Is outdoor-resting behaviour in malaria vectors consistent? Short report from northern Ghana.

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Abstract

Background

Recent reports of a change in the resting behaviour of malaria vectors, from predominantly indoor resting to outdoor resting following blood feeding, have been attributed to selection pressure from use of vector control tools such as indoor residual spraying and long-lasting insecticide-treated nets. Recent studies have observed vectors resting predominantly outdoors in settings where anti-vector tools are extensively deployed. This present study examined if the outdoor resting behaviour in the vector population, is random or indicative of a consistent preference of one resting site over the other.

Methods

Mark-release-recapture (MRR) experiments were conducted with outdoor-resting *Anopheles gambiae* and *An. funestus* mosquitoes collected from pit shelters, animal houses and granaries in two villages in Northern Ghana during rainy and dry seasons. Mosquitoes were marked with fluorescent dyes and released indoors. The experiments were controlled with indoor-resting mosquitoes, which were marked and released outdoors. Species of all recaptured mosquitoes were identified and assessed for consistency in their resting behaviour.

Results

A total of 4,460 outdoor-resting mosquitoes comprising *An. gambiae sensu lato (s.l.*) (2,630, 59%) and *An. funestus* complex (1,830, 41%) were marked and released. Overall, 31 (0.7%) mosquitoes were recaptured mostly from outdoor location comprising 25 (81%) *An. gambiae s.l.* and 6 (19%) *An. funestus* complex. Only 3 (10%) of the recaptured mosquitoes were found resting indoors where they were released. The majority of the outdoor-recaptured mosquitoes were *An. arabiensis* (11, 39%), followed by *An. coluzzii* (7, 25%); whereas all indoor-recaptured mosquitoes were *An. coluzzii*. For the control experiment, 324 indoor-resting mosquitoes constituting 313 (97%) *An. gambiae s.l.* and 11 (3%) *An. funestus* complex were marked and released. However, none of these was recaptured neither indoors nor outdoors. More mosquitoes were significantly captured and recaptured during rainy season ($Z = 6.579$, $P < 0.0001$).

Conclusions

The results obtained suggested the tendency for the mosquitoes to retain their outdoor-resting behaviour. Further investigations are required to ascertain if emerging preference for outdoor resting behaviour in malaria vector populations is consistent or a random occurrence.

Background

Vector control with indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) has contributed largely to reduction in malaria incidence and mortality [1]. These tools are both insecticide and indoor-based and target vectors that feed indoors (endophagic) and rest indoors (endophilic) [2–4]. However, recent reports are indicating that vector control tools are inducing behavioural changes in mosquitoes such that some vector species have shifted from indoor-resting behaviour to resting outdoors (exophilic). This was as documented from studies in Ethiopia [5], Ghana [6, 7], Kenya [8,9)], Libreville [10] and Tanzania [11, 12]. Such behavioural diversification in vector population can be detrimental to vector control [13, 14], which mainly target vectors resting indoors. Therefore, efforts to understand emergence of such behaviour are highly essential for elimination agenda.

Outdoor resting behaviour in vector populations may be triggered by heavy use of insecticide-based indoor intervention in settings where control tools are widely implemented [11, 15, 16]. Vectors in these settings avoid contact with insecticides and rest outdoors where no insecticide is used [11, 15–17]. Such behaviour promotes outdoor transmission as vectors can bite unprotected humans outdoors [11, 18].

Mark-release-recapture (MRR) studies are a standard experimental method extensively employed to investigate mosquito biology, ecology, life history and behaviour, measuring parameters including dispersal and flight distance; survival, population size and density, as well as blood feeding, host seeking and reproductive behaviour [19–22]. MRR experiments have been used particularly for field and laboratory studies involving mosquito species such as *Aedes* [23, 24], *Culex* [25, 26] and *Anopheles* [19, 20, 22]; to collect data highly relevant in understanding pathogen transmission, gene flow and development and optimization of vector intervention tools [20, 22, 27].

Northern Ghana has documented outdoor resting in *An. gambiae sensu lato (s.l.*) population [6, 7] but it is not clear whether this behavior only occurs randomly in the vector population. This present study examined if the preference for outdoor resting in the vector population was consistent or not. MRR experiments were conducted using field-collected mosquitoes of unknown age in two villages in northern Ghana, where coverage of IRS and LLINs are high [7, 28, 29].

Materials And Methods

Study sites

The MRR experiments were conducted in two villages in northern Ghana, Kpalsogu (9.33^0 N, 1.02^0 W) and Libga (9.35^0 N, 0.51^0 W) during the rainy season between July-November 2017 and dry season in December 2017-January 2018. The region has a unimodal rainfall pattern with monthly density between 150–250 mm and a forest vegetation zone with mean daily temperature ranging between 25–30°C; and an average relative humidity between 65–75% [30]. .

