Barrier bednets target malaria vectors and expand the range of usable insecticides

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Transmission of *Plasmodium falciparum* malaria parasites occurs when nocturnal *Anopheles* mosquito vectors feed on human blood. In Africa, where malaria burden is greatest, bednets treated with pyrethroid insecticide were highly effective in preventing mosquito bites and reducing transmission, and essential to achieving unprecedented reductions in malaria until 2015[1]. Since then, progress has stalled [2] and with insecticidal bednets losing efficacy against pyrethroid-resistant *Anopheles* vectors[3,4], methods that restore performance are urgently needed to eliminate any risk of malaria returning to the levels seen prior to their widespread use throughout sub-Saharan Africa[5]. Here we show that the primary malaria vector *Anopheles gambiae* is targeted and killed by small insecticidal net barriers positioned above a standard bednet, in a spatial region of high mosquito activity but zero contact with sleepers, opening the way for deploying many more insecticides on bednets than currently possible. Tested against wild pyrethroid-resistant *Anopheles gambiae* in Burkina Faso, pyrethroid bednets with organophosphate barriers achieved significantly higher killing rates than bednets alone. Treated barriers on untreated bednets were equally effective, without significant loss of personal protection. Mathematical modelling of transmission dynamics predicted reductions in clinical malaria incidence with barrier bednets that matched those of 'next-generation' nets recommended by WHO against resistant vectors. Mathematical

models of mosquito-barrier interactions identified alternative barrier designs to increase performance. Barrier bednets that overcome insecticide resistance are feasible using existing insecticides and production technology, and early implementation of affordable vector control tools is a realistic prospect.

Sleeping under a long-lasting insecticidal net (LLIN) is the most effective way of preventing malaria in Africa, where the widespread use of LLINs was the main contributor to 50% and 40% reductions in malaria prevalence and clinical disease incidence respectively between 2000 and 2015[1]. Those first generation 'standard' LLINs used pyrethroids, fast-acting insecticides with minimal health risks for bednet users. By 2017 however the annual reduction was gone, replaced by an increase of 3.5 million malaria cases in the ten highest burden African countries[2]. Although its contribution to this alarming development is unclear, pyrethroid resistance is widespread in *Anopheles spp.* vector populations[4,5] and standard LLINs have lost efficacy against resistant vectors[3-6]. Hence, overcoming resistance is a global priority, demanding insecticides that do not share resistance mechanisms with pyrethroids, or methods that reduce dependency on insecticides[7-9]. Recent trial results identified insecticide combinations that impact pyrethroid-resistant vectors[3,10,11], but toxicity restrictions on risks to occupants, especially infants, and higher cost of new insecticides limit bednet treatment choices.

Previous studies showed that *Anopheles gambiae* hostseeking activity predominates on a bednet roof, typically above the supine host's torso[12-15]. We also reported high numbers of flight paths traversing the space above the bednet roof, comprising flights with minimal ('visiting') or zero net contact ('swooping')[12,13]. To target these flights, we proposed intercepting mosquitoes with insecticidal net barriers projecting vertically from the bednet roof, where the insecticide would be beyond the reach of children, never touched by the bednet's occupants and rarely touched during routine human activity. If effective, then small net targets might control malaria vectors using a greater range of insecticides than possible with standard bednets[16].

As proof of concept, we evaluated a single transverse barrier (0.5m tall, 0.9m wide) above a standard pyrethroid LLIN (Permanet® 2.0, 'P2'), positioned off-centre above the sleeper's torso (Fig 1a,b). Barriers comprised P2 ('P2B', deltamethrin), or untreated netting dipped in fenitrothion ('OPB', 0.02g/m^2), an organophosphate widely used for indoor residual spraying against pyrethroid resistant mosquitoes (IRS)[17-19], but never deployed on standard bednets.

In initial laboratory bioassays (Fig. 1c), the unmodified P2 bednet killed 77% and 56% of insecticide-susceptible and resistant *An. gambiae* strains respectively, within 48h of exposure. Adding a P2 barrier (P2B) did not affect mortality rates with either strain but the fenitrothion barrier (OPB) was significantly better, killing 100% of resistant mosquitoes within 48h (90% at 24h; $P<0.01$).

In a malaria-endemic setting in Cascades region, Burkina Faso, where *Anopheles gambiae s.l.* vectors are highly resistant to deltamethrin but susceptible to fenitrothion (Extended data Table 1), we tested in a hut trial, three different transverse barriers (Fig 1d): Permanet® 2.0 ('P2B'); fenitrothion-dipped netting ('OPB'; 0.5g/m^2, 20x higher than previous lab tests, equivalent to 25% of the target dose of IRS treatment); non-pyrethroid mixture (indoxacarb, fenazaquin, each at 3-5%; 'NPB'). The results show all treatments significantly reduced mosquito entry rates and increased exit rates compared to untreated bednets (Fig 1e; Extended Data Table 2; $P<0.001$). All three non-pyrethroid barriers increased killing, particularly the OP barriers: OPB on P2 bednets killed 28.8% more than unmodified P2 and increased personal protection by 23% and 66% relative to unmodified P2 ($P<0.001$) and untreated bednets ($P=0.008$), respectively. Remarkably, OP barriers on untreated bednets increased killing by nearly 34% over unmodified P2 ($P=0.008$), without significant loss in personal protection ($P=0.954$).

We explored these encouraging field results in a malaria transmission dynamics mathematical model, to estimate the expected public health impact in Cascades region if existing nets were replaced with barrier bednets. By necessity, the model simplifies malaria transmission into a series of mechanistic processes based on assumptions about the probability of transmission[20-22].The impact of nets was modelled to: i) reduce numbers of mosquitoes entering the house to feed; ii) reduce the feeding success of mosquitoes that enter houses; iii) increase mosquito mortality, relative to a scenario without nets. LLINs reduce malaria infections in mosquitoes and humans by impacting on vector survival and feeding rates, the strength and duration of which are specific to each LLIN type and parameterized from experimental hut data[4,23]. There are limitations to the model's capacity to predict LLIN impact (see Supplementary Information), particularly when considering net durability, though this can be simulated by washing nets [4,20,24].

Hut trial data (Extended Data Table 2) were converted into summary estimates of the probability of mosquitoes being killed, repeating host searching behaviour or successfully feeding on each attempt, for each net/barrier type tested (Extended Data Table 3),with reductions in prevalence continuing until the active ingredient (AI) had waned. Over three years following replacement of

P2 with P2+OPB nets, the mathematical model predicted relative reductions in clinical malaria incidence of 10.4% (0 – 34.47%, 95%CI), 13.3% (0 – 37.12%, 95%CI) and 16.4% (1.15 – 39.76%, 95%CI), at net coverage rates of 60%, 80% and 95%, respectively. With OP barriers on untreated nets (UT+OPB), predicted impacts were even greater, at 13.8% (0 – 37.30%, 95% CI),18.4% (4.62 – 40.71%, 95% CI) and 21.4% (11.66 – 43.67%, 95% CI) for the same coverage levels. We compared this result with PBO-nets, next-generation pyrethroid LLINs that are co-treated with piperonyl butoxide (PBO) to disable resistance mechanisms, and recommended by WHO where pyrethroid resistance is confirmed[23,25]. From equivalent values calculated using the association between experimental hut mortality and bioassay mortality data[4], and similar vector resistance (99% survival in WHO bioassays), PBO-nets were predicted to reduce clinical incidence by 13.0% (0 – 36.09%, 95% CI), 16.2% (0 – 39.14%, 95% CI) and 18.4% (0 – 41.66%, 95%CI) at similar respective coverage levels (Fig. 2b). These, and the 12% reduction reported with another new pyrethroid LLIN (Olyset duo, containing pyriproxyfen) also in Cascades region, are matched by the predictions for barrier bednets.

We investigated how barriers target mosquitoes, using infra-red video tracking to map and quantify mosquito-netting contact (a proxy for insecticide exposure) using defined behavioural modes[12,13]. Contact predominated at the LLIN roof in all treatments (60-95% of total contact; Extended Data Table 4), demonstrating that barriers did not alter this characteristic behaviour at standard LLINs[12,13]. Adding P2 barriers increased overall activity compared to unmodified LLINs ($P<0.001$) (Fig 3a,b), but not contact; P2 barriers increased flight activity in behaviour modes with zero or minimal contact ($P<0.001$) (Extended Data Table 6; Fig 3c,d).

OP barriers killed resistant mosquitoes at contact durations of 12.5, 6.6 and 9s/ mosquito for P2+OPB (laboratory), P2+OPB (Africa) and UT+OPB respectively. Though too brief to kill immediately, these times are similar to the minimum levels of contact accrued by susceptible *An. gambiae* during the critical first 10-minutes of activity at pyrethroid LLINs (range 11-57s/ mosquito), after which few survive[12]. A lethal dose of entomopathogenic fungus can be acquired from treated netting in only 5 seconds[26].

Fenitrothion surface residues can be strongly repellent[19], whereas P2 netting (deltamethrin) exerts a far weaker effect[12]. Thus without deltamethrin (P2+OPB vs. UT+OPB; Fig 3c) contact increased with the untreated surface (Fig 3c; $P=0.048$), but not with the treated barrier (Fig 3e). All barrier treatments resulted in higher activity but lower contact overall (*i.e.* Visiting or Swooping: 60-95% of total activity; Supplementary Video) compared with unmodified P2 LLINs

(12- 27%)(Fig 3e). The exception was the low dosage P2+OPB (0.02g/m^2 fenitrothion) where low-contact (53.3% total) was not significantly different to unmodified P2 (*P*=0.298), but markedly lower than with higher dosages in the field (0.5g/m^2; 85-95%; Fig 3e). Elevated flight without contact most likely combines a response to an insecticide's inherent repellent properties with the ability of *An. gambiae* to avoid net collisions[12] and may typify behaviour at barriers, requiring careful selection of net and barrier treatments to maximise lethality.

