

Partial connectivity and fine-scale population structure in Honduran *Anopheles albimanus* inferred from microsatellite markers

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Abstract

Background

Understanding population connectivity in malaria vectors is important for surveillance in elimination settings, where local dispersal and population subdivision may influence residual transmission and the spread of adaptive traits. *Anopheles albimanus* is the major malaria vector in Honduras, yet its population structure remains poorly characterized. This study aimed to assess the genetic diversity and population structure of Honduran *An. albimanus* populations using microsatellite markers.

Methods

Adult mosquitoes collected from ten populations across Honduras ($n = 176$) were genotyped at eight species-specific microsatellite loci. Genetic diversity was estimated using number of alleles, effective number of alleles, observed and expected heterozygosity, and Wright's fixation index. Population differentiation was evaluated using pairwise F_{ST} estimates and analysis of molecular variance (AMOVA). A hierarchical AMOVA was used to assess regional structure, and Bayesian clustering implemented in STRUCTURE was used to infer genetic clusters, with the most likely number of clusters determined using the Evanno ΔK method.

Results

All loci were polymorphic and showed moderate to high genetic diversity ($H_e = 0.513\text{--}0.817$). Observed heterozygosity was consistently lower than expected, with widespread heterozygote deficits and frequent departures from Hardy–Weinberg equilibrium across populations. Pairwise F_{ST} values ranged from 0.008 to 0.195, indicating heterogeneous levels of genetic connectivity. AMOVA showed that 88% of genetic variation occurred within populations and 12% among populations ($\Phi_{IPT} = 0.117$, $p < 0.001$), consistent with moderate but significant differentiation. Hierarchical AMOVA indicated minimal differentiation between La Mosquitia and the rest of Honduras ($\Phi_{IRT} = 0.012$), while Bayesian clustering supported two inferred genetic clusters ($K = 2$) with evidence of admixture across populations, and Mantel analysis detected no evidence of isolation by distance.

Conclusions

Honduran *An. albimanus* populations harbour substantial genetic diversity while exhibiting moderate differentiation and fine-scale substructure despite overall connectivity. These findings support a model of partial connectivity shaped primarily by local population-level processes and highlight the utility of microsatellite markers for detecting contemporary population structure relevant to malaria vector surveillance and control in elimination settings.

Background

Malaria elimination increasingly requires surveillance approaches that integrate entomological, epidemiological, and molecular data to better understand residual transmission dynamics [1]. In addition to monitoring vector abundance and species composition, characterizing population connectivity among malaria vectors can provide insight into dispersal, local demographic processes, and the potential spread of adaptive traits, including insecticide resistance [2, 3, 4, 5]. This is particularly relevant in elimination settings, where focal transmission may persist despite declining incidence, and where localized vector populations may sustain residual malaria through processes not readily detected by conventional surveillance alone [4, 6].

Anopheles albimanus is the major malaria vector in much of Mesoamerica and one of the most broadly distributed anopheline species in the Neotropics. Its ecological plasticity, opportunistic feeding behaviour, and ability to occupy diverse larval habitats have contributed to its persistence across heterogeneous transmission settings [7, 8, 9]. Although *An. albimanus* has often been considered broadly connected genetically, several studies suggest that population subdivision may arise at regional or local scales, potentially shaped by ecological heterogeneity, demographic history, and partial barriers to dispersal [10, 11, 12]. Understanding whether such substructure exists is relevant because even moderate population subdivision may influence the spatial spread of adaptive alleles and the operational effectiveness of vector control interventions.

Microsatellite or short tandem repeat (STR or SSR) markers have long been used to investigate fine-scale population structure in malaria vectors because their high polymorphism provides resolution for detecting contemporary differentiation. Across multiple *Anopheles* species from the Americas, Africa and Asia, microsatellite studies have repeatedly documented moderate differentiation, high within-population diversity, recurrent heterozygote deficits, and weak or absent isolation by distance, suggesting that these may represent common features of mosquito populations shaped by interacting demographic and ecological processes [13, 14, 15, 16, 17]. In several systems, these studies have also revealed hidden substructure not readily captured by mitochondrial markers alone, underscoring the value of rapidly evolving nuclear markers for assessing contemporary population processes [18, 19]. Despite growing interest in genome-wide approaches [20, 21, 22, 23], STR-based analyses remain practical and informative tools for vector population surveillance.

In Honduras, where malaria transmission has historically been concentrated in ecologically diverse regions including La Mosquitia and other lowland transmission foci [24, 25, 26], the population structure of *An. albimanus* remains poorly characterized. Previous mitochondrial evidence suggested broad connectivity among *An. albimanus* populations in Honduras [8, 9], yet this does not preclude the existence of finer-scale contemporary subdivision detectable with more polymorphic nuclear markers. Moreover, recent studies from the broader region have highlighted both ecological complexity and subtle population differentiation within *An. albimanus* [11, 12]. However, no study has evaluated microsatellite-based population structure across geographically distinct Honduran populations, leaving unresolved

whether vector populations in the country behave as a largely panmictic system or exhibit detectable subdivision.

