

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 migration³. 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 surge⁶ 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 perennial³. 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.

51 Mosquito dispersal, hereafter referred to as migration¹², has been extensively studied because it directly
52 impacts disease transmission, the spread of adaptations (e.g., insecticide resistance), and control
53 strategies, such as insecticide barriers^{13,14}. Although tracking mosquitoes over large scales has seldom
54 been attempted^{13,14}, the prevailing view is that the dispersal of malaria mosquitoes does not exceed 5
55 km¹³⁻¹⁶ and the alternative view¹⁷⁻²⁰ is typically considered to pertain to “accidental events” of minimal
56 epidemiological importance¹³. Nonetheless, the prediction of long-distance migration of anophelines in
57 the Sahel prompted us to question this dogma. Our study is the first to systematically sample insects
58 migrating at high altitude over multiple seasons in Africa to determine if malaria vectors engage in wind-
59 assisted movements, and if so, assess the epidemiological relevance by addressing the following
60 questions: what species are involved? how frequently and at what heights do they fly? how many
61 mosquitoes migrate and how likely are they to carry *Plasmodium*? Then, using simulations, we estimate
62 how far mosquitoes may have travelled and from where.

63 During 617 aerial sampling nights, we caught 461,100 insects at heights between 40–290 m agl, in four
64 villages in the Sahel of Mali, West Africa (ED Fig. 1), including 2,748 mosquitoes, of which 235 were
65 anophelines (Table 1). These mosquitoes belonged to 10 species: *Anopheles coluzzii*, *An. gambiae*, *An.*
66 *pharoensis*, *An. coustani*, *An. squamosus*, *An. rufipes*, *An. namibiensis* and three distinct but currently
67 undetermined *Anopheles* (Table 1). The first two are the primary malaria vectors in Africa, with the next
68 four of secondary importance²¹. Mosquitoes were not among the 564 insects captured on 508 control nets
69 (Table 1, and Methods), confirming that these *Anopheles* were intercepted at altitude rather than near the
70 ground during deployment. The maximum anophelines/night was three, indicating that migration
71 occurred over many nights. Consistent with Poisson distributions, the values of the variance to mean ratio
72 were all near one (Table 1 and Supplementary Discussion). Unless otherwise specified, quantitative
73 results presented hereafter refer to the five most abundant *Anopheles* species, represented by >20
74 individuals (Table 1).

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

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

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

96 27 (*An. gambiae*) and 3,719 (*An. squamosus*, Fig. 1d) per night. When interpolated over a 100-km line
97 joining our sampling sites (ED Figs. 1a, 2c), annual migrations exceeded 80,000 *An. gambiae*, 6.25
98 million *An. coluzzii*, and 44 million *An. squamosus* in that region alone (Fig 1d). Thus, windborne
99 migration in the Sahel occurs on a massive scale.

100 For each mosquito capture event, flight trajectories for two- and nine-hour flight durations were estimated
101 using HYSPLIT²⁵ (using the most accurate assimilated meteorological data available: ERA5), assuming
102 that mosquitoes ascend by their own flight but are passively carried by the wind at altitude (Methods).
103 The mean nightly displacements (straight-line distances) were 30 and 120 km (maxima 70 and 295 km),
104 respectively (Table 2 and Fig. 2). Notably, maximal 9-hour nightly flight displacements ranged between
105 257–295 km for all anophelines with sample size >20 (Table 2). These backwards trajectories exhibited a
106 south-westerly origin (Rayleigh test; mean bearing = 212°, $r = 0.54$, $P < 0.0001$, Table 2), corresponding
107 to the prevailing winds during peak migration (August–September, Fig. 2). Trajectories of most species
108 originated from a broad arc (>90 degrees, Fig. 2), suggesting migrants emanated from multiple sites
109 across a large region. Migration from this direction fits with the presence of high-density populations due
110 to perennial larval sites and earlier population growth following the monsoon rains. The back-trajectories
111 with a strong northerly component, observed during the sparsely sampled period of October–December
112 (Fig. 2) might indicate southward “return flights”, on the Harmattan winds prevailing during this season.

113 Contrary to the conventional view that dispersal of African anophelines is <5 km^{13,15,16,26}, our results
114 provide compelling evidence that primary and secondary malaria vectors regularly engage in windborne
115 migration spanning tens to hundreds of kilometers per night. With massive numbers of females that had
116 taken at least one blood-meal, this migration probably involves human *Plasmodium* among other
117 pathogens. Separate outbreaks of malaria in Egypt and Israel have been attributed to *An. pharoensis*
118 traveling over 280 km¹⁷. Assuming, a conservative^{23,27}, 1% infection rate in migrating females of *An.*
119 *coluzzii*, *An. gambiae*, *An. coustani*, and *An. pharoensis* and 0.1% in the remaining anophelines
120 (excluding the unknown *An. sp.* Mali 1 and 2, Supplementary Discussion), a total of 286,700 infected
121 migrant mosquitoes are expected to cross a 100-km line perpendicular to the wind at altitude every year.
122 Accordingly, *An. pharoensis*, *An. coustani*, and *An. coluzzii*, contributed 41%, 25%, and 17%,
123 respectively, to the malaria transmission by infected windborne mosquitoes. Although these estimates are
124 relatively coarse, this suggests that migratory secondary vectors could be a major infection source and
125 should be included in studies of transmission as well as in control programs.

126 Contrary to our initial prediction³, *An. coluzzii* was more common than *An. gambiae* among the migrants.
127 This expectation was based on data suggesting that *An. coluzzii* aestivates locally and thus may not
128 require long-distance migration to recolonize the Sahel. Indeed, windborne migration occurs from the end
129 of July to October, well after the surge of Sahelian *An. coluzzii* following the first rain (May–June)^{3,6}. The
130 northward and southward oscillations of the Intertropical Convergence Zone during the wet season
131 continually create better mosquito resource-patches with the rains. Additionally, wet-season droughts
132 endanger local mosquito populations every decade or two²⁸. Thus, selection pressures to track fresh-water
133 resources by riding the winds that bring rain²⁹ may explain why Sahelian residents such as *Oedaleus*
134 *senegalensis* grasshoppers and *An. coluzzii* have a mixed strategy of migration³⁰ and local dormancy.
135 *Anopheles gambiae*, which presumably recolonizes the Sahel every wet season is relatively rare in
136 Sahelian villages³, and thus only one specimen was captured by our nets. It may migrate on fewer nights
137 and constitute a smaller fraction of windborne migrants (Supplementary Discussion).

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

141 infected mosquitoes. For example, north-eastern South Africa has the highest incidence of persistent
142 malaria in the country with many cases not associated with human travel, which are concentrated in an arc
143 extending over ~150 km from the borders with Zimbabwe and Mozambique, where transmission is still
144 high. This area includes the Kruger National Park where roads are scarce and vehicular transport of
145 infected mosquitoes³¹ may be hampered. Testing the correlation of such infection events with
146 corresponding winds will help to assess this hypothesis. If confirmed, incorporation of disease control
147 efforts in source populations to minimize or block migration are likely to be an essential element of the
148 elimination strategy.

