Trends in
Parasitology

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Spotlight

Dried out but alive: how mosquitoes survive 8 months

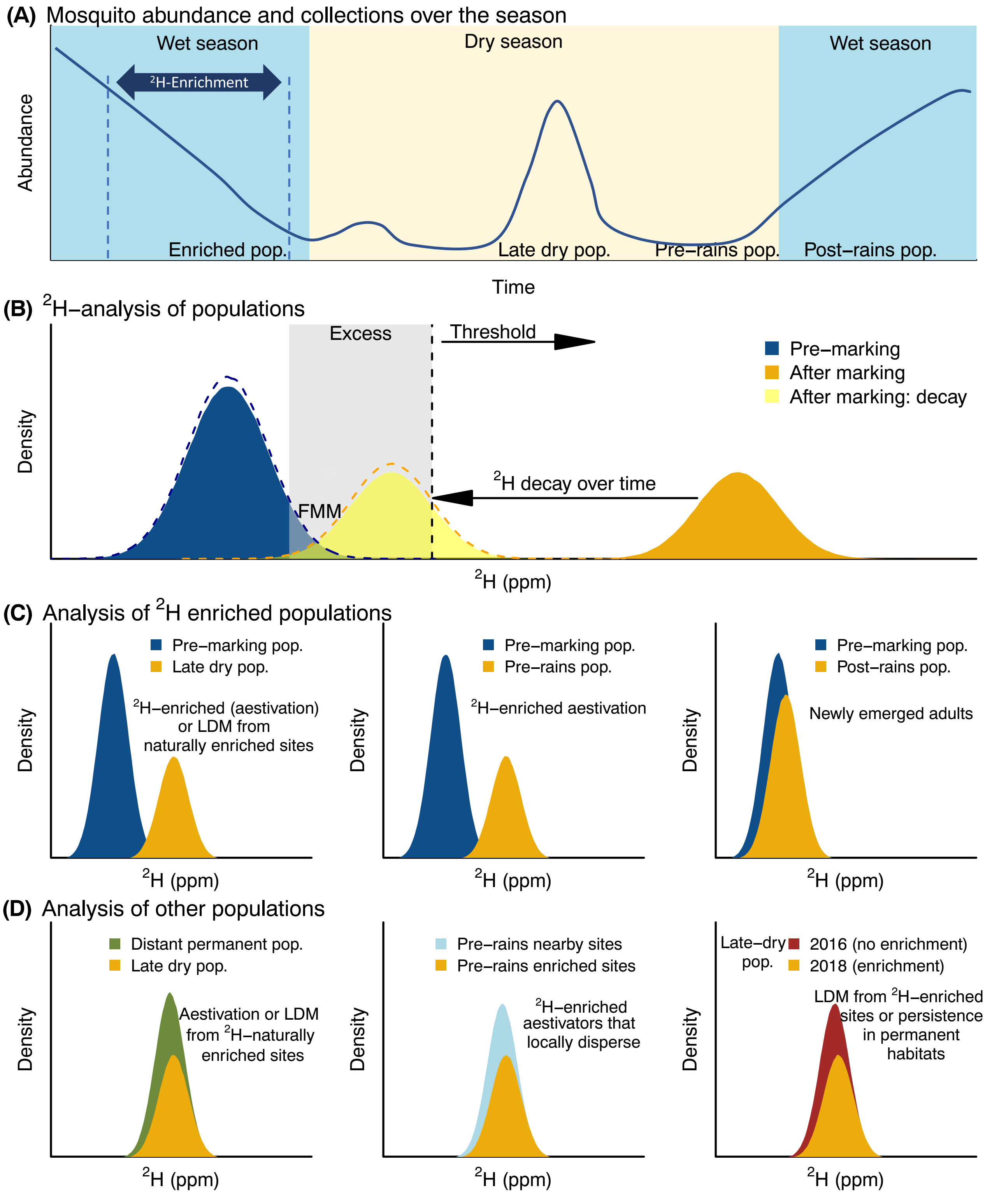
Francesco Baldini [1,*] and Mafalda Viana [1,*]

How malaria mosquitoes persist during the dry season in the Sahel and rapidly rebound at the onset of rains is unclear. Recently, Faiman and colleagues demonstrated that aestivation, a summer dormancy mechanism, is a major persistence strategy of *Anopheles* mosquitoes, which could be targeted by vector control.

