

Temporal and biting dynamics of the malaria vectors *Anopheles gambiae* and *Anopheles coluzzii* harboring the 2La inversion in Bangui, Central African Republic

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May 27, 2022

Abstract

The chromosomal rearrangement 2La has been directly involved in the ecological and deadly epidemiological success of the malaria mosquitoes *Anopheles gambiae* and *Anopheles coluzzii* in sub-Saharan Africa. However, little is known about the biological and ecological factors that drive the local and temporal dynamics of this inversion in both species. Here, we performed a year-round longitudinal survey in Bangui, Central African Republic. We monthly sampled *An. gambiae* and *An. coluzzii* mosquitoes indoor and outdoor using human landing catches for 48 hours non-stop. We molecularly karyotyped all specimens to study the 2La inversion frequency variations, and monitored the mosquito spatial and temporal biting behavior throughout the year. In total, we successfully karyotyped 5121 *An. gambiae* and 986 *An. coluzzii* specimens. The 2La inversion frequency was higher in *An. coluzzii* than in *An. gambiae* across the year. In *An. gambiae* and *An. coluzzii*, the inversion frequency or karyotypes did not influence the biting behavior, either location or time. Moreover, the inversion frequency variation in both species was also independent of local climatic changes. Overall, our results revealed that in Bangui, the 2La inversion segregates at different frequency in each species, but this is not influenced by their trophic behavior. Studying the impact of urban settings and the population genetic structure of these two *An. gambiae* complex members could bring insights into the intrinsic relationship between 2La inversion and local conditions. More studies are needed to understand the polymorphic equilibrium of this inversion in Bangui.

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Keywords : inversion 2La, biting behavior, population dynamics, *Anopheles* , malaria, Bangui

Abstract

The chromosomal rearrangement 2La has been directly involved in the ecological and deadly epidemiological success of the malaria mosquitoes *Anopheles gambiae* and *Anopheles coluzzii* in sub-Saharan Africa. However, little is known about the biological and ecological factors that drive the local and temporal dynamics of this inversion in both species. Here, we performed a year-round longitudinal survey in Bangui, Central African Republic. We monthly sampled *An. gambiae* and *An. coluzzii* mosquitoes indoor and outdoor using human landing catches for 48 hours non-stop. We molecularly karyotyped all specimens to study the 2La inversion frequency variations, and monitored the mosquito spatial and temporal biting behavior throughout the year. In total, we successfully karyotyped 5121 *An. gambiae* and 986 *An. coluzzii* specimens. The 2La inversion frequency was higher in *An. coluzzii* than in *An. gambiae* across the year. In *An. gambiae* and *An. coluzzii* , the inversion frequency or karyotypes did not influence the biting behavior, either location or time. Moreover, the inversion frequency variation in both species was also independent of local climatic changes. Overall, our results revealed that in Bangui, the 2La inversion segregates at different frequency in each species, but this is not influenced by their trophic behavior. Studying the impact of urban settings and the population genetic structure of these two *An. gambiae* complex members could bring insights into the intrinsic relationship between 2La inversion and local conditions. More studies are needed to understand the polymorphic equilibrium of this inversion in Bangui.

Background

The ability to rapidly adapt to environmental and anthropogenic conditions allowed some members of the *Anopheles gambiae* complex to become the most prominent malaria vectors in Africa (White, Cheng et al. 2010). Within the *Anopheles gambiae* complex, two species, *Anopheles gambiae* and *Anopheles coluzzii* , stand out due to their ecological success, as indicated by their faculty to colonize a myriad of habitats across sub-Saharan Africa and their efficient transmission of malaria parasites (Costantini, Ayala et al. 2009, Simard, Ayala et al. 2009, Sinka, Bangs et al. 2010). Their success is rooted in their virtually limitless genetic polymorphism (Fontaine, Pease et al. 2015, Miles, Harding et al. 2017), including chromosomal inversions (Ayala, Ullastres et al. 2014, Ayala, Acevedo et al. 2017).

