LETTERS
https://doi.org/10.1038/s41564-019-0607-2
nature microbiology

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

Gregory P. D. Murray[1], Natalie Lissenden[1], Jeff Jones[1], Vitaly Voloshin[2], K. Hyacinthe Toé[3], Ellie Sherrard-Smith[4], Geraldine M. Foster[1], Thomas S. Churcher[4], Josephine E. A. Parker[1], Catherine E. Towers[2], Sagnon N'Falé[3], Wamdaogo M. Guelbeogo[3], Hilary Ranson[1], David Towers[2] and Philip J. McCall[1*]

Transmission of *Plasmodium falciparum* malaria parasites occurs when nocturnal *Anopheles* mosquito vectors feed on human blood. In Africa, where malaria burden is highest, 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 (ref. [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 before 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 is currently possible. Tested against wild pyrethroid-resistant *A. 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 the World Health Organization 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 replaced by an increase of 3.5 million malaria cases in the ten African countries[2] with the highest burden. 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]. Thus, 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 have identified insecticide combinations effective against pyrethroid-resistant vectors[3,10,11], but toxicity restrictions on risks to occupants, especially infants, and the higher cost of new insecticides limit bednet-treatment options.

Previous studies have shown that *A. gambiae* host-seeking 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 ('swooping') net contact[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 wider range of insecticides than possible with standard bednets[16].

As a proof of concept, we evaluated a single transverse barrier (0.5 m tall, 0.9 m 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) or untreated netting dipped in fenitrothion (OPB, 0.02 g m^{-2}), an organophosphate widely used for indoor residual spraying (IRS) against pyrethroid-resistant mosquitoes[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 *A. gambiae* strains, respectively, within 48 h of exposure. Adding the P2B did not affect mortality rates with either strain, but the OPB was significantly better, killing 100% of resistant mosquitoes within 48 h (90% at 24 h; *t*-test, $P < 0.01$).

In a hut trial in a malaria-endemic setting in the Cascades region, Burkina Faso—where *A. gambiae* sensu lato (s. l.) vectors are highly resistant to deltamethrin but susceptible to fenitrothion (Extended Data Fig. 1)—we tested three different transverse barriers (Fig. 1d): P2B; fenitrothion-dipped netting (OPB; with 0.5 g m^2 fenitrothion, 20× higher than in the previous laboratory tests, equivalent to 25% of the target dose of IRS treatment); non-pyrethroid mixture (NPB; comprising indoxacarb and fenazaquin, each at 3–5%). The results show that all treatments significantly reduced mosquito entry rates

[1]Vector Biology Department, Liverpool School of Tropical Medicine, Liverpool, UK. [2]School of Engineering, University of Warwick, Coventry, UK. [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso. [4]School of Public Health, Faculty of Medicine, Imperial College, London, UK. *e-mail: philip.mccall@lstmed.ac.uk

