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Reconstructing the Evolution of Vertebrate Sex Chromosomes

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ABSTRACT

Sex chromosomes and their evolution have captivated researchers since their discovery. For over 100 years, the dominant model of sex chromosome evolution has held that differentiated sex chromosomes, such as the X and Y chromosomes of mammals or the Z and W chromosomes of birds, evolved from ordinary autosomes, primarily through the degeneration of the sex-specific Y chromosome or W chromosome. At the same time, the sex chromosomes shared between sexes, the X chromosome and Z chromosome, are expected to remain essentially untouched. This model was based on limited cytogenetic and genetic data. Only in the last decade, with the advent of genomics, has the complete sequence of any sex chromosome pair become available. High quality finished sequences of the human and chimpanzee Y chromosomes, as well as the human X chromosome, have revealed sequence features unanticipated by the traditional model of sex chromosome evolution. Large, highly identical, tandem and inverted arrays of testis-expressed genes are major sources of innovation in gene content on sex-specific as well as sex-shared chromosomes. Accounting for the emergence of these ampliconic structures presents a challenge for future studies of sex chromosome evolution.

INTRODUCTION

Since the discovery of sex chromosomes, researchers have sought to explain the evolutionary forces that could produce a pair of chromosomes that differed between the sexes. During the twentieth century, the fields of classical genetics, evolutionary and population genetics, and cytology converged on a single explanation for the evolution of heteromorphic sex chromosomes: sex chromosomes evolved from autosomes primarily through the degeneration of the sex-specific Y or W chromosome, while the X or Z chromosome faithfully preserved the gene content of the ancestral autosome pair. X and Z chromosomes were museums; Y and W chromosomes were ruins, destined to be lost to the sands of time.

In the last ten years, genomics has revolutionized the study of evolution. Evolution changes the sequence of DNA molecules, and comparing DNA sequences allows us to reconstruct evolutionary events from the past. The availability of DNA sequences from multiple vertebrates has confirmed that the process of sex chromosome evolution envisioned by theorists has played out multiple times in the evolution of vertebrate sex chromosomes. However, complete, high-quality sequences of sex chromosomes have led to discoveries that were unanticipated by existing theory. Sex-specific chromosomes are not doomed to decay, but selection can act to preserve their gene content over long time scales. Amplicons, massive and highly identical arrays of duplicated genes, are sources of innovation in gene content on sex-specific as well as sex-shared chromosomes. These arrays consist of genes expressed exclusively or predominantly in the testis.

The unexpected results of genomic analyses have challenged long-standing assumptions about the evolution of sex chromosomes. It is now clear that sex chromosomes are subject to constant remodeling; they resemble Theseus' ship rather than museums or ruins. The dramatic nature of innovation in gene content on sex chromosomes presents major theoretical challenges for the field of sex chromosome evolution. What selective forces can generate ampliconic structures? What is the relationship between ampliconic genes and male reproduction? As more sex chromosome sequences become available, including those of multiple mammals as well as the Z and W chromosomes of birds, they will enhance our ability to address these questions.

THEORETICAL MODELS OF SEX CHROMOSOME EVOLUTION

The study of sex chromosome evolution shares its origin with that of genetics, in Thomas Hunt Morgan’s fly room at Columbia University. In 1913, Alfred Sturtevant produced the first genetic map, consisting of six sex-linked genes (Sturtevant 1913). The following year, his colleague, Calvin Bridges, combined Sturtevant's linear map of sex-linked genes with his own work on non-disjunction of sex chromosomes to demonstrate that Sturtevant's map was that of the X chromosome, and the chromosomes were the material of heredity (Bridges 1914). This suggested that sex chromosomes were not merely a sign, but instead the root cause of sexual dimorphism. The following year, a third member of Morgan's lab, Herman Muller, established the linkage of a gene with the fourth chromosome, the smallest Drosophila autosome (Muller 1914). With Muller’s publication, all Drosophila chromosomes, with the exception of the Y chromosome, had at least one known gene. This fact troubled Muller, who explained it with the first theory of sex chromosome evolution: the X chromosome and the Y chromosome evolved from an ordinary pair of autosomes, but the Y chromosome, unable to recombine in males, had accumulated deleterious mutations, eliminating all of its genes. This simple theory, that heteromorphic sex chromosomes evolve from autosomes through the decay of the sex-specific chromosome, has been fundamental to the study of sex chromosome evolution for nearly 100 years.

