Windborne long-distance migration of malaria mosquitoes in the Sahel

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Over the past two decades, control efforts have halved malaria cases globally, yet burdens remain high in much of Africa and elimination has not been achieved even where extreme reductions have occurred over many years, such as in South Africa[1,2]. Studies seeking to understand the paradoxical persistence of malaria in areas where surface water is absent for 3–8 months of the year, suggested that certain *Anopheles* mosquitoes employ long-distance migration[3]. Here, we confirmed this hypothesis by aerial sampling of mosquitoes 40–290 m above ground, providing the first evidence of windborne migration of African malaria vectors, and consequently the pathogens they transmit. Ten species, including the primary malaria vector *Anopheles coluzzii,* were identified among 235 anophelines captured during 617 nocturnal aerial collections in the Sahel of Mali. Importantly, females accounted for >80% of all mosquitoes collected. Of these, 90% had taken a blood meal before their migration, implying that pathogens will be transported long distances by migrating females. The likelihood of capturing *Anopheles* species increased with altitude and during the wet seasons, but variation between years and localities was minimal. Simulated trajectories of mosquito flights indicated mean nightly displacements of up to 300 km for 9-hour flight durations. Annually, the estimated numbers of mosquitoes at altitude crossing a 100-km line perpendicular to the winds included 81,000 *An. gambiae* s.s., 6 million *An. coluzzii*, and 44 million *An. squamosus*. These results provide compelling evidence that millions of previously blood-fed, malaria vectors frequently migrate over hundreds of kilometers, and thus almost certainly spread malaria over such distances. Malaria elimination success may, therefore, depend on whether sources of migrant vectors can be identified and controlled.

In Africa, malaria spans the humid equatorial forest to the semi-arid zones in the north and south. In regions where surface water, essential for larval development, is absent during the 3–8 month dry season, mosquito densities and disease transmission drop dramatically[3–8]. Yet, shortly after the first rain, vector populations surge[6] and transmission recommences. Recent studies suggest that Sahelian *Anopheles coluzzii* survives the long dry season by aestivation (dormancy)[3,6,9–11], whereas *An. gambiae s.s.* (hereafter, *An. gambiae*), and *An. arabiensis* re-establish populations by migration from distant locations where larval sites are perennial[3]. However, direct evidence, including the capture of aestivating adults in their shelters or the recapture of marked-mosquitoes hundreds of kilometers from their release sites, remains elusive.

Mosquito dispersal, hereafter referred to as migration[12], has been extensively studied because it directly impacts disease transmission, the spread of adaptations (e.g., insecticide resistance), and control strategies, such as insecticide barriers[13,14]. Although tracking mosquitoes over large scales has seldom been attempted[13,14], the prevailing view is that the dispersal of malaria mosquitoes does not exceed 5 km[13–16] and the alternative view[17–20] is typically considered to pertain to "accidental events" of minimal epidemiological importance[13]. Nonetheless, the prediction of long-distance migration of anophelines in the Sahel prompted us to question this dogma. Our study is the first to systematically sample insects migrating at high altitude over multiple seasons in Africa to determine if malaria vectors engage in wind-assisted movements, and if so, assess the epidemiological relevance by addressing the following questions: what species are involved? how frequently and at what heights do they fly? how many mosquitoes migrate and how likely are they to carry *Plasmodium*? Then, using simulations, we estimate how far mosquitoes may have travelled and from where.

During 617 aerial sampling nights, we caught 461,100 insects at heights between 40–290 m agl, in four villages in the Sahel of Mali, West Africa (ED Fig. 1), including 2,748 mosquitoes, of which 235 were anophelines (Table 1). These mosquitoes belonged to 10 species: *Anopheles coluzzii*, *An. gambiae*, *An. pharoensis*, *An. coustani*, *An. squamosus*, *An. rufipes*, *An. namibiensis* and three distinct but currently undetermined *Anopheles* (Table 1). The first two are the primary malaria vectors in Africa, with the next four of secondary importance[21]. Mosquitoes were not among the 564 insects captured on 508 control nets (Table 1, and Methods), confirming that these *Anopheles* were intercepted at altitude rather than near the ground during deployment. The maximum anophelines/night was three, indicating that migration occurred over many nights. Consistent with Poisson distributions, the values of the variance to mean ratio were all near one (Table 1 and Supplementary Discussion). Unless otherwise specified, quantitative results presented hereafter refer to the five most abundant *Anopheles* species, represented by >20 individuals (Table 1).

Females outnumbered males by >4:1 (Table 1). Critically, with 87.5% fully gravid, 0.7% semi-gravid, and 2.9% blood-fed, >90% of the anopheline females had taken a blood meal prior to their high-altitude flights (Table 1), suggesting likely exposure to malaria and other pathogens. Although 31% of bloodmeals came from humans, no *Plasmodium*-infected mosquitoes were detected amongst the 23 *An. gambiae s.l.* or the 174 secondary vectors (Table 1). Considering typical rates of *Plasmodium* infections in primary (1–5%) and secondary (0.1–1%) vectors[5,22–24], our results probably reflect the small sample size, with likelihood for zero infected mosquitoes being >30% and >18% (assuming the highest rates in each range), in the primary and secondary vectors, respectively (Supplementary Discussion). Hence, unless infection reduces migratory capacity or migrants are resistant to parasites (there is no evidence for either), *Plasmodium* and other pathogens are almost certainly transported by windborne mosquitoes that may infect people post-migration.

Mosquitoes were intercepted flying between 40 and 290 m agl (Fig. 1a). Overall panel and aerial density increased with altitude, with a significant effect across species on mean panel density ($P<0.037$, $F_{1/24}=4.9$, ED Fig. 2b), suggesting that anopheline migration also occurs >290 m agl. The similar species distributions across years and villages (ED Fig. 2c; non-significant effects of year and village across species, ED Table 1), combined with its marked seasonality (aerial mosquito captures occurred between July–November, peaking between August–October, Fig. 1b, ED Table 1), all attest to the regularity of windborne migration of *Anopheles* mosquitoes.

Using mean aerial densities and wind speeds at altitude (4.8 m/s, Fig. 1c), and conservatively assuming mosquitoes fly in a layer between 50 and 250 m agl (see above), we estimated the nightly expected numbers of migrants crossing a 1-km line perpendicular to the wind direction. Estimates ranged between

27 (*An. gambiae*) and 3,719 (*An. squamosus*, Fig. 1d) per night. When interpolated over a 100-km line joining our sampling sites (ED Figs. 1a, 2c), annual migrations exceeded 80,000 *An. gambiae*, 6.25 million *An. coluzzii*, and 44 million *An. squamosus* in that region alone (Fig 1d). Thus, windborne migration in the Sahel occurs on a massive scale.

For each mosquito capture event, flight trajectories for two- and nine-hour flight durations were estimated using HYSPLIT[25] (using the most accurate assimilated meteorological data available: ERA5), assuming that mosquitoes ascend by their own flight but are passively carried by the wind at altitude (Methods). The mean nightly displacements (straight-line distances) were 30 and 120 km (maxima 70 and 295 km), respectively (Table 2 and Fig. 2). Notably, maximal 9-hour nightly flight displacements ranged between 257–295 km for all anophelines with sample size >20 (Table 2). These backwards trajectories exhibited a south-westerly origin (Rayleigh test; mean bearing = 212°, r = 0.54, P < 0.0001, Table 2), corresponding to the prevailing winds during peak migration (August–September, Fig. 2). Trajectories of most species originated from a broad arc (>90 degrees, Fig. 2), suggesting migrants emanated from multiple sites across a large region. Migration from this direction fits with the presence of high-density populations due to perennial larval sites and earlier population growth following the monsoon rains. The back-trajectories with a strong northerly component, observed during the sparsely sampled period of October–December (Fig. 2) might indicate southward "return flights", on the Harmattan winds prevailing during this season.

Contrary to the conventional view that dispersal of African anophelines is <5 km[13,15,16,26], our results provide compelling evidence that primary and secondary malaria vectors regularly engage in windborne migration spanning tens to hundreds of kilometers per night. With massive numbers of females that had taken at least one blood-meal, this migration probably involves human *Plasmodium* among other pathogens. Separate outbreaks of malaria in Egypt and Israel have been attributed to *An. pharoensis* traveling over 280 km[17]. Assuming, a conservative[23,27], 1% infection rate in migrating females of *An. coluzzii, An. gambiae*, *An. coustani*, and *An. pharoensis* and 0.1% in the remaining anophelines (excluding the unknown *An. sp.* Mali 1 and 2, Supplementary Discussion), a total of 286,700 infected migrant mosquitoes are expected to cross a 100-km line perpendicular to the wind at altitude every year. Accordingly, *An. pharoensis*, *An. coustani*, and *An. coluzzii*, contributed 41%, 25%, and 17%, respectively, to the malaria transmission by infected windborne mosquitoes. Although these estimates are relatively coarse, this suggests that migratory secondary vectors could be a major infection source and should be included in studies of transmission as well as in control programs.

