Genetic Engineering for Species Conservation Applications in Hawai'i

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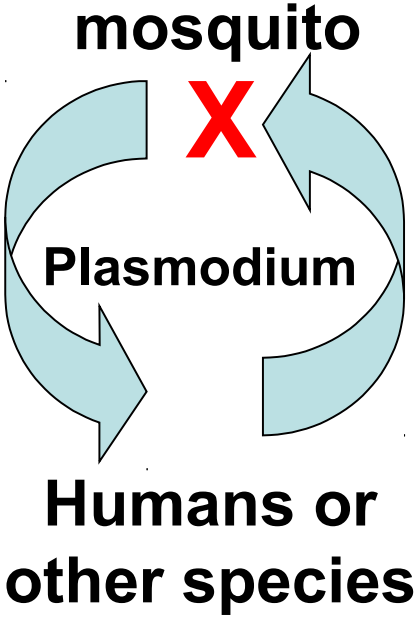
http://www.hawaiireedlab.com/presentations

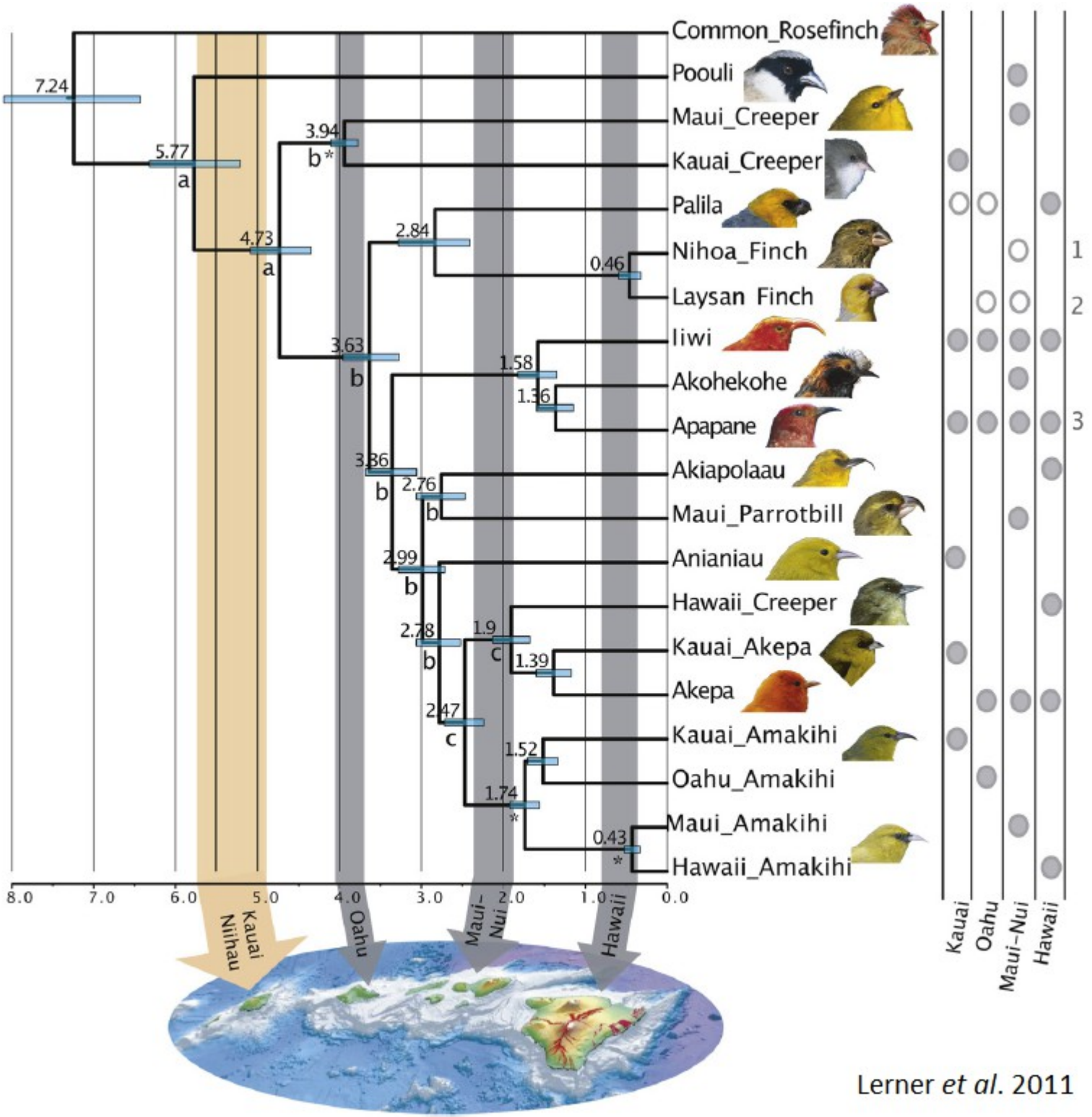
Motivation

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).



Lerner *et al*. 2011

Culex mosquitoes were accidentally introduced to Hawai'i in the early 19th century and are infected with avian malaria.

Culex mosquitoes were accidentally introduced to Hawai'i in the early 19th century and are infected with 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 Species[3]	Critically Endangered Species[3]
Kona Grosbeak (*Chloridops kona*) ʻUla-ʻai-Hawane (*Ciridops anna*) Lanai Hookbill (*Dysmorodrepanis munroi*) Hawaii Mamo (*Drepanis pacifica*) Black Mamo (*Drepanis funerea*) Oʻahu ʻAkialoa (*Hemignathus ellisianus*) Hawaiʻi ʻAkialoa (*Hemignathus obscurus*) Lanai ʻAlauahio (*Paroreomyza montana montana*) Greater Amakihi (*Hemignathus sagittirostris*) Kakawahie (*Paroreomyza flammea*) Lesser Koa-Finch (*Rhodacanthis flaviceps*) Greater Koa-Finch (*Rhodacanthis palmeri*)	Nukupuu (*Hemignathus lucidus*), probably extinct ʻAkekeʻe (*Loxops caeruleirostris*) Poʻo-uli (*Melamprosops phaeosoma*), probably extinct ʻAkikiki (*Oreomystis bairdi*) ʻAkohekohe (*Palmeria dolei*) Oʻahu ʻAlauahio (*Paroreomyza maculata*), probably extinct Maui Parrotbill (*Pseudonestor xanthophrys*) ʻŌʻū (*Psittirostra psittacea*), probably extinct Nihoa Finch (*Telespiza ultima*)
Endangered Species[3]	**Vulnerable Species[3]**
ʻAkiapolaʻau (*Hemignathus munroi*) Palila (*Loxioides bailleui*) ʻAkepa (*Loxops coccineus*) Hawaiʻi Creeper (*Oreomystis mana*) Maui Nui ʻAlauahio (*Paroreomyza montana*)	Oʻahu ʻAmakihi (*Hemignathus flavus*) Kauaʻi ʻAmakihi (*Hemignathus kauaiensis*) ʻAnianiau (*Hemignathus parvus*) Laysan Finch (*Telespiza cantans*) ʻIʻiwi (*Vestiaria coccinea*)
Near Threatened Species[3]	**Species of Least Concern[3]**
	Common ʻAmakihi (*Hemignathus virens*) ʻApapane (*Himatione sanguinea*)

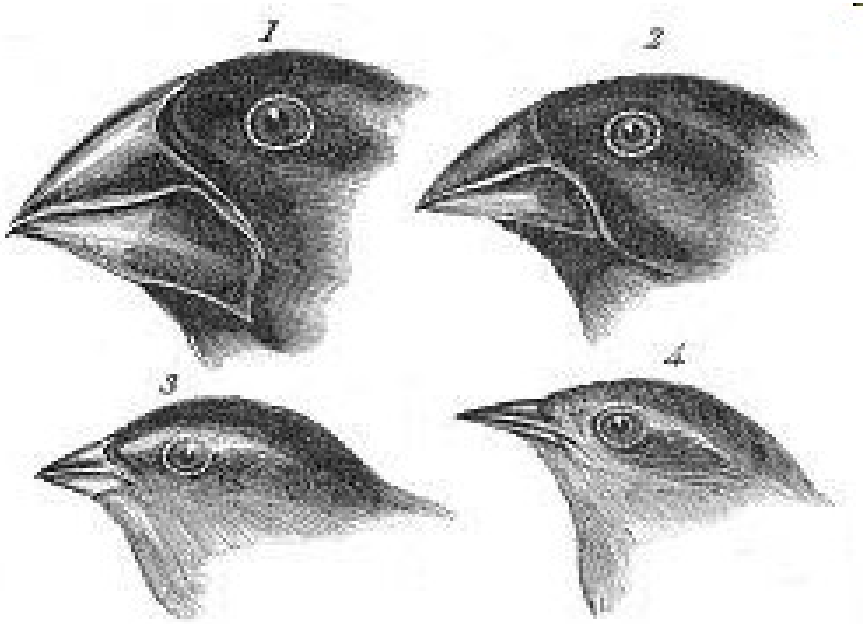
http://en.wikipedia.org/wiki/Hawaiian_honeycreeper_conservation

Maui Parrotbill (rediscovered!)

If Darwin was impressed with the Galapagos finches...

http://naturenotesblog.blogspot.com/2011/05/book-review-birds-of-hawaii-and.html

BTW - Culex mosquitoes are also now established on the Galapagos islands, and avian malaria has recently been reported there (http://whc.unesco.org/en/news/445 , July 1, 2008).



Finches from Galapagos Archipelago

http://www.kiwifoto.com/

Elevation limits avian malaria, but climate change...

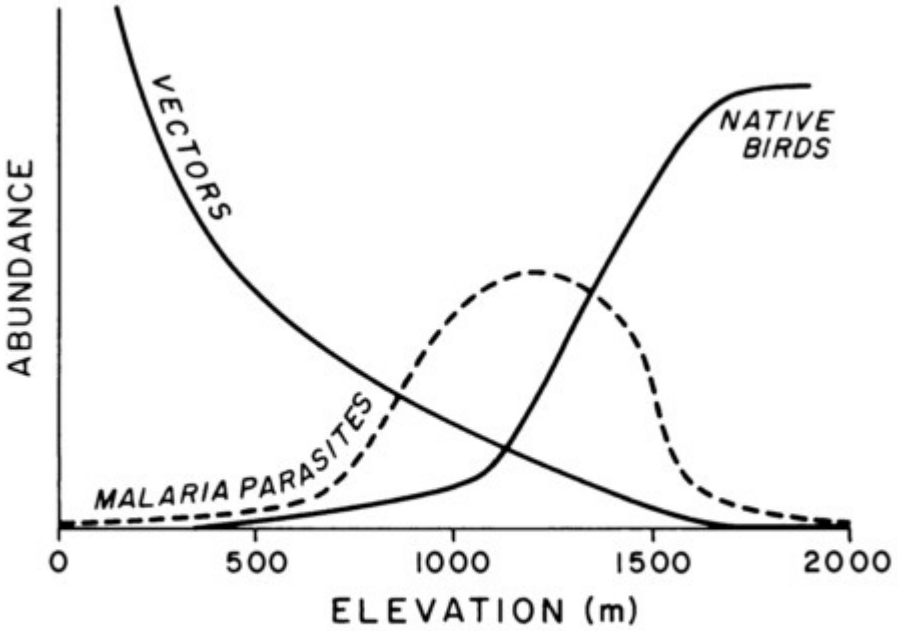
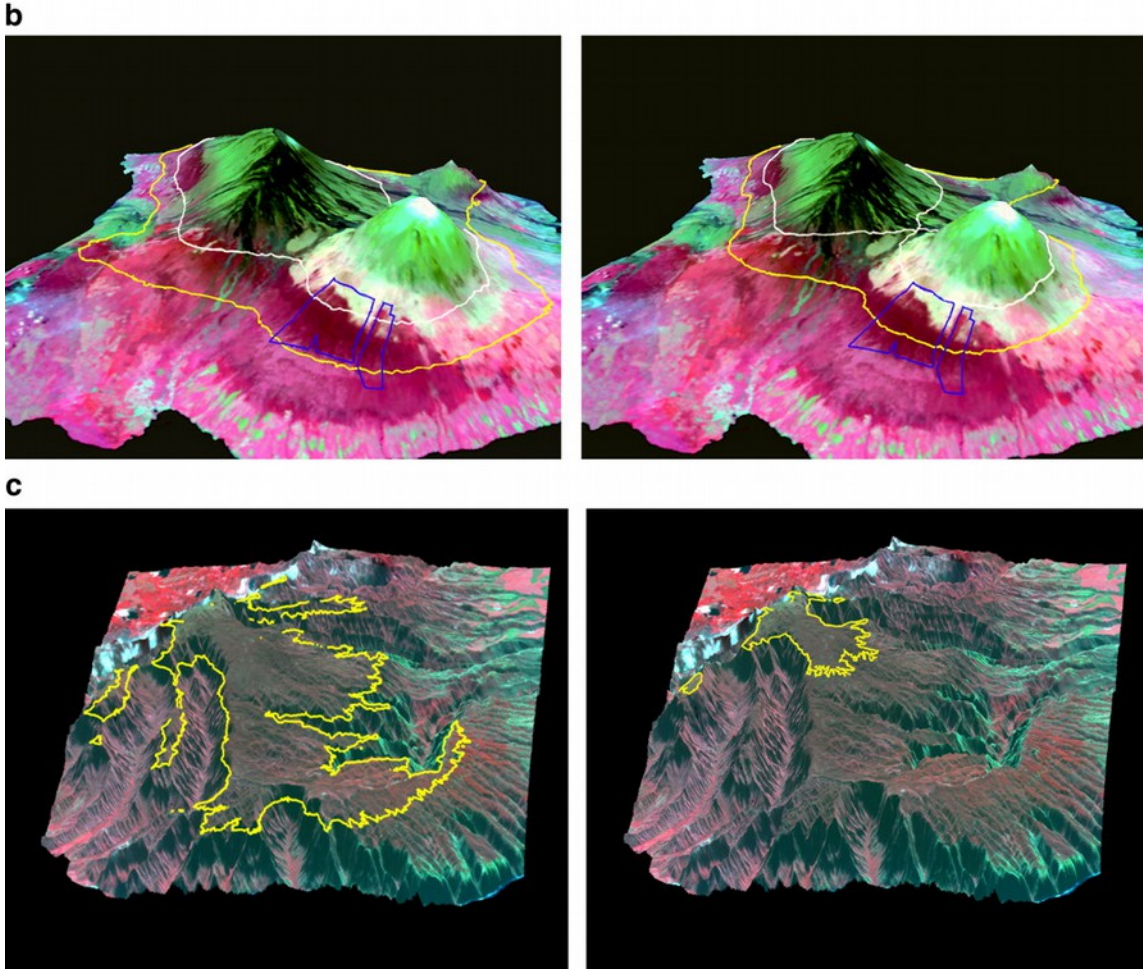


FIG. 11. A generalized model of native bird abundances, malarial parasite incidence, and mosquito vector levels along an elevation gradient on Mauna Loa, Hawaii.

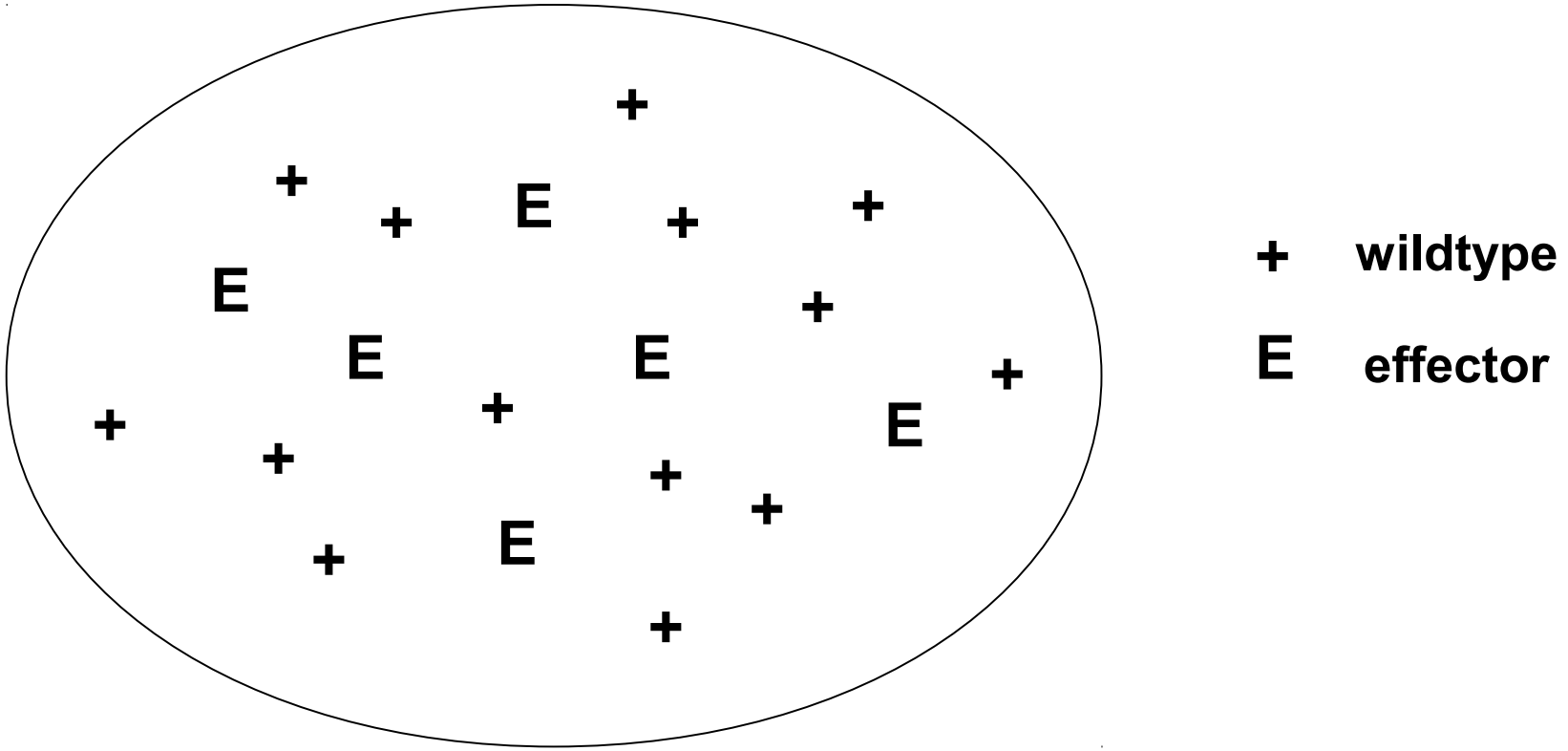
van Riper et al. (1986)



Benning *et al*. (2002)

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

Effectors can be released into a wild population:



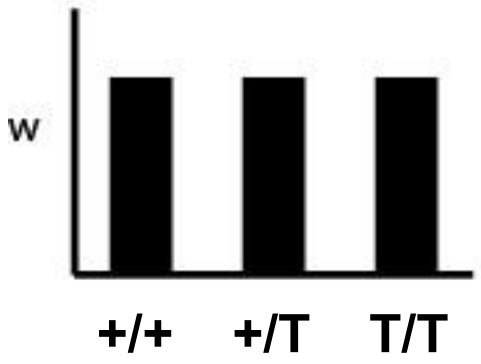
But, if there is no fitness advantage, and especially likely, if there is a fitness cost, they are unlikely to reach fixation and may be quickly lost from the wild.

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

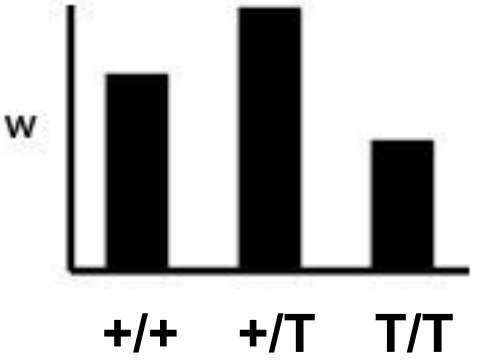
However, we might utilize “drive” mechanisms linked to effector constructs to push effectors to high frequency or fixation in the wild without increasing fitness (population transformation).

Two alleles give three genotypes and four different fitness configurations.

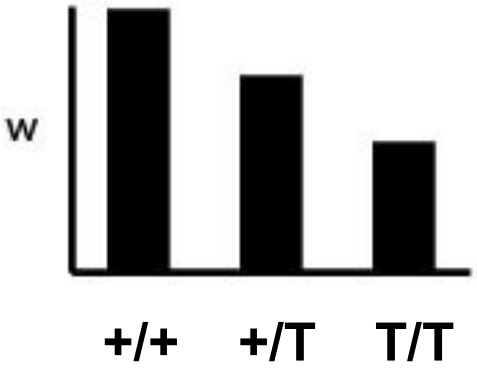
1) Neutrality



3) Overdominance

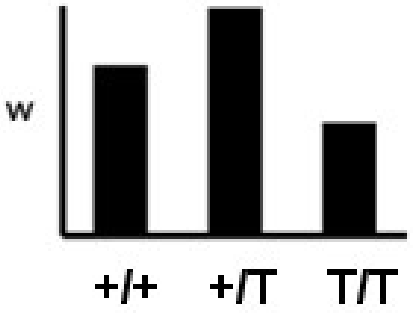


2) Directional

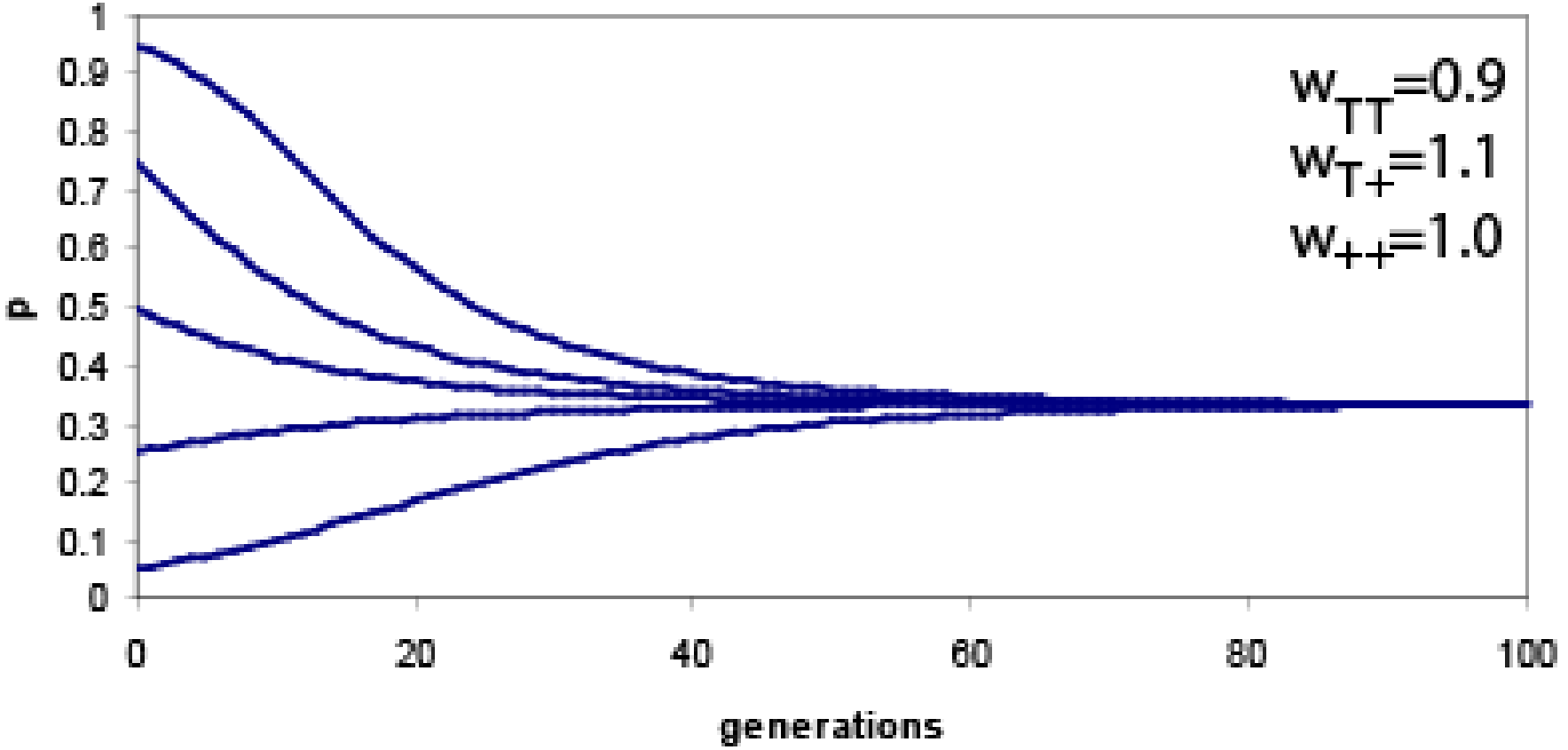


4) Underdominance

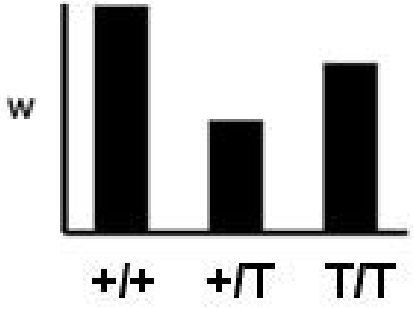
3) Overdominance



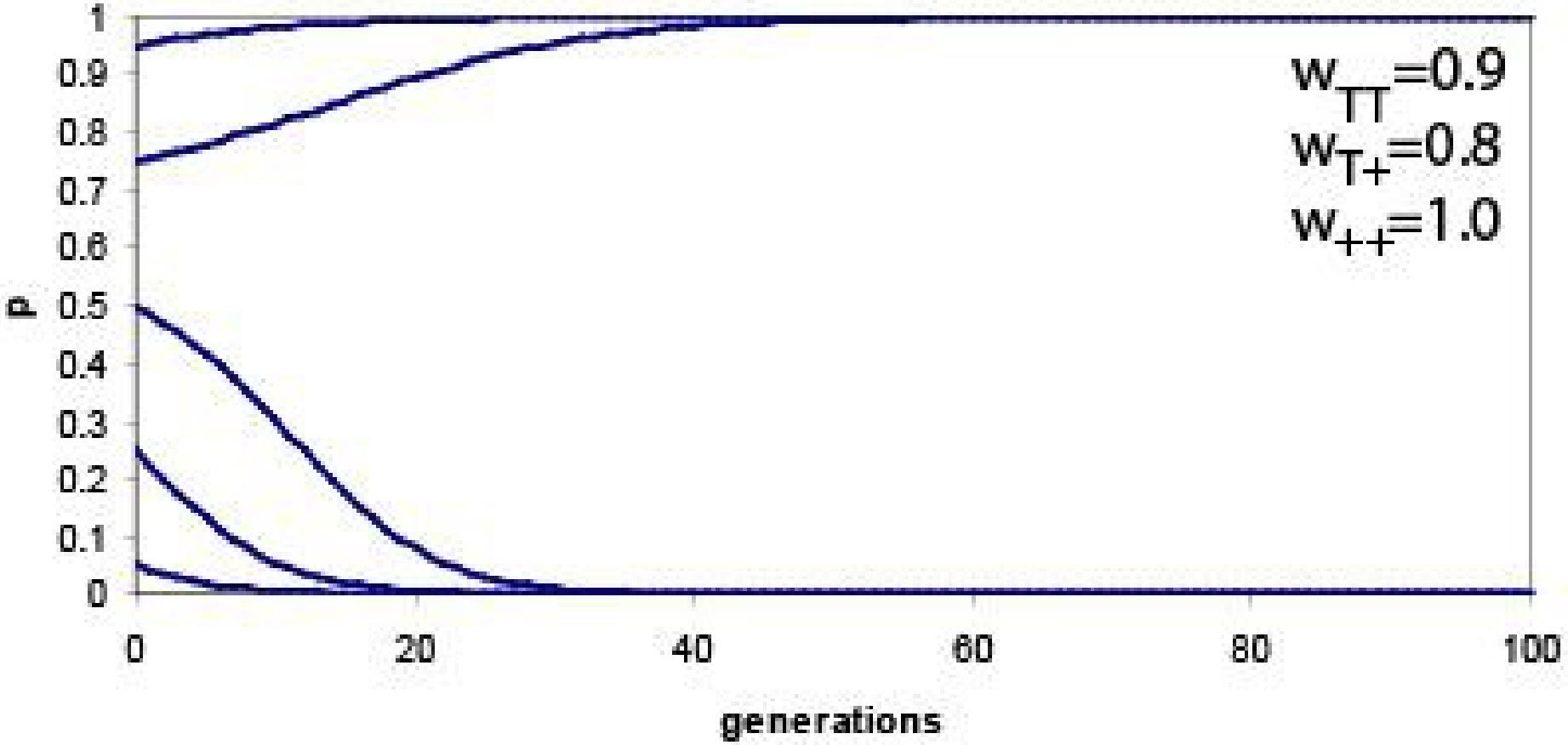
A heterozygote advantage leads to a stable equilibrium.



