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GENETICS OF BIOCONTROL, A LONG IGNORED SCIENCE

Population Genetics and Biological Control

- Population genetics has been ignored in biocontrol
- Relatively easy gains in biocontrol efficiency can be made by applying the lessons of population genetics
- Next gen sequencing can be an important source of markers to test the importance of some of these lessons
- Ode to High Resolution Melt curves for easy recognition of genetic variants

Why has it been ignored?

- Rarely shown to be important in biocontrol using natural enemies (Contrast with SIT applications)

- Emphasis on the production of large numbers of offspring during the mass rearing phase
  - In CBC for being able to release in many sites, higher release numbers more success
  - In ABC higher numbers more natural enemies to sell
  - How do you get higher numbers in mass-rearing?

The axis of evil: Aspects of prolonged mass rearing

- Initial genetic variation in starting population
  - Low is a problem: inbreeding
  - High is a problem: domestication
- Seasonal reduction in population size
  - Severe reduction in population size in "off-season"
- Number of generations reared
  - The longer a population reared inbreeding may increase
  - The longer a population reared the more domestication takes place

Experiments with *Drosophila melanogaster* show effects of long-term captive rearing on fitness

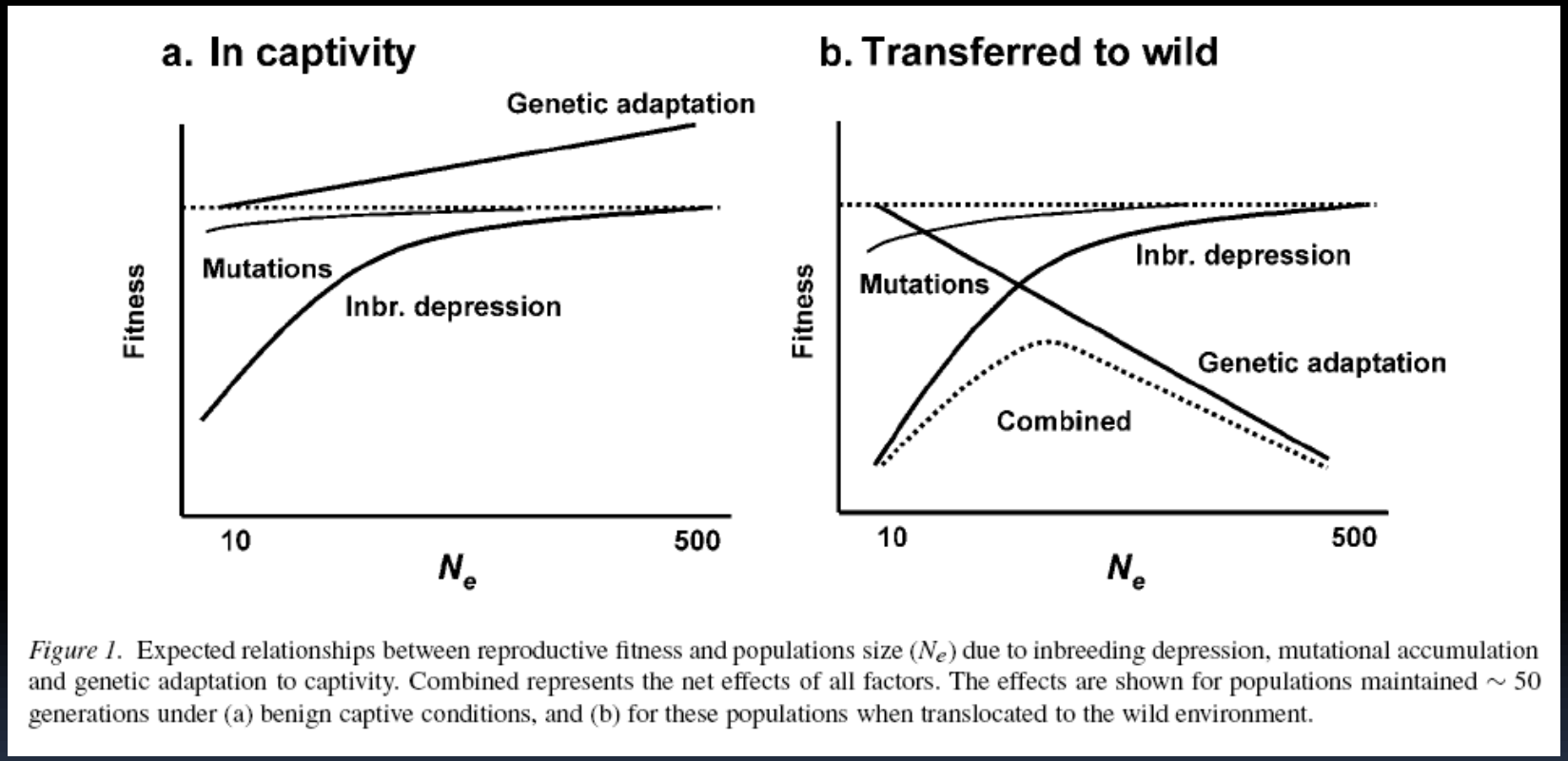


Figure 1. Expected relationships between reproductive fitness and populations size (N_e) due to inbreeding depression, mutational accumulation and genetic adaptation to captivity. Combined represents the net effects of all factors. The effects are shown for populations maintained ~ 50 generations under (a) benign captive conditions, and (b) for these populations when translocated to the wild environment.

Woodworth et al 2003. Conservation Genetics 3:277-288

Decline in fitness happens fast

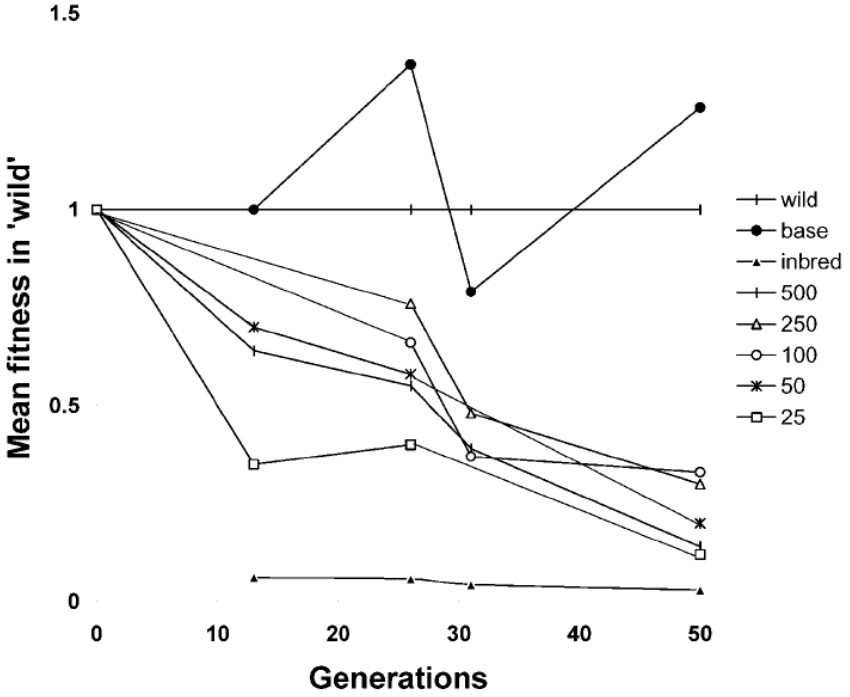


Figure 3. Reproductive fitness over time in the Experimental pedigreed populations (500, 250, 100, 50 and 25) maintained under benign captive conditions, but translocated to 'wild' conditions, compared to a wild control population, a marked inbred control (C*spa*D) and their base population. The T92 base population was maintained under conditions akin to the 'wild' environment.

