Molecular Characterization and Genotype Distribution of Thioester-containing Protein 1 Gene, A Key Regulator of Malaria Transmission in *An. Gambiae* Mosquitoes in Western Kenya

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Keywords: Anopheles gambiae, thioester-containing protein 1, genetic diversity, population structure, signature of selection, malaria transmission

Posted Date: December 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1171980/v1>

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Abstract

Background

Evolutionary pressures lead to the selection of efficient malaria vectors either resistant or susceptible to *Plasmodium* parasites. These forces may elevate the introduction of new species genotypes that adapt to new breeding habitats which could have serious implications on malaria transmission. Thioester-containing protein 1 (TEP1) of *Anopheles gambiae* plays an important role in innate immune defenses against parasites. This study aims to characterize the distribution pattern of TEP1 polymorphisms determining vector competence and subsequently malaria transmission in western Kenya.

Methods

Anopheles gambiae adult and larvae were collected using pyrethrum spray catches (PSC) and plastic dippers respectively from Homa Bay, Kakamega, Bungoma, and Kisumu counties between 2017 and 2020. Collected adults and larvae reared to the adult stage were morphologically identified and then identified to sibling species by PCR. TEP1 alleles were determined using restriction fragment length polymorphisms-polymerase chain reaction (RFLP-PCR) and to validate the TEP1 genotyping results, a representative sample of alleles was sequenced.

Results

Two TEP1 alleles (TEP1*S1 and TEP1*R2) and three corresponding genotypes (*S1/S1, *R2/S1, and *R2/R2) were identified. TEP1*S1 and TEP1*R2 with their corresponding genotypes, homozygous *S1/S1 and heterozygous *R2/S1 were widely distributed across all sites with allele frequencies of approximately 80% and 20%, respectively both in *An. gambiae* and *An. arabiensis*. There was no significant difference detected among the population and between the two mosquito species in TEP1 allele frequency and genotype frequency. The overall low levels in population structure ($F_{ST} = 0.019$) across all sites corresponded to an effective migration index ($N_m = 12.571$) and low Nei's genetic distance values (< 0.500) among the subpopulation. The comparative fixation index values revealed minimal genetic differentiation between species and high levels of gene flow among populations.

Conclusion

There is a low genetic diversity and population structure in western Kenya. TEP1*R2 and TEP1*S1 were the most common alleles in both species which may have been maintained through generations in time. However, the TEP1*R2 allele was in low frequencies and may be used to estimate malaria prevalence. Continued surveillance of the distribution of TEP1 is essential for monitoring the population dynamics of local vectors and their implications on malaria transmission and hence designing targeted vector interventions.

Background

Anopheles gambiae mosquitoes are competent vectors for malaria in sub-Saharan Africa (1, 2). Ongoing vector control interventions (3, 4), climate change (5-9), and environmental modifications modulate the mosquito abundance promoting the abundance of efficient malaria vectors either refractory or susceptible to

Plasmodium parasites (10). These factors may cause vectorial rearrangement exacting selection pressure that could change TEP1 allele frequencies and subsequently have serious implications on malaria transmission. Despite the increased vector densities, malaria transmission is dependent on infectious parasites and competent vectors to influence susceptibility to infections in local vector populations. A vector's susceptibility and/or resistance to *Plasmodium* parasites is a determining factor for vector competence and is in part influenced by the thioester containing protein 1 (TEP1).

In *Anopheles gambiae*, TEP1 exhibits allelic variations that alter vector competence and subsequently influence malaria infectivity (11, 12). These variations may be as a result of selective pressures such as climate change and vector control interventions acting on the TEP1 gene that eventually influence the vector's ability to transmit the *Plasmodium* parasite (12). The TEP1 gene was reported to target the *Plasmodium* parasite in the early stages of infection in the mosquito host mostly the ookinetes (13, 14) either by melanization or lysis (15, 16) effectively reducing oocysts and sporozoite numbers in the vector. However, there is a lack of knowledge on how these allelic polymorphisms in vector competence affect malaria transmission (17, 18). Therefore, understanding molecular mechanisms underlying mosquito genotypes and *Plasmodium* adaptations to different *Anopheles* species is essential and can be used to monitor infection trends in vectors that directly have an impact on malaria transmission.