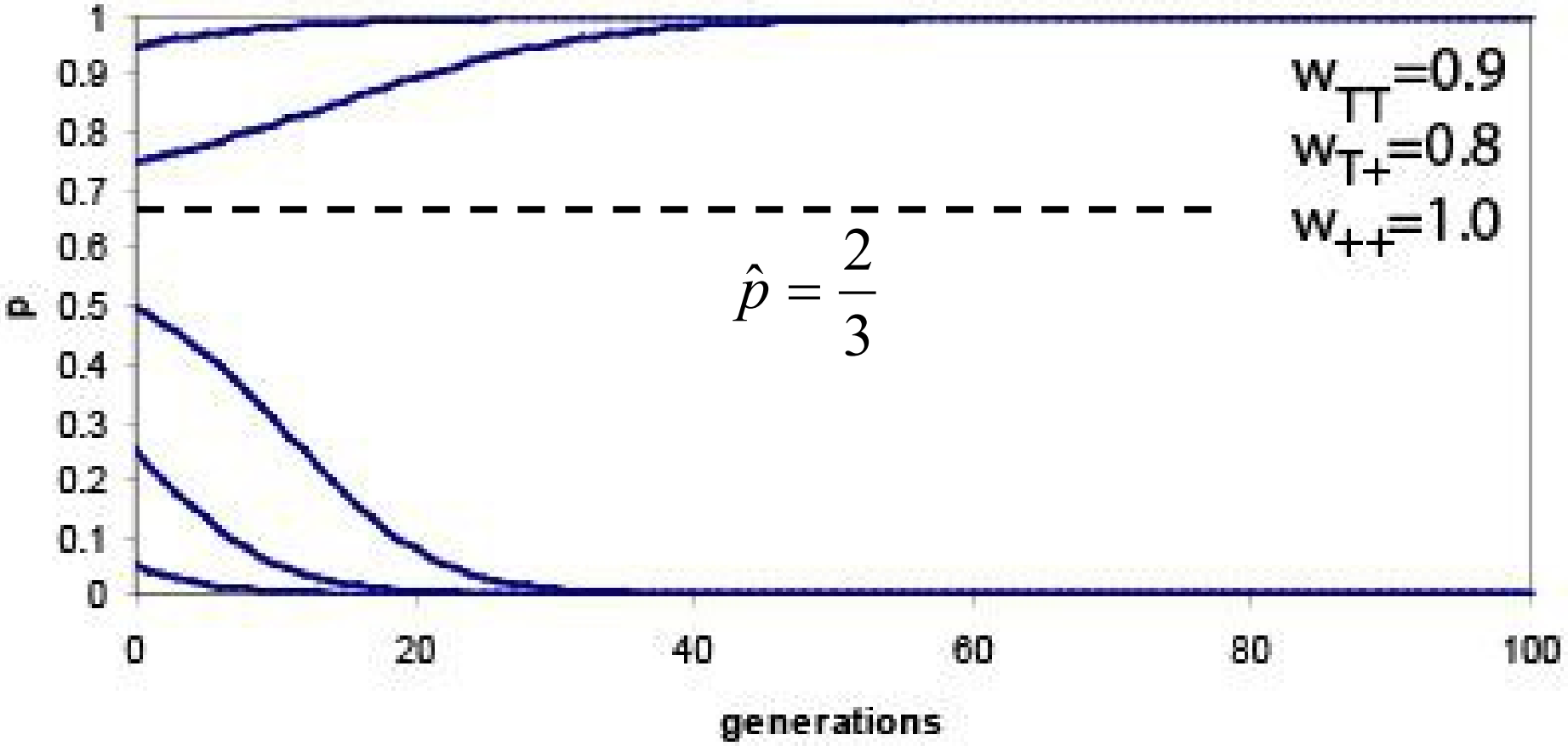
4) Underdominance



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.



The Hardy-Weinberg Principle

An allele is most often paired with another allele that is common in the population.

Rare alleles are not paired with themselves (often).

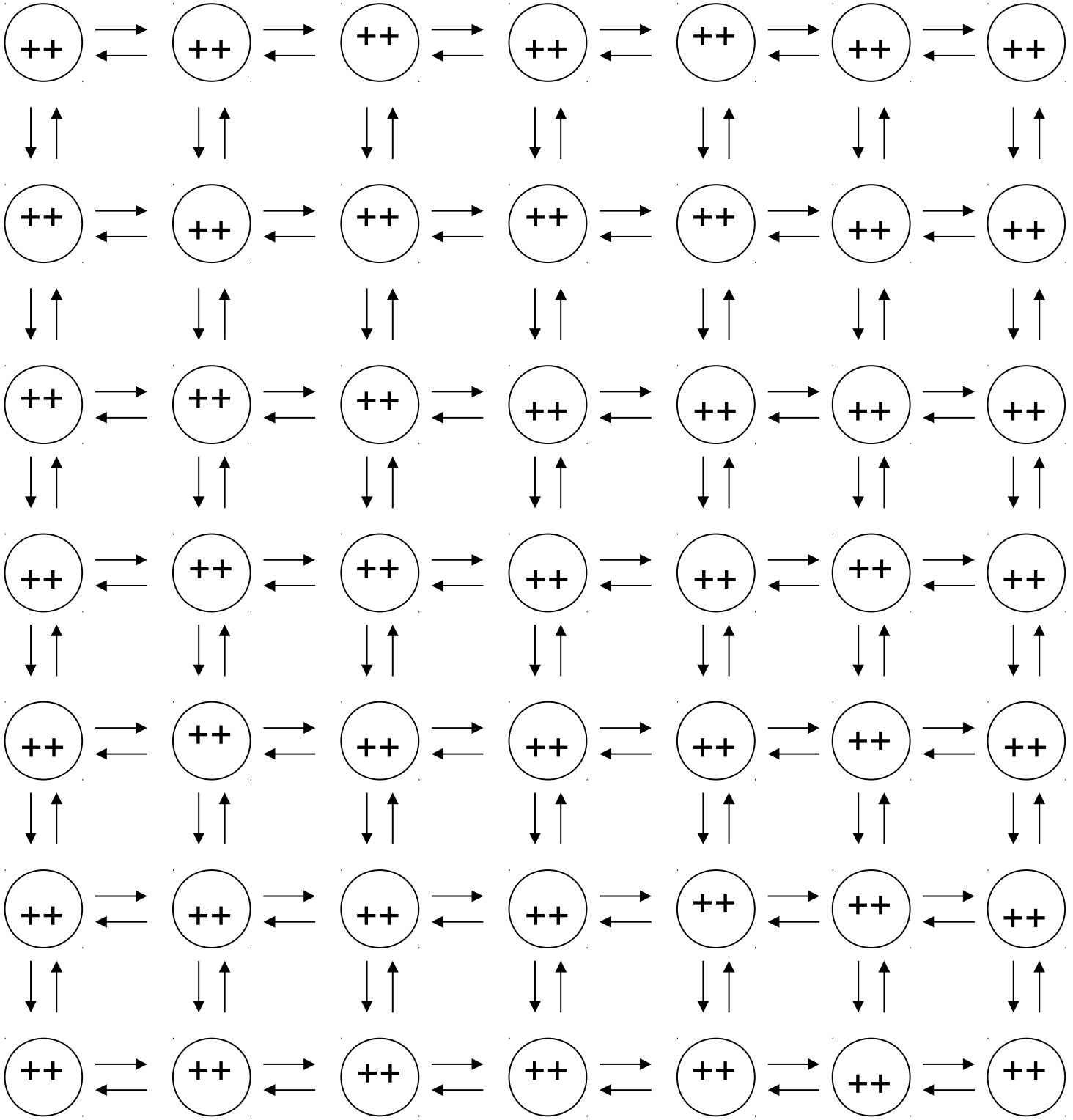
Rare alleles tend to be heterozygotes.

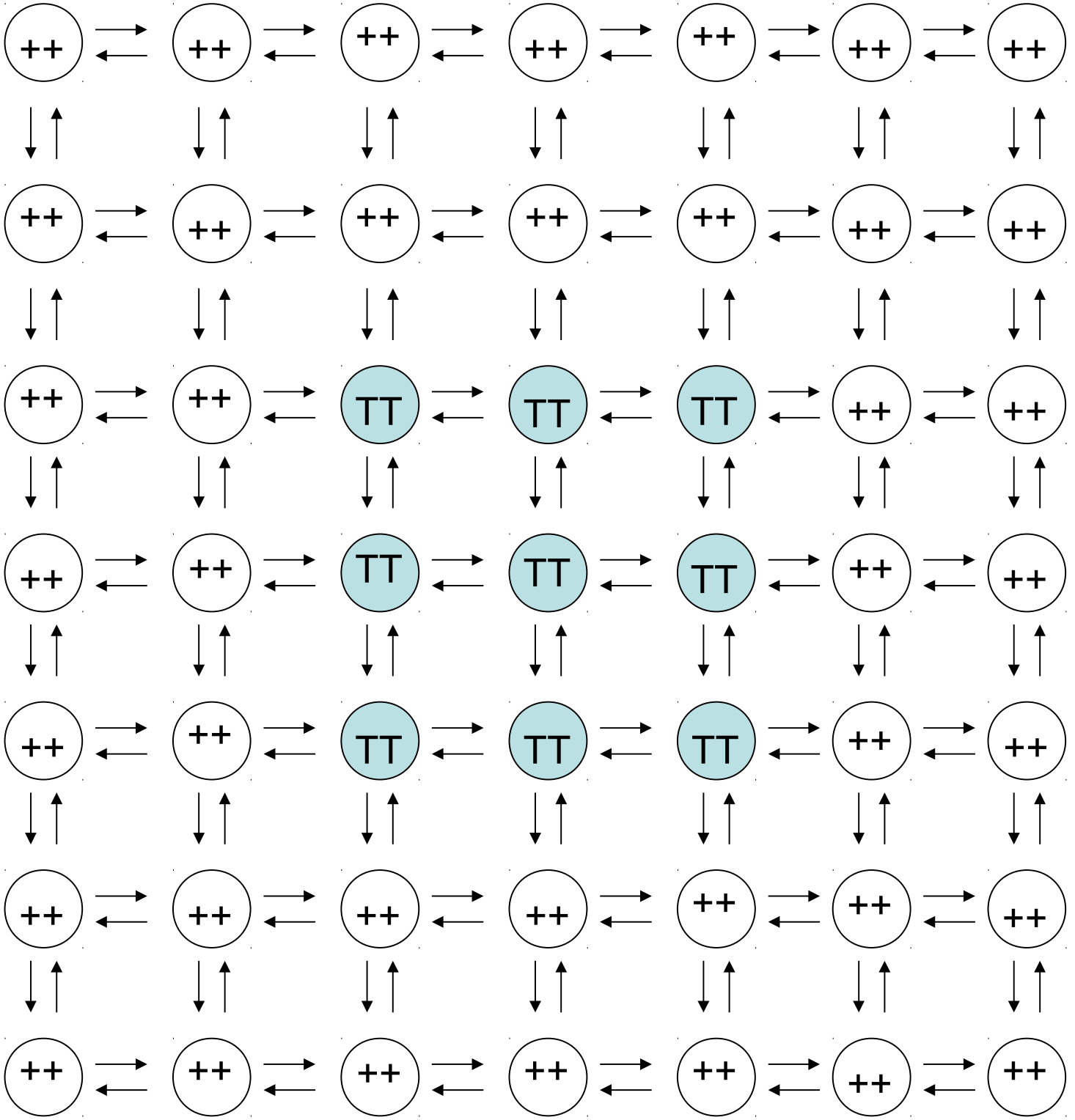
Underdominance is a rare allele disadvantage.

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

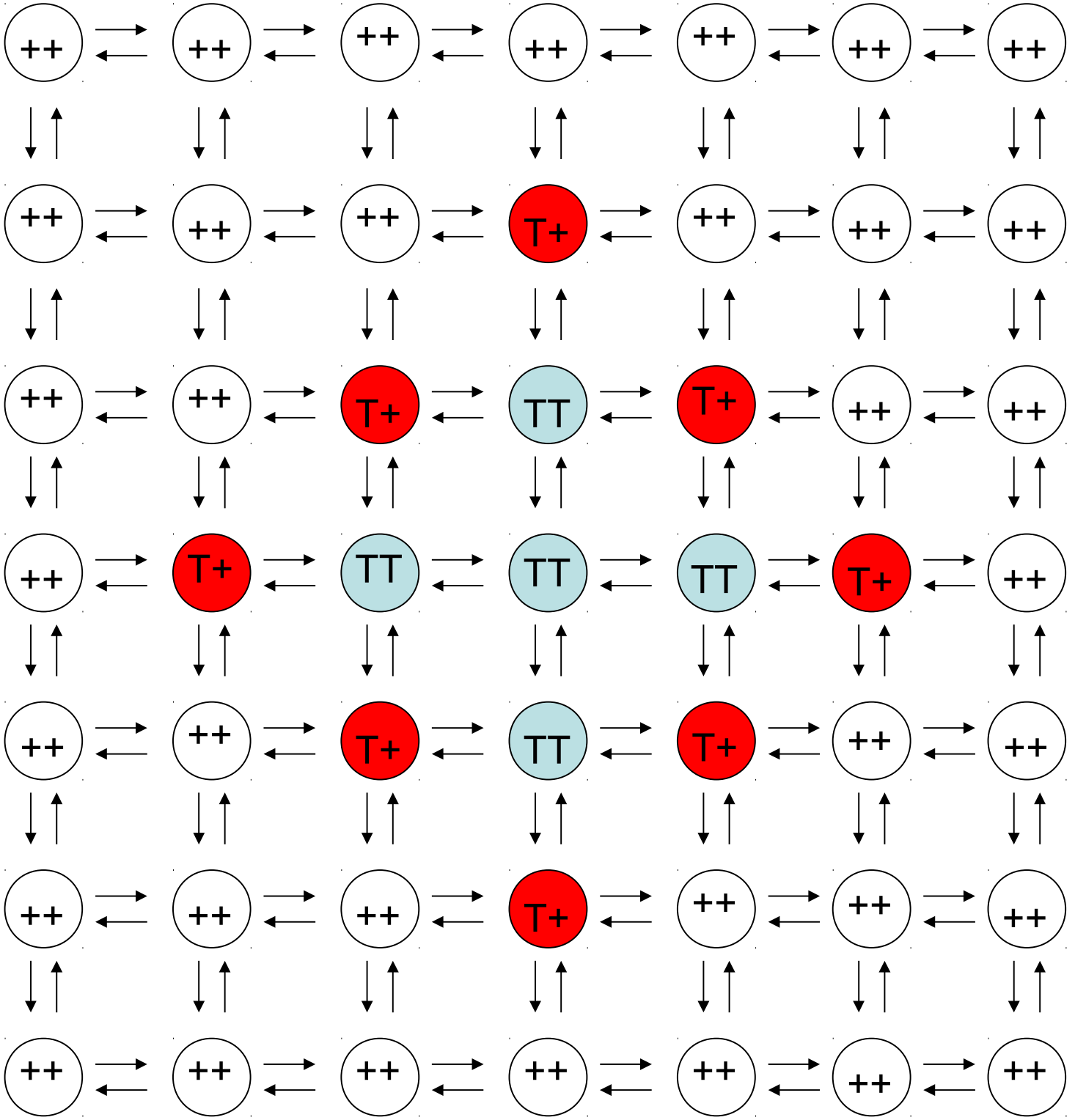
- Geographic Stability
- Reversibility

I will appeal to an intuitive abstracted example to illustrate.



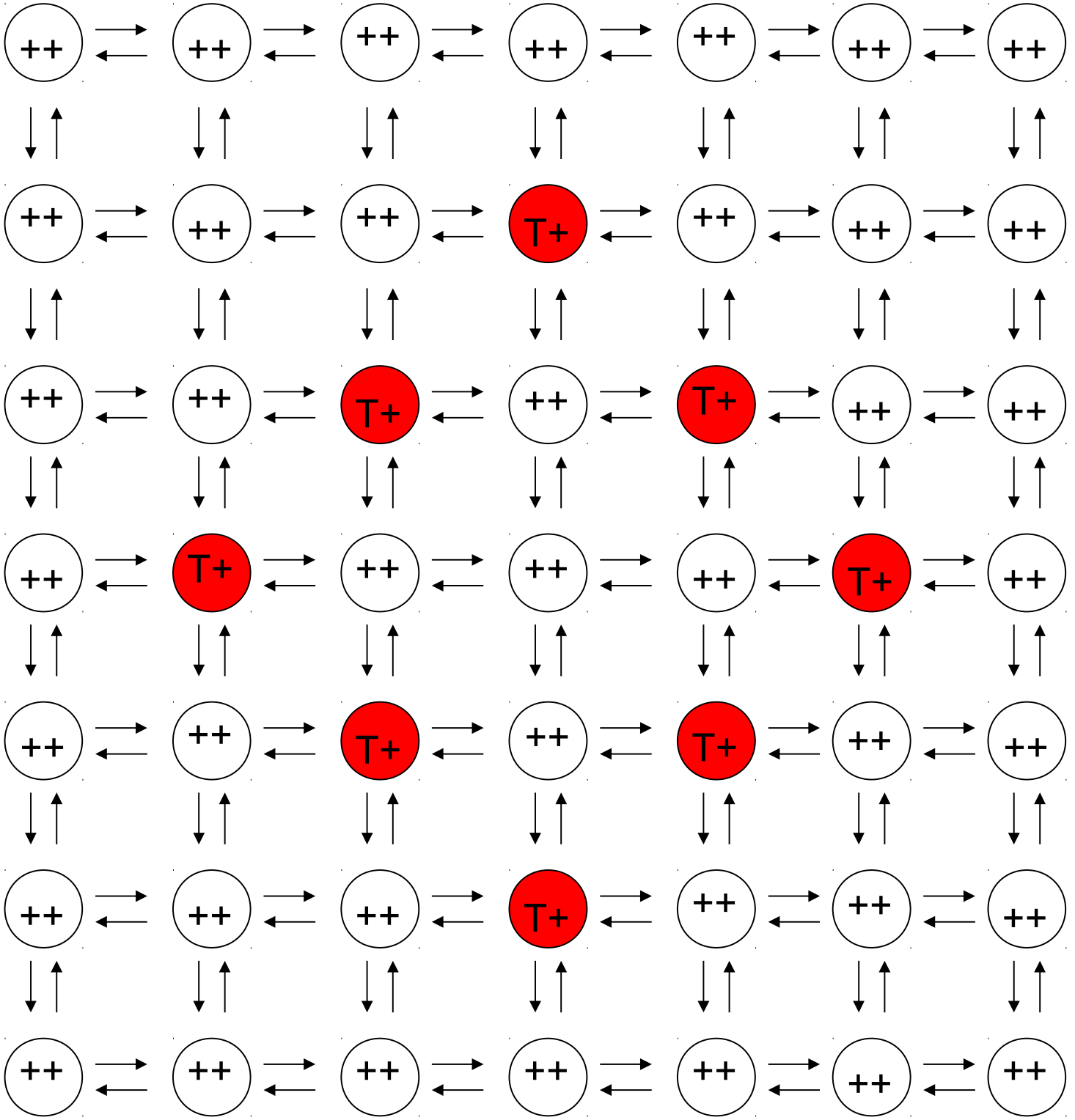


TT release

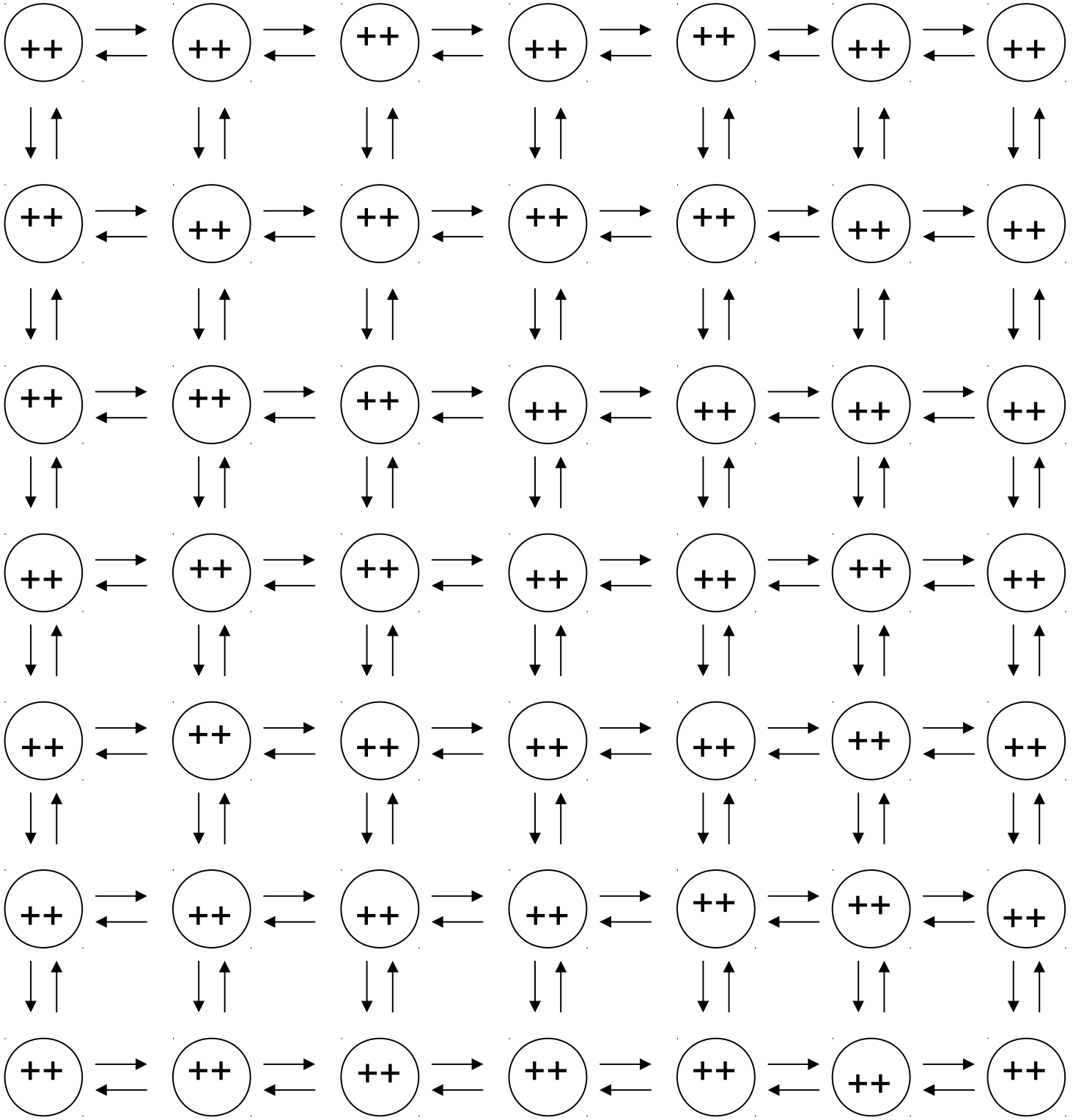


Migration-Selection Equilibrium

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.