Contrary to our initial prediction[3], *An. coluzzii* was more common than *An. gambiae* among the migrants. This expectation was based on data suggesting that *An. coluzzii* aestivates locally and thus may not require long-distance migration to recolonize the Sahel. Indeed, windborne migration occurs from the end of July to October, well after the surge of Sahelian *An. coluzzii* following the first rain (May–June)[3,6]. The northward and southward oscillations of the Intertropical Convergence Zone during the wet season continually create better mosquito resource-patches with the rains. Additionally, wet-season droughts endanger local mosquito populations every decade or two[28]. Thus, selection pressures to track fresh-water resources by riding the winds that bring rain[29] may explain why Sahelian residents such as *Oedaleus senegalensis* grasshoppers and *An. coluzzii* have a mixed strategy of migration[30] and local dormancy. *Anopheles gambiae*, which presumably recolonizes the Sahel every wet season is relatively rare in Sahelian villages[3], and thus only one specimen was captured by our nets. It may migrate on fewer nights and constitute a smaller fraction of windborne migrants (Supplementary Discussion).

In areas approaching elimination, malaria cases without a history of travel are presumed to represent indigenous transmission. We propose that a substantial fraction of such cases, especially those that occur within ~300 km from high malaria transmission areas, arise from the bites of exogenous-windborne-

infected mosquitoes. For example, north-eastern South Africa has the highest incidence of persistent malaria in the country with many cases not associated with human travel, which are concentrated in an arc extending over ~150 km from the borders with Zimbabwe and Mozambique, where transmission is still high. This area includes the Kruger National Park where roads are scarce and vehicular transport of infected mosquitoes[31] may be hampered. Testing the correlation of such infection events with corresponding winds will help to assess this hypothesis. If confirmed, incorporation of disease control efforts in source populations to minimize or block migration are likely to be an essential element of the elimination strategy.

Table 1. Summary of mosquitoes collected in aerial samples on standard and control panels (2013-2015)

	Standard Panels[a] (N=1,894)											Control Panels[b] (N=508)		
Taxa	Total Captured	Mean Panel Density	L95%CL Poisson[c]	U95%CL Poisson[c]	Max/ Panel	Nightly Presence (%)	Var/Mean ratio	% Female (n)	% Post Blood Feed[d] (n)	% Infected[e] (n)	% Anthro-pophily[h]	Total Captured	Mean Panel Density	Max/ Panel
An. squamosus	100	0.053	0.042	0.063	3	11.02	1.37	76.0 (96)	93.2 (73)	0 (73)	41.1 (17)	0	0	0
An. pharoensis	40	0.021	0.015	0.028	2	6.00	1.08	82.5 (40)	100 (33)	0 (33)	33.3 (6)	0	0	0
An. coustani	30	0.016	0.01	0.022	2	4.38	1.05	88.9 (27)	87.5 (24)	0 (24)	14.3 (7)	0	0	0
An. rufipes	24	0.013	0.008	0.018	2	3.24	1.16	80 (20)	93.8 (16)	0 (16)	0 (4)	0	0	0
An. coluzzii	23	0.012	0.007	0.017	2	3.08	1.16	95.5 (22)	90.5 (21)	0 (21)	100 (1)	0	0	0
An. (Ano.) sp. Mali 1	2	0.001	0	0.003	1	0.32	1	100 (2)	100 (2)	0 (2)	nd	0	0	0
An. gambiae s.s.	1	0.0005	0	0.002	1	0.16	1	100 (1)	100 (1)	0 (1)	nd	0	0	0
An. sp. nr concolor[g]	1	0.0005	0	0.002	1	0.16	1	0 (1)	na[f]	na	na	0	0	0
An. sp. Mali 2	1	0.0005	0	0.002	1	0.16	1	100 (1)	100 (1)	0 (1)	nd	0	0	0
An. namibiensis	1	0.0005	0	0.002	1	0.16	1	100 (1)	100 (1)	0 (1)	nd	0	0	0
Anopheles unidentified	12	0.006	0.003	0.01	1	1.78	0.99	33.3 (6)	100 (2)	0 (2)	nd	0	0	0
Culicinae	2340	1.236	1.185	1.286	22	58.19	4.83	86.4 (1866)	96.7 (1629)	nd	nd	0	0	0
Culicid unidentified	173	0.091	0.078	0.105	8	17.18	1.92	62.9 (116)	91.8 (73)	nd	nd	0	0	0
Total Culicidae	2748	1.451	1.397	1.505	23	64.18	4.92	84.5 (1876)	96.2 (1804)	nd	nd	0	0	0
Total Insects	461100	243.58	242.88	244.29	2601	100	314.75	nd[f]	nd	na	na	564	1.110	31

[a] Nightly aerial sampling using sticky nets (panels, usually 3/balloon) launched and retrieved at 17:00 and 07:00, respectively. Nets were raised to set altitudes between 40 and 290 m above ground (see Methods).

[b] Control panels were raised to 40 -120 m agl and immediately retrieved during the launch and retrieval of the standard panels to estimate the number of insects captured during the ascent and descent (see Methods).

[c] Estimated using the normal approximation of the Poisson distribution. Low negative values < -0.0001, when a single mosquito/taxon were captured, were rounded to zero.

[d] Only a few bloodfed and half-gravid females (see text for percentages) were pooled with gravids to reflect those which were evidently exposed to at least one blood meal. In these mosquito species blood feeding is required for egg development as indicated by the gravid state. Unfed mosquitoes consisted of the rest.

[e] Infection with human *Plasmodium* species was tested as described in the Methods.

[f] na and nd denote not applicable and not determined, respectively.

[g] This species was identified based on male genitalia

[h] Identified via PCR (see Methods) with additional confirmations by sequencing. Nonhuman hosts include cow, goat, and possibly unknown rodents.

Table 2. Summary of displacement distance and source direction based on 2 and 9 hour flight trajectories of mosquitoes produced using HYSPLIT (see Methods and Figure 2).

	Trajectories: 2-hour flight				Trajectories: 9-hour flight								
Taxa	Trajectories N[a]	Displace mean	Displace 95%CLM	Displace min-max	Trajectories N[a]	Displace mean	Displace 95%CLM	Displace min-max	Hourly Disp. mean[c]	Actual Hourly Disp. Mean[d]	mean Bearing Final[e]	R[f] [bearing]	$P_{[R]}$
An. squamosus	1100	27.7	27-29	2-68	400	109.1	103-115	4-265	13.3	12.1	213	0.516	0.0000
An. pharoensis	440	31.1	30-33	1-65	160	125.3	116-134	24-260	14.7	13.9	214	0.660	0.0000
An. coustani	330	28.5	27-30	2-60	120	125.8	114-138	16-295	14.5	14.0	199	0.270	0.0802
An. rufipes	264	26.1	24-28	2-70	96	109.2	97-121	24-257	12.5	12.1	199	0.454	0.0003
An. coluzzii	253	38.6	37-41	3-69	92	154.1	140-168	47-270	17.3	17.1	217	0.815	0.0000
An. sp. Mali 1	22	20	14-26	6-52	8	94.3	52-136	51-172	10.2	10.5	223	0.947	0.0000
An. gambiae s.s.	11	33.5	ND[b]	ND[b]	4	131.1	ND[b]	ND[b]	15.9	14.6	254	ND[b]	ND[b]
An. sp. nr concolor	11	17.2	ND[b]	ND[b]	4	48.2	ND[b]	ND[b]	8.4	5.4	184	ND[b]	ND[b]
An. sp. Mali 2	11	29.9	ND[b]	ND[b]	4	104.4	ND[b]	ND[b]	13.1	11.6	234	ND[b]	ND[b]
An. namibiensis	11	40.1	ND[b]	ND[b]	4	149.3	ND[b]	ND[b]	16.7	16.6	241	ND[b]	ND[b]
Anopheline Overall	2453	29.4	28.8-30.0	1-70.4	892	118.8	115-123	4-295	14.1	13.2	212	0.540	0.0000

[a] The number of unique nightly trajectories assumes all possible nightly interception times, given flight duration and flight start and end between 18:00 and 06:00, respectively. Thus, for each night with a captured mosquito there were eleven unique 2-hour-flight trajectories and four 9-hour-flight trajectories.

[b] Not determined for species with a single specimen captured.

[c] Hourly displacement between successive 1-hour points along the 9-hour trajectory.

[d] Effective hourly displacement computed by as the quotient of the total 9-hour trajectory displacement by 9.

[e] The mean bearing (angle) between the interception point (zero) and the final point of the 9-hour trajectory computed from the North.

[f] A measure of angular dispersion which varies from 0 (uniform dispersion from all directions) to 1 (a single angle where all points align to.

