Problem formulation consultations for gene drive modified mosquitoes designed to reduce malaria transmission in Africa

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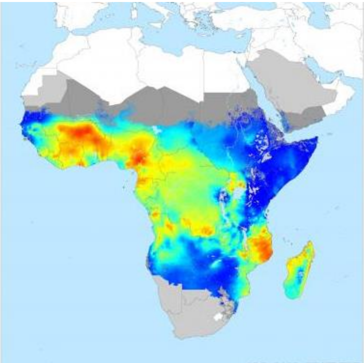
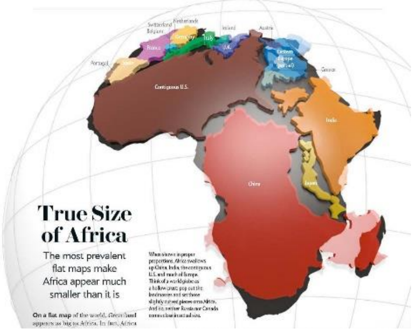
Different types of drive

- Self-limiting
  - Transient drive
    - Unable to pass the modification on indefinitely
    - Will eventually be eliminated due to fitness costs
- Self-sustaining
  - Heritable modifications can persist and spread
    - Threshold-dependent
      - Must exceed a critical frequency in the population to spread
    - Threshold-independent
      - Able to spread from very low initial population frequencies
- Different characteristics are suited for different situations





Focus on threshold-independent drive for control of malaria transmission in Africa

Problem Formulation Workshop, 2016

- 3-day workshop held in Reston, Virginia, May 25-27, 2016
- > 40 international experts including vector biologists, malaria researchers, public health officials, and regulatory officials
- Exercise in problem formulation to identify plausible risks of *Anopheles gambiae* mosquitoes modified with threshold-independent gene drive systems
- Background on technology and mosquito biology; introduction to problem formulation; discussion of hypothetical case studies; consideration of protection goals, potential hazards and pathways to harm

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Perspective Piece

Results from the Workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes"

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Abstract. Reducing the incidence of malaria has been a public health priority for nearly a century. New technologies and associated vector control strategies play an important role in the prospect of sustained reductions. The development of the CRISPR/Cas9 gene editing system has generated new possibilities for the use of gene-drive constructs to reduce or alter vector populations to reduce malaria incidence. However, before these technologies can be developed and exploited, it will be necessary to understand and assess the likelihood of any potential harms to humans or the environment. To begin this process, the Foundation for the National Institutes of Health and the International Life Sciences Institute Research Foundation organized an expert workshop to consider the potential risks related to the use of gene drives in *Anopheles gambiae* for malaria control in Africa. The resulting discussion yielded a series of consensus points that are reported here.

INTRODUCTION

The control of malaria has been a global public health priority for almost 100 years.[1] Concerted efforts in the 21st century by national governments, international bodies, and civil society organizations supporting public health programs to reduce the spread and impact of malaria have reduced the incidence of infection by 37% globally and mortality by 60% since 2000. In Africa, it is estimated that measures against malaria vectoring mosquitoes, notably the use of long-lasting insecticidal bed nets (LLINs) and house spraying with residual insecticides (IRS), have contributed ~78% of all gains accrued against malaria since 2000. Nevertheless, more than 3 billion people remain at risk for malaria infection, and more than 200 million cases and over 400,000 deaths have been attributed to malaria in 2015.† This burden falls disproportionately on sub-Saharan Africa, where the deadliest malaria parasite, *Plasmodium falciparum*, coexists with the most efficient malaria vectors, including *Anopheles gambiae* and *Anopheles funestus*.

Despite the key successes already achieved by major malaria vector control tools like LLINs and IRS, new complementary technologies are constantly being developed and evaluated for use in control programs to bridge existing gaps and accelerate progress toward eventual malaria elimination. Practical advances in molecular biology, particularly the successful use of the CRISPR/Cas9 gene editing machinery to create gene drive,‡ are envisioned to make more practical and realizable large-scale campaigns to drastically suppress malaria vector populations or alter these populations so they no longer transmit disease.[2,3] However, before these methods can be developed and deployed, it will be necessary to assess their potential to cause harm to human health and the environment.

