Is massive introgression driving species radiation at the range limit of *Anopheles gambiae*?

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Supplementary Figures and Tables

Supplementary Table 1| Microsatellites used in the study.

Chromosome	Locus	Repeat	Dye	Primers	T_A
X	X6U2[a]	$(CA)_{13}$	HEX	F*-TTGTTGCTCGGCTTGAAGTA R-GAAGGAATCGAGGGTGCTCT	60
	X5D1[a]	$(CA)_{11}$	NED	F*-GGAAACCGACACCACAAAG R-TGCCATTGAATGATGATGATG	55
	X678	$(TC)_7$	HEX	F*-CCTCTCCCCAGAATCGGTAC R-AAGAGCAGAAACAACCGCAG	54
	X5C1[a]	$(GT)_{10}$	6-FAM	F*-TCGCTTCGACAAATCATCAC R-GGGCGAAAATTCGTACAGAG	60
	X5B1[a]	$(CA)_{10}$	NED	F*-CAACAGCGAAAGGGTTATCG R-CAGCAGAACATACACGCACA	62
	XH07	$(AT)_3(GT)_7$	HEX	F*-CACGATGGTTTTCGGTGTGG R-ATTTGAGCTCTCCCGGGTG	54
	XH25	$(GT)_9$	6-FAM	F*-GCCGAAAACATTCCAACAGG R-CAGTTATGTCGGCATGCTAC	54
	XH77	$(GT)_{10}$	6-FAM	F*-TGGGACTGTAAGTGTCTCCC R-TATCAGTGAGGCCGAGTTGC	54
	X145	$(GT)_{11}$	HEX	F*-TGGTGGAATGTGAGACACAG R-ATGATGGTCGATCCTTGTCC	54
3L	H577	(GT)16	6-FAM	F-TTCAGCTTCAGGTTGGTCTC R*-GGGTTTTTTGGCTGCGACTG	56
	H758	(GT)11	6-FAM	F*-TGATTTGCCAGTTCTGCCAG R-GTGATTGGAGTGGCTAGTGG	54
	H242	(GT)8	HEX	F*-TTCATTTCCACCGCAGCTGC R-GGCGACACTCAATCCTTCC	56
	H45C[b]	(TG)4+7+4	HEX	F*-AAAAGTGGTGACCGAGTGAC R- ATCTTCAACACTTCAGCACG	54
3R	H555	(GT)8	NED	F*-GCAGAGACACTTTCCGAAAC R-TGTCAACCCACATTTTGCGC	54
	H119	(GT)6	HEX	F*-GGTTGATGCTGAAGAGTGGG R-ATGCCAGCGGATACGATTCG	54
	H249	(GT)15	NED	F*-ATGTTCCGCACTTCCGACAC R-GCGAGCTACAACAATGGAGC	54
	H059	(GT)9	HEX	F*-CCCCTATTAAACCCTGGACG R-TGTTGTTGCCCTGCGTTACC	54
	H128	(GT)21	6-FAM	F*-CGGGACGGCTAGATAAAGCG R-CCGGGCGACATAACCCACCC	56
	H093	(GT)4+7	NED	F*-TCCCCAGCTCACCCTTCAAG R-GGTTGCATGTTTGGATAGCG	54

Dye: Applied Biosystems® fluorescent dye used to label the primer. * Primer fluorescently labeled; T_A: annealing temperature; [a] Stump *et al*. 2005. *Genetics* 169, 1509-1519, [b] Lehmann *et al.* 1998. *Mol. Biol. Evol.* 15, 264-276, remaining loci were described in Zheng *et al*. 1996. *Genetics* 143, 941-952.

Supplementary Table 2| Genetic diversity estimates per microsatellite locus and locality.

