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

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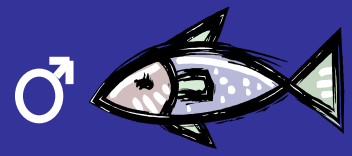
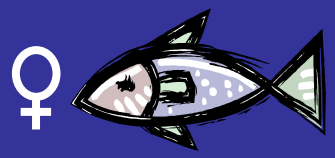
Division of Aquaculture

XY Sex-Determination



XY

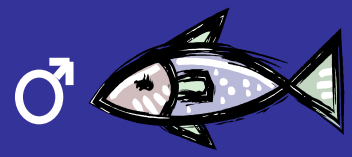


XX

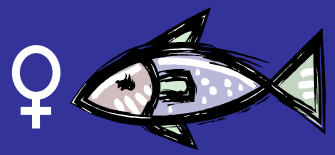
	X	Y
X	XX	XY
X	XX	XY

Males/Females
Ratio 1:1

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

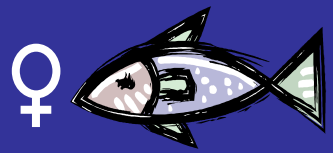


Mxy



Fxx

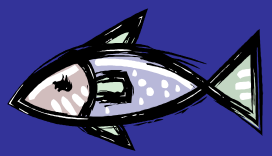
	X	Y
X	XX	XY
X	XX	XY



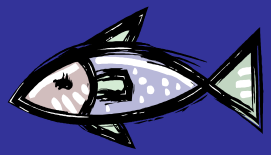
Fyy

	X	Y
Y	XY	YY
Y	XY	YY

Myy males are viable and produce only male offspring

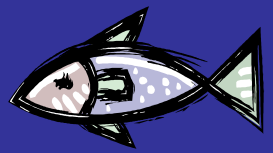
♀ 

Fxx

♂ 

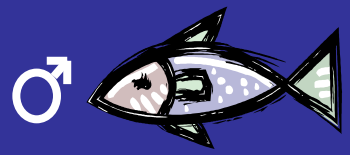
Mxy

	X	Y
X	XX	XY
X	XX	XY

♀ 

Fyy

	X	Y
Y	XY	YY
Y	XY	YY

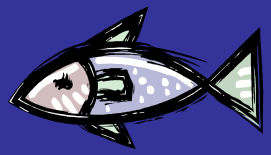
♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

