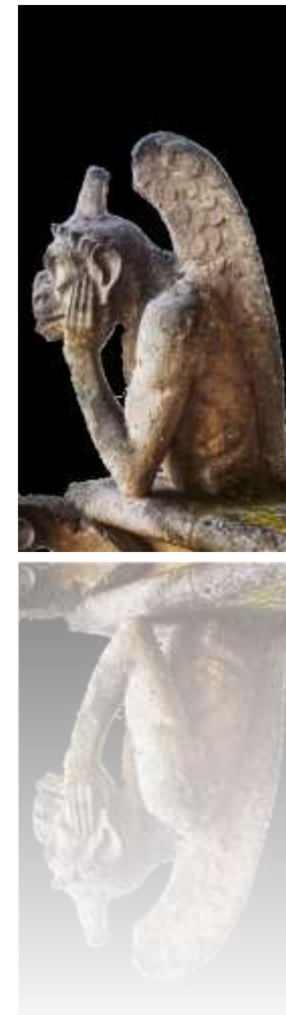
IRSN
INSTITUT
DE RADIOPROTECTION
ET DE SÛRETÉ NUCLÉAIRE

Faire avancer la sûreté nucléaire

Radiosensitivity and transgenerational effects in non-human species



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On behalf of the related ALLIANCE topical Working Group

ICRP 4TH INTERNATIONAL SYMPOSIUM ON THE SYSTEM OF RADIOLOGICAL PROTECTION

October 10-12 2017, Paris, France

ERPW 2ND EUROPEAN RADIOLOGICAL PROTECTION RESEARCH WEEK

15 groups involved from 11 countries

Prefered pet organism

Group	Species of interest
HZ-IRE	Bacteria, C. elegans , Danio rerio, plant cell cultures
UIAR	Plants: A thaliana, Pinus
CEH-MEEG	Earthworms, C. elegans
CEH-CG	Large mammals
IRSN	C.elegans, Danio rerio, Daphnia magna, autochtonous fish sp.
UoP	Fish: stickle backs+ autochtonius
UGent	Daphnia
SCK•CEN	Plants: Ath, Oryza sativa, L minor
RIRA	Plants: scots Pine
NMBU	Danio rerio, C.elegans
SU	Interacting species (small ecosystems/foodwebs)
CEA	bacteria







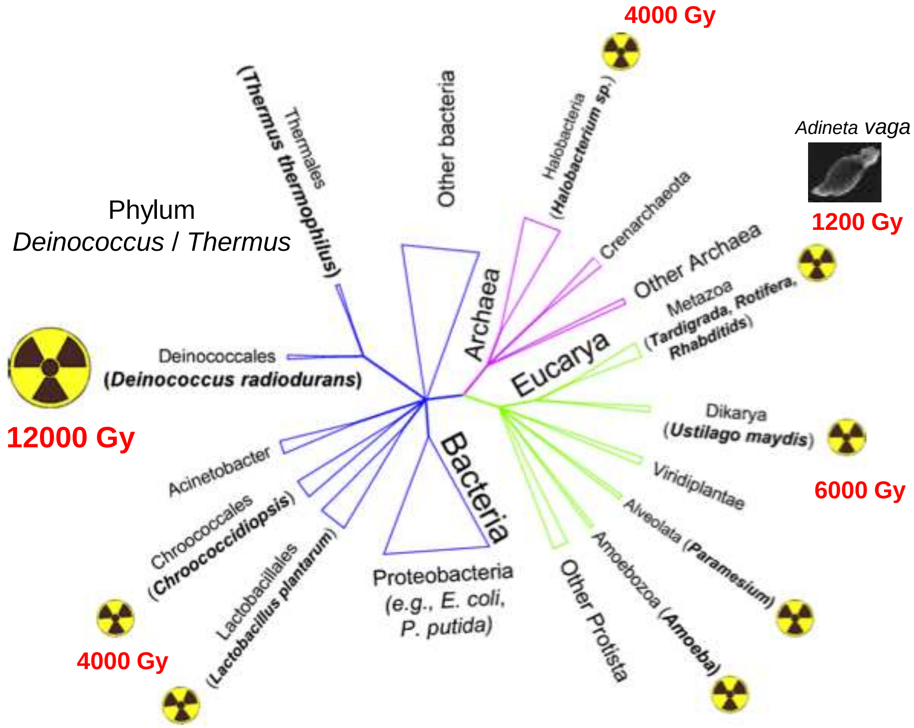

Actions

- Inter-comparison on the analysis of biomarkers of effects
- Project submission to international calls (COMET European project (2013-2017); BIOMARKER (CONCERT))
- Roadmap
- Position paper on "DNA methylation as a potential biomarker for environmental radiological contamination", Horemans et al. (to be submitted)
- Paper for the proceedings of this congress "Radiosensitivity and transgenerational effects in non-human species", Adam-Guillermin et al.

Evolution (and directed evolution) of radioresistance

Radioresistant organisms found in the three orders

Deinococcus: highest radiation resistance to **acute** exposure



D_{10}
(10% survival)

Directed evolution

Bacteria :

Bacillus pumibus, *Salmonella typhimurium*, *E. coli* => Highly radiation resistant *E. coli* mutants (3000 Gy)

