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Spatial distribution of the 2La chromosomal inversion in *Anopheles gambiae* populations across Nigeria: ecological associations and implications for malaria control

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Abstract

Background Chromosomal inversions are key drivers of local adaptation and ecological diversification in *Anopheles gambiae*, the principal malaria vector in sub-Saharan Africa. Among these, the 2La inversion is associated with tolerance to aridity, behavioural variation, and insecticide resistance. However, large-scale data on inversion frequency and ecological association remain scarce in Nigeria. This study investigated the spatial distribution of 2La inversion frequencies in *An. gambiae* and their relationship with ecological zones across the country.

Methods A total of 1200 *Anopheles* mosquitoes were collected across 12 states representing Nigeria's major ecozones. 2La inversion karyotypes were determined by PCR, and allele frequencies were analysed in relation to ecological zones, latitude, and ecological gradients. Spatial mapping and statistical analyses were performed using R version 4.4.

Results Morphological identification and species-specific PCR revealed 999 *An. gambiae* s.l., comprising *An. gambiae* s.s. (39.7%), *An. coluzzii* (51.5%), and *An. arabiensis* (8.7%). A total of 399 *An. gambiae s.s.* was karyotyped. From the *An. gambiae s.s.* population karyotyped, a distinct south–north cline in inversion frequency was observed. The 2La/2La homokaryotype predominated in arid northern ecozones (Sahelian and Sudan savannas), whereas 2La + /2La + was dominant in humid southern forests and mangrove regions. The heterokaryotype 2La/2La + occurred at moderate frequencies in transitional zones (Osun–Taraba corridor). Linear regression analyses confirmed significant correlations between inversion frequency and latitude ($R^2 = 0.68–0.73$, $P < 0.001$). Generally, the allele frequencies from 2La karyotyping did not deviate significantly from Hardy–Weinberg equilibrium expectations ($P > 0.05$) except in Bayelsa and Kebbi States.

Conclusion This study provides the first nationwide evidence linking 2La inversion polymorphism to ecological adaptation of *An. gambiae* in Nigeria. The inversion likely enhances vector survival across diverse habitats, contributing to spatial variation in malaria transmission. These findings have direct operational relevance for Nigeria's National Malaria Elimination Programme (NMEP), indicating that vector-control effectiveness may vary by ecological context. Integrating ecotype- and region-specific evidence into NMEP planning could improve intervention targeting. Continuous genomic surveillance will be essential to sustain malaria control gains.

Keywords Malaria, *Anopheles gambiae*, 2La inversion, Polymorphism, Vector control, Nigeria

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Background

Malaria remains a major public health challenge in sub-Saharan Africa, accounting for about 94% of global cases and deaths, with Nigeria contributing approximately 25.9% of the global burden [1]. Despite substantial gains from long-lasting insecticidal nets, indoor residual spraying, and chemoprevention, transmission remains intense due to complex interactions among vector ecology, parasite biology, and environmental factors. The sustainability of malaria control is increasingly threatened by vector behavioural change, insecticide resistance, and genetic adaptation to control interventions [2, 3].

Among malaria vectors, members of the *Anopheles gambiae* complex are considered the most efficient vectors of *Plasmodium* parasites in Africa. This group of morphologically indistinguishable sibling species, including *An. gambiae* sensu stricto, *An. coluzzii*, and *An. arabiensis*, has undergone rapid adaptive radiation, enabling them to thrive across diverse ecological settings [4, 5]. Their ecological success is attributed to extensive genetic diversity and capacity for local adaptation to environmental and anthropogenic pressures [6, 7].

These adaptations encompass physiological resistance to insecticides, plasticity in resting and biting behaviours, and tolerance to climatic extremes [8, 9]. Such traits not only sustain vector populations under control pressure but also influence the epidemiology of malaria transmission. At the genomic level, these phenotypic responses are associated with structural variants that promote stable local adaptation, particularly through chromosomal inversions. These inversions play a central role by maintaining advantageous combinations of alleles within regions of suppressed recombination, thereby facilitating ecological differentiation and adaptive responses [10–12]. The *Anopheles gambiae* genome is extensively polymorphic for inversions, especially on chromosome 2, where multiple inversions contribute to ecological and behavioural differentiation among populations [9, 13, 14]. These structural variations function as genomic regions facilitating local adaptation by suppressing recombination and preserving co-adapted allelic combinations that confer fitness advantages under specific environmental conditions [15].

Among these, the 2La chromosomal inversion is one of the well-studied and most ecologically significant polymorphisms in *An. gambiae* s.s. [16, 17]. The inversion comprises two alternative arrangements, 2La and 2L+a, whose frequencies vary predictably along Africa's latitudinal climatic gradient [16]. The 2La arrangement predominates in arid and semi-arid savanna regions, whereas the 2L+a arrangement is common in humid forest and coastal zones [13, 18]. These patterns are thought to reflect selection by climatic factors such as temperature, humidity, and aridity, with alternative karyotypes occupying distinct adaptive peaks along environmental gradients [16, 19]. Seasonal shifts in inversion frequencies, often higher during the dry season, further support the role of environmental pressures in maintaining polymorphism [17] (Ayala et al. 2014). However, contrasting results from Central Africa [20] suggest that local ecological dynamics may influence these associations.

The polymorphism of the 2La inversion has been linked to multiple aspects of vector biology, including desiccation resistance [21, 22], thermal tolerance, larval habitat preference [17, 23], feeding and resting behaviour [16], and insecticide resistance [24, 25], indicating that inversion karyotypes represent integrated adaptive strategies shaped by local ecological conditions. The inversion is also associated with variations in *Plasmodium* infection rates, suggesting that it may influence vector competence [26]. Climate-based predictive models have demonstrated that inversion frequencies can be used to forecast the presence or absence of certain *Anopheles* karyotypes with high accuracy [27], buttressing their adaptive importance.

