CHAPTER 11

# Evolutionary interactions between sex chromosomes and autosomes

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## 11.1 Introduction

The sex chromosomes offer a genetic apparatus involved in the sex determination in many dioecious organisms. There can be heterogametically defined males and homogametically defined females (the X–Y systems, e.g. humans and *Drosophila*) or vice versa as heterogametically females and homogametically males (the Z–W systems, e.g. chickens and silkworms). The origin and evolution of sex chromosomes has been a classic topic in evolutionary genetics that has led to many interesting observations and various theories with predicting powers. From a retrospective view, three stages of pursuit with respect to the evolution of sex chromosomes have provided much progress in understanding of the process, patterns, and evolutionary forces involved. In the first stage, attention was paid more often to the member of the sex chromosomal pair with genetically suppressed recombination, Y and W. It was proposed that these highly diverged, often degenerate, chromosomes originated from autosomes (Muller 1932; Ohno 1967; Charlesworth 1978, 1991; Lucchesi 1994), with mounting evidence recently from various genetic and genomic comparisons (Charlesworth and Charlesworth 2000). In the second stage, an active exploration examined the evolutionary changes that occurred on the X chromosome. For example, the rapid-X hypothesis, with its evolutionary dynamics that interpret the rapid change of X-linked genes (Charlesworth et al. 1987), explained whether or not sexual antagonistic mutations prefer an X-linked environment (Rice 1984). These two stages of exploration gave insight into the process and mechanisms of chromosomal evolution. Since several reviews (e.g. Vicoso and Charlesworth 2006; Ellegren and Parsch 2007; Ellegren 2011) provide clear overviews of these major lines of research, we will not simply repeat what these reviews have already summarized but will focus on the discussion of a new picture that is recently emerging in a third stage of sex chromosome evolution research: the interaction between sex chromosomes and autosomes.

Whereas investigations based on the specific biology of sex chromosomes gave exciting insight and generated valuable data about the evolution of sex chromosomes, this third stage of research—exploring the interaction between coevolving sex chromosomes and autosomes—started a decade ago when a directional copying process through retroposition was observed (Betran et al. 2002). The central question raised was no longer how the two members of the sex chromosome pair and the genes encoded in them evolve by themselves, or how the sex chromosomes affect each other during evolution. The new question is whether or not the sex chromosomes and autosomes directly affect each other over evolutionary timescales. In other words, how is the evolution of the entire genome determined by evolutionary interactions between the sex chromosomes and autosomes? Three questions were derived from this newly defined problem: (1) what is the global genome-wide pattern associated with this interaction process? (2) How does the evolution of sex chromosomes globally change the gene content across the whole genome? (3) What is the evolutionary mechanism that underlies sex chromosome–autosome interactions? The pursuit of these questions presented a new angle to view, together, sex chromosome and genome

evolution. Evidence revealed that the evolution of sex chromosomes was no longer solely a consequence of the unique genetics of the sex chromosomes themselves, but a result of global interactions between sex chromosomes and autosomes. In this chapter, we will provide an overview of this emerging area of genome evolution, often rapid in nature, which has been driven by evolutionary interactions between the sex chromosomes and the autosomes.

## 11.2 Gene traffic between sex chromosome and autosomes

Male-biased genes are a class of rapidly evolving elements with high rates of origination (e.g. Swanson et al. 2001, 2004; Ellegren and Parsch 2006; Vicoso and Charlesworth, 2006). Early theoretical works described predictions of the chromosome locations of mutations from various genetic models. Notably, Rice (1984) discussed the genetic conditions in which the mutation for sexual antagonism with advantageous male but disadvantageous female effects would more likely be X-linked if it was recessive. Charlesworth et al. (1987) compared the fixation probabilities between sex chromosomal and autosomal mutations in various genetic models, showing that for recessive mutations, X-linked loci possess a fixation probability higher than autosomal loci. These theoretical results led to a conventional belief in the early 2000s that most genes for male functions might be on the X-chromosome with very limited data of genomic locations (e.g. Wang et al. 2001; Bainbridge 2003). However, such predictions on the chromosomal locations of male-biased genes were soon put into question with the analyses of genome sequence data from *Drosophila melanogaster* and humans. These genomic sequence data and analyses revealed unexpected interaction between the X chromosome and autosomes, which also impacted the genomic locations of sex-biased genes and the evolution of sex chromosomes.

### 11.2.1 Gene traffic in *Drosophila*

Soon after *D. melanogaster*, the first multicellular organism, was sequenced in 2000 (Adams et al. 2000), a computational approach to identify new genes was developed (Betran et al. 2002). Because there was only one fruit fly assembly available, the team decided to focus on the paralogous comparisons for identifying new genes created from RNA-based duplication, e.g. retroposition (Brosius 1993), in which a parental gene transcribes, processes out introns, and then adds a poly-A tail to the 3′ end of the retrogene and a pair of short duplicate sequences flanking the retrosequence. The ancestral relationship between parental and new copies can be easily discriminated by looking at the exon–intron structures: the parental copy contain introns whereas the new copy is intronless and may carry a poly-A track. Through a pairwise comparison of all annotated genes, recent gene duplicates with protein sequence identities higher than 70% were identified in the *D. melanogaster* genome. Further inspection of intron presence or absence in these duplicates identified 24 pairs of new retrogenes and parental gene pairs (Betran et al. 2002). A reduction to 50% protein identity uncovered 81 interchromosomal retroposition events (Dai et al. 2007).