Among the polymorphic chromosomal rearrangements, the 2La inversion is particularly interesting in both species. This inversion spans along almost half (~ 22 Mb) of the left arm of chromosome 2, and is polymorphic only in *An. gambiae* and *An. coluzzii* within the *An. gambiae* complex (Coluzzi, Sabatini et al. 1979, Sharakhov, White et al. 2006). It is acquired by adaptive introgression from the sister species *Anopheles arabiensis* where it is fixed (Sharakhov, White et al. 2006, Fontaine, Pease et al. 2015), and has been associated with many adaptive traits that influence malaria transmission (Ayala, Ullastres et al. 2014). For decades, the 2La inversion has been essentially associated with a latitudinal cline in many parts of Africa (Coluzzi, Sabatini et al. 1979, Costantini, Ayala et al. 2009, Simard, Ayala et al. 2009). This reflects an aridity gradient, with increasing polymorphism from humid habitats (standard karyotype) to dry savannas (inverted karyotype) in Africa (Ayala, Acevedo et al. 2017). This aridity adaptation has been also associated with phenotypic traits, such as thermal tolerance in larvae and desiccation resistance in adults (Rocca, Gray et al. 2009, Fouet, Gray et al. 2012). A recent study on the genetic basis of this adaptation proposed that some candidate genes within the 2La inversion may explain the stronger desiccation resistance conferred by this inversion (Ayala, Zhang et al. 2019). Moreover, seasonal fluctuations of the 2La inversion frequency (from high frequency in the dry season to low frequency in the wet season) have been recorded in different parts of the African continent (Toure, Petrarca et al. 1994, Ayala, Ullastres et al. 2014). Several studies showed that the 2La inversion could also affect the choice of biting and resting sites, leading to higher frequency of indoor than outdoor captures (Coluzzi, Sabatini et al. 1979, Ayala, Ullastres et al. 2014). This non-random association

between 2La inversion and temporal/spatial behaviors may affect the effectiveness of vector control tools, such as indoor residual spraying and long-lasting insecticidal nets, at the local scale, increasing the level of malaria transmission in many parts of sub-Saharan Africa (Riehle, Bukhari et al. 2017). This is particularly true in areas where this inversion is highly polymorphic and both *Anopheles* species are the major malaria vectors.

In Bangui, the Central African Republic capital, two studies showed that *An. gambiae* and *An. coluzzii* are the only members of the *An. gambiae* complex present in the city, and that *An. gambiae* is the predominant vector (Ndiath, Eiglmeier et al. 2016, Ole Sangba, Sidick et al. 2017, Kamgang, Tchapgá et al. 2018). Ndiath et al., in 2016 also showed that the 2La inversion frequency is ~ 40% (Ndiath, Eiglmeier et al. 2016). To better understand the role of the 2La inversion in the population dynamics and behavior of these two mosquito species, we carried out an extensive longitudinal survey in Bangui throughout one year. We molecularly karyotyped both species and analyzed how the 2La inversion frequency varied across the year, and in function of the collection locality (indoor and outdoor), and biting time. These data are essential to better understand the local adaptation of these two malaria vectors and to assess the effectiveness of frontline interventions for malaria control in Bangui, Central African Republic.

Material and Methods

Ethical approval

This study and the methods employed for mosquito collection, including human landing catch (HLC), were approved by the Institutional Review Board of the Health Sciences Faculty of Bangui University (authorization number 01/UB/FACSS/CSCVPER/17).

Study sites and climatic variables

Mosquitoes were collected at four sites in Bangui: Gbanikola (4.3311 N, 18.5400 W), Îles de singes (4.3549 N, 18.5667 W), Ouango (4.3767 N, 18.6082 W), and Pk10 (4.4304 N, 18.5468 W) (Figure 1A). Bangui is located at the southern border of the country and lies on the northern banks of the Ubangui River, along the Democratic Republic of Congo border. The climate is tropical with relative humidity varying from 47% in December to 72% in August. The rainy season extends from May to October, and the dry season from November to April.

During the sampling period, meteorological data (rain, temperature, and relative humidity) were collected monthly by the Meteorological Station of Bangui (Table S1).

Mosquito collection and morphological identification of species.

All mosquitoes were collected during 11 months (from June 2016 to May 2017, except December due to logistical reasons). Each month, adult mosquitoes were field-collected by HLC during two consecutive days (non-stop for 48h). At each site, collections were carried out by two volunteers, inside and outside each house, for 6 hours, who were then replaced by two new volunteers. The volunteers' location and sampling time were changed several times to avoid bias in the collections. All samples were transported to the Institut Pasteur de Bangui. Mosquitoes were morphologically identified using the taxonomical identification keys described by Gillies and De Meillon (Gillies and De Meillon 1968) and Gillies and Coetzee (Gillies and Coetzee 1987).

