

Genomic insights into adaptive divergence and speciation among malaria vectors of the *Anopheles nili* group

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Abstract

Ongoing speciation in the most important African malaria vectors gives rise to cryptic populations, which differ remarkably in their behavior, ecology, and capacity to vector malaria parasites. Understanding the population structure and the drivers of genetic differentiation among mosquitoes is crucial for effective disease control because heterogeneity within vector species contributes to variability in malaria cases and allow fractions of populations to escape control efforts. To examine population structure and the potential impacts of recent large-scale control interventions, we have investigated the genomic patterns of differentiation in mosquitoes belonging to the *Anopheles nili* group—a large taxonomic group that diverged ~3 Myr ago. Using 4,343 single nucleotide polymorphisms (SNPs), we detected strong population structure characterized by high- F_{ST} values between multiple divergent populations adapted to different habitats within the Central African rainforest. Delineating the cryptic species within the *Anopheles nili* group is challenging due to incongruence between morphology, ribosomal DNA, and SNP markers consistent with incomplete lineage sorting and/or inter-specific gene flow. A very high proportion of loci are fixed ($F_{ST} = 1$) within the genome of putative species, which suggests that ecological and/or reproductive barriers are maintained by strong selection on a substantial number of genes.

KEYWORDS

Anopheles nili, divergent selection, high- F_{ST} regions, speciation

1 | INTRODUCTION

One of the principal goals of population genetics is to summarize the genetic similarities and differences between populations (Wright, 1984). This task can be relatively straightforward for some taxa, but the genetic relationship among populations can also be difficult to summarize, especially for species whose evolutionary history is complex and reticulate. The best known mosquito species of the genus *Anopheles*—which includes all vectors of human malaria parasites—exhibit very complex rangewide population structure due to the combined effects of cryptic speciation, adaptive flexibility and ongoing gene flow across strong but incomplete reproductive barriers (Harbach, 2013;

Krzywinski & Besansky, 2003). For example, almost all major malaria vectors of the Afrotropical region belong to large taxonomic groups encompassing multiple incipient species relatively isolated reproductively and geographically from one another (reviewed by Sinka et al., 2010; Antonio-Nkondjio & Simard, 2013; Coetzee & Koekemoer, 2013; Dia, Guelbeogo, & Ayala, 2013; Lanzaro & Lee, 2013). These characteristics make them promising model systems to study speciation and the processes which contribute to reproductive barriers (e.g., Turner, Hahn, & Nuzhdin, 2005; Lawniczak et al., 2010; Neafsey et al., 2010; Fontaine et al., 2015; Weng, Yu, Hahn, & Nakhleh, 2016), but can also have far-reaching practical consequences. Both spatial and temporal variabilities in malaria cases and the effectiveness of vector

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control measures are greatly impacted by heterogeneity within vector species (Molineaux & Gramiccia, 1980; Van Bortel et al., 2001). For these reasons, research on the genetic structure among the major African malaria vector mosquitoes has intensified over the last few decades (Antonio-Nkondjio & Simard, 2013; Coetzee & Koekemoer, 2013; Dia et al., 2013; Lanzaro & Lee, 2013).

The recent scaling up of insecticide-treated nets usage and indoor insecticide spraying to a lesser extent have led to a dramatic reduction in malaria morbidity and mortality across the continent (WHO, 2016). However, other consequences of these large-scale interventions include increased insecticide resistance (reviewed by Hemingway et al., 2016; Ranson & Lissenden, 2016), range shift (e.g., Bøgh, Pedersen, Mukoko, & Ouma, 1998; Derua et al., 2012; Mwangangi et al., 2013) and profound evolutionary changes among vector populations. In contrast to insecticide resistance and range shift, which have been extensively studied, the recent adaptive changes among mosquito populations have yet to be addressed significantly. These changes—which involve local adaptation and genetic differentiation, introgressive hybridization, and selective sweeps across loci conferring resistance to xenobiotics—are particularly evident in the most anthropophilic species (Barnes et al., 2017; Clarkson et al., 2014; Kamdem, Fouet, Gamez, & White, 2017; Norris et al., 2015).

The ecology, taxonomic complexity, geographic distribution, role in transmission, and evolutionary potential of each vector species are unique. Consequently, further research is needed to specifically resolve population structure and the genomic targets of natural selection at a fine scale in all of the important taxa including currently understudied species. The present work focused on a group of malaria vector species representing a large taxonomic unit named *Anopheles nili* group. Despite the significant role some of its species play in sustaining high malaria transmission, this group has received little attention. To date, four species that occur in forested areas of Central and West Africa and are distinguishable by slight morphological variations are known within the *An. nili* group: *An. nili sensu stricto* (hereafter *An. nili*), *An. ovengensis*, *An. carnevalei*, and *An. somalicus* (Awono-Ambene, Kengne, Simard, Antonio-Nkondjio, & Fontenille, 2004; Gillies & Coetzee, 1987; Gillies & De Meillon, 1968). These species are characterized by reticulate evolution and complex phylogenies that have been challenging to resolve so far (Awono-Ambene et al., 2004, 2006; Kengne, Awono-Ambene, Antonio-Nkondjio, Simard, & Fontenille, 2003; Ndo et al., 2010, 2013; Peery et al., 2011; Sharakhova et al., 2013). Populations of *An. nili* and *An. ovengensis* are very anthropophilic and efficient vectors of *Plasmodium* in rural areas where malaria prevalence is particularly high (Antonio-Nkondjio et al., 2006).

To delineate genomic patterns of differentiation, we sampled mosquito populations throughout the range of species of the *An. nili* group in Cameroon and used reduced representation sequencing to develop genomewide SNP markers that we genotyped in 145 individuals. We discovered previously unknown subpopulations characterized by high pairwise differentiation within *An. ovengensis* and *An. nili*. We further explored the genetic differentiation across the genome and revealed the presence of a very high number of outlier loci that are targets of selection among locally adapted subpopulations. These

findings provide significant baseline data on the genetic underpinnings of adaptive divergence and pave the way for further genomic studies in this important group of mosquitoes. Notably, a complete reference genome will enable us to conduct in-depth studies in order to decipher the functional and phenotypic characteristics of the numerous differentiated loci as well as the contribution of recent selective events in ongoing adaptation.

2 | MATERIALS AND METHODS

2.1 | Mosquito species

We surveyed 28 locations within the geographic ranges of species of the *An. nili* group previously described in Cameroon (Figure 1) (Antonio-Nkondjio et al., 2009; Awono-Ambene et al., 2004, 2006; Ndo et al., 2010, 2013). The genetic structure of *Anopheles* species is most often based on macrogeographic or regional subdivisions of gene pools, but can also involve more subtle divergence between larvae and adults, or between adult populations found in or around human dwellings (e.g., Riehle et al., 2011). To effectively estimate

