

Faster-Z Evolution Is Predominantly Due to Genetic Drift

Judith E. Mank,^{*,1,2} Kiwoong Nam,² and Hans Ellegren²

¹Department of Zoology, Edward Grey Institute, University of Oxford, Oxford, United Kingdom

²Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

*Corresponding author: E-mail: judith.mank@zoo.ox.ac.uk.

Associate editor: John H. McDonald

Abstract

Genes linked to sex chromosomes may show different levels of functional change than autosomal genes due to different evolutionary pressures. We used whole-genome data from zebra finch–chicken orthologs to test for Faster-Z evolution, finding that Z-linked genes evolve up to 50% more rapidly than autosomal genes. We combined these divergence data with information about sex-specific expression patterns in order to determine whether the Faster-Z Effect that we observe was predominantly the result of positive selection of recessive beneficial mutations in the heterogametic sex or primarily due to genetic drift attributable to the lower effective population size of the Z chromosome compared with an autosome. The Faster-Z Effect was no more prevalent for genes expressed predominantly in females; therefore, our data indicate that the largest source of Faster-Z Evolution is the increased levels of genetic drift on the Z chromosome. This is likely a product of sexual selection acting on males, which reduces the effective population size of the Z relative to that of the autosomes. Additionally, this latter result suggests that the relative evolutionary pressures underlying Faster-Z Evolution are different from those in analogous Faster-X Evolution.

Key words: faster-X, female-biased expression, male-biased expression, avian genomics.

Introduction

The X chromosome has several properties that distinguish it from the autosomes (Vicoso and Charlesworth 2006) and that have the potential to influence the rate and pattern of evolution of X-linked genes (Charlesworth et al. 1987). Recent genomic scans in both *Drosophila* (Counterman et al. 2004; Begun et al. 2007; Baines et al. 2008; Vicoso et al. 2008) and mammals (Khaitovich et al. 2005; Lu and Wu 2005; Torgerson and Singh 2006; Baines and Harr 2007) have shown that X-linked genes exhibit accelerated rates of functional change compared with autosomes. These studies provide a broad consensus for Faster-X Evolution; however, the underlying evolutionary mechanism remains a subject of debate. The topic is of some importance, as the alternative explanations have profoundly different implications for the nature of mutation and selection, as well as their role in genomic evolution.

For male-heterogametic lineages such as mammals and *Drosophila*, males have only one copy of the X chromosome and therefore only one copy of X-linked genes. Faster-X Evolution may result from the expression of recessive ($h < 0.5$) mutations on the X chromosome in the heterogametic sex, as X-linked recessive alleles are directly exposed to selection when present in males (Charlesworth et al. 1987). By contrast, autosomal recessive alleles are directly exposed to selection only when homozygous, a rare event for nascent mutations. Therefore, recessive beneficial alleles will be more rapidly fixed by positive selection when on the X chromosome, thereby providing one explanation for Faster-X Evolution.

Alternatively, Faster-X may result from genetic drift and the fixation of mildly deleterious mutations. For every

reproductive pair, the effective population size of the X (N_{eX}) is three-quarters that of an autosome (N_{eA}). This reduced effective population size can produce higher levels of genetic drift, increasing the proportion of alleles that act as neutral, as purifying selection is less powerful at lower effective population sizes (Caballero 1995; Laporte and Charlesworth 2002), and leading to the fixation of more mildly deleterious alleles on the X chromosome than on the autosomes. This scenario for Faster-X Evolution suggests that the phenomenon is a neutral, nonadaptive process.

Birds have a female-heterogametic system of sex chromosome inheritance, where males have a ZZ karyotype and females are ZW, and the avian Z chromosome would be expected to exhibit analogous Faster-Z Evolution. Previously, we used expressed sequence tag (EST) data from genes expressed in the avian brain to show that orthologous chicken–zebra finch genes on the Z chromosome evolve roughly 30% more rapidly than autosomal genes (Mank, Axelsson, and Ellegren 2007; Mank, Hultin-Rosenberg, et al. 2007). There was some evidence, based on available polymorphism data, that the Faster-Z Effect was due to positive selection; however, we may have simply lacked the statistical power to identify the signature of genetic drift from this limited data set.

Animal mating systems often involve significant levels of sexual selection (Andersson 1994), typically acting on males. This increases the variance in male reproductive success and in effect reduces the number of males contributing to each generation. For male-heterogametic systems, sexual selection on males increases N_{eX} above $3/4N_{eA}$, reducing the influence of genetic drift on Faster-X Evolution and suggesting that a significant portion of the Faster-X Effect in mammals and *Drosophila* is due to positive selection

acting on recessive beneficial mutations (Vicoso and Charlesworth 2009; Mank et al. 2010). By contrast, increased variance in male mating success in birds decreases the effective population size of the Z chromosome (N_{eZ}) below the $3/4N_{eA}$ expected if females and males have equal variance in reproductive success (Caballero 1995; Charlesworth 2001; Laporte and Charlesworth 2002; Ellegren 2009). This is supported by estimates of N_{eZ} in birds significantly below $3/4N_{eA}$ (Sundström et al. 2004; Borge et al. 2005) and suggests that a larger proportion of the Faster-Z Effect may be due to genetic drift (Vicoso and Charlesworth 2009; Mank et al. 2010).

It stands to reason that if the majority of Faster-Z Evolution is due to positive selection acting on recessive mutations, the Faster-Z Effect will be greatest for those genes predominantly expressed in females, as it is only in females that Z-linked genes are hemizygotously exposed. Additionally, there would be little to no Faster-Z Effect for genes expressed primarily in males, as there is no difference in the homogametic sex in the exposure to selection of Z and autosomal loci. By the same line of argument, genes expressed in both sexes would show an intermediate Faster-Z Effect, as they are exposed directly in females one-third of the time. Alternatively, if Faster-Z Evolution is due primarily to genetic drift, female-biased genes would not be expected to show the greatest Faster-Z Effect, and the relationship between sex-biased expression and Faster-Z Evolution would be governed by other factors such as the strength of purifying selection and the deleterious effect of dominant mutations.

Here, we perform the first test of Faster-Z evolution using two complete avian genomes, those of the zebra finch (Wesley C. Warren, David F. Clayton, Hans Ellegren et al. in preparation) and chicken (International Chicken Genome Sequencing Consortium 2004). We combine these data with transcriptome profiles to test the relative importance of positive selection and genetic drift in the Faster-Z Effect.

Materials and Methods

Divergence Estimates

We downloaded annotated coding sequence from the chicken (WASHUC2) and zebra finch genomes (Tea-Gut3.2.4) from biomart (www.biomart.org), and determined 1:1 orthology between the two species using Inparanoid3.0 (O'Brien et al. 2005) with reciprocal Blast searches and bootstrapping, employing 90% support cutoffs. For genes with more than one transcript, we selected the longest transcript for this analysis. Selected coding sequences were then translated into protein sequences prior to alignment, and aligned amino acid sequences were then translated back into DNA using MUSCLE (Edgar 2004), using custom-made PERL scripts. Using this method, we obtained 11,176 pairwise 1:1 orthologs and for each 1:1 ortholog we calculated the number of nonsynonymous and synonymous sites, the number of nonsynonymous and synonymous substitutions using codeML in PAML 4.1 (Yang 1997). From these estimates, we calculated the number of nonsynonymous substitutions per nonsynonymous site (d_N) and the number of synonymous

substitutions per synonymous site (d_S). The ratio of these two metrics (d_N/d_S) can be used as a measure of functional evolutionary change in the protein structure, correcting for underlying variation in mutation rate.

