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Forum

Anopheles stephensi ecology and control in Africa

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The encroachment and rapid spread of *Anopheles stephensi* across Africa presents a significant challenge to malaria control and elimination efforts. Understanding the ecology and behavior of *An. stephensi* will critically inform control measures and provide prerequisite knowledge for exploring new larval and adult control tools to contain its spread.

The rapid spread of *An. stephensi* in Africa and knowledge gaps

The invasion into Africa of *An. stephensi* Liston 1901, a malaria vector from South Asia, and its rapid expansion in its new territory pose a significant threat to malaria control and elimination on the continent. Since it was first detected in Djibouti in 2012, *An. stephensi* has been found in Ethiopia, Sudan, Somalia, Kenya, Ghana, and Nigeria [1,2]. The invasion of *An. stephensi* has been linked to malaria outbreaks in Djibouti and Ethiopia [3,4]. Due to the gravity of the situation, the World Health Organization (WHO) has called for urgent action to halt the spread of *An. stephensi* in Africa [2]. In Ethiopia, where most sites of *An. stephensi* invasion in Africa have been reported [5], larviciding is currently implemented at selected sites for *An. stephensi* control [6]. However, to effectively plan and implement larval and adult mosquito control programs, a better understanding of *An. stephensi* ecology and behavior is essential. Extensive research has been conducted on the ecology and behavior of *An. stephensi* in its native range in Asia. Although *An. stephensi* is known to prefer breeding in containers, its larvae have also been found in natural habitats in Asia [7–9]. The dire situation of *An. stephensi* invasion in Africa has prompted many recent recommendations, laboratory studies, and field studies [1,10–12]. However, there remain significant knowledge gaps regarding the ecology of *An. stephensi* in Africa. Furthermore, the scientific literature on *An. stephensi* ecology (e.g., types of breeding habitats, resting and biting behaviors, etc.) is often confounding and contradictory [1,7,10,11], likely due to the lack of evidence about vector ecology in different eco-epidemiological settings in different countries worldwide. More importantly, very few previous published works have discussed potential new *An. stephensi* control tools or have addressed future research directions for containing the spread of *An. stephensi* in Africa.

The focus of this study is to provide a succinct overview of the fundamental ecology of three major native East African malaria vectors, namely *Anopheles gambiae sensu stricto* (hereafter referred to as *An. gambiae*), *Anopheles funestus sensu lato* (*An. funestus*), and *Anopheles arabiensis*, as well as the recently invasive vector *An. stephensi*. We aim to identify the distinctions and similarities between the two groups (i.e., native and invasive malaria vectors) so as to introduce potential new *An. stephensi* larval and adult control tools and inform prospective future research directions. We emphasize that most of the ecological studies on *An. stephensi* were conducted in Asia, as knowledge of *An. stephensi* ecology in Africa is currently limited.

Adult ecology of native African versus invasive malaria vectors

All four species of *Anopheles* mosquitoes discussed in this study (*An. gambiae, An. arabiensis, An. funestus,* and *An. stephensi*) transmit both *Plasmodium falciparum* and *Plasmodium vivax* parasites (Table 1). Native African malaria vectors are primarily active in rural areas, although malaria transmission also occurs in urban areas. By contrast, invasive *An. stephensi* transmits malaria parasites primarily in urban areas and less commonly in rural areas. The dynamics of vector populations are strongly seasonal in many places for all four species. African malaria vectors occur perennially, with strong seasonality, in many places, and *An. stephensi* is perennial in Asia. A key characteristic of *An. stephensi* is that it has three biological forms that are distinguished by the number of ridges on the egg float: *mysorensis*, intermediate, and type form [9]. In Asia, the type form is an efficient vector of urban malaria parasite, while the *mysorensis* form is found mainly in rural areas and is an important vector in certain places such as Iran [9]. The intermediate form is typically found in rural and peri-urban areas in Asia, and its vector status has not yet been confirmed. So far, the biological forms of *An. stephensi* in Africa are unclear. In addition, the behavior of *An. stephensi* may have adapted to its newly invaded African environment.

