Antimalarials in mosquitoes overcome *Anopheles* and *Plasmodium* resistance to malaria control strategies

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Abstract

The spread of insecticide resistance in *Anopheles* mosquitoes and drug resistance in *Plasmodium* parasites is contributing to a global resurgence of malaria, making the generation of control tools that can overcome these issues an urgent public health priority. We recently showed that the transmission of *Plasmodium falciparum* parasites can be efficiently blocked when exposing *Anopheles gambiae* females to antimalarials deposited on a treated surface, with no negative consequences on mosquito fitness. Here, we demonstrate this approach can overcome the hurdles of insecticide resistance in mosquitoes and drug resistant in parasites. We show that the transmission-blocking efficacy of mosquito-targeted antimalarials is maintained when field-derived, insecticide resistant *Anopheles* are exposed to the potent cytochrome b inhibitor atovaquone, demonstrating that this drug escapes insecticide resistance mechanisms that could potentially interfere with its function. Moreover, this approach prevents transmission of field-derived, artemisinin resistant *P. falciparum* parasites (*Kelch13* C580Y mutant), proving that this strategy could be used to prevent the spread of parasite mutations that induce resistance to front-line antimalarials. Atovaquone is also highly effective at limiting parasite development when ingested by mosquitoes in sugar solutions, including in ongoing infections. These data support the use of mosquito-targeted antimalarials as a promising tool to complement and extend the efficacy of current malaria control interventions.

Significance Statement

Effective control of malaria is hampered by resistance to vector-targeted insecticides and parasite-targeted drugs. This situation is exacerbated by a critical lack of chemical diversity in both interventions and, as such, new interventions are badly needed. Recent laboratory studies have shown that an alternative approach based on treating *Anopheles* mosquitoes directly with antimalarial compounds can render the vector incapable of transmitting the *Plasmodium* parasites that cause malaria. While promising, showing that mosquito-targeted antimalarials remain effective against wild parasites and mosquitoes, including drug- and insecticide-resistant populations, respectively, is crucial to the future viability of this approach. In this study, carried out in the US and Burkina Faso, we show that antimalarial exposure is highly effective, even against extremely resistant mosquitoes, and can block transmission of drug-resistant parasites. By combining lab, and field-based studies in this way we have demonstrated that this novel approach can be effective in areas where conventional control measures are no longer as effective.

Introduction

Human malaria, a parasitic disease caused by unicellular eukaryotic *Plasmodium* parasites and spread through the bite of *Anopheles* mosquitoes, remains a substantial cause of global morbidity and mortality (1). Malaria control programs rely on preventative measures focused on mosquito control and on therapeutic measures based on the use of antimalarial drugs. Mosquito-targeted interventions are the most effective tools at reducing the transmission of *Plasmodium* parasites, with long-lasting insecticide-impregnated nets (LLINs) and indoor residuals spraying (IRS) as primary methods for malaria prevention. LLINs alone are predicted to have contributed to 68% of malaria cases averted between 2000 and 2015 (2). Alongside these preventative interventions, artemisinin combination therapies (ACT) have been the cornerstone of human malaria treatment since their widespread

55 introduction at the beginning of this century (3, 4) and have contributed substantially to the reduction
56 in malaria mortality and morbidity observed since then (2).

57 Despite sizeable investment, malaria control and elimination efforts are, however, faltering due to
58 reduced operational effectiveness of these key control tools, largely caused by mosquito resistance to
59 insecticides and parasite resistance to drugs (5-8). In the malaria hyperendemic regions of southern
60 Mali and southwest Burkina Faso, for example, resistance to pyrethroids is extreme (9), driven by
61 multifactorial and synergistic resistance mechanisms including enhanced metabolic detoxification
62 through upregulated cytochrome P450s (metabolic resistance), and reduced tarsal uptake through
63 cuticular thickening (cuticular resistance) (10-13). Similarly, the emergence and spread of artemisinin
64 resistance to sub-Saharan Africa — a region where as many as 93% of annual malaria deaths occur (1)
65 — is a major concern. Until recently, resistance to these first line antimalarials was limited
66 geographically to the Greater Mekong Subregion (GMS), however *de novo* mutations in *PfKelch13*
67 associated with *in vitro* resistance have now been detected in Uganda, Tanzania, and Rwanda (8, 14-
68 19). Of further concern is the recent invasion and spread of the Asian vector *Anopheles stephensi* to
69 the horn of Africa (20), as this mosquito species is highly competent for the transmission of *P.*
70 *falciparum* parasites endemic to the GMS and therefore, invasive populations may facilitate the spread
71 of parasites harboring artemisinin resistance mutations from Asia to Africa. Besides insecticide
72 resistance, an additional hurdle to malaria elimination is represented by residual malaria. Defined as
73 malaria transmission in the presence of universal effective LLIN coverage, residual malaria is driven
74 by mosquitoes that exhibit outdoor or daytime biting preferences and is a considerable hurdle to
75 malaria eradication efforts (21). Increased focus on residual malaria has stimulated interest in the use
76 of attractive toxic, or targeted (22), sugar baits (ATSBs) to attract and kill adult mosquitoes irrespective
77 of blood-feeding behavior, which in field trials have shown some promise as a tool for suppressing
78 vector populations (23, 24).

Thus, a control strategy that prevents insecticide resistant *Anopheles* populations from transmitting malaria parasites, including parasite strains carrying drug resistance mutations, regardless of mosquito feeding behavior and without imposing strong selective pressure on mosquitoes could overcome the limitations of current mosquito-targeted interventions. In an effort to generate such a strategy, we recently demonstrated that transmission of *P. falciparum* parasites can be prevented when *Anopheles gambiae* are exposed to the antimalarial atovaquone (ATQ) through direct contact — analogous to the mode of insecticide exposure on LLINs or IRS (25). Contact with ATQ-coated surfaces completely abrogated parasite development when it occurred around the time of infection (between 24 hours (h) before and 12 h post feeding on *P. falciparum*-infected blood), preventing onward transmission of the parasite. Importantly, mathematical models based on these results showed that integrating antimalarial ingredients into existing mosquito-targeted interventions could considerably reduce malaria transmission in areas of widespread insecticide resistance, empowering our best malaria prevention tools (25).

Here we show that targeting *P. falciparum* with antimalarials during its development in the *Anopheles* female circumvents the hurdles of insecticide and drug resistance, providing a critical addition to the malaria elimination toolkit. Parasite development is substantially reduced when wild, as well as recently lab adapted *Anopheles coluzzii* (a sibling species of *An. gambiae*) that are highly resistant to pyrethroids are exposed to ATQ prior to feeding on blood taken from *P. falciparum*-infected donors in Burkina Faso. ATQ is also fully active against field-derived *P. falciparum* parasites from Cambodia that are resistant to artemisinin. When using distinct drug targets in humans and mosquitoes, this method is therefore capable of both overcoming insecticide resistance mechanisms and stopping transmission of parasite mutations that confer resistance to frontline antimalarials. Finally, we show that delivering ATQ via sugar solutions causes a striking reduction in both parasite numbers and growth, proving that antimalarials could also be incorporated into interventions that target outdoor malaria transmission, such as ATSB. Targeting *Plasmodium* parasites in the mosquito vector is

104 therefore a promising strategy that circumvents key limitations of current malaria control and
105 preventative interventions.

106 **Results**

107 **Exposure to ATQ substantially reduces infection with field *P. falciparum* isolates in insecticide**
108 **resistant *An. coluzzii***

109 To determine whether antimalarial exposure can maintain efficacy in insecticide-resistant mosquitoes,
110 we took *An. coluzzii* collected as pupae from larval breeding sites in Bama, Burkina Faso and reared
111 them to adults at the IRSS, Burkina Faso. Adult mosquitoes were infected using *P. falciparum*
112 gametocyte positive blood obtained from a malaria infected human donor on the day of infection. The
113 *An. coluzzii* mosquitoes endemic to this part of Burkina Faso – hereafter named AcVK5 – are highly
114 resistant to pyrethroids (9, 11, 26). AcVK5 mosquitoes were exposed to ATQ for 6 minutes (min) at
115 two concentrations (100 µmol- or 1 mmol/m^2) or a mock-treated blank control surface prior to feeding
116 on infectious blood samples. Infection outcomes were assayed at 7 days (d) post infectious blood meal
117 (pIBM) by dissection of the mosquito midgut to determine the prevalence and intensity of parasite
118 oocysts (Fig. 1 a). Control-exposed AcVK5 females were robustly infected with 81.3% harboring at
119 least one *P. falciparum* oocyst, and median infection intensity in infected females of 19 oocysts per
120 midgut. In contrast, AcVK5 mosquitoes exposed to either dose of ATQ had significantly reduced *P.*
121 *falciparum* infection both in terms of prevalence and intensity (Fig 1b). At the highest concentration,
122 we observed a 99% overall reduction in infection relative to the control (84.6% reduction in prevalence
123 and 94.8% reduction in median intensity), while at the lower dose inhibition of infection reached 96%
124 overall (65.9% reduction in prevalence and 89.5% reduction in median intensity). These results
125 demonstrate that direct tarsal antimalarial exposure, for instance incorporating antimalarials in LLINs
126 and IRS, can effectively block transmission of circulating west African *P. falciparum* parasites in
127 highly insecticide resistant endemic *Anopheles* mosquitoes. Although our previous findings had shown

that both ATQ doses tested above are capable of complete inhibition of parasite transmission using the combination of standard *P. falciparum* (NF54) and insecticide-susceptible *Anopheles* (G3) populations (25), our results confirm that parasite development can be considerably impaired when exposing mosquitoes to antimalarials.

The observation of few parasites surviving exposure may indicate that parasite or mosquito factors in our Burkinabe populations could be reducing the efficacy of ATQ in this assay, potentially including interference from extant insecticide resistance mechanisms in AcVK5 mosquitoes, or reduced ATQ drug sensitivity in *P. falciparum* in this region. To test these possibilities, we initially assayed the efficacy of ATQ against insecticide resistant, Burkina Faso-derived *An. coluzzii* (hereafter Bama-R) with the lab standard *P. falciparum* strain NF54. Reared under pyrethroid selective pressure under otherwise standard laboratory conditions, Bama-R have maintained the parental trait of 100% resistance to permethrin at the WHO discriminating concentration (DC, 696 μmol/m^2) and exhibit appreciable acute survival at five times this dose (SFig. 1b). Bama-R mosquitoes are segregating for the *kdr* mutation in *para* conferring target site resistance (SFig. 1c), but constitutively overexpress CytochromeP450 genes associated with both metabolic resistance through enhanced small molecule detoxification (SFig. 1d(27)), and cuticular thickening (26) Bama-R females were exposed to the maximal effective concentration for tarsal ATQ (EC_{99}, 100 μmol/m^2, 6 min (25)) or to a vehicle control immediately preceding infection, and parasite prevalence and intensity were determined at 7 d pIBM. While control females were highly infected, with a median of 12 oocysts per infected midgut and 81.25% overall prevalence of infection, no oocysts were observed in females exposed to ATQ, suggesting that insecticide-resistance mechanisms found in highly resistant, natural *Anopheles* populations do not interfere with the transmission blocking activity of ATQ (Fig. 1c).

Next, we established an *in vitro P. falciparum* culture from a polyclonal isolate (P5) collected from a gametocytemic donor from Burkina Faso (28)) and infected the laboratory standard, insecticide susceptible mosquito strain *An. gambiae* (G3). P5 development was 100% suppressed in females

153 treated with the EC_{99} of ATQ (Fig. 1d) such that zero oocysts were observed in midguts, compared to
154 heavy infections—both in terms of infection intensity (median 59.5 oocysts per midgut) and infection
155 prevalence (95.7%)—in controls. Delivery of ATQ to mosquitoes is therefore fully effective against
156 field-derived *P. falciparum* isolates currently circulating in West Africa.

157 **ATQ prevents the transmission of an artemisinin-resistant *P. falciparum* isolate from the GMS**

158 Given the results obtained with field-derived parasites from Africa, we next tested the ability of ATQ
159 to kill artemisinin resistant parasites from the GMS, where mutations conferring artemisinin resistance
160 occur in a high proportion of *P. falciparum* isolates, constituting a major public health threat. We
161 reasoned that these experiments would also allow us to test the concept of directly targeting drug
162 resistant *P. falciparum* during mosquito development, removing resistance mutations from the parasite
163 population, and thereby "rescuing" ACT efficacy in human treatment. To this end, we used a
164 Cambodian *P. falciparum* patient clone (KH001_029 (5), hereafter ART29) carrying the C580Y
165 mutation in *PfK13* conferring resistance to artemisinin (Fig. 2a). We used the major Asian malaria
166 vector *An. stephensi* for these experiments as initial tests with *An. gambiae* did not produce appreciable
167 infections (SFig. 2). ART29 generated robust infections in control, mock-exposed *An. stephensi*,
168 (median 16 oocysts per midgut, 100% prevalence of infection). Conversely, no oocysts were detected
169 in females exposed to ATQ prior to infection (100 μmol/m^2, 6 min) (Fig. 2b). These data show that
170 mosquito exposure to antimalarials, such as by incorporation in bed nets, indoor residual sprays (or
171 other contact methods such as eaves tubes (29), could be an effective strategy for reducing the spread
172 of artemisinin resistance both within and between malaria endemic areas, including sub-Saharan
173 Africa.