The above two-species methods measure the functional divergence that has occurred between chicken and zebra finch, which last shared a common ancestor roughly 100 Ma (van Tuinen et al. 2000). As our gene expression information, described below, is taken from chicken, we were also interested in the pattern substitution that has occurred on the chicken lineage alone. In order to do this, we expanded the d_N/d_S calculations to include several more taxa: Anolis (ANOCAR1), Human (NCBI36), Mouse (NCBIM37), and Opossum (MonDom5), downloaded through Biomart. These genomes, combined with chicken and zebra finch, yielded 7,789 1:1:1:1:1 orthologs, which was reduced to 7,733 orthologs after the removal of those loci where gap positions eliminated all informative sites. We performed codon-based alignments and substitution calculations in the same manner as the pairwise alignments, identifying only those substitutions that occurred on the chicken lineage after it diverged from the common ancestor with zebra finch.

Divergence Data Treatment

In both data sets, we removed all loci <100 bp, as maximum likelihood estimates of divergence can be problematic for short transcripts. This resulted in the removal of 23 genes in the two-species data and 53 in the six-species data. Additionally, we filtered the data to remove all orthologs where $d_S > 2$, as it has been previously shown that the transition–transversion ratio at 4-fold degenerate sites is linear for $d_S \leq 2$, and is nonlinear for values >2 (Axelsson et al. 2008). This resulted in the removal of 417 loci from the two-species data set, and 70 from the six-species data set. We also removed all loci with premature stop codons.

Genomic location was taken from the current chicken (GalGal3) and zebra finch (TaeGut3) assemblies (www.emsembl.org), and orthologs were parsed into autosomal and Z categories, and all orthologs with other genomic locations were removed from further analysis. Only orthologs that were in the same category in both avian species were used for further analysis, and this was the majority of orthologs as the chicken and zebra finch genomes are highly syntenic (Shetty et al. 1999; Itoh and Arnold 2005; Backström et al. 2006; Itoh et al. 2006).

Mean d_N (defined as the number of nonsynonymous substitutions per nonsynonymous site) and d_S (the number of synonymous substitutions per synonymous site) were calculated by dividing the sum over all genes in a genomic category of the number of substitutions by the sum of the number of sites for the Z chromosome and autosomes separately. This has the advantages of weighting data by alignment length and circumventing the problem of infinitely high d_N/d_S values arising from genes with no synonymous substitutions. Ninety-five percent confidence intervals were determined via bootstrapping (1,000 repetitions), and a permutation test with 1,000 repetitions was used to assess significant

differences for each metric (d_N , d_S , and d_N/d_S) between chromosomal categories. Significant Faster-Z Evolution was also computed with Fisher's Exact Test for each comparison.

The avian karyotype exhibits large variation in chromosome size, with several large chromosomes (designated macrochromosomes and intermediate chromosomes) and numerous small (<20 Mb) microchromosomes. Microchromosomes differ from larger chromosomes in many ways, including gene density, repeat number, recombination rate, intron length, and GC content (International Chicken Genome Sequencing Consortium 2004), and some of these characteristics, particularly recombination, can influence the nature and efficacy of selection. The Z chromosome is most similar to chicken chromosomes 5 and 6 in size, and a fairer, though less statistically powerful, Faster-Z comparison is therefore between the Z and similarly sized chicken autosomes 1–10.

Chicken and zebra finch chromosomes 1–10 are largely syntenic (Burt et al. 1999; Griffin et al. 2007), with the exception of the p arm of chromosome 4, which represents a fusion of a microchromosome to a macrochromosome at intervening point after passerine and galliform radiation (International Chicken Genome Sequencing Consortium 2004). Therefore, we removed the genes from this region in our comparison of the Z chromosome with chicken chromosomes 1–10.

In order to verify that we had sufficient statistical power with sufficient observations to avoid undue risk of falsely accepting the null hypothesis, we performed a power analysis to determine whether our data were sufficient at $\alpha = 5\%$ (5% chance of incorrectly rejecting the null hypothesis that the categories are equal) using `pwr.t2n.test` function in the `pwr` package (Cohen 1988) with the R platform. For the analysis of Faster-Z Evolution, we calculated power using the mean d_N/d_S for Z and autosomal loci, the standard deviation for these values based on bootstrapping (1,000 replicates), and the sample sizes of each category. In all comparisons, we used a two-tailed power analysis.

Expression Data

Whole-transcriptome profiling was done on adult chicken gonads to determine sex-specific expression patterns. Adult gonads were chosen for two reasons. First, the adult gonad is the only tissue with sufficient numbers of female-biased genes (Mank and Ellegren 2009b) needed to overcome the overall lack of Z chromosome dosage compensation in birds (Ellegren et al. 2007; Itoh et al. 2007). Additionally, although the Z chromosome is inactivated in females due to meiotic sex chromosome inactivation (Schoenmakers et al. 2009), the phenomenon is ephemeral in birds, with the Z reactivated in adult females. Adult gonads therefore maximize the number of female-biased Z-linked genes while minimizing the effects of meiotic sex chromosome inactivation.

The handling and statistical methods for the expression data have been previously described (Mank and Ellegren 2009b), and only the most relevant details are presented here in order to aid comprehension of the experiments. Briefly, an-

imals were purchased from OVA Productions (Morgongåva, Sweden) as newly fertilized eggs and derived from the same white leghorn genetic background that has been reared under standardized conditions for several generations. Adults were collected at 26 weeks posthatching after sexual maturity was achieved, and the left gonad from each individual extracted, homogenized, and used for RNA preparation. After RNA samples passed Agilent 2100 Bioanalyzer quality control, six non-overlapping pools of RNA were established, three for each sex, each pool composed of five individuals. Pooled RNA was prepared and hybridized to Affymetrix GeneChip expression arrays, and washed and stained arrays were scanned with the GeneChip scanner 3000 7G. All arrays passed Affymetrix quality control criteria.

The resulting CEL files were RMA preprocessed (Irizarry et al. 2003) and then subjected to a series of normalizations to adjust for local, global, and experimental variation. Data were then filtered for significant expression, with those probe sets without significant expression in three of the six replicates removed from further analysis. Genomic location of significantly expressed probes was determined with the GalGal3 assembly of the chicken genome (www.ensembl.org), and all probes that mapped either to the Z chromosome or autosomes were used in subsequent analysis. Sex-biased expression was defined as \log_2 (average male expression)/(average female expression), resulting in positive values for male-biased genes and negative values for female-biased genes. The lack of Z chromosome dosage compensation in birds means that most genes on the Z are by default of gene dose expressed at higher levels in males (on average 1.5-fold male biased) (Ellegren et al. 2007; Itoh et al. 2007), and therefore, we set thresholds to define sex bias above this level. Male-biased and female-biased genes were defined for this analysis as significantly greater than 2-fold different between the sexes, with significance determined with a modified *t*-test and corrected for a 0.05% false discovery rate (FDR) with the Benjamini–Hochberg calculation. This 2-fold difference represents a balance between the need to correct for the lack of dosage compensation and the need to maximize the number of genes defined as sex-biased for statistical power.

