Exposing Anopheles mosquitoes to antimalarials blocks Plasmodium parasite transmission

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Bites of Anopheles mosquitoes transmit Plasmodium falciparum parasites that cause malaria, which kills hundreds of thousands of people every year. Since the turn of this century, efforts to prevent the transmission of these parasites via the mass distribution of insecticide-treated bed nets have been extremely successful, and have led to an unprecedented reduction in deaths from malaria1. However, resistance to insecticides has become widespread in Anopheles populations2–4, which has led to the threat of a global resurgence of malaria and makes the generation of effective tools for controlling this disease an urgent public health priority. Here we show that the development of P. falciparum can be rapidly and completely blocked when female Anopheles gambiae mosquitoes take up low concentrations of specific antimalarials from treated surfaces—conditions that simulate contact with a bed net. Mosquito exposure to atovaquone before, or shortly after, P. falciparum infection causes full parasite arrest in the midgut, and prevents transmission of infection. Similar transmission-blocking effects are achieved using other cytochrome b inhibitors, which demonstrate that parasite mitochondrial function is a suitable target for killing parasites. Incorporating these effects into a model of malaria transmission dynamics predicts that impregnating mosquito nets with Plasmodium inhibitors would substantially mitigate the global health effects of insecticide resistance. This study identifies a powerful strategy for blocking Plasmodium transmission by female Anopheles mosquitoes, which has promising implications for efforts to eradicate malaria.

Substantial strides have been made in malaria control with the introduction of insecticide-based strategies that target the Anopheles mosquito species that transmit Plasmodium parasites. Long-lasting insecticide-treated bed nets (LLINs) alone are predicted to be responsible for 68% of all malaria cases that have been averted since the beginning of the twenty-first century and—together with indoor residual-insecticide spraying of house walls—represent a cornerstone of efforts to control malaria2. However, the pervasive use of these strategies has caused the spread of resistance to insecticides in all major Anopheles populations in malaria-endemic countries2–5. Containment and management of this issue has been undermined by the lack of approved active ingredients for LLINs, which until recently6–8 were limited to pyrethroids. Undoubtedly, the rapid decline in insecticide efficacy constitutes a pressing public health emergency, which threatens to roll back much of the progress that has been made towards eliminating malaria since the introduction of LLINs. After a period of steady decline in annual clinical cases, sub-Saharan Africa and other geographical areas have experienced a plateau—or even an increase—in malaria incidence9. As many countries move towards a focus not only on the control of malaria but also on its elimination, it is imperative that more, and improved, tools to stop parasite transmission by the Anopheles mosquito are generated.

Besides LLINs and indoor residual-insecticide spraying, strategies for the control of malaria rely heavily on drugs to cure Plasmodium infections in humans; the current gold-standard treatment is the use of artemisinin-based combination therapy9. We reasoned that it might be possible to use antimalarial compounds to clear Plasmodium infections directly in the Anopheles mosquito, and to do so using delivery methods that are equivalent to mosquito contact with insecticides on a bed net or wall. This rationale exploits the fact that generally fewer than 100 P. falciparum ookinetes successfully cross the midgut epithelium to form oocysts, which represents a notable bottleneck to transmission. To test this approach, we coated a glass substrate with the potent parasite cytochrome b inhibitor atovaquone (ATQ) and allowed A. gambiae female mosquitoes to rest on this surface immediately before P. falciparum infection. This tarsal exposure (that is, via the legs of the mosquito) is based on a modified WHO (World Health Organization) insecticide assay10 that simulates how mosquitoes take up insecticides on LLINs and from indoor residual-insecticide spraying. Owing to its highly lipophilic nature, we hypothesized that ATQ would be capable of traversing the insect cuticle and killing the parasite during sporogony. No P. falciparum oocysts were detected in ATQ-treated female mosquitoes (1 mmol per m² for 60 min) at 7 days after an infectious blood meal, whereas control mock-exposed individuals showed a high prevalence and intensity of infection (Fig. 1a). To characterize the protective effect of ATQ, we performed a dilution series of exposures and observed a complete blockade of P. falciparum development when a tenfold-lower ATQ concentration (100 μmol per m²) was used; at concentrations as low as 10 μmol per m², we still found significant inhibition of the prevalence (87.6% inhibition) and intensity (87.5% inhibition) of infection (Fig. 1b). Further dilutions of ATQ had a progressively reduced, dose-dependent inhibitory effect (Fig. 1b). By interpolating these data onto a dose–response curve, we calculated that the half-maximal inhibitory concentration (IC₅₀) of ATQ exposure, as a surface concentration, was 1.77 μmol per m² (Fig. 1c). This is comparable to the half-maximal lethal concentration of permethrin, a potent neurotoxic insecticide used in LLIN, for susceptible A. gambiae (63 μmol per m² for a 60-min tarsal exposure11).

Importantly, the killing of P. falciparum parasites was similarly effective when the exposure time was reduced to 6 min (Fig. 2a), which indicates that transmission-blocking doses of ATQ are taken up across the insect cuticle within a timeframe that is compatible with reported contact times for host-seeking mosquitoes on LLINs12. Parasites were killed at the early zygote–ookinete transition, as determined by immunofluorescent assays of infected midguts (Fig. 2b). These data are consistent with previous studies that show that ookinetes are arrested when mosquitoes feed on mice infected with Plasmodium berghei and injected with ATQ13, or when parasites are cultured in vitro in the presence of this antimalarial drug14. Parasite development was also completely aborted when mosquitoes were exposed to ATQ 24 h before or 12 h after infection (Fig. 2c, d). These findings indicate that ATQ-like antimalarials could be incorporated into control interventions other than treated nets, such as attractive toxic sugar baits, in which female mosquitoes become exposed to chemicals while feeding on sugar15, or indoor residual-insecticide spraying, in which contact occurs while female mosquitoes are resting before or after blood feeding. Notably,
ATQ exposure had no fitness cost to the mosquito in terms of survival and reproductive output (Extended Data Fig. 1).

