



Transgenic Insect-Killing fungi for versatile mosquito control

Etienne Bilgo, Brian Lovett, Raymond St Leger and Abdoulaye Diabate

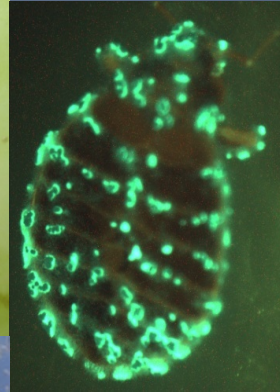
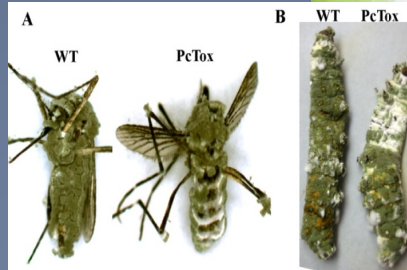
15th Annual Meeting Vector Control Working
Geneva, 3 February 2020



Biocontrol with insect-killing fungi

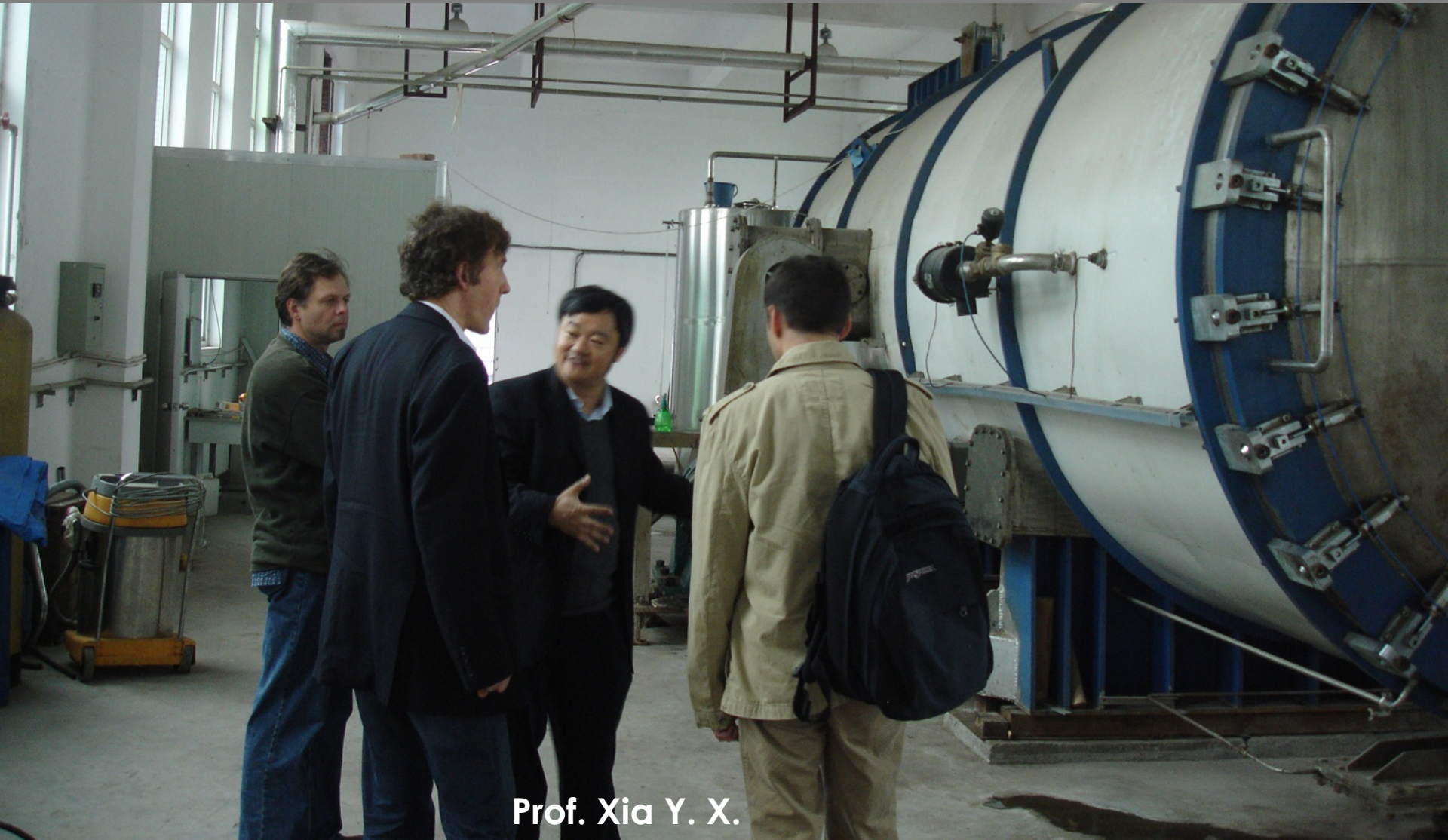


Chitoumou



• Unlike bacteria and viruses, fungi attack insects actively by directly penetrating insect cuticle i.e. they function as “contact” insecticides