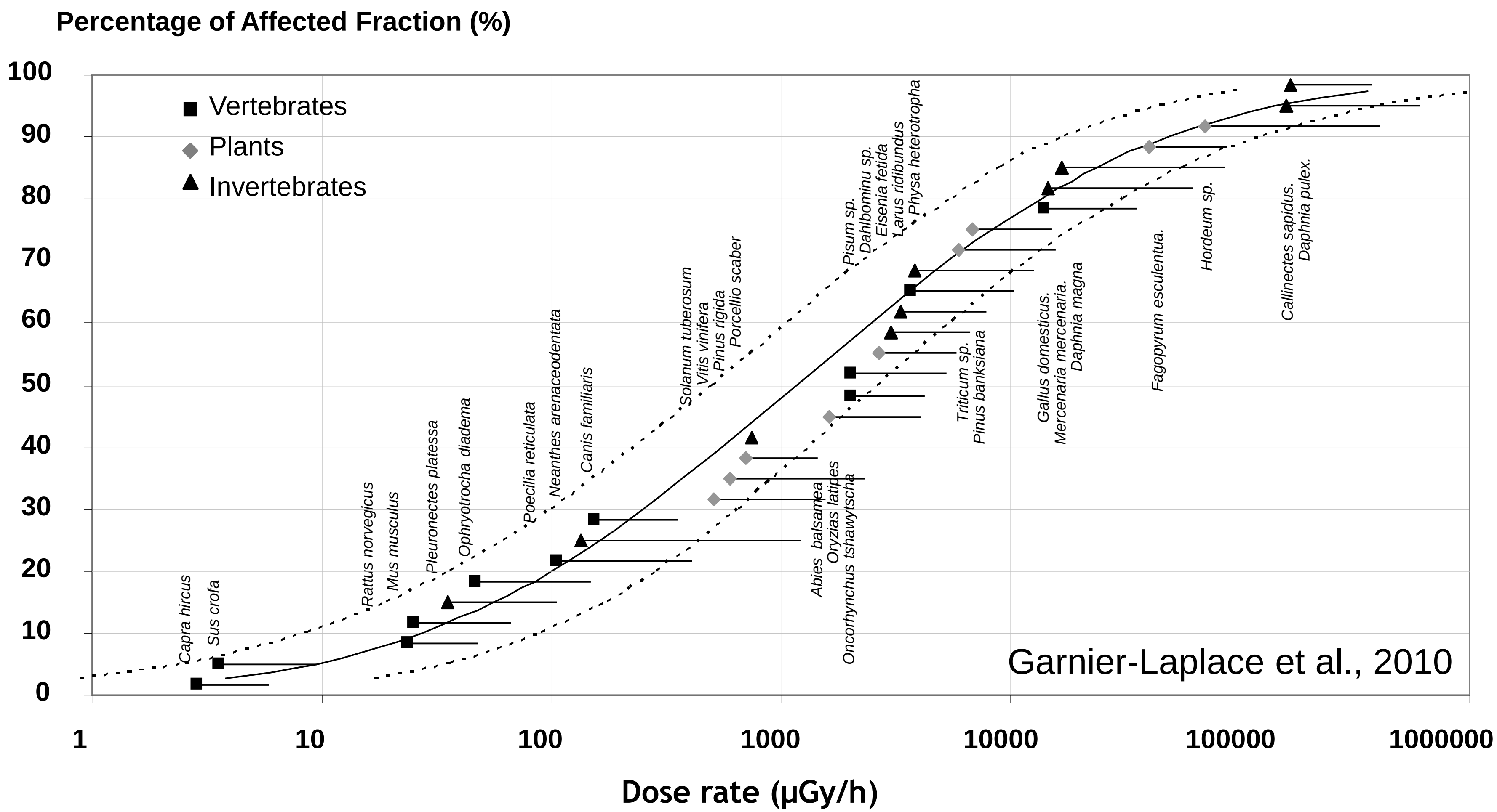
Byrne *et al.*, (2014) eLiFE

Cancer cells :

radioresistance developed during fractionated exposures to γ rays

Daly MJ, (2012) *DNA Repair* (Rev)
and ref. therein
Blanchard and de Groot, 2017

Radiosensitivity between species ranges over 6 orders of magnitude under **chronic** γ controlled exposure (Effect Dose Rate$_{10}$)



Topic 1 : radiosensitivity

- Mechanistic understanding of ≠ in radiation sensitivity across species
- Identification of sensitive species that may require special attention in monitoring and radiation protection
- How do differences in sensitivity between species lie behind overall effects at higher levels (community, ecosystem) ?

Topic 1 : radiosensitivity

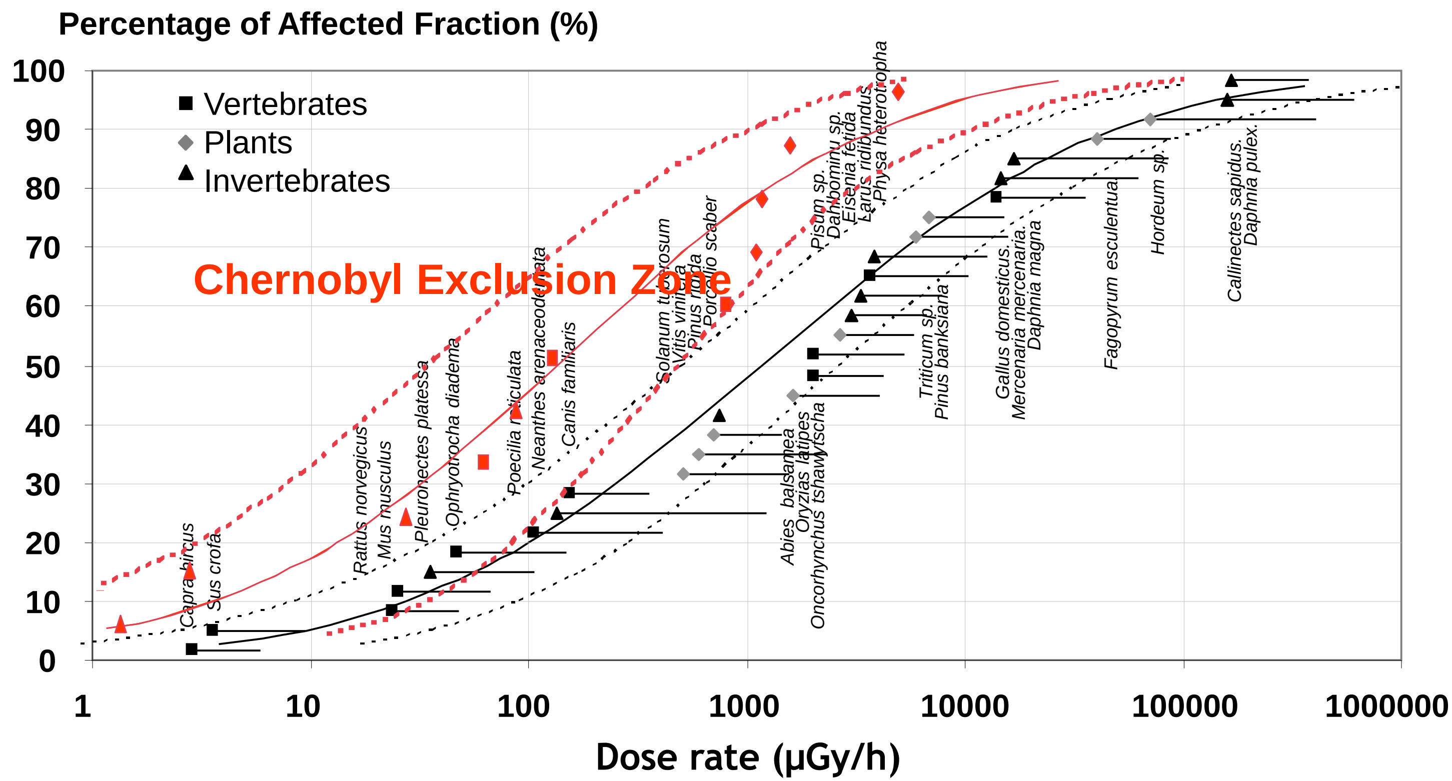
- **Genome size** ; but **highly condensed nucleoid** may prevent the dispersion of the DNA fragments generated by irradiation thus facilitating DNA repair (e.g. *Deinococcus* or spz)

