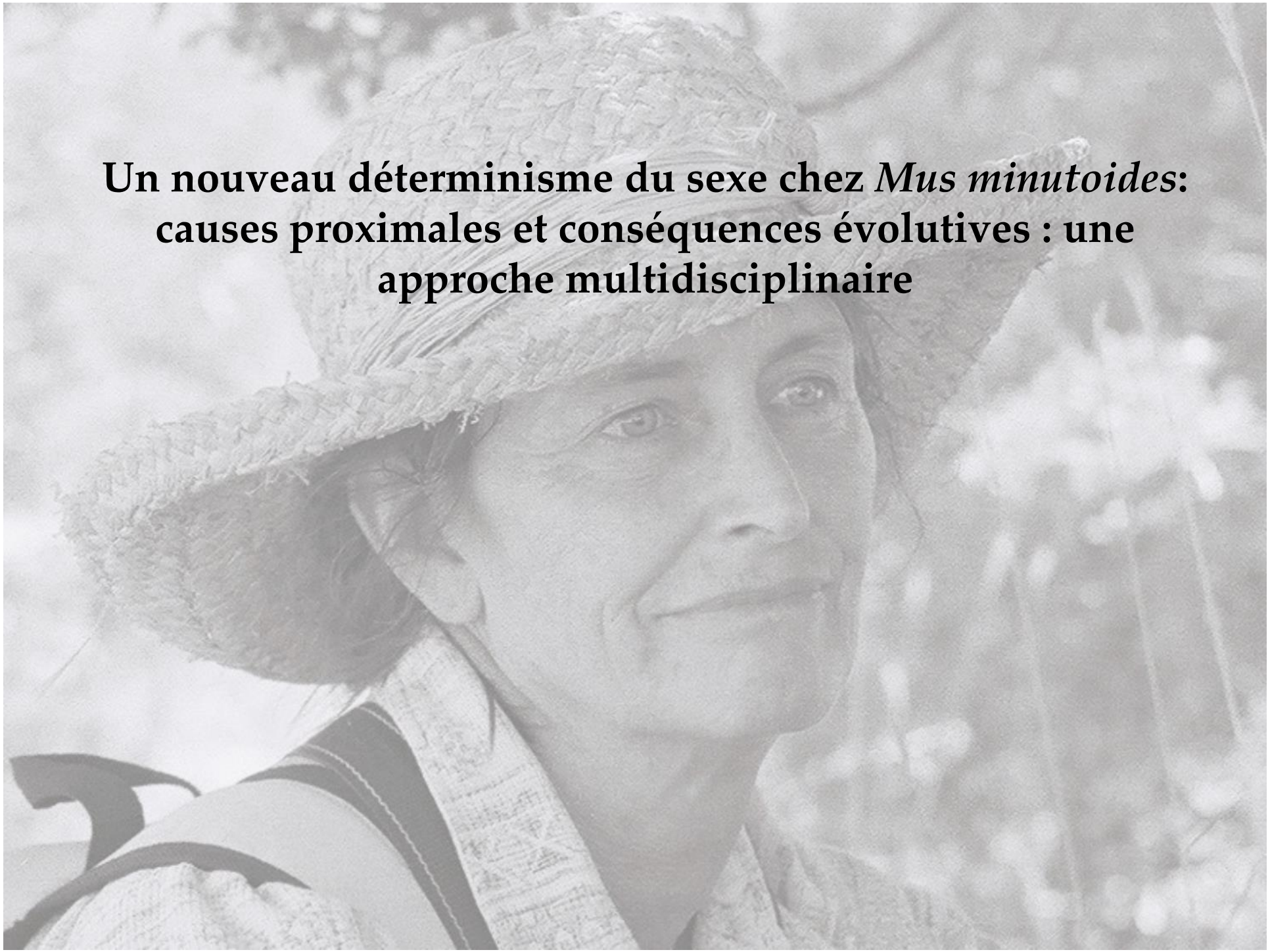


Un nouveau déterminisme du sexe chez *Mus minutoides*: causes proximales et conséquences évolutives : une approche multidisciplinaire

Sex determination is a fundamental process, but mechanisms are diverse

In many taxa: high rates of turnover

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

Rana rugosa
- XX/XY Populations
-ZW/ZZ Populations

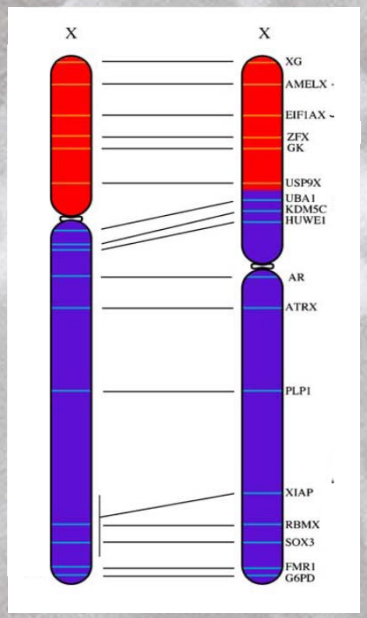
Oreochromis niloticus
- XX/XY species with influence of the temperature

…in others: highly conserved

♂ XY ♀XX

♂ ZZ ♀ZW

Mammals have an extremely conserved sex chromosome system



And any modifications generally lead to sterility

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

But some exceptions exist…

- A dozen of mammalian species that represent Darwinian paradoxes

Microtus oregoni
XO / XY

Dicrostonyx torquatus
♀ XY

Ellobius lutescens
XO / XO

Ornithorhynchus anatinus

X1X1X2X2X3X3X4X4X5X5 / X1Y1X2Y2X3Y3X4Y4X5Y5

- They constitute invaluable models to better understand standard mammalian sex determination

Biological Model

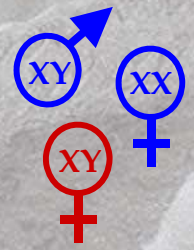
- **African pygmy mouse *Mus minutoides***

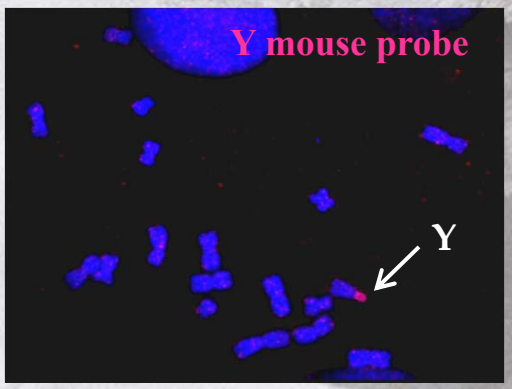
- Same genus as the laboratory mouse

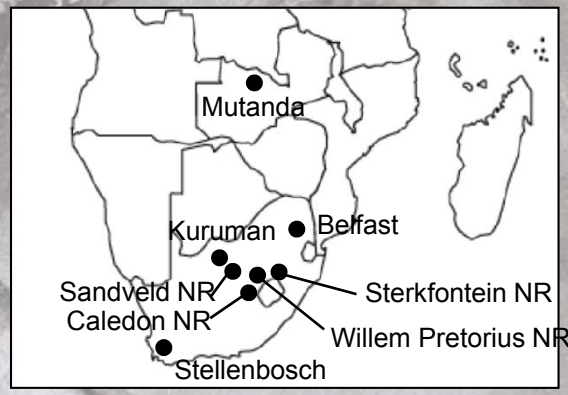
Britton-Davidian et al 2012, *Acta Oecol*

A novel Sex Determination System (1)

- Very high proportion of fertile sex-reversed females







Localities	Females XY	Females XX	Males XY
Belfast	0	1	0
Caledon NR	15	4	14
Kuruman	1	0	1
Mutanda	2	0	1
Sandveld NR	1	1	3
Stellenbosch	3	4	3
Sterkfontein NR	2	0	1
Willem Pretorius NR	0	1	0
Total	**24**	**11**	**23**

Veyrunes et al *Proc R Soc B* 2010

A novel Sex Determination System (2)

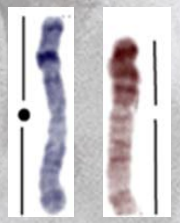
- Sex reversal is not due to a mutation on *SRY* gene nor a Y-linked gene

