Residual Transmission of Malaria: An Old Issue for New Approaches

Lies Durnez and Marc Coosemans

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55925

1. Introduction

Malaria is one of the most serious vector-borne diseases, affecting millions of people mainly in the tropics. Recently, a substantial decline in malaria incidence has been observed all over the world. Vector control is one of the key elements in achieving this world-wide malaria decline, with scaling up of Insecticide Treated Nets (ITNs) and the expansion of Indoor Residual Spraying (IRS) programmes contributing significantly. Besides the personal protection, ITNs confer a community protection when wide coverage is assured, meaning that unprotected persons benefit from the large scale intervention [1]. IRS is only meaningful when applied at a large coverage. In the 2011 World Malaria Report [2], the percentage of households owning at least one ITN in sub-Saharan Africa is estimated to have risen from 3% in 2000 to 50% in 2011 while the percentage protected by indoor residual spraying (IRS) rose from less than 5% in 2005 to 11% in 2010. Household surveys indicate that 96% of persons with access to an ITN within the household actually use it [2]. Although these numbers might overestimate the real ITN use, they show that in recent years, several vector control measures were scaled up substantially. Despite these large increases in coverage, a widely held view is that with the currently available tools, namely vector control tools, intermittent preventive treatment, and early diagnosis and treatment, much greater gains could be achieved, including elimination from a number of countries and regions [3].

When considering vector control tools, even when hypothesizing a full coverage of ITNs and IRS, malaria transmission may still continue. Indeed, IRS only affects endophilic\textsuperscript{1} mosquitoes and ITNs only target night biting mosquitoes. Moreover both intervention methods will mainly affect anthropophilic\textsuperscript{2} mosquitoes that are endophagic\textsuperscript{3}. This leaves ample opportunity

\textsuperscript{1} Endophily is the tendency for mosquitoes to prefer resting indoors
for more exophilic, zoophilic and/or exophagic vectors to escape from contact with insecticide treated surfaces and to maintain a certain level of transmission. Independently of the ITN and/or IRS coverage, outdoor and early malaria transmission occurs in many malaria endemic regions. In the west of Eritrea for example over a two year sampling period 36.4% of infective bites were acquired outdoors [4], in southern Tanzania this was 10% for non ITN users [5]. A study in northeastern Tanzania showed that 12% of the malaria transmission occurred before sleeping time [6]. In Uganda, in 6 sentinel sites throughout the country, up to 36% of indoor transmission and 49% of outdoor transmission occurred before sleeping time, with the highest proportion of early in- and outdoor transmission in the suburban area of Jinja where An. gambiae was the main vector [7]. In central Vietnam, where ITNs are used at large scale, 69% of the infective bites in forest plots were acquired before sleeping time [8]. In a study conducted in the east and west of Cambodia before widespread ITN use, 29% of the bites occurred before sleeping time in villages and forest plots [9]. In North-East India, 21% of the indoor infective bites occurred before 21h [10]. Also in Nicaragua, in an area with mainly Vivax malaria, 50% of the infective bites were acquired before sleeping time [11]. This part of the malaria transmission has the possibility to continue despite high coverage of ITNs and IRS, and is defined for the purpose of this review as ‘residual transmission’.

Controlling residual transmission requires a different approach as compared to the currently used vector control measures. This is not new and was already perceived as a major obstacle in the previous malaria eradication era [12]. In 2007 malaria eradication was put as the ultimate goal [3] and renewed attention was given to residual transmission, with vector control models also incorporating outdoor and zoophilic malaria vectors. Recently, an established mathematical model adjusted for human in- and outdoor movements was used to illustrate that even with 50% outdoor biting vectors, transmission suppression can be achieved by a large ITN coverage [13]. However the authors assumed a uniform exposure so that the ITN induced mortality affects equally in- and outdoor biting vectors. When assuming a uniform exposure all individuals of the vector population (belonging to the same or to different species), will exhibit at each gonotrophic cycle a random behaviour (e.g. exo- or endophily, exo-or endophagy, anthropo- or zoophily, early- or late-biting), so that all individual mosquitoes are equally affected by indoor-based vector control measures. In case of non-uniform exposure, two or more subpopulations of vectors (belonging to the same or to different species) are assumed, each exhibiting a specific behaviour. Therefore, each of these subpopulations is affected differently by indoor-based vector control measures [14]. As a result, a fraction of vectors will persist in the presence of these control measures and can be responsible for residual transmission. It was shown that pre-intervention variables reflecting behavior, such as the degree

2 Anthropophily is the tendency for mosquitoes to prefer feeding on human hosts
3 Endophagy is the tendency for mosquitoes to prefer biting indoors
4 Exophily is the tendency for mosquitoes to prefer resting outdoors
5 Zoophily is the tendency for mosquitoes to prefer feeding on animal hosts
6 Exophagy is the tendency for mosquitoes to prefer biting outdoors
7 In this paper, s.l. (sensu lato) is added to the species name when referred to the species complex (An. gambiae s.l., An. minimus s.l., An. dirus s.l.). In the absence of s.l., the species is concerned (e.g. An. gambiae, An. minimus, An. dirus).
of exophily, may predict the efficacy of a specific intervention [15,16]. Assuming non-uniform exposure, the exophagic fractions of vectors will be less exposed to ITNs, the probability of survival and the vectorial capacity of this subpopulation will be weakly affected, and malaria transmission cannot be reduced further. The model developed in [17] takes into account the non-uniform exposure of the different anopheline species, i.e., the anthropo-endophilic vector species An. gambiae and An. funestus, and the more zoo-exophilic vector An. arabiensis. As would be intuitively expected, this model predicts that even the combination of very effective ITN distribution, twice yearly mass screening and treatment campaigns, and IRS will not succeed in getting the parasite prevalence rate below the 1% threshold if the zoo-exophilic An. arabiensis is present. When only An. gambiae or An. funestus are present, the same combination of interventions are successful in this model [17]. Moreover even within a well-defined species different subpopulations may occur exhibiting different behavioural patterns, resulting in non-uniform exposure within a species.

Therefore, when designing and applying vector control strategies it would be essential to have a good knowledge of the vector behavioural traits particularly those relevant to the chosen control method. However, entomological findings for one region or one anopheline species do not necessarily hold true for the same or different anopheline species encountered in the same or different malaria-endemic regions. In this chapter we will show that even before widespread use of vector control measures, a heterogeneity in behaviour between and within species was present. Because of the heterogeneity in behaviour, mosquitoes have different opportunities to escape from the killing or excito-repellent actions of insecticides used in ITNs or IRS. We will give examples of species shifts, shifts to outdoor- or early biting, shifts to zoophily or to exophily from different malaria-endemic regions linked to the use of ITNs and IRS. Although the causes and mechanisms behind these shifts are not yet well understood, we will argue that ITNs and IRS may select for vector populations that predominantly feed early or outdoors, rest outdoors, or that are able to change their behaviour in response to the presence of these insecticides. Therefore, residual transmission will be dominated by vectors that bite outdoors, early or on animals, and that rest outdoors. These vectors require different control strategies, which might also be based on reducing host-vector contact, or target other key environmental resources.

The concept of uniform versus non-uniform exposure is illustrated in Figure 1.

2. Heterogeneity in anopheline behaviour

Heterogeneity in behaviour of anopheline mosquitoes between and within species is present in all malaria endemic regions. In Africa, the two most efficient malaria vector species, An. gambiae and An. funestus, are very anthropophilic, endophilic, endophagic, and late-night biting [18]. In contrast, An. arabiensis, a species belonging to the same complex as An. gambiae, is more plastic in its behaviour, exhibiting more often zoophily, exophily, exophagy, and early-night biting as compared to An. gambiae and An. funestus. However, different factors can influence the behaviour of the anophelines. Host availability for example plays an important
factor in the final host choice of the vector. This has been shown for *An. gambiae* in several study sites. In Burkina Faso for example, a double choice experiment shows that 88% of the *An. gambiae* choose for a human odour baited trap and only 12% for a cattle odour trap. In contrast, the human blood index of indoor-resting *An. gambiae* collected in the same locality was only 40% [19], showing that this population of *An. gambiae* will adapt its host choice in case of a lower availability of human hosts. *An. gambiae* in São Tomé feeds more on dogs and was observed to be extremely exophagic most probably due to a combination of preference and the ease to reach the dogs sleeping outside under pillar houses [20]. On the Bioko Island (Equatorial Guinea), *An. gambiae* was also observed to be partly exophagic and early-biting [21]. This means that when humans are not available inside, e.g. because of a high bed net use, some populations of *An. gambiae* are observed to feed outside or on animal hosts. In those cases, the frequency of human-vector contact will be lowered although humans will still be bitten in the evening. As a consequence, the longevity of these exophagic or zoophilic vectors will slightly, or not, be affected by ITNs, meaning that the vectorial capacity is not affected and malaria transmission continues.

Also in South-East Asia, heterogeneity of behaviour is observed for the primary and secondary vector species [22]: *An. dirus* is for example very anthropophilic, whereas *An. minimus*,

---

**Figure 1.** Effect of control measures on mosquito populations in the assumption of uniform exposure and non-uniform exposure. The density of a uniform population (belonging to the same or to different species) A. before applying the control measure. B. after applying the control measure. The control measure reduces the density of the whole population by 80%. The density of a non-uniform population C. before applying the control measure. D. after applying the control measure. The population consists of two subpopulations (Subpopulations 1 and 2, belonging to the same or to different species) each with a different behavioural tendency. Limited contact with the insecticide due to its behavioural tendency makes that Subpopulation 1 is reduced by 20% only, while Subpopulation 2 is reduced by 80% of its initial density. As a result, a fraction of vectors will persist in the presence of these control measures and can be responsible for malaria transmission.
depending on the geographical region, has both anthropophilic and zoophilic tendencies. *An. maculatus* has a high tendency for early biting as compared to *An. dirus* or *An. minimus*, but there are large differences between localities. Different populations of *An. minimus* observed in various localities also differ in their endophilic and endophagic tendencies [22]. Whereas *An. dirus* is generally observed to be very exophagic and exophilic, populations in Lao PDR have shown highly endophilic and endophagic trends [23]. Moreover, as reviewed in [24], *An. dirus* s.l. can even take blood-meals during daylight in the jungle.

In Latin-America, one of the most efficient vectors, *An. darlingi* is mainly anthropophilic, whereas the other dominant vectors, such as *An. albimanus*, *An. nuneztovari*, and *An. aquasalis* also have zoophilic tendencies or are more opportunistic. Most of the vectors in Latin America are mainly exophilic, but within each species, the degree of exophily can vary between geographical regions. *An. albimanus* for example is predominantly exophagic and exophilic, as observed in the Dominican Republic, Colombia, and Haiti. However, in Mexico and Central America, 80% of the *An. albimanus* was observed to have an endophilic resting behaviour [25]. Also the time and place of biting differs between sites for most of the species. In some localities for example, *An. darlingi* bites mostly during sleeping hours, or early in the morning [26], whereas in other localities, the main biting peak is early in the evening [27]. In French Guiana, *An. darlingi* was endo-exophagous with a clear predilection for biting outdoors [28].

3. How can the indoor use of insecticides select for exophilic, exophagic, zoophilic and/or early biting mosquito populations?

Insecticides can elicit different actions with different results on mosquitoes [29–31]. These various modes of action are important when talking about selection of ‘insecticide avoiding’ mosquitoes. Toxic or cidal actions result in knockdown or death after contact with the insecticide. Excito-repellent actions, including contact irritancy and non-contact repellency, result in above-normal levels of undirected movements coupled with loss of responsiveness to host cues. The insecticidal actions and their results depend among others on the insecticidal product used and on the mosquito species present. Large differences in actions of insecticides used in IRS have been observed: dieldrin for example only elicits a cidal action, while alphacypermethrin has both contact-irritant and killing actions, and DDT elicits mainly a repellent effect and secondarily a toxic action. [30]. Pyrethroids, the only family of insecticides used on ITNs, have well-documented excito-repellent actions [21] which are dose-dependent, but with for example higher toxic actions of alphacypermethrin as compared to deltamethrin and permethrin [31].

