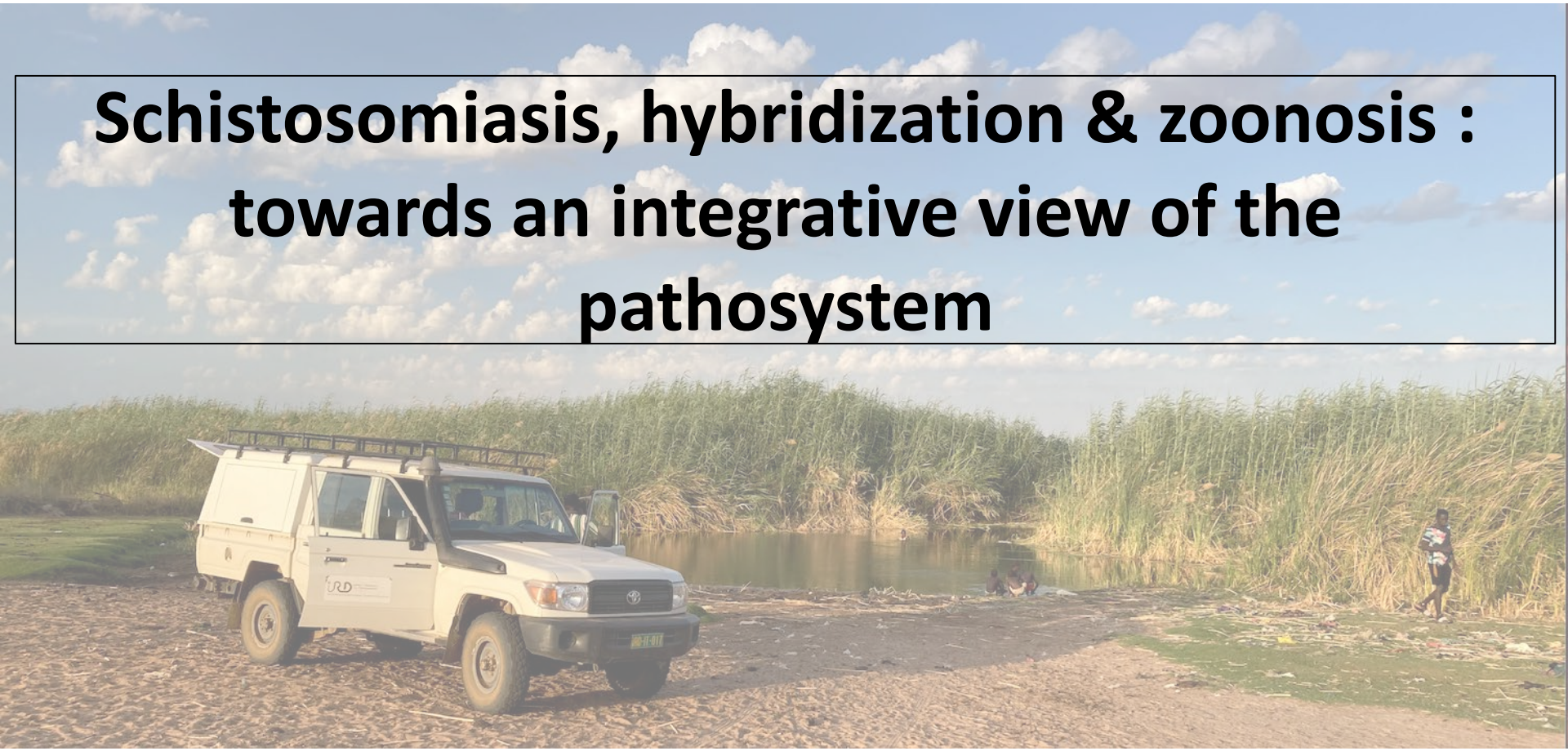
Séminaire CBGP
12/05/2022

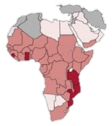
Schistosomiasis, hybridization & zoonosis : towards an integrative view of the pathosystem



Julien Kincaid-Smith
kincaid-smith.julien@ird.fr

Previous research activities

From populations



L3 IHPE :
Cellular support – Immune response - *Biomphalaria sp*
Flow-cytometer

1

M2 IHPE:
Sexual biology - Parental imprinting
Transcriptomics

3

Postdoc 1 RVC-UK:
Clinical trial - morbidity hotspots - childhood fibrosis - treatment
Genetics / genomics
Uganda