We next incorporated these results into a mathematical model of malaria transmission that includes mosquito population dynamics and human malaria infection (Extended Data Fig. 2). The effects of the introduction of either conventional insecticide-treated nets, or nets combining insecticides with a compound that has ATQ-like properties, were modelled in populations with a varying intensity of malaria transmission (20–80% human infection prevalence), at varying intervention coverage (0–100%) and with varying levels of insecticide resistance (Extended Data Fig. 3). ATQ-like compounds considerably increased the effectiveness of the control intervention under a broad range of scenarios, which indicated that these compounds would facilitate malaria suppression across transmission settings that are relevant in Africa and other malaria-endemic regions (Fig. 3a, Extended Data Fig. 4). Moreover, we modelled 567 specific locations in West and East Africa for which recent (2013–2018) insecticide-resistance data are available, and incorporated estimates of Plasmodium falciparum prevalence and LLIN coverage from 2015. Consistent with our other model outputs, these data predict that adding an ATQ-like compound to LLINs would appreciably reduce Plasmodium falciparum prevalence in these areas (Fig. 3b). The incorporation of ATQ-like compounds would therefore markedly expand the lifespan of insecticide-based strategies; this factor is particularly important in transmission hot spots in which resistance to pyrethroids is nearly total.

ATQ acts by displacing ubiquinone from the QO site of complex III (cytochrome bc1) of the mitochondrial electron transport chain, disrupting the mitochondrial membrane potential and thus inhibiting both mitochondrial ATP production and de novo pyrimidine synthesis. We next tested additional compounds with anti-cytochrome b activity that are not in clinical use, but have the potential to be rapidly adapted for use in mosquito-targeting interventions: the registered insecticides acequinocyl (ACE) and hydramethylnon (HYD), and the veterinary drug decoquinate (DEC). We also included the dihydrofolate reductase inhibitor pyrimethamine (PYR), a compound with extremely potent transmission-blocking activity in humans that acts by disrupting parasite DNA replication (Extended Data Table 1). All of these compounds have nanomolar activity against Plasmodium falciparum asexual stages in vitro, and they lack acute insecticidal activity against A. gambiae, as determined in our experiments (Extended Data Fig. 5a). HYD and ACE showed strong Plasmodium falciparum killing activity: they reduced oocyst prevalence by 63.9% and 64.3%, respectively, relative to control prevalence, calculated by interpolation, is indicated. Mean inhibition relative to control prevalence is indicated. Error bars are 95% confidence intervals (CI). Dashed portions of the sigmoidal fit are estimated. NS, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. For a and b, n indicates the number of biologically independent mosquito samples. For c, n indicates the number of biologically independent mosquito samples. For d, n indicates the number of biologically independent mosquito samples.
Fig. 2 | The transmission-blocking activity of ATQ is maintained at an exposure time of six minutes and at time points of exposure before and after infection. a, P. falciparum parasites are completely eliminated (0% oocyst intensity and 0% prevalence of infection; shown in the pie charts) in female mosquitoes exposed to 1 mmol per m² or 100 μmol per m² ATQ for 6 min. Prevalence: two-sided chi-squared test; 1 mmol per m², n = 113, df = 1, χ² = 91.00, P < 0.0001; 100 μmol per m², n = 102, df = 1, χ² = 80.59, P < 0.0001. At 10 mmol per m², prevalence of infection (n = 149, df = 1, χ² = 55.58, P < 0.0001) and median oocyst intensity (two-sided Mann–Whitney U test, n = 149, df = 1, U = 258, P = 0.0349) are significantly reduced in the ATQ-treated group. Medians are indicated. b, Immunofluorescent assays of mosquito midgut lumens 21 h after P. falciparum infection, using parasite-specific antibodies (anti-PfS25, green) and DNA staining (DAPI, blue). Example images from 14 independent mosquito midgut samples (7 control and 7 treated with ATQ; P. falciparum forms are shown. Left image, mature ookinete in controls. Right image, zygote (asterisk) and retort forms (white arrows) in ATQ-treated female mosquitoes. ATQ-treated female mosquitoes have few ookinetes (1.2% total parasites) and a large proportion of zygotes (88.5% total parasites), which indicates parasite arrest; controls contain a significantly larger proportion of normal ookinetes (40.1%, nominal logistic regression, n = 5,691, df = 14, χ² = 1,620.88, P < 0.0001). Scale bar, 10 μm. c, d, P. falciparum parasites are also completely eliminated when female mosquitoes are exposed to ATQ (1 mmol per m², 6 min) either 24 h before (c) (two-sided chi-squared test with Bonferroni correction, n = 152, df = 1, χ² = 116.74, P < 0.0001) or 12 h after (d) (two-sided chi-squared test with Bonferroni correction, n = 141, df = 1, χ² = 75.11, P < 0.0001) an infectious blood meal. Medians are indicated. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. For a, c and d, n indicates the number of biologically independent mosquito samples. For b, n indicates the number of independent parasite forms.

to controls (Fig. 4). By contrast, DEC and PYR had no detectable effect on infection (Extended Data Fig. 5b), possibly owing to their higher polar surface area relative to ATQ, ACE and HYD (and insecticides used in LLINs), which may negatively affect their uptake by mosquitoes (Extended Data Table 1).