DNA extraction, species identification, and molecular karyotyping of the 2La inversion.

Total genomic DNA was extracted from all mosquitoes morphologically identified as *An. gambiae* complex members using 2% cetyl-trimethyl-ammonium-bromide following the protocol described by Morlais *et al.*, (Morlais, Poncon et al. 2004). DNA pellets were dissolved in 100 μ l sterile water at 4°C for 24 hours, and then stored at -20°C. *An. gambiae* complex members were then identified using species-specific PCR assays according to the protocols developed by Santolamazza *et al.*, (Santolamazza, Mancini et al. 2008). The molecular karyotyping of the 2La inversion was performed according to White *et al.*, (White, Santolamazza et al. 2007) and based on amplicon band sizes that correspond to the standard homokaryotype (2L+^a/2L+^a, her-

eafter standard), the inverted homokaryotype (2La/a, hereafter inverted), or the heterokaryotype (2La/+^a, hereafter heterozygote).

Genetic, statistical and modeling analyses

Conformity to the Hardy-Weinberg equilibrium (HWE) was assessed using the ‘HardyWeinberg’ package (Graffelman and Morales-Camarena 2008, Graffelman 2015) in R (R 2021). The HWE was tested for each species at each site, location, and month. When the number of individuals was <10, the test was not performed (‘NA’).

Circular statistical analyses were carried out in R according to Sangbakembi et al., (Sangbakembi-Ngounou, Costantini et al. 2022) and Pewsey *et al.* (Pewsey, Neuhäuser et al. 2013) and the functions available therein. As mosquitoes were aggregated over 1-hour time slots at collection, biting events were transformed into a pseudo-continuous variable by adding a uniform random component over the [0, 1] interval of each time slot. Bites were grouped by species and by karyotype to compare the daily bite rhythmicity in the four grouped collection sites using circular statistics.

Then, to determine the variables that play a role in the 2La inversion and biting behavior dynamics in *An. coluzzii* and *An. gambiae*, a generalized linear model with a binomial distribution was parameterized (Hosmer, Jovanovic et al. 1989). The response variable was the 2La inversion frequency in each species, and the explanatory variables were the climatic parameters listed in Table S1.

All statistical analyses were performed using the R software, version 4.0.2 (<http://cran.r-project.org/>), and the package “stats” in R (R 2021).

Results

Frequency of the 2La inversion in Bangui. We collected 6198 mosquitoes belonging to the *An. gambiae* complex at the four sites in Bangui (Figure 1A, Table 1, Table S2). We molecularly identified 5187 *An. gambiae sensu stricto* (hereafter *An. gambiae*), 991 *An. coluzzii*, and 20 hybrids. Only *An. gambiae* and *An. coluzzii* specimens were retained for the 2La inversion analysis. In total, we successfully molecularly karyotyped 5121 *An. gambiae* and 986 *An. coluzzii* samples (Table 1). The mean frequency of the arrangement across the year was significantly different between *An. gambiae* and *An. coluzzii* (0.38 and 0.51; Wilcoxon test, $W = 120$, $p < 0.001$) (Figure 1A). Moreover, 48.70% and 31.74% of samples harbored the standard, 24.74% and 34.69% the inverted, and 26.56% and 33.57% the heterozygote karyotype in *An. gambiae* and in *An. coluzzii*, respectively (Table 1). We observed a significant deficit of heterozygotes in both *An. gambiae* and *An. coluzzii* across the year (HWE, exact test Chi-Square = 974.31, $p < 0.001$, Table 1). When we estimated the HWE deviation for each month, we observed a constant deficit of heterozygotes in *An. gambiae*, and a deficit in more than the half of the months in *An. coluzzii* that coincided mostly with the dry season (Table S3). We obtained similar results when we computed the HWE deviation by month and location for each species (Table S4), or by month, location, and site (Table S5).

Species	Location	Standard	Heterozygote	Inverted	Total	Frequency	HWE
<i>An. coluzzii</i>	Indoor	190	202	213	605	0.52	<0.001
	Outdoor	123	129	129	381	0.51	<0.001
<i>An. gambiae</i>	Indoor	1546	786	794	3126	0.38	<0.001
	Outdoor	948	574	473	1995	0.38	<0.001

Table 1. Summary of mosquito specimens collected and karyotyped in Bangui, Central African Republic.