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

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

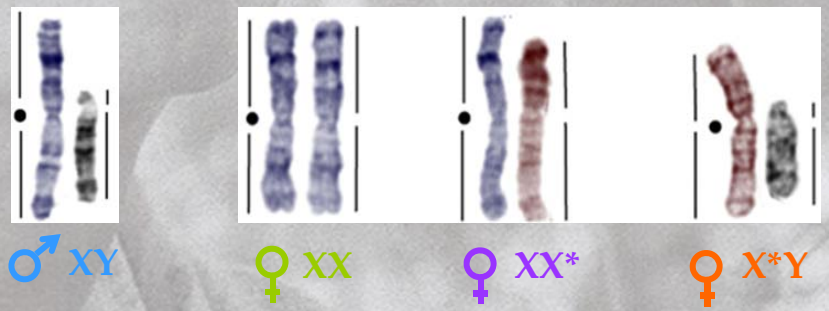
- … but rather on a X-linked mutation

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



X X*

- 1 type of Males, 3 types of Females

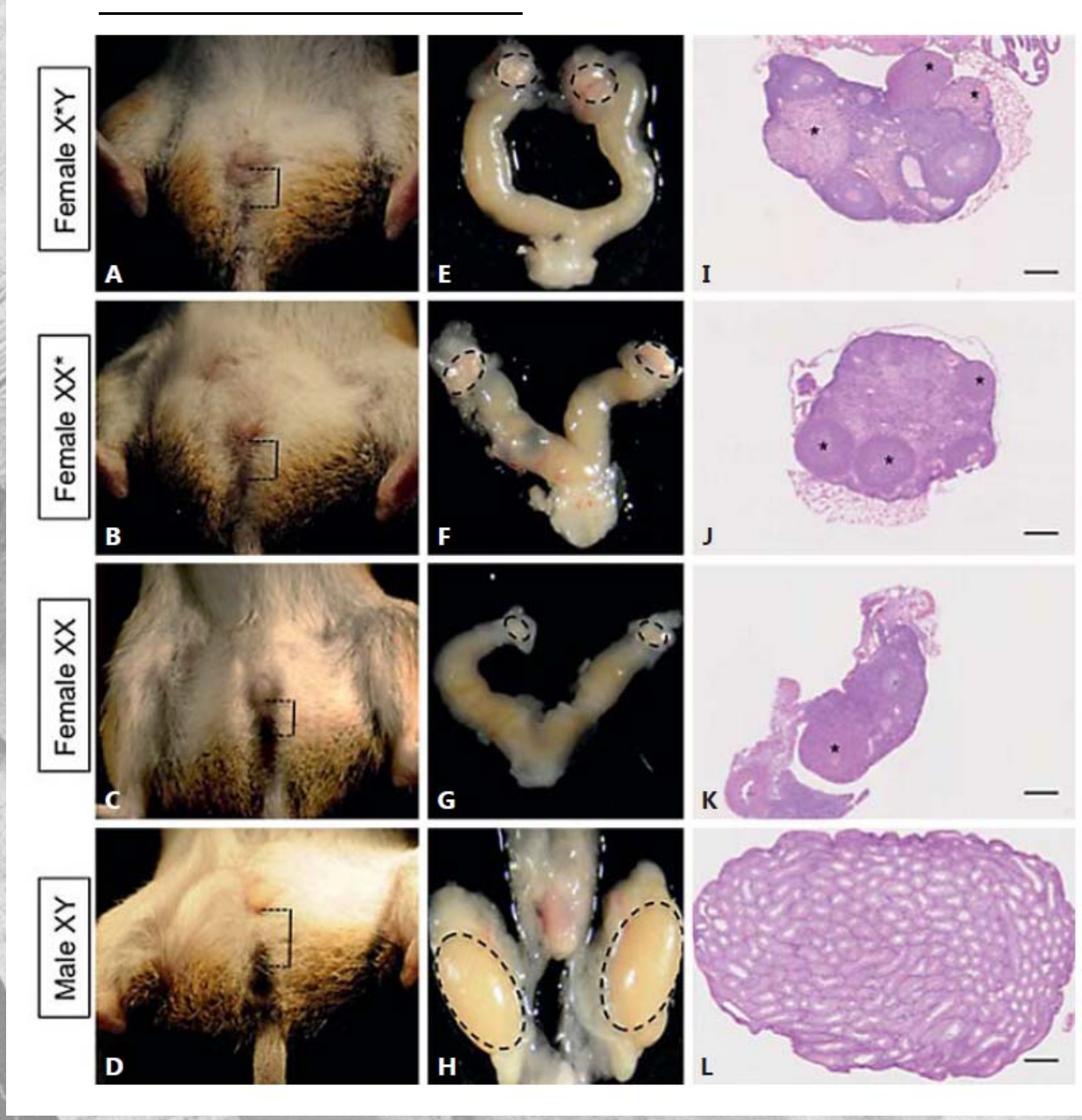


♂ XY ♀ XX ♀ XX* ♀ X*Y

Veyrunes et al *Proc R Soc B* 2010

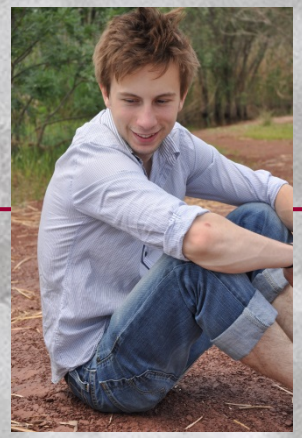
Anatomical analyses of XY ovaries:

- Complete sex-reversed phenotype of X*Y females



Rahmoun et al. (2014) *Sex Dev*

Turnover in SDS: evolution, characterization of the genetic basis



Paul Saunders
(PhD student)

- **Evolutionary Approach**

Evolution of such system is a paradox

Loss YY

Fitness advantage?

Genomic conflicts?

BREEDING PROGRAM

- Life History Traits (reproduction)
- Behavioral ecology
- Sex chromosome transmission

- **Functional Approach**

Identification of the mutation

TEST ON CANDIDATE GENES

- Expression patterns of sex-reversal genes
- ID chromosomal rearrangement changing the X into X*

The breeding program

- June 2010:

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

Evolutionary Approach: Life History Traits (1)

Loss of YY

Fitness advantage?

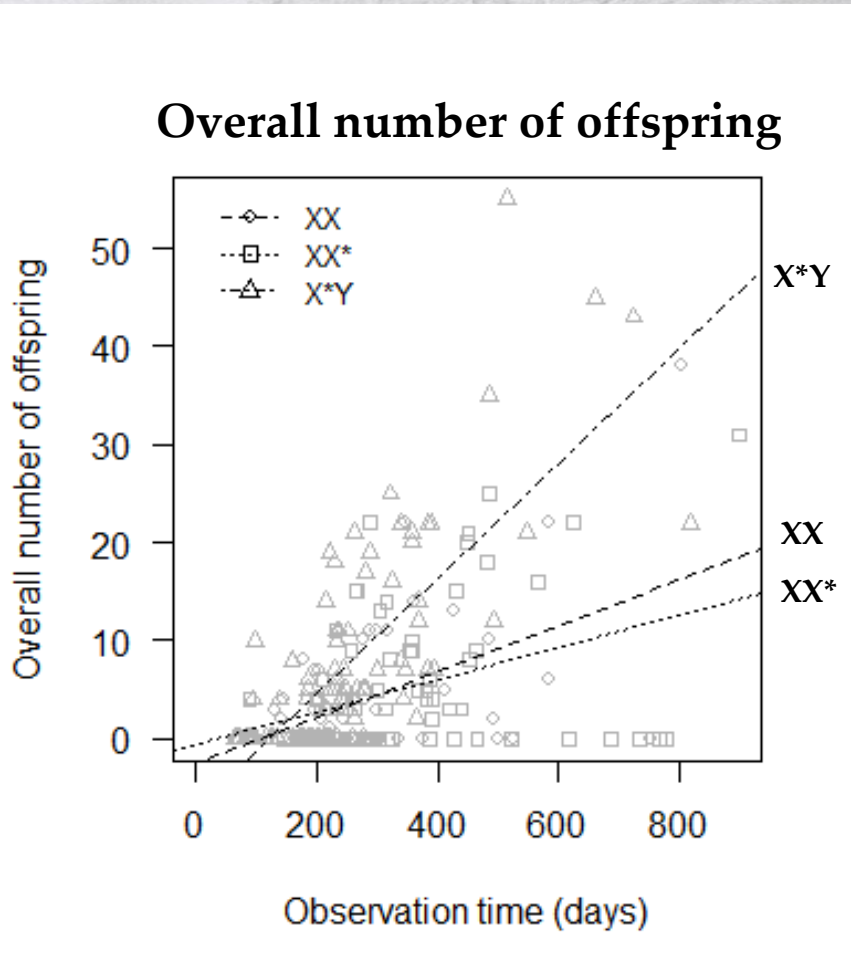
- **Could X*Y females avoid the expected loss of fertility?**

Bull & Charnov 1977, Van Doorn & Kirkpatrick 2007, 2010

Evolutionary Approach: Life History Traits (2)

Fitness advantage?

