Vector control

CellPress
OPEN ACCESS

Trends in
Parasitology

Review

Vector control: agents of selection on malaria parasites?

Catherine E. Oke [1,*] Victoria A. Ingham [2] Craig A. Walling [1] and Sarah E. Reece [1,3]

Insect vectors are responsible for spreading many infectious diseases, yet interactions between pathogens/parasites and insect vectors remain poorly understood. Filling this knowledge gap matters because vectors are evolving in response to the deployment of vector control tools (VCTs). Yet, whilst the evolutionary responses of vectors to VCTs are being carefully monitored, the knock-on consequences for parasite evolution have been overlooked. By examining how mosquito responses to VCTs impact upon malaria parasite ecology, we derive a framework for predicting parasite responses. Understanding how VCTs affect the selection pressures imposed on parasites could help to mitigate against parasite evolution that leads to unfavourable epidemiological outcomes. Furthermore, anticipating parasite evolution will inform monitoring strategies for VCT programmes as well as uncovering novel VCT strategies.

Highlights

Vector control tools (VCTs) affect diverse aspects of mosquito biology and are a driver of vector evolution.

VCTs change parasite ecology by exposing parasites to insecticides and to vectors with altered genotypes and phenotypes (e.g., lifespan, behaviour, immunity, metabolism, microbiome).

Parasite activities are affected by the ways that VCTs alter parasite–vector interactions, and this can drive parasite evolution.

Parasite responses to VCTs are likely to include plastic and evolutionary changes to transmission traits expressed during infections in hosts/vectors.

Parasite responses could undermine gains made towards malaria elimination and may have knock-on consequences for parasite–host interactions.

Knowledge of parasite responses to the selection pressures imposed by VCTs could offer new approaches to reduce disease transmission that are robust to parasite evolution.

Why consider parasite evolution in response to vector control tools?

Vector control is one of the most effective methods to curb vector-borne diseases, with insecticide-based interventions predicted to have averted the majority of malaria cases since 2000 [1]. However, because they reduce vector survival and reproduction, the continued and widespread use of chemical-based vector control tools (VCTs) has contributed to the evolution of insecticide resistance (IR) and evasion, particularly in Anopheline mosquito **populations** (Box 1) [2,3], and threatens progress towards malaria elimination targets [4]. It is therefore not surprising that the responses of insect vectors to VCTs receive intensive investigation [5,6]. However, remarkably little attention has been paid to how VCTs alter parasite–vector–host interactions and how parasites are responding to the selection pressures imposed by the consequences of VCTs.

Just like drugs or vaccines, VCTs are an ecological perturbation that decreases parasite fitness by reducing vectorial capacity (the rate at which a vector can transmit a pathogen from a currently infectious case; Box 2) [7]. History illustrates that attempts to reduce the survival and/or transmission of parasites/pathogens are readily met with counter-evolution. For example, malaria parasites have evolved resistance against all classes of antimalarial drugs [8], and can alter **life history traits** (see Glossary) to partially compensate for fitness lost due to drug treatment [9]. Parasites transmitted via vectors targeted by VCTs face diverse perturbations to their ecology (reviewed in [10]). In the short term, at the start of a control programme, parasites experience a dramatic drop in the health, abundance, and lifespan of vectors, and in the longer term, parasites encounter vectors that have altered genotypes and phenotypes, as well as alternative vector species.

Predicting parasite responses to VCT-driven changes to their ecology requires: (i) uncovering how VCTs affect parasite fitness, both directly and indirectly via their impacts on vectors; (ii) establishing which aspects of VCTs impose constraints on parasite activities and/or provide opportunities to better exploit vectors; and (iii) considering how *de novo* mutation, standing genetic variation, and **phenotypic plasticity** can contribute to the adaptation of parasites to

[1]Institute of Ecology and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3FL, UK
[2]Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Im Neuenheimer Feld 324, 69210 Heidelberg, Germany
[3]Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3FL, UK

*Correspondence:
catherine.oke@ed.ac.uk (C.E. Oke).

Box 1. Vector responses to insecticide-based VCTs

Mechanisms of resistance

Insecticide target-site mutations reduce insecticide toxicity by causing structural modifications to target proteins, and include knockdown resistance mutations (*kdr*) in the *para sodium channel* gene (pyrethroid/DDT resistance), an *Rdl* gene mutation (dieldrin resistance), and an acetylcholinesterase enzyme (*ace-1*) mutation (organophosphate and carbamate resistance) [15]. Increased metabolism and clearance via overexpression of detoxification gene families, including cytochrome P450-associated monooxygenases (P450s), glutathione S-transferases (GSTs), and carboxylesterases, enhance insecticide detoxification [33]. Other mechanisms include reducing insecticide penetration via a thicker cuticle [75], and sequestration by chemosensory proteins in the legs [76].

Insecticide exposure

Due to insecticide decay and insecticide resistance (IR) mechanisms, exposure to sublethal insecticide doses occur. In the short term, this stimulates changes to detoxification and redox metabolism gene expression [30,33], and reduces host-seeking and blood feeding [20,77]. Longer term effects include reduced survival of both IR and insecticide-susceptible (IS) mosquitoes [77,78], but lifespan is not affected in some highly resistant populations [79]. Furthermore, older mosquitoes are more susceptible to insecticides [80].

Methods for evasion

Avoidance is an alternative to coping with insecticides and occurs by blood foraging less frequently or at times of day when hosts are not protected by bed nets [5], in the early evening [81], or early morning [82]. ITNs target anthropophilic species that bite indoors at night, rather than less specialist feeders that bite at any time of day and/or outdoors. Thus, sustained ITN use is associated with increased outdoor biting [83] and resting [84], as well as seeking a higher proportion of blood meals from non-human vertebrate hosts [84,85].

Further considerations

Identifying the genetic basis and **heritability** of IR traits is challenging for complex behaviours such as a biting time-of-day, habitat choice, and host preference, but some behavioural resistance strategies are heritable [86,87]. In general, mechanisms conferring resistance are costly when expressed in the absence of insecticides. For example, biochemical resistance is associated with costs across both larval and adult stages [27,88–90], including reduced fecundity and lifespan, differing across vector genotypes and resistance mechanisms [15,16]. Trade-offs may limit avoidance; changes to biting time-of-day affect reproductive schedule [39] and may cause 'jet lag' between feeding rhythms and other circadian-regulated processes, such as detoxification and immune responses [38], increasing susceptibility to insecticides at certain times of day [91]. Furthermore, blood meals from non-preferred host species reduce mosquito fitness [36], due to differences in haematological properties [51].

VCT-imposed alterations of parasite–host–vector interactions. Here, we give an overview of (i) and (ii), which are more thoroughly covered in [10], and we focus on providing a framework to address (iii), including monitoring and mitigation strategies.

Due to the wealth of knowledge about the effects of insecticide-based VCTs on Anopheline vectors of malaria, we focus on this system, but the concepts will be generalisable to other vector-borne infectious diseases and other VCTs, including tools currently in development, such as gene drives and endectocides (compounds administered to mammalian hosts to render blood meals toxic to mosquitoes) [11,12]. Moreover, opening the black box of parasite–vector interactions may reveal how to make VCTs robust to clinically and epidemiologically unfavourable parasite counter-evolution as well as uncover new approaches for VCTs.

VCTs: mosquito responses and the consequences for parasites

Insecticide-based VCTs, such as **insecticide-treated bed nets** (**ITNs**) and **indoor residual spraying** (**IRS**), are highly effective, but their widespread deployment and limited chemical nature has selected for the evolution of multiple IR mechanisms [13]. Resistance mechanisms can be biochemical, morphological, or behavioural [5], and underpinned by genetic evolution and/or deployment of pre-existing adaptive phenotypic plasticity (APP) (summarised in Box 1).