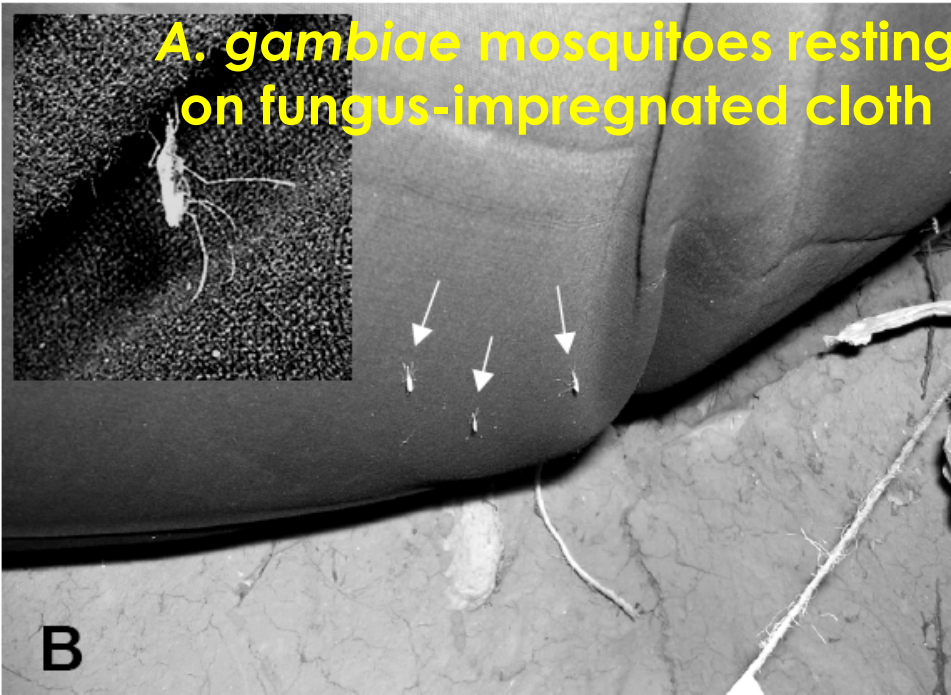
Spraying *M. acridum* achieved 65-97% reductions within 2 weeks in populations of the oriental migratory locust in China. The cost of producing Metarhizium is competitive with chemical insecticides



Prof. Xia Y. X.



A Position of black cloth treated with *M. anisopliae* inside a Tanzanian house.



B *A. gambiae* mosquitoes resting on fungus-impregnated cloth

Scholte et al., 2005
Science 308: 1641-1642

An Entomopathogenic Fungus for Control of Adult African Malaria Mosquitoes

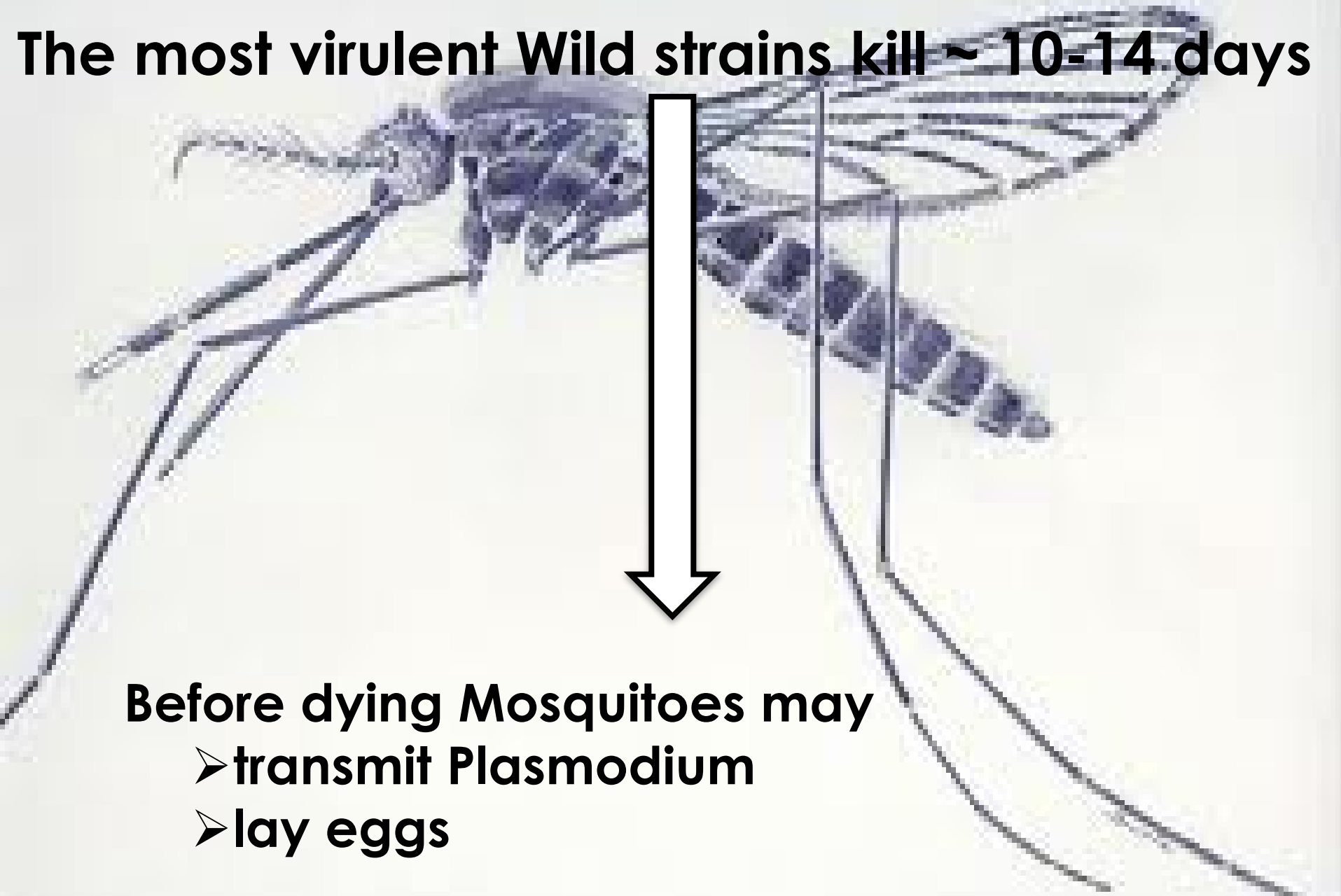
Ernst-Jan Scholte,¹ Kija Ng'habi,² Japheth Kihonda,²
Willem Takken,¹ Krijn Paaijmans,¹ Salim Abdulla,²
Gerry F. Killeen,^{2,3} Bart G. J. Knols^{1,4*}

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.