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Authors Contributions

The project was conceived by TL and DLH. Field methods and operations were designed by DLH with input from DRR and JWC. Field work, protocol optimization, data acquisition and management, and initial specimens processing including tentative species identification was performed by AD, ASY, MD, SD, and YO and subsequent processing by AK, JF, and LV with inputs from ET and LC. Species identification and molecular analysis of specimens were conducted primarily by Y-ML, RM, AK, and BJK with contributions by DW, RF, and MJD. Data analysis and HYSPLIT simulations were carried out by TL with inputs from all authors, especially RF, BJK, DRR, JWC, ES and Y-ML. BJK mapped simulated trajectories. The manuscript was drafted by TL and revised by all authors. Throughout the project, all authors have contributed key ingredients and ideas that have shaped the work and the final paper.

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Figure Legend

Figure 1. Flight altitude, seasonality, wind speed, and abundance of migratory anopheline species. **a)** The relationship of altitude (panel height) and panel- (blue) and aerial- (orange, mosquitoes/10^6 m^3 of air) density for the five most common anopheline species (Table 1). Bubble size is proportional to density (x 10^3 is shown in the bubble), thus no bubble is shown with zero value. The number of sampling nights (Nights) per panel height is shown on the left. **b**) Monthly panel density (N=1,894 panels) for the five most common species (Table 1. Note: values of *An. squamosus* were divided by three to preserve scale) overlaid by the length of migration period (dashed lines). Sampling month of species collected once or twice is shown by letters. **c)** Distribution of mean nightly wind speed at flight height in nights with one or more anopheline collected. Wind speed data were taken from ERA5 database after matching panel height to the nearest vertical layer (Methods). Corresponding box-whisker plot (top) shows the median, mean, quartiles and extreme values overlaid by arrows indicating the mean, 10 and 90, percentiles (red). **d)** The number of mosquitoes per species crossing at altitude (50–250 m agl) imaginary lines perpendicular to wind (see legend). Migrants per night per 1 km (right Y axis) are superimposed on the annual number per 100 km line (left Y axis, Main text).

Figure 2. Backward flight trajectories for each anopheles capture event. Backward nine-hour trajectories were estimated by HYSPLIT (Table 2) and overlaid on a map showing parts of Mali and neighboring countries (Map data: Google, Landsat / Copernicus 2019). Each line represents one of 4 simulated trajectories of one (or more) mosquitoes intercepted at that location and night; The area encompassed by the four trajectories is shadowed. Migration season is shown by different line color. *Anopheles* species is indicated above each panel. The seasonal wind rose diagrams reflecting wind conditions at 180 m agl averaged from 2013 to 2015 are shown at the right.

Methods

Study area Aerial sampling stations were located in four Sahelian villages in Mali (Fig. S1): Thierola (13.6586, -7.2147) from March 2013 to November 2015, Siguima (14.1676, -7.2279) from March 2013 to October 2015; Markabougou (13.9144, -6.3438) from June 2013 to April 2015; and Dallowere (13.6158, -7.0369) from July 2015 to November 2015. This study area has been described in detail previously[3,6,9,11,32–34]. Briefly, the region is rural, characterized by scattered villages with traditional mud-brick houses, surrounded by fields. A single growing season (June–October) allows the farming of millet, sorghum, maize, and peanuts, as well as subsistence vegetable gardens. Over 90% of the annual rains fall during this season (~550mm). Cattle, sheep, and goats graze in the savannah that consists of grasses, shrubs, and scattered trees. The rains form small puddles and larger seasonal ponds that usually are totally dry by the end of November. From November until May, rainfall is absent or negligible (total precipitation < 50mm), and by December water is available only in deep wells.

Aerial sampling and specimen processing Aerial sampling stations were placed ~0.5 km from the nearest house of the village in open areas away from large trees. The method of aerial insect collection was adapted from a study on high-altitude mating flights in ants[35]. Rectangular 3 x 1m nets ($3m^2$), cut from a roll of tulle netting (mesh: 8 holes/cm; hole diameter of 1.2 mm), were sewn to form four narrow sleeves 1m apart along the net (ED Fig. 3). A 1m carbon rod was inserted into each sleeve and glued to the net using Duco Cement Glue (Devcon, FL, ED Fig 3). Three nets were spread over each other on a clean large wooden table topped by a 3.5 x 1.5m plywood and coated with a thin film of insect glue (Tanglefoot, Tropical Formula, Contech Enterprises Inc., BC) by rolling a PVC pipe smeared with this glue over them, while applying moderate pressure downward. The pipe was held at each end (from each side of the long table) by two persons and repeatedly rolled (and smeared) until a uniform thin layer of glue coated the net (but did not block its holes). After coating, the sticky nets were immediately rolled individually, and kept in two tightly secured plastic bags indoors, to avoid accidental contact with insects prior to setup.

Prior to the launch, polyurethane balloons (3m in diameter; Mobile Airship & Blimps, Canada, or Lighter than Air, FL, USA), were inflated to full capacity with balloon-grade helium (>98.5%) and topped up to ensure full capacity as needed, usually every 1–3 days based on the balloon condition (ED Fig. 3). Typically, balloons were launched over ~10 consecutive nights per month. The balloon was kept stationary at ~200 m agl by a cord (AmSteel®Blue, synthetic rope sling, Southwest Ocean Services, TX) secured to a $1m^3$ cement block inserted under the ground. The cord then went through a horizontal manually-rotating drum made of a garden-hose reel used for reeling it. A larger 3.3 m diameter balloon (Lighter than Air, FL) was used between July and September 2015, and launched to ~300 m agl.

A team of five trained technicians operated each aerial sampling station. During the launch of a balloon, one team member held the cord under the balloon with heavy-duty gloves and manually controlled its ascent and descent, another controlled the reel, while the other three added or removed the sticky nets to and from their specified positions on the cord. The nets were attached to Velcro panels previously placed on the cord at desirable positions and spaced to fit each of the matching Velcro pieces on the four carbon rods (ED Fig. 3). A knot was made below the top-most Velcro and above the bottom-most Velcro, ensuring that the nets would remain stretched even in strong winds (rather than slip on the cord). Additionally, the team secured the balloons over a “landing patch,” padded by tires covered by a tarpaulin. The balloon was secured to the ground through its main cord by a central hook, at the middle of the landing patch, and by a large tarpaulin that covered it from the top and secured to the ground using 14 large stakes. Team members inspected the nets upon launch to verify that they were free of insects. Upon retrieval of the balloon, the team worked in reverse order and immediately rolled each sticky net

(hereafter, called a panel) and placed it in clean labeled plastic bags, inserted in another bag, each tightened with a cord until inspection.

Each balloon typically carried three sticky nets. Initially, they were suspended at 40, 120, and 160 m agl, but from August 2013, the typical altitude was set to 90, 120, 190 m agl. When the larger balloon was deployed in the Thierola station (August–September 2015), two additional nets were added at 240 and 290 m agl. Balloons were launched approximately 1 hour before sunset (~17:00) and retrieved 1 hour after sunrise (~07:30), the following morning. To control for insects trapped near the ground as the nets were raised and lowered, control nets were raised up to 40 m agl and immediately retrieved (between September and November 2014 the control nets were raised to 120 m agl) during the launch and retrieval operations. The control nets spent 5 minutes in the air (up to 10 minutes when raised to 120 m). Once retrieved they were processed as other nets. Following panel retrieval, inspection for insects was conducted between 09:00 and 11:30 in a dedicated clean area. The panel was stretched between two posts and scanned for mosquitoes, which were counted, removed using forceps, and preserved in 80% ethanol before all other insects were similarly processed and placed in other tubes. Depending on their condition, the sticky panels were sometimes reused the subsequent night.

Species identification Glue attached to the insects was washed off with 100% chloroform. The mosquitoes were gently agitated (<30 sec) to loosen them from one another. Individual mosquitoes were transferred into consecutive wells filled with 85% ethanol. Using a dissecting scope, the samples were morphologically sorted by mosquito subfamily (*Anophelinae, Culicinae*), and tentative identifications to *Anopheles* species /species group undertaken. All *An. gambiae* s.l. visually classified (and two identified based on molecular barcode analysis, see below), were identified to species based on fragment-size differentiation after amplification of the nuclear ITS2 region and digestion of the product[36]. Validation was carried out in LSTM (DW's laboratory) where each specimen was washed with 500µL heptane followed by two further washes with ethanol. DNA was then extracted using the Nexttec (Biotechnologie, GmbH) DNA isolation kit according to manufacturer's instructions. Species identification using a standard PCR method, including all primers[37] with products visualized on 2% agarose gel. *Anopheles gambiae* s.l. samples were further identified to species by SINE insertion polymorphism[38]. In cases where no species-specific bands were detected using the first method, approximately 800 bp region of the mtDNA cytochrome oxidase I genes was amplified using the primers C1_J_2183 and TL2_N_3014[39]. PCR products were purified using the QIAquick PCR-Purification kit (QIAgen) and sequenced in both directions using the original PCR primers by MacroGen Inc. (Amsterdam, Netherlands). Sequences were aligned using CodonCode Aligner (CodonCode Corporation, Dedham, MA) and compared to existing sequences in GenBank to identify species. All other *Anopheles* mosquitoes were identified by the retrospective correlation of DNA barcodes, with morphologically–verified reference barcodes compiled by Walter Reed Biosystematics Unit and the Mosquito Barcoding Initiative in Y-ML's lab. Head-thorax portions of all samples were separated and used for DNA extraction using the Autogen® automated DNA extraction protocol. MtDNA COI barcodes were amplified using the universal LCO1490 and HCO2198 barcoding primers[40], and amplified, cleaned and bi-directionally sequenced according to previously detailed conditions[41]. All DNA barcodes generated from this study are available under the project "MALAN – Windborne *Anopheles* migrants in Mali" on the Barcode of Life Database (www.boldsystems.org) and in GenBank under accession numbers MK585944–MK586043. *Plasmodium* infection status was determined following previously described protocol[42] using DNA extracts from the whole body for *An. coluzzii* and, for all other specimens, for thorax and head (n=190) as well as separated abdomens (n=156) extracted and tested individually using published protocols[43,44]. Due to the nature of the collections, all body parts were not available for each specimen, accounting for the discrepancy in numbers. Bloodmeal identification was carried out following published protocol[45].

