Interplay Between Plasmodium Infection and Resistance to Insecticides in Vector Mosquitoes

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Despite its epidemiological importance, the impact of insecticide resistance on vector-parasite interactions and malaria transmission is poorly understood. Here, we explored the impact of Plasmodium infection on the level of insecticide resistance to dichlorodiphenyltrichloroethane (DDT) in field-caught Anopheles gambiae sensu stricto homozygous for the kdr mutation. Results showed that kdr homozygous mosquitoes that fed on infectious blood were more susceptible to DDT than mosquitoes that fed on noninfectious blood during both ookinete development (day 1 after the blood meal) and oocyst maturation (day 7 after the blood meal) but not during sporozoite invasion of the salivary glands. Plasmodium falciparum infection seemed to impose a fitness cost on mosquitoes by reducing the ability of kdr homozygous A. gambiae sensu stricto to survive exposure to DDT. These results suggest an interaction between Plasmodium infection and the insecticide susceptibility of mosquitoes carrying insecticide-resistant alleles. We discuss this finding in relation to vector control efficacy.

Keywords. malaria; cost of infection; Anopheles gambiae; insecticide exposure; kdr; insecticide resistance; Plasmodium falciparum.

Human malaria is caused by a parasitic protist of the genus Plasmodium, which is transmitted through the bites of infected mosquitoes of the Anopheles genus. Plasmodium parasites are dependent on completing a complex life cycle in the Anopheles mosquito vector for transmission to occur. Thus, reducing the mosquito abundance or interfering with its ability to support the parasite cycle can interrupt malaria transmission. Consequently, malaria control mainly relies on targeting the mosquito vector by using insecticides, but its efficacy has been challenged by the emergence of insecticide resistance [1]. Insecticide resistance in the main malaria vector species, such as Anopheles gambiae, is spreading dramatically in Africa [2]. Insecticides impose intense selection pressure on mosquito populations as a result of crop protection practices and increasing coverage of disease vector controls required for public health purposes [3]. Two main mechanisms have been described for resistance: target site mutations and enhanced metabolic detoxification. The molecular basis of target site insensitivity has been characterized in many insect species [3] and has demonstrated conserved resistant mutations across insect vectors. Point mutations in the gene coding for the voltage-gated sodium channel, named kdr for knockdown resistance, confer resistance to pyrethroids and dichlorodiphenyltrichloroethane (DDT) insecticides [4, 5]; and mutations in the ace-1 gene, which encodes the acetylcholinesterase enzyme, confer cross-resistance to carbamates and organophosphate insecticides [6]. Metabolic resistance is the result of elevated levels of detoxifying enzymes such as cytochrome P450 monooxygenases, esterases, or glutathione-S-transferases [3]. Currently, insecticide resistance is widespread, and multiple mechanisms are selected together as a result of increasing insecticide
selective pressure. Because resistant mosquitoes are the only mosquitoes able to survive in the presence of lethal dose of insecticides, pathogen transmission is ensured mostly by resistant vectors in areas where vector control is implemented.

The selection of insecticide resistance in mosquito vectors is thought to interfere with the development and transmission of parasites because of pleiotropic effects on vector longevity, vector competence, and vector feeding behavior [7]. In agreement with this, we recently demonstrated the impact of insecticide resistance on parasite infection in mosquitoes, using the natural system A. gambiae–Plasmodium falciparum; target-site mutations responsible for insecticide resistance (ace-1 G119S and kdr L1014F) increased the prevalence of P. falciparum infection in A. gambiae sensu stricto and probably its transmission through increased sporozoite prevalence [8]. However, no investigations have been made on the impact of infection on the susceptibility to insecticide in this epidemiologically relevant vectorial system.

P. falciparum sets up complex interactions and undergoes various developmental stages before the sporozoites reach the salivary glands of the mosquito vector and can be transmitted to human hosts. Although the cost of infection in natural vector-parasite associations is still in debate, development of the Plasmodium parasite is thought to reduce the overall fitness of its arthropod vector [9] because of mosquito cell damage when parasites cross the midgut epithelium [10, 11] or the cost of immune system activation [12]. Indeed, Plasmodium parasites are associated with increased oxidative stress in the mosquito midgut because of the production of reactive oxygen species (ROS) [13]. These ROS enhance immunity but are also detrimental to the mosquitoes, which respond by modifying expression of detoxifying enzymes [14, 15]. Insecticides, similar to other xenobiotics, induce a detoxification response through the overexpression of specific enzymes involved in binding, transport, and breakdown of exogenous compounds, resulting in an increase in tolerance [16]. This detoxification response is also observed in mosquitoes carrying mutations responsible for insecticide resistance [17]. Because enzymatic detoxification is implicated in both response to infection and insecticide exposure, we hypothesized that Plasmodium infection could then impact the susceptibility to insecticides. Previous works have demonstrated the interaction of insecticide exposure and infection on host life history traits [18, 19]. For example, infection with entomopathogenic fungi increases insecticide-induced mortality among resistant mosquitoes [18].