Divergence and Expression Analysis

Expression data were combined with divergence data described above, and loci were parsed into three categories for both the Z and the autosomes: significantly female-biased (\log_2 male/female < −1, 0.05% FDR), significantly male-biased (\log_2 male/female > 1, 0.05% FDR), and unbiased genes. For each expression category, we tested whether d_N , d_S , and d_N/d_S were significantly different between autosomal and Z chromosome loci with both permutation testing (1,000 repetitions) and Fisher's Exact test.

The strength of the Faster-Z Effect was calculated for each expression category, defined as the average Z chromosome divergence (d_{NZ}/d_{SZ}) divided by the average autosomal divergence (d_{NA}/d_{SA}). Where $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA}) = 1$, Z-linked and autosomal loci evolve at similar rates. $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA}) > 1$ indicates Faster-Z evolution, and $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA}) < 1$ indicates Slower-Z Evolution. The

Table 1. Number of Synonymous and Nonsynonymous Substitution for Autosomal and Z-Linked Orthologs.

	Autosomes	Autosomes 1–10	Z Chromosome
Chicken–zebra finch comparison			
Number of nonsynonymous substitutions	783,650	505,263	45,713
Number of synonymous substitutions	1,913,703	1,118,893	94,558
Chicken lineage–specific			
Number of nonsynonymous substitutions	179,299	123,443	15,262
Number of synonymous substitutions	536,895	348,835	31,285

strength of the Faster-Z Effect is proportional to the degree that $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA})$ exceeds 1. In order to maximize statistical power, we assessed the strength of the Faster-Z Effect only for autosomal and Z-linked genes, omitting the category autosomes 1–10 as it contained too few genes to be informative.

As in the overall analysis of Faster-Z Evolution, we performed a power analysis in order to verify that we had sufficient power in these comparisons for $\alpha = 5\%$ (5% chance of incorrectly rejecting the null hypothesis that the categories are equal) using `pwr.t2n.test` function in `pwr` package (Cohen 1988) with the R platform.

Gene Ontology

In order to investigate whether nonrandom distribution of functional gene classes influenced our results, we used the chicken Gene Ontology (Gene Ontology Consortium 2000). Using Ontologizer 2.0 (Robinson et al. 2004; Bauer et al. 2008), we tested whether female-biased, male-biased (defined as >2-fold expression difference, 5% FDR), or unbiased genes showed significant differences in gene functionality from the pool of all expressed genes. We used parent–child analysis, correcting for multiple comparisons with the Westfall–Young Single Step method (Grossman et al. 2007). We also report all Gene Ontology terms that were significantly different before correction ($P < 0.01$).

Results

Whole-Genome Assessment of Faster-Z Evolution

After filtering, we had divergence estimates for 10,626 1:1 zebra finch–chicken orthologs, encompassing 16.37 Mb and covering roughly 63% of the ENSEMBL-modulated protein-coding genes from the chicken genome. Within these data, there were 829,363 nonsynonymous substitutions between zebra finch and chicken lineages and 2,008,588 synonymous differences (table 1). The pattern of synonymous and nonsynonymous substitution was significantly different between the Z chromosome and all autosomal genes (Fisher’s Exact Test, $P < 0.0001$).

The avian karyotype is heterogeneous with regards to chromosome size. In addition to large chromosomes, the chicken and zebra finch genomes contain many small chromosomes (<20 Mb), and these “microchromosomes” differ from larger chromosomes in having higher gene density, higher rates of recombination, greater GC content, as well as fewer repeats and shorter introns (International Chicken Genome Sequencing Consortium 2004). These factors influence the overall divergence pattern for avian genes

on microchromosomes relative to larger chromosomes (Axelsson et al. 2005), and so a fairer, though less powerful, comparison, is between the Z chromosome and chromosomes of similar size. As the chicken Z chromosome is intermediate in size to chromosomes 5 and 6, we used a subset of chromosomes, autosomes 1–10, as a comparison to the Z. This comparison is congruent with that using the full autosomal complement, as the pattern of substitution differed between the Z and autosomes 1–10 (Fisher’s Exact Test, $P < 0.0001$).

Overall, the data indicate a significantly greater d_N/d_S ratio for genes linked to the Z chromosome when compared with all genes linked to autosomes and to genes linked to autosomes 1–10 ($P = 0.050$ and $P = 0.018$, respectively, based on permutation testing, 1,000 replicates), as shown in table 2. In the comparison with loci located on autosomes 1–10, Faster-Z Evolution was due to an increase in Z chromosome d_N ($P = 0.011$). For the comparison with the full autosomal complement of genes, Faster-Z Evolution was due to a drop in Z chromosome d_S ($P = 0.015$), although there was also a marginally nonsignificant ($P = 0.074$) increase in Z chromosome d_N that also contributed to Faster-Z Evolution. Overall, the Z chromosome shows a 15–20% increase in d_N/d_S ratio compared with autosomal loci (fig. 1a).

Because our expression data are taken from chicken (described below), it is useful to examine the divergence pattern of the chicken lineage alone, and to do this, we used six-species alignments (chicken, zebra finch, anolis, human, mouse, and opossum) to identify the divergence pattern on the chicken lineage since its split from the common ancestor with zebra finch. These alignments yielded divergence estimates for 7,548 1:1:1:1:1:1 orthologs after data filtering, covering 10.43 Mb and roughly 45% of known chicken protein-coding loci. There were 194,561 nonsynonymous substitutions on the chicken lineage and 568,180 synonymous substitutions on this branch (table 1). There was a significant difference in the pattern of substitution between the Z and both categories of autosome (Fisher’s Exact Test, $P < 0.0001$ in both cases), with more nonsynonymous substitutions on the Z than expected.

The divergence data for the chicken lineage based on the six-species data are qualitatively consistent with the two-species data, with more nonsynonymous substitutions on the Z than either class of autosomes (Fisher’s Exact Test, $P < 0.0001$ in both cases, table 1), and with loci linked to the Z chromosome showing a significant increase in overall d_N/d_S compared with genes linked to all autosomes or autosomes 1–10 ($P = 0.0060$ and $P = 0.043$, respectively,

Table 2. Divergence for Chromosomal Classes of 1:1 Orthologs Based on Zebra Finch–Chicken Alignments.

	Number of Loci	Mb	d_N	d_S	d_N/d_S
Autosomes	10,092	15.55	0.0675	0.485*	0.139*
Autosomes 1–10	6,734	10.58	0.0645*	0.433	0.149*
Z chromosome	534	0.82	0.0755	0.442	0.171

Autosomal estimates that are significantly different ($P < 0.05$, based on 1,000 permutations) from that of the Z chromosome are indicated (*).

permutation testing, 1,000 repetitions), as shown in **table 3**. Loci linked to autosomes 1–10 exhibited significantly higher d_N and d_S ($P = 0.012$ and $P = 0.0070$, respectively). In the comparison between Z-linked genes and all autosomal loci, this was due entirely to an increase in Z chromosome d_N ($P = 0.0070$). For the six-species data, the Z chromosome shows a 40–50% increase in d_N/d_S ratio compared with autosomal loci (**fig. 1b**).

