The Rift Valley Complex as a Barrier to Gene Flow for *Anopheles gambiae* in Kenya

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Recent studies of *Anopheles gambiae,* the principal mosquito vector of malaria in Africa, suggested that the eastern Rift Valley and its surrounding areas act as a barrier to gene flow. To quantify the unique effect of these areas on gene flow, we measured genetic variation within and between populations from each side of the Rift. Low differentiation was measured between populations on each side of the Rift (mean $F_{ST} < 0.008$, mean $R_{ST} < 0.002$). However, high differentiation was measured across the Rift (mean $F_{ST} = 0.104$; mean $R_{ST} = 0.032$). Genetic diversity within populations was lower in eastern populations, suggesting that the effective population sizes (N_e) of these populations were lower than those of western populations. We partitioned the overall differentiation across the Rift into three factors: variation in N_e between populations contributed 7–20%; distance contributed 10–30%, and the remainder, corresponding to the unique effect of the Rift was 50–80%. The Rift's effect was highly significant based on F_{ST}. The greater sensitivity of F_{ST} in measuring differentiation indicated that drift and not mutation generated the differences between populations. Restricted gene exchange across several hundred kilometers on the face of intense human transportation implies that active mosquito dispersal is the major form of migration, and that migration is a multistep process, where step length is relatively short.

Anopheles gambiae is the principal vector of malaria in Africa. Knowledge of its population structure is needed for better management of insecticide resistance and for evaluation of genetic control strategies aimed at reducing the vectorial capacity of the mosquitoes (Collins and Besansky 1994). Recent studies have resulted in apparently contradictory descriptions of the population structure of *A. gambiae.* Low differentiation was measured between populations from western Kenya and from Senegal (6000 km apart) based on microsatellites ($F_{ST} = 0.016$ and $R_{ST} = 0.036$), allozymes ($F_{ST} = 0.036$), and mtDNA ($F_{ST} = 0.085$), and negligible differentiation was measured by these markers up to 50 km away (Besansky et al. 1997; Lehmann et al. 1996, 1997). However, western and eastern Kenyan populations (700 km apart) showed entirely distinct RFLP arrays of the rDNA intergenic spacer (McLain et al. 1989) and levels of differentiation ($F_{ST} = 0.08$) based on microsatellites (Kamau et al. 1998) considerably higher than that of populations across the continent. Significant differences in the effective population size (N_e) of western and eastern populations (Lehmann et al. 1998) complicated the measurement and the interpretation of differentiation. Finding higher differentiation at a smaller geographic scale suggested that (1) one set of results is wrong or applies only to specific populations, (2) differences in N_e between the two Kenyan populations inflated the differentiation between them, and (3) western and eastern Kenyan populations are separated by a barrier to gene flow. These hypotheses are not mutually exclusive, however, the third hypothesis was favored by Kamau et al. (1998) who proposed that the eastern Rift Valley is that barrier. These studies, however, could not rule out either of the first two hypotheses because they included only one population from each side of the Kenyan Rift Valley and did not consider the effect of the difference in N_e between populations nor the effect of distance in a quantitative way.

The Rift system forks to eastern and western Rift Valleys on either side of Lake Victoria. The eastern Rift Valley in Kenya and northern Tanzania is a system of arid valleys that are sparsely populated by man and surrounded by highlands. The arid valleys and the cool highlands do not support *A. gambiae* populations. Dry savanna, probably too dry to support *A.*

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gambiae, extends between the narrow coastal plain along the Indian Ocean and the highlands flanking the eastern Rift Valley. These series of geographical strips, all inhospitable for *A. gambiae,* are hereafter referred to as the Rift Valley complex (RVC). The western Rift cuts through Uganda; it is characterized by a wetter climate and is dominated by moist savanna and woodland (Kingdom 1989, pp. 14–20). Thus the western Rift is probably a favorable environment for *A. gambiae.*

Identifying a barrier to gene flow between allopatric populations requires not only a demonstration of significant difference in the genetic composition of populations across it, but especially a demonstration that other factors cannot explain this genetic difference (e.g., Barbujani and Sokal 1991). Few studies demonstrated the existence of barriers to gene flow by ruling out the effects of confounding factors. Confounding factors include distance, differences in N_e between populations or a bottleneck effect, and possibly some form of selection.

In this study we assessed the unique effect of the RVC on gene flow in *A. gambiae* (savanna chromosomal form). We measured genetic variation at nine microsatellite loci between several populations from each side of the RVC and partitioned the overall differentiation across the RVC into distance, the difference in N_e between populations, and the remainder, which we attributed to the unique effect of the Rift Valley on gene flow. Since we are not aware of any selection or other factor that can contribute to the overall differentiation, we assumed that the RVC is responsible for the remainder. We compared different indices of differentiation (F_{ST} and R_{ST}) which differ in their sensitivity to drift and mutation to assess the robustness of the results and to determine which evolutionary force generated the genetic differences between populations.

Materials and Methods

Study Sites

West of the RVC, mosquitoes were collected from four sites including Awendo, Asembo Bay (hereafter referred to as Asembo),Wathrego, and Kimilili (Figure 1). East of the RVC, mosquitoes were collected from eight sites including Embu, Chegoria, Thika, Voi, Wundanyi, Malindi, Jego, and Taveta. *A. gambiae* was found at all sites west of the RVC, but only at two sites east of the RVC, located along the Kenyan coast, Jego and Goshi (Figure 1). In this

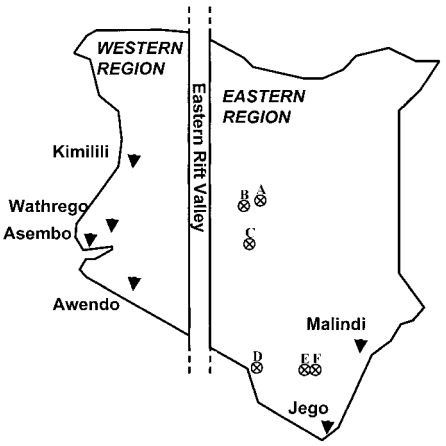

Figure 1. A schematic map of Kenya showing sampling sites (except for the population from Senegal, see text) and location of the eastern Rift Valley. Sites where *A. gambiae* were found are shown by an arrow near the site name. Sites where no *A. gambiae* were found are shown by a circled X near a letter (A = Embu; B = Chegoria; C = Thika; D = Taveta; E = Wundanyi; F = Voi). See text for details.

article, region refers to the collection of sites or populations on each side of the RVC. All sites are densely populated, occupying a rolling landscape. Most inhabitants live in traditional houses made of wood and mud walls and thatch or corrugated sheet metal roofs surrounded by farmland. During the major rainy season (April–July), many breeding sites are available and mosquito density is high, but during the dry season most breeding sites disappear and density is remarkably lower. In some years, a shorter rainy season may occur around November.