Woodworth et al 2003. Conservation Genetics 3:277-288

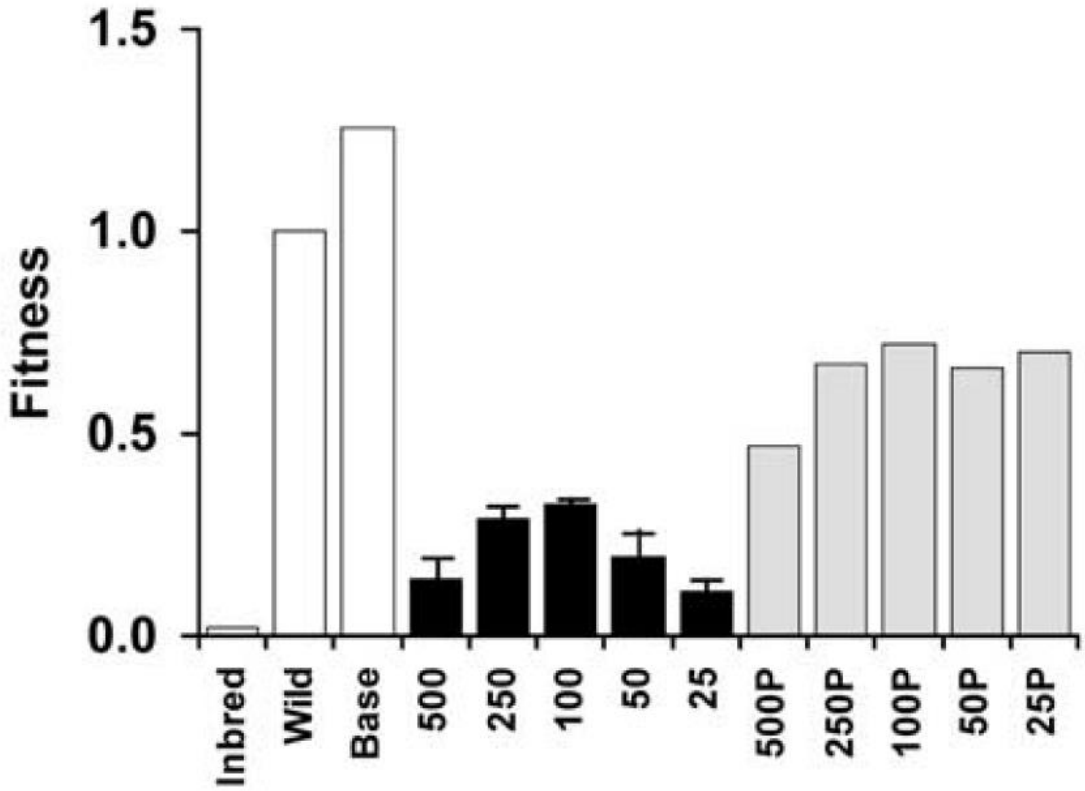


Figure 4. Reproductive fitness of the pedigreed populations (500, 250, 100, 50 and 25) and *Pooled* populations (500*P*, 250*P*, 100*P*, 50*P* and 25*P*) at generation 50, when measured in 'wild' conditions, compared to a wild population control, a genetically marked, highly inbred control (C*spa*D) and their T92 base population.

Woodworth et al 2003. Conservation Genetics 3:277-288

If this is the case then what are the solutions?

- Avoiding domestication
  - By maintaining large number of isolines of species
    - Within line inbreeding and loss of genetic variation, inhibits possibility of domestication
    - Mixing lines takes away the effects of inbreeding and restores population without the negative effects of domestication
  - Mixing populations kept at a relatively high population sizes
  - Reinitiating mass reared populations with field selected individuals. (Not adding a few individuals to an existing mass rearing population)

While solutions are clear they are not applied- why?

- To the users of ABC the quality of the product – natural enemy is difficult to assess
- How good is the parasitoid I am releasing?
- No clear ABC examples of failure of BC– in ABC quality can be compensated by numbers

Economy- Producers will only try to improve when there is a profit to be made, or if forced to.

- Quality control labels
  - Based on realistic tests- how do they perform in the field
- Cheap solutions that do not interfere with the production costs
  - Some of the solutions mentioned are not expensive to apply
- Patentable natural enemies
  - Selected breeding or genetic modification
- Research needed to show improvements are possible and worthwhile

How can we show that domestication is bad and takes place?

- Start mass reared populations with identical genetic variation and mass rear for different numbers of generations test their performance in the field

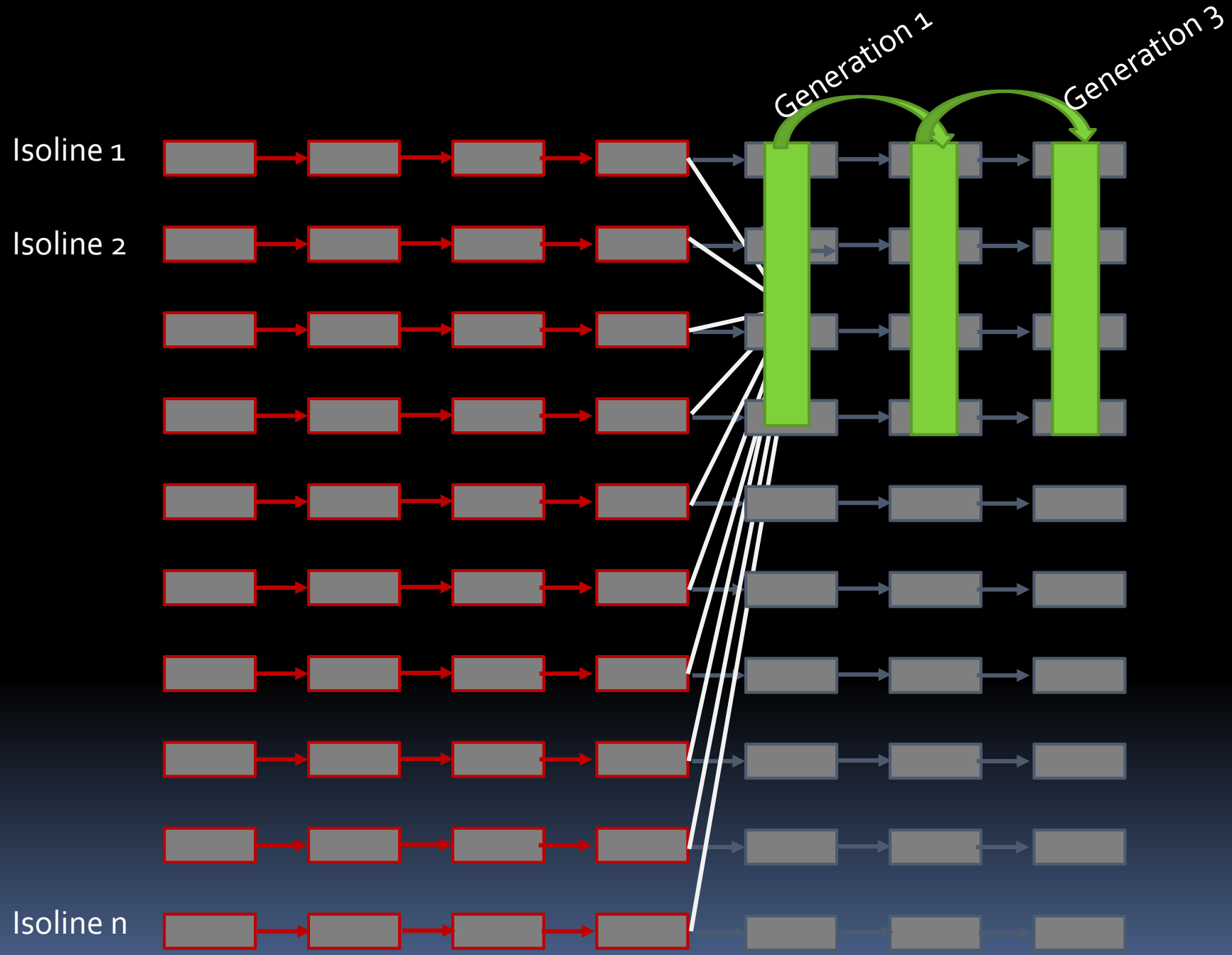
- How can this be tested?

