Minisatellites and microsatellites – similar names but different biology

Yuri E Dubrova

Department of Genetics
University of Leicester

Tandem repeat DNA loci

- **Consist of repeats** **$GAACA_n$**

  _GAACA_GAACA_GAACA_GAACA_GAACA ….

- **Highly variable** **up to 100+ alleles per locus**

- **High mutation rate** **up to 0.15 per locus**

- **Because of this they are widely used in:**
  - **forensics (paternity testing, identity identification)**
  - **population genetics**
  - **gene mapping**
  - **mutagenesis (germline & somatic mutation induction)**
  - **cancer studies (stability of cancer cells)**

Tandem repeat DNA loci

- **Microsatellite loci**
  PubMed 26,529 items
- **Minisatellite loci**
  PubMed 3,120 items
- **Expanded Simple Tandem Repeat loci (ESTR)**
  PubMed 63 items

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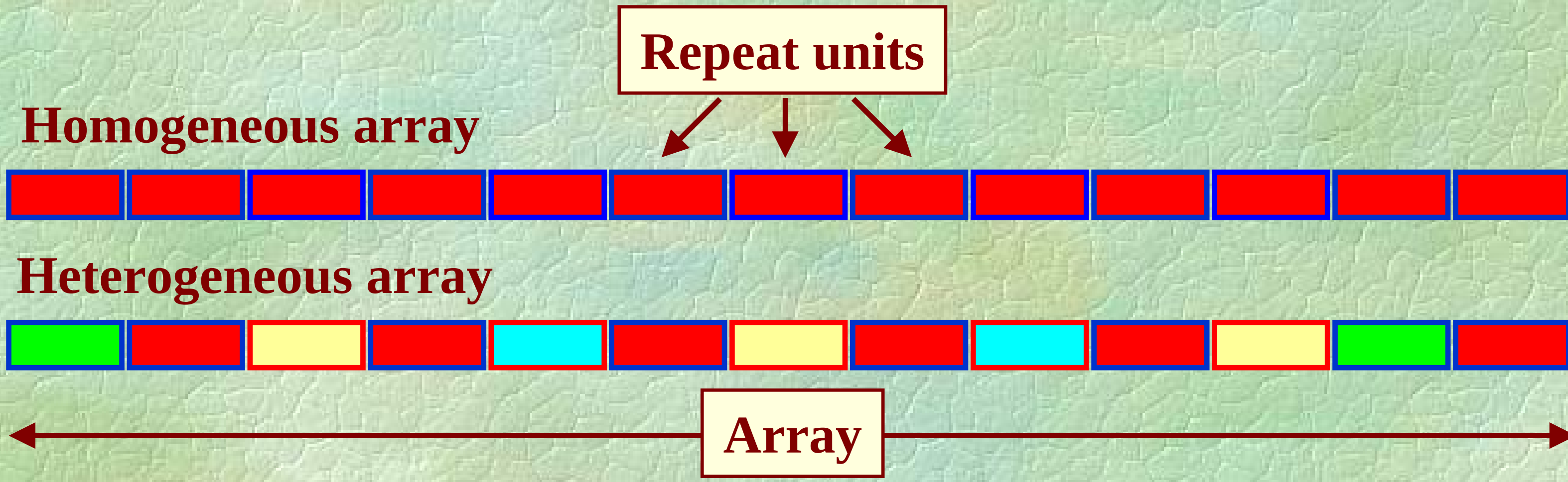
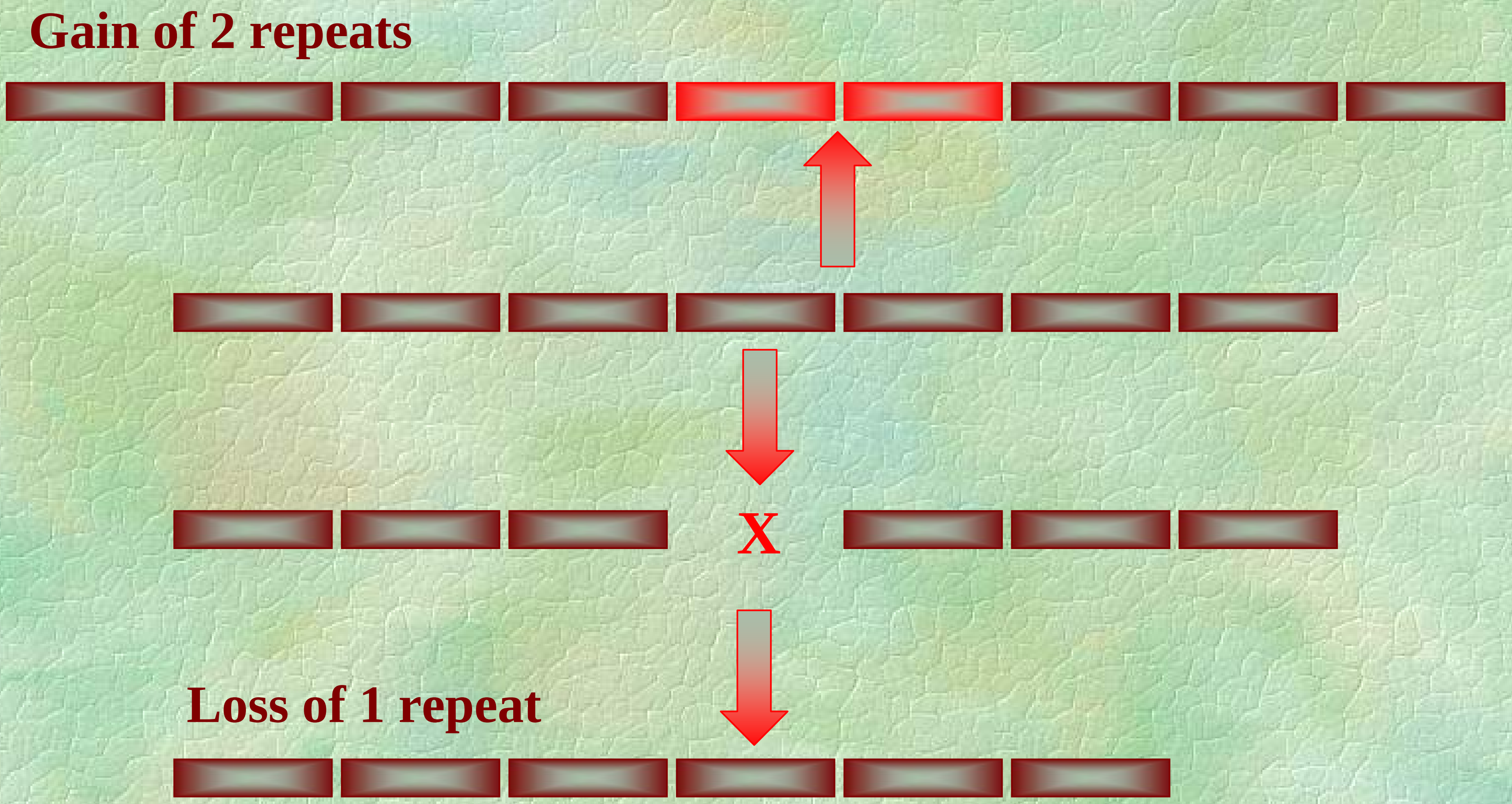
Tandem repeat DNA loci



	Minisatellites	*ESTRs*	*Microsatellites*
➢ Repeat unit	10 – 60 bp	4 – 10 bp	2 – 6 bp
➢ Size of array	0.5 – 15 kb	0.1 – 20 kb	10 – 1000 bp
	10 – 1,500 rpts	10 – 2,000 rpts	5 – 200 rpts
➢ Complexity of array	heterogeneous	mostly homogeneous	mostly homogeneous

Schematic examples of mutations at tandem repeat DNA loci



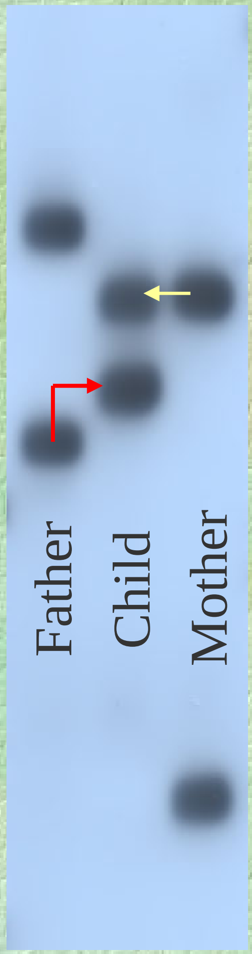
Germline mutations at human minisatellite loci

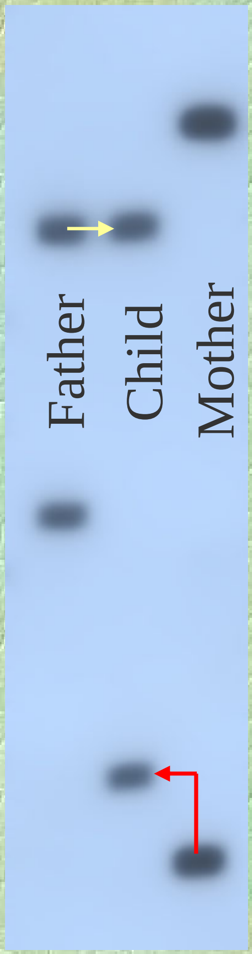
MS31
paternal/gain

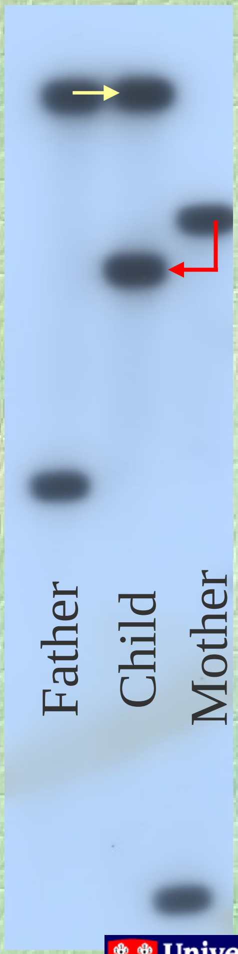
MS32
paternal/loss

MS1
maternal/gain

CEB25
maternal/loss







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Spontaneous germline mutation rates at tandem repeat DNA loci and protein-coding genes