Glossary

Genotype-by-environment interaction (G × E): different genotypes differ in the extent of their plastic response to a change in environmental conditions, demonstrating genetic variation for plasticity.
Gonotrophic cycle: the cycle of blood feeding, egg development, and oviposition of female mosquitoes.
Heritability: the proportion of phenotypic variance in a trait that has an additive genetic basis, which is a key determinant of the evolutionary potential of a trait.
Indoor residual spraying (IRS): an intervention that targets indoor-biting mosquitoes by coating indoor wall surfaces of a house with an insecticide that kills mosquitoes when they rest on these surfaces after feeding.
Insecticide-treated bed nets (ITNs): bed nets that have been treated with a pyrethroid insecticide and protect the user against biting mosquitoes. New-generation bed nets include additional chemistry, such as a pyrrole insecticide, the synergist piperonyl butoxide (PBO) which enhances the efficacy of pyrethroids, or the insect growth regulator pyriproxyfen.
Life history trade-off: a trade-off exists when an increase in one life history trait is coupled to an unavoidable decrease in a second trait. Trade-offs can be mediated at the physiological level (e.g., competitive allocation of resources to different traits) and/or have a genetic basis (alleles having antagonistic pleiotropic effects or linkage disequilibrium between loci). Genetic trade-offs can constrain evolution.
Life history traits: traits which affect organismal fitness. Life history theory is the branch of evolutionary theory developed to explain how selective forces shape the traits/strategies/phenotypes of multicellular organisms to optimise survival and reproduction. For multicellular organisms, the target of natural selection is usually considered as a single organism, but in single-celled parasites, the target is best viewed as a single genotype within an infection, because the fitness interests of closely related parasite cells are aligned.
Phenotypic plasticity: the phenomenon by which a given genotype can produce different traits/phenotypes in response to a change in environmental conditions. Plasticity is considered adaptive when a plastic genotype has

Box 2. R_0, vectorial capacity and transmission dynamics

R_0, also known as the Ross-MacDonald equation, describes the expected number of infected hosts generated from a single infected host in a completely susceptible population. It is defined using the equation [92]:

$$R_0 = \frac{ma^2bc}{(-\ln p)r}p^v \quad [I]$$

The equation for vectorial capacity derives from the above. It excludes *r*, the daily rate that each human recovers from infection because it contains the purely entomological concepts of R_0 [92]:

$$V = \frac{ma^2bc}{(-\ln p)}p^v \quad [II]$$

Density of vectors per vertebrate hosts (*m*). Transmission is positively correlated to the number of vectors per host and is shaped by the reproductive output of vector species and how this interacts with environmental factors.

Human-biting rate (*a*). The rate that humans are bitten varies across vector species due to species specificity in host preference and whether preference depends on the availability of different kinds of host [93].

Vector competence (*bc*). The proportion of bites by an infectious mosquito that infect a human (*b*) and the probability that a mosquito becomes infected after biting an infected human (*c*) make up vector competence [94]. The ability of a parasite to survive within the vector during development into the form which is infective to a new human host reflects the combined effects of parasite infectivity to vectors and vector susceptibility [95].

Duration of parasite extrinsic incubation period (EIP) (*v*). The time it takes for the parasite to develop from the point of ingestion to the form infective to a new human host is shaped by parasite intrinsic factors [96] as well as environmental temperature [97] and resource availability within the vector [64]. Because *v* and *p* interact exponentially, small changes in EIP can dramatically affect vectorial capacity.

Daily probability of adult survival (*p*). The EIP lasts for a long proportion of vector lifespan, and transmission requires that the vector survives throughout this period.

All vectorial capacity parameters (even including *m*) are a product of how parasites and vectors interact and are subject to alteration by VCTs. Based on observations in the literature, Table I illustrates potential impacts of the consequences of VCTs on these parameters. Increases are denoted by '+', reductions are denoted by '–', and scenarios that are yet to be investigated or for where specific details matter and general principles are unlikely are indicated by '?'. Particularly noteworthy is that the effects of VCTs on parasite contributions to vectorial capacity are largely unknown.

Table I. The consequences of VCT use and their potential effect on vectorial capacity parameters

Impact of VCT on vectors	Potential change to vectorial capacity parameter			
	+	–	?	No change
Reduction in population size	–	*m, a*	–	*bc, v, p*
Insecticide exposure	–	*m, a, bc, p*	*v*	–
Insecticide resistance mechanisms	*m, p*	*p*	*a, bc, v*	–
Altered resource allocation	–	–	*a, bc, v, p*	*m*
Change in species composition	–	*m, a, bc*	*v, p*	–
Biting time shifts	*a*	*m, p*	*bc, v*	–
Change to outdoor biting	*a*	*m, p*	*bc, v*	–

During the mosquito phase of the *Plasmodium* life cycle (**sporogony**, Figure 1A), Anopheline vectors undergo multiple **gonotrophic cycles**, temporally coupling activities that must be undertaken by parasite and vector [14] (Figure 1B). Thus, parasites will likely be directly exposed to insecticides at regular intervals during sporogony when host-seeking mosquitoes interact with ITNs/IRS, as well as facing the effects that insecticides have on vectors (including alterations to lifespan, resource acquisition and allocation, metabolism, oxidative state, and immune responses; Box 1). Furthermore, the consequences of insecticide exposure include disrupted

higher fitness following a (often predictable) change in environmental conditions compared to a nonplastic genotype, but plasticity can also be maladaptive or have a neutral impact on an organism's fitness.
Population: a group of individuals (i.e., parasite genotypes) that can interbreed.
Reaction norm: the shape of the relationship between the phenotypes (e.g., different values for a life history trait) produced by a specific genotype and the environmental conditions inducing these phenotypes. When the reaction norms for different genotypes are compared across the same environmental conditions, different slopes indicate genotype-by-environment interactions.
Sporogony: the obligate phase of sexual reproduction, development, and replication of *Plasmodium* parasites within the insect vector.