Nonetheless, increased mosquito-netting contact directly increases insecticide exposure and we explored whether alternative barrier designs and sizes could increase frequency of contact. We used an agent-based, 3D spatio-temporal model of mosquitoes at an occupied LLIN in a virtual insectary to compare with the 50cm transverse barrier (Fig. 4). With untreated netting on bednet and barrier, transverse barriers only modestly increased contact duration over unmodified bednets (42.75 and 40.71 min respectively; 25 mosquitoes, 1hr), whereas the complex bilateral diagonal cross accrued 103.08 min (Extended Data Table7). When both bednet and barrier were insecticide-treated, contact and kill rates increased with greater barrier surface area and complexity (Extended data figure1a). However, as larger complex barriers increase manufacturing costs, barrier area was weighted by cost/m^2, and the 30cm longitudinal barrier performed almost as well as the 50cm bilateral vertical cross (Extended data Fig. 1b). Encouraged by our semi-field trial result (Fig. 1e), we modelled performance where only barriers delivered insecticide, elevating the hypothetical dosage such that barrier-only contacts killed all mosquitoes within a 1hr simulation time window. Again, complex designs killed the population more rapidly, but performance levelled off at 20cm height. (Extended data Table 7). Weighted by surface area however, and with the transverse barrier as reference, a simple 40cm longitudinal barrier was nearly as effective as the more complex bilateral cross designs (Fig. 5) and a lead candidate for further development.

These results demonstrate that simple net barriers mounted on standard bednets can target *Anopheles gambiae*. With appropriate insecticide, potentially including heretofore excluded classes, barriers significantly improve bednet performance, essentially restoring efficacy against pyrethroid-resistant mosquitoes. More effective barrier designs are possible, as are net and barrier treatment combinations to maximise lethality and improve durability with significant public health benefits[27].

We emphasise that we are not specifically proposing organophosphate-treated barriers. We used fenitrothion primarily for its availability and efficacy against malaria vectors in west Africa[18,20], and expect comparable or better killing/repellency, net adherence, wash resistance from many

insecticides or from non-insecticidal treatments[26,28]. Considerable industry and public sector investment in the past decade have delivered three new LLIN classes, all comprising a pyrethroid combined with a synergist[3], second insecticide[11] or insect growth regulator[10]. If new or additional insecticides make LLINs more expensive, treating only barriers would reduce costs. The barrier's position might permit relaxation of constraints on AIs for bednets (*e.g.* knockdown rate or oral toxicity if ingested by infants), increasing the range of possible treatments. Furthermore, the potential to switch barrier treatments as resistance patterns shift would benefit resistance management and reduce insecticide waste. From manufacturing technology to correct nightly usage by communities in endemic settings, minimal change from existing LLIN processes and behaviours would be required to implement barrier bednets as an appropriate, safe and affordable method to extend LLIN lifespan in the fight against malaria**.**

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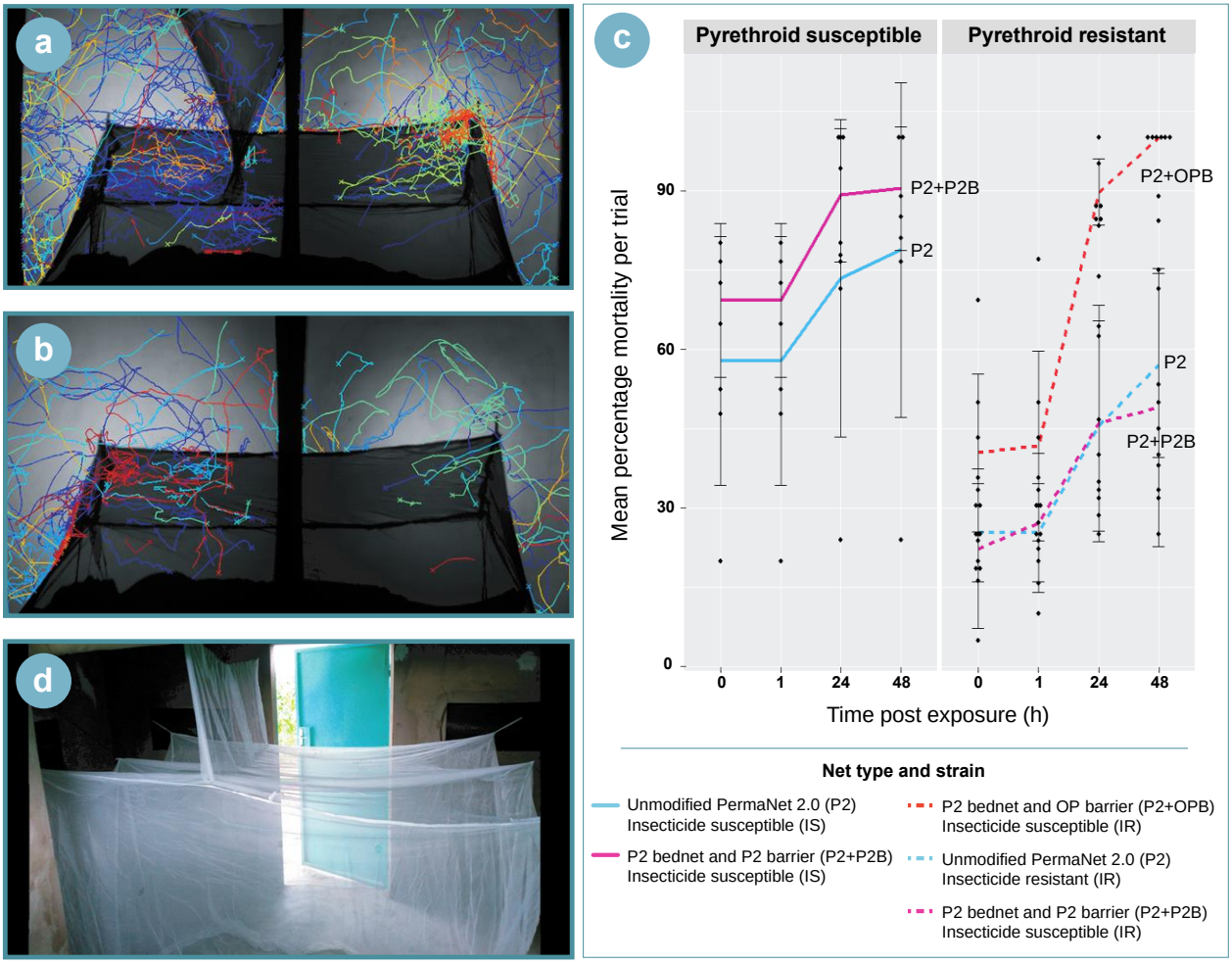
Author contributions: P.J.M. conceived the barrier bednet and designed the study. G.M. collected most experimental data, with assistance from N.L., K.H.T., S.N., W.M.G. and G.M.F. D.T., C.E.T. designed the video tracking capture and analysis systems, which V.V. and J.E.A.P. actualized. T.C. and E.S.S. performed malaria impact predictions. J.J. performed barrier design simulations. G.M. performed statistical analyses with P.J.M. G.M., P.J.M. interpreted results with H.R. and G.F. P.J.M. wrote the paper with input from G.M., G.M.F. and other authors. All authors approved the submitted version.

Competing interests: a patent application (WO2015063455A1) that names PJM was filed by LSTM in respect of the barrier bednet, initially in UK (7th May 2015), but has now entered the PCT process. LSTM has a research agreement with Vestergaard SA, who provided LLIN materials but had no role in study design, data collection, analysis and interpretation, report writing, or publishing. The authors declare no other competing interests.

Data availability: The hut trial dataset is available on Dryad Digital Repository under accession number https://doi.org/10.5061/dryad.hqbzkh1b7. All data analysed during this study are available as detailed in the text. The authors declare that all other data supporting the findings of this study, are available within the article or the Supplementary Information files or are available from the authors on reasonable request.


a
b
d
c
Pyrethroid susceptible
Pyrethroid resistant
Mean percentage mortality per trial
0
30
60
90
P2+P2B
P2
P2+OPB
P2
P2+P2B
0
1
24
48
Time post exposure (h)
Net type and strain
Unmodified PermaNet 2.0 (P2)
Insecticide susceptible (IS)
P2 bednet and P2 barrier (P2+P2B)
Insecticide susceptible (IS)
P2 bednet and OP barrier (P2+OPB)
Insecticide susceptible (IR)
Unmodified PermaNet 2.0 (P2)
Insecticide resistant (IR)
P2 bednet and P2 barrier (P2+P2B)
Insecticide susceptible (IR)