Locus		Quinhamel	Safim	Antula	Mansoa	Mandingará	Gambana	Comuda	Leibala	Mean
X6U2	$Ar_{(100)}$	8.1	9.2	8.3	8.6	9.7	6.2	11.2	14.4	9.4
	H_e	0.660	0.661	0.669	0.801	0.817	0.732	0.838	0.832	0.751
	F_{IS}	0.101	0.007	0.052	0.054	0.059	0.062	-0.009	-0.008	0.040
X5D1	$Ar_{(100)}$	7.0	5.7	5.0	7.6	7.5	6.2	6.3	6.5	6.5
	H_e	0.543	0.526	0.614	0.587	0.661	0.618	0.709	0.640	0.612
	F_{IS}	0.130	-0.084	0.107	0.122	-0.072	0.003	-0.070	0.008	0.018
X678	$Ar_{(100)}$	14.7	15.2	15.7	15.7	10.2	7.8	15.3	20.0	14.3
	H_e	0.805	**0.785**	0.796	0.834	0.646	0.531	0.870*	0.886	0.769
	F_{IS}	0.044	0.124	-0.054	0.170	0.240	0.068	**0.180**	0.041	0.102
X5C1	$Ar_{(100)}$	12.5	10.8	13.7	12.7	11.3	11.6	10.9	11.3	11.9
	H_e	0.825	0.801	0.819	0.843	0.842	0.835	0.819	0.846*	0.829
	F_{IS}	0.044	0.092	0.055	0.063	0.106	-0.077	**0.236**	0.175	0.087
X5B1	$Ar_{(100)}$	13.8	14.6	13.4	14.0	15.9	17.2	15.0	12.0	14.5
	H_e	0.851	0.810	0.817	**0.836***	0.865	0.869	0.846	0.799	0.837
	F_{IS}	0.031	0.138	0.019	**0.346**	0.102	0.023	-0.012	0.112	0.095
XH07	$Ar_{(100)}$	8.1	8.2	7.8	7.9	10.2	7.9	8.2	7.4	8.2
	H_e	0.741	0.686	0.653	0.701	0.711	0.684	0.727*	**0.735***	0.705
	F_{IS}	-0.009	0.233	0.095	0.172	0.157	0.151	**0.261**	**0.410**	0.184
XH25	$Ar_{(100)}$	8.2	8.4	8.1	7.8	7.3	7.0	8.4	8.5	8.0
	H_e	0.720	0.730	0.714	0.764*	0.707	0.734	0.770	0.743	0.735
	F_{IS}	-0.038	0.102	- 0.008	0.199	0.130	0.023	0.102	0.104	0.077
XH77	$Ar_{(100)}$	8.6	8.5	8.8	11.5	9.5	8.4	9.6	8.9	9.2
	H_e	0.779	0.803	0.797	0.822	0.805	0.811	0.790	0.797	0.801
	F_{IS}	0.041	0.036	-0.025	-0.040	-0.009	-0.019	-0.057	0.110	0.005
X145	$Ar_{(100)}$	6.9	7.8	7.1	9.3	7.7	7.2	7.8	7.4	7.7
	H_e	0.743	0.743	0.727	0.745	0.725	0.740	0.739	0.717	0.735
	F_{IS}	-0.066	0.147	-0.020	-0.061	-0.057	0.031	-0.056	0.011	-0.009
Mean	$Ar_{(100)}$	9.8	9.8	9.8	10.6	9.9	8.8	10.3	10.7	
X-loci	H_e	0.741	0.727	0.734	0.770	0.753	0.728	0.790	0.777	
	F_{IS}	0.031	0.088	0.025	0.114	0.073	0.029	0.064	0.107	

$Ar_{(100)}$: Allele richness. H_e: expected heterozygosity (in bold: significant heterozygote deficit). F_{IS}: inbreeding coefficient (in bold: significant value). Asterisks indicate presence of null alleles determined by Micro-Checker. X-loci: chromosome-X microsatellites; 3-loci: chromosome-3 microsatellites.

Supplementary Table 2 (continued).