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

150

Taxa	Standard Panels ^a (N=1,894)											Control Panels ^b (N=508)		
	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
<i>An. squamosus</i>	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
<i>An. pharoensis</i>	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
<i>An. coustani</i>	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
<i>An. rufipes</i>	24	0.013	0.008	0.018	2	3.24	1.16	80 (20)	93.8 (16)	0 (16)	0 (4)	0	0	0
<i>An. coluzzii</i>	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
<i>An. (Ano.) sp. Mali 1</i>	2	0.001	0	0.003	1	0.32	1	100 (2)	100 (2)	0 (2)	nd	0	0	0
<i>An. gambiae s.s.</i>	1	0.0005	0	0.002	1	0.16	1	100 (1)	100 (1)	0 (1)	nd	0	0	0
<i>An. sp. nr concolor^g</i>	1	0.0005	0	0.002	1	0.16	1	0 (1)	na ^f	na	na	0	0	0
<i>An. sp. Mali 2</i>	1	0.0005	0	0.002	1	0.16	1	100 (1)	100 (1)	0 (1)	nd	0	0	0
<i>An. namibiensis</i>	1	0.0005	0	0.002	1	0.16	1	100 (1)	100 (1)	0 (1)	nd	0	0	0
<i>Anopheles</i> 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

151

152 ^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
153 290 m above ground (see Methods).

154 ^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
155 during the ascent and descent (see Methods).

156 ^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.

157 ^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
158 these mosquito species blood feeding is required for egg development as indicated by the gravid state. Unfed mosquitoes consisted of the rest.

159 ^e Infection with human *Plasmodium* species was tested as described in the Methods.

160 ^f na and nd denote not applicable and not determined, respectively.

161 ^g This species was identified based on male genitalia

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

163

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

166

Taxa	Trajectories: 2-hour flight				Trajectories: 9-hour flight								
	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]
<i>An. squamosus</i>	1100	27.7	27-29	2-68	400	109.1	103-115	4-265	13.3	12.1	213	0.516	0.0000
<i>An. pharoensis</i>	440	31.1	30-33	1-65	160	125.3	116-134	24-260	14.7	13.9	214	0.660	0.0000
<i>An. coustani</i>	330	28.5	27-30	2-60	120	125.8	114-138	16-295	14.5	14.0	199	0.270	0.0802
<i>An. rufipes</i>	264	26.1	24-28	2-70	96	109.2	97-121	24-257	12.5	12.1	199	0.454	0.0003
<i>An. coluzzii</i>	253	38.6	37-41	3-69	92	154.1	140-168	47-270	17.3	17.1	217	0.815	0.0000
<i>An. sp. Mali 1</i>	22	20	14-26	6-52	8	94.3	52-136	51-172	10.2	10.5	223	0.947	0.0000
<i>An. gambiae s.s.</i>	11	33.5	ND ^b	ND ^b	4	131.1	ND ^b	ND ^b	15.9	14.6	254	ND ^b	ND ^b
<i>An. sp. nr concolor</i>	11	17.2	ND ^b	ND ^b	4	48.2	ND ^b	ND ^b	8.4	5.4	184	ND ^b	ND ^b
<i>An. sp. Mali 2</i>	11	29.9	ND ^b	ND ^b	4	104.4	ND ^b	ND ^b	13.1	11.6	234	ND ^b	ND ^b
<i>An. namibiensis</i>	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

167

168 ^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
 169 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.

170 ^b Not determined for species with a single specimen captured.

171 ^c Hourly displacement between successive 1-hour points along the 9-hour trajectory.

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

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

174 ^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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262 the purpose of providing specific information and does not imply recommendation or endorsement by the
263 USDA.

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265

266 **Authors Contributions**

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

277

278 **Competing Interests:** All authors declare no competing financial interests.

279

280

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318

319 **Figure Legend**

320 **Figure 1. Flight altitude, seasonality, wind speed, and abundance of migratory anopheline species.**

321 **a)** The relationship of altitude (panel height) and panel- (blue) and aerial- (orange, mosquitoes/ 10^6 m³ of
322 air) density for the five most common anopheline species (Table 1). Bubble size is proportional to density
323 ($\times 10^3$ is shown in the bubble), thus no bubble is shown with zero value. The number of sampling nights
324 (Nights) per panel height is shown on the left. **b)** Monthly panel density (N=1,894 panels) for the five
325 most common species (Table 1. Note: values of *An. squamosus* were divided by three to preserve scale)
326 overlaid by the length of migration period (dashed lines). Sampling month of species collected once or
327 twice is shown by letters. **c)** Distribution of mean nightly wind speed at flight height in nights with one or
328 more anopheline collected. Wind speed data were taken from ERA5 database after matching panel height
329 to the nearest vertical layer (Methods). Corresponding box-whisker plot (top) shows the median, mean,
330 quartiles and extreme values overlaid by arrows indicating the mean, 10 and 90, percentiles (red). **d)** The
331 number of mosquitoes per species crossing at altitude (50–250 m agl) imaginary lines perpendicular to
332 wind (see legend). Migrants per night per 1 km (right Y axis) are superimposed on the annual number per
333 100 km line (left Y axis, Main text).

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

341

342 **Methods**

343 **Study area** Aerial sampling stations were located in four Sahelian villages in Mali (Fig. S1): Thierola
344 (13.6586, -7.2147) from March 2013 to November 2015, Siguima (14.1676, -7.2279) from March 2013 to
345 October 2015; Markabougou (13.9144, -6.3438) from June 2013 to April 2015; and Dallowere (13.6158,
346 -7.0369) from July 2015 to November 2015. This study area has been described in detail
347 previously^{3,6,9,11,32–34}. Briefly, the region is rural, characterized by scattered villages with traditional mud-
348 brick houses, surrounded by fields. A single growing season (June–October) allows the farming of millet,
349 sorghum, maize, and peanuts, as well as subsistence vegetable gardens. Over 90% of the annual rains fall
350 during this season (~550mm). Cattle, sheep, and goats graze in the savannah that consists of grasses,
351 shrubs, and scattered trees. The rains form small puddles and larger seasonal ponds that usually are totally
352 dry by the end of November. From November until May, rainfall is absent or negligible (total
353 precipitation < 50mm), and by December water is available only in deep wells.

354 **Aerial sampling and specimen processing** Aerial sampling stations were placed ~0.5 km from the nearest
355 house of the village in open areas away from large trees. The method of aerial insect collection was
356 adapted from a study on high-altitude mating flights in ants³⁵. Rectangular 3 x 1m nets (3m²), cut from a
357 roll of tulle netting (mesh: 8 holes/cm; hole diameter of 1.2 mm), were sewn to form four narrow sleeves
358 1m apart along the net (ED Fig. 3). A 1m carbon rod was inserted into each sleeve and glued to the net
359 using Duco Cement Glue (Devcon, FL, ED Fig 3). Three nets were spread over each other on a clean
360 large wooden table topped by a 3.5 x 1.5m plywood and coated with a thin film of insect glue
361 (Tanglefoot, Tropical Formula, Contech Enterprises Inc., BC) by rolling a PVC pipe smeared with this
362 glue over them, while applying moderate pressure downward. The pipe was held at each end (from each
363 side of the long table) by two persons and repeatedly rolled (and smeared) until a uniform thin layer of
364 glue coated the net (but did not block its holes). After coating, the sticky nets were immediately rolled
365 individually, and kept in two tightly secured plastic bags indoors, to avoid accidental contact with insects
366 prior to setup.

367 Prior to the launch, polyurethane balloons (3m in diameter; Mobile Airship & Blimps, Canada, or Lighter
368 than Air, FL, USA), were inflated to full capacity with balloon-grade helium (>98.5%) and topped up to
369 ensure full capacity as needed, usually every 1–3 days based on the balloon condition (ED Fig. 3).
370 Typically, balloons were launched over ~10 consecutive nights per month. The balloon was kept
371 stationary at ~200 m agl by a cord (AmSteel@Blue, synthetic rope sling, Southwest Ocean Services, TX)
372 secured to a 1m³ cement block inserted under the ground. The cord then went through a horizontal
373 manually-rotating drum made of a garden-hose reel used for reeling it. A larger 3.3 m diameter balloon
374 (Lighter than Air, FL) was used between July and September 2015, and launched to ~300 m agl.

