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## Spatial Patterns of *Plasmodium falciparum* Clinical Incidence, Asymptomatic Parasite Carriage and *Anopheles* Density in Two Villages in Mali

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**Abstract.** Heterogeneity in malaria exposure is most readily recognized in areas with low-transmission patterns. By comparison, little research has been done on spatial patterns in malaria exposure in high-endemic settings. We determined the spatial clustering of clinical malaria incidence, asymptomatic parasite carriage, and *Anopheles* density in two villages in Mali exposed to low- and mesoendemic-malaria transmission. In the two study areas that were < 1 km<sup>2</sup> in size, we observed evidence for spatial clustering of *Anopheles* densities or malaria parasite carriage during the dry season. *Anopheles* density and malaria prevalence appeared associated in some of our detected hotspots. However, many households with high parasite prevalence or high *Anopheles* densities were located outside the identified hotspots. Our findings indicate that within small villages exposed to low- or mesoendemic-malaria transmission, spatial patterns in mosquito densities and parasite carriage are best detected in the dry season. Considering the high prevalence of parasite carriage outside detected hotspots, the suitability of the area for targeting control efforts to households or areas of more intense malaria transmission may be limited.

### INTRODUCTION

The burden of malaria is unequally distributed in malaria-endemic settings. Substantial differences in malaria transmission intensity exist between regions within a country, between villages and even between individual households within malaria-endemic villages.<sup>1–3</sup> It is estimated that 80% of the morbidity and transmission of malaria is present in less than 20% of the population.<sup>1,4</sup> These patterns of local heterogeneity in malaria transmission have received considerable interest in recent years.<sup>5,6</sup> Although human genetic<sup>7</sup> and behavioral<sup>5,8,9</sup> factors contribute to differences in disease incidence and outcome, small-scale heterogeneity in malaria burden is probably largely explained by micro-epidemiological variations in exposure to malaria-infected mosquitoes.<sup>6</sup> Those who are bitten most have the highest chance of being infected and amplify transmission by transmitting malaria parasites to a large proportion of mosquitoes.<sup>10</sup> The variation in mosquito exposure can partly be explained by the variation in the presence of water bodies, and thus potential mosquito breeding sites, which have been related to malaria morbidity in various urban<sup>5,11,12</sup> and rural<sup>8,13</sup> settings.

It has been hypothesized that by targeting malaria control efforts to areas of intense malaria transmission the community impact of interventions can be maximized.<sup>6</sup> In Mali, western Africa, the coverage and use of insecticide-treated nets (ITNs) in the general population tripled from approximately 20% in 2006 to over 60% in 2008.<sup>10</sup> Still, malaria remains a leading cause of morbidity and mortality, especially in children under the age of 5 years.<sup>14</sup> The use of ITNs remains one of the most valuable malaria control methods, alongside indoor residual spraying and case management with

efficacious artemisinin-based combination therapy. However, these measures are costly and may need large scale implementation with coverage levels nearing 100% to maximize their impact on transmission.<sup>1,10,13,14</sup> If hotspots of higher malaria exposure can be identified and maximum coverage with interventions can be achieved in these hotspots that may fuel malaria transmission to the wider area,<sup>6</sup> this could support malaria control efforts.

Micro-epidemiological variations in malaria exposure are most readily appreciated in areas with low- to moderate-transmission patterns.<sup>1,5,8,13</sup> By comparison, little research has been done on spatial patterns in malaria transmission in high-endemic settings<sup>9,15</sup> that characterize large parts of Mali.<sup>16</sup> We hypothesized that heterogeneity in *Anopheles* density and associated malaria morbidity and asymptomatic parasite carriage can be detected in the peak of transmission in Malian settings and that spatial clustering of malaria cases persists during the subsequent dry season. To test our hypothesis, we concurrently determined symptomatic malaria, asymptomatic malaria parasite carriage, and mosquito densities in two areas with different malaria transmission patterns and vector compositions in Mali.

