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Microhabitat Partitioning of *Aedes simpsoni* (Diptera: Culicidae)

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Abstract

Yellow fever virus is a reemerging infection responsible for widespread, sporadic outbreaks across Africa. Although *Aedes aegypti* (L.) is the most important vector globally, in East Africa, epidemics may be vectored by *Aedes bromeliae* (Theobald), a member of the *Aedes simpsoni* (Theobald) species complex. The *Ae. simpsoni* complex contains 10 subspecies, of which *Ae. bromeliae* alone has been incriminated as a vector of yellow fever virus. However, morphological markers cannot distinguish *Ae. bromeliae* from conspecifics, including the sympatric and non-anthropophilic *Aedes lili* (Theobald). Here, we used three sequenced nuclear markers to examine the population structure of *Ae. simpsoni* complex mosquitoes collected from diverse habitats in Rabai, Kenya. Gene trees consistently show strong support for the existence of two clades in Rabai, with segregation by habitat. Domestic mosquitoes segregate separately from forest-collected mosquitoes, providing evidence of habitat partitioning on a small spatial scale (<5km). Although speculative, these likely represent what have been described as *Ae. bromeliae* and *Ae. lili*, respectively. The observation of high levels of diversity within Rabai indicates that this species complex may exhibit significant genetic differentiation across East Africa. The genetic structure, ecology, and range of this important disease vector are surprisingly understudied and need to be further characterized.

Keywords

Aedes simpsoni; yellow fever virus; genetic differentiation; domestication

Yellow fever virus (family *Flaviviridae*, genus *flavivirus*, YFV) is a reemerging pathogen owing to declining population immunity, habitat destruction, urbanization, population movements, and climate change (Barrett and Higgs 2007). The Kaiser Foundation reported 2,600 cases in 2011, with the burden of disease falling disproportionately in Africa (Kaiser Foundation 2013); however, many countries do not report cases and the realized burden is likely much greater. Recent large outbreaks in East Africa—in Kenya in 1992–1993 and Sudan in 2003 and 2005—were caused by novel virus genotypes unreported for >40 yr and demand a renewed attention to YFV detection and prevention in the region (Ellis and Barrett 2008).

YFV is maintained in three transmission cycles. In the urban cycle, YFV is transmitted between humans by domesticated *Aedes (Stegomyia) aegypti* (L.). In the sylvatic cycle, the virus is maintained by tree hole-breeding *Aedes (Stegomyia) africanus* (Theobald) mosquitoes feeding on monkeys (Haddow et al. 1948, Smithburn et al. 1949, Mutebi and Barrett 2002). In the less understood, intermediate, or savannah cycle, humans become infected through spillover from the sylvatic cycle, which then allows anthropophilic mosquitoes to vector large human outbreaks (Ellis and Barrett 2008).

The ecology of East African YFV is unique owing to the absence of urban transmission because *Ae. aegypti* has never been incriminated as a vector in the region (Sanders et al. 1998, Onyango et al. 2004). Instead, the intermediate transmission cycle results in sporadic, widespread epidemics in a largely susceptible population (Ellis and Barrett 2008). In East African emergence zones, erratic spillover events result in human outbreaks vectored by *Aedes bromeliae* (Theobald), a member of the *Aedes simpsoni* (Theobald) species complex (Mahaffy et al. 1942, Barrett and Monath 2003). *Ae. bromeliae* was implicated as the vector of the largest recorded YFV outbreak of an estimated 200,000 cases in Ethiopia in 1961–1962 (Sérié et al. 1964), and in laboratory studies it is the most competent vector for the newly emergent East or Central African viral genotype (Ellis et al. 2012).

Strikingly, despite the increasing public health threat posed by YFV, the ecology of YFV in East Africa remains understudied. The biology and population structure for the members of the *Ae. simpsoni* complex remain uncharacterized and it is currently impossible to distinguish competent YFV vectors from conspecifics (Mukwaya et al. 2000). Since the incrimination of *Ae. bromeliae* as a vector a century ago, there has been continued controversy (Huang 1979, 1986; Lutwama and Louis 1994) over the systematics of the three sister species originally described by Theobald—*Ae. simpsoni* sensu stricto, *Aedes lilii*, and *Ae. bromeliae* (Theobald 1905, 1910, 1915). Huang (1979) developed a morphological key to distinguish *Ae. simpsoni* sensu stricto, *Ae. lilii*, and *Ae. bromeliae*. Specifically, *simpsoni* had simple tarsal claws and *lilii* and *bromeliae* had toothed tarsal claws, with leg markings used to differentiate *lilii* and *bromeliae* (Huang 1979). However, Lutwama and Louis (1994) reexamined the characters described by Huang (1979) and found that the morphological trait differences between subspecies were continuous, rendering them insufficient for species assignment (Huang 1979, Lutwama and Louis 1994, Mukwaya et al. 2000).

