.5 cm long), a normal penis, but the urethra orifice was located next to the anus. In case 4 a rudimentary penis with spines and glans penis (but not covered with a prepuce) was observed. For all cases cytogenetic, molecular and histological analyses of the gonads were performed. The cytogenetic analysis, with the use of Giemsa staining, G-banding and fluorescence in situ hybridization (FISH) with a BAC probe specific for the *SRY* gene, revealed a normal male karyotype (38,XY) in all cases. The PCR detection of the *SRY* and the *ZFY* genes confirmed the presence of the Y chromosome derived sequences in all studied cats. Moreover, sequencing of three candidate genes (sex determining region Y—*SRY*, androgen receptor—*AR* and steroid-5-alpha-reductase—*SRD5A2*) was carried out. In the *SRY* of case 4 a known missense substitution g.389G>C, p.Arg130Thr was identified. In the *AR* a common tandem repeat polymorphism (CAG) in exon 1 was found, while in the *SRD5A2* gene 5 SNPs (a silent one in exon 1, 2 in introns and 2 in 3′UTR) and 2 intronic indels were observed. Histological examination of the gonads facilitated their classification as testes. In details, only Sertoli cells in seminiferous tubules were found in case 1, while in cases 2 and 3 testes with normal spermatogenetic activity were present. In case four no sperms were found in normally developed seminiferous tubules. Taking into consideration the obtained results we conclude that karyotypes of the studied cats were not altered. Thus, the cases were classified as testicular DSD (38,XY and *SRY*-positive). Further analyses of other candidate genes are postulated.

P12

Exclusion of SOX9 duplication and over expression in a horse with XX, SRY-Negative Ovotesticular DSD

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Disorders of sex development (DSD), include a broad range of atypical sexual phenotypes and have also been described in domestic animals. We investigated an equine case, which after molecular, cytogenetic, anatomic and histopathological analysis was classified as an XX, SRY-Negative Ovotesticular DSD. Since there is some empirical evidence in humans and lab animals that duplication and/or over-expression of the SOX9 gene may explain the presence of testicular tissue in absence of the testis determining factor (i.e. SRY), we explored this possibility in such an individual. DNA was extracted from blood samples, while cDNA was obtained by reverse transcription of total RNA from vulvar skin biopsies of the animals in the study. SOX9 copy number and gene expression were determined by real time PCR,

using the single copy gene CG and the ubiquitously expressed gene GAPDH, respectively, as the reference genes. Neither results gave indications that the clinical case had an abnormal gene copy variation ($P \geq 0.05$) and/or over-expression ($P \geq 0.05$) for the SOX9 gene. We excluded these possibilities as explanation for the development of the described sexual disorder in the horse. Further investigations are warranted in order to identify the etiology for the XX, SRY-Negative Ovotesticular DSD syndrome in horses.

Molecular Cytogenetics, Gene Mapping and Genomics

L6

Centromere repositioning during evolution

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

L7

Genomes are doomed to repeat: satellite DNA as modulators of chromosome reshuffling and genome regulation

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Are Genomes Doomed to Repeat? Why? Repetitive DNAs are, most likely, the genetic factors responsible for promoting genomic plasticity and higher rates of chromosome mutation. Satellite DNA (SatDNA) is a highly dynamic repetitive genomic fraction that evolves "rapidly" by diverse mechanisms that induce a high turnover of these sequences. This dynamic nature occurs at two levels: at the molecular organization of sequences (nucleotide sequence alterations and copy number variation), but also at the physical localization. Evidences suggest that these SatDNAs are transcribed and can adopt different genome functions, as gene silencing and maintenance of chromosomal integrity. However, data regarding the transcription of SatDNA repeats is still very scarce, but from the recent advances it is clear that is mandatory to disclose the "genuine" function of these transcripts.

In this talk we will present new data that could be the starting point to unveil why these sequences are maintained in some genomes, and are depleted or relocated in others and how they assist chromosome rearrangements and different genome mammalian architectures. Is our goal to understand how SatDNA modulate chromosome reshuffling and genome regulation in evolution and cancer and then, we will understand why our genomes are full of "junk" DNA and are in fact, doomed to repeat!

L8

Y-chromosome linked genes and their expression in cattle

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In mammals, males have one X-chromosome and one Y-chromosome while females have two X-chromosomes. The Y-chromosome varies from species to species but, in general, it contains only about 30 genes, which code for proteins. Many of the Y-linked genes are present in multiple copies and are only expressed in reproductive tissues, while a few (15 %) have homologues on the X-chromosome and are expressed in a variety of tissues. To gain a better understanding of this chromosome we have studied multiple copy (eg TSPY, HSFY,) and single copy genes (SRY, DDX3Y, EIF1AY, HSFY, SRY, TSPY, USP9Y, ZFY, and ZRSR2Y) on the bovine Y chromosome. TSPY (testis specific protein Y encoded) was found to vary from about 30 copies to 200 copies among bulls, while individuals appear to be mosaics with copy number varying significantly from tissue to tissue. Furthermore, we found an association between the number of copies and fertility. HSFY (heat shock factor Y encoded) consistently had approximately 75 copies and did not vary significantly among individuals. All Y-linked genes examined were expressed in bull testis. To determine if these genes are transcribed prior to development of the fetal gonad, we screened embryos for the presence of their transcripts from the two cell stage (30–40 h after fertilization) up to the blastocyst stage (8 days after fertilization). We found that of the nine genes examined, 6 (DDX3Y, EIF1AY, TSPY, USP9Y, ZFY, and ZRSR2) showed the presence of their mRNAs at the blastocyst stage embryos. Moreover, *USP9Y* transcripts were observed from the 4-cell stage to the blastocyst stage, while *ZFY* transcripts were observed from the 8-cell stage to the blastocyst stage. Knock down of TSPY and USP9Y by siRNA led to reduced developmental rates, although both males and females were found among those that developed to blastocyst stage.

These studies showed that the Y-chromosome is a dynamic chromosome that plays a role in bovine embryo development and is associated with fertility in adult bulls. Y-chromosome linked genes are potential markers for fertility and embryo viability.

Funding provided by NSERC and the CRC program.

L9

The Buffalo Genome: Structure and Function

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The publication of the human genome sequence in 2001 was a major step forward in knowledge necessary to understand the variations between individuals. For farmed species, genomic sequence information will facilitate the selection of animals optimised to live and be productive in particular environments. In the future we can envisage that the sequence of an individual will allow us to predict its phenotype with high accuracy. With the availability of the cow genome sequence for and knowledge of genome variations, the cattle breeding industry has taken the first steps towards predicting phenotypes from genotypes by estimating "genomic breeding values" (gEBV) for bulls using genome-wide DNA markers.

The international buffalo consortium (IBGC) has completed the assembly of the draft genome sequence of the river buffalo and the outline sequence of the swamp buffalo, and the river buffalo genome has been annotated. The genomes of several buffalo of different breeds have been sequenced a low coverage, which has facilitated the identification of millions of sequence variants among buffalo genomes. Based on frequencies of variants within and among buffalo breeds, and their distribution across the genome, 90,000 putative single nucleotide polymorphisms (SNP) were selected to create an Axiom® Buffalo Genotyping Array 90K. This "SNP Chip" has been tested in several buffalo populations to study genetic diversity geographically. The SNP panel has also been used in a pilot association analysis to identify loci affecting milk production traits.

O7

Tandemly repeated satellite DNA in the Artiodactyla

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Tandemly repeated, satellite, DNA sequences are an abundant component of the genome of most species, including the Artiodactyla. Multiple DNA familes are present, each in long tandem arrays, with members of each family present on one or more chromosomes at characteristic positions. In particular, several familes are located at the centromeres of most chromosomes, including acrocentrics, metacentrics and the sex chromosomes. Individual arrays are made up of variants of particular sequence motifs, which may be longer than 1,500 bp. In this presentation, we will discuss aspects of the evolution of repetitive sequences within and between chromosomes, with comparative data between different species. With pig, we will show details of the localization of tandem repeats at meiosis, and how these sequences relate to sequence amplification and loss, as well as the epigenetic behaviour of the resulting heterochromatin. In the Bovinae, we will show how molecular cytogenetic methods are essential to build up a full picture of the behaviour and distribution of satellite DNA where current sequencing methods are unable to assemble the sequences blocks accurately.

Further details, references and slides are available from www.molcyt.com.

O8

3D nuclear organization of the CMH complex in control and LPS-activated porcine macrophages

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It is now clearly established that 3D nuclear organization plays an essential role in the regulation of cellular functions. While numerous studies have demonstrated the major role of nuclear architecture in several contexts including cell differentiation, embryonic development and tumor progression, the situation is different for immune response, another important biological process for which information remains still limited. So, we focused our interest on pig macrophages which are essential components of the innate immune response. In a first study combining transcriptomic and molecular cytogenetic approaches, we demonstrated that when macrophages are activated by LPS/IFNg, some up-regulated genes tended to relocate in the nucleus space and/or relatively to their chromosome territories while down-regulated genes did not show any significant changes. In the present study, we examined the large-scale chromatin architecture of the major histocompatibility complex region (MHC) during macrophage activation as one of the most important region for immune response. We used BACs containing MHC class I, II or III genes and we performed 3D-FISH experiments on macrophages (quiescent and LPS-IFNg activated) in which nuclear morphology was preserved. Confocal microscopy and 3D image analyses using NEMO software allowed us to investigate the nuclear organization of the MHC complex in macrophages and the effect of LPS-activation on this organization. We also analyzed the chromatin condensation state in this region by measuring the 3D distances between the MHC Class I, II and III genes.

O9

The application of molecular cytogenetic approaches to genomic mapping and sequencing of the pig Y chromosome

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The mammalian Y-chromosomes carry genes critical for spermatogenesis and male-specific functions. However, determining the sequence and gene content of the Y chromosome has presented a daunting challenge to genome researchers due to its low gene content and highly repetitive nature at sequence level. So far, only a few mammalian Y-chromosomes have been characterised at DNA sequence level, with the human Y having the most advanced maps. Due to its economical importance, there has been a long-lasting interest to map and sequence the Y chromosome of the domestic pig. Recently, a fosmid library derived from flow-sorted Y-chromosomes of the pig has been established, and several hundreds of clones that potential contain Y-specific genes were sequenced using shotgun approach. In spite of the ensuing difficulties in assembling repetitive sequences, a highly tentative pig Y map has been proposed. To further validate this pig Y map, we have carried out extensive multicolour fluorescent in situ hybridisation (FISH) at three levels of resolution (i.e. metaphase, interphase and DNA-fibre). The FISH results have led to an significant improvement of the pig Y assembly by enabling the determination of correct gene order and gap sizes as well as gene copy number. Furthermore, our results demonstrate that Molecular Combing is a robust tool for characterizing genomic region enriched in repeats. This presentation will highlight the contribution of molecular cytogenetics to our understanding of genomic structure and evolution of the pig Y chromosome.

