Role of Testis-Specific Gene Expression in Sex-Chromosome Evolution of *Anopheles gambiae*

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ABSTRACT Gene expression in *Anopheles gambiae* shows a deficiency of testis-expressed genes on the *X* chromosome associated with an excessive movement of retrogene duplication. We suggest that the degeneration of sex chromosomes in this monandrous species is likely the result of pressures from *X* inactivation, dosage compensation, and sexual antagonism.

IN a heterogametic sex determination system, the sex chromosomes (*X* or *Z*) spend a disproportionate amount of evolutionary time in one gender over the other (Vicoso and Charlesworth 2006). For example, relative to autosome evolution, the *X* chromosome occurs twice as frequently in females compared to males; thus, new sex-linked mutations will be exposed to selection two-thirds of the time in females and only one-third in males. One expected consequence of this relationship is that the sex-related content of autosomes and sex chromosomes should be unequally distributed through the genome as a result of antagonism between the sexes (Rice 1984). It is predicted that dominant or partially dominant mutations beneficial to females (and detrimental to males) spread more easily if they are *X*-linked, whereas recessive mutations beneficial to males (and detrimental to females) should accumulate on the *X* chromosome because sexual antagonism will be masked in heterozygous females.

Even though the outcome of sexual antagonism is expected to be highly dependent on the dominance of mutations in question, several genomic studies have found significant differences in the gene content of autosomes and the *X* chromosome. First, sex-biased gene expression in somatic and germline tissue is often nonrandomly distributed among chromosomes (Parisi *et al.* 2003; Ranz *et al.* 2003; Khil *et al.* 2004). Second, several studies have reported an excess of duplicated genes moving from the *X* chromosome to the autosomes associated with testis expression (Betran *et al.* 2002; Emerson *et al.* 2004; Meisel *et al.* 2009; Vibranovski *et al.* 2009a). While sexual antagonism no doubt contributes to the observed deficiency of sex-biased genes in somatic tissues, a further consideration within the male germline is *X* inactivation. Evidence from *Drosophila* indicates that gene duplications escaping the *X* chromosome have a higher likelihood of testis expression and are retained on the autosomes because they are required during spermatogenesis (Betran *et al.* 2002; Meisel *et al.* 2009; Vibranovski *et al.* 2009a,b). Thus, *X* inactivation can generate selection for increased dosage and favor the fixation of autosomal gene duplications, whether or not they are in fact sexually antagonistic.

Heteromorphic sex chromosomes have evolved independently multiple times in the Diptera. In the mosquito *Anopheles gambiae*, males and females have fully differentiated heteromorphic sex chromosomes, whereas in *Aedes aegypi* the sex chromosomes are homomorphic and largely homologous (Krzywinski *et al.* 2004; Nene *et al.* 2007). By classifying the origin of retro-transposition events between these species, Toups and Hahn (2010) found evidence for an excess of *X*-to-*A* turnover, primarily in the *Anopheles* lineage, indicating that the *Anopheles* common ancestor had homomorphic sex chromosomes. Yet, in contrast to higher Diptera, Toups and Hahn (2010) found no evidence that *X*-to-*A* duplications are associated with male-biased gene expression, leading to the conclusion that sex-chromosome

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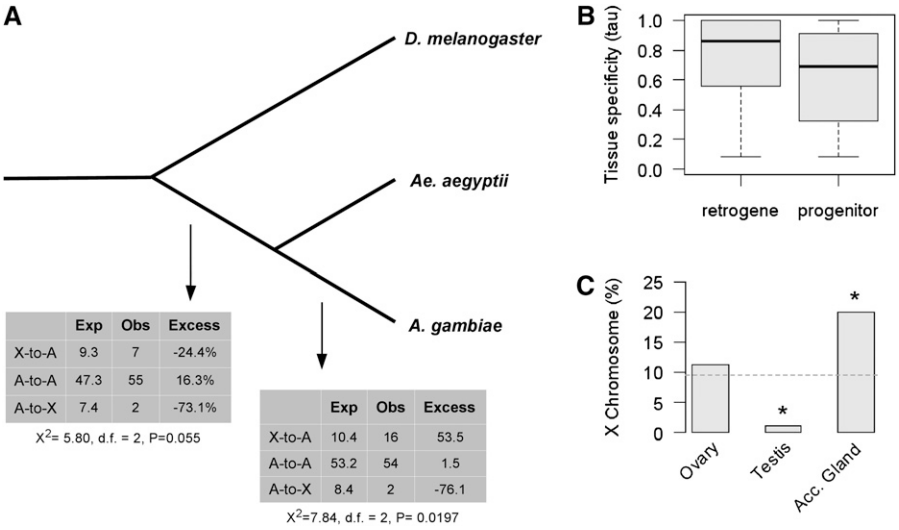