Number of mosquitoes for each 2La inversion karyotype (standard, heterozygote, and inverted). Frequency of the 2La inversion in each group. HWE: Conformity to the Hardy Weinberg Equilibrium exact test (package ‘HardyWeinberg’) (Graffelman and Morales-Camarena 2008, Graffelman 2015).

The temporal dynamics of the 2La inversion. We calculated the 2La inversion frequency during one year

(Figure 1B) and did not observe any significant difference among months for both *An. gambiae* (ANOVA: F-statistic= 1.2, $p > 0.05$) and *An. coluzzii* (ANOVA: F-statistic= 1.4, $p > 0.05$), and also between species (Wilcoxon test, $p > 0.05$) (Figure 1B). We obtained similar results when comparing the inversion karyotype frequencies for each month and for both species (Wilcoxon test, $p > 0.05$). Analysis of the karyotypes in each species across the year revealed different temporal patterns between species, particularly for the standard karyotype (Figure 1C).

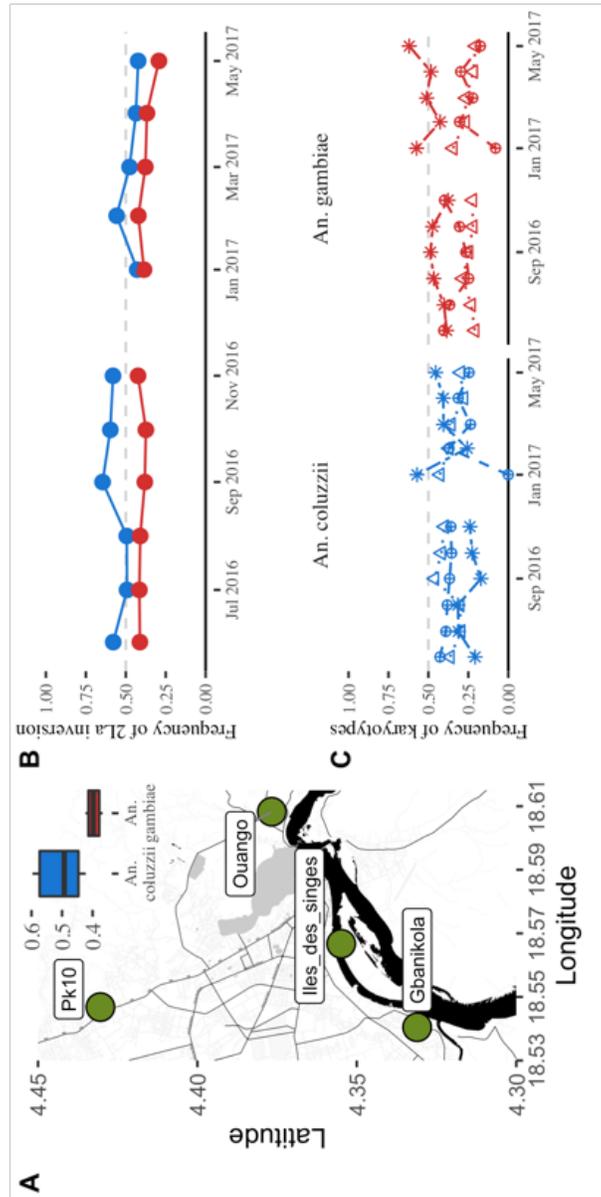


Figure 1 . Collection sites and temporal dynamics of the 2La inversion frequency in Bangui.

(A) Green circles indicate the mosquito collection sites in the city of Bangui (source: OpenStreetMap.org). The inset shows the mean frequency of the 2La inversion in the two species during the collection year. (B) Linear representation of the 2La inversion frequency variations for each species (blue line, *An. coluzzii* ; red

line, *An. gambiae*) during the collection year. C) Linear representation of each karyotype (mean frequency) per species across the year. Blue lines, *An. coluzzii* ; red lines, *An. gambiae* . Asterisks, standard karyotype; circles with a cross, heterozygote karyotype; triangles, inverted karyotype.

