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Population structure of *Anopheles gambiae* along the Kenyan coast

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Abstract

In the tropics, *Anopheles* mosquito abundance is greatest during the wet season and decline significantly during the dry season as larval habitats shrink. Population size fluctuations between wet and dry seasons may lead to variation in distribution of specific alleles within natural *Anopheles* populations, and a possible effect on the population genetic structure. We used 11 microsatellite markers to examine the effect of seasonality on population genetic structure of *Anopheles gambiae* s.s. at two sites along the Kenyan coast. All loci were highly polymorphic with the total number of alleles for pooled samples ranging from 7 (locus ND36) to 21 (locus AG2H46). Significant estimates of genetic differentiation between sites and seasons were observed suggesting the existence of spatio-temporal subpopulation structuring. Genetic bottleneck analysis showed no indication of excess heterozygosity in any of the populations. These findings suggest that along the Kenyan coast, seasonality and site specific ecological factors can alter the genetic structure of *A. gambiae* s.s. populations.

Keywords

Anopheles gambiae; Seasonality; Population structure; Kenyan coast

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1. Introduction

In Africa, members of the *Anopheles gambiae* complex are the most widespread, and the most effective vectors of *Plasmodium falciparum* malaria. *A. gambiae* s.s. (hence forth referred to as *A. gambiae*) is the most dominant member of the complex and accounts for the largest proportion of malaria transmission in sub-Saharan Africa. This is partly because it has the ability to adapt and remain stable in a wide range of bio-ecological and seasonal conditions (Coluzzi et al., 2002)

Along the Kenyan coast, this species occurs in sympatry and share larval habitats with another malaria vector, *Anopheles funestus*, particularly during the wet season. However, during the dry season when small and transient larval habitats preferred by *A. gambiae* shrink, *A. funestus* continue to breed in permanent and vegetated habitats thus sustaining malaria transmission during the dry season (Mbogo et al., 2003). The seasonal rise and fall of *A. gambiae* populations can lead to loss of some alleles thereby altering the genetic structure of this species.

There has been no field study conducted to establish whether larval habitat re-colonization after the dry season is due to egg survival through the dry season, or due to new migrants arriving from neighbouring areas. As a result, it is not clear whether the adult population undergoes local extinction, aestivation or employs egg survival strategies to maintain itself during the dry season. Previous studies have shown that depending on soil type, *A. gambiae* eggs could survive for up to 15 days under stressful dry conditions (Shililu et al., 2004). Such a limited desiccation tolerance of *A. gambiae* eggs may only contribute to short-term survival in moist or dry soil. Direct observations of individual movement through mark release recapture (MRR) data along the Kenyan coast has been informative in estimating how far mosquitoes are able to move (Midega et al., 2007). However, these methods are inefficient at detecting long distance dispersal and are rarely able to demonstrate if dispersal has resulted in effective breeding. In this study, we used population genetics measurements to determine whether there are changes in the population genetic structure of *A. gambiae* between the wet and dry seasons. Two *A. gambiae* populations from Kilifi District collected during the wet and dry seasons were genotyped using 11 microsatellite loci to determine: (a) whether there is any population differentiation between *A. gambiae* populations in the two study sites; (b) whether there are any changes in the population genetic structure of the *A. gambiae* populations between seasons; and (c) the levels of gene flow between *A. gambiae* populations as measured indirectly using microsatellite DNA markers.

2. Materials and methods

2.1. Study area

Adult mosquitoes were collected in Jaribuni and Mtepeni; two villages located approximately 50km apart in Kilifi District, along the Kenyan coast. Jaribuni is located 03°37.3′ South and 039°44.6′ East. The Jaribuni River runs across Jaribuni village and during the rainy season, water levels rise and temporary larval habitats are formed at the edge of the river. These habitats expand and contract with the rise and fall of water, and may disappear when the water levels reduce to extremely low levels. Mtepeni is located 03°54.5″ South and 039° 43.6″ East. The site has a hilly terrain and a seasonal stream crosses through the site, contributing the bulk of mosquito larval habitats. Households in both study sites are sparsely distributed and most homesteads consist of two or more houses. The rains are generally bimodal with long rains falling from April to June (with peak in May) and short rains in October to December. Average annual rainfall varies between 400 and 1200mm. The mean daily minimum andmaximumtemperatures averages 22 and 30 °C, respectively

and the average relative humidity is approximately 70% (Mtwapa meteorological Station, 2010).

