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Chromosomal localization of actin genes in the malaria mosquito *Anopheles* darlingi

L. C. BRIDI[1], **M. V. SHARAKHOVA**[2], **I. V. SHARAKHOV**[2], **J. CORDEIRO**[3], **G. M. AZEVEDO JUNIOR**[1], **W. P. TADEI**[4], and **M. S. RAFAEL**[4,*]

[1]Programa de Pós- Graduação em Genética, Conservação e Biologia Evolutiva – PP-G GCBEv, Instituto Nacional de Pesquisas da Amazônia - INPA

[2]Department of Entomology, Fralin Life Science Institute, West Campus Drive, MC 0346, Virginia Tech, Blacksburg, VA, 24061, USA

[3]Programa de Pós Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, CEP 91501-970, Porto Alegre, RS

[4]CSAS - Coordenação de Sociedade, Ambiente e Saúde, Laboratório de Vetores da Malária e Dengue / INPA, Av. André Araújo, 2936, Aleixo, CEP 69060-000, Manaus, Amazonas state, Brazil

Abstract

Physical and genetic maps have been used for chromosomal localization of genes in vectors of infectious diseases. The availability of polytene chromosomes in malaria mosquitoes provides a unique opportunity to precisely map genes of interest. We report physical mapping of two actin genes on polytene chromosomes of the major malaria vector in Amazon *Anopheles darlingi*. The clones with the actin genes sequences were obtained from a cDNA library constructed from RNA isolated from adult females and males of *An. darlingi*. Each of the two clones was mapped to a unique site on the chromosomal arm 2L in subdivisions 21A (clone pl05-A04) and 23B (clone pl17-G06). The obtained results together with previous mapping data provide a suitable basis for comparative genomics and for establishing chromosomal homologies among major malaria vectors.

Keywords

physical mapping; chromosomal homologies; FISH; malaria vector

Anopheles (*Nyssorhynchus*) *darlingi* Root, 1926 is a major human malaria vector and the most anthropophillic and endophagous species of *Anopheles* in the neotropical region and especially in the Brazilian Amazon Basin (Tadei *et al.,* 1998). The relevance of *An. darlingi* as vector of malaria, its geographic distribution and population structure have been demonstrated in several studies (dos Santos *et al.,* 1999; Gilman *et al.,* 2006; Schlichting *et al.,* 2003; Tadei *et al.,* 1998). However, crucial genetic and genomic studies on *An. darlingi* have been lagged behind because of the lack a laboratory colony for this vector. Knowledge of the chromosomal location of genes has important applications for comparative genomics, map-based cloning, and genetic manipulations. *Anopheles darlingi* has been a neglected species in cytogenetic research, because the source of polytene chromosomes is limited to

Corresponding author: Míriam S. Rafael, CSAS - Coordenação de Sociedade, Ambiente e Saúde, Laboratório de Vetores da Malária e Dengue, Av. André Araújo, 2936, Aleixo, CEP 69060-000, Manaus, Amazonas state, Brazil, Office phone (+55-92) 3643 3066; Fax: (+55-92) 3643 3061; msrafael@inpa.gov.br.

the wild-caught larvae, and because of the lack of the high-resolution chromosomal map. The first photo map of polytene chromosomes from salivary glands of the *An. darlingi* larvae was developed by Kreutzer *et al.* (1975). However, this map was not divided into numbered and lettered regions, and, therefore, was not useful for detailed physical mapping. More recently, we created a new cytogenetic map with positions of inversion breakpoints for this species and with numbered and lettered regions (Rafael *et al.,* 2010). The new photomap can serve as a tool to perform evolutionary genetic studies, to localize genes of interest on chromosomes, and to guide a genome assembly effort for *An. darlingi*.