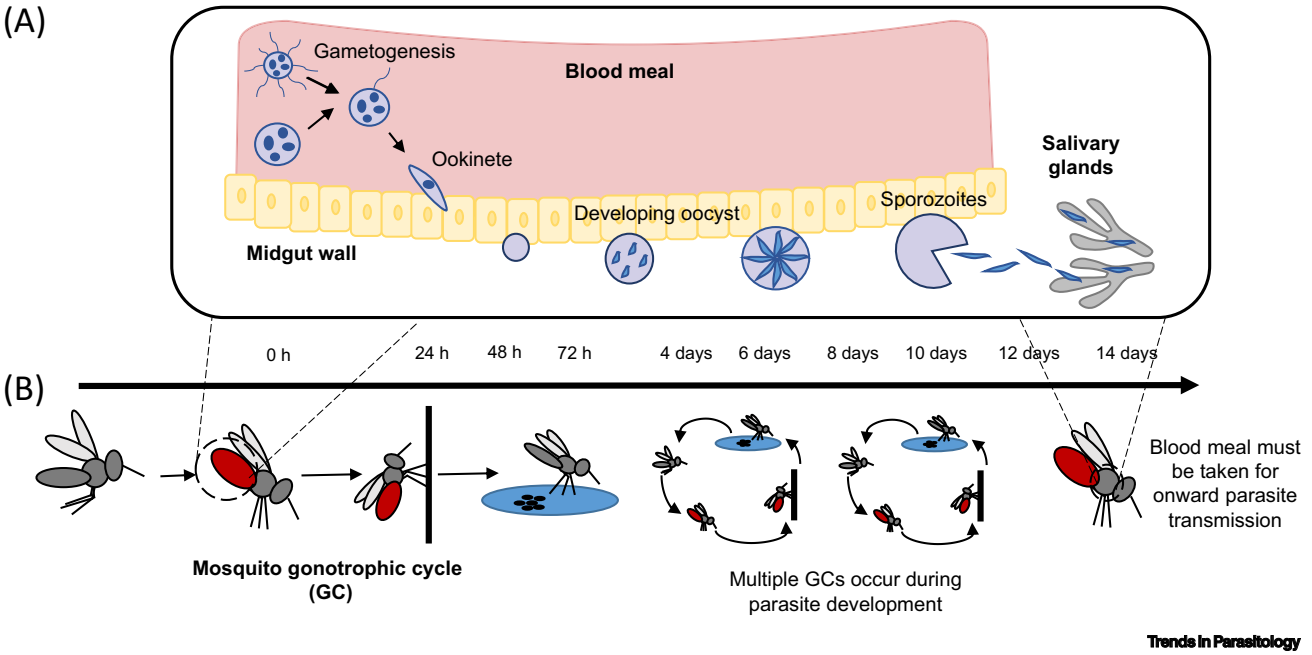

Figure 1. Mosquito and malaria parasite ecology are closely interlinked. (A) Malaria transmission is initiated in the vertebrate host when a small proportion of parasites during each intraerythrocytic developmental cycle commit to producing sexual stages (gametocytes). Upon ingestion in a blood meal, parasites undergo sexual reproduction in which gametocytes rapidly differentiate into gametes, undergo a single round of sexual reproduction, and develop into motile ookinetes. Ookinetes traverse the midgut wall and become oocysts, within which parasites undergo many rounds of asexual replication over multiple days to produce thousands of sporozoites. Sporozoites are released upon oocyst egress and must migrate through the haemocoel to the salivary glands where they reside until injected into a new host upon blood feeding. (B) Female *Anopheles* mosquitoes seek a blood meal from a human. After blood feeding, mosquitoes spend approximately 48–72 h resting while their eggs develop and then seek an oviposition site to lay eggs, sometimes ingesting a sugar meal for energy before they begin host-seeking again. The gonotrophic cycle (blood-feed, egg development, oviposition) can repeat approximately two or three times during the period of oocyst development.Vector control tools (VCTs) can affect the gonotrophic cycle in a variety of ways and therefore affect parasite as well as mosquito ecology.

blood-feeding schedules and changes in the species composition of vectors, which also impact host–parasite interactions during onwards transmission.

The additive, antagonistic, or interacting effects of VCT-driven ecological perturbations on parasite–vector–host interactions are poorly understood, with many key open questions [10]. The few studies available suggest that the individual impacts of these factors on transmission are complex and that variation in IR mechanisms and vector genotype add further complexities [15,16]. In this section, we outline how interactions with vectors that possess IR mechanisms, exhibit altered behaviours, and belong to different species all affect parasite ecology, and we relate these impacts to transmission and vector competence (Box 2).

Insecticide exposure and IR mechanisms

Due to the near ubiquitous nature of IR, modern parasites frequently encounter vectors altered by IR mechanisms (Box 1). These parasites also face the effects of direct exposure to insecticides through vectors that have been exposed to sublethal doses; this occurs because IR vectors can withstand contact with high doses and insecticide-susceptible (IS) vectors may survive contact with degraded insecticide. Because the duration of sporogony [the extrinsic incubation period (EIP)] is long relative to mosquito lifespan, even small effects of IR vectors and/or insecticide exposure on the survival and development of parasites can have large effects on parasite fitness (Box 2).

Few studies have considered the direct consequences of insecticide exposure for parasite fitness, but observations include reduced mating and/or impaired early development [17,18] (N. Hill, PhD thesis, London School of Hygiene and Tropical Medicine, 2002). Pyrethroid insecticides, which are neurotoxic, are efficacious against some Apicomplexans [19], but the direct impact on *Plasmodium* is currently unknown. The multiplicity of IR mechanisms could provide protection for the parasite from the direct action of the insecticide through lowering concentration of the toxic insecticide; however, indirect effects of these mechanisms on the parasite are likely to be costly.

Insecticide exposure is also expected to reduce transmission by inhibiting host-seeking activity of IR mosquitoes, lengthening the gonotrophic cycle, and potentially decreasing mosquito lifespan [20]. However, whilst some studies demonstrate that IR mechanisms reduce the intensity of malaria infections [21], others show that IR mechanisms make mosquitoes more susceptible to infection [22,23], or have no effect [24]. The lack of consensus for how IR mosquitoes affect parasite fitness [25] is likely due to the wide range of IR mechanisms (Box 1) that can also interact in complex ways, the difficulties of controlling for the confounding effects of insecticide exposure and age in field studies, and because malaria infection may exacerbate mosquito susceptibility to insecticides [26].

Altered resource allocation

Like all organisms, mosquitoes have a finite amount of resources, and face **life history trade-offs** where they must differentially allocate resources between life history traits (e.g., immune defences vs. reproduction, antioxidants vs. immune defences). Life history trade-offs may explain in part the reduced fecundity of IR mosquitoes compared to their susceptible counterparts [27], as well as their differences in microbiota [28], immune gene expression [29], respiration rate [30], and lifespan [31]. Additionally, most IR mechanisms involve overproduction of proteins, which requires the investment of resources. For example, to ameliorate the oxidative costs of overexpressed P450s (Box 1), mosquitoes may invest relatively more in redox management, which could trade off against investment in immune defences, fecundity, and/or lifespan.

How mosquitoes divide resources between IR mechanisms and managing their consequences versus other traits is likely to have complex consequences for parasites. For example, oxidative stress through the production of reactive oxygen species (ROS) negatively affects parasites [32] and so IR involving ROS could provide a form of immune defence against infection. However, if these mosquitoes reduce their fecundity to cope with infection and/or insecticide exposure [27], resources may be released for parasites to scavenge, enhancing parasite productivity. Furthermore, if investment in fecundity is prioritised over non-ROS forms of immune defence, parasites also benefit. Yet, if maintaining physiological health and fecundity comes at the expense of lifespan, parasites may suffer from premature vector mortality. Thus, the net effects of mosquito life history strategies on parasite transmission may pivot on mechanisms that affect ROS levels in potentially contrasting ways [30,33]. This complexity is highlighted by the contrasting effects of the detoxification enzymes, the P450s and GSTs (Box 1), which elevate and reduce oxidative stress respectively [10].

Behavioural avoidance

Insecticide evasion behaviours of mosquitoes include biting at a time of day when humans are not protected by ITNs, resting outdoors, and blood feeding from alternative host species (Box 1). In one sense, behavioural avoidance of ITNs and IRS is beneficial for parasites because, by reducing the chance of insecticide-induced vector mortality, parasites have more opportunities for onwards transmission. However, this benefit may be eroded if behavioural avoidance delays

blood-feeding activity because parasites may receive resources from the blood meal less often or at less useful points during sporogony [34,35]. Similarly, if mosquitoes shift to feeding on non-human hosts, they may receive a blood meal they are less capable of utilising, and so both vectors and parasites become resource-limited [36].

Daily rhythms dominate malaria transmission; parasites enter and exit the vector at specific times of day. Parasites are confronted with daily rhythms in mosquito physiology, including immune responses and insecticide detoxification [37] for the duration of infections. If day-biting leads to mosquito rhythms being temporally decoupled from the parasite's developmental schedule, parasites could benefit if key transitions in the life cycle, such as ookinete migration, shift to a time of day when mosquito immune responses are suppressed [38]. However, temporal dysregulation of the mosquitoes' own rhythms as a consequence of a shift in the timing of blood feeding could reduce mosquito fitness and, consequently, decrease vector population size and reduce lifespan [39]. Environmental rhythms may also play a role; when reared under realistic daily temperature regimes, mosquitoes feeding in the early evening are more competent vectors [40]. This is likely due to an interaction between the effects of mosquito rhythms on susceptibility and the temperature-sensitive mating of parasites.