2.2. Adult mosquito sampling and species identification

Mosquito samples were collected for a period 4 months covering the wet (April and October 2003) and the dry (February and July 2003) seasons. Each month, weekly collections of adult females were done indoors by manual aspiration (DRI) and human landing catches and outdoors by human landing catches. DRI was conducted between 08:00 and 11:00 h, while human landing catches were conducted between 19:00 and 24:00 h. Samples were transported to the laboratory and identified morphologically to species using taxonomic keys of Gillies and De Meillon (1968). Legs and wings from each sample were preserved in 70% ethanol for DNA extraction (Severson, 1997) and sibling species identification by rDNA PCR method (Scott et al., 1993). The DNA was stored at −20 °C until ready for use. Non-amplifying samples were recorded as unknown after two unsuccessful trials.

2.3. Microsatellite amplification

Only *A. gambiae* was included in the microsatellite analysis. Eleven microsatellite loci representing all five chromosomal arms (Table 1)were selected from the published genetic map of Zheng et al. (1996) and cytogenetic maps developed by Coluzzi and Sabatini (1967).Multilocus genotypes of at least 150 specimens were scored per site (Tables 2 and 3). A standard PCR was run in a PTC-100 Thermal cycler (MJ Research Water town, MA) in a 10μL PCR reaction volume containing 8.0μl of 2× PCR master mix (Promega WI, USA), 0.5μl of 20 pmol of each primer, 0.5μL of template DNA and 1.0μL of nuclease free water. The tailed primer method was used to label the forward primer with M13 fluorescent label added to the 5′ end of the oligonucleotide (Sharakhov et al., 2004). The DNA amplification profile was conducted through 35 cycles as follows: An initial denaturation step of 2min at 95 °C; followed by 35 cycles of 30 s annealing at 55 °C, 20 s extension at 72 °C and a 45 s final extension step. Each PCR product was prepared for electrophoresis by mixing with sequencing dye followed by denaturation for 5min. A Li-Cor Model 4200 Automated DNA Analyzer (Li-Cor Inc., Lincoln, NE) was used for gel electrophoresis using 1.5μl of the final mixture in a 5% acrylamide gel. Microsatellite Allele sizes was determined using the Gene ImagIR 3.55 fragment analysis software (Li-Cor Inc., Lincoln, NE).

2.4. Statistical analysis

Genetic analysis was conducted using GenAlex version 6.1 (Peakall & Smouse, 2006) and GENEPOP version 3.4 (Rousset, 2008). Fragment sizes were entered into the CONVERT software (Slatkin and Barton, 1989) to facilitate data conversion into formats required for subsequent population genetic software packages.

Genetic diversity of mosquito populations was assessed by the number of observed (Na) and effective (Ne) alleles per locus; expected heterozygosity (He), and unbiased genetic diversity (H_E). Analysis of molecular variance (AMOVA) was performed to determine the genetic variation portioned at two levels: among individuals within populations and among populations. Estimates of gene flow (*Nm*) among population pairs were undertaken using the private alleles method with correction for sample size (Slatkin and Barton, 1989) and inbreeding coefficient (*Fis*) were carried out, using GENEPOP. Probability tests for Hardy Weinberg equilibrium (HWE) and for genotypic linkage disequilibrium (LD) were also done using GENEPOP with 10,000 iterations. Samples from the two sites were also examined for evidence of any recent severe decrease in population size (genetic bottleneck). Because recently bottle-necked populations exhibit heterozygosity excess at a majority of loci, the Wilcoxon Signed-Rank Test for heterozygosity excess was employed to detect bottlenecks under the infinite alleles model (IAM), stepwise mutation model (SMM) and two-phase

model (TPM), using BOTTLENECK version 1.2.02 (Cornuet & Luikart, 1996). Evidence for allele frequency distortion which occurs at neutral loci during bottlenecks was also assessed using the “mode-shift” indicator (Luikart et al., 1998).

3. Results

3.1. Species composition

A total of 417 *A. gambiae* s.l. samples were collected in the two study sites. These comprised 211 specimens from Jaribuni and 206 from Mtepeni. In Jaribuni, 50% of the specimens were collected during the dry season (February and July) and the remaining half were collected during the wet season (April and October). PCR analysis identified all specimens in Mtepeni as *A. gambiae* whereas in Jaribuni there were 203 *A. gambiae*, 4 *A. merus* and 4 indeterminate specimens.