The general concepts of stress-induced variation in evolution [32] can be applied to the effect of insecticides on mosquito populations. Indoor use of insecticides will pose a stress on the female anopheline population, but only when the insecticides present a barrier for indoor feeding or indoor resting. At least three processes can be at the origin of perceived shifts in mosquito behaviour by insecticides:
1. A first protective mechanism can be behavioural plasticity in response to the presence of the insecticide. The ability to actively remove from the insecticide by either relocation or avoidance requires an ability to detect (either by contact or non-contact) or anticipate the presence of the insecticide and the ability to exhibit insecticide avoidance strategies or adjustments [32]. The insecticide, or the unavailability of the host, can then trigger the expression of gene variants that have been accumulated, but were phenotypically neutral under a normal range of environments [32]. Many mosquitoes indeed naturally possess a high degree of irritability or repellency which is evident at the very first exposure of the population to residual insecticides [29]. Where this irritation is such that mosquitoes settling on the insecticide deposit are activated before they have absorbed a lethal dose of insecticide, and are able to avoid further contact and to escape unharmed, the term “protective avoidance” has been suggested. In the presence of a high coverage of IRS or ITNs, mosquitoes exhibiting this protective avoidance should then be able to redirect their behaviour to low-risk behaviour which also can lower their survival. For example, for a species that is normally endophilic changing its behaviour to resting outdoors, the external environment may be unfavourable to the survival of the species [12].

2. A second protective mechanism for the mosquito is a consistent “protective behaviour” [29] such as exophily, exophagy, zoophily or early-biting resulting in a minimal contact with the insecticides used indoors. As mentioned above, some mosquito populations naturally exhibit this kind of protective behaviour, which is probably genetically determined (see further). Also differences in responses to the insecticides can result in diverse exposure rates of different species or subpopulations to the insecticide. An. minimus for example, shows very strong repellency responses to several insecticides and would have a higher survival chance in the presence of insecticides as compared to An. harrisoni which shows a much lower repellency response [33]. In this case, insecticides will favour the (sub) populations of mosquitoes that have this innate preference for protective behaviour or for avoidant strategies by which they will escape the exposure to the insecticide. This is probably the mechanism that is occurring for many of the perceived species shifts that are illustrated below.

3. Where these phenomena of protective avoidance or protective behaviour are not evident at the very first exposure of the population to the insecticides, but develop only gradually, perhaps over several years under continued insecticide pressure, the term “behaviouristic resistance” is employed [29]. The presence of the insecticide will in that case result in the selection of mutations and recombination that favour the survival of the mosquito in the presence of the insecticide, eventually leading to a directional selection. This can be compared to the development of insecticide resistance, although selections of many mutations will probably be required before an appropriate behavioural change may occur. Classification as “behaviouristic resistance” is only valid on the basis of accurate comparisons made before and subsequent to the widespread use of residual insecticides in any particular area. As shown below, very few behaviour shifts observed so far, would fit this definition of behaviouristic resistance.
4. Shifts observed in the presence of indoor insecticidal pressure

In the following paragraphs we will review the shifts that were observed in the presence of IRS and ITNs. For the purpose of this review, a ‘shift’ means an observed change, including relative changes, with a reasonable link to the indoor use of insecticides (ITNs or IRS). A distinction is made between different kinds of shifts: species shifts describe changes in the species composition which can also be within species complexes, whereas shifts to early biting, exophagy, zoophily or exophily describe changes in biting time, biting place, host, or resting place within a species, or within a species complex if no species information was available. Because a large part of the shifts in literature are described in the Afrotropical region, this region will be handled separately.

5. Afrotropical region

5.1. Species shifts

An IRS campaign resulted in the elimination of An. funestus from the South Pare District (at the Tanzania-Kenya border), at the same time reducing the numbers of indoor-resting An. gambiae s.l. [34]. In the years immediately following this IRS campaign, populations of endophilic An. gambiae s.l. slowly regained their former levels, whereas gradual resurgence of An. funestus was not observed until almost 10 years after the campaign was abandoned. IRS campaigns in two Kenyan villages resulted in a large decrease (up to total disappearance) of An. funestus, with an increase in the more exophagic An. rivulorum [35] or An. parensis [36], both not considered as malaria vectors in the study sites. In Niger, nation-wide Long-lasting insecticidal net (LLIN) distribution caused a marked decrease of An. funestus, without effect on An. gambiae s.l. abundance[37]. Following an IRS campaign, An. gambiae was completely eliminated from Pemba Island (Tanzania), leaving the salt-water breeding An. merus, an exophilic mosquito with a preference for cattle [38]. In Kenya and Tanzania, large scale ITN use significantly decreased the proportion of indoor-resting An. funestus [39] and An. gambiae [39–42] while the proportion of An. arabiensis increased. The shift from An. gambiae to An. arabiensis was also observed in the larval collections [40,41]. As larvae of An. gambiae and An. arabiensis show no habitat segregation, larval sampling reflects true proportions of the two species. The change from sub-populations dominated by An. gambiae to those dominated by An. arabiensis took about a decade, as would be expected if caused by a constant ITN selection pressure [43].

In contrast, in Kenya and on the Bioko Island (Equatorial Guinea), the same species compositions were observed regardless of the use of ITNs or IRS [21,44]. Moreover, in the north-east of Tanzania, a species shift has been observed in the absence of insecticide selective pressure, in a region without organized vector control activities reported [45]: An. gambiae, the most dominant in the past, was replaced by An. arabiensis without any known reason.

5.2. Shifts to early-evening or early-morning biting

Studies have shown that widespread ITN use increases the proportion of early bites by An. gambiae [46] and An. funestus [42,46] in Tanzania. Such shift was not observed for Culex
quinquefasciatus which is highly resistant against pyrethroids [46]. According to the authors [46], this suggests that for anophelines, where there is considerable killing by contact with ITNs, several years of selection has begun to produce an upward shift in the proportions of insects biting at a time when people are accessible. Also in southern Benin, a significant change in host seeking behaviour of An. funestus was observed after achieving a universal coverage of ITNs. The shift in biting time was here not to the early evening but to the early morning. Moreover in one locality about 26% of the An. funestus bites were observed after sunrise [47].

The use of ITNs resulted in a shift towards earlier biting of An. gambiae s.l. in Kenya [48] and Tanzania [42,49], possibly [48,49] or certainly [42] related to a species shift from An. gambiae to An. arabiensis.

In other studies however, no evidence for a shift in biting time after the introduction of ITNs or IRS was obtained for An. gambiae s.l. in Tanzania, Kenya, The Gambia and Nigeria [44,50–52], for An. gambiae the Bioko Island (Equatorial Guinea) [21], or for An. funestus in Kenya [44]. Widespread use of mostly untreated bed nets did not result in more early biting of An. gambiae [5].

| Country         | Vector control measure | Insecticide b | Collection methods c | Species shift d | Shift to early-biting d | Shift to exophagy e | Shift to zoophily d | Reference |
|-----------------|------------------------|--------------|----------------------|-----------------|------------------------|---------------------|---------------------|-----------|
| Benin           | ITN                    | Deltamethrin | Indoor/outdoor HLC   | ND              | Yes                    | Yes                 | ND                  | [47]      |
| Burkina Faso    | ITC                    | Permethrin   | Indoor/outdoor CDC, LT | ND              | ND                     | Not observed        | Not observed        | [53]      |
| Equatorial Guinea | IRS                 | Unspecified | IRC, Odor-baited traps | ND              | ND                     | ND                  | Yes                 | [19]      |
| Benin           | ITN                    | Deltamethrin, alphacypermethrin, bendiocarb. Unspecified LLINs | Indoor/outdoor HLC | Not observed | Not observed | Yes | ND | [21] |
| Benin           | ITN                    | Deltamethrin, alphacypermethrin, bendiocarb. Unspecified LLINs | Indoor/outdoor HLC | Not observed | Not observed | Yes | ND | [21] |
| Kenya           | IRS                    | Dieldrin     | ORC, IRC, LD, HLC    | Yes             | ND                     | ND                  | ND                  | [35]      |
| Kenya           | IRS                    | DDT          | Indoor/outdoor HLC   | Yes             | ND                     | ND                  | ND                  | [36]      |
| Kenya           | IRS                    | Dieldrin     | IRC, ORC             | Yes             | ND                     | ND                  | Not observed, Yes, but not significant | [34]      |
| Kenya           | ITN                    | Permethrin   | IRC, indoor and outdoor HLC | ND          | Yes                    | Yes                 | ND                  | [48]      |
| Kenya           | ITN                    | Permethrin   | IRC, ORC             | ND              | ND                     | ND                  | ND                  | [54]      |
| Kenya           | ITN                    | Permethrin   | WET, IRC, outdoor bed net traps | Not observed | Not observed | ND | ND | [44] |
| Kenya           | ITN                    | Permethrin, alphacypermethrin, Unspecified LLINs | IRC, IRC, LD | Yes | ND | ND | ND | [39] |
| Kenya           | ITN                    | Permethrin, alphacypermethrin, Unspecified LLINs | IRC, IRC, LD | Yes | ND | ND | Not observed | [41] |
| Country     | Vector control measure | Insecticide b | Collection methods c | Species shift d | Shift to early-biting d | Shift to exophagy d | Shift to zoophily d | Reference |
|-------------|------------------------|---------------|----------------------|----------------|------------------------|---------------------|---------------------|-----------|
| Kenya       | ITN, ITC               | Permethrin, alpha cypermethrin, deltamethrin | Bed net traps, IRC, LD | Yes            | ND                     | ND                  | ND                  | [43]      |
| Kenya       | ITN                    | Unspecified   | IRC, ORC, LD         | Yes            | ND                     | ND                  | ND                  | [40]      |
| Niger       | ITN                    | Unspecified   | IRC, indoor/ outdoor HLC, indoor/ outdoor CDC LT | ND             | ND                     | Yes                 | ND                  | [37]      |
| Nigeria     | IRS                    | Propoxur      | Indoor/ outdoor HLC, IRC | ND             | ND                     | Yes                 | ND                  | [14]      |
| Nigeria     | IRS                    | Propoxur      | Indoor/ outdoor HLC, IRC | Not observed | Not observed | Yes                 | Not observed | [52]      |
| Tanzania    | IRS                    | Dieldrin      | IRC, ORC, IRC, WET   | Yes            | ND                     | Not clear | Not clear | [38]      |
| Tanzania    | ITN+IRS                | Permethrin or lambda cyhalothrin, DDT | Indoor CDC LT, outdoor HLC, IRC, ORC | ND             | Inconclusive | Not observed | Inconclusive | [50]      |
| Tanzania    | ITN                    | Lambda cyhalothrin, deltamethrin | IRC, WET, indoor HLC | ND             | Yes                    | ND                  | ND                  | [49]      |
| Tanzania    | ITN                    | Majority untreated nets | Indoor/ outdoor HLC | ND             | Not observed | Not observed | ND                  | [5]       |
| Tanzania    | ITN                    | Unspecified   | Indoor CDC LTs, Mbita traps | ND             | Yes                    | ND                  | ND                  | [46]      |
| Tanzania    | ITN                    | Unspecified   | Indoor/ Outdoor HLC  | Yes            | Yes                    | Yes                 | ND                  | [42]      |
| The Gambia  | ITN                    | Permethrin    | Outdoor HLC, IRC, indoor CDC LT, bed net searches | ND             | ND                     | Yes                 | Not observed | [55]      |
| The Gambia  | ITN                    | Permethrin    | Indoor/ outdoor HLC, IRC, WET | ND             | Not observed | Not observed | Yes, but not significant | [51]      |

a ITN: Insecticide treated nets; IRC: Indoor residual spraying; ITC: Insecticide treated curtains
b LLINs: Long lasting insecticidal nets
c IRS: Indoor resting collection; ORC: Outdoor resting collection; CDC LT: Center for Disease Control light trap; HLC: Human landing collection; WET: Window exit trap; LD: Larval dipping; CMR: Capture-Mark-Recapture
d ND: Not done

Table 1. Review of the effect of insecticide based indoor vector control measures on malaria vectors in the Afrotropical region
5.3. Shifts to exophagy

In Nigeria, IRS resulted in a threefold increase of the proportion of *An. gambiae* s.l. biting outdoors [14,52]. Several years of vector control by IRS and later ITNs in the Bioko Island, increased the trend for outdoor biting of *An. gambiae* [21] as compared to historical data in the same region of preferred behaviour for indoor biting. Also in Tanzania, high ITN-use resulted in an increased outdoor biting for *An. funestus* [42]. In the latter study the proportion of indoor contact with *An. funestus* bites had dropped to only half of the indoor contact before widespread ITN-use. In southern Benin as well, after achieving universal ITN coverage, a higher proportion of outdoor biting was observed for *An. funestus* [47], although this was only observed in one out of two localities that were studied.

