The Challenge of Arthropod Biocontainment in the Non-model Organism World: Mosquitoes, Gene Drive, and Beyond

Zach N. Adelman, Dept of Entomology

Insect
Molecular
Biology

Sindbis virus-induced silencing of dengue viruses in mosquitoes

Z. N. Adelman, C. D. Blair, J. O. Carlson, B. J. Beaty, K. E. Olson

First published: 20 December 2001 | https://doi.org/10.1046/j.1365-2583.2001.00267.x |

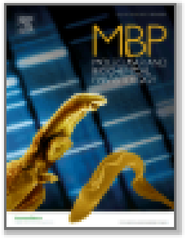
JOURNAL OF VIROLOGY, Dec. 2002, p. 12925–12933 Vol. 76, No. 24
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.24.12925–12933.2002

RNA Silencing of Dengue Virus Type 2 Replication in Transformed C6/36 Mosquito Cells Transcribing an Inverted-Repeat RNA Derived from the Virus Genome

Zach N. Adelman,† Irma Sanchez-Vargas, Emily A. Travanty, Jon O. Carlson, Barry J. Beaty, Carol D. Blair, and Ken E. Olson*

Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

Molecular and Biochemical Parasitology
Volume 121, Issue 1, 30 April 2002, Pages 1-10



Review: Insect vector biology and genetics. 1

Development and applications of transgenesis in the yellow fever mosquito, *Aedes aegypti*

Zachary N Adelman, Nijole Jasinskiene, Anthony A James

ELSEVIER

Methods

Volume 69, Issue 1, 15 August 2014, Pages 38-45

Targeted genome editing in *Aedes aegypti* using TALENs

Azadeh Aryan, Kevin M. Myles, Zach N. Adelman

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
DOUBLE-STRAND DNA BREAKS
GENE TARGETING
TRANSGENIC ORGANISMS
NON-MODEL ORGANISMS

Germline excision of transgenes in *Aedes aegypti* by homing endonucleases

Azadeh Aryan, Michelle A. E. Anderson, Kevin M. Myles & Zach N. Adelman

Fralin Life Science Institute and Department of Entomology, Virginia Tech, Blacksburg, VA 24061.

PNAS

Silencing of end-joining repair for efficient site-specific gene insertion after TALEN/CRISPR mutagenesis in *Aedes aegypti*

Sanjay Basu[a,1], Azadeh Aryan[a,1], Justin M. Overcash[a], Glady Hazitha Samuel[a], Michelle A. E. Anderson[a], Timothy J. Dahlem[b], Kevin M. Myles[a,2], and Zach N. Adelman[a,2]

[a]Fralin Life Science Institute and Department of Entomology, Virginia Tech, Blacksburg, VA 24061; and [b]Mutation Generation and Detection Core, Health Science Center Core Research Facility, University of Utah, Salt Lake City, UT 84132

Edited by Anthony A. James, University of California, Irvine, CA, and approved February 24, 2015 (received for review February 6, 2015)

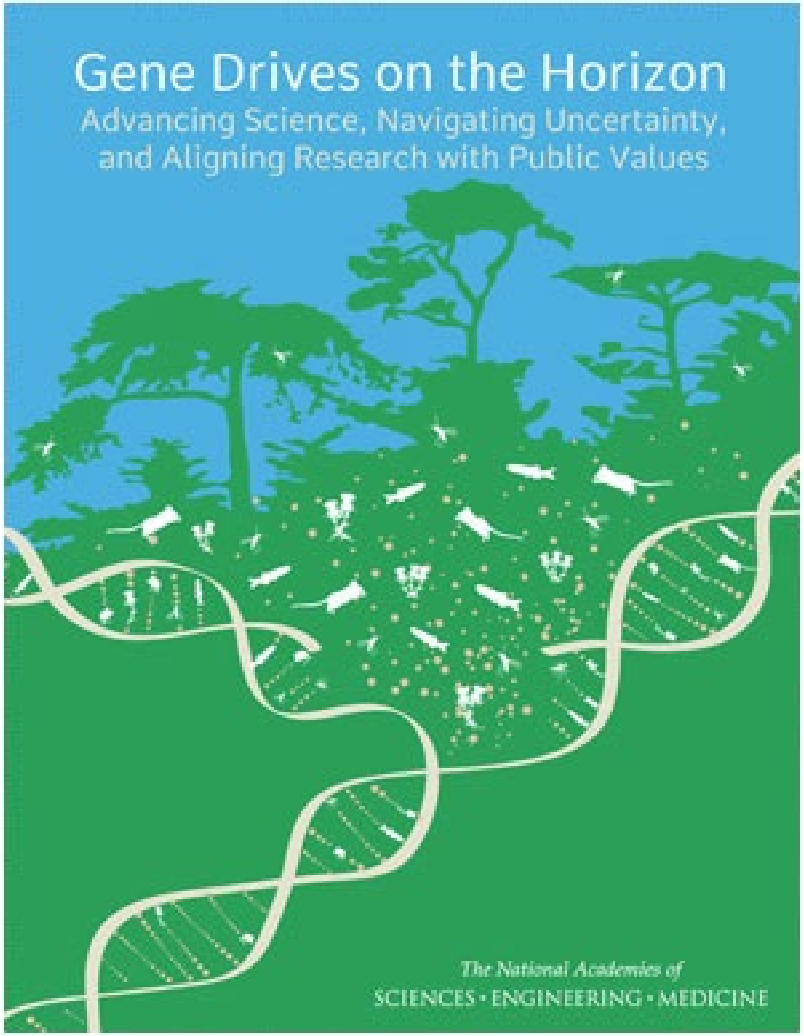
Genetic alteration of mosquito populations

Sex Determination: Conversion to males that do not bloodfeed.
→ *Nix is a dominant M-factor*

Physiology: Unable to digest blood or complete vitellogenesis.
→ Salivary proteins important in blood meal acquisition
→ Midgut proteins important in digestion

Immunity: Unable to support pathogen replication/transmission.
→ RNAi and the intertwined nature of small regulatory RNAs

DNA repair: Engineering the mosquito genome and improving gene
→ drive approaches



"Although there is insufficient evidence available at this time to support the release of gene-drive modified organisms into the environment, ***the likely benefits of gene drives for basic and applied research are significant and justify proceeding with laboratory research and highly-controlled field trials****."*

http://nas-sites.org/gene-drives/

Gene Drive

Gene drive in the news

ScienceNews
MAGAZINE OF THE SOCIETY FOR SCIENCE & THE PUBLIC

News: Genetics, Ecology

In lab tests, this gene drive wiped out a population of mosquitoes

Success with the genetic engineering tool raises hopes of eliminating the malaria carrier

By Tina Hesman Saey 11:20am, September 24, 2018

Perspective

Received: 29 March 2018 Revised: 6 July 2018 Accepted article published: 12 July 2018 Published online in Wiley Online Library: 18 September 2018

(wileyonlinelibrary.com) DOI 10.1002/ps.5137

Gene drive systems: do they have a place in agricultural weed management?

Paul Neve*

Gene drives in our future: challenges of and opportunities for using a self-sustaining technology in pest and vector management

James P. Collins

From Environmental Release of Engineered Pests: Building an International Governance Framework Raleigh, NC, USA. 5-6 October 2016

Gene drive in the news

Vox

Gene drives could end malaria. And they just escaped a UN ban.

The most important international summit you haven't heard of, explained.

By Dylan Matthews | @dylanmatt | dylan@vox.com | Dec 7, 2018, 9:30am EST

The Economist

Extinction on demand

The promise and peril of gene drives

A new genetic-engineering technology should be used with care

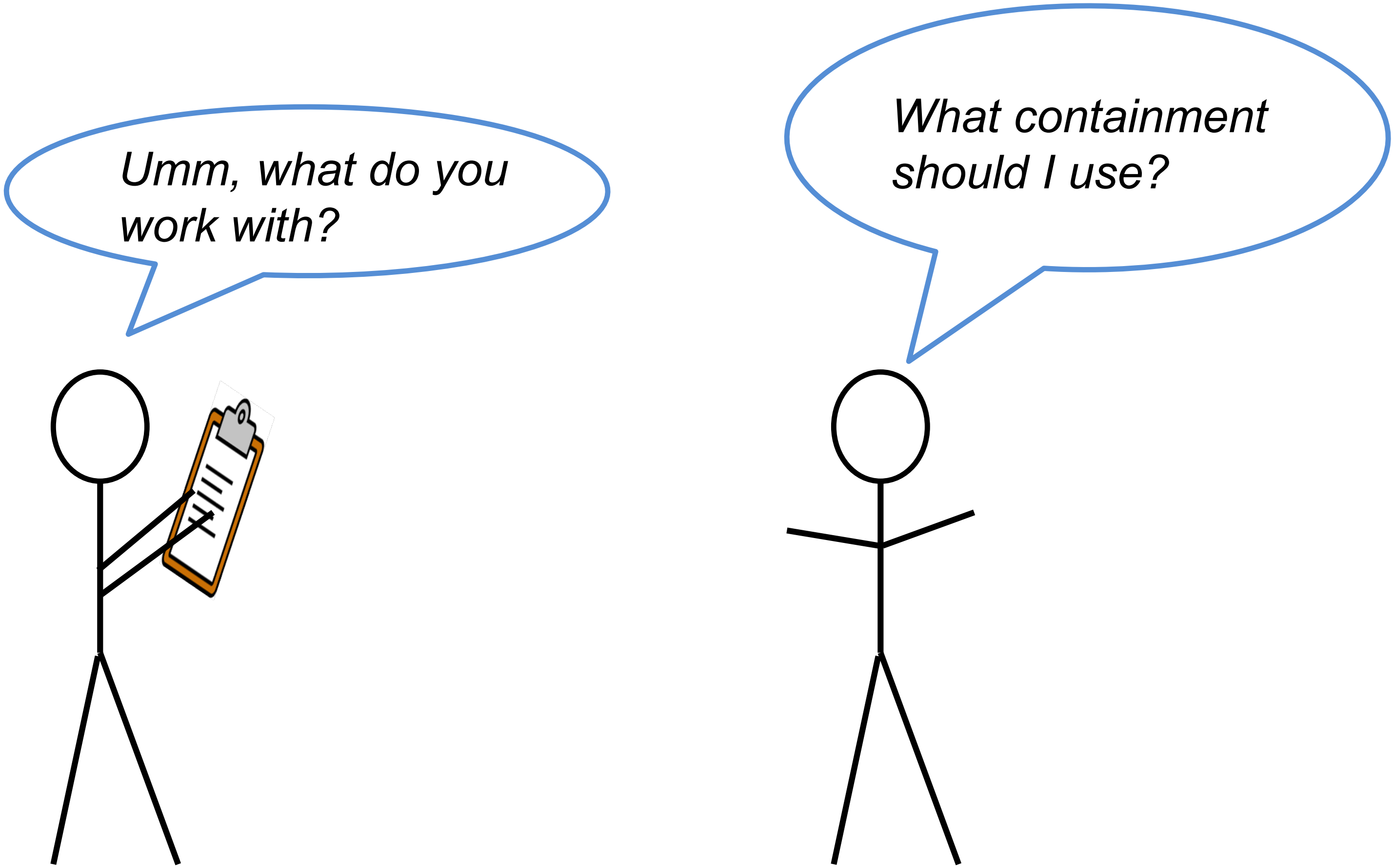
Gene Drive is:

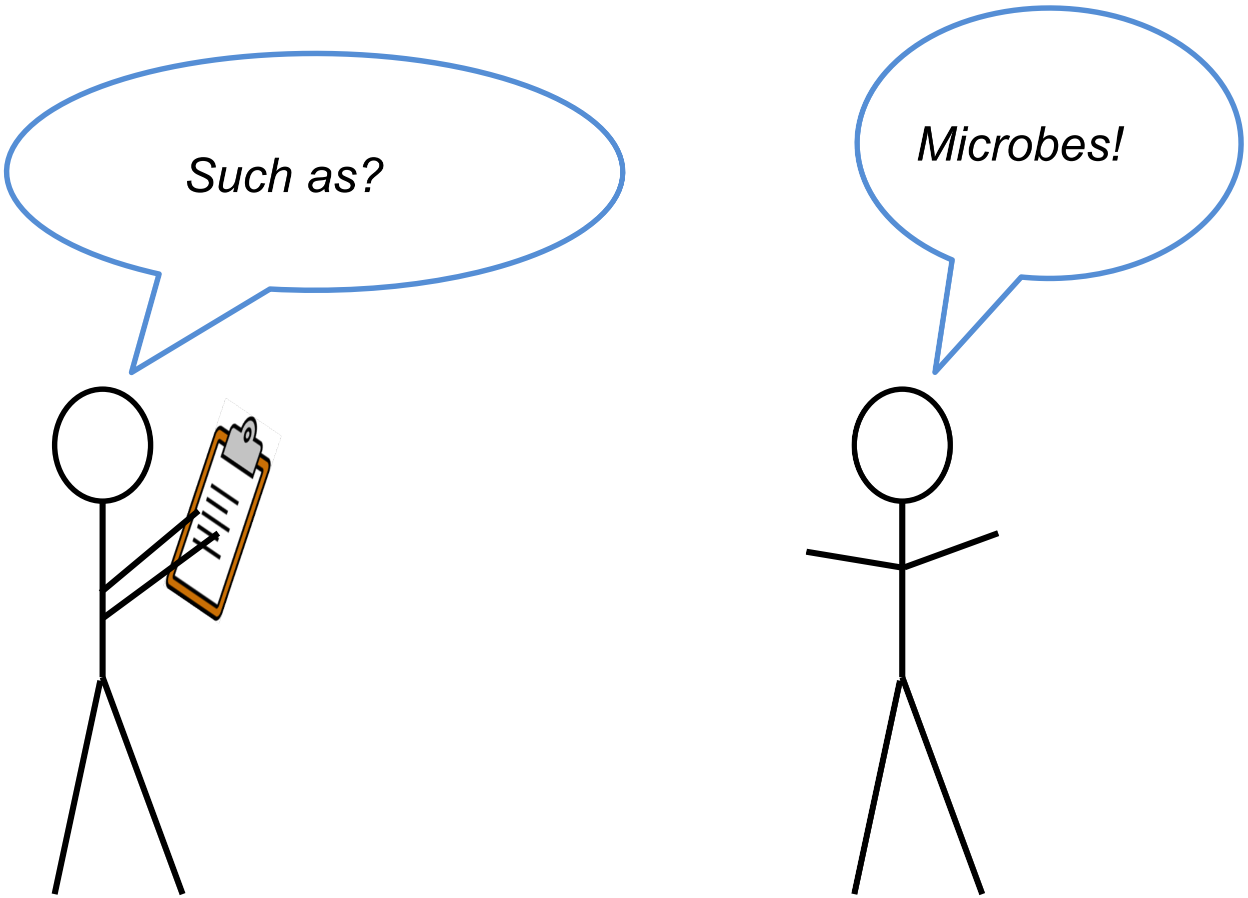
1) A completely new phenomenon in laboratory research

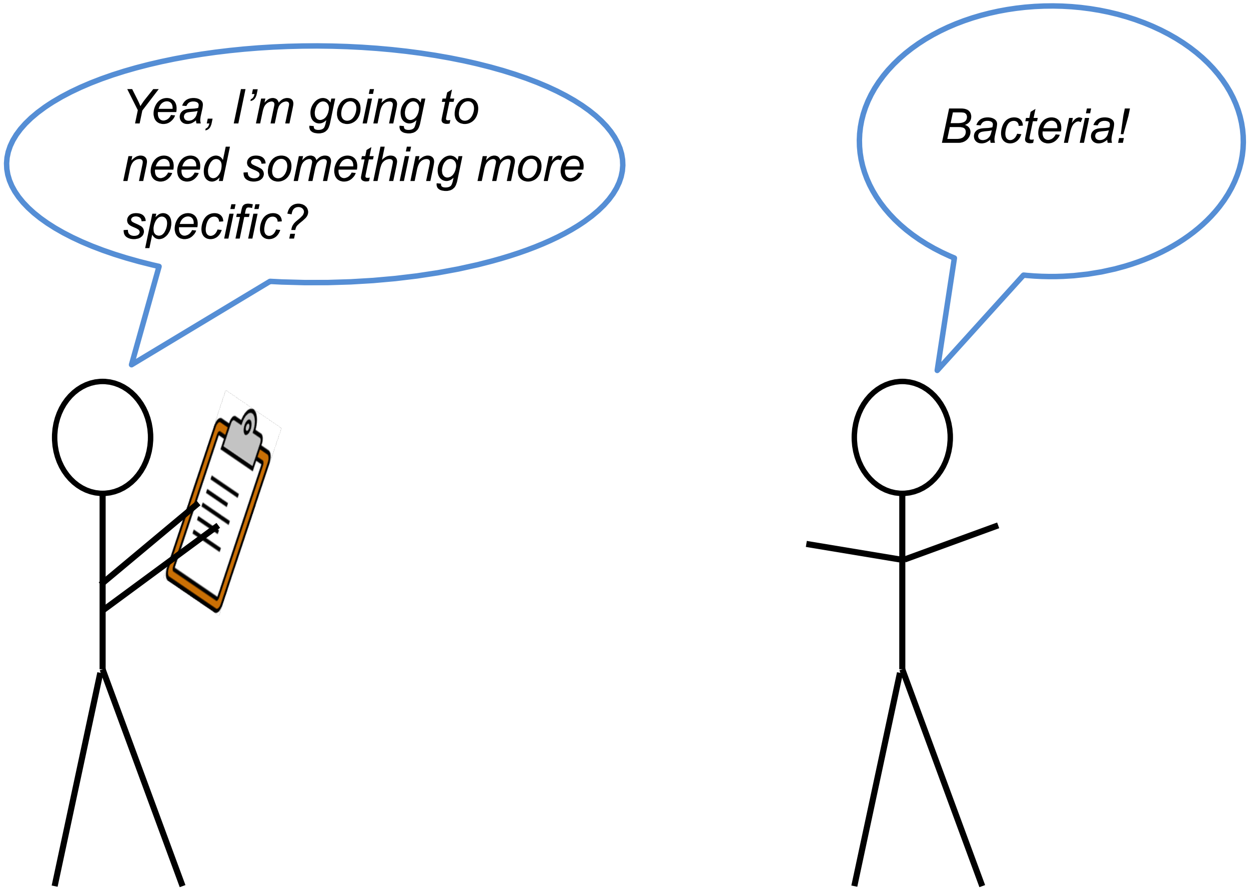
2) A process that completely breaks all laws of inheritance

