Interactions between transposable elements and the W-chromosome influence evolution and speciation

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Abstract

This literature-research paper gives insights of the most recent findings considering transposable elements and the evolution of the W-chromosome. Transposable elements are mobile genes that move through the genome from one location to another. Their ability to change DNA strands and control epigenetic expression makes them essential in the diversification and speciation of organisms. In this paper, the impact of transposons on the host together with the evolved defense mechanisms are discussed. According to various studies, it has been hypothesized that incomplete silencing of LTR-transposons (mostly endogenous retroviruses) increases the mutational load suggesting that this contribute to the 'toxic' W-chromosome and the reduced life spam of female birds. The accumulation and activity of transposable elements (TEs) on the W chromosome indicate that it serves as a reservoir for potentially active TEs. This phenomenon has significant evolutionary implications, such as enhancing genetic diversity and impacting reproductive isolation and speciation. This paper will provide information about the current state of the knowledge, there is about TEs and the further prospects that still need to be studied.

Introduction

A large portion of the genome is called ‘junk DNA’ and doesn't encode for proteins. This junk DNA consists regulatory sequences, introns and repetitive DNA elements like transposable elements. Another name for transposable elements in the genome of organisms are the so called: ‘jump genes’. These TEs have the ability to move throughout the genome and can cause mutations, recombination events, alterations in gene-expression or even disruptions of genes. There are two main classes of transposons, retrotransposons and DNA transposons, *figure 1*. The retrotransposons move via an RNA intermediate and are converted back into DNA with the help of a reverse transcriptase (copy-paste). DNA transposons move directly in the form of DNA and propagate by a cut-and-paste mechanism. Within these classes, different types can be further separated and classified. The two classes retrotransposons can be further separated in Long Terminal Repeat transposons and Non-LTR transposons. These are classified based on their length in terminal repeats. LTR transposons often contain endogenous retroviruses. Non-LTR transposons can be further classified in LINEs (Long Interspersed Nuclear Elements) and SINEs (short Interspersed Nuclear Elements) [9]. The main classes of the DNA transposons are, TIR elements, Helitrons and Miniature Inverted-repeat Transposable Elements (MITEs). TIR elements have inverted repeats at the ends and encode for a transposase that facilitates their movement. Helitrons replicate using a rolling-circle mechanism instead of the traditional cut-and-paste method. Unlike other transposons, they do not depend on terminal inverted repeats for their mobility. Helitrons are commonly found in both plants and animals. MITEs are small, non-autonomous transposable elements derived from TIR transposons. They rely on the transposase enzymes of related autonomous elements to move within the genome. These elements are particularly common in plants [15].

In this paper an investigation will be done to get more clarity about the various TE types that are the most relevant in evolutionary and regulatory perspective in birds. Due to the instability that can be created by the TEs, they could reduce the host fitness and cause numerous diseases in all kinds of organisms. TEs can be the molecular origin for various diseases, for example human cancer, hemoglobinopathies neurological and metabolic diseases [3]. Even aging is considered to be influenced by TEs. This genome instability caused by TEs, is countered by several defence mechanisms that have evolved to supress this TE activity. Similar to the different types of transposons also various defence mechanisms have evolved with different molecular pathways to tackle the mobility of TEs. One of these evolved strategies is cystine methylation to silence this mobility by altering the chromatin structure and recruiting proteins that repress gene expression [19, 34]. It is broadly known that avian genomes harbour lower percentages of repetitive DNA than other vertebrates like mammals [26].

In this literature-review the focus lies on the evolutionary and regulatory role of these TEs and the influence they have on physiology and reproductive isolation of birds. In order to structure this research, the following questions have been formulated.

- *What is the interaction of transposons and the W-chromosome (sex-limited chromosome) in an evolutionary perspective?*
- *What is the effect of TEs activity on the host genome of different organisms?*
- *What is the role of the high-density TEs of W-chromosome on the reproductive isolation and physiology of birds?*

