J Mol Evol (2010) 70:129–136
DOI 10.1007/s00239-009-9315-5

The Chicken Z Chromosome Is Enriched for Genes with Preferential Expression in Ovarian Somatic Cells

Libor Mořkovský · Radka Storchová · Jiří Plachý · Robert Ivánek · Petr Divina · Jiří Hejnar

Received: 25 September 2009 / Accepted: 15 December 2009 / Published online: 27 December 2009

Abstract Theory predicts that sexually antagonistic mutations will be over- or under-represented on the X and Z chromosomes, depending on their average dominance coefficients. However, as little is known about the dominance coefficients for new mutations, the effect of sexually antagonistic selection is difficult to predict. To elucidate the role of sexually antagonistic selection in the evolution of Z chromosome gene content in chicken, we analyzed publicly available microarray data from several somatic tissues as well as somatic and germ cells of the ovary. We found that the Z chromosome is enriched for genes showing preferential expression in ovarian somatic cells, but not for genes with preferential expression in primary oocytes or non-sex-specific somatic tissues. Our results suggest that sexual antagonism leads to a higher abundance of female-benefit alleles on the Z chromosome. No bias toward Z-linkage for oocyte-enriched genes can be explained by lower intensity of sexually antagonistic selection in ovarian germ cells compared to ovarian somatic cells. An alternative explanation would be that meiotic Z chromosome inactivation hinders accumulation of oocyte-expressed genes on the Z chromosome. Our results are consistent with findings in mammals and indicate that recessive rather than dominant sexually antagonistic mutations shape the gene content of the X and Z chromosomes.

Keywords Z chromosome · Sexual antagonism · Meiotic sex chromosome inactivation · Birds · Oogenesis · Gene expression

Libor Mořkovský and Radka Storchová have contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00239-009-9315-5) contains supplementary material, which is available to authorized users.

L. Mořkovský · R. Storchová (✉)
Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, 128 44 Prague 2, Czech Republic
e-mail: radkas@natur.cuni.cz

L. Mořkovský
Department of Cell Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic

J. Plachý · R. Ivánek · P. Divina · J. Hejnar
Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Introduction

Homogametic sex chromosomes, X and Z, are characterized by nonrandom content of genes with preferential or specific expression in one sex (hereafter sex-biased genes). This phenomenon has been described in various organisms, including human (Hurst and Randerson 1999; Lercher et al. 2003; Saifi and Chandra 1999), mouse (Divina et al. 2005; Khil et al. 2004; Mueller et al. 2008; Wang et al. 2001), Drosophila (Parisi et al. 2003; Ranz et al. 2003; Sturgill et al. 2007), *C. elegans* (Reinke et al. 2004), chicken (Kaiser and Ellegren 2006; Storchova and Divina 2006) and silkworm (Arunkumar et al. 2009). Interestingly, the proportion of sex-biased genes on the sex chromosomes is not consistent across various tissues and/or taxa (reviewed in Gurbich and Bachtrog 2008; Vicoso and Charlesworth 2006). For instance, the mammalian X chromosome is enriched for male-biased genes expressed in somatic tissues, while genes expressed in spermatogenic cells during meiosis are less abundant on this chromosome (Khil et al. 2004). In contrast, the Drosophila X chromosome is

depleted of male-biased genes regardless of their tissue expression patterns (Parisi et al. 2003; Sturgill et al. 2007). In chicken, the Z chromosome is depleted of female-biased genes in somatic as well as germinal tissues (Kaiser and Ellegren 2006; Storchova and Divina 2006).

The observed inconsistencies indicate that the sex chromosomes are shaped by several evolutionary forces whose impact differs among tissues and/or taxa. One of these forces is sexually antagonistic selection that leads to nonrandom genomic distribution of mutations with sex-biased fitness effects (Rice 1984). The accumulation of sexually antagonistic mutations in a gene will eventually lead to selection for modifier mutations that alter expression in a sex-specific manner, thus leading to genes with sex-biased expression (Connallon and Knowles 2005; Mank 2009). Sexually antagonistic selection can lead either to over-representation or under-representation of sex-biased genes on the X/Z chromosome, depending on the average dominance of sexually antagonistic mutations (Ellegren and Parsch 2007). The other factors that can affect the genomic distribution of sex-biased genes include meiotic sex chromosome inactivation in the heterogametic sex (Khil et al. 2004; Mueller et al. 2008), epigenetic modifications of the X chromosome associated with dosage compensation (Rogers et al. 2003; Vicoso and Charlesworth 2009) and incomplete dosage compensation in ZW species (Arnold et al. 2008; Ellegren et al. 2007; Itoh et al. 2007; Zha et al. 2008).

