PROGRESS IN MAPPING THE YELLOW FEVER MOSQUITO GENOME

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Mosquito-borne diseases cause significant problems for the human health. For this reason, the genomes of three most dangerous species of mosquitoes, including the yellow fever mosquito *Aedes aegypti*, were sequenced in last decade. The efficient vector of arboviruses. *Ae. aegypti*, is also a convenient model for laboratory research. The intensive genetic mapping of morphological and molecular markers conducted for this mosquito in the past was very successful. This mapping was also used as a tool to localize a number of quantitative trait loci related to the mosquito's ability to transmit various pathogens. However, physical mapping of the *Ae. aegypti* genome is difficult due to the lack of high-quality polytene chromosomes. Here, we review different mapping approaches that help improving genome sequence assembly and also integrate linkage, chromocome and genome maps the yellow fever mosquito.

Key words: mosquito, *Aedes aegypti*, yellow fever, genome map.

Abbreviations: BAC — bacterial artificial chromosome, cDNA — complementary DNA, cM — centimorgan, FISH — fluorescent *in situ* hybridization, iMap — integrated map, QTLs — quantitative trait loci, RAPD — random amplified polymorphism DNA, RFLP — restriction fragment length polymorphism.

Mosquitoes are vectors of numerous human pathogens such as malaria parasites transmitted by the subfamily Anophelinae; lymphatic filarial worms transmitted by both Anophelinae and Culicinae subfamilies; and arboviruses whose transmission is largely associated with the subfamily Culicinae. *Aedes aegypti* is recognized as a principal vector of dengue and yellow fever viruses. These two diseases have a significant world-wide impact on human health. Dengue fever is currently considered the most important vector-borne arboviral disease of the 21st century (Gubler, 2012). Because of the absence of a vaccine or drug treatment, the prevention of this disease largely relies on controlling its major vector mosquito *Ae. aegypti*.

Ae. aegypti represent both efficient vector of arboviruses and also convenient species for the experimental laboratory research. It can be easily colonized and very tolerant to inbreeding (Severson, 2008). As a result of these advantages, genetic mapping conducted on *Ae. aegypti* was very successful. The genetic mapping was originally inspired from the study of inheritance of insecticide resistance as a single dominant trait (Coker, 1958). A similar mechanism of inheritance, as a single gene or a single block of chromosome material, was demonstrated for the sex determination (McClelland, 1962). The sex determination alleles were described as *Mm* in males and *mm* in females and linked to homomorphic chromosome 1 (McDonald, Rai, 1970). Later from 87 morphological mutations described for *Ae. aegypti* 28 were mapped to the 3 linkage groups corresponding to the three chromosomes of mosquito (Craig, Hickey, 1967). This map was extended by additional morphological, physiological and enzyme loci (Munstermann, Craig, 1979). The final classical genetic map included about 70 loci of morphological mutants, insecticide resistance, and isozyme markers (Munstermann, 1990).

A possibility of using DNA molecular markers opened a new era in genetic mapping of mosquito genomes. The first molecular marker-based genetic map for *Ae. aegypti* was constructed using restriction fragment length polymorphism (RFLP) of complementary DNA (cDNA) clones (Severson et al., 1993). This map included 50 DNA markers and covered 134 centimorgan (cM). Random amplified polymorphism DNA (RAPD) map, constructed later, consisted of 96 markers covering 168 cM (Antolin et al., 1996). A composite map for RFLP, single-strand conformation polymorphism (SSCP), and single nucleotide polymorphism (SNP) markers incorporated 146 loci and covered 205 cM (Severson, 2008). Finally, the genetic map of *Ae. aegypti* was extended by mapping microsatellite loci (Chambers et al., 2007). The genetic map of *Ae. aegypti* was also used as a tool to localize several quantitative trait loci (QTLs) related to pathogen transmission: the filarioid nematode, *Brugia malayi* (Severson et al., 1994), the avian malaria parasite, *Plasmodium gallinaceum* (Severson et al., 1995), and dengue virus (Gomez-Machorro et al., 2004). Among all mosquitoes, the genetic map developed for *Ae. aegypti* is the most densely populated.