Here, we used species-specific microsatellite markers to characterize the genetic diversity and population structure of *An. albimanus* across multiple Honduran populations. We evaluated allelic diversity, Hardy–Weinberg equilibrium, genetic differentiation, hierarchical variance partitioning, and Bayesian clustering to assess the extent of connectivity and subdivision. Based on evidence from regional and global studies, we hypothesized that Honduran populations would show high genetic diversity accompanied by moderate differentiation and evidence of fine-scale substructure despite overall connectivity. By testing this hypothesis, this study seeks to contribute to a broader understanding of malaria vector population dynamics and inform vector surveillance strategies in elimination settings.

Methods

Study design and sampling sites

A cross-sectional descriptive-analytical study was conducted to evaluate the genetic diversity and population structure of *An. albimanus* in Honduras. Mosquitoes were collected from 10 sampling sites distributed across seven departments representing different ecological regions of the country, including coastal, lowland, and inland areas (Fig. 1).

Sampling sites were part of routine entomological surveillance activities conducted by the Honduran Ministry of Health in malaria-endemic areas. Geographic coordinates of each collection site were recorded using a handheld global positioning system (GPS) (**Supplementary material 1**).

Mosquito collection and identification

Adult *Anopheles* mosquitoes were collected between 2019 and 2021. One-per-site adult mosquito collections were conducted exclusively outdoors in both peridomestic and extra domicile environments. Sampling was performed in 2–5 selected households per locality using CDC light traps and Shannon-type tent traps positioned approximately 10–50 m from each dwelling. Traps operated from 18:00 to 06:00 h for two to three consecutive nights at each collection site. Additionally, outdoor resting mosquitoes were collected using manual aspirators between 18:00 and 21:00 h. Following collection, specimens were preserved in conical microtubes containing silica gel and transported to Tegucigalpa for morphological identification and further molecular analysis

Specimens were morphologically identified as *An. albimanus* using standard taxonomic keys for Central America and Mexico [27]. Individual mosquitoes were stored in 1.5 mL microtubes and preserved at –20°C until molecular analysis. A total of 176 specimens were selected for genotyping, representing ten populations across different regions of Honduras (Table 1).

Table 1

Sampling locations of *Anopheles albimanus* populations analysed in this study and number of genotyped specimens. The table includes population codes, administrative divisions (department and municipality), and sample sizes (n) for each collection site across Honduras. A total of 176 specimens were successfully genotyped.

Code	Department	Municipality	Samples (n)
EP01	El Paraíso	Morocelí	18
GD02	Gracias a Dios	Puerto Lempira	20
GD03	Gracias a Dios	Puerto Lempira	20
IB04	Bay Islands	Roatán	20
OL05	Olancho	Catacamas	18
YO06	Yoro	Santa Rita	20
CO07	Colón	Sonaguera	15
CM08	Comayagua	Comayagua	20
KK09	Gracias a Dios	Puerto Lempira	17
DP10	Gracias a Dios	Puerto Lempira	8
			176

DNA extraction

Genomic DNA was extracted from individual mosquitoes using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. For each specimen, the head and thorax were dissected and homogenized. Tissue lysis was performed by overnight incubation at 56°C. DNA was eluted in 150 µL of elution buffer and stored at – 20°C until further use.

Microsatellite amplification

Eight microsatellite loci previously described for *An. albimanus* were selected for genotyping, based on their polymorphism and informativeness [7, 28]. PCR amplifications were performed in a final reaction volume of 25 µL containing 2× Taq Master Mix (Promega), template DNA, primers labelled with fluorescent dyes, and nuclease-free water. To optimize amplification efficiency, loci were grouped into four multiplex PCR panels based on fragment size and annealing temperature conditions.

Thermal cycling conditions varied slightly among multiplex groups but generally included an initial denaturation step (95°C, 2–5 min), followed by 35–37 cycles of denaturation (95°C, 30 s), annealing

(54–57°C, 30–45 s), and extension (72°C, 1–2 min), with a final extension step at 72°C for 5–7 min (Table 2). PCR products were initially verified by electrophoresis on 3% agarose gels.

Table 2

Microsatellite loci used for genotyping *Anopheles albimanus* populations in Honduras. The table summarizes the characteristics of each marker, including primer names and sequences (5'–3'), fluorescent labels, repeat motifs, annealing temperatures, and expected fragment sizes (bp). Forward (+) and reverse (–) primers are indicated for each locus.