Mosquito Collection

Mosquitoes were collected from Wathrego in June 1994, and from all other sites in July 1996. In Asembo, indoor resting mosquitoes were collected by the pyrethrum spray method, while at all other sites, indoor resting mosquitoes were caught using aspirators. At each site, mosquitoes were collected over 2–7 days from houses in an area of less than 8 km in diameter. At least 15 households were thoroughly examined at each site in which no *A. gambiae* s.l. was found.

DNA Extraction and Microsatellite Genotype Scoring

The procedures and loci were described in detail previously (Lehmann et al. 1996, 1997, 1998). Only *A. gambiae* were included in the analysis after species identification was carried out (Scott et al. 1993). Microsatellite loci were mapped to sites outside polymorphic chromosomal inversions in *A. gambiae* (Table 1). Microsatellite alleles were PCR amplified with one fluorescent-labeled primer and their size was determined using the ABI 377 semi-automated sequencing system.

Data Analysis

Goodness-of-fit tests with Hardy–Weinberg expectations in each population were performed using exact tests available in GENEPOP 3.1 (Raymond and Rousset 1995). Exact tests (available in GENEPOP) of genotypic linkage disequilibrium between all pairs of loci within all populations were performed to evaluate the independence of loci and whether the gene pool is subdivided in each population.

Differentiation between populations was assessed by *F* statistics (Wright 1978), calculated according to Weir and Cockerham (1984) and also according to Nei and Chesser (1983), as shown in Nei (1987, equation 7.36 based on equations 7.38, 7.39, and 7.40). Certain attributes of our populations better met the assumptions of the method of Nei and Chesser (see below). The microsatellite's equivalent *R* statistics (Slatkin 1995) were calculated according to Kimmel et al. (1996, equations 17 and 18). For R_{ST} calculation, noncanonical alleles (alleles whose size did not fit a series predicted by the size of the repeat motif and a constant flanking sequence, e.g., an allele of an odd size of 137 bp in a series consisting of alleles with even sizes such as 134, 138, and 140, which were found mostly at loci *2A1* and *AG3H88,* and rarely at loci *33C1* and *AG3H45*; Figure 2) were treated as described previously (Lehmann et al. 1996, 1997). Estimates of migration, N_m, between populations were calculated using equation 15a for R_{ST} and equation 15b for the F_{ST} [equations after Slatkin (1995)]. The N_m values for X-linked loci were adjusted assuming a ratio of 4:3 of the N_e of autosomes to sex chromosomes, respectively. Deviation of a single locus F_{ST} from 0 that can be tested by using the usual chi-square test of heterogeneity of gene frequencies (Nei 1987, p. 165) was evaluated instead by the corresponding exact test (available in GENEPOP). The significance of the R_{ST} values were evaluated using a nested ANOVA on the repeat number in a model, including the individual and the population as factors (Slatkin 1995). To evaluate the significance of the average F_{ST} or R_{ST}, two "global" tests were employed. The sequential Bonferroni procedure (Holm 1979) can detect a significant departure of a single locus that may represent a locus-specific departure, whereas the binomial test (which estimates the

Table 1. Genetic diversity of microsatellite loci in each population

Locus (cyt)[a]	Parameter	Awendo	Asembo	Wathrego	Kimilili	Jego	Malindi
1D1 (X:1D)	N[b]	113	101	77	64	110	123
	Alleles	5	4	3	4	3	3
	H_E[c]/H_{eq}[d]	50.0/67.0	54.3/59.1	47.8/46.6	50.7/59.6	28.7/44.6	18.2/45.4
	H_o[e] (%)	42.2	50.0	57.6	46.4	27.8	18.6
	F_{IS}[f]	+0.191	+0.081	−0.226	+0.078	+0.048	−0.067
2A1 (X:2A)	N	112	92	71	71	98	98
	Alleles	7	9	6	7	4	4
	H_E/H_{eq}	65.4/76.4	66.4/82.7	59.7/73.2	64.9/77.7	58.8/58.7	65.4/58.4
	H_o (%)	76.0	53.3	48.5	50.0	35.4	43.8
	F_{IS}	−0.099	+0.195	+0.200	+0.239**	+0.401*	+0.336*
AGXH99 (X:1-3)	N	114	107	81	74	106	123
	Alleles	5	7	4	5	5	5
	H_E/H_{eq}	72.6/66.6	75.4/76.6	73.8/59.0	71.5/68.1	56.3/66.8	44.3/66.4
	H_o (%)	61.5	60.4	62.9	57.6	58.5	40.0
	F_{IS}	+0.148	+0.202**	+0.149	+0.184*	−0.040	+0.105
AG2H46 (II:7A)	N	122	102	92	82	104	114
	Alleles	11	15	10	10	10	9
	H_E/H_{eq}	85.7/85.5	88.6/84.5	84.4/84.6	87.0/84.7	78.0/84.4	79.9/82.3
	H_o (%)	77.0	80.4	84.8	90.2	63.5	71.9
	F_{IS}	+0.101	+0.076	−0.004	−0.038	**+0.204*** **	+0.101
29CI (III:29C)	N	126	108	86	82	107	132
	Alleles	2	2	2	2	2	2
	H_E/H_{eq}	43.1/21.5	34.9/22.4	47.8/23.3	42.9/23.5	44.3/23.3	48.1/22.3
	H_o (%)	40.0	26.0	48.8	46.3	40.0	45.5
	F_{IS}	+0.080	+0.259	−0.021	−0.081	+0.085	+0.056
33D1 (III:33D)	N	123	110	92	77	104	134
	Alleles	13	14	13	12	5	6
	H_E/H_{eq}	76.8/88.1	79.1/89.2	79.5/88.6	81.4/87.7	41.0/67.0	44.9/72.7
	H_o (%)	68.9	76.4	82.6	81.6	40.4	44.8
	F_{IS}	+0.100**	+0.035	−0.040	−0.004	+0.015	+0.003
AG3H88 (III:35-38)	N	112	101	84	82	108	122
	Alleles	13	16	13	11	10	10
	H_E/H_{eq}	75.0/88.3	81.6/90.8	85.5/88.8	79.7/86.2	57.0/84.3	74.9/84.0
	H_o (%)	44.6	51.2	54.8	51.2	37.0	63.3
	F_{IS}	**+0.407*** **	**+0.379*** **	**+0.363*** **	**+0.360*** **	**+0.352*** **	+0.154**
AG3H127 (III:39A)	N	124	106	89	80	100	108
	Alleles	10	10	9	9	8	7
	H_E/H_{eq}	40.0/84.0	42.4/84.1	40.9/82.6	39.6/82.9	57.7/79.7	53.7/76.8
	H_o (%)	29.0	35.8	43.2	32.5	44.0	25.9
	F_{IS}	**+0.275*** **	+0.155**	−0.045	+0.181**	**+0.239*** **	**+0.456*** **
45D1 (III:45)	N	106	102	90	81	104	112
	Alleles	7	11	8	8	7	5
	H_E/H_{eq}	74.5/76.7	80.8/86.0	80.6/79.9	81.4/80.1	61.6/76.4	51.2/66.8
	H_o (%)	80.0	68.6	82.2	75.0	51.9	45.5
	F_{IS}	−0.071	**+0.177*** **	−0.020	+0.082*	+0.159	+0.088*
Population mean	N	116.9	103.2	84.7	77.0	104.6	118.4
	Alleles	8.1	9.8	7.6	7.6	6.1	5.7
	H_E/H_{eq}	64.8/72.7	67.1/75.0	66.7/69.6	66.6/72.3	53.7/65.0	53.4/63.9
Regional mean	N		95.4			111.5	
	Alleles		8.3 (8.4)[h]			5.9 (5.6)[h]	
	H_E/H_{eq}		66.3/72.4			53.6/64.5	
	$N\mu$[g]		0.976 (0.492)			0.456 (0.289)	