Data Analysis Although aerial collections started in April 2012, protocol optimization and standardization took most of that year, and data included in the present analysis covers only the period March 2013–November 2015. Nights when operations were interrupted by storms or strong winds (e.g., the balloon was retrieved during darkness) were also excluded.

The total number of mosquitoes per panel represents 'net density' of each species. Aerial density was estimated based on the species' panel density and total air volume that passed through that net that night, i.e.,

Aerial density = panel density / volume of air sampled, and

volume of air sampled = panel surface area * mean nightly wind speed * sampling duration,

Net surface area was 3 m^2. Wind speed data were obtained from the atmospheric re-analyses of the global climate, ERA5. Hourly data available at 31 km surface resolution with multiple vertical levels including ground, 2, 10, 32, 55, 85, 115 180, 215, 255, and 300 m agl. Overnight records (18:00 through 06:00) for the nearest grid center were used to calculate the nightly direction and mean wind speed at each village: Siguima, Markabougou and Thierola. Dallowere, located 25 km south of Thierola, was included in the same grid cell of Thierola. The mean nightly wind speed at panel height was estimated based on the nearest available altitude layer.

To evaluate clustering in mosquito panel density and the effects of season, panel height, year and locality, mixed linear models with either Poisson or negative binomial error distributions were implemented by proc GLIMMIX[46]. The clustering at the levels of the panel and the night of sampling were evaluated as random effects as was the case for the year of sampling and locality. These models accommodate counts as non-negative integer values. The ratio of the Pearson χ^2 to the degrees of freedom was used to assess overall "goodness of fit" of the model, with values of >2 indicating a poor fit. The significance of the scale parameter estimating k of the negative binomial distribution was used to choose between Poisson and negative binomial models. Sequential model fitting was used, starting with random factors before adding fixed effects. Lower Bayesian Information Criterion (BIC) values and the significance of the underlying factors were also used to select the best fitting model of each species.

The magnitude of windborne migration was expressed as the expected minimum number of migrants per species crossing an imaginary line of 1 km perpendicular to the wind at altitude. This commonly used measure of abundance assumes that the insects fly in a layer that is 1 km wide and does not require knowledge of the distance or time the insects fly to or from the interception point[47–49]. We used the mean wind speed at altitude (4.8 m/s, see below) and assumed that mosquitoes fly in a layer depth of 200 m between 50 and 250 m agl, conservatively reflecting that mosquitoes were captured between 40-290 m (see below). Accordingly, this nightly migration intensity was computed as the product of the mean aerial density across the year (conservatively including periods when no migrants were captured) by the volume of air passing over the reference line during the night. The corresponding annual index was estimated by multiplying the nightly index by the period of windborne migration estimated from the difference between the first and last day and month a species was captured over the three years. Species that were captured once were assumed to migrate during a single month. The annual number of migrants per species crossing a line of 100 km was used because of the similar species composition across our sampling sites spanning 100 km (Fig. S1a and see below).

Like most insects in their size range[48,50,51], the flight speed of mosquitoes does not typically exceed 1 m/s[52,53]. Because winds at panel altitude attain speeds considerably higher than the mosquito's own speed, flight direction and speed are governed by the wind[47,48] and thus, flight trajectory can be simulated based

on the prevailing winds during the night of capture at the relevant locations and altitudes as has been done previously[54–56]. Accordingly, backward flight trajectories of mosquitoes were simulated using HYSPLIT: Hybrid Single-Particle Lagrangian Integrated Trajectory model[25] based on ERA5 meteorological reanalysis data. Data available in ERA5 present the highest spatial and temporal resolution available for that region. Comparisons with the lower spatial and temporal resolution data available from the MERRA2 reanalysis data[57] and the Global Data Assimilation System available at 0.5 degree spatial resolution showed good agreement in trajectory direction and overall distance (not shown). Trajectories of each captured mosquito were simulated starting at its capture location, altitude, and all multiple interception (full) hours during the night of the collection. Because anophelines are nocturnal, we conservatively assumed that flights started at or after 18:00 and ended by 06:00 the following morning and computed trajectories for every hour that allowed for a total of two or nine h flight. For example, to complete 9 hours flight by 06:00, a mosquito could have started at 18:00, 19:00, 20:00, or 21:00. Total flight duration of tethered female *An. gambiae* s.l. and *An. atroparvus* reached or exceeded 10 hours with average speed of 1 km/h[52] in accord with other studies[53,58,59]. Likewise, *An. vagus* and *An. hyrcanus* caught 150 m agl after midnight over India would have been migrating for >6 hours, assuming they took off around dusk[20]. Thus, we conservatively assumed that windborne long-distance migrant anopheline mosquitoes fly between two and nine hours per night although longer duration is possible. Each trajectory consisted of the global positions of the mosquitoes at hourly intervals from the interception time. In addition to plotting trajectories[60–67], the linear distance from the interception site and the azimuth (angle between interception site and mosquito simulated position from the North, projected on a plane) were computed for all trajectories. To evaluate distance range and dominant directions of flight, the mean and 95% CI of the distance and azimuth (as a circular statistic) were computed for the two- and nine-hours flight trajectories. The dispersion of individual angles (azimuths) around the mean was measured by the mean circular resultant length 'r', which can vary from 0 to 1, with higher values indicating tighter clustering around the mean. Rayleigh's test was used to test that there was no mean direction, as when the angles form a uniform distribution over a circle[68].

Data and Code Availability

1. Data on anopheline capture, identification, sex, and gonotrophic status are available from www.boldsystems.org (Project code: MALAN) and in Genbank (MK585944–MK586043).

2. SAS code used for statistical analyses (and data manipulations) and 9-hour backward trajectories data for each mosquito capture event based on HYSPLIT are available from TL upon request.

3. Plotting trajectories (code available at https://github.com/benkraj/anopheles-migration)

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Extended Data Legends

Extended Data Figure 1. Study area and aerial sampling effort. a) Map of the study area with aerial sampling villages and the number of sampling nights per village under a schematic map of Africa showing the Sahel region (source: Wikipedia, https://pt.m.wikipedia.org/wiki/Ficheiro:Sahel_Map-Africa_rough.png). **b)** Nightly sampling effort by year. Fringe under zero indicates the sampling nights (by village) and needles denote the total number of mosquitoes per night regardless of the number of panels per night. Dry and wet seasons are indicated by yellow and green in the ruler under the X-axis.

Extended Data Figure 2. Regularity of migratory flights, flight altitude, and variability among years and localities in species aerial presence. a) Relationship between mosquito presence (fraction of positive nights) and mean panel density to evaluate if appearance can be accounted by overall abundance

rather than by unique migratory nights. **b)** The relationship between panel height and mean mosquitoes density/panel ($x10^3$, regression line with shading denotes 95% CI) showing mean panel density by species. Inset summarizes the covariance analysis (ANCOVA), underlying this regression, which includes the species and panel height. Number of nights per panel altitude is given in blue along the X axis (see Figure 1a). **c)** Variation in mosquito presence (fraction of positive nights) by species between years (top) and villages (bottom) with their 95% CI. Sampling effort expressed as the number of panels per year/village is shown adjacent to the legend.

Extended Data Table 1. Variation in mosquito capture rate between years, localities, and heights above ground (GLIMMIX models of random and fixed variables, total number of panels was 1,894).

Extended Data Figure 3. A photo showing a tethered sticky panel setup and attachment. A sticky panel (3x1m net) on a test helium balloon (lower volume/capacity), showing attachment of net covered with glue to the cord tethering the balloon to the ground. Note the four carbon poles and Velcro attachment points (see text for details). A close-up of the attachment of the panel to the cord and preparing to launch a standard 3 m balloon.

Figure 1.