N	Panel	Primer	Sequence (5'–3')	Fluorophore	Repeat motifs	Annealing temperature (°C)	Size (bp)	Reference
1	A	1–90+	GCA TAA ATA ATA GCC AA CA	FAM	ACAG	48	149	[7]
		1–90–	GTC ACA CTT CCG ACT ACA AA			55		
2	A	2–14+	GCC CTT GCC AAG ATA AAA TGG AAA	FAM	AG	60	93	[7]
		2–14–	TCA AAT AAT CCT AAA ACA CCG TCC			57		
3	B	2–25+	GGT TTC CAG CCT CCA TTC TC	FAM	AG	58	106	[7]
		2–25–	CCT TAC TGT GCT GGA ACA CG					
4	B	6–41+	CGG CAT CCA TCC TTT CTC TG	HEX	ACAG	58	184	[7]
		6–41–	GAC CTC GCG CCT TGT CAT AA			60		
5	C	0086+	CCG GAA CAC GAT ACC TGT C	TAMRA	TG	55	88– 100	[28]
		0086–	GGG TAG AAC CGA AAC CGA AC					

N	Panel	Primer	Sequence (5'–3')	Fluorophore	Repeat motifs	Annealing temperature (°C)	Size (bp)	Reference
6	C	0078+	GTC ATT TTA CGG CGG AAC TG	TAMRA	CT	55	71– 83	[28]
		0078-	GCG CAC CTA ATG GAC GTA G					
7	D	0129+	AAT GAT GCC GCT TGA TC	ROX	GA	57	237– 289	[28]
		0129-	TAG TTC TCA CGG CAC GAT G					
8	D	0008+	TCC GTA GCG TTC AGA GGA GT	ROX	TG	55	102– 110	[28]
		0008-	AAA ATG GGG GAG GTT TAT GG					

Fragment analysis and allele scoring

For fragment size analysis, multiplex PCR products were pooled and diluted in water at a 1:15 ratio. For each microliter of the diluted mixture, 0.5 µl of the GS 600 LIZ v2.0 internal size standard (Applied Biosystems) and Hi-Di formamide were added to reach a final volume of 10 µl. Fragment separation was carried out by capillary electrophoresis using an ABI 3500® Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific). Allele sizes were determined relative to the internal size standard and scored using GeneMapper® software (Applied Biosystems). The resulting allele calls were used to construct a multilocus genotype matrix (**Supplementary material 2**).

Quality control procedures included the detection of potential null alleles, which were excluded from downstream analyses when necessary.

Genetic diversity and population structure analyses

Genetic diversity was assessed using GenAlEx v6.5 [29]. For each locus and population, the following parameters were calculated: Number of alleles (Na), Effective number of alleles (Ne), Observed

heterozygosity (H_o), and Expected heterozygosity (H_e).

Population genetic structure was evaluated using pairwise F_{ST} values to estimate genetic differentiation among populations. An analysis of molecular variance (AMOVA) was performed to partition genetic variation within and among populations. A hierarchical AMOVA was also conducted by grouping populations into two regions: La Mosquitia and the rest of the country. Statistical significance was assessed using 9,999 permutations.

Isolation by distance (IBD) was evaluated using a Mantel test to assess the relationship between pairwise linearized genetic differentiation ($F_{ST}/(1 - F_{ST})$) and log-transformed geographic distances among populations [30]. Geographic distances were calculated from sampling-site coordinates using great-circle distances (km). The Mantel test was performed using Pearson's correlation with 9,999 permutations to assess statistical significance. Analyses were conducted under the null hypothesis of no association between genetic and geographic distance.

Population structure analysis

Population genetic structure was inferred using a Bayesian clustering approach implemented in STRUCTURE v2.3.4 [31]. Analyses were conducted using the admixture model and location prior based on sampling sites (10 iterations from K_1 to K_5).

For each value of K (number of genetic clusters), ranging from 1 to 5, ten independent runs were performed with a burn-in period of 200,000 iterations followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations.

The most likely number of clusters (K) was determined using the ΔK method described by Evanno et al. [32], as implemented in STRUCTURE HARVESTER. To account for label switching and to obtain a consensus clustering solution across runs, replicate runs were aligned using the LargeKGreedy algorithm implemented in CLUMPP, and graphical visualization of cluster membership was generated in CLUMPAK.

Individual assignment probabilities (Q -values) were used to assess ancestry proportions among inferred clusters. Individuals with $Q \geq 0.80$ were considered assigned to a single cluster, whereas those with lower probabilities were considered admixed.

Results

Genetic diversity across microsatellite loci

A total of 176 *An. albimanus* specimens from ten populations in Honduras were successfully genotyped at eight microsatellite loci, all of which were polymorphic (100%). Across loci, the number of alleles per locus ranged from 4.1 to 9.7, while the effective number of alleles varied from 2.43 to 6.06. Expected

heterozygosity was moderate to high ($H_e = 0.513\text{--}0.817$), indicating substantial genetic variability among loci. However, observed heterozygosity was consistently lower than expected at several loci, particularly 1–90 and 0129, which also exhibited high fixation indices ($F > 0.70$), suggestive of heterozygote deficits. The Shannon information index ranged from 0.960 to 1.949, supporting the high informativeness of the markers used (Table 3).

Table 3
Genetic diversity parameters by locus in *Anopheles albimanus* (8 SSR loci, $n = 176$).