Locus		Quinhamel	Safim	Antula	Mansoa	Mandingará	Gambana	Comuda	Leibala	Mean
H093	$Ar_{(100)}$	15.2	16.5	14.0	13.8	14.5	14.2	13.2	11.6	14.1
	H_e	0.843*	**0.836***	**0.822**	0.779	0.842	0.825*	0.841*	0.816	0.825
	F_{IS}	**0.197**	**0.242**	0.163	0.215	0.142	**0.205**	0.177	0.091	0.179
H128	$Ar_{(100)}$	22.0	23.3	25.7	19.9	21.5	21.7	22.9	22.3	22.4
	H_e	0.923	0.930	0.937*	0.923*	0.929*	**0.915***	0.918	0.925	0.925
	F_{IS}	-0.036	0.018	0.094	**0.180**	**0.194**	**0.142**	0.069	0.082	0.093
H059	$Ar_{(100)}$	8.9	9.8	9.3	8.6	7.7	9.2	10.4	8.3	9.0
	H_e	0.797	0.808	0.797	0.818	0.779	0.759	0.806	0.814	0.797
	F_{IS}	0.049	0.042	0.082	0.153	0.191	0.136	0.100	0.115	0.109
H249	$Ar_{(100)}$	15.5	16.0	18.0	15.1	12.9	19.7	16.0	14.2	15.9
	H_e	0.871	0.883	0.861	0.870	0.874	0.865	0.854	0.873	0.869
	F_{IS}	0.093	-0.023	0.038	0.092	0.139	**0.136**	0.061	0.040	0.072
H119	$Ar_{(100)}$	14.6	15.4	14.6	15.0	16.1	14.5	14.6	13.7	14.8
	H_e	0.875	0.858	0.850	0.852	0.879	0.886*	0.865	0.833	0.862
	F_{IS}	0.033	0.023	0.026	-0.003	0.144	0.146	0.099	0.058	0.066
H555	$Ar_{(100)}$	9.0	12.6	9.6	9.8	11.3	9.1	9.9	11.6	10.4
	H_e	0.827	0.815	0.810	0.823*	0.832	0.837	0.815	0.821	0.822
	F_{IS}	0.057	-0.082	0.078	0.198	-0.017	-0.026	0.056	-0.022	0.030
H577	$Ar_{(100)}$	11.4	11.6	13.8	9.3	11.8	11.0	12.6	10.8	11.5
	H_e	0.686	0.709	**0.761**	0.574	0.651	0.642	0.684	0.636	0.668
	F_{IS}	-0.074	-0.016	0.144	-0.069	- 0.016	0.041	-0.028	-0.014	-0.004
H758	$Ar_{(100)}$	14.8	18.4	15.4	18.0	14.6	15.6	14.8	16.7	16.0
	H_e	**0.896***	0.911	0.901	0.907*	0.894	**0.892***	0.894*	0.881*	0.897
	F_{IS}	**0.179**	**0.141**	0.085	**0.183**	0.158	**0.143**	**0.168**	**0.137**	0.149
H242	$Ar_{(100)}$	7.4	8.8	8.2	9.2	7.3	7.1	7.9	9.0	8.1
	H_e	0.648	**0.739**	0.620	0.668	0.744	0.651	0.686	0.671	0.678
	F_{IS}	0.102	0.142	0.098	0.156	0.133	0.194	0.071	-0.009	0.111
H45C	$Ar_{(100)}$	6.9	7.2	6.3	7.6	7.0	7.2	7.6	8.2	7.3
	H_e	0.795	0.782	0.769	0.746	0.788	0.746	0.770	0.775	0.771
	F_{IS}	-0.024	0.125	0.057	0.180	0.044	0.066	0.001	0.043	0.062
Mean 3-loci	$Ar_{(100)}$	12.6	14.0	13.5	12.6	12.5	13.0	13.0	12.6	
	H_e	0.816	0.827	0.813	0.796	0.821	0.802	0.813	0.804	
	F_{IS}	0.058	0.061	0.087	0.129	0.111	0.118	0.077	0.052	
Mean All loci	$Ar_{(100)}$	11.2	12.0	11.7	11.6	11.3	11.0	11.7	11.7	
	H_e	0.780	0.780	0.776	0.784	0.789	0.767	0.802	0.792	
	F_{IS}	0.045	0.074	0.057	0.122	0.093	0.076	0.071	0.078	

