



Paradigm shift in sex chromosome evolution

Abstracts of the workshop held in Berlin, 19-22 September 2019

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Abstract

Explaining the variation in sex-determining mechanisms across the tree of life and understanding the evolution of sex chromosomes represent important goals in evolutionary biology. Many papers have followed the classical paradigm of differentiation pathways of sex chromosomes from autosomes. However, the evolutionary drivers and many other aspects of the differentiation of sex chromosomes still remain controversial. Thanks especially to the impact of new genomic techniques, the always vibrant field of sex chromosome evolution research is currently boosting. Results from recent functional and comparative studies across both model and especially non-model organisms challenge the traditional paradigms of sex chromosome evolution. Therefore, this is the perfect time to open a forum for discussion, summary and reappraisal of our understanding of this field. The aim of the meeting is to gather evolutionary biologists with different backgrounds (developmental biology, population genetics, genomics, evolutionary ecology) to formulate and address fundamental questions and to uncover gaps in our understanding of sex chromosome evolution as well as applications (e.g. in endocrine disruption research, aquaculture, and species evolution/biodiversity).

Workshop organizers

PD Dr. Matthias Stöck, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Germany Prof. Dr. Lukáš Kratochvíl, Charles University Prague, Czech Republic Prof. Dr. Werner Kloas, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Germany Prof. Dr. Dr. Manfred Schartl, University of Würzburg, Germany (and Texas A&M University, USA)

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The evolution of gonad plasticity and AMH in vertebrates: New insight from the medaka, *Oryzias latipes*

In vertebrates, the reproductive organs are generally specialized to perform a male or female function. Acquisition of the Müllerian duct, which gives rise to the oviduct, together with emergence of the Amh/Amhr2 system favored evolution of viviparity in jawed vertebrates. Teleosts lost the Müllerian duct during evolution and developed for gamete release a complete independence from the urinary system, creating optimal anatomic and developmental preconditions for physiological sex change. The common and probably ancestral role of Amh is related to survival and proliferation of germ cells in early and adult gonads of both sexes rather than induction of Müllerian duct regression. The relationship between germ cell maintenance and sex differentiation is most evident in species in which Amh became the master male sex-determining gene, like in pejerrey and tilapia. Complete ablation of the amh gene by CRISPR/Cas9 in medaka (Oryzias latipes) resulted in male-to-female sex reversal in over 99% of the cases, and no phenotype was observed in heterozygote fish. Already at early larval stages both XX and XY animals presented increased numbers of undifferentiated germ cells. mRNA expression and protein localization analysis suggest that the role of Amh in the early gonad is maintained in the adults. In addition, temperature stress and cortisol treatments have a direct effect on germ cell number and lead to masculinization of XX medaka fish. This points to the importance of regulating the germ cell number in the process of sex determination in the presence or absence of the genetic trigger for male or female gonad development.

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Tempo and mode of sex chromosome turnovers in an adaptive radiation

Sexual reproduction is ancient and almost universal in the eukaryotic kingdom. However, the mechanisms that actually determine male or female sex are highly divers encompassing a variety of extrinsic and intrinsic drivers. Intrinsic factors, commonly referred to as genetic sex determination, comprise systems as simple as a single base pair difference between the sexes to highly differentiated sex chromosomes. Why sex-determining systems change frequently in some taxa, whereas the same sex chromosome pair persists over long evolutionary time in others, remains an open question. With ~3000 species, cichlid fishes are one of the largest vertebrate families and an extraordinary example of adaptive radiations. If different sex-determining mechanisms contribute to the species richness of this fish lineage is largely unknown. The oldest and most diverse African cichlid assemblage is the one of Lake Tanganyika, which consists of 250 endemic species. Using whole genome sequencing, comparative genomics and expression data, we investigate the genetic basis of sex determination and the evolution of sex chromosomes in this radiation. We identified at least 27 sex chromosome transitions in cichlids with a transition rate of 0.19 per million years. To the best of our knowledge this is the highest transition rate so far described. We show that closely related species can have different sex chromosomal systems indicative of recent turnover events. On the other hand, we also found cases of convergently evolved sex chromosomes in distantly related cichlid lineages. Chromosomal fusions likely played a role in cichlid sex chromosome evolution. We compare patterns of transitions in sex chromosomes but also heterogamety between cichlids and other ay-finned fishes. Cichlids perfectly exemplify the diversity as well as the constraints of sex chromosome evolution and can reveal the forces driving the evolution of sex-determining systems. Sex chromosomes appear to be one of the ultra fast evolving traits in this astonishing group of fishes.

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Sex chromosomes and sex determining system in *Xenopus tropicalis*: a meiotic mess

Xenopus tropicalis is the only diploid representative of the genus. This characteristic has prompted the adoption of this species as amphibian model. However, although the sex determination of this species is genetic, not much is known about its sex determing gene. The ovary determining gene in *X. laevis, dmw*, is not present in the genome of *X. tropicalis*. On the other hand, as in 95% of amphibian species, *X. tropicalis* has homomorphic sex chromosomes not differentiable by cytogenetic techniques, hindering the analysis of the sex determination and gonadal differentiation processes.

Genetic analysis of laboratory colonies shows that, at least, three different sex chromosomes exist in this species: Y, W and Z, observed in YZ, YW and ZZ males, and ZW and WW females. This sex chromosome system produces males and females that can be either homogametic or heterogametic. Also, the sex ratios obtained can be different from 1:1 depending of the sex chromosome constitution of the parents.

Meiosis in this species adds another level of complexity. Some females produce triploid offspring with a frequency as high as 80%. These triploid individuals are generated from diploid eggs produced by non-segregation in meiosis II.

Triploid individuals are fertile, although notable differences are observed between males and females. While males produce offspring with a high frequency of aneuploidies, females produce normal diploid offspring.

The analysis of the clutches obtained from several triploid females indicate that triploid females produce haploid gametes. Also, from the frequency of sex-linked markers it can be deduced that there are differences between sex chromosomes in their preference to pair with other sex chromosomes. These differences affect the transmission of the sex chromosome and thus, the sex ratio of the offspring.

Gynogenic YY, ZZ and WW individuals are viable, indicating that the sex chromosomes of this species are at the initial steps of differentiation and no lethal mutations have arisen. However, the offspring of triploid females show the existence of differences between these sex chromosomes.

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Characterization of the sex determining system(s) of the Mexican tetra, Astyanax mexicanus.

In vertebrates, a remarkable diversity of sex determining mechanisms is observed, ranging from environmental to genetic sex determination (ESD and GSD). In teleosts, sex determination mechanisms include both environmental and genetic regulation and are extremely diverse, changeable, and labile. In addition, many sex-determining genes have been already found in this group of vertebrates showing that sex determination genes can be extremely variable even between closely related organisms. Hence, teleost fish have emerged as interesting models to study the macroevolution of sex determining mechanisms within vertebrates.