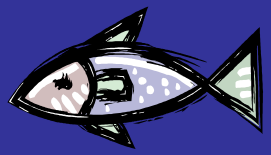
Males/Females Ratio 1:0

Four different matings are possible, leading to increased male production

♂ 

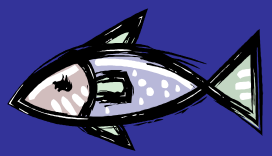
Mxy

	X	Y
X	XX	XY
X	XX	XY

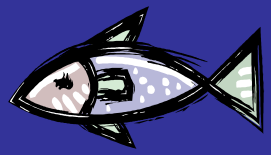
♂ 

Myy

	Y	Y
X	XY	XY
X	XY	XY

♀ 

Fxx

♀ 

Fyy

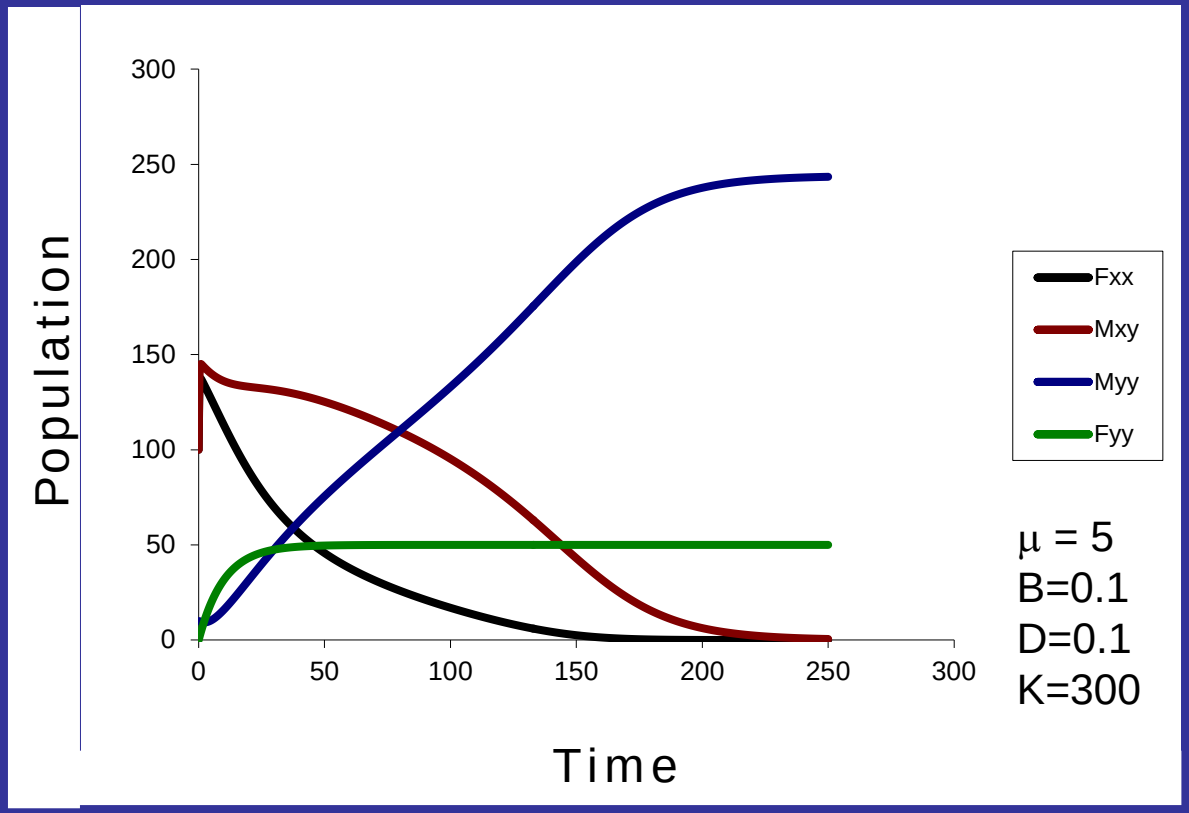
	X	Y
Y	XY	YY
Y	XY	YY

	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.



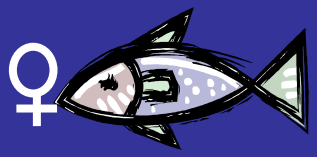
The carrying capacity of the system becomes occupied by Myy fish (males with two Y chromosomes).

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

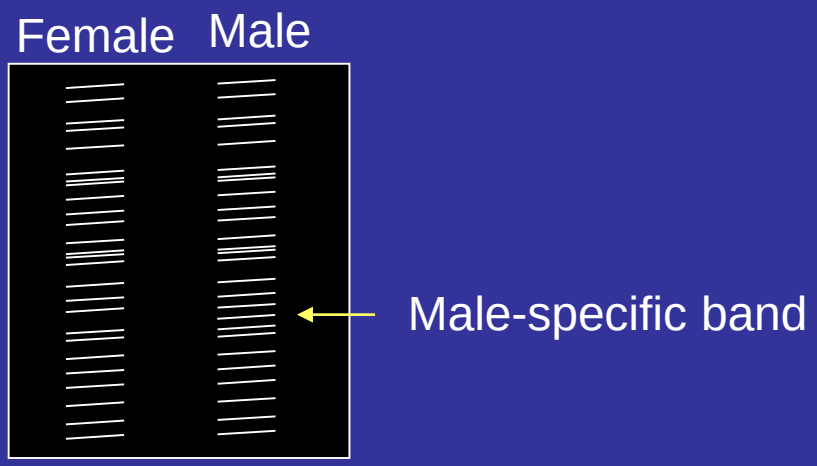
RAPD PCR

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.



Three invasive fish species were screened for sex-specific DNA markers using RAPD PCR.