O10

Meiotic recombination analysis for individual chromosomes in male pigs

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For the first time in the domestic pig, immunolocalization of MLH1 protein allowed the direct study of meiotic recombination along the 18 porcine autosomes. Overall, 7,848 synaptonemal complexes from 436 spermatocytes were analyzed, and 13,969 recombination sites were mapped. For 2,034 out of the 7,848 synaptonemal complexes, immunostaining was combined with fluorescence in situ hybridization (FISH) in order to identify the chromosomes individually. The average total autosomal synaptonemal compexes length per cell was 190.3 μm, with on average 32.0 recombination sites (crossing-over) per cell. Significant intra- (i.e. between cells) and inter-individual variations were observed for the number of crossing-over and the length of the autosomal synaptonemal complexes. The distributions of the recombination sites were similar within each chromosomal category: for metacentrics and submetacentrics, the crossing-over concentrated in the telomeric regions of p- and q-arms, whereas for the acrocentrics, the two hotspots were located near the centromere and in the telomeric region. The lack of MLH1 foci mainly concerns the smaller chromosomes particularly *Sus scrofa* chromosome 18 (SSC18) and the sex chromosomes. Positive interference was demonstrated for all the autosomes, with an important variability between chromosomes.

O11

Copy Number Variations in Canadian swine populations

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Variability in genomes (including single nucleotide polymorphisms (SNP), copy number variations (CNV) and chromosomal rearrangements (CA)) is responsible for a significant proportion of the diverse phenotypes associated with many important traits including fertility. CAs have proven their potential detrimental effects through the identification of numerous infertile or subfertile animals. Smaller scale structural alterations represent the territory of CNVs that are estimated to occur more frequently than SNPs, thus can be considered as a prominent source of individual genetic

variation and candidate markers for diseases or economically important traits. We have chosen the Porcine SNP60 Genotyping Chip (Illumina) as a screening tool for CNVs. A group of 21 boars showing either very low or high Direct Boar Effect on litter size was identified as a set of candidate animals for a preliminary analysis. We identified 125 CNV regions distributed across the 18 autosomes and encompassing 360 genes. Several regions showed association with fertility. In the 2nd part of this work we have analyzed more than 4,000 boar samples and found 11 CNVs per animal on average, although this number ranges from 1 to 68. These will serve as candidate regions for future association studies to provide novel molecular markers of fertility.

Research support was obtained from NSERC, Agriculture and Agri-Food Canada, and the Canada Research Chairs program.

O12

Physical mapping of paralogs of Hox paralogs in duplicated genome of the North American paddlefish

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The North American Paddlefish (Polyodon spathula) belongs, together with sturgeons, to the most ancient extant lineage of ray-finned fishes (Actinopterygii). Recently, an analysis of Hox gene sequences (Hox-A and Hox-D clusters) confirmed an independent whole genome duplication (WGD) in the paddlefish lineage dated approximately 42 Myr ago. We used cloned paralogs alpha and beta of both of these Hox clusters investigated in the aforementioned study and mapped them onto paddlefish chromosomes using fluorescence in situ hybridization (FISH). The Hox-A alpha paralog was localized to the telomeric region of two large metacentric chromosomes. The Hox-A beta paralog was also localized telomerically but to a different pair of large metacentric chromosomes. There were no overlapping hybridization signals resulting from co-localization of these two paralogs. The Hox-D beta paralog was localized to the telomeric region of one pair of the largest acrocentric chromosomes. However, the Hox-D alpha paralog yielded a rather interspersed and inconclusive FISH signal. It was impossible to localize this region. As in the Hox-A paralogs, no overlapping hybridization signals could be observed.

We interpret these results as suggestive of an advanced stage of rediploidization in these two Hox gene clusters; this will be discussed in the context of consequences of WGD identifiable by means of molecular cytogenetics.

This study was supported by projects P506/11/P596 and 14-02940S (Czech Science Foundation, Czech Republic), CENAKVA CZ.1.05/2.1.00/01.0024 and LO1205 (with financial support from the MEYS of the CR under the NPU I program).

O13

Molecular Cytogenetic Studies on Indian mithun, gaur, mithun cattle crossbred and cattle

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Mithun (*Bos frontalis*) is a unique bovine species with three hypotheses for ancestry of Indian mithun. The mithun and Gaur are 2n=58, whereas cattle is 2n=60. The first autosomal pair and X chromosomes are submetacentric, the remaining chromosomes being acrocentrics in these two species. The aim is to study the chromosomal localization of repetitive DNA sequences by FISH in mithun and ancestral related species that

ultimately help in physical mapping of genes in these less explored species. The investigation was carried out on 40 mithuns, 6 mithun cattle crossbreds, 8 cattles and 8 gaurs. The metaphase spreads prepared conventionally were hybridized with centromeric and telomeric probes. The chromosomal localization was highlighted by FISH signals in all species. In addition, the bovine chromosome painting probes were also used to delineate the chromosomal rearrangements. The centromeric DNA sequences were similar in all acrocentric mithun chromosomes; no signals in submetacentric chromosome of mithun crossbred and gaur were observed, while in cattle the signals were present in all chromosomes. The preliminary study supports that the Indian mithun and gaur are more closely related ancestral species than cattle.

O14

Multicolor FISH with 10 specific painting probes for the rapid identification of the sub-metacentric river buffalo autosomes (*Bubalus bubalis,* 2n=50)

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Multiplex-FISH (M-FISH), spectral karyotyping (SKY) and combined binary ratio labeling FISH (COBRA-FISH) are the current methods used for the simultaneous visualization of chromosomes in different colors. Their application in human clinical cytogenetics makes easier the identification of chromosomal abnormalities. In animal cytogenetics the use of these methods is still very limited, mainly for the lack of species specific-probes. In this work we propose a multi-color approach based on the simultaneous hybridization of specific river buffalo chromosome painting probes. Ten specific autosomal probes were prepared through conventional microdissection and DOP-PCR. Probes were labeled with Spectrum Green and Spectrum Orange in the second DOP-PCR step. Five sequential rounds of FISH were performed on the same slides. Each round was realized by using two probes simultaneously hybridized on the mitosis. Slides were counterstained with DAPI in antifade. After each hybridization, the slide was washed twice in PBST for 10 min, air dried and reused again for the subsequent step. Digital images were captured in gray-scale and pseudo-colored by the software. All the five pairs of biarmed river buffalo autosomes (BBU 1p, 1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p and 5q) were identified with a different color. The simultaneous hybridization of these probes allowed to develop the first multi-color FISH in the river buffalo species. Given the remarkable extent of chromosome banding homology within the Bovidae family, these probes might be utilized for cross-species hybridization experiments within the family, thus opening further opportunities for cytogenetic investigation also in other species.

This study was supported by CISIA-VARIGEAV project, National Research Council (CNR) of Italy.

O15

Genomics and Evolution of TR loci in *Tursiops truncates*

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The dolphin (*Tursiops truncatus*) is a mammal that has fully adapted to the aquatic environment during its evolution. Despite its popularity and even iconic status, our knowledge about dolphin genomics and evolution is very limited. We employed the GenBank and the EMBL-EBI draft genome assemblies (Ttru_1.4 and turTru1, respectively) to identify T cell receptor (TR) loci in Tursiops truncatus. The dolphin gamma (TRG) locus is the smallest and simplest of all mammalian TRG loci yet studied because it only contains a single constant (TRGC), two variable (TRGV1, V2) and three joining (TRGJ1, J2, J3) genes only. Expression analysis to evaluate the gamma chain repertoire in the peripheral blood from animals in a marine monitored environment identified all the possible V-J rearrangements, some of which are preferentially expressed. Although there is not a definitive scenario because of gaps in the available assemblies, the dolphin beta (TRB) locus is also very simple in comparison to human and ovine. A significant bias for expression of the variable TRBV17S1 gene,

located downstream of the constant (TRBC) gene in an inverted transcriptional orientation, was observed. However, we have found no evidence of preferential rearrangement with regard to diversity (TRBD) and joining (TRBJ) genes, respectively. In addition, dolphin delta (TRD) genes are clustered within the alpha (TRA) locus and, as expected in artiodactyls, a recent genomic duplication involving genes belonging to the TRDV1 subgroup was evident. Finally phylogenetic analysis further confirms that cetaceans and artiodactyls are strictly related.

P13

Identification of a causative mutation for XXSR syndrome in dog

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The process of sex determination involves the coordinated steps of several genetic factors; the right process will lead to the development of testes in XY zygotes and ovaries in XX ones. The development of the testes is controlled by the SRY gene. Its activation induces a genetic pathway that lead to the development of a functional testis, involving another important gene: SOX9. SOX9 coordinates the activity of other genetic factors indispensable for testis development. However, we sometimes observe a testicular development in the absence of SRY: in this case we refer to subjects having XX sex reversal (XXSR). This syndrome is known in many species: human, pig, goat, llama, horse and dog. In dogs, however, this syndrome occurs with a higher frequency than in other species so much that has been reported in many breeds, including mongrels. Despite the high frequency no causative mutation has been identified until now. In this work we report the results of our analyses performed on different XXSR subjects by Array-CGH. We identified two dogs showing SOX9 gene alterations. This work opens a mean to improve our knowledge about sex determination pathway.

P14

Bioinformatic and molecular analysis of SOX9 locus in dog

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The SOX9 gene plays a key role in testicular development. After the activation of the process by SRY, SOX9 is the gene responsible for coordinating the activities of other genetic factors involved in testis development. In human, the investigation of XXSR patients has led to identify a region 5′ to SOX9 gene that seems to assume importance in the mechanism of gene regulation. This region, called *RevSex,* is positioned at about 50 kb from the start of the transcription region of SOX9. Contrary to what expected, the homologous region in dog is located very far to the SOX9 gene and additionally is located 3′. Finally this region is described as a polymorphic CNV in many breeds. Now the question to be answered is: in dog, has this region lost its regulatory function of sexual development as result of the shift of its position in the genome or it is due to the differences that exist in different species at the molecular level? Using FISH technology we were able to establish that the dog genome for this region has not been well assembled and even in this specie the *RevSex* region is located 5′ and close to the SOX9 gene. This work has allowed us to understand the need to verify the accuracy of genomic assemblies, obtained by bioinformatics tools, using molecular biology techniques useful to confirm the available data.

P15

Cytogenetic description of chicken microchromosomes at the lampbrush phase

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An asymmetrical karyotype of the chicken (*Gallus gallus domesticus*) (2n=78) comprises 28 pairs of microchromosomes, which represent the gene-rich part of the genome. In their most condensed state at the metaphase microchromosomes are less than 2 μm long, the smallest ones appearing as dots at the highest magnification of a light microscope. Due to their size, high GC content and apparently high density of repeated DNA, some chicken microchromosomes stay cytogenetically undistinguishable, are unidentifiable in a flow karyotype and are missing in the chicken genome assembly (GGA 29-31, 33-38). As a result of the transcription activation, at the diplotene stage of female meiosis all chicken chromosomes transform into greatly elongating structures known as lampbrush chromosomes. Their distinctive chromomere-loop patterns enable recognition of each chromosome, including the smallest ones.

We applied to chicken lampbrushes a set of BAC clones specific to medium-sized microchromosomes (GGA 17-28), which allowed us to identify them and construct their cytogenetic maps. We also ordered lampbrush microchromosomes carrying PO41 tandem repeats. All chromosomes carrying PO41 are monochiasmatic; chromatin of the smallest ones is packed into two chromomeres. Even at the lampbrush stage the smallest microchromosomes are less than 2 μm in length and probably correspond to uncharacterised chromosomes in the chicken karyotype.

The work is supported by SPbU grant 1.37.153.2014 and grant for Leading Scientific Schools HIII-3553.2014.4.