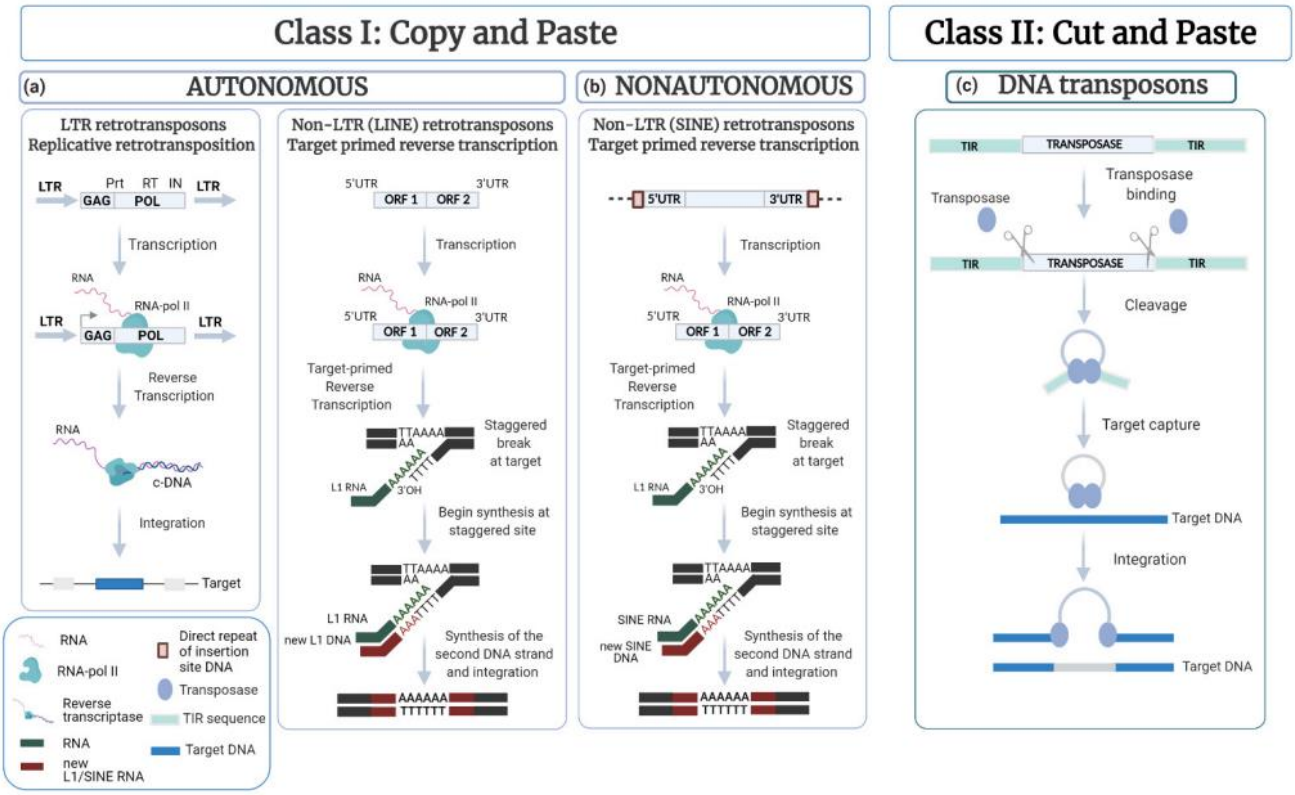

Figure 1: Mechanisms for different types of transposons. Transposons can be separated into two types of TE classes in which class 1 transposons propagate via a copy-paste mechanism. The class 2 transposons propagate directly via DNA, without the RNA intermediate. Class 1 can be further divided in autonomous and non-autonomous transposons. Autonomous transposons can move independently because they encode for the essential enzymes needed for their own transposition. They typically encode a transposase enzyme for DNA transposons or reverse transcriptase and integrase enzymes for retrotransposons. Non-autonomous transposons cannot move independently and rely on enzymes produced by autonomous TEs. Class II (DNA transposons) have a protein called transposase (TPase) that is surrounded by terminal inverted repeats (TIRs). TPase helps move transposable elements within the genome in two ways. The first way, called the "cut and paste" or "non-replicative pathway," cuts the TE out of its original spot and inserts it somewhere else. The second way, known as the "replicative pathway," copies the TE and moves the copy to a new location, leaving the original in place. [9]

Research Analysis

Evolution of W and TEs

Despite the fact that avian genomes harbour low percentages of repetitive DNA, the sex-limited W chromosome shows high amounts of repetitive elements including TEs. The density of TEs on the W chromosome is 55% whereas the density on the wide genome is less than 10% [14, 27]. This can be explained by the way sex limited chromosomes evolve. In general sex chromosomes evolve from autosomes with the sex determining genes. Around the location of the genes on the sex-limited chromosomes (W and Y chromosome in mammals), the recombination is actively suppressed. Therefore the accumulation of TEs on the W/Y chromosomes is hypothesized to begin with insertions close to crucial heterogametic sex-linked genes, benefiting from the hitchhiking effect of favourable mutations. These TEs can avoid removal due to their proximity to important genes and the restricted recombination on W/Y chromosomes, which leads to a reduced pressure selection against deleterious mutations. This environment allows TEs to spread and overpopulate these chromosomes, especially as functional TEs near important genes are less likely to become heterochromatized. For both the W and Y the retained genes are known to be critical and highly conserved [1, 16]. These genes play a crucial role in certain biological functions including cell cycle control, transcription and translation, as their retention prevents deleterious effects and gene loss. To protect these essential genes a combination of structural features and biochemical pathways have evolved for example, heterochromatin, palindromic sequences, and satellite DNA provide physical protection [17]. Besides that, epigenetic upregulation of these genes was observed to counterattack gene loss [22]. This can be established via DNA methylation, histone modifications, and non-coding RNAs. One of the features that go along with the lack of recombination, is accumulation of repetitive sequences, transposable elements, and mutations. The moment that the recombination rate decreased, TEs become more successful to insert themselves in the W/Y chromosome and they spread out further over time. Eventually due to hitchhiking with beneficial mutations, Muller's ratchet (process in which accumulation of deleterious mutations happen irreversibly), and interactions with silencing proteins, the rate of insertion accelerated and TEs colonized parts of the Y/W chromosome. All the TE transcripts hindered the genomic defense mechanism to counter this colonization of TEs. Therefore other processes became active that could remove entire sections of the sex-limited chromosomes which explains the shrinkage of both W and Y chromosomes, *figure 2*.

Considering the genomic defense against TE activity, it becomes clear that the negative effects of transposable elements on host fitness may not persist constantly. When talking about retrotransposons, each new insertion can slightly diminish the effectiveness of the genome's defense mechanisms against all other functional transcripts. This can also lead to an increasing cell's energetic expenditure and therefore additional proteins are needed to maintain the efficiency of genome defense. As new sources (the sequences of transposons) of functional TE transcripts emerge, the overall cost of genome defense rises. With each functional TE insertion, negative selection for other silenced elements in the genome can increase. Over time, the energetic costs of maintaining genome defense may grow as the number of functional TEs producing transcripts increases. As the number of retrotransposon transcripts increases, they compete for binding sites, which can exhaust host factors required for genome defense, therefore lowering host fitness. This competition for host factors can result in their unavailability for other essential processes. This is one of the factors that cause the removal of the transposable elements. The mechanisms for removing deleterious loads include heterochromatization [17]. So this accumulation ultimately leads to the degeneration and the gene-poor state of these chromosomes, in contrast to the more gene-rich autosomes and recombining sex chromosomes like the X and Z. The evolution of the interaction between TEs and sex chromosomes are sex specific. Some species exhibit a high tolerance for TE accumulation, while others have evolved mechanisms to suppress TE activity.

