DOI: 10.1534/genetics.107.072033

Bird and Mammal Sex-Chromosome Orthologs Map to the Same Autosomal Region in a Salamander (Ambystoma)

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Manuscript received February 12, 2007
Accepted for publication June 26, 2007

ABSTRACT

We tested hypotheses concerning the origin of bird and mammal sex chromosomes by mapping the location of amniote sex-chromosome loci in a salamander amphibian (Ambystoma). We found that ambystomatid orthologs of human X and chicken Z sex chromosomes map to neighboring regions of a common Ambystoma linkage group 2 (ALG2). We show statistically that the proportion of human X and chicken Z orthologs observed on ALG2 is significantly different from the proportion that would be expected by chance. We further show that conserved syntenies between ALG2 and amniote chromosomes are identified as overlapping conserved syntenies when all available chicken ($N = 3120$) and human ($N = 14{,}922$) RefSeq orthologs are reciprocally compared. In particular, the data suggest that chromosomal regions from chicken chromosomes (GGA) Z and 4 and from human chromosomes (HSA) 9, 4, X, 5, and 8 were linked ancestrally. A more distant outgroup comparison with the pufferfish *Tetraodon nigroviridis* reveals ALG2/GGAZ/HSAX syntenies among three pairs of ancestral chromosome duplicates. Overall, our results suggest that sex chromosomal regions of birds and mammals were recruited from a common ancestral chromosome, and thus our findings conflict with the currently accepted hypothesis of separate autosomal origins. We note that our results were obtained using the most immediate outgroup to the amniote clade (mammals, birds, and other reptiles) while the currently accepted hypothesis is primarily based upon conserved syntenies between in-group taxa (birds and mammals). Our study illustrates the importance of an amphibian outgroup perspective in identifying ancestral amniote gene orders and in reconstructing patterns of vertebrate sex-chromosome evolution.

A classic problem in evolution concerns the origin of sex chromosomes among amniote vertebrates (Ohno 1967). In mammals, females have two identical (XX) sex chromosomes while males have an X and Y (XY). In contrast, birds have a ZZ–ZW determination system wherein females are the heterogametic sex (ZW). The mammalian Y and chicken W chromosomes are conspicuously smaller than their X and Z counterparts and they contain fewer loci. Presumably, these sex-chromosome homologs have undergone extreme, divergent evolution since their recruitment as sex-determining factors, a pattern observed broadly among animals and plants (Ohno 1967; Bull 1983; Lahn *et al.* 2001; Ayling and Griffin 2002; Charlesworth and Charlesworth 2005; Charlesworth *et al.* 2005; Khil and Camerini-Otero 2005). Ohno (1967) proposed nearly four decades ago that bird and mammalian sex chromosomes are homologous. Recent comparative genomic analyses have observed that HSAX contains many orthologs of GGA4 genes (Ross *et al.* 2005) and that GGAZ contains many orthologs of HSA9 genes and fewer orthologs of HSA5 and 8 (Fridolfsson *et al.* 1998; Burt *et al.* 1999; Nanda *et al.* 2002). Because HSAX and GGAZ share few if any orthologs, these comparative data have been interpreted as strong evidence that the sex chromosomes of birds and mammals evolved independently through separate recruitments of bird and mammalian sex chromosomes from independent ancestral autosomes (*e.g.*, Fridolfsson *et al.* 1998; Nanda *et al.* 1999, 2000, 2002; Ellegren 2000; Graves *et al.* 2002; Handley *et al.* 2004; Kohn *et al.* 2004, 2006; Khil and Camerini-Otero 2005).