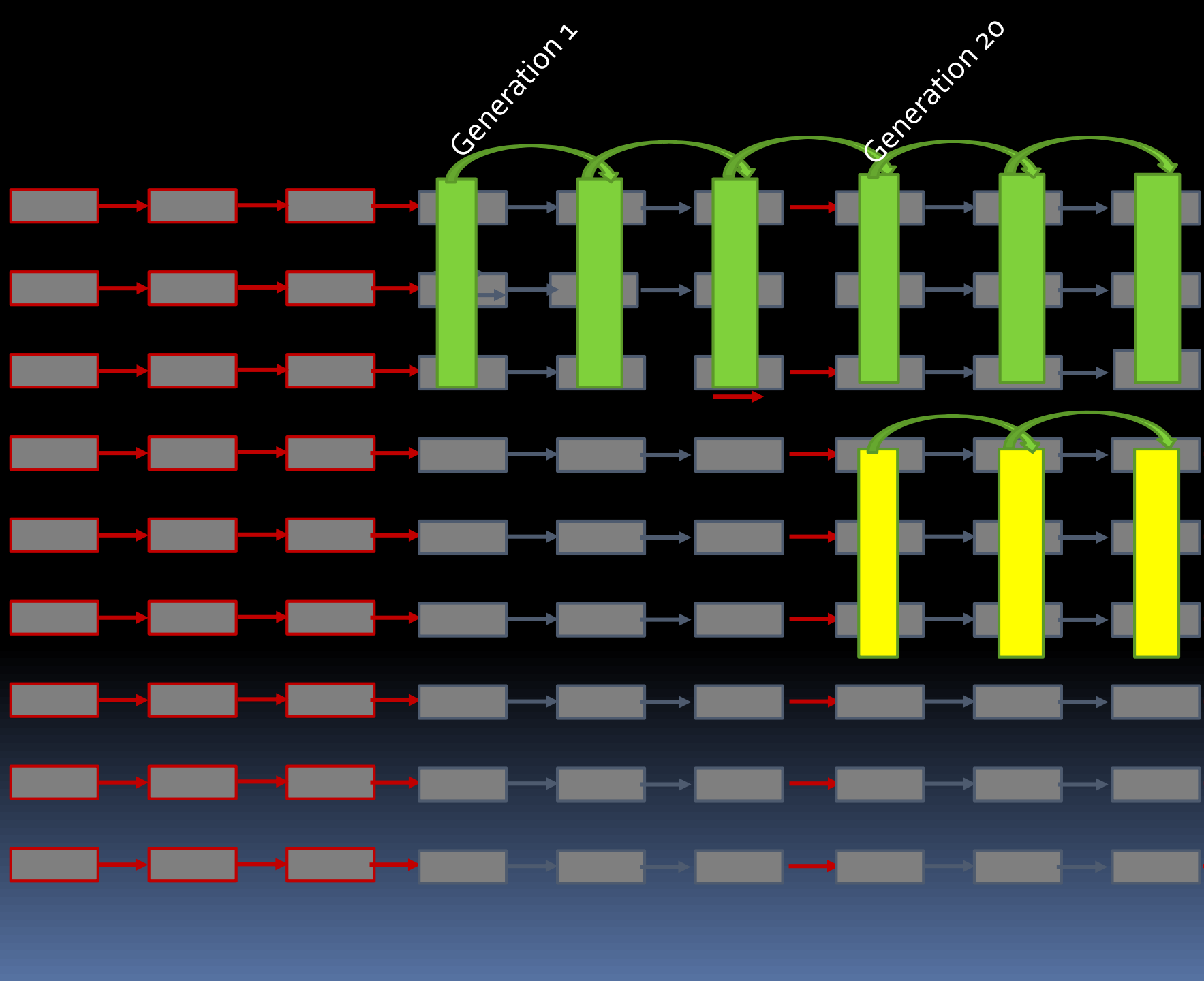
Methods to determine effects of prolonged rearing on biocontrol performance

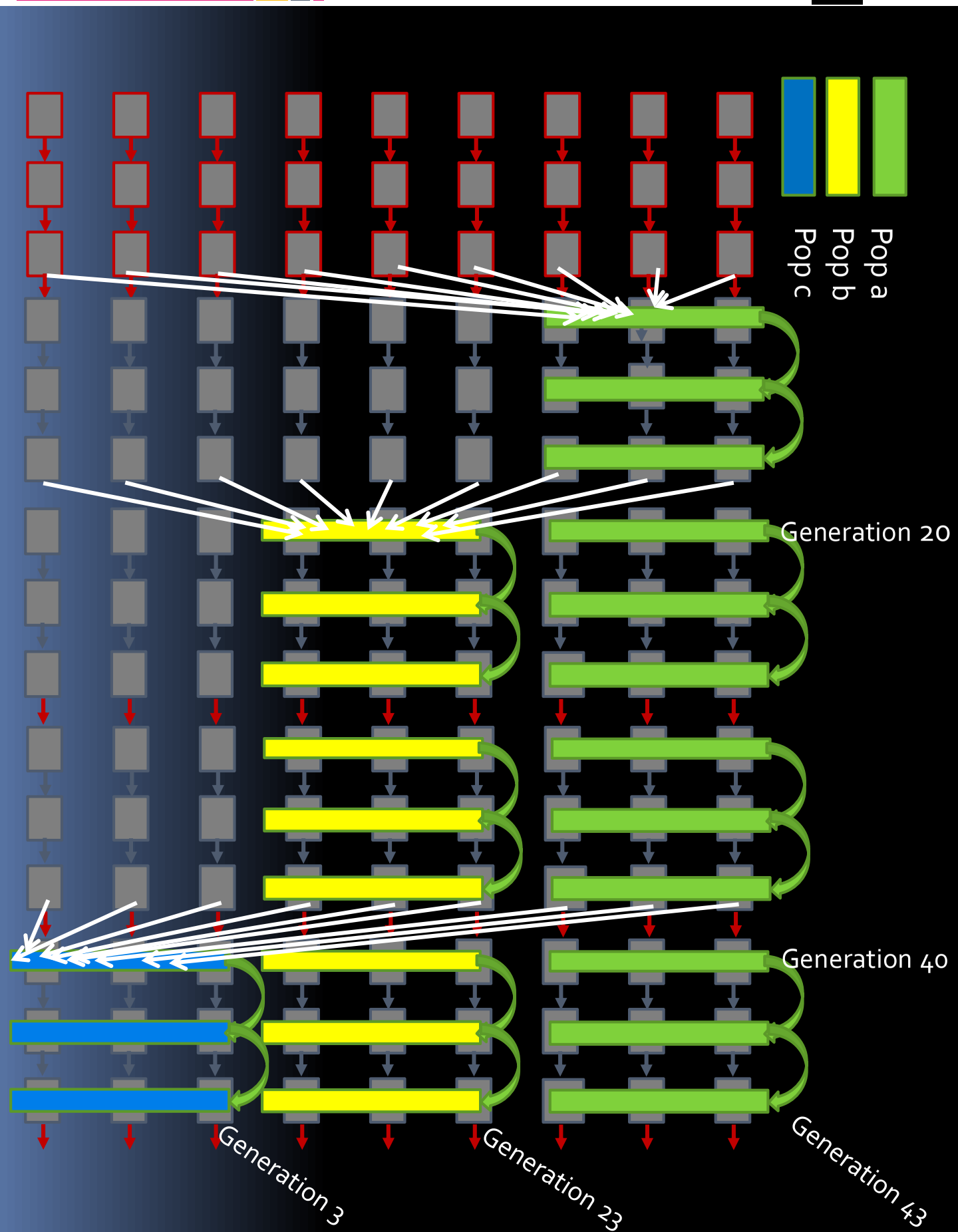
- Start with isofemale lines collected from the field
- Use is female lines to create replicate populations
- Expose these populations to different lengths of mass rearing
- Test field performance of these different populations
- Expectation is that population reared the shortest should perform the best in the field

How to create replicate populations?