The complement-like thioester-containing protein 1 (TEP1) plays a key role in immunity against pathogens (18-21). TEP1 is a highly polymorphic protein (22-24) located in the thioester domain (TED) on chromosome 3L coding for 1338 amino acids long protein contributing to phenotypic divergence and demonstrates genetic variations associated with distinct genotypes in its refractoriness to *Plasmodium* parasites. Six allelic classes; TEP1*S1, TEP1*S2, TEP1*S3, TEP1*R1, TEP1*R2, and TEP1*R3 have recently been characterized in the *An. gambiae* complex in Africa (14, 15, 25). TEP1*S1 and TEP1*R2 are the most common TEP1 alleles identified across Africa. The TEP1*S1 however lacks a defined geographical structure. The TEP1*S2 allele identified in the 4Arr strain is specific to *An. coluzzii* (25) and gets rid of the damaged sperm cells in the male mosquitoes (26) bringing forth varying *Anopheles* population abundance. TEP1*S3 allele closely related to TEP1*S1 is fixed in the G3 strain associated with susceptibility of infection to *P. berghei* (14). TEP1*R1 identified in the L3-5 strain depicts the highest level of resistance to *Plasmodium* associated with melanization (14, 26, 27) and documented in *An. coluzzii* in West Africa (25). A newly identified allele TEP1*R3 is specific to the saline water mosquito, *An. merus* found at the Kenyan coast. Selective pressures influence these variations in the genetic structure of the natural *An. gambiae* populations in different ecological settings and differences in their refractoriness to *Plasmodium* parasites are not clear. Genotyping TEP1 in local vector populations is therefore critical for monitoring changes in abundance that explain sporozoite rates and potential malaria prevalence in varying levels of endemicities and is a potential tool for developing vector control interventions. This study was designed to elucidate the characterization and distribution of TEP1 alleles circulating in *An. gambiae* vectors in malaria-endemic regions in western Kenya.

Methods

Study sites and design

This study was conducted in four counties in western Kenya namely, Bungoma, Kakamega, Kisumu, and Homa Bay (Fig. 1). Two malaria epidemic-prone highland sites including Kimaeti (00.6029° N, 034.4073° E; altitude 1,430–1545 m above sea level) in Bungoma, and Iguhu (34°45'E, 0°10'N; 1,430–1,580 m above sea level) in Kakamega, and two lowland sites located around Lake Victoria; Kombewa (34°30'E, 0°07'N; 1,150–1,300 m above sea level) in Kisumu and Kendu Bay (34.64190°E-0.38000°S; 1134-1330 m above sea level) in Homa Bay. The climate in western Kenya consists of long and short rainy seasons that malaria transmission peaks between March to May and October to November respectively. Temperature ranges from a minimum of 14-18⁰C to a maximum of 30-36⁰C and average rainfall ranges between 1740mm and 1940mm annually. *Plasmodium falciparum* is the most common cause of malaria and is transmitted by *An. arabiensis*, *An. gambiae* and *An. funestus* (28, 29). The key vector control interventions are long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) (30). Indoor residual spray was conducted in Homa Bay County once a year in 2017 and 2018, unlike the other sampling sites.

Adult Sampling

Anopheles mosquitoes were collected in a cross-sectional study design using Pyrethrum Spray catch (PSC) from 30 randomly selected houses per site between 2017 and 2020 during the dry and rainy seasons. Collections were conducted between 0630 and 1000hrs in the morning and transported to the Sub-Saharan Africa International Center of Excellence for Malaria Research (ICEMR), Homa Bay, Kenya. Samples were stored at -20°C in 1.5 ml Eppendorf tubes containing silica gel and assigned a unique code for further molecular processing.