Biting behavior and 2La inversion. To assess the role of the 2La inversion in the biting behavior of both malaria mosquito species, we estimated the inversion frequency in specimens collected indoor and outdoor in Bangui. We observed slight, but not significant, differences in its frequency between indoor and outdoor collections for each species during the sampling months (ANOVA: F-statistic= 0.007, $p > 0.05$) (Figure 2A). We did not detect any significant interaction between the inversion frequency and the sampling location for both species (ANOVA: F-statistic= 4.20, $p > 0.05$). Moreover, we asked whether the different inversion karyotypes were associated with the host-seeking behavior. Analysis of the sampling data showed no difference in the frequency of the different karyotypes per month between specimens captured indoor and outdoor for both species (Wilcoxon test, $p > 0.05$) (Figure 2B). For both species, mosquitoes harboring the standard karyotype were as likely to be captured indoor and outdoor as mosquitoes carrying the inverted karyotype (*An. coluzzii* : ANOVA: F-statistic= 9.618e-29, $p > 0.05$; *An. gambiae* : ANOVA: F-statistic= 5.349e-29, $p > 0.05$) (Figure 2B).

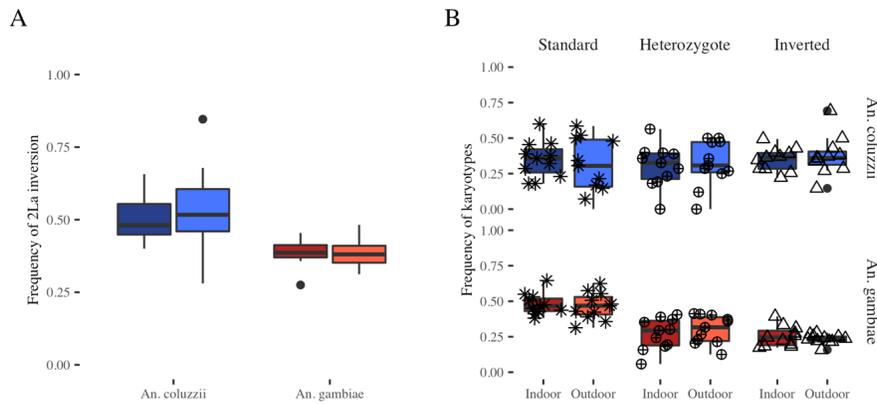


Figure 2 . Spatial distribution of the 2La inversion in function of the collection location.

(A) Boxplots show the 2La inversion frequency in mosquitoes collected indoor (darker colors) and outdoor (lighter colors) throughout the year (*An. gambiae* in red, *An. coluzzii* in blue). (B) Frequency of each 2La inversion karyotype (asterisk, standard; circle with cross, heterozygote; triangle, inverted) for *An. gambiae* and *An. coluzzii* specimens captured indoor and outdoor.

Then, we analyzed the daily mosquito bite rhythmicity as a continuous distribution of bite events that occurred during the entire diel cycle of 24h. Despite the slight differences observed (Sangbakembi-Ngounou, Costantini et al. 2022), we assumed that each karyotype exhibited similar spatial biting distributions, although more studies are needed to investigate variations according to spatial and temporal heterogeneities. We then estimated the trigonometric moments, such as the mean direction ([?]) and the mean length (R), of each karyotype in the two species and the locations throughout the year and for each month. The [?] computes the arithmetic mean of the different bite events during the 24h cycle, and the resulting length varies between 0 and 1 and represents the variation –dispersion– in values of the angles (Pewsey, Neuhauser et al. 2013, Sangbakembi-Ngounou, Costantini et al. 2022). The estimation of these two parameters (Table 2) showed that mosquitoes (all karyotypes) tended to bite after midnight and before 3 a.m. (Table 2, Figure 3). In *An. coluzzii* , all mosquitoes (the three inversion karyotypes) exhibited the same aggressivity pic, independently of the location. In *An. gambiae* , all mosquitoes (the three inversion karyotypes) showed the same aggressivity pic; however, mosquitoes tended to bite earlier outdoor than indoor (Figure 3). As previously observed (Sangbakembi-Ngounou, Costantini et al. 2022), outdoor biting events were more concentrated compared with indoor biting events, independently of the karyotype (Table 2, Figure 3). This pattern re-

mained stable across the year with slight differences, mainly related to the low sample size (Figures S1-S3). Overall, we did not observe any difference in biting rhythm in function of the karyotype in both species. We also asked whether biting events were uniformly distributed around the 24-hour diel cycle. The uniformity hypothesis was rejected for all groups, implying that there are different biting pics during the diel cycle (Table 2). Then, we tested whether the different biting distributions were reflective symmetric throughout the day. This hypothesis was systematically rejected for all *An. gambiae* specimens collected indoor (all karyotypes), and for *An. gambiae* and *An. coluzzii* specimens harboring the inverted karyotype and collected outdoor (Table 2), suggesting the existence of multiple distributions (Sangbakembi-Ngounou, Costantini et al. 2022).