Species	Haploid genome size (Mbp)	D_{10} survival (Gy) (10% survival)	Number of survivable DSBs per haploid genome[a]	DSB/Gy/Mbp (approximate linear density of DSBs in vivo)
E. coli (MG1655)	4.6	700	6	0.002
D. radiodurans	3.3	12,000	118	0.003

- Efficiency of checkpoint control mechanisms and **DNA repair** (HR vs NHEJ, UNSCEAR 2000 ; "extended synthesis-dependent strand annealing" (ESDSA) Zahradka et al., 2006)

- **Reactive oxygen species scavenging** :
  - Higher [Mn]/[Fe] ratios in Deinococcus sp. (de Groot et al.; 2009)
  - Melanized pigments in radioresistant fungi in Chernobyl (Dadachova and Casadevall, 2009)
  - Carotenoid pigments in bacteria (e.g. Rubrobacter radiotolerans) and birds (Møller et al., 2005)
  - Pheomelanin-based colorations in birds and mammals (Galvan et al., 2011 ; Boratynski et al., 2014)
- Induction of cell death ; tissue regeneration ; life stage
- **Metabolic rate** (high metabolic rate=sensitive species, Baas and Koijman, 2015)

Organisms in their natural environmental are 8 times more sensitive than in laboratory controlled conditions (Garnier-Laplace et al., 2013)



- Combination of factors such as gene diversity, competition, predation, and abiotic factors.
- Evolutionary adaptation or increased sensitivity in different environmental extremes, for **long-term exposure.**

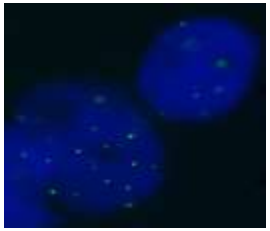
- Increase of sensitivity :

Chronic exposure of microcrustaceans to Am-241 (15 mGy/h max). Altered survival and fecundity in F1 and F2 but not in F0 (Alonzo et al., 2008). **Effects higher in F2 than in F1** (5-fold)

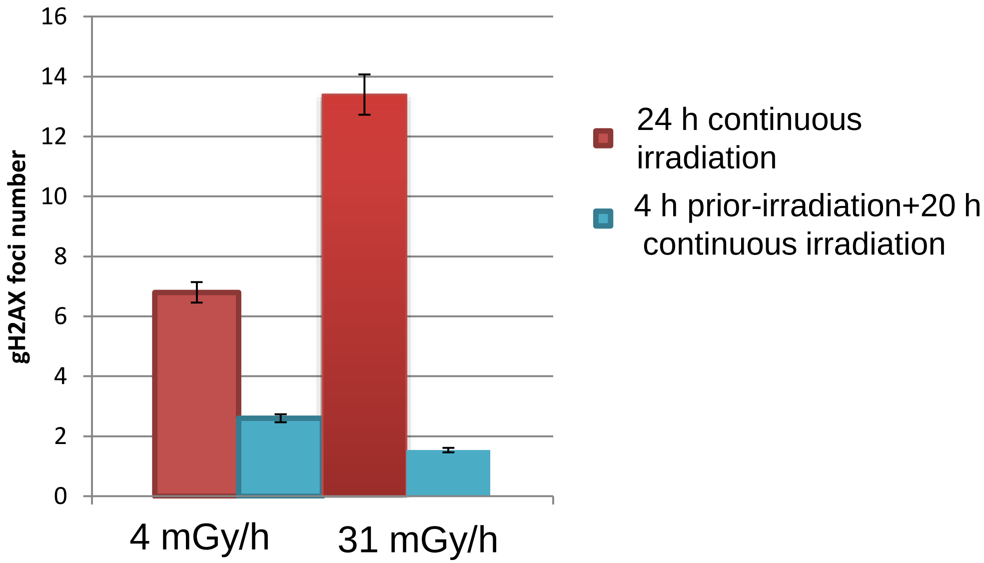
Field studies: observation of morphologic abnormalities in butterflies exposed at Fukushima at 3 µSv/h. **Worse morphologic abnormalities observed in the following generations** obtained in laboratory controlled conditions (Hiyama et al., 2012, 2015)

- Decrease of sensitivity :

Chronic exposure of nematodes to uranium. Drastic decrease of fertility until F3, then, from F3 to F21, **selection** of individuals being the more fertile and having the fastest growth (Dutilleul et al., 2014)



Radioadaptation effect observed in ZF4 cells (zebrafish fibroblasts), exposed to a prior irradiation dose (Pereira et al., 2014)



- Role of genetic mechanisms (mutation rates and types ; genetic diversity)…

Genetic…Decrease of fertility and increase of mutation rates, DNA damages or to chromosomes in sparrows (Ellegren et al., 1997 ; Bonisoli-Alquati et al., 2010) and mice in Chernobyl (Pomerantseva et al., 1997) or in daphnids in the lab (>7 µGy/h) (Parisot et al., 2015).

- …but not only

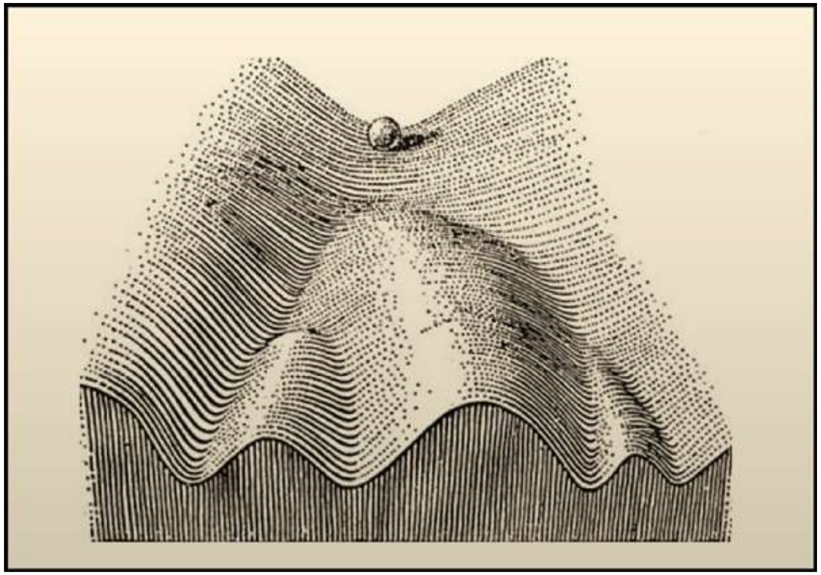
Adaptation of pines to high doses in Chernobyl despite deformities and DNA damages (Kovalchuck et al., 2003). These adaptive mechanism can not be only genetic (10^{-5}-10^{-6} mutation per germ cell)…increase of DNA methylation : **epigenetic mechanisms**

