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Spatial and Temporal Genetic Structure of *Anopheles arabiensis* in Southern Zambia over Consecutive Wet and Drought Years

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Abstract

No studies have addressed the spatial complexity of *Anopheles arabiensis* populations in Zambia or the effects of drought on the genetic structure of this species. We genotyped approximately 420 *An. arabiensis* at 12 microsatellite loci representing 18 collections from the Southern Province of Zambia. Collections spanned three transmission seasons and covered a wet year–drought year–wet year cycle. *Anopheles arabiensis* within the 2,000 km² of the Macha study region were panmictic, with high gene flow between Macha and Namwala, Zambia, which are 80 km apart. There was little evidence for genetic structuring among years, with no significant shifts in allele frequency distributions or observed heterozygosity, and no evidence for a genetic bottleneck despite a drastic reduction in mosquito numbers during the drought year. *Anopheles arabiensis* in southern Zambia has a large deme size, and the regional genetic structure of this species was little affected by an extended drought period.

INTRODUCTION

Anopheles gambiae s.s. Giles and *An. arabiensis* Patton are two of the most widespread and important malaria vectors in Africa.^{1,2} The need to understand the population structure of these vectors has generated increasing interest towards the interpretation of ecologic and biologic events in a genetic context. For example, data generated by population genetic studies on *An. gambiae* s.s. or *An. arabiensis* can be directly applied towards monitoring and predicting the spread of genetic elements conferring insecticide resistance^{3–5} or the spread of transgenes potentially introduced as control strategies for vector populations.^{6,7} Genetic studies on *An. gambiae* s.s. in west Africa have uncovered extraordinary intraspecific genetic complexity; this species represents not one homogenous gene pool but is comprised of five discrete chromosomal forms.^{8,9} In addition to cytogenetics, microsatellite markers

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Note: Supplementary Table 1, Observed and Expected Heterozygosity for *Anopheles arabiensis* Collections in Macha, Zambia, appears online at www.ajtmh.org.

developed for *An. gambiae* s.s.^{10,11} have been integral to studies aimed at understanding the genetic structure and patterns of gene exchange among geographically and seasonally diverse mosquito populations.^{8,12–15} More recently, microsatellites identified in *An. gambiae* have been applied with varying degrees of success in studies targeting *An. arabiensis*.^{15–22} Although relatively less studied, *An. arabiensis* serves as a major, and sometimes the primary, vector of *Plasmodium falciparum* throughout much of sub-Saharan Africa.^{23–25}

Much less structure is evident among and within *An. arabiensis* populations compared with its sister taxon, *An. gambiae* s.s. However, there is evidence of genetic differentiation within this species according to habitat cline, topography, and geographic distance across Africa.^{17–22} In general, deme sizes of *An. arabiensis* appear to be large, with little genetic differentiation among mosquito collections separated by less than 200–250 km.^{15–17,21} Given the limited flight range observed in *An. arabiensis*,^{26,27} studies have attributed the findings of high gene flow between widely separated mosquito collections to large effective population sizes and/or recent range expansion rather than extensive contemporary gene flow or mass migration.^{15–17} In comparison, Donnelly and Townson¹⁷ found extensive differentiation among collections of *An. arabiensis* separated by > 200 km along a north-south transect in east Africa. Onyabe and Conn²² also reported significant population structuring along a cline of ecologic zones in Nigeria. These studies demonstrate that geographic distance as well as changes in habitat contribute to the differentiation observed among *An. arabiensis* from different locations. These findings of genetic differentiation across Africa are consistent with observations of differential blood host preference and resting behavior in *An. arabiensis* from east Africa, west Africa, and Madagascar.^{25,28–30} In some instances, landforms such as mountainous terrain and islands have been noted to limit genetic exchange between *An. arabiensis* populations from geographically similar locations.^{17,18,20}

Apart from physical distance and topography influencing population structure, climate can exert a significant selective pressure on the genetic make-up of a mosquito population. Although microsatellites are characteristically neutral to selection, chromosome inversions present in the *An. gambiae* complex such as 2Rbc and 2La were more commonly observed in mosquitoes inhabiting arid versus wet climates in Nigeria.³¹ Similarly in Mali, the Mopti chromosomal form of *An. gambiae* s.s. was more abundant during the dry season and early rainy season whereas the Bamako and Savanna forms predominated during the late rainy season.³² Inversion 2Rbc varied in frequency from nearly 90% during the dry season to 30% in the wet season.³² Thus, shifts in microsatellite allelic diversity and frequency may be evident at loci located within chromosomal inversions that are subject to selection.