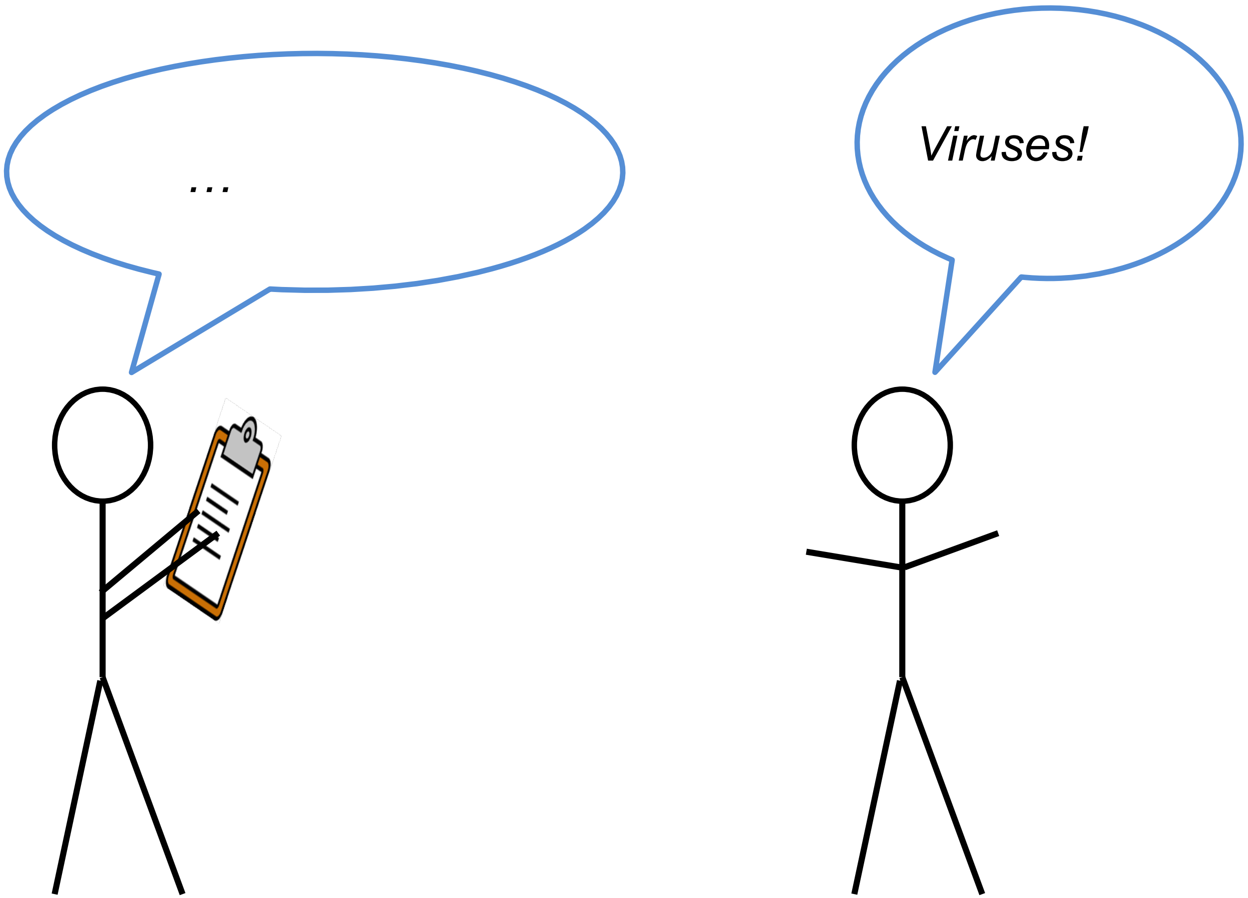
3) A really good way to get around town

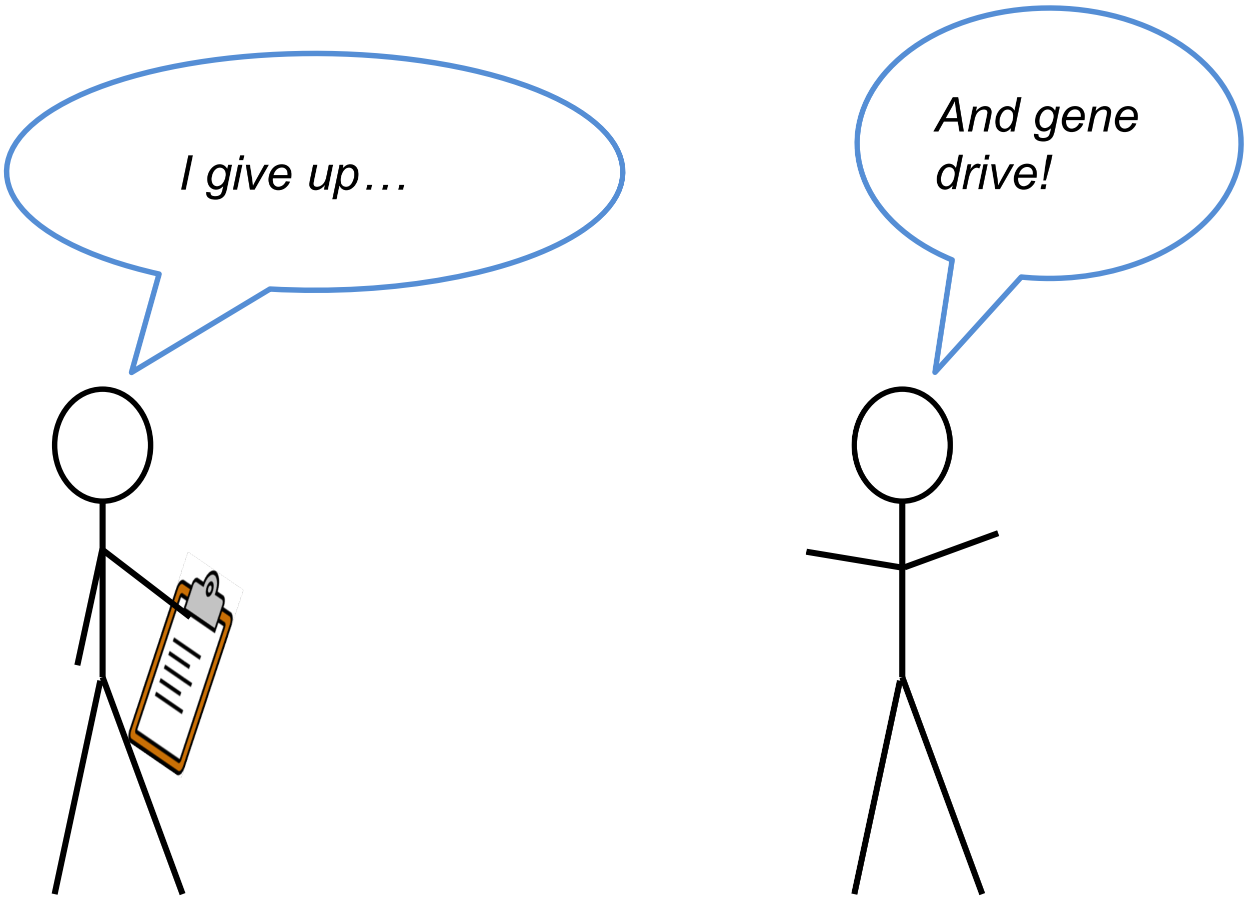
4) A term that has limited utility as a starting point for risk assessment.


Umm, what do you work with?
What containment should I use?


Such as?
Microbes!

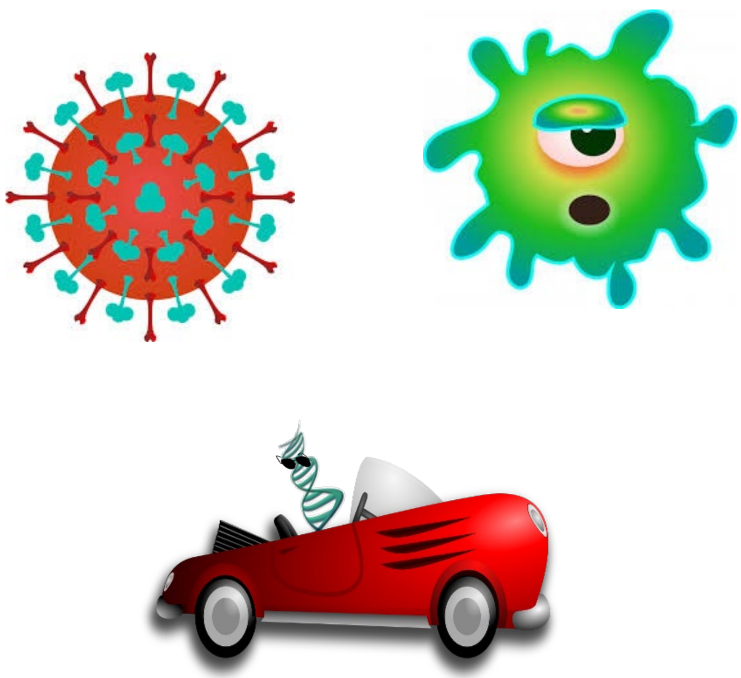

Yea, I'm going to need something more specific?
Bacteria!

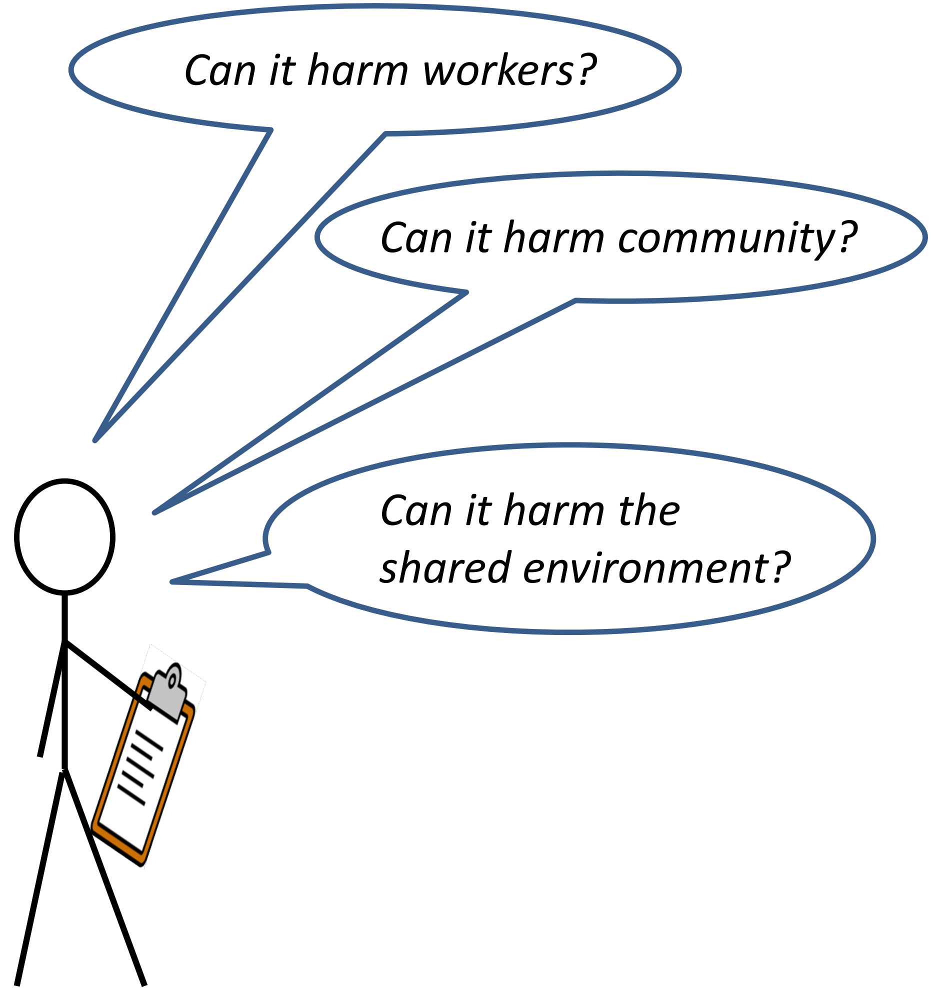

…
Viruses!


I give up…
And gene drive!

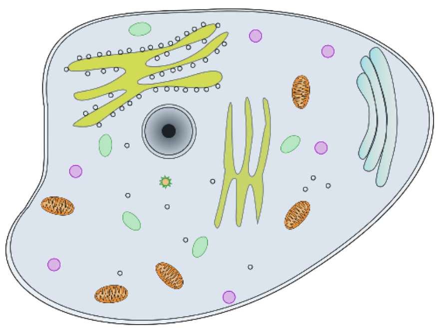
Agent

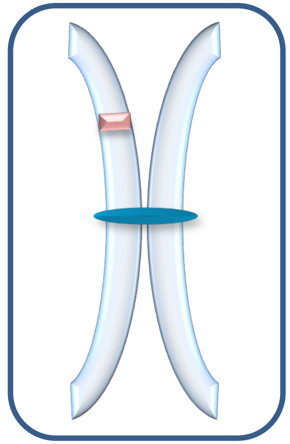
Risk Assessment



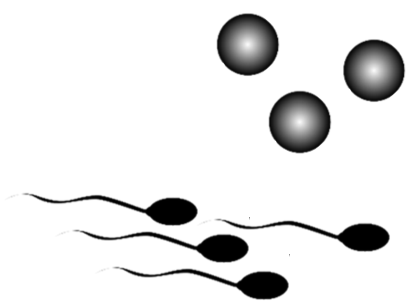


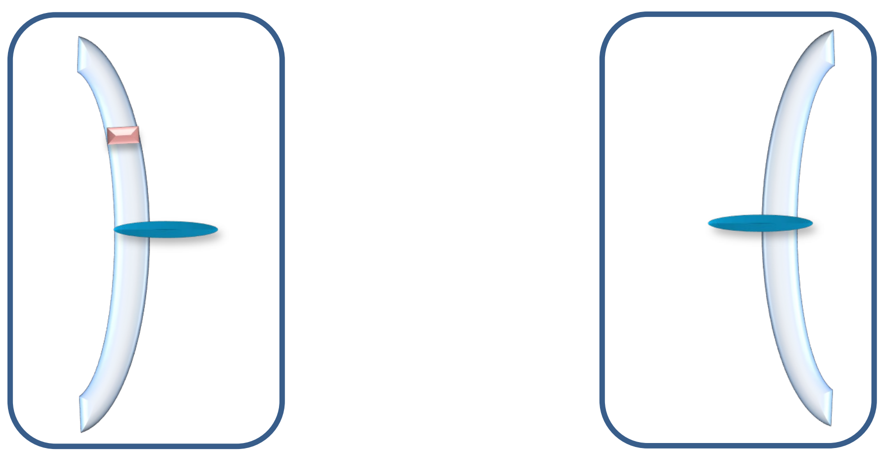
Mendelian inheritance of genes





Diploid (2 copies of each chromosome)