With the goal to inform research programs, public health and donor organizations and government regulators about plausible risks related to potential uses of gene-drive technology in mosquitoes, the Foundation for the National Institutes of Health convened a 3-day workshop involving expert participants with diverse perspectives to identify the hazards. The workshop, which was facilitated by the International Life Sciences Institute Research Foundation's Center for Environmental Risk Assessment, applied a problem formulation approach to identify plausible risks using case studies. The case studies were developed to illustrate realistic applications of gene drive for malaria vector control in sub-Saharan Africa, particularly focusing on *A. gambiae*.

CONDUCT OF THE WORKSHOP

International experts including researchers, public health officials, and regulatory officials participated in the 3-day program. The first day consisted of background lectures on 1) the use of CRISPR/Cas-9 system to drive genetic constructs,[4,5] 2) the biology of *A. gambiae*,[6] and 3) the problem formulation process for planning risk assessment and identifying relevant concerns.[7–9] Participants then conducted a problem formulation exercise based on case studies providing background information and hypothetical examples illustrating potential uses of gene-drive strategies for population suppression and population alteration by either introducing genes encoding novel proteins, or editing endogenous genes in the mosquito. The end result was the identification of pertinent environmental/ecological protection goals that could plausibly be impacted by releasing genetically modified mosquitoes, followed by discussion of how those protection goals might reasonably be impacted. Consensus from these discussions was then drafted to inform ongoing

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†http://www.who.int/malaria/en/

‡"Gene drive" here refers to the altered inheritance of any allele such that it is transmitted to most offspring, rather than the 50% expected through Mendelian inheritance. This can be achieved, theoretically, through a variety of mechanisms including, for example, naturally occurring selfish genetic elements.

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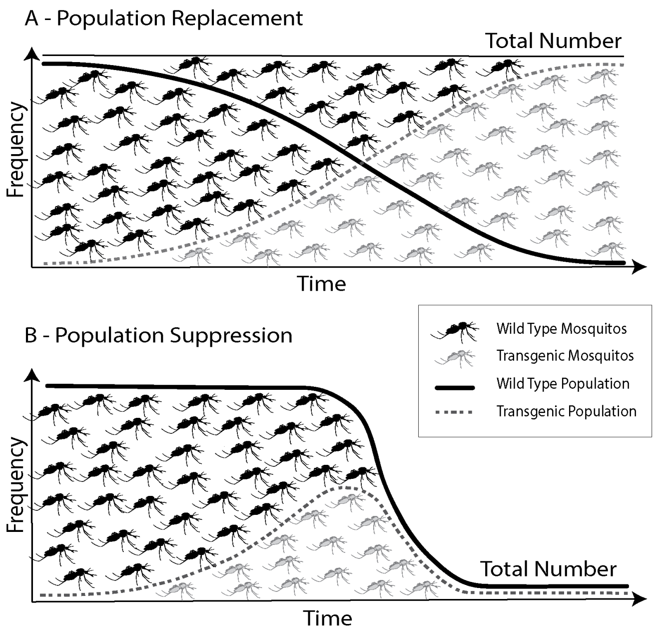
https://fnih.org/what-we-do/programs/gene-drive-guidance-documents-reports

Case studies

- Considered 4 *hypothetical* case studies
  - Population replacement or population suppression
  - Introduction or editing
- Recognized different implications for environmental interactions
  - Gene-drive mosquitoes for population replacement are designed to persist in the environment over a relevant time
  - Gene-drive mosquitoes for population suppression are designed to eventually reduce in numbers in the environment over a relevant time

Gene drive strategies:



Review of Protection Goals

- Pertinent Broad Protection Goals
  - **Human Health**
  - **Animal Health (livestock)**
  - **Biodiversity**
    - Including threatened and valued species as well as ecologically important organisms
    - Ecosystems through interactions with other organisms
  - **Water Quality** – identified as plausible but unlikely