The Southern Province of Zambia experiences hyperendemic transmission of *P. falciparum* by both *An. arabiensis* and *An. funestus* s.s. Giles in the apparent absence of *An. gambiae* s.s.³³ To date, no studies have examined the genetic structure of *An. arabiensis* populations within Zambia, and until recently, no studies had been published on the entomologic parameters of malaria transmission in Zambia in more than 25 years.²⁵ Characterizing the population structure of mosquitoes in this region would help guide future malaria research and control efforts.

In conjunction with studies on seasonal malaria transmission dynamics in this region, our specific aims were to 1) evaluate additional microsatellite markers for use in *An. arabiensis*, 2) characterize the genetic structure of *An. arabiensis* collected throughout the 2000-km² Macha region in the Southern Province of Zambia to determine any barriers to gene flow, and 3) examine the data for evidence of genetic drift across a wet year–drought year–wet year cycle. We hypothesized that *An. arabiensis* in this study area would represent one panmictic population given no obvious topo-graphic barriers that might restrict gene flow. However, highly focal transmission intensity recorded among closely situated villages (5 km)²⁵ suggested minimal movement of mosquitoes between villages in this region. Considering temporal fluctuations in the mosquito population density, we also sought to determine whether drought conditions extending throughout the 2004–2005 rainy season resulted in a genetic bottleneck in *An. arabiensis*.

MATERIALS AND METHODS

Study area

The Johns Hopkins Malaria Research Institute's field station in Macha, Zambia is located in the Southern Province at an elevation approximately 1,000 meters above sea level. The habitat is characterized as miombo woodland. Average annual rainfall varies greatly in Zambia, but averages 600–1,000 mm (24–40 inches) in the southern parts of the country.² There is one rainy season each year that lasts from approximately November to April, followed by cool dry (April–August), and hot dry (August–November) seasons. During 2004–2005, mosquito activity was substantially reduced because of an extended regional drought.²⁵ Total rainfall in Macha between November 2004 and May 2005 was 457mm (18 inches) and concentrated at the beginning of the season; total rainfall between November 2005 and May 2006 was 915 mm (36 inches). The dry conditions during the 2004–2005 season coupled with temperatures averaging more than 25°C between December 2004 and March 2005 resulted in 40–90% loss of the maize crop and scarce water availability in the Southern Province during 2004–2005.³⁴ Namwala, Zambia is located approximately 80 km northwest of Macha on the Kafue River delta and was not as severely affected by the drought.

Mosquito collection and handling

To aid in the random selection of collection sites, the greater Macha region (2,000 km²) was divided into 5 km × 5 km grids (Figure 1). *Anopheles arabiensis* mosquitoes were collected by pyrethrum spray catch³⁰ from 15–20 representative houses in each of 20 randomly-selected grids. Spray catches were executed with locally purchased DOOM Super® (Adcock Ingram Ltd., Bryanston, South Africa) combination synthetic pyrethroid (D-phenothrin, 0.92g/kg; prallethrin, 0.4 g/kg; and imiprothrin, 0.25 g/kg). Two or three village areas were sampled per day over a period of two weeks during early (November 2004 and 2005), middle (January 2005 and 2006), and late (March 2005 and 2006) rainy season. Additionally, preliminary collections were obtained by spray catch from 10 village areas during the 2003–2004 rainy season. All spray catches were performed between 7:00 AM and 10:00 AM. The houses selected varied throughout the course of the season. Mosquitoes in Namwala were collected in CDC light traps³⁵ in February 2006 as part of a preliminary

sampling effort for an independent project. Immediately after collection, specimens were killed by freezing, morphologically identified,^{1,36} and packed individually in tubes containing silica gel desiccant (J. T. Baker, Phillipsburg, NJ) and cotton and stored at room temperature. Mosquito samples included in genetic analysis are listed in Table 1.