Mosquitoes lay their eggs in aquatic habitats, where they develop as larvae and then emerge as adults. This is why, during the rainy season, mosquito abundance peaks, and during the dry season they seemingly disappear [1]. As eggs die without water, and adult mosquitoes have a short life span of maximum 3–6 weeks in the wild [2], how can mosquitoes survive the 3–8 month dry season of the semi-arid Savannah and Sahel regions of Africa in the absence of aquatic habitats? Beyond an ecological curiosity that has lasted decades, understanding the seasonal fluctuations of mosquitoes could provide opportunities to improve control of mosquito-borne diseases. This is particularly relevant for malaria which, in sub-Saharan Africa, is transmitted predominantly by four species of *Anopheles* mosquitoes: *A. gambiae* s.s., *A. coluzzii*, *A. arabiensis*, and *A. funestus*. During the dry season, malaria cases almost cease but they spike back at the start of the wet season [1], following the abundance of mosquitoes.

The rapid rebound of *Anopheles* populations with the first rains in the dry savannah has been hypothesised by two main strategies. (i) Long-distance migration (LDM) occurs when mosquitoes are transported for hundreds of kilometres by winds from sites where larval habitats are permanent. Direct evidence of LDM has been shown by capturing *A. gambiae* s.s. and *A. coluzzii* with high-altitude sticky traps attached to helium balloons [3]. (ii) Aestivation, also known as summer dormancy, occurs when mosquitoes survive several months in a state of suppressed reproduction and prolonged longevity during harsh conditions [4]. Direct evidence of aestivation has been sparse but following a mark–recapture study of *A. coluzzii*, a single marked mosquito was recaptured 7 months later. Indirectly, longitudinal monitoring of *A. coluzzii* abundance showed local persistence during the dry season and rapid rebound at the onset of the first new rains. On the contrary, *A. gambiae* s.s. seems to be completely absent in the dry season and reappears 2 months after the start of the wet season, which is more compatible with LDM [5]. An alternative hypothesis relies on the existence of few permanent larval habitats, such as underground water sources, allowing dry season persistence at very low abundances, but evidence has not been found yet. These hypotheses are not necessarily mutually exclusive but the contribution of each of these strategies to the persistence and recolonization of different *Anopheles* species at the onset of the wet season – when malaria transmission resumes – is unknown.

Here we highlight the recent work by Faiman *et al.* [6] which showed that aestivation is a major persistence mechanism of *A. coluzzii* over the dry season in the Sahel region in Mali. This exciting new evidence is supported by cross-checking a new mosquito-tracking technique with multiple data analysis methods, and a comprehensive characterisation of the local habitats and mosquito ecology. In the search for a tracking strategy that could be applied to small organisms like mosquitoes, and persist long enough to be detected after several months, the authors creatively added deuterium (^2H) isotope, in the form of heavy water (^2H_2O), to larval habitats during the last 2 months of the rainy season (Figure 1A). Deuterium becomes incorporated in larvae and is subsequently detectable in the tissues of emerging adults, as previously tested in the laboratory [7]. Thus, by monitoring the fraction of ^2H-enriched mosquitoes during the dry season and at the onset of new rains, Faiman *et al.* [6] quantified the proportion of aestivating mosquitoes. However, this technique poses some challenges: (i) deuterium decays over time in mosquito tissues, thus marked mosquitoes that survive several weeks might become indistinguishable to non-marked ones [7]; (ii) deuterium levels can vary spatially, making it difficult to distinguish recaptures of ^2H-marked mosquitoes from those from a distant site that is ^2H-naturally enriched [6]; and (iii) deuterium levels can vary temporally, for example, as heavy water (^2H_2O) evaporates less than protium water (H_2O) [8], the amount of deuterium in larval habitats might change seasonally. To overcome these challenges, the authors adopted three different analyses to quantify ^2H-enriched mosquitoes (Figure 1B), as follows. (i) Threshold: the fraction of mosquitoes whose ^2H levels were above the highest ^2H value before the enrichment. (ii) Excess: the proportion of mosquitoes in the marked population whose ^2H values exceeded the third quartile (75th) of the non-marked mosquito population distribution, leading to an excess over the expected of 25% (iii) Finite mixed-distribution model (FMM) to assess whether a population consisted of two subpopulations with low and high ^2H values, potentially corresponding to non-marked and marked mosquitoes, respectively. These three approaches were then used to compare populations before, during, and at the end of the dry season, from two enriched villages. The authors found that, after enrichment of