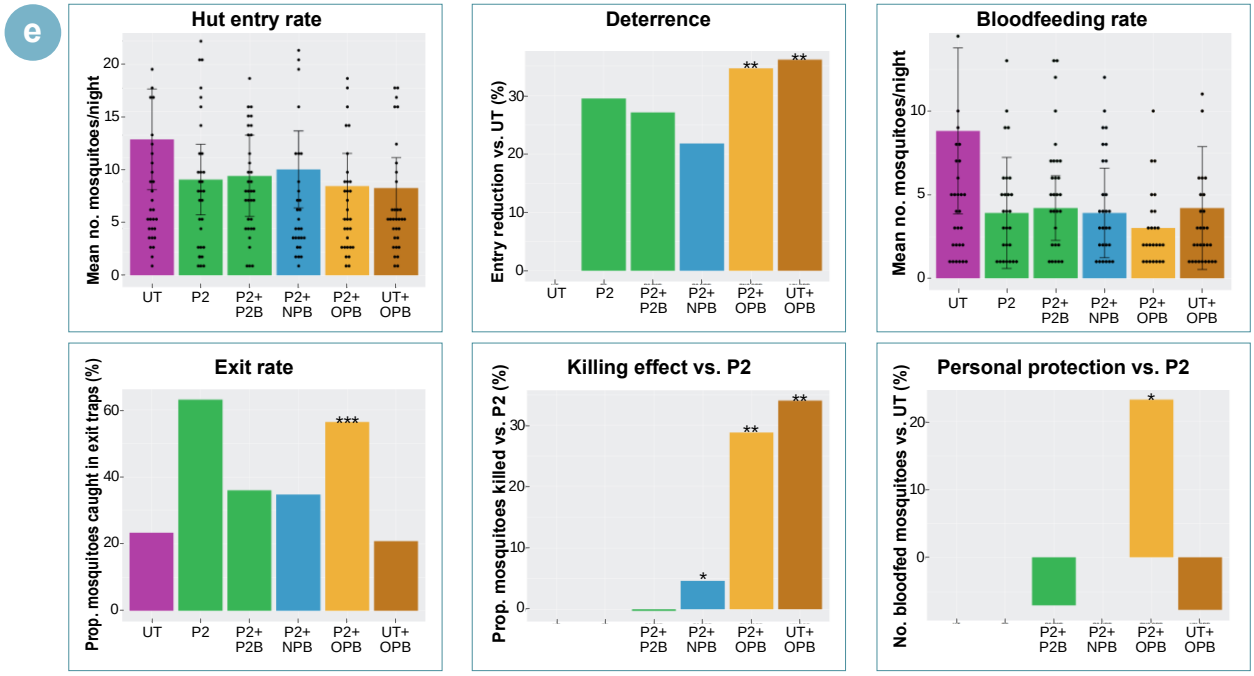

e
Hut entry rate
Mean no. mosquitoes/night
Deterrence
Entry reduction vs. UT (%)
Bloodfeeding rate
Mean no. mosquitoes/night
Exit rate
Prop. mosquitoes caught in exit traps (%)
Killing effect vs. P2
Prop. mosquitoes killed vs. P2 (%)
Personal protection vs. P2
No. bloodfed mosquitoes vs. UT (%)
UT
P2
P2+ P2B
P2+ NPB
P2+ OPB
UT+ OPB

Figure 1. Performance of barrier bednets in laboratory and semi-field trials. (a) Infra-red tracks of mosquito flights at P2 bednets with 50cm high transverse barrier (positioned off-centre, above sleeper's torso) and (b) unmodified P2 (recorded during bioassays; 25 mosquitoes, 60min). (c) Mean (± SD, n=6 trials/treatment) mortality rates of *Anopheles gambiae* strains susceptible (Kisumu) or resistant (Tiassalé) to pyrethroids, following free-flight exposure to human-baited P2 nets, with or without barriers.

P2 and P2+P2B mortality rates were not significantly different for susceptible (t-test, n=82, df =5.3, t=0.75, P=0.48) and resistant strains (t-test, n=109, df=8.7, t=0.62, P=0.55). P2+OPB mortality at 24 (90%) and 48hrs (100%) significantly exceeded unmodified P2 (IR 24h, 45%; t-test, n=91, df =6.1, t=5.21, P<0.01; IR 48h, 57%; t-test, n=31, df =5.1, t=6.5, P<0.01) and P2+P2B (IR 24,46%; t-test, n=91, df =5.8, t=4.61, df=5.8, P<0.01; IR 48h, 49%; t-test, n=41, df =5.1, t=4.74, df=5.1, P<0.01).

(d) Barrier bednet *in situ,* Burkina Faso. (e) Summary of key results from the hut trial; all comparisons *vs.* UT, unless stated otherwise; asterisks denote significant differences (P=0.05-0.01*; 0.01-0.001**;<0.001***) Error bars of estimates are based on standard deviation around the arithmetic mean and the number of independent samples (Extended Data Table 2).

Non-pyrethroid barriers (P2+NPB, P2+OPB, UT+OPB) killed significantly more than untreated controls (Poisson regression GLM; n=44, df=5, Z=2.12, P=0.03; n=133, df=5, Z=7.61, P<0.001; n=152, df=5, Z=8.32, P<0.001, respectively). Personal protection (no. bloodfed mosquitoes prevented relative to untreated nets) was significantly higher with P2-OPB (66%; Negative Binomial GLM; n=109, df=5, Z=-2.649, P<0.01): the reduction with UT+OPB was not significant (Negative Binomial GLM; n=153, df=5, P=0.954). Killing effects of test net *vs.* unmodified P2 were higher with P2+NPB ((Poisson regression GLM; n=44, df=5, Z=1.82, P=0.043), P2+OPB (n=133, df=5, Z=5.91; P=0.008) and UT+OPB (n=152, df=5, Z=7.53, P=0.044)(Extended Data Table 2).

Treatment codes: UT (Untreated unmodified bednet), P2 (unmodified Permanet 2.0 bednet, deltamethrin 55mg/), P2+P2B (Permanet 2.0 and P2 barrier); P2+OPB (P2 and fenitrothion barrier, 0.02g/m^2 in laboratory, 0.5g/m^2 in field). Treatments P2+NPB (P2 net and non-pyrethroid barrier [indoxacarb/ fenazaquin, 3-5%]) and UT+OPB (untreated bednet and fenitrothion-dipped barrier) were tested in the field only.

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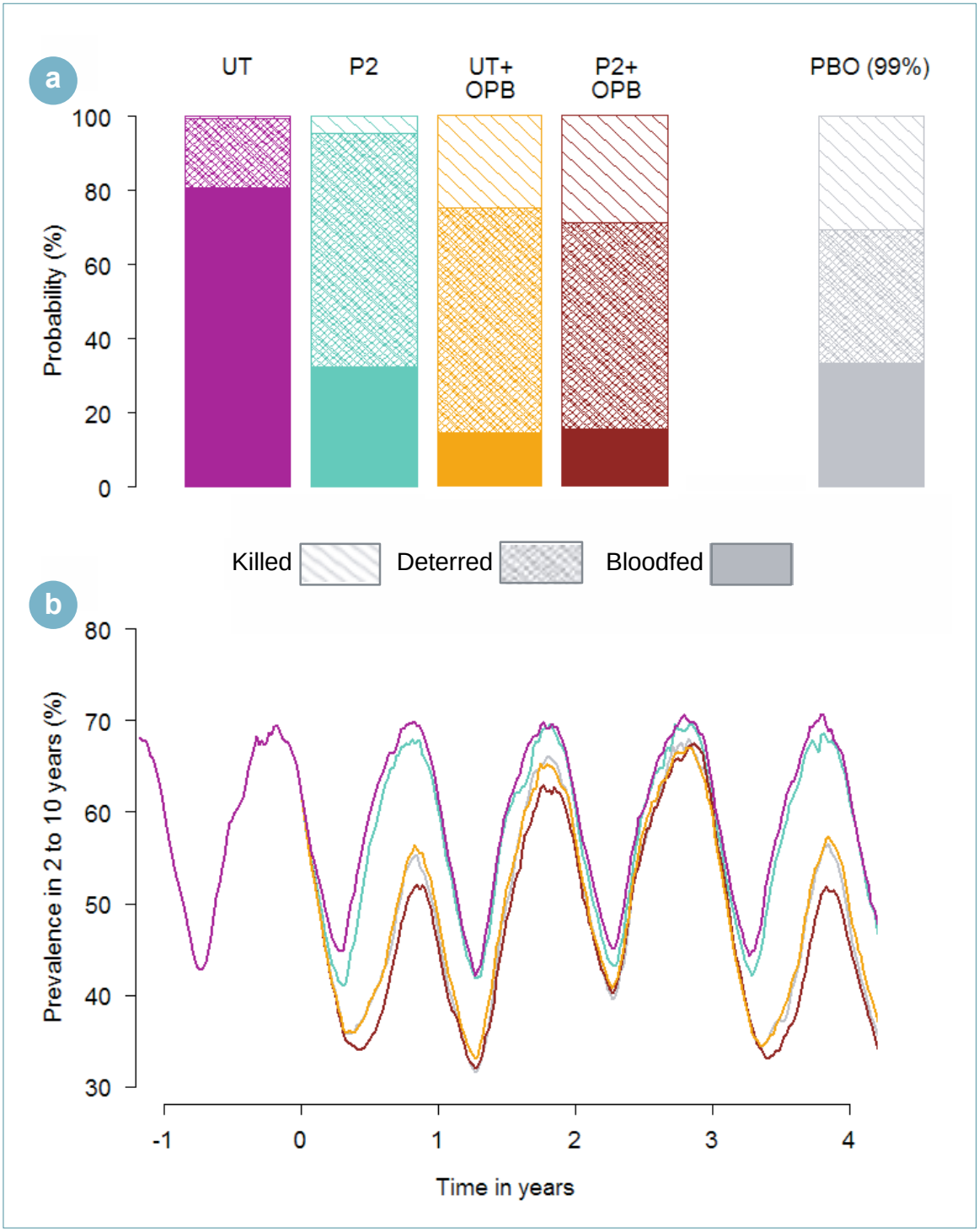
a
UT
P2
UT+ OPB
P2+ OPB
PBO (99%)
Probability (%)
100
80
60
40
20
0
Killed
Deterred
Bloodfed
b
Prevalence in 2 to 10 years (%)
80
70
60
50
40
30
-1
0
1
2
3
4
Time in years

Figure 2. Summary of efficacy estimates of different bednet barrier combinations, and comparison with estimates for PBO bednets at high pyrethroid resistance.

(a) The probable outcome of a mosquito feeding attempt is determined for each net intervention: mosquitoes are either killed, deterred but return to feed again, or bloodfeed successfully. Summary estimates were generated from hut trial data for untreated nets (UT), pyrethroid only nets (P2) with or without an organophosphate barrier (OPB) (Extended Data Table 2). At a pyrethroid resistance level of 99%, the probability of an OPB barrier bednet killing mosquitoes was comparable to that of the PBO-nets, with fewer mosquitoes bloodfeeding, regardless of whether the bednet was treated (UT+OPB) or untreated (P2+OPB).

(b) The efficacy of these five bednet barrier combinations drives the contrasting predicted reductions in prevalence among 2 to 10-year old children for the years following net distribution campaigns at Time zero and Time three. Colour codes match the different bednet barrier combinations in (a). Model was parameterized to reflect the seasonality, entomology and endemicity of malaria in Cascades Region, Burkina Faso.