**Reduces number of infectious
bites from 256 to 52 per year.**

Weakness for the use of Wild fungi for vector control

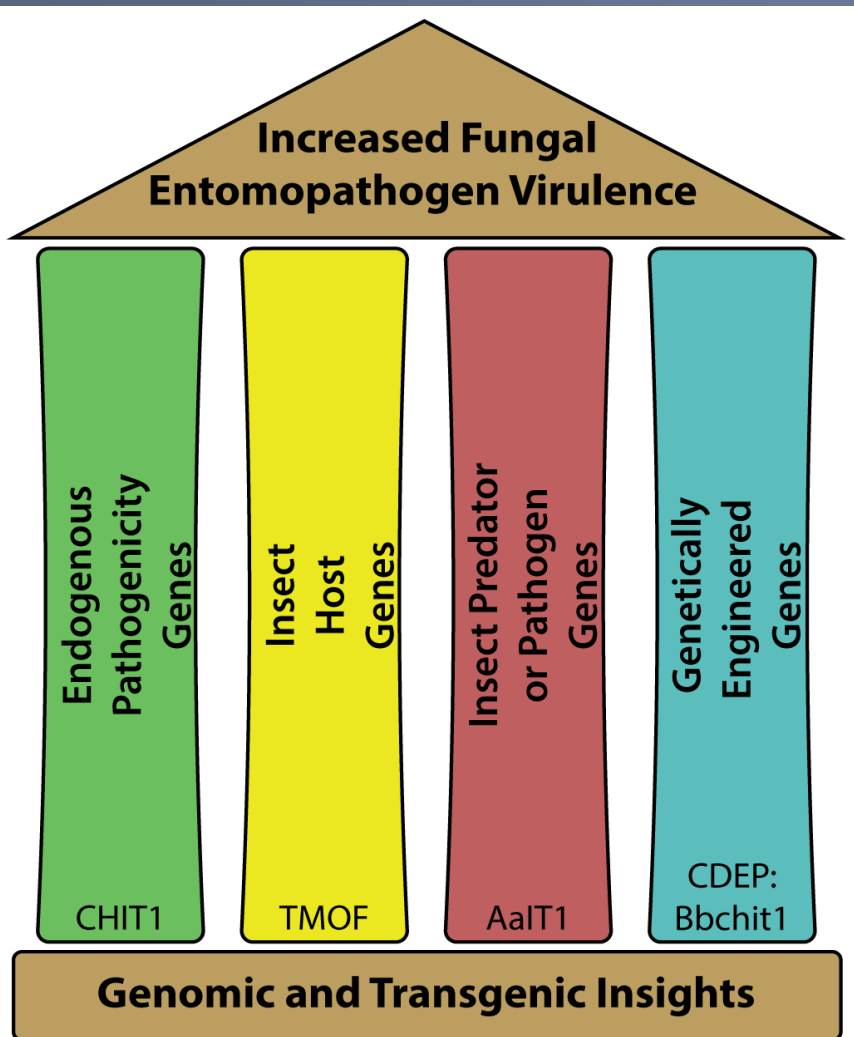
The most virulent Wild strains kill ~ 10-14 days



