Note

Retrogenes Reveal the Direction of Sex-Chromosome Evolution in Mosquitoes

Melissa A. Toups* and Matthew W. Hahn*,†,1

*Department of Biology and †School of Informatics and Computing, Indiana University, Bloomington, Indiana 47405

ABSTRACT

The mosquito *Anopheles gambiae* has heteromorphic sex chromosomes, while the mosquito *Aedes aegypti* has homomorphic sex chromosomes. We use retrotransposed gene duplicates to show an excess of movement off the *An. gambiae* X chromosome only after the split with *Ae. aegypti*, suggesting that their ancestor had homomorphic sex chromosomes.

HETEROMORPHIC sex chromosomes, both XX/ XY and ZZ/ZW systems, have evolved independently multiple times in both animals and plants (BULL 1983; CHARLESWORTH 1996; RICE 1996). Sex chromosomes are thought to evolve from a pair of autosomes that acquire a new sex-determining locus. Theory suggests that natural selection will favor tight linkage between the newly arisen sex-determining locus and sexually antagonistic alleles (*i.e.*, genes that are beneficial in one sex, but detrimental in the other), which favors the suppression of recombination near the sex-determining locus (CHARLESWORTH *et al.* 2005). In some species, this nonrecombining region includes only a small portion of the sex chromosome (hereafter referred to as homomorphic sex chromosomes), whereas in other species, this region encompasses most of the sex chromosomes (heteromorphic sex chromosomes). In many species the nonrecombining region progressively expands from only the portion near the sex-determining locus to nearly the full extent of the sex chromosomes (LAHN and PAGE 1999; LAWSON HANDLEY *et al.* 2004; NICOLAS *et al.* 2005). However, the broad phylogenetic distribution of homomorphic sex chromosomes suggests that this progression does not happen in every species (*e.g.*, MATSUBARA *et al.* 2006; TSUDA *et al.* 2007), although why it should occur in some lineages and not in others is unknown. As noted by GILCHRIST and HALDANE (1947, p. 187): "It is a striking fact that this [the suppression of recombination across the sex chromosome] has not happened in many large and successful groups."

Within the order Diptera, there are a wide variety of sex chromosomes and sex-determination mechanisms, including XY, ZW, multiple-X, and homomorphic systems, often varying within the same family (MARIN and BAKER 1998; SCHUTT and NOTHIGER 2000; SANCHEZ 2008). The mosquito *Anopheles gambiae* (a species in the subfamily Anophelinae) has fully differentiated heteromorphic X and Y chromosomes that show no evidence of recombination (KRZYWINSKI *et al.* 2004). The mosquito *Aedes aegypti* (subfamily Culicinae) has a nonrecombining sex-determining region that spans only a few megabases on chromosome 1; this chromosome is homologous to chromosomes X and 2R of *An. gambiae* (NENE *et al.* 2007). *An. gambiae* and *Ae. aegypti* diverged ∼150 million years ago (KRZYWINSKI *et al.* 2006).

Because of the rapid turnover of sex-chromosome systems among the Diptera, it is not clear if the common ancestor of *Ae. aegypti* and *An. gambiae* had only a sex-determining region (*i.e.*, homomorphic sex chromosomes) or fully differentiated heteromorphic sex chromosomes (RAI and BLACK 1999). The generally accepted model of sex-chromosome evolution, in which homomorphic sex chromosomes progressively suppress recombination and become heteromorphic, predicts that the common ancestor of *Ae. aegypti* and *An. gambiae* had homomorphic sex chromosomes (Figure 1A). This implies that evolution of heteromorphic sex chromosomes in *An. gambiae* occurred in a short period of time after the split between these two lineages and before the radiation of the Anophelines and that the homomorphic sex chromosomes of *Ae. aegypti* have been nearly static over evolutionary time. Alternatively, the common ancestor may have had nearly or fully differentiated sex chromosomes, and *Ae. aegypti* evolved from heteromor-

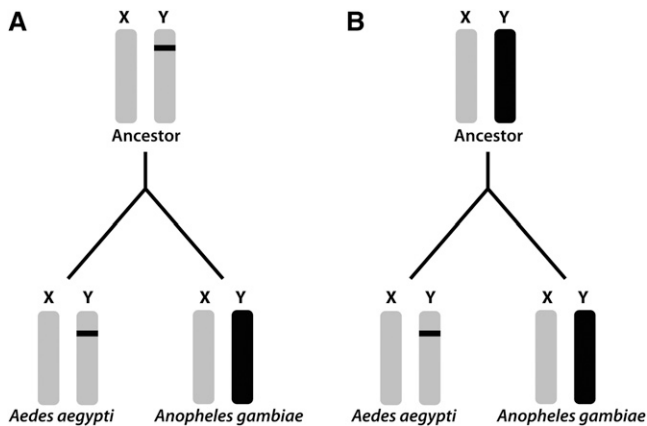¹*Corresponding author:* 1001 E. Third St., Indiana University, Bloomington, IN 47405.   E-mail: mwh@indiana.edu

