

## The Impact of Periodic Distribution Campaigns of Long-Lasting Insecticidal-Treated Bed Nets on Malaria Vector Dynamics and Human Exposure in Dielmo, Senegal

Seynabou Sougoufara,<sup>1,2\*</sup> Omar Thiaw,<sup>1</sup> Aurélie Cailleau,<sup>3,4</sup> Nafissatou Diagne,<sup>1</sup> Myriam Harry,<sup>5</sup> Charles Bouganali,<sup>1</sup> Pape M. Sembène,<sup>2</sup> Souleymane Doucoure,<sup>1</sup> and Cheikh Sokhna<sup>1</sup>

<sup>1</sup>Aix Marseille University, Institut de Recherche pour le Développement (IRD) (Dakar, Marseille, Papeete), AP-HM, Institut Hospitalo-Universitaire-Méditerranée Infection, UMR Vecteurs—Infections Tropicales et Méditerranéennes (VITROME), Marseille, France; <sup>2</sup>Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop de Dakar, Dakar Fann, Sénégal; <sup>3</sup>Unité d'Entomologie Médicale (UME), Institut Pasteur Dakar, Dakar, Sénégal; <sup>4</sup>Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS), Yopougon, Abidjan, Côte d'Ivoire; <sup>5</sup>UMR Évolution, Génomes, Comportement, Écologie (EGCE) Centre National de la Recherche Scientifique (CNRS), IRD-University Paris-Sud, IDEEV, University Paris-Saclay, Gif-sur-Yvette Cedex, France

**Abstract.** The implementation of long-lasting insecticidal-treated bed nets (LLINs) has contributed to halving the mortality rate due to malaria since 2000 in sub-Saharan Africa. These tools are highly effective against indoor-feeding malaria vectors. Thus, to achieve the World Health Assembly's new target to reduce the burden of malaria over the next 15 years by 90%, it is necessary to understand how the spatiotemporal dynamics of malaria vectors and human exposure to bites is modified in the context of scaling up global efforts to control malaria transmission. This study was conducted in Dielmo, a Senegalese village, after the introduction of LLINs and two rounds of LLINs renewals. Data analysis showed that implementation of LLINs correlated with a significant decrease in the biting densities of the main malaria vectors, *Anopheles gambiae* s.l. and *Anopheles funestus*, reducing malaria transmission. Other environment factors likely contributed to the decrease in *An. funestus*, but this trend was enhanced with the introduction of LLINs. The bulk of bites occurred during sleeping hours, but the residual vector populations of *An. gambiae* s.l. and *An. funestus* had an increased propensity to bite outdoors, so a risk of infectious bites remained for LLINs users. These results highlight the need to increase the level and correct use of LLINs and to combine this intervention with complementary control measures against residual exposure, such as spatial repellents and larval source management, to achieve the goal of eliminating malaria transmission.

### INTRODUCTION

The burden of malaria transmission has dramatically decreased all over the world in recent years, with an estimated 18% reduction in malaria cases and a 48% reduction in malaria deaths since 2000.<sup>1</sup> However, the African continent still remains the most endemic area, and it is estimated that 90% of malaria deaths take place there, despite substantial control efforts being undertaken. Since 2000, the proportion of the population in sub-Saharan Africa sleeping under long-lasting insecticidal-treated bed nets (LLINs) rose from an estimated less than 2% to 55% in 2015.<sup>1</sup> The implementation of vector control tools that reduce human exposure to malaria vectors is followed by a decrease in the frequency of mosquitoes' blood feeding, which has led to a drop in their density.<sup>2,3</sup> Consequently, a decrease has been observed in malaria transmission. In several sub-Saharan African countries, the use of LLINs has contributed to the reduction of the malaria-associated morbidity.<sup>4–6</sup> Furthermore, the use of LLINs has contributed toward achieving very low levels of parasites transmission and moving from perennial to short seasonal transmission periods in some endemic malaria areas. In addition, Bhatt et al.<sup>7</sup> reported that since 2000, 663 million clinical cases have been avoided and that 68% of this has resulted from the use of LLINs. These impressive results have revived the hope of eliminating malaria and have led the World Health Organization (WHO), through the World Health Assembly, to adopt a technical report for the period of 2016–2030 to achieve an ambitious new target for reducing the global malaria burden by 90% over the next 15 years from

the 2015 baseline.<sup>8</sup> To reach this goal, the World Health Assembly recommends strengthening malaria control by achieving universal coverage for first-line vector control tools i.e., LLINs supported by other interventions such as indoor residual spraying for all at-risk populations. Indeed, Griffin et al.<sup>9</sup> reported through a mathematical model of malaria that the incidence of malaria would increase by 21% from 2015 to 2030 if the current level of LLIN coverage combined with suitable diagnostic and treatment of malaria cases is not increased. Indeed, resurgences in malaria transmission have been reported in many areas in the sub-Saharan Africa after the implementation of LLINs.<sup>10–15</sup> This situation justifies the efforts to increase the LLIN coverage, renew them on a regular basis to maintain their effectiveness, and to improve their level of use. Furthermore, the effectiveness of these indoor interventions is also threatened by the spread of pyrethroid resistance in the main vectors.<sup>16,17</sup> Pyrethroids are the only compound approved by WHO to treat bed nets. In addition, the behavioral changes of vector and the shift in species composition resulting from the use of LLINs may also compromise the efficacy of malaria control tools.<sup>18–24</sup>

Indeed, the strategy to use LLINs is mainly effective against those malaria vectors that are mostly anthropophilic, and bite and rest inside rooms.<sup>25</sup> These are the distinctive characteristics of the main African malaria vectors that belong to the *Anopheles gambiae* complex and the *Anopheles funestus* group. In sub-Saharan Africa, malaria vectors that can occur in sympatry have differential vectorial capacities and, consequently, the effectiveness of vector control interventions could depend on the species occurring in a given place.

Because of these various pitfalls, the WHO technical report for 2016–2030<sup>8</sup> emphasizes the prerequisite to perform high-quality surveillance data collection to implement effective vector management to achieve the goal of eliminating malaria. Thus, entomological monitoring in areas where LLIN

\* Address correspondence to Seynabou Sougoufara, VITROME, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin 13385 Marseille Cedex 05, France. E-mail: seynabou.sougoufara@ird.fr

coverage has been implemented for several years is needed to provide critical information to tackle the current deficiencies of the use of bed nets that could maintain residual malaria transmission. For this purpose, the present study was undertaken to investigate the longitudinal dynamics of malaria vectors before and after three LLIN distribution campaigns.