### METHODS

**Study areas and populations.** The peri-urban settlement of Sotuba is situated in the outskirts of Bamako on the bank of the Niger River with a population of ~6,500 (Figure 1; latitude 12.66200, longitude -7.91601) and characterized by low-intensity malaria transmission (annual entomologic inoculation rate [EIR] < 15 infective bites per person). Malaria incidence in 2000 was 0.8 in 0–5 year olds and 1.3 in 6–10 year olds.<sup>17</sup> Kollé is situated 55 km south of Bamako and is a typical rural Savannah village with a population of ~2,500 (Figure 1; latitude 12.13380, longitude -8.24455) with mesoendemic and seasonal malaria transmission. Average rainfall during the wet season (June–October<sup>16</sup>) was 177 mm in

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FIGURE 1. Map of Africa, Mali with capital Bamako, and study sites Sotuba and Kollé.

Sotuba and 176 mm in Kollé, during the subsequent dry season hardly any rain fell with on average 5 mm and 3 mm, respectively.

Data on mosquito density, human parasite carriage, and malaria incidence were collected from July (Sotuba) and May (Kollé) to December 2009. At both sites, all households were geo-located using a handheld global position system (GPS) (Garmin 62S; Garmin International, Inc., Olathe, KS) and characterized using a questionnaire on household characteristics, the use of preventive measures and socioeconomic factors. Ethical approval for the study protocol was obtained from the Ethics Committee of the Faculty of Medicine, Pharmacist and Dentistry, University of Bamako, Mali.

**Entomological data.** In the wet season, water bodies were identified, geo-located and monitored for productivity based on larval collections from 10 dips with a routine dipper.<sup>18</sup> *Anopheles* breeding sites were defined as water bodies where larvae were present. Permanent water bodies were identified in the dry season and not assessed for productivity. To strategically sample adult mosquitoes by light traps, households in the study areas were stratified into “buffer zones” that were formed by circles around these permanent water bodies, leading to three buffer zones (0–200 m, 201–400 m, > 400 m). For subsequent mosquito catches, 15 houses per buffer zone were randomly selected from the village census list. In case of refusal, another house was selected in the same household or in the next household. Mosquitoes were sampled by placing Centers for Disease Control (CDC) light traps (Model 512; John W. Hock Company, Gainesville, FL) near the sleeping area from dusk till dawn as detailed elsewhere<sup>8</sup> aiming for at least 2–3 households per buffer zone per night. Full details of the number of *Anophelines* caught and the number of trapping nights are given in the supporting information.

**Malaria morbidity data.** Malaria incidence data were collected by passive case detection; the general population was

asked to consult the local health center in case of any signs or symptoms of malaria infection. This system of passive case detection has been operating in the study areas for more than 15 years and, although some clinical episodes may be missed, is deemed efficient in reflecting clinical malaria episodes in the community since the health facility is very accessible and within 1 km of all households. Malaria parasite carriage was also determined by cross-sectional surveys during wet season (July) and dry season (December). All members from households enrolled in entomological data collection were invited to the health center during these surveys and gave informed consent before the start of the study. We aimed for 300 individuals per study area; this number was based on logistical feasibility and not driven by formal sample size considerations that were challenging considering the absence of prior information on spatial patterns in parasite carriage in the study area. For both study areas, a census register is available with all households including family members (name, age, and sex), GPS coordinates, household ID number, and family name; and this data was used to randomly select individuals for consenting until exactly 300 individuals consented for Sotuba and for Kollé; the actual number of participating individuals differed depending on attendance of the central sampling point and the fieldworker ability to locate individuals. In both passive case detection and the cross-sectional surveys, household location (based on the name of the attendee and confirmed by the family name), axillary temperature, age, and sex were recorded and the presence of *Plasmodium* parasites was determined by microscopic thick blood smear performed at the health facility. Participants who tested positive for malaria were treated according to national guidelines with artesunate/amodiaquine.<sup>16</sup>

