Molecular and metabolic mechanisms of transgenerational effects

in *Daphnia* exposed to radionuclides

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IRSN INSTITUT DE RADIOPROTECTION ET DE SÛRETÉ NUCLÉAIRE

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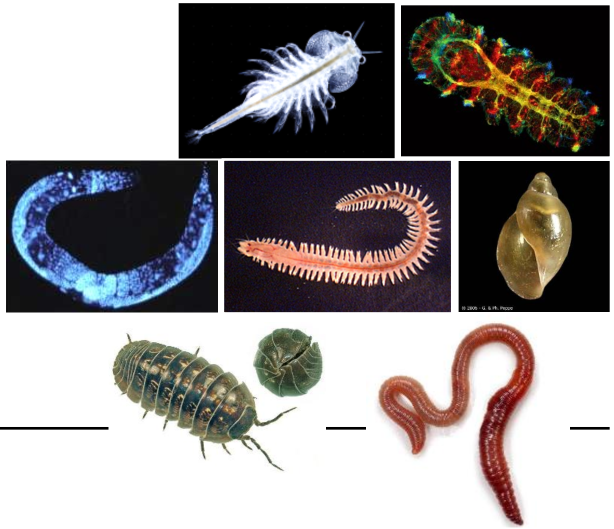
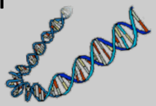
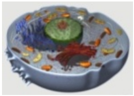
Toxicity of chronic ionizing radiation



In invertebrates…

Biological scale	Effect description	Species (reference)
Molecular 	DNA alterations	Arenicola marina (Hingston et al., 2003) Mytilus sp. (Al Amri et al., 2012) Tigriopus japonicus (Han et al., 2014)
Cellular 	Oxydizing stress, chromosomal aberrations	Artemia salina (Iwasaki, 1973) Neanthes arenaceodentata (Harrison et al., 1987) Paracyclopina nana (Won & Lee, 2014)
Organism 	Reduction in survival and fecundity	Physa heterostropha (Cooley & Nelson, 1970) Neanthes arenaceodentata (Harrison & Anderson, 1988) Ophryotrocha diadema (Knowles & Greenwood, 1994) Porcelio scaber (Hingston et al., 2004)
Population 	Transgenerational effects or adaptive response	Neanthes arenaceodentata (Harrison & Anderson, 1994) Eisenia fœtida (Hertel-Aas et al., 2007) Caenorhabditis elegans (Buisset-Goussen et al., 2014)

Challenges of a mechanistic approach to transgenerational effects…

- Need for studies addressing toxicity over several generations
- Need for studies addressing several biological scales at the same time

↗ Identify underlying biological mechanisms involved in multigenerational responses

↗ Link radiation effects among different scales

↗ Explain the dynamics of effects across generations

The cladoceran *Daphnia magna* as a test species

- Sensitive freshwater invertebrate species commonly used in ecotoxicology
  - Standard tests (OCDE, 2008)
- Easy to raise at the laboratory
  - Small size (~5mm)
  - Short life cycle (10 days) and high fecundity (~100 larvae after 20 days)
  - Clonal reproduction (small genetic variability)
- Molecular tools
  - DNA alterations using RAPD (Atienzar et al., 1999, 2000)
  - Epigenetic changes (Vandegehuchte et al., 2009; Asselman et al., 2017)
- Mechanistic modelling (DEBtox)
  - Original formulation (Kooijman & Bedaux, 1996)
  - Revised version (Billoir et al., 2008)
  - Recent reformulation (Jager et Zimmer, 2012)

Daphnia magna in ecotoxicology of radionuclides

Multigenerational exposure experiments

- Waterborne Am-241 (Alonzo et al., 2008)
- Waterborne depleted U (Massarin et al., 2010; Plaire et al. 2013)
- External Cs-137 (Parisot et al., 2015; Trijau et al., 2018)

Molecular alterations

- Waterborne depleted U (Plaire et al., 2013)
- External Cs-137 (Parisot et al., 2015; Trijau et al., 2018)

Objectives

↗ To test whether toxic effects of ionising radiation (and depleted U) varied in intensity among generations

↗ To investigate the role of genetic and epigenetic processes in the transgenerational changes

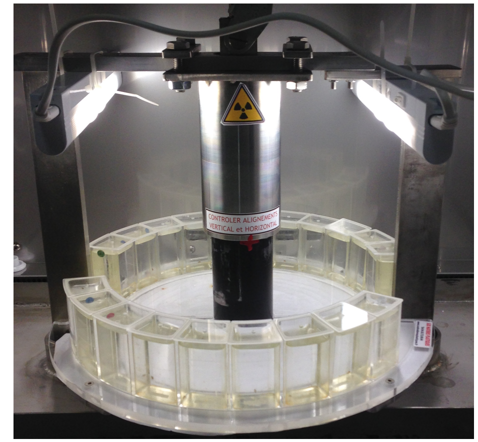
↗ To explain the mechanisms underlying the transgenerational response using a DEBtox approach

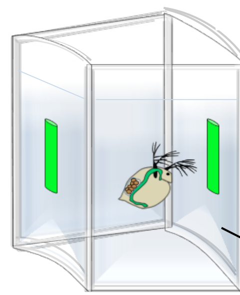
Cs-137 Gamma irradiation set up for *Daphnia magna*

- 22 experimental units placed in circle around a liquid or solid Cs-137 source
- Sources of various activities delivering different dose rates ranging from environmentally relevant to significantly toxic

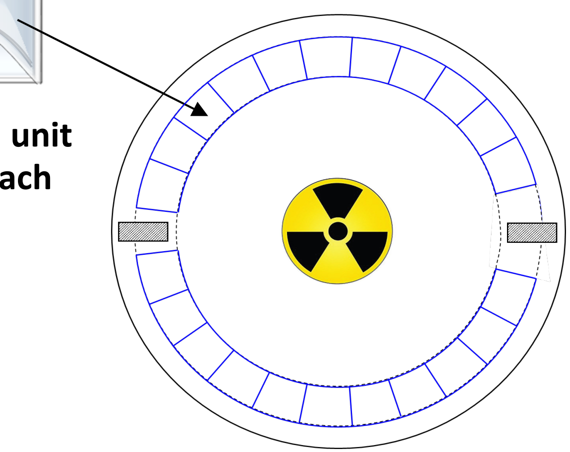
10 μGy h^{-1}

- 6.5 – 7.0 μGy h^{-1}
- 70 μGy h^{-1}
- 650 μGy h^{-1}
- 4.7 mGy h^{-1}
- 35 – 41 mGy h^{-1}





