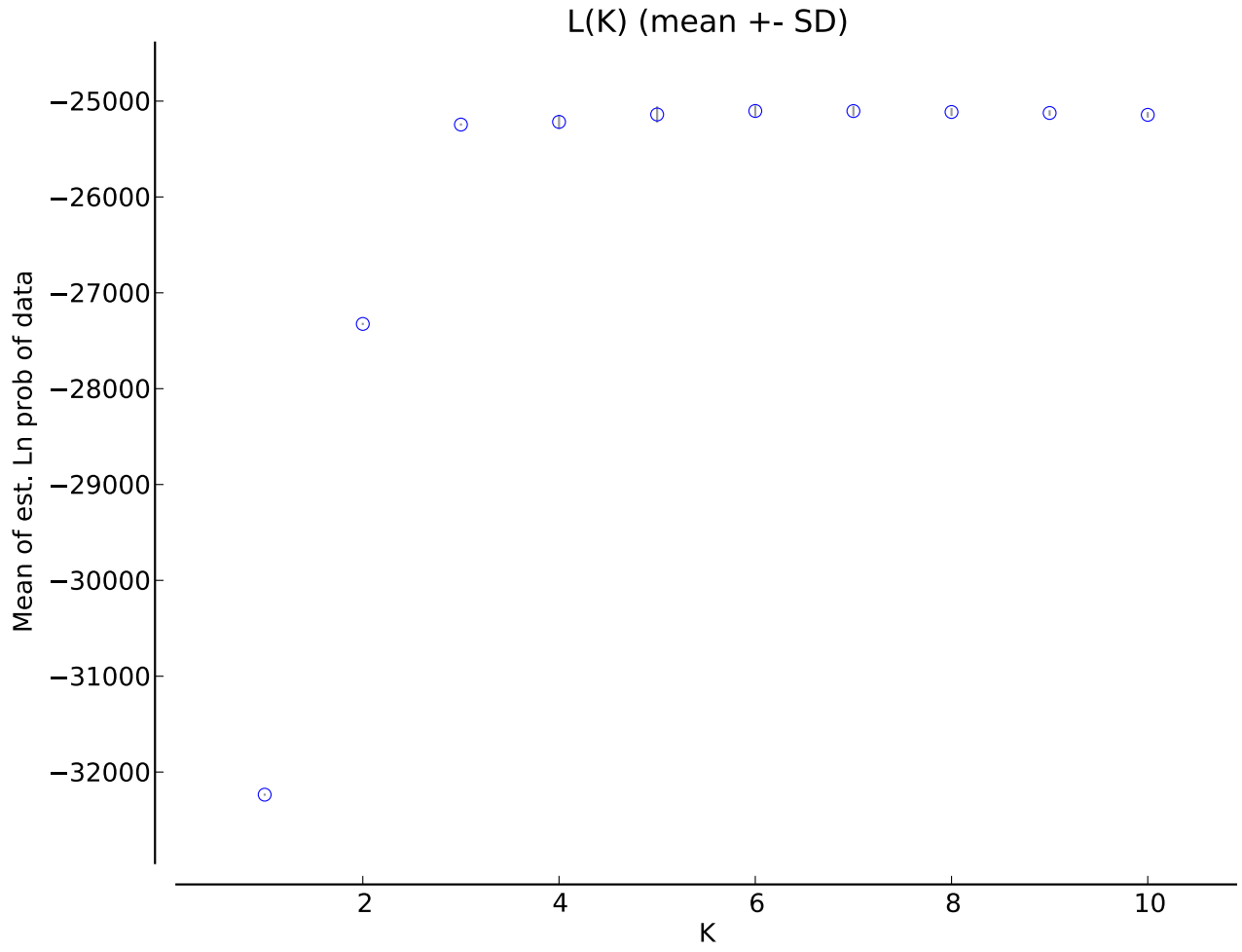

Figure S1 Results of Structure Harvester (Earl 2011), indicating likelihood (LnP(K)) scores of *K* populations (1-10).