[a] Chromosome location and subdivision as determined by in situ hybridization and division when location was approximated by genetic mapping (Zheng et al. 1996).

[b] Number of chromosomes scored. Odd numbers resulted from scoring X-linked loci (*1D1, 2A1,* and *AGXH99*) in males and from rare cases where confident genotype scores were made only for one allele, hence the other score was omitted. The frequency of missing alleles at Awendo was 0.018 in *2A1*, 0.008 in *33C1,* and 0.051 in *45C1*; at Asembo 0.01 in *2A1* and 0.029 in *AG3H88*; at Wathrego 0.033 in *AG3H127*; at Kimilili 0.013 in *33C1* and 0.012 in *45C1*; at Jego 0.009; and at Malindi 0.016 in *AG3H88* and 0.018 in *45C1.*

[c] Unbiased expected heterozygosity (Nei 1978; in percent).

[d] Expected heterozygosity under equilibrium between mutation and drift and the stepwise mutation model, based on the number of alleles and sample size estimated by BOTTLENECK (Cornuet and Luikart 1996). See text for details.

[e] Observed heterozygosity.

[f] Calculated according to Weir and Cockerham (1984) using GENEPOP 3.1. Significance level represents an individual test of the heterozygote deficit (one-sided test). Bold values represent significant tests on the multitest level under the sequential Bonferroni procedure (see Materials and Methods) considering a total of 54 tests.

[g] Estimated $N\mu$ (the product of N_e and mutation rate) based on the SMM (Nei 1987), $N\mu = \{[1/(1 - H_E)]^2 - 1\}/8$, where H_E is the expected heterozygosity. Estimated $N\mu$ is in parenthesis, based on the IAM (Nei 1987), $N\mu = H_E/(4 - 4H_E)$.

[h] Least squares means adjusted for the difference in sample size (see Table 3).

probability of obtaining the observed number of significant tests at the 0.05 level given the total number of tests) can detect weaker departures of several loci that may represent genomewide effects. Bootstrap over loci was also performed to construct 95% confidence intervals (CIs) around the mean values. Significance of the mean F_{ST} and R_{ST} values was determined based on whether the CI included 0 or overlapped with each other when testing the difference between two values. The number of bootstrap replications was 5,000 (1,000 for bootstrapping regression analyses, below). Calculations not available in GENEPOP were carried out using programs written by T.L. in SAS language (SAS Institute 1990).

Results

Hardy–Weinberg Equilibrium

Within-population deviations from Hardy–Weinberg (HW) expectations were found in 17 ($p < .05$, single test level) out of 54. This is a highly significant result based on the binomial test ($p < .001$). All significant tests were associated with positive F_{IS} values (except locus *33C1* in Wathrego, $p = .023$). Tests of heterozygote deficits (available in GENEPOP) verified that the deviations from HW expectations were the result of heterozygote deficits (Table 1). Heterozygote deficits in multiple loci indicated that samples may represent pooling several subpopulations (Wahlund effect), inbreeding, or the effect of null alleles (Callen et al. 1993). Null alleles are locus specific, whereas the Wahlund effect and inbreeding affect the entire genome. Significant deficits were clustered in particular loci. For example, significant deficits across all populations were found in locus *AG3H88* and across five of the six populations in locus *AG3H127* (Table 1), whereas an excess of heterozygotes or insignificant deficits across all populations were found in loci *1D1, 29C1,* and *33C1.* Moreover, of the 10 significant deficits at the multitest level (using the sequential Bonferroni method; bold values in Table 1), 8 were in loci *AG3H88* and *AG3H127.* In addition, a few individuals failed to amplify a PCR product for only a particular locus (mostly in loci *AG3H88* and *AG3H127*) on repeated PCR amplifications, suggesting that they represented homozygotes for null alleles. To further evaluate this explanation, linkage disequilibrium analysis was performed within each population (Table 2). If the Wahlund effect or inbreeding were the cause of the heterozygote def-