Experimental unit
1 daphnid each
in 50 mL



(Gilbin et al., 2008; Parisot et al., 2015; Trijau et al., 2018)

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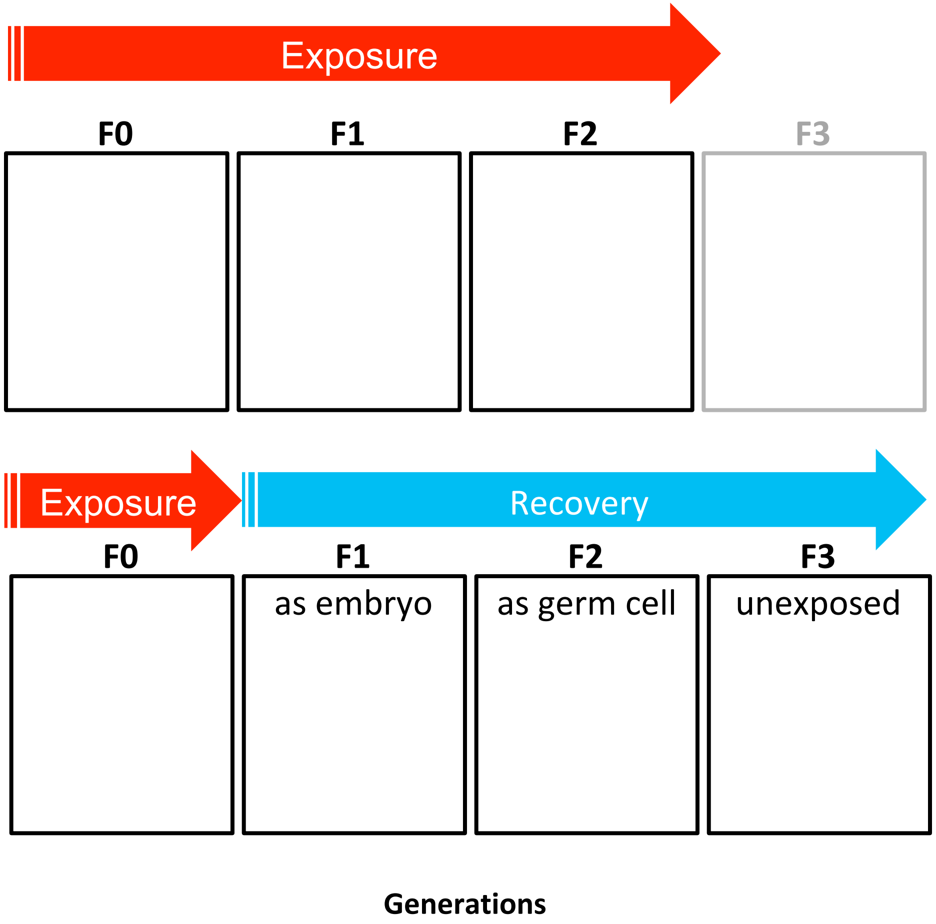
Two regimes of external gamma irradiation across generations

- A chronic exposure over 3 generations F0, F1 and F2
  - ↗ to monitor growth and reproduction curves and analyze DNA alterations
- A chronic exposure in generation F0 followed by recovery in offspring generations F1, F2 and F3
  - ↗ To test the inheritance of epigenetic modifications by unexposed generations



(Parisot et al., 2015; Trijau et al., 2018)

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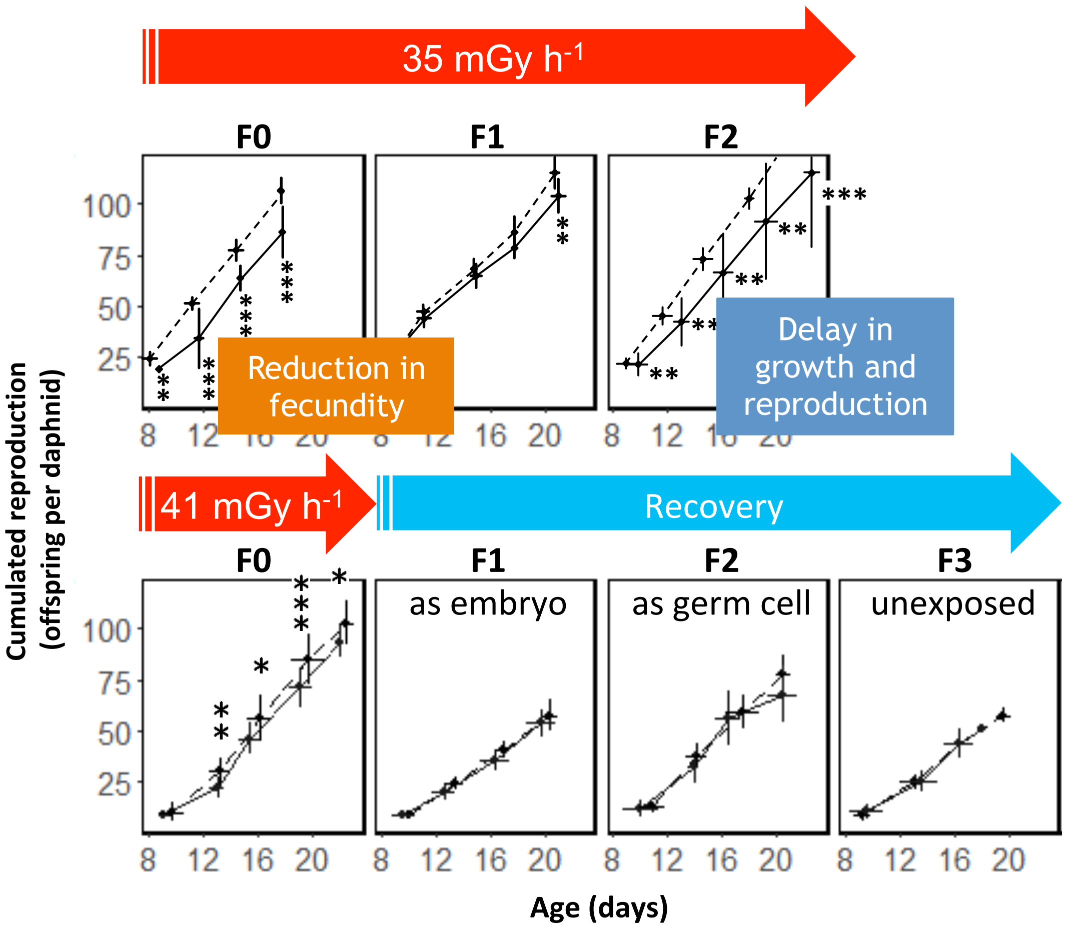
Toxic effects of external gamma irradiation across generations

- Effects on reproduction (and growth) increase in severity between generations F0 and F2
- Transcient smaller effects in generation F1
- Undetected effects as early as the F1 generation during recovery

? Contribution of genetic and epigenetic processes?