## METHODS

**Study area.** The study was conducted in Dielmo, a village located 280 km southeast of Dakar, in Senegal. The climate in the village is typical of the Sudanian Savanna and the rainy season occurs from June to October with an annual average rainfall ranging from 400 to 900 mms. Most of the inhabitants in Dielmo belong to the Sereer ethnic group and are cattle raisers and agricultural workers. They live in close contact with their livestock, which is kept in their houses at night. In 1990, the village was selected for a longitudinal survey to investigate the determinants of malaria transmission, because of its high malaria prevalence.<sup>26</sup> In 2006, Artemisinin-based Combination Therapy was introduced in Dielmo as the first-line treatment against malaria. In addition, campaigns to encourage a general coverage of LLINs (active ingredient: deltamethrin; PermaNet® 2.0, Vestergaard Frandsen Group SA, Lausanne, Switzerland) have been implemented since 2008. These strategies have been followed by a decrease in the prevalence of infection with malaria parasites provided by thick blood films, which fell between 2006 and 2008 from 36.8% to 12.3% in children aged 0–14 years, and from 27.6% to 9.0% in people aged 15 years and older.<sup>27</sup> However, two episodic resurgences of malaria transmission were observed in the village in 2010 and 2013.<sup>10,28</sup> After each resurgence of malaria, LLIN coverage was renewed, in July 2011 and in August 2014, respectively. In May 2016, the Senegalese National Malaria Control Program (NMCP) renewed all LLINs in the country, including the ones in Dielmo that are in line with the WHO recommendations to achieve the objective of eliminating the transmission of malaria. Therefore, according to the distribution of LLINs, four periods were defined: period 1 (P1) before LLIN coverage in Dielmo, from July 2006 to June 2008; period 2 (P2), from July 2008 to June 2011; period 3 (P3), from July 2011 to June 2014; and period 4 (P4), from August 2014 to April 2016. Period 1 lasted 2 years, whereas P2 and P3 lasted for 3 years each, with one rainy season per year. In P4, the LLINs were renewed by the Senegalese NMCP in May 2016; indicating that P4 lasted less than 2 years (21 months) and covered two rainy seasons.

**Field study and laboratory processing.** For each study period, mosquitoes were collected every month using human landing catches (HLCs). Human landing catches were performed indoors and outdoors at two sentinel sites over two or three consecutive nights per month between 7 PM and 7 AM. All mosquitoes were morphologically identified using keys of Gilles and De Meillon<sup>29</sup> and kept individually in Eppendorf tubes with silica. The presence of the *Plasmodium falciparum* circumsporozoite protein (CSP) antigen in each *Anopheles* specimen was detected in the crushed head and thorax using the enzyme-linked immunosorbent assay-CSP technique.<sup>30</sup>

Monthly rainfall records were obtained using a rain gage in the village.

**Statistical analyses.** *Variations in per person per night biting rates.* Variations in mosquitoes' biting rates summed

across nights were analyzed for *An. gambiae* s.l. and *An. funestus* populations using a general linear mixed model (GLMM) assuming a negative binomial distribution of the response variable (mosquito bites) with the fixed factors being, the species, rainfall, or month (these two variables, being highly correlated, were not included in the same models), period (P1, P2, P3, or P4), and location site (indoors or outdoors). We also included random factors: the date of capture (two to three nights per month, making 357 levels) and the house (two houses per year, making 22 levels). The dual interaction between all fixed factors was also analyzed in the model, and the triple interactions involving the species, to compare the dynamics of bites between the *An. gambiae* s.l. and the *An. funestus* populations.

The model was fitted using the glmmADMB library<sup>31,32</sup> in the R Software Version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria),<sup>33</sup> on a data set of 2,632 rows. Random effects significance was assessed with a likelihood ratio test (with the anova function in R), whereas fixed effects significance was assessed via model selection (Akaike Information Criterion [AIC] comparisons), where more than one reasonably well-fitting models (the difference in their AIC is less than 10)<sup>34</sup> were identified, but the simplest was preferred. Where *P* values were used, statistically significant was defined as *P* < 0.05.

The human biting rates according to the mosquito species, the period, and the site of collection were calculated using the *predict* R function on the best selected model. Post hoc comparisons were made by running glmm similar to the previously identified best model, on subsets of the data set (in particular, on subsets by species and by periods). The correlation between the abundance of *An. gambiae* s.l. and *An. funestus* and the rainfall data was also measured using Spearman correlation coefficients in the R software.

**Variations in nocturnal activity.** To analyze the effect of nocturnal activity, we desegregated the data set (so far aggregated by the night of capture) to count the number of bites when people are awake (from 7 PM to 10 PM and 5 AM to 7 AM, according to our observations), and when people are sleeping (from 10 PM to 5 AM). We refitted the best model identified in the previous analysis to this desegregated data set (5,264 rows), and then added the new binary variable (sleeping/awake) and its interactions, with the other variables, sequentially. Again, a given term was kept only if AIC was diminished by more than 10.

The variation in the global, *An. gambiae* s.l., and *An. funestus* exposures across periods was then analyzed following the Killeen et al.<sup>35</sup> methodology which is as follows: the mean biting rate experienced by an unprotected individual (an individual not using mosquito nets);  $B_u$ , was calculated by summing the expected number of bites when the person is awake and outdoors; and the expected number of bites when the person is sleeping and indoors. Similarly, the mean biting rate experienced by a protected individual (an individual always using mosquito nets),  $B_p$ , was calculated by summing the number of bites when the person is awake and outdoors (from 7 PM to 10 PM and 5 AM to 7 AM) and the expected number of bites when the person is sleeping indoors under the net (assuming a protective effect of 80%, which means the number of bites under the net is 20% of the indoor biting rate, from 10 PM to 5 AM). The relative biting rate for insecticide-treated bed nets (ITN) users,  $\lambda_p$ , is then the ratio between these two values ( $B_p/B_u$ ), and the protective efficacy of an ITN

against a given species of *Anopheles* ( $P^*$ ) is  $1 - \lambda_p$ . Eventually, a last indicator, that is useful to interpret  $P^*$ , is  $\pi_i$ , the proportion of bites occurring indoors for an unprotected individual, which can be calculated as the number of bites predicted to occur indoors, divided by the total number of bites predicted to occur both indoors and outdoors.

**Infection and entomological inoculation rates.** The occurrence of infection (positive/negative) in collected mosquitoes was analyzed as a function of species and period using a GLM with a binomial distribution.

