

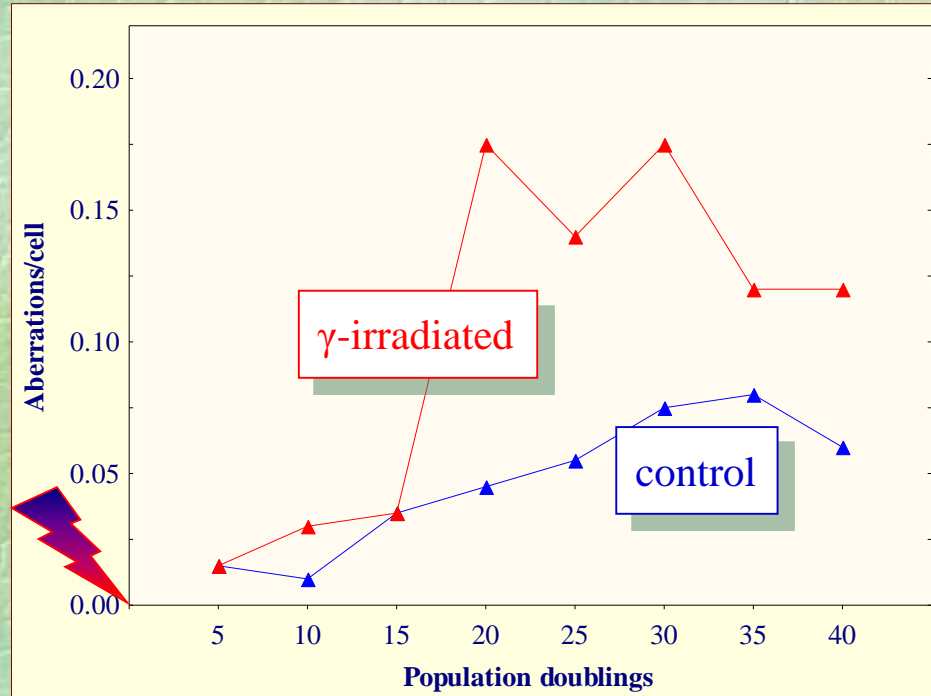
Transgenerational effects of parental exposure

Yuri E Dubrova

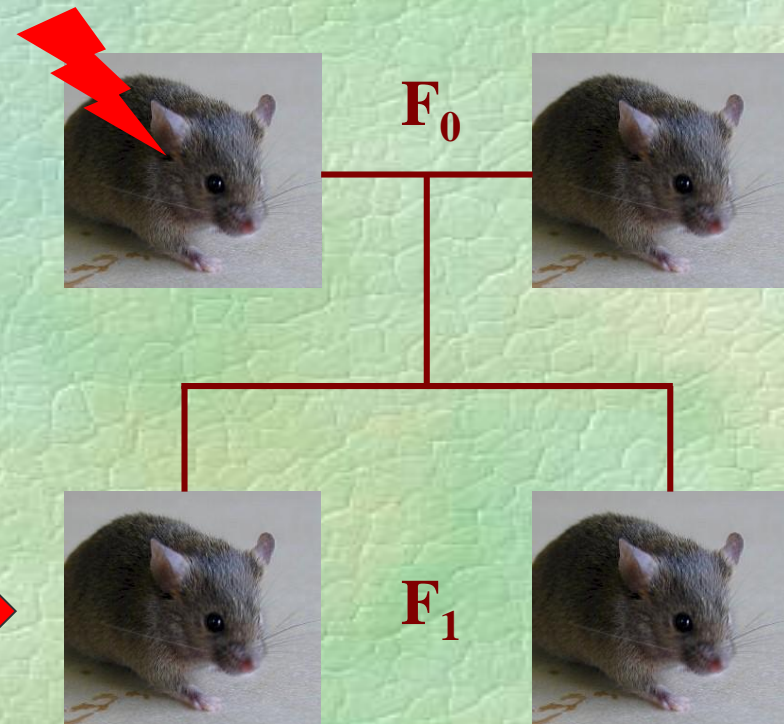
yed2@le.ac.uk

*Department of Genetics
University of Leicester, UK*

Radiation-induced genomic instability

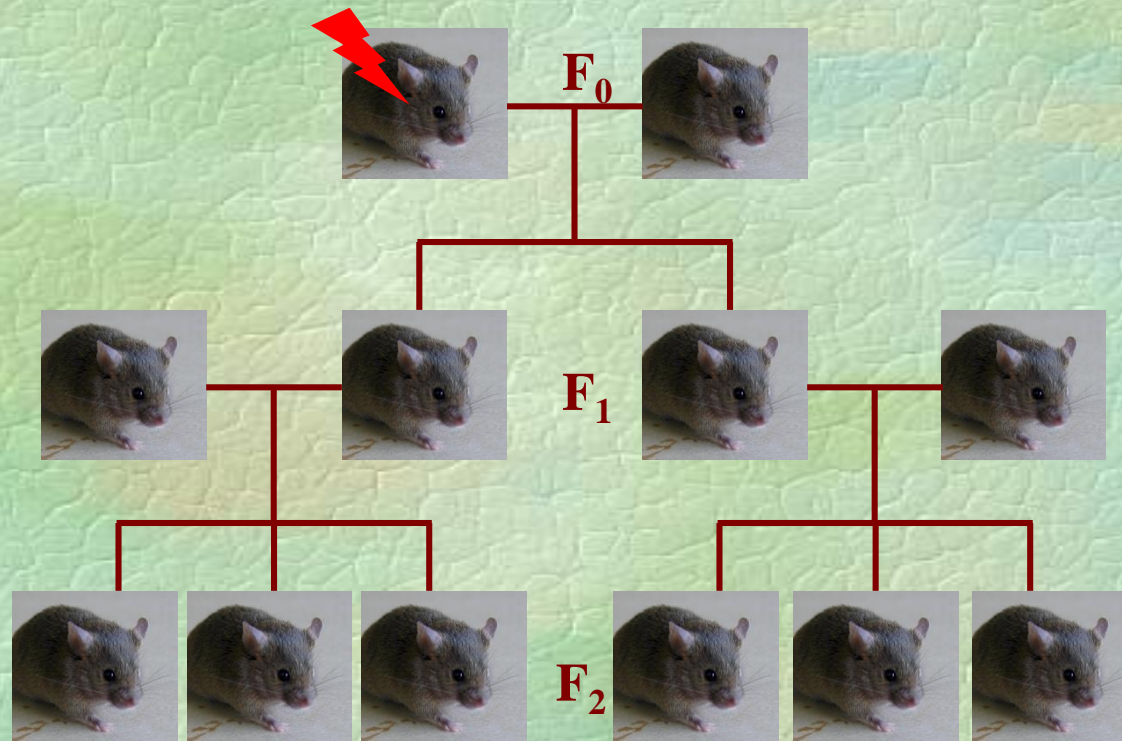


Are they unstable? 

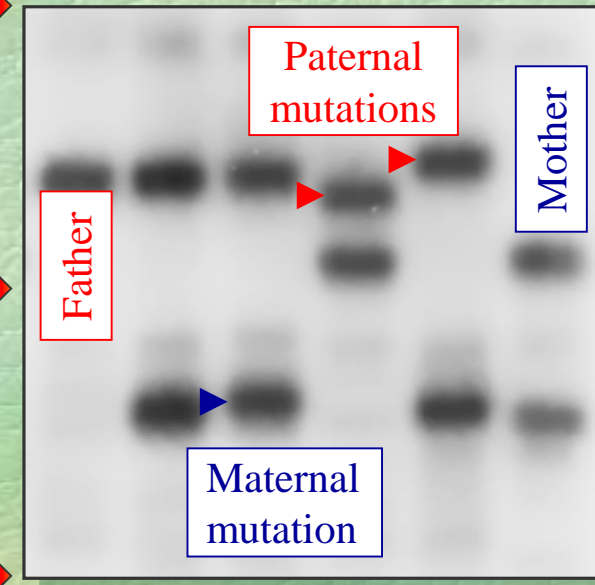


Let's go transgenerational...

**0.5 Gy of
fission neutrons**



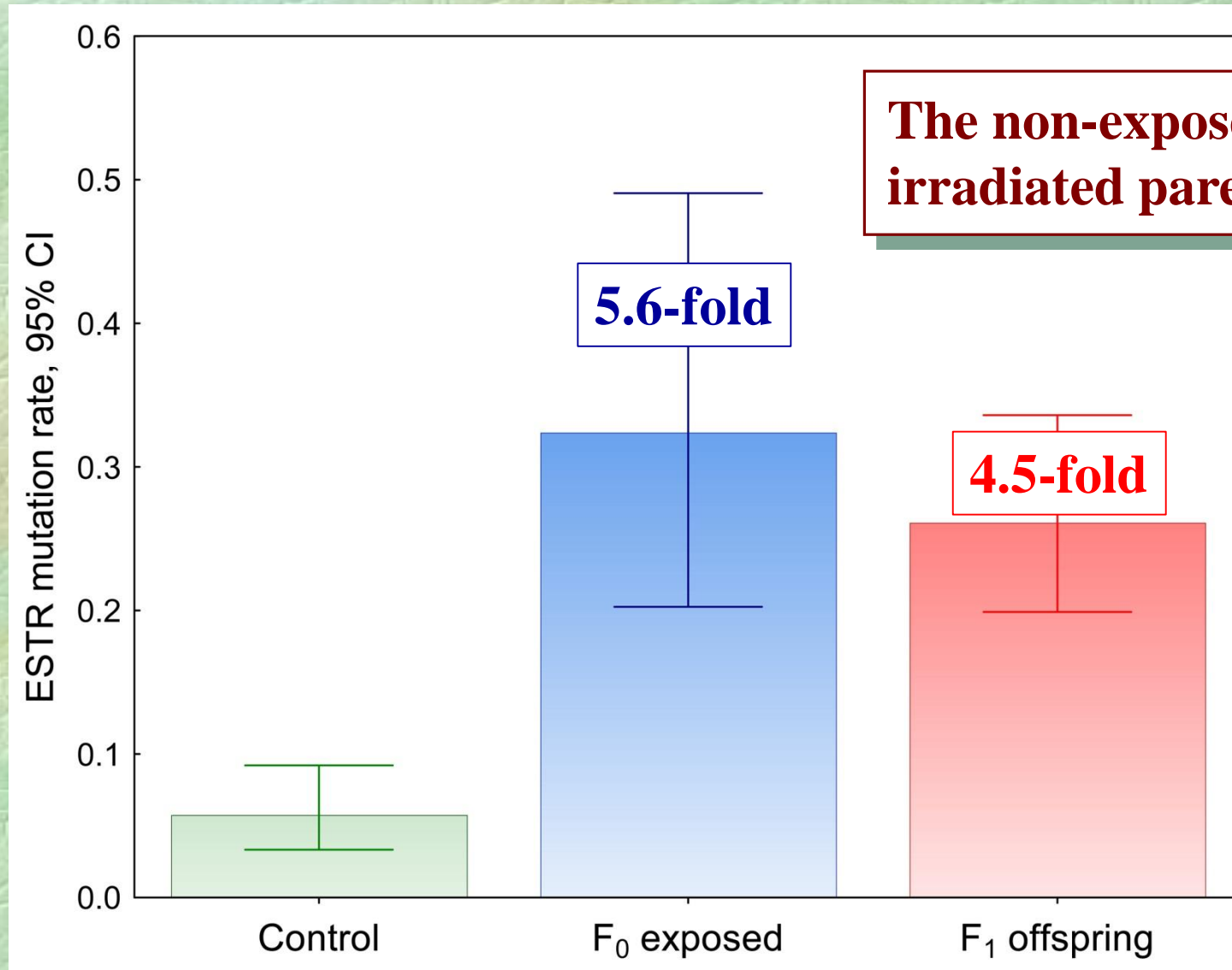
**Expanded simple tandem
repeat (ESTR) loci
= microsatellite loci**



ESTR mutation rates were evaluated in the germline of:

- **irradiated F₀ males = mutation induction**
- **non-exposed F₁ offspring = transgenerational instability**

Transgenerational germline instability in the F₁ offspring of CBA/H male mice exposed to 0.5 Gy of fission neutrons