DNA preparation and polymerase chain reaction (PCR)

DNA was extracted from mosquitoes by a modified salt procedure as described.³⁷ Prior to homogenization, dry field specimens were rehydrated at room temperature in 20 μ L of double-distilled H₂O for 20 minutes. The relative quality of all DNA extractions was checked by PCR amplification of a fragment of the mitochondrial NADH dehydrogenase subunit 4 (ND4) using arthropod-specific primers.³⁸ The identity of all specimens morphologically identified as *An. gambiae* s.l. was molecularly confirmed by PCR.³⁹ All DNA amplifications were completed on a MJ Research® PTC-200 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA) and visualized on 2% agarose gels stained with ethidium bromide. All gels were run with a GeneRuler 100-basepair DNA ladder (Fermentas Life Science, Hanover, MD).

Microsatellite amplification

Twenty microsatellite loci originally identified in *An. gambiae* s.s. were evaluated, and 12 were ultimately optimized for use with *An. arabiensis* (Table 2). Loci were used if they amplified successfully from all samples and were polymorphic (contained greater than one allele). Selected loci spanned chromosomes 2 and 3 and included microsatellites presumed to be located both within and outside inversions. Four hundred twenty-two specimens representing 19 collections were genotyped at all 12 micro-satellite loci. All PCRs were performed in 20- μ L reaction volumes containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 1.0 mM dNTPs, 1.0 units of *Taq* polymerase, and 25 pmol each of forward and reverse primer. An initial denaturation of 2 minutes at 95°C was followed by 29 cycles of 94°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The final 72°C extension step was 1 hour. The forward primer in each reaction was labeled with a fluorescent marker (FAM, TET, or HEX) compatible with ABI PRISM (Perkin-Elmer, Norwalk, CT) capillary electrophoresis. Single locus PCR products were mixed for multiplexed analysis in the following combinations: 119/750/59, 788/249/ 143, 93/128/746, and 95/79/811.

Statistical analysis

Multiplexed PCR products were evaluated on an ABIPrism 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA) and data analyzed using GeneScan and GenoTyper Fragment Analysis software packages to derive microsatellite genotypes and allele sizes (Applied Biosystems). Arlequin version 2.0⁴⁰ was used to calculate observed (H_o) and expected heterozygosity (H_e), allele frequencies, test for compliance to Hardy-Weinberg equilibrium (HWE), and estimate F_{ST} ⁴¹ and N_m ⁴² values. Departures from HWE at each locus were evaluated using the Markov chain algorithm of Guo and Thompson⁴³ with 100,000 steps in the Markov chain and 1,000 dememorization steps. The proportion of observed heterozygote deficiencies (D), the frequencies of null alleles explaining those

deficiencies (r), and the expected number of individuals homozygous for a null allele for each collection (F) were calculated according to the methods of Chakraborty and others.⁴⁴ Linkage equilibrium for all pairs of loci was evaluated by exact test.⁴⁵

To avoid artifacts caused by the Wahlund effect,⁴⁶ collections were partitioned by both year and grid location, as listed in Table 1. The initial 12 loci were reduced to a set of nine, for which at least 15 of 19 of the collections were in compliance with HWE after Bonferroni correction. Collections were evaluated for genetic structuring across all nine loci, or for loci only on chromosome 2 or 3, or for loci located either inside or outside of inversions. *Anopheles arabiensis* from Namwala, Zambia were included as a geographic outgroup, and *An. quadriannulatus* (Theobald) served as a sympatric, taxonomic outgroup because *An. gambiae* s.s. Giles is not present in this area.²⁵ Structural analysis of molecular variance (AMOVA)⁴¹ was performed on Macha *An. arabiensis* collections grouped by year for each scenario (all loci, by chromosome, by inversion) to test the hypothesis that there is no difference in genotype frequency among collections between wet and drought years.

Analyses were performed with all individuals listed in Table 1, as well as a random sample of 32 mosquitoes (from 2 or 3 randomly selected collections) from each year to reduce sampling bias. Allele frequency distributions for loci located outside inversions, inside fixed inversions and inside polymorphic inversions and for Macha *An. arabiensis* were analyzed by the Mann-Whitney U test to test the hypothesis that allele frequency distributions at each locus were the same between collections from 2004 through 2005, 2005 through 2006, and 2004 through 2006. Observed heterozygosity for Macha *An. arabiensis* was similarly analyzed by the Mann-Whitney U test to test the hypothesis that heterozygosity was the same between collections from 2004 through 2005, 2005 through 2006, and 2004 through 2006. To evaluate temporal fluctuations in gene flow, Macha collections (excluding *An. quadriannulatus*) from the same year were pooled after confirmation of infinite gene flow among collections on a spatial scale.