P16

Genomic characteristic of copy number variations in Polish cattle

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Genome structural variation like copy number variation (CNV) is recently in a scope of animal genetics as an important source of genetic variation with a potential large effect on phenotypic traits. In this study, we used data generated with Bovine SNP50 assay in 859 Holstein (HO) and 301 Polish Red (RP) cattle to generate CNV calls and to create the map of natural variation in genomes of those breeds. Within the genome of HO cattle we found 91 copy number variable regions (CNVRs) covering in total 168 Mb of sequence, corresponding to 6.7 % of the autosomal genome. Deletion events had a mean size of 941 kb, but the mean size of amplifications was much higher and was estimated at 3.4 Mb. In RP breed, 37 unique variable regions were detected. The regions had a mean size of 602 kb and together covered 22.3 Mb of sequence, corresponding to 0.89 % of the autosomal genome. Comparison of CNVRs coordinates between the studied breeds revealed that as much as 71 % of the regions identified in RP breed overlapped with 22 % regions found in HO breed. The overlapping segments covered in total 14.3 Mb. In HO breed, the CNVRs encompassed more than one thousand RefSeq genes. The highest coverage in CNVRs was found on BTA14 (almost 33 %) an the lowest on chromosome 16 (0.13 % of chromosome genomic sequence). No CNVRs were detected on BTA27 in both breeds.

P17

Chromosomal assignment of the small heat protein gens in the sheep genome

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Changes in small heat shock gene (sHsps) and protein expression are associated with prion protein deposition, gliosis, spongliosis and prion diseases. sHsps exhibit also a clear neuroprotective effect against the stress related to ovine encephalopathy. The aim of this study was the cytogenetic localization of five small heat shock protein genes: *HspB1*, *HspB2*, *HspB5*, *HspB6* and *HspB8* in the sheep genome with the use of the CHORI - 240 Bovine BAC Library clones as probes. Comparative, indirect mapping was performed due to ambiguous localization of the available BACs for these genes in the sheep. Prior to FISH, carried out on DAPI-FISH banded metaphase chromosomes, the presence of the genes of interest in the selected clones was

confirmed by PCR. The following physical location was established—the *HspB1* was mapped to ovine chromosome OAR24q22; *HspB6* to OAR14q24; *HspB8* to OAR17q24-q25 and two adjacent genes *HspB2* and *HspB5* were assigned to the same chromosome region 15q14-q21. The identical bovine BAC clones were previously used to locate studied genes in the homologous bovine genome regions, respectively, 25q22, 18q24, 17q24-q25 and 15q14-q21. Comparative cytogenetic localization confirmed the high conservation of autosomal chromosomes among bovid species and extended sheep cytogenetic map.

Research work financed from National Science Centre funds, author's project no. N N311 082540.

P18

Physical mapping of the *HSPB* genes in the domestic and wild pigs

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The small heat shock proteins (HSPB) display cytoprotective functions involving apoptosis and inflammatory response as well as chaperone activity concerning the protein folding and aggregation control. Expression of HSPB is essential for differentiation of nervous cells whereas the mutations of genes encoding these proteins are responsible for desmin-related myopathies or neurodegenerative and autoimmune diseases. The aim of this study was cytogenetic assignment of five small heat shock protein genes: *HspB1*, *HspB2*, *HspB5*, *HspB6* and *HspB8* in the domestic and wild pigs genomes with the use of the CHORI-242 Porcine BAC Library clones as probes. The clones were selected by using information on BAC-end sequences (BES). The presence of the genes in the chosen clones was confirmed by PCR. Chromosome localization was performed by FISH on DAPI-FISH banded metaphase plates. The studied genes were assigned to the following chromosome regions of the domestic and wild pigs: *HspB1* - SSC3p15; *HspB6* - SSC6q12; *HspB8* - SSC14q21; *HspB2* and *HspB5*, due to their proximity, to the same chromosome band—SSC9p21. Comparative FISH mapping confirmed the high chromosome homology and conservation of studied *Suidae* species.

Research work financed from National Science Centre funds, author's project no. N N311 082540.

P19

Molecular-genetic certification of kazakh sheep breeds

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Sheep breeding is a priority direction of agricultural development in Kazakhstan. However, in recent decades the system of scientific control over the selection process in sheep breeding was affected. Currently, individual farms are carrying out selection relying only on phenotypic characteristics of animals. As a result, there is a large heterogeneity of the sheep breeds, which reduces economic value of the species. One of the ways of genetic improvement of sheep herds is cytogenetic and molecular-genetic control of animals that are recommended for reproduction.

In this research we aim to study cytogenetic and molecular genetic characteristics of commercially valuable species of sheep in Kazakhstan (Edilbay and Kazakh arharomerinos) in order to create a genomic passport of breeds.

The selection and tribal documents of Edilbay and Kazakh arkharomerinos breeds, which presented in 4 breeding farms, were studied to date. The necessary zoo technical measurements were done, and rams and ewes with the best breed characteristics were selected. DNA samples presenting 69 Edilbay sheep, which have been selected by excellent zoo technical parameters, were well genotyped on 21 microsatellite loci. Also 30 Kazakh arharomerinos sheep were genotyped on 32 microsatellite loci.

P20

Comparative physical mapping of genes associated with meat production traits in the wild pig genome

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Uncoupling proteins *UCP2, UCP3* and ghrelin (*GHRL*) play a functional role in global energy metabolism in the body, growth and obesity as well as organoleptic meat quality. The studies of genes encoding these proteins, involving the genetic variation underlying economically important traits, may be useful for searching of markers associated with meat production in the wild pig to further integrate genomics of *Suidae* species. The aim of this study was comparative cytogenetic mapping of *GHRL* as well as *UCP2* and *UCP3* genes in the wild pig genome (*Sus scrofa scrofa*) with the use of commercial human probes (Vysis, Q-BIOgene), specific for human chromosome regions HSA3p25-26 and HSA11q13-14 comprising *loci* of these genes. Chromosome localization was performed by FISH on DAPI-FISH banded metaphase plates. The following physical location was established—the *GHRL* was assigned to wild pig chromosome SSC13q31-32, and due to their proximity, *UCP2* and *UCP3* were mapped to the same chromosome region—SSC9p21-24. Cross-species in situ hybridizations confirmed conservation of the linkage groups and high degree of homology of chromosome regions containing *GHRL*, *UCP2* and *UCP3 loci* in compared species.

This study was conducted as part of NRIAP statutory activity, project no. 04-006.1.

P21

FISH-based comparative mapping of the *Hsp27* gene on chromosomes of the domestic *Bovids*

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The Hsp27 (HspB1) protein, from the family of small heat shock proteins (sHsps), plays a functional role in regulation of many intracellular processes and protection from environmental stress factors. Mutations of *Hsp27* gene lead to neuronal cells dysfunction associated with myopathies, motor neuropathies and neurodegenerative disorders. The aim of this study was comparative mapping of *Hsp27* gene on cattle, sheep and goats chromosomes with the use of the CHORI-240 Bovine BAC Library clone as probe. Prior to FISH, carried out on DAPI-FISH banded metaphase chromosomes, the presence of this gene in the selected clone was confirmed by PCR. Precise physical location was established—the *Hsp27* was assigned to bovine BTA22q22 and caprine CHI22q22 genome regions as well as ovine chromosome band OAR24q22. The results obtained by FISH mapping with *locus*-specific bovine probe were verified by cross-species in situ hybridization with commercial human probe (Vysis), specific for human chromosome region HSA7q11.23 containing *Hsp27* gene. Corresponding human location of this gene is in agreement with comparative mapping data between bovids and humans. The studies confirmed conservative nature of linkage groups and homology of bovine, ovine, caprine and human chromosome regions comprising *Hsp27 locus*.

This study was conducted as part of NRIAP statutory activity, project no. 04-006.1.

P22

Genomic analysis of the ovine T cell receptor alfa/delta (TRA/TRD) locus deduced by comparative and expression analyses

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Recent advances in DNA sequencing technology have facilitated the availability of quite a large number of complete genomes from different species of mammals. To make sense to sequence data, the genomes should be annotated by characterizing regions on a single one based on some property and content information, and compared by defining relationships between regions in

one or more genomes. In this context, we have identified the genomic organization and the gene content of the TRA/TRD locus in *Ovis aries*, defined as a "γδ T cell high" species. For this purpose, a sequence of about 0.9 Mb was retrieved directly from the reference genome sequence available at NCBI database and analysed. Using a collection of sheep cDNA and genomic clones, the TRA/TRD genes were identified and annotated. The analysis has shown that although the general organization and the size of the sheep TRA/TRD locus does not differ greatly from that of the "γδ T cell low" species, differences in the gene content can be observed considering the large extension of the TRD germline repertoire mainly due to multiple duplication events. These data demonstrate that the sheep is able to generate a highly diversified repertoire of T cell receptor delta chain molecules. Our study contributes to gain a better understanding of the yet not well-defined role of γ/δ T cells in the cell-mediated immune response.

P23

Genome-wide SNP assay in the Nero Siciliano pig to discover potential wild boar introgression

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Genetic characterization is a fundamental prerequisite for managing animal genetic resources. Nowadays high-throughput commercial SNP platforms allow the genetic characterization of intra and inter-breeds diversity. The Illumina porcine SNP60 genotyping BeadChip was recently used for the genetic analysis of the autochthonous *Nero Siciliano* black pig, reared in the natural park of the Nebrodi Mountains. The comparison of 92 individuals well distributed in 22 farms with cosmopolitan breeds suggested the uniqueness of this breed. Moreover 100 randomly chosen SNP seemed enough to distinguish the investigated breeds, offering a useful tool for the traceability of typical products. Since cytogenetic investigation suggested a possible cross with wild boar in at least one *Nero Siciliano* pig, showing a rob(15;17), we compared the 92 individuals with 88 wild boars from a recent study on Northwest European breeds.

The genetic structure of this dataset (180 individuals) was investigated by a Bayesian clustering approach with the ADMIXTURE software, which highlighted a low level of admixture between the wild boar and the domestic pig in almost all samples (81 out of 92 pigs). This admixture could be derived both from SNP recently introgressed or from common ancestors. In particular two *Nero Siciliano* pigs showed a higher proportion of admixture. Analyses are in progress to assess the origin of the introgression in these two subjects.

Acknowledgements. The study was supported by the "CISIA-GenePig" project.

P24

Characterization of Leptin Receptor Gene in *Bubalus bubalis*

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The present study aimed to characterize the *leptin receptor* gene (*LEPR*) in *Bubalus bubalis* and to identify single-nucleotide polymorphism (SNP) sites in different coding and non-coding regions. Starting from the bovine whole genome shotgun sequence encoding the complete sequence of the *LEPR* gene, we had designed primers to amplify part of 5′ flanking region and the whole coding region. A group of 64 unrelated Mediterranean Italian buffaloes from Animal Production Research Centre (CRA-PCM) were genotyped by direct sequencing at 17 *LEPR* loci. Nucleotide sequence of different regions of bubaline *LEPR* was submitted to GenBank (Acc. Num: JQ235651, JQ235652, JQ235654, JQ235660, JQ235662). Twenty-eight SNPs were detected, genotype and minor allele frequencies of all animals were calculated. Linkage Disequilibrium between these SNPs were evaluated by Haploview software using the parameter Lewontin's D′ between all pairwise combinations of markers. The analysis highlights three haplotype blocks of linkage between polymorphisms. Sequence analysis revealed the presence of nine interesting SNPs. In the 5′ flanking region a triallelic SNP and one SNP that fall in the core sequence of PAR/bZIP family transcription

factor binding site (Genomatix software for transcriptional factor binding site search). In the exonic region we identified seven missense mutations, and SIFT software analysis showed that two of these mutations affect on protein function. Finally, in this study we report for the first time the molecular characterization of bubaline *leptin receptor* gene.