This dataset of interchromosomal retropositions revealed two unexpected patterns (Table 11.1). First, there were 53% retrogenes that originated from the X-linked (X) parental copies. The proportion of X→A retropositions was remarkably higher than the proportion of genes on the X (17% in the fly genome). An expectation of neutrality that assumed the random generation and insertion of retrosequences predicted that the frequency of interchromosomal retroposition should be proportional to the numbers of genes and the lengths of chromosomes (the formula for calculating expectation was developed in Betran et al. (2002)). Thus, the observed and expected rates of retroposition across chromosomes differed significantly. Second, it was observed that 90% of new genes from the X→A retroposition evolved testis expression. This suggests that the origin of retrogenes would be related to the evolution of *de novo* testis function. By using genomic sequences of multiple *Drosophila* species, Bai et al. (2007) estimated the rate of retroposition throughout different evolutionary periods in ancestral genomes of *Drosophila* and detected no origination bursts,

**Table 11.1** Retrogenes prefer autosomal locations in *Drosophila* (Dai et al. 2007)

| | X→A | A→X | Ai → Aj | Total |
|---|---|---|---|---|
| Observation | 43 | 10 | 28 | 132 |
| Expectation | 18 | 16 | 47 | |
| Excess (%) | 132 | −37 | −40 | |
| | $\chi^2 = 39.13$, df = 2, P = $2 \times 10^{-8}$ | | | |

indicating that the process of retrosposition is a stable process with a constant rate within *Drosophila* lineages.

Retroposition also occurs within chromosomes, i.e. both retrogene and parent copies are located on the same chromosome. Dai et al. (2007) showed that retroposition events within autosomes 2 and 3 in *D. melanogaster* were actually more frequent than the retroposition between autosomes (46:28). However, contrary to the autosomes, the parental genes that are located on the X appeared to avoid inserting its retrogenes onto the X chromosome. Among 44 X-derived retrogenes, only one was re-inserted onto the X whereas the other 43 moved to autosomes 2 and 3. Thus, these within-chromosomal data further supported the interchromosomal analysis: the X chromosome tended to be avoided as an insertion site of retrogenes while a large excess of its genes fathered the retrogenes.

These described studies were primarily conducted on the retrogenes found from a single species, *D. melanogaster*, so the system was underutilized: the *Drosophila* genus consists of more than 2000 species (Powell 1997). Is the X→A retrogene traffic a general phenomenon in the entire genus? After the genomes of 12 *Drosophila* species, representative of the species in the two subgenera of *Drosophila*, were sequenced (Clark et al. 2007), Vibranovski et al. (2009a) and Meisel et al. (2009) independently investigated this problem. The former study took advantage of a gene relocation database including RNA-based duplicates independently identified by Bhutkar et al. (2007) in the 12 species and the latter created their own retrogene database via a comparison of the 12 genome sequences. Both studies revealed significant X→A retrogene traffics in non-*D. melanogaster* lineages, suggesting that this is a general phenomenon in the genus.

### 11.2.2 Gene traffic in mammals

Soon after the initial observations of X→A traffic in *Drosophila*, attempts were made to investigate whether or not a similar process of retrogene origination also existed in the genomes of humans and other mammalian species. However, two issues from previous analyses of the genomes of *Drosophila* and humans had to be considered. First, Venter et al. (2001) failed to find a pattern in their genomic analysis of retroposition between the X chromosome and autosomes, because no attempt was made to construct a theoretical expectation as a baseline for comparison with the observation. Second, in the derivation of the expected chromosomal distribution of the *Drosophila* retrogenes, the expectation that retroposition number was proportional to both the gene number and the length of donor and recipient chromosomes assumed random mutation. It was unlikely to directly test this hypothesis in *Drosophila* because of a lack of functionless retrogenes, i.e. the processed pseudogenes (Harrison et al. 2003), although it seems to be so in an indirect inference (Betran et al. 2004) by examining the distribution of the LINE-like retrotransposons (Kaminker et al. 2002).

