THE MYSTERY OF ZEBRAFISH GONAD DEVELOPMENT

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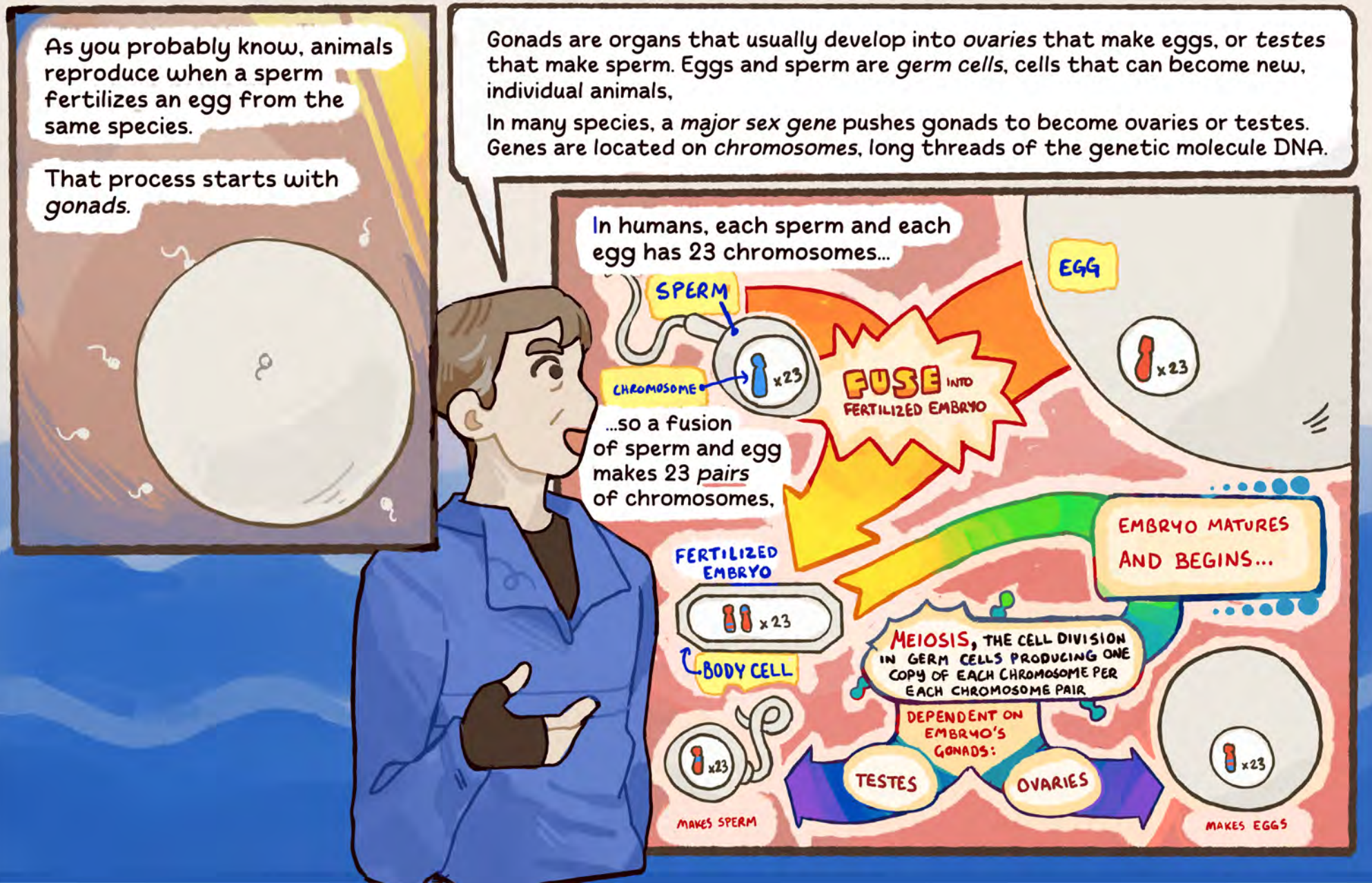
EDITED BY AUDRA McNAMEE

THIS COMIC WAS CREATED THROUGH THE UNIVERSITY OF OREGON SCIENCE AND COMICS INITIATIVE.