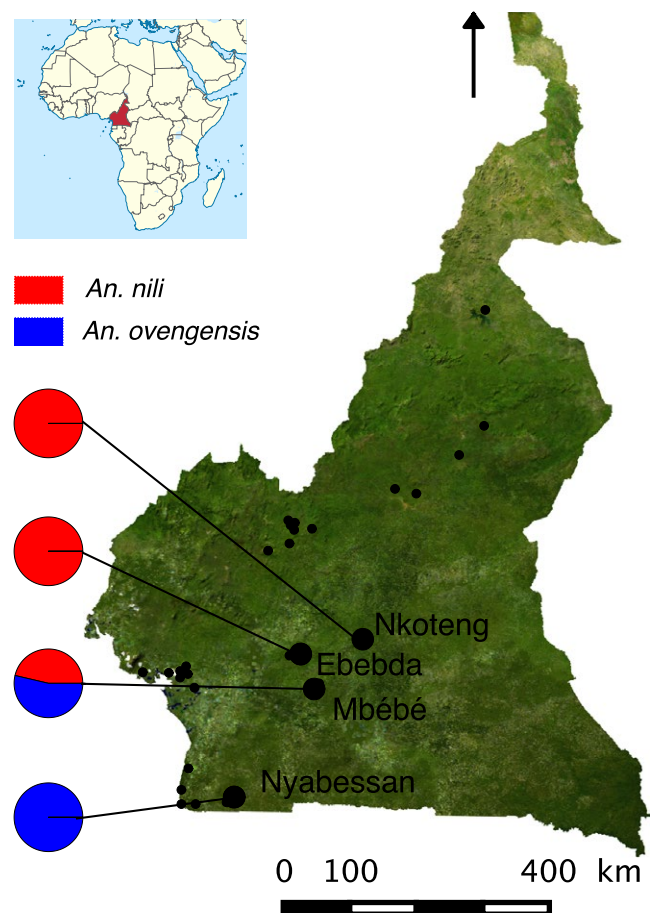


FIGURE 1 Map showing the sampling locations and the relative frequencies of the morphologically defined species *An. nili* and *An. ovengensis* in Cameroon. Small and large black dots indicate, respectively, the 28 locations surveyed and the four sampling sites where mosquitoes were collected

the genetic diversity and identify potential cryptic populations within species, we collected larvae and adult mosquitoes within and around human dwellings using several sampling techniques (Service, 1993) in September–October 2013 (Table S1). To identify the four currently known members of the *An. nili* group, we used morphological keys and a diagnostic PCR, which discriminates species based on point mutations of the ribosomal DNA (Awono-Ambene et al., 2004; Gillies & Coetzee, 1987; Gillies & De Meillon, 1968; Kengne et al., 2003).

2.2 | Library preparation, sequencing, and SNP discovery

We created double-digest restriction site-associated DNA (ddRAD) libraries as described in Kamdem et al. (2017) using a modified version of the protocol designed by Peterson, Weber, Kay, Fisher, and Hoekstra (2012). Briefly, genomic DNA of mosquitoes was extracted using the DNeasy Blood and Tissue kit (Qiagen) and the Zymo Research MinPrep kit for larvae and adult samples, respectively. Approximately 50 ng (10 μ l) of DNA of each mosquito was digested simultaneously with *MluC1* and *NlaIII* restriction enzymes. Digested products were ligated to adapter and barcode sequences enabling identification of individuals. Samples were pooled, purified, and 400-bp fragments selected. The resulting libraries were amplified via PCR and purified, and fragment size distribution was checked using the BioAnalyzer. PCR products were quantified, diluted and single-end sequenced to 100 base reads on Illumina HiSeq2000.

2.3 | SNP discovery and genotyping

The *process_radtags* program of the Stacks v 1.35 pipeline (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013; Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011) was used to demultiplex and clean Illumina sequences. Reads that passed quality filters were aligned to the *An. nili* Dinderesso draft genome assembly (Giraldo-Calderon et al., 2015) made up of 51,048 short contigs (~200–30,512 bp long) using Gsnap (Wu & Nacu, 2010). To identify and call SNPs within consensus RAD loci, we utilized the *ref_map.pl* program of Stacks. We set the minimum number of reads required to form a stack to three and allowed two mismatches during catalogue creation. We generated SNP files in different formats for further downstream analyses using the *populations* program of Stacks and Plink v1.09 (Purcell et al., 2007).

2.4 | Population genomics analyses

We analyzed the genetic structure of *An. nili sensu lato* (s.l.) populations using a principal component analysis (PCA) and an unrooted Neighbor-Joining tree (NJ). We also examined ancestry proportions and admixtures between populations in Admixture v1.23 (Alexander, Novembre, & Lange, 2009) and Structure v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). We used the package *adegenet* (Jombart, 2008) to implement the PCA in R (R Development Core Team 2016). The individual-based NJ network was generated from SNP allele frequencies via a matrix

of Euclidian distance using the R package *ape* (Paradis, Claude, & Strimmer, 2004). We ran Admixture with 10-fold cross-validation for values of k from 1 through 8. Similarly, we analyzed patterns of ancestry from k ancestral populations in Structure, testing five replicates of $k = 1-8$. We used 200,000 iterations and discarded the first 50,000 iterations as burn-in for each Structure run. Clumpp v1.1.2 (Jakobsson & Rosenberg, 2007) was used to summarize assignment results across independent runs. To identify the optimal number of genetic clusters in our sample, we applied simultaneously the lowest cross-validation error in Admixture, the ad hoc statistic deltaK (Earl & VonHoldt, 2012; Evanno, Goudet, & Regnaut, 2005) and the discriminant analysis of principal component (DAPC) method implemented in *adegenet*. To examine the level of genomic divergence among populations, we assessed genetic differentiation (F_{ST}) across SNPs using the *populations* program of the Stacks pipeline. Mean F_{ST} values were also used to quantify pairwise divergence between populations. To infer the demographic history of different populations, we used the diffusion approximation method implemented in the package *daadi* v 1.6.3 (Gutenkunst, Hernandez, Williamson, & Bustamante, 2009). Single-population models were fitted to allele frequency spectra, and the best model was selected using the lowest likelihood and Akaike information criterion as well as visual inspections of residuals.

3 | RESULTS

3.1 | SNP genotyping

We collected mosquitoes from four locations out of 28 sampling sites (Figure 1, Table S1) and sequenced 145 individuals belonging, according to morphological criteria and diagnostic PCRs, to two species (*An. nili* [$n = 24$] and *An. ovengensis* [$n = 121$]). We assembled 197,724 RAD loci that mapped to unique positions throughout the reference genome. After applying stringent filtering rules, 408 loci present in all populations and in at least 50% of individuals in each population were retained. Within these loci, we identified 4,343 high-quality biallelic markers that were used to analyze population structure and genetic differentiation.

3.2 | Morphologically defined species do not correspond to genetic clusters

The PCA and the NJ tree show that the genetic variation across 4,343 SNPs is best explained by more than two clusters, implying subdivisions within *An. nili* and *An. ovengensis* (Figure 2). Three subgroups are apparent within *An. nili* while two distinct clusters segregate in *An. ovengensis* (hereafter referred to as *An. nili* group 1, *An. nili* group 2, *An. nili* group 3, *An. ovengensis* group 1 and *An. ovengensis* group 2). These five subpopulations are strongly correlated with the different sampling sites suggesting local adaptation of divergent populations. Importantly, Structure and Admixture analyses reveal that, at $k = 2$, one population identified by morphology and the diagnostic PCR as *An. nili* has almost the same ancestry pattern as the largest *An. ovengensis* cluster (Figure 3). Such discrepancies between morphology-based and

