11. Heterogeneity in malaria transmission: underlying factors and implications for disease control

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Abstract

In this chapter, we describe the current evidence for the existence of hotspots of malaria transmission. Heterogeneity is a common element of many infectious diseases, whereby infection and disease are concentrated in a small proportion of individuals and not distributed evenly across the population. In malaria this heterogeneity is manifested as small groups of households, or hotspots, within malaria endemic communities that are at a substantially increased risk of malaria transmission compared to surrounding households. These hotspots exist in all transmission settings, but are most easily detected at low transmission. The ecological, human and entomological factors that influence the occurrence of hotspots are currently not fully understood. Human genetic components are strongly related to the risk of (severe) clinical disease but their role in determining the location and intensity of hotspots remains uncertain. The roles of factors related to mosquito exposure are more apparent in defining geographical patterns in transmission intensity. The importance for malaria control and elimination lies in the fact that hotspots maintain transmission in low transmission seasons, representing the source of infection to the general community when vector densities increase. Hotspots of malaria transmission thereby form small geographical areas where malaria transmission is more intense and from where malaria may spread to the remainder of the community. Interventions targeted to hotspots of malaria transmission hold promise to reduce transmission intensity in the community as a whole. Before hotspots can be targeted, operationally attractive approaches to identify them need to be defined. Some of these approaches are described in this chapter together with a tool-box for hotspot-targeted interventions.

Keywords: *Plasmodium* falciparum, heterogeneity, hotspots, elimination, transmission, Anopheles

Spatial variation in malaria incidence

The occurrence of clinical malaria attacks is not equally distributed in space in time. In many endemic regions, malaria incidence shows striking spatio-temporal variation. The temporal variation in malaria exposure is well described for areas of seasonal malaria transmission. The extent of seasonality differs between regions and can be quantified as the proportion of all malaria episodes occurring in the peak transmission season. Marked seasonality can be defined as a transmission pattern in which more than 75% of malaria episodes occur in 6 or fewer months (Roca-Feltrer *et al.* 2009); in some areas ~90% of all malaria episodes may occur in a period as short as 3 months (Giha *et al.* 2000). Malaria transmission is practically absent in months when drought and/or temperatures are less suitable for mosquito propagation or the development of malaria parasites inside their mosquito vectors (Giha *et al.* 2000, John *et al.* 2009, Shililu *et al.* 2003).

In addition to this variation in time, geographical variation can be very pronounced. Spatial variation in malaria exposure exists between neighbouring villages (Bejon *et al.* 2010, Bousema *et al.* 2007, Kreuels *et al.* 2008) or sub-villages (Bousema *et al.* 2010a, Drakeley *et al.* 2003) and even between households in the same village (Bejon *et al.* 2010, Bousema *et al.* 2010a, Gaudart *et al.* 2006, Ghebreyesus *et al.* 1999). In longitudinal studies where the incidence of clinical malaria

attacks is quantified at an individual level, it is commonly observed that some individuals can remain malaria-free for more than one year while others experience multiple malaria attacks during the same period (Bousema *et al.* 2010a, Clark *et al.* 2008, Mwangi *et al.* 2008). In an area of 16 km^2 exposed to low and unstable malaria transmission in the highlands of Kenya, small geographical areas were identified where malaria risk was up to 40-fold higher than elsewhere (Ernst *et al.* 2006). In studies conducted in areas of low to moderate endemicity in Uganda and Tanzania, 47-69% of children remained malaria free during a period of >20 months while others experienced up to 9-14 malaria attacks (Bousema *et al.* 2010a, Clark *et al.* 2008).

It has long been assumed that this heterogeneity is absent or at least far less evident in areas of higher endemicity (Carter *et al.* 2000). It is, however, likely that even in highly endemic regions considerable variation in the exposure to malaria infected mosquitoes exists (Carter *et al.* 2000, Kreuels *et al.* 2008, Trape *et al.* 2002). This heterogeneity in exposure may not lead to easily detectable variation in malaria incidence in these regions, because many infections in high endemic regions do not cause clinical symptoms (Males *et al.* 2008, Proietti *et al.* 2011) and even the individuals with the lowest relative exposure may experience at least one malaria episode in a year (Trape *et al.* 2002). As a consequence, variation in malaria exposure within these regions may remain undetected. Despite this, micro-epidemiological variations in disease risk can be detected in areas with an entomological inoculation rate (EIR) of >100 infected bites per person per year (ibpy) if studies prospectively quantify malaria incidence, or the incidence of malaria infections regardless of symptoms (Bousema *et al.* 2011, Gaudart *et al.* 2006), and link incidence to individual geo-located households. In a region of intense malaria transmission in Ghana (EIR ~400 ibpy), some children experienced malaria attacks at a rate that was five-fold higher than the village average while one-third of children remained malaria-free over a period of 21 months (Kreuels *et al.* 2008). Our current understanding is that spatial variation in malaria exposure is present across all levels of transmission intensity but is more easily recognised at lower endemicity.

Defining hotspots of malaria transmission

Global trends of reducing malaria transmission intensity (Barnes *et al.* 2009, Bhattarai *et al.* 2007, Ceesay *et al.* 2008, Kleinschmidt *et al.* 2009, O'Meara *et al.* 2008), have changed the epidemiology of malaria. These changes have uncovered heterogeneity in disease transmission in areas that were previously exposed to intense and apparently homogeneous malaria transmission. In these areas, small localities of intense transmission intensity can exist (or persist) in regions with a lower average level of malaria exposure (Bejon *et al.* 2010, Bousema *et al.* 2010a). These findings have fuelled the academic and public health interest in defining hotspots of malaria transmission intensity (Dolgin 2010). For this, it is essential to define what is meant by *hotspots of malaria transmission*. The current literature is inconsistent; entire countries or islands are sometimes classified as malaria hotspots (Singh *et al.* 2009, Toty *et al.* 2010), while some studies reserve the term hotspots of malaria transmission for small geographical areas that form part of a larger endemic region (Bejon *et al.* 2010, 2011a, Bousema *et al.* 2010a). Two related but distinct geographical elements in malaria transmission should be separated:

1. The World Health Organization definition of a *focus of malaria transmission* is a defined and circumscribed locality situated in a currently or former malarious area and containing the continuous or intermittent epidemiological factors necessary for malaria transmission. Foci of malaria transmission can be classified as residual active, residual non-active, cleared up, new potential, new active, endemic or pseudo foci (World Health 2007). In more academic terms, an active focus of malaria transmission is a geographical area that supports malaria transmission, where the local *Anopheles* population sustains R_0, the average number of secondary cases

arising in a susceptible population as a result of a single human case over the course of their malaria infection, to a level >1 (Bousema *et al.* 2010a, Carter *et al.* 2000). A mosquito breeding site forms the centre of a focus of malaria transmission. The size of the focus of malaria transmission depends on the maximum effective dispersal range of vector mosquitoes; the border is the location furthest away from the breeding site where malaria is still supported by this breeding site.

