

# Ménage à trois in the African pygmy mouse, *Mus minutoides*.

## A third sex chromosome and a third sexual phenotype

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# Sex determination is a fundamental process, but **mechanisms are diverse**

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In many taxa: **high rates of turnover**



*Gekkonidae*

- environmental sex determination
- sex chromosomes (XX/XY; ZW/ZZ)

...in others: **highly conserved**



♂ XY ♀ XX



♂ ZZ ♀ ZW



*Rana rugosa*

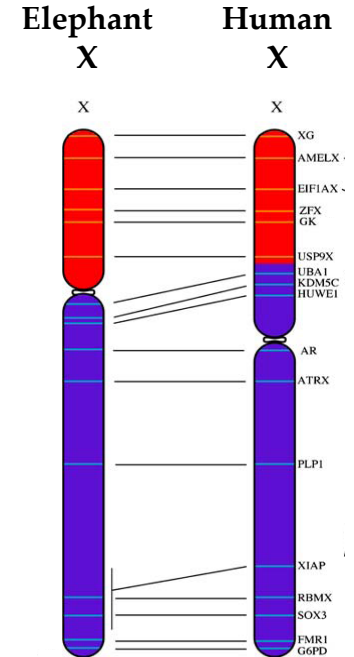
- XX/XY Populations
- ZW/ZZ Populations



*Oreochromis niloticus*

- XX/XY species with influence of the temperature

# Mammals have an **extremely conserved sex chromosome system**



And any modifications generally lead to **infertility**

- Human patients with Klinefelter syndrome (XXY)  
or Turner (XO)





## But some exceptions exist...

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- A dozen of mammalian species escape convention



*Microtus oregoni*  
XO / XY



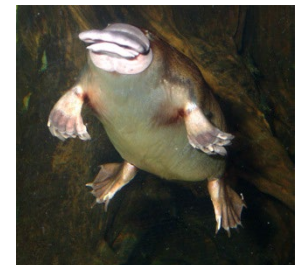
*Dicrostonyx torquatus*  
♀ XY



*Ellobius lutescens*  
XO / XO



*Mus minutoides*  
♀ XY



*Platypus*  
10 sex chromosomes !

- They constitute **invaluable models to better understand sex chromosome evolution and the mammalian sex determining pathway**

# Biological Model

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- African pygmy mouse *Mus minutoides*

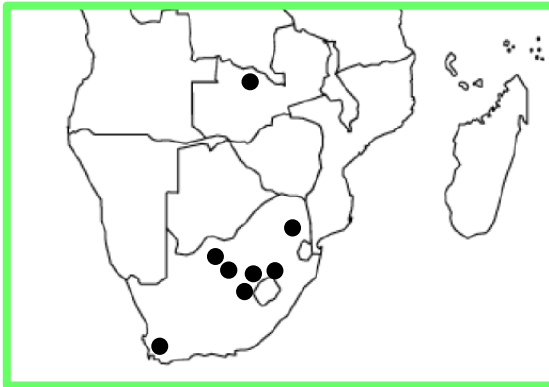
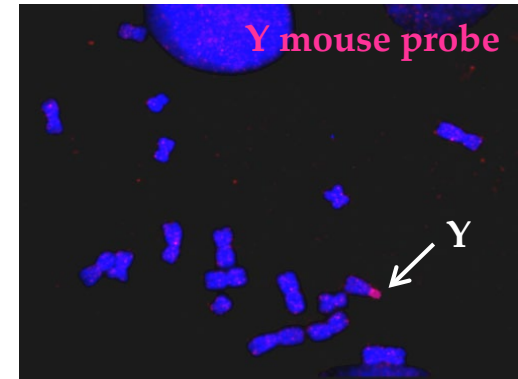
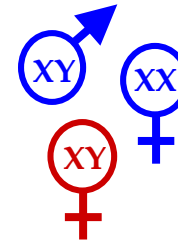


- Same genus as the house mouse

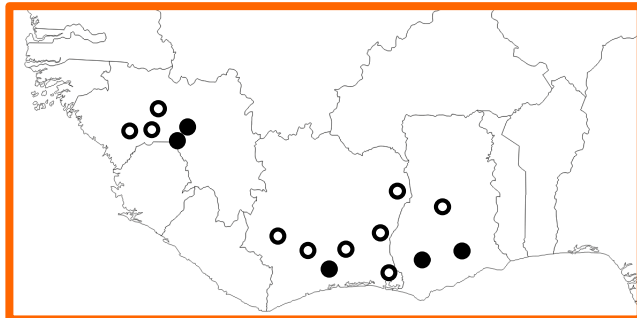


# A novel Sex Determination System

- Very high proportion of fertile sex-reversed females

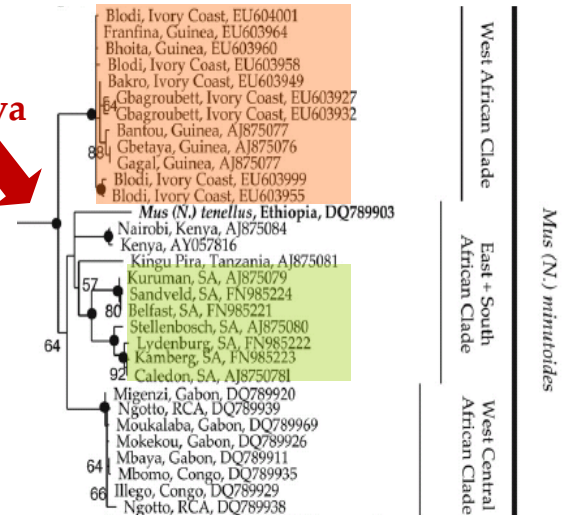


X\*Y females : 29/44 (Southern Africa)



X\*Y females : 10/72 (Western Africa)

0.9 Mya



# A novel Sex Determination System

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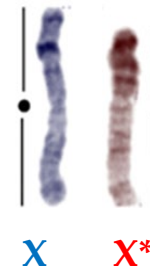
- Sex reversal is not due to a mutation on *SRY* gene nor a Y-linked gene