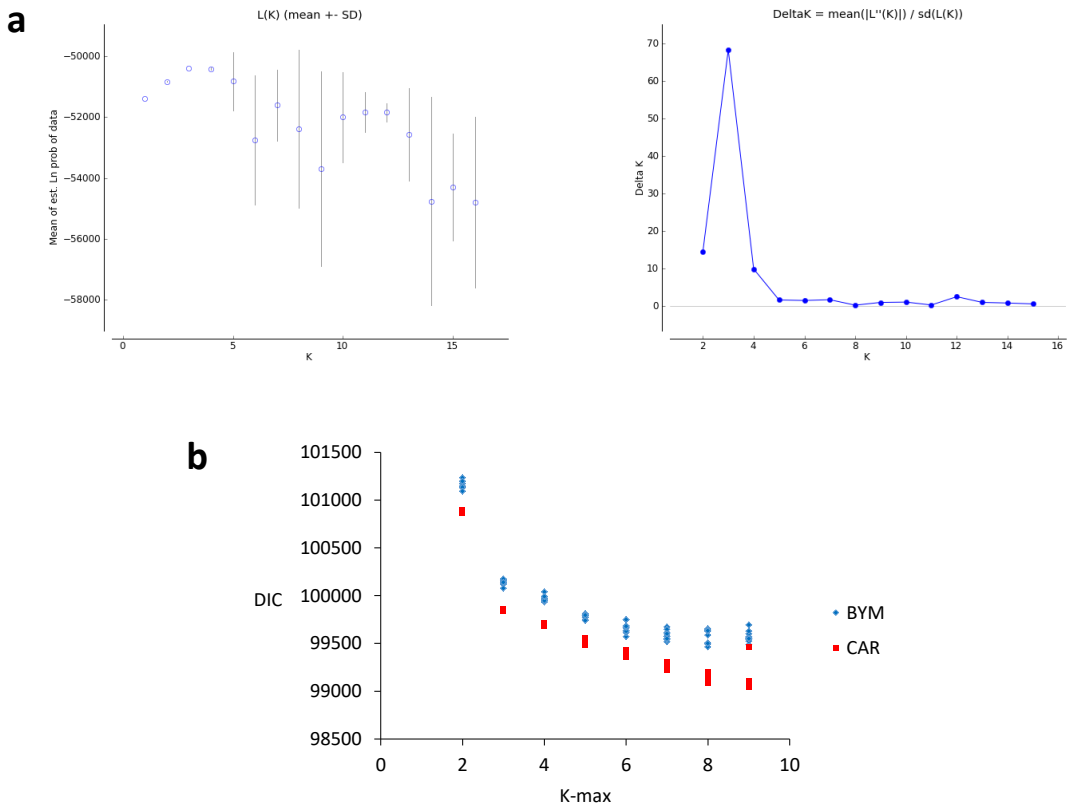
Supplementary Table 3| Accession numbers, Mean sequencing depth (autosomes), karyotype and *kdr* genotypes for the individuals sequenced.