The efficiency of these mechanisms and the differences are influenced by the evolutionary history and ecological niches of the species. However, because of the fact that the sex-limited chromosomes are gene poor and have high amount of repeats, it was thought that the W chromosome did not had any further functions beside the sex determination and gonadal development. This will be further discussed in the paper.

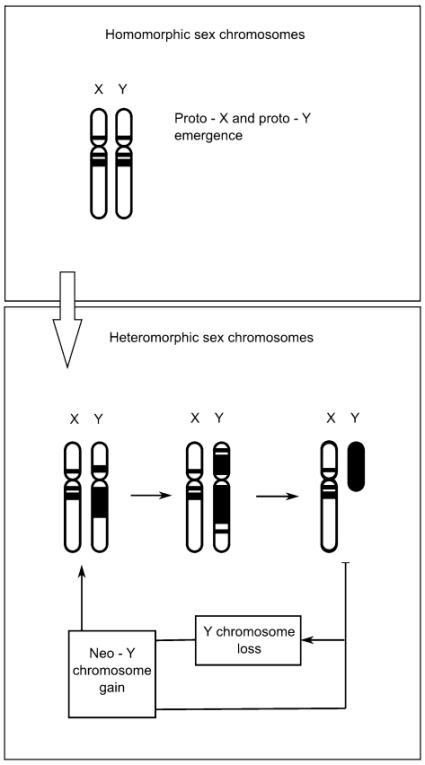

Figure 2: The evolutionary degeneration of the sex-limited in the X and Y or Z and W system. TEs colonize the sex-limited chromosomes due to the low recombination rate. [17]

The genomic and epigenetic instability

Transposons in Diseases

TEs are present in genomes of almost all living organisms. Their widespread presence highlights their significant role in shaping the genome along with their ability to move in the genome. The mutations created by TEs, involve different types of transposons and many forms of rearrangement. The TE insertions often cause deleterious mutations, but can also cause insertions or chromosomal mutations. These changes in DNA sequences can be very disruptive and influence host fitness, especially if these alterations occur in DNA regions that play roles in DNA repair systems. Aside from the fact that TEs are found in the genomes of nearly all living organisms, diseases caused by TEs are primarily studied in humans. As a result, most examples of diseases caused by TEs in this paper will highlight the working principle of the TEs in humans. Nonetheless it's still relevant to understand molecular mechanisms in whatever organism and understand what the effect is on the host. A way TEs can create genomic instability is when they enter alteration sides of the DNA that are import for regulation, intron- and exon regions, and change these regions (*figure 3)*. The effects of retrotransposon insertions have been extensively studied, showing that about 0.3% of all mutations are caused by these insertions [25]. When TEs insert themselves into coding regions of the genome, they can create frameshift

mutations, which result in early termination of protein production. This can create missense which is the incorrect placement of an amino acid or nonsense when a unintended stop codon terminate further transcription. For example, Alu elements (SINE retrotransposons) inserted into exonic regions of mRNA can alter the open reading frame (ORF), impacting gene expression. Additionally, when TEs are inserted into intronic regions, they can introduce new splice sites, leading to alternative splicing that disrupts normal gene transcription. This is again seen with Alu elements when they are inserted within introns, but it is also seen with LINE-1 insertions. TEs can also affect gene expression by inserting into the 5' (upstream) or 3' (downstream) regions of genes [2]. The cumulative effect of these changes in gene expression due to TE insertions has been related to many diseases, including cancer and genetic disorders. An example where similarity in mechanism can be observed, is the recombination-activating gene 1 and 2 recombinases that play an essential role in V(D)J recombination in the development of lymphocytes [6]. Both transposons and recombination-activating genes (for inducing different potential cancer cells) promote deleterious mutations and chromosomal rearrangements. These alterations caused by recombinases are realized via a similar mechanism as reactions catalysed by transposases. The presence of transposable element sequences, even if they can no longer move around, can still disrupt the genome's stability. TEs can attract epigenetic modifiers, which not only change the TE sequences but also affect nearby regions. For example, methylation of CpG-rich TE inserts can lead to the loss of CpG sites in the surrounding DNA which could lead to loss of functional genes [34]. The methylation status of DNA is strongly related to diseases like cancer [23]. In cancer cells, global hypomethylation and epigenetic dysfunction often lead to the activation of non-LTR retrotransposons during the development of tumors [4]. For example, the insertion of Alu elements into DNA repair genes such as breast cancer 1 and 2 genes[29], and the insertion of LINE-1 elements into tumor suppressor genes like adenomatous polyposis coli [18] and retinoblastoma 1[24], can disrupt their functions and promote tumorigenesis. It is well-established that active retrotransposons like Alu and LINE-1 are associated with the development of cancer.[4]