Despite accounting for more than a quarter of global malaria cases, Nigeria lacks a comprehensive, nationwide assessment of 2La chromosomal inversion frequency and its ecological correlates in *Anopheles gambiae* populations across its diverse ecozones. Addressing this knowledge gap, the present study investigates the spatial distribution, frequency, and environmental associations of 2La inversion polymorphism across Nigeria. By linking inversion frequency to environmental gradients, this study aims to explain the role of chromosomal inversions in shaping adaptive landscapes of *An. gambiae* and to inform vector ecology and malaria control efforts in Nigeria.

Methods

Study area

Nigeria covers 923,768 km^2 in West Africa (9.0820° N, 8.6753° E) and is the most populous country in Africa, with an estimated population exceeding 218 million in 2021. Approximately 51.7% of the population lives in rural areas and 48.3% in urban centres, with a population density of about 167 persons per km^2.The country experiences two main seasons: rainy (April–October) and dry (November–March), with marked regional variation in rainfall. Southern coastal areas along the 853-km Atlantic coastline receive 1500–2000 mm annually, whereas northern regions receive 600–1000 mm. This rainfall gradient drives a transition from mangrove forests and rainforests in the south to Guinea, Sudan, and Sahel savannahs in the north [28]. These ecological differences

shape environmental dynamics and vector distribution. Mosquito breeding sites are semi-permanent in the south but largely seasonal in the north [16], with larvae occurring in pools, puddles, tyre tracks, ponds, and footprints [25]. These vectors are predicted to have wide adaptive coverage across Nigeria [29, 30]. *Anopheles* larvae were randomly collected from 12 states representing the six geopolitical zones (Fig. 1).

Mosquitoes specimen

Larval stages of anopheline mosquitoes were collected from 12 locations spanning Nigeria's mangrove swamp to savanna ecozones using standard dipping methods. Sampling was conducted between April and September 2021 at identified breeding sites georeferenced with a Garmin eTrex® GPS 10. Larvae and pupae were collected following established protocols (Service et al., 1971), transferred into labelled collection containers, and transported to the Molecular Entomology and Vector-Control Research Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. Samples were reared under standard insectary conditions (25–28 °C, 70–80% relative humidity, 12 h light–dark cycle) [32], with larvae fed daily on yeast and emergent adults maintained on 10% glucose solution.

Species identification

Adult mosquitoes were morphologically identified using the Gilles and Coetzee guide [33]. Genomic DNA was extracted using a NIMR (Nigeria) kit, including positive controls from the Molecular Entomology and Vector Control Unit, NIMR Lagos. PCR amplification was performed using 1 µl of DNA template. Species within the *Anopheles gambiae* complex, including M and S forms, were identified following Wilkins et al. [34] using species-specific intentional mismatch primers (Table 1). Reactions (12.5 µl) contained standard PCR buffer, dNTPs, $MgCl_2$, Taq polymerase, and primers. Thermocycling