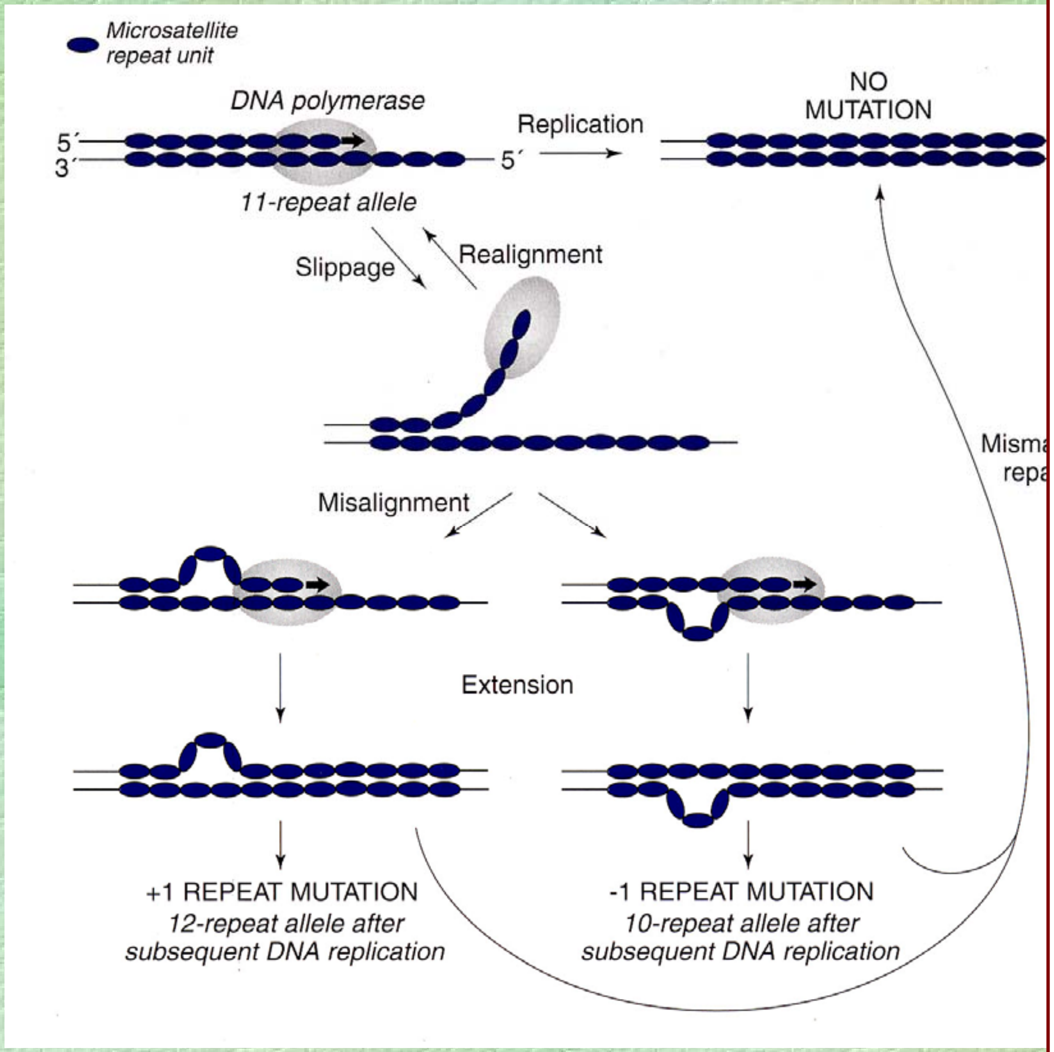
Probe (Locus)	*Paternal*	*Maternal*	*Total*
	Mutation rate per gamete		
➢ **Human minisatellite loci**			
✓ CEB1 (*D2S90)*	**0.161**	**0.003**	**0.082**
✓ B6.7 (20q13)	**0.076**	**0.012**	**0.044**
✓ MS1 (*D1S7)*	**0.055**	**0.049**	**0.052**
✓ CEB25 (*D10S180)*	**0.035**	**0.019**	**0.027**
✓ CEB36 (*D10S473)*	**0.018**	**0.018**	**0.018**
✓ MS31 (*D7S21)*	**0.012**	**0.003**	**0.008**
✓ MS32 (*D1S8)*	**0.009**	**0.006**	**0.007**
➢ **Mouse ESTR loci**			
✓ *Ms6-hm*	**0.10-0.18**	**0.06-0.09**	**0.09-0.13**
✓ *Hm-2*	**0.02-0.07**	**0.04-0.14**	**0.04-0.11**
➢ **Human microsatellite loci**	mean ~ **0.002** (0.0001 – 0.01)		
➢ **Protein-coding genes**	**10^{-6} – 10^{-5} (< 1 per 100,000)**		

Data from numerous original publications

Mechanisms of mutation

Microsatellites

Replication slippage



Predictions

- ➢ Mutate in dividing cells only
- ➢ No germline specificity, *i.e.* mutations frequently occur in somatic tissues
- ➢ Germline mutation rate in males > females
- ➢ Age-related accumulation of mutations
- ➢ Simple mutation spectrum

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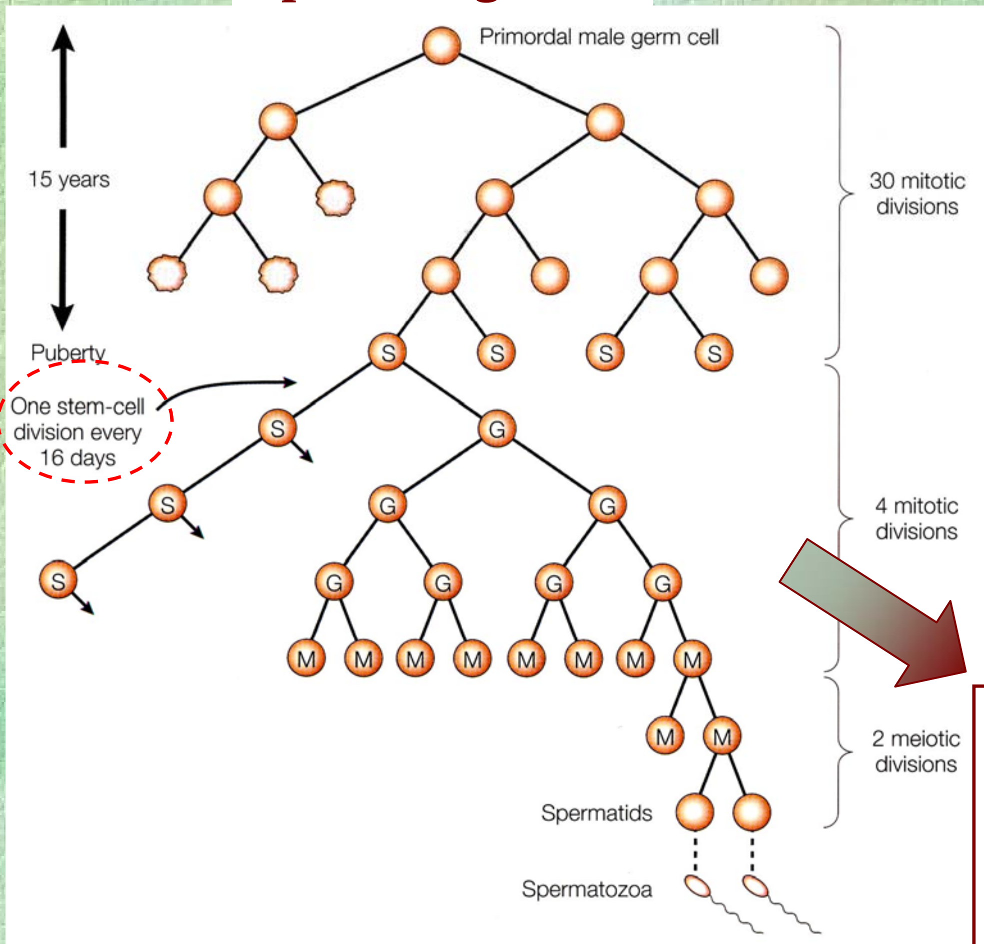
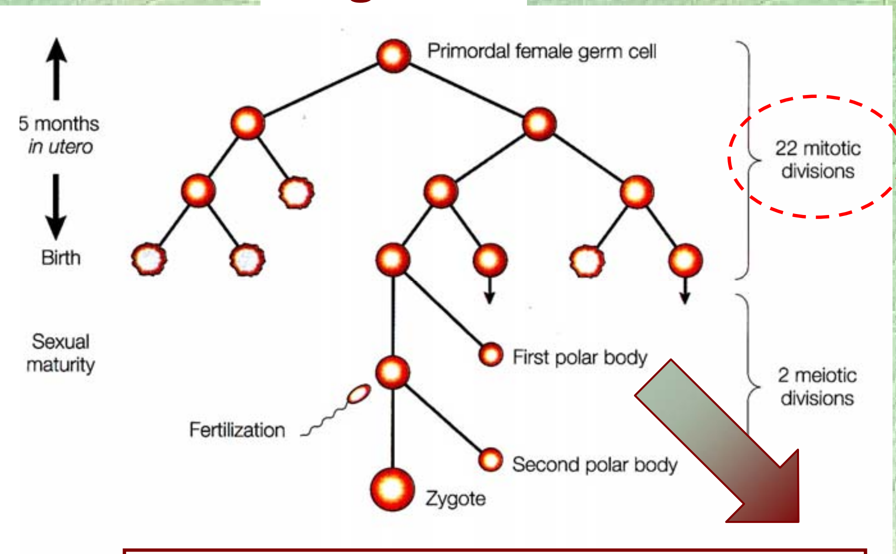
Cell divisions during spermatogenesis & oogenesis in humans