- Keep isofemale lines
- At generation 1 (Pop A) mix 100 individuals of isoline 1-line 30 together and rear the resulting population in mass rearing continuously
- At generation 20 (Pop B) mix 100 individuals of isoline 1-isoline 30 together and rear the resulting population in mass rearing continuously
- At generation 40 (Pop C) mix 100 individuals of isoline 1-isoline 30 together and rear the resulting population in mass rearing continuously
- At generation 43 field test Pop A, versus Pop B versus Pop C


Generation 1
Generation 3
Isoline 1
Isoline 2
Isoline n


Generation 1
Generation 20


Pop a
Pop b
Pop c
Generation 20
Generation 40
Generation 3
Generation 23
Generation 43

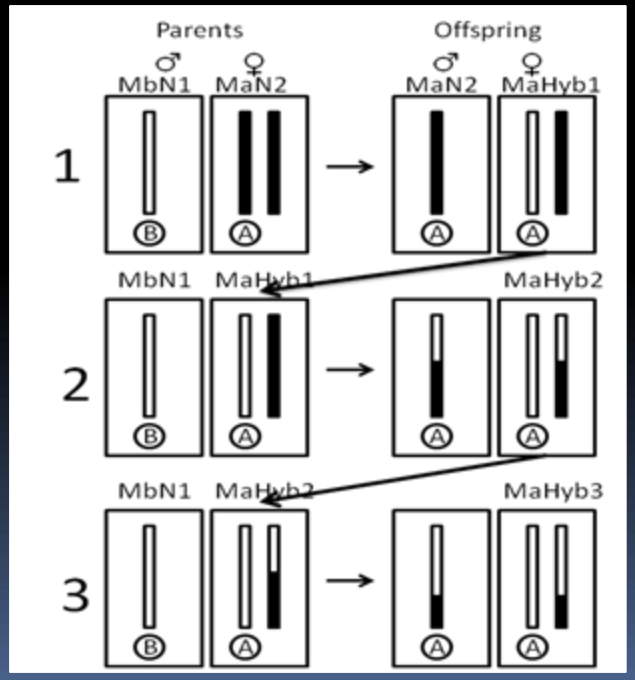
Test populations with different levels of domestication against each other

- Compare biocontrol performance against each other in different plots by determining the offspring produced by each population: by f.i. hanging up trapcards with hosts

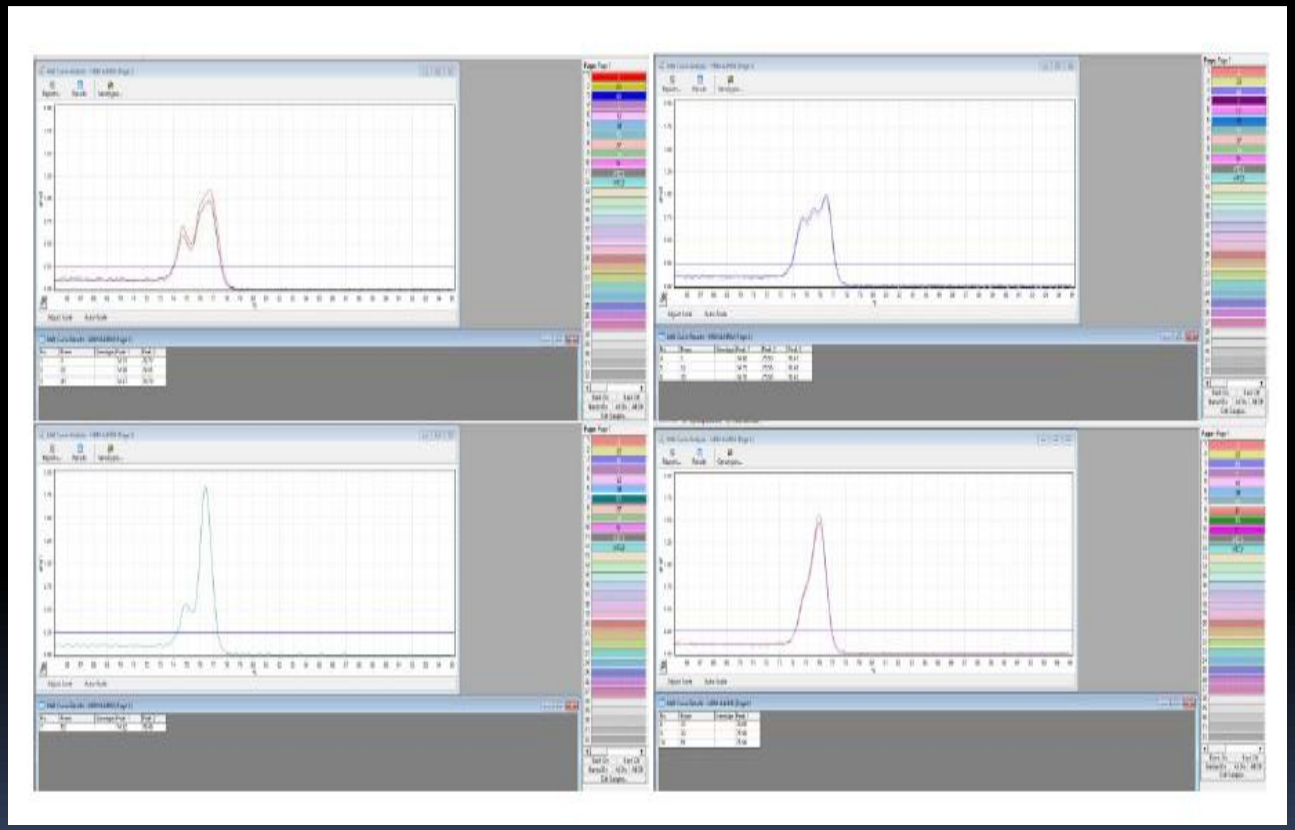
- Alternative: Test different populations against each other in same plot….. But how can we distinguish the performance of the different populations?

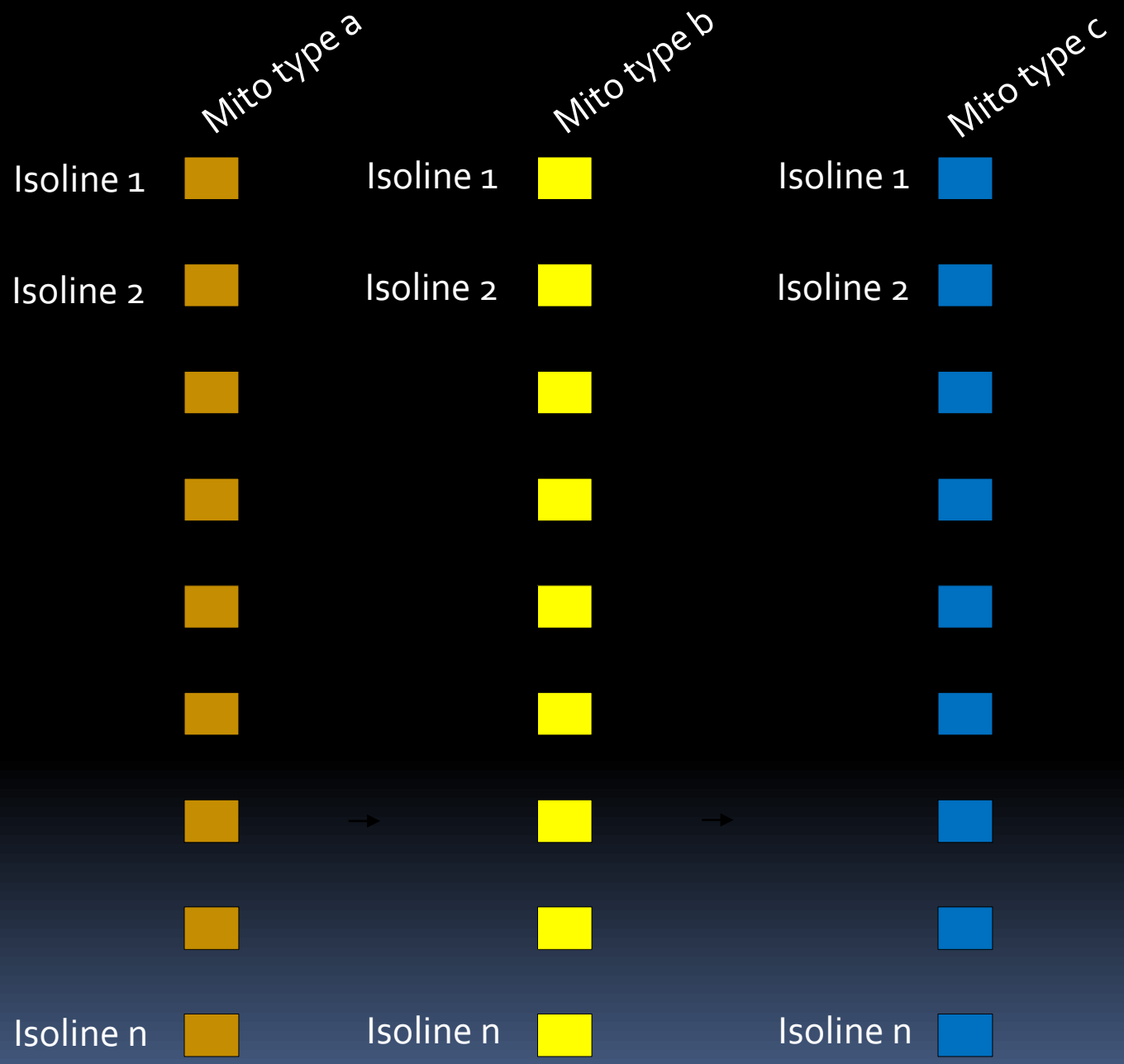
Use mitochondrial sequences to mark the lines and resulting populations