Therefore, in this study, we aimed to determine whether P. falciparum infection influences the level of insecticide resistance in wild-caught A. gambiae. Female mosquitoes were allowed to feed on control or gametocyte-infected blood, and mortality due to 4% DDT was tested at various times following blood feeding, corresponding to different parasite stages of the Plasmodium sporogony.

MATERIALS AND METHODS

Ethical Statement

Ethical approval was obtained from the Centre Muraz Institutional Ethics Committee under the ethical clearance number 003-2009/cE-cM. All human volunteers were enrolled after receipt of written informed consent from the participant and/or their legal guardians.

Mosquito Sampling

Anopheline larvae were sampled in Soumousso village, located 40 km southeast of Bobo-Dioulasso, Burkina Faso, during the rainy season (from August to September) in 2011 and 2012. This site was selected because anopheline mosquitoes are highly resistant to DDT. During the rainy season, this site is composed predominantly of Anopheles gambiae S molecular form, now called A. gambiae sensu stricto [40], with a high frequency of the kdr allele (f_{kdr} = 0.77), a low frequency of the ace-1^R allele (f_{ace-1^R} = 0.15), and absence or low levels of amplified detoxification enzymes [21]. Larvae were collected from typical A. gambiae sensu latro breeding sites, including gutters, swallow wells, and pools of standing water. Larvae were brought back to the insectary and reared to adults. Emerging adults were morphologically identified using standard identification keys as A. gambiae sensu latro females and were further used for blood feeding.

P. falciparum Experimental Infection by Membrane Feeding Assay

Membrane feeding assays were performed as described by Alout et al [8]. Briefly, P. falciparum gametocyte carriers were selected by examining thick blood smears of blood samples from children aged 5–11 years from 2 villages in southwestern Burkina Faso (Dandé and Soumousso, located 60 km north and 40 km southeast, respectively, of Bobo-Dioulasso). Children with a gametocyte density of >20 gametocytes/µL of blood were selected, and a venous blood sample (8 mL) was taken after a second measure of the gametocyte density for confirmation. Blood serum was replaced with European naive AB serum to limit the potential effect of human transmission blocking immunity [39]. Reconstituted blood samples were divided in 2 batches, from which one was used directly as infectious blood, and the other was heated at 42°C for 15 minutes as noninfectious control blood. This treatment results in the inactivation of gametocytes [41, 42] and does not affect the fitness of mosquitoes [43]. Membrane feeders were filled with 500 µL of reconstituted blood and maintained at 37°C by water jackets. Female mosquitoes aged 3–5 days were allowed to feed separately on infected or heat-inactivated (control) blood through a Parafilm membrane for up to 30 minutes. A total of 2–3 feeders were used for each blood type (infectious or control), to limit potential feeder effect. Unfed female mosquitoes were discarded, and only fully fed mosquitoes were maintained in a large cage (area,
30 × 30 × 30 cm) under standard insectary conditions on a 5% sucrose solution. This procedure was repeated 9 times, with each feeding assay using a different gametocyte-infected blood.

**Insecticide Exposure**

Insecticide exposure was performed on female mosquitoes, using the World Health Organization standard tube protocol [44]. Adult females were exposed for 1 hour to 4% DDT-impregnated paper, a discriminating concentration that kills all individuals that do not carry alleles conferring insecticide resistance [44]. Exposure to insecticides was performed on days 1, 7, and 14 after the blood meal. For each replicate, a batch of adult females was exposed to a nonimpregnated paper to ensure that natural mortality was <10%. Mortality was recorded 24 hours after insecticide exposure. Mosquitoes that died and those that survived after insecticide exposure were then stored separately on silica gel for subsequent DNA extraction and polymerase chain reaction (PCR) analysis.