Spermatogenesis



Oogenesis



22 mitotic divisions for all ages

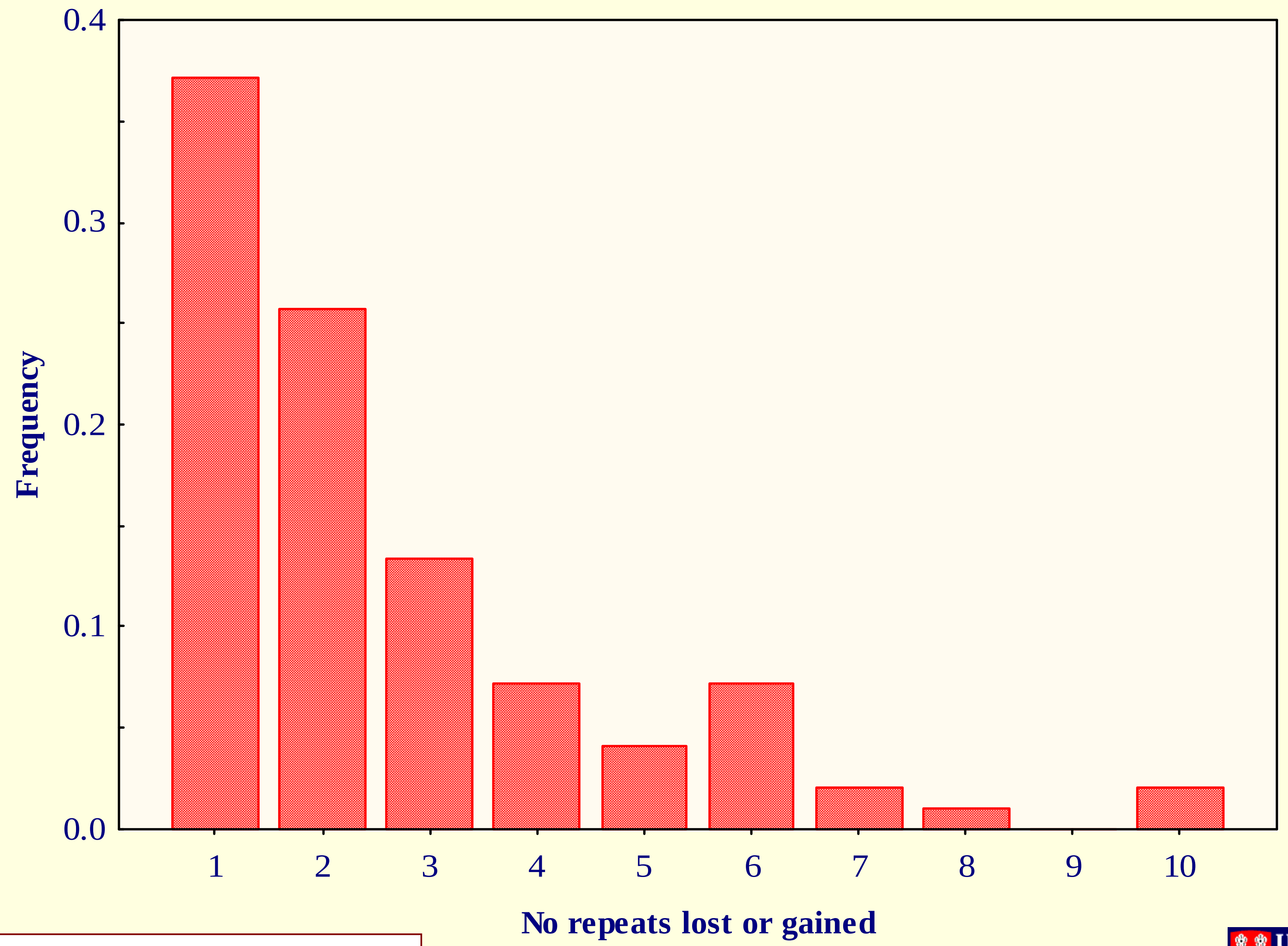
No male germ-cell divisions

Age, years	*No replications*
15	35
20	150
30	380
40	610
50	840

Microsatellite mutation rates
Male/Female ratio 5 : 1

From: Crow, 2000, *Nature Rev Genet*, **1,** 40-7; Ellegren, 2000, *TIG* **16,** 551-8

Spectrum of spontaneous microsatellite mutation in humans (362 loci, 53 pedigrees, 630 subjects, 97 mutational events)

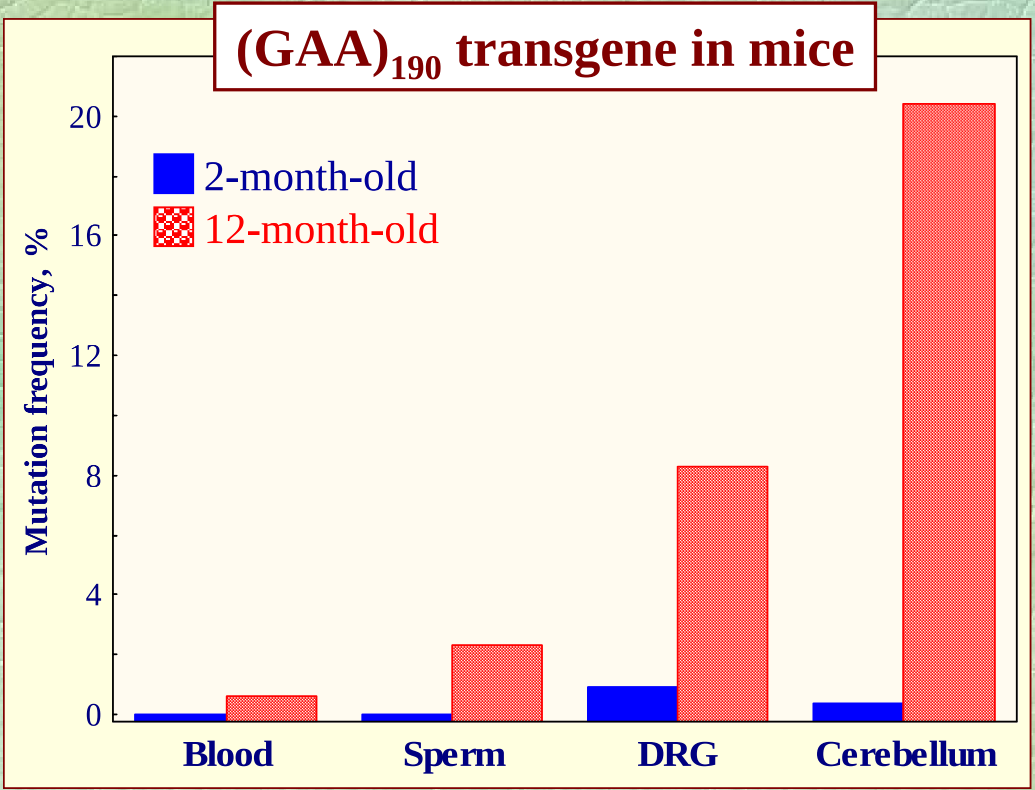


From: Huang *et al.*, 2002, *Am J Hum Genet* **70,** 625-34

The big, the bad, the ugly microsatellites

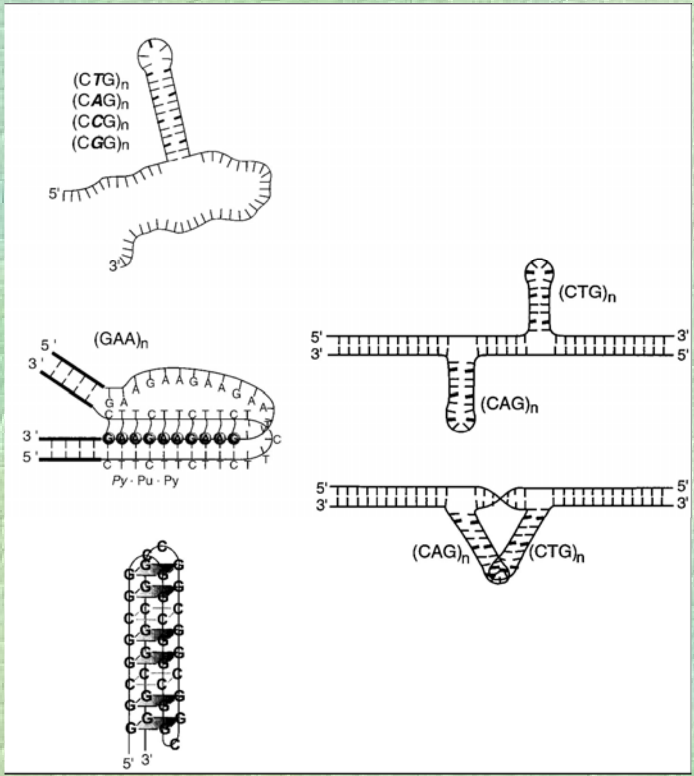
- Long arrays are highly unstable, ‘100%’ mutation rate
- Associated with some human diseases