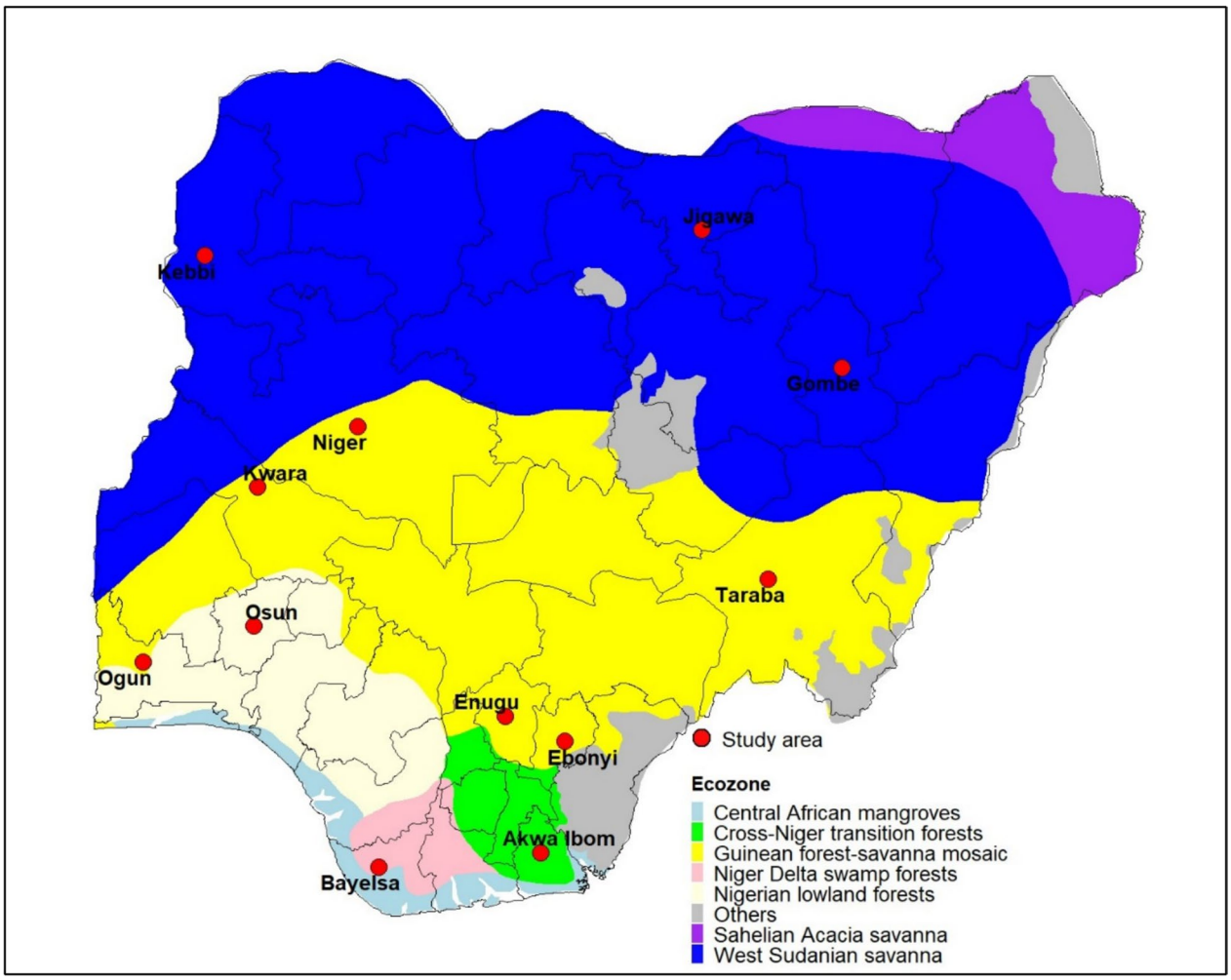

Fig. 1 Map of Nigeria showing major ecotypes and the study sites

consisted of initial denaturation at 95 °C for 5 min; 30 cycles of 95 °C for 30 s and 72 °C for 30 s; and a final extension at 72 °C for 5 min. Amplified products were resolved on 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination.

2La inversion karyotyping

Molecular karyotyping of the 2La inversion was performed on *Anopheles gambiae* specimens from all study sites following White et al. [15]. Primer sequences are provided in Table 2. PCR reactions (12 µl) contained 10× buffer, $MgCl_2$, dNTPs, primers (2L + a, 2La, and universal), DreamTaq DNA polymerase, and 0.5 µl of template DNA. Thermocycling conditions were: 94°C for 2 min; 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s; followed by a final extension at 72 °C for 10 min and a 4 °C hold. Primers were sourced from Inqaba Biotec™ (South Africa).

PCR products were resolved on 1.5% agarose gel stained with ethidium bromide. The assay generated diagnostic fragments of 207 bp (2L + a allele) and 492 bp (2La allele), allowing classification into standard homokaryotype (2L + a/2L + a), inverted homokaryotype (2La/2La), or heterokaryotype (2La/2L + a).

Data analysis

Allele frequencies were analysed in relation to ecological zones, latitude, and ecological gradients. Genotype frequencies and observed numbers were calculated and correlated with ecotype using linear regression analysis. Karyotype numbers from each population were further subjected to Hardy–Weinberg estimates using Chi-square test and Bonferroni correction was applied for multiple testing. All spatial mapping and statistical analyses were performed using R version 4.4.

Results

Spatial distribution of 2La inversion frequencies across Nigerian states

A total of 999 *Anopheles gambiae* s.l. were successfully identified morphologically from the 1200 *Anopheles* specimens collected across Nigeria. PCR identification of sibling species revealed that 399 (39.7%; 95% CI = 36.7–42.8) were *An. gambiae* s.s., 513 (51.5%; 95% CI = 48.4–54.6) were *An. coluzzii*, and 87 (8.7%; 95% CI = 7.1–10.6) were *An. arabiensis*. The karyotyped counts and frequencies are presented in Table 3. The frequency of the 2La/2La ranged from 0 in Akwa Ibom to 0.93 in Taraba, whereas the $2La^+/2La^+$ allele ranged from 0 (in Niger, Jigawa and Taraba) to 0.90 in Bayelsa. The heterozygous $2La/2La^+$ allele occurred at intermediate frequencies, ranging from 0.01 (in Bayelsa and Kebbi) to 0.17 (in Osun). The 2La + /2La + arrangement was absent in populations from Jigawa, Niger, and Taraba States, while the 2La/2La form was not detected in Akwa Ibom State (Table 3).

Spatial ecozone overlay revealed distinct geographic structuring in inversion frequencies across Nigeria. The homozygous 2La/2La arrangement predominated in the northern regions, especially in the Sahelian and West Sudanian savanna ecozones, with frequencies

Table 1 Primer name, primer sequence 5′–3′ and primer type used for target-site modifications

Primer name	Primer type	Target species/form	Sequence (5′–3′)	Notes
IMP-UN	Universal forward	*An. gambiae complex*	GCTGCGAGTTGTAGAGATGCG	Common forward primer
QD-3 T	IMP reverse	*An. quadriannulatus*	GCATGTCCACCAACGTAAATCC	Species-specific
ME-3 T	IMP reverse	*An. merus*	CAACCCACTCCCTTGACGATG	Species-specific
GA-3 T	IMP reverse	*An. gambiae s.s.*	GCTTACTGGTTTGGTCGGCATGT	Species-specific
AR-3 T	IMP reverse	*An. arabiensis*	GTGTTAAGTGTCCTTCTCCGTC	Species-specific
IMP-SI	IMP reverse	S-form (*An. gambiae*)	CCAGACCAAGATGGTTCGCTG	Molecular form
IMP-M1	IMP reverse	M-form (*An. coluzzii*)	TAGCCAGCTCTTGTCCACTAGTTTT	Molecular form

Table 2 PCR primers for molecular karyotyping of 2La and 2L + a chromosomes

Primer name	Direction	Target	Sequence (5′–3′)
23A2	Forward (Universal)	2L inversion	CTCGAAGGGACAGCGAATTA
27A2	Reverse	2La	ACACATGCTCCTTGTGAACG
23A2	Forward (Universal)	2L inversion	CTCGAAGGGACAGCGAATTA
DPCross5	Reverse	2L + [a]	GGTATTTCTGGTCACTCTGTTGG

