# Organization and evolution of piRNA clusters in mosquitoes

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The Piwi-interacting RNA (piRNA) pathway is an important mechanism in the defense against transposable element (TE) mobilization in many species including *Drosophila melanogaster* (the fruit fly) [1], *Aedes aegypti* (the Yellow Fever mosquito) [2], and *Mus musculus* (the mouse) [3]. In *Drosophila*, it has been shown that clusters of piRNA produce transcripts that, when interacting with PIWI proteins, create a complex that can recognize and silence retrotransposable elements in the germ line. In *Aedes* mosquitoes and the mouse, the piRNA clusters appear to involve a higher number of protein coding genes. The aims of our study were 1) to see if the piRNA mechanism in *Anopheles gambiae* is more closely related to the *Drosophila* pathway or to the *Aedes* pathway and 2) to test if incipient species of *An. gambiae* differ in the structure of piRNA clusters. The recent emergence of two molecular forms, M and S, as incipient species of *An. gambiae* has provided a unique opportunity to explore how the piRNA mechanism has diverged in terms of piRNA cluster content and positioning. The M and S forms are morphologically indistinguishable, yet behaviorally and ecologically dissimilar. We hypothesize that the piRNA transcripts generated by these forms may differ to combat the form-specific TE mobilization. We sequenced small RNAs from M (Mali) and S (Zanu) forms of *An. gambiae,* and have created a database of uniquely mapping piRNAs using the NucBase software. To identify clusters of piRNAs, all unique piRNAs have been mapped to the PEST reference genome, a mixture of genomic sequences of the M and S forms. The most abundant clusters of piRNAs were found primarily in high TE-content areas—the intercalary and pericentromeric heterochromatin. Our results demonstrate that piRNAs, much like in *Aedes* and *Drosophila*, are present and active in Anopheline mosquitoes. Cluster locations and content suggest that the piRNA pathway in *Anopheles* compares more favorably to the *Drosophila* pathway than to the *Aedes* pathway. The top 15

clusters identified in *Anopheles gambiae* potentially produce ~74% of the total number of piRNAs; all 15 of these clusters map primarily to TE vestiges. Our study has also detected divergence in piRNA sequences and cluster composition between the M and S forms. Our data indicate that although speciation between the two forms is recent, there are differences in TE vestiges that make up the clusters, as well as the piRNA sequences that can be present and absent when mapped to their respective genomes. We hypothesize that the defense mechanism in the two incipient species has begun to rapidly evolve to protect its respective genome against novel TE invaders that have not incorporated into both populations.

1. Brennecke, J., et al. (2007) Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. *Cell*. **128**:1089-1103.
2. Arensburger, P., et al. (2011) The mosquito *Aedes aegypti* has a large genome size and high transposable element load but contains a low proportion of transposon-specific piRNAs. *BMC Genomics*. **12**:606.
3. Aravin, A.A., et al. (2008) A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Mol Cell*. **31**:785-799.