Some studies have shown that distribution of ITNs in Niger, Kenya, and The Gambia decreased the endophagic rate of *An. gambiae* s.l. [37,48,55], and to a lesser extend of *An. funestus* [37]. However, as the species of the *An. gambiae* complex were not determined in these studies, a possible reason for this decrease would be a species shift from *An. gambiae* to *An. arabiensis*.

In other studies however, no evidence for a shift to outdoor biting of *An. gambiae* s.l. due to widespread IRS or ITNs use was found in Tanzania [42,50], Burkina Faso [53] and The Gambia [51]. Also widespread use of mostly untreated bed nets did not result in a higher outdoor biting rate of *An. gambiae* [5].

5.4. Shifts to zoophily

In Kenya, ITN-use caused a shift in host selection of *An. gambiae* s.l. and *An. funestus* [54] from humans towards cattle or other animals. Similar observations were made in Burkina Faso with *An. gambiae* [19]. In other studies in Kenya and The Gambia, the use of ITNs caused only small and insignificant decreases in human blood index (HBI) for *An. gambiae* s.l. [40,48,51] and *An. funestus* [40].

The use of ITNs, IRS, or insecticide treated curtains caused no shift in host selection (or decrease in HBI) for *An. arabiensis* in Zambia [56], for *An. gambiae* s.l. in Nigeria, Burkina Faso, The Gambia, Tanzania and Kenya [34,50,52,53,55], and for *An. funestus* in Tanzania and Kenya [34,50].

5.5. Shifts to exophily

As summarized in [57], different populations of *An. arabiensis*, e.g. in the Pare-Taveta malaria scheme, Mauritius, Madagascar, Zanzibar, Nigeria and other West African localities, became either completely exophilic or, at most, remained only partially endophilic after IRS campaigns. ITN distribution reduced the indoor resting fraction of *An. gambiae* s.l. in Niger and Kenya [37,48], and of *An. funestus* in Kenya [48]. No evidence for a resting place shift after introduction of ITNs or after IRS was observed in Tanzania [50].
6. Australasian, Oriental, and Neotropical Regions

6.1. Species shifts

In the Solomon Islands, IRS in the 1960s has nearly eliminated the major malaria vectors *An. koliensis* and *An. punctulatus*, which are mainly endophagic and late-biters. The density of *An. farauti*, a more exophagic and early-biting malaria vector, remained quite high, particularly in outdoor man-biting situations [58]. The latter species is now the primary vector in the Solomon Islands, with the former major malaria vectors being totally absent. *An. hinesorum*, which is not considered a vector, has now occupied the breeding sites commonly used by *An. koliensis* [59].

In the forested hilly areas of Thailand, IRS resulted in a higher proportional decrease of *An. dirus* s.l. as compared to *An. minimus* s.l. [60]. Widespread use of IRS resulted in a different behaviour of the *An. minimus* s.l. present [61], which probably reflects a species shift from *An. minimus* to *An. harrisoni*, as also observed in Vietnam as a result of widespread use of ITNs [62]. Residual spraying did effectively control indoor resting species in Nepal such as *An. annularis, An. culicifacies, An. splendidus* and *An. vagus*. The abundance of the partially outdoor resting species, *An. fluviatilis* s.l. and *An. maculatus* s.l. also decreased markedly after the spray application, but then rebounded rapidly within 1 or 2 months after treatment [63]. ITN use in China caused a higher decrease of the endophilic and anthropophilic *An. lesteri* (syn. *An. anthropophagus*) [64] and *An. minimus* s.l. [65] than of the exophagic and zoophilic *An. sinensis*.

In British Guiana, the primary malaria vector *An. darlingi* (both larvae and adults) was rapidly eliminated by IRS, whereas larvae and adults of a zoophilic species, *Anopheles aquasalis*, a possible malaria vector, were completely unaffected [66]. In Guatemala, *An. vestitipennis* decreased in abundance in communities with a wide distribution of ITNs, while *An. albimanus* did not change. Whether this change was an effect of the ITNs could not be concluded as the study was not designed for answering that question [67].

6.2. Shifts to early biting

In Papua New Guinea, ITN distribution immediately changed the biting cycles of both *An. farauti* and *An. koliensis* from a post-midnight peak towards a pre-midnight peak [68]. Also on the Solomon Islands, intervention and longitudinal studies have shown that IRS, ITNs, or a combination of both, changed the biting cycle of *An. farauti* to an earlier biting peak [58,69,70].

IRS changed the indoor biting peak of *An. dirus* s.l. in the forested hilly areas of Thailand to one hour earlier. Outdoors, the peak remained the same, but a higher proportion bite earlier. Also for *An. minimus* s.l., a shift to earlier biting was observed [60]. In the foothills on the other hand, where *An. minimus* s.l. was the main vector, no effect of DDT was seen on the already early biting *An. minimus* s.l. population [71]. Also recent studies in Vietnam have shown that in the prolonged presence of impregnated bed nets, 45% of the *Anopheles* bites are acquired before sleeping time in the forest, and 64% before sleeping time in the village [8]. In Cambodia,
in a period when ITN coverage was still low, already 29% of the Anopheles bites were acquired before sleeping time [9].

Although we have not encountered studies in Latin-America with evidence for shifts to earlier biting, some studies indicated that also in this region, early biting vectors can maintain residual transmission. In an area in Brazil covered by IRS for example, blood-feeding of An. darlingi started at sunset, remained high during the first half of the night, and decreased gradually until early morning [72]. Also in the Bolivian Amazon, in an area with high ITN use, peak outdoor biting of An. darlingi occurred between 19:00 and 21:00 hours, when 48% of the total night's biting took place, and 83% of the night's biting had occurred by 22:00 hours when most local people go to bed [73].

6.3. Shifts to exophagy

On different islands of the Solomon, proportional shifts to outdoor biting (from 47% to 67%) were observed for An. farauti after IRS [58]. Moreover, compared to An. koliensis and An. punctulatus, the exophagic An. farauti population recovered completely within nine months after the spraying campaign. However, in other intervention and longitudinal studies on the Solomon Islands, the shift to outdoor biting of An. farauti due to ITNs and/or IRS was not so obvious [59,69].

IRS increased the outdoor biting rate of An. dirus s.l. [60,74], and of An. minimus s.l. in forested and foothill regions in Thailand [60,61]. In contrast, in another foothill region of Thailand, an initial effect of DDT was seen on the malaria transmission, but this was not sustained for this already outdoor biting An. minimus s.l. population [71]. Also wide scale use of ITNs caused a higher decrease in the indoor biting populations as compared to the outdoor biting populations of An. sinensis, An. lesteri (syn. An. anthropophagus) and An. minimus s.l. in China [64,65]. In Vietnam, after prolonged ITNs distribution, outdoor biting densities of the main vectors, An. dirus, An. maculatus s.l. and An. minimus s.l. were significantly higher than indoor biting density [8]. In Laos, in contrast, the use of ITNs did not stop An. dirus from entering the houses [75].

In an IRS area in Brazil, An. darlingi fed more frequently outdoors, whereas in earlier years before IRS this species mainly fed indoors [72]. In contrast, in Colombia, IRS did not stop malaria vectors to bite both indoors and outdoors [76]. The combined use of ITNs and IRS has preceded the collapse of a mainly exophagic An. darlingi population in Suriname. However, this collapse can also be attributed to an unusual, extensive flooding which coincided with the onset of the control interventions [77].

6.4. Shifts to zoophily

A significant decrease in HBI of An. farauti was observed immediately after the distribution of ITNs in Papua New Guinea, although this shift could be due to a slightly changed sampling method [68].

In Thailand, in the prolonged presence of DDT use in IRS, An. minimus s.l. exhibited a marked zoophily, whereas in villages with lower DDT pressure, no preference was observed [61],
although this apparent ‘change in behaviour’ could have been due to a species shift within the *An. minimus* complex as observed in Vietnam [62]. In an intervention study in India, the HBI of *An. culicifacies* was lower in areas with ITNs as compared to areas with untreated bed nets or no nets [78].

In Mexico, a much lower HBI was observed in areas where IRS was implemented as compared to historical data [79]. Also in areas covered by IRS in Brazil, *An. darlingi* was mostly zoophilic [80].

### 6.5. Shifts to exophily

A very low endophily rate was observed for *An. farauti* after several DDT spraying campaigns in the Solomon Islands [58].

IRS also significantly reduced the indoor resting abundance of all anopheline species except for *An. fluviatilis* s.l. in Nepal [63], and of *An. dirus* s.l. in Thailand [74]. In India, *An. culicifacies* s.l. has been observed to be highly exophilic in areas where residual spraying with DDT was widely used [81]. Also in areas with wide scale use of ITNs in India fewer *An. culicifacies* s.l. were collected indoors (resting collections) as compared to control areas. However, in this area more *An. culicifacies* s.l. were found indoor-resting in individual houses with untreated bed nets as compared to houses with ITNs, both located in the ITN-area [78]. This suggests that this mosquito population did not shift entirely to exophily, but that this behaviour mainly reflects the excito-repellent effect of the permethrin.