P25

Synaptic behavior and chromatin remodeling of the multiple sex chromosomes in bats

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The usual sex-chromosome system among eutherian mammals is XX/XY. However, many exceptions from this general pattern were discovered. One of these sex-chromosome mechanisms is the *multiple sex-chromosome system*, which is evolutionary-fixed among many bat species of the family Phyllostomidae, and has arisen by a translocation between one original gonosome (X or Y chromosome), and an autosome, giving rise to a "Neo-XY body". The aim of this work is to study the synaptic behavior and chromatin remodeling of multiple sex chromosomes in different species of phyllostomids. Testicular tissues from adult males of the species *Artibeus lituratus*, *Artibeus planirostris*, *Uroderma bilobatum* and *Vampyrodes caraccioli* are analyzed by optical/electron microscopy and immunofluorescence of meiotic proteins involved in synapsis (SYCP3, SYCE3), sister-chromatids cohesion (SMC3), early recombination (RAD51) and chromatin silencing (BRCA1, γ-H2AX). The common features shared by all of them are the presence of RAD51, BRCA1 and γ-H2AX-labelled chromatin domains on asynaptic regions of rearranged chromosomes. The presence of asynaptic segments throughout pachynema in many species of phyllostomids—that are fertile with normal spermatogenesis—contradicts the claim that silent asynaptic autosomal segments necessarily lead to spermatogenic impairment as it is known in humans.

P26

Comparative genomic mapping between two species of the *Neacomys* genus (Rodentia, Sigmodontinae, Oryzomyini)

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The *Neacomys* genus (Sigmodontinae, Oryzomyini) is composed of eight species with a distribution predominantly in the Amazon region. Most species have only the diploid number described. In this work we compare the karyotypes of two species, *Neacomys* sp. A (NSP-A, 2n=58/FNa=64) and *Neacomys* sp. B (NSP-B, 2n=54/FNa=66) by G-banding and Zoo-FISH with probes of *Hylaeamys megacephalus* (HME, 2n=54/FNa=62), besides comparison with *Cerradomys langguth* (CLA, 2n=46/FNa=62). NSP-A shows 33 hybridization signals in the haploid set with 15 autosomes and X shows conserved synteny (eight autosomes hybridized to whole chromosomes and five associated with other chromosomes), and the remaining eight probes give multiple signals on different chromosomes. NSP-B shows 39 hybridization signals in the haploid set with 12 autosomes and the X shows conserved synteny (6 autosomes hybridized to whole chromosomes and 6 associated with other chromosomes), and the remaining 11 probes give multiple signals on different chromosomes. A comparative analysis of NSP-A, NSP-B and CLA karyotypes show that the differences are due to chromosomal fusions, fissions and pericentric/paracentric inversions, with associations 20/[13,22]/4, 12/[16,17] and fission of 1 (1a and 1b), probably synapomorphic for *Neacomys*, and the associations 6/21 and 20/[13,22] probably ancestral traits of Oryzomyini tribe.

P27

Syntenic relationship among species of Oryzomyini and Akodontini Tribes (Rodentia: Sigmodontinae)

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Sigmodontinae rodents are one of the most diverse and complex group of mammals in South America, comprising 380 species. About 60 % belongs to Oryzomyini and Akodontini tribes. The high specific diversification observed in both tribes is accompanied by a remarkable morphological and numerical diversity of chromosome complements, from 2n=10–86. Developing a consistent hypothesis on the origin of this diversification depends on the correct establishment of chromosomal synteny analyzed in a suitable phylogenetic framework. We analyzed the species Akodon montensis (2n=24, AMO) and Thaptomys nigrita (2n=52, TNI) with chromosome probes from Hylaeamys megacephalus (2n=56, HME) of the tribe Oryzomyini. At least 16 of the 26 autosomal chromosome pairs of HME show conserved synteny in AMO and 17 in TNI. AMO, as in some species of the Akodon cursor group, has a highly fused karyotype, where many syntenic associations probably represent synapomorphies in this species group or, alternatively, AMO autapomorphies. A set of such associations (HME 3/25; 11/16,17; 6/21; 13,22/11; 13,22/20; 11/16,17; and, 14/19), which are also present in TNI, could represent possible synapomorphies of Akodontini. Extension of these observations to other members of the Akodontini and Oryzominyi tribes are necessary to confirm these assumptions.

P28

Cytogenetic evaluation in a Miniature Schnauzer with Persistent Müllerian Duct Syndrome (*PMDS*)

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A 3-years old male Miniature Schnauzer dog has been cytogenetically and genetically examined for a suspect of Persistent Müllerian Duct Syndrome (PMDS), a form of inherited male pseudohermaphroditism due to failure of the regression of Mullerian ducts in male during embryo development. In pure breed Miniature Schnauzer it is due to a point mutation in the exon 3 of MISRII gene. Chromosome stability tests like SCE and CA in lymphocytes are being used extensively to evaluate the presence and extent of chromosomal damage in human and animal populations. Very few studies have been performed to evaluate the association between sexual developmental disorders and chromosome stability. Aim of this work is to confirm the suspect of PMDS by sequencing the exon 3 of MISRII gene, to evaluate karyotype profile and, for the first time, chromosome stability before and 6 months after the orchidectomy and hysterectomy. Five clinically healthy dogs have been used as control group. Lymphocytes cultures for Chromosome Aberration (CA) test and treated for RBA-banding were set up and incubated at 37.5 °C for 72 h. BrdU was added to cultures for RBA-banding 6 h before the harvesting and Colcemid was added 1 h before harvesting. One hundred and 20 metaphases were examined from slides treated for CA test and RBA-banding respectively. Karyotype analysis was performed according to standard karyotype. Sequence analyses of MISRII exon 3 of the Miniature Schnauzer showed the homozygous C241T transition while karyotype was normal. A statistically significant difference has been found between the aneuploidy values of the PMSD dog before (21 %) and 6 month after (10 %) surgery and between the PMSD dog before (21 %) and the control group (11 %). Mean values of gaps and chromatid breaks in PMSD dog before orchidectomy and hysterectomy were 1.34±1.12 and 0.04±0.20, respectively; 6 months after surgery were 1.06±1.17 and 0.04±0.20, respectively; and in control group were 1.33±1.17 and 0.04±0.20, respectively, being the differences not statistically significant.

P29

Comparative FISH-mapping of *TNF*, *STAT5A* and *MNTR1A* fecundity genes on river buffalo, cattle, sheep and goat

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The aim of "CISIA" project, funded by National Research Council, was to improve the valorization and sustainability of Southern Italy agrifood products. One of the area of application of this project is to genotype Mediterranean Italian Buffaloes breeding in order to detect the genes involved in fertility and reproductive seasonality. For this reason, our contribute to this project has been to perform physical maps using bovine artificial chromosomes (BAC) clones containing genes related to fecundity. BACs were selected taking in account the data available on the Bov Map database (http://locus.jouy.inra.fr/cgibin/bovmap/intro2.pl), considering their physical position and the data obtained from banding experiments. Fluorescent in situ hybridization (FISH) was performed on three gene sequences: tumor necrosis factor-α (TNF), correlated to male fertility; signal transducer and activator of transcription 5A (STAT5A) important for its influence on milk production and reproduction activity; melatonin receptor 1A (MNTR1A) important for reproductive seasonality. BAC probes were hybridized on RB-banded of river buffalo (*Bubalus bubalis*, 2n=50, BBU), for the first time and to relate bovid species: cattle (Agerolese breed), sheep (Laticauda breed) and goat (Cilentana breed). TNF was assigned to BTA/CHI23q21-22, OAR20q21-22 and BBU 2p21-22; STAT5A was assigned to BTA/CHI19q17-21, OAR11q17-21 and BBU3p15-21; MTNR1A was assigned to BTA/CHI27q14-15, OAR11q17-21 and BBU1p21-22, underling the high degree of chromosome homologies among Bovids and extending the cytogenetic maps of this economically important species.

Acknowledgements. This study was in part supported by CISIA-VARIGEAV project and in part by RARECA-project, PSR, Misure 214 e2 of Campania region.

P30

Phylogenetic reconstruction in Phyllostomini tribe (*Chiroptera, Phyllost*omidae) based on cross-species chromosome painting

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Phyllostominae presents different rates of chromosomal evolution between genera, with *Phyllostomus* probably retaining the ancestral karyotype for this subfamily. We performed multi-directional chromosome painting, hybridizing whole chromosome paint probes from *Carollia brevicauda* (CBR) and *Phyllostomus hastatus* (PHA) onto chromosomes of *Lophostoma silvicola* (LSI, 2n=34, FN=60), *Phyllostomus discolor* (PDI, 2n=32, FN=60) and *Tonatia saurophila* (TSA, 2n=16, FN=20) from tribe Phyllostomini of Amazon region, to reconstruct the phylogenetic relationships within this tribe. LSI has 2n=34, FN=60; PDI has 2n=32, FN=60; TSA has 2n=16, FN=20. Comparative analysis using G-banding and chromosome painting show that the karyotypic complement of TSA is highly rearranged relative to LSI, PHA, while LSI, PHA and PDI have similar karyotypes, differing by only three chromosome pairs. Nearly all chromosomes of PDI and PHA were conserved in toto, except for chromosome 15 that changed by a pericentric inversion. A strongly supported phylogeny (bootstrap=100), confirms the monophyly of Phyllostomini. In agreement with molecular topologies, TSA was in basal position, while PHA and LSI formed sister taxa. A few ancestral syntenies are

conserved without rearrangements and most associations are apomorphic traits for TSA or plesiomorphic (PHA-11, 14 and 15) for the three genera analyzed here.

Environmental Cytogenetics and Mutagenesis

L10

Chromosomal mutations in captive ungulates in zoological gardens

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Numerous large mammals are kept and bred in zoological gardens. Even though there are breeding programmes for a number of species, cytogenetic testing is not commonplace.

Congenital balanced structural chromosomal aberrations (reciprocal and Robertsonian translocations) usually do not manifest themselves in the phenotype of their carriers and can be transmitted from generation to generation. However, such a chromosomal rearrangement might affect the meiotic process and fertility of the carrier. While examining Burchell's zebras we found a female animal with a constitutional translocation t(8;13)(p;p). We also found the same translocation in a proband's female offspring and this offspring's male offspring, but not in parents. Thus, the translocation originated de novo in the proband and was transmitted by females to the next two generations. To the best of our knowledge, no translocation has been described in zebras to date. In a Mountain Reedbuck we identified a de novo Robertsonian translocation and in a Forest Buffalo centric fission of a submetacentric chromosome. The situation in captive animals of family Bovidae is complicated by centric fusion polymorphism, which is very common in some species. In the present study, we described the way of transmission and prevalence of this polymorphism in captive populations of impalas and waterbucks. Furthermore, in captive animals, we described numerical aberrations of sex chromosomes, such as XYY/XY mosaic in Mountain Reedbuck and trisomy of chromosome X in a Red Lechwe. The study demonstrates the important role of cytogenetic screening in captive animals at zoological gardens.