- Non-Pertinent Broad Protection Goals
  - **Soil Quality**
  - **Air Quality**
  - **Natural Resources (other than biodiversity)**
    - Biogeochemical process, minerals, forestry, fisheries
  - **Agricultural Production (excluding animal health)**

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Health

- **The relevant interaction for human health is biting**
  - Consider
    - Potential for increased transmission of malaria parasites or other locally transmitted blood-borne pathogens, or alterations in pathogen virulence
    - Toxicity/allergenicity of novel proteins expressed in *Anopheles gambiae* saliva, including components of the gene drive and markers
  - Unlikely
    - Harm from incidental exposure through inhalation, ingestion, etc.
    - Horizontal gene flow to humans

CDC/James Gathany

- **Similar conclusions for animal health**

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Biodiversity

- ***Anopheles gambiae* is considered a public health threat**
- ***An. gambiae* interacts with other species by feeding on them, serving as prey, or as a competitor**
  - Consider
    - Known interactions with threatened, endangered, or valued species
    - Species for which *An. gambiae* is known to be a crucial food source
    - Toxicity of introduced proteins
  - Unlikely
    - Harm to ecosystem services by reduction/elimination of *An. gambiae*
      - Not considered a keystone species
      - Not known to provide non-redundant ecosystem services
      - No environmental impact noted in areas where *An. gambiae* has been greatly reduced
    - Harm from horizontal gene transfer to a non-target species
      - Gene flow to some members of the *An. gambiae* complex is possible but unlikely to cause harm
      - Gene flow to other species unlikely on a relevant time scale
    - Harm due to incidental contact with other organisms

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Other considerations

- Gene drive-modified mosquitoes should be used as part of integrated vector management
- Potential harms should be considered in the context of other vector and malaria control methods
- Failure to sustain any form of vector control may result in malaria resurgence; gene drive-modified mosquitoes are not different in this regard

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African regional consultations

- Organized by the African Union Development Agency – New Partnership for Africa’s Development
    - Accra, Ghana, Oct. 17-19, 2016
    - Nairobi, Kenya, June 20-22, 2017
    - Gabarone, Botswana June 26-28, 2017
    - Libreville, Gabon, Feb. 20-22, 2018
- Representatives from regional human health and environmental agencies, local and international scientists, other government officials
- Same hypothetical case studies; introduction to problem formulation; brief discussion of protection goals and hazards – **not definitive**
- Manuscript submitted for publication

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Summary outcomes

- **Relevant protection goals**
  - Human health and biodiversity emphasized
  - Animal health and water quality identified by some groups
  - Soil quality, air quality, natural resources never/rarely raised

- **Suggestions of possible hazards**
  - Increased prevalence of mosquito-borne diseases
    - Expansion of other vectors due to vacant ecological niche (population suppression)
    - Increased transmission of other pathogens, increased mosquito fitness, increased virulence of malaria parasite (population replacement)
  - Harm to biodiversity
    - Decrease in predator populations (population suppression)
    - Displacement of other mosquito populations (population replacement)
  - Water quality
    - Increase in aquatic algae or bacteria (population suppression)
    - Reduced quality of drinking water
  - Animal health
    - Increase in livestock diseases
    - Effect on aquaculture

Other sources of information for hazard identification – *genetically modified mosquitoes*

- **Guidance Framework for testing genetically modified mosquitoes, WHO 2014**
  http://www.who.int/tdr/publications/year/2014/guide-fmrk-gm-mosquit/en/

- **Biosafety for human health and the environment in the context of the potential use of genetically modified mosquitoes, WHO 2015**
  https://apps.who.int/iris/handle/10665/180388

- **Guidance on Risk Assessment of Living Modified Organisms, Cartagena Protocol on Biosafety, 2016**
  https://www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf

Other sources of information for hazard identification – *gene drive*

- **Gene Drives on the Horizon, NASEM 2016**
  http://nas-sites.org/gene-drives/
- **Synthetic Gene Drives in Australia: Implications of Emerging Technologies, Australian Academy of Sciences 2017**
  https://www.science.org.au/files/userfiles/support/documents/gene-drives-discussion-paper-june2017.pdf
- **Pathway to Deployment of Gene Drive Mosquitoes as a Potential Biocontrol Tool for Elimination of Malaria in Sub-Saharan Africa: Recommendations of a Scientific Working Group AJTMH 2018**
  http://www.ajtmh.org/content/journals/10.4269/ajtmh.18-0083

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Health considerations

TABLE 1
Prominent safety considerations related to human and animal health

Potential harm	Example hazards	Assessment Parameters*
Increased disease transmission	Increased abundance of vector mosquitoes	Fitness components including[15]: Growth rate Mating success Fecundity Adult, egg, or larval survival Environmental tolerances
	Increased vectorial capacity	Host seeking and biting activity[15] Vector competence* (*Plasmodium* or other pathogens carried by *Anopheles gambiae*) Change in temperature tolerance that could affect environmental niche or range
	Reduced control capability	Insecticide resistance*
Increased direct pathology	Increased allergenicity	Known allergenic sequences expressed by construct; construct-encoded proteins detected in saliva
	Increased toxicity	Standard toxicity test on construct-encoded proteins
	Increased parasite virulence (population replacement)[96]	Genotypic or phenotypic changes in parasites after passage through gene drive mosquitoes†

* Changes to be assessed in comparison to local wild-type mosquitoes of the same genetic background.
† This would best be performed with gametocytes collected from the field testing site to reflect the diversity of parasite strains circulating at the location and will not predict the evolutionary consequences of ongoing interactions of the parasite with the mosquito and vertebrate host over time.

Biodiversity considerations

TABLE 2

Some considerations for possible effects of *Anopheles gambiae* containing gene drive constructs, extrapolated from consideration of biocontrol agents on nontarget species

Potential effect	Concern	Relevance for *An. gambiae*	Trigger for concern
First-order genetic	Construct might spread to a second species through interspecific mating.	May be anticipated and a deliberate part of the implementation strategy—for example, a gene introduced into *An. gambiae* s.s. is expected to spread into closely related vector species such as *Anopheles coluzzii* or *Anopheles arabiensis*. This would be useful for preventing malaria transmission by a second malaria vector, but the possibility of more distant gene transfer through interspecific mating also must be considered in risk assessment	Genetic evidence for low-frequency intraspecific mating outside the *An. gambiae* complex
Second-order genetic	Construct might spread through some other, non-mating, process to a second species	For example, the construct might move into a mobile genetic element that could be transferred through a microbial vector	Genomic evidence for the transfer of genetic material between mosquitoes and distantly related species
First order ecological	Removal of a species from a community might harm species that directly feed on it or which rely on the species for pollination.	The extent to which a predator or plant relies on *An. gambiae*. The harm done to *Plasmodium* through the removal of its vector is an example of a deliberate, anticipated, and beneficial first order ecological effect, but the possibility of detrimental effect on other, more valued, species also should be considered	Evidence that *An. gambiae* s.l. makes up a considerable fraction of the diets of specific predators in the same ecosystem, or that particular plants are largely pollinated by these species
Second order ecological	An indirect ecological effect resulting from removal of a species allows an increase in the density of another species (or resource) on which it fed (first order effect), which in turn allows a competitor species to increase in density by utilizing the unused resource	Removal of *An. gambiae* might result in increased abundance of another species, with detrimental effects	Presence in the same larval habitats as *An. gambiae* of other species of mosquito that share the same food source and pose a worse threat to human health; evidence of indirect ecological effects, including adaptation of the malaria parasite that have arisen after other successful interventions that have reduced *An. gambiae* density (such as bed nets)
Higher order ecological	An ecological perturbation causes further effects that ripple through the ecological community, and which are amplified rather than being damped	Addition or removal of a keystone species have major effects in ecological communities	A plausible mechanism based on comparative ecological studies showing how *An. gambiae* could act as a keystone species