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a
An. gambiae s.s laboratory colonies
Pyrethroid susceptible
Pyrethroid resistant
P2
P2+P2B
P2+OPB
Mean no. contacts/trial
c
Wild An. gambiae s.l Burkina Faso
Wild An.gambiae population pyrethroid-resistant
P2+OPB
UT+OPB
Mean contact duration/trial (s)
Barrier(1)
Other regions
b
Bednet
Barrier
d
Mean duration of swooping/trial (s)
4,6,7,9
13,15
Region of activity
e
Level of flight track contact
HIGH
LOW
Wild population pyrethroid resistant
Total time active (seconds)
Time (minutes)

Figure 3. Behaviour at barrier bednets of *Anopheles gambiae s.l.* laboratory colonies and wild population in Burkina Faso

Mean number (a) and duration (b) per test of flights contacting bednet or barrier for each treatment and mosquito laboratory strain; (c) Mean duration of barrier or bednet contact, in regions shown in the inset key in fig 3c and (d) mean total time spent in swooping mode (no net contact) for wild mosquitoes. Error bars based on standard deviation around the arithmetic mean and the number of independent samples in Extended Data Tables 4 (a,b); 5 (c) and 6 (d).

(e) Activity at 5min intervals during 60 (laboratory) or 120 (field) min assays, showing mean durations of flight in High (Resting, Bouncing) or Low (Visiting, Swooping) contact behaviour modes; pie charts show relative proportions of total duration per category. Treatment codes as Fig. 1.

Behaviour modes[12]: Swooping - tracks without net contact; Visiting - relatively lengthy flights with infrequent net contacts, trajectory turns of ≥80° and 0.4s minimum interval between contacts; Bouncing - multiple rapid contact, intervals <0.4s or unbroken contact, never static; Resting - static $\geq$ 0.75seconds, velocity < 1.33mm/s, unbroken net contact.

Flight activity increased significantly with P2 barriers (mean flight activity per trial; IS: 5012±1975s and 1341.6±741s; Wilcoxon rank sum test; n=25, df=1, W=5422, P<0.001; IR, 577.2±79s and 464.4±30s; n=65, df=1, W= 23017, P<0. 001), but not OP barriers (371.2±45s and 464.4±30s; n=65, df=1, W=23689.5, P=0.155, P2 and P2+OPB respectively).

Low contact activity increased with P2 barriers in IR (t-test, n=65, df=176, t=3.50, P<0.001) and IS (t-test, n=37, df=73, t=2.519, P=0.01) mosquitoes, but not with OP barriers (P=0.298).

Significantly more swooping activity occurred over the host's torso, proximal to the barrier; t-test, n=5, df=7.61, t=2.6976, P=0.028). Swooping (*i.e.* zero contact) was significantly higher in both OPB barriers in the field (P2+OPB, 79.5% of all flights; Pearson's chi-squared test; n=125, df=3, χ^2 = 163.4; UT+OPB, 64.2%; n=124 , df=3, χ^2 = 86.7; P<0.001).

Netting contact duration (bednet plus barrier) was higher with OP barriers on an untreated bednet than on a P2 (t-test, n=5, df=12, t=-2.19, P=0.048).

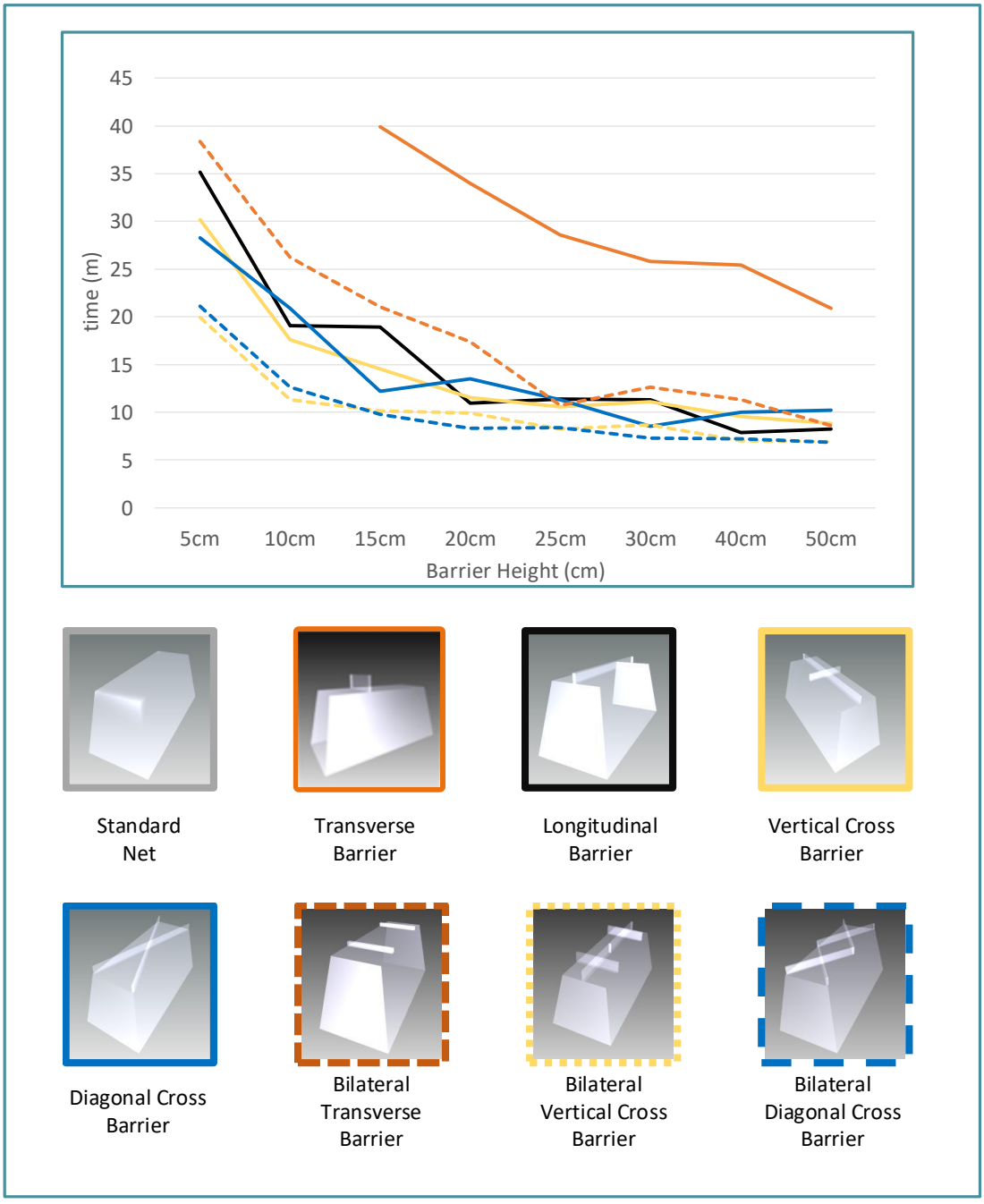
45
40
35
30
25
20
15
10
5
0
time (m)
5cm
10cm
15cm
20cm
25cm
30cm
40cm
50cm
Barrier Height (cm)
Standard Net
Transverse Barrier
Longitudinal Barrier
Vertical Cross Barrier
Diagonal Cross Barrier
Bilateral Transverse Barrier
Bilateral Vertical Cross Barrier
Bilateral Diagonal Cross Barrier

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Figure 4. Comparing different barrier designs and heights by evaluating performance *in silico.* Population kill time (total time needed to achieve complete population death, mins) by different barrier bednets when the bednet is untreated and insecticide is deployed only on the barrier. Values are weighted by surface area, using a transverse barrier with an equivalent height as reference. The eight designs are illustrated and include a standard (unmodified) bednet and the transverse barrier bednet tested in our experiments. Frame colour and pattern on the illustrations correspond with the lines on the graph.

METHODS

Ethics review and research permission. All research methods were performed in accordance with approved guidelines for those procedures and written informed consent was obtained from all volunteers sleeping in experimental huts and laying under bednets during tracking experiments. The study was approved by the Research Ethics Committees at the Liverpool School of Tropical Medicine (LSTM Research Protocol 16-38, 11th October 2016, Liverpool) and Centre National de Recherche et de Formation sur le Paludisme (CNRFP Deliberation no. 2016-9-097, 20th September 2016, Ouagadougou). No adverse effects of treatment or mosquito-borne infections were reported by volunteers during the course of the study.

Bed net and barrier materials. In all tests, rectangular bed nets measuring 2m x 0.9m x1.5m tall were used as the standard bednet. To facilitate image capture, the net roof was tilted on its long axis when facing the cameras, to ensure activity on the roof was visible (Fig. 2B,C). Hence, the net height was 0.93m near the camera and 1.19m at the rear. Pyrethroid-treated nets were Permanet® 2.0 (75 denier polyester net impregnated with deltamethrin at 55mg/m^2; Vestergaard, Lausanne, Switzerland). New LLINs were hung for four weeks prior to use and tested for insecticidal activity using the standard WHO cone test and two laboratory strains (n=4 repeats per mosquito strain-LLIN combination; see next section).

The barrier comprised a vertical net panel positioned transversely on the net roof (Fig. 1A), one of the simplest barrier designs[16]. The barrier was 0.9m wide (extending edge to edge across the LLIN) and as it was fitted above the tilted roof of the rectangular LLIN. It measured 0.8m high (front) and 0.54m (rear) to ensure the top edge was horizontal at a total height of 1.9m from the floor. The lower edge was pinned to the roof of the net slightly off-centre, at 0.8m from the head end (*i.e.* 0.2m from mid-point) (Fig. 2B, C). To facilitate video tracking, creases, sagging and wrinkles were minimized by suspending the barrier from the ceiling using string and supporting the net and barrier edges with 5mm carbon fibre rods.