Muller's theory that heteromorphic sex chromosomes were the result of degradation of the sex-specific chromosome was corroborated by the lack of credible Y-linked phenotypes in humans. As was the case in Drosophila, the first traits mapped to a

human chromosome were mapped to the X chromosome (Morgan 1911a; Morgan 1911b; Wilson 1911). By the middle of the century, X-linked inheritance had been reported for dozens of traits, while only a handful of traits had been mapped to the Y chromosome (Stern 1957; McKusick 1962). In 1957, Curt Stern, another former student of Morgan's and the President of the American Society of Human Genetics, addressed the society's annual meeting (Stern 1957). Stern used his address to systematically debunk every reported case of Y-linkage in humans. Stern noted that no Y-linked trait had been discovered in experimental mammals, but cautioned investigators not to give up the search for Y-linked traits. Two years later it was discovered that the human and mouse Y chromosomes contained the male sex-determining gene (Ford et al. 1959; Jacobs and Strong 1959; Welshons and Russell 1959), but the reputation of the Y chromosome had been irreparably damaged. Apart from sex-determination, geneticists viewed the sex-specific Y chromosome as a “dud” (McKusick 1962).

The idea of the sex-specific chromosome as a degenerate autosome was not only in accord with the genetic data from flies and mammals; it could also account for the diverse sex determining mechanisms of vertebrates. Many vertebrate species have no sex chromosomes; in these species, sex is determined by an environmental cue such as temperature. Some species have homomorphic sex chromosomes. Homomorphic sex chromosomes are not cytologically distinguishable, but they can be revealed by experiments with artificially sex-reversed animals. Heteromorphic sex chromosomes of the type seen in Drosophila predominate in three vertebrate lineages: mammals, birds, and snakes. Susumu Ohno argued that these three states, the absence of sex chromosomes, homomorphic sex chromosomes, and heteromorphic sex chromosomes,

represented a continuum that revealed the evolutionary trajectory of the heteromorphic vertebrate sex chromosomes (Ohno 1967). Ohno conjectured that the common ancestor of vertebrates possessed no sex chromosomes, but that in some lineages, a mutation had arisen which caused an ordinary pair of autosomes to behave as homomorphic sex chromosomes, and after this event, the sex-specific chromosome decayed producing heteromorphic sex chromosomes like those of mammals, birds, and snakes.

Ohno also modified Muller's theory to account for differences in recombination between Drosophila and vertebrates. Muller's theory relied on the absence of crossing over between homologous chromosomes in Drosophila males to automatically isolate any Y chromosome from crossing over, but because recombination occurs in both sexes in vertebrates, the sex-specific chromosomes of vertebrates would not spontaneously begin to degenerate. After the emergence of a new sex-determining gene in a vertebrate, a second event is required to suppress crossing over. Ohno proposed that a pericentric inversion on the sex-specific chromosome that encompassed the region of the sex determining gene could suppress crossing over between sex chromosomes in the heterogametic sex (Ohno 1967). If crossing over occurs within the boundaries of a pericentric inversion, the recombinant chromosomes will be duplicated in part of the inversion and deficient in the other; if essential genes fall within the boundaries of the inversion, recombinant progeny will die and only those whose sex chromosomes that did not recombine will survive. Once the sex-specific Y chromosome or W chromosome was isolated, it would begin to diverge from the shared X chromosome or Z chromosome by losing its gene content as Muller had predicted.

As the study of population genetics emerged, it became clear that Muller's explanation for the degeneration of the sex-specific chromosome was inadequate. Inspired by his work on chromosomes carrying balanced lethal mutations, Muller initially proposed that a lack of crossing over was sufficient to lead to genetic decay. Each chromosome in a pair carrying balanced lethal mutations exists only in the heterozygous state; recessive mutations on one chromosome are not exposed to selection so long as the other chromosome maintains the ancestral allele. Thus, both chromosomes can accumulate complementary recessive mutations. Muller believed that Y and W chromosomes, held in a heterozygous state by linkage to the sex determining locus, would be sheltered from selection by their partner, while X and Z chromosomes were exposed to selection against recessive mutations in the homogametic sex (Muller 1918). Fisher demonstrated that this explanation could not account for the degeneration of the sex-specific chromosome, because mutation must affect incipient sex chromosomes equally (Fisher 1935). If an X-linked or Z-linked gene suffered a loss of function, the result would be selection against a parallel loss of function in the Y-linked or W-linked counterpart. Fisher showed that for an infinite population, degeneration of the type Muller described could only occur if the mutation rate is much higher on the sex-specific chromosome than in the rest of the genome. In light of this difficulty, it was necessary to modify Muller's theory to explain why only the sex-specific chromosome was subject to degeneration.