3.2. Genetic diversity among populations

All the 11 loci displayed high polymorphism. The total number of alleles (Na) for pooled samples ranged from 7 (locus ND36) to 21 (locus AG2H46); mean ± S.D. = 13.8 ± 4.5. However, the number of alleles for each of the two populations separately was lower than that of the pooled population [Jaribuni: Na range = 3.0 (locus ND36) to 13 (locus H46), mean ± S.D. = 9.0 ± 3.8; Mtepeni: Na range = 4 (locus H131) to 16 (loci H46 & H249), mean ± S.D. = 10.5 ± 4.2]. In Jaribuni, the number of alleles per locus (Na) for the two dry seasons ranged from 3 to 13 and 3 to 9 (mean ± S.D. = 6.5 ± 2.8 and 6.0 ± 2.1) and from 3 to 13 and 3 to 11 alleles per locus (mean ± S.D. = 6.3 ± 2.9 and 6.9 ± 3.4; Table 2) for the two wet seasons. In Mtepeni, Na per locus ranged from 2 to 8 and 2 to 9 alleles in the dry seasons (mean ± S.D. = 5.4 ± 2.4 and 5.2 ± 2.3); and from 2 to 7 and 3 to 13 alleles in the wet seasons (mean ± S.D. = 4.6 ± 1.6 and 7.7 ± 2.9). Ne for all populations was much lower than Na, reflecting a high frequency of rare alleles (Table 3).

3.3. Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD)

Of the 44 tests that were conducted for the wet and dry seasons in Jaribuni, 82% (36/44) deviated from HWE. However, after Bonferroni correction for multiple comparisons, the number reduced to 68.2% (30/44); 5 loci in February (dry), 11 loci in April (wet), 8 loci in July (dry), and 6 loci in October (Table 2). In Mtepeni, 59.1% (26/44) of the tests deviated from HWE but after Bonferroni correction, this deviation reduced to 50% (22/44) of the tests; 4 loci in February, 11 loci in April, 4 loci in July and 3 loci in October (Table 3). Of the 55 Bonferroni-corrected pairwise linkage disequilibrium tests undertaken at Jaribuni, LD was observed only in February (6/55, 11%) and April (21/55, 38.2%); while at Mtepeni, LD was observed only in April (7/55, 12.7%). In a few cases, we observed LD among locus pairs located on chromosomes 2 or 3. These were found in Jaribuni in February (2/55; 3.6%) and April (7/55, 12.7%) and in Mtepeni in April (2/55, 3.6%). This low level of LD involving the same contig and the complete absence of LD during some seasons indicates that the observed LD was not due to physical linkage among loci. Significant LD occurred in 23/55 (41.8%) locus pairs across all seasonal populations, but when all populations across all sites were pooled and analyzed as a single population, LD was observed among all locus pairs.

3.4. Gene flow and population structure

Analysis of molecular variance (AMOVA) showed significant difference between the two populations (Table 4). Monthly comparisons of data from the two populations produced low *Nm* values (0.08–1.35) and significant *Fst* values (0.03–0.42) suggesting the existence of population differentiation and some restriction to gene flow. Even for each of the two

populations, there was a significant difference in *Fst* values between the wet and dry months, with a slightly increased level of gene flow during the dry seasons compared to the wet seasons. Overall, there was 17% variation among populations, and 83% variation within population ($Fst = 0.171$, $P < 0.001$).

3.4.1. Bottleneck analysis—The infinite allele model (IAM) showed evidence of bottlenecks in majority of cases but TPM and SMM did not support this ($P > 0.05$). Overall, the alleles showed a normal, L-shaped distribution across all populations indicating the lack of sufficient evidence for population bottlenecks (Table 5).