174 **ATQ exposure during an ongoing infection delays oocyst growth and decreases sporozoite**
175 **prevalence**

In the field, mosquitoes that contact an antimalarial compound through mosquito-targeted interventions may harbor parasites from a previous blood meal that have already traversed the midgut lumen and formed oocysts. We therefore investigated the effects of ATQ on parasites in which oocyst development is already underway, exposing G3 mosquitoes 6d pIBM (NF54, Fig. 3a). In contrast to females exposed before infection, ATQ had no effect on the prevalence or intensity of infection, as measured at 10d pIBM, suggesting ATQ acts differently on oocysts compared to zygote and ookinetes (Fig. 3b). However, when we measured the size of the developing oocysts, we observed a significant, 45% decrease in the mean oocyst cross-sectional area (Fig. 3c). Oocyst size is a good proxy for rate of growth (30) and as such, when we sampled mosquitoes at a later time point when sporozoite invasion of salivary glands has already occurred (14 d pIBM), we observed a 33% reduction in the prevalence of sporozoites in the salivary glands of ATQ-treated females (Fig. 3d). Similar results were obtained when ATQ exposure instead occurred at 3 d pIBM (SFig. 3). Taken together, these results suggest that ATQ exposure after oocyst formation has a partial cytostatic effect on *P. falciparum*.

Ingestion of an ATQ-glucose solution blocks the establishment of *P. falciparum* infection

To determine whether anti-parasitic compounds in sugar could suppress *Plasmodium* development in the mosquito, we began by testing the efficacy of mosquito ATQ-glucose ingestion against the transmission of *P. falciparum* parasites isolated from gametocytemic donor blood samples collected from gametocyte carriers in Nasso, near Bobo Dioulasso, Burkina Faso. Adult female *An. gambiae,* collected as pupae from larval breeding sites, were denied sugar for 24 h, then given access to an ATQ-treated sugar solution (100 µM ATQ/0.5% v/v DMSO/10% w/v glucose) *ad libitum* for the 24 h preceding infection (Fig. 4a). We observed a striking, 85% reduction in the prevalence of wild *P. falciparum* infection in female mosquitoes that had access to ATQ-glucose prior to infection (Fig. 4b). Importantly, median mosquito survival between control and ATQ treatment groups was not significantly different (SFig. 4), confirming previous findings that atovaquone is not toxic to

mosquitoes at parasiticidal concentrations (25). This implies that other *Plasmodium*-specific inhibitors would therefore not impose selective pressures leading to resistance mechanisms in the mosquito.

Using the same conditions with *in vitro* cultured *P. falciparum* (NF54) and lab-adapted *An. gambiae* (G3) resulted in a remarkably similar infection outcome, with a 92.5% reduction in oocyst prevalence in female *An. gambiae* given access to ATQ-glucose solution relative to controls (Fig. 4c). When ATQ concentration was reduced to 10- and 100-fold ATQ dilutions, we observed progressively reduced, dose dependent effects on prevalence (Fig. 4d).

ATQ ingestion during an ongoing *P. falciparum* infection impairs sporogony

As mosquitoes may often visit a sugar bait after acquiring an infectious blood meal, we also investigated the impact of ATQ ingestion on ongoing *P. falciparum* (NF54) infections, providing ATQ-treated sugar to G3 females from 2 d pIBM, when ookinetes have escaped the midgut lumen and formed oocysts on the midgut basal lamina (Fig. 5a). This time, as based on our previous results (Fig. 3) we expected a possible cytostatic effect on oocyst growth, we performed a sampling time course to capture oocyst development through mid- to late sporogony (7d, 10d and 14 d pIBM). We also counted salivary gland sporozoites, the end point of parasite development in the mosquito, at 14 d pIBM. In agreement with our previous results, we observed no change in oocyst prevalence and intensity because of ATQ ingestion (SFig. 5). However, we observed an 80.8% decrease in oocyst cross-sectional area relative to controls at 7 d pIBM, which persisted at later time points (89% and 76.3% decreases at 10- and 14 d pIBM, respectively) (Fig. 5b). By 14 d pIBM, ATQ-exposed oocysts had a similar size to 7 d pIBM control oocysts, suggesting a remarkable suppression of growth. Inspection of DAPI-stained infected midguts revealed a stark decrease in the number of nuclear foci, with a single, diffuse DNA signal compared to many condensed foci in oocysts in controls (Fig. 5c). Strikingly, we detected no sporozoites in the salivary glands of mosquitoes given access to ATQ-glucose 14 d pIBM, despit robust infection in controls (Fig. 5d). Combined, these data point to a strong suppression of oocyst growth,

DNA replication and sporozoite differentiation after ATQ ingestion, suggesting that besides preventing new mosquito infection, this delivery method would be extremely effective at curbing ongoing infections and transmission.

Discussion

In this study we demonstrate the strong potential of incorporating antimalarials into LLINs, IRS and ATSBs to stop transmission of endemic parasites by insecticide-resistant *Anopheles*. Our proof of principle compound ATQ was very effective at killing a polyclonal, field-derived parasite isolate from sub-Saharan Africa, showing that an antimalarial-based LLIN or IRS could suppress transmission even in areas of malaria hyperendemicity, where vector pyrethroid resistance is exceptionally high, and multiple parasite haplotypes coexists (31, 32).

Tarsal ATQ exposure against wild *An. coluzzii* (AcVK5) collected as pupae from breeding sites was able to strongly suppress the transmission of *P. falciparum* isolates circulating in children. Interestingly, in these infections, carried out in Burkina Faso, we observed a small number of "breakthrough" oocysts at ATQ doses that are non-permissive in tests using, respectively, lab-adapted and susceptible NF54 parasites and G3 mosquitoes (25). We reasoned that this marginal reduction in efficacy could be due to extant insecticide resistance circulating in wild *Anopheles gambiae* s.l populations in Burkina Faso, including the wild AcVK5 mosquitoes used for these experiments. While clearly target-site resistance mechanisms are unlikely to impact the activity of antimalarials like ATQ, both cuticular and metabolic resistance could potentially interfere with function by limiting compound uptake and stability, respectively. The CYP450 monooxygenases associated with pyrethroid metabolic detoxification in *An. gambiae* s.l. have been shown to confer some degree of resistance to a structurally and functionally diverse array of compounds including the insecticide DDT, the juvenile hormone agonist pyriproxyfen (33), and several arthropod mitochondrial complex I inhibitors including the otherwise promising insecticide fenpyroximate (34). Similarly, cuticular resistance, where the waxy

248 exocuticle of the mosquito has thickened as an adaptation to insecticide pressure (11), could slow or
249 eliminate uptake of other small molecules. However, tarsal ATQ (at the EC_{99}, 100 µmol/m^2 for 6 min)
250 completely abrogated parasite infection in Bama-R mosquitoes, which are derived from the parental
251 AcVK5 population and exhibit a similar, high level of insecticide resistance combining the additive
252 effects of metabolic and cuticular resistance components (SFig. 1, (26)). This observation suggests that
253 mosquito pyrethroid resistance status, or other vector factors, did not affect tarsal antimalarial efficacy
254 in our experiments.

255 Similarly, in the reciprocal experiment, transmission of the culture-adapted Burkinabe *P. falciparum*
256 isolate P5 was blocked in an insecticide susceptible *An. gambiae* lab strain (G3), again indicating that
257 parasite factors are likely not responsible for the observed reduction in efficacy. Clinically-induced
258 resistance to ATQ in *P. falciparum* is associated with mutation at position 268 of the mitochondrial
259 gene *cytochrome B* (35), while *in vitro* selection typically results in mutation elsewhere in the gene
260 (36-38). ATQ is not in clinical use in sub-Saharan Africa, so it is unlikely that ATQ resistance-
261 conferring mutations are circulating in Burkina Faso at any appreciable frequency. However, while
262 certain ATQ-resistance mutations induce a transmission defect in both *P. berghei* and *P. falciparum* -
263 resulting in failure to establish infection in mosquitoes (37) there are contradictory findings in the
264 literature (38), and it has been shown that naturally occurring ATQ resistance-conferring mutations
265 can persist at ultra-low frequency in parasite populations through mitochondrial heteroplasmy (39).
266 Thus while the Burkinabe isolate (P5) used in our lab-based studies was susceptible to ATQ *in vitro*,
267 and Sanger sequencing of *Cytochrome B* from both P5 asexual blood stages and oocysts showed that
268 these parasites are wild-type, we cannot rule out the possibility that either cryptic parasite factors, lost
269 during the short P5 laboratory adaptation period, or specific vector-parasite interactions in wild
270 populations are responsible for the observed reduction in ATQ efficacy in our Burkina-based
271 experiments. The observed decrease in *P. falciparum* numbers remains extremely significant (above
272 96% total inhibition at 100 µmol/m^2). Taken together, these data demonstrate that mosquito-targeted

antimalarial exposure can bypass currently circulating insecticide resistance mechanisms, maintaining activity even in areas where conventional insecticides have ceased to be effective — an essential trait of any new mosquito-targeted tool.

ATQ exposure also killed artemisinin-resistant *P. falciparum* parasite ART29 in *An. stephensi*. *De novo PfK13* mutations associated with *in vitro* resistance, already highly prevalent in Cambodia and other part of the GMS, have now been detected in Uganda, Tanzania and Rwanda (14-19) and the recent invasion and spread of *An. stephensi* to the horn of Africa (20) may facilitate the spread of these parasites. Widespread artemisinin resistance in Africa, a region where malaria prevention is already challenging due to insecticide resistance, would be a major public health concern. Although our results were somewhat expected given ART29 parasites are ATQ-sensitive *in vitro* during asexual development (40), they are an important proof of concept, both that drug-based mosquito-targeted interventions could be developed to specifically contain and eliminate parasite haplotypes conferring resistance to human antimalarial therapeutics, and that tarsal uptake of antimalarials function similarly in *An. stephensi*. Antimalarial pressure directed at different drug targets in the human and mosquito life stages could effectively suppress the spread of resistance mutations selected in either host and to either target, allowing the possibility for close integration of human- and mosquito-targeted antimalarial interventions in the future. Importantly, mosquito-targeted antimalarials attack the parasite during an extreme population bottleneck and in a non-cyclical stage in its life cycle (41-43), reducing the probability of selection of both extant and *de novo* resistance mutations during mosquito-stage drug challenge compared to treatment during the human asexual cycle. Nevertheless, even the possibility for selection of resistance during parasite sporogonic development means, as a fundamental principle, any mosquito-targeted antimalarial compound integrated into LLIN or IRS must not share a mode of action with current human antimalarial therapeutics. Thus, despite its efficacy in our studies, ATQ could not be responsibly incorporated into a mosquito-targeted intervention due to its use as a

297 human prophylactic and therapeutic drug (44), making identification of additional active compounds
298 in diverse mode of action classes a priority.

299 Ingestion of sugar solutions containing ATQ before *P. falciparum* infection blocked transmission in
300 both field-derived and *in vitro* cultured *P. falciparum*. Such close agreement between these
301 experiments, carried out at different sites with different parasites and mosquitoes, is a testament to the
302 promise of this strategy, demonstrating its high effectiveness in spite of the inherent variability of
303 mosquito sugar feeding behavior (45). Furthermore, continued mosquito access to ATQ-sugar post
304 infection reduced oocyst growth resulting in absence of salivary gland sporozoites. These results
305 support the use of antimalarials in ATSB-like strategies to reduce residual malaria transmission carried
306 out by mosquitoes that predominantly rest and feed outside and thereby avoid both LLINs and IRS.
307 While current proposed ATSB designs rely on insecticidal ingredients (23, 24, 46), the use of
308 antimalarials in sugar baits could act as a more specific and environmentally benign paradigm for this
309 promising intervention should suitable antimalarial ingredients be identified (47-49). ATQ uptake via
310 tarsal contact at 3- or 6-days post infection also significantly reduced oocyst growth, resulting in an
311 appreciable reduction in the proportion of salivary gland sporozoite-positive mosquitoes. *P.*
312 *falciparum* is therefore also vulnerable to inhibition through tarsal exposure during the oocyst stage,
313 which is by far the longest developmental stage in the mosquito, taking between 7-10 days depending
314 on the frequency of blood feeding (30), and thus is the parasite life stage most likely to encounter a
315 mosquito-targeted intervention. As such, the ability to stall or kill oocysts and sporozoites is a highly
316 desirable quality for mosquito-targeted antimalarials.