FIGURE 1.—Hypotheses for sex-chromosome evolution in *Anopheles gambiae* and *Aedes aegypti*. (A) The ancestor of *An. gambiae* and *Ae. aegypti* had homomorphic sex chromosomes and heteromorphism evolved along the Anopheline lineage. (B) The ancestor of *An. gambiae* and *Ae. aegypti* had heteromorphic chromosomes and homomorphism evolved along the Culicine lineage.
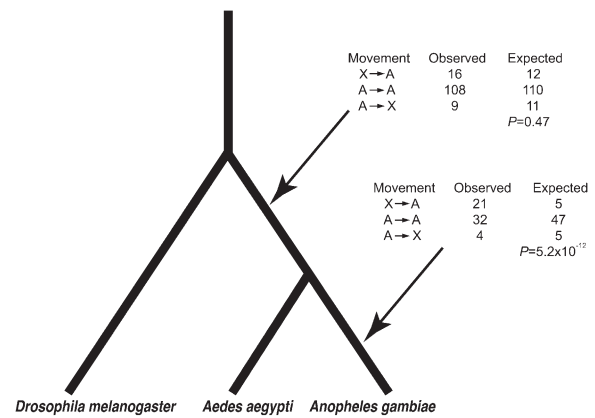

FIGURE 2.—Retroposition events off the X chromosome. There is an excess of genes moving off the X chromosome on the *An. gambiae*-specific lineage, but not on the branch leading to the common ancestor of *An. gambiae* and *Ae. aegypti*.

phic sex chromosomes to having only a small sex-determining region (Figure 1B; RAO and RAI 1987). We imagine this transition may have occurred by one of two mechanisms: either the sex-determining locus was transposed from the ancestral sex chromosome to an autosome or, in an XO sex-determination system, one of the "numerator" genes located on the X chromosome sustained an inactivating mutation, effectively making a karyotypic XX individual into a genetically male XO individual. (The precise mechanism of sex determination in *Ae. aegypti* is not known.)

To determine the state of the mosquito common ancestor, we examined genes duplicated by retrotransposition in the *An. gambiae* genome. Several organisms with heteromorphic sex chromosomes, including mammals and Drosophila, have an excess of retrotransposed genes moving from the X chromosome to autosomes compared to genes moving between autosomes or from the autosomes to the X (BETRAN *et al.* 2002; EMERSON *et al.* 2004; VINCKENBOSCH *et al.* 2006; MEISEL *et al.* 2009). This pattern is further found to be strongly associated with the origin of new X chromosomes in both mammals and Drosophila (POTRZEBOWSKI *et al.* 2008; MEISEL *et al.* 2009), although it continues long after X chromosomes arise. While there are many hypotheses for the evolutionary forces that drive gene movement off X chromosomes—including sexual antagonism and meiotic sex-chromosome inactivation (*e.g.*, HENSE *et al.* 2007)—it is likely that all of these forces also act in mosquitoes, implying excess movement off the heteromorphic X in this clade as well. We reasoned that if the common ancestor of *Ae. aegypti* and *An. gambiae* had homomorphic sex chromosomes (Figure 1A), there should be an excess of retrogene movement off the X chromosome in *An. gambiae* only after the divergence of the two lineages (*i.e.*, since *An. gambiae* evolved a

differentiated X chromosome). In contrast, if the common ancestor had fully heteromorphic chromosomes (Figure 1B), then our prediction is that there will be an excess of gene movement off the *An. gambiae* X on both the shared ancestral branch and the *Anopheles*-specific branch after the split with *Aedes*. (Note that the *Ae. aegypti* genome is largely not assembled onto chromosomes, precluding a similar analysis in this species.)