How blood-feeding rhythms affect transmission is further complicated by the role of daily rhythms exhibited by hosts and parasites. For example, *Anopheles stephensi* mosquitoes infected in their rest phase (i.e., daytime for nocturnal mosquitoes) are more susceptible to *Plasmodium chabaudi*, but this seeming advantage is negated by gametocytes being less infective during the daytime [41]. Whilst host rhythms do not affect transmission to mosquitoes in laboratory models [39], this might not be the case in nature. For example, dengue virus replicates faster and exhibits a shorter EIP when its *Aedes aegypti* vector receives a blood meal with a high blood glucose concentration [42]. In keeping with this, malaria parasite gametogenesis and mating are glucose-hungry processes [43] and day-feeding Anophelines will coincide with the daytime peak in blood glucose of human hosts. However, any advantage of entering a mosquito at an unusual time of day might be eroded by entering a new human host at this time of day because mammalian hosts are generally more susceptible to infection in their rest phase (night for humans) [44]. However, predicting whether transmission is affected by VCT-induced shifts in the timing of blood seeking requires information on how much vector biting rhythms can shift.

Shifts in vector species

A consequence of ITNs and IRS primarily targeting indoor, night-biting, anthropophilic species is that transmission shifts to less anthropophilic vector species which are experiencing less of a decline in areas of high ITN use [45,46]. This imposes a change in both the composition and the relative abundance of different species that can be used as vectors. The constraints and opportunities presented to parasites by alternative vectors are even less well studied than the previously discussed consequences of VCTs. However, because vector species differ in their activity rhythms and their preferences for host species, it is likely that parasites may encounter similar problems as described in the previous section [47,48].

Other important behaviours, such as the number of blood meals taken during sporogony, also vary between species [49], affecting both transmission opportunities and the number of parasite genotypes that sequentially coinfect mosquitoes [50]. Furthermore, sporogony is less productive when vectors take blood meals from novel host species [51]. In addition to interspecific variation in behaviours that affect transmission, differences in immune regulation [52] across vector species affect vector competence. While *Plasmodium* has adapted to evade the immune system of its local vector across its wide geographical range, parasites perform less well in nonsympatric

vectors [53]. Thus, if parasites are decreasingly able to rely on a single vector species, vector specialists will not transmit as successfully as generalists. Large-scale comparative experiments are required to reveal which parasite–vector interactions underpin successful sporogony across vector species and to predict the impacts of vector shifts on transmission.

Parasite responses to the consequences of VCTs

Assuming that evolution and/or APP allows parasites to alter transmission traits in response to the consequences of VCTs, how might parasites be changing? In an ideal world, parasites would infect long-lived, frequently blood-feeding vectors, and produce large numbers of highly infective sporozoites after a short EIP. However, constraints and trade-offs limit parasite transmission, many of which are imposed by their hosts and vectors. The latter include host/vector immune responses, the type and amount of host/vector resources available, vector lifespan, and interactions with other organisms sharing the vector, all of which are likely to be altered by VCTs as illustrated in the previous sections (also see Box 2).

Parasite traits underpinning transmission include the density and sex ratio of gametocytes, which are traits expressed within the host, and all the stages of sporogony which include mating and zygote development, ookinete migration, oocyst development, sporozoite egress and migration to the salivary glands (Figure 1A). In the following sections, we illustrate how these traits could be altered in response to the consequences of VCTs and identify the likely costs, constraints, and limits to these responses. Each parasite trait can be fit into the framework outlined in Box 3, in which the environments refer to aspects of vector traits before and during deployment of a VCT. Rather than exploring responses to each of the impacts of insecticide VCTs discussed in the preceding text, we collate them into key overarching agents of selection on parasites in the following sections.

Infection genetic diversity

Reducing transmission is expected to lower the probability of genetically mixed infections and reduce the number of coinfecting genotypes in vectors and hosts [54]. The multiplicity of infection (MOI) determines the degree of competition each genotype faces, and within-host competition is costly enough to select for APP in reproductive strategies and favour more virulent parasites [55,56]. Thus, theory predicts that a reduction in MOI allows parasites to increase investment in transmission [55] and may lead to less severe infections. Furthermore, because within-host competition exacerbates the fitness costs of drug resistance, a reduction in MOI lessens this constraint. This suggests that parasites and drug resistance alleles may spread better, but infections would be less severe.

Whether and how MOI affects within-vector ecology and shapes parasite evolution are open questions. Intuition suggests that competition between unrelated parasites' genotypes is more intense within the vector due to limited amounts of nutrients, space, and time, compared to within a human. If so, a reduction in MOI will also release parasites from within-vector competition, but the opposite has been noted in certain circumstances [50]. Heterospecific interactions, such as between parasites and the mosquito microbiome, can also reduce parasite transmission via altered mosquito immune defences (i.e., 'apparent competition') [57]. Thus, IR-induced changes to the microbiome [58] may drive selection for immune evasion or strategies to perturb microbiota composition.

Vector longevity

Whilst insecticide exposure generally reduces lifespan, parasites may encounter mosquitoes with a broader distribution of lifespans because IR mechanisms affect longevity differentially [31]. To

Box 3. Parasite evolution framework

How, and over what timescales, parasite populations can respond to VCTs are unknown. Yet, this information can direct monitoring programmes to mitigate against the most likely, or concerning, parasite responses. Just as vector responses to VCTs occur via evolution by natural selection and APP, parasite responses can also take these forms.

If an ecological perturbation imposes a strong enough selective pressure, and there is genetic variation for fitness-related traits within the population, evolution occurs. Genetic variation is well documented for transmission traits that parasites express in the host (e.g., investment into, and sex ratio of, gametocytes [55]), though heritability is infrequently reported. Observations are consistent with intraspecific genetic variation for within-vector parasite phenotypes (e.g., infectivity to a given mosquito strain [53] and effects on mosquito survival and fecundity [98]) in both laboratory models and natural infections, and interspecific genetic variation in EIP [74].

APP in traits expressed during sporogony has been overlooked, but EIP is sensitive to resource availability [14,35,99,100], temperature [97], and parasite density [101]. Whether this is due to the parasite adopting APP or simply due to physiological constraints imposed by the vector upon parasite development is unknown. Given the utility of APP within-host [55], it would be surprising if parasites were unable to use APP to cope with the variation in vectors they encounter.

If parasites use APP to alter traits during sporogony to maintain fitness according to, for example, whether or not their vector is affected by a VCT, predicting evolution becomes more complex. This is because APP itself is subject to evolution by natural selection, and APP can facilitate or constrain evolution in non-mutually exclusive ways, dependent on the **reaction norms** exhibited by genotypes in a population, and whether there are **genotype-by-environment interactions** (Figure I) [102]. Ecological perturbations generally increase the extent of genetic variation exposed to selection, elevating evolutionary potential [103]. In contrast, APP could constrain evolution; by mitigating against the loss of fitness caused by VCTs, APP weakens selection on parasites [102]. Selection can also be constrained if trade-offs between traits have a genetic basis and/or if multiple traits face opposing selection pressures [104].

Testing for APP and quantifying genetic variation remain key objectives to infer the consequences of VCTs for parasite evolution. Highly controlled laboratory studies can inform which traits and genetic markers should be prioritised for monitoring in the field, as well as provide forecasts to integrate into epidemiological models.

