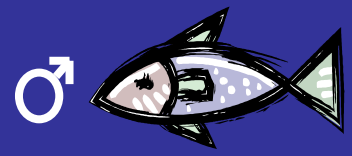
Trojan Y Chromosome Eradication of Invasive Fish: Sex-specific DNA Markers for Tilapia

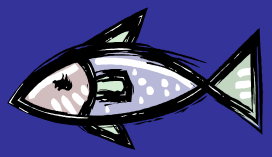
John Teem, Florida Department of Agriculture and Consumer Services
Division of Aquaculture

XY Sex-Determination



♂

XY



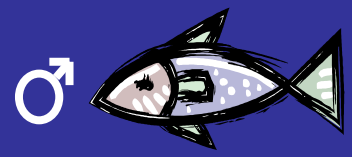
♀

XX

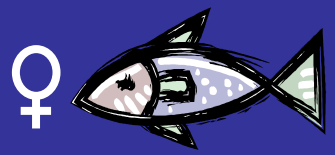
	X	Y
X	XX	XY
X	XX	XY

Males/Females
Ratio 1:1

Females with Two Y chromosomes Produce Only Male Progeny, Half of Which are Myy

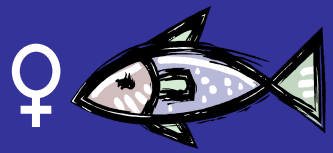


Mxy



Fxx

	X	Y
X	XX	XY
X	XX	XY



Fyy

	X	Y
Y	XY	YY
Y	XY	YY

Myy males are viable and produce only male offspring

♀ Fxx

♂ Mxy

	X	Y
X	XX	XY
X	XX	XY

♀ Fyy

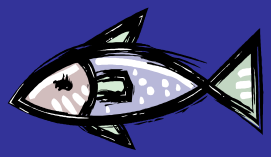
	X	Y
Y	XY	YY
Y	XY	YY

♂ Myy

	Y	Y
X	XY	XY
X	XY	XY

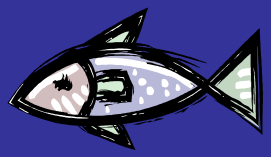
Males/Females Ratio 1:0

Four different matings are possible, leading to increased male production

♂ 

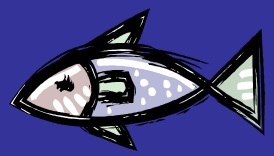
Mxy

	X	Y
X	XX	XY
X	XX	XY

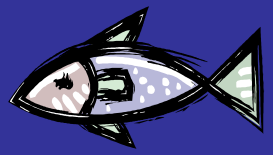
♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