**Molecular Analysis**

Genomic DNA was extracted using the CTAB protocol [45] from all individual *A. gambiae* sensu lato mosquitoes that survived or died after DDT exposure. They were identified to the species and molecular level by PCR, as described elsewhere [46]. The presence of the L1014F *kdr*-west mutation, responsible for resistance to DDT, was determined using the diagnostic test of Martinez-Torres et al [4]. *P. falciparum* infection was determined by PCR with PF1/PF2 primers [47] for females that were exposed to DDT on day 7 and 14 after the blood meal.

**Statistical Analysis**

To analyze the effect of *P. falciparum* infection on resistance phenotype, the mortality rate 24 hours after insecticide exposure was used as the response variable. Here, only mosquitoes belonging to *A. gambiae* sensu stricto and identified as homozygous for the *kdr* mutation (predominant in the studied sample) were included, to avoid confounding effects (n = 839 among 9 feeding assays). We used 4 explanatory variables: blood meal (a 2-level categorical variable, defined as infectious or control), donor (a categorical variable, with each gametocyte carrier representing a level), gametocyte density of the blood donor (a numerical variable), and day (a numerical variable, defined as the day after the blood meal when mosquitoes were exposed to insecticide). To explore the impact of *P. falciparum* infection in detail, mosquitoes that were not infected following an infectious blood meal (ie, *P. falciparum*–negative PCR result) were removed, and only those positive for *P. falciparum* infection were compared to the control group. Therefore, only those that were exposed to DDT on day 7 and day 14 after the blood meal were included (n = 556 among 7 feeding assays) because we could not determine by PCR on day 1 after the blood meal whether mosquitoes were infected, because of the potential presence of parasite DNA in the remaining blood in the midgut. A second analysis was then performed, in which the blood meal variable was replaced by the infection variable (a 2-level categorical variable, defined as infected or control). Both maximal models included the variables blood meal or infection, gametocyte density, and day and their interactions as fixed effects. The donor variable was used as a random variable to account for the correlation between individuals from the same feeding assay. Statistical analyses were performed with Statistical Analysis Software (SAS Institute, Cary, NC). For each analysis, the relevance of the random structure was compared with a generalized linear model with no random effect, based on the lowest Akaike information criterion. The mortality rate was analyzed using the GLIMMIX procedure with a binomial error structure.

**RESULTS**

***Species Identification and kdr Genotyping***

Of the 888 blood-fed female mosquitoes exposed to DDT over 9 replicates, 872 were identified as *A. gambiae* sensu stricto, and 16 were identified as *Anopheles arabiensis*. Molecular diagnosis of the *kdr* mutation revealed that 839 *A. gambiae* sensu stricto mosquitoes (>96%) were homozygous for the L1014F mutation; 13 were heterozygous and 10 were homozygous for the susceptible allele. Only 1 of 16 *A. arabiensis* specimens was heterozygous for the *kdr* mutation; all others were homozygous for the susceptible allele (Table 1). Regardless of species, all mosquitoes heterozygous or homozygous for the susceptible allele died after insecticide exposure. Thus, we analyzed only individuals homozygous for the *kdr* mutation, which included only *A. gambiae* sensu stricto (n = 839 females) and no *A. arabiensis* mosquitoes.

**Impact of Plasmodium Infection on Insecticide Resistance**

Our first analysis looked at the influence of the presence of infectious gametocytes in the blood but not at the outcome of infection in mosquitoes (Table 2). The type of blood meal (infectious or not), day of exposure, and the interaction of both variables had a significant effect on DDT-associated mortality.

<table>
<thead>
<tr>
<th>Anopheles Species</th>
<th>kdr Genotype, <em>f</em> No. (f)</th>
<th>Total, No. (f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. gambiae sensu stricto</td>
<td>RR: 839 (0.96) RS: 23 (0.03) SS: 10 (0.01)</td>
<td>872 (1)</td>
</tr>
<tr>
<td>A. arabiensis</td>
<td>0: 1 (0.06) 15 (0.94) 16 (1)</td>
<td></td>
</tr>
</tbody>
</table>

* RR and SS indicate mosquitoes homozygous for the resistant allele and the susceptible allele, respectively, and RS indicates mosquitoes heterozygous for the *kdr* mutation.