Comparisons between chickens and humans are powerful for identifying features of the ancestral amniote genome that have been conserved in both lineages, but they provide no evolutionary insight about features that have changed within amniote lineages. To determine whether the precursors of GGAZ and HSAX were or were not linked ancestrally, it is necessary to consider the condition of these ancestral regions within an appropriate outgroup species (Stevens 1980; Watrous and Wheeler 1981; Maddison *et al.* 1984; Futuyma 1998; Martin 2001; Bourque *et al.* 2005). In general, the most appropriate outgroup is the taxon that is most closely related to the last common ancestor of the clade but not included within the clade (the most proximate outgroup). In the case of amniote/amniote

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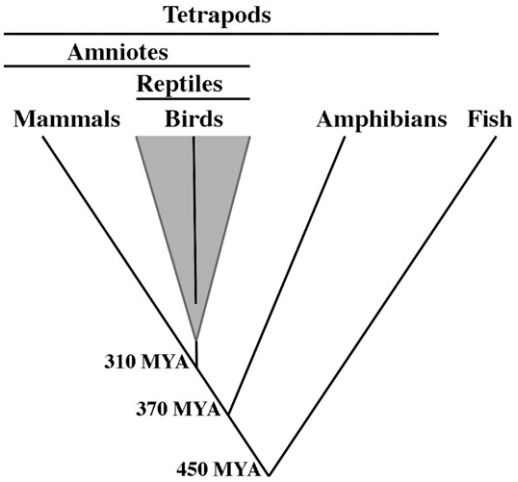

Figure 1.—An abridged phylogeny of the major groups of bony vertebrates. Divergence times were obtained from the literature (Kumar and Hedges 1998; Ruta *et al.* 2003) Birds represent an ancient reptile lineage that diverged from other reptilian groups ~220 MYA (Kumar and Hedges 1998).

comparisons, amphibians represent the most proximate living outgroup (Figure 1). Until recently, there were few amphibian gene order data available for comparative analyses of vertebrate genome structure (Voss *et al.* 2001; Smith and Sinclair 2004; Ohta *et al.* 2006). However, the recently developed genetic linkage map for the salamander genus Ambystoma provides a new outgroup perspective for reconstructing amniote genome evolution (Smith *et al.* 2005; Smith and Voss 2006). The ambystomatid genome contains relatively few large chromosomes that show extensive synteny conservation with chromosomes from fish and amniote genomes (Smith *et al.* 2005; Smith and Voss 2006).

A few studies of amniote sex-chromosome evolution have used teleost (ray finned) fish to provide an outgroup perspective (Kohn *et al.* 2004, 2006). The results of these studies have been interpreted as supporting the hypothesis of separate autosomal recruitments because amniote sex-chromosome orthologs are observed to be distributed among several fish chromosomes. However, these studies have not explicitly tested for the presence or absence of the ancestral association of amniote sex chromosomes. Indeed, analyses across deep phylogenetic distances have rarely used statistical approaches to investigate the possibility of conserved syntenies (but see Danchin and Pontarotti 2004). Moreover, it is generally accepted that the ancestor of most teleosts experienced a whole-genome duplication, which was followed by massive losses of paralogous duplicates (Amores *et al.* 1998; Postlethwait *et al.* 1998; Jaillon *et al.* 2004; Woods *et al.* 2005). Such events, especially in combination with several hundred million years of independent evolution, would be expected to distribute ancestral syntenies among chromosomes.

Interestingly, a recent study of the sex-determining chromosomes of a monotreme (egg laying) mammal seemingly lends support to the idea that Z and W chromosome loci may have been linked in the ancestral amniote genome. The deepest split within the mammalian lineage is between monotremes (platypus and echidna) and therians (all other mammals, *i.e.*, marsupial and placental mammals) (Van Rheede *et al.* 2006). The platypus X1 chromosome contains many genes from the mammalian X conserved region (XCR) (Graves 1995; Ross *et al.* 2005) and is linked, via a meiotic translocation chain of five X and five Y chromosomes, to a chromosome that harbors the DMRT1 gene (Grützner *et al.* 2004; Rens *et al.* 2004). The gene DMRT1 is located within the sex-determining region of the avian Z chromosome and is a primary candidate for the avian sex-determining gene, along with two W-linked genes: ASW and FET1 (Smith and Sinclair 2004). Currently, it is unclear whether the localization of Z and X orthologs to the platypus sex-determining chromosomes is representative of the condition in the ancestral amniote genome or of rearrangements that were derived after the monotreme/therian divergence (Grützner *et al.* 2004; Rens *et al.* 2004; Charlesworth and Charlesworth 2005; Ezaz *et al.* 2006).