♀ 

Fxx

♀ 

Fyy

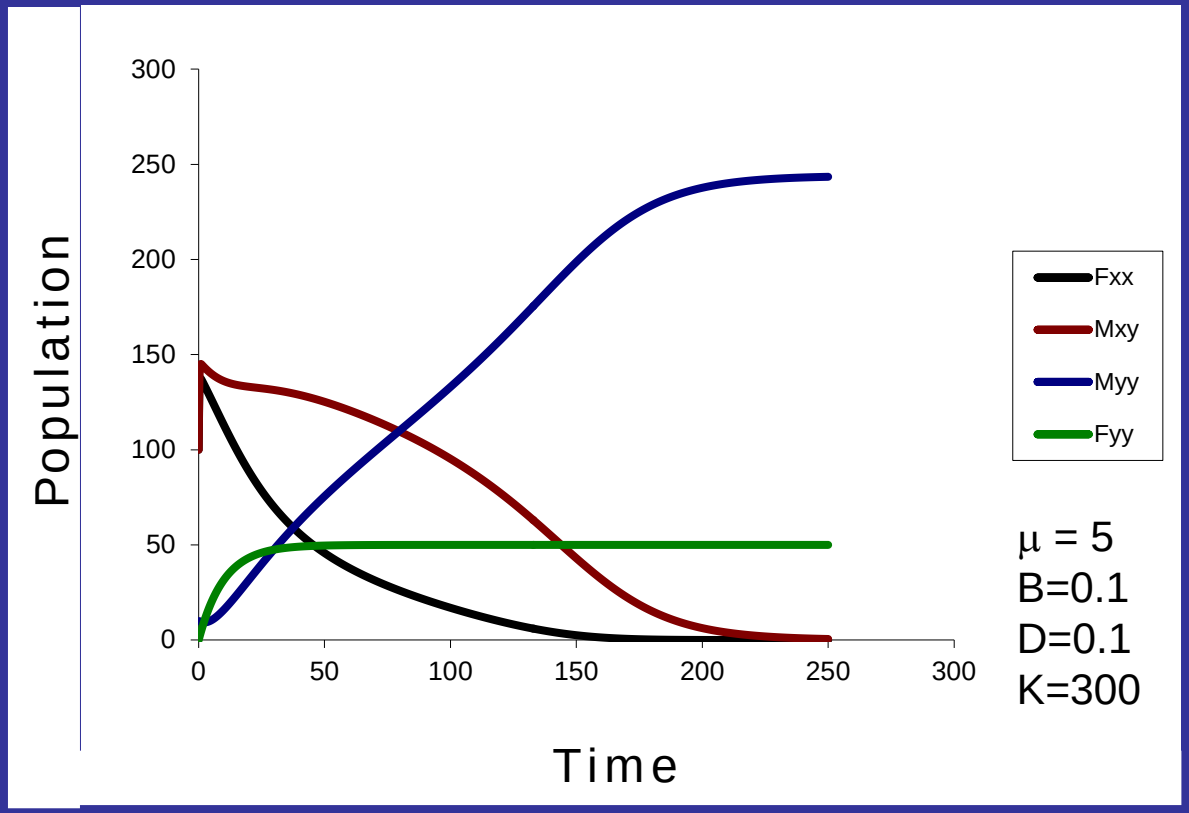
	X	Y
Y	XY	YY
Y	XY	YY

	Y	Y
Y	YY	YY
Y	YY	YY

Males/Females Ratio 7:1

Male/Female ratio will increase over time if Fyy added.

The addition of a Trojan Y female (Fyy) to a target population will cause females (Fxx) to go to extinction over time.



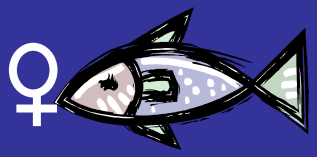
The carrying capacity of the system becomes occupied by Myy fish (males with two Y chromosomes).

The production of YY fish requires selective breeding and the use of hormone-induced sex reversal techniques.

YY genotypes are verified by test crosses and evaluation of the sex distribution in progeny.

Sex-specific DNA markers can greatly reduce the time required to generate YY fish by allowing YY genotypes to be detected by DNA analysis (instead of test crosses).

For some fish, sex-specific DNA markers have been Identified by using the RAPD PCR method.



Fyy

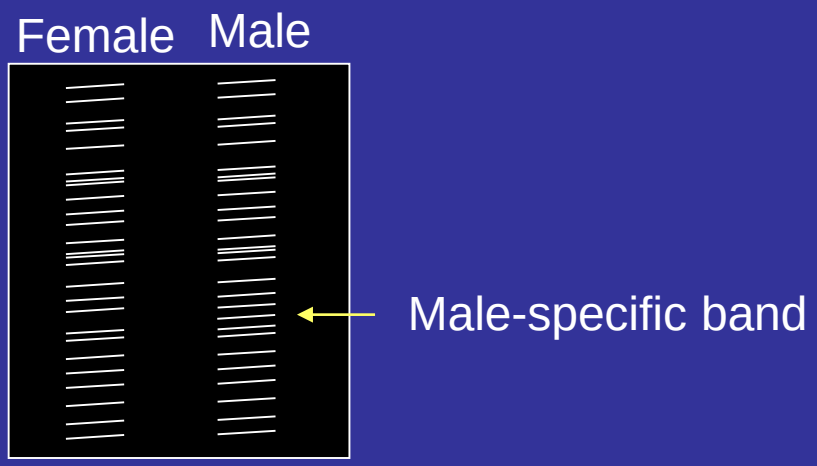
RAPD PCR

Create a DNA pool from only females and another from only males.

Test each pool with PCR using a collection of short DNA primers that will amplify sequences at different locations in the genome.

