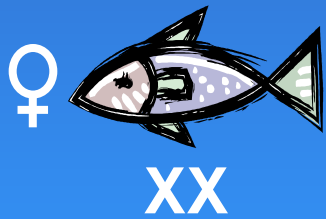
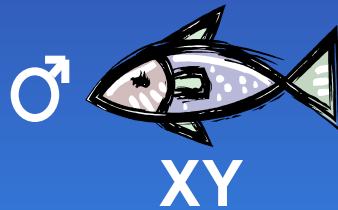


# **A Trojan Y Chromosome Strategy for the Eradication of Invasive Fish**

**John Teem, Division of Aquaculture, Florida Department of Agriculture  
Juan B. Gutierrez, Dept. of Mathematics, University of Miami**

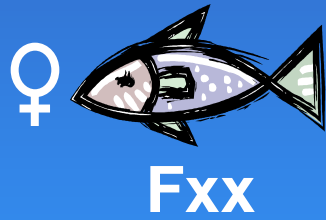
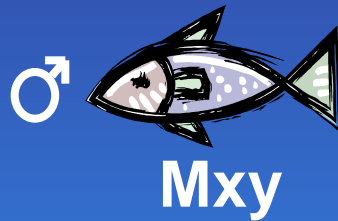
# XY Sex-Determination



	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

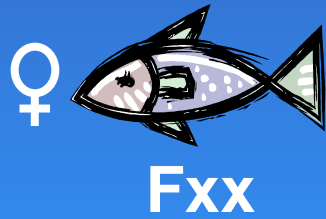
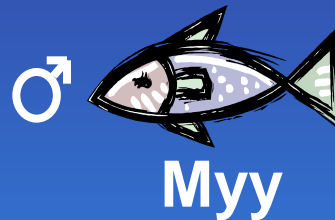
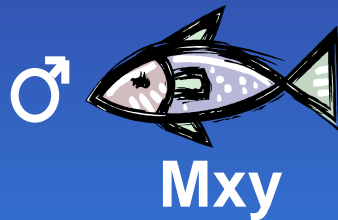


	X	Y
X	XX	XY
X	XX	XY



	X	Y
Y	XY	YY
Y	XY	YY

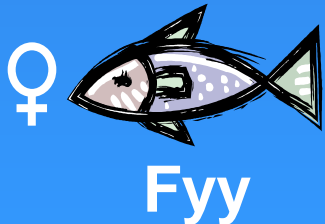
# Myy Males are Viable and Produce Only Male Offspring



	X	Y
X	XX	XY
X	XX	XY

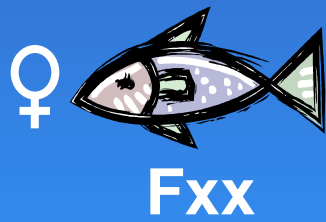
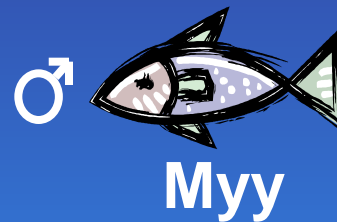
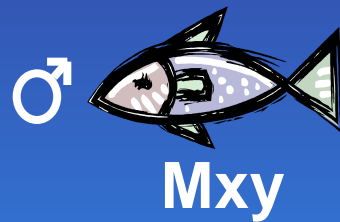
	Y	Y
X	XY	XY
X	XY	XY

Males/Females  
Ratio 1:0



	X	Y
Y	XY	XY
Y	XY	YY

# Four Different Matings are Possible, Leading to Increased Male Production

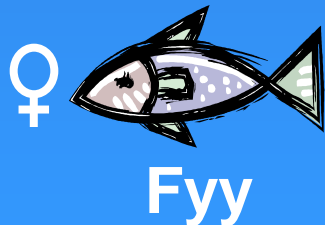


	X	Y
X	XX	XY
X	XX	XY

	Y	Y
X	XY	XY
X	XY	XY

Males/Females  
Ratio 7:1

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

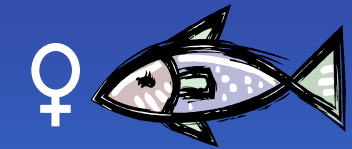


	X	Y
Y	XY	YY
Y	XY	YY

	Y	Y
Y	YY	YY
Y	YY	YY

Fxx will go extinction.

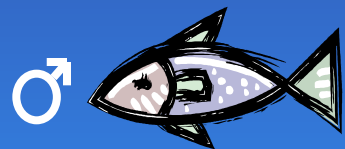
# Modeling the Trojan Y Chromosome System Using Differential Equations



♀ **100**  $F_{xx}$



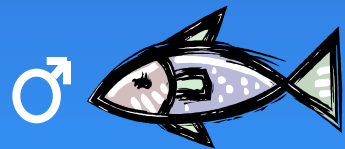
$$\frac{d}{dt} F_{xx} = 0.5BF_{xx}M_{xy}L - DF_{xx}$$



♂ **100**  $M_{xy}$



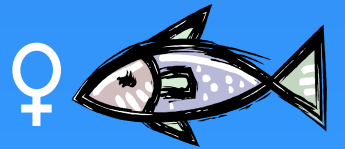
$$\frac{d}{dt} M_{xy} = (0.5BF_{xx}M_{xy} + 0.5BF_{yy}M_{xy} + BF_{xx}M_{yy})L - DM_{xy}$$