This region has previously documented malaria incidence rate of about 40% in under-five children [29, 31] and LLINs coverage is > 70% in both villages [7, 29]. Kpasolgu has been under IRS implementation since 2008 [7]. Extensive farming is practiced in both villages, occurring throughout the year as the villages are close to irrigation dams.

Mosquito collection

Live outdoor-resting adult female *Anopheles* mosquitoes were collected from pit shelters, granaries and animal resting shelters. The pit shelters were positioned about 5-10m outside six randomly selected compounds in each village. The mosquitoes were carefully collected using mouth and prokopack electrical aspirators so that they remain alive.

To compare the trend in behaviour of mosquitoes when resting indoors and also as a control for the experiments, indoor-resting mosquitoes were also collected from sleeping rooms in the compounds where the pit shelters were situated, using the protocols described above. Collections were carried out during the early mornings (05.00–07.00 hr).

Mosquito processing, marking and subsequent release.

Captured mosquitoes were immediately transported to the insectary for morphological identification as female *An. gambiae s.l.* and *An. funestus* complex [32]. Following identification, indoor and outdoor mosquitoes were carefully kept in separate cages in the insectary, irrespective of their abdominal status. The mosquitoes were fed with 10% glucose until experiment time at dusk of the same day between 18:00–19:00 hr.

The marking of mosquitoes was carried out 3 hours to release time. Batches of 15–20 mosquitoes from the same compounds were kept in the same paper cup. The outdoor-collected mosquitoes, designated as 'test' mosquitoes, were dusted with red fluorescent dye while the indoor mosquitoes, serving as control, were similarly marked with green dye. All mosquitoes were left in the cup to rest after marking, until when transported to the study sites for release.

Two compounds were selected near the pit traps for each release event. About 200 test mosquitoes were released in five sleeping rooms (indoors) in each of these compounds. The mosquitoes were released indoors to assess if they would remain indoors to rest or return outdoors where they were originally collected from.

The control mosquitoes were released outdoors; outside one of the selected compounds. Two release events were conducted for the control mosquitoes where about 150 specimens were released in each event. These mosquitoes were released outdoors to ascertain if an alternate trend in the resting behaviour would be demonstrated.

Recapture of released mosquitoes

The mosquitoes were left for 48 hours before recapture was initiated. This offered sufficient time for the mosquitoes to redistribute in their new environment and enabled us to assess if the mosquitoes opted to return to their initial collection location.

To recapture, mosquitoes were sought from sleeping rooms, animal houses, clay pots, granaries and pit traps deploying light traps, exit traps, pyrethrum spray catches as well as mouth and prokopak aspirations. All rooms and compounds in each of the study sites were sampled to recapture the released mosquitoes. Recapturing was done for 7 consecutive days before the next release event.

In all, twelve (12) rounds of MRR experiments were conducted for the test mosquitoes within the study period, with six experiments performed per study site. Most of the experiments during the rainy season were done at Kpalsogu, whereas most of those done in the dry season took place at Libga. Two control MRR experiments were also conducted, one each during rainy and dry season at Kpalsogu.

Processing and identification of recaptured mosquitoes

All recaptured mosquitoes were morphologically identified as marked and unmarked. Only the marked *Anopheles* mosquitoes were selected for analyses and assessed for change in resting behaviour. All unmarked mosquitoes were counted and recorded.

The marked recaptured mosquitoes were processed to discriminate the sibling members of *An. gambiae s.l.* and *An. funestus* complex. DNA was extracted from these mosquitoes and molecular species were genotyped using the protocols previously described [33, 34].

Ethical considerations

Ethical approval

was obtained from the Ghana Institutional Review Boards (IRB) of Noguchi Memorial Institute for Medical Research (NMIMR). Village heads were also engaged in discussions where concerns on release of mosquitoes into sleeping rooms were clarified. MRR experiment presents no potential risks as mosquitoes were released in the compounds where they were initially captured and a proportion was to be recaptured.

Data analysis

The number of recaptured mosquitoes was counted and proportion determined. The different release events were considered as replicates. Data from both study sites were merged together as there was no significant difference in the results obtained from the different replicates. Z-test was used to compare the difference in proportion of the recaptured mosquitoes between the rainy and dry season.

Results

Recaptured mosquitoes and their species

Overall, a total of 4,460 outdoor-resting test mosquitoes were released to indoor space, while 324 indoor-resting control mosquitoes were released outdoors. The morphological identification of test mosquitoes (outdoor) revealed that 2, 636 (59 %) were *An. gambiae s.l.* while 1, 824 (41%) were *An. funestus* complex (Figure 1). Whereas the control mosquitoes comprised 313 (97%) *An. gambiae s.l.* and 11 (3%) *An. funestus* complex.