Individual code	ERS Accession	Mean sequencing depth	Country	Region	Species (IGS/SINE assays)	2L Karyotype	2Rb Karyotype	2Rd Karyotype	*kdr* genotype
AJ0001-C	ERS040121	29,8	Guinea Bissau	Leibala	*A. gambiae*	+/+	+/+	+/+	L/L
AJ0007-C	ERS040133	26,6	Guinea Bissau	Leibala	*A. gambiae*	+/+	+/+	+/+	L/L
AJ0009-C	ERS040129	31,5	Guinea Bissau	Leibala	*A. gambiae*	a/a	b/+	+/+	L/L
AJ0011-C	ERS040122	29,8	Guinea Bissau	Leibala	*A. gambiae*	a/a	b/+	+/+	L/L
AJ0013-C	ERS040137	32,7	Guinea Bissau	Safim	*A. gambiae*	+/+	+/+	d/d	L/L
AJ0014-C	ERS040120	20,8	Guinea Bissau	Safim	*A. gambiae*	a/a	b/b	+/+	L/L
AJ0016-C	ERS040130	33,4	Guinea Bissau	Safim	*A. gambiae*	+/+	+/+	d/+	L/L
AJ0018-C	ERS040123	22,7	Guinea Bissau	Safim	*A. gambiae*	+/+	+/+	d/d	L/L
AJ0020-C	ERS040132	25,4	Guinea Bissau	Safim	*A. gambiae*	+/+	+/+	+/+	L/L
AA0006-C	ERS012670	24,7	Ghana	Greater Accra	*A. gambiae*	-	-	-	F/F
AA0007-C	ERS012671	21,9	Ghana	Greater Accra	*A. gambiae*	-	-	-	F/F
AA0008-C	ERS012672	24,9	Ghana	Greater Accra	*A. gambiae*	-	-	-	F/F
AA0009-C	ERS012673	17,8	Ghana	Greater Accra	*A. gambiae*	-	-	-	F/F
AJ0043-C	ERS242776; ERS254362	27,9	Guinea Bissau	Antula	*A. gambiae*	-	-		L/L
AJ0047-C	ERS242791; ERS254358	20,2	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0059-C	ERS242792; ERS254321	31,9	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0061-C	ERS242801; ERS254310	25,7	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0071-C	ERS224332	34,1	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0076-C	ERS224284	35,4	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0085-C	ERS242803; ERS254298	87,2	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0096-C	ERS224313	35,1	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0098-C	ERS224312	33,6	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0100-C	ERS224272	32,5	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0107-C	ERS224805	29,7	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L
AJ0113-C	ERS224286	31,7	Guinea Bissau	Antula	*A. gambiae*	-	-	-	L/L

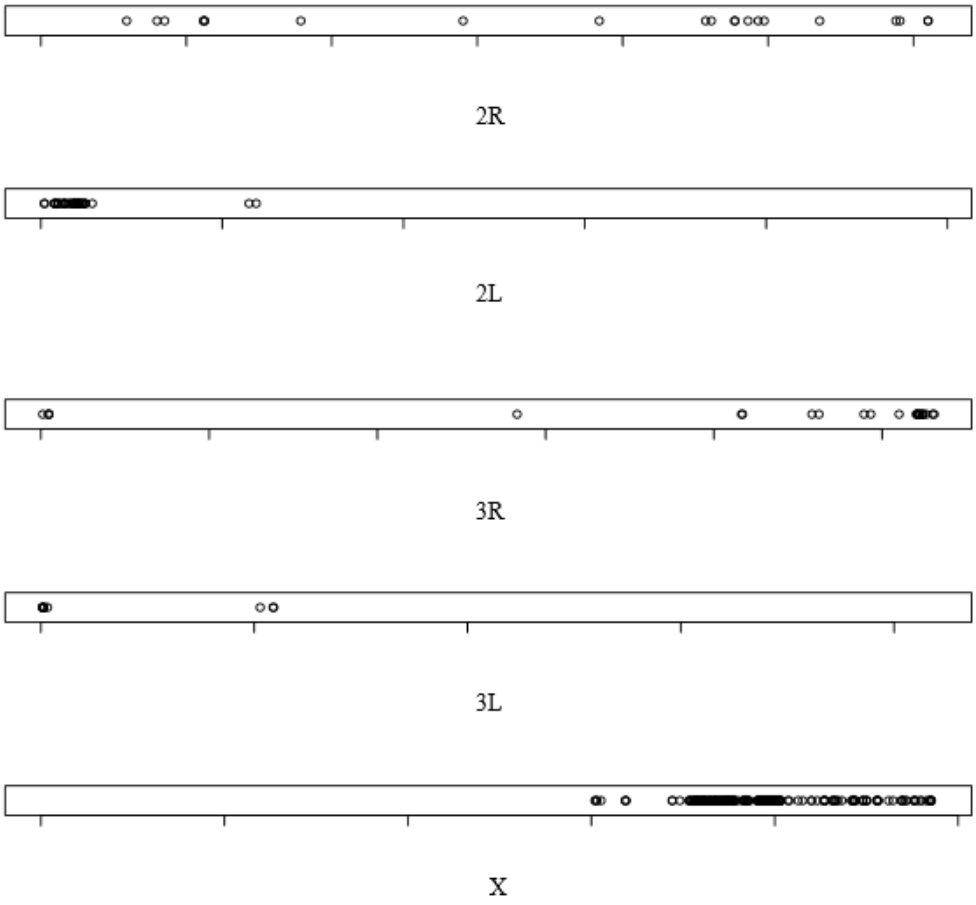
Supplementary Table 4| Blood meal identification by ELISA according to STRUCTURE genetic clusters.