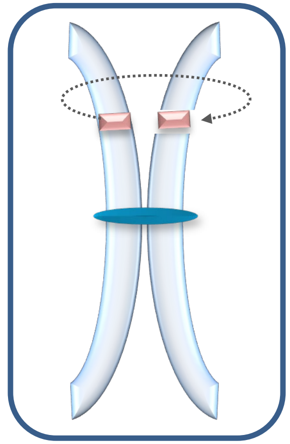




Half (50%) of gametes (eggs/sperm) carry the transgene

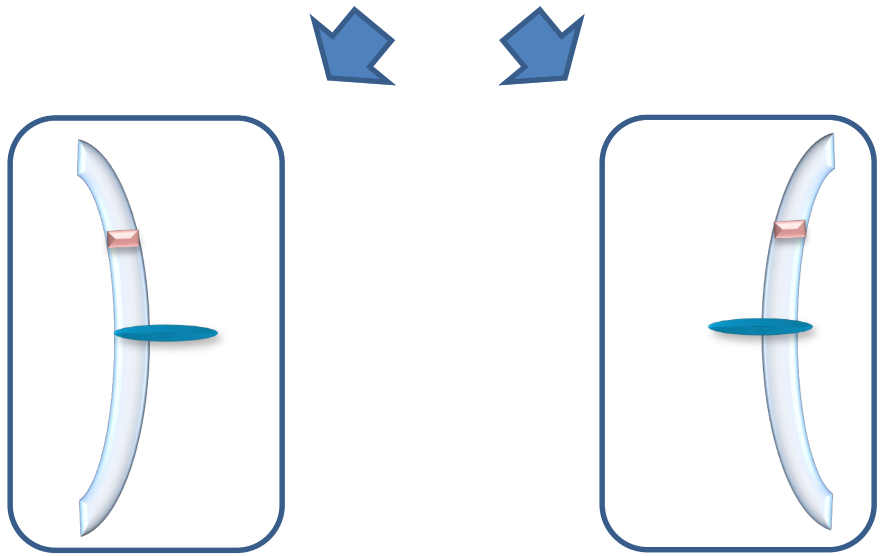
Haploid (1 copy of each chromosome)

Homing-based Gene Drive



Mode of inheritance is still the same

Diploid (2 copies of each chromosome)



All (100%) of gametes (eggs/sperm) carry the transgene

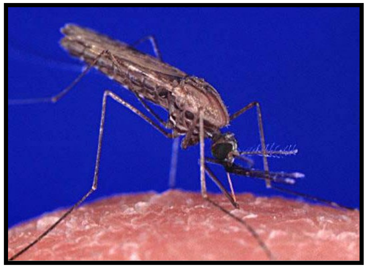
Haploid (1 copy of each chromosome)

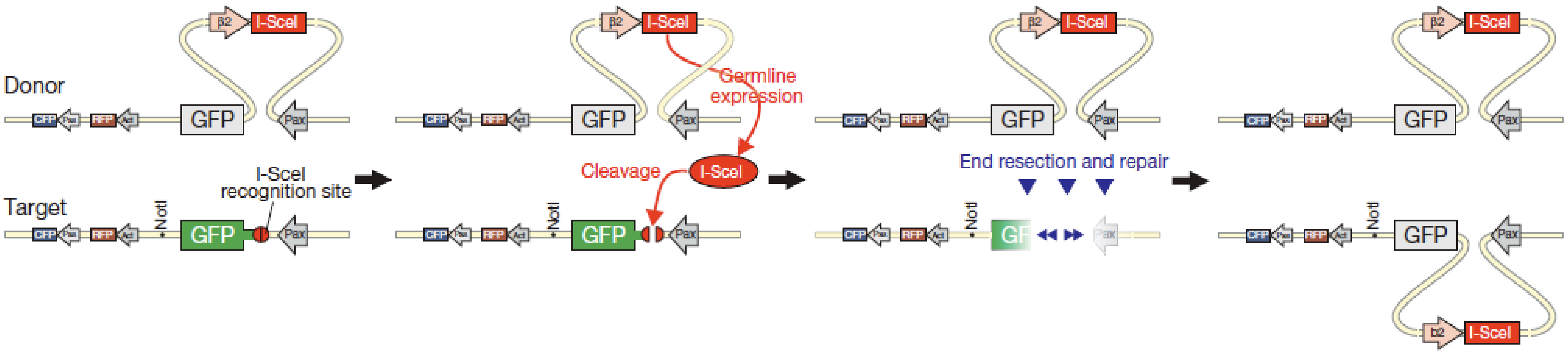
Homing-based Gene Drive

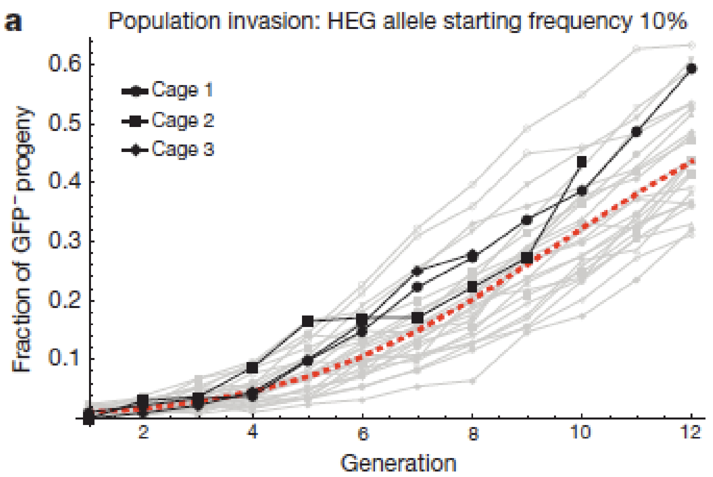
A synthetic homing endonuclease-based gene drive system in the human malaria mosquito

Nikolai Windbichler[1], Miriam Menichelli[1], Philippos Aris Papathanos[1], Summer B. Thyme[2,3], Hui Li[4], Umut Y. Ulge[4,5], Blake T. Hovde[6], David Baker[2,3,7], Raymond J. Monnat Jr[4,5,6], Austin Burt[1,8]* & Andrea Crisanti[1,9]*

2 1 2 | N AT U R E | VO L 4 7 3 | 1 2 M AY 2 0 1 1







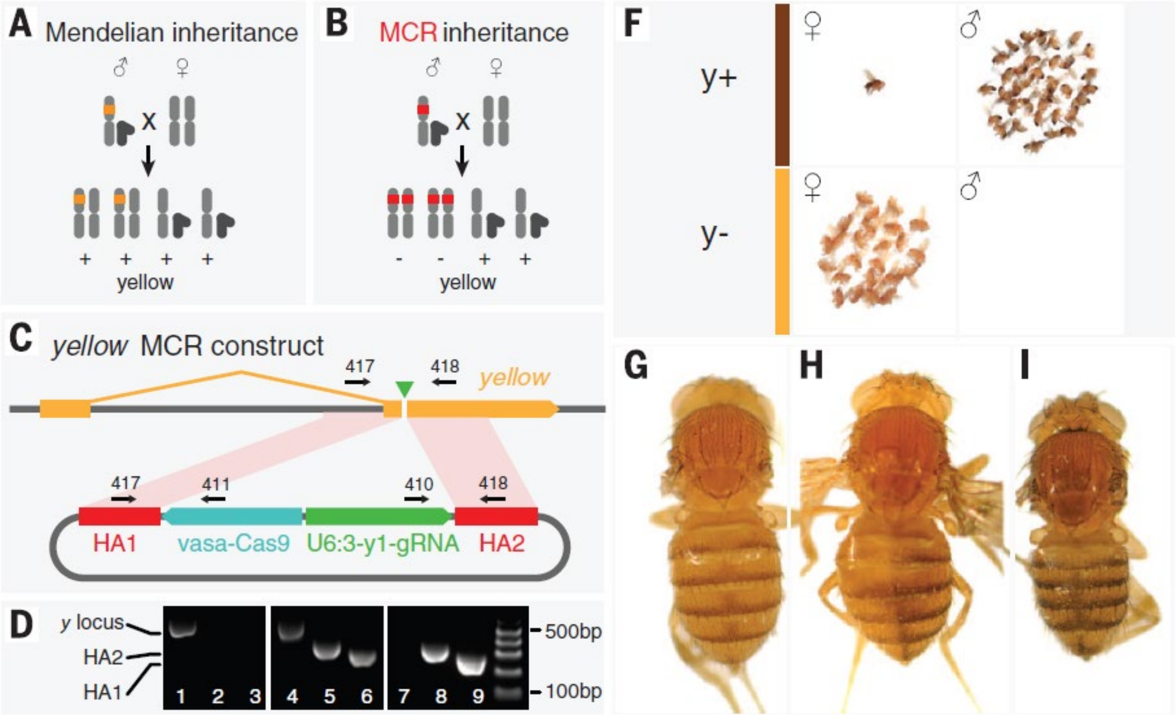
Homing-based Gene Drive

GENOME EDITING

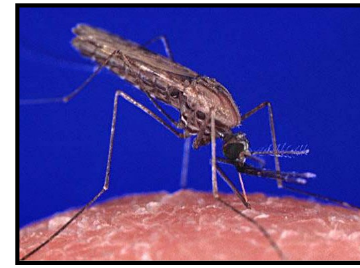
The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations

Valentino M. Gantz* and Ethan Bier*

2015 Science ;348(6233):442-4.



Homing-based Gene Drive



nature biotechnology

A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*

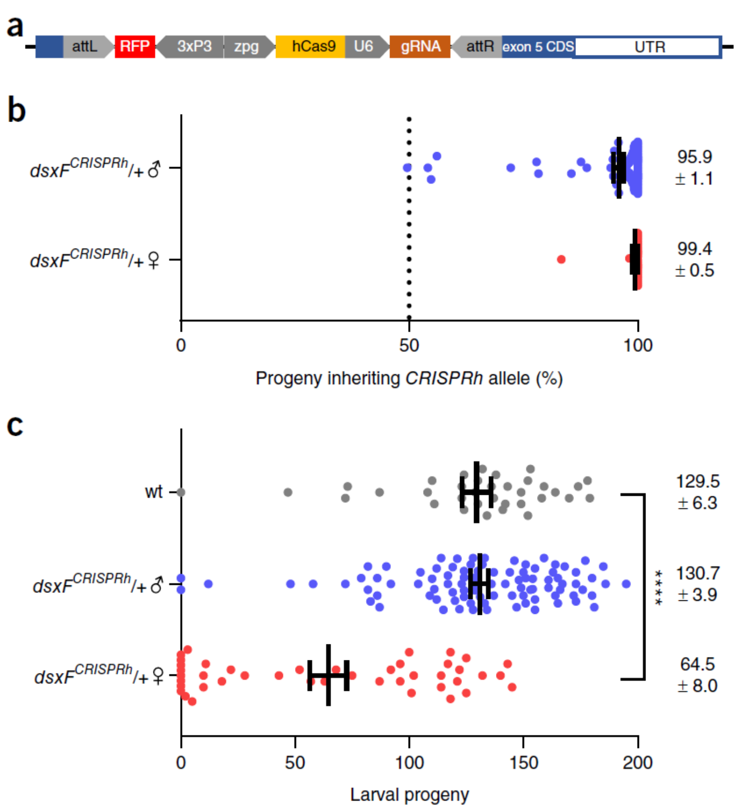
Andrew Hammond[1], Roberto Galizi[1], Kyros Kyrou[1], Alekos Simoni[1], Carla Siniscalchi[2], Dimitris Katsanos[1], Matthew Gribble[1], Dean Baker[3], Eric Marois[4], Steven Russell[3], Austin Burt[1], Nikolai Windbichler[1], Andrea Crisanti[1] & Tony Nolan[1]

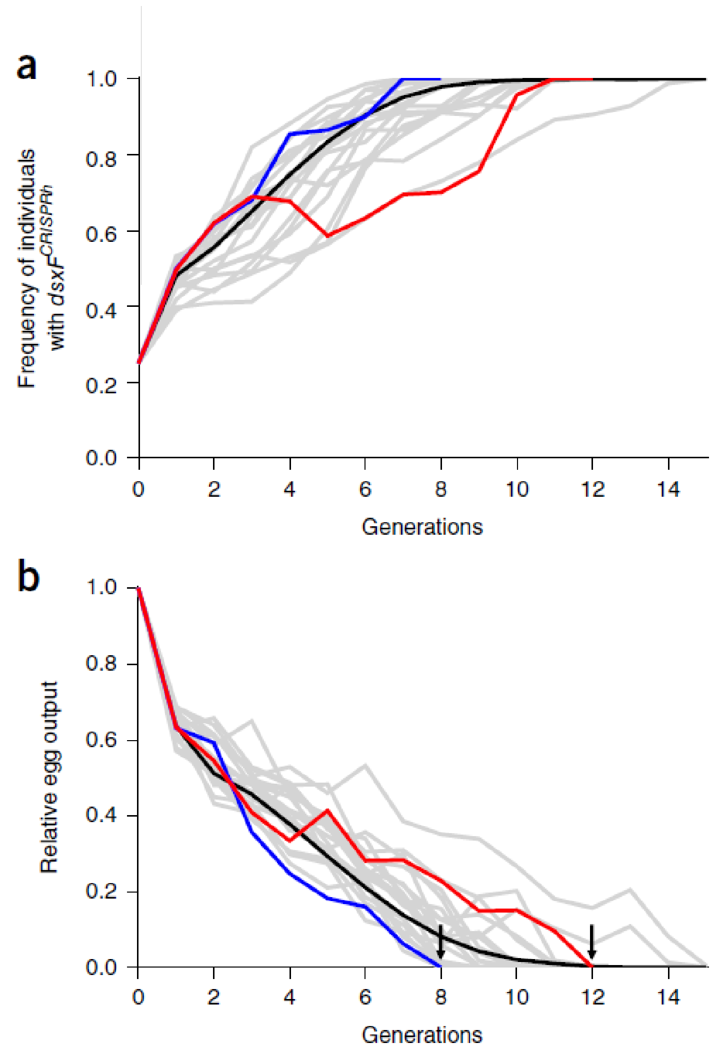
"For each targeted locus we observed a strong gene drive at the molecular level, with transmission rates to progeny of 91.4 to 99.6%."

A new gene drive target shows no signs of resistance development

A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes

Kyros Kyrou[1,2], Andrew M Hammond[1,2], Roberto Galizi[1], Nace Kranjc[1], Austin Burt[1], Andrea K Beaghton[1], Tony Nolan[1] & Andrea Crisanti[1]



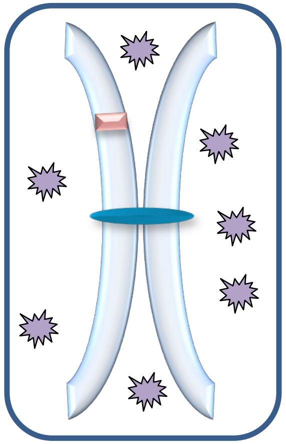


Homing-based gene drive: Same mechanism, completely different risk profiles

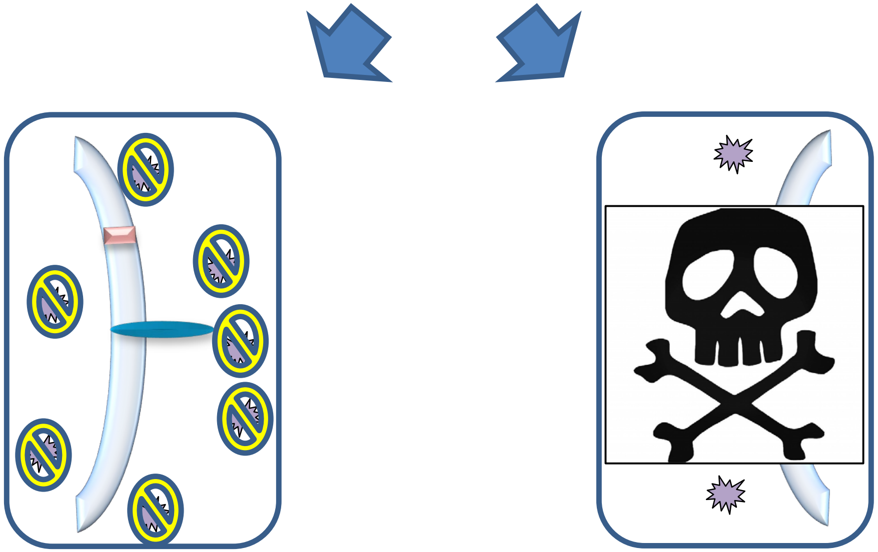
Nuclease	*Target*		*Potential for spread in environment*
I-SceI	I-SceI target		**None**, target site not present in any natural population
CRISPR	yellow		**Limited to none**, as gene is not essential and resistance was selected for rapidly
CRISPR	Gene involved in reproduction		**Limited**, even though gene is essential, resistance was rapidly selected for
CRISPR	Gene involved in female sex determination		**Possible**, resistance was not selected for in laboratory populations. Target site conserved in wild populations.