In both the two-species and six-species data, our analysis indicates that we had 100% power at $\alpha = 5\%$ for the comparisons between the Z and all autosomal genes, as well as the Z and genes on autosomes 1–10. Therefore, it seems unlikely that the evidence for Faster-Z Evolution that we observed is due to statistical error.

Strength of the Faster-Z Effect

We had divergence estimates for 9,322 loci from the two-species data for which we also had expression data. We divided these data into three expression categories, corresponding to those genes expressed more in females or

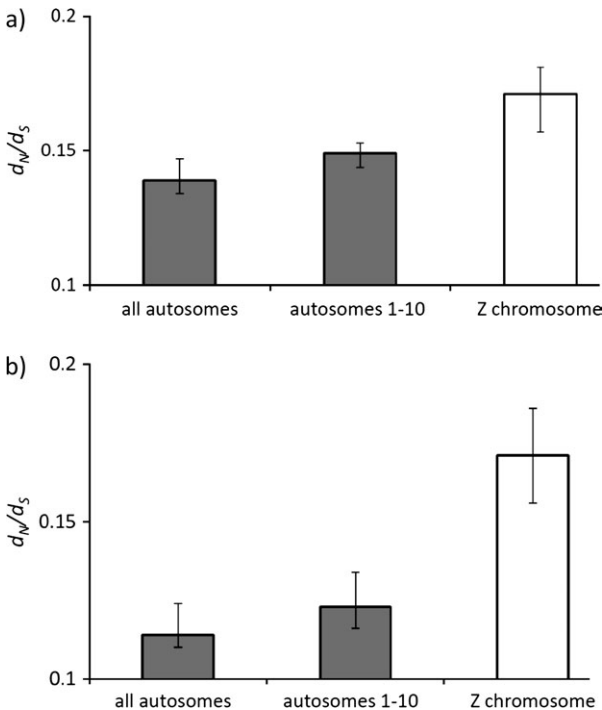


Fig. 1. Estimates of d_N/d_S for loci on autosomes, autosomes 1–10, and the Z chromosome for zebra finch–chicken alignments (a) and for the chicken lineage alone based on six-species alignments (b). Autosomal classes are shown in gray, Z chromosome in white. Whiskers indicate 95% confidence intervals based on bootstrapping (1,000 replicates).

Table 3. Divergence Estimates for the Chicken Lineage for Chromosomal Classes, Based on Six-Species 1:1:1:1:1 Orthologs Alignments.

	Number of Loci	Mb	d_N	d_S	d_N/d_S
Autosomes	7,150	9.86	0.0244*	0.213	0.114*
Autosomes 1–10	4,874	6.88	0.0242*	0.197*	0.123*
Z chromosome	398	0.57	0.0363	0.212	0.171

Autosomal estimates that are significantly different ($P < 0.05$, based on 1,000 permutations) from that of the Z chromosome are indicated (*).

males (defined as 2-fold difference in expression between the females and males, corrected for a 0.05 FDR) and those genes expressed similarly between the sexes. For female-biased, male-biased, and unbiased genes, the pattern of substitution was significantly different between Z-linked and autosomal genes (Fisher’s Exact Test $P < 0.0001$ in all cases, shown in **table 4**).

There was evidence of Faster-Z Evolution for female-, male-, and unbiased genes (**table 5**); however, Faster-Z was significant only for female- and male-biased genes ($P = 0.050$ and $P = 0.0040$, respectively, based on permutation testing with 1,000 repetitions). Z-linked unbiased genes exhibit a significantly lower d_S than similarly expressed

Table 4. Number of Synonymous and Non-synonymous Substitution for Autosomal and Z-Linked 1:1 Zebra Finch–Chicken Orthologs, Categorized by Expression Class, Where Sex Bias Is Defined As >2 -Fold Difference between Male and Female Expression (0.05 FDR).

Expression Pattern	Autosomes	Z Chromosome
Two-species comparison		
Female biased		
Nonsynonymous substitutions	96,082	3,850
Synonymous substitutions	257,540	8,649
Unbiased		
Nonsynonymous substitutions	500,401	26,669
Synonymous substitutions	1,261,990	59,402
Male biased		
Nonsynonymous substitutions	119,654	10,729
Synonymous substitutions	252,280	17,509
Six-species comparison		
Female biased		
Nonsynonymous substitutions	23,313	1,144
Synonymous substitutions	74,349	2,581
Unbiased		
Nonsynonymous substitutions	117,456	9,706
Synonymous substitutions	357,849	20,312
Male-biased		
Nonsynonymous substitutions	26,616	3,028
Synonymous substitutions	69,363	5,285

Table 5. Divergence Estimates for Chromosomal Classes with Different Expression Categories for 1:1 Zebra Finch–Chicken Orthologs.

Expression Pattern	Chromosome Class	Number of Loci	Mb	d_N	d_S	d_N/d_S
Female-biased	Autosomes	1,592	2.10	0.0616	0.486	0.126*
	Z chromosome	57	0.075	0.0692	0.438	0.158
Unbiased	Autosomes	5,931	10.29	0.0652	0.483*	0.135
	Z chromosome	298	0.52	0.0696	0.437	0.159
Male-biased	Autosomes	1,339	2.11	0.0763*	0.463	0.165*
	Z chromosome	105	0.16	0.0934	0.437	0.214

Sex-biased expression refers to genes with >2-fold differences between males and females at 0.05 FDR. Autosomal classes that are significantly different ($P < 0.05$ based on permutation testing, 1,000 replicates) from Z chromosome are indicated (*).

autosomal genes ($P = 0.017$); however, this did not result in a significant overall difference in d_N/d_S . The overall strength of the Faster-Z Effect, defined as $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA})$, did not differ significantly among the expression classes (fig 2a), suggesting that Faster-Z Evolution is primarily driven by genetic drift in both sexes rather than positive selection in females.

For all significantly expressed genes in the adult gonad, we had chicken-specific divergence estimates based on the six-species alignments for 6,801 loci. The results from the six-species data were qualitatively identical to the two-species data, with Z chromosome substitution patterns (table 4) significantly different for all expression classes than that of the autosomes ($P < 0.0001$ in all cases, Fisher’s Exact Test). Additionally, Z chromosome d_N/d_S was greater in all expression classes than autosomal d_N/d_S , though significantly so only for female- ($P = 0.0080$, permutation testing with

1,000 repetitions) and male-biased genes ($P < 0.001$), as shown in table 6. Overall, there was no significant difference among expression categories in the strength of the Faster-Z Effect ($P < 0.1$, fig. 2b).

Our power analysis indicates that we had sufficient statistical power in the majority of the comparisons regarding the strength of the Faster-Z Effect. Because our comparisons indicate that there was no difference in $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA})$, the β parameter is most relevant, as it represents the probability of incorrectly failing to reject the null hypothesis that the categories are equal. Our power analysis for the two-species data indicate that we had 100% power, defined as $1 - \beta$, for all comparisons of the strength of Faster-Z among expression categories. For the chicken lineage alone based on the six-species data, the male female-biased, female-unbiased, and male-unbiased comparisons also had 100% at $\alpha = 5\%$, suggesting that overall our data set is powerful enough to accept the null hypothesis, that there is no difference among expression classes in the strength of Faster-Z Evolution, without undue risk of type II error. This points to an overall strong influence of genetic drift.