Loss of YY



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

Saunders et al. (2014) *Evolution*

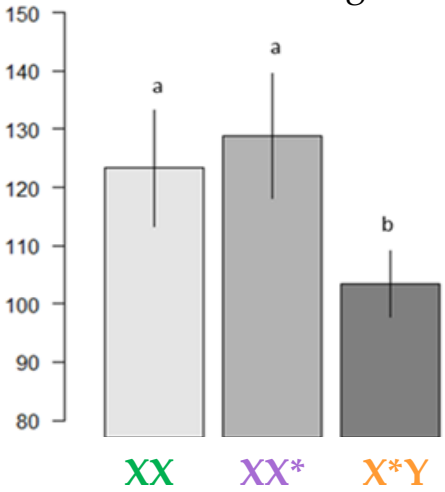
Evolutionary Approach: Life History Traits (3)

Fitness advantage?

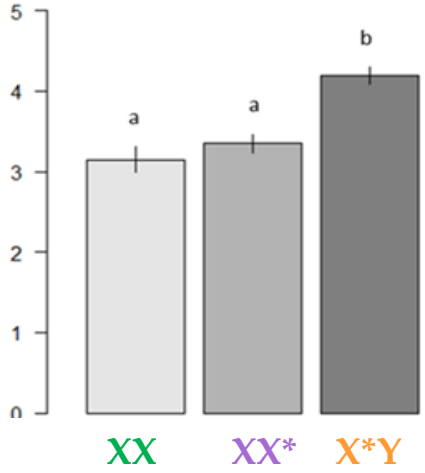
Loss of YY

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

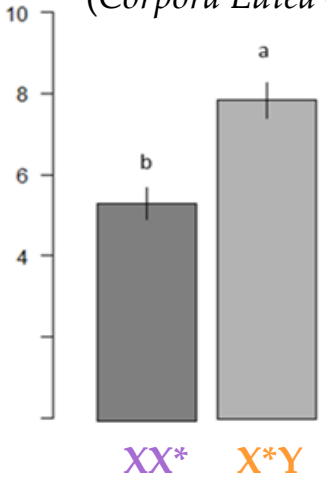
First litter age



Mean litter size



Ovulation rate
(*Corpora Lutea* count)

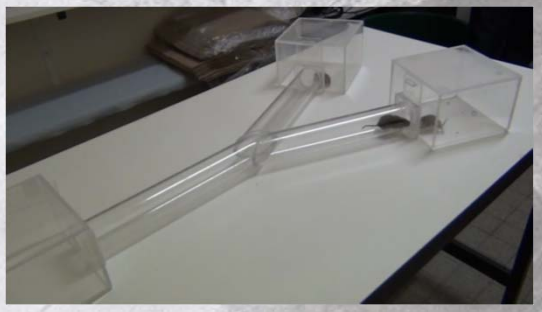


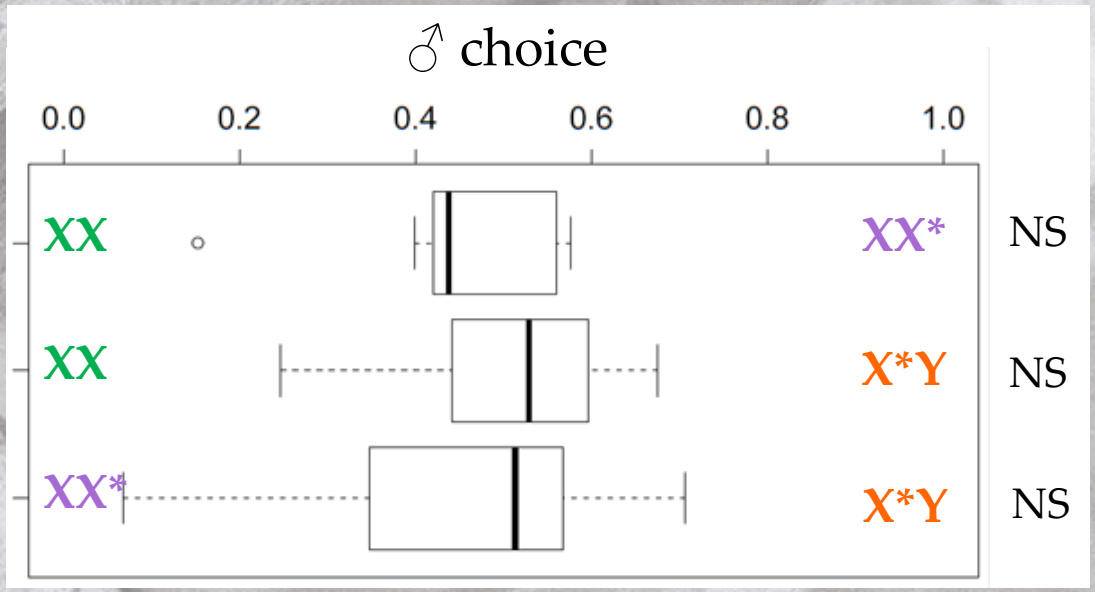
- X*Y females :
  - **More females succeed to breed**
  - **1st reproduction earlier (20 days)**
  - **Larger litters (almost one more pup)**
  - **One and a half times more ova per cycle**

Saunders et al. (2014) *Evolution*

Evolutionary Approach: Behaviour (1)

- **Do ♂ prefer X*Y ♀?**



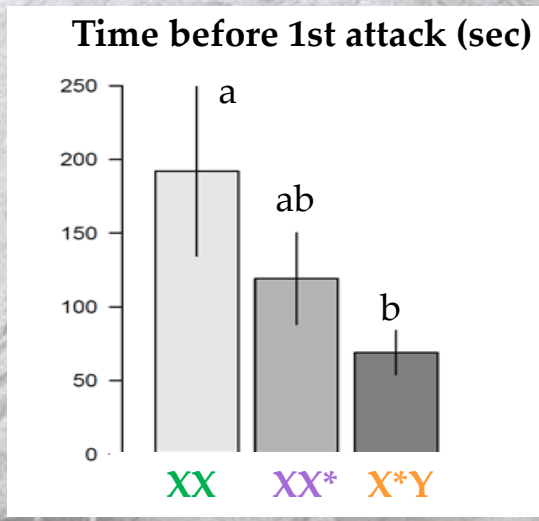


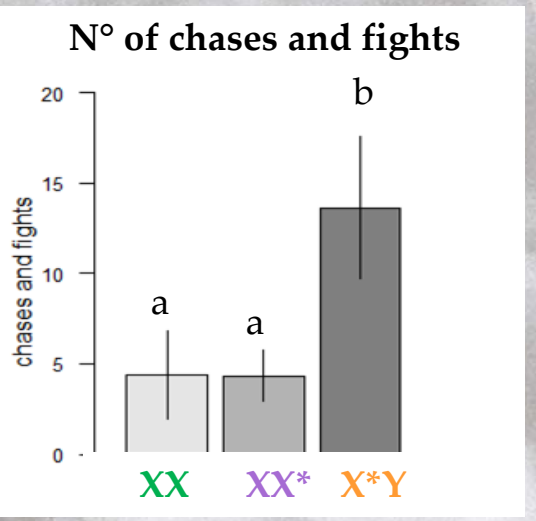
Saunders et al. (2015) *Scientific Reports*

Evolutionary Approach: Behaviour (2)