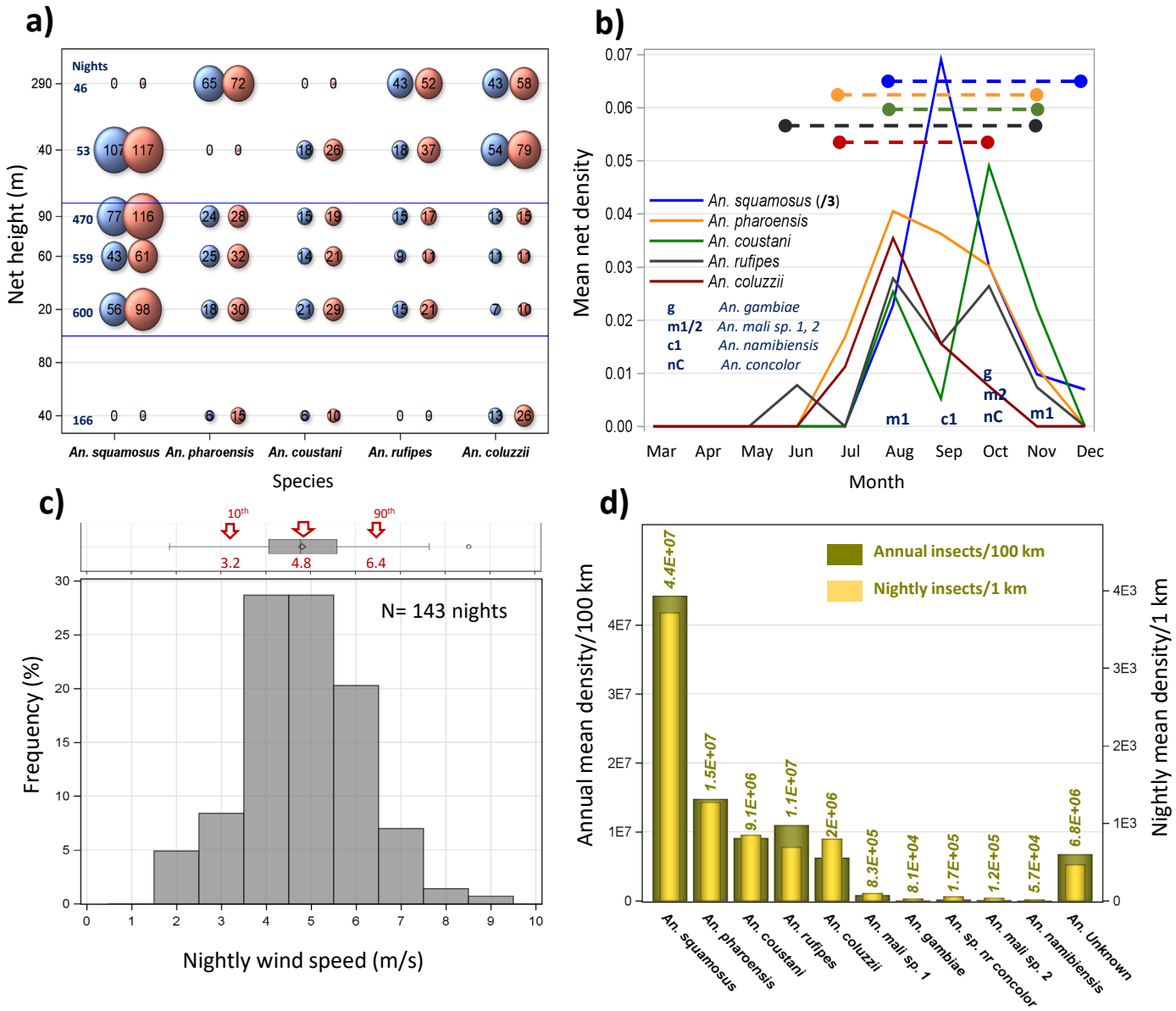
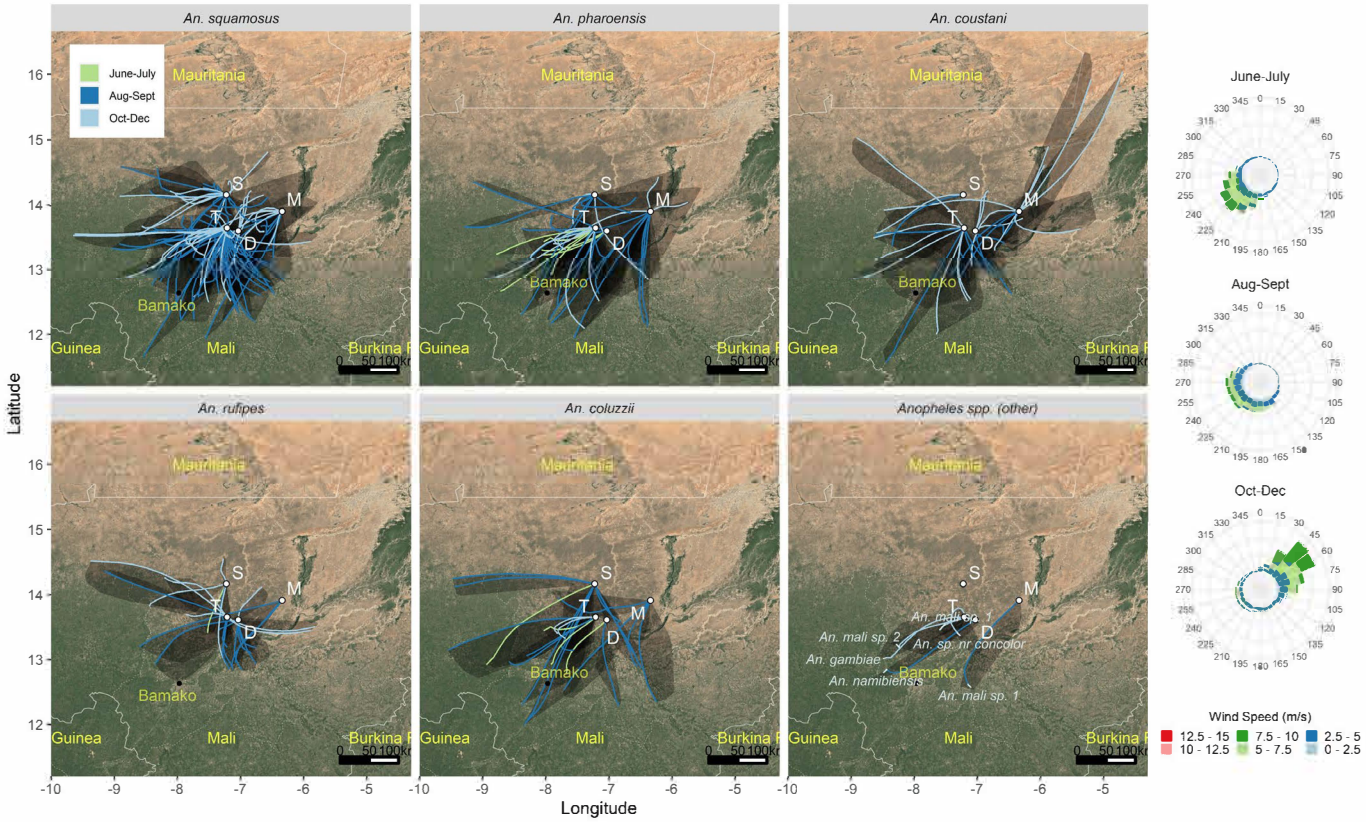
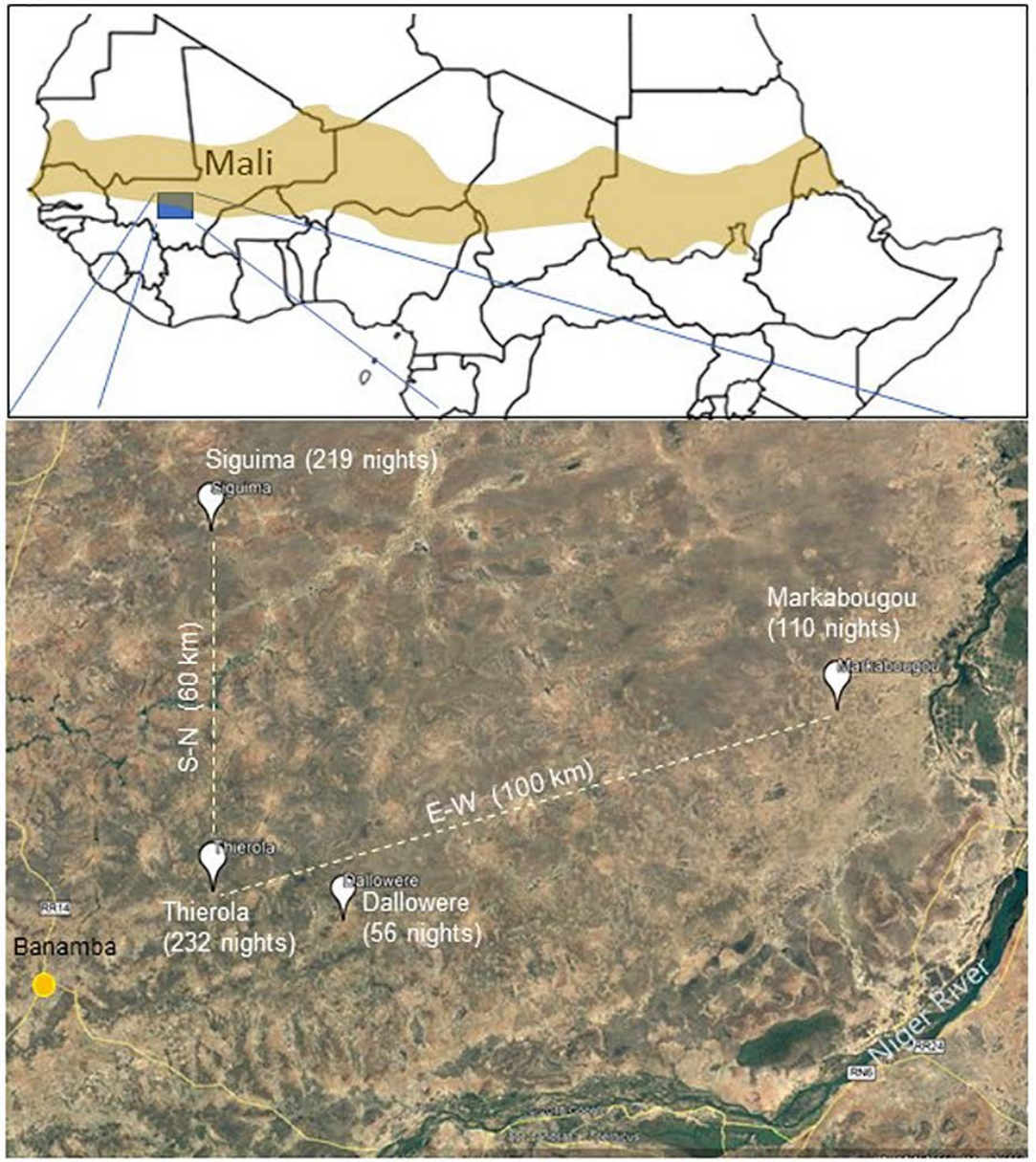

