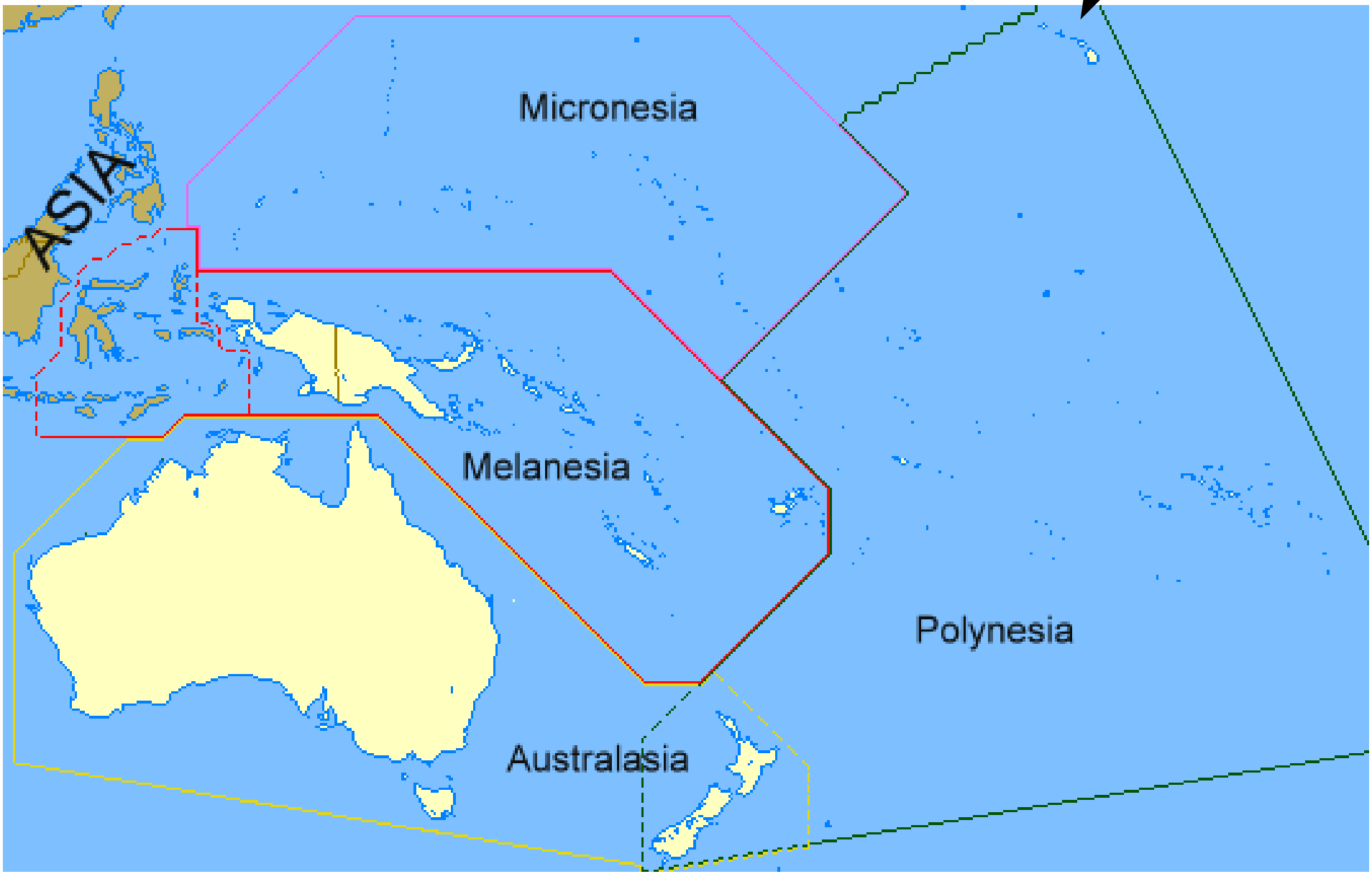
Genetic Engineering for Species Conservation Applications in Hawai'i

Floyd A. Reed and Jolene T. Sutton

Department of Biology, University of Hawai'i at Mānoa, Honolulu, HI, USA

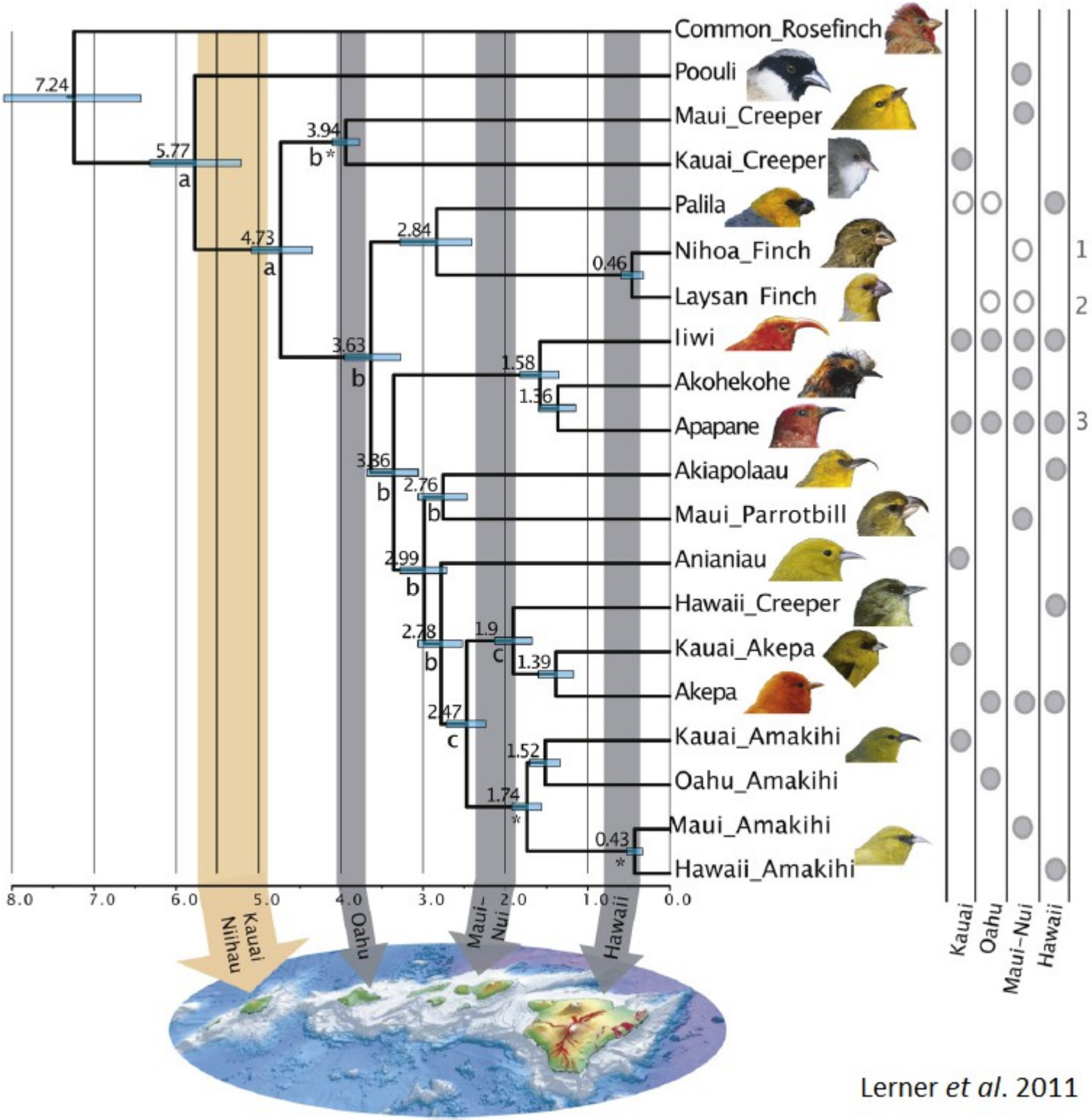
http://www.hawaiireedlab.com/presentations

Polynesian neighbors* of Australasia



*N.Z. is both. So, we are closer than neighbors!

http://en.wikipedia.org/wiki/Australasia



Lerner *et al*. 2011

Culex mosquitoes were accidentally introduced to Hawai'i in the early 19th century and vector the Plasmodium that causes avian malaria.

Culex mosquitoes were accidentally introduced to Hawai'i in the early 19th century and vector the Plasmodium that causes avian malaria.

One bite by an infected Culex is likely to kill a juvenile i'iwi (Atkinson *et al*. 1993)

Hawaii Mamo (Kulemans 1893)

Probable Extinction Dates

1891 1892
1894 1895
1898 1901
1907 1918
1933 1937
1940 1963
1985 1989
1998 2004

Conservation Status

Extinct (EX)	Threatened (EW, CR, EN, VU)	Least Concern (NT, LC)
Extinct Species[3]		
Kona Grosbeak (*Chloridops kona*) 'Ula-'ai-Hawane (*Ciridops anna*) Lanai Hookbill (*Dysmorodrepanis munroi*) Hawaii Mamo (*Drepanis pacifica*) Black Mamo (*Drepanis funerea*) O'ahu 'Akialoa (*Hemignathus ellisianus*) Hawai'i 'Akialoa (*Hemignathus obscurus*) Lanai 'Alauahio (*Paroreomyza montana montana*) Greater Amakihi (*Hemignathus sagittirostris*) Kakawahie (*Paroreomyza flammea*) Lesser Koa-Finch (*Rhodacanthis flaviceps*) Greater Koa-Finch (*Rhodacanthis palmeri*)		
Endangered Species[3]		
'Akiapola'au (*Hemignathus munroi*) Palila (*Loxioides bailleui*) 'Akepa (*Loxops coccineus*) Hawai'i Creeper (*Oreomystis mana*) Maui Nui 'Alauahio (*Paroreomyza montana*)		
Near Threatened Species[3]		

Extinct (EX)	Threatened (EW, CR, EN, VU)	Least Concern (NT, LC)
Critically Endangered Species[3]		
	Nukupuu (*Hemignathus lucidus*), probably extinct 'Akeke'e (*Loxops caeruleirostris*) Po'o-uli (*Melamprosops phaeosoma*), probably extinct 'Akikiki (*Oreomystis bairdi*) 'Akohekohe (*Palmeria dolei*) O'ahu 'Alauahio (*Paroreomyza maculata*), probably extinct Maui Parrotbill (*Pseudonestor xanthophrys*) 'Ō'ū (*Psittirostra psittacea*), probably extinct Nihoa Finch (*Telespiza ultima*)	
Vulnerable Species[3]		
	O'ahu 'Amakihi (*Hemignathus flavus*) Kaua'i 'Amakihi (*Hemignathus kauaiensis*) 'Anianiau (*Hemignathus parvus*) Laysan Finch (*Telespiza cantans*) 'I'iwi (*Vestiaria coccinea*)	
Species of Least Concern[3]		
	Common 'Amakihi (*Hemignathus virens*) 'Apapane (*Himatione sanguinea*)	

http://en.wikipedia.org/wiki/Hawaiian_honeycreeper_conservation

Elevation limits avian malaria, but climate change is predicted to reduce the refuge dramatically.