Larval Sampling

Larval sampling was conducted using 350 ml standard dippers and hand pipettes (31). A maximum of 10 dips was taken at each habitat and the presence or absence of larvae was recorded. To avoid collecting siblings from the same pool, larvae were randomly sampled from different breeding habitats. Collected larvae were labeled by habitat type and identified morphologically using the referenced keys (32). Only *Anopheles* larvae were sorted and transported to the ICEMR insectary. The larvae were reared to adults using standardized rearing methods (33). Emerged adults were anesthetized using chloroform and identified using the morphological key in the laboratory as described by Gillies and Coetzee to species (34, 35).

Molecular Identification Of Mosquito Species

Genomic DNA was extracted from randomly selected single *An. gambiae* female adult using the Chelex resin (chelex® -100) method following a protocol by Musapa *et al* (36). Briefly, deionized water was added into single mosquito sample tubes and ground into a uniform suspension. Phosphate buffer saline 1X and 10% Saponin was then added to sample homogenates, mixed gently, and incubated at room temperature for 20 minutes. The suspension was then centrifuged and the supernatant discarded. The pellets were then resuspended in PBS 1X and centrifuged, supernatant discarded, and gently vortexed. The pellets were then suspended in sterile deionized water and 20% Chelex-resin suspension in deionized water. The samples were

incubated at 85°C for 10 minutes, centrifuged at 20,000 x g for a minute, and DNA transferred into prelabelled storage vials. *Anopheles gambiae* was identified to sibling species using polymerase chain reaction (PCR) as described by Scott *et al.* (37).

Genotyping and DNA sequencing of TEP1 alleles in *Anopheles gambiae* mosquitoes

Genotyping of TEP1 alleles was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described by Gildenhard *et al.* (25). Briefly, the initial PCR was conducted using Nest 1 primers - VB3 and VB4- targeting 892 base pairs, followed by a second PCR performed on 5µl of the resulting product from Nest 1 with Nest 2 primers VB1 and VB2 producing a final fragment length of 758 base pairs. Both PCR reaction conditions were set as denaturation at 95°C for 3 minutes, 35 cycles of 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and a final step at 72°C for 6 min using DreamTaq Green Master Mix (Thermo Fisher Scientific). PCR products were digested by restriction enzymes *Bam* HI, *Hind* III, or *Bse* NI (New England Biolabs Inc) (**S1 Table**) according to the manufacturer's instructions and analyzed the result with 2.5% agarose gel electrophoresis. The TEP 1 allelic classes were then determined by fragment size of restriction enzyme digestion (**S1 Table**). A subset of samples with identified TEP1 alleles was further confirmed through sequencing of respective Nested II amplicons. Sequencing was done using 3700/3730 BigDye® Terminator v3.1 Sequencing Standard kit (ABI PRISM® 3700 DNA Analyzer).

Statistical analysis

Descriptive statistical analyses were performed using GraphPad Prism v.8.0.1 Software and SPSS version 25 for Windows. Statistical significance was set at $P \leq 0.05$. TEP1 allele frequencies observed heterozygosity (*Ho*), and expected heterozygosity (*He*), the inbreeding coefficients (F_{IS}), departure from Hardy-Weinberg expectations were analyzed using GenEalex version 6.053 software (38). DNA sequences of TEP1 haplotypes were compared with published sequences. Basic Local Alignment Search Tool (BLASTN) was used to retrieve sequences from the National Center for Biotechnology Information (NCBI) database with a high similarity index to each of the haplotype sequences. The retrieved sequences with accession numbers AF291654.1, FN431783.1, FN431782.1, FN431785.1, FN431784.1, and MF098591.1 together with the identified haplotype sequences in this study were aligned. MView web-based tools (39) were used to conduct the alignment of the sequences and to calculate pairwise sequence identity and similarity. Phylogenetic analysis of the representative sequenced and GenBank retrieved TEP1 sequences was performed using MEGA 7.0 software (40)

Results

Species composition of *An. gambiae* s. l across western Kenya

A total of 627 *An. gambiae* s.l. adults were collected and molecularly identified to sibling species based on species-specific PCR. Overall, the species identified were *An. gambiae* and *An. arabiensis* constituting 49.28% (309/627) and 50.72% (318/627) of the total samples genotyped respectively (Table 1). A significant difference in species abundance (*An. gambiae* versus *An. arabiensis*) was observed in all study sites ($\chi^2 = 592.012$ df=1, $P < 0.0001$).