Selective survival gene drive

Mode of inheritance
is still the same



Diploid (2 copies of each chromosome)



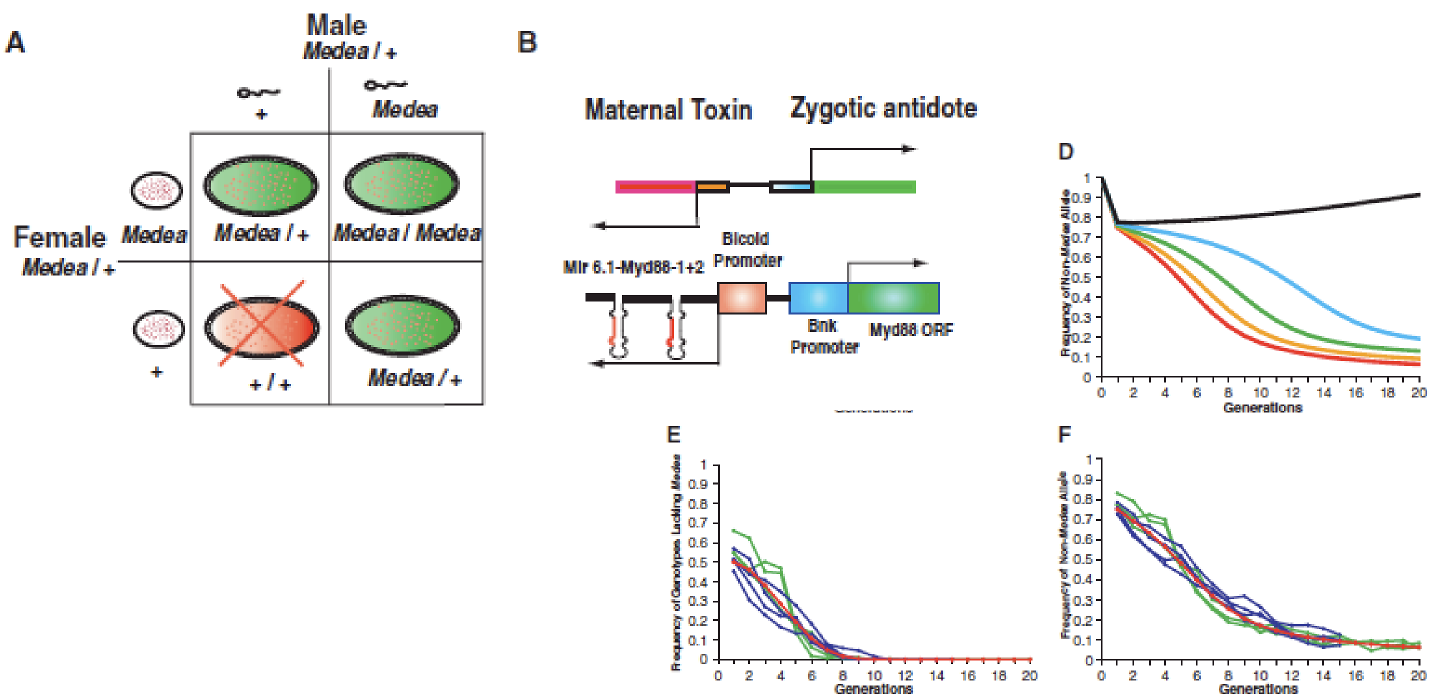
All (100%) of gametes (eggs/sperm) carry the transgene

Haploid (1 copy of each chromosome)

Gene Drive: MEDEA

A Synthetic Maternal-Effect Selfish Genetic Element Drives Population Replacement in *Drosophila*

Chun-Hong Chen,[1] Haixia Huang,[1] Catherine M. Ward,[1] Jessica T. Su,[1]
Lorian V. Schaeffer,[1] Ming Guo,[2] Bruce A. Hay[1]*



Concept can be adapted for targeting any maternally deposited transcript vital for embryo survival; Very stable, highly invasive.

Selective Survival: X-shredding in *An. gambiae*

ARTICLE

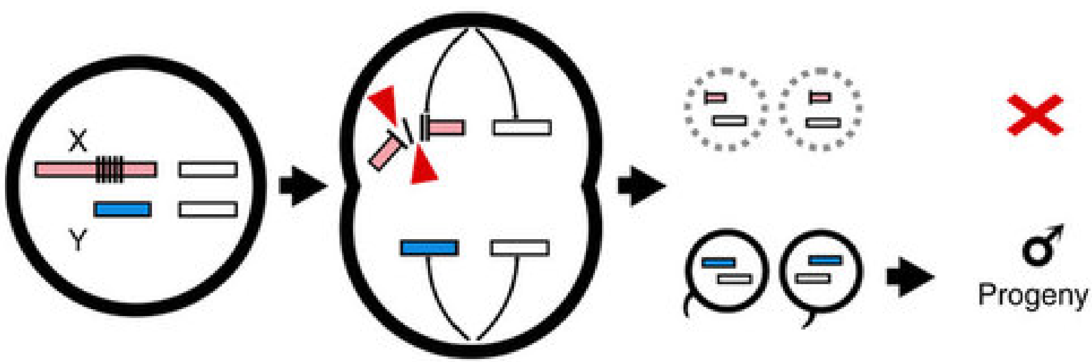
Received 12 Mar 2014 | Accepted 28 Apr 2014 | Published 10 Jun 2014

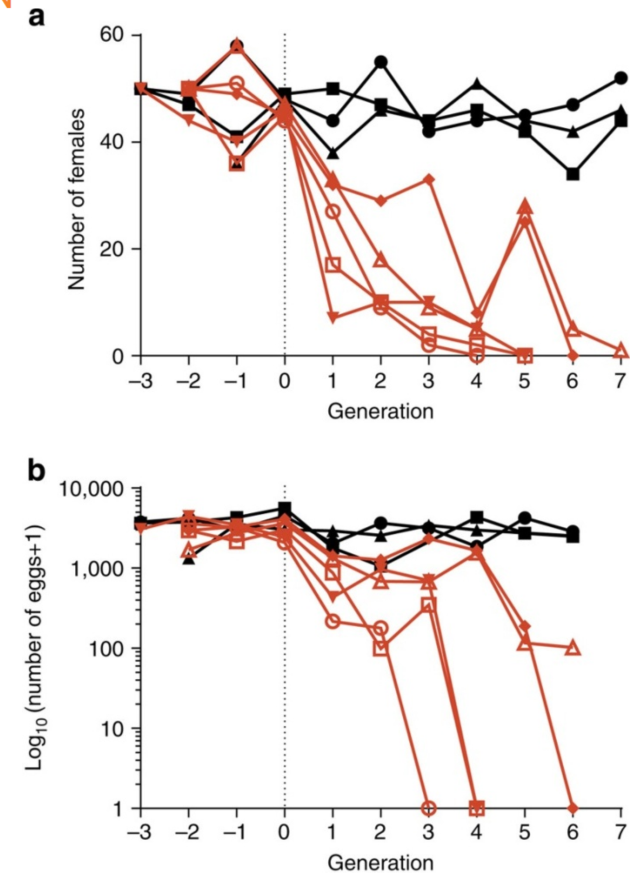
DOI: 10.1038/ncomms4977

OPEN

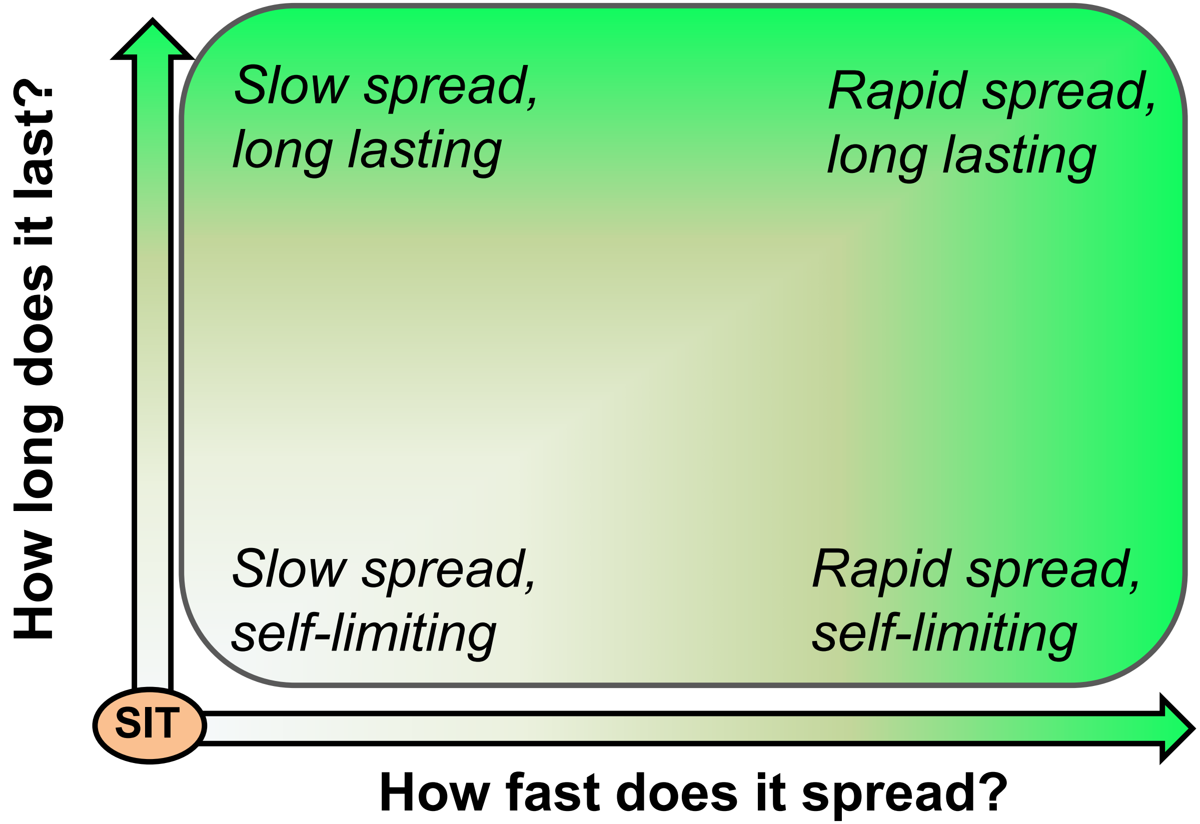
A synthetic sex ratio distortion system for the control of the human malaria mosquito

Roberto Galizi[1,2], Lindsey A. Doyle[3], Miriam Menichelli[1], Federica Bernardini[1], Anne Deredec[1], Austin Burt[1], Barry L. Stoddard[3], Nikolai Windbichler[1,*] & Andrea Crisanti[1,2,*]