Following all the 12 MRR experiments conducted, a total of 3, 950 *Anopheles* mosquitoes were collected from outdoor location, of which 31(0.8%) were marked and 3,919 (99.2%) were unmarked, including males and non-Anophelines. Subsequent analyses of the outcome of the MRR experiments were focused only on the marked mosquitoes that were recaptured. Recapture of the test mosquitoes was achieved from nine (9) out of twelve release experiments.

Thirty-one (31) marked test mosquitoes were recaptured from 4,460 released. This indicates a recapture rate of 0.7%. More mosquitoes (17, 55%) were recaptured from Kpalsogu during the rainy season than from Libga (14, 45%), where MRR was mostly done during the dry season. The difference in recapture rate between the study sites, as well as the rainy and dry seasons was significant ($Z=6.579$, $P<0.0001$).

The breakdown of the recaptured test mosquitoes comprised 25 (81%) *An. gambiae s.l.* and 6 (19%) *An. funestus* complex (Figure 1), from which 28 (90%) were resting outdoors, and 3 (10%) indoors. The majority of the mosquitoes recaptured outdoors were *An. arabiensis* (11, 39%), followed by *An. coluzzii* (7, 25%) and *An. funestus s.s.* (6, 21%). *An. gambiae s.s.* constituted the least outdoor-recaptured species (4, 15%) (Table 1). The three mosquitoes recaptured indoors were all identified as *An. coluzzii*. None of the control samples that were released outdoors was recaptured from any location, indoor or outdoor.

Table 1: Molecular species of the recaptured test mosquitoes

	# Recaptured	
Mosquito species	Indoor	Outdoor
An. arabiensis	0	11
An. coluzzii	3	7
An. gambiae s.s.	0	4
An. funestus s.s.	0	6

These were the marked test mosquitoes only, 28 of which were recaptured outdoors and 3 indoors

Discussion

Behavioural shift from predominantly indoor to outdoor-resting is becoming widespread in vector populations in settings where anti-vector vector interventions are extensively deployed [6, 15, 16, 35, 36]. The aim of this study was to examine if outdoor resting behaviour of the malaria vector populations described in the study settings [6, 7] was consistent or it was just a random occurrence. The study observed that among the test mosquitoes recaptured, the majority was collected outdoor. This suggested that these mosquitoes returned outdoors to rest after being released indoors. This pattern of preference for outdoor resting in mosquitoes was consistently observed from nine out of the twelve experiments, despite a low recapture rate. As outdoor resting behaviour in vector population could counteract control efforts [18, 37, 38], research assessing the extent of this behaviour is well-timed and necessary to guide decision making in malaria control programmes.

Switch to outdoor-resting behaviour in the vectors may be as a result of avoidance behaviour from indoor interventions [38, 39], which were heavily used in the study sites. Most sleeping rooms had LLINs and they were also sprayed with pirimiphos methyl, the insecticide that was being used for IRS at the time of the experiments [7]. These mosquitoes might be avoiding contact with the insecticide indoors and chose to rest outdoors, where they may be safe, as there was no outdoor intervention in the villages. This could have also accounted for the predominant outdoor resting preference documented in the vectors in a previous study from this setting [6] and others all over Africa [6, 8, 12, 36, 37], which prompted this investigation.

The highly endophilic vectors: *An. gambiae s.s., An. coluzzii* and *An. funestus s.s.* were recaptured outdoors after being released indoors. This suggests a likely switch to outdoor resting behavior because these vector species may be avoiding contact with insecticide indoors and exiting to rest outdoors [40–42]. Change from endophily to exophily in response to control intervention is increasingly being documented in these highly endophilic vectors [8, 12, 16, 35, 43, 44]. This could have negative impact on malaria control as it can promote outdoor and residual transmission in these settings [11, 38, 45].

Anopheles arabiensis was found to predominate the mosquito species found resting outdoors from the recaptured test mosquitoes. This may be because *An. arabiensis* is known to be highly exophilic and zoophilic (preference for animal blood meal) [46–48], where it tends to stays outdoors to rest and feed on animals when there is intervention indoors [4, 49]. This vector species has also been observed to display insecticide avoidance and early-exiting behaviour [12, 18, 42], which make them difficult to control [11]

In this study, the recapture rate was much lower than most MRR studies reported. Several reasons could have accounted for this. One of such reasons is that the intervention indoors could have could have killed the mosquitoes while attempting to rest post-feeding [50, 51]. Another reason is the age of mosquitoes [20, 22, 52], which was unknown in this study. Plausibly, if the mosquitoes were old, they may have died within the period of recapture. Indeed, senescence is a factor associated with reduction of mosquito daily survival in the wild [53, 54]. Age was also previously suggested to be responsible for the low recapture rate in a similar experiment [20]. Moreover, predators such as spiders were particularly common in the study areas, and were found inside animal houses that provide favourable environment for the mosquitoes to rest. Likewise, as some of the released mosquitoes were blood fed as well as semi-gravid and gravid, they might divert to seeking favorable oviposition spots which naturally occurs in the night. These mosquitoes may not return to rest within the same locality or might have been exposed to predators or died naturally. Other factors including emigration from the study area, climate condition, stress and negative effect of the experimental procedures could have also contributed to this low recapture success as previously documented in other MRR experiments [20, 22, 27].