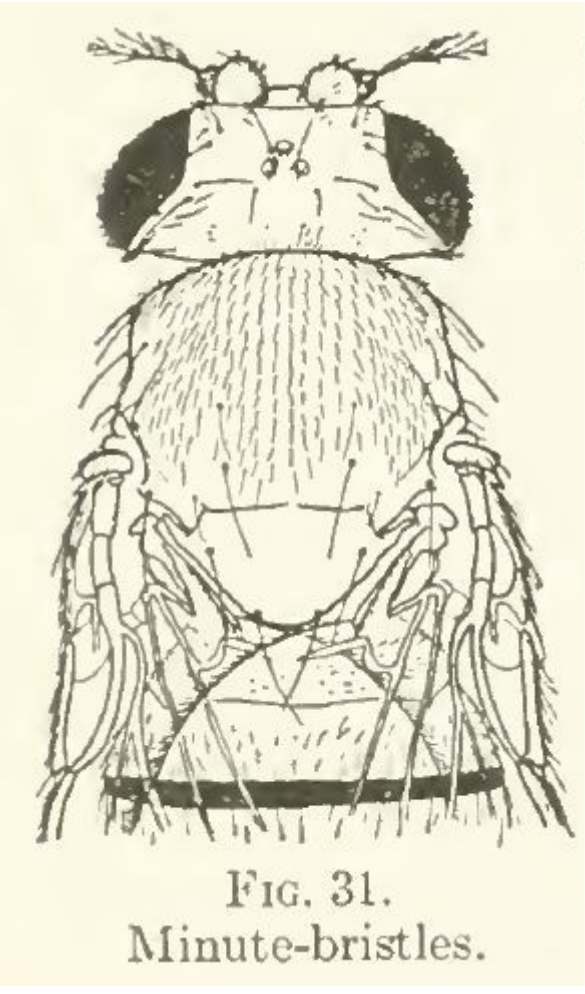


FIG. 31.
Minute-bristles.

Bridges & Morgan 1923^

From the beginning we wanted a system that is likely to be portable across species (not only Drosophila).

"Minutes" are a class of mutant genes across The genome that are reported in a wide range of species.

Thin bristles; also, most common is a **delay in development**.

Marygold *et al*. 2007 →

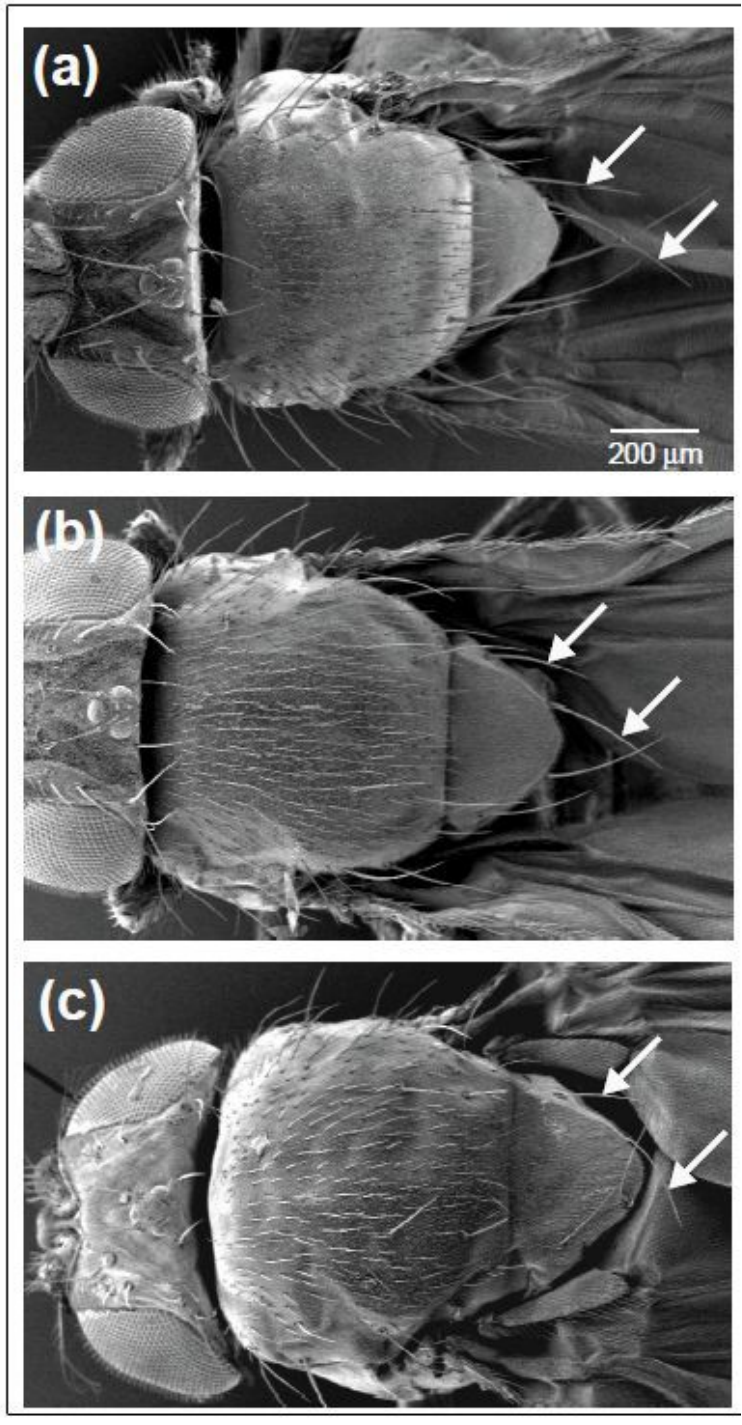


Figure 3
The Minute bristle phenotype. Minute flies have shorter and thinner bristles
bristles, indicated here by the arrows and pseudocoloring. (a, a') Wild type.

Minute loci are ribosomal proteins, are homozygous lethal and haploinsufficient.

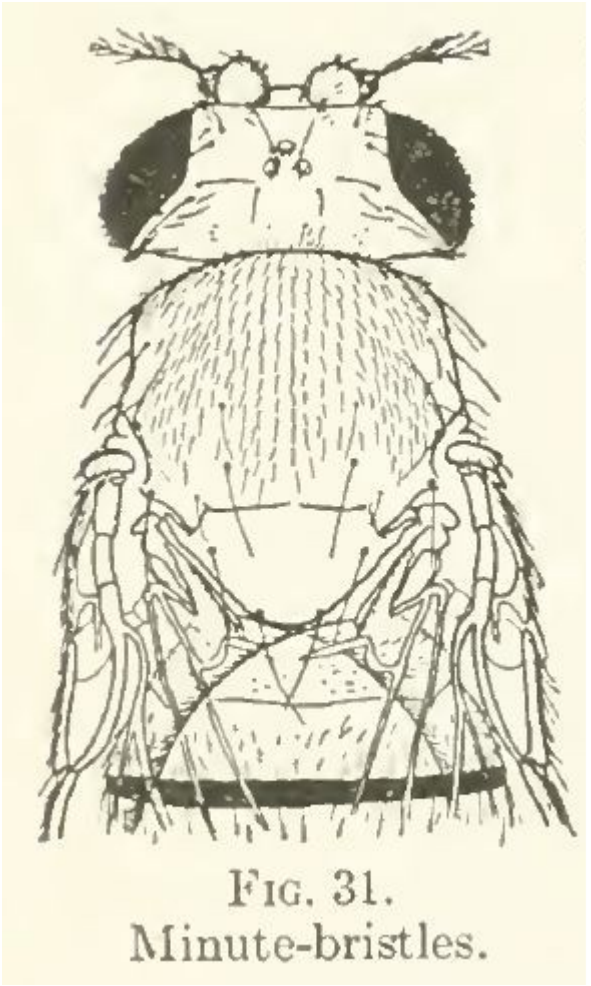
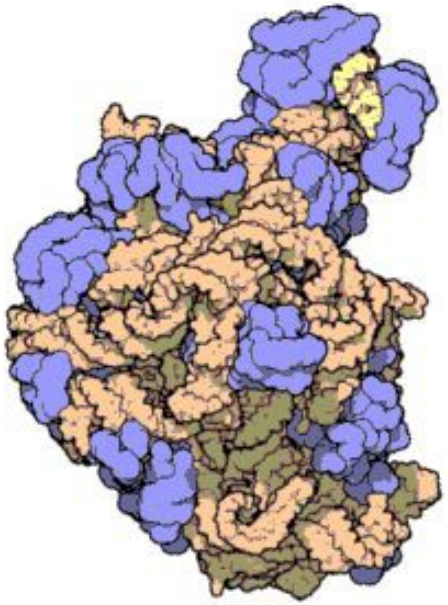
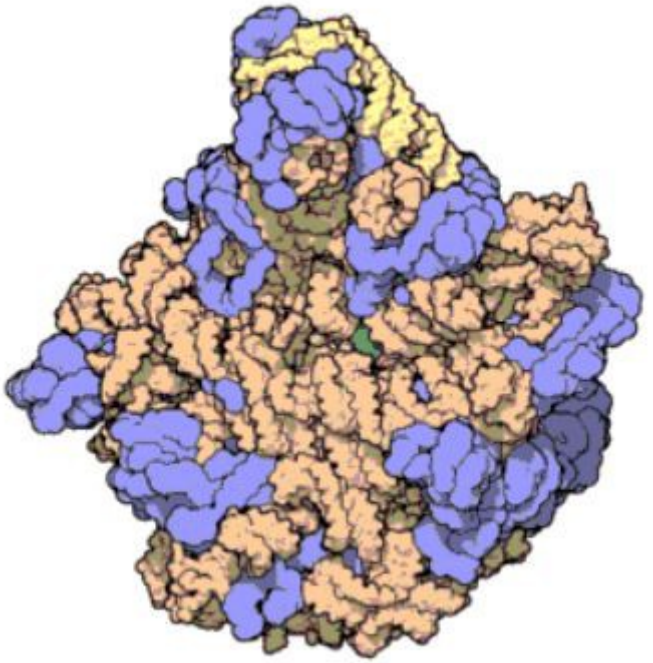
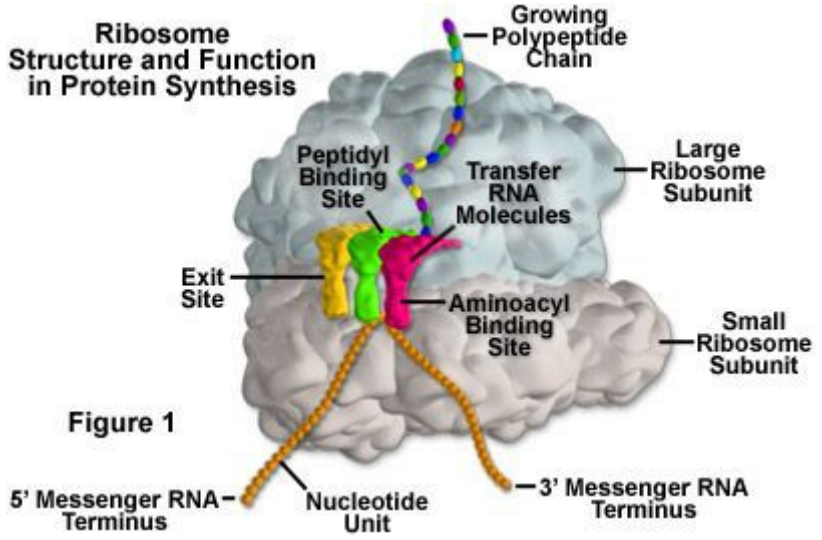


FIG. 31.
Minute-bristles.







Many mutations are recessive and haplo-sufficient.

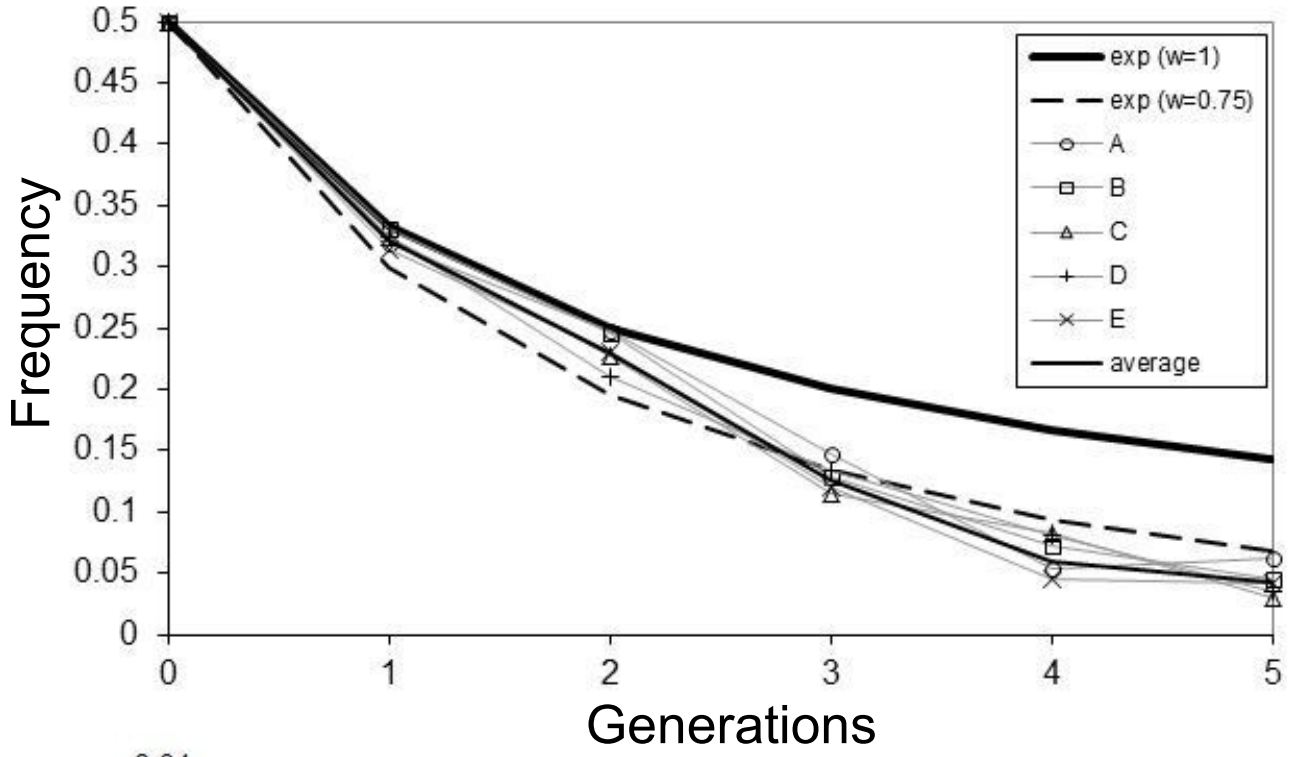
A female Drosophila just needs one copy of the wildtype alllele to have normal 'red' eyes.

http://theconversation.com/animals-in-research-drosophila-the-fruit-fly-13571

Haploinsufficiency of Cytoplasmic Ribosomal Proteins (CRPs) across eukaryotes

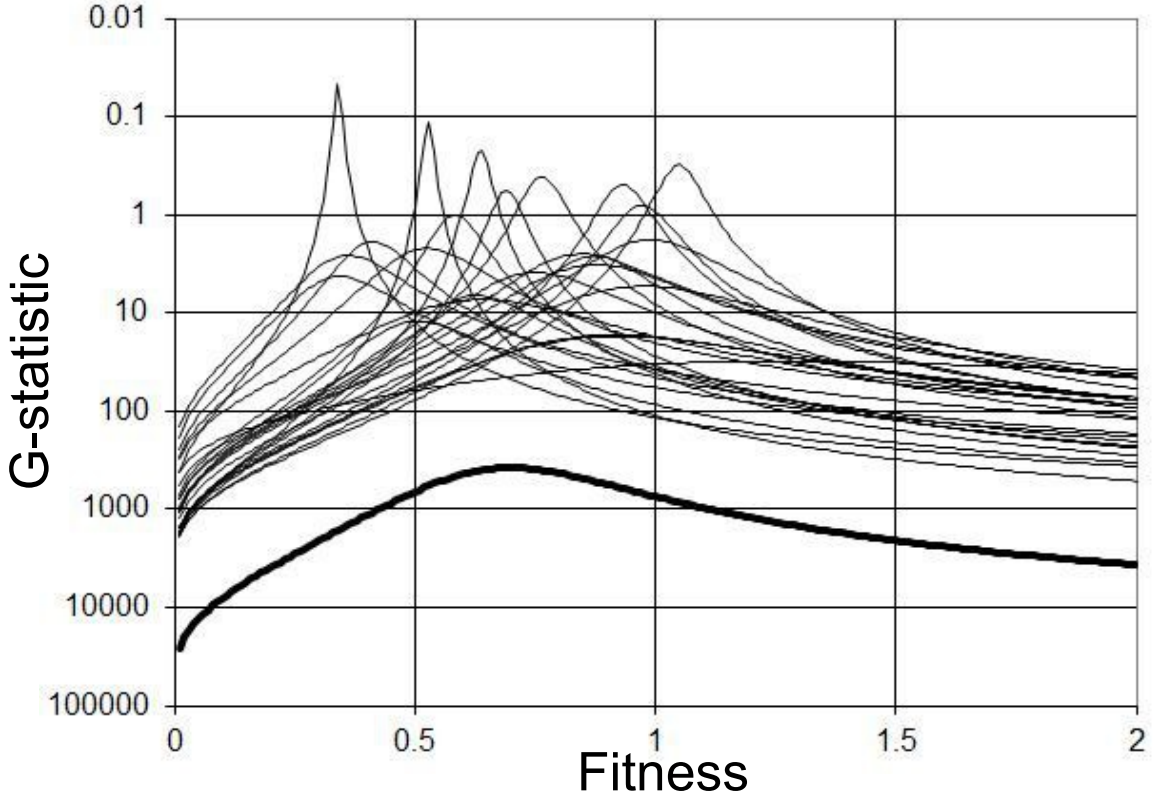
Animals	*Drosophila melanogaster*	64 CRPs	(Marygold *et al*. 2007)
	Mus musculus	*RpL*24	(Oliver *et al*. 2004)
	Homo sapiens	*RpS*19	(Choesmel *et al*. 2007)
	Danio rerio	11 CRPs	(Amsterdam *et al.* 2004)
Fungi	*Saccharomyces cerevisiae*	72 CRPs	(Deutschbauer *et al*. 2005)
	Schizosaccharomyces pombe	8 CRPs	(Kim *et al*. 2010)
Plants	*Arabidopsis thaliana*	*AtRpS*5a/b	(Weijers *et al*. 2001)

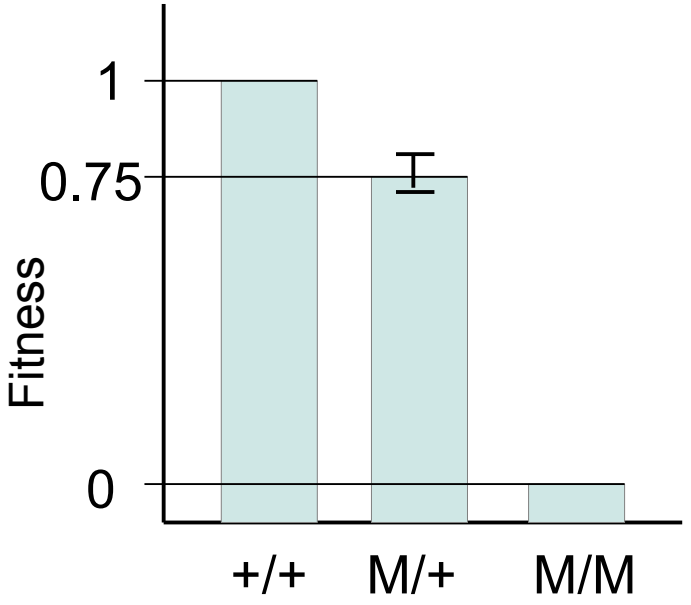
*RpL*19



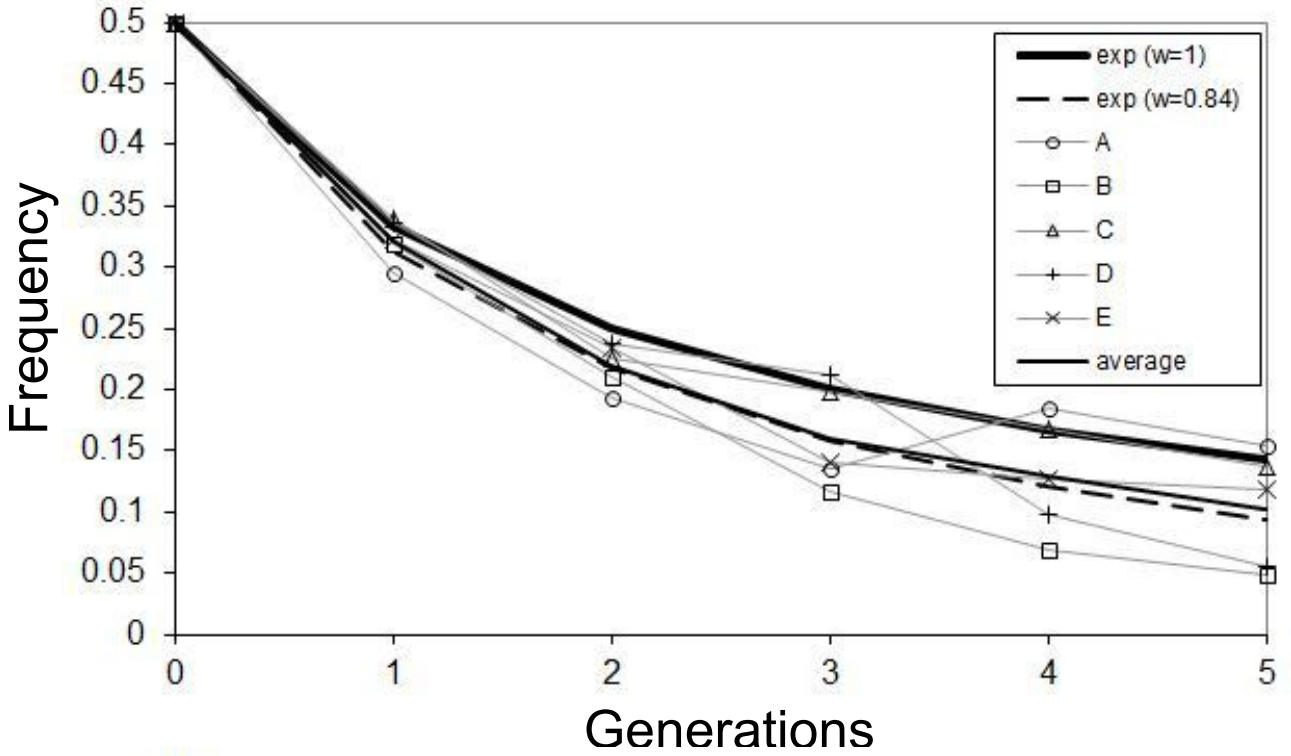
Relate change in frequency to relative heterozygote fitness