317 ATQ targets the ubiquinol oxidation (Qo) site of cytochrome b (50), a key element of the
318 mitochondrial electron transport chain (mtETC) which has dual roles in both ATP generation through
319 oxidative phosphorylation and DNA replication through ubiquinone-mediated redox of dihydroorotate
320 dehydrogenase (51). Thus, inhibition of either DNA replication or ATP production, or both, could
321 explain the cytostatic effect observed here. Consistent with our findings, previous studies have shown

that disruption of ATP production through knock-out of components of the tricarboxylic acid cycle in *P. falciparum* (52) and mtETC in *P. berghei* (53-55) can cause oocyst arrest. Moreover, chemical inhibition of *P. vivax* DNA replication during sporogony was also sporontocidal (56), suggesting mitochondrial inhibitors could also be utilized to prevent transmission of these widespread human malaria parasites. Identifying the specific mechanism by which ATQ and other mtETC inhibitors affect sporogonic development in *Plasmodium* is an interesting area for further study.

Although at an early stage, mosquito-targeted antimalarials have the potential to be an effective element to drive malaria incidence down. To this end, identifying more compounds with strong antiparasitic activity during the mosquito stages of *P. falciparum* development – and in particular compounds with sporogony-specific activity – will be a crucial next step, and one that should leverage the extensive libraries of known antimalarials. Indeed, one of the key strengths of this approach is the potential to exploit and repurpose compounds that are otherwise unsuitable for human therapeutic use, whether due to toxicity, poor bioavailability, poor kinetics, or other limiting factors. By integrating human and mosquito-based interventions, this strategy will extend and protect the efficacy of human therapeutics and vector control strategies, giving malaria control efforts renewed vigor.

Materials and Methods

Mosquito lines, insecticide resistance selection and husbandry

Anopheles spp. mosquito populations used in this study were: 1) wild *An. coluzzii* captured as pupae from breeding sites, described below. 2) Laboratory-reared *An. gambiae* obtained from an outbred colony established in 2019 and repeatedly replenished with F1 from wild-caught females collected in Soumousso (11°23′14″N, 4°24′42″W).3) *An. gambiae* G3 ("G3"), a highly lab-adapted, insecticide-susceptible strain competent for *P. falciparum* of African origin. 4) *An. stephensi* (Anst-S), a similarly lab-adapted, insecticide-susceptible strain competent for *P. falciparum* of both African and Asian origin received as a gift from The Institute of Molecular Medicine, University of Lisbon, Portugal. 5) *An. coluzzii.* Bama-R ("Bama-R") a colony established through hybridization of the F1 progeny of female *An. coluzzii* collected from Vallee du Kou, Burkina Faso, with our G3 colony. Since establishment, Bama-R has been kept under frequent permethrin selection pressure and exhibits a consistent pyrethroid resistance phenotype. At the time of this study, (F17-20) Bama-R females where highly resistant to pyrethroids, exhibiting 97% survival in standard WHO insecticide resistance assays — briefly, 1 h exposure to the WHO discriminating concentration (DC, 275 mg/m^2) of permethrin-impregnated papers with mortality scored at 24 h post-exposure — and 43% survival at 5x the DC. Except for selection of resistance in Bama-R, all mosquito colonies were maintained identically at 26 ºC ± 2 ºC and 80% ± 10%. relative humidity (RH). Larvae were cultured in 2-liter (l) catering pans in 500 ml distilled water (dH_2O) under an optimized density and feeding regimen. At the onset of pupation, pupae were separated from larvae using a vacuum aspirator, collected in dH_2O, and placed in a 30x30x30 cm cage (Bugdorm, Megaview Science Co, Ltd, Thailand). After emergence, adult mosquitoes had access to separate sources of 10% glucose (Sigma Aldrich, US) and dH_2O *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, UK). For mosquito colony 2) females were maintained on rabbit blood by direct feeding (protocol approved by the national committee of Burkina

Faso; IRB registration #00004738 and FWA 00007038) and adult males and females fed with a 5% glucose solution. Larvae were reared at a density of about 300 first-instar larvae in 700 ml of water in plastic trays and fed with Tetramin Baby Fish Food (Tetrawerke, Melle, Germany).

P. falciparum strains and culture

P. falciparum strains used in this study were: 1) *P. falciparum* NF54. NF54 is the drug-susceptible standard strain for mosquito transmission studies, obtained from BEI Resources in 2014. This parasite culture was received through a material transfer agreement (MTA) with the laboratory of Dr. Carolina Barillas-Mury. 2) *P. falciparum* P5. P5 is polyclonal (n=3, KMMM, KMKM, RMMM), as determined by MSP1 PCR genotyping following standard procedures (28), and has been culture-adapted from a blood sample contributed by a gametocytemic malaria donor in Burkina Faso in 2017. 3) *P. falciparum* KH001_029 "ART29". ART29 is a *P. falciparum* monogenomic parasite isolate obtained from an infected human patient in Pursat, Cambodia (KH1 clade) between 2011 and 2013 as part of the TRAC I initiative (5). This parasite carries the *PfK13* mutation C580Y associated with resistance to artemisinin. ART29 has clear phenotypic artemisinin resistance, both as determined by *in vivo* clearance time (11.8 h) and *in vitro* ring-stage survival (22.6%) (57), alongside resistance to other antimalarial drugs, including mefloquine and chloroquine, but is not resistant to piperaquine or ATQ (40). These parasites generate robust infections in *An. stephensi* mosquitoes, but not *An. gambiae* (SFig. 2).

For mosquito infection with gametocytemic donor blood samples, *P. falciparum* samples were collected and prepared for infection as described previously (58). Briefly, *P. falciparum* gametocyte-positive whole blood samples were collected from 5–13-year-old donors from the villages surrounding Bobo Dioulasso, Burkina Faso as part of a separate study. Red blood cells were isolated by centrifugation of a 4 ml aliquot of donor blood followed by resuspension in *Plasmodium* naïve human AB serum. Blood samples were then provided to mosquitoes using a custom blown, water heated glass feeder. For mosquito infection with *in vitro* cultured parasites, females were transferred to a secure

malaria infection facility and provided a 14-21 d post-induction stage V *P. falciparum* gametocyte culture using a custom blown, water heated glass feeder. For all infection experiments, within 24 h of infection, partially engorged or unfed mosquitoes were collected by vacuum aspiration and discarded. To determine oocyst burden, between 7 and 14 d pIBM, infected mosquitoes were collected by vacuum aspiration, and dissected to isolate the midgut. The oocyst burden was determined after staining midguts with 0.2% w/v mercurochrome and examination under a 40x air objective inverted compound light microscope (Olympus, US). For sporozoites, at 14 d pIBM infected mosquitoes were collected by vacuum aspiration and beheaded. The mosquito salivary glands were extracted into RPMI media by applying pressure to the lateral thorax. *P. falciparum* sporozoites were isolated by homogenization and centrifugation of salivary gland material, followed by resuspension in a known volume of RPMI. Sporozoites were counted using a disposable haemocytometer under a 20x air objective inverted compound light microscope (Olympus, US). All *P. falciparum* strains were cultured and induced to form gametocytes using standard protocols (59, 60). All strains have been confirmed to be *P. falciparum* by PCR followed by DNA sequencing of the amplified products (61) and have been confirmed free of mycoplasma infection.

Pre- and post-infection tarsal contact infection assays

Tarsal exposure plates were prepared as described previously (25). Briefly, for 100 µmol/m^2 plate concentrations, 0.1 ml of this solution of a 0.1% w/v solution of ATQ in acetone was diluted with 1 ml additional acetone and spread onto a 6 cm diameter (0.002628 m^2) glass petri dish. For 1 mmol/m^2, 1 ml of 0.1% w/v ATQ/acetone solution was added directly to the plate. Plates dried for a minimum of 4 h, with agitation on a lateral shaker at room temperature. For pre-infection exposure, 30 min prior to infection, 3-5 d old virgin female mosquitoes were incubated on either ATQ-coated plates, or an acetone treated control, for 6 min. To prevent crowding and agitation, a maximum of 25 mosquitoes were exposed per plate, with all exposures occurring in parallel. For post-infection exposure, due to

411 biosafety considerations, compound exposures were carried out in serial, with a maximum of 10
412 infected mosquitoes per plate.

413 *Pre- and post-infection sugar feeding infection assays*

414 For experiments involving compound-treated sugar solutions, 20 mM stock solutions of each active
415 ingredient (AI) were prepared in 100% DMSO. Each 20 mM stock solution was diluted 200-fold in
416 10% w/v glucose to achieve the final working concentration of 100 µM AI/0.5% DMSO/10% w/v
417 glucose. For pre-infection sugar feeding experiments, 2-4 d post-emergence females were denied
418 access to glucose or water for 24 h and then provided access to either the test solution, or a control
419 solution of 0.5% v/v DMSO/10% glucose, for 24 h. After this time had elapsed, all mosquitoes were
420 provided with an infectious *P. falciparum* blood meal as described above. Infected mosquitoes were
421 provided with untreated 10% w/v glucose *ad libitum* for the remainder of the experiment. For post-
422 infection sugar-feeding experiments, 3-5 d post-emergence female mosquitoes were infected with *P.*
423 *falciparum* and denied access to glucose or water for 48 h pIBM. After this time, infected mosquitoes
424 were continuously provided either 100 µM ATQ/0.5% DMSO/10% w/v glucose or 0.5% DMSO/10%
425 w/v glucose as control, *ad libitum.* Sugar feeders were replaced every 48 h for the remainder of the
426 experiment, up to 14 d pIBM.

427 *Statistical analyses*

428 Statistical analyses were carried out using GraphPad Prism v8.4.2 for MacOSX (GraphPad Software
429 Inc., USA) and JMP Pro 15 (SAS Corp. US).

430 *Data generated from donor isolated gametocytes*

431 For infections with donor isolated gametocytes (Fig. 1(b), Fig. 4(b)), prevalence and intensity of
432 infection were analyzed using more complex statistics to account for between-replicate effects of
433 different human gametocyte donors. **For prevalence:** we constructed a General Linear Model as
434 follows, independent variable/y "Infected?" (Two-level, categorical (yes/no)), with cofactors

"Treatment" (Two-level, categorical (Control/ATQ)) and "Gametocyte Donor" (Two-level, categorical (Donor 1/Donor 2)), we also included the interaction term Treatment*Gametocyte Donor to detect higher level effects. As the output was categorical, the GLM model was run with a binomial distribution and logit link-function. To achieve the best model fit, we iteratively removed cofactors from the model, and selected the model output with the lowest corrected Akaike information criterion (AICc). In all cases, the best model fit included both cofactors, but excluded the interaction term. **For intensity:** Independent variable "Oocyst Count" (Continuous, positive integer) with cofactors "Treatment" (Two-level, categorical (Control/ATQ)) and "Gametocyte Donor" (Two-level, categorical (Donor 1/Donor 2)), and the interaction term Treatment*Gametocyte Donor. To account for the overdispersion typical of parasite count data, we again took an iterative approach to model construction. Data were analyzed using both a GLM using a Poisson distribution, (link function: log; overdispersion parameter estimated by Pearson Chi-square/DF) and Generalized Regression with a Negative Binomial distribution. Relative model quality was determined by comparison of AICc for each distribution function (For GLM, with and without the overdispersion correction) and by iterative removal of cofactors. The highest quality model fit was an overdispersion-corrected Poisson/Log GLM with both cofactors but without the interaction term.

Data generated from in vitro experiments

For all other infections, differences in prevalence were analyzed by Chi^2. In experiments where both treatment groups had individuals that produced >0 oocysts, differences in median oocyst burden between groups (intensity of infection) was analyzed using a Mann-Whitney Mean Ranks test. For multiple comparisons, differences in prevalence between multiple groups were determined using pairwise Chi^2 corrected for multiple comparisons (Bonferroni). Similarly, multiple comparisons of intensity were carried out using a Kruskal-Wallis test with Dunn's *post hoc.*

Author Contributions:

459 DGP and FC wrote the manuscript. DGP, TL and FC designed the experiments. DGP and TL carried
460 out statistical analysis. DGP, ASP, EM, DFSH, PP, RSY, TL and NS carried out infection experiments.
461 DFSH, RSY, TL, WRS, SKV, and SB collected, established, maintained, and assayed parasite lines.
462 KLA established and reared insecticide-resistant mosquito lines and carried out resistance assays. TL,
463 AD, RKD, DFW, and FC, provided funding and oversight. All authors read and approved the
464 manuscript prior to submission.