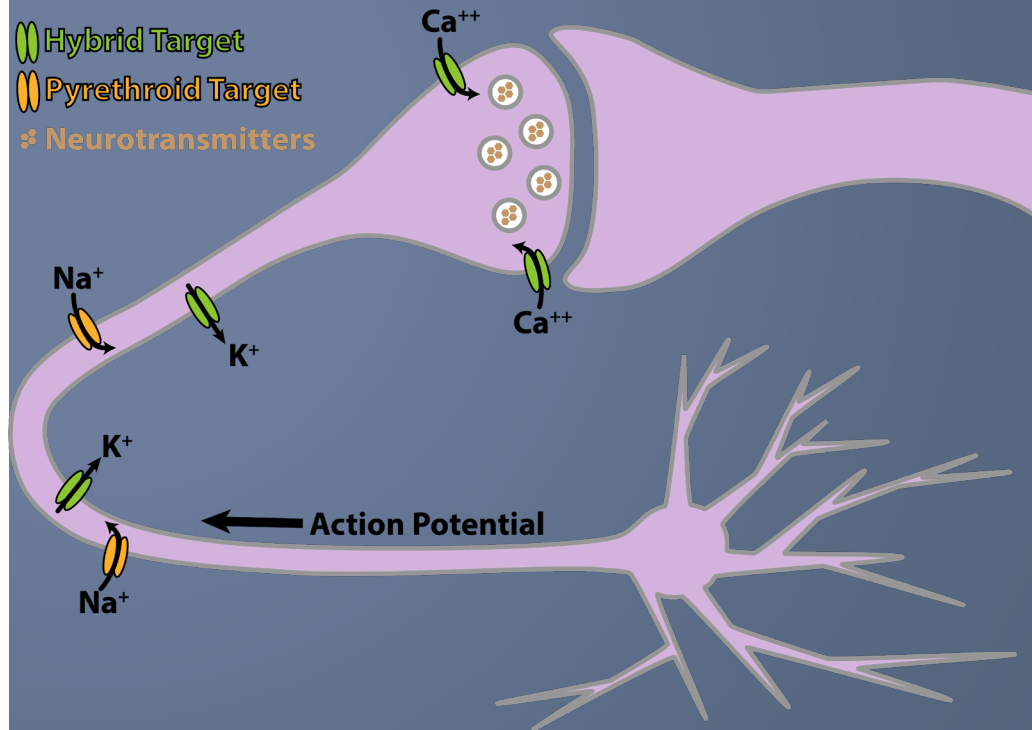
Before dying Mosquitoes may

- transmit Plasmodium
- lay eggs

Transgenic strategies to controlling malaria with Fungi

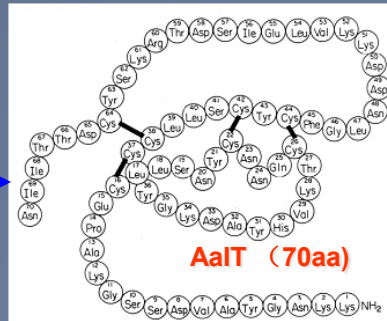


Expression of Hybrid toxin



Genetically engineering *Metarhizium*

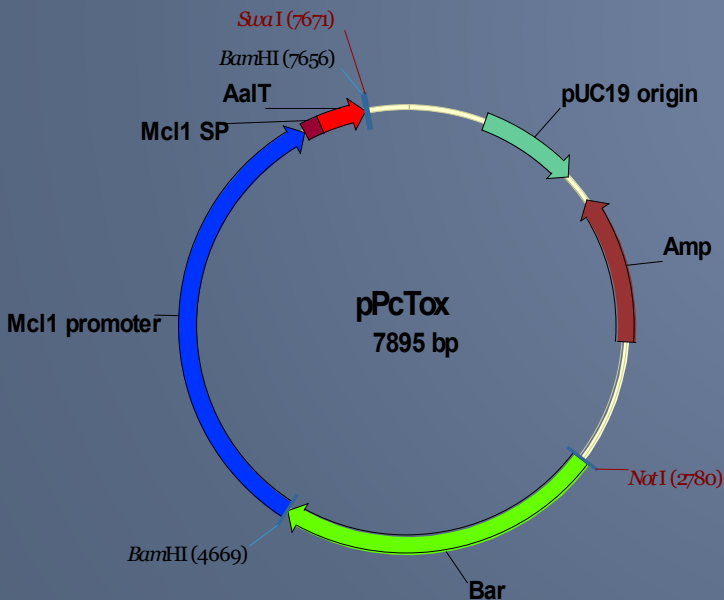
Wang and St. Leger. *Nature Biotechnology*, 2007. 25:1455.



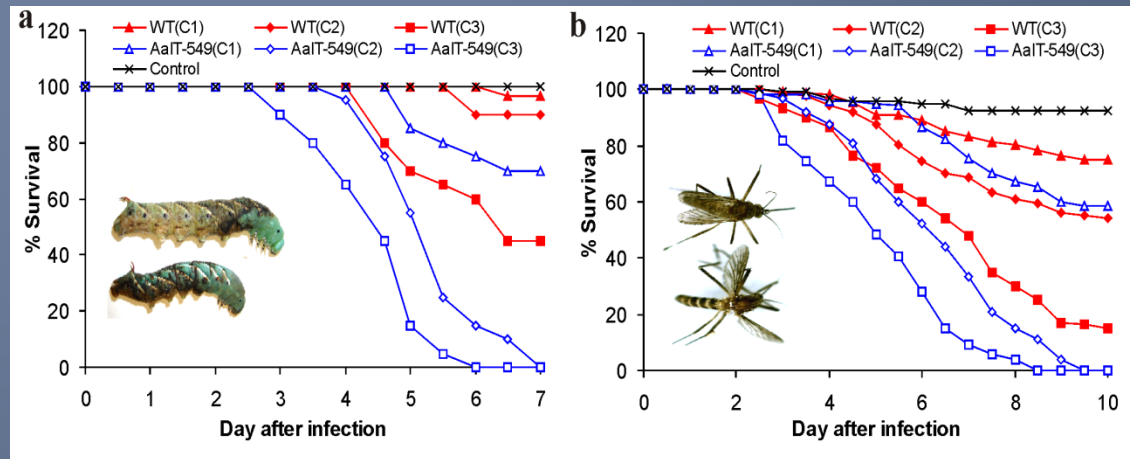
A

```

1  CCGGATCCGGTTCATGGAACATCACACTCGCTGACTCTGGACACCAACTGTATTCACTCG
   (BamHI)
61  CTAATTCCGTTCCCTGGCTCAAATCTTTTCGGTTCCTAGACCATCATGCGTGAACCTTCT
   M R E L S
121 TCGGTTCTCGCCCTTTCGGGCTTGGCTGGCCCTGGCGTAAAGAACGGCTACGCC
   S V L A L S G L L A L A S A K K N G Y A
181 GTCGATAGCAGCGGCAGGCCCGCCAGTGCCTGCTGAGCAACTACTGCAACAACCAAGTGC
26  V D S S G K A P E C L L S N Y C N N Q C
241 ACCAAGTCCACTACGCCGATAAGGCTACTGCTGCCTGCTGAGCTGCTACTGCTTCGGCC
46  T K V H Y A D K G Y C C L L S C Y C F G
301 CTGAACGATGATAAG AAGTCTTGGAGATCAGCGATACCCGTAAGAGCTACTGCGATACC
66  L N D D K K V L E I S D T R K S Y C D T
361 ACCATCATCAACTAAAGGATCCCG
86  T I I N * (BamHI)
    
```