Resident-intruder Test

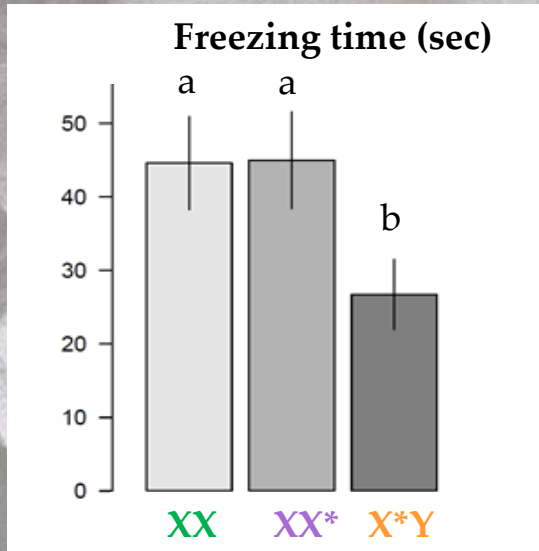
Aggressiveness & Social interactions

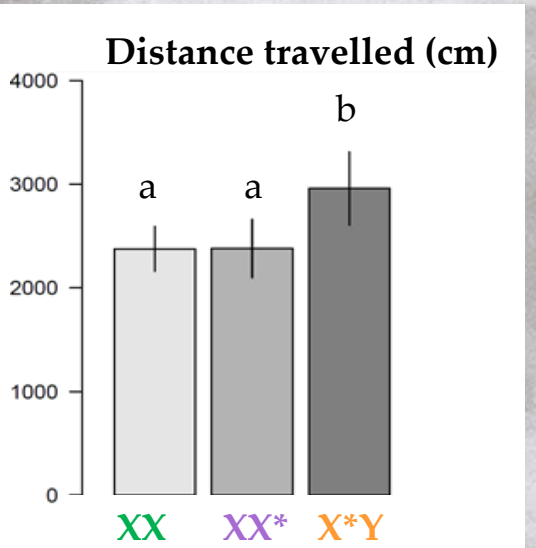




Open Field

Anxiety & Motor activity



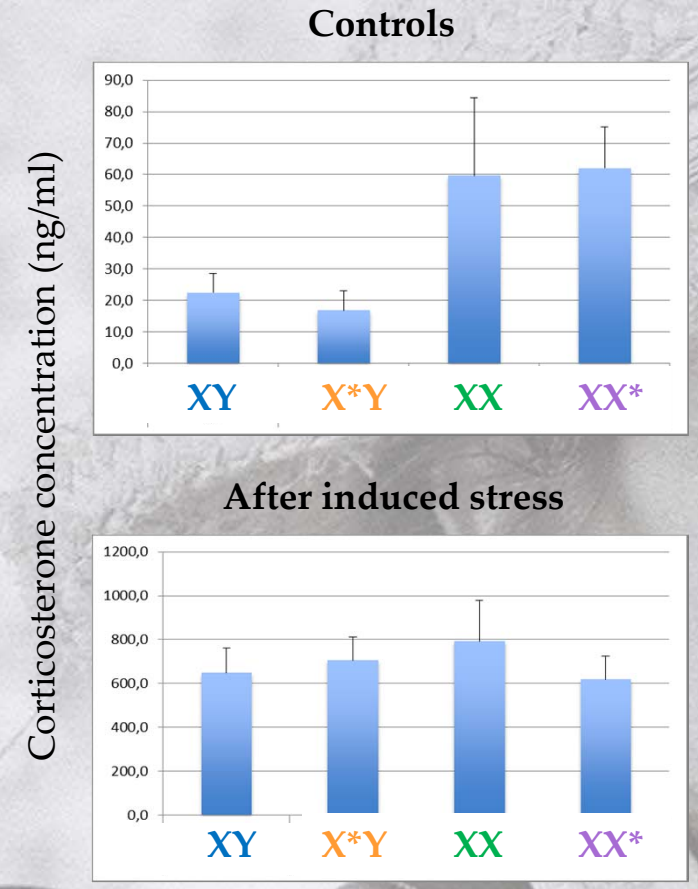


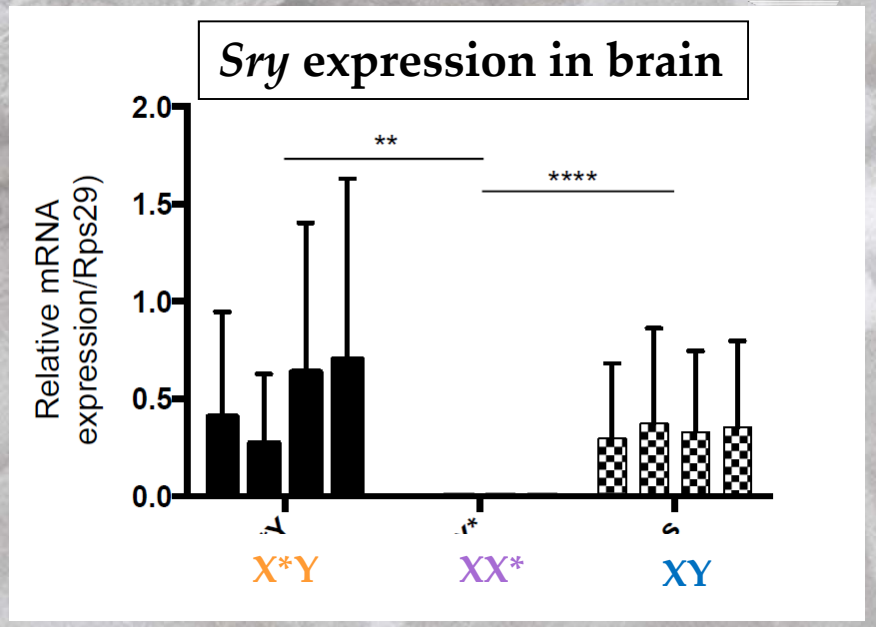
- **Masculinised behaviour of X*Y females**
- **Direct of effects of sex chromosomes on Behaviour**

Saunders et al. (2015) *Scientific Reports*

Evolutionary Approach: Behaviour & Hormones and Gene Expressions

Corticosterone level





- Correspondance between anxiety test and Cortico level (involved in stress response)
- Sry in brain has been shown to influence aggressiveness (Guillot et al. 1995)
- To do list: testosterone dosage, expression of *Maoa*, …

Life History Traits & Behaviour

Loss of YY

Fitness advantage?

- Whereas in mammals, sex-reversed individuals are usually sterile
- In *Mus minutoides*, X*Y females do better than XX and XX*
- **Fitness advantage of emerging genotypes hypothesis**
- Masculinised behaviour of X*Y females
- Direct of effects of sex chromosomes on Behaviour

Sex-ratio

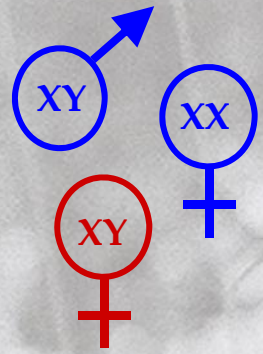
Genomic conflict?

Loss of YY

X* : female biased sex-ratio

- **Could the X* have evolved in response to a selfish genetic element ?**

Werren & Beukeboom 1998, Kozielska et al. 2010



Sex-ratio

Genomic conflict?

Loss of YY

XX

XY		X
	X	♀
	Y	♂

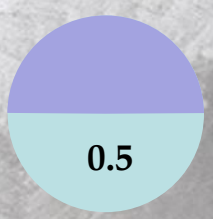
XX*

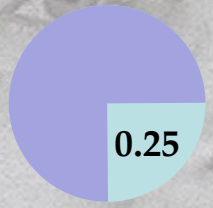
	X*	X
X	♀	♀
Y	♀	♂

X*Y

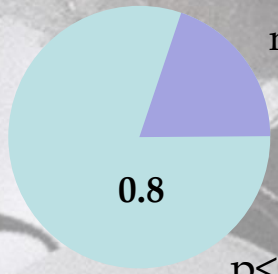
	X*	Y
X	♀	♂
Y	♀	†

Expected sex-ratios (Mendelian segregation):



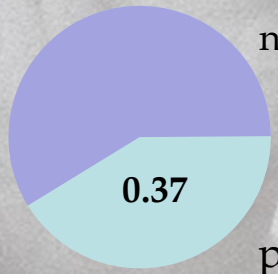


Observed sex-ratios :



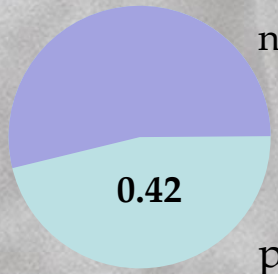
n=206

p<0.001



n=370

p<0.001



n=670

p<0.001

➡ More ♂ than expected : sex chromosome transmission distortion

Sex chromosome transmission (1)

Genomic conflict?