Gene Ontology

We investigated whether nonrandom distribution of gene functionalities across the genome could be influencing our results. There is some evidence of a nonrandom distribution of gene function on the Z compared with autosomes, possibly resulting from unbalanced sex-specific selection regimes (Mank and Ellegren 2009a) or residual meiotic sex chromosome inactivation in females (Schoenmakers et al. 2009). In order to determine whether our measurements of the strength of the Faster-Z effect for different expression classes was influenced by these processes, we used the chicken Gene Ontology to determine significant over or underenrichment of gene classes on the Z for female-, male-, and unbiased genes (table 7). Only one ontology term was significantly overrepresented on the Z chromosome after multiple testing correction: There were more male-biased genes on the Z that related to the endo-membrane system than would be expected of male-biased genes overall ($P_{adj} = 0.042$). Other terminologies were not significantly different after multiple testing correction.

Discussion

Here, we present the first whole-genome analysis of Faster-Z Evolution, made possible by the recent draft sequence of the zebra finch genome (Warren et al. in preparation),

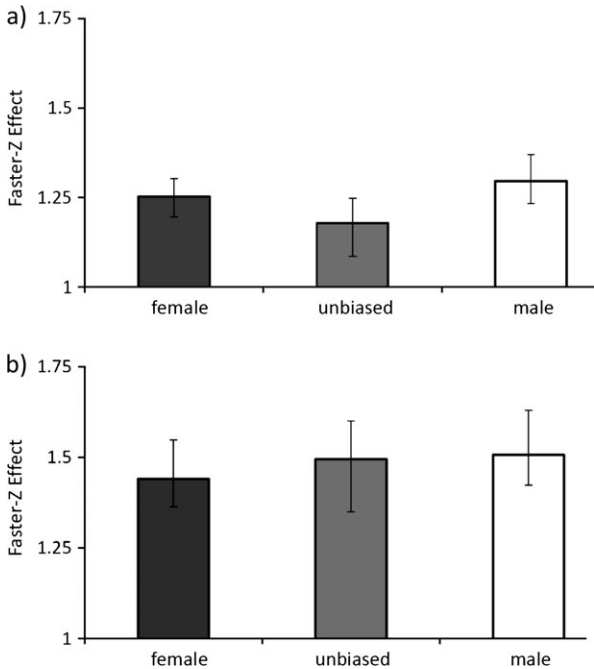


FIG. 2. Strength of Faster-Z Effect for different gene-expression classes. Shown is the relative Z to autosomal divergence $((d_{NZ}/d_{SZ})/(d_{NA}/d_{SA}))$ for each expression class of genes. Female-biased (\log_2 male/female < -1 , 0.05% FDR) genes are shown in dark gray, male-biased genes (\log_2 male/female > 1 , 0.05% FDR) are in white, and unbiased genes in light gray. Whiskers represent 95% confidence intervals based on bootstrapping (1,000 repetitions). Zebra finch–chicken data are shown in panel a, chicken-specific based on six-species alignments in panel b.

Table 6. Divergence Estimates for Chromosomal Classes within the Chicken Lineage with Different Expression Categories, Based on Six-Species Orthologs.

Expression Pattern	Chromosome Class	Number of Loci	Mb	d_N	d_S	d_N/d_S
Female-biased	Autosomes	1,153	1.34	0.0232	0.217	0.107*
	Z chromosome	41	0.050	0.0308	0.201	0.153
Unbiased	Autosomes	4,292	6.57	0.0240	0.213	0.113
	Z chromosome	236	0.38	0.0349	0.208	0.168
Male-biased	Autosomes	1,002	1.36	0.0263*	0.198	0.133*
	Z chromosome	77	0.099	0.0414	0.207	0.200

Sex-biased expression refers to genes with >2-fold differences between males and females at 0.05% FDR. Autosomal classes that are significantly different (based on permutation testing, 1,000 replicates) from Z chromosome are indicated (*).

demonstrating that Z-linked loci exhibit accelerated rates of functional evolution compared with autosomal genes (fig. 1), as predicted by theory (Charlesworth et al. 1987), and in agreement with our previous EST data (Mank et al. 2007). The majority of our data suggests that this is due to an increase in d_N for genes on the Z, rather than a decrease in Z chromosome d_S that could result from codon bias differences between the sex chromosomes and the autosomes (McVean and Charlesworth 1999), weak selection at synonymous sites (Lu and Wu 2005), or other violations of the assumptions of synonymous substitution neutrality that may preferentially influence the Z chromosome (Vicoso et al. 2008). The Z chromosome decrease in d_S seen in the comparison to all autosomes is not unexpected because the numerous microchromosomes have unusually high neutral substitution rate (Axelsson et al. 2005).

Faster-Z Evolution may result either from positive selection of hemizygotously exposed beneficial mutations in females or due to the fixation of mildly deleterious alleles due to genetic drift. We tested these alternative explanations with gene expression data in order to identify which of these evolutionary factors was the predominant force driving Faster-Z Evolution. We employed adult gonad tissue for our transcription measurement assays, as this tissue both maximizes the number of female-biased Z-linked genes (Mank and Ellegren 2009b) and minimizes the effect of meiotic Z chromosome inactivation, as the available evidence suggests that the Z is transcriptionally reactivated in females shortly after hatching (Schoenmakers et al. 2009). If Faster-Z Evolution is due to positive selection in the hemizygous sex, we would expect genes expressed predominantly in females (female-biased genes) to show the largest Faster-Z Effect, as females are heterogametic in birds. These predictions also suggest that genes expressed predominantly in males would show the least Faster-Z Effect, as there is no difference in

exposure to selection for genes on the Z and the autosomes in males. Genes expressed in both sexes equally would show an intermediate Faster-Z Effect, as they are present in females one-third of the time. Alternatively, if Faster-Z Evolution is due to genetic drift, the strength of the Faster-Z Effect would not exhibit the predicted female-biased > unbiased > male-biased evolutionary pattern and would instead be shaped by the power of purifying selection and the deleterious effect of nascent mutations, presumably affecting each expression class similarly.

Our data suggest that Faster-Z Evolution is primarily due to genetic drift, as the strength of the Faster-Z Effect is not significantly different among sex-biased expression classes (fig. 2). Our data are not suggestive of the female-biased > unbiased > male-biased predictions for the strength of Faster-Z, indicating that lack of evidence for positive selection is not due to a lack of statistical power, but rather the absence of the pattern itself. Additionally, our power analysis indicates that this lack of significant difference is not due to a lack of statistical power. This is incongruent with previous expression studies of Faster-X Evolution in *Drosophila* (Baines et al. 2008) and suggests that Faster-Z Evolution may not result from the same underlying mechanisms as Faster-X.

Is Faster-Z Evolution the Same As Faster-X?

Even though the data from *Drosophila* (Betancourt et al. 2002; Thornton and Long 2005; Begun et al. 2007; Baines et al. 2008; Vicoso et al. 2008) and mammals (Torgerson and Singh 2003; Khaitovich et al. 2005; Lu and Wu 2005; Torgerson and Singh 2006) indicate Faster-X Evolution that is analogous to Faster-Z, there are several lines of evidence suggesting that the underlying mechanisms of Faster-X and Faster-Z are not the same. Specifically, the role of genetic drift in Faster-X evolution may be less than in Faster-Z.