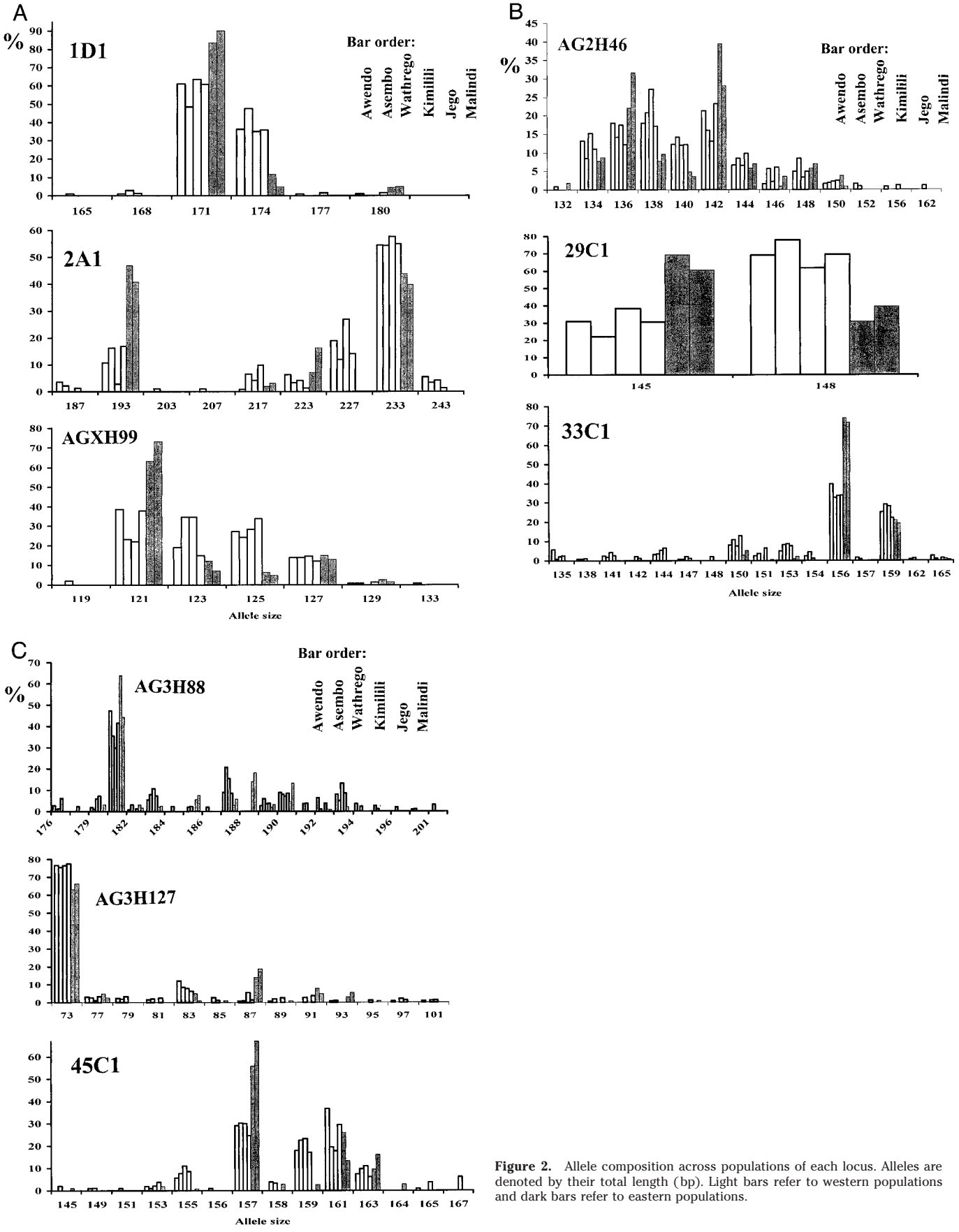

Figure 2. Allele composition across populations of each locus. Alleles are denoted by their total length (bp). Light bars refer to western populations and dark bars refer to eastern populations.

Table 2. Loci showing significant linkage disequilibrium in each population (individual test level of significance)

Awendo	Asembo	Wathrego	Kimilili	Jego	Malindi
33C1-29C1*	33C1-1D1**	33C1-1D1**	—	1D1-45C1*	29C1-2A1*
1D1-AGXH99*	AG2H46-AG3H88***	AG2H46-45C1**		AG2H46-45C1*	
1D1-AG3H127***	29C1-2A1*	33C1-AGXH99			
AG3H88-54C1*					

Table 3. Differences between western and eastern populations in genetic diversity (ANOVA results)

Dependent variable	Source	df	Mean square	p
Alleles	Model	10	63.73	.0001
R^2_{Model} = 86%	Error	43	2.51	—
	Locus[a]	8	69.83	.0001
	Sample size	1	6.2	.12
	Region	1	70.4	.0001
Unbiased H_E	Model	9	0.14	.0001
R^2_{Model} = 78%	Error	44	0.008	—
	Locus	8	0.14	.0001
	Region	1	0.19	.0001

[a] The factor "locus" is used as blocks.

icits, it should cause linkage disequilibrium between loci because members of the different subpopulations have different probabilities to carry certain combinations of alleles. But if null alleles caused the heterozygote deficits, linkage equilibrium is not expected, because all individuals are equally likely to carry a null allele, and the association between alleles from different loci is not disturbed. Thirteen significant linkage disequilibrium tests were found of 216 (6%), which is insignificant based on the binomial test ($p > .19$), and none of the tests was significant based on the sequential Bonferroni method. Loci which showed strong heterozygote deficits (*AG3H88* and *AG3H127*) were not more likely to be involved in a significant disequilibrium (Table 2), and populations with a higher number of significant heterozygote deficiencies did not have a higher number of significant tests showing disequilibrium ($r = -0.6$, $p > .17$, df = 4). These results provided further support that the heterozygote deficits were caused by null alleles and not by the Wahlund effect or inbreeding. Nevertheless, insignificant results my reflect the limited power of this test.

If the frequency of null alleles is the same across populations they will have a minimal effect on differentiation. But if their frequency varies between populations, they may contribute to differentiation. As variation in their frequency represents a component of differentiation, loci *AG3H88* and *AG3H127* were included in all subsequent analyses. However, results were recalculated excluding these loci to assess the influence of null alleles on the conclusions.