O16

Illegitimate Recombination between T Cell Receptor Genes

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T cells express either the αβ or γδ type of antigen-specific receptor encoded by T cell receptor (TCR) genes (*TRA*, *TRB*, *TRD* and *TRG*) which reside in three loci on mammalian chromosomes. During the maturation of T cells, TCR genes are rearranged by site-specific recombination. Occasionally, interlocus recombination of different TCR genes takes place, resulting in chromosome rearrangements. It has been suggested that the absolute number of these *trans*-rearrangements correlates with the risk of lymphoma.

Using fluorescence in situ hybridization with painting probes we assessed the frequencies of rearrangements with breakpoints in TCR genes in pig peripheral blood lymphocytes (PBLs), separated T lymphocytes and γδ T lymphocytes. The frequencies of two translocations involving the *TRA*/*TRD* locus in pigs were significantly higher than the frequency of translocations with breakpoints in the *TRB* and *TRG* genes and the frequencies of all corresponding rearrangements in human lymphocytes. The increase was detected in porcine PBLs and separated T cells but not in γδ T cells.

To study the effect of evolutionary chromosome reshuffling on accessibility of TCR genes for interlocus recombination, we investigated these trans-rearrangements in equids. The results suggest the impact of rearrangements of chromosomal segments bearing TCR gene and their orientation with respect to the centromere on illegitimate recombination between TCR genes.

O17

Environmental impact on sheep pastured in some polluted areas of Sardinia island: preliminary results with SCE-test

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Sardinia is the second largest island in the Mediterranean sea. Although it is famous for very beautiful landscapes not only along the coast, there are some polluted areas. A special project supported by the Sardinian Region and involving several research groups is trying to study these areas by using several approaches. In the present study, we report the preliminary results obtained from six sheep herds (Sardinian breed) grazing on natural pasturelands in the vicinity of possible polluted areas. Two herds were located in the Nordern area (industrial and military zones) and four herds were located in the Souther area (military, industrial and mine zones) of the Iceland.

Peripheral blood samples were collected from 20 adult sheep (over 4 years) randomly selected from each of six herds grazing around above mentioned areas, as well as from three herds grazing in areas retained far from possible polluted areas and used as control. Two types of cell cultures were performed: without (normal cultures) and with addition of BrdU during the last 28 h: the former, to study the AC-test (chromosome and chromatid breaks), the latter for the SCE-test. In this report only data from SCE-test are reported. A total of 92 sheep from polluted areas and 37 sheep from control areas were studied. Upon 35 cells studied for each animal, SCE-mean values were 8.65±3.40, 8.10±3.50, 8.05±3.08, 7.42±3.34, 9.28±3.56 and 8.38±3.29 in the exposed areas, as well as 7.86±3.31 in the control group. Significant increasing ($P<0.01$) of SCEs were found only in three areas of Southern area. Cytogenetic analyses using AC-test on the same animal groups are in progress.

Acknowledgements. The study has been supported by "Regione Autonoma della Sardegna", project CRP-24901, L.R. 7/2007.

P31

Chromosome stability in Agerolese cattle heterozygous for rob(1;29)

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Agerolese cattle is an autochtonous endangered breed reared in Campania region included in the RARECA project of protection and safeguard (PSR Campania 2007–2013—Misura 214). Rob(1;29) has been found in several Italian autochthonous breeds with very different frequency ranging from 0.9 % in the Grey Alpine and 23.3 % in the Cinisara. In the present study, five Agerolese cattle (4 females and 1 male) heterozygous carriers of rob(1;29) (group 1) and nine Agerolese cattle (7 females and 2 males) with normal karyotype (control group), reared in different farms, have been analyzed to asses chromosome stability by SCE test using peripheral blood samples. Mean values of SCE/cell were 6.34±2.72 and 5.44±2.50 in group 1 (carriers) and control, respectively being the difference statistically significant ($p<0.05$); mean values of SCE/X chromosome were 0.45±0.67 and 0.27±0.59 in group 1 and control group respectively being the difference statistically significant ($p<0.05$); mean values of SCE in t1 and t29 were 0.22±0.47 and 0.02±0.15, respectively. The mean value of SCE/cell in the control group (5.44±2.50) is almost comparable with the one earlier found in Agerolese cattle breed showing that, despite the selection programs currently carried out, no changes in genome stability have been occurred. Although mean number of SCE/cell is higher in Agerolese cattle carrying rob(1;29) than in the control, the observed SCEs number (42) on the rob(1;29) was considerately lower than those expected (72) on the basis of relative chromosome length. On the contrary, the SCEs number observed in the X (78) was much higher than expected (51.07). At the moment

there are no data in literature to explain this condition however it has been demonstrated that in Agerolese cattle carrying rob(1;29) there is one fragile site more than in other animals, future studies will evaluate a possible correlation between the increase in SCEs on X chromosome and the presence of this fragile site.

This research was supported by PSR Campania 2007–2013—Misura 214, RARECA project.

P32

Sister chromatid exchange (SCE) test in river buffalo cells treated with Furocoumarins

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Cytogenetic test can be very useful to detect chromosome (genome) fragility in both animal and human cells. Furocoumarin derivatives constitute a class of compounds widely investigated for the development of photo-chemotherapeutic drugs and effective in treating many diseases. The interest inside this research field originated from the effectiveness of PUVA therapy, realized by oral or topical administration of a linear furocoumarin (psoralen) followed by irradiation with UVA light, for the treatment of psoriasis and cutaneous T-cell lymphoma. Furocoumarins are also present in *Psoralea* plants elected to be also used as alternative feed for animals considering that it's a perennial leguminous and, more important, it's green during the summer time. In the present study we report the preliminary results obtained using the SCE-test in river buffalo cells exposed in vitro to furocoumarin extracts from a Sardinian population of *Posoralea morisana* (L.) Stirton (Punta Giglio,). Peripheral blood samples from five young river buffaloes (2 males and 3 females) were incubated at 38 °C for 72 h in presence of different quantities of furocoumarin extracts: 0 (control), 50, 100, 200 and 400 μg/ml. Thirty cells for each cell culture (and furocoumarin dose) were analyzed. Although the cell growth appeared normal in both treated and untreated (control) cells, a significant ($P<0.01$) higher number of SCEs observed in treated cells, compared to those achieved in the control. On the basis of these results, cells from five river buffalo cows were treated with 0 (control), 100 and 200 μg/ml of furocoumarins for only 3 h after 24 h of incubation, in presence and absence of S9. No statistical differences were found between treated and untreated cells with furocoumarins both in presence and absence of S9.

Acknowledgements. The study has been supported by CISIA-VARIGEAV project, National Research Council (CNR) of Italy.

P33

Chromosomal abnormalities in cattle induced by aflatoxin contamination of forages

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During the last year, in the south-eastern part of Romania, a high aflatoxin contamination of milk was identified in many cattle farms. The values of aflatoxin concentration of milk was higher then permitted (0.05 ppb), ranging from 0.06 to 0.228 ppb. In this context a cytogenetic investigation was carried out for 45 Romanian Black and Spotted cows from two cattle farms: one with aflatoxin contamination and one free of aflatoxin contamination. Our study revealed that four cows from contaminated farm presented a large number of mono-and bicromatidic breackages on autosomes and heterosomes, loss of chromosome fragments and gaps. Our investigation continued through SCEs-test, which is a specific test for identifying the effects of toxic agents on the genetic material integrity. For animals with many chromosomal breakages the number of sister chromatid exchanges (SCEs) was extremely high (11.7±2.56 SCEs/cell) compared to the normal animals (6.73± 2.47 SCEs/cell). Chemical analyses were performed on feed samples (alfaalfa hay, corn silage, mixed fodder and bran) used for feeding of the cows raised in the two farms. In the contaminated farm it was identified a level of aflatoxin content more than maximum allowed (<4.0) in 2 of the 4 forages analyzed as follows: alfaalfa hay (4.93±0.03) and bran (5.17±0.05). With this results, we can appreciate that the chromosomal abnormalities we

found are related to the aflatoxin contamination identified in milk and forages. The assessment of the aflatoxin effects on the genetic material integrity of investigated cows emphasizes the role of animals as biological indicators of the environmental pollution.

Acknowledgements: *This study was supported by the Sectorial Project 7.3.1./2011. We are grateful to Prof. L. Iannuzzi, ISPAAM Napoli, Italy, for the scientific and technical support.*

P34

Chromosomal instability in Italian Friesian cows exposed to dioxins and raised in proximity of an industrial area producing steel in Taranto city (Southern-Italy)

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Dioxins are a large family of congeners which can be fitted in three main groups: polychloro-dibenzo-dioxins (PCDDs), polychloro-dibenzo-furans (PCDFs) and dioxin-like polychlorobiphenyls (DL-PCBs). These chemicals are considered highly toxic, especially the tetrachloro-dibenzo-*p*-dioxin (TCDD). Most PCDDs, PCDFs are produced by both industrial processes and illegal waste burning, while DL-PCBs are produced only during some industrial process like those producing steel. Dioxins are also high persistent in the environment, especially when entering the human or animal body due to their ability to be absorbed by fat tissue where they can remain for long time being their half-life in the body for years. Fifty-six randomly selected Italian Friesian cows from two farms located in vicinity and far (control) from a metallurgic factory of Taranto city (Southern-Italy) underwent cytogenetic investigations to ascertain possible differences in their chromosome fragility. The farm located close to the industrial area was under legal sequestration due to the presence in the milk mass of higher mean values of dioxins (mainly DL-PCBs) than those permitted. As control, samples of cows of same breed reared far (65 Km) from the industrial area, were employed. The two cytogenetic test we used (chromatid and chromosome breaks, SCE) revealed a significantly ($P<0.01$) higher chromosome fragility in cells of exposed cows compared to those of control, thus suggesting a new politic of animal breeding to prevent contamination of the food chain and human diseases in urban areas, especially close to metallurgic factories.

Supported by PON 2007–2013, 01_01145, Project "Sviluppo tecnologico e innovazione per la sostenibilità e competitività della cerealicoltura meridionale" (ISCOCEM).

P35

Citogenetic investigations in two endangered pig breeds raised in Southern-Italy

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Pig from Casertana and Siciliana breeds are two endangered breeds raised in the southern-Italy (Campania and Sicily regions, respectively) and characterized to have a black skin. Special projects are trying to save and characterize both of them by using several approaches. In this study we report the preliminary results we obtained after a cytogenetic investigation we performed by using both C- and R-banding techniques and the sister chromatid exchange (SCE) test to verify their chromosome stability under the environmental conditions. Fifty-two pigs from Casertana breed and 19 pigs from Siciliana breed were investigated. All animals from both breeds showed a normal karyotype, with the exception of two male pig from Siciliana breed which were found heterozygous carrier of rob(15;17) (2n=37, XY), probably being hybrids with the wild pig (2n=36) present in the

Nebrodi mountains where this breed is raised in Sicily. SCE-test applied on 42 pigs from Casertana breed (22 males and 20 females) and 19 pigs from Siciliana breed (8 males and 11 females) revealed no statistical differences between the SCE-mean number in Casertana pig (7.13±3.20) than that (6.87±3.12) achieved in Siciliana pig. Statistical differences were found between males (7.26±3.38) and females (6.59±2.90) of Siciliana pig breed, as well as between females of Casertana (7.24±3.26) and Siciliana (6.59±2.90) breeds, while no statistical differences were found between males of the breeds, as well as between males and females of Casertana breed.

Acknowledgements. The study has been supported by project "RARECA, PSR, Misura 214 e2" of Campania region and "CISIA-GenePig" project, National Research Council (CNR) of Italy.