IRS has brought the disappearance of *An. darlingi* from the interior of houses in Brazil and French Guiana [28,80]. However, outdoor-resting still persists, either in the vicinity of the houses [80] or outside the peridomestic environment [28]. ITNs as well caused less indoor-resting in an intervention trial in Guatemala [67]. In contrast, in Mexico, after prolonged use of DDT no deterrence was observed anymore for *An. pseudopunctipennis*, with as many mosquitoes seeking shelter in sprayed huts as in unsprayed huts [82].

| Country                  | Vector control measure | Insecticide | Collection methods | Species shift | Shift to early-biting | Shift to exophagy | Shift to zoophily | Reference |
|-------------------------|------------------------|-------------|--------------------|---------------|-----------------------|-------------------|-------------------|-----------|
| **Australasian Region** |                        |             |                    |               |                       |                   |                   |           |
| Papua New Guinea        | ITN                    | Permethrin  | Outdoor HLC        | Not observed  | Yes                   | ND                | Yes?              | [68]      |
| Solomon Islands         | IRS                    | DDT         | HLC                | Yes           | Yes                   | Yes               | ND                | [58]      |
| Solomon Islands         | IRS, ITN               | DDT, permethrin | Outdoor HLC, indoor CDC, LT, outdoor pig baited traps | ND | Yes | Not clear | ND | [69] |
| **Oriental region**     |                        |             |                    |               |                       |                   |                   |           |
| Solomon Islands         | IRS, ITN               | DDT, lambda cyhalothrin, Permethrin, unspecified LLIN | Indoor/ outdoor HLC, LD, animal baited trap | Yes | ND | ND | ND | [59] |
| Solomon Islands         | ITN, IRS               | Deltamethrin, lambda cyhalothrin | Indoor/ outdoor HLC, IRC, WET, LD | ND | yes | Yes, small | ND | [59] |
| Country       | Vector control measure * | Insecticide b | Collection methods c | Species shift d | Shift to early-biting d | Shift to exophagy d | Shift to zoophily d | Reference |
|---------------|---------------------------|--------------|----------------------|----------------|------------------------|-------------------|-------------------|-----------|
| China         | ITN                       | Deltamethrin | Indoor/ outdoor man-baited nets | Yes           | ND                     | Yes               | ND                | [64]      |
| China         | ITN                       | Deltamethrin | ?                    | Yes           | ND                     | Yes               | ND                | In [65]   |
| India         | ITN                       | Lambda cyhalothrin | IRC, Indoor HLC, Outdoor Cattle collection | ND           | ND                     | ND                | Yes               | [78]      |
| Nepal         | IRS                       | DDT, bendiocarb, malathion | Indoor/ outdoor HLC, IRC, ORC, cattle collections, LD | Yes           | ND                     | Yes               | ND                | [63]      |
| Thailand      | IRS                       | DDT          | Indoor/ outdoor HLC | Yes           | Yes                    | Yes               | ND                | [60]      |
| Thailand      | IRS                       | DDT          | Indoor/ outdoor HLC | Not observed  | Not observed           | Not observed      | ND                | [71]      |
| Thailand      | IRS                       | DDT          | Indoor/ outdoor HLC, boid-baited trap, IRC, ORC | Probably       | ND                     | Yes               | Yes               | [61]      |
| Thailand      | IRS                       | DDT, fenitrothion | Indoor/ outdoor HLC, IRC | ND           | ND                     | Yes               | ND                | [74]      |
| Vietnam       | ITN                       | Permethrin   | Indoor/ outdoor HLC, IRC, CDC LT | Yes          | ND                     | ND                | ND                | [62]      |

**Neotropical Region**

| Country       | Vector control measure | Insecticide b | Collection methods c | Species shift d | Shift to early-biting d | Shift to exophagy d | Shift to zoophily d | Reference |
|---------------|-------------------------|--------------|----------------------|----------------|------------------------|-------------------|-------------------|-----------|
| Brazil        | IRS                     | DDT          | IRC, ORC, animal baited trap, | ND           | ND                     | ND                | Yes?              | In [80]   |
| Brazil        | IRS                     | DDT          | Indoor/ outdoor HLC, outdoor animal baited trap | ND           | ND                     | Yes               | ND                | [72]      |
| British Guiana| IRS                     | DDT          | IRC, LD              | Yes           | ND                     | ND                | ND                | [66]      |
| Guatemala     | ITN                     | Permethrin   | Indoor/ outdoor HLC, IRC | Yes?          | ND                     | Not observed      | ND                | [67]      |
| Mexico        | IRS                     | DDT, bendiocarb | IRC, ORC             | ND           | ND                     | ND                | Yes               | [79]      |
| Mexico        | IRS                     | DDT (dieldrin before) | Entry traps, WET | ND           | ND                     | ND                | ND                | [82]      |

*ITN: Insecticide treated nets; IRS: Indoor residual spraying; ITC: Insecticide treated curtains
*LLINs: Long lasting insecticidal nets
*IRC: Indoor resting collection; ORC: Outdoor resting collection; CDC LT: Center for Disease Control light trap; HLC: Human landing collection; WET: Window exit trap; LD: Larval dipping; CMR: Capture-Mark-Recapture
*ND: Not done

**Table 2.** Review of the effect of insecticide based indoor vector control measures on malaria vectors in the Australasian, Oriental and Neotropical regions
7. Discussion

7.1. The importance of residual transmission by outdoor and early biting malaria vectors

In this chapter we have shown that outdoor and early biting malaria vectors are widespread among malaria endemic countries and, as relative shifts to outdoor, early or animal-biting and outdoor resting vectors occur due to the use of IRS and ITNs, these vectors will increasingly contribute to malaria transmission in regions with a high coverage of ITNs and IRS. However the reported shifts are not always well documented: species identification of complexes are often missing, and confounding factors such as changes of the environment, habitat, human behaviour and occupation are not considered.

In Africa, most of the species shifts observed resulted in a large decrease of the important endophagic, endophilic and anthropophilic malaria vectors, *An. funestus* and *An. gambiae*, while the more exophagic, exophilic, and/or zoophilic species *An. arabiensis* persists. Reports on such species shift are recently increasing, with most of these shifts described in East-Africa. But also in the other geographical regions, shifts in species abundances have been observed. It is however important to note that the majority of shifts described are shifts in relative abundances, where the more endophagic, endophilic and/or anthropophilic species declines more (or is being eliminated) while the more exophagic, exophilic and/or zoophilic species maintains at the same density or declines less. Only in some cases, the density of the latter species actually increases (e.g. the non-vector species *An. rivulorum* [35] or *An. parensis* [36]), probably because they take over the breeding sites of the declining species. Moreover, as also mentioned in [83], the vectorial capacity of the species predominating after the intervention does not necessarily increase, but persisting species that are malaria vectors, such as *An. arabiensis*, will be responsible for the residual malaria transmission, while the role of e.g. *An. gambiae* or *An. funestus* decreases.

Therefore, one of the most plausible reasons for species shifts to occur in the presence of ITNs or IRS is the non-uniform exposure of the different species to the insecticides, as described above. This hypothesis is supported by a study in Kenya in which the persisting *An. arabiensis* in an area with high ITN coverage had little to no pyrethroid resistance compared to the declining *An. gambiae*, with moderate to high levels of pyrethroid resistance [41,43]. Moreover, in experimental hut trials on northeast Tanzania, the mortality of *An. arabiensis* measured in experimental huts was consistently lower than that of *An. gambiae* and *An. funestus* [83], which probably is a major contributing factor to the species shifts observed in East Africa following scale up of ITNs. The authors state that, as cone tests on the nets prior to the trials produced rather similar levels of mortality among *An. gambiae* and *An. arabiensis*, the most likely explanation for lower *An. arabiensis* mortality was behavioural avoidance of treated net surfaces. As feeding inhibition in this experiment was similar for *An. arabiensis* and *An. gambiae*, outdoor blood-feeding would be the major mechanism to which *An. arabiensis* avoids contact with the ITN, as opposed to abandoning host-searching when confronted with ITNs.

Besides the species shifts, shifts to earlier-, outdoor-, and animal-biting have been observed for primary vectors such as *An. gambiae, An. funestus, An. farauti, An. koliensis, An. dirus* s.l., *An.
Anopheles mosquitoes - New insights into malaria vectors

minimus s.l., An. culicifacies, and An. darlingi. These shifts might also be linked to the non-random exposure of subpopulations of vectors to insecticide treated surfaces (ITNs or IRS). Several studies have indeed shown that the feeding and resting behaviour of anophelines is consistent in certain subpopulations and/or linked to certain genetic markers. Most of the studies on genetic determination of biting and resting behaviour are based on chromosomal inversions. Alleles captured within chromosome rearrangements are protected from recombination and can as such favour local adaptation by capturing sets of locally adapted genes which might lead to reproductive isolated entities or subpopulations [84]. In the Garki District in Nigeria, chromosomal arrangements in An. arabiensis and An. gambiae have been associated with exophagy and exophily [85,86] and with zoophily [87]. Exophagy and exophily were associated with the standard chromosomal arrangements 2R+ for An. arabiensis and 2R+b for An. gambiae, and the inverted arrangement 2Rbc for An. arabiensis. Moreover, the chromosome arrangements associated with indoor biting or resting are the ones adapted to drier environments, while arrangements more frequent in outdoor collected specimens are those associated with more humid environments [85]. In the Zambesi valley, 2Rc An. arabiensis heterozygotes were associated with exophily and zoophily [57]. In Ethiopia An. arabiensis heterozygotes of the 2La and/or 2Rb chromosomal arrangements tended to bite later at night than the double homozygotes [88]. Also in laboratory experiments an association between chromosomal arrangements and circadian flight activity has been found [89]: female An. stephensi homozygotes for the 2Rb inversion showed more activity following light-on (corresponding to early morning) as compared to homozygous females for the standard 2R+b arrangement. Other field-based evidence on the existence of subpopulations showing consistent behaviour was obtained by studying behaviour of An. balabacensis in a capture-mark-recapture experiment in Borneo (Malaysia) [90]. This study revealed significant trends of An. balabacensis to be recaptured on the same host or resting site of the original capture. In contrast, a similar capture-mark-recapture study on resting behaviour of An. gambiae s.l. in Tanzania showed no faithful tendencies of endo- or exophily [91]: the same individuals within the An. gambiae s.l. population mixed indoor and outdoor resting. More recent genetic studies are based on the frequencies of enzyme polymorphisms. In the Malaysian study [90], faithfully indoor and outdoor-resting populations showed significant differences in isozyme frequencies (loci Est-3 and Idh-3). Also in Burundi, isozyme frequencies were significantly different between in- and out-door biting An. arabiensis (locus Mdh-2) and in- and out-door resting An. gambiae (Mpi and Got-2 loci) [92]. Such differences were not observed for An. gambiae in Burkina Faso [93]. Moreover, mosquitoes carrying a specific genotype [93] or chromosome karyotypes [87] were found to be significantly more infected with sporozoites, suggesting the occurrence of subpopulations having different vector behaviours. These independent genetic studies, either based on karyotyping or on genotyping, provide evidence that active choice for the best place, time or host to bite, or the best place to rest can be associated with specific genotypes. This suggests the existence of subpopulations characterized by specific behavioural patterns which implies a non-uniform exposure to IRS or ITNs. Selection of specific behavioural patterns can then not be excluded.

However, other mechanisms can also explain these kinds of shifts. More early biting could occur as females that fail to obtain a blood meal during the previous night, might be more likely to commence host seeking in the early evening [44]. By disrupting the feeding behaviour,
the ITNs would increase the length of the oviposition cycle of the overall population [68]. This mechanism could explain the immediate change in biting cycles of both *An. farauti* and *An. koliensis* after ITN distribution in Papua New Guinea. Both species shifted from a post-midnight biting peak towards a pre-midnight peak [68], with an extended oviposition cycle. Also in the Solomon Islands, the oviposition cycle was extended from 3 to 4 days due to ITN use, possibly explaining the higher tendency for early biting observed in the village with ITN use [69]. Shifts to outdoor biting by *An. farauti* also occurred immediately after DDT spraying [58]. This first effect would be caused by the deterrent effect of DDT, while only in second instance the endophilic fraction of *An. farauti* is being killed. Moreover, compared to *An. koliensis* and *An. punctulatus*, the *An. farauti* population recovered completely within nine months after the spraying campaign, indicating that this change of behaviour is due to a plastic response to the deterrent effect of DDT. Moreover, it has been shown that the occurrence of a shift in host selection does not necessarily reflect a selection of a more zoophilic vector subpopulation, but can also indicate plasticity in host selection. The *An. gambiae* population in Burkina Faso that showed a high proportion of cattle feeding (HBI of only 40%), had an innate preference for humans (88%) in a choice experiment using an odour-baited trap [19]. The weak accessibility of humans due to the use of ITNs, forces the mosquitoes to feed on cattle. According to the authors of the study, this suggests that in this area a plastic foraging strategy could provide greater benefits than a specialist strategy for this species.