Locus	Na	Ne	I	Ho	He	F
2–25_A1	8.30	5.07	1.774	0.528	0.777	0.315
1–90_A1	9.50	5.26	1.793	0.213	0.753	0.727
2–14_A1	9.70	5.75	1.943	0.281	0.811	0.657
6–41_A1	6.00	3.72	1.423	0.578	0.694	0.157
0078_A1	6.50	4.35	1.582	0.489	0.753	0.348
0086_A1	6.30	4.15	1.529	0.433	0.731	0.393
0008_A1	9.70	6.06	1.949	0.600	0.817	0.261
0129_A1	4.10	2.43	0.960	0.129	0.513	0.705

Na = number of alleles; **Ne** = effective number of alleles; **I** = Shannon’s information index; **Ho** = observed heterozygosity; **He** = expected heterozygosity; **F** = Wright’s fixation index.

Genetic diversity within populations

Genetic diversity estimates across the ten *An. albimanus* populations revealed moderate to high levels of intrapopulation variation. The mean number of alleles per population ranged from 5.25 (KK09) to 10.00 (IB04), while the effective number of alleles varied from 2.93 to 6.28. Expected heterozygosity remained relatively high across populations ($H_e = 0.638\text{--}0.825$), consistent with substantial genetic diversity within localities. However, observed heterozygosity was consistently lower than expected ($H_o = 0.360\text{--}0.542$), resulting in positive fixation indices across all populations ($F = 0.309\text{--}0.531$), indicative of widespread heterozygote deficits. Several populations also showed departures from Hardy–Weinberg equilibrium at multiple loci, most notably IB04 (100% of loci), EP01 (87.5%), and GD03 and CM08 (75%), whereas DP10 showed the lowest proportion of loci deviating from equilibrium (25%) (Tables 4 and 5).

Table 4

Genetic diversity parameters by population of *Anopheles albimanus* estimated from eight microsatellite loci (SSR) in 176 individuals.

Population	n	Na	Ne	I	Ho	He	F
EP01	18	8.50	5.76	1.872	0.382	0.809	0.531
GD02	20	7.00	4.01	1.516	0.363	0.701	0.496
GD03	20	9.75	6.28	1.961	0.425	0.825	0.490
IB04	20	10.00	6.17	1.922	0.431	0.803	0.468
OL05	18	8.13	4.83	1.765	0.542	0.786	0.309
YO06	20	7.88	4.07	1.622	0.413	0.727	0.427
CO07	15	5.50	3.54	1.320	0.383	0.644	0.472
CM08	20	7.38	4.28	1.519	0.375	0.697	0.448
KK09	17	5.25	2.93	1.245	0.360	0.638	0.447
DP10	8	5.75	4.12	1.451	0.391	0.681	0.366
Note: n = sample size; Na = mean number of alleles per locus; Ne = effective number of alleles; I = Shannon's information index; Ho = observed heterozygosity; He = expected heterozygosity; F = Wright's fixation index. Values correspond to means calculated across eight SSR loci.							

Table 5 Number and percentage of microsatellite loci departing from Hardy–Weinberg equilibrium by population of *Anopheles albimanus*.

Population	Loci departing from HWE	% departing from HWE
EP01	7/8	87.5%
GD02	5/8	62.5%
GD03	6/8	75%
IB04	8/8	100%
OL05	5/8	62.5%
YO06	5/8	62.5%
CO07	4/8	50%
CM08	6/8	75%
KK09	4/8	50%
DP10	2/8	25%

HWE = Hardy–Weinberg equilibrium; SSR = microsatellites. Deviations were determined using exact tests of Hardy–Weinberg equilibrium for each locus by population. Values represent the number and percentage of loci showing significant deviations from equilibrium expectations under random mating.

Genetic differentiation and population structure

Pairwise genetic differentiation among populations was variable, with F_{ST} values ranging from 0.008 to 0.195, indicating heterogeneous levels of connectivity across localities. The lowest differentiation was observed between EP01 and GD03, whereas the highest occurred between KK09 and DP10 (Table 6).

Table 6
Pairwise F_{ST} values among *Anopheles albimanus* populations (n = 176).

EP1	EP1	GD2	GD3	IB4	OL5	YO6	CO7	CM8	KK9	DP10
	-									
GD2	0.103	-								
GD3	0.008	0.071	-							
IB4	0.045	0.118	0.051	-						
OL5	0.039	0.083	0.040	0.086	-					
YO6	0.072	0.089	0.068	0.105	0.033	-				
CO7	0.087	0.152	0.062	0.095	0.096	0.089	-			
CM8	0.071	0.118	0.088	0.074	0.079	0.040	0.108	-		
KK9	0.077	0.138	0.091	0.155	0.131	0.126	0.167	0.166	-	
DP10	0.082	0.117	0.068	0.058	0.082	0.115	0.085	0.092	0.195	-
Note: Values correspond to pairwise fixation index (F_{ST}) estimates calculated from the complete dataset (10 populations, n = 176).										