465

466 The authors state that they have no conflicting interests.

Figure 1

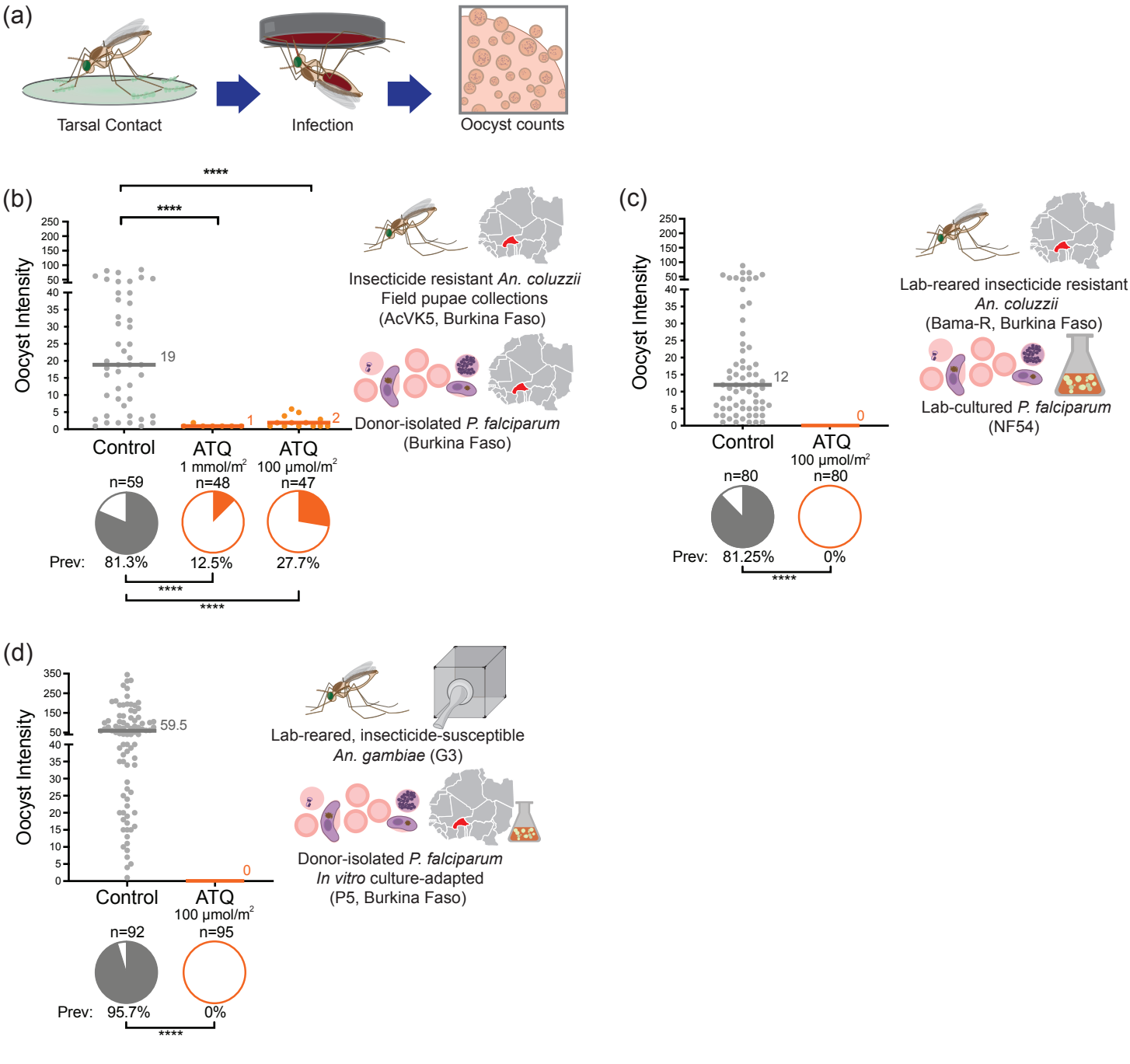

(a)
Tarsal Contact
Infection
Oocyst counts
(b)
Oocyst Intensity
Control
ATQ
1 mmol/m²
ATQ
100 µmol/m²
n=59
n=48
n=47
Prev:
81.3%
12.5%
27.7%
Insecticide resistant An. coluzzii
Field pupae collections
(AcVK5, Burkina Faso)
Donor-isolated P. falciparum
(Burkina Faso)
(c)
Oocyst Intensity
Control
ATQ
100 µmol/m²
n=80
n=80
Prev:
81.25%
0%
Lab-reared insecticide resistant
An. coluzzii
(Bama-R, Burkina Faso)
Lab-cultured P. falciparum
(NF54)
(d)
Oocyst Intensity
Control
ATQ
100 µmol/m²
n=92
n=95
Prev:
95.7%
0%
Lab-reared, insecticide-susceptible
An. gambiae (G3)
Donor-isolated P. falciparum
In vitro culture-adapted
(P5, Burkina Faso)

Figure 1: Exposing *An. gambiae* mosquitoes to ATQ suppresses *P. falciparum* development in field-derived parasites and insecticide-resistant mosquitoes. (a) Experimental scheme. **(b)** Female AcVK5 mosquitoes, collected as pupae from breeding sites in southwestern Burkina Faso, were exposed to ATQ by tarsal contact at either the maximal effective concentration (EC_{99}) for insecticide-susceptible mosquitoes (100 µmol/m^2, EC_{99}) or 10x EC_{99} (1 mmol/m^2) for 6 minutes, prior to infection with donor-isolated "wild" *P. falciparum* (Burkina Faso), collected the same day. ATQ treatment resulted in a significant reduction in both infection prevalence (GLM (Binomial;Logit): 100 µmol/m^2, n=106, df=1, X^2=31.997, p<0.0001; 1 mmol/m^2, n=107, df=1, X^2=50.240, p<0.0001) and intensity (GLM (Poisson;Log): 100 µmol/m^2: n=53, df=1, X^2=24.687, p<0.0001, 1 mmol/m^2: n=59, df=1, X^2=31.997, p<0.0001) at both doses, as measured by determination of *P. falciparum* oocyst burden at 7 d pIBM. **(c)** In contrast, transmission of *in vitro*-cultured *P. falciparum* (NF54) is completely blocked when field-derived, lab-adapted insecticide-resistant *An. gambiae* (Bama-R) are exposed to ATQ for 6 min to the maximal effective concentration (EC_{99}) for insecticide-susceptible mosquitoes (100 µmol/m^2) prior to infection. In ATQ-exposed mosquitoes, prevalence was zero, in contrast to the robust infection in mock-exposed controls (Chi^2, n=160, df=1, X^2=93.545, p<0.0001). **(d)** Similarly, transmission of a polyclonal *P. falciparum* West African donor isolate (P5 – *in vitro* culture adapted) was blocked (zero evidence of infection at 7 d pIBM) after ATQ exposure prior an infectious blood meal, despite a strong (prevalence 95.7%, median intensity 59.5 oocysts per midgut) infection in controls (Chi^2, n=187, df=1, X^2=167.995, p<0.0001). Median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst Prevalence and Oocyst Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Figure 2

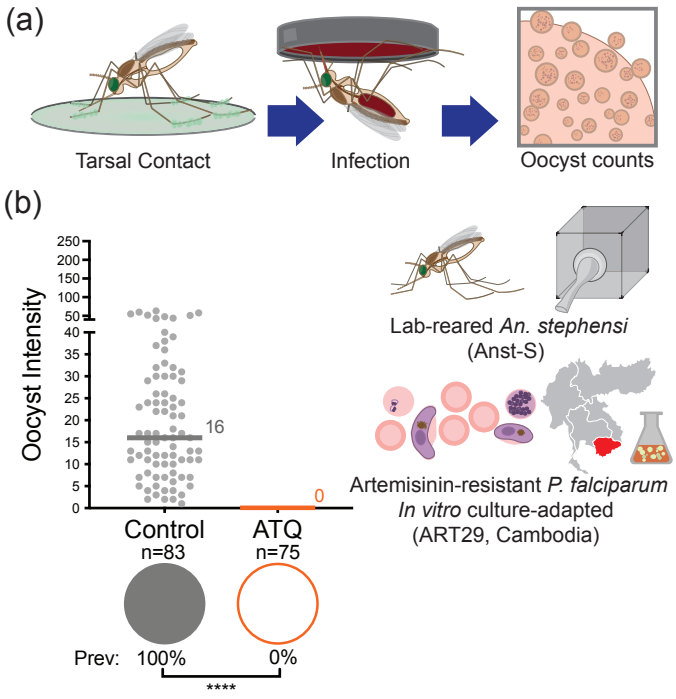

(a)
Tarsal Contact
Infection
Oocyst counts
(b)
Oocyst Intensity
250
200
150
100
50
40
35
30
25
20
15
10
5
0
16
0
Control
n=83
ATQ
n=75
Prev: 100%
0%
****
Lab-reared An. stephensi
(Anst-S)
Artemisinin-resistant P. falciparum
In vitro culture-adapted
(ART29, Cambodia)

Figure 2: Exposing *An. stephensi* to ATQ blocks an artemisinin-resistant *P. falciparum* patient isolate from Cambodia. (a) Experimental scheme. **(b)** Transmission of artemisinin resistant *P. falciparum* (ART29) is completely blocked when *An. stephensi* females (Anst-S) are exposed to ATQ for 6 min to the maximal effective concentration (EC_{99}) for insecticide-susceptible mosquitoes. In ATQ exposed mosquitoes, prevalence (indicated by pie charts) was zero despite robust infection in mock-exposed controls (Chi^2, n=158, df=1, X^2=156, p<0.0001). Median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst Prevalence and Oocyst Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Figure 3

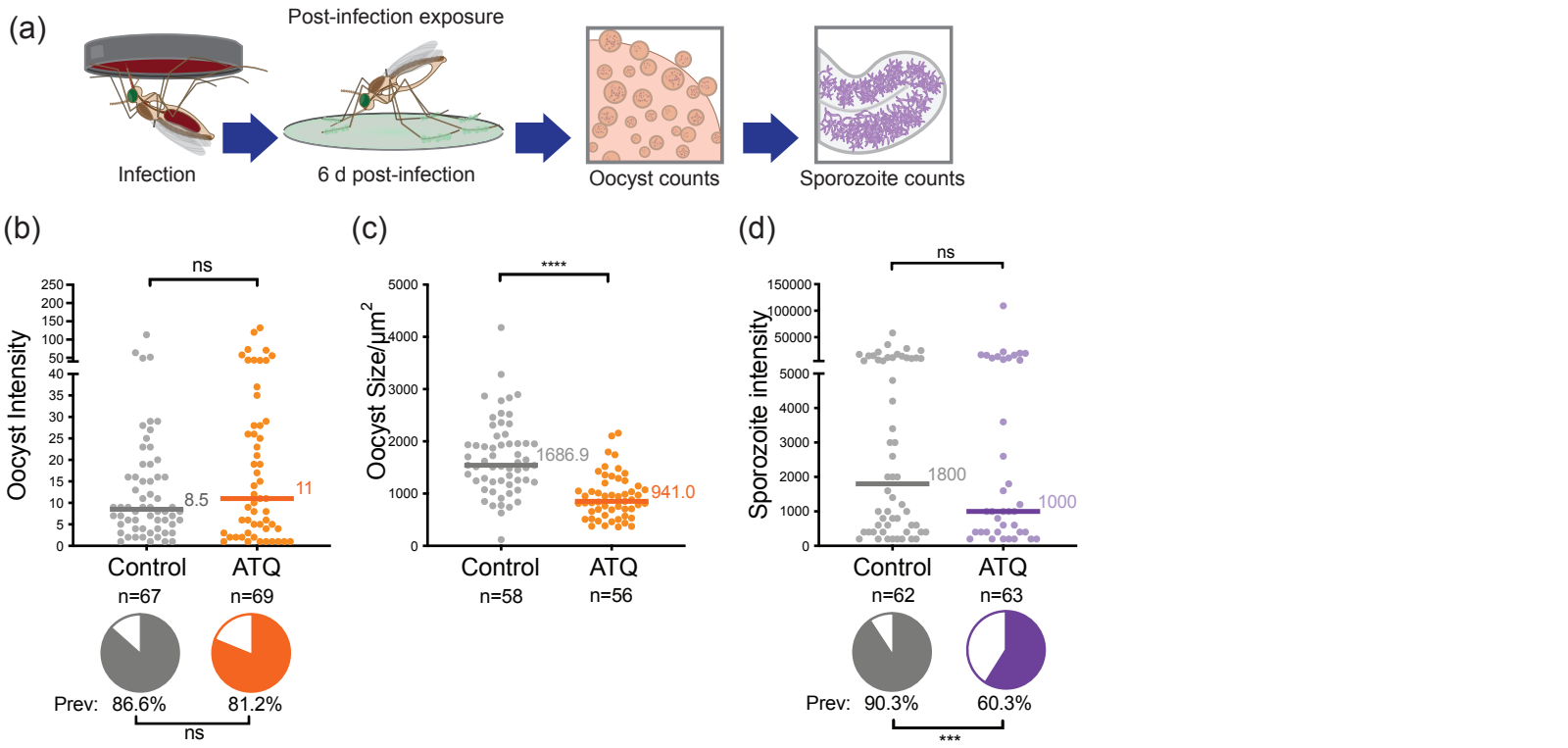

(a)
Post-infection exposure
Infection
6 d post-infection
Oocyst counts
Sporozoite counts
(b)
Oocyst Intensity
ns
8.5
11
Control
ATQ
n=67
n=69
Prev: 86.6%
81.2%
ns
(c)
Oocyst Size/um2
****
1686.9
941.0
Control
ATQ
n=58
n=56
(d)
Sporozoite intensity
ns
1800
1000
Control
ATQ
n=62
n=63
Prev: 90.3%
60.3%
***