Genetic Diversity Within Populations

Genetic diversity within populations was summarized by the number of alleles observed and by the expected heterozygosity (H_E). Moderate to high levels of polymorphism were found in all loci across all populations (Table 1). Previous results indicated that the H_E in Jego (samples taken in 1987 and 1996) were lower than that in Asembo (samples taken in 1987 and 1994); differences that were part of the evidence for smaller N_e in Jego (Lehmann et al. 1998). We sampled multiple populations from each region to determine whether the differences were population specific or represented differences between the eastern and western regions. Both the number of alleles and H_E were significantly lower in the eastern populations (Table 3). The number of alleles depends on sample size, but because sample size did not vary much in this study, its effect was insignificant and the least-squares means are very similar to the unadjusted means (Table 1). Furthermore, higher numbers of alleles were found in western populations, where average sample sizes were lower. These results established that genetic diversity in eastern populations is lower than in western populations.

Differentiation Between Populations

Allele composition varied to a limited extent among populations within regions but varied considerably between regions (Figure 2). Differentiation between populations in the western region was very low, as indicated by an average F_{ST} value of 0.003 (Table 4). The average R_{ST} value was even lower (0.002; Table 4). These low values were insignificant by bootstrapping (over loci) tests. However, allele composition significantly differed among populations at four of the nine loci ($p < .001$, binomial multisample test) and significant R_{ST} values were found in two of the loci ($p < .009$, binomial multisample test). Low differentiation was also measured between populations in the eastern region (average F_{ST} values were 0.0077 and 0.0065; Table 4). The corresponding average R_{ST} value was the lowest (0.001; Table 4). Bootstrapping tests of the average values were insignificant (at the multitest level), but significant differences in allele distributions were measured in two loci, and one R_{ST} value was significant. These results showed marginal subdivision between populations up to 220 km apart, within both regions. Because the 95% CI of the mean differentiation indices in each region overlapped to a considerable extent, we concluded that this marginal within-region differentiation was similar in both regions.

In contrast to the low differentiation within regions, the values of F_{ST} and R_{ST} measuring differentiation between regions were 0.1 and 0.032, respectively. These values are more than 10 times higher than their corresponding within-region values (Table 4). Highly significant differences in allele distributions were detected across all loci and R_{ST} values were significant in all but two loci (Table 4). All the multisample tests showed a highly significant subdivision of the gene pool between regions.

The F_{ST} values calculated by Nei and Chesser (1983) and by Weir and Cockerham (1984) were nearly identical (Table 4; $r = 0.99$, df = 25). We used Nei and Chesser's method throughout subsequent analyses (see more below). Weaker correlation was found between F_{ST} and R_{ST} ($r = 0.64$, $p < .001$, df = 25). Although R_{ST} is thought to be a more sensitive index of differentiation for microsatellites (Slatkin 1995), F_{ST} values were approximately three times higher than corresponding R_{ST} values and the difference between these indices was highly significant ($p < .006$, df = 26, paired signed rank test, and Table 4).

The Unique Effect of the RVC on Differentiation

The results above demonstrate that populations were heterogeneous with respect to both N_e and the level of differentiation between them. Variation in N_e violates the island model, which assumes a large set of populations, all with identical demography and history. While the original formulation of F_{ST} and R_{ST} assumed the island model, Nei and Chesser (1983) relaxed some of these assumptions and explicitly accommodated populations with different N_e, provided that the relative size of the N_e values is known. We used Nei and Ches-

Table 4. Differentiation between populations within and between regions

	Within regions				Between regions: west vs. east pooled[a]					
	Western (4 populations)		Eastern (2 populations)		Unadjusted		Adjusted		Percent reduction	
Locus	F_{ST}[b]	R_{ST}[c]	F_{ST}	R_{ST}	F_{ST}	R_{ST}	F_{ST}[d]	R^{w}_{ST}[d]	F_{ST}	R_{ST}
1D1	0.0069^{ns} (0.0063)	−0.0026^{ns}	0.0126^{ns} (0.0131)	−0.0024^{ns}	0.1951*** (0.1815)	0.0141***	0.1741	0.0107	10.8	24.1
2A1	0.0058* (0.0020)	0.0076*	−0.0021^{ns} (−0.0026)	−0.0032^{ns}	0.0896*** (0.1046)	0.0964***	0.0843	0.0773	5.9	19.8
AGXH99	0.0163* (0.0163)	−0.0033^{ns}	0.0047^{ns} (0.0066)	0.0023^{ns}	0.1483*** (0.1498)	0.0426***	0.1360	0.0346	8.3	18.8
AG2H46	−0.0021^{ns} (−0.0023)	0.0015^{ns}	0.0049^{ns} (0.0051)	−0.0004^{ns}	0.0309*** (0.0306)	−0.0001^{ns}	0.0289	−0.0010	6.5	—
29C1	0.0098^{ns} (0.0095)	−0.0018^{ns}	0.0071^{ns} (0.0057)	0.0037^{ns}	0.2087*** (0.2090)	0.0695***	0.2013	0.0530	3.6	23.7
33C1	−0.0047^{ns} (−0.0037)	0.0057^{ns}	−0.0068^{ns} (−0.0067)	0.0003^{ns}	0.1074*** (0.1023)	0.0211***	0.0960	0.0145	10.6	31.3
AG3H88	0.0021*** (0.0052)	−0.0005^{ns}	0.0258* (0.0268)	0.0190*	0.0238*** (0.0398)	0.0064*	0.0218	0.0046	8.4	28.1
AG3H127	−0.0076^{ns} (−0.0057)	0.0001^{ns}	−0.0063^{ns} (−0.0064)	−0.0048^{ns}	0.0382*** (0.0390)	0.0350***	0.0374	0.0292	2.1	16.6
45C1	0.0032* (0.0030)	0.0101*	0.0183** (0.0273)	−0.0036^{ns}	0.0996*** (0.0964)	−0.0001^{ns}	0.0913	−0.0033	8.3	—
Mean[e]	0.0033^{ns} (0.0034)	0.0019^{ns}	0.0065^{ns} (0.0077)	0.0012^{ns}	0.1041*** (0.1059)	0.0317***	0.0968***	0.0244***	7.2***	23.2***
U 95% CI[f]	0.0077 (0.0079)	0.0048	0.0128 (0.0162)	0.0058	0.1468 (0.1457)	0.0537	0.1351	0.0408	8.9	26.7
L 95% CI[f]	−0.0007 (−0.0014)	−0.0009	0.0001 (0.0002)	−0.0023	0.0633 (0.0682)	0.0124	0.0594	0.0090	5.3	19.8

[a] Populations within a region were pooled.