**Statistical analyses.** The location of all participating households was mapped using ArcGIS 9.2 and permanent water bodies were added manually in ArcGIS using aerial photographs from Google Maps. All statistical analyses were done using SPSS version 20 (IBM Corp. Armonk, NY) and SAS version 9.2 (SAS Institute, Cary, NC), and data were categorized for wet and dry seasons. A clinical malaria episode was defined as measured temperature  $\geq 38^{\circ}\text{C}$  and  $\geq 1,000$  parasites/ $\mu\text{L}$ . In passive case detection, episodes that were > 28 days separated from the previous episode were considered independent episodes. McNemar’s test was used for paired nominal data and Wilcoxon signed rank tests for continuous variables. Because of the highly skewed “*Anopheles* per catch” distribution with excess zeroes, median, and interquartile range (IQR) were considered uninformative descriptive metrics; therefore, means and standard deviations were presented while nonparametric tests were used for statistical comparisons. Quintiles of *Anopheles* densities were calculated for all households in the wet season for Kollé and Sotuba separately; for this mean *Anopheles* densities were calculated per household if mosquitoes were sampled over multiple nights. Nonparametric trend tests were used for trends in malaria parasite carriage, clinical episodes, and *Anopheles* density in relation with proximity to a permanent water body (i.e., present in the dry season). Spatial analysis was performed using SaTScan (Boston, MA) to assess possible circular clusters of malaria clinical incidence (Poisson model), *Plasmodium falciparum* carriage (Bernoulli model), and quintiles of *Anopheles* per catch (nominal model) as described previously<sup>1,19,20</sup>; the maximum radius size was set to 50% of the population and 1 km.

Multivariable logistic regression was used to identify the risk of being in a cluster based on statistically significant explanatory variables in the crude analysis. To adjust for the clustering of data (multiple individuals per household), the general estimating equations method was used.

## RESULTS

**Characteristics of malaria transmission in the study areas.** In the peri-urban area of Sotuba, the only permanent water body, present throughout the year, was formed by a sidearm of the river Niger. In rural Kollé, a swampy area that bordered the village in the north, east, and south was the

main permanent water body. Wet season *Anopheles*-breeding sites were present throughout the study area in Sotuba (Figure 2A), but more related to the borders of the village, and thus the permanent water bodies, in Kollé (Figure 2B). Malaria transmission intensity was markedly different in the two study areas, reflected by differences in malaria parasite prevalence in humans and mosquito density. A total of 297 individuals who consented were successfully sampled in the wet season in Sotuba and 291 in Kollé; in the subsequent dry season these numbers were 265 and 260, respectively. Cross-sectional parasite prevalence in children below 15 years of age in Sotuba was 8% (13/171) in the wet season and 8% (12/158) in the subsequent dry season; in Kollé these figures were 21%