Table 7. Significantly Enriched Gene Ontology Terms for Sex-Biased Expression Categories on the Z Chromosome Compared with All Genes in Each Expression Class.

Expression Class	GO ID	Name	Process	P	P_{adj}
Female-biased	6508	Proteolysis	Biological	0.0090	0.42
Male biased	12505	Endomembrane system	Cellular	0.0012	0.042
	5643	Nuclear pore	Cellular	0.0024	0.11
	46930	Pore complex	Cellular	0.0033	0.16
	31975	Envelope	Cellular	0.0058	0.30
	31967	Organelle envelope	Cellular	0.0058	0.30
	5942	Phosphoinositide 3-kinase complex	Cellular	0.0061	0.55
Unbiased					

When the variance in reproductive success is equal between the sexes, the effective population size of the X (N_{eX}) or Z (N_{eZ}) chromosome is $3/4$ that of an autosome (N_{eA}). Sexual selection, acting predominantly on males and increasing the variance in male mating success, affects this ratio in opposite directions for male- and female-heterogametic species (Caballero 1995; Charlesworth 2001; Laporte and Charlesworth 2002; Vicoso and Charlesworth 2009). For male-heterogametic species, sexual selection in males would increase N_{eX} relative to N_{eA} , and this is consistent with the majority of the data demonstrating that the N_{eX} is greater than $3/4 N_{eA}$ in mammals (International SNP Map Working Group 2001; Gerald et al. 2008; Hammer et al. 2008; Lau et al. 2009; Mank et al. 2010), approaching $1N_{eA}$ in *Drosophila* (Andolfatto 2001; Connallon 2007; Singh et al. 2007). This suggests that much of Faster-X Evolution is due to positive selection in the hemizygous sex and that genetic drift is not a major contributor to the phenomenon.

Overall, the proportion of nascent mutations that are recessive and beneficial may be rather small, as suggested by the available data from male-heterogametic lineages where sexual selection narrows the difference between N_{eX} and N_{eA} and therefore the role of genetic drift. First, the virtual equality of N_{eA} and N_{eX} in *Drosophila*, combined with the overall low Faster-X Effect in this clade, suggests that the number of emergent mutations that are recessive and beneficial is limited. The Faster-X Effect is somewhat higher in eutherian mammals (Khaitovich et al. 2005; Lu and Wu 2005; Torgerson and Singh 2006; Baines and Harr 2007), and this may be due at least in part to the fact that random X chromosome inactivation in this clade renders females functionally hemizygous for cell autonomous genes, and increasing the proportion of genes subject to positive selection through hemizygous exposure (Charlesworth et al. 1987).

By contrast, sexual selection acting on males in female-heterogametic lineages reduces N_{eZ} below $3/4 N_{eA}$, consistent with polymorphism data for the Z and autosomes (Sundström et al. 2004; Borge et al. 2005). This suggests that genetic drift has the potential to play a much greater role in Faster-Z Evolution than Faster-X Evolution, as our analysis indicates and that a smaller fraction of Faster-Z Evolution is due to hemizygous exposure of recessive beneficial alleles.

Drosophila and mammals also differ from birds in that both male-heterogametic clades exhibit sex chromosome dosage compensating mechanisms (Fagegaltier and Baker 2004; Payer and Lee 2008). In contrast, birds lack global mechanisms of sex chromosome dosage compensation (Ellegren et al. 2007; Mank 2009). The lack of dosage compensation for avian sex chromosomes is predicted to strengthen the Faster-Z Effect for deleterious mutations and weaken it for beneficial variations in the coding sequence (Charlesworth et al. 1987). This prediction, which is circumstantially supported by our data, also suggests that drift is a more important force in Faster-Z than Faster-X evolution, as the Faster-X has only been investigated in clades with X chromosome dosage compensation.

The greater role of genetic drift in Faster-Z also provides a possible explanation for why sex-biased expression data

of Faster-X Evolution in *Drosophila* (Baines et al. 2008) are not convergent with our results from birds. Specifically, this type of analysis cannot differentiate the precise contribution of both positive selection and genetic drift to Faster-Z or Faster-X Evolution, but rather identify which of these two forces is more powerful overall in shaping the phenomenon. As the role of genetic drift in *Drosophila* Faster-X Evolution is negligible due to the similarity in N_{eA} and N_{eX} , positive selection is therefore the most powerful force shaping Faster-X Evolution in the clade. Our analysis cannot rule out the possibility that there is some contribution of positive selection in Faster-Z Evolution; however, the data indicate that the majority of the phenomenon is the result of increased levels of genetic drift acting on the Z chromosome, consistent with what is known about avian polymorphism patterns (Sundström et al. 2004; Borge et al. 2005) and mating systems (Bennett and Owens 2002).

Nonrandom Distribution of Gene Functionality

The rate of evolution is influenced by gene function, as some classes of genes evolve more rapidly than others, particularly genes related to immune defense and reproduction (Meiklejohn et al. 2003; Cutter and Ward 2005; Pröschel et al. 2006; Ellegren and Parsch 2007; Mank et al. 2007). Our use of the $(d_{NZ}/d_{SZ})/(d_{NA}/d_{SA})$ statistic for different types of sex-biased genes means that our measure of the Faster-Z Effect was relative, effectively normalizing across sex-bias expression categories and eliminating the potential problems associated with accelerated divergence from some classes of sex-biased genes. However, differential regulation of the sex chromosomes compared with the autosomes could influence our results.

Females experience meiotic sex chromosome inactivation in birds (Schoenmakers et al. 2009), which is analogous to male meiotic sex chromosome inactivation in male-heterogametic species (Hense et al. 2007; Turner 2007). This means that genes located on the Z chromosome are transcriptionally inactivated in meiotic cells in females. However, unlike *Drosophila* and mammals, the inactivation appears to be ephemeral in birds, with the Z reactivated in female hatchlings. Therefore, although meiotic X chromosome inactivation could influence Faster-X estimates due to demasculinization of the X chromosome (Parisi et al. 2003; Ranz et al. 2003; Emerson et al. 2004; Vinckenbosch et al. 2006; Sturgill et al. 2007; Potrzebowski et al. 2008), the expected defeminization of the avian Z is likely far less, as suggested by the fact that we did not observe differences in Gene Ontology terms between the Z and autosomes for genes functioning in female gametogenesis.

Sex-biased genes are more tissue specific than unbiased genes (Mank et al. 2008), and the excess of male-biased genes on the Z due to both the lack of dosage compensation (Ellegren et al. 2007; Itoh et al. 2007) and unbalanced sexually antagonistic selection pressure (Rice 1984; Mank and Ellegren 2009a) may mean that expression for Z-linked genes is narrower on average than that for autosomal genes. Genes with narrower expression breadth tend to have higher rates of divergence than more broadly expressed genes (Duret and

Mouchiroud 2000; Yang et al. 2005), suggesting that some of the overall Faster-Z Effect is due to this difference in expression breadth. However, it is not expected that expression breadth would affect the observed sex-specific Faster-Z Effect.