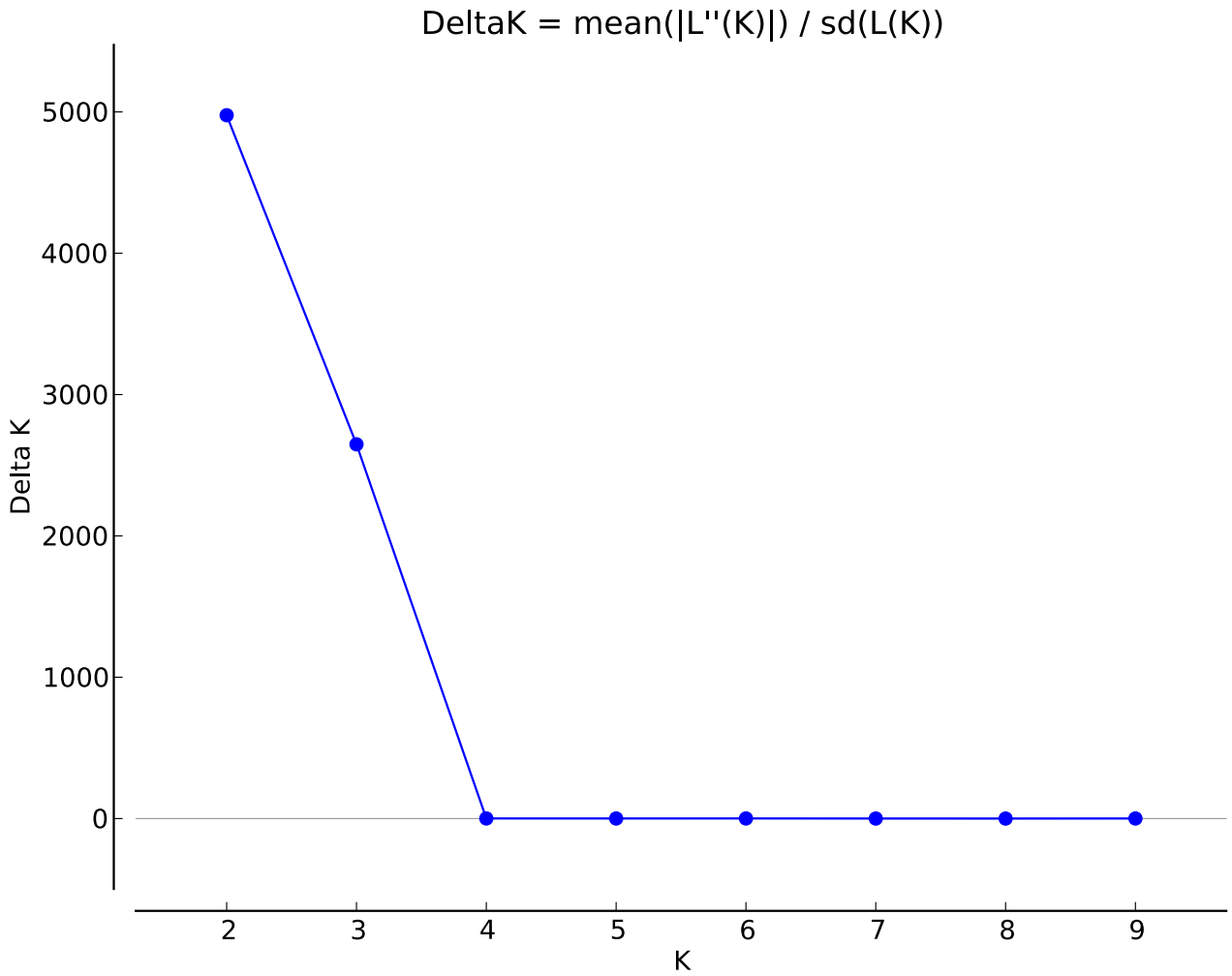

Figure S2 Results of Structure Harvester (Earl 2011), indicating Delta*K* values for *K* populations (1-9).

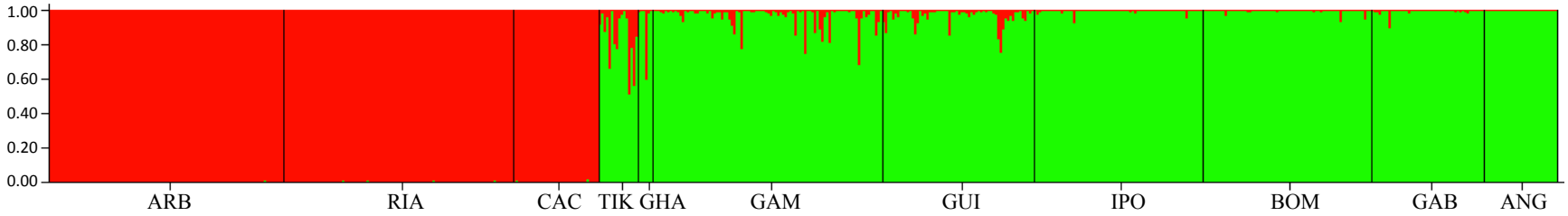

Figure S3 Results of the Bayesian assignment test for two putative populations (*K*=2) based upon microsatellite DNA data implemented in the program Structure (Pritchard *et al.* 2000). Each vertical bar corresponds to a single individual, and colors represent the proportion of the genome that is assigned to a particular cluster based upon the admixture model. Sample populations are annotated according to population abbreviations defined in Table 1.