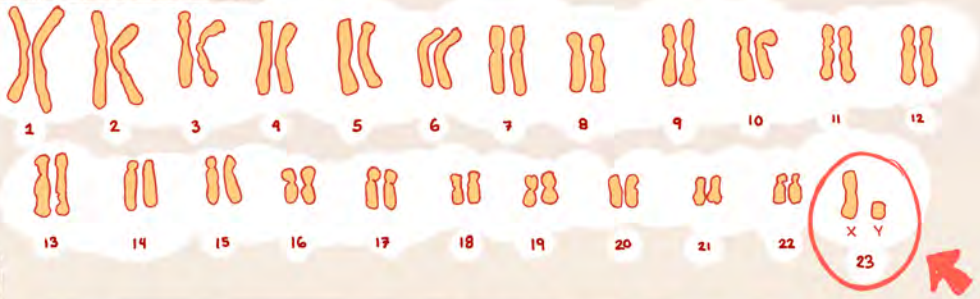
COMPLETED JULY 31ST, 2025


As you probably know, animals reproduce when a sperm fertilizes an egg from the same species.
That process starts with *gonads*.
Gonads are organs that usually develop into *ovaries* that make eggs, or *testes* that make sperm. Eggs and sperm are *germ cells*, cells that can become new, individual animals.
In many species, a *major sex gene* pushes gonads to become ovaries or testes. Genes are located on *chromosomes*, long threads of the genetic molecule DNA.
In humans, each sperm and each egg has 23 chromosomes...
SPERM
CHROMOSOME
x23
...so a fusion of sperm and egg makes 23 *pairs* of chromosomes.
FUSE INTO FERTILIZED EMBRYO
EGG
x23
FERTILIZED EMBRYO
x23
BODY CELL
EMBRYO MATURES AND BEGINS...
MEIOSIS, THE CELL DIVISION IN GERM CELLS PRODUCING ONE COPY OF EACH CHROMOSOME PER EACH CHROMOSOME PAIR
DEPENDENT ON EMBRYO'S GONADS:
TESTES
OVARIES
x23
MAKES SPERM
x23
MAKES EGGS

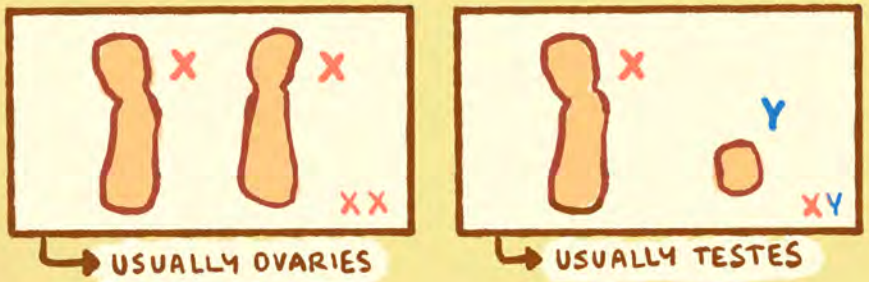
22 of the 23 pairs of human chromosomes have roughly the same size, shape, and gene content.

But one pair, the sex chromosomes X and Y, have different size, shape, and gene content.

THE Y CHROMOSOME IS SO SMALL COMPARED TO X?



HUMAN SEX CHROMOSOME PAIRS



Most female humans have two X chromosomes, resulting in an XX chromosome pair.

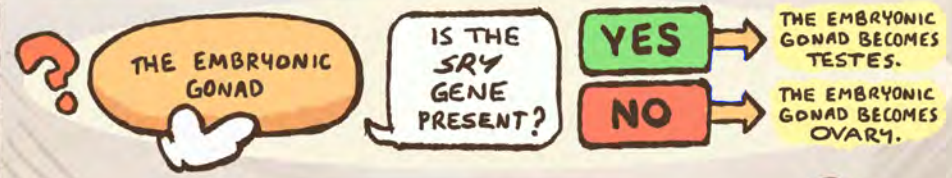
Most male humans have one X chromosome and one Y chromosome, resulting in a XY pair.

Like many things in this comic, there are complexities beyond this simple explanation, but this is what we have room for!

The human X chromosome has 864 protein-coding genes while the Y has only 63 protein-coding genes.

Among those few 63 genes is the major sex gene that causes human gonads to become testes instead of ovaries.

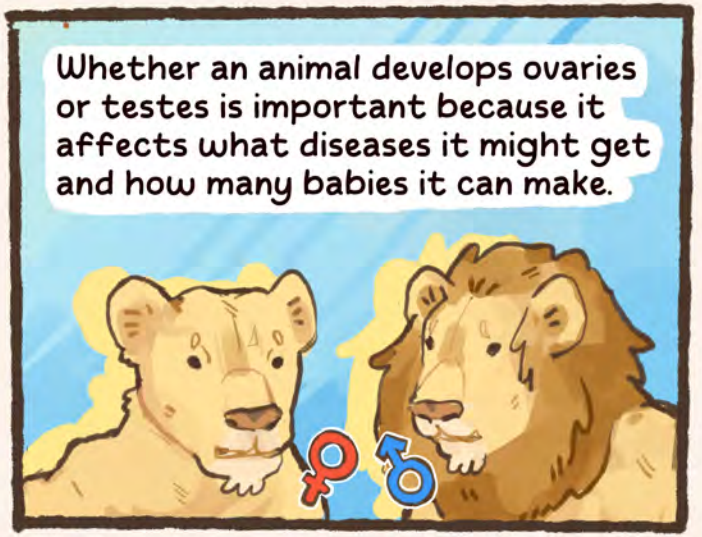
This major sex gene encodes the *sex-determining region Y protein* (a.k.a *SRY*). The *SRY* gene determines gonads in nearly all mammals like dogs! Without *SRY*, their gonads become ovaries.