Analysis of molecular variance (AMOVA) showed that most genetic variation was distributed within populations (88%), while 12% was attributable to differences among populations, yielding a moderate but significant level of differentiation ($\Phi_{IPT} = 0.117$, $p < 0.001$) (Table 7). A hierarchical AMOVA considering two geographic regions (La Mosquitia and the rest of Honduras) showed that only 1% of the total genetic variation was attributable to differences between regions, whereas 11% was explained by differences among populations within regions and 88% remained within populations (Table 8). The low but significant regional differentiation ($\Phi_{IRT} = 0.012$, $p < 0.001$), together with the higher differentiation among populations within regions ($\Phi_{PR} = 0.111$), indicates that genetic structure is driven primarily by local population-level differences rather than broad regional subdivision. These findings suggest that the geographic separation of La Mosquitia explains only a small fraction of the observed genetic variation.

Table 7
Analysis of molecular variance (AMOVA) for *Anopheles albimanus* populations (n = 176).

Source of variation	df	Variance component	%
Among populations	9	1.202	12
Within populations	166	9.047	88
Total	175	10.249	100
PhiPT = 0.117; p < 0.001			

PhiPT = genetic differentiation index obtained from AMOVA; p = significance value estimated in GenAlEx v6.5.

Table 8
Analysis of molecular variance (AMOVA) considering two geographic regions in *Anopheles albimanus* populations (n = 176).

Source of variation	df	Estimated variance	%
Among regions	1	0.128	1
Among populations within regions	8	1.135	11
Within populations	166	9.047	88
Total	175	10.310	100
PhiRT = 0.012; PhiPR = 0.111; PhiPT = 0.123 (p < 0.001)			

Isolation by distance analysis

Mantel analysis showed no evidence of isolation by distance among Honduran *An. albimanus* populations. No significant correlation was detected between pairwise linearized genetic differentiation and log-transformed geographic distance ($r = -0.2047$, $P = 0.9482$; 9,999 permutations). The negative and non-significant correlation indicates that geographic distance does not explain the observed genetic structure, suggesting that differentiation is not organized as a simple clinal pattern.

Bayesian clustering analysis

Bayesian clustering analysis implemented in STRUCTURE identified $K = 2$ as the most likely number of genetic clusters according to the Evanno ΔK method ($\Delta K = 16.676$), with lower support for $K = 3$ and $K = 4$ (Fig. 2). Although likelihood-based probability values increased with higher K values, including $K = 5$, this pattern was consistent with overpartitioning at larger K and did not provide a more biologically informative clustering solution. The CLUMPAK consensus plot for $K = 2$ showed two inferred ancestral components across the ten populations, with evidence of admixture in several localities. Although some populations showed differences in ancestry proportions, the inferred clusters were not completely segregated by geographic region. These results are consistent with the AMOVA and pairwise F_{ST}

analyses, supporting moderate genetic structuring with substantial shared ancestry among Honduran *An. albimanus* populations.

Discussion

The present study reveals that *Anopheles albimanus* populations in Honduras harbor moderate to high levels of genetic diversity, accompanied by significant but moderate population differentiation and widespread heterozygote deficits. The high levels of expected heterozygosity and allelic richness observed across loci and populations indicate substantial standing genetic variation, consistent with the ecological flexibility and broad dispersal capacity described for this species [7, 8]. At the same time, the predominance of within-population genetic variance (88%) relative to among-population variance (12%) and the moderate overall differentiation ($\Phi_{PT} = 0.117$) suggest a pattern of incomplete genetic homogenization rather than strong fragmentation. Notably, the Bayesian inference of two ancestral clusters ($K = 2$), coupled with admixture across localities, supports a scenario in which genetic connectivity persists but is spatially heterogeneous. Taken together, these results suggest that Honduran populations of *An. albimanus* may function as a partially connected system in which dispersal, local differentiation, and admixture coexist.

The magnitude and structure of differentiation observed here are remarkably consistent with prior studies in *An. albimanus* and other malaria vectors. In Colombian *An. albimanus*, Gutiérrez et al. (2009) [10] reported comparable levels of pairwise differentiation and similarly widespread heterozygote deficits, attributing these patterns partly to hidden substructure and semi-permeable barriers to gene flow. Likewise, Molina-Cruz et al. (2004) [7] documented high diversity and substantial regional connectivity across Central America, while suggesting that local differentiation may still arise within a broader connected genetic background. Our findings extend this regional framework by showing that even within a single country, local-scale structure can be detectable despite evidence of shared ancestry. Similar patterns have also been reported in other Neotropical vectors, including *Anopheles marajoara* and *An. darlingi*, where moderate differentiation, predominant within-population variance, and cryptic substructure have repeatedly been observed [13, 33, 34]. Collectively, these studies support the interpretation that the population structure observed here is not anomalous, but rather consistent with recurrent patterns in neotropical anophelines.