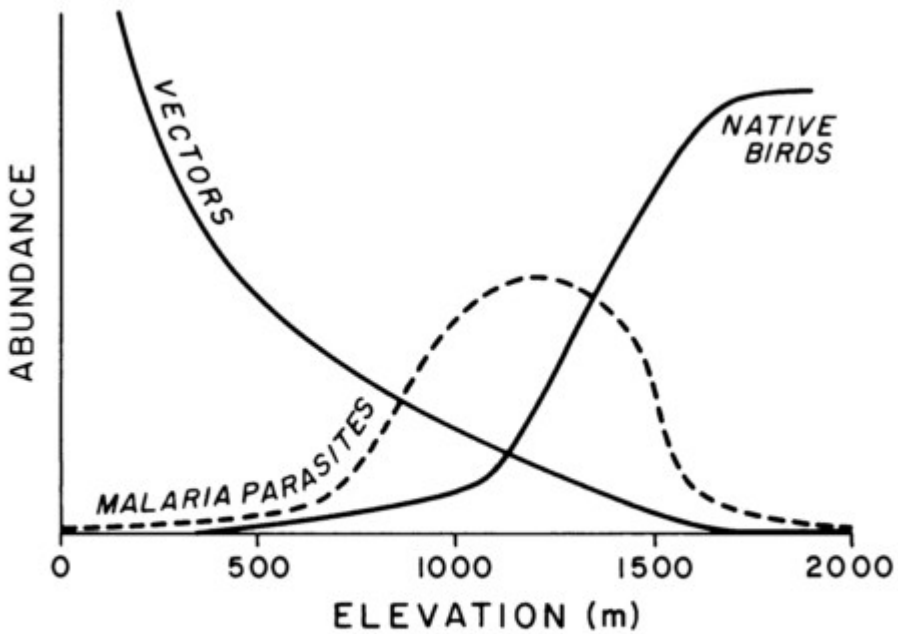
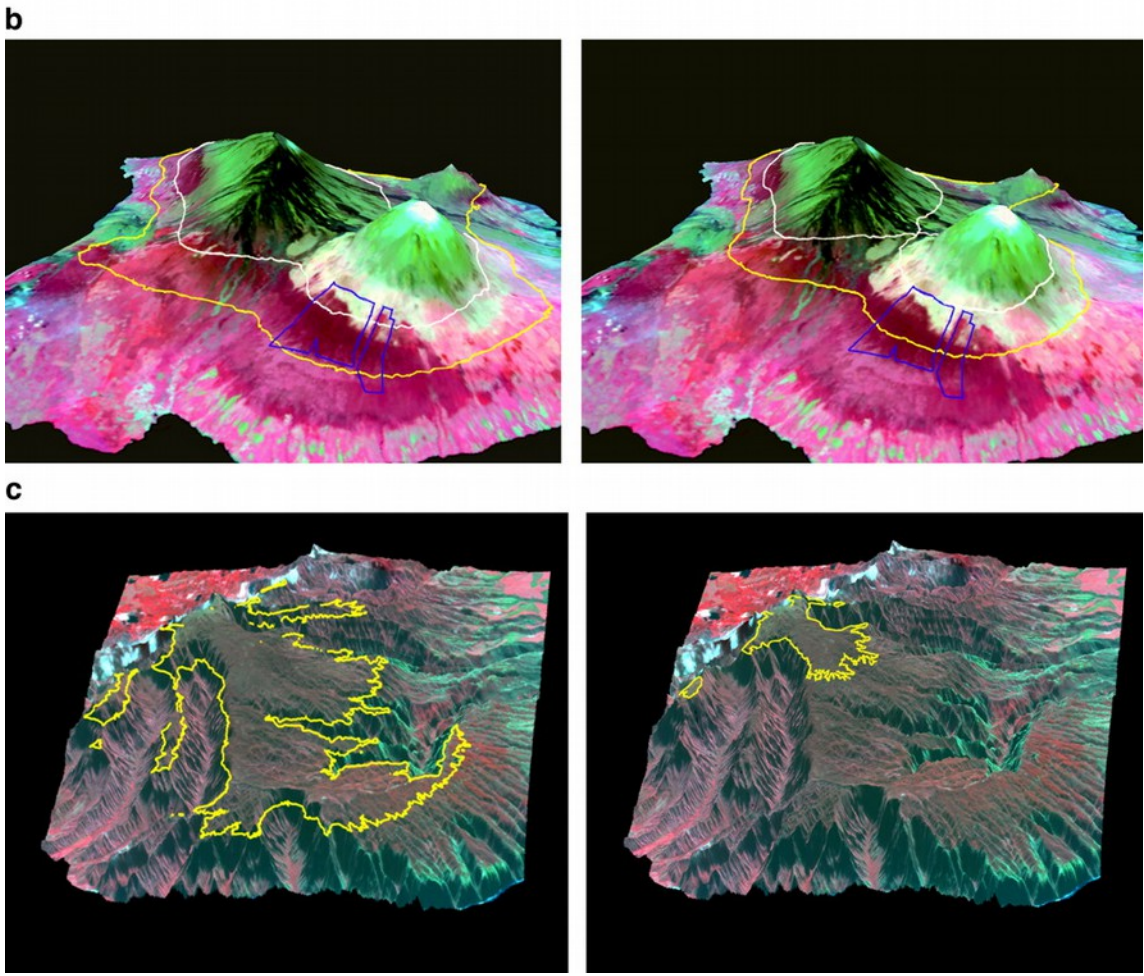
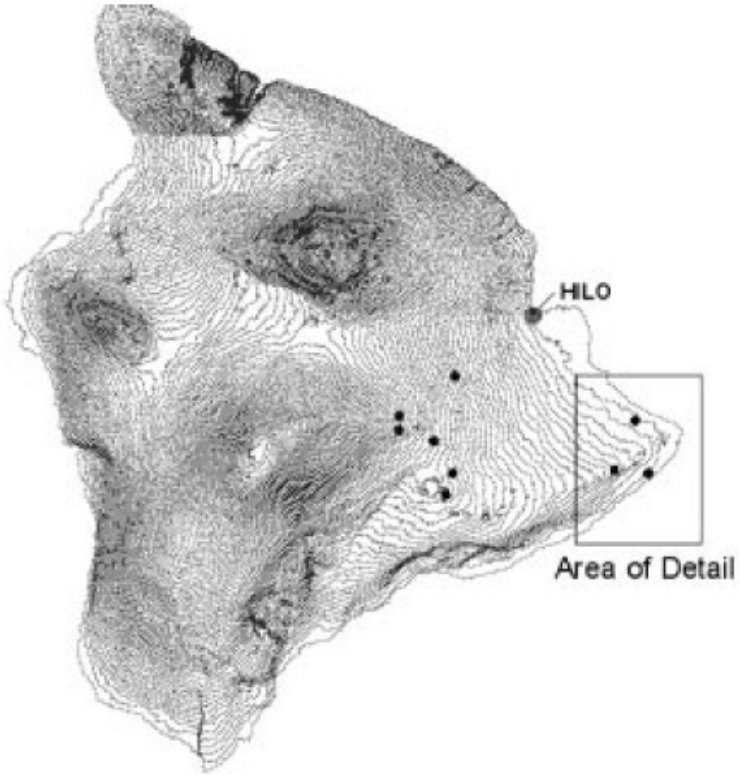


FIG. 11. A generalized model of native bird abundances, malarial parasite incidence, and mosquito vector levels along an elevation gradient on Mauna Loa, Hawaii.

van Riper et al. (1986)



Benning *et al*. (2002)



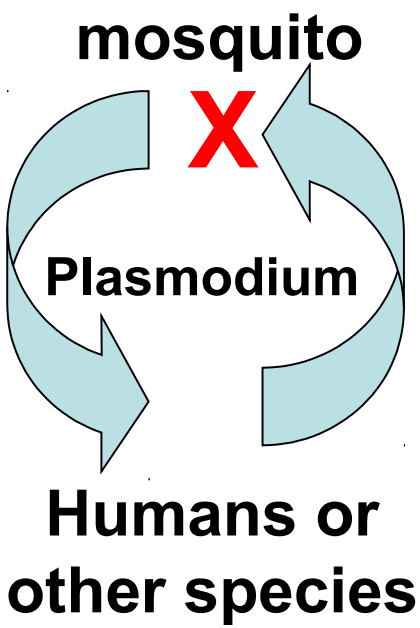
However, there may be some long term hope! One species, the 'amakihi, appears to finally be evolving resistance to and/or tolerance of Plasmodium on the island of Hawai'i (Woodworth *et al*. 2005 PNAS).

We are interested in buying the other birds time.

http://kaunewsbriefs.blogspot.se/2010_12_03_archive.html

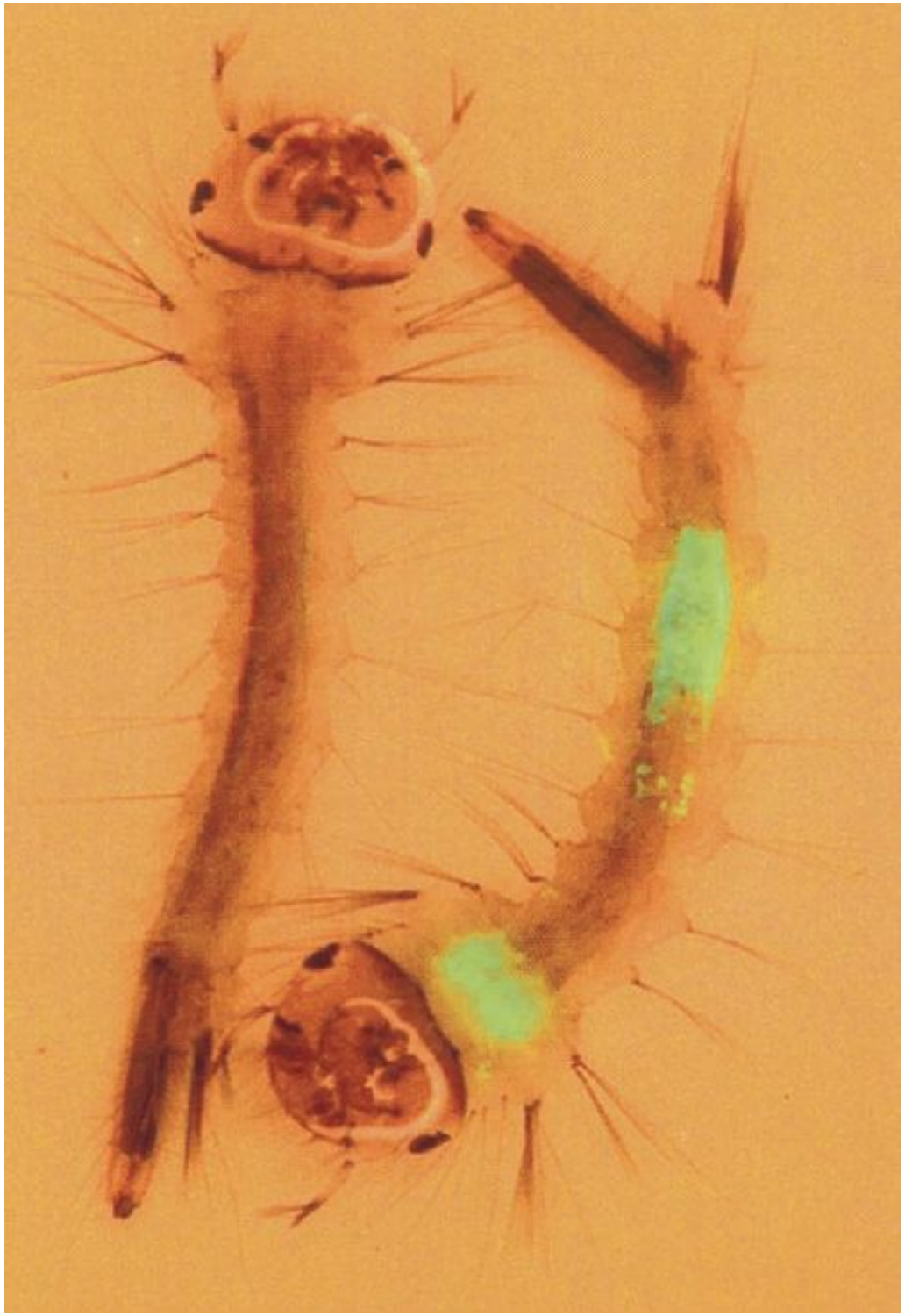
Motivation

Reducing disease transmission



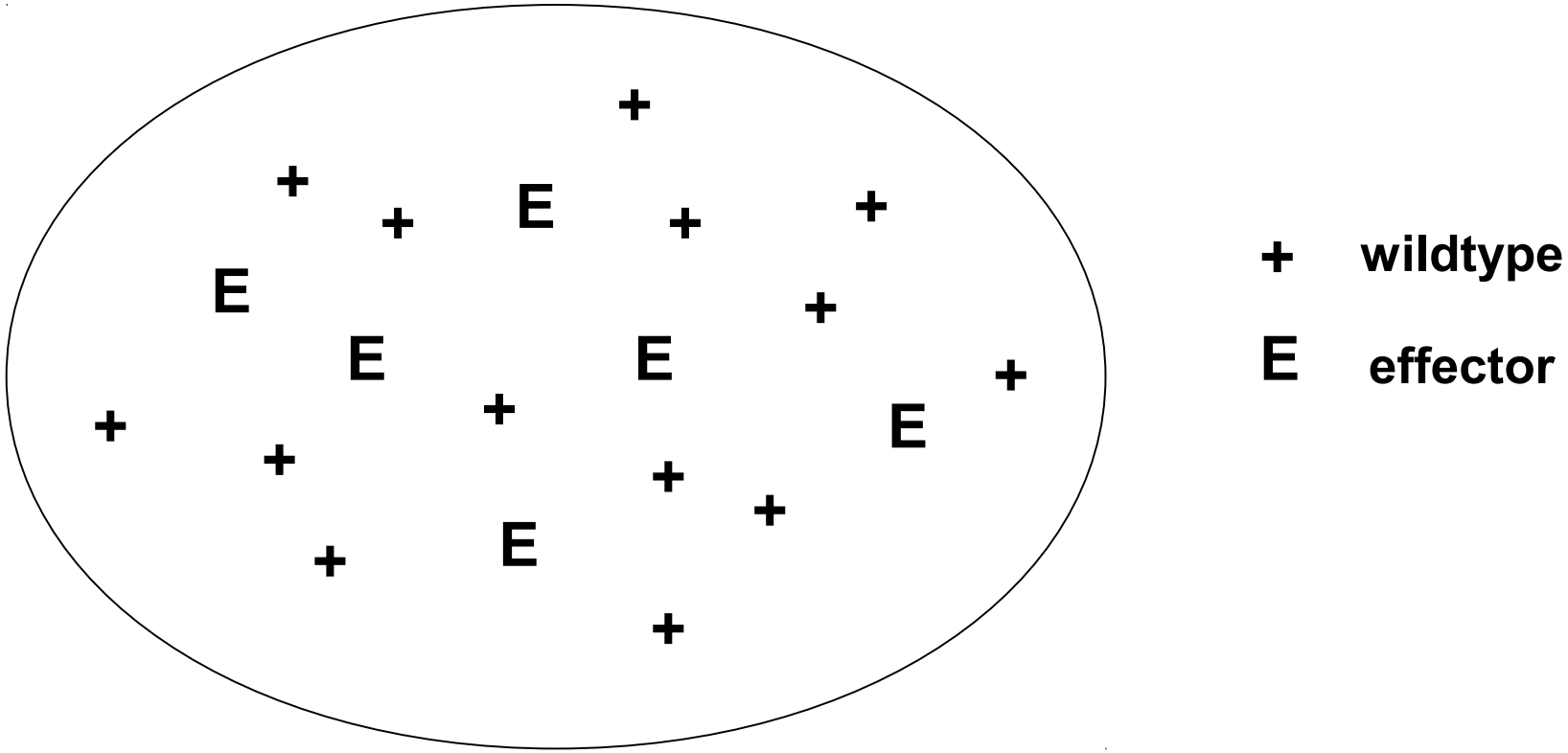
Engineered Effector Genes: Reduce Plasmodium Transmission