Although Muller's initial explanation for the degeneration of the sex-specific chromosome proved inadequate, population genetic theories designed to explain the benefits of sex and recombination became the source of alternative models which could

account for the degeneration of a non-recombining chromosome. Muller proposed that genetic drift could account for the degeneration of non-recombining chromosomes through a mechanism which is now known as "Muller's ratchet" (Muller 1964; Felsenstein 1974). Muller’s ratchet is the idea that, in the absence of crossing over, a population cannot generate chromosomes with a smaller mutational load than those that currently exist within the population. If the least-mutated class of chromosomes is lost to drift, it is replaced by one that carries more mutations, and the 'ratchet' has clicked irreversibly towards the decay of the non-recombining chromosome.

Alternative models of degeneration rely on the absolute linkage between all the sites on a non-recombining chromosome. Selection at one site interferes with selection at linked sites, preventing the efficient elimination of deleterious mutations and slowing the spread of beneficial mutations (Felsenstein 1974). Strongly beneficial mutations can sweep through a population, dragging many weakly deleterious mutations along with them (Genetic Hitchhiking) (Maynard Smith and Haigh 1974; Rice 1987); chromosomes with strongly deleterious alleles will be lost from the population before they can spread, increasing the chances that weakly deleterious alleles will become fixed by drift (Background Selection) (Charlesworth et al. 1993; Charlesworth 1994). Both of these models predict reductions in the effective population size of a non-recombining chromosome, increasing the effects of genetic drift (Charlesworth 1978). Thus, both genetic hitchhiking and background selection should act synergistically with Muller's ratchet to hasten the degeneration of a non-recombining chromosome (Charlesworth 1978; Bachtrog 2008).

Theoretical models of sex chromosome evolution based on population genetics implicitly assumed that the sex-shared X chromosome and Z chromosome were unchanging; Susumu Ohno codified this as an explicit prediction. Ohno predicted that the X chromosome and Z chromosome should preserve the gene content of the ancestral autosome pair from which they evolved (Ohno 1967). As a corollary, the sex chromosomes of species that share a common origin are expected to share the same ancestral gene content. This concept is now most familiar as "Ohno's Law," that genes that are X-linked in one mammal should be X-linked in all others, but Ohno applied his predictions equally to the Z chromosomes of birds and snakes. Ohno and others reasoned that the degeneration of the sex-specific chromosome would result in the evolution of dosage compensation on the sex chromosome shared between the sexes (Ohno 1967; Charlesworth 1978; Jegalian and Page 1998). Once genes were lost from the sex-specific chromosome, the heterogametic sex would only have half the original dose of X-linked genes (Ohno 1967; Charlesworth 1978; Jegalian and Page 1998). A system of dosage compensation would evolve to provide males with the correct expression level for X-linked genes (Ohno 1967; Charlesworth 1978; Jegalian and Page 1998). Ohno argued that autosomal genes could not be added to the X chromosome because they would be expressed at too low a level in males, and X-linked genes could not move to autosomes because they were dependent on the dosage compensation mechanism for proper expression (Ohno 1967). Thus, while Y chromosomes and W chromosomes were subject to drastic changes in gene content, X chromosomes and Z chromosomes were locked into stably retaining their ancestral genes.