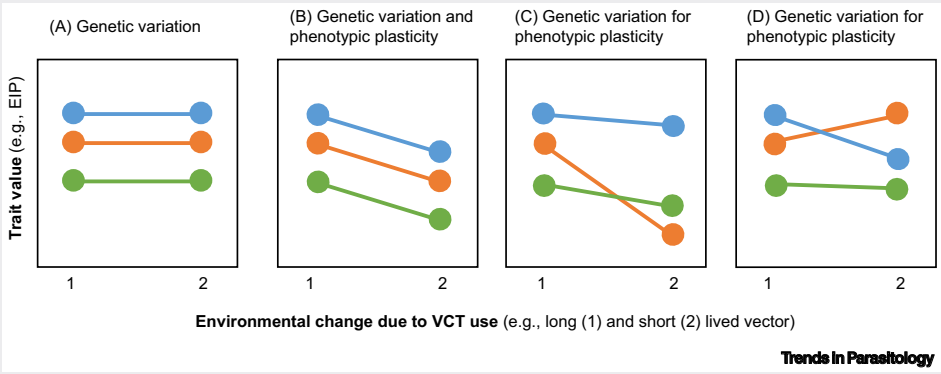

Figure I. The evolutionary potential of parasites. A hypothetical parasite population is composed of three different genotypes which adopt different values for a fitness-related trait in different environmental conditions. The spread of intercepts indicates the extent of genetic variation exposed to selection in each environment, and the slope dictates the degree of plasticity of each genotype (i.e., how much a genotype's trait changes across environments). The four possible patterns are: genotypes exhibit no plasticity but do differ from each other (A), all genotypes are plastic and respond by the same extent (B) and by differing extents (C), and genotypes respond in different manners (D). Applying this to a scenario where the trait is the extrinsic incubation period (EIP) and in condition 1 (long-lived vector unaffected by avector control tool, VCT) a long EIP is optimal, but in condition 2 (vector with reduced lifespan due to impacts of a VCT) a shorter EIP returns higher fitness, we can predict the following. (i) The relative abundance of the genotypes changes because the fitness ranks alter (blue is fittest and green is least fit in long-lived vectors, but in short-lived vectors, green generally performs best). (ii) Compared with scenarios (A) and (D), selection is more effective in (C) because the spread of the genotypes' trait values is greater in condition 2 than 1. (iii) Aside from the switch in fitness ranks, selection is constrained in (B) because plasticity allows all genotypes to alter EIP in the direction that maintains fitness.

cope with increased variation in vector lifespan, it would be beneficial for parasites to use APP to alter their EIP to match vector lifespan on a mosquito-by-mosquito basis. If parasites can alter the EIP, this is most likely achieved by curtailing the oocyst stage, which is relatively long compared to sexual reproduction and sporozoite migration (Figure 1). However, if parasites are unable to detect reliable cues for vector lifespan, then bet-hedging is the best strategy [59], which could be achieved by producing oocysts that burst at different times to generate a range of EIPs. Furthermore, moderation of EIP via APP according to the circumstances of individual mosquitoes would allow parasites to avoid the costs of a shorter EIP when they encounter mosquitoes unaffected by VCTs.

How much the EIP can shift is unclear; since EIP is close to mosquito life expectancy, it is assumed that constraints imposed by the vector and/or benefits to the parasite must be maintaining this duration [60]. Perhaps a short EIP means that fewer rounds of mitosis are undertaken within oocysts, reducing sporozoite numbers [61]. Alternatively, speeding up replication to maintain sporozoite production during a short EIP might interfere with the rate of lipid acquisition, resulting in less infective sporozoites [34]. However, these costs might be worth paying in dire circumstances; ensuring some level of transmission from a short-lived vector is better than none.

An alternative view is that resource availability mediates the EIP. For example, when additional resources are available, oocysts can afford to speed up development [35] but must avoid exploiting mosquito resources too rapidly and risk premature vector mortality, as observed for dengue [62]. Whereas, in resource-limited conditions, oocysts exhibit dormancy, with growth rescued by providing additional resources [63]. In this scenario, dormancy is an adaptive strategy when vectors are resource-limited but likely to survive long enough to gain additional resources, but maladaptive in short-lived vectors with little chance of survival.

Resource allocation and immune defences

Due to VCT use leading to evolution of IR mechanisms, exposure to insecticides, and changes to vector behaviours and species composition, parasites are likely to encounter mosquitoes with differing resource allocation patterns [64]. For example, pyriproxyfen is an insect growth regulator added to new-generation ITNs which directly impacts mosquito reproduction. Allocation to reproduction has complex effects on parasites; lower investment in reproduction associates with a lower oocyst burden but faster development [14], suggesting that parasites scavenge resources during/after egg development [34]. Thus, changes in mosquito resource allocation (due to trade-offs) or acquisition (due to altered gonotrophic cycles) could favour parasites that adjust EIP and/or that can manipulate mosquito reproductive processes. Interactions between different IR mechanisms and resource allocation to various forms of immunity are likely to result in multiple, potentially conflicting selection pressures on parasites. Such complexity might constrain the evolution of mean trait values, and instead, favour APP in which parasites adjust vector exploitation activities (e.g., rate of oocyst growth) according to the type and strength of immune responses mounted by individual mosquitoes. For example, a stronger immune response may select for faster replicating (more harmful) parasites as observed in laboratory models [64], as well as increased immune evasion. Such a strategy would be costly in low-resourced mosquitoes with a suppressed immune response that will die prematurely if exploited too aggressively. In which case, undergoing a form of programmed cell death to regulate the intensity of infection, which has been observed for ookinetes, is beneficial [65].

As well as facing IR-driven changes to the physiology of preferred vector species, parasites encounter different immune responses between alternative vector species. For example, mosquito immune responses exert their greatest impact on ookinetes traversing the midgut,

and haplotypes of the surface protein *Pfs47* allow *Plasmodium falciparum* isolates to avoid immune detection by their local vector [53]. Whilst it is unlikely that a given parasite genotype will be able to effectively evade immune responses across multiple mosquito species, parasite genotypes with the 'wrong' haplotype for a particular mosquito species can infect other species, albeit at a lower prevalence and intensity [53]. Thus, selection to adapt to an alternative vector or become generalists might drive the evolution of *Pfs47* haplotypes that are not optimal to infect any individual vector population but do reduce detection across multiple species.

Mosquito behaviours

As well as IR mechanisms and insecticide exposure, shifts to less anthropophilic vector species and altered biting time expose parasites to mosquitoes with different behaviours. A consequence of transmission via less anthropophilic mosquito species is that parasites will encounter non-human hosts more often. Alongside adaptation to novel vector species, parasites may become host generalists or undertake host shifts because the receptors that parasites use for red blood cell invasion vary across vertebrate species [66]. An alternative solution is to manipulate vectors to preferentially bite humans and manipulate humans to attract preferred vector species [67,68]. Vector manipulation abilities would also benefit parasites facing vectors with longer gonotrophic cycles [69] to ensure transmission opportunities as soon as sporozoites become infectious.

Altered biting time-of-day (via behavioural avoidance or vector species shifts) causes parasite activities to be out of synch with rhythms within the vector and with the abiotic environment. First, parasites may have to adjust their developmental schedule to align with mosquito rhythms. For example, if ookinete invasion of the midgut is most successful during the night-time (i.e., approximately 20 h post blood meal), ookinetes may have to accelerate development or wait until the following evening to invade. Both options are likely to incur costs; fast ookinetes are likely to be of lower quality, and waiting may increase the risk of being digested. Second, day-biting forces parasites to undergo gametogenesis and fertilisation during warmer parts of the circadian cycle than night-biting. High thermal sensitivity during early sporogony is widely observed across human, murine, and avian malaria parasites [70–72]. Thus, if day-biting mosquitoes do not find cool places to rest following blood feeding, high temperatures may be a physiological constraint that parasites cannot adapt to cope with. Therefore, VCTs that are most likely to be evaded by vectors biting in the daytime have good potential to be robust against parasite counter evolution.

In areas of seasonal transmission, a consequence of VCTs reducing vector density is that fewer mosquitoes are likely to be present at the start of the transmission season to stimulate parasites into increasing transmission investment, as observed for avian malaria parasites [73]. If a similar phenomenon occurs in human parasites, they may not receive stimulation by sufficient mosquitoes until further into the transmission season, leading to a shorter transmission window. In this case, parasites would benefit by becoming more sensitive to mosquito bites or evolving to use a different proxy for seasonality, such as seasonal changes in host hormones, to schedule their transmission activities according to vector availability. However, whether parasites detect the activities of vectors or use alternate proxies for seasonality is unknown. Alternatively, this problem may strengthen selection for manipulation of humans to be more attractive to mosquitoes.