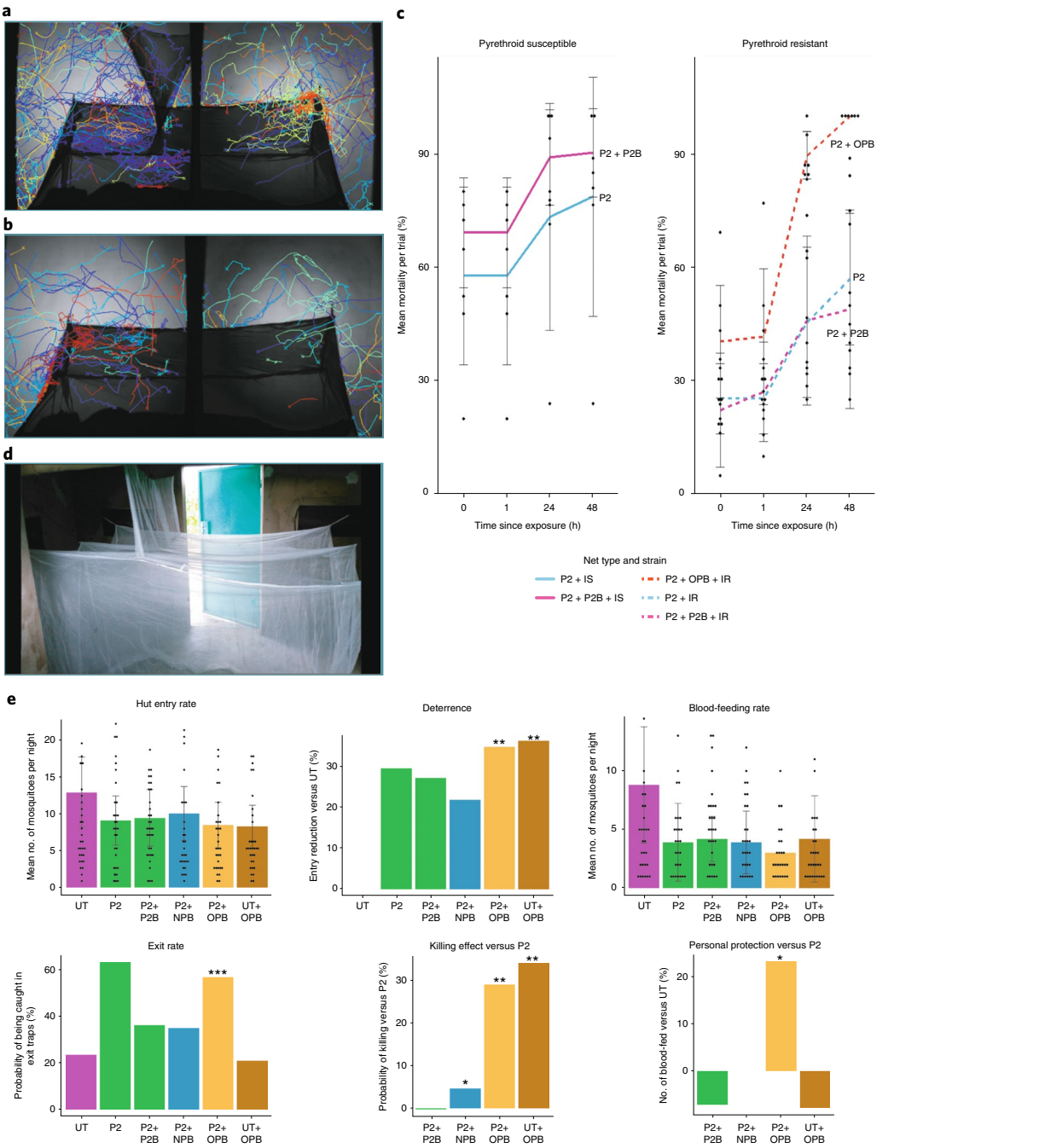


Fig. 1 | Performance of barrier bednets in laboratory and semi-field trials. a,**b**, Infrared tracks of mosquito flights at P2 bednets with a 50-cm-high transverse barrier (positioned off-centre, above the sleeper's torso) (**a**) and unmodified P2 (**b**). Tracks were recorded during bioassays; 25 mosquitoes, 60 min. **c**, Mean (±s.d., $n=6$ trials per treatment) mortality rates of *A. gambiae* strains susceptible (IS) or resistant (IR) 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 IS (*t*-test, $n=82$, d.f. = 5.3, $t=0.75$, $P=0.48$) and IR (*t*-test, $n=109$, d.f. = 8.7, $t=0.62$, $P=0.55$). P2 + OPB mortality at 24 h (90%) and 48 h (100%) significantly exceeded unmodified P2 mortality (IR 24 h, 45%; *t*-test, $n=91$, d.f. = 6.1, $t=5.21$, $P<0.01$; IR 48 h, 57%; *t*-test, $n=31$, d.f. = 5.1, $t=6.5$, $P<0.01$) and P2 + P2B (IR 24 h, 46%; *t*-test, $n=91$, d.f. = 5.8, $t=4.61$, $P<0.01$; IR 48 h, 49%; *t*-test, $n=41$, d.f. = 5.1, $t=4.74$, d.f. = 5.1, $P<0.01$). **d**, Barrier bednet in situ, Burkina Faso. **e**, Summary of key results from the hut trial; all comparisons versus UT, unless stated otherwise; asterisks denote significant differences ($0.05 \geq *P > 0.01$, $0.01 \geq **P > 0.001$ and $***P < 0.001$). Data are mean ± s.d. (Extended Data Fig. 2). Non-pyrethroid barriers (P2 + NPB, P2 + OPB and UT + OPB) killed significantly more than untreated controls (Poisson regression generalized linear model; $n=44$, d.f. = 5, $Z=2.12$, $P=0.03$; $n=133$, d.f. = 5, $Z=7.61$, $P<0.001$; and $n=152$, d.f. = 5, $Z=8.32$, $P<0.001$, respectively). Personal protection (number of blood-fed mosquitoes prevented relative to untreated nets) was significantly higher with P2 + OPB (66%; negative binomial GLM; $n=109$, d.f. = 5, $Z=-2.649$, $P<0.01$); the reduction with UT + OPB was not significant (negative binomial GLM; $n=153$, d.f. = 5, $P=0.954$). Killing effects of test net versus unmodified P2 were higher with P2 + NPB (Poisson regression GLM; $n=44$, d.f. = 5, $Z=1.82$, $P=0.043$), P2 + OPB ($n=133$, d.f. = 5, $Z=5.91$; $P=0.008$) and UT + OPB ($n=152$, d.f. = 5, $Z=7.53$, $P=0.044$) (Extended Data Fig. 2). Treatments: UT, untreated unmodified bednet; P2, unmodified Permanet 2.0 bednet with 55 mg m^{-2} deltamethrin; P2 + P2B (Permanet 2.0 and P2 barrier); P2 + OPB, P2 and fenitrothion barrier (0.02 g m^{-2} in laboratory, 0.5 g m^{-2} in field. Treatments P2 + NPB (P2 net and non-pyrethroid barrier (3–5% indoxacarb and fenazaquin)) and UT + OPB (untreated bednet and fenitrothion-dipped barrier) were tested in the field only.

and increased exit rates compared with untreated bednets (Fig. 1e and Extended Data Fig. 2; GLM, $P<0.001$). All three non-pyrethroid barriers increased killing, particularly OPB; OPB on P2 bednets killed 28.8% more mosquitoes 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, OPB on untreated bednets increased killing by nearly 34% over unmodified P2 ($P=0.008$), without significant loss in personal protection ($P=0.954$).

We investigated these encouraging field results using a mathematical model of malaria transmission dynamics to estimate the expected public health impact in the Cascades region if existing nets were replaced with barrier bednets. By necessity, the model simplifies malaria transmission as a series of mechanistic processes on the basis of assumptions about the probability of transmission[20–22]. Replacement with barrier bednets was modelled to determine how this would: (1) reduce the numbers of mosquitoes entering the house to feed; (2) reduce the feeding success of mosquitoes that enter houses and; (3) increase mosquito mortality relative to a scenario without nets. LLINs reduce malaria infections in mosquitoes and humans by affecting 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 Fig. 2) were converted to summary estimates of the probability of mosquitoes being killed, repeating host-searching behaviour or successfully feeding on each attempt, for each tested net and barrier type (Extended Data Fig. 3), with reductions in prevalence continuing until the active ingredient had waned. Over three years following replacement of P2 nets with P2 + OPB nets, the mathematical model predicted relative reductions in clinical malaria incidence of 10.4% (95% confidence interval (CI) 0–34.47%), 13.3% (95% CI 0–37.12%) and 16.4% (95% CI 1.15–39.76%), at net coverage rates of 60%, 80% and 95%, respectively. With OPBs on untreated (UT) nets (UT + OPB), predicted impacts were even greater, at 13.8% (95% CI 0–37.30%), 18.4% (95% CI 4.62–40.71%) and 21.4% (95% CI 11.66–43.67%) for the same coverage levels. We compared this result with next-generation pyrethroid LLINs that are co-treated with piperonyl butoxide (PBO) to disable resistance mechanisms, which are recommended by the World Health Organization (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% (95% CI 0–36.09%), 16.2% (95% CI 0–39.14%) and 18.4% (95% CI 0–41.66%) at similar respective coverage levels (Fig. 2b). These results, and the 12% reduction reported with another new pyrethroid LLIN (Olyset duo, containing pyriproxyfen) also in the Cascades region, are similar to the predictions for barrier bednets.

We investigated how barriers target mosquitoes using infrared 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 Fig. 4), demonstrating that barriers did not alter this characteristic behaviour at standard LLINs[12,13]. Adding P2Bs increased overall activity compared with unmodified LLINs ($P<0.001$) (Fig. 3a,b), but not contact; P2Bs increased flight activity in behaviour modes with zero or minimal contact ($P<0.001$) (Fig. 3c,d and Extended Data Fig. 6).