Figure 1 (A) Dipteran retrogene events. We find an excess of *X*-to-*A* retrogene events occurring within the *Anopheles* lineage, but not on the branch prior to the *Anopheles* and *Aedes* split. X, *X* chromosome. A, autosomes. Excess = $[(O - E)/E] \times 100$. (B) Tissue-specificity of retrogenes and progenitors. Retrogenes show a higher incidence of tissue-specific gene expression. Unpaired Wilcoxon test: $P < 0.05$. (C) *X*-chromosome enrichment of genes expressed in reproductive tissue. Consistent with previous studies, there is a deficiency of testis-specific genes on the *Anopheles X* chromosome, but an over-representation of male accessory gland genes (expected *X*: 9.6%; χ^2 test: $^*P < 0.05$).

evolution is not driven by patterns of sex-specific gene expression in *Anopheles* (Hahn and Lanzaro 2005; Toups and Hahn 2010). However, an important difference between *Anopheles* and other species investigated to date is that females mate only once during their life time (Tripet *et al.* 2003), a key attribute affecting male testis size (Hosken and Ward 2001). Whereas much of the sex-biased expression displayed by *Drosophila* and other polygonous species results directly from expression in the gonads (Parisi *et al.* 2003), in *Anopheles* the testis are proportionately much smaller compared to body mass, and consequently, when transcription levels are assessed at a whole-body level, male-biased expression is less pronounced.

A recently published catalog of tissue-specific expression in *Anopheles* (MozAtlas, http://www.tissue-atlas.org) reveals that <10% of testis-enriched genes are male-biased in expression because they are largely undetected in whole-body samples (Baker *et al.* 2011). This contrasts with the ovaries in which >50% of genes have female-biased expression, a finding that is likely to account for elevated levels of female transcription observed in whole-body samples (Hahn and Lanzaro 2005). As we have reported previously (Baker *et al.* 2011), this data set reveals that there is also an under-representation of genes on the *X* chromosome expressed within the testis, consistent with studies in higher Diptera (Parisi *et al.* 2003).

In light of these findings, we have re-examined the relationship between *Anopheles* retrogene duplication and male-related gene expression (supporting information, File S1 for methods). Using recent orthology assignments (Vilella *et al.* 2009), and in agreement with Toups and Hahn (2010), we observe an excess of retrogene relocations out of the *X* chromosome after the dipteran split (Figure 1A and Table S1). Importantly, while our data set is more stringent, we also find that the majority of *X*-to-*A* duplication events occur within the *Anopheles* lineage ($n = 16/23$; 53% excess). However, in contrast to earlier findings, when male-related gene expression is compared with retrogene movement, we find that testis expression is a common feature of duplications derived from the *X* chromosome (*X* to *A* = 17/18; 95%), whereas retrogenes originating from the autosomes are less likely to be expressed in the testis (*A* to *A* = 18/37; 48%) ($\chi^2 = 9.08$, d.f. = 1, $P = 0.002$). Thus, we find support for a link between the movement of gene duplications and male-related gene expression.