Table 3 Observed numbers and frequencies of 2La karyotypes in sampled *An. gambaie* s.s. across 12 States in Nigeria

S/N	State from south to north	Number karyotyped	Allele number (frequency) per state		
			2La/2La	$2La^+/2La^+$	$2La/2La^+$
0	Bayelsa	31	2 (0.07)	28 (0.90)	1 (0.03)
1	Akwa Ibom	40	0 (0.00)	27 (0.68)	13 (0.32))
2	Enugu	36	4 (0.11)	21 (0.58)	11 (0.31)
3	Ebonyi	30	1 (0.03)	23 (0.77)	6 (0.20)
4	Ogun	32	2 (0.06)	16 (0.50)	14 (0.44)
5	Osun	30	9 (0.30)	4 (0.13)	17 (0.57)
6	Kwara	32	13 (0.41)	3 (0.09)	16 (0.50)
7	Niger	31	21 (0.68)	0 (0.00)	10 (0.32)
8	Kebbi	30	27 (0.90)	2 (0.07)	1 (0.03)
9	Jigawa	31	21 (0.68)	0 (0.00)	10 (0.32)
10	Gombe	30	19 (0.63)	2 (0.07)	9 (0.30)
11	Taraba	46	43 (0.93)	0 (0.00)	3 (0.07)
	Total	399	**162 (0.41)**	**126 (0.32)**	**111 (0.28)**

exceeding 0.7 in Kebbi, Niger, Taraba, and Gombe States. In contrast, 2La + /2La + (standard arrangement) was largely confined to the southern forest and coastal ecozones, including Bayelsa, Akwa Ibom, and parts of Enugu, where frequencies approached or exceeded 0.8. The heterozygous 2La/2La + form showed intermediate and uneven distribution, with high frequency occurring mainly in central transition zones such as Osun, Kwara, and Niger (Fig. 2), corresponding to the Guinean forest–savanna mosaic.

When mapped over ecological regions, 2La inversion patterns clearly reflected Nigeria's south–north ecological gradient. The 2La/2La arrangement was strongly associated with arid and semi-arid ecozones (Sahelian and West Sudanian savannas), while 2La + /2La + was linked to humid lowland and mangrove forests of southern Nigeria. These patterns highlight adaptive differentiation among *An. gambiae* populations occupying distinct ecotypes (Fig. 3).

Separate maps display state-level frequencies of the standard homokaryotype (2L + a/2L + a), inverted homokaryotype (2La/2La), and heterokaryotype (2La/2L + a). Colour gradients represent karyotype frequency, with darker shades indicating higher values. Red circles mark state centroids, and larger circles denote frequencies ≥ 0.5. Frequencies are presented across the surveyed states to illustrate geographic variation in inversion distribution.

State-level frequencies of 2La inversion karyotypes are overlaid on a map of Nigeria's major ecological zones. Background shading represents national ecozone classifications. Red circles indicate state centroids, with circle size proportional to karyotype frequency. Separate panels display the distribution of the standard homokaryotype (2L + a/2L + a), inverted homokaryotype (2La/2La), and heterokaryotype (2La/2L + a).

Latitudinal patterns of 2La inversion frequencies along a south–north cline of Nigeria

A ridge plot illustrating latitudinal patterns of 2La inversion frequencies along a south–north cline is presented in Fig. 4. The results show that the frequency of 2La/2La arrangement increases sharply toward the north, whereas 2La + /2La + declines in the same direction, suggesting a strong climatic gradient influencing karyotype distribution across ecozones. The point of inflection (around Osun to Taraba) marks the transition between the southern forest and northern savanna climes and is characterized by a slightly higher frequency of $2La/2La^+$ heterokaryotypes. These patterns indicate directional selection or environmental filtering along the climatic gradient (Fig. 4).

Furthermore, the inversion frequencies of each allele type (2La/2La, $2La^+/2La^+$ and $2La/2La^+$) plotted against latitude (States arranged from south to north) revealed statistically significant patterns (Fig. 5). A clear south-to-north clinal shift in inversion frequencies was observed. No significant variation in frequency was detected for the 2La/2La + heterokaryotype, while the inverted 2La/2La arrangement increased significantly toward the north ($R^2 = 0.68$, $P < 0.001$). Conversely, 2La + /2La + decreased significantly with increasing latitude ($R^2 = 0.73$, $P < 0.001$). In addition, the allele frequencies from 2La karyotyping did not deviate significantly from Hardy–Weinberg equilibrium expectations ($P > 0.05$) except in Bayelsa and Kebbi States, where significant departures were observed (Table 4).

Discussion

This study demonstrates that the spatial distribution of the 2La chromosomal inversion in *Anopheles gambiae* s.s. across Nigeria is strongly structured along ecological gradients. The significant association between latitude and inversion frequency indicates that a substantial proportion of spatial variation in karyotype distribution is explained by eco-climatic conditions, supporting adaptive differentiation rather than random population structure. Chromosomal inversions promote local adaptation by suppressing recombination and preserving co-adapted gene complexes, particularly along humidity–aridity gradients [9, 18]. Our findings therefore reinforce the role of the 2La inversion as a key genomic architecture underlying ecological specialization in this major malaria vector.