Nile Tilapia

African Jewelfish

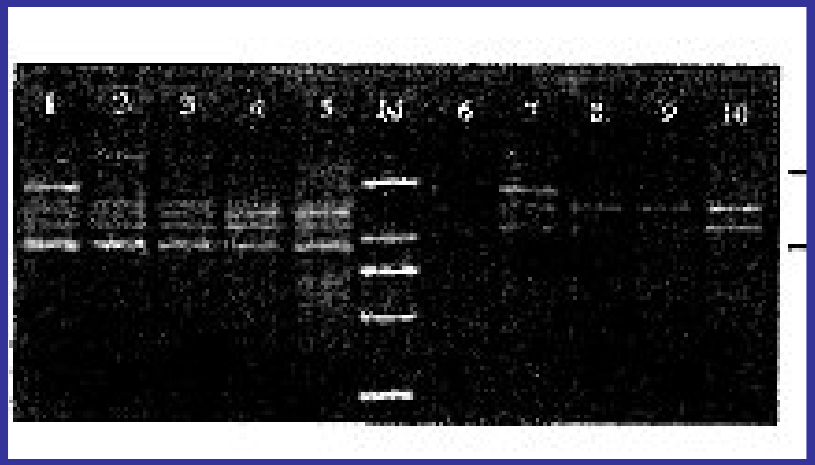
Silver Carp

A Male-specific DNA Marker for Common Carp

Male

Female

Male-specific DNA band



Male-specific DNA band

(Chen et al., 2009)

Could this same DNA marker be used to identify males in silver carp, tilapia or African jewelfish?

