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Year manuscript completed

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Dr. Jinsong ZhuCommittee Member Dr. Daniela Cimini

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The organization and evolution of heterochromatin in the *Anopheles gambiae* complex

Phillip George

Dissertation submitted to the faculty of the Virginia Polytechnic Institute
and State University in partial fulfillment of the requirements for the
degree of

Doctor of Philosophy
In
Entomology

Igor Sharakhov, Chair
Daniela Cimini
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Carlyle Brewster
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The organization and evolution of heterochromatin in the *Anopheles gambiae* complex

Phillip George

Abstract

The *Anopheles gambiae* complex is comprised of the most important vectors of malaria in Sub-Saharan Africa. Most current control methods involve the use of chemicals that help to limit potential contact with these mosquitoes. However, these control methods still have risks that include insect resistance, environmental toxicity, human health, as well as animal health. In order to develop new strategies that either produce novel targeted insecticides or transgenic mosquitoes that can replace current mosquito populations, it is important to acquire as much biological information about the vector as possible. The reduction in cost and speed of high-throughput sequencing has brought forth many new sequenced genomes that can provide a wealth of information about individual populations as well as their respective evolutionary histories. However, in order to fully understand a genome, these sequences must be assembled properly.

One of the largest challenges toward fully assembling a genome is the abundance of repetitive sequences. These sequences, typically part of gene poor regions known as heterochromatin, are generally left as unassembled scaffolds that are neglected in many genomic studies. Heterochromatin is a biologically important chromatin state that has roles in gene regulation and genome stability. Exclusion of these chromatin domains from experimental assays can provide an incomplete picture in regards to organismal biology. A lack of information regarding heterochromatin, even in *An. gambiae*, necessitates further understanding and characterization of this chromatin type that can provide valuable information about the mosquito's biology.

Heterochromatin is organized differently amongst different species. Some species with compact genomes, like *Drosophila melanogaster*, exhibit rigid organization of heterochromatin, with repetitive elements being confined to peri-centromeric and sub-telomeric regions of the chromosome. Larger genomes such as *Aedes aegypti*, have a much less structured heterochromatin pattern, with repetitive elements being dispersed across the genome. However, *An. gambiae*'s genome is more intermediate in size as well as transposable element content. These factors may have an impact in controlling how heterochromatin is organized within the *An. gambiae* genome. Does *An. gambiae* compensate for the increased genome size by expanding past the peri-centromeric heterochromatin into new intercalary compartments?

In *An. gambiae*, heterochromatin had yet to be identified separately from euchromatin. Morphologically, some regions of *An. gambiae* chromosomes exhibited characteristics similar to transcriptionally active puffs or peri-centromeric heterochromatin. We characterize these regions, as well as the rest of the genomic landscape, by using morphological and genetic features to identify various chromatin types. Peri-centromeric heterochromatin and new regions of intercalary heterochromatin were identified. Genomic coordinates representing the transition from euchromatin to heterochromatin were also identified. By finding these heterochromatin-euchromatin boundaries, various genetic features could be assigned to either heterochromatin or euchromatin. Critical genes associated with heterochromatin formation and basic genomic functions were identified. These data help to better understand features that are associated with the different environments created by chromatin compaction.

This study also looks at the Piwi-interacting RNA (piRNA) pathway and its role in *An. gambiae*. The piRNA pathway is associated with transposable element (TE) suppression in many species, where clusters of vestigial TEs provide some of the RNA necessary for the pathway to function. These clusters are primarily associated with heterochromatin in *Drosophila melanogaster*. We identify piRNA clusters in *An. gambiae* and see a similar shift from primarily peri-centromeric compartmentalization toward the presence of intercalary regions located within the euchromatin. Transposable elements are maintained in secondary heterochromatin regions that exhibit similar morphology and features to peri-centromeric

heterochromatin. The piRNA pathway also has implications in gene regulation, germline development, and anti-viral immunity. Three candidate genes associated with spermatogenesis and embryogenesis have been identified. These genes showed piRNA enrichment, and upon further analysis show up-regulation after a blood meal is taken. These genes could potentially prove useful in vector control as targets of transgenic experiments.

Heterochromatin is an important, yet neglected aspect of the genome. These studies attempt to provide data to stimulate the study of heterochromatin through characterization of heterochromatin-related genomic features.