Mutate in non-dividing tissues



From: Clark *et al.*, 2007, *Hum Genet* **120,** 633

Secondary structures
DNA-repair is involved



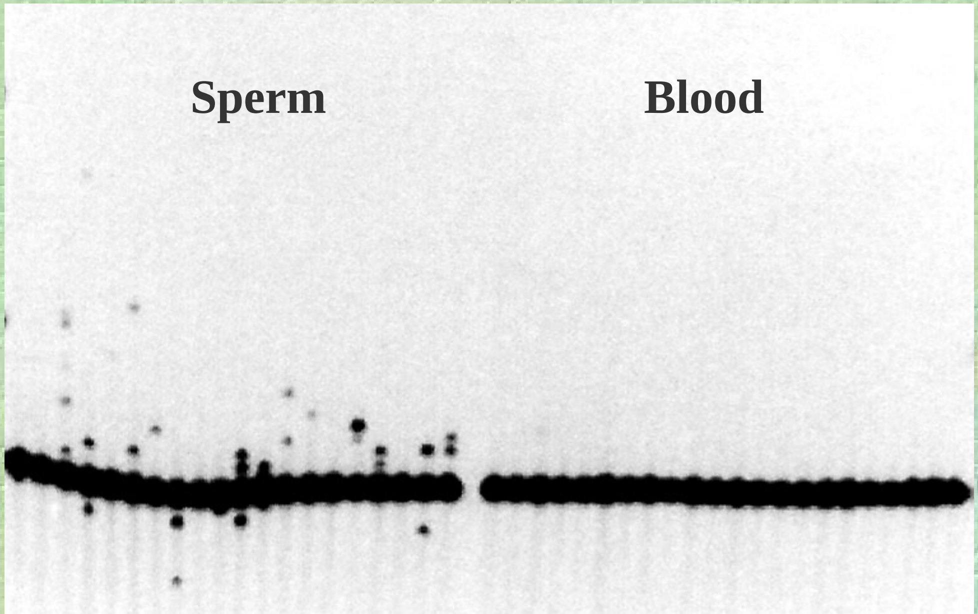
From: Sinden, 1999, *Am J Hum Genet* **64,** 346-53

Minisatellites

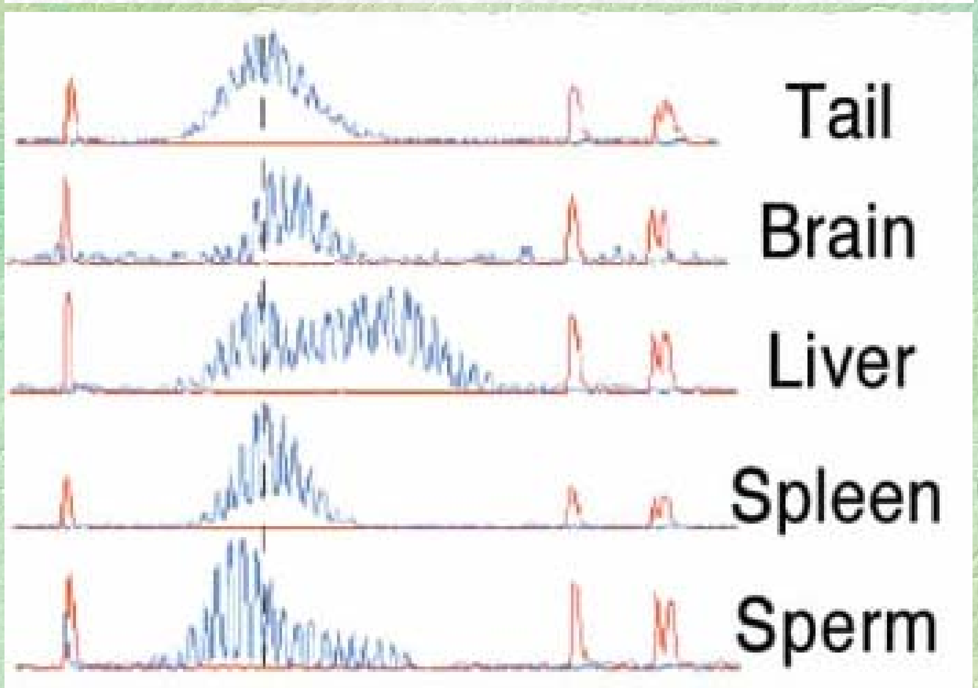
- Very unstable in the germline
  but ~ dead in somatic tissues

- Very complex mutations in the male germline
  and very simple in somatic tissues

Germline *vs.* somatic mutation at minisatellite loci



Human minisatellite B6.7



CAG_{118} human microsatellite transgene in mice

From: Tamaki *et al.*, 1999, *Hum Mol Genet* **8,** 879-88
Kovtun & McMurray, 2001, *Nat Genet* **27,** 407-11

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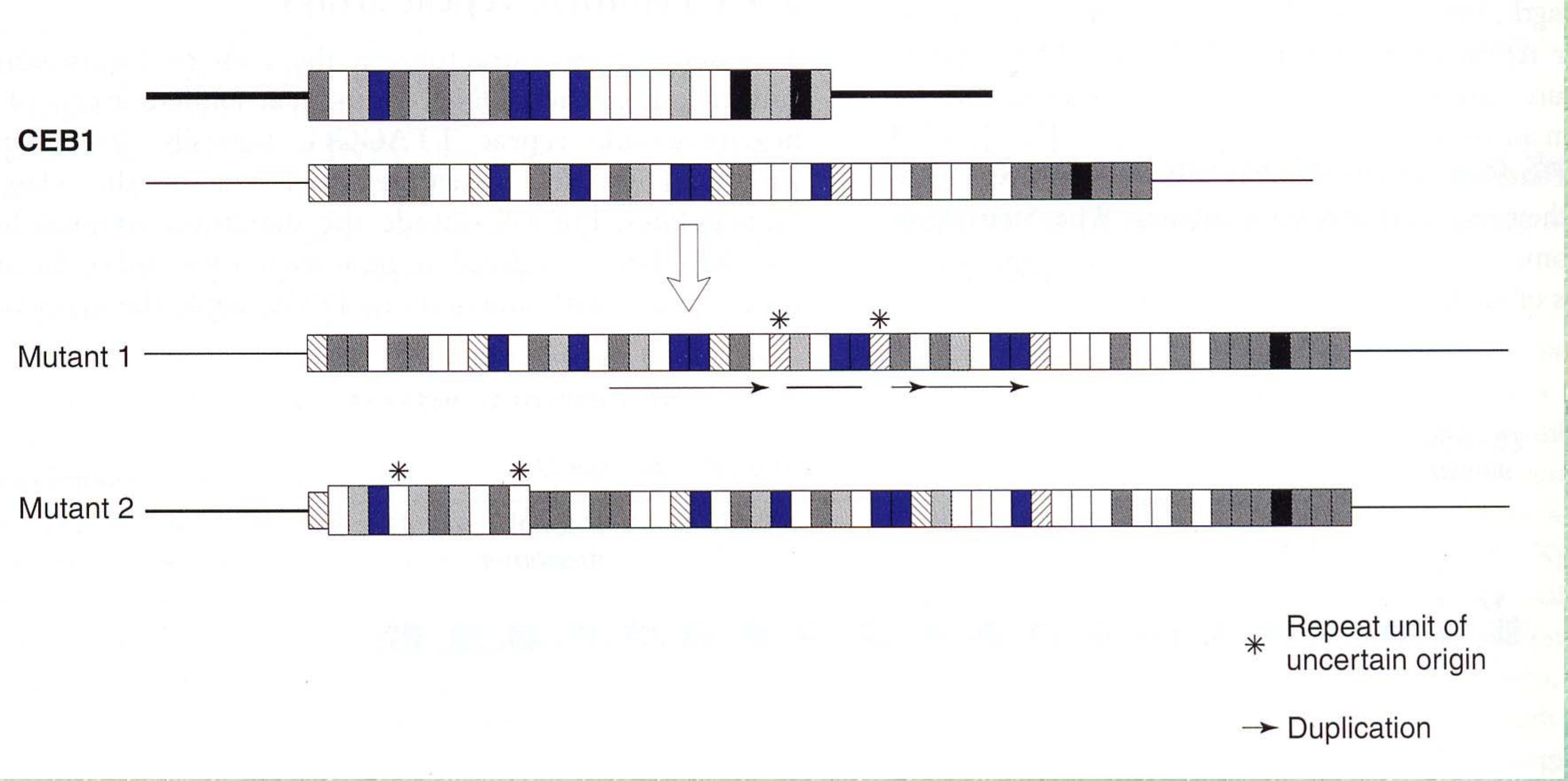
Spectrum of minisatellite mutants in sperm