Eventually, annual entomological inoculation rates were calculated for each period based on the infection rates and the exposure of the bed net users (protected individuals) and the nonusers (unprotected individuals), respectively. The global protective efficacy of an ITN (against infected species of *Anopheles*),  $P^{*f}$  is  $1 - (EIR_p/EIR_u)$ .

## RESULTS

**Mosquito composition collection and abundance.** A total of 41,637 female mosquitoes were collected using HLCs between July 2006 and April 2016 with 1,324 person-nights (Table 1). The anophelines represented 37.3% (15,536) of the mosquitoes collected and the culicines, represented here by the *Culex*, *Aedes* and *Mansonia* genera represented 62.7% (26,101).

Regarding the *Anopheles* species, *An. gambiae* s.l. and *An. funestus* were the major species captured with 24.7% and 11.8% of the mosquitoes' collection, respectively. Other anophelines such as *Anopheles nili*, *Anopheles pharoensis*, *Anopheles pretoriensis*, *Anopheles welcomei*, *Anopheles zeimani* and *Anopheles brunnipes* were also collected but at low density and represented less than 1%. It is interesting to note that the number of specimens collected for these minority *Anopheles* species ranged annually from 1 to 49 individuals over the course of the whole study, apart from 2009, 1 year after the first implementation of LLINs, when at least 100 specimens were collected (Table 1).

**Variations in per person per night biting rates. Model selection.** According to the GLMM analysis, species, period, site of collection, rain, and month all had a significant effect on the number of bites (Table 2). The effect of rain being highly correlated with the effect of month, and the effect of month explaining much more variance, the effect of month was

selected over the effect of rain in model selection (but note that when processing model selection with rain instead of month, the same terms were deleted). The best model was *model\_1e* that is close to the full model except that the triple interactions Species  $\times$  Site of collection  $\times$  Month and Species  $\times$  Site of collection  $\times$  Period are removed ( $\alpha = 2.0774$ , zero inflation:  $P = 0.015$ ). Both random factors (house and night of capture) had a significant effect (house variance = 0.072, night of capture variance = 0.242), as well as the Species  $\times$  Period  $\times$  Month interaction, i.e., the effect of months differ by period but differently for *An. gambiae* s.l. and *An. funestus*.

**Differences between periods.** Before the implementation of the LLINs at P1, both *An. gambiae* s.l. and *An. funestus* were present in Dielmo. During that period, *An. funestus* was the prevalent species with 12.5 bites per person per night (b.p.n), while *An. gambiae* s.l. accounted for 8.7 b.p.n (Figure 1). However, during the P2 period which coincided with the first implementation of the LLINs, the human biting rates of *An. funestus* dropped substantially to 1.0 b.p.n, and that of *An. gambiae* s.l. increased slightly (12.3 b.p.n). The biting density of *An. funestus* remained steady between 1 and 2 b.p.n in P3 and P4 respectively. At the same periods, *An. gambiae* s.l. biting rates fell from 4.8 b.p.n in P3 to 1.7 b.p.n in P4. In summary, *An. funestus* was originally predominant, but *An. funestus* bites were much less frequent beginning in P2, while the number of *An. gambiae* s.l. bites first increased in P2, such that it was prevalent in this period, and then decreased steadily in P3 and P4. In P4, the number of bites of both species was very low compared with P1.

**Seasonal variations.** Monthly variations must be described taking into account rainfall (Figure 2) and, according to the model, depend greatly on the period and species (month  $\times$  period  $\times$  species interaction).

As previously mentioned, rainfall and month were highly correlated but the effect of month better explained the variance in the model. The correlation between the number of bites and rainfall was low but significant for *An. funestus* (Spearman coefficient  $r = 0.088$ ,  $P = 0.001$ ), and moderate and significant for *An. gambiae* s.l. (Spearman coefficient  $r = 0.402$ ,  $P < 0.001$ ). Indeed, *An. funestus* was the most abundant species during and after the rainy season in 2006 (Figure 2). The abundance of the species decreased after the rainy season of 2007 and remained low in the beginning of 2008. It seems to drop even lower right after the implementation of the

TABLE 1  
Number of mosquitoes collected between July 2006 and April 2016

Year	<i>Anopheles gambiae</i> s.l.	<i>Anopheles funestus</i>	Other <i>Anopheles</i>	<i>Aedes</i> sp.	<i>Culex</i> sp.	<i>Mansonia</i> sp.	Total <i>Aedes</i> , <i>Culex</i> , and <i>Mansonia</i>	Total <i>Anopheles</i>	Total	PN	Rainfall (mm)
2006	753	1,894	9	1,279	1,172	–	2,451	2,656	5,107	72	432.4
2007	966	1,074	9	651	671	–	1,322	2,049	3,371	96	426.3
2008	1,450	300	36	929	2,491	–	3,420	1,786	5,206	104	838.4
2009	2,398	156	117	4,658	672	2	5,332	2,671	8,003	140	903.2
2010	1,869	123	49	1,761	361	0	2,122	2,041	4,163	144	745.6
2011	971	262	21	201	2,532	764	3,497	1,254	4,751	144	667.7
2012	395	145	17	96	3,050	354	3,500	557	4,057	144	949.2
2013	781	281	35	34	660	474	1,168	1,097	2,265	144	949.7
2014	354	353	40	143	682	197	1,022	747	1,769	144	629.1
2015	316	248	25	18	1,908	43	1,969	589	2,558	144	813.9
2016	23	65	1	0	258	40	298	89	387	48	0
Total	10,276 (24.7)	4,901 (11.8)	359 (0.9)	9,770 (23.5)	14,457 (34.7)	1,874 (4.5)	26,101 (62.7)	15,536 (37.3)	41,637	1,324	7,896

PN = number of person-nights per year.

Value in parentheses show the relative proportions in percentages of species sampled.