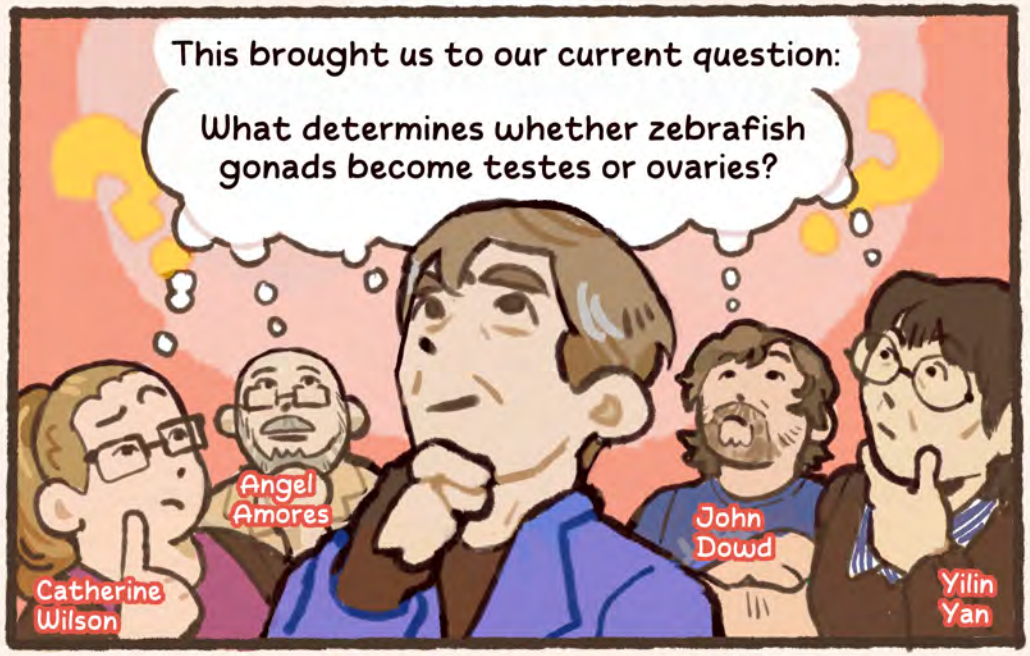


Birds fly in a different direction: Hens have a ZW chromosome pair and roosters are ZZ. The Z chromosome has a weak testis- specifying gene (*DMRT1*) not on the W.

Because it takes two copies of *DMRT1* to make a testis, ZW birds have ovaries.

SPECIES AND THEIR GONAD DETERMINING FACTORS!
While genes are gonad determinants in some species, environment determines gonads in other species.
SEA TURTLE
↳ TEMPERATURE
CLOWNFISH
↳ SOCIAL ENVIRONMENT
TIGER
↳ SRY GENE
PLATYFISH
↳ POLYGENIC
(DETERMINED BY MULTIPLE GENES)


Whether an animal develops ovaries or testes is important because it affects what diseases it might get and how many babies it can make.
♀
♂


This brought us to our current question:
What determines whether zebrafish gonads become testes or ovaries?
Catherine Wilson
Angel Amores
John Dowd
Yilin Yan

You might ask:
Why zebrafish?
XY
THE SIMPLE ZEBRAFISH
WELL,

HERE'S: A QUICK INTRO TO ZEBRAFISH

A MAJOR MODEL FOR GENE ACTION, DEVELOPMENT, AND PHYSIOLOGY WORLDWIDE ✩

Has most but not all of the same organs as humans (e.g. it has a brain, liver, and intestines).

Has transparent embryos! This makes it easy to observe internal development.

Develops rapidly! It only takes a couple days for embryos to develop into free living youngsters.

THE ACTUALLY SUPER COOL ZEBRAFISH

They have high *fecundity* (they can make a lot of children very fast).

Female zebrafish can make up to ~200 babies per week!

Over 60,000 research papers in medical literature mention zebrafish!

Has been domesticated in labs for ~40 years.

Originates in India!

