Supplementary Information:

Windborne long-distance migration of malaria mosquitoes in the Sahel

Huestis DL[a], Dao A[b], Diallo M[b], Sanogo ZL[b], Samake D[b], Yaro AS[b], Ousman Y[b], Linton Y-M[f], Krishna A[a], Veru L[a], Krajacich BJ[a], Faiman R[a], Florio J[a], Chapman JW[c], Reynolds DR[d], Weetman D[e], Mitchell R[g], Donnelly MJ[e], Talamas E[h,j], Chamorro L[h], Strobach E[k] and Lehmann T[a]

[a] Laboratory of Malaria and Vector Research, NIAID, NIH. Rockville, MD, USA
[b] Malaria Research and Training Center (MRTC)/Faculty of Medicine, Pharmacy and Odonto-stomatology, Bamako, Mali
[c] Centre for Ecology and Conservation, and Environment and Sustainability Inst., University of Exeter, Penryn, Cornwall, UK and College of Plant Protection, Nanjing Agricultural University, Nanjing, P. R. China.
[d] Natural Resources Institute, University of Greenwich, Chatham, Kent ME4 4TB, and Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[e] Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK
[f] Walter Reed Biosystematics Unit, Smithsonian Institution Museum Support Center, Suitland MD, USA and Department of Entomology, Smithsonian Institution, National Museum of Natural History, Washington DC, USA
[g] Smithsonian Institution - National Museum of Natural History, Washington DC, USA
[h] Systematic Entomology Laboratory - ARS, USDA, Smithsonian Institution - National Museum of Natural History, Washington DC, USA
[j] Florida Department of Agriculture and Consumer Services, Department of Plant Industry, Gainesville FL, USA
[k] Earth System Science Interdisciplinary Center, University of Maryland, College Park, MD, USA

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.

References (Supplementary Information)

1. Drake, V. A. & Reynolds, D. R. *Radar entomology : Observing insect flight and migration*. (CABI International., 2012).

2. Hay, S. I., Rogers, D. J., Toomer, J. F. & Snow, R. W. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, Internet access and review. *Trans. R. Soc. Trop. Med. Hyg.* **94,** 113–27 (2000).

3. Kent, R. J. & Norris, D. E. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. *Am. J. Trop. Med. Hyg.* **73,** 336–42 (2005).

4. Pedgley, D. E. *Windborne pests and diseases: Meteorology of airborne organisms*. (Ellis Horwood Ltd., 1982).

5. Dao, A. *et al.* Signatures of aestivation and migration in Sahelian malaria mosquito populations.

Nature **516,** 387–390 (2014).

6. Lehmann, T. *et al.* Aestivation of the African Malaria Mosquito, *Anopheles gambiae* in the Sahel. *Am. J. Trop. Med. Hyg.* **83,** 601–606 (2010).

7. Antonio-Nkondjio, C. *et al. Complexity of the Malaria Vectorial System in Cameroon: Contribution of Secondary Vectors to Malaria Transmission*. *J. Med. Entomol* **43,** (2006).

8. Lemasson, J.-J. *et al.* Comparison of behavior and vector efficiency of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae) in Barkedji, a Sahelian area of Senegal. *J. Med. Entomol.* **34,** 396–403 (1997).

9. Fontenille, D. *et al.* Four years' entomological study of the transmission of seasonal malaria in Senegal and the bionomics of *Anopheles gambiae* and *A. arabiensis*. *Trans. R. Soc. Trop. Med. Hyg.* **91,** 647–652 (1997).

10. Toure, Y. T. *et al.* Perennial transmission of malaria by the *Anopheles gambiae* complex in a north Sudan Savanna area of Mali. *Med. Vet. Entomol.* **10,** 197–199 (1996).

11. Chapman, J. W., Reynolds, D. R. & Wilson, K. Long-range seasonal migration in insects: Mechanisms, evolutionary drivers and ecological consequences. *Ecol. Lett.* **18,** 287–302 (2015).

12. Taylor, L. R. Mortality and viability of insect migrants high in the air. *Nature* **186,** 410 (1960).

13. Sanders, C. J., Selby, R., Carpenter, S. & Reynolds, D. R. High-altitude flight of Culicoides biting midges. *Vet. Rec.* **169,** 208 (2011).

14. Ming, J. *et al.* Autumn southward 'return' migration of the mosquito Culex tritaeniorhynchus in China. *Med. Vet. Entomol.* **7,** 323–327 (1993).

15. Reynolds, D. R. *et al.* Atmospheric transport of mosquitoes in northeast India. *Med. Vet. Entomol.* **10,** 185–186 (1996).

16. Glick, P. A. *The distribution of insects, spieders, and mites in the air*. *United States Department of Agriculture, Technical Bulletin* **673,** (1939).