*SRY* sequencing = male & female have the same haplotype

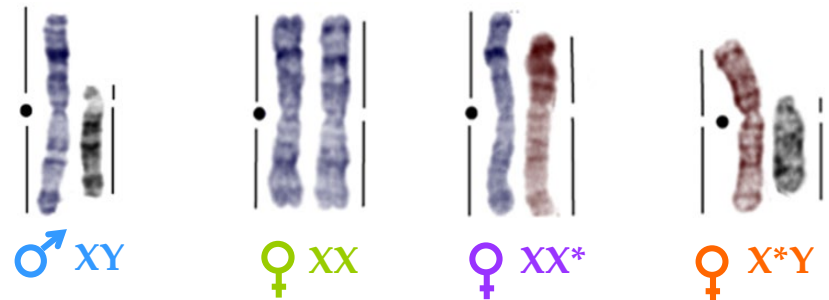
Breeding program = X\*Y females give their Y chromosome to their sons

- ... but rather on a X-linked mutation

Two morphologically different X chromosomes,  
one always associated to sex-reversed females, X\*

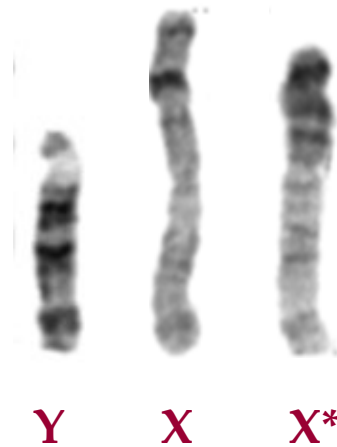


- 1 type of Males, 3 types of Females



# A novel Sex Determination System

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- A **polygenic** sex determination system
  - with **three sex chromosomes**: Y, X and X\*
  - and **two sex determining genes**:
    - the regular mammalian male determinant *Sry* on the Y
    - and a still unknown dominant female determinant on the X\*



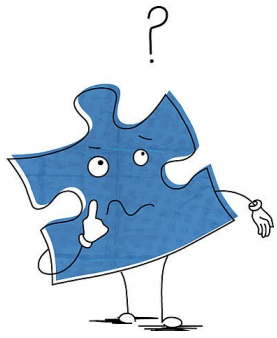
# The breeding colony

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● June 2010:



200 traps, 10 nights of trapping = 13 specimens (8F 5M)



**Evolutionary forces at play**

**Causes & Consequences of this new Sex Determination System**

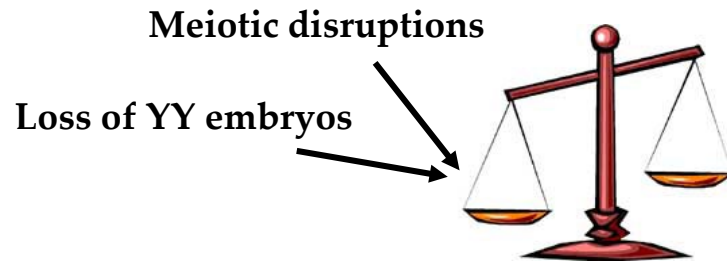
**An Integrative, Multidisciplinary Approach**

**Life History  
Traits &  
Reproduction**

# I. *Life History Traits & Reproduction*

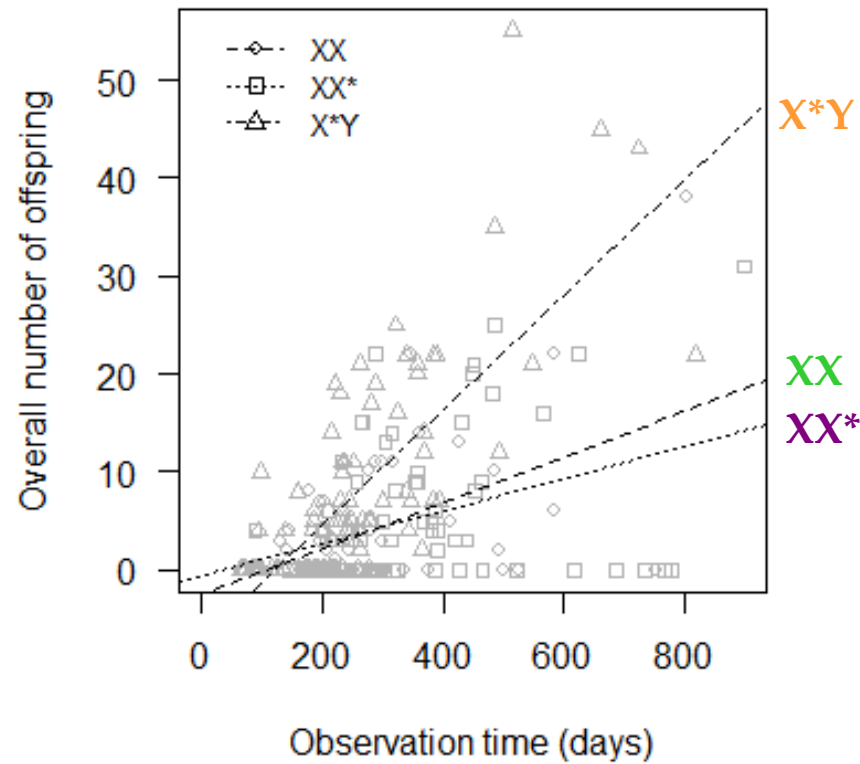
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Evolution of such a system is a paradox