However, significant phenotypic and genetic variation within the complex has been observed. A previous population study using ribosomal DNA sequence variation found distinct anthropophilic and nonanthropophilic clades (Mukwaya et al. 2000). Importantly, *Ae. bromeliae* is the only anthropophilic member of the complex (Huang 1986), and thus is the only conspecific that is a significant human disease vector. Therefore, genetic diagnostic tools are needed to correctly identify potential YFV vectors so that the distribution of competent mosquito vectors can be characterized and human risk better understood (Mukwaya et al. 2000).

Domestication, or association with human-modified environments, may play an important role in defining vectorial capacity. Evidence of habitat segregation may indicate the existence of domesticated mosquito populations with a greater potential to vector outbreaks

of human pathogens. Discriminating between human-associated populations and sylvatic populations within the same species complex will importantly inform targeted vector control efforts. Here, we examined sequence variation at three nuclear markers to determine the population structure of *Ae. simpsoni* complex mosquitoes collected from a variety of ecological habitats in Rabai, Kenya. We used comparative sequence analysis to test for genetic differentiation between ecologically divergent populations of *Ae. simpsoni* (*s.l.*).

Materials and Methods

Mosquito Collection and Species Identification

Ae. simpsoni mosquitoes were collected from six sites across Rabai, Kenya, in 2009 as part of a larger *Ae. aegypti* sampling project (Fig. 1). Collections were conducted within three microhabitats: domestic, peridomestic, and forest. Domestic specimens were collected as larvae in artificial containers or in ovitraps placed within or immediately outside homes. Peridomestic specimens were collected with ovitraps placed within villages, but outside of the immediate domestic environment. Forest specimens were collected outside of villages from tree holes, as well as from ovitraps attached to trees. Mosquitoes were collected as eggs and larvae and reared to adults in the laboratory. Adults were identified as *Ae. simpsoni* (*s.l.*) using the Walter Reed Biosystematics Unit “Key for Medically Important Mosquito Species” (The Walter Reed Bio-systematics Unit 2013). Adults were preserved in 70% EtOH. In total, 200 *Ae. simpsoni* samples were collected, of which 30 mosquitoes were used in the current study.

Genetic Methods

Whole genomic DNA was extracted individually from a sample of 30 *Ae. simpsoni* mosquitoes by using a Qiagen DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Several nuclear and mitochondrial markers used in *Ae. aegypti* (Morlais and Severson 2003, Paduan and Ribolla 2009) were evaluated for potential use on *Ae. simpsoni* mosquitoes. Primers developed by Brown et al. (2013) for use in *Ae. aegypti* were used in this study. Three nuclear markers—apolipoprotein 2 (*apoLp2*), cytochrome p450 (*CYPJ92*), and short-chain dehydrogenase–reductase (*SDR*)—were found to be variable and amplify successfully across *Ae. simpsoni* populations. Although mitochondrial markers are often preferable for within-species phylogenetics, the presence of long mtDNA copies in nuclear genomes of several *Aedes* mosquitoes precludes their use as informative markers (Hlaing et al. 2009). The presence of nuclear copies of mtDNA was detected in many of our *Ae. simpsoni* (*s.l.*) individuals during the marker selection and primer optimization phase of this study.

All loci were amplified in a 20 μ l PCR reaction containing 1 $\times$ GoTaq Reaction Buffer (Promega, Madison, WI), 0.5 mM each dNTP, 0.5 μ M each primer, 2 mM MgCl₂, and 1U GoTaq DNA Polymerase (Promega). Loci were amplified according to the following thermocycling conditions: initial denaturation at 95°C for 2 min, followed by 38 cycles of 95°C for 30 s, loci-specific annealing at 52°C for 30 s, at 72°C for 1 min, and with a final extension at 72°C for 5 min. Sanger sequencing was performed on all products from both directions. Sequences are available on GenBank, accession numbers KF478942–KF478991.