♂ **0**  $M_{yy}$



$$\frac{d}{dt} M_{yy} = (0.5BF_{yy}M_{xy} + BF_{yy}M_{yy})L - DM_{yy}$$



♀ **10**  $F_{yy}$



$$\frac{d}{dt} F_{yy} = \mu - DF_{yy}$$

$$L = \left( 1 - \frac{F_{xx} + F_{yy} + M_{xy} + M_{yy}}{K} \right)$$

**B** = Rate of births. Is proportional to the fecundity.

**D** = Rate of deaths. Takes into account the life span.

**K** = Carrying capacity of the ecosystem.

**$\mu$**  = **Fyy Trojan fish added**

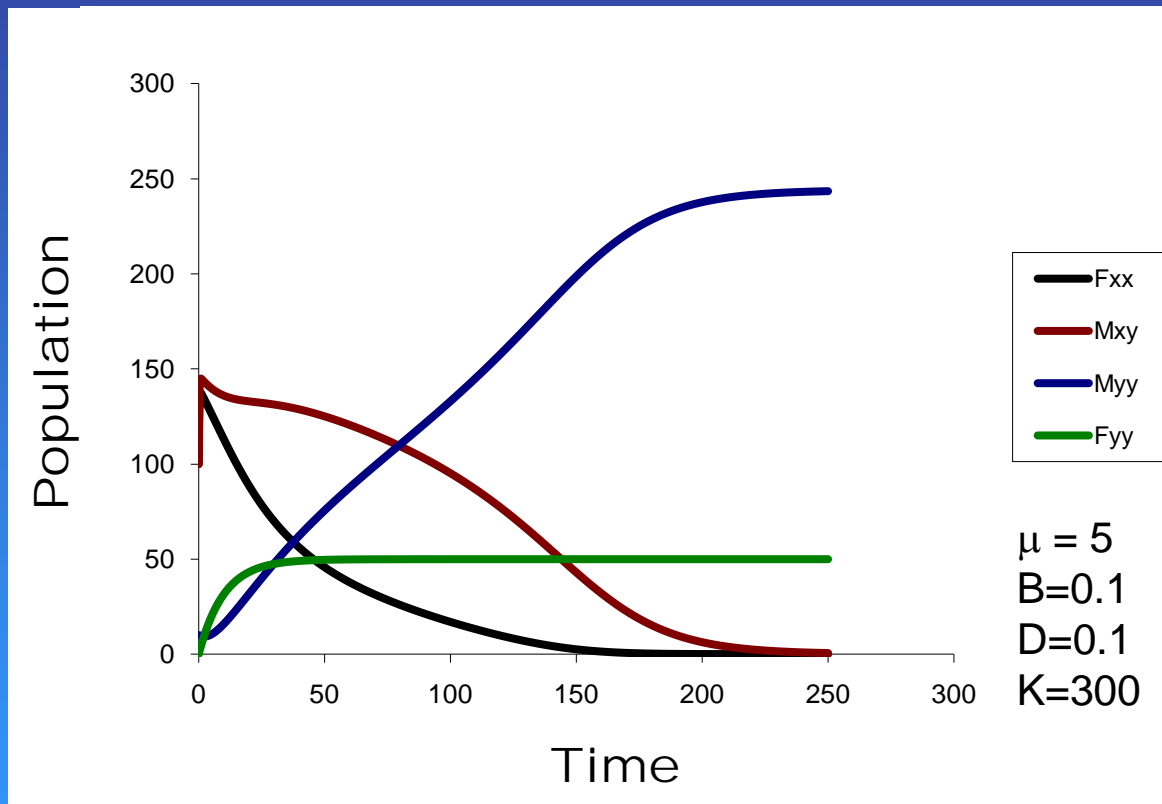
**$B=[0.01, 10]$**

**$D=[0.1, 3]$**

**$K= 300$**

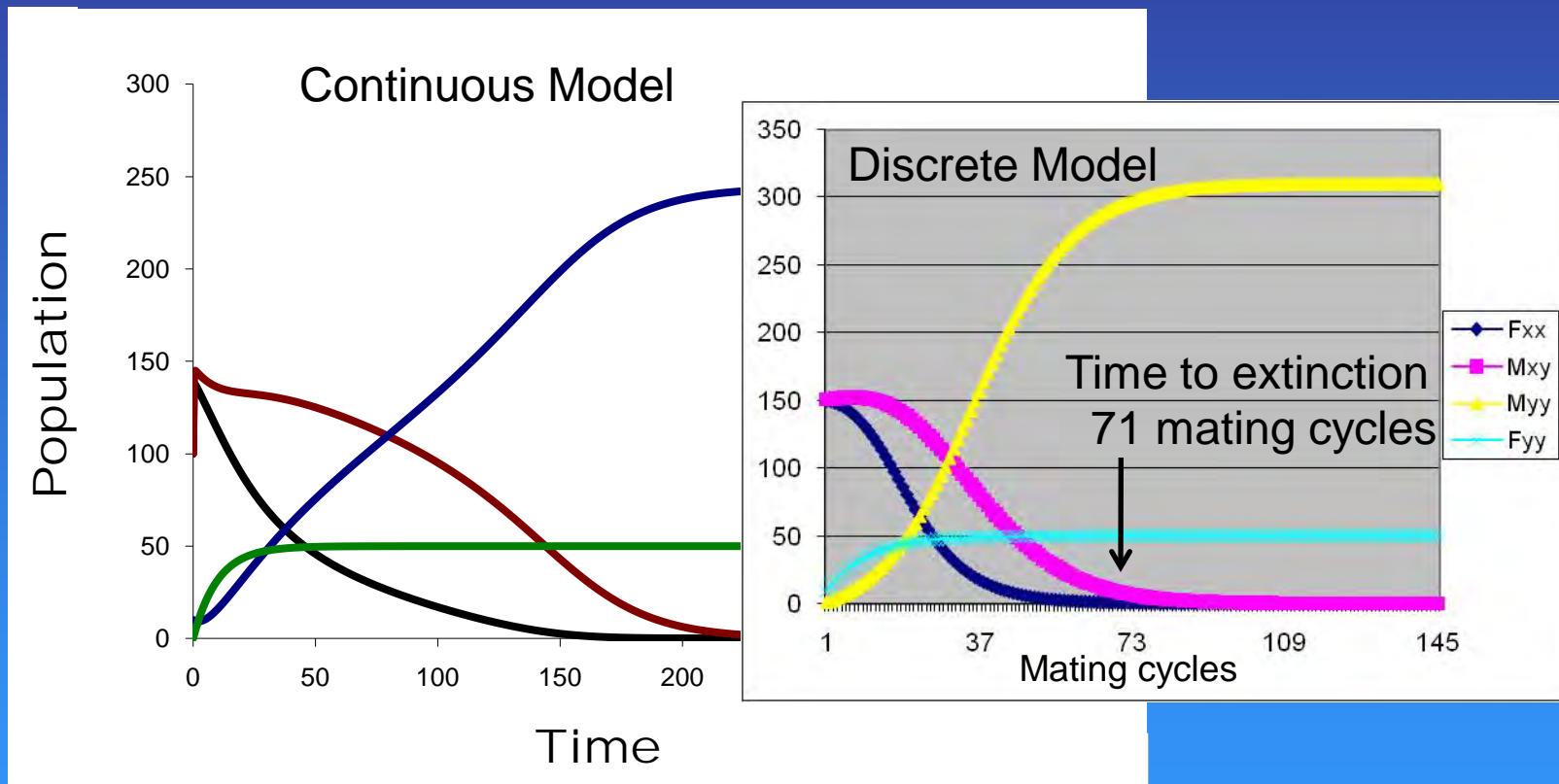
**$\mu= [0, 100]$**

**As the constant influx of Fyy ( $\mu$ ) increases,  
normal females (Fxx) decline**



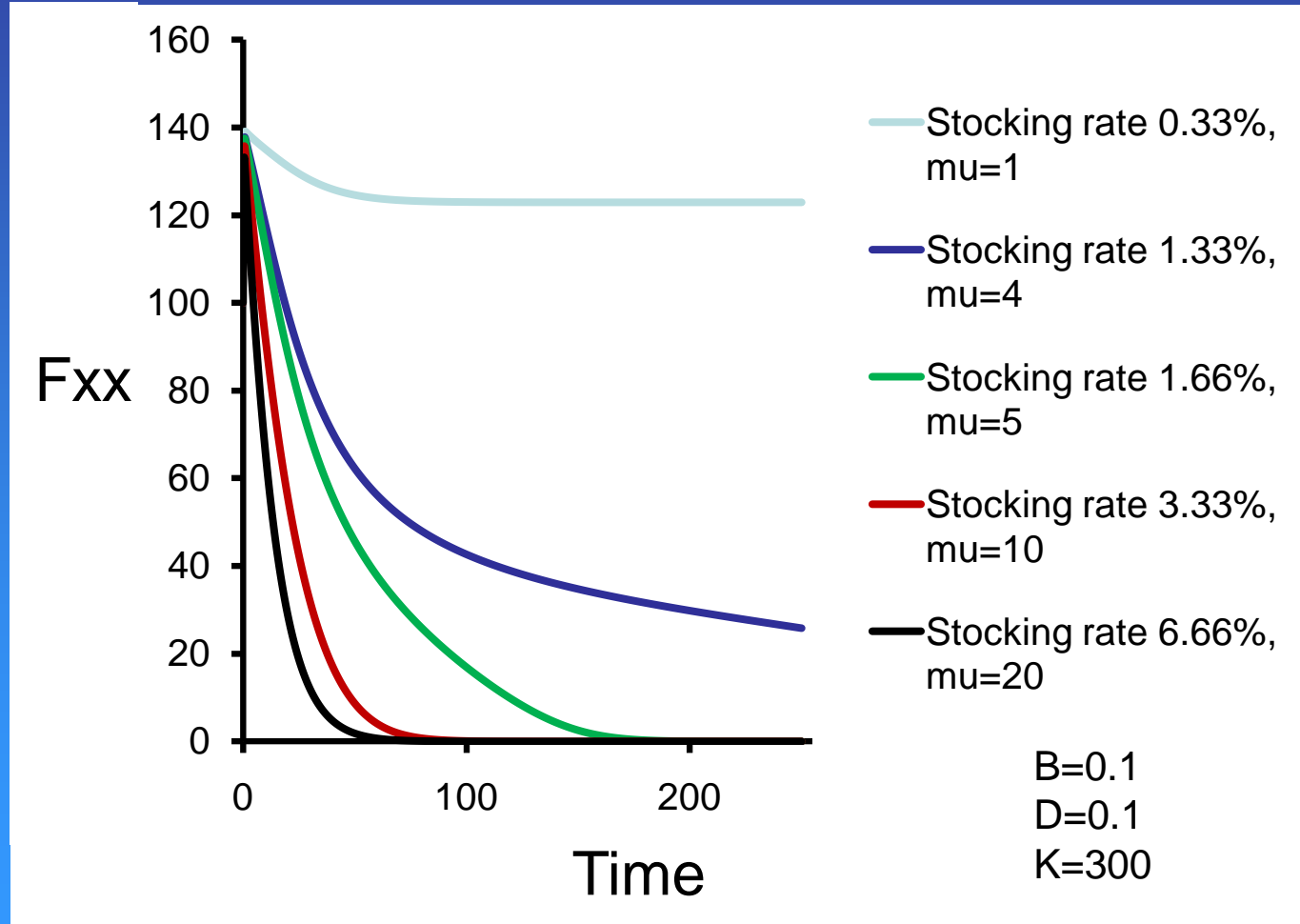
**Normal females (Fxx) go to extinction at  $\mu = 4$  or greater**

# The Continuous Model Can be Calibrated Using a Discrete Model Where Time is Represented by Mating Cycles



For a fish that matures and reproduces once a year, the timeframe for extinction is about 70 years if the Trojan fish is stocked at 1.66% ( $\mu=5$ ).

# Extinction Occurs Faster if the Number of $F_{yy}$ is Increased.



# TYC Eradication Strategy

For a target fish utilizing a simple XY sex-determination system, the addition of YY female sex-reversed fish (Fyy) to a normal population of exotic fish can cause extinction by reducing the numbers of normal females (Fxx) to zero over time.

Fyy fish must be added in an amount that represents at least 1.07 % of the total population, and the addition must be continuous over time.

The shift in the sex ratio of the population requires many generations, thus extinction may require decades.

