Population Genetic Structure of the Philippine Malaria vector, *Anopheles flavirostris*

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Anopheles flavirostris is a principal malaria vector in the Philippines and widely distributed throughout the country. However, to understand the variability in behavior reported for this taxon, we conducted an investigation of its species status and population structure. This knowledge is essential for management of insecticide resistance and can assist in targeted vector control measures. Allozymes and partial sequences of ribosomal DNA i.e. the internal transcribed spacer 2 (ITS2) (483 bp) and third domain (D3) (332 bp) of the 28S subunit, cytochrome c oxidase subunit 1(COI) (261 bp) of mitochondrial DNA and microsatellite markers (8 loci) were used to investigate the genetic structure of *An. flavirostris* population from the Philippines. Mosquitoes collected from 34 sites throughout the Philippines were used to test the hypothesis that this taxon is a single panmictic species. No fixed differences were observed for allozymes and no sequence variation was observed for ITS2 and only one base pair difference was observed between Philippine and Indonesian D3 sequences. Fixed ITS2 and D3 sequence differences between *An. flavirostris* and other members of the Minimus Subgroup confirms the diagnostic value of these molecular markers.
Sixteen COI haplotypes from 20 sites were identified with 33 polymorphic sites of which 25 were parsimony informative sites. Neighbor-Joining, Maximum Parsimony, Maximum Likelihood and Bayesian phylogenetic analysis of COI sequences for *An. flavirostris* and outgroup taxa revealed strong branch support for the monophyly of *An. flavirostris,* thus confirming the separate taxonomic status of Philippine *An. flavirostris* within the Minimus Group. Variation in the behavior of *An. flavirostris* is likely to be intraspecific rather than interspecific in origin.
For microsatellites, we studied the distribution of genetic diversity of 14 populations of *An. flavirostris* using eight microsatellite loci. One hundred and thirty six alleles were detected across the eight loci in the analysis of 200 *An. flavirostris*. High levels of genetic diversity were observed with number of alleles /locus ranging from 9-26, mean =17. The significant deviation from Hardy-Weinberg equilibrium with heterozygote deficits observed could be due to the presence of null alleles. Low level of inter-population gene flow (Nm = 1.66-4.10) between populations of *An flavirostris* is suggested by low Fst values (Fst = 0.066-0.277). Both Fst (0.066-0.277)and Rst values (0.00-0.856) showed significant differentiation between populations Mantel tests revealed that the spatial genetic structure can be partly explained by isolation –by-distance and limited gene flow among populations.
Both mitochondrial and microsatellite markers demonstrated significant genetic differentiation among individuals within sampling sites (φst for COI =0.622, $P<0.001$ and Fst for microsatellite = 0.144, $P<0.001$) than among sampling sites. Higher level of intra-population than inter-population diversity was found in *An flavirostris* as demonstrated by AMOVA. AMOVA revealed that variation within population was higher

(microsatellites =85%; mtCOI =38%) than that observed among populations (microsatellites =13%; mtCOI = 8%). Both markers demonstrated significant genetic differentiation among individuals within sampling sites (φst for COI =0.622, $P<0.001$ and Fst for microsatellite = 0.144, $P<0.001$) than among sampling sites. Both markers showed low estimates of gene flow (mt COI = 0.04-0.84; microsatellites =1.66-4.10).

Luzon samples showed significant heterozygote deficiencies under SMM and TPM indicating population expansion. Luzon had the highest estimate of the imbalance index β_1 indicating population expansion. Population expansion within Luzon samples was also detected for the mtCOI data. These results could be explained by past demographic change or a selective sweep. The present study suggests the presence of barriers to gene flow. Geographic distance, historical processes and sea barriers have helped shape the genetic structure of *An. flavirostris*.