- **Could X\*Y females avoid the expected loss of fertility?**

## Overall number of offspring



- X\*Y females have a better reproductive output than XX and XX\*



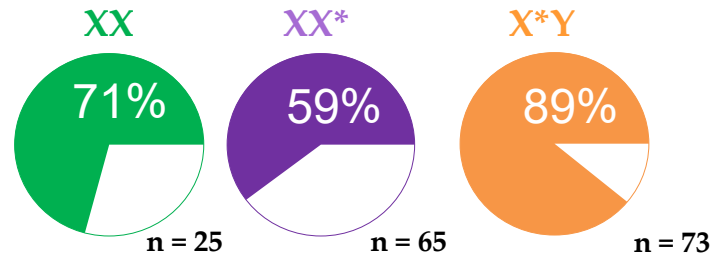
Data on > 500 litters



# Life History Traits & Reproduction

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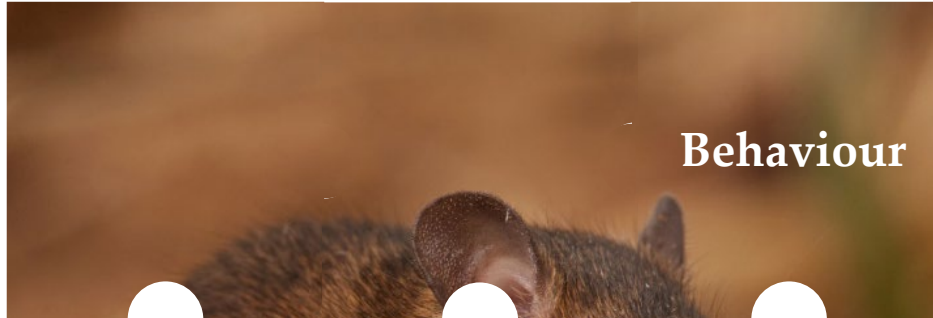
Proportion of females having at least one litter (after 6 months)



- **X\*Y females easily compensate, and even overpass the cost of the embryo's loss**

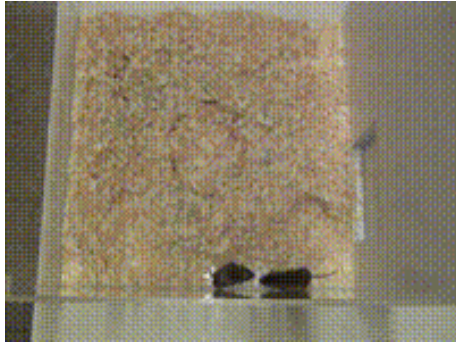


# An Integrative, Multidisciplinary Approach



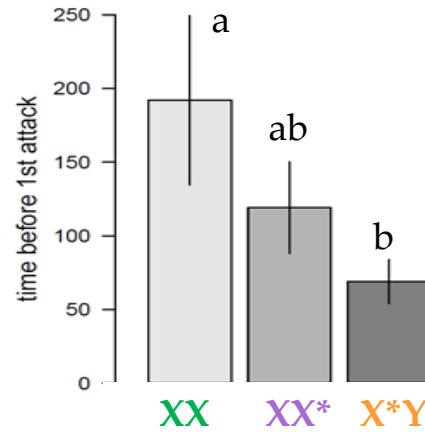
# Behaviour

## Resident-intruder Test

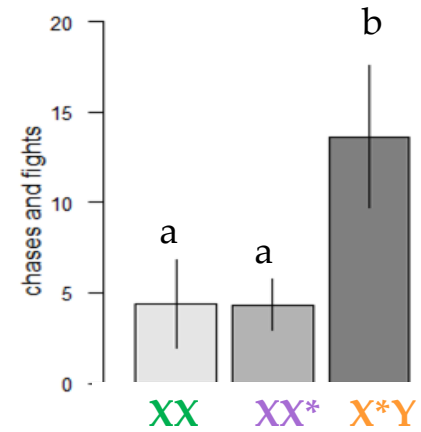


*Aggressiveness & Social interactions*

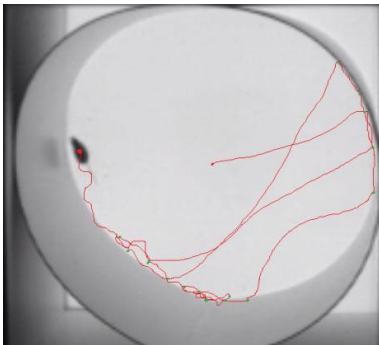
### Time before 1st attack (sec)