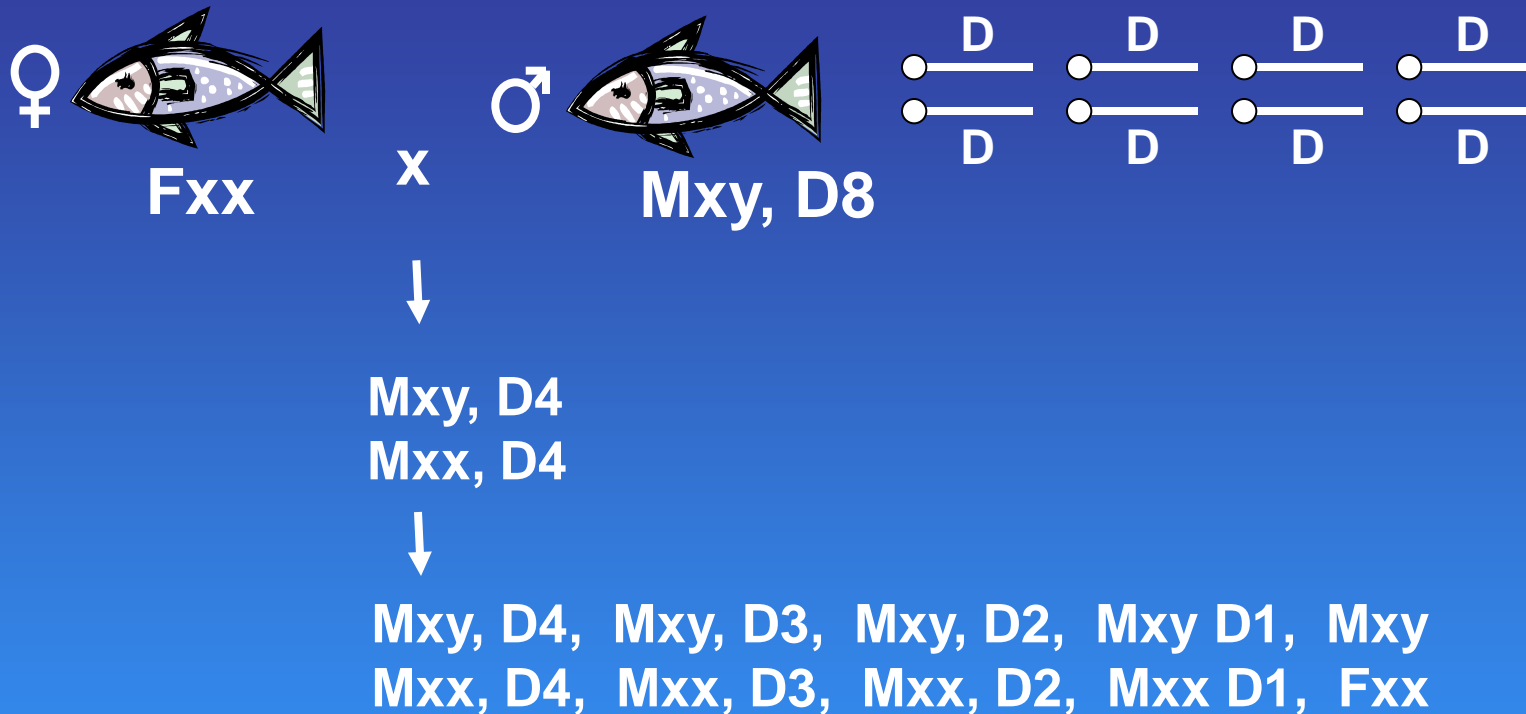
# How Does the Time to Extinction with the TYC Strategy Compare With Daughterless?

Is the Fyy Trojan Fish Equivalent to a  
Daughterless Fish with Two Copies of  
the Aromatase Inhibitor Gene?

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



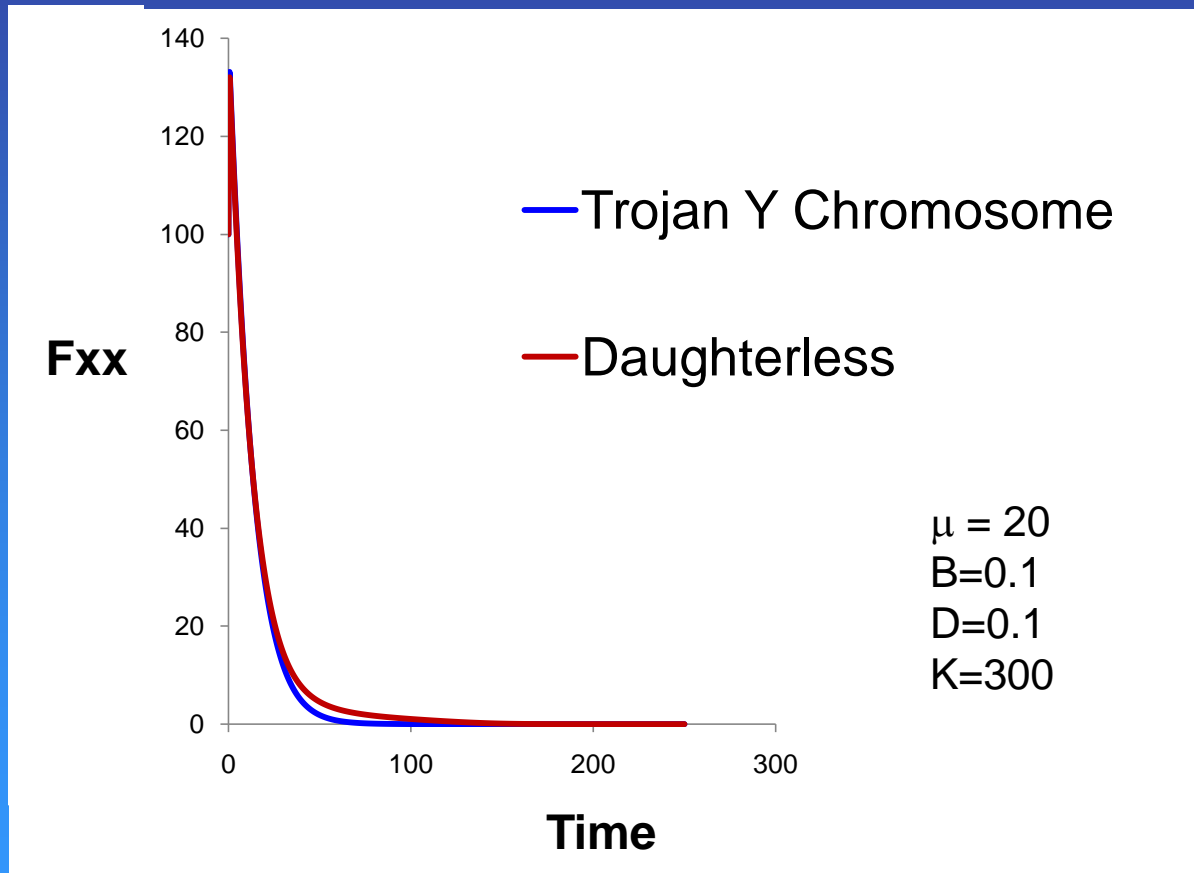
# Using Differential Equations to Model Daughterless with 8 Gene Copies



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.