Coping with insecticide exposure

Whether direct exposure to insecticide agents imposes selection pressure on parasites is unclear but does raise the question of whether parasites can evolve resistance to insecticides, as they do against antimalarial drugs. Ookinetes appear to be sensitive to insecticides (M. Kristan, PhD thesis, London School of Hygiene and Tropical Medicine, 2018) and may contact insecticides picked up the previous night during blood feeding. On one hand, because this stage is short-

lived, protecting it from insecticide toxicity might be possible (e.g., by dormancy), especially because exposure appears to be relatively transient (N. Hill, PhD thesis, London School of Hygiene and Tropical Medicine, 2002), depending on how much this increases the risk of being digested. On the other hand, ookinete survival is a major bottleneck in sporogony, suggesting that there are many ecological constraints ookinetes are unable to overcome. Understanding the risks of direct exposure to insecticides faced by parasites requires knowledge of how long clearance takes in IR mosquitoes. This is further complicated by the addition of chemicals, such as piperonyl butoxide (PBO) and chlorfenapyr, to new-generation ITNs which prolong the window of insecticide efficacy on mosquitoes and potentially on parasites.

For parasites to encounter an insecticide, their vector must have encountered it too, and it is possible that coping with both direct exposure plus the effects of insecticides on the vector constrains parasite evolution. Furthermore, analogous to antimalarial drug resistance and IR in mosquitoes, parasite IR will involve fitness costs. Thus, unless the IR mechanism is expressed only during sporogony or occurs via APP, absence of insecticide pressure in a vertebrate host will cause IR mutants to be outcompeted by sensitive parasites. If parasites do evolve to evade insecticides, the options available are likely to depend on the stage of sporogony when detrimental exposure occurs. The most obvious tactic would be to enter dormancy until the insecticide is no longer active. This is unlikely to be feasible during sexual reproduction and may be too costly for ookinetes but could be deployed following exposure during the oocyst stage.

Addressing parasite evolution in response to VCTs

Predicting and monitoring parasite responses to VCTs should not be a secondary aim of programmes, only to be undertaken once the primary goal of vector control is achieved. Clearly, this is a huge and multidisciplinary challenge, but progress is possible. Laboratory studies are an efficient way to test for proof-of-concept for fundamental evolutionary predictions as well as determine how the most promising hypotheses should be tested in endemic settings, given the parameters and nuances of natural infections. Animal models are invaluable for estimating parasite fitness and testing whether there are clinical consequences of genetic correlations underpinning traits expressed in sporogony and traits expressed in the vertebrate host. Genetic variation and APP (Box 3) are hard to assess without undertaking common-garden experiments that have considerable statistical power, which is possible using laboratory and semi-field settings. For example, comparing traits across parasite genotypes when each is represented by multiple replicate infections in which the age/strain/condition of vectors, density/sex ratio of gametocytes, and environmental perturbations are tightly controlled. Selection experiments, where parasites are serially 'passaged' through different types of vector, can also reveal how parasite genotypes and phenotypes evolve and provide genetic markers for field monitoring. However, the most acute need is to ascertain the role that individual parasite traits play in vector competence and transmission (Box 2).

Concluding remarks

VCTs reduce vectorial capacity and therefore transmission. Keeping transmission under control requires anticipating and preventing parasite counter-evolution, especially given that almost all parameters underpinning vectorial capacity are indirectly or directly influenced by parasite activities (Box 2) [74]. Thus, how VCTs affect selection on, and the potential evolutionary responses of, parasites is a major knowledge gap (see Outstanding questions). We have explored the diverse manners in which VCTs affect malaria parasite transmission and illustrated how parasites could meet these challenges. The scenarios we cover are not an exhaustive treatment but highlight the diverse potential outcomes of VCTs for parasite ecology and evolution.

Outstanding questions

How do the myriad ways that vector biology is affected by VCTs directly and/or indirectly affect parasite interactions with insect vectors and human hosts? For example, how does IR affect resource allocation in vectors, and how does this affect parasite development?

Do the ecological perturbations caused by VCTs impose constraints on parasite activities that reduce fitness and/or can VCTs provide opportunities for parasites to exploit to enhance fitness? For example, do the costs of IR cause vectors to mount weaker immune responses against parasites, facilitating transmission?

How much heritable genetic variation is there for parasite traits that are exposed to selection as a result of VCTs, and are these traits subject to genetic and resource allocation trade-offs? For example, do parasite genotypes that have the fastest development in the vector produce the fewest host-infective stages?

Will parasite plasticity and genotype-by-environment interactions facilitate or constrain parasite evolutionary responses to selection driven by VCTs? For example, if parasites possess the ability to plastically adjust development time according to variation in vector lifespan, does this reduce the strength selection to alter developmental duration?

How do parasite traits expressed in the vector link to clinical/epidemiological outcomes for human hosts? For example, are parasite genotypes with a faster development in the vector less infective/virulent to human hosts?

Can knowledge of how plasticity and evolution help parasites cope with the consequences of VCTs be harnessed to improve the efficacy of VCTs? For example, if VCTs with differing modes of action impose antagonistic selection pressures on parasites, could combining these tools in specific combinations retard unfavourable parasite evolution?

How parasites have evolved to exploit vectors and cope with the constraints they impose have largely remained a black box since mosquito transmission was discovered over a century ago. Yet, a better understanding of vector–parasite interactions in the presence and absence of VCTs will reveal approaches that are most robust to parasite counter-evolution, including directing interventions to target the most evolutionarily constrained parasite traits, as well as informing programmes designed to monitor parasite responses to VCTs.

Acknowledgments

We thank Tsukushi Kamiya for helpful comments. S.E.R. and C.E.O. are supported by the Wellcome Trust (202769/Z/16/Z), the Royal Society (URF/R/180020 and RGF\EA\181046), and the University of Edinburgh's School of Biological Sciences.

Declaration of interests

The authors declare no competing interests.