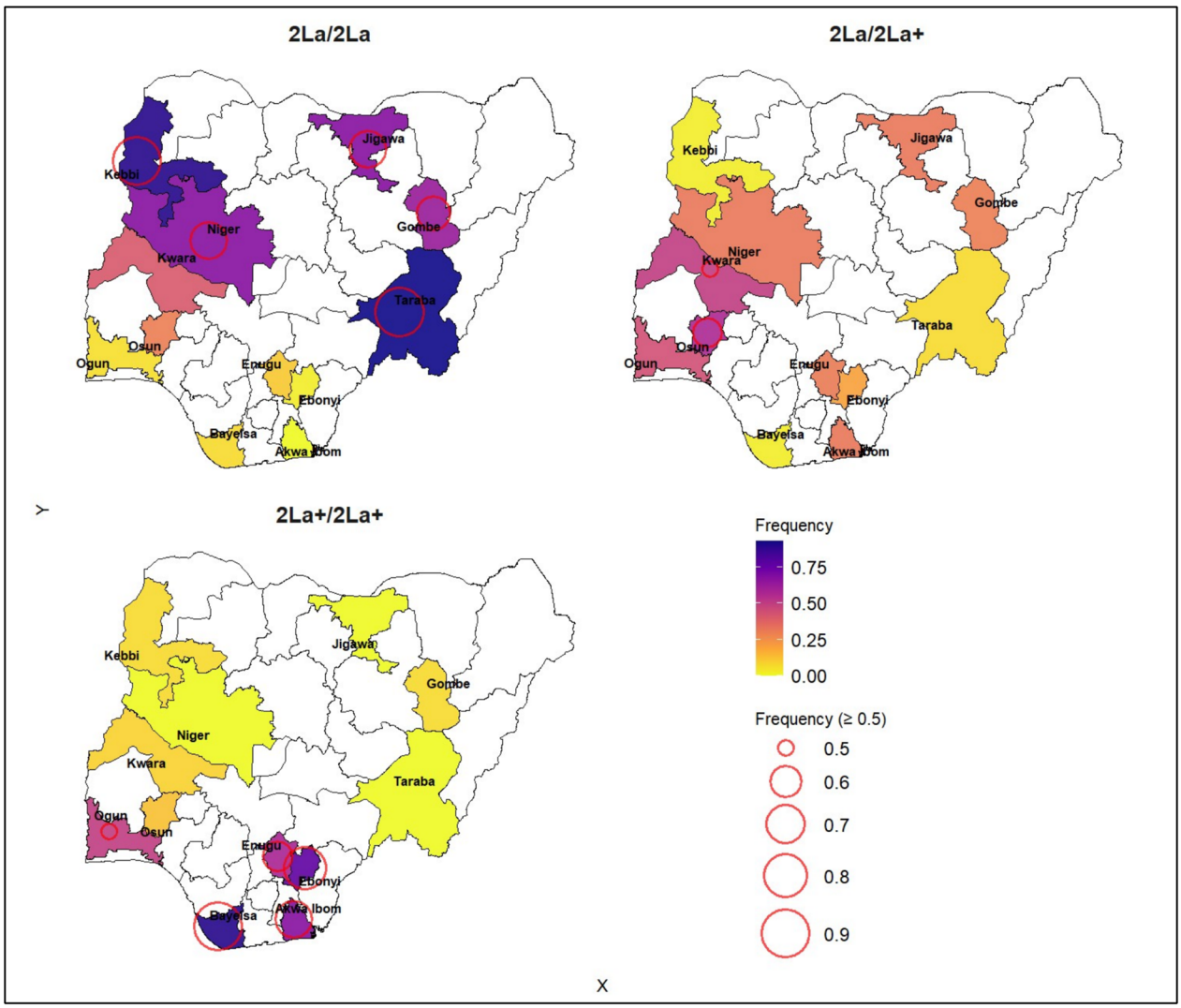

Fig. 2 Spatial distribution of 2La inversion karyotypes in *Anopheles gambiae* s.s. across 12 Nigerian states

The observed latitudinal cline aligns with patterns reported across sub-Saharan Africa [5, 37]. The predominance of the inverted homokaryotype (2La/2La) in northern, drier ecozones suggests adaptation to low humidity and evaporative stress, consistent with evidence linking this arrangement to enhanced desiccation resistance and survival in arid environments [18, 21, 38]. In contrast, the standard homokaryotype (2L + a/2L + a) is more frequent in humid southern forest and mangrove regions. The 2La inversion influences ecologically relevant traits—including desiccation tolerance, insecticide resistance, and biting and resting behaviour [9, 16, 18, 24, 26]—and likely contains genes involved in cuticular hydrocarbon composition and stress-response pathways [36]. Despite its importance, inversion frequency remains under-characterized in Nigerian populations [14].

Transitional zones between Osun and Taraba show coexistence of both homokaryotypes, suggesting maintenance of polymorphism through balancing selection or gene flow. Environmentally driven shifts in inversion frequency have been documented across ecological races of the *An. gambiae* complex [18, 21, 38]. However, our findings contrast with Ibrahim et al. [9], who reported no association between 2La and thermotolerance in northern Nigeria. Although thermotolerance may become increasingly relevant under climatic variability [39, 40], adaptation to aridity likely reflects interactions among humidity, temperature, and behaviour. Indeed,