Example: Single chain antibodies targeting sporozoite surface proteins and expressed in mosquito salivary glands can reduce transmission 2 to 4 orders of magnitude in the avian model.
(Jasinskiene *et al*. 2007 *Am. J. Trop. Med. Hyg.* 76:1072).



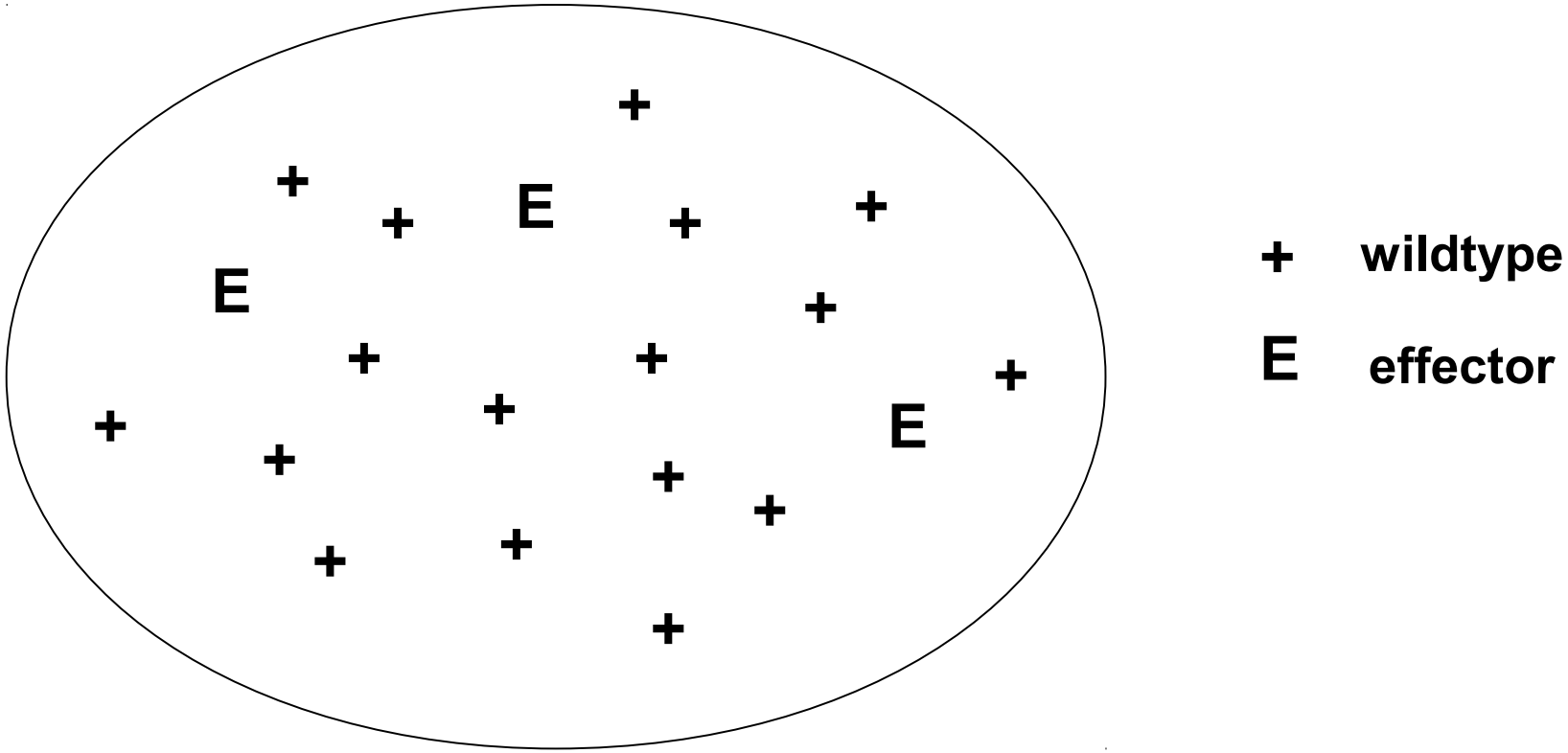
Genetic transformation of *Culex* is possible (Allen *et al*. 2001 *J. Med. Entomol.* 38:701).

Effectors can be released into a wild population:



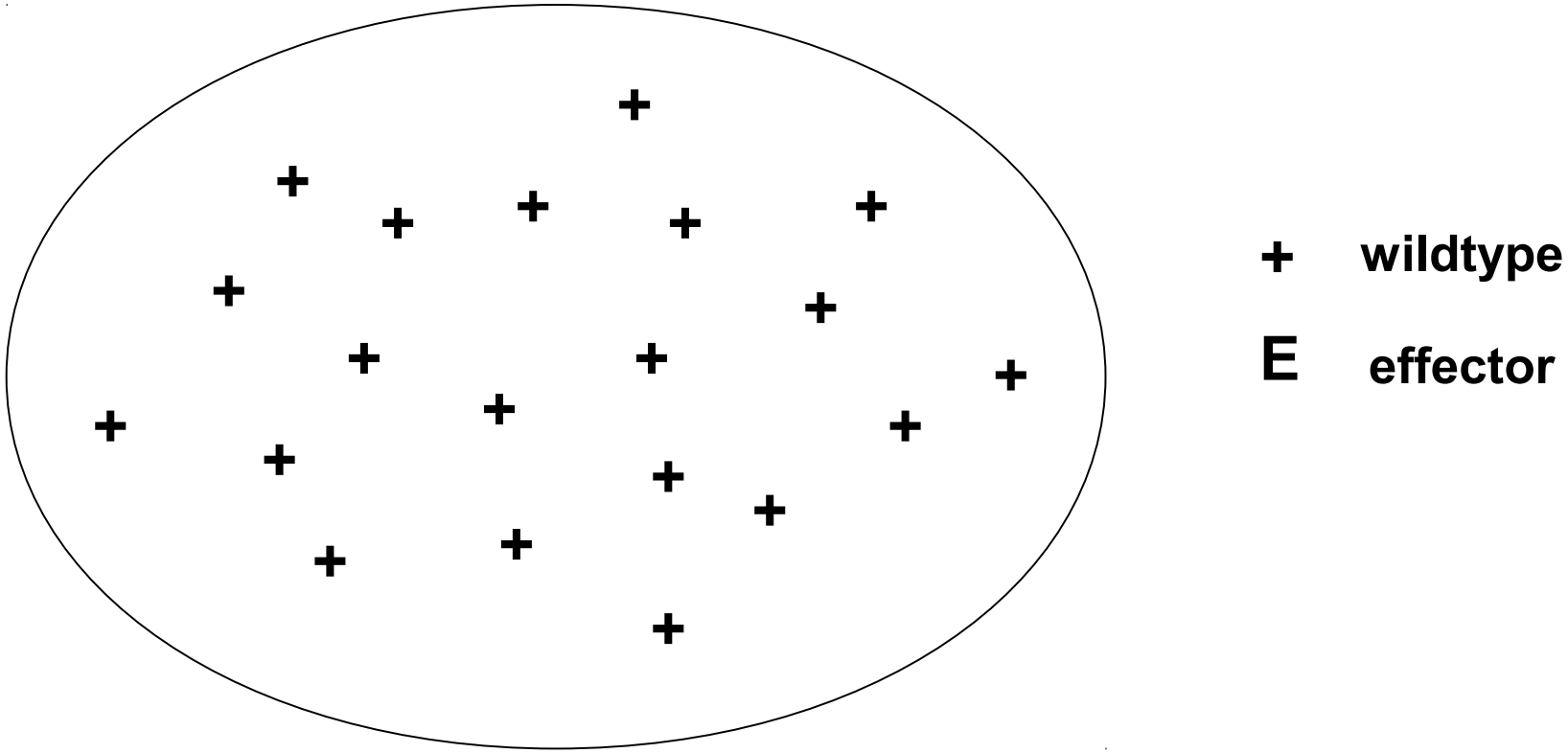
But, if there is no fitness advantage, and especially likely, if there is a fitness cost, they are unlikely to reach fixation and may be quickly lost from the wild.

Effectors can be released into a wild population:



But, if there is no fitness advantage, and especially likely, if there is a fitness cost, they are unlikely to reach fixation and may be quickly lost from the wild.

Effectors can be released into a wild population:



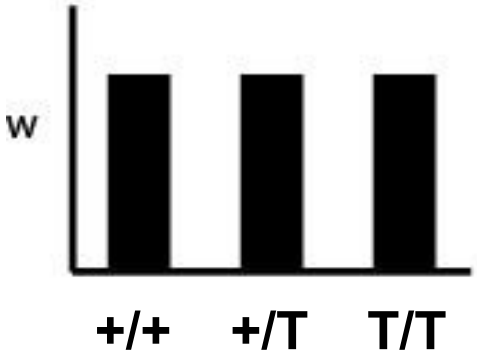
But, if there is no fitness advantage, and especially likely, if there is a fitness cost, they are unlikely to reach fixation and may be quickly lost from the wild.

It may be next to impossible to engineer a construct with higher fitness than wildtype (in the adaptive sense).

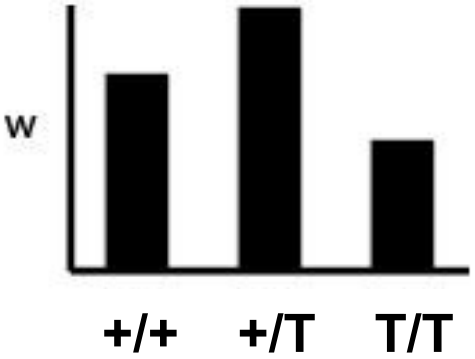
However, we might utilize “drive” mechanisms linked to effector constructs to push effectors to high frequency or fixation in the wild without increasing fitness (population transformation).

Two alleles give three genotypes and four different fitness configurations.