Cytogenetic mapping on *Ae. aegypti* and other culcine is difficult because of the absence of high-quality, easily spreadable polytene chromosomes (Sharma et al., 1978; Campos et al., 2003). The majority of cytogenetic studies for *Ae. aegypti* were conducted on mitotic chromosomes from brain ganglia or meiotic chromosomes from male testis (Rai, 1963; Newton et al., 1974; Motara, Rai, 1977). These studies led to the conclusion that *Ae. aegypti* has a kariotype of three pairs of metacentric chromosomes (Motara et al., 1985). The chromosomes were fist numbered as chromosomes I, II and III in order of increasing size (Rai, 1963), but later renumbered as chromo-

somes 1, 2 and 3 in correspondence to the linkage map developed for *Ae. aegypti* and the longest chromosome III became chromosome 2 (McDonald, Rai, 1970). Chromosomes from brain ganglia of *Ae. aegypti* were first utilized for the successful unfluorescent *in situ* hybridization of two ribosomal genes (Kumar, Rai, 1990). Fluorescent *in situ* hybridization (FISH) technique was developed on mitotic chromosomes from the ATC-10 cell line of *Ae. aegypti* resulting in direct positioning of 37 cosmid clones onto chromosomes (Brown et al., 1995). In addition 21 cDNA genetic markers, and 8 cosmid clones contained the RFLP markers were also mapped to the chromosomes from ATC-10 cell line (Brown et al., 2001). This map was the first attempt to integrate genetic and physical maps for *Ae. aegypti*.

Recent progress in *Ae. aegypti* cytogentics is associated with using mitotic chromosomes from imaginal discs of 4th instar larvae as alternative source of the chromosomes (Sharakhova et al., 2011). This method utilized live larva and does not require using cell lines which usually accumulate chromosomal rearrangements (Steiniger, Mukherjee, 1975). Each slide preparation from one ID contains ~175 chromosomal spreads which is 6-folds more than in two brain ganglia. Clearly visible banding patterns of mitotic chromosomes from IDs allowed to construct preliminary idiograms without numbered divisions for the chromosomes at mid-metaphase (Sharakhova et al., 2011). A FISH technique was optimized for using BAC clones as probes (Timoshevskiy et al., 2012), resulting in assigning of 10 BAC clones and ribosomal 18S DNA to the bands on the idiograms. Recently, more for early metaphase chromosome with 23 numbered divisions and 94 subdivisions. These idiorgams represent the fist cytogenetic map developed for yellow fever mosquito and can be used for the detailed physical mapping.

The genome of *Ae. aegypti* was among first mosquito genomes sequenced in last decade (Nene et al., 2007). As compared to malaria vector *Anopheles gambiae* (Holt et al., 2002) and West Nile fever vector *Culex quinquefasciatus* (Arensburger et al., 2010) the genome of *Ae. aegypti* is the largest and consist of 1.376 Mb. The availability of *Ae. aegypti* genome finally provides an opportunity to integrate linkage, chromosome and genome maps for this mosquito. In order to do this a total of 106 bacterial artificial chromosome (BAC) clones carrying major genetic markers were identified from the BAC library using PCR approach (Jimenez et al., 2004). One hundred of them were mapped to the chromosomes *Ae. aegypti* by FISH (Timoshevskiy et al., 2013). For the detailed ordering of the BAC clones «two-step» physical mapping approach was used. In the first step, BAC clones were assigned to the specific band on chromosome idiograms. In the second step, BAC clones within the same band were ordered by higher resolution mapping using prophase or polytene chromosomes. This study developed the first integrated genetic, cytogenetic and genome map — iMap — for the yellow fever mosquito *Ae. aegypti*, which incorporated 94 cytogenetic bans, 100 molecular genetic markers, and 183 Mb of the genome sequences.

The assignment of the significant portion of *Ae. aegypti* genome supercontigs associated with genetic markers to the specific positions on the iMap will help to identify candidate genes within QTL that might be important targets for developing advanced genome-based strategies for vector control. However, because only 13.3 % of the genome was placed to the chromosomes future mapping efforts are needed to improve the quality of the genome assembly for the yellow fever mosquito *Ae. aegypti*.

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КАРТИРОВАНИЕ ГЕНОМА КОМАРА-ПЕРЕНОСЧИКА ЖЕЛТОЙ ЛИХОРАДКИ

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Болезни, переносимые комарами, являются причиной серьезных проблем здоровья людей в мире. Поэтому геномы трех наиболее опасных видов комаров, включая переносчика желтой лихорадки *Aedes aegypti*, были просеквенированы за последнее десятилетие. Эффективный переносчик арбовирусов *Ae. aegypti* является также удобной моделью для лабораторных исследований. Интенсивное генетическое картирование морфологических и молекулярных маркеров, ранее проведенное на этом комаре, было очень успешным. На основе этого картирования целый ряд локусов количественных признаков, связанных со способностью комара переносить различные патогены, были также выявлены. Однако в связи с отсутствием качественных политенных хромосом физическое картирование генома *Ae. aegypti* является затруднительным. В данной статье мы рассматриваем альтернативные способы картирования, которые позволили объединить генетическую, хромосомную и геномную карты комара-переносчика желтой лихорадки.

Ключевые слова: комар *Aedes aegypti*, желтая лихорадка, картирование генома.