Disentangling the relative importance of various selective forces shaping the X/Z chromosome gene content might be a difficult task. Recently, Mank and Ellegren (2009b) introduced the first attempt to decouple the effect of incomplete dosage compensation and sexually antagonistic selection on the Z chromosome gene content in chicken. They studied genomic distribution of genes with differential expression between adult and embryonic gonads, which are expected to differ in the intensity of sexually antagonistic selection. Their results revealed that genes that are up-regulated in the adult ovary (i.e., female-benefit genes) are less abundant on the Z chromosome, while genes down-regulated in the adult ovary (i.e., female-detriment genes) are enriched on Z (Mank and Ellegren 2009b). These results are consistent with a dominant mode of inheritance for sexually antagonistic mutations. Under such scenario, female-benefit genes are expected to be under-represented on the Z chromosome, since this chromosome spends less time in females than in males. However, there is an alternative explanation that meiotic Z chromosome inactivation, i.e. transcriptional silencing of the Z chromosome during oogenesis (Schoenmakers et al. 2009), results in paucity of genes expressed in adult ovary on the Z chromosome. Meiotic Z inactivation begins in pachytene that occurs just after hatching and lasts a few days until early diplotene (Schoenmakers et al. 2009). Although reactivation of the Z chromosome has been demonstrated in the later stages of oogenesis, it is possible that some epigenetic modifications persist and influence gene expression also in adult ovaries (Schoenmakers et al. 2009).

In this study, we analyzed publicly available microarray data from several somatic tissues as well as somatic and germ cells of the adult ovary. These data allowed us to distinguish between the effects of sexually antagonistic selection and meiotic sex chromosome inactivation on the Z chromosome gene content. We assumed that sexually antagonistic selection will result in nonrandom genomic distribution of genes with preferential expression in somatic as well as germ cells of the ovary, as both groups of genes are expected to have female-benefit effect. These genes can be either less or more abundant on the Z chromosome depending on the average dominance of sexually antagonistic mutations. On the other hand, meiotic Z chromosome inactivation is expected to result in lower abundance of genes expressed in ovarian germ cells but should not affect genomic distribution of genes expressed in ovarian somatic cells or other somatic tissues.

Methods

Chicken Microarray Data

We used chicken microarray data publicly available in Gene Expression Omnibus repository (http://www.ncbi.nlm.nih.gov/GEO/—Affymetrix Chicken Genome Array platform GPL3213) from several somatic tissues including brain, heart, and liver (Ellegren et al. 2007; Itoh et al. 2007) and sorted populations of primary oocytes and granulosa cells (Elis et al. 2008). All somatic tissues were obtained from 14 or 18 days old embryos. Granulosa cells were collected from mature preovulatory (F1) follicles of adult hens. Primary oocytes came from F1 follicles and just ovulated oocytes. Since populations of F1 oocytes were contaminated with granulosa cells (Elis et al. 2008), we used only data from ovulated primary oocytes that were contamination-free in our analysis. Only microarray data from female individuals were included in the analysis to eliminate the effect of differential expression of Z-linked genes in males and females due to incomplete dosage compensation. The extent of local gene-specific dosage compensation on the Z chromosome is similar across the somatic tissues and gonads (Mank and Ellegren 2009a; Melamed and Arnold 2007) and should not influence the selection of tissue-enriched genes. The GEO samples used in this study are listed in Table 1.