Figure 3: Sporozoite prevalence is significantly reduced after tarsal ATQ exposure during oocyst development when *An. gambiae* (G3) are infected with *P. falciparum* (NF54). (a) Experimental scheme. **(b)** There was no effect of 6 d pIBM ATQ exposure on either prevalence (indicated by pie charts) or intensity (indicated by points) of infection determined at 10 d pIBM. Prevalence: Chi^2, n=136, df=1, X^2=0.733, p=0.3919, intensity: Mann-Whitney, n=114, df=1, U=1482, p=0.4335. **(c)** ATQ exposure at 6 d pIBM significantly reduced the median cross-sectional area of oocysts relative to control (n=114, df=1, U=531, p<0.0001). **(d)** The prevalence, but not the median intensity of sporozoites in salivary glands was significantly reduced in mosquitoes exposed to ATQ at 6 d pIBM (Chi^2, n=125, df=1, X^2=7.190, p=0.0073). Median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst Prevalence and Oocyst Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Figure 4

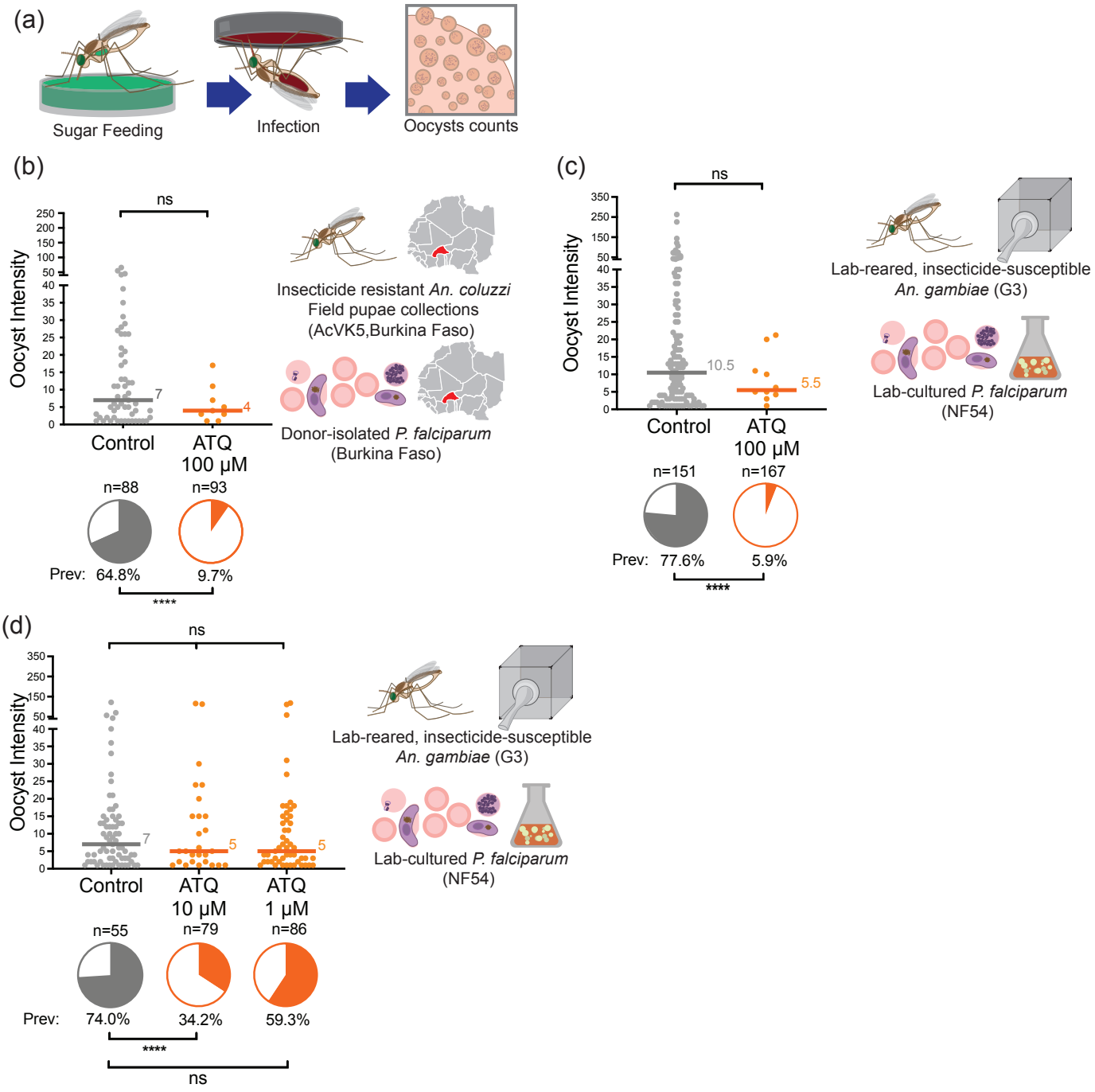

(a)
Sugar Feeding
Infection
Oocysts counts
(b)
Oocyst Intensity
ns
7
4
Control
ATQ
100 µM
n=88
n=93
Prev: 64.8%
9.7%
****
Insecticide resistant An. coluzzi
Field pupae collections
(AcVK5,Burkina Faso)
Donor-isolated P. falciparum
(Burkina Faso)
(c)
Oocyst Intensity
ns
10.5
5.5
Control
ATQ
100 µM
n=151
n=167
Prev: 77.6%
5.9%
****
Lab-reared, insecticide-susceptible
An. gambiae (G3)
Lab-cultured P. falciparum
(NF54)
(d)
Oocyst Intensity
ns
7
5
5
Control
ATQ
10 µM
ATQ
1 µM
n=55
n=79
n=86
Prev: 74.0%
34.2%
59.3%
****
ns
Lab-reared, insecticide-susceptible
An. gambiae (G3)
Lab-cultured P. falciparum
(NF54)

Figure 4: Ingestion of an ATQ-glucose solution prior to *P. falciparum* infection significantly reduces transmission. (a) Experimental scheme. **(b)** AcVK5, collected as pupae from field sites in Bama, Burkina Faso, mosquitoes were significantly less likely to become infected with *P. falciparum* (human gametocyte donor sample) after ingestion of 100 µM ATQ/10% w/v glucose (access to treated sugar 24 h prior to infection). The proportion of AcVK5 mosquitoes infected with *P. falciparum* oocysts at 7 d pIBM was reduced from 64.8% in the control group, to 9.7% in mosquitoes given access to ATQ/sugar solution (85.0% reduction, GLM (Binomial;Logit), n=181, df=1, X^2= 59.242, p<0.0001). While median intensity of infection in mosquitoes harboring ≥1 oocyst was reduced in ATQ-treated mosquitoes, this reduction was not significant. **(c)** Infection with culture adapted NF54 *P. falciparum* in insecticide susceptible G3 *An. gambiae* produced remarkably similar results. Ingestion of 100 µM ATQ/0.5% DMSO/10% w/v glucose (access to treated sugar 24 h prior to infection) significantly reduced the prevalence of infection at 7 d pIBM (92.4% reduction Chi^2, n=318, df=1, X^2=162.467, p<0.0001) but had no detectable effect on the median intensity of infection (Mann-Whitney, n=128, df=1, *U*=480.5, p=0.3364). **(d)** Significant, dose-dependent inhibition of oocyst prevalence was observed with a 10-fold reduction in ATQ concentration (10 µM: Chi^2, n=134, df=1, X^2=19.275, p<0.0001). A non-significant reduction in prevalence was also observed at 1 µM (Chi^2, n=141, df=1, X^2=2.642, p=0.1041). Median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst Prevalence and Oocyst Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Figure 5

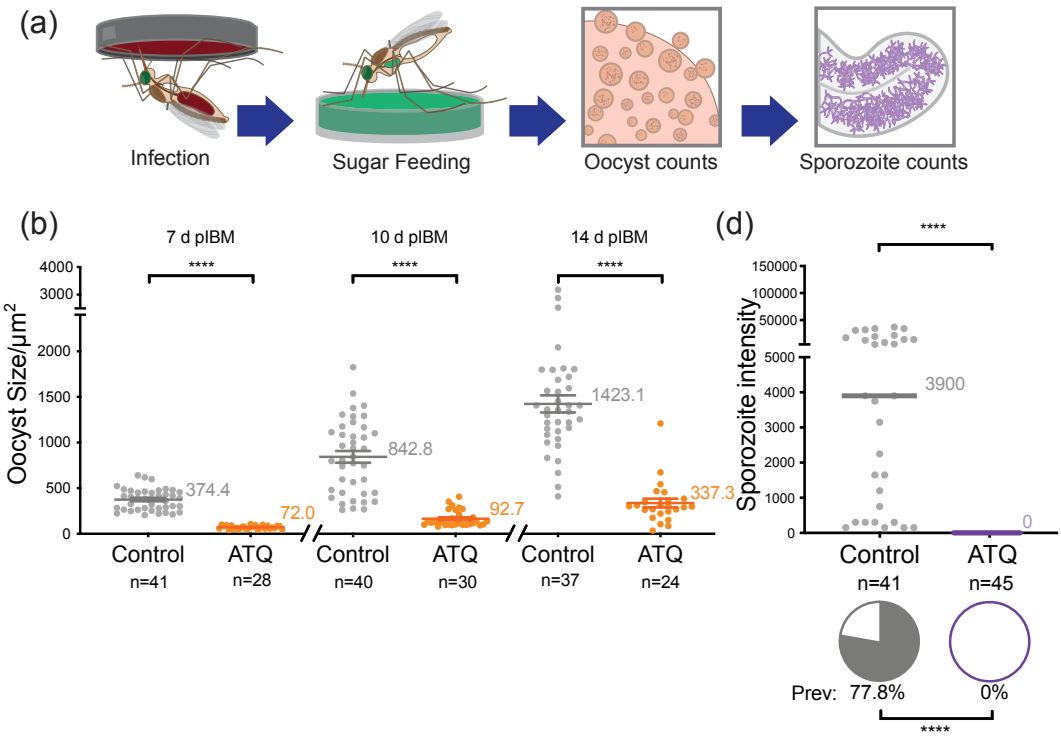
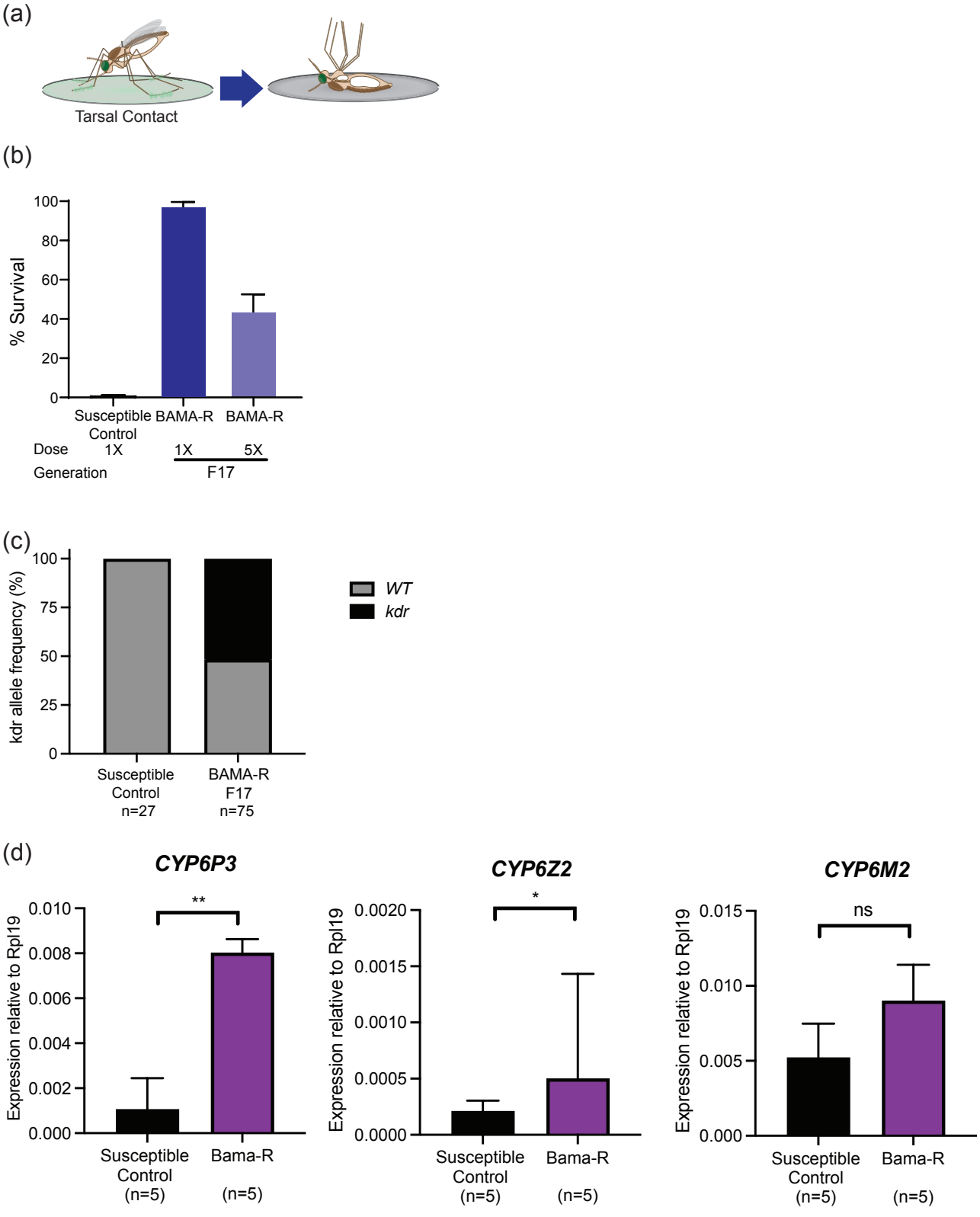