Stimulated by these considerations, Emerson et al. (2004) investigated the chromosomal distribution of retroposition mutations by surveying the distribution of the retropseudogenes and their parents in humans. Because retropseudogenes are not functional, their fixation probabilities should follow the prediction of the neutral theory of molecular evolution (Kimura 1983), that mutation rate is equal to the rate of neutral substitution. An examination of 1859 retropseudogenes and their parents in the human genome revealed a highly significant linear regression with the number of genes per chromosome as donors and chromosome length

as recipients. This finding strongly suggested that retroposition in mammalian genomes is a random process with respect to their chromosomal distribution. Comparing 94 and 105 functional retrogenes in, respectively, human and mouse, created by interchromosomal retroposition with expected random frequencies, Emerson et al. (2004) revealed patterns unexpected from the previous analysis with *Drosophila*. Similar to *Drosophila*, there is an excess of X-linked parental genes that were copied as a retrogene onto autosomes. Different from *Drosophila*, there is an excess of retrogenes on the X-chromosome, in sharp contrast to a low rate of retroposition between autosomes (Table 11.2). Thus, the gene traffic in mammals are two-way processes between the X chromosome and autosomes. However, looking at the expression of these genes unveiled interesting patterns: the vast majority of the autosomal retrogenes which originated from the X-linked parental genes were found to be expressed in testis while an excess of the retrogenes on the X were non-sexually expressed or female-expressed (Potrzebowski et al. 2008). These bidirectional movements of retrogenes revealed the mutual impact of the X chromosome and autosomes in the fixation of new retrogenes and reorganizing the landscape of sex genes and non-sexual genes in the mammalian genome, as also seen in the mouse genome (Emerson et al. 2004).

When did the retrogene traffic start to emerge? Comparative genomic analysis of multiple mammalian species mapped the retroposition events on various branches of the mammalian phylogenetic tree (Potrzebowski et al. 2008). A high rate of retrogene origination (also see Vinckenbosch et al. 2006) was observed close to the eutherian–marsupial split 180 million years ago (mya), coincidently when the nascent sex chromosomes were formed. The expression analysis of these retrogenes out of the X chromosome was found to compensate for the silencing of their X-linked parental genes during male meiotic sex chromosome inactivation (MSCI), indicating that the MSCI is a main selective target to drive the retrogenes into the autosomes.

### 11.2.3 The cause and consequence of gene traffic

The non-random distribution of retrogenes and their parental genes discussed in the earlier sections in mammals and flies indicated that the mutation distribution was not the cause. Further functional analyses based on tissue expression revealed a potential target for natural selection: compensation for MSCI on the X chromosome that often silences the expression of X-linked parental genes is likely a selective advantage that can directionally fix the retrogenes on the autosomes. A recent population genomic analyses using the McDonald–Kreitman test (McDonald and Kreitman, 1991) on retroposed loci in *D. melanogaster* detected positive selection responsible for the significant excess of fixed X-origination events of retroposition (Schrider et al. 2011).

Retrosposition represents a copying mechanism that can transfer genes between ectopic chromosomal locations, e.g. between the X chromosome and autosomes. There are other copying mechanisms that can also facilitate gene movement to autosomes, including DNA-based duplication (e.g. Vibranovski et al. 2009a; Zhang et al. 2010a) and

**Table 11.2** Retroposition between the X chromosome and autosomes in humans (Emerson et al. 2004)

| Retroposition | Expected | Observed | Excess (%) | *P-value* |
|---|---|---|---|---|
| Parental copies from chromosomes: | | | | |
| X→ | 3.76 | 15 | 299 | 0.00012 |
| A→ | 90.24 | 79 | −12 | |
| Retrogene insertion into chromosomes: | | | | |
| →X | 3.61 | 13 | 260 | 0.00244 |
| →A | 90.39 | 81 | −10 | |

selective gene extinction on the X chromosome (Sturgill et al. 2007). These copying mechanisms, if under positive selection over a long evolutionary timescale, predict an enrichment of male expression genes on autosomes. This predicted consequence has been detected in the genomes of mammals (Khil et al. 2004) and *Drosophila* (Parisi et al. 2003; Ranz et al. 2003) in which an under-representation of male genes was observed on the X chromosome, resulting in dominant male genes on the autosomes. Recently, it was also observed that excess female genes moved to autosomes in birds (Ellegren 2011), which can be interpreted as the earlier observed depletion of female-biased genes on the Z (Kaiser and Ellegren 2006; Storchova and Divina 2006; Mank and Ellegren 2008). The bidirectional retrogene movement between the Z chromosome and autosomes was recently found to be associated with an excess of female retrogenes from the Z chromosome and an excess of male retrogenes onto the Z chromosome (Wang et al. 2011), which confirms a previously observed over-representation of testis-specific genes on the Z chromosome in these organisms (Arunkumar et al. 2009). Thus, the evolution of sex chromosomes clearly impacted the numbers and functional properties of genes in autosomes; these two chromosomes extensively interacted in the past.

## 11.3 The generality of gene traffic out of the X in the genus *Drosophila*

Gene traffic associated with testis expression raised the possibility that natural selection may have played an essential role in the distribution of sex-biased genes, suggesting that the 'out of the X' movement pattern should not be limited on the particular lineage toward *D. melanogaster* or on the particular molecular mechanism to generate new gene duplicates. Similar gene traffic should also be observed in non-*D. melanoagster* species and non-RNA-based duplication such as DNA-based duplication. Testing the generality of gene traffic requires a multiple-species genomic comparison in order to assess the ancestral and derived states of gene duplicates, which fortunately is supported by the availability of the 12 sequenced *Drosophila* species (Clark et al. 2007).