Here, we use the Ambystoma genetic map to provide an outgroup perspective on the origin of bird and mammalian sex chromosomes. We observe that genes from the XCR and GGAZ map to adjacent regions of ALG2, and we further demonstrate that the proportion of sex chromosome orthologies observed on ALG2 is dramatically different from the proportion that would be expected by chance. Further comparisons between chicken and human genomes, and with the draft genome of the pufferfish *Tetraodon nigroviridis*, support the Ambystoma outgroup perspective and reveal further traces of this common ancestry.

MATERIALS AND METHODS

Linkage mapping and QTL analysis: Linkage analyses were performed using the previously described mapping panels AxTg (Voss 1995) and WILD2 (Voss and Smith 2005). Primers and probes for all genetic markers have been reported previously (Smith *et al.* 2005), except for 13 markers on ALG2. Primer sequences, diagnostic polymorphisms, and polymorphism detection assays for these 13 markers are summarized in supplemental Table 1 at http://www.genetics.org/supplemental/. Linkage mapping and association analyses were performed using MapManagerQTXb21 (Meer *et al.* 2004).

Identification of orthologs: We identified presumptive orthologies by aligning salamander, human RefSeq, chicken RefSeq, and *T. nigroviridis* transcripts to human, chicken, and *T. nigroviridis* genome assemblies. Similarity searches and sequence alignments were accomplished using the program BLAT (Kent 2002). Source sequences for human (International Human Genome Sequencing Consortium 2001), chicken (International Chicken Genome Sequencing Consortium 2004), and *T. nigroviridis* (Jaillon *et al.* 2004) (hg17 build 35, galGal3, and tetNig1) genomes were downloaded from the UCSC Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway). Cumulative bitscores were calculated for

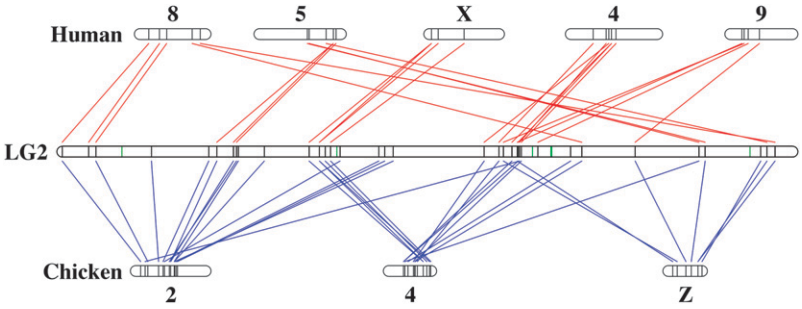

FIGURE 2.—A comparative map of Ambystoma LG2 and syntenic chromosomes from humans and chickens. Vertical bars within ALG2 represent the position of Ambystoma transcripts that yielded an alignment in either the human or the chicken genome. ALG2 loci that do not correspond to orthologs on GGA Z and 4 or on HSA 4, 5, 8, 9, and X are highlighted in green. Red lines connect human/Ambystoma orthologies and blue lines connect chicken/Ambystoma orthologies. The sex chromosomes of human (X) and chicken (Z) are both syntenic with Ambystoma LG2.

alignments between transcripts and full genome sequences by summing across presumptive exons. This was accomplished by summing bitscores for otherwise continuous alignments that were interrupted by gaps of 10,000 or fewer bases using the program MapToGenome (PUTTA *et al.* 2007). Positions of transcripts within conspecific genomes required an alignment with ≥98% nucleotide sequence identity. A genomic region was considered to be orthologous to a transcript if translated sequences yielded an alignment bitscore of ≥99% of the highest alignment bitscore for that transcript. Positions of orthologous loci were plotted using MapChart 2.1 (VOORRIPS 2002).