$$w = \frac{p'(1-p)}{p(1-2p')}$$





*RpL*14



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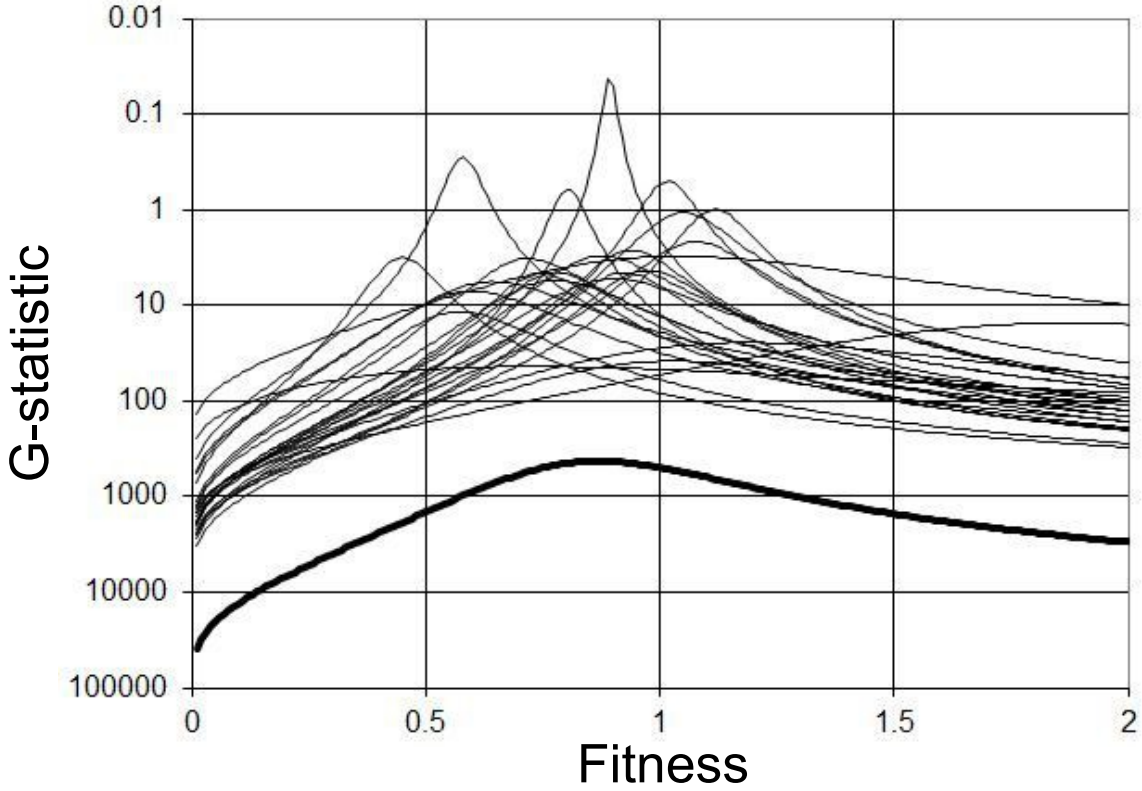
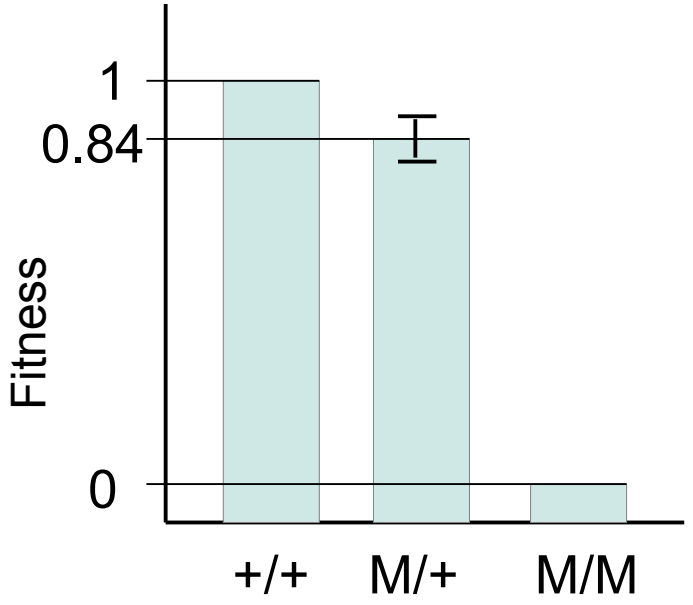
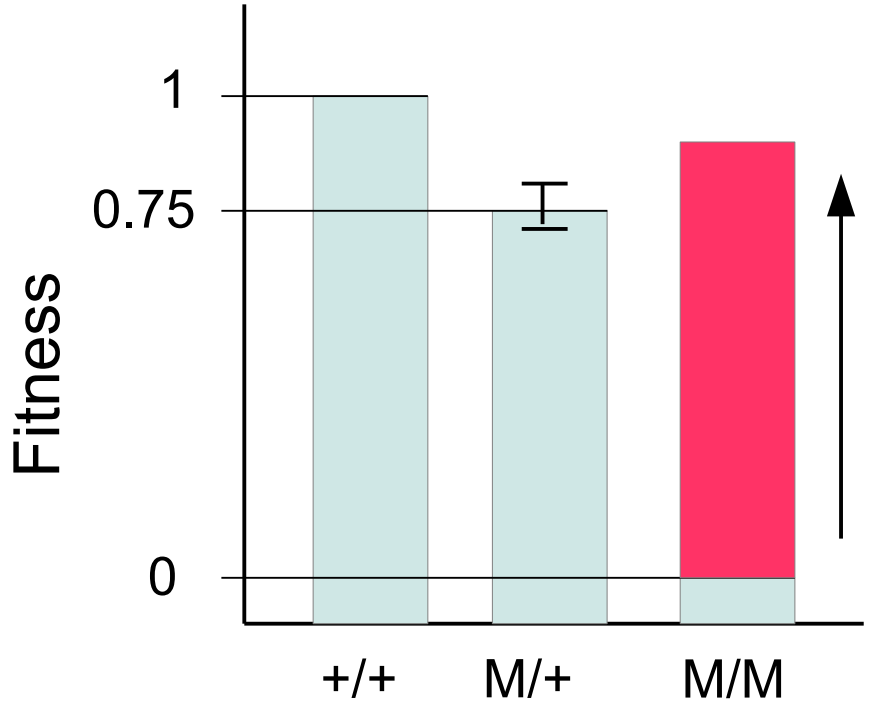
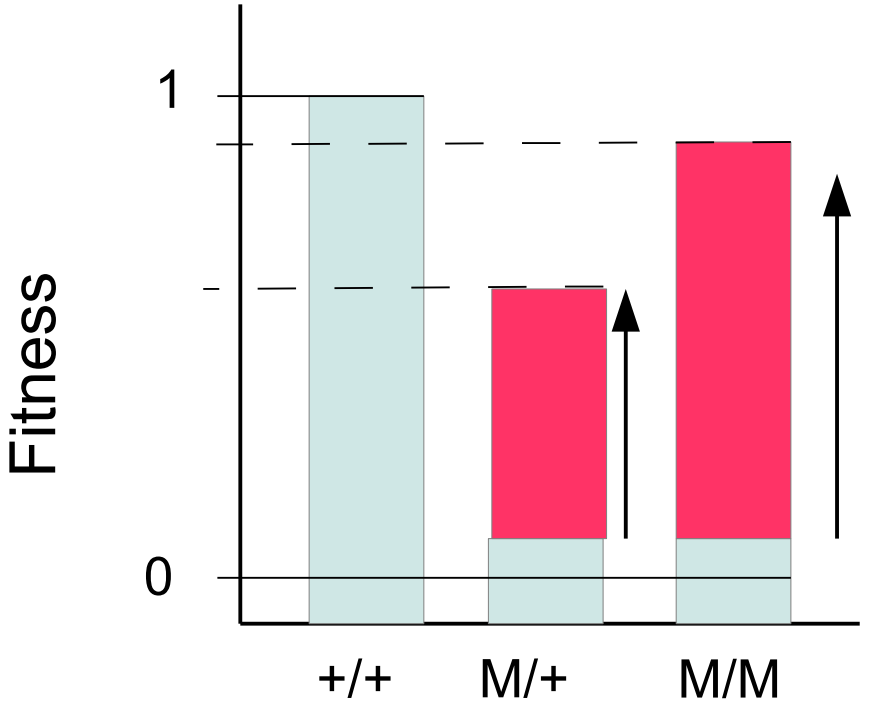




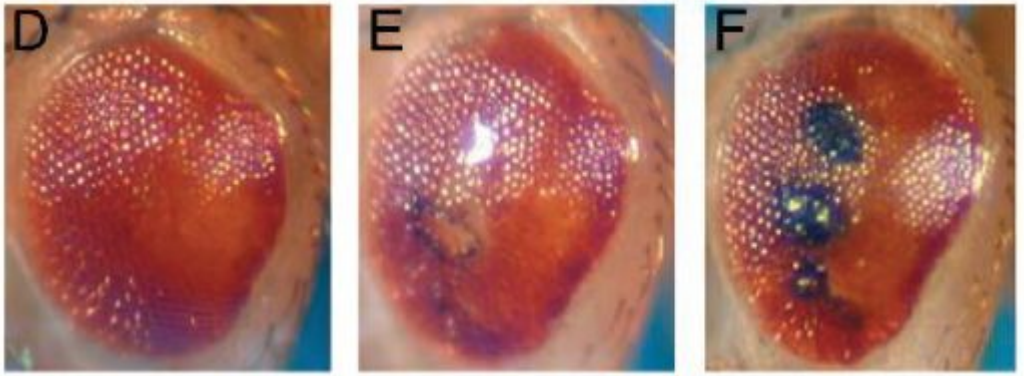
What we want to do is rescue homozygote fitness to result in underdominance



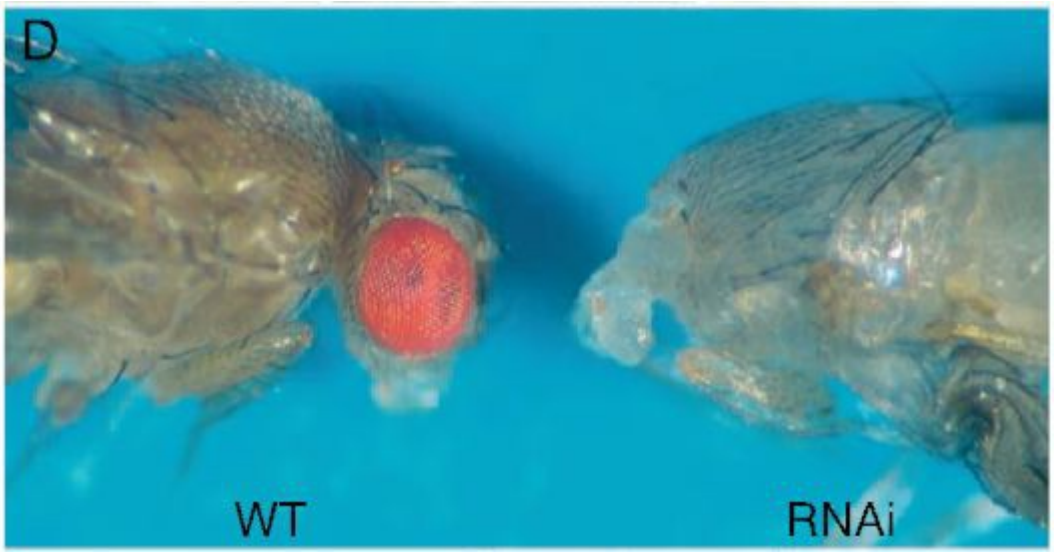
What we needed to do is incompletely rescue heterozygote fitness to result in underdominance (due to haploinsufficiency).



Knocking down CRP expression can dramatically affect tissues

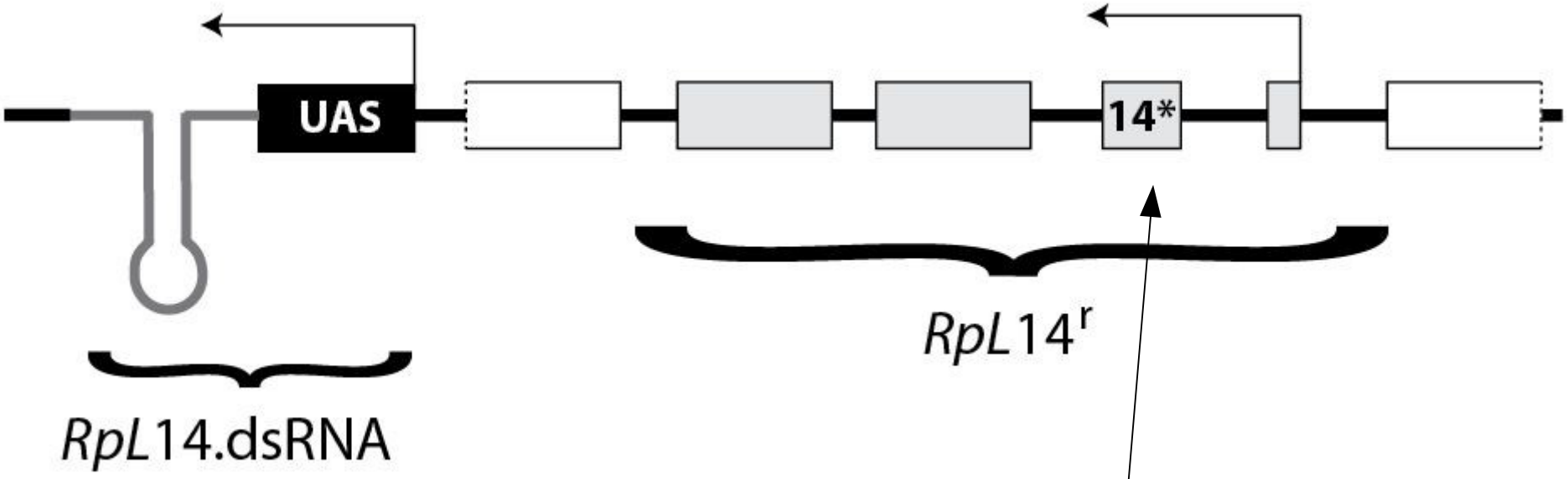


ey.H-GAL4/UAS-RNAi.RpL14

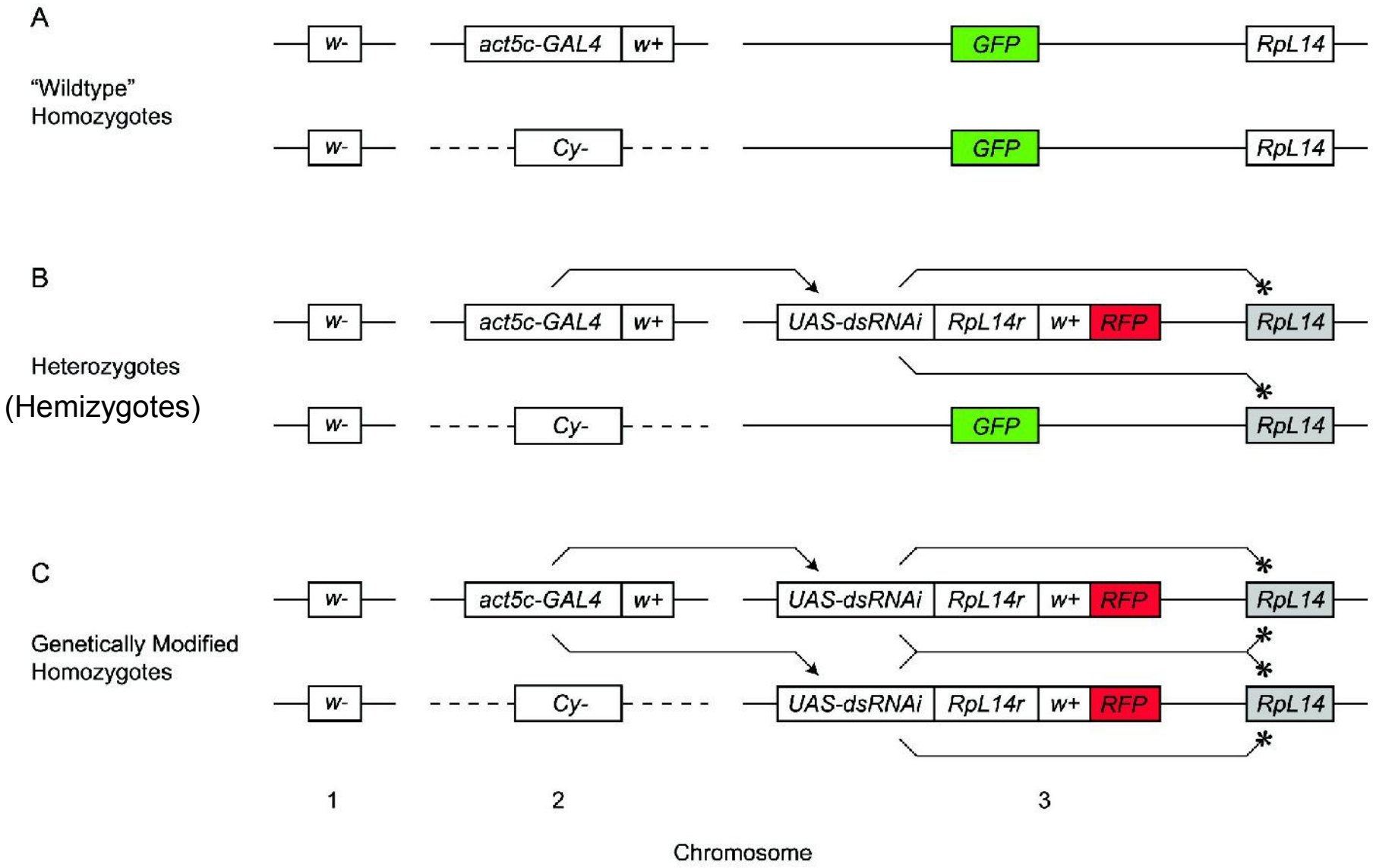


Enerly *et al*. 2003
Gene 320:41

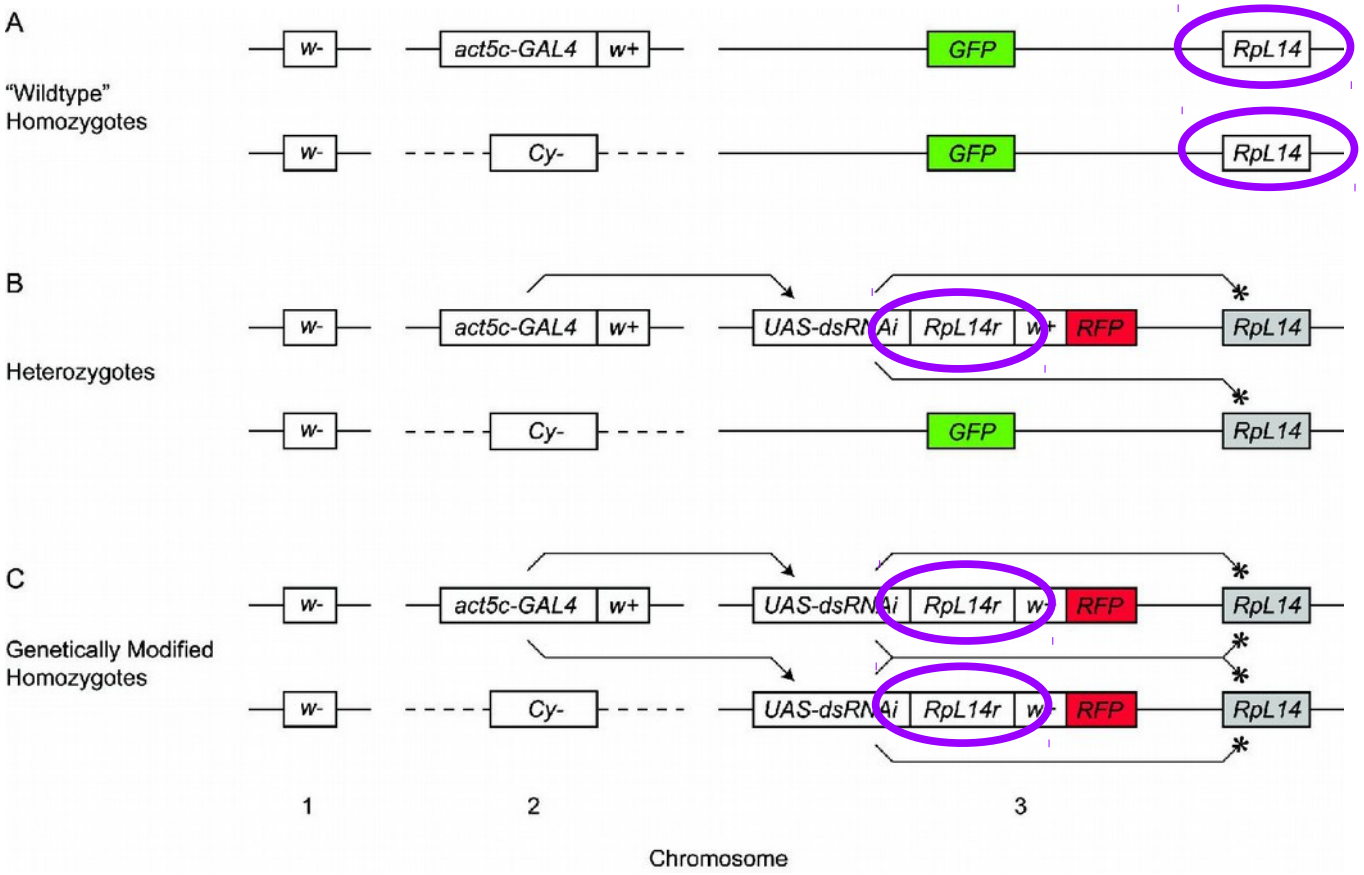
“Poison” “Rescue”



Second exon engineered to evade RNAi knockdown


A
"Wildtype"
Homozygotes
w-
act5c-GAL4
w+
GFP
RpL14
w-
Cy-
GFP
RpL14
B
Heterozygotes
(Hemizygotes)
w-
act5c-GAL4
w+
UAS-dsRNAi
RpL14r
w+
RFP
RpL14
w-
Cy-
GFP
RpL14
C
Genetically Modified
Homozygotes
w-
act5c-GAL4
w+
UAS-dsRNAi
RpL14r
w+
RFP
RpL14
w-
Cy-
UAS-dsRNAi
RpL14r
w+
RFP
RpL14
1
2
3
Chromosome