Pooled collections within each year were analyzed as above, as well as evaluated for evidence of a genetic bottleneck using the software Bottleneck.⁴⁷ Bottleneck compares the observed heterozygosity against the expected equilibrium heterozygosity at each locus based on the number of alleles and sample size to determine if there is a gene diversity excess or deficit.⁴⁷ Populations that have recently undergone a genetic bottleneck should display excess heterozygosity as compared with what would be expected for populations under mutation-drift equilibrium. The same set of 9 loci ultimately used in the genetic analysis were evaluated for excess heterozygosity by the sign test and the Wilcoxon sign-rank test using 1,000 permutations.⁴⁷ Calculations were performed under the Infinite Alleles Model (IAM), Stepwise Mutation Model (SMM), and Two-Phased Model of Mutation (TPM) with 85% of SMM in TPM.

RESULTS

Hardy-Weinberg equilibrium and linkage disequilibrium

Approximately 420 *An. arabiensis* from Macha and Namwala, Zambia, and 15 *An. quadriannulatus* from Macha were geno-typed at 12 microsatellite loci (Tables and 2).

Microsatellite loci that were tested but either did not amplify well or gave ambiguous results were AG2H802, AG3H817, AGXH412, AG3H83, AG2H26, AG3H311, AG3H544, and AG2H637. Of the 228 tests (12 loci $\times$ 19 populations) for conformance to HWE at the locus level within populations, 17 tests (7.46%) showed significant deviation from HWE after Bonferroni correction for multiple comparisons. These deviations were predominantly due to loci AG3H746 and AG2H788. The expected frequency of null alleles for these two loci ranged from 0.13 to 1.00 for AG2H788 and from 0.01 to 0.32 for AG2H746. Therefore, these two loci were excluded from all further analyses. AG2H79 was either significant or borderline significant in seven populations, and was also excluded. In exact tests for linkage disequilibrium, only 8 (1.16%) of 684 comparisons were significant after Bonferroni correction.

Observed proportional heterozygote deficiencies, the expected frequency of null alleles, and the estimated number of mosquitoes homozygous for a null allele in each collection were all very low for the nine analyzed loci (Supplementary Table 1). The expected frequency of null alleles was generally less for these microsatellite loci in *An. arabiensis* than for other loci previously examined in *An. arabiensis*.^{21,22} These parameters were also similar between *An. arabiensis* and *An. quadriannulatus* in this study. A complete table of observed and expected heterozygosity and null allele data for the nine loci in all 19 populations can be found in the supplementary data (Supplementary Table 1).

Spatial analysis of genetic differentiation

As expected, *An. arabiensis* populations sampled throughout the Macha region and between Macha and Namwala were panmictic (Table 3). There was infinite gene flow among the 18 collections across all nine analyzed loci (Table 3). There was modest structuring among collections when loci on chromosome 2 or inside inversions were analyzed as compared with loci on chromosome 3 or outside inversions. However, gene flow remained high and these observations were not significant. Two *An. arabiensis* collections were consistently identified as having reduced gene flow relative to all others. These two collections, E02_2006 and E06_2005, accounted for the low N_m values estimated for these population comparisons. For the analyses of chromosome 2 and inside inversions, collection E02_2006 had reduced gene flow, and for chromosome 3 and outside inversions, collection E06_2005 had reduced gene flow relative to the other collections. It is unclear whether there is a biologic explanation for these unexpected observations, or if they represent technical anomalies.

As expected, F_{ST} values were significant ($P < 0.0003$) between Macha *An. arabiensis* and *An. quadriannulatus* on chromosome 2 (Table 3). Gene flow between *An. quadriannulatus* and *An. arabiensis* was reduced to 1.7–9.3 migrants per generation when considering only loci on chromosome 2 or to 4.2–86.6 migrants per generation for loci inside inversions. *Anopheles quadriannulatus* contained 14 unique alleles in seven loci compared with more than 400 specimens of *An. arabiensis*. These were AG2H95: alleles 108 and 128; AG3H119: 194 and 216; AG3H128: 96 and 82; AG2H143: 149 and 147; AG3H750: 76 and 78; AG2H788: 67, 71, and 75; and AG3H746: 83.