From: Jobling *et al*., 2004, *Human Evolutionary Genetics*

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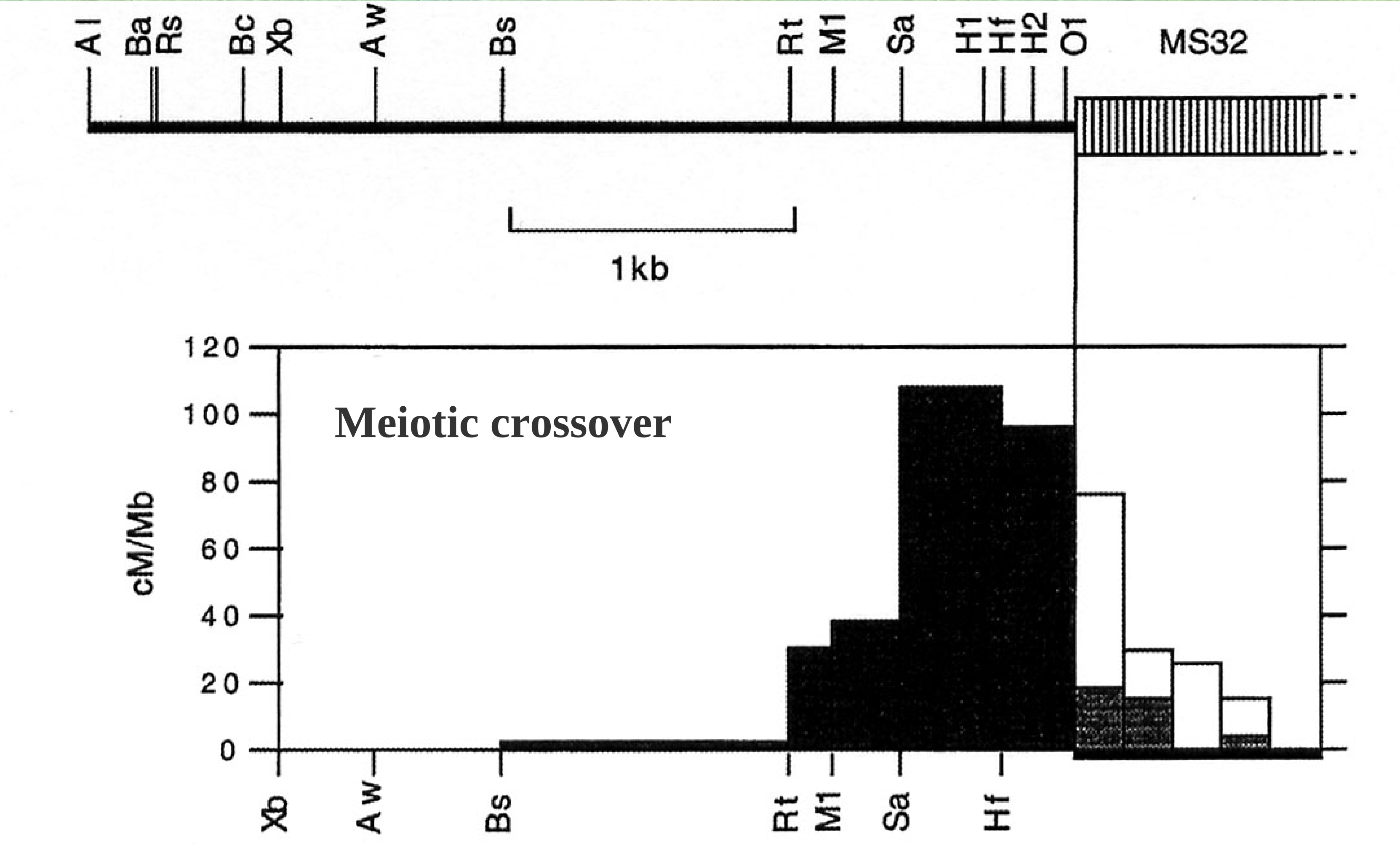
Minisatellite mutation is somehow related to what is going on at meiotic crossover hot-spots

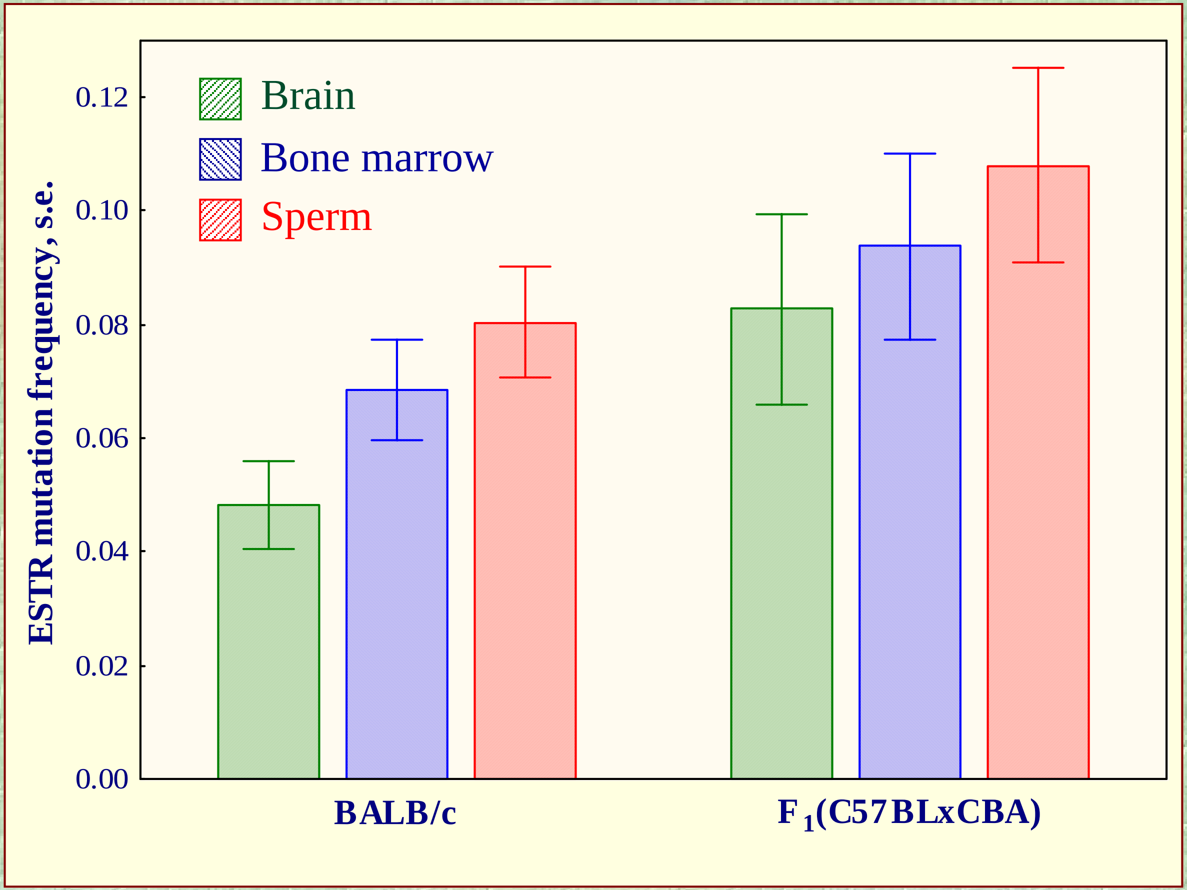


From: Jeffreys *et al.*, 1999, *Electrophoresis* **20,** 1665-75

<u>E</u>xpanded <u>S</u>imple <u>T</u>andem <u>R</u>epeat loci or ESTR

ESTR's

- **Repeat size: 4-10 bp, *i.e.* between micro- (1-4bp) & mini-**
- **Array size: 10-2000 rpts, *i.e.* more mini-like**
- **Unstable in somatic tissues, *i.e.* micro-like**



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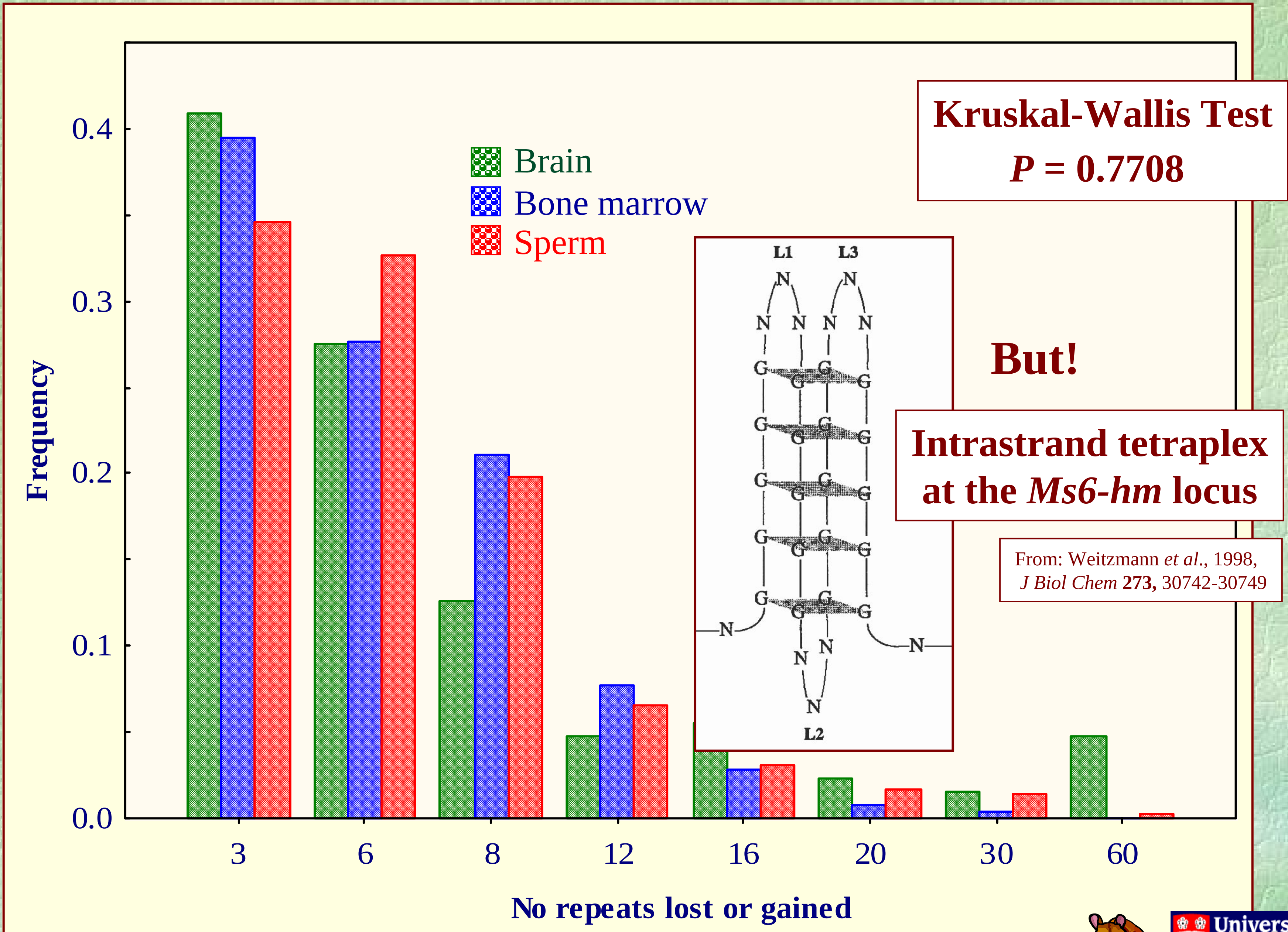
Similar spectra of spontaneous ESTR mutation in mouse tissues