Regardless of the mechanism that causes these behavioural shifts, the case studies show that in several areas the proportion of outdoor-, early- and/or animal biting primary vectors are relatively increasing, which will then be responsible for residual transmission. Moreover, in a similar way, transmission by ‘secondary’ vectors that have outdoor or early biting behaviour might become more important than transmission by primary vectors in contexts of high coverage of ITNs and IRS. In a malaria endemic region of Thailand, one specimen of the Barbirostris Subgroup (*An. barbirostris/campestris*) was found to contain *Plasmodium* oocysts, in the prolonged absence of the main malaria vectors, showing that *An. barbirostris s.l.*, an outdoor biting mosquito [94], might be responsible for maintaining malaria transmission in the absence of the main vectors [95]. As secondary vectors are often less anthropophilic, and might be more exophagic and early biting, planning of vector control should also take into account their behaviour. Moreover, as pointed out in [8], secondary vectors might be better vectors of *P. vivax* as compared to *P. falciparum*, as the extrinsic incubation period of *P. vivax* is shorter. In British Guiana, for example, *An. aquasalis*, a mostly zoophilic and exophilic mosquito species breeding in brackish water, was vector of several Vivax malaria outbreaks after *An. darlingi* was eliminated by DDT spraying [96]. Also more recently in Vietnam, *An. sawadwongporni*, a very early biting secondary vector, was found positive for *P. vivax* [8].

7.2. ITNs and IRS are very effective, but additional measures are needed for reaching malaria elimination

ITNs and IRS have been shown to have a large impact on malaria infection and disease [97,98]. Moreover, several entomological studies have also shown that where the vectors are mostly endophagic, endophilic and anthropophilic, ITNs and IRS are very effective in reducing their
population density. This was for example shown for *An. minimus* in India [99] and for *An. dirus* in Laos [100], both of them being anthropophilic, indoor- and late-biting in the respective study sites. A recent study in Zambia also showed that even at a high coverage of ITNs and IRS, the highest probability for malaria transmission based on human and vector behaviour, still occurs indoors [101], making ITNs and IRS valuable tools.

ITNs can also have an effect on malaria transmitted by more zoophilic and exophagic mosquitoes. In Sao Tomé for example, where *An. gambiae* is zoophilic and very exophagic, increased bed net use decreased the malaria prevalence in both bed net users and non-users [102]. The differences in prevalence between users and non-users were greatest in children under 5 years old, who are more likely to use the bed nets in the evening, showing that indeed the bed nets were the cause of the decrease. However, in older age groups, that are more likely to remain outside in the evening, no such difference was observed. Moreover, even at an almost 80% ITN coverage, still a 30% malaria prevalence was observed among bed net users. This means that, as expected, a part of transmission by these zoophilic and exophagic mosquitoes could not be prevented by ITNs [102]. Also in other parts of the world it has been shown that ITNs are less performing in areas with outdoor biting or resting vectors, for example in Peru and Nicaragua [11]. In the Garki District (Nigeria), the impact of the IRS campaign with propoxur was related to the prespraying ratio between the man-biting density and the indoor-resting density and to intraspecific cytogenetic variation [52]. Moreover, as reviewed in [103], even low levels of exophagy, exophily or zoophily may attenuate the impact of ITNs and IRS because this allows mosquitoes to obtain blood while avoiding fatal contact with insecticides.

As we have shown that outdoor-, animal- and early biting behaviour, as well as outdoor resting behaviour is widespread among malaria vectors all over the world and might be increasing as a result of widespread IRS or ITN use, there is an urgent need for additional control measures tackling malaria transmission by these vector populations [103–106]. In other words, there is a ‘gap’ in protection, not only before sleeping time, but also for people that remain outdoors during the night (Figure 2) and this gap needs to be tackled by additional vector control measures. There are many ways of additionally reducing host-vector contact, including the use of topical repellents, spatial repellents, insecticide treated clothing, long lasting insecticidal hammocks, etc. Recently much research is carried out on the effectiveness of these kind of tools. For example, in the Bolivian Amazon, where the primary vectors *An. darlingi* has a peak biting activity before sleeping time, a household based cluster randomized trial has shown that the combined use of a topical repellent (para-menthane-3,8-diol, PMD) and ITNs can reduce the incidence of malaria by 80%, which was only significant for *P. vivax* and not for *P. falciparum*, as compared to the use of ITNs alone [107]. DEET-based repellents also had an additional protective efficacy against malaria disease in a small scale community based trial in India [108], and DEET-based repellent soap against *P. falciparum* malaria in a household randomized trial in a refugee camp in Pakistan [109]. In an ongoing study in Cambodia, Picaridin based repellents are shown to provide a protection of more than 90% against the bites of the main malaria vectors *An. dirus* and *An. minimus* (MalaResT project led by ITM-Antwerp, preliminary results). Whether the mass use of this repellent will result in a decrease of malaria infection is currently under investigation using a cluster-randomized controlled trial in
Ratanakkiri province in Cambodia. In a refugee camp in Kenya, permethrin treated clothing and blankets reduced malaria infection significantly [110]. In Southeast Asia, long lasting insecticidal hammocks have been shown to be effective against malaria disease [111] and against *An. minimus* bites, but not *An. dirus* bites [112]. For zoophilic mosquitoes, intervening in the host-vector contact could be more efficient by focusing on its preferred hosts, e.g. by insecticide treatment of cattle. However, killing partly zoophilic mosquitoes in sufficient numbers to suppress malaria transmission would require high protective coverage of both human and animal blood sources [104]. Moreover, it has been observed in Ethiopia that more than 90% of the blood meals taken by zoophilic vectors were taken from the legs of cattle [113], which are more difficult to treat.

Alternative personal protection measures are also of interest for people that work or reside in the forest, a risk area of malaria transmission in Southeast Asia [114]. For temporary shelters in the forest, insecticide treated plastic sheeting could be useful as this has proven to be effective in protecting against malaria disease in emergency camps [115]. Their effectiveness will rely both on their repelling effect and their killing effect, and whether mosquitoes will rest on this sheeting. Alternatively, other more accepted insecticide treated bed net-designs (V-shaped
nets, long lasting insecticidal hammocks, etc.), could provide protection for people staying in the forest during the night.

The more zoophilic, exophagic, or early biting a mosquito species or population, the more personal protection will act simply by blocking host-vector contact (through lethal or repellent effects). As shown by a mathematical model, malaria transmission involving zoophilic vectors (with 10% feeding on humans) can only be significantly decreased if the personal protection measures confer high levels of individual protection to users (80%) and be used by the majority of human population (80%) [116]. Therefore, the success of any intervention in this context will depend on its entomological efficacy, but also on the human behaviour, including acceptance and adherence to the preventive measures within the community. In São Tomé for example, many people watch communal television outdoors, posing them at risk for early-evening malaria transmission [117]. In Thailand, people do not take their ITN from the village to their farm plot [118]. Also in Vietnam, people often combine living in the village with a second home at their fields located in the forest [119], creating other malaria control needs, such as, for example, long lasting insecticidal hammocks. Taking into account human behaviour when adapting vector control strategies will then be crucial. In Bioko Island (Equatorial Guinea) for example, an increased trend of outdoor biting was observed for the main malaria vector *An. gambiae* [21]. However, the main malaria risk group, namely children under 15 years old, rarely

| Tool                                                                 | Mosquito behaviour that is targeted | Personal (P) or community (C) protection |
|----------------------------------------------------------------------|-------------------------------------|----------------------------------------|
| **Tools relying on host-vector contact**                             |                                     |                                        |
| ITNs                                                                 | N A I I P & C                        |                                        |
| Long lasting insecticidal hammocks & other net designs adapted to outdoor conditions | (E & N) A O O P & C                  |                                        |
| Insecticide treated plastic sheeting for shelters in the forest     | E & N A & Z I & O O P               |                                        |
| Personal protection including Topical & spatial repellents, Insecticide treated clothing | E & N A I & O I & O P & C*           |                                        |
| Insecticide treatment of cattle                                      | E & N Z I & O I & O C*               |                                        |
| **Tools not relying on vector-host contact**                         |                                     |                                        |
| IRS                                                                  | E & N A & Z I & O I C               |                                        |
| Larval source management                                             | E & N A & Z I & O I & O C*           |                                        |
| Toxic sugar baits                                                    | E & N A & Z I & O I & O C*           |                                        |
| Treatment of outdoor resting places, e.g. with fungal biopesticides | E & N A & Z I & O O C*               |                                        |

* E: Early evening & morning biting; N: Night biting; A: Antropophilic; Z: Zoophilic; I: Indoor; O: Outdoor
* Community protection can only be achieved if the coverage of the intervention is large enough.
* Community protection is assumed or shown in a limited number of studies, but more evidence is required for confirmation of community protection.

Table 3. Vector control tools and their targets.
stay outdoors when it is dark, and there is no evidence that children who report to stay outdoors during the night are at higher risk for malaria infection as compared to those who do not [120]. Implementing control measures that target outdoor biting mosquitoes in this age group would then provide no additional benefit and would be a waste of resources, as personal protection tools might be very expensive to implement.

Also other tools not relying on the host-vector contact can supplement ITNs and IRS as they are not specific for indoor biting and indoor resting mosquito populations [105,106]. Vector control tools could for example target key environmental resources such as the aquatic larval habitat, sugar sources, and resting behaviour. Very little is known about how to manipulate these environmental resources so that malaria transmission is interrupted [105]. Knowledge on vector ecology and behaviour therefore remains crucial. However, despite large knowledge gaps, several examples exist of malaria control by targeting non-blood meal related steps of the mosquito cycle. Larval source management has indeed shown to be effective where vectors breed in large water bodies [121]. However, when larval habitats are more dispersed and not permanent, this approach is considered less feasible. Renewed attention has been given to larval source management as complementary tool to ITNs as recent studies in Africa have shown that it provides substantial additional protection with a high cost-effectiveness in specific settings [122]. Moreover, other innovative ideas combined with knowledge on the vector behaviour can lead to successful vector control. Toxic sugar baits for example were successfully used in a targeted way for the control of the cistern dwelling malaria vector *An. claviger* in the desert oases of Israel [123]. Fungal biopesticides also have the potential to significantly reduce densities of malaria vectors [124] as well as associated malaria transmission [125]. These fungi could be delivered through outdoor odour-baited stations, and in this way slowly eliminate a high proportion of outdoor-resting vectors [126].

8. Conclusion

For malaria eradication to succeed, all elements in the transmission cycle must be sufficiently targeted. With the current vector control tools, only indoor- and late-biting, and indoor-resting vectors are tackled. In this paper, we have shown that there is a ‘gap’ in protection, not only before sleeping time, but also for people that remain outdoors during the night. Moreover, by describing different shifts in vector species, and vector behaviour within species or species complexes, we have shown that the importance of this gap can increase as a result of widespread ITN or IRS use. Therefore, to eliminate residual malaria transmission, additional vector control tools will be needed. These new vector control tools should be designed to target outdoor and early feeding mosquitoes. Moreover, they should be accessible and acceptable for the populations at risk. A specific mosquito behaviour assuring its vectorial status is only relevant in relation to a specific human behaviour and the relation people have with their surrounding environment. Interrupting malaria transmission may than require different combinations of mosquito control methods addressing each mosquito behaviour at risk for transmission, but also taking into account possible changes in soil occupation, housing conditions, sleeping habits, and outdoor occupation. In conclusion, there is no ‘silver bullet’
in vector control and malaria prevention. New paradigms for controlling and/or interrupting malaria transmission should then be explored for their protective efficacy and adapted to the local context for a good efficiency. Although implementation of such new approaches might be very expensive, they will be crucial if malaria elimination is the final aim.