A Male-specific Carp Marker Can be Used to Design 10-mer RAPD PCR Primers

GCACGCAATCCACCCATTAGAAC GGCCTTCATTTCTGAACAACATTATG

GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
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GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
GCACGCAATCCACCCATTAGAAC	GGCCTTCATTTCTGAACAACATTATG
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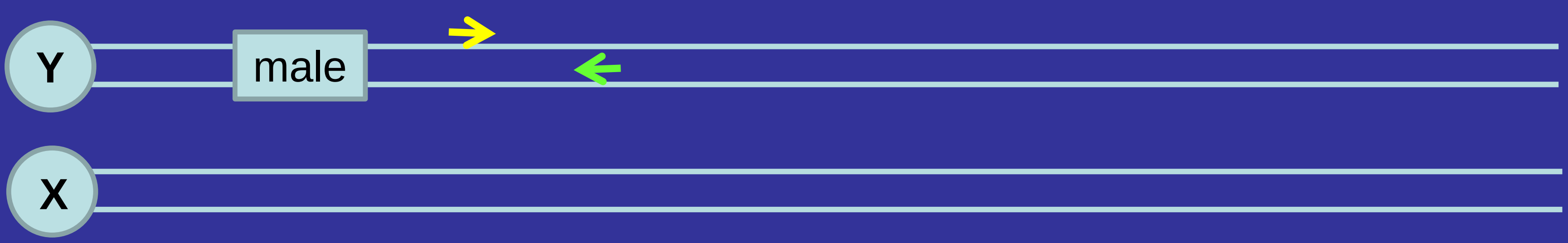
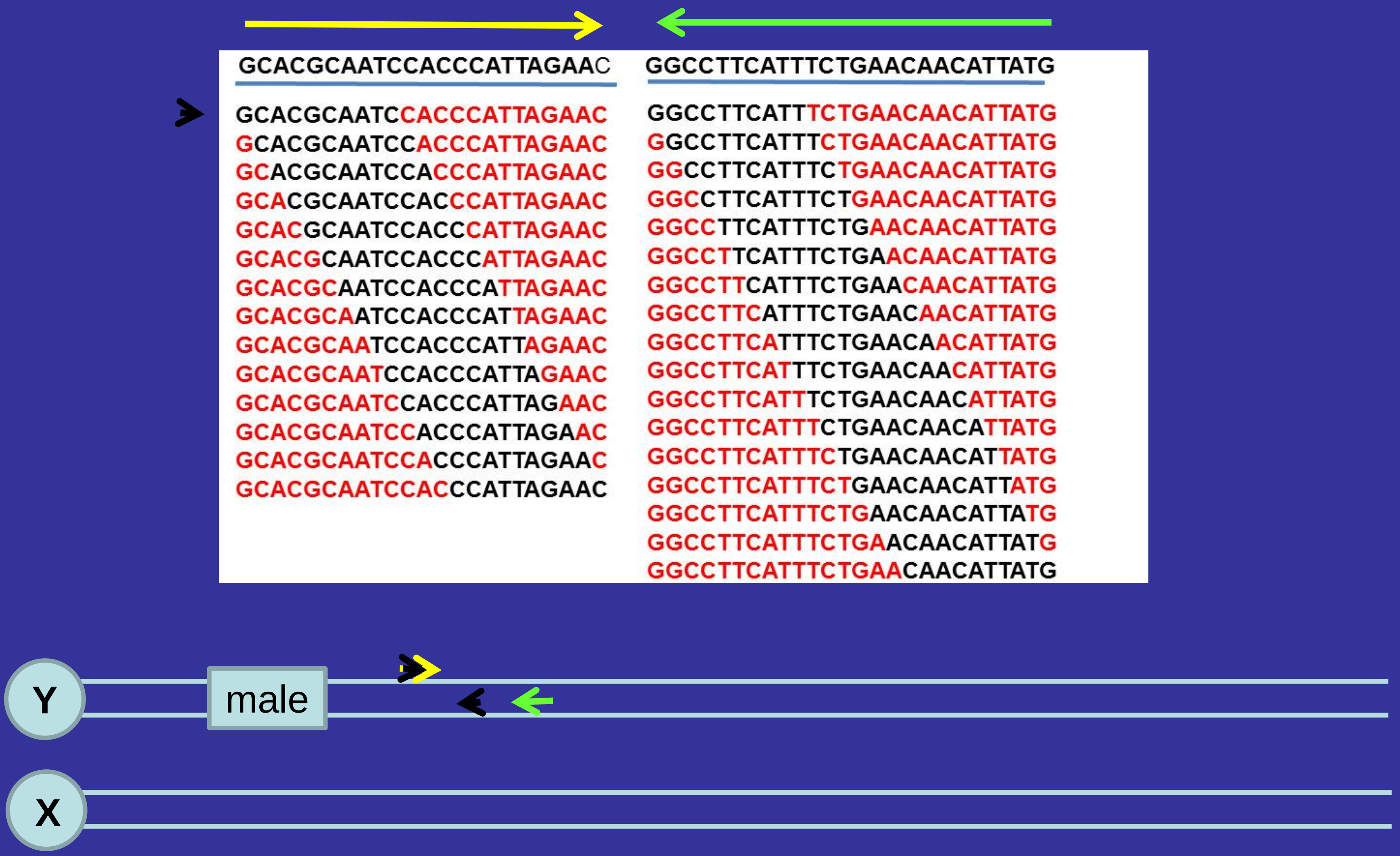
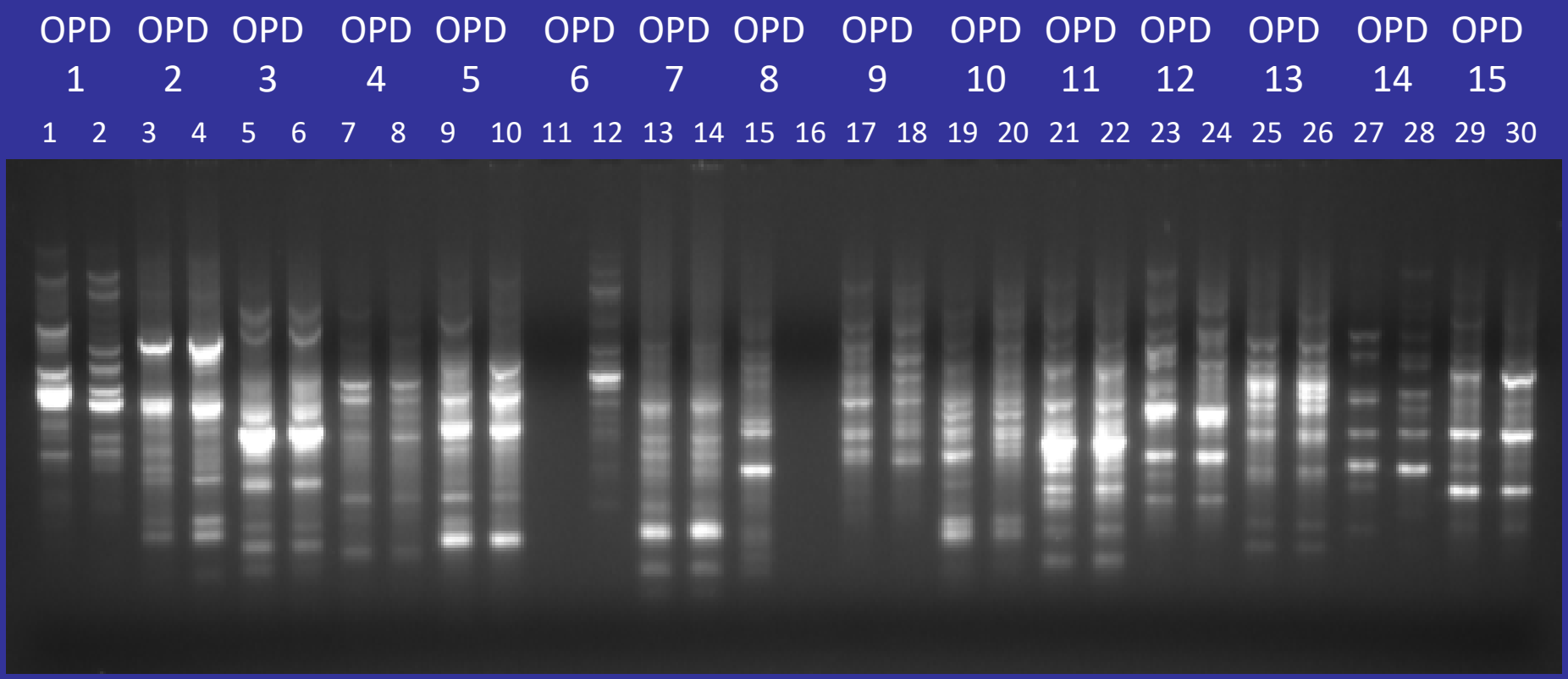