Biological Frequency

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mortality (Table 3). Regardless of the blood meal type, DDT-associated mortality changed depending on the day of exposure. Mortality increased significantly between day 7 and day 14 after the blood meal (mean mortality [±SEM], 13.02% ± 7.98% and 60.09% ± 14.88%, respectively; \( P = .0143 \)) but not between day 1 and day 7 after the blood meal (16.09% ± 9.53% and 13.02% ± 7.98%, respectively; \( P = .804 \)). Across all days of exposure, ingestion of an infectious blood meal increased DDT-associated mortality (mean mortality [±SEM], 17.17% ± 5.89% to 37.27% ± 9.27%; \( P < .001 \)). Mortality among young noninfected mosquitoes (12.8% on day 1 after the blood meal) is consistent with previously described DDT-associated mortality among \textit{Anopheles gambiae} sensu stricto mosquitoes from the same sample site in 2010 [20, 21]. On day 1 and day 7 after the blood meal, DDT-associated mortality was greater among female mosquitoes that took an infectious blood meal, compared with those that fed on heat-inactivated blood (mean mortality [±SEM], 31.7% ± 4.24% for the infectious group and 12.8% ± 3.44% for the control group on day 1 [\( P = .004 \)] and 28.3% ± 3.74% for the infectious group and 8.7% ± 2.31% for the control group on day 7 [\( P < .001 \)]).

**Table 3. Statistical Analysis of Dichlorodiphenyltrichloroethane-Induced Mortality in Relation to the Blood Meal Type**

<table>
<thead>
<tr>
<th>Source</th>
<th>df, Numerator</th>
<th>df, Denominator</th>
<th>F</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious blood meal</td>
<td>1</td>
<td>823</td>
<td>24.55</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Day</td>
<td>2</td>
<td>823</td>
<td>3.79</td>
<td>.023</td>
</tr>
<tr>
<td>Infectious blood meal × day</td>
<td>2</td>
<td>823</td>
<td>4.23</td>
<td>.0148</td>
</tr>
</tbody>
</table>

Significance of variables obtained after selection of the minimal mixed effect model is presented.

When mosquitoes were exposed to insecticide on day 14 after the blood meal, there was no difference in DDT-associated mortality between blood meal types (mean mortality [±SEM], 50.3% ± 3.74% for the infectious group and 45.9% ± 3.66% for the control group [\( P = .2536 \); Figure 1]).

We ran a second analysis in which only females positive for \textit{P. falciparum} infection were compared to females that fed on a noninfectious blood (infected vs control). The data consisted only of females that were exposed on day 7 and day 14 after the blood meal (\( n = 556 \)), as we were not able to confirm the success of infection on day 1 after the blood meal. The results of this second analysis agreed with those of the first analysis (Table 4).

**Figure 1.** Dichlorodiphenyltrichloroethane (DDT)–induced mortality among blood-fed female mosquitoes. The mortality rate among \textit{Anopheles gambiae} sensu stricto females that fed on gametocyte-infected blood (red) or heat-inactivated blood (blue) was recorded 24 hours after DDT exposure at different times after the blood meal. Bars above and below the means represent standard errors of the mean. **\( P < .01 \), ***\( P < .001 \). Abbreviation: NS, nonsignificant.

**Table 4. Statistical Analysis of Dichlorodiphenyltrichloroethane-Induced Mortality in Relation \textit{Plasmodium falciparum} Infection**

<table>
<thead>
<tr>
<th>Source</th>
<th>df, Numerator</th>
<th>df, Denominator</th>
<th>F</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmodium infection</td>
<td>1</td>
<td>545</td>
<td>24.11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Day</td>
<td>2</td>
<td>545</td>
<td>4.87</td>
<td>.0227</td>
</tr>
<tr>
<td>Infection × day</td>
<td>2</td>
<td>545</td>
<td>6.29</td>
<td>.0125</td>
</tr>
</tbody>
</table>

Significance of variables obtained after selection of the minimal mixed effect model is presented.
This suggests that the age of mosquitoes has a pronounced effect (ie, day 14 after the blood meal). However, several experimental differences may account for this discrepancy, such as the use of different vector-parasite combination, the use of an insecticide-susceptible strain with no history of insecticide exposure, or the use of a different insecticide (permethrin vs DDT). Additionally, an important difference is the presence of insecticide-resistant alleles (L1014F) in our sampled mosquitoes. Also, these collected samples may have experienced insecticide exposure in the field as larvae or in previous generations, which could affect various life history traits, particularly vector competence [25, 26]. Previous exposures of kdr homozygous mosquitoes to insecticides may have induced a detoxification response resulting in a variable insecticide resistance phenotype. Although we did not test for metabolic resistance mechanisms in the sampled mosquitoes, glutathione-S-transferases may be present in mosquitoes from the village where they were collected [21].