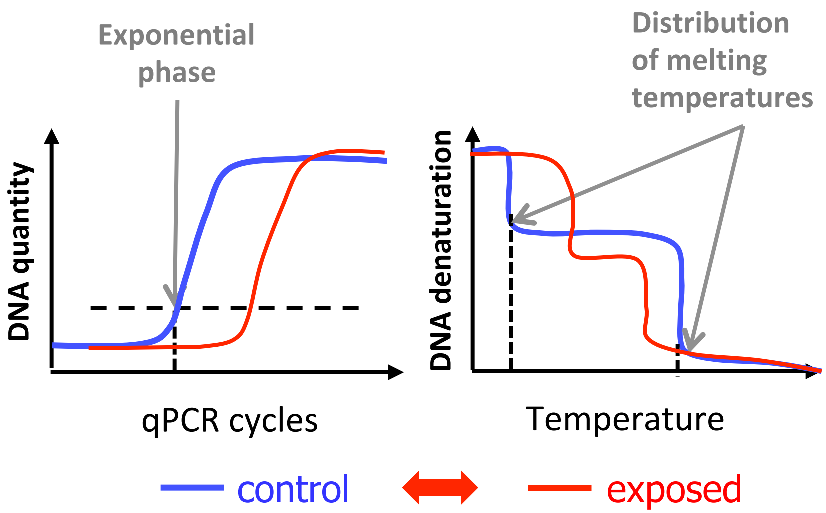


(Parisot et al., 2015; Trijau et al., 2018)

Two methods to address genetic and epigenetic changes

RAPD analyses by qPCR

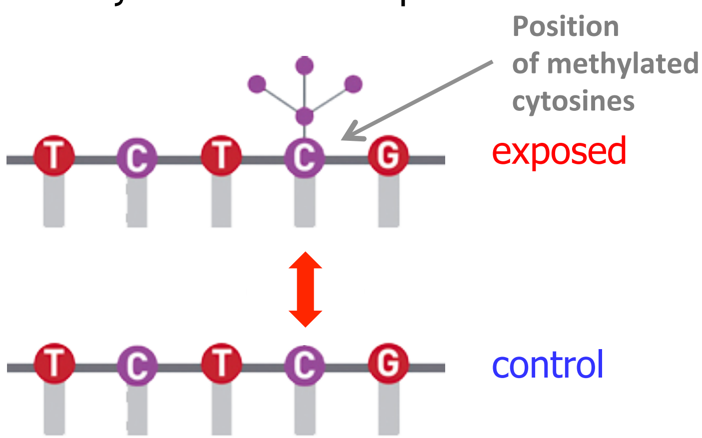
- Kinetics of DNA amplification
- Composition in PCR products



↗ **Any change reflects DNA alterations (creation /loss /modifications of hybridation sites)**

Whole genome bisulfite sequencing

- DNA methylation at the sequence level

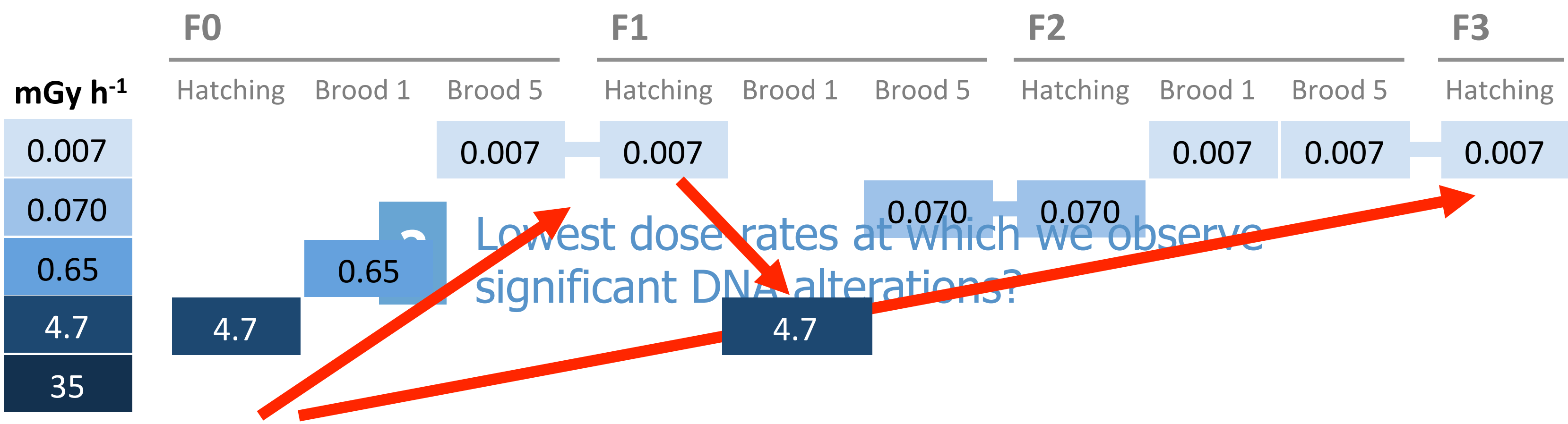


↗ **Any modification might affect the regulation of gene expression**

Changes in DNA methylation are reported in irradiated mice and pines

(Plaire et al., 2013; Parisot et al., 2015; Trijau et al., 2018)

Dynamic of DNA alterations across generations



- Significant DNA alterations at decreasing dose rates over the course of F0
  - ↗ reflecting a gradual induction of DNA damage in the first generation
- Significant DNA alterations at decreasing dose rates across generations
  - ↗ reflecting an accumulation and transmission across generations
- Significant DNA alterations at higher dose rate during F1
  - ↗ reflecting a transient elimination associated with repair processes?

(Parisot et al., 2015)

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Methylation changes detected in both irradiated daphnids and their unexposed offspring!