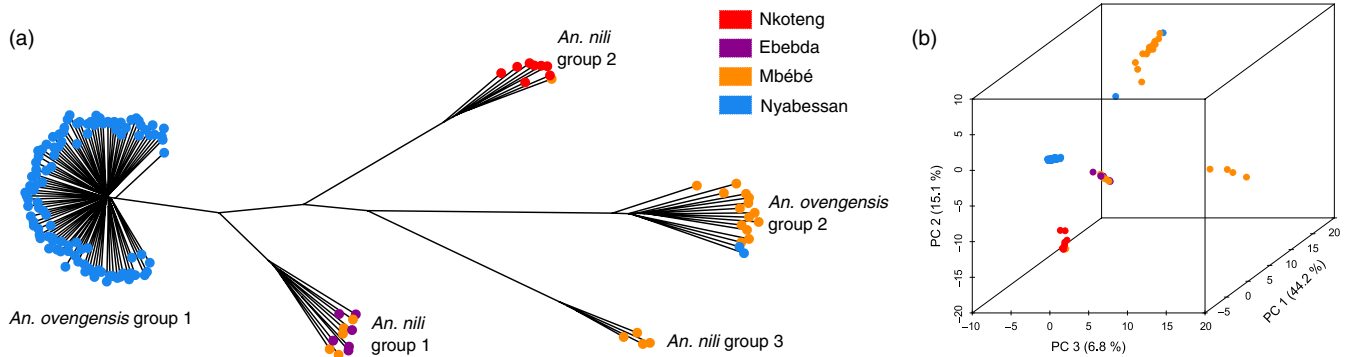


FIGURE 2 Population genetic structure inferred from 4,343 SNPs using a PCA (a) and a neighbor-joining tree (b). The percentage of variance explained is indicated on each PCA axis. Note the strong association between the five genetic clusters and the different sampling locations

molecular taxonomies can be due to a variety of processes including phenotypic plasticity, introgressive hybridization, or incomplete lineage sorting (i.e., when independent loci have different genealogies by chance) (Arnold, 1997; Combosch & Vollmer, 2015; Fontaine et al., 2015; Weng et al., 2016). At $k = 2$ and $k = 3$, some populations also exhibit half ancestry from each morphological species suggestive of gene flow. We found a conflicting number of genetic clusters in our samples likely reflecting the complex history of subdivisions and admixtures among populations (Figure 4). The Evanno et al. (2005) method, which highlights the early stages of divergence between *An. nili* and *An. ovengensis*, indicates two probable ancestors. DAPC and the Admixture cross-validation error, which are more sensitive to recent hierarchical population subdivisions, show five or more distinct clusters as revealed by the PCA and the NJ tree (Figure 4).

As suggested by the long internal branches, which connect subpopulations on the NJ tree, there is strong differentiation between and within morphological species characterized by globally high- F_{ST}

values (Table 1). Relatively lower F_{ST} values observed between certain clusters may be due to greater interpopulation migration and intermixing or more recent divergence. The F_{ST} values do not reflect the morphological delimitation of species. Indeed, the level of genetic differentiation is higher between some subpopulations within the same morphological species. Overall, patterns of genetic structure and differentiation reveal a group of populations whose phylogenies and species status are likely confounded by hybridization and/or incomplete lineage sorting. We argue that the current taxonomy based on morphology and ribosomal DNA does not capture the optimal reproductive units among populations of this group of mosquitoes.

3.3 | Genomic signatures of divergent selection and demographic history

We analyzed patterns of genetic differentiation across SNP loci throughout the genome. Pairwise comparisons are based on filtered variants that satisfy all criteria to be present in both populations, which explains the discrepancy in the number of SNPs observed between specific paired comparisons (Figure 5). The distribution of locus-specific F_{ST} values between the five subpopulations revealed a U-shape characterized by two peaks around 0 and 1. The large majority of SNPs have low-to-moderate divergence, but a substantial number of variants are extremely differentiated between populations. The maximum F_{ST} among SNPs is 1, and the proportion of loci with $F_{ST} = 1$ varies from 6.52% between the populations we termed *An. nili* group 1 and *An. ovengensis* group 1 to 44.74% between the subgroups called *An. nili* group 2 and *An. nili* group 3 (Figure 5). This pattern of genomewide divergence suggests that a very high number of sites with abrupt differentiation—which likely contain genes that contribute to divergent selection and/or reproductive isolation—coexist with regions of weak divergence that can be freely exchanged between species. As is the case with the overall genetic differentiation, morphology is not a reliable predictor of locus-specific divergence. Precisely, the lowest percentage of fixed SNPs is found between *An. ovengensis* from Nyabessan and *An. nili* collected from Mbébé and Ebebda (Figures 1 and 5). In contrast, the largest proportion of fixed loci is observed between locally adapted subgroups within the same morphological species: *An. nili*. The draft reference genome made

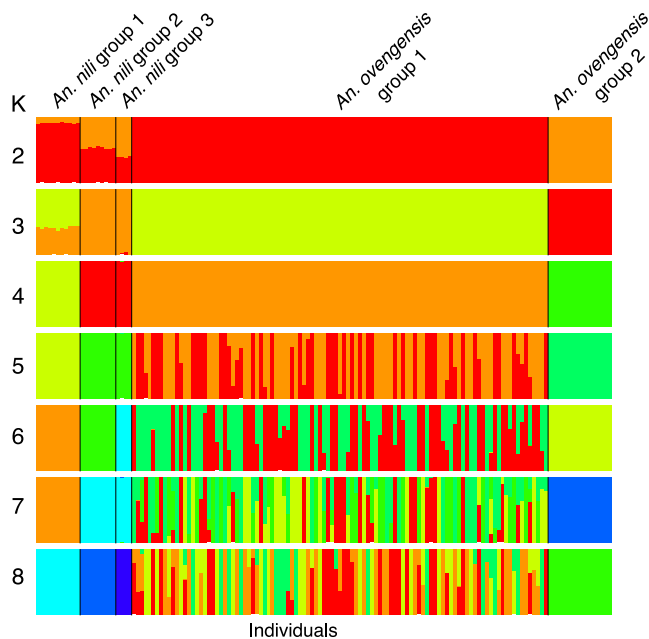


FIGURE 3 Ancestry proportions inferred in Admixture with $k = 2-8$

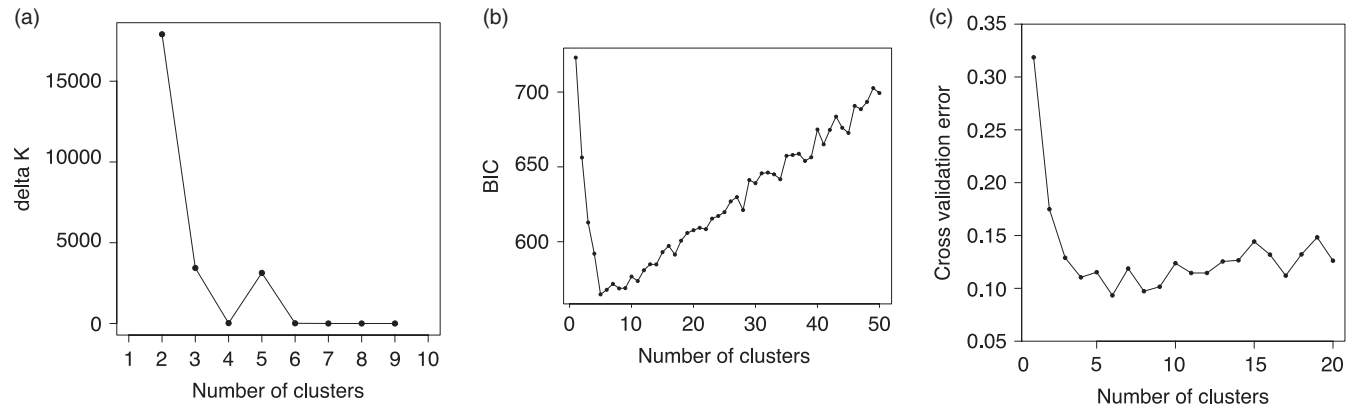


FIGURE 4 Identification of the optimal number of genetic clusters using the delta k method of Evanno et al. (2005) (a), DAPC (b) and 10-fold cross-validation in Admixture (c). The lowest Bayesian information criterion (BIC) and cross-validation error and the highest delta k indicate the most probable number of clusters

TABLE 1 Pairwise F_{ST} between divergent subpopulations of *An. nili* s.l.