a)
Nights
Net height (m)
An. squamosus
An. pharoensis
An. coustani
An. rufipes
An. coluzzii
Species
b)
Mean net density
An. squamosus (/3)
An. pharoensis
An. coustani
An. rufipes
An. coluzzii
g An. gambiae
m1/2 An. mali sp. 1, 2
c1 An. namibiensis
nC An. concolor
Mar Apr May Jun Jul Aug Sep Oct Nov Dec
Month
c)
10th
90th
3.2
4.8
6.4
N= 143 nights
Frequency (%)
Nightly wind speed (m/s)
d)
Annual insects/100 km
Nightly insects/1 km
Annual mean density/100 km
Nightly mean density/1 km
4.4E+07
1.5E+07
9.1E+06
1.1E+07
2E+06
8.3E+05
8.1E+04
1.7E+05
1.2E+05
5.7E+04
6.8E+06
An. squamosus
An. pharoensis
An. coustani
An. rufipes
An. coluzzii
An. mali sp. 1
An. gambiae
An. sp. nr concolor
An. mali sp. 2
An. namibiensis
An. Unknown

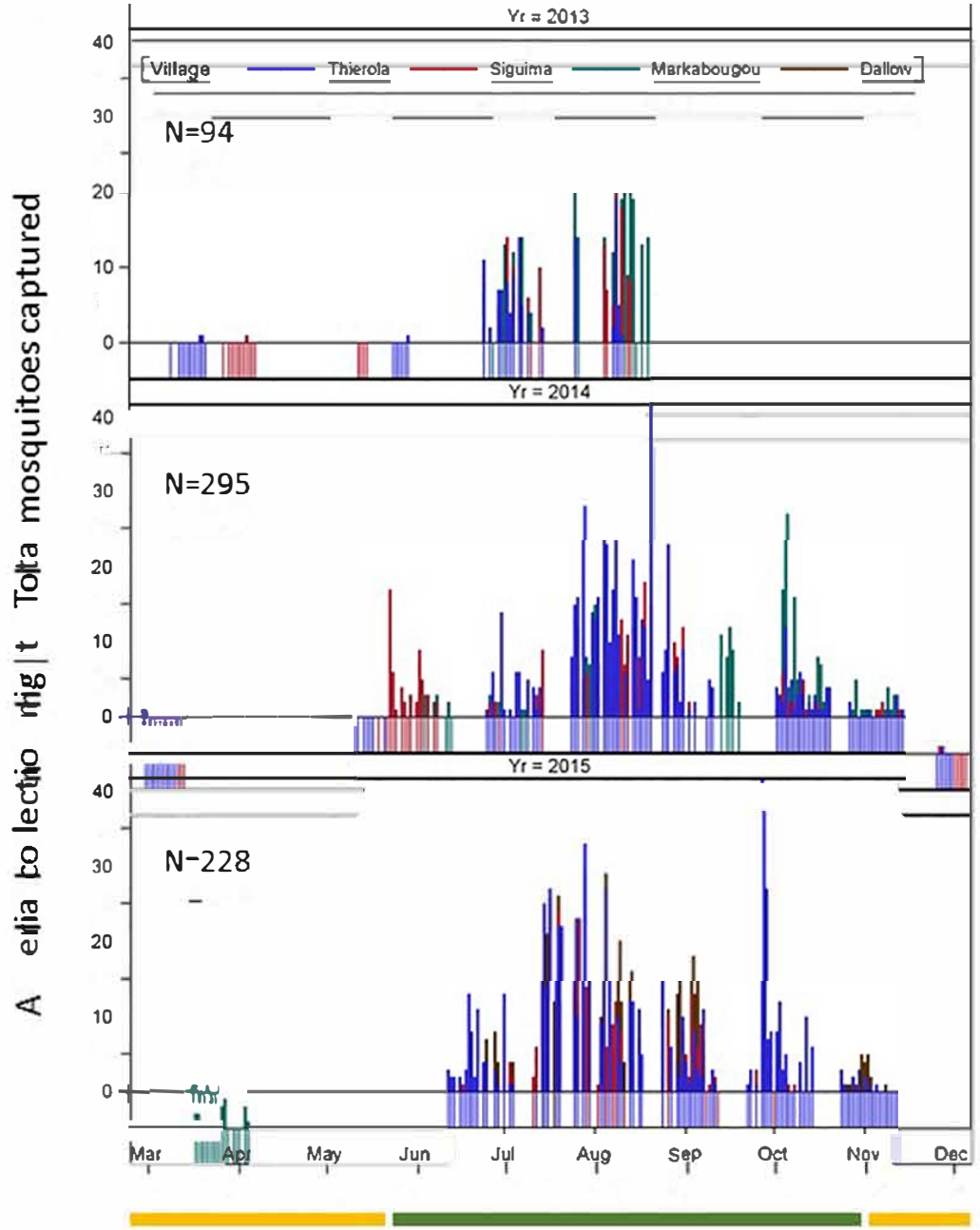
Figure 2.


An. squamosus
An. pharoensis
An. coustani
An. rufipes
An. coluzzii
Anopheles spp. (other)
June-July
Aug-Sept
Oct-Dec
Mauritania
Guinea
Mali
Bamako
S
M
T
D
An. mali sp. 2
An. gambiae
An. namibiensis
An. mali sp. 1
An. sp. nr concolor
Latitude
Longitude
Wind Speed (m/s)
12.5 - 15
10 - 12.5
7.5 - 10
5 - 7.5
2.5 - 5
0 - 2.5