While there are differences in adult ecology between *An. stephensi* and native African malaria vectors, adult *An. stephensi* and the African malaria vector *An. arabiensis* share many ecological traits (Table 1). There are also major differences in ecology between different species of malaria vectors native to Africa (Table 1). *An. gambiae* and *An. funestus* are endophilic (with a preference for human dwellings), endophagic and night-biting, and primarily anthropophilic, although recent studies have found zoophilic and early-evening/early-morning biting behaviors, likely due to the increased protection of humans by indoor interventions such as indoor

Table 1. Vector ecology, behavior, and malaria transmission potential of native East African malaria vectors and *Anopheles stephensi*

Parameters		Native African malaria vectors		*An. stephensi*
		Anopheles gambiae s.s., *Anopheles funestus*	*Anopheles arabiensis*	
Transmission	Parasites	*Plasmodium falciparum*, *Plasmodium vivax*[a]		
	Ecological settings	Primarily rural, less in urban areas[b]		Primarily urban or peri-urban, less in rural areas of Africa
	Dynamics	Strong seasonality depending on region[a]		
Adult ecology and behavior	Resting behavior	Endophilic	Endophilic and exophilic	Endophilic and exophilic
		Human dwellings	Animal sheds and human dwellings	Primarily animal sheds
	Feeding behavior	Endophagic	Exophagic and endophagic	Endophagic and exophagic
		Night-biting	Night-biting	Dusk- and night-biting
	Host preference	Anthropophilic, less zoophilic	Zoophilic, less anthropophilic	Zoophilic and anthropophilic
	Desiccation	Does not tolerate extreme conditions[b]		Tolerates extreme hot and dry conditions
Larval ecology	Habitats	Primarily natural habitats[b]		Man-made and natural habitats
		Water bodies in rural fields, less common in urban areas[b]		Mainly in urban areas
	Water quality	Clean water[b]		Clean and polluted water
	Co-breeding	Share habitats among themselves[b]		Do not share habitats[c]
Insecticide resistance		Adults resistant to the four classes of commonly used insecticides[a]		

[a]Same for both native African malaria vectors and *An. stephensi*.
[b]Shared by all native African *Anopheles* mosquitoes.
[c]Do not share habitats with native African *Anopheles*, based on larval surveys in Ethiopia.

residual spraying (IRS) and insecticide-treated nets (ITNs). By contrast, both *An. arabiensis* and *An. stephensi* exhibit behavioral plasticity – endophilic and exophilic behaviors with a preference for animal sheds or other outbuildings or structures, exophagic and endophagic feeding with night-biting behavior, and zoophilic tendencies, although *An. arabiensis* also rests inside human dwellings [10]. Studies have found that *An. stephensi* tolerates extreme hot and dry conditions, while native African malaria vectors cannot tolerate extreme conditions [10]. As a result, *An. stephensi* has invaded and established its populations in hot, dry areas of the Middle East over the past 50 years and more recently in dry areas of the Horn of Africa, including Somalia, Sudan, Djibouti, northern and eastern Ethiopia, and northern Kenya [1].

Larval ecology of African native versus invasive malaria vectors

The major difference between native African malaria vectors and *An. stephensi* is their larval ecology (Table 1). Native African malaria vectors breed primarily in non-container man-made habitats or natural habitats with clean water in mainly rural and some peri-urban areas. In Africa, *An. stephensi* breeds primarily in man-made habitats (e.g., artificial water containers) in urban areas [3]. Like native African malaria vectors, *An. stephensi* breeds in rural areas and in different types of natural habitats such as irrigation channels in Asia [7,9]. In addition, *An. stephensi* can breed in clean, polluted, or brackish saline water [5,9]. African malaria vectors frequently occur in sympatry and share habitats among themselves, and *An. stephensi* similarly shares habitats with other Asian *Anopheles* larvae. However, co-occurrence of *An. stephensi* and native African malaria vector larvae in breeding sites in Africa needs to be confirmed [5,6].