What are epigenetic mechanisms

- "The causal interactions between genes and their products, which bring the phenotype into being" Waddington 1942

- "Epigenetics, in a broad sense, is a bridge between genotype and phenotype— a phenomenon that changes the final outcome of a locus or chromosome without changing the underlying DNA sequence" Goldberg et al 2007

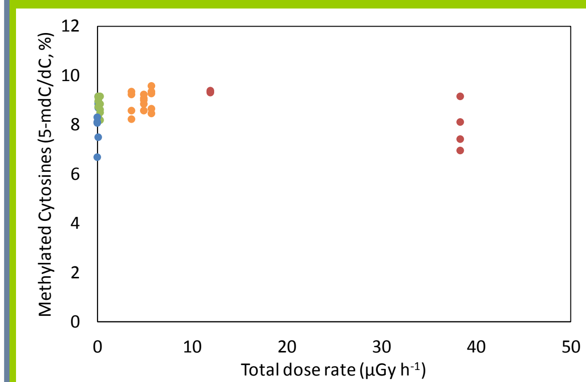
- Three interacting players:
  - DNA methylation
  - Histone modifications
  - Non-codingRNA



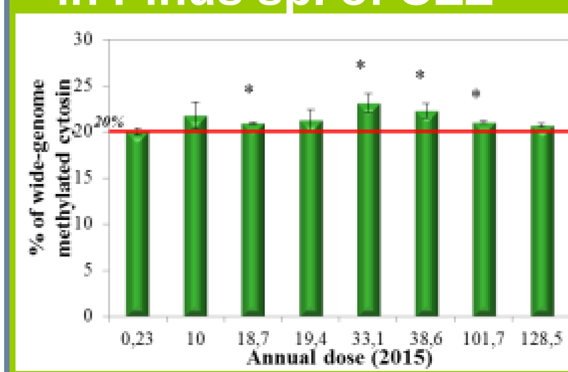
Goldberg et al. 2007 reprinted from Waddington 1957