The cavefish Astyanax mexicanus belongs to the Characiform group. Different populations of A. mexicanus have been described, including pigmented riverdwelling and several depigmented blind cave populations. These populations are still inter-fertile and cave populations are known to have evolved from an ancestral surface population less than 100,000 years ago. In addition, these Astyanax populations can be also reared under laboratory conditions quite easily and are amenable to genetic manipulations. Altogether this makes surface and cave populations a particularly interesting evolutionary genetic model system for comparative and microevolution studies. We thus initiated studies on the evolution of sex determination and sex determining genes in *A. mexicanus* using restriction site-associated DNA sequencing (RAD-Seq) and Pool-sequencing (Pool-Seq) approaches to identify sex-biased molecular markers in both cavefish originating from the Pachón cave and surface fish populations.

Our first results led to the identification of a XX/XY sex determination system and a candidate master sex-determining gene in the Pachón cave population. But surprisingly this sex determination system is not completely conserved in the surface population as we found in this population a very strong but not complete sex linkage

for the Pachón cave master sex-determining gene with all females being negative and 2 groups of males that have or do not have the Pachón cave sex determining gene. These results show that sex determination in *Astyanax mexicanus* has already evolved between surface and cave populations suggesting a quick evolutionary transition between what could be a polygenic sex determination system in the surface population and a simple monofactorial system in the cave population. <u>Amaury Herpin^{1,2}</u>, Cornelia Schmidt², Susanne Kneitz², Clara Gobé³, Martina Regensburger², Aurélie Le Cam¹, Jérome Montfort¹, Mateus C. Adolfi², Christina Lillesaar², Dagmar Wilhelm⁴, Michael Kraeussling², Brigitte Mourot¹, Béatrice Porcon¹, Maëlle Pannetier³, Eric Pailhoux³, Laurence Ettwiller⁵, Dirk Dolle⁵, Yann Guiguen¹ and Manfred Schartl^{2, 6, 7}

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Transcriptional rewiring, post-transcriptional regulation and neo-functionalization: how the master sex-determining gene of medaka was born

The medaka Oryzias latipes has genetic sex determination of the XX/XY type. Dmrt1bY, the male sex-determining gene, arose through a segmental gene duplication event from an autosomal gene, dmrt1a. Both genes are co-orthologs of the highly conserved dmrt1 gene, which acts downstream in the sex determining cascade of mammals, similar to its Drosophila and C. elegans homologues. In Medaka, the duplication of the chromosomal fragment that included dmrt1a occurred approximately 10 million years ago. After insertion into the proto-Y chromosome a massive degeneration of all genes except dmrt1bY occurred. Subsequent loss of dmrt1bY in several medaka strains reveals the high degree of plasticity of this sex determining mechanism.

From the molecular evolution point of view the main question is how in medaka dmrt1bY could become the master regulator of male development after acquiring an upstream position in the sex-determining cascade. Remarkably, this evolutionary novelty, requiring a rewiring of the regulatory network, was brought about by co-optation of pre-existing cis-regulatory elements contributed by transposable elements. On another level of regulation, differential gene expression of the two dmrt1 co-orthologs seems to be also mediated by post-transcriptional regulation through a highly conserved cis-regulatory motif that directs differential gonadal synexpression of dmrt1 transcripts during gonad development. While the autosomal dmrt1a medaka gene is essential for testis maintenance, in contrast, dmrt1bY was shown to be responsible for male-specific primordial germ cell (PGC) mitotic arrest in the developing gonad at the sex-determination stage, suggesting neofunctionalization.

For a detailed analysis of expression, regulation and function of dmrt1a and dmrt1bY in medaka, we generated several transgenic fluorescent reporter lines (BAC clones). Strikingly, we observed a very early expression of dmrt1bY in the PGCs long before the somatic gonadal primordium is committed. This PGC-specific expression then progressively vanishes while the somatic expression of dmrt1bY rises only at hatching stage. Concomitantly we observed that an HMG-box gene shows a strictly inverse PGC-specific expression pattern in relation to dmrt1bY. Interestingly, also some other gonad development genes showed transient sex-specific expression in the pre-committed gonad primordium. Using in vivo whole tissue ChIP and in vitro gene regulation assays, we found clear evidences that the specific expression pattern of dmrt1bY is controlled by differential cross-regulations of dmrt1a, dmrt1bY and sox5 as well as by specific stabilization/degradation of dmrt1 transcripts via two RNA-binding proteins.

Our results suggest that after local gene duplication, transcriptional rewiring and neo-functionalization of the dmrt1 co-orthologs were necessary in order to establish dmrt1bY as the master sex-determining gene of medaka. Our data also indicate that sex determination in medaka occurs much earlier than previously thought and assigns a more important role to germ cell identity in the process of commitment towards testis or ovary development.

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So easy to abandon sex? On the interconnection between asexuality, hybridization and speciation

Genetic machinery controlling production of recombined gametes is very conserved, but in many animal and plant taxa it has been repeatedly disrupted giving rise to organisms, whose gametogenetic aberrations more or less alleviate the classical sexual reproduction. Despite their importance for general biology, very little is known about the proximate mechanisms making these so-called asexual lineages switching from sex to asexuality.

In this talk, I will briefly summarize state-of-the-art knowledge about molecular and cytogenetic mechanisms triggering the emergence of asexual reproduction. In particular, I will focus on a question why the distortion of reproductive modes towards asexuality is often bound to interspecific hybridization. Subsequently, I will present our recent research on the model organism of loach fishes (Cobitidae) and show how it contributed to advance in understanding of sex, asexuality and hybridization. Cobitidae comprise several species pairs forming independent hybrid zones and producing wide array of hybrid forms ranging from sexual, through (hemi)clonal to sterile ones. Our comparative analysis of hybrids and parental species employed combination of phylogenomics, gene expression profiling and experimental crossings along with functional analyses of the gametogenetic pathways. It provided some general clues explaining the link between hybridization and asexuality. First, clonal gametogenesis is not restricted to only some 'preadapted' parental genomes, but evolves gradually as a side-effect of accumulation of reproductive incompatibilities between species. Hybrid's asexuality may thus be viewed as a special type of Bateson-Dobzhansky-Muller incompatibility.

Second, successful establishment of hybrid clones requires certain level of cisregulatory divergence between parental genomes, which ensures partially independent execution of co-inherited developmental programs in hybrids. We further revealed that the efficiency of trans regulatory pathways in a hybrid were systematically affected by polyploidization, which explain why polyploidy is so common among asexuals. Finally, asexuality typically emerges in one hybrid sex but evolves hand-in-hand with sterility of the other hybrid's sex. Although various types of epistatic incompatibilities are likely involved in independent cases of sterility and clonality, they are developmentally canalized into only several types of gametogenetic aberrations.

Our study points at tight association between asexuality and other postzygotic reproductive barriers and proposes that emergence of hybrid asexuality probably stems from deregulation of molecular tools preexisting in parental organisms before genome merging.

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Sex-chromosome recycling and turnover in frogs

We introduce two unique cases of sex chromosome evolution in frogs. If turning to be a heteromorphic state, the sex chromosomes are evolutionarily trapped, meaning that they go to be almost eternally conserved. Here, we show a case of recycling heteromorphic sex chromosomes in hybridization between geographic populations with different sex chromosome systems (XX-XY and ZZ-ZW) in a Japanese frog. The W chromosomes that once are dead revive from the X chromosomes. On the other hand, as long as staying in homomorphic state, the sex-chromosomes can turnover during the phylogenetic history and it is repeated among six different chromosomes in frogs. Here, we show a rare case of multiple sex-chromosomes in a Taiwanese frog. The male-specific triangular translocations among three chromosomes, which all belong to the members of the potential sex-chromosomes, created a new system comprising six sex-chromosomes. We discuss about a structural kinship relation among the potential sex chromosomes to turnover in frogs.