Acknowledgements

This review was initiated under the impulse of the Roll Back Malaria - Vector Control Working Group – Work Stream Outdoor Malaria Transmission (http://www.rbm.who.int/mechanisms/vcwgWorkstream2.html), the MalaResT research Project (B&M Gates Foundation OPP1032354) and the Third ITM-DGCD Framework Agreement Programme. We would like to thank Vincent Sluydts and Sylvie Manguin for their critical review of this paper.

Author details

Lies Durnez¹ and Marc Coosemans¹,²*

*Address all correspondence to: mcoosemans@itg.be; ldurnez@itg.be

1 Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

2 Department of Biomedical Sciences, Faculty of Pharmaceutical, Veterinary and Biomedical Sciences, University of Antwerp, Antwerpen (Wilrijk), Belgium

References

[1] Killeen, G F, Smith, T A, Ferguson, H M, Mshinda, H, Abdulla, S, Lengeler, C, & Kachur, S P. Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. PLoS Medicine (2007). 4, e229.

[2] WHO. World Malaria Report 2011. World Health Organisation (2012).

[3] Alonso, P L, Brown, G, Arevalo-herrera, M, Binka, F, Chitnis, C, Collins, F, Doumbo, O K, Greenwood, B, Hall, B F, Levine, M M, Mendis, K, Newman, R D, Plowe, C V, Rodriguez, M H, Sinden, R, Slutsker, L, & Tanner, M. A research agenda to underpin malaria eradication. PLoS Medicine (2011). 8, e1000406.

[4] Shililu, J, Ghebremskel, T, Seulu, F, Mengistu, S, Fekadu, H, Zerom, M, Asmelash,G, Sintasath, D, Mbogo, C, Githure, J, Brantly, E, Beier, J, & Novak, R. Seasonal abundance, vector behaviour, and malaria parasite transmission in Eritrea. Journal of the American Mosquito Control Association (2004). 20, 155-64.
[5] Killeen, G F, Kihonda, J, Lyimo, E, Oketch, F R, Kotas, M E, Mathenge, E, Schellenberg, J A, Lengeler, C, Smith, T A, & Drakeley, C J. Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. BMC Infectious Diseases (2006). 6, 161.

[6] Maxwell, C A, Wakibara, J, Tho, S, & Curtis, C F. Malaria-infective biting at different hours of the night. Medical and Veterinary Entomology (1998). 12, 325-7.

[7] Okello, P E, Van Bortel, W, Byaruhanga, A M, Correwyn, A, Roelants, P, Talisuna, A, D’Alessandro, U, & Coosemans, M. Variation in malaria transmission intensity in seven sites throughout Uganda. The American Journal of Tropical Medicine and Hygiene (2006). 75, 219-25.

[8] Van Bortel, W, Trung, H D, Hoi, L X, Van Ham, N, Van Chut, N, Luu, N D, Roelants, P, Denis, L, Speybroeck, N, D’Alessandro, U, & Coosemans, M. Malaria transmission and vector behaviour in a forested malaria focus in central Vietnam and the implications for vector control. Malaria Journal (2010).9, 373.

[9] Durnez, L, Mao, S, Van Bortel, W, Denis, L, Roelants, P, Sochantha, T, & Coosemans, M. Outdoor malaria transmission in forested villages of Cambodia. Manuscript in preparation.

[10] Prakash, A, Bhattacharyya, D, Mohapatra, P, & Mahanta, J. Malaria transmission risk by the mosquito Anopheles baimaii (formerly known as An. dirus species D) at different hours of the night in North-east India. Medical and Veterinary Entomology (2005). 19, 423-7.

[11] Kroeger, A, González, M, & Ordóñez-González, J. Insecticide-treated materials for malaria control in Latin America: to use or not to use? Transactions of the Royal Society of Tropical Medicine and Hygiene (1999). 93, 565-70.

[12] Mouchet, J, & Hamon, J. Difficulties in malaria eradication campaigns due to the behavior of the vectors. Geneva: World Health Organisation (1963). WHO/Mal/394; WHO/Vector Control/35.

[13] Govella, N J, Okumu, F O, & Killeen, G F. Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. The American Journal of Tropical Medicine and Hygiene (2010). 82, 415-9.

[14] Molineaux, L, Shidrawi, G R, Clarke, J L, Boulzaguet, J R, & Ashkar, T S. Assessment of insecticidal impact on the malaria mosquito’s vectorial capacity, from data on the man-biting rate and age-composition. Bulletin of the World Health Organization (1979). 57, 265-74.

[15] Molineaux, L, Shidrawi, G R, Clarke, J L, Boulzaguet, R, Ashkar, T, & Dietz, K. The impact of propoxur on Anopheles gambiae s.l. and some other anopheline populations,
and its relationship with some pre-spraying variables. Bulletin of the World Health Organization (1976). 54, 379-89.

[16] Smits, A, Coosemans, M, Van Bortel, W, Barutwanayo, M, & Delacollette, C. Readjustment of the malaria vector control strategy in the Rusizi Valley, Burundi. Bulletin of Entomological Research (1995). 85, 541-8.

[17] Griffin, J T, Hollingsworth, T D, Okell, L C, Churcher, T S, White, M, Hinsley, W, Bousema, T, Drakeley, C J, Ferguson, N M, Basáñez, M-G, & Ghani, A C. Reducing Plasmodium falciparum malaria transmission in Africa: a model-based evaluation of intervention strategies. PLoS Medicine (2010). 7, e1000324.

[18] Sinka, M E, Bangs, M J, Manguin, S, Coetzee, M, Mbogo, C M, Hemingway, J, Patil, A P, Temperley, W H, Gething, P W, Kabaria, C W, Okara, R M, Van Boeckel, T, Godfray, H C J, Harbach, R E, & Hay, S I. The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasites & Vectors (2010). 3, 117.

[19] Lefèvre, T, Gouagna, L-C, Dabiré, K R, Elguero, E, Fontenille, D, Renaud, F, Costantini, C, & Thomas, F. Beyond nature and nurture: phenotypic plasticity in blood-feeding behaviour of Anopheles gambiae s.s. when humans are not readily accessible. The American Journal of Tropical Medicine and Hygiene (2009). 81, 1023-9.

[20] Sousa, C A, Pinto, J, Almeida, A P, Ferreira, C, Do Rosário, V E, & Charlwood, JD. Dogs as a favored host choice of Anopheles gambiae sensu stricto (Diptera: Culicidae) of São Tomé West Africa. Journal of Medical Entomology (2001). 38, 122-5.

[21] Reddy, M R, Overgaard, H J, Abaga, S, Reddy, V P, Caccone, A, Kiszewski, A E, & Slotman, M A. Outdoor host seeking behaviour of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. Malaria Journal (2011). 10, 184.

[22] Trung, H D, Van Bortel, W, Sochantha, T, Keokenchanh, K, Briët, O J T, & Coosemans, M. Behavioural heterogeneity of Anopheles species in ecologically different localities in Southeast Asia: a challenge for vector control. Tropical Medicine & International Health (2005). 10, 251-62.

[23] Vythilingam, I, Sidavong, B, Chan, S T, Phonemixay, T, Vanisaveth, V, Sisoulad, P, Phetsouvanh, R, Hakim, S L, & Phompida, S. Epidemiology of malaria in Attapeu Province, Lao PDR in relation to entomological parameters. Transactions of the Royal Society of Tropical Medicine and Hygiene (2005). 99, 833-9.

[24] Obsomer, V, Defourny, P, & Coosemans, M. The Anopheles dirus complex: spatial distribution and environmental drivers. Malaria Journal (2007). 6, 26.

[25] Sinka, M E, Rubio-Palis, Y, Manguin, S, Patil, A P, Temperley, W H, Gething, P W, Van Boeckel, T, Kabaria, C W, Harbach, R E, & Hay, S I. The dominant Anopheles...
vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. Parasites & Vectors (2010). 3, 72.

[26] Hiwat, H, Issaly, J, Gaborit, P, Somai, A, Samjhawan, A, Sardjoe, P, Soekhoe, T, & Girod, R. Behavioural heterogeneity of Anopheles darlingi (Diptera: Culicidae) and malaria transmission dynamics along the Maroni River, Suriname, French Guiana. Transactions of the Royal Society of Tropical Medicine and Hygiene (2010). 104, 207-13.

[27] Moutinho, P R, Gil, LHS, Cruz, R B, & Ribolla, P E M. Population dynamics, structure and behaviour of Anopheles darlingi in a rural settlement in the Amazon rainforest of Acre, Brazil. Malaria Journal (2011). 10, 174.

[28] Girod, R, Gaborit, P, Carinci, R, Issaly, J, & Fouque, F. Anopheles darlingi bionomics and transmission of Plasmodium falciparum, Plasmodium vivax and Plasmodium malariae in Amerindian villages of the Upper-Maroni Amazonian forest, French Guiana. Memórias Do Instituto Oswaldo Cruz (2008). 103, 702-10.

[29] Muirhead-Thomson, R C. The significance of irritability, behaviouristic avoidance and allied phenomena in malaria eradication. Bulletin of the World Health Organization (1960). 22, 721-34.

[30] Grieco, J P, Achee, N L, Chareonviriyaphap, T, Suwonkerd, W, Chauhan, K, Sardelis, M R, & Roberts, D R. A new classification system for the actions of IRS chemicals traditionally used for malaria control. PloS One (2007). 2, e716.

[31] Siegert, P Y, Walker, E, & Miller, J R. Differential behavioural responses of Anopheles gambiae (Diptera: Culicidae) modulate mortality caused by pyrethroid-treated bed-nets. Journal of Economic Entomology (2009). 102, 2061-71.

[32] Badyaev, A V. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proceedings. Biological Sciences / The Royal Society (2005). 272, 877-86.

[33] Pothikasikorn, J, Chareonviriyaphap, T, Bangs, M J, & Prabaripai, A. Behavioural responses to DDT and pyrethroids between Anopheles minimus species A and C, malaria vectors in Thailand. The American Journal of Tropical Medicine and Hygiene (2005). 73, 343-9.

[34] White, G. Blood feeding habits of malaria vector mosquitoes in the South Pare district of Tanzania 10 years after cessation of a dieldrin residual spraying campaign. Geneva: World Health Organisation (1969). WHO/MAL/69.684 – WHO/VBC/69.144.

[35] Gillies, M, & Smith, A. The effect of a residual house-spraying campaign in East Africa on species balance in the Anopheles funestus group. The replacement of A. funestus Giles by A. rivulorum Leeson. Bulletin of Entomological Research (1960). 51, 243-52.
[36] Gillies, M, & Furlong, M. An investigation into the behaviour of *Anopheles parensis* Gillies at Malindi on the Kenya coast. Bulletin of Entomological Research (1963). 55, 1-16.

[37] Labbo, R, Czeher, C, Djibrila, A, Arzika, I, Jeanne, I, & Duchemin, J-B. Longitudinal follow-up of malaria transmission dynamics in two villages in a Sahelian area of Niger during a nationwide insecticide-treated bednet distribution programme. Medical and Veterinary Entomology (2012). 26, 386-95.

[38] Iyengar, R. The bionomics of salt-water *Anopheles gambiae* in East Africa. Bulletin of the World Health Organization (1962). 27, 223-9.

[39] Lindblade, K, Gimnig, J, Kamau, L, Hawley, W, Odhiambo, F, Olang, G, TerKuile, F O, Vulule, J, & Slutsker L. Impact of sustained use of insecticide-treated bednets on malaria vector species distribution and culicine mosquitoes. Journal of Medical Entomology (2006). 43, 428-32.

[40] Mutuku, F M, King, C H, Mungai, P, Mbogo, C, Mwangangi, J, Muchiri, E M, Walker, E D, & Kitron, U. Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. Malaria Journal (2011). 10, 356.