P36

Cytogenetic analyses in rabbits feed in presence of Verbascoside: SCE-test

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Phenylpropanoid glycosides (PPG), like other phenolic compounds, are powerful antioxidants. Beside phenolic compounds, verbascoside, shows the highest scavenger activity in the PPG and has high antioxidant power in comparison with other phenolic compounds. Previous studies by using in vitro exposure of human blood lymphocytes to verbascoside reported a significant increasing of chromosome fragility compared to control. In the present study four homogeneous groups of rabbits (six animals per group) were used to test in vivo the verbascoside by feeding the animals without Verbascoside and Licopene (*control – group A*), with lycopene (*5 mg/Kg of feeding, group B*), with verbascoside (*5 mg/Kg of feeding, group C*) with verbascoside and lycopene (*5 mg/Kg of feeding each, group D*). Peripheral blood cultures were performed in three different times: at 0, 40 and 80 days of the experiment. Two types of cell cultures were performed: without (normal cultures) for the AC-test (chromosome and chromatid breaks) and with BrdU (10 μg/ml), the latter added 26 h before harvesting, for the SCE-test. In the present study only data from SCE-test are presented. Mean number of SCEs were generally lower at both 40 and 80 days in groups B, C and D, compared with the same groups at zero day. In particular, they were statistically ($P<0.01$) lower at 40 and 80 days when using lycopene. In conclusion, on the basis of SCE-test applied on cells of rabbits treated in vivo with verbascoside or/and with lycopene, no chromosome fragility increasings were observed in cells of rabbit feed with verbascoside. However, a final conclusion will be done when data from AC-test will be available.

Evolutionary and Comparative Cytogenetics

L11

Avian cytogenetics goes functional

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Whole chromosomes (and sub-chromosomal homologous synteny blocks (HSBs)) have great significance in molecular studies of genome evolution. In birds, our ability to define chromosomes and HSBs precisely has however been impeded by a near intractable karyotype and so has focused primarily on comparative molecular cytogenetics (zoo-FISH) of the largest chromosomes (1–10+Z). Availability of multiple avian genome sequence assemblies has however allowed us, for the first time, to identify chromosomal syntenies across species. In recent work we have made use of comparative maps for 20+ avian genome assemblies (plus out-groups) and presented them on "Evolution Highway" an open-access, interactive freely available comparative chromosome browser designed to store and visualise comparative chromosome maps.

This browser (http://evolutionhighway.ncsa.uiuc.edu) is used to visualize comparative genome organization and to identify and visualize the different types of evolutionary breakpoint regions (EBRs) in chromosomes, e.g., lineage specific, ordinal, superordinal, and reuse. Comparative analysis of all available genomes is providing insight into the mechanisms of chromosome change through correlation of EBRs with transposable elements and non-allelic homologous recombination. Gene ontology analysis is revealing interesting correlations with avian specific phenotype and function. Focus on six genomes (chicken, turkey, duck, zebra finch, ostrich and budgerigar) with both the largest N50s and supporting molecular cytogenetic information, has allowed us to assemble a putative ancestral avian karyotype and identify the key changes that led to the gross genome organization of representatives in the major avian clades (Palaeognathae, Galliformes, Anseriformes and Neoaves). We describe, for the first time, numerous inter-chromosomal rearrangements in a Paleoganthaeous bird (the ostrich), plus rearrangements in the budgerigar (Psattaciformes) and 15 other species. Intra-chromosomal evolutionary change in all species studied, can be derived, most parsimoniously, by a series of inversions, inter-chromosomal rearrangements by fissions and fusions. Increased chromosome rearrangement is associated with differentiation in certain clades, with the most intrachromosomal changes (primarily inversions) occurring in the zebra finch (Passeriformes) since its divergence from its sister group, the Psittaciformes 54MYA, This is coincident with the evolution of passerine-specific phenotypes e.g. vocal learning. Results also suggest that the Galloanserae (especially chicken) underwent the fewest changes compared to the ancestral karyotype; notably these birds appear, from fossil evidence, to be the most similar to ancient avian ancestors. We thus present the most comprehensive analysis of chromosomal rearrangements in birds to date and draw novel conclusions about their mechanisms of origin and association with avian-specific phenotypic features.

L12

Evolution and molecular dynamics of centromeres in the genus Equus

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The centromere is the locus directing chromosome segregation at cell division. The mechanism by which centromere identity is specified on chromosomal DNA sequences has been deeply enigmatic, with a clear dependence on the epigenetic inheritance of the centromeric histone, CENP-A. While a degree of autonomy of centromere placement along the chromosome has been established by studies of human neocentromeres and observation of evolutionary centromere repositioning, a role for DNA sequence in driving centromere location remains to be elucidated. The typical association of mammalian centromeres with extensive arrays of highly repetitive satellite DNA, has so far hampered a detailed molecular dissection of centromere function and evolution.

In previous work, we discovered that, in the genus Equus (horses, asses and zebras), centromere repositioning during evolution was exceptionally frequent and that satellite DNA and centromeres are often uncoupled in this genus. We then described the first native satellite-free centromere discovered in a mammal, that of horse chromosome 11; using a combination of molecular and cytogenetic approaches we recently demonstrated that the precise positioning of this native mammalian centromere is highly variable, even on the two homologous chromosomes in a single individual. These results corroborate the hypothesis that CENP-A is the principal determinant of centromere identity, but they make a much deeper point: CENP-A location along the DNA polymer is not fixed but rather exhibits a diffusion-like behavior.

We are now characterizing a number of satellite-less centromeres in asses and zebras; preliminary observations on the molecular organization of centromeres, based on the exploitation of this powerful model system, will be presented.

O18

New evolutionary differences between cattle and goat

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Cattle (*Bos taurus*) and goat (*Capra hircus*) have the same number of chromosomes: 2n=60. Many studies based on the application of the banding techniques have highlighted the great homology between the two sets of chromosomes and have confirmed the hypothesis that lays centric fusions as the main instrument of the evolution of karyotypes among bovids. To date it was highlighted only one exception to this rule: a centromeric region of BTA9 does not map on goat homologue chromosome (CHI9) but is both located on CHI14 chromosome and inverted.

In this preliminary work we applied a bioinformatic strategy to highlight any other differences and then FISH technology to confirm or reject the alleged evolutionary divergences. Following this strategy, we have identified many evolutionary divergences until now unknown; some of them have been confirmed and therefore we can say that do not represent errors in the genomes assembly but real evolutionary divergences.

This study has opened a new mean to improve the knowledge about genomic animal evolution. It should be investigated further in order to create genetic maps more and more accurate.

O19

Numerous interchromosomal rearrangements in spite of high synteny conservation between duck and chicken

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The Beijing duck (*Anas platyrhynchos*, APL) karyotype presents most of the characteristics of the typical avian karyotype: a large number of chromosomes (2n=80), the presence of numerous microchromosomes, the female is the heterogametic sex (ZW) and male the homogametic one (ZZ). Detailed genomic studies have been developed for this species, as it is a natural reservoir of all influenza A viruses and is used a food source, culminating with the publication of its genome in 2013.

Several evolutionary studies have shown that birds seem to have a very stable organization of their genomes when compared to mammals, with only very rare interchromosomal rearrangements corresponding to fusion/fission events. The phylogenetic distance between chicken and duck is 80 million years. To date, only one interchromosomal difference was reported between the chicken (*Gallus gallus domesticus*, GGA) and the duck karyotypes, with APL4 and APL10 corresponding to GGA4q and GGA4p respectively. Only very few intrachromosomal rearrangements were observed. The construction of RH maps allowed ordering the scaffolds of the duck assembly along the corresponding duck chromosomes and to compare the duck genome sequence with the chicken genome chromosome wise. This establishment of high resolution comparative maps led to the discovery of a higher number of intrachromosomal rearrangements than expected considering the previous published data. These rearrangements have been confirmed by heterologous FISH using chicken BAC clones from the Wageningen library. This suggests a specific evolutionary mechanism in bird genomes based mainly on complex intrachromosomal rearrangements.

O20

An update to the chromosomal data of the European bitterling *Rhodeus amarus* (Teleostei: Cypriniformes: Acheilognathinae)

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A cytogenetic analysis was performed on the European bitterling *Rhodeus amarus* using conventional Giemsa staining, C-banding, silver nitrate (Ag-NOR) and chromomycin A_3 (CMA_3) staining. Physical mapping of the 28S and 5S ribosomal genes, and the telomeric sequence on the chromosomes were also performed. All analyzed specimens showed the same chromosomal constitution of 2n=48 (8m+20sm+20st-a). C-banding revealed the constitutive heterochromatin at the pericentromeric regions in most of the chromosomes, and some of the chromosomes had completely heterochromatic short arms. NORs were located at the p-arms of two submetacentric chromosomes and the size polymorphism of NORs was found. GC-rich chromatic blocks were observed on the short arms of the two NOR bearing chromosomes. Size polymorphism of the CMA_3 positive-sites was identified. No DAPI-positive signals were detected on the chromosomes. 28S rDNA was placed on short (or sometimes both) arms of three submetacentric chromosomes and on p-arms from three to five acrocentric chromosomes, while 5S rDNA sites were found on the short arms of two subtelocentric chromosomes. The telomeric repeats were localized at the ends of all the chromosomes. Obtained results may suggest that some inter-population chromosomal diversity may occur in *Rhodeus amarus*. This data may also be useful in a conservation of this endangered species.

O21

Comparative genomics between pig, cattle and human: establishing precise chromosomal syntenies using GenAlyzer and FISH

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The study of chromosomes (cytogenetics) is of utmost significance in many scientific areas such as medical research and diagnostics as well as genome evolution. Research from an evolutionary perspective has provided much insight into the origin of disease mechanisms. For example, changes in chromosome structure can lead to infertility in patients, and thereafter, reproductive isolation. There have been many improvements over the years in cytogenetic techniques such as chromosome spreading, banding and Fluorescence in situ Hybridisation (FISH). The availability of assembled whole genome sequences has however taken this analysis a step further and permitted global analysis of genomes, pinpointing chromosomal changes more precisely. Significant progress in pig and cattle genomics has also been made in recent years with the pig genome assembly now being in its 10th iteration. GenAlyzer was used to establish chromosomal syntenies between pig, human and cattle to identify evolutionary breakpoint regions (EBRs), homologous synteny blocks (HSBs) and intrachromosomal translocations (ICTs) between the three species.

In total 115 evolutionary breakpoints were identified between pig and human, 184 between pig and cattle and 149 between human and cattle. Selected pig/human syntenies were confirmed independently by FISH.

Data generated had provided us with reasonable confidence that GenAlyzer data represents an accurate indicator of the real biological comparisons between the species in this study.

P37

A comparative study of meiotic recombination in domestic cattle, sheep, goat and barbary sheep

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Homologous recombination in sexually reproducing organisms plays crucial role in correct segregation of chromosomes into gametes and creates genetic diversity for natural selection. Despite quite similar mammalian genome size, great variability in recombination rates is observed.

In our study we evaluated crossover number, their position on chromosomes, as well as effect of a crossover interference in three domestic species—bull, sheep, goat (*Bos taurus*, 2n=60; *Ovis aries*, 2n=54; *Capra hircus*, 2n=60) and one wildlife species—barbary sheep (*Ammotragus lervia*, 2n=58). Different chromosome count in sheep, which is caused by three Robertsonian fusions of ancestral bovid chromosomes, gave us an opportunity for comparison of distribution of homologous recombination.