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

Fenitrothion surface residues can be strongly repellent[19], whereas P2 netting (deltamethrin) exerts a far weaker effect[12]. Thus without deltamethrin (P2 + OPB versus 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 less contact overall (that is, 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 (0.02 g m^{-2} fenitrothion), where low-contact

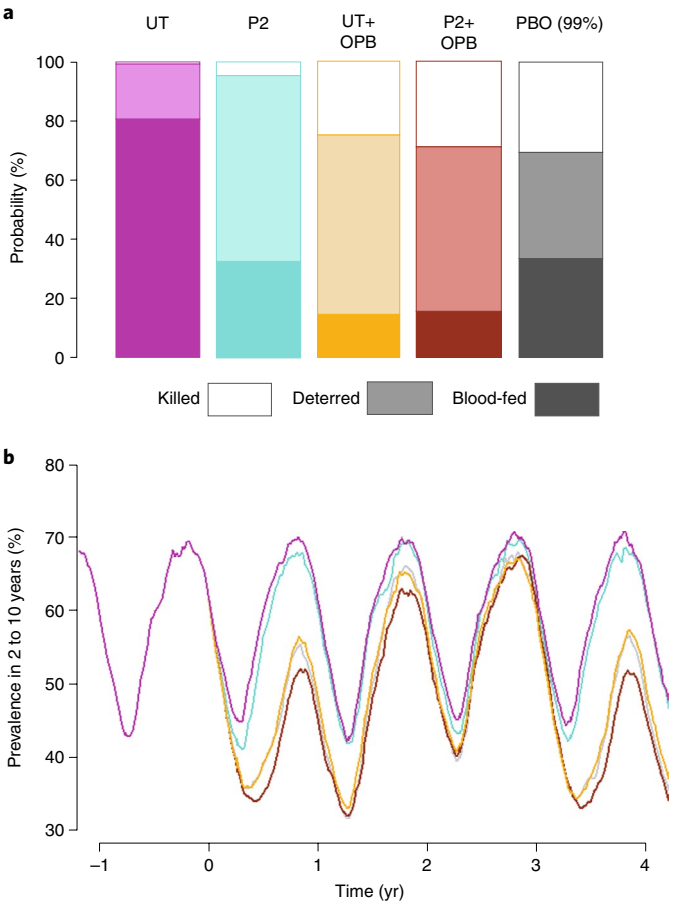


Fig. 2 | Summary of efficacy estimates of different bednet-barrier combinations and comparison with estimates for PBO bednets at high pyrethroid resistance. a, The probable outcome of a mosquito feeding attempt is determined for each net intervention: mosquitoes are either killed, deterred but return to feed again, or blood-feed successfully. Summary estimates were generated from hut trial data for UT and P2 with or without OPB (Extended Data Fig. 2). At a pyrethroid resistance level of 99%, the probability of an OPB bednet killing mosquitoes was comparable to that of the PBO nets, with fewer mosquitoes blood-feeding, regardless of whether the bednet was untreated (UT + OPB) or treated (P2 + OPB). **b**, The efficacy of these five bednet-barrier combinations drives the contrasting predicted reductions in prevalence among two- to ten-year-old children for the years following net-distribution campaigns at year zero and year three. Colour codes match the different bednet-barrier combinations in **a**. The model's parameters reflected the seasonality, entomology and endemicity of malaria in Cascades region, Burkina Faso.