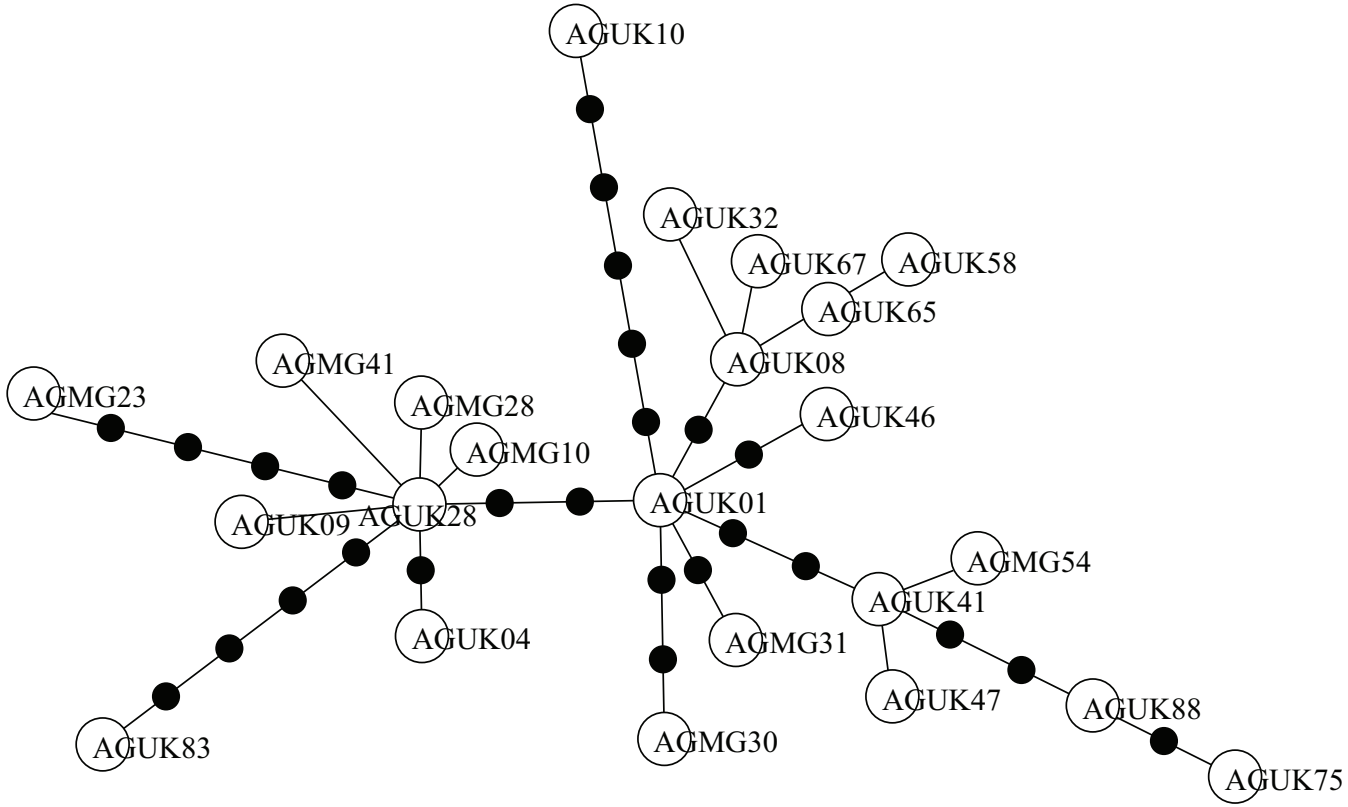

Figure S4 *An. gambiae* minimum spanning tree (Kruskal 1956, Prim 1957) constructed in the program Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010), and visualized using HapStar (Teacher and Griffiths 2011). White circles represent sampled haplotypes and black intermediate circles represent ancestral or unsampled haplotypes. Haplotypes represent *An. gambiae* collected from Mongola (Bioko Island, E.G., AGMG##) and Ukomba (mainland, E.G., AGUK##), and do not show any geographic clustering.

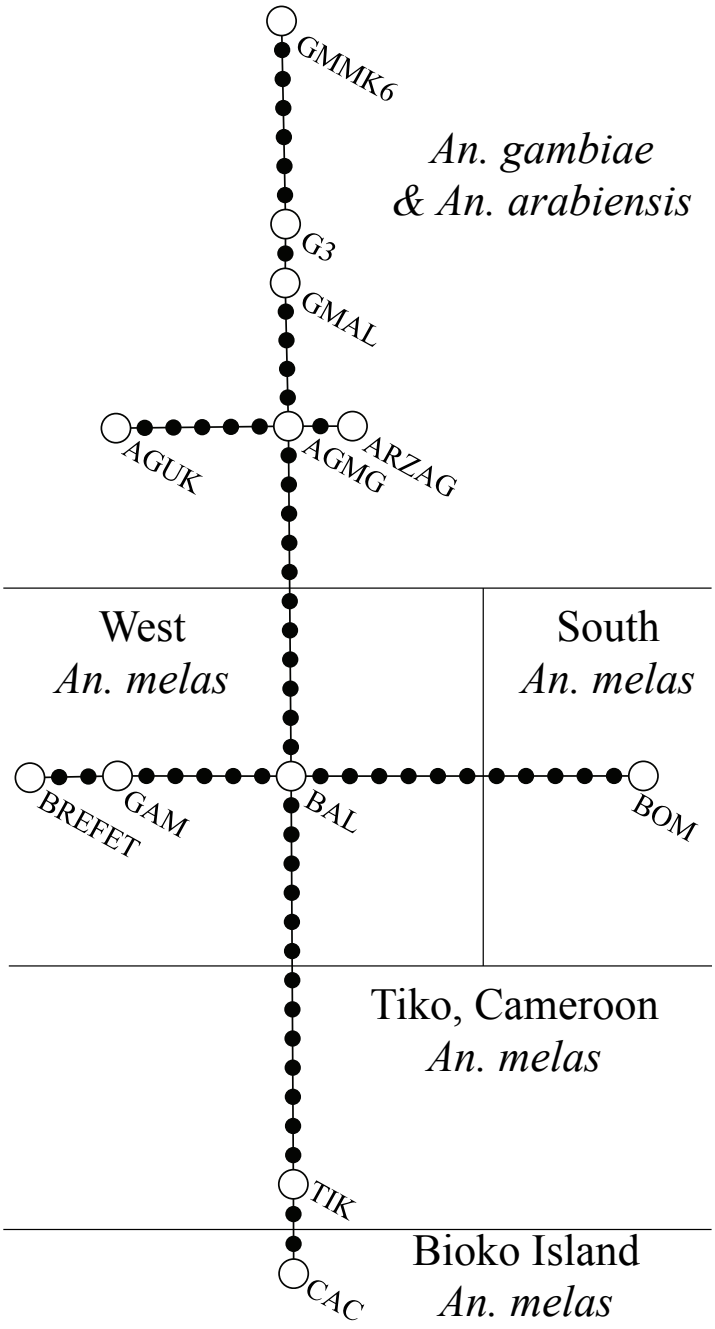

Figure S5 *An. gambiae* complex minimum spanning tree (Kruskal 1956, Prim 1957) constructed in the program Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010), and visualized using HapStar (Teacher and Griffiths 2011). White circles represent sampled haplotypes and black intermediate circles represent ancestral or unsampled haplotypes. Population and strain abbreviations refer to those defined in Table 1 and Table S1, respectively. Population and species clusters are annotated according to *An. melas* population cluster or species (if different from *An. melas*).