Crossover frequency and positions were analyzed by immunofluorescence labeling of pachytene spermatocytes, using antibodies against SCP3, MLH1 and centromeric proteins. In total, 10 sheep, 4 bulls, 4 goats and one barbary sheep were examined. FISH method was utilized for recognition of corresponding chromosomes in cattle and sheep.

The mean numbers of MLH1 foci were 47.2 in bulls, 60.9 in goat, 62.2 in sheep and 57.6 in barbary sheep. As our data suggest there are significant differences in recombination rates between bulls and animals from tribe Caprini. Also, we observed great interindividual variability in recombination rates (sheep 58.2–67.3, bull 46.3–50.4).

P38

Chromosomal mapping of 5S rDNA in two species from the subfamily Alburninae (Teleostei: Cypriniformes: Cyprinidae)

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Two species of the family Alburninae *viz*. bleak *Alburnus alburnus* and spirlin *Alburnoides bipunctatus* were cytogenetically examined by fluorescence in situ hybridization (FISH) with 5S rDNA as a probe. The range from three to five of 5S rDNA signals were observed in *A. alburnus*, and from four to nine in *A. bipunctatus*. The 5S rDNA sites were mainly found on the short (p) arms of two submetacentric and two subtelo-acrocentric chromosomes in bleak, while the most common hybridization pattern in spirlin consisted of six signals also located on the short (p) arms of two submetacentric and four subtelocentric chromosomes.

The presented results indicate high cytogenetic similarities of both fish species to the characters specific for subfamilies Alburninae and Leuciscinae. Thus, observed chromosome features supported a connection of bleak and spirlin into one group according to the phylogenetic relationships suggested by other authors.

P39

Cytogenetic Architecture of Indian mithun (*Bos frontalis*), mithun cattle crossbred by conventional staining, G-, C and R-banding techniques

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Mithun (*Bos frontalis*) is conserved in the north-eastern states of India, used mainly for meat purpose. The prime objective is to construct the cytogenetic profile for complete chromosomal characterization of mithun.

The investigation performed on 90 mithuns and 6 mithun cattle crossbred (100 spreads/per animal). It was found that 2n=58 both in mithun and crossbred. Apart from karyotype and ideogram, the cytogenetic analysis indicated that first autosome and X chromosome were submetacentric (centromeric index=29.48 %, 28.02 %, arm ratio=2.40, 2.03, respectively), the rest of the autosomes being acrocentric, while the Y chromosome is the smallest metacentric (CI=46.51 %, AR=1.65). The average relative length was 5.51 % with range from 1.79 to 5.70 %, and in Y chromosome it is 1.59 %. The R- and C-banded karyotype suggested that the X chromosome is second largest sub metacentric and the largest is first pair of autosome. The average number of dark bands is 3 and 5, whereas light bands are 4 and 7 in submetacentric and acrocentric chromosome respectively with R-banding.

P40

Chromosomal mapping of 5 classes of repetitive DNAs in three species of the genus *Eigenmannia* (Gymnotiformes, Sternopygidae)

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Eigenmannia Jordan & Evermann (1896) is a genus of electric fish endemic of the Neotropical region. *Eigenmannia* is composed by eight valid species that

may be subdivided in two main groups, the microstoma and virences group. Cytogenetic studies in this genus indicate the occurrence of variation of the karyotypic macrostructure, describing karyotypes ranging from 24 to 40 chromosomes. Despite this wide variation, the most common dipliode number corresponds to the 38 chromosomes and can differentiate only by their karyotype morphology. Here, we report the chromosomal locations of the five classes of repetitive DNAs revealed by fluorescent in situ hybridization (5S-18S rDNA, H3/H4 Histone cluster and U2snRNA) in three species of the *Eigenmannia* genus, *E. microstoma*, *E. limbata* and *E. virescens,* all with karyotypes composed for 38 chromosomes. For the three species, the 18S rDNA was located on a single pair of chromosomes (ST), while the sites for 5S rDNA were found in the pericentromeric region on its chromosomes carrying, distributed in a particular way in each species. The location of the H3/H4 histones was widely dispersed throughout the genome of three species. On the other hand, the U2 snRNA follows a pattern similar to that exhibited by 5S rDNA distribution, presenting blocks on specific chromosomes in each species, however, contrasting the patterns observed in others fishes with only one site. Little is known about the structure and organization of Gymnotiforms genomes and the knowledge of the chromosomal distribution of repetitive DNA sequences in *Eigenmannia* represents the first step for achieving an integrated view of their genomes.

P41

Karyotype variability and molecular heterogeneity in *Xyrichthys novacula* (Labridae, Perciformes) possibly disclosing cryptic species

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Xyrichthys novacula (Linnaeus, 1758) is a benthic species, distributed in both sides of the Atlantic Ocean (including the Caribbean Sea) and in the Mediterranean Sea. A cytogenetic and molecular survey on specimens collected in Margarita Island (Venezuela) reveals variability in the diploid number (2n=45–48, NF=51–56), due to variable number of both acrocentric (35–41) and large metacentric (1–3) chromosomes. The observed pattern is likely due to centric fusions, as it cannot be attributed to the presence of B chromosomes or of sex-chromosomes. Molecular comparison of COI sequences obtained in this study with sequences retrieved from GenBank provides a phylogenetic tree with two groups, one exclusive of some of the specimens from Venezuela, the second clustering the remaining specimens from Isla de Margarita and individuals collected in Mexico, Panama and Virgin Islands. A parallel survey has been undertaken on samples from Italian coasts, where *X. novacula* is the only representative of the genus and where previous literature data reported a constant diploid number of 48 chromosomes (8SM+40A, NF=56). Molecular data show that the Italian samples belong to a third, different clade. These preliminary results suggest that specimens morphologically identified as *X. novacula* might belong to a species complex that requires to be unravelled.

Cytogenetics of non-Mammalian Vertebrates and Invertebrates

L13

Cytogenomic advances in teleosts: state of the art

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While teleosts (26 300 species) comprise 96 % of fishes (*sensu lato*) and 46 % of all vertebrates, less than 15 % have had their karyotypes published. In teleost comparative cytogenetics, banding techniques cannot be used to ascertain interspecific chromosomal homologies. On the basis of molecular syntenies in the protokaryotypes of several recently-sequenced, and distantly-related vertebrates, the ancestral teleost chromosome number has been estimated at n=24–25, a value corresponding well with the teleost mean chromosome number. Chromosome rearrangements can therefore be polarized within monophyletic groups, but only used as indicators of "possible karyo-evolutionary trends", since

interspecific chromosonal homologies are not taken into account. This may explain why classical cytogenetics has been neglected as a tool for understanding teleost fish genome structure and evolution. Most specialists readily combine it with molecular approaches in order to track the chromosomal homologies by comparative FISH mapping of sequenced markers. Such mapping has been often incorporated in applied research projects: fishes provide the basis for numerous medical models: zebrafish and medaka (evolutionary developmental biology and sex determination); platyfish (melanoma); icefish (anemia and osteopenia). In addition, species of economic or agronomical interest such as Nile tilapia, salmonids, turbot, etc.…, are also the subjects for genomic projects including whole genome sequencing and physical mapping. In genome assembly programmes, extended metaphase FISH mapping can be used to anchor and orient contigs, and to obtain an overview of repeated sequence chromosomal distributions. Genomic in situ hybridization (GISH) and whole chromosome painting after micro-dissection or flow sorting, are also starting to be used for cross-species hybridization in teleosts. This can help to delineate syntenic chromosome regions among closely- and sometimes distantly-related species in phylogenetic reconstruction and in studying the evolution of sex chromosome systems. The term "cytogenomics" which combines molecular studies on the whole genome structure, with physical mapping at chromosome level, seems therefore appropriate to most developing fish cytogenetic research.

O22

Comparative Chromosome Mapping of Repetitive Sequences in two Species of Mactridae (Bivalvia)

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Although the family Mactridae includes some of the world most commercially important clam species, very little is known about their genomic organization and phylogeny. In order to increase the knowledgement of these clams we performed molecular and cytogenetic studies in the European surf clams *Spisula subtruncata* and *S. solida*. Chromosome spreads were obtained from gill and gonadic tissues of juvenile specimens. Genomic compartmentalization was studied by DAPI/PI/CMA3 staining. Major and minor ribosomal RNA and histone gene clusters and a GC-rich satellite DNA were mapped by fluorescent in situ hybridization (FISH) using specific probes generated by PCR. Differences in the number and the chromosomal location of the ribosomal and histone gene clusters were found. *S. solida* shows only two terminal GC-rich regions, coincident with the major rDNA clusters, while in *S. subtruncata* GC-rich bands were detected on eight chromosome pairs. These GC-rich bands are coincident with the GC-rich satellite signals. The deep differences in both karyotype and genome organization between *S. subtruncata* and *S. solida* could help to improve our understanding on the chromosomal evolutionary process within this family.

O23

Light shone on northern cod fish cytogenetics as a contribution to the knowledge of Arctic marine biodiversity

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Gadids (cod fish) are ecologically prominent within the Arctic marine fauna. Many of the species are of commercial value, having been targeted by commercial fisheries, and, in the last decades, some of them have also been considered for aquaculture purposes.

Despite their ecological importance, and the commercial relevance, logistic constraints and fragmentary non coherent time-series have historically limited the knowledge of Arctic cods. The rapidity of the ongoing climate changes have stressed the urgency to complete the picture of Arctic marine biodiversity as a mandatory step to identify trends and to draw conservation actions. The TUNU-Programme, coined in 2002, addresses the Arctic fish diversity at the community, species and intra-species levels, in a long-term perspective. Classic taxonomy and molecular approaches are combined to provide comprehensive time-related snapshots.

As a contribution to the programme, we undertook the cytogenetic study of three gadids: the Boreal-Arctic *Gadus morhua* (Atlantic cod), and the Arctic *Boreogadus saida* (polar cod) and *Arctogadus glacialis* (ice cod). The first information on the species specific karyotype in the two Arctic species, and an in depth description of the Atlantic cod chromosomal set are among the most relevant achievements. Of particular interest, intra-specific polymorphisms were detected in the polar and arctic cods, whose nature and origin is different in the two species, thus revealing an unexpected chromosomal complexity and pointing out how much still need to be known about cod fishes, also at the chromosomal level.

O24

Comparative cytogenetics in crustacean decapods by telomeric and ribosomal sequences mapping

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To date, the knowledge on the karyology of decapods is limited, and the available data supply general information on chromosome complements and meiotic configurations. The diploid values have been reported to range from 2n=54 in *Liocarcinus vernalis* to 2n=376 in *Astacus trowbridgii*. The occurrence of large number of small sized chromosomes in most species and, in some cases, of B chromosomes, made difficult the cytogenetic analysis and the comparison among species. Therefore, the physical mapping of genes and sequences could provide useful information about the evolutionary relationships among Decapods. In this study, the location of 45S rDNA and of the pentameric telomeric repeat (TTAGG), have been compared among nine species of decapods belonging to the Palinuridae, Scyllaridae, Nephropidae and Cambaridae families (Crustacea, Decapoda). Mitotic and meiotic chromosomes have been prepared from gonads and hepatopancreas of males by the air-drying technique; 45S rDNA and TTAGG telomeric repeat have been mapped by FISH and the co-localization of these DNA sequences have been investigated by double FISH. In all species the pentanucleotide sequence labelled the telomeres, confirming that this is the Decapoda telomeric repeat and that it could be the ancestral telomere motif for arthropods. In some species, this telomeric probe hybridized on interstitial chromosome regions and sometimes co-localized with the 45SrDNA. NORs were mapped in two to six chromosome pairs; in three of the Palinuridae species 45SrDNA has been located also on B chromosomes. Our findings provide chromosome markers for cytogenomic analyses in decapods suggesting the usefulness of this approach in this taxon.