EVOLUTIONARY STRATA: RECONSTRUCTING THE DEGENERATION OF SEX-SPECIFIC CHROMSOMES

As DNA sequences from vertebrate sex chromosomes became available, researchers interpreted them in the context of the theories built on Muller's ideas. Pairing and crossing over between the human X and Y chromosomes at meiosis implied that some vestige of the original autosomal homology between them remained (Solari and Tres 1970; Rasmussen and Holm 1978). This suspicion was confirmed by the discovery of pseudoautosomal genes on the mammalian X chromosome and Y chromosome (Cooke et al. 1985; Simmler et al. 1985; Goodfellow et al. 1986). The first sequence map of the Y chromosome showed that even outside the PAR, the human X chromosome and Y chromosome carried homologous genes (Foote et al. 1992; Vollrath et al. 1992). The sequence of these Y-linked genes, when compared to the sequence of their X-linked homologs, revealed a pattern that suggested a pathway for X-Y evolution (Lahn and Page 1999b). Nucleotide divergence between X-linked and Y-linked gene copies was strongly correlated with the position of the X-linked gene copy, such that X-Y pairs formed several groups of increasing divergence from the short arm to the long arm of the X chromosome. Bruce Lahn likened the surviving gene pairs to fossils preserved in layers of stone from different periods in the past, and christened these groups "evolutionary strata." Each stratum contains genes isolated from recombination by the same event, thus the genes share similar levels of divergence. Lahn postulated at least four inversion events on the Y chromosome to account for his observations, in accordance with Ohno's

prediction that inversion events would initiate Y chromosome divergence and that the X chromosome would remain untouched.

Subsequent work on the human X chromosome and the chicken Z and and W chromosomes provided further evidence for the degeneration of the sex-specific chromosome. The finished sequence of the human X chromosome was presented as a foil for the Y chromosome, revealing further details of Y chromosome degeneration (Ross et al. 2005). Ross and colleagues confirmed the existence of the strata identified by Lahn, and identified an additional, more recent stratum. As was the case for the X chromosomes and Y chromosomes of mammals, the first sequence data from the chicken sex chromosomes showed that the Z chromosome and the W chromosome shared genes, suggesting that they too had evolved from a homologous pair of autosomes (Fridolfsson et al. 1998). As more W-linked genes were identified, Handley and colleagues compared them to their Z-linked homologs, and identified strata (Handley et al. 2004). The sex-specific Y chromosome and W chromosome evolved from autosomes along the same pathway of progressive isolation from recombination followed by degeneration.

CONSERVATION, RECOMBINATION, AND INNOVATION ON THE Y CHROMOSOME

The finished sequence of the human Y chromosome, published almost ninety years after Muller's original paper anticipating the degeneration of the non-recombining sex chromosome, represented the first sequence of any sex-specific chromosome (Skaletsky et al. 2003). The human Y chromosome sequence was assembled from individual BAC (Bacterial Artificial Chromosome) clones from a single man’s Y chromosome, allowing a greater degree of completeness in repetitive regions than has been achieved for other human chromosomes (Skaletsky et al. 2003). This effort enabled genomic comparisons that could, for the first time, rigorously test theoretical predictions of the course of sex chromosome evolution. While it was clear that the human X chromosome and Y chromosome had evolved from autosomes, unanticipated findings called into question some of the core assumptions of sex chromosome evolutionary theory. The human Y chromosome appeared to be a mosaic of different sequence classes that had different evolutionary trajectories (Skaletsky et al. 2003). The divergence evident in X-degenerate sequences had defined the evolutionary strata, but subsequent work would show that selection was more effective at preserving the surviving genes from degeneration than had been anticipated. The Y chromosome also gained genes in X-transposed and ampliconic sequences; these sequences demonstrated that Y chromosomes evolved not only by degeneration, but also by growth and elaboration.

Nearly half of the human Y chromosome is composed of X-degenerate sequences that contain genes that have survived the stepwise process of Y degeneration from the

ancestral autosome pair that gave rise to the X chromosome and Y chromosome (Skaletsky et al. 2003). The X-degenerate portion of the Y chromosome has unquestionably lost most genes that were present on the ancestral autosome pair; only 16 single-copy genes have survived out of the hundreds which are inferred to have been present on the ancestor of the X and Y chromosomes (Skaletsky et al. 2003). This has led to prominent claims that the Y chromosome is decaying at such a rapid pace that it will be devoid of genes in 10 million years (Aitken and Graves 2002). However, there is abundant evidence the Y chromosome will not "self-destruct" any time soon. Rozen and colleagues examined variation in these surviving genes across a panel of 105 men representing worldwide Y chromosome diversity (Rozen et al. 2009). They discovered that there is remarkably little variation in X-degenerate protein coding sequences -- on average, two randomly chosen Y chromosomes differ by only a single amino acid change (Rozen et al. 2009). They found that both nucleotide diversity and the proportion of variant sites are higher for silent substitutions than for substitutions which would lead to amino acid changes, implying that natural selection has operated effectively to preserve the coding sequences of the X-degenerate genes during human history (Rozen et al. 2009). Non-recombining sequences can be stable over even longer time scales. Hughes and colleagues systematically compared the human X-degenerate genes to those of the chimpanzee. They found that the human Y has preserved all X-degenerate genes that were present in the common ancestor of humans and chimps (Hughes et al. 2005). Thus, the X degenerate sequences of the human Y chromosome have been stable for at least the past 6 million years.