[b] F_{ST} (unweighted) calculated according to Nei and Chesser (1983). F_{ST} in parentheses are calculated according to Weir and Cockerham (1984). Significance level for individual loci is based on exact test of homogeneity of allele frequency.

[c] Significance level for individual loci R_{ST}'s is based on a nested ANOVA on allele size including both the individual and population as factors.

[d] Weighted F_{ST} calculated according to Nei and Chesser (1983). Weighted R_{ST}, R^{w}_{ST} was calculated by weighted ANOVA (see text for details).

[e] Simple mean over loci. Significance level is determined by bootstrapping over loci (5,000 replications) and adjusted to the number of tests performed, that is, three tests (one test for each region).

[f] The 2.5 and 97.5 percentiles of the bootstrap generated distribution (unadjusted to the number of tests performed).

ser's formulation, weighting populations according to their N_e ratio. We followed Nei and Chesser's approach and calculated a weighted R_{ST} (R_{ST}^{w}) using a weighted ANOVA to obtain weighted variance estimates (MS_{tot} and MS_{with}) instead of the "unweighted" estimates.

Current (deme) N_e is the size of an idealized population which would give rise to the same variance in gene frequency observed in the actual population under consideration. Based on temporal variation in allele frequencies, deme N_e values were estimated in Asembo and in Jego and their N_e ratio was 1.6:1 (Lehmann et al. 1998). Estimates of $N\mu$ (the product of N_e and the mutation rate), calculated based on the average H_E and the number of alleles and sample size (Table 1), support that the effective population size in the western region is higher than that in the eastern region. However, such estimates are sensitive to departures of populations from mutation-drift equilibrium, to incorrect mutation process (see below), to low selection effects, and especially to low migration between regions. Because the N_e ratio based on temporal variation in allele frequencies is a more robust estimate, we used it to test the unique role of the RVC on differentiation.

The contribution of the variation in N_e to the overall differentiation across the RVC was calculated as the difference between the unadjusted and adjusted (for the variation in N_e among populations) index of differentiation, divided by the unadjusted index (Table 4). Approximately 7% of the overall differentiation (unadjusted) measured by F_{ST} was contributed by the variation in N_e among populations. The corresponding value for R_{ST} was 23% (Tables 4 and 5).

The contribution of distance to the overall differentiation across the RVC was evaluated assuming the isolation by distance model (Slatkin 1994; Wright 1951). This model quantifies the effect of distance on differentiation and allows prediction of the differentiation between populations given the distance between them. Using population pairs located *within* each side of the RVC, we predicted the differentiation across it, assuming that migration is equally likely in all directions in two-dimensional space and that distance is the main determinant of differentiation. The difference between the observed differentiation (adjusted for the variation in N_e) and that predicted by distance represents the unique contribution of the RVC to the overall differentiation. Because the distance across the RVC (700 km) is larger than the distance between any pair of populations sampled on each side of the RVC (up to 220 km between Awendo and Kimilili), an extrapolation was required. To avoid extrapolation, we included a population from Senegal located approximately 6000 km west of Asembo in the western region that had similar genetic diversity to other populations in the western region [see Lehmann et al. (1996) for a description of this population]. The Mantel test was used to assess the significance of the effect of distance on differentiation, and bootstrapping based on residual resampling (Mooney and Duval 1993) was used to determine the 95% CI of the predicted F_{ST} and R_{ST} values across the RVC.

Differentiation measured by both F_{ST} and R_{ST} increased with distance ($p < .001$, Mantel tests, and Figure 3). The expected value of F_{ST} for populations 700 km apart was 0.0100 and the 95% CI was 0.0065–0.0138. The corresponding estimate of R_{ST} was 0.0096 and its 95% CI was 0.0072–0.0124. The observed F_{ST} and R_{ST} averages (adjusted for the variation in N_e) were higher than the upper 95% CI limits of the expected values due to distance (Figure 3). The 95% CI of the observed (adjusted) F_{ST} mean did not overlap with the 95% CI of the value predicted by distance, dem-

Table 5. Partition of the mean overall differentiation across the RVC to its contributing factors

		Factors contributing to the overall differentiation		
	Overall (unadjusted)	Variation in N_e	Distance 700 km	Net effect of the RVC
F_{ST}	0.1041	0.0075	0.0100	0.0866
Percent	100	7.2	9.6	83.2
R_{ST}	0.0317	0.0074	0.0096	0.0147
Percent	100	23.2	30.3	46.5