ATQ, in combination with proguanil, is used extensively for prophylaxis in travellers to malaria-endemic areas, and as a stopgap therapy in the case of treatment failure with artemisinin-based combination or other therapies. A handful of mutations that confer resistance to ATQ in the P. falciparum erythrocytic cycle have been shown to cause parasite arrest early in mosquito infection23, although partial transmissibility has previously been observed for some ATQ-resistant P. berghei parasites23,24. Similar developmental arrest of Plasmodium parasites during the mosquito stages is associated with the functional knockout of other components of the mitochondrial electron transport chain, such as type II NADH dehydrogenase25, succinate dehydrogenase (complex II)26.
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Transmission across a broad range of transmission settings.

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The model considers an intermediate 45% prevalence of human infection (effects at lower and higher malaria prevalence are described in Extended Data Fig. 4). The effectiveness of the interventions is defined as 1 – proportion reduction in malaria transmission relative to no intervention, and is represented as colours ranging from yellow (no change in malaria transmission) to dark blue (elimination of malaria transmission) at varying levels of coverage (x axis) and insecticide resistance (y axis). Insecticide resistance is the percentage of mosquitoes that are impervious to insecticide; coverage is the probability of a mosquito encountering an intervention during a single feeding episode. Predicted effects of adding ATQ to existing insecticide-treated nets in 567 African locations with available insecticide-resistance data (indicated by black dots on the map of Africa). For each location, the model considers the estimated bed-net coverage and \( P. falciparum \) prevalence in 2–10-year-old children reported in 2013 \(^1\), and insecticide-resistance levels reported between 2013 and 2018 \(^6\). The graphs show mean malaria prevalence for bed nets treated with a combination of insecticides and ATQ (INS + ATQ), relative to bed nets treated only with insecticide (dotted line at \( y = 1 \)), for sampled sites in West and East Africa (red boxes, \( n = 186 \) and \( n = 381 \), respectively). Error bars represent one s.d. from the mean prevalence. In a and b, the model outputs demonstrate that the addition of ATQ substantially increases the ability of treated nets to reduce malaria transmission across a broad range of transmission settings.

and ATP synthase (complex V) \(^{27} \), all of which are non-essential for asexual growth. These observations demonstrate that the mitochondrial electron transport chain has a critical role during \( P. falciparum \) development within female \( Anopheles \), which makes mitochondrial function an attractive target for killing parasites. However, the use of ATQ in mosquito-targeting interventions may not be advisable: in the event of the widespread incorporation of ATQ on LLINs, mutations could arise that confer resistance to this compound, which would compromise its efficacy as a therapeutic agent in treatments of malaria in humans. A priority area for future research is, therefore, the identification of additional effective compounds that can kill \( P. falciparum \) in its mosquito stages and that use modes of action different to those of ATQ. By contrast, mosquito resistance to ATQ or other parasite inhibitors would be unlikely, given the lack of observed fitness costs—in terms of either fecundity or survival—that are associated with exposure to the compound in female \( A. gambiae \).

Our study demonstrates the potential of anti-parasitic compounds for methods of controlling malaria that are aimed at the \( Anopheles \) vector, and greatly expands the library of compounds that can be considered for use on bed nets and in other interventions such as indoor residual-insecticide spraying and attractive toxic baits. It is important to note that there is a considerable gap between the proof-of-concept demonstrated here and implementation of a field-ready product. Although we were able to demonstrate here that exposure to ATQ deposited on a net substrate was also able to completely block infection (Extended Data Fig. 6), many additional parameters—including compound toxicity, formulation, cost and stability—will need to be determined before this strategy can be deployed. Once these hurdles are overcome, the use of \( Plasmodium \) inhibitors on LLINs or in indoor residual-insecticide spraying could rapidly be integrated into the extensive manufacturing and distribution pipelines that are already in operation in all malaria-endemic regions, and thereby provide an effective and safe tool in eliminating malaria.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-019-0973-1.

Received: 28 August 2018; Accepted: 29 January 2019;
Published online 27 February 2019.

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Acknowledgements We thank N. Singh, E. Lund and K. Thornburg for Plasmodium and Anopheles culture; M. Bernardi for help with graphics; D. Wirth, S. Bopp, H. Ranson, and the members of the Catteruccia laboratories for comments and suggestions on the manuscript; Malaria prevalence and LLIN coverage map data were retrieved from the Malaria Atlas Project (www.mapox.ac.uk). Insecticide resistance data were retrieved from the IR Mapper database (www.irmapper.com). F.C. is funded by a Faculty Research Scholar Award by the Howard Hughes Medical Institute (HHMI) and the Bill & Melinda Gates Foundation (BMGF) (Grant ID: OPP1158190), and by the National Institutes of Health (NIH) (R01 AI124165, R01 AI104956). L.M.C. is supported by Simons Foundation Collaboration Grant 524390. C.O.B is supported by NIGMS Maximizing Investigator’s Research Award (MIRA) R35GM124715-02. The findings and conclusions within this publication are those of the authors and do not necessarily reflect positions or policies of the HHMI, the BMGF, Simons Foundation or the NIH.

Reviewer information Nature thanks Jaime Gerardin, Janet Hemingway, Elizabeth Winzeler and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions D.G.P. carried out and analysed infection and fitness experiments. M.A.I. carried out immunofluorescent assays, L.M.C. and I.E.H. generated code and carried out mathematical modelling. C.O.B. and F.C. supervised the study.