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Epigenetic regulation of sexual development

DNA methylation is an epigenetic modification that regulates gene expression without changes in the underlying nucleotide sequence. DNA methylation patterns vary across different groups of amniote vertebrates and thus it is believed that it has contributed to sex chromosome evolution and dosage compensation. However, heteromorphic sex chromosomes are less abundant in anamniote vertebrates, where, in addition to "pure" genetic (i.e., chromosomal) sex determination (GSD) systems, other systems such as polygenic sex determination (PSD) and environmental sex determination (ESD) are frequent. Furthermore, since epigenetic regulatory mechanisms integrate genetic and environmental variation, attention is increasingly being paid to the epigenetic regulation of sex, particularly in fish and reptiles with ESD, as well as in sex-changing hermaphrodite fish. Thus, there is increasing evidence for the contribution of epigenetics in gene expression regulation during different stages of sex differentiation. Using two fish species with PSD, the zebrafish (laboratory strains) and the European sea bass, data is presented on the genetic component of epigenetic variation and the influence of temperature and population density in shaping DNA methylation patterns in key genes related to sexual development. Combining these data from data gathered in other vertebrate species, the Conserved Epigenetic Regulation of Sex (CERS) model will be presented. A derivate of this model is the possibility of identifying Essential Epigenetic Markers (EEM) that can have practical applications, for example, for sex prediction and for uncovering hidden effects of environmental perturbations in otherwise apparently morphologically normal gonads. Finally, I will present the idea that epigenetic mechanisms make transitions possible, both among different sexual systems (gonochorism and different forms of hermaphroditism) and also between different mechanisms of sex determination, favoring and regulating sexual phenotypic plasticity. Supported by Spanish Ministry of Science, Innovation and Universities grant AGL2016-78710-R to F.P.

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How (postcopulatory) sexual selection shapes the genetic diversity on the Z chromosome and contributes to the large Z-effect for reproductive isolation in birds

The disproportionately large role of the X and Z sex chromosomes in reproductive isolation is one of a few general rules in evolutionary biology. The underlying mechanisms of this large X/Z effect are, however, still poorly understood. Here we investigated a possible interconnection between intensity of sexual selection acting on males, patterns of molecular evolution on the Z chromosome, and the large Z effect in two closely related passerine birds, the Common Nightingale (Luscinia megarhynchos) and the Thrush Nightingale (L. luscinia). We found that the two nightingale species differ markedly in levels of postcopulatory sexual selection, with L. megarhynchos showing higher levels as was indicated by significantly longer spermatozoa as well as lower between-male variation in sperm length in this species compared to L. luscinia. The Z chromosome in L. megarhynchos, but not in L. luscinia, showed reduced levels of within-species polymorphism and at the same time an excess of non-synonymous polymorphisms relative to non-synonymous substitutions, suggesting increased levels of genetic drift acting on this chromosome. Interestingly, we also revealed asymmetrical introgression on the Z chromosome with lower levels from L. megarhynchos to L. luscinia than in the opposite direction, indicating that the Z chromosome of L. megarhynchos accumulated more hybrid incompatibilities. Together, our results are consistent with the view that high levels of (postcopulatory) sexual selection acting on males can significantly reduce the effective population size of the Z chromosome and thus lead to higher levels of genetic drift on this chromosome. This can result in relatively faster accumulation of hybrid incompatibilities on the Z chromosome and thus contribute to the large Z effect. Our results challenge the prevailing view that positive selection is the main driver of the origin of hybrid incompatibilities and highlight the importance of genetic drift in evolution of reproductive isolation and the large Z effect.

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Evolution of sex chromosomes and mechanisms of dosage compensation in amniotes

Amniotes possess variability in sex determination, ranging from environmental to genotypic sex determination with differentiated sex chromosomes. Genotypic sex determination evolved within amniotes independently at least 40 times, resulting in systems varying from poorly to highly differentiated sex chromosomes under both female (ZZ/ZW) and male (XX/XY) heterogamety. Recently, it was demonstrated that the same parts of genome play the function of differentiated sex chromosomes in different amniote lineages. The independent co-option of the same regions for the role sex chromosomes in different lineages might be caused by limited multiple choices. Nevertheless, an intriguing possibility is that only particular regions can meet the requirements to become differentiated sex chromosomes such as low recombination rates, gene content involved in gonad differentiation, development of sex-linked traits and epigenetic regulation. Particularly, we will review how the amniote lineages cope with differences between males and females in copy numbers of genes linked to paired sex chromosomes (X or Z) missing on the degenerated unpaired sex chromosomes (Y or W). We will summarize the recent advances in transcriptomics and genomics and conceptual approaches allowing us to identify the sex-specific gene content in details and to shed light on the evolution of sex chromosomes and dosage compensation mechanisms in amniotes.

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Sex chromosome evolution in Poeciliid fish

The outstanding diversity of sex determination mechanisms and the plasticity of the underlying molecular pathways is a hallmark of fish biology. In a few species the description of the varying mechanisms and pathways has already proceeded to high resolution. However, neither the biological meaning of this diversity nor the evolutionary forces, which drive it, are understood. So far, our information mainly comes from a handful of species representing distant branches of the fish tree of life. To contribute the databases on which hypotheses can be built, we are studying sex determining mechanisms and sex chromosome structures in closely related species of three genera of Poeciliids: Poecilia, Gambusia and Xiphophorus. For our comparative work we are using RAD-sequences, pool sequencing, whole genome assemblies and RNA-seq. We find independent development of separate pairs of ancestral chromosomes to opposing heterogametic sex determination mechanisms in two sister species of *Gambusia* with strongly differing effects on sex chromosome evolution. In the genus *Xiphophorus*, the same ancestral chromosome pair became the sex chromosome in all species analysed so far. Surprisingly, this chromosome pair acts as WZ in some species, as XY in others, and as W, X and Y in the platyfish, Xiphophorus maculatus. Looking at candidate sex determination genes and sex chromosome-linked genes, we aim to reconstruct the evolution of sex chromosomes on a micro-evolutionary scale.

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Co-option of the same syntenic blocks in vertebrate sex chromosome evolution

Application of high-throughput sequencing methods and the improvement of genomic assemblies have facilitated new discoveries in the field of comparative genomics of sex chromosomes and sex determination systems. There is a growing body of evidence that fish, amphibians and many reptile groups demonstrate high plasticity of the sex determination systems. However, the latest data suggest that both the syntenic blocks from which the sex chromosomes originate and the sex triggering genes are not random, which leads to a large number of cases of evolutionary parallelisms. The discoveries of sex chromosome systems and sex determining genes in new species allow us to reveal the general patterns of evolution of sex chromosomes.