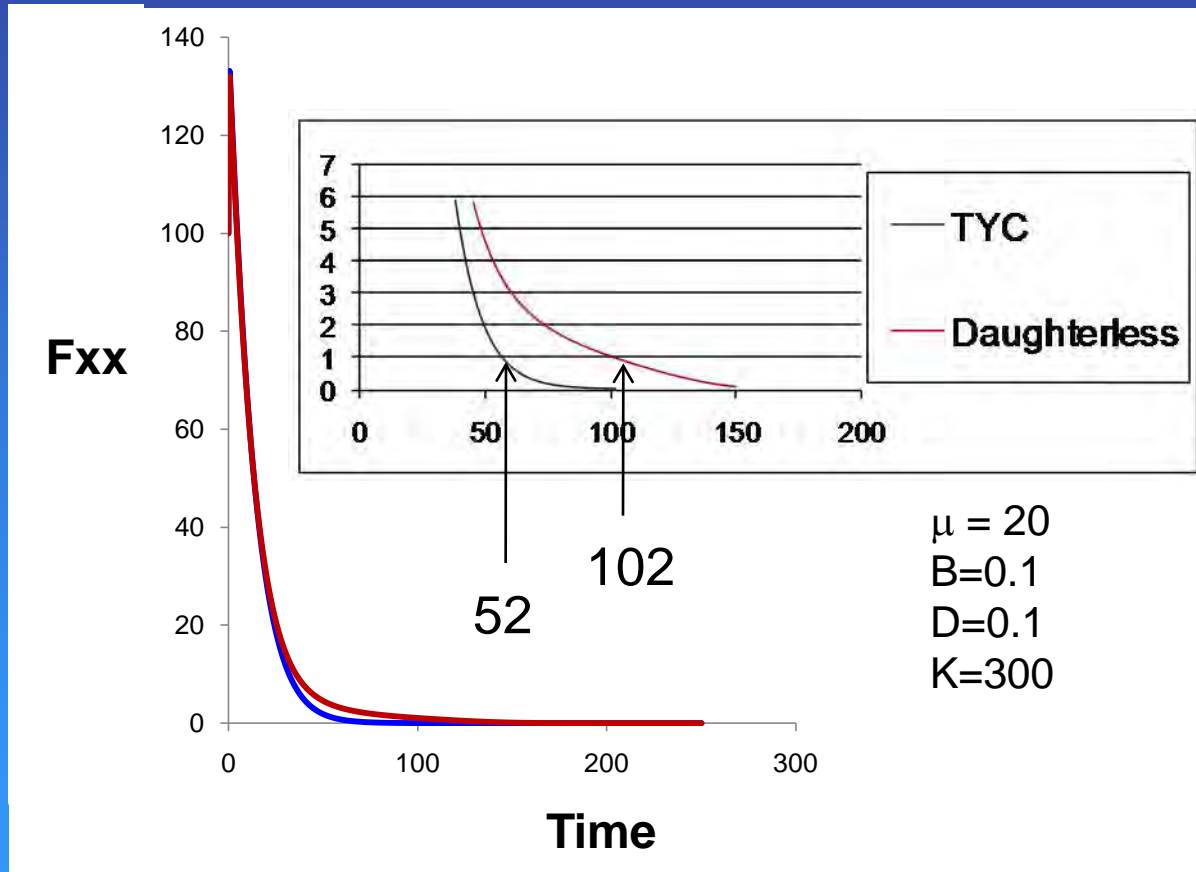
Each of the 11 fish in the population can be represented by an ordinary differential equation.

# Comparison of the TYC strategy to Daughterless



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

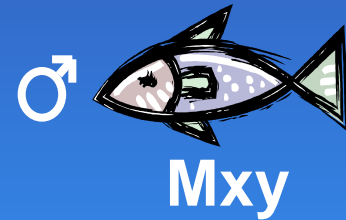
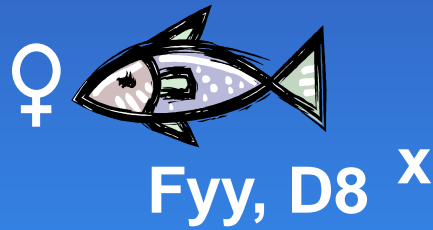
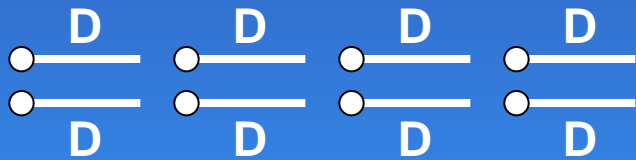
# Comparison of the TYC strategy to Daughterless



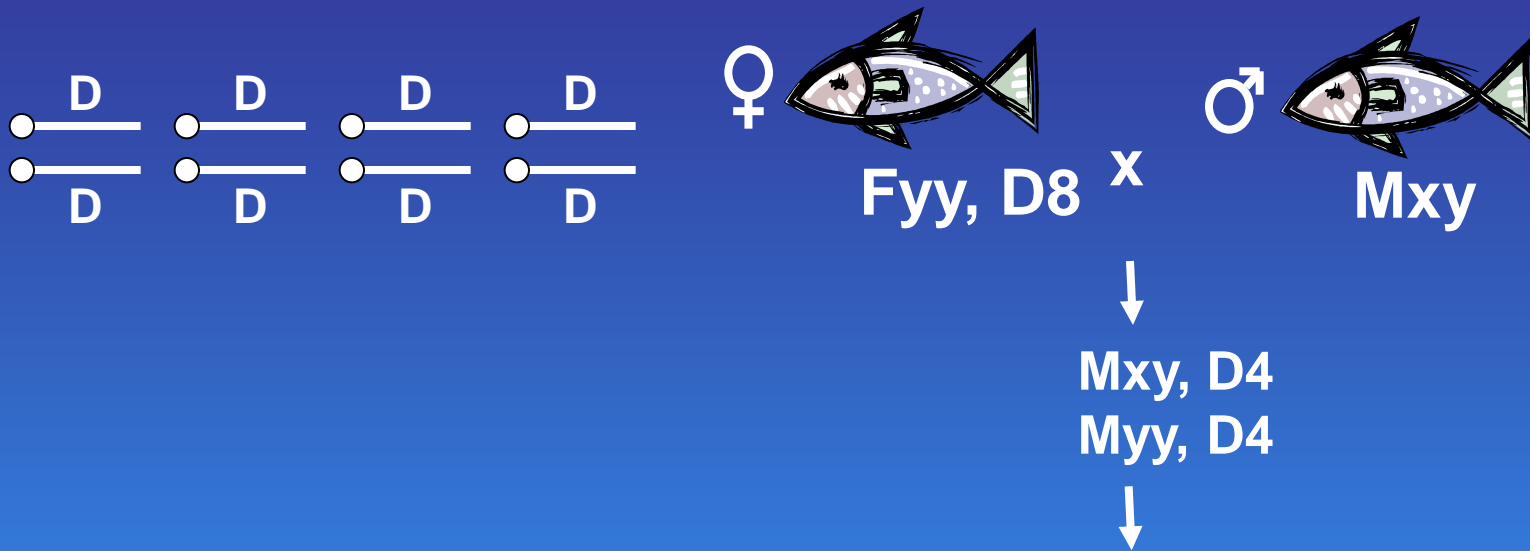
Under identical conditions, the initial rate of decline of females is similar.