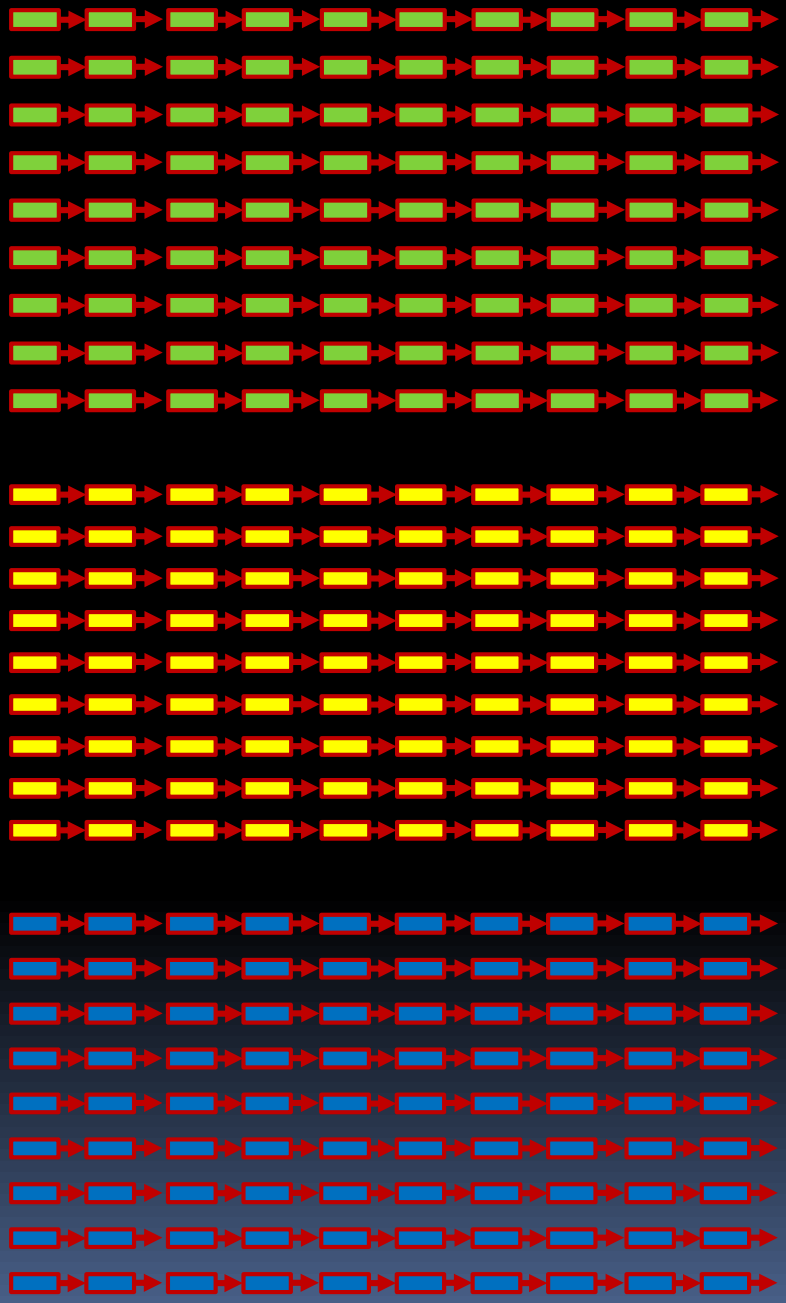
- Introgress each isofemale lines nuclear background into different mitochondrial backgrounds



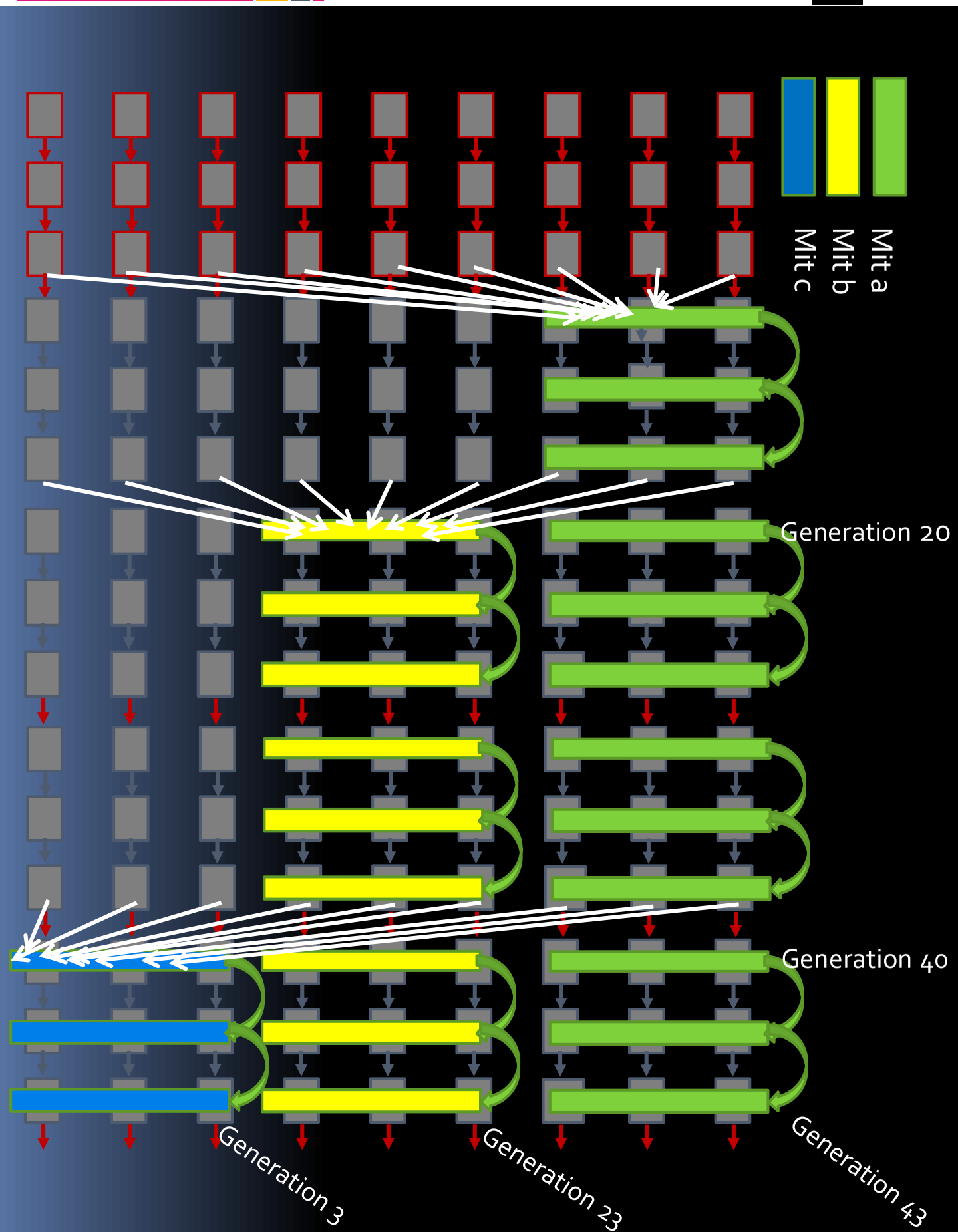
	Mit a	Mit b	Mit c
Nuc isoline 1	MaN1	MbN1	McN1
Nuc isoline 2	MaN2	MbN2	McN2
Nuc isoline 3	MaN3	MbN3	McN3
Nuc isoline n	MaNn	MbNn	McNn




Mito type a
Mito type b
Mito type c
Isoline 1
Isoline 2
Isoline n
Isoline 1
Isoline 2
Isoline n
Isoline 1
Isoline 2
Isoline n



Maintain all isolines for the durationof the experiments


Mit a
Mit b
Mit c
Generation 20
Generation 40
Generation 3
Generation 23
Generation 43

Mitochondrial markers can also be used for determining impact of augmentative releases into existing populations

- Problem what is the impact of releases of *Trichogramma platneri* against light brown apple moth in popualtions where native *T. platneri* is present?
- Sample the native population
- Establish isofemale lines from collected wasps
- Determine the sequence of mitochondrial COI

Are the released wasps doing anything?