### N° of chases and fights

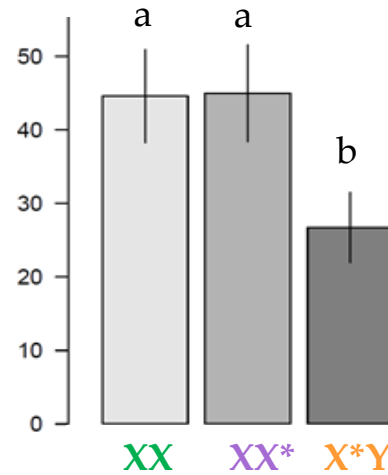


## Open Field

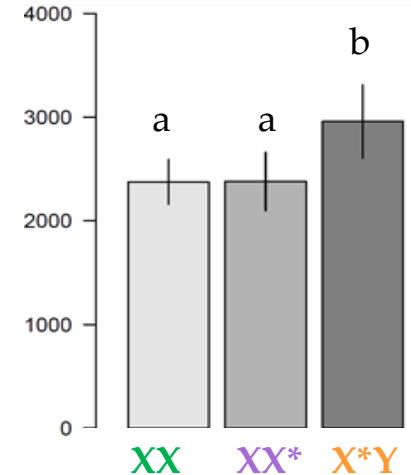


*Anxiety & Motor activity*

### Freezing time (sec)



### Distance travelled (cm)

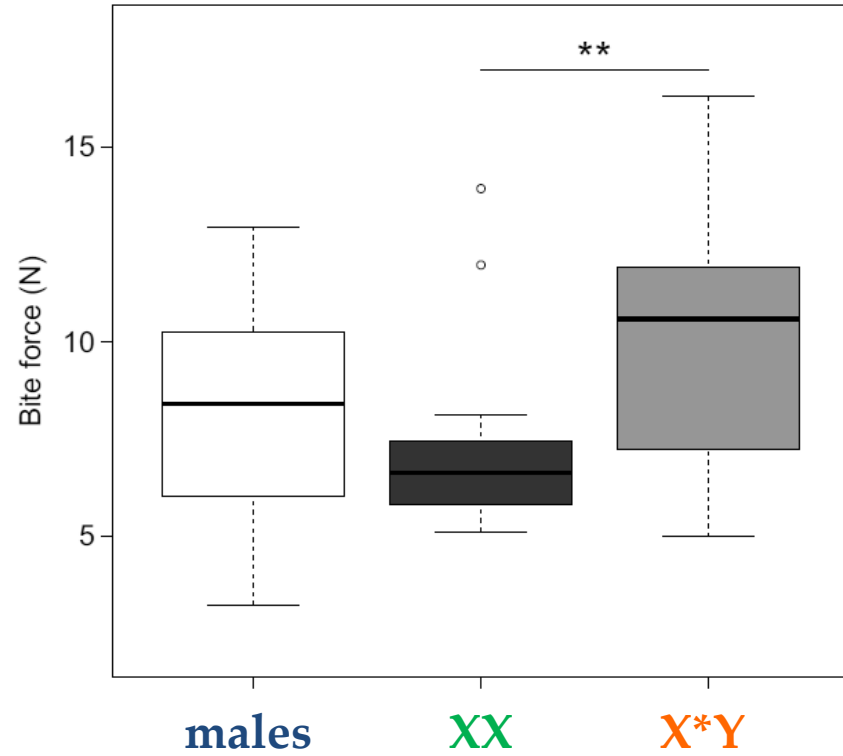


- Masculinised behaviour of X\*Y females

**May suggest Direct effects of sex chromosomes on Behaviour**



Biting force



- Higher bite force in X\*Y females

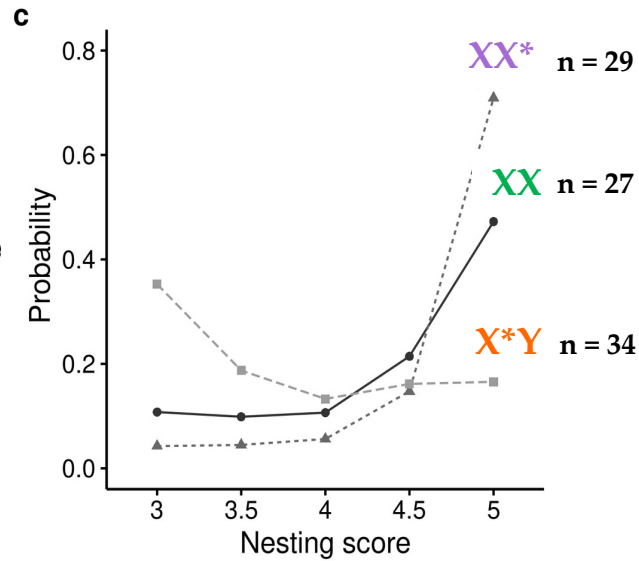
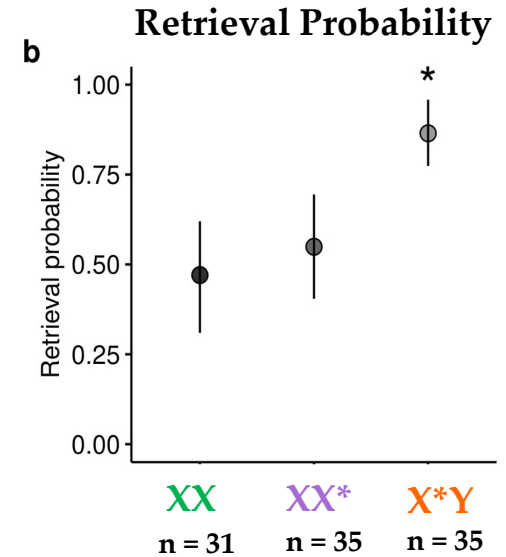
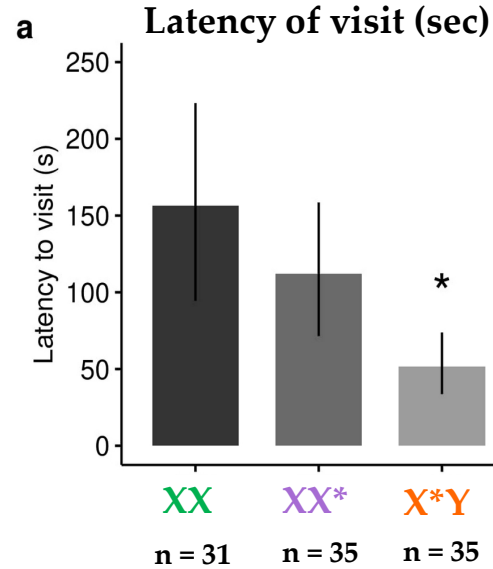
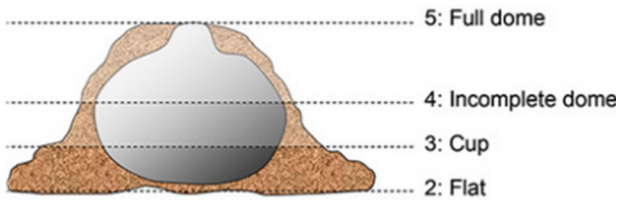