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

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

389 Each balloon typically carried three sticky nets. Initially, they were suspended at 40, 120, and 160 m agl,
390 but from August 2013, the typical altitude was set to 90, 120, 190 m agl. When the larger balloon was
391 deployed in the Thierola station (August–September 2015), two additional nets were added at 240 and
392 290 m agl. Balloons were launched approximately 1 hour before sunset (~17:00) and retrieved 1 hour
393 after sunrise (~07:30), the following morning. To control for insects trapped near the ground as the nets
394 were raised and lowered, control nets were raised up to 40 m agl and immediately retrieved (between
395 September and November 2014 the control nets were raised to 120 m agl) during the launch and retrieval
396 operations. The control nets spent 5 minutes in the air (up to 10 minutes when raised to 120 m). Once
397 retrieved they were processed as other nets. Following panel retrieval, inspection for insects was
398 conducted between 09:00 and 11:30 in a dedicated clean area. The panel was stretched between two posts
399 and scanned for mosquitoes, which were counted, removed using forceps, and preserved in 80% ethanol
400 before all other insects were similarly processed and placed in other tubes. Depending on their condition,
401 the sticky panels were sometimes reused the subsequent night.

402 **Species identification** Glue attached to the insects was washed off with 100% chloroform. The
403 mosquitoes were gently agitated (<30 sec) to loosen them from one another. Individual mosquitoes were
404 transferred into consecutive wells filled with 85% ethanol. Using a dissecting scope, the samples were
405 morphologically sorted by mosquito subfamily (*Anopheles*, *Culicinae*), and tentative identifications to
406 *Anopheles* species /species group undertaken. All *An. gambiae* s.l. visually classified (and two identified
407 based on molecular barcode analysis, see below), were identified to species based on fragment-size
408 differentiation after amplification of the nuclear ITS2 region and digestion of the product³⁶. Validation
409 was carried out in LSTM (DW's laboratory) where each specimen was washed with 500µL heptane
410 followed by two further washes with ethanol. DNA was then extracted using the Nexttec (Biotechnologie,
411 GmbH) DNA isolation kit according to manufacturer's instructions. Species identification using a
412 standard PCR method, including all primers³⁷ with products visualized on 2% agarose gel. *Anopheles*
413 *gambiae* s.l. samples were further identified to species by SINE insertion polymorphism³⁸. In cases where
414 no species-specific bands were detected using the first method, approximately 800 bp region of the
415 mtDNA cytochrome oxidase I genes was amplified using the primers C1_J_2183 and TL2_N_3014³⁹.
416 PCR products were purified using the QIAquick PCR-Purification kit (QIAGEN) and sequenced in both
417 directions using the original PCR primers by MacroGen Inc. (Amsterdam, Netherlands). Sequences were
418 aligned using CodonCode Aligner (CodonCode Corporation, Dedham, MA) and compared to existing
419 sequences in GenBank to identify species. All other *Anopheles* mosquitoes were identified by the
420 retrospective correlation of DNA barcodes, with morphologically-verified reference barcodes compiled
421 by Walter Reed Biosystematics Unit and the Mosquito Barcoding Initiative in Y-ML's lab. Head-thorax
422 portions of all samples were separated and used for DNA extraction using the Autogen® automated DNA
423 extraction protocol. MtDNA COI barcodes were amplified using the universal LCO1490 and HCO2198
424 barcoding primers⁴⁰, and amplified, cleaned and bi-directionally sequenced according to previously
425 detailed conditions⁴¹. All DNA barcodes generated from this study are available under the project
426 "MALAN – Windborne *Anopheles* migrants in Mali" on the Barcode of Life Database
427 (www.boldsystems.org) and in GenBank under accession numbers MK585944–MK586043. *Plasmodium*
428 infection status was determined following previously described protocol⁴² using DNA extracts from the
429 whole body for *An. coluzzii* and, for all other specimens, for thorax and head (n=190) as well as separated
430 abdomens (n=156) extracted and tested individually using published protocols^{43,44}. Due to the nature of
431 the collections, all body parts were not available for each specimen, accounting for the discrepancy in
432 numbers. Bloodmeal identification was carried out following published protocol⁴⁵.
433

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

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

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

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

443 Net surface area was 3 m². Wind speed data were obtained from the atmospheric re-analyses of the
444 global climate, ERA5. Hourly data available at 31 km surface resolution with multiple vertical levels
445 including ground, 2, 10, 32, 55, 85, 115 180, 215, 255, and 300 m agl. Overnight records (18:00 through
446 06:00) for the nearest grid center were used to calculate the nightly direction and mean wind speed at each
447 village: Siguima, Markabougou and Thierola. Dallowere, located 25 km south of Thierola, was included
448 in the same grid cell of Thierola. The mean nightly wind speed at panel height was estimated based on the
449 nearest available altitude layer.

450 To evaluate clustering in mosquito panel density and the effects of season, panel height, year and locality,
451 mixed linear models with either Poisson or negative binomial error distributions were implemented by
452 proc GLIMMIX⁴⁶. The clustering at the levels of the panel and the night of sampling were evaluated as
453 random effects as was the case for the year of sampling and locality. These models accommodate counts
454 as non-negative integer values. The ratio of the Pearson χ^2 to the degrees of freedom was used to assess
455 overall “goodness of fit” of the model, with values of >2 indicating a poor fit. The significance of the
456 scale parameter estimating k of the negative binomial distribution was used to choose between Poisson
457 and negative binomial models. Sequential model fitting was used, starting with random factors before
458 adding fixed effects. Lower Bayesian Information Criterion (BIC) values and the significance of the
459 underlying factors were also used to select the best fitting model of each species.

460 The magnitude of windborne migration was expressed as the expected minimum number of migrants per
461 species crossing an imaginary line of 1 km perpendicular to the wind at altitude. This commonly used
462 measure of abundance assumes that the insects fly in a layer that is 1 km wide and does not require
463 knowledge of the distance or time the insects fly to or from the interception point⁴⁷⁻⁴⁹. We used the mean
464 wind speed at altitude (4.8 m/s, see below) and assumed that mosquitoes fly in a layer depth of 200 m
465 between 50 and 250 m agl, conservatively reflecting that mosquitoes were captured between 40-290 m
466 (see below). Accordingly, this nightly migration intensity was computed as the product of the mean aerial
467 density across the year (conservatively including periods when no migrants were captured) by the volume
468 of air passing over the reference line during the night. The corresponding annual index was estimated by
469 multiplying the nightly index by the period of windborne migration estimated from the difference
470 between the first and last day and month a species was captured over the three years. Species that were
471 captured once were assumed to migrate during a single month. The annual number of migrants per
472 species crossing a line of 100 km was used because of the similar species composition across our
473 sampling sites spanning 100 km (Fig. S1a and see below).