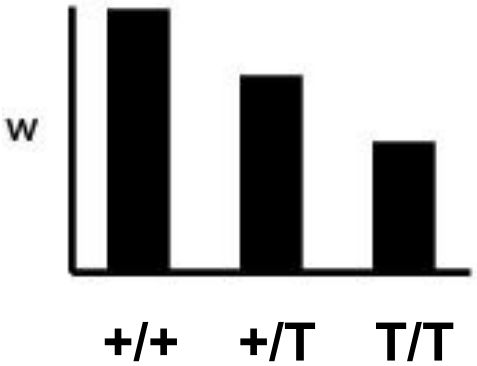
1) Neutrality



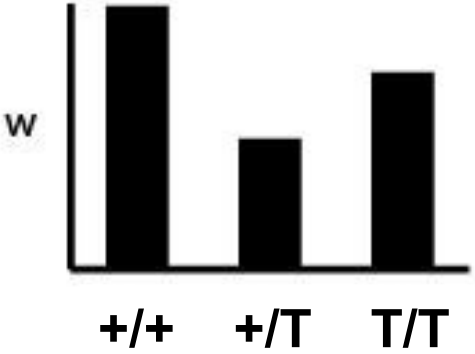
3) Overdominance



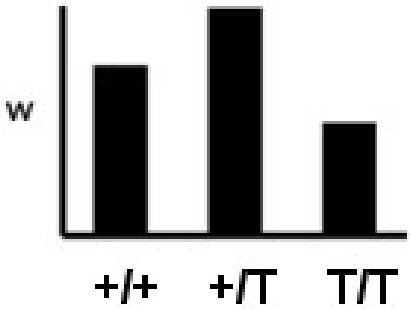
2) Directional



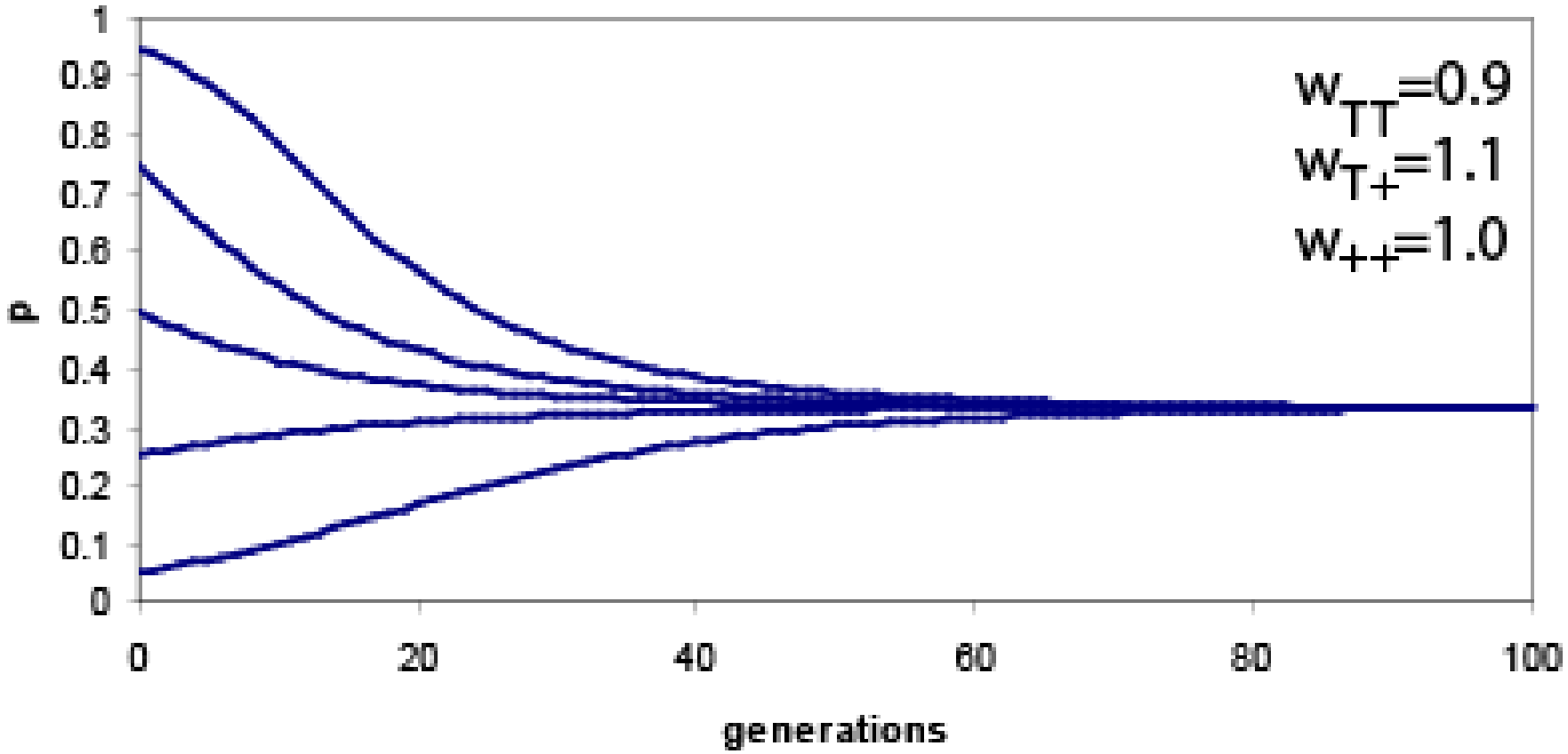
4) Underdominance



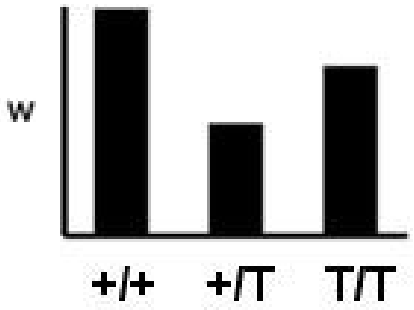
3) Overdominance



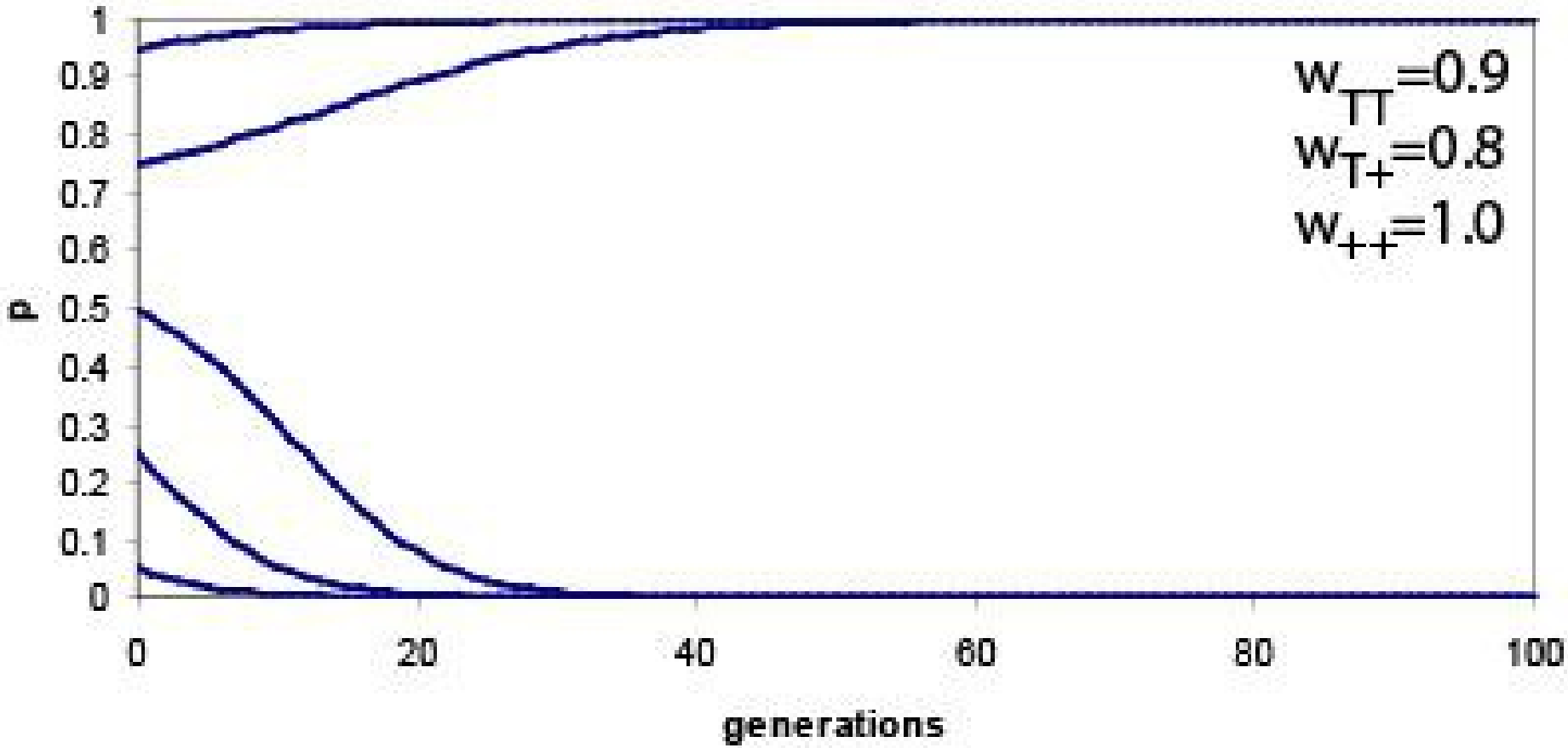
A heterozygote advantage leads to a stable equilibrium.



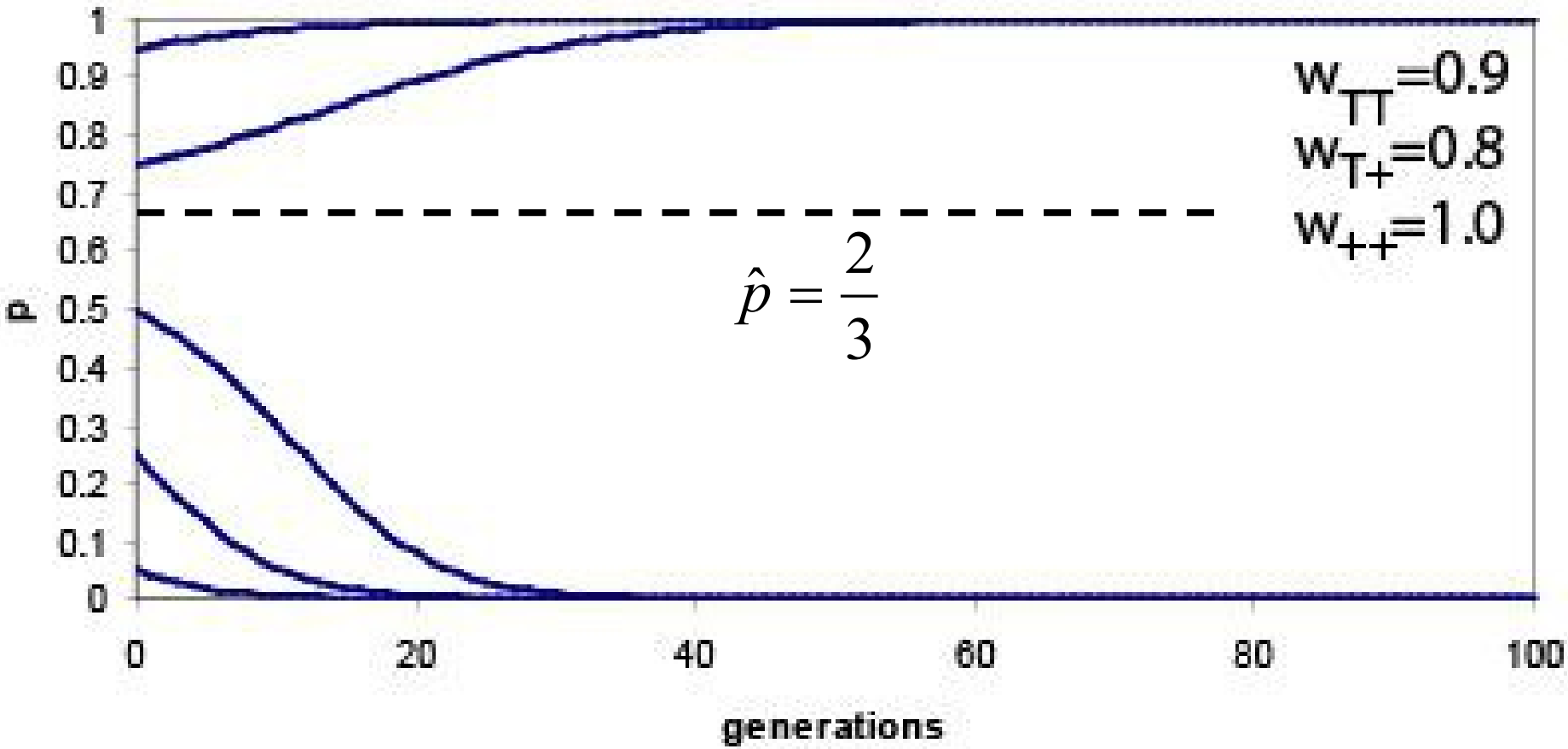
4) Underdominance



A heterozygote disadvantage leads to an unstable equilibrium.