For each primer, compare female-specific DNA amplified products with male-specific amplified products using gel electrophoresis.

Find a primer that gives a band in one DNA pool, but not the other.



Getting Started on RAPD PCR for Nile Tialpia

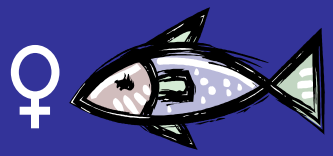
Collaborators at Auburn University will provide male-specific and female-specific DNA for Nile tilapia.

Screening for sex-specific PCR bands will begin shortly.

How Does the Time to Extinction with the Trojan Y Chromosome (TYC) Strategy Compare With Daughterless Carp (DC)?

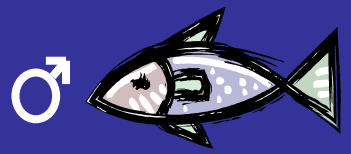
How Does the Time to Extinction with the TYC Strategy Compare with Daughterless Carp?

Using Differential Equations to Model Daughterless with 8 Gene Copies

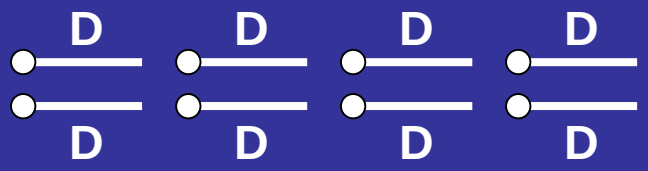


♀ Fxx

x



♂ Mxy, D8



↓

Mxy, D4
Mxx, D4

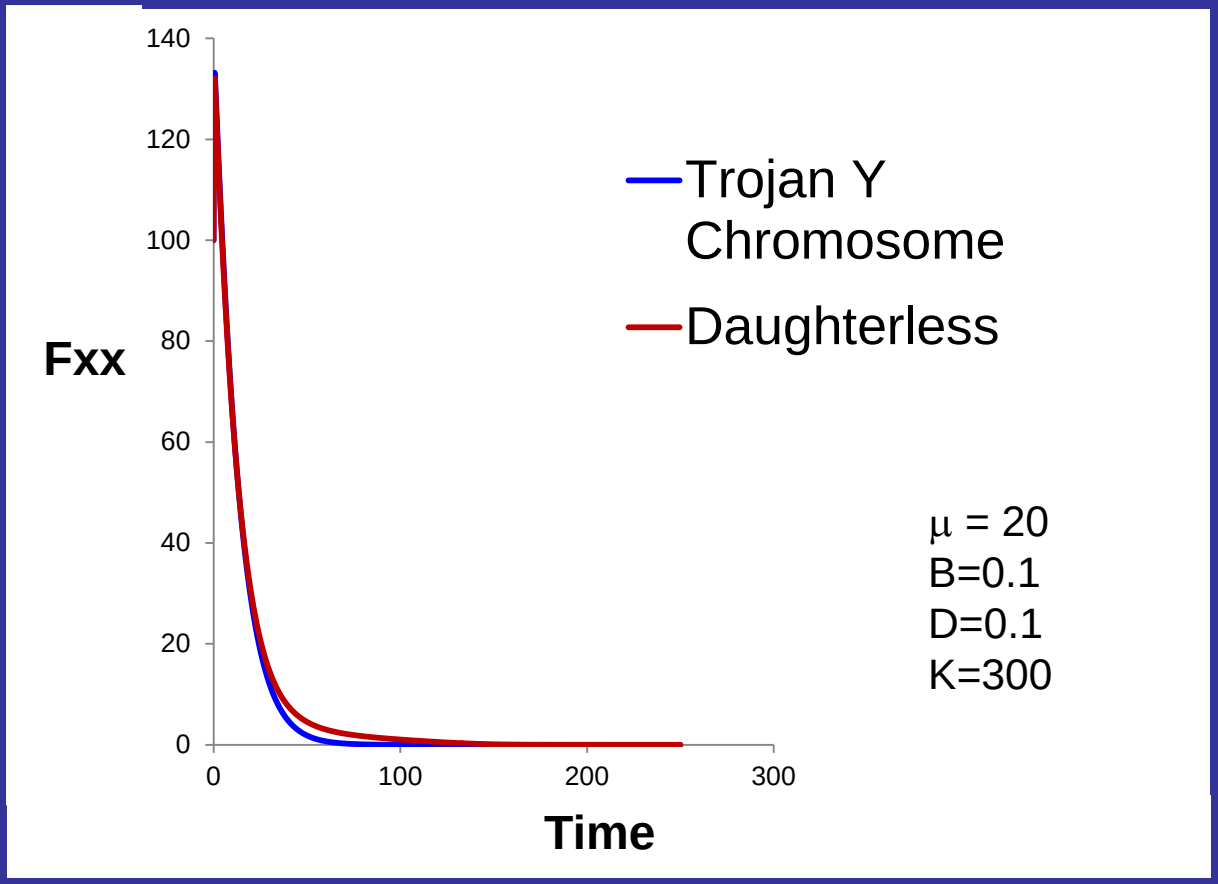
↓

Mxy, D4, Mxy, D3, Mxy, D2, Mxy D1, Mxy
Mxx, D4, Mxx, D3, Mxx, D2, Mxx D1, Fxx

There are 10 types of mating pairs that can occur in the population, and the genotypes of progeny from each mating can be determined using a binomial distribution.

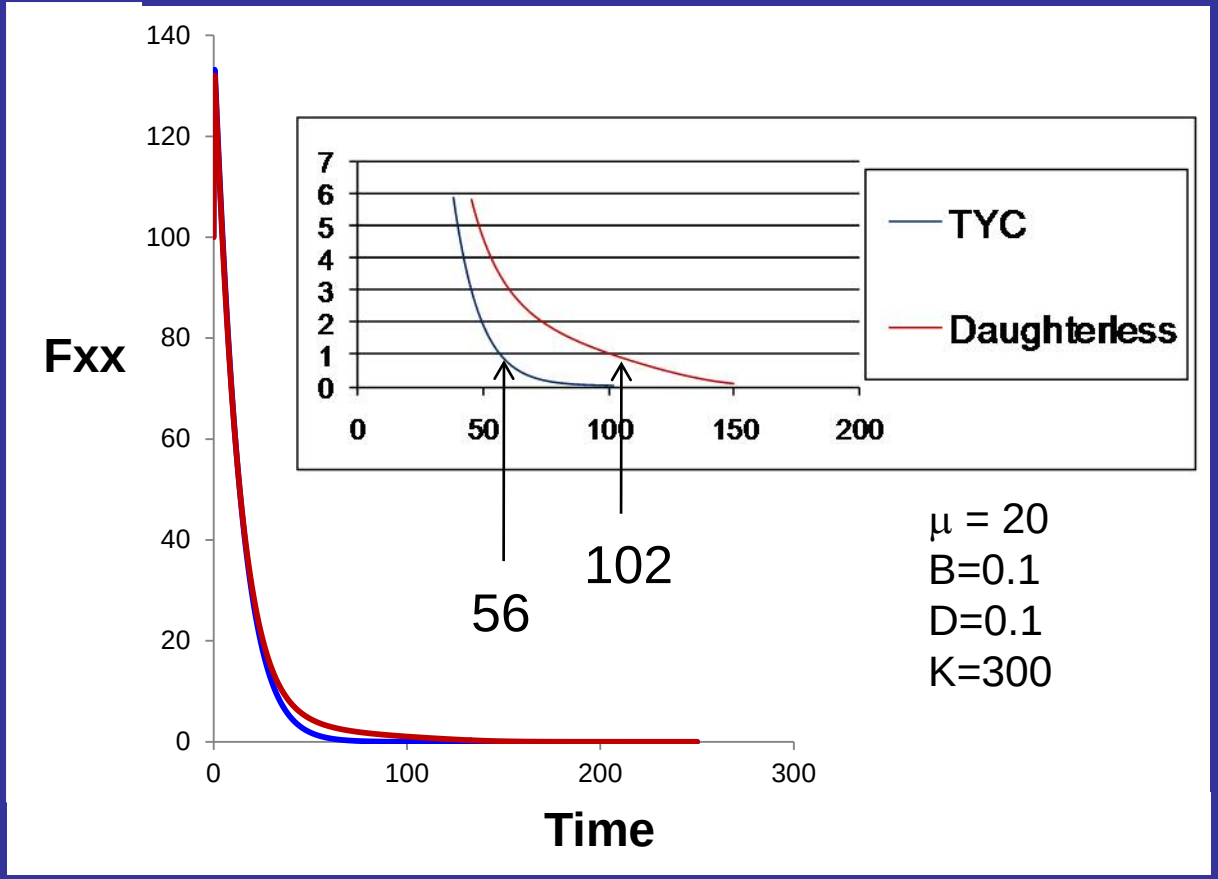
Each of the 11 fish in the population can be represented by an ordinary differential equation.