F_{ST}	<i>An. nili</i> group 1	<i>An. nili</i> group 2	<i>An. nili</i> group 3	<i>An. ovengensis</i> group 1	<i>An. ovengensis</i> group 2
<i>An. nili</i> group 1	–				
<i>An. nili</i> group 2	0.374	–			
<i>An. nili</i> group 3	0.506	0.552	–		
<i>An. ovengensis</i> group 1	0.135	0.275	0.364	–	
<i>An. ovengensis</i> group 2	0.432	0.458	0.492	0.349	–

up of short contigs did not enable us to test hypotheses about the genomic distribution of differentiated loci. For example, it remains unknown whether the numerous SNPs that are fixed among populations are spread throughout the entire genome or clustered within genomic regions of low recombination including chromosomal inversions and chromosome centers (Nosil & Feder, 2012; Roesti, Hendry, Salzburger, & Berner, 2012).

Models of population demography indicate that all subgroups have experienced an increase in effective size in a more or less recent past (Table 2). Nevertheless, confidence intervals of population parameters are high in some populations, and our results should be interpreted with the necessary precautions. The population growth is less significant in *An. nili* group 1.

4 | DISCUSSION

4.1 | Genetic differentiation

Advances in sequencing and analytical approaches have opened new avenues for the study of genomes of disease vectors. We have focused on malaria mosquitoes of the *An. nili* group, whose taxonomy and population structure have been challenging to resolve with low-resolution markers. We analyzed genetic structure using genomewide SNPs and found strong differentiation and local adaptation among populations belonging to the two morphologically defined species *An. nili* and *An.*

ovengensis. The exact number of subpopulations remains contentious, with the suggested number of divergent clusters varying from two to five. Significant population structure at eight microsatellite loci has been described among *An. nili* populations from Cameroon, with F_{ST} values as high as 0.48 between samples from the rainforest area (Ndo et al., 2013). By contrast, *An. ovengensis* was discovered recently and the genetic structure of this vector remains understudied. This species was initially considered as a sibling of *An. nili* (Awono-Ambene et al., 2004, 2006; Kengne et al., 2003), but more recent studies have started to challenge the assumed relatedness between the two species due to the high divergence revealed by polytene chromosomes (Sharakhova et al., 2013). Our findings call for a careful review of the current taxonomy within this group of species, which is a necessary first step for accurately delineating the role played by the different subpopulations in malaria transmission.

Our samples were collected from locations characterized by a more or less degraded forest within the rainforest area of Cameroon. In these habitats, larvae of *An. nili* s.l. exploit relatively similar breeding sites consisting of slow-moving rivers (Antonio-Nkondjio et al., 2009). The ecological drivers of genetic differentiation remain unknown, and will be difficult to infer from our data given the apparent similarity of habitats among the divergent populations we described. Further study is needed to clearly address the environmental variables that may be correlated with ongoing adaptive divergence at adult and larval stages. One of the most expected outcomes of current large-scale malaria