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Turtle genomic insights into the evolution of sex chromosomes and their dosage compensation

Sexual fate of developing vertebrates can be established by a wide spectrum of mechanisms, spanning from highly canalized systems under the control of sex chromosomes (genotypic sex determination or GSD) to plastic systems under environmental control such as those driven by incubation temperature (temperature-dependent sex determination or TSD) as is common in reptiles, including turtles. Our understanding of sex chromosome evolution in reptiles and turtles is growing fast as molecular cytogenetic and genomic techniques are enabling rapid advances in the identification of sex chromosome systems, their content, structure, and evolution. The degeneration of the Y or W sex chromosome may result in gene dosage imbalance which can induce suboptimal phenotypes or death. Dosage Compensation (DC) mechanisms remedy this by equalizing the activity of Xor Z-linked genes between sex chromosomes and autosomes, and between males and females. DC is important in development, genome evolution and speciation. Yet, the diversity of dosage compensation and evolution remain unclear, as new data continually debunk purported patterns. Turtles are an ideal system to study these issues because XX/XY and ZZ/ZW have evolved independently multiple times in various lineages. Here we examine the evolution of sex chromosomes across turtles via the co-option of distinct ancestral autosomes, the evolution of their content, including chromosomal rearrangements that may have facilitated the transition from TSD to GSD, and the deep homology they share with a putative vertebrate proto sexchromosome(s). We present a comparative analysis of dosage compensation across multiple turtle lineages that revealed diverse patterns of dosage compensation, some supporting and some refuting current theoretical models. Reconciling these contrasting data leads to a revised paradigm of sex chromosome evolution.

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Sex chromosome evolution of birds and bird-like mammals: from karyotype to 3D genome

Sex chromosomes are the outliers of genome given their unique evolutionary history and regulatory programs. Their diversity across species remain largely unexplored due to their usually repetitive sequence nature despite the status quo of thirdgeneration sequencing. I will present our recent work on birds and platypus, whose genomes are assembled into a chromosome-level by Hi-C technique with corroboration of published fluorescence in situ hybridization (FISH) data. During the analyses of 15 Paleognathae and 12 songbird genomes, we found consistent with our previous results, bird sex chromosomes only shared one time of recombination suppression encompassing the male-determining DMRT1 gene, but independently form evolutionary strata after the rapid species radiation. We found several cases of genomic regions that exhibit excessive female heterozygosities, but a conserved synteny between the Z/W chromosomes according to FISH data. This indicates recombination suppression occurred without chromosomal inversions. In the case of platypus, we traced some of the sex chromosome origin to the microchromosomes of chicken. To our surprise, platypus sex chromosome show unexpected interchromosomal interactions that are not observed on any other chromosomes. We further confirmed the pattern with mitotic cell imaging showing indeed certain pairs of labelled sex chromosomes tend to have more contacts with each other. And such an interaction pattern seems to be conserved with human. These results highlight the fact that in the post-genomic era, karyotypic resources and techniques become in fact more important than before in the studies of sex chromosome evolution. And higher order of chromatin structure maybe conserved even involved chromosomes have changed their identity.

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Sex determination without sex chromosomes: Temperature-dependent regulation of *Kdm6b* controls sex determination in a turtle species

In many reptilian species, no sex chromosomes have been identified. Instead, many reptiles undergo a mode of sex determination that is contingent upon the temperature of the nest. In the 50 years since temperature dependent sex determination was first reported in reptiles, the underlying mechanism has remained a mystery. Several studies have proposed that epigenetic regulation mediates the effects of temperature on gene expression to initiate male or female sex determination pathways. Recently, we showed that an epigenetic regulator, KDM6B, is upregulated in the bipotential gonad at the male producing temperature. Using a knock-down approach, we showed that KDM6B is required for expression of DMRT1, a gene that is required to initiate the male pathway and testis development. In the absence of KDM6B, male genes are not activated and the female pathway is initiated, leading to formation of an ovary. Here we show that STAT3, a known repressor of KDM6B, is expressed in T. scripta gonads at both the male and female producing temperatures, but is only phosphorylated and bound to the *KDM6B* locus at the female producing temperature. Inhibition of phosphorylated STAT3 at the female producing temperature leads to upregulation of KDM6B and its downstream target DMRT1. We propose that temperature-mediated influx of calcium at 31°C drives phosphorylation of STAT3, which represses transcription of Kdm6b and downstream activation of male sex determination pathways. While these data provide a causal link between temperature sensing and STAT3 phosphorylation, it is probable that there are other inputs into differential activation of STAT3 that may be employed to regulate sex determination without sex chromosomes in other species. This work was supported by Bridge Funds from the Duke School of Medicine and the National Science Foundation.

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Developmental systems drift and the drivers of sex chromosome evolution

Phenotypic invariance -- the outcome of purifying selection -- is a hallmark of biological importance. However, invariant phenotypes might be controlled by diverged genetic systems in different species. In this seminar I will discuss how an important and invariant phenotype -- the development of sexually differentiated individuals -- is controlled in over two dozen species in the frog family Pipidae. We uncovered evidence in different species for (i) a W chromosome that is not found in a minority of females and is found in a minority of males, (ii) independent losses and (iii) autosomal segregation of this W chromosome, (iv) changes in male versus female heterogamy, and (v) substantial variation among species in recombination suppression on sex chromosomes. We further provide evidence of, and evolutionary context for, the origins of at least seven distinct systems for regulating sex determination. These systems are distinct in their genomic locations, evolutionary origins, and/or male versus female heterogamy. Our findings demonstrate that the developmental control of sexual differentiation changed via loss, sidelining, and empowerment of a mechanistically influential gene, and offer insights into novel factors that impinge on the diverse evolutionary fates of sex chromosomes.

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High throughput sex chromosome discovery in lizards and snakes

Sex chromosomes play a significant role in many biological processes including speciation, genetic conflict, sexual dimorphism, and perhaps most importantly, sex determination. However, sex chromosomes remain one of the most poorly studied regions of the genome and most of our knowledge concerning sex chromosome structure and evolution has been derived from studying just a handful of species. Expanding studies of sex chromosome evolution to include additional clades, particularly clades with repeated transitions among sex chromosome systems, will facilitate the study of sex chromosome evolution. Indeed, studying sex chromosomes in a wider array of taxa will help separate general principles of sex chromosome evolution from patterns unique to specific lineages. I will discuss recent advances that use next-generation sequencing data to identify sex chromosome systems (XY or ZW) in species with cryptic, homomorphic sex chromosomes. I will also discuss of how whole genome sequencing has accelerated the pace of sex chromosome discovery using lizard and snake examples. Finally, I will argue that greater knowledge about squamate genomes will enhance their utility as a model clade as well as improve our understanding of the origins and evolution of sex chromosomes and sex-determining systems.