The study also recaptured more mosquitoes during the rainy season relative to the dry season. This might be because more mosquitoes were released during this rainy season as expected. However, none of the control mosquitoes was recaptured throughout the experiments. The possible explanations could be that the mosquitoes might have died due to the residual effect of insecticides [50, 51] from their initial contact before collection. It may also be due to predators attack and other factors as suggested above.

Conclusions

This study suggests that preference for outdoor resting in malaria mosquitoes may be an emerging consistent behaviour. There is a need for further studies to establish this observation in settings where interventions are extensively deployed. Furthermore, a probe into the genetic basis underlying this behavioural change will also be highly essential. This is important as malaria control moves to the elimination phase in sub-Saharan African countries.

Abbreviations

MRR: Mark, release and recapture

DNA: Deoxyribonucleic Acid

IRS: Indoor residual spraying

LLINs: Long-lasting insecticidal nets

SIT : Sterile insect techniques

s.l.: *sensu lato*

s.s: *sensu stricto*

Declarations

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Authors' contributions

MHA designed, performed the field and laboratory work, analyzed data and drafted the manuscript. YAA conceived and supervised the study, analyzed data and revised the manuscript. AAN and DN supervised study and revised the manuscript. IS, ARM and OKA performed field and laboratory experiments.

Competing Interests

The authors have declared that no competing interests exist.

Ethical Approval

Ethical approval was given by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. Verbal consent was also obtained from village heads before all field work.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are presented within the paper. No supporting information is available.

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Figures

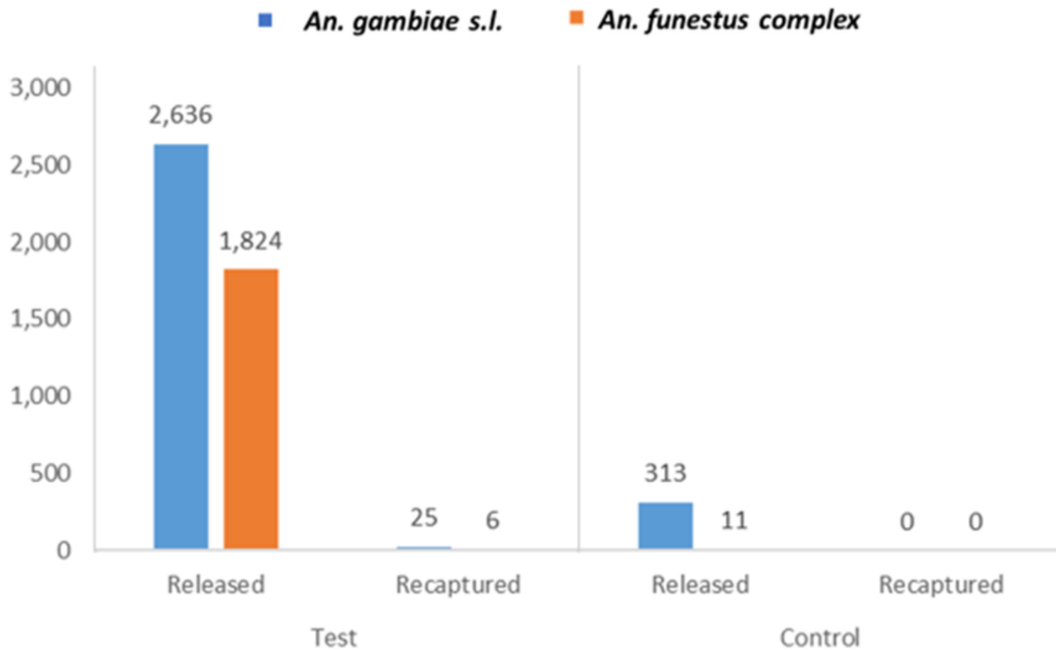

Figure 1

Composition of released and recaptured marked test and control mosquitoes "Test" (left) are mosquitoes collected from outdoor and released indoors, whereas "Control" (right) are those mosquitoes collected from indoor and released outdoors. "Test" mosquitoes are the mosquitoes being investigated for consistency or randomness in resting behaviour. "Control" mosquitoes are the counterpart used to confirm alternate trend in the behaviour of the “test” mosquitoes.