Table 1
Molecular determined species composition in western Kenya.

Sampling sites	<i>An. arabiensis</i> , n (%)	<i>An. gambiae</i> , n (%)
Bungoma	39 (37.5)	65 (62.5)
Kakamega	29 (34.1)	56 (65.9)
Homa Bay	186 (82.7)	39 (17.3)
Kisumu	55 (25.8)	158 (74.2)
Grand Total	309 (49.3)	318 (50.7)

Tep1 Allele Distribution In Western Kenya

Overall, two TEP1 alleles (TEP1*S1, and TEP1*R2) were identified across western Kenya with average frequencies of 84.9% and 15.1%, respectively. *Anopheles arabiensis* populations from Homa Bay had the highest TEP1*S1 allele frequency (89%) followed by *An. arabiensis* from Kisumu (86.4%), Kakamega (84.5%), and Bungoma (74.4%) respectively. Among *An. gambiae* s.s, populations from Bungoma displayed the highest TEP1*S1 allele frequency (93.1%) followed by Homa Bay (84.6%), Kakamega (83.9%), and Kisumu (83.5%) respectively (Fig. 2A). The highest TEP1*R2 allele frequency among *An. arabiensis* was observed on vector populations from Bungoma (26%) followed by Kakamega (15.5%), Kisumu (13.6%), and Homa Bay (11%). In *An. gambiae* the TEP1*R2 allele frequency was highest in populations from Kisumu and Kakamega displaying allele frequencies of 16.5% and 16.1% respectively, followed by Homa Bay (15.4%) and Bungoma (7%) respectively. No significant differences in allele frequency were observed between species ($P=0.799$).

Tep1 Genotype Distribution In Western Kenya

A total of 3 genotypes were identified in western Kenya *An. gambiae* populations. Out of the 3 genotypes, 2 were homozygous (TEP1*S1/S1 and TEP1*R2/R2) and 1 heterozygous (TEP1*R2/S1). Homozygote TEP1*S1/S1 and heterozygote TEP1*R2/S1 genotypes had distinct frequencies (Fig. 2B). TEP1*S1/S1 and TEP1*R2/S1 genotypes were commonly present among species in all sites at an average frequency of 71.75% and 26.61% respectively. TEP1*R2/R2 although rare, was only present in *An. arabiensis* from Bungoma (2.6%), Kakamega (3.4%) and Homa Bay (1.6%) and *An. gambiae* s.s from Kakamega (3.6%) and Kisumu (1.9%) but in the lowest average frequency of 1.64% (Fig. 2B). The TEP1*S1/S1 genotype was predominant followed by TEP1*R2/S1 but in low varied frequencies among species across all sampling sites. The TEP1*S1/S1 genotype frequency was highest in *An. gambiae* as compared to *An. arabiensis* from all sites except Kakamega populations that displayed higher TEP1*S1/S1 frequencies in *An. arabiensis* (75.9%) than in *An. gambiae* (53.6%) (Fig. 2B). On the contrary, the distribution of TEP1*R2/S1 genotypes was highest in *An. arabiensis* than *An. gambiae* in all sites except populations from Kakamega where higher genotype frequencies (42.9%) were observed in *An. gambiae* than in *An. arabiensis* (20.7%). The observed RFLP results for each TEP1 allele were confirmed by respective sequences upon alignment with reference sequences from the NCBI database. The TEP1*S1 and TEP1*R2 sequences had 100% identity matrix to AF291654.1 and

FN431784.1 respectively. A significant difference in genotype frequency was observed among sites in *An. gambiae* populations (Fisher's exact test two-sided p-value < 0.001, n=309) whereas no significant difference was observed among sites in *An. arabiensis* population (Fisher's exact test two-sided p-value =0.0727, n=318).