2. A *hotspot of malaria transmission* is defined as a geographical part of a focus of malaria transmission where malaria transmission exceeds the average level. Micro-epidemiological conditions for malaria transmission are favourable in a hotspot of malaria transmission, resulting in R_0 estimates that exceed the average for the focus of malaria transmission. The size of a hotspot of malaria transmission is smaller than the maximum dispersal range of vector mosquitoes; its borders are defined by the distance from the centre of the hotspot where transmission intensity is no longer (statistically significantly) higher than the average for the focus of malaria transmission (Bejon *et al.* 2010, Bousema *et al.* 2010a).

Human factors and heterogeneity in malaria transmission

The occurrence of clinical malaria and, to a lesser extent, asymptomatic infection is influenced by innate and acquired protective responses. Several human genetic polymorphisms have been identified that have been associated with protection against (severe) clinical malaria An additional aspect of human genetic polymorphisms that is receiving increasing attention is their potential impact on gametocyte carriage and the transmission of malaria to mosquitoes (Gouagna *et al.* 2010, Robert *et al.* 1996). This results in a double interest in human genetic polymorphisms in the context of heterogeneity in malaria transmission: they may explain part of the variation in malaria incidence and may also result in differences in malaria transmission potential in the human population. Their impact on malaria incidence may influence the accuracy of detecting hotspots of malaria transmission (see paragraph on operational approaches to detecting hotspots of malaria transmission), their impact on transmission potential may contribute to the formation of hotspots of malaria transmission.

Genetic factors related to human susceptibility to malaria infection and clinical malaria

The malaria parasite depends on human red blood cells (RBC) for shelter and the provision of nutrients for the duration of its infection in humans. It is therefore logical that it is sensitive to variations in RBCs. Over one hundred RBC-related genetic polymorphisms have been described, several of which have well described effects on the erythrocyte phenotype (Min-Oo and Gros 2005). For haemoglobin alterations affecting the β-chains (e.g. HbS, HbC and HbE) or conditions in which the balance between α and β chains is altered (e.g. α- and β-thalassaemia), there is sufficient evidence for a role in protection against (severe) malaria. The same is true for alterations in levels of a key enzyme in red blood cells, glucose-6-phosphate deficiency, and structural alterations leading to physical structural changes on the RBC membrane like the Duffy blood group (Kwiatkowski 2005). With the exception of the Duffy blood group, none of these polymorphisms seem to protect against initial infection with malaria but rather convey ways to keep the infection controlled and prevent progression to high density infections or severe disease. Although these genetic polymorphisms have direct beneficial effects for the human host, their epidemiological consequences for malaria transmission may not necessarily be equally beneficial.

Haemoglobinopathies: haemoglobin variants S, C and E

Haemoglobin (Hb) is a tetrameric molecule which comprises one of the main structural and functional elements of erythrocytes. It consists of two α-chains and two β-chains. In an altered form of haemoglobin, which is described as haemoglobin S (HbS) a mutation occurs which leads to the modification of one or both β-chains. Heterozygote carriers (HbAS) have one normal and one altered β-chain. Their red blood cells function relatively normal. This contrasts with RBCs of homozygous individuals (HbSS) in whom both β-chains are abnormal; HbSS cells assume a typical sickle shape under low oxygen conditions which leads to increased cell lysis and obstruction in the micro-vascular system. In countries where advanced medical care lacks HbSS homozygote individuals often die during early childhood. HbAS does not have this detrimental effect but protects against severe clinical consequences of malaria infection, but not against infection itself (Williams 2006). HbAS individuals may have a 90% lower risk of severe and lethal malaria compared to normal (HbAA) individuals (Aidoo *et al.* 2002, Williams *et al.* 2005b). Infected HbAS cells, sickle at a higher frequency compared to uninfected cells; sickle cells are known to be cleared in the spleen at higher frequencies. This results in a direct reduction of parasite densities and may also confer enhanced antigen presentation in the spleen, resulting in an improved acquisition of immune responses (Williams *et al.* 2005a). This immune component is supported by findings that the extent of the protective effect conferred by HbAS increases with age (Williams *et al.* 2005a). Sickle haemoglobin is also described to have a negative effect on the cytoadherence of RBCs infected with *Plasmodium falciparum*. This seems to correlate with an altered display of *P. falciparum* erythrocyte membrane protein-1 (Pf-EMP-1), a protein of great significance in terms of virulence and the adherence of infected RBC's to micro vascular veins (Cholera *et al.* 2008).

Haemoglobin C and E are both alternations in the β-globin chain but affect the erythrocytes in different ways. HbC is mainly found in West Africa and in higher frequencies in specific parts of West Africa like Ghana and Burkina Faso. The protective effect of HbC can especially be observed in HbCC homozygotes (Williams 2006) and may be related to a reduced ability of *P. falciparum* parasites to grow and multiply in these variant RBCs (Fairhurst *et al.* 2003, Olson and Nagel 1986, Williams 2006); in HbAC cells parasite growth may be at the same level as normal cells (Hutagalung *et al.* 1999). An alternative mechanism for the protective effect of HbC may be a reduced expression of *P*f-EMP-1, resulting in reduced cytoadherence of infected cells and a lower risk of severe (but not of uncomplicated) malaria (Fairhurst *et al.* 2003, Olson and Nagel 1986, Williams 2006).

Haemoglobin E is also associated with a reduced risk of severe malaria (Williams 2006). There is some evidence of reduced parasite growth in cells in both HbAE and HbEE individuals; both cell types also seem to be phagocytosed at higher frequencies when infected (Chotivanich *et al.* 2002). Similar to HbCC, HbEE is relatively benign and it is possible that selection favours both homo- and heterozygotes (Williams 2006).

Haemoglobinopathies: thalassaemias

Thalassaemias are polymorphisms resulting in an imbalance in the synthesis of α and β-globin chains of the haemoglobin molecules. α-thalassaemia is commonly found in regions of sub-Saharan Africa and Asia where malaria is or has been endemic. α-thalassaemia results from a deletion of one or more α-globulin genes. The clinical consequences depend on how many genes are still operational and how severe the imbalance between α and β globulin is. Absence of all 4 genes results in stillborns and is therefore not observed in malaria-endemic populations. Deletion of one or two of the genes may result in lower haemoglobin levels (Veenemans *et al.* 2008).

α-thalassaemia is associated with protection against severe anaemia during asymptomatic malaria infections (Veenemans *et al.* 2008), protection against severe disease but not against asymptomatic infection (Williams 2006) and probably not against uncomplicated disease (Wambua *et al.* 2006). There seems to be a strong correlation with age dependant factors determining to what degree α-thalassaemia offers protection against malaria (Mockenhaupt *et al.* 2004, Veenemans *et al.* 2011).

