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Impact of visual features on capture of *Aedes aegypti* with host decoy traps (HDT)

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Running head: Visual cues in mosquito trap performance

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Abstract

The host decoy trap (HDT) is a surveillance trap that presents a combination of heat, visual, and odor stimuli to attract bloodmeal-seeking mosquitoes. Here we employed a semi-field study to

25 demonstrate the role of the visual attributes present on the HDT on the effectiveness of *Aedes aegypti*
26 capture. Our results show that the HDT is an effective means of capturing *Ae. aegypti* mosquitoes in
27 semi-field conditions, with a per trial capture rate of up to 69% across four visually distinct HDTs. The
28 solid black colored HDT captured more mosquitoes than HDTs with black-white stripes, black-white
29 checkerboard patches, or solid white color by a factor of 1.9, 1.7, and 1.5 respectively. In all cases,
30 mosquito capture was not evenly distributed on the HDT surface, with captures on the HDT's outer
31 downwind half, away from the odor delivery, exceeding captures on the inner upwind half. We
32 conclude that the solid black surface of the original HDT design is more effective than the other
33 surfaces (white or black/white patterns) for the capture of *Ae. aegypti*. Our results demonstrate that
34 mosquito attraction to the thermal and odorant cues of the HDT is modulated by visual information.

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Introduction

38 The oppressive human diseases of yellow fever, dengue fever, chikungunya, and Zika are all
39 vectored by the *Aedes aegypti* mosquito, creating a heavy disease burden in *Ae. aegypti*-endemic
40 regions (Bhatt *et al.*, 2013; Musso *et al.*, 2015). Control of *Ae. aegypti* and other vector species include
41 insecticide-treated nets (ITN), indoor-residual spraying (IRS), and larvicide techniques. Interventions
42 such as these knock down local mosquito populations, thereby curbing the transmission of a number of
43 mosquito-borne diseases (Hawley *et al.*, 2003; Mabaso *et al.*, 2004; Weeratunga *et al.*, 2017; Che-
44 Mendoza *et al.*, 2018).

45 *Aedes aegypti*'s geographical distribution necessarily informs the application of vector control.

46 *Aedes* species traditionally were considered to propagate in tropical and subtropical climates but

47 recently have expanded into temperate regions (Kraemer *et al.*, 2015). Global climate change and

48 exploding rates of transcontinental human travel and interconnectedness could benefit *Ae. aegypti* and
49 its propagation worldwide, necessitating diligent vector surveillance and refinement of distribution
50 maps (Brown PT; Glaesser *et al.*, 2017).

51 Effective surveillance traps apply principles of *Ae. aegypti*'s navigation, oviposition, and host-
52 seeking mechanisms. With respect to female host-seeking, laboratory studies have described the effects
53 of a range of sensory stimuli on host-seeking behaviors. The major contributor to long-range host
54 detection is the olfactory system, with CO₂ plumes originating from hosts dictating the flight direction
55 of *Ae. aegypti* females from a distance (Carde, 2015). Once the mosquitoes have closed the distance to
56 the source of stimuli, however, visual and thermal cues begin to play a significant role, with dark,
57 warm, and visually contrasting objects being most attractive to the *Ae. aegypti* females (Wood &
58 Wright, 1968; Muir *et al.*, 1992; van Breugel *et al.*, 2015). Moreover, studies have shown that these
59 cues act in concert. For instance, CO₂ detection is a prerequisite for female mosquitoes' responses to
60 host-derived odors such as lactic acid (McMeniman *et al.*, 2014), whereas the detection of dark visual
61 cues is a prerequisite for thermotaxis (Liu & Vosshall, 2019). Although male *Ae. aegypti* do not exhibit
62 host-seeking drives, their ability to navigate towards a target has been shown to be influenced by a
63 similar set of stimuli, including a positive correlation with the visual complexity of the target's
64 environment (Staunton *et al.*, 2020).

65 Surveillance traps make use of multiple sensory cues relevant to the behavior of target
66 mosquitoes that is induced by particular physiological statuses. One such trap, the host decoy trap
67 (HDT), primarily targets female mosquitoes seeking human targets for bloodmeals. The HDT, in its
68 original design, is a black cylindrical structure presenting a warm surface and the odors of a live host
69 to mosquitoes. The surface of the HDT is adhesive, allowing mosquitoes to be captured if they land on
70 the HDT. The effectiveness of the HDT has been established for the capture of the malaria mosquito

71 *Anopheles gambiae* s.l., as well as *Mansonia* and *Culex* species (Abong'o *et al.*, 2018). Iyaloo, et al.
72 (Iyaloo *et al.*, 2017) demonstrated *Aedes albopictus* are captured by a different multisensory
73 cylindrical trap, the BG-Sentinel. In the latter study, traps composed of black cylinders and black lids
74 were shown to capture more mosquitoes than traps containing white cylinders/lids and traps presenting
75 contrasting black and white colors on the cylinder and lids.