The effects of transposable elements after they are inserted into the genome can affect the genome in many different ways in for example: overall structure, behaviour, and function of chromosomes. When TEs insert into untranslated regions like introns, or areas upstream or downstream of genes, they can act as enhancers or promoters, influencing the activity of nearby target-genes. Additionally events following these insertions, like non-allelic homologous recombination (type of genetic recombination that occurs between sequences of DNA that are similar, homologous, but not alleles of the same gene), can lead to deletions, duplications, and inversions of DNA segments. Additionally, the abnormal expression of LINE-1 sequences is believed to contribute to the development of tumors. [4]

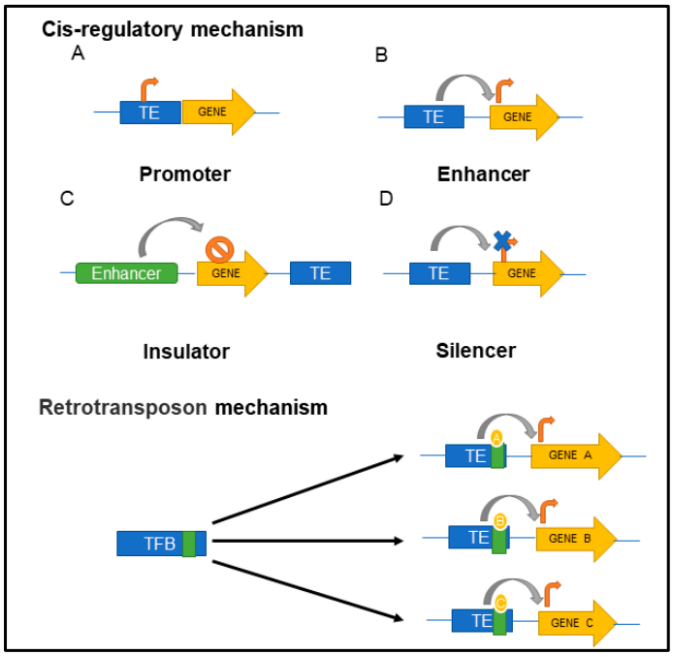

Figure 3: TE regulated control in the host cell. A, Promoter integrating specific transcription factors. B , Enhancer boosts gene expression by integrating TE. C, Insulator blocks the expression of the gene with a chromatin barrier or enhancer-blocking. D, Silencer supresses gene expression. [3]

Transposons as Symbionts

That transposable elements can influence the genome at various levels (genes, chromatin, and chromosomes) is clear. When TEs are integrated into new locations in the genome, they can modify new regulatory programs by adding enhancers, alternative promoters, or silencers. They can also create new exons that provide useful functions to gene products. Recent studies have shown that TEs often function as active regulatory elements. While transposons can be the molecular origin for diseases, they also interact with valuable regulatory sequences in a way that they control or promote gene expression. This process is called "molecular domestication." It has helped the development of complex transcriptional regulation systems [9]. For example, the gene that encodes for the tumor suppressor protein p53 which is expressed when DNA damage and stress signals are detected. The open reading frame of this gene has many DNA-binding sites enriched with ERV-LTR elements. These elements affect the expression of p53 target genes and contribute to species-specific regulatory networks. From an evolutionary perspective, the reactivation or co-option of TE sequences can benefit the host by becoming coding or non-coding elements [31]. Recent genome sequencing efforts have identified many exapted TEs that benefit the host. [20] In addition to providing regulatory elements, TEs can produce a wide range of non-coding RNA transcripts, such as micro RNAs and long non-coding RNAs, which can regulate gene expression. For example, TE-derived ncRNAs play roles in adaptive immunity, nervous system development, and placenta formation. Some env genes from Endogenous Retroviruses (ERVs) have undergone positive selection in mammals, leading to the expression of syncytin 1 and 2 genes, essential for placenta development. [13]

That transposable elements can act as ‘parasites’ by modifying gene expression and regulatory networks is well studied. However most insertions aren’t harmful and is not even selected against by certain environmental pressures. They can enhance the functions of genes and contribute to the host's fitness. Thus, they may remain in a population as polymorphisms, and through genetic drift, some of them may become common alleles or even become permanently fixed in species. In this context TEs can be seen as an biological inducers for biodiversity, but more important as ‘symbionts’ of the genome. [9]

The impact of TEs on the W chromosome

Trade-off between silencing and genome function

Although Y chromosomes in mammals and flies have been extensively studied, the evolutionary roles of W chromosomes across different organisms remain largely unknown. There is speculation about the effect of the high TE densities on the W chromosome and reduced fitness of the heterogametic sex [5, 33]. Research in various species has shown that TE activity is not completely silenced and can occur in healthy adult tissues. This ongoing activity suggests that the mechanisms responsible for TE silencing are often incomplete or imperfect, allowing TEs to remain active in certain contexts [12, 27]. It may suggest a trade-off between epigenetic TE silencing and host genome function, because silencing marks can spread from TE to nearby genes, creating a repressive chromatin environment which ultimately can results in phenotypic variation and reduced fitness. Considering this incomplete silencing and the high densities of TEs on the sex limited chromosome, it can significantly influence the genome of the heterogametic sex. To give an example, in Drosophila (which has an X/Y system), the incomplete silencing of transposons on the Y chromosome is believed to increase the mutational load in males, resulting in a "toxic" Y chromosome [33]. This increased mutational burden may contribute to male-specific aging.