In summary, we demonstrate here that Faster-Z Evolution in birds is primarily due to genetic drift. The relative role of genetic drift in shaping Faster-Z Evolution compared with Faster-X may result from the prevalence of sexual selection pressures acting on males in birds (Kempnaers et al. 1992; Sheldon and Ellegren 1999; Pizzari and Birkhead 2000; Rutstein et al. 2004). Unlike male-heterogametic systems, where sexual selection on males increases N_{eX} above $3/4 N_{eA}$, sexual selection on males in female heterogametic systems decreases N_{eZ} below $3/4 N_{eA}$, thereby increasing the potential role of genetic drift.

Acknowledgments

We thank Björn Brunström, Hanna Göransson, Maria Rydåker, Pelle Wejaker, and Oddvar Fossum for assistance with tissue sample preparation and microarray analysis. This project was supported by the Wenner-Gren Foundation, the Swedish Research Council, and the Royal Society.

References

- Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.
- Andolfatto P. 2001. Contrasting patterns of X-linked and autosomal nucleotide variation in *Drosophila melanogaster* and *Drosophila simulans*. *Mol Biol Evol*. 18:279–290.
- Axelsson E, Hultin-Rosenberg L, Brandstrom M, Zwahlen M, Clayton DF, Ellegren H. 2008. Natural selection for avian protein-coding genes expressed in brain. *Mol Ecol*. 17:3008–3017.
- Axelsson E, Webster MT, Smith NGC, Burt DW, Ellegren H. 2005. Comparison of the chicken and turkey genomes reveals a higher rate of nucleotide divergence on microchromosomes than macrochromosomes. *Genome Res*. 15:120–125.
- Backström N, Brandström M, Gustafsson L, Qvarnström A, Cheng H, Ellegren H. 2006. Genetic mapping in a natural population of collared flycatchers (*Ficedula albicollis*): conserved synteny but gene order rearrangements on the avian Z chromosome. *Genetics* 174:377–386.
- Baines JF, Harr B. 2007. Reduced X-linked diversity in derived populations of house mice. *Genetics* 175:1911–1921.
- Baines JF, Sawyer SA, Hartl DL, Parsch J. 2008. Effects of X-linkage and sex-biased gene expression on the rate of adaptive protein evolution in *Drosophila*. *Mol Biol Evol*. 25:1639–1650.
- Bauer S, Grossman S, Vingron M, Robinson PN. 2008. Ontologizer 2.0—a multifunction tool for GO term enrichment analysis and data exploration. *Bioinformatics* 24:1650–1651.
- Begun DL, Holloway AK, Stevens K, et al. (13 co-authors). 2007. Population genomics: whole-genome analysis of polymorphism and divergence in *Drosophila simulans*. *Plos Biol*. 5:e310.
- Bennett PM, Owens IPF. 2002. Evolutionary ecology of birds: life history, mating systems, and extinction. Oxford: Oxford University Press.
- Betancourt AJ, Presgraves DC, Swanson WJ. 2002. A test for faster X evolution in *Drosophila*. *Mol Biol Evol*. 19:1816–1819.
- Borge T, Webster MT, Andersson G, Saetre GP. 2005. Contrasting patterns of polymorphism and divergence on the Z chromosome and autosomes in two *Ficedula* flycatcher species. *Genetics* 171:1861–1873.
- Burt DW, Bruley C, Dunn IC, et al. (13 co-authors). 1999. The dynamics of chromosome evolution in birds and mammals. *Nature* 402:411–413.
- Caballero A. 1995. On the effective size of populations with separate sexes, with particular reference to sex-linked genes. *Genetics* 139:1007–1011.
- Charlesworth B. 2001. The effect of life-history and mode of inheritance on neutral genetic variability. *Genet Res*. 77:157–166.
- Charlesworth B, Coyne JA, Barton NH. 1987. The relative rates of evolution of sex chromosomes and autosomes. *Am Nat*. 130: 113–146.
- Cohen J. 1988. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates.
- Connallon T. 2007. Adaptive protein evolution of X-linked and autosomal genes in *Drosophila*: implications for faster-X hypotheses. *Mol Biol Evol*. 24:2566–2572.
- Counterman BA, Ortiz-Barrientos D, Noor MAF. 2004. Using comparative genomic data to test for fast-X evolution. *Evolution* 58:656–660.
- Cutter AD, Ward S. 2005. Sexual and temporal dynamics of molecular evolution in *C. elegans* development. *Mol Biol Evol*. 22:178–188.
- Duret L, Mouchiroud D. 2000. Determinants of substitution rates in mammalian genes: expression pattern affects selection intensity but not mutation rate. *Mol Biol Evol*. 17:68–74.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 32:1792–1797.
- Ellegren H. 2009. The different levels of genetic diversity in sex chromosomes and autosomes. *Trends Genet*. 25:278–284.
- Ellegren H, Hultin-Rosenberg L, Brunström B, Dencker L, Kultima K, Scholtz B. 2007. Faced with inequality: chicken does not have general dosage compensation of sex-linked genes. *BMC Biol*. 5:40.
- Ellegren H, Parsch J. 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nat Rev Genet*. 8:689–698.
- Emerson JJ, Kaessmann H, Betran E, Long M. 2004. Extensive gene traffic on the mammalian X chromosome. *Science* 303:537–540.
- Fagegaltier D, Baker BS. 2004. X chromosome sites autonomously recruit the dosage compensation complex in *Drosophila* males. *Plos Biol*. 2:1854–1861.
- Gene Ontology Consortium. 2000. Gene ontology: a tool for the unification of biology. *Nat Genet*. 25:25–29.
- Geraldes A, Basset P, Gibson B, Smith KL, Harr B, Yu HT, Bulatova N, Ziv Y, Nachman MW. 2008. Inferring the history of speciation in house mice from autosomal, X-linked, Y-linked, and mitochondrial genes. *Mol Ecol*. 17:5349–5363.
- Griffin DK, Robertson LBW, Tempest HG, Skinner BM. 2007. The evolution of the avian genome as revealed by comparative molecular cytogenetics. *Cytogenet Genome Res*. 117:64–77.
- Grossman S, Bauer S, Robinson PN, Vingron M. 2007. Improved detection of overrepresentation of gene-ontology annotations with parent child analysis. *Bioinformatics* 23:3024–3031.
- Hammer MF, Mendez FL, Cox MP, Woerner AE, Wall JD. 2008. Sex-biased evolutionary forces shape genomic pattern of human diversity. *Plos Genet*. 4:e1000202.
- Hense W, Baines JF, Parsch J. 2007. X chromosome inactivation during *Drosophila* spermatogenesis. *Plos Biol*. 5:2288–2295.
- International Chicken Genome Sequencing Consortium. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432:695–716.
- International SNP Map Working Group. 2001. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 409:928–933.
- Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4:249–264.