Insecticidal barrier panels (0.6m^2) were cut from new Permanet® 2.0 LLINs or untreated polyester netting treated with the organophosphate fenitrothion ('OPB'). We selected this low fenitrothion concentration (100 times less than that used in IRS) to minimize any potential repellent effects of organophosphate residues. OPB barriers (0.02g/m2) were prepared by immersing eight pre-cut untreated net barriers (plus 0.2m^2 fragment to ensure all liquid was absorbed) into a 224ml aqueous emulsion containing 0.1g of fenitrothion (Greyhound Chromatography and Allied

Chemicals, Birkenhead, UK). Unmodified Permanet® 2.0 LLINs were used for comparison. Fresh barriers were used for each test repeat (6 for Tiassalé (IR), 5 for Kisumu (IS).)

Evaluation of barrier net performance in the laboratory. Initial tests were conducted on human-occupied bednets in a dedicated insectary in UK (5.6m x 3.6m in area, 2.3m high; climate controlled at 27±2°C, 70±10% RH), using *An. gambiae sensu lato* strains from Liverpool School of Tropical Medicine (LSTM) colonies of "Kisumu" (*Anopheles gambiae s. str.*.; insecticide susceptible 'IS', n=9) or "Tiassalé" (*An. gambiae s. str.* and *Anopheles coluzzii* mix; resistant to pyrethroids and the majority of other insecticides used in public health, 'IR', n=17[25]. Three to five-day-old unfed adult female mosquitoes (25 per experiment) were deprived of sugar and water for 4 hours prior to transfer to the experimental room to acclimatize (1 hour) before testing. All tests were conducted within 1-3 hours of the start of scotophase.

Human volunteers lay uncovered on a fresh sheet over a 2m x 0.9m mattress (0.18m thick; surface at 0.48m above the floor). Mosquitoes were recorded using a video-tracking system of paired identical camera setups (one each for the upper or lower body of a supine human), each comprising a single infrared LED (850mm wavelength, 1000mA minimum; M850L2, Thorlabs, UK) aligned with a pair of Fresnel lenses (mounted either side of the bed, with a 43cm gap between the lens and mattress on each side) and monochrome camera with 12.5mm imaging lens (Baumer HXC40NIR, Camera Link, 4Mpix; Lambda Photometrics, UK). Video was recorded at 50 FPS, using StreamPix software (www.norpix.com), and data saved as .seq files.30 minutes after the volunteer entered the bed, recording was started and mosquitoes were released from a paper cup at a height of 2m, 1.4m from the net. Activity was recorded for 60 minutes.

Bioassays of mosquito behaviour at human-occupied bednets. Eighteen human volunteers, 9 males and 9 females of different ethnicities, aged between 22 and 49, were recruited from staff and students at LSTM. Volunteers were clothed and barefoot and lay on their backs, as immobile as comfort permitted during the 1-hour test. All were asked to eschew scented toiletries when testing. The majority were tested with both barrier-modified (P2 or OP barrier) and unmodified P2 nets on different days, with an average interval of 41 days between their tests. After each 1-hour test, the number of live and dead mosquitoes in the room was recorded. Living mosquitoes were maintained with sugar and water and mortality recorded at 1, 24 and 48 hours.

Video tracking mosquitoes in the laboratory. Tracking individual mosquitoes or determining the number of responders of the 25 released was not possible as the entire room was not visible.

Each flight track, from entry to exit of the field of view, was analysed individually, using segmentation and tracking algorithms through bespoke software in the Matlab framework (Mathworks). Data were extracted and interpreted to quantify the number and duration of contacts with different bednet regions and flight activity in spatial regions around the barrier.

Mosquito flight tracks were categorized in four behaviour modes, using previously reported quantification algorithms[13,14]: Swooping - flight tracks without net contact; Visiting - extended flight tracks with infrequent net contacts; Bouncing - multiple rapid contacts with the bednet surface; including short flights between contacts, 'walking' and 'probing' behaviour; Resting - static or slow movement.

The field of view recorded by the cameras was divided into specific regions on the surface of barrier and bednet, or in the airspace surrounding it. The limits of each region were delineated accurately to fit every barrier/bednet assembly, as shown in Fig 2A and Fig 3A. The number and duration of events in each behaviour mode were determined for every net and spatial region. When a single track included more than one behaviour mode, the time spent in each mode was recorded separately.

Quantifying mosquito contact at barriers and bednet regions. Bednet contact comprised all flight tracks in bouncing, visiting and resting behaviour modes. The number and duration of contacts were calculated for each test as total values and mean values per trial. Tracking individual mosquitoes throughout an entire assay is not possible with this system as the entire room was not visible, and plausible estimates of minimum and maximum values of net contact per individual were calculated. The minimum value was total contact duration divided by the total number of released mosquitoes (n=25); maximum net contact time per individual was calculated as the total contact duration divided by the maximum number of mosquitoes observed simultaneously ($n \leq 4$).

Evaluation of barrier bednets in the field. Between July and October 2017, barrier nets were tested against adult female mosquitoes morphologically identified as *Anopheles gambiae* complex, reared from wild larvae collected at Tengrela (10°40'N, 4°50'W) near Banfora, Burkina Faso. Species identification[29] conducted on a random selection of adult females tested identified 87.41% (n = 437) of samples to be *Anopheles coluzzii* Coetzee & Wilkerson, previously found to be highly resistant to pyrethroids at this site[30].

Barrier bednets were assembled as described for the laboratory study, with the exception of OPB. These fenitrothion-dipped barriers were prepared by immersing pre-cut netting (0.65m^2 or

456 0.8m^2) in a solution of fenitrothion, prepared by adding 7.3ml or 9ml of fenitrothion stock solution
457 (0.044g/ml acetone; AK Scientific, California, USA) to 22ml or 27ml acetone, giving 29.3ml and
458 36ml of 0.01g fenitrothion/ml acetone respectively. At an absorbency rate of 45ml/m^2, this
459 deposited 0.5g/m^2 on the netting surface, equivalent to 25% of the target dose for IRS treatment.
460 We selected this concentration, 25 times higher than in the initial laboratory experiment, based on
461 the absence of evidence for repellency in the initial laboratory experiments, and out of concern that
462 durability of dipped nets at lower concentrations might be compromised in harsher field
463 conditions.

464 Barriers (0.5m high x 1.3-1.6m) were placed across the full roof width of standard rectangular
465 Permanet® 2.0 (1.6 x 1.8 x 1.5m) or untreated polyester nets (1.3 x 1.5 x 1.8m), at an off-centre
466 position, 0.7m from the sleeper's head, 1.1m from the foot of the net (Fig. 3A). Unlike the
467 laboratory study, the bednet was not tilted to aid video tracking.

468 **Hut trial design and protocol.** The trial followed WHO guidelines[31] in six WHO standard
469 cement huts of the West African design (3.5 × 2 × 2m high) that had been used previously for
470 evaluation of vector control tools, including PBO-nets[32]. The cement walls stand on concrete
471 platforms with water-filled moats to minimize entry by ants and other scavengers. The roof is
472 corrugated metal with a polythene sheet ceiling. Window and veranda traps were open during
473 tests. To permit mosquito entry, holes were cut in all bednets as defined in WHOPES guidelines:
474 six 4cm x 4cm holes, two on the long sides and one on the short sides, were cut in each net. The
475 experiment comprised six treatment arms:

476 1. Untreated control bed net (UT): untreated polyester netting of similar denier and mesh size as LLINs in
477 other treatments; no insecticidal properties; no barrier.

478 2. Permanet® 2.0 LLIN (P2): a WHOPES recommended standard size double LLIN (1.6m x 1.8m x 1.5m)
479 treated with deltamethrin at 55mg/m^2; no barrier.

480 3. Permanet® 2.0 LLIN with Permanet 2.0 barrier (P2+P2B): Standard LLIN with a barrier element of
481 identical Permanet 2.0 netting.

482 4. Permanet® 2.0 LLIN with Non-Pyrethroid Insecticide (NPI) Barrier (P2+NPB): Standard P2 LLIN with
483 an added barrier element treated with a combination of two non-pyrethroid insecticides: Indoxacarb
484 (oxadiazine 3-5%) and Fenazaquin (quinazoline 3-5%).

485 5. Permanet®2.0 LLIN with fenitrothion (OP) barrier (P2+OPB): Standard LLIN with an added barrier
486 element treated with the organophosphate fenitrothion, at a concentration of 0.5g/m^2, equivalent to 25%
487 of the level applied in IRS.

6. Untreated net with OP barrier (UT+OPB): untreated polyester bed net with an added barrier element treated with 0.5g/m^2 of fenitrothion.

To complete a full rotation for this comparison of six treatment arms, 36 experimental nights were required. Treatments were rotated between the huts weekly and the sleepers were allocated to different huts each night (Hut trial rotation plan, Supplementary Information). A new set of treated and untreated nets were prepared and used in each week of the trial. Prior to use, all manufactured LLINs and untreated control nets for use in any particular week were removed from packaging, aired for seven days. OP barrier nets were dipped in fenitrothion as described above and aired for three days before use. To ensure the dipping process was successful, barrier samples were bioassayed before and after the trial (Supplementary text). Human volunteers were recruited from the local community and each aided once with each treatment. After the clothed, but bare foot volunteer had entered the bed, research staff checked the net to ensure it was secure. Sleepers remained under the net between 20:00 and 05:00 hours. Seated at a distance of 10m or more, a supervisor was on duty throughout the trial, to ensure behaviour complied with the protocol, and to assist the volunteers if required. At 05.00, volunteers collected mosquitoes inside their nets (using glass universal tubes with cotton wool plugs) before exiting the net and closing the veranda traps to prevent mosquito movement between the veranda and hut. Mosquitoes were then collected from the main hut and veranda, before research staff entered huts to check for remaining mosquitoes.