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

477 on the prevailing winds during the night of capture at the relevant locations and altitudes as has been done
478 previously^{54–56}. Accordingly, backward flight trajectories of mosquitoes were simulated using [HYSPLIT](#):
479 Hybrid Single-Particle Lagrangian Integrated Trajectory model²⁵ based on ERA5 meteorological
480 reanalysis data. Data available in ERA5 present the highest spatial and temporal resolution available for
481 that region. Comparisons with the lower spatial and temporal resolution data available from the [MERRA2](#)
482 reanalysis data⁵⁷ and the [Global Data Assimilation System](#) available at 0.5 degree spatial resolution
483 showed good agreement in trajectory direction and overall distance (not shown). Trajectories of each
484 captured mosquito were simulated starting at its capture location, altitude, and all multiple interception
485 (full) hours during the night of the collection. Because anophelines are nocturnal, we conservatively
486 assumed that flights started at or after 18:00 and ended by 06:00 the following morning and computed
487 trajectories for every hour that allowed for a total of two or nine h flight. For example, to complete 9
488 hours flight by 06:00, a mosquito could have started at 18:00, 19:00, 20:00, or 21:00. Total flight duration
489 of tethered female *An. gambiae* s.l. and *An. atroparvus* reached or exceeded 10 hours with average speed
490 of 1 km/h⁵² in accord with other studies^{53,58,59}. Likewise, *An. vagus* and *An. hyrcanus* caught 150 m agl
491 after midnight over India would have been migrating for >6 hours, assuming they took off around dusk²⁰.
492 Thus, we conservatively assumed that windborne long-distance migrant anopheline mosquitoes fly
493 between two and nine hours per night although longer duration is possible. Each trajectory consisted of
494 the global positions of the mosquitoes at hourly intervals from the interception time. In addition to
495 plotting trajectories^{60–67}, the linear distance from the interception site and the azimuth (angle between
496 interception site and mosquito simulated position from the North, projected on a plane) were computed
497 for all trajectories. To evaluate distance range and dominant directions of flight, the mean and 95% CI of
498 the distance and azimuth (as a circular statistic) were computed for the two- and nine-hours flight
499 trajectories. The dispersion of individual angles (azimuths) around the mean was measured by the mean
500 circular resultant length ‘r’, which can vary from 0 to 1, with higher values indicating tighter clustering
501 around the mean. Rayleigh’s test was used to test that there was no mean direction, as when the angles
502 form a uniform distribution over a circle⁶⁸.

503

504 **Data and Code Availability**

- 505 1. Data on anopheline capture, identification, sex, and gonotrophic status are available from
506 www.boldsystems.org (Project code: MALAN) and in Genbank ([MK585944–MK586043](#)).
- 507
- 508 2. SAS code used for statistical analyses (and data manipulations) and 9-hour backward trajectories data
509 for each mosquito capture event based on HYSPLIT are available from TL upon request.
- 510 3. Plotting trajectories (code available at <https://github.com/benkraj/anopheles-migration>)

511

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585

586 **Extended Data Legends**

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

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

596 rather than by unique migratory nights. **b)** The relationship between panel height and mean mosquitoes
597 density/panel ($\times 10^3$, regression line with shading denotes 95% CI) showing mean panel density by
598 species. Inset summarizes the covariance analysis (ANCOVA), underlying this regression, which includes
599 the species and panel height. Number of nights per panel altitude is given in blue along the X axis (see
600 Figure 1a). **c)** Variation in mosquito presence (fraction of positive nights) by species between years (top)
601 and villages (bottom) with their 95% CI. Sampling effort expressed as the number of panels per
602 year/village is shown adjacent to the legend.

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

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

Figure 1.