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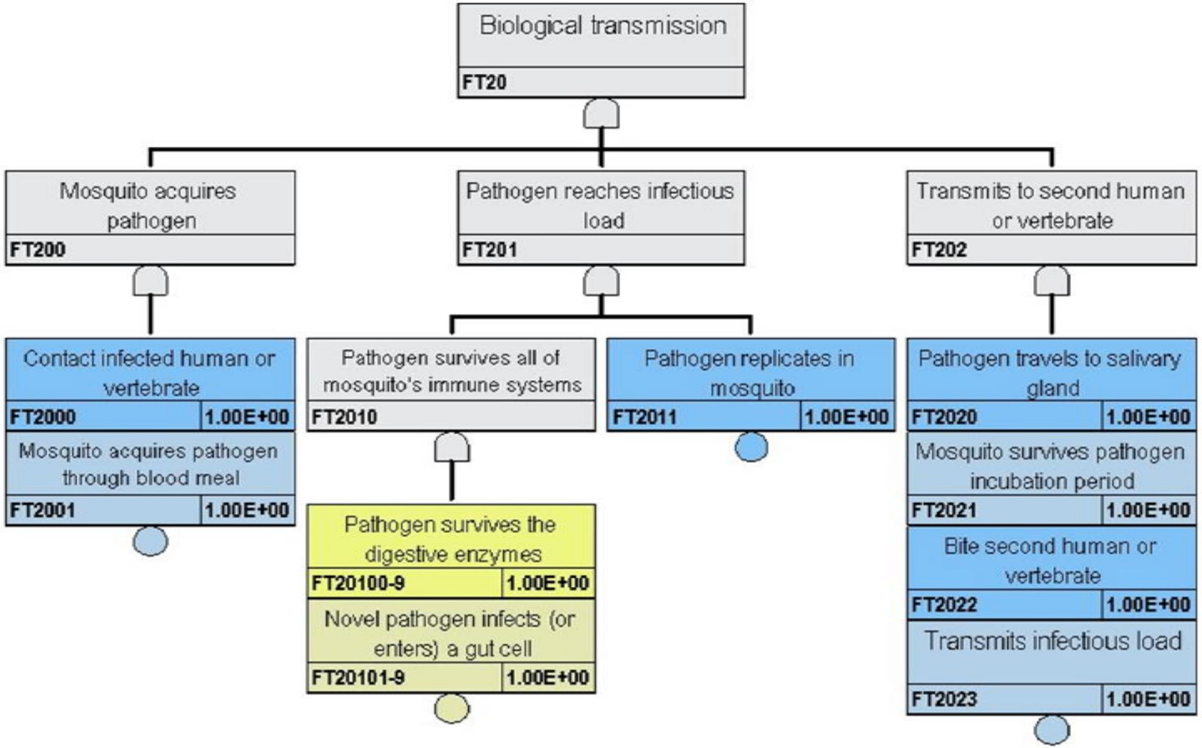
Example Fault Tree Analysis - Probability that a mosquito transmits a novel blood borne pathogen



- Transmission of a novel pathogen
- Spread of construct in non-target eukaryotes
- Spread of construct in non-eukaryotes

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(https://fnih.org/what-we-do/programs/gene-drive-guidance-documents-reports; https://targetmalaria.org/resources/)

Final thoughts

- Multiple early efforts to identify concerns regarding gene drive-modified mosquitoes by diverse experts
  - Technical documents discuss relevant hazards for genetically modified and gene drive mosquitoes
  - Consultative workshops, while limited, reached a broader group of stakeholders
  - Probabilistic risk assessments for GM sterile male *An. gambiae* strain
  - More to be done
- Most discussed protection goals involved human health and biodiversity
  - Human health considerations were most often related to altered pathogen transmission
  - Potential for harm to mosquito predators was a widely raised biodiversity concern
- African consultations identified similar protection goals and pathways to US workshop, but
  - More interest in potential changes in mosquito behavior
  - More interest in potential harm to aquatic habitats
- Results will inform future environmental risk assessment by identifying:
  - Potential harms of broad concern
  - Data that will be required to decrease uncertainties

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