76 In this study, we evaluated both the effectiveness of the HDT as a means of capturing *Ae.*
77 *aegypti* and the effect of different visual stimuli on the HDT surface in attracting *Ae. aegypti*. To
78 determine this, we compared the capture rates of four HDTs with varying visual appearances. These
79 alterations included a solid black surface, a black-white striped surface, a black-white checkerboard
80 surface, and a solid white surface. In doing so, this study demonstrates the importance of visual cues in
81 the host-seeking behavior of *Ae. aegypti*.

82

83

Materials and Methods

84 **Study Site.** This study was conducted during May and June 2019, using semi-field screen house arenas
85 at the Kisian Campus, Centre for Global Health Research (CGHR) of the Kenya Medical Research
86 Institute (KEMRI) in Kisian, Kisumu County, Kenya.

87 **Setup of Host Decoy Traps.** All HDTs used in this study were manufactured by BioGents AG and
88 used primarily as described in Abong'o *et al.* (2018) with the following modifications. In addition to
89 the original solid black HDT, we employed HDTs with a solid white surface (HDT W), a striped
90 pattern consisting of evenly alternating black and white stripes each of 6 cm width (HDT S), and a
91 checkerboard pattern consisting of 6 cm x 6 cm alternating black and white square patches (HDT P).
92 To create HDT S and P, the black areas were printed onto the white vinyl fabric, and the fabric
93 wrapped around, and also placed on the upper surface, of each of the drums (Figure 1A). HDT W was

94 created using the same white vinyl fabric. The sides of all four drums were then wrapped with
95 transparent adhesive plastic sheets (FICS Film, Barrettine Environmental Health, Bristol, UK) so that
96 identical tactile surfaces able to capture mosquitoes were present on all HDTs.

97 *Semi-field arena.* The arena used in this study was constructed using untreated Optinet netting
98 material permeable to air and draped over a 20 m long x 8 m wide x 3 m high galvanized steel frame.
99 Figure 1B depicts the arrangement of PVC pipes and connectors directing airflow to the four HDTs in
100 the arena. To ensure that the sensory cues of each HDT were as spatially distinct as possible, the traps
101 were symmetrically positioned such that the distance between each pair of HDTs was at least 4 m, and
102 that the distance between the HDTs and the arena walls was at least 2 m. Mosquitoes were released
103 from the center of the arena, equidistant from each of the HDTs. Due to the terrain over which the
104 semi-field arena was constructed, the four corners of the arena possessed different vegetation cover and
105 soil types. Moreover, the direction of the sunset cast varying levels of light intensity upon each trap
106 position. To minimize the impact of these and other variables on observed capture rates, we employed
107 a Latin square design in which all four HDTs were systematically rotated to different positions for each
108 trial such that each HDT was placed at each position the same number of times.

109 Natural host odors were transferred to the HDTs from a ventilated canvas tent (Pop Up Tent,
110 Sports God) 2 m outside the arena (Figure 1C). The same human volunteer sat in the tent for the
111 duration of each of the 16 trials conducted. A high-speed 12 V, DC fan (Delta) powered by a 12 V, DC
112 7 ah rechargeable lead acid batteries (ExpertPower) was attached via duct tape to a PVC pipe (10 cm in
113 diameter). This end of the pipe was placed inside the tent. The PVC pipe was directed into the arena
114 and sealed at its entry point with cement. Each of the four pipe exit points was sealed with untreated
115 mosquito netting. An HDT unit was positioned 10 cm away from each opening to allow odors and CO₂
116 from the tent to ventilate over each HDT. The 12 V fan provided a wind speed of approximately 1.26

117 m/s at each exit point, delivering approximately 600 l/min of human odors to each HDT. These
118 parameters were set to approximate those used by Abong'o et al. (Abong'o *et al.*, 2018).