The non-exposed offspring of irradiated parents are unstable

**Is transgenerational instability
strain-specific?**



CBA/H

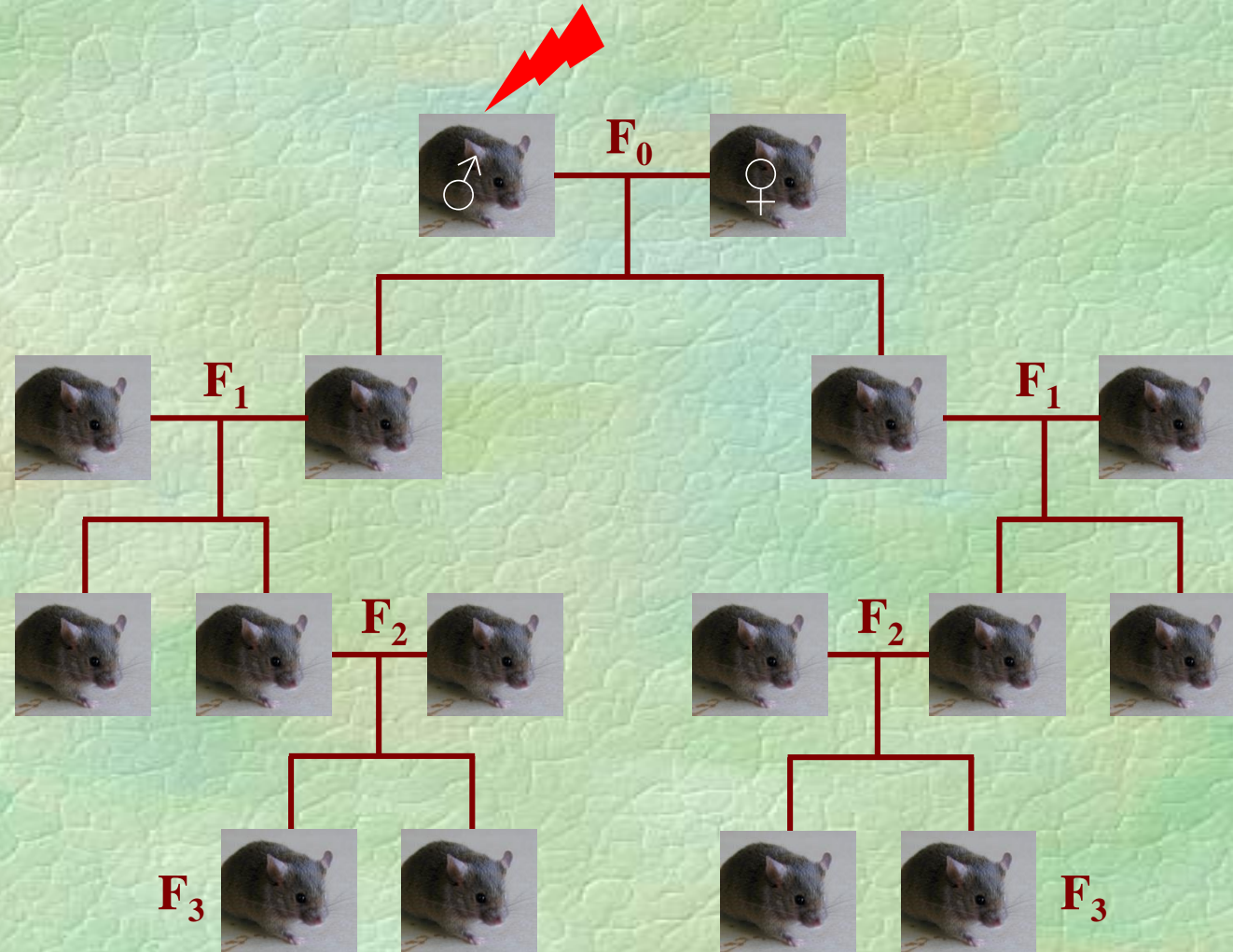


BALB/c

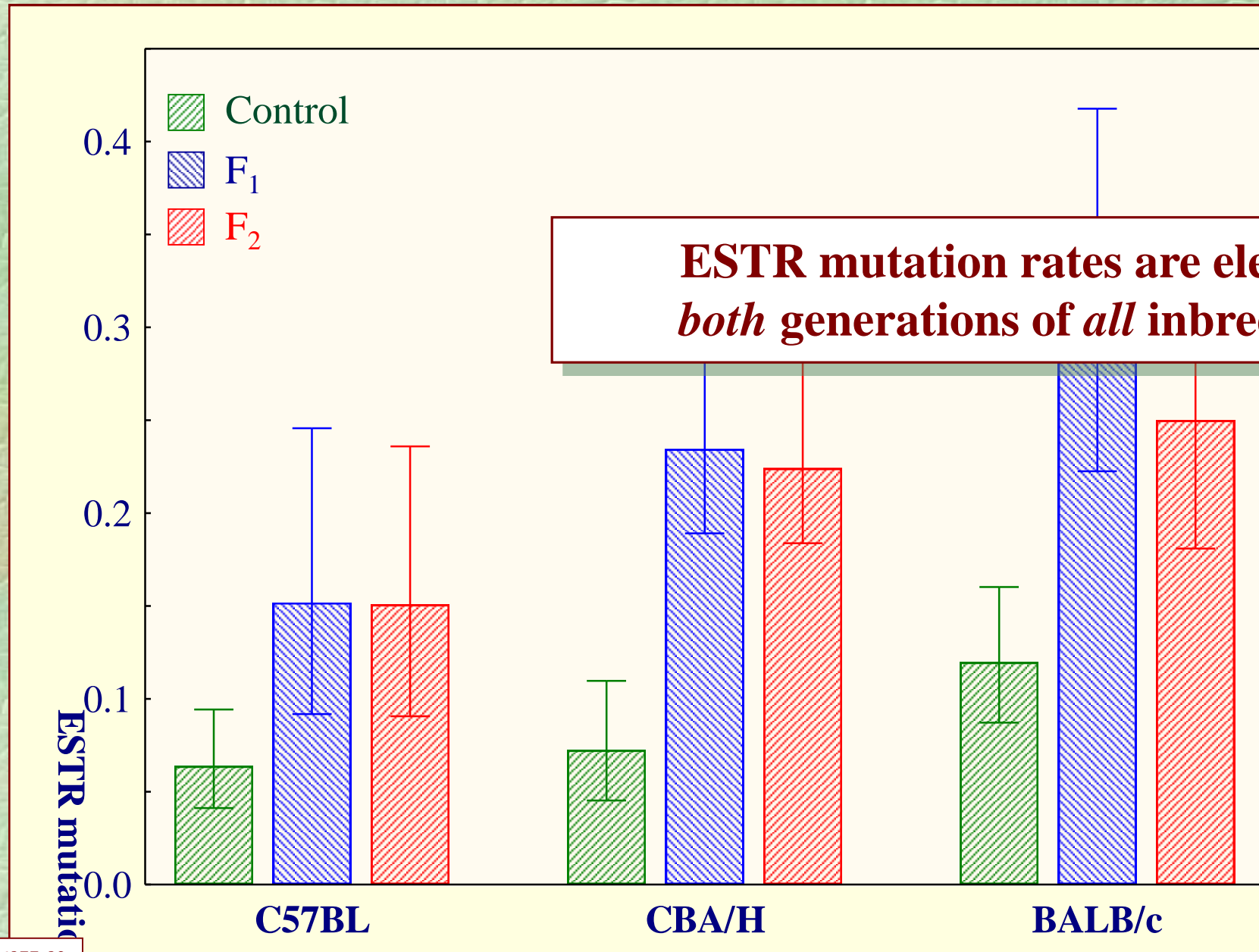


C57BL/6J

Fission neutrons, 0.4 Gy: CBA/H; C57BL/6
 Acute X-rays, 2 Gy: CBA/H
 Acute X-rays, 1 Gy: BALB/c

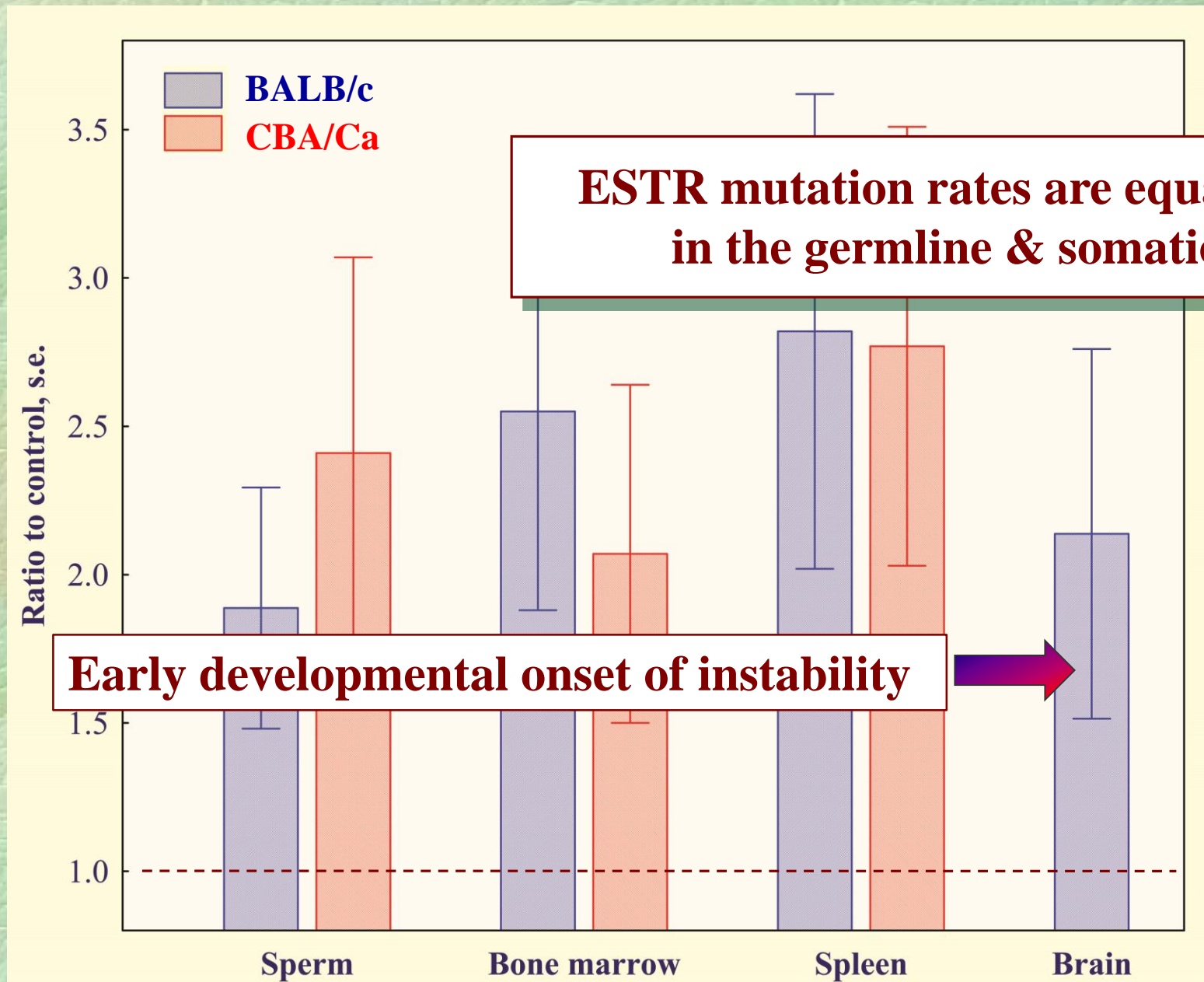


Transgenerational instability in three inbred mouse strains



**Is transgenerational instability
tissue-specific?**

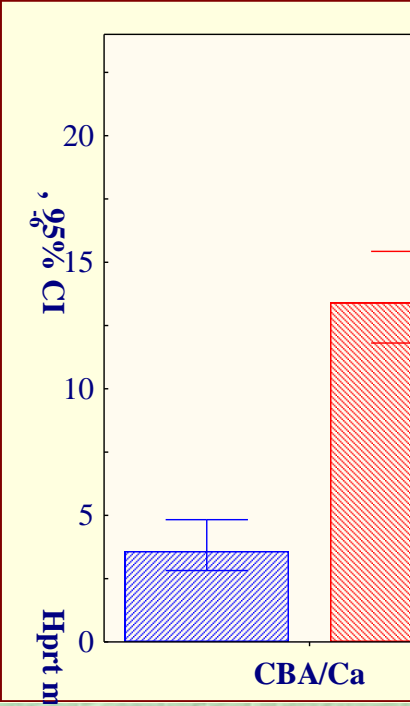
Transgenerational instability in the germline & somatic tissues



**Is transgenerational instability
specific for tandem repeat loci?**

Transgenerational instability at the mouse *hprt* locus

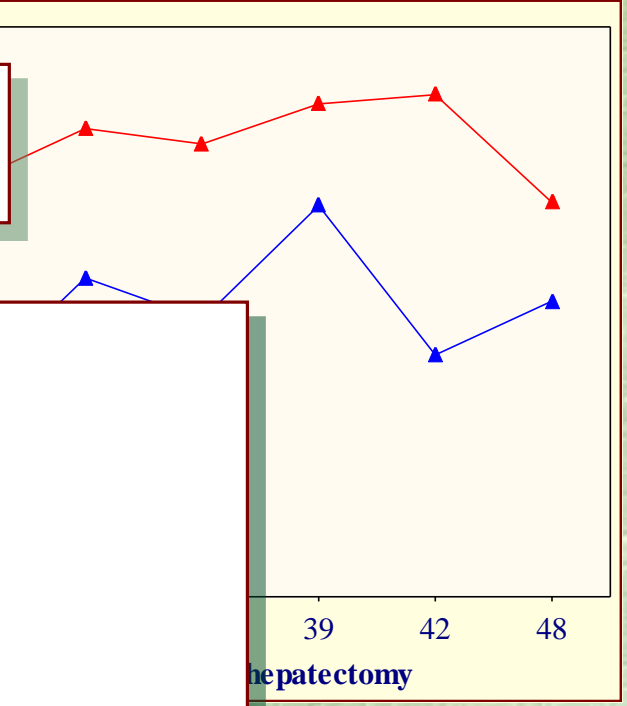
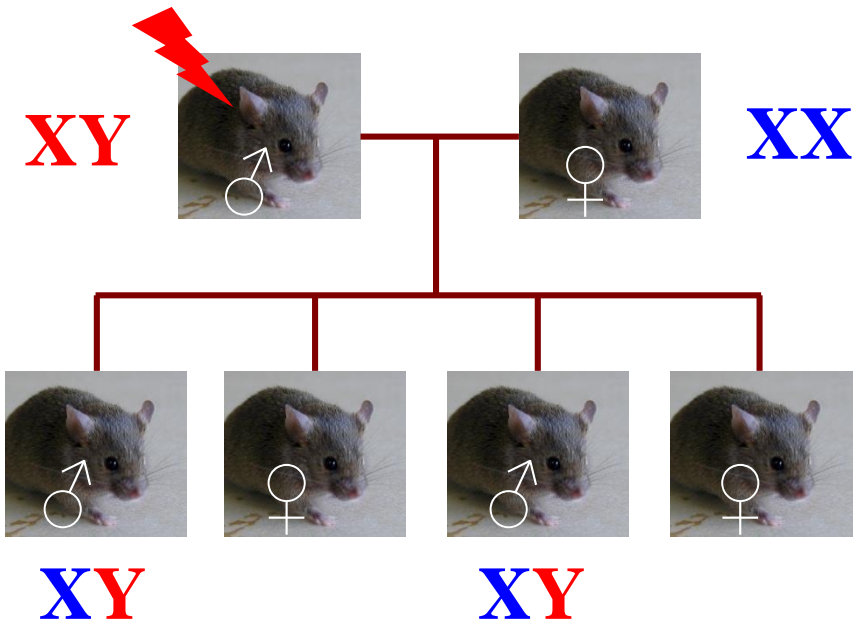
Chromosome aberrations in the F₁ offspring of irradiated rats