Distribution of orthologies: Adjusted *G*-statistics (SOKAL and ROHLF 1995) were used to test for nonrandom distribution of sex-chromosome orthologs and orthologs from human and chicken chromosomes that share ancestry with sex chromosomes from the other species (reciprocal amniote sex-chromosome orthologs) on ALG2. We first asked the following question: Are the frequencies of X or Z orthologies on ALG2 significantly higher than expected by chance? The ALG2 loci (the ones that have orthologs) fall into two classes: sex-chromosome orthologs and non-sex-chromosome orthologs (supplemental Table 2 at http://www.genetics.org/supplemental/). The observed numbers of orthologs on ALG2 from the human alignment were 5 from the XCR and 38 from other chromosomes. If ALG2 orthologs were randomly drawn from the human genome, we would expect a proportion of XCR orthologs on ALG2 that approximates the proportion within the human genome. This expected proportion (0.030) was based on the number of genes that are located between 60 and 155 Mb of the X chromosome ($N = 810$) relative to the total number of genes for the Human Genome Assembly build 36.2 (http://www.ncbi.nih.gov/mapview/stats/BuildStats.cgi?taxid=9606&build=36&ver=2) ($N = 28,617$), excluding mitochondrial and HSAY genes. A similar statistic was also calculated on the basis of the expected proportion (0.053) of GGAZ genes ($N = 840$) relative to the total number of genes for the Chicken Genome Assembly build 2.1 (http://www.ncbi.nih.gov/mapview/stats/BuildStats.cgi?taxid=9031&build=2&ver=1) ($N = 15,928$), excluding mitochondrial and GGAW genes.

On the basis of extensive comparisons between chicken and human genomes (FRIDOLFSSON *et al.* 1998; NANDA *et al.* 1999, 2000, 2002; ELLEGREN 2000; GRAVES *et al.* 2002; HANDLEY *et al.* 2004; KOHN *et al.* 2004, 2006; KHIL and CAMERINI-OTERO 2005), we also asked the question: Is the frequency of reciprocal amniote sex-chromosome orthologies on ALG2 significantly higher than expected by chance? Here, ALG2 loci fall into two classes: reciprocal sex-chromosome orthologs and non-sex-chromosome orthologs. The observed numbers based on the human set (HSAX, 9, 5, 8) are 26 ALG2/sex-chromosome orthologs and 17 ALG2/non-sex-chromosome orthologs. If ALG2 orthologs were randomly drawn from the human genome, we would expect a proportion of HSAX, 9, 5, and 8 orthologs on ALG2 that approximates the proportion within the human genome. This expected proportion (0.165) was based on the number of genes on HSA X, 5, 8, and 9 ($N = 4736$) relative to the total number of genes for the Human Genome Assembly build 36.1 ($N = 28,617$). Again, a similar statistic was also calculated on the basis of the expected proportion (0.128) of GGAZ/4 genes ($N = 2043$) relative to the total number of genes for the Chicken Genome Assembly build 2.1 ($N = 15,928$).

Adjusted *G*-statistics (SOKAL and ROHLF 1995) were also used to test for nonrandom distribution of *T. nigroviridis* orthologs among human and chicken chromosomes (supplemental Tables 3–6 at http://www.genetics.org/supplemental/). The frequency of orthologies that were identified on all amniote/*T. nigroviridis* chromosome pairs was tested for goodness of fit to the frequencies of all orthologs on each of the two chromosomes, relative to the grand total of orthologies that were identified. A similar adjusted *G*-statistic was also calculated to test for nonrandom distribution of orthologs from the human RefSeq/chicken genome comparison (925 reciprocal amniote sex-chromosome orthologies and 11,116 non-sex-chromosome orthologs; supplemental Table 7) on three ancestrally duplicated pairs of *T. nigroviridis* chromosomes (H, A, B; *sensu* JAILLON *et al.* 2004), relative to all other *T. nigroviridis* chromosomes.