β-thalassaemia is associated with a lower parasite density but not prevalence (Willcox *et al.* 1983) and is more prevalent in the Mediterranean and Middle East than in sub-Saharan Africa. When infected with malaria parasites, β-thalassaemic cells show a reduced parasite growth *in vitro* when exposed to oxidant stress. Individuals with a single a-thalassaemic gene deletion seem to support parasite growth at normal rates; there is some evidence that infected thalassaemic cells to show enhanced antigen presentation (Williams *et al.* 2005c), suggesting an immune component in thalassaemia-associated protection.

RBC enzymes: glucose-6-phosphate dehydrogenase deficiency

Since erythrocytes lack nuclei and active translation machinery they are greatly dependent on some key long lived enzymes to create and maintain an appropriate environment for the cell to exhibit its function. Glucose-6-phosphate dehydrogenase (G6PD) is one of these enzymes; it metabolizes glucose through the pentose phosphate pathway and plays a key role in synthesizing NADPH. The gene coding for this enzyme is located on the X-chromosome and therefore autosomal; explaining why the more severe forms G6PD are mostly found in males in whom a mutation in a single gene results in a less efficient or completely deficient enzyme. Different mutations are responsible for varying degrees of G6PD deficiency across the globe (Ruwende *et al.* 1995), the most common African variant resulting in less severe G6PD deficiency than the Mediterranean variant (Tripathy and Reddy 2007).

The protection against malaria conferred by G6PD deficiency involves the early phagocytosis of infected RBCs. G6PD-deficient infected RBCs are phagocytized more efficiently than infected normal cells through a mechanism that may involve human immune components and the fact that they are more prone to oxidative stress. (Cappadoro *et al.* 1998) This results in a similar risk of infection while the time to reach densities that cause symptomatic or severe malaria would be longer for G6PD deficient individuals (Missinou *et al.* 2003).

Membrane proteins: ovalocytosis

Ovalocytosis is a disorder which affects the cytoskeleton of the erythrocyte. The typical round shape of RBCs is changed to a more oval shape. This condition is predominantly found in South East Asia; and although the condition is rare in most regions, it can be found in up to 15% of the population of some Asian countries. Ovalocytosis is associated with a more rigid RBC and an increased RBC adherence to endothelium receptors that are not present in the vascular endothelium of the brain (Cortes *et al.* 2005). Ovalocytosis has also been associated with resistance against invasion by some, but not all, parasite lines *in vitro* (Cortes *et al.* 2004). Ovalocytosis is associated with a reduced risk to the development of severe (cerebral) malaria (Allen *et al.* 1999, Cortes *et al.* 2005) but not against asymptomatic infection (Genton *et al.* 1995, Williams 2006).

Membrane proteins: Duffy blood group negativity

Lack of the Duffy antigen or Duffy blood group negativity, is associated with protection against *Plasmodium vivax*. This parasite is largely absent in much of sub-Saharan African countries while prevalent in Asia and South America. This geographical pattern has largely been attributed to a single nucleotide polymorphism leading to the absence of the Duffy binding protein from RBCs in most African populations. This Duffy binding protein was long thought to be absolutely essential for the binding and entering of *P. vivax* merozoites of RBCs. In more recent years, transmission of *P. vivax* has been reported in Duffy negative individuals (Menard *et al.* 2010, Ryan *et al.* 2006). This would indicate that the parasite has found an alternative pathway to invade red blood cells within individuals negative for the Duffy antigen (Mendes *et al.* 2011). Nevertheless, the Duffy antigen is a striking illustration of how human genetic polymorphisms can shape the geographical map of malaria transmission.

Other genetic related factors

The correlation between genetically defined RBC polymorphisms and malaria has fuelled ideas around the burden of disease and selective pressure. Host gene polymorphisms in relation with malaria may not be restricted to those affecting RBCs. Cohort studies in Kenya indicated that the genetic contribution to variability of malaria incidence is well beyond that explained by the anticipated effects of the haemoglobinopathies alone; genetic and unidentified household factors may each account for around one quarter of the total variability in malaria incidence (Mackinnon *et al.* 2005). Associations with severe malaria and high parasitaemia have also been found with human leukocyte antigens (HLA), a highly polymorphic family of molecules that play a crucial role in immune responses (Hill *et al.* 1992). Also other specific chromosomal regions contributing to the hosts immune response have been identified (Abel *et al.* 1992, Garcia *et al.* 1998). Differences have been found in populations of same ancestral origin but living at higher altitudes where malaria was not endemic (Terrenato *et al.* 1988). Genome wide linkage and association studies have shown several associations with genetic immune-related traits and host responses to malaria infections (Griffiths *et al.* 2005). All these combined findings prove that malaria parasites have imposed a strong selective pressure on the human genome in former and current malaria endemic regions. The list of genetic factors associated with (severe) clinical malaria is becoming longer and as more results from genome wide linkage and association studies become available, the complex pathophysiology of malaria is likely to be progressively revealed (Terrenato *et al.* 1988).

Human genetic factors related to human infectiousness to mosquitoes

There are several ways in which human genetic polymorphisms may influence transmission potential. Reductions in the risk of infection with malaria parasites or the density of malaria parasites will plausibly result in a reduction in transmission potential (Bousema and Drakeley 2011) while a longer duration of infections will in turn increase transmission potential. These conflicting possible outcomes warn against strong conclusions based on cross-sectional data that largely miss effects that become apparent with time.

There is little evidence for a reduced risk of malaria infection associated with any of the above described polymorphisms (with the exception of Duffy antigen); instead they may lower parasite density or slow parasite growth which may translate in a longer duration of asymptomatic infections. This will increase the development and duration of gametocyte carriage, which seems

to be supported by the suggestion that there is a significant human genetic contribution to gametocyte carriage in asymptomatic but not in symptomatic infections (Lawaly *et al.* 2010).

There are several indications that human genetic factors may influence gametocyte carriage and malaria transmission. Differences in gametocyte carriage were observed between tribes in West Africa that could not be explained by innate differences in immunity against asexual parasites (Paganotti *et al.* 2006). Most detailed studies on human genetic polymorphisms in relation to malaria transmission potential have focused on HbC and HbS. HbAS was associated with increased gametocyte carriage and increased density of gametocytes (Lawaly *et al.* 2010) and with an increased transmission of malaria to mosquitoes (Robert *et al.* 1996). In line with this, Gouagna and colleagues observed an association between the protective HbC and HbS genotypes and increased transmission to mosquitoes, an effect estimated as up to twofold in *in vivo* and fourfold in *ex vivo* studies (Gouagna *et al.* 2010). It was hypothesized that HbS and HbC might promote sexual differentiation, reduce human transmission blocking immune responses or increase gametocyte carriage as a result of the longer duration of parasite carriage in these individuals (Gouagna *et al.* 2010).