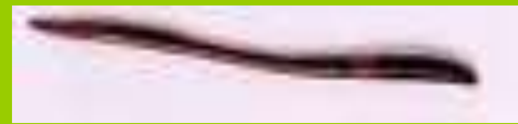
European Project COMET

DNA methylation in *C. bursa* pastoris of FEZ



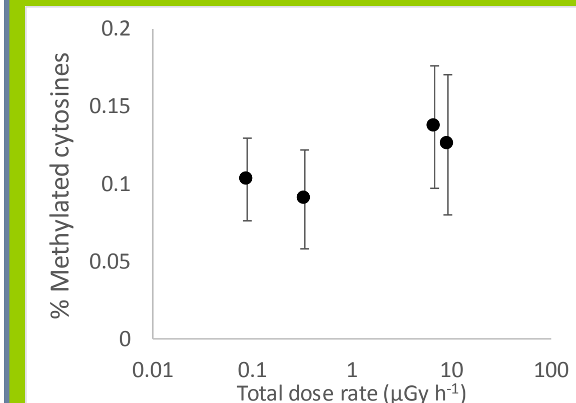
DNA methylation in Pinus sp. of CEZ



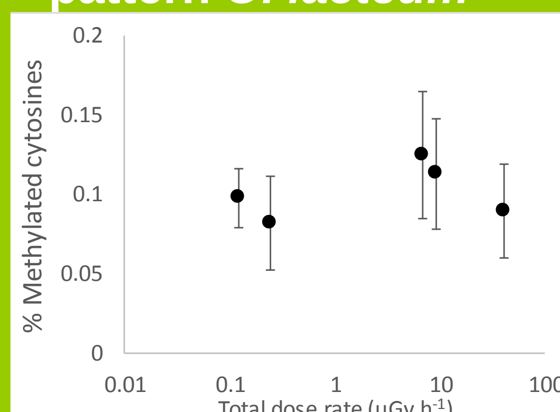


CEZ

DNA methylation pattern *A. caliginosa*



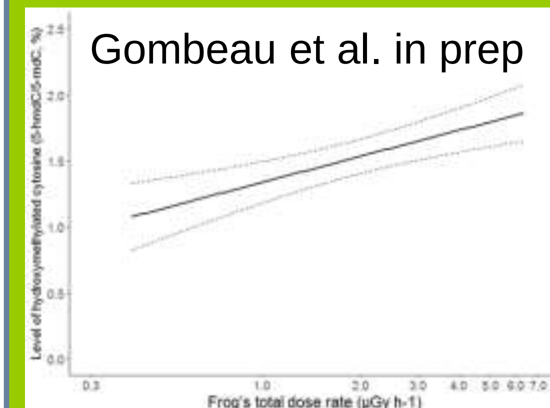
DNA methylation pattern *O. lacteum*



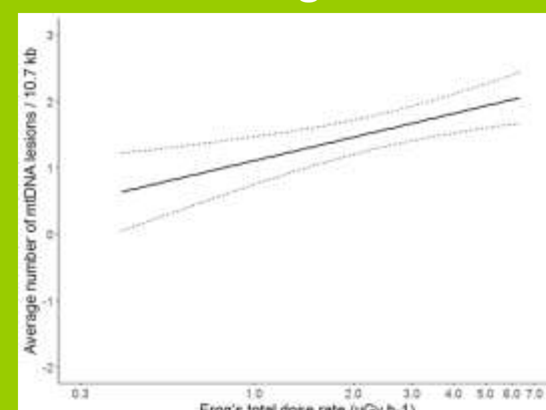
Fukushima

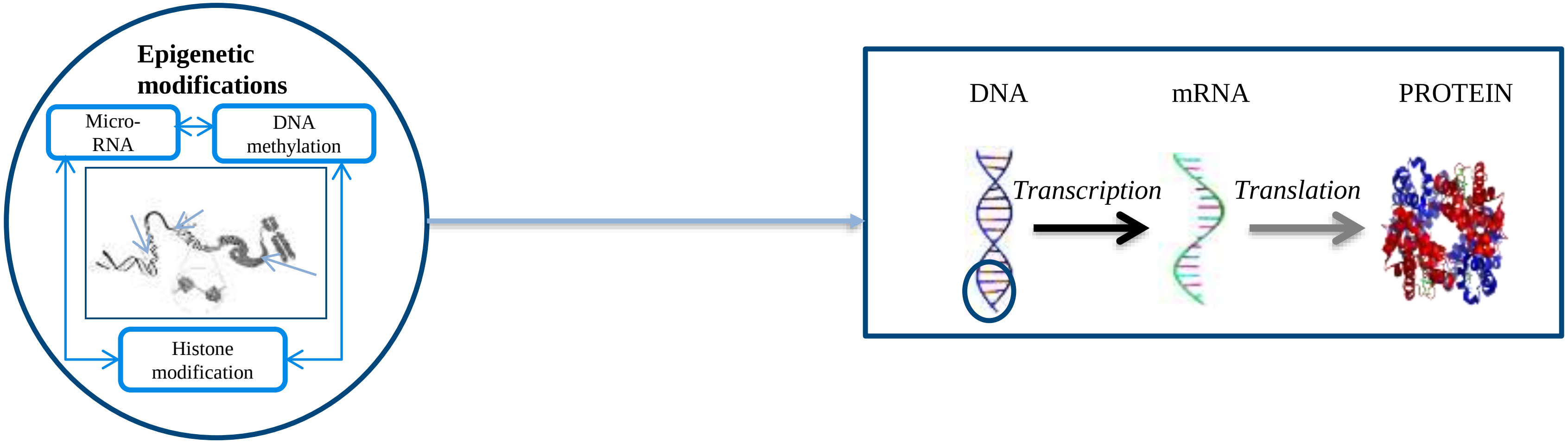
DNA methylation

Gombeau et al. in prep

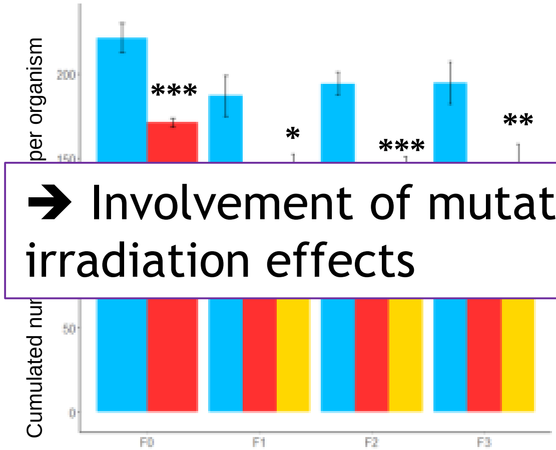


DNA damages





C. elegans: Methylations of histone H3K4 and adenines jointly control the epigenetic inheritance of phenotypes



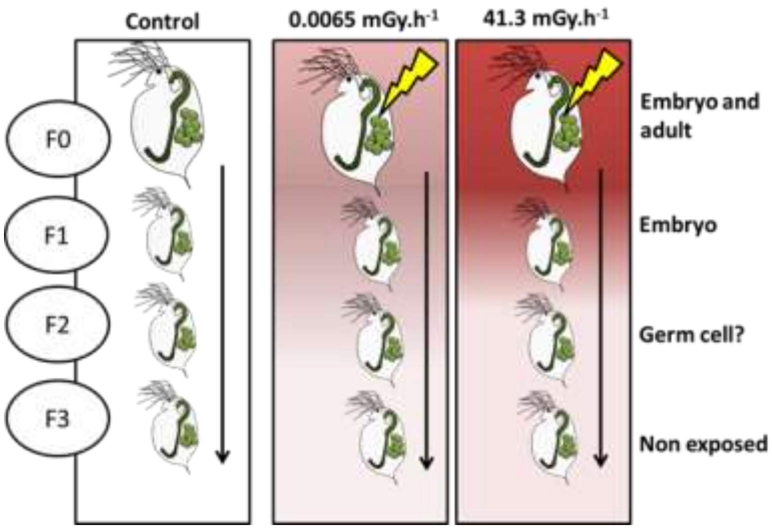
➔ Involvement of mutated pathways in the sensitivity and heritability of irradiation effects

Wild type N2

Control | Recovery | Exposed

*P-value : *** < 0,001 ; **<0,01 ; *<0,05 ; 0,05<.<0,1*

Specific methylation of genome (bisulfite sequencing)



Trijau et al., in prep

Decrease of F0 daphnid fecundity (exposed to IR)

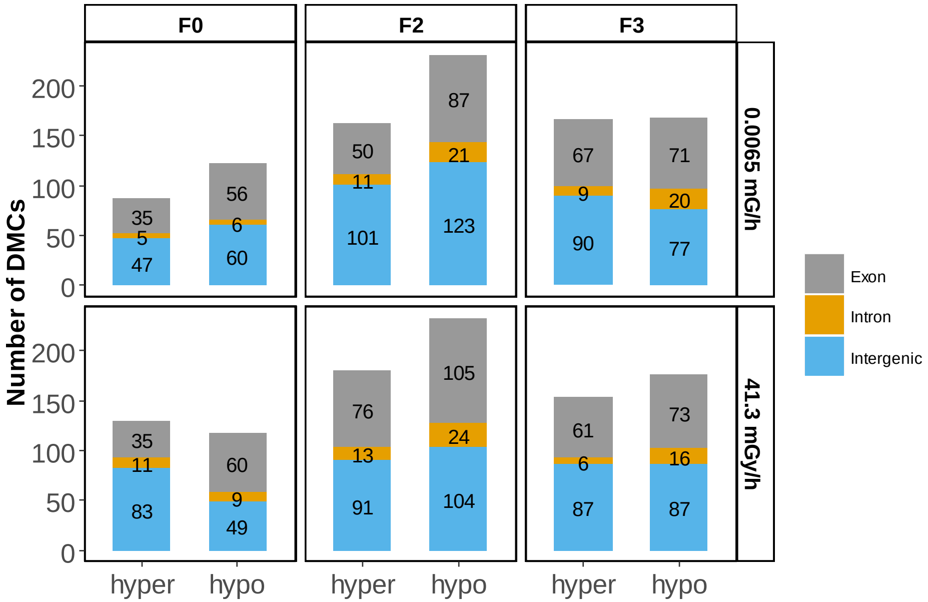
	F0		F1		F2		F3	
mGy/h	0.007	40	0.007	40	0.007	40	0.007	40
Survival	ø	ø	ø	ø	ø	ø	ø	ø
Growth	ø	ø	ø	ø	ø	ø	ø	ø
Reproduction	ø	+	ø	ø	ø	ø	ø	ø

IRSN

Topic 2 : long term and transgenerational effects

- **No significant effect on average global methylation levels was observed in generations F0, F2 and F3, at 0.007 and 40 mGy/h**

- **Differentially (hypo- or hyper-) methylated cytosines (DMC) were detected in various genomic locations in generations F0, F2 and F3 after F0 exposure to 0.0065 and 41.3 mGy/h**



- **Hypomethylation of DNA** in generations F2 and F3 coming from mother exposed to IR and **heritability of this methylation pattern between F2 and F3 generations : β-mpp** (biogenesis of mitochondrial proteins), **Hsp 70** (protein repair, **IR**), **rpl28** (protein synthesis, **IR**)

- → Epigenetic biomarkers for IR exposure and effect

	D. magna	*C. elegans*
Multigenerational effects on reproduction	✓ 35 mGy/h in F0 ✓ 70 µGy/h in F2	✓ 26 mGy/h in F0 ✓ 7 mGy/h in F2
Transgenerational effects on reproduction	× F1, F2, F3 (41mGy/h)	✓ F1, F2, F3 (50 mGy/h)
Epigenetic changes	DMCs: ↗ F0, F2, F3	Increase in radioresistance of mutants depleted on 6mA and H3K4me2

(Parisot et al., 2015 ; Buisset-Goussen et al., 2014 ; Lecomte-Pradines et al., 2017 ; Trijau et al., in prep.)

