SAMPLE R21 SPECIFIC AIMS

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Anopheline mosquitoes include the primary vectors of malaria, which is one of the deadliest and most costly diseases in human history. There is no effective vaccine for malaria. Current strategies to control malaria rely on prompt diagnosis, treatment with anti-malarial drugs, and control of the mosquito vectors (Morel et al., 2002). These measures are becoming less effective as insecticide and drug resistance increases. Recent developments in mosquito research, that were highlighted by the publication of a draft genome sequence of the primary African malaria vector *Anopheles gambiae* (Holt *et al.*, 2002), provide exciting opportunities to greatly enhance our understanding of mosquito genetics, behavior, and physiology. New insights in mosquito biology will undoubtedly facilitate the development of novel strategies to combat malaria and other mosquito-borne diseases. The basic concept underlying this proposal is to combine comparative inquiry with modern genomic technology to help realize the promise of the *An. gambiae* genome project. Genomic comparison between *An. gambiae* and *Drosophila melanogaster* has revealed many interesting differences that are likely related to their biological adaptations (e.g., Zdobnov et al., 2002; Hill et al., 2002). Genomic comparison between a wide-range of mosquitoes will provide an even greater amount of information that will reveal both the genetic conservation underlying the common features of their biology and the genetic divergence underlying their diverse behavior, physiology, and ecological adaptations. Therefore, the establishment and application of effective comparative genomics approaches will contribute toward **our long-term objectives,** which are to illuminate the genetic determinants of vector-pathogen and vector-host interactions, the genetic basis for mosquito speciation and their ecological and physiological adaptations, and the evolutionary dynamics of mosquito genomes.

The major thrust of this exploratory R21 project is to develop an efficient method for multi-species comparison of targeted genomic regions between Anopheline mosquitoes that will provide high resolution identification of gene regulatory elements, detect local gene expansions/loss/rearrangements, and reveal correlations between these genetic changes and biological adaptations. This targeted approach will efficiently complement future genome projects and provide preliminary data to facilitate the selection of species for genome sequencing. Similar targeted comparative approaches have been used in *Drosophila* and vertebrates (Bergman et al., 2002; Boffelli et al., 2003; Thomas et al., 2003; Berezikov et al., 2004). In our preliminary study, we constructed a bacterial artificial chromosome (BAC) genomic library from *An. stephensi*, a species in the same subgenus *Cellia* as *An. gambiae*. We sequenced three randomly picked *An. stephensi* BAC clones that cover approximately 350 kilobases. When compared to *An. gambiae*, these sequences showed prevailing but not absolute gene order conservation, high levels of identity in coding sequences, and islands of conservation in low identity non-coding regions. Our comparison has also revealed potentially new genes or exons that are not identified in the current Ensembl annotation of the *An. gambiae* genome. Building upon our preliminary success, we will pursue the following **Specific Aims:**

- **Specific Aim 1.** Sequence 12 randomly picked BAC clones from *An. stephensi* to obtain baseline information on the relative genomic organization and evolutionary divergence between *An. stephensi* and *An. gambiae.*
- **Specific Aim 2.** Target six regions that contain odorant receptor (GPRor) genes or gene clusters for pair-wise comparisons between *An. stephensi* and *An. gambiae* to examine the evolutionary divergence, conserved regulatory elements, and expansions/loss/rearrangements of these genes.
- **Specific Aim 3.** Further target two GPRor regions for multi-species comparisons to efficiently identify potential regulatory elements, detect local gene expansions/loss/rearrangements, and examine possible correlations between these genetic changes and biological adaptations.