A major feature of our data is the recurrent deficit of heterozygotes and frequent departures from Hardy–Weinberg equilibrium. These patterns may reflect hidden population subdivision (Wahlund-like effects), non-random mating, or contributions from locus-specific phenomena such as null alleles or segregation distortion. Importantly, these alternatives should not be treated as mutually exclusive. Both Sunil et al. (2013) [15] and Ma et al. (2011) [16] explicitly proposed that heterozygote deficits in mosquito microsatellite studies may arise from combined biological and technical causes, rather than a single mechanism. In our case, the presence of two inferred ancestral clusters and heterogeneous pairwise F_{ST} values lend support to cryptic fine-scale subdivision as at least a partial explanation. The absence of IBD further suggests that the observed heterozygote deficits are unlikely to be explained by simple

geographic isolation alone, lending additional support to hidden substructure (Wahlund-like effects) as one plausible contributing mechanism. This interpretation is further strengthened by comparable findings in *An. funestus* and *An. gambiae*, where moderate differentiation and substructure have been documented even in the absence of strong isolation by distance [14, 17, 18]. Notably, the convergence of AMOVA partitions in our study (88/12) with those reported independently in Africa and Asia strongly suggests that moderate differentiation combined with recurrent HWE deviations may represent a common feature of mosquito populations analyzed using microsatellite markers, rather than an artifact unique to this system.

The low regional variance attributable to La Mosquitia versus the rest of Honduras ($\Phi_{RT} = 0.012$), together with stronger differentiation among populations within regions, suggests that broad geographic partitioning explains little of the observed structure and that differentiation emerges primarily at local scales. This interpretation is reinforced by the absence of isolation by distance in the Mantel analysis, which showed no relationship between genetic differentiation and geographic distance. Thus, populations separated by greater distances were not consistently more differentiated than geographically proximate populations, arguing against a stepping-stone model of dispersal. This is consistent with models in which ecological heterogeneity, rather than simple geographic distance, contributes to fine-scale substructure. Environmental heterogeneity, variable habitat suitability, and differential demographic histories may generate partially isolated subunits even in broadly distributed vectors [16, 35]. This interpretation is also compatible with previous Honduran mitochondrial evidence suggesting broad historical connectivity [8, 9], whereas the microsatellite markers used here reveal finer contemporary population subdivision. Similar contrasts between broad connectivity inferred from mitochondrial markers and finer structure detectable with higher-resolution approaches have been discussed elsewhere in *An. albimanus* [11]. Such a pattern is compatible with a metapopulation-like framework, where recurrent dispersal, local demographic fluctuations, and incomplete homogenization coexist. Importantly, recent comparative genomic analyses have shown that even geographically distant strains of *An. albimanus* remain genomically cohesive without evidence of cryptic speciation [12], suggesting that the moderate differentiation observed here should be interpreted as local population subdivision within a connected species, rather than deep evolutionary fragmentation.

Finally, our results have implications for future vector surveillance, population genomic research, and genomic based vector control innovation. Although the eight-locus microsatellite panel detected robust signals of diversity and structure, evidence from *An. darlingi* suggests that conventional STR markers may underestimate subtle differentiation detectable through genome-wide SNP approaches [19]. Thus, the structure observed here may represent a conservative estimate of underlying population subdivision. Future studies incorporating denser geographic sampling and genome-wide markers could help determine whether the absence of IBD observed here reflects neutral demographic processes, local adaptation, or both. This question is relevant not only for evolutionary inference but also for vector control, as partial substructure may influence the spread of adaptive alleles, including those associated with insecticide resistance, vector competence, or limits potential effectiveness of self-limiting transgenic suppression or similar genomic based approaches. Together, these findings suggest that

population structure in Honduran *An. albimanus* is shaped by interacting demographic, ecological, and potentially adaptive processes, with implications for malaria vector surveillance in elimination settings.

Conclusions

This study provides the first microsatellite-based evidence that populations of *Anopheles albimanus* in Honduras harbour substantial genetic diversity while exhibiting moderate but significant population differentiation and evidence of fine-scale substructure despite overall connectivity. The predominance of within-population genetic variation, together with heterogeneous pairwise differentiation, Bayesian clustering, and recurrent heterozygote deficits, supports a model of partial connectivity in which dispersal, admixture, and localized differentiation coexist. The low contribution of broad regional partitioning further suggests that genetic structure is driven primarily by local population-level processes rather than major geographic separation or isolation by distance, supporting a non-clinal pattern not explained by isolation by distance. These findings highlight the value of microsatellite markers for detecting contemporary population structure in malaria vectors and suggest that partial substructure in Honduran *An. albimanus* may have implications for vector surveillance and control in malaria elimination settings.