Temporal analysis of genetic differentiation

Examinations for population differentiation across wet and dry transmission seasons used three strategies. First, because there was infinite gene flow among collections throughout the Macha region on a spatial scale, collections from the same year were pooled to analyze genetic structure and gene flow among Macha *An. arabiensis* from year to year. There were modest fluctuations in gene flow observed from year to year when data were analyzed by chromosome or inversion status, although N_m values were still high (Table 3). Second, an AMOVA was performed on all as well as a random set of 32 mosquitoes from each year. Data were structured by both collection grid and year. All of the observed variation was within groups (years); there was no significant structuring evident among *An. arabiensis* collections from year to year. Mann-Whitney U tests on allele frequency distributions showed no significant shifts in allele frequencies between wet and drought years for loci located outside inversions, inside fixed inversions, or inside polymorphic inversions ($P > 0.05$). Observed heterozygosity also did not fluctuate significantly between wet and drought years ($P > 0.05$).

Finally, pooled collections from each year were analyzed for evidence of a genetic bottleneck at each locus and across all loci. Despite some isolated locus-specific events of excess heterozygosity, there was no significant evidence of a genetic bottleneck in *An. arabiensis* collections from any of the three years under any of the mutation models. None of the years contained significant excess heterozygosity by the sign test for any of the mutation models ($P > 0.05$). Heterozygosity for each of the three years conformed to an L-shaped distribution typical of populations in mutation-drift equilibrium.

DISCUSSION

We examined the spatial and temporal genetic structuring of *An. arabiensis* in Macha, Zambia. Of the 20 loci initially selected, we obtained consistent heterologous amplification of alleles that were easily scored in 12 of these loci. Unlike Donnelly and others,¹⁶ we had good success with AG3H93 and AH3H249. These observations suggest that the utility of some loci in *An. arabiensis* may vary from region to region due to intrinsic variations in priming site sequence among different mosquito populations. Such sequence divergences are expected when locus-specific primers designed for one species are applied to a closely-related species.^{17,48}

As expected, significant genetic differentiation was evident between *An. arabiensis* and *An. quadriannulatus* on chromosome 2. However, estimates of gene flow among our collections of *An. arabiensis* and *An. quadriannulatus* were high compared with previously reported gene flow estimates between sibling species in the *An. gambiae* complex.^{8,14,22} Infinite gene flow between *An. arabiensis* and *An. quadriannulatus* is biologically implausible because these species are considered to be largely reproductively isolated taxa. Hybrid specimens of *An. arabiensis* × *An. quadriannulatus* in nature have been documented from Zimbabwe, although at a frequency < 0.1% of the total sample.⁴⁹ High estimates of gene flow between *An. arabiensis* and *An. quadriannulatus* are not necessarily the result of contemporary introgression, but may reflect either shared ancestral alleles or convergence back to shared alleles due to limitations in the allele model. Although gene flow between these two species

was unexpectedly high, there were several unique alleles identified in *An. quadriannulatus* in seven of the 12 loci, which is indicative of reproductive isolation and speciation. Speciation of members of the *An. gambiae* complex is thought to be relatively recent, as demonstrated by shared ancestral ND5 haplotypes between *An. gambiae* s.s. and *An. arabiensis*.⁵⁰ Large numbers of shared mitochondrial haplotypes have also been documented between *An. gambiae* s.s. and *An. bwambe*.⁵¹ Still, we observed only slightly less amplification success of alleles in *An. quadriannulatus* relative to *An. arabiensis* across the set of nine loci, which extended the usefulness of these loci into this previously unexplored taxon. Furthermore, there was a relatively low expected frequency of null alleles for each of these two species.

Despite physical distance and very different habitat types, there was infinite gene flow among *An. arabiensis* populations within the Macha region and between Macha and Namwala, which predicted a deme size that covers an area greater than 2,000 km², with a radius greater than 80 km. These estimates are consistent with those previously reported for *An. arabiensis*, in which population differentiation using microsatellites was not detected at distances less than 200–250 km^{15–17,21} where no topographic barrier was present. Limited structuring detected for loci analyzed on chromosome 2 and within inversions is consistent with most inversions being present on chromosome 2, thus limiting genetic exchange through recombination.^{8,19} Lanzaro and others⁸ reported greatly reduced gene flow among chromosomal forms of *An. gambiae* s.s. on chromosome 2 ($N_m = \sim 3\text{--}4$) compared with chromosome 3 ($N_m = \sim 20\text{--}infinity$). Temu and Yan¹⁹ also observed greater structuring when loci were analyzed relative to inversion. Microsatellite loci within polymorphic inversions showed 4–7 times higher genetic differentiation by comparison of F_{ST} than markers within fixed inversions or outside of inversions. Therefore, the careful selection of particular microsatellite loci appears to be a critical determinant in the degree of population sub-structure ultimately showed by genetic analysis.