The sequence of the human Y chromosome showed that not only has the human Y avoided destruction, but it is also undergoing growth and innovation in gene content. The rest of the human Y chromosome is composed of two sequence classes, X-transposed and ampliconic, many of whose genes have been added to the Y chromosome since it began to diverge from the X (Skaletsky et al. 2003). After the divergence of humans and chimpanzees, a transposition event restored a block of two-single copy X-transposed genes to the human Y chromosome (Skaletsky et al. 2003). Ampliconic sequences form highly identical (>99.9% nucleotide identity) tandem arrays and inverted repeats that could only be resolved by BAC-based finishing strategies. The largest was a nearly perfect palindrome almost three megabases across (Kuroda-Kawaguchi et al. 2001; Skaletsky et al. 2003). The ampliconic portion of the Y chromosome contains nine multi-copy gene families, totaling approximately 60 transcription units (Skaletsky et al. 2003). Two gene families are survivors of Y chromosome decay that have become amplified, while others appear to have moved to the Y chromosome from autosomes (Saxena et al. 1996; Lahn and Page 1999a; Skaletsky et al. 2003). All of these genes are expressed in the testis (Skaletsky et al. 2003), and deletions in these sequences are the most common known genetic cause of spermatogenic failure in humans (Kuroda-Kawaguchi et al. 2001; Repping et al. 2002; Repping et al. 2003). Muller's theory did not predict the existence of this crucial part of the Y chromosome.

Further characterization of mammalian Y chromosomes demonstrated that ampliconic sequences represent a major exception to Muller's theory. The high nucleotide identity between the genes in palindromes on the human Y chromosome could be interpreted as evidence that the ampliconic sequences evolved relatively recently in

human evolution, within the last 100,000 years. However, Rozen and colleagues used comparative sequencing in great apes to show that at least six of the eight human Y chromosome palindromes predate the divergence of chimpanzees and humans over six million years ago (Rozen et al. 2003). To explain this result, they hypothesized that the arms of these palindromes must engage in gene conversion, driving the paired arms to evolve in concert. They confirmed this by surveying the diversity of human Y chromosomes to capture instances of gene conversion within the human lineage (Rozen et al. 2003). Muller and others had assumed that the Y chromosome could not engage in recombination and would inevitably decay, but gene conversion allows for productive recombination between palindrome arms as though they were two alleles on homologous autosomes (Rozen et al. 2003; Skaletsky et al. 2003). This has allowed the ampliconic genes of the Y chromosomes to survive and expand during primate evolution while many single-copy genes have decayed.

Not only are ampliconic regions capable of recombination, this recombination results in the continual remodeling of Y chromosome sequence. Since ampliconic regions are, by definition, highly identical sequences in tandem or inverted repeats, they are prone to rearrangements that lead to variations in copy number as well as inversions. Repping and colleagues surveyed a panel of diverse Y chromosomes and observed extensive structural variation among human Y chromosomes (Repping et al. 2006). Using the phylogentic tree of human Y chromosomes, they were able to place a lower bound on the rate of rearrangements; most rearrangements occur on the order of 10^{-4} events per father-to-son transmission (Repping et al. 2006). This high rate of rearrangement causes the structure of ampliconic sequences to evolve much more rapidly than X-degenerate

sequences. Hughes and colleagues found that only 6 of 9 ampliconic gene families are conserved between humans and chimpanzees, and chimpanzee ampliconic sequences have experienced many more rearrangements than the X-degenerate sequences, producing a completely different structure (Hughes et al. 2010). Unlike the X-degenerate regions of the Y, the ampliconic regions are a source of continual growth and change.