Recently, we developed a cDNA library from total RNA isolated from adult females and males pool of *An. darlingi* collected in Coari, Amazonas state (M. Rafael, unpublished observations). This library is a suitable source of gene sequences that can be directly mapped to chromosomes of this species. In this study, we identified two actin genes sequences in the *An. darlingi* Contig 167, ADLSDA03021A01.g00 (GenBank accession number: Actin_Ad1 JQ307420) and Contig 152, ADELSDA03017G06.g00 (GenBank accession number: Actin_Ad2 JQ307421). Both actin genes sequences were mapped on polytene chromosomes of *An. darlingi* to determine their chromosomal locations and to establish chromosomal homologies between major malaria vectors. Actin is a highly conserved gene in eukaryotes (Hennessey *et al.,* 1993), which functions include the determination of cell shape, cell motility, cytokinesis, intracellular transport and construction of microfibrils in muscle cells. It was recently demonstrated that engineering of late-acting, repressible, tissue-specific, and female-specific transgene expression to cause a flightless phenotype in female *Ae. aegypti* is possible (Fu *et al.,* 2010). This system was based on the promoter derived from the *Ae. aegypti actin-4* gene, which leads to the expression of tTA in a stage-, tissue-, and sex-specific manner.

We used *Anopheles darlingi* collected in Coari, (4o06'S, 63o03'W), Amazonas state, Brazil for the physical mapping. We dissected salivary glands of fourth instar larvae in Fixative I (Carnoy's solution and water), Fixative II (Carnoy's solution and water) and Fixative III (95% lactic acid, P.A., acetic acid and water). We analyzed the bandingpattern of 10 chromosome preparations of *An. darlingi* under a Zeiss Axioplan phase contrast Microscope 100x objective and 10x/25 objective (Carl Zeiss MicroImaging, Inc., USA). For *in situ* hybridization, we used two clones from a cDNA library constructed in pCMVsport6.0 plasmid vector using total RNA isolated from adult females and males pool of *An. darlingi* collected in Coari, Amazonas state, Brazil (GenBank accession numbers: Actin_Ad1 JQ307420 and Actin_Ad2 JQ307421). We labeled the isolated DNA with Cy5-AP3-dUTP (GE Healthcare UK Ltd., Buckinghamshire, England) or with Biotin-16-dUTP using a modified Nick-Translation Mix protocol (Roche Applied Science) and hybridized to the chromosomes at 39°C overnight in hybridization solution (Invitrogen Corporation, Carlsbad, CA, USA). We detected fluorescent signals using an ACCORD Automatic Fluorescent Imaging System (BioView (USA), Inc., Billerica, MA, USA) and determined localization within a subdivision, using a standard cytogenetic map for *An. darlingi* (Rafael *et al.,* 2010).

In this study, two cDNA clones were mapped to polytene chromosomes of *An. darlingi* using fluorescence (FISH) and non-fluorescence *in situ* hybridization. The new cytogenetic photomap of *An. darlingi* (Rafael et al., 2010) allowed, for the first time, the identification of chromosomal positions of the probes within subdivisions. The probe pl05-A04 (GenBank accession number: Actin_Ad1 JQ307420) was mapped to section 21A on the left arm of chromosome 2 of *An. darlingi* (Fig. 1A). The clone pl17-G06 (GenBank accession number: Actin_Ad2 JQ307421) was hybridized to section 23B on 2L (Fig. 1B). We used TBLASTX to search against transcripts of the AgamP3.6 Gene Build, which is available at VectorBase (Lawson *et al.,* 2009), to identify homologous sequences in *An. gambiae*. Accordingly, the *An. darlingi* cDNA clone pl05-A04 (1378 bp) (GenBank accession number: Actin_Ad1

JQ307420) had the highest similarity to the *An. gambiae* actin gene AGAP011514 (e-value=8e-15). The *An. darlingi* cDNA clone pl17-G06 (779 bp) (GenBank accession number: Actin_Ad2 JQ307421) had the highest similarity to the *An. gambiae* actin genes AGAP011516 and AGAP005095 (e-value=1e-146). Gene AGAP005095 is located in subdivision 21D of the 2L arm. Genes AGAP011516 and AGAP011514 are located close to each other in the *An. gambiae* genome, in subdivision 43C of 3L arm (Lawson *et al.,* 2009). However, the homologous sequences of pl05-A04 and pl17-G06 are separated by four cytological subdivisions on the *An. darlingi* 2L chromosome (Fig. 2). These results suggest that tandem organization of the actin genes was disrupted by inversions or transpositions in the *An. darlingi* lineage. A previous study demonstrated that paracentric inversions and whole-arm translocations are the major types of chromosome rearrangements in Anopheles (Xia et al., 2010).