One expectation for models involving *X* inactivation as the underlying cause of male-related genes moving off the *X* chromosome is that ancestral *X*-linked genes should have low expression levels in the testis when compared to autosomal retrogenes. Indeed, we find that derived retrogenes have a higher incidence of tissue-specific expression than ancestral genes (Figure 1B; unpaired Wilcoxon test; $P < 0.05$). Unfortunately, this difference in itself cannot differentiate the effects of *X* inactivation *vs.* sexual antagonism because the latter also predicts a spread of male beneficial dominant mutations to the autosomes (including testis-expressed genes). However, we find that testis enrichment, in particular, is higher for retrogenes in *X*-to-*A* relocations compared to retrogenes in *A*-to-*A* duplication events, whereas ancestral genes show the opposite effect; *i.e.*, ancestral genes in *X*-to-*A* duplications have significantly lower testis enrichment in ancestral genes when compared to *A*-to-*A* duplication events (Table 1). This finding was repeated specifically for genes within the *Anopheles* lineage on the basis of testis log-fold enrichment *vs.* the carcass (Figure S1). Since sexual antagonism does not predict a difference in gene expression between autosomes on the basis of where the ancestral gene was located, deviation in testis expression of *X*-to-*A* *vs.* *A*-to-*A* retro-duplicated genes suggests the involvement of *X* inactivation (Vibranovski *et al.* 2009a).

However, a third mechanism that we must consider that could deplete male-related genes on the *X* chromosome is the action of dosage compensation systems. Dosage compensation acts to balance gene expression between the *X* chromosome and autosomes of the heterogametic sex and may contribute to the paucity of male-biased genes in

Table 1 Testis gene expression enrichment

	Progenitors (*Culicidae/Anopheles*)		Retrogenes (*Culicidae/Anopheles*)	
Direction	Enriched	Not enriched	Enriched	Not enriched
X to *A*	0/0	3/12	3/6	0/6
A to *A*	2/6	12/5	2/2	12/9

Testis enrichment calls for the *Culicidae* and *Anopheles* branches. Testis enrichment is significantly higher for retrogenes in *X*-to-*A* relocations when compared to *A*-to-*A* duplications across branches, whereas testis enrichment of ancestral genes (progenitors) in *X*-to-*A* relocations is significantly lower than in *A*-to-*A* duplications. Significant differences within progenitor and retrogene sets were determined by pooling branch samples and comparing the observed χ^2 statistic with 100,000 permuted χ^2 values via Monte Carlo simulation. See File S1 and File S2 for expression values and significance tests. For progenitors, $P(\chi^2) < 0.032$; for retrogenes, $P(\chi^2) < 0.004$.

a number of ways. For instance, when the *X* chromosome is hyper-activated in males, increased testis expression may be harder to achieve for single *X*-linked genes if transcription rates are limited, thus reducing the accumulation of male-biased genes on the *X* chromosome (Vicoso and Charlesworth 2009; Bachtrog *et al.* 2010). Alternatively, if the *X* chromosome lacks dosage compensation in males, selection for increased dosage may favor the fixation of male-related genes on the autosomes in a fashion similar to *X* inactivation during spermatogenesis. We find that while male and female somatic tissues are expressed at the same level on autosomes and on the *X* chromosome in *Anopheles* (soma: X_{female}/A_{female}: 1.07–1.29; X_{male}/A_{male}: 1.12–1.26; see Table S2), male germline expression on the *X* chromosome is half of the autosomal levels, as would be expected in the absence of dosage compensation (germline: X_{female}/A_{female}—1.05; X_{male}/A_{male}—0.48; unpaired Wilcoxon test—$P < 2.38 \times 10^{-12}$). Subsequently, unlike *Drosophila* (Gupta *et al.* 2006), we detect *X* chromosome hyper-activation only in the male soma, indicating that an absence of male germline dosage compensation may contribute to a deficiency of male-related genes on the *X* chromosome in *Anopheles*.