Effectively



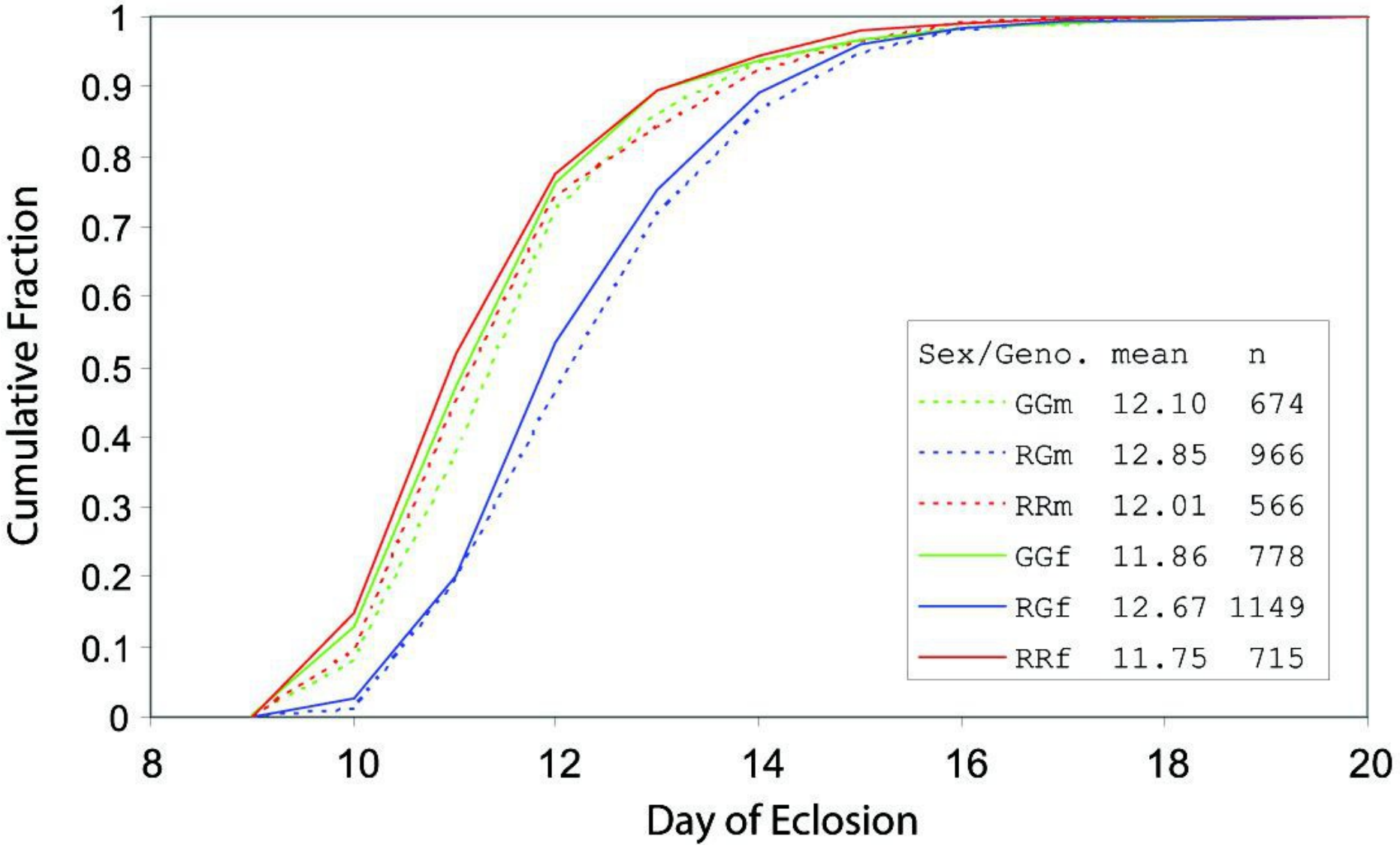
Homzyg: 2 CRPs

Hetzyg: 1 CRP

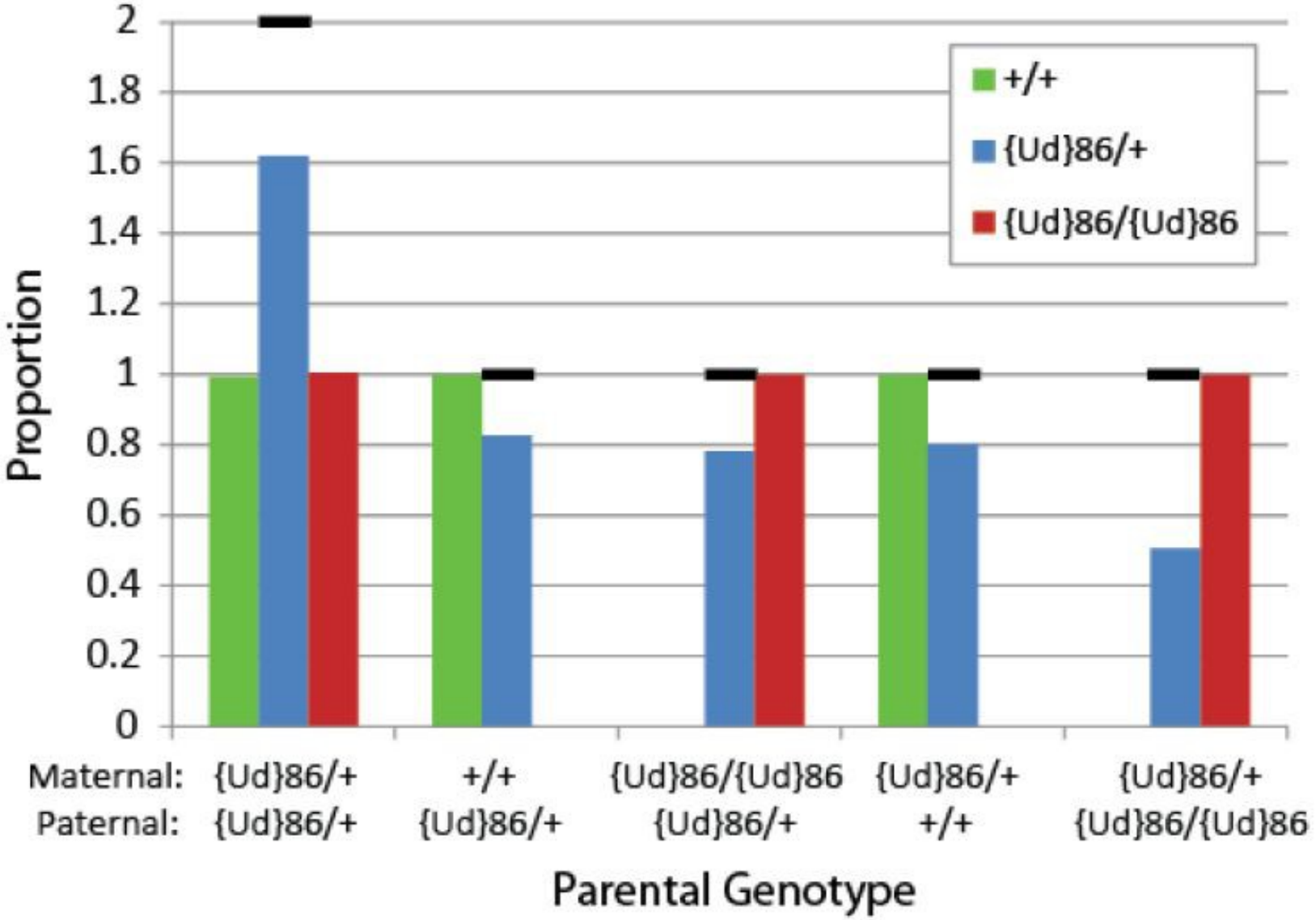
Homzyg: 2 CRPs

Heterozygotes are least fit because of haploinsufficiency

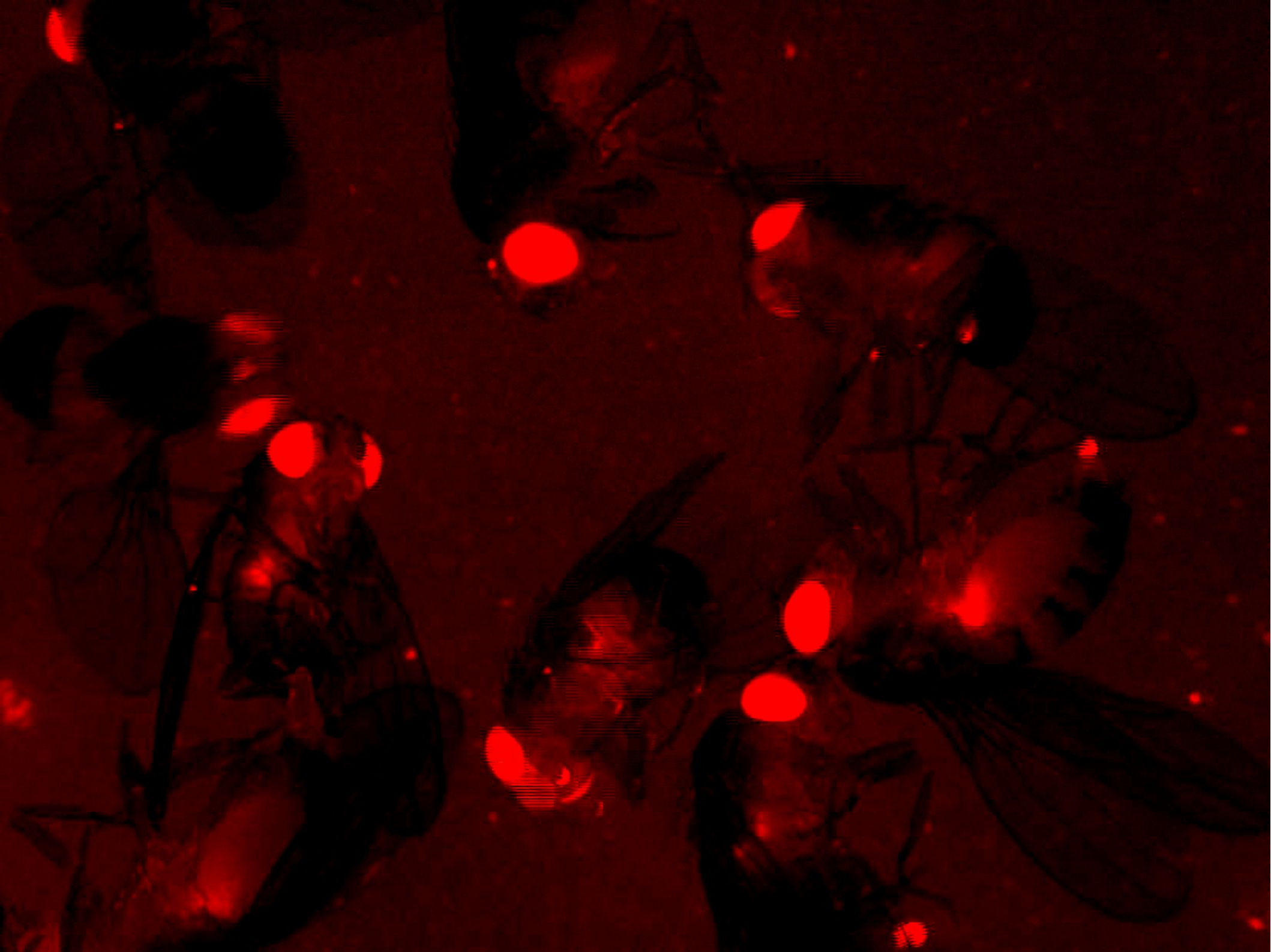
Development Time

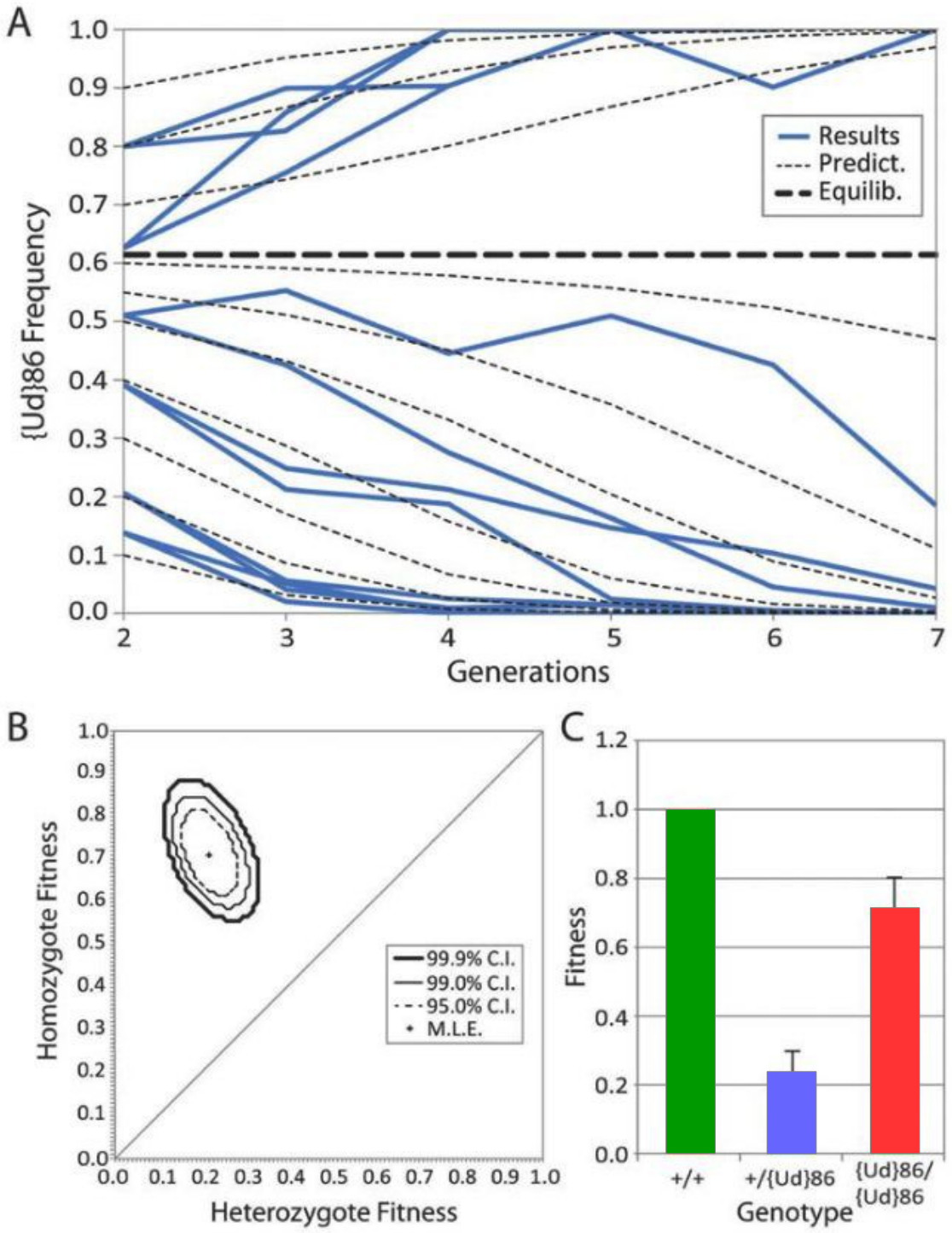


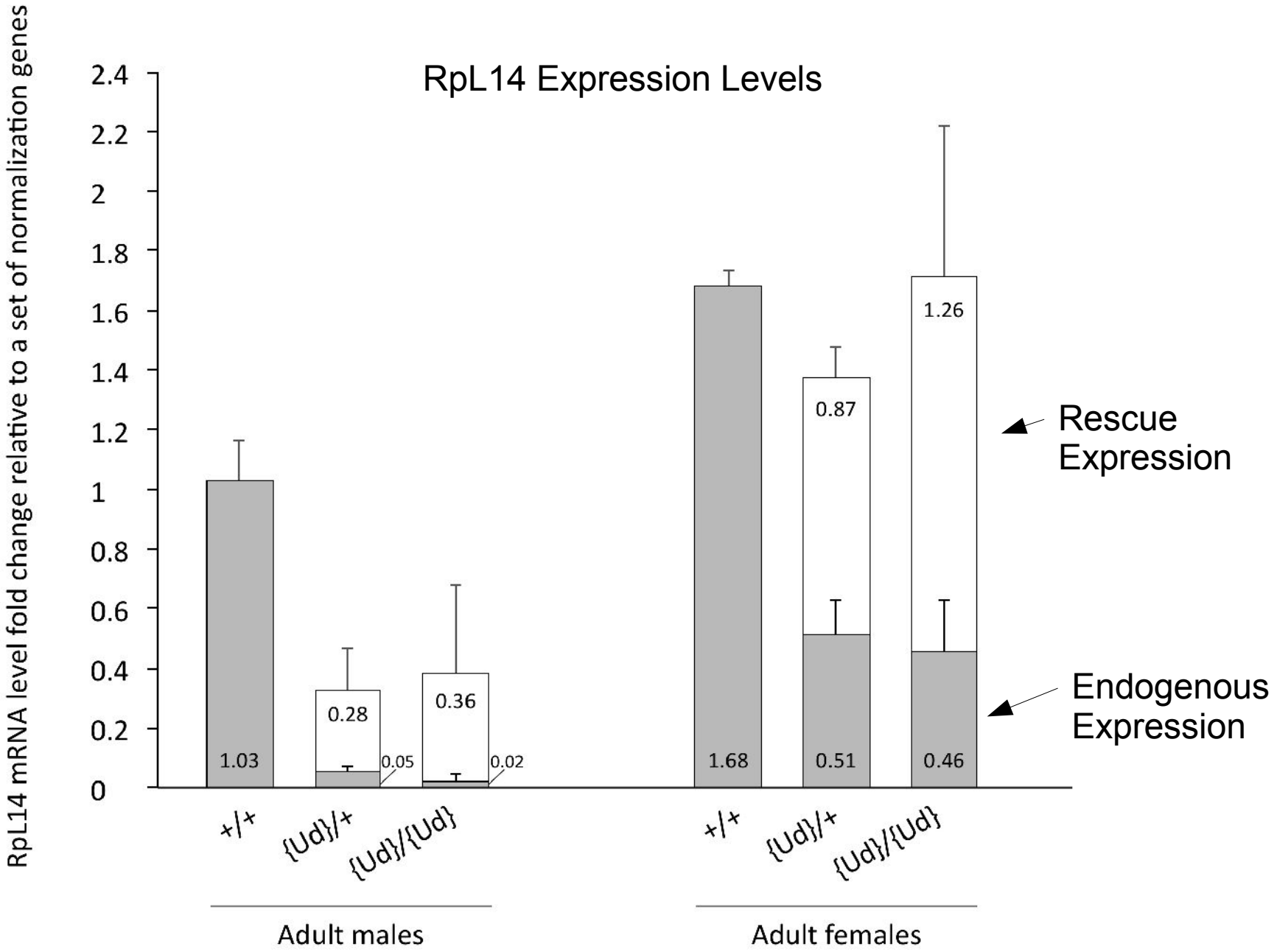
Egg-to-adult viability (apparent Mendelian distortion)

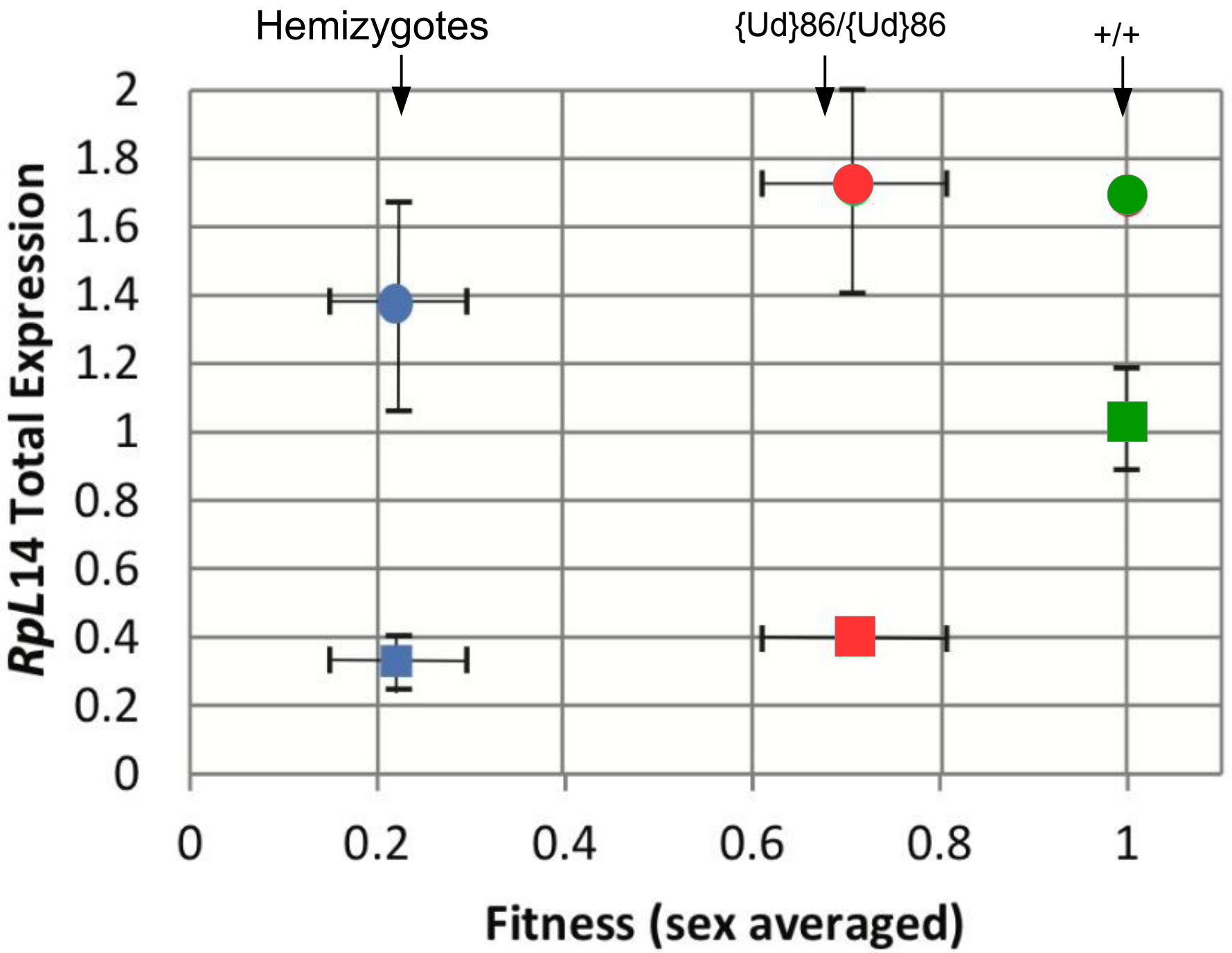


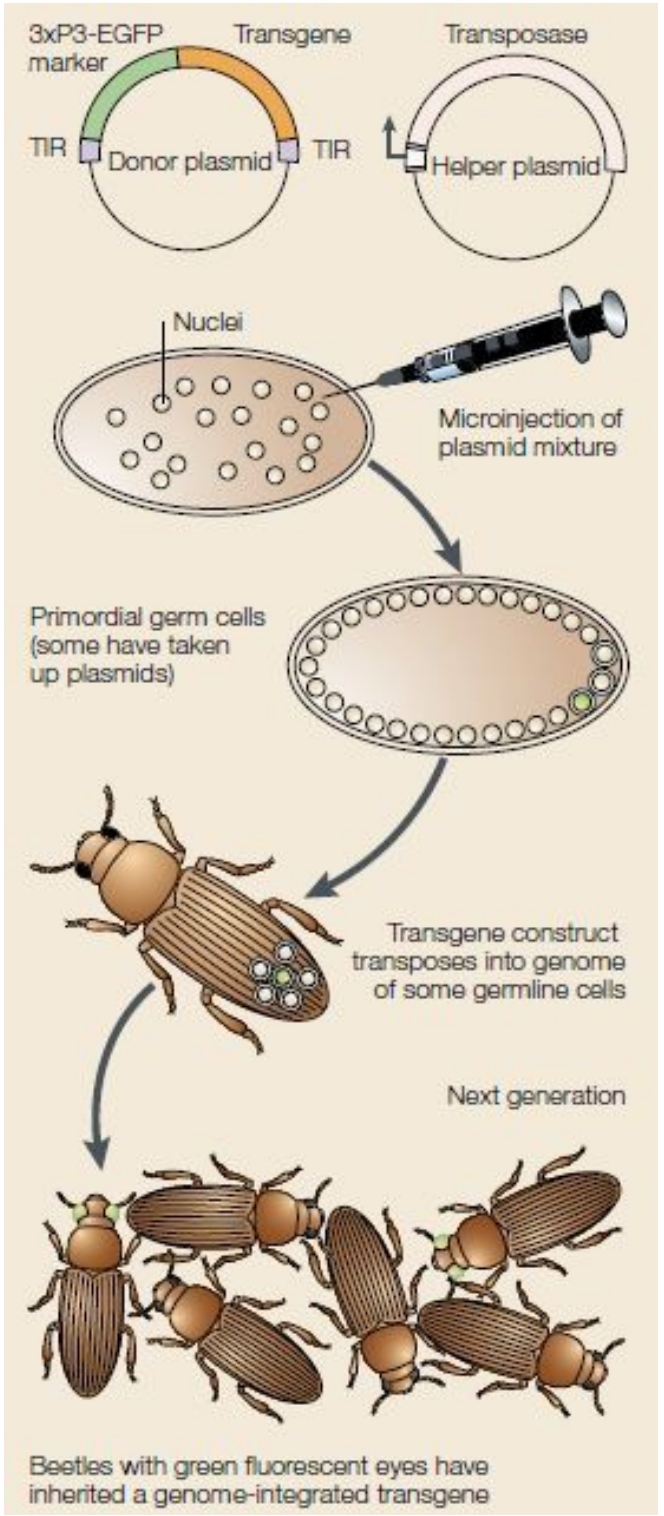
In each case heterozygotes are missing



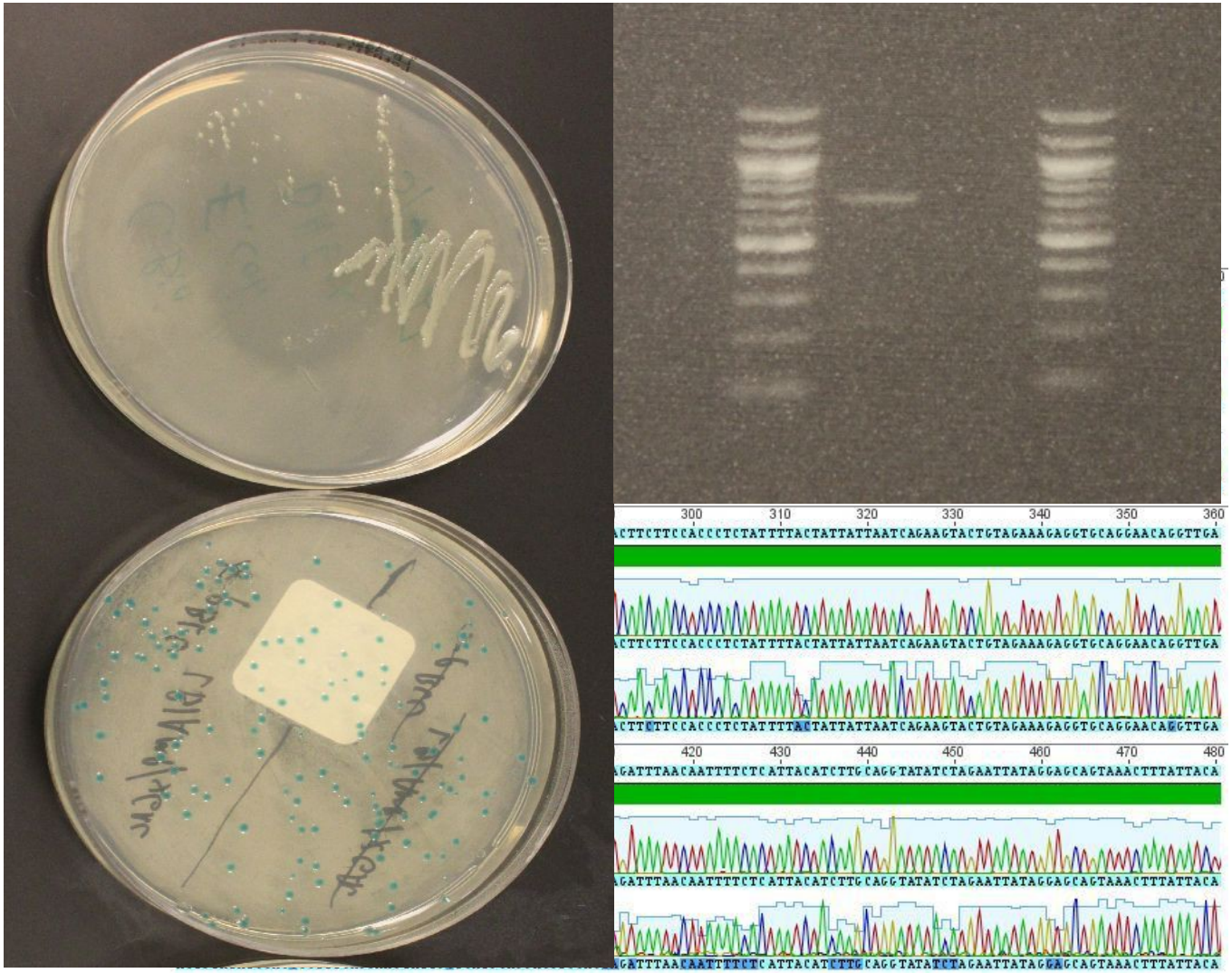

A
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
{Ud}86 Frequency
Results
Predict.
Equilib.
2
3
4
5
6
7
Generations
B
Homozygote Fitness
Heterozygote Fitness
99.9% C.I.
99.0% C.I.
95.0% C.I.
M.L.E.
C
Fitness
1.2
1.0
0.8
0.6
0.4
0.2
0.0
+/+
+/{Ud}86
{Ud}86/
{Ud}86
Genotype


RpL14 Expression Levels
RpL14 mRNA level fold change relative to a set of normalization genes
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.03
0.28
0.05
0.36
0.02
1.68
0.87
0.51
1.26
0.46
Rescue Expression
Endogenous Expression
+/+
{Ud}/+
{Ud}/{Ud}
+/+
{Ud}/+
{Ud}/{Ud}
Adult males
Adult females


Hemizygotes
{Ud}86/{Ud}86
+/+
RpL14 Total Expression
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
Fitness (sex averaged)