From: Barber *et al.*, 2006, *Oncogene*

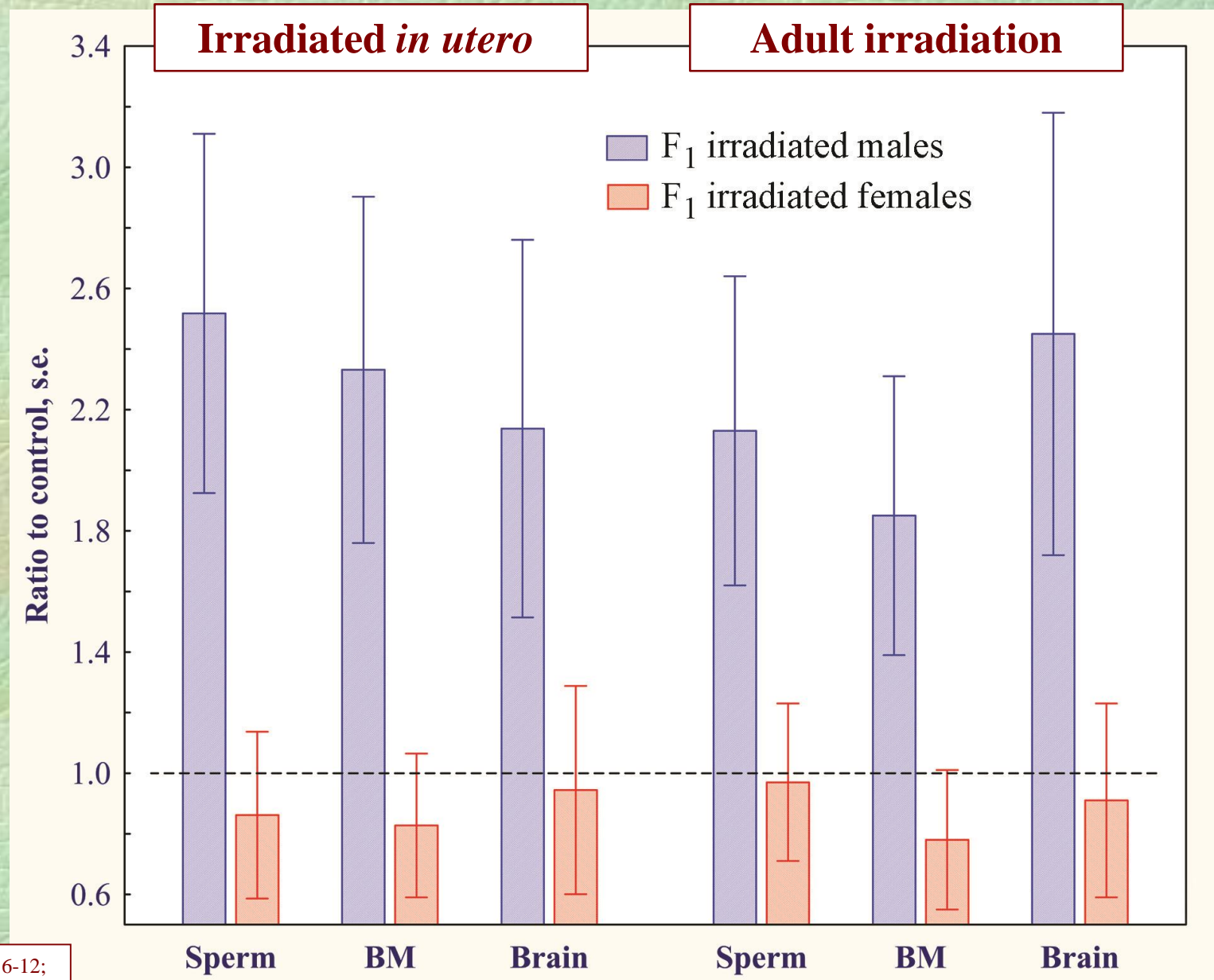
A genome-wide destabilisation

hprt is X-linked gene



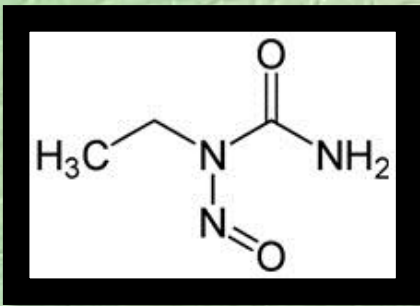
**Is transgenerational instability
sex-specific?**

The offspring of irradiated females are stable



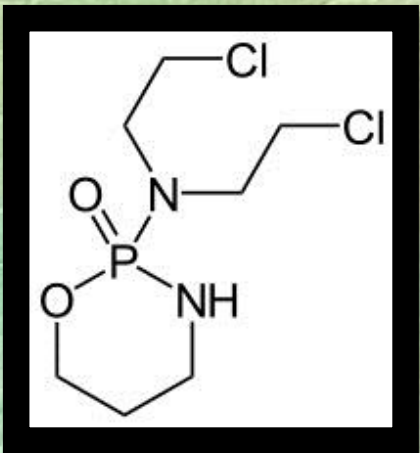
From: Barber *et al.*, 2009, *Mutat Res* **664**, 6-12;
Abouzeid Ali *et al.*, 2012, *Mutat Res* **732**, 21-5

**Can paternal exposure to chemical mutagens
destabilise the F_1 genomes?**



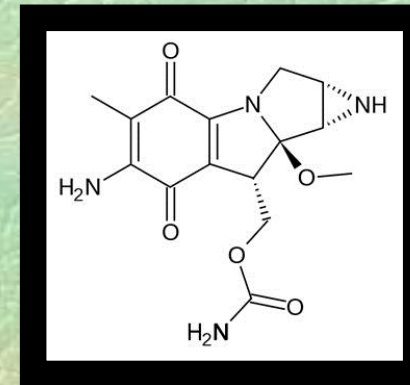
Alkylating agent ethylnitrosourea, ENU

- mostly base damage
- results in base substitutions
- ~ no ENU-induced DSBs



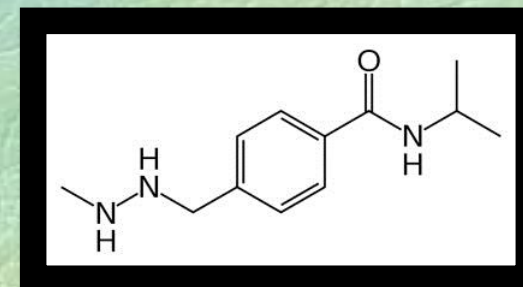
Anticancer drug cyclophosphamide, CPP

- alkylated monoadducts & crosslinks
- results in base substitutions
- crosslinks can result in DSBs after replication/repair



Anticancer drug mitomycin C, MMC

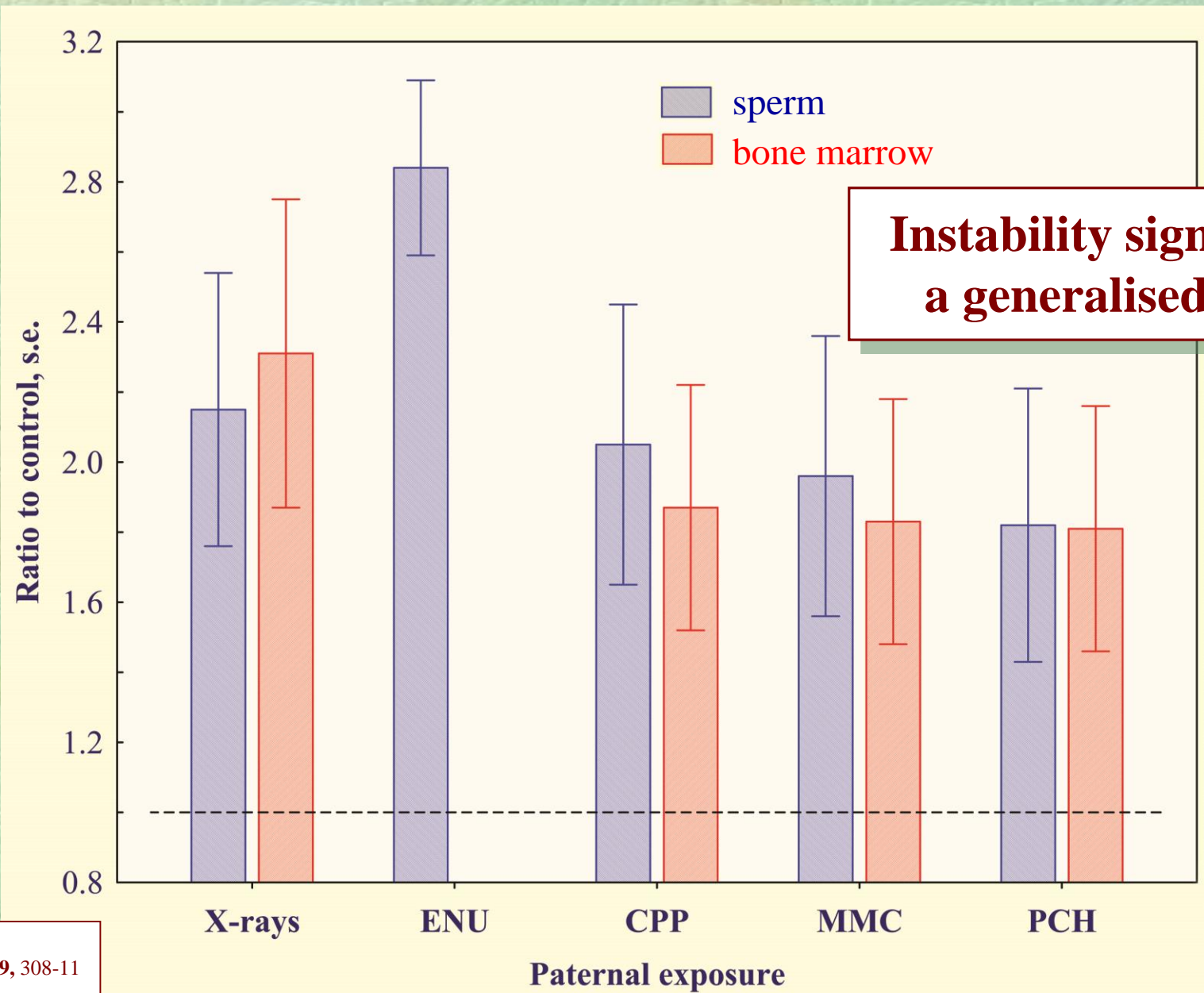
- alkylated monoadducts & crosslinks
- base substitutions
- crosslinks can result in DSBs



Anticancer drug procarbazine, PCH

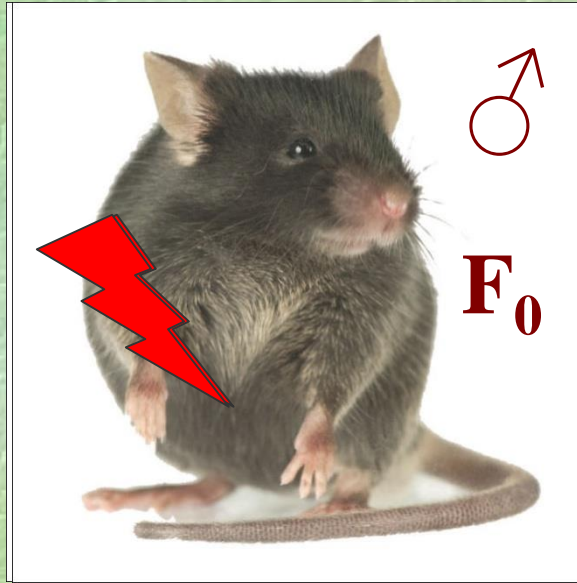
- alkylated monoadducts
- free radical species
- base substitutions & SSBs