### 11.3.1 Gene traffic in Drosophilidae and RNA-based and DNA-based duplication

Vibranovski et al. (2009b) analyzed the duplicate gene database from an independent group (Bhutkar et al. 2007) who identified all duplicate events by comparing the genome sequences of the 12 *Drosophila* species (Clark et al. 2007). They differentiated between newly created copies derived from RNA-based and DNA-based duplications and mapped the traffic patterns between the X chromosome and autosomes onto the phylogenetic tree of this genus (Fig. 11.1). The distributions of the RNA-based and DNA-based duplication events in the phylogenetic tree compared to neutral expectations (Fig. 11.1) revealed that: (1) RNA-based duplication events in the non-*D. melanogaster* lineages showed significant X→A movements, as was previously found in the paralogous analysis in the *D. melanogaster* lineage (Betran et al. 2002). This analysis suggests that the gene traffic generated by RNA-based duplication is not a specific property of the *D. melanogaster* genome, but a general phenomenon in the *Drosophila* genus as represented by the sequenced twelve species. (2) Surprisingly, DNA-based duplication events identified from the 12 species also showed significant out-of-X moment. By pooling all 2003 events, 85 moved from the X chromosome to autosomes, significantly more frequently than expected at a 61.7% excess (Table 11.3).

### 11.3.2 Independent tests of gene traffic

Meisel et al. (2009) also generated a gene duplicate database using the 12 *Drosophila* species' genomes. In this valuable effort, they provided an independent test of similar issues. First, they confirmed the X–A patterns in the RNA-based duplication in these species. Second, they reported no significant X→A excess in interchromosomal distribution of DNA-based duplication events except for the excess of DNA-based movement out of the neo-X chromosome in *D. pseudoobscura*. Thus, while most observations in the Vibranovski et al. (2009b) were confirmed, there was a difference regarding most lineages in the DNA-based duplicates in this study. This difference was embedded in the different tests

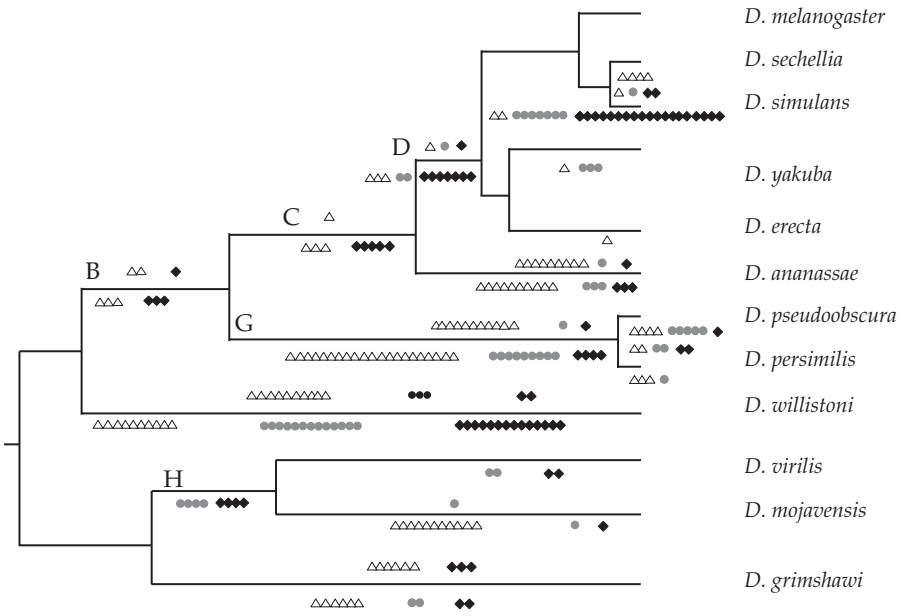


**Figure 11.1** The phylogenetic distribution of new genes created by RNA-based and DNA-based duplication in the *Drosophila* genus (Vibranovski et al. 2009). Relocations based on RNA and DNA are located above and below the branch lines, respectively. Movements between chromosomes are presented as follows: (△) X→ A; (●) A→X; (◆) Ai→Aj. The average expected proportions of these relocations are 21:23:56, respectively. For species bearing neo-X chromosome the average expected proportions are 35:34:31.

**Table 11.3** The analysis of the new genes which originated through RNA-based duplication and DNA-based duplication within the *Drosophila* genus

| | Observation | RNA-based duplication Expectation | Excess | Observation | DNA-based duplication Expectation | Excess |
|---|---|---|---|---|---|---|
| X→A | 39 | 18 | 121% | 85 | 53 | 62% |
| A→X | 9 | 16 | −43% | 52 | 62 | −16% |
| Ai→Aj | 11 | 26 | −57% | 66 | 89 | −26% |
| | | $x^2 = 36.29$, df = 2, $P = 1.32 \times 10^{-8}$ | | | $x^2 = 27.28$, df = 2, $P = 1.19 \times 10^{-6}$ | |

Note: all the branches toward *D. melanogaster* were excluded for the RNA-based duplication.

that dealt with sample size and lineage distribution. In the Meisel et al. (2009) study, individual lineages were tested individually, many of which had very small numbers of duplication events rendering the tests with very low statistical power. For example, in *D. melanogaster*, only 9–12 events were detected and used in a statistical analysis while in *D. grimshawi*, a sample size as small as four events was used. Even so, when checked, the excess in the analysis of 15 individual cases for DNA-based duplication, most of the cases (11) demonstrated a positive excess for X→A, implicating a pattern in support of the conclusion drawn by Vibranovski et al. (2009a). It appears to be safe to conclude that the two different databases independently created by Bhutkar et al. (2007) and Meisel et al. (2009) support the same conclusion: X→A gene traffic is a general property in the *Drosophila* genus, independent of duplication mechanisms (i.e. RNA-based vs. DNA-based duplication).

