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Signatures of aestivation and migration in Sahelian malaria mosquito populations

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During the long Sahelian dry season, mosquito vectors of malaria are expected to perish when no larval sites are available; yet, days after the first rains, mosquitoes reappear in large numbers. How these vectors persist over the 3–6 month-long dry season has not been resolved, despite extensive research for over a century[1–3]. Hypotheses for vector persistence include dry-season diapause (aestivation) and long-distance migration (LDM); both are facets of vector biology that have been highly controversial due to lack of concrete evidence. Here we show that certain species persist by a form of aestivation, while others engage in LDM. Based on time-series analyses, the seasonal cycles of *Anopheles coluzzii*, *Anopheles gambiae s.s.*, and *Anopheles arabiensis* were estimated, and their effects were found to be significant, stable, and highly species-specific. Contrary to all expectations, the most complex dynamics occurred during the dry season, when the density of *A. coluzzii* fluctuated dramatically, peaking when migration would seem highly unlikely, while *A. gambiae s.s.* was undetected. The population growth of *A. coluzzii* followed the first rains closely, consistent with aestivation, whereas the growth phase of both *A. gambiae s.s.* and *A. arabiensis* lagged by two months. Such a delay is incompatible with local persistence, but fits LDM. Surviving the long dry season *in situ* allows *A. coluzzii* to predominate and become the primary force of malaria transmission. Our results reveal profound ecological divergence between *A. coluzzii* and *A. gambiae s.s.*, whose standing as distinct species have been challenged, and suggest that climate is one of the selective pressures that led to their speciation. Incorporating vector dormancy and LDM is key to predicting changes in the range of malaria due to global climate change[4] and to the elimination of malaria from Africa.

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Author's Contributions and Statements

TL conceived the study and together with AD and ASY designed it. AD, ASY, MD, ST, DLH, YK, AIT, ZLS, and DS performed the research, both in the field and the laboratory. All authors have discussed and interpreted the results as well as made decisions on various field and laboratory operations. AD led the field operations and data management; TL analyzed the data and wrote the paper, with extensive input from AD and DLH.

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Over half a million malarial deaths still occur annually, mostly in sub-Saharan Africa[5]. Transmitted by *Anopheles gambiae s.s.*, *A. coluzzii* (previously known as the *A. gambiae* S and M molecular forms[6]), *A. arabiensis*, and *A. funestus*, malaria is widespread, including dry savannahs and semi-arid areas. Persistence of malaria in areas where the surface waters required for larval development are absent for several months a year[2,7–12] has been the subject of much interest, as it has long been recognized that, during the dry season, reproductively quiescent adult mosquitoes are especially vulnerable to control[13,14]. Recent findings suggested that aestivation is used by *A. coluzzii* to persist throughout the dry season[14–18]; yet, more definitive evidence is required to fully resolve this question.

Data from a five-year study of Sahelian *A. coluzzii*, *A. gambiae s.s.*, and *A. arabiensis* population densities at an unparalleled resolution were subjected to time-series analyses to isolate the seasonal components, assess their magnitude, and determine if they were stable or time-varying (Methods). This statistical framework allowed identification of salient elements of the seasonal cycle of each species, providing unique ecological signatures, which were then deciphered to determine if populations endured the dry season locally or if populations recolonized the area by migration.

From September 2008 to August 2013, a total of 40,195 *A. gambiae s.l.* (28,547 females and 11,648 males) were collected in the Sahelian village of Thierola, Mali during 511 collection days (Figs. 1 and ED-1; Table ED-1, Supplementary Information). The complexity of the population dynamics of *A. gambiae s.l.* was epitomized by dramatic fluctuations during the dry season (Figs. ED-2 and ED-3). Putative seasonal elements were visually identified (Methods; Table ED-2), providing a descriptive framework and expectations, to aid the interpretation of the statistical results. Briefly, the population growth phase (June–August) started ~3 weeks after the first rain, resulting in the wet-season peak (September–October). Density declined as larval sites dried (November), reaching its dry-season minima in February–March. Surprisingly, density started rising halfway into the dry season (March) and culminated in a dramatic dry-season peak lasting <7 days, returning to the typical low density weeks later (April–May), and ending with the first rain surge, 3–7 days after the first rains (Fig. ED-3; Table ED-2).