One longitudinal study that tried to determine a direct link between α-thalassaemia and gametocyte carriage or density failed to show a significant impact (Lawaly *et al.* 2010). Gametocyte rates were reported to be lower in children carrying the β-thalassaemia trait although the cause for this association remains to be established (Willcox *et al.* 1983). In summary, data are very limited but for HbC and S there is some evidence that the individual protection conferred by these haemoglobinopathies comes at the epidemiological cost of a higher transmission potential to the wider community.

Human genetic factors and hotspots of malaria transmission

Genetic factors that render individuals more or less susceptible to malaria infection or clinical malaria attacks can strongly influence intra-individual differences in malaria incidence. If human genetic factors cluster geographically, i.e. in compounds or villages, this clustering may be epidemiologically relevant for malaria transmission. Using the terminology of foci and hotspots of malaria transmission: neighbouring villages may differ in genetic background (Dolo *et al.* 2005) and hence their risk of malaria (i.e. contribute to the intensity of transmission in a focus of malaria transmission). In some exceptional areas, individuals who are genetically more prone to produce gametocytes during their infection may cluster geographically in such a way that they infect mosquitoes which subsequently spread the infection to populations with a different genetic make-up. Human genetic components could therefore contribute to the formation of a hotspot of malaria transmission. This has been suggested for areas in Burkina Faso where the susceptibility for malaria, and potentially a different infectiousness of different tribes to mosquitoes, has been described (Dolo *et al.* 2005, Paganotti *et al.* 2006). This scenario may prove to be less exceptional once genetic elements determining the human transmission potential are identified at household or family level and if local 'super spreaders', people who are disproportionally infectious to mosquitoes (Bousema and Drakeley 2011, Lawaly *et al.* 2010), can be identified. In this respect, the authors believe there will be an important role for human genetic factors that determine their attractiveness to mosquitoes; these factors may explain heterogeneity in malaria exposure in villages where all known risk factors for malaria are apparently homogeneously distributed. Until these factors are revealed, most studies on heterogeneity in malaria transmission justifiably focus on factors related to spatial variation in mosquito exposure.

Heterogeneity in mosquito exposure

Factors determining spatial heterogeneity in mosquito exposure

Given the nature of malaria transmission, it is unsurprising that heterogeneity in malaria incidence has long been associated with the vicinity of mosquito breeding sites. Observations from ancient Egypt and Greece already noted the association between fevers and wet ground and anti-malaria regulations in Italy in the early nineteenth century required that irrigated land had to be at least 500 meters away from general housing and at least 8 kilometres from the capital of a kingdom (Carter *et al.* 2000, Watson 1949). More recent studies described an association between malaria incidence and the distance to the forest (Kreuels *et al.* 2008), river (Lindsay *et al.* 1993b, Oesterholt *et al.* 2006), water body (Bousema *et al.* 2010a, Ghebreyesus *et al.* 1999) or confirmed *Anopheles* breeding site (Bousema *et al.* 2010a, Thomas and Lindsay 2000). The strength of these associations will depend on the productivity of breeding sites and the effective mosquito dispersal range. The productivity of mosquito breeding sites is variable and is influenced by factors including the type (Bogh *et al.* 2003, Edillo *et al.* 2004, Fillinger *et al.* 2009b, Majambere *et al.* 2008, Mutuku *et al.* 2006), size (Majambere *et al.* 2008) and stability of habitats (Mutuku *et al.* 2006), temperature (Edillo *et al.* 2004, Kirby and Lindsay 2009), rainfall (Paaijmans *et al.* 2007), vegetation (Bogh *et al.* 2003, Fillinger *et al.* 2009b, Majambere *et al.* 2008), salinity (Bogh *et al.* 2003), presence of larvae of other mosquito species (Majambere *et al.* 2008) and other micro-environmental characteristics. The presence of water in a potential focus of malaria transmission therefore does not automatically translate in an epidemiologically important source of malaria vectors. Similarly, the apparent absence of evident water bodies does not exclude the presence of a source of mosquito emergence. This was recently illustrated by the association of hotspots of malaria transmission with soil moisture content (Bejon *et al.* 2010) despite an apparent homogeneous ecology (Bejon *et al.* 2010, Dolgin 2010). Obviously, findings of higher mosquito abundance in endemic areas do not equal increased malaria transmission intensity; in northern Tanzania a highly significant cluster of higher mosquito exposure was not associated with a higher malaria incidence (Bousema *et al.* 2010a).

The effective mosquito dispersal range from breeding sites will depend on their localisation in relation to potential blood meal sources. If humans are the primary source of blood meals for mosquitoes, the dispersal of mosquitoes from their breeding sites will strongly depend on the human population density in the area surrounding this breeding site. The *Anopheles* dispersal range may generally be less than 1 km in densely populated areas (Carter *et al.* 2000, Manga *et al.* 1993, Midega *et al.* 2007, Trape *et al.* 1992) but low population densities motivate mosquitoes to extend their flying range in search of a blood meal, potentially leading to ranges of ≥2 kilometres (Carter *et al.* 2000, Ejerctto and Urbino 1951, Lindsay *et al.* 1995). In densely populated areas, there can be a strong gradient in relative mosquito exposure and distance to a (potential) breeding site. In an urban area in Senegal, mosquito exposure approximately halved with every 200 m increase in distance from a known mosquito breeding site (Trape *et al.* 1992). In a Malian village with one permanent breeding site and dense human inhabitation at 50-1000 m from this breeding site, there were evident hotspots of mosquito exposure in the dry season close to the main breeding site but mosquitoes were more dispersed during the wet season (Figure 1).

The pattern described in Figure 1 is typical for areas of seasonal malaria transmission, where mosquito exposure is commonly most clustered in the dry season when few permanent breeding sites exist (Lindsay *et al.* 1995, Trape *et al.* 1992). After seasonal rains the permanent breeding sites become more productive but alternative breeding sites also arise that are more widely distributed across the transmission focus, leading to a wider dispersal of mosquitoes. Seasonal mosquito

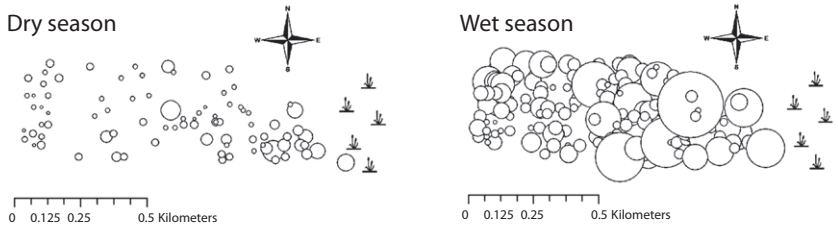

Figure 1. Mosquito catches in an area of moderate and seasonal malaria transmission in Mali. Mosquitoes were sampled in every household on a monthly basis. The size of the circles represents the average number of mosquitoes caught per households. Mosquito exposure was low and clustered near the main permanent breeding site in the dry season (left) but was higher and more widely dispersed during the wet season (right) (Bousema, unpublished observations).

dispersal patterns may also change under the influence of seasonal variations in wind patterns (Lindsay *et al.* 1995).

