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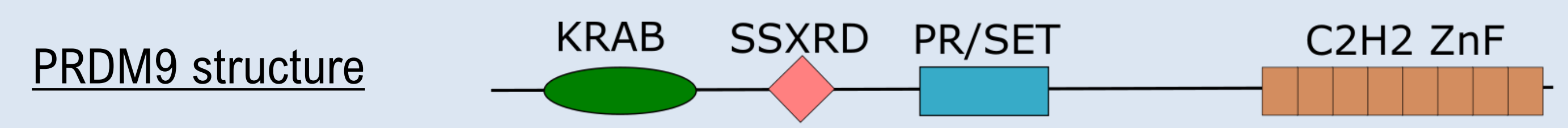
# Investigating PRDM9 function in invertebrates

Lucie Murphy, Amélie Rudler, Richard Galinier, Jean-François Allienne, Anne Rognon, Julie A.J. Clément

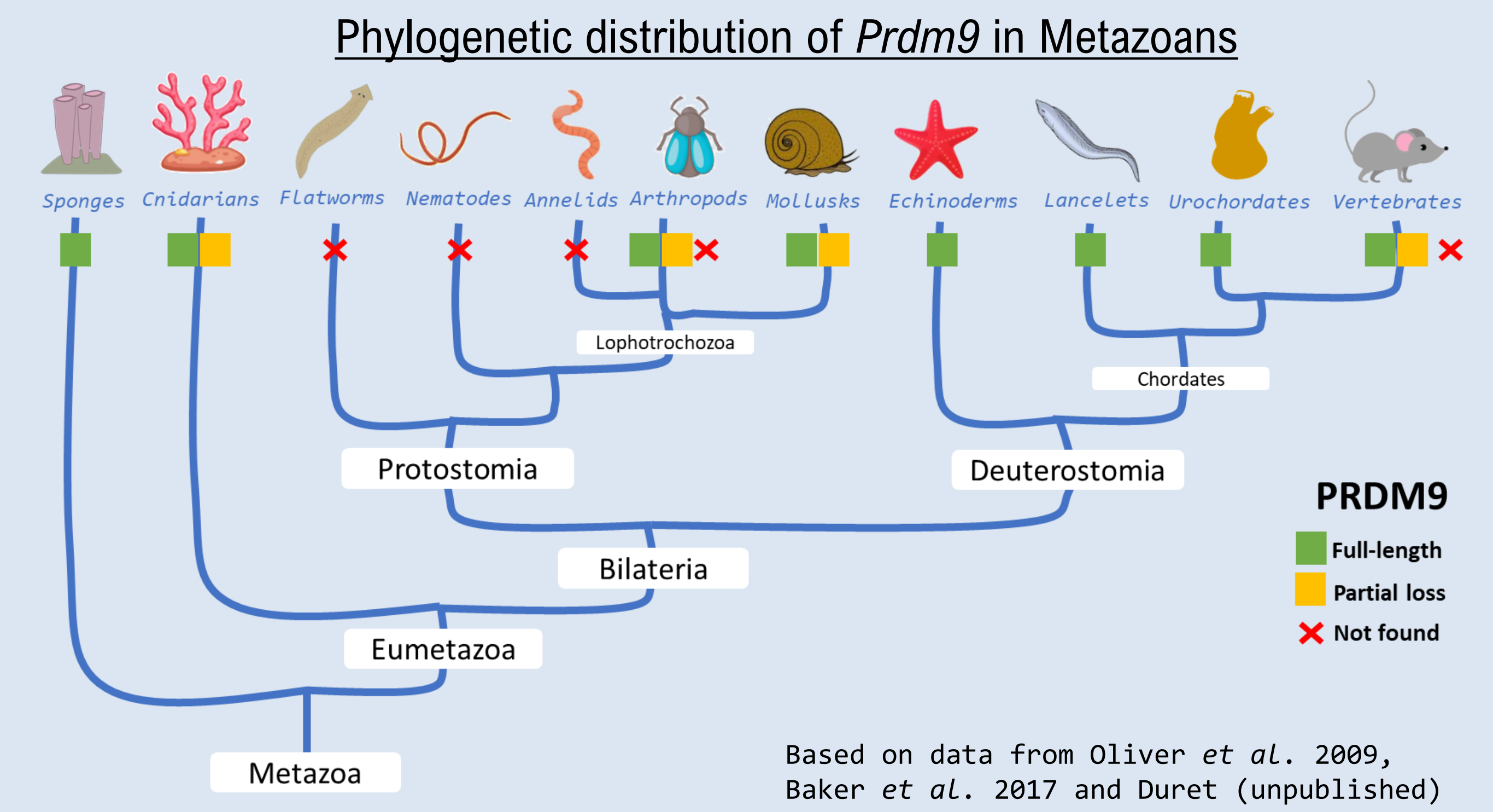
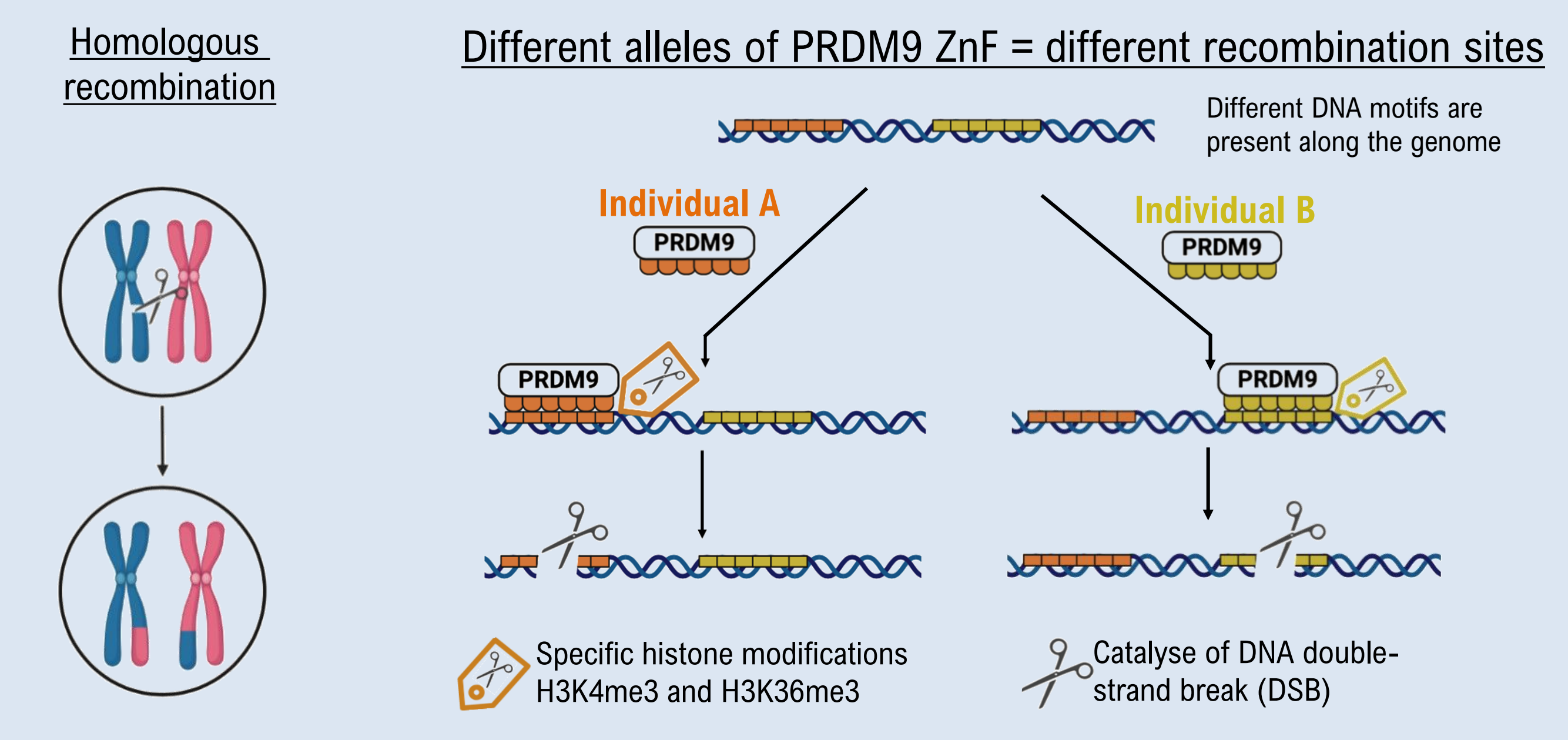
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If I am not around, this picture should help to find me!