Sequences were aligned and heterozygotes identified using CLC DNA Workbench version 6.5 (CLCbio, Aarhus, Denmark). PHASE was used to infer phase and separate haplotypes in heterozygous individuals (Stephens et al. 2001, Stephens and Donnelly 2003). SeqPHASE was used to convert between FASTA and PHASE file formats (Flot 2010).

Analyses

Multiple sequence alignments were created in MEGA5 (Tamura et al. 2011) using ClustalW (Larkin et al. 2007). Diversity and polymorphism statistics were examined using DNAsp v5 (Librado and Rozas 2009). The number of polymorphic sites (S), number of haplotypes, haplotype diversity (H_d), mean nucleotide diversity per site (π), average number of nucleotide differences (k), and genetic diversity (θ) were calculated for each nuclear locus. Bootstrapped neighbor-joining trees were created in MEGA5 for each nuclear gene (Tamura et al. 2011). Trees were rooted using three *Ae. aegypti formosus* samples (constituting six unique alleles) collected from the same region in Kenya as an outgroup. Trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

A common problem for arthropod nuclear gene phylogeography is the large number of alleles segregating at each locus, resulting in low power to reconstruct haplotypes (Garrick et al. 2010). The large number of alleles per locus (Table 1) observed here may result in limited power in resolving the haplotypes in heterozygous samples using PHASE. To address this potential source of bias, we constructed neighbor-joining trees excluding all heterozygous samples for comparison.

Results

Phylogenetic Structure

Three nuclear markers were found to amplify successfully and exhibit high variability across sampled populations. Each of the three gene trees (*apoLp2*, *CYP9J2*, and *SDR*) demonstrates a high level of bootstrap support for the existence of two clades within Rabai (Figs. 2–4). The *apoLp2* and *SDR* gene trees shows 100 and 99%, respectively, support for the existence of two *Ae. simpsoni* clades, whereas *CYP9J2* shows 99% bootstrap support for each clade. *Ae. aegypti formosus* alleles form an outgroup clade with 100% support in each gene tree. The peridomestic alleles (from samples As8 and As12) are distributed in the domestic clade of the *apoLp2* tree and do not form a distinct lineage. The strong differentiation observed is robust to the inclusion of phased heterozygote haplotypes. Neighbor-joining gene trees excluding all heterozygous samples (which may contain unresolved haplotypes, see Methods) were consistent with gene trees including all samples, indicating that the low statistical power to infer haplotype phase did not distort our observation of strong differentiation.

In the *CYP9J2* gene tree, a single forest allele clusters with the domestic clade. The existence of a one rare allele in the unexpected clade may represent an ancestral allele, not observed in this small sample; a rare, recent hybridization event; or an ongoing, low-level gene flow between domestic and sylvan populations.

Each gene tree indicates some evidence of population structuring within the sylvan clade. On the *CYP9J2* tree, the forest clade is divided into two subclades with strong (>90%) support. The structuring apparent in the forest population may be because of spatial structure or may reflect further undescribed microhabitat partitioning associated with breeding sites or available sylvatic hosts. Larger sample sizes are needed to further investigate structuring within each observed clade.

Nucleotide Diversity and Haplotype Analysis

Polymorphism statistics were calculated for each gene in addition to each sampling habitat (forest or domestic–peridomestic) and are presented in Table 1. Because of the small sample size of peridomestic-collected mosquitoes ($n = 2$) and their position within the domestic clade, we collectively referred to peridomestic and domestic-collected samples as “domestic” for diversity measures. Each locus exhibits a high level of nucleotide diversity: 12.8% variable sites (*apoLp2*), 15.3% (*CYP9J2*), and 10.3% (*SDR*). Each locus exhibits a large number (7–13) of haplotypes and a high degree of haplotype uniqueness (H_d) for a small sample size (28 alleles for *apoLp-2*, 34 alleles for *CYP9J2*, and 20 for *SDR*).