- Determine which mitochondrial type is rare in the population
- Mass rear the relatively rare mitochondrial type and release these wasps
- Determine the frequency of rare mitochondrial type before and after releases using trap hosts
- Difference is the impact of the released wasps

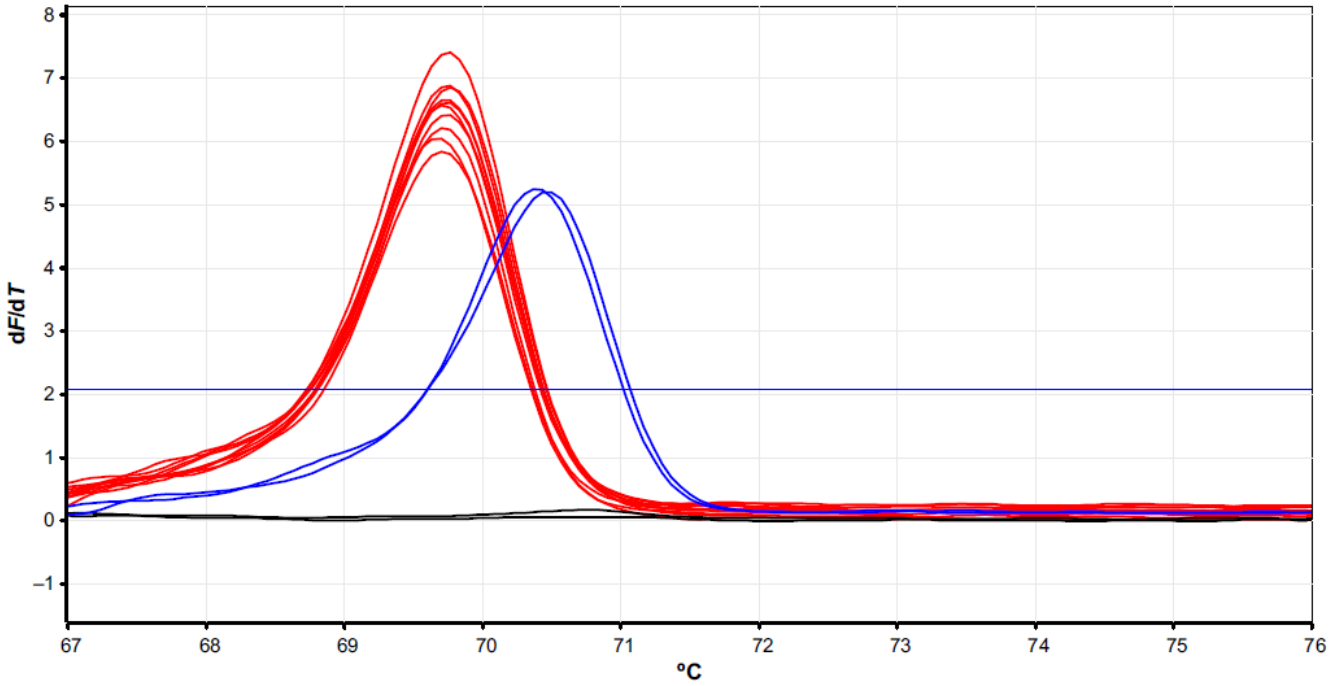
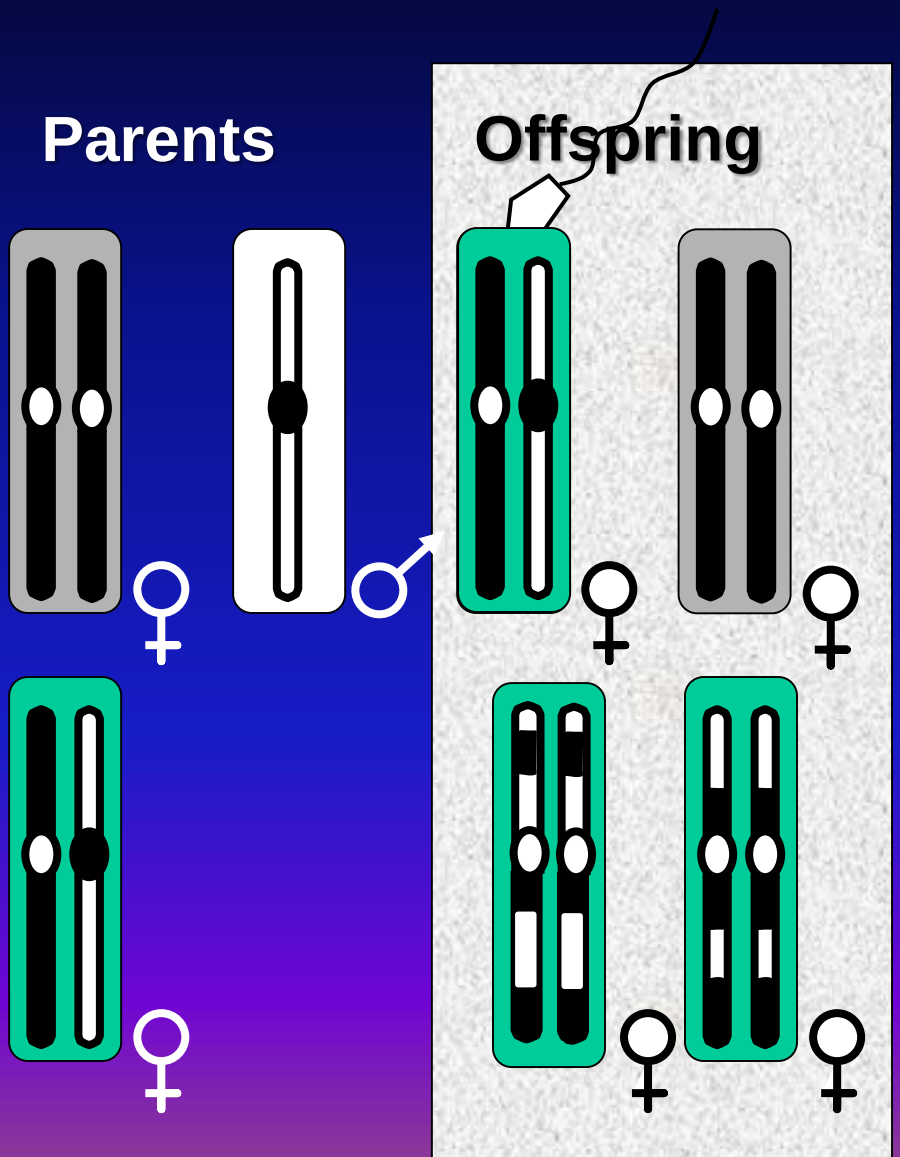


Fig. 2 Diagnostic melt curves of a native *Trichogramma platneri* haplotype (blue) mass-reared for augmentative biological control releases against LBAM, relative to five further native *T. platneri* haplotypes (red). Two individuals are shown per haplotype plus no-template controls [black]. HRM was performed on a Rotor-Gene RG-3000 qPCR instrument (QIAGEN), immediately after production of a 134-bp amplicon of the COI gene directly from the body of a whole wasp. Temperature is shown on the *x*-axis, and the *y*-axis represents change in fluorescence converted to a distinct melting peak by plotting the first negative derivative of the fluorescence as a function of temperature (-dF/dT).