On the 2L chromosome photomap of *An. darlingi,* breakpoints of the inversion 2La are located in subdivisions 20A and 23C, and breakpoints of a complex inversion 2Lab are located in subdivisions 21B and 23C. Of the two clones containing actin genes, pl05-A04 was mapped inside both inversions and close to the 2Lb proximal breakpoint (Fig. 2). Clone pl17-G06 was mapped outside these inversions. Reduced recombination and selection can influence loci within inversions or near inversion breakpoints, resulting in estimates of gene flow that may depart significantly from loci located outside inversions (Lanzaro *et al.,* 1998; Tripet *et al.,* 2005). Therefore, we can expect higher differentiation between naturally occurring alleles of actin gene of pl05-A04 than that of actin gene of pl17-G06.

According to the mapping of actin genes, arm 2L of *An. darlingi* is homologous to arm 3L of *An. gambiae,* 2L in *An. stephensi,* 3L in *An. funestus*, and 3R in *An. albimanus* (Cornel and Collins 2000; Xia *et al.,* 2010). The results indicate that whole-arm translocations are common not only in subgenus *Cellia* (*An. gambiae, An. stephensi, An. funestus*) (Xia *et al.,* 2010) but also in subgenus *Nyssorhynchus* (*An. albimanus, An. darlingi*). Previously we mapped rDNA to the proximal end (5C region) of the X chromosome of *An. darlingi* (Rafael *et al.,* 2003). rDNA is also found in the X heterochromatin of *An. gambiae* and other mosquitoes (Collins *et al.,* 1987; Rafael *et al.,* 2006). The physical location of the Hsp70-12A and Hsp70-14A genes on 2R chromosome (subdivisions 12A and 14A) of *An. darlingi* was also useful for establishing chromosome homology (Rafael *et al.,* 2004). Hsp70 has been also mapped to two locations on 2R in *An. albimanus* (11C and 13C) (Benedict *et al.,* 1993) and to three locations on 2R in *An. gambiae* indicating that 2R arms are homologous in multiple mosquito species. Thus, physical maps are a useful tool for establishing chromosome arm homology and evolutionary genomics studies among *Anopheles* species.