(a)
Infection
Sugar Feeding
Oocyst counts
Sporozoite counts
(b)
7 d pIBM
10 d pIBM
14 d pIBM
****
Oocyst Size/µm2
4000
3000
2000
1500
1000
500
0
374.4
72.0
842.8
92.7
1423.1
337.3
Control
ATQ
n=41
n=28
n=40
n=30
n=37
n=24
(d)
****
Sporozoite intensity
150000
100000
50000
5000
4000
3000
2000
1000
0
3900
0
Control
ATQ
n=41
n=45
Prev: 77.8%
0%
****
(c)
Control bright-field
Control fluorescent
ATQ bright-field
ATQ fluorescent

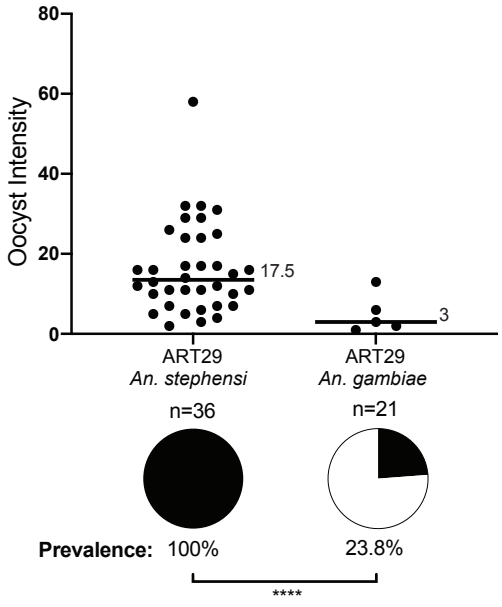
Figure 5: Ingestion of ATQ-glucose after *P. falciparum* (NF54) infection blocks parasite development in *An. gambiae* (G3). (a) Experimental scheme. **(b)** Oocyst size over time. Access to ATQ-glucose from 2 d pIBM caused a significant reduction in mean oocyst cross-sectional area ("size"/m^2) relative to control at 7 d pIBM (2-way T-test, n=69, df=67, t=13.63, p<0.0001), 10 d pIBM (2-way T-test, n=70, df=68, t=8.998, p<0.001), and 14 d pIBM (2-way T-test, n=61, df=59, t=8.793, p<0.0001). Means are indicated, error bars represent the standard error of the mean (SEM). **(c)** Example brightfield, and fluorescent micrographs of control and ATQ-exposed bright-field and DAPI-stained oocysts at 10 d pIBM (outline indicated by dashed line). At this timepoint, the control oocyst is large, and contains many discrete nuclear foci, indicating nuclear division and sporozoite differentiation. In contrast, the ATQ-exposed oocyst is small relative to control and contains a single nuclear focus. Dense hemozoin crystals (white triangle), typically associated with younger oocysts, are visible. Scale bars represent 10 µm, **(d)** Salivary gland sporozoite prevalence and intensity at 14 d pIBM. No sporozoites (0% prevalence, median intensity = 0) were observed in salivary glands samples collected from mosquitoes given access to 100 µM ATQ/0.5% DMSO/10% w/v glucose *ad libitum* (Chi^2, n=86, df=1, X^2=53.202, p<0.0001). Where relevant, median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst/Sporozoite Prevalence and Oocyst/Sporozoite Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Supplementary Figure 1



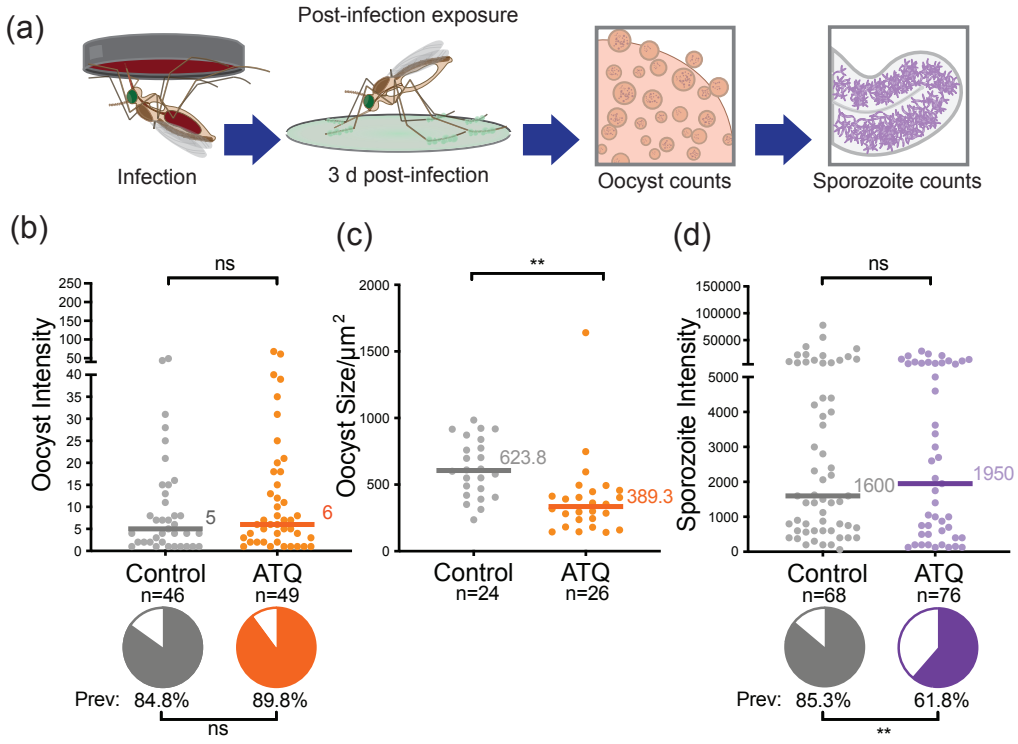
Supplementary Figure 1: *An. gambiae* Bama-R mosquitoes are highly resistant to permethrin. **(a)** Experimental scheme. **(b)** G3 females were 0% resistant at the DC, while 97% of exposed Bama-R females survived at the same dose, and 43% survived exposure to 5xDC, indicating extreme permethrin resistance. Mean survival ± SEM from 3 replicates is indicated. **(c)** Bama-R mosquitoes are segregating for the *kdr* allele conferring target site resistance to pyrethroids. Allele frequency at generation F17 was 51.7% (n=75), indicating that the observed resistance phenotype is the result of additional modalities. **(d)** qPCR analysis shows that key cytochrome P450 genes associated with metabolic insecticide resistance are constitutively upregulated in Bama-R females compared to a susceptible control (G3). Median expression level normalized to the *Anopheles* housekeeping gene *rpl19* are shown, error bars represent the interquartile range (IQR). Expression levels for *Cyp6P3* and *Cyp6Z2* were significantly elevated compared to a phenotypically susceptible control (*Cyp6P3*, Mann-Whitney, n=10, df=1, U=0, p=0.0079; *Cyp6Z2*, Mann-Whitney, n=10, df=1, U=2, p=0.0317) while *Cyp6M2* was not significantly upregulated. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Supplementary Figure 2



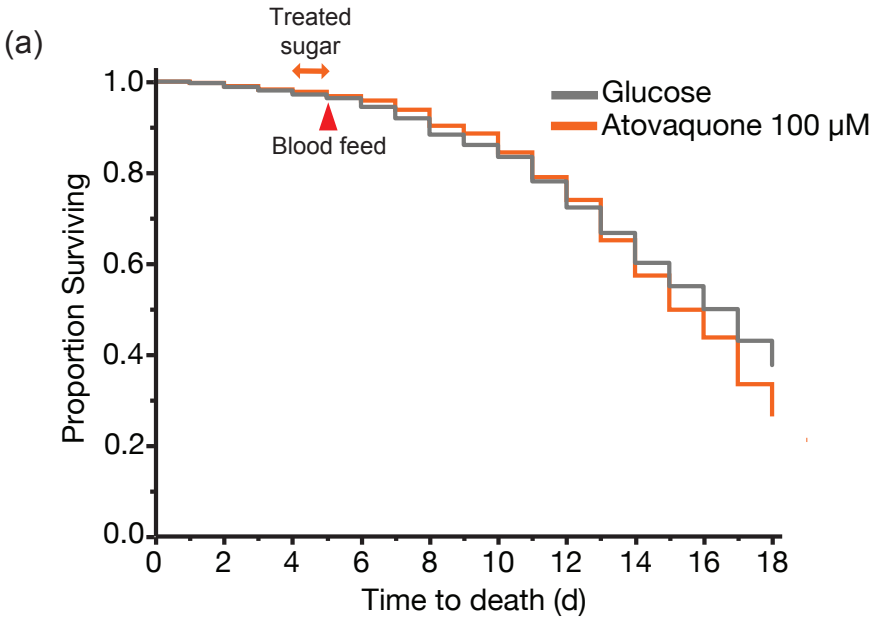
Supplementary Figure 2: Development of the artemisinin-resistant *P. falciparum* parasite ART29 in *An. stephensi* and *An. gambiae*. Female G3 (*An. gambiae*) and Anst (*An. stephensi*) were provided with a blood meal containing mature ART29 gametocytes. Outcome of infection was determined at 7 d pIBM by oocyst count. While ART29 exhibited poor infectivity in *An. gambiae* (23.8% infection prevalence), they established robust infections in *An. stephensi* (100% infection prevalence).

Supplementary Figure 3



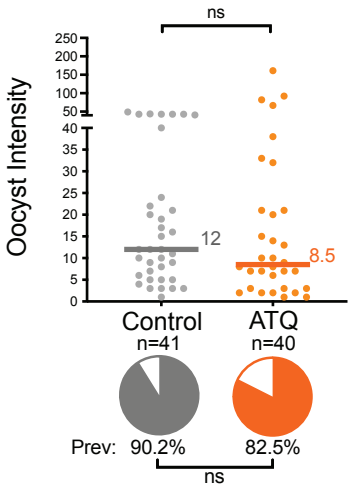
Supplementary Figure 3: The proportion of *An. gambiae* mosquitoes with salivary gland sporozoites is significantly reduced after tarsal ATQ exposure at 3 d pIBM. (a) Experimental scheme. **(b)** There was no effect of 3 d pIBM ATQ exposure on either prevalence (indicated by pie charts) or intensity (indicated by points) of infection determined at 10 d pIBM. Prevalence: Chi^2, n=95, df=1, X^2=0.540, p=0.4623, intensity: Mann-Whitney, n=80, df=1, U=717.5, p=0.4530. **(c)** ATQ exposure at 3 d pIBM significantly reduced the median cross-sectional area of oocysts at 7 d pIBM relative to control (Chi^2, n=50, df=1, *U*=110, p<0.0001). **(d)** The prevalence, but not the median intensity of *P. falciparum* sporozoites in mosquito salivary glands was significantly reduced in mosquitoes exposed to ATQ at 3 d pIBM (Chi^2, n=144, df=1, X^2=9.995, p=0.0016). Median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst/Sporozoite Prevalence and Oocyst/Sporozoite Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Supplementary Figure 4



584 **Supplementary Figure 4: AcVK5 survival and infectivity after ingestion of ATQ/glucose. (a)**
585 Survival prior to- and following 24 h access to 100 µM/10% w/v Glucose/0.5 v/v/ DMSO (4 d post
586 emergence, orange arrow), followed by *P. falciparum* (donor blood) infection (5 d post emergence,
587 red arrow). Ingestion of ATQ/glucose had no impact on the survival of AcVK5 mosquitoes relative to
588 a control group provided with 10% w/v glucose/0.5% v/v DMSO (Log-Rank Survival, n=1374, df=1,
589 X^2=1.3795, p=0.2402).