Competing interests A patent application (US provisional application no. 62/726,757) covering the concept of the application of antimarial compounds to mosquitoes has been filed on behalf of F.C. and D.G.P. by the President and Fellows of Harvard University. The authors state that they have no other competing interests.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-019-0973-1. Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-0973-1. Reprints and permissions information is available at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to F.C. Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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METHODS

Power analysis was used to determine the minimum sample size required to detect a 50% reduction in oocyst intensity (n = 21 for power 0.9) and to detect a 50% reduction in prevalence (n = 18, power 0.9) based on a priori hypotheses. For randomization, mosquitoes were allocated to treatment group by random aspiration after cage agitation; for infections, blood-feeder–treatment cage combinations were rotated systematically to account for potential non-random feeder effects. For blinding, blinding was not always used during data inspection owing to the biosafety regulations of the Plasmodium infection facility. Under these rules, all infected groups must be clearly labelled with the treatment undergone by the mosquitoes, from the point of infection to when slides are assessed for infection prevalence and intensity. To ensure impartiality in reporting of the results, random slides from each group were selected and counted by more than one investigator. Moreover, images of each infected gut were captured during counting and stored to allow verification.

Insect lines and rearing. Mosquitoes used for this study were A. gambiae sensu stricto, G3 strain. Adult and larval mosquitoes were maintained in a purpose-built insectary at 27 ± 2 °C and 80% relative humidity. Larvae were reared from hatching in 1 l D2O using an optimized density and feeding regimen. Pupae were collected and placed in cages (Bugdorm, Megaview Science), and after eclosion adult mosquitoes were provided with water and 10% w/v glucose solution ad libitum. For colony maintenance, 5–7-day-old adults were provided a blood meal of donated human blood using an artificial membrane feeding system (Hemotek). Compound exposures. Compounds (ATQ, ACE, HYD, PYR and permethrin (PER), Millipore-Sigma) were dissolved in a suitable, volatile vehicle (Extended Data Table 1; concentrations of 3–10 mg ml⁻¹ (0.3–1%/v/v). Working concentrations of each compound were created through serial dilutions. To generate a compound-coated surface, a volume of working solution containing a known quantity of compound was added to 1 ml excess vehicle and transferred to a 6-cm diameter glass Petri dish (0.0283 m²). Treated dishes were placed on a lateral shaker and left for 4 h or overnight until the volatile vehicle evaporated, coating the compound to the glass substrate. Control plates were treated identically, using only the vehicle. A translucent plastic cup was placed over the coated surface to contain mosquitoes during exposure. A flap was cut into the base of the cup to allow the introduction of mosquitoes. Plates were used for 1 day and discarded. For exposures, 15–25 mosquitoes were introduced through the cup flap using a mouth aspirator (J. W. Hock) and incubated on the treated surface for 6–60 min depending on the experimental parameters. Exposure plates were agitated once during exposure to discourage resting on the untreated walls and base of the cup. After exposure, mosquitoes were transferred to a clean 17.5-cm² cage (Bugdorm, Megaview Science). For net exposures, 10 × 10 cm squares of 100-denier polyester netting were dipped in a 0.5 mg ml⁻¹ solution of ATQ in acetone (or acetone alone) and allowed to air-dry for 10 min. Mosquito exposure was carried out as described above.

P. falciparum infection assays. Infections were carried out using the NF54 P. falciparum cell line provided by C. Barillas-Mury via a BSL-II MTA from BEI Resources. The original source of this cell line is BEI Resources (MRA-1000). The parasite line was authenticated using a nested PCR protocol that uses primers specific for P. falciparum. Our NF54 cultures were confirmed to be free of any mycoplasma contamination. A. gambiae female mosquitoes (five days old) were exposed to compounds as described above. Immediately after exposure, female mosquitoes were transferred to a sealed, secure infection glovebox and provided an in vitro culture of P. falciparum (NF54) gametocytes28,29 through a custom-made, glass, water-heated membrane feeder. After 60 min, female mosquitoes that failed to engorge fully were vacuum-exposed to ATQ, PYR, ACE, HYD and PER for 1 h at a concentration of 4% paraformaldehyde (PFA) for 15 min. Slides were then rinsed with 0.05% ethanol, and discarded. At 7–9 days after an infectious blood meal, female mosquitoes that had blood-fed were vacuum-aspirated into 80% ethanol, incubated for 10 min at −20 °C, and transferred out of the secure feeding box into PBS on ice. Midguts were dissected out in PBS and stained with 0.2% w/v mercuricchrome (in DdH₂O) for 17 min. After staining, midguts were mounted on glass microscope slides in 0.02% w/v mercuricchrome, and oocyst prevalence and intensity were determined by examination at 40 × air objective on an inverted compound light microscope (Olympus).

Ookinete immunofluorescent staining. Twenty-one hours after an infectious blood meal, female mosquitoes (either ATQ- or mock-exposed) were aspirated into PBS at 4 °C, beheaded and transferred to a dissecting microscope. Female midguts, including the blood bolus, were isolated and transferred to 20 μl PBS on ice. Guts were disrupted by repeated pipetting and the crude isolate homogenized by vortexing briefly (5 s). Ten microlitres of the homogenate was spotted onto a poly-l-lysine–coated slide and air-dried. Once dry, the tissues were fixed by incubation with 4% paraformaldehyde (PFA) for 15 min. Slides were then rinsed with 0.05% w/v BSA in PBS and stained with a mouse antibody raised against the P. falciparum surface protein PfSP52 (BEI Resources). Secondary staining was carried out with a FITC-donkey-anti-mouse antibody (Thermo Fisher Scientific). After staining and rinsing, tissues were mounted in Vectashield with DAPI (Vector Laboratories) and examined under oil at 63 × magnification using a Zeiss Observer.Z1 inverted fluorescent microscope (Carl Zeiss Microscopy).