IgG-positive	*A. coluzzii* (13)	*A. gambiae*-inland (34)	*A. gambiae*-coast (65)	Admixed (23)
Human	38.5	82.4	24.6	34.8
Bovine	30.8	5.9	43.1	52.2
Porcine	15.4	8.8	9.2	0.0
Canine	7.7	0.0	4.6	4.3
Bovine/porcine	0.0	0.0	4.6	0.0
Human/porcine	0.0	0.0	4.6	0.0
Human/bovine	0.0	0.0	0.0	4.3
Human/caprine	0.0	0.0	1.5	0.0
Caprine/porcine	0.0	0.0	0.0	4.3
Other/negative	7.7	2.9	7.7	0.0

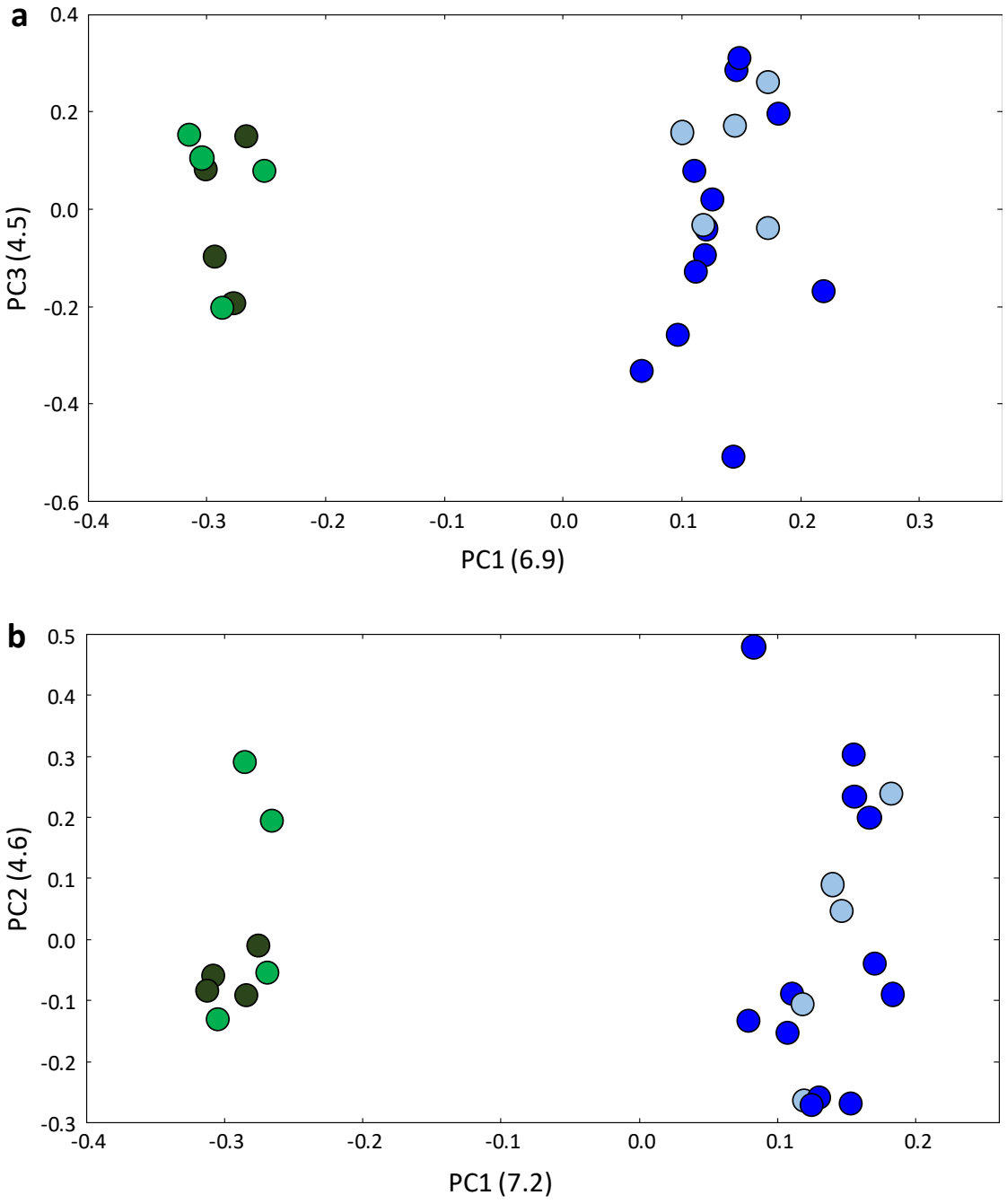
In parenthesis: number of blood meals analyzed for each genetic cluster. Eight individuals with blood meal from Antula (6 *A. gambiae*; two admixed by IGS/SINE) were not included in microsatellite analysis.