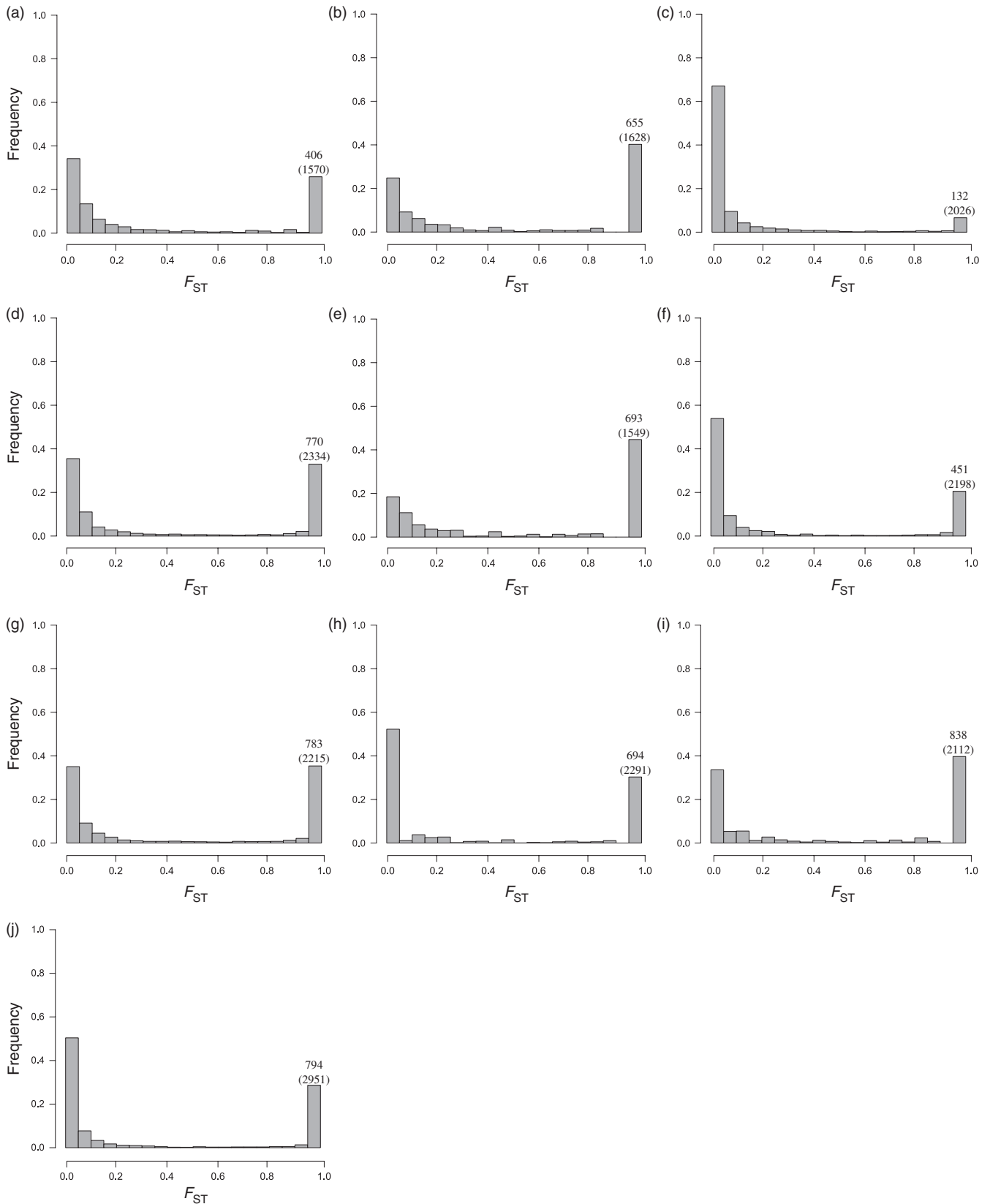


FIGURE 5 Distribution of F_{ST} values throughout the genome between *An. nili* group 1 and *An. nili* group 2 (a); *An. nili* group 1 and *An. nili* group 3 (b); *An. nili* group 1 and *An. ovengensis* group 1 (c); *An. nili* group 1 and *An. ovengensis* group 2 (d); *An. nili* group 2 and *An. nili* group 3 (e); *An. nili* group 2 and *An. ovengensis* group 1 (f); *An. nili* group 2 and *An. ovengensis* group 2 (g); *An. nili* group 3 and *An. ovengensis* group 1 (h); *An. nili* group 3 and *An. ovengensis* group 2 (i); *An. ovengensis* group 1 and *An. ovengensis* group 2 (j). The number of SNPs with $F_{ST} = 1$ is indicated in each pairwise comparison as well as the total number of SNPs in parenthesis

TABLE 2 Demographic models of different subgroups of *An. nili* s.l.

Population	Best model	Log-likelihood	Final population size ^a (95% CI)	Time ^b (95% CI)
<i>An. nili</i> group 1	Growth	-18.42	6.41 (5.326–20.71)	3.70 (1.11–13.31)
<i>An. nili</i> group 2	Two-epoch	-19.97	17.87 (9.33–35.50)	11.27 (4.93–19.64)
<i>An. ovengensis</i> group 1	Growth	-112.18	13.04 (12.15–17.26)	0.70 (0.58–1.08)
<i>An. Ovengensis</i> group 2	Growth	-22.98	19.95 (14.45–45.70)	5.11 (2.33–15.13)

^aRelative to ancestral population size.^bExpressed in units 2Ne generations from start of growth to present.

control measures that are underway in sub-Saharan African countries concerns the effects of increased insecticide exposure on the genetic diversity and population demography of vectors. A substantial population decline that may considerably affect the adaptive potential of vector species has been occasionally reported following a major insecticide-treated bed net distribution campaign and/or indoor residual house spraying (e.g., Athrey et al., 2012). The inferred demographic history of the different subpopulations within the *An. nili* group does not reveal signatures of bottlenecks that can be potentially correlated with increased usage of insecticides and insecticide-treated nets. This result is consistent with the demography of several other important malaria vectors of the Afrotropical region, including *An. gambiae*, *An. coluzzii*, *An. funestus* and *An. moucheti*, which reveals a substantial population increase suggesting that intense insecticide exposure has yet to leave deep or detectable impacts on patterns of genetic variation among mosquito populations (Fouet, Kamdem, Gamez, & White, 2017; Kamdem et al., 2017; O'Loughlin et al., 2014).

4.2 | Genomic architecture of geographic and reproductive isolation

Understanding the genomic architecture of reproductive isolation may reveal crucial information on the sequence of events that occur from the initial stages of divergence among populations to the onset of strong reproductive barriers between species (e.g., Turner et al., 2005; Harr, 2006; Nadeau et al., 2012; Ellegren et al., 2012; Carneiro et al., 2014; Burri et al., 2015). One influential concept of speciation coined the “genic view of species” proposes that boundaries between species are properties of individual genes or genome regions and not of whole organisms or lineages (Barton & Hewitt, 1985; Harrison & Larson, 2014; Harrison, 1990; Key, 1968; Nosil & Feder, 2012; Rieseberg, Whitton, & Gardner, 1999; Wu, 2001). We have discovered a substantially high number of SNPs that are strongly differentiated between populations and often fixed within subgroups of *An. nili* s.l. Interpreting this intriguing pattern of genomic differentiation is not straightforward due to the complex interactions between numerous forces—including positive or negative selection, recombination, introgressive hybridization and incomplete lineage sorting—that can affect the level of divergence among SNPs (Begun & Aquadro, 1992; Cutter & Payseur, 2013; Harrison & Larson, 2016; Nachman & Payseur, 2012; Roesti et al., 2012). Some of these variants exhibiting high divergence among populations certainly contain markers of ecological and/or reproductive isolation. However, as far as reproductive

barriers are concerned, recent studies have indicated a complex relationship between the degree of genetic differentiation and gene flow at the genome level (e.g., Gompert et al., 2012; Hamilton, Lexer, & Aitken, 2013a,b; Larson, Andrés, Bogdanowicz, & Harrison, 2013; Larson, White, Ross, & Harrison, 2014; Parchman et al., 2013; Taylor, Curry, White, Ferretti, & Lovette, 2014). Highly divergent genomic regions do not necessarily coincide with regions of reduced gene flow among established or emerging species. Several alternative interpretations exist for the numerous high- F_{ST} regions we detected in all pairwise comparisons (Cruickshank & Hahn, 2014; Delmore et al., 2015; Nachman & Payseur, 2012; Noor & Bennett, 2009). Nevertheless, careful examination of these outliers of differentiation may reveal significant insights into the wide range of genes and traits that contribute to ecological divergence and/or reproductive isolation between subgroups of *An. nili* s.l. A complete genome assembly will be necessary to better delineate specific regions of the genome under natural selection, and therefore clarify the genomic basis of phenotypic fitness differences between divergent populations. This will also help understand the extent to which recent selection associated with human interventions contribute to local adaptation and genetic differentiation as observed in *An. gambiae* and *An. coluzzii* (Kamdem et al., 2017).

Signals consistent with gene flow between *An. nili* and *An. ovengensis* are apparent in our data although it has been proposed that the two morphological species diverged ~3 Myr ago (Ndo et al., 2013). Some individuals display almost half ancestry from each morphological species. The disagreement between morphology/PCR and molecular taxonomies observed in Structure and Admixture analyses also suggests that incongruent genealogies may be widespread along chromosomes due to hybridization. However, hybridization can be difficult to detect because other factors such as incomplete lineage sorting or technical artifacts can leave signatures that are similar to those of interspecific gene flow (Liu et al., 2014; Patterson et al., 2012). A complete reference genome is also needed to analyze the detailed distribution of genealogies across small genomic windows and to disentangle the relative contribution of processes that generate the putative admixtures and species confusion observed among divergent populations (Fontaine et al., 2015; Martin et al., 2013; Weng et al., 2016).

5 | CONCLUSIONS AND IMPLICATIONS

Delineating the fine-scale population structure of mosquito populations is crucial for understanding their epidemiological significance

and their potential response to vector control measures. Moreover, recent malaria control efforts affect interspecific gene flow, genetic differentiation, population demography and natural selection in mosquitoes (Athrey et al., 2012; Barnes et al., 2017; Clarkson et al., 2014; Kamdem et al., 2017; Norris et al., 2015). Deciphering the signatures of these processes across mosquito genomes is important to minimize their negative impacts on vector control. Our findings shed some light on the complex evolutionary history and provide a framework for future investigations into the genetic basis of ecological and reproductive barriers among species of the *An. nili* group.

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DATA ARCHIVING STATEMENT

Raw data (fastq files) for 145 *Anopheles nili* individuals are available at: <https://doi.org/10.5061/dryad.5d5b3>

AUTHOR CONTRIBUTIONS

CF, CK, and BJW conceived and designed the experiments. CF, CK, SG, and BJW performed the experiments. CF and CK analyzed the data. CF, CK, and BJW wrote the manuscript.