Research example 1 using the sequences of Eurasian crows [32]

One study used RNA-seq to show that female (Eurasian) crows have a greater abundance ~9.5% of the total TE transcripts than male crows and that most of those transcripts ~85% originated on the W chromosome. LTR retrotransposons occupied a similar proportion of genomic sequence as LINE elements on the autosomes and Z chromosome, but they were the most dominant type of transposable element on the W chromosome. Besides the measured densities of the transcripts, it was also shown that W-linked TEs were significantly more expressed than TEs located on the other chromosomes. The proportions of the active transcription for the autosomal, Z-chromosomal and W-chromosomal TEs were ~6.4%, ~6.9% and ~23.9%, *figure 4*. Several autosomes also contained regions with a high density of transcribed TEs. However, these high-density regions were often near sub-telomeric areas, and the maximum density of transcribed TEs on any autosome was still lower than the average density of transcribed TEs on the W chromosome. Additionally, young transposable elements are often still intact and functional, retaining their ability to move within the genome [30]. The analysis of that study shows that a significant proportion of LTRs across all chromosome types are young (0%-1% divergence) and these young LTRs were highly transcribed in comparison with older LTRs (>10% divergence) on all three chromosomes. LINEs are primarily older (>10% divergence) and the transcription of LINEs does not appear to be changed by the age of the TE, *figure 5*. The abundance and the proportion of transcribed TEs can be used to get understanding in further research of regulatory control mechanisms and TE function. The outcome of this study suggest that the W-chromosome indeed is a source for potentially active transposable elements.

Research example 2 using the sequences of six different species [21]

Another study used protein mass-photometry and RNA-seq to analyze reference-quality genomes of six species spanning the avian Tree of Life from both Paleognathae (emu with homomorphic sex chromosomes) and Neognathae (Anna's hummingbird, chicken, kakapō, zebra finch, paradise crow with heteromorphic sex chromosomes), *figure 6* [21]. The results of that study showed that autosomes contained between 6% and 12% TEs on average, whereas the Z chromosome showed similar or slightly higher TE densities in a range from 5% to 17%. In contrast, the W chromosome had a significantly higher TE content, between 22% and 80%. Remarkably, the homomorphic W chromosome of the emu also had a higher TE content of 22% compared to its autosomes and Z chromosome 6.4% and 5.6%, respectively [21]. The TE landscape of the Z chromosome was more similar to that of the autosomes, both in abundance and types of TEs. Long interspersed elements (LINEs) from the Chicken Repeat 1 superfamily were dominant on autosomes and the Z chromosome, while endogenous retroviruses were the major component of the W chromosome, accounting for more than 50% of the chromosome [21]. Endogenous retroviruses are LTR-retrotransposons that originate from retrovirus integrations inherited through the germline. They exist primarily in two forms: full-length elements with terminal repeats (LTRs) flanking protein-coding genes essential for retrotransposition, and solo-LTRs, which result from homologous recombination between flanking LTRs. Only the full-length elements can undergo retrotransposition. For birds, the toxicity index was calculated by comparing the number of full-length ERVs (fl-ERVs) in diploid females to those in diploid males. Results showed that females with heteromorphic sex chromosomes had 20-90% more fl-ERVs than males [21]. Even emus, with mostly homomorphic sex chromosomes, had 7-16% more fl-ERVs in females than in males. This indicates that the non-recombining region of the W chromosome, regardless of its size, consistently accumulates a large number of new transposable elements.

In this study two indexes were determined to test the refugium theory and the toxic-W hypothesis, the refugium index and toxicity index as mentioned above. The refugium index, which represents the density of TE-derived base pairs on the sex-limited chromosome (SLC) relative to other chromosomes, and the toxicity index, which represents the number of intact TEs (full-length copies of

LTRs, LINEs, and DNA transposons) in the heterogametic sex compared to the other sex. Past research recently explored the theory of the toxic-Y hypothesis in vertebrates with both XY and ZW systems. This hypothesis lies contrary to the 'unguarded-X' hypothesis, which suggests that shorter lifespans in the heterogametic sex, are because of the expression of recessive mutations on X/Z chromosomes. Notably, reduced female lifespans have been observed in many bird species. Some studies used the relative sizes of Y and W chromosomes to X and Z as proxies for toxicity, assuming smaller sex-limited chromosomes are more repetitive [28]. While a strong correlation was found between Y chromosome size and lifespan in mammals, no such correlation was found for the W chromosome in birds. The authors of this study suggest that their toxicity index, which considers the load of intact and potentially active TEs, might be a better predictor of female lifespans [21]. When the six bird species are compared, it can be obviously seen that the emu and Anna's hummingbird had the lowest and highest toxicity indexes, respectively. This indicates the need for more research and measurements (more species and sequencing) to validate the theory behind the measurements.