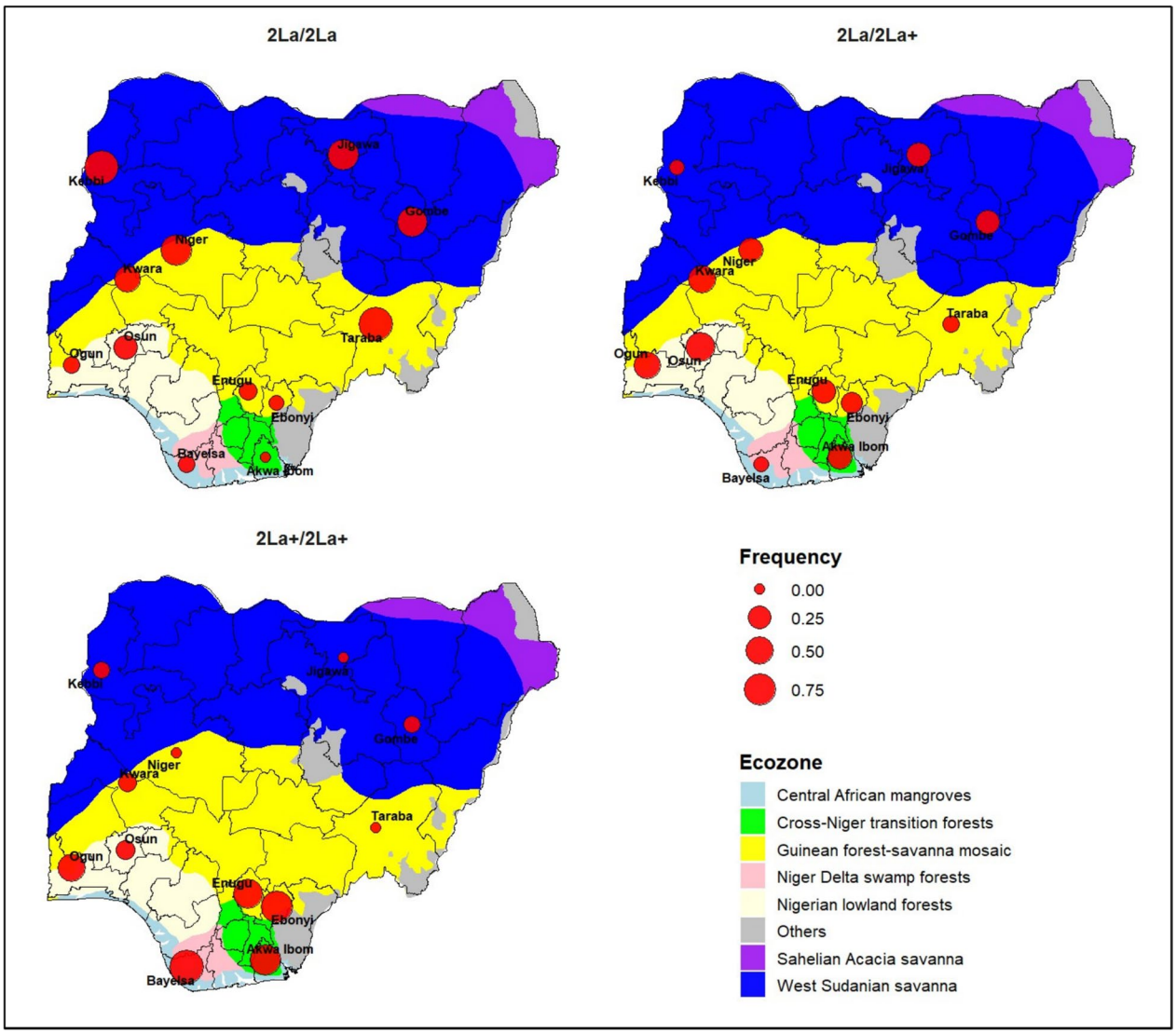

Fig. 3 Geographic distribution of 2La inversion karyotypes in *Anopheles gambiae* s.s. across Nigerian ecozones

experimental studies show that heat tolerance does not consistently align with aridity adaptation, for example, 2L+a/2L+a larvae may tolerate acute heat stress better than 2La/2La individuals [21, 41], supporting context-dependent fitness effects of inversion-linked alleles [42]. Behavioural differences, including greater exophily in dry-adapted populations, may further contribute to ecological divergence [43].

The moderate frequency of heterokaryotypes (2La/2L+a) across sites indicates maintenance of balanced polymorphism [45]. While the 2La inversion is not directly associated with pyrethroid resistance used in ITNs [24], chromosome 2 inversions have been linked to insecticide resistance in *Anopheles*. These include associations between the 2Rb inversion and DDT resistance in northern Nigeria [24], and between 2La and resistance to dieldrin in southern populations [25] and pyrethroids in northern Nigeria [9]. Although the mechanistic basis remains unclear, inversions may function as genomic reservoirs of adaptive alleles, contributing to spatial heterogeneity in intervention outcomes.

Most states conformed to Hardy–Weinberg equilibrium, suggesting stable population structure. Deviations in Bayelsa and Kebbi may reflect localized selection or demographic processes. In Bayelsa (humid coastal zone), deviation could indicate selection favouring the standard homokaryotype or demographic expansion linked to ecological disturbance. In Kebbi (Sahelian zone), directional selection favouring the inverted homokaryotype under arid conditions is plausible. Alternatively, migration,