Taken together, these observations suggest that retrogene movement, as a result of expression in the male testis, may be a general feature of sex-chromosome evolution in the Diptera. However, lower expression of the *X* chromosome in the male germline could correspond to an absence of dosage compensation or meiotic *X* inactivation in *Anopheles*. In the future, it will be of considerable interest to measure *X* chromosome and autosomal expression in mitotic and meiotic cells of this species to help resolve which mechanisms contribute to this trend. Since *Y* chromosome degeneration appears to have evolved rapidly in *Anopheles* after its divergence from *Aedes*, further study is likely to yield important insight into how these processes and modes of sexual antagonism contribute to sex-chromosome evolution in monandrous species like the mosquito. Given that *Anopheles* appears to have evolved sex chromosomes independently of *Drosophila*, much is to be gained from understanding the generality of phenomena that we have observed.

For example, if we extend our original analysis of germline and somatic expression to consider the chromosomal distribution of genes expressed only in single tissues, unlike *Drosophila melanogaster* (Mueller *et al.* 2005), we find an excess of genes detected solely in the male accessory glands of *Anopheles* (Figure 1C and Table S3). Male accessory gland (MAG) proteins are an essential component of seminal fluid deposited into the female reproductive tract during copulation, often associated with male reproductive success (Klowden and Russell 2004; Rogers *et al.* 2008). While the relative importance of such proteins to sexual conflict in the mosquito is still debated, an over-representation of MAG proteins on the *X* chromosome suggests that at least some components of male reproductive biology are potentially antagonistic in this species. Indeed, it is predicted that recessive genes conferring a male advantage at the expense of females should be located on the *X* chromosome (Rice 1984). However, if sexually antagonistic mutations are often dominant, this could explain a deficiency of male-biased genes on the *X* chromosome, including somatically expressed genes.

Acknowledgments

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Literature Cited

Bachtrog, D., N. R. Toda, and S. Lockton, 2010 Dosage compensation and demasculinization of X chromosomes in *Drosophila*. Curr. Biol. 20: 1476–1481.

Baker, D. A., T. Nolan, B. Fischer, A. Pinder, A. Crisanti *et al.*, 2011 A comprehensive gene expression atlas of sex- and tissue-specificity in the malaria vector, *Anopheles gambiae*. BMC Genomics 12: 296.

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

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

Gupta, V., M. Parisi, D. Sturgill, R. Nuttall, M. Doctolero *et al.*, 2006 Global analysis of X-chromosome dosage compensation. J. Biol. 5: 3.

Hahn, M. W., and G. C. Lanzaro, 2005 Female-biased gene expression in the malaria mosquito *Anopheles gambiae*. Curr. Biol. 15: R192–R193.

Hosken, D. J., and P. I. Ward, 2001 Experimental evidence for testis size evolution via sperm competition. Ecol. Lett. 4: 10–13.

Khil, P. P., N. A. Smirnova, P. J. Romanienko, and R. D. Camerini-Otero, 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.

Klowden, M. J., and R. C. Russell, 2004 Mating affects egg maturation in *Anopheles gambiae* Giles (Diptera: Culicidae). J. Vector Ecol. 29: 135–139.

Krzywinski, J., D. R. Nusskern, M. K. Kern, and N. J. Besansky, 2004 Isolation and characterization of Y chromosome sequences from the African malaria mosquito *Anopheles gambiae*. Genetics 166: 1291–1302.

Meisel, R. P., M. V. Han, and M. W. Hahn, 2009 A complex suite of forces drive gene traffic from Drosophila X chromosomes. Genome Biol. Evol. 1: 176–188.

Mueller, J. L., K. Ravi Ram, A. McGraw, M. C. Bloch Gazi, E. D. Siggia *et al.*, 2005 Cross-species comparison of *Drosophila* male accessory gland protein genes. Genetics 171: 131–143.

Nene, V., J. R. Wortman, D. Lawson, B. Haas, C. Kodira *et al.*, 2007 Genome sequence of *Aedes aegypti*, a major arbovirus vector. Science 316: 1718–1723.

Parisi, M., R. Nuttall, D. Naiman, G. Bouffard, J. Malley *et al.*, 2003 Paucity of genes on the *Drosophila* X chromosome showing male-biased expression. Science 299: 697–700.

Ranz, J. M., C. I. Castillo-Davis, C. D. Meiklejohn, and D. L. Hartl, 2003 Sex-dependent gene expression and evolution of the Drosophila transcriptome. Science 300: 1742–1745.