[41] Bayoh, M N, Mathias, D K, Odiere, M R, Mutuku, F M, Kamau, L, Gimnig, J E, Vulule, J M, Hawley, W A, Hamel, M J, & Walker, E D. *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bednets in western Nyanza Province, Kenya. Malaria Journal (2010). 9, 62.

[42] Russell, T L, Govella, N J, Azizi, S, Drakeley, C J, Kachur, S P, & Killeen, G F. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malaria Journal (2011). 10, 80.

[43] Mathias, D K, Ochomo, E, Atieli, F, Ombok, M, Bayoh, M N, Olang, G, Muhia, D, Kamau, L, Vulule, J M, Hamel, M J, Hawley, W A, Walker, E D, & Gimnig, J E. Spatial and temporal variation in the kdr allele L1014S in *Anopheles gambiae* s.s. and phenotypic variability in susceptibility to insecticides in Western Kenya. Malaria Journal (2011). 10, 10.

[44] Mathenge, E M, Gimnig, J E, Kolczak, M, Ombok, M, Irungu, L W, & Hawley, W A. Effect of permethrin-impregnated nets on exiting behaviour, blood feeding success, and time of feeding of malaria mosquitoes (Diptera: Culicidae) in western Kenya. Journal of Medical Entomology (2001). 38, 531-6.

[45] Derua, Y A, Alifrangis, M, Hosea, K M, Meyrowitsch, D W, Magesa, S M, Pedersen, E M, & Simonsen, P E. Change in composition of the *Anopheles gambiae* complex and its possible implications for the transmission of malaria and lymphatic filariasis in northeastern Tanzania. Malaria Journal (2012). 11, 188.

[46] Braimah, N, Drakeley, C, Kweka, E, Mosha, F, Helinski, M, Pates, H, Maxwell, C, Massawe, T, Kenward, M G, & Curtis, C. Tests of bednet traps (Mbita traps) for mon-
itoring mosquito populations and time of biting in Tanzania and possible impact of prolonged insecticide treated net use. International Journal of Tropical Insect Science (2005). 25, 208-13.

[47] Moiroux, N, Gomez, M B, Pennetier, C, Elanga, E, Djènontin, A, Chandre, F, Djègbé, I, Guis, H, & Corbel, V. Changes in Anopheles funestus biting behaviour following universal coverage of Long-Lasting Insecticidal Nets in Benin. The Journal of Infectious Diseases (2012). 206, 1622-9.

[48] Mbogo, C N, Baya, N M, Ofulla, A V, Githure, J I, & Snow, R W. The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. Medical and Veterinary Entomology (1996). 10, 251-9.

[49] Njau, R, Mosha, F, & Nguma, J. Field trials of pyrethroid impregnated bednets in northern Tanzania 1. Effect on malaria transmission. International Journal of Tropical Insect Science (1993). 14, 575-84.

[50] Magesa, S M, Wilkes, T J, Mnzava, A E, Njunwa, K J, Myamba, J, Kivuyo, M D, Hill, N, Lines, J D, & Curtis, C F. Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. Acta Tropica (1991). 49, 97-108.

[51] Quiñones, M L, Lines, J D, Thomson, M C, Jawara, M, Morris, J, & Greenwood, B M. Anopheles gambiae gonotrophic cycle duration, biting and exiting behaviour unaffected by permethrin-impregnated bednets in The Gambia. Medical and Veterinary Entomology (1997). 11, 71-8.

[52] Molineaux, L, & Gramiccia, G. The Garki Project: Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa. World Health Organisation (1980), 331pp.

[53] Ilboudo-Sanogo, E, Cuzin-Ouattara, N, Diallo, D A, Cousens, S N, Esposito, F, Habbuetzel, A, Sanon, S, & Ouédraogo, A P. Insecticide-treated materials, mosquito adaptation and mass effect: entomological observations after five years of vector control in Burkina Faso. Transactions of the Royal Society of Tropical Medicine and Hygiene (2001). 95, 353-60.

[54] Bøgh, C, Pedersen, E M, Mukoko, D A, & Ouma, J H. Permethrin-impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. Medical and Veterinary Entomology (1998). 12, 52-9.

[55] Lindsay, S W, Alonso, P L, Armstrong Schellenberg, JR, Hemingway, J, Adiamah, J H, Shenton, FC, Jawara, M, & Greenwood, B M. A malaria control trial using insecticide treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, west-Africa. 7. Impact of permethrin-impregnated bed nets on malaria vectors. Transactions of the Royal Society of Tropical Medicine and Hygiene (1993). Suppl 2, 45-51.
[56] Fornadel, C M, Norris, L C, Glass, G E, & Norris, D E. Analysis of *Anopheles arabiensis* blood feeding behaviour in southern Zambia during the two years after introduction of insecticide-treated bed nets. The American Journal of Tropical Medicine and Hygiene (2010). 83, 848-53.

[57] White, G. *Anopheles gambiae* complex and disease transmission in Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene (1974). 68, 278-301.

[58] Taylor, B. Changes in the feeding behaviour of a malaria vector, *Anopheles farauti* Lav., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. Transactions of the Royal Entomological Society of London (1975). 127, 277-92.

[59] Bugoro, H, Iro’ofa C, Mackenzie, D O, Apairamo, A, Hevalao, W, Corcoran, S, Bobogare, A, Beebe, N W, Russell, T L, Chen, C-C, & Cooper, R D. Changes in vector species composition and current vector biology and behaviour will favour malaria elimination in Santa Isabel Province, Solomon Islands. Malaria Journal (2011). 10, 287.

[60] Ismail, I A, Notananda, V, & Schepens, J. Studies on malaria and responses of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT residual spraying in Thailand. Acta Tropica (1975). 32, 206-31.

[61] Nustsathapana, S, Sawasdiwongphorn, P, Chitprarop, U, & Cullen, J R. The behavior of *Anopheles minimus* Theobald (Diptera: Culicidae) subjected to differing levels of DDT selection pressure in northern Thailand. Bulletin of Entomological Research (1986). 76, 303-12.

[62] Garros, C, Marchand, R P, Quang, N T, Hai, N S, & Manguin, S. First record of *Anopheles minimus* C and significant decrease of *An. minimus* A in central Vietnam. Journal of the American Mosquito Control Association (2005). 21, 139-43.

[63] Reisen, W K, Pradhan, S P, Shrestha, J P, Shrestha, S L, Vaidya, R G, & Shrestha, J D. Anopheline mosquito (Diptera: Culicidae) ecology in relation to malaria transmission in the inner and outer terai of Nepal, 1987-1989. Journal of Medical Entomology (1993). 30, 664-82.

[64] Li, Z Z, Zhang, M C, Wus, Y G, Zhong, B L, Lin, G Y, & Huang, H. Trial of deltamethrin impregnated bed nets for the control of malaria transmitted by *Anopheles sinensis* and *Anopheles anthropophagus*. The American Journal of Tropical Medicine and Hygiene (1989). 40, 356-9.

[65] Zhang, Z, & Yang, C. Application of deltamethrin-impregnated bednets for mosquito and malaria control in Yunnan, China. The Southeast Asian Journal of Tropical Medicine and Public Health (1996). 27, 367-71.
[66] Giglioli, G. Nation-wide malaria eradication projects in the Americas III. Eradication of Anopheles darlingi from the inhabited areas of British Guiana by DDT residual spraying. Journal of the National Malaria Society (1951). 10, 142-61.

[67] Richards, F, Flores, R Z, Sexton, J D, Beach, R F, Mount, D L, Cordon-Rosales, C, Gatica, M, & Klein, R E. Effects of permethrin-impregnated bed nets on malaria vectors of Northern Guatemala. Bulletin of PAHO (1994). 28, 112-21.

[68] Charlwood, J D, & Graves, P M. The effect of permethrin-impregnated bednets on a population of Anopheles farauti in coastal Papua New Guinea. Medical and Veterinary Entomology (1987). 1, 319-27.

[69] Hii, J L, Birley, M H, Kanai, L, Foligeli, A, & Wagner, J. Comparative effects of permethrin-impregnated bednets and DDT house spraying on survival rates and oviposition interval of Anopheles farauti (Diptera: Culicidae) in Solomon Islands. Annals of Tropical Medicine and Parasitology (1995). 89(1), 521-9.

[70] Bugoro, H, Cooper, R D, Butafa, C, Iro’ofa, C, Mackenzie, DO, Chen, C-C, & Russell, T L. Bionomics of the malaria vector Anopheles farauti in Temotu Province, Solomon Islands: issues for malaria elimination. Malaria Journal (2011). 10, 133.

[71] Ismail, I A, Phinichpongse, S, & Boonrasri, P. Responses of Anopheles minimus to DDT residual spraying in a cleared forested foothill area in central Thailand. Acta Tropica (1978). 35, 69-82.

[72] Lourenço-de-Oliveira, R, Guimarães, A E, Arlé, M, Da Silva, T F, Castro, M G, Motta, M A, & Deane, L M. Anopheline species, some of their habits and relation to malaria in endemic areas of Rondônia State, Amazon region of Brazil. Memórias Do Instituto Oswaldo Cruz (1989). 84, 501-14.

[73] Harris, A F, Matias-Arnéz, A, & Hill, N. Biting time of Anopheles darlingi in the Bolivian Amazon and implications for control of malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene (2006). 100, 45-7.

[74] Suwonkerd, W, Amg-Ung, B, Rimwangtrakul, K, Wongkattiyakul, S, Kattiymongkoel, B, Chitprarop, U, & Takagi, M. A Field Study on the Response of Anopheles dirus to DDT and Fenitrothion Sprayed to Huts in Phetchabun Province, Thailand. Tropical Medicine (Nagasaki) (1990). 32, 1-5.

[75] Pongvongsa, T, Ha, H, Thanh, L, Marchand, R P, Nonaka, D, Tojo, B, Phongmany, P, Moji, K, & Kobayashi, J. Joint malaria surveys lead towards improved cross-border cooperation between Savannakhet province, Laos and Quang Tri province, Vietnam. Malaria Journal (2012). 11, 262.

[76] Elliott, R. Studies on man vector contact in some malarious areas in Colombia. Bulletin of the World Health Organization (1968). 38, 239-53.

[77] Hiwat, H, Mitro, S, Samjhawan, A, Sardjoe, P, Soekhoe, T, & Takken, W. Collapse of Anopheles darlingi populations in Suriname after introduction of insecticide-treated
nets (ITNs); malaria down to near elimination level. The American Journal of Tropical Medicine and Hygiene (2012). 86, 649-55.

[78] Sampath, T R, Yadav, R S, Sharma, V P, & Adak, T. Evaluation of lambdacyhalothrin impregnated bednets in a malaria endemic area of India. Part 2. Impact on malaria vectors. Journal of the American Mosquito Control Association (1998). 14, 437-43.

[79] Loyola, E G, Rodríguez, M H, González, L, Arredondo-Jimenez, J I, Bown, D N, & Vaca, M A. Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of Anopheles pseudopunctipennis in Mexico. Journal of the American Mosquito Control Association (1990). 6, 635-40.

[80] Giglioli, G. Biological variations in Anopheles darlingi and Anopheles gambiae; their effect on practical malaria control in the neotropical region. Bulletin of the World-Health Organization (1956). 15, 461-71.

[81] Barai, D, Hyma, B, & Ramesh, A. The scope and limitations of insecticide spraying in rural vector control programmes in the states of Karnataka and Tamil Nadu in India. Ecology of Disease (1982). 1, 243-55.

[82] Martinez-Palacios, A, & De Zulueta, J. Ethological changes in Anopheles pseudopunctipennis in Mexico after prolonged use of DDT. Nature (1964). 203, 940-1.