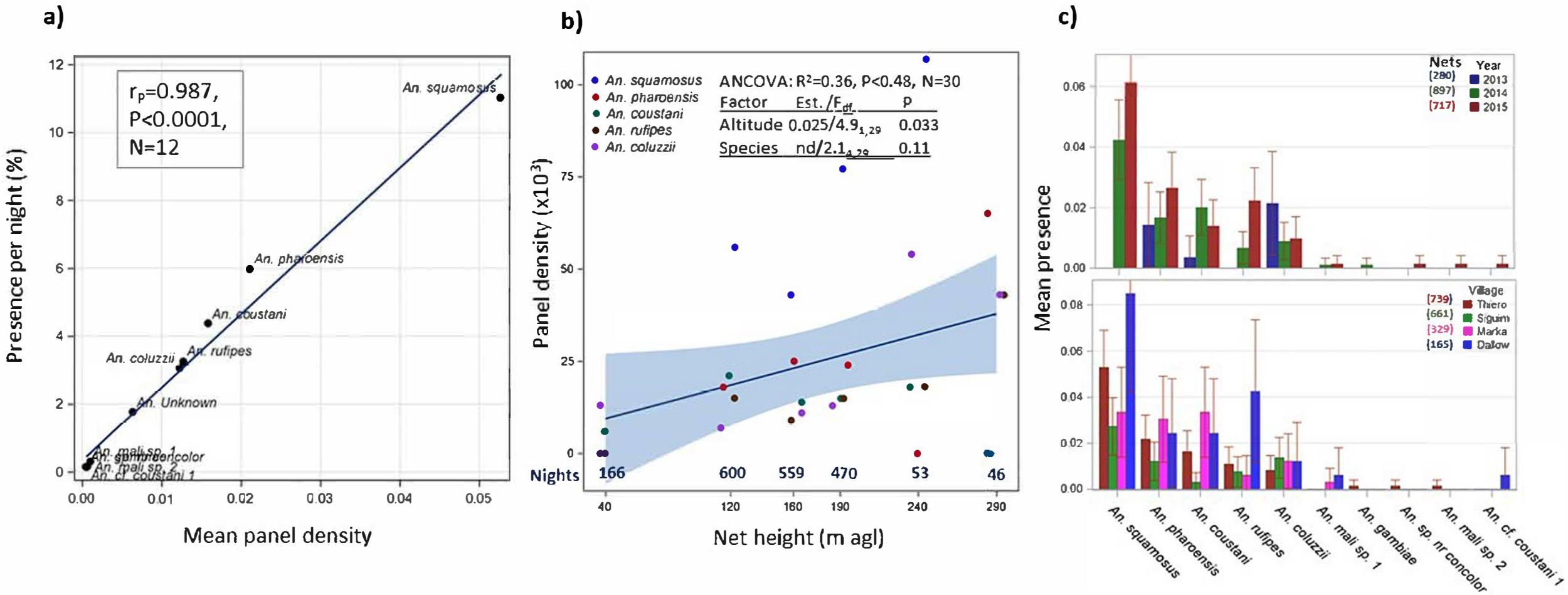
ED Fig.1 a)


Mali
Siguima (219 nights)
Siguima
S-N (60 km)
Markabougou
(110 nights)
Markabougou
E-W (100 km)
Thierola
Dallowere
Thierola
(232 nights)
Dallowere
(56 nights)
Banamba
Niger River

b)


Yr = 2013
Village
Thierola
Siguima
Markabougou
Dallow
N=94
Yr = 2014
N=295
Yr = 2015
N=228
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

Extended Data Figure 2



Extended Data Figure 3

Extended data Table 1 .

Dependent: Panel Density	Parameter	*A. squamosus*	*A. pharoensis*	*A. coustani*	*A. rufipes*	*A. coluzzii*
Random vars only: Poisson	Pearson $\chi 2$/df (BIC)	1.13 (793.5)	**1.04 (394.4)**	**0.90 (306.52)**	**1.11 (260.4)**	**1.16 (252.8)**
Random vars only:	Pearson χ^2/df, Scale[a] (BIC)	**0.83, 5.98*** (756.2)**	0.97, 3.84^{ne} (391.4)	0.87, 2.09^{ns} (306.7)	0.99, 10.6^{ne} (254.5)	0.98, 15^{ns} (246.7)
Negative Binomial	intercept[mean] (SD)	-4.06^{ns} (1.23)	-3.9** (0.226)	-4.4* (0.63)	-4.7*** (0)	-4.4** (0.23)
	Year (SD)	3.24^{ns} (4.36)	0^{ns} (0.06)	0.09^{ns} (0.31)	0.55^{ns} (0.56)	0^{ne}
	Locality[b] (SD)	0.075^{ns} (0.116)	0.04^{ns} (0.15)	0.73^{ns} (3.19)	0^{ne}	0^{ne}
Random vars only: Poisson	Night[c] (SD)	**4.02** (1.42)**	**1.78* (0.99)**	6.57^{ns} (7.3)	**29.0* (16.8)**	**32.0* (17.9)**
Random vars only: Neg. Bin.	Night[c] (SD), scale	3.9** (1.5), 0.74^{ns}	1.6^{ns} (1.1), 0.34^{ns}	0.5^{ne} (ne), 0^{ne}	30.1* (17.5), 0.7^{ns}	33.5* (18.7), 0.76^{ns}
Fixed and random: Poisson	Pearson χ^2/df (BIC)	0.37 (700)	0.6 (403)	0.2 (308)	0.09 (258)	0.08 (243)
	Night	1.4** (0.0)	0.78^{ns} (0.8)	1.9* (1.1)	14.0^{ns}(13.3)	21.9^{ns} (15.2)
	Period[d]	Aug-Oct*	Aug-Oct*	Aug-Oct^{ns}	Aug-Oct^{ns}	Aug-Oct***
	Panel height (m)	0.001***(0)	0.003***(0)	-0.007***(0)	0.001***(0)	0.014*(0.006)
Dependent: Aerial Density	Pearson χ^2/df (BIC)	0.42 (938)	0.41 (503)	0.2 (378)	0.1 (304)	0.09 (283)
Fixed and random: Poisson	Night	2.9*** (0.8)	2.6* (1.2)	5.2^{ns} (3.9)	26.8*(16.0)	31.5* (17.6)
	Period[d]	Aug-Oct^{ns}	Aug-Oct*	Aug-Oct^{ns}	Aug-Oct^{ns}	Aug-Oct***
	Panel height (m)	-0.003***(0)	-0.002***(0)	-0.008*(0.004)	-0.001***(0)	0.01*(0.005)

[a] - For negative bionomial scale parameter estimates the k parameter of this distribution.

[b] - The effects locality was estimated considering only 3 locations after pooling Dallowere and Thierola which are only 20 km apart (see Methods).

[c] The significance of clustering by night (across locations) estimated as the only random effect (using subject statement) after finding insignificant variance componenets of Year and Location.

[d] Periods included: March-May, June-July, August-October, and November-December. The period of highest panel density is shown with its statistical significance.

[e] Panel height levels inlcuded 40, 120 (90-120), 160, 190, and 250, (220-290) m agl due to small sample sizes (nights) of certain altitudes.

***, **, *, ^{ns}, and ^{ne} refer to significance probability of 0.001, 0.01 and 0.05, >0.05, and to parameters that could not be estimated, respectively.

Supplementary Information:

Windborne long-distance migration of malaria mosquitoes in the Sahel

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Supplementary Discussion

Seasonality and altitude as sources of variation in mosquito capture and between-species correlations:

Abundance measured by mean panel density (insects/net), varied more than 100-fold between *An. squamosus* and *An. gambiae*. The frequency with which anophelines were caught varied between 0.2 and 11% per night (Table 1) and was highly correlated with the overall mean density of species (r=0.987, P<0.0001, N=11, Fig. S2), indicating that species caught less frequently were the least abundant, rather than exhibiting more clustered timing of flights. Clustering of capture events on panels was detected only for *An. squamosus* by a significant scale parameter of the negative binomial distribution (Table S1). The inclusion of the sampling night in the model, however, rendered no remaining support for clustering at the panel level even in *An. squamosus* (Table S1) and indicated that mosquitoes do not fly together in a swarm but as separate individuals as is typical of nocturnally-migrating insects[1]. Even after accommodating seasonality, sampling night was a significant source of variation in all species except for *An. coustani* (Table S1), indicating that although migration occurred over many nights, particular nights had higher migration activity (Table S1 and below). Correlations between species' nightly aerial densities during the migration period (July-November) were modest with the highest (r = 0.26, P<0.001, nights = 221) between *An. coluzzii* and *An. pharoensis* followed by that between *An. squamosus* and *An. coustani* (r = 0.15, P<0.025, nights = 221), indicating mostly independent species migration events. Elucidating the contributions of the species abundance in source locations and favorable conditions for migration in the air (or the ground) to nights with elevated flight activity awaits further studies. All but one of the species (*An. coustani*) analyzed showed a significant positive effect of altitude on panel density, but this relationship was reversed in the analyses of aerial density in three of the species (*An. squamosus*, *An. pharoensis*, and *An. rufipes*; Table S1). Similarly, in the cross-species ANCOVA (analysis of covariance), the effect of panel height on panel density was significant (ED Fig 2b) as was its effect on aerial density, but unlike the former, the latter was not statistically significant (slope=0.0001/m, P=0.093, $F_{1/24}$=3.07), nor was the effect of species (P=0.085, $F_{4/24}$=2.33), suggesting that once corrected for wind speed, the effect of elevation was minimal. Thus, the greater volume of air passing through the higher panels may account for the increased abundance of the latter three species but not that of *An. coluzzii*, which shows increased abundance in higher altitudes after accommodating for the effect of air volume.

Estimation of Plasmodium *infection likelihood:*

To compute the binomial probabilities of obtaining zero infected mosquitoes, we conservatively used the upper 95% infection rate (4.1%) based on Hay and colleagues[2] who compiled 125 studies in Africa, focused on *An. gambiae* s.l. and *An. funestus* (mean infection rate = 3.4%). For secondary vectors, we used a 1% infection rate based on the sources listed in the main text. Because infection rate determined by ELISA is expected to be lower than that determined by PCR, our calculation might have overestimated the likelihood of zero infection rate in our samples. However, data on ELISA-based measurements of mosquito infection rates is extensive and unmatched by the few studies using PCR. Moreover, infection rates during our study (2013–2015) were significantly lower[3] than that during the period covered by Hay and colleagues[2] and ELISA is known to excessively produce false positives[4]. Moreover, our aerial sampling concentrated on the early rainy season (June–August) and the late dry season peak (March–April[5]), when infection rates are lowest, therefore, although we relied on infection rates measured by ELISA, the likelihood of finding uninfected mosquitoes based on PCR may not be much lower than our estimates reveal.