Table 1 A list of chicken microarray data used in the analysis

Tissue	GEO Sample IDs	Publication
Brain	GSM157823, GSM157824, GSM157825, GSM157826, GSM157827	Itoh et al. (2007)
Liver	GSM157879, GSM157881, GSM157882, GSM157884, GSM157886	Itoh et al. (2007)
Heart	GSM157809, GSM157811, GSM157813, GSM157815, GSM157817	Itoh et al. (2007)
Brain	GSM215459, GSM215460, GSM215461, GSM215462	Ellegren et al. (2007)
Heart	GSM215473, GSM215474, GSM215475, GSM215476	Ellegren et al. (2007)
Primary oocytes	GSM189423, GSM189424	Elis et al. (2008)
Granulosa cells	GSM189428, GSM189427	Elis et al. (2008)

Normalization and Annotation of Microarray Data

Data analysis was performed using the software Bioconductor 2.1 (http://www.bioconductor.org/) (Gentleman et al. 2004) and R project for statistical computing (version 2.6.0, http://www.r-project.org/). The probes were annotated to Ensembl gene identifiers (Ensembl release 46) using the custom chip description file (cdf) "ggchickenggensg" version 10.0.0 (Dai et al. 2005) from the Bioconductor repository. This file contains 11,998 probe sets assigned to Ensembl genes with known chromosomal location. Expression levels were calculated using GC robust multi-array average (gcRMA) with the help of the probe sequence (Irizarry et al. 2003). For the comparison of microarray data from different experiments, the gcRMA preprocessing step was performed for all the data in one batch.

Statistical Analysis

We used Linear Models for Microarray Data Package, "limma" version 2.12.0 (Smyth 2005), for statistical evaluations of expression differences. A linear model was fitted for each gene in a given series of arrays using the lmFit function. To rank the differential expression of genes, we applied the eBayes function of the empirical Bayes method. A correction for multiple testing was performed using the Benjamini and Hochberg false-discovery-rate (FDR) method. Adjustment for multiple testing between tissues was done by the nested *F*-test approach (decideTests parameter method set to "nestedF" in limma).

Tissue-enriched genes (also referred to as genes with preferential/predominant expression in a given tissue) were defined as genes fulfilling the following criteria: (1) Significantly higher expression in a given tissue compared to any other tissue in the analysis, (2) fold-change value higher than three or five in a given tissue than in any other tissue in the analysis, and (3) average expression level higher than 100. This level is often used to discriminate between true expression and signal noise in microarray data. Because of a limited number of tissues used in our analysis and embryonic origin of non-sex-specific tissues, our selection of tissue-enriched genes should be considered as approximate. The observed and expected numbers of tissue-enriched genes on individual chromosomes were compared using the χ^2 test. Expected gene counts were calculated using total numbers of annotated genes on GPL3213 GeneChips using "ggchickenggensg" annotation.

The MicroArray Quality Control (MAQC) project has demonstrated high consistency among data from Affymetrix GeneChips prepared in different laboratories, which justifies intraplatform comparisons (MAQC Consortium 2006). Nevertheless, to independently asses whether differences among microarray data prepared in different laboratories had not introduced any bias into the analysis, we performed normalization and selection of tissue-enriched genes in two analyses, each time using microarray data partially prepared in different laboratories. In the first analysis, we included expression data from brain and heart prepared in the laboratory of A. P. Arnold (Itoh et al. 2007). In the second analysis, we included the data from brain and heart prepared in the laboratory of H. Ellegren (Ellegren et al. 2007). The data from liver, oocytes and granulosa cells were the same in both analyses (Itoh et al. 2007; Elis et al. 2008). Our results show that 52–82% of tissue-enriched genes were identical in both analyses (Supplementary Fig. 1). We then performed the third analysis with the combined data set. All statistical analyses were performed separately for all three data sets. Because we obtained the same results in terms of genomic distribution of tissue-enriched genes for all three data sets, only results from the combined data set are presented. Results for the data set 1 and data set 2 are shown in Supplementary material. Consistent results across analyses suggest that the observed patterns in genomic distribution of tissue-enriched genes are unlikely to be caused by differences in microarray data prepared in different laboratories.

Results

To clarify the effects of sexually antagonistic selection and meiotic Z chromosome inactivation on the Z

chromosome gene content, we analyzed genomic distribution of genes with preferential expression in several non-sex-specific tissues as well as somatic and germ cells of the ovary (hereafter tissue-enriched genes). Tissue-enriched genes were selected using two different fold-change thresholds (fold-change 3 and fold-change 5) (see Methods). Our results show that genes with preferential expression in granulosa cells, which represent ovarian somatic cells, are 3.4 times (for fold-change 3) or 4.1 times (for fold-change 5) more common on the Z chromosome than expected assuming random genomic distribution. This represents significant enrichment (χ^2 test, $p < 0.001$, Table 2). Interestingly, such enrichment was not found for genes with preferential expression in primary oocytes (χ^2 test, $p > 0.05$, Table 2). Genes with predominant expression in non-sex-specific tissues, including brain, heart and liver, also did not show any significant deviations from expected distribution (χ^2 test, $p > 0.05$, Table 2). Enrichment of the Z chromosome for genes with preferential expression in granulosa cells was also significant after applying Bonferroni correction for multiple comparisons ($p < 0.01$; in total 20 tests were performed).