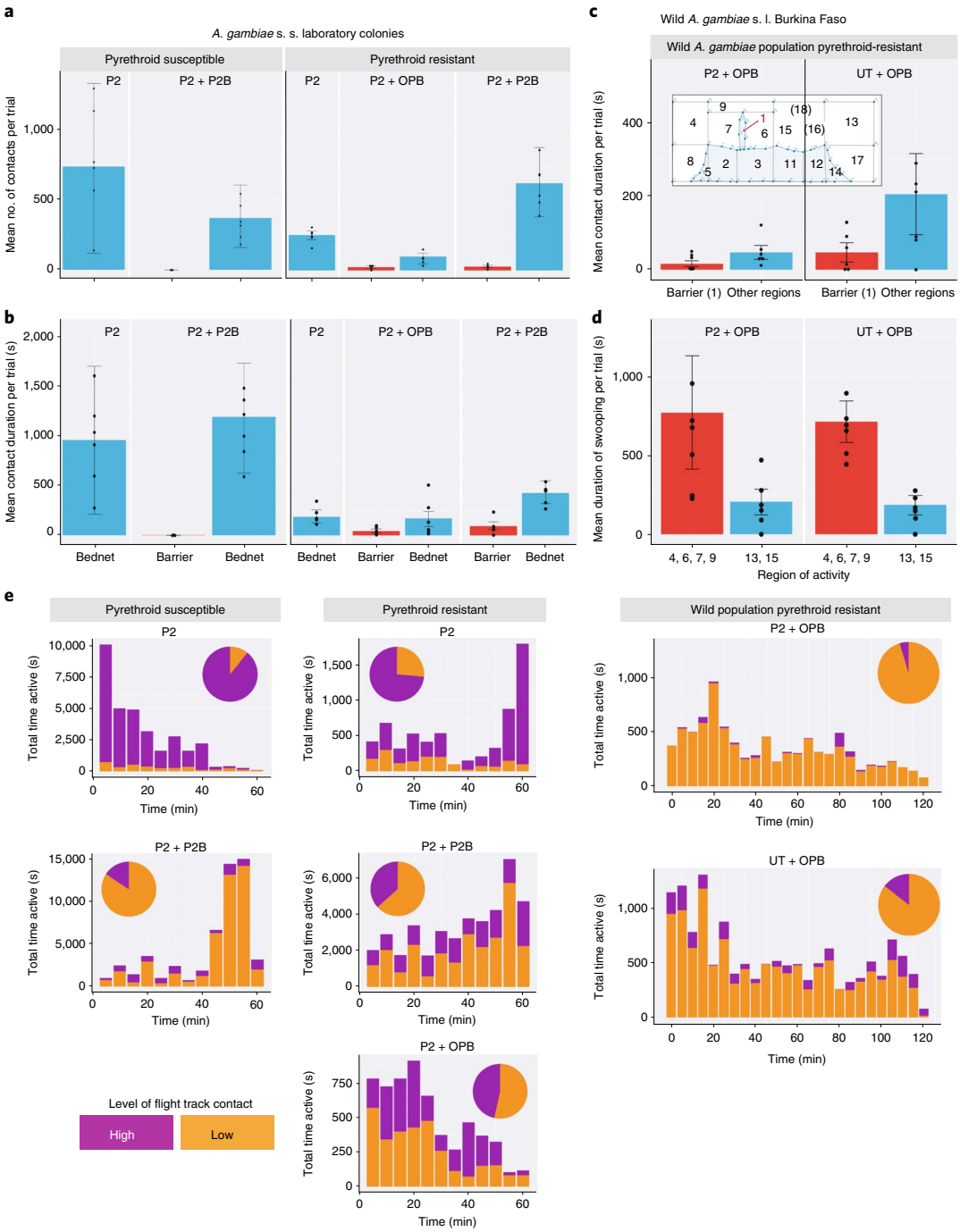


Fig. 3 | Behaviour at barrier bednets of *A. gambiae* s. l. laboratory colonies and wild population in Burkina Faso. a,**b**, Mean number (**a**) and duration (**b**) per test of flights contacting bednet or barrier for each treatment and mosquito laboratory strain. **c**,**d**, Mean duration of barrier or bednet contact in regions shown in the inset key (**c**) and mean total time spent in swooping mode (no net contact) (**d**) for wild mosquitoes. Inset: regions 16 and 18 correspond with 6 and 9, respectively, but activity in 15, 16 and 18 was pooled for analysis. Data are mean ± s.d.; number of independent samples is shown in Extended Data Figs. 4a,b, 5c and 6d. **e**, Activity at 5 min intervals during 60 min (laboratory) or 120 min (field) 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 in Fig. 1. Behaviour modes[12]: 'Swooping', tracks without net contact; 'Visiting', relatively lengthy flights with infrequent net contacts, trajectory turns of ≥ 80° and 0.4 s minimum interval between contacts; 'Bouncing', multiple rapid contact, intervals < 0.4 s or unbroken contact, never static; 'Resting', static > 0.75 seconds, velocity < 1.33 mm s^{-1}, unbroken net contact. Flight activity increased significantly with P2Bs (mean flight activity per trial; IS: 5,012 ± 1,975 s and 1,341.6 ± 741 s; Wilcoxon rank-sum test; $n = 25$, d.f. = 1, $W = 5422$, $P < 0.001$; IR, 577.2 ± 79 s and 464.4 ± 30 s; $n = 65$, d.f. = 1, $W = 23{,}017$, $P < 0.001$), but not OPBs (371.2 ± 45 s and 464.4 ± 30 s; $n = 65$, d.f. = 1, $W = 23{,}689.5$, $P = 0.155$, P2 and P2 + OPB respectively). Low contact activity increased with P2Bs in IR (*t*-test, $n = 65$, d.f. = 176, $t = 3.50$, $P < 0.001$) and IS (*t*-test, $n = 37$, d.f. = 73, $t = 2.519$, $P = 0.01$) mosquitoes, but not with OPBs ($P = 0.298$). Significantly more swooping activity occurred over the host's torso proximal to the barrier; *t*-test, $n = 5$, d.f. = 7.61, $t = 2.6976$, $P = 0.028$). Swooping (that is, zero contact) was significantly higher in both OPBs in the field (P2 + OPB, 79.5% of all flights; Pearson's χ^2 test; $n = 125$, d.f. = 3, $\chi^2 = 163.4$; UT + OPB, 64.2%; $n = 124$, d.f. = 3, $\chi^2 = 86.7$; $P < 0.001$). Netting contact duration (bednet plus barrier) was higher with OPBs on an untreated bednet than on a P2 bednet (*t*-test, $n = 5$, d.f. = 12, $t = -2.19$, $P = 0.048$).

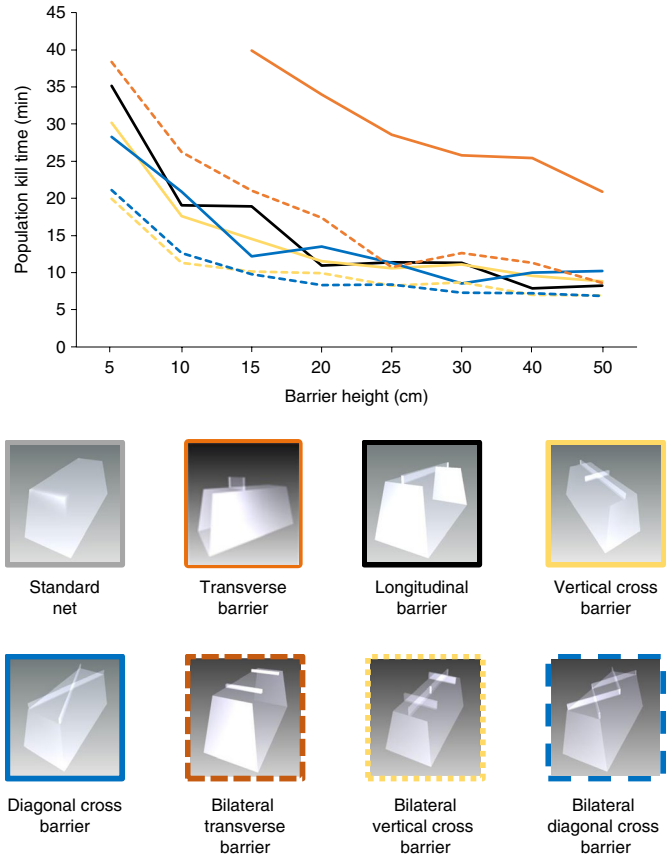


Fig. 4 | Comparing different barrier designs and heights by evaluating performance in silico. Population kill time (total time needed to achieve complete population death in minutes) for different barrier bednets when the bednet is untreated and insecticide is deployed only on the barrier. Values are weighted by surface area, using a transverse barrier of 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, other than the standard net.

activity (53.3% total) was not significantly different from that with unmodified P2 ($P=0.298$), but markedly lower than with the higher dosages in the field trial (0.5 g m^{-2}; 85–95%; Fig. 3e). Increased flight without contact most probably combines a response to an insecticide's inherent repellent properties with the ability of *A. gambiae* to avoid net collisions[12] and may typify behaviour at barriers; thus careful selection of net and barrier treatments is required to maximize lethality.

Nevertheless, increased mosquito–netting contact directly increases insecticide exposure and we investigated whether alternative barrier designs and sizes could increase the frequency of contact. We used an agent-based, 3D spatiotemporal model of mosquitoes at an occupied LLIN in a virtual insectary to determine the effect of the 50 cm transverse barrier (Fig. 4). With untreated netting on bednet and barrier, transverse barriers showed only modestly increased contact duration over unmodified bednets (42.75 and 40.71 min, respectively; 25 mosquitoes, 1 h), whereas the complex bilateral diagonal cross barrier accrued 103.08 min of contact (Extended Data Fig. 7). When both bednet and barrier were treated with insecticide, contact and kill rates increased with greater barrier surface area and complexity (Extended Data Fig. 8). However, as larger complex barriers increase manufacturing costs, barrier area was weighted by cost per m^2, and the 30 cm longitudinal barrier performed almost as well as the 50 cm bilateral vertical cross (Extended Data Fig. 8). Encouraged by our semi-field trial result (Fig. 1e), we modelled performance where only barriers delivered insecticide, increasing the hypothetical dosage such that barrier-only contacts killed all mosquitoes within a 1 h simulation time window. Again, complex designs killed the population more rapidly, but performance levelled off at 20 cm barrier height. (Extended Data Fig. 7). Weighted by surface area however, and with the transverse barrier as reference, a simple 40 cm longitudinal barrier was nearly as effective as the more complex bilateral cross designs (Fig. 4) and was therefore a lead candidate for further development.