[83] Kitau, J, Oxborough, R M, Tungu, P K, Matowo, J, Malima, R C, Magesa, S M, Bruce, J, Mosha, F W, & Rowland, M W. Species shifts in the Anopheles gambiae complex: do LLINs successfully control Anopheles arabiensis? PloS One (2012). 7, e31481.

[84] Ayala, D, Fontaine, M C, Cohuet, A, Fontenille, D, Vitalis, R, & Simard, F. Chromosomal inversions, natural selection and adaptation in the malaria vector Anopheles funestus. Molecular Biology and Evolution (2011). 28, 745-58.

[85] Coluzzi, M, Sabatini, A, Petrarca, V, & Di Deco, M A. Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the Anopheles gambiae complex. Nature (1977). 266, 832-3.

[86] Coluzzi, M, Sabatini, A, Petrarca, V, & Di Deco MA. Chromosomal differentiation and adaptation to human environments in the Anopheles gambiae complex. Transactions of the Royal Society of Tropical Medicine and Hygiene (1979). 73, 483-97.

[87] Coluzzi, M. Malaria vector analysis and control. Parasitology Today (1992). 8, 113-8.

[88] White, G B. Biological effects of intraspecific chromosomal polymorphism in malaria vector populations. Bulletin of the World Health Organization (1974). 50, 299-306.

[89] Jones, M. Inversion polymorphism and circadian flight activity in the mosquito Anopheles stephensi List. (Diptera, Culicidae). Bulletin of Entomological Research (1974). 64, 305-11.

[90] Hii, J L, Chew, M, Sang, V Y, Munstermann, L E, Tan, S G, Panyim, S, & Yasoothornsrikul, S. Population genetic analysis of host seeking and resting behaviours in the
malaria vector, *Anopheles balabacensis* (Diptera: Culicidae). Journal of Medical Entomology (1991). 28, 675-84.

[91] Lines, J D, Lyimo, E O, & Curtis, C F. Mixing of indoor- and outdoor-resting adults of *Anopheles gambiae* Giles s.l. and *A. funestus* Giles (Diptera: Culicidae) in coastal Tanzania. Bulletin of Entomological Research (2009). 76, 171.

[92] Smits, A, Roelants, P, Van Bortel, W, & Coosemans, M. Enzyme polymorphisms in the *Anopheles gambiae* (Diptera: Culicidae) complex related to feeding and resting behaviour in the Imbo Valley, Burundi. Journal of Medical Entomology (1996). 33,545-53.

[93] Coosemans, M, Smits, A, & Roelants, P. Intraspecific isozyme polymorphism of *Anopheles gambiae* in relation to environment, behaviour, and malaria transmission in southwestern Burkina Faso. The American Journal of Tropical Medicine and Hygiene (1998). 58, 70-4.

[94] Sinka, M E, Bangs, M J, Manguin, S, Chareonviriyaphap, T, Patil, A P, Temperley, W H, Gething, P W Elyazar, I R F, Kabaria, C W, Harbach, R E, & Hay, S I. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionic précís. Parasites & Vectors (2011). 4, 89.

[95] Limrat, D, Rojruthai, B, Apiwathnasorn, C, Samung, Y, & Prommongkol, S. *Anopheles barbirostris /campestris* as a probable vector of malaria in Aranyaprathet, Sa Kaeo Province. The Southeast Asian Journal of Tropical Medicine and Public Health (2001). 32,739-44.

[96] Giglioli, G. Ecological change as a factor in renewed malaria transmission in an eradicated area. A localized outbreak of *A. aquasalis*-transmitted malaria on the Demerara river estuary, British Guiana, in the fifteenth year of *A. darlingi* and malaria eradication. Bulletin of the World Health Organization (1963). 29, 131-45.

[97] Lengeler, C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database of Systematic Reviews (2004). 2, CD0003632009.

[98] Pluess, B, Tanscer, F C, Lengeler, C, & Sharp, B L. Indoor residual spraying for preventing malaria. Cochrane Database of Systematic Reviews (2010). 4, CD006657.

[99] Jana-Kara, B R, Jihullah, W A, Shahi, B, Dev, V, Curtis, C F, & Sharma, V P. Delta-methrin impregnated bednets against *Anopheles minimus* transmitted malaria in Assam, India. The Journal of Tropical Medicine and Hygiene (1995). 98, 73-83.

[100] Kobayashi, J, Phompida, S, Toma, T, Looareesuwan, S, Toma, H, & Miyagi, I. The effectiveness of impregnated bed net in malaria control in Laos. Acta Tropica (2004). 89, 299-308.

[101] Seyoum, A, Sikaala, C H, Chanda, J, Chinula, D, Ntamatungiro, A J, Hawela, M, Miller, J M, Russell, T L, Briët, O J, & Killeen, G F. Human exposure to anopheline mos-
quitoes occurs primarily indoors, even for users of insecticide-treated nets in Luangwa Valley, South-east Zambia. Parasites & Vectors (2012). 5, 101.

[102] Charlwood, J D, Alcântara, J, Pinto, J, Sousa, C A, Rompão, H, Gil, V, & Rosário, V E. Do bednets reduce malaria transmission by exophagic mosquitoes? Transactions of the Royal Society of Tropical Medicine and Hygiene (2005). 99, 901-4.

[103] Govella, N J, & Ferguson, H. Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination? Frontiers in Physiology (2012).3, 199.

[104] Kiware, S S, Chitnis, N, Devine, G J, Moore, S J, Majambere, S, & Killeen, G F. Biologically meaningful coverage indicators for eliminating malaria transmission. Biology Letters (2012). 8, 874-877.

[105] Ferguson, H M, Dornhaus, A, Beeche, A, Borgemeister, C, Gottlieb, M, Mulla, M S, Gimnig, J E, Fish, D, & Killeen, G F. Ecology: a prerequisite for malaria elimination and eradication. PLoS Medicine (2010). 7, e1000303.

[106] The malERA Consultative Group on Vector Control. A research agenda for malaria eradication: vector control. PLoS Medicine (2011). 8, e1000401.

[107] Hill, N, Lenglet, A, Arnéz, A M, & Carneiro, I. Plant based insect repellent and insecticide treated bed nets to protect against malaria in areas of early evening biting vectors: double blind randomised placebo controlled clinical trial in the Bolivian Amazon. BMJ (Clinical Research Ed.) (2007).335, 1023.

[108] Dutta, P, Khan, A M, Khan, S A, Borah, J, Sharma, C K, & Mahanta, J. Malaria control in a forest fringe area of Assam, India: a pilot study. Transactions of the Royal Society of Tropical Medicine and Hygiene (2011). 105, 327-32.

[109] Rowland, M, Downey, G, Rab, A, Freeman, T, Mohammad, N, Rehman, H, Durrani, N, Reyburn, H, Curtis, C, Lines, J, & Fayaz, M. DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. Tropical Medicine & International Health (2004). 9, 335-42.

[110] Kimani, E W, Vulule, J M, Kuria, I W, & Mugisha, F. Use of insecticide-treated clothes for personal protection against malaria: a community trial. Malaria Journal (2006). 5, 63.

[111] Thang, N D, Erhart, A, Speybroeck, N, Xa, N X, Thanh, N N, Van Ky, P, Hung, L X, Thuan, L K, Coosemans, M, & D’ Alessandro, U. Long-lasting Insecticidal Hammocks for controlling forest malaria: a community-based trial in a rural area of central Vietnam. PloS One (2009). 4, e7369.

[112] Sochantha, T, Van Bortel, W, Savonnaroth, S, Marcotty, T, Speybroeck, N, & Coosemans, M. Personal protection by long-lasting insecticidal hammocks against the bites of forest malaria vectors. Tropical Medicine & International Health (2010). 15,336-41.
[113] Habtewold, T, Prior, A, Torr, S J, & Gibson, G. Could insecticide-treated cattle reduce Afrotropical malaria transmission? Effects of deltamethrin-treated Zebu on *Anopheles arabiensis* behaviour and survival in Ethiopia. Medical and Veterinary Entomology (2004). 18, 408-17.

[114] Coosemans, M, & Van Bortel, W. Malaria Vectors in the Mekong Countries: a Complex Interaction between Vectors, Environment and Human Behaviour. In: International Conference Hubs, Harbours and Deltas in Southeast Asia. Royal Academy of Overseas Sciences (2006). 551-569.

[115] Burns, M, Rowland, M, Guessan, R, Carneiro, I, Beeche, A, Ruiz, S S, Kamara, S, Takken, W, Carnevale, P, & Allan, R. Insecticide-treated plastic sheeting for emergency malaria prevention and shelter among displaced populations: an observational cohort study in a refugee setting in Sierra Leone. The American Journal of Tropical Medicine and Hygiene (2012). 87, 242-50.

[116] Kiware, S S, Chitnis, N, Moore, S J, Devine, G J, Majambere, S, Merrill, S, & Killeen, G F. Simplified models of vector control impact upon malaria transmission by zoophasic mosquitoes. PloS One (2012). 7, e37661.

[117] Charlwood, J D, Pinto, J, Ferrara, P R, Sousa, C A, Ferreira, C, Gil, V, & Do Rosário V E. Raised houses reduce mosquito bites. Malaria Journal (2003). 2, 45.

[118] Somboon, P, Lines, J, Aramrattana, A, Chitrarotrop, U, & Prajakwong, S. Entomological impregnated evaluation of community-wide use of lambdacyhalothrin-bed nets against malaria in a border area of north-west Thailand. Transactions of the Royal Society of Tropical Medicine and Hygiene (1995). 89, 248-54.

[119] Grietens, K P, Xuan, X N, Ribera, J M, Duc, T N, Van Bortel, W, Ba, N T, Van, K P, Xuan, H L, D’Alessandro, U, & Erhart, A. Social Determinants of Long Lasting Insecticidal Hammock-Use Among the Ra-Glai Ethnic Minority in Vietnam: Implications for Forest Malaria Control. PLoS One (2012). 7, e29991.

[120] Bradley, J, Matias, A, Schwabe, C, Vargas, D, Monti, F, Nseng, G, & Kleinschmidt, I. Increased risks of malaria due to limited residual life of insecticide and outdoor biting versus protection by combined use of nets and indoor residual spraying on Bioko Island, Equatorial Guinea. Malaria Journal (2012). 11, 242.

[121] Fillinger, U, & Lindsay, S W. Larval source management for malaria control in Africa: myths and reality. Malaria Journal (2011). 10, 353.

[122] Worrall, E, & Fillinger, U. Large-scale use of mosquito larval source management for malaria control in Africa: a cost analysis. Malaria Journal (2011). 10, 338.

[123] Müller, G C, & Schlein, Y. Efficacy of toxic sugar baits against adult cistern-dwelling *Anopheles claviger*. Transactions of the Royal Society of Tropical Medicine and Hygiene (2008). 102, 480-4.
[124] Scholte, E-J, Ng’habi, K, Kihonda, J, Takken, W, Paaijmans, K, Abdulla, S, Killeen, GF, & Knols, BGJ. An entomopathogenic fungus for control of adult African malaria mosquitoes. Science (2005). 308, 1641-2.

[125] Blanford, S, Chan, BHK, Jenkins, N, Sim, D, Turner, RJ, Read, AF, & Thomas, MB. Fungal pathogen reduces potential for malaria transmission. Science (2005). 308, 1638-41.

[126] Lwetoijera, D W, Sumaye, R D, Madumla, E P, Kavishe, D R, Mnyone, L L, Russell, T L, & Okumu, F O. An extra-domiciliary method of delivering entomopathogenic fungus, *Metharizium anisopliae* IP 46 for controlling adult populations of the malaria vector, *Anopheles arabiensis*. Parasites & Vectors (2010). 3, 18.