

Maria Francesca Piras
Department of Genetics and Microbiology "A. Buzzati Traverso"
Characterization of evolutive neocentromeres in the genus *Equus*
Prof.ssa Elena Giulotto

The genus *Equus*, family of Equidae, order of Perissodactyla, comprises horses, zebras and asses. These animals are morphologically very similar but their karyotypes are quite different even if their separation occurred only recently. We observed that centromere repositioning (CR) is a frequent event in the evolution of donkey and horse. With this event, a neocentromere appears in a new position along the chromosome after the inactivation of the old one. In a previous work¹ we demonstrated that at least seven CR events occurred in the donkey and one in the horse chromosome 11 (ECA11), the only evolutionary neocentromere that we observed in the horse. All these neocentromeres were created recently on an evolutionary timescale by "repositioning" and not by rearrangement; they are stable but not associated to centromeric satellite DNA.

In collaboration with the Broad Institute (MIT, Boston) and the University of Bologna we are studying the ECA 11 neocentromere in order to identify the sequence necessary for the centromeric function. To do this we constructed a physical map of the region of ECA 11 containing the centromere using a panel of BAC clones in FISH experiments and identified the DNA sequence contained in this region using bioinformatic tools. We then constructed an oligonucleotide array from the centromeric region of ECA11 identified by FISH and hybridised the array with the DNA obtained by coimmunoprecipitation of horse genomic DNA with the centromeric protein CENP-A. Other ChIP experiments are under way using antibodies against CENP-C and CENP-H. The preliminary results are very encouraging in fact we identified two DNA fragments highly enriched in binding sites for CENP-A.

Another aspect of my research is to find new events of centromere repositioning in Perissodactyla using rhinoceros and tapir as outgroup and to define the ancestral conformation of the neocentromere containing chromosomes, including ECA11.

References

1. L. Carbone, S. G. Nergadze, E. Magnani, D. Misceo, M. F. Cardone, R. Roberto, L. Bertoni, C. Attolini, **M. F. Piras**, P. de Jong, T. Raudsepp, B. P. Chowdhary, G. Guerin, N. Archidiacono, M. Rocchi, E. Giulotto, Evolutionary movement of centromeres in horse, donkey and zebra, *Genomics* 87 (2006) 777.

Full papers

1. Carbone L, Nergadze S.G, Magnani E, Misceo D, Cardone M.F, Roberto R, Bertoni L, Attolini C, **Piras M.F**, De Jong P, Raudsepp T, Guérin G, Archidiacono N, Rocchi M, Giulotto E. "Evolutionary movement of centromeres in horse, donkey, and zebra". *Genomics* 87 (2006) 777 – 782.

2. Herszberg B, Mata X, Giulotto E, Decaunes P, **Piras M.F.**, Chowdhary B.P., Chaffaux S, Guérin G. (2007) "Characterization of the equine glycogen debranching enzyme gene (AGL): Genomic and cDNA structure, localization, polymorphism and expression". *Gene* 404 (2007) 1-9.

Abstracts

1. Magnani E, Nergadze SG, **Piras MF**, Bertoni L, Attolini C, Giulotto E (2004) "Rapid evolution of satellite DNA in Equidae". 6° FISV Congress, Riva del Garda, Italy September 30 - October 3, 2004.
2. Magnani E, Nergadze SG, Carbone L, **Piras MF**, Bertoni L, Attolini C, Raudsepp T, Guérin G, Archidiacono N, Rocchi M, Giulotto E. Comparative analysis of the horse (*Equus caballus*), donkey (*Equus asinus*) and zebra (*Equus grevyi*) karyotypes. Plant & Animal Genomes XIII Conference, San Diego, California, January 15-19 2005.
3. Giulotto E, Magnani E, Nergadze SG, Carbone L, Misceo D, Cardone MF, Roberto R, Bertoni L, Attolini C, **Piras MF**, de Jong P, Raudsepp T, Chowdhary BP, Guérin G, Archidiacono N, Rocchi M. "Evolutionary centromere repositioning in the genus *Equus*" Sixth International Gene Mapping Workshop Dublin, Ireland, July 11-14, 2005.
4. **Piras MF**, Magnani E, Giulotto E (2006) "The centromeric function is dissociated from satellite DNA in donkey chromosomes". 8° FISV Congress, Riva del Garda, Italy September 28 - October 1, 2006.