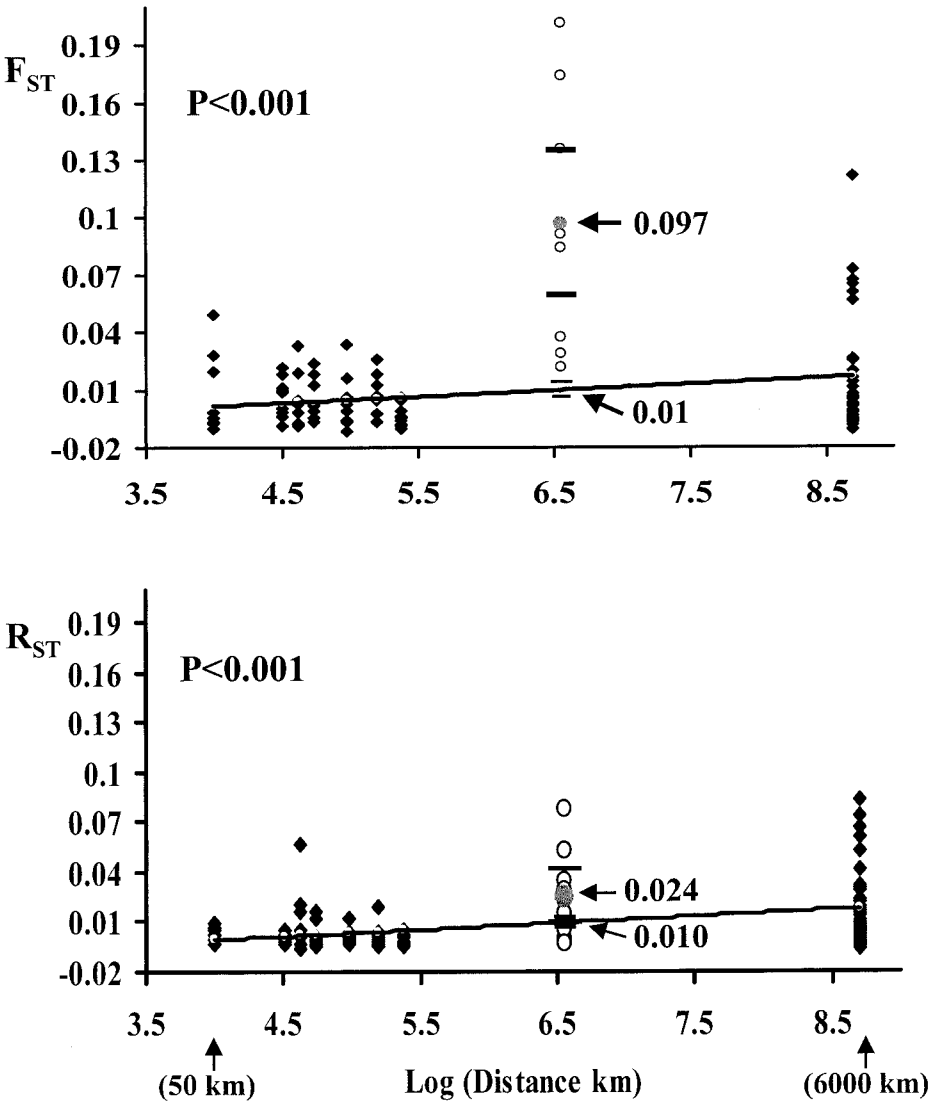

Figure 3. The effect of distance on differentiation between populations. The regression line was calculated based on F_{ST} (upper panel) and R_{ST} (lower panel) for all pairs of populations *within* the region at individual loci (closed diamonds) and tested using the Mantel test (see text for details). Differentiation indices between regions of all loci were adjusted to the variation in N_e (open circles). The observed average differentiation between regions is shown by a closed circle with their values (see arrow) and the predicted value on the regression line marked with an arrow. The 95% CI of the observed (thick lines) and the predicted (thin lines) means across 700 km were calculated using bootstrapping. Note that the 95% CIs do not overlap in the case of F_{ST}, but do overlap in the case of R_{ST}.

onstrating high significance for the effect of the RVC (Figure 3). However, these CI intervals overlapped for the R_{ST} (Figure 3). Testing the effect of the RVC on differentiation by R_{ST} using a one side test (i.e., the effect of the RVC is greater than that predicted by distance) showed that the effect is significant only at the 10% level. Partitioning of the differentiation measured by F_{ST}, therefore, established the role of the RVC as a geographical barrier to gene flow, whereas a similar, but insignificant trend was found using R_{ST}. The net effect of the RVC on differentiation after accounting for the effects of distance and the variation in N_e between populations is equivalent to an F_{ST} of 0.0866 and to an R_{ST} of 0.0147 (Table 5). Differentiation due to the RVC as measure by F_{ST} was 6 times higher than that measured by R_{ST}, and its relative contribution was 80% and 50% based on F_{ST} and R_{ST}, respectively. The results demonstrated substantial differences in the sensitivity of F_{ST} and R_{ST} to measure the differentiation of these populations.

Discussion

The RVC comprises areas that do not support *A. gambiae* populations, including the arid valley itself, its flanking highlands, and probably the dry savanna stretching between the highland and the narrow coastal plain along the Indian Ocean. Our collection records concur with this account as *A. gambiae* was not found in Embu, Chegoria, Thika, Wundanyi, Voi, and Taveta (Figure 1), while it was abundant at all other sites. As the total width of the RVC spreads across 400–500 km, distance contributes to differentiation across it. Populations within region, over 200 km apart, were marginally differentiated, in agreement with previous studies, indicating that distance has only a weak effect on gene flow (Besansky et al. 1997; Kamau et al. 1998; Lehmann et al. 1996, 1997). However, differentiation across the RVC was 10- to 40-fold larger and highly significant. In addition, variation in N_e (Lehmann et al. 1998) may have further increased the estimate of differentiation across the RVC. Partitioning the differentiation among these factors strongly suggested that the RVC is a geographical barrier to gene flow.

Nine polymorphic loci spread throughout the genome (outside polymorphic inversions) ensured high resolution in measuring differentiation and that locus-specific effects (selection) would not dominate the pattern of variation. However, sampling a larger number of *A. gambiae* populations from the eastern region spanning 600–900 km would strengthen our interpretation. Significant heterozygote deficits, probably caused by null alleles, did not affect these results as exclusion of loci *AG3H88* and *AG3H127* did not change the trends in the results or their statistical significance (data not shown).

Genetic diversity, measured by both H_E and the number of alleles, was significantly higher in the western region, corroborating that deme and long-term N_e of a population from Asembo was higher than that of Jego (Lehmann et al. 1998). The present results showed that this difference is region specific and not population specific. The possibility that continental populations differ in their N_e is usually ignored in studies of population structure because reliable estimates of their N_e ratio are rarely available. Nonetheless, variation in N_e indicates ecological and/or historical heterogeneity between populations, and if overlooked may result in erroneous estimates of gene flow and divergence time. The larger the differences in N_e among populations, the worse its effect on such inferences. To test the unique role of the RVC as a barrier to gene flow we used the N_e ratio based on temporal variation in allele frequencies (Lehmann et al. 1998). The temporal method, however, refers to the series of generations between sam-