TABLE 2  
Per person per night dynamic model selection

Model terms	Triple interactions	Double interactions	Simple effects	Random factors	Deviance	AIC	ΔAIC	df
Full	Sp:OI:Period + Sp:OI:Month + Sp:Period:Month	Sp:OI + Sp:Period + Sp:Month + OI:Period + OI:Month + Month:Period	Sp + OI + Period + Month	1 CaptDate + 1 House	0	11,744	0	130
Full_rain	Sp:OI:Period + Sp:OI:Rain + Sp:Period:Rain	Sp:OI + Sp:Period + Sp:Rain + OI:Period + OI:Rain + Rain:Period	Sp + OI + Period + Rain	1 CaptDate + 1 House	1,803	12,054.1	310.1	30
Model 0a	–	–	–	Full – 1 House	77.88	$P < 2.2e-16$		129
Model 0b	–	–	–	Full – 1 CaptDate	191.56	$P < 2.2e-16$		129
Model 0c	–	–	–	Full – 1 House – 1 CaptDate	278.2	$P < 2.2e-16$		128
Model 1a	Full – Sp:Period:Month	–	–	–	551.2	11 930.86	186.58	97
Model 1b	Full – Sp:OI:Month	–	–	–	10.38	11,732.66	–11.62	119
Model 1c	Full – Sp:OI:Period	–	–	–	523.56	11,750.92	6.64	127
Model 1d	Full – Sp:OI:Month – Sp:Period:Month	–	–	–	552.88	11,921.52	177.24	86
Model 1e	Full – Sp:OI:Month – Sp:OI:Period	–	–	–	23.62	11,739.90	–4.38	116
Model 2a	–	Model 1b – OI:Month	–	–	49.02	11 749.30	5.02	108
Model 3a	–	Model 1e – OI:Month	–	–	61.54	11,755.82	11.54	105
Model 3b	–	Model 1e – OI:Period	–	–	121.66	11,831.94	87.66	113
Model 3c	–	Model 1e – OI:Sp	–	–	25.14	11,739.42	–4.86	115
Best model: Model 1e	Sp:Period:Month	Sp:OI + Sp:Period + Sp:Month + OI:Period + Month:Period	Sp + OI + Period + Month	1 CaptDate + 1 House	–	11,739.90	–	116

CapDate = date of capture; OI = indoor/outdoor; Sp = species.

We first compared two full models, one with rain, and the other with month as explanatory variables. Month was explaining much more variability than rain alone and was therefore privileged as an explanatory variable in further steps. For model comparison, the difference between the AIC of each model and the AIC of the full model was calculated ( $\Delta AIC$ ). Two models, Model 1b and Model 1e, fit better than the full model ( $\Delta AIC < 0$ ) and both fit reasonably well (the difference between their AIC is less than 10). We choose the simpler of those two models as our best model (Model 1e). This model is close to the full model except the triple interactions Sp:OI:Month and Sp:OI:Period are removed.

LLINs in July 2008. Compared with *An. funestus*, *An. gambiae* s.l. was less abundant, particularly during dry seasons before, but also after the implementation of LLINs in 2008 (Figure 2). The peak of aggressiveness of *An. gambiae* s.l. was usually observed during rainy seasons. It started to decrease substantially during the rainy season of July 2013, coinciding with the beginning of P3, the period of the renewal of LLINs. During the following years, the aggressiveness of this species was low but a small peak still occurred during the rainy seasons.

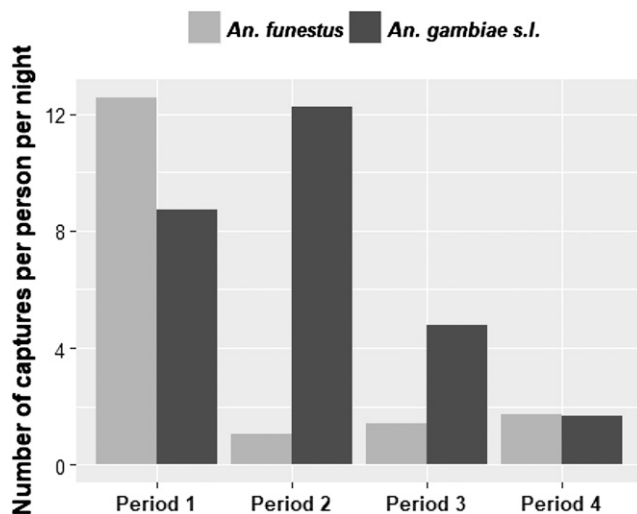


FIGURE 1. Variations in the number of captures of *Anopheles gambiae* s.l. and *Anopheles funestus* per period as predicted by the model: period 1 (July 2006 to June 2008), period 2 (July 2008 to June 2011), period 3 (July 2011 to June 2014), and period 4 (August 2014 to April 2016).

**Site of collection (indoor/outdoor) effect.** As shown in Figure 3, the effect of the site of collection (indoor/outdoor) depended on the species (site of collection  $\times$  species interaction) and the period (site of collection  $\times$  period interaction). Both species were collected as much indoors as outdoors during P1 and P2, and switched to be collected outdoors during P3 and P4 (post hoc model selection steps are provided in Supplemental Material 1).

**Variations in nocturnal activity. Hourly dynamic.** In P1, the number of mosquitoes collected increased progressively indoors and outdoors for both species from 7 PM up to 3 AM indoors and up to 4 AM outdoors (Figure 4, left panels). It then started to decrease (although a smaller peak can be seen around 5 AM) but was not back to the number of mosquitoes that were collected in the first hours on the night, when captures end at 7 AM. In P2 the nightly biting dynamic of these species remained unchanged, although a new peak of activity appeared at 10 PM for *An. gambiae* s.l., both indoors and outdoors. The peak activity of both the species switched to occur a bit earlier, around 12 PM and 1 AM (with the second, smaller peak occurring between 4 AM and 5 AM) in P3 and P4 both indoors and outdoors. However, these changes did not translate into differences in the number of bites while people are awake versus when they are sleeping, as none of the interactions between the binary variables counting the number of bites when people are awake versus sleeping and other variables (species, indoor/outdoor, period, or month) are significant (the best model is model 4, Table 3). Hence, although catches decreases progressively from P1 to P4, 76.9% occurred when humans are asleep from P1 to P4 (see Supplemental Material 2).