INNOVATION ON THE X CHROMOSOME

Although the finished sequence of the human Y chromosome led to discoveries that challenged the traditional model of the Y chromosome as a rotting autosome by showing growth and change on the Y chromosome, it also reinforced the view of the X chromosome as unchanging. Muller's theory predicts that the decay of genes on Y chromosomes and W chromosomes constrains X chromosomes and Z chromosomes to stably maintain the gene content of the autosomes from which they evolved. In formulating Ohno's Law, Ohno reasoned that an elaborate chromosome-wide mechanism of dosage compensation would also stabilize the gene content of X chromosomes and Z chromosomes, since genes which translocated to or from an X chromosome or Z chromosome would become misregulated (Ohno 1967). As a result, most genomic studies have treated the X chromosome as a control to show the dramatic changes on the Y chromosome, leaving the question of changes in X chromosome gene content unexamined. Only comparisons among X chromosomes or between X chromosomes and the autosomes of other species can test whether the gene content of the X chromosome has changed through the course of X chromosome evolution.

Initial comparisons of X chromosomes and Z chromosomes among species have generally supported Muller and Ohno's predictions of conservation. Comparative mapping experiments have repeatedly shown that the genes of the X chromosome are well conserved among placental mammals (O'Brien et al. 1993; Carver and Stubbs 1997; Chowdhary et al. 1998; Ross et al. 2005). While mammalian X chromosomes have experienced a number of rearrangements, particularly in the rodent lineage, over the

course of mammalian evolution they have sustained fewer interchromosomal translocations than mammalian autosomes (Carver and Stubbs 1997). Outside of mammals, comparative mapping of Z-linked genes in birds by FISH has indicated that the Z chromosome is conserved among avian species (Nanda et al. 2008). Similar results have been reported in comparisons of several snake species (Matsubara et al. 2006). Because comparative mapping experiments are designed to locate the orthologs of the genes from one species on the chromosomes of another, the results of these experiments are biased towards finding conservation rather than novelty.

In line with the predictions of Ohno's law, PARs (pseudoautosomal regions have not been as well-conserved as the rest of the X chromosome. Several genes in the mammalian PAR have moved from the PAR to autosomes in mice (Palmer et al. 1995; Carver and Stubbs 1997). Wilcox and colleagues examined the locations of human X-linked genes in marsupials, and monotremes (Wilcox et al. 1996). They discovered that the genes composing the short arm of the human X were present on the autosomes of monotremes and marsupials (Wilcox et al. 1996). This gene traffic to and from the mammalian X chromosome seems like a violation of Ohno's law, but is actually in accord with Ohno's predictions. The region added to the X in eutherian mammals falls into the three most recent strata of the human sex chromosomes; when it translocated to the ancestral eutherian X chromosome, it was added to the PAR, and shared with the Y chromosome. Because PARs still participate in crossing over, Y-linked gene copies do not decay and the X-linked copies are not subject to dosage compensation. The genes in PAR are free to move between autosomes and the sex chromosomes until they are locked

in by an event that expands the region of suppressed recombination between the sex chromosomes.

Even outside of the PARs, the gene content of the mammalian X chromosome is not completely stable. Genomic data from human and mouse have allowed researchers to systematically identify gene movement to and from the mammalian X chromosome. Emerson and colleagues found that the mouse and human X chromosomes have both generated and received an excess of genes through retrotransposition (Emerson et al. 2004). By comparing the human and mouse X chromosomes, they found that this process began before humans and mice diverged, and has continued after that divergence in both lineages. Mammalian X chromosomes have also gained genes through the duplication of existing X-linked genes. Warburton and colleagues found that the human X chromosome is enriched for amplicons that contain testis-expressed genes (Warburton et al. 2004). These X chromosome amplicons primarily contain the cancer-testis antigen (CTA) genes. Comparative studies have shown that several CTA gene families expanded in the primate lineage (De Backer et al. 1999; Aradhya et al. 2001; Kouprina et al. 2004). Other CTA gene families, including the MAGE genes, the most abundant gene family on the human X chromosome, have independently expanded in both rodent and primate lineages (Chomez et al. 2001; Chen et al. 2003; Birtle et al. 2005; Ross et al. 2005). Mueller and colleagues found that the mouse X chromosome contained 33 multi-copy gene families, which, like human CTA genes, are expressed in the testis (Mueller et al. 2008). These multi-copy families were arranged in elaborate ampliconic structures covering 19 megabases of the mouse X chromosome (Mueller et al. 2008). Just as ampliconic gene

families are a source of unexpected novel gene content on mammalian Y chromosomes, they are a source of innovation on X chromosomes as well.