To explore whether the nonrandom genomic distribution of genes with preferential expression in granulosa cells is specific for the Z chromosome, we compared proportions of tissue-enriched genes on individual chromosomes (Fig. 1). Only chromosomes with more than 350 annotated genes were included into the analysis. These chromosomes involved the Z chromosome and autosomes 1–9. χ^2 test was then used to compare the observed and expected numbers of tissue-enriched genes on individual chromosomes. The only tissue that showed significant deviations from random genomic expectations when Bonferroni correction for multiple comparisons was applied were granulosa cells (χ^2 test, df = 9, $p < 0.00001$). The significant deviation from expected gene counts was mostly due to over-representation of genes with preferential expression in granulosa cells on the Z chromosome (Fig. 1 and Table 3). When the Z chromosome was excluded from the analysis, no significant deviations from expected gene counts were observed (χ^2 test, df = 8, $p > 0.05$). Our results thus suggest that nonrandom distribution of genes with predominant expression in granulosa cells is specifically related to the Z chromosome.

Table 2 Observed and expected numbers of tissue-enriched genes on the autosomes (A) and the Z chromosome (Z)

Tissue	Fold-change	Observed			Expected		p*
		A	Z	%Z	A	Z	
Brain	3	423	19	4.30	421	21	0.8714
	5	324	14	4.14	322	16	0.8518
Heart	3	205	6	2.84	201	10	0.4445
	5	141	5	3.42	139	7	0.7681
Liver	3	455	23	4.81	455	23	0.8799
	5	364	11	2.93	357	18	0.2558
Granulosa cells	3	212	41	16.21	241	12	**0.0000**
	5	125	29	18.83	147	7	**0.0002**
Primary oocytes	3	316	14	4.24	314	16	0.8518
	5	158	8	4.82	158	8	0.7978

* χ^2 test, df = 1, significant values are indicated in bold

The observed enrichment of the Z chromosome for genes with preferential expression in granulosa cells could in principle be caused by a higher duplication rate of these genes on the Z chromosome rather than more frequent fixation of new female-benefit mutations on this chromosome. To exclude this possibility, we analyzed chromosomal location and homology of 41 Z-linked genes showing preferential expression in granulosa cells (fold-change 3) (Supplementary Table 3). Using Ensembl genome browser, we found that only two of these genes, ENSGALG00000012575 and ENSGALG00000015184, were paralogous. Both genes are located next to each other on the chromosome, suggesting that they might have originated by tandem duplication. Nevertheless, the presence of these two paralogues cannot explain the marked over-representation of granulosa cell-enriched genes on the Z chromosome.

Discussion

To elucidate the selective forces that shape the Z chromosome gene content in chicken, we analyzed genomic distribution of genes with preferential expression in several non-sex-specific tissues as well as somatic and germ cells of the ovary. We found that the Z chromosome is enriched for genes with preferential expression in granulosa cells—the ovarian somatic cells—but not for genes with predominant expression in non-sex-specific tissues or genes with preferential expression in primary oocytes. The observed enrichment of the Z chromosome for genes with preferential expression in granulosa cells cannot be attributed to recent duplications of these genes on the Z chromosome. Instead, we suggest that sexually antagonistic selection resulting in higher abundance of female-benefit alleles on the Z chromosome is responsible for the observed pattern. Below, we compare our results with previous studies on the X and Z chromosome gene content and discuss our findings in the light of theories on the evolution of sex chromosome gene content.