119 ***Mosquito Rearing.*** *Aedes aegypti* mosquitoes present at sites around Kisian, Kisumu County were
120 captured using ovitraps and used to establish a breeding colony within the rearing facility. Larvae were
121 fed on Super Brewers Yeast Tablets (Pharmadass Ltd., Healthaid House, Marlborough Hill, Harrow,
122 Middlesex, HA1 1UD, United Kingdom) ground into powder and deposited in water as required. The
123 resulting F1 male and female adults were fed a 10% solution of sugar. These adults were aged for five
124 days to provide an opportunity for females to mate. The sugar solution was removed 6 hours prior to
125 the collection of females.

126 ***Trial Operation and Data Collection.*** KEMRI insectary staff maintained production of adult mosquito
127 rates sufficient to provide 200 female mosquitoes per trial, the same number used in previous semi-
128 field studies (Dugassa *et al.*, 2014; Batista *et al.*, 2017). One hundred females were aspirated into each
129 of two paper cups (a total of 200) before each experimental trial and transported to the arena. Each
130 HDT was heated with hot water as described by Abong'o et al. (2018). At the start of each trial, the
131 temperature of the HDT surface, the wind speeds at pipe exit points, the light intensity in the arena, and
132 the arena's temperature and humidity were measured and recorded using an infrared spot thermometer,
133 anemometer, photometer, and a temperature-humidity sensor respectively. The mosquitoes were then
134 released from the center of the screen house (Figure 1C). To align with the day-biting behavior of *Ae.*
135 *aegypti*, each trial began between 12:00 h and 13:00 h, and ended at 18:00 h, lasting a total of 5-6
136 hours. The same set of HDT-specific and environmental parameters were re-measured at the
137 conclusion of each trial. Mosquitoes not captured on the HDTs during the trials were collected from the
138 arena using a battery-powered aspirator (Prokopack Model 1419, The John W. Hock Company).

139 At the end of each trial, the adhesive sheet on the surface of each HDT was wrapped with
140 plastic food wrap, sandwiching the captured mosquitoes between the adhesive sheet and the wrap. The
141 adhesive sheets were labeled using permanent marker to denote the HDT type, the position of the black
142 and white regions on the striped and patched HDTs, and the location of the pipe outlet.

143 **Data Analysis.** Mosquitoes captured on the HDT adhesive sheets and recaptured from the arena were
144 killed in a -20°C freezer overnight. The number of captured mosquitoes on each HDT type were then
145 counted. For all adhesive sheets, the count of mosquitoes on each HDT's inner half (facing the pipe
146 outlet) and outer half (facing away from the pipe outlet) was determined. For HDTs S and P, the count
147 of mosquitoes captured on their black and white regions was also determined.

148 The data were analyzed using SPSS Statistics software, version 26.0.0. Confounding variables
149 were assessed via ANOVA (analysis of variance) and Kruskal-Wallis analyses. Linear regression
150 assessed the effect of ambient environmental variables, odor wind speeds, and HDT surface
151 temperatures on the catch of mosquitoes on HDTs. To address the primary research question (i.e.,
152 which HDT type had the highest capture rate), χ^2 goodness of fit analysis determined if differences in
153 capture rates between the HDT types were statistically significant. Then, pairwise post hoc
154 comparisons between the traps were conducted among pairs of HDT types to determine the source of
155 the capture rate incongruence, and thus statistical differences between HDT types. Similar pairwise
156 comparisons were made to determine whether there were landing preferences on the black and white
157 regions of the striped and patched HDTs (S and P). An independent samples comparison was used to
158 assess mosquito preference for landing on the outer half of the HDT surface.

159

160

Results

161 ***HDT B is more effective at capturing Ae. aegypti females than HDTs S, P, and W.*** Table 1
162 summarizes the capture data from the 16 replicates of this study. Of the total of 3200 released
163 mosquitoes, 1096 (34%) were captured by the HDTs. χ^2 goodness of fit analysis of the capture counts
164 of the four HDT types revealed that there is a significant overall difference between the HDT types'
165 mean capture rates ($\chi^2 = 77.82, p = 9.014 \times 10^{-17}$), and 11 out of 16 χ^2 GOF tests on individual
166 trials revealed significant differences in the HDTs' capture rates (Table 1).

167 HDT B captured the most mosquitoes, with a mean of 24.81 per trial (95% CI: 18.54-31.09)
168 compared to the 12.81 captured by HDT S (95% CI: 9.14-16.49), the 14.81 by HDT P (95% CI: 10.49-
169 19.13), and the 16.06 by HDT W (95% CI: 12.44-19.69) (Figure 2A). Paired analyses of differences in
170 captures amongst HDT types in each trial revealed that HDT B's capture counts significantly exceeded
171 those of HDTs S, P, and W by a mean difference of 12 (95% CI: 6.6-17.4), 10 (95% CI: 5.3-14.7), and
172 8.75 (95% CI: 2.7-14.8) respectively (Figure 2B). These analyses revealed no significant differences
173 between the mean capture counts per trial between HDTs S, P, and W (Figure 2B).