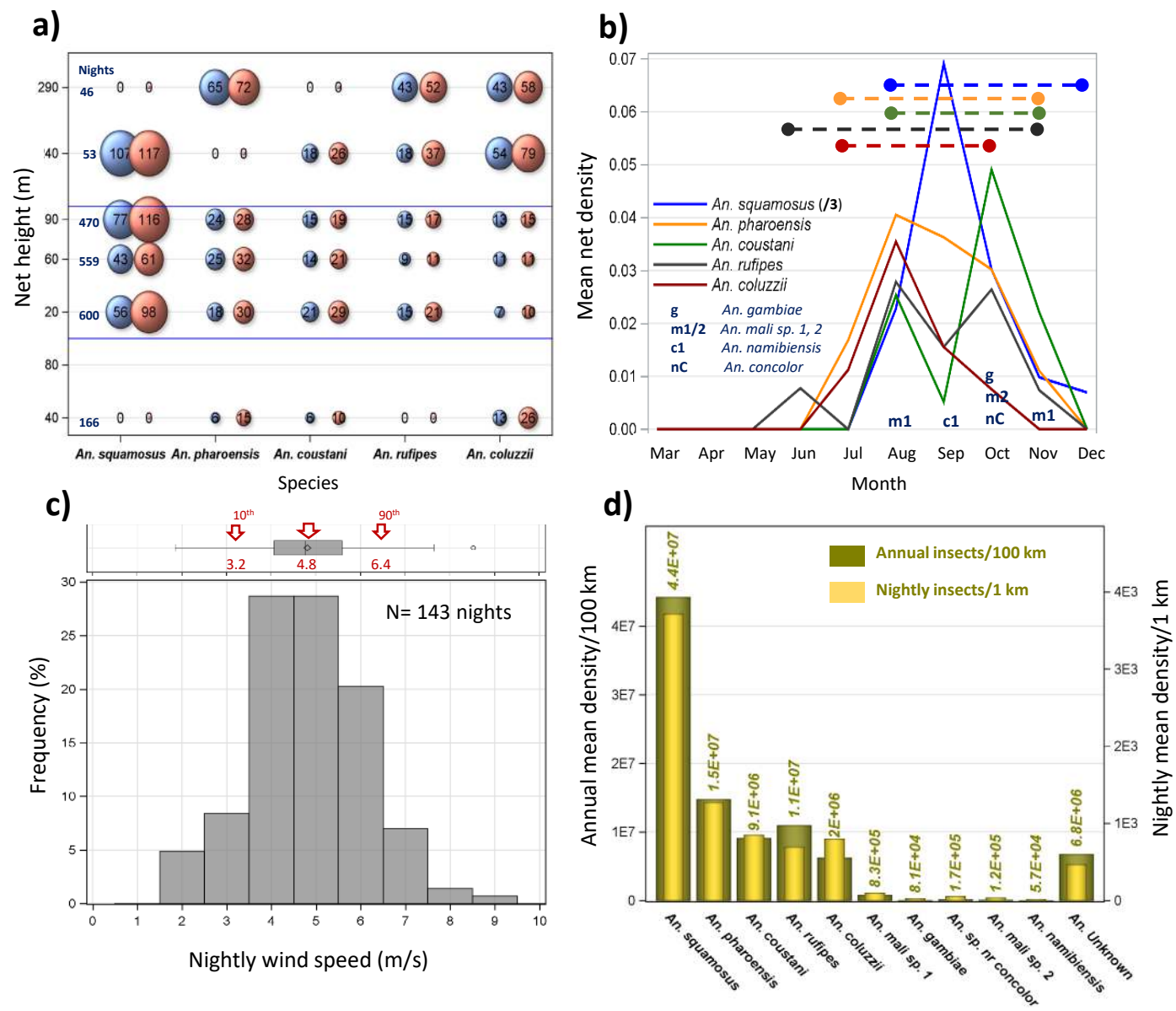
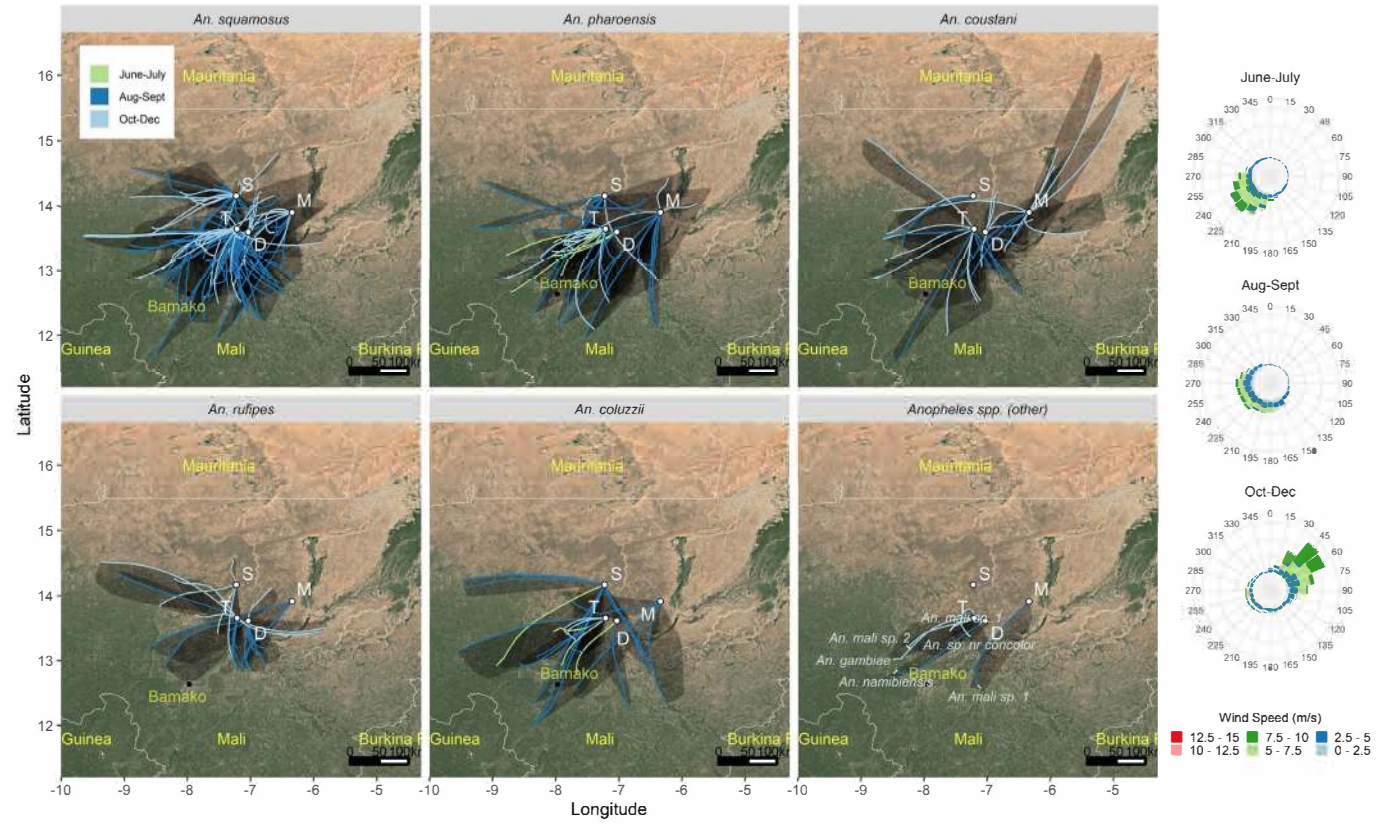
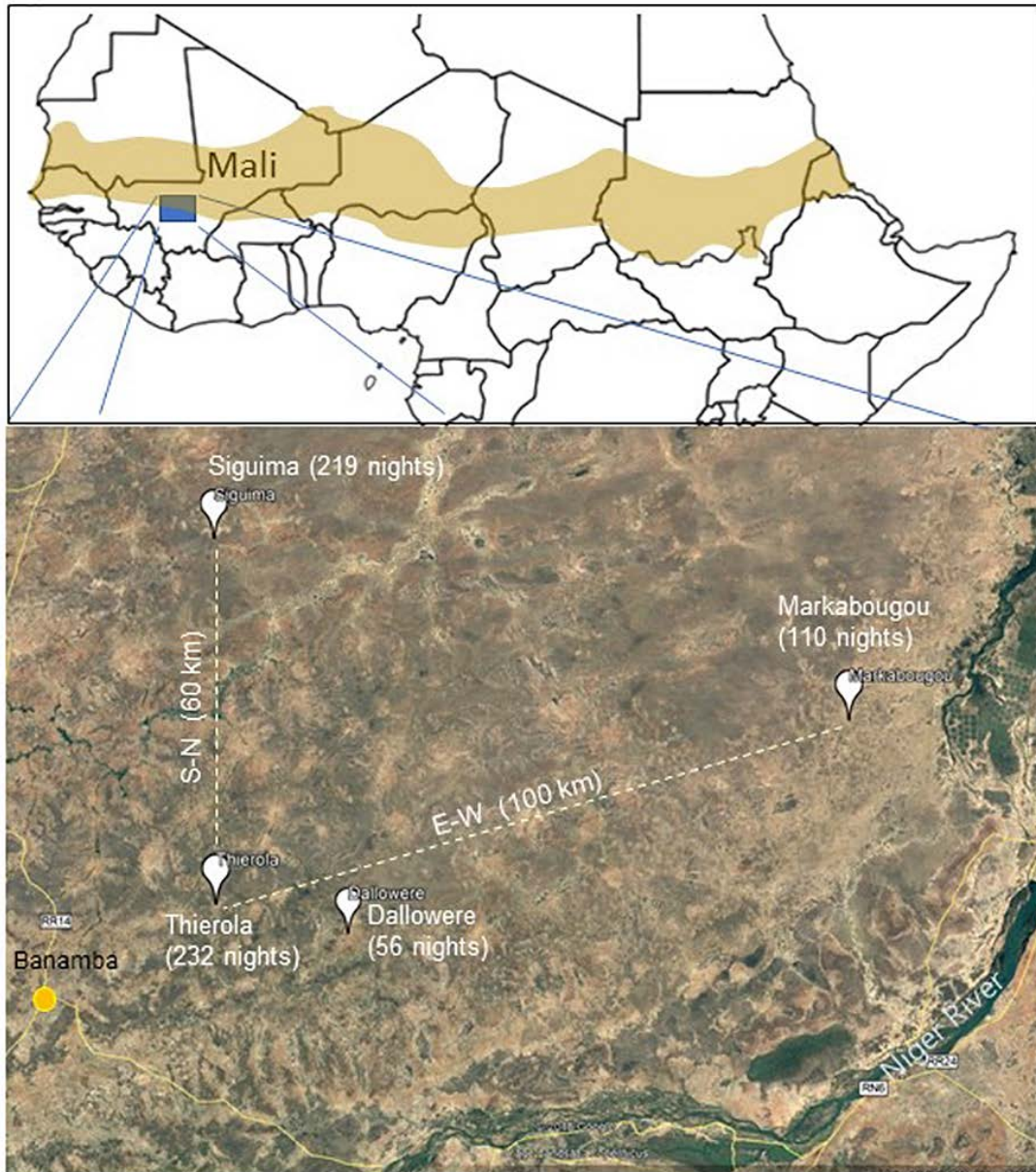