However, the time to extinction of females is less for TYC (52) as compared to Daughterless (102).

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



# Modeling TYC/Daughterless with 8 Gene Copies

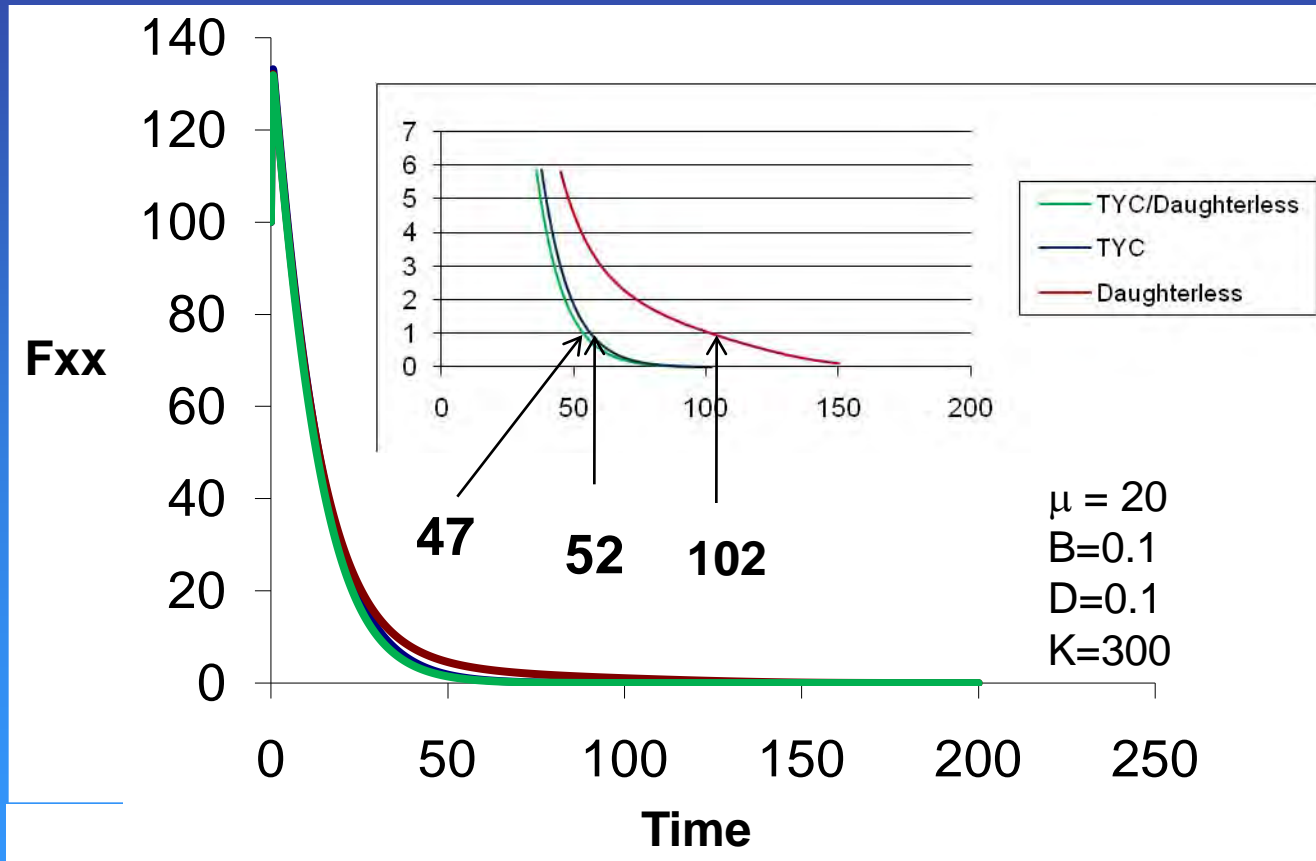


$M_{xy}, D_8, M_{xy}, D_7, M_{xy}, D_6, M_{xy}, D_5, M_{xy}, D_4, M_{xy}, D_3, M_{xy}, D_2, M_{xy}, D_1, M_{xy}, M_{xx}, D_4, M_{xx}, D_3, M_{xx}, D_2, M_{xx}, D_1, M_{yy}, D_8, M_{yy}, D_7, M_{yy}, D_6, M_{yy}, D_5, M_{yy}, D_4, M_{yy}, F_{xx}$