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Convergent recruitment of chromosomes for sex determination via an unprecedented rate of turnover in true frogs

Most amphibians, and especially frogs, have homomorphic sex chromosomes, where differentiation between gametologs (e.g. X and Y) is difficult to see. We have previously shown that one major reason for this in true frogs (Ranidae) might be the rapid rate of transitions between sex chromosome systems. However the mechanisms of these transitions and, perhaps more importantly their evolutionary drivers, are not entirely clear. To address the former, we performed targeted sequencing of 12 genes known to be important for sex determination, across 6 species, in order to generate hypotheses for the mutations underlying the sex chromosome transitions between them. As for their evolutionary drivers, based on somewhat anecdotal evidence, we have hypothesised that they are driven primarily by rapid mutation load build-up as a result of rapid loss of recombination across incipient Y chromosomes. One major source of deleterious mutation is repeat element insertion and we know that frog genomes are full of highly active transposable element (TE) families. In light of this, we also attempted to quantify the extent and speed of repeat element build-up on new frog sex chromosomes following a transition using a new genome assembly and pooled sequencing data of males and females from the European common frog (Rana temporaria). I will discuss these results in the context of how amphibian sex chromosomes help us understand the evolution of sex chromosomes and sex determination more generally.

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Revisited sex chromosome evolution in moths and butterflies (Lepidoptera)

Current reviews provide a clear picture of sex chromosome evolution in Lepidoptera, which along with their sister order caddisflies (Trichoptera) constitute the most species rich taxon with female heterogamety. According to the prevailing hypothesis, early diverging lepidopteran lineages together with their sister order caddisflies (Trichoptera) share an ancestral Z0/ZZ sex chromosome constitution. A WZ/ZZ system then supposedly evolved upon a Z chromosome-autosome fusion, which occurred in a common ancestor of the family Tischeriidae and the clade Ditrysia comprising vast majority of extant Lepidoptera. However, this inference was drawn mostly from indirect cytogenetic evidence and some contradictory data were overlooked in the reviews. Here we revise the sex chromosome evolution in Lepidoptera using both molecular cytogenetic and genomic analyses. Surprisingly, comparative studies showed no evidence for the Z-autosome fusion in early diverging Lepidoptera, which suggests that the W chromosome evolved via a noncanonical mechanism such as B chromosome adoption. Moreover the W chromosome most likely occurred independently in different clades, as its presence was recently confirmed in one of the earliest diverging lepidopteran families, Hepialidae. Together these results rebut most of our inferences on W chromosome differentiation in Lepidoptera, except for few studies of neo-sex chromosomes. It may seem that sex chromosome-autosome fusions are as exceptional in Lepidoptera as in vertebrate female heterogametic taxa. However, many neo-sex chromosomes have been recently identified in Lepidoptera. The fusion origin of the W is still plausible in the family Tischeriidae, where comparative analysis uncovered multiple Z chromosome-autosome rearrangements. Detailed studies of lepidopteran neo-sex chromosomes revealed that autosomes repeatedly involved in fusions often correspond to synteny blocks, which comprise clusters of ovary-specific genes in a reference genome of a silkworm, Bombyx mori (Bombycoidea). Since ovary specific expression is one way to resolve sexual conflict, we can hypothesize that the neo-sex chromosome formation in Lepidoptera is driven by sexual antagonism. While this hypothesis may explain the origin of the W chromosome in Tischeriidae, independent evolution of the Ws from B chromosomes in Hepialidae and Ditrysia could have been driven by acquisition of novel sex determining mechanism. Indeed, analysis of a B chromosome in a Mediterranean flour moth, Ephestia kuehniella (Pyraloidea), showed that it contains sequences of the intracellular bacterium Wolbachia, which is ovarially transmitted and can induce feminization. It is tempting to envision a scenario in which *Wolbachia* incorporated into a B chromosome, took over sex determination, and thus gave rise to a W chromosome.

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Sequencing the threespine stickleback Y chromosome

Heteromorphic sex chromosomes (i.e. XY and ZW) have evolved repeatedly and independently in both plants and animals. Suppression of recombination between young X and Y (or Z and W) chromosomes can lead to rapid sequence divergence and degeneration of the Y (or W) chromosome. The early stages of degeneration are still not well understood, as complete Y or W chromosome assemblies have only been generated for a handful of mammalian and avian systems, which are 180 and 140 million years old, respectively. This knowledge gap is in part due to the technical challenges associated with assembling a non-recombining Y chromosome that has accumulated repetitive DNA. We have been conducting a long-term project to fully assemble the threespine stickleback (Gasterosteus aculeatus) Y chromosome, which is less than 16 million years old. Our previous work has shown that the nonrecombining region between the X and the Y spans ~17.5 Mb on the X chromosome, and that this young Y chromosome harbors at least three inversions as well as large arrays of repetitive sequences. To assemble the Y chromosome, we combined high coverage long-read PacBio sequencing with a Hi-C-based proximity guided assembly to generate a 17.48 Mb assembly of the non-recombining region of the Y chromosome. Our assembly was concordant with cytogenetic maps and an assembly of over 100 Y-chromosome specific clones from a bacterial artificial chromosome (BAC) library made from two males from the Paxton Benthic population. We found three evolutionary strata on the Y chromosome, consistent with the three inversions identified by our previous cytogenetic analyses. Although the threespine stickleback Y chromosome is at the early stages of evolution relative to mammalian sex chromosomes, we found the overall genetic architecture is already being shaped by many of the same evolutionary forces that have been documented on ancient sex chromosomes. First, many of the genes retained on the Y chromosome in the oldest stratum have signatures of haploinsufficiency, suggesting selection is maintaining functional alleles of essential genes on both sex chromosomes. Second, gene traffic onto the Y chromosome appears to arise early, as we identified paralogs of many autosomal genes present in each of the strata. Third, some genes are already amplified within the Y chromosome. Both genes of autosomal origin and recently duplicated genes show testis-biased expression, indicating male beneficial functions may be driving their accumulation on the Y chromosome. Finally, the Y chromosome contains a duplicate Amh gene (Amhy), which differs in many amino acids from the ancestral Amh gene on stickleback chromosome 8 and is an excellent candidate for the master sex-determination locus in threespine stickleback.

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Sex-chromosome differentiation and 'sex races' in common frogs (*Rana temporaria*)

Sex chromosomes have followed diverse evolutionary trajectories among vertebrates. Contrasting with the highly heteromorphic chromosomes found in mammals (XY) and birds (ZW), many fishes, amphibians and non-avian reptiles present homomorphic sex chromosomes. Reasons for these differences remain obscure, but species such as common frogs, with sex chromosomes at early stages of evolution and showing variable levels of differentiation, have the potential to deliver important insights. Seminal studies dating back to the early 20th century had revealed so-called 'sex races', differing in the patterns and rate of testis development, and seemingly distributed across climatic gradients throughout Europe (Witschi 1921, 1929, 1930). With the rise of molecular markers, Chr01 was later identified as the sex-chromosome pair, and Dmrt1 as a serious candidate sexdetermining gene. Moreover, genetic differentiation between X and Y was shown to range from extensive (XY males with Y-specific alleles at most markers along Chr01), to limited (XY° males with proto-sex chromosomes, only differing from females by their Y-specific Dmrt1 alleles), to null (XX males, genetically identical to females), possibly also distributed across a climatic gradient.