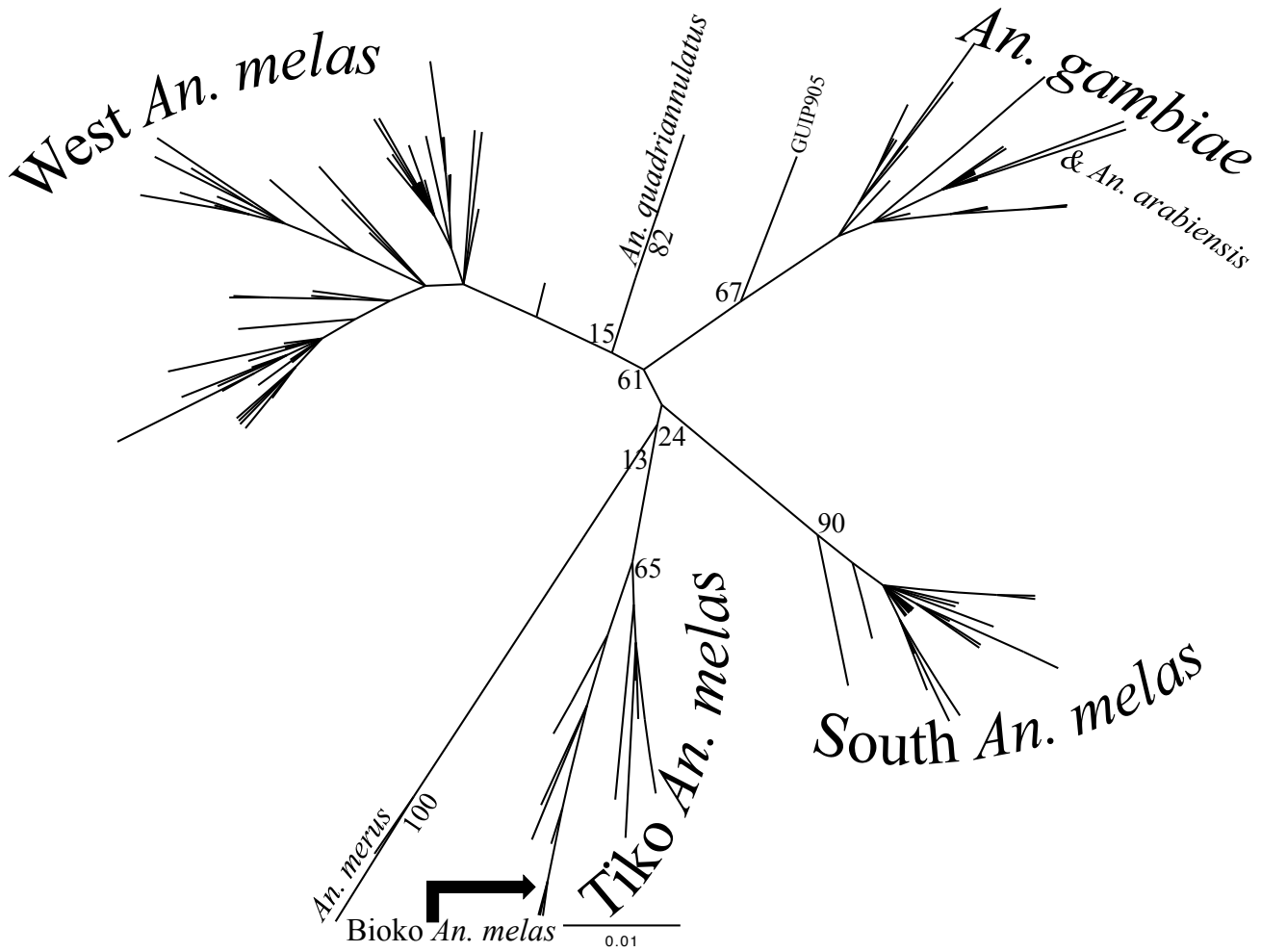

Figure S6 Unrooted maximum likelihood tree of *An. gambiae* complex member species including Central, West, and Bioko Island *An. melas* populations. Node annotations indicate bootstrap support resulting from 1000 replicates. Population and species clusters are annotated according to *An. melas* population cluster or species (if different from *An. melas*).