This study was in parts supported by the Fondazione Banco di Sardegna (grant n°2013-0367).

O25

Characterization and in situ hybridization mapping of Helinoto, a DNA transposon isolated in the genome of the antarctic icefish *Chionodraco hamatus*

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The recently discovered family of helitrons, a class of RC (rolling-circle) DNA transposons particularly widespread in eukaryote kingdom, seems to be virtually very active in reshuffling the transcriptome of organisms. These elements are believed to have an important role in the host genome evolution, owing to their frequent capture of host genes. We isolated and characterized a novel helitron, HeliNoto (8.9 kb), from the genome of the icefish species *Chionodraco hamatus*, belonging to the Channichthyidae, the most derived Notothenioidei, a group of Perciformes that currently dominate the Antarctic waters by virtue of their remarkable cold-adaptation ability.

We conducted an accurate structural analysis of Helinoto, including chromosomal localization, and investigated its distribution among Notothenioidei. Finally, we provided, for the first time, evidence for a transcriptional activity of Helinoto in several tissues of *C. hamatus*.

O26

Evidences of DNA Methylation Changes in the Early Development and Metamorphosis of the Sea Lamprey

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The sea lamprey (*Petromyzon marinus*) is an anadromous species, which spends most of their life as filter-feeding larva, undergoing metamorphosis to become parasitic adults that depends on marine hosts. Recent studies in the sea lamprey have revealed the existence of genomic rearrangements affecting almost all the genome and including the loss of millions of base pairs.

In the present study, we have focused on DNA methylation alterations associated with two crucial processes in the development of the sea lamprey: (1) genome rearrangements and (2) metamorphosis. In order to find alterations in the distribution of DNA methylation, immunolocation of 5-methylcytosine was performed in combination with DAPI/PI staining on meiotic and mitotic chromosomes. Furthermore, methylation-sensitive amplified polymorphism techniques were used to search for differences in genome-wide DNA methylation patterns between larval and adult stages.

Differences in number and brightness of 5 m-cytosine signals were found comparing meiotic and mitotic chromosomes. In addition, MSAP (methylation-sensitive AFLP-derived method) analysis resulted in significant differences between larvae and adults pointing out changes in the methylation pattern associated to metamorphosis.

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Cytogenetic examination and histological analysis of the gonadal development of the sea trout (*Salmo trutta* L.) × Atlantic salmon (*Salmo salar* L.) hybrids

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Despite large karyotypic differences, hybrid progenies of the sea trout (*Salmo trutta*) (2n=80, FN=100) and the Atlantic salmon (*Salmo salar*) (2n=58, FN=74) are viable and usually fertile. Prevalence of males observed among the sea trout x Atlantic salmon F_1 hybrids in the present survey suggested disturbances of the sex differentiation process in the females. As incompatibility between parental sets of chromosomes may result in reduced fertility or even sterility in the interspecific fish hybrids, cytogenetic analysis and gonadal histology were applied to the sea trout x Atlantic salmon hybrids. In all fish studied here diploid chromosome number equaled 69 and the chromosome arm number was 87. Among 12 hybrids, 8 males, 2 intersex individuals and 1 female were described. In one specimen, gonads were not found. In both intersex individuals, predominance of the testicular component in the gonads was observed. Homogeneity of the chromosome number and chromosome arm number in the hybrid individuals showing normal and abnormal gonadal development suggested interactions between particular chromosomes or even genes rather than unpairing of the parental chromosomes might have impaired of the ovarian development in the offspring provided in the course of the interspecific hybridization between sea trout and Atlantic salmon.

P43

Production of androgenetic and gynogenetic haploid brown trout (*Salmo trutta* L.) embryos

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Haploid embryos enable studies concerning recessive traits and provide source for the haploid embryonic stem

cells. Thus, the main goal of this research was to induce gynogenetic and androgenetic haploid development of the brown trout (2n=80, FN=100). In the androgenetic variant of our experiment, brown trout eggs subjected to radiation-induced (420 Gy of X-rays) inactivation of the nuclear DNA were inseminated with the normal brown trout sperm. In turn, induction of the gynogenetic development included insemination of the untreated brown trout eggs with genetically inactivated by UV-irradiation (energy output 2,075 μW/cm2 applied for 12 min.) sperm. Androgenetic and gynogenetic haploid brown trout were developing efficiently during embryogenesis however, only few haploids hatched. Haploid brown trout embryos exhibited 40 chromosomes and 50 chromosome arms (FN). Apart from the intact chromosomes, radiation-induced small chromosome fragments were identified in about 21 % and 14 % of the cytogenetically examined androgenetic and gynogenetic embryos, respectively. Embryos with chromosome fragments were mosaics what means that not every cell of an individual possessed the same number of fragments. The number of chromosome fragments varied intraindividually from 1 to 9 and from 1 to 4 within the androgenetic and gynogenetic haploid embryos, respectively.

P44

Bursts of retrotransposons and extensive chromosomal repatterning within the Antarctic teleost fish species flock Trematominae

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Stress due to climatic changes can lead to quick adaptation and diversification to multiple niches in the new environment. Species flocks are characterized by a high level of morphological, ecological diversity, endemism and habitat dominance, and often accompanied by extensive chromosome repatterning. This leads to reproductive isolation and genome expansion due to correlative bursts of active transposable elements (TEs). We have started to investigate whether the presence of TEs in the genomes can boost chromosomal rearrangements involved in rapid speciation mechanisms, using as model the marine Antarctic teleost fish species flock Trematominae and some representatives of their outgroups. Trematominae have highly diversified karyotypes ranging from 2n=24 to 2n=58. Three major lineages of retrotransposons have already been identified in their genomes: *copia*, *gypsy*, and tyrosine recombinase elements (DIRS). Their sequences show a striking conservation within species of this notothenioid clade. These TEs have been FISH-mapped onto the chromosomes of most trematomine species. Whilst *copia* and *gypsy* produce practically no FISH signals, DIRS display clear patterns of accumulation in pericentromeric, centromeric and intercalary positions in the chromosomes of all species examined. So chromosomal rearrangements within trematomine species could occur at DIRS hot spot insertions.

P45

Meiotic Surface-spread and Immunodetection for Synaptonemal Complex Protein in Teleost fish

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Studies of meiotic chromosomes provide important information about the behavior and the formation of sex chromosomes, origin and evolution of supernumerary chromosomes, and chromosomal rearrangements involved in the karyotype evolution. The main difficulty in obtaining good preparations of synaptonemal complex (SC), is the need for obtaining dividing cells in the pachytene stage. This requires the determination of the exact gonad maturation phase of the studied organism. The preparations obtained with this protocol can be used for other techniques, for example Silver Nitrate staining, immunostaining and localization of DNA markers by fluorescence in situ hybridization combined with immunostaining, in order to visualize simultaneously the behavior of chromosomes, unpaired regions, as well as the location of molecular markers or whole chromosomes painting during pachytene Our protocol allows the use of specific antibodies for fish, as well as commercial

antibodies derived from mammals, once performed a pretreatment with citrate buffer, leaving the sites for complex of proteins most exposed to binding to the antibodies. Positive results were obtained using antibodies for SYCP3 protein found in the lateral elements of the SC in different fish species, such as *Oreochromis niloticus*, *Eigenmannia sp*., *Astyanax paranae*, and *Moenkhausia sanctaefilomenae*, demonstrating that this protocol can be used in various teleost fish groups.

P46

Fishing for sex genes by chromosome microdissection in two tilapia species with different sex determining systems

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In the tilapia species group, the major sex determining factors have been located on Linkage Group 1 (LG1), on a small chromosome (Chr) pair in *Oreochromis niloticus*, on LG3 (the largest Chr pair) in *O. aureus* or on both, depending on populations/strains. LG3 has all the traits of an old sex chromosome, whereas LG1 seems to be an emerging one. Taking advantage of its large size, LG3-Chr has been microdissected to search for sex-linked genes. It was isolated from metaphase spreads of XX-female and YY-male in *O. niloticus* and of ZZ-male in *O. aureus*. Using cDNA capture and direct cDNA selection procedures we isolated various transposons but a reduced number of genes. We therefore compared three different whole genomic amplification (WGA) methods (DOP-PCR, GenomePlex and GenomiPhi) using a pool of 30 microdissected chromosomes, to evaluate the best LG3-Chr representation. Loci from 5 microsatellites, 2 genes and 2 uncoded fragments located on LG3-Chr have been searched by PCR on the DNA obtained by the 3 methods. GenomePlex and GenomiPhi gave 60 % loci amplification. GenomePlex probe produced the best painting probe, entirely covering the two LG3-Chrs with weaker signals in the gene-rich pericentromeric region, in both species, confirming that this pair is essentially composed of conserved and specific repeated sequences. This will allow to trace its history within the tilapia group.

P47

FISH localization of 5S ribosomal gene family in Atlantic and Indopacific morays (*Anguilliformes: Muraenidae*)

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Muraenidae is a large and cosmopolitan anguilliform family of teleost, ecologically important as consumers; nevertheless they are poorly known and taxonomic uncertainties are still present in this group. Cytogenetically, less than 10 % of morays have been karyotyped and available data show a basal 2n=42 and morphologically diversified karyotypes mainly due to pericentric inversion, robertsonian rearrangements and heterochromatin changes. Large amount of heterochromatin and a single terminal NOR cluster are also the most frequent chromosomal features. Till now, FISH mapping of major ribosomal genes and telomeric sequences was obtained for few species and no data about the chromosomal location of 5S rDNA is available. In this study, 5S ribosomal genes have been hybridized on metaphase chromosomes, obtained from lymphocyte cultures, of Muraenidae species coming from both Atlantic and Indo-Pacific oceans and representative of *Muraena* and *Gymnothorax* genera. After FISH experiments, a species-specific distribution of different number of 5S positive signals was detected. Most of them were interstitially located and in some cases were associated to heterochromatic regions and/or sintenic to major ribosomal genes. Furthermore, some of the 5S clusters were similarly located among species. These first data on chromosomal mapping of the 5S rDNA could be very useful in understanding the karyotype evolution of Muraenidae family.

P48

Characterization of a highly conserved repetitive DNA family in Molluscs

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A repetitive DNA family, designated PcBrep, was identified by Bgl II digestion in the genome of *Patella caerulea* (Mollusca: Gastropoda). Southern blotting analysis, performed on several *Patella* species, suggested a high degree of conservation for this repeated sequence, which in addition to interspersed signals also is arranged in short (di-tri) tandem repeats. Moreover, PCR amplifications with primers designed on our sequences showed a high level of conservativeness of the PcBrep even in species belonging to different classes of molluscs. The analysis of PcBrep clones of *P. caerulea* revealed two clusters of sequences that differ by an insertion/deletion of about 10 bp. Similar organization was found in clones isolated in *Lepidochitona caprearum*. Fluorescence in-situ hybridizations (FISH) on metaphase chromosomes showed that Bgl II family is interspersed along all the chromosome arms of *Patella*, while its distribution on *Nuttallochiton mirandus* and *L. caprearum* spermatids is peculiar because the hybridization signals are located at one end of the nuclei.

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