Although spatial analysis did not demonstrate any significant barriers to gene flow among regional collections of *An. arabiensis*, extreme drought conditions during the study period presented the opportunity to analyze the data for evidence of genetic drift events. When data were examined for evidence of a genetic bottleneck, there was no convincing pattern of excess heterozygosity in any of the years. Structure analysis also did not show any significant differentiation in *An. arabiensis* collections between years. Certain polymorphic chromosomal inversions are known to be influenced by arid climate.^{31,32} An alternative arrangement to 2Rbc in Sudan populations of *An. arabiensis*,⁵² 2Rb is common in *An. arabiensis* populations inhabiting arid environments, and varies in frequency between wet and dry seasons.^{31,32} Inversion 2Rb has also been associated with indoor biting and resting behaviors in *An. arabiensis*,^{31,53} an observation consistent with the highly anthropophilic nature of *An. arabiensis* at our study site.²⁵ Because locus AG2H79 is located within inversion 2Rb, we questioned whether there would be a shift in allele frequencies at AG2H79 as a result of climate induced selection pressures on inversion 2Rb during extended drought conditions. However, no significant shifts in allele frequency were observed.

In conclusion, relatively high estimates of gene flow, constant observed heterozygosity among years, no significant shifts in allele frequency distributions, and a large deme size collectively argue that the 2004–2005 drought in southern Zambia had little overall impact on the genetic diversity of *An. arabiensis* in the Southern Province. Therefore, heterogeneous transmission intensity among closely-situated villages during this time period was not due to genetically partitioned mosquito populations.²⁵ Our results are in accord with those of Simard and others¹⁵ who demonstrated a large deme size, relatively high effective population size, and constant mean observed heterozygosity in populations of *An. arabiensis* in Senegal, west Africa, between rainy and dry seasons over three consecutive years. Furthermore, additional estimates of large effective population sizes of *An. arabiensis* during the dry season in Mali, Nigeria, and Burkina Faso support the hypothesis that *An. arabiensis* populations are maintained continuously throughout the year with seasonal reductions rather than severe bottlenecks or extinctions.⁵⁴ Therefore, neither predictable annual dry seasons nor unexpected extended drought periods appear to have an appreciable effect on the genetic diversity of this arid-adapted mosquito.

There are several possible explanations for the lack of a genetic bottleneck in spite of extreme and extended drought conditions. First, too few generations may have been completed due to mosquitoes entering estivation or reproducing infrequently to see any temporal effects of genetic drift. Second, the effective population size may be so large that there is no evident effect of the drought on the overall genetic constitution of *An. arabiensis* over a large spatial scale.^{15,54} Third, because of drought conditions and the extensive elimination of surface water, mosquitoes may have migrated farther in search of breeding sites.⁵⁵ In this case, the high N_m values we observed may be in part due to actual mosquito movement across the Macha catchment region. In agreement with those results from west Africa, *An. arabiensis* in Macha, Zambia most likely persisted throughout the extended drought in a diffused deme, in which mosquitoes survived the dry conditions in small numbers below a sampling threshold in individual villages, but were still part of a large overall population.^{15,56} It is tempting to speculate on extended drought conditions wiping out mosquito populations given the reduction in malaria transmission. However in this case, extended drought conditions appeared to have little observable impact on the regional genetic diversity of *An. arabiensis* and malaria transmission in the following season despite greatly reduced mosquito numbers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Map of *Anopheles arabiensis* collection sites in Macha, Zambia. + represent rural health centers and triangles depict mapped households comprising the village areas in grids randomly selected for study. Mapped anopheline breeding sites are shown as points enclosed inside triangles, and rivers are depicted as lines. Each of these village areas was sampled during regional pyrethrum spray collections in 2004–2005 and 2005–2006 and corresponds to samples listed in Table 1. The location of Namwala, Zambia is represented as a dot to the northwest of Macha on inset map of Zambia.