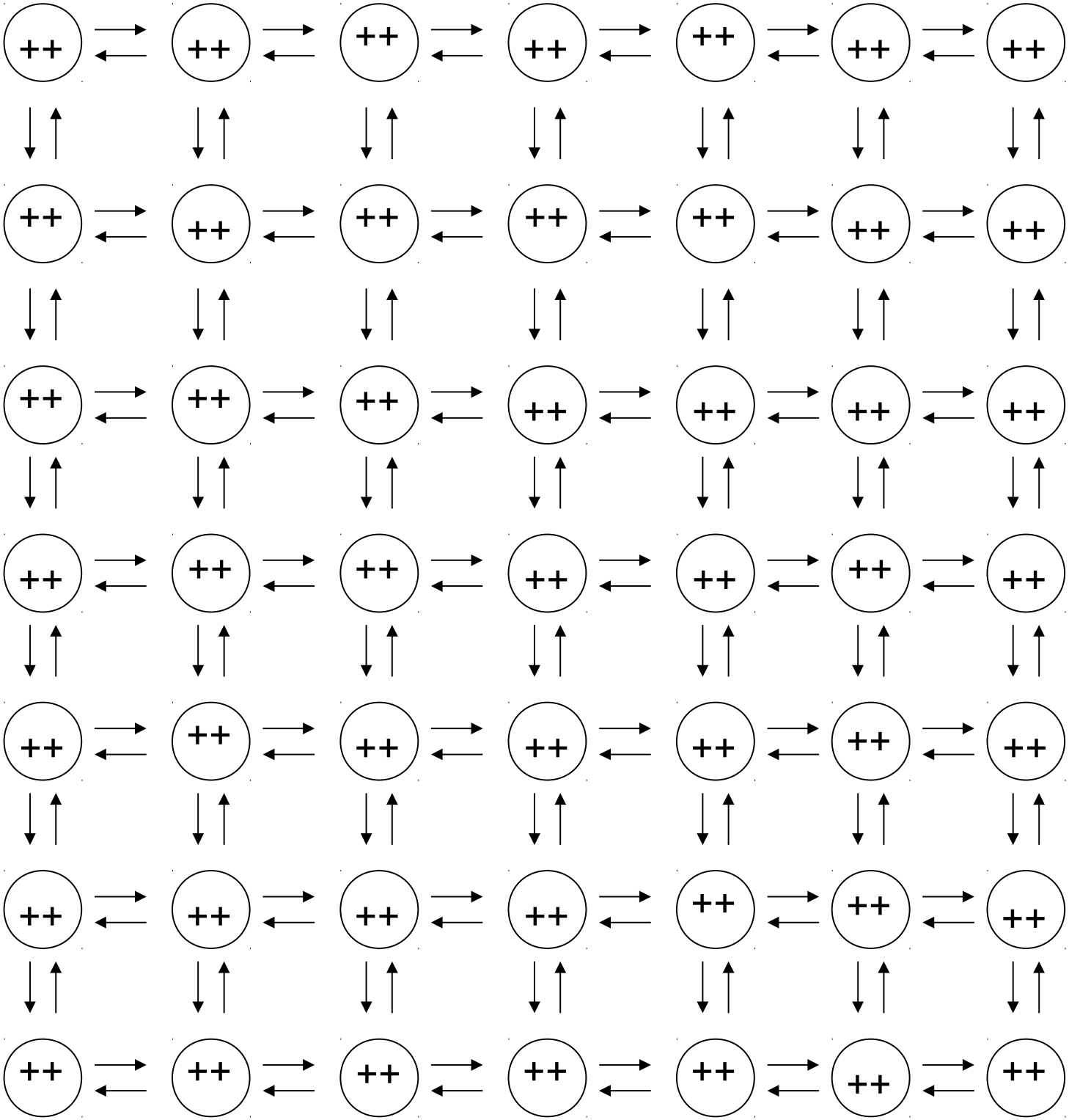
If starting at a frequency above this equilibrium value, an allele <u>less fit than wildtype</u> can stably fix in a population.

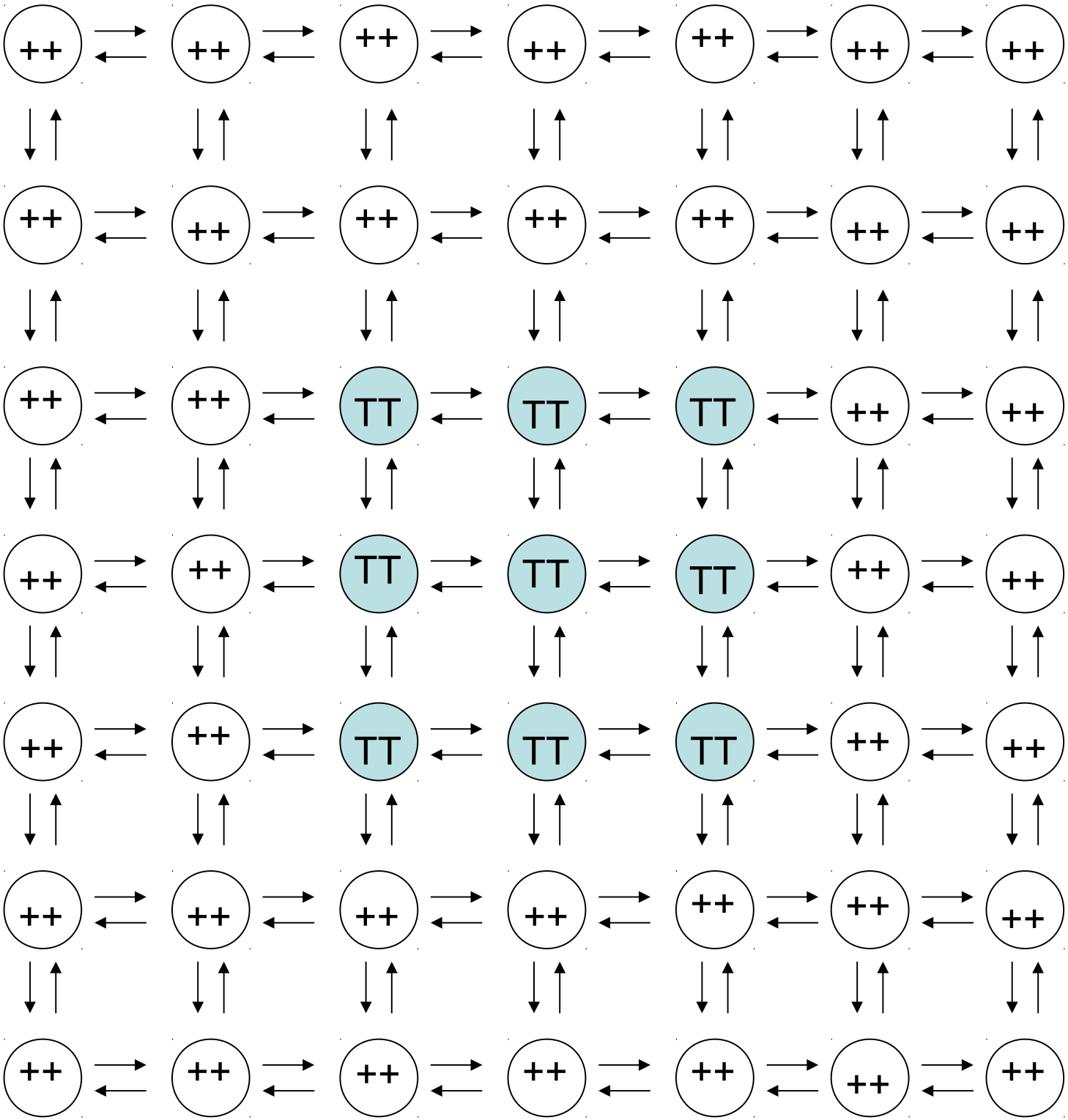


Underdominance has useful spatial properties for testing effector systems (and is tractable in island—Hawai'i!—models).

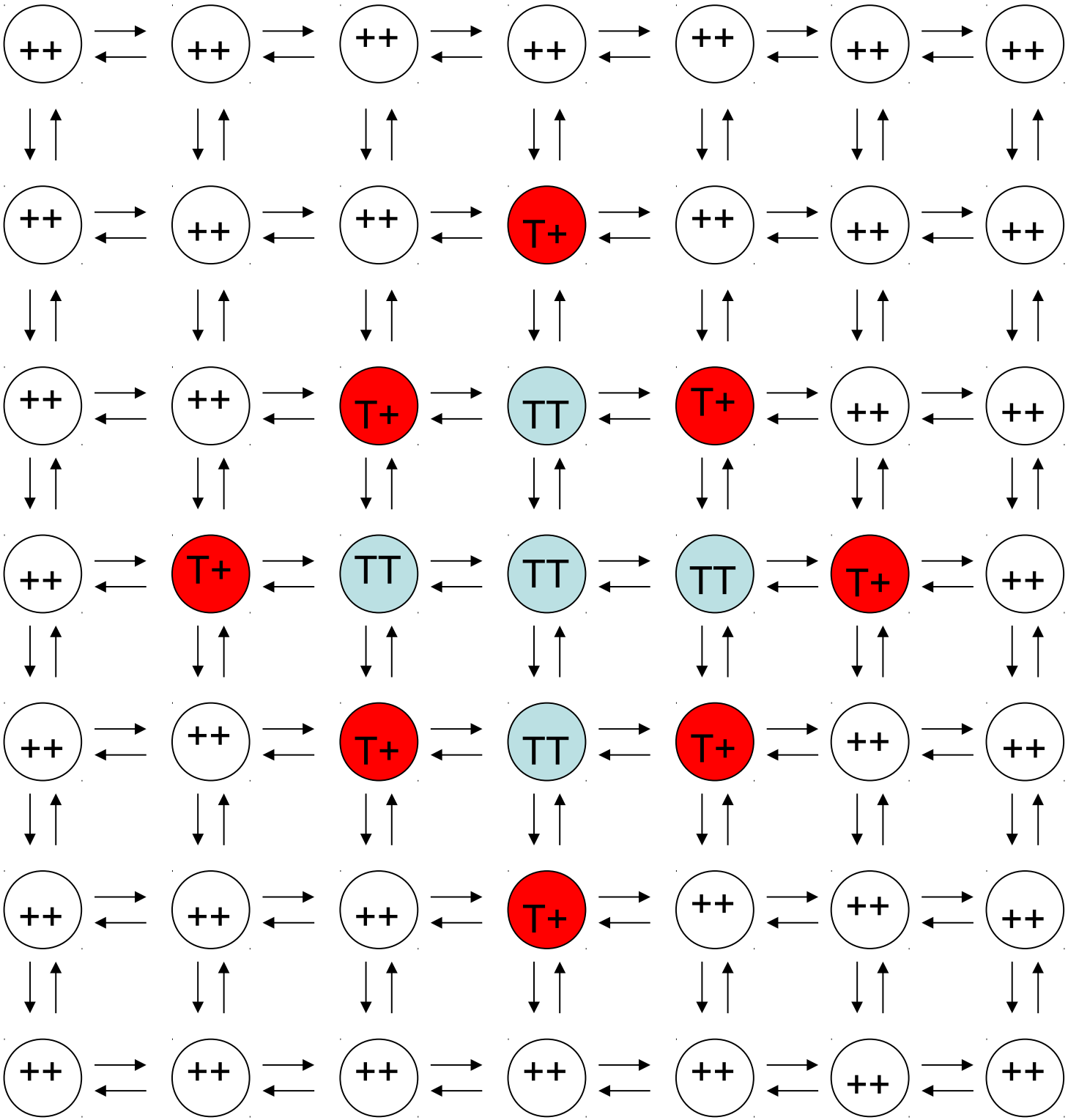
- Geographic Stability
- Reversibility

I will appeal to an intuitive abstracted example to illustrate.