Loss of YY

XX

♂ \ ♀	X
X	♀ XX
Y	♂ XY

XX*

♂ \ ♀	X*	X
X	♀ XX*	♀ XX
Y	♀ X*Y	♂ XY

X*Y

♂ \ ♀	X*	Y
X	♀ XX*	♂ XY
Y	♀ X*Y	†

Sex chromosome transmission (1)

Genomic conflict?

Loss of YY

XX

♂ \ ♀	X
X	43
Y	163

XX*

♂ \ ♀	X*	X
X	37	52
Y	135	142

X*Y

♂ \ ♀	X	
X	248	83
Y	119	†

- **Transmission distortion in males (p<0.001):**

Y transmitted in 79% **76%**

Sex chromosome transmission (1)

Genomic conflict?

Loss of YY

XX

♂ \ ♀	X
X	43
Y	163

XX*

♂ \ ♀	X	X*
X	37	
Y		

X*Y

♂ \ ♀	X*	Y
X	248	283
Y	139	†

- **Transmission distortion in males (p<0.001):**

Y transmitted in 79% **76%** **36%**

Sex chromosome transmission (1)

Genomic conflict?

Loss of YY

XX

♂ \ ♀	X
X	43
Y	163

XX*

♂ \ ♀	X*	X
X	37	52
Y	135	142

X*Y

♂ \ ♀	X*	Y
X	248	283
Y	139	†

- **Transmission distortion in males (p<0.001):**

Y transmitted in 79% **76%** **36%**

- **No transmission distortion in females (50/50)**

→ First documented ♀ genotype-dependent distorsion

Genomic conflicts between the sex chromosomes

Sex chromosome transmission (2)

Genomic conflict?

Loss of YY

- Yes, presence of genomic conflicts,
  with sex chromosome transmission distorsion in ♂

- An analytical approach suggests that this peculiar distortion would allow the evolution and maintenance of the X*

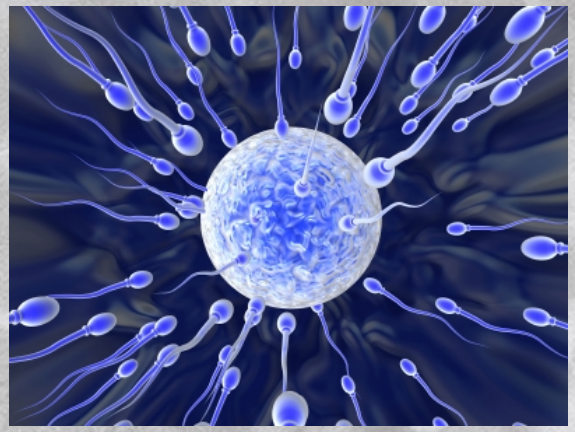
- Modeling and simulation analysis combining empirical data to address questions of formation/fixation/evolution of this atypical SDS

Sex chromosome transmission (2)

Genomic conflict?

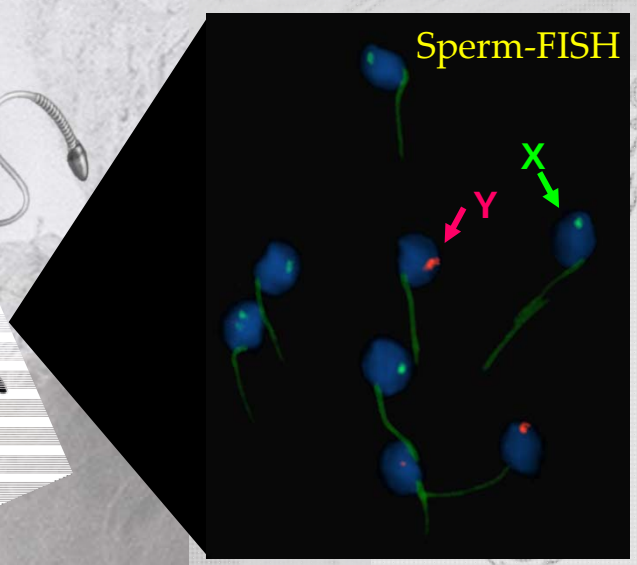
Loss of YY

- What are the proximal causes of the ♀-dependent transmission distortion?



Chronology of the Conception

Male genital tract	Female tract, pre-zygotic	Female tract, post-zygotic



50:50 transmission to female

(capacitation)

rate

≠ embryonic mortality

≠ juvenile mortality

Chronology of the Conception

Male genital tract	Female tract, pre-zygotic	Female tract, post-zygotic

Meiotic Drive

Sex-ratio in Embryos
~10-15 dpc

XX* mother =0,38 (n=109)
X*Y mother =0,42 (n=113)

Sex-ratio at weaning

XX* mother =0,37 (n=370)
X*Y mother =0,42 (n=670)

Bimodal test: p>0,9

(capacitation)

≠ juvenile mortality

Chronology of the Conception

Male genital tract	Female tract, pre-zygotic	Female tract, post-zygotic

N° of expulsed ovocytes = N° embryos
⟶ NO loss of embryos

Sex-ratio in Embryos
~10-15 dpc

XX* mother =0,39 (n=27)

≠ embryonic mortality

≠ fecondation rate

≠ sperm transport
(capacitation)

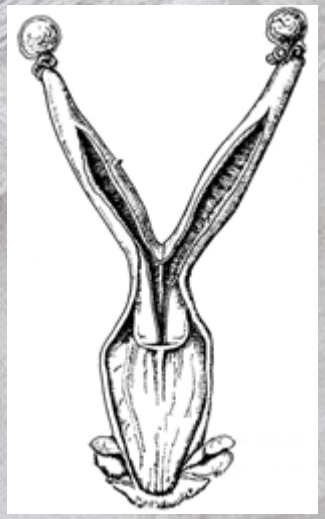
≠ juvenile mortality

Chronology of the Conception

Male genital tract	Female tract, pre-zygotic	Female tract, post-zygotic

Meiotic Drive

≠ fecondation rate



≠ sperm transport (capacitation)

Fecondation In Vitro
Manuel Avilés - U. de Murcia

Tunnel assay

≠ embryonic mortality

≠ juvenile mortality

Turnover in SDS: evolution, characterization of the genetic basis

- **Evolutionary Approach**

Loss YY

Fitness advantage?

Genomic conflicts?

Evolution of such system is a paradox

Massilva Rahmoun (postdoc)

- **Functional Approach**

Identification of the mutation

TEST ON CANDIDATE GENES

- Expression patterns of sex-reversal genes
- ID chromosomal rearrangement changing the X into X*

Functional Approach:

Identification of the sex-reversal Mutation

➢ Expression of genes involved in sex determination

Molecular & Cellular Biology

qRT-PCR

IF

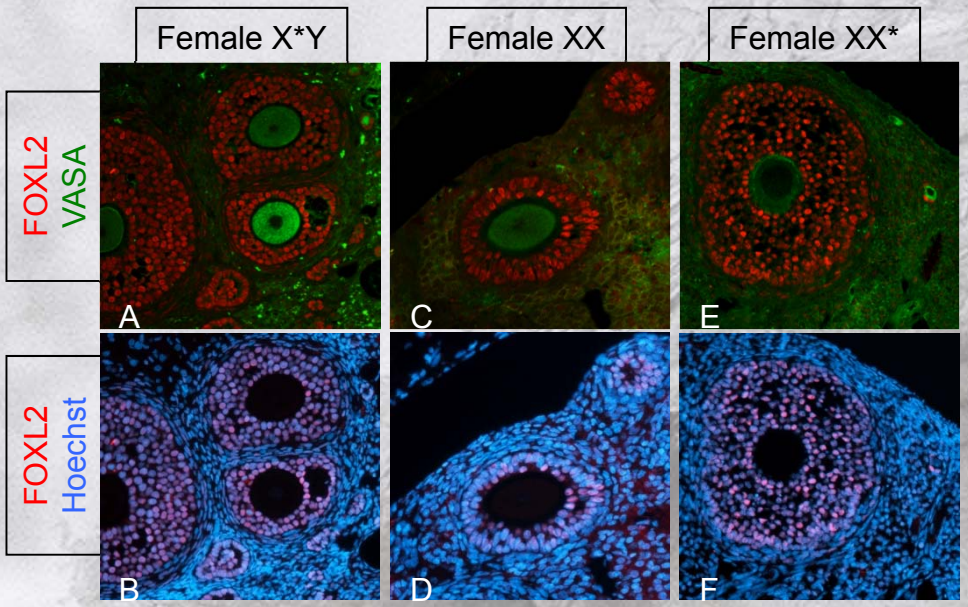
➢ Identification of the chromosomal rearrangement changing X into X*