REFERENCES

- Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, 19, 1655–1664.
- Antonio-Nkondjio, C., Kerah, C. H., Simard, F., Awono-Ambene, P., Chouaibou, M., Tchuinkam, T., & Fontenille, D. (2006). Complexity of the malaria vectorial system in Cameroon: Contribution of secondary vectors to malaria transmission. *Journal of Medical Entomology*, 43, 1215–1221. [https://doi.org/10.1603/0022-2585\(2006\)43\[1215:COTMVS\]2.0.CO;2](https://doi.org/10.1603/0022-2585(2006)43[1215:COTMVS]2.0.CO;2)
- Antonio-Nkondjio, C., Ndo, C., Costantini, C., Awono-Ambene, P., Fontenille, D., & Simard, F. (2009). Distribution and larval habitat characterization of *Anopheles moucheti*, *Anopheles nili*, and other malaria vectors in river networks of southern Cameroon. *Acta Tropica*, 112(3), 270–276. <https://doi.org/10.1016/j.actatropica.2009.08.009>
- Antonio-Nkondjio, C., & Simard, F. (2013). Highlights on *Anopheles nili* and *Anopheles moucheti*, Malaria Vectors in Africa. In S. Manguin (Ed.), *Anopheles mosquitoes - New insights into malaria vectors (INTECH)* (pp. 828). Croatia, European Union: InTech. ISBN 978-953-51-1188-7.
- Arnold, M. L. (1997). *Natural hybridization and evolution*. Oxford: Oxford University Press.
- Athrey, G., Hodges, T. K., Reddy, M. R., Overgaard, H. J., Matias, A., & Ridl, F. C., ... Slotman, M. A. (2012). The effective population size of malaria mosquitoes: Large impact of vector control. *PLoS Genetics*, 8(12), e1003097. <https://doi.org/10.1371/journal.pgen.1003097>
- Awono-Ambene, H. P., Kengne, P., Simard, F., Antonio-Nkondjio, C., & Fontenille, D. (2004). Description and bionomics of *Anopheles (Cellia) ovengensis* (Diptera: Culicidae), a new malaria vector species of the

- Anopheles nili* group from south Cameroon. *Journal of Medical Entomology*, 41, 561–568. <https://doi.org/10.1603/0022-2585-41.4.561>
- Awono-Ambene, H. P., Simard, F., Antonio-Nkondjio, C., Cohuet, A., Kengne, P., & Fontenille, D. (2006). Multilocus enzyme electrophoresis supports speciation within the *Anopheles nili* group of malaria vectors in Cameroon. *The American Journal of Tropical Medicine and Hygiene*, 75, 656–658. <https://doi.org/10.1093/ajtmh/75.4.656> [pii]
- Barnes, K. G., Weedall, G. D., Ndula, M., Irving, H., Mzihalowa, T., Hemingway, J., & Wondji, C. S. (2017). Genomic Footprints of Selective Sweeps from Metabolic Resistance to Pyrethroids in African Malaria Vectors Are Driven by Scale up of Insecticide-Based Vector Control. *PLoS Genetics*, 13(2), e1001122. <https://doi.org/10.1371/journal.pgen.1006539>
- Barton, N., & Hewitt, G. (1985). Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, 16, 113–148.
- Begun, D., & Aquadro, C. (1992). Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. *Nature*, 356, 519–520.
- Bøgh, C., Pedersen, E. M., Mukoko, D. A., & Ouma, J. H. (1998). Permethrin-impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. *Medical and Veterinary Entomology*, 12(1), 52–59.
- Burri, R., Nater, A., Kawakami, T., Mugal, C. F., Olason, P. I., & Smeds, L., ... Ellegren, H. (2015). Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of *Ficedula* flycatchers. *Genome Research*, 25(11), 1656–1665. <https://doi.org/10.1101/gr.196485.115>
- Carneiro, M., Albert, F. W., Afonso, S., Pereira, R. J., Burbano, H., & Campos, R., ... Ferrand, N. (2014). The genomic architecture of population divergence between subspecies of the European Rabbit. *PLoS Genetics*, 10(8), e1003519. <https://doi.org/10.1371/journal.pgen.1003519>
- Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and genotyping Loci de novo from short-read sequences. *G3 (Bethesda, Md.)*, 1(3), 171–182. <https://doi.org/10.1534/g3.111.000240>
- Catchen, J., Hohenlohe, P. a., Bassham, S., Amores, A., & Cresko, W. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, 22, 3124–3140. <https://doi.org/10.1111/mec.12354>
- Clarkson, C. S., Weetman, D., Essandoh, J., Yawson, A. E., Maslen, G., & Manske, M., ... Donnelly, M. J. (2014). Adaptive introgression between *Anopheles* sibling species eliminates a major genomic island but not reproductive isolation. *Nature Communications*, 5, 4248. <https://doi.org/10.1038/ncomms5248>
- Coetzee, M., & Koekemoer, L. (2013). Molecular Systematics and Insecticide Resistance in the Major African Malaria Vector *Anopheles funestus*. *Annual Review of Entomology*, 58, 393–412.
- Combosch, D. J., & Vollmer, S. V. (2015). Trans-Pacific RAD-Seq population genomics confirms introgressive hybridization in Eastern Pacific *Pocillopora* corals. *Molecular Phylogenetics and Evolution*, 88, 154–162. <https://doi.org/10.1016/j.ympev.2015.03.022>
- Cruikshank, T. E., & Hahn, M. W. (2014). Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Molecular Ecology*, 23(13), 3133–3157. <https://doi.org/10.1111/mec.12796>
- Cutter, A. D., & Payseur, B. A. (2013). Genomic signatures of selection at linked sites: Unifying the disparity among species. *Nature Reviews Genetics*, 14(4), 262–274. <https://doi.org/10.1038/nrg3425>
- Delmore, K. E., Hübner, S., Kane, N. C., Schuster, R., Andrew, R. L., & Câmara, F., ... Irwin, D. E. (2015). Genomic analysis of a migratory divide reveals candidate genes for migration and implicates selective sweeps in generating islands of differentiation. *Molecular Ecology*, 24(8), 1873–1888.
- Derua, Y. a., Alifrangis, M., Hosea, K. M., Meyrowitsch, D. W., Magesa, S. M., Pedersen, E. M., & Simonsen, P. E. (2012). Change in composition of the *Anopheles gambiae* complex and its possible implications for the transmission of malaria and lymphatic