Time-series analysis of the log-transformed density (Fig. ED-2), using an unobserved components model (Methods), was fitted for *A. gambiae s.l.* (Table 1). The model selected had a fixed level (equivalent to intercept) and no slope (trend), reflecting a stable mosquito density over the study. An additional non-seasonal cycle with a long period was also included (Methods and Supplementary Information). The variance of the seasonal component was insignificant, indicating it was not time-varying; thus, it was modeled as a fixed component, simplifying its interpretation. The seasonal component of *A. gambiae s.l.* population dynamics was highly significant ($P<0.0001$, Table 1). The estimated seasonal variation (Fig. 2A) revealed a large gap between the 95% CIs of the wet-season peak and that of the mid-dry-season low; thus, these elements and the decline between them are statistically well-supported. Likewise, large gaps were found between the 95% CIs of the mid-dry-season low and the late-dry-season peak, between this peak, the end-dry-season low, and the following wet-season peak, indicating that these elements (and the transitional

phases connecting them) were statistically supported. Other putative elements (Table ED-2) had insufficient statistical support.

The putative elements of each species' seasonal cycle were identified (Table ED-2). The seasonal component of all species was fixed (its variance was insignificant) and was highly significant ($P<0.0001$, Table 1). The time-series model selected for *A. coluzzii* was structurally similar to that of *A. gambiae s.l.* (Table 1). Based on their 95% CIs, one wet-season peak and two dry-season peaks, which were observed in all years (Fig. 1), were statistically supported (Fig. 2B). The early wet-season decline of *A. coluzzii* produced the pre-dry-season trough in mid-November, before the last larval site dried, which was followed by an early dry-season peak in late December (Fig. 2B). Subsequently, its seasonal component was virtually identical to that of *A. gambiae s.l.* (Fig. 2), consistent with its predominance in species composition (Fig. ED-1C). The model for *A. gambiae s.s.* included two non-seasonal cycles as well as an autoregressive (lag 1) error (Table 1). Only a single wet-season peak and a long dry-season trough were statistically discerned in *A. gambiae s.s.* (Fig. 2C). The model for *A. arabiensis* was structurally similar to that of *A. coluzzii* (Table 1). A single wet-season peak and the long dry-season trough were supported (Fig. 2D), whereas changes during the dry season were not distinguished from noise.

The species-specific signatures manifested by their population dynamics provide compelling evidence that *A. coluzzii* persists locally in the Sahel during the dry season, whereas *A. gambiae s.s.* recolonizes via LDM after the first rains; the evidence is less clear for *A. arabiensis*. Firstly, *A. coluzzii* was present throughout the dry season (albeit in small numbers), whereas *A. gambiae s.s.* was undetected from January to May (Figs. 2 and ED-4), consistent with previous studies[9,14,19–22]. Secondly, the density of *A. coluzzii* rose dramatically (ten- to ninety-fold from their preceding phase) twice during the dry season (Fig. 2). Since these peaks preceded the first rain by at least six weeks, any potential migrant mosquitoes would likely perish before reproductive opportunities were available, given the absence of surface waters in the area (ruling out dry-season reproduction). Thirdly, the most crucial evidence relates to the period when population growth starts with respect to the first rain. The onset of population growth can be defined as the first time when the lower 95% CI of the seasonal component is greater than the upper 95% CI during the preceding dry season's low phase (red arrows, Fig. 2). This phase started in June for *A. coluzzii* but in August for *A. gambiae s.s.* and *A. arabiensis* (Fig. 2). A delay of six to eight weeks in the onset of population growth for the latter two species corroborated our previous results in two other Sahelian villages, 10–25 km away from Thierola[14]. Commencing population growth shortly after the first rain fits well with local persistence (e.g., aestivation), but a two-month "delay" in that phase cannot be reconciled with it, especially contrasted with its rapid onset in *A. coluzzii*. Arrival of migrants from distant locations, on the other hand, may take several weeks, consistent with this "delay." The earlier (August vs. October) and higher wet-season peak of *A. coluzzii* is explained by the two-month "advantage" it had in building its density (Figs. 2 and ED-4). The prompt population growth of *A. coluzzii* is consistent with previous studies showing that its density surged over tenfold, five days after the first rain[14,16] (egg-to-adult developmental time is ≥8 days) and with the recapture of one marked female that was released seven months earlier in the same village[16]. Fourthly, density of *A. coluzzii* was

declining by October, at least 4 weeks before the last larval sites dried up, reaching its pre-dry-season trough in November whereas *A. arabiensis* and *A. gambiae s.s.* continued to reproduce (Figs. 1–2 and ED–4). This early decline in *A. coluzzii* is consistent with another hallmark of diapause, the initiation phase[23–25], in which insects change their behavior and physiology and move into shelters before unfavorable conditions unfold.