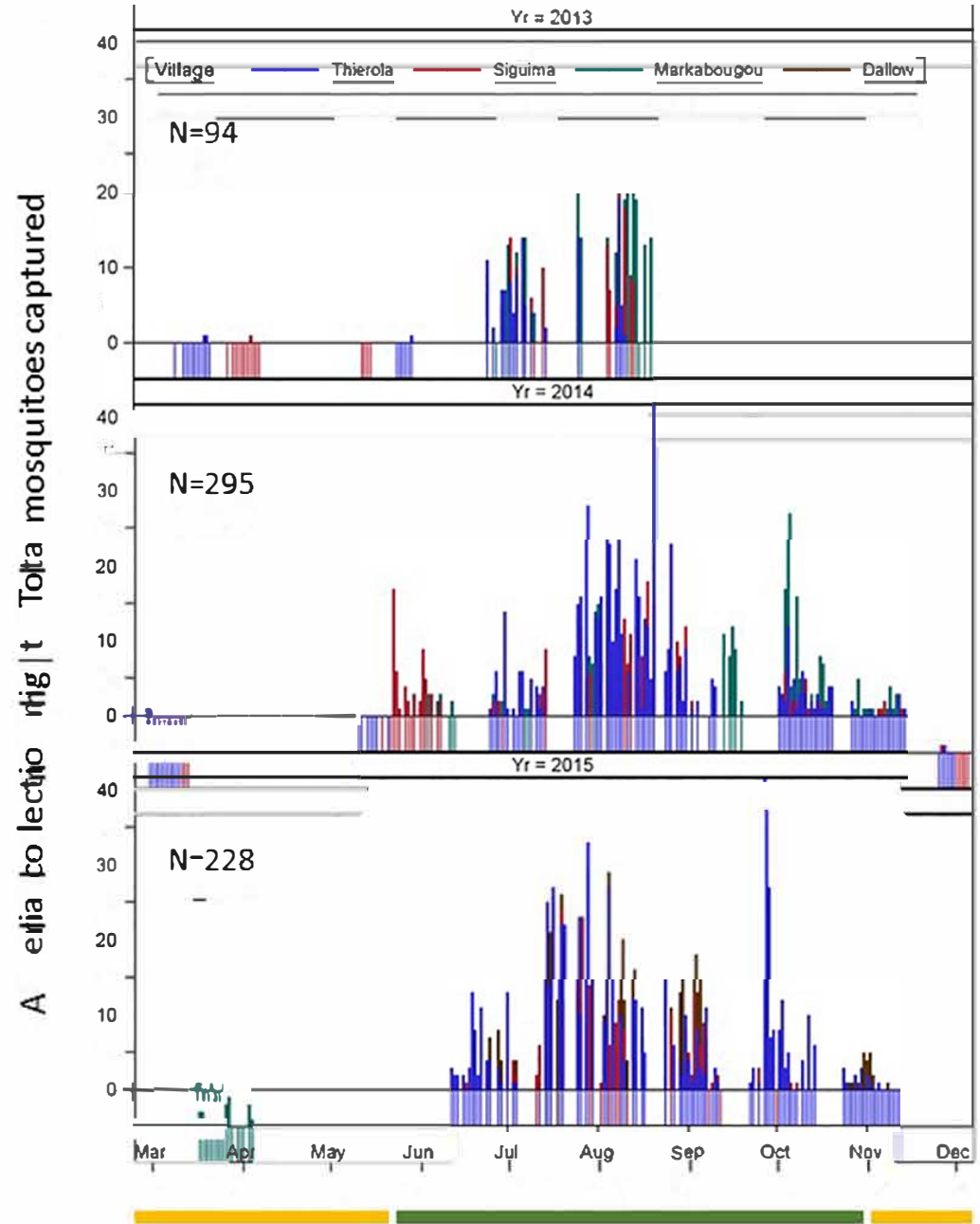
Figure 2.



ED Fig.1 a)

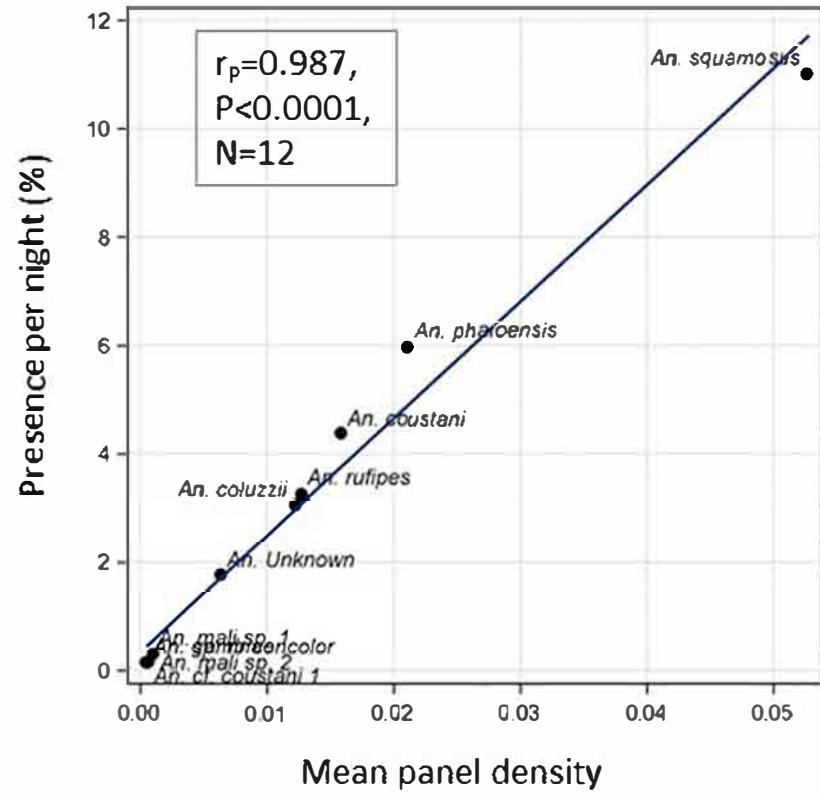


b)