Abbreviations

AMOVA: Analysis of Molecular Variance

bp: Base pairs

CLUMPAK: Cluster Markov Packager Across K

CLUMPP: Cluster Matching and Permutation Program

DNA: Deoxyribonucleic acid

F: Wright's fixation index

FST: Fixation index (genetic differentiation among populations)

GenAlEx: Genetic Analysis in Excel

GPS: Global Positioning System

He: Expected heterozygosity

Ho: Observed heterozygosity

HWE: Hardy–Weinberg equilibrium

I: Shannon's information index

IBD: Isolation by distance

K: Number of inferred genetic clusters

MCMC: Markov Chain Monte Carlo

Na: Number of alleles

Ne: Effective number of alleles

PCR: Polymerase Chain Reaction

PhiPR: Genetic differentiation among populations within regions

PhiPT: Overall genetic differentiation estimated by AMOVA

PhiRT: Genetic differentiation among regions

Q-values: Individual assignment probabilities in Bayesian clustering

SSR: Simple Sequence Repeats

STR: Short Tandem Repeats

STRUCTURE: Bayesian clustering software for population structure inference

Declarations

Ethics approval and consent to participate.

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

GF conceptualized the study, designed the methodology, drafted the original manuscript, acquired funding, provided resources, and supervised the project. AP performed the experiments, validated the results, contributed to project administration, and participated in supervision. DE contributed to methodological design, conducted laboratory and field work, validated results, performed statistical and bioinformatic analyses, contributed software tools, participated in supervision, and contributed to the original draft. AM performed laboratory and field work, curated the data, and conducted statistical analyses. All authors reviewed, edited, and approved the final manuscript.