Table 1

Anopheles arabiensis mosquito collections used in genetic analysis from 2003–2004 (wet), 2004–2005 (drought), and 2005–2006 (wet) transmission seasons^{*}

Year	Collection grid	GPS location	No. of mosquitoes tested
2003–2004	A09	S –16.53310, E 26.90134	9
	C10	S –16.47858, E 26.94370	19
	D10	S –16.39383, E 26.92084	2
	E02	S –16.36836, E 26.58828	38
	E06	S –16.36319, E 26.76670	6
2004–2005	C07	S –16.42302, E 26.79061	7
	E06	S –16.36319, E 26.76670	15 [†]
	E06	S –16.36319, E 26.76670	25
2005–2006	A06	S –16.52354, E 26.76617	9
	A10	S –16.53292, E 26.95307	16
	B05	S –16.50541, E 26.76616	26
	C10	S –16.47858, E 26.94370	31
	D10	S –16.39383, E 26.92084	22
	E02	S –16.36836, E 26.58828	12
	E06	S –16.36319, E 26.76670	10
	E09	S –16.34218, E 26.88820	50
	G03	S –16.39430, E 26.79049	53
	H09	S –16.20747, E 26.89604	12
	Namwala	S –15.75879, E 26.43274	48

^{*} Collection grid column refers to reference grids depicted in Figure 1. GPS = global positioning system.

[†] One collection of *An. quadriannulatus*.

Table 2

Microsatellite loci described by Zheng and others¹⁰ selected for population genetic analysis of *Anopheles arabiensis* in Zambia*

Locus	Chromosome	Repeat motif ¹⁰	Location relative to inversion	Total no. of alleles	Observed allele size range
AG2H143	2L	(TC) 9	Inside fixed 2La ²⁰	10	151–167
AG2H95	2R	(GT) 5 + 2 + 2	Outside	8	100–118
AG2H788	2R	(GT) 8	Inside polymorphic 2Ra	5	77–87
AG2H79	2R	(GT) 20	Inside polymorphic 2Rb ²³	5	167–175
AG3H59	3R	(GT) 9	Outside	9	112–136
AG3H119	3R	(GT) 6	Inside polymorphic 3Ra	6	174–186
AG3H128	3R	(GT) 21	Outside	21	76–128
AG3H750	3R	(GT) 8	Outside	6	80–92
AG3H811	3R	(TG) 9	Outside	34	122–190
AG3H249	3R	(GT) 15	Outside	21	106–158
AG3H746	3R	(GT) 14	Outside	32	83–157
AG3H93	3R	(gt) 4 + 7	Outside	25	122–174

When reference is not given for location relative to inversion, this information was surmised by comparison of linkage maps presented by Coluzzi and others,⁹ Zheng and others,¹⁰ and Petrarca and others.⁵²

Table 3

Spatial and temporal comparison of estimated F_{ST} and N_m values for all collections across all nine loci by inversion and by chromosome *

Comparison	All nine loci				Outside inversions				Inside inversions				Chromosome 2				Chromosome 3			
	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m	F_{ST}	N_m
Within Macha arab	0.00000	Inf	-0.00008	-0.0000	31838-inf	-0.04926	-0.11622	3.8-inf	-0.0697	-0.1485	2.9-inf	-0.0004	-0.0003	2198-inf						
Macha-Namwala arab	0.00000	Inf	-0.00002	-0.0000	22287-inf	-0.04018	-0.00487	10-inf	-0.0281	-0.0725	6.4-inf	-0.0002	-0.0002	2287-inf						
Macha arab-quad	0.00000	Inf	0.00000		2235-inf	0.00574	-0.0503	4.2-86.6	0.0512	-0.2231	1.7-9.3	0.000	-0.00022	2235-inf						
Macha 2003/4-2004/5	0.00000	Inf	0.00011		4403	-0.00035		Inf	0.01084		45.6	0.00023		2204						
Macha 2004/5-2005/6	0.00000	Inf	0.00018		2772	0.00374		133.2	0.00116		430	0.00017		8388						
Macha 2003/4-2005/6	-0.00001	Inf	0.00005		10866	-0.00121		Inf	0.01214		40.7	0.00006		2926						

* Only the F_{ST} for *Anopheles arabiensis* (arab) and *An. quadrimaculatus* (quad) on chromosome 2 were significant after Bonferroni correction ($P < 0.0003$). These values are in bold. Inf = infinity.