Comparison of the TYC strategy to Daughterless



Under identical conditions, the initial rate of decline of females is similar.

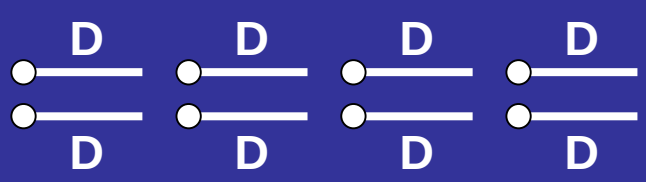
Comparison of the TYC strategy to Daughterless

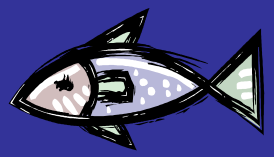
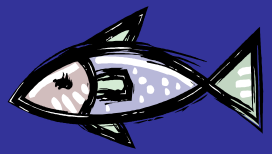


Under identical conditions, the initial rate of decline of females is similar.

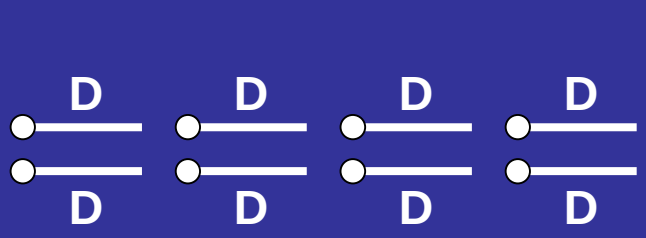
However, the time to extinction of females is less for TYC (56) as compared to Daughterless (102).

Can the TYC Strategy be Combined Together With the Daughterless Strategy to Cause Extinction to Occur Faster?