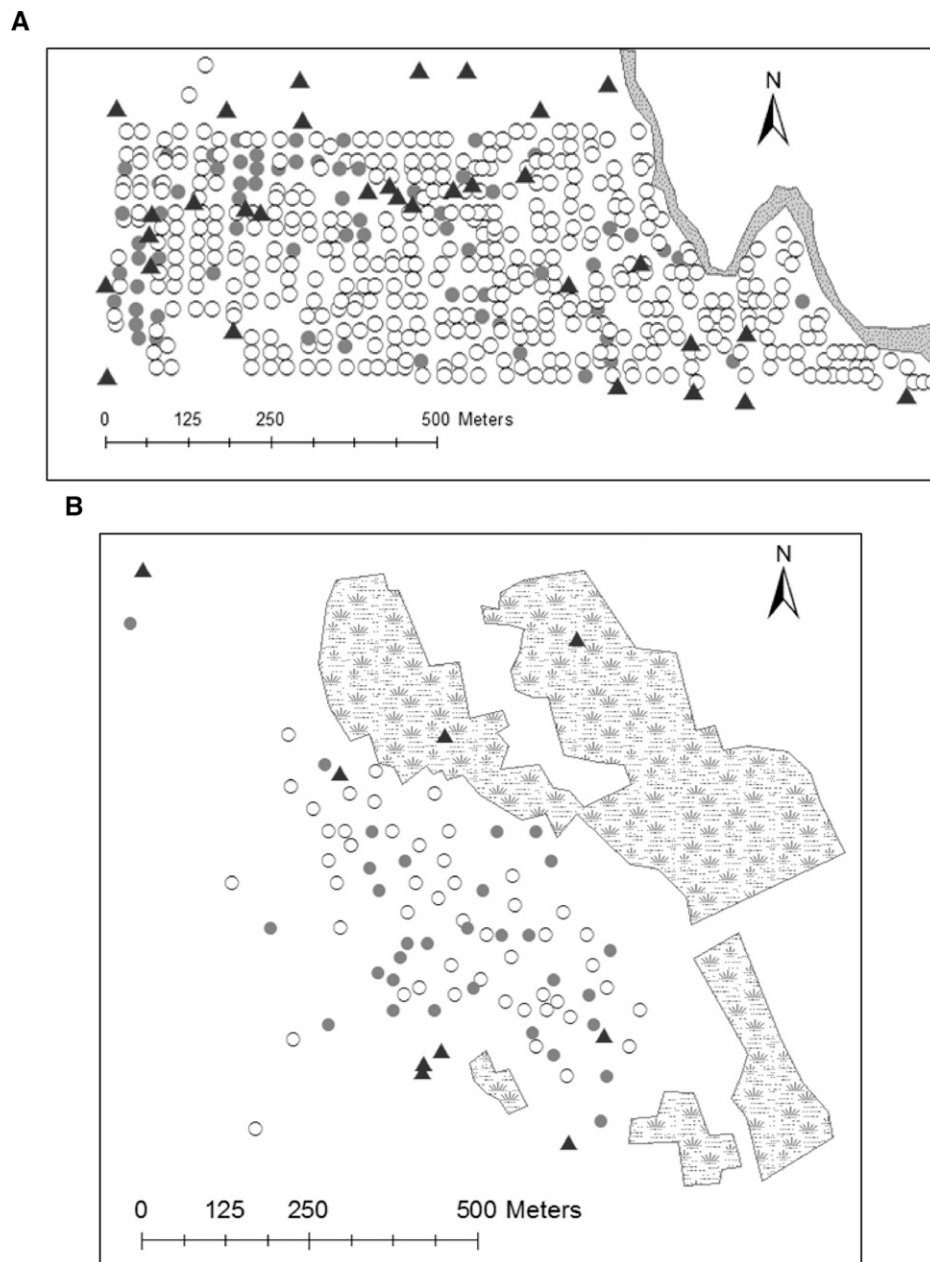


FIGURE 2. Map of Sotuba with (A) a sidearm of the Niger River. Black triangles indicate wet season *Anopheles* breeding sites, dots indicate households and gray dots indicate households with individuals participating in the cross-sectional surveys (B) Map of Kollé surrounding swamps. Black triangles indicate wet season *Anopheles* breeding sites, dots indicate households and gray dots indicate households with individuals participating in the cross-sectional surveys.



(44/207) and 35% (66/191). On the basis of parasite prevalence, malaria transmission was classified low in Sotuba while Kollé was classified as mesoendemic. During the wet season, 204 malaria episodes (defined as measured temperature  $\geq 38^{\circ}\text{C}$  and  $\geq 1000$  parasites/ $\mu\text{L}$ ) were detected in Sotuba (~6,500 inhabitants) and 190 in Kollé (~2,500 inhabitants). This results in an estimated wet season incidence of seven cases per 1,000 person months in Sotuba and 16 in Kollé. Mosquito density also suggested lower malaria transmission intensity in Sotuba compared with Kollé. In Sotuba, 90% (298/330) of all mosquito traps contained  $\geq 1$  *Anopheles* in the wet season with a mean of 12 *Anopheles* per catch. In the subsequent dry season, only 32% (37/117) of the traps contained  $\geq 1$  *Anopheles* with a mean of one per trap ( $P < 0.001$  for wet season versus subsequent dry season). In Kollé, 98% (664/676) of all traps contained  $\geq 1$  *Anopheles* in the wet season with a mean of 26 compared with 58% (152/262) of the traps containing  $\geq 1$  *Anopheles* and a mean of one per trap in the dry season ( $P < 0.001$  for wet season versus subsequent dry season). In the wet season, *Anopheles gambiae* s.l. comprised  $\geq 96\%$  of all caught *Anopheles* in both Sotuba and Kollé. In the dry season, 97% of all *Anopheles* were *An. gambiae* s.l. in Sotuba whereas in Kollé 45% of all *Anopheles* were *An. funestus* (Table 1).