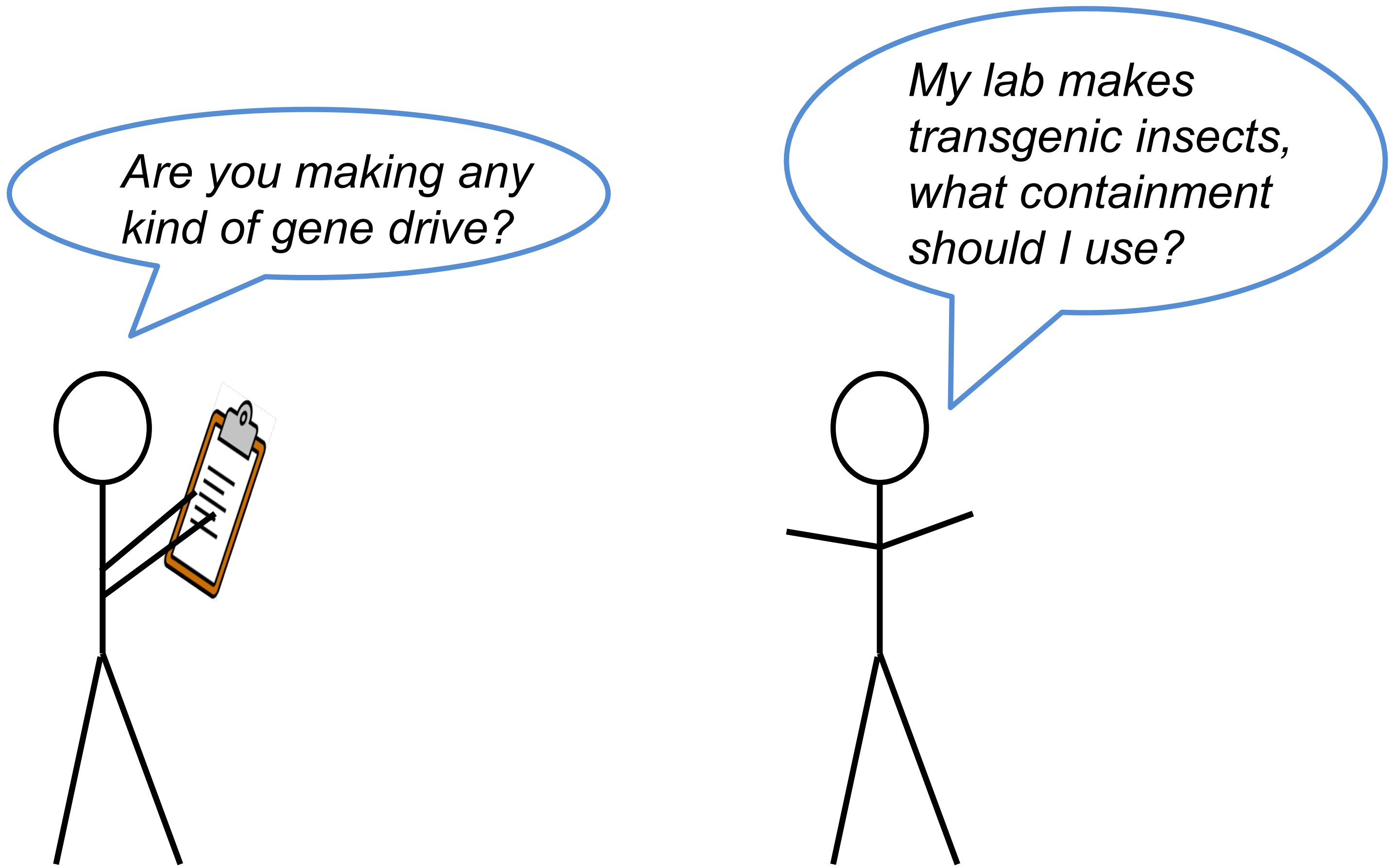


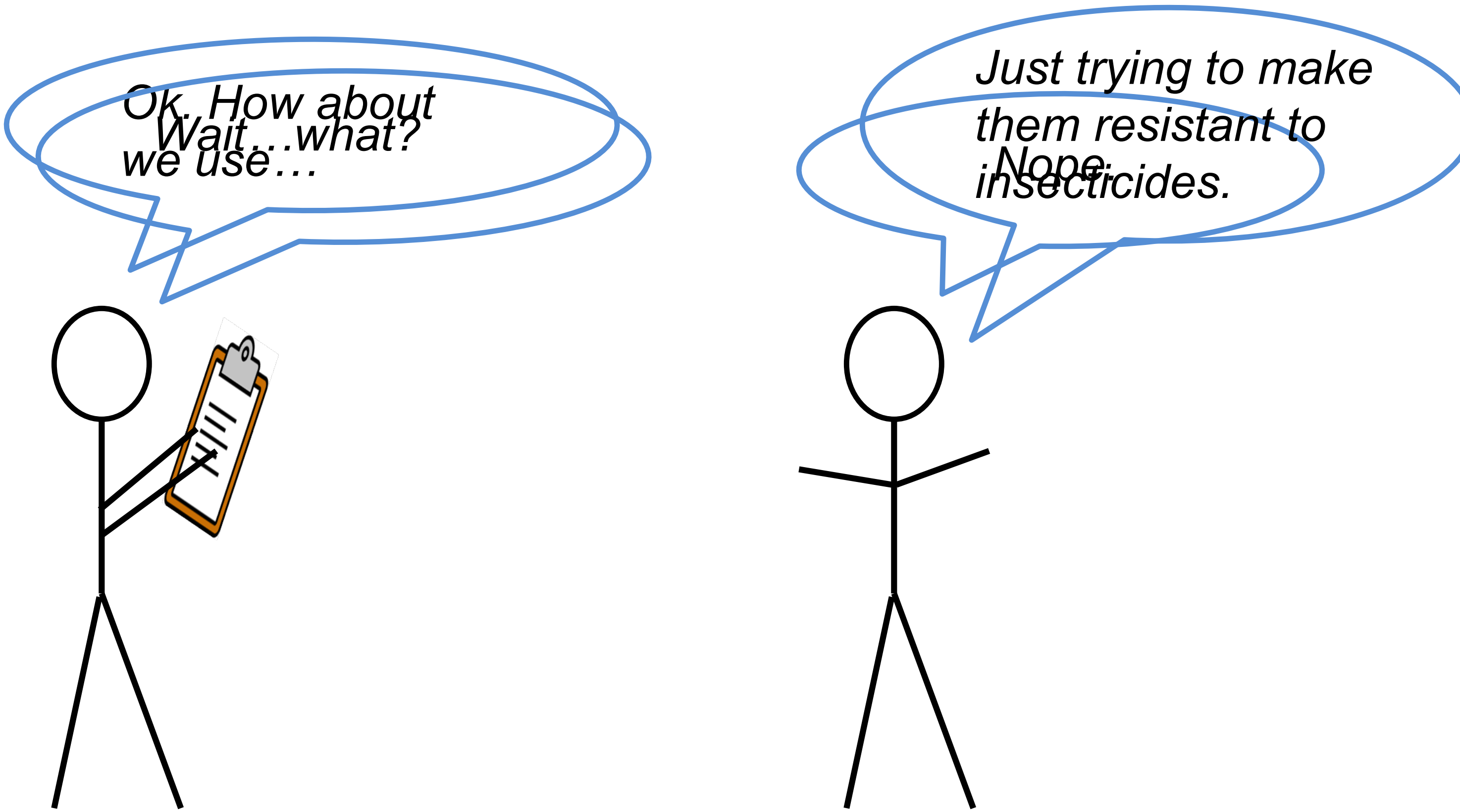


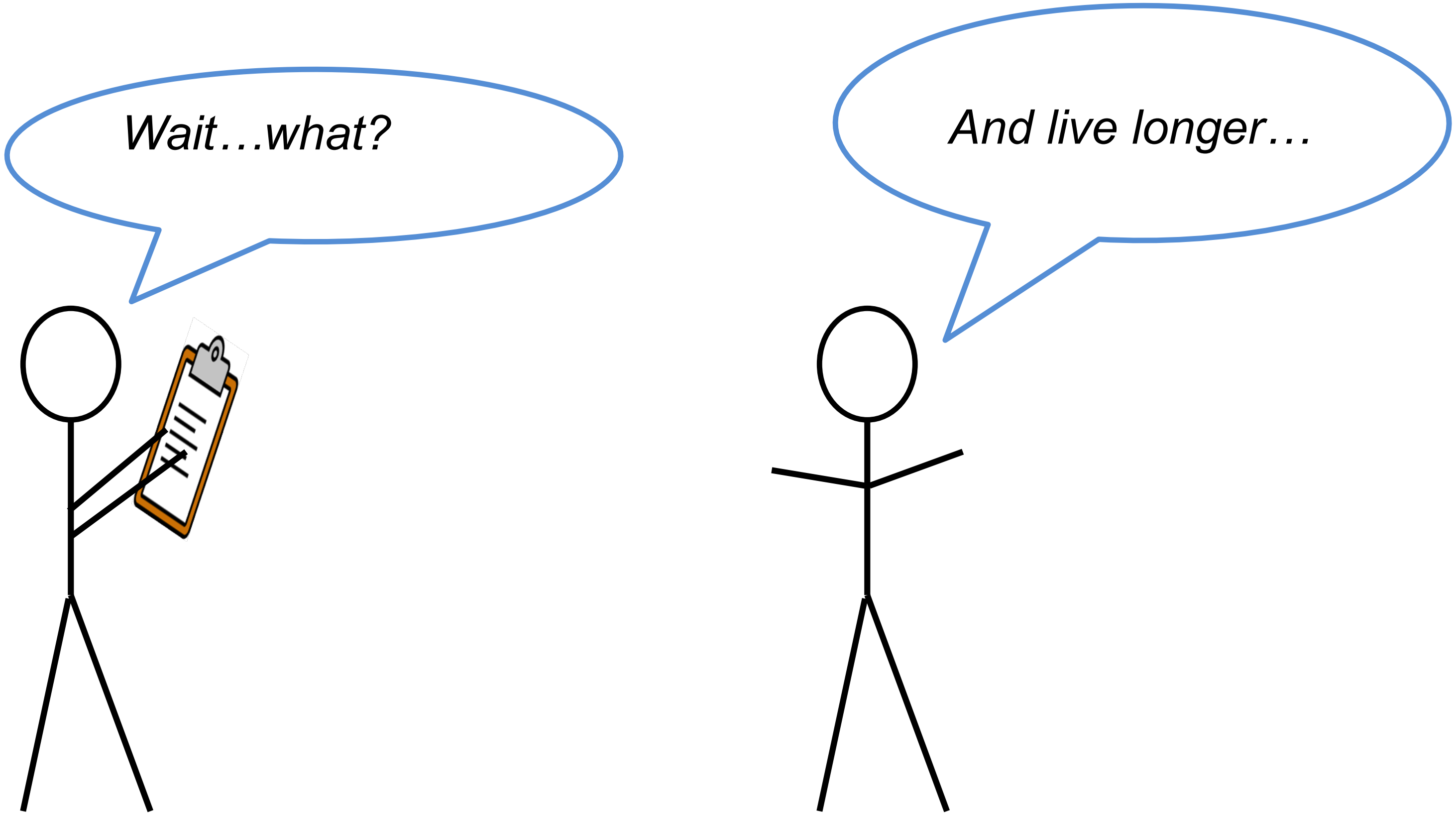
Any attempt to begin risk assessment based on the use of a particular technology has little chance of keeping up

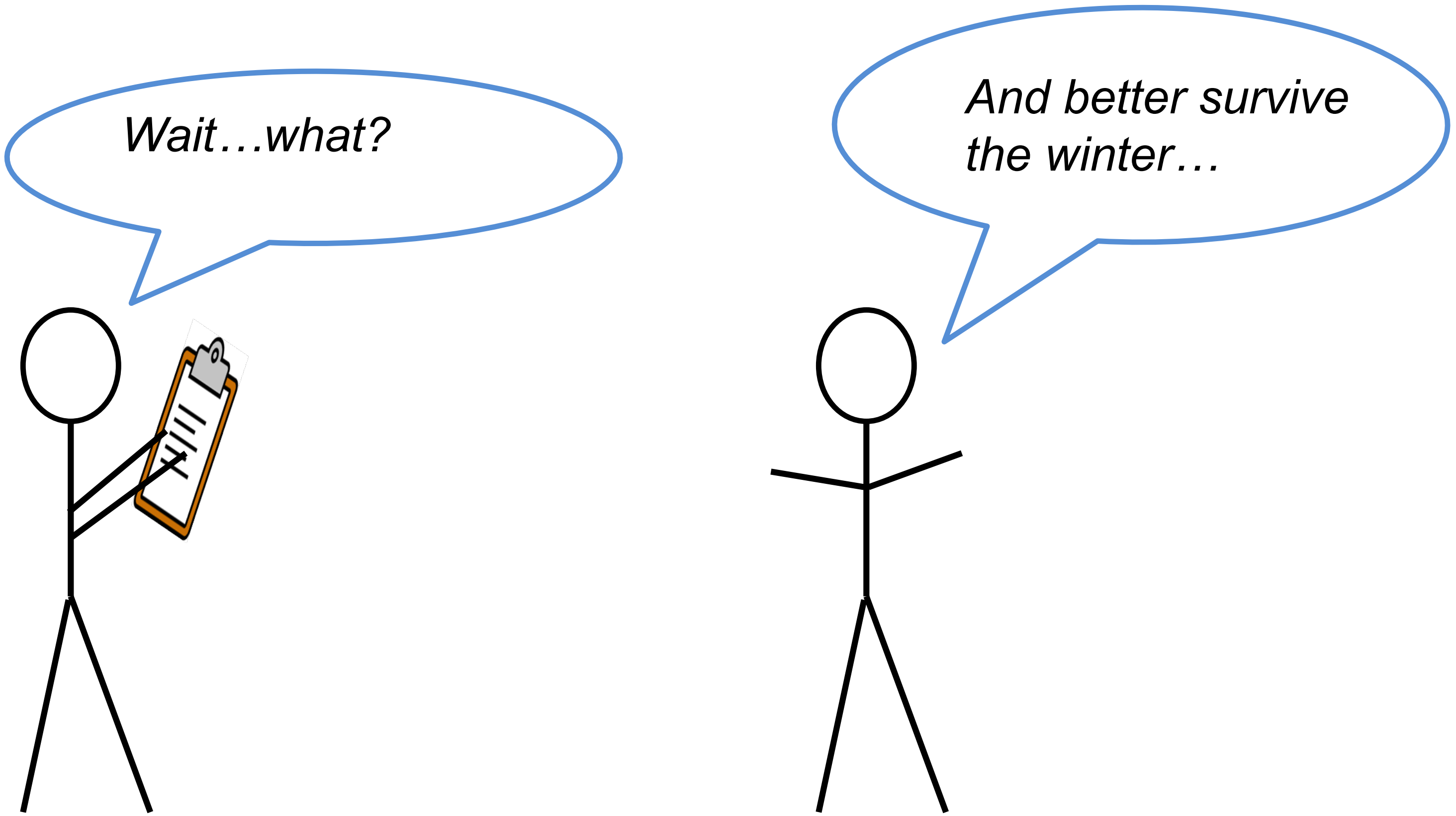


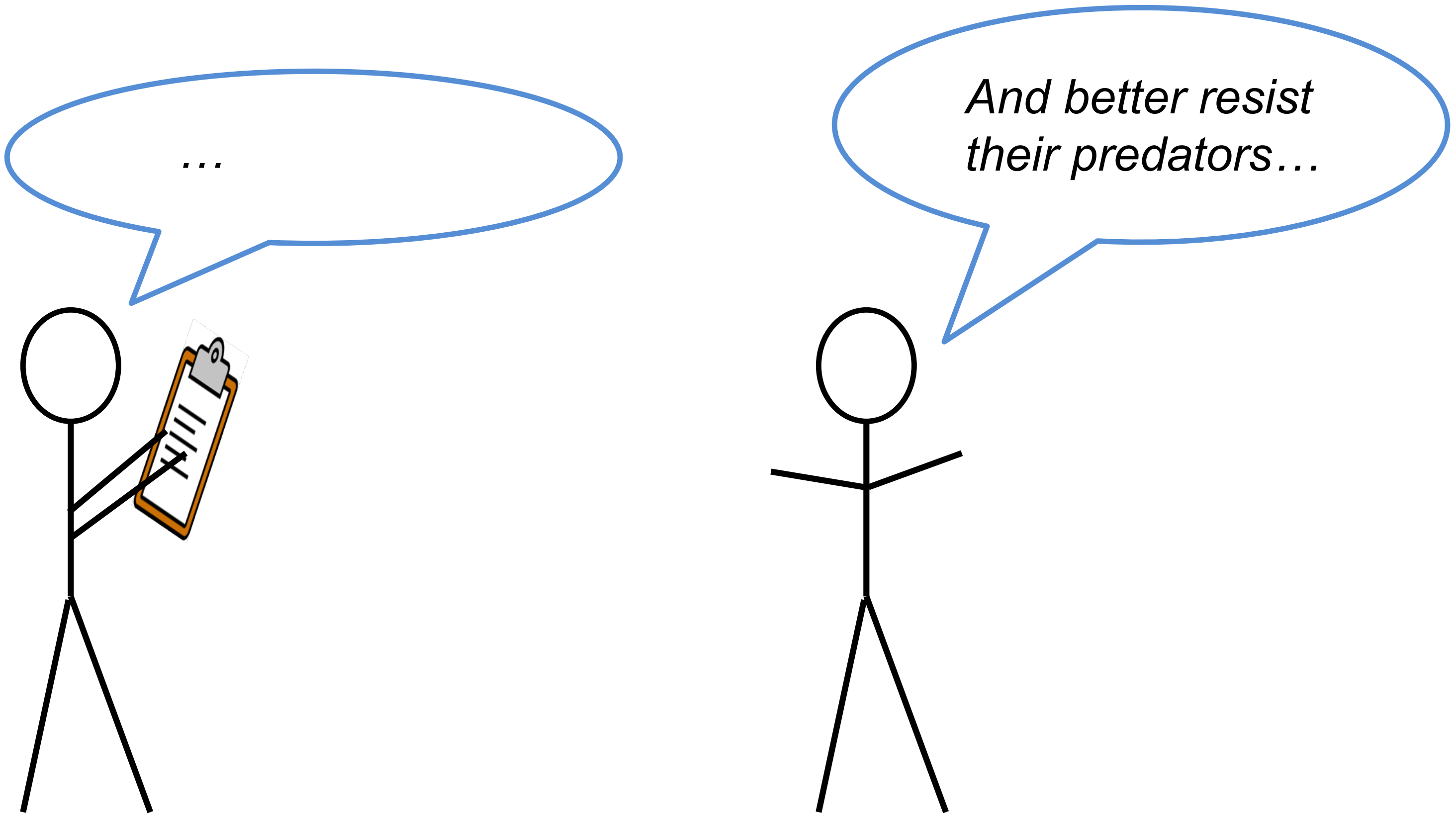
New technologies that might also result in gene drive have likely not been built yet


Are you making any kind of gene drive?
My lab makes transgenic insects, what containment should I use?


Ok. How about we use…
Wait…what?
Just trying to make them resistant to insecticides.
Nope.


Wait…what?
And live longer…


Wait…what?
And better survive the winter…


…
And better resist their predators…

A updated starting point for risk assessment of laboratory-based transgenic organisms

- Is the introduced transgene (or combination of transgenes) likely to persist or spread through a natural population if introduced?

Yes

Includes some gene drive transgenes, but also transgenes that provide a net benefit

No

Includes some gene drive transgenes, but also transgenes that are neutral or confer a disadvantage

Risk Assessment– Infectious Agents

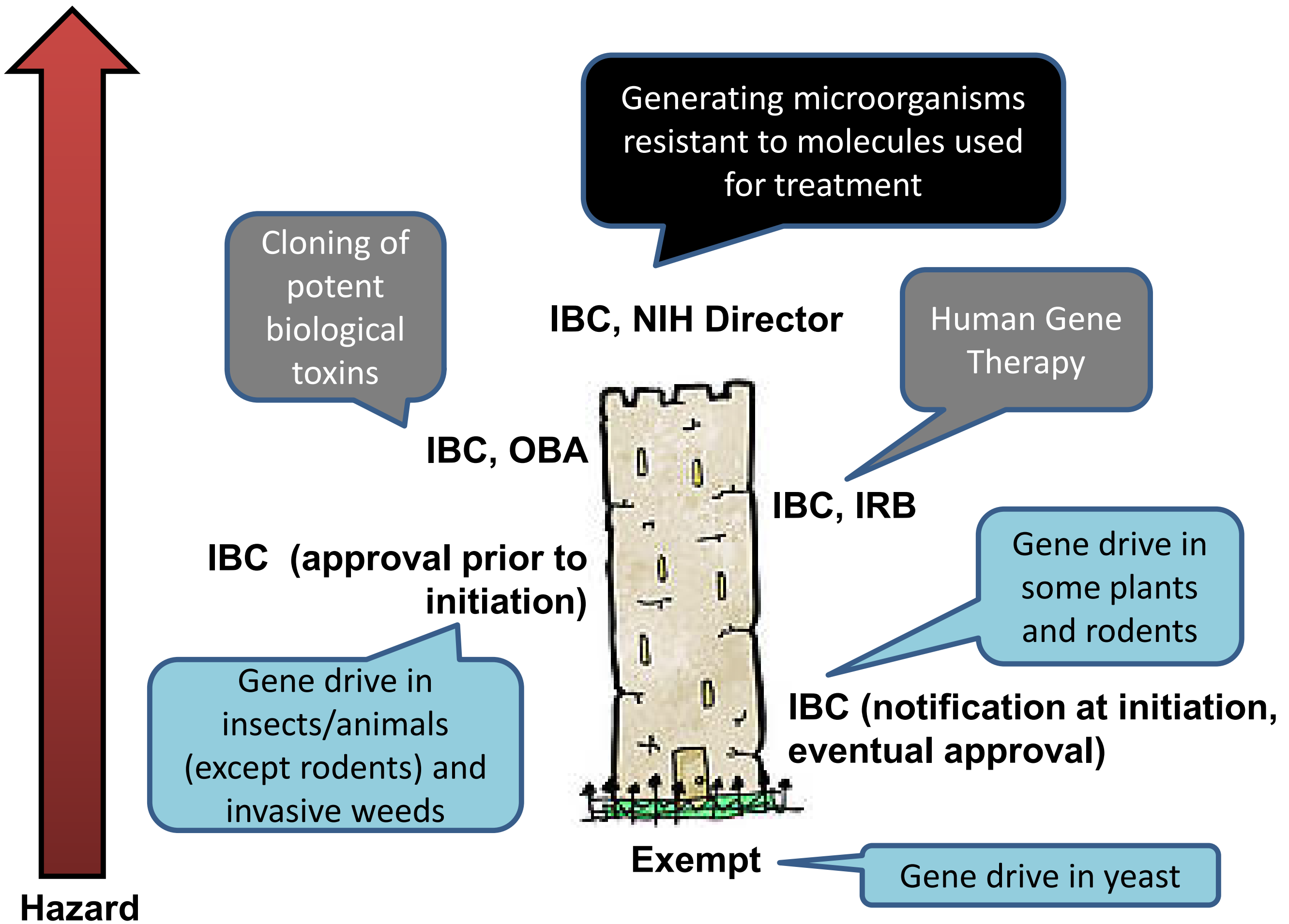
Risk Group	Definition	Examples
1	Agents that are not associated with disease in healthy adult humans	*B. subtilis*
2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are ***often*** available	*Salmonella*
3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions ***may*** *be* available (high individual risk but low community risk)	Prions, HIV types 1 and 2
4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are ***not usually*** available (high individual risk and high community risk)	Lassa virus, Ebola virus;

Safety Considerations – Transgenes

Risk Group	Definition	Gene Drive	No Gene Drive
?	Transgenes that are less fit than wild-type and cannot persist/spread in the wild	*Homing-drive (no target), Underdominance*	*EGFP inserted into vital gene*
?	Transgenes that may persist in the wild in the short term, but cannot spread	*Homing-drive (resistance alleles can be selected, target site limited)*	*EGFP inserted into neutral location*
?	Transgenes that may spread/persist in the wild in the long-term, but cannot transfer to new species	*Homing-drive (resistance alleles cannot be selected)*	Gene than confers increased disease/pesticide resistance (no hybridization)
?	Transgenes that are likely to spread/persist in the wild and present a significant risk of horizontal transfer to new species.	*Homing-drive (resistance alleles cannot be selected), target site conserved in related species*	Gene than confers increased disease/pesticide resistance (hybridization)

Containment conditions/practices set on case-by-case basis

Regulatory Landscape for Gene Drive in Laboratory Containment



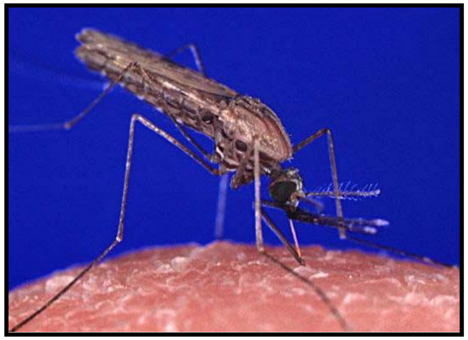
Individual Entities may require additional review

Entities receiving no NIH money may not require any review

To drive or not to drive (in arthropods)…

It doesn't matter according to the current NIH guidelines, it falls under:

Section III-D-4: Experiments involving whole animals



Challenges for IBC review of transgenic arthropod research

Transgenic arthropods alone present little risk to the health and safety of laboratory workers and thus may not be given as thorough a review as pathogen-based work or human gene therapy.