During the dry season, no surface waters were available near Thierola for at least a 30 km radius, and in the distant localities where surface waters did exist, overall density was very low and *A. gambiae s.s.* was not detected until the wet season[14,16]. The nearest high-density source is in the Niono rice cultivation area (~150 km ENE of Thierola), but it consists exclusively of *A. coluzzii*[26]. Therefore, LDM spanning hundreds of kilometers is necessary to explain the re-colonization of *A. gambiae s.s.* Alternative explanations, including desiccation-tolerant dormant eggs, larvae, or pupae, as well as larval growth in deep, underground water sources, should not be altogether dismissed, despite being contradictive to available knowledge.

Population dynamics of *A. arabiensis* exhibited mixed signatures. Statistically, it is similar to *A. gambiae s.s.*, and the long delay in population growth after the rains (Figs. 1, 2, and ED-4) indicates that it too persists by LDM. Yet, throughout the dry season, sporadic individuals were found every year, as opposed to zero *A. gambiae s.s.* Possibly, the dominant strategy of *A. coluzzii* is expressed in a small fraction of *A. arabiensis*, consistent with previous reports of local persistence of *A. arabiensis* by aestivation in the East African Sahel[2,7] and perhaps in other parts of the West African Sahel[10,27]. Alternatively, the occasional *A. arabiensis* recovered in the dry season could represent backcrossed hybrids between *A. coluzzii* and *A. arabiensis*.

These results provide fresh insights that dramatically change our understanding of the ecology of African malaria vectors and resolve the “dry-season paradox.” Ignoring the pervasive effects of dormancy and LDM limits our understanding of malaria transmission and its response to control and elimination strategies. Dormancy shapes vector composition in the Sahel, where *A. coluzzii* comprised 75% of the overall indoor vector density with its wet-season peak being at least twice as high and broad than that of either *A. gambiae s.s.* or *A. arabiensis* (Fig. 1, Supplementary Information, and references[14,16]). Arguably, dormancy underlies the heavy burden of malaria transmission in such areas by exponential amplification of human-vector cycles that lead to intense late-wet-season transmission. Although *A. gambiae s.s.* and *A. arabiensis* predominate during the end of the wet season (October–November), we doubt that they alone can sustain the high rate of malaria transmission had *A. coluzzii* not amplified infections from June to September. Therefore, vector-control strategies that eliminate *A. coluzzii* alone may cut peak malaria transmission to very low levels. Targeting *A. coluzzii* while in its hidden shelters during the dry season is probably the most efficient control strategy, if these sites are found, but the indoor population during the early wet season and the late-dry-season peaks also represent promising targets. Thus, a single residual spraying indoors in the late dry-season (e.g., March) that is effective for 4 months may achieve dramatic reduction in malaria transmission in the following wet season[14]. Moreover, the spread of introduced genes by

genetically modified mosquitoes may be hindered or aided by dormancy and LDM, as would other forms of malaria control and elimination campaigns.

Divergent strategies of persistence through the dry season were revealed by species-specific seasonal dynamics: local persistence of *A. coluzzii*, as opposed to annual recolonization by LDM for *A. gambiae s.s.* and *A. arabiensis*. They signify a multitude of behavioral, physiological, and molecular divergence processes and thus probably represent the most striking phenotypic differences between the species found so far[28,29], lending support for the elevated taxonomic status of the molecular forms to species[6]. Consistent with previous interpretations[6,9,12,19,20], the adaptation to exploit arid environment such as the Sahel via aestivation may represent the central dimension in the adaptive divergence between the species. The implications of these differences for understanding speciation and for explaining their geographical range[11,12] are just beginning to be appreciated.

Methods

The study was performed between September 2008 and August 2013 in Thierola (13.6583°N, 7.2155°W), a small rural village in the Malian Sahel. The village populations, ethnic composition, agricultural activities, and house structure were described previously[18]. During the wet season, the rains fill two large ponds and many small puddles near the village. The small puddles require frequent rains, as they dry within a week without additional rain. The last rain typically falls in October and usually all surface water dries by December. From November until May, rainfall is altogether absent or negligible (total precipitation <30 mm). In the course of this study, dry-season “mango” rains (<20 mm) fell in the area in March 2009, but no rain fell during the dry season in any of the subsequent years, at least over a 30 km radius. During the dry season, water is only available in four deep wells (~25 m deep). Seepage of water around wells and troughs for animals was monitored every dry season, but no mosquito larvae were found in these small puddles, which typically dry up every evening. A few trees may be irrigated by buckets every several days, but all water dries within hours. Annual precipitation is approximately 500 mm (543 mm in Segou, which lies 30 km south and 100 km east of Thierola). For this study, the dry season refers to December–May and the wet season to July–October; the transition periods (June and November) are marked by climatic irregularity (surface water may or may not be available). In this paper, a year is defined as the period spanning from after the first rains (July 1st) to the end of the following dry season (June 30th). On-the-ground searches for surface waters during the dry season were conducted every year, in consultation with herdsmen and hunters, and a detailed examination of the satellite photographs available in Google Earth was also performed. Tree holes containing water that last until January (and rarely into February) were also monitored, but no anopheline larvae were found. Except after the mango rains of March 2009[16], no surface waters during the dry season have been found in a distance up to 30 km around Thierola.