By genotyping and rearing families from a range of populations along altitudinal and latitudinal gradients across Europe, we provide support for a direct link between these two phenomena, likely mediated by a polymorphism at the candidate sex-determining gene *Dmrt1*. On the one hand, the rate of testes development in juvenile males depends on which *Dmrt1* haplotype lies on their Y (testes development is much delayed in XX males). On the other hand, the presence of genetically differentiated Y chromosomes is conditional to that of Y-specific *Dmrt1* haplotypes. The probability of having a differentiated Y chromosome, however, also depends on haplotype identity, possibly due to differences in the penetrance of different *Dmrt1* alleles: some Y-specific haplotypes seem more regularly associated with sex reversal (leaky GSD), leading to XY recombination in XY females (and thereby XY° sons). In addition, our results cast some doubts on the importance of climate in the distribution of sex races and patterns of sex-chromosome differentiation, which might actually depend more on the phylogeographic distribution of *Dmrt1* haplotypes.

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How to determine sex reversal? Troubles with molecular and cytogenetic markers in the bearded dragon (*Pogona vitticeps*)

The understanding of the transitions between environmental sex determination (ESD) and genotypic sex determination (GSD) is crucial for comprehension of the evolution of sex determination. Whereas the transition from ESD to GSD has apparently occurred in amniotes many times, the sole well-documented transition in the opposite direction, from ancestral GSD to derived ESD, was proved in the laboratory in the bearded dragon (Pogona vitticeps), where very high incubation temperature led to the production of females with male-typical (ZZ) genotype. Such sex-reversed females then produced offspring with sex ratio depending on its incubation temperature. Sex-reversed animals identified by molecular and cytogenetic markers were reported also from the field and their production was put into the connection with the global warming. In our study we cross-bred sexreversed individuals focusing on the transmission of known W-specific markers and the results demonstrate, that the used markers are not consistent possibly due to the recombination between the Z and W chromosomes. These events are likely rare as the sex-specificity of these markers can be surprisingly traced back to 14-25 million years ago as it was found in three species of the genus Pogona and in the related genus Diporiphora. We suggest that currently available sex-linked, either even apparently sex-specific markers are not a reliable tool for identification of sexreversed individuals in a population.

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Evolution of sex chromosomes in boas and pythons: a molecular-cytogenetic perspective

For over 50 years, it was believed that all snake lineages share homologous ZZ/ZW sex chromosomes: homomorphic and poorly differentiated in "basal" snakes such as boas and pythons, but heteromorphic and well differentiated in "advanced" (caenophidian) snakes. Recent studies revealed that homologous and differentiated sex chromosomes are indeed shared among all families of caenophidian snakes, but two species of boas (Boa imperator, B. constrictor) and a python (Python bivittatus) independently evolved XX/XY sex chromosomes. Additionally, heteromorphic ZZ/ZW sex chromosomes were recently revealed in another boa species (Acrantophis sp. cf. dumerili). Since the evolution of sex chromosomes in "basal" snakes is apparently more complex than previously thought, we examined 11 species of pythons and boas by conventional and molecular-cytogenetic methods, aiming to reveal their sex chromosomes. We applied Comparative Genome Hybridization (CGH) to uncover sex-specific regions of the genome and Fluorescence in situ hybridization (FISH) to test the distribution of repetitive elements frequently accumulated in differentiated sex chromosomes in vertebrates. Our results show that all examined species don't possess sex-specific differences in their genomes, at least up to the detection efficiency of our cytogenetic methods. Therefore, we conclude that these species have poorly differentiated sex chromosomes. Interestingly, FISH with telomeric repeats revealed extensive distribution of interstitial telomeric repeats (ITRs) in eight species. Cross-species comparisons indicate that the ITRs distributions can be the result of intra-chromosomal rearrangements. We conclude that evolution of sex chromosomes in "basal" snakes should be further explored with highthroughput methodologies.

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Sex Chromosomes, Genes and Palindromic Segmental Duplication

The large number of newly sequenced genomes during these last decades highlighted the large proportion of duplicated sequences – at many scales and scopes – they nearly all contain. Among these duplications, SDs (segmental duplications) are especially important, as they play a major role during the creation of genetic variation, both at the species and at the individual level. To ease in silico exploration of these new datasets, we developed ASGART, a fast and efficient tool designed toward precise mapping of duplicated areas in DNA fragments.

We will present a first set of results in the field of the evolutionary patterns obtained in sex chromosomes among multiple species obtained thanks to ASGART. Alessio Iannucci^{1*}, Marie Altmanová^{2,3*}, Claudio Ciofi¹, Malcolm Ferguson-Smith⁴, Massimo Milan⁵, Jorge Claudio Pereira⁴, James Pether⁶, Ivan Rehák⁷, Michail Rovatsos², Roscoe Stanyon¹, Petr Velenský⁷, Petr Ráb³, Lukáš Kratochvíl², <u>Martina</u> Johnson Pokorná^{2,3}

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Karyotype evolution and conserved sex chromosomes in monitor lizards (Varanidae)

Despite their long history with the basal split dating back to the Eocene, all species of monitor lizards studied so far share the same, among squamates derived, chromosome number 2n = 40. However, there are differences in the morphology of the macrochromosome pairs 5-8. Further, sex determination, which revealed ZZ/ZW sex microchromosomes, was studied only in a few varanid species and only with techniques not allowing a test of their homology. We investigated karyotype evolution and sex chromosome homology across varanids by hybridizing flow sorted chromosome paints from Varanus komodoensis to metaphases of nine species of monitor lizards. The results show that differences in the morphology of the chromosome pairs 5-8 can be attributed to intrachromosomal rearrangements, which led to transitions between acrocentric and metacentric chromosomes in both directions. We also documented the first case of spontaneous triploidy among varanids in Varanus albigularis. The triploid individual was fully grown, which demonstrates that polyploidisation is compatible with life in this lineage. We found that the W chromosome differs between species in size, heterochromatin and repeat motifs. In contrast, the varanid Z chromosome is clearly conserved in all the analysed species. Next to iguanas, caenophidian snakes and lacertid lizards, varanids are thus other squamate group with proven highly conserved sex chromosomes for a long evolutionary time.

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Sex differences in gene expression across multiple tissues in Lake Tanganyika cichlids

In organisms with separate sexes, many genes differ in their expression between the sexes across time and tissues. This sex bias is the foundation for phenotypic differences between males and females within a species. Sex-biased gene expression can be tissue- and time-specific, and sexual selection might be held responsible for the evolution of sexual dimorphism. Phenotypic sexual dimorphism can reflect sex-specific trait optima, which are based on sex-biased gene expression. In other words, the evolution of sex-biased gene expression is a mean to resolve intralocus sexual conflict.

The cichlid fishes in Darwin's dream pond Lake Tanganyika are a great example of the substantial levels of morphological variation among and within species, as well as between sexes. This makes them an ideal system to study differences in sex-biased gene expression. Here, we investigate the evolution of sex-biased gene expression in 73 representative species of the Lake Tanganyika cichlid assemblage across five tissues: brain, gills, lower pharyngeal jaw, liver and gonads. Preliminary results showed that gonads are the only tissue that has sex-biased genes in all studied species. In the gonads, the number of sex-biased genes is higher in males than in females. Astonishingly, only a handful of sex-biased genes are commonly shared within all males and within all females, 21 and 113 respectively. These shared genes are enriched for gene ontology terms related to gamete generation, such as spermatogenesis or female gamete generation in males and females respectively, as was expected. A hierarchical clustering analysis revealed that the strongest sex-biased genes in gonads are grouped first by sex and then phylogenetically, whilst for the other tissues the opposite pattern is observed.