Species	Location	Karyotype	n	[?]	R	Uniformity	Uniformity	Reflective symmetry	R
						Rayleigh	p-value	z	p
<i>An. coluzzii</i>	Indoor	Heterozygote	202	0.41	0.39	0.39	< 0.01	0.467	0.
		Inverted	213	1.17	0.19	0.19	< 0.01	0.292	0.
		Standard	190	0.59	0.14	0.15	0.014	0.525	0.
	Outdoor	Heterozygote	129	0.49	0.71	0.71	< 0.01	1.761	0.
		Inverted	129	0.43	0.66	0.66	< 0.01	1.993	0
		Standard	123	0.39	0.70	0.70	< 0.01	0.551	0.
<i>An. gambiae</i>	Indoor	Heterozygote	786	1.50	0.33	0.33	< 0.01	3.316	<
		Inverted	794	2.14	0.31	0.31	< 0.01	3.147	<
		Standard	1546	1.46	0.35	0.35	< 0.01	2.732	<
	Outdoor	Heterozygote	574	0.36	0.75	0.75	< 0.01	0.251	0.
		Inverted	473	0.54	0.80	0.80	< 0.01	2.017	0
		Standard	948	0.49	0.76	0.76	< 0.01	0.993	0.

Table 2. Summary of the circular statistic results for each species, location, and 2La inversion karyotype across the year.

n, number of mosquitoes; [?], mean direction in hour and minutes; R, bias-corrected mean length or dispersion; Uniformity, uniformity tests (Rayleigh statistic and p-value) of biting events during the 24 hours; Reflective symmetry tests (Z statistic and p-value) of biting events during the 24 hours.

Hosted file

image3.emf available at <https://authorea.com/users/485464/articles/570840-temporal-and-biting-dynamics-of-the-malaria-vectors-anopheles-gambiae-and-anopheles-coluzzii-harboring-the-2la-inversion-in-bangui-central-african-republic>

Figure 3. Circular representation of biting events for each karyotype and species.

Biting events are represented by points, the kernel distributions by lines, and the mean directions ([?]) and resulting lengths (R) are depicted as arrow vectors on the unit circle. Darker colors represent indoor collections, and lighter colors outdoor collections.

Environmental drivers of the 2La inversion. The 2La inversion frequency variations are governed by environmental factors (Simard, Ayala et al. 2009). This is particularly relevant at the macrogeographical scale (Ayala, Acevedo et al. 2017). Therefore, we determined whether the 2La inversion frequency was affected by the temporal variations of the local climatic conditions in Bangui. Using a generalized linear model approach, we correlated the 2La inversion frequency with different climate parameters (Table S1). Overall, our models revealed that the local changes in inversion frequency in both species were not related to variations in the local climatic factors (Table S6). This remained true also when we analyzed the 2La inversion frequency variations in function of the sampling location (indoor or outdoor). Similarly, when we

analyzed the frequency variations of the different karyotypes in each species, we observed the same pattern, without significant correlations with the local climatic factors in Bangui (Table S6).

