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## *Reconstructing the Evolution of Vertebrate Sex Chromosomes*

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<b>Citation</b>	Bellott, D.W. and D.C. Page. "Reconstructing the Evolution of Vertebrate Sex Chromosomes." Cold Spring Harbor Symposia on Quantitative Biology, 2009. 74: 345-353.
<b>As Published</b>	<a href="http://dx.doi.org/10.1101/sqb.2009.74.048">http://dx.doi.org/10.1101/sqb.2009.74.048</a>
<b>Publisher</b>	Cold Spring Harbor Laboratory
<b>Version</b>	Author's final manuscript
<b>Citable link</b>	<a href="http://hdl.handle.net/1721.1/66577">http://hdl.handle.net/1721.1/66577</a>
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Reconstructing the Evolution of Vertebrate Sex Chromosomes

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10 ABSTRACT

11

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

28 INTRODUCTION

29

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

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

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

60 THEORETICAL MODELS OF SEX CHROMOSOME EVOLUTION

61

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

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

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

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

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

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

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

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

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

159         Alternative models of degeneration rely on the absolute linkage between all the  
160 sites on a non-recombining chromosome. Selection at one site interferes with selection at  
161 linked sites, preventing the efficient elimination of deleterious mutations and slowing the  
162 spread of beneficial mutations (Felsenstein 1974). Strongly beneficial mutations can  
163 sweep through a population, dragging many weakly deleterious mutations along with  
164 them (Genetic Hitchhiking) (Maynard Smith and Haigh 1974; Rice 1987); chromosomes  
165 with strongly deleterious alleles will be lost from the population before they can spread,  
166 increasing the chances that weakly deleterious alleles will become fixed by drift  
167 (Background Selection) (Charlesworth et al. 1993; Charlesworth 1994). Both of these  
168 models predict reductions in the effective population size of a non-recombining  
169 chromosome, increasing the effects of genetic drift (Charlesworth 1978). Thus, both  
170 genetic hitchhiking and background selection should act synergistically with Muller's  
171 ratchet to hasten the degeneration of a non-recombining chromosome (Charlesworth  
172 1978; Bachtrog 2008).

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

195 EVOLUTIONARY STRATA: RECONSTRUCTING THE DEGENERATION OF SEX-  
196 SPECIFIC CHROMSOMES

197

198           As DNA sequences from vertebrate sex chromosomes became available,  
199 researchers interpreted them in the context of the theories built on Muller's ideas. Pairing  
200 and crossing over between the human X and Y chromosomes at meiosis implied that  
201 some vestige of the original autosomal homology between them remained (Solari and  
202 Tres 1970; Rasmussen and Holm 1978). This suspicion was confirmed by the discovery  
203 of pseudoautosomal genes on the mammalian X chromosome and Y chromosome (Cooke  
204 et al. 1985; Simmler et al. 1985; Goodfellow et al. 1986). The first sequence map of the  
205 Y chromosome showed that even outside the PAR, the human X chromosome and Y  
206 chromosome carried homologous genes (Foote et al. 1992; Vollrath et al. 1992). The  
207 sequence of these Y-linked genes, when compared to the sequence of their X-linked  
208 homologs, revealed a pattern that suggested a pathway for X-Y evolution (Lahn and Page  
209 1999b). Nucleotide divergence between X-linked and Y-linked gene copies was strongly  
210 correlated with the position of the X-linked gene copy, such that X-Y pairs formed  
211 several groups of increasing divergence from the short arm to the long arm of the X  
212 chromosome. Bruce Lahn likened the surviving gene pairs to fossils preserved in layers  
213 of stone from different periods in the past, and christened these groups "evolutionary  
214 strata." Each stratum contains genes isolated from recombination by the same event, thus  
215 the genes share similar levels of divergence. Lahn postulated at least four inversion  
216 events on the Y chromosome to account for his observations, in accordance with Ohno's

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

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

232 CONSERVATION, RECOMBINATION, AND INNOVATION ON THE Y  
233 CHROMOSOME

234

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

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

255 ancestral autosome pair that gave rise to the X chromosome and Y chromosome  
256 (Skaletsky et al. 2003). The X-degenerate portion of the Y chromosome has  
257 unquestionably lost most genes that were present on the ancestral autosome pair; only 16  
258 single-copy genes have survived out of the hundreds which are inferred to have been  
259 present on the ancestor of the X and Y chromosomes (Skaletsky et al. 2003). This has led  
260 to prominent claims that the Y chromosome is decaying at such a rapid pace that it will  
261 be devoid of genes in 10 million years (Aitken and Graves 2002). However, there is  
262 abundant evidence the Y chromosome will not “self-destruct” any time soon. Rozen and  
263 colleagues examined variation in these surviving genes across a panel of 105 men  
264 representing worldwide Y chromosome diversity (Rozen et al. 2009). They discovered  
265 that there is remarkably little variation in X-degenerate protein coding sequences -- on  
266 average, two randomly chosen Y chromosomes differ by only a single amino acid change  
267 (Rozen et al. 2009). They found that both nucleotide diversity and the proportion of  
268 variant sites are higher for silent substitutions than for substitutions which would lead to  
269 amino acid changes, implying that natural selection has operated effectively to preserve  
270 the coding sequences of the X-degenerate genes during human history (Rozen et al.  
271 2009). Non-recombining sequences can be stable over even longer time scales. Hughes  
272 and colleagues systematically compared the human X-degenerate genes to those of the  
273 chimpanzee. They found that the human Y has preserved all X-degenerate genes that  
274 were present in the common ancestor of humans and chimps (Hughes et al. 2005). Thus,  
275 the X degenerate sequences of the human Y chromosome have been stable for at least the  
276 past 6 million years.