Supplementary Figure 5



Supplementary Figure 5: Ingestion of an ATQ-glucose solution after *P. falciparum* infection has no effect on oocyst prevalence or intensity. There was no difference relative to controls in either intensity (n=68, df=1, U=495.5, p=0.3257) or prevalence (n=81, df=1, X^2=2.441, p=0.1182) of oocysts at 10 d pIBM in females with continued access to 100 µM ATQ/0.5% DMSO/10% w/v glucose from 2 d pIBM - 14 d pIBM. Median lines and values are indicated, "n" indicates the number of independent samples. To isolate Oocyst Prevalence and Oocyst Intensity, midgut samples with zero oocysts have been excluded from intensity analysis. Statistical significance is indicated where relevant as follows: ns=not significant, * = p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

References

1. World Malaria Report 2020. World Health Organisation. 2020.
2. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle K, Moyes CL, Henry A, Eckhoff PA, Wenger EA, Briet O, Penny MA, Smith TA, Bennett A, Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray CLJ, Smith DL, Hay SI, Cibulskis RE, Gething PW. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526(7572):207-11. doi: 10.1038/nature15535. PubMed PMID: 26375008; PMCID: PMC4820050.
3. Lin JT, Juliano JJ, Wongsrichanalai C. Drug-Resistant Malaria: The Era of ACT. Curr Infect Dis Rep. 2010;12(3):165-73. Epub 2011/02/11. doi: 10.1007/s11908-010-0099-y. PubMed PMID: 21308525; PMCID: PMC3058555.
4. Ogbonna A, Uneke CJ. Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. Trans R Soc Trop Med Hyg. 2008;102(7):621-7. Epub 2008/05/24. doi: 10.1016/j.trstmh.2008.03.024. PubMed PMID: 18499204.
5. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, Rahman MR, Hasan MM, Islam A, Miotto O, Amato R, MacInnis B, Stalker J, Kwiatkowski DP, Bozdech Z, Jeeyapant A, Cheah PY, Sakulthaew T, Chalk J, Intharabut B, Silamut K, Lee SJ, Vihokhern B, Kunasol C, Imwong M, Tarning J, Taylor WJ, Yeung S, Woodrow CJ, Flegg JA, Das D, Smith J, Venkatesan M, Plowe CV, Stepniewska K, Guerin PJ, Dondorp AM, Day NP, White NJ, Tracking Resistance to Artemisinin C. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2014;371(5):411-23. Epub 2014/07/31. doi: 10.1056/NEJMoa1314981. PubMed PMID: 25075834; PMCID: PMC4143591.
6. Staedke SG, Gonahasa S, Dorsey G, Kamya MR, Maiteki-Sebuguzi C, Lynd A, Katureebe A, Kyohere M, Mutungi P, Kigozi SP, Opigo J, Hemingway J, Donnelly MJ. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign. Lancet. 2020;395(10232):1292-303. Epub 2020/04/20. doi: 10.1016/S0140-6736(20)30214-2. PubMed PMID: 32305094; PMCID: PMC7181182.
7. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, Sam B, Dek D, Try V, Amato R, Blessborn D, Song L, Tullo GS, Fay MP, Anderson JM, Tarning J, Fairhurst RM. Dihydroartemisinin-piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. The Lancet Infectious diseases. 2016;16(3):357-65. doi: 10.1016/S1473-3099(15)00487-9. PubMed PMID: 26774243; PMCID: 4792715.
8. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana SI, Yamauchi M, Opio W, Emoto S, Anywar DA, Kimura E, Palacpac NMQ, Odongo-Aginya EI, Ogwang M, Horii T, Mita T. Evidence of Artemisinin-Resistant Malaria in Africa. N Engl J Med. 2021;385(13):1163-71. Epub 2021/09/23. doi: 10.1056/NEJMoa2101746. PubMed PMID: 34551228.
9. Toé KH, Jones CM, N'Fale S, Ismail HM, Dabiré RK, Ranson H. Increased Pyrethroid Resistance in Malaria Vectors and Decreased Bed Net Effectiveness, Burkina Faso. Emerging Infectious Diseases. 2014;20(10):1691-6. doi: 10.3201/eid2010.140619. PubMed PMID: PMC4193182.
10. Samantsidis GR, Panteleri R, Denecke S, Kounadi S, Christou I, Nauen R, Douris V, Vontas J. 'What I cannot create, I do not understand': functionally validated synergism of metabolic and target site insecticide resistance. Proc Biol Sci. 2020;287(1927):20200838. Epub 2020/05/27. doi: 10.1098/rspb.2020.0838. PubMed PMID: 32453986; PMCID: PMC7287358.
11. Balabanidou V, Kampouraki A, MacLean M, Blomquist GJ, Tittiger C, Juarez MP, Mijailovsky SJ, Chalepakis G, Anthousi A, Lynd A, Antoine S, Hemingway J, Ranson H, Lycett GJ, Vontas J. Cytochrome P450 associated with insecticide resistance catalyzes cuticular hydrocarbon production in Anopheles gambiae. Proc

Natl Acad Sci U S A. 2016;113(33):9268-73. doi: 10.1073/pnas.1608295113. PubMed PMID: 27439866; PMCID: PMC4995928.

12. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol Biol. 2000;9(5):491-7. Epub 2000/10/13. doi: 10.1046/j.1365-2583.2000.00209.x. PubMed PMID: 11029667.

13. Stevenson BJ, Bibby J, Pignatelli P, Muangnoicharoen S, O'Neill PM, Lian LY, Muller P, Nikou D, Steven A, Hemingway J, Sutcliffe MJ, Paine MJ. Cytochrome P450 6M2 from the malaria vector Anopheles gambiae metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed. Insect Biochem Mol Biol. 2011;41(7):492-502. Epub 2011/02/18. doi: 10.1016/j.ibmb.2011.02.003. PubMed PMID: 21324359.

14. Tacoli C, Gai PP, Bayingana C, Sifft K, Geus D, Ndoli J, Sendegeya A, Gahutu JB, Mockenhaupt FP. Artemisinin Resistance-Associated K13 Polymorphisms of Plasmodium falciparum in Southern Rwanda, 2010-2015. Am J Trop Med Hyg. 2016;95(5):1090-3. Epub 2016/11/04. doi: 10.4269/ajtmh.16-0483. PubMed PMID: 27573632; PMCID: PMC5094222.

15. Bwire GM, Ngasala B, Mikomangwa WP, Kilonzi M, Kamuhabwa AAR. Detection of mutations associated with artemisinin resistance at k13-propeller gene and a near complete return of chloroquine susceptible falciparum malaria in Southeast of Tanzania. Sci Rep. 2020;10(1):3500. Epub 2020/02/28. doi: 10.1038/s41598-020-60549-7. PubMed PMID: 32103124; PMCID: PMC7044163.

16. Uwimana A, Legrand E, Stokes BH, Ndikumana JM, Warsame M, Umulisa N, Ngamije D, Munyaneza T, Mazarati JB, Munguti K, Campagne P, Criscuolo A, Ariey F, Murindahabi M, Ringwald P, Fidock DA, Mbituyumuremyi A, Menard D. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020;26(10):1602-8. Epub 2020/08/05. doi: 10.1038/s41591-020-1005-2. PubMed PMID: 32747827; PMCID: PMC7541349.

17. Asua V, Conrad MD, Aydemir O, Duvalsaint M, Legac J, Duarte E, Tumwebaze P, Chin DM, Cooper RA, Yeka A, Kamya MR, Dorsey G, Nsobya SL, Bailey J, Rosenthal PJ. Changing Prevalence of Potential Mediators of Aminoquinoline, Antifolate, and Artemisinin Resistance Across Uganda. J Infect Dis. 2021;223(6):985-94. Epub 2020/11/05. doi: 10.1093/infdis/jiaa687. PubMed PMID: 33146722; PMCID: PMC8006419.

18. Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyaneza T, Habimana RM, Rucogoza A, Moriarty LF, Sandford R, Piercefield E, Goldman I, Ezema B, Talundzic E, Pacheco MA, Escalante AA, Ngamije D, Mangala JN, Kabera M, Munguti K, Murindahabi M, Brieger W, Musanabaganwa C, Mutesa L, Udhayakumar V, Mbituyumuremyi A, Halsey ES, Lucchi NW. Association of Plasmodium falciparum kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. The Lancet Infectious diseases. 2021;21(8):1120-8. Epub 2021/04/18. doi: 10.1016/S1473-3099(21)00142-0. PubMed PMID: 33864801.

19. Straimer J, Gandhi P, Renner KC, Schmitt EK. High prevalence of P. falciparum K13 mutations in Rwanda is associated with slow parasite clearance after treatment with artemether-lumefantrine. J Infect Dis. 2021. Epub 2021/07/04. doi: 10.1093/infdis/jiab352. PubMed PMID: 34216470.

20. Sinka ME, Pironon S, Massey NC, Longbottom J, Hemingway J, Moyes CL, Willis KJ. A new malaria vector in Africa: Predicting the expansion range of Anopheles stephensi and identifying the urban populations at risk. Proceedings of the National Academy of Sciences of the United States of America. 2020;117(40):24900-8. Epub 2020/09/16. doi: 10.1073/pnas.2003976117. PubMed PMID: 32929020; PMCID: PMC7547157.

21. Sherrard-Smith E, Skarp JE, Beale AD, Fornadel C, Norris LC, Moore SJ, Mihreteab S, Charlwood JD, Bhatt S, Winskill P, Griffin JT, Churcher TS. Mosquito feeding behavior and how it influences residual malaria transmission across Africa. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(30):15086-95. Epub 2019/07/10. doi: 10.1073/pnas.1820646116. PubMed PMID: 31285346; PMCID: PMC6660788.

22. Fraser KJ, Mwandigha L, Traore SF, Traore MM, Doumbia S, Junnila A, Revay E, Beier JC, Marshall JM, Ghani AC, Muller G. Estimating the potential impact of Attractive Targeted Sugar Baits (ATSBs) as a new vector control tool for Plasmodium falciparum malaria. Malar J. 2021;20(1):151. Epub 2021/03/19. doi: 10.1186/s12936-021-03684-4. PubMed PMID: 33731111; PMCID: PMC7968277.