Mosquito Collection

Live collections using mouth aspirators inside all houses (n~120), were conducted throughout the study period as described previously[18]. The number of houses sampled (n=

511; median=119; 95% CI=103–125) varied because houses were not accessible when their owners were away from the village (and the actual number of houses changed over the five-year study as some were destroyed and others were built). Typically, collections were made every day (dry season) or every-other day (wet season) for two weeks per month. Each house was visited by two trained collectors, both searching for mosquitoes for 10–15 minutes (and until no mosquitoes were collected for 3–5 minutes). The same collectors were used throughout the study and rotated across all houses. During certain periods (e.g., the wet season of 2008 and 2009, dry season of 2010, and dry season of 2012), collected mosquitoes were marked and released about 1 hour after sunset on the day of collection. During other periods, mosquitoes were not released after capture but used for various experiments (reported separately). Because the recapture rate was low (<3%), the effect of removing mosquitoes on the subsequent density, as opposed to releasing them (i.e., sampling with and without replacement), was assumed to be negligible.

Additional methods used to collect mosquitoes outdoors included clay pots (with or without water/sugar), CDC light traps, fruit/flower baited traps, oviposition traps, emergence traps from larval sites, fence traps, and traps over domestic animals (calves, goats, sheep, and chickens), pit latrines, wells, or rodent burrows. Although some of these traps were useful during the wet season (e.g., emergence traps), they all yielded virtually no mosquitoes during the dry season, as opposed to indoor collections (above), and therefore were discontinued after various intervals (ranging from weeks to years). Several of these methods were described previously[16,17]. To evaluate congruence between adult and larval composition, larval collections using dippers were conducted during the wet season of 2009 and 2010 from multiple larval sites and multiple positions in each site.

Occasionally other anopheline species were collected by the different methods including, *A. rufipes* and *A. pharoensis*, but their numbers were insufficient for analysis. *A. funestus* was also observed in small numbers during the first year of the study[16] (2008–2009), but virtually vanished during the subsequent years, presumably as a result of the mass distribution of insecticide-impregnated bednets that started in 2008 in the region.

Data Analysis

The *Anopheles gambiae s.l.* indoor collection records, consisting of 511 collection days in all (~120 accessible) houses from September 2008 through August 2013, were used to produce mean daily density per house (dividing the total collected in each day by the number of houses searched). Mean daily density/house was transformed to stabilize the variance into natural log density as follows: Ln(density) = mean density + [0.9/(no. of houses sampled)]. Although statistically equivalent to a transformation using 1, 0.9 was used to signify that the drop of density from 1 to zero, which probably reflects, biologically, “more” than a change from 1.1 to 0.1.

To provide a descriptive framework as part of the data exploration, putative elements of the annual (seasonal) cycle were visually identified if they appeared in two or more years, after scanning both the linear and logarithmic scales (Figure ED-2 and Table ED-2) using two 15-day-long frames that were shifted horizontally along the figures. These time series of daily density were converted into an equidistant, 5-day interval time series consisting of 362

intervals using Proc Expand[30], after fitting a continuous curve to the data by connecting successive straight-line segments between non-missing input values using the 'join' method. This procedure interpolated missing values in the time series. The 5-day interval estimates produced showed the best fit to the observed data based on visual inspection and the sum of the difference between the observed and expected values, when compared with 10-day, 14-day, and 1 month intervals. Moreover, if the interpolated (missing) value differed from the corresponding 10 d mean by more than 30% of that mean, it was replaced by the latter. If no 10-day mean density was available for that period, the same was carried out with the corresponding global 10-day mean density (across the five years). Less than 5% of values required such substitutions. The fit between the equidistant time series and the observed daily mean density is depicted in Fig. ED-2. Statistical analyses were performed on the equidistant log-density time series.

