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An Entomopathogenic Fungus for Control of Adult African Malaria Mosquitoes

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mosquitoes is very unlikely, fungal transgenes would be much easier to control than mosquito transgenes.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/308/5728/1638/DC1
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An Entomopathogenic Fungus for Control of Adult African Malaria Mosquitoes

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Biological control of malaria mosquitoes in Africa has rarely been used in vector control programs. Recent developments in this field show that certain fungi are virulent to adult *Anopheles* mosquitoes. Practical delivery of an entomopathogenic fungus that infected and killed adult *Anopheles gambiae*, Africa's main malaria vector, was achieved in rural African village houses. An entomological inoculation rate model suggests that implementation of this vector control method, even at the observed moderate coverage during a field study in Tanzania, would significantly reduce malaria transmission intensity.

Mosquito vector control is an integral part of controlling malaria (*1*). In Africa, this is almost exclusively based on the use of chemical insecticides for indoor residual spraying and impregnation of bednets for killing adult mosquitoes (*2*–*6*). The high efficacy achieved at modest coverages results from the exquisite sensitivity of malaria transmission intensity to the daily survival rate of adult mosquitoes (*7*). However, the continuing use of both public health and agricultural insecticides has led to a substantial increase in physiological resistance of mosquitoes in recent years (*8*, *9*). These problems have increased interest in alternative and integrated implementation of vector control methods that include biological control. Although several effective biological larvicides exist (*10*), there have been no biological control agents effective against adult mosquitoes. Addressing this gap, we have recently reported encouraging results with entomopathogenic fungi from the Hyphomycetes (Imperfect Fungi) infecting and killing adults of the African malaria vector *Anopheles gambiae sensu stricto* through tarsal contact in laboratory containers (*11*, *12*). Unlike other mosquitocidal biocontrol agents, such as bacteria, microsporidia, and viruses, these fungi can infect and kill insects without being ingested. Tarsal contact alone is enough to kill the insect, a characteristic shared with insecticidal chemicals. Moreover, Hyphomycetous insect-pathogenic fungi, such as *Metarhizium anisopliae* and *Beauveria bassiana*, are produced commercially and used against several agricultural insect pests worldwide (*13*).

Here, we report the results of a field study in a rural village in Tanzania in which we assessed whether wild mosquitoes became infected and had reduced life spans after resting on 3 m^2 *M. anisopliae*–impregnated black (*14*) cotton sheets ("targets") suspended from ceilings in traditional houses (fig. S1). Pre- and postintervention mosquitoes were collected, and equal numbers of untreated and treated houses were included (*15*).

In the 10 study houses, we collected a total of 2939 mosquitoes, 1052 during the preintervention (3 weeks) and 1887 during the intervention period (3 weeks). These were maintained on a 10% glucose diet in paper cups until death, after which fungal infections were detected, retrospectively, by observation of emerging hyphae from mosquito cadavers (*16*). We found that 88.9% were *A. gambiae s.l.* (*17*) and 10.7% *Culex quinquefasciatus*. Overall, 53.6% of the mosquitoes were caught on the targets, and 46.4% elsewhere in the rooms (*18*). None of the mosquitoes that had been collected during the preintervention period, nor any of the mosquitoes collected from the control houses during the entire experimental study period were found to be infected with the fungus. Of the 580 female *A. gambiae s.l.* that were collected in the five treatment houses during the intervention period, 132 were infected with *M. anisopliae*.

There was no significant difference in longevity between mosquitoes that were collected before and uninfected mosquitoes that were caught after the intervention ($F = 2.903$, $P = 0.088$). Similarly, longevity of mosquitoes caught in the control houses was not different from that of noninfected mosquitoes collected in the treatment houses during the intervention period ($F = 0.91$, $P = 0.3411$). By contrast, fungus-infected *A. gambiae s.l.* had significantly shorter life spans compared with those of noninfected mosquitoes (Fig. 1; overall effect pooling both sexes, $F = 178.9$, $P < 0.001$). Median lethal times (LT_{50}) values were 3.70 and 3.49 days for *M. anisopliae*–infected males and females, respectively, and 5.88 and 9.30 days for uninfected males and females, respectively. Of the 188 infected

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mosquitoes, most were caught in the first 2 weeks after the start of the intervention; 80, 79, and 29 in the first, second, and third week, respectively. This decline in infectivity was consistent with results of the conidial viability checks during the intervention period. Although conidia that were kept in suspension barely lost viability (from 96.3 ± 0.88% germinating at day 1 to 93.7 ± 0.88% after 3 weeks, where error is SD), we found that conidia that were impregnated on the sheets gradually lost viability (from 95.0 ± 1.0% germinating after 1 day to 82.7 ± 6.17% after 1 week, 70.7 ± 7.35% after 2 weeks, and 63.0 ± 6.7% after 3 weeks).