Survival assays. To assess acute survival following exposure, 40 female mosquitoes were exposed (as described above) to ATQ, PYR, ACE, HYD and PER for 1 h at a dosage of 1 mmol per m² for each compound. Each exposure had an independent negative control of 40 mock-exposed female mosquitoes. Immediately after exposure, each treatment group was transferred to a 500-μg paper cup and provided with 4% glucose. At 48 h after exposure, the proportion of surviving mosquitoes in each group was determined. Differences in survival between control- and compound-exposed mosquitoes were detected using chi-squared analysis. For long-term survival, ~100 ATQ- or control-exposed female mosquitoes were placed in 17.5-cm² cages (Bugdorm, Megaview Science). Water and a 10% w/v glucose solution were provided ad libitum. Cages were checked daily for mortality, and dead mosquitoes were removed and counted. Each experiment continued until all mosquitoes had died. Differences in median time-to-death between treatment groups were analysed using a log-rank Mantel–Cox test.

Egg development assay. Females (five days old) were exposed to ATQ at 1 mmol per m² for 60 min and provided with an infectious blood meal as described above. Gravid female mosquitoes were collected at three days after an infectious blood meal and the ovaries dissected out in 1 × PBS. Developed eggs were liberated from the ovarian tissue by gentle agitation with a fine dissection needle and counted.

Modelling. We built upon a discrete time model of the mosquito life cycle and malaria transmission36. We used a simple model to distinguish the qualitative effect on transmission of adding ATQ to bed nets in the presence of insecticide resistance, in comparison with standard approaches. For clarity we used a single, well-mixed mosquito population without spatial structure, reasoning that efficacy of interventions, seasonality or outdoor biting behaviour. In brief, mosquitoes progressed through egg, larval and adult stages, which included four-day gonotrophic cycles (feeding, two days of resting, laying), with a time step of one day (Extended Data Fig. 2a). Malaria transmission was incorporated through a simple susceptible–infectious–susceptible–infected framework for human malaria infection. Modifications of the model included (i) the possibility for exposure in every feeding compartment; (ii) a revised formulation of age-dependent adult daily mortality; and (iii) updated computations of the mosquito–human transmission risk functions ($\beta_M$ and $\beta_H$). All simulations were carried out using MATLAB 2016a.

During every feeding, mosquitoes could be exposed to insecticide, alone or in combination with a second insecticide. The principles to insecticide-induced mortality are described above. ATQ is known to interfere with P. falciparum infection (that is, 100% protection against infection on the same day of exposure). Age-dependent mortality for adult mosquitoes was determined by a Gompertz distribution36 (Extended Data Fig. 1a) with scale parameter $b = 0.1868$ and shape parameter $r = 0.0293$ (Extended Data Fig. 2b), such that the survival function (that is, one minus the cumulative distribution function) was $S(x) = exp(-x^r)$. The daily risk of a human becoming infected was computed using $\beta_M(t) = 1 - (1 - b^H)^{t\beta_M}$, in which $b = 0.55$ is the probability of infection given a bite from an infectious mosquito, $n$ is the bites per human per mosquito, and $r(t)$ is the number of infectious feeders on day $t$ (Extended Data Fig. 2c, subpanel (i)). We fitted the biting rate $r$ to give the desired transmission setting in the absence of intervention, assuming a larval carrying capacity of $K = 5 \times 10^5$ mosquitoes and a human recovery rate of 25 days. We found $a = 1.1 \times 10^{-3}$ in moderate transmission (45% human infection prevalence (HIPP)). For other transmission settings, we found $a = 4.2 \times 10^{-4}$ at 20% HIPP, $a = 6.4 \times 10^{-4}$ at 30% HIPP, $a = 9.2 \times 10^{-4}$ at 40% HIPP, $a = 1.34 \times 10^{-4}$ at 50% HIPP, $a = 1.96 \times 10^{-4}$ at 60% HIPP, $a = 3.04 \times 10^{-4}$ at 70% HIPP and $a = 5.32 \times 10^{-4}$ at 80% HIPP.