In practice, some of the statistical analyses that are outlined above can be implemented as a *G*-test or a permutation test (*e.g.*, Fisher's exact test). Both tests are appropriate for the question at hand and will generally give similar results (SOKAL and ROHLF 1995). We have chosen to use *G*-tests for the analysis of ALG2 orthologies because they permit us to incorporate external data on gene frequencies from the human and chicken genome databases to better approximate sampling probabilities. For consistency, we report *G*-statistics throughout this article. We note, however, that comparing *G*-statistics or Fisher's exact tests among different contrasts does not provide insight into the relative strength of association among contrasts. That is, the statistics provide an estimation of the probability of obtaining a given distribution of orthologies under a random sampling scheme, but the values of these statistics are not directly interpretable in a probabilistic sense (FISHER 1938; GOODMAN and KRUSKAL 1954; KENDALL and STUART 1967).

RESULTS AND DISCUSSION

We were able to meiotically map 20 amniote sex-chromosome orthologs to Ambystoma linkage groups. We found that the majority of Z orthologs (85%) mapped to a single Ambystoma linkage group (ALG2) (Figure 2; supplemental Table 2 at http://www.genetics.org/supplemental/). The frequency of Z orthologs on ALG2 was greater than would be expected by chance

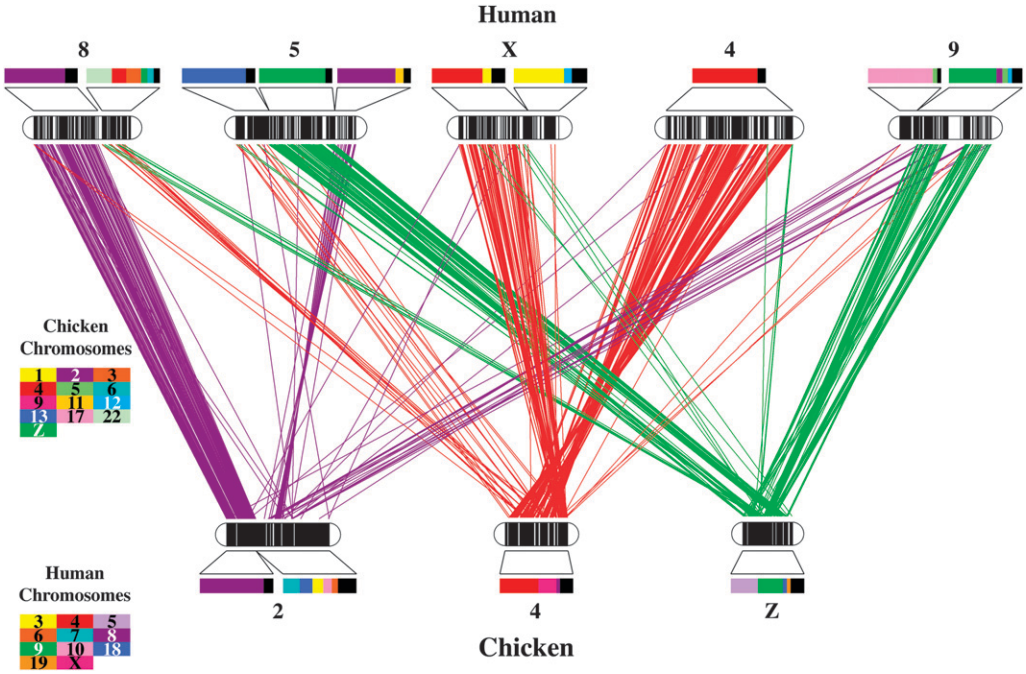

FIGURE 3.—A comparative map of the human and chicken chromosomes that are syntenic with Ambystoma LG2. Vertical bars within chromosomes represent the position of mapped human RefSeq transcripts or chicken orthologs. Lines connect the positions of human/chicken orthologies. Bars above human chromosomes show the proportions of chicken chromosome orthologs that were identified within a given segment, and bars below chicken chromosomes show the proportions of human chromosome orthologs that were identified within a given segment. Note that the chicken chromosomes Z and 4 map to adjacent and overlapping regions of HSA8 and HSA5.