ESTR instability in the F₁ offspring of mutagen-treated male mice

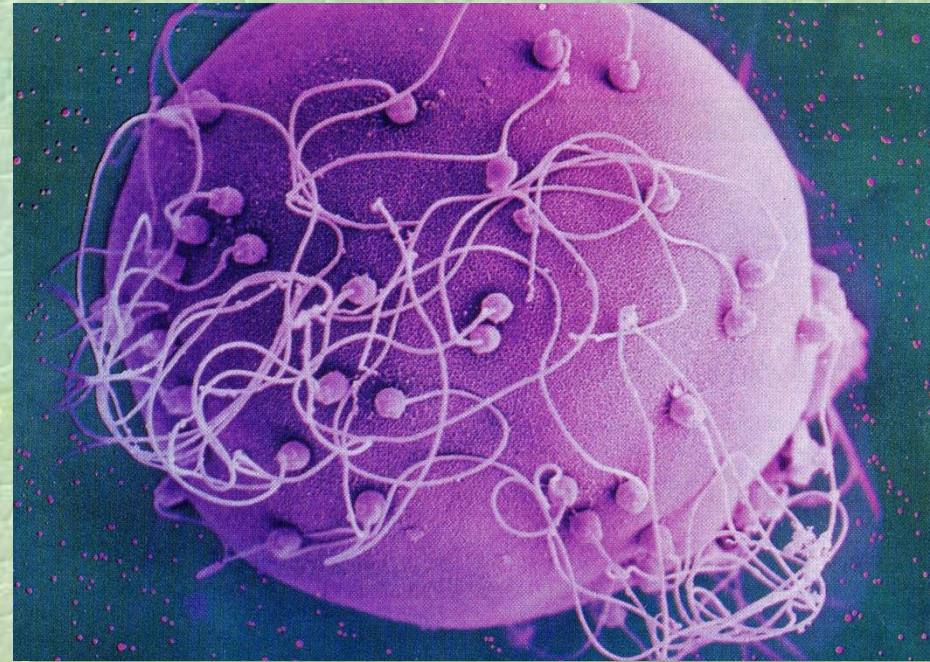


From: Barber *et al.*, 2002, *PNAS* **99**, 6877-82
Dubrova *et al.*, 2008, *Environ Mol Mutagen* **49**, 308-11
Glen, Dubrova 2012, *PNAS* **109**, 2984

Mechanisms



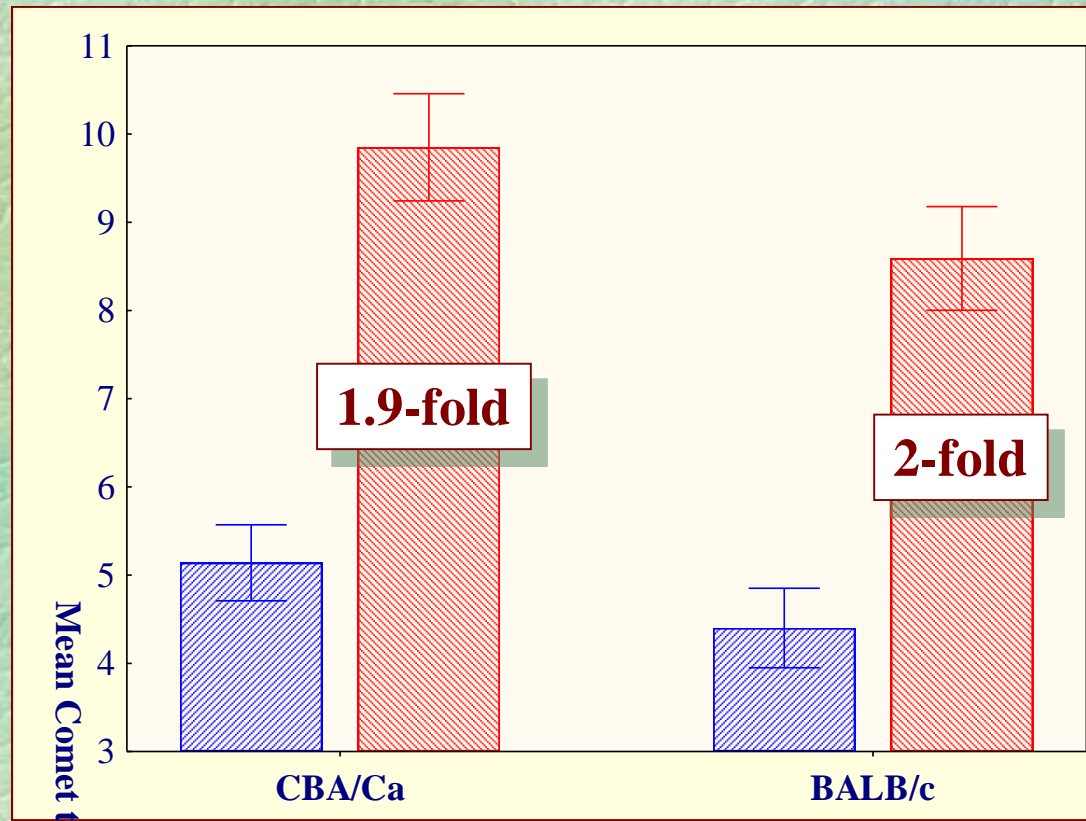
Initiation of an epigenetic instability signal in the directly exposed male germ cells



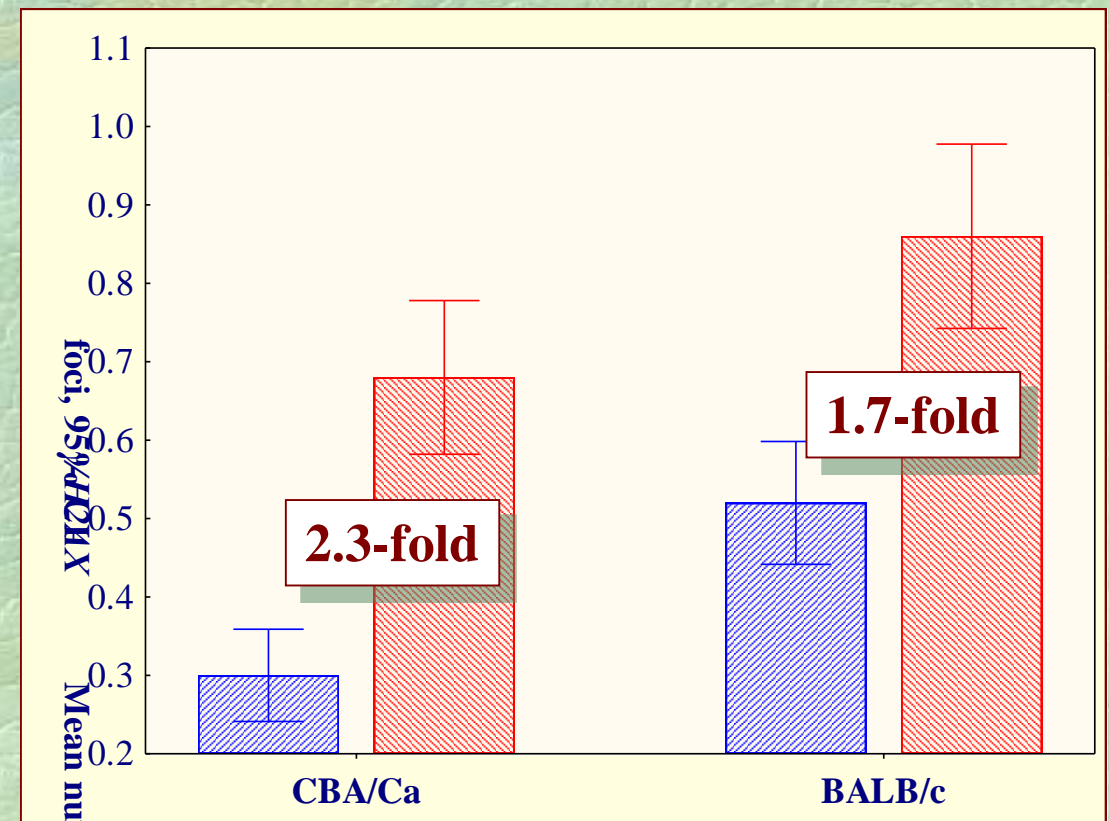
Transmission of an epigenetic instability signal to the offspring & its manifestation

Endogenous DNA damage in controls & the F₁ offspring of irradiated males

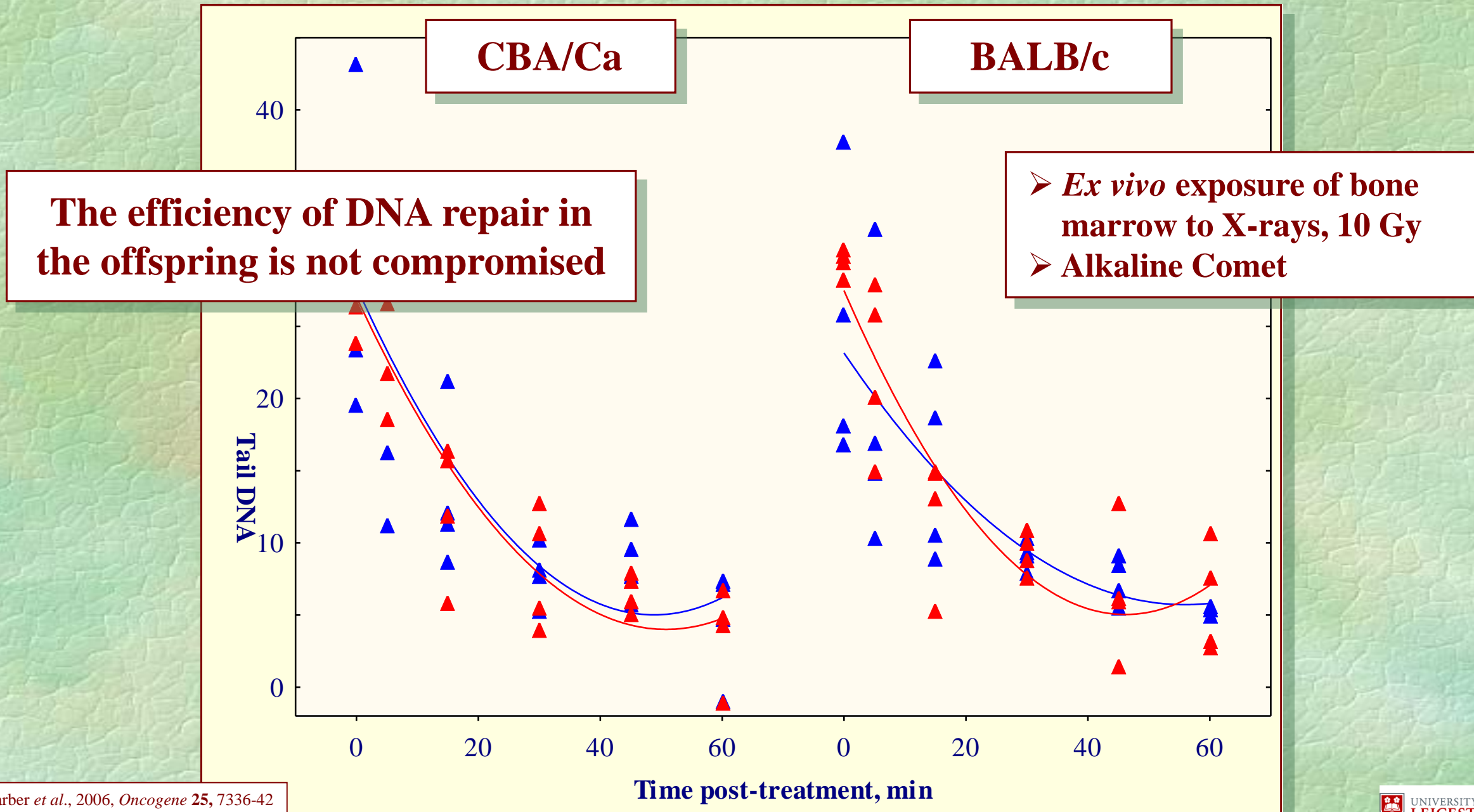
Single-strand DNA breaks
Comet assay, bone marrow



Double-strand DNA breaks
 γ -H2AX assay, spleen



DNA repair in the F₁ offspring of irradiated males



Oxidative DNA damage in the F₁ offspring (FPG Comet)

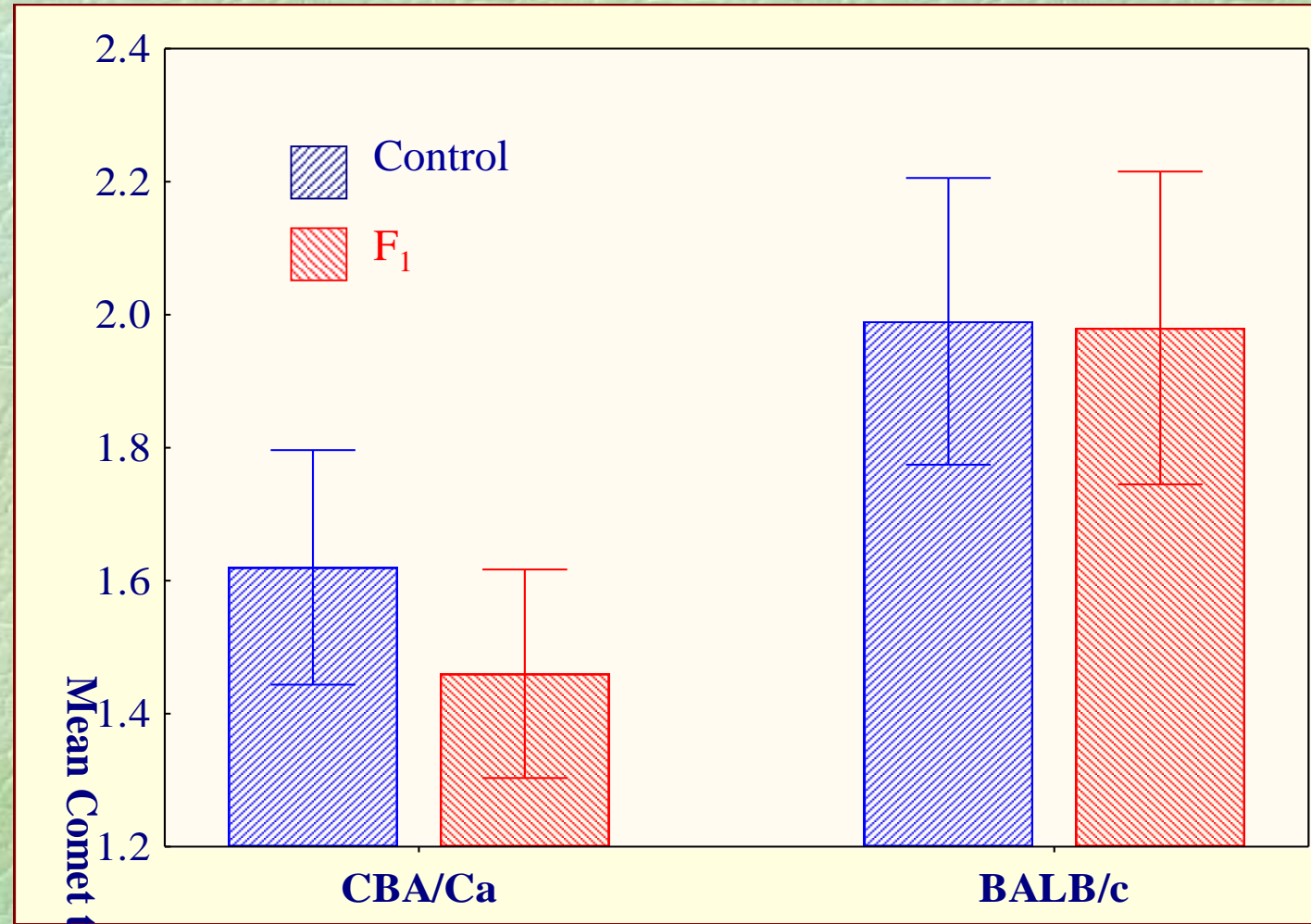
Oxidative stress

DNA damage:

- modified bases
- single-strand breaks
- double-strand breaks

Hallmark:

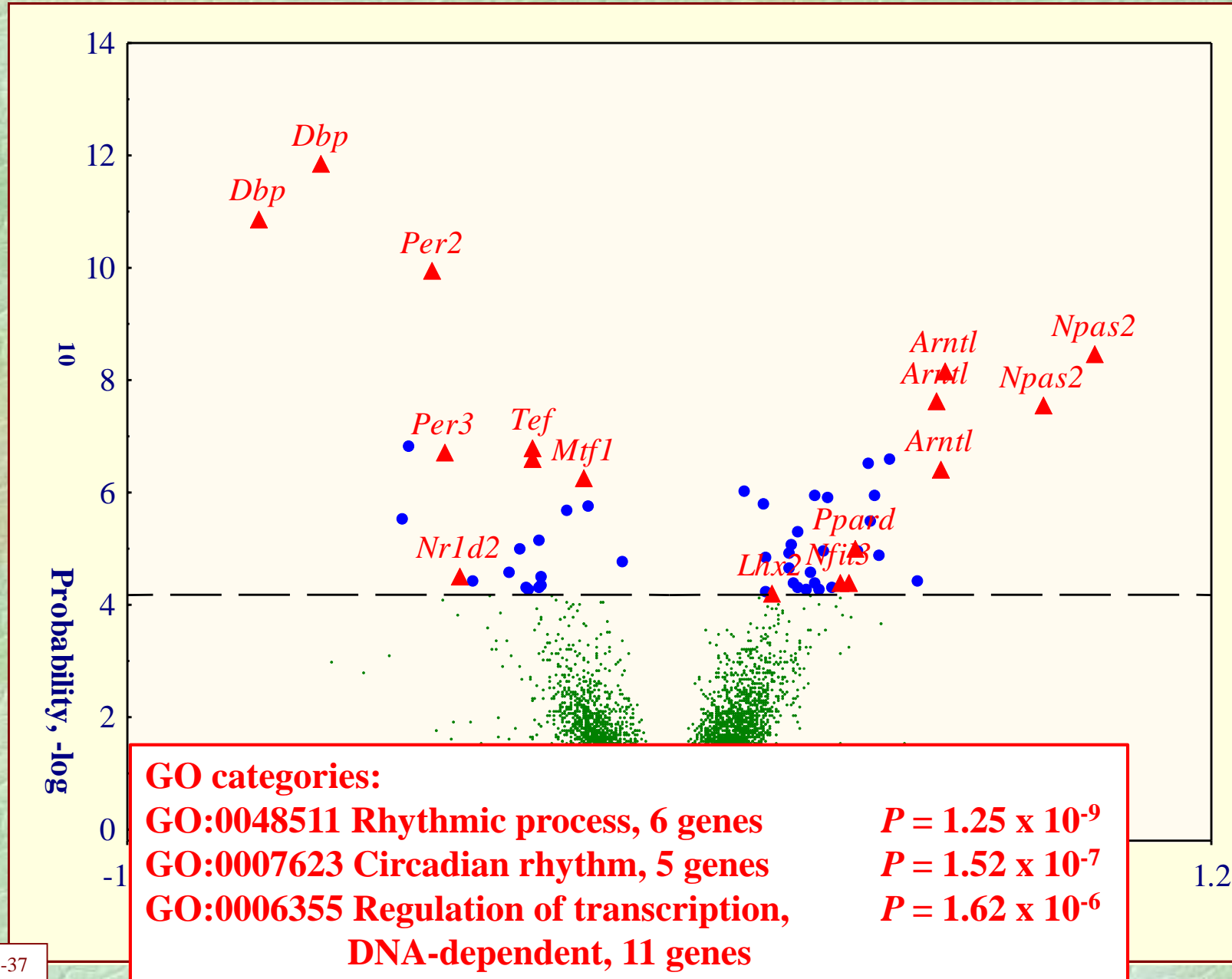
Accumulation of oxidatively damaged nucleotides in DNA



- The efficiency of DNA in the F₁ offspring is OK
- No sign of oxidative stress in the F₁ offspring
- What else?

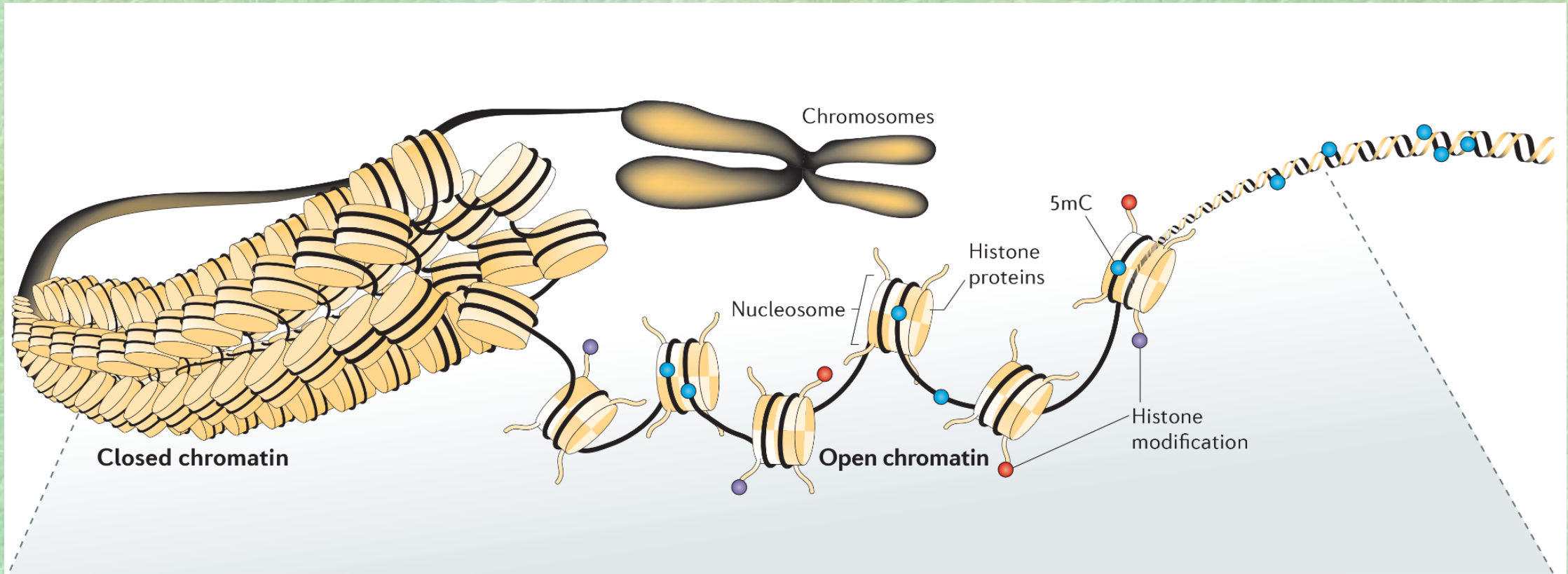
From: Barber *et al.*, 2006, *Oncogene* 25, 7336-42

The effects of paternal irradiation on F₁ gene expression



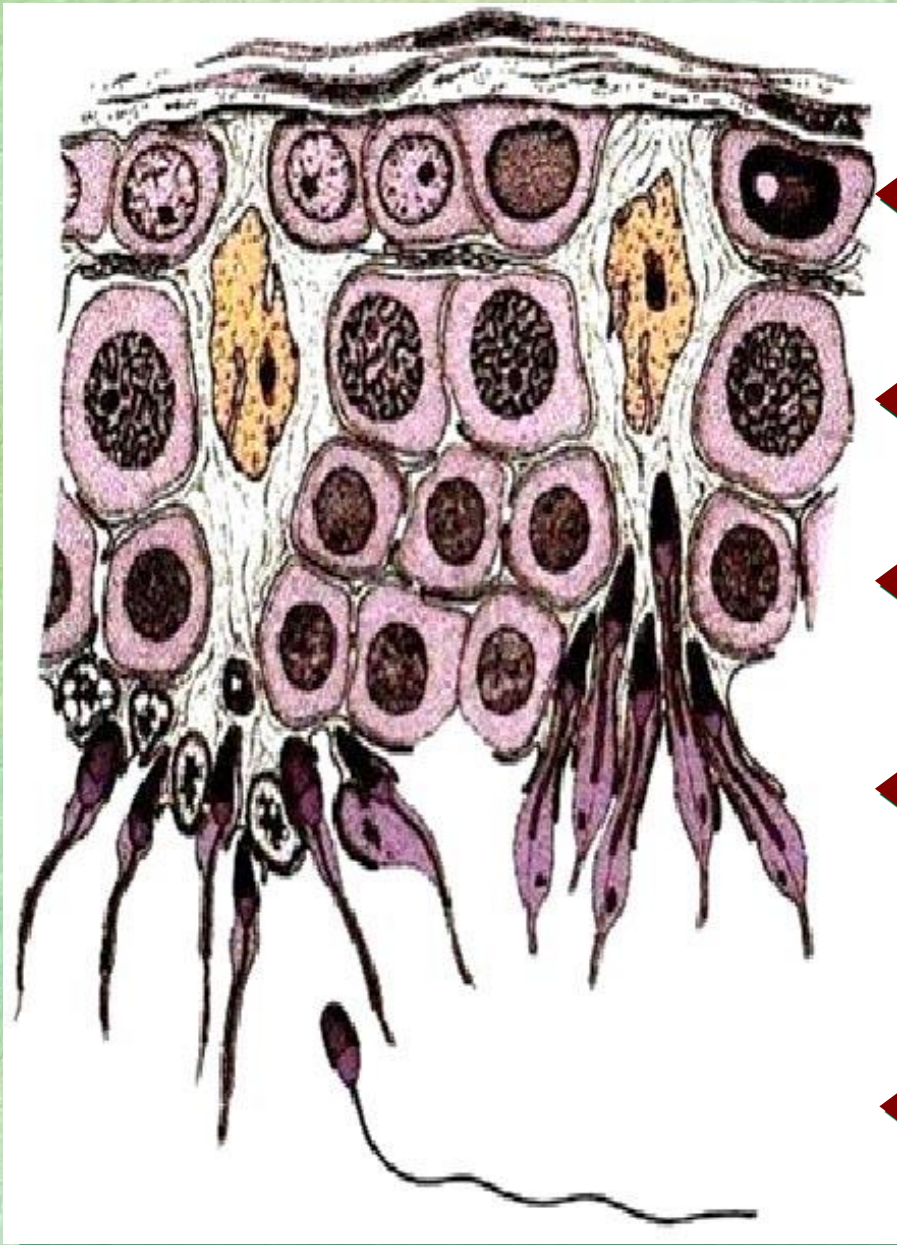
And what is transmitted?

What can we expect to find?