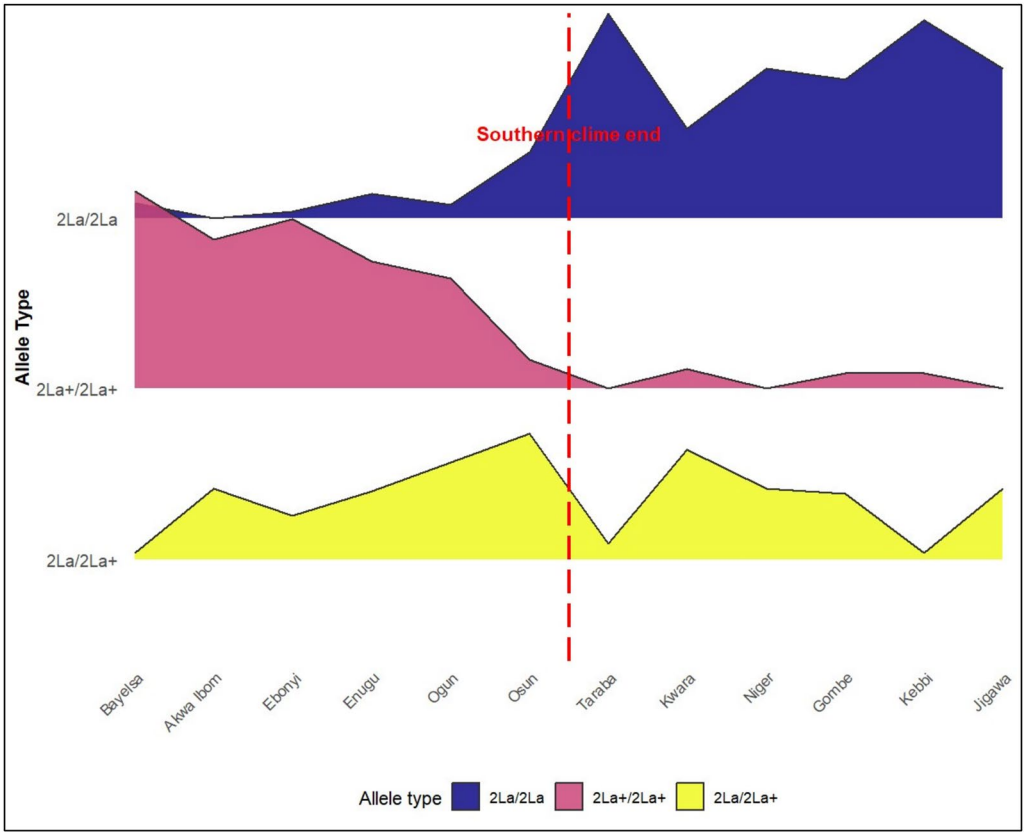

Fig. 4 Latitudinal distribution of 2La inversion frequencies in *Anopheles gambiae* s.s. Ridge plots illustrating the distribution of 2La inversion frequencies across states arranged from southern to northern Nigeria. Separate panels display the standard homokaryotype (2L + a/2L + a), inverted homokaryotype (2La/2La), and heterokaryotype (2La/2L + a). The dashed vertical red line indicates the approximate geographic transition between southern and northern regions (Osun–Taraba boundary). Frequencies are shown as state-level estimates along the latitudinal gradient

sub-structuring (Wahlund effect), or sampling heterogeneity may contribute. Further genomic and demographic analyses are needed to disentangle these drivers.

From a public health perspective, inversion-driven ecological differentiation has direct implications for malaria control. Populations enriched for the inverted homokaryotype (2La/2La) in northern savanna and Sahel regions may exhibit more exophilic or exophagic tendencies, potentially reducing exposure to indoor interventions such as LLINs and IRS [9]. Southern populations dominated by the standard homokaryotype may remain more responsive to indoor tools. Integrating inversion surveillance into genomic monitoring frameworks could improve detection of ecological or behavioural shifts that compromise intervention effectiveness [44].

For Nigeria's National Malaria Elimination Programme, these findings support climate-informed, ecozone-specific vector control. Strategies should include: (i) integration of inversion genotyping into routine entomological and resistance surveillance; (ii) deployment of complementary outdoor-targeted interventions in northern regions; and (iii) incorporation of ecological and genomic data into stratified planning and resource allocation. Continuous genomic surveillance will be essential to support adaptive, evidence-based malaria control as environmental conditions evolve.

Conclusion

This study provides the first nationwide evidence linking 2La chromosomal inversion polymorphism to ecological adaptation of *Anopheles gambiae* s.s. in Nigeria. The 2La inversion likely enhances vector survival across diverse ecological settings, contributing to spatial heterogeneity in malaria transmission and intervention effectiveness. These findings have direct operational relevance for Nigeria's National Malaria Elimination Programme (NMEP), highlighting that vector-control performance may vary across ecological zones.

Future research should prioritize whole-genome sequencing of mosquitoes representing different 2La karyotypes and ecozones to identify causative alleles within the inversion and to disentangle the genetic