- ~5.4 millions cytosines analyzed for methylation…

Epigenetic modifications as a molecular mechanism for transgenerational effects in *Daphnia magna* exposed to radionuclides

Marie TRIJAU, Jana ASSELMAN, Olivier ARMANT,
Christelle ADAM-GUILLERMIN, Karel de SCHAMPHELAERE and Frédéric ALONZO

(Trijau et al., 2018)

How do DNA alterations link with radiotoxicity at the individual level?

↗ Similar trends between DNA alterations and macroscopic effects across generations

	Gamma irradiation 0.007 – 35 mGy h^{-1}			Waterborne depleted U 2 – 50 µg L^{-1}		
	F0	F1	F2	F0	F1	F2
Increase in effects on growth and reproduction	↗	↘	↗↗	↗	↗↗	↗↗↗
Accumulation and transmission of DNA alterations	↗	↘	↗↗	↗	↗↗	↗↗↗

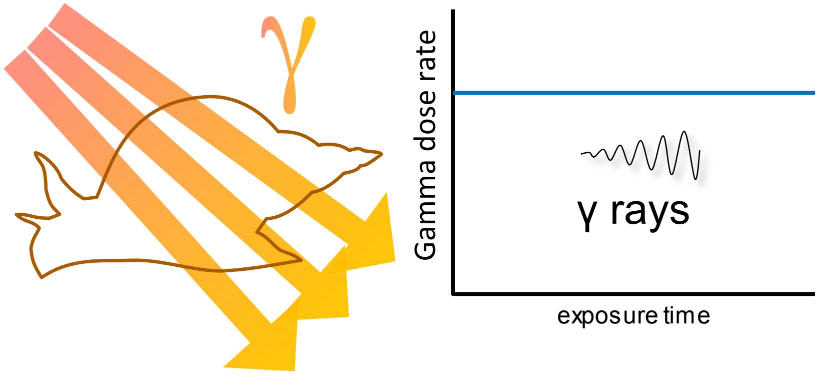
? Can we describe these mechanisms using a mechanistic approach?

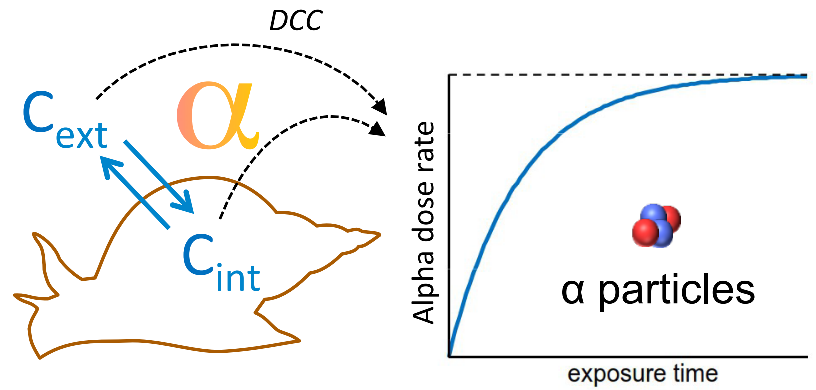
Parisot et al. (2015)

Plaire et al. (2013)

Need to define the adequate « dose metrics » for radiotoxicity

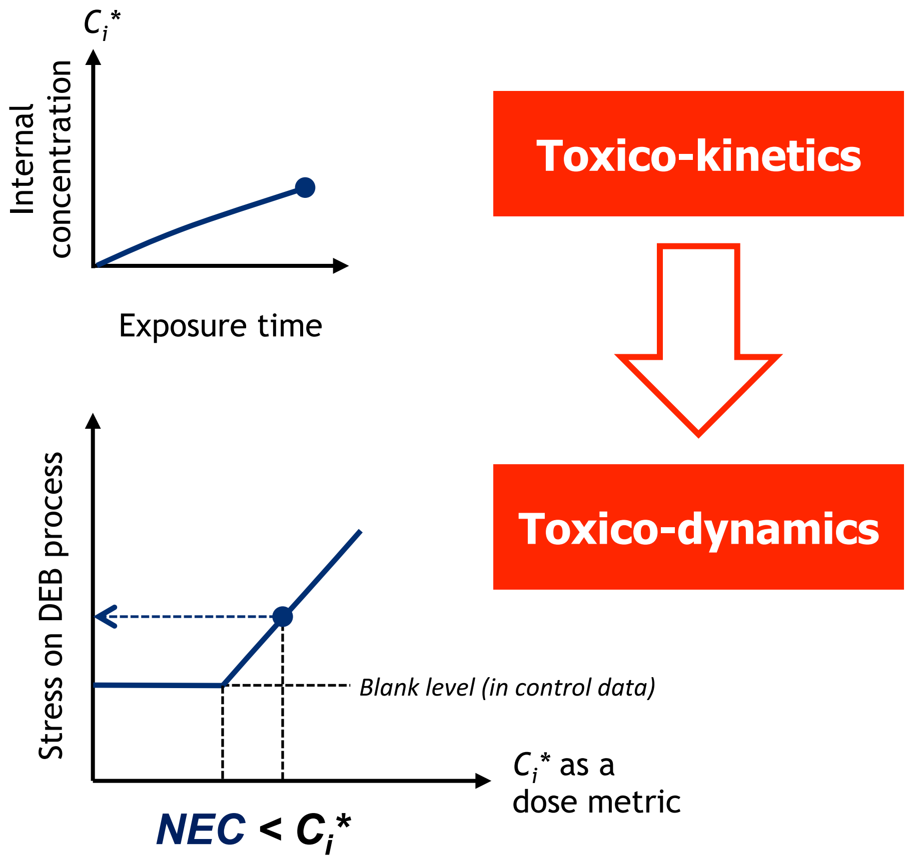
- Dose rate (energy deposited per unit volume) is a suitable dose metric
  - As shown in the nematode *Caenorhabditis elegans*
- Gamma dose rate (Cs-137) constant over time during external irradiation
  - A simple exposure situation with no internalization
  - Make it possible to explore the toxicodynamics more closely
- Alpha radiation is harmful when the emitter (Am-241) is internalized
  - Water and internal concentrations converted to alpha dose rate using conversion coefficients (DCC)





(Lecomte-Pradines et al., 2017)