Our results show that Plasmodium infection increases DDT-associated mortality of kdr-resistant mosquitoes during the first week after infection (ie, from day 1 to day 7 after the blood meal) but not later. This suggests a trade-off between mounting an effective immune response and surviving DDT exposure. About 18–24 hours after the infectious blood meal, ookinete cross the midgut epithelium and trigger the activation of the mosquito immune system [48], which is mediated in part by the production and detoxification of ROS in the mosquito midgut [13, 14]. Plasmodium infection has been shown to decrease the expression of a large number of genes coding detoxification enzymes at the ookinete stage, particularly cytochrome P450 and the glutathione-S-transferases [15]. This may explain the increased susceptibility to DDT in our experiment. Thus, we hypothesize that the change in expression of detoxification genes induced by infection would lead to a trade-off between the control of ROS levels and the elimination of insecticides. This would trigger immune defenses against Plasmodium that may induce a cost on the ability of kdr-homozygous A. gambiae to survive insecticide exposure. In line with this hypothesis, Farnehorst et al [18] have demonstrated that fungal infection increases insecticide susceptibility of resistant mosquitoes that is probably due to a reallocation of insecticide-detoxifying enzymes toward fungal toxins. An alternative hypothesis might be that insecticide exposure affects the susceptibility to infection, leading to a greater Plasmodium-induced mortality rate, which is consistent with the immunotoxic effect of insecticides in vertebrates [27, 28].

The main method to control malaria transmission is through insecticide, as it reduces the longevity of the vector, the most important parameter of the vectorial capacity for a mosquito population [29]. Insecticide resistance limits the efficacy of vector control, as mosquitoes are able to survive high doses of insecticide. Alleles responsible for insecticide resistance may also impact other life history traits of the vectors, including vector competence and other parameters of the vectorial capacity, such as biting frequency, and vector density [7]. We recently showed that target-site resistant mutations affect vector competence, particularly the kdr mutation, which increases the probability of

DISCUSSION

In this study, we investigated the impact of Plasmodium infection on the insecticide resistance phenotype of wild-caught A. gambiae. We determined the impact of different stages of Plasmodium development on the ability of kdr homozygous mosquitoes to survive DDT exposure. The results clearly showed that kdr mosquitoes that fed on infectious blood were less able to survive after DDT exposure during oocyst development (day 1 after the blood meal) and oocyst maturation (days 7 after the blood meal), compared with those that fed on control blood. These results were confirmed when comparing only P. falciparum–positive individuals on day 7 after the blood meal to noninfected controls. However, DDT-induced mortality was not different between sporozoite-infected and control mosquitoes on day 14 after the blood meal. As reported in other studies, the level of insecticide resistance decreases with age in field-collected and laboratory-colonized Anopheles mosquitoes [22, 23]. This suggests that the age of mosquitoes has a greater impact on insecticide susceptibility than sporozoite infection (ie, day 14 after the blood meal).

The results presented here contrast with that of Anopheles stephensi infected by Plasmodium chabaudi, for which no increase of mortality was observed after permethrin exposure [24]. However, several experimental differences may account for this discrepancy, such as the use of different vector-parasite combination, the use of an insecticide-susceptible strain with no history of insecticide exposure, or the use of a different insecticide (permethrin vs DDT). Additionally, an important difference is the presence of insecticide-resistant alleles (L1014F) in our sampled mosquitoes. Also, these collected samples may have experienced insecticide exposure in the field as larvae or in previous generations, which could affect various life history traits, particularly vector competence [25, 26]. Previous exposures of kdr homozygous mosquitoes to insecticides may have induced a detoxification response resulting in a variable insecticide resistance phenotype. Although we did not test for metabolic resistance mechanisms in the sampled mosquitoes, glutathione-S-transferases may be present in mosquitoes from the village where they were collected [21].