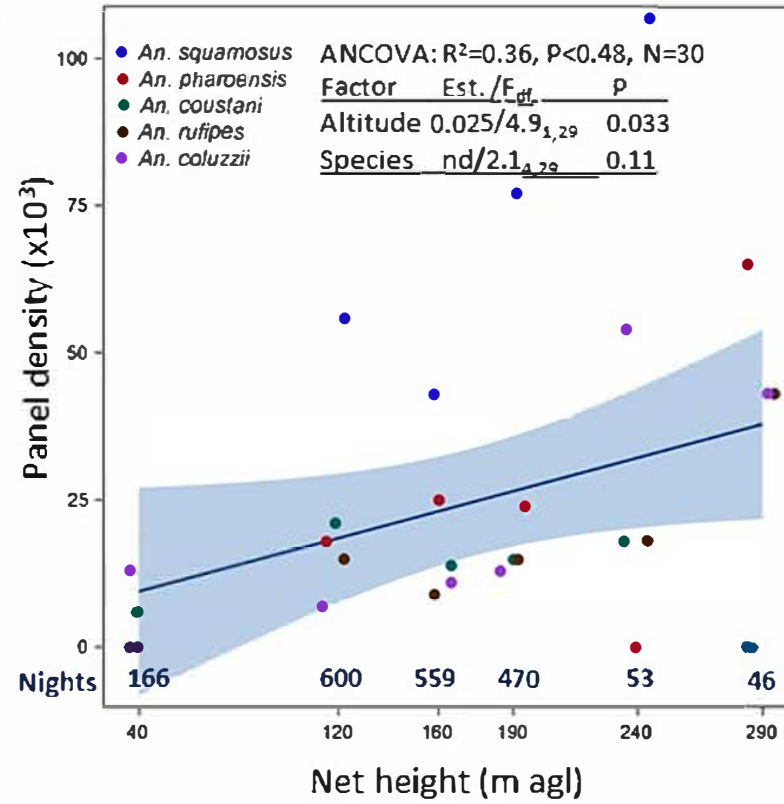


Extended Data Figure 2

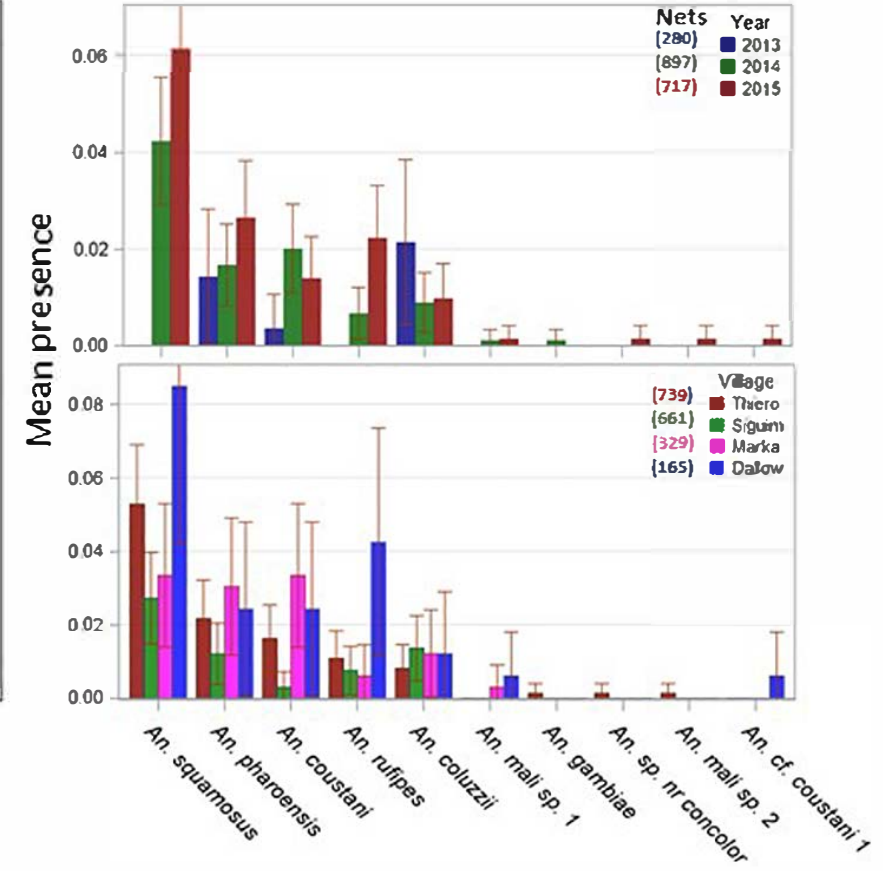
a)



b)



c)



Extended Data Figure 3



Extended data Table 1 .

Dependent: Panel Density	Parameter	<i>A. squamosus</i>	<i>A. pharoensis</i>	<i>A. coustani</i>	<i>A. rufipes</i>	<i>A. coluzzii</i>
Random vars only: Poisson	Pearson χ^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 binomial 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 components 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 included 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.

1 **Supplementary Information:**

2 **Windborne long-distance migration of malaria mosquitoes in the Sahel**

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21

22 **Supplementary Discussion**

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

24 Abundance measured by mean panel density (insects/net), varied more than 100-fold between *An.*
25 *squamosus* and *An. gambiae*. The frequency with which anophelines were caught varied between 0.2 and
26 11% per night (Table 1) and was highly correlated with the overall mean density of species ($r=0.987$,
27 $P<0.0001$, $N=11$, Fig. S2), indicating that species caught less frequently were the least abundant, rather
28 than exhibiting more clustered timing of flights. Clustering of capture events on panels was detected only
29 for *An. squamosus* by a significant scale parameter of the negative binomial distribution (Table S1). The
30 inclusion of the sampling night in the model, however, rendered no remaining support for clustering at the
31 panel level even in *An. squamosus* (Table S1) and indicated that mosquitoes do not fly together in a
32 swarm but as separate individuals as is typical of nocturnally-migrating insects¹. Even after
33 accommodating seasonality, sampling night was a significant source of variation in all species except for
34 *An. coustani* (Table S1), indicating that although migration occurred over many nights, particular nights
35 had higher migration activity (Table S1 and below). Correlations between species' nightly aerial densities
36 during the migration period (July-November) were modest with the highest ($r = 0.26$, $P<0.001$, nights =
37 221) between *An. coluzzii* and *An. pharoensis* followed by that between *An. squamosus* and *An. coustani*
38 ($r = 0.15$, $P<0.025$, nights = 221), indicating mostly independent species migration events. Elucidating the
39 contributions of the species abundance in source locations and favorable conditions for migration in the
40 air (or the ground) to nights with elevated flight activity awaits further studies. All but one of the species
41 (*An. coustani*) analyzed showed a significant positive effect of altitude on panel density, but this
42 relationship was reversed in the analyses of aerial density in three of the species (*An. squamosus*, *An.*
43 *pharoensis*, and *An. rufipes*; Table S1). Similarly, in the cross-species ANCOVA (analysis of covariance),
44 the effect of panel height on panel density was significant (ED Fig 2b) as was its effect on aerial density,
45 but unlike the former, the latter was not statistically significant (slope=0.0001/m, $P=0.093$, $F_{1/24}=3.07$),
46 nor was the effect of species ($P=0.085$, $F_{4/24}=2.33$), suggesting that once corrected for wind speed, the
47 effect of elevation was minimal. Thus, the greater volume of air passing through the higher panels may
48 account for the increased abundance of the latter three species but not that of *An. coluzzii*, which shows
49 increased abundance in higher altitudes after accommodating for the effect of air volume.

50 *Estimation of Plasmodium infection likelihood:*

51 To compute the binomial probabilities of obtaining zero infected mosquitoes, we conservatively used the
52 upper 95% infection rate (4.1%) based on Hay and colleagues² who compiled 125 studies in Africa,
53 focused on *An. gambiae* s.l. and *An. funestus* (mean infection rate = 3.4%). For secondary vectors, we
54 used a 1% infection rate based on the sources listed in the main text. Because infection rate determined by
55 ELISA is expected to be lower than that determined by PCR, our calculation might have overestimated
56 the likelihood of zero infection rate in our samples. However, data on ELISA-based measurements of
57 mosquito infection rates is extensive and unmatched by the few studies using PCR. Moreover, infection
58 rates during our study (2013–2015) were significantly lower³ than that during the period covered by Hay
59 and colleagues² and ELISA is known to excessively produce false positives⁴. Moreover, our aerial
60 sampling concentrated on the early rainy season (June–August) and the late dry season peak (March–
61 April⁵), when infection rates are lowest, therefore, although we relied on infection rates measured by
62 ELISA, the likelihood of finding uninfected mosquitoes based on PCR may not be much lower than our
63 estimates reveal.