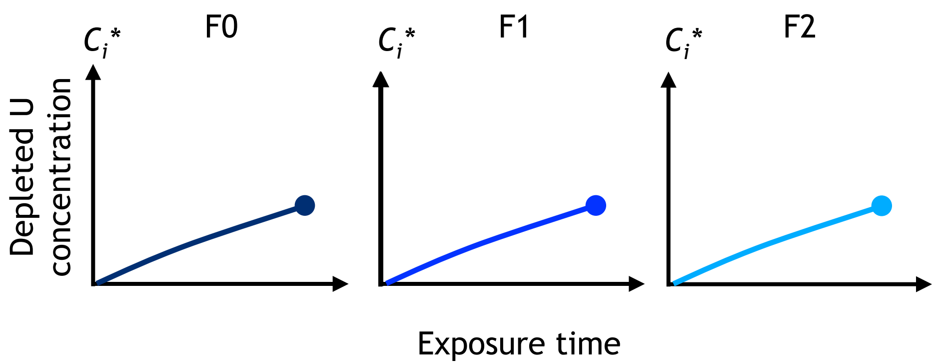
DEBtox models consider toxicity as a dynamic process varying over time

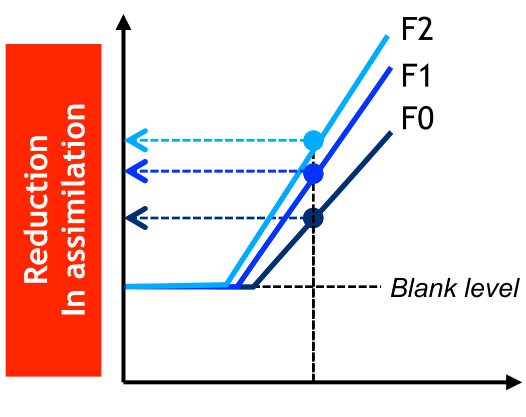


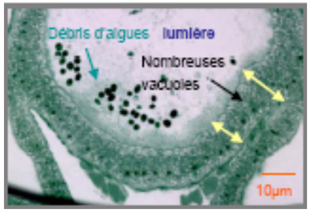
- DEBtox models first describe changes in toxicant concentration in the organism over time, using a simple kinetic model
- DEBtox models then describe how the internalised toxicant alters a DEB process

(Kooijman and Bedaux, 1996; Jager and Zimmer, 2012)

A DEBtox analysis of effects of depleted U in a multigenerational context







- A reduction in assimilation as the most likely mode of action
- In agreement with observations of digestive tract and carbon assimilation
- Each generation analyzed separately (same toxicokinetics)
- Different stress functions to describe the increase in toxicity

NEC (µg L^{-1})	F0	F1	F2
	10,0	5,8	2,0

↗ Reason why TKTD parameters varied across generations unclear…

(Massarin et al., 2011)

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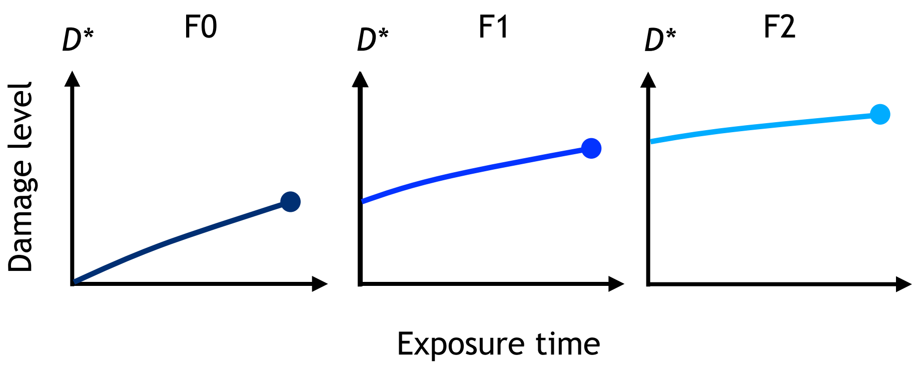
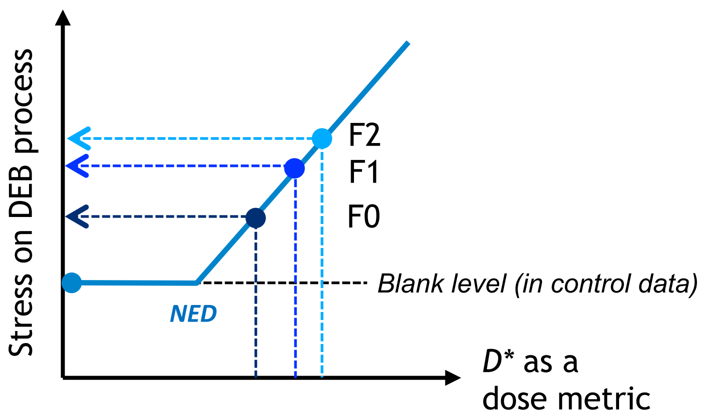
A transgenerational damage to address increasing toxicity over generations



- A damage compartment is introduced, with a level that is transmitted from one generation to the next

$$D^{*}/dt = \boldsymbol{k}_{\boldsymbol{r}} (DR - D^{*})$$

Damage repair rate



- The damage level increases over generations and can explain why toxicity is stronger in the progeny than in parents
- One single stress function sufficient to describe toxicity in all generations

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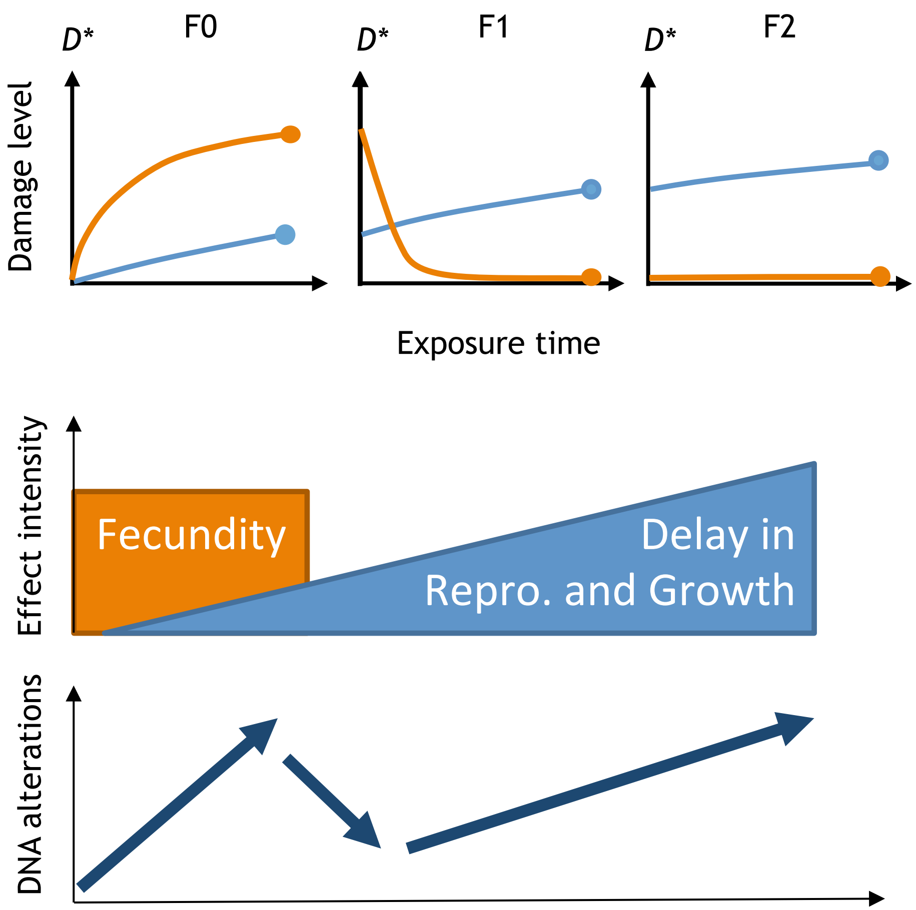
Two modes of action with their separate toxicokinetics for gamma irradiation



