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# A retroposon-based view on the temporal differentiation of sex chromosomes

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**Retroposon presence/absence patterns in orthologous genomic loci are known to be strong and almost homoplasy-free phylogenetic markers of common ancestry. This is evidenced by the comprehensive reconstruction of various species trees of vertebrate lineages in recent years, as well as the inference of the evolution of genes via retroposon-based gene trees of paralogous genes. Recently, it has been shown that retroposon markers are also suitable for the inference of differentiation events of gametologous genes, i.e., homologous genes on opposite sex chromosomes. This is because sex chromosomes evolved via stepwise cessation of recombination, making the presence or absence of a particular retroposon insertion among the two different gametologs in more or less closely related species a clear-cut indicator of the timing of differentiation events. Here, I examine the advantages and current limitations of this novel perspective for understanding avian sex chromosome evolution, compare the retroposon-based and sequence-based insights into gametolog differentiation and show that retroposons promise to be equally applicable to other sex chromosomal systems, such as the human X and Y chromosomes.**

**Keywords:** rare genomic change, retroposon, LTR, CR1, *Alu*, cladistic marker, sex chromosome, interchromosomal recombination, evolutionary stratum, gametolog

**Abbreviations:** RGC, rare genomic change; RE, retroposed element; mya, million years ago; indel, insertion/deletion; LTR, long terminal repeat element; LINE, long interspersed element; CR1, chicken repeat 1, *CHD1*, chromodomain helicase DNA–binding protein 1; *NIPBL*, *Drosophila* Nipped-B homolog; *ATP5A1*, ATP synthase α-subunit isoform 1; *MXRA5*, matrix-remodeling-associated protein 5

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## The Utility of Retroposed Elements as Cladistic Markers

Rare genomic changes are powerful and independent tools for the reevaluation of molecular phylogenetic hypotheses based on nucleotide sequence analyses.[1] Of particular interest are retroposed elements, RNA-derived repetitive sequences that are almost randomly scattered throughout the genome and constitute straightforward synapomorphies of shared ancestry. Due to the wealth of unique character states[2] in such a marker system of retroposon presence/absence patterns, homoplasious phylogenetic signals caused by parallel insertions (i.e., featuring an identical RE subtype, RE orientation, RE truncation, insertion site and target site duplication) or precise deletions occur very rarely.[3,4] Consequently, retroposon insertions have been widely used as reliable cladistic markers for the inference of the phylogeny of several vertebrate lineages, inter alia, settling controversies regarding the relationships among placental[5] and marsupial[6] mammals. Among birds, the long-standing phylogenetic enigma of the passerine sister group has been recently reappraised by the identification of unambiguous retroposon support[7] for the close relationship of passerines to parrots and their second-closest affinities to falcons,[8] termed the Psittacopasserae and Eufalconimorphae hypotheses.[7]

In addition to the utility of REs for the resolution of phylogenetic relationships among species, the presence or absence of a given retroposon insertion within different paralogs of a gene provides a phylogenetic signal for the unambiguous reconstruction of gene trees, for example, of snake phospholipase $A_2$ genes[9] or genes within the segmentally duplicated human chromosome 1q22 region.[10] Another application was recently proposed and reported by Suh et al.,[11] as the presence/absence analysis of CR1 and LTR retroposon insertions in homologous genes on opposite sex chromosomes

(i.e., gametologs[12]), permitted the reconstruction of sex chromosomal differentiation events and an independent view on the evolution of sex chromosomes in birds.

## The Retroposon Chronology of Avian Sex Chromosome Differentiation

In birds, sex is determined via a ZW sex chromosomal system: Female birds possess one Z plus one W chromosome and males exhibit two Z chromosomes. Comparable to the situation of the Y chromosome of the human XY sex chromosomal system, the female-specific W chromosome is more or less degenerated as a result of regional cessation of interchromosomal Z-W recombination.[13] Thus, except for the pseudoautosomal region where Z-W recombination still occurs, W-chromosomal gametologous genes exhibit Z-W divergence levels that reflect the spatiotemporal evolutionary history of avian sex chromosome differentiation. Interestingly, about 99% of all bird species (Neognathae; e.g., chicken and zebra finch) exhibit a high degree of W chromosome degeneration, but within Palaeognathae, the ratites (e.g., ostrich) exhibit largely homomorphic sex chromosomes except for a small nonrecombining region,[14,15] and within ratites,[16] the tinamous feature various stages of more or less pronounced moderate degeneration of their W chromosome.[17,18]