Gene synthesis



Bioassays

Transgenic fungi in the Lab

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Improved efficacy of an arthropod toxin expressing fungus against insecticide-resistant malaria-vector mosquitoes

Etienne Bilgo¹, Brian Lovett², Weiguo Fang³, Niraj Bende⁴, Glenn F. King⁴, Abdoulaye Diabate¹ & Raymond J. St. Leger²

Received: 18 January 2017

Accepted: 27 April 2017

Published online: 13 June 2017

A single spore of Transgenic fungus Expressing Hybrid/AAIT is Sufficient to Kill mosquitoes

PLOS ONE

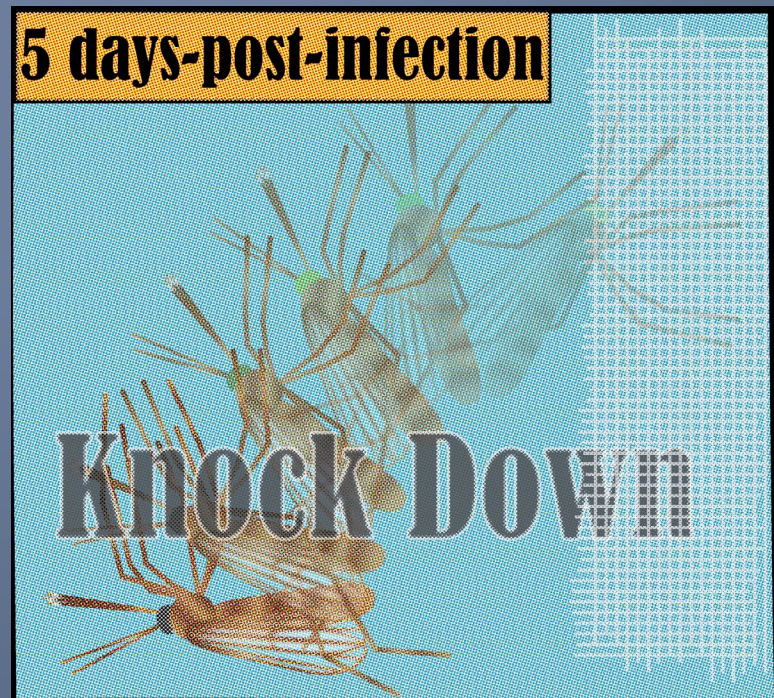
RESEARCH ARTICLE

Transgenic *Metarhizium pingshaense* synergistically ameliorates pyrethroid-resistance in wild-caught, malaria-vector mosquitoes

Etienne Bilgo^{1,2}, Brian Lovett³, Koama Bayili^{1,4}, Abel Souro Millogo¹, Issiaka Saré^{1,4}, Roch K. Dabiré¹, Antoine Sanon², Raymond J. St. Leger^{2*}, Abdoulaye Diabate¹