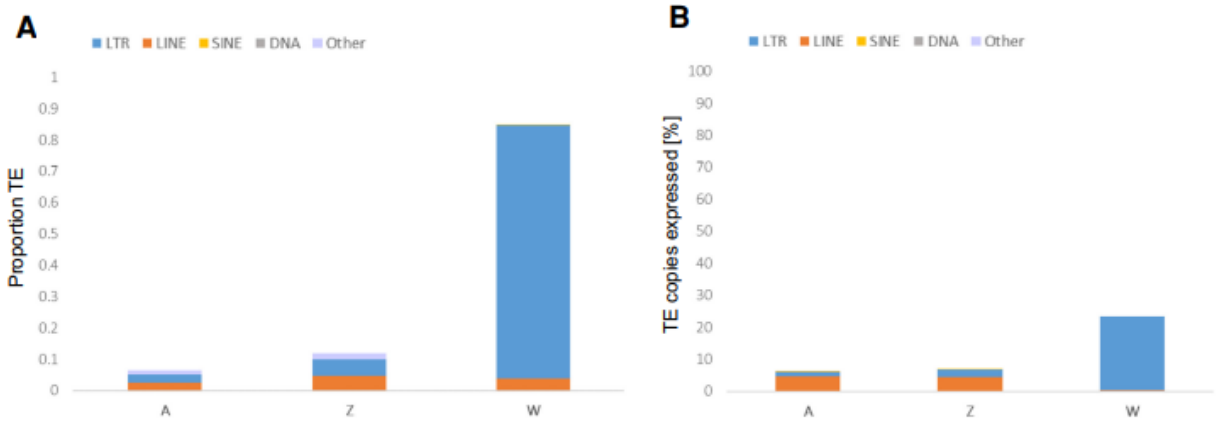

Figure 4: The abundance and transcprition of different type of TEs is shown in this figure of the hooded crow per chromosomal class (Autosomal, Z-chromosomal, W-chromosomal). ***A****. TE content is expressed as the proportion of TE sequences relative to the total sequence length.* ***B*** *The proportion of transcribed TEs relative to the total number of TE copies. [32]*

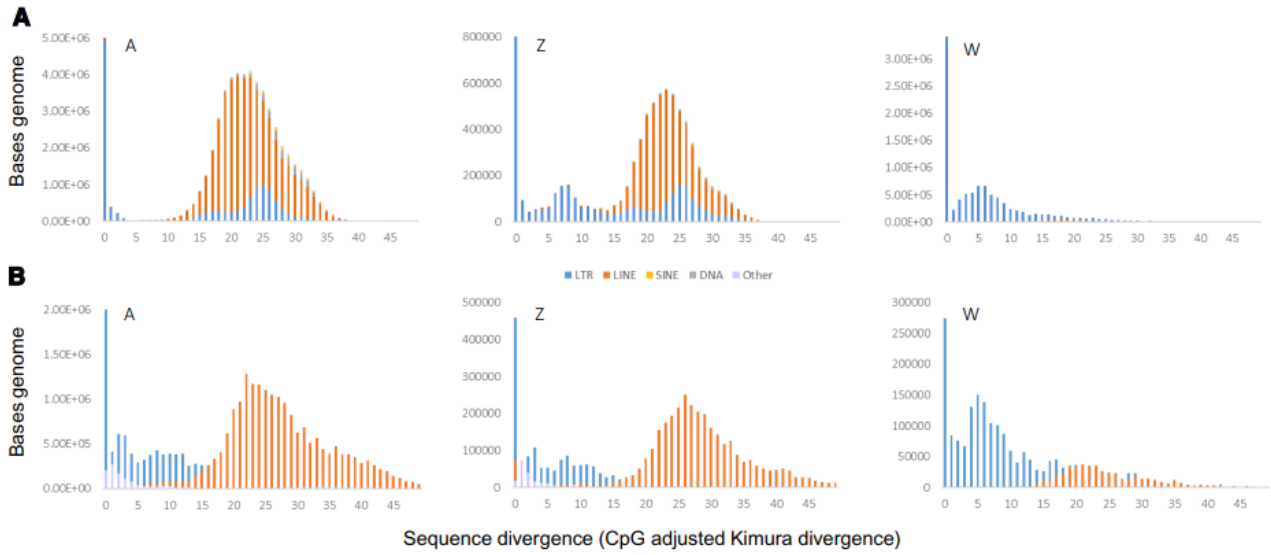

Figure 5: Frequency distribution of TE sequences that increases with the divergence on the X-as. The CpG adjusted Kimura divergence is a metric used to measure the evolutionary distance between DNA sequences. By

correcting for these CpG-specific mutation rates, the CpG adjusted Kimura divergence provides a more accurate estimate of the evolutionary changes in transposons, which helps in understanding their age, activity, and evolutionary dynamics in the genome. [32]