The proportion of fungus-infected mosquitoes observed in the study was combined with baseline data from a well-characterized nearby village in an adapted malaria transmission model (*19*) to estimate the impact of fungus-treated targets on the intensity of malaria transmission [entomological inoculation rate (EIR)] (SOM text). The model estimates show that fungus-impregnated sheets would have a significant impact on parasite transmission. Even with just 23% of the mosquitoes in houses acquiring an infection, as obtained in this experimental study, the EIR could be reduced from a baseline level of 262 infective bites per person per year to 64 (i.e., 75% reduction of transmission intensity; Fig. 2). The proportion of mosquitoes with sporozoites in the overall population would decline from 0.011 to 0.0036 (fig. S2). Relatively simple modifications such as larger sized sheets, higher conidial dosages, and improved efficacy of the conidial formulation are all expected to increase considerably the overall proportion of mosquitoes that become infected and therefore the effectiveness of the intervention. For example, increased coverage of mosquito resting sites could improve impact further such that a still modest proportion of 50% of mosquitoes becoming infected would reduce the EIR by 96%.

We conclude that the application of fungal pathogens to kill adult malaria vectors could significantly reduce parasite transmission and therefore lead to reduced malaria risk. This finding, together with the reported reduced bloodfeeding propensity of fungus-infected female mosquitoes (*12*, *20*) and possible negative effects of fungal infection on *Plasmodium* development in the mosquito (*12*), demonstrates that this method of biological control has potential as a new strategy for malaria control.

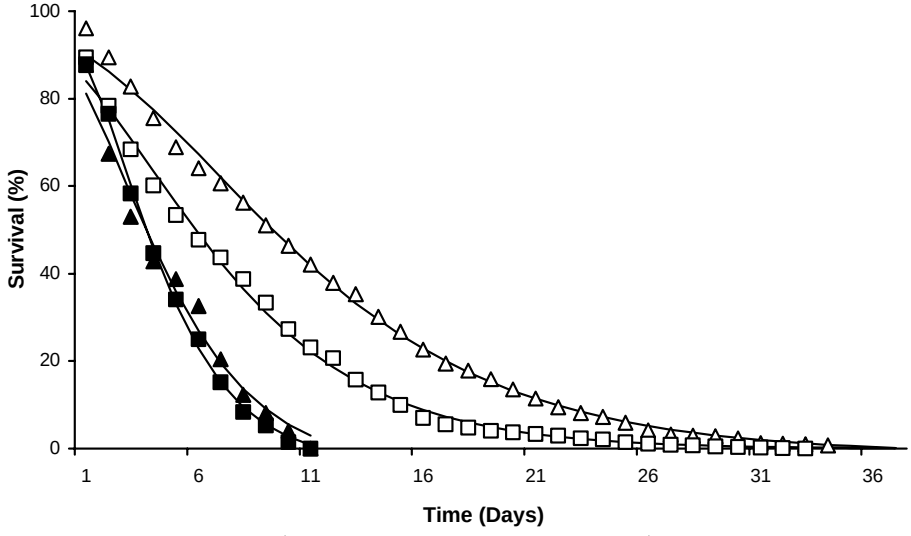

Fig. 1. Survival of uninfected (open symbols: Δ = females, □ = males) and *M. anisopliae*–infected (closed symbols: ▲ = females, ■ = males) wild *A. gambiae s.l.* mosquitoes collected from rural Tanzanian houses. Data fit to the Gompertz survival distribution model.

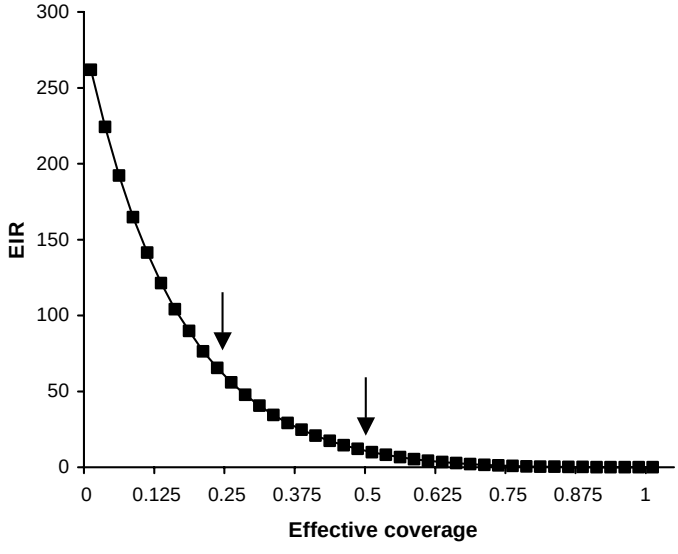

Fig. 2. Predicted relationship between effective coverage with *M. anisopliae*–treated cloths and reduction in EIR. Arrows show the EIR at coverage of 0.228 as achieved in the field trial (left arrow) and the anticipated effect of increasing it to 50% (right arrow).

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