Developing Markers for *Ae. simpsoni*

In total, 30 mosquitoes were used in analysis, including 17 forest samples, 11 domestic samples, and 2 peridomestic samples. Despite multiple optimization attempts, of the 30 samples tested, several samples failed or sequences were truncated at each loci (Table 2). Because fulllength amplicons were not always produced for each of the three loci, the same individuals are not always represented in every tree. The failure of loci to amplify may be because of 1) low-quality DNA from old, field-collected samples; 2) variability in primer binding sites, as these primers were designed for *Ae. aegypti*; or 3) laboratory error. However, the strong differentiation observed in a low sample size suggests that we can confidently describe strong genetic differentiation within Rabai.

Discussion

Comparative sequence analysis shows strong support for the existence of two clades of *Ae. simpsoni* complex mosquitoes in Rabai, Kenya, with domestic and peridomestic mosquitoes segregating separately from forest-collected mosquitoes. Genetic divergence at three nuclear loci with high levels of bootstrap support is consistent with the existence of two sympatric, highly differentiated subspecies within Rabai. In addition, a high level of nucleotide diversity and high number of haplotypes are observed across forest and domestic populations at a small spatial scale (5 km). The two identified clades likely represent previously described subspecies *Ae. bromeliae* (domestic or peridomestic) and *Ae. lillii* (forest), both members of the *Ae. simpsoni* species complex are known to be present throughout Kenya (Mukwaya et al. 2000).

Although previous work by Mukwaya et al. (2000) examined genetic differentiation within this species complex, this early work focused on a single trait, anthropophily, defined as propensity to bite human hosts, and used a single locus approach. The current work expands upon and differs from this earlier study, further characterizing diversity within the under-

described *Ae. simpsoni* complex. First, we use a multilocus approach, expanding on the single locus approach used by the earlier paper. Second, we investigate genetic differentiation with regards to habitat preference, whereas the earlier paper investigated anthropophily; genetic variation associated with both phenotypes need to be investigated to assess variation in vectorial competence across the species complex. Third, we investigated differentiation at small spatial scales (<5 km) within a single city, Rabai, Kenya, whereas the previous work investigated differentiation across three countries in Africa.

The three neighbor-joining gene trees indicate significant genetic differentiation in sympatry. Our findings of nearly complete lineage sorting and high levels of support for sylvan and domestic clades across gene trees indicate much stronger differentiation than that observed between domestic and sylvan *Ae. aegypti* populations from East Africa (Brown et al. 2011). However, because of our small sample sizes and evidence of low levels of ongoing gene flow—for example, the presence of forest alleles of CYP9J2 in the domestic clade—we do not have sufficient evidence to describe these clades as distinct species. We conclude that gene flow between domestic and sylvatic forms of *Ae. simpsoni* mosquitoes is restricted, evidence of distinct habitat preferences. However, our small sample size cannot unambiguously define the observed clades as distinct species, and we suggest that more definitive future genetic studies explore species delineation within *Ae. simpsoni* complex mosquitoes.

Domestication

Human-modified environments impose multiple selective pressures and have driven genetic differentiation and speciation across a range of taxa. For mosquito vectors, human-modified habitats offer both constant access to food (human blood) as well as plentiful oviposition sites (Lyimo and Ferguson 2009, Brown et al. 2011). Previous work on the dengue and YFV vector, *Ae. aegypti*, has shown that mating often occurs near the host, resulting in positive assortative mating between domestic and sylvan forms (McClelland and Weitz 1963, Hartberg and Craig 1968). The selective pressure for specialization to domestic environments may result in reproductive isolation from sylvatic forms and drive differentiation and subsequent speciation across mosquito taxa (Tabachnick et al. 1979, Lyimo and Ferguson 2009). Population genetics provides important tools for studying domestication events (Tabachnick and Iv 1995), and previous work on *Ae. aegypti* has employed population genetics to identify a single historic domestication event, enabling global human-mediated spread of the domestic form of *Ae. aegypti* (Brown et al. 2011). Here, we found genetic evidence of domestication within *Ae. simpsoni*, a known YFV vector. Differentiation between domestic and sylvan subspecies may indicate that the domestic clade is an YFV vector, whereas the sylvatic clade may not play a significant role in virus transmission.