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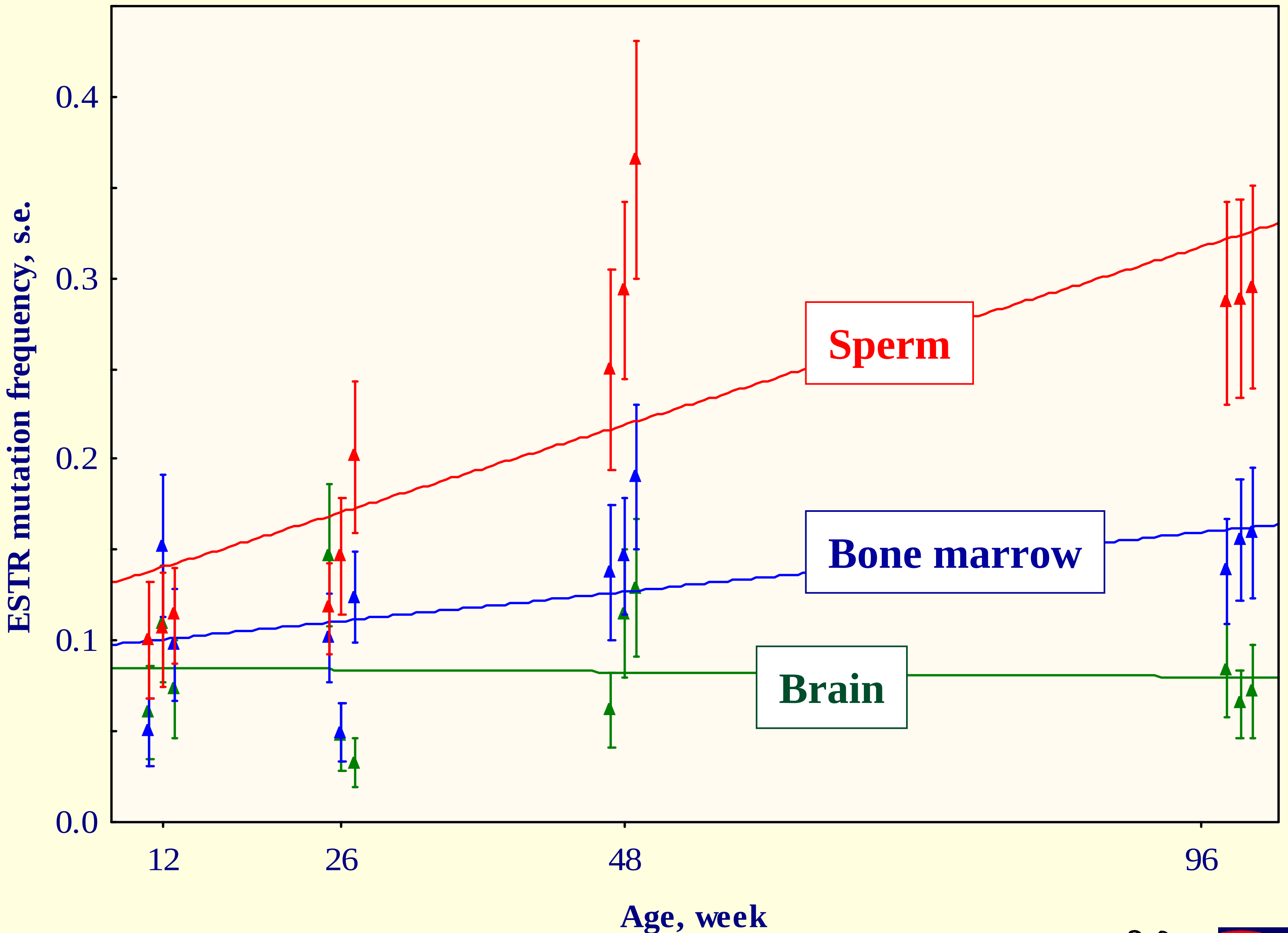
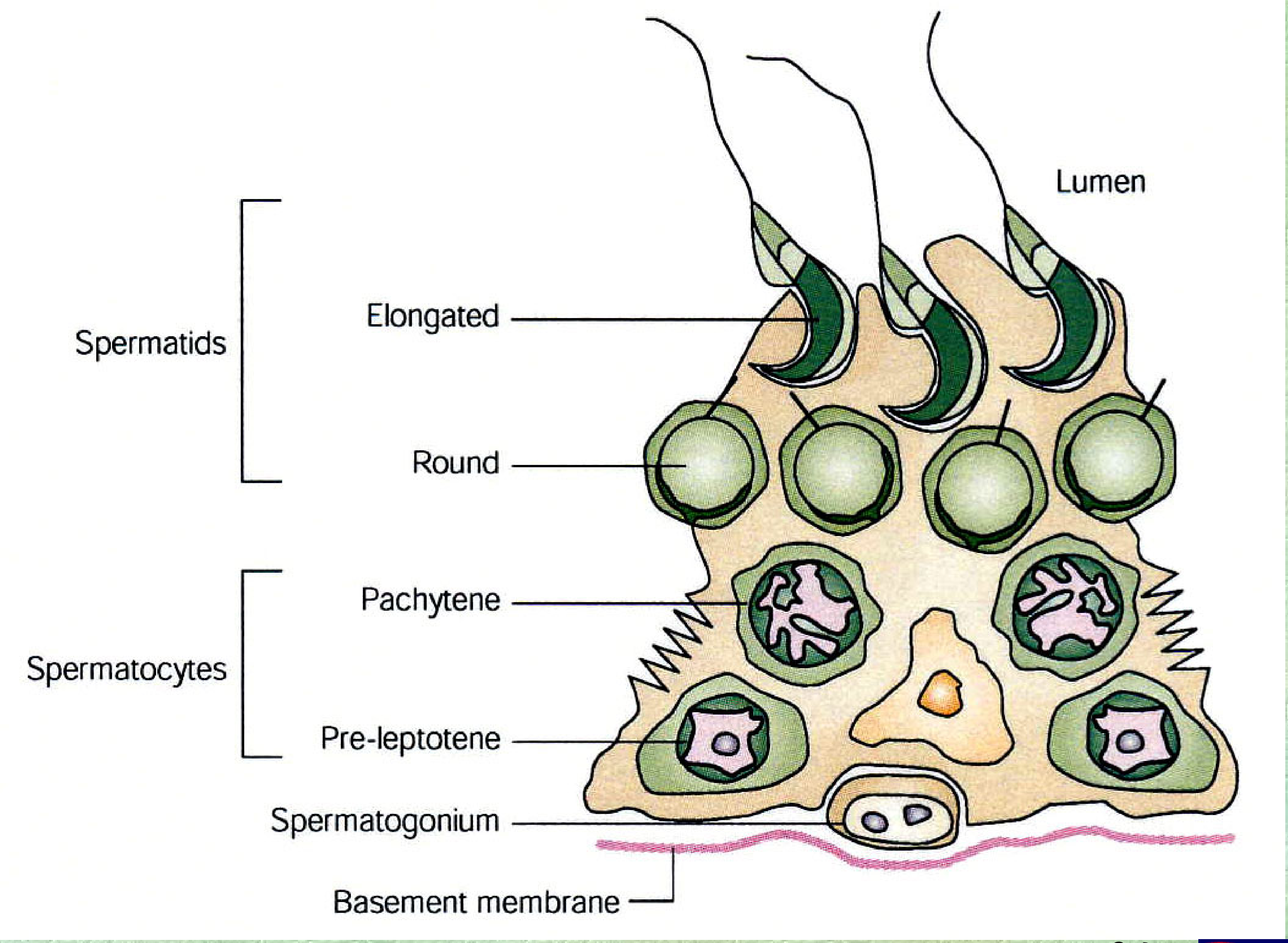
If ESTR loci ‘behave’ like true microsatellites then:

- **Age-related increases in replication-proficient tissues**
- **No age-related changes in non-dividing tissues**
- **Stage-specific pattern of spontaneous ESTR mutation in the male germline**

Age-related changes in ESTR mutation frequency in mice



A single Sertoli cell with its associated germ cells



From: Cooke & Saunders, 2002, *Nat Rev Genet* **3,** 790

Meiotic events

Pre-meiotic spermatogonia → **Meiotic spermatocytes I** | Round spermatids | Elongated spermatids