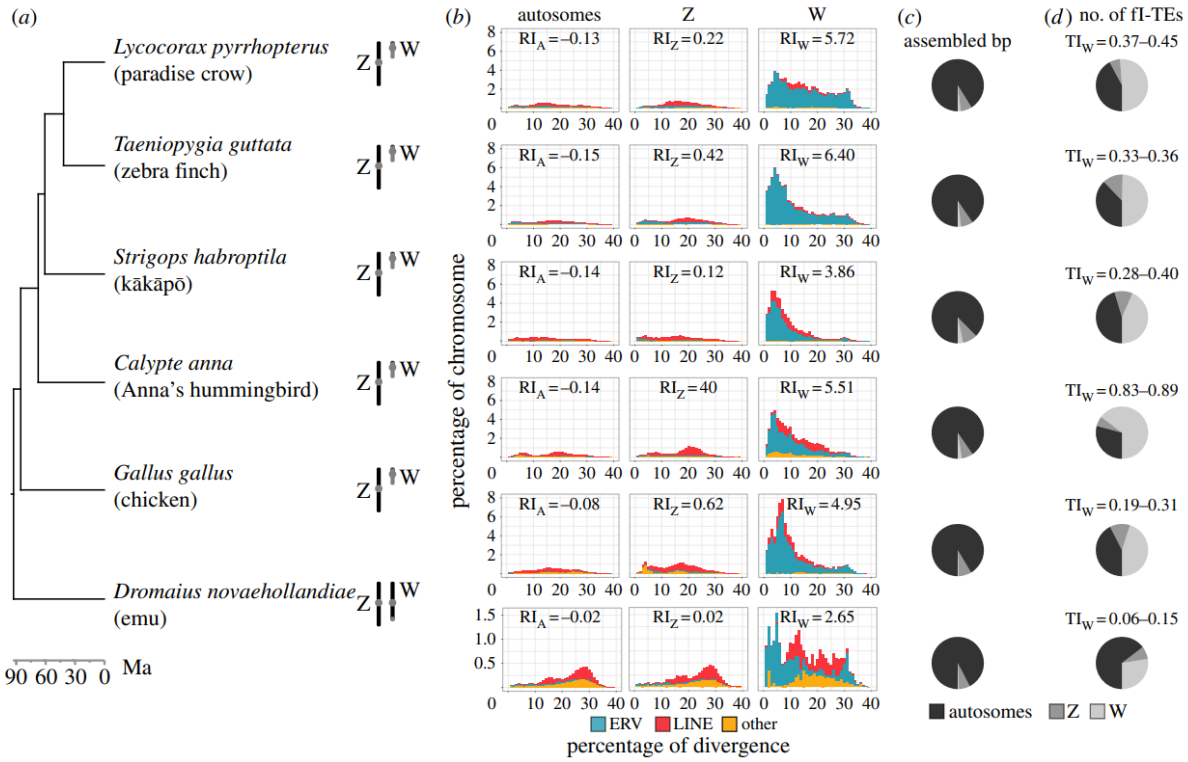

Figure 6: Avian Time Tree that shows the different abundances of ERV, LINE and 'other'transposons in six different species. Massive accumulation of ERVs on W chromosomes of six female reference-quality genome assemblies spanning the avian Tree of Life. [21]

Conclusion

The goal of this paper was to get a better understanding of the relation between TEs and the evolution of the W-chromosome. Therefore a few questions were formulated in the introduction to get some overview on this topic. Out of the findings in literature, it is obvious that the evolution of the W-chromosome is significantly influenced by the presence of TEs. To protect and maintain the crucial genes that play a role in certain biological functions including, cell cycle control, transcription and translation , the recombination rate went down and TEs could easier insert themselves. Because the TEs became more active and spread out over time, the transcripts of these TEs started to hinder the genome's defence mechanisms (CpG-methylation, chromatin alterations etc.), failing to prevent their colonization. Consequently, other processes were activated that could remove entire sections of the sex-limited chromosomes, leading to the observed shrinkage of both the W and Y chromosomes. The evolution of the sex chromosomes is sex-specific. While some species demonstrate a high tolerance for TE accumulation, others have evolved mechanisms to suppress TE activity. These variations are shaped by each species' unique evolutionary history and ecological niches.

Apart from the fact that diseases related to TEs are virtually only studied in humans, the point is that TEs can both negatively and positively impact the host fitness (parasites or symbionts). The effects of transposable elements safter they insert into the genome affect the overall structure, behaviour, and

function of chromosomes. These mutations can be very disruptive and have influence on the host fitness. When transposable elements insert into untranslated regions such as introns or areas upstream or downstream of genes, they can function as enhancers or promoters, thereby influencing the activity of nearby target genes. They may persist in a population as alleles when they aren't harmful, and through genetic drift, some may become common or even permanently fixed within a species. In this context, transposable elements act as biological inducers for biodiversity and can be considered 'symbionts' of the genome, playing a significant role in genomic evolution and diversity.

The evolutionary roles of W chromosomes and their interaction with transposable elements, are complex and not fully understood compared to the extensively studied Y chromosomes in mammals and flies. Research indicates that TEs on the W chromosome are not completely silenced, leading to ongoing TE activity in healthy adult tissues. This suggests that the mechanisms for silencing TEs are often incomplete, allowing these elements to remain active under certain conditions. The high densities of TEs on the W chromosome can lead to a significant mutational load in the heterogametic sex, as seen in Drosophila where the Y chromosome's incomplete silencing of TEs increases the mutational burden in males which may contribute to the male-specific aging. Both studies showed that the abundance of TEs is higher in females and that especially LTR-transposons are highly transcribed on the W-chromosome [21, 32]. They suggest that the W chromosome acts as a source for potentially active transposable elements. This can have significant evolutionary implications, such as contributing to genetic diversity and influencing reproductive isolation and speciation.

To conclude, insights from multiple species reveal diverse regulatory mechanisms controlling TE activity. Understanding these mechanisms across species is crucial, as the underlying processes and the impact of certain alterations in gene regulatory networks on W chromosome evolution remain largely unknown. Further research that studies the toxicity of TEs and the efficacy of various defense mechanisms against them, will provide valuable information about the evolutionary balance between genomic innovation and stability. This will also help predicting the different lifespans of the sexes in bird species.