## 11.4 Mechanisms underlying gene traffic out of the X: the detection of meiotic sex chromosome inactivation

A few evolutionary genetic models have been proposed to discuss the roles of related evolutionary mechanisms that drive the accumulation of male-biased genes on the autosomes and can be used to interpret the gene traffic between sex chromosomes and autosomes. These include models built at the population genetic level and the molecular mechanistic level.

### 11.4.1 Evolutionary genetic models

The evolutionary models commonly discussed include sexual antagonism, faster-X evolution, and the meiotic drive model. All these models, under the assumptions of certain genetic conditions, can provide interpretations for the observed interactions between the sex chromosome and autosomes. However, no statistical tests were developed for the quantitative analyses of gene movement. In sexual antagonism, the original version, as proposed in Rice (1984), predicted the X-enrichment of antagonistic alleles that favor males and were undesirable for females if such alleles were recessive. This was not the case for the distribution of male-biased genes (Parisi et al. 2003; Ranz et al. 2003), which might represent the resolution of the conflict (Innocenti and Morrow 2010). But this, similar to the faster-X evolution for recessive advantageous alleles (Charlesworth et al. 1987), is consistent with the initial stage of the traffic, a temporal excess of young male genes, as demonstrated by Zhang et al. (2010a). Assuming the dominance of antagonistic alleles, a prediction is a higher fixation probability in autosomes, which provides an explanation of the excess X→A traffic. Recently, duplication was proposed as a mechanism to resolve the sexual antagonism in which different copies can evolve male- and female-specific functions (Ellegren and Parsch 2007; Gallach et al. 2010; Gallach and Betran 2011). An analysis of the duplication model of sexual antagonism revealed that dominance was not needed to interpret these patterns of gene movement (Connallon and Clark 2011). The meiotic-drive alternative proposed by Tao (2007a, b) predicted that autosomal retrogenes might serve as an autosomal repressor to suppress the X-linked distorter in order to ensure a normal sex ratio. In this model, the excess of autosomal retrogenes can be a result of selection against meiotic-drive.

### 11.4.2 Molecular mechanistic models

Currently, there are two mechanistic processes which may serve as target selection to avoid: MSCI which was used in Betran et al. (2002) and dosage compensation (DC) recently proposed by Vicoso and Charlesworth (2009) and Bachtrog et al. (2010). Both hypotheses are based on the idea that if some functional process is occurring on the X that prevent or reduce the expression of male-biased genes, then natural selection will favor those mutations which relocate these genes onto autosomes. The DC and MSCI hypotheses complement each other by restricting the localization of male-biased genes on the X because both are not complete processes, as shown in the observation that those X-linked regions expressing MSCI were in the regions less compensated between the sites initiating DC in *D. melanogaster* (Bachtrog et al. 2010).

The phenomenology of MSCI has been well established in mammals (Richler et al. 1992; Ayoub et al. 1997) and observed in nematodes (Kelly et al. 2002; Reinke et al. 2004) and birds (Shoenmakers et al. 2009). The recent origination of MSCI in therian was found to correlate with the starting stage of gene movement out of the X in the similar period (Potrzebowski et al. 2008) (Fig. 11.1). It should be noted that the inactivation is by no means complete, showing various degrees of reduction in the expression level in different chromosomal regions and different organisms. However, the same phenomenon and its evolutionary role were not so straightforward in the exploration.

The possibility that MSCI may exist in *Drosophila* can be traced back to the early 1970s when Lifschytz and Lindsley (1972) analyzed the relationship between sterility and chromosomal translocations in *Drosophila*. While MSCI has been identified in mammals and nematodes (Richler et al. 1992; Kelly et al. 2002), it was not until recently that supporting evidence for MSCI in *Drosophila* and chicken has been demonstrated (Hense et al. 2007; Vibranovski et al. 2009a; Schoenmakers et al. 2009). In *Drosophila*, two studies used different