- Two damage compartments needed to drive the early effect on fecundity and the late delay in growth and reproduction
- Damage level should follow the trends observed in DNA alterations
- Different values for the repair rate to make Damage 1 drop in F1:

  k_{r1} in F0 < k_{r1} in F1
- Slower kinetics for Damage 2:

  $k_{r2} < k_{r1}$

Bayesian inference to compare likelihood and quantify uncertainty

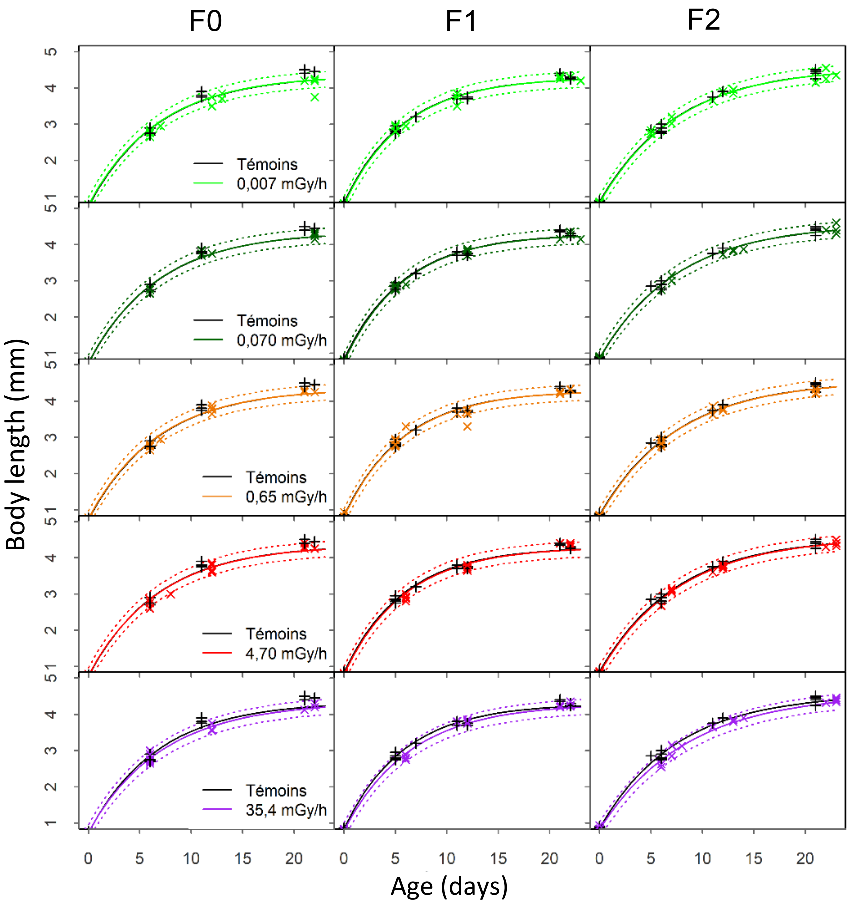
- Bayesian modelling achieved using the R software with the Rjags and Coda packages (R Core team, 2012; Plummer, 2016a, 2016b)
- Convergence of MCMC is evaluated using Gelman and Rubin (1992) test modified by Brooks and Gelman (1998)
- Likelihood is compared among modes of action based on DIC (Deviance Information Criterion) (Spiegelhalter et al., 2002, 2003)

Radionuclide	Tested modes of action	Gelman	DIC
Depleted U chemotoxicity (metal)	Assimilation + Growth	1.03	15783 ←
	Assimilation + Maintenance	1.01	15845
	Assimilation + Assimilation	1.01	15817
Cs-137 gamma radiotoxicity	Cost + Growth	1.00	6461 ←
	Cost + Maintenance	1.00	6492
	Cost + Assimilation	1.00	6488
	Hazard + Growth	1.00	6461 ←
	Hazard + Maintenance	1.01	6491
	Hazard + Assimilation	1.01	6488
Am-241 alpha radiotoxicity	Cost + Growth	1.03	3299 ←
	Cost + Maintenance	1.03	3299 ←
	Cost + Assimilation	1.02	3300 ←
	Hazard + Growth	1.02	3313
	Hazard + Maintenance	1.04	3312
	Hazard + Assimilation	1.01	3305
	Growth	1.01	3304 ←
	Hazard	1.02	3302 ←