In areas where the human population is on average located further away from the breeding site, more sparsely distributed or where mosquitoes feed on human and non-human hosts, geographical patterns may be less distinct. In a rural area in Tanzania where households were located 800-2,000 m from a permanent river, where large numbers of cattle were present and *Anopheles arabiensis* Patton was the main vector, there was no clear association between mosquito exposure and distance to the river (Oesterholt *et al.* 2006).

Factors determining the contact rate between man and mosquito

In addition to the distance to mosquito breeding sites, elements that influence the contact rate between humans and mosquitoes have an obvious effect on the exposure to (infected) mosquitoes. The incidence of malaria and/or the exposure to mosquitoes has been associated with poorer housing conditions, especially incomplete housing (Gamage-Mendis *et al.* 1991), mud walls (Bousema *et al.* 2010a, Gamage-Mendis *et al.* 1991, Kirby *et al.* 2008), uneven wall structure (Bousema *et al.* 2010a), thatched roofs and ceiling (Bousema *et al.* 2010a, Gamage-Mendis *et al.* 1991, Lindsay *et al.* 1995, Ye *et al.* 2006), window screening (Oesterholt *et al.* 2006), window size (Oesterholt *et al.* 2006), presence of eaves (Kirby *et al.* 2008, Lindsay and Snow 1988, Lindsay *et al.* 1995), presence of animals (Kirby *et al.* 2008, Yamamoto *et al.* 2009) and the use of smoke or local incense to repel mosquitoes (Kirby *et al.* 2008, Lindsay *et al.* 1995, Yamamoto *et al.* 2009). Improvements in the house conditions, notably mosquito screening and closure of eaves, therefore lead to substantial reductions in mosquito exposure and can contribute to a reduction in anaemia in human inhabitants (Kirby *et al.* 2009). Other household protective measures such as indoor residual spraying (IRS) and insecticide treated nets (ITNs) have also been associated with reductions in malaria incidence (Bousema *et al.* 2010a, Bradley *et al.* 1986, Hawley *et al.* 2003, Pluess *et al.* 2010). In addition to a direct protective effect, reducing the risk of malaria in people sleeping under an ITN, there is evidence for a community effect of ITNs. In an area of intense malaria transmission in Western Kenya, it was described that ITNs exert a protective effect in compounds lacking ITNs that are located within 300 m of compounds with ITNs (Hawley *et al.*

2003). This indirect beneficial effect of ITNs is evident for child mortality, moderate anaemia and high-density parasitaemia (Hawley *et al.* 2003).

Regardless of the usage of protective measures, the attractiveness of humans also differs. Intra-individual mosquito attractiveness is related to variability in carbon dioxide (CO_2) release, ammonia, lactic acid, and other aliphatic carboxylic acids (Smallegange and Takken 2010). Pregnancy may increase attractiveness to mosquitoes by a factor 1.7-4.5 (Ansell *et al.* 2002), possibly as a result of differences in body surface, related to CO_2 release, temperature and odour. Non-pregnant individuals also differ in their attractiveness to mosquitoes and in their response to being bitten, resulting in differences in the number of bites experienced by individuals (Lindsay *et al.* 1993a). The intrinsic variation in attractiveness is probably largely mediated by sweat-associated human volatiles (Smallegange *et al.* 2011, Verhulst *et al.* 2009). This human odour profile has a genetic background (Roberts *et al.* 2005), explaining the genetic component that was previously associated with differential attractiveness (Kirk *et al.* 2000). Human leukocyte antigen (HLA) genes in particular may be involved in determining the intrinsic differential attractiveness of humans to mosquitoes (Verhulst *et al.* 2010) through their influence on the human body odour profile. The role of olfaction in vector-borne diseases is described in detail in a previous book in this series (Takken and Knols 2010).

Stability of hotspots of malaria transmission over time

To be of public health relevance, the geographical location of hotspots should be identifiable and show a certain consistency over time. If hotspots of malaria transmission change between seasons or years, it will become costly and logistically challenging to identify and target hotspots to have a beneficial impact on the burden of disease. The temporal stability and logistical identifiability are therefore crucial for utilizing hotspots of malaria transmission for effective malaria control. Some studies have reported that clusters of higher malaria incidence may change with time (Bejon *et al.* 2010, Coleman *et al.* 2009) while others indicated that they are remarkably stable over months or even years (Bautista *et al.* 2006, Bejon *et al.* 2010, Bousema *et al.* 2010a, Coleman *et al.* 2009, Ernst *et al.* 2006, Gaudart *et al.* 2006, Nourein *et al.* 2011). Unstable clusters of malaria incidence may reflect a problem in using malaria incidence for detecting hotspots, something that is explained in more detail in the Section 'Operational approaches to detecting hotspots of malaria transmission'. In areas of unstable malaria transmission, an unpredictable influx of malaria-infected individuals into an area with a suitable climate for malaria may also lead to different hotspots of malaria transmission over time (Coleman *et al.* 2009). Contrary to these important but relatively uncommon observations, most published reports indicate that hotspots of malaria transmission or mosquito exposure are stable over time. The most convincing evidence for this temporal stability of geographically defined hotspots comes from areas where transmission intensity decreased over time as a consequence of climatic changes or untargeted interventions but where hotspots of intense malaria transmission persisted (Bautista *et al.* 2006, Ernst *et al.* 2006, Gaudart *et al.* 2006, Nourein *et al.* 2011).

The most readily available data to describe the temporal consistency of hotspots of malaria transmission comes from entomological studies. Although we argued that the presence of mosquitoes does not automatically equal elevated malaria transmission intensity, field studies that sampled mosquitoes in the same households over several months provide valuable information on the consistency in exposure to malaria vectors. In Table 1, the findings from some of these studies from West and East Africa are summarised. Although the average number of mosquitoes differs tremendously between the wet and dry season, the same households are exposed to

Table 1.Consistency in mosquito exposure in three African settings.

	Ifakara, Tanzania (Akim *et al.* 2000, Drakeley *et al.* 2003)	Korogwe, Tanzania (Bousema *et al.* 2010a)	Sotuba, Mali
Location	Latitude 8° 8′ S Longitude 36° 41′ E	Latitude 5° 9′ S Longitude 38° 29′ E	Latitude 12° 40′ N Longitude 7° 55′ W
Parasite prevalence 2-9 year old children	55.1 (53.3-56.9)	28.6 (23.3-33.7)	6.1% (3.4-10.4)
Households sampled	32	185	254
Area sampled	4×5 km	5×6 km	0.8×0.7 km
Average *Anopheles* mosquito count wet season (range)	137.8 (4-1,266)	5.8 (0-129)	16.0 (0-184)
Average anopheles mosquito count early dry season/cool season (range)	1.4 (0-10.7)	1.3 (0-24)	7.9 (0-142)
Average *Anopheles* mosquito count end dry season/hot season (range)	0.4 (0-5.2)	0.50 (0-30)	1.2 (0-13)
Correlation household catches wet – early dry (*P*-value)	0.54 (0.0015)	0.30 (<0.001)	0.41 (<0.001)
Correlation household catches wet – end dry (*P*-value)	0.45 (0.01)	0.15 (0.05)	0.38 (<0.001)
Correlation household catches early – end dry (i-value)	0.72 (<0.001)	0.32 (<0.001)	0.42 (<0.001)

the highest relative number of mosquitoes. One could argue that this (statistically significant) consistency is unsurprising in relatively large geographical areas where environmental differences make certain sub-villages consistently more exposed to malaria than others (e.g. Korogwe and Ifakara in Table 1). However, the findings from households in a single village in Mali indicate that also at micro-epidemiological level, relative exposure to anophelines may be highly consistent over time despite temporal fluctuations in mosquito abundance.