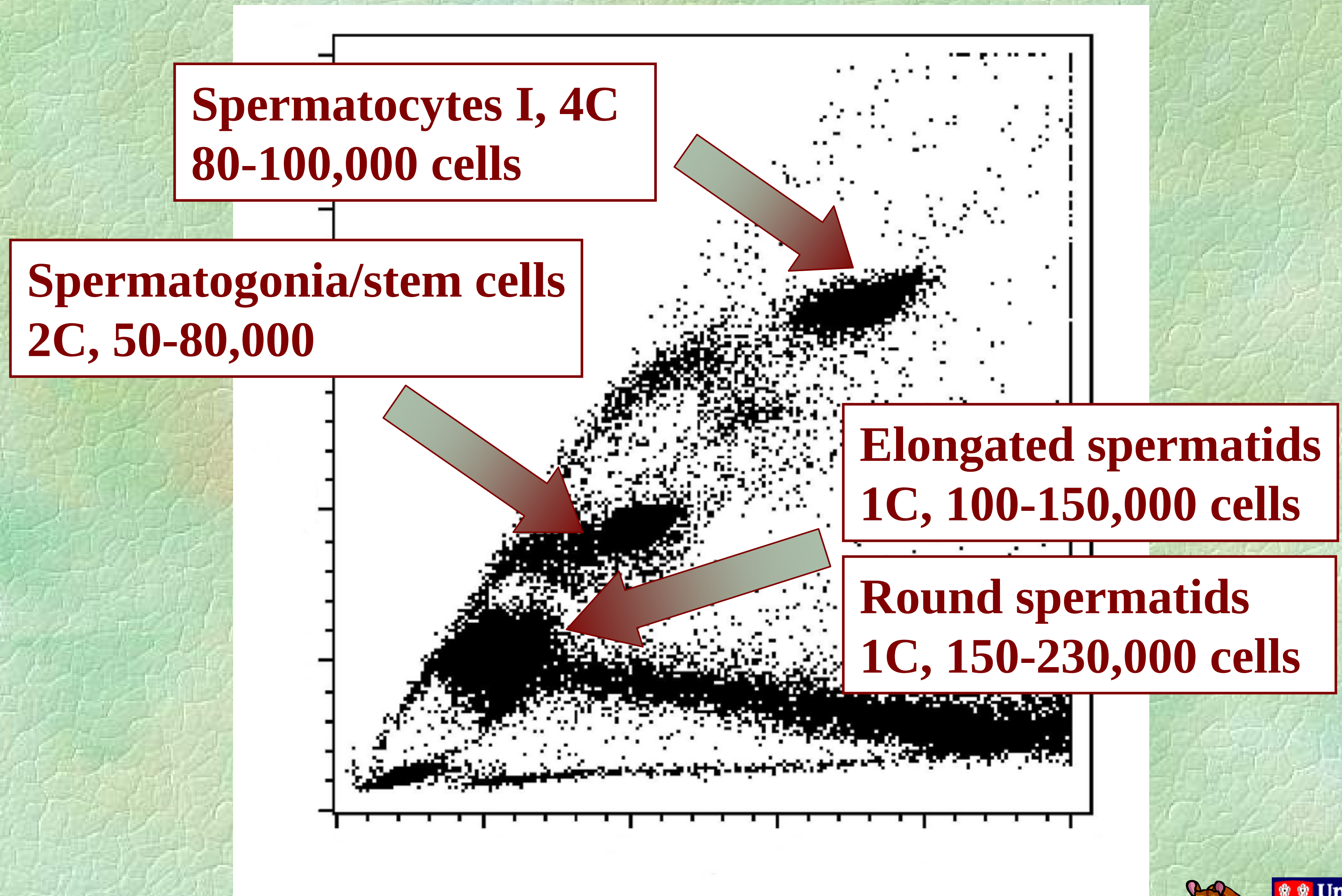
Late post-meiotic events

Pre-meiotic spermatogonia | Meiotic spermatocytes I | Round spermatids → **Elongated spermatids**

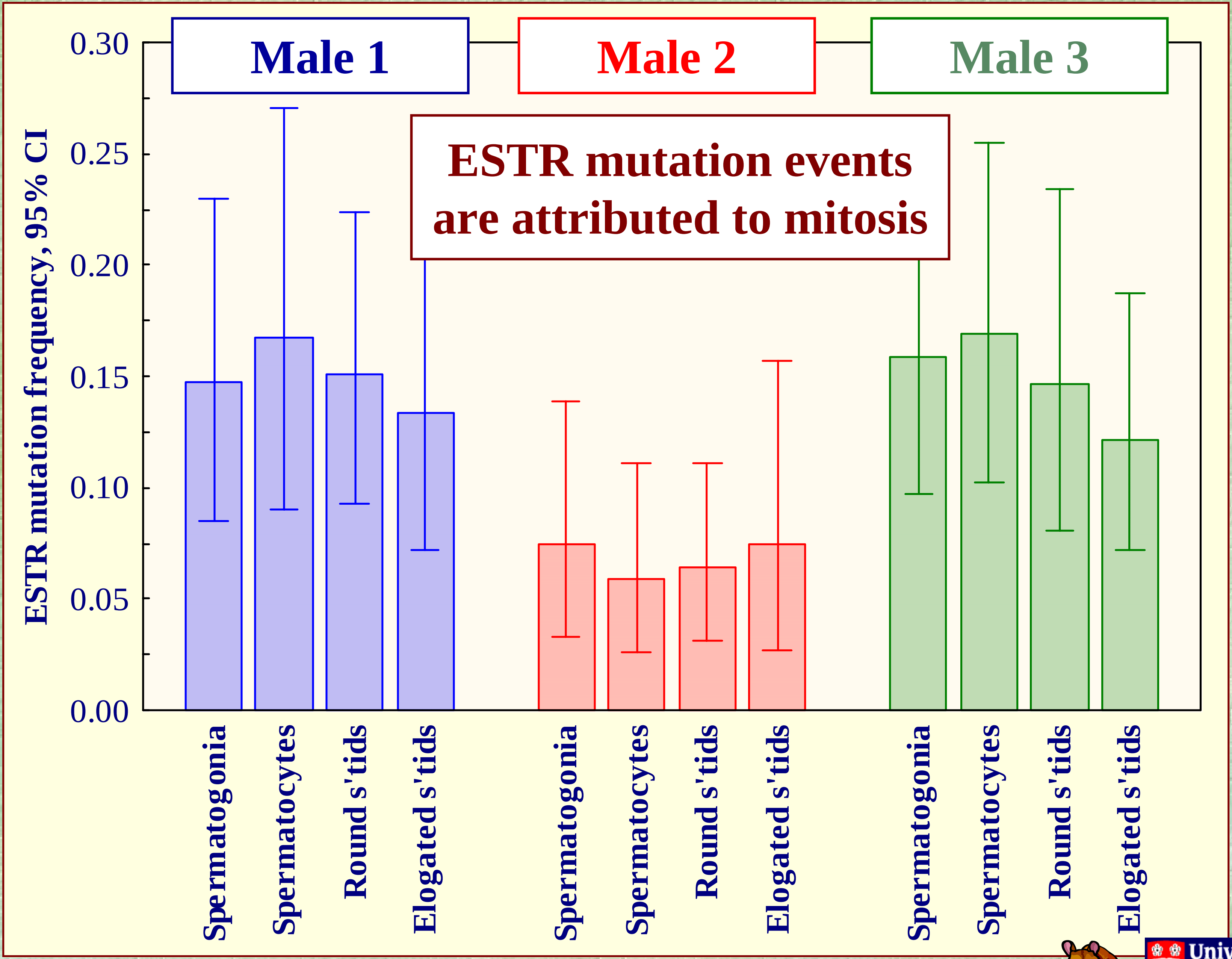
Mitotic events

Pre-meiotic spermatogonia | Meiotic spermatocytes I | Round spermatids | Elongated spermatids

Separation of mouse germ cells by flow cytometry



ESTR mutation frequencies in mouse germ cells



Problems

We observe changes in mutation frequencies, p

***p* = *f*(*u*, *N*, *s*)**

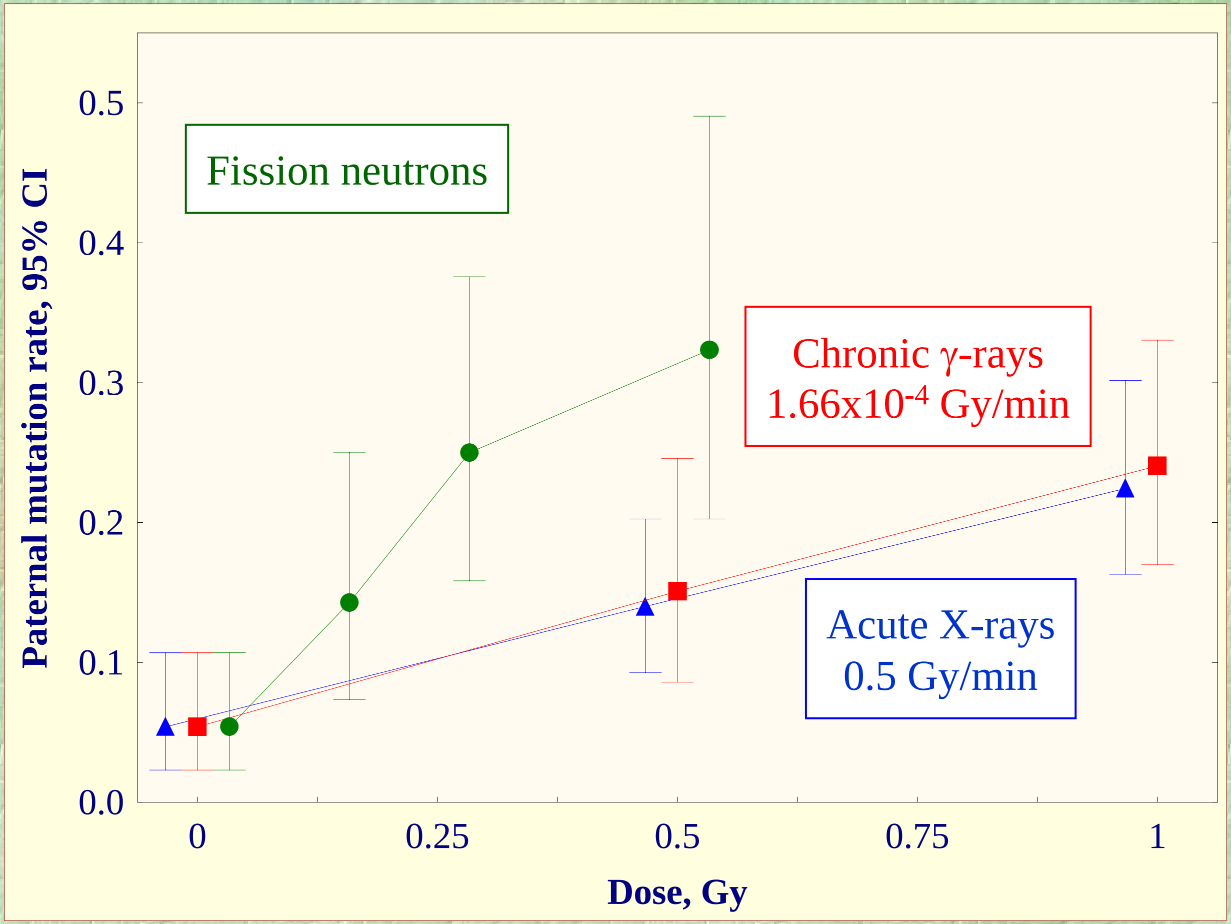
***u* – mutation rate per DNA replication/cell division**