Supplementary Figure 1| Inference of the number of clusters obtained from microsatellite-based Bayesian clustering (STRUCTURE) and spatially explicit (TESS) analyses (**a**) Plots for inferring *K* from STRUCTURE. Left plot is for the ln[Pr(X|K)] (Pritchard *et al.* 2000. *Genetics* 155, 945-959) and right plot for the ΔK (Evanno *et al.* 2005. *Mol. Ecol.* 14, 2611-2620). (**b**) Deviance Information Criterion (DIC) plot for BYM and CAR models to infer the optimal number of clusters obtained by TESS (Chen *et al.* 2007. *Mol. Ecol. Notes* 7, 747-756).

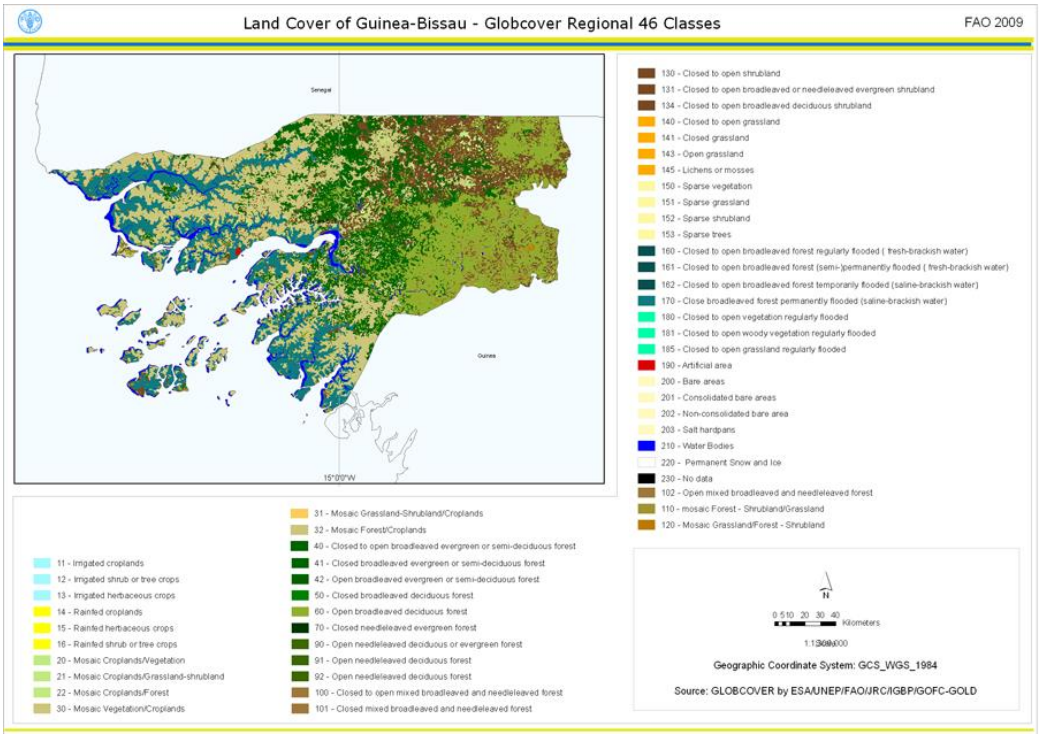


Supplementary Figure 2| Distribution of ancestry informative markers (AIMs). Each bar represents a chromosome within which the relative location of the SNPs used in the AIM analysis is marked by circles.

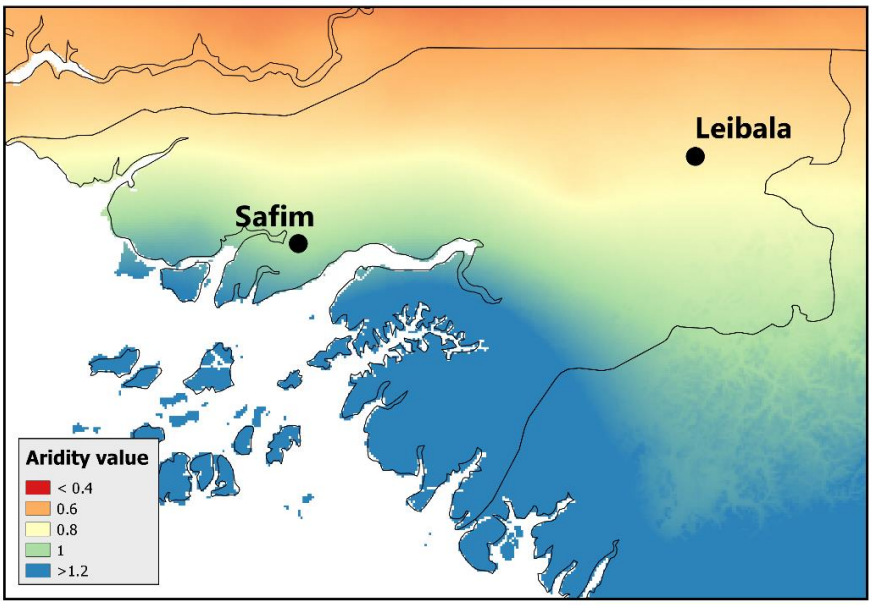


Supplementary Figure 3| Principal Components Analysis for whole genome sequenced *A. gambiae*. (**a**) Plot between principal components 1 and 3 for chromosome 3L variants. (**b**) Plot between principal components 1 and 2 for chromosome 3R variants. Sequenced sample are labelled as follows: Antula (blue), Safim (light blue), Leibala (green) and Accra-Ghana (dark green).

a



b



Supplementary Figure 4| Landcover and aridity maps of Guinea Bissau. (**a**) GlobCover 2009 land cover map of Guinea Bissau. Copyright notice: © ESA 2010 and UCLouvain. Available at: http://www.fao.org/geonetwork/srv/en/metadata.show?id=37189&currTab=simple. (**b**) aridity map for Guinea Bissau based on the CGIAR-CSI Global Aridity Index database. The map shows location of the two sites for which chromosomal data are available. Values are the mean Aridity Index from the 1950-2000 period at 30' spatial resolution (Trabucco, A., & Zomer, R.J. 2009. *Global Potential Evapo-Transpiration (Global-PET) and Global Aridity Index (Global-Aridity) Geo-Database*. CGIAR Consortium for Spatial Information. Available from the CGIAR-CSI GeoPortal at: http://www.csi.cgiar.org)