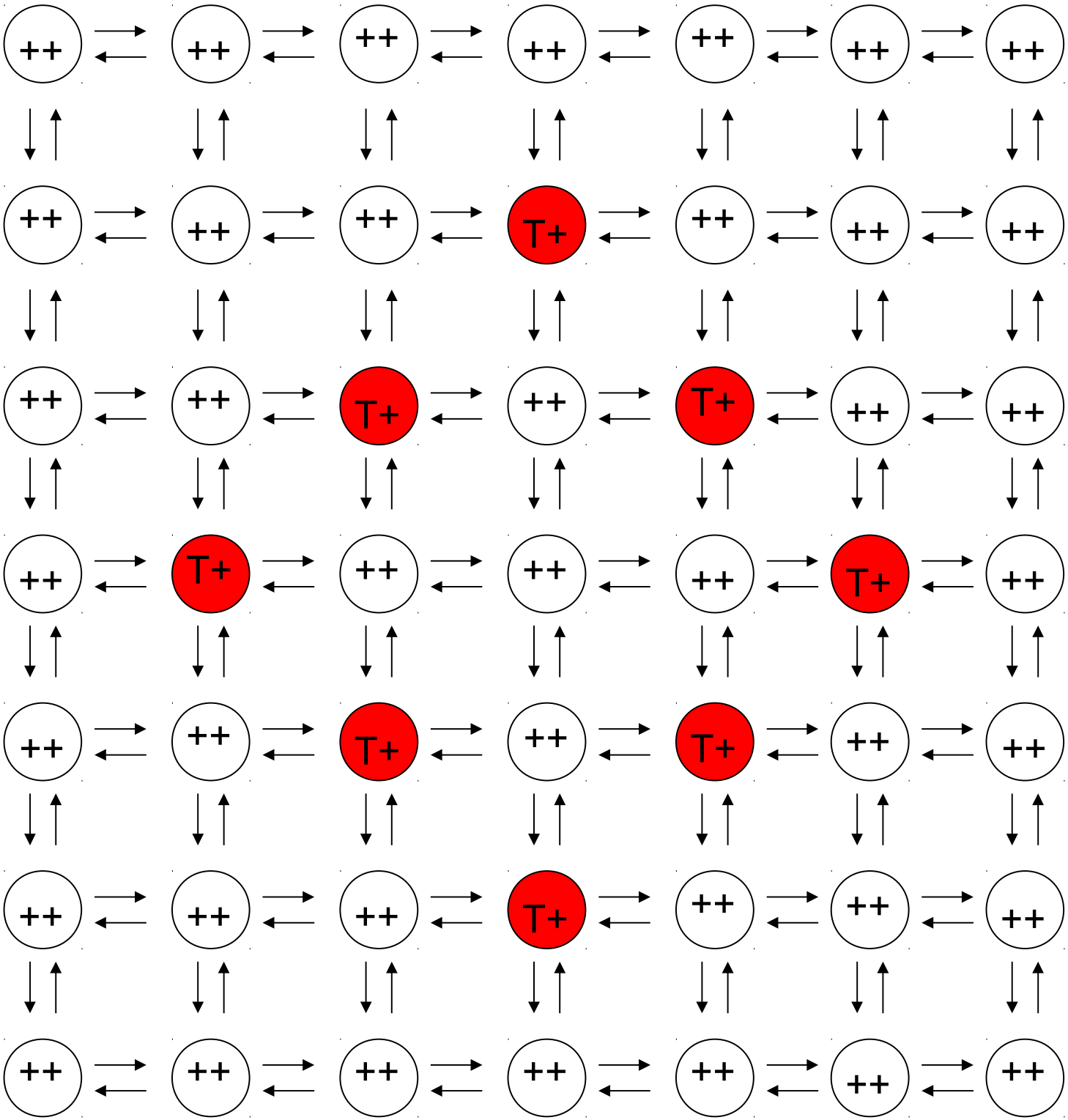


TT release

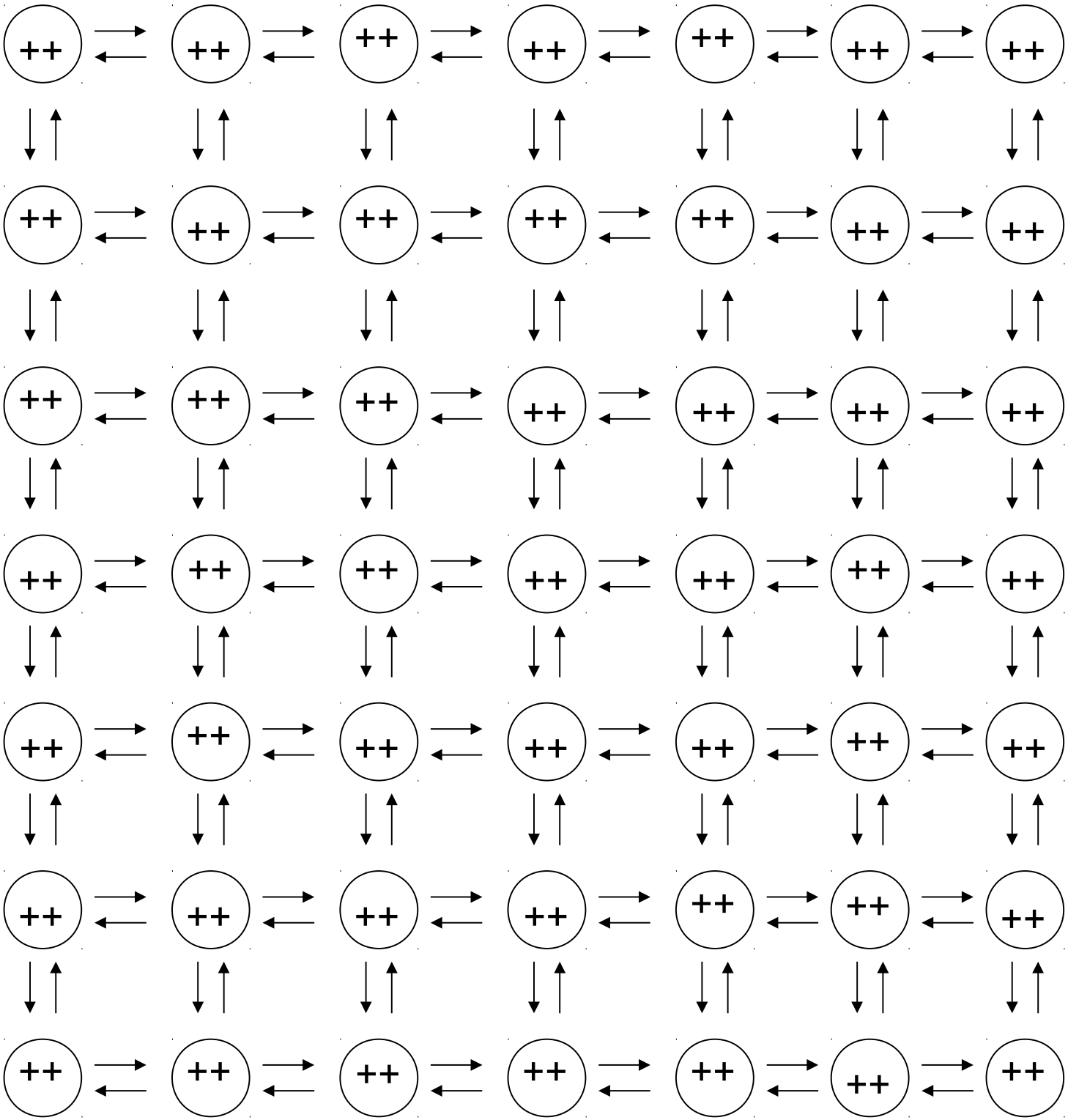


Migration-Selection Equilibrium

Geographic Stability



++ release



Migration-
Selection
Equilibrium

Reversibility

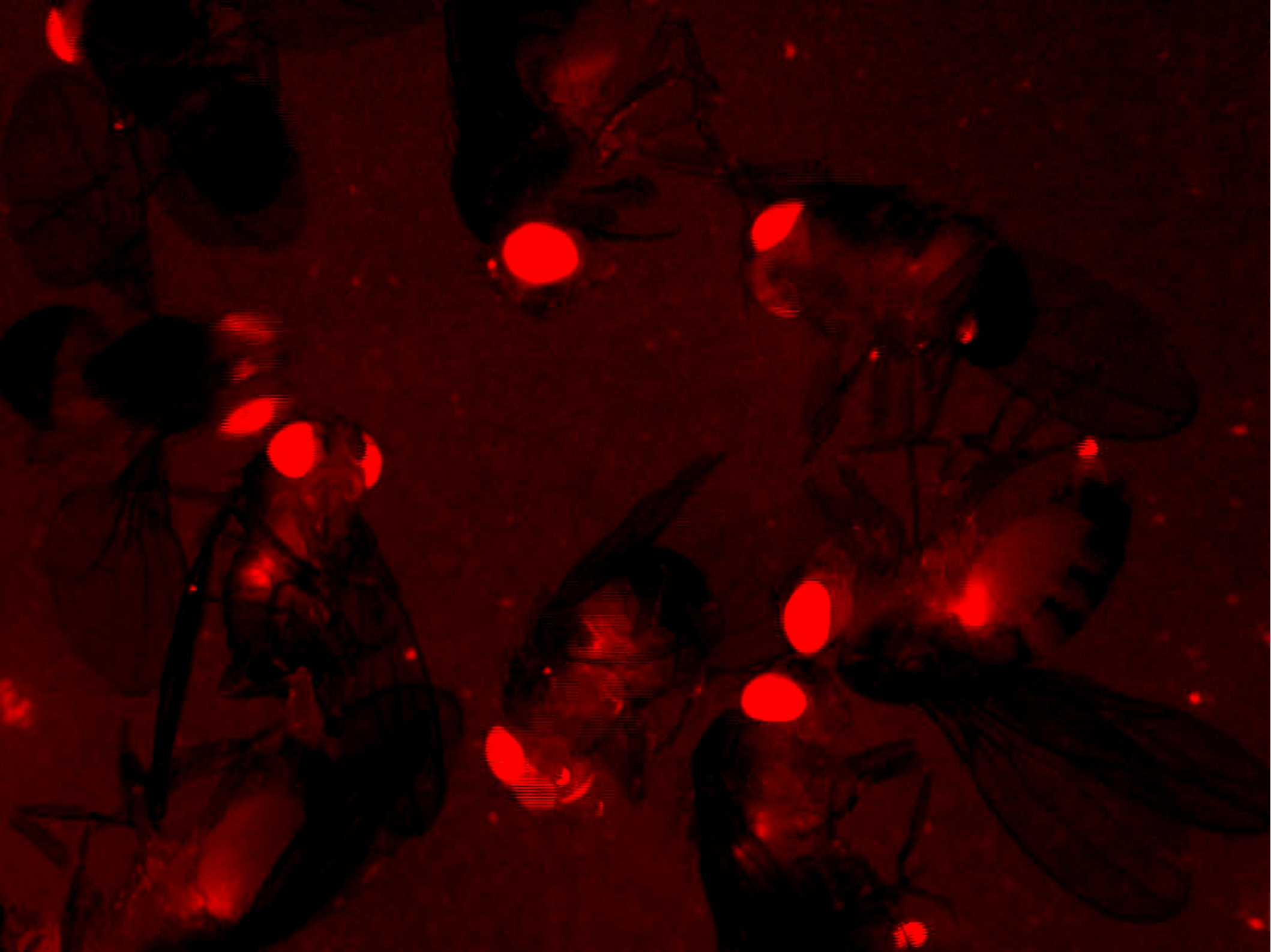
NATURE, VOL. 218, APRIL 27, 1968

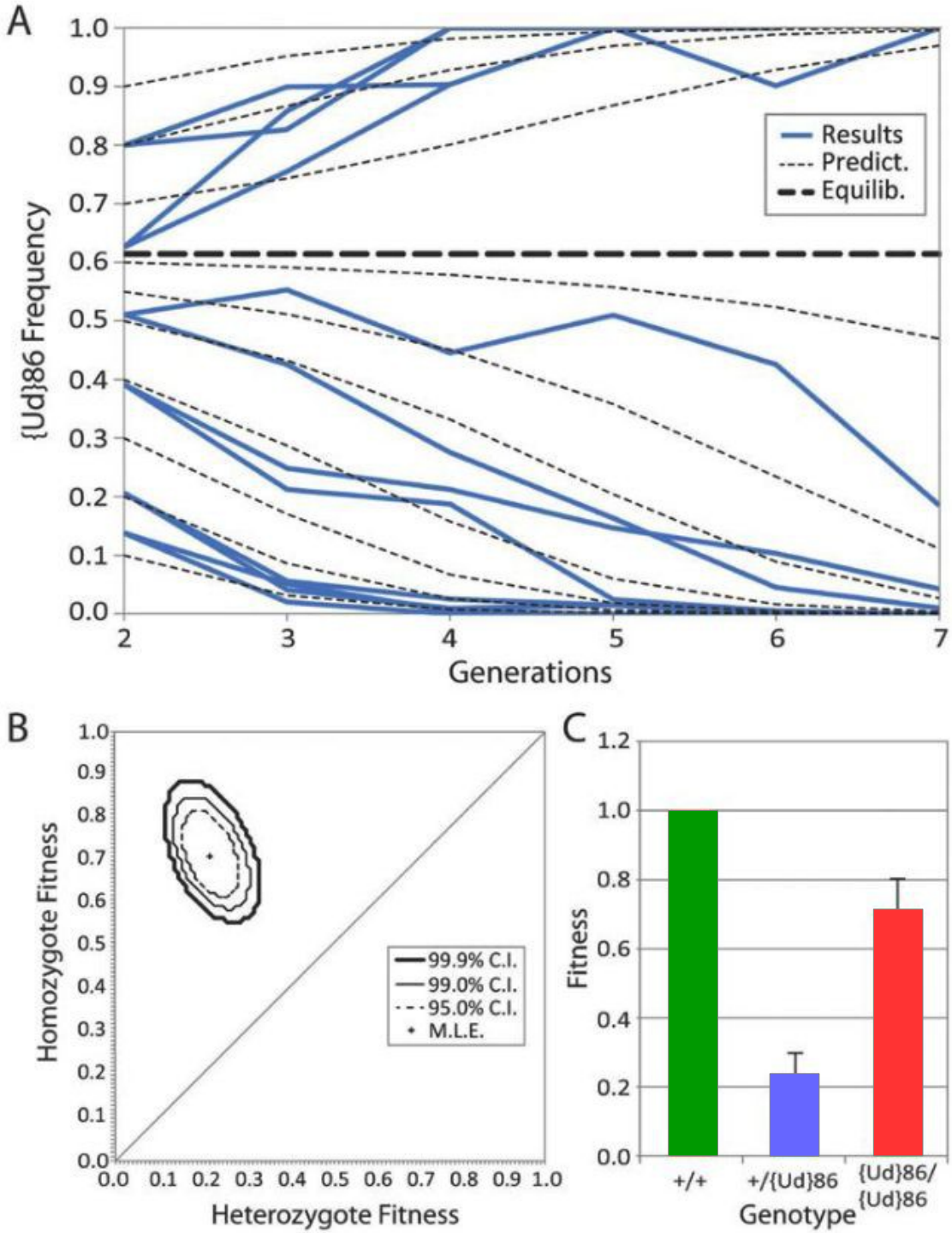
Possible Use of Translocations to fix Desirable Genes in Insect Pest Populations

CHROMOSOME translocation heterozygotes ($T/+$) are usually semisterile, but translocation homozygotes (T/T) if viable are usually fully fertile. If such a viable translocation were produced in an insect pest, T/T insects could be reared in captivity and released into the wild, where matings with wild types ($+/+$) would produce $T/+$ progeny. ...