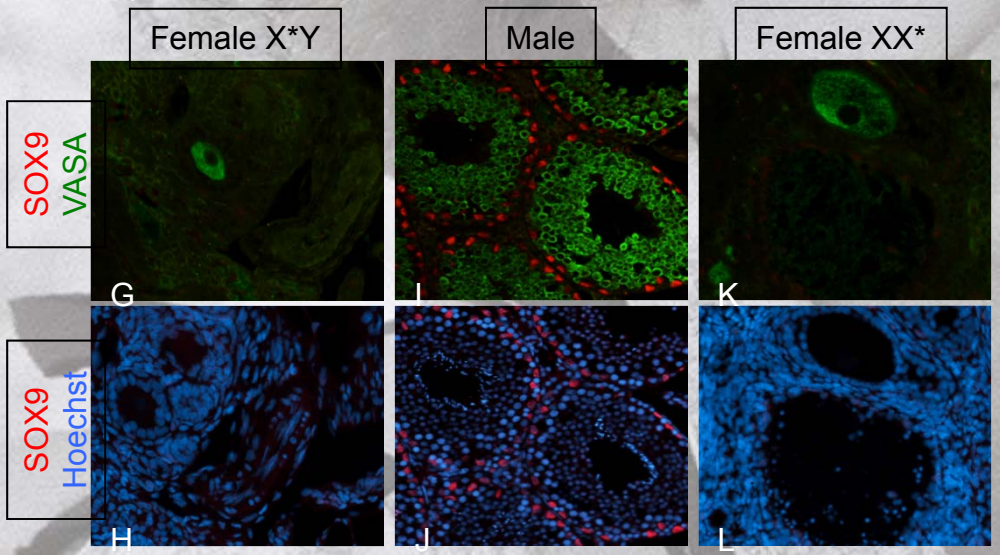
Cytogenomics

Bac-mapping (deletions, inversions…)

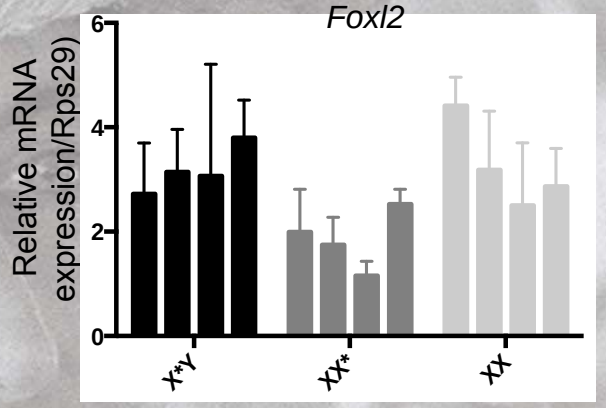
CGH-array (deletions, duplications…)

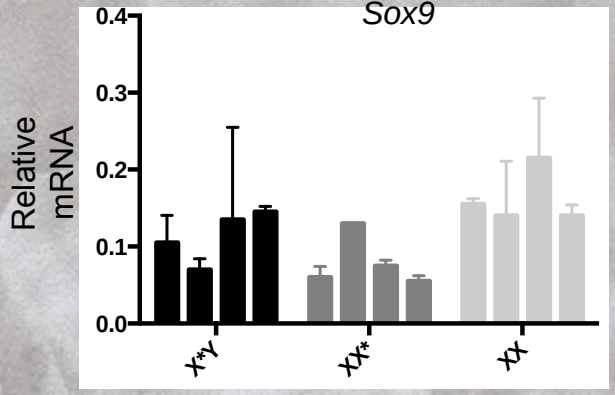
Functional Approach: Molecular & Cellular Biology (1)





- Expression of key sex differentiation genes in adult gonads

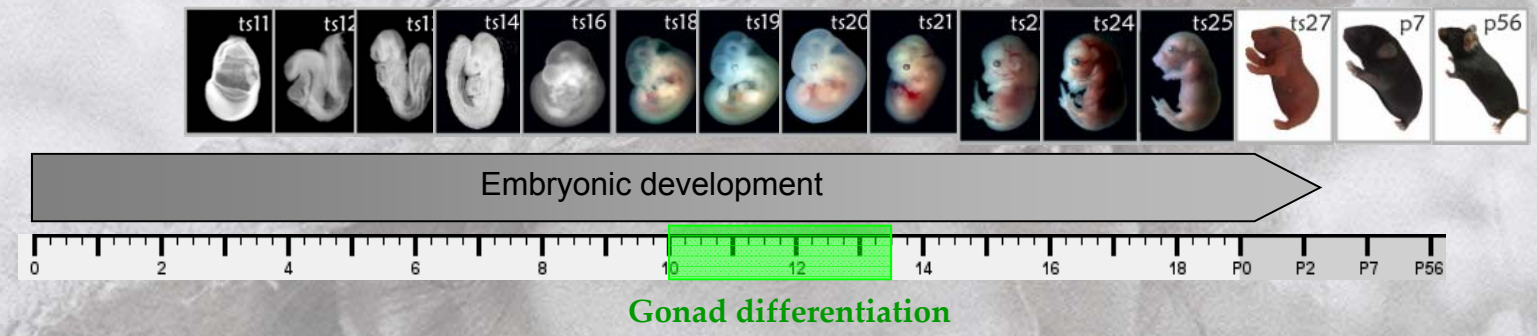


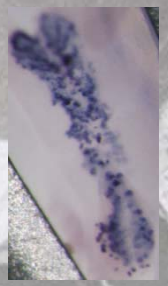


Rahmoun et al. (2014) *Sex Dev*

Functional Approach: Molecular & Cellular Biology (2)

- Expression of known key genes at different stages in testes and ovaries from 11.5 to 14 dpc