Mosquitoes that were morphologically identified as members of *A. gambiae s.l.* complex were subjected to molecular identification to determine their species[31]. To estimate species composition, we pooled specimens collected from Thierola and nearby villages (up to a 6 km radius) into 10-d intervals based on the day of the month (1–10, 11–20 and 21–31) and separately into monthly intervals. In a few cases with small sample sizes ($n<15$), we pooled two consecutive 10-d intervals. The resulting series had variable gaps representing missing values either because no mosquitoes were collected despite extensive collection effort (e.g., January–February 2012), or because no collections were made (e.g., December 2008–March 2009). When no composition data were available for the whole month, the monthly mean fractions of each species estimated for that month across the five years were imputed for the missing values. Composition data for 10/61 months (16%) were imputed in that way. The compositional series consisting of 10 d estimates and imputed monthly values were thereafter interpolated using Proc Expand[30] to 10-d intervals (without changing observed compositional values) for each species separately. The interpolated values were restricted to values between 0 and 100. The species-specific (absolute) mean density was then estimated as the product of the proportion of each species at that 10-day time interval by the density of *A. gambiae s.l.* at the corresponding 5-day time intervals described above.

Time-series analysis of the log density of each species was carried out using the Unobserved Components Model in SAS[30] (Proc UCM), which accommodates time-varying parameters of the trend, seasonal, and cycle components derived from decomposition of the time series, as well as various methods of incorporating autoregressive processes. It estimates both deterministic and stochastic parameters and provides tests of the parameters' variance to determine if these parameters are time-varying. Overall goodness-of-fit measures, such as Akaike information criterion (AIC) used to compare models were computed, as well as extensive tests of the residuals and diagnostic graphics. We tested whether overall seasonal variation was statistically significant and if so, determined if it was time-varying or constant, before identifying its salient elements. The seasonal component is a unique cycle with a strictly annual periodicity (whose parameters sum to zero over a year). Seasonality was modeled as a series of 73 dummy variables, each representing a 5-day interval. Starting with the basic structural model[32] that includes stochastic slope, level, and seasonal components (in addition to the irregular element), we removed or added one parameter at a time and

evaluated the significance of all parameters, the overall fit of the model, and the residual diagnostics for serial correlation, heteroscedasticity, and normality. Additional cycles or auto-regressive functions may be required to model the interdependencies of the data between time-points until the distribution of the residuals complies with white noise (Supplementary Text). This approach led to selecting a parsimonious model that accounted well for the pattern of the time series and met the required assumptions. The seasonal component extracted from the selected model and its 95% CI were used to identify elements (phases) with statistical support. Thus, a peak whose 95% CI did not overlap with its adjacent minima had statistical support. All tests and P-values are based on two-sided tests.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Donnelly MJ, Simard F, Lehmann T. Evolutionary studies of malaria vectors. Trends Parasitol. 2002; 18:75–80. [PubMed: 11832298]
2. Omer SM, Cloudsley-Thompson JL. Dry season biology of Anopheles gambiae Giles in the Sudan. Nature. 1968; 217:879–880.
3. Holstein, MH. Research in French West Africa. World Health Organization; Geneva: 1954. Biology of Anopheles gambiae.
4. Siraj AS, et al. Altitudinal changes in malaria incidence in highlands of Ethiopia and Colombia. Science. 2014; 343:1154–1158.10.1126/science.1244325 [PubMed: 24604201]
5. WHO. World Malaria Report 2013. Vol. 178. World Health Organization; Geneva, Switzerland: 2013.
6. Coetzee M, et al. Anopheles coluzzii and Anopheles amharicus, new members of the Anopheles gambiae complex. Zootaxa. 2013; 3619:246–274.10.11646/zootaxa.3619.3.2 [PubMed: 26131476]
7. Omer SM, Cloudsley-Thompson JL. Survival of female Anopheles gambiae Giles through a 9-month dry season in Sudan. Bull World Health Organ. 1970; 42:319–330. [PubMed: 5310144]
8. Taylor CE, Toure YT, Coluzzi M, Petrarca V. Effective population size and persistence of Anopheles arabiensis during the dry season in west Africa. Med Vet Entomol. 1993; 7:351–357. [PubMed: 8268490]
9. Toure YT, et al. Ecological genetic studies in the chromosomal form Mopti of Anopheles gambiae s.s. in Mali, West Africa. GENETICA. 1994; 94:213–223. [PubMed: 7896141]
10. Simard F, Lehmann T, Lemasson JJ, Diatta M, Fontenille D. Persistence of Anopheles arabiensis during the severe dry season conditions in Senegal: an indirect approach using microsatellite loci. Insect Mol Biol. 2000; 9:467–479. [PubMed: 11029665]
11. Coetzee M, Craig M, le Sueur D. Distribution of African malaria mosquitoes belonging to the Anopheles gambiae complex. Parasitol Today. 2000; 16:74–77. [PubMed: 10652493]
12. della Torre A, Tu Z, Petrarca V. On the distribution and genetic differentiation of Anopheles gambiae s.s. molecular forms. Insect Biochem Mol Biol. 2005; 35:755–769. [PubMed: 15894192]