***N* – number of cell divisions**
known for the male germ cells

***s* – selection against mutants, *s* = 0**

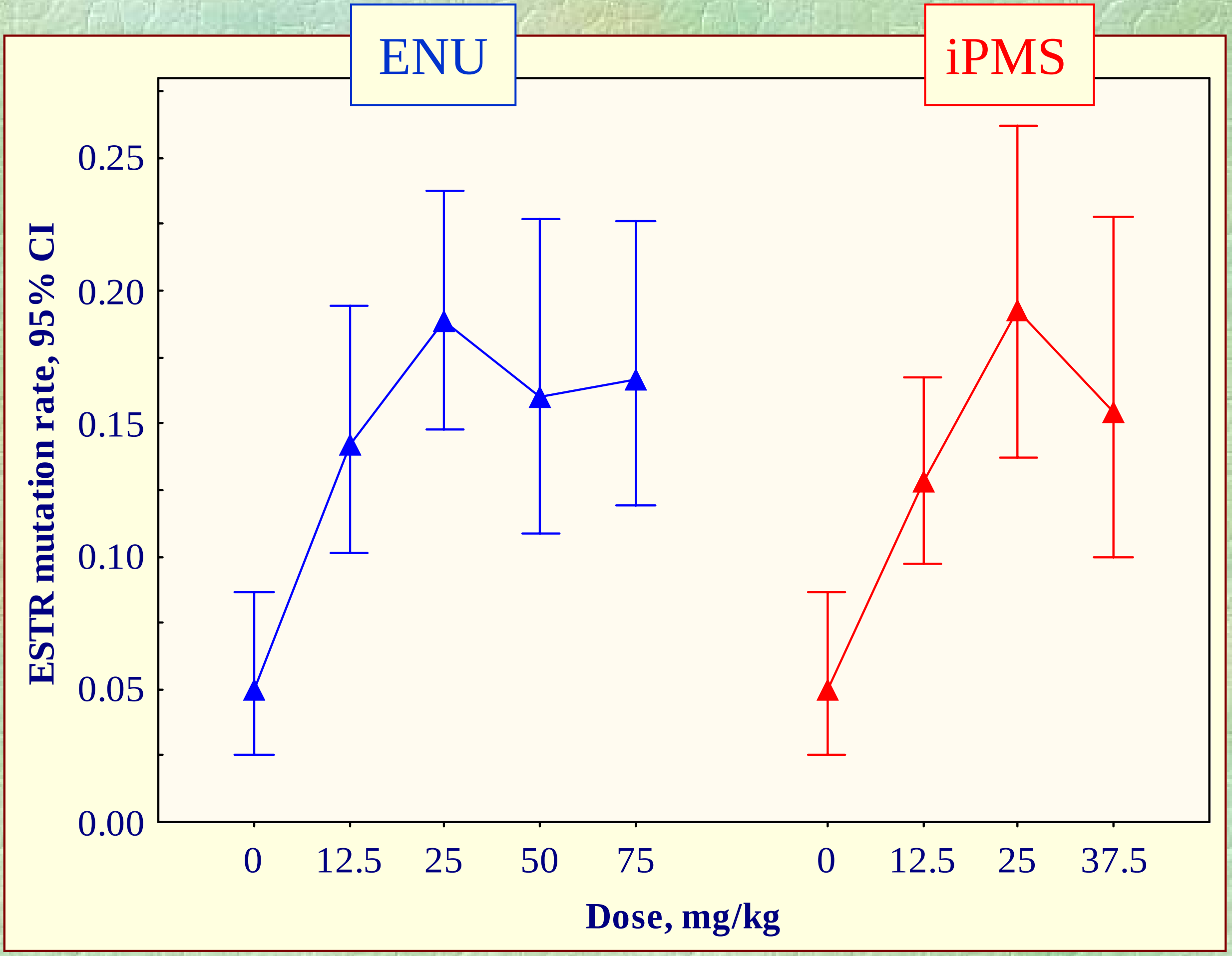
?

Mutation induction at mouse ESTR loci



From: Dubrova *et al.*, 1998, *PNAS* **95,** 6251-6255
2000, *Mutat Res* **453,** 17-24

Mutation induction at mouse ESTR loci



From: Vilariño-Güell *et al.*, 2003, *Mutat Res* **526,** 63-73

Non-targeted mutation induction at mouse ESTR loci

Expected genome damage from mouse ESTR data

➢ Spontaneous mutation rate (*Ms6-hm* + *Hm-2*)	0.055 per locus
➢ Induced paternal mutation rate (1 Gy)	0.225 per locus
➢ Radiation-induced increase in mutation rate	0.170 per locus
➢ Mean size for *Ms6-hm* & *Hm-2* (CBA/H mice)	5×10^3 bp
➢ Genome size	3×10^9 bp
➢ Damage to the whole genome	$0.17 \times (3 \times 10^9)/(5 \times 10^3) =$ 100,000

Radiation-Induced Damage in Eukaryotic Cells

➢ Base damage	2000 per 1Gy
➢ Single-strand breaks	1000 per 1Gy
➢ DNA-protein links	150 per 1Gy
➢ Double-strand breaks	40 per 1Gy
➢ Bulky lesions	40 per 1Gy
➢ Total	3300 per 1Gy $\ll$ 100,000

From: Dubrova *et al.*, 1998, *PNAS* **95,** 6251-6255
Frankenberg-Schwager, 1990, *Radiat Environ Biophys* **29**, 273-292

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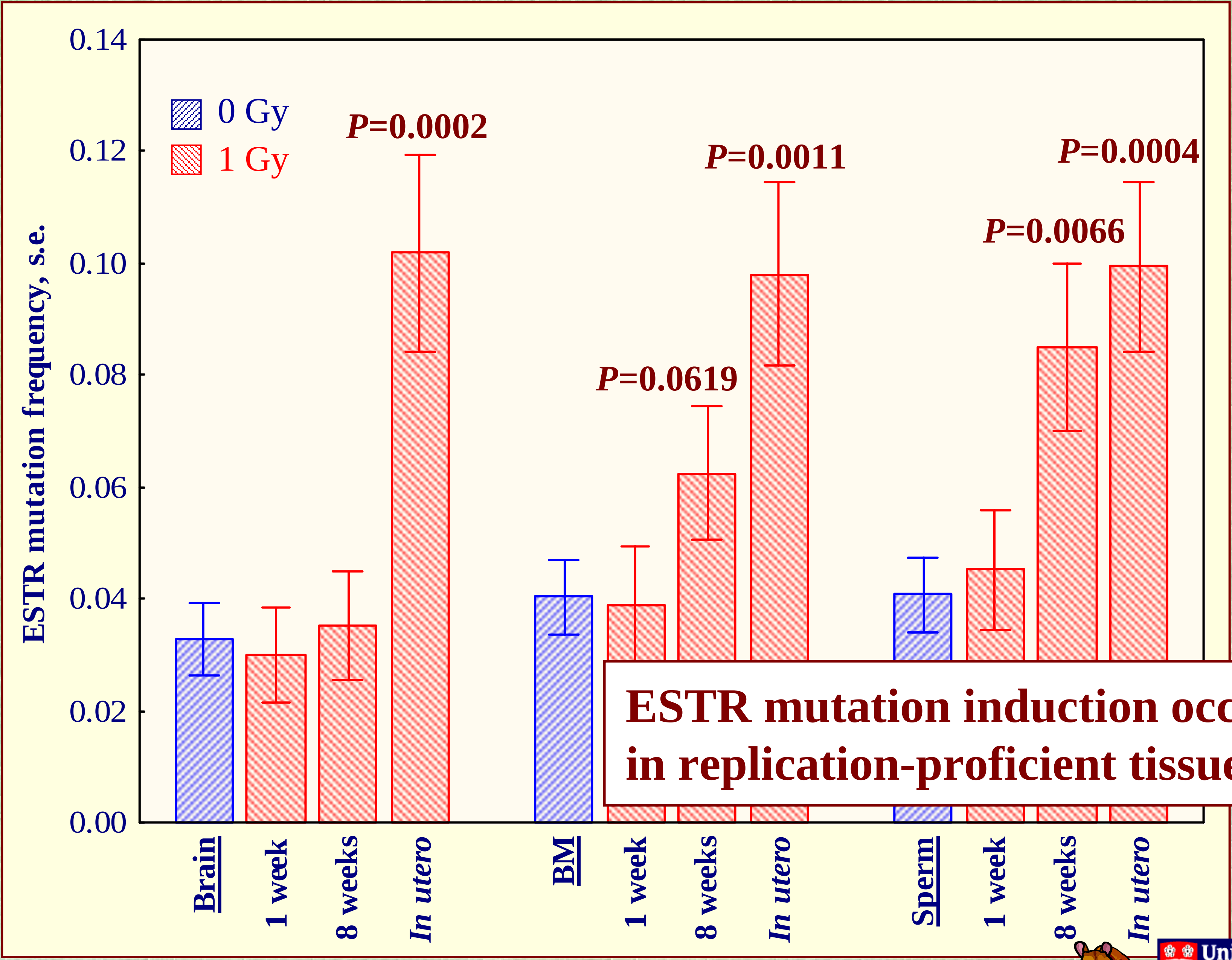
ESTR mutation induction and cell division

Stage of irradiation	Tissue, proliferation capacity: Brain	Bone marrow	Male germ cells
Embryo males 12 days of gestation	+	+	+
Adult males 1 week after exposure	-	-	-
Adult males 10 weeks after exposure	-	+/-	+

ESTR mutation induction in male mice exposed to 1 Gy X-rays



ESTR mutation induction occurs in replication-proficient tissues

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Problems

- **We observe changes in mutation <u>frequency</u>**

- **The magnitude of changes in <u>mutation rate</u> per cell division which corresponds to the observed increase**