- filariasis in north-eastern Tanzania. *Malaria Journal*, 11(1), 188. <https://doi.org/10.1186/1475-2875-11-188>
- Dia, I., Guelbeogo, M., & Ayala, D. (2013). Advances and Perspectives in the Study of the Malaria Mosquito *Anopheles funestus*. In S. Manguin (Ed.), *Anopheles mosquitoes - New insights into malaria vectors (INTECH)* (pp. 828). Croatia, European Union: InTech.
- Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(359–361).
- Ellegren, H., Smeds, L., Burri, R., Olason, P. I., Backström, N., & Kawakami, T., ... Wolf, J. B. W. (2012). The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature*, 491(7426), 756–760. <https://doi.org/10.1038/nature11584>
- Evanno, G., Goudet, J., & Regnaut, S. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, 14, 2611–2620.
- Fontaine, M. C., Pease, J. B., Steele, a., Waterhouse, R. M., Neafsey, D. E., & Sharakhov, I. V., ... Besansky, N. J. (2015). Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science*, 347(6217), 1258524. <https://doi.org/10.1126/science.1258524>
- Fouet, C., Kamdem, C., Gamez, S., & White, B. J. (2017). Extensive genetic diversity among populations of the malaria mosquito *Anopheles moucheti* revealed by population genomics. *Infection, Genetics and Evolution*, 48, 27–33. <https://doi.org/10.1016/j.meegid.2016.12.006>
- Gillies, M. T., & Coetzee, M. (1987). *A supplement to the Anophelinae of Africa south of the Sahara*. Johannesburg: The South African Institute for Medical Research.
- Gillies, M. T., & De Meillon, B. (1968). *The Anophelinae of Africa South of the Sahara*, 2nd edn. Johannesburg: Publications of the South African Institute for Medical Research.
- Giraldo-Calderon, G. I., Emrich, S. J., MacCallum, R. M., Maslen, G., Dialynas, E., & Topalis, P., ... Lawson, D. (2015). VectorBase: An updated bioinformatics resource for invertebrate vectors and other organisms related with human diseases. *Nucleic Acids Research*, 43(D1), D707–D713. <https://doi.org/10.1093/nar/gku1117>
- Gompert, Z., Lucas, L. K., Nice, C. C., Fordyce, J. A., Forister, M. L., & Buerkle, C. A. (2012). Genomic regions with a history of divergent selection affect fitness of hybrids between two butterfly species. *Evolution*, 66(7), 2167–2181. <https://doi.org/10.5061/dryad.f0b2f083>
- Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, 5(10), e1000695. <https://doi.org/10.1371/journal.pgen.1000695>
- Hamilton, J. a., Lexer, C., & Aitken, S. N. (2013a). Differential introgression reveals candidate genes for selection across a spruce (*Picea sitchensis* × *P. glauca*) hybrid zone. *New Phytologist*, 197(3), 927–938. <https://doi.org/10.1111/nph.12055>
- Hamilton, J. A., Lexer, C., & Aitken, S. N. (2013b). Genomic and phenotypic architecture of a spruce hybrid zone (*Picea sitchensis* × *P. glauca*). *Molecular Ecology*, 22(3), 827–841. <https://doi.org/10.1111/mec.12007>
- Harbach, R. E. (2013). The Phylogeny and Classification of *Anopheles*. In S. Manguin (Ed.), *Anopheles mosquitoes - New insights into malaria vectors (INTECH)* (pp. 828). Croatia, European Union: InTech. ISBN 978-953-51-1188-7.
- Harr, B. (2006). Genomic islands of differentiation between house mouse subspecies, 730–737. <https://doi.org/10.1101/gr.5045006.entiation>
- Harrison, R. G. (1990). Hybrid zones: Windows on evolutionary process. *Oxford Surveys in Evolutionary Biology*, 7, 69–128.
- Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, 105(S1), 795–809. <https://doi.org/10.1093/jhered/esu033>
- Harrison, R. G., & Larson, E. L. (2016). Heterogeneous genome divergence, differential introgression, and the origin and structure of hybrid zones. *Molecular Ecology*, 25, 2454–2466. <https://doi.org/10.1111/mec.13582>
- Hemingway, J., Ranson, H., Magill, A., Kolaczinski, J., Fornadel, C., & Gimnig, J., ... Hamon, N. (2016). Averting a malaria disaster: Will insecticide resistance derail malaria control? *The Lancet*, 387(10029), 1785–1788. [https://doi.org/10.1016/s0140-6736\(15\)00417-1](https://doi.org/10.1016/s0140-6736(15)00417-1)
- Jakobsson, M., & Rosenberg, N. (2007). CLUMPP: A cluster matching and permutation program for dealing with multimodality in analysis of population structure. *Bioinformatics*, 23, 1801–1806.
- Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405.
- Kamdem, C., Fouet, C., Gamez, S., & White, B. J. (2017). Pollutants and insecticides drive local adaptation in African malaria mosquitoes. *Molecular Biology and Evolution*, 34(5), 1261–1275.
- Kengne, P., Awono-Ambene, H. P., Antonio-Nkondjio, C., Simard, F., & Fontenille, D. (2003). Molecular identification of the *Anopheles nili* group African malaria vectors. *Medical and Veterinary Entomology*, 17, 67–74.
- Key, K. H. L. (1968). The concept of stasipatric speciation. *Systematic Zoology*, 17, 14–22.
- Krzywinski, J., & Besansky, N. J. (2003). Molecular systematics of *Anopheles*: From subgenera to subpopulations. *Annual Review of Entomology*, 48(1), 111–139. <https://doi.org/10.1146/annurev.ento.48.091801.112647>
- Lanzaro, G. C., & Lee, Y. (2013). Speciation in *Anopheles gambiae* — The Distribution of Genetic Polymorphism and Patterns of Reproductive Isolation Among Natural Populations. In S. Manguin (Ed.), *Anopheles mosquitoes - New insights into malaria vectors (INTECH)*. (pp. 828). Croatia, European Union: InTech.
- Larson, E. L., Andrés, J. A., Bogdanowicz, S. M., & Harrison, R. G. (2013). Differential introgression in a mosaic hybrid zone reveals candidate barrier genes. *Evolution*, 67(12), 3653–3661. <https://doi.org/10.1111/evo.12205>
- Larson, E. L., White, T. A., Ross, C. L., & Harrison, R. G. (2014). Gene flow and the maintenance of species boundaries. *Molecular Ecology*, 23(7), 1668–1678. <https://doi.org/10.1111/mec.12601>
- Lawniczak, M. K. N., Emrich, S. J., Holloway, A. K., Regier, A. P., Olson, M., & White, B., ... Besansky, N. J. (2010). Widespread divergence between incipient *Anopheles gambiae* species revealed by whole genome sequences. *Science (New York, N.Y.)*, 330(6003), 512–514. <https://doi.org/10.1126/science.1195755>
- Liu, K. J., Dai, J., Truong, K., Song, Y., Kohn, M. H., & Nakhleh, L. (2014). An HMM-Based Comparative Genomic Framework for Detecting Introgression in Eukaryotes. *PLoS Computational Biology*, 10(6), e1003649. <https://doi.org/10.1371/journal.pcbi.1003649>
- Martin, S. H., Dasmahapatra, K. K., Nadeau, N. J., Salazar, C., Walters, J. R., Simpson, F., ... Jiggins, C. D. (2013). Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Research*, 23(11), 1817–1828. <https://doi.org/10.1101/gr.159426.113>
- Molineaux, L., & Gramiccia, G. (1980). *The Garki Project. Research on the epidemiology and control of malaria in the sudan savanna of West Africa*. Geneva: World Health Organization, 311 pp. ISBN: 9241560614, 9789241560610.
- Mwangangi, J. M., Mbogo, C. M., Orindi, B. O., Muturi, E. J., Midega, J. T., Nzovu, J., ... Beier, J. C. (2013). Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. *Malaria Journal*, 12, 13. <https://doi.org/10.1186/1475-2875-12-13>
- Nachman, M. W., & Payseur, B. A. (2012). Recombination rate variation and speciation: Theoretical predictions and empirical results from rabbits and mice. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367, 409–421. <https://doi.org/10.1098/rstb.2011.0249>
- Nadeau, N. J., Whibley, A., Jones, R. T., Davey, J. W., Dasmahapatra, K. K., & Baxter, S. W., ... Jiggins, C. D. (2012). Genomic islands of divergence in hybridizing *Heliconius* butterflies identified by large-scale targeted