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

We emphasize that we are not specifically proposing the use of 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 and wash resistance from many insecticides or from non-insecticidal treatments[26,28]. Considerable industry and public sector investments 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 position of the barrier might enable relaxation of constraints on active ingredients for bednets (for example, 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.

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, 11 October 2016, Liverpool) and Centre National de Recherche et de Formation sur le Paludisme (Deliberation no. 2016-9-097, 20 September 2016, Ouagadougou). No adverse effects of treatment or mosquito-borne infections were reported by volunteers during the course of the study.

Bednet and barrier materials. In all tests, rectangular bednets measuring 2 m × 0.9 m × 1.5 m 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. 1 and 2b,c). Hence, the net height was 0.93 m near the camera and 1.19 m at the rear. Pyrethroid-treated nets were Permanet 2.0 (75 denier polyester net impregnated with deltamethrin at 55 mg m^{-2} (Vestergaard)). New LLINs were hung for four weeks before 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 Evaluation of barrier net performance in the laboratory).

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 0.9 m wide (extending edge-to-edge across the LLIN) and was fitted above the tilted roof of the rectangular LLIN. It measured 0.8 m high (front) and 0.54 m (rear) to ensure the top edge was horizontal at a total height of 1.9 m from the floor. The lower edge was pinned to the roof of the net slightly off-centre, at 0.8 m from the head end (that is, 0.2 m from the midpoint) (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 5 mm carbon fibre rods.

Insecticidal barrier panels (0.6 m^2) were cut from new Permanet 2.0 LLINs or untreated polyester netting treated with the organophosphate fenitrothion (to make the OPB). We selected this low fenitrothion concentration (100 times less than that used in IRS) to minimize any potential repellent effects of organophosphate residues. OPBs (0.02 g m^{-2}) were prepared by immersing eight pre-cut untreated net barriers (plus 0.2 m^2 fragment to ensure all liquid was absorbed) into a 224 ml aqueous emulsion containing 0.1 g of fenitrothion (Greyhound Chromatography and Allied Chemicals). Unmodified Permanet 2.0 LLINs were used for comparison. Fresh barriers were used for each test repeat (six IR and five for IS).

Evaluation of barrier net performance in the laboratory. Initial tests were conducted on human-occupied bednets in a dedicated insectary in the UK (5.6 m × 3.6 m × 2.3 m high; climate controlled at 27 ± 2 °C, 70 ± 10% relative humidity), using *A. gambiae* s. l. strains from LSTM colonies of Kisumu (*A. gambiae* senso stricto (s. s.); IS, $n = 9$) or Tiassalé (*A. gambiae* s. s. and *Anopheles coluzzii* mix; resistant to pyrethroids and the majority of other insecticides used in public health, IR, $n = 17$ (ref. [25]). Three- to five-day-old unfed adult female mosquitoes (25 per experiment) were deprived of sugar and water for 4 h before transfer to the experimental room to acclimatize (1 h) 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 2 m × 0.9 m mattress (0.18 m thick; surface at 0.48 m 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 (850 mm wavelength, 1,000 mA minimum; M850L2, Thorlabs) aligned with a pair of Fresnel lenses (mounted either side of the bed, with a 43 cm gap between the lens and mattress on each side) and monochrome camera with 12.5 mm imaging lens (Baumer HXC40NIR, Camera Link, 4 megapixels; Lambda Photometrics). Video was recorded at 50 frames s^{-1} using StreamPix software (www.norpix.com), and data were saved as .seq files. Thirty minutes after the volunteer entered the bed, recording was started and mosquitoes were released from a paper cup at a height of 2 m, 1.4 m from the net. Activity was recorded for 60 min.

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 h test. All were asked to eschew scented toiletries when testing. The majority were tested with both barrier-modified (P2B or OPB) and unmodified P2 nets on different days, with an average interval of 41 d between their tests. After each 1 h test, the number of live and dead mosquitoes in the room was recorded. Living mosquitoes were maintained with sugar and water and mortality was recorded at 1, 24 and 48 h.

Video tracking mosquitoes in the laboratory. Tracking individual mosquitoes or determining the number of responders among the 25 released was not possible as it was not possible to view the entire room. 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; and '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 Figs. 2a and 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 it was not possible to view the entire room, 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 ($n < 4$).

Evaluation of barrier bednets in the field. Between July and October 2017, barrier nets were tested against adult female mosquitoes morphologically identified as *A. 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 *A. coluzzii* Coetzee and Wilkerson, which have previously been 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 (0.65 m^2 or 0.8 m^2) in a solution of fenitrothion, prepared by adding 7.3 ml or 9 ml of fenitrothion stock solution (0.044 g ml^{-1} in acetone; AK Scientific) to 22 ml or 27 ml acetone, giving 29.3 ml or 36 ml of 0.01 g fenitrothion ml^{-1} acetone, respectively. At an absorbency rate of 45 ml m^{-2}, this deposited 0.5 g m^{-2} on the netting surface, equivalent to 25% of the target dose for IRS treatment. We selected this concentration, 25 times higher than in the initial laboratory experiment, on the basis of the absence of evidence for repellency in the initial laboratory experiments, and out of concern that durability of dipped nets at lower concentrations might be compromised in harsher field conditions.