Using hotspots for targeted malaria control

Do hotspots fuel wider malaria transmission?

There are several reasons to assume that hotspots form important reservoirs for further malaria transmission. In areas of very low and unstable malaria transmission, hotspots can be present throughout the seasons and form the only likely source of parasites for seasonal or epidemic increases in malaria transmission in the wider community (Ernst *et al.* 2006, Nourein *et al.* 2011). Mathematical models also consistently show that the overall level of transmission intensity is increased by heterogeneity in malaria transmission, suggesting a fuelling effect of hotspots of malaria transmission. In a seminal study by Woolhouse and colleagues, R_0 was 2-4 fold increased when heterogeneity in mosquito exposure was included in malaria transmission models (Woolhouse *et al.* 1997). It was later demonstrated that the impact of heterogeneity in mosquito exposure on transmission efficiency may differ between different settings (Smith *et al.* 2007): heterogeneity in mosquito exposure may augment malaria transmission in low endemic settings by allowing

mosquitoes to source their infections efficiently from hotspots; in areas of very high transmission intensity heterogeneity in mosquito exposure may result in segregation of populations where the clustering of mosquito exposure hinders instead of stimulates the spread of malaria. In more general terms, mosquitoes have to preferentially source their parasites from hotspots and subsequently spread their infection geographically to form a source of malaria transmission to a wider community. For this, the entire population of a focus of malaria transmission should be exposed to the same mosquito population while individuals living in hotspots should have more encounters with an otherwise randomly mixing mosquito population.

A study in Tanzania where mosquitoes were captured, marked with fluorescent powder, released and recaptured observed that 68% of mosquitoes returned to the same household as where they were initially captured (McCall *et al.* 2001) but these findings were not confirmed in follow-up experiments. In Papua New Guinea mosquitoes appeared to have a 'memorized' home range and limited dispersal range in the focus of malaria transmission they are accustomed to (Charlwood *et al.* 1988). These indications that mosquito populations may not mix randomly in villages need confirmation but would have important epidemiological consequences (Dye and Hasibeder 1986). In an extreme and highly unlikely scenario, no dispersal of mosquito populations from hotspots could result in intense transmission intensity inside hotspots of malaria transmission without consequences for the rest of the focus of malaria transmission that are exposed to a different mosquito population. A more likely scenario would be that there is a certain level of site fidelity and mosquitoes acquiring infections in hotspots transmit this infection to humans living outside hotspots but at a lower rate than is currently assumed by mathematical simulation models.

Will targeted interventions reduce malaria transmission?

Untargeted control efforts are relatively inefficient if heterogeneous transmission is assumed and high-risk households are missed (Carter *et al.* 2000, Dye and Hasibeder 1986). A disproportionate exclusion of hotspots of malaria transmission from malaria control measures is not a hypothetical scenario since several of the factors that have been associated with increased mosquito exposure are related to a lower socio-economic status which is in turn an important predictor of low participation in control methods. Population averaged coverage levels may therefore not accurately reflect the 'effective coverage' with malaria control measures.

There are several reasons why interventions targeted to hotspots of malaria transmission will be more efficient in reducing the burden of malaria than untargeted approaches. The most obvious is to protect individuals living in areas where the risk of (severe) malaria is highest. The largest, and most cost-efficient, impact on (severe) disease can be expected if those individuals who are most at risk preferentially receive protective measures. However, this does not reflect the full potential of hotspot targeted interventions. The additional and perhaps most attractive benefit of targeting hotspots of malaria transmission is that it can result in community-wide beneficial effects. By reducing or interrupting transmission in those households that contribute most to malaria transmission, community-wide malaria control may be improved in a cost-efficient manner. For this, a paradigm shift in thinking about malaria control is needed that focuses on epidemiologically relevant malaria cases instead of clinically vulnerable individuals (MalERA Consultative Group on Diagnoses and Diagnostics 2011). If the main objective of targeted interventions would be to protect individuals at risk of severe disease, an approach may be chosen where the known risk groups of (severe) disease are preferentially included in interventions. Although this will reduce morbidity and mortality in clinically relevant risk groups, the public health impact may be limited because other parts of the population can be equally important for disease transmission

(Bousema and Drakeley 2011, Drakeley *et al.* 2000). Asymptomatic individuals of all age groups play an important role in maintaining malaria transmission (Okell *et al.* 2008, Ouedraogo *et al.* 2009) and are particularly common in hotspots of malaria transmission (Bejon *et al.* 2010, Ernst *et al.* 2006, Stresman *et al.* 2010). To maximize the impact of targeted interventions, these interventions should therefore aim to eliminate malaria transmission in and from the hotspot. For this, a comprehensive approach is needed where conventional vector control methods such as ITNs and IRS can be combined with more laborious but efficacious vector control tools such as larviciding (Fillinger *et al.* 2009a) and interventions that aim to reduce the human infectious reservoir of malaria. Interventions that aim to clear the human infectious reservoir of malaria may include tools that are currently deemed less suitable for community-wide coverage such as mass drug administration with antimalarial drugs (Okell *et al.* 2011), focal screening of asymptomatic individuals followed by antimalarial treatment (Okell *et al.* 2011, Stresman *et al.* 2010) and employment of (transmission-blocking) malaria vaccines in all age groups (MalERA Consultative Group on Vaccines 2011, Sauerwein 2007).

Mathematical simulations suggest that perfectly targeted malaria control efforts can have an impact that is up to 4-fold higher than that of untargeted control efforts (Carter *et al.* 2000, Smith *et al.* 2007). As discussed in the previous paragraph, these estimates are influenced by epidemiological characteristics of the transmission setting (notably spatial patterns in population density and mixing patterns in mosquito populations (Dye and Hasibeder 1986, Smith *et al.* 2007)) and may need to be adjusted in the context of mosquito site fidelity. Nevertheless, all current evidence suggests a beneficial effect of targeted control efforts if (1) hotspots of malaria transmission can be operationally identified; (2) this information allows logistically feasible targeting; (3) the benefit of a higher efficiency of interventions financially outweighs the costs of detecting hotspots of malaria transmission (Carter *et al.* 2000). The first hurdle to take is to define an operational approach for detecting hotspots of malaria transmission.