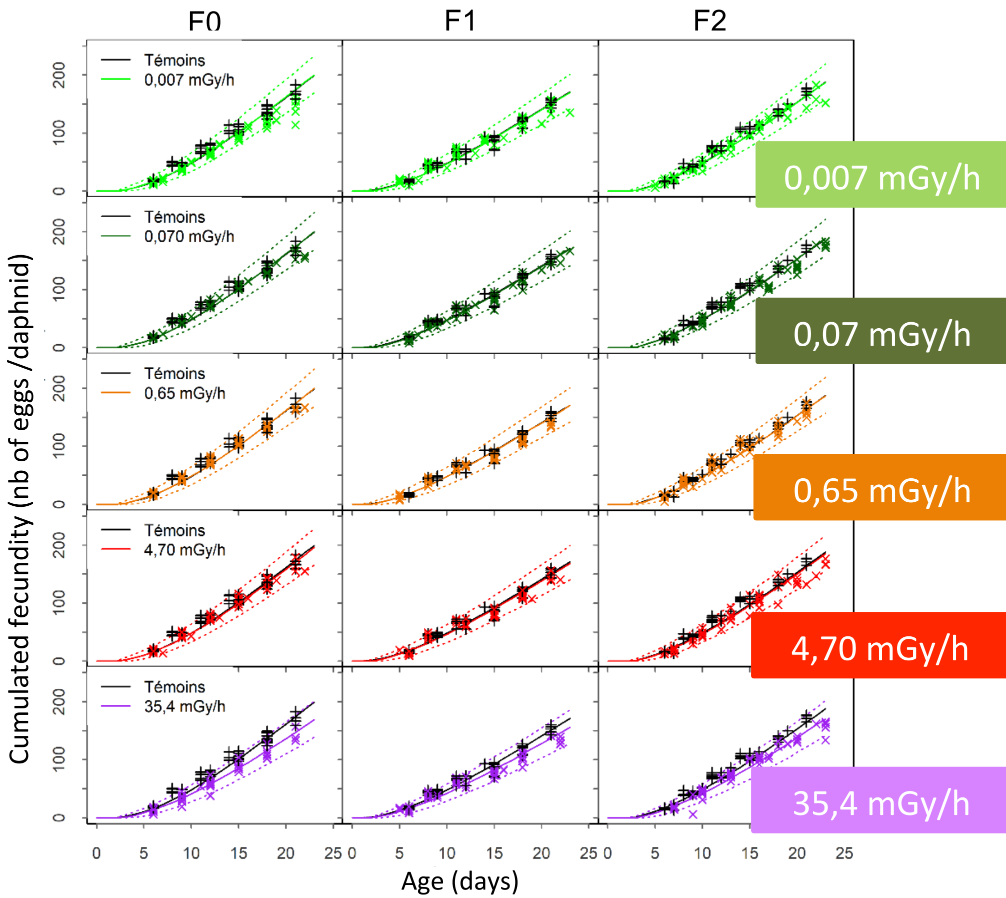
(Alonzo et al., in prep; Billoir et al., in prep; Trijau et al., in prep)

Goodness of fits to data

Growth

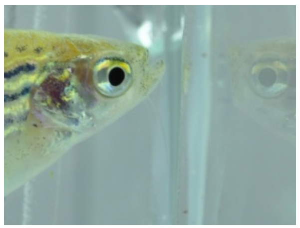
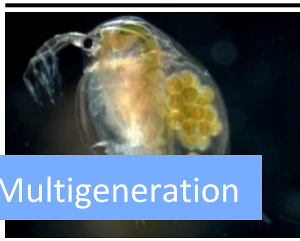


Reproduction



(Trijau et al., in prep)

Similar modes of action of Cs-137 and depleted U among species

	Depleted U	↗Assimilation + Growth
	External gamma Depleted U	↗Sperm mortality + Growth ↗Assimilation
 Multigeneration	External gamma Depleted U	↗Cost or Hazard + Growth ↗Assimilation + Growth

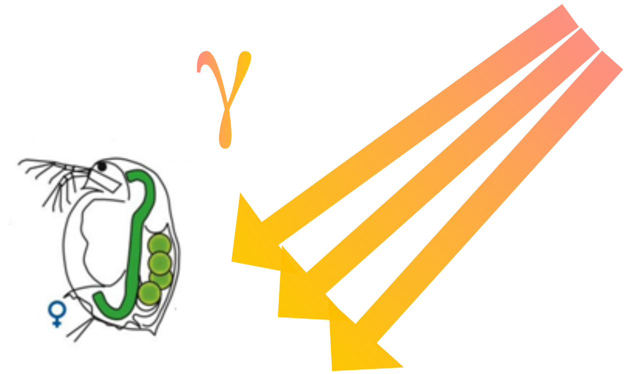
(Massarin et al., 2011; Augustine et al., 2012; Goussen et al., 2015; Lecomte-Pradines et al., 2017)

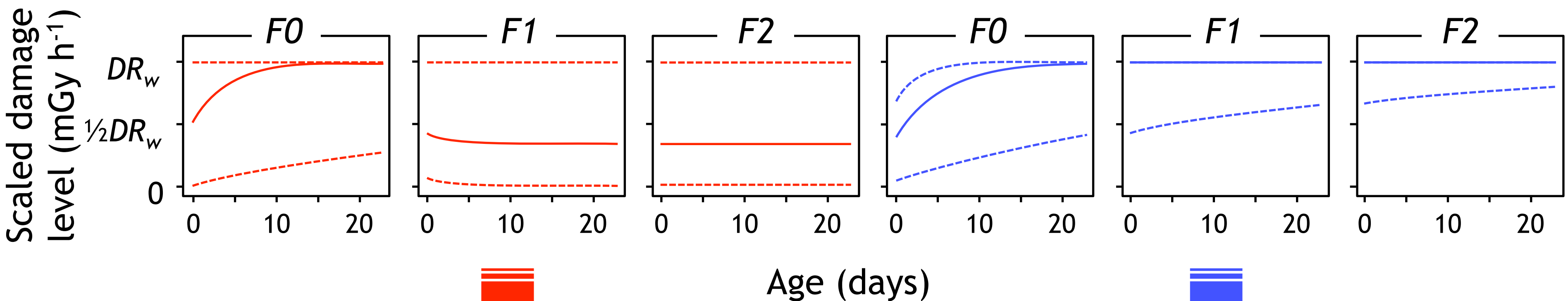
In conclusion…

- Effects of depleted U, Cs-137 and Am-241 on survival, growth and reproduction vary in severity across 3 generations
- DNA alterations are accumulated and transferred to offspring generations during multigenerational exposure to depleted U and Cs-137
- DEBtox models with transgenerational damage compartments are most helpful to analyse toxicity across generations and build mechanistic links among biological scales

↗ Need for more informative data to reduce uncertainty in damage levels and confirm modes of actions

Thanks for your attention!
…Any question?