**Exposure of protected and unprotected individuals.** Given the mosquito's nocturnal activities (model 4 predictions, Figure 4 right panels), the number of bites expected for bed net

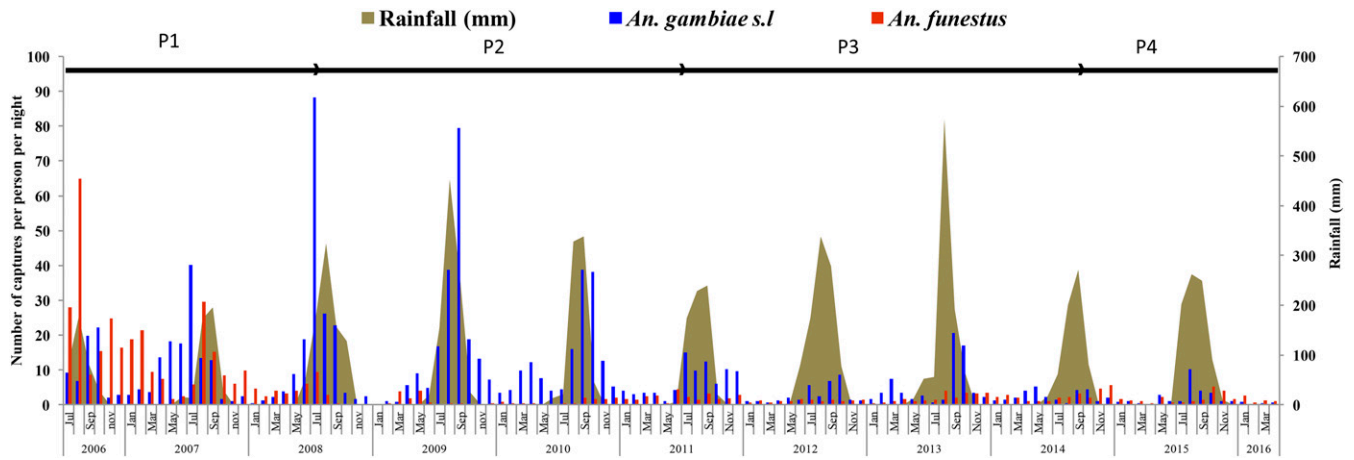


FIGURE 2. Monthly variations in the number of captures per night for *Anopheles gambiae* s.l. and *Anopheles funestus* species and monthly rainfall (mm) between July 2006 and April 2016 in Dielmo. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

users and nonusers are given for each period and each species in Table 4 (the whole calculation process is provided in Supplemental Material 2). The number of bites decreases for both net users and nonusers from P1 ( $B_p = 8.08$  and  $B_u = 22.05$ ) to P4 ( $B_p = 1.41$  and  $B_u = 2.59$ ). The proportion of those bites that are occurring outdoors for an unprotected individual (nonuser) increases from P1 ( $1 - \pi_i = 0.20$ ) to P4 ( $1 - \pi_i = 0.43$ ).

**Sporozoitic infection and entomological inoculation rates.** *Infection rates.* Among all the anophelines tested, the presence of the *P. falciparum* circumsporozoite protein antigen was detected only in *An. gambiae* s.l. and *An. funestus*. The infection rate varied significantly depending on the periods of the study ( $\chi^2 = 10.648$ ,  $P = 0.014$ ), and the interaction between the species and the period ( $\chi^2 = 19.219$ ,  $P < 0.001$ ). For *An. gambiae* s.l., the infection rate increased progressively from 1.0% in P1 to 2.2% in P3 and then decreased to 0.5% in P4. Inversely, for *An. funestus*, the infection rate decreased progressively from 1.6% in P1 to 0.2% in P3 and increased to 0.8% in P4 (Table 4).

**Entomological inoculation rates.** Taking into account the infections rates, we can calculate that in P1, where few people

were using bed nets, the number of infected bites was around 110 infected bites per year per (unprotected) individual. At this time, a bed net user would have received 40.4 infected bites per year. A decrease can be seen in P2, P3, and P4, and comparatively, in P4, a bed net user is expected to encounter 3.2 infected bites per year, whereas a nonuser is expected to encounter 5.8 infected bites per year (Table 4).

The relative hazard for users compared with nonusers,  $\lambda_p$ , almost steady between P1 (~37%) and P2 (~38%), increased up to 47% in P3 and 55% in P4. Consequently, the true protective efficacy of the net  $P^{sf}$  decreased from 63% in P1–45% in P4 (Table 4).

## DISCUSSION

In this study, a significant decline in the biting densities of *An. gambiae* s.l. and *An. funestus* was observed after the introduction of LLINs. The same trend was observed in the human experienced bites, however the protective efficacy of LLINs decreased due to the exposure to outdoor biting

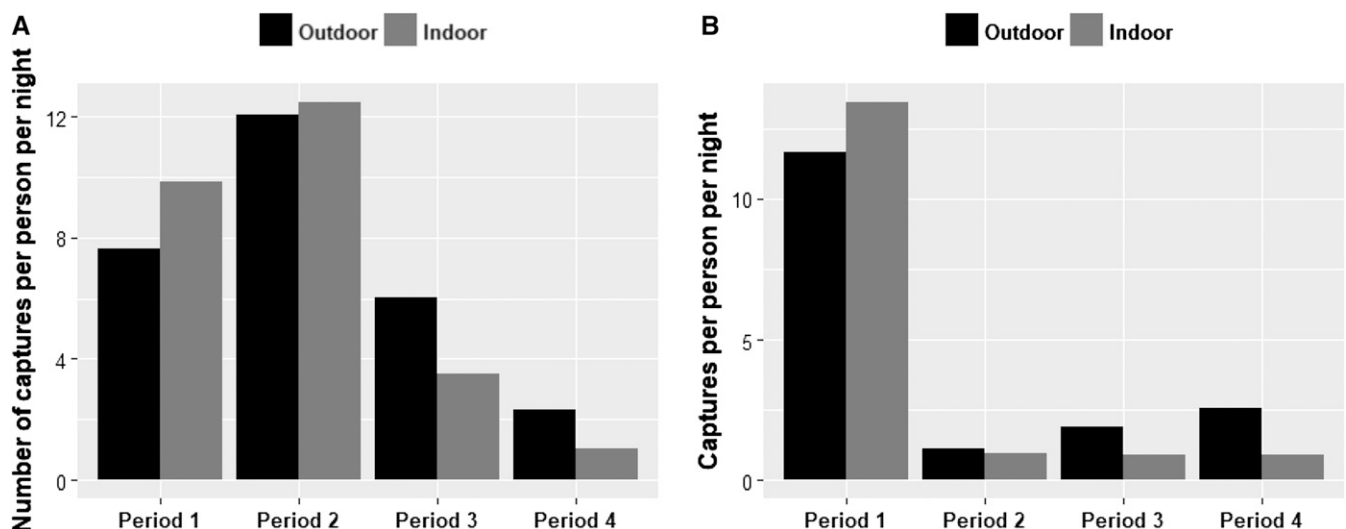


FIGURE 3. Variations in the indoor and outdoor biting rates of *Anopheles gambiae* s.l. (A) and *Anopheles funestus* (B) per period according to the model: period 1 (July 2006 to June 2008), period 2 (July 2008 to June 2011), period 3 (July 2011 to June 2014), and period 4 (August 2014 to April 2016).