The daily risk of a mosquito becoming infected was computed with $\beta_H(t) = k_H(t - k_M(t) + k_s)$, in which $k_H = 0.2$ which restricts the maximum risk to ~18%22,33 (Extended Data Fig. 2c, subpanel (ii)). To calculate a rough estimate of the possible effect of adding alaminarial compounds to insecticide-treated nets in Africa, we examined locations from which there were available data on insecticide resistance, malaria prevalence and bed-net coverage. To this end, insecticide-resistance measurements in sub-Saharan Africa from 2013 to 2018 (as per IR Mapper) were combined with estimates from 2015 for bed-net coverage and P. falciparum parasite rate in 2–10-year-old children from the Malaria Atlas Project37. We then projected to 2020 by assuming a linear increase to give a grid square size of 5 km by 5 km at the equator. We considered each grid square to be a location, and only included these grid squares in the analysis, because insecticide-resistance is likely to be highly spatially heterogeneous. In total, we
found 2,641 insecticide-resistance measurements from 597 locations. For 10 locations there were insufficient data on *Plasmodium falciparum* parasite rate and bed-net coverage to fit the model. We additionally excluded a further 20 sites outside of our selected geographical range, for a final total of 567 locations. Insecticide resistance, bed-net coverage and *P. falciparum* parasite rate ranged between 0 and 100%, 0 and 100% and 0.13 and 7.44, respectively, in these locations. When there were multiple insecticide-resistance measurements in a location, we used the average level of insecticide resistance. For simplicity, and because we calculated a relative effect, we assumed that *P. falciparum* parasite rate in 2–10-year-old children reflected the overall prevalence in the population. For each location, given the level of bed-net coverage and insecticide resistance, we fitted the biting rate to return the reported *P. falciparum* parasite rate value as the prevalence for the entire population of that grid square. We compared the *P. falciparum* parasite rate with the prevalence predicted by the model when including an ATQ-like compound (as above, considering 100% blocking of new *Plasmodium* infections on the same day of exposure to the antimalarial compound, and no effects on ongoing *Plasmodium* infections) on all insecticide-treated bed nets predicted from the Malaria Atlas Project. We considered the relative reduction in prevalence in each location and grouped them by West Africa (186 locations) and East Africa (381 locations), to account for broad differences in ecology and epidemiology. This approach is necessarily simplistic, and does not account for the fact that the relationship between current prevalence, bed-net use and insecticide resistance may not be at equilibrium, or that the measurements for the underlying data may not have been taken during the same time period. Further, the estimated effect of our approach may not be generalizable to areas outside of our sample area for which insecticide-resistance data are not available. Overall, however, this calculation gives a rough estimate for the relative effect of adding antimalarial compounds to bed nets in the regions for which data exist.

**Statistics and reproducibility.** Statistical analyses were carried out using GraphPad Prism v.7.0 for MacOSX (GraphPad) unless otherwise stated. For infections, differences in prevalence were analysed by chi-squared test. In experiments in which both treatment groups had individuals that produced >0 oocysts, differences in median oocyst burden between groups (intensity of infection) was analysed using a Mann–Whitney mean ranks test. For multiple comparisons (for example, Fig. 4), differences in prevalence between multiple groups were determined using pairwise chi-squared test, corrected for multiple comparisons (Bonferroni). Similarly, multiple comparisons of intensity were carried out using Wilcoxon with Dunn’s post hoc test. To determine IC50 values from dose–response data, the mean relative inhibition (ATQ-exposed prevalence/control prevalence) was calculated for each tested dose, and fit with a sigmoidal curve function using nonlinear regression. To compare the relative proportions of each parasite form detected in the mosquito midgut at 21 h after an infectious blood meal, we constructed a logistic regression model using JMP Pro 14 (SAS Institute) with ‘parasite form’ (ookinete, retort or zygote) as the independent variable and ‘treatment’ (ATQ or control) as the dependent variable. We also included the term ‘mosquito sample’ (n = 7 per treatment) to account for random between-sample variation. This cofactor was nested within treatment. All infection experiments were replicated a total of three times as independent biological replicates. Survival and fitness experiments were replicated independently twice. All collected data are included in the presented figures.

**Code availability.** All custom computer code used in this study has been uploaded to GitHub and can be accessed from the following URL: https://github.com/lau-renchilds/ATQAnopheles.

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

**Data availability**

Raw data for infection experiments are available as Supplementary Data. All further data are available upon request.

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Extended Data Fig. 1 | Effects of ATQ exposure on survival and post-blood-feeding egg production in *A. gambiae* female mosquitoes. a, ATQ exposure has no effect on the acute or long-term survival of *A. gambiae* female mosquitoes (two-sided log-rank Mantel–Cox, *n* = 189, df = 1, $\chi^2 = 0.00, P = 0.9951$). The sigmoidal fit used for subsequent modelling is shown. b, The production of eggs after an infectious blood meal is unaffected by ATQ exposure (two-sided, unpaired Student’s *t*-test, *n* = 75, df = 1, $t = 0.826, P = 0.4115$). Means and 95% confidence intervals of the mean are indicated. ns, not significant; *n* indicates the number of biologically independent mosquito samples.
Extended Data Fig. 2 | Model structure and population parameters.

a, Schematic of the mosquito life cycle model with the time step of one day. Mosquitoes spend three days as eggs ($E_i$) and ten days as larvae ($L_i$) (which includes the pupal stage). Adult female mosquito compartments fall within the dashed box, and begin with a rest day ($R_0$) followed by mating ($M$) or feeding ($F$). After feeding, female mosquitoes undergo two days of rest ($R_1$), followed by a day for egg laying (EL). Then, the cycle repeats. Shaded boxes denote when exposure to insecticide or ATQ could occur. These are the same compartments in which mosquitoes can become infected or transmit infections, assuming they have been infected for a period longer than the incubation time.

b, Survival of the mosquito population as a function of age. The curve is a Gompertz distribution with scale parameter $b = 0.1868$ and shape parameter $\eta = 0.0293$.