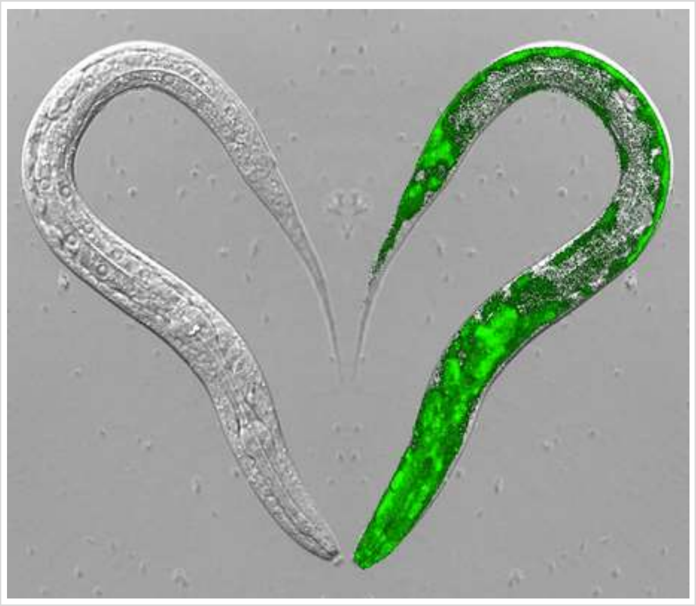
→ Chronic effects has to be considered in a multigenerational context
→ Epigenetic mechanisms play a major role in these effects
→ Need to understand the link between molecular process and phenotype changes (Adverse Outcome Pathway)

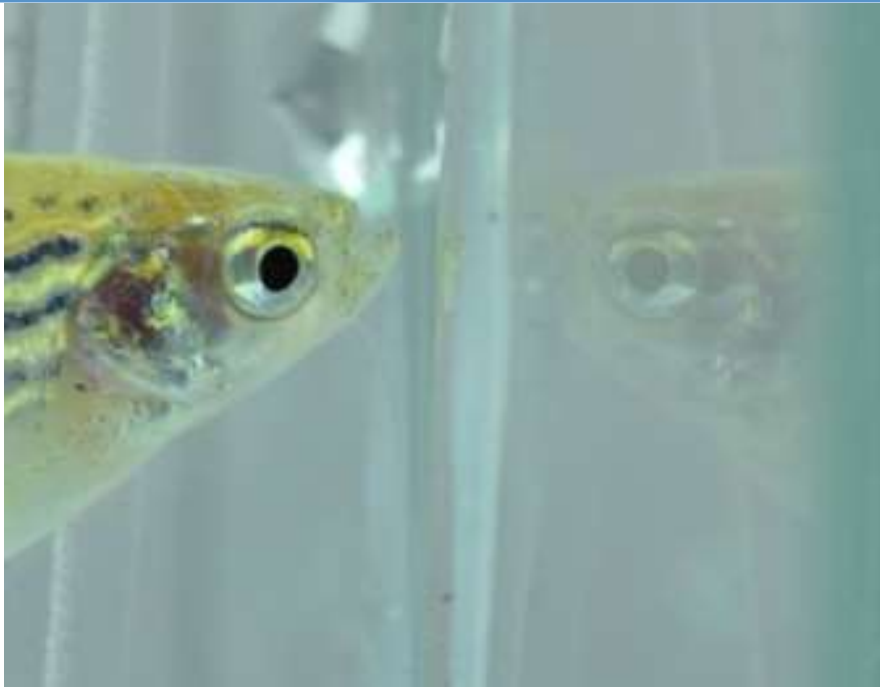
Perspectives:

→ High throughput analyses to identify finderprints of ionising radiation effects and early and sensitive biomarkers

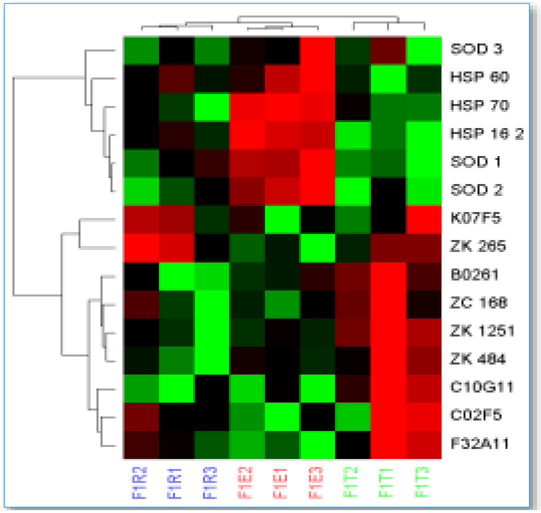
→ Most realistic conditions of exposure (lower dose rates, more generations, use of complex systems)

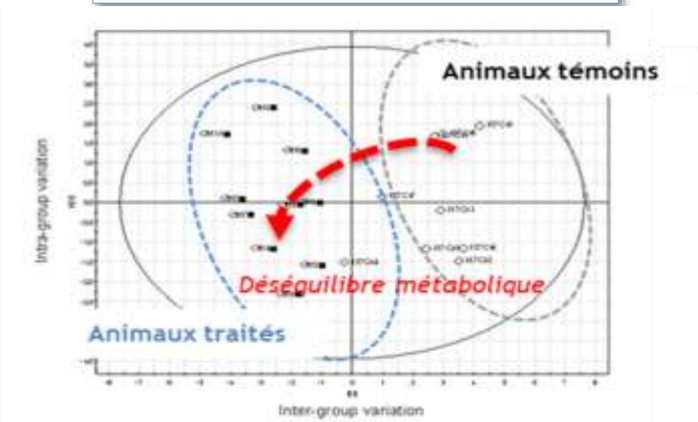
Thank you !