An additional source of potential bias in estimates of infection rate of secondary vectors is that available data are based on sampling in rural communities, where humans are concentrated, rather than in the wild. However, the elevated concentration of cows, goats, sheep, dogs, cats, chickens, guinea fowl, ducks, rodents, and other domestic and sylvatic animals around these communities provide even larger access to a non-human host. Successful PCR bloodmeal amplification[3] was obtained from 38 of 159 specimens (mostly gravid, Table 1), showing that overall, 31% of bloodmeals were wholly or partially human in origin, with the remainder being from goat and cattle sources. These results show that, as expected, the degree of anthropophagy is lower in secondary than in primary malaria vectors, yet they confirm that these windborne secondary malaria vectors are exposed to human blood and therefore, include potentially infected mosquitoes.

High-altitude flight of mosquitoes is a deliberate species-specific activity

As has been established for other windborne migrant insects, ample evidence suggests that mosquitoes deliberately ascend into and descend out of the winds at altitude and thus, manifest some control over their long-range movements[1,4]. In addition to the non-random composition of the sexes and female gonotrophic states (Main Text), the species composition at altitude (Table 1, Fig. 1) also differs from expectations based on ground sampling. The high-altitude collections were dominated by secondary malaria vectors, e.g., *An. squamosus* and *An. pharoensis* (Table 1), whereas, on the ground using indoor collections, outdoor clay-pot traps, and larval collections in the vicinity of the same villages, >90% of *Anopheles* captured were *An. gambiae* s.l.[5,6]. Different sampling methods, e.g., animal baited traps, would yield a higher abundance of the zoophilic taxa (e.g., *An. rufipes*), but it remains unclear if this ground composition will resemble the aerial one because larval collections are similar to composition indoors, indicating that the composition of anopheline species on the ground and at altitude are distinct. Most species found at altitude are expected to be found on the ground, but the reverse may not be true because not all species engage in windborne migration. However, even considering sampling bias, it is puzzling that our ground collections consisting of many thousands of anophelines, failed to identify a single *An. squamosus* or *An. coustani*. The differences between altitude and ground collections of the anthropophilic members of *An. gambiae* s.l. are more robust because they share similar larval, biting, and resting sites[5,7–10] and thus are less affected by sampling bias (above). Ground collections in the same villages show that *An. coluzzii* predominates throughout the year, except between late September and early November, when the other sibling species together often exceed 70%[5]. In that window *An. coluzzii* typically drops below 30% of the ground collection and some years dips below 10%, before it regains its dominance by mid-

November. Despite their abundance on the ground during October, aerial sampling collected just a single *An. gambiae*, no *An. arabiensis*, and one *An. coluzzii* suggesting species-specific differences in high altitude flight behavior (Main Text). Species represented by a single specimen may be accidental or less abundant regular windborne migrants. More data are needed to resolve this, yet the low efficiency of the aerial sampling method implies that aerial density must be substantial even for a single capture.

Because insect windborne migration starts and ends on the ground, sampling at lower elevations, e.g., 40 or 90 m may reflect ascent and descent in addition to the horizontal 'transmigration' phase. Accordingly, if migrants fly homogenously at all altitudes between 50 and 250, we expect to find more at low altitudes especially if transmigration is relatively short. However, the results suggest the reverse, indicating that transmigration is long and mosquitoes concentrate at altitudes above 100 m, further solidifying the view that windborne migration is a deliberate activity of mosquitoes as it is in many other insects[1,4,11].

Concerns about viability of windborne migrant insects have been settled long ago by many studies. For example, Taylor[12] compared survival and reproduction in a live collection of insects, including some small Diptera (using non-sticky nets, at altitudes similar to our panels) with those captured on the ground. After finding similar survival and reproductive success, Taylor concluded that "This seems to establish the viability of high-level migrants beyond reasonable doubt." Furthermore, the mosquitoes caught by aerial netting in China and India by one of the present authors (Reynolds DR)[13,14] were alive and active upon capture. On a few occasions during removal from the sticky nets in our study, *Anopheles* mosquitoes were observed moving their limbs despite the glue, substantiating their capture as live insects. Further, to test survival of mosquitoes at high altitudes, we placed female *Anopheles gambiae* s.l. collected the same morning indoors (from villages near aerial sampling stations) individually, in modified 50 ml tubes (both ends opened covered with mesh) affixed to the net's frame, so that wind passed through the tubes. There was no difference in survival (Likelihood Ratio Chi Square Test: $P>0.38$, $\chi^2_1=0.75$) of these females kept at altitude (>100m, 58% N=26) vs. on the ground (71%, N=17) from launch (17:30) to retrieval (07:00, the next morning). These experiments affirm Taylor's conclusion (above) specifically for mosquitoes.

Role of windborne migration in Anophelines:

Our results affirm anecdotal observations of anophelines flying at high altitudes in North America, South Asia, and Australia[15–17], and inferences of long-distance windborne migration of *An. pharoensis*[4,18,19] and *An. squamosus*[20]. However, the significance of these movements has been largely disregarded by vector biologists, malariologists, and epidemiologists[19,21] who maintain that the dispersal of malaria mosquitoes does not exceed 5 km[19,22–24], with mean distances of 0.54, 0.85, and 1.1 km (S.D. ~0.4 km) reported for the genus *Anopheles*, *An. gambiae* s.l., and *An. pharoensis*, respectively[25]. Long-distance migration provides a powerful explanation for the puzzling shallow genetic structure of *An. gambiae* and *An. coluzzii* over large geographical distances[26–30] and for the persistence of certain Sahelian vector populations, as revealed by comprehensive modeling[31]. The importance of long distance migration to malaria control and elimination is arguably linked to the success of those African countries near elimination, (so-called "E-2020"[32]), because they are all surrounded by >200 km "migration barriers": Cabo Verde and Comoros (oceans), Algeria (Sahara Desert and Mediterranean Sea), Botswana (Kalahari Desert) and South Africa and Swaziland (Ocean, Kalahari Desert, and the near-elimination areas), supporting the role of windborne migration in "residual" transmission. Separating the roles of Odyssean malaria[21] (transmission via infected mosquitoes transported by vehicles) from windborne migrants necessitates further studies (Main Text). Whether windborne migration has limited the success of past interventions, such as the Garki project, that included intensive use of insecticides and drugs[33], remains to be answered. It is noteworthy that the Onchocerciasis Control Programme (OCP) in West Africa, had to

be restructured because large numbers of blackflies *Simulium damnosum* s.s. and *S. sirbanum* engaged in wind-assisted migration (closely associated with the northward movement of the Inter Tropical Convergence Zone) over distances of over 400 km, resulting in recolonization of the control areas[34,35]. Most migrants were post blood feeding and included flies infected with *Onchocerca volvulus*. Other vectors like *S. yahense* and *S. squamosum* traveled only a few kilometers, indicating that migratory behavior was highly species-specific.

Our results reveal that similar to many other insects[1,36,37] anophelines exhibit two modalities of movements: appetitive movements in their 'flight boundary layer', within approximately the first 5 m agl[38,39] and long-range windborne movements in altitudes that include 100–300 m agl. Unlike most long-distance flying insects, which are post-teneral (i.e. newly-emerged, typically pre-reproductive, adults)[36], our results show that anopheline female mosquitoes engage in such flights after taking a blood meal. What primes these mosquitoes to undertake high-altitude flights and whether migrants have already deposited an egg batch in their provenance area prior to their journey remain to be explored, as well as if they embark on more than a single night of windborne migration. Although significant species-specific differences in displacement distances were detected (Table 2), the scale of displacement distance was similar among species. The West African Sahel is dotted with human settlements seldom separated by more than 7 km, suggesting that appetitive flights would suffice to land a migrant in a village even if it descended from altitude in between them. However, distances between villages were longer a hundred years ago, raising the question of whether windborne migration in anthropophilic mosquitoes is recent. The proposed recolonization of the Sahel by species such as *An. gambiae* from southern source populations (Main Text) follows a "source-sink model" that requires "return migration" to maintain this strategy[40]. We have detected few such movements (Fig. 2), possibly because such return flights occur in large numbers only over a few nights (e.g., the grasshopper *Oedaleus senegalensis*[41]), every several years, or because our sampling sites were located closer to the northern edge of the migration zone instead of near its center; hence, there are fewer source populations that can produce migrants to be detected by our sampling method. Accordingly, aerial sampling ~150 km south of our current locations may be used to test this hypothesis. With many questions awaiting answers, we believe the evolution of windborne migration in mosquitoes, its drivers, mechanisms, and impacts present a new and important scientific frontier. The implications of these investigations will improve our understanding of disease transmission, disease modeling, and malaria control and elimination efforts.

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