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Chromosome-level genome of emu provides new insights into evolution of avian karyotypes and sex chromosomes

Paleognathous birds have attracted great attentions due to their unique flightless features and homomorphic sex chromosomes. However, the lack of high-quality genomes from this clade has hampered our understandings into the evolution of avian karyotypes and sex chromosomes. Here, we present a new version of emu (Dromaius novaehollandiae) genome assembly, built by PacBio sequencing, 10X linked reads and chromatin conformation technologies (Hi-C). The resulting assembly has high contiguity (contig N50 14Mb and scaffold N50 82Mb) and completeness (94% BUSCO value), including the mostly assembled pericentromeric and telomeric regions. We have also assembled over 8Mb highly repetitive W chromosome including the region that has recently suppressed recombination with the Z chromosome. We compared the emu genome together with the other five chromosome-level avian genomes against that of rattlesnake, and defined a conserved set of chromosomes throughout Reptilian, as well as individual rearrangements, particularly on microchromosomes of each species. Both *paleognathous* genomes (emu and ostrich) showed less genome rearrangements and longer synteny blocks, and resembled more closely to the ancestral avian genome due to a lower evolutionary rate. Using the published RNA-seq data from multiple tissues, we confirmed the sex chromosome of emu is undergoing incomplete dosage compensation and identified a total of 78 broad-spectrum dosage compensated (DC) genes. Their orthologous genes in green anole showed a higher expression level than the rest, suggesting the important function of DC genes. Furthermore, we showed Z and W chromosomes have a different topologically associated domain at the region that has recently suppressed recombination between Z/W. Such a divergence probably promotes the expression differentiation, and the sequence degeneration of W-linked genes.

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Sex is determined by XX/XY sex chromosomes in Australasian side-necked turtles (Testudines: Chelidae)

Turtles demonstrate a remarkable variability in sex determination and constitute an excellent model for the evolution of sex chromosomes. Notably, the cytogenetic traits as well as sex determination of turtles of the family Chelidae are still poorly explored. This family includes more than 50 species of freshwater turtles distributed in South America, Australia, New Guinea and Indonesia in two major lineages: the South American and the Australasian. We prepared chromosome suspensions from representatives of the Australasian genera Chelodina, Emydura and Elseya. We performed both classical and molecular cytogenetic analyses, such as comparative genome hybridization (CGH) and fluorescence in situ hybridization (FISH) with probes specific for rDNA genes, (GATA)₈ microsatellite motif and telomeric-like sequences. These repetitive elements often accumulated on vertebrate differentiated sex chromosomes. We were able to identify XX/XY sex chromosomes in all studied species. Surprisingly, the turtles from the genera Chelodina have different morphology of sex chromosomes than Emydura and Elseya. In the genus Chelodina, the sex chromosomes are represented by cryptic microchromosomes, while in the genera Emydura and Elseya, the sex chromosomes are medium-sized. In both clades, the Y chromosomes contain a male-specific region characterized by accumulation of repetitive elements, detectable by FISH and CGH, crucial in the identification of Chelodina sex chromosomes. Despite the differences in morphology might indicate a non-homology of sex chromosomes among these two groups, we can conclude that male was likely already present in the common ancestor of the genera Chelodina, *Emydura* and *Elseya*.

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A cnidarian sex determination locus

Sexual reproduction is ubiquitous among eukaryotes, with sex determination strategies varying greatly between species. Little is known, however, about the mechanisms that determine sex in non-bilaterians. Here, we report the identification of a ~3 Mb genomic region that co-segregates with sex in the cnidarian, Hydractinia symbiolongicarpus. To identify this region, we adopted a strategy based on the construction of a linkage map and subsequent analysis for QTLs linked to sex. We first crossed two outbred colonies to create a mapping population of 87 animals, performed whole-genome resequencing of these offspring and their parents, used a GATK-based pipeline to call variants, and filtered the variants for high quality markers that satisfied the requirements of a pseudo-testcross. We then used these data to generate high-density linkage maps reflecting recombination in each parent. In both maps, we recovered 15 linkage groups, corresponding to the haploid chromosome number for Hydractinia. We then used these maps to perform quantitative trait locus analysis in R/QTL, searching for markers linked to sex. No sexlinked QTLs were identified on the female map, but a single region on one chromosome of the male map was found to be significantly correlated with sex (LOD = 20.5, p << 0.001). This region includes four SNPs spanning ~3 Mb that cosegregated with sex in 84 of the 87 offspring. The remaining three offspring each changed sex from male to female during our study. Our results are consistent with a genetic sex determination system in which males are the heterogametic sex (XX/XY), although the observation of sex changes in three animals suggests the existence of sex-modifying loci, environmental influences, or some combination of both. We are currently analyzing this genomic region to identify candidate sex-determination genes.

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Sex chromosome variability vs. conservativism in Neotropical fishes: Molecular cytogenetic insights from *Hoplias malabaricus* species complex and the family Triportheidae

Teleost fishes exhibit remarkable diversity of sex determination and differentiation mechanisms. They include at least nine different male- or female-heterogametic sex chromosome systems with different stages of differentiation, high rate of inter- and intraspecific variability and frequent turnovers. Neotropical freshwater ichthyofauna offers many vital models to investigate the patterns and extent of such variability along with underlying evolutionary forces. Here, we present two contrasting trajectories of sex chromosome evolution gained through the study of Neotropical characiform models: Hoplias malabaricus (Erythrinidae) and Triportheus (Triportheidae). While different male-heterogametic sex chromosome systems found among seven karyotype forms (karyomorphs) of H. malabaricus have evolved independently at least four different times, representatives of Triportheus show highly conserved ZZ/ZW sex chromosome system of a monophyletic origin. During last few years, we applied a suite of molecular-cytogenetic methods, with comparative genomic hybridization (CGH) and whole chromosome painting (WCP) being the most productive ones; with an aim to gain a comprehensive view of mechanisms of origin and extent of differentiation and homology of sex chromosomes in above-mentioned fish models. In H. malabaricus, CGH delimited a the putative (and rather small-sized) male-specific regions in four out of six karyomorphs, while the last seventh karyomorph (E) could not be included into the analysis. Cross-species WCP with painting probes corresponding to various H. malabaricus sex chromosomes confirmed a homology between XY and XY_1Y_2 systems of karyomorphs F and G, respectively; and it revealed lack of homology with sex chromosomes of remaining karyomorphs and several related erythrinid species. We supposed a parallel origin of sex chromosomes of karyomorphs F and G from the common ancestor via a (likely tandem) fusion, which got fixed in a heterozygous form in endemic karyomorph G. Our data also strenghten the view of advanced stage of post-zygotic reproductive isolation between karyomorphs of *H. malabaricus*. On the other hand, similar cytogenetic investigation in *Triportheus* species unmasked highly conserved Z chromosome, contrasting with high extent of phylogenetically unrelated variability concerning size and repetitive DNA content of W chromosome. CGH displayed mostly extensive female specific region on W, pointing on striking differences in dynamics of degeneration in male- vs. female-heterogametic sex chromosome systems in fishes. Most importantly, chromosome painting probes derived from Z and W chromosomes of T. auritus confirmed not only that ZW sex chromosome system of a single origin is conserved among all *Triportheus* species analyzed to date, but that this conservativism encompass also other Triportheidae genera and, surprisingly, a certain degree of synteny is retained also with ZW sex chromosomes found in closely-related family Gasteropeleacidae. Such situation has no parallel among fishes and it also points on considerable ancestry of this ZW sex chromosome system. Taken together, molecular cytogenetic analyses brought important new insights into the two opposing scenarios of sex chromosome differentiation in Neotropical fishes. Research aimed at DNA sequence analysis of microdissected sex chromosomes is underway.