698 23. Muller GC, Beier JC, Traore SF, Toure MB, Traore MM, Bah S, Doumbia S, Schlein Y. Successful field
699 trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the Anopheles
700 gambiae complex in Mali, West Africa. Malar J. 2010;9:210. Epub 2010/07/29. doi: 10.1186/1475-2875-9-210.
701 PubMed PMID: 20663142; PMCID: PMC2914067.
702 24. Beier JC, Muller GC, Gu W, Arheart KL, Schlein Y. Attractive toxic sugar bait (ATSB) methods decimate
703 populations of Anopheles malaria vectors in arid environments regardless of the local availability of favoured
704 sugar-source blossoms. Malar J. 2012;11:31. doi: 10.1186/1475-2875-11-31. PubMed PMID: 22297155;
705 PMCID: PMC3293779.
706 25. Paton DG, Childs LM, Itoe MA, Holmdahl IE, Buckee CO, Catteruccia F. Exposing Anopheles mosquitoes
707 to antimalarials blocks Plasmodium parasite transmission. Nature. 2019;567(7747):239-43. Epub 2019/03/01.
708 doi: 10.1038/s41586-019-0973-1. PubMed PMID: 30814727.
709 26. Adams KL, Sawadogo SP, Nignan C, Niang A, Paton DG, Robert Shaw W, South A, Wang J, Itoe MA,
710 Werling K, Dabire RK, Diabate A, Catteruccia F. Cuticular hydrocarbons are associated with mating success and
711 insecticide resistance in malaria vectors. Commun Biol. 2021;4(1):911. Epub 2021/07/28. doi:
712 10.1038/s42003-021-02434-1. PubMed PMID: 34312484; PMCID: PMC8313523.
713 27. Kwiatkowska RM, Platt N, Poupardin R, Irving H, Dabire RK, Mitchell S, Jones CM, Diabate A, Ranson
714 H, Wondji CS. Dissecting the mechanisms responsible for the multiple insecticide resistance phenotype in
715 Anopheles gambiae s.s., M form, from Vallee du Kou, Burkina Faso. Gene. 2013;519(1):98-106. Epub
716 2013/02/06. doi: 10.1016/j.gene.2013.01.036. PubMed PMID: 23380570; PMCID: PMC3611593.
717 28. Kaneko O, Kimura M, Kawamoto F, Ferreira MU, Tanabe K. Plasmodium falciparum: allelic variation in
718 the merozoite surface protein 1 gene in wild isolates from southern Vietnam. Exp Parasitol. 1997;86(1):45-57.
719 doi: 10.1006/expr.1997.4147. PubMed PMID: 9149240.
720 29. Knols BG, Farenhorst M, Andriessen R, Snetselaar J, Suer RA, Osinga AJ, Knols JM, Deschietere J,
721 Ng'habi KR, Lyimo IN, Kessy ST, Mayagaya VS, Sperling S, Cordel M, Sternberg ED, Hartmann P, Mnyone LL,
722 Rose A, Thomas MB. Eave tubes for malaria control in Africa: an introduction. Malar J. 2016;15(1):404. Epub
723 2016/08/16. doi: 10.1186/s12936-016-1452-x. PubMed PMID: 27515306; PMCID: PMC4982263.
724 30. Shaw WR, Holmdahl IE, Itoe MA, Werling K, Marquette M, Paton DG, Singh N, Buckee CO, Childs LM,
725 Catteruccia F. Multiple blood feeding in mosquitoes shortens the Plasmodium falciparum incubation period
726 and increases malaria transmission potential. PLoS Pathog. 2020;16(12):e1009131. Epub 2021/01/01. doi:
727 10.1371/journal.ppat.1009131. PubMed PMID: 33382824; PMCID: PMC7774842.
728 31. Soulama I, Nebie I, Ouedraogo A, Gansane A, Diarra A, Tiono AB, Bougouma EC, Konate AT, Kabre GB,
729 Taylor WR, Sirima SB. Plasmodium falciparum genotypes diversity in symptomatic malaria of children living in
730 an urban and a rural setting in Burkina Faso. Malar J. 2009;8:135. Epub 2009/06/24. doi: 10.1186/1475-2875-
731 8-135. PubMed PMID: 19545390; PMCID: PMC2705376.
732 32. Sondo P, Derra K, Rouamba T, Nakanabo Diallo S, Taconet P, Kazienga A, Ilboudo H, Tahita MC, Valea
733 I, Sorgho H, Lefevre T, Tinto H. Determinants of Plasmodium falciparum multiplicity of infection and genetic
734 diversity in Burkina Faso. Parasit Vectors. 2020;13(1):427. Epub 2020/08/21. doi: 10.1186/s13071-020-04302-
735 z. PubMed PMID: 32819420; PMCID: PMC7441709.
736 33. Mitchell SN, Stevenson BJ, Muller P, Wilding CS, Egyir-Yawson A, Field SG, Hemingway J, Paine MJ,
737 Ranson H, Donnelly MJ. Identification and validation of a gene causing cross-resistance between insecticide
738 classes in Anopheles gambiae from Ghana. Proc Natl Acad Sci U S A. 2012;109(16):6147-52. doi:
739 10.1073/pnas.1203452109. PubMed PMID: 22460795; PMCID: PMC3341073.
740 34. Lees RS, Ismail HM, Logan RAE, Malone D, Davies R, Anthousi A, Adolfi A, Lycett GJ, Paine MJI. New
741 insecticide screening platforms indicate that Mitochondrial Complex I inhibitors are susceptible to cross-
742 resistance by mosquito P450s that metabolise pyrethroids. Sci Rep. 2020;10(1):16232. doi: 10.1038/s41598-
743 020-73267-x. PubMed PMID: 33004954; PMCID: PMC7530702.
744 35. Musset L, Bouchaud O, Matheron S, Massias L, Le Bras J. Clinical atovaquone-proguanil resistance of
745 Plasmodium falciparum associated with cytochrome b codon 268 mutations. Microbes Infect.
746 2006;8(11):2599-604. doi: 10.1016/j.micinf.2006.07.011. PubMed PMID: 16962361.
747 36. Bopp SE, Manary MJ, Bright AT, Johnston GL, Dharia NV, Luna FL, McCormack S, Plouffe D, McNamara
748 CW, Walker JR, Fidock DA, Denchi EL, Winzeler EA. Mitotic evolution of Plasmodium falciparum shows a stable

749 core genome but recombination in antigen families. PLoS Genet. 2013;9(2):e1003293. doi:
750 10.1371/journal.pgen.1003293. PubMed PMID: 23408914; PMCID: PMC3567157.
751 37. Goodman CD, Siregar JE, Mollard V, Vega-Rodriguez J, Syafruddin D, Matsuoka H, Matsuzaki M,
752 Toyama T, Sturm A, Cozijnsen A, Jacobs-Lorena M, Kita K, Marzuki S, McFadden GI. Parasites resistant to the
753 antimalarial atovaquone fail to transmit by mosquitoes. Science. 2016;352(6283):349-53. doi:
754 10.1126/science.aad9279. PubMed PMID: 27081071; PMCID: PMC5149070.
755 38. Blake LD, Johnson ME, Siegel SV, McQueen A, Iyamu ID, Shaikh AK, Shultis MW, Manetsch R, Kyle DE.
756 Menoctone Resistance in Malaria Parasites Is Conferred by M133I Mutations in Cytochrome b That Are
757 Transmissible through Mosquitoes. Antimicrob Agents Chemother. 2017;61(8). doi: 10.1128/AAC.00689-17.
758 PubMed PMID: 28584157; PMCID: PMC5527649.
759 39. Siegel S, Rivero A, Adapa SR, Wang C, Manetsch R, Jiang RHY, Kyle DE. Mitochondrial heteroplasmy is
760 responsible for Atovaquone drug resistance in Plasmodium falciparum. bioRxiv. 2017:232033. doi:
761 10.1101/232033.
762 40. Bopp S, Magistrado P, Wong W, Schaffner SF, Mukherjee A, Lim P, Dhorda M, Amaratunga C, Woodrow
763 CJ, Ashley EA, White NJ, Dondorp AM, Fairhurst RM, Ariey F, Menard D, Wirth DF, Volkman SK. Plasmepsin II-
764 III copy number accounts for bimodal piperaquine resistance among Cambodian Plasmodium falciparum. Nat
765 Commun. 2018;9(1):1769. Epub 2018/05/04. doi: 10.1038/s41467-018-04104-z. PubMed PMID: 29720620;
766 PMCID: PMC5931971.
767 41. Graumans W, Jacobs E, Bousema T, Sinnis P. When Is a Plasmodium-Infected Mosquito an Infectious
768 Mosquito? Trends Parasitol. 2020;36(8):705-16. doi: 10.1016/j.pt.2020.05.011. PubMed PMID: 32620501;
769 PMCID: PMC7386819.
770 42. Sinden RE, Billingsley PF. Plasmodium invasion of mosquito cells: hawk or dove? Trends Parasitol.
771 2001;17(5):209-12. doi: 10.1016/s1471-4922(01)01928-6. PubMed PMID: 11323288.
772 43. Smith RC, Vega-Rodriguez J, Jacobs-Lorena M. The Plasmodium bottleneck: malaria parasite losses in
773 the mosquito vector. Mem Inst Oswaldo Cruz. 2014;109(5):644-61. PubMed PMID: 25185005; PMCID:
774 PMC4156458.
775 44. Burrows J, Fidock DA, Miller RS, Rees S. Blocking Plasmodium Development in Mosquitoes: A Powerful
776 New Approach for Expanding Malaria Control Efforts. Am J Trop Med Hyg. 2019;101(4):734-5. Epub
777 2019/07/03. doi: 10.4269/ajtmh.19-0318. PubMed PMID: 31264564; PMCID: PMC6779223.
778 45. Kessler S, Vlimant M, Guerin PM. The sugar meal of the African malaria mosquito Anopheles gambiae
779 and how deterrent compounds interfere with it: a behavioural and neurophysiological study. J Exp Biol.
780 2013;216(Pt 7):1292-306. Epub 2012/12/25. doi: 10.1242/jeb.076588. PubMed PMID: 23264482.
781 46. Qualls WA, Muller GC, Traore SF, Traore MM, Arheart KL, Doumbia S, Schlein Y, Kravchenko VD, Xue
782 RD, Beier JC. Indoor use of attractive toxic sugar bait (ATSB) to effectively control malaria vectors in Mali, West
783 Africa. Malar J. 2015;14:301. Epub 2015/08/06. doi: 10.1186/s12936-015-0819-8. PubMed PMID: 26242186;
784 PMCID: PMC4524285.
785 47. Hien D, Pare PSL, Cooper A, Koama BK, Guissou E, Yameogo KB, Yerbanga RS, Farrell IW, Ouedraogo
786 JB, Gnankine O, Ignell R, Cohuet A, Dabire RK, Stevenson PC, Lefevre T. Contrasting effects of the alkaloid
787 ricinine on the capacity of Anopheles gambiae and Anopheles coluzzii to transmit Plasmodium falciparum.
788 Parasit Vectors. 2021;14(1):479. doi: 10.1186/s13071-021-04992-z. PubMed PMID: 34526119; PMCID:
789 PMC8444468.
790 48. Paaijmans K, Fernandez-Busquets X. Antimalarial drug delivery to the mosquito: an option worth
791 exploring? Future Microbiol. 2014;9(5):579-82. doi: 10.2217/fmb.14.30. PubMed PMID: 24957084.
792 49. Ferreira TN, Pita-Pereira D, Costa SG, Brazil RP, Moraes CS, Diaz-Albiter HM, Genta FA. Transmission
793 blocking sugar baits for the control of Leishmania development inside sand flies using environmentally friendly
794 beta-glycosides and their aglycones. Parasit Vectors. 2018;11(1):614. doi: 10.1186/s13071-018-3122-z.
795 PubMed PMID: 30501613; PMCID: PMC6271627.
796 50. Srivastava IK, Rottenberg H, Vaidya AB. Atovaquone, a broad spectrum antiparasitic drug, collapses
797 mitochondrial membrane potential in a malarial parasite. J Biol Chem. 1997;272(7):3961-6. PubMed PMID:
798 9020100.

799 51. Painter HJ, Morrisey JM, Mather MW, Vaidya AB. Specific role of mitochondrial electron transport in
800 blood-stage Plasmodium falciparum. Nature. 2007;446(7131):88-91. doi: 10.1038/nature05572. PubMed
801 PMID: 17330044.
802 52. Ke H, Lewis IA, Morrisey JM, McLean KJ, Ganesan SM, Painter HJ, Mather MW, Jacobs-Lorena M, Llinas
803 M, Vaidya AB. Genetic investigation of tricarboxylic acid metabolism during the Plasmodium falciparum life
804 cycle. Cell Rep. 2015;11(1):164-74. doi: 10.1016/j.celrep.2015.03.011. PubMed PMID: 25843709; PMCID:
805 PMC4394047.
806 53. Boysen KE, Matuschewski K. Arrested oocyst maturation in Plasmodium parasites lacking type II
807 NADH:ubiquinone dehydrogenase. J Biol Chem. 2011;286(37):32661-71. doi: 10.1074/jbc.M111.269399.
808 PubMed PMID: 21771793; PMCID: PMC3173203.
809 54. Hino A, Hirai M, Tanaka TQ, Watanabe Y, Matsuoka H, Kita K. Critical roles of the mitochondrial
810 complex II in oocyst formation of rodent malaria parasite Plasmodium berghei. J Biochem. 2012;152(3):259-
811 68. doi: 10.1093/jb/mvs058. PubMed PMID: 22628552.
812 55. Sturm A, Mollard V, Cozijnsen A, Goodman CD, McFadden GI. Mitochondrial ATP synthase is
813 dispensable in blood-stage Plasmodium berghei rodent malaria but essential in the mosquito phase. Proc Natl
814 Acad Sci U S A. 2015;112(33):10216-23. doi: 10.1073/pnas.1423959112. PubMed PMID: 25831536; PMCID:
815 PMC4547259.
816 56. Terzian LA, Stahler N, Dawkins AT, Jr. The sporogonous of Plasmodium vivax in Anopheles mosquitoes
817 as a system for evaluating the prophylactic and curative capabilities of potential antimalarial compounds. Exp
818 Parasitol. 1968;23(1):56-66. doi: 10.1016/0014-4894(68)90042-8. PubMed PMID: 4876903.
819 57. Mukherjee A, Bopp S, Magistrado P, Wong W, Daniels R, Demas A, Schaffner S, Amaratunga C, Lim P,
820 Dhorda M, Miotto O, Woodrow C, Ashley EA, Dondorp AM, White NJ, Wirth D, Fairhurst R, Volkman SK.
821 Artemisinin resistance without pfkelch13 mutations in Plasmodium falciparum isolates from Cambodia. Malar
822 J. 2017;16(1):195. Epub 2017/05/13. doi: 10.1186/s12936-017-1845-5. PubMed PMID: 28494763; PMCID:
823 PMC5427620.
824 58. Marcenac P, Shaw WR, Kakani EG, Mitchell SN, South A, Werling K, Marrogi E, Abernathy DG, Yerbanga
825 RS, Dabire RK, Diabate A, Lefevre T, Catteruccia F. A mating-induced reproductive gene promotes Anopheles
826 tolerance to Plasmodium falciparum infection. PLoS Pathog. 2020;16(12):e1008908. Epub 2020/12/22. doi:
827 10.1371/journal.ppat.1008908. PubMed PMID: 33347501; PMCID: PMC7785212.
828 59. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193(4254):673-5.
829 Epub 1976/08/20. doi: 10.1126/science.781840. PubMed PMID: 781840.
830 60. Ifediba T, Vanderberg JP. Complete in vitro maturation of Plasmodium falciparum gametocytes.
831 Nature. 1981;294(5839):364-6. Epub 1981/11/26. PubMed PMID: 7031476.
832 61. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, Thaithong S, Brown KN. High
833 sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol
834 Biochem Parasitol. 1993;61(2):315-20. PubMed PMID: 8264734.

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