Evolutionary Relationship Based On Tep1 Gene

The phylogenetic analysis of TEP1 sequences showed that alleles were clustered into susceptible and resistant groups with high bootstrap values, ranging from 72–100%. Out of the sequences retrieved from the gene bank, TEP1*S1 alleles identified in western Kenya have a common lineage with TEP1*S1 (AF291654) from Suakoko, Liberia. TEP1*S1 evolved as a result of a mutation on the mosquito strain G3 with TEP1*S3 (FN431782) which had a close ancestral lineage with strain 4Arr that had the TEP1*S2 (FN431783) allele. TEP1*R2 from western Kenya and TEP1*R1 independently evolved from TEP1*R3 (MF035809) which shared common ancestral lineage with the Susceptible (S) alleles (Fig. 3).

Heterozygosity And Departure From Hardy Weinberg Equilibrium (Hwe)

The overall mean observed heterozygosity of TEP domain in *An. gambiae* and *An. arabiensis* across all sites was 0.270 ± 0.035 and a mean (H_e) expected heterozygosity of 0.251 ± 0.025 . There were slight variations between H_o ranges 0.188-0.462 in *An. arabiensis* and in *An. gambiae* ranges 0.138-0.321. *An. gambiae* populations from Bungoma, Kakamega, and Homa bay, and *An. arabiensis* from Bungoma and Kisumu also showed similar trends of higher observed heterozygosity than the expected with negative F_{IS} values (Table 2). A deviation was observed among *An. gambiae* from Kisumu and *An. arabiensis* from Kakamega and Homa Bay which displayed slightly higher expected heterozygosity than observed signifying the presence of inbreeding among these populations (Table 2).

Table 2
Genetic diversity of *An. gambiae* (GA) and *An. arabiensis* (AR) in western Kenya.

Population	N	Na	Ho	He	F
AR-Bungoma	39	2.000	0.462	0.381	-0.210
AR-Homa Bay	186	2.000	0.188	0.196	0.041
AR-Kakamega	29	2.000	0.241	0.262	0.079
AR-Kisumu	55	2.000	0.236	0.236	-0.004
GA-Bungoma	65	2.000	0.138	0.129	-0.074
GA-Homa Bay	39	2.000	0.308	0.260	-0.182
GA-Kakamega	56	2.000	0.321	0.270	-0.191
GA-Kisumu	158	2.000	0.266	0.275	0.033
N represents the total number of mosquitoes sampled per study site, Na- Number of alleles per site, Ho- Observed heterozygosity, He- Expected heterozygosity, F- Fixation index					

The F_{IS} showed a negative and non-significant value in *An. arabiensis* population from Bungoma (-0.210) and Kisumu (-0.004) and *An. gambiae* from Bungoma (-0.074), Kakamega (-0.191), and Homa Bay (-0.182). These results indicate a slight departure from HWE and excess of heterozygotes in these populations. The F_{IS} results for *An. arabiensis* from Homa Bay (0.041) and Kakamega (0.079) and *An. gambiae* from Kisumu (0.033) infer possible inbreeding. None of the analyzed population was at HWE as all the computed values were nonsignificant ($P > 0.05$). The computed HWE values for *An. arabiensis* across the four localities ranged from 0.001 to 0.307 whereas for *An. gambiae* ranged 0.174 to 2.053 which was > 1 .

Population Structure

The pairwise Wright's fixation index (F_{ST}) values revealed a low genetic differentiation among *An. arabiensis* and *An. gambiae*. Sub-populations in western Kenya. The F_{ST} values $0 \leq 0.05$ were interpreted as low differentiation, $0.05 \geq 0.15$ moderate differentiation and $0.15 \geq 0.25$ high levels (41). Zero value represented complete Panmixis between species in the subpopulations. The F_{ST} values ranged from no subdivision to moderate differentiation (0.000 - 0.036) among *An. arabiensis* from the four study sites (Table 3). A moderate differentiation in *An. arabiensis* was observed between Bungoma and Homa bay subpopulations ($F_{ST} = 0.036$). The F_{ST} values ranged from 0.000 to 0.022 among the *An. gambiae* subpopulations across the four regions. No population differentiation was observed between Kakamega and Homa Bay, Kisumu and Homa Bay, and Kakamega and Kisumu subpopulations ($F_{ST} = 0$). All pairwise F_{ST} values for *An. gambiae* and *An. arabiensis* from all regions across western Kenya demonstrated low population differentiation ($0 \leq 0.05$) except *An. arabiensis* and *An. gambiae* from Bungoma that showed moderate differentiation ($0.05 \geq 0.15$). The overall low levels in population structure ($F_{ST} = 0.019$) across all sites were supported by the high level of gene flow ($N_m = 12.571$) and low Nei's genetic distance values (< 0.5) among the subpopulation.