References

1. Bhatt, S. *et al.* (2015) The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526, 207–211
2. Huijben, S. and Paaijmans, K.P. (2018) Putting evolution in elimination: winning our ongoing battle with evolving malaria mosquitoes and parasites. *Evol. Appl.* 11, 415–430
3. Killeen, G.F. *et al.* (2017) Developing an expanded vector control toolbox for malaria elimination. *BMJ Glob. Health* 2, e000211
4. World Health Organisation (2021) *Global Technical Strategy for Malaria 2016–2030, 2021 Update*, WHO
5. Carrasco, D. *et al.* (2019) Behavioural adaptations of mosquito vectors to insecticide control. *Curr. Opin. Insect Sci.* 34, 48–54
6. Ranson, H. and Lissenden, N. (2016) Insecticide resistance in African *Anopheles* mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol.* 32, 187–196
7. MacDonald, G. (1956) Epidemiological basis of malaria control. *Bull. World Health Organ.* 15, 613–626
8. Hyde, J.E. (2005) Drug-resistant malaria. *Trends Parasitol.* 21, 494–498
9. Schneider, P. *et al.* (2018) Adaptive plasticity in the gametocyte conversion rate of malaria parasites. *PLoS Pathog.* 14, e1007371
10. Rivero, A. *et al.* (2010) Insecticide control of vector-borne diseases: when is insecticide resistance a problem? *PLoS Pathog.* 6, e1001000
11. Namias, A. *et al.* (2021) The need for practical insecticide-resistance guidelines to effectively inform mosquito-borne disease control programs. *eLife* 10, e65655
12. Dabira, E.D. *et al.* (2021) Mass drug administration of ivermectin and dihydroartemisinin–piperaquine against malaria in settings with high coverage of standard control interventions: a cluster-randomised controlled trial in The Gambia. *Lancet Infect. Dis.* 22, 519–528
13. Toé, K.H. *et al.* (2014) Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness Burkina Faso. *Emerg. Infect. Dis.* 20, 1691–1696
14. Werling, K. *et al.* (2019) Steroid hormone function controls non-competitive *Plasmodium* development in *Anopheles*. *Cell* 177, 315–325.e14
15. Alout, H. *et al.* (2016) Interactive cost of *Plasmodium* infection and insecticide resistance in the malaria vector *Anopheles gambiae*. *Sci. Rep.* 6, 29755
16. Okoye, P.N. *et al.* (2007) Relative developmental and reproductive fitness associated with pyrethroid resistance in the major southern African malaria vector, *Anopheles funestus*. *Bull. Entomol. Res.* 97, 599–605
17. Alout, H. *et al.* (2014) Insecticide exposure impacts vector–parasite interactions in insecticide-resistant malaria vectors. *Proc. R. Soc. B Biol. Sci.* 281, 20140389
18. Kristan, M. *et al.* (2016) Exposure to deltamethrin affects development of *Plasmodium falciparum* inside wild pyrethroid resistant *Anopheles gambiae* s.s. *mosquitoes* in Uganda. *Parasit. Vectors* 9, 100
19. Symington, S.B. *et al.* (1999) Characterization of pyrethroid action on ciliary calcium channels in *Paramecium tetraurelia*. *Pestic. Biochem. Physiol.* 65, 181–193
20. Thiévent, K. *et al.* (2019) The interaction between permethrin exposure and malaria infection affects the host-seeking behaviour of mosquitoes. *Malar. J.* 18, 79
21. Lo, T.M. and Coetzee, M. (2013) Marked biological differences between insecticide resistant and susceptible strains of *Anopheles funestus* infected with the murine parasite *Plasmodium berghei*. *Parasit. Vectors* 6, 184
22. Ndiath, M.O. *et al.* (2014) Effects of the kdr resistance mutation on the susceptibility of wild *Anopheles gambiae* populations to *Plasmodium falciparum*: a hindrance for vector control. *Malar. J.* 13, 340
23. Alout, H. *et al.* (2013) Insecticide resistance alleles affect vector competence of *Anopheles gambiae* s.s. for *Plasmodium falciparum* field isolates. *PLoS One* 8, e63849
24. Wolie, R.Z. *et al.* (2021) Evaluation of the interaction between insecticide resistance-associated genes and malaria transmission in *Anopheles gambiae* sensu lato in central Côte d'Ivoire. *Parasit. Vectors* 14, 581
25. Minetti, C. *et al.* (2020) Effects of insecticide resistance and exposure on *Plasmodium* development in *Anopheles* mosquitoes. *Curr. Opin. Insect Sci.* 39, 42–49
26. Alout, H. *et al.* (2014) Interplay between *Plasmodium* infection and resistance to insecticides in vector mosquitoes. *J. Infect. Dis.* 210, 1464–1470
27. Tchouakui, M. *et al.* (2020) Cytochrome P450 metabolic resistance (CYP6P9a) to pyrethroids imposes a fitness cost in the major African malaria vector *Anopheles funestus*. *Heredity (Edinb)* 124, 621–632
28. Cansado-Utrilla, C. *et al.* (2021) The microbiome and mosquito vectorial capacity: rich potential for discovery and translation. *Microbiome* 9, 111
29. Vontas, J. *et al.* (2005) Gene expression in insecticide resistant and susceptible *Anopheles gambiae* strains constitutively or after insecticide exposure. *Insect Mol. Biol.* 14, 509–521
30. Ingham, V.A. *et al.* (2021) Integration of whole genome sequencing and transcriptomics reveals a complex picture of the reestablishment of insecticide resistance in the major malaria vector *Anopheles coluzzii*. *PLoS Genet.* 17, e1009970
31. Brown, F. *et al.* (2020) A steroid hormone agonist reduces female fitness in insecticide-resistant *Anopheles* populations. *Insect Biochem. Mol. Biol.* 121, 103372
32. Kumar, S. *et al.* (2003) The role of reactive oxygen species on *Plasmodium* melanotic encapsulation in *Anopheles gambiae*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14139–14144
33. Ingham, V.A. *et al.* (2021) Transcriptomic analysis reveals pronounced changes in gene expression due to sub-lethal pyrethroid exposure and ageing in insecticide resistance *Anopheles coluzzii*. *BMC Genomics* 22, 337