Any question?





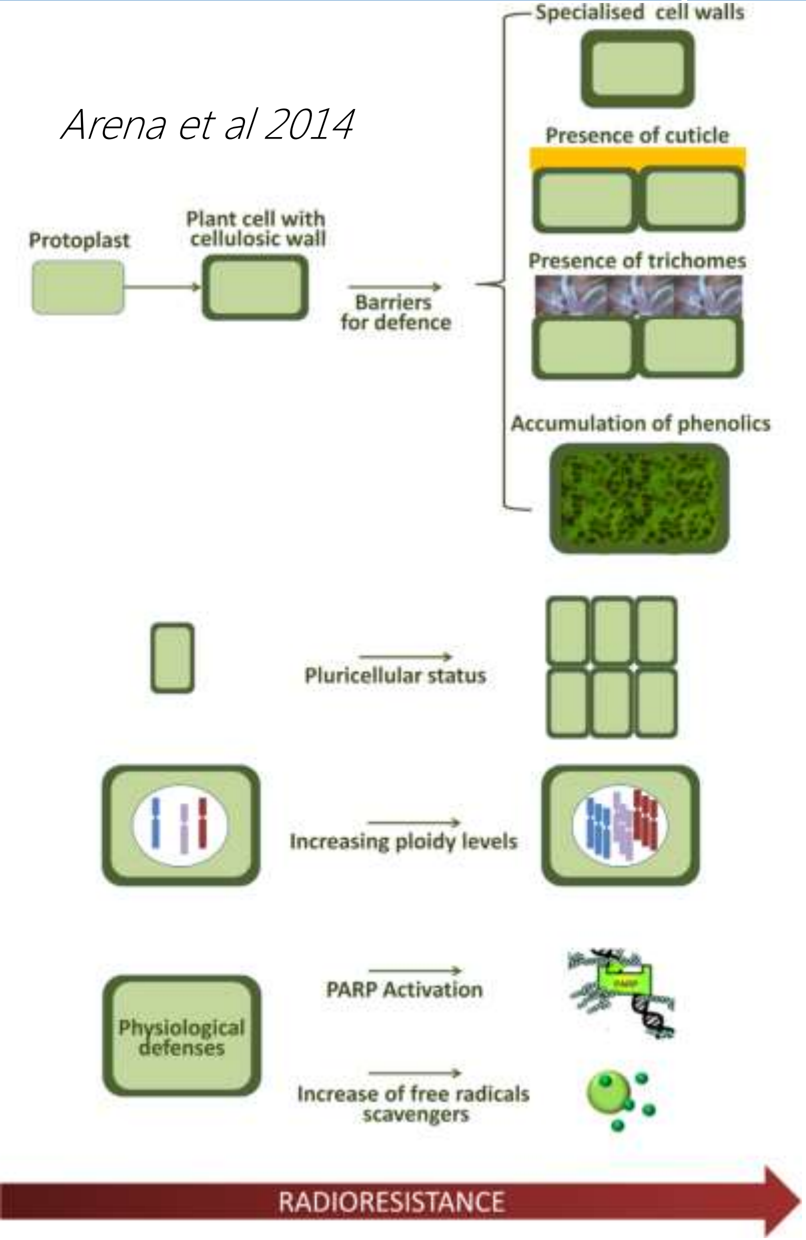
Fingerprints/signatures
« OMICS »

Sensitive and early biomarkers

Highlighting (or not) long term effects

Key species
Radiosensitvity factors

Arena et al 2014

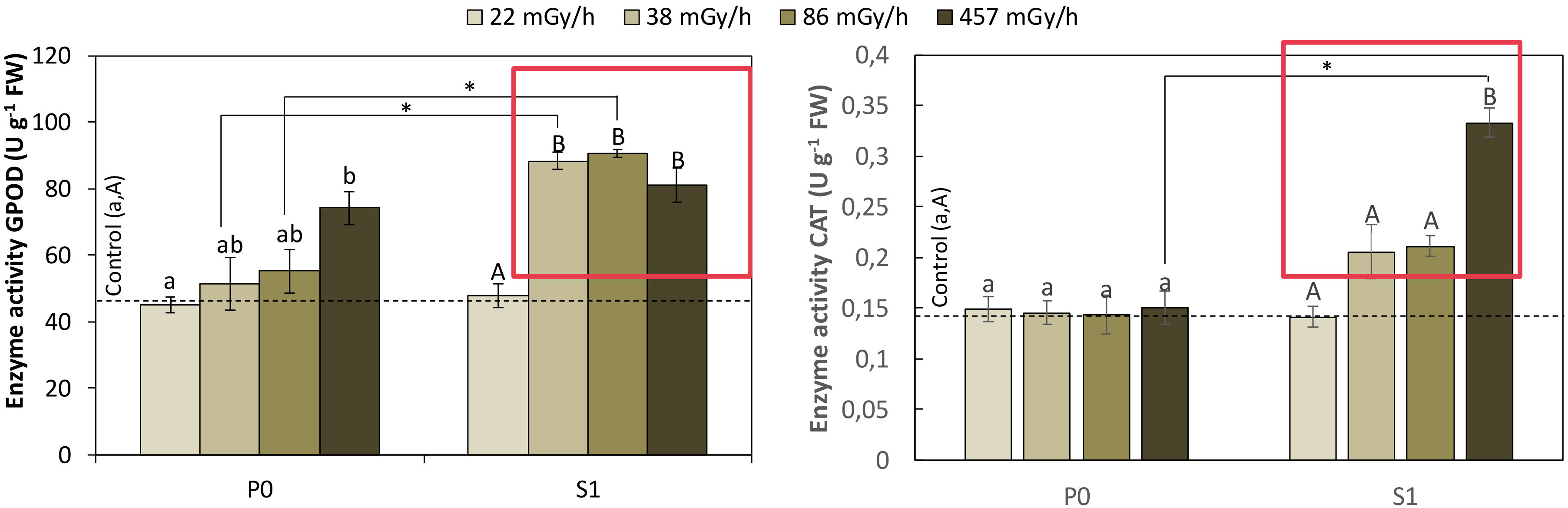


What explains radiosensitivity in plants ?