64 An additional source of potential bias in estimates of infection rate of secondary vectors is that available
65 data are based on sampling in rural communities, where humans are concentrated, rather than in the wild.
66 However, the elevated concentration of cows, goats, sheep, dogs, cats, chickens, guinea fowl, ducks,
67 rodents, and other domestic and sylvatic animals around these communities provide even larger access to
68 a non-human host. Successful PCR bloodmeal amplification³ was obtained from 38 of 159 specimens
69 (mostly gravid, Table 1), showing that overall, 31% of bloodmeals were wholly or partially human in
70 origin, with the remainder being from goat and cattle sources. These results show that, as expected, the
71 degree of anthropophagy is lower in secondary than in primary malaria vectors, yet they confirm that
72 these windborne secondary malaria vectors are exposed to human blood and therefore, include potentially
73 infected mosquitoes.

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

75 As has been established for other windborne migrant insects, ample evidence suggests that mosquitoes
76 deliberately ascend into and descend out of the winds at altitude and thus, manifest some control over
77 their long-range movements^{1,4}. In addition to the non-random composition of the sexes and female
78 gonotrophic states (Main Text), the species composition at altitude (Table 1, Fig. 1) also differs from
79 expectations based on ground sampling. The high-altitude collections were dominated by secondary
80 malaria vectors, e.g., *An. squamosus* and *An. pharoensis* (Table 1), whereas, on the ground using indoor
81 collections, outdoor clay-pot traps, and larval collections in the vicinity of the same villages, >90% of
82 *Anopheles* captured were *An. gambiae* s.l.^{5,6}. Different sampling methods, e.g., animal baited traps, would
83 yield a higher abundance of the zoophilic taxa (e.g., *An. rufipes*), but it remains unclear if this ground
84 composition will resemble the aerial one because larval collections are similar to composition indoors,
85 indicating that the composition of anopheline species on the ground and at altitude are distinct. Most
86 species found at altitude are expected to be found on the ground, but the reverse may not be true because
87 not all species engage in windborne migration. However, even considering sampling bias, it is puzzling
88 that our ground collections consisting of many thousands of anophelines, failed to identify a single *An.*
89 *squamosus* or *An. coustani*. The differences between altitude and ground collections of the anthropophilic
90 members of *An. gambiae* s.l. are more robust because they share similar larval, biting, and resting sites^{5,7–}
91 ¹⁰ and thus are less affected by sampling bias (above). Ground collections in the same villages show that
92 *An. coluzzii* predominates throughout the year, except between late September and early November, when
93 the other sibling species together often exceed 70%⁵. In that window *An. coluzzii* typically drops below
94 30% of the ground collection and some years dips below 10%, before it regains its dominance by mid-

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

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

106 Concerns about viability of windborne migrant insects have been settled long ago by many studies. For
107 example, Taylor¹² compared survival and reproduction in a live collection of insects, including some
108 small Diptera (using non-sticky nets, at altitudes similar to our panels) with those captured on the ground.
109 After finding similar survival and reproductive success, Taylor concluded that “This seems to establish
110 the viability of high-level migrants beyond reasonable doubt.” Furthermore, the mosquitoes caught by
111 aerial netting in China and India by one of the present authors (Reynolds DR)^{13,14} were alive and active
112 upon capture. On a few occasions during removal from the sticky nets in our study, *Anopheles*
113 mosquitoes were observed moving their limbs despite the glue, substantiating their capture as live insects.
114 Further, to test survival of mosquitoes at high altitudes, we placed female *Anopheles gambiae* s.l.
115 collected the same morning indoors (from villages near aerial sampling stations) individually, in modified
116 50 ml tubes (both ends opened covered with mesh) affixed to the net’s frame, so that wind passed through
117 the tubes. There was no difference in survival (Likelihood Ratio Chi Square Test: $P > 0.38$, $\chi^2_1 = 0.75$) of
118 these females kept at altitude (>100m, 58% N=26) vs. on the ground (71%, N=17) from launch (17:30) to
119 retrieval (07:00, the next morning). These experiments affirm Taylor’s conclusion (above) specifically for
120 mosquitoes.

121 *Role of windborne migration in Anophelines:*

122 Our results affirm anecdotal observations of anophelines flying at high altitudes in North America, South
123 Asia, and Australia¹⁵⁻¹⁷, and inferences of long-distance windborne migration of *An. pharoensis*^{4,18,19} and
124 *An. squamosus*²⁰. However, the significance of these movements has been largely disregarded by vector
125 biologists, malariologists, and epidemiologists^{19,21} who maintain that the dispersal of malaria mosquitoes
126 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
127 the genus *Anopheles*, *An. gambiae* s.l., and *An. pharoensis*, respectively²⁵. Long-distance migration
128 provides a powerful explanation for the puzzling shallow genetic structure of *An. gambiae* and *An.*
129 *coluzzii* over large geographical distances²⁶⁻³⁰ and for the persistence of certain Sahelian vector
130 populations, as revealed by comprehensive modeling³¹. The importance of long distance migration to
131 malaria control and elimination is arguably linked to the success of those African countries near
132 elimination, (so-called “E-2020”³²), because they are all surrounded by >200 km “migration barriers”:
133 Cabo Verde and Comoros (oceans), Algeria (Sahara Desert and Mediterranean Sea), Botswana (Kalahari
134 Desert) and South Africa and Swaziland (Ocean, Kalahari Desert, and the near-elimination areas),
135 supporting the role of windborne migration in “residual” transmission. Separating the roles of Odyssean
136 malaria²¹ (transmission via infected mosquitoes transported by vehicles) from windborne migrants
137 necessitates further studies (Main Text). Whether windborne migration has limited the success of past
138 interventions, such as the Garki project, that included intensive use of insecticides and drugs³³, remains to
139 be answered. It is noteworthy that the Onchocerciasis Control Programme (OCP) in West Africa, had to

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

146 Our results reveal that similar to many other insects^{1,36,37} anophelines exhibit two modalities of
147 movements: appetitive movements in their ‘flight boundary layer’, within approximately the first 5 m
148 agl^{38,39} and long-range windborne movements in altitudes that include 100–300 m agl. Unlike most long-
149 distance flying insects, which are post-teneral (i.e. newly-emerged, typically pre-reproductive, adults)³⁶,
150 our results show that anopheline female mosquitoes engage in such flights after taking a blood meal.
151 What primes these mosquitoes to undertake high-altitude flights and whether migrants have already
152 deposited an egg batch in their provenance area prior to their journey remain to be explored, as well as if
153 they embark on more than a single night of windborne migration. Although significant species-specific
154 differences in displacement distances were detected (Table 2), the scale of displacement distance was
155 similar among species. The West African Sahel is dotted with human settlements seldom separated by
156 more than 7 km, suggesting that appetitive flights would suffice to land a migrant in a village even if it
157 descended from altitude in between them. However, distances between villages were longer a hundred
158 years ago, raising the question of whether windborne migration in anthropophilic mosquitoes is recent.
159 The proposed recolonization of the Sahel by species such as *An. gambiae* from southern source
160 populations (Main Text) follows a “source-sink model” that requires “return migration” to maintain this
161 strategy⁴⁰. We have detected few such movements (Fig. 2), possibly because such return flights occur in
162 large numbers only over a few nights (e.g., the grasshopper *Oedaleus senegalensis*⁴¹), every several years,
163 or because our sampling sites were located closer to the northern edge of the migration zone instead of
164 near its center; hence, there are fewer source populations that can produce migrants to be detected by our
165 sampling method. Accordingly, aerial sampling ~150 km south of our current locations may be used to
166 test this hypothesis. With many questions awaiting answers, we believe the evolution of windborne
167 migration in mosquitoes, its drivers, mechanisms, and impacts present a new and important scientific
168 frontier. The implications of these investigations will improve our understanding of disease transmission,
169 disease modeling, and malaria control and elimination efforts.

170

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