NIH/BMBL provides little to no specific guidance on containment for arthropods.

PIs may be less familiar with the NIH guidelines, principles of biosafety.

Expertise typically found on IBCs

Bacteriology
Virology
Cell culture
Gene therapy
Occupational/Public Health

Animal Expert
Plant Expert

Community (Public Health)

PIs familiar with IBC process

Expertise not typically found on IBCs

Entomology
Biological Control
USDA Quarantine
Ecology
Invasive species

PIs not familiar with IBC process

Risk assessment for laboratory research using transgenic arthropods

Transgenic arthropod

^

Section V-M. Determination of whether a ~~pathogen~~ has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of ~~plant diseases, crops~~, and ecosystems in the geographic area of the research. ???

Challenges for IBC review of transgenic arthropod research

Transgenic arthropods alone present little risk to the health and safety of laboratory workers and thus may not be given as thorough a review as pathogen-based work or human gene therapy.

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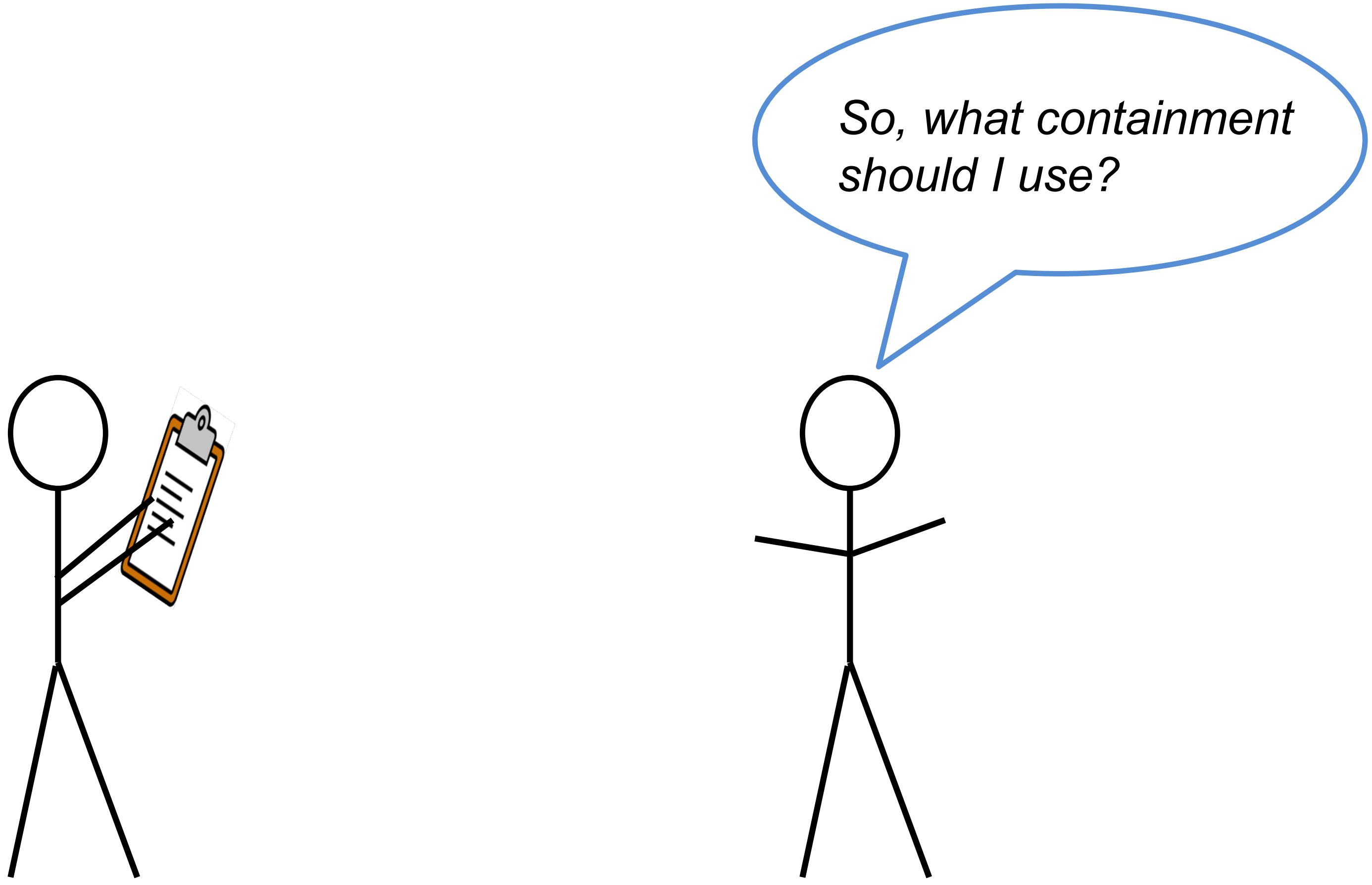
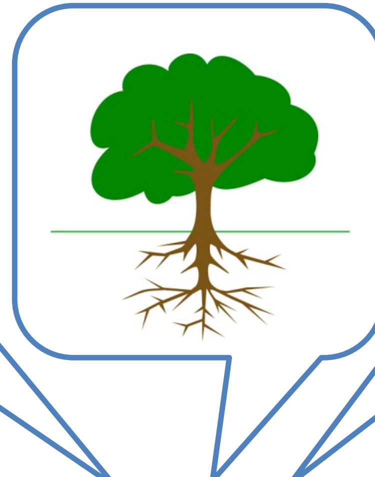
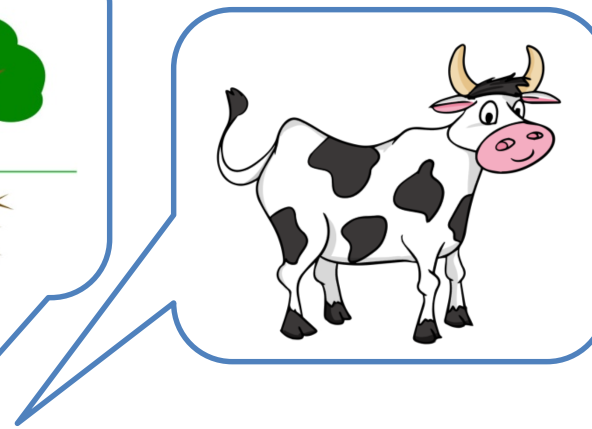

So, what containment should I use?

Containment practices





- **Physical (Appendix G, P, Q)**
  - **Practices**
  - **Equipment**
  - **Facilities**
- **Biological (Appendix I)**
  - **Survival**
  - **Transmission**

No specific guidance for arthropod containment

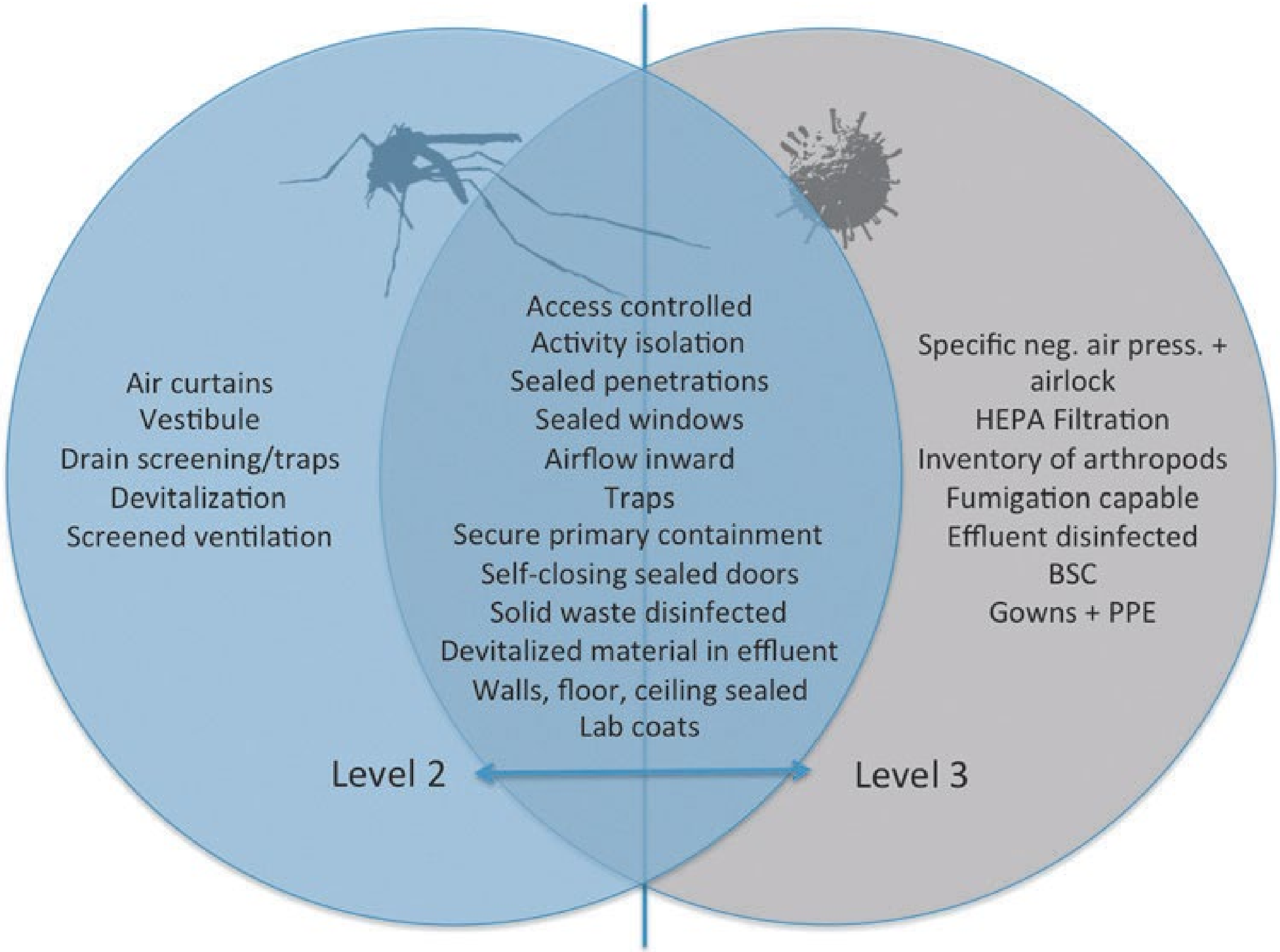
Modified from: NIH/OBA

Arthropod Containment Guidelines

- Developed by a subcommittee of the American Society of Tropical Medicine and Hygiene in 2003.
- Containment levels 1-4 to mirror handling pathogen-infected arthropods (based on agent BSL)
- Containment ACL-2 designated for genetically-modified arthropods.
- ACG do not mention gene drive, but current interpretations utilize ACL-2 as well.

ACG are not binding and may or may not be utilized by PIs/IBCs

ACGs are structured to contain both the vector and the microbial pathogen



Benedict et al (2018) VBZD

Arthropod Containment Guidelines

Arthropod containment level:	1		2	3	4
Arthropod distribution, escaped arthropod fate	Exotic, inviable or transient	Indigenous	Exotic with establishment, indigenous, and transgenic		
Infection status	Uninfected or infected with non-pathogen		Up to BSL-2	Up to BSL-3	BSL-4
Active VBD cycling	No	Irrelevant			
Practices	ACL-1 Standard Arthropod-Handling Practices		ACL-1 plus more rigorous disposal, signage, and limited access	ACL-2 with more highly restricted access, training and record-keeping	ACL-3 with high access restriction, extensive training, full isolation
Primary Barriers	Species-appropriate containers		Species-appropriate containers	Escape-proof arthropod containers, glove boxes, BSC	Escape-proof arthropod containers handled in cabinet or suit laboratory
Secondary Barriers			Separated from laboratories, double doors sealed electrical/ plumbing openings. Breeding containers and harborages minimized	BSL-3	BSL-4

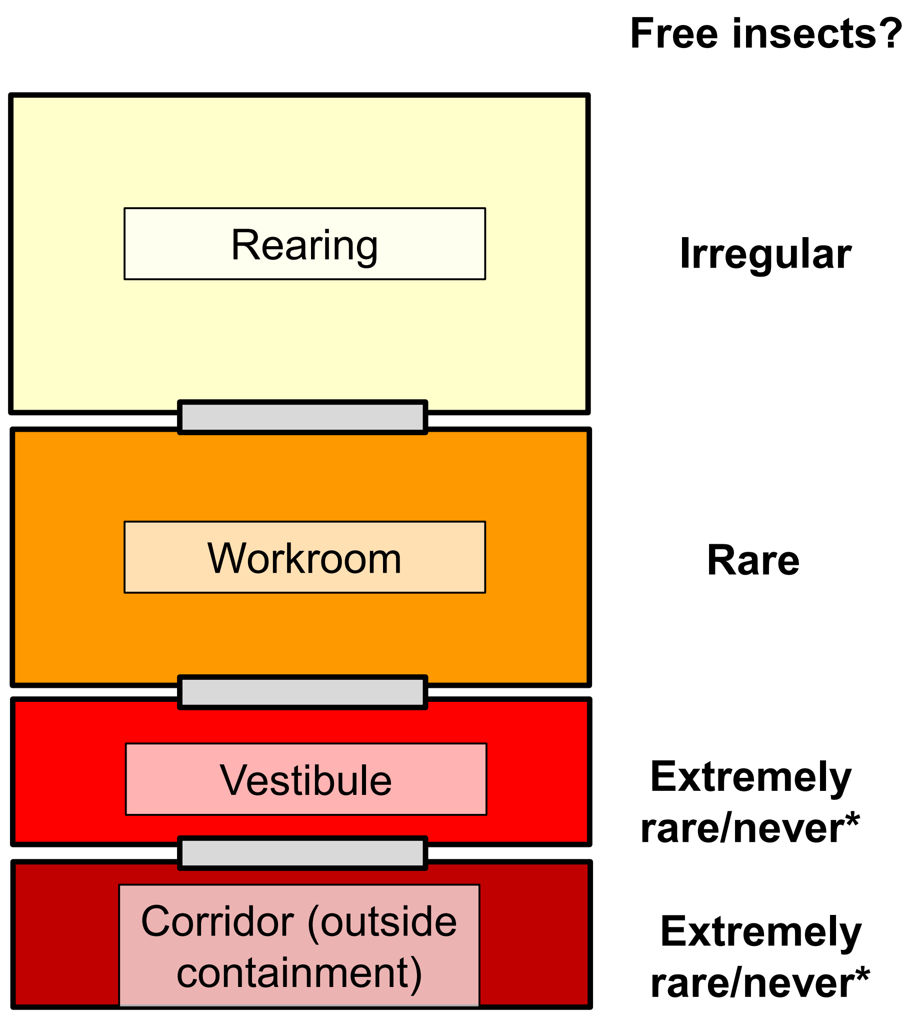
VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 3, Number 2, 2003
Mary Ann Liebert, Inc.