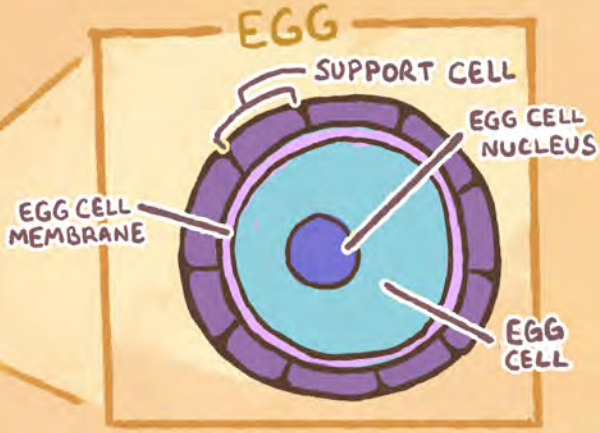
NOW, LET'S GET INTO THE RESEARCH!

✩ BASICALLY, USED FOR LOTS OF RESEARCH. KIND OF A BIG DEAL! ∞ KRIS

Here's what we know about how the gonad develops in domesticated (i.e. lab-bred) zebrafish.

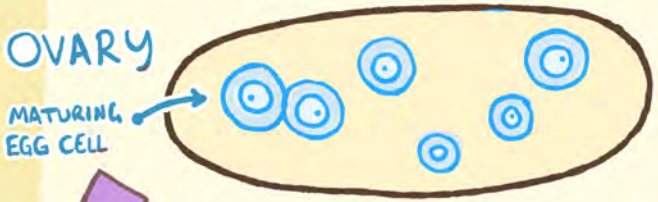
At 19 days post-fertilization (dpf), a juvenile zebrafish has bipotential gonads that can develop into either ovaries or testes.

At 22 dpf, gonads in each lab zebrafish start to form egg cells - creating a juvenile ovary.



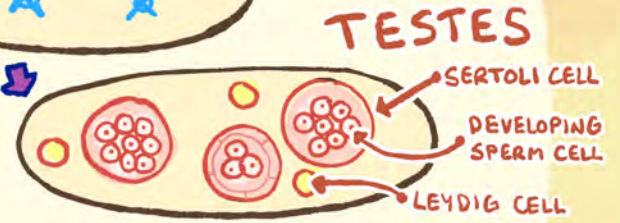
AT 25 DPF

In some individual zebrafish, egg cells continue to increase in number and size. The support cells become estrogen-producing "granulosa cells".



The juvenile ovary continues to develop, resulting in a female zebrafish.

In other zebrafish, young egg cells die.



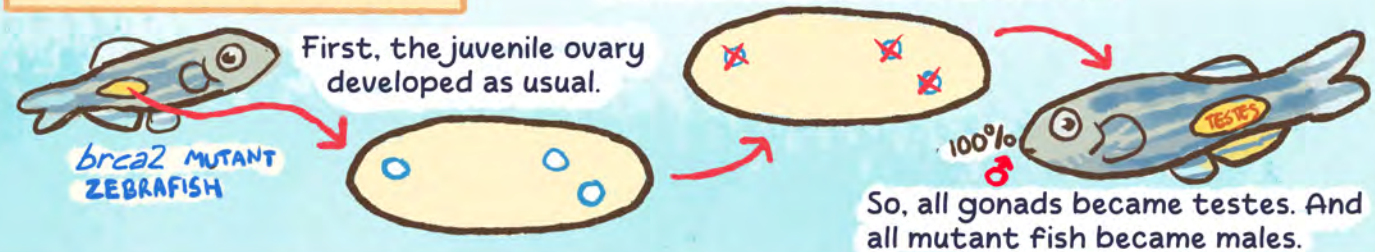
In this case, support cells become Sertoli cells that nourish growing sperm. Other cells become testosterone-producing Leydig cells.

The juvenile ovary has changed into a testis, resulting in a male zebrafish.