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

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

1. Untreated control bednet (UT): untreated polyester netting of similar denier and mesh size as LLINs used in other treatments, no insecticidal properties and no barrier
2. Permanet 2.0 LLIN (P2): a WHO Pesticide Evaluation Scheme-recommended standard-size double LLIN (1.6 m × 1.8 m × 1.5 m) treated with deltamethrin at 55 mg m^{-2} with no barrier
3. Permanet 2.0 LLIN with Permanet 2.0 barrier (P2 + P2B): standard LLIN with a barrier element of identical Permanet 2.0 netting
4. Permanet 2.0 LLIN with non-pyrethroid insecticide barrier (P2 + NPB): standard P2 LLIN with an added barrier element treated with a combination of two non-pyrethroid insecticides: indoxacarb (3–5% oxadiazine) and fenazaquin (3–5% quinazoline)
5. Permanet 2.0 LLIN with fenitrothion barrier (P2 + OPB): standard LLIN with an added barrier element treated with the organophosphate fenitrothion, at a concentration of 0.5 g m^{-2}, equivalent to 25% of the level applied in IRS
6. Untreated net with OP barrier (UT + OPB): untreated polyester bednet with an added barrier element treated with 0.5 g 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 on each night (see Supplementary Information, Hut trial rotation plan). A new set of treated and untreated nets was prepared and used in each week of the trial. Before use, all manufactured LLINs and untreated control nets for use in any particular week were removed from their packaging and aired for seven days. OPB 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 was used once with each treatment. After the clothed, barefoot 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. Seated at a distance of 10 m 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 and hut, location (inside net, in hut or in veranda), alive or dead, sex and abdominal status (blood fed, semi-blood fed, unfed, gravid or semi-gravid). Live *A. gambiae* s. l. were sorted by hut and held in paper cups (5 mosquitoes per 250 ml 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 2 h of the test ending and at 24 h intervals thereafter until no mosquitoes remained alive. We quantified and compared a range of outcomes incorporating the standard parameters recommended by the WHO for evaluating LLINs[31]:

- Deterrence: the reduction in hut entry relative to control huts (untreated nets)
- Exophily or repellency: the proportion of mosquitoes found in the veranda traps

- Blood-feeding inhibition: the reduction in blood-feeding compared 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:

$$\text{Personal protection}\,(\%) = \frac{100\times(B_u - B_t)}{B_u}$$

where B_u is the total number blood-fed mosquitoes in huts with untreated nets and B_t 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; that is, 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:

$$\text{Mortality} = \frac{100\times(K_t - K_u)}{T_u}$$

where K_t is the number of mosquitoes killed in huts with treated nets, K_u is the number of mosquitoes killed in huts with untreated nets, and T_u 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[1], as per ref. [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 to 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 Figs. 2 and 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, while 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. PBO synergist nets are the first next-generation nets to reach the market. PBO inhibits specific metabolic enzymes in mosquitoes that can detoxify pyrethroids, thereby extending the active life 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 Fig. 2 outlines the parameter changes made in the model to represent the predicted impact of organophosphate panels on prevalence in two- to ten-year-old children and all clinical cases in the Cascades administrative region in Burkina Faso. In the absence of wash data (used to simulate the natural wearing of the active ingredient of nets and to determine net durability)[4,23], we assumed a conservative estimate for the half-life of barrier nets based on maximum mortality estimates from the experimental hut data. This corresponds to approximately six months for the two barrier nets tested (P2 + OPB and UT + OPB). We compared the effect of PBO nets and barrier nets (P2 + OPB and UT + OPB) relative to P2 nets.

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 6 m × 4 m in area and 3 m 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 (± s.d.) temperature and humidity during recording of 28 °C (± 3.1 °C) and mean (± s.d.) relative humidity of 75% (± 12.5%). Thirty minutes before tests, the volunteer entered the bednet, the mosquitoes were placed in a paper cup resting on the lip of the eave, 2 m above the ground, and the room was closed.
A section of eave screen was cut to enable 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 min of tests to acclimatize to the hut interior environment. All tests were run during the night, starting at or shortly after 19:30.

Five of each bednet–barrier combination (that is, P2 + OPB and UT + OPB) that had previously been used in the hut trial over six nights were used. Human volunteers lay on a 2 m × 0.88 m sleeping mat, with the bednet evenly tucked under by one of the researchers before filming. The recording period lasted 2 h 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 was swept to eliminate or recover any dead or knocked-down mosquitoes. The collected mosquitoes were maintained under ambient conditions in a separate hut nearby, were provided sucrose solution ad libitum and assessed (dead, knocked-down or alive) immediately at collection and 1, 24 and 48 h later. Video was recorded at 50 frames s^{-1} using StreamPix software (www.norpix.com) and 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 (>200 Gb video files). Following this, the video files were compressed with bespoke software using the .mp4 container and a dedicated video card (<5 Gb). 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. We developed an agent-based 3D spatiotemporal model of mosquito behaviour at a human-occupied LLIN in a virtual insectary to compare designs for optimizing barrier-net performance. Indoor vector control testing system (InVeCTS) 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 bednet and human host. For the experiments presented in this document an arena of size 5.6 × 3.6 × 2.3 m was used, corresponding to the experimental insectary at LSTM used previously[10,11]. Barrier bednets were designed from 3D triangular meshes, building on standard 'reference' simple unmodified bednet design (Fig. 4). The standard bednet design measured 2 m long x 0.9 m wide (at its widest point on the floor) and 0.8 m high. Barrier bednets of different designs and heights (5, 10, 15, 20, 25, 30, 40 and 50 cm) were assessed. The bednets were placed in the centre of a virtual insectary (5.6 m long × 3.6 m wide × 2.3 m high) and a population of 25 virtual mosquitoes were released from a wall-mounted position halfway along the longest axis (2.8 m) at a height of 2 m. A human-bait stimulus profile was centred in the bednet design with the head region furthest away from the release location. Each experiment was run for the equivalent of 1 h 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. Welch's independent two-sample unequal variances *t*-tests were used. For all tests, an α-threshold of 0.05 was used. Statistical analyses were performed using R v.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, blood-feeding 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 v.3.1.2 (http://www.R-project.org/). Comparisons of mortality and activity levels were performed on the basis of Welch's two-sample (unequal variances) *t*-test; when the assumption of normality was not met, they were based on a Shapiro–Wilk test, and 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.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The hut trial dataset generated during the current study is available at Dryad Digital Repository (https://doi.org/10.5061/dryad.hqbzkh1b7). All data analysed during this study are available as described in the paper. All other data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the authors on reasonable request.

Code availability

Data handling scripts and video segmentation and tracking software are available from the authors on reasonable request.