IBC (with BSO/Office of Biosafety)

Review:

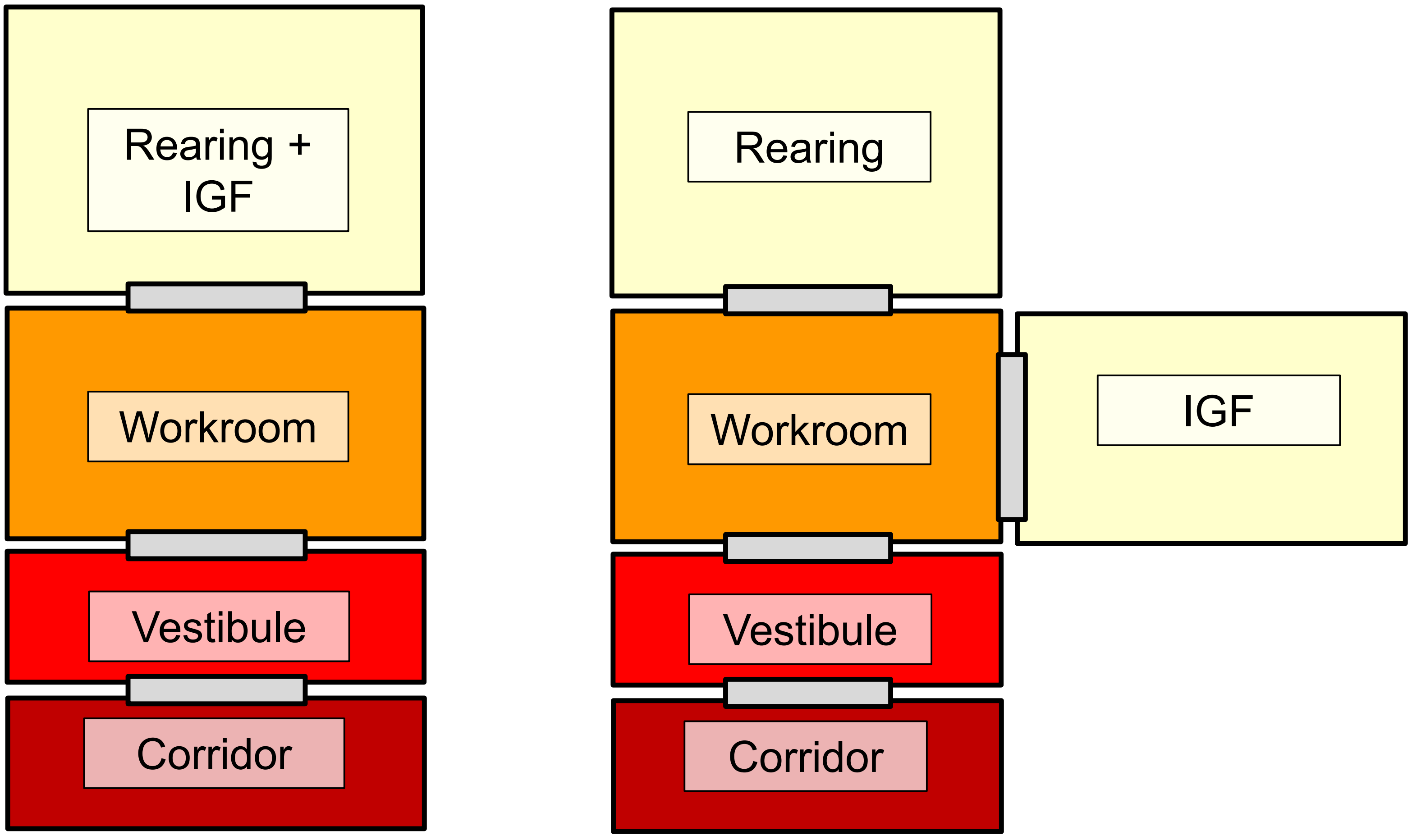
- Work practices (SOPs, biosafety manuals)
- Safety equipment
- Personal protective equipment
- Training needs
- Facility design
- Security

Containment is multi-layered for a reason



* For some common insects, it is possible for wild relatives to enter from the outside

Segregate insects with invasive genetic factors (IGFs) from other transgenic and stock strains

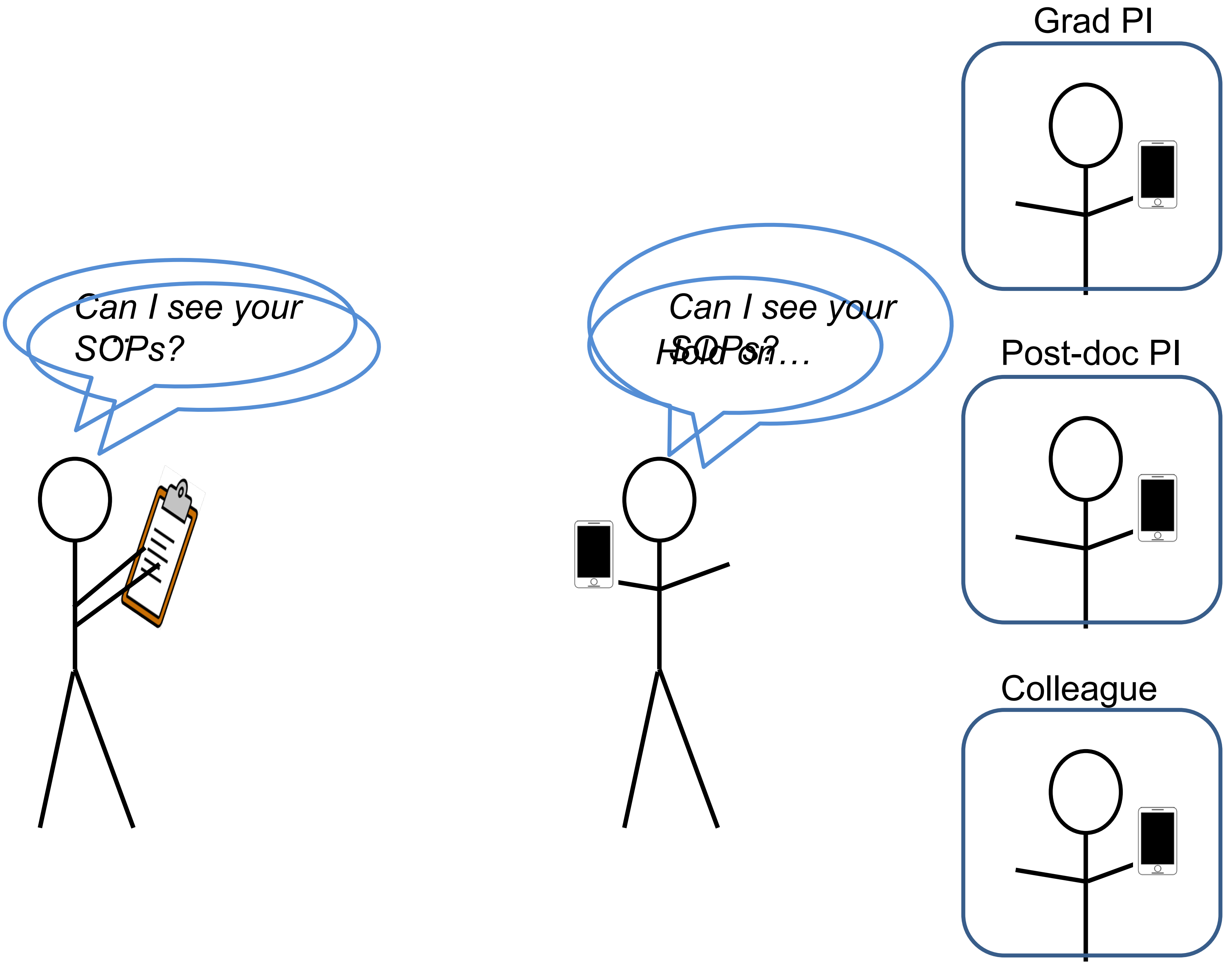


Challenges for IBC review of transgenic arthropod research

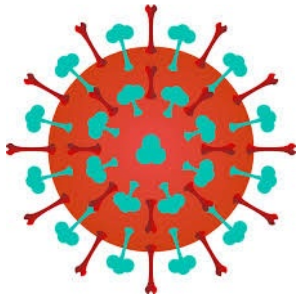
Transgenic arthropods alone present little risk to the health and safety of laboratory workers and thus may not be given as thorough a review as pathogen-based work or human gene therapy.

NIH/BMBL provides little to no specific guidance on containment for arthropods.

PIs may be less familiar with the NIH guidelines, principles of biosafety.

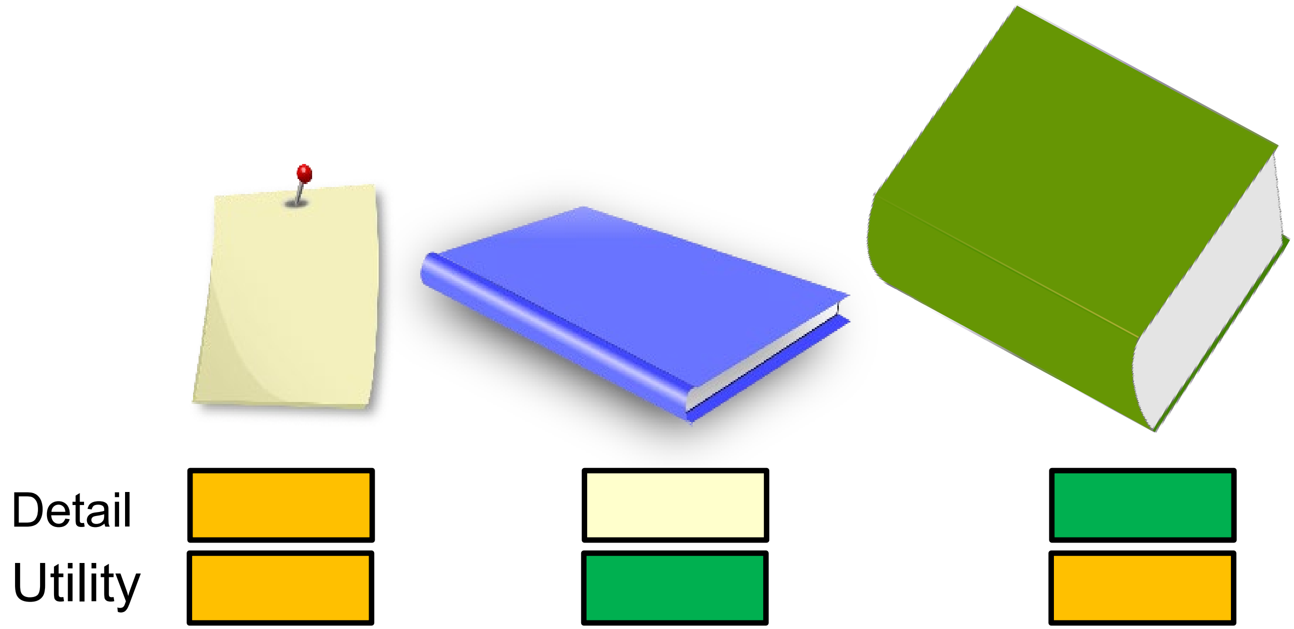

Can I see your SOPs?
Can I see your SOPs?
Hold on…
Grad PI
Post-doc PI
Colleague

IBC/Biosafety



APHIS

Standard Operating Procedures



Procedures insufficiently documented

Procedures too difficult to remember and keep updated

Having a great facility means little if it is no one knows or follows the rules…

Written SOPs may or may not equal actual practices

Are actual practices working?

If so, document them.

If not, get them working, then document them.

Questions for lab members, staff, students (inspection, tabletop exercise).

All SOPs worked out using non-transgenic versions with effective monitoring.

Access SOP:

Restricted to authorized, trained personnel only

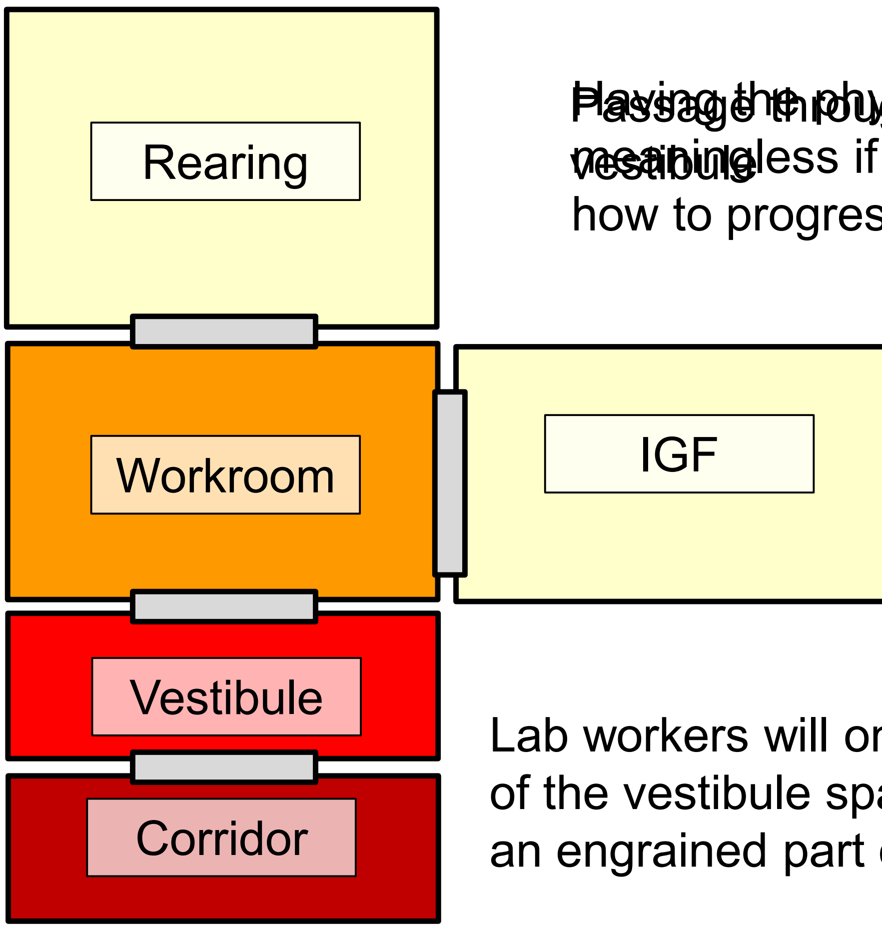
What is your key? Who gets one? Who gives them out?

Entry/Exit SOP:

Insectary is separated from corridor via at least two self-closing doors

Passage through a relatively small double-door vestibule

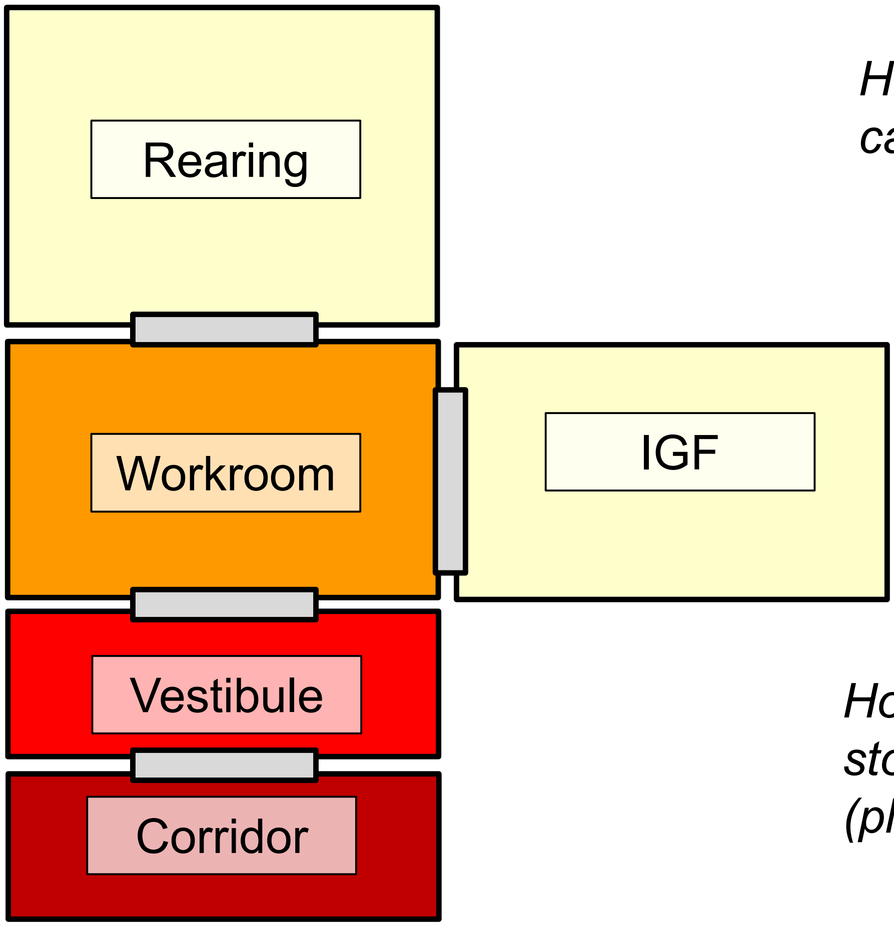
Having the physical structure of a vestibule is meaningless if there are no procedures for how to progress through this area



Lab workers will only perform a diligent examination of the vestibule space during every entry/exit if it is an engrained part of the safety culture

Facility integrity SOP:

The facility is evaluated annually for compliance to the ACL-2 level



How often are screens, caulking, traps inspected?

How will work be suspended or stopped for facility maintenance (planned or unplanned)?

Waste SOP:

Devitalization, waste disposal, and routine decontamination

drawception.com

Arthropods with IGFs should be killed multiple times, just to make sure they are dead…

All solid waste autoclaved.
No living stages placed in solid waste stream (autoclave bag).

