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Name of applicant and applicant institute:

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Project title:

Molecular cytogenetic approach to evolution of sex-determining mechanisms in lizards: from dependency on thermal environment to genetic control of a crucial life-history trait

Name of host and host institute:

Willem Rens
Molecular Cytogenetics Laboratory, Centre for Veterinary Science, Department of Veterinary Medicine, University of Cambridge

Purpose of the visit:

The right decision of an individual concerning its sex is one of the most important determinants of lifetime fitness in gonochoristic organisms. Moreover, such decisions have dramatic effects also at the population level, because appropriate sex ratio is an important population characteristic directly influencing susceptibility to extinction. Research of sex-determining mechanisms and their evolution is thus more than solving challenging theoretical issues, but it has an important conservation consequences. The aim of the project was to conduct the first molecular-cytogenetic comparison of genomes of related vertebrate species with contrasting sex-determining mechanism and hence to explore karyotypic evolution and synteny of chromosomes among gecko species with temperature-dependent (TSD) and newly evolved genotypic sex determination (GSD).

Description of the work carried out during the visit:

During my visit in the Molecular Cytogenetics Laboratory, I focused on mastering of cytogenetic methods including establishment of cell (fibroblast) cultures from embryos of several eye-lid geckos species, sorting of chromosomes on flow cytometer, subsequent fluorescent marking of individual chromosomes and their fluorescent *in situ* hybridization (FISH) to metaphase spreads of the other species. Moreover, I worked on interspecific FISH with chromosomal probes derived from a related organism with well-known genome (chicken) on gecko chromosomes to obtain information about synteny/gene content of gecko autosomes and sex chromosomes. I focused also on PCR gene mapping on sorted chromosomes. Using DNA from sorted chromosomes, it is possible to map position of important conservative genes in karyotype. I mapped especially conservative genes known as a part of gene network responsible for sex determination/differentiation in some vertebrate species. Comparison of the position of these genes between species with different sex determining mechanisms is important for consideration of synteny and homology of sex chromosomes and thus can give us important information about evolution of GSD as well as TSD.

Description of the main results obtained:

1) Cell cultures, chromosome sorting and FISH

For chromosome sorting, it is necessary to prepare fibroblast cell cultures. Before and during my stay in the Molecular Cytogenetics Laboratory, we established cell cultures from embryos of nine gecko species. These cultures are preserved in liquid nitrogen in the Molecular Cytogenetics Laboratory ready for further analyses.

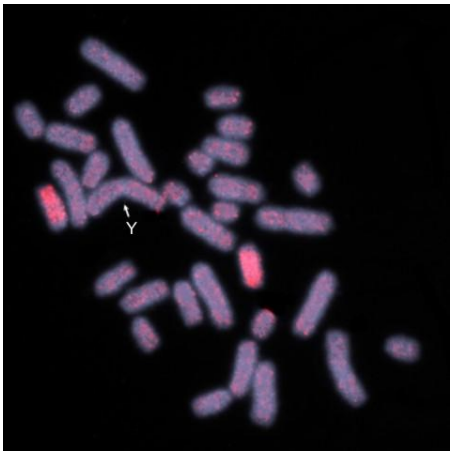
One of the main successes of the project was sorting of chromosomes in several species of eye-lid geckos including species with GSD as well as species possessing TSD. We focused especially on sorting of chromosomes in *Coleonyx elegans* possessing multiple sex chromosome system (X1X2Y) and the TSD species *Eublepharis macularius*. We paid higher attention to these two species, but we sorted also chromosomes from another seven species of geckos. From sorted chromosomes, we prepared probes for fluorescent *in situ* hybridization and tested quality of these probes on metaphases of the same species. High quality probes were used for cross-species hybridization.

Comparison of genome of closely related species with different sex chromosomes can give us information about homology or no homology of sex chromosomes and thus about evolution of sex determining system in general. For example, we successfully hybridized probe from one of the X chromosomes of *C. elegans* with metaphase spreads of *Coleonyx variegatus*. *C. variegatus* is a GSD species with homomorphic sex chromosomes. We chose this species especially because it is closely related to *C. elegans* with undifferentiated sex chromosomes. From our experiments it is obvious that X chromosome is homologous to the second largest pair of chromosomes in karyotype of *C. variegatus*. This finding confirms suitability of the technique used for comparison of genomes among gecko species. The material collected and prepared during the project will enable further comparative mapping among species with different sex determining systems.

2) Interspecific FISH with probe from chicken Z

Using a probe from chicken (*Gallus gallus*) Z chromosome for hybridisation with metaphase chromosomes of several species representing several different lineages of squamate reptiles including studied geckos, we obtained information about synteny among bird and reptile sex chromosomes and autosomes. In *C. elegans,* the chicken Z probe painted a pair of middle-sized autosomes (Fig. 1). The same pattern was confirmed also in *E. macularius* as well as in *C. variegatus*. As also in other squamate reptiles probe from chicken Z painted autosomes and not sex chromosomes, we can say that sex chromosomes in reptiles are not homologous to those in birds. Moreover, this approach also allowed us to find syntenic regions of chromosomes among gecko species with different sex determining systems.

Fig.1 FISH with chicken Z probe on metaphase of *Coleonyx elegans*. Probe painted a pair of autosoms.



3) PCR gene mapping

We have been successfully mapping several genes lying on chicken Z chromosome in the karyotype of *C. elegans*. For example, *DMRT1* gene, which paralogues are known as sex determining gene in several vertebrate lineages and which is involved in testis development, mapped on the Y chromosomes of *C. elegans*. On the other hand, other chicken Z-linked genes lie according to our results on different chromosomal pairs in *C. elegans*. In *C. variegatus* we mapped *DMRT1* on the same chromosomal pair painted by probe from *C. elegans* X chromosome. Results from this PCR gene mapping suggest that homologue/paralogue of *DMRT1* can play a role in sex determination in gecko species with newly evolved sex chromosomes. Further study will be devoted to the situation (copy number, position within the genome) in closely related TSD species and can help in reconstruction of events connected with transitions between sex determining systems.

List of planned publications and planned further collaboration:

First, we plan to publish results about hybridization of chicken Z on chromosomes of squamate lineages and also results from the PCR gene mapping. We will continue in close collaboration with Dr. Willem Rens and with the whole team in Molecular Cytogenetics Laboratory in Cambridge. Newly, we established collaboration with Dr. Massimo Giovannotti from Universita Politechnica delle Marche in Ancona, Italy, who worked in the Cambridge laboratory at the time of my stay. The material collected during the project (e.g. chromosomal probes, sorted chromosomes) and the skills I obtained allow further comparison of genomes in reptile lineages with different sex determining modes in near future.