At present, the chicken Z and W chromosomes are the only full set of sequenced avian sex chromosomes available,[19] complemented by the recent sequencing of the zebra finch Z chromosome.[20] Based on sequence comparisons of the 12 known pairs of gametologous genes in the chicken, Nam and Ellegren[21] identified three Z-chromosomal regions that show a discrete interval of Z-W divergences, respectively: The oldest region, namely evolutionary stratum 1, differentiated 150–132 mya and comprises two gametologous gene pairs. The remaining two evolutionary strata appear to be considerably younger, as stratum 2 contains four genes that diverged 99–71 mya and stratum 3 consists of six genes that ceased recombining 57–47 mya.

Suh et al.[11] studied presence/absence patterns of retroposons in three gametologous gene pairs, namely *CHD1Z/CHD1W*, *NIPBLZ/NIPBLW* and *ATP5A1Z/ATP5A1W*. The remaining nine gametologous gene pairs could not be studied from a retroposon-based perspective, either due to paucity of retroposon insertions in the respective gene pairs or due to the many gaps in the assembly of the W chromosome.[22] Another limiting factor was the suitability of retroposon insertion loci for PCR-based experimental screening across the breadth of avian taxa, i.e., loci with two well-conserved retroposon-flanking regions at less than 1.5 kb distance to each other. Additionally, cases of retroposon Z-absence/W-presence were not considered, as the successful PCR