Contrary to the expectations of Muller's theory and Ohno's Law, recent research has shown that the gene content of X chromosomes is not static. On the one hand, conservation of gene content is observed throughout the majority of the mammalian X chromosome, where gene loss from the Y and the subsequent evolution of dosage compensation restrict the flow of genes off of and onto the X. On the other hand, PARs have been sites of gene movement to and from the X chromosome, the most dramatic being the X added region of placental mammals, which accounts for nearly the entire short arm of the human X chromosome. Even outside of PARs, retrotransposition and gene duplication have reshaped the gene content of mammalian X chromosomes, creating amplicons of testis-expressed genes parallel to those observed on mammalian Y chromosomes. The changes to X chromosomes are as impressive as their conservation.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

For nearly 100 years the evolution of sex chromosomes has been described in the context of Muller's theory that sex chromosomes evolve from autosomes through the degeneration of the sex-specific chromosome. This hypothesis accounts for nearly all the data that were available before the sequences of sex chromosomes were completed. However Muller's theory does not account for the degree to which gene movement and duplication have shaped the evolution of sex chromosomes. The ampliconic sequences of the human Y chromosome are essential for male fertility, and therefore for the continued survival of the Y chromosome, but they were unanticipated in Muller’s theory. Amplicons on X chromosomes represent unexpected innovations in gene content on what was presumed to be an unchanging chromosome. In the same way that the development of population genetics reshaped the description of Y degeneration under Muller's theory, it is necessary to amend Muller's hypothesis in light of genomic data.

A greater understanding of the forces that generate amplicons will result from a more complete description of their function. One possibility is that the high copy number of ampliconic genes reflects selection for increased expression. Ampliconic genes might be duplicated to facilitate high levels of transcription, as has been proposed for ribosomal RNAs, transfer RNAs, and histone genes (Finnegan et al. 1978; Kedes 1979; Long and Dawid 1980). The high frequency of transcription of mouse X ampliconic genes despite the general post-meiotic silencing of single-copy genes on the X chromosome would be consistent with this hypothesis. The universal expression of ampliconic genes in the testis

provides a second possible explanation: that repetitive DNA structures provide a chromatin environment that is permissive for gene expression in germ cells. As an alternative to hypotheses based on gene expression, amplicons may play a role in preserving functional gene copies in regions where crossing over with a homologous chromosome rarely, if ever, occurs. The amplicons on the Y chromosome of primates engage in gene conversion, providing a mechanism to preserve the function of genes in the face of chromosome-wide degradation. Ideally, a unified theory would explain why amplicons are more prevalent on sex chromosomes than in the rest of the genome, but it is possible that amplicons are present on different sex chromosomes for different reasons.

Escape from post-meiotic silencing on sex chromosomes could serve as a compelling explanation for the location of amplicons in mammals, but silencing of sex chromosomes is far from universal. Unlike XY male mammals, ZW female birds do not appear to silence unpaired chromosomes during meiosis (Solari 1977). During the diplotene stage of female meiosis, the Z chromosome and W chromosome of chickens are highly transcriptionally active, forming lamp-brush chromosomes (Hutchison 1987). If ampliconic sequences exist in birds, they will require an alternative explanation.

An alternative to the avoidance of meiotic silencing is that sex-linked amplicons are the result of sexually antagonistic selection. Sexually antagonistic genes are those that produce a phenotype which benefits one sex more than the other. These traits are more likely to become fixed on sex chromosomes than on autosomes because the sex chromosomes are not evenly exposed to selection in both sexes (Rice 1984). Male benefit genes should accumulate on Y chromosomes, and female benefit genes should accumulate on W chromosomes. The case for X chromosomes and Z chromosomes is

454 more complex. Dominant traits that benefit the homogametic sex should accumulate
455 because they are exposed to selection twice as often in the homogametic sex. Recessive
456 traits that benefit the heterogametic sex should accumulate because they are always
457 exposed to stronger selection in the heterogametic sex than in the homogametic sex,
458 where they can be masked by other alleles. Eventually sexually antagonistic genes are
459 expected to evolve sex-limited expression to avoid costs to the sex where they are not
460 beneficial (Rice 1984). As a result, one would expect to find that sex chromosomes
461 would become enriched for genes expressed only in one sex.