From: Taudt *et al.*, 2016, *Nat Rev Genet* 17, 319-332

Spermatogenesis in mice & humans



Diploid spermatogonia

Meiotic primary spermatocytes

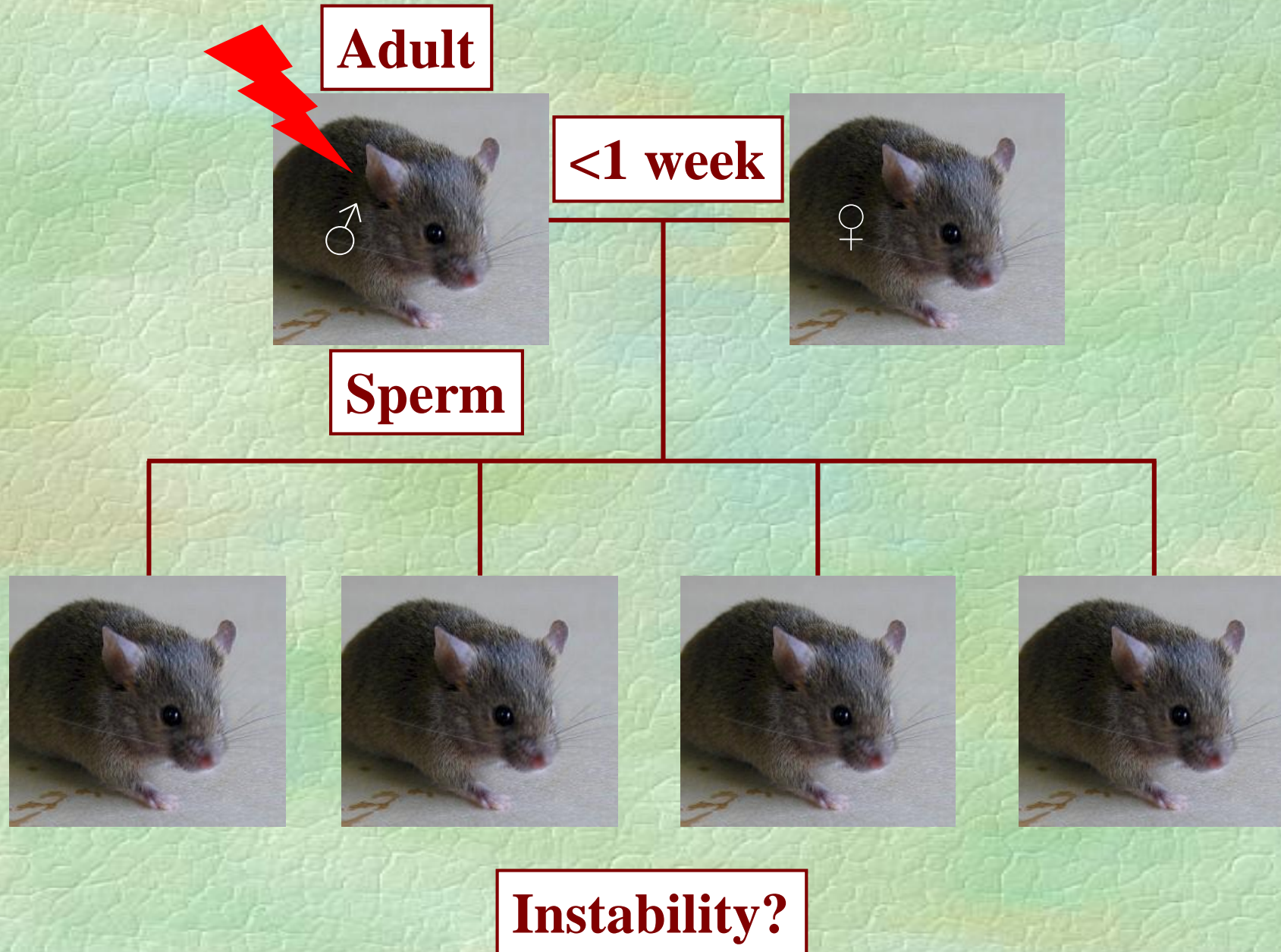
Meiotic secondary spermatocytes

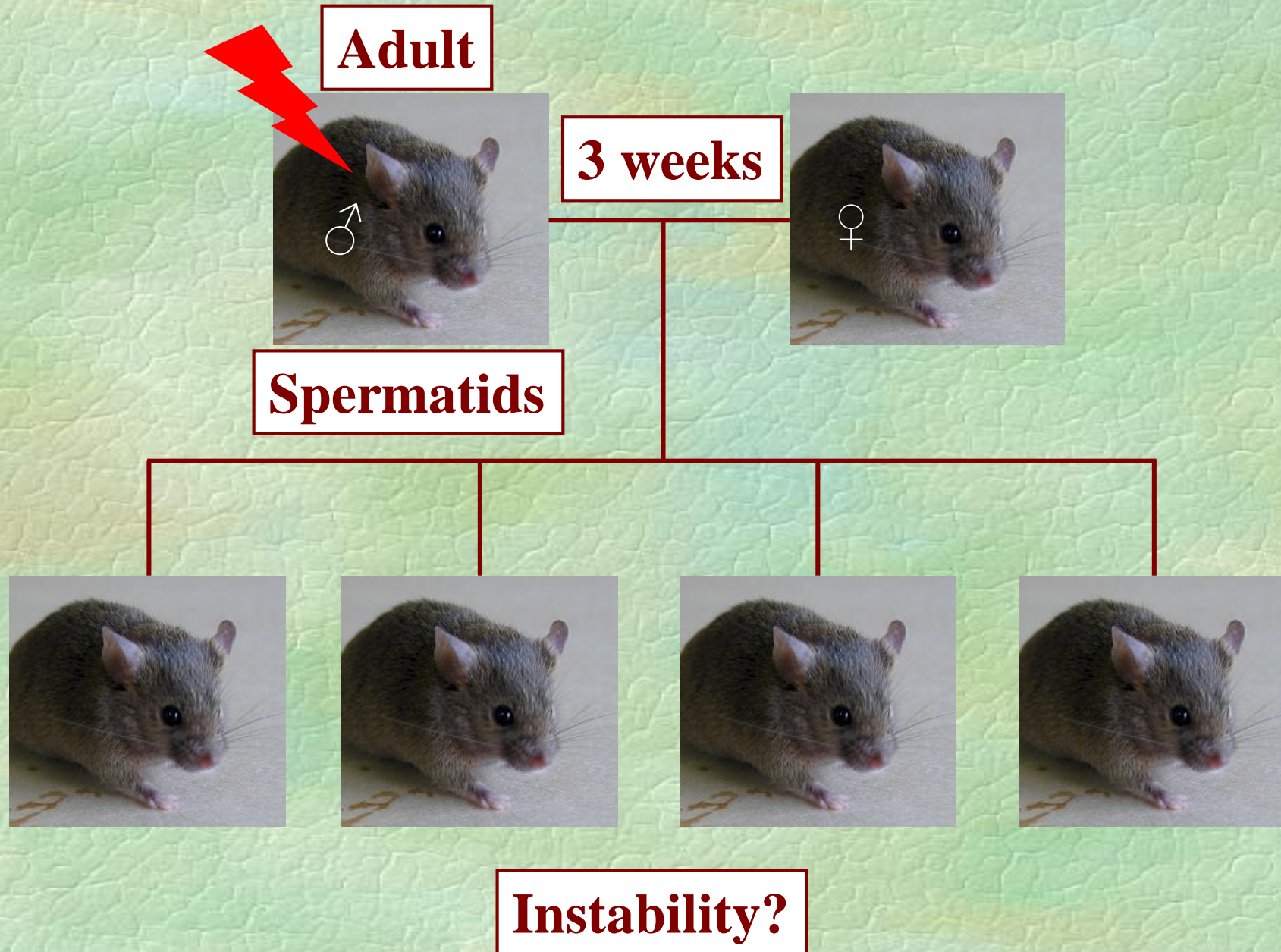
Post-meiotic spermatids

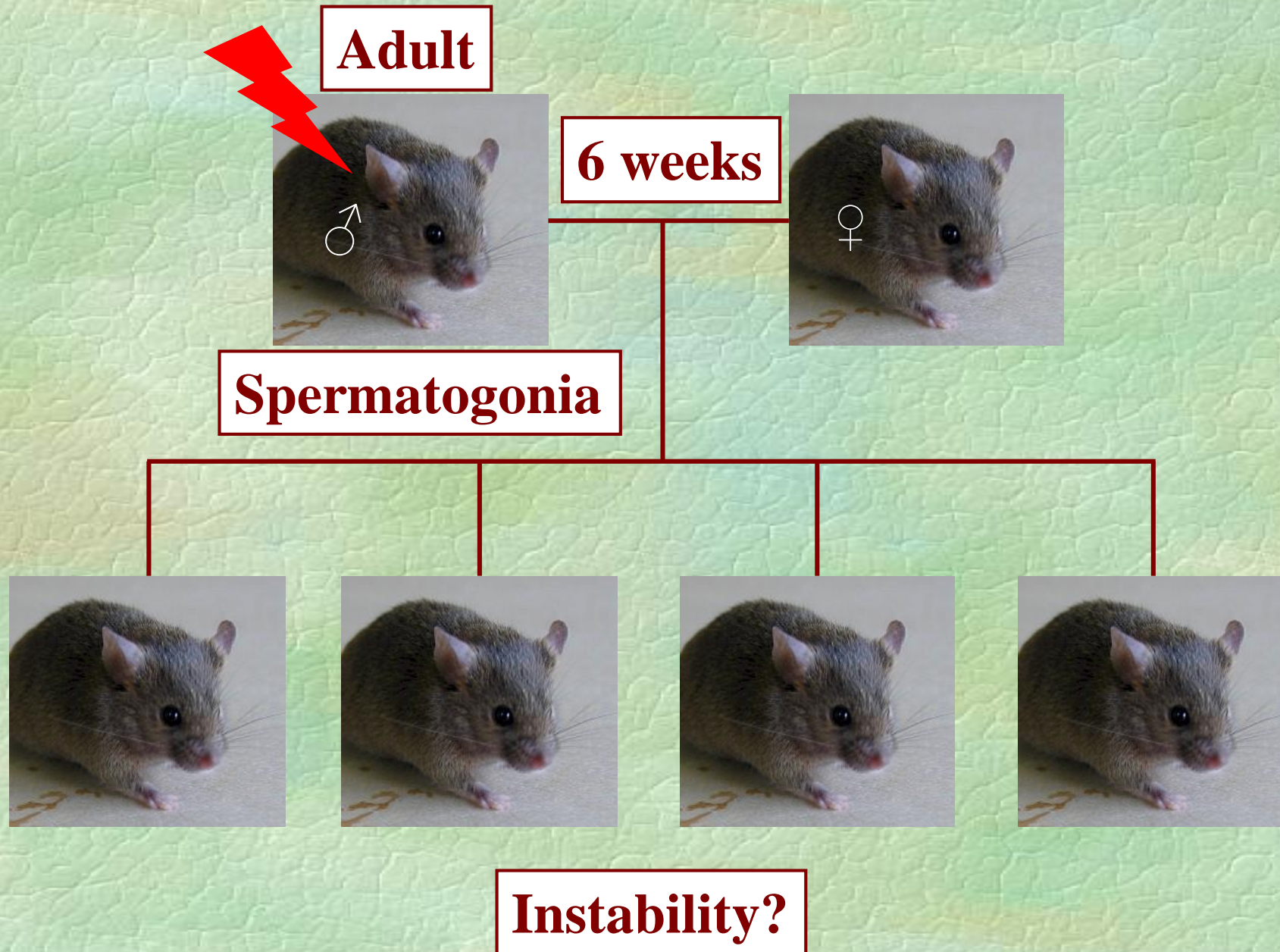
~~Sperm~~ cells

Transcription

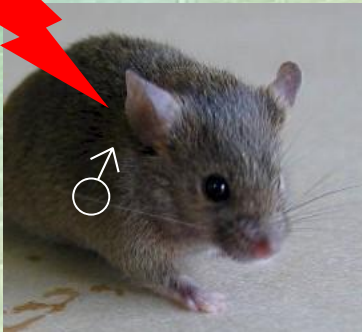








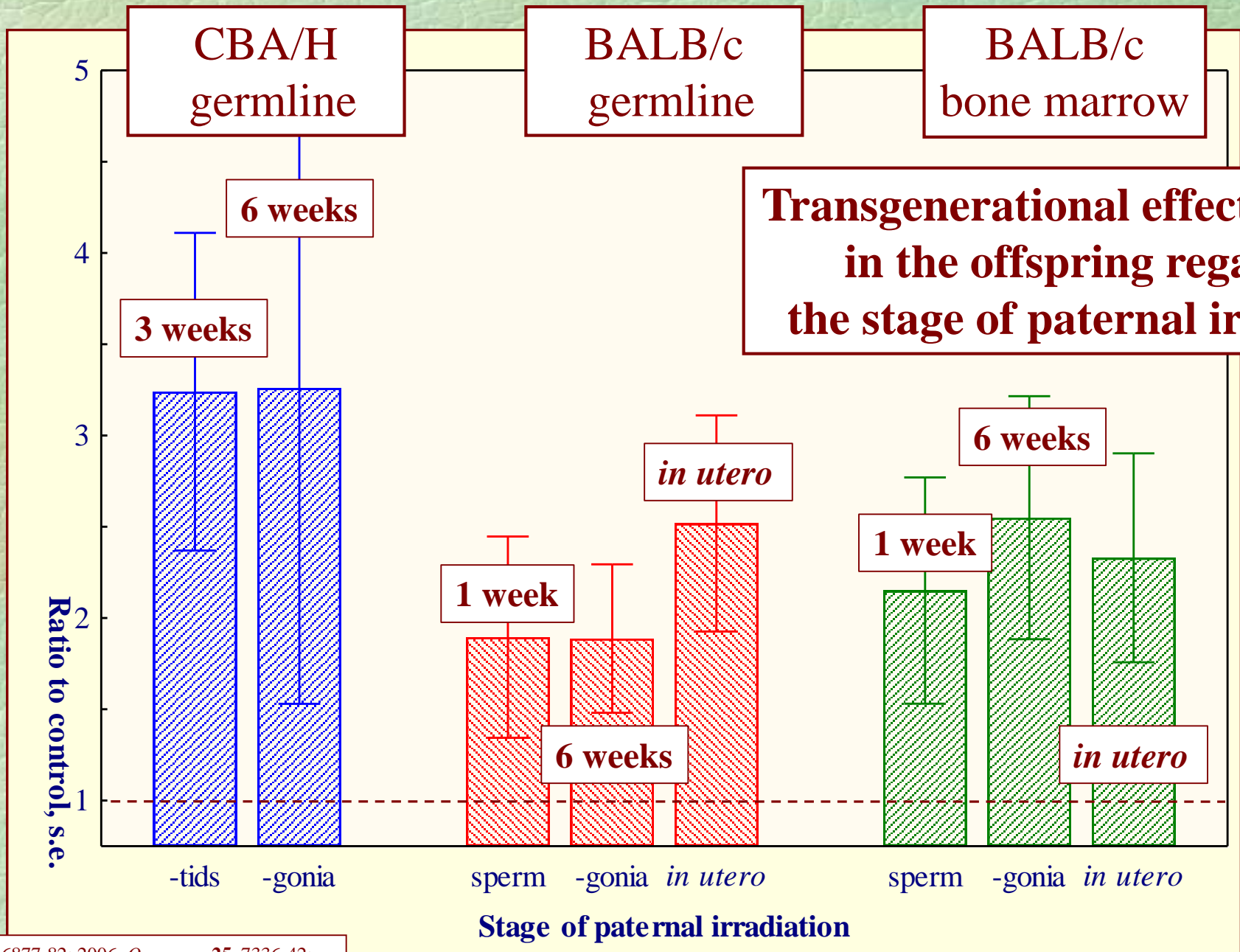
in utero



Primordial stem cells

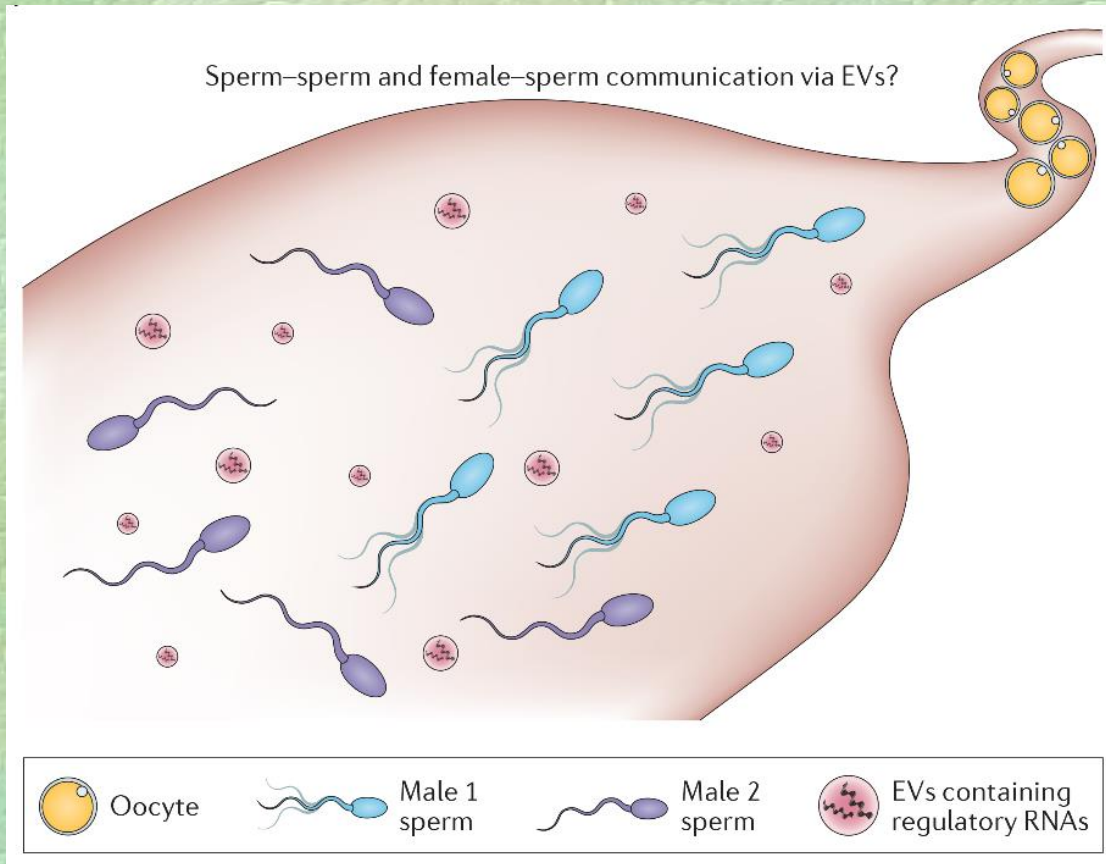