- sequencing, *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 367(1587), 343–353.
- Ndo, C., Antonio-Nkondjio, C., Cohuet, A., Ayala, D., Kengne, P., Morlais, I., ... Simard, F. (2010). Population genetic structure of the malaria vector *Anopheles nili* in sub-Saharan Africa. *Malaria Journal*, 9, 161. <https://doi.org/10.1186/1475-2875-9-161>
- Ndo, C., Simard, F., Kengne, P., Awono-Ambene, P., Morlais, I., Sharakhov, I., ... Antonio-Nkondjio, C. (2013). Cryptic Genetic Diversity within the *Anopheles nili* group of Malaria Vectors in the Equatorial Forest Area of Cameroon (Central Africa). *PLoS One*, 8(3), 1–12. <https://doi.org/10.1371/journal.pone.0058862>
- Neafsey, D. E., Lawniczak, M. K. N., Park, D. J., Redmond, S. N., Coulibaly, M. B., Traoré, S. F., ... Muskavitch, M. A. T. (2010). SNP genotyping defines complex gene-flow boundaries among African malaria vector mosquitoes. *Science (New York, N.Y.)*, 330(6003), 514–517. <https://doi.org/10.1126/science.1193036>
- Noor, M. A. F., & Bennett, S. M. (2009). Islands of speciation or mirages in the desert? Examining the role of restricted recombination in maintaining species. *Heredity*, 103(6), 439–444. <https://doi.org/10.1038/hdy.2010.13>
- Norris, L. C., Main, B. J., Lee, Y., Collier, T. C., Fofana, A., Cornel, A. J., & Lanzaro, G. C. (2015). Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proceedings of the National Academy of Sciences*, 2014, 18892. <https://doi.org/10.1073/pnas.1418892112>
- Nosil, P., & Feder, J. L. (2012). Widespread yet heterogeneous genomic divergence. *Molecular Ecology*, 21(12), 2829–2832. <https://doi.org/10.1111/j.1365-294x.2012.05580.x>
- O'Loughlin, S. M., Magesa, S., Mbogo, C., Mosha, F., Midega, J., Lomas, S., & Burt, A. (2014). Genomic analyses of three malaria vectors reveals extensive shared polymorphism but contrasting population histories. *Molecular Biology and Evolution*, 1, 14. <https://doi.org/10.1093/molbev/msu040>
- Paradis, E., Claude, J., & Strimmer, K. (2004). Analyses of Phylogenetics and Evolution in R language. *Bioinformatics*, 20(2), 289–290.
- Parchman, T. L., Gompert, Z., Braun, M. J., Brumfield, R. T., McDonald, D. B., Uy, J. A. C., ... Buerkle, C. A. (2013). The genomic consequences of adaptive divergence and reproductive isolation between species of manakins. *Molecular Ecology*, 22(12), 3304–3317. <https://doi.org/10.1111/mec.12201>
- Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., ... Reich, D. (2012). Ancient admixture in human history. *Genetics*, 192(3), 1065–1093. <https://doi.org/10.1534/genetics.112.145037>
- Peery, A., Sharakhova, M. V., Antonio-Nkondjio, C., Ndo, C., Weill, M., Simard, F., & Sharakhov, I. V. (2011). Improving the population genetics toolbox for the study of the African malaria vector *Anopheles nili*: Microsatellite mapping to chromosomes. *Parasites & Vectors*, 4(1), 202. <https://doi.org/10.1186/1756-3305-4-202>
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS One*, 7(5), e37135. <https://doi.org/10.1371/journal.pone.0037135>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M., Bender, D., ... Sham, P. (2007). PLINK: A toolset for whole-genome association and population-based linkage analysis. *American Journal of Human Genetics*, 81(3), 559–575.
- R Development Core Team (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ranson, H., & Lissenden, N. (2016). Insecticide Resistance in African *Anopheles* Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends in Parasitology*, 32(3), 187–196. <https://doi.org/10.1016/j.pt.2015.11.010>
- Riehle, M. M., Guelbeogo, W. M., Gneme, A., Eiglmeier, K., Holm, I., Bischoff, E., ... Vernick, K. D. (2011). A cryptic subgroup of *Anopheles gambiae* is highly susceptible to human malaria parasites. *Science (New York, N.Y.)*, 331(6017), 596–598. <https://doi.org/10.1126/science.1196759>
- Rieseberg, L. H., Whitton, J., & Gardner, K. (1999). Hybrid Zones and the Genetic Architecture of a Barrier to Gene Flow Between Two Sunflower Species.
- Roesti, M., Hendry, A. P., Salzburger, W., & Berner, D. (2012). Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. *Molecular Ecology*, 21(12), 2852–2862. <https://doi.org/10.1111/j.1365-294x.2012.05509.x>
- Service, M. W. (1993). *Mosquito ecology: Field sampling methods*. London: UK Elsevier Applied Science, Ed.
- Sharakhova, M. V., Peery, A., Antonio-Nkondjio, C., Xia, A., Ndo, C., Awono-Ambene, P., ... Sharakhov, I. V. (2013). Cytogenetic analysis of *Anopheles ovengensis* revealed high structural divergence of chromosomes in the *Anopheles nili* group. *Infection, Genetics and Evolution*, 16, 341–348. <https://doi.org/10.1016/j.meegid.2013.03.010>
- Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., ... Hay, S. I. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: Occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*, 3(1), 117. <https://doi.org/10.1186/1756-3305-3-117>
- Taylor, S. A., Curry, R. L., White, T. A., Ferretti, V., & Lovette, I. (2014). Spatiotemporally consistent genomic signatures of reproductive isolation in a moving hybrid zone. *Evolution*, 68(11), 3066–3081. <https://doi.org/10.1111/evo.12510>
- Turner, T. L., Hahn, M. W., & Nuzhdin, S. V. (2005). Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology*, 3(9), 1572–1578. <https://doi.org/10.1371/journal.pbio.0030285>
- Van Bortel, W., Harbach, R. E., Trung, H. D., Roelants, P., Backeljau, T., & Coosemans, M. (2001). Confirmation of *Anopheles varuna* in vietnam, previously misidentified and mistargeted as the malaria vector *Anopheles minimus*. *The American Journal of Tropical Medicine and Hygiene*, 65(6), 729–732.
- Weng, D., Yu, Y., Hahn, M. W., & Nakhleh, L. (2016). Reticulate evolutionary history and extensive introgression in mosquito species revealed by phylogenetic network analysis. *Molecular Ecology*, 25(11), 2361–2372.
- WHO. (2016). *World malaria report 2016*. Geneva: WHO
- Wright, S. (1984). *Evolution and the genetics of populations: Genetics and biometric foundations* v. 1. Chicago, IL: University of Chicago. 480 pp. ISBN-13: 978-0226910383.
- Wu, C. (2001). The genic view of the process of speciation. *Journal of Evolutionary Biology*, 14, 851–865.
- Wu, T. D., & Nacu, S. (2010). Fast and SNP-tolerant detection of complex variants and splicing in short reads. *Bioinformatics*, 26(7), 873–881.

SUPPORTING INFORMATION

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