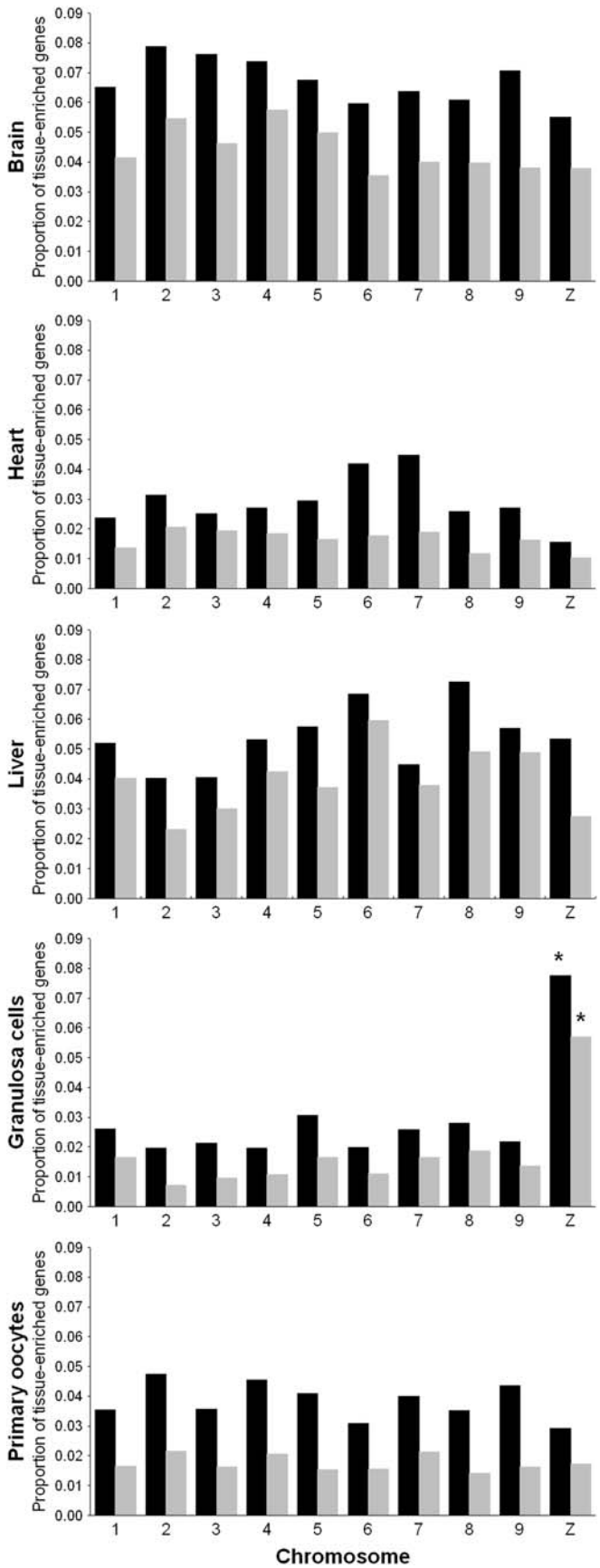

Fig. 1 The proportions of tissue-enriched genes on individual chromosomes. Only chromosomes with more than 350 annotated genes are shown. Black and gray bars represent genes with fold-change three and five, respectively. Granulosa cells were the only tissue showing significant deviations from random genomic expectations when Bonferroni correction for multiple testing was applied (χ^2 test, df = 9, $p < 0.00001$). This deviation was mostly due to a higher abundance of tissue-enriched genes on the Z chromosome

Table 3 Observed (O) and expected (E) numbers of genes with preferential expression in granulosa cells on individual chromosomes

Chr	Fold-change 3			Fold-change 5		
	O	E	$(O - E)^2/E$	O	E	$(O - E)^2/E$
1	38	36	0.15	25	22	0.50
2	16	24	2.41	7	14	3.75
3	15	22	2.16	8	13	2.12
4	17	19	0.30	8	12	1.24
5	21	17	1.21	10	10	0.00
6	7	10	0.68	4	6	0.57
7	7	9	0.42	6	5	0.06
8	8	9	0.11	6	5	0.05
9	6	8	0.40	5	5	0.02
Z	41	12	67.68	29	7	62.41
Sum	176	164	75.52	108	100	70.70

* χ^2 test, df = 9, $p < 0.000001$

Only chromosomes with more than 350 annotated genes are shown

Sexually Antagonistic Selection and the Dominance of Sexually Antagonistic Mutations

Theoretical models predict that sexually antagonistic selection should result in a higher abundance of male-advantageous alleles on the X chromosome if new beneficial mutations are on average recessive (Rice 1984). The reason is that male-advantageous recessive mutations are more likely to become fixed on the X chromosome than on autosomes, because they are immediately exposed to selection in hemizygous males. On the other hand, if mutations are on average dominant, the X chromosome is expected to become enriched for female-advantageous alleles and depleted of male-advantageous alleles because this chromosome spends two-thirds of its time in males, but only one-third in females (Rice 1984). By analogy, the Z chromosome should become enriched for female-benefit alleles if new mutations are mostly recessive, but depleted of female-benefit alleles if new mutations are mostly dominant. Since the level of dominance of new sexually antagonistic mutations is not known, the effect of sexually antagonistic selection is difficult to predict (van Doorn 2009; Vicoso and Charlesworth 2006).