Instability?

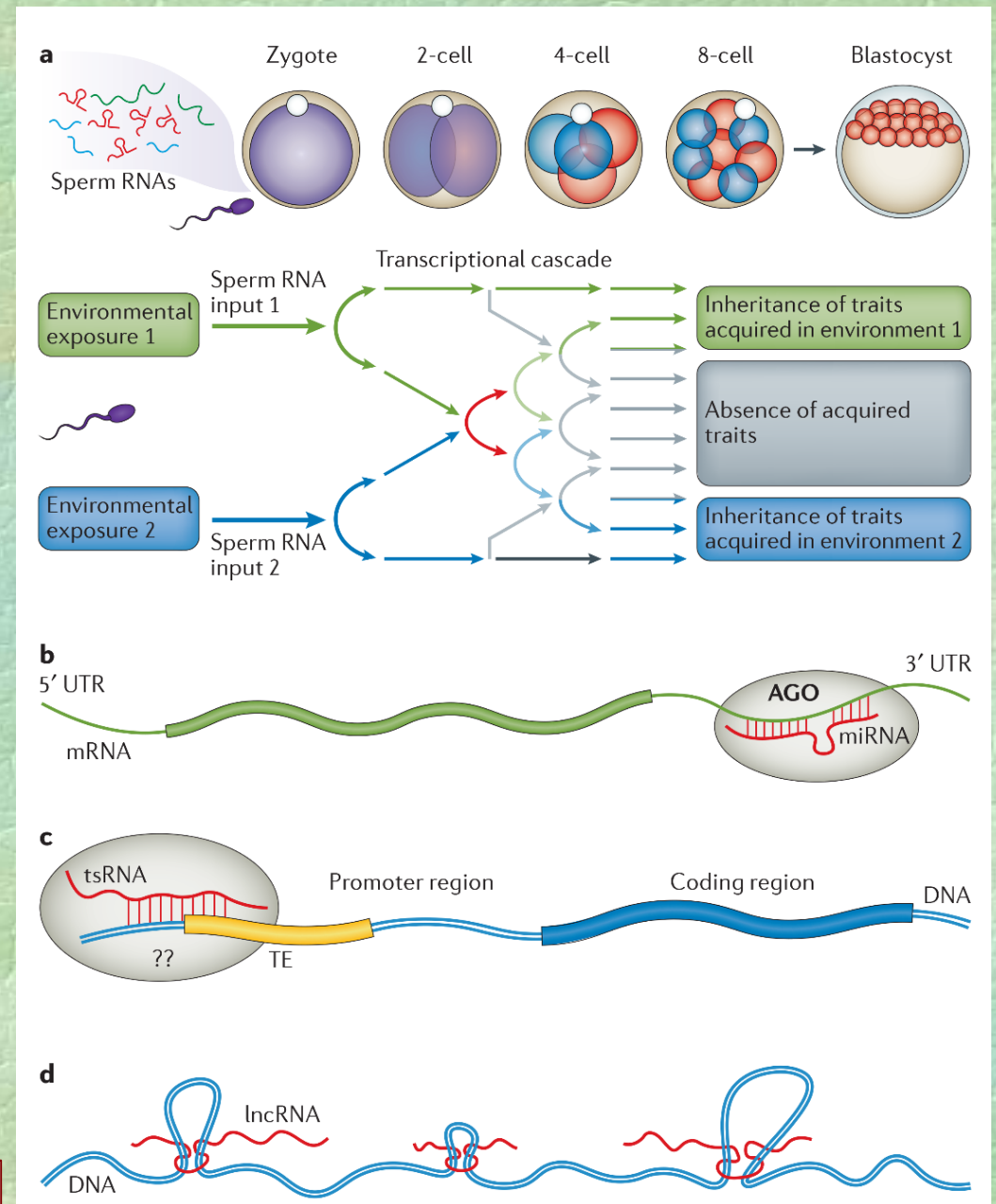


From: Barber *et al.*, 2002, *PNAS* **99**, 6877-82; 2006, *Oncogene* **25**, 7336-42; 2009, *Mutat Res* **664**, 6-12; Hatch *et al.*, 2007, *Oncogene*, **26**, 4720-4

Potential transgenerational mechanisms involving sperm RNAs



Extracellular vesicles, Evs may be regarded as the transgenerational messenger

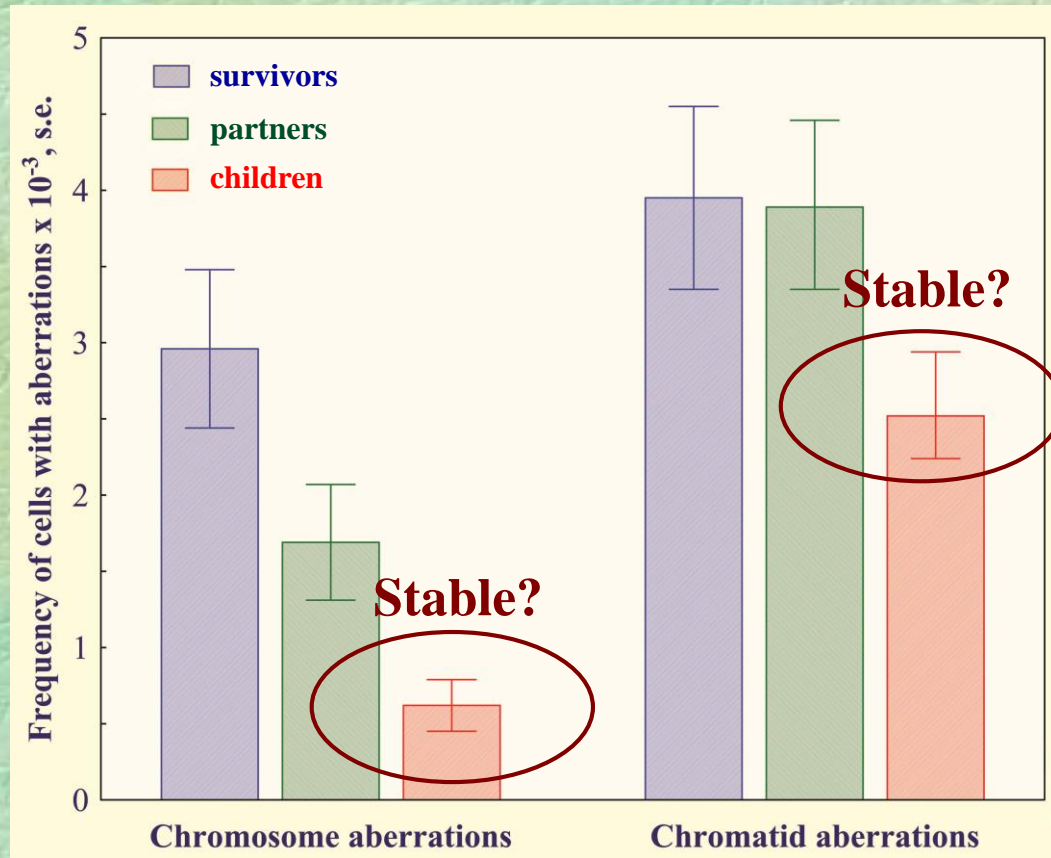


From: Chen *et al.*, 2016, *Nat Rev Genet* **17**, 733-743

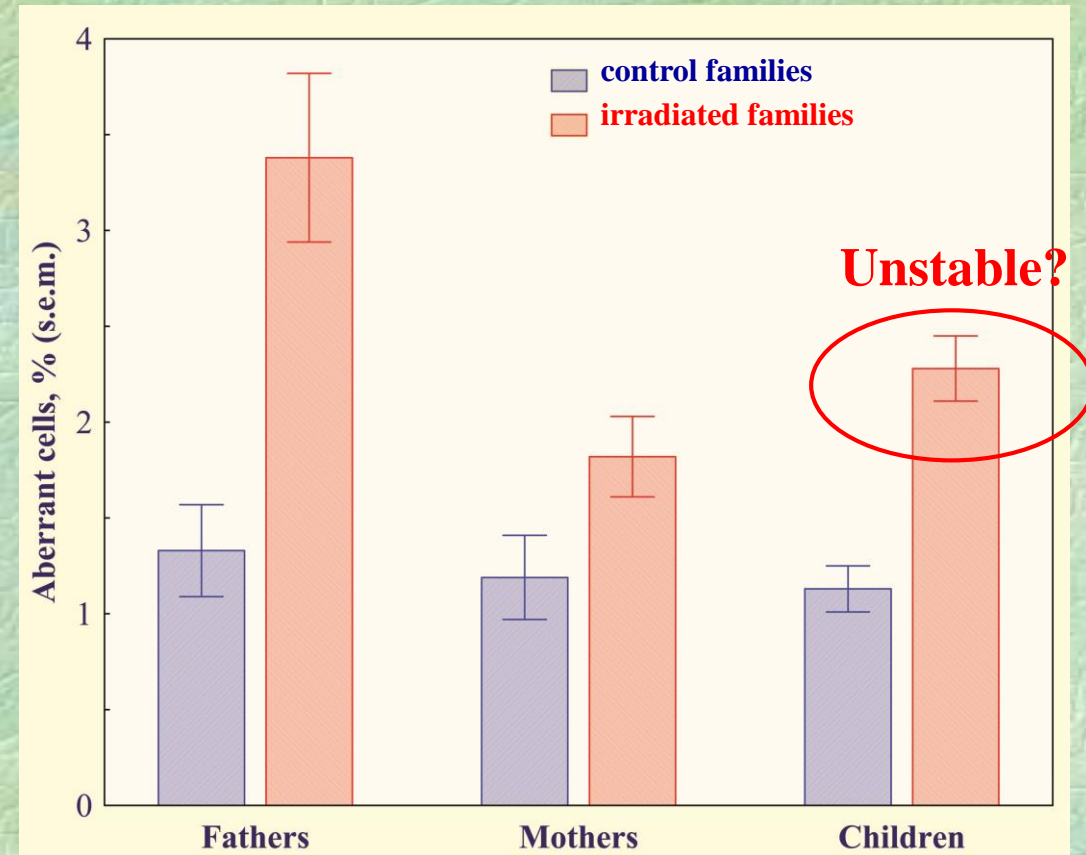
And so what?