# Parental Care

## Pup retrieving Test



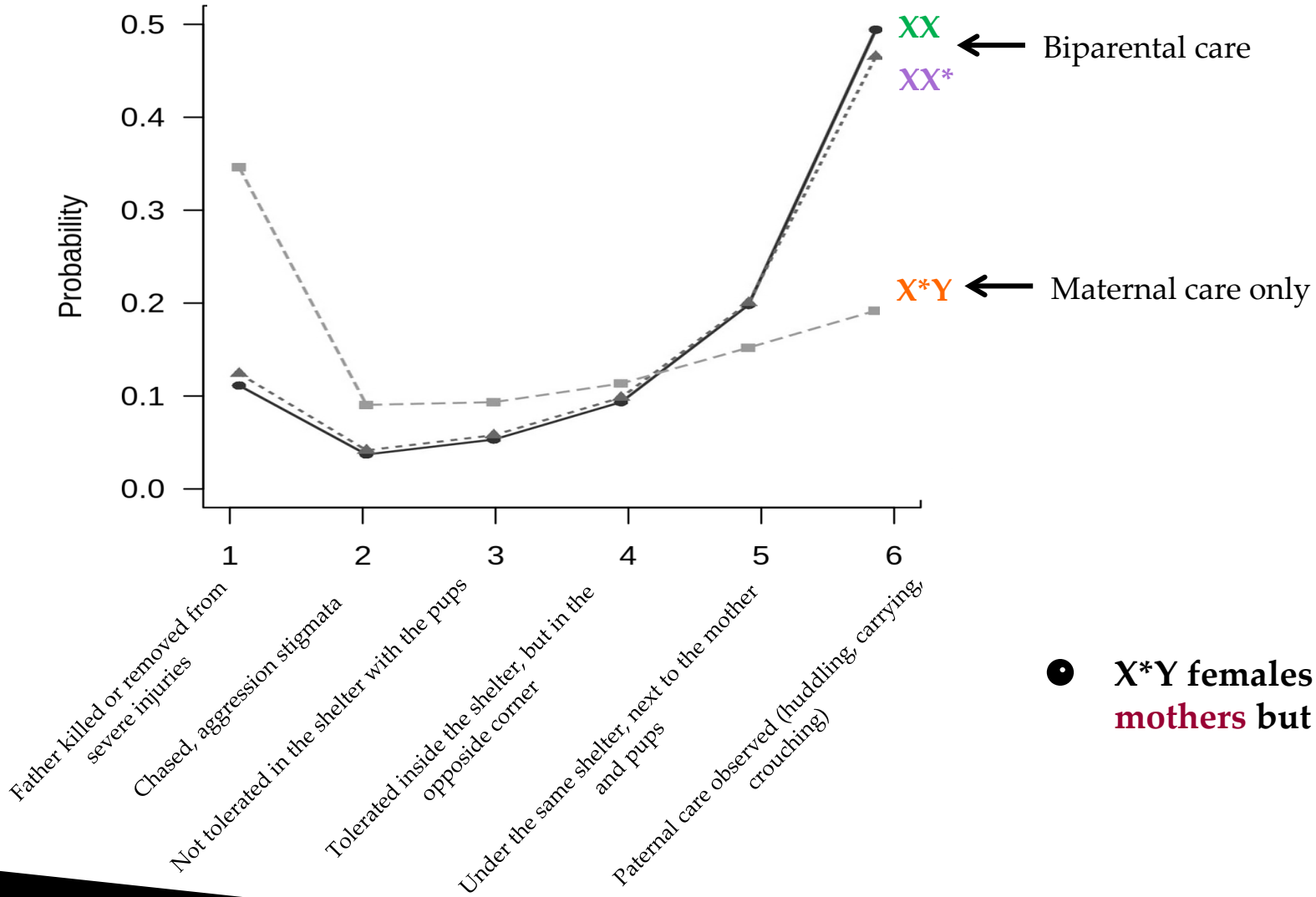
## Nesting Test



● **X\*Y females perform better at retrieving, but are poor nest builders**

# Parental Care

## Maternal Care Strategy (n = 376 observations)



Agonistic Behaviours

# An Integrative, Multidisciplinary Approach



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- **What are the proximal causes of these phenotypic differences?**

**Hormonal concentrations *vs.* sex chromosome effects**

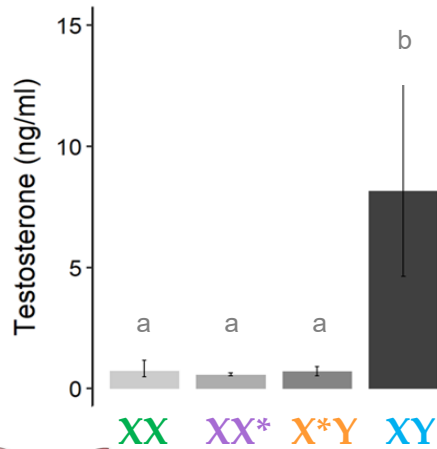



In *M. minutoides*: **Gonadal sex** and **chromosomal sex** are partially uncoupled

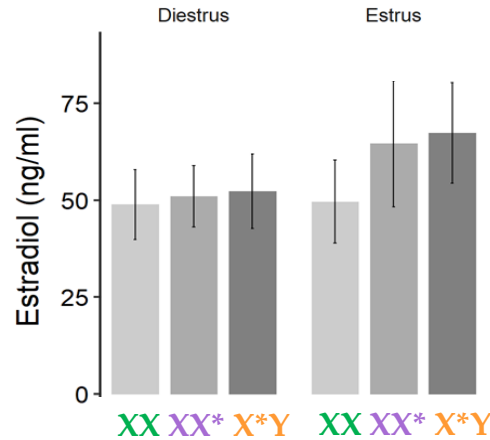
♂	♀	♀	♀
XY	X*Y	XX	X*Y


➔ **Dosage of 3 steroid hormones to disentangle the influence of sex chromosomes and sex hormones on behaviors.**

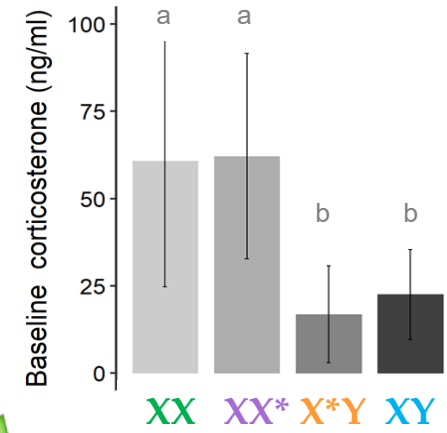
# Hormonal Profiles





 TESTOSTERONE and Aggressiveness of X\*Y



 ESTRADIOL and enhanced reproductive performance and maternal care behaviors of X\*Y