Genetic and reproductive factors influencing the sensitivity of plants to radiation (Sparrow and Miksche, 1961)

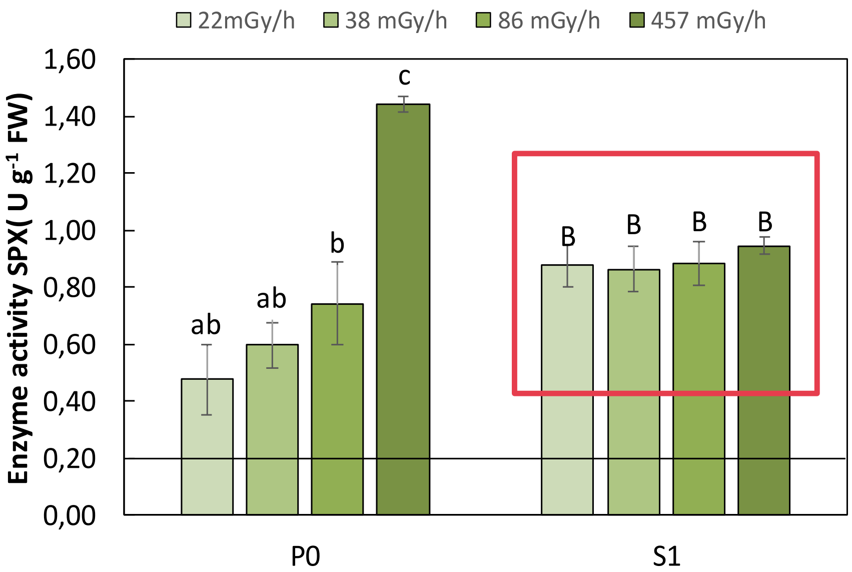
Increasing Sensitivity	Decreasing Sensitivity
Large nucleus	Small nucleus
Large chromosomes	Small chromosomes
Acrocentric chromosomes	Metacentric chromosomes
Low chromosome number	High chromosome number
Diploid or haploid	High polyploid
Sexual reproduction	Asexual reproduction
Long intermitotic time	Short intermitotic time
Long dormant period	Short or no dormant period

Enzyme capacities



→ Higher activity of ROS detoxifying enzymes in S1

Enzyme capacities



SPX + GPX ↑ in S1

↓

Syringaldazine = Lignin precursor

+

GPX involved in cell wall metabolism

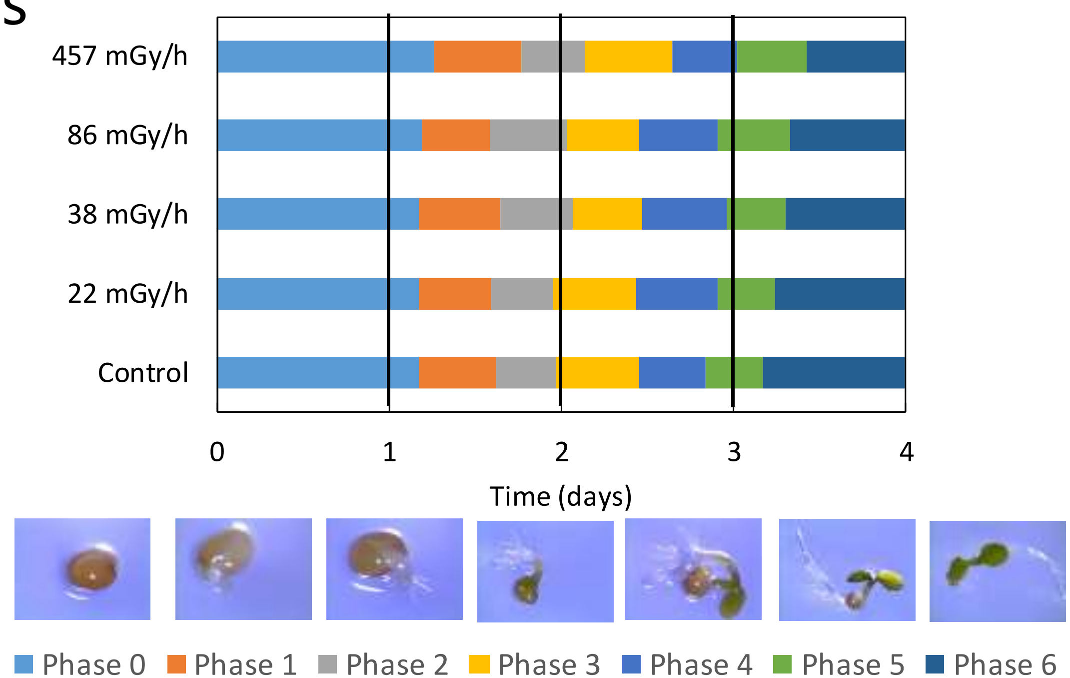
↓

Cell wall strengthening as defense

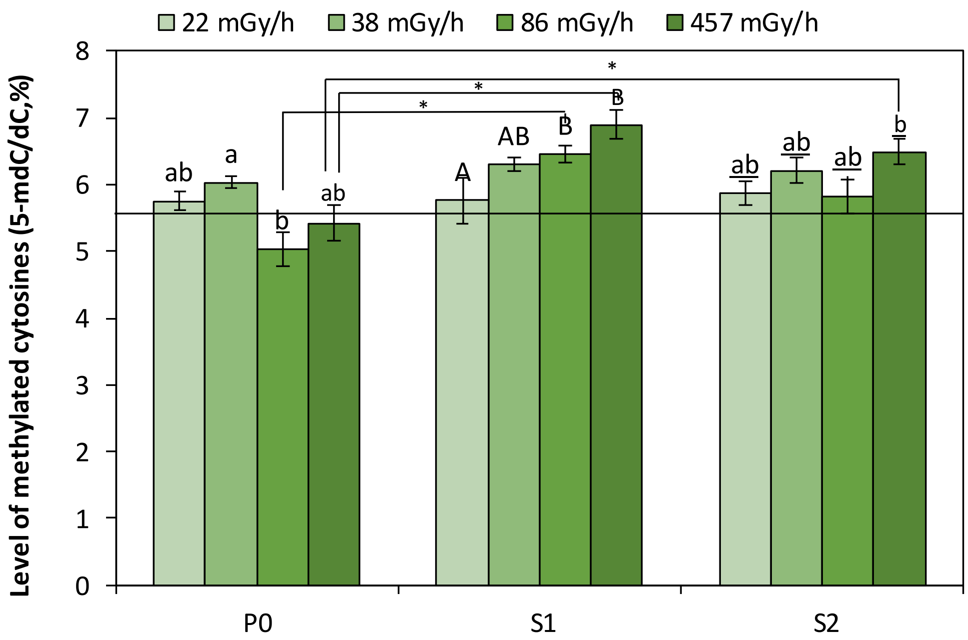
Arabidopsis thaliana - Phenotypical characterization

- Number of leaves on the rosette, rosette diameter, inflorescence frequency, inflorescence height, amount of seed pods, seed weight, germination capacity.

- Delay of inflorescence day in irradiated plants and of germination in irradiated plants maintained in control conditions



→ Global hypermethylation of DNA in S1 and S2



→ What about field studies?