Discussion

Understanding the chromosomal 2La inversion dynamics in the malaria mosquitoes *An. gambiae* and *An. coluzzii* is required to better explain their capacity to adapt to local environmental conditions. Previous studies positioned the city of Bangui in the middle of the 2La inversion gradient (Ayala, Acevedo et al. 2017), with a mean frequency of 40% (Ndiath, Eiglmeier et al. 2016). In agreement, we found 2La inversion frequencies of 38% and 51% in *An. gambiae* and *An. coluzzii*, respectively. However, *An. coluzzii* exhibited significantly higher frequency than its sister species across the year (Figure 1). Although this frequency difference between species in favor of *An. coluzzii* has been already observed in other parts of Africa (Ayala, Acevedo et al. 2017), it was not investigated. Indeed, the fitness advantage for desiccation (Fouet, Gray et al. 2012, Ayala, Zhang et al. 2019) and thermal tolerance linked to the 2La inversion (Gray, Rocca et al. 2009) have been empirically demonstrated only in *An. gambiae*. Looking at the existing gene flow between mosquitoes (we found 20 hybrids, Table S1), we do not expect significant genetic differences. However, these results need to be confirmed by phenotypic tests in both species collected at the same location, and Bangui offers a compelling opportunity to this end. The expected similar frequencies in both species could be distorted also by the 2Rb inversion segregation. The 2Rb inversion is in linked disequilibrium with the 2La inversion (Costantini, Ayala et al. 2009, Simard, Ayala et al. 2009, Ayala, Acevedo et al. 2017), and exhibits signals of selection concerning desiccation resistance (Fouet, Gray et al. 2012, Ayala, Zhang et al. 2019). Unfortunately, in our study we molecularly karyotyped only the 2La and not the 2Rb inversion and therefore, we cannot say whether this rearrangement segregates differently between species. Finally, the strategies to survive during the dry season are different in these two mosquito species, and they could affect the frequency of this rearrangement through the movement of populations. Indeed, *An. gambiae* migrate, whereas *An. coluzzii* stay in the same location during the dry season (Dao, Yaro et al. 2014). We detected Hardy Weinberg Disequilibrium across most of the months in both species, revealing that populations experience asymmetric gene flow levels (Table S3-S5). Although adaptation to local environments may affect also the gene flow (Lenormand 2002), we could expect different connectivity levels for each mosquito species with the surroundings populations. Population genetic analysis across different spatial and time points could help to understand the population dynamics of both vectors across the year.

The 2La inversion exhibits a latitudinal gradient in *An. gambiae* and *An. coluzzii* across Africa that is significantly related to climatic variables (Ayala, Acevedo et al. 2017). We did not observe any significant correlation between the 2La inversion frequency and variations of local environmental factors (including precipitations) in both species (Table S5). Moreover, we investigated whether seasonal changes in inversion frequencies were associated with environmental variables, as described for other Diptera species and also mosquitoes (Hoffmann, Sgro et al. 2004, Guelbeogo, Sagnon et al. 2009, Ayala, Ullastres et al. 2014, Kapun and Flatt 2019). Rishikesh et al. (Rishikesh, Di Deco et al. 1985) detected an association between the 2La inversion frequency in *An. gambiae* and the season, with higher frequency in the dry season. In our study, the 2La inversion frequency remained stable throughout the year. Slight frequency variations were not consistent with the dry (November to April) and rainy seasons (May to October) (Figure 1). Similarly, we did not observe any karyotype-season association (Figure 1). One potential explanation could be the local adaptation to urban habitats. Urban settings offer more stable habitats where mosquitoes can breed in all seasons (i.e. availability of suitable breeding sites). Therefore, aridity selection could be less strong than in rural areas. However, we observed a reduction of mosquitoes during the dry season (Table S2). A comparison with rural sites close to Bangui could elucidate the role of urban settings in the 2La inversion frequency temporal dynamics.

Variations at the local scale can affect the behavioral patterns of mosquito populations. It has been shown that within the same mosquito population, individuals differ in their choice of biting or resting locations (Carnevale, Robert et al. 2009). These changes are governed by intrinsic (genetic) and extrinsic (environmental) factors (Pates and Curtis 2005). The 2La inversion frequency may follow microclimatic changes.

Coluzzi and co-workers (Coluzzi, Sabatini et al. 1979) found that dryer indoor conditions favor the collections of individuals carrying the 2La inversion. In Bangui, we did not observe any significant inversion and karyotype frequency variation between outdoor and indoor locations (Figure 2). Therefore, indoor and outdoor microclimatic conditions seem not to have any effect. However, a recent study in Western Kenya showed the impact of insecticide-treated nets (ITN) on the 2La inversion frequency (Matoke-Muhia, Gimnig et al. 2016), although it did not find any significant difference between indoor and outdoor collections (Matoke-Muhia, Gimnig et al. 2016). In the city of Bangui, two successive mass distribution of ITN occurred in 2006 and 2015 (MSF 2020) that might have influenced the 2La inversion frequency dynamics. Unfortunately, we do not have data on the inversion frequency before bed-net implementation in order to appreciate the ITN potential effect on the inversion frequency. We then studied whether fluctuations in relative humidity during the day could explain changes in the biting times. To this end, we performed a circular analysis of the biting events by mosquitoes harboring each karyotype (Table 2, Figure 2, Figures S1,S2 and S3). Our results did not highlight any biting pattern difference among karyotypes. As previously reported (Sangbakembi-Ngounou, Costantini et al. 2022), mosquitoes bite earlier outdoor than indoor, independently of their karyotype. However, we observed slight differences across the months. Future studies should investigate potential spatial and temporal effects on the biting activity period.