5 days-post-infection

Knock Down

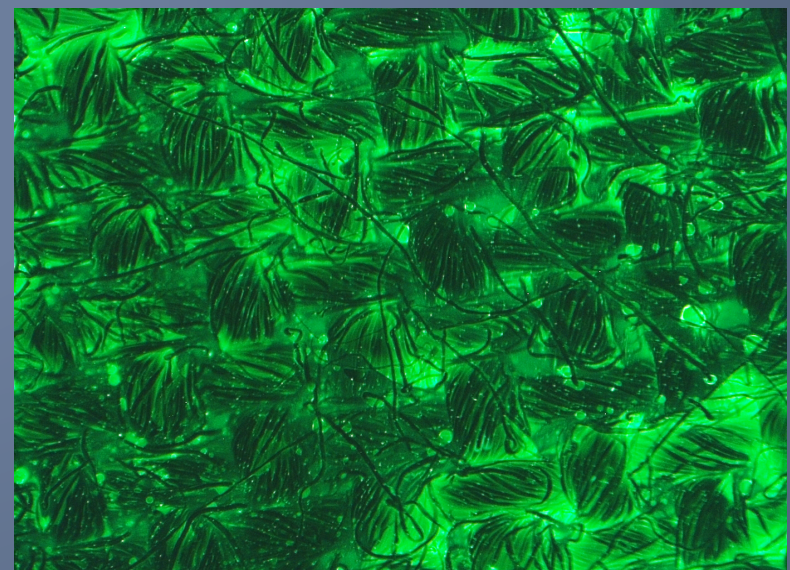




MosquitoSphere with with WHO Huts



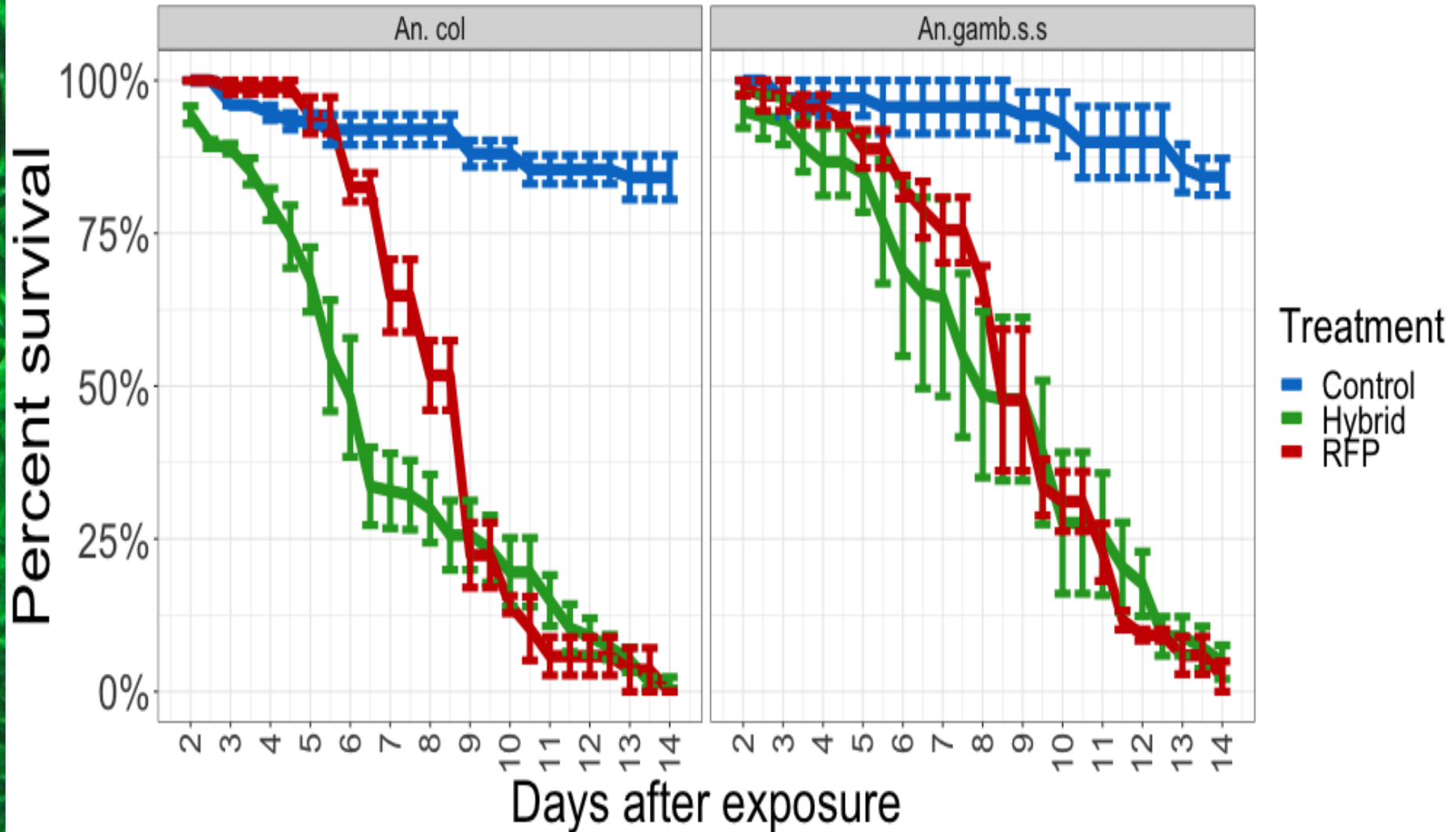
Release of mosquitoes



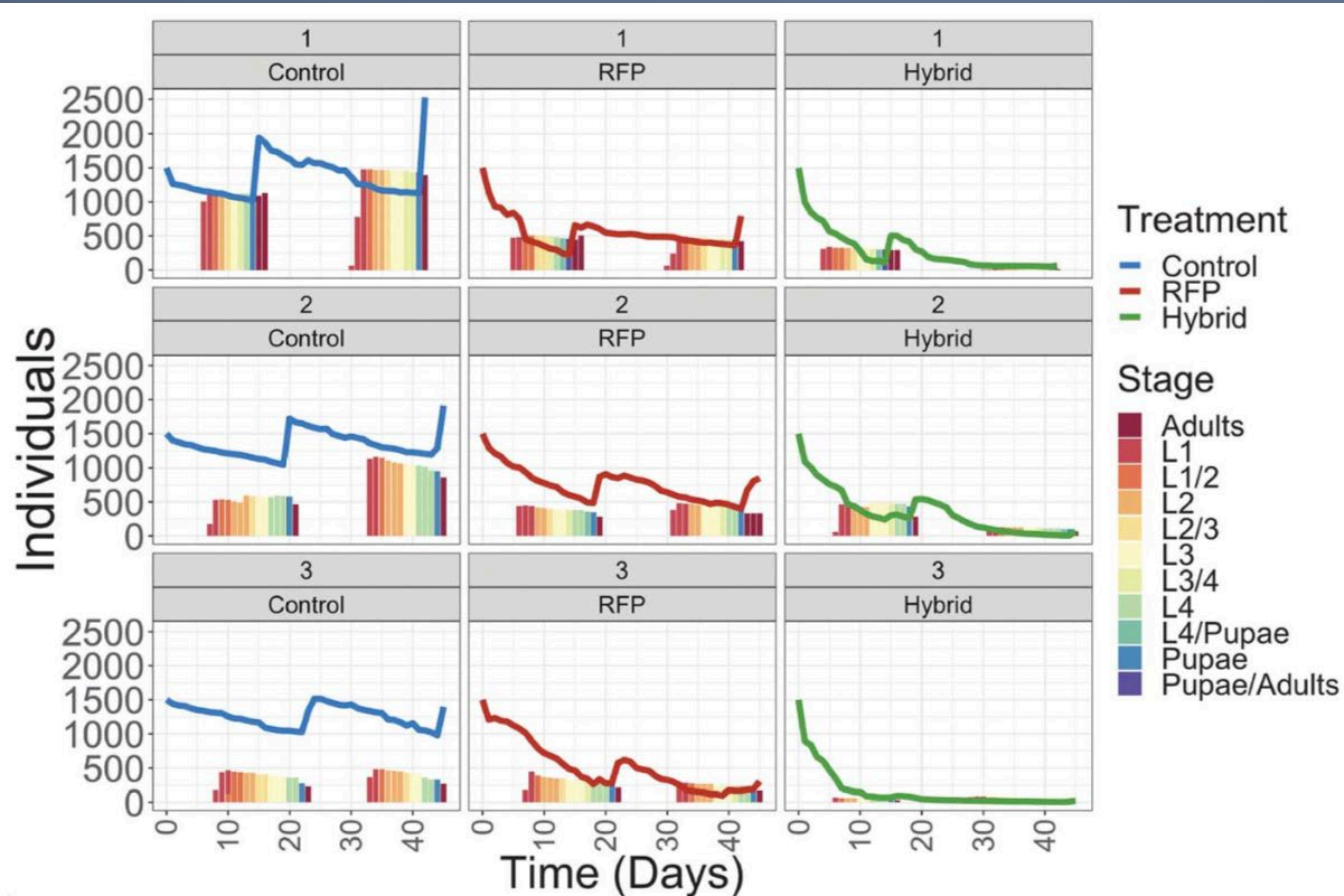
**8% oil formulation
 3.7×10^9 conidia/meter**