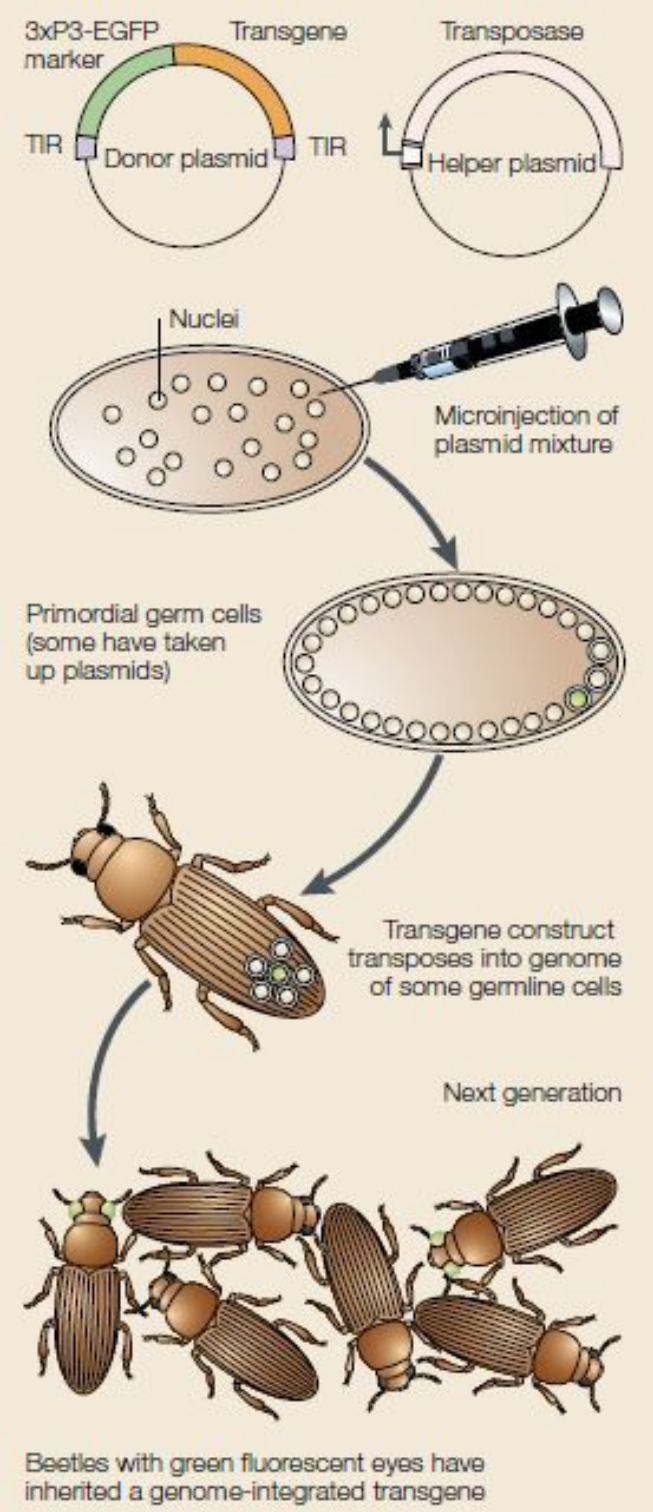
C. F. CURTIS

Tsetse Research Laboratory,
University of Bristol,
Langford, near Bristol.

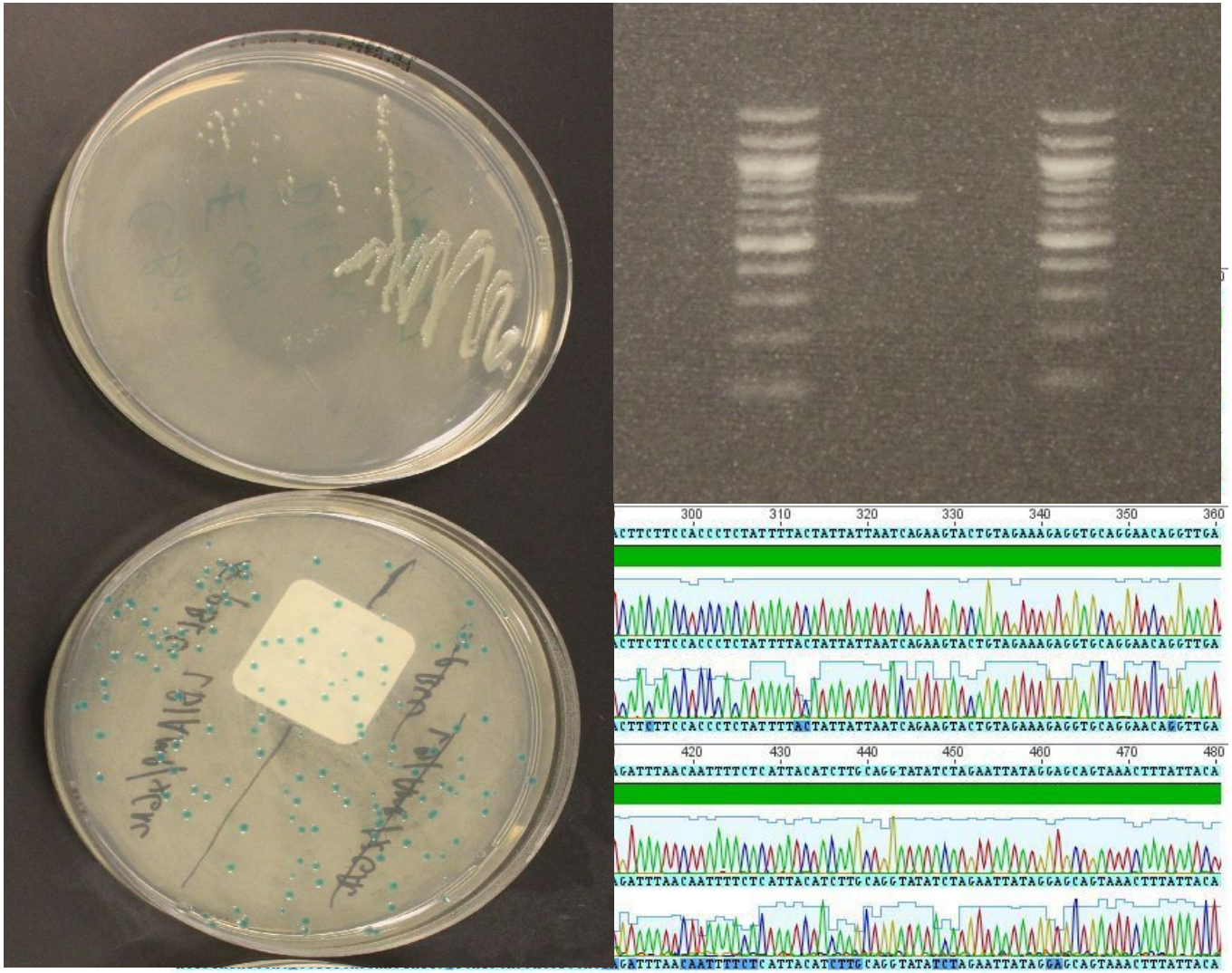




Reeves *et al*. 2014 *PLoS ONE*

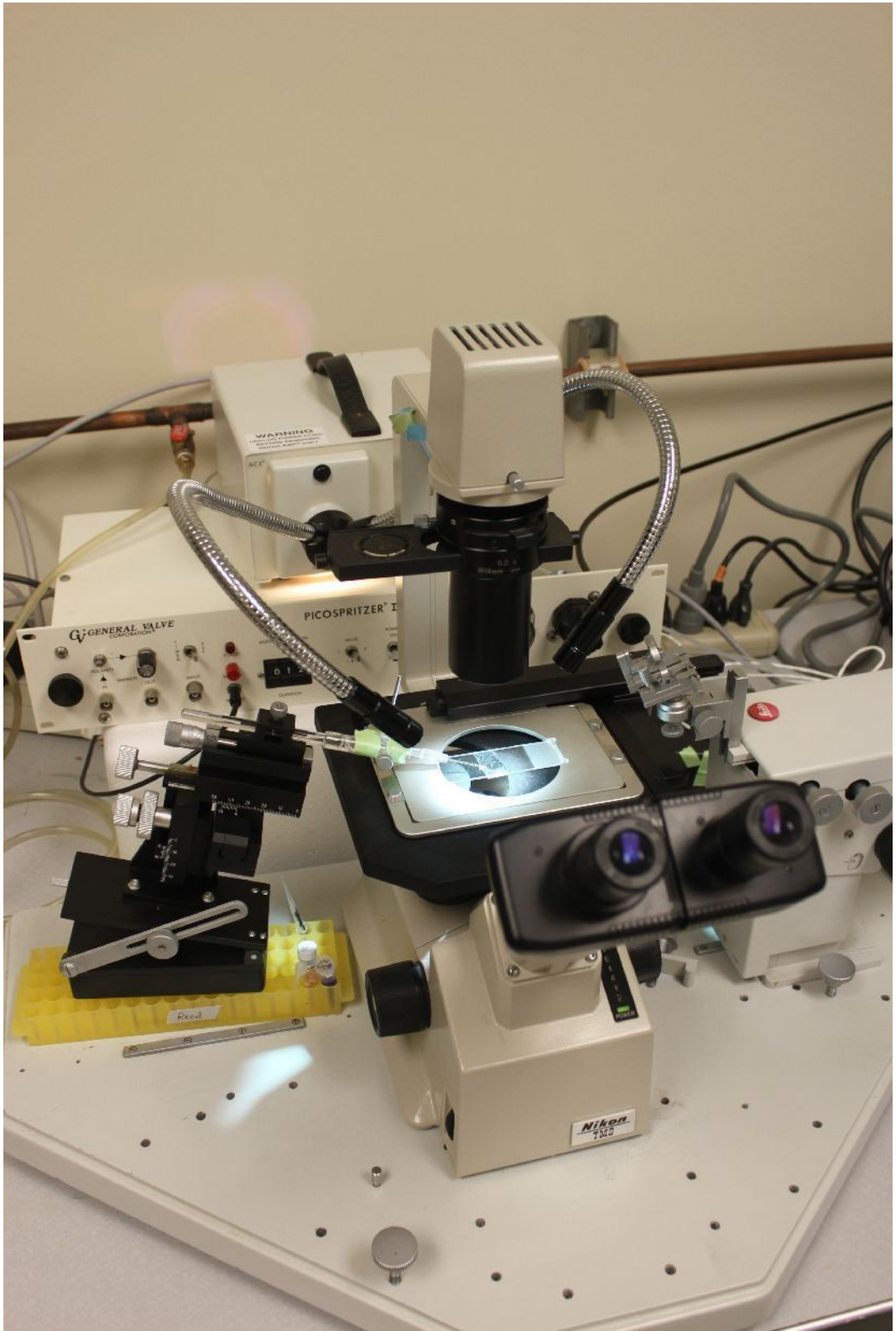


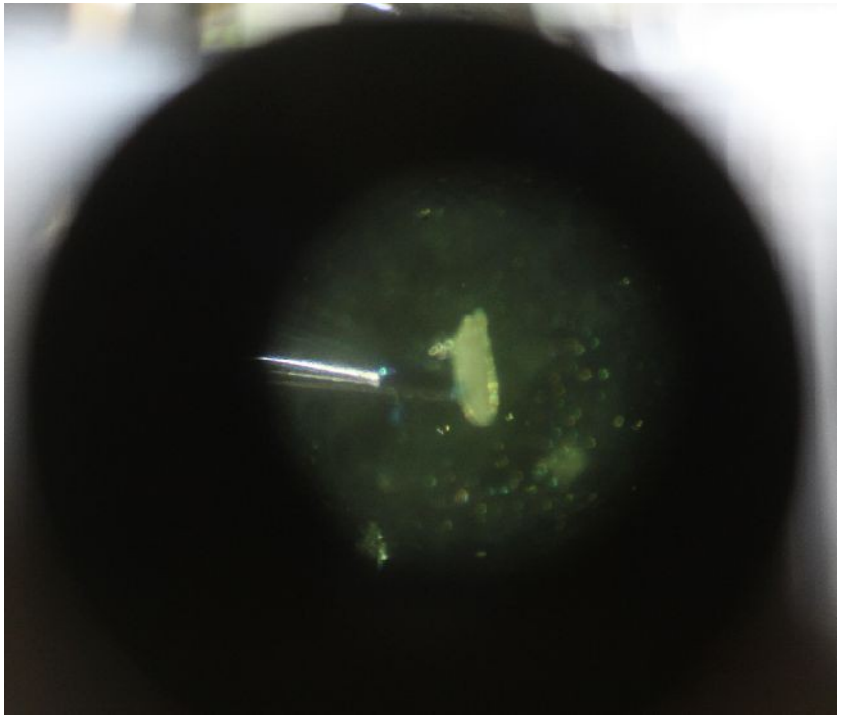
Current direction: Port our system to Culex mosquitoes.