XX → Rspo1 → Wnt4, Stra8 FoxL2 →

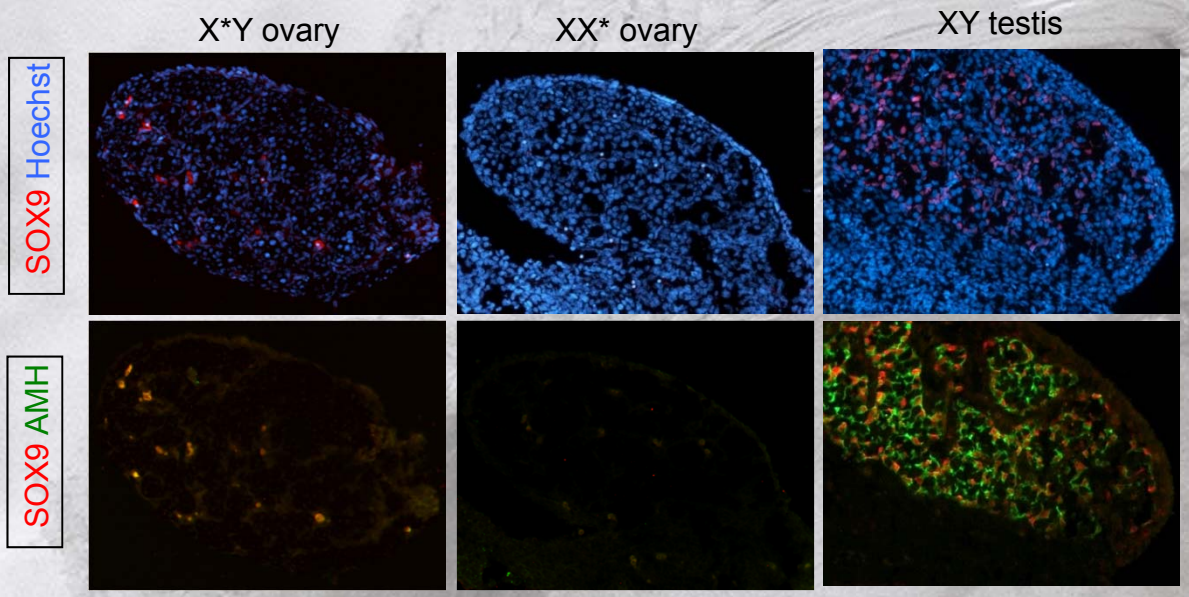
ovaries

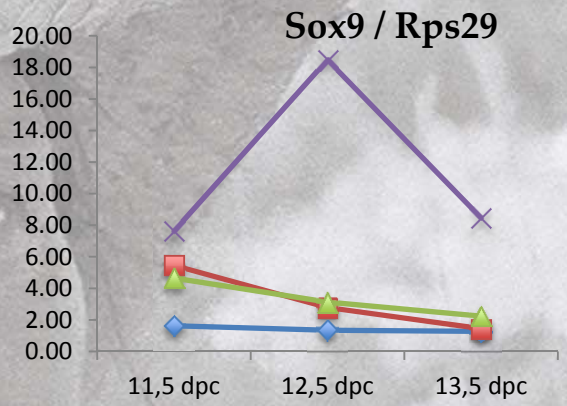
XY → Sry → Sox9 → Amh …

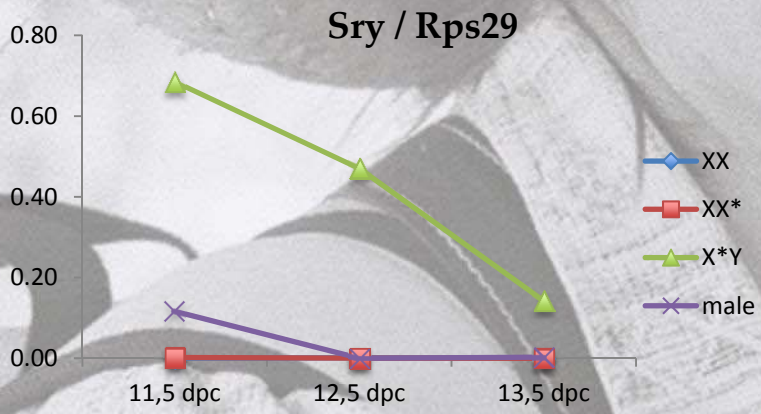
testes

Functional Approach: Molecular & Cellular Biology (3)

Embryonic gonads at 12.5 dpc







- All these data confirm that male pathway is not triggered in X*Y gonads
- This suggests that the mammal X carries one gene (still unknown) as important as *Sry* for the testis determining cascade

Functional Approach:

Identification of the sex-reversal Mutation

➢ Expression of genes involved in sex determination

Molecular & Cellular Biology

qRT-PCR

IF

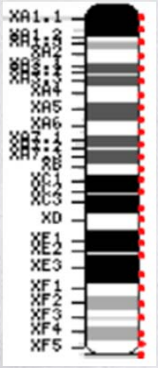
➢ Identification of the chromosomal rearrangement changing X into X*

Cytogenomics

Bac-mapping (deletions, inversions…)

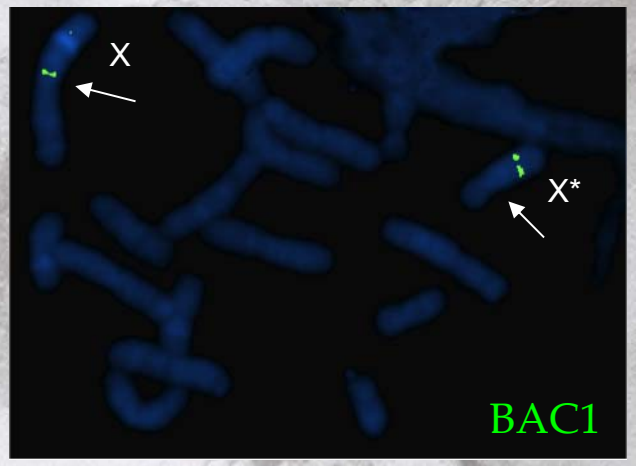
CGH-array (deletions, duplications…)

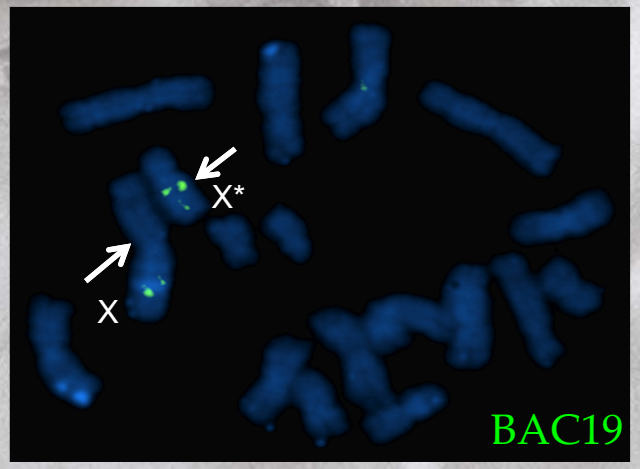
Functional Approach: Cytogenomics (1)

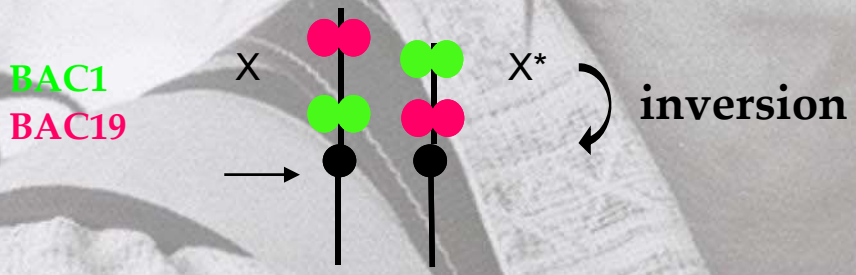


BAC-mapping onto XX* females

BACs = 100-200 kb sequences covering every 4-5 Mb of the mouse X chromosome.





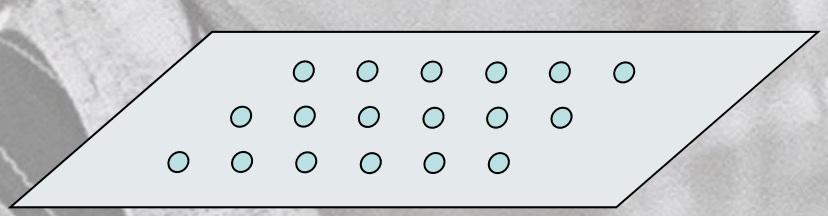


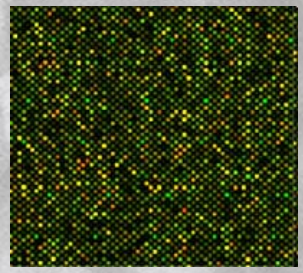
Functional Approach: Cytogenomics (2)

Comparative Genomic Hybridization (CGH-array)