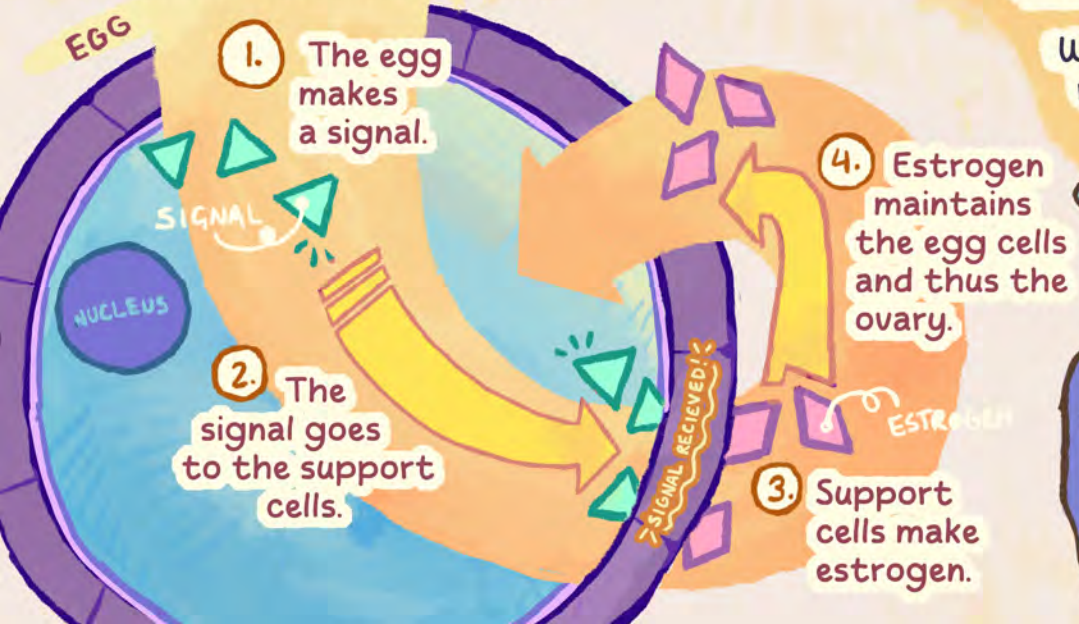
To study transformation of the juvenile ovary, we created zebrafish with a mutation in the *brca2* gene. This mutation makes cells unable to repair DNA breaks, and in humans, it leads to breast cancer.

(WHAT HAPPENED TO THE FISH?)

This is what happened with the mutated lab zebrafish:

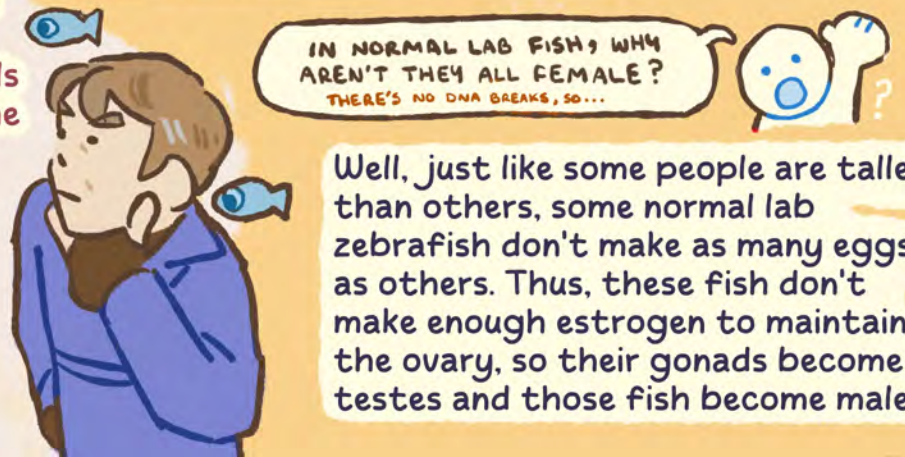


Based on this result, we hypothesize that in normal lab zebrafish:



In contrast, in the *brca2* mutant, all egg cells die so there's no estrogen to maintain the ovary and its egg cells.

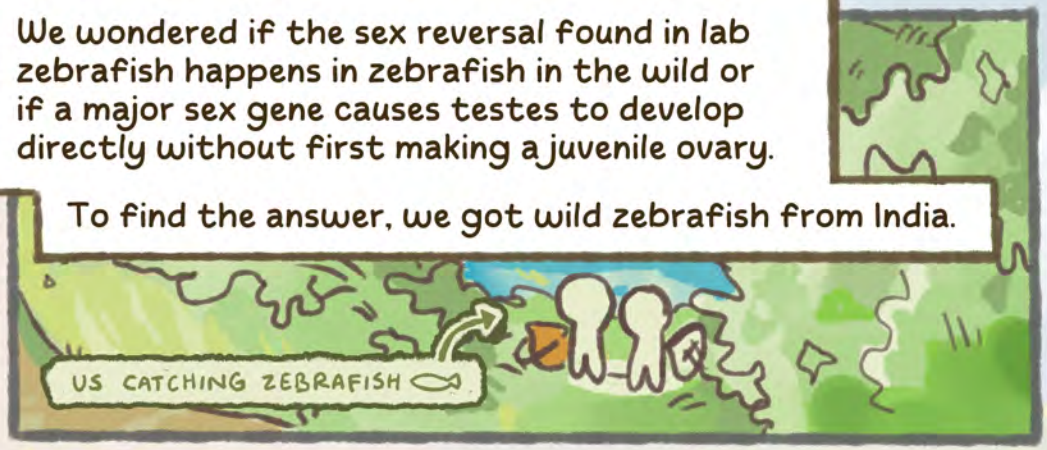
Without estrogen, the ovary becomes a testis, resulting in only male zebrafish. This is 'sex reversal': juvenile females change into males.