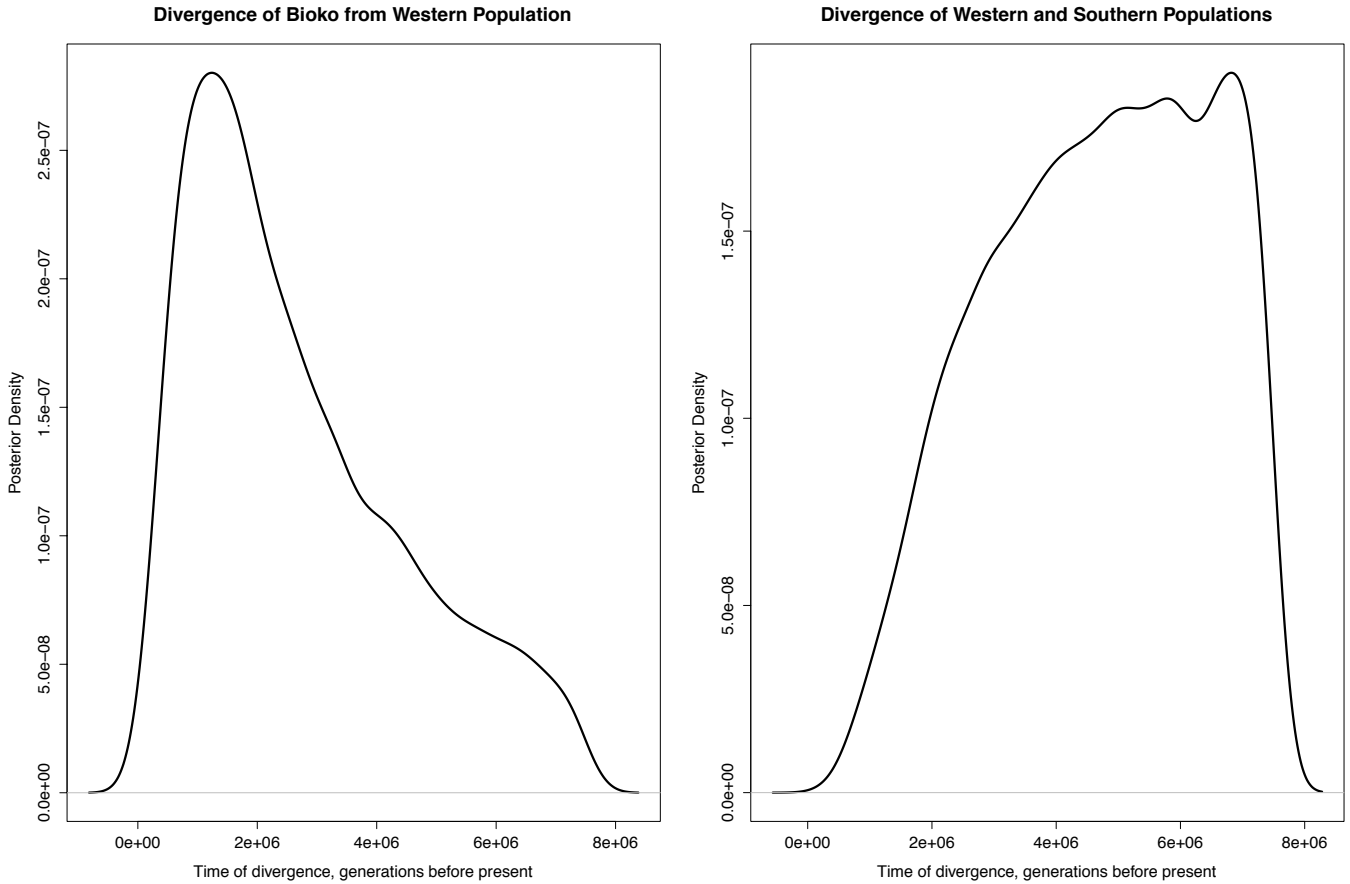

Figure S7 Posterior density plots for the timing of divergence events (generations before present) of a) Bioko Island from the western cluster and b) of the split between the western and southern clusters, as estimated by approximated Bayesian computation.