Community ecology of African native versus invasive malaria vectors

The community ecology of African malaria vectors has been well studied. Larval and adult community structure, species diversity, and cryptic species have been described in different ecological settings across Africa. In addition, many studies have reported on larval habitat characteristics and the impact of habitat types on larval survival and development, as well as on larval aquatic competitors and predators. The regulation of population growth by competitors, predators, and environment has also been studied extensively. By comparison, studies on the community ecology or biological of *An. stephensi* are limited in Asia and nearly absent in Africa [10].

Insecticide susceptibility of *An. stephensi*

In Asia, *An. stephensi* adults have displayed high resistance intensity to all four classes of insecticides commonly used for malaria control [13]. The mechanisms of resistance involve metabolic resistance caused by increased esterase, glutathione *S*-transferase (GST), and cytochrome P450 metabolic enzyme activity. Knockdown resistance (*kdr*) has been detected in populations of *An. stephensi* from several Asian countries [13]. In Sudan and Ethiopia, *An. stephensi* adults were highly resistant to organochlorines (DDT), organophosphates (malathion, pirimiphos-methyl), carbamates (propoxur, bendiocarb), and pyrethroids (α-cypermethrin, deltamethrin, permethrin). Exposure to piperonyl butoxide (PBO) synergist nearly fully restored *An. stephensi* susceptibility to pyrethroid insecticides, suggesting the existence of a metabolic resistance mechanism [5,13]. Very low frequencies of *kdr* mutations have been detected in Ethiopian *An. stephensi* populations. Based on the PMI VectorLink 2021 report, some populations of *An. stephensi* in Ethiopia exhibit possible resistance to the pyrrole insecticide chlorfenapyr, a class of agricultural pesticide and a candidate insecticide for public health. A recent study in Ethiopia found that *An. stephensi* larvae are fully susceptible to temephos, an organophosphate larvicide, and to the microbial larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) [6].

Implications for interventions

The following key characteristics of *An. stephensi* ecology and behavior in Africa may be useful for designing and implementing vector control strategies in Africa.

(i) *An. stephensi* adult females mainly rest inside animal sheds, and biting occurs mainly indoors at dusk and night.
(ii) *An. stephensi* adult females have a high animal blood meal index.
(iii) *An. stephensi* adult females are resistant to nearly all insecticides commonly used for public health, but their susceptibility to pyrethroids can be fully restored by adding the synergist PBO.
(iv) *An. stephensi* larvae are primarily found in man-made habitats such as artificial water containers and tanks, cisterns, and construction pits.
(v) *An. stephensi* larvae are susceptible to temephos and the microbial larvicide *Bti*.

Although new generations of ITNs and IRS have been tested in the field, these indoor (inside human dwellings) intervention measures may have only a limited impact on *An. stephensi* populations if *An. stephensi* does not rest inside human dwellings [1]. However, residual spraying targeting the key resting place of *An. stephensi*, for example, inside animal sheds or cryptic resting sites (Box 1), may be effective in reducing *An. stephensi* adult population abundance. Similarly, larviciding using temephos and *Bti* may be effective in reducing immature population abundance (Box 1). In fact, a slow-release and long-lasting (4–6 months) microbial larvicide containing *Bti* and *Bacillus sphaericus* (*Bs*) was shown in the field to be effective for indoor and outdoor malaria vector control in Africa [14]. Many *An. stephensi* larval habitats, such as water tanks and construction pits, are permanent, which is ideal for long-lasting larviciding. PBO-synergized pyrethroid ultra-low volume (ULV) sprayings have been widely used against *Aedes* and *Culex* mosquitoes in the Americas for West Nile and dengue control; residual spraying of these synergized insecticides has not yet been tested for malaria vector control.