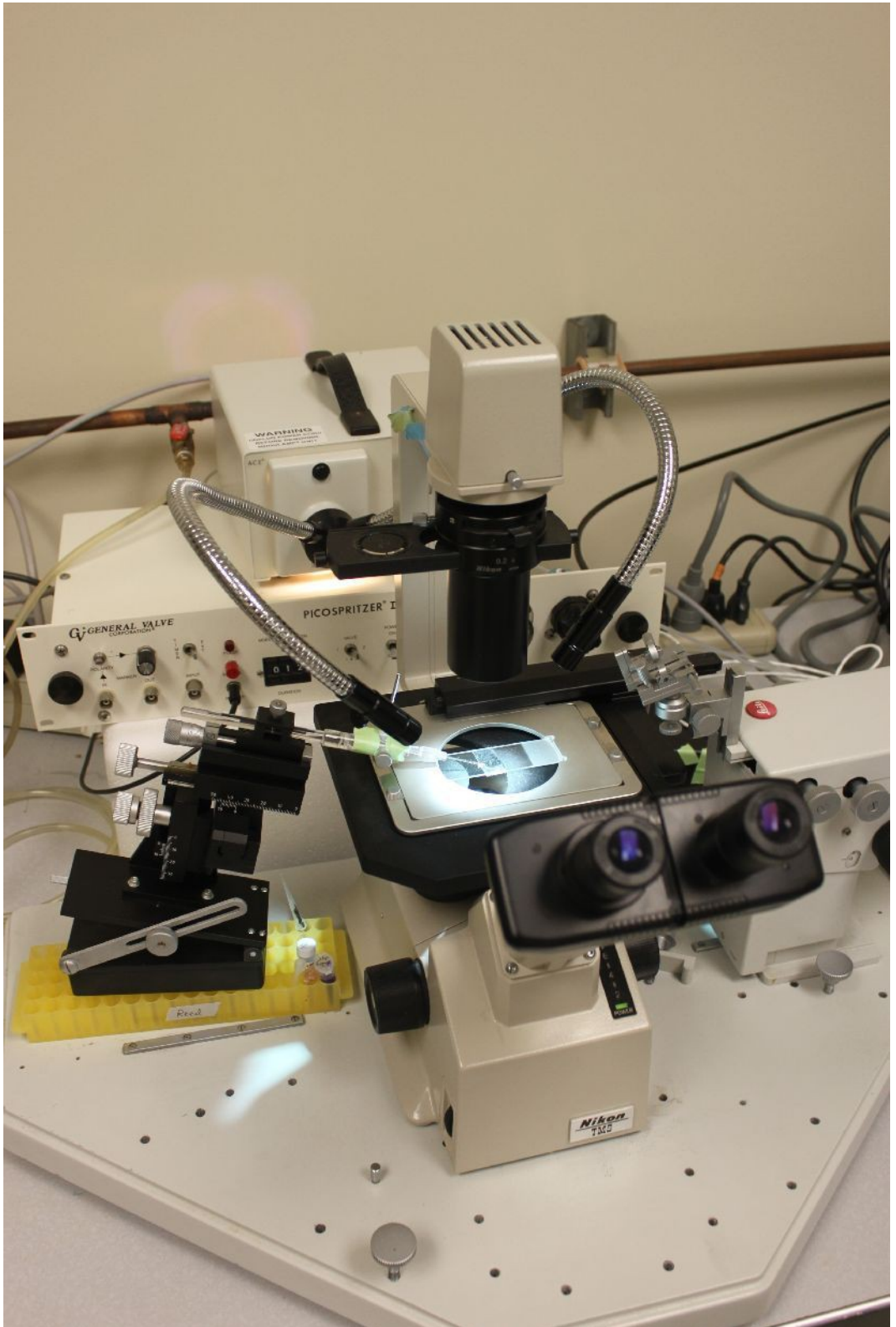


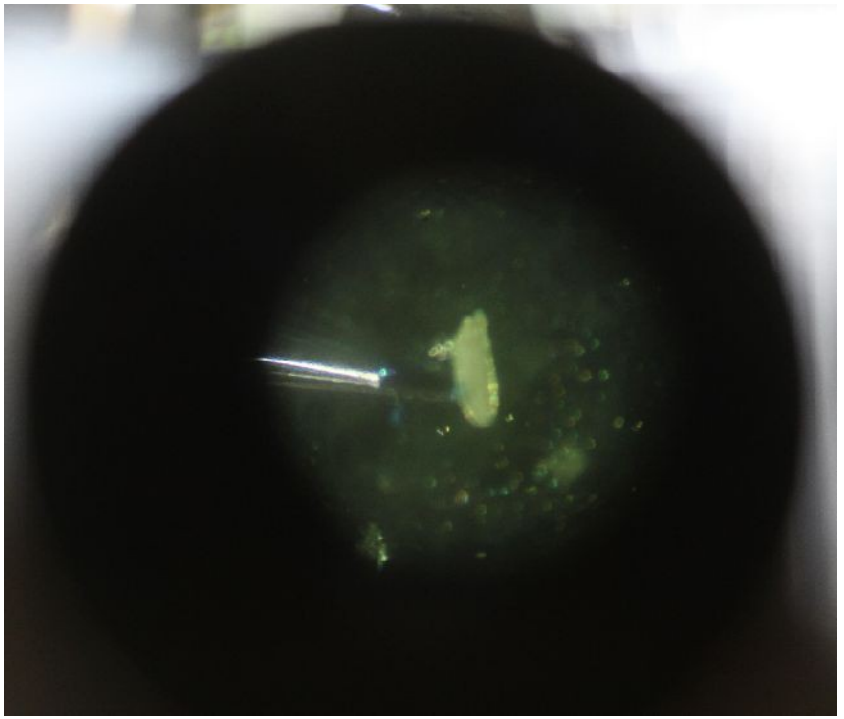
Current direction: Port our system to Culex mosquitoes.

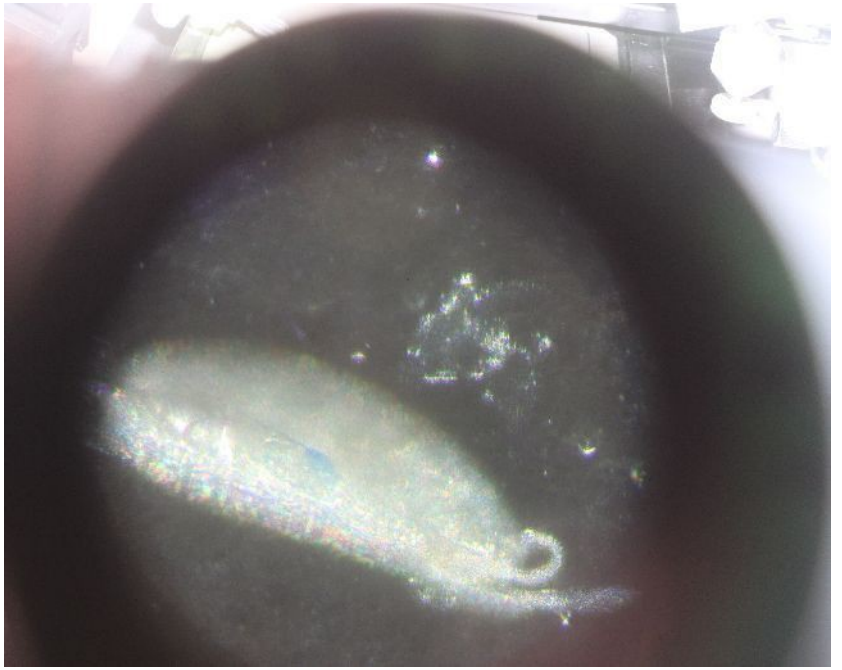


← Wimmer 2003 Nat. Rev. Genet. 4:225

Embryo Microinjection

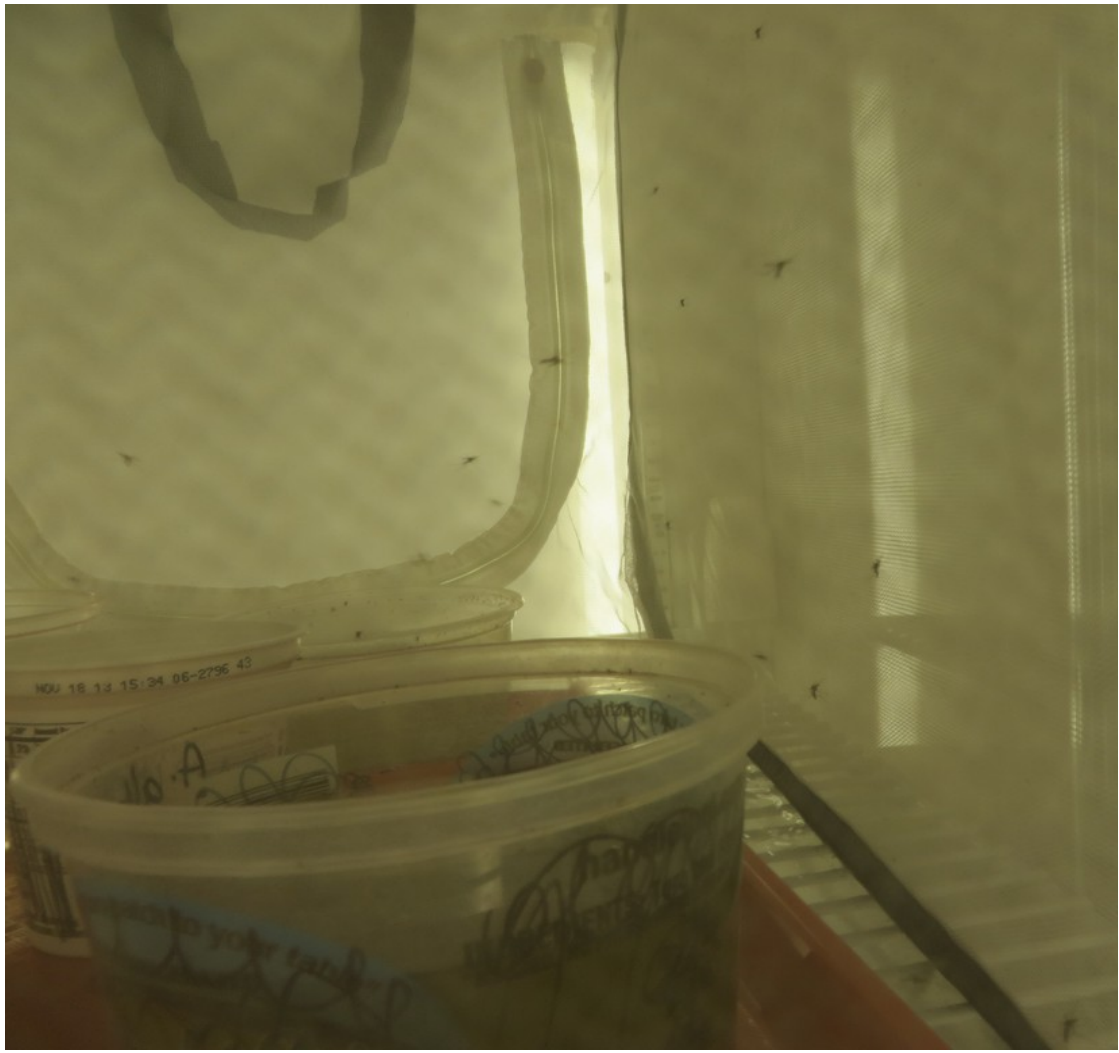






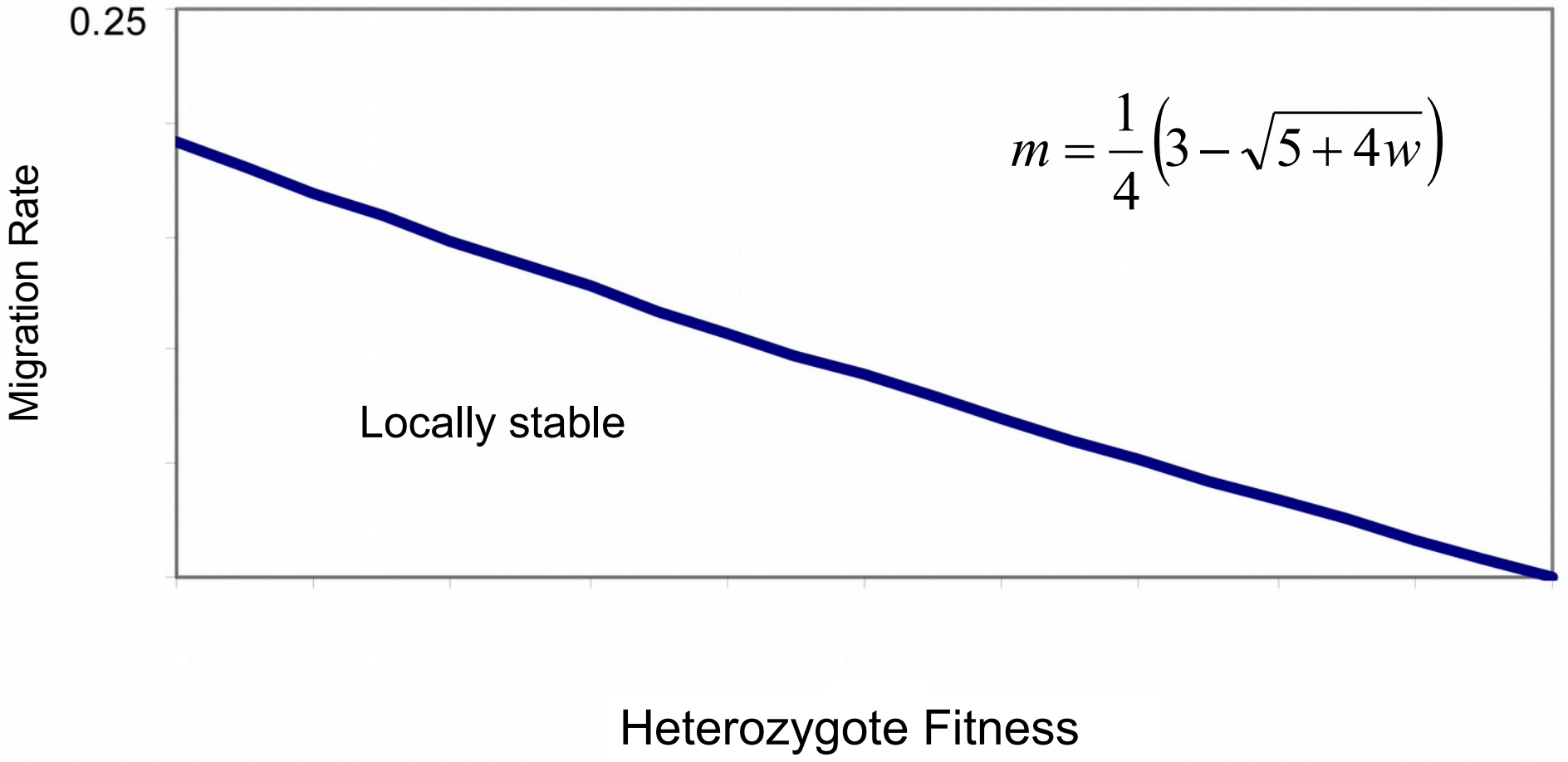
Culex Larvae are Easily Collected on Campus...

Our current step is optimize raising them in the lab.

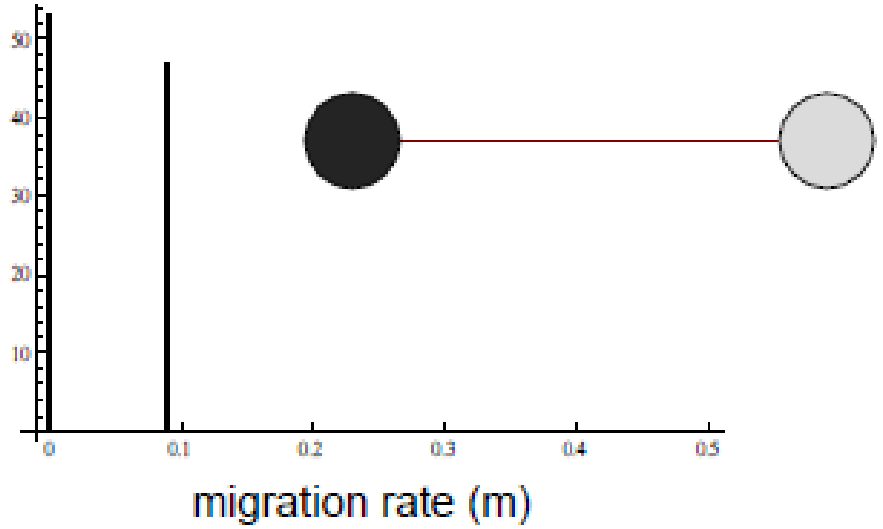


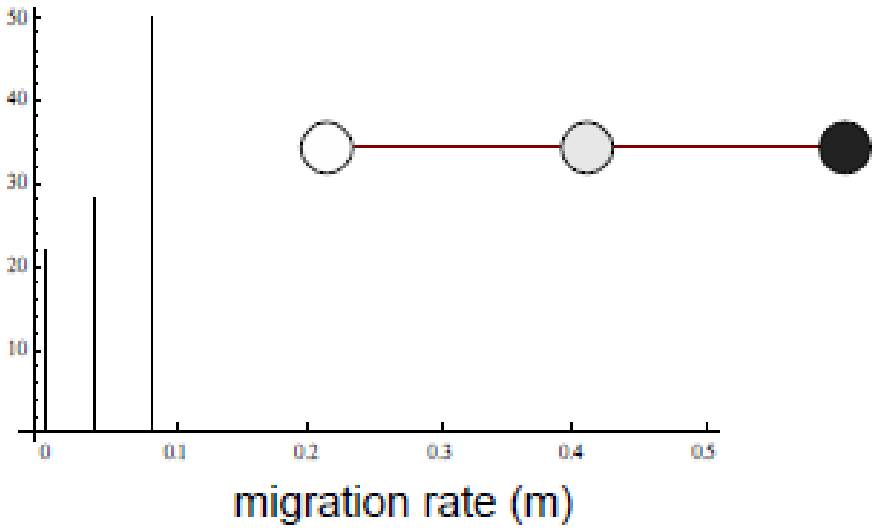
A little theory then let's talk about Hawai'i

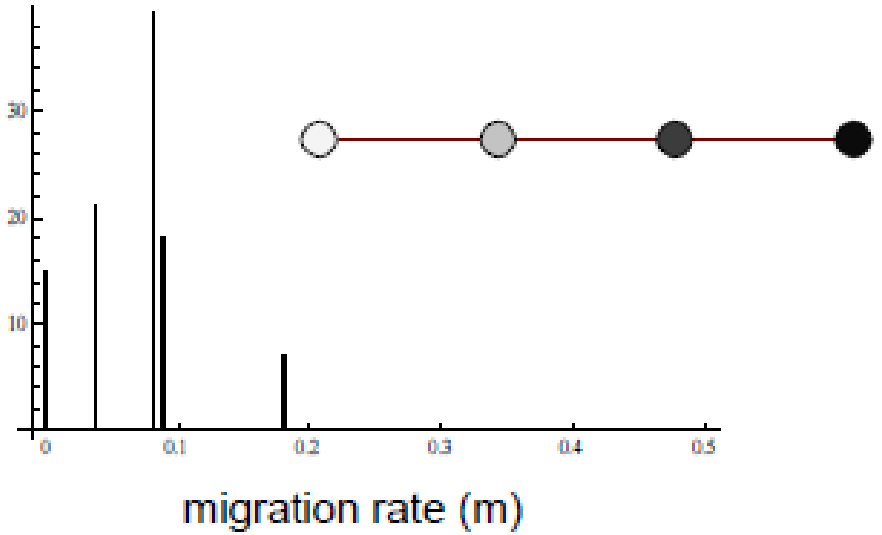
The maximum migration rate that allows local underdominant stability between <u>two</u> population exchanging migrants.

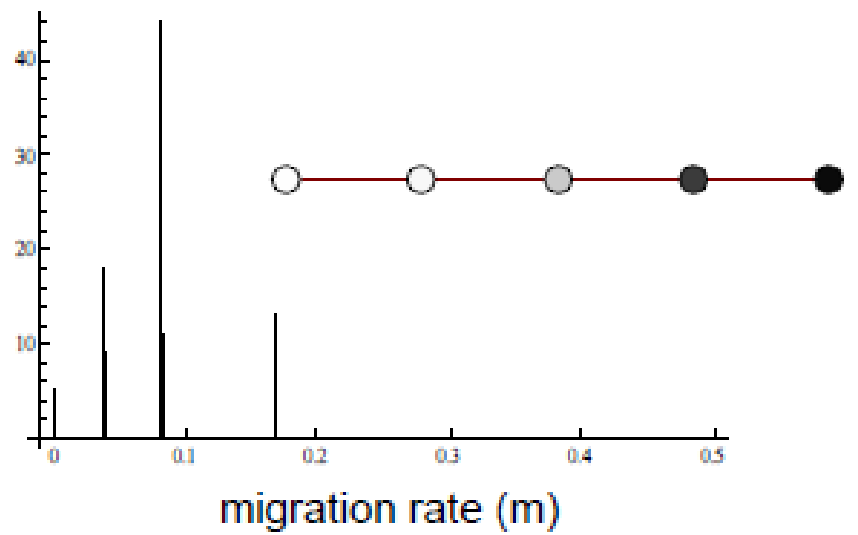


Extending from two populations in one dimension (w=0.5).