Well, just like some people are taller than others, some normal lab zebrafish don't make as many eggs as others. Thus, these fish don't make enough estrogen to maintain the ovary, so their gonads become testes and those fish become males.

We wondered if the sex reversal found in lab zebrafish happens in zebrafish in the wild or if a major sex gene causes testes to develop directly without first making a juvenile ovary.

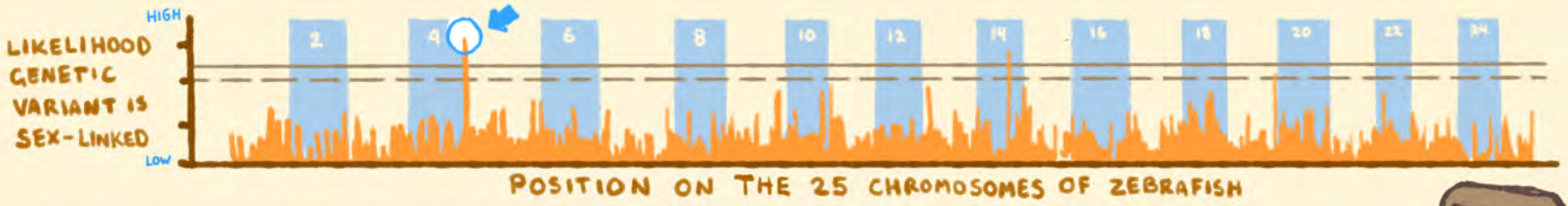
To find the answer, we got wild zebrafish from India.



We seperated the males and females.

Then, we extracted their DNA to identify DNA sequences that were present in one sex but not in the other.

Finally, we mapped the likelihood that a DNA sequence is associated more often with one sex and not the other vs. the location of that DNA sequence on the fish chromosomes.



Results showed that a single place along all 25 chromosomes of wild zebrafish was statistically linked to sex: at the tip of chromosome-4. We called it '*sex-associated region on chromosome-4*', or *sar4*.

When doing the same analysis in lab zebrafish, we found no site in the genome linked to sex.

We concluded that wild zebrafish have a major sex gene, but lab zebrafish lost it in domestication.

The data showed that:

Wild zebrafish males have two Z chromosomes...

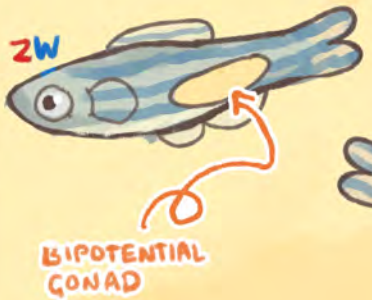
...and wild females have one Z and one W chromosome.

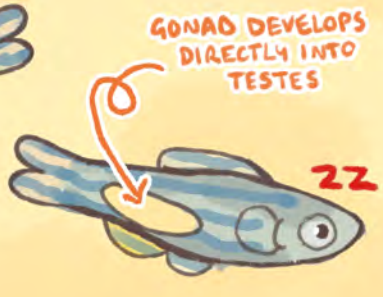
So, wild zebrafish sex chromosomes are similar to those in chickens.

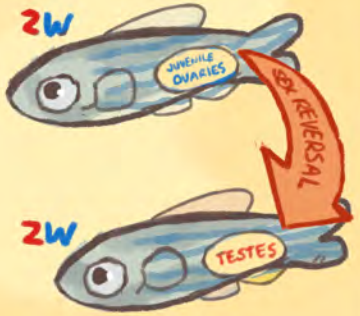
ZW wild fish, like all lab zebrafish, have bipotential gonads that become juvenile ovaries.

Gonads in ZZ wild fish always develop directly into testes and ZZ fish always become males.

Most ZW fish become females but some experience sex reversal and their juvenile ovaries become testes.

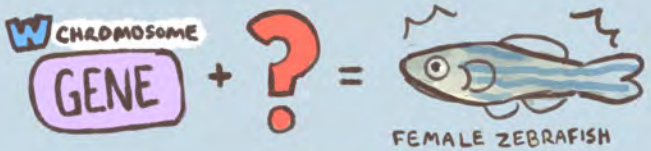






WE CONCLUDED THAT EITHER:

1. The W has a gene that is necessary to make a female but that gene is not sufficient to make a female (since some ZW fish undergo sex reversal).