174

175 ***Aedes aegypti showed no preference for landing on black areas of HDT S and HDT P.*** We
176 separately counted the captures on the black and white areas of HDTs S and P to determine if *Aedes*
177 mosquitoes showed a preference for landing on one of these surfaces. χ^2 goodness of fit analysis of
178 HDT P's capture counts on the black patches (84 captured) and white patches (85 captured) indicated
179 no significant difference in captures ($\chi^2 = 0.0059, p = 0.9387$). The same analysis performed on
180 HDT S showed a modest trend towards increased capture within the black stripes (133 captured)
181 relative to the white stripes (107 captured) but this difference was not statistically significant ($\chi^2 =$
182 2.8167, $p = 0.0933$).

183

184 ***Ae. aegypti* prefers to land on the outer half of the HDT.** During post-trial analysis, the location of
185 each captured mosquito was categorized by whether they landed on the inner, facing the odor delivery,
186 or outer half of the HDT that faced away from the odor delivery site (Figure 3A). Trials 1 and 3 were
187 excluded from this analysis due to heavy rain that washed away the labels on the sticky sheets. In the
188 remaining 14 trials, the distribution of *Ae. aegypti* landings on the HDTs' surfaces indicated significant
189 skew towards the outer half landings compared to the inner half landings for each HDT type (Figure
190 3B). The inner halves of HDTs B, S, P, and W caught an average of 4.36, 3.36, 2.94, and 2.79
191 mosquitoes respectively, whereas the outer halves of B, S, P, and W caught 20.79, 12.50, 10.86, and
192 14.29 respectively. In total, capture on the outer halves was significantly greater than capture on the
193 inner halves ((11.25 mosquitoes/trial, 95% CI: 9.05 – 13.45, $p < 0.0001$). A similar analysis
194 performed individually for each HDT type also showed a significant excess of captures on the outer
195 halves.

196
197 ***Capture of Ae. aegypti by HDTs was not significantly affected by environmental variables.*** To
198 determine if the position of the HDT within the screen house influenced capture rates, the data for all
199 HDT types were stratified by location (Table 2). When no distinction was made with respect to the
200 HDT type at each position, the differences in captures at each position was not significant ($F =$
201 $2.185, p = 0.099$). For three of the four HDT types, differences in capture rate based on position in the
202 screen house showed no statistical significance. The fourth, HDT S, did show a significant difference
203 in capture between positions 2 and 3, the positions closest to the entrance of the arena. However, given
204 the lack of statistical significance for all other tests involving positions 2 and 3, this finding was not
205 expected to alter conclusions regarding the mosquito's attraction to HDT trap type.

206 We found no evidence that ambient light intensity, temperature, and humidity conditions
207 influenced the total mosquito capture during the 16 trials. Linear regression analysis indicates no
208 significant correlation between a trial's total HDT capture and: (1) the mean light intensity ($p =$
209 0.697), (2) the trial's mean ambient temperature ($p = 0.669$), and (3) the mean relative humidity ($p =$
210 0.521) respectively. Similarly, a lack of significant correlation was observed with regression analyses
211 of the values of each of these environmental variables at the beginning and end of each trial. Moreover,
212 for each HDT type, no correlation was observed between capture counts and the mean surface
213 temperatures ($p = 0.157$) and wind speed ($p = 0.853$) at the pipe exit points.

214

215

Discussion

216 The goal of this study was to characterize the impact of altering visual characteristics on the
217 landing frequency of *Ae. aegypti* females. The HDT paradigm allows for a host mimic target to present
218 variations in the parameters of host-associated stimuli. The original HDT design outlined in Hawkes et
219 al. (Hawkes *et al.*, 2018) used a solid black surface color. This choice is supported by laboratory
220 studies that point to *Ae. aegypti*'s visual preferences. Muir et al. (1992) demonstrated *Ae. aegypti*'s
221 preference for landing on dark targets. More recent studies uncovered details on the interactions
222 between different types of stimuli. Van Breugel et al. (2015) demonstrated that CO₂ detection activates
223 a strong attraction for dark targets on a light background. Liu & Vosshall (2019) also showed *Ae.*
224 *aegypti*'s preference for landing on a single dark spot on light background in the presence of CO₂.
225 These studies suggested that contrasting surface features might enhance the HDT's effectiveness. To
226 test this, we designed HDTs having different visual features but possessing identical nonvisual cues
227 (detectable heat signature, host odors, and CO₂) known to attract *Ae. aegypti* (van Breugel *et al.*, 2015;
228 Liu & Vosshall, 2019).