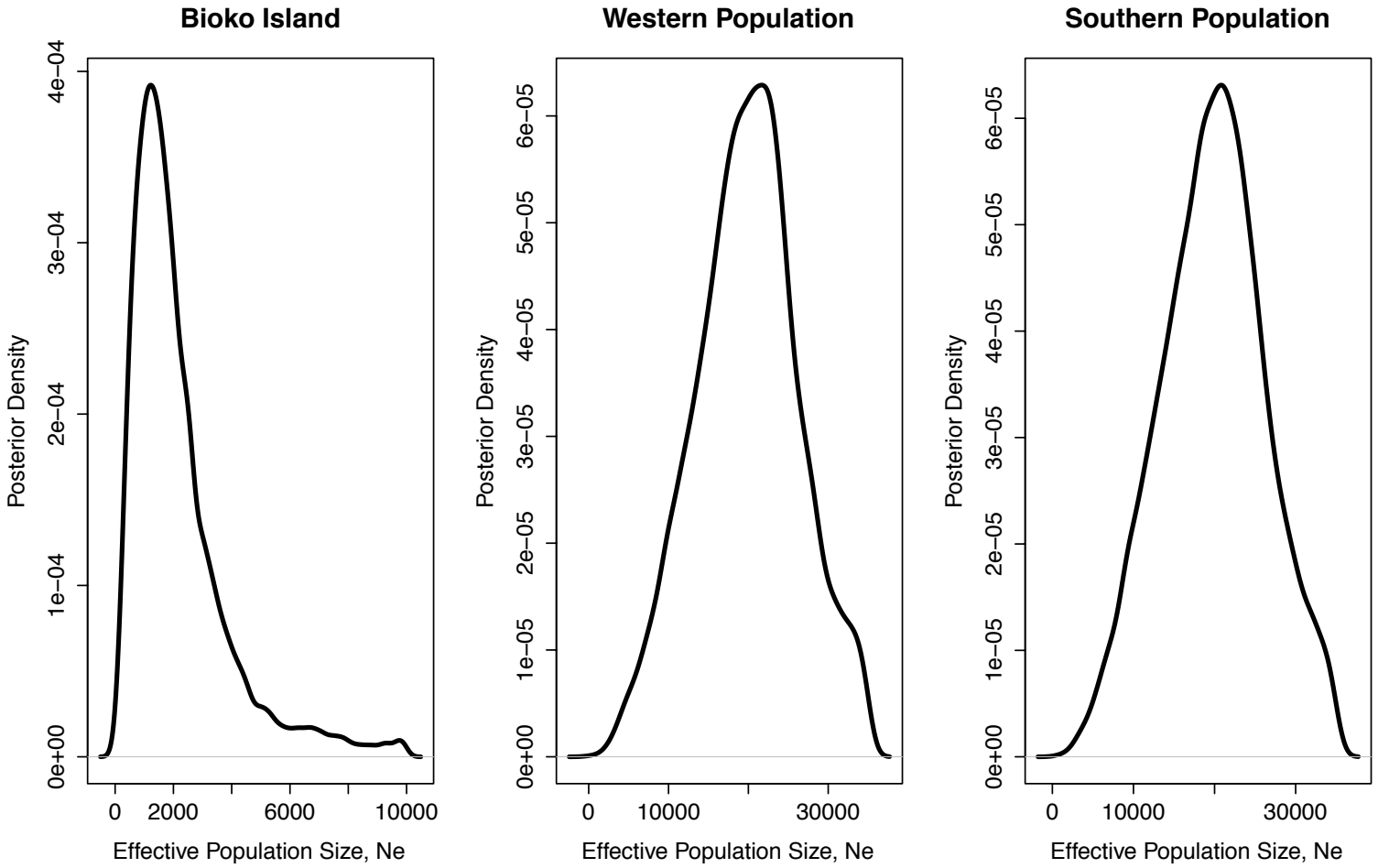

Figure S8 Posterior density plots of effective population size (N_e) estimates for the three main populations considered in ABC analysis – the Western and Southern population clusters and Bioko island.

SUPPLEMENTARY TABLES

Table S1 *An. gambiae* Complex ND4-ND5 mtDNA sequences that represent 5 different species from the *An. gambiae* complex and were originally published by Besansky *et al.* (1994).

Species	Abbreviation	GenBank Accession No.	Geographical Origin
An. melas	BAL	U10123	The Gambia
An. arabiensis	GMAL	U10124	Sudan
An. arabiensis	ARZAG	U10125	Burkina Faso
An. gambiae	G3	U10126	The Gambia
An. gambiae	GMMK6	U10127	Burkina Faso
An. gambiae	MUHEZA	U10128	Tanzania
An. melas	BREFET	U10129	The Gambia
An. merus	V12	U10130	Kenya
An. merus	ZULU	U10131	Zululand
An. quadriannulatus	CHIL	U10132	Zimbabwe
An. quadriannulatus	SQUAD	U10133	Unknown

Table S2: Description of Individual Scenarios: (Note: although coalescent simulations go backwards in time and were coded as such, to simplify interpretation, they are described as if occurring forwards in time, as in reality). For prior distributions of parameters, refer to Table S3

.

Scenario	Description	Parameters	Explanation
1	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance, with the Southern population being ancestral. More recently, the Bioko Island population (Nb) split off from the western cluster through a vicariance event.	Nb Nw Ns Nw+Ns T1b T1a 0	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Present
2	An ancestral population (Nanc) gave rise to the Western and Southern clusters via founding events, creating bottlenecked populations Nwf (Western) and Nsf (Southern), which expanded to contemporary populations Nw, and Ns. The ancestral population was in the south. More recently, the Bioko island population split off from the Western cluster through vicariance.	Nb Nw Ns Nanc T1b T1a T1c 0 Nwf Nsf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W&S founder event Present Ne of Western following founding event Ne of Southern following founding event

3	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (western) and Nsf (southern), which expanded to contemporary populations Nw and Ns. The ancestral population was from the south. More recently, the Bioko island population was created through a founding event (Nbf) from the Western cluster. The Bioko population expanded to Nb following this founding event.	Nb Nw Ns Nanc T1b T1a T1c T1d 0 Nwf Nsf Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event
4	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (western) and Nsf (southern), which expanded to contemporary populations Nw and Ns. The ancestral population was from the south. More recently, the Bioko island population was created through a founding event (Nbf) from the Western cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko to Nbb.	Nb Nw Ns Nanc T1b T1a T1c T1d T1e 0 Nwf Nsf Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event Ne of Bioko at recent bottleneck

5	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance, with the Southern being ancestral. More recently, the Bioko island population was created during a founding event (Nbf) from the Western cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb). .	Nb Nw Ns Nw+Ns T1b T1a T1d T1e 0 Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Bioko at founder event Ne of Bioko at recent bottleneck
6	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance, with the southern being ancestral. More recently, the Bioko island population was created during a founding event (Nbf) from the Western cluster. The Bioko population expanded to Nb after this initial founding event.	Nb Nw Ns Nw+Ns T1b T1a T1d 0 Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Present Ne of Bioko at founder event
7	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance, with the Western being ancestral. More recently, the Bioko Island population (Nb) was split off from the Western cluster through vicariance.	Nb Nw Ns Nw+Ns T2b T2a 0	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Present

8	An ancestral population (Nanc) gave rise to the Western and Southern clusters via founding events, creating bottlenecked populations Nwf (Western) and Nsf (Southern), which later expanded to contemporary populations Nw and Ns. The ancestral population was in the west. More recently, the Bioko island population split off from the western cluster through vicariance.	Nb Nw Ns Nanc T2b T2a T2c 0 Nwf Nsf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W&S founder event Present Ne of Western following founding event Ne of Southern following founding event
9	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (western) and Nsf (southern), which later expanded to contemporary populations Nw and Ns. The ancestral population was in the west. More recently, the Bioko island population was created during a founding event (Nbf) from the Western cluster. The Bioko population subsequently expanded to Nb.	Nb Nw Ns Nanc T2b T2a T2c T2d 0 Nwf Nsf Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event

10	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (Western) and Nsf (Southern), which expanded to contemporary populations Nw and Ns. The ancestral population was in the west. More recently, the Bioko island population was created through a founding event (Nbf) from the Western cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb).	Nb Nw Ns Nanc T2b T2a T2c T2d T2e 0 Nwf Nsf Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event Ne of Bioko at recent bottleneck
11	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance. The ancestral population was in the west. More recently, the Bioko island population was created though a founding event (Nbf) from the Western cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb).	Nb Nw Ns Nw+Ns T2b T2a T2d T2e 0 Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Bioko at founder event Ne of Bioko at recent bottleneck