approaches to measure gene activity in spermatogenesis to show the downregulation of X-linked genes (Hense et al. 2007; Vibranovski et al. 2009a). In the first study, a testis-specific reporter gene construct was inserted into different positions of the genome (Hense et al. 2007). Revealed by $\beta$- galactosidase enzymatic assays and RT-PCR (reverse transcription polymerase chain reaction) in whole *Drosophila* testis, the X-linked insertions showed significantly lower expression than those of the autosomal-linked ones, thus supporting the MSCI in *Drosophila* (Hense et al. 2007). The insertion positions were later expanded to construct a fine-scale map of the X-chromosome demonstrating that the inactivation phenomenon is spread along the entire chromosome (Kemkemer et al. 2011). In the second study, a global gene expression profile from mitotic and meiotic and postmeiotic cells from male germline was characterized via microarrays (Vibranovski et al. 2009b). Although the cells from the two first stages of spermatogenesis were not completely separated, the Bayesian analysis provided a more powerful approach than regular means-based comparisons (e.g. Sturgill et al. 2007; Meiklejoin et al. 2011) detecting a significant downregulation of X-linked genes in meiosis (Fig. 11.2). Furthermore, this analysis also revealed the compensatory expression between parental genes and retrogenes in mitotic and meiotic stages. These data, based on expression differences between mitotic and meiotic stages, provided the first opportunity to place the MSCI phenomenon in a specific meiotic phase of spermatogenesis (Vibranovski et al. 2009b). Recently, the reanalysis of *Drosophila* testis transcriptional profiles in a mutant that terminated the development of spermatogenesis in early stages of mitosis revealed a significant reduction in the expression of the X-linked genes compared to autosomes in the wild-type males, suggesting both dosage compensation in mitosis and X-inactivation in meiosis (Deng et al. 2011).

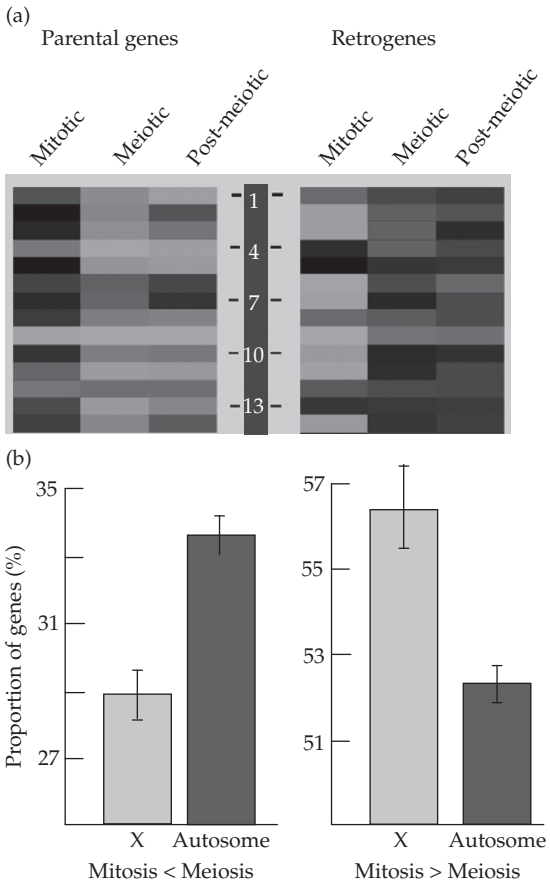


**Figure 11.2** The gene locations and MSCI. (a) Mouse parental and retrogenes (from Potrzebowski et al. 2008). The transcription profile for the 14 genes in spermatogenesis which show that the retrogenes, which are copied onto autosomes, express in the MSCI stages of meiosis and postmeiosis while the parental genes on the X-chromosome express only in the mitosis stage before MSCI. (b) *Drosophila* genes that are expressed in spermatogenesis (from Vibranovski et al. 2009b). Bayesian comparison of the genes that show higher expression in meiosis compared to mitosis revealed a significant enrichment on autosomes (the left panel: meiosis > mitosis). In contrast, a comparison of the genes that show a higher expression in mitosis than meiosis revealed that most genes are X-linked (the right panel: meiosis < mitosis). Significantly more genes show the complementary expression patterns between the mitosis and meiosis stages, as shown in mouse (a).

## 11.5 The X-recruitment of young male-biased genes and gene traffic out of the X chromosome

The previous analyses revealed that male-biased genes are under-represented on the X chromosome of *D. melanogaster* (Parisi et al. 2003; Ranz et al. 2003). In mammals, Khil et al. (2004) found that the genes expressed during the meiotic stage in the male germline were also under-represented on the X-chromosome. This genome-wide pattern was further confirmed in multiple *Drosophila* species (Sturgill et al. 2007). Consistently, gene traffic studies showed an out-of-X gene traffic pattern

where both DNA- and RNA-level autosomal duplicates tend to be male-biased if they have X-linked parental genes (Betran et al. 2002; Betran et al. 2004; Vibranovski et al. 2009a). These results may be interpreted in the sexual antagonistic model of dominant male-beneficial and female-undesirable alleles (Rice 1984) or the MSCI (Vibranovski et al. 2009b). However, a number of studies identified X-linked young testis-specific genes including *Sdic* and *Hun* originated by DNA-level duplication (Nurminsky et al. 1998; Arguello et al. 2006) and *Hydra* and four other *de novo* genes (Levine et al. 2006; Chen et al. 2007). Are these observations contradictory with the observed out-of-X gene traffic?