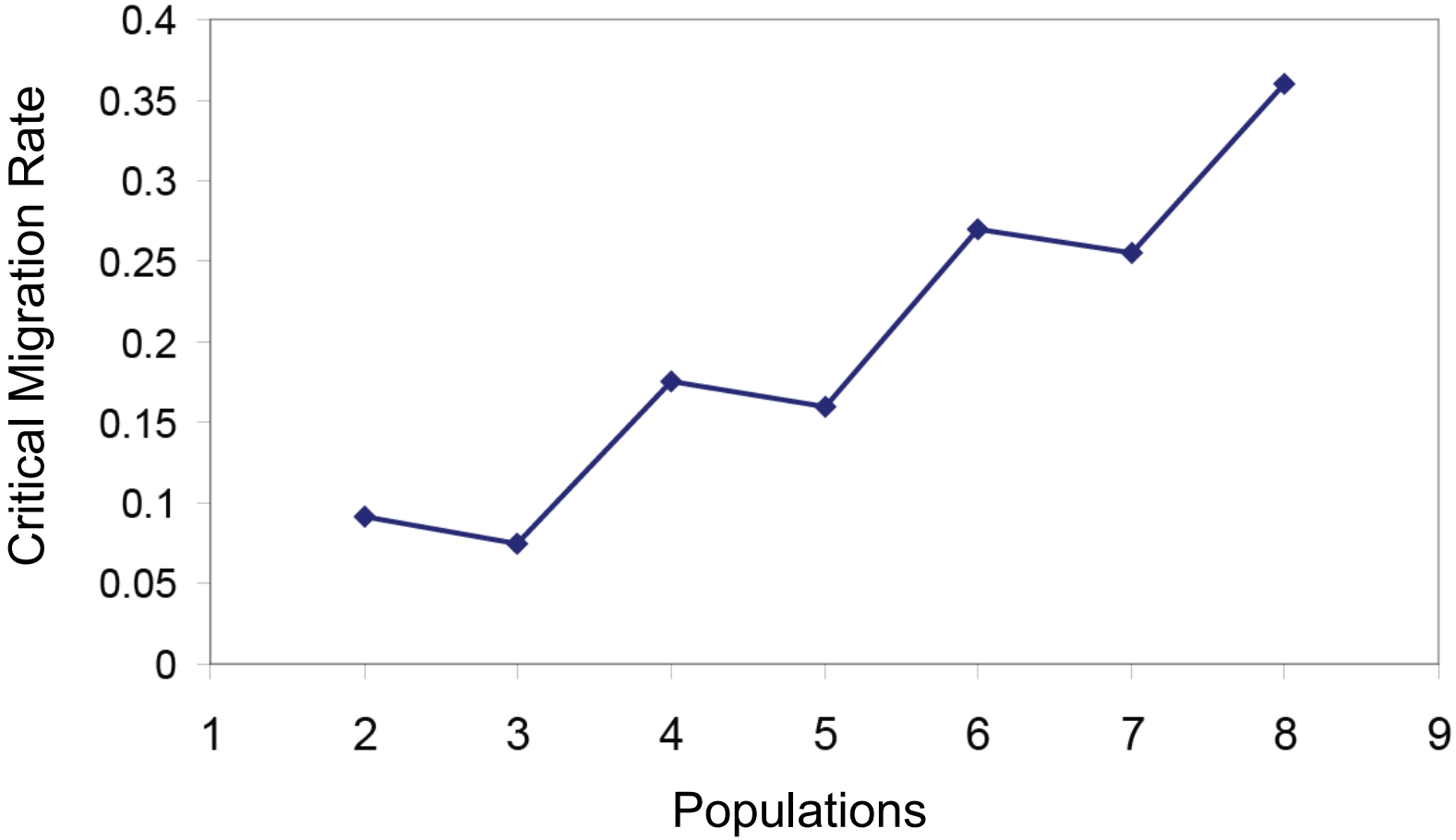




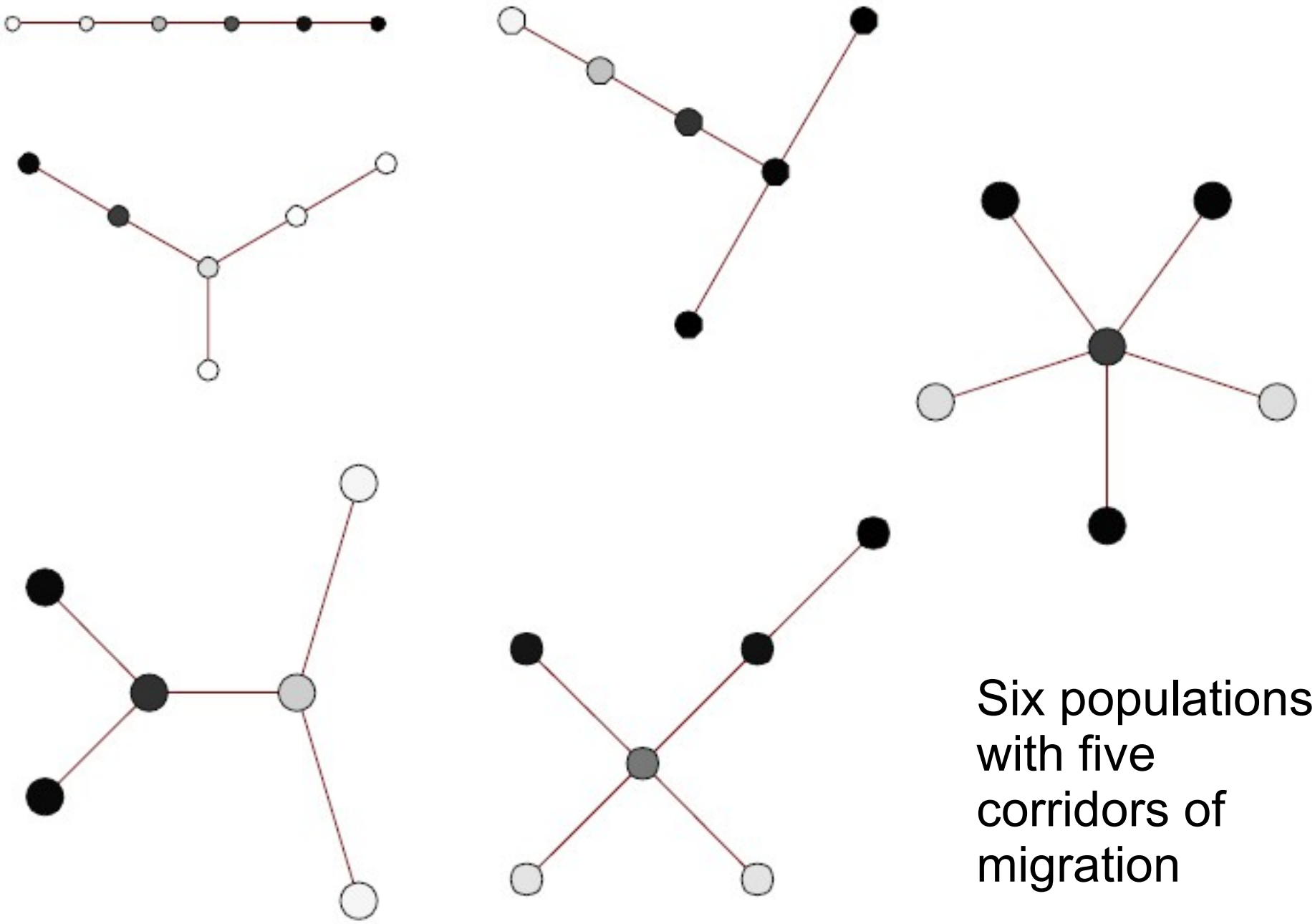




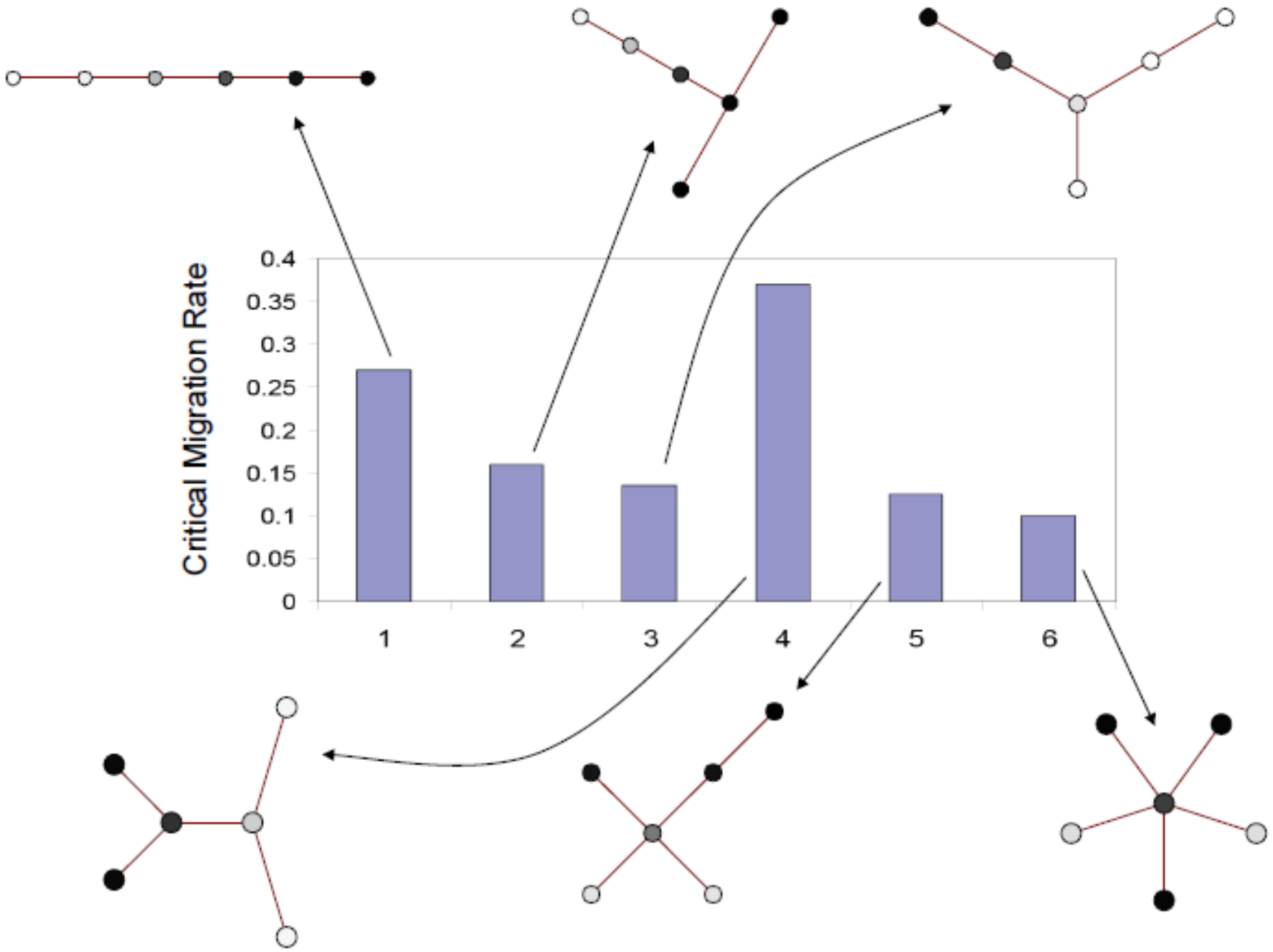
An even-odd sawtooth stability pattern appears.

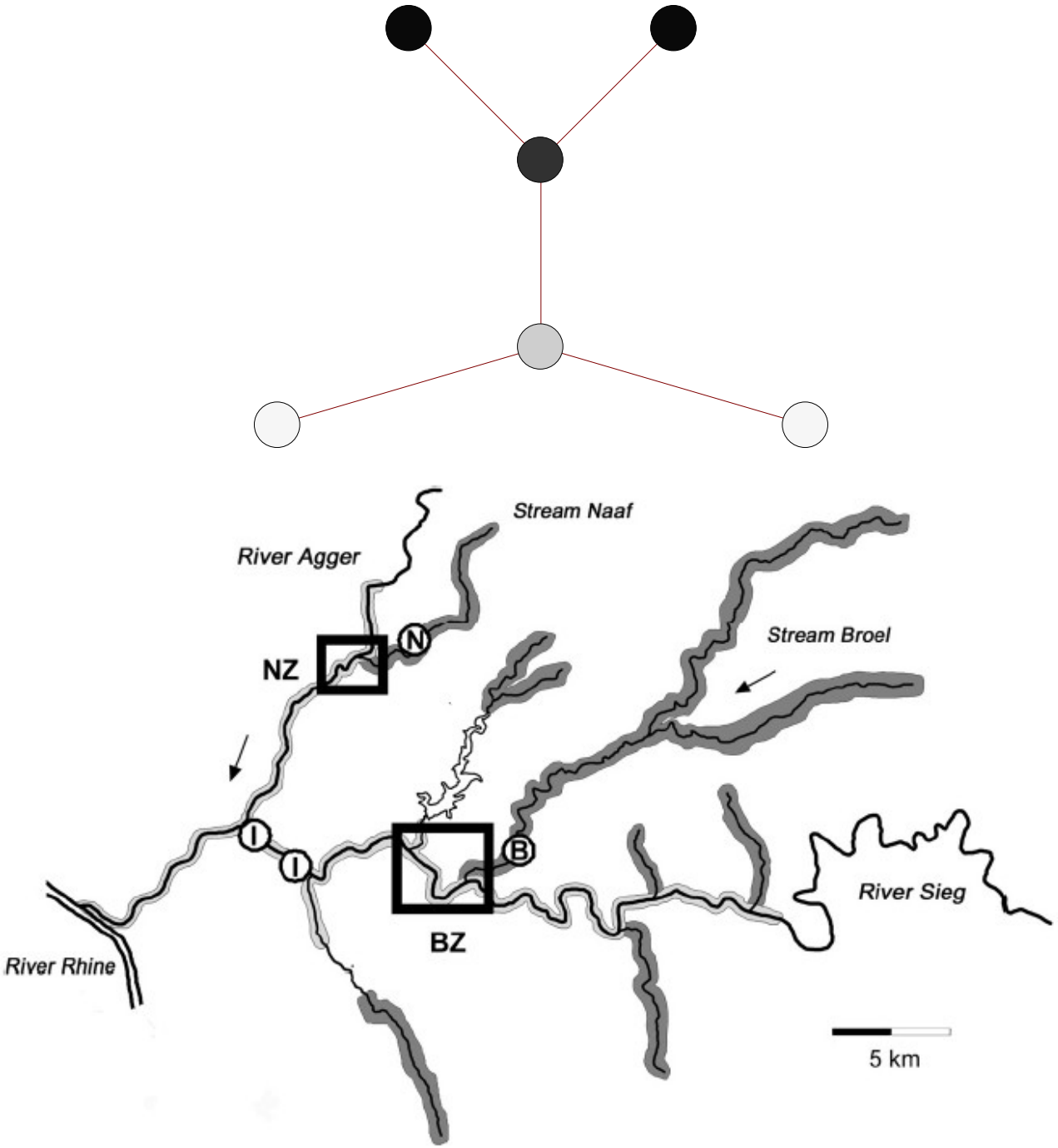


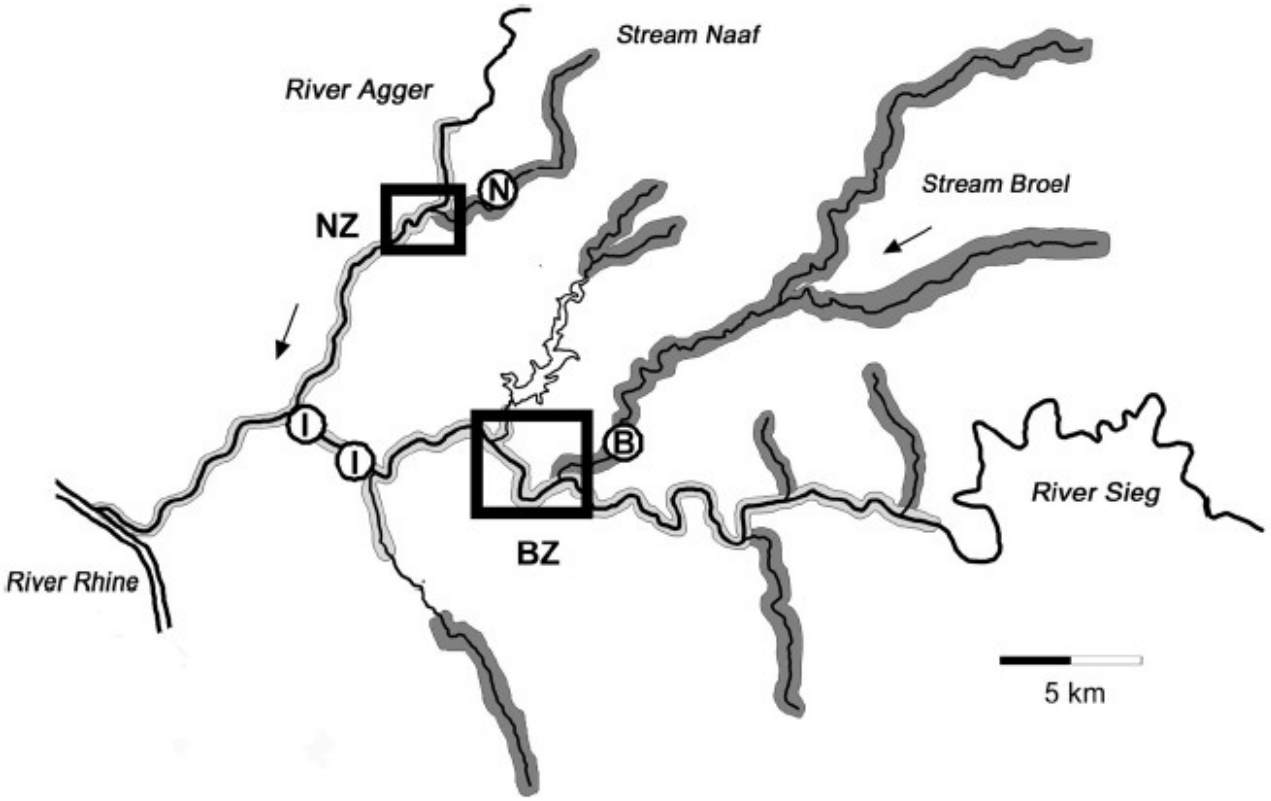
Two buffer populations are more stable than one or three?



Six populations with five corridors of migration


Critical Migration Rate
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6


Stream Naaf
River Agger
N
NZ
Stream Broel
I
I
B
BZ
River Sieg
River Rhine
5 km


Stream Naaf
River Agger
N
NZ
Stream Broel
I
I
B
BZ
River Sieg
River Rhine
5 km

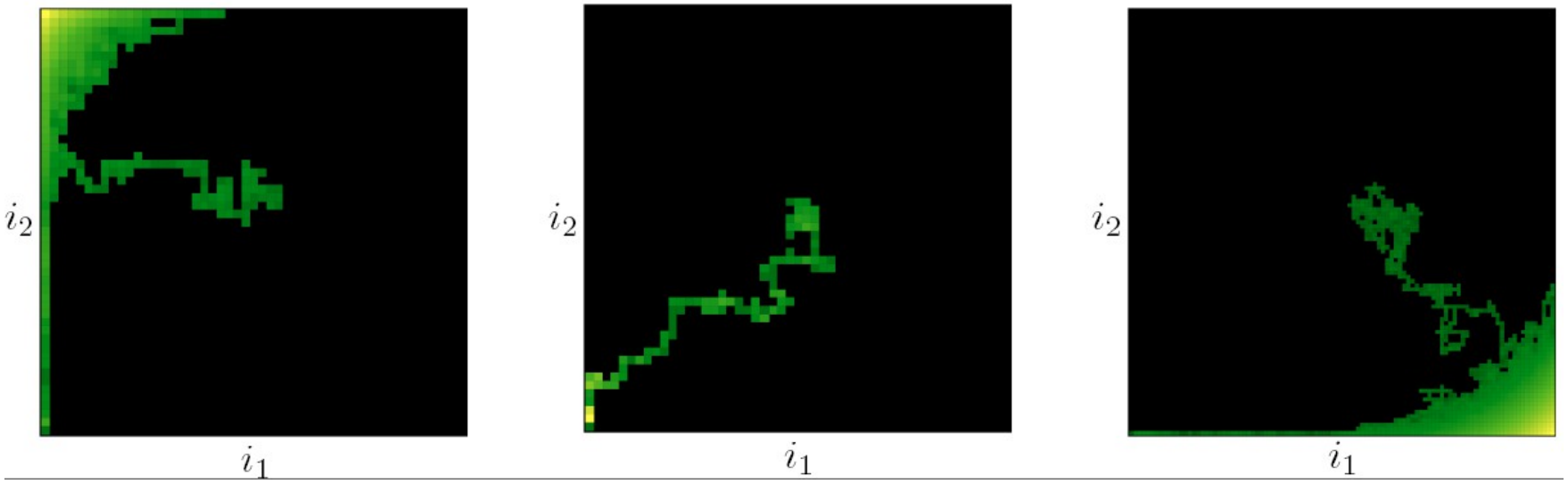
Molecular Ecology (2009) 18, 2615–2627

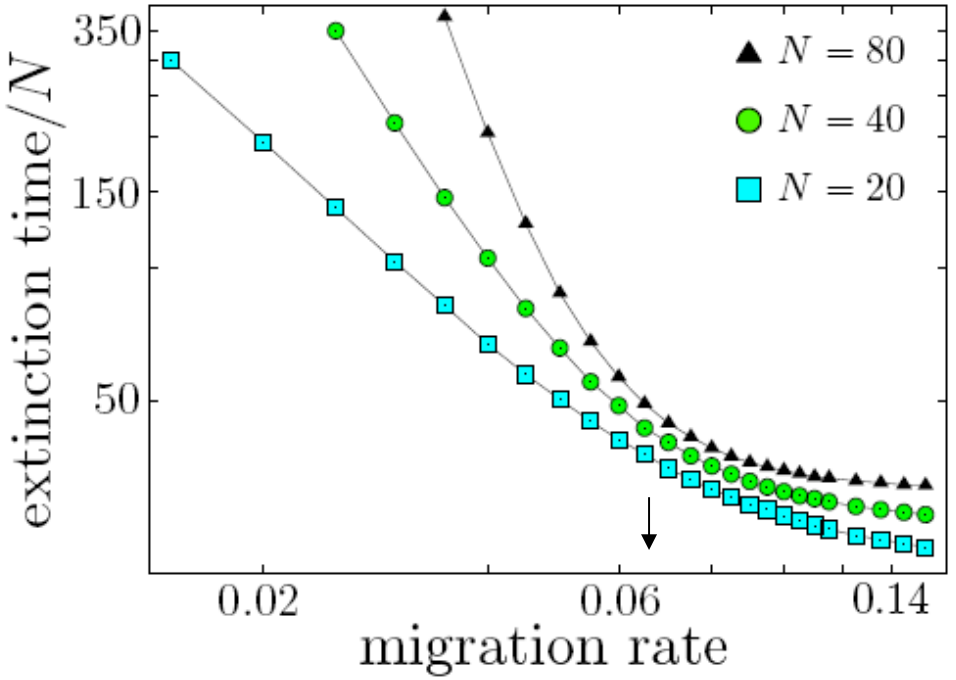
Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations

A. W. NOLTE,* Z. GOMPERT† and C. A. BUERKLE†

Results suggest widespread underdominance between Cottus populations.

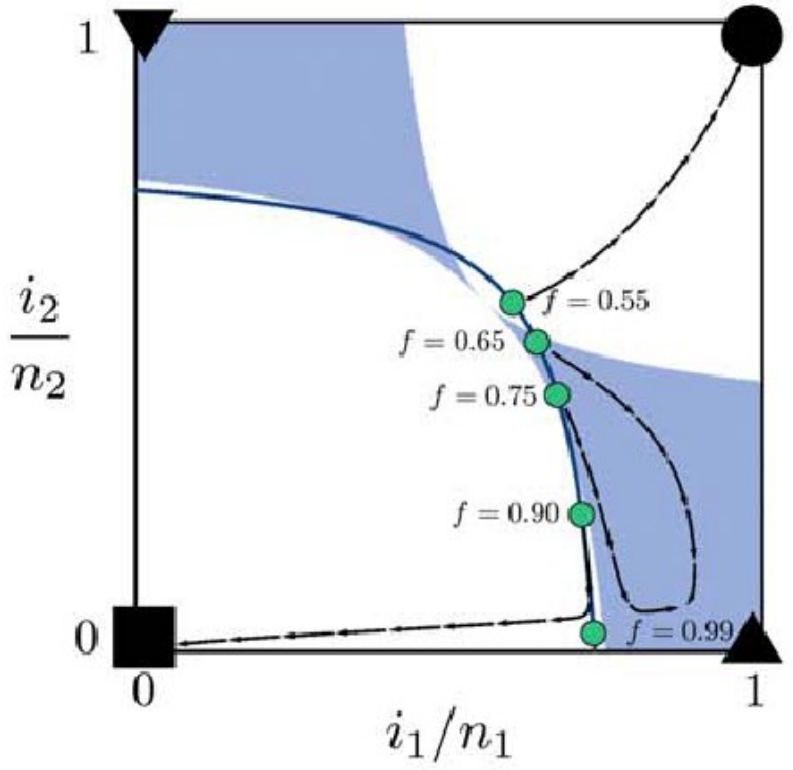
Stochastic/Finite Population Predictions





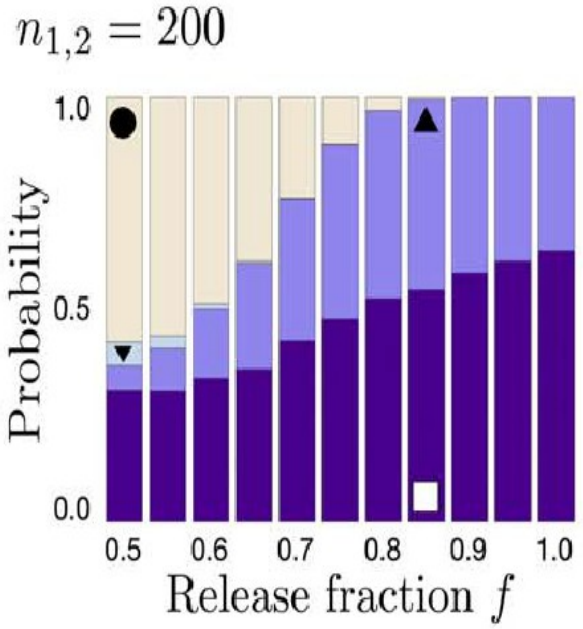
Range of release strategies

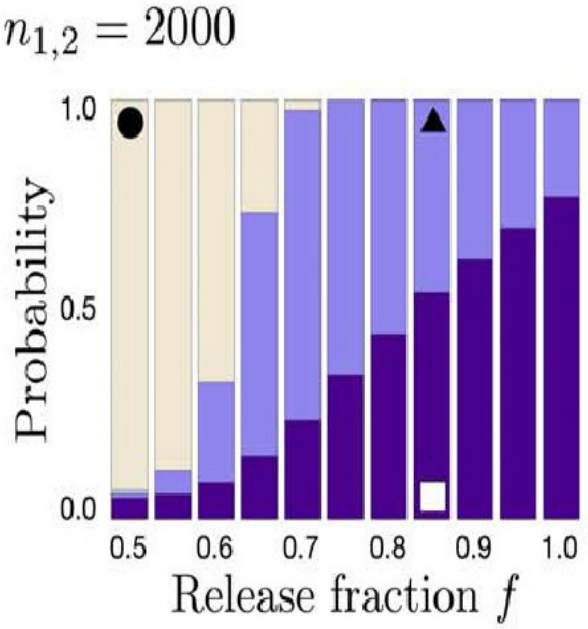
For a constant release size of R=2.75N

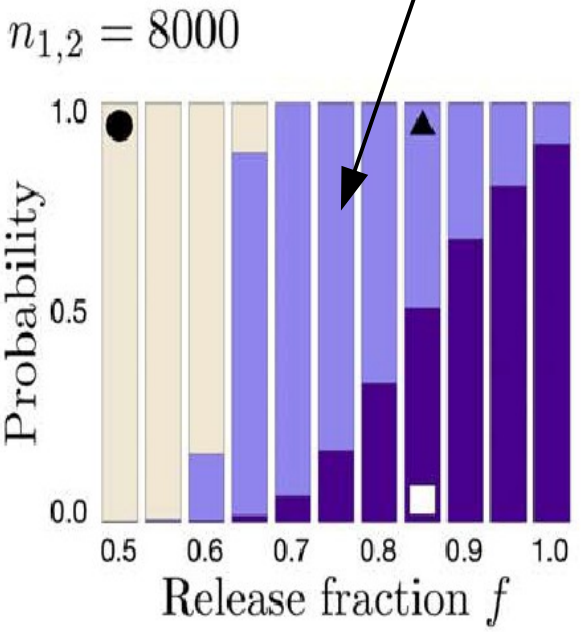


w=0.5
v=0.9
m=0.055

More likely to succeed if you release some into the non-target population!?