Conclusion

Our study investigated the impact of the 2La inversion on the population dynamics and biting behavior of *An. gambiae* and *An. coluzzii*, the two main malaria vectors in Bangui, Central African Republic. This inversion has a key role in the ecological success of both mosquitoes across Africa (Ayala, Acevedo et al. 2017) and also influences malaria parasite transmission (Riehle, Bukhari et al. 2017). By its position in the middle of the frequency gradient of this inversion, Bangui provides a compelling opportunity to study changes at the local level. Although it is highly polymorphic, the 2La inversion frequency segregated non-randomly, evidencing gene flow barriers. Moreover, we observed significant inversion frequency differences between species, raising questions about different fitness effects or population structure in each species. Conversely, the inversion frequency seemed not to be affected by changes in the local environmental conditions. Moreover, in Bangui, we did not observe spatial and temporal biting changes in function of the inversion karyotype. However, anthropogenic factors related to urban habitats, such as light, temperature, humidity, may disturb the inversion frequency pattern at the micro-geographical level. Future research should compare urban and rural conditions.

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Supplementary tables

Table S1. Monthly summary of climatic data.

Table S2 . List of *Anopheles gambiae* and *Anopheles coluzzii* specimens used in this study.

Date: Year and month of collection; inv2La: 2La inversion karyotype.

Table S3. Hardy-Weinberg Equilibrium tests for each species and month.

The p-value was computed with the exact test. Date: Year and month of collection; number of mosquitoes with the indicated 2La inversion karyotype; Freq_2La: Frequency of the inversion 2La; HWE: Hardy-Weinberg Equilibrium.

Table S4. Hardy-Weinberg Equilibrium tests for each species, month, and location.

The p-value was computed with the exact test.

Table S5. Hardy-Weinberg Equilibrium tests for each species, month, location, and site.

The p-value was computed with the exact test.

Table S6. Generalized linear model to assess the 2La inversion frequency correlation with different climate parameters in the two species (*An. gambiae* and *An. coluzzii*) and in function of their 2La inversion karyotype.

Supplementary Figures.

Supplementary Figure S1. Circular representation of biting events for each month in each species (standard 2La inversion karyotype).

Biting events are represented by points, the kernel distributions by lines, and the mean directions ([?]) and resulting lengths (*R*) are depicted as arrow vectors in the unit circle. Darker colors represent indoor collections, and lighter colors are outdoor collections.

Supplementary Figure S2. Circular representation of biting events for each month, in each species (heterozygote 2La inversion karyotype).

Biting events are represented by points, the kernel distributions by lines, and the mean directions ([?]) and the resulting lengths (*R*) are depicted by arrow vectors in the unit circle. Darker colors represent indoor collections, and lighter colors are outdoor collections.

Supplementary Figure S3. Circular representation of biting events for each month, in each species (inverted 2La inversion karyotype).

Biting events are represented by points, the kernel distributions by lines, and the mean directions ([?]) and the resulting lengths (*R*) are depicted by arrow vectors in the unit circle. Darker colors represent indoor collections, and lighter colors are outdoor collections.

Data Accessibility Statement

All data are included in Supplemental Material

Competing Interests Statement

The authors declare that they have no competing interests.

Authors contributions

CSN and DA designed and conceptualized the study. CSN and CN coordinated the fieldwork. CSN and RAE performed the molecular analysis. CSN, CN, PK and DA performed the statistical analysis and discussed

the results. CSN and DA wrote the first draft of the manuscript. CN, EN and DA provided funding. All authors provided critical feedback on the manuscript.

Acknowledgements

Funding was provided by the International Division of Institut Pasteur Paris, Institut Pasteur in Bangui, and the Institut de Recherche pour le Developpement. The funders had no role in the study design, data collection or analysis, decision to publish, or preparation of the manuscript. We are grateful to Carlo Costantini for R-scripts development for circular statistics of biting events. We thank the volunteers in Gbanikola, Iles des singes, Pk10, and Ouango in Bangui for their collaboration and their compliance during the field investigations. We also thank Neil Longo Pendy, Judicael Obame, Guy-Andre Kpilimbi, Jonas Mbiko-Tanza, Gildas Gonetomy, and Roger Detol for their assistance and discussions.