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Figures

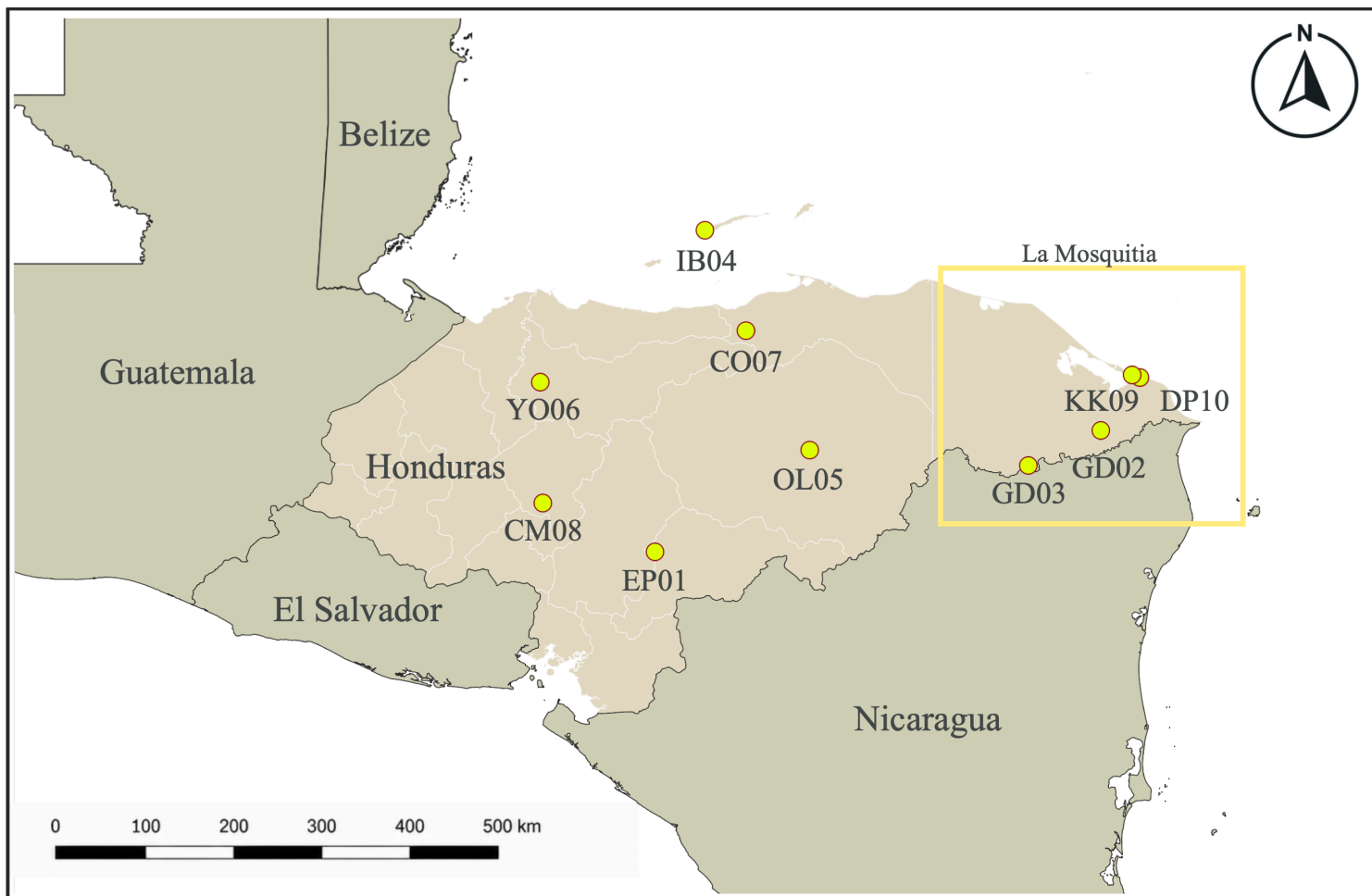


Figure 1

Geographic distribution of ten *Anopheles albimanus* populations sampled across seven departments of Honduras, including mainland, insular, and eastern endemic regions (Gracias a Dios, “La Mosquitia”). Yellow circles indicate collection localities. The boxed inset highlights clustered sampling sites in La Mosquitia, where four populations (GD02, GD03, KK09 and DP10) are shown at enlarged scale for clarity.

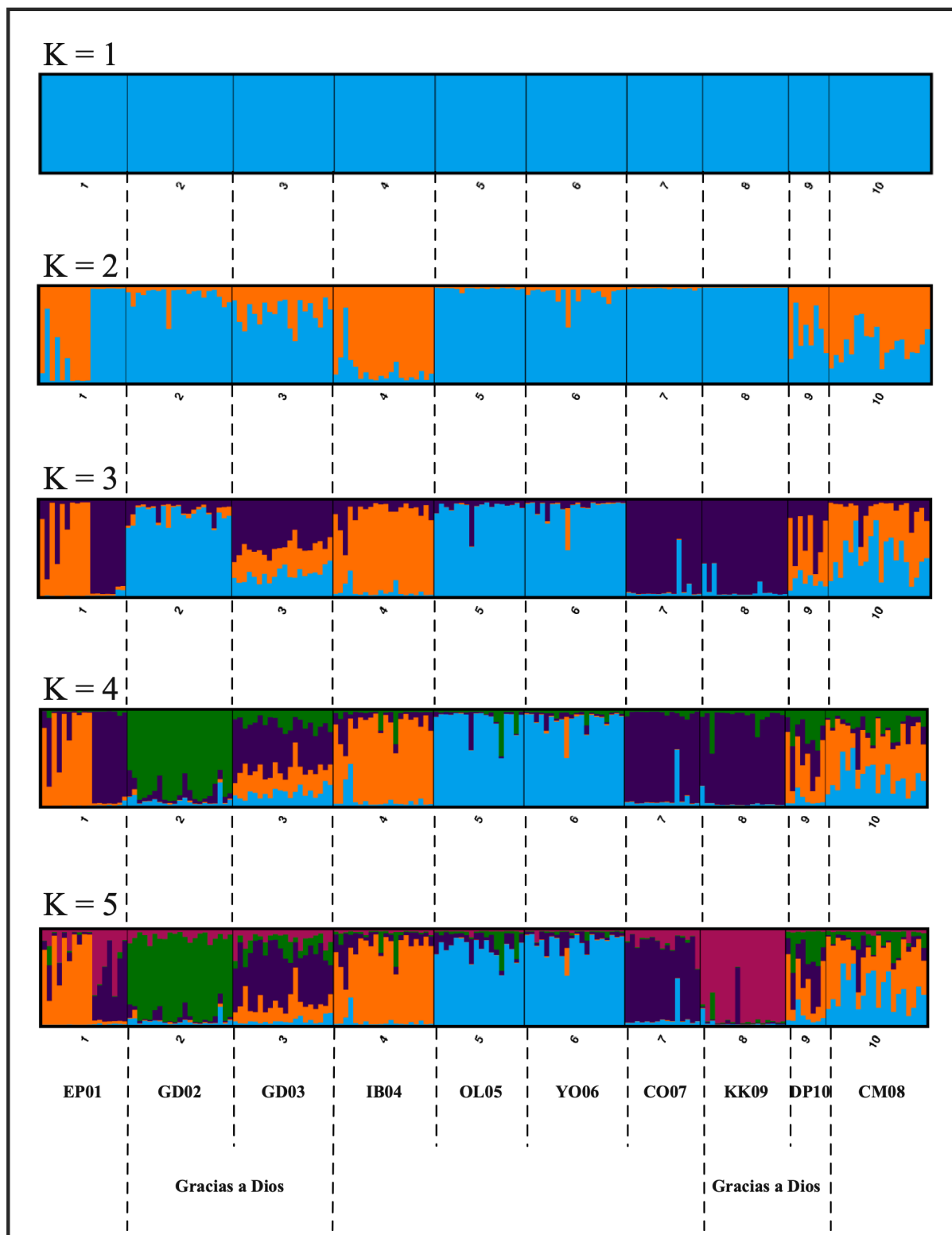


Figure 2

Bayesian clustering analysis of *Anopheles albimanus* populations from Honduras inferred with STRUCTURE. Each vertical bar represents an individual, and colours indicate proportional membership coefficients (Q-values) to inferred genetic clusters. Results are shown for K = 2, the most likely number of clusters according to the Evanno ΔK method. Populations are ordered according to Table 1.

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