5

2013 — Schistosoma — **2022**

2

M1 IHPE:
Patterns of chronobiology – host adaption
Population genomics
Oman

4

Thesis IHPE:
Hybridization - life history traits modification - Molecular mechanisms.
Genet/Geno/Transcripto
Cameroon/Corsica

6

Postdoc 2 CBGP:
Epidemiology - reservoir hosts - Zoonosis-
Pop genet/genomics
Senegal

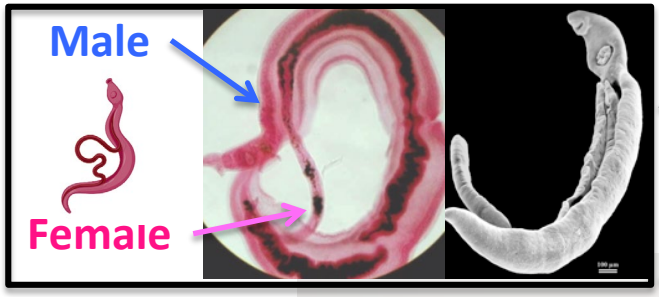
To molecules

Schistosomes: causative agents of schistosomiasis

Phylum **Platyhelminths,** Class **Trematoda**

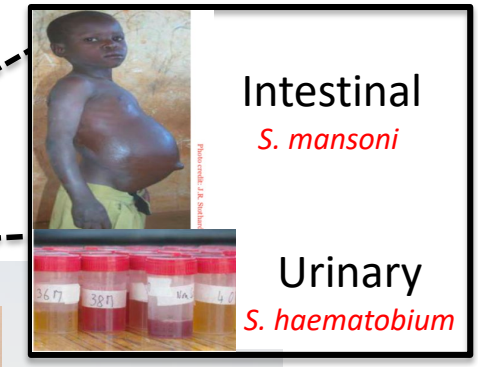
Schistosoma sp : 23 species that infect **humans**, les **animals**, **zoonotic**

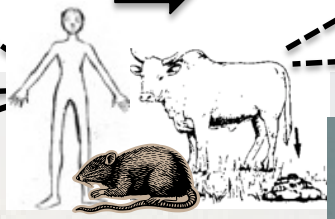
Schistosome adulte worms



- Gonochoric
- Female heterogametic (ZZ – ZW)