Table 3
Pairwise comparison of F_{ST} among *An.gambiae* and *An. arabiensis* populations in western Kenya

Population	AR-Bungoma	AR-Homa Bay	AR-Kakamega	AR-Kisumu	GA-Bungoma	GA-Homa Bay	GA-Kakamega	GA-Kisumu
AR-Bungoma	0							
AR-Homa Bay	0.036	0						
AR-Kakamega	0.016	0.004	0					
AR-Kisumu	0.023	0.002	0.001	0				
GA-Bungoma	0.064	0.005	0.019	0.012	0			
GA-Homa Bay	0.016	0.004	0	0.001	0.018	0		
GA-Kakamega	0.014	0.005	0	0.001	0.021	0	0	
GA-Kisumu	0.013	0.006	0	0.002	0.022	0	0	0

The AMOVA results revealed that 99% of the observed variations in allele frequency were among individuals within respective populations, and a 1% variation was observed among populations and within individuals (Table 4). These results show that the level of genetic differentiation among populations was very low.

Table 4
Analysis of molecular variance of the TEP1 gene in *An. gambiae* populations circulating in western Kenya

Source	df	SS	MS	Est. Var.	%
Among Pops	7	2.306	0.329	0.001	1%
Among Individuals	619	71.992	0.116	0.000	0%
Within Individuals	627	77.000	0.123	0.123	99%
Total	1253	151.298		0.124	100%
<i>df</i> , degrees of freedom; SS, sum of squares; MS, mean squares.					

Discussion

Plasmodium falciparum triggers an immune response in *An. gambiae* mosquitoes (42). Following infections with *P. falciparum* in *An. gambiae*, the midgut mounts specific and nonspecific immune responses to minimize epithelial damage (43) A key component of the immune system is the thioester containing protein 1 (TEP1)

that displays allelic variations associated with distinct genotypes in their ability to eliminate *Plasmodium* parasites. Originally, TEP1 variations have been characterized into five allelic classes including *R1, *R2, *R3 *S1, and *S2 (14, 23). This study identified two alleles (TEP1*R2 and, TEP1*S1) in *An. gambiae* s.l from western Kenya. These alleles were characterized from western Kenya regions with different malaria endemicities. A high similarity index was observed among sequenced alleles that were initially identified by RFLP-PCR and sequences retrieved from NCBI. Consistent with previous reports, TEP1*R2 and TEP1*S1 were the most common identified alleles (25, 44) circulating in western Kenya and did not display a defined distribution in sampled regions implying that they are conserved and may represent ancestral alleles maintained over generations in time. Furthermore, why these alleles have been maintained in the local populations and their roles and significance in vector competence is still not clear (14, 22, 23, 45).

Low TEP1*R allele frequencies observed in these malaria-endemic areas in our study sites may be a product of selective pressures in the TEP1 gene resulting in functional variations that select

for susceptible mosquitoes to *Plasmodium* infection (12, 44, 46) as well as encourage evolutionary processes in the TEP1 loci (22). Consequently, these regions still record relatively high malaria prevalence. Implemented vector control interventions such as insecticide treated nets, indoor residual spraying, and environmental factors determine the population structure. In Africa, commonly used vector control interventions are ITNs and IRS (47). In 2017, IRS was deployed in Homa Bay to supplement the existing malaria interventions. The pre-spray period constituted 83% *An. funestus* and 16% *An. gambiae* s.l. However, there was adrift in the local species composition with 99% of mosquitoes in the post-spray period being *An. arabiensis* in 2018 in the same site (Kenya Annual Entomological Monitoring Report, October 2017-September 2018). Furthermore, ecological niches contributing towards selection forces acting on genetic variations shape the population structure of the local species populations in time and hence the adaptations of these malaria vectors to available breeding habitats (48). Understanding the underlying molecular mechanisms that determine vector competence is crucial and will thereafter contribute towards developing new vector control interventions and also complement existing control methods.