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

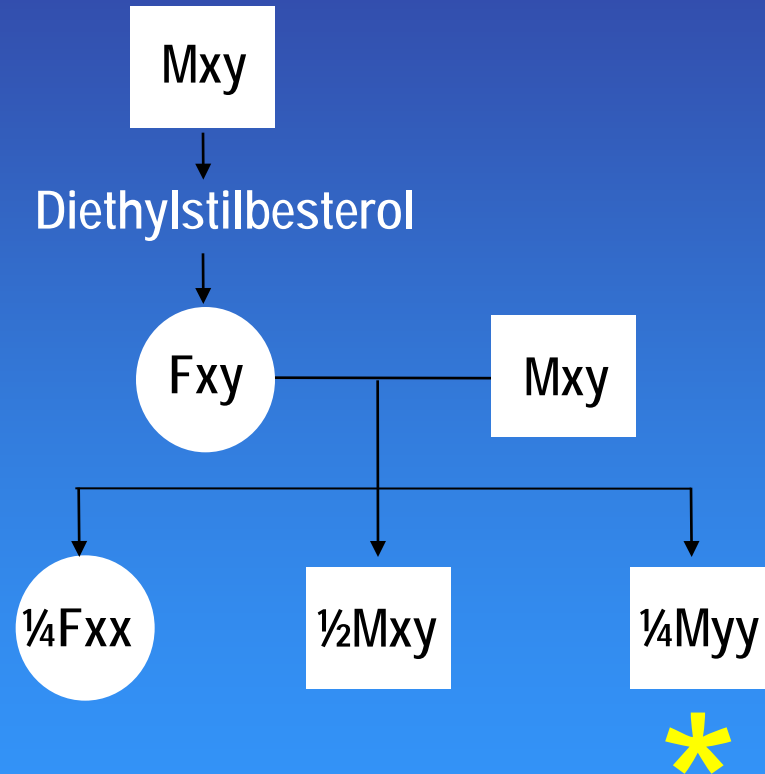


The combined TYC/Daughterless strategy also reduces the number of fish required to achieve extinction.