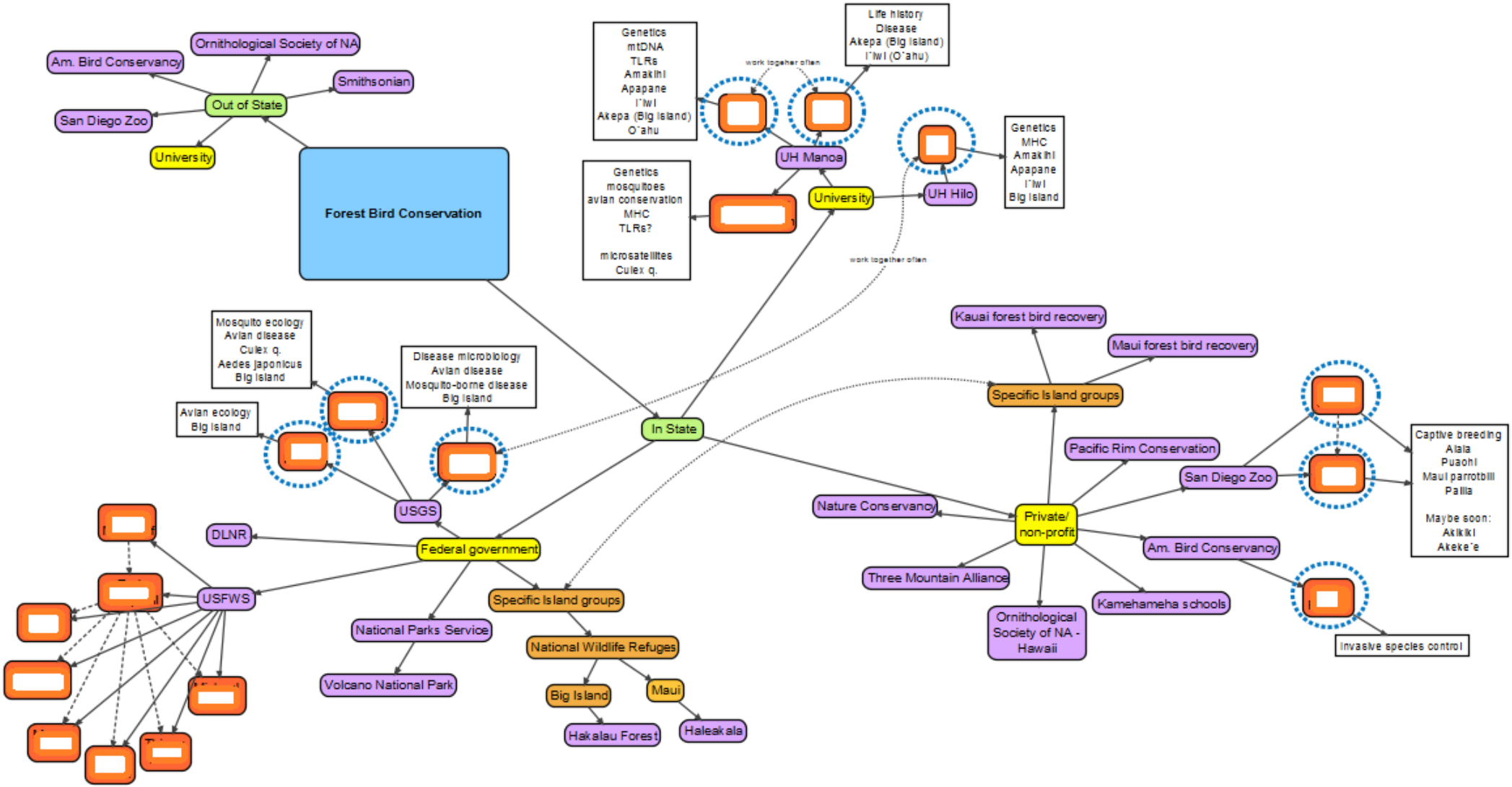






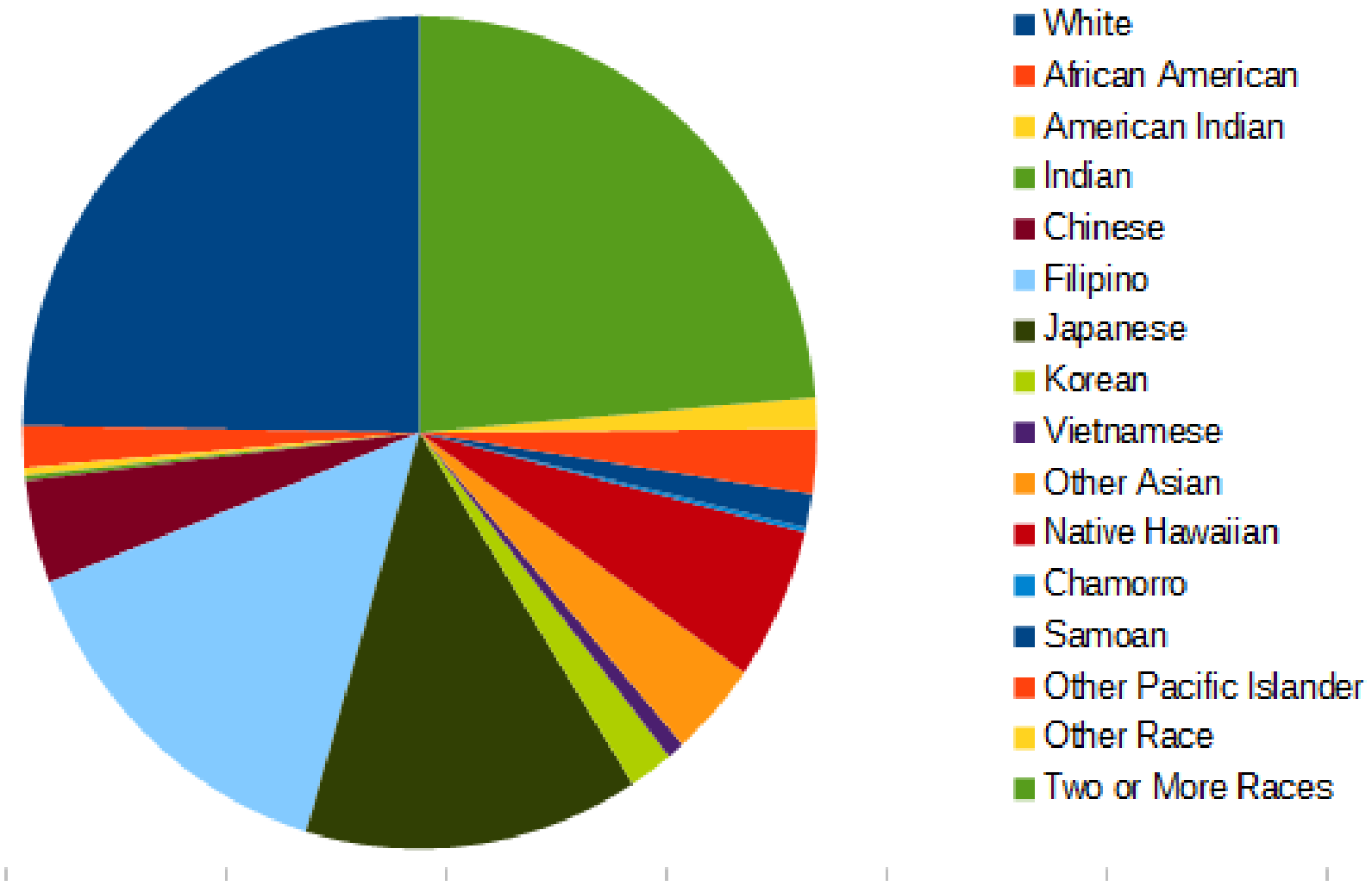
The social landscape in Hawai'i

Hawai'i Forest Bird Conservation—some major player organizations.



Disclaimer: a work in progress, not fully accurate or complete, illustrative only.

2010 Federal Census Data for Hawai'i



http://factfinder2.census.gov/faces/tableservices/jsf/pages/productview.xhtml?pid=DEC_10_DP_DPDP1

When I talk about this work to other scientists in Hawai'i I have had enthusiasm from some and have been openly laughed at by other people as impossible.

I asked to meet with Native Hawaiians to ask for advice and have been told that what I am proposing is connected to issues of “social justice” in Hawai'i.

So, I have been collecting information along these lines. However, I am not an expert in this area and some of you likely know more than I do about this.

http://csleps.dasa.ncsu.edu/asb/hawaii

Hawai'i and the Western World: A Brief Sketch

1778 Capt. J. Cook came upon the islands and communicated this to the west.

1810 Kamehameha I united the islands.

1817 Kaua'i was a protectorate of the Russian Empire

1843 Royal Navy Admiral Paulet ordered Hawai'i to submit to Great Britain, burned flags, *etc*. July 26, Admiral Thomas declared an end to the occupation and established **international recognition of Hawai'i as a sovereign nation**.
Kamahameha III "*Ua Mau ke Ea o ka 'Āina i ka Pono*"
"The (continuing) life/breath of the land is perpetuated in righteousness/fairness/respect"

1853 The Russian fort on Kaua'i closed.

1856 Sikaiana joined Hawai'i.

Location of Sikaiana Atoll in the Pacific Ocean

1875 The "reciprocity treaty," established trade between Hawai'i and the US, allowed the US to temporarily use Pearl Harbor as a military base.

1887 US citizens in Hawai'i formed a "reform party" and a militia, then seized control of the palace and forced King David Kalākaua to sign the "bayonet constitution."

King David Kalākaua refused to renew the reciprocity treaty which now allowed a permanent military base at Pearl Harbor.

1889 The reform militia stopped the "Wilcox rebellion" to replace King Kalākaua with Princess Lili'uokalani.

1891 King Kalākaua died and Princess Lili'uokalani became the new monarch and drafted a new constitution.

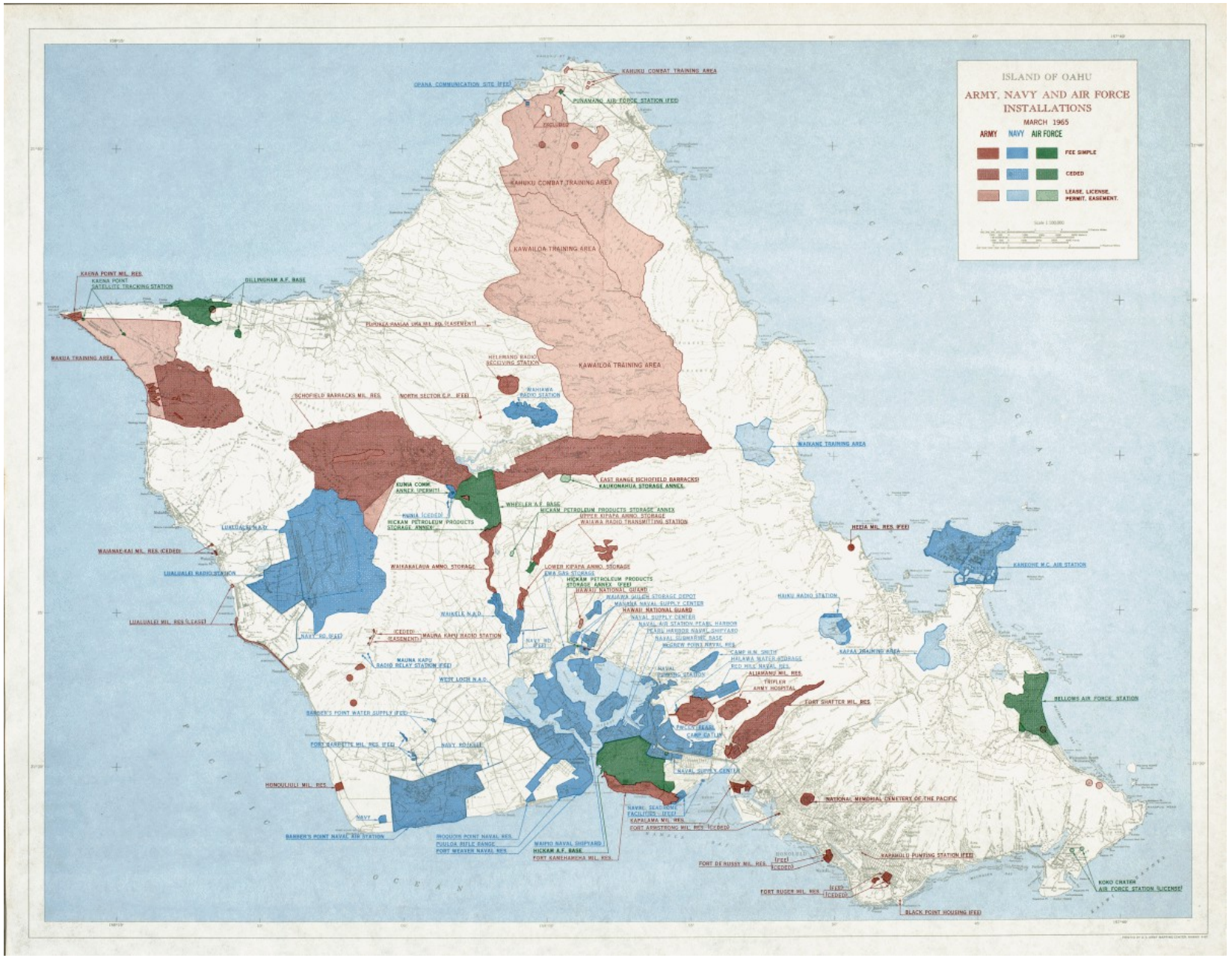
1893 Some Europeans and Americans formed a "committee of safety," US Marines and sailors landed. The reform party militia forced Queen Lili'uokalani to abdicate citing superior force backing of the militia by the US military.

1894 A new government was formed with Sanford **Dole** as president.

1897 US president McKinley proposed a treaty annexing Hawai'i, which was not approved by the senate because of petitions from Hawai'i.

1898 McKinley annexed Hawai'i by a resolution he signed.

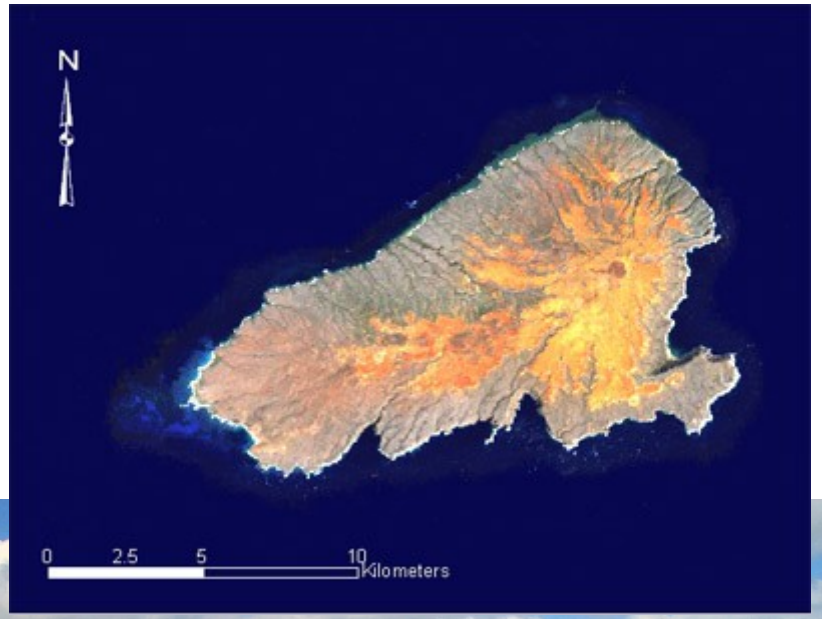
¼ of O'ahu is a US military base.



http://digicoll.manoa.hawaii.edu/savedmaps/Pages/viewtext.php?s=browse&tid=70&route=browseby.php&start=8&by=year#

Kaho'olawe

In the 1900's the US military used the entire tropical island for bombing practice.



"The (continuing) life/breath of the land is perpetuated in righteousness/fairness/respect"
- Kamehameha III 1843

http://earthfirstjournal.org/newswire/2013/04/15/indigenous-hawaiians-take-on-monsanto-and-gmos/

Genetic pest management is likely to be initially interpreted in terms of classical bio-control and GMO crops.

...and this may be seen in the context of what has been done to Hawai'i by outsiders from the mainland US; thus, connected to issues of sovereignty and self determination.

Biocontrol in Hawai'i, examples

1932 Cane Toad (*Rhinella marina*)
To eat sugar cane beetles.
Collected from Hawai'i to introduce in Australia

Biocontrol in Hawai'i, examples

There were many species of tree snail. Today all the remaining species are endangered. Some are down to a single known specimen.

1936 Giant African Snail (Achatina fulica)
Brought as a garden ornamental.
A crop pest

1955 Cannibal Wolfsnail (*Euglandina rosea*)
To control giant African snails.
Hunted <u>many</u> species of tree snail to extinction

Biocontrol in Hawai'i, examples

Barn Owls and Cattle Egrets released in the 1950's to control insects and rodents

1929 Japanese white eyes as insect control.

All three are threatening native bird species in various ways.

http://en.wikipedia.org/wiki/File:Japanese_White-eye.jpg

http://www.killerculture.com/environmental-health-your-culture-is-killing-earth/save-hawaiian-birds/save-hawaiis-egrets-and-owls/

Biocontrol in Hawai'i, examples

1883 Mongoose (*Herpestes javanicus*) to eat nocturnal rates. The mongoose is active during the day and eats ground nesting bird eggs among many other things.

There are many other examples. Some are considered successes (e.g., *Eurytoma erythrinae* and the wiliwili) but, honestly, I want to be cautious about saying that here.

http://mauiinvasive.org/2012/04/18/moving-on-from-the-mongoose-the-succuss-of-biological-control-in-hawaii/

GMO Kalo, Haloa (Taro)

Haloa (Kalo) is the older brother of humans and Kalo plays an extremely important role in Hawaiian culture.

Taro leaf blight (*Phytophthora colocasiae*) is present in Hawai'i and efforts were made to engineer resistant varieties.

2002-2003 UH begins genetic work on Kalo

2006 People chain the doors of the UH medical building in protest

2007 Bills are introduced in the state legislature to place a moratorium on GMO Kalo.

2008 Hawai'i State Legislature established the Taro Security and Purity Task Force

http://hawaiiseed.org/local-issues/taro/
http://islandbreath.org/2008Year/01-farming/0801-01SaveHaloa.html
http://www.ediblearoids.org/portals/0/taropest/lucidkey/taropest/media/Html/Fungi/Pcolocasiae/Pcolocasiae6.htm
http://www.hawaiimagazine.com/blogs/hawaii_today/2009/1/15/Hawaii_poi_on_the_Mainland

http://ecowatch.com/2013/11/21/hawaii-bans-gmo-biotech-companies/

“'āina” - land; “kama'āina” people of the land, long term residents

http://occupy-monsanto.com/tag/gmo-free-hawaii/

"pono" - respectful, the right way to do things.

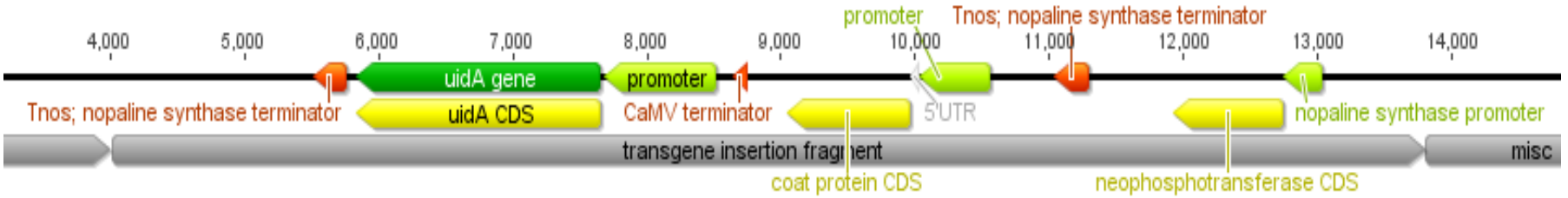
http://kahea.org/blog?ph_field_1=Land%20and%20Cultural%20Rights&b_start:int=133
http://www.flickr.com/photos/hdoug/7999273946/

Rainbow Papaya

Developed by a former Hawaiian resident Dennis Gonsalves at Cornell.

Resistant to ringspot virus, which devistated the papya industry in the 1990's—essentially saved the industry.

Widespread use in Hawai'i today.

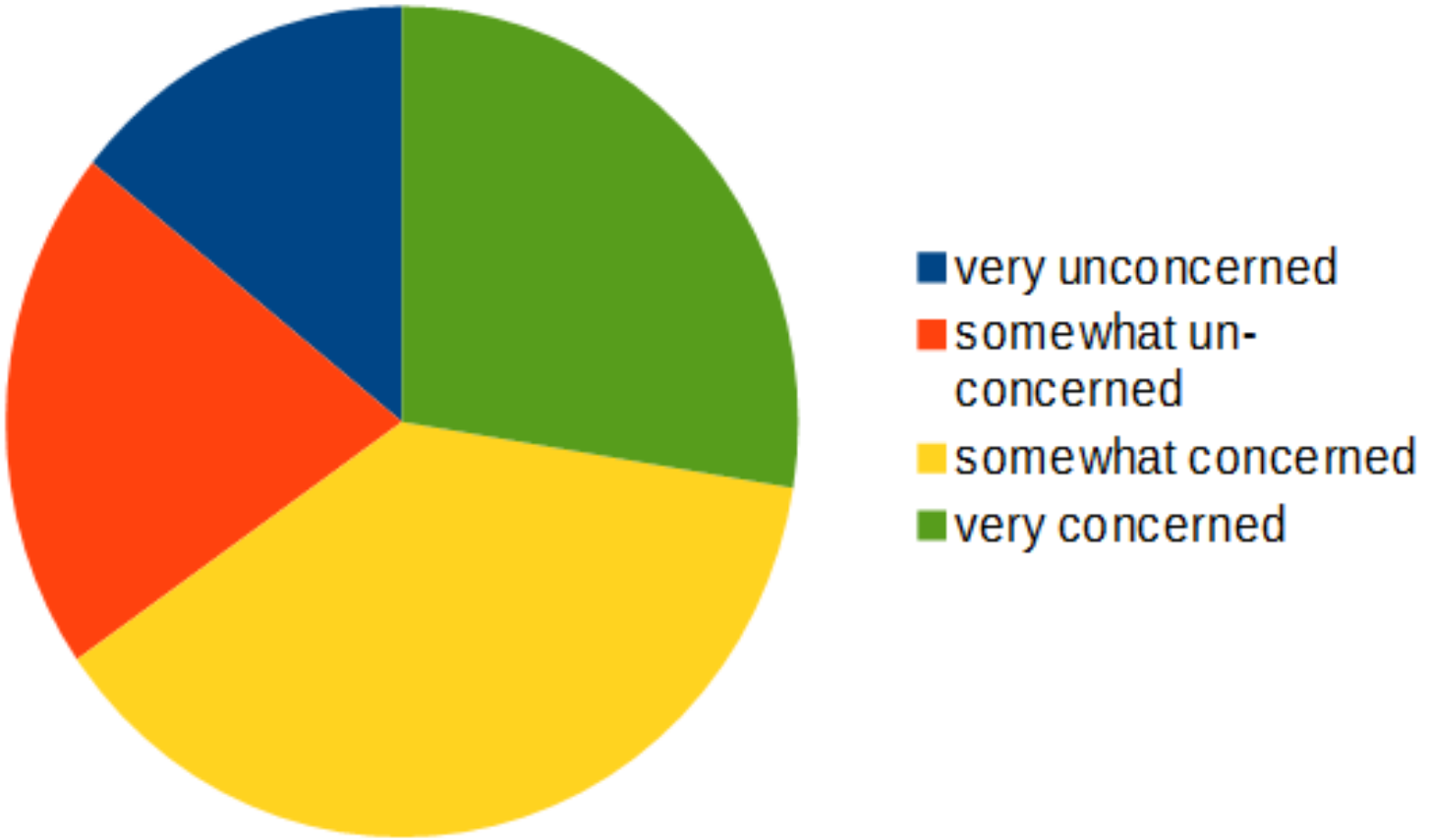


“GMO 4 EVA” Many local farmers support GMO Crops.

http://voteno113.wordpress.com/

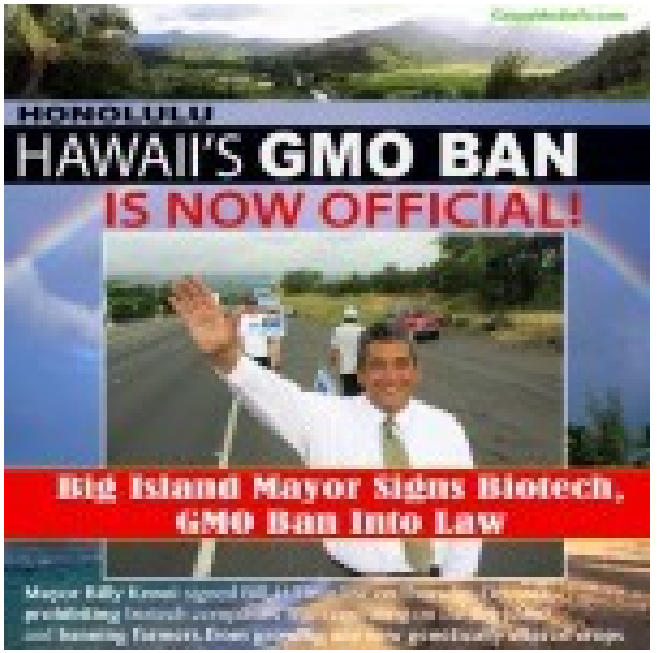
http://www.nytimes.com/2014/01/05/us/on-hawaii-a-lonely-quest-for-facts-about-gmos.html?_r=0

Hawaii News Now/Star Advertiser
"Campaign 2014 Hawaii Poll"
Residents' opinion about GMO crops in Hawaii



Bills to ban GMO crops

This is THE hot topic in Hawai'i now and I believe likely to be in a state of flux for some time to come—and likely to be a mistake to try to summarize here. I encourage you to keep an eye on the news over the next year.



http://vaccineliberationarmy.com/2013/12/08/breatking-news-mayor-signs-bill-to-ban-gmos-on-the-big-island-of-hawaii-kauii-anti-gmo-bill-safe-also/

http://www.truth-out.org/news/item/20256-on-the-front-lines-of-hawaiis-gmo-war-part-two

What do you call brown fuzz that comes out at night, roots around in mud, and eats worms?—and doesn't even have arms!

What do you call brown fuzz that comes out at night, roots around in mud, and eats worms?—and doesn't even have arms!

Embraced as a synonym for a nation.

What do you call brown fuzz that comes out at night, roots around in mud, and eats worms?—and doesn't even have arms!

Embraced as a synonym for a nation.

On one hand Hawai'i and New Zealand have a lot of similarities, yet, the public awareness of, and identity with, conservation issues are very different...

The i'iwi may be the closest thing to an icon of endemic Hawai'i birds.

Perhaps they could serve a role to focus and rally general public support?

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