Rugman-Jones PF, Stouthamer R. 2017. High-resolution melt analysis without DNA extraction affords rapid genotype resolution and species identification Molecular ecology resources 17 (4), 598-607

Selection of the best biocontrol line of unisexual *Trichogramma pretiosum*

- Unisexuality in *Trichogramma* is caused by infection with Wolbachia
- Cytogenetic mechanism that allows females to produce daughters without mating is gamete duplication
- Simply haploid unfertilized eggs become diploid by a fusion of two identical mitotic copies of the genome
- Result diploid individuals that are completely homozygous



Wolbachia infected females sometimes will use sperm of males

Recombinant offspring of virgin hybrid Wolbachia infected females. Use to start Recombinant Isofemale Line (RIL)

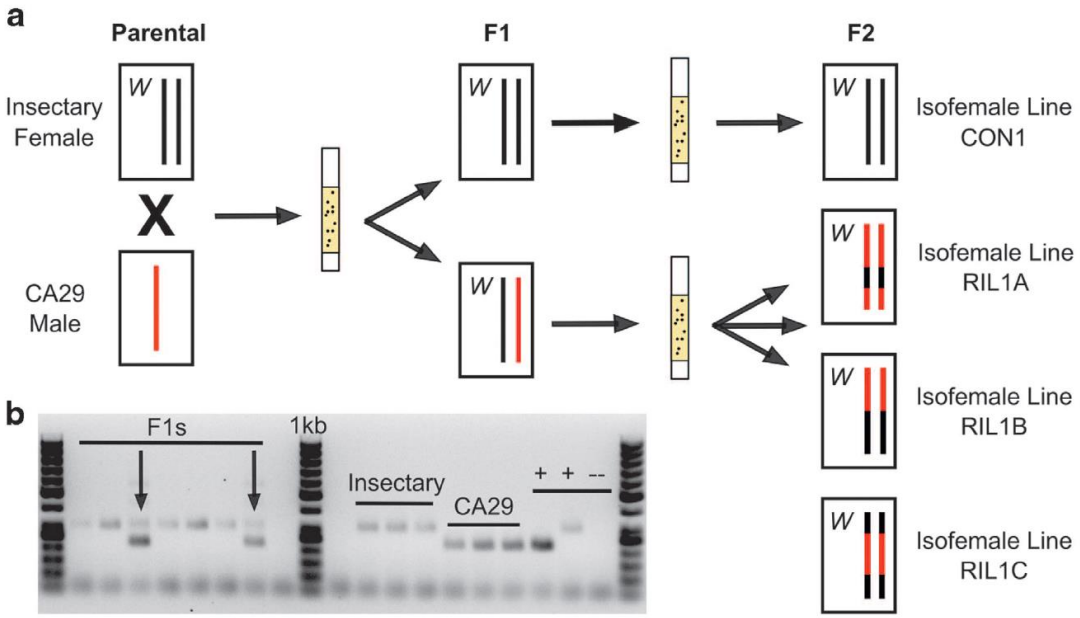


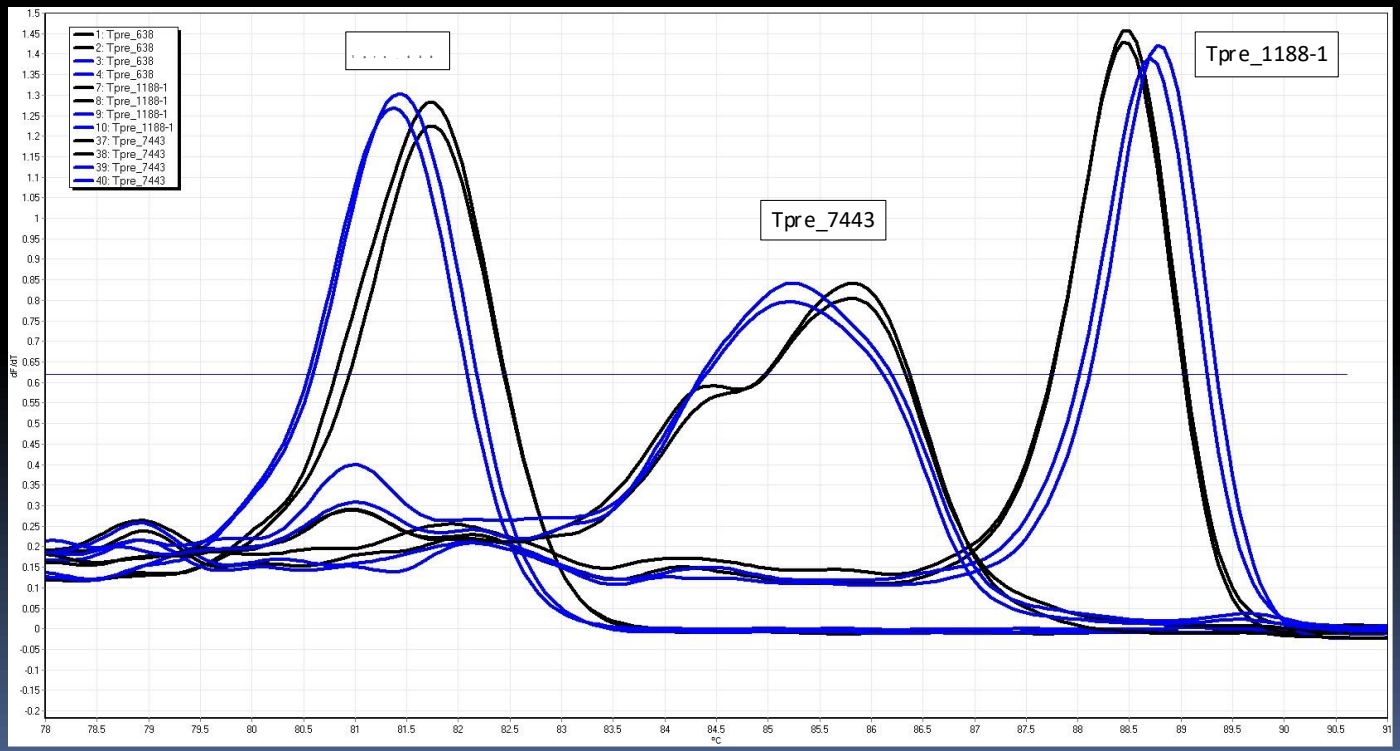
Figure 1 Experimental design for creating recombinant and control lines. (**a**) Crossing scheme. '*W*' indicates infection with *Wolbachia*, inherited maternally. Yellow bars with black dots represent parasitized egg cards. F1 offspring were screened for zygosity while F2s develop. (**b**) Microsatellite PCR assay for determining zygosity. Arrows on the gel image point to heterozygote F1s, from which individual offspring were isolated to initiate Recombinant Isofemale Lines (RILs). Positive controls are previously amplified samples from the Insectary and CA-29 lines, where as the Insectary and CA-29 labels indicate exact parents used in crosses.

