Abstract

The influence of chromosomal inversions on *Anopheles gambiae* complex ecology and malaria transmission potential in Ghana

Background: *Anopheles gambiae* complex mosquitoes are rapidly adapting to the selection pressures from insecticides, urbanization, deforestation, intensive agriculture, environmental pollution, and climate variability. A key mechanism underlying this adaptive flexibility is chromosomal inversions. Inversions occur when a chromosome segment flips 180°, reversing gene order, and influencing traits that can enhance vector survival under selection pressure. Inversion-driven adaptive heterogeneities may help vectors persist across diverse environments, evade insecticidal interventions, sustain malaria transmission, and expand into new and previously unfavourable habitats. Unfortunately, little is known about current inversion distribution patterns and their epidemiological implications. This study therefore investigated associations between chromosomal inversion polymorphism (2La, 2Rb, and 2Rc), the ecology of *Anopheles gambiae* complex and their malaria transmission potential in Ghana.

Methods: Immature *Anopheles* mosquitoes were sampled from breeding habitats and resting adults were sampled in and around human habitations using prokopack aspirations. The sampling was undertaken in different sites in different ecological zones of Ghana from January 2023 to June 2025. Adult *Anopheles* mosquitoes were morphologically identified using the keys of Coetzee. PCR was used to molecularly identify the species of the *Anopheles gambiae* complex and karyotype 2La, 2Rb, and 2Rc inversion polymorphisms in each mosquito. Oocyst infected and uninfected adult *Anopheles* mosquitoes from an ongoing *Plasmodium* infectivity study were karyotyped to determine the association of inversion polymorphism and *Plasmodium falciparum* parasite development in *Anopheles gambiae* complex. Prospectively collected *An. gambiae* s.l. from communities where indoor residual sprays, piperonyl butoxide bednets and standard bednets are used were also karyotyped to determine the impact of the vector control interventions on inversion polymorphism frequencies. Furthermore, *An. gambiae* s.l. from highland (over 600m above sea level) and lowland (less than 30m above sea level) communities were karyotyped to study vector adaptation in these environments. Descriptive statistics were used to generate frequency distributions and presented as tables and graphs. The chi-square test of independence and logistic regression were used to statistically analyze the data generated.

Results: A high number of *An. coluzzii* sampled in the Guinea-savannah ecological zone [2La homozygotes (53.33%, 112/210), 2La/2L+a heterozygotes (30%, 63/210)] and from the

Coastal ecological zone [2La homozygote (30%, 60/200), 2La/2L+a heterozygotes (31%, 62/200)] had inverted 2La karyotypes, possibly to adapt and survive the warm environmental conditions. The inverted 2Rb homozygote karyotype was observed in 76.67% (69/90) of *An. gambiae* s.s sampled in the Forest ecological zone, possibly as an adaptive mechanism to the changing microclimate of the Forest ecological zone. In the Coastal-savannah ecological zone, 47.59% (69/145) of the *An. coluzzii* sampled were 2Rc/2R+c heterozygotes, suggestive of an ongoing adaptive selection process. There was a higher number of *An. coluzzii* mosquitoes that had inverted 2La inversion karyotypes to survive the hot dry season (62.22%, 195/315) compared to during the rainy season (54.36%, 162/298).

Inverted 2La homozygote *An. gambiae* s.l. mosquitoes were observed to be significantly likely to rest outdoors (Guinea-savannah zone [64%, 64/100]), (Coastal zone [54%, 27/50]), and (Forest zone [30.77%, 4/13]). *Anopheles coluzzii* vectors that had the inverted 2Rb homozygote karyotype (Coastal ecological zone= 16.67%, 7/42, Guinea-savannah=57.89%, 11/19) were more susceptible to *Plasmodium falciparum* infection than those that had not inverted (Coastal ecological zone= 2.38%, 1/42, Guinea-savannah=0.00%, 0/19). *Anopheles coluzzii* mosquitoes in the communities with standard bednets both in the rural site of Ada (48.75%, 39/80) and urban site of Tuba (52.50%, 42/80) had inverted 2La karyotypes. In contrast, majority of the vectors sampled in the communities with no intervention in the Island areas had the standard (uninverted) 2L+a homozygote karyotype (64.79%, [46/71]). *Anopheles gambiae* s.s. mosquitoes in the highland sites had higher frequencies of the inverted 2Rb homozygote karyotype (68%, 51/75), compared to those in the lowland site (2Rb homozygotes (15.79%, 15/95)), possibly as an adaptive mechanism to surviving the cooler conditions of the highland.

Conclusion: Study findings show a high number of *An. gambiae* s.l. in Ghana had inverted 2La, 2Rb and 2Rc chromosomes as adaptive response to unfavourable conditions, likely aiding their survival and disease transmission. Integrating inversion monitoring into routine vector surveillance could inform adaptive malaria control strategies.