($G_{ajd} = 6.2$, $P = 0.013$). Many orthologs from the XCR (42%) mapped to neighboring, but nonoverlapping, regions of ALG2 and no more than two X chromosome orthologs were identified on any other Ambystoma linkage group. The frequency of XCR orthologs on ALG2 was also greater than would be expected by chance ($G_{ajd} = 6.3$, $P = 0.009$). When we searched all ALG2 genes against the full genome assemblies for human and chicken, we found them to be distributed nonrandomly among human chromosomes ($G_{ajd} = 42.6$, $P = 6.9e^{-11}$) and chicken chromosomes ($G_{ajd} = 32.9$, $P = 9.7e^{-9}$) that harbor reciprocal amniote sex-chromosome loci. Although some chromosomal associations might be expected by chance, given that the salamander genome is composed of relatively few large chromosomes, statistical analysis shows that the distribution of sex-chromosome orthologies on ALG2 is not likely to have occurred by chance. This pattern of orthologies on ALG2 is consistent with the idea that the X–Y and Z–W chromosomal regions were linked on an ancestral chromosome prior to the divergence of the amphibian and amniote lineages. The pattern of orthologies on ALG2 also provides support for all other conserved syntenies that have been previously identified between amniote sex chromosomes and autosomes (FRIDOLFSSON *et al.* 1998; BURT *et al.* 1999; NANDA *et al.* 2002; INTERNATIONAL CHICKEN GENOME SEQUENCING CONSORTIUM 2004; BOURQUE *et al.* 2005; ROSS *et al.* 2005). The ALG2 region defined by Z orthologs also includes orthologs from HSA 4, 5, 8, and 9 and the ALG2 region defined by the XCR included loci from GGA4. Our comparisons show that many of the gene orders conserved between the sex chromosomes and autosomes of chickens and humans are interspersed but conserved on the same salamander chromosome. Thus, ALG2 apparently retains some of the gene content of an ancestral chromosome that gave rise to the X and Z sex chromosomes.

To further test the idea that the sex chromosomes of birds and mammals were derived from the same ancestral chromosome, we identified the location of ($N = 14{,}922$) human RefSeq orthologs in the chicken genome and ($N = 3{,}120$) chicken RefSeq orthologs in the human genome (supplemental Tables 7 and 8 at http://www.genetics.org/supplemental/). As has been reported previously (FRIDOLFSSON 1998; BURT *et al.* 1999; NANDA *et al.* 2002), we identified a large region of conserved synteny between GGAZ and HSA9 ($N = 211$ loci) (Figure 3) and smaller, but very confined, regions of conserved synteny between GGAZ and HSA8 ($N = 19$ loci) and between GGAZ and HSA5 ($N = 227$ loci). In the reciprocal comparison of humans to chickens, we identified a large region of synteny between XCR and GGA4 ($N = 272$). Thus, when only amniote sex-chromosome loci are considered, there is seemingly no support for the idea of a common autosomal origin for mammalian and bird sex chromosomes, because conserved syntenies are not observed between X and Z loci. However, regions of synteny are observed between GGA4, HSAX, and Y and between GGA4 and human autosomes (HSA 5 and 8) that show synteny with GGA Z (Figure 3). Thus, syntenies that are observed in comparisons of ALG2 and amniote genomes are also observed in comparisons between human and chicken genomes. This pattern supports the idea that amniote chromosomal *regions* from GGA Z and 4 and from HSA 9, 4, X, 5, and 8 were linked ancestrally.

To investigate a deeper outgroup perspective on the evolution of amniote sex chromosomes, we asked the