### 11.5.1 Age-dependence in *Drosophila*

This line of evidence suggests that the X chromosome is actively recruiting new male-biased genes regardless of its overall paucity of male-biased genes and out-of-X male-biased gene traffic. In order to test this hypothesis, we developed a genome-wide dating strategy to infer gene ages based on syntenic genomic alignments (Zhang et al. 2010a). We classified 12,856 protein-coding genes into seven different age groups and 947 (7%) young genes originated after *Sophophora* and *Drosophila* subgenus split.

We next profiled the transcriptional bias of new genes based on FlyAtlas microarray data (Chintapalli et al. 2007). After removing probes mapping to both parental gene and daughter genes (Dai et al. 2005) and identifying genes differentially expressed between testis and ovary (Gentleman et al. 2004; Smyth 2004), a stage-specific distribution of new genes with distinctive expression pattern was observed. As shown in Fig. 11.3, X-linked young genes are significantly more male-biased compared to autosomal young genes. Interestingly, a majority (70%) of recently evolved X-linked genes postdating the *D. melanogaster* and *D. yakuba* split are male-biased. With the elapse of evolutionary time, this proportion steadily declined. In contrast, autosomal young genes show a relatively stable proportion of male-biased genes. We also performed a genome-wide analysis without partitioning genes into different age groups and confirmed the overall demasculization of the X-chromosome where only 19% of X-linked genes are male-biased in contrast to 26% of autosomal genes which were

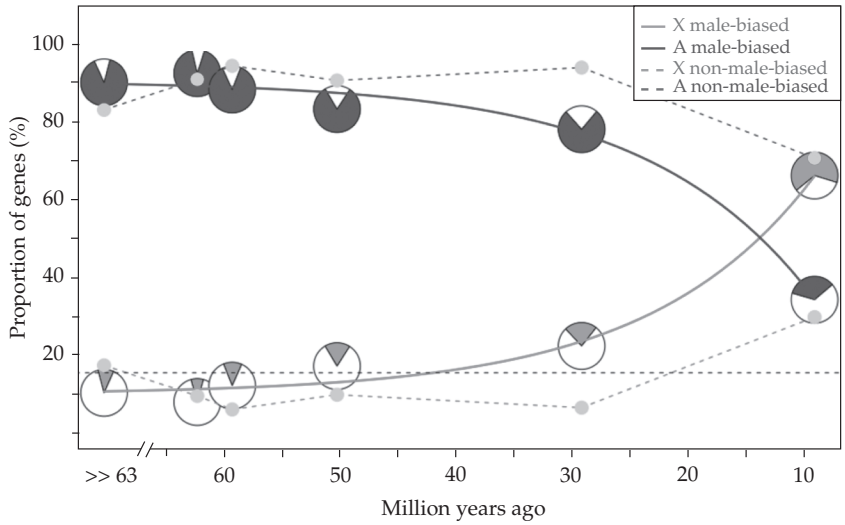


**Figure 11.3** The shift of gene expression between the X-chromosome and autosomes over evolutionary time in the *Drosophila* genus, as shown by the proportions of male-biased and non-male-biased (female-biased and unbiased combined) genes originating in different evolutionary periods (Zhang et al. 2010a). For male-biased genes, we calculated the male-biased proportion as the number of male-biased genes in a given chromosome out of the whole genome. Analogously, we calculated non-male-biased gene proportions. Pie plots mark chromosomal proportions of male-biased genes. The proportions were calculated across six different evolutionary timeframes (0–6) from ancestral lineages towards present-day *D. melanogaster*.

male-biased. We, thus, detected the early stages in which X-linked male-biased genes are dominant: X-linked dominance then decreases over time until autosomal male-biased genes establish their dominance.

### 11.5.2 Age-dependence in mammals

Since mammals and flies possess a similar XY system that may be subject to similar evolutionary processes such as sexual antagonism, faster-X, and MSCI, we expect a similar, if not identical, pattern of new gene origination with their sex-biased expression as observed in evolution of *Drosophila* new genes. Indeed, both human and mouse data show that young X-linked genes are enriched with male-biased genes and the trend becomes reversed as gene age increases until evolutionary old male-biased genes become dominant on the autosomes (Table 11.3; Zhang et al. 2010b). However, because the number of young genes (hominoid-specific or primate-specific) is only 10% the fraction of the older male-biased genes, the general pattern seen from whole testis transcriptomes in humans is that autosomal male-biased genes are in significant excess over X-linked male-biased genes. Consistent with the previous genome-wide analysis (Namekawa et al. 2006), the majority of X-linked genes (i.e. 489 old X-linked genes) are subject to MSCI where significantly more autosomal genes are transcribed in spermatocytes. This trend extends later to spermatids. In contrast, 35 young genes are not subject to MSCI where a similar proportion of young genes are expressed in spermatocyte (29% vs. 23%) and a significantly higher proportion of X-linked young genes are expressed in spermatids (71% vs. 29%). In rodent genomes, 55% of the testis genes which showed the X-linkage originated in the 10 million years (my) after mouse diverged from rat and the rest, 25%, are rodent-specific although they originated after mouse–rat divergence (Mueller et al. 2008; Zhang et al. 2010b).