Reference List

1. Bellott, Daniel W., et al. "Avian W and mammalian Y chromosomes convergently retained dosage-sensitive regulators." Nature genetics 49.3 (2017): 387-394.
2. Belancio, Victoria P., Dale J. Hedges, and Prescott Deininger. "LINE-1 RNA splicing and influences on mammalian gene expression." Nucleic acids research 34.5 (2006): 1512-1521.
3. Bhat, Audesh, et al. "Role of transposable elements in genome stability: implications for health and disease." International journal of molecular sciences 23.14 (2022): 7802.
4. Bojang Jr, Pasano, and Kenneth S. Ramos. "Epigenetic reactivation of LINE-1 retrotransposon disrupts Nu RD corepressor functions and induces oncogenic transformation in human bronchial epithelial cells." Molecular Oncology 12.8 (2018): 1342-1357.
5. Brown EJ, Nguyen AH, Bachtrog D. 2020. "The Y chromosome may contribute to sex-specific ageing in Drosophila." Nat Ecol Evol 4: 853–862. doi:10 .1038/s41559-020-1179-5
6. Rommel, Philipp C., et al. "RAG1/2 induces genomic insertions by mobilizing DNA into RAG1/2-independent breaks." *Journal of Experimental Medicine* 214.3 (2017): 815-831.
7. Chénais, Benoît. "Transposable elements and human diseases: mechanisms and implication in the response to environmental pollutants." International journal of molecular sciences 23.5 (2022): 2551.
8. Choi, Jae Young, and Yuh Chwen G. Lee. "Double-edged sword: The evolutionary consequences of the epigenetic silencing of transposable elements." PLoS genetics 16.7 (2020): e1008872.
9. Colonna Romano, Nunzia, and Laura Fanti. "Transposable elements: major players in shaping genomic and evolutionary patterns." Cells 11.6 (2022): 1048.
10. Cui, Jie, et al. "Low frequency of paleoviral infiltration across the avian phylogeny." *Genome biology* 15 (2014): 1-13..
11. Derks, Martijn FL, et al. "Gene and transposable element methylation in great tit (Parus major) brain and blood." Bmc Genomics 17 (2016): 1-13.
12. Dong Y, Huang Z, Kuang Q, Wen Z, Liu Z, Li Y, Yang Y, Li M. 2017. "Expression dynamics and relations with nearby genes of rat transposable elements across 11 organs, four developmental stages and both sexes." BMC Genomics 18: 666. doi:10.1186/s12864-017-4078-7
13. Dupressoir, Anne, Christian Lavialle, and T. Heidmann. "From ancestral infectious retroviruses to bona fide cellular genes: role of the captured syncytins in placentation." Placenta 33.9 (2012): 663-671.
14. Kapusta A, Suh A. 2017. "Evolution of bird genomes—a transposon's-eye view." Ann. N.Y. Acad. Sci. 1389, 164–185. (doi:10.1111/nyas.13295)
15. Lin, Lianyu, Anupma Sharma, and Qingyi Yu. "Recent amplification of microsatellite-associated miniature inverted-repeat transposable elements in the pineapple genome." BMC Plant Biology 21 (2021): 1-13.
16. Mank JE. 2012. "Small but mighty: the evolutionary dynamics of W and Y sex chromosomes." Chromosome Res. 20, 21–33. (doi:10.1007/s10577-011-9251-2)
17. **Martykaauthor, Rafa, Ewa B. Sliwinska and Piotr Tryjanowskiauthor. "Evolutionary interaction between W/Y chromosome and transposable elements." (2015).**
18. Miki, Yoshio, et al. "Disruption of the APC gene by a retrotransposal insertion of L1 sequence in a colon cancer." Cancer research 52.3 (1992): 643-645.
19. Miura, Asuka, et al. "Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis." Nature 411.6834 (2001): 212-214.
20. Nicolau, Melody, Nathalie Picault, and Guillaume Moissiard. "The evolutionary volte-face of transposable elements: from harmful jumping genes to major drivers of genetic innovation." Cells 10.11 (2021): 2952.
21. **Peona, Valentina, et al. "The avian W chromosome is a refugium for endogenous retroviruses with likely effects on female-biased mutational load and genetic incompatibilities." Philosophical Transactions of the Royal Society B 376.1833 (2021): 20200186.**
22. Ponnikas, Suvi, et al. "Why do sex chromosomes stop recombining?." Trends in Genetics 34.7 (2018): 492-503.
23. Robertson, Keith D. "DNA methylation and human disease." Nature Reviews Genetics 6.8 (2005): 597-620.
24. Rodriguez-Martin, Carlos, et al. "Familial retinoblastoma due to intronic LINE-1 insertion causes aberrant and noncanonical mRNA splicing of the RB1 gene." Journal of human genetics 61.5 (2016): 463-466.
25. Rommel, Philipp C., et al. "RAG1/2 induces genomic insertions by mobilizing DNA into RAG1/2-independent breaks." Journal of Experimental Medicine 214.3 (2017): 815-831.
26. Smeds L et al. 2015 "Evolutionary analysis of the female-specific avian W chromosome." Nat. Commun. 6, 7330. (doi:10.1038/ncomms8330)
27. Sotero-Caio, Cibele G., et al. "Evolution and diversity of transposable elements in vertebrate genomes." Genome biology and evolution 9.1 (2017): 161-177.

28. Sultanova, Zahida, Philip A. Downing, and Pau Carazo. "Genetic sex determination and sex-specific lifespan in tetrapods–evidence of a toxic Y effect." bioRxiv (2020): 2020-03.
29. Teugels, Erik, et al. "De novo Alu element insertions targeted to a sequence common to the BRCA1 and BRCA2 genes." Human mutation 26.3 (2005): 284-284.
30. Vijay, Nagarjun, et al. "Evolution of heterogeneous genome differentiation across multiple contact zones in a crow species complex." Nature communications 7.1 (2016): 13195.
31. Wang, Ting, et al. "Species-specific endogenous retroviruses shape the transcriptional network of the human tumor suppressor protein p53." Proceedings of the National Academy of Sciences 104.47 (2007): 18613-18618.
32. Warmuth, Vera M., Matthias H. Weissensteiner, and Jochen BW Wolf. "Accumulation and ineffective silencing of transposable elements on an avian W Chromosome." Genome research 32.4 (2022): 671-681.
33. Wei, Kevin H-C., Lauren Gibilisco, and Doris Bachtrog. "Epigenetic conflict on a degenerating Y chromosome increases mutational burden in Drosophila males." Nature Communications 11.1 (2020): 5537.
34. Zhou, Wanding, et al. "DNA methylation enables transposable element-driven genome expansion." Proceedings of the National Academy of Sciences 117.32 (2020): 19359-19366.