277           The sequence of the human Y chromosome showed that not only has the human Y  
278 avoided destruction, but it is also undergoing growth and innovation in gene content. The  
279 rest of the human Y chromosome is composed of two sequence classes, X-transposed and  
280 ampliconic, many of whose genes have been added to the Y chromosome since it began  
281 to diverge from the X (Skaletsky et al. 2003). After the divergence of humans and  
282 chimpanzees, a transposition event restored a block of two-single copy X-transposed  
283 genes to the human Y chromosome (Skaletsky et al. 2003). Ampliconic sequences form  
284 highly identical (>99.9% nucleotide identity) tandem arrays and inverted repeats that  
285 could only be resolved by BAC-based finishing strategies. The largest was a nearly  
286 perfect palindrome almost three megabases across (Kuroda-Kawaguchi et al. 2001;  
287 Skaletsky et al. 2003). The ampliconic portion of the Y chromosome contains nine multi-  
288 copy gene families, totaling approximately 60 transcription units (Skaletsky et al. 2003).  
289 Two gene families are survivors of Y chromosome decay that have become amplified,  
290 while others appear to have moved to the Y chromosome from autosomes (Saxena et al.  
291 1996; Lahn and Page 1999a; Skaletsky et al. 2003). All of these genes are expressed in  
292 the testis (Skaletsky et al. 2003), and deletions in these sequences are the most common  
293 known genetic cause of spermatogenic failure in humans (Kuroda-Kawaguchi et al. 2001;  
294 Repping et al. 2002; Repping et al. 2003). Muller's theory did not predict the existence of  
295 this crucial part of the Y chromosome.

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

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

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

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

328 INNOVATION ON THE X CHROMOSOME

329

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

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

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

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

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

375         Even outside of the PARs, the gene content of the mammalian X chromosome is  
376 not completely stable. Genomic data from human and mouse have allowed researchers to  
377 systematically identify gene movement to and from the mammalian X chromosome.  
378 Emerson and colleagues found that the mouse and human X chromosomes have both  
379 generated and received an excess of genes through retrotransposition (Emerson et al.  
380 2004). By comparing the human and mouse X chromosomes, they found that this process  
381 began before humans and mice diverged, and has continued after that divergence in both  
382 lineages. Mammalian X chromosomes have also gained genes through the duplication of  
383 existing X-linked genes. Warburton and colleagues found that the human X chromosome  
384 is enriched for amplicons that contain testis-expressed genes (Warburton et al. 2004).  
385 These X chromosome amplicons primarily contain the cancer-testis antigen (CTA) genes.  
386 Comparative studies have shown that several CTA gene families expanded in the primate  
387 lineage (De Backer et al. 1999; Aradhya et al. 2001; Kouprina et al. 2004). Other CTA  
388 gene families, including the MAGE genes, the most abundant gene family on the human  
389 X chromosome, have independently expanded in both rodent and primate lineages  
390 (Chomez et al. 2001; Chen et al. 2003; Birtle et al. 2005; Ross et al. 2005). Mueller and  
391 colleagues found that the mouse X chromosome contained 33 multi-copy gene families,  
392 which, like human CTA genes, are expressed in the testis (Mueller et al. 2008). These  
393 multi-copy families were arranged in elaborate ampliconic structures covering 19  
394 megabases of the mouse X chromosome (Mueller et al. 2008). Just as ampliconic gene

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

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

408

409 CURRENT CHALLENGES AND FUTURE DIRECTIONS

410

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

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

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

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

447       An alternative to the avoidance of meiotic silencing is that sex-linked amplicons  
448 are the result of sexually antagonistic selection. Sexually antagonistic genes are those that  
449 produce a phenotype which benefits one sex more than the other. These traits are more  
450 likely to become fixed on sex chromosomes than on autosomes because the sex  
451 chromosomes are not evenly exposed to selection in both sexes (Rice 1984). Male benefit  
452 genes should accumulate on Y chromosomes, and female benefit genes should  
453 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

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

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

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

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

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

526

#### 527 ACKNOWLEDGEMENTS

528 We thank H. Skaletsky, J. Hughes, J. Mueller, A. Larracuenta, S. Soh, and K. Romer for  
529 comments on the manuscript. Our work is supported by the National Institutes of Health  
530 and the Howard Hughes Medical Institute.

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