Retrieved mosquitoes were sorted by treatment/hut, location (inside net/in hut/in veranda), alive/dead, sex and abdominal status (bloodfed/ semi-bloodfed/ unfed; gravid/ semi-gravid). Live *An. gambiae s.l.* were sorted by hut and held in paper cups (5mosq /250ml cup), separated by feeding status and location, provided with 10% sugar solution on cotton wool pads and retained in a nearby hut until natural death. Mortality was assessed within two hours of the test ending and at 24-hour intervals thereafter until no mosquitoes remained alive.

We quantified and compared a range of outcomes incorporating the standard parameters recommended by WHO for evaluating LLINs[31]:

- Deterrence: the reduction in hut entry relative to control huts (untreated nets)
- Exophily/Repellency: the proportion of mosquitoes found in the veranda traps
- Blood-feeding inhibition: the reduction in blood-feeding in comparison with the control huts (untreated nets)

- Immediate and delayed mortality: the proportions of mosquitoes entering the hut that are found dead in the morning (immediate mortality) or after being caught alive and held for 48 h with access to a sugar solution (delayed mortality)

Since deterrence and blood feeding inhibition are indicators of personal protection, the personal protection effect of a treated net was calculated as:

$$\text{Personal protection (\%)} = \frac{100 * (Bu - Bt)}{Bu}$$

where Bu is the total number blood-fed mosquitoes in huts with untreated nets and Bt is the total number of blood-fed mosquitoes in huts with treated nets.

Mortality (immediate and delayed) is an indicator of the potential mass killing effect of LLIN use, *i.e.* a reduction in the density and/or longevity of mosquitoes in areas with high net coverage, resulting in community-wide protection that also benefits non-users of LLINs. The potential killing effect of a treated net was estimated from:

$$\text{Mortality} = \frac{100 * (Kt - Ku)}{Tu}$$

where Kt is the number of mosquitoes killed in huts with treated nets, Ku is the number of mosquitoes killed in huts with untreated nets, and Tu is the total number of mosquitoes collected from huts with untreated nets.

Predicting barrier bednet effectiveness for malaria control in a highly endemic context. An individual-based transmission dynamics model of malaria[20, 22, 33-34] was used to explore the public health impact of nets with organophosphate barrier panels fitted to the roof section. This model tracks *P. falciparum* infection in people and mosquitoes. Susceptible people are exposed to infectious mosquito bites at a rate dependent on local mosquito density and infectivity. Mosquito dynamics describe the effects of mosquito control and the resulting decline in egg laying[22].

The specific seasonal profiles[35] and historic scale-up of IRS and LLIN interventions from 2000 to 2015 were matched for the Cascades administration region in Burkina Faso (Malaria Atlas Project, MAP[1]as per[36]. The mosquito density was adjusted to capture the underlying transmission intensity which is high in the Cascades region. We used 60% prevalence in 2 – 10-year old children at peak

transmission as the baseline prevalence in this exercise. For all model simulations, the same baseline parameters were applied but the parameters that determine net efficacy were estimated from the experimental hut data (Extended data tables 2, 3). Uncertainty in model predictions was generated by running the model 50 times with randomly drawn estimates from the posterior distribution of each model parameter, whilst fixing net parameter estimates as recorded in the experimental hut trials.

Next-generation nets are being developed to mitigate the potential lost impact of indoor interventions in the context of pyrethroid resistance. Piperonyl butoxide (PBO) synergist nets are the first next-generation nets to reach the market. PBO inhibits specific metabolic enzymes within mosquitoes that can detoxify pyrethroids, thereby extending the active life-length of the insecticide in LLINs. We investigated how well barrier nets might perform relative to these PBO-nets. Given that the average mortality in experimental huts for standard nets (unmodified Permanet® 2.0) during the 8-week monitoring period was just 7.4%, and the relationship between discriminatory dose bioassay and experimental hut mortality determines that this corresponds to 99% resistance[4] we compared nets at this level of pyrethroid resistance. Extended data table 2 outlines the parameter changes made within the model to represent the predicted impact of organophosphate panels on prevalence in 2 – 10-year old children and all clinical cases in the Cascades administrative region in Burkina Faso. In the absence of wash data (used for simulating the natural wearing of the active ingredient of nets and to determine net durability)[4,23], we assumed the conservative estimate for the half-life of barrier nets that is based on maximum mortality estimates from the experimental hut data. This corresponds to approximately 6 months for the two barrier nets tested (P2+OPB and an UT+OPB). We compared the effect of PBO- and barrier nets (P2+OPB and UT+OPB) relative to P2 nets alone.

Video tracking mosquito flight in Burkina Faso. A dedicated experimental hut was constructed adjacent to the WHO huts at Tengrela, to accommodate a video-tracking system based on a previously described system[37]. The room measured 6m x 4m in area and 3m high, with a corrugated steel roof. Steel-shuttered windows and eaves were also present on two walls that were closed during recording to limit the movement of mosquitoes, airflow and external light sources. Conditions inside the hut were similar to ambient, with a mean temperature and humidity during recording of 28ºC (SD=3.1) and 75% relative humidity (SD=12.5). Half an hour before tests, the volunteer entered the bednet, the mosquitoes were placed in a paper cup resting on the lip of the eave, 2m above the ground and the room was closed. A section of eave screen was cut to allow a

researcher to release the mosquitoes by uncovering and emptying the cup at the start of the trial before the eave screen and shutter were closed.

Unfed females, insectary-reared from larvae collected at Tengrela and aged 4-7 days post-eclosion, were used in all tests. Mosquitoes were transferred to the experimental hut within 30 minutes of tests to acclimatize to the hut interior environment. All tests were run during the night, starting at or shortly after 19:30hrs.

Five of each bednet-barrier combination(*i.e.* P2+OPB, UT+OPB) that had previously been used in the hut trial over 6 nights were used. Human volunteers lay on a 2m x 0.88m sleeping mat, with the bed net evenly tucked under by one of the researchers prior to filming.

The recording period lasted 2 hours from the time of mosquito release. Throughout, a researcher monitored the recording system from an adjacent control room. Before and after recording, mosquitoes in the room were collected with aspirators and the floor swept to eliminate or recover any dead or knocked-down mosquitoes. The collected mosquitoes were maintained under ambient conditions in a separate hut nearby, provided sucrose solution *ad libitum* and assessed (dead, knocked-down or alive) immediately at collection and at 1, 24 and 48 hours later.

Video was recorded at 50 FPS, using StreamPix software (www.norpix.com), with data saved as .seq files. Initial analysis was performed using segmentation and tracking algorithms through bespoke software in the Matlab framework (Mathworks) using these large files (>200Gb video files). Following this, the video files were compressed using bespoke software using the .mp4 container and a dedicated video card (<5Gb). This compression was designed to be compatible with the segmentation algorithms, allowing subsequent analysis to be performed on the compressed or re-rendered video files with negligible loss of information. All recorded video was then stored on multiple, redundant external drives.

Optimization of barrier size and shape - *in silico* models. We developed an agent-based 3D spatio-temporal model of mosquito behaviour at a human-occupied LLIN in a virtual insectary to compare designs for optimizing barrier net performance. InVeCTS (Indoor Vector Control Testing System) is an attempt to create a virtual environment in which to assess mosquito populations' interactions with their host and their environment. This is a multi-agent approach using a fine-grained spatial representation in which a mosquito population can interact with a human host over time. Mosquito flight occurs in real time and all mosquito flight paths and interactions with the environment are recorded for subsequent analysis.

A population of mobile virtual mosquito insects are created. These individuals fly in a continuous 3D space representation inside a discretized spatial arena, representing an insectary or hut containing a bed net and human host. For the experiments presented in this document an arena of size 5.6 x 3.6 x 2.3m was used, corresponding to the experimental insectary at LSTM used previously[10,11].

Barrier bednets were designed from 3D triangular meshes, building upon standard 'reference' simple unmodified bed net design (Fig. 5). The standard bed net design measured 2m long x 0.9m wide (at its widest point on the floor) and 0.8m high. Barrier bednets of different designs and heights (5, 10, 15, 20, 25, 30, 40 and 50cm) were assessed. The bed nets were placed in the centre of a virtual insectary (5.6m long x 3.6m wide x 2.3m high) and a population of 25 virtual mosquitoes were released from a wall-mounted position halfway along the longest axis (2.8m) and at a height of 2m. A human bait stimulus profile was centred in the bed net design with the head region furthest away from the release location. Each experiment was run for the equivalent of 1hr and results were recorded for further analysis. Five runs were performed at each barrier height. Experiments were performed under two treatment conditions. The untreated net condition was used to assess the contact time of the different net designs. The treated net condition was used to assess the effectiveness of the designs in reducing the activity of the virtual mosquito population.

Statistical Analyses. Random effects generalized linear models were used for analyses of activity time, behavioural modes, region preferences, tortuosity, number of tracks, activity decay and effects of treatment type. Non-normality of data was tested for using Shapiro–Wilk tests. t-tests were Welch's independent Two-sample unequal variances) t-tests. For all tests, an α threshold of 0.05 was used. Statistical analyses were performed using R (R version 2.15.1) (R Development Core Team 2012).

In the hut trial, analysis was performed to assess the performance of the barrier bednet relative to the untreated control and standard PermaNet 2.0, with the extra arms allowing for a description of the relative benefits of the different insecticide treatments. The number of mosquitoes found inside the huts, bloodfeeding rates and mortality were compared using Poisson regression Generalized Linear Models or Negative Binomial Generalized Linear Models to account for over-dispersion.

In modelling barrier design and height, all statistical analyses were performed using R version 3.1.2 (http://www.R-project.org/). Comparisons of mortality and activity levels were based on Welch's two-sample (unequal variances) t-test; when the assumption of normality was not met,

based on a Shapiro–Wilk test, then a one-sided Wilcoxon signed-rank test was used. Generalized linear models with Poisson distribution were used to compare hut trial outcomes, except in cases of over-dispersion, where Negative Binomial GLMs were used. For all tests, an α threshold of 0.05 was used. Unless stated otherwise, data are reported as arithmetic means and associated standard deviation.