Vertebrate definitive hosts



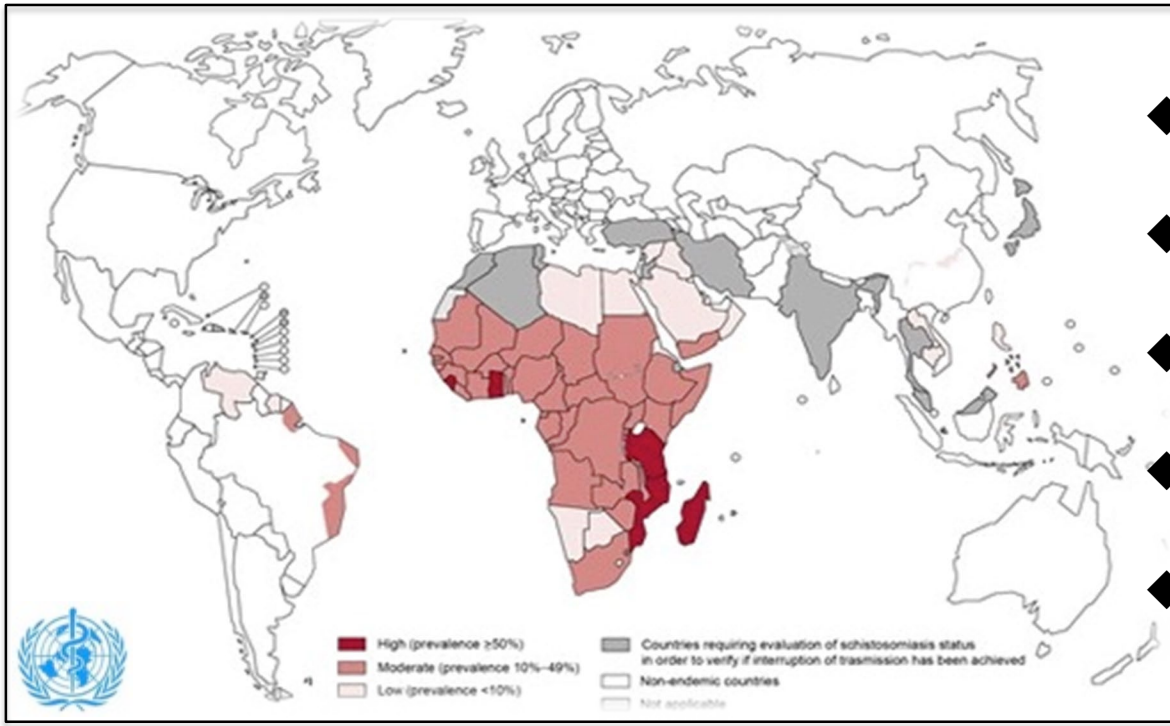


Reproduction

Mollusc intermediate hosts (vectors)

Schistosomiasis : a public health problem in the tropics

2nd human parasitic disease (Neglected Tropical Disease)



- **78 endemic countries**
- **>800 million people at risk**
- **>240 million people infected**
- **90% in sub-Saharan Africa**
- **>200 000 death / year**

Global changes (climate + human movements) may promote **modifications in hosts and parasites distribution**

Disease spread and emergence

IHPE
Intéractions-Hôtes-Pathogènes-Environnements

UMR5244 – IHPE
CNRS-UPVD-UM2-IFREMER

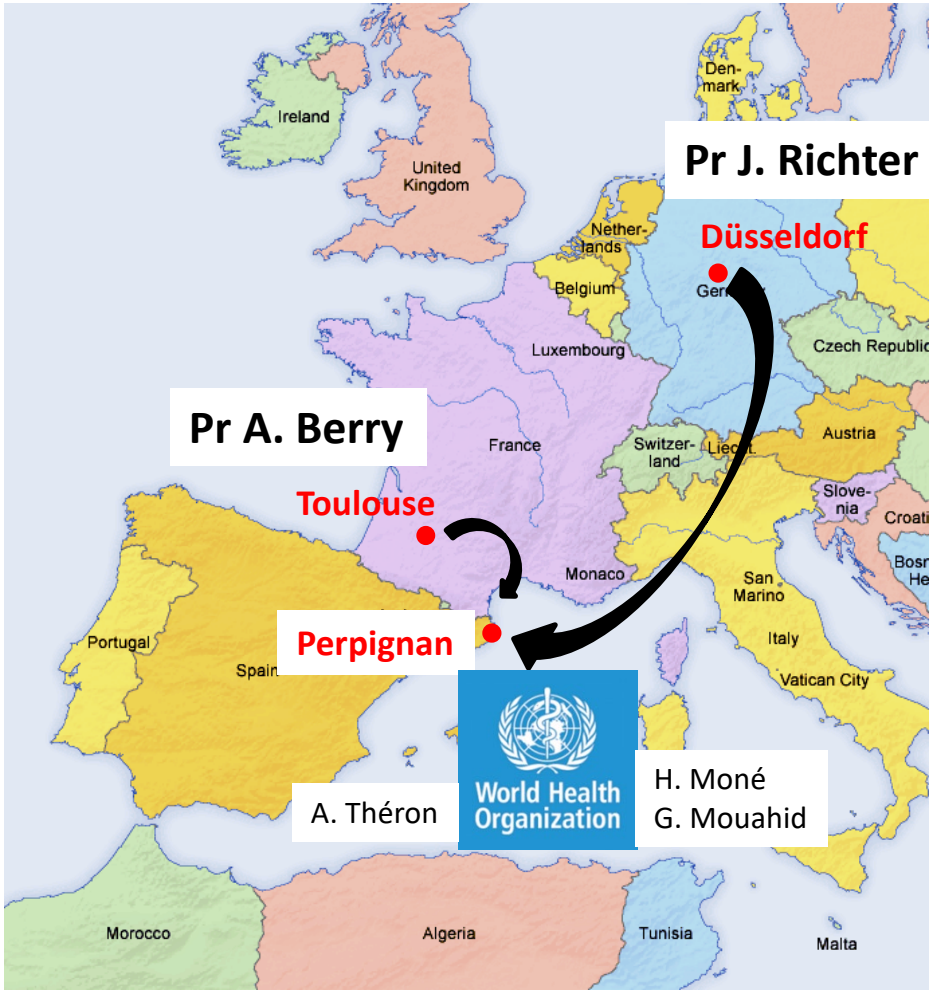
WHO collab center

Emergence of urinary schistosomiasis in Corsica : biological invasion & sanitary risks