Many ways of killing (either within cages or once free from cages should be available)

Tracking/ responding to escapes SOP:

Escaped arthropod handling, monitoring, and accidental release reporting

"Remember, there is no problem so bad that you cannot make it worse"

-Canadian astronaut Chris Hadfield

Tracking/ responding to escapes SOP:

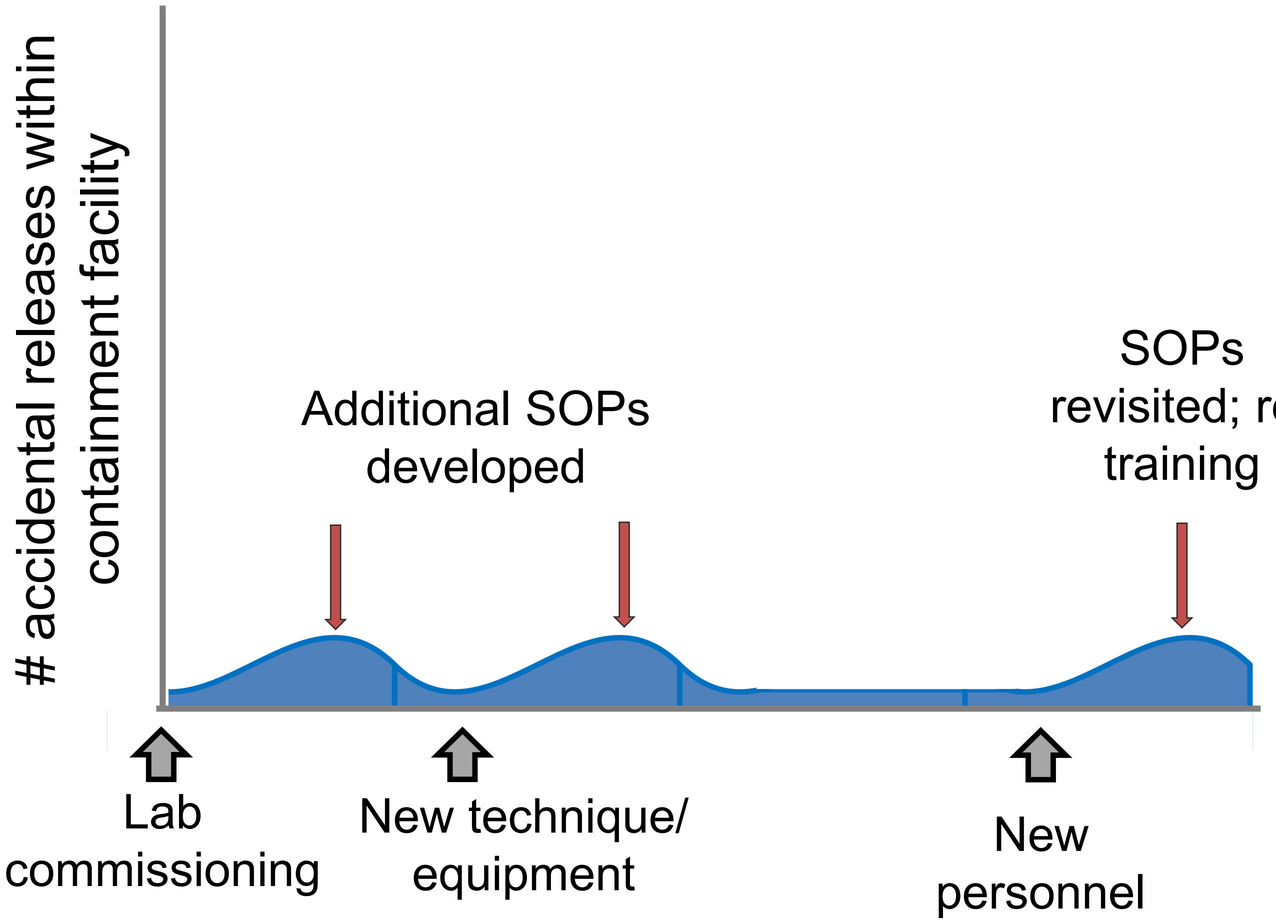
Escaped arthropod handling, monitoring, and accidental release reporting

Some escaped arthropods will find you, many others will not

Escaped arthropods are everyone's concern

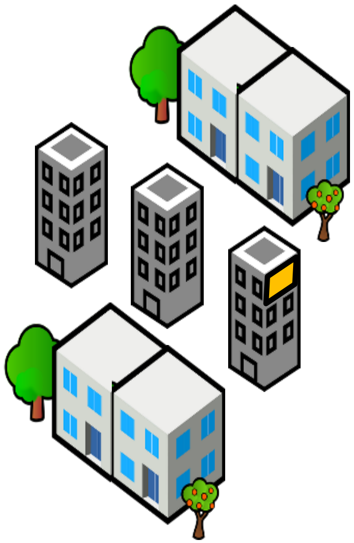
Every attempt must be made to link an escape event to a work practice

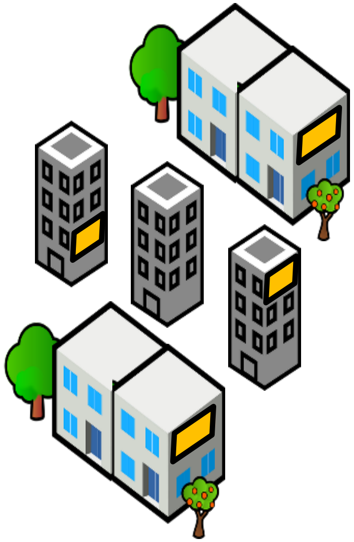
SOPs are living documents, and must be revised based on how things are going

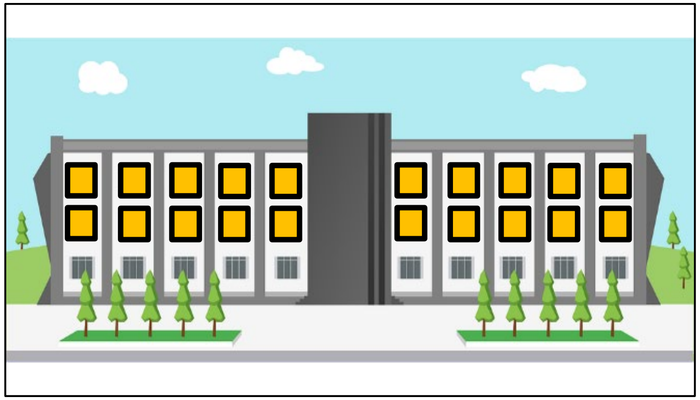


As IGF activities grow, consider dedicated space

Single lab	Multiple labs	Dedicated multi-user building







	Single lab	Multiple labs	Dedicated multi-user building
Access/Security	Few individuals	Many individuals	Centralized access
Operating Procedures	Single PI SOPs	Multi, variable SOPs	Centralized SOPs
Training	Single PI	Multi PI	Centralized training
Day-to-Day Oversight	Single PI	Multi PI	Independent Biosafety
Maintenance	No dedicated personnel	No dedicated personnel	Dedicated personnel
Cost	Single renovation	Multi-renovation	Dedicated building

Further reading

PATHOGENS AND GLOBAL HEALTH, 2017
VOL. 111, NO. 8, 436–447
https://doi.org/10.1080/20477724.2018.1424514

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Developing standard operating procedures for gene drive research in disease vector mosquitoes

Zach N. Adelman, David Pledger and Kevin M. Myles

Department of Entomology, Texas A&M University, College Station, TX, USA

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 18, Number 1, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2017.2121

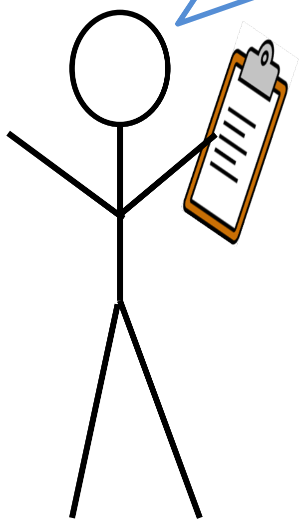
ORIGINAL ARTICLES

Recommendations for Laboratory Containment and Management of Gene Drive Systems in Arthropods

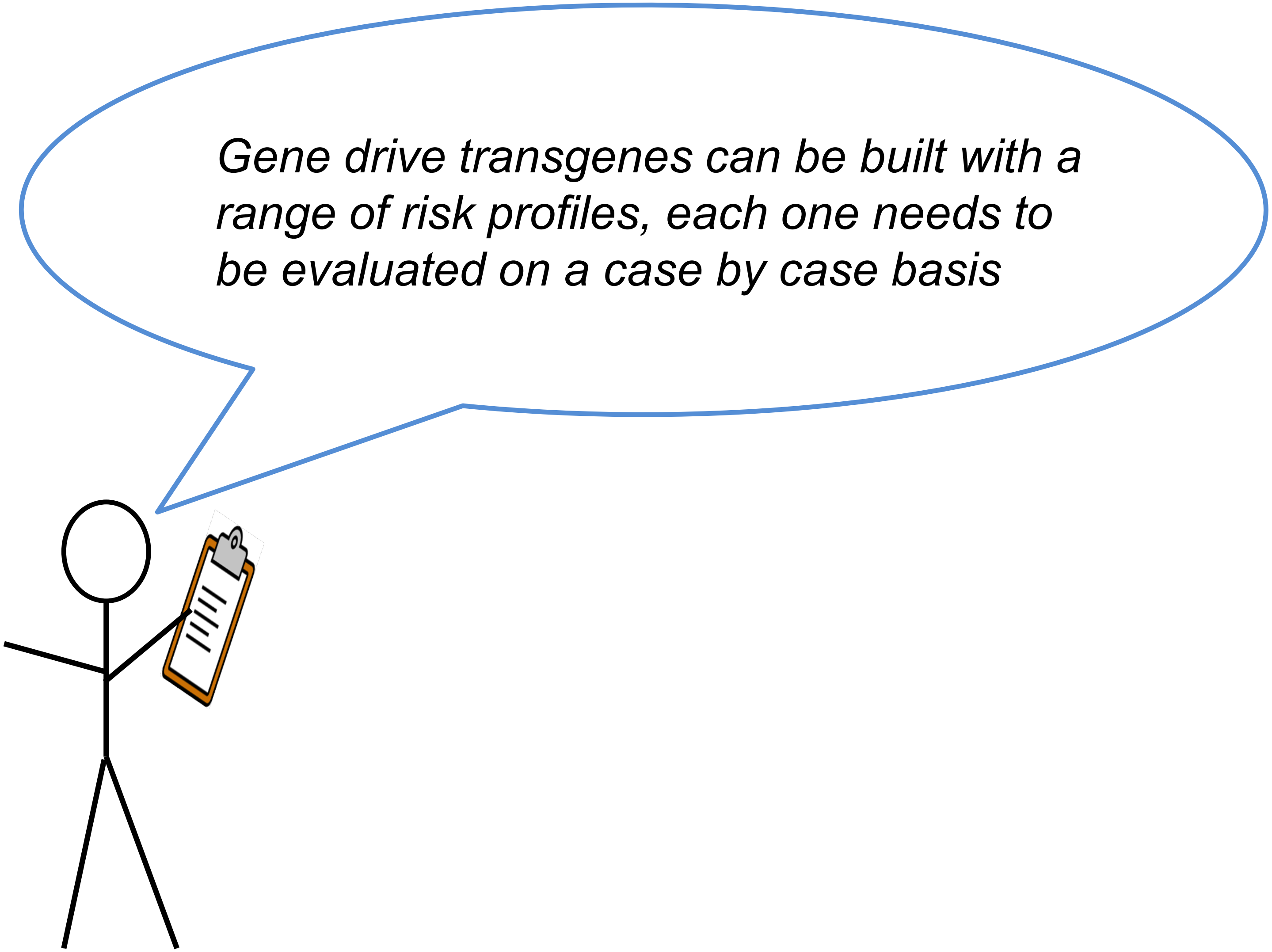
Mark Q. Benedict,[1] Austin Burt,[2] Margareth L. Capurro,[3,4] Paul De Barro,[5] Alfred M. Handler,[6] Keith R. Hayes,[7] John M. Marshall,[8] Walter J. Tabachnick,[9] and Zach N. Adelman[10]

Summary

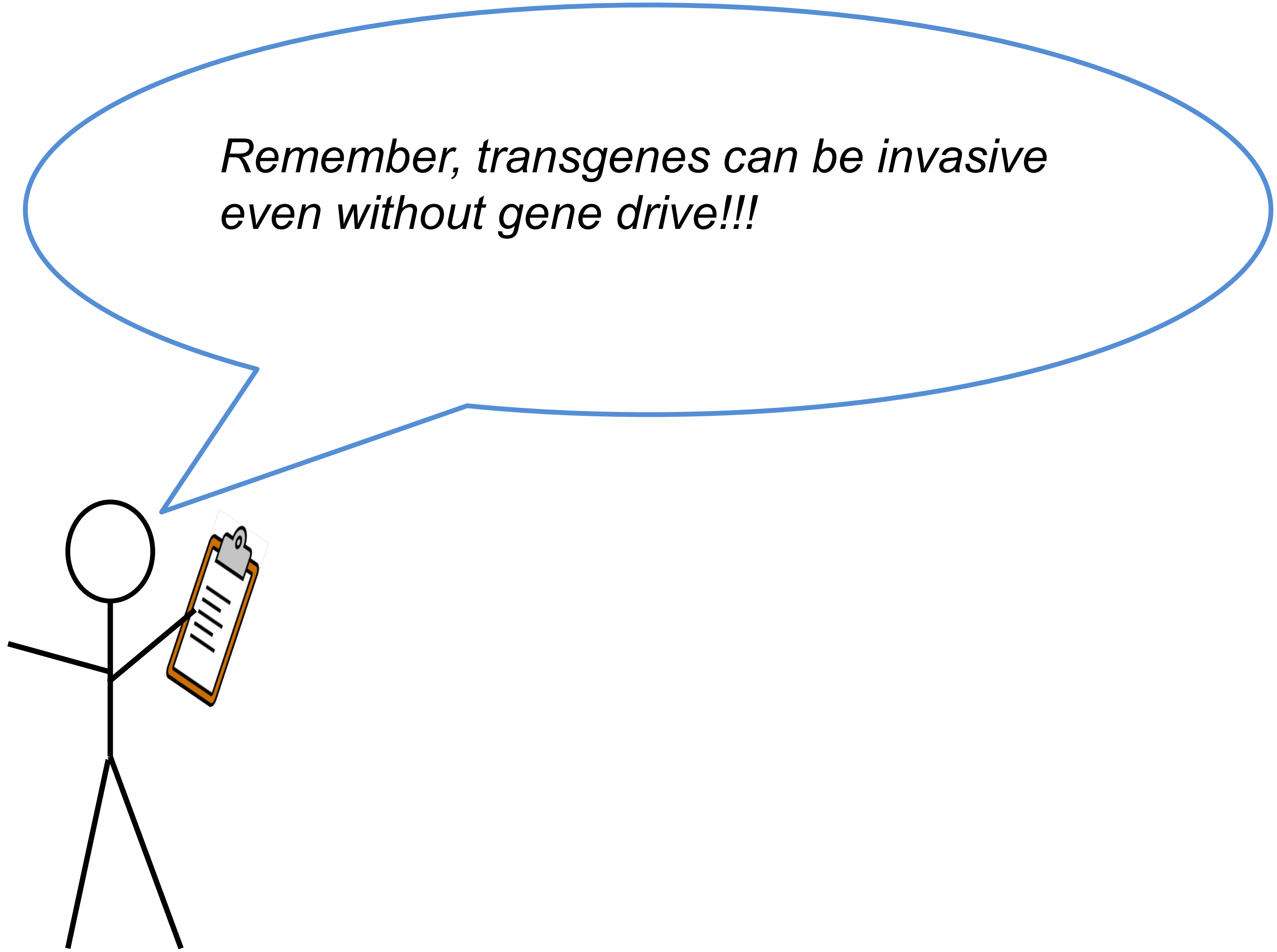
Gene drive refers to introduced genetic material capable of increasing its frequency in a given population in spite of providing no benefit or even a fitness detriment



Summary


Gene drive transgenes can be built with a range of risk profiles, each one needs to be evaluated on a case by case basis

Summary


Remember, transgenes can be invasive even without gene drive!!!

Novel and Exceptional Technology and Research Advisory Committee

The Novel and Exceptional Technology and Research Advisory Committee is a federal advisory committee that provides recommendations to the NIH Director and a public forum for the discussion of the scientific, safety, and ethical issues associated with emerging biotechnologies. NExTRAC proceedings and reports are posted to the OSP Web site to enhance their accessibility to the scientific and lay public.

- Charter of the Novel and Exceptional Technology and Research Advisory Committee
- Novel and Exceptional Technology and Research Advisory Committee Roster

Announcements about the NExTRAC:

- NIH Director's Statement
- *Under the Poliscope Blog*

Inaugural NExTRAC Meeting:

December 5-6, 2019
The John Edward Porter Neuroscience Research Center
NIH Campus, Building 35A, Room 620/630
9000 Rockville Pike
Bethesda, MD 20892

The Novel and Exceptional Technology and Research Advisory Committee (NExTRAC) will meet to discuss 1) pathways for responsible innovation in emerging biotechnologies; 2) characteristics of emerging biotechnologies, including presentations on horizon scanning, gene editing in the clinic, gene drives, neurotechnology, artificial intelligence, and synthetic biology; and 3) proactively addressing scientific and societal implications of emerging biotechnologies. In addition, charge(s) to the committee will be presented.

Questions?