A Male-specific Carp Marker Can be Used to Design 10-mer RAPD PCR Primers



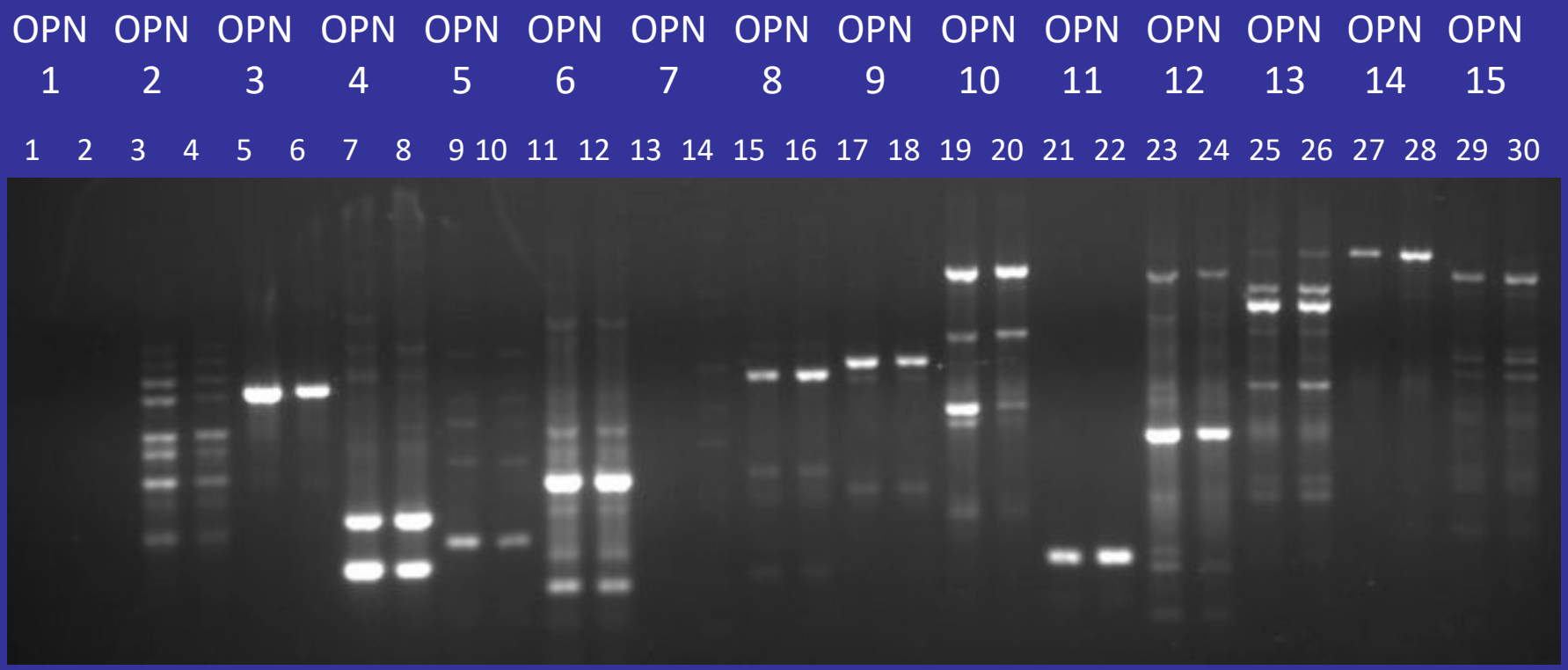
PCR Screening for Sex-Specific DNA Markers in African Jewelfish



Odd # lanes = pooled male-specific DNA from African Jewelfish
Even # lanes = pooled female-specific DNA from African Jewelfish

DNA fragments from PCR reactions using RAPD primers OPD1–OPD15 are separated on a 1.5% agarose gel.