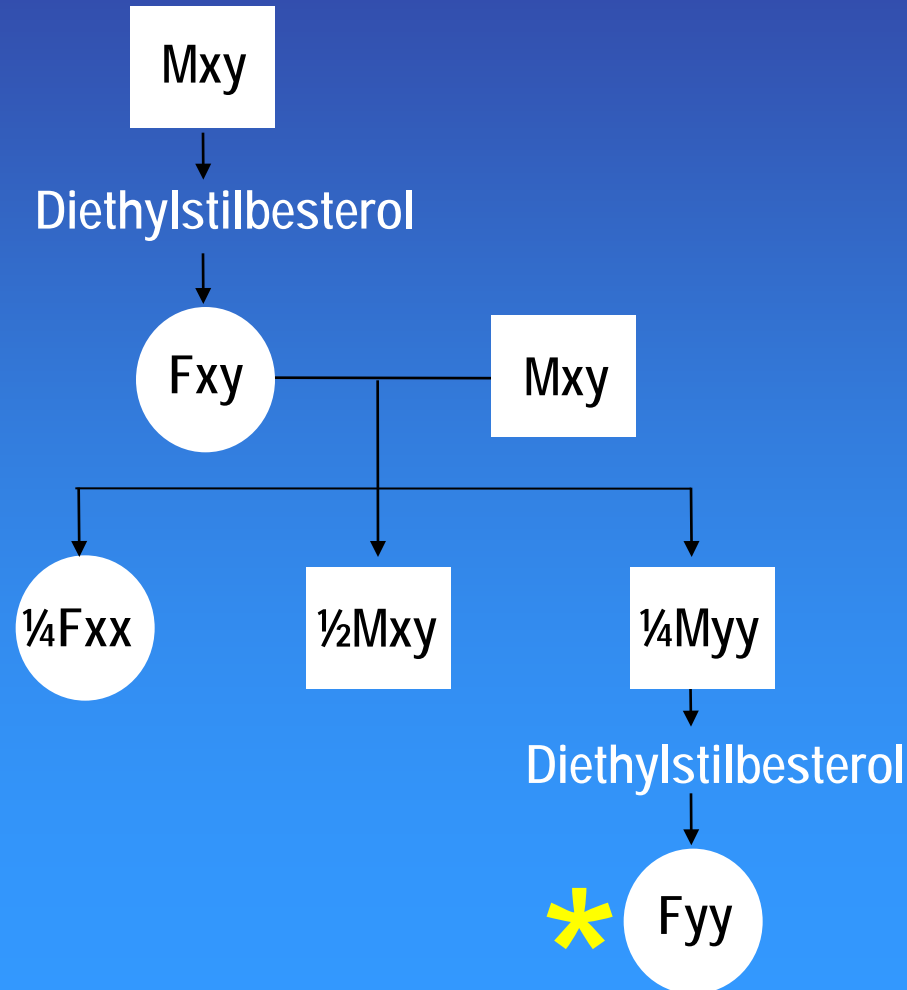
# **TYC Species Requirements**

- 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**

# Hormone Manipulation Can Be Used to Generate Male Fish with Two Y chromosomes (Myy).

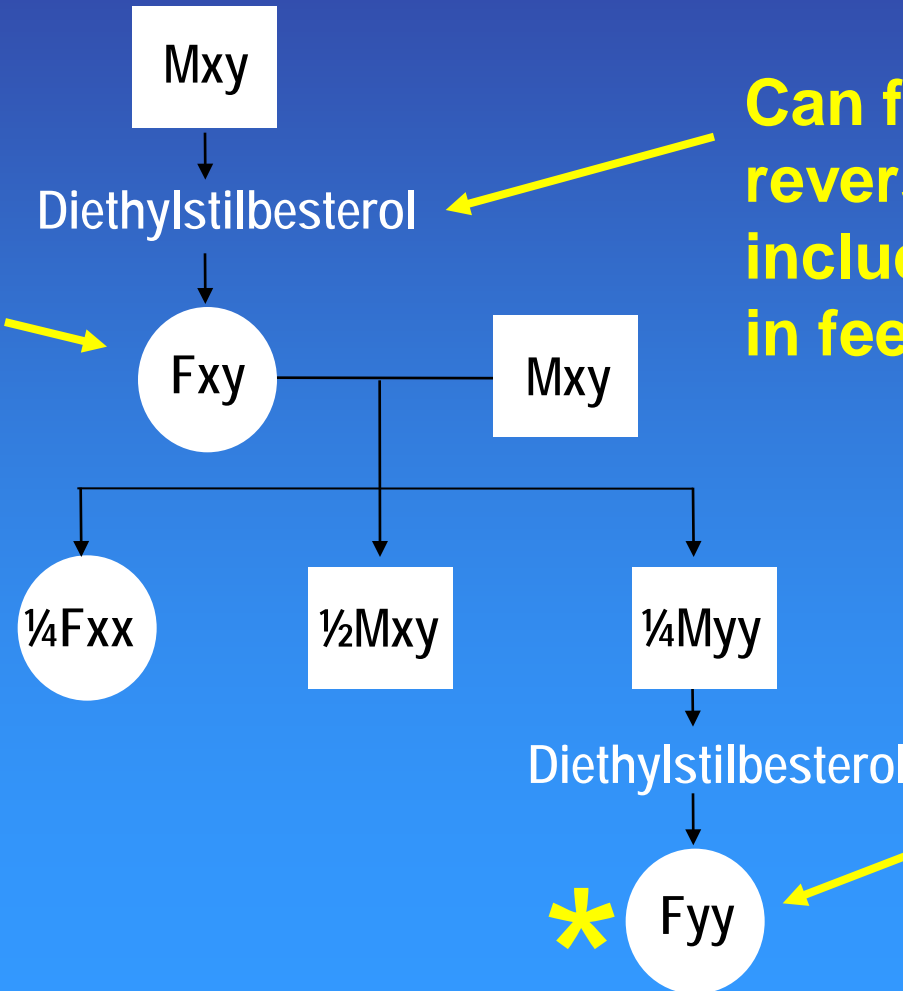


# Hormone Manipulation Can Also be Used To Generate Female Fish With Two Y Chromosomes (Fyy).



# Hormone Manipulation Can Also be Used To Generate Female Fish With Two Y Chromosomes (Fyy).

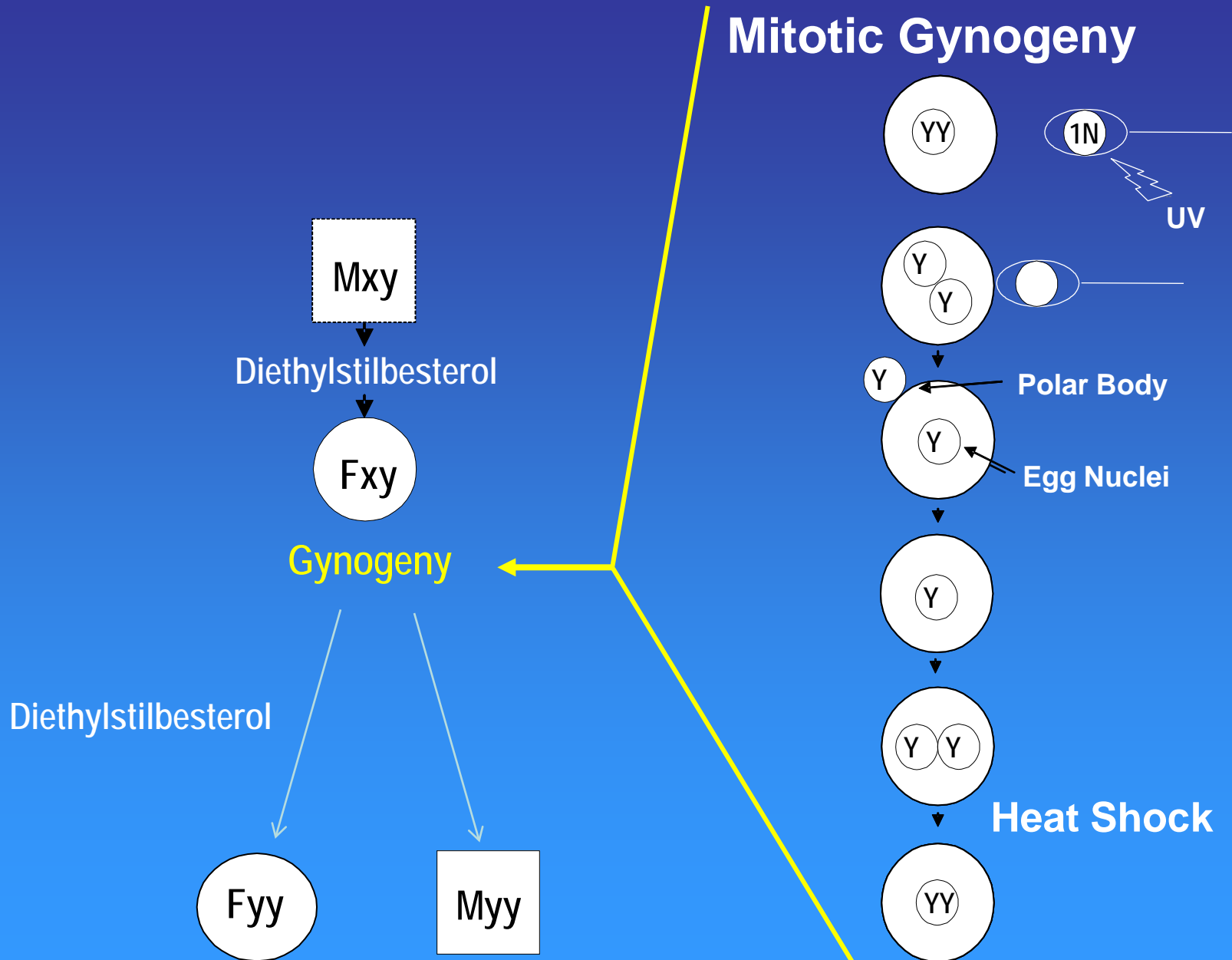
Can DNA testing be used to identify the Y chromosome in progeny?



Can fish be sex-reversed by including hormone in feed?

How efficiently does this Fyy fish mate?

# Shortcut for production of YY Broodstock



# 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.

Development of a TYC strategy for other invasive fish species (Asian carps, snakehead) will require basic research to characterize the respective features of sex-determination, YY viability, sex-reversal, gynogeny, and suitability for aquaculture for each species.

# How can genetic biocontrol strategies be further refined and improved?

Under identical modeling conditions, extinction of females occurs faster with the Trojan Y Chromosome approach as compared to Daughterless.

A combined strategy of TYC and Daughterless results in the extinction of females in the shortest time and requires the lowest stocking rate (as compared to either TYC or Daughterless alone).

Additional research is needed to identify additional modifications to Trojan fish strategies that will improve efficiency and shorten extinction times.