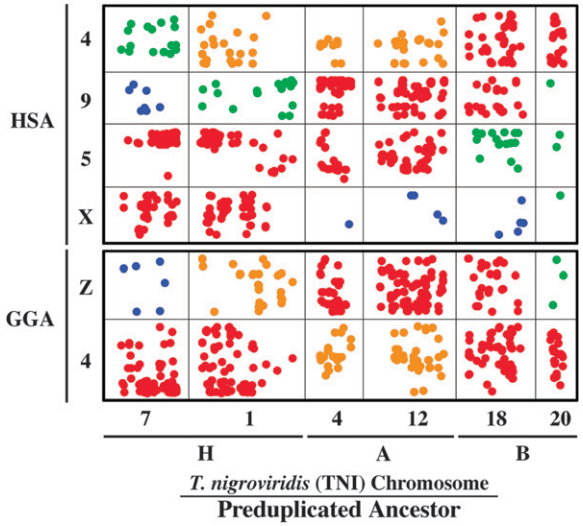

FIGURE 4.—Oxford plot of the position of amniote sex-chromosome loci in the *T. nigroviridis* genome. The *y*-axis represents the relative position of orthologs on human (HSA) and chicken (GGA) chromosomes. The *x*-axis represents the relative position of orthologs on *T. nigroviridis* chromosomes. "H", "A," and "B" correspond to preduplicated ancestral chromosomes (JAILLON *et al.* 2004). Cells containing an excess of orthologies are highlighted in red ($P < 0.005$) and yellow. Cells containing a deficiency of orthologies are highlighted in blue ($P < 0.005$) and green.

question: Do comparisons with *T. nigroviridis* provide evidence for deep conservation of HSAX/GGAZ/ALG2 syntenies? Although these comparisons span an additional 670 MY of independent evolution (KUMAR and HEDGES 1998) and a teleost whole-genome duplication (AMORES *et al.* 1998; POSTLETHWAIT *et al.* 1998; JAILLON *et al.* 2004; WOODS *et al.* 2005), we detected statistically significant, nonrandom distributions of reciprocal amniote sex-chromosome orthologies among three pairs of *T. nigroviridis* chromosomes (Figure 4; supplemental Tables 2 and 3 at http://www.genetics.org/supplemental/). These chromosome pairs correspond to the proposed duplicates of ancestral teleost chromosomes A, B, and H (JAILLON *et al.* 2004). To more directly address the common ancestry of sex-chromosome orthologs among these three duplicate chromosome pairs, we took into consideration the distribution of sex-chromosome orthologs that were identified in comparisons between human RefSeq and the chicken genome (supplemental Table 7). These sex-chromosome orthologs correspond to 1194 human RefSeq genes that fall on HSA 5, 8, 9, or X and correspond to orthologs on GGA 4 or Z. Of these genes, 925 identify orthologs in the *T. nigroviridis* genome and the overwhelming majority of these ($n = 724$) fall on the three chromosome pairs (H, A, B) that contain a significant excess of reciprocal amniote sex-chromosome loci (Figure 4). Of 11,116 non-sex-chromosome/*T. nigroviridis* orthologies, 2906 fall on H, A, and B chromosome pairs and 8210 fall on other *T. nigroviridis* chromosomes. The distribution of sex-chromosome *vs.* non-sex-chromosome orthologs on *T. nigroviridis* H, A, B, chromosome pairs is very unlikely to have occurred by chance ($G_{ajd} = 959.8$, $P = 9.8e^{-211}$). Thus, a large fraction the orthologous loci that are associated in the *T. nigroviridis* duplicates of A, B, and H are from the same amniote chromosomal regions that support ancestral XCR/GGAZ linkage in the salamander genome. Of particular importance is the observation that GGAZ and GGA4 orthologs are linked in both *T. nigroviridis* and Ambystoma genomes (Figures 2 and 4). Because conserved syntenies also reveal common ancestry of GGA4 and XCR chromosomal regions, the most parsimonious interpretation of *T. nigroviridis* comparative mapping data is ancestral linkage of Z and X chromosomal regions in the tetrapod and amniote lineages. The distribution of ancestral amniote sex-chromosome regions among different *T. nigroviridis* chromosomes also reveals the confounding effects of genome duplications and rearrangements that have occurred within the lineage that gave rise to *T. nigroviridis* subsequent to the diversification of the bony vertebrate (euteleost) ancestor.