17. Kay, B. H. & Farrow, R. A. Mosquito (Diptera: Culicidae) Dispersal: Implications for the epidemiology of Japanese and Murray Valley encephalitis viruses in Australia. *J. Med. Entomol.* **37,** 797–801 (2000).

18. Garrett-Jones, C. The possibility of active long-distance migrations by *Anopheles pharoensis* Theobald. *Bull. World Health Organ.* **27,** 299–302 (1962).

19. Service, M. W. Mosquito (Diptera: Culicidae) dispersal - the long and the short of it. *J. Med. Entomol.* **34,** 579–588 (1997).

20. White, G. B. Evidence for *Anopheles squamosus* migration ? *Nature* **227,** 739–740 (1970).

21. Frean, J., Brooke, B., Thomas, J. & Blumberg, L. Odyssean malaria outbreaks in Gauteng Province, South Africa, 2007 - 2013. *SAMJ South African Med. J.* **104,** 335–338 (2014).

22. Service, M. W. *Mosquito Ecology Field Sampling Methods*. (Elsevier Applied Science, 1993).

23. Costantini, C. *et al.* Density, survival and dispersal of *Anopheles gambiae* complex mosquitoes in a west African Sudan savanna village. *Med. Vet. Entomol.* **10,** 203–219 (1996).

24. Toure, Y. T. *et al.* Mark-release-recapture experiments with *Anopheles gambiae* s.l. in Banambani Village, Mali, to determine population size and structure. *Med. Vet. Entomol.* **12,** 74–83 (1998).

25. Verdonschot, P. F. M. & Besse-Lototskaya, A. A. Flight distance of mosquitoes (Culicidae): A metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. *Limnologica* **45,** 69–79 (2014).

26. Lehmann, T. *et al.* Population structure of *Anopheles gambiae* in Africa. *J. Hered.* **94,** 133–147 (2003).

27. Lehmann, T. *et al.* Genetic differentiation of *Anopheles gambiae* populations from East and west Africa: comparison of microsatellite and allozyme loci. *Heredity (Edinb.).* **77 (Pt 2),** 192–200 (1996).

28. Reidenbach, K. R. *et al.* Patterns of genomic differentiation between ecologically differentiated M and S forms of Anopheles gambiae in West and Central Africa. *Genome Biol. Evol.* **4,** 1202–1212 (2012).

29. Miles, A. *et al.* Genetic diversity of the African malaria vector *Anopheles gambiae*. *Nature* **552,** 96 (2017).

30. Lehmann, T. *et al.* Tracing the origin of the early wet-season *Anopheles coluzzii* in the Sahel. *Evol. Appl.* **10,** 704–717 (2017).

31. North, A. R. & Godfray, H. C. J. Modelling the persistence of mosquito vectors of malaria in Burkina Faso. *Malar. J.* **17,** 140 (2018).

32. WHO | World malaria report 2017. *WHO* (2018).

33. Molineaux, L. & Gramiccia, G. *The Garki project*. (1980).

34. Garms, R. & Walsh, J. F. Studies on the reinvasion of the Onchocerciasis Control Programme in the Volta River Basin by *Simulium damnosum* s.l. with emphasis on the south-western areas. *Tropenmed. Parasitol*. **30,** 345–362 (1979).

35. Walsh, J. F., Davies, J. B. & Garms, R. Further studies on the reinvasion of the Onchocerciasis Control Programme by *Simulium damnosum* s.l.: The Effects of an Extension of Control Activities into Southern Ivory Coast during 1979. **32,** 269–273 (1981).

36. Dingle, H. & Drake, A. What is migration? *Bioscience* **57,** 113-121 (2007).

37. Hu, G. *et al.* Mass seasonal bioflows of high-flying insect migrants. *Science* **354,** 1584–1587 (2016).

38. Gillies, M. T. & Wilkes, T. J. The vertical distribution of some West African mosquitoes (Diptera, Culicidae) over open farmland in a freshwater area of the Gambia. *Bull. Entomol. Res.* **66,** 5 (1976).

39. Gillies, M. T. & Wilkes, T. J. The effect of high fences on the dispersal of some West African mosquitoes (Diptera: Culicidae). *Bull. Entomol. Res.* **68,** 401 (2009).

40. Chapman, J. W. *et al.* Seasonal migration to high latitudes results in major reproductive benefits in an insect. *Proc. Natl. Acad. Sci.* **109,** 14924–14929 (2012).

41. Cheke, R. A. *et al.* A migrant pest in the Sahel: the Senegalese grasshopper *Oedaleus senegalensis*. *Philos. Trans. R. Soc. B* **328,** 539–553 (1990).