Empirical results provide mixed evidence on the role of sexually antagonistic selection in the evolution of sex chromosome gene content. Consistently with a dominant mode of inheritance for sexually antagonistic genes, Mank and Ellegren (2009b) found that genes that are up-regulated in the adult ovary relative to the embryonic ovary (i.e., female-benefit genes) are under-represented on the avian Z chromosome, while genes down-regulated in the adult ovary compared to the embryonic ovary (i.e., female-detriment genes) are over-represented on the Z chromosome. The paucity of male-biased genes on the Drosophila X chromosome is also consistent with the dominant nature of sexually antagonistic mutations (Parisi et al. 2003; Ranz et al. 2003; Sturgill et al. 2007). The data from mammals, however, show just the opposite pattern. Male-biased genes expressed in somatic tissues or pre-meiotic germ cells are more common on the X chromosome, indicating that sexually antagonistic mutations are on average recessive (Divina et al. 2005; Khil et al. 2004; Lercher et al. 2003; Wang et al. 2001).

The inconsistency of these results could be explained by various epigenetic effect that shape the X/Z chromosome gene content alongside with sexually antagonistic selection. Thus, under-representation of male-biased genes on the Drosophila X chromosome might be caused by up-regulation of the single X chromosome in males owing to dosage compensation (Baker et al. 1994). This up-regulation might limit further increase in X-linked gene expression in males, resulting in the paucity of male-biased genes on the X chromosome (Rogers et al. 2003; Vicoso and Charlesworth 2009). In chicken, the paucity of genes up-regulated in the adult ovary relative to the embryonic ovary as well as enrichment of genes down-regulated in the adult ovary compared to the embryonic ovary on the Z chromosome could be related to meiotic inactivation of the Z chromosome (Schoenmakers et al. 2009) rather than sexually antagonistic selection. Indeed, the observation that genes with differential expression between embryonic and adult gonads show nonrandom genomic distribution only in females but not in males (Mank and Ellegren 2009b) is more compatible with the action of meiotic Z chromosome inactivation than with sexually antagonistic selection that is expected to affect both female-effect and male-effect genes (Ellegren and Parsch 2007).

In this study, we show that genes with preferential expression in granulosa cells, that are expected to have female-benefit effect, are more abundant on the Z chromosome. This is consistent with results from mammals (Divina et al. 2005; Khil et al. 2004; Lercher et al. 2003; Wang et al. 2001) and indicates that recessive rather than dominant sexually antagonistic mutations drive the evolution of the X and Z chromosomes. The question still remains why the Z chromosome is not enriched also for genes with preferential expression in primary oocytes. Because primary oocytes and granulosa cells together form one functional unit, the ovary follicle, the level of sexual antagonism in both cell types is expected to be similar. We thus suggest that meiotic sex chromosome inactivation might hinder accumulation of oocyte-expressed genes on the Z chromosome.

Meiotic Sex Chromosome Inactivation and Defeminization of the Z Chromosome

In organisms with heterogametic males, the X chromosome is subject to meiotic sex chromosome inactivation in the male germline (McKee and Handel 1993; Turner 2007). In mammals, meiotic X inactivation begins at pachytene and although partial reactivation occurs after the prophase I, about 87% of X-linked genes remain suppressed post-meiotically (Namekawa et al. 2006). Transcriptional silencing of the X chromosome during male meiosis has also been observed in Drosophila (Hense et al. 2007) and *C. elegans* (Kelly et al. 2002). It has been suggested that meiotic X chromosome inactivation is the most likely explanation for the paucity of spermatocyte- and spermatid-expressed genes on the mammalian X chromosome (Emerson et al. 2004; Khil et al. 2004; Mueller et al. 2008; Potrzebowski et al. 2008) and might contribute to under-representation of male-biased genes on the X in Drosophila (Betran et al. 2002; Parisi et al. 2003; Ranz et al. 2003; Sturgill et al. 2007; Vibranovski et al. 2009) and *C. elegans* (Reinke et al. 2004).