13. Sogoba N, et al. Monitoring of larval habitats and mosquito densities in the Sudan savanna of Mali: implications for malaria vector control. Am J Trop Med Hyg. 2007; 77:82–88. [PubMed: 17620634]
14. Adamou A, et al. The contribution of aestivating mosquitoes to the persistence of Anopheles gambiae in the Sahel. Malar J. 2011; 10:151. 1475-2875-10-151 [pii]. 10.1186/1475-2875-10-151 [PubMed: 21645385]
15. Huestis DL, et al. Seasonal variation in metabolic rate, flight activity and body size of Anopheles gambiae in the Sahel. J Exp Biol. 2012; 215:2013–2021. 215/12/2013 [pii]. 10.1242/jeb.069468 [PubMed: 22623189]
16. Lehmann T, et al. Aestivation of the African Malaria Mosquito, Anopheles gambiae in the Sahel. American Journal of Tropical Medicine and Hygiene. 2010; 83:601–606.10.4269/ajtmh.2010.09-0779 [PubMed: 20810827]
17. Yaro AS, et al. Dry season reproductive depression of Anopheles gambiae in the Sahel. J Insect Physiol. 2012; 58:1050–1059.10.1016/j.jinsphys.2012.04.002 [PubMed: 22609421]
18. Lehmann T, et al. Seasonal Variation in Spatial Distributions of Anopheles gambiae in a Sahelian Village: Evidence for Aestivation. Journal of Medical Entomology. 2014; 51:27–38.10.1603/me13094 [PubMed: 24605449]
19. Coluzzi M, Petrarca V, Di Deco MA. Chromosomal inversion intergradation and incipient speciation in Anopheles gambiae. Bollettino di Zoologia. 1985; 52:45–63.
20. Coluzzi M, Sabatini A, Petrarca V, Di Deco MA. Chromosomal differentiation and adaptation to human environments in the Anopheles gambiae complex. Trans R Soc Trop Med Hyg. 1979; 73:483–497. [PubMed: 394408]
21. Sogoba N, et al. Spatial distribution of the chromosomal forms of anopheles gambiae in Mali. Malar J. 2008; 7:205.10.1186/1475-2875-7-205 [PubMed: 18847463]
22. Toure YT, et al. Perennial transmission of malaria by the Anopheles gambiae complex in a north Sudan Savanna area of Mali. Med Vet Entomol. 1996; 10:197–199. [PubMed: 8744717]
23. Denlinger DL. Dormancy in tropical insects. Annual Review of Entomology. 1986; 31:239–264.
24. Denlinger DL, Armbruster PA. Mosquito diapause. Annu Rev Entomol. 2014; 59:73–93.10.1146/annurev-ento-011613-162023 [PubMed: 24160427]
25. Tauber, MJ.; Tauber, CA.; Masaki, S. Seasonal Adaptations of Insects. Oxford University Press; 1986.
26. Sogoba N, et al. Malaria transmission dynamics in Niono, Mali: the effect of the irrigation systems. Acta Trop. 2007; 101:232–240. [PubMed: 17362859]
27. Lemasson JJ, 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. 1997; 34:396–403. [PubMed: 9220672]
28. Huestis DL, Lehmann T. Ecophysiology of Anopheles gambiae s.l.: persistence in the Sahel. Infect Genet Evol. 2014 In Press.
29. Lehmann T, Diabate A. The molecular forms of Anopheles gambiae: A phenotypic perspective. Infection Genetics and Evolution. 2008; 8:737–746.10.1016/j.meegid.2008.06.003
30. SAS, I., Inc. Sas Institute; Cary, NC: 2011.
31. Fanello C, Santolamazza F, della Torre A. Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. Med Vet Entomol. 2002; 16:461–464. [PubMed: 12510902]
32. Harvey, AC. Forecasting, Structural Time Series Models and the Kalman Filter. Cambridge Univ. Press; 1989.