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Sex determination of the Nile tilapia shows polymorphism between two wild populations from East and West Africa

Sex control is essential for tilapia aquaculture, but it is currently based on hormone treatments with negative environmental effects. Sex determination in these species is still poorly understood. It is generally accepted that the Nile tilapia, Oreochromis niloticus, has an XX/XY system of sex determination, but minor genetic factors and high temperatures may also be involved. On the basis of studies mainly carried out on captive stocks, the Y chromosome was located on either LG1 or LG23. On LG23, the *amhY* (anti-Müllerian hormone) gene has been identified as the master sex determiner. The major gene on LG1 remains to be discovered. However, X and Y chromosome markers based on the *amh* genes do not always correlate with the sex of fish from wild populations. This suggests the existence additional polymorphisms in the "major" sex determining region. To understand this complex system better, we have performed both ddRAD genotyping on families with wild-caught parents raised under controlled conditions, and whole genome sequencing from several wild-caught individuals. Our results have shown that: 1) The genetic basis of sex determination is not the same in the two populations. One population showed a strong association with LG23 as expected. The other had a strong sex-linkage to both LG23 and LG03. 2) The amh gene has been identified as the major sex determining gene in at least one of the two populations, but the sequence of the *amh* region may differ between populations. 3) The existence of a B chromosome has been found for the first time in the Nile tilapia in both wild populations. This selfish chromosome might play a role in sex determination in Nile tilapia.

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Evolutionary stability of sex chromosomes in lacertid lizards

Amniotes possess variability in sex determination, from environmental sex determination (ESD), where no sex chromosomes are present, to genotypic sex determination (GSD) with highly differentiated sex chromosomes. Some evolutionary scenarios postulate high stability of differentiated sex chromosomes and rare transitions from GSD to ESD. However, sex chromosome turnovers and two independent transitions from highly differentiated ZZ/ZW sex chromosomes to ESD were previously reported in the lacertid lizards. Here, we examined the homology of sex chromosomes in the wide phylogenetic spectrum of lacertids and their outgroups by comparing gene copy numbers between sexes in genes previously found to be Z-specific in some lacertids. Our current sampling covers 45 species from 26 genera including lineages supposed to possess a derived sex determining systems. We found that all tested lacertids share homologous differentiated ZZ/ZW sex chromosomes, which were present already in their common ancestor living around 85 million years ago. These differentiated sex chromosomes are not present in amphisbaenians and teiid lizards, the close relatives of lacertids. Our study demonstrates how inaccuracies in data can influence the outcome of phylogenetic reconstructions of evolution of sex determination, in this case they overestimated the number of shifts from GSD to ESD and the rate in turnovers of sex chromosomes.

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Sex chromosome evolution in Palaeognathae

Avian species are categorized into two large groups: the Palaeognathae (flightless ratites and volant tinamous) and the Neognathae. Although Palaeognathae only include a small number of species, they are characterized by male parental care, which involves reversed patterns of the sex roles and size dimorphism. Palaegonaths usually have homomorphic sex chromosomes with cytologically indistinguishable Z and W. The reason for this slow differentiation of sex chromosomes is unclear. Moreover, the phylogeny and evolutionary trajectory and of sex chromosomes across the Palaeognathae remains unresolved.

Here we sequenced the female genomes of 9 tinamou and 3 ratite birds, and analyzed a total of 15 Palaeognathae genomes. We identified about 85 Mb Z-linked and different lengths of W-linked scaffolds per species. This indicates a great diversity of sequence differentiation of sex chromosomes across Palaeognathae. We found that almost all ratite birds have long pseudo-autosomal regions (PARs), suggesting these species have a W chromosome that retains the ancestral status of bird sex chromosomes. In contrast, the PARs and non-recombining regions of tinamou species exhibit a complex pattern, resulting from the suppression of recombination in an independent stepwise manner. We concluded that all Palaeognathae share one stratum with any other birds, and independently formed from one to three strata after their rapid speciation. Contrary to the classic notion, the youngest evolutionary stratum of Elegant crested tinamou formed without chromosomal inversion, and some of its encompassing W-linked genes in Chilean tinamou have unexpectedly upregulated their expression level in ovary, probably due to the female-specific selection. Intriguingly, we found that some CR1 subtypes (i.e. CR1-J1/2 Pass) are only enriched in tinamou Z chromosomes, which indicates the formation of evolutionary strata has also reshaped the TE landscape of the Z chromosomes.

We proposed that the reduced intensities of sexual selection derived from the unique male-only parental care system of Palaeognathae has important contribution to their low rate of sex chromosome divergence evolution. This study uncovers an unexpected complexity of sex chromosomes in Palaeognathae at the genomic level, thus greatly expands our knowledge of vertebrate ZW sex chromosomes evolution.

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The evolution of sex chromosome turnover in tilapia revealed by genome sequencing

Unlike the scenario in mammals or birds, sex chromosome turnover has been frequently observed in teleost fish, but the underlying evolutionary processes remain unclear. A classic example is seen in tilapia where both intra- and interheterogamety turnover of sex chromosomes have occurred in the past 5 million years. Here we assembled the genomes of both sexes of Nile tilapia (Oreochromis niloticus, XX and YY) and blue tilapia (O. aureus, ZZ and WW) at chromosome levels using long-read sequencing and chromatin conformation capture technology. YY and WW karyotype individuals were produced through crosses of sex-reversed and normal fish. This allows for the direct sequencing of the Y and W chromosomes that presumably contain sex determining genes. The Y chromosome assembly (LG23) of Nile tilapia unveils the complete sequence of the tandemly duplicated *amhy* that has been identified as a male-determining gene in Nile tilapia. In another family of Nile tilapia, by using population genetic approaches, we mapped the sex-determining loci to a ~2 Mb region on LG1. This region coincides the gene csmd1, a previously reported candidate gene. In addition, the sex-determining loci has been mapped to a ~500 kb interval on LG3, the Z/W chromosome in blue tilapia. This region contains a duplicated gene banf1 that shows ovary biased expression. The high-quality genomes and transcriptomes of developing gonads newly produced in this study empower a detailed study of sex chromosome evolution in tilapias, and our analyses demonstrate the role of recent gene duplications in driving the turnover of sex chromosomes.



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