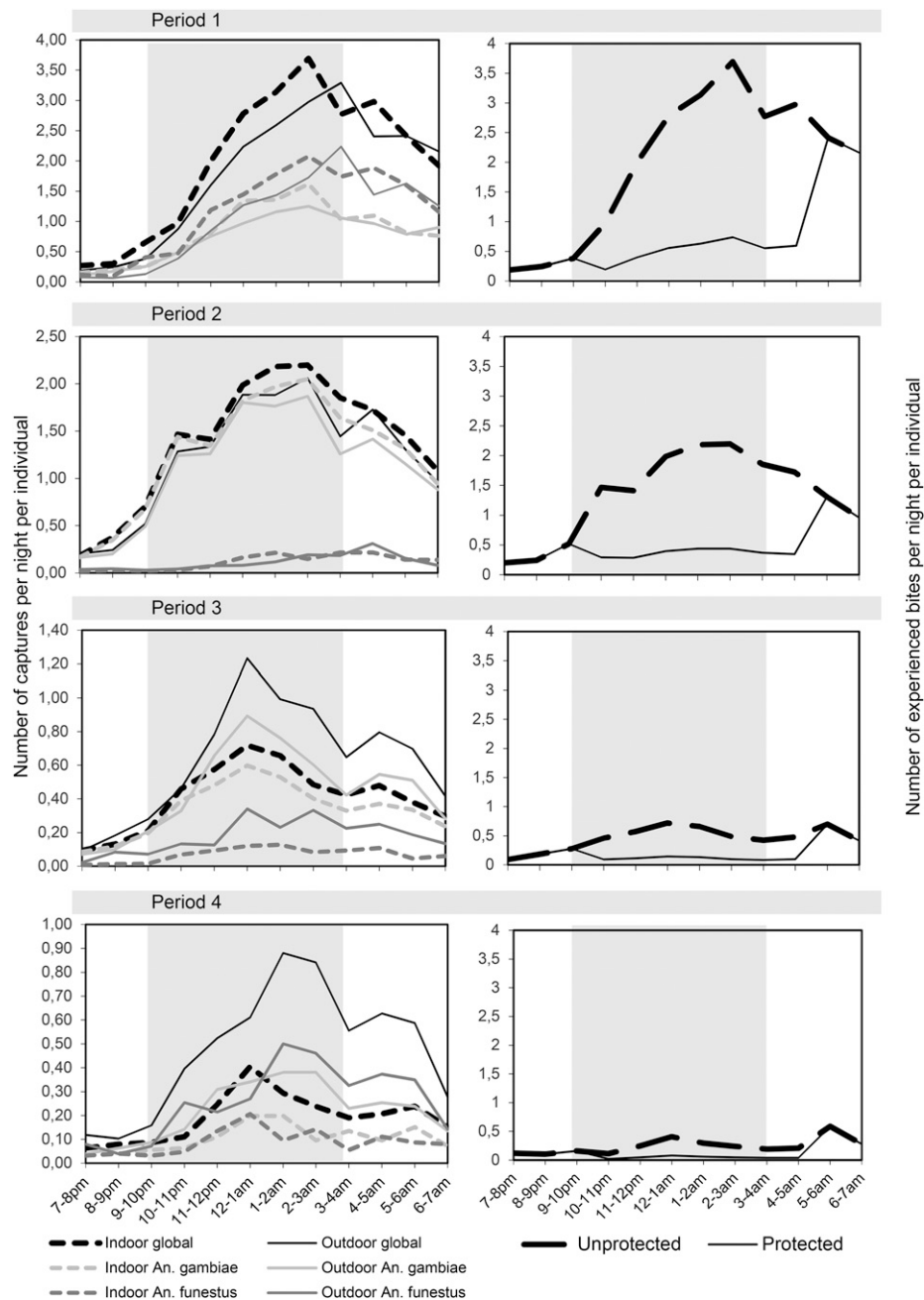


FIGURE 4. Nocturnal activity. Left: variations in the hourly indoor and outdoor number of catches. Right: biting rates experienced by bed net users (plain) and nonusers (bold) by period.

displayed by both species that may maintain the residual malaria transmission in Dielmo. During the pre-intervention P1, *An. gambiae* s.l. and *An. funestus* were both present in Dielmo, with *An. funestus* being the dominant species. Previous studies have already reported the presence of this species in the village and its involvement in malaria transmission throughout the rainy and dry season.<sup>36</sup> In this study, the abundance of *An. funestus* decreased dramatically between P1 and P2. Our detailed analysis shows that the abundance of *An. funestus* started to decline during P1, more specifically in 2007, 1 year before the implementation of the LLINs. The decrease of *An. funestus* populations before the implementation of the LLINs could be attributed to the low rainfall from 2006 to 2007 compared with other years, which

has reduced the presence of water required for the formation of breeding sites. A disappearance of *An. funestus* was also observed in the north of Senegal after the droughts of the 1970, causing a substantial decline in malaria transmission.<sup>37,38</sup> In our study, the presence of *An. funestus* in the following years has not been observed despite the heavy rainfall since the year 2008 in Dielmo. Instead, just after the implementation of LLINs, the abundance of this species has further decreased and has never come back to its previous state. By contrast, *An. gambiae* s.l. populations showed high densities after the introduction of the LLINs in P2 (60–80% of Dielmo inhabitants stating that they always used their LLINs during this period)<sup>25</sup> but decreased substantially after renewing the LLINs in P3 and P4. We could hypothesize that the rise in *An. gambiae* s.l. population's

TABLE 3  
Nocturnal activity model selection

Model terms	Terms added	Deviance	AIC	$\Delta$ AIC	df
Model 1e	–	0	17,876.46	1,052.86	116
<b>Model 4</b>	<b>SleepingAwake</b>	<b>1,054.9</b>	<b>16,823.60</b>	<b>0</b>	<b>117</b>
Model 4a	SleepingAwake:Sps	1,055.5	16,824.94	–1.36	118
Model 4b	SleepingAwake:Period	1,059.2	16,825.28	–1.68	120
Model 4c	SleepingAwake:OI	1,055	16,825.42	1.82	118
Model 4d	SleepingAwake:Month	1,079.2	16,821.24	–2.36	128

A new variable, hereafter named SleepingAwake, was added sequentially to the former best model, refitted on the desaggregated data set. For model comparison, the difference between the AIC of each model and the AIC of Model 4 which is the best model eventually selected, is provided ( $\Delta$ AIC).

dynamics in P2 could be explained by the increase in rainfall since 2008, a paramount factor required for the development of the larval habitat of *An. gambiae* s.l. or a rapid decrease in physical integrity or insecticidal activity of LLINs that has occurred during P2, as it was the first time that the villagers had used bed nets. Indeed, although the WHO specifies that LLINs should have a relative lifespan of around 20 washes under laboratory conditions and should last for 3 years of use,<sup>39</sup> the physical damage to half of the LLINs in several areas in Ethiopia was reported after only 3–6 months of use, which rose to 93% after 26–32 months of use.<sup>40</sup> However, if this hypothesis is true, then mosquito nets cannot alone be held responsible for the maintenance of a low density of *An. funestus* populations in P2.