Ae. simpsoni complex mosquitoes are known to be associated with cultivated microhabitats (Lounibos 1981) and preferentially breed in phytotelma, i.e., water reservoirs held by plants, including tree holes and plant axils (Haddow 1948, Teesdale 1957, Pajot 1983, Lutwama and Louis 1994), although they are also found in artificial water containers (Trpis et al. 1971, Bown and Bang 1980). Gillett (1951) proposed that *Ae. simpsoni* were tree hole

breeders in the ancestral state; the large-scale cultivation of bananas, faro, and pineapples may have provided new breeding sites with continually available water sources, resulting in spatial reproductive isolation owing to oviposition site (Teesdale 1957).

Further reproductive isolation may result from the timing of oviposition. Breeding of the sylvan ecotype is closely correlated to rainfall and oviposition rates markedly decline during the dry season, when phytotelma are unavailable for the mosquitoes to oviposit (Bown and Bang 1980). The availability of oviposition sites for the domestic ecotype during the dry season does not pose the same seasonal constraints on breeding of the domestic clade, potentially enhancing the level of reproductive isolation between sylvan and domestic forms.

Population differentiation within *Ae. simpsoni* mosquitoes has been previously described (Gibbins 1942, Haddow 1945, Gillett 1951). Gillett (1951) proposed that the existence of both anthropophilic and nonanthropophilic populations in Uganda represented “separate races,” which he proposed evolved because of the geographic isolation of plantation habitats. Mukwaya (1977) found that host preference was genetically determined and found that the two genetic lineages of *Ae. simpsoni* segregated based on host preference (Mukwaya et al. 2000). The microhabitat partitioning observed here suggests that reproductive isolation owing to breeding site may have been a driving selective force in the sympatric divergence of the observed clades.

Further Directions

YFV remains a significant public health risk in East Africa, where little is known about the drivers of epidemic emergence from the sylvatic cycle (Mutebi and Barrett 2002, Ellis and Barrett 2008). The potential for more explosive emergence events or establishment of an urban transmission cycle of YFV in largely immune populations poses a serious threat (Ellis and Barrett 2008). However, it is impossible to understand the dynamics of YFV emergence without basic knowledge of the significant vectors and vector distribution. *Ae. simpsoni* complex mosquitoes were implicated as a competent YFV vector in 1942 (Mahaffy et al. 1942) and are known to serve as a critical bridge vector between sylvatic and human cycles. That we are still unable to distinguish the disease vector from sister species is problematic. Because *Ae. simpsoni* mosquitoes cannot be differentiated morphologically, genetic diagnostic tools are needed to correctly assign species as well as to explore population structure within known YFV vectors.

It is clear that we need to expand the molecular toolbox available for *Ae. simpsoni* complex mosquitoes. Our work has been limited to a subset of variable loci in *Ae. aegypti*, which amplify in *Ae. simpsoni*. Development of additional multilocus or single nucleotide polymorphism markers will give us higher resolution to examine patterns of population structure and insight onto the species status of *Ae. simpsoni* clades. These genomic markers will allow us to examine the genetics of multiple characteristics involved in domestication and relevant to understanding epidemiological risk including habitat and host preference, vectorial competence, and olfaction.

Further mosquito sampling and population genetic investigations should focus on regions with previous YFV epidemic activity across Sudan and Ethiopia (Kirk 1941, Sérié et al. 1964). Work is also needed in potential emergence zones, often characterized by a jungle or savannah mosaic where peridomestic vectors are abundant (Mutebi and Barrett 2002), to understand the extent of diversity within *Ae. simpsoni* complex mosquitoes and the range and abundance of competent vector populations. The high level of diversity observed in Rabai (Mukwaya et al. 2000) suggests the existence of much unsampled diversity across other habitats. Defining the distribution of relevant YFV vectors should be a priority as it will enable vector control and vaccination campaigns to be targeted to high-risk populations.