**Clustering of parasite carriage and mosquito density.** Spatial scanning of the two study areas was undertaken to detect clusters of higher or lower parasite prevalence or *Anopheles* density (in quintiles); spatial scans were performed for the wet and dry seasons separately. These scans revealed one statistically significant cluster of higher parasite prevalence (“hotspot”) in the dry season in Sotuba (Figure 3;  $P = 0.011$ ), one cluster of lower *Anopheles* density (“coldspot”) in the dry season in Sotuba ( $P = 0.029$ ) and one hotspot of higher *Anopheles* density in the dry season in Kollé ( $P = 0.040$ ). In the wet season, no clusters of higher or lower parasite prevalence or mosquito density were detected in either of the two study areas. The hotspot of higher parasite prevalence in the dry season in Sotuba was located near the side-arm of the river Niger, contained only two households with 10 sampled individuals and was characterized by 50% (5/10) parasite-positive individuals in the dry season compared with 3% (9/273) among other villagers ( $P < 0.001$ ) and a significantly higher mean number of *Anopheles* per catch (Table 2;  $P = 0.023$ ). The coldspot of lower mosquito density in the dry season in Sotuba was characterized by significantly

lower parasite prevalence in the human population in the dry season cross-sectional survey (Table 2; 2% inside versus 12% outside the coldspot,  $P = 0.013$ ) and a lower mean number of *Anopheles* per trap ( $P = 0.003$ ). The hotspot of high *Anopheles* density in Kollé in the dry season was surrounded by swampy areas and was not characterized by a significantly higher parasite prevalence in the human population (40% inside versus 30% outside the hotspot,  $P = 0.129$ ), but significantly higher mosquito density for all used (and related) indices of mosquito density (Table 2). The proportion of households with at least one clinical malaria episode did not differ between hotspots and coldspots. We observed no statistically significant geographical clustering of clinical incidence cases in Sotuba and Kollé (data not shown).

**Factors explaining parasite prevalence and mosquito density.** The hotspot of higher parasite prevalence in Sotuba had lower reported bed net use in the previous night (Table 2;  $P = 0.032$ ), whereas the coldspot of *Anopheles* density presented with no statistically significant differences in human and household risk factors. Households in the hotspot of *Anopheles* density in Kollé presented with a significantly lower number of open eaves (OR = 0.62; 95% CI = 0.50–0.78;  $P < 0.001$ ) and smaller window surfaces (OR per  $\text{m}^2 = 0.89$ , 0.80–0.998;  $P = 0.046$ ), after adjusting for potential confounders. Other factors such as wall structure, floor structure, livestock, or water near the house did not differ between clusters and the surrounding areas. Proximity to a permanent water body was nonsignificantly related to higher *P. falciparum* carriage in both Sotuba (Table 3;  $P = 0.070$ ) and Kollé ( $P = 0.058$ ), and significantly related to *Anopheles* density in Kollé ( $P < 0.001$ ).

## DISCUSSION

There is an increasing awareness that malaria exposure is highly heterogeneous across endemic settings. This heterogeneity is most easily recognized at a larger spatial scale (e.g., differences in transmission intensity between regions), but also present within geographically confined regions such as individual villages.<sup>1–3</sup> Although most research on heterogeneity in malaria transmission has focused on low endemic areas or areas where transmission declined recently,<sup>1,5,8,15</sup> we determined spatial patterns in *Anopheles* density and malaria parasite prevalence in two areas in Mali where malaria transmission is by comparison intense and highly seasonal.<sup>21</sup> We selected two areas with

TABLE 1  
Parasitological and entomological characteristics of both study areas

	Sotuba			Kollé		
	Low transmission			Mesoendemic transmission		
	Wet season	Dry season	<i>P</i> value	Wet season	Dry season	<i>P</i> value
Parasite prevalence, % ( <i>n/N</i> )						
< 5	14 (4/28)	8 (2/24)	1.000	14 (8/59)	35 (19/54)	0.019
5–14	6 (9/143)	7 (10/134)	1.000	24 (36/148)	34 (47/137)	0.059
$\geq 15$	3 (4/126)	2 (2/107)	1.000	13 (11/84)	26 (18/69)	0.035
Number of catches (number of households)	330 (69)	117 (59)	–	676 (31)	262 (27)	–
Catches with $\geq 1$ <i>Anopheles</i> , % ( <i>n/N</i> )	90 (298/330)	32 (37/117)	< 0.001	98 (664/676)	58 (152/262)	< 0.001
Mean number of <i>Anopheles</i> /catch (SD)	12 (18)	1 (2)	< 0.001	26 (19)	1 (1)	< 0.001
<i>Anopheles</i> species composition*						
<i>An. gambiae</i> s.l., % ( <i>n/N</i> )	99 (13856/14,016)	97 (401/415)	< 0.001	96 (142,070/147,699)	55 (1,623/2,939)	< 0.001
<i>An. funestus</i> , % ( <i>n/N</i> )	1 (160/14,016)	3 (14/415)		4 (5,629/147,699)	45 (1,316/2,939)	

SD = standard deviation.