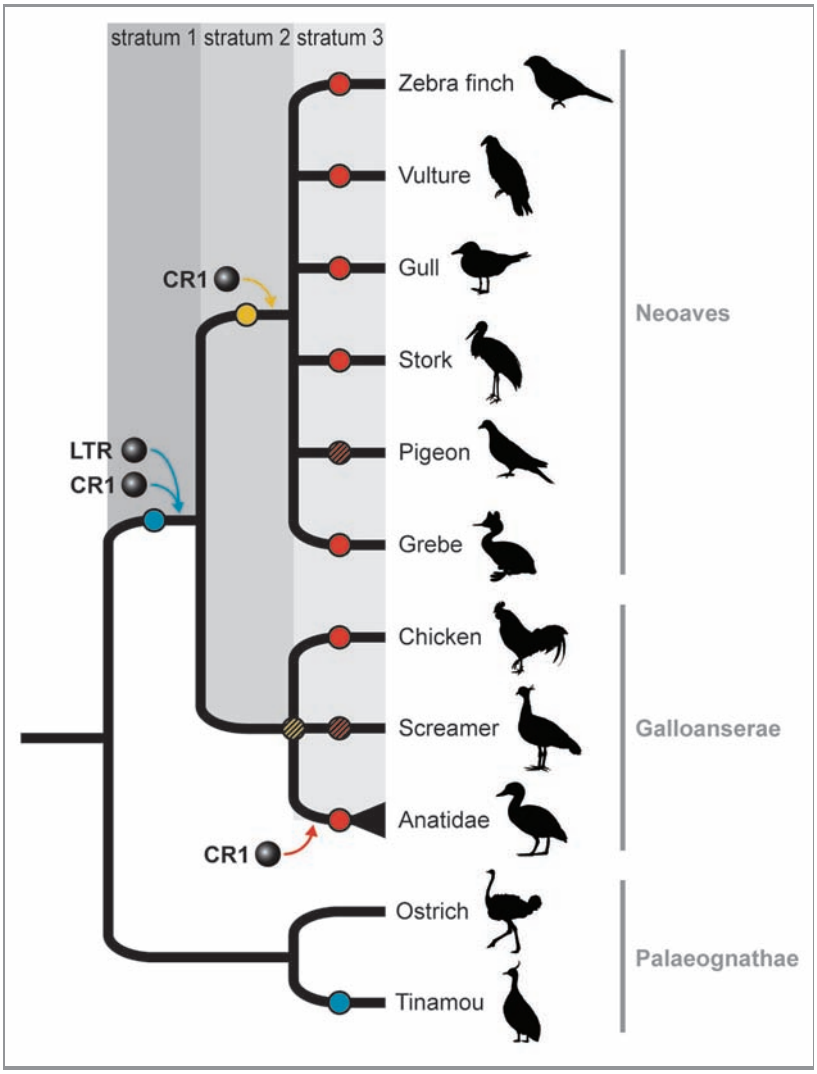


**Figure 1.** Retroposon evidence for the sex chromosomal differentiation events (circles) of *CHD1Z/CHD1W* (blue), *NIPBLZ/NIPBLW* (orange) and *ATP5A1Z/ATP5A1W* (red) during the evolution of birds. The species tree topology (congruent with the current understanding of bird phylogeny[8]) is based on RGCs[11] and illustrates the putative temporal emergence of the three evolutionary strata on the Z chromosomes of neognathous birds (Neoaves + Galloanserae). Colored arrows (colors correspond to those in the colored circles) indicate retroposition events (gray balls) of LTR or CR1 retroposons that either occurred prior to (i.e., Z-/W-presence) or after (i.e., Z-presence/W-absence) the divergence of the respective pair of gametologous genes. Hatched circles denote uncertainty regarding differentiation events either due to lack of RGC data (hatched orange circle) for the particular parts of the tree or due to absence of a W-specific sequence (hatched red circles). The latter case might be the result of W-chromosomal gene loss or ongoing interchromosomal Z-W recombination.

amplification of the female-specific W gametolog might be less likely when the W gametolog is considerably larger than the Z gametolog.

Together with other RGCs, namely random indels, the retroposon-based gametologous gene trees[11] provide an independent corroboration of the three evolutionary strata *sensu* Nam and Ellegren[21] on the Z chromosomes of neognathous birds (**Fig. 1**). *CHD1* belongs to stratum 1 in Neognathae (congruent with ref. 12) and independently diverged in the tinamid lineage (corroborating ref. 17), where no evolutionary strata have yet been identified. In contrast to this, *NIPBL* appears to be undifferentiated in tinamous, but diverged in the ancestor of Neoaves (i.e., stratum 2) and at least once within Galloanserae, although the exact timing could not be elucidated (but see ref. 21). At least nine independent cessations of recombination (more than previously hypothesized[23]) occurred in the neognathous *ATP5A1*, including two potential losses from the W chromosome of the pigeon and the screamer. The inclusion of this gene in stratum 3 *sensu* Nam and Ellegren[21] corresponds to its proximity to the pseudoautosomal region of the Z chromosome and highlights the evolutionary forces of stepwise cessation of recombination that independently acted upon the sex chromosomes of the majority of lineages of birds.

## The Potential Impact of Retroposons on Sex Chromosomal Recombination

In mammals and other XY sex chromosomal systems, the male-specific Y chromosome typically exhibits a higher amount of REs than the X chromosome (reviewed by ref. 13), as the Y-chromosomal nonrecombining regions appear to rapidly accumulate repetitive sequences after cessation of X-Y recombination. In contrast to this, the ZW chromosomes of birds appear to have no sex-biased density of CR1 retroposons.[24]

The abundance and distribution of REs on sex chromosomes has been attributed to cause several momentous phenomena during sex chromosome differentiation: Recombination events between different retroposon insertions due to shared RE sequence similarity often lead to the loss of genes from the sex-specific W or Y chromosome that are retained on the respective counterpart.[13,25] Such retroposon-based recombination events might also lead to sex chromosomal rearrangements, such as the inversion of a large nonrecombining region on one of the two sex chromosomes.[26] Notably, the recombination between non-orthologous retroposon insertions on opposite sex chromosomes might even lead to secondary Z-W or X-Y recombination, homogenizing the sequences of previously diverged gametologous genes, for example, on human[26,27] or feline[28] sex chromosomes. In birds, this form of gene conversion has probably not played a detectable role in the evolution of their Z and W gametologs.[21]