Transgenerational effects in the children of irradiated parents

Childhood cancer survivors




Chernobyl clean-up workers




From: Tawn *et al.*, 2005, *Mutat Res* 523, 198-206; Aghajanyan & Suskov, 2009, *Mutat Res* 523, 52-7

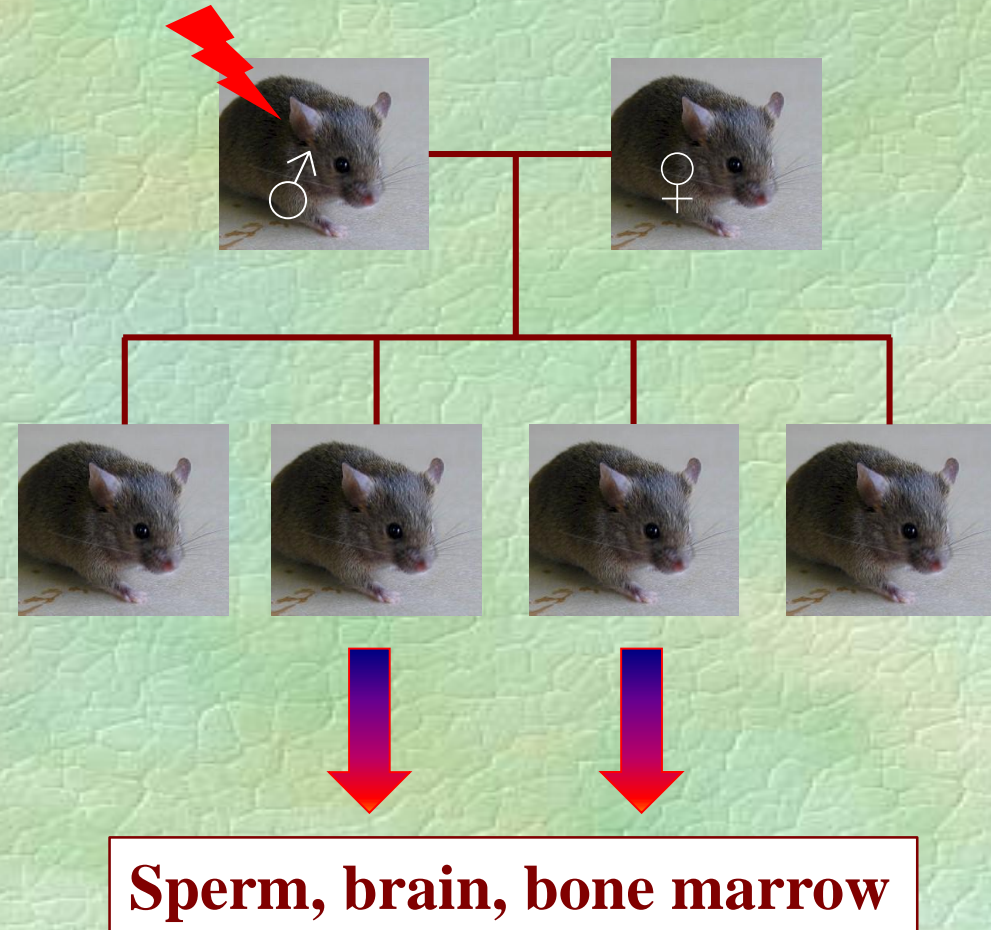
From mice to humans....



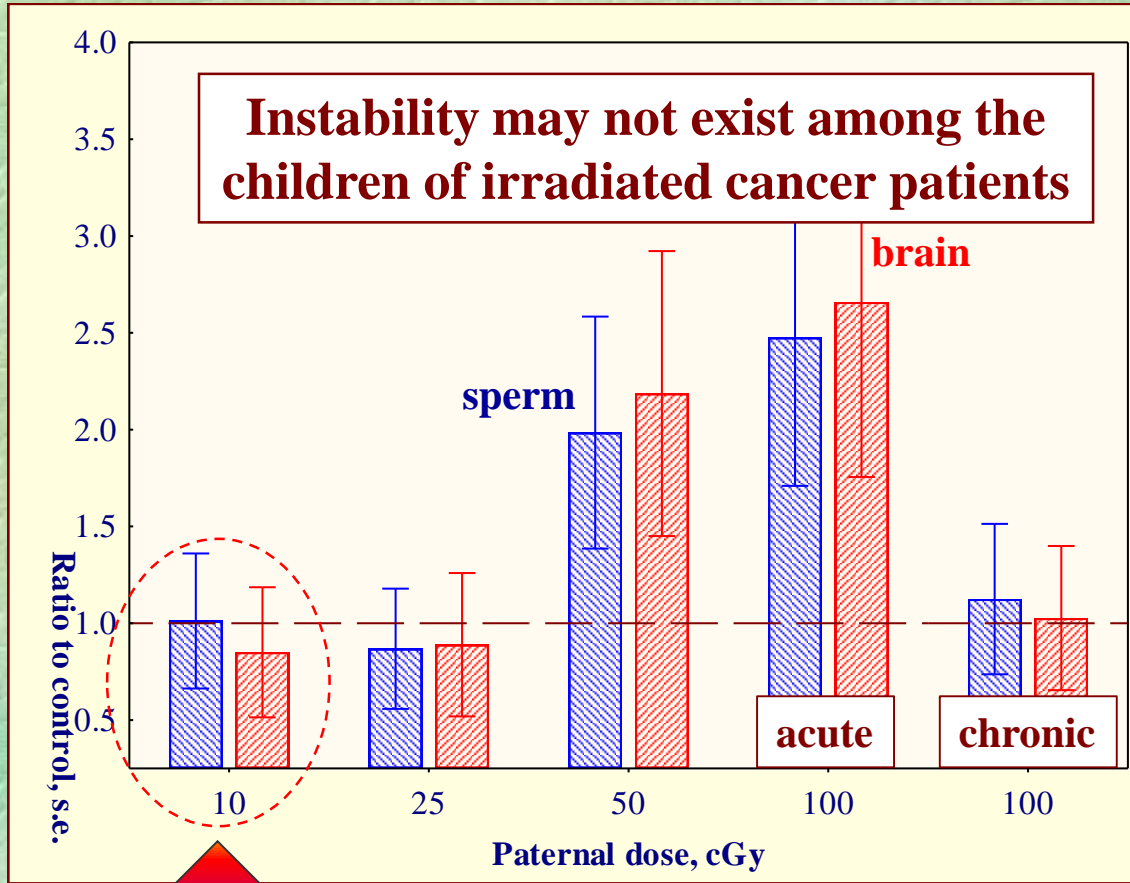
Experiment one:
Male mice exposed to
10 – 100 cGy acute γ -rays
or 100 cGy chronic γ -rays



Experiment two:
Male mice exposed to
clinically-relevant doses
of 3 anticancer drugs:
Cyclophosphamide
Mitomycin C
Procarbazine



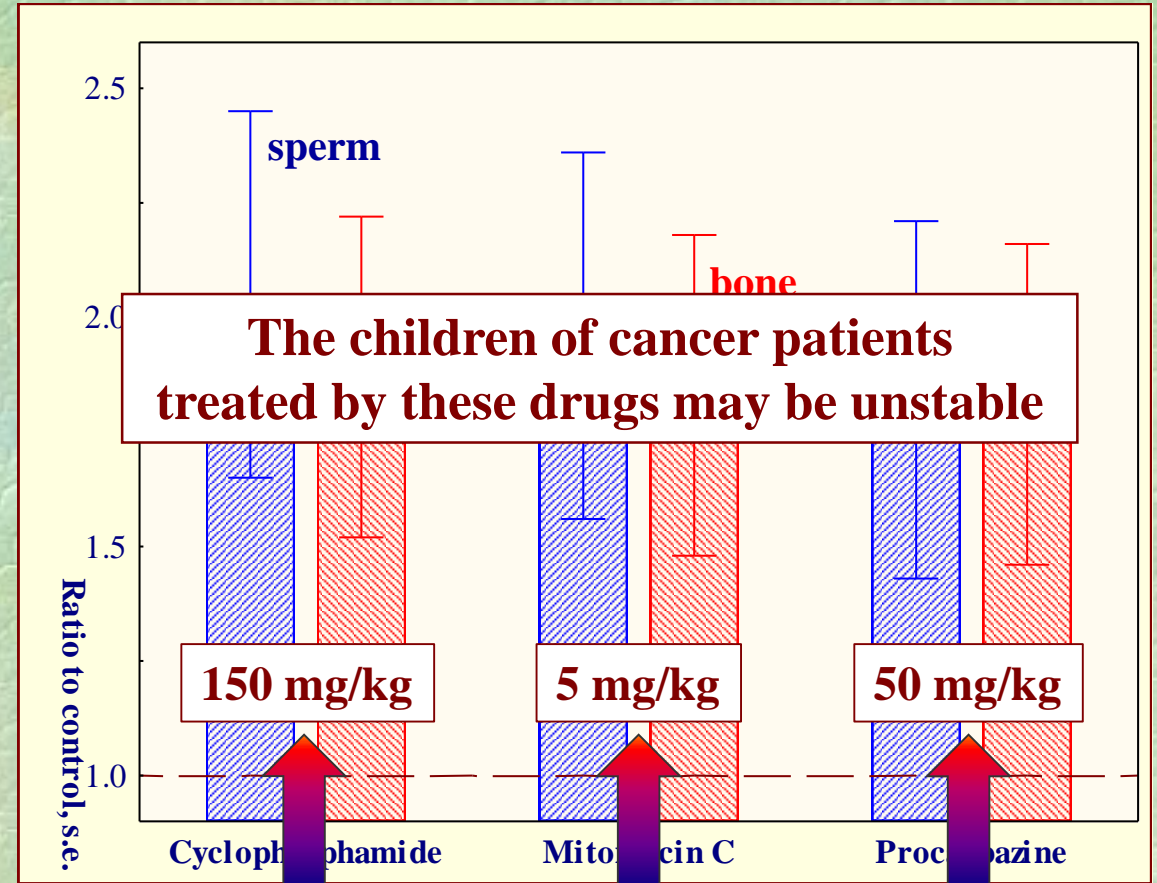
Paternal exposure to acute & chronic γ -rays



From: Mughal et al., 2012, *PLoS ONE* 7, e41300

Dose per single radiotherapy procedure

Paternal exposure to anticancer drugs



From: Glen & Dubrova, 2012, *PNAS* 109, 2984

Doses per single chemotherapy procedure

Conclusions

- **High-dose acute paternal exposure to a number of mutagens can significantly destabilise the genomes of their offspring**
- **Transgenerational instability is a genome-wide phenomenon which affects the frequency of chromosome aberrations and gene mutations**
- **Transgenerational instability is triggered in the directly exposed germ cells by a stress-like response to a generalised DNA damage**
- **Transgenerational instability is attributed to the presence of a persistent subset of endogenous DNA lesions**
- **Transgenerational instability is attributed to the epigenetic changes affecting the expression of a subset of genes, involved in rhythmic process & regulation of transcription**
- **Transgenerational instability may represent an important component of the long-term genetic risk of human exposure to mutagens, but we need HUMAN data to prove it!**

Acknowledgements

➤ **Dubrova's lab**

Ruth Barber

Dominic Kelly

Peter Hickenbotham

Karen Burr

Demetria Pavlou

Colin Glen

Robert Hardwick

Morag Shanks

Karen Monger

Adeolu Adewoye

Safeer Mughal

Mariel Voutounou

Carles Vilarino-Guell

Julia Brown

Andre Gomes

Carole Yauk

Bruno Gutierrez

Natalya Topchiy

Hamdy Ali Abouzeid

Tim Hatch

Isabelle Roux

Peter Black

➤ **MRC Radiation and Genome Stability Unit, Harwell, UK**

Mark Plumb

Emma Boulton

Jan Fennelly

Dudley Goodhead

➤ **Department of Cancer Studies and Molecular Medicine, University of Leicester, UK**

George "Don" Jones

Gabriela Almeida

➤ **NI Vavilov Institute of General Genetics, Moscow, Russia**

Alexander Rubanovich

Andrey Myazin

➤ **Medical Radiological Research Centre, Obninsk, Russia**

Leonid Zhavoronkov

Yuri Semin

Albert Brovin

Valentina Glushakova

Valentina Posadskaya

Olga Izmet'eva

➤ **MRC Toxicology Unit, Leicester, UK**

Andy Smith

➤ **Centre for Molecular Genetics and Toxicology, University of Wales, Swansea, UK**

George Johnson

James Parry

➤ **Catholic University of Nijmegen, The Netherlands**

Peter de Boer

Alwin Derijck

Godfried van der Heijden

➤ **Gray Cancer Institute, Northwood, UK**

Kai Rothkamm

Comet Assay

Chronic irradiation

Chronic irradiation

Anticancer drugs

Hprt assay

Sperm irradiation

γ H2AX assay