In birds, the Z chromosome becomes transcriptionally silenced during pachytene and remains inactive until early diplotene (Schoenmakers et al. 2009). This period occurs just after hatching and lasts approximately 5–6 days. It has been suggested that reactivation of the Z chromosome at early diplotene may be necessary to allow expression of genes that are required for further maintenance and growth of the large and long-living oocytes (Namekawa and Lee 2009). However, it is possible that, similarly as in mammals, the Z chromosome is not reactivated completely and that some Z-linked genes might be transcriptionally suppressed even in later stages of oogenesis. This could result in the relative paucity of genes with preferential expression in adult oocytes on the Z chromosome. Alternatively, even a short period of Z chromosome inactivation might create selective pressure against Z-linkage of genes expressed in the female germline.

Our results thus suggest that genes expressed in primary oocytes can be exposed to two evolutionary forces with opposite effects: Sexual antagonism that increases Z-linkage and meiotic Z inactivation that decreases it. Balance between these two forces might explain why we did not observe any bias in Z-linkage for oocyte-expressed genes. Further studies on gene expression changes during the course of oogenesis should shed more light on the evolutionary forces shaping the Z chromosome gene content.

Acknowledgments We thank Šárka Takáčová for linguistic revision of the manuscript. This work was supported by junior grant GAAV (KJB501110701) to RS and by the Ministry of Education, Youth and Sports of the Czech Republic (MSM0021620828).

References

Arnold AP, Itoh Y, Melamed E (2008) A bird's-eye view of sex chromosome dosage compensation. Annu Rev Genom Hum Genet 9:109–127

Arunkumar KP, Mita K, Nagaraju J (2009) Silkworm Z chromosome is enriched in testis-specific genes. Genetics 182:493–501

Baker BS, Gorman M, Marin I (1994) Dosage compensation in Drosophila. Annu Rev Genet 28:491–521

Betran E, Thornton K, Long M (2002) Retroposed new genes out of the X in Drosophila. Genome Res 12:1854–1859

Connallon T, Knowles LL (2005) Intergenomic conflict revealed by patterns of sex-biased gene expression. Trends Genet 21:495–499

Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, Bunney WE, Myers RM, Speed TP, Akil H, Watson SJ, Meng F (2005) Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res 33:e175

Divina P, Vlcek C, Strnad P, Paces V, Forejt J (2005) Global transcriptome analysis of the C57BL/6 J mouse testis by SAGE: Evidence for nonrandom gene order. BMC Genom 6:29

Elis S, Batellier F, Couty I, Balzergue S, Martin-Magniette ML, Monget P, Blesbois E, Govoroun MS (2008) Search for the genes involved in oocyte maturation and early embryo development in the hen. BMC Genom 9:110

Ellegren H, Parsch J (2007) The evolution of sex-biased genes and sex-biased gene expression. Nat Rev Genet 8:689–698

Ellegren H, Hultin-Rosenberg L, Brunstrom B, Dencker L, Kultima K, Scholz B (2007) Faced with inequality: Chicken do not have a general dosage compensation of sex-linked genes. BMC Biol 5:40

Emerson JJ, Kaessmann H, Betran E, Long M (2004) Extensive gene traffic on the mammalian X chromosome. Science 303:537–540

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J (2004) Bioconductor: Open software development for computational biology and bioinformatics. Genome Biol 5:R80

Gurbich TA, Bachtrog D (2008) Gene content evolution on the X chromosome. Curr Opin Genet Dev 18:493–498

Hense W, Baines JF, Parsch J (2007) X chromosome inactivation during Drosophila spermatogenesis. PLoS Biol 5:e273

Hurst LD, Randerson JP (1999) An eXceptional chromosome. Trends Genet 15:383–385

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, Melamed E, Yang X, Kampf K, Wang S, Yehya N, Van Nas A, Replogle K, Band MR, Clayton DF, Schadt EE, Lusis AJ, Arnold AP (2007) Dosage compensation is less effective in birds than in mammals. J Biol 6:2

Kaiser VB, Ellegren H (2006) Nonrandom distribution of genes with sex-biased expression in the chicken genome. Evolution 60:1945–1951