Mode of action 1
(direct on reproduction)

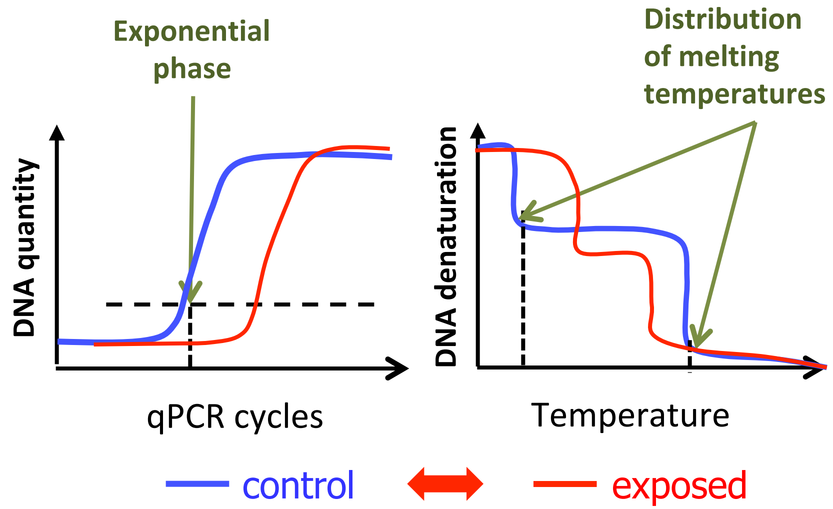
Increase in cost
for growth and maturation

Transgenerational increase in effects on growth and reproduction

(Trijau et al., in prep)

Analyses of DNA alterations in *Daphnia* across generations

- RAPD analyses by qPCR:
  - Kinetics of DNA amplification
  - Composition in PCR products

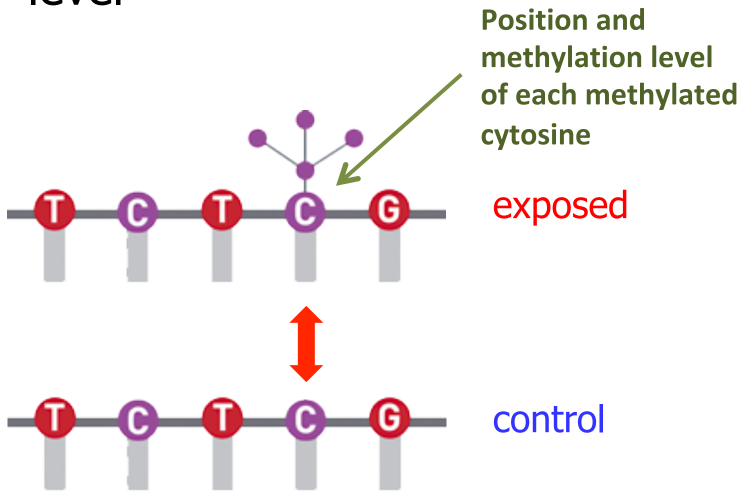


Any change reflects DNA alterations
(creation /loss /modifications of hybridation sites)

(Plaire et al., 2013; Parisot et al., 2015)

Analyses of epigenetic profiles in *Daphnia* across generations

- Whole genome bisulfite sequencing:
  - DNA methylation at the sequence level



Any change might affect the regulation of gene expression

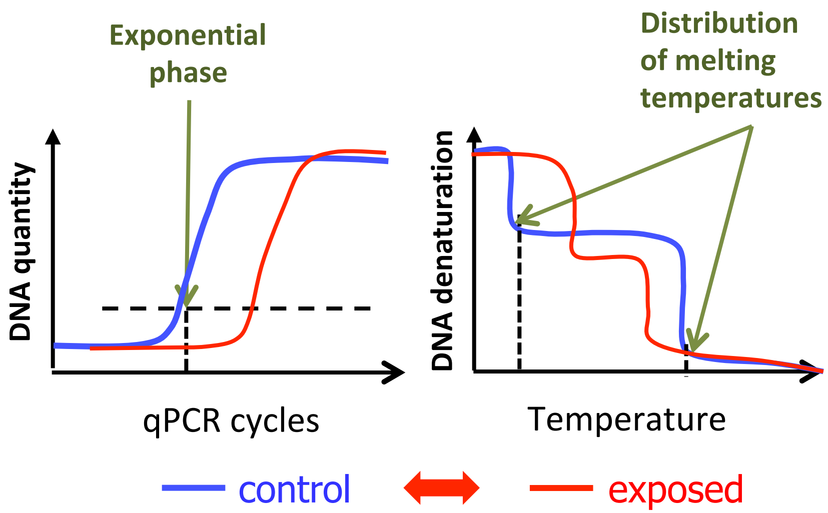
- DNA methylation contributes to the regulation of gene expression
- In *Daphnia*, 1% only of cytosines are methylated (against 70 to 80 % in mammals)
- in many species (including *Daphnia*), DNA methylation can be modified by environmental stress and other ecological factors
- Exposure to ionising radiation changes DNA methylation:
  → *in vivo* and *in vitro* studies in mice
  → in the field in pines from Chernobyl

(Tawa et al., 1998; Koturbash et al., 2006; Kovalchuk et al., 2003, 2004; Asselman et al., 2015; Trijau et al., 2018)

Analyses of DNA alterations in *Daphnia* across generations

- RAPD analyses by qPCR:
  - Kinetics of DNA amplification
  - Composition in PCR products

2 endpoints x 2 probes (OPA9 and OPB10)



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(Plaire et al., 2013; Parisot et al., 2015)

Dynamic of DNA alterations across generations

mGy h⁻¹	F0			F1			F2			F3
	Hatching	Brood 1	Brood 5	Hatching	Brood 1	Brood 5	Hatching	Brood 1	Brood 5	Hatching
0.007	/	/	0.007	0.007	/	/	/	0.007	0.007	0.007
0.070	/	/	**/*	*/*	/	0.070	0.070	/*	**/**	/
0.65	/	0.65	/*	/**	/	/	/	*/**	**/**	*/*
4.7	4.7	*/	/**	**/*	4.7	/	/	/*	**/**	/*
35	*/**	**/**	*/**	*/	*/*	*/*	/	*/*	**/**	*/**

- Significant DNA alterations at decreasing dose rates over the course of F0
  → reflecting a gradual induction of DNA damage in the first generation

- Significant DNA alterations at decreasing dose rates across generations
  → reflecting an accumulation and transmission across generations

- Significant DNA alterations at higher dose rate during F1
  → reflecting a transient elimination associated with repair processes?

(Parisot et al., 2015)

New developments in DEBtox models to address toxicity across generations

- Definition of the adequate « dose metric » for radiological toxicity (dose rate)
- Exposure of egg stage considered without adding parameter
- Thorough account of differences in exposure among generations
- Combination of two modes of action
- Transgenerational damage compartment

(Lecomte-Pradines et al., 2017)

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