**Prdm9 determines where meiotic recombination takes place in many mammals<sup>1</sup>**  
 During meiosis, DNA double-strand breaks (DSBs) initiates homologous recombination at specific loci called **hotspots**. In many mammals, their localization corresponds to specific DNA sequences bound by the zinc finger (ZnF) array of PRDM9. A remarkable property of PRDM9 is the **high diversity and fast evolution of its ZnF domain**. The consequence is the fast evolution of recombination map for species having a PRDM9-directed system of hotspot localization.



**Prdm9 was lost many times since its appearance in the LCA of Metazoans<sup>2,3</sup>**  
 PRDM9 appears in the **Last Common Ancestor (LCA)** of metazoans but its partial or complete loss have been surprisingly reported in many taxa. Species lacking a full-length PRDM9 have evolutionary stable hotspots that are located near promoter-like regions, that are evicted by PRDM9. The involvement of PRDM9 in meiotic recombination has not been yet explored outside vertebrate species, one reason being that classical invertebrate model species (drosophila, nematode) have no PRDM9.

## Is the role of Prdm9 conserved and does it shape recombination landscape in invertebrates ?

**Full-length Prdm9 found in many Gastropods**

► Prdm9 identification and phylogenetic analysis across Gastropods

Still need exploration for Prdm9 in *P. acuta*

+ co-evolving proteins<sup>4</sup>: ZCWPW1 ; ZCWPW2 ; TEX15 ; FBXO47

+ PRDM9's partner<sup>5,6</sup>: HELLS

Collaboration with Laurent Duret, LBBE Lyon

**PRDM9 zinc finger diversity and evolution**

*Bi. glabrata*

*Bu. truncatus*

3 hypervariable positions involved in DNA contact

► Individual number and population number to extend

**Prdm9 transcription**

► Prdm9 is specifically transcribed in ovotestis of *Bi. glabrata*

Data from Randall J DeJong, VectorBase rel.1.47

► To be done for all the four species: **Transcription level**

Using Long-read RNA sequencing in Ovotestis and control tissues

► To be done for *Bi. glabrata*: **Transcript localisation**

Using RNAscope on ovotestis sections

AMPLIFY signal → VISUALIZE with morphology → QUANTIFY single-cell expression

source: ACDBio

*Mei@nail*

**How meiotic recombination is controlled in freshwater snails? Does it depend on Prdm9?**

**Prdm9 functional validation**

► siRNA-RNA interference approach<sup>7</sup> to knock-down Prdm9

Microinjection through the shell at ovotestis location

How RNA interference works

What to measure?

- Fertility : clutch number; viable eggs
- Meiotic progression (cytology)
- DSB formation (cytology)
- DSB localisation (molecular approaches)

Collaboration with David Duval, IHPE Perpignan

**Where are meiotic hotspots?**

► **Population genomic approach<sup>8</sup>**

- 10-20 individuals from natural populations
- Whole genome sequencing (~30X)
- SNPs identification
- Linkage disequilibrium (LD) analysis

→ Identification of recombination hotspots and estimation of recombination rate (along the genome, at genomic features, ...)

► **Molecular approaches**

- Selecting different *Bi. glabrata* lines with different Prdm9 alleles

- Using ChIP-seq to map PRDM9 binding sites and DSBs<sup>9,10</sup>
- Expectation if Prdm9 designed meiotic DSBs, from Mouse data<sup>11</sup>

Collaboration with Bernard de Massy, IGH Montpellier

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