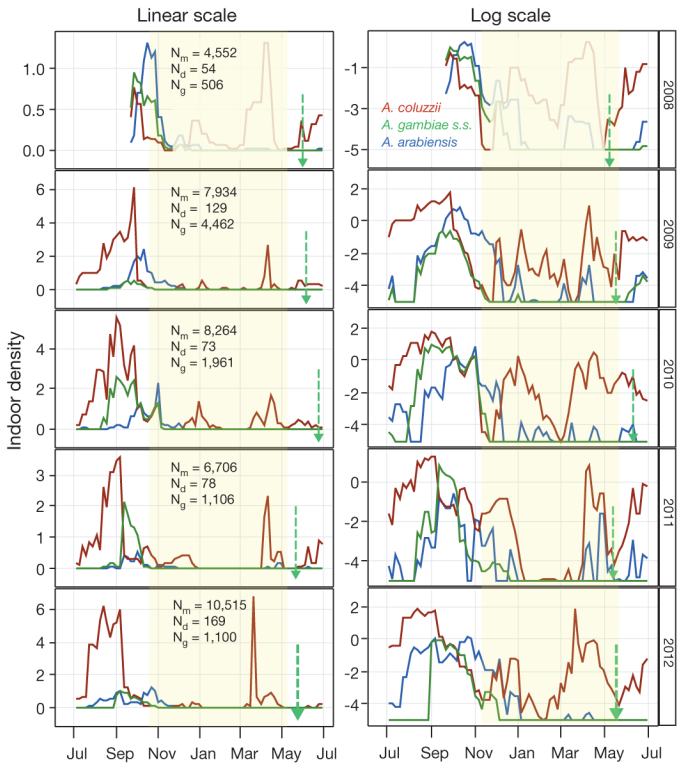

Figure 1. Species-specific population dynamics of the members of *Anopheles gambiae s.l.*

Average densities of *Anopheles coluzzii* (red), *A. gambiae s.s.* (green), and *A. arabiensis* (blue) are shown on linear and natural logarithm scales from July to June of every year, portraying changes both at low and high density ranges. Green arrows mark the first rain and tan background denotes the dry season. N_m, N_d, and N_g denote sample size of *A. gambiae* s.l., collection days, and the number genotyped to species, respectively (Methods). Shading indicates a gap in sampling (December–March 2008) when imputed values were used (Methods).