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larval habitats, most mosquitoes collected at the end of the wet season and at the start of the dry season were ^{2}H-enriched, suggesting that isotopic marking had worked. Three–four months after the last larval habitats have dried out, as well as at the turn of the new wet season, they found that the newly collected *A. coluzzii* mosquitoes were ^{2}H-enriched, showing a potential signature of aestivation. Then, when larval breeding sites were established after the rains restarted, ^{2}H levels returned to similar pre-marking levels, as expected from the emergence of new non-marked adults (Figure 1C). Combining all evidence, the authors concluded that at least 18% of the mosquitoes had survived the dry season as aestivators.

To further confirm the results, the authors also compared the marked population with a past collection from a different late dry season. This analysis revealed similar ^{2}H levels (higher threshold but lower excess) between them, suggesting that the origin of these mosquitoes cannot be fully explained by aestivation, and LDM from distant permanent natural ^{2}H-enriched habitats could be hypothesised. Indeed, ^{2}H levels of populations from the late dry season were similar to populations sampled 140 km northeast, where rice irrigation allows all-year larval habitats, and adult mosquitoes could be brought by winds. Nevertheless, just before the new rains, ^{2}H levels differed between the studied villages and those with permanent larval habitats, precluding the LDM hypothesis at this time and further suggesting aestivation as a more plausible strategy of perseverance in this case. Finally, at the onset of rains, populations from marked and nearby villages had similar ^{2}H levels (lower threshold but higher excess); this could be explained by marked aestivated mosquitoes dispersing to nearby villages, and/or LDM mosquitoes arrived in the late dry season from perennial habitats that survived more than 2 months, possibly by aestivation (Figure 1D).

More work remains to be done to confirm and quantify the contribution of the different strategies that allow mosquito populations to persist during the dry season. The approach taken by Faiman *et al.* opens exciting opportunities. Determining the best timing for deployment and the spatial–temporal variation of ^{2}H could help to disentangle the impacts of LDM and potential confounding effects arising from within and between site heterogeneities (e.g., multiple small larval habitats). Stable isotope analyses that describe and compare isotopic niches in the presence of high levels of variation are available in other fields of ecology (e.g., for dietary analysis), and could provide opportunities to develop a holistic statistical framework for the challenging ^{2}H data [9,10]. Notwithstanding, Faiman *et al.* [6] provides the most concrete evidence of *A. coluzzii* aestivation so far, and it has important implications for the timing of malaria control. For example, applying vector-control tools, such as indoor spraying, in the dry season and immediately after the first rains, could prevent the aestivators from restarting the population; additionally releasing genetically modified mosquitoes before they aestivate could ensure that the required traits spread across seasons.

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Declaration of interests

The authors declare no competing interests.

[1]School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, UK

*Correspondence:
Francesco.Baldini@glasgow.ac.uk (F. Baldini) and
Mafalda.Viana@glasgow.ac.uk (M. Viana).

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Figure 1. Schematic of experiment with deuterium isotope-marked mosquitoes in the Sahel showing signals of aestivation in *Anopheles coluzzii* during the dry season. (A) Faiman *et al.* [6] used deuterium (^{2}H) isotopes in larval aquatic habitats to mark mosquitoes at the end of the wet season and recaptured them during the dry season and at the turn of the new wet season. (B) As deuterium decays over time, the authors used three analyses to distinguish ^{2}H-marked mosquitoes from pre-marked populations [^{2}H threshold, excess in 75th interquartile, finite mixed populations (FMM)]. (C) *A. coluzzii* collected during the late dry season (left panel) and before the onset of rains (pre-rains, middle panel) showed ^{2}H-enriched populations, compatible with an aestivation strategy. When the rains re-established aquatic habitats (post-rains), newly emerged mosquitoes showed pre-marking ^{2}H levels (right panel). (D) Similar ^{2}H levels in the late dry season between mosquitoes collected in ^{2}H-enriched villages and in distant sites (left panel) or in a different dry season (right panel) does not preclude that *A. coluzzii* persists also by long-distance migration (LDM) and/or in permanent habitats. Mosquito ^{2}H levels in enriched villages were similar to nearby villages before the new rains (pre-rains, middle panel), suggesting local dispersion of aestivators. Abbreviation: pop., population.