Considering the tendency of these species to bite indoors or outdoors, neither *An. gambiae* s.l. nor *An. funestus* had any preference for feeding indoors or outdoors in P1 and P2. The decrease of *An. funestus* densities in P2 concerned both the mosquitoes biting indoors and outdoors. A shift was observed in P3 and P4, where more mosquitoes were caught outdoors than indoors. For *An. gambiae* s.l., the indoor biting decreased faster than the outdoor one and for *An. funestus* the outdoor biting increased from P2 to P4. Two hypotheses could explain these behavioral changes in the indoors to outdoors ratio of catches, they are as follows: the occurrence of phenotypic plasticity within a mosquito population able to bite both indoors and outdoors and look for blood meals; and the selection of mosquito adapted to outdoor biting. The pattern observed with *An. funestus* populations, with a number of catches drastically diminished both indoors and outdoors, fits

more the phenotypic plasticity hypothesis, instead of two populations specialized to bite indoors or outdoors. However, when regarding the steady increase in outdoors biting from between P2 and P4, the fact that a minority lineage specialized in biting outdoors might rise and occupy the niche left by the plastic lineage cannot be excluded. Regarding *An. gambiae* s.l., however, the pattern we observed could result from the occurrence of several species of *An. gambiae* s.l. which exhibit different behaviors in their responses to vector control tools. Indeed, in the *An. gambiae* complex, *An. gambiae* sensu stricto which has recently been split into *An. gambiae* and *Anopheles coluzzii*,<sup>41</sup> and their sibling species *Anopheles arabiensis*, are known as the main vectors of malaria transmission in sub-Saharan Africa.<sup>42</sup> A recent study conducted in Dielmo has reported a shift in these species' composition, with *An. arabiensis* being the most prevalent species after the introduction of the LLINs.<sup>24</sup> These three species exhibit differences in their biting behaviors. More specifically, *An. gambiae* and *An. coluzzii* are primarily endophilic and anthropophilic, whereas *An. arabiensis* displays a behavioral plasticity in its biting activities by feeding on humans and animals outdoors.<sup>43</sup> Thus, the latter species could be less exposed to vector control tools used indoors than *An. gambiae* and *An. coluzzii*, which are more restricted in their biting activities, and the implementation of the LLINs would lead to selective pressure on *An. gambiae* and *An. coluzzii*, with high mortalities in both species, whereas *An. arabiensis* survives. If so, in the future, there is a risk that *An. arabiensis* might fill what is left of the ecological niche left empty by its sister species in the

TABLE 4

Sporozoite rates, mean number of bites across nights expected for bed net users and nonusers, annual EIR, relative hazard and true protective efficacy of an ITN ( $P^*$ ) according to the following periods: P1 (July 2006 to June 2008), P2 (July 2008 to June 2011), P3 (July 2011 to June 2014), and P4 (August 2014 to April 2016)

Species	Periods	Exposure		Infection rates			EIR/year protected		EIR/year unprotected		Relative hazard	True protective efficacy of an ITN ( $P^*$ )
		Protected ( $B_p$ )	Unprotected ( $B_u$ )	Tested	Positive	$s = \text{positive}/\text{tested}$	$\text{EIR}_p = B_p \times 365 \times s$		$\text{EIR}_u = B_u \times 365 \times s$		$\lambda_p = B_p/B_u = \text{EIR}_p/\text{EIR}_u$	$P^* = 1 - \lambda_p$
<i>Anopheles gambiae</i> s.l.	P1	3.32	9.28	1,044	11	1.05%	12.76		35.68		36.65%	64.24%
	P2	4.46	11.71	4,820	58	1.20%	19.61		51.43		38.37%	61.87%
	P3	1.92	4.12	2,077	46	2.21%	15.55		33.30		47.44%	53.30%
	P4	0.69	1.33	448	2	0.45%	1.12		2.16		54.31%	48.02%
<i>Anopheles funestus</i>	P1	4.76	12.77	2,075	33	1.59%	27.65		74.14		37.29%	62.71%
	P2	0.40	0.97	506	3	0.59%	0.87		2.10		41.37%	58.63%
	P3	0.58	1.17	645	1	0.16%	0.33		0.66		50.06%	49.94%
	P4	0.72	1.26	507	4	0.79%	2.06		3.64		56.75%	43.25%
		$B_p (\text{An. gambiae s.l.}) + B_p (\text{An. funestus})$	$B_u (\text{An. gambiae s.l.}) + B_u (\text{An. funestus})$	Tested	Positive	$s$	$\text{EIR}_p (\text{An. gambiae s.l.}) + \text{EIR}_p (\text{An. funestus})$		$\text{EIR}_u (\text{An. gambiae s.l.}) + \text{EIR}_u (\text{An. funestus})$		$\text{EIR}_p/\text{EIR}_u$	$P^* = (1 - \text{EIR}_p/\text{EIR}_u)$
Total	P1	8.08	22.05	3,119	44	1.41%	40.41		109.82		36.79%	63.21%
	P2	4.87	12.68	5,326	61	1.15%	20.48		53.53		38.25%	61.75%
	P3	2.51	5.29	2,722	47	1.73%	15.88		33.96		46.77%	53.23%
	P4	1.41	2.59	955	6	0.63%	3.19		5.80		54.97%	45.03%

EIR = entomological inoculation rates; P1 = period 1; P2 = period 2; P3 = period 3; P4 = period 4.

future, and the situation would be worrying as it could prevent progress in malaria control. Such an issue should be addressed by studying which are the most limiting factors determining the carrying capacity of the *An. arabiensis* populations. As for the late decrease of *An. gambiae* s.l., the question remains why these shifts toward more outdoor bites did not happen in P2, but only in P3 and P4, as LLINs were implemented at the beginning of this period and widely used. The most likely explanation is that, as hypothesized previously, mosquito net integrity was decreased in P2. If so, *An. funestus* was maintained at a low level during P2 by another environmental factor affecting equally mosquitoes biting indoors and outdoors. However, it is difficult to further test this hypothesis as these indicators of the effectiveness of LLINs were not tested in Dielmo during these periods.