Received: 2 May 2019; Accepted: 11 October 2019;
Published online: 2 December 2019

References

1. Bhatt, S. et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* **526**, 207–211 (2015).
2. *World Malaria Report 2018* (WHO, 2018).
3. Protopopoff, N. et al. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. *Lancet* **391**, 1577–1588 (2018).
4. Churcher, T. S., Lissenden, N., Griffin, J. T., Worrall, E. & Ranson, H. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. *eLife* **5**, e16090 (2016).
5. Hemingway, J. The way forward for vector control. *Science* **358**, 998–999 (2017).
6. Pryce, J., Richardson, M. & Lengeler, C. Insecticide-treated nets for preventing malaria. *Cochrane Database Syst. Rev.* **11**, CD00063 (2019).
7. Ranson, H. & Lissenden, N. Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol.* **32**, 187–196 (2016).
8. Ranson, H. & Killeen, G. F. Insecticide-resistant malaria vectors must be tackled. *Lancet* **391**, 1551–1552 (2018).
9. The malERA Consultative Group on Vector Control. A research agenda for malaria eradication: vector control. *PLoS Med.* **8**, e1000401 (2011).
10. Tiono, A. B. et al. Efficacy of Olyset Duo, a bednet containing pyriproxyfen and permethrin, versus a permethrin-only net against clinical malaria in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomised controlled trial. *Lancet* **392**, 569–580 (2018).
11. Bayili, K. et al. Evaluation of efficacy of Interceptor G2, a long-lasting insecticide net coated with a mixture of chlorfenapyr and α-cypermethrin, against pyrethroid resistant *Anopheles gambiae* s. l. in Burkina Faso. *Malar. J.* **16**, 190 (2017).
12. Parker, J. E. A. et al. Infrared video tracking of *Anopheles gambiae* at insecticide-treated bed nets reveals rapid decisive impact after brief localised net contact. *Sci. Rep.* **5**, 13392 (2015).
13. Parker, J. E. A. et al. Host-seeking activity of a Tanzanian population of *Anopheles arabiensis* at an insecticide treated bed net. *Malar. J.* **16**, 270 (2017).
14. Lynd, A. & McCall, P. J. Clustering of host-seeking activity of *Anopheles gambiae* mosquitoes at the top surface of a human-baited bednet. *Malar. J.* **12**, 267 (2013).
15. Sutcliffe, S. & Yin, S. Behavioural responses of females of two anopheline mosquito species to human-occupied, insecticide-treated and untreated bed nets. *Malar. J.* **13**, 294 (2014).
16. McCall, P. J. Mosquito bed net assembly. International patent WO2015063455A1 (2015).
17. Agossa, F. R. et al. Efficacy of various insecticides recommended for indoor residual spraying: pirimiphos methyl, potential alternative to bendiocarb for pyrethroid resistance management in Benin, West Africa. *Trans. R. Soc. Trop. Med. Hyg.* **108**, 84–91 (2014).
18. Gnanguenon, V. et al. Malaria vectors resistance to insecticides in Benin: current trends and mechanisms involved. *Parasit. Vectors* **8**, 223 (2015).
19. Akogbéto, M. et al. Bendiocarb, a potential alternative against pyrethroid resistant *Anopheles gambiae* in Benin, West Africa. *Malar. J.* **9**, 204 (2010).
20. Griffin, J. T. et al. Reducing *Plasmodium falciparum* malaria transmission in Africa: a model-based evaluation of intervention strategies. *PLoS Med.* **7**, e1000324 (2010).
21. Griffin, J. T. et al. Potential for reduction of burden and local elimination of malaria by reducing *Plasmodium falciparum* malaria transmission: a mathematical modelling study. *Lancet Infect. Dis.* **16**, 465–472 (2016).
22. White, M. T. et al. Modelling the impact of vector control interventions on *Anopheles gambiae* population dynamics. *Parasit. Vectors* **4**, 153 (2011).
23. *Conditions for Deployment of Mosquito Nets Treated with a Pyrethroid and Piperonyl Butoxide* (WHO, 2017).
24. Mahama, T. et al. Effectiveness of permanet in Côte d'Ivoire rural areas and residual activity on a knockdown-resistant strain of *Anopheles gambiae*. *J. Med. Entomol.* **44**, 498–502 (2017).
25. Toé, K. H. et al. Do bednets including piperonyl butoxide offer additional protection against populations of *Anopheles gambiae* s. l. that are highly resistant to pyrethroids? An experimental hut evaluation in Burkina Faso. *Med. Vet. Entomol.* **32**, 407–416 (2018).
26. Sternberg, E. D., Waite, J. L. & Thomas, M. B. Evaluating the efficacy of biological and conventional insecticides with the new 'MCD bottle' bioassay. *Malar. J.* **13**, 499 (2014).
27. Janko, M. M. et al. Strengthening long-lasting insecticidal nets effectiveness monitoring using retrospective analysis of cross-sectional, population-based surveys across sub-Saharan Africa. *Sci. Rep.* **20**, 17110 (2018).
28. Paton, D. G. et al. Exposing *Anopheles* mosquitoes to antimalarials blocks *Plasmodium* parasite transmission. *Nature* **567**, 239–243 (2019).
29. Edi, C. V. et al. Multiple-insecticide resistance in *Anopheles gambiae* mosquitoes, Southern Côte d'Ivoire. *Emerg. Infect. Dis.* **18**, 1508–1511 (2012).
30. Coetzee, M. et al. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa* **3619**, 246–274 (2013).
31. *Guidelines for Laboratory and Field Testing of Long-lasting Insecticidal Nets* (WHO, 2013).
32. Toé, K. H. et al. Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso. *Emerg. Infect. Dis.* **20**, 1691–1696 (2014).
33. Griffin, J. T., Ferguson, N. M. & Ghani, A. C. Estimates of the changing age-burden of *Plasmodium falciparum* malaria disease in sub-Saharan Africa. *Nat. Commun.* **5**, 1–10 (2014).
34. Griffin, J. T. et al. Gradual acquisition of immunity to severe malaria with increasing exposure. *Proc. Royal Soc. B* **282**, 2657–2657 (2015).
35. *Climate Prediction Center* (National Weather Service, accessed 24 March 2016); http://www.cpc.ncep.noaa.gov/products/international/
36. Winskill, P. et al. Malaria Initiative, *Plasmodium falciparum* transmission and mortality: a modelling study. *PLoS Med.* **14**, e1002448 (2017).
37. Angarita-Jaimes, N. et al. A novel video-tracking system to record behaviour of nocturnal mosquitoes attacking human hosts. *J. Royal Soc. Interface* **13**, 20150974 (2016).

Acknowledgements

We are grateful to the community at Tengrela, Burkina Faso, who continue to host our studies at their village. This research was jointly funded by awards from the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat Agreement (MR/M011941/1 and MRC Confidence in Concept award MC_PC_13069), and the Wellcome Trust (200222/Z/15/Z). We thank M. Bernardi for her help preparing the figures.

Author contributions

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

Competing interests

A patent application (WO2015063455A1) that names P.J.M. was filed by LSTM in respect of the barrier bednet, initially in the UK (7 May 2015), but has now entered the Patent Cooperation Treaty process. LSTM has a research agreement with Vestergaard, which 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.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41564-019-0607-2.

Supplementary information is available for this paper at https://doi.org/10.1038/s41564-019-0607-2.

Correspondence and requests for materials should be addressed to P.J.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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 Fig. 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 Fig. 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 simulation						
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 Fig. 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 (*for example*. 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.