Increased Field Persistence

Week: 0



Generational control of mosquito populations with Transgenic *Metarhizium* in Semi field



F₀: 1000 virgin Males
X
500 virgin Females

Hut with Transgenic
Mp

F₁: ~400 Adults

F₂: ~13 Adults

Hut with a wild Mp

F₁: 436 Adults

F₂: 455 Adults

Control hut: cloth treated with
sesame oil alone

F₁: increased by ~900 Adults

F₂: increased by ~1400 Adults

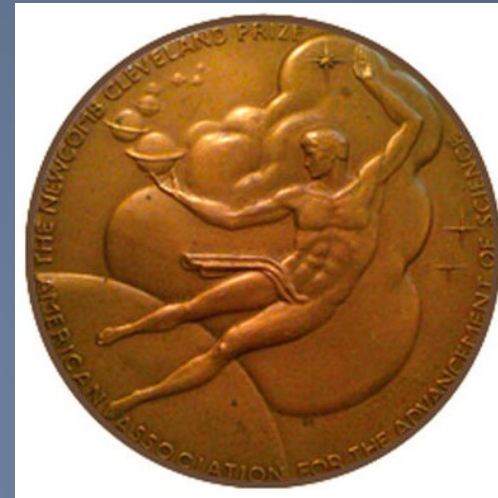
MALARIA CONTROL

Transgenic *Metarhizium* rapidly kills mosquitoes in a malaria-endemic region of Burkina Faso

Brian Lovett^{1*}, Etienne Bilgo^{2*}, Souro Abel Millogo², Abel Kader Ouattarra², Issiaka Sare², Edounou Jacques Gnambani², Roch K. Dabire², Abdoulaye Diabate^{2†}, Raymond J. St. Leger^{1†}

This is an important milestone building off decades of ongoing research into the biosafety and biology of these transgenic fungi

AAAS Newcomb Cleveland Prize



OPEN ACCESS Freely available online



Enhanced UV Resistance and Improved Killing of Malaria Mosquitoes by Photolyase Transgenic Entomopathogenic Fungi

Weiguo Fang^{1*}, Raymond J. St. Leger²

Bilgo et al. *Parasites & Vectors* (2018) 11:209
<https://doi.org/10.1186/s13071-018-2796-6>

Parasites & Vectors

SHORT REPORT

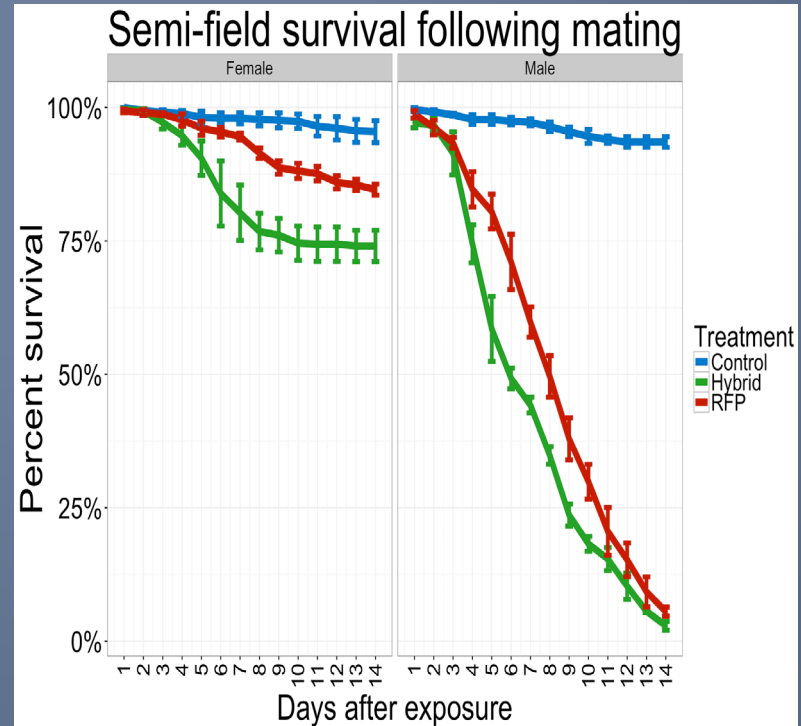
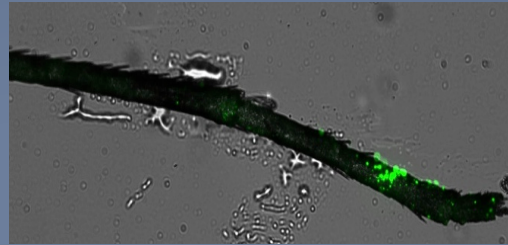
Open Access

