Microgeographic differentiation in Anopheles gambiae from seven villages less than 10 km apart in Asembo Bay, western Kenya was estimated by analysis of variability in seven microsatellite loci. Results from the Asembo Bay villages were compared with specimens collected in Kilifi, coastal Kenya, 700 km to the east. Allele frequency distribution was very similar in all villages in Asembo Bay, but differed for the Kilifi population. Genetic differentiation among villages was low with loci-specific Fst falling within the range of 0.0000-0.0085. These low estimates of differentiation correspond to among-village migration indices greater than 5.66, suggesting a high level of gene flow within the Asembo population. The Nm value between Kilifi and Asembo Bay was 1.54, indicating much lower levels of gene flow. Average observed heterozygosity among the seven villages was in all but one case less than the expected heterozygosity, most likely indicating the presence of null alleles, but possibly the presence of randomly mating units (demes) smaller than the village. We conclude that there is likely no genetic structure at the level of the village in Asembo Bay but that gene flow is restricted between western and coastal Kenya, probably by the high elevation rift.