12	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance. The ancestral population is in the west. More recently, the Bioko island population was created though a founding event (Nbf) from the Western cluster. It subsequently expanded to Nb after this initial founding event.	Nb Nw Ns Nw+Ns T2b T2a T2d 0 Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Present Ne of Bioko at founder event
13	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance. The ancestral population was in the south. More recently, a vicariance event divided the Bioko Island population (Nb) and the Southern cluster.	Nb Nw Ns Nw+Ns T3b T3a 0	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Present
14	An ancestral population (Nanc) gave rise to the two population clusters via founding events, creating bottlenecked populations Nwf (Western) and Nsf (Southern). These later expanded to contemporary populations Nw, and Ns. The ancestral population was from the south. More recently, the Bioko island population split off from the Southern cluster through vicariance.	Nb Nw Ns Nanc T3b T3a T3c 0 Nwf Nsf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W&S founder event Present Ne of Western following founding event Ne of Southern following founding event

15	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (western) and Nsf (southern). These later expanded to contemporary populations Nw and Ns. The ancestral population was from the south. More recently, the Bioko island population was created through a founding event (Nbf) from the Southern cluster. The Bioko population expanded to Nb after this initial founding event.	Nb Nw Ns Nanc T3b T3a T3c T3d 0 Nwf Nsf Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event
16	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (Western) and Nsf (Southern). These expanded to contemporary populations Nw and Ns. The ancestral population was from the south. More recently, the Bioko island population was created through a founding event (Nbf) from the Southern cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb).	Nb Nw Ns Nanc T3b T3a T3c T3d T3e 0 Nwf Nsf Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event Ne of Bioko at recent bottleneck

17	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters by vicariance, The ancestral population was in the south. More recently, the Bioko island population was created during a founding event (Nbf) from the Southern cluster. Following this initial founding, a very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb).	Nb Nw Ns Nw+Ns T3b T3a T3d T3e 0 Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Bioko at founder event Ne of Bioko at recent bottleneck
18	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance. The ancestral population was in the south. More recently, the Bioko island population was created during a founding event (Nbf) from the South. The Bioko population expanded to Nb after this initial founding event.	Nb Nw Ns Nw+Ns T3b T3a T3d 0 Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Present Ne of Bioko at founder event
19	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance. The ancestral population was in the west. More recently, the Bioko Island population (Nb) split off from the Southern cluster though a vicariance event.	Nb Nw Ns Nw+Ns T4b T4a 0	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Present

20	An ancestral population (Nanc) gave rise to the Western and Southern clusters via founding events, creating bottlenecked populations Nwf (Western) and Nsf (Southern). These expanded to contemporary populations Nw and Ns. The ancestral population was from the west. More recently, the Bioko island population split off from the Southern cluster through vicariance.	Nb Nw Ns Nanc T4b T4a T4c 0 Nwf Nsf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W&S founder event Present Ne of Western following founding event Ne of Southern following founding event
21	An ancestral population (Nanc) gave rise to the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (western) and Nsf (southern). These later expanded to contemporary populations Nw and Ns. The ancestral population was from the west. More recently, the Bioko island population was created during a founding event (Nbf) from the Southern cluster. The Bioko population expanded to Nb after this initial founding event.	Nb Nw Ns Nanc T4b T4a T4c T4d 0 Nwf Nsf Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event

22	An ancestral population (Nanc) was divided into the Western and Southern clusters through founding events, creating bottlenecked populations Nwf (western) and Nsf (southern]. These later expanded to contemporary populations Nw and Ns. The ancestral population was from the west. More recently, the Bioko island population was created through a founding event (Nbf) from the Southern cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb).	Nb Nw Ns Nanc T4b T4a T4c T4d T4e 0 Nwf Nsf Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population to W & S Time of split between W &S Time of split of Bioko Time of W & S founder events Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Western at founder event Ne of Southern at founder event Ne of Bioko at founder event Ne of Bioko at recent bottleneck
23	An ancestral cluster (Nw+Ns) was divided into the Western (Nw) and Southern (Ns) clusters through vicariance. The ancestral population was from the west. More recently, the Bioko island population was created through a founding event (Nbf) from the Southern cluster. A very recent vector-control induced bottleneck reduced population sizes on Bioko (Nbb).	Nb Nw Ns Nw+Ns T4b T4a T4d T4e 0 Nbf Nbb	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Time of recent Bioko bottleneck Present Ne of Bioko at founder event Ne of Bioko at recent bottleneck

24	An ancestral cluster (Nw+Ns) split equally into the Western (Nw) and Southern (Ns) clusters by vicariance, where the Southern split off from the Western. At a more recent time, the Bioko island population was created during a founding event (Nbf) when it diverged from the Southern cluster. The Bioko population expanded to Nb after this initial founding event.	Nb Nw Ns Nw+Ns T4b T4a T4d 0 Nbf	Ne of Bioko Ne of Western Ne of Southern Ancestral Population before vicariance Time of split between W &S Time of split of Bioko Time of Bioko founder event Present Ne of Bioko at founder event

Table S3: Notations of parameters listed in Table S2 and prior ranges and parameterization conditions used in simulations.