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 Fig. 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

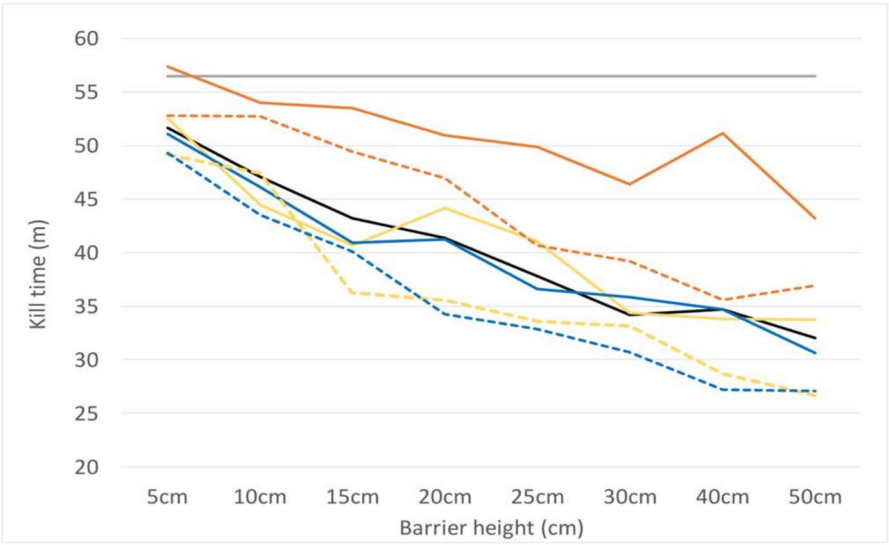
Extended Data Fig. 5 | Frequency and duration of contact at bednets with OP- treated barriers by wild *Anopheles coluzzii* in Banfora, Burkina Faso. The number, location and duration of mosquito contact on barrier bednets recorded during tests (Fig. 1b). Data refer to 2hr video recordings, with 25 female 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).

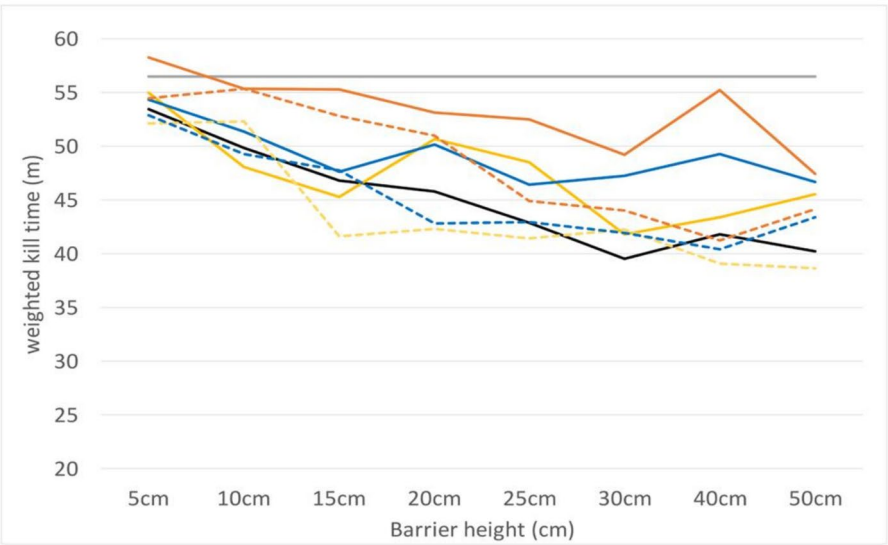
		Low contact		High contact	
Mosquito strain	**Treatment**	**Swooping**	**Visiting**	**Bouncing**	**Resting**
IS	P2	11.2 (0-36.7)	31.6 (0-117.8)	292.6 (0-1350.5)	11.2 (0-303.3)
IS	P2+P2B	1013.3 (0-3755.5)	194.8 (0-639.7)	45.0 (0-113.2)	1013.3 (0-1183.25)
IR	P2	13.9 (0-27.8)	22.2 (0-46.6)	78.6 (0-222.0)	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)
	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 Fig. 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, *for example*. '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 Fig. 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. 8 | 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 bednet as reference. Plot colours correspond to barrier design borders in Fig. 4.

nature research

Corresponding author(s): Philip McCall

Last updated by author(s): Sep 30, 2019

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Video was recorded using StreamPix V8 software (www.norpix.com). Segmentation and analysis of video footage was performed using bespoke software under the Matlab framework (Mathworks) and C++ programming languages.
Data analysis	Video files were compressed using bespoke software and the .mp4 container. Statistical analyses were performed using R (R version 3.1.2) (R Development Core Team 2014).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The hut trial dataset generated during the 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 manusript. The authors declare that all other data supporting the findings of this study, are available within the article and its Supplementary Information files, or are available from the authors upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Hut trial sample size was based on WHO guidelines - 28) World Health Organization. "Guidelines for laboratory and field testing of long-lasting insecticidal nets" http://apps.who.int/iris/bitstream/handle/10665/80270/9789241505277_eng.pdf?sequence=1 (2013). Tracking and modelling studies were based on power calculations, with estimated effect sizes unless limited by the availability of biological samples.
Data exclusions	No data were excluded from the analysis.
Replication	Experimental findings were replicated across repeated trials, with general patterns conserved between laboratory and field trials. The key outcome, the hut trial, was based on standard WHO guidelines with reproducibility as a major consideration.
Randomization	Treatments and participants were assigned at random based on a blinded selection
Blinding	Investigators were not blinded to the groups during filming or hut trial work due to the ease with which bednet designs could be distinguished visually.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The study used two laboratory strains of mosquito: Anopheles gambiae s.s. Kisumu (insecticide susceptible) and a hybrid of Anopheles gambiae/ coluzzii Tiassale (pyrethroid resistant)
Wild animals	The study reared adult Anopheles gambiae s.l. from aquatic stages collected at the study site in Burkina Faso. Adult female mosquitoes were used in the experiments
Field-collected samples	No samples were collected in the field.
Ethics oversight	All research methods were performed in accordance with approved guidelines. 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).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	For laboratory tests 18 human volunteers, 9 males and 9 females of different ethnicities, aged between 22 and 49, were

Population characteristics	recruited from institutional staff and students. For hut trial and field-based filming, 6 volunteers were recruited from the local community, aged between 21 and 35.
Recruitment	Participants were asked to volunteer to participate in the study, field-based research participants were recruited with the assistance of a local guide as some previous experience of collecting mosquitoes was required.
Ethics oversight	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.

Note that full information on the approval of the study protocol must also be provided in the manuscript.