Rice, W. R., 1984 Sex chromosomes and the evolution of sexual dimorphism. Evolution 38: 735–742.

Rogers, D. W., M. M. Whitten, J. Thailayil, J. Soichot, E. A. Levashina *et al.*, 2008 Molecular and cellular components of the mating machinery in *Anopheles gambiae* females. Proc. Natl. Acad. Sci. USA 105: 19390–19395.

Toups, M. A., and M. W. Hahn, 2010 Retrogenes reveal the direction of sex-chromosome evolution in mosquitoes. Genetics 186: 763–766.

Tripet, F., Y. T. Toure, G. Dolo, and G. C. Lanzaro, 2003 Frequency of multiple inseminations in field-collected *Anopheles gambiae* females revealed by DNA analysis of transferred sperm. Am. J. Trop. Med. Hyg. 68: 1–5.

Vibranovski, M. D., Y. Zhang, and M. Y. Long, 2009a General gene movement off the X chromosome in the *Drosophila* genus. Genome Res. 19: 897–903.

Vibranovski, M. D., H. F. Lopes, T. L. Karr, and M. Y. Long, 2009b Stage-specific expression profiling of *Drosophila* spermatogenesis suggests that meiotic sex chromosome inactivation drives genomic relocation of testis-expressed genes. PLoS Genet. 5: e1000731.

Vicoso, B., and B. Charlesworth, 2006 Evolution on the X chromosome: unusual patterns and processes. Nat. Rev. Genet. 7: 645–653.

Vicoso, B., and B. Charlesworth, 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.

Vilella, A. J., J. Severin, A. Ureta-Vidal, L. Heng, R. Durbin *et al.*, 2009 EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. Genome Res. 19: 327–335.

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FILE S1

Supporting Methods

Identifying retro-duplication events.

Using Ensembl Metazoa gene-tree reconstructions (April 2011 Release 8; Villella et al., 2009), we screened for gene families in which one gene contained multiple exons (parental gene), while one or more other members of the same family contained a single exon (retrogenes). We make the assumption that genes without introns represent retro-duplication events, and that the most closely related parental gene by amino acid sequence similarity is the progenitor. Branches were assigned on the basis of tree topology and classified as diverging either before or after the *Culicidae* split. All duplication events occurring prior to the Dipteran split were not included in our analysis. Furthermore, unlike the analysis of Toups and Hahn (2010), we restricted our dataset to gene pairs in which progenitors share at least 20% sequence similarity, and exclude families of Odorant Binding proteins, given that such gene families are known to evolve by a mechanism other than retro-duplication. Subsequently, our retro-duplication dataset is more stringent than previously published by Toups and Hahn (2010) and includes 54 fewer duplication events. See File S2 for the complete gene set.

Excess of retrogene events.

We re-calculated the expected frequency of retro-duplication events among chromosomes in *Anopheles*, using the equation outlined in Betran et al. (2002) and the estimated size of chromosomes (euchromatin) from Sharakhova et al. (2010). The expected frequency (P_{KL}) indicates the direction of gene duplication events from the parental gene to the new gene (i.e. $P_{A\text{-}X}$, $P_{X\text{-}A}$, $P_{A\text{-}A}$):

$$P_{KL} = \frac{\sum Ni\, Lj\, fij}{\sum\sum Ni\, Lj\, fij}$$

Ni is the proportion of genes at source chromosome *i*, and *Lj* is the proportion of euchromatin in the target chromosome *j*. The frequency of retro-transposition type to chromosomes in the population is given by *fij*. According to *Anopheles* genomic data and the existence of males and females in the population the relative population sizes of the X chromosome and autosomes are displayed in Table S1. The expected frequency of $P_{X\text{-}A}$, $P_{A\text{-}A}$ and $P_{A\text{-}X}$ retro-duplication events are 14.5%, 73.9% and 11.6% respectively.

Testis expression breadth and enrichment.