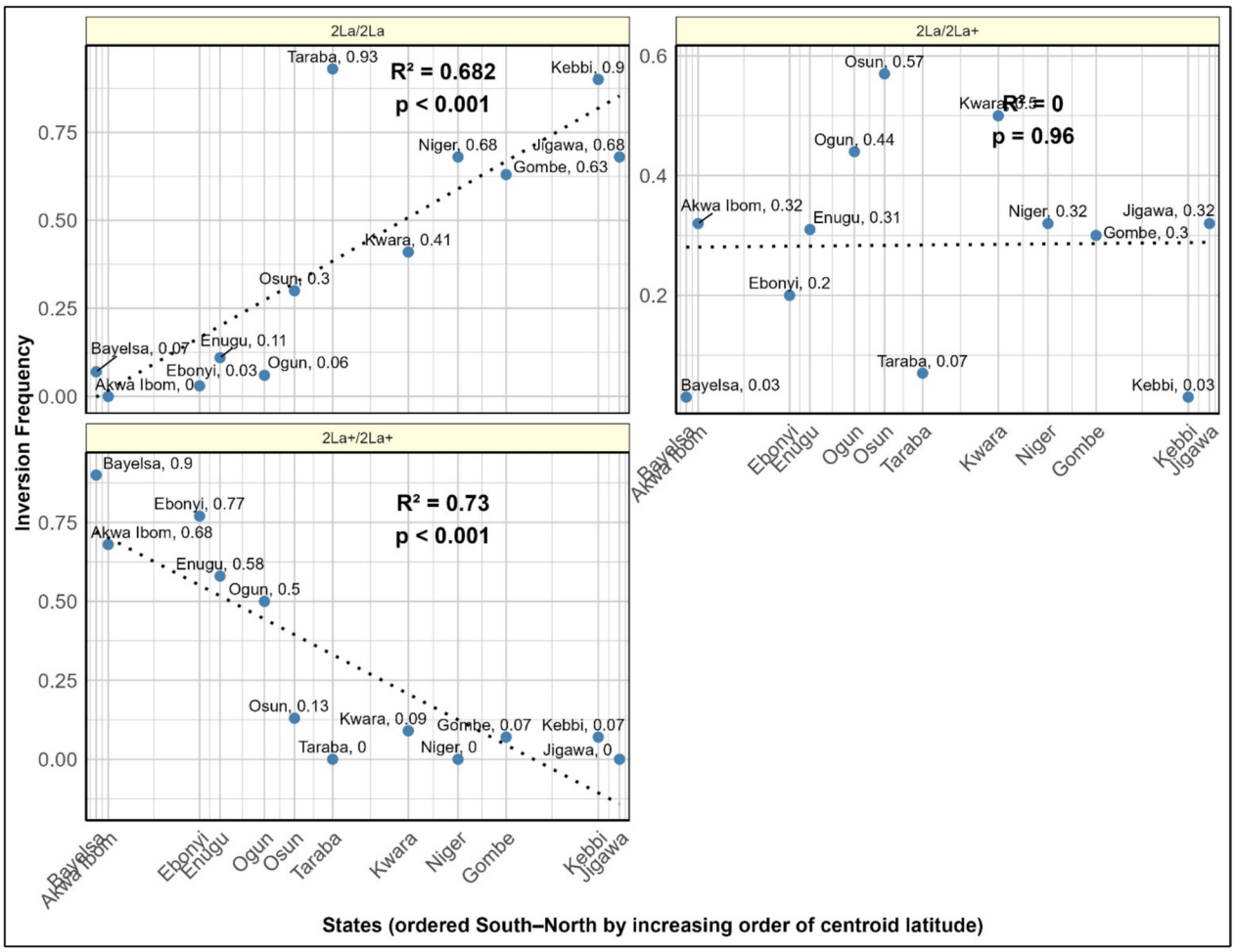

Fig. 5 Latitudinal cline of 2La inversion frequency in *Anopheles gambiae* s.s. Scatterplots showing the relationship between inversion frequency and latitude across 12 Nigerian states. Separate panels display frequencies of the standard homokaryotype (2L + a/2L + a), inverted homokaryotype (2La/2La), and heterokaryotype (2La/2L + a). Points represent state-level frequencies ordered from south to north. Dotted lines indicate fitted linear regression models, with corresponding coefficients of determination (R^2) shown in each panel

Table 4 Observed and expected (Hardy–Weinberg) karyotype frequencies for polymorphic chromosome inversion $2La^+$ and 2La in *An. gambiae* from the sampled states in Nigeria

States	Counts	2La + /2La +	2La/2La +	2La/2La	χ^2	P value
Bayelsa	No. observed	28	1	2	18.979	< 0.0001*
	Expected H–W	26.2	4.6	0.2		
Akwa Ibom	No. observed	27	13	0	1.506	0.471
	Expected H–W	28.06	10.89	1.06		
Enugu	No. observed	21	11	4	1.641	0.44
	Expected H–W	19.51	13.99	2.51		
Ebonyi	No. observed	23	6	1	0.544	0.762
	Expected H–W	22.54	6.93	0.53		
Ogun	No. observed	16	14	2	0.216	0.898
	Expected H–W	16.53	12.94	2.53		
Osun	No. observed	4	17	9	0.824	0.662
	Expected H–W	5.21	14.58	10.21		
Kwara	No. observed	3	16	13	0.375	0.829
	Expected H–W	3.78	14.44	13.78		
Niger	No. observed	0	10	21	1.146	0.564
	Expected H–W	0.81	8.39	21.81		
Kebbi	No. observed	2	1	27	18.337	< 0.0001*
	Expected H–W	0.21	4.58	25.21		
Jigawa	No. observed	0	10	21	1.146	0.564
	Expected H–W	0.81	8.39	21.81		
Gombe	No. observed	2	9	19	0.405	0.817
	Expected H–W	1.41	10.18	18.41		
Taraba	No. observed	0	3	43	0.052	0.97
	Expected H–W	0.05	2.9	43.05		

Hardy–Weinberg equilibrium was assessed using a Chi-square (χ^2) goodness-of-fit test with 2 degrees of freedom. $P < 0.05$ was considered statistically significant (*). Bonferroni correction for multiple testing ($\alpha = 0.05/13 = 0.0038$) was considered; only Bayelsa and Kebbi remained significant after correction

mechanisms underlying ecological adaptation, behavioural variation, and insecticide resistance.

Abbreviations

WHO	World Health Organization
LLIN	Long-lasting insecticide net
IRS	Indoor residual pray
NIMR	Nigerian Institute of Medical Research
NMSP	Nigeria National Malaria Strategic Plan
DNA	Deoxyribose nucleic acid
PCR	Polymerase chain reaction
IMP	Intentional mismatch primer
MgCl	Magnesium chloride
dNTP	Dinitrogen triphosphate
Taq	*Thermus aquaticus*
Bp	Base pair
DDT	Dichloro-diphenyl-trichloroethane

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Author contributions
Conceptualization—AOA, RTI, ASB, OA. Supervision—AOA, ASB, TAO. Investigation—AOA, RTI, ASB, TAO, OA. Software and visualization—ASB. Data collection—RTI, OA, JW, LOB, OAA. Formal analysis—ASB, AOA. Writing original draft—AOA, RTI, ASB. Writing review and editing—AOA, RTI, ASB, TAO, OA, OAA, JW, LOB, GA, SA, JC, STA.

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Availability of data and materials
All data generated and analysed during the study are present in the article.

Declarations

Ethics approval and consent to participate
Ethical clearance for this study was obtained from the Ethics review committee, Federal Ministry of Health. All methods including mosquito larva collection and breeding, laboratory analysis and data management were performed in accordance with the 1964 Declarations of Helsinki. Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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