- Itoh Y, Arnold AP. 2005. Chromosomal polymorphism and comparative painting analysis in the zebra finch. *Chromosome Res.* 13:47–56.
- Itoh Y, Kampf K, Arnold AP. 2006. Assignment of human X-linked genes to a zebra finch microchromosome by in situ hybridization of BAC clones. *Cytogenet Genome Res.* 112:342M.
- Itoh Y, Melamed E, Yang X, et al. (13 co-authors). 2007. Dosage compensation is less effective in birds than in mammals. *J Biol.* 6:2.
- Kempnaers B, Verheyen GR, Vandenbroeck M, Burke T, Vanbroeckhoven C, Dhondt AA. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature* 357:494–496.
- Khaitovich P, Hellmann I, Enard W, Nowick K, Leinweber M, Franz H, Weiss G, Lachmann M, Pääbo S. 2005. Parallel patterns of evolution in the genomes and transcriptomes of humans and chimpanzees. *Science* 309:1850–1854.
- Laporte V, Charlesworth B. 2002. Effective population size and population subdivisions in demographically structured populations. *Genetics* 162:501–519.
- Lau AN, Peng L, Goto H, Chemnick L, Ryder OA, Markova KD. 2009. Horse domestication and conservation genetics of Przewalski's horse inferred from sex chromosomal and autosomal sequences. *Mol Biol Evol.* 26:199–208.
- Lu J, Wu CI. 2005. Weak selection revealed by the whole-genome comparison of the X chromosome and autosomes of human and chimpanzee. *Proc Natl Acad Sci USA.* 102:4063–4067.
- Mank JE. 2009. The W, X, Y and Z of sex chromosome dosage compensation. *Trends Genet.* 25:226–233.
- Mank JE, Axelsson E, Ellegren H. 2007. Fast-X on the Z: rapid evolution of sex-linked genes in birds. *Genome Res.* 17:618–624.
- Mank JE, Ellegren H. 2009a. Sex linkage of sexually antagonistic genes is predicted by female, but not male, effects in birds. *Evolution* 63:1464–1472.
- Mank JE, Ellegren H. 2009b. All dosage compensation is local: gene-by-gene regulation of sex-bias in chicken Z chromosome expression. *Heredity* 102:312–320.
- Mank JE, Hultin-Rosenberg L, Axelsson E, Ellegren H. 2007. Rapid evolution of female-biased, but not male-biased, genes expressed in avian brain. *Mol Biol Evol.* 24:2698–2706.
- Mank JE, Hultin-Rosenberg L, Zwahlen M, Ellegren H. 2008. Pleiotropic constraint hampers the resolution of sexual antagonism in vertebrate gene expression. *Am Nat.* 171:35–43.
- Mank JE, Vicoso B, Berlin S, Charlesworth B. 2010. Effective population size and the Faster-X effect: empirical evidence. *Evolution* doi: 10.1111/j.1558-5646.2009.00853.x.
- McVean GT, Charlesworth B. 1999. A population genetic model for the evolution of synonymous codon usage: patterns and predictions. *Genet Res.* 74:145–158.
- Meiklejohn CD, Parsch J, Ranz JM, Hartl DL. 2003. Rapid evolution of male-biased gene expression in *Drosophila*. *Proc Natl Acad Sci USA.* 100:9894–9899.
- O'Brien KP, Remm M, Sonnhammer ELL. 2005. Inparanoid: a comprehensive database of eukaryotic orthologs. *Nucleic Acids Res.* 33:476–480.
- Parisi M, Nuttall R, Naiman D, Bouffard G, Malley J, Andrews J, Eastman S, Oliver B. 2003. Paucity of genes on the *Drosophila* X chromosome showing male-biased expression. *Science* 299: 697–700.
- Payer B, Lee JT. 2008. X chromosome dosage compensation: how mammals keep the balance. *Annu Rev Genet.* 42:733–772.
- Pizzari T, Birkhead TR. 2000. Female feral fowl eject sperm of subdominant males. *Nature* 405:787–789.
- Potrzebowski L, Vinckenbosch N, Marques AC, Chalmel F, Jegou B, Kaessmann H. 2008. Chromosomal gene movements reflect the recent origin and biology of the therian sex chromosomes. *Plos Biol.* 6:e80.
- Pröschel M, Zhang Z, Parsch J. 2006. Widespread adaptive evolution of *Drosophila* genes with sex-biased expression. *Genetics* 174:893–900.
- Ranz JM, Castillo-Davis CI, Meiklejohn CD, Hartl DL. 2003. Sex-dependent gene expression and evolution of the *Drosophila* transcriptome. *Science* 300:1742–1745.
- Rice WR. 1984. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* 38:735–742.
- Robinson PN, Wollstein A, Böhme U, Beattie B. 2004. Ontologizing gene-expression microarray data: characterizing clusters with gene ontology. *Bioinformatics* 20:979–981.
- Rutstein AN, Gilbert L, Slater PJB, Graves JA. 2004. Mate attractiveness and primary resource allocation in the zebra finch. *Anim Behav.* 68:1087–1094.
- Schoenmakers S, Wassenaar E, Hoogerbrugge JW, Laven JSE, Grootegoed JA, Baarends WM. 2009. Female meiotic sex chromosome inactivation in chicken. *Plos Genet.* 5:e1000466.
- Sheldon BC, Ellegren H. 1999. Sexual selection resulting from extrapair paternity in collared flycatchers. *Anim Behav.* 57:285–298.
- Shetty S, Griffin DK, Graves JAM. 1999. Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. *Chromosome Res.* 7:289–295.
- Singh ND, Macpherson JM, Jensen JD, Petrov DA. 2007. Similar levels of X-linked and autosomal nucleotide variation in African and non-African populations of *Drosophila melanogaster*. *BMC Evol Biol.* 7:202.
- Sturgill D, Zhang Y, Parisi M, Oliver B. 2007. Demasculinization of X chromosomes in the *Drosophila* genus. *Nature* 450:238–242.
- Sundström H, Webster MT, Ellegren H. 2004. Reduced variation on the chicken Z chromosome. *Genetics* 167:377–385.
- Thornton K, Long M. 2005. Excess of amino acid substitutions relative to polymorphism between X-linked duplications in *Drosophila melanogaster*. *Mol Biol Evol.* 22:273–284.
- Torgerson DG, Singh RS. 2003. Sex-linked mammalian sperm proteins evolve faster than autosomal ones. *Mol Biol Evol.* 20:1705–1709.
- Torgerson DG, Singh RS. 2006. Enhanced adaptive evolution of sperm-expressed genes on the mammalian X chromosome. *Heredity* 96:39–44.
- Turner JMA. 2007. Meiotic sex chromosome inactivation. *Development* 134:1823–1831.
- van Tuinen M, Sibley CG, Hedges SB. 2000. The early history of modern birds inferred from DNA sequences of nuclear and mitochondrial ribosomal genes. *Mol Biol Evol.* 17:451–457.
- Vicoso B, Charlesworth B. 2006. Evolution on the X chromosome: unusual patterns and processes. *Nat Rev Genet.* 7:645–653.
- Vicoso B, Charlesworth B. 2009. Effective population size and the Faster-X Effect: an extended model. *Evolution* 63:2413–2426.
- Vicoso B, Haddrill PR, Charlesworth B. 2008. A multispecies approach for comparing sequence evolution of X-linked and autosomal sites in *Drosophila*. *Genet Res.* 90:421–431.
- Vinckenbosch N, Dupanloup I, Kaessmann H. 2006. Evolutionary fate of retroposed gene copies in the human genome. *Proc Natl Acad Sci USA.* 103:3220–3225.
- Yang J, Su AI, Li WH. 2005. Gene expression evolves faster in narrowly than in broadly expressed mammalian genes. *Mol Biol Evol.* 22:2113–2118.
- Yang Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput Appl Biosci.* 13:555–556.