462 Sexually antagonistic selection is an attractive explanation for the enrichment of
463 amplicons on the sex chromosomes, but there are incongruities with the existing data.
464 There do not appear to be any female-benefit amplicons on X chromosomes, where they
465 might be expected to arise because the X chromosome is exposed to more frequent
466 selection in females than in males. All known ampliconic sequences, including those on
467 X chromosomes, are expressed in the testis. The presence of testis-expressed amplicons
468 on X chromosomes is striking because gene duplication was classically imagined as a
469 dominant gain of function mutation (Muller 1932), but the theory of sexually
470 antagonistic selection predicts that only recessive male-benefit alleles should accumulate
471 on X chromosomes. If sexually antagonistic selection is responsible for the generation of
472 testis-expressed amplicons, then gene duplication on the X chromosome may be preceded
473 by the evolution of male-limited expression, so that duplications are only subjected to
474 selection in males.

475 Amplicons could also be involved in intragenomic conflict through segregation
476 distortion in the germline. Autosomal segregation distortion due to the t-haplotype of

chromosome 17 in mice is well known (Silver 1993). On the sex chromosomes, a segregation-distorting locus could function as a sex ratio distorter. Since most organisms are constrained to a 1:1 sex ratio, any sex ratio distorter that meets with success immediately increases the selective advantage for a second distorter to restore the sex ratio to equilibrium (Fisher 1930; Nur 1974). This could lead to an evolutionary arms race between sex chromosomes. There are indications that the mouse X chromosome and Y chromosome are involved in segregation distortion; deletions on the long arm of the mouse Y chromosome lead to an excess of female offspring, suggesting that the multi-copy genes on the mouse Y chromosome may suppress X chromosome segregation distortion (Conway et al. 1994). If amplicons are primarily generated as a result of intragenomic conflict between the sex chromosomes, birds and snakes would be expected to accumulate genes that are expressed during female meiosis to influence the partition of the Z and W chromosomes between the oocyte and the first polar body (Rutkowska and Badyaev 2008).

In the past ten years, genomic data from vertebrate sex chromosomes have allowed reconstructions of the process of sex chromosome evolution, and these reconstructions have revealed surprising exceptions to Muller's theory. We can look forward to the availability of additional sex chromosome sequences that will enable us to extend our analyses of sex chromosomes. Sequencing efforts for several mammalian Y chromosomes are underway. These will allow us to extend our comparisons of Y chromosomes from the divergence of human populations through primate evolution, to the very base of the mammalian tree. The sequences of the chicken sex chromosomes will allow us to extend our evolutionary comparisons even further. The chicken sex

chromosomes have evolved independently of mammalian sex chromosomes for over 300 million years. As a result, the chicken sex chromosomes and the human sex chromosomes represent the outcome of two parallel experiments of nature. Reciprocal comparisons of the finished sequences of the chicken Z and human X chromosomes to the orthologous autosomal regions in the other species will enable us to trace changes that occurred on the Z chromosome and X chromosome during the course of sex chromosome evolution. Intra-specific comparisons between the finished sequences of the Z and W chromosomes will reveal whether the course of W evolution has been parallel to that of the degeneration and elaboration of the human Y chromosome. The description of ampliconic sequences on the W chromosome is also likely to be revealing. There are at least two multi-copy gene families on the W chromosome, but they are ubiquitously expressed and their genomic structure is unknown. W amplicons, if they exist, may show a functional coherence like that of the human Y, revealing genes that are essential for female fertility.

Additional insights on par with those obtained from the sequence of the human X and Y chromosomes can only come with additional high quality finished sequencing efforts. Ampliconic sequences could not have been described without the BAC-based, “clone-by-clone” methods used to determine the sequence of the human sex chromosomes. Shotgun sequencing technologies collapse highly identical repeats into single contigs, obscuring rather than revealing their structure and organization. This deficiency of shotgun methods only worsens with shorter read lengths. Only BAC-based sequencing provides the positional information needed to disentangle long repeats. While these BAC-based sequencing technologies are slower and more expensive than their

whole genome shotgun counterparts, they have resulted in insights that would have been impossible to obtain in any other way, and which were unanticipated by a century of theory.

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