

DEVELOPMENT OF A MICROSATELLITE-ENRICHED LIBRARY FOR THE NEW WORLD SCREWORM, *Cochliomyia hominivorax* (DIPTERA: CALLIPHORIDAE)

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The New World Screwworm (NWS), *Cochliomyia hominivorax*, is one of the most important parasitic insect pests causing invasive myiasis in warm-blooded vertebrates, particularly livestock, and therefore, affecting the economic development of the agricultural sector throughout Neotropical regions. Due to the substantial economic losses caused by this pest, an international effort has been involved in the program for the eradication of NWS from endemic areas and the prevention and rapid response to invasions into screwworm-free areas. To investigate specific evolutionary patterns of evolution, to enhance the efficiency of the implementation of the eradication program for screwworm and to reduce risk associated with the introduction into new areas it is necessary to determine the genetic variability and population structure of the NWS across its current geographic distribution. Simple Sequence Repeats (SSR), or microsatellites, are short tandem repeats of nucleotides widely distributed in eukaryotic genomes. Among the several classes of molecular markers, microsatellite loci stand out as codominant markers with high number of alleles per locus, high polymorphism and high expected heterozygosity value. Due to these characteristics, they were the molecular markers chosen for this study. The aim of this work was to develop an (AC)_n enriched genomic library for the NWS. Genomic DNA extracted from colony-bred pupae was digested with *Sau3A* I and fragments in the range of 200 to 800 were recovered and linked to specific oligonucleotide adapters. Fragments containing AC repeats were selected by hybridization to biotinylated oligonucleotides bound to magnetic beads. This fraction was used to construct a small insert genomic library enriched for the dinucleotide sequence motif poli-AC/TG. We are currently screening this library for SSR-containing clones by hybridization of colony plaques with a (TG)₁₃ probe. Positive clones will be further screened by anchor-PCR and later sequenced by automated fluorescent cycle sequencing in an ABI Prism 377. Sequences flanking the repeats will be used to design specific primers pairs. Our goal is to develop a set of a minimum of 10 SSR based markers amplifiable by a single PCR protocol and providing loci with expected heterozygosity higher than 0.7. The isolation and characterisation of microsatellite markers opens a new perspective for the generation of fundamental population genetic data of NWS from South America populations and for monitoring the expansions of the pest into screwworm-free areas.

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