M. minutoides genomic DNA

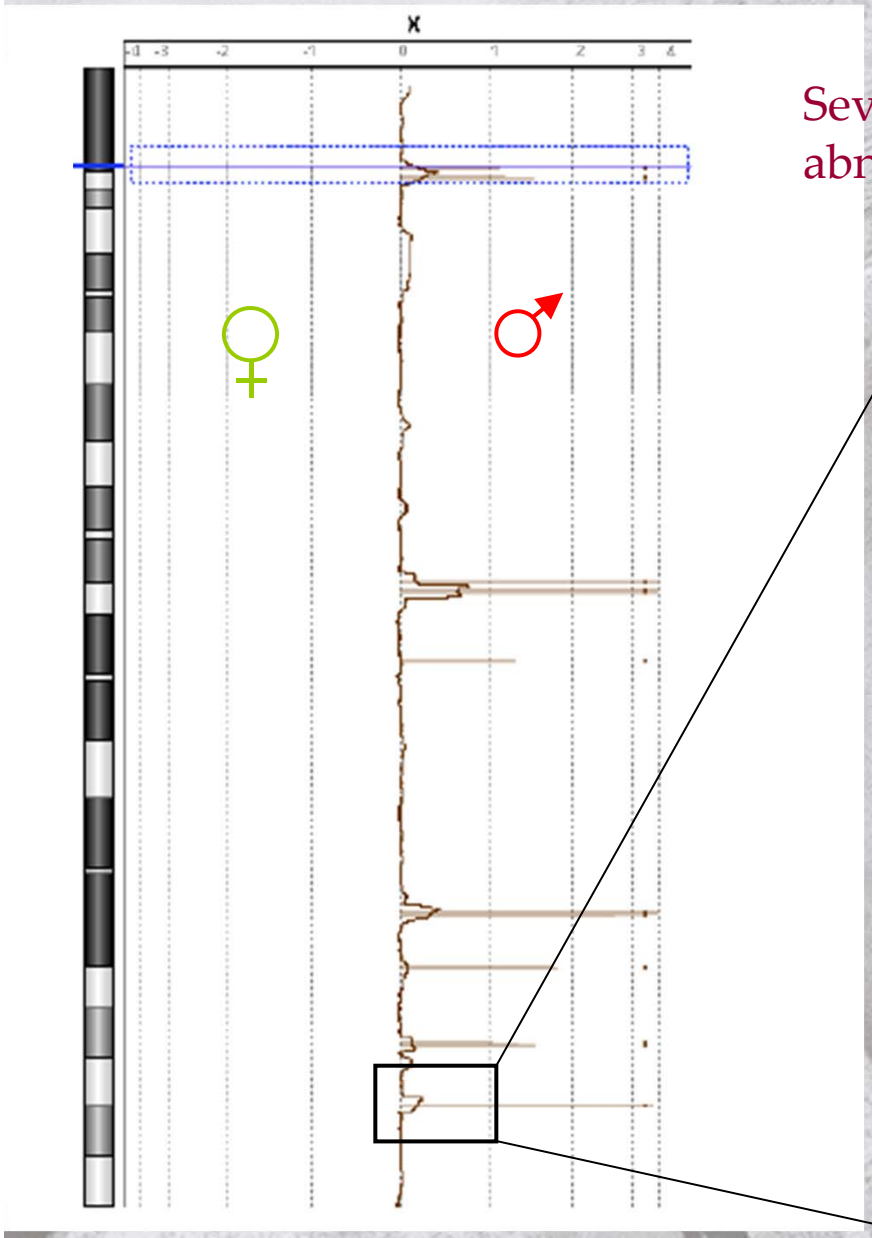
Hybridization



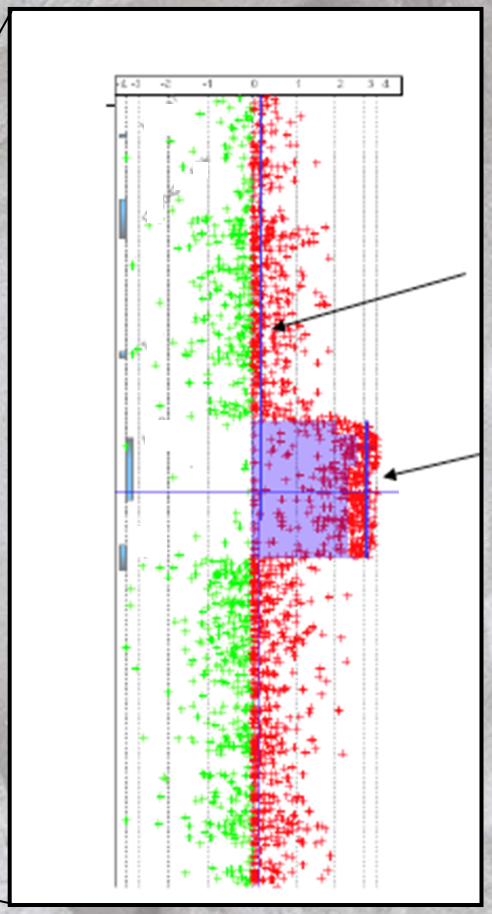


M. musculus X chromosome chip
(1M probes, Agilent Technologies)

Functional Approach: Cytogenomics (3)



Seven chromosomal regions with abnormalities have been found



14 occurrences corresponding to :

9 genes,
4 predicted genes,
1 pseudogene

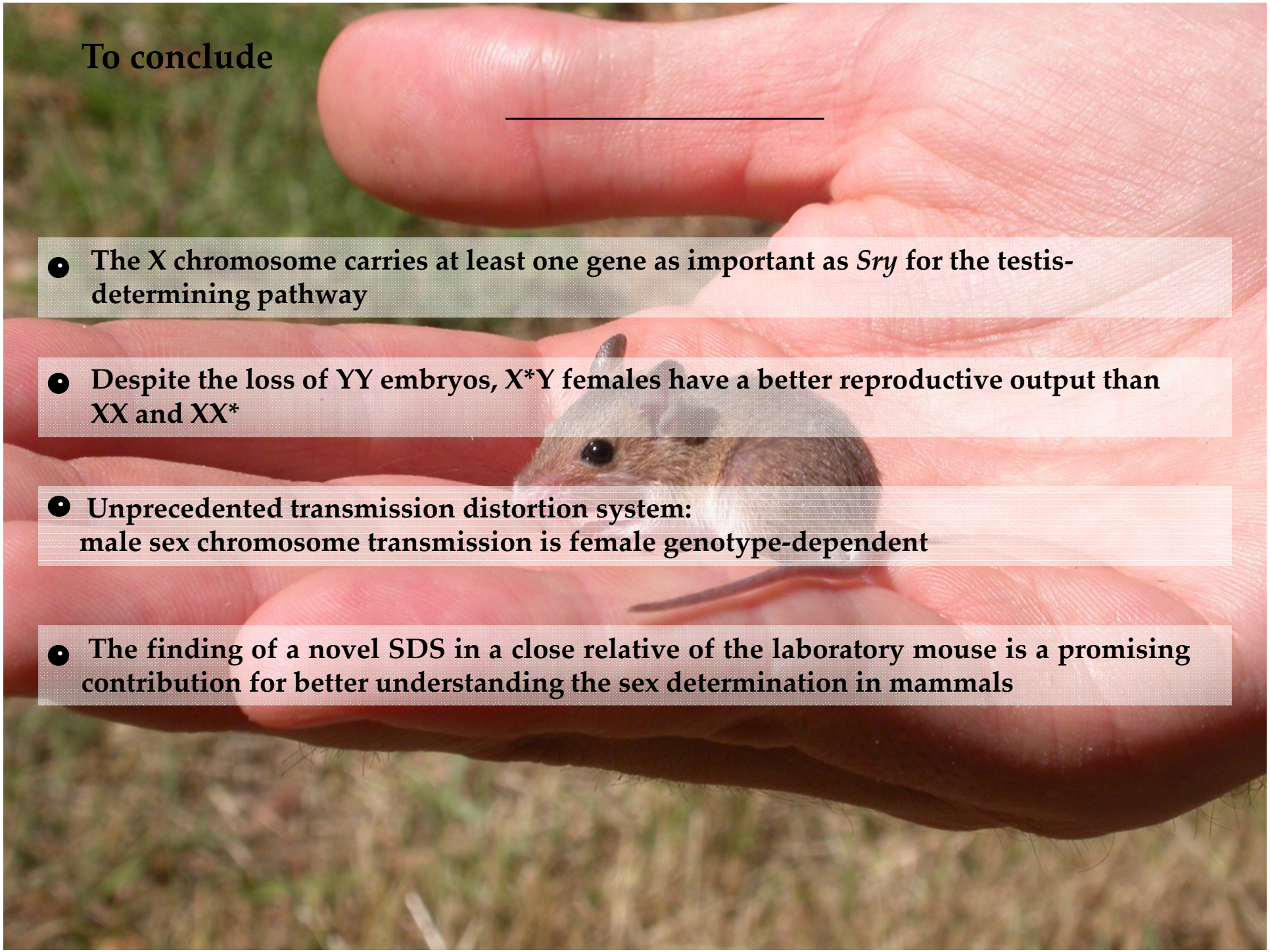
…but none already known to be involved in sex determinination

Future Prospects

- **Complete sequence assembly of the X and X* chromosomes**
  - → To confirm CGH results
  - → Sequence evolution of a "third" sex chromosome

Modification of evolutionary trajectories of the sex chromosomes

X	Y	X*
↘ Effective size	Loss of ♂-specific transmission	♀-specific transmission
↘ Recombination rate		Arrest recombination over a large region
Modif Sex Antagonistic selection (spends more time in males)		Expected to evolve like an heterogametic sex chromosome (degeneration, feminisation…)



To conclude

- The X chromosome carries at least one gene as important as *Sry* for the testis-determining pathway
- Despite the loss of YY embryos, X*Y females have a better reproductive output than XX and XX*
- Unprecedented transmission distortion system:
  male sex chromosome transmission is female genotype-dependent
- The finding of a novel SDS in a close relative of the laboratory mouse is a promising contribution for better understanding the sex determination in mammals

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