\*Species composition of all anophelines caught in this village and season.

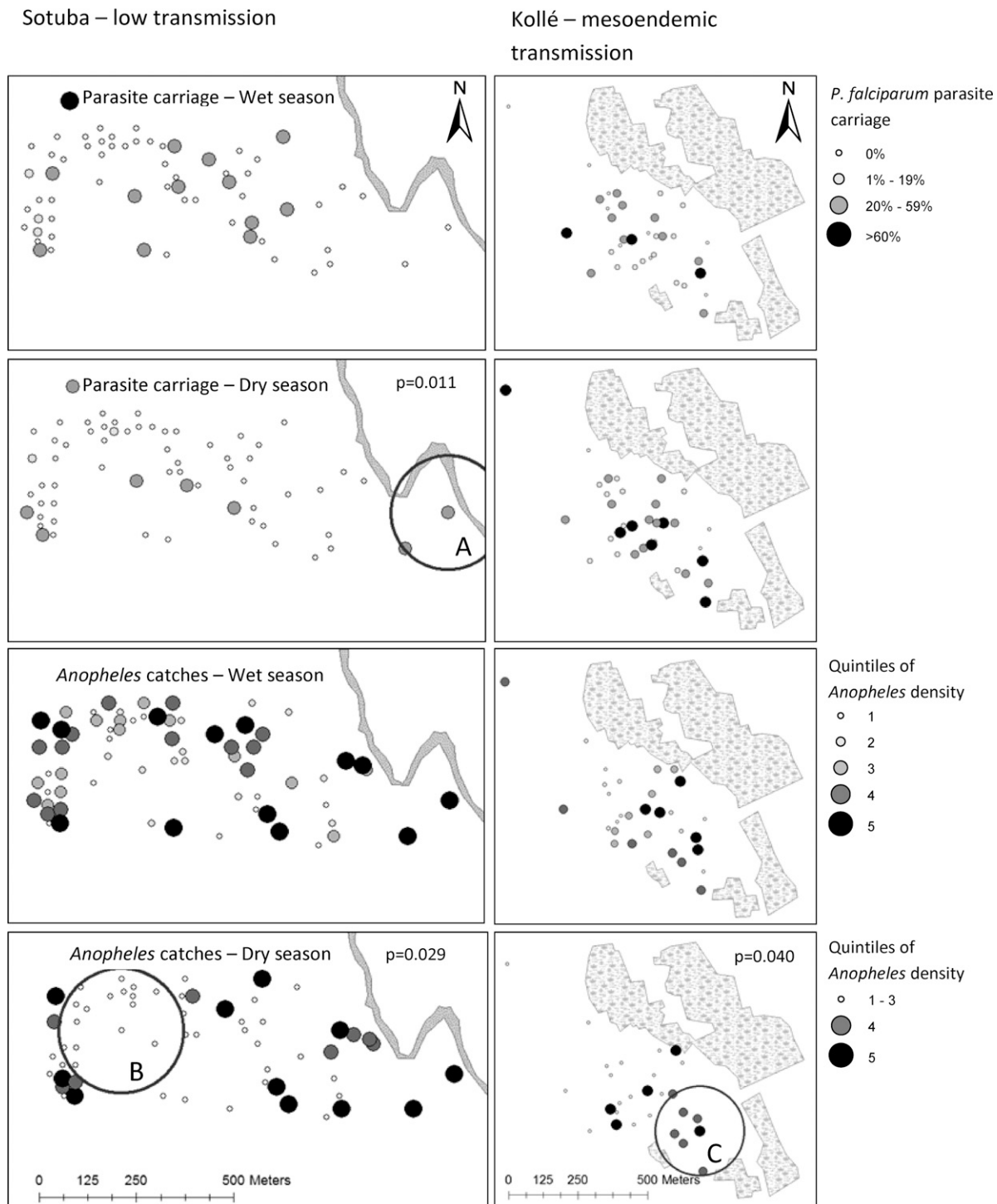


FIGURE 3. Household *Plasmodium falciparum* parasite carriage and *Anopheles* density in quintiles prevalence maps in both the wet season and subsequent dry season. On the left side of the panel Sotuba and on the right side of the panel Kollé. Maps show only households with individuals included in the cross-sectional surveys and mosquito prevalence maps are based on quintiles of *Anopheles* density. One hotspot of *P. falciparum* parasite carriage is shown in Sotuba (cluster A;  $P = 0.011$ ), one coldspot of *Anopheles* density is shown in Sotuba (cluster B;  $P = 0.029$ ) and one hotspot of *Anopheles* density is shown in Kollé (cluster C;  $P = 0.040$ ); all during dry season.