♀  Fyy, D8 x ♂  Mxy

Modeling TYC/Daughterless with 8 Gene Copies



Fyy, D8 x Mxy

↓

Mxy, D4
Myy, D4

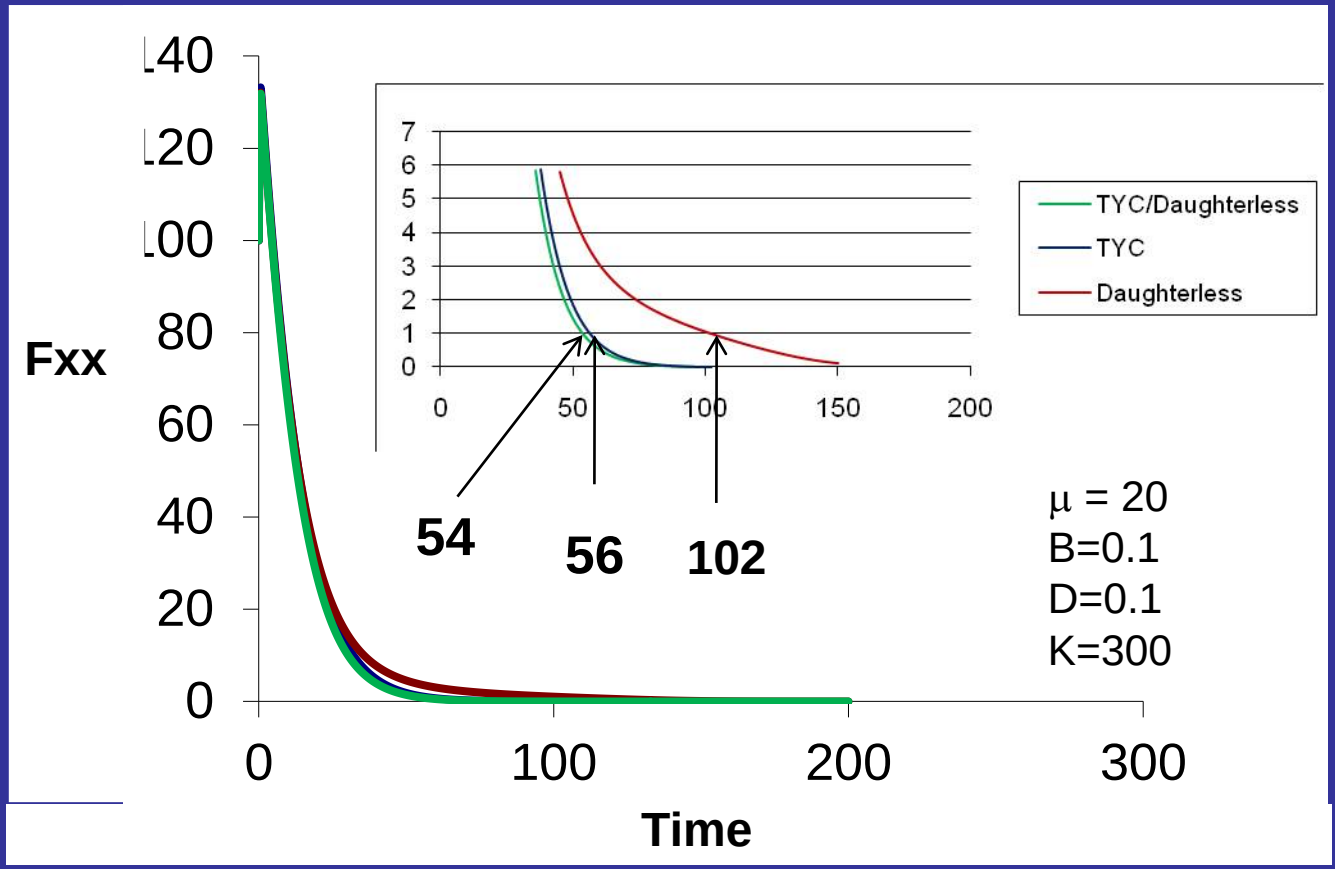
↓

Mxy, D8, Mxy, D7, Mxy, D6, Mxy D5, Mxy, D4, Mxy, D3, Mxy, D2, Mxy, D1, Mxy, Mxx, D4, Mxx, D3, Mxx, D2, Mxx, D1, Myy, D8, Myy, D7, Myy, D6, Myy, D5, Myy, D4, Myy, Fxx

There are 36 different mating pairs that can occur in the population, and the genotypes of progeny from each mating can be determined using a binomial distribution.

Each of the 20 fish genotypes in the population can be represented by an ordinary differential equation.

Extinction Occurs Slightly Faster with a Combined TYC/Daughterless Strategy



The combined TYC/Daughterless strategy also reduces the number of fish required to achieve extinction.

TYC Species Requirements

1. The target fish must have a XY sex-determination system

2. The target fish must be amenable to hormone-induced sex reversal

3. A female fish with two Y chromosomes (Fyy) must be viable and mate at the same efficiency as wildtype

4. The target fish must be amenable to propagation via aquaculture

Why a Trojan Y Chromosome strategy might be an appropriate technique for controlling invasive species

- Species specific
- Requires no new technology development
- Involves standard aquaculture techniques, no recombinant DNA
- Trojan Y chromosome fish have been already been produced in one species (*Oreochromis niloticus*)
- Reversible

Risks

- The invasive species is maintained at the carrying capacity of the system until females are eliminated. The harmful affects of the invasive species thus persist until females are eliminated.

- If insufficient numbers of Trojan Y Chromosome fish are added to the system, extinction will not occur.

- The strategy must be employed for decades to reach extinction.

What is the timeline for the TYC strategy to be used for control or elimination of a non-native species in the wild?

An test of the system on Nile tilapia (*Oreochromis niloticus*) could be done at the present time if existing YY broodstock were made available.

The identification of sex-specific DNA markers For Nile tilapia will allow YY will greatly facilitate the development of YY broodstock.