All estimates of gene expression were obtained from the MozAtlas dataset (www.tissue-atlas.org) re-mapped onto AgamP3.6 gene build (December 2010). Testis-specific transcription was first determined using probe detection in which genes were classified as specific if at least 3 out of 4 mismatch calls were apparent from Affymetrix microarray chips. To explore expression between retrogenes and their progenitors, a second measure of tissue breadth was calculated by normalizing transcription amongst tissues against maximal expression to generate the tau-statistic (Yanai et al., 2005). The resulting values fall with the range of 0 to 1, in which higher values indicate greater tissue-specific expression. Testis expression enrichment was determined with a linear model of gene expression fit to male testis and carcass samples using the R statistics package, LIMMA (Smyth, 2004). Only probes giving

at least 3 out of 4 mismatch calls in dissected tissues were used in the analysis.

Comparison of testis enriched expression in ancestral and retrogenes was achieved with a) testis enrichment calls among branches (Table 2), and b) log fold expression changes between testis and carcass in the *Anopheles* lineage (Fig. S1). On the basis of differential expression, we first identified genes as testis enriched where there was a significant 2-fold change of intensity, in addition to statistical significance at a FDR Q<0.05 level (P<0.042) (Storey & Tibshirani, 2003). Please see Suppl. File 2 for expression values and significance tests. Significant differences within progenitor and retrogene sets was determined by pooling branch samples together and comparing the observed X^2 statistic with 100,000 permuted X^2 values via Monte Carlo simulation (Table 2). Each re-sampled dataset was selected using the same proportion of genes found in branches from the original dataset to omit bias. In the *Anopheles* lineage, we also compared the distribution of testis gene expression changes against the carcass in ancestral and retrogenes (Fig. S1). Significance levels were determined with 10,000 bootstrapped datasets (Unpaired Wilcoxon test).

Dosage compensation.

The presence of dosage compensation in germline and somatic tissue was determined with male and female expression of *X*-linked versus autosomal genes in the testis/ovary, carcass, head, salivary glands, midgut and Malpighian tubules (Table S2). For each tissue, estimates of normalized gene expression obtained from the MozAtlas dataset were used in the analysis if expression was detected in both sexes (i.e. 3 out of 4 mismatch calls). Divergence of X chromosome and autosome expression intensity was determined using Wilcoxon unpaired tests.

Tissue-specific chromosome enrichment.

Tissue-specific transcription was determined for the ovary, testis and the male accessory gland using probe detection. Genes were classified as tissue-specific if at least 3 out of 4 mismatch calls were apparent from Affymetrix microarray chips, but only in a single tissue. This analysis provided gene sets for analysing expected versus observed chromosome distributions amongst the autosomes and X chromosome via chi-squared tests (Table S3).

REFERENCES

Baker, D. A., T. Nolan, B. Fischer, A. Pinder, A. Crisanti and S. Russell, 2011 A comprehensive gene expression atlas of sex- and tissue-specificity in the malaria vector, *Anopheles gambiae*. BMC Genomics **12:** 296.

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

Sharakhova, M.V., P. George, I.V. Brusentsova, S.C. Leman, J.A Bailey, *et al.*, 2010 Genome mapping and characterization of the *Anopheles gambiae* heterochromatin. BMC Genomics **11:** 459.

Smyth, G.K., 2004 Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. **3:** Article 3.

Storey, J. D., and R. Tibshirani, 2003 Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. U. S. A. **100:** 9440-9445.

Toups, M. A., and M. W. Hahn, 2010 Retrogenes Reveal the Direction of Sex-Chromosome Evolution in Mosquitoes. Genetics **186:** 763-766.

Vilella, A. J, J. Severin, A. Ureta-Vidal, L. Heng, R. Durbin and E. Birney, 2009 EnsemblCompara GeneTrees: Complete, duplication-

aware phylogenetic trees in vertebrates. Genome Res. **19:** 327-335.

Yanai, I., Benjamin H., Shmoish M., Chalifa-Caspi V., Shklar M., *et al.*, 2005 Genome-wide midrange transcription profiles reveal expression level relationships in human tissue specification. Bioinformatics **21:** 650-659.