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### Figure 2. Dichlorodiphenyltrichloroethane (DDT)–induced mortality among Plasmodium falciparum–infected and control female mosquitoes. The mortality rate among P. falciparum–positive (red) and noninfected control (blue) Anopheles gambiae sensu stricto females was recorded 24 hours after DDT exposure at different times after the blood meal. Bars above and below the means represent the standard errors of the mean. ***P < .001. Abbreviation: NS, nonsignificant.

29.1% ± 14.3% for the infected group and 5.9% ± 5.2% for the control group; P < .001), but the difference was not significant on day 14 after the blood meal (mean mortality [±SEM], 71.2% ± 14.6% for the infected group and 57.3% ± 17.5% for the control group; P = .304) as illustrated in Figure 2.
*P. falciparum* infection and decreases the intensity of infection in *A. gambiae* [8]. Therefore, insecticide resistance would lead to an increase of malaria transmission. However, because of the widespread application of insecticides for agricultural and public health purposes, insecticide-resistant mosquitoes are likely to be in contact with insecticides several times during their feeding cycles. Mosquitoes carrying insecticide-resistant alleles will be more susceptible to insecticides as they get older [22, 23], and *Plasmodium* infection may even reduce their insecticide resistance level. This might keep malaria vector control efficient, as older and infected females would be killed more easily even in insecticide-resistant populations. Consistently, by modeling the effect of late-life-acting insecticides, Read et al showed that an age-dependent biocide (ie, an insecticide that kills disproportionately older mosquitoes) would keep vector control efficient while slowing the evolution of resistance [30]. Moreover, if this insecticide is more effective against *Plasmodium*-infected mosquitoes, the evolution of resistance would be further slowed.

While insecticides affect directly the vectorial capacity of a mosquito population and, probably, pathogen transmission through the longevity of mosquitoes and their vector competence (ie, the ability to support parasite development) [31], they also have indirect effects through the selection of insecticide resistance mechanisms [8]. Indeed, declined insecticide concentrations over time on insecticide-treated surfaces would lead to multiple exposures to sublethal doses among resistant populations. This could induce the production of detoxification enzymes in the mosquito, affecting vector competence [7, 14]. Last, other ecological/environmental factors may influence the insecticide resistance phenotype, such as humidity, temperature [32], larval diet [33], or urban pollutant [34]. For example, susceptibility to organophosphate insecticides in natural populations of *A. aegypti* increases with increasing larval rearing temperature [32]. Taken together, these observations reveal the complex ecological consequence of insecticide application on the selection of resistance and on malaria transmission. A better understanding of ecological and environmental factors affecting mosquito resistance to pyrethroids may thus represent a way to better manage vector population and insecticide resistance.

In conclusion, we provide evidence that *P. falciparum* infection can reduce the level of resistance to DDT among field-caught mosquitoes homozygous for the *kdr* mutation. Insecticides that are no longer active on younger *kdr* homozygous mosquitoes may be still partially efficient in controlling disease transmission because infected mosquitoes carrying insecticide-resistant alleles have less chance to survive after insecticide exposure. This might explain why selection of insecticide resistance in *A. gambiae* by operational control was not associated with an increase in malaria prevalence. Indeed, only 1 case of control failure due to pyrethroid resistance, in South Africa during 2000, has been reported [35]. Data from other studies suggest that insecticide resistance may have a smaller effect than expected on malaria control, such as in Bioko Island, where the frequency of *kdr* increases but transmission index and malaria cases decrease [2]. In Burundi and Côte d’Ivoire, distribution of insecticide-treated nets led to a reduction in malaria incidence despite a high *kdr* frequency (>80%) [36, 37]. Future studies in various ecological settings should help to determine the impact of both insecticide resistance and vector control programs on malaria incidence. These results support the importance of environmental stresses (ie, insecticides) on the ability of mosquitoes to transmit *Plasmodium* parasites [38] and emphasizes the need to better understand the relationships between insecticide selection, insecticide resistance, and *Plasmodium* infection, which is critical for effective implementation of vector control strategies.

**Notes**

**Acknowledgments.** We thank the participating children and their parents, for their involvement in this study; the local authorities, for their support; the IRS staff in Burkina Faso, for technical assistance; and the Laboratoire Mixte International LAMIVECT in Bobo-Dioulasso, Burkina Faso, for technical support.

**Financial support.** This work was supported by the European Community’s Seventh Framework Program FP7/2007-2013 (grants 242095 and 223736) and the Institut de recherche pour le développement (financial support and fellowship to H. A.).

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


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