Code availability: Data handling scripts and video segmentation and tracking software are available from the authors upon reasonable request.

Data and materials availability: The hut trial dataset generated during the current study is available on Dryad Digital Repository under accession number: https://doi.org/10.5061/dryad.hqbzkh1b7.

All data analysed during this study are available as detailed in the present paper. The authors declare that all other data supporting the findings of this study, are available within the article and its Supplementary Information files are available from the authors upon reasonable request.

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Supplementary Information

38) Date	Insecticide	Knockdown at 1hr (%)	Mortality at 24hr (%)	No. mosquitoes tested
Aug 2016	Pyrethroid control	0	0	23
	Deltamethrin 0.05%	14.89	9.57	94
	Organophosphate control	0	5.26	19
	Fenitrothion 1%	0	94.44	90
Jun 2017	Pyrethroid control	--	0	57
	Deltamethrin 0.05%	--	35.67	157
Oct 2017	Organophosphate control	0	0	25
	Fenitrothion 1%	98.98	100	98
Mar 2018	Pyrethroid control	--	1.61	62
	Deltamethrin 0.05%	--	17.39	69
Sep 2018	Pyrethroid control	--	0	311
	Deltamethrin 0.05%	--	0	125

Extended data table 1 | Insecticide susceptibility status of the wild *Anopheles gambiae s.l.* population at Tengrela, Banfora in Cascades region of Burkina Faso. Adult female mosquitoes were tested using the WHO tube test. Mortality rates of less than 95% are indicative of resistance.

Outcome	UT	P2	P2 + P2B	P2 + NPB	P2 + OPB	UT + OPB
Total no. caught	522	368	381	408	341	334
Mean no. caught per night	14.5	10.2	10.6	11.3	9.5	9.3
% Deterrence	-	29.5	27.1	21.8	34.6**	36.1**
Total no. bloodfed	320	142	152	142	109	153
Mean no. bloodfed per night	8.8	3.9	4.2	3.9	3.0	4.2
Personal protection (%)	-	55.6	52.5	55.6	65.9**	52.1
Number dead on collection	8	27	26	44	133	152
Killing effect (%)	-	3.6	3.4	6.8*	23.9***	27.6***
Mean survival post collection (days)	12.0	11.6	11.3	11.1	11.4	10.6
% Exiting	23.4***	63.1	36.0	34.8	56.5	20.8
% collected inside net	31.6*	36.5	24.3	25.7	38.3	20.2
Killing effect (%) *vs.* P2	-	-	-0.27	4.61*	28.8**	33.96**
Personal protection (%) *vs.* P2	-	-	-7.04	0	23.23*	-7.74

Extended data table 2 | Complete results summary of the hut trial in Tengrela, Cascades Region, Burkina Faso. Treatment codes: UT (Unmodified untreated polyester bednet), P2 (unmodified Permanet 2.0), P2+P2B (Permanet 2.0 bednet and barrier of P2.0); P2+NPB (P2 net and non-pyrethroid mixture [indoxacarb/ fenazaquin, 3-5%]); P2+OPB (P2 and fenitrothion-dipped barrier, 0.5g/m^2); UT+OPB (untreated bednet and fenitrothion-dipped barrier). Outcomes are defined in Methods. Asterisks denote significant differences between treatments (*P*=0.05-0.01*; 0.01-0.001**;<0.001***). All comparisons *vs.* UT, unless stated otherwise.

Percentage Deterrence: Poisson regression GLM; P2+OPB, *n*=6, df=5, Z=3.02 *P*=0.02; UNT+OPB, *n*=6, df=5, Z=2.21, *P*= 0.03.

Personal protection: Negative Binomial GLM; P2+OPB, *n*=109, df=5, Z=-2.649, *P*=0.008.

Killing effect: Poisson regression GLM; P2+NPB, *n*=44, df=5, Z= 2.127, *P*= 0.03; P2+OPB, *n*=133, df=5, Z= 7.612, *P*<0.001; UT+OPB, *n*=152, df=5, Z=8.320, *P*<0.001.

Percentage exiting: Negative Binomial GLM; UT, n=121, df=5, Z= -5.805 P<0.001.

Percentage collected inside net: Negative Binomial GLM; UT, n=163, df=5, Z=-2.047 P<0.0407.

Killing effect vs. unmodified P2: Poisson regression GLM; P2+NPB, n=44, df=5, Z= 1.921, P= 0.04; P2+OPB, n=133, df=5, Z= 2.644, P=0.008; UT+OPB, n=152, df=5, Z=5.322, P=0.005.

Personal protection vs. unmodified P2: Negative Binomial GLM; P2+OPB, n=109, df=5, Z=1.61, P=0.03.

Parameters	Parameter estimates for Tengrela, Cascades Region simulations						
Baseline prevalence	60% (at peak transmission season)						
Assumed proportion *An. gambiae s.s.*	0.577						
Assumed proportion *An. funestus s.s.*	0.223						
Assumed proportion *An. arabiensis*	0.200						
Net coverage in 2015	95.7%						
Parameterization data from (*5*) *Estimated*				**Parameterization from experimental hut data** *Observed*			
	Permanet 2.0 (P2)		**PBO-net**	**Untreated net**	**P2 nets**	**P2+OPB**	**UT+OPB**
Assumed level of pyrethroid resistance	0%	99%	99%	-	-	-	-
Probability of repeating on encounter with net, r_{N0}	0.310	0.373	0.415	0.187	0.629	0.608	0.556
Probability of dying upon encounter with net, d_{N0}	0.510	0.140	0.203	0.007	0.047	0.247	0.288
Net half-life (years), γ_N	2.640	0.355	0.551	0.222	0.253	0.551	0.644

Extended data table 3 | Transmission model parameter estimates used to test the effect of organophosphate panels on bednets in the Cascades administration region of Burkina Faso. All other parameters match those previously reported *(21,29,30,33)*. Parameter estimates are noted for: i) standard nets (*e.g.* Permanet 2.0) working optimally; ii) standard nets working as predicted for the resistance scenario where 99% of mosquitoes survive during a discriminatory dose WHO bioassay test in the presence of pyrethroid insecticides; iii) Permanet 2.0 with an organophosphate barrier, and; iv) an untreated net with an organophosphate barrier.

Mosquito Strain (net treatment)	Net Region	Number of net contacts			Duration of contact (s)		
		Total	mean/test (SD)	% of total contact	Total	mean/test (SD)	% of total contact
Susceptible (unmodified P2)	Roof	943	380.1 (311.2)	55.0	1159	579.6 (551.5)	60.4
	Sides	771	385.5 (531.1)	45.0	759.9	379.9 (481.1)	39.6
Resistant (unmodified P2)	Roof	1103	220.6 (61.1)	90.6	881	176.2 (132.5)	95.2
	Sides	114	28.8 (11.1)	9.4	44	8.8 (12.3)	4.8
Susceptible (P2+P2B)	Barrier	15	3.0 (1.2)	0.5	1	0.28 (0.4)	0.0
	Roof	2182	436.4 (359.1)	70.7	4605	921.1 (736.4)	83.8
	Sides	887	117.4 (285.9)	28.8	889	177.9 (299.8)	16.2
Resistant (P2+P2B)	Barrier	154	29.6 (15.9)	3.9	461	92.3 (87.7)	15.1
	Roof	3287	534.6 (390.4)	83.7	2313	374.3 (265.1)	75.6
	Sides	468	83.2 (118.8)	12.4	286	52.8 (111.9)	9.3
Resistant (P2+OPB)	Barrier	150	24.1 (18.3)	10.5	249	41.5 (39)	14.4
	Roof	1187	95.5 (108.2)	82.8	1445	167.5 (192.3)	83.1
	Sides	49	4.0 (2.4)	6.7	44	3.8 (7.8)	2.5

Extended Data Table 4 | Frequency and duration of mosquito contact with bednets and barriers in the laboratory. The number, location and duration of mosquito contact at unmodified and barrier bednets; data from video recordings of the bioassays in Fig 1b (25 female mosquitoes, 1hr). The bednet roof was the primary contact location in all treatments (t-test: IS, P=0.45; IR, P=0.19; IR/OPB, P=0.93). Contact with treated netting (bednet+barrier) was similar between treatments for IS (mean±SD contact/ trial: 959±1032s and 1099±1035s; t-test, P=0.839) and IR mosquitoes (185±144.8 vs. 519±455.7, t-test, P=0.478; Fig. 2G); and between P2 and P2+OPB (185.0±144.8 vs. 212.8±239.1, t-test, P=0.309) or number (249.4±7.2 and 123.5±13; t-test, P=0.056).

Net treatment	Net Region	Number of net contacts			Duration of contact (s)		
		Total	mean/test (SD)	% of all contact	Total	mean/test (SD)	% of all contact
P2+OPB	Barrier	40	10 (4.1)	10.9	78.8	19.7 (19.8)	26
	Net	329	82.3 (70.5)	89.1	224.1	56 (47.5)	74
UT+OPB	Barrier	174	43.5 (46.8)	6.3	220.7	55.2 (64.7)	17.7
	Net	2607	651.6 (915.6)	93.7	1024.9	256.3 (301.6)	82.3

Extended data Table 5 | Frequency and duration of contact at bednets with OP-treated barriers by wild *Anopheles coluzzii* in Banfora, Burkina Faso. The number, location and duration of mosquito contact on barrier bednets recorded during tests (Fig. 1B). Data refer to 2hr video recordings, with 25 female mosquitoes released. Comparisons of number or duration of contacts between treatments were not significant for the bednet or barrier, based on t-tests (normality tested using Shapiro-Wilk test). When bednet and barrier contacts were combined, duration was significantly higher in UT+OPB (t-test; n=5, df=12, t = -2.19, P=0.048).