PCR Screening for Sex-Specific DNA Markers in Nile Tilapia

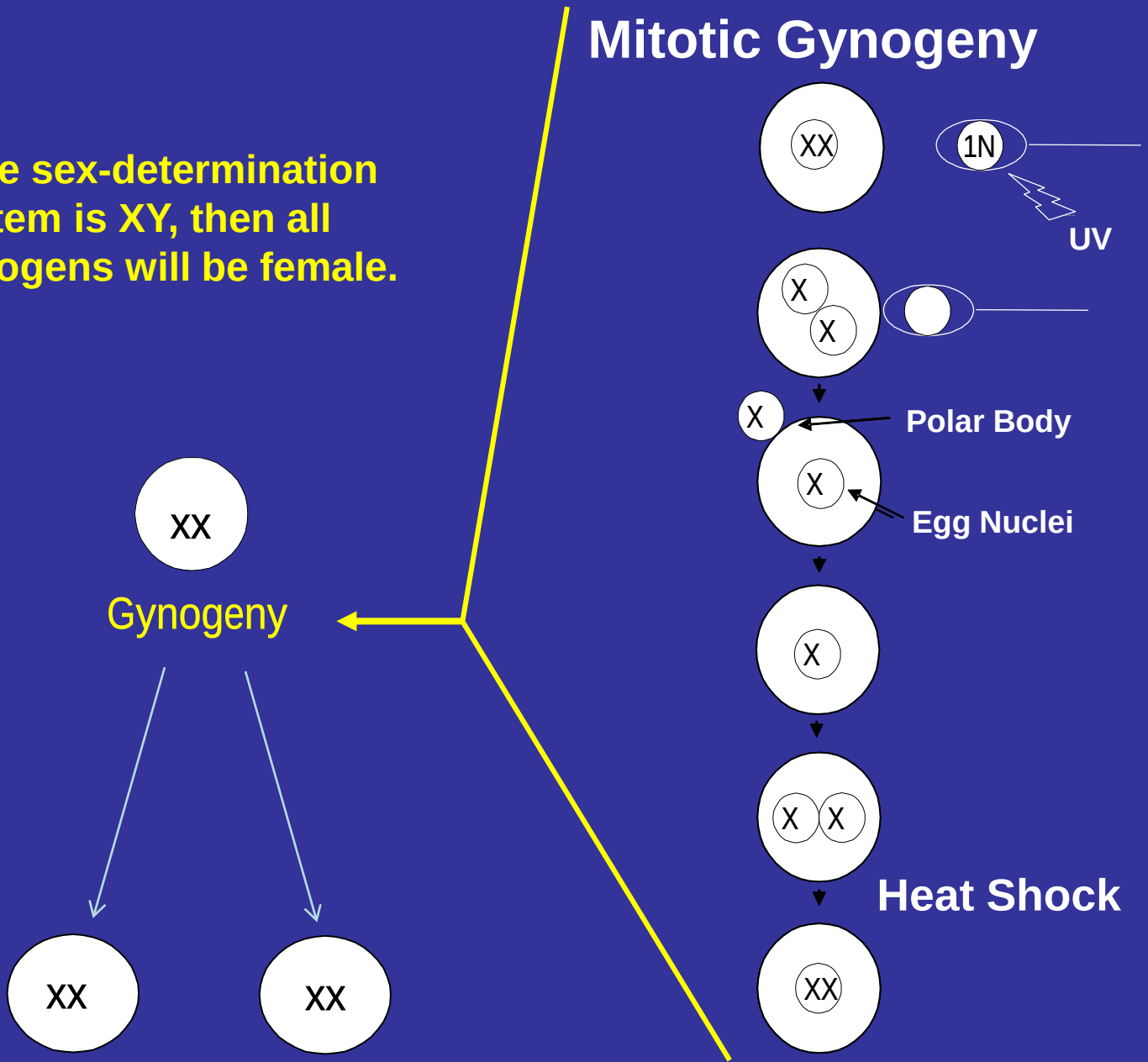


Odd # lanes = pooled male-specific DNA from Nile Tilapia
Even # lanes = pooled female-specific DNA from Nile Tilapia

DNA fragments from PCR reactions using RAPD primers OPN1–OPN15are separated on a 1.5% agarose gel.

Making Gynogens from African Jewelfish

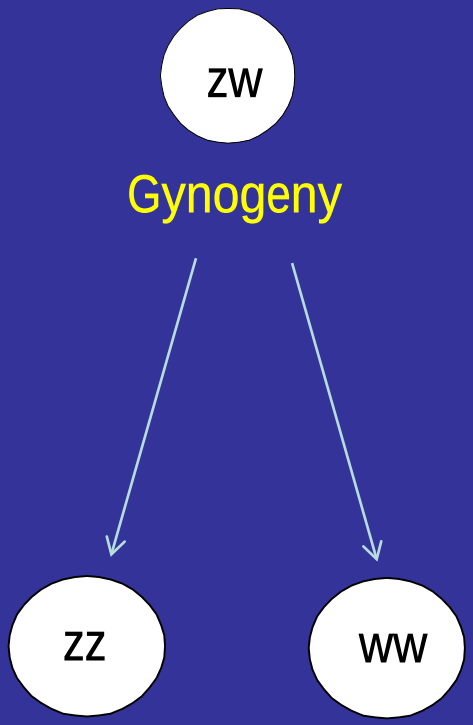
If the sex-determination system is XY, then all gynogens will be female.



Making Gynogens from African Jewelfish

In a ZW sex-determination system the female determines the sex of the progeny. Progeny that inherit the W chromosome are female.

If the sex-determination system is ZW, then half of the gynogens will be male.



Conclusions

Screening for sex-specific DNA markers has been done with African Jewelfish, Nile Tilapia and Silver Carp.

African Jewelfish have been the first priority because broodstock are being developed for this species by USGS.

No sex-specific markers have been identified as yet for any of the three species.

Experiments to determine the sex-determination system for African Jewelfish are in progress in collaboration with USGS.