Symbol	Priors	Steps	Conditions
Nb	[0:35000]	100	
Nn	[0:35000]	100	
Ns	[0:35000]	100	
Nbf	[0:35000]	100	
Nwf	[0:35000]	100	
Nsf	[0:35000]	100	
Nbb	[0:35000]	100	
Nanc	[0:35000]	100	Nanc>Nwf Nanc>Nsf
t1a, t2a, t3a, t4a	[0:7500000]	1000	
t1b, t2b, t3b, t4b	[0:7500000]	1000	
t1c, t2c, t3c, t4c	[11000:7500000]	1000	>t1a (t2a, t3a, t4a) ≤t1b (t2b, t3b, t4b)
t1d, t2d, t3d, t4d	[11000:7500000]	1000	≤t1a (t2a, t3a, t4a)
t1e, t2e, t3e, t4e	[0:11000]	50	

Table S3 Population diversity estimates at each of 15 *An. melas* specific loci amplified in four *An. melas* populations. Bold indicates significance after Bonferroni correction (p-val.<0.0003). Grey shaded HWE p-val. indicates that null alleles were detected by Micro-Checker for the denoted locus/population comparison. *Ar*, allelic richness, rarefied to *N*=16. H_O, observed heterozygosity. H_E, expected heterozygosity. HWE p-val., Hardy-Weinberg Equilibrium test p-value. Population abbreviations correspond with those defined in Table 1.

	ARB				RIA				CAC			
Locus	*Ar*	H_O	H_E	HWD P-val.	*Ar*	H_O	H_E	HWD P-val.	*Ar*	H_O	H_E	HWD P-val.
AMXH25	2.99	0.60	0.64	0.1562	2.83	0.12	0.47	**0.0000**	2.80	0.49	0.50	0.8168
AMXH38	2.43	0.44	0.42	0.7327	2.22	0.41	0.36	0.1708	2.22	0.23	0.36	0.0483
AM2H46	3.37	0.48	0.52	0.7424	3.81	0.57	0.63	0.6130	3.37	0.46	0.43	0.6431
AM3H93	2.14	0.18	0.19	0.2905	3.00	0.32	0.36	0.0008	1.64	0.09	0.08	1.0000
AM3H127	4.06	0.72	0.73	0.0697	4.10	0.80	0.74	0.3990	4.25	0.69	0.72	0.7677
AM2H143	2.78	0.51	0.57	0.6549	3.00	0.60	0.58	0.0414	2.92	0.54	0.58	0.2347
AM2H157	1.30	0.02	0.04	0.0321	1.32	0.04	0.04	1.0000	1.23	0.03	0.03	1.0000
AM2H215	2.89	0.57	0.56	0.6469	3.04	0.58	0.58	0.5854	2.88	0.59	0.59	0.3941
AMXH293	3.59	0.65	0.65	0.9527	3.69	0.63	0.64	0.7088	4.16	0.71	0.68	0.2587
AM3H555	3.31	0.53	0.49	0.7807	3.42	0.36	0.47	0.0024	3.06	0.40	0.53	0.3244
AM2H603	1.00	0.00	0.00	N/A	1.00	0.00	0.00	N/A	1.00	0.00	0.00	N/A
AM3H753	1.09	0.01	0.01	1.0000	1.65	0.12	0.11	1.0000	1.00	0.00	0.00	N/A
AMXH755	2.84	0.59	0.58	0.4411	3.03	0.61	0.60	1.0000	2.84	0.48	0.52	0.4672
AM2H793	2.66	0.27	0.26	0.7239	2.40	0.19	0.20	0.0608	2.34	0.30	0.27	1.0000
AMXH808	2.00	0.44	0.42	0.8117	2.17	0.43	0.40	0.8823	2.00	0.45	0.42	0.6909
Mean	2.56	0.40	0.41	–	2.71	0.39	0.41	–	2.51	0.36	0.38	–

71 **Table S3** (continued)

	TIK				GHA				GAM			
Locus	*Ar*	H_O	H_E	HWD P-val.	*Ar*	H_O	H_E	HWD P-val.	*Ar*	H_O	H_E	HWD P-val.
AMXH25	5.84	0.75	0.80	0.4043	3.82	0.67	0.74	1.0000	3.94	0.61	0.64	0.3185
AMXH38	8.03	0.81	0.90	0.1086	6.17	0.67	0.86	0.2652	8.80	0.87	0.90	0.0604
AM2H46	6.83	0.81	0.86	0.8170	6.45	0.67	0.91	0.1745	6.32	0.81	0.83	0.0836
AM3H93	4.47	0.75	0.65	0.7415	5.33	1.00	0.82	0.2001	7.80	0.88	0.87	0.1035
AM3H127	5.48	0.88	0.81	0.4102	4.80	0.83	0.83	0.5589	4.24	0.63	0.72	0.1778
AM2H143	6.76	0.47	0.78	0.0061	4.65	0.83	0.80	0.7802	6.24	0.82	0.82	**0.0003**
AM2H157	6.88	0.88	0.84	0.3942	3.80	0.83	0.65	1.0000	6.36	0.88	0.82	0.5503
AM2H215	6.89	0.81	0.84	0.3752	4.00	0.60	0.78	0.6942	5.98	0.83	0.80	0.9723
AMXH293	7.93	0.88	0.88	0.7982	5.48	0.67	0.85	0.0193	6.56	0.83	0.83	0.6922
AM3H555	6.75	0.75	0.84	0.2627	3.82	0.83	0.71	1.0000	5.69	0.65	0.73	0.1193
AM2H603	3.99	0.50	0.50	1.0000	1.83	0.17	0.17	1.0000	2.03	0.19	0.18	1.0000
AM3H753	2.65	0.25	0.28	0.3057	1.98	0.33	0.30	1.0000	2.41	0.22	0.21	1.0000
AMXH755	5.83	0.56	0.76	0.1868	3.00	0.20	0.51	0.1106	3.44	0.67	0.60	0.8216
AM2H793	9.03	0.69	0.91	0.0387	7.82	1.00	0.94	1.0000	8.85	0.91	0.90	0.9744
AMXH808	6.32	0.73	0.82	0.6382	5.64	0.50	0.88	0.0614	5.90	0.84	0.80	0.1858
Mean	6.25	0.70	0.77	–	4.57	0.65	0.72	–	5.64	0.71	0.71	–