Lindsey & Stouthamer , 2017, Heredity

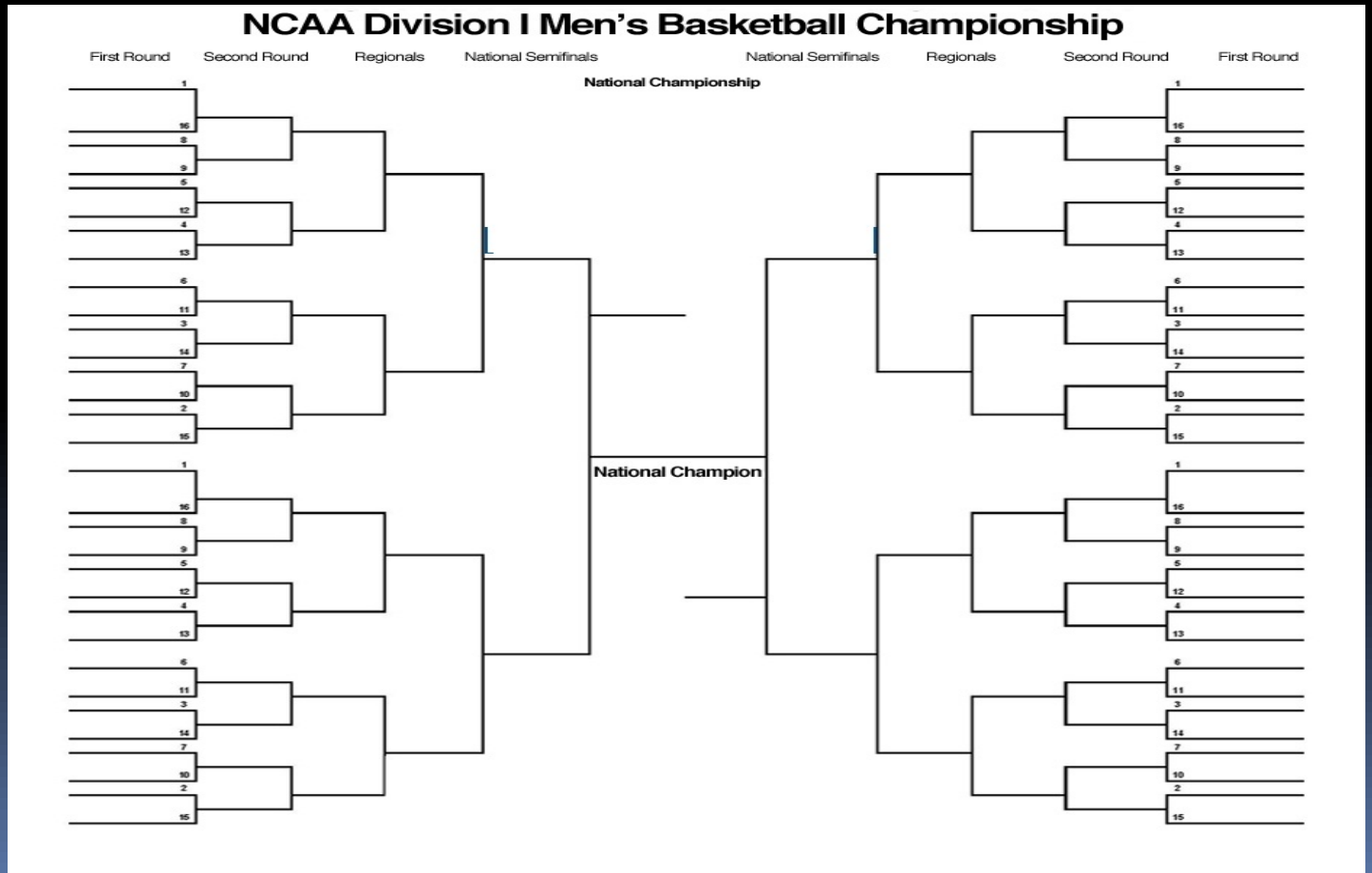
Each recombinant isofemale line is: 1. completely homozygous and 2. its genome consists of a unique combination of the genome of the parental lines in this case the "insectary" line and CA-29 line

Both genomes have been sequenced and were used to chose DNA stretches where both lines differ from each other and we can develop a high resolution melt curve

- We are using 5 nuclear markers to distinguish the lines, here three are shown



Which RIL is most suitable for biocontrol? Compete each line against another line and find the winner (worldcup for *Trichogramma* lines)



- Number of RI lines that can be distinguished using n loci with 2 alleles per locus: N= (number of alleles per locus)$^{(\text{number of loci})}$
- $N= 2^3 = 8$
- We aim to have 5 loci that can be used and will test 20 lines against each other

Line	Locus		
	A	B	C
RIL 1	1	1	1
RIL 2	1	1	0
RIL 3	1	0	1
RIL 4	0	1	1
RIL 5	0	0	1
RIL 6	0	1	0
RIL 7	1	0	0
RIL 8	0	0	0

Any two lines in this set can be distinguished from each other using one locus

- Release equal numbers of females of two lines together in an environment and determine which line produces the most offspring

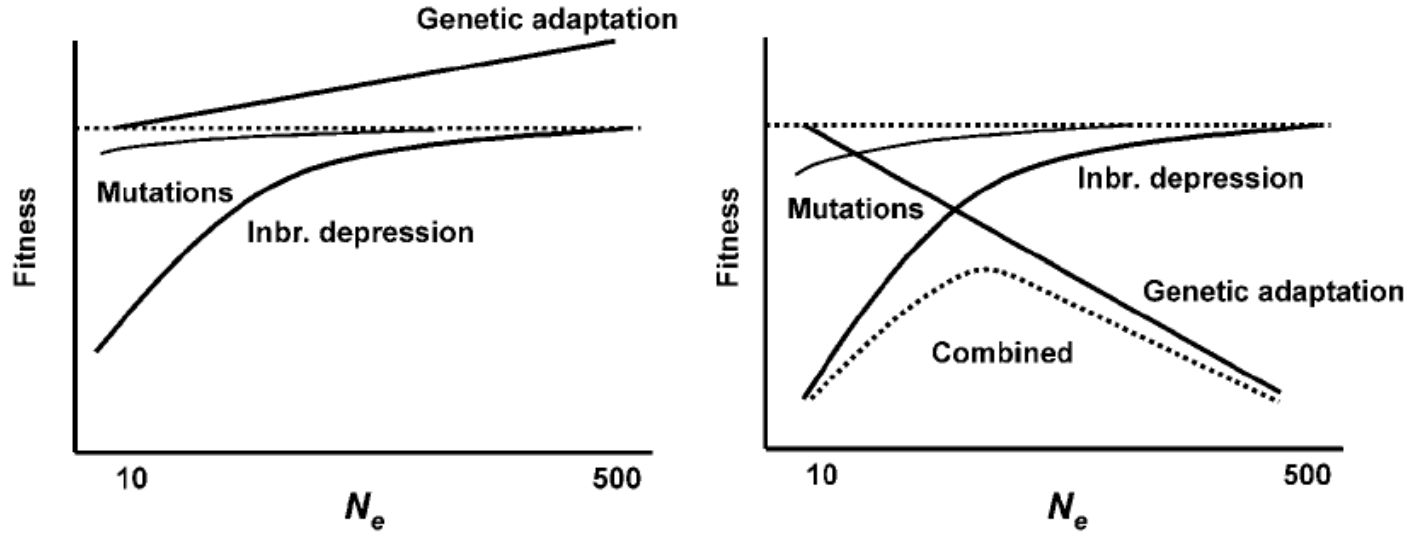
- If I compete RIL 1 against RIL 2 the only locus I need to test is locus C, RIl1 against Ril 6 either locus A or locus C

- Offspring of each line can be distinguished from each other by testing a single locus

Line	Locus		
	A	B	C
RIL 1	1	1	1
RIL 2	1	1	0
RIL 3	1	0	1
RIL 4	0	1	1
RIL 5	0	0	1
RIL 6	0	1	0
RIL 7	1	0	0
RIL 8	0	0	0

Summary

- Mass reared natural enemies used in biological control can be improved vastly by paying attention to population genetics theory, and needed changes are not expensive



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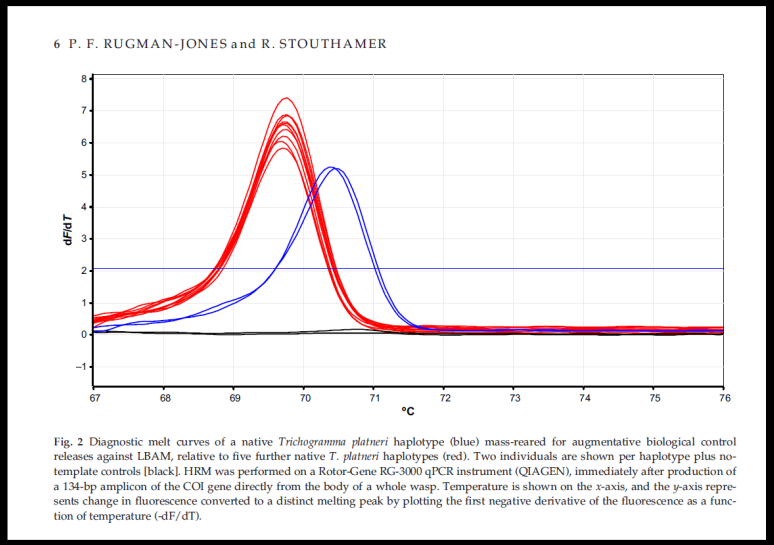
Summary 2

- Next gen sequencing allows for the discovery and use of many markers that can be used to distinguish populations that have undergone different treatments to determine the importance of such treatments .
  - Time in rearing
  - Do the released natural enemies do anything
  - Which of these lines perform best in the field
- HRM is a great method for rapidly typing many different individuals in populations experiments

Acknowledgements

- Paul Rugman-Jones
- Amelia Lindsey
- Jack Werren
- Len Nunney



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