Julien Kincaid-Smith, Eve Toulza, Olivier Rey, Anne Rognon, Jean-Francois Allienne & Jérôme Boissier

University of Perpignan, FRANCE

April 2014: Clusters of urogenital schistosomiasis diagnosed in French and German hospitals



- Düsseldorf (Pr J. Richter)

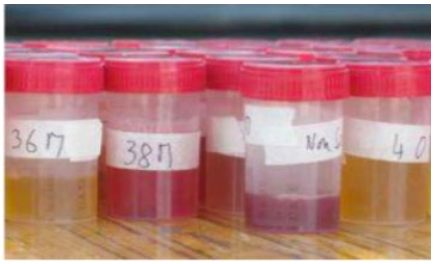
Boy, 12yo with macroscopic haematuria

Richter et al. 2016

- Toulouse (Pr A. Berry)

Girl, 4yo avec with macroscopic haematuria schistosomes eggs in the bladder (biopsy) + urine

Berry et al 2014



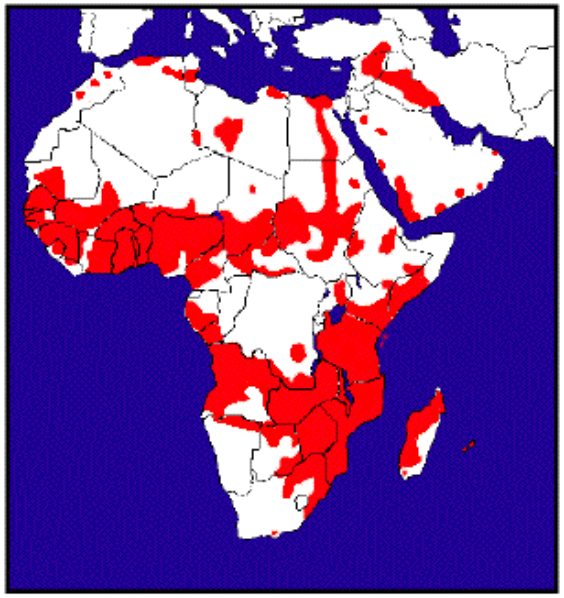
S. haematobium
(human urine)

Elliptical shape
Terminal spine

Imported cases of schistosomiasis in Europe ?

Patients had no travel history of schistosomiasis endemic countries

Distribution of *S. haematobium*



Anamnesis of patients :

-Tunisia
-Ile Maurice
-Iles des Baléares
-Brazil
-Albania
-Other European countries…

But, one common destination…

They had spent summer 2013 in Corsica: a popular French Mediterranean island

North Porto-Vecchio
The Cavu river

Most frequented river in Southern Corsica :
3.000 to 5.000 people/day in summer

The vector snails (*Bulinus truncatus)* are present in Corsica

Brumpt 1930

CYCLE ÉVOLUTIF COMPLET DE *SCHISTOSOMA BOVIS*

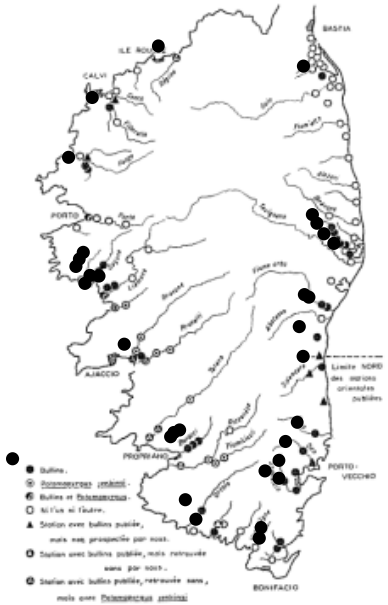
Infection naturelle en Corse et infection expérimentale de *Bullinus contortus*

Par E. BRUMPT

Bullins et bilharzioses en Corse

Répartition, fréquence et biologie de « Bulinus truncatus »

Par J.-M. DOBY, B. RAULT, S. DEBLOCK et A. CHABAUD (1)



"Le premier cas certain de bilharziose humaine autochtone n'est donc pas inconcevable…"

Doby et al. 1966

2014: Cavu river

Bulinus truncatus

Where have the contaminations occurred?