### 11.5.3 The slow enrichment of X-linked female genes

In both flies and mammals, the enrichment of female-biased genes on the X-chromosome was observed in the older gene group. In *Drosophila*, for all the genes older than 63 my (predating the divergence of the two subgenera, *Sophophora* and *Drosophila*), the proportion of female genes on the X chromosome is 11% higher than autosomal females genes. On the other hand, for new genes that have originated within 63 my, only 9% of X-linked new genes evolved female-biased expression and only a few new autosomal genes were detected to have female-biased expression (Fig. 11.4). These data revealed a slow pace of female gene evolution and their preferential fixation onto the X-chromosome. A similar evolutionary process of ovary-biased genes was also

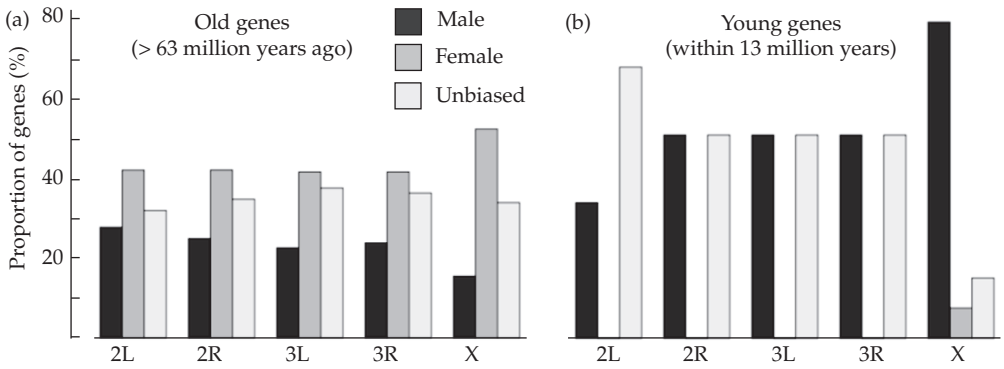


**Figure 11.4** The female enrichment of genes in *Drosophila*, as shown in the chromosomal distributions of male-biased, female-biased, and unbiased *D. melanogaster* genes. (a) Evolutionary older genes that originated 63 mya before the *Sophophora–Drosophila* subgenus split. (b) Evolutionary recent genes that originated within recent 13 my.

observed in mammalian genomes, leading to significantly higher proportion of ovary-biased genes fixed in the X than autosomes, ~180 mys (before the placental–marsupial split) (Zhang et al. 2010). However, for the genes which originated within primates, more ovary-biased genes are fixed in autosomes than the X-chromosome. In genes originating between the therian and primate divergence, there are no significant differences in female-biased gene fixation between the X and autosomes. This pattern is consistent with the detected low rate of copying autosomal parental genes onto the X by the mechanism of retroposition (Emerson et al. 2004), revealing a long evolutionary time before the X-chromosome establishes a significant enrichment of female-biased genes. Interestingly, in the chicken genome, it was observed that testis-biased genes originating during avian evolution appeared to have moved to the Z-chromosomes, thus leading to over-representation on the Z chromosome (Ellegren, 2011), consistent with the female-biased gene fixation patterns in XY sex chromosomal systems. However, it was observed that the excess of old genes which are expressed in somatic ovarian cells (Granulosa) were enriched on the Z chromosome of chicken too (Morkovsky et al. 2010). These data suggest that X/Z chromosomes have been experiencing a similar functional reorganization towards an enrichment of heterogametic sex functions, since their origination from ancestral autosomes.

## 11.6 Concluding remarks

In this chapter, we summarize over a decade of major findings from the cross-chromosomal gene traffic literature, after the initial findings that an excess of retrogenes was found copied onto autosomes from X-linked parental genes in *Drosophila*. Through these observations and analyses, extended from *Drosophila* to mammals, birds, silkworms, and nematodes, a new concept is emerging: the interaction between the sex chromosomes and autosomes has impacted the evolution of genes and genomes, continuously changing the structure of genomes in terms of gene content and their reproductive functions in the sex chromosomes and autosomes. Over a longer evolutionary timescale, genes with heterogametic sex-biased expression will establish a dominant presence in autosomes and a lower but significant excess of genes with homogametic sex-biased expression in the sex chromosomes, X and Z. In *Drosophila* and mammalian genomes with independent origins of sex chromosomes, processes of gene evolution share an evident pattern: both started from the X-linkage of dominant young male-biased genes before the trend shifted towards an autosomal dominance of male-biased genes. Furthermore, the diverse genomes of XY and ZW genetic systems evolved via symmetrical patterns of gene movements through long evolutionary processes even though their heterogameties (or homogameties) define opposite sexes. However, although it may be safe to conclude that the underlying evolutionary force to drive the interaction is positive selection, the evolutionary genetic mechanisms and selective targets are far from clear. The current data reveal that there are likely multiple factors responsible, including population genetic processes and molecular mechanisms. Mechanistic processes such as meiotic sex chromosomal inactivation and dosage compensation are better understood than population genetic processes in which no explicit statistical tests have been developed. These leave new and challenging questions to pursue the understanding of evolutionary interactions between the sex chromosome and autosomes and their roles in driving the evolution of genes, genomes, and genetic systems such as sex and reproduction.

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