229

230 ***The original black HDT showed the highest capture rates.*** Our results indicated that the black surface
231 was more effective than either the white surface or the black/white contrasting surfaces of the S and P
232 HDTs. Importantly, these results establish that the *Ae. aegypti* attraction to the HDT is influenced by
233 the visual attributes of the trap. However, the results are contrary to our expectation in that the S and P
234 HDTs would improve the HDT's capture rate. These two HDTs were outperformed by both HDT B and
235 HDT W. One consideration is that the width of the stripes and patches were set at 6 cm on the S and P
236 HDTs. *Ae. aegypti* requires a minimum optical angle of between 4° and 8° to perceive distinct objects
237 (Bidlingmayer, 1994). At a distance of 1 m, the angle of perception of a 6 cm wide stripe is 3.4°. For
238 this reason, the black/white regions of these traps are not likely to be visible to a mosquito until they
239 are less than one meter away from the trap. At greater distances, the solid black trap would present the
240 strongest contrast to the surrounding environment than the other HDTs. Thus, it is possible that HDT
241 performance could be improved by increasing the size of the contrasting black and white regions so
242 that *Ae. aegypti* could discern the contrasting surface of the HDT at greater distances.

243

244 ***Mosquito capture was not evenly distributed on the HDT.*** In this study we found a greater
245 concentration of mosquitoes captured on the HDTs outer half, facing away from the odor delivery
246 pipe. This suggests that the three-dimensional surface of the HDT was not a uniform surface for
247 capture. Clearly there was a nonuniform distribution of odors across the surface of the HDTs since the
248 odors were vented from a single exit point directed toward the HDT. Because odors were deposited at
249 10 cm away from the base of the HDT, the surface of the HDT facing the exit point received the odor
250 immediately as it was dispensed from the pipe. An important consideration is that the odor was vented
251 from the pipe at a speed of approximately $1.26 \pm 0.18 \text{ m}^{\text{s}}$, which may have created sufficient

252 directional air flow to induce upwind flight in *Ae. aegypti*. Geier *et al.* (1999) showed that *A. aegypti*
253 increase their upwind flight activity upon exposure to plumes of both CO₂ and host odors both
254 independently and in combination. Because odor delivery through the pipe creates a wind current,
255 mosquitoes would have first encountered the HDT surface facing away from the pipe due to this
256 tendency to fly upwind while tracking odor plumes. Additionally, the wind velocity resulting from
257 airflow out of the pipe may have limited the ability of the mosquito to reach the surface facing the
258 pipe. Further investigation of the dynamics of airflow onto and around the HDT's cylindrical drum
259 may illuminate these mechanisms as well as alternative methods of odor delivery to improve overall
260 capture rates.

261

262 ***The HDT is a useful tool for both mosquito surveillance and behavioral studies.*** This study
263 investigated the role of visual stimuli on HDT capture of female *Ae. aegypti* likely engaged in host-
264 seeking behavior. The compact materials of the HDT allows it to be transported to the vicinities of
265 remote human communities with difficult terrains and climates where surveillance of mosquito
266 prevalence is most lacking, and little supervision is required once set up is complete. Data provided by
267 the HDT can effectively serve as important indicators of regional species prevalence and host
268 preferences and will contribute to further refinements of probability models of *Ae. aegypti* and other
269 species' geographical distributions (Kraemer *et al.*, 2015). The importance of such models was
270 demonstrated in a study that aligned the known distributions of several *Aedes* species throughout
271 Thailand and the distributions of human incidences of dengue, chikungunya, and Zika infections
272 (Suwanmanee *et al.*, 2018). Continuously updating information on vector prevalence is crucial to
273 combatting short-term disease outbreaks and to observing long-term trends in geographical shifts in
274 vector species distribution.