 High CORTICO predicts low fitness, chronic stress in XX & XX\*

 **YES, BUT...**  
Sex chrom. may have an impact on steroids, which in turn may influence behaviors

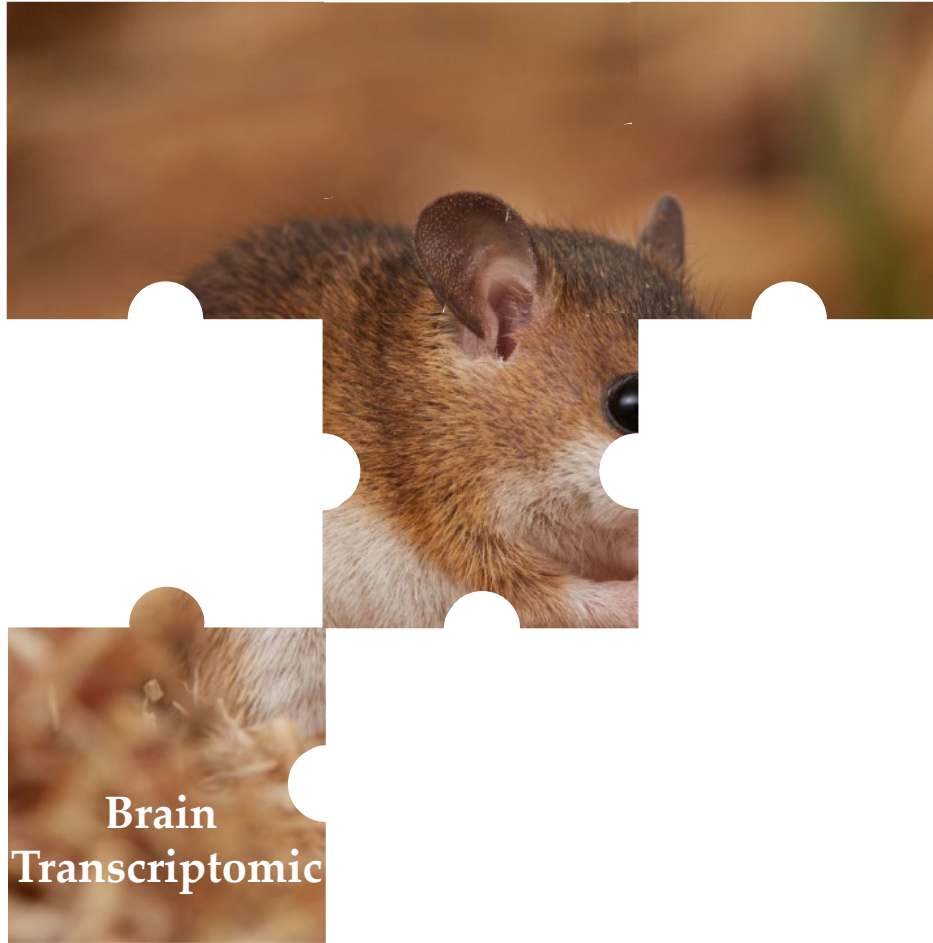
- May suggest that most of the behaviors are driven by sex chromosome factors rather than steroid hormones.
- Need to reconsider the classical dogma of hormone predominance in the induction of sexual dimorphism.

- X\*Y females are systematically different from XX and XX\* females
- The X\* effect goes well beyond sex reversal only, and is at the origin of a third sexual phenotype

- With opposite results : gonad feminization and partial masculinization of behaviors.



# An Integrative, Multidisciplinary Approach



Brain  
Transcriptomic

**Gonadal sex** *vs.* **Chromosomal sex**  
(ovaries or testes) (XX or XY)

- Whole Brain Transcriptomes  
(adults, 5 XX, 5 XX\*, 5 X\*Y, 5 XY)
- > 13 000 genes, of which 1310 are  
**Differentially Expressed genes**



- Hierarchical clustering Method WGCNA  
8 clusters of DE genes



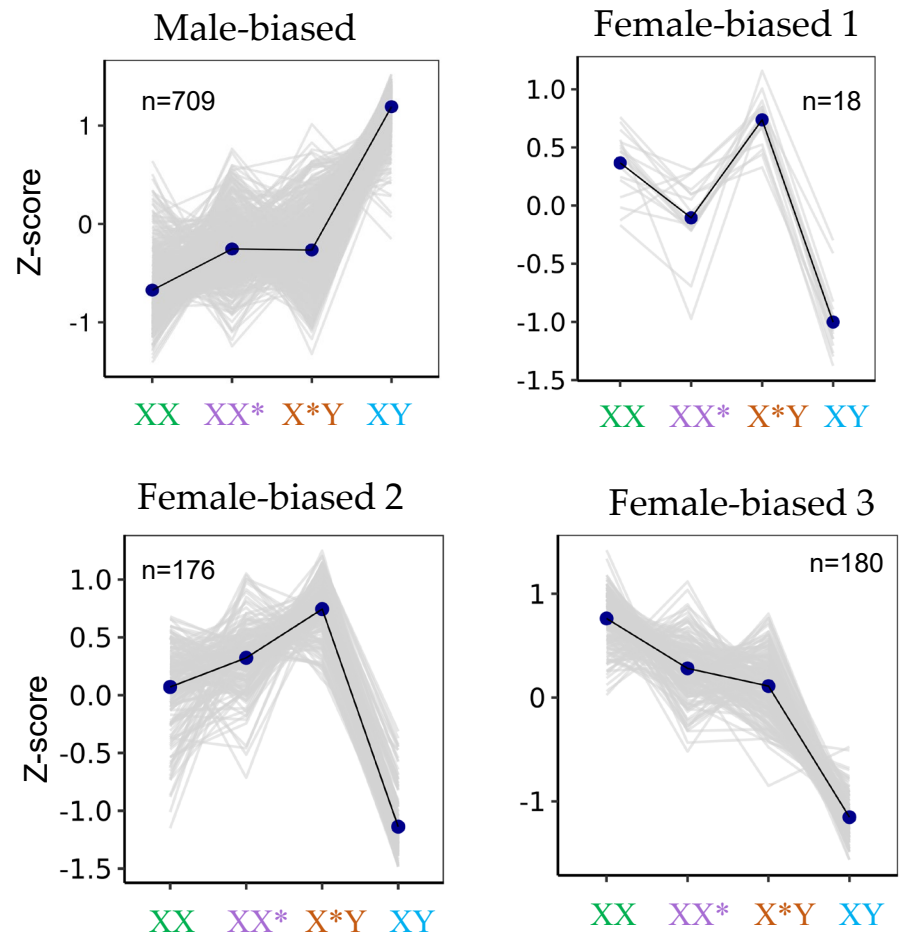
● Hierarchical clustering Method WGCNA

4 clusters correlated to  
**Gonadal Sex**

(~ 83% of DE genes)