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References

- Barrett AD, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annu Rev Entomol.* 2007; 52:209–229. [PubMed: 16913829]
- Barrett AD, Monath TP. Epidemiology and ecology of yellow fever virus. *Adv Virus Res.* 2003; 61:291–315. [PubMed: 14714435]
- Bown DN, Bang YH. Ecological studies on *Aedes simpsoni* (Diptera: Culicidae) in southeastern Nigeria. *J Med Entomol.* 1980; 17:367–374. [PubMed: 7420363]
- Brown JE, McBride CS, Johnson P, Ritchie S, Paupy C, Bossin H, Lutomiah J, Fernandez-Salas I, Ponlawat A, Cornel AJ, et al. Worldwide patterns of genetic differentiation imply multiple “domestications” of *Aedes aegypti*, a major vector of human diseases. *Proc Biol Sci.* 2011; 278:2446–2454. [PubMed: 21227970]
- Brown J, Evans B, Zheng W, Obas V, Barrera-Martinez L, Egizi A, Zhao H, Caccone A, Powell JR. Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. *Evolution.* 2013; 68:514–525. [PubMed: 24111703]
- Ellis BR, Barrett AD. The enigma of yellow fever in East Africa. *Rev Med Virol.* 2008; 18:331–346. [PubMed: 18615782]
- Ellis BR, Sang RC, Horne KM, Higgs S, Wesson DM. Yellow fever virus susceptibility of two mosquito vectors from Kenya, East Africa. *Trans R Soc Trop Med Hyg.* 2012; 106:387–389. [PubMed: 22521217]
- Flot JF. SEQPHASE: a web tool for interconverting phase input/output files and fasta sequence alignments. *Mol Ecol Resour.* 2010; 10:162–166. [PubMed: 21565002]
- Garrick RC, Sunnucks P, Dyer RJ. Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. *BMC Evol Biol.* 2010; 10:118. [PubMed: 20429950]
- Gibbins EG. On the habits and breeding-places of *Aedes simpsoni* Theobald in Uganda. *Ann Trop Med Parasitol.* 1942; 36:151–160.
- Gillett JD. The habits of the mosquito *Aedes (Stego-myia) simpsoni* Theobald in relation to the epidemiology of yellow fever in Uganda. *Ann Trop Med Parasitol.* 1951; 45:0–6.
- Haddow AJ. The mosquitoes of Bwamba County, Uganda; the vertical distribution of mosquitoes in a banana plantation and the biting cycle of *Aedes (Stegomyia) simpsoni*, Theo. *Bull Entomol Res.* 1945; 36:297–304. [PubMed: 21003326]
- Haddow AJ. The mosquitoes of Bwamba County, Uganda; mosquito breeding in plant axils. *Bull Entomol Res.* 1948; 39:185–212. [PubMed: 18886058]