markedly different malaria transmission characteristics: a peri-urban area exposed to low-transmission intensity and a rural mesoendemic village. In the two study areas that were  $< 1 \text{ km}^2$  in size, we observed evidence for spatial clustering of *Anopheles* densities or malaria parasite carriage. Although spatial clustering in Malian settings has been described before,<sup>16</sup> our findings are

striking since the study areas were relatively small in size and mosquitoes could easily reach the extremities of these settings.<sup>22</sup>

We hypothesized a contraction of the parasite reservoir in humans from the wet season into the dry season, both in terms of the number of parasitaemic individuals and their locality.<sup>6</sup> However, seasonality in human parasite prevalence was

TABLE 2

Parasitological, clinical, and entomological characteristics of statistically significant spatial clusters of *Plasmodium falciparum* parasite carriage or *Anopheles* density

Study area (transmission level)		Cluster A			Cluster B			Cluster C		
		Sotuba (low)			Sotuba (low)			Kollé (mesoendemic)		
Description (season)		Hotspot of <i>P. falciparum</i> parasite carriage (dry)			Coldspot of <i>Anopheles</i> density (dry)			Hotspot of <i>Anopheles</i> density (dry)		
Radius of cluster in meters ( <i>P</i> value)		140 (0.011)			160 (0.029)			160 (0.040)		
Number of sampled households		Inside cluster 2			25			10		
		Outside cluster 71			48			28		
Malaria parasite carriage and morbidity		Inside cluster 50 (5/10)			2 (2/84)			40 (29/72)		
Parasite prevalence, % ( <i>n/N</i> )		Outside cluster 3 (9/273)			12 (21/181)			30 (59/194)		
		<i>P</i> value < 0.001			0.013			0.129		
Households with ≥ 1 malaria episode, % ( <i>n/N</i> )		Inside cluster 10% (2/2)			60 (15/25)			100 (10/10)		
		Outside cluster 23 (15/66)			65 (28/43)			89 (25/28)		
		<i>P</i> value 0.060			0.673			0.552		
<i>Anopheles</i> density		Inside cluster 100 (6/6)			0 (0/31)			100 (76/76)		
Catches with ≥ 1 <i>Anopheline</i> , % ( <i>n/N</i> )		Outside cluster 28 (31/111)			43 (37/86)			41 (76/186)		
		<i>P</i> value 0.002			< 0.001			< 0.001		
Households in 4th or 5th quintile of <i>Anopheles</i> density, % ( <i>n/N</i> )		Inside cluster 100 (2/2)			0 (0/25)			70 (7/10)		
		Outside cluster 29 (17/59)			40 (19/48)			14 (4/28)		
		<i>P</i> value 0.187			0.002			0.004		
Mean number of <i>Anopheles</i> /catch (SD)		Inside cluster 2.8 (0.3)			0.1 (0.2)			1.8 (0.6)		
		Outside cluster 0.7 (1.5)			1.1 (1.7)			1.2 (0.9)		
		<i>P</i> value 0.023			0.003			0.013		
Household factors potentially explaining malaria morbidity and <i>Anopheles</i> density		Inside cluster 50 (4/8)			81 (64/79)			73 (52/71)		
Bed net use, % ( <i>n/N</i> )		Outside cluster 84 (223/266)			83 (138/167)			80 (154/193)		
		<i>P</i> value 0.032			0.757			0.254		
Household with open eaves, % ( <i>n/N</i> )		Inside cluster 100 (2/2)			73 (16/22)			11 (1/8)		
		Outside cluster 70 (46/66)			70 (32/46)			73 (16/22)		
		<i>P</i> value 1.000			0.789			0.004		
Median window surface in m <sup>2</sup> (IQR)		Inside cluster 225 (–)			438 (300–750)			500 (313–975)		
		Outside cluster 375 (300–750)			375 (300–800)			3,600 (1,506–8,575)		
		<i>P</i> value 0.094			0.406			< 0.001		