=

**Main factor**



● Hierarchical clustering Method WGCNA

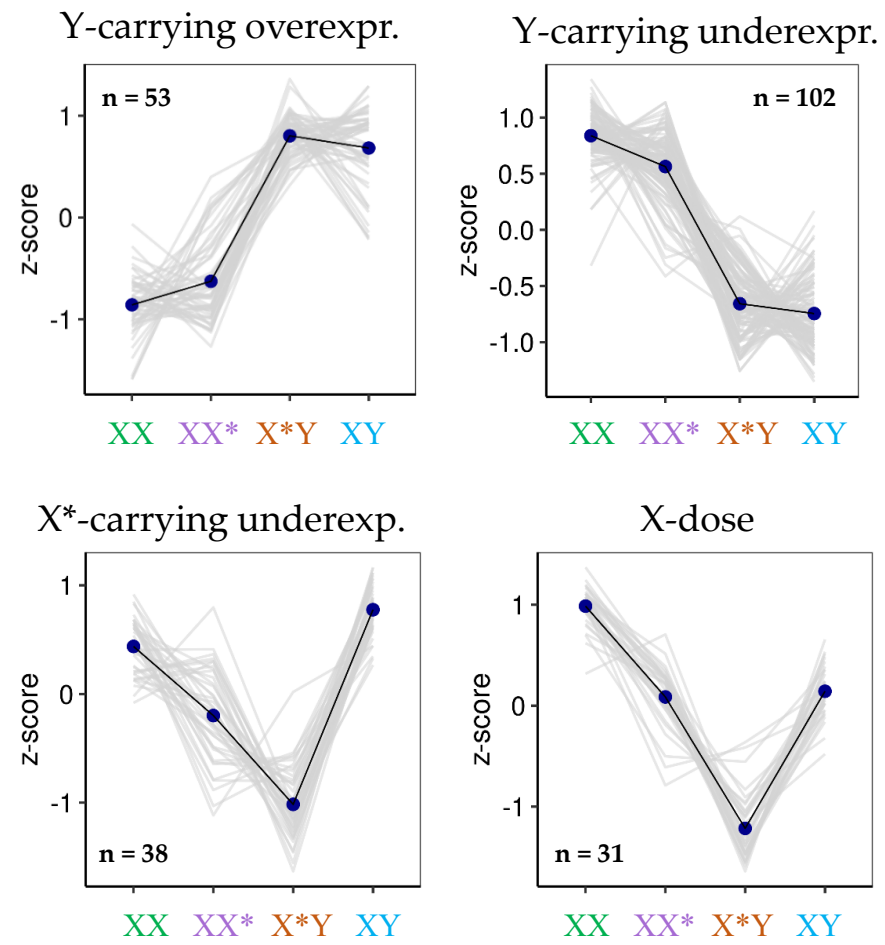
4 clusters correlated to Chromosomal Sex (~ 17% of DE genes)

(of which > 10% XX, XX\* vs. XY, X\*Y)

≠

0,06% of DE genes showed a genotype effect in sex-reversed Rana temporaria frogs (Ma et al. 2018)

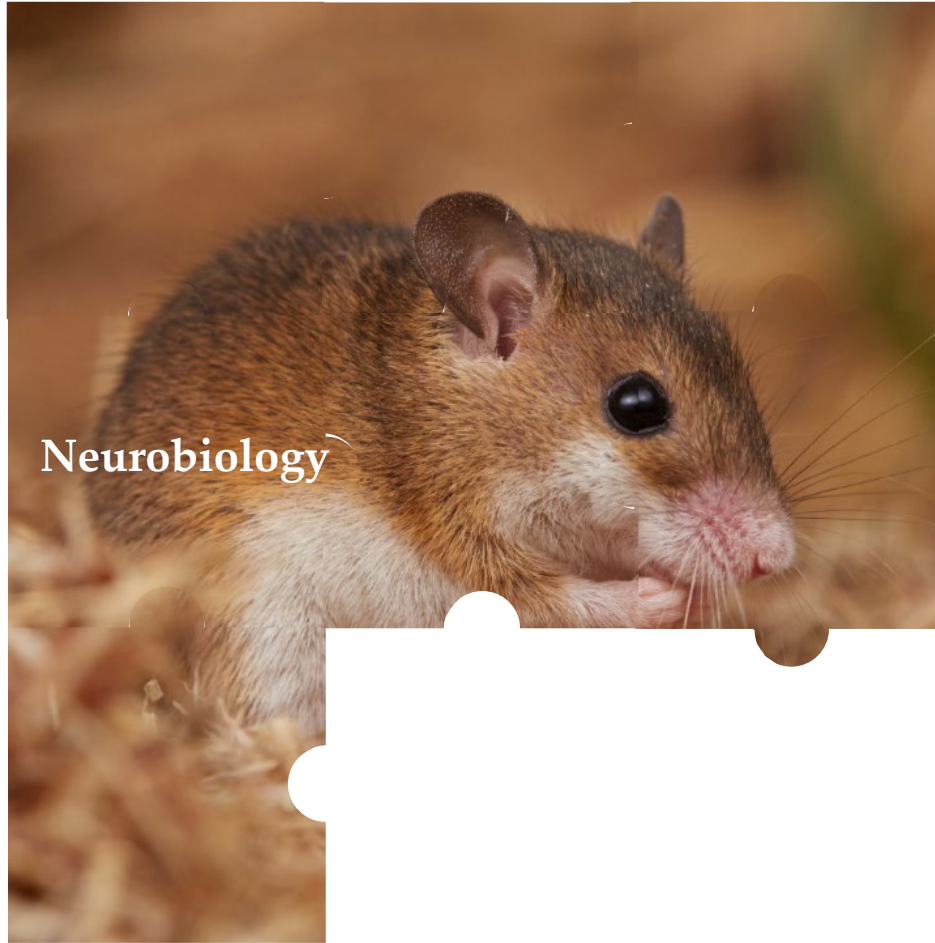
0,02% in sex-reversed transgenic mice (Arnold 2019)



Sex chromosomes impact brain differentiation. Chromosomal sex matters...