- Haddow AJ, Smithburn K, Dick G, Kitchen S, Lumsden W. Implication of the mosquito *Aedes (Stegomyia) africanus* Theobald in the forest cycle of yellow fever in Uganda. *Ann Trop Med Parasitol.* 1948; 42:218–223. [PubMed: 18891448]
- Hartberg WK, Craig GB. Reproductive isolation in *Stegomyia* mosquitoes. I. Sexual isolation between *Aedes aegypti* and *A. mascarensis*. *Ann Entomol Soc Am.* 1968; 61:865–870. [PubMed: 5758054]
- Hlaing T, Tun-Lin W, Somboon P, Socheat D, Setha T, Min S, Chang MS, Walton C. Mitochondrial pseudogenes in the nuclear genome of *Aedes aegypti* mosquitoes: implications for past and future population genetic studies. *BMC Genet.* 2009; 10:11. [PubMed: 19267896]
- Huang YM. *Aedes (Stegomyia) simpsoni* complex in the Ethiopian region with lectotype designation for *simpsoni* (Theobald) (Diptera: Culicidae). *Mosq Syst.* 1979; 11:221–234.
- Huang YM. *Aedes (Stegomyia) bromeliae* (Diptera: Culicidae), the yellow fever virus vector in East Africa. *J Med Entomol.* 1986; 23:196–200. [PubMed: 3701804]
- Kaiser Foundation. Yellow fever deaths WHO Vaccine-Preventable Diseases: Monitoring System 2013 Global Summary. 2013. http://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidenceyellowfever.html
- Kirk R. An epidemic of yellow fever in the Nuba mountains, Anglo-Egyptian Sudan. *Ann Trop Med Parasitol.* 1941; 35:35–67.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. Clustal W and Clustal X, version 2.0. *Bioinformatics.* 2007; 23:2947–8294. [PubMed: 17846036]
- Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 2009; 25:1451–1452. [PubMed: 19346325]
- Lounibos LP. Habitat segregation among African treehole mosquitos. *Ecol Entomol.* 1981; 6:129–154.
- Lutwama JL, Louis GM. Variation in morphological characters of adults of the *Aedes (Stegomyia) simpsoni* complex from Uganda, Kenya, and South Africa (Diptera: Culicidae). *Mosq Syst.* 1994; 26:145–157.
- Lyimo IN, Ferguson HM. Ecological and evolutionary determinants of host species choice in mosquito vectors. *Trends Parasitol.* 2009; 25:189–196. [PubMed: 19269900]
- Mahaffy AF, Smithburn KC, Jacobs HR, Gillett JD. Yellow fever in western Uganda. *Trans R Soc Trop Med Hyg.* 1942; 36:9–20.
- McClelland GA, Weitz B. Serological identification of the natural hosts of *Aedes aegypti* (L.) and some other mosquitoes (Diptera, Culicidae) caught resting in vegetation in Kenya and Uganda. *Ann Trop Med Parasitol.* 1963; 57:214–224. [PubMed: 14042652]
- Morlais I, Severson DW. Intraspecific DNA variation in nuclear genes of the mosquito *Aedes aegypti*. *Insect Mol Biol.* 2003; 12:631–639. [PubMed: 14986924]
- Mukwaya LG, Kayondo JK, Crabtree MB, Savage HM, Biggerstaff BJ, Miller BR. Genetic differentiation in the yellow fever virus vector, *Aedes simpsoni* complex, in Africa: sequence variation in the ribosomal DNA internal transcribed spacers of anthropophilic and non-anthropophilic populations. *Insect Mol Biol.* 2000; 9:85–91. [PubMed: 10672075]
- Mutebi JP, Barrett ADT. The epidemiology of yellow fever in Africa. *Microbes Infect.* 2002; 4:1459–1468. [PubMed: 12475636]
- Onyango CO, Grobbelaar AA, Gibson GV, Sang RC, Sow A, Swaneopel R, Burt FJ. Yellow fever outbreak, southern Sudan, 2003. *Emerg Infect Dis.* 2004; 10:1668–1670. [PubMed: 15498174]
- Paduan KS, Ribolla P. Characterization of eight single nucleotide polymorphism markers in *Aedes aegypti*. *Mol Ecol Resour.* 2009; 9:114–116. [PubMed: 21564575]
- Pajot, FX. Phytotelmata and mosquito vectors of sylvatic yellow fever in Africa. In: Frank, JH.; Lounibos, LP., editors. *Phytotelmata Terrestrial Plants as Hosts Aquatic Communities*. Plexus, Medford, NJ: 1983. p. 79-99.
- Sanders EJ, Marfin AA, Tukei PM, Kuria G, Ademba G, Agata NN, Ouma JO, Cropp CB, Karabatsos N, Reiter P, et al. First recorded outbreak of yellow fever in Kenya, 1992–1993. I. Epidemiologic investigations. *Am J Trop Med Hyg.* 1998; 59:644–649. [PubMed: 9790446]
- Sérié C, Andral L, Lindrec A, Neri P. Epidémie de fièvre jaune en Ethiopie (1960–1962). *Bull OMS.* 1964; 30:299–319.

- Smithburn KC, Haddow AJ, Lumsden WH. An outbreak of sylvan yellow fever in Uganda with *Aedes (Stegomyia) africanus* Theobald as principal vector and insect host of the virus. *Ann Trop Med Parasitol.* 1949; 43:74–89. [PubMed: 18121272]
- Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 2001; 68:978–989. [PubMed: 11254454]
- Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Human Gen.* 2003; 73(5):1162–1169.
- Tabachnick W, Black WC. Making a case for molecular population genetic studies of arthropod vectors. *Trends Parasitol.* 1995; 11:27–30.
- Tabachnick W, Munstermann L, Powell J. Genetic distinctness of sympatric forms of *Aedes aegypti* in East Africa. *Evolution (N Y).* 1979; 33:287–295.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* 2011; 28
- Teesdale C. The genus *Musa* Linn. and its role in the breeding of *Aedes (Stegomyia) simpsoni* (Theo.) on the Kenya coast. *Bull Entomol Res.* 1957; 50:251–260.
- The Walter Reed Biosystematics Unit. Keys to the medically important mosquito vectors: *Aedes* mosquitoes, Afrotropical (AFRICOM). 2013
- Theobald FV. A new *Stegomyia* from Transvaal. *Entomologist.* 1905; 38:101–104.
- Theobald, FV. A Monograph of the Culicidae or Mosquitoes. British Museum of Natural History; London, United Kingdom: 1910.
- Theobald, FV. Novae Culicidae, Part 1. Wye, Kent: 1915. Uganda Culicidae including thirteen new species.
- Trpis M, Hartberg WK, Teesdale C, McClelland GA. *Aedes aegypti* and *Aedes simpsoni* breeding in coral rock holes on the coast of Tanzania. *Bull World Health Organ.* 1971; 45:529–531. [PubMed: 5317088]