Native entomopathogenic *Metarhizium* spp. from Burkina Faso and their virulence against the malaria vector *Anopheles coluzzii* and non-target insects



Etienne Bilgo^{1,3*}, Brian Lovett², Raymond J. St. Leger², Antoine Sanon³, Roch K. Dabiré¹ and Abdoulaye Diabaté¹

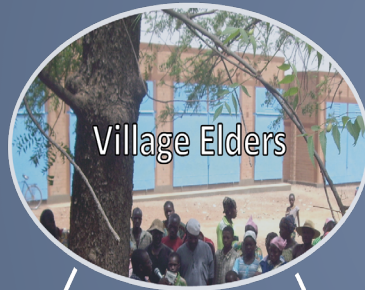
Metarhizium as an STD

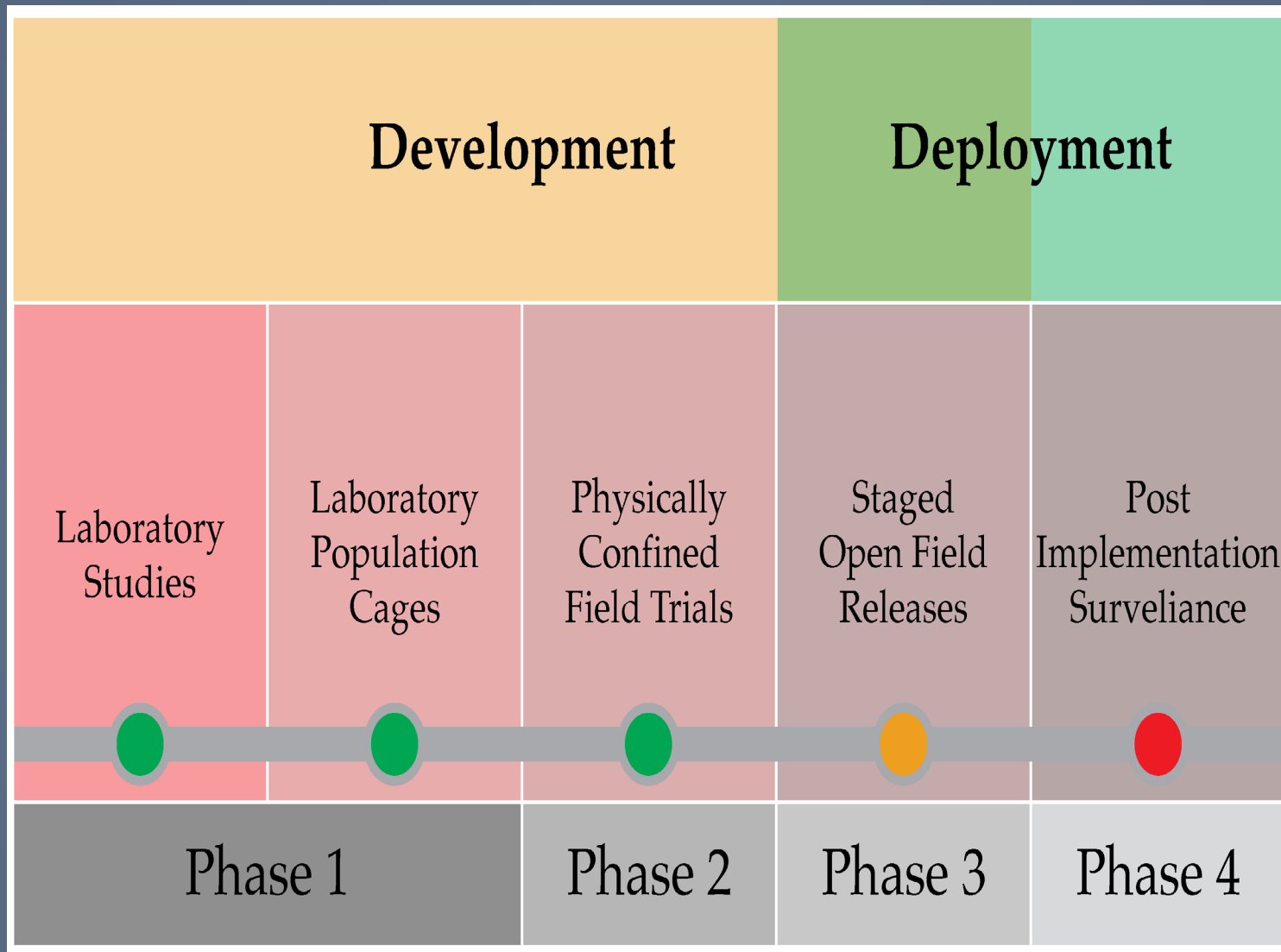


Social Anthropology: Entry Points Into Villages



Administrative and
Traditional Authorities
(Mayor, Counsellor, Prefect,
Chief of Village or Land)





Transition Go/No-Go Criteria: Efficacy and safety endpoints, regulatory approvals, social acceptance