New interventions can be designed based on *An. stephensi* ecology studies and interventions for controlling *Aedes* mosquitoes – another urban disease vector (Box 1). For example, mosquito female auto-dissemination of pyriproxyfen (PPF), a juvenile growth regulator which slowly kills mosquito larvae, has proved to be efficient for controlling *Aedes* larvae in urban areas [15]. The interiors of animal shelters (the main *An. stephensi* resting area) are sprayed with PPF, where ovipositing *An. stephensi* females then carry and spread PPF to breeding habitats (Box 1). Before implementing any large-scale application of these new vector larval and adult control tools, semi-field and field testing in systematically designed trials should be conducted to evaluate the efficacy of vector control intervention tools in reducing *An. stephensi* population abundance in urban areas. Integration of larval and adult controls with improved surveillance may achieve better results in combating the spread of *An. stephensi* in Africa.

Prospective future research

Most of the *An. stephensi* ecological observations have been made in Asia. Thus, there are key knowledge gaps in *An. stephensi* ecology in Africa that require further investigation (Box 1). For example, *An. stephensi* often shares breeding habitats with other Asian malaria vectors, but no evidence to date has reported shared habitats with native African malaria vectors. *An. stephensi* is also rarely found in rural Africa. Effective vector surveillance tools with minimal bias and maximal catch efficiency are necessary to better understand the bionomics and population dynamics of *An. stephensi* in Africa. The relative contribution of *An. stephensi* to malaria transmission in areas where it co-occurs with native African malaria vectors is currently unknown. Last but not least, the development and evaluation of potential new *An. stephensi* larval and adult control tools deserves special attention.

Concluding remarks

The invasion of *An. stephensi* into Africa and its rapid spread pose significant challenges for malaria control on the continent, especially in urban areas. The ecological

Box 1. Examples of prospective research regarding *An. stephensi* ecology and control in Africa

Larval ecology

Why do *An. stephensi* larvae rarely share habitats with native African malaria vectors? Is it due to interspecific competition, habitat biological and physicochemical characteristics, or habitat organic matter?

Why is *An. stephensi* rarely found in rural Africa? Is the presence or absence of *An. stephensi* related to its bioforms?

What are the environmental, climatic, and biotic factors that regulate *An. stephensi* larval development, survival, and population dynamics?

Adult ecology

Does *An. stephensi* rest and/or bite humans inside human dwellings?

Does *An. stephensi* rest and bite outdoors (as per the WHO, animal sheds are considered as indoors)?

What is the best method, with minimal bias and maximal catch efficiency, for *An. stephensi* adult surveillance?

What factors regulate population dynamics?

Community ecology

Key larval predators.

Interactions between *An. stephensi* and native African malaria vectors and other mosquitoes such as *Culex* and *Aedes* spp.

Species composition, diversity, and predominant species in different ecological settings.

Factors shaping community structures.

Insecticide resistance

Why is *An. stephensi* resistant to the agricultural pesticide chlorfenapyr (pyrrole class) in invaded countries such as Ethiopia? Is the observed resistance due to multiple cross-resistance or to other unknown mechanisms?

In general, what are the *An. stephensi* insecticide resistance mechanisms in Africa?

Systematic evaluation of new and existing larval and adult control measures

Long-lasting *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* (*Bti*/*Bs*) and other larvicides.

Indoor residual spraying (IRS)/space spray targeting key *An. stephensi* resting places such as inside animal shelters.

Animal-baited insecticide-treated tents (ITTs), namely the use of animals as an attractant combined with insecticides to kill mosquitoes.

Female auto-dissemination of pyriproxyfen (PPF), namely by spraying the inside of animal shelters with PPF and letting ovipositing female mosquitoes auto-disseminate PPF into breeding habitats.

characteristics of *An. stephensi* carry essential implications for the design of effective vector control strategies. However, there are important knowledge gaps, warranting extensive future studies. New mosquito larval and adult intervention control strategies need to be explored and evaluated for their potential field implementations. The contribution of *An. stephensi* to malaria transmission in Africa requires further investigation, given its potential for undermining the gains made in reducing the burden of the disease. Therefore, there is an urgent need to contain the spread of this invasive species.

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Declaration of interests

The authors declare no competing interests.

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