Operational approaches to detecting hotspots of malaria transmission

Hotspots of malaria transmission have been detected based on variations in the human and in the vector components of malaria transmission. Micro-epidemiological elevations in malaria incidence are often used as evidence for malaria hotspots (Bejon *et al.* 2010, 2011a, Bousema *et al.* 2010a, Brooker *et al.* 2004, Clark *et al.* 2008). In addition, elevations in (asymptomatic) parasite prevalence (Bejon *et al.* 2010, Cook *et al.* 2011, Pullan *et al.* 2011), serological responses to malaria-specific antigens (Bousema *et al.* 2010a,c, Cook *et al.* 2011), mosquito abundance (Bousema *et al.* 2010a) and exposure to infected mosquitoes (Bousema *et al.* 2010a) have been utilized in attempts to quantify micro-epidemiological variations in malaria risk.

Entomological indicators

The most direct evidence of a hotspot of malaria transmission would be an increased exposure to infected mosquito bites. This gold standard measure for defining transmission intensity is difficult to assess at micro-epidemiological level: it depends on intensive sampling of mosquitoes over time and space and the detection of parasite stages in the mosquito salivary glands by microscopical examination or enzyme-linked immunosorbent assay (ELISA) (Wirtz *et al.* 1987). This makes entomological evaluations very laborious, especially in areas with low vector densities where currently available mosquito sampling tools lose sensitivity (Hamad *et al.* 2002, Oesterholt *et al.* 2006). An additional problem is the current uncertainty about the best mosquito sampling tool. Repeated sampling over time and at multiple locations require simple and affordable tools

such as miniature light traps, odour baited traps or pyrethrum spray catches. Water storage clay pot traps were piloted as low-cost affordable approach that can be used for large-scale sampling (Odiere *et al.* 2007) but have serious limitations in reliably sampling mosquitoes in field settings (Van den Bijllaardt *et al.* 2009). Importantly, sampling strategies for outdoor biting and resting mosquitoes are poorly standardized. This creates a risk of ignoring important vector populations that have a preference for outdoor biting and resting (Riehle *et al.* 2011). These limitations make it operationally unattractive to depend on entomological assessments of malaria exposure to define hotspots of malaria transmission in most endemic settings.

Human indicators

More indirect but more easily accessible evidence for hotspots of malaria transmission may come from detecting (the consequences of) malaria infections in humans. The overarching advantage of relying on infections in humans is that this circumvents any problems in low densities or indoor/outdoor biting mosquitoes. Heterogeneity in malaria incidence is a frequently used indicator of increased exposure but its validity is affected by the differential acquisition of immunity inside and outside hotspots and treatment seeking behaviour. In areas that are consistently exposed to higher levels of malaria transmission, immunity may be acquired at a faster rate and as a consequence fewer infections result in clinical malaria. Clinical malaria and high density parasitaemia may therefore be lowest in areas exposed to higher levels of transmission intensity where people have acquired protective immunity more rapidly (Clarke *et al.* 2002, Thomas and Lindsay 2000). Clinical incidence may therefore give inaccurate estimates if measured in age-groups where residence in a hotspot of malaria transmission has resulted in an effective immune response.

This confounding effect of immunity may be less prominent for asymptomatic parasite carriage. Asymptomatic parasite carriage may last several months (Falk *et al.* 2006) making estimates more robust in settings where the clinical infrastructure allows rapid treatment of symptomatic infections. Most importantly, immune responses that prevent infection are acquired later in life compared to clinical immunity (Smith *et al.* 2005); low density infections in hyper immune adults suggest that immunity effectively preventing malaria infection may actually be very rare (Okell *et al.* 2009, Proietti *et al.* 2011). This suggests that clustering of asexual parasite carriage may form a more stable indicator of transmission intensity than clinical malaria episodes (Bejon *et al.* 2010).

A third option to utilize malaria infections in humans as indirect indicator of higher malaria exposure is formed by serological markers of malaria exposure. Antibody responses to malaria specific antigens are acquired in response to (cumulative) exposure and can be used to define small-scale variations in malaria exposure (Bousema *et al.* 2010a,b, Drakeley *et al.* 2005). Because antibody responses are relatively long-lived, serological markers of malaria exposure are likely to be most suitable for detecting stable hotspots of malaria transmission (Bousema *et al.* 2010a) and most sensitive in areas of low endemicity (Corran *et al.* 2007). The strong age-dependency of antibody responses necessitates an analysis of an age-dependent conversion rate from sero-negative to sero-positive (Bousema *et al.* 2010a, Drakeley *et al.* 2005) or an age-adjusted antibody density (Bousema *et al.* 2010c, Wilson *et al.* 2007). Human genetic polymorphisms that modulate the risk of clinical and asymptomatic parasite carriage (see above) may also influence malaria specific antibody responses (Sarr *et al.* 2006). The importance of this immune modulating effect remains to be established but could alter the sensitivity of serological markers of exposure in identifying hotspots of malaria transmission in areas where human genetic polymorphisms show spatial heterogeneity.

Spatial analysis on entomological or human indicators

The level of statistical significance is a relevant factor in determining whether a certain geographical area forms a plausible hotspot of malaria transmission. A powerful statistical tool that is frequently used to analyse spatial and spatio-temporal patterns is SaTScan (SatScan). The SaTScan software is freely available online and uses a Kulldorf spatial scan statistic (Coleman *et al.* 2009) to detect clusters in space and (if requested) time. A scanning window is used that moves across space and counts the observed and expected number of cases or attributes of cases for each location and size of the window. For the sake of simplicity, the remainder of the text will assume a case-control approach (Bernouilli model) although SaTScan also allows scans on continuous or categorical variables and allows the detection of hotspots (higher count than expected) as well as coldspots (lower count than expected). In the case-control approach, the window with the greatest ratio of observed to expected cases is noted. The statistical significance of this hotspot or coldspot is then evaluated taking into account the multiple tests for the many potential cluster locations and sizes evaluated. The output of the SaTScan analysis gives the location, size and level of statistical significance of the most likely clusters.

One important characteristic of the SaTScan approach is that it calculates the number of expected cases by considering an even distribution of cases across the population and is very susceptible to the restrictions given by the user (e.g. maximum window size, allowance of overlapping clusters). This means that a spatial scan on a large geographical area will only pick up the most extreme hotspots. An additional complication with spatial scans on patterns in the occurrence of malaria is formed by the strong seasonal fluctuations in malaria incidence. Spatial scans on variables that are less susceptible to these seasonal fluctuations such as cumulative malaria incidence over several seasons (Bejon *et al.* 2010, Bousema *et al.* 2010a), cumulative parasite prevalence (Bejon *et al.* 2010) or long-lived antibodies to malaria antigens (Bejon *et al.* 2010, 2011b, Bejon *et al.* 2010, Bousema *et al.* 2010a,b) are likely to produce most robust results. The scans can be performed separately for individual villages to increase the likelihood of detecting hotspots that are of local relevance but that would not have been detected in an area-wide scan.