We collected data on all functional, intact duplicates in the *An. gambiae* genome and all orthologs between *An. gambiae* and *Ae. aegypti* from Ensembl version 54. When genes are retrotransposed there will be introns in the parental copy, but no introns in the daughter copy, allowing us to polarize gene movement. Although introns may be lost—and more rarely gained—over time, the rate of such changes is quite low (COULOMBE-HUNTINGTON and MAJEWSKI 2007). Nevertheless, unless a parental gene loses all of its introns and the daughter gene gains introns, such changes will merely cause us to miss events rather than to assign them to an incorrect chromosome. Using gene-tree/species-tree reconciliation (GOODMAN *et al.* 1979), we identified retrotransposition events in the *An. gambiae* genome that have occurred since the split with *Drosophila melanogaster* and assigned them to a branch on the basis of the timing of the inferred duplication event in the gene tree. Calculating the expected number of movements on the basis of the equations presented in BETRAN *et al.* (2002), we find that an excess of movement off the X chromosome has in fact occurred since the split with *D. melanogaster* ($\chi^2 = 23.83$, d.f. $= 2$, $P = 6.7 \times 10^{-6}$). We then divided the retrotransposition events into those that occurred before the divergence of *An. gambiae* and *Ae. aegypti* and those that occurred only in *An. gambiae* since the split. We determined that there is a 400% excess of retrotransposition events off the X chromosome since the *An. gambiae* and *Ae. aegypti* split (Figure 2: $\chi^2 = 51.97$, d.f. $= 2$, $P = 5.2 \times 10^{-12}$). However, there is no excess of retrotransposition off the X chromosome prior

to the split between *An. gambiae* and *Ae. aegypti* (Figure 2: $\chi^2 = 1.51$, d.f. = 2, $P = 0.47$). This strongly suggests a recent origin of fully differentiated heteromorphic sex chromosomes in *An. gambiae*.

The deepest split between species within the subfamily Anophelinae—all of which have fully differentiated sex chromosomes—occurs soon after the split with the Culicinae (KRZYWINSKI *et al.* 2006). This implies that the evolution of heteromorphic sex chromosomes must have occurred very soon after the split with *Ae. aegypti*. To determine whether there was a burst of retrotransposition off the X following this split, we examined the amino acid sequence identity between X-to-autosome retrotransposed proteins and their parental paralogs. A comparison of these distributions indicates that there is no difference in the percentage of identity of genes retrotransposed off the *An. gambiae* X chromosome and one-to-one orthologs between *An. gambiae* and *Ae. aegypti* (71.1% *vs.* 70.7%, *t*-test, $P = 0.92$; JTT amino acid distances, 0.508 *vs.* 0.436, *t*-test, $P = 0.57$). Given the fact that functional retrotransposed genes have been found to evolve more rapidly than single-copy genes (BETRAN *et al.* 2002), these results support the idea that these duplication events occurred soon after the split between *An. gambiae* and *Ae. aegypti*.

Our results have important implications for two further areas of research. First, a recent article (MOYLE *et al.* 2010) proposed that X-to-autosome duplication events could be partly responsible for the large X-effect—the disproportionate effect of the X chromosome on reproductive isolation (COYNE and ORR 2004). This is because gene movement between chromosomes can itself cause reproductive isolation (*e.g.*, MASLY *et al.* 2006), and any excess movement involving the X will lead to an excess of reproductive isolation loci mapping to this chromosome. One prediction of this model is that species showing the large X-effect should also show an excess of X-to-autosome gene movement. As *An. gambiae* does in fact exhibit patterns consistent with the large X-effect (SLOTMAN *et al.* 2005), our demonstration of an excess of movement off the X supports this model.

Second, it has been proposed that the excess movement off the X in Drosophila is the cause of the deficit of male-biased genes on the X in the same species (*e.g.*, VIBRANOVSKI *et al.* 2009), although the number of retrotransposed genes is much smaller than the number of missing male-biased genes (BETRAN *et al.* 2002; PARISI *et al.* 2003). We have previously shown that there is no deficit of male-biased genes on the *An. gambiae* X chromosome, at any significance level (HAHN and LANZARO 2005). Given the observed excess of gene movement off the X presented here, we therefore find little support for a causal link between movement and genome-wide patterns of male-biased gene expression.

Our results suggest that retrogene movement is a general feature of sex-chromosome evolution and support the hypothesis that the common ancestor of *An.* *gambiae* and *Ae. aegypti* had homomorphic sex chromosomes. It appears that the nonrecombining region around the sex-determining locus in *An. gambiae* expanded rapidly after the divergence with *Ae. aegypti*. Further investigation into the causes of the rapid expansion in the *An. gambiae* lineage and the long-term stasis in the *Ae. aegypti* lineage is clearly warranted.

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