pling time points and ignores population sizes preceding the first sampling point. Consequently a bottleneck that occurred a short period prior to the first time point will not be detected despite its potential effect on differentiation. If population experienced a recent bottleneck, its expected heterozygosity under equilibrium (H_{eq}) based on the number of alleles and sample size is expected to be lower than its expected heterozygosity based on allele frequencies (H_E; Table 1), because allele number is reduced faster by bottleneck than expected heterozygosity (Cornuet and Luikart 1996). These parameters are expected to be the same in populations at equilibrium, provided that the correct mutation model is used. The difference between these quantities was tested using the BOTTLENECK software (Cornuet and Luikart 1996). The two-phase mutation model (TPM) is considered as the most suitable model for microsatellites (e.g., Cornuet and Luikart 1996; Di Rienzo et al. 1994; Jarne and Lagoda 1996), hence we calculated H_{eq} based on the TPM with fractions of multistep mutations set to 30%, 20%, and 10%, and also based on IAM (equivalent to TPM with a frequency of multistep mutations of 100%) and SMM (equivalent to TPM with a frequency of multistep mutations of 0%). Insignificant deviations were found under the IAM and the TPM with 30% and 20% multistep mutations. Only Jego was significant ($p = .027$, single-test level) under the TPM with the 10% multistep mutation, whereas Jego, Malindi, Asembo, and Awendo significantly departed from the SMM H_{eq} ($.05 > p > .014$; Wilcoxon test). All significant deviations, however, were associated with H_E less than H_{eq}, indicating that they were not caused by a recent bottleneck but that the strict SMM is an inadequate model for these loci. These results showed no obvious violation of equilibrium and therefore suggested no recent bottleneck.

While R_{ST} was expected to be a more-sensitive measure of differentiation for microsatellites (e.g., Kimmel et al. 1996; Slatkin 1995), the overall differentiation measured by R_{ST} was only a third of that measured by F_{ST} (0.032 versus 0.104; Tables 4 and 5). Lower sensitivity of R_{ST} in comparison to F_{ST} was reported in humans by Perez-Lezaun et al. (1997), in bears by Paetkau et al. (1997), in *A. gambiae* by Lehmann et al. (1998), and in *A. arabiensis* by M. Donnelli and others (unpublished data). R_{ST} was especially designed to capture the effect of high mutation rate under the stepwise mutation model, which is a function of time and degree of isolation (Slatkin 1995). The lower sensitivity of R_{ST} suggests that differentiation between regions was primarily generated by drift and not by mutation. Further evidence for the effect of drift is that there were no unique alleles (with frequency greater than 5%; Figure 2) in eastern populations but only in western populations. Why then is mutation effect not detected? We considered the following hypotheses: (1) mutation rate in these loci is too low compared with drift, and (2) constraints on allele size arrest allele distributions. The upper bound mutation rate in dinucleotide repeats of *A. gambiae* was estimated as 3.4×10^{-5} [Lehmann et al. (1998) based on previous data; Zheng et al. (1996)]. This is not an unusually low rate since a lower mutation rate of 6.3×10^{-6} was measured in *Drosophila melanogaster* (Schug et al. 1997). Because no other estimates are available for dipteran insects, it is reasonable to assume that the average mutation rate in *A. gambiae* microsatellites is around 10^{-5} but not much higher. Temporal variation in allele frequency due to drift is expected to be $1/(2N_e)$ per generation (Waples 1991), which was estimated as 10^{-4} (Lehmann et al. 1998). Under these conditions, drift effect would be nearly 10 times stronger than mutations. Drift would also have a greater effect than mutation if populations became separated only recently or if eastern populations passed a recent bottleneck (but see above). Alternatively, constraints on allele size that bound the range of allele size would also minimize the role of mutation. However, the effect of constraints depends on high mutation rate (e.g., Nauata and Weissing 1996), which is not supported by the available data.

The index of effective migration, N_m, based on F_{ST} ($N_m = 2.9$) and R_{ST} ($N_m = 11.1$), is greater than 1. Although these values should be considered cautiously, they suggest that the RVC is a partial barrier to gene flow. Migration across the RVC is likely to take place through southern Tanzania or even further south, where the topography of the eastern Rift Valley and its flanking highlands becomes milder and there is moist savanna and woodland, which can support *A. gambiae* populations (Kingdom 1989, pp. 14–20). From this area northward on the eastern side of the RVC, only the narrow coastal plain along the Indian Ocean is a suitable habitat for *A. gambiae*. The coastal plain, flanked by dry savanna and the Indian Ocean, extends like a finger northward and migration between populations in this region probably occurs in a linear dimension. Linear migration and a smaller N_e would result in higher differentiation between populations in the eastern region of the RVC than between populations separated by the same distance in the western region. The observed pattern (Table 4) is consistent with this explanation, but additional samples are required to test it. Other regions that do not support *A. gambiae* populations need to be identified and their effect on gene flow evaluated.

The strong effect of the RVC on gene flow despite extensive human traffic (trains, airplanes, and cars) crossing it implies that transportation of *A. gambiae* by human activity (passive dispersal) is rather low. This conclusion negates our previous explanation that the high gene exchange across the continent is mediated by passive dispersal (Lehmann et al. 1996). A similar argument is valid against the possibility that *A. gambiae* dispersal is made by long one-step movements such as those made by some spiders and small day-flying insects aided by wind (Service 1997). Migration seems to be a multistep process, where the step length is rather short, possibly shorter than 100 km. This view agrees with that of Soper and Wilson (1943), mapping the expansion of *A. gambiae* s.l. after its introduction into Brazil, and with direct mark-release-recapture studies (Constantini et al. 1996; Gillies 1961). Indeed, with the exception of the RVC, the distribution of suitable habitats for *A. gambiae* (as can be inferred based on maps of vegetation and climate) is more or less continuous throughout its range, or at least it is maintained through some corridors of suitable habitat.

In conclusion, we attempted to quantify the unique effect of the RVC on the gene flow of *A. gambiae*. We compared the level of differentiation between populations on each side of the RVC and across it and used weighted analyses of F and R statistics to assess differentiation between populations that evidently differed in their N_e. Using an isolation by distance model for within-region populations, we predicted the effect of distance on differentiation across the RVC and then partialed it out from the overall effect of the RVC. After removal of the effects of the difference in N_e and distance on differentiation, the remainder, attributed to the unique effect of the RVC, was significant when measured by F_{ST}. Contrary to expectation, F_{ST} values were substantially higher than those of R_{ST}, suggesting that drift but not mutation is the main force that generated this dif-

ferentiation. The weak effect of mutation was ascribed to a relatively low mutation rate in microsatellites of *A. gambiae*. The restricted gene exchange across several hundred kilometers in spite of extensive human transportation implies that active mosquito dispersal is the major form of migration and that migration is a multistep process where step length is relatively short.

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