34. Costa, G. *et al.* (2018) Non-competitive resource exploitation within mosquito shapes within-host malaria infectivity and virulence. *Nat. Commun.* 9, 3474
35. Shaw, W.R. *et al.* (2020) Multiple blood feeding in mosquitoes shortens the *Plasmodium falciparum* incubation period and increases malaria transmission potential. *PLoS Pathog.* 16, e1009131
36. Lyimo, I.N. *et al.* (2013) The impact of host species and vector control measures on the fitness of African malaria vectors. *Proc. R. Soc. B Biol. Sci.* 280, 20122823
37. Rund, S.S.C. *et al.* (2011) Genome-wide profiling of diel and circadian gene expression in the malaria vector *Anopheles gambiae*. *Proc. Natl. Acad. Sci. U. S. A.* 108, E421–E430
38. Rund, S.S.C. *et al.* (2016) Daily rhythms in mosquitoes and their consequences for malaria transmission. *Insects* 7, 14
39. O'Donnell, A.J. *et al.* (2019) Time-of-day of blood-feeding: effects on mosquito life history and malaria transmission. *Parasit. Vectors* 12, 301
40. Suh, E. *et al.* (2020) The influence of feeding behaviour and temperature on the capacity of mosquitoes to transmit malaria. *Nat. Ecol. Evol.* 4, 940–951
41. Schneider, P. *et al.* (2018) Adaptive periodicity in the infectivity of malaria gametocytes to mosquitoes. *Proc. R. Soc. B Biol. Sci.* 285, 20181876
42. Weng, S.-C. *et al.* (2021) Blood glucose promotes dengue virus infection in the mosquito *Aedes aegypti*. *Parasit. Vectors* 14, 376
43. Talman, A.M. *et al.* (2014) Proteomic analysis of the *Plasmodium* male gamete reveals the key role for glycolysis in flagellar motility. *Malar. J.* 13, 315
44. Westwood, M.L. *et al.* (2019) The evolutionary ecology of circadian rhythms in infection. *Nat. Ecol. Evol.* 3, 552–560
45. Sanou, A. *et al.* (2021) Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors in the Cascades region of Burkina Faso. *Sci. Rep.* 11, 17569
46. Tedrow, R.E. *et al.* (2019) *Anopheles* mosquito surveillance in Madagascar reveals multiple blood feeding behavior and *Plasmodium* infection. *PLoS Negl. Trop. Dis.* 13, e0007176
47. Sangbakembi-ngounou, C. *et al.* (2022) Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be 'out of control'. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2104282119
48. Carnevale, P. and Manguin, S. (2021) Review of issues on residual malaria transmission. *J. Infect. Dis.* 223, S61–S80
49. Guelbéogo, W.M. *et al.* (2018) Variation in natural exposure to *Anopheles* mosquitoes and its effects on malaria transmission. *eLife* 7, e32625
50. Pollitt, L.C. *et al.* (2015) Existing infection facilitates establishment and density of malaria parasites in their mosquito vector. *PLoS Pathog.* 11, e1005003
51. Emami, S.N. *et al.* (2017) The transmission potential of malaria-infected mosquitoes (*An.gambiae*-Keele, *An.arabiensis*-Ifakara) is altered by the vertebrate blood type they consume during parasite development. *Sci. Rep.* 7, 40520
52. Simões, M.L. *et al.* (2017) Immune regulation of *Plasmodium* is *Anopheles* species specific and infection intensity dependent. *mBio* 8, e01631–17
53. Molina-Cruz, A. *et al.* (2015) *Plasmodium* evasion of mosquito immunity and global malaria transmission: the lock-and-key theory. *Proc. Natl. Acad. Sci. U. S. A.* 112, 15178–15183
54. Karl, S. *et al.* (2016) Spatial effects on the multiplicity of *Plasmodium falciparum* infections. *PLoS One* 11, e0164054
55. Schneider, P. and Reece, S.E. (2021) The private life of malaria parasites: strategies for sexual reproduction. *Mol. Biochem. Parasitol.* 244, 111375
56. Bell, A.S. *et al.* (2006) Within-host competition in genetically diverse malaria infections: parasite virulence and competitive success. *Evolution* 60, 1358
57. Gao, H. *et al.* (2020) Mosquito microbiota and implications for disease control. *Trends Parasitol.* 36, 98–111
58. Omoke, D. *et al.* (2021) Western Kenyan *Anopheles gambiae* showing intense permethrin resistance harbour distinct microbiota. *Malar. J.* 20, 77
59. Starrfelt, J. and Kokko, H. (2012) Bet-hedging-a triple trade-off between means, variances and correlations. *Biol. Rev.* 87, 742–755
60. Koella, J.C. (1999) An evolutionary view of the interactions between anopheline mosquitoes and malaria parasites. *Microbes Infect.* 1, 303–308
61. Gerald, N. *et al.* (2011) Mitosis in the human malaria parasite *Plasmodium falciparum*. *Eukaryot. Cell* 10, 474–482
62. Ye, Y.H. *et al.* (2016) Evolutionary potential of the extrinsic incubation period of dengue virus in *Aedes aegypti*. *Evolution* 70, 2459–2469
63. Habtewold, T. *et al.* (2021) *Plasmodium* oocysts respond with dormancy to crowding and nutritional stress. *Sci. Rep.* 11, 3090
64. Shaw, W.R. *et al.* (2022) *Plasmodium* development in *Anopheles*: a tale of shared resources. *Trends Parasitol.* 38, 124–135
65. Reece, S.E. *et al.* (2011) The meaning of death: evolution and ecology of apoptosis in protozoan parasites. *PLoS Pathog.* 7, e1002320
66. Lim, C. *et al.* (2013) Expansion of host cellular niche can drive adaptation of a zoonotic malaria parasite to humans. *Nat. Commun.* 4, 1638
67. Robinson, A. *et al.* (2018) *Plasmodium*-associated changes in human odor attract mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 115, E4209–E4218
68. Vantaux, A. *et al.* (2021) Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*. *Peer Community J.* 1, e13
69. Osoro, J.K. *et al.* (2022) Insecticide resistant *Anopheles gambiae* have enhanced longevity but reduced reproductive fitness and a longer first gonotrophic cycle. *Sci. Rep.* 12, 8646
70. Noden, B.H. *et al.* (1995) The impact of variations in temperature on early *Plasmodium falciparum* development in *Anopheles stephensi*. *Parasitology* 111, 539–545
71. Vanderberg, J.P. and Yoeli, M. (1966) Effects of temperature on sporogonic development of *Plasmodium berghei*. *J. Parasitol.* 52, 559–564
72. Ball, G.H. and Chao, J. (1964) Temperature stresses on the mosquito phase of *Plasmodium relictum*. *J. Parasitol.* 50, 748–752
73. Cornet, S. *et al.* (2014) Evolution of plastic transmission strategies in avian malaria. *PLoS Pathog.* 10, e1004308
74. Lefevre, T. *et al.* (2017) Transmission traits of malaria parasites within the mosquito: genetic variation, phenotypic plasticity, and consequences for control. *Evol. Appl.* 11, 456–469
75. Balabanidou, V. *et al.* (2016) Cytochrome P450 associated with insecticide resistance catalyzes cuticular hydrocarbon production in *Anopheles gambiae*. *Proc. Natl. Acad. Sci. U. S. A.* 113, 9268–9273
76. Ingham, V.A. *et al.* (2020) A sensory appendage protein protects malaria vectors from pyrethroids. *Nature* 577, 376–380
77. Glunt, K.D. *et al.* (2018) Empirical and theoretical investigation into the potential impacts of insecticide resistance on the effectiveness of insecticide-treated bed nets. *Evol. Appl.* 11, 431–441
78. Viana, M. *et al.* (2016) Delayed mortality effects cut the malaria transmission potential of insecticide-resistant mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 113, 8975–8980
79. Hughes, A. *et al.* (2020) *Anopheles gambiae* populations from Burkina Faso show minimal delayed mortality after exposure to insecticide-treated nets. *Parasit. Vectors* 13, 17
80. Jones, C.M. *et al.* (2012) Aging partially restores the efficacy of malaria vector control in insecticide-resistant populations of Anopheles gambiae s.l. from Burkina Faso. *Malar. J.* 11, 24
81. Thomsen, E.K. *et al.* (2017) Mosquito behavior change after distribution of bednets results in decreased protection against malaria exposure. *J. Infect. Dis.* 215, 790–797
82. Moiroux, N. *et al.* (2012) Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in benin. *J. Infect. Dis.* 206, 1622–1629
83. Russell, T.L. *et al.* (2011) Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar. J.* 10, 80
84. Kreppel, K.S. *et al.* (2020) Emergence of behavioural avoidance strategies of malaria vectors in areas of high LLIN coverage in Tanzania. *Sci. Rep.* 10, 14527

85. Ndenga, B.A. *et al.* (2016) Malaria vectors and their blood-meal sources in an area of high bed net ownership in the western Kenya highlands. *Malar. J.* 15, 76
86. Govella, N.J. *et al.* (2021) Heritability and phenotypic plasticity of biting time behaviors in the major African malaria vector *Anopheles arabiensis*. *bioRxiv* Published online May 17, 2021. https://doi.org/10.1101/2021.05.17.444456
87. Main, B.J. *et al.* (2016) The genetic basis of host preference and resting behavior in the major African malaria vector, *Anopheles arabiensis*. *PLoS Genet.* 12, e1006303
88. Nkahe, D.L. *et al.* (2020) Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon [version 2; peer review: 2 approved, 1 not approved]. *Wellcome Open Res.* 5, 171
89. Assogba, B.S. *et al.* (2016) The ace-1 locus is amplified in all resistant *Anopheles gambiae* mosquitoes: fitness consequences of homogeneous and heterogeneous duplications. *PLoS Biol.* 14, e2000618
90. Otali, D. *et al.* (2014) Increased production of mitochondrial reactive oxygen species and reduced adult life span in an insecticide-resistant strain of *Anopheles gambiae*. *Bull. Entomol. Res.* 104, 323–333
91. Balmert, N.J. *et al.* (2014) Time-of-day specific changes in metabolic detoxification and insecticide resistance in the malaria mosquito *Anopheles gambiae*. *J. Insect Physiol.* 64, 30–39
92. Smith, D.L. *et al.* (2012) Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLoS Pathog.* 8, e1002588
93. Takken, W. and Verhulst, N.O. (2013) Host preferences of blood-feeding mosquitoes. *Annu. Rev. Entomol.* 58, 433–453
94. Cator, L.J. *et al.* (2020) The role of vector trait variation in vector-borne disease dynamics. *Front. Ecol. Evol.* 8, 189
95. Lefèvre, T. *et al.* (2013) Non-genetic determinants of mosquito competence for malaria parasites. *PLoS Pathog.* 9, e1003365
96. Ohm, J.R. *et al.* (2018) Rethinking the extrinsic incubation period of malaria parasites. *Parasit. Vectors* 11, 178
97. Shapiro, L.L.M. *et al.* (2017) Quantifying the effects of temperature on mosquito and parasite traits that determine the transmission potential of human malaria. *PLoS Biol.* 15, e2003489
98. Ferguson, H.M. *et al.* (2003) Mosquito mortality and the evolution of malaria virulence. *Evolution* 57, 2792–2804
99. Hien, D.F.d.S. *et al.* (2016) Plant-mediated effects on mosquito capacity to transmit human malaria. *PLoS Pathog.* 12, e1005773
100. Shapiro, L.L.M. *et al.* (2016) Larval food quantity affects the capacity of adult mosquitoes to transmit human malaria. *Proc. R. Soc. B Biol. Sci.* 283, 20160298
101. Childs, L.M. and Prosper, O.F. (2020) The impact of within-vector parasite development on the extrinsic incubation period. *R. Soc. Open Sci.* 7, 192173
102. Chevin, L.M. *et al.* (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* 8, e1000357
103. Paaby, A.B. and Rockman, M.V. (2014) Cryptic genetic variation: evolution's hidden substrate. *Nat. Rev. Genet.* 15, 247–258
104. Stearns, S.C. (1989) Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259–268