c(i), Functions relating infection levels of humans and mosquito to risk of infection. Subpanel (i) shows the risk of a human becoming infected, $\beta_H$, as a function of the number of infectious feeders, $f$. Subpanel (ii) shows the risk of a mosquito becoming infected, $\beta_M$, as a function of the fraction of the human population that is infected, $I_H$. 
Extended Data Fig. 3 | Sensitivity of model results to variation in prevalence, coverage and insecticide resistance. The graphs show the enhanced effectiveness of insecticide combined with ATQ (relative to insecticide alone) in reducing human prevalence under varying levels of coverage (across panels), prevalence (along x axis), and insecticide resistance (IR) (bar colour). The enhanced effectiveness of the interventions is defined as human prevalence with only insecticide — human prevalence with insecticide and ATQ, divided by human prevalence with insecticide alone, and is represented by positive values when the addition of ATQ is beneficial. Prevalence is quantified after ten years of simulation. The coverage is varied from 20–80% (top left panel 20%; top right panel 40%; bottom left panel 60%; and bottom right panel 80%). In each panel, the position of the bars determines the malaria prevalence under no intervention, from 20–80%. In the complete absence of insecticide resistance, all mosquitoes that contact insecticide are killed; all dark-green bars equal zero.
Extended Data Fig. 4 | Malaria transmission model predicting the effects of adding ATQ to insecticide-treated nets in additional malaria prevalence settings. a, b. The heat maps show changes in malaria transmission for bed net-like interventions using insecticide alone or insecticide plus an ATQ-like compound, relative to no intervention at varying coverage and varying insecticide resistance levels. The model considers both 20% (a) and 70% (b) prevalence of malaria. The effectiveness of the interventions is defined as \((1 - \text{proportion reduction in malaria transmission relative to no intervention})\), and is represented as colours ranging from yellow (no change in malaria transmission) to dark blue (elimination of malaria transmission) at varying levels of coverage (x axis) and insecticide resistance (y axis). Insecticide resistance is the percentage of mosquitoes that are impervious to insecticide. Coverage is the probability of a mosquito encountering an intervention during a single feeding episode. The model output demonstrates that addition of ATQ significantly increases the ability of an LLIN-like intervention to reduce and even eliminate malaria transmission.
Extended Data Fig. 5 | Testing additional compounds for fitness costs and transmission-blocking activity through tarsal contact.

a, Mosquito survival relative to an untreated control 48 h after exposure to ATQ, DEC, PYR, HYD, ACE and PER. The proportion of female *A. gambiae* surviving exposure to each compound (1 mmol per m², 60 min) relative to the proportion of individuals surviving exposure to an untreated control is shown. PER exposure causes almost complete mortality (proportionate survival relative to controls = 0.055, pairwise two-sided chi-squared test with Bonferroni correction, *n* = 80, df = 1, $\chi^2 = 76.10, P < 0.0001$), whereas all other compounds behave comparably to controls. b, Neither PYR nor DEC (1 mmol per m², 6 min) are capable of reducing the prevalence of *P. falciparum* through tarsal contact, relative to controls (pairwise two-sided chi-squared test with Bonferroni correction: DEC, *n* = 93, df = 1, $\chi^2 = 2.42, P = 0.12$; PYR, *n* = 92, df = 1, $\chi^2 = 0.55, P = 0.46$). Similarly, DEC and PYR had no effect on the intensity of infection, compared to a mock-treated control (Wilcoxon with Dunn’s post hoc test, *n* = 183, df = 3, $P = 0.31$ (DEC) and $P = 0.99$ (PYR)). Letters indicate groups that are statistically different from one another. ****$P < 0.0001$. Medians are indicated; *n* denotes the number of biologically independent mosquito samples.
Extended Data Fig. 6 | ATQ exposure via a netting substrate completely inhibits \( P. falciparum \) development. \( A. gambiae \) female mosquitoes were allowed to rest for 60 min on 100-denier polyester netting that had been treated with either a 0.5 mg ml\(^{-1}\) (0.05% w/v) solution of ATQ in acetone, or acetone alone. Females exposed to ATQ in this way failed to become infected after an infectious \( P. falciparum \) blood meal, demonstrating that a netting substrate is also capable of delivering sufficiently high doses of ATQ to inhibit infection (two-sided chi-squared test, \( n = 98 \), df = 1, \( \chi^2 = 75.55, P < 0.0001 \)). ****P < 0.0001. Medians are indicated; \( n \) denotes the number of biologically independent mosquito samples.
Extended Data Table 1 | Chemical properties and structures of study compounds and bed-net-approved chemicals

| Compound          | Vehicle     | H Donors | H Acceptors | Rotational Bonds | Polar Surface Area (Å²) | Active Tarsally? | Target                                         | Chemical Structure |
|-------------------|-------------|----------|-------------|------------------|-------------------------|-------------------|------------------------------------------------|---------------------|
| Permethrin        | Acetone     | 0        | 3           | 7                | 35.5                    | Yes               | Para sodium-gated ion channel (cell membrane) | ![Permethrin Structure](image1) |
| Deltamethrin      | Acetone     | 0        | 4           | 7                | 59.3                    | Yes               | Para sodium-gated ion channel (cell membrane) | ![Deltamethrin Structure](image2) |
| Pyriproxifen      | Acetone     | 0        | 4           | 7                | 40.6                    | Yes               | Methoprene Tolerant (nucleus)                   | ![Pyriproxifen Structure](image3) |
| Chlorfenapyr      | Acetone     | 0        | 5           | 4                | 38                      | Yes               | Mitochondrial inter-membrane space              | ![Chlorfenapyr Structure](image4) |
| Piperonyl butoxide| Acetone     | 0        | 5           | 13               | 46.2                    | Yes               | Cytochrome P450s                                | ![Piperonyl Butoxide Structure](image5) |
| Atovaquone        | Acetone     | 1        | 3           | 2                | 54.4                    | Yes               | Cytochrome B (mitochondrial inner membrane)    | ![Atovaquone Structure](image6) |
| Hydramethylnon    | Acetone     | 2        | 8           | 6                | 48.8                    | Yes               | Cytochrome B (mitochondrial inner membrane)    | ![Hydramethylnon Structure](image7) |
| Acequinocyl       | Acetone     | 0        | 4           | 13               | 60.4                    | Yes               | Cytochrome B (mitochondrial inner membrane)    | ![Acequinocyl Structure](image8) |
| Decoquinate       | Chloroform  | 1        | 6           | 15               | 73.89                   | No                | Cytochrome B (mitochondrial inner membrane)    | ![Decoquinate Structure](image9) |
| Pyrimethamine     | Acetone (sparingly) | 2 | 4 | 2 | 77.8 | No | Dihydrofolate Reductase-Thymidylate Synthase (cytosol) | ![Pyrimethamine Structure](image10) |