File S2

Supporting Data

File S2 is available for download as an Excel file at http://www.genetics.org/content/suppl/2011/09/02/genetics.111.133157.DC1.

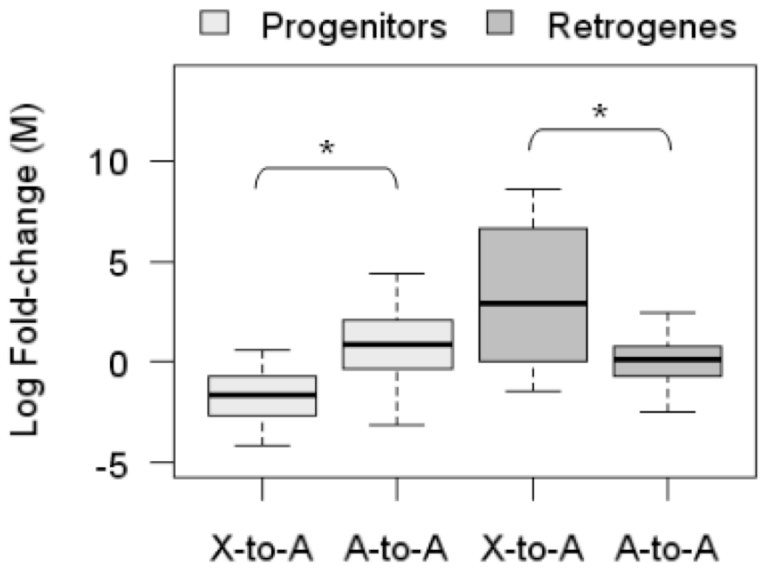

Figure S1 A boxplot with mean log-fold gene expression of testis enrichment versus the carcass (M) in progenitor and retrogene datasets from X-to-A and A-to-A branches within the Anopheles lineage. As in Table 1, we find testis enrichment is significantly higher for retrogenes in X-to-A relocations compared to A-to-A duplications, whereas testis enrichment of ancestral genes (progenitors) in X-to-A relocations is significantly lower than in A-to-A duplications. Significance was determined with 10,000 bootstrapped datasets; Progenitors $P < 0.031$; Retrogenes: $P < 0.035$.

Table S1 Figures used to determine the expected frequency of retro-transposition events.

Chr	Gene No.	Euchromatin (kb)	*Ni*	*Lj*	*fij*
X	1094	20,009.76	0.088	0.094	0.75
2	6636	104,888.5	0.534	0.492	1
3	4697	88,430.84	0.378	0.414	1

Table S2 Evidence for hyper-activation of the X chromosome in male somatic tissue, but not the male germline.

	Germline	Carcass	Head	Salivary	Midgut	Malpighian
Male log2(A)						
Autosomal (median)	7.08	6.77	6.47	6.78	6.95	7.04
X-linked (median)	6.03	6.61	6.22	6.44	6.78	6.87
X_{male}/A_{male}	**0.48**	1.12	1.18	1.26	1.12	1.12
P-value	**2.380e-12**	0.480	0.115	0.309	0.188	0.443
Female log2(A)						
Autosomal (median)	7.33	6.85	6.61	6.76	6.98	7.13
X-linked (median)	7.41	6.56	6.24	6.65	6.76	6.78
X_{female}/A_{female}	1.05	1.23	1.29	1.07	1.16	1.27
P-value	0.924	0.419	0.057	0.216	0.250	0.065
Genes (n)	*4071*	*3874*	*4039*	*3216*	*3179*	*3904*

Significance levels were determined with an Unpaired Wilcoxon test.

Table S3 Chromosome enrichment for genes with tissue-specific expression in *Anopheles*.

Tissue	Genes (n)	Autosomes (obs/exp)	X Chromosome (obs/exp)	P value
Ovary	133	118/120	15/13	0.51
Testis	746	737/674	9/72	7.13×10^{-15}
Acc. Glands	55	44/50	11/5	< 0.008

Tests for enrichment were conducted with a Chi-squared test (Exp: A=90.4%; X=9.6%).