4. Discussion

High levels of genetic variation were found at 11 microsatellite loci of *A. gambiae* from coastal Kenya. The observed number of alleles ranged from 7 for locus ND36 to 21 for locus AG2H46. When samples from the two sites were analyzed together on the assumption of being a single panmictic population, significant deviations s from HWE with heterozygote deficiency and linkage disequilibrium between pairs of loci were observed, suggesting the existence of subpopulation structuring. This observation was unexpected because previous reports from microsatellite-based studies suggest that *A. gambiae* has a deme size greater that 50km (Lehmann et al., 1996; Kamau et al., 1998). In fact, some studies that used microsatellite markers have reported extensive gene flow between *A. gambiae* populations as far as 5000km apart (Lehmann et al., 1996, 1999; Kamau et al., 1998) except in regions that are separated by a remarkable geographical barrier, such as The Great Rift Valley (Simard et al., 2000). Other than physical distance, topography and climate are two other factors associated with subpopulation differentiation (Simard et al., 2000; Touré et al., 1998b). The absence of an apparent topographical barrier between the two study sites provides no convincing evidence to associate the observed genetic differentiation with this factor. Perhaps the short flight range of *A. gambiae* (Midega et al., 2007) coupled with selection due to local ecological factors could have led to local adaptation resulting in subpopulation differentiation. This hypothesis is supported by the high number of private alleles detected in Jaribuni than in Mtepeni, indicating that this species can easily adapt to a wide range of ecological pressures. Jaribuni site is characterized by a river whose water level rises during the rainy season, forming temporary larval habitats at its edge. These habitats expand and contract in response to rainfall events and disappear when the water levels reduce to extremely low levels. At Mtepeni, there is a seasonal stream that dries up at the end of the rainy season. Thus, while gravid females in Jaribuni might find an opportunity to oviposit during the dry season, this opportunity hardly exists in Mtepeni where larval habitats are rare during the dry season. A good example of how *A. gambiae* can adapt to local ecological conditions is illustrated by the five chromosomal forms of this species in West Africa (Coluzzi et al., 1979, 2002).

Unlike other population genetics studies that based their conclusions on samples collected over short periods of time, sampling for our study was spread across all seasons of the year, focusing on obtaining female mosquitoes during the dry and the wet seasons. This study design allowed us to assess whether there was notable seasonal variation in genetic structure of the two populations. Results from HWE, LD, Nei's Fst and AMOVA revealed significant genetic differentiation between the dry and the wet season. Previous studies using the sister species (*Anopheles arabiensis*) did not detect significant genetic differentiation between the dry and the wet seasons (Touré et al., 1998b). The significant differentiation observed in this study can be attributed to inbreeding and presence of null alleles. However, if null alleles were the main cause of observed deficiency in heterozygosity, we would not have detected significant LD because all individuals have equal probability of carrying null alleles. Thus, it is possible that certain genotypes were more adapted to the dry and wet conditions,

respectively. Such observations have been reported for *A. gambiae* where certain chromosomal inversions for instance, 2Rbc and 2La are associated with mosquitoes inhabiting arid areas (Coluzzi et al., 1979). However, all the markers used in the present study are located outside the inversion, suggesting that markers in other regions of the genome are also likely to be under selection. Interestingly, we did not find any evidence of annual population bottleneck in any of the two populations. This observation seems to suggest that *A. gambiae* populations are maintained continuously throughout the year with seasonal reduction rather than severe bottleneck or extinction. Assuming that this premise holds, how were the observed alleles maintained throughout the year given that there was apparent seasonal genetic differentiation? As proposed by Simard et al. (2000) for *A. arabiensis*, perhaps mosquitoes adapted to the wet season "hide" exceptionally well during the dry season, making it difficult to collect them using conventional mosquito collection techniques or that they survive the dry season as eggs contrary to previous reports (Beier et al., 1990). These assumptions are supported by the short flight range observed for this species in the two study sites (Midega et al., 2007).

Due to the rapid spread of *Anopheles* and *Plasmodium* strains resistant to commonly used insecticides and antimalaria drugs, respectively, there have been proposals to control malaria by releasing in nature transgenic mosquitoes that are refractory to infection with *Plasmodium* parasites (Collins and Besansky, 1994). This method requires that the target mosquito population has a large deme size to facilitate the spread of transgenes. The high amounts of genetic differentiation observed in the current study suggest that this method has lower chances of success in the study area. However, such restricted gene flow is advantageous because it acts as a barrier to the spread of insecticide resistant genes and may partly explain why unlike other regions, widespread occurrence of insecticide resistance genes has not been reported in the study area.

5. Conclusion

These studies found significant evidence of restricted gene flow between two *A. gambiae* populations separated by a distance of 50 km. Despite the observed genetic differentiation between the wet and dry seasons, there was no evidence of a significant reduction in effective population size. These findings suggest that even at shorter distances, ecological factors may induce selection pressures that may lead to reproductive isolation between *A. gambiae* populations. The results further suggest that *A. gambiae* could be surviving the dry weather either as eggs or aestivating adults that are probably missed during routine mosquito sampling.