OR

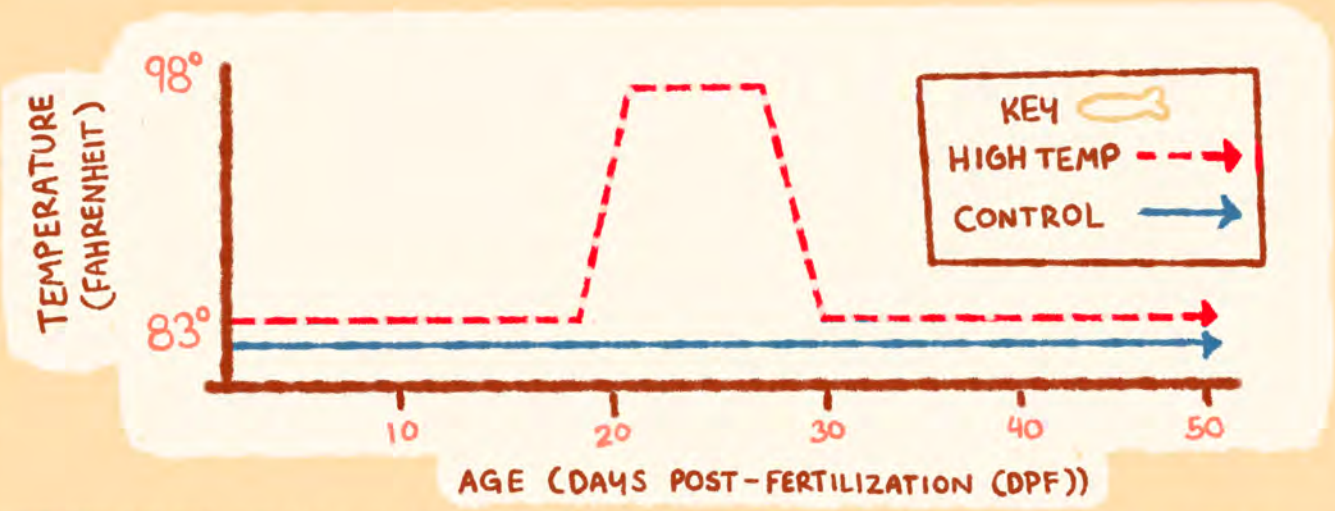
2. Two Zs are necessary to make a male, like in birds.

Regardless of which hypothesis is correct, we figured out lab zebrafish are different from wild zebrafish because lab zebrafish lost the Z chromosome during domestication and are now chromosomally WW.

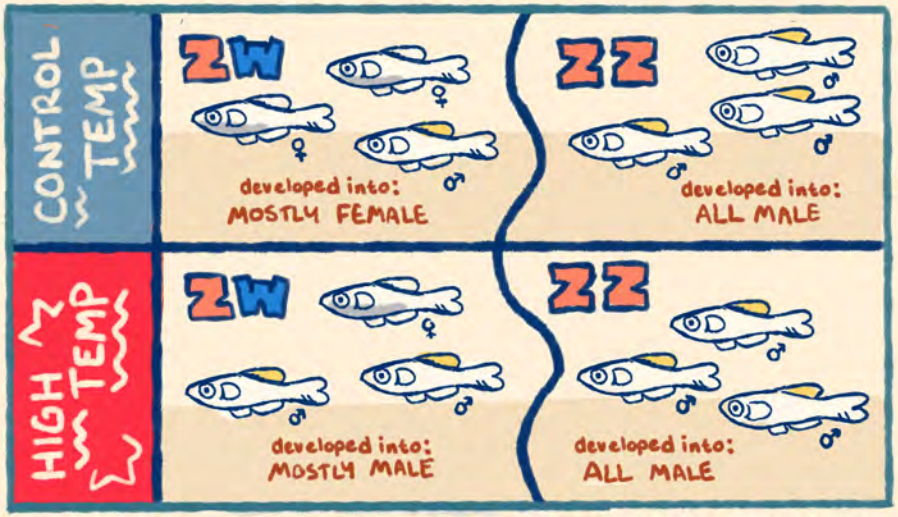
WHY DO SOME ZW JUVENILE OVARIES CHANGE TO TESTES?

We thought it might be environmental, like how turtle gonad development depends on temperature.

So we raised some wild zebrafish at a high temperature (98F, 36C) during the period of sex determination and others at the normal temperature (83F, 28C) as a control.



THE RESULTS!



We observed that most ZW zebrafish developed ovaries at the control temperature; meanwhile, ZW zebrafish raised at the higher temperature underwent sex reversal and developed testes.