Date	Insecticide	Knockdown at 1hr (%)	Mortality at 24hr (%)	No. mosquitoes tested
Aug 2016	Pyrethroid control	0	0	23
	Deltamethrin 0.05%	14.89	9.57	94
	Organophosphate control	0	5.26	19
	Fenitrothion 1%	0	94.44	90
Jun 2017	Pyrethroid control	--	0	57
	Deltamethrin 0.05%	--	35.67	157
Oct 2017	Organophosphate control	0	0	25
	Fenitrothion 1%	98.98	100	98
Mar 2018	Pyrethroid control	--	1.61	62
	Deltamethrin 0.05%	--	17.39	69
Sep 2018	Pyrethroid control	--	0	311
	Deltamethrin 0.05%	--	0	125

Extended data table 1 | Insecticide susceptibility status of the wild *Anopheles gambiae s.l.* population at Tengrela, Banfora in Cascades region of Burkina Faso. Adult female mosquitoes were tested using the WHO tube test. Mortality rates of less than 95% are indicative of resistance.

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		Low contact		High contact	
Mosquito strain	**Treatment**	**Swooping**	**Visiting**	**Bouncing**	**Resting**
IS	P2	11.2 (0-36.7)	31.6 (0-117.8)	292.6 (0-1350.5)	11.2 (0-303.3)
IS	P2+P2B	1013.3 (0-3755.5)	194.8 (0-639.7)	45.0 (0-113.2)	1013.3 (0-1183.25)
IR	P2	13.9 (0-27.8)	22.2 (0-46.6)	78.6 (0-222.0)	13.9 (0-64.9)
IR	P2+P2B	20.8 (0-55.4)	45.7 (0-96.0)	77.7 (0-167.7)	20.8 (0-64.2)
IR	P2+OPB	16.5 (0-34.1)	25.7 (0-61.7)	25.5 (0-79.4)	16.5 (0-44.9)
Wild	P2+OPB	62.1 (0-138.0)	12.6 (0-36.6)	2.6 (0-13.5)	62.1 (57.8-66.47)
Wild	UN+OPB	82.7 (0-173.1)	23.7 (0-64.7)	12.9 (0-39.1)	82.7 (68.2-96.6)

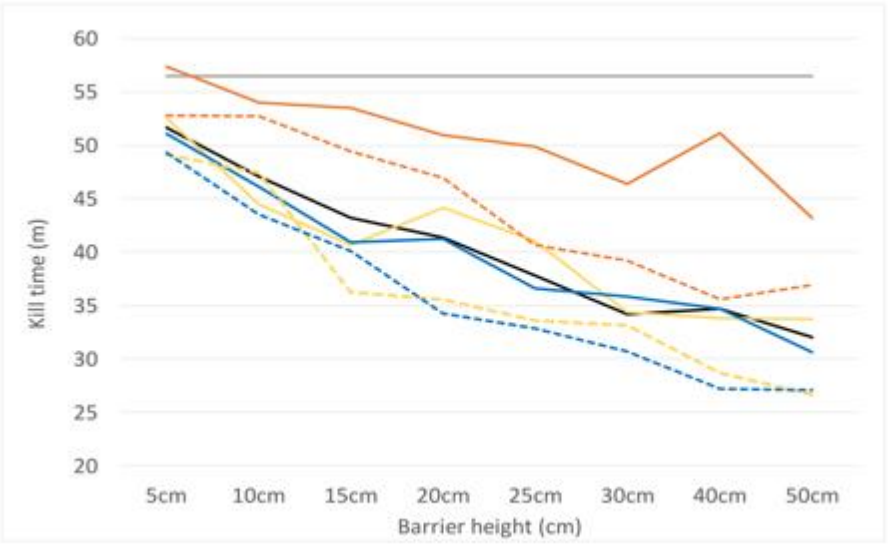
Extended data Table 6 | Behaviour modes of *Anopheles gambiae* at bednets with or without barriers Duration of activity in each behaviour mode; data from video recording of activity of 25 adult female *Anopheles gambiae s.l.* over 60min (pyrethroid susceptible [IS] or resistant [IR] strains; top) or 120min (wild Burkina Faso population, bottom). Total duration of all tracks classed in each behaviour mode (geometric mean ±SD, seconds). Since multiple mosquitoes were often active simultaneously in the field of view, the total activity times could exceed 60 minutes.

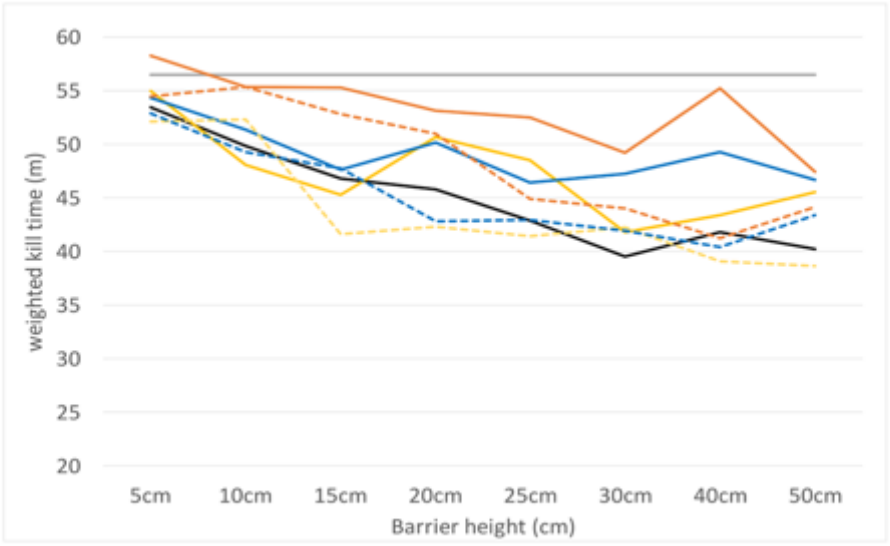
Behaviour modes, defined previously[12], were as follows: Swooping - tracks that did not contact netting; Visiting - tracks of relatively long flight period interspersed with infrequent bednet contacts, characterized by sharp trajectory turns of ≥80° and 0.4s minimum interval between multiple contacts; Bouncing - tracks of multiple rapid netting contact, at intervals of less than 0.4s, including short flights between contacts, or unbroken contact without being static, *e.g.* 'walking' and 'probing'; Resting - static for at least 0.75 seconds, or velocity less than 1.33mm/s, unbroken contact with net.

Barrier Height (cm)	**Standard unmodified bednet**	**T Barrier**	**L Barrier**	**V Cross**	**D Cross**	**Bi T Barrier**	**Bi V Cross**	**Bi D Cross**
A. Mean total mosquito population contact time (min)								
0	40.71	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5	N/A	39.98	44.77	44.36	49.60	43.46	44.36	51.58
10		41.06	49.86	51.31	53.23	47.69	51.31	58.15
15		40.79	56.13	56.83	58.04	51.97	56.83	66.98
20		41.45	60.33	62.00	62.89	55.64	62.00	73.09
25		41.63	64.65	66.49	65.74	57.87	66.49	80.23
30		42.23	68.52	69.45	67.41	61.86	69.45	84.06
40		42.50	72.61	73.94	73.09	66.35	73.94	94.29
50		42.75	77.01	78.11	75.73	69.96	78.11	103.08
B. Mean time to kill the entire mosquito population, when both bednet and barrier are insecticide-treated (min)								
0	56.50	N/A	N/A	N/A	N/A	N/A	N/A	N/A
5	N/A	54.17	51.67	52.56	51.11	52.78	49.17	49.33
10		60.00	47.11	44.50	46.11	52.72	47.44	43.56
15		56.00	43.22	40.67	40.89	49.44	36.28	40.11
20		56.28	41.33	44.17	41.22	46.94	35.56	34.28
25		53.28	37.83	41.06	36.61	40.67	33.61	32.83
30		54.17	34.17	34.39	35.83	39.22	33.11	30.67
40		53.00	34.67	33.83	34.67	35.61	28.67	27.22
50		51.83	32.00	33.72	30.61	36.94	26.67	27.06
C. Mean population kill time when only the barrier is insecticide-treated (min)								
5		N/A	34.55	29.31	27.02	37.78	19.11	19.98
10		N/A	18.31	16.53	19.06	25.36	10.42	11.32
15		39.89	17.71	13.26	10.60	20.03	8.96	8.35
20		33.96	10.06	10.19	11.27	16.25	8.48	6.74
25		28.54	10.20	9.09	9.07	9.87	6.81	6.49
30		25.79	9.93	9.27	6.56	11.44	6.90	5.44
40		25.40	6.60	7.54	7.15	9.90	5.20	4.94
50		20.89	6.69	6.66	6.78	7.31	4.82	4.34

Extended data Table 7 | Comparison of simulated performances of different barrier designs and heights. (A) Mean total mosquito population contact time (duration of all contact and resting events; minutes) per experiment for a standard untreated bednet and different untreated barrier designs at different heights. Note: with no negative impact from untreated net contact, virtual mosquitoes revisit the net *ad infinitum*, hence high contact rates within 1hr. (**B)** Mean time in minutes to kill the entire mosquito population, when both bednet and barrier are insecticide-treated, by each barrier design and barrier height on treated nets. All net contact areas deliver a dose of 0.05 units per contact. The insecticide treatment is identical on every surface treated, and equivalent to a Permanet 2.0 in terms of repellency. The agent response to contacting a *treated* net is to decrement health and to select a new random direction and fly away. Thus, the insecticide

approximates contact irritancy and not spatial repellency. **(C)** Mean population kill time when only the barrier is insecticide-treated (dose=1 unit per contact). Note: 5 and 10cm T-barriers did not kill the entire mosquito population in all runs.





Extended Data Fig. 1 | Comparing different barrier designs and heights by evaluating performance *in silico*. **(A)** Population kill time (total time needed to achieve complete population death) when insecticide is delivered by both bednet and barrier, for different barrier designs at increasing barrier height. **(B)** Population kill time as in A, weighted by surface area with a standard unmodified bed net as reference. Plot colours correspond to barrier design borders in Fig 4.