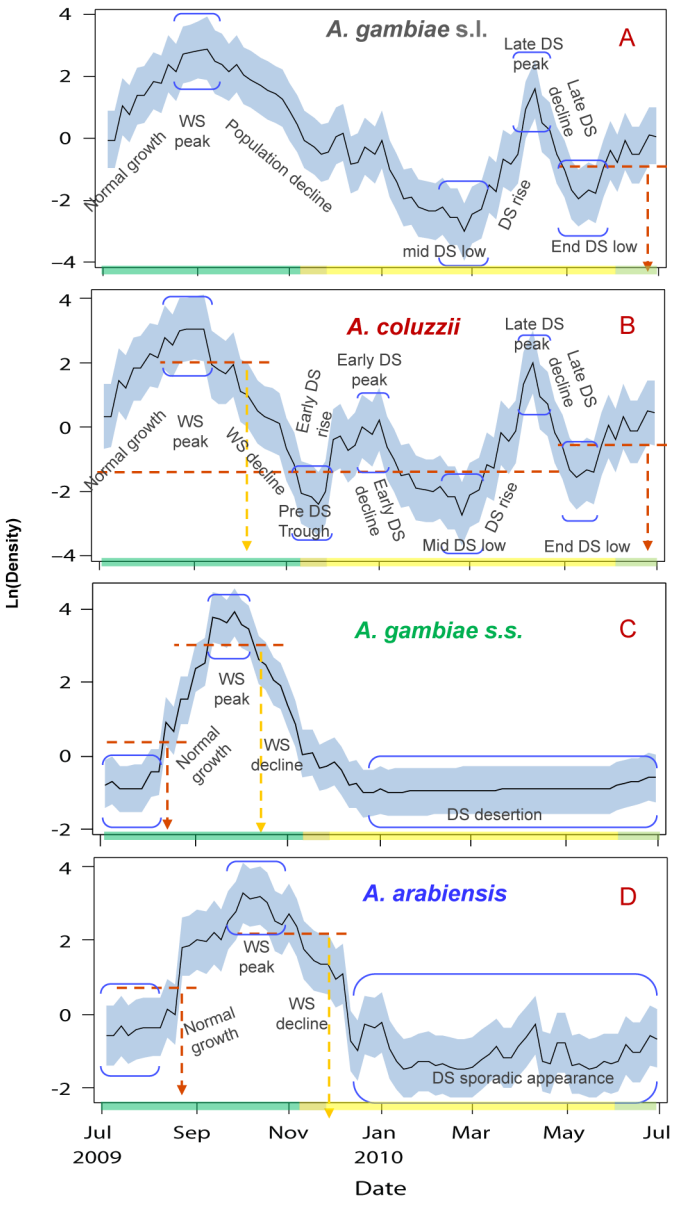

Figure 2. Seasonal population dynamics of the members of *Anopheles gambiae s.l*

The seasonals were estimated using unobserved component time-series models (Table 1, and Methods). Bands denote 95% CI, while blue brackets surround peaks and troughs whose 95% CIs do not overlap. Red and orange arrows denote the onset and decline of population growth, respectively; defined as the earliest time when the 95% CI of the population growth (or decline) phase does not overlap with that of the preceding phase (horizontal red line). Population phase names correspond with putative elements (Table ED-2). Sample sizes are based on Fig. 1.

Table 1

Unobserved component time-series (final) models of the population dynamics for each taxon (Methods).

Taxon[a]	Parameter	Var (Stochastic)[b]	P [var]	Determ. Est.[c]	P [effect]
A. gambiae s.l.	**Seasonals**[d]	0 (-fixed)	na	see Fig. 2	0.0001
R^2=0.76	Level[e] E var	0 (-fixed)	na	−1.23	0.0001
AIC=793.5	Cycle[f] DampF	0.7	0.0001		
BIC=808.1	Cycle[f] Period	5758.7	0.99		
	Cycle[f] E var	0.56	0.0001		0.99
	Irreg. E var	0.0112	0.85	na	0.95
A. coluzzii	**Seasonals**	0 (-fixed)	na	see Fig. 2	0.0001
R^2=0.72	Level E var	0 (-fixed)	na	−1.68	0.0001
AIC=844	Cycle DampF	0.7	0.0001		
BIC=858	Cycle Period	47.1	0.5		
	Cycle E var	0.65	0.0001		0.99
	Irreg. E var	0.011	0.92	na	0.95
A. gambiae s.s.	**Seasonals**	0 (-fixed)	Na	see Fig. 2	0.0001
R^2=0.89	Level E var	0 (-fixed)	Na	−4.1	0.0001
AIC=515	Cycle DampF	0.76	0.0001		
BIC=545	Cycle Period	16.1	0.0001		
	Cycle E var	0.14	0.0017		0.0005
	Cycle2 DampF	1	0.0001		
	Cycle2 Period	41.1	0.0001		
	Cycle2 E var	0.0001	0.54		0.21
	Irreg. E var	0.042	0.28		
	Irreg. AR(1)[g]	0.94	0.0001		0.0003
A. arabiensis	**Seasonals**	0 (-fixed)	na	see Fig. 2	0.0001
R^2=0.77	Level E var	0 (-fixed)	na	−3.52	0.0001
AIC=742	Cycle DampF	0.69	0.0001		
BIC=757	Cycle Period	11966	0.99		

Taxon[a]	Parameter	Var (Stochastic)[b]	P [var]	Determ. Est.[c]	P [effect]
	Cycle E var	0.48	0.0001		0.08
	Irreg. E var	0.00001	0.99	na	0.99

[a] All models (species) include 362 observations (5-day means from 22/9/08 and 1/9/13, based on all *A. gambiae s.l.* and those genotyped, see Methods and Fig ED-1).

[b] Stochastic variance and test of significance (P [var]) indicate whether the parameter is time varying.

[c] Effect size and test of significance (P [effect]) measure the overall deterministic effects.

[d] Seasonal component was modeled by 73 dummy variables. Individual effect of each of these parameters and 95% CI are shown in Figure 2 (Text and Methods).

[e] Level is equivalent to intercept (in UCM framework, if time varying, it results in a “random walk” between successive time points), and was found to be fixed in all analyses.

[f] Non-seasonal stochastic (trigonometric) cycles, each defined by three parameters: a period (Period, time difference between two successive peaks; here in units of 5-day intervals), cycle damping factor (DampF, decay in amplitude between cycles over time), and the variance of the error of the cycle over successive periods (E var, Methods and Supplementary Text).

[g] One-lag autoregressive (AR1) parameter was modeled as part of the irregular component of *A. gambiae s.s.*