As a single retroposition event constitutes a large mutation that is likely to affect the structure of its genomic insertion locus, another potential role of retroposons during sex chromosome evolution might be the reduction of the frequency of interchromosomal recombination events. If several retroposition events occurred shortly after each other in one of the two gametologs of a previously pseudoautosomal, frequently recombining locus, they might reduce the gametologs' sequence identity drastically, ultimately leading to a complete cessation of recombination at that particular locus. In this context, it is an interesting coincidence that in Suh et al.,[11] all of the three Z-specific REs (two in *CHD1Z* and one in *NIPBLZ*) were inserted shortly after the divergence of the respective gametologous gene pairs. At present, it remains speculative whether or not this observation is in fact due to a causal relationship between the differential presence of retroposon insertions among two gametologs and the progression of sex chromosome differentiation. Nevertheless, in the comparable situation of the evolution of paralogs via autosomal gene duplication, studies on gene families (such as α-globins[29] and lysozymes[30]) suggest that insertions of retroposons and other large sequences in only one of several


**Figure 2.** Retroposon evidence for the sex chromosomal differentiation event (circle) of *MXRA5X/MXRA5Y* (pink) during the evolution of simian primates. (A) The alignment of a part of *MXRA5* intron 2 (Alexander Suh, unpublished data) comprises sequences from human (hg19), chimp (panTro3), rhesus (rheMac2) and marmoset (calJac3) genomes available in Genome Browser. The insertion of an *Alu*Yf2 retroposon (lowercase letters on gray background) is flanked by a 15-nt target site duplication (direct repeats, in black boxes). The *Alu* insertion is present in the orthologous insertion site of the X chromosomes of catarrhine primates (human, chimp and rhesus), but absent in the X chromosome of platyrrhine primates (marmoset) and the available Y chromosomes. (B) Thus, the X-chromosomal retroposition of this *Alu* element (gray ball) occurred in the common ancestor of human, chimp and rhesus; more precisely, shortly after the divergence of the *MXRA5* gametologs in the youngest of the five human X chromosomal strata (29–32 million years ago[35]).

paralogs might act as a barrier for genetic interchange between these paralogous genes.[31] To address such a putatively similar role of retroposon insertions in the divergence of gametologous genes, the recently differentiated sex chromosomes of tinamous[18] or the neo-sex chromosomes of sylvioid songbirds[32] might be ideal model systems for the future.

## The Future of Sex Chromosomal Retroposon Markers

Considering the successful reconstruction of differentiation events of gametologous genes in birds,[11] it is obvious that this methodology could be equally applicable to other sex chromosomal systems, such as those of mammals, snakes and the many other independently emerged sex chromosomes of further animals or plants. The human XY sex chromosomal system is a very promising example for future studies, as a large fraction of the human genome consists of repetitive DNA,[33] providing a wealth of retroposon insertions for any kind of RE-based study. A retroposon presence/absence screening (Alexander Suh, unpublished data) in one of the human gametologous gene pairs (i.e., *MXRA5X*/*MXRA5Y*) using the primate genome sequences available in Genome Browser (http://genome.ucsc.edu/cgi-bin/hgBlat[34]) revealed an X-chromosomal *Alu* retroposon insertion in catarrhine primates (**Fig. 2**) that inserted shortly after the differentiation of *MXRA5* in the ancestor of Catarrhini. This observation is congruent with the sequence-based inclusion of this gene in stratum five of the human X chromosome[35] and indicates the utility of retroposon presence/absence patterns for the future reconstruction of the evolution of mammalian sex chromosomes from a retroposon-based perspective.

Likewise, the use of REs for the study of avian sex chromosomes is not yet fully exploited. It is to hope that the chicken W chromosome is sequenced at a higher coverage via targeted sequencing[22] and the W-chromosomal sequence of a second bird species (preferably the zebra finch) becomes available, each providing previously unavailable sex chromosomal retroposon insertions for the study of additional gametologous gene pairs. Additionally, the targeted enrichment of CR1 retroposon insertion loci[36] might also yield information about gametologous REs. For as more taxa and gametologous genes are added, the chronology of gametolog differentiation reveals the many aspects of the complex evolutionary history of avian sex chromosome evolution.

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