The overall F_{ST} values for the pairwise comparison for all populations demonstrate very minimal genetic differentiation between species and sites representing the western Kenya highlands (Bungoma and Kakamega) and lowlands (Homa Bay and Kisumu) suggesting the absence of barriers across regions. This observation does not support that ongoing intervention and ecological changes impacted on allele frequency of TEP1 in the region. The low levels of genetic differentiation correspond to an effective migration index ($Nm=12.571$) indicating high levels of gene flow across the sampling sites. Expected heterozygosity values were higher than the observed heterozygosity among *An. arabiensis* from Homa Bay and Kakamega and *An. gambiae* from Kisumu implies the presence of null alleles and maybe as a result of inbreeding and non-random mating of individuals within those populations (F_{IS} 0.041, 0.079, and 0.033 respectively). The insignificant deviations from HWE imply that the TEP1 loci are under strong selection and confirm other forces such as natural mutations (49, 50) and gene flow that may directly be shaping TEP1 alleles in *An. gambiae* s. l mosquitoes in western Kenya.

Conclusion

This study reveals minimal genetic differentiation and a low population structure in the highland and lowland regions of western Kenya with different malaria transmission patterns. TEP1*R2 and TEP1*S1 were the most common alleles across all regions indicating that *An. gambiae* and *An. arabiensis* may have had these specific alleles before inhabiting new ecological niches. However, TEP1*R allele frequencies observed especially in the highlands may be used to estimate malaria prevalence as compared to the lowlands. Therefore, continued TEP1 surveillance is essential for monitoring the population dynamics of local malaria vectors especially those that have implications on malaria transmission then contribute towards developing targeted vector interventions.

Abbreviations

RT-PCR: Real-time polymerase chain reaction

LLIN: Long-lasting insecticide-treated net

IRS: Indoor residual spraying

DBS: Dried blood spots

DNA: Deoxyribonucleic acid

PCR: Polymerase chain reaction

Declarations

Ethics approval

This study was approved by the University of California, Irvine Institutional Review Board (UCI IRB) and Maseno University Ethics Review Committee (MUERC protocol No. 00456) and authorized by the Ministry of Health.

Consent for publication

Not applicable

Availability of data and materials

The data sets generated and analyzed during this study are available from the corresponding authors on reasonable requests

Conflict of interest

All authors declare that they have no conflict of interest.

Funding

The study was supported by grants from the National Institutes of Health (U19 AI129326, D43 TW001505, R01 AI050243, and R01 A1123074).

Authors' contributions

Project conceptualization: SAO, KOO and DZ, Project implementation: SAO, SOO, and AKG, Data collection and sample analysis: SAO, MGM, CJO, ID, and JOO, Formal analysis: SAO, KOO, and DZ. Drafting manuscript: SAO. Editing and revising manuscript: KOO, MGM, AKG, SOO, ML, GZ, EK, JWK, YAA, DZ, and GY. Funded project: JWK and GY

Acknowledgments

We thank all the community health workers and field assistants and the entire ICEMR staff who participated in the entomological surveys and the village residents for their continued support.

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Figures

Figure 1

Geographical location of the mosquito collection sites in Western Kenya

2A

2B

Figure 2

Distribution of TEP1 genotypes and alleles circulating in *An. gambiae* and *An. arabiensis* in Bungoma, Kakamega, Homa Bay, and Kisumu Counties in western Kenya.

Figure 3

The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model [1]. The tree with the highest log likelihood (-1872.22) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log-likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7700)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 43.64% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 15 nucleotide sequences. There were a total of 873 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2]. Red and green dots indicate haplotypes identified in this study; squares with different colors represent reference haplotypes extracted from GenBank.

Supplementary Files

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