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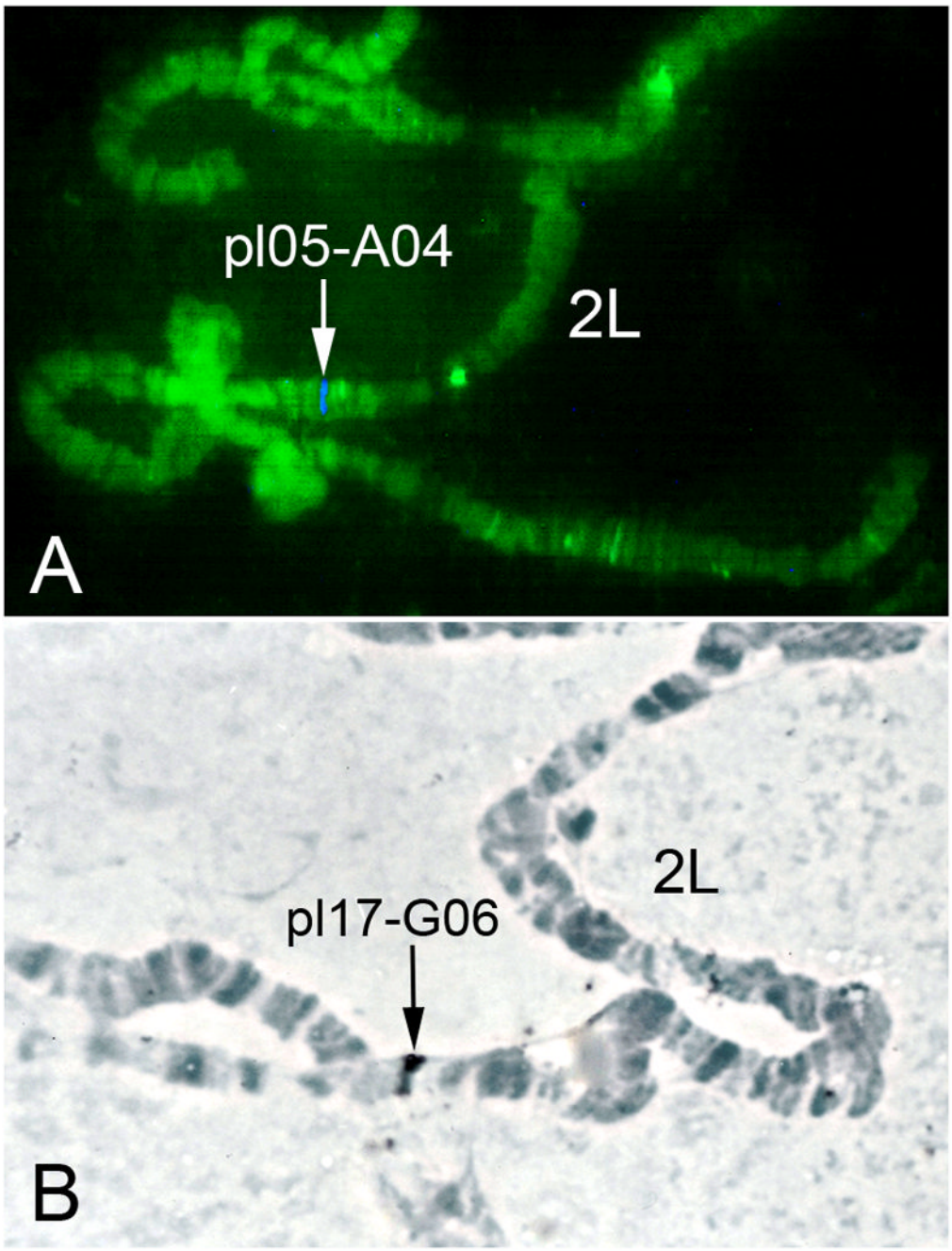

Fig. 1.
Mapping of actin genes to polytene chromosomes of the malaria mosquito *An. darlingi.* A) FISH of *An. darlingi* cDNA pl05-A04 labeled with Cy5. B) Non-fluorescent *in situ* hybridization of *An. darlingi* cDNA pl17-G06 labeled with biotin. Arrows indicate the signal of hybridization in subdivision 21A, 2L (A) and subdivision 23B, 2L (B).

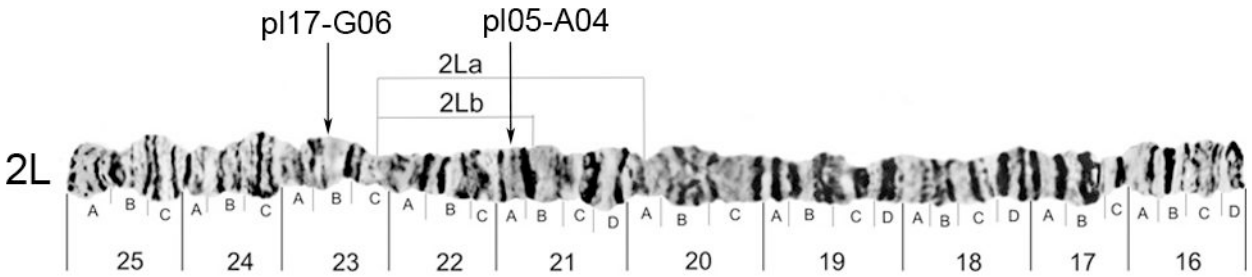

Fig. 2.
The photomap of chromosome arm 2L of *An. darlingi* (Rafael *et al.,* 2010) showing the positions of pl05-A04 and pl17-G06 in relation to the polymorphic inversions 2La and 2Lb.