Schistosomiasis reaches Europe

Boissier et al. Lancet ID 2015

✓ **Pictures from tourists**

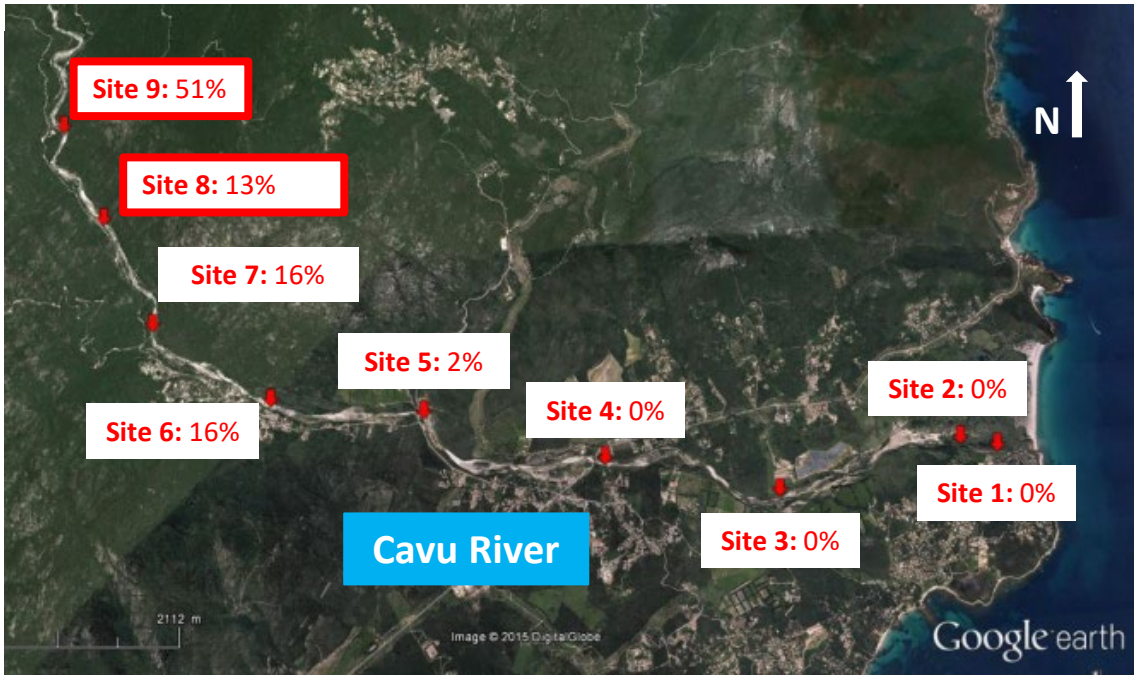
4 families

✓ **Meeting along the river with locals**

✓ 31 oral testimonies

Boissier et al Lancet ID 2016

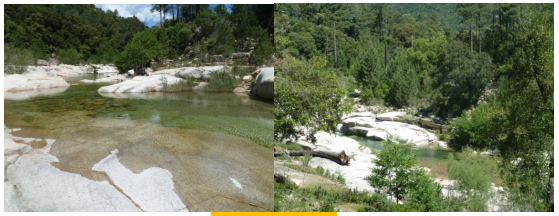
9 potential transmission sites (12km long river)



National survey : 54% and 38% of infected patients – frequented sites 9 and 8, respectively

Noel et al. 2017

Site 8



Site 9

No snails were found naturally infected

Summer 2014 : 3,544 molluscs tested (cercarial emission)

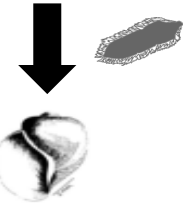
UMR5244 – IHPE : mandated by the Regional Health Agency (ARS) to monitor the snail populations

Summer 2015 : 1,965 molluscs tested (cercarial emission)
Summer 2016 : 3,453 molluscs tested (PCR)
Summer 2017 : 5,364 molluscs tested (PCR)
Summer 2018 : 4,329 molluscs tested (PCR)
Summer 2019 : 5,100 molluscs tested (PCR)
Summer 2020 : 3,500 molluscs tested (PCR)
Summer 2021 : ….

0 positif / > 30 000

Experimental infections confirm the role of local snails

Infected patient from Corsica



13-24% positive

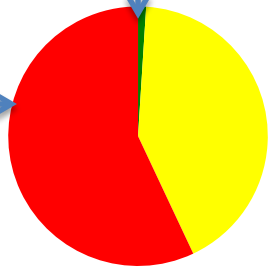
Boissier et al. 2016
Kincaid-Smith et al. 2018

What is the parasite's origin ?