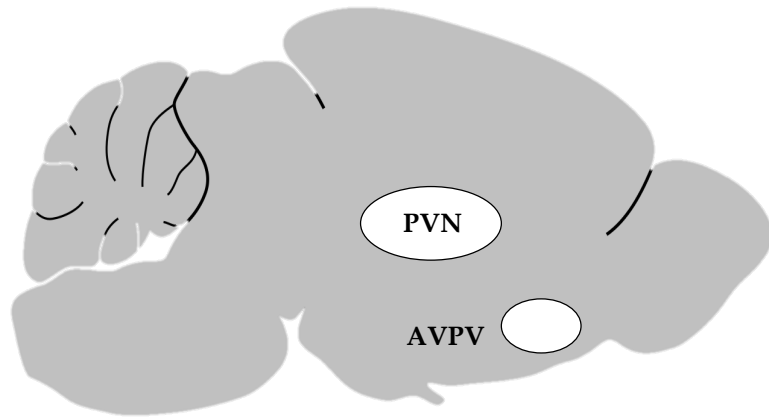


## An Integrative, Multidisciplinary Approach



# Candidate neural circuits involved in behaviours

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**Oxytocin** in PVN → Pup retrieving  
→ Maternal Aggression

**Vasopressin** in PVN —| Nest building

**Thyrosin Hydroxylase (Th)** in AVPV → Pup retrieving

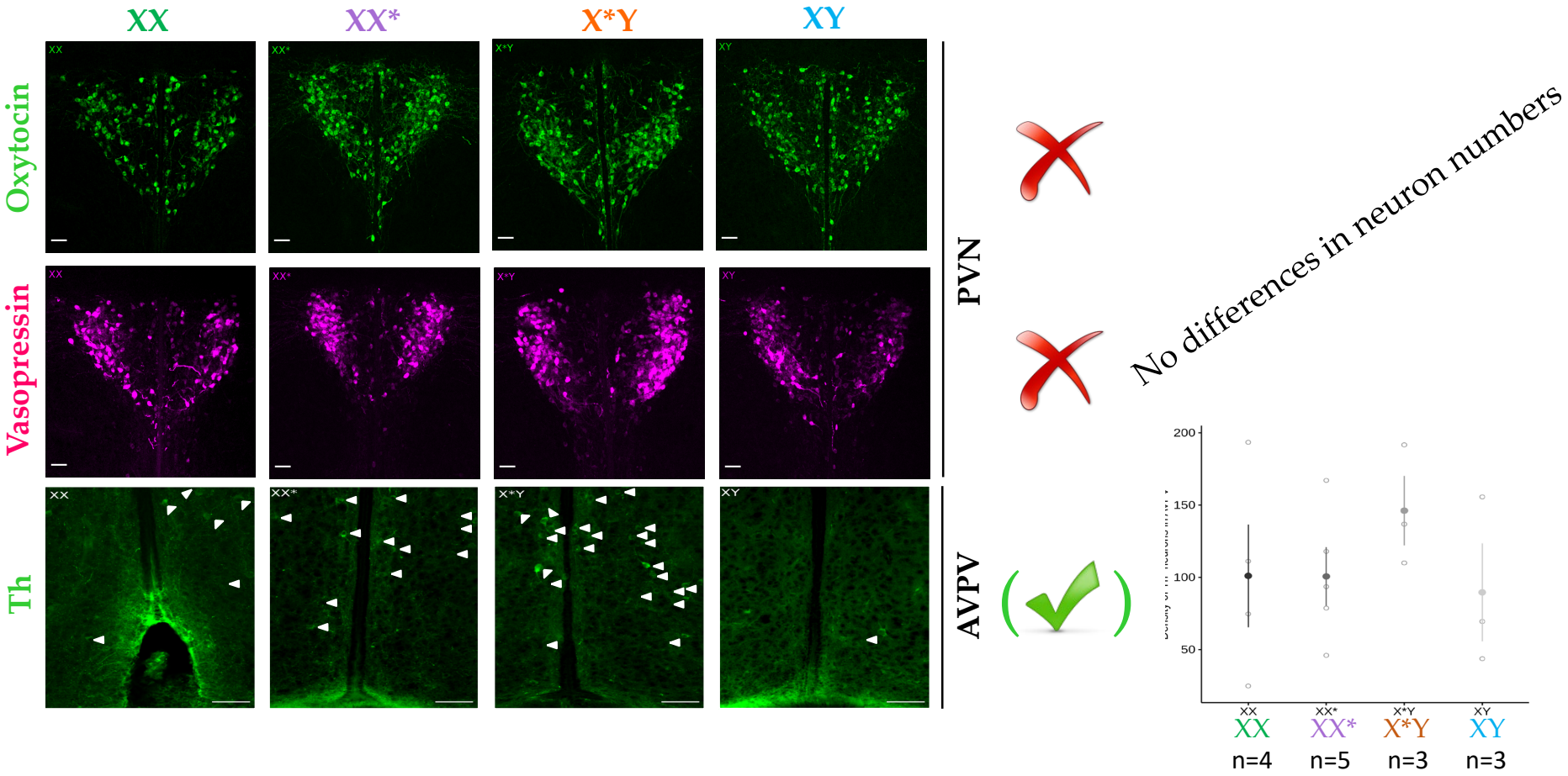
Scott *et al.* 2015; Bendesky *et al.* 2017

## ● Predictions

Greater number of **Oxytocin**, **Vasopressin** & **Th** neurons in X\*Y

# Candidate neural circuits involved in behaviours

- Immunostaining and neuron counts



- Tendency for greater density of Th neurons in X\*Y ➡ Pup retrieving



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