275 This study further documents the potential value of the HDT as an experimental tool in field
276 settings. In terms of capture effectiveness, cattle-baited HDTs were demonstrated to capture
277 significantly more *Anopheles* mosquitoes in the wild than HLCs, though further studies must take
278 place to replicate this finding for *A. aegypti* and human hosts. More importantly, in terms of design,
279 HDTs have the intrinsic advantage over HLCs in that they present negligible risk of mosquito bites to
280 researchers. The properties of the HDTs can also be modified to present different stimuli that may arise
281 from hosts, manipulating experimental variables related to thermal, olfactory and visual properties of
282 hosts so that the effect of these properties on host-seeking behavior of field populations can be studied.
283 We have demonstrated the HDT's ability to safely and effectively capture *Ae. aegypti* females,
284 extending the work of Abong'o et al. (2018) showing effective capture of *Anopheles* and *Culex* species
285 in field settings.

286

287 ***The presence of attractive visual cues on traps can contribute to mosquito control.*** Many current
288 vector control methods such as insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS),
289 though effective at hampering human contact with mosquitoes, do not eliminate mosquito populations
290 (van den Berg, 2011). The sustained use of insecticides consistently leads to mosquito resistance
291 (Barrera *et al.*, 2013; Moyes *et al.*, 2017; Dusfour *et al.*, 2019). Mosquito surveillance traps could be
292 adapted as alternative vector control tool. The BG-Sentinel, for example, is a multisensory surveillance
293 trap that was shown to be effective at controlling populations of *Ae. mediovittatus*, a mosquito vector
294 native to the Caribbean (Barrera *et al.*, 2013). Moreover, with regards to its visual design, Iyaloo et al.
295 (Iyaloo *et al.*, 2017) demonstrated *Ae. albopictus*' preference for landing on a solid black BGS over
296 other black-white surface patterns. Similarly, optimizing the HDT's visual design could be an avenue
297 of introducing it as a vector control tool. This study's findings constitute a step towards not only

298 optimizing the visual design of the HDT, but also elucidating general patterns of vision-based behavior
299 that can inform the design of novel vector control methods. Ultimately, any vector control methods that
300 take advantage of sensory cues instead of or in addition to the traditional chemical cues would
301 diversify the set of stimuli that place selective pressure on mosquito evolution and mitigate the
302 development of resistance to vector control (Amelia-Yap *et al.*, 2018).

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Data Availability Statement

305 The raw data that were reported as averages in this study are available from the corresponding author
306 upon reasonable request.

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Table 1.

HDT type	Black (B)	Striped (S)	Patched (P)	White (W)	Total captured on HDTs	χ^2	p-value
Trial 1	14	2	2	4	22	18.00	<0.001
Trial 2	40	17	16	17	90	18.18	<0.001
Trial 3	31	10	12	14	67	16.64	0.001
Trial 4	16	10	15	10	51	2.41	0.491
Trial 5	9	6	7	16	38	6.42	0.093
Trial 6	11	18	17	22	68	2.11	0.302
Trial 7	37	10	22	16	87	11.67	<0.001
Trial 8	36	30	12	17	95	6.32	0.001
Trial 9	18	14	9	17	57	3.38	0.337
Trial 10	20	11	15	19	65	3.12	0.373
Trial 11	24	4	15	10	52	16.21	0.001
Trial 12	32	18	16	14	80	10.00	0.019
Trial 13	11	10	5	23	49	14.27	0.003
Trial 14	49	19	36	34	138	13.13	0.004
Trial 15	23	9	25	15	72	9.11	0.028
Trial 16	26	17	13	9	65	9.77	0.021
Mean	24.81	12.81	14.81	16.06	68.5	78.64	<0.001
Total	397	205	237	257	1096		

396

397 **Table 1.** Compilation and χ^2 GOF statistical analysis* of mosquito capture data in the 16 trials of the
398 study.

399 *11 (bolded) out of 16 demonstrate significant difference in capture counts between the four HDTs.

400 All tests were performed with three degrees of freedom, with a null hypothesis of expected counts

401 being equal for all four HDT types.

402

403 **Table 2.**

Trap Position	Black (B)	Striped (S)	Patched (P)	White (W)	Total
1	52	65	56	55	228
2	120	33	29	50	232
3	115	75	84	60	334
4	110	32	74	92	308
Total	397	205	237	257	1096
Statistical Significance	No (p = 0.112)	Yes (p = 0.031)	No (p = 0.149)	No (p = 0.107)	

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405

406 **Table 2.** Mosquito capture rates analyzed* with respect to position of the HDTs in the screen house.