Kelly WG, Schaner CE, Dernburg AF, Lee MH, Kim SK, Villeneuve AM, Reinke V (2002) X-chromosome silencing in the germline of *C. elegans*. Development 129:479–492

Khil PP, Smirnova NA, Romanienko PJ, Camerini-Otero RD (2004) The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. Nat Genet 36:642–646

Lercher MJ, Urrutia AO, Hurst LD (2003) Evidence that the human X chromosome is enriched for male-specific but not female-specific genes. Mol Biol Evol 20:1113–1116

Mank JE (2009) Sex chromosomes and the evolution of sexual dimorphism: Lessons from the genome. Am Nat 173:141–150

Mank JE, Ellegren H (2009a) All dosage compensation is local: Gene-by-gene regulation of sex-biased expression on the chicken Z chromosome. Heredity 102:312–320

Mank JE, Ellegren H (2009b) Sex-linkage of sexually antagonistic genes is predicted by female, but not male, effects in birds. Evolution 63:1464–1472

MAQC Consortium (2006) The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol 24:1151–1161

McKee BD, Handel MA (1993) Sex chromosomes, recombination, and chromatin conformation. Chromosoma 102:71–80

Melamed E, Arnold AP (2007) Regional differences in dosage compensation on the chicken Z chromosome. Genome Biol 8:R202

Mueller JL, Mahadevaiah SK, Park PJ, Warburton PE, Page DC, Turner JMA (2008) The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. Nat Genet 40:794–799

Namekawa SH, Lee JT (2009) XY and ZW: Is meiotic sex chromosome inactivation the rule in evolution? PLoS Genet 5:e1000493

Namekawa SH, Park PJ, Zhang LF, Shima JE, McCarrey JR, Griswold MD, Lee JT (2006) Postmeiotic sex chromatin in the male germline of mice. Curr Biol 16:660–667

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

Potrzebowski L, Vinckenbosch N, Marques AC, Chalmel F, Jegou B, Kaessmann H (2008) Chromosomal gene movements reflect the recent origin and biology of therian sex chromosomes. PLoS Biol 6:e80

Ranz JM, Castillo-Davis CI, Meiklejohn CD, Hartl DL (2003) Sex-dependent gene expression and evolution of the Drosophila transcriptome. Science 300:1742–1745

Reinke V, Gil IS, Ward S, Kazmer K (2004) Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. Development 131:311–323

Rice WR (1984) Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742

Rogers DW, Carr M, Pomiankowski A (2003) Male genes: X-pelled or X-cluded? Bioessays 25:739–741

Saifi GM, Chandra HS (1999) An apparent excess of sex- and reproduction-related genes on the human X chromosome. Proc Biol Sci 266:203–209

Schoenmakers S, Wassenaar E, Hoogerbrugge JW, Laven JS, Grootegoed JA, Baarends WM (2009) Female meiotic sex chromosome inactivation in chicken. PLoS Genet 5:e1000466

Smyth G (2005) Limma: Linear models for microarray data. In: Gentleman R, Carey VJ, Huber W, Dudoit S, Irizarry RA (eds) Bioinformatics and computational biology solutions using R and bioconductor. Springer, New York, pp 397–420

Storchova R, Divina P (2006) Nonrandom representation of sex-biased genes on chicken Z chromosome. J Mol Evol 63:676–681

Sturgill D, Zhang Y, Parisi M, Oliver B (2007) Demasculinization of X chromosomes in the Drosophila genus. Nature 450:238–241

Turner JM (2007) Meiotic sex chromosome inactivation. Development 134:1823–1831

van Doorn GS (2009) Intralocus sexual conflict. Ann N Y Acad Sci 1168:52–71

Vibranovski MD, Zhang Y, Long M (2009) Out of the X chromosomal gene movement in the Drosophila genus. Genome Res 19:897–903

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) The deficit of male-biased genes on the *D. melanogaster* X chromosome is expression-dependent: A consequence of dosage compensation? J Mol Evol 68:576–583

Wang PJ, McCarrey JR, Yang F, Page DC (2001) An abundance of X-linked genes expressed in spermatogonia. Nat Genet 27: 422–426

Zha X, Xia Q, Duan J, Wang C, He N, Xiang Z (2008) Dosage analysis of Z chromosome genes using microarray in silkworm, *Bombyx mori*. Insect Biochem Mol Biol 39:315–321