*Approved for use in long-lasting insecticide treated nets

†only in combination with permethrin/deltamethrin

§against Plasmodium falciparum infection

Chemical properties of insecticides and synergists currently approved for bed-net use (permethrin, deltamethrin, pyriproxifen, piperonyl butoxide and chlorfenapyr), and of all compounds tested in this study (atovaquone, hydramethylnon, acequinocyl, decoquinate and pyrimethamine).
Corresponding author(s): Flaminia Catteruccia

Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of 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 statistics 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
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection  
No software was used

Data analysis  
Statistical analyses were carried out using graphpad prism v7.0 and JMP Pro 13.0; Modeling was carried out using Matlab 2016a; Image analysis was carried out using Image v1.48. Custom code available at https://github.com/laurenchilds/ATQAnopheles

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Life sciences study design

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

| Sample size | Power analysis was to determine the minimum sample size required to detect a 50% reduction in oocyst intensity (n=21 for power 0.9, μa 20, μb 10, SD 10, equal variance) and to detect a 50% reduction in prevalence (n=18, power 0.9, PA 0.9, PB 0.45) based on a priori hypotheses. |
| Data exclusions | No data were excluded |
| Replication | All infection experiments were replicated 3 times. Survival and fitness experiments were replicated twice. All attempts at replication were successful. |
| Randomization | Mosquitoes were allocated to treatment group by random aspiration after cage agitation; blood feeder/treatment cage combinations were rotated systematically to account for potential feeder effects |
| Blinding | Blinding was not always utilized during data collection due to the biosafety regulations of the Plasmodium infection facility. Under these rules, all infected groups must be clearly labeled with the treatment undergone by the mosquitoes from the point of infection to when slides are assessed for infection prevalence and intensity. To ensure impartiality in reporting of the results, random slides from each group were selected and counted by more than one investigator. Moreover, images of each infected gut were captured during counting and stored to allow verification. |

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|--------------------------------|---------|
| n/a: Involved in the study | n/a: Involved in the study |
| [ ] Unique biological materials | [ ] ChIP-seq |
| [ ] Antibodies | [ ] Flow cytometry |
| [ ] Eukaryotic cell lines | [ ] MRI-based neuroimaging |
| [ ] Palaeontology | |
| [ ] Animals and other organisms | |
| [ ] Human research participants | |

Antibodies

| Antibodies used | Primary Antibody: BEI Resources NIAID, NIH: Monoclonal Antibody Anti-Plasmodium falciparum 25 kDa Gamete Surface Protein (Pfs 25), Cat. # MRA-28. |
|----------------|----------------------------------------------------------------------------------------------------------------------------------|
|                | Secondary Antibody: Invitrogen Donkey Anti-Mouse IgG, Alexa Fluor 488 conjugate. Cat. # A-21202. Lot # 1022448 |

| Validation | Pfs25: Specificity: Pfs25 (no known cross reactivity) Immunogen: Recombinant Pfs25 Source of antibody: mouse ascites Mouse monoclonal antibody 487 recognizes the 25 kDa gamete surface protein (Pfs25) of Plasmodium falciparum (P. falciparum). The hybridoma was produced by fusion of spleen cells from a BALB/c mouse immunized twice with recombinant vaccinia virus expressing Pfs25, and boosted with whole P. falciparum gametes. Barr, P. J., et al. "Recombinant Pfs25 Protein of Plasmodium falciparum Elicits Malaria Transmission-Blocking Immunity in Experimental Animals." J. Exp. Med. 174 (1991): 1203-1208. PubMed: 1940798. Donkey Anti-Mouse IgG, Alexa Fluor 488 conjugate: Lot-specific validation statement available here: https://assets.thermofisher.com/TFS-Assets/LSG/certificate/Certificates%20of%
**Eukaryotic cell lines**

Policy information about **cell lines**

**Cell line source(s)**

NF54: provided by Carolina Barillas-Mury at the NIH via a BSL-II MTA from BEI Resources. The original source of this cell line is from BEI Resources (MRA-1000).

**Authentication**

NF54 was authenticated using a nested PCR protocol that uses primers specific for *P. falciparum* (G. Snounou et al., 1993 Mol Biochem Parasitol). An initial amplification uses primers specific to the *Plasmodium* genus, yielding an approximately 1200 bp fragment. Nested PCR using *P. falciparum*-specific primers on this product generates a 205 bp product that we have confirmed as *P. falciparum* by Sanger DNA sequencing.

**Mycoplasma contamination**

NF54 was tested for mycoplasma contamination using the MycoFind Kit from Clongen Laboratories, LLC which utilizes multiplex PCR for detection of mycoplasma. Our NF54 cultures were confirmed to be free of any mycoplasma contamination.

**Commonly misidentified lines**

(See ICLAC register)

N/A

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**Animals and other organisms**

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

**Laboratory animals**

*Anopheles gambiae* G3 strain, female, 5-7 days old

**Wild animals**

No wild animals were used in this study

**Field-collected samples**

No field-collected samples were used in this study