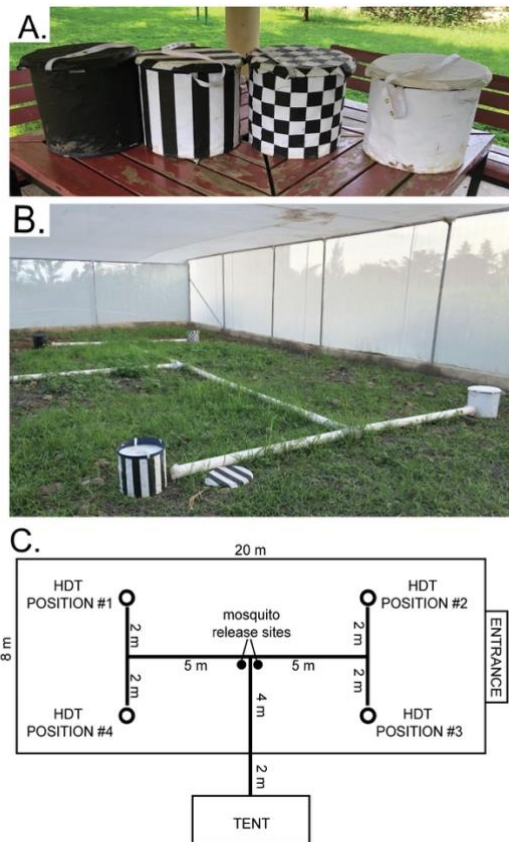
407

408 *Two different statistical tests were used to determine if position was responsible for differences in
 409 capture rates. For HDT S and HDT W, because variances were equal at the four positions, ANOVA
 410 analysis was used, while for HDT B and HDT P, where the variances were unequal, Kruskal-Wallis
 411 was employed. The statistical analysis showed that only the HDT S data showed significant differences
 412 in capture due to trap position. Post-hoc tests determined that this difference was due to the HDT S
 413 data collected from positions 2 and 3.