In contrast, ZZ zebrafish developed testes under both circumstances.

NOTE:

Other environmental stressors (like population density or lack of food) also caused sex reversal in developing ZW zebrafish.

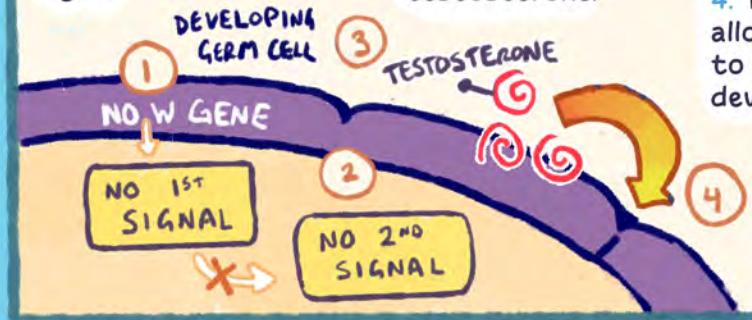
SO: HERE'S HOW ZEBRAFISH SEX GENES LIKELY CONTROL GONAD DEVELOPMENT

ZW ZEBRAFISH

1. A W gene in support cells causes them to send a feminizing signal to germ cells.
2. Germ cells send a second signal to support cells.
3. Support cells make estrogen
4. Estrogen maintains the egg cells and thus, the ovary

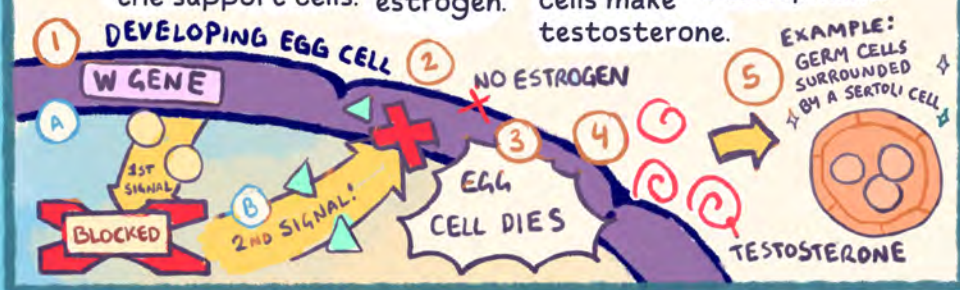
ZZ ZEBRAFISH

1. Without the W gene, there's no feminizing signal.
2. There's no second signal.
3. Support cells make testosterone.
4. Testosterone allows germ cell to begin sperm development.



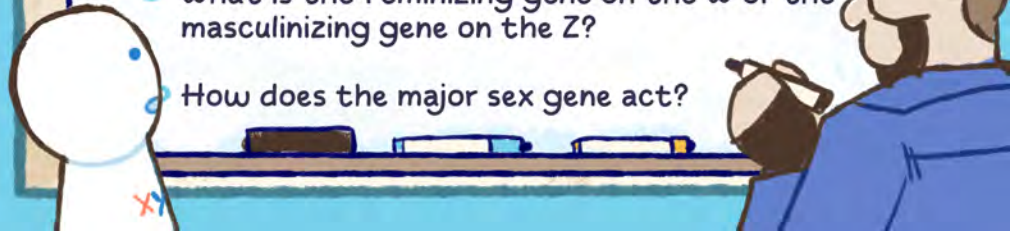
ZW ZEBRAFISH + HIGH TEMP

1. Heat blocks either: A. the feminizing signal to the germ cell or B. the second signal to the support cells.
2. Without the signal, there is no estrogen.
3. The egg cells die.
4. Support cells make testosterone.
5. Germ cells begin sperm development.



Questions for the future:

- If climates warm, will all wild zebrafish become male?
- How does high temperature block signals?
- What is the feminizing gene on the W or the masculinizing gene on the Z?
- How does the major sex gene act?



RESEARCHER

JOHN POSTLETHWAIT

John Postlethwait is a professor of biology and a zebrafish researcher. He's been cited over 50,000 times with 103 publications cited more than 103 times (Google Scholar)! You can find his research on zfin.org/ZDB-LAB-970408-67.

ARTIST

KRISTOPHER CHU

Kristopher Chu is a recent UO Art+Tech graduate. He drew 36 zebrafish (and five zebrafish researchers) in this comic! You can find more of his work at kristopherchu.carrd.co.

EDITOR

AUDRA McNAMEE

Audra McNamee is a cartoonist, editor at Crucial Comix, and program coordinator for the University of Oregon Science and Comics Initiative.

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Key primary lab work performed by Angel Amores, John Dowd, Catherine Wilson, and Yilin Yan.

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