Regarding the nocturnal activity, the fact that the nightly biting peak of *An. funestus* and *An. gambiae* s.l. seemed to change a bit in Dielmo was not an issue, given that most human hosts were still protected by bed nets at this time. Indeed, our analysis of the nocturnal activity showed that the rate of bites occurring while people were sleeping versus awake did not change significantly between periods (it was always around 76.9%). This observation showed that *Anopheles* vectors did not change their nocturnal activity when bed nets were used. In our study, the relative protection provided by LLINs to malaria exposure in users relative to nonusers ( $P^*$ ) decreased from ~63% in P1 to ~45% in P4, due to the increased proportion of outdoor biting compared with indoor biting in both vectors in P3 and P4. Note that these estimates might be a bit underevaluated, as our observations of people going to bed at 10 PM and awaking at 5 AM are rough, and because people might go to sleep a bit earlier or wake up a bit later (this would increase the proportion of bites when people are asleep, and therefore, increase the difference between bed net users and nonusers, and thus,  $P^*$  and  $P^{*f}$ ). However, the fact that captures stopped at 7 AM, although the biting rate is not back to zero would on the contrary lead to underestimate the number of bites got while outdoors and awake, and therefore to overestimate  $P^*$ , which likely compensates. Our finding that 76.9% of catches occur when people are asleep is consistent with a study conducted in Tanzania that reported the large majority of *An. gambiae* bites during sleeping hours.<sup>35</sup> However, in the latter study, the relative protection of bed net users compared with nonusers ( $P^*$ ) was more than 70%, because the proportion of bites occurring outdoors was much lower ( $1 - \pi_i = 0.08$  in 1997 and  $1 - \pi_i = 0.10$  in 2004) compared with our study. Another study in Benin, in Lokohoué reported that the protection provided by LLINs was 62.5% for early risers (10 PM to 5 AM), which is consistent with our results in P1.<sup>44</sup> In this study, however,  $P^*$  was more than 80% on average for the global population despite a shift to the diurnal and outdoors feeding of *An. funestus* were being reported.

It should be noted that in P2 and P3, a fall and then rise in malaria was observed in Dielmo.<sup>10,26</sup> This situation reflected seasonal malaria transmission in the village after the introduction of vector control tools, whereas the transmission of malaria was perennial previously.<sup>26,36</sup> The low level of mosquito bites to which Dielmo inhabitants have been exposed to could be a factor explaining a decrease in the protective immunity after the implementation of LLINs, as reported by Diop et al.<sup>45</sup> Such a decrease in protective immunity, associated

with the maintenance of a low level of infectious bites in mosquitoes, could be sufficient to explain the resurgence. Unsurprisingly, most of the resurgent malaria cases were noted in younger adults who declared that they had not used their LLINs and they had remained outdoors to watch television.<sup>28</sup> This situation illustrates that the behavior of human populations and the residual outdoor biting are still a challenge to eliminating malaria using LLINs alone as a vector control tool.

This study has some limitations which can be improved in the future. The analysis conducted with retrospective data and surveys about the human behavior relative to the time they were indoors at night under their LLINs to sleep, and the time they woke up and left the bedroom in the morning have been conducted based on our own observations. Accurate socio-anthropological studies should record these important data. Furthermore, molecular data for distinguishing sibling species would improve our knowledge of the involvement of each species that specifically contributed to the main observations in this study.

As a conclusion, a substantial decrease in the human exposure and malaria transmission was noted after the implementation of LLINs, showing that this vector control tool confers an effective personal protection against malaria exposure. However, the protective efficacy of LLINs decreased due to outdoor biting exhibited by residual *An. gambiae* s.l. and *An. funestus* populations that ensure residual transmission. Because of the remarkable increase of *An. gambiae* s.l. after the introduction of LLINs in P2 and the resurgence of malaria in villagers not using bed nets and staying outdoors even after the renewal of LLINs showed that the level attained is not sufficient for elimination. Indeed, as recommended by Griffin et al.<sup>9</sup> the level of coverage of such interventions needs to be increased to 80–90% to reduce the burden of malaria between 2016 and 2030. This means that a more widespread and effective use of these tools and other tools must be promoted in Dielmo and in other areas where the level of use remains low.

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Authors' addresses: Seynabou Sougoufara, Aix Marseille University, IRD (Dakar, Marseille, Papeete), AP-HM, IHU-Méditerranée Infection, UMR Vecteurs—Infections Tropicales et Méditerranéennes (VITROME), Marseille, France, and Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop de Dakar, Dakar Fann, Sénégal, E-mail: [seynabou.sougoufara@gmail.com](mailto:seynabou.sougoufara@gmail.com). Omar Thiaw, Nafissatou Diagne, Charles Bouganali, Souleymane Doucoure, and Cheikh Sokhna, Aix Marseille University, IRD (Dakar, Marseille, Papeete), AP-HM, IHU-Méditerranée Infection, UMR Vecteurs—Infections Tropicales et Méditerranéennes (VITROME), Marseille, France, E-mails: [thiawomar185@yahoo.com](mailto:thiawomar185@yahoo.com), [nafissatou.diagne@ird.fr](mailto:nafissatou.diagne@ird.fr), [charles.bouganali@ird.fr](mailto:charles.bouganali@ird.fr), [souleymane.doucoure@ird.fr](mailto:souleymane.doucoure@ird.fr), and [cheikh.sokhna@ird.fr](mailto:cheikh.sokhna@ird.fr). Aurélie Cailleau, Unité d'Entomologie Médicale (UME), Institut Pasteur Dakar, Dakar, Sénégal, and Centre Suisse de Recherches Scientifiques en Côte



d'Ivoire (CSRS), Yopougon, Abidjan, Côte d'Ivoire, E-mail: a.cailleau@csrs.ci. Myriam Hary, UMR Évolution, Génomes, Comportement, Écologie (EGCE) CNRS, IRD-University Paris-Sud, IDEEV, University Paris-Saclay, Gif-sur-Yvette Cedex, France, E-mail: myriam.hary@u-psud.fr. Pape M. Sembène, Département de Biologie Animale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop de Dakar, Dakar Fann, Sénégal, E-mail: mbacke.sembene@ird.fr.

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