Our comparative analyses provide the first amphibian outgroup perspective on the evolution of amniote sex chromosomes and are most parsimoniously interpreted as evidence for ancestral linkage of XCR and GGAZ regions. However, we recognize that the most parsimonious evolutionary scenario may not always be correct. It is possible (although we think less probable) that these associations are derived from rearrangements that occurred independently within the amniote, amphibian, and fish lineages. Resolution of this issue will necessitate additional comparative data from amphibian outgroups and, to some extent, perspective from the preduplicated fish genome. Ongoing progress toward improving linkage (SMITH *et al.* 2005) and physical (http://genome.jgi-psf.org/Xentr4/Xentr4.home.html) maps for representative amphibian species will likely result in more accurate reconstructions of the ancient events that have structured vertebrate genomes. We note, however, that even with the modest number of Ambystoma/amniote orthologies that are currently mapped, statistical analyses strongly support conserved synteny of ALG2/amniote sex-chromosome orthologs. We therefore expect that additional mapping studies will tend to support our primary findings.

Our study does not resolve the question of what type of sex-determining system was present in the ancestral amniote lineage and how modifications on this ancestral system gave rise to the mammalian XY and avian ZW sex-determining systems. From a theoretical standpoint; it is thought that chromosomal sex-determining systems should evolve from ancestors with environmental (*e.g.*, temperature dependent) sex determination (BULL 1983; JANZEN and KRENZ 2004; CHARLESWORTH *et al.* 2005). However, the distribution of ZW and XY systems within fish and amphibian phylogenies suggests that direct

transitions between ZW and XY systems may be a relatively common occurrence (reviewed by Ezaz *et al.* 2006). The unusual sex chromosomes of platypus have been interpreted as evidence supporting the idea that the mammalian XY system evolved from something very similar to the avian ZW (Ezaz *et al.* 2006; Waters *et al.* 2007). Here again, information from appropriately positioned outgroups (amphibians) and a whole-genome perspective will be critical for testing alternate hypotheses that platypus sex chromosomes represent an ancestral state *vs.* a state that was derived independently within the monotreme lineage (Grützner *et al.* 2004; Rens *et al.* 2004; Charlesworth and Charlesworth 2005; Ezaz *et al.* 2006).

In conclusion, our gene mapping data show that amphibian orthologs for loci on chicken and human sex chromosomes are linked in the Ambystoma genome. We interpret this pattern of linkage, which is revealed by including an amphibian outgroup perspective, as a signature of shared ancestry between avian and mammalian sex chromosomes. We believe this signature is retained as a vestige for two reasons:

1. In comparison to amniotes with chromosomal sex determination, our mapping results show that sex in Ambystoma is determined by a single locus on a chromosome with autosomal characteristics (J. J. Smith and S. R. Voss, unpublished data). Gene orders on ALG2 have not been disrupted by amniote specific rearrangements or mechanisms that are associated with the divergence of dimorphic sex chromosomes (Ohno 1967; Bull 1983; Lahn *et al.* 2001; Ayling and Griffin 2002; Charlesworth and Charlesworth 2005; Charlesworth *et al.* 2005; Khil and Camerini-Otero 2005).
2. In comparison to amniote genome evolution, Ambystoma has experienced relatively lower rates of genome rearrangement and fission (Smith and Voss 2006). The signature of shared sex-chromosome ancestry is difficult to see when comparing only bird and mammalian genomes because mutational processes have fractured and rearranged gene orders within these groups, especially in the mammalian lineage (Burt *et al.* 1999; Bourque *et al.* 2005; Smith and Voss 2006).

Our study shows that clarity in comparative vertebrate genomics can be greatly increased by including relevant and phylogenetically well-positioned outgroups like Ambystoma.

Comments of several anonymous reviewers substantially improved the quality and clarity of this manuscript. This project was supported by the Kentucky Spinal Cord Injury Research Trust and grant number 5-R24-RR016344-06 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH. The project was also supported by a National Science Foundation (NSF) CAREER Award (IBN-0242833; IBN-0080112). This project also utilized resources and facilities provided by the Kentucky Bioinformatics Research Infrastructure Network, the Spinal Cord and Brain Injury Research Center, and the NSF-supported *Ambystoma* Genetic Stock Center (DBI-0443496).

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Communicating editor: A. D. Long