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416 **Figures and Legends**



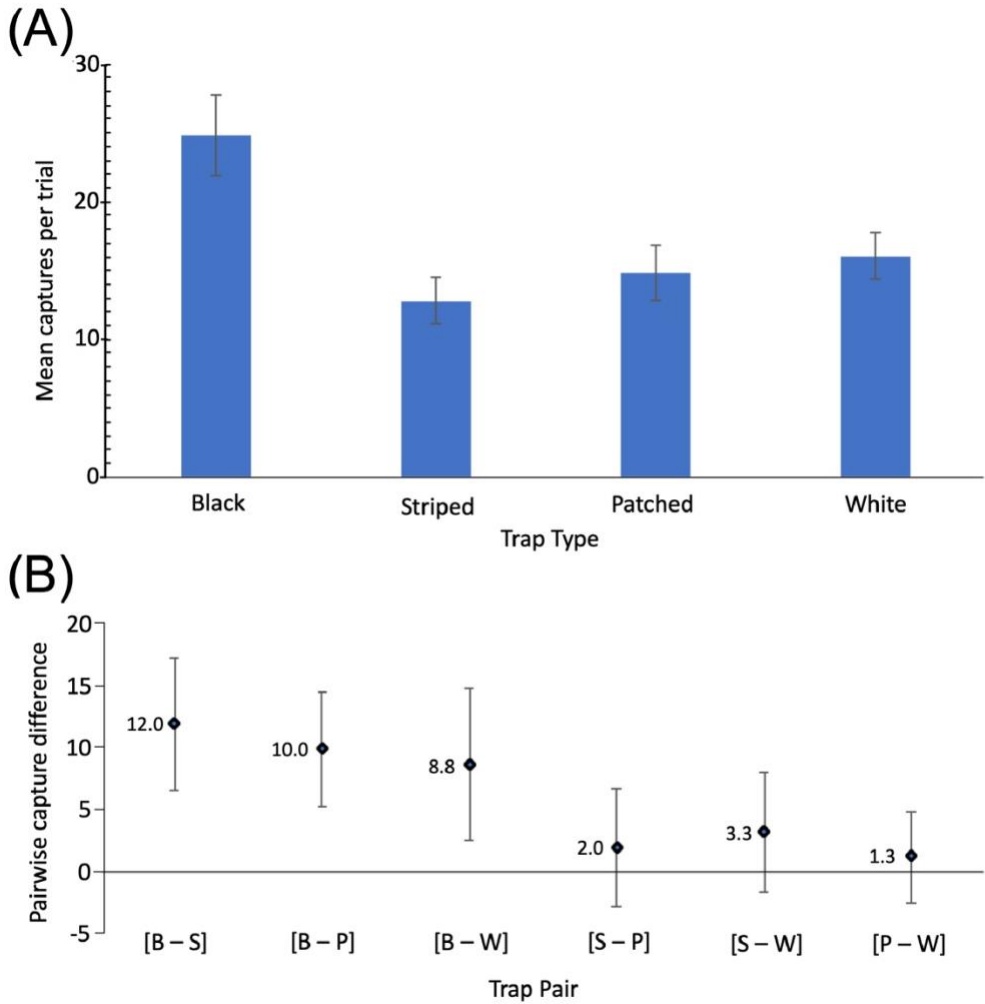
417 **Figure 1.**

418 **Figure 1.** Visual features on the HDTs and layout of the experimental facility.

419 (A) Test traps showing the visual feature designs for (left to right) the black, striped, patched and white
420 HDTs.

421 (B) Example arena set up of the four HDTs and the pipework delivering the human odors from the tent
422 to each of the HDTs.

423 (C) Diagram showing the dimensions of the screen house and other elements of the experimental set
424 up. A North arrow indicates the approximate cardinal orientation of the arena. The pipework delivers
425 odors from a human subject residing in the tent to symmetrically placed HDTs within the screen house.



426

Figure 2.

427

Figure 2. Comparisons between mean capture rates of HDTs B, S, P, and W.

428

(A) Mean capture rates of each HDT type. Error bars indicate standard deviation.

429

(B) Pairwise differences in mean capture by trial between each pair of HDTs depicting the 95%

430

confidence bands. Bands that do not include 0 indicate with 95% confidence that there is a non-zero

431

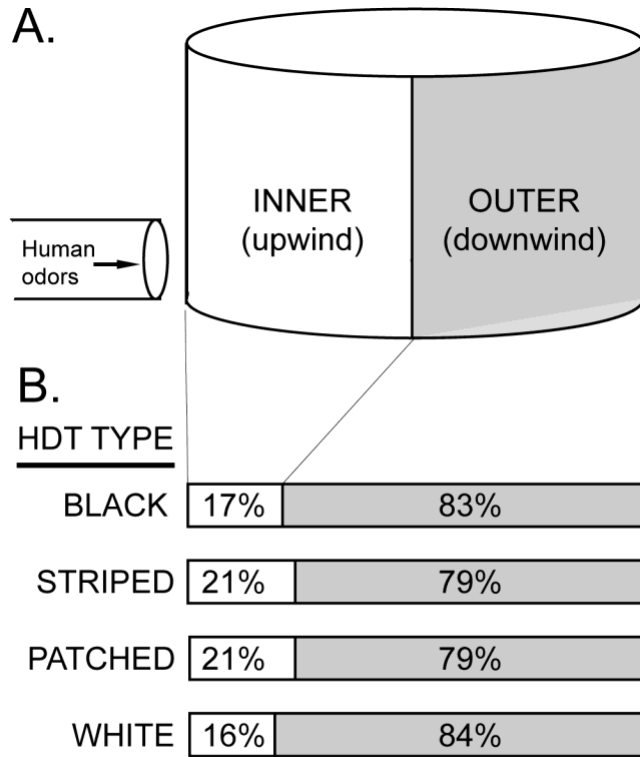
difference in captures between the pair of HDT types, i.e. a statistically significant difference. By this

432

metric, HDT B captured significantly more mosquitoes than the other three DHT types, while the

433

differences between HDTs S, P, and W are not significant.



434 Figure 3.

435 **Figure 3.** Comparisons of mosquito capture on outer and inner halves of the HDTs.

436 (A) An illustration designating the inner and outer halves of the HDTs. The terms upwind and
 437 downwind denote the direction of the wind currents generated by odor delivery through the pipe.

438 (B) The percent capture determined for the inner and outer surfaces of each HDT type.

439 **Abbreviations**

440 KEMRI: Kenya Medical Research Institute

441 CGHR: Centre for Global Health Research

442 PVC: polyvinyl chloride

443 HDT: host decoy trap

444 HDT B: solid black HDT

445 HDT S: black/white striped HDT

446 HDT P: black/white patched HDT

447 HDT W: solid white HDT

448 HLC: human landing catch

449 χ^2 GOF: chi-square goodness of fit statistical test

450 RNA: ribonucleic acid

451 BGS: Biogents-Sentinel trap

452 ITN: insecticide-treated bed nets

453 IRS: indoor residual spraying

454 ANOVA: analysis of variance