IQR = interquartile range; SD = standard deviation.

unpronounced in our low endemic village Sotuba and parasite prevalence was unexpectedly higher in the dry season in the mesoendemic village of Kollé. The latter observation could be explained by the timing of our surveys; we conducted our dry season survey 1 month after the end of the wet season. This was potentially too early; parasite prevalence in humans reflects transmission in the preceding months and may increase following a peak in exposure to infected *Anopheles* mosquitoes, and therefore take several months to decline after mosquito density has declined.<sup>8</sup> It is therefore possible that a contraction in parasite carriage would have been detectable at a later time point in the dry season. In the peak transmission season, we observed no statistically significant clustering of *Anopheles* density or parasite carriage, possibly related to the

abundant mosquito breeding sites that we detected throughout the study areas in the wet season and the small size of the villages that allowed mosquitoes to reach the extremities.<sup>4</sup> *Anopheles* densities were approximately more than 10-fold higher than in the subsequent dry season. In this dry season, we observed one hotspot of higher parasite carriage in our low endemic village, close to the river. Households in this hotspot were also more likely to have a clinical malaria episode and mosquito traps indicated higher *Anopheles* densities; however, this finding should be interpreted with caution since this hotspot only includes two household with 10 sampled individuals. In the same village, we also detected a coldspot of lower *Anopheles* densities, located furthest away from the river. Household members in this coldspot were less likely to

TABLE 3

*Plasmodium falciparum* parasite prevalence and *Anopheles* density relative to proximity to a permanent mosquito breeding site. *P. falciparum* parasite carriage episodes in either the wet season or the dry season and *Anopheles* density during the wet season

% ( <i>n/N</i> )	Sotuba		Kollé	
	Low transmission		Mesoendemic transmission	
Distance to breeding site (m)	<i>P. falciparum</i> parasite prevalence	Proportion in 4th or 5th quintile of <i>Anopheles</i> density	<i>P. falciparum</i> parasite prevalence	Proportion in 4th or 5th quintile of <i>Anopheles</i> density
0–200	26 (5/19)	52 (12/23)	73 (11/15)	80 (16/20)
201–400	9 (5/53)	41 (26/64)	43 (67/155)	57 (74/129)
> 400	9 (18/196)	35 (68/193)	39 (39/100)	9 (8/88)
<i>P</i> value for trend	0.070	0.105	0.058	< 0.001

be parasite positive in the dry season. The only household factor that was associated with this clustering of malaria risk was reported bed net use, which was lower in the hotspot of higher malaria parasite prevalence. In our mesoendemic village, we detected one hotspot of higher *Anopheles* densities, surrounded by swampy areas. Household members in this hotspot had a numerically higher parasite prevalence compared with surrounding areas but this was not statistically significant. Households in this hotspot had smaller windows and were more likely to have closed eaves. The reasons for these apparently counter intuitive associations are unclear. One may speculate that these households may have had a more favorable temperature for mosquitoes, as shown before,<sup>23</sup> or were adapted in response to high mosquito exposure in that part of the village. Alternatively, it may be a chance finding and houses were coincidentally of higher quality in this area. Our findings indicate that within small villages exposed to low- or mesoendemic-malaria transmission, spatial patterns in mosquito densities, and parasite carriage are best detected in the dry season. Although statistically significant clustering was detected, it is debatable whether this clustering is sufficient to justify targeted interventions. Many households with high parasite prevalence or high *Anopheles* densities were located outside the identified hotspots and parasite carriage and mosquito exposure were abundant throughout the examined villages. It is our opinion that malaria transmission may be too widely dispersed in our two villages to expect an impact of hotspot-targeted interventions on malaria transmission outside these hotspots.

In conclusion, this study presents insight into malaria transmission dynamics in two areas in Mali exposed to low- and mesoendemic-malaria transmission. We observed spatial clustering in *Anopheles* densities and *P. falciparum* parasite carriage. *Anopheles* exposure and malaria prevalence appeared associated in some of our detected hotspots. However, since malaria transmission was this widespread, the suitability of the area for targeting control efforts to households or areas of more intense malaria transmission may be limited.

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