Fig. 1. Collection sites. Map of Africa with inset showing mosquito collection sites depicted as blue points. Rabai, Kenya, is indicated with a red star in each map for reference. (Online figure in color.)

Fig. 2.

Gene tree for *apoLp-2*. Alleles from forest and domestic-collected mosquitoes are bracketed. The scale bar indicates 0.01 nuclear substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Three *Ae. aegypti formosus* (Aef) samples (constituting six unique alleles) collected from the same region in Kenya constitute the outgroup.

Fig. 3.

Gene tree for *SDR*. Alleles from forest and domestic-collected mosquitoes are bracketed. The scale bar indicates 0.02 nuclear substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Three *Ae. aegypti formosus* samples (constituting six unique alleles) collected from the same region in Kenya constitute the outgroup.

Fig. 4.

Gene tree for *CYP9J2*. Alleles from forest and domestic-collected mosquitoes are bracketed. The two forest-collected alleles which cluster with the domestic clade are indicated by a circle at the node. The scale bar indicates 0.02 nuclear substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Three *Ae. aegypti formosus* samples (constituting six unique alleles) collected from the same region in Kenya constitute the outgroup.

Table 1

Nucleotide diversity and polymorphism statistics

Measure	apoLp-2 (671 bp)			CYP9J2 (484 bp)			SDR (464 bp)		
	Total	For	Dom	Total	For	Dom	Total	For	Dom
No. of alleles	28	14	14	34	22	12	20	13	7
No. polymorphic sites	86	26	31	74	73	1	48	13	13
No. of haplotypes	13	7	6	11	9	2	7	5	3
Hd (haplotype diversity)	0.952	0.923	0.879	0.872	0.887	0.303	0.832	0.705	0.667
π (nucleotide diversity)	0.047	0.014	0.019	0.067	0.031	0.001	0.068	0.010	0.021
k (avg. nucleotide differences)	31.556	9.582	12.835	32.175	15.004	0.303	20.979	4.333	6.476
θ (per site from Eta)	0.034	0.012	0.015	0.038	0.043	0.001	0.051	0.010	0.017

Table 2

Mosquito sampling information

Sample information				Amplified loci		
Sample ID	Habitat type	In- or outdoors	Lifestage	APO	SDR	CYP
As1	DOM	In	Larvae	x	x	x
As3	DOM	In	Larvae		x	x
As4	FOR	Out	Eggs	x	x	x
As6	FOR	Out	Eggs	x	x	x
As8	PER	Out	Eggs	x		
As9	DOM	Out	Larvae	x		
As11	DOM	Out	Larvae	x	x	x
As12	PER	Out	Eggs	x		
As13	DOM	In	Larvae			x
As14	FOR	Out	Eggs			x
As19	FOR	Out	Eggs		x	
As20	FOR	Out	Eggs			x
As23	FOR	Out	Eggs			x
As24	DOM	In	Larvae			x
As25	DOM	In	Larvae	x		
As27	FOR	Out	Eggs	x	x	x
As28	FOR	Out	Eggs	x		
As29	FOR	Out	Eggs		x	x
As30	DOM	Out	Larvae	x		
As31	DOM	Out	Larvae		x	x
As32	FOR	Out	Eggs	x	x	x
As33	FOR	Out	Eggs	x	x	x
As35	FOR	Out	Eggs		x	x
As36	FOR	Out	Eggs	x	x	x

Sequences are available on GenBank, accession numbers KF478942–KF478991.

Habitat type: DOM, domestic; FOR, forest; PER, peridomestic.