Environmental models

Environmental factors have long been associated with individual malaria risk. The simplest environmental models for detecting spatial variation in malaria risk incorporate distance to plausible mosquito breeding sites. These models have some value in predicting malaria risk (Carter *et al.* 2000, Clark *et al.* 2008, Kreuels *et al.* 2008, Oesterholt *et al.* 2006) but failed to explain hotspots of malaria transmission in two recent studies (Bejon *et al.* 2010, Bousema *et al.* 2010a). In reality, the prediction of hotspots of malaria transmission is relatively straightforward in foci of malaria transmission with a single or very limited number of plausible sources of mosquito emergence are present. In other settings the correlation between malaria risk and distance to water may be weak (Bousema *et al.* 2010a) and additional factors have to be incorporated in models to reach a sensitivity that justifies rationally targeting malaria control. In these circumstances site-specific models may have to be prepared to encapsulate all locally relevant predictors of malaria risk. This makes environmental models less attractive from a public health perspective.

A more sophisticated form of environmental modelling that may partly overcome the necessity for on-site data-collection to define local malaria risk factors is an approach that utilizes remote sensing data to determine factors such as elevation, daytime and night-time temperature, humidity, vegetation, soil moisture content, etc. The Malaria Atlas Project incorporates

epidemiological data on parasite prevalence, environmental covariates and human settlement data and has resulted in malaria risk maps for different endemic regions (Hay *et al.* 2009, 2010, Malaria Atlas Project undated). A current limitation for utilizing environmental data for detection hotspots of malaria transmission is that is routinely available remote sensing data has limited spatial resolution, typically 8 km^2 or 1 km^2 per pixel (Bejon *et al.* 2010, Hay *et al.* 2006). Some data is available at a higher resolution but have been validated less widely and currently do not benefit from processing by a temporal algorithm (Fourier), an approach that normalizes readings while preserving the seasonal variation in measures (Hay *et al.* 2006). As a consequence, environmental models are currently not validated for detecting hotspots of malaria transmission with malaria transmission foci.

Operational approaches to target hotspots of malaria transmission

Once hotspots of malaria transmission are detected, they can be targeted with conventional and less conventional malaria control tools. Hotspot-targeted interventions are likely to be beneficial for people living inside and people living outside the targeted hotspots (see Section 'Will targeted interventions reduce malaria transmission?'). The most straightforward approach of targeting hotspots is formed by the local up-scaling of efficacious conventional vector control tools such as ITNs and IRS. However, this approach will only target indoor biting and/or resting vectors while there is accumulating evidence that outdoor biting vectors are becoming increasingly important for malaria transmission (Reddy *et al.* 2011, Russell *et al.* 2011). The very essence of hotspot targeted interventions, focal interventions to protect the community at large, allows more laborious interventions that become operationally attractive because these require implementation in a fraction of the malaria endemic area only. Vector control tools that form attractive components of intensive hotspot-targeted interventions include larviciding of mosquito breeding sites (Fillinger *et al.* 2008) and the use of entomopathogenic fungi (Knols *et al.* 2010) for the control of adult mosquitoes. Both tools require frequent re-application that makes them less attractive for community-wide interventions but this shortcoming does not necessarily hinder targeted implementations. Similarly, the use of odour-baited mosquito traps (Jawara *et al.* 2011, Okumu *et al.* 2010) as part of community-wide interventions is currently unattractive but a targeted push-pull approach where mosquitoes are deterred from households in hotspots with a repellent and lured into traps with synthetic human odours may hold promise.

Intensive vector control in hotspots can be supported by interventions that aim to reduce the transmission potential of the human host. The human infectious reservoir can be reduced by improving malaria case management in hotspots, thereby reducing the duration of parasite and gametocyte carriage in clinical malaria cases (Bousema *et al.* 2010b). Because a large proportion of parasite carriers in hotspots may harbour their parasites without experiencing symptoms (Stresman *et al.* 2010), and therefore without actively seeking treatment, a more aggressive approach to clear the human infectious reservoir will be beneficial to reduce malaria transmission. The most inclusive approach is formed by mass treatment campaigns where all individuals, regardless of symptoms, receive a full therapeutic dose of antimalarials (Von Seidlein and Greenwood 2003). Apart from ethical issues with mass drug administration, an operational drawback is that it may require several rounds to be efficacious (Okell *et al.* 2011). This makes deployment of mass treatment campaigns most attractive in the form of targeted interventions. Mass drug administrations are laborious and ethically challenging in asymptomatic individuals who do not experience a personal benefit from treatment. In targeted interventions, the additional efforts to clear the human infectious reservoir are justified by the increased importance of the targeted parasite carriers for overall malaria transmission.

A last operational element that is important in identifying and targeting hotspots of malaria transmission concerns human movement patterns. Parasitaemic individuals may contribute to hotspots of malaria transmission in low endemic settings, as was suggested for the Kenyan highlands (Ernst *et al.* 2006). In addition, the effect of interventions targeted to the human population (e.g. treatment campaigns) may be diminished by the migration of parasitaemic and untargeted individuals. A comprehensive approach where parasites are targeted in humans and mosquitoes is likely to be less affected by human movement patterns.

Conclusions

- Spatial variation in malaria incidence and exposure to malaria-infected mosquitoes is present at all levels of transmission intensity.
- Hotspots of malaria transmission are geographical parts of foci of malaria transmission that are characterised by an increased level of transmission intensity compared to the average value of the focus.
- Human genetic factors contribute considerably to individual variation in malaria risk but with exception of areas where genetic traits cluster at micro-epidemiological level, do not explain hotspots of malaria transmission.
- There is some evidence that human genetic factors contribute specifically to transmission stages of malaria parasites and their infectivity to mosquitoes. These findings require confirmation in future studies but could be of epidemiological relevance in explaining malaria transmission patterns and planning malaria control efforts.
- Spatial variation in mosquito exposure is remarkably consistent despite large seasonal fluctuations in mosquito densities. Within regions and individual villages, the same households can be exposed to consistently higher mosquito numbers.
- The spread of malaria infections from hotspots to the wider community is biologically plausible but needs prospective confirmation and quantification to support the planning of hotspot-targeted interventions.
- Hotspot-targeted interventions should aim at reducing or preventing malaria transmission in and from the hotspot, not at protecting vulnerable individuals living in a hotspot of malaria transmission.
- Hotspots of malaria transmission can be identified by entomological and human parameters. Malaria incidence is an unreliable indicator of hotspots of malaria transmission unless restricted to age-groups that have limited clinical immunity; malaria parasite carriage or the presence or density of serological markers of malaria exposure may be more robust indicators of heterogeneity in malaria exposure.

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