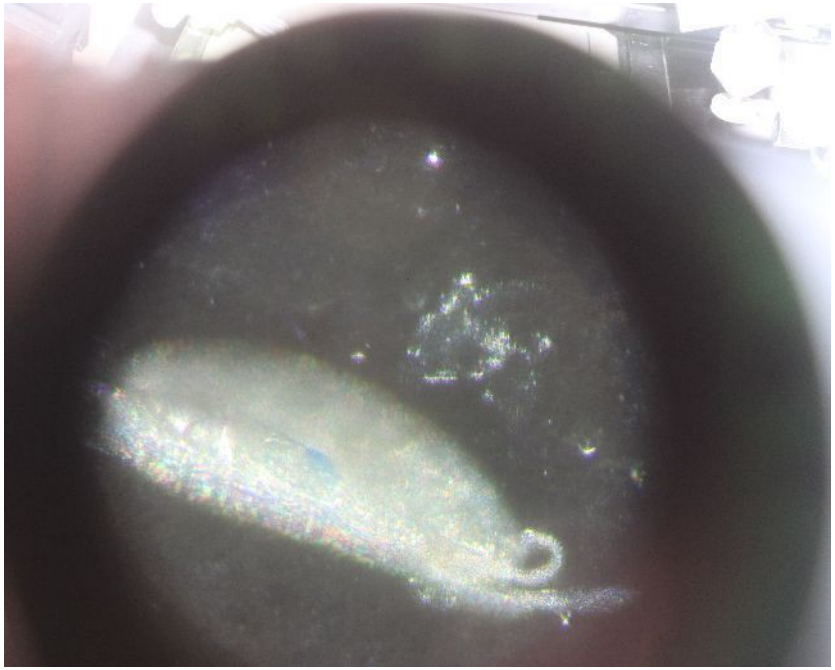


← Wimmer 2003 Nat. Rev. Genet. 4:225

Embryo Microinjection



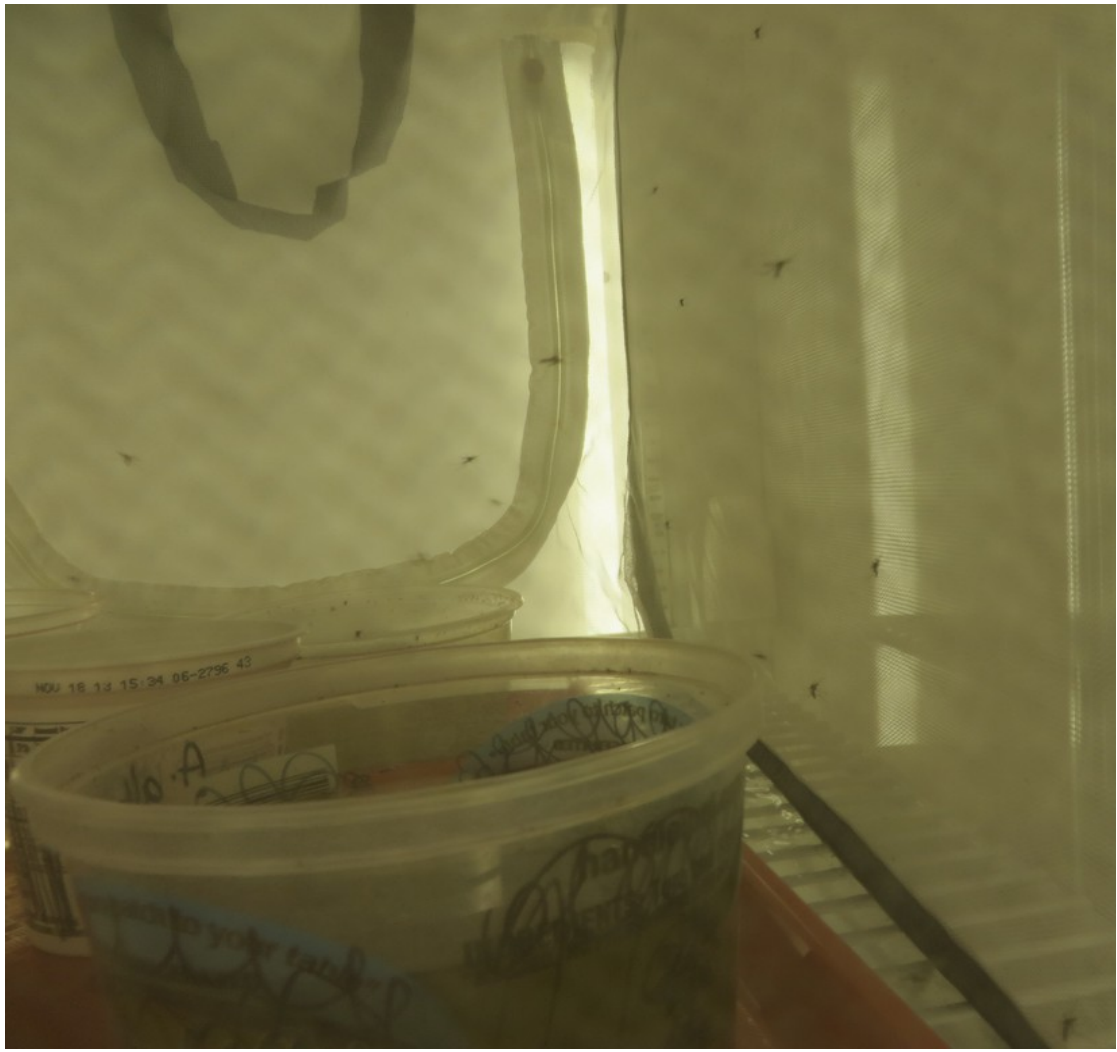




Culex Larvae are Easily Collected on UH Campus...

We have only been able to work with mosquitoes over the last ten months.

Our current focus is to optimize raising them in the lab, plasmid design, and practice microinjection techniques.



Other directions:

Study the genetic basis of evolving immune response in the 'amakihi. (Dr. Sutton presented initial results yesterday.)

Explore the potential to use Wolbachia to suppress Plasmodium (and viruses) in Hawaiian Culex (*cf*. O'Neill, Monash U.).

Explore population suppression approaches using cytoplasmic incompatibility (*cf*. Laven 1967 Nature 216:383)

Acknowledgements

	In Germany	In Hawai'i
Support:	Max-Planck-Society DFG (Deutshe Forschungsgemeinschaft)	University of Hawai'i, College of Natural Sciences Hawaiian Community Foundation
People:	**Guy Reeves** Jai Denton Jarek Bryk Philipp Altrock Anita Möller	**Jolene Sutton** Natasha Isaac Áki J. Láruson

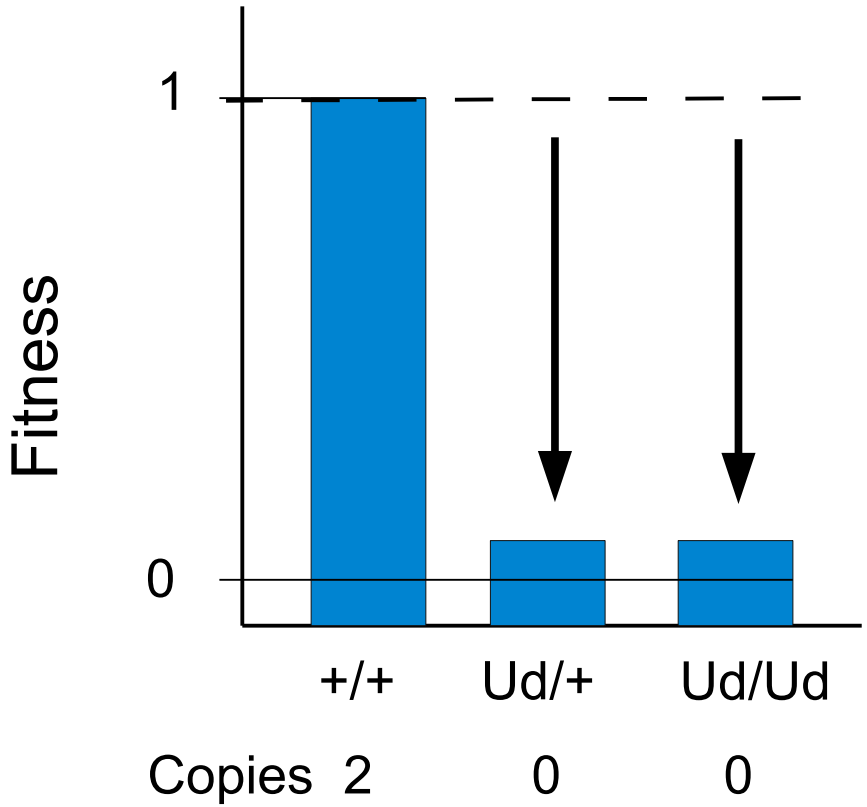
http://www.hawaiireedlab.com/presentations

Grad. Students Wanted

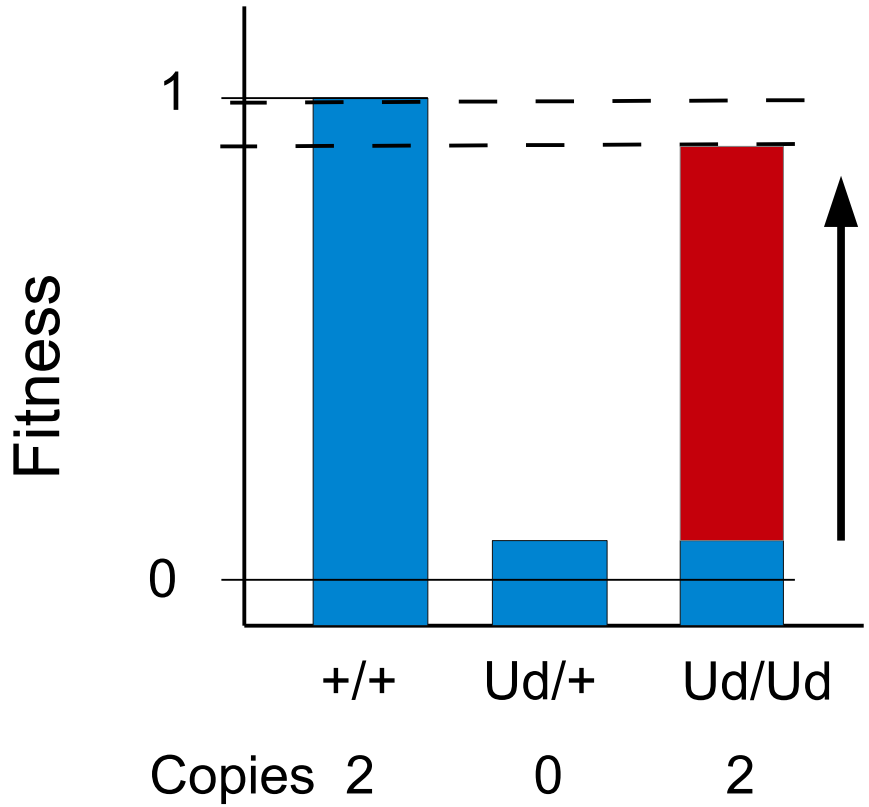
Masters and Ph.D.

floydr@hawaii.edu

Our approach is to dominantly knock-down expression of a gene that significantly impacts fitness.



Then we rescued the gene with a modified copy that evades knockdown. (The rescue homozygote has two copies.)



The key was to rescue in a fashion that is **haploinsufficient** (one copy is not enough in the hemizygote).