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Table 1

Microsatellite loci studied in *Anopheles gambiae*.

Locus	Chromosomal location	No. of repeats	Allele size (bp)
AGXH1D1	X	CCA	172
AGXH131	X	GT	175
AGXH678	X	AG	153
AG2H46	2R	GT	138
AG2H143	2L	TC	160
ND30	2R	GT	184
ND36	2L	GT	92
AG3H555	3L	GT	81
AG3H88	3L	GT	176
AG3H249	3R	GT	129
AG3H29	3R	TGA	148

Table 2

Locus by locus sample size (N), number of Alleles (Na), effective number of alleles (Ne), observed heterozygosity (H_o), expected (He) and Nei's unbiased heterozygosity (H_E) and inbreeding coefficients (Fis) for samples of *A. gambiae* from Jaribuni, Kilifi district, Kenya.

Season		H29	H131	H143	H1D1	H46	ND30	H249	ND36	H678	H555	H88	Mean ± S.D.
Dry (February)	N	57	52	55	58	56	48	49	57	50	55	54	53.7 ± 3.5
n = 58	Na	7	7	7	5	13	5	6	3	10	4	5	6.5 ± 2.8
	Ne	2.65	3.42	4.44	1.24	6.56	2.68	3.18	1.48	4.57	3.14	3.44	3.3 ± 1.5
	Ho	0.74	0.44	0.53	0.21	0.48	0.52	0.39	0.40	0.68	0.60	0.28	0.05 ± 0.2
	He	0.62	0.71	0.77	0.19	0.85	0.63	0.69	0.33	0.78	0.68	0.71	0.63 ± 0.2
	H_E	0.63	0.71	0.78	0.19	0.86	0.63	0.69	0.33	0.79	0.69	0.72	0.64 ± 0.2
	Fis	0.175	**0.383**	**0.328**	0.065	**0.438**	0.180	**0.442**	0.231	0.139	0.129	**0.614**	
Dry (July)	N	38	38	40	41	31	30	38	38	30	37	39	36.4 ± 4.0
n = 43	Na	8	6	7	4	9	3	8	3	8	5	5	6.0 ± 2.1
	Ne	3.17	3.00	3.91	1.25	4.17	2.25	3.89	1.57	4.99	2.99	3.02	3.1 ± 1.1
	Ho	0.32	0.42	0.45	0.20	0.48	0.40	0.47	0.18	0.57	0.38	0.36	0.38 ± 0.1
	He	0.68	0.67	0.74	0.20	0.76	0.56	0.74	0.36	0.80	0.67	0.67	0.62 ± 0.2
	H_E	0.69	0.68	0.75	0.20	0.77	0.57	0.75	0.37	0.81	0.67	0.68	0.63 ± 0.2
	Fis	**0.548**	**0.380**	**0.406**	0.043	**0.378**	0.296	**0.374**	0.501	**0.307**	**0.442**	**0.474**	
Wet (April)	N	55	46	50	51	51	45	54	54	47	48	45	49.6 ± 3.7
n = 60	Na	5	9	7	6	11	3	10	3	13	4	5	6.9 ± 3.4
	Ne	2.53	4.65	4.39	1.89	4.84	2.92	5.12	1.97	7.50	3.67	3.91	3.9 ± 1.6
	Ho	0.29	0.43	0.24	0.25	0.35	0.31	0.54	0.20	0.53	0.25	0.31	0.34 ± 0.1
	He	0.60	0.78	0.77	0.47	0.79	0.66	0.80	0.49	0.87	0.73	0.74	0.70 ± 0.1
	H_E	0.61	0.79	0.78	0.48	0.80	0.66	0.81	0.50	0.88	0.74	0.75	0.71 ± 0.1
	Fis	**0.526**	**0.455**	**0.694**	**0.467**	**0.562**	**0.535**	**0.341**	**0.593**	**0.395**	**0.662**	**0.589**	
Wet (October)	N	27	27	25	22	25	19	24	27	24	25	25	24.6 ± 2.4
n = 42	Na	3	9	5	5	10	4	9	3	11	5	5	6.3 ± 2.9
	Ne	2.57	5.48	4.55	1.90	6.19	2.93	4.22	1.92	6.94	2.68	3.87	3.9 ± 1.7
	Ho	0.37	0.59	0.60	0.41	0.48	0.53	0.54	0.30	0.58	0.44	0.24	0.46 ± 0.1
	He	0.61	0.82	0.78	0.47	0.84	0.66	0.76	0.48	0.86	0.63	0.74	0.70 ± 0.1
	H_E	0.62	0.83	0.80	0.49	0.86	0.68	0.78	0.49	0.87	0.64	0.76	0.71 ± 0.1

Season		H29	H131	H143	H1D1	H46	ND30	H249	ND36	H678	H555	H88	Mean ± S.D.
	Fis	**0.410**	**0.293**	**0.250**	0.160	**0.444**	0.227	0.309	**0.398**	0.337	0.316	**0.687**	

Table 3

Locus by locus sample size (N), number of Alleles (Na), effective number of alleles (Ne), observed heterozygosity (H_O), expected (He) and Nei's unbiased heterozygosity (H_E) and inbreeding coefficients (Fis) for samples of *A. gambiae* from Mtepeni, Kilifi district, Kenya.

Season		H29	H131	H143	H1D1	H46	ND30	H249	ND36	H678	H555	H88	Mean ± S.D.
Dry (February)	N	51	50	46	49	49	34	51	49	44	47	44	46.7 ± 4.9
n = 52	Na	2	4	7	5	9	2	8	3	7	5	7	5.4 ± 2.4
	Ne	1.86	2.39	2.76	2.14	2.51	1.97	3.84	1.69	5.36	2.60	4.62	2.9 ± 1.2
	Ho	0.49	0.54	0.59	0.71	0.43	0.53	0.69	0.37	0.73	0.40	0.55	0.55 ± 0.1
	He	0.46	0.58	0.64	0.53	0.60	0.49	0.74	0.41	0.81	0.61	0.78	0.61 ± 0.1
	H_E	0.47	0.59	0.64	0.54	0.61	0.50	0.75	0.41	0.82	0.62	0.79	0.61 ± 0.1
	Fis	0.050	**0.081**	0.090	−0.033	**0.298**	−0.059	0.082	0.110	0.117	**0.352**	**0.314**	
Dry (July)	N	38	33	35	45	36	30	40	43	33	40	38	37.4 ± 4.5
n = 46	Na	2	4	6	3	8	3	8	3	8	6	6	5.2 ± 2.3
	Ne	1.63	2.30	3.19	1.25	3.69	2.00	3.85	1.51	5.57	3.35	2.22	2.8 ± 1.3
	Ho	0.53	0.33	0.66	0.22	0.36	0.50	0.70	0.33	0.79	0.30	0.16	0.44 ± 0.2
	He	0.39	0.56	0.69	0.20	0.73	0.50	0.74	0.34	0.82	0.70	0.55	0.56 ± 0.2
	H_E	0.39	0.57	0.70	0.20	0.74	0.51	0.75	0.34	0.83	0.71	0.56	0.57 ± 0.2
	Fis	0.345	0.422	0.058	−0.101	**0.515**	0.016	0.067	0.042	**0.055**	**0.581**	0.719	
Wet (April)	N	28	41	43	46	37	36	43	39	39	39	40	39.2 ± 4.7
n = 49	Na	7	3	10	7	13	5	9	6	11	8	6	7.7 ± 2.9
	Ne	4.16	2.74	5.91	3.27	6.60	3.69	4.28	3.72	7.28	5.17	3.44	4.6 ± 1.5
	Ho	0.64	0.37	0.65	0.52	0.51	0.50	0.67	0.72	0.79	0.49	0.30	0.56 ± 0.2
	He	0.76	0.63	0.83	0.69	0.85	0.73	0.77	0.73	0.86	0.81	0.71	0.76 ± 0.1
	H_E	0.77	0.64	0.84	0.70	0.86	0.74	0.78	0.74	0.87	0.82	0.72	0.77 ± 0.1
	Fis	**0.171**	**0.433**	**0.227**	**0.258**	**0.406**	**0.327**	**0.131**	**0.031**	**0.091**	**0.407**	**0.585**	
Wet (October)	N	41	54	51	51	53	53	52	48	50	53	49	50.5 ± 3.6
n = 57	Na	5	3	4	6	5	2	6	3	4	6	7	4.6 ± 1.6
	Ne	3.71	1.68	2.70	3.60	2.11	1.79	1.33	2.58	3.02	1.90	3.64	2.6 ± 0.9
	Ho	0.66	0.37	0.59	0.63	0.42	0.51	0.23	0.60	0.64	0.30	0.55	0.50 ± 0.1
	He	0.73	0.41	0.63	0.72	0.53	0.44	0.25	0.61	0.67	0.47	0.73	0.56 ± 0.2
	H_E	0.74	0.41	0.64	0.73	0.53	0.45	0.25	0.62	0.68	0.48	0.73	0.57 ± 0.2

Season		H29	H131	H143	H1D1	H46	ND30	H249	ND36	H678	H555	H88	Mean ± S.D.
	Fis	0.111	0.097	0.076	0.141	0.219	−0.142	0.074	0.023	**0.053**	**0.372**	**0.250**	

Table 4

Analysis of molecular variance (AMOVA) among *A. gambiae* populations in two sites of coastal Kenya.

Month/season	Source	df	SS	MS	Est. Var	%	*Fst*	*P*	*Nm**
February/Dry	Jaribuni vs. Mtepeni								
	Among Pops	1	36.329	36.329	0.338	9%	0.089	0.001	1.21
	Within Pops	198	680.452	3.437	3.437	91%			
	Total	199	716.781		3.774	100%			
April/Wet	Jaribuni vs. Mtepeni								
	Among Pops	1	23.058	23.058	0.197	5%	0.047	0.001	0.08
	Within Pops	192	760.799	3.962	3.962	95%			
	Total	193	783.857		4.160	100%			
July/Dry	Jaribuni vs. Mtepeni								
	Among Pops	1	13.068	13.068	0.111	3%	0.033	0.001	1.35
	Within Pops	174	575.116	3.305	3.305	97%			
Total	175	588.184		3.416	100%			
	Jaribuni vs. Mtepeni								
October/Wet	Among Pops	1	150.430	150.430	1.924	36%	0.362	0.001	0.16
	Within Pops	168	569.192	3.388	3.388	64%			
	Total	169	719.622		5.312	100%			
February vs. April	Jaribuni								
	Among Pops	1	25.160	25.160	0.183	5%	0.047	0.001	N/A
	Within Pops	232	859.894	3.706	3.706	95%			
	Total	233	885.055		3.890	100%			
July vs. October	Jaribuni								
	Among Pops	1	12.142	12.142	0.124	3%	0.033	0.001	N/A
	Within Pops	140	510.903	3.649	3.649	97%			
	Total	141	523.045		3.773	100%			
February vs. April	Mtepeni								
	Among Pops	1	54.321	54.321	0.505	12%	0.117	0.001	N/A
	Within Pops	198	751.803	3.797	3.797	88%			
	Total	199	806.123		4.302	100%			
July vs. October	Mtepeni								

Month/season	Source	df	SS	MS	Est. Var	%	*Fst*	*P*	*Nm*[*]
	Among Pops	1	234.973	234.973	2.295	42%	0.423	0.001	N/A
	Within Pops	202	633.405	3.136	3.136	58%			
	Total	203	868.377		5.431	100%			
Overall	All populations and seasons								
	Among Pops	7	523.215	74.745	0.734	17%	0.171	<0.001	N/A
	Within Pops	772	2756.005	3.570	3.570	83%			
	Total	779	3279.220		4.304	100%			

Table 5

Bottleneck analysis of *A. gambiae* populations from two areas 50km apart in the coastal Kilifi district of the Kenya, by season.

Area and season[a]	Model[b]			Mode-shift
	IAM	TPM	SMM	
Jaribuni				
February (*n* = 58)	0.05371	0.41309	0.06738	Normal
April (*n* = 59)	0.00146	0.12305	0.63770	Normal
July (*n* = 42)	0.05371	0.70020	0.01221	Normal
October (*n* = 29)	0.00244	0.12305	0.83105	Normal
Mtepeni				
February (*n* = 51)	0.00684	0.32031	0.46484	Normal
April (*n* = 49)	0.00490	0.00684	0.70020	Normal
July (*n* = 46)	0.01611	0.76465	0.08301	Normal
October (*n* = 56)	0.10156	0.46484	0.83105	Normal

n = number of samples tested.

[a] February and July are dry seasons, while April and October are rainy seasons.

[b] The Wilcoxon test was used to test for heterozygosity excess under infinite allele (IAM), stepwise mutation (SMM) and two-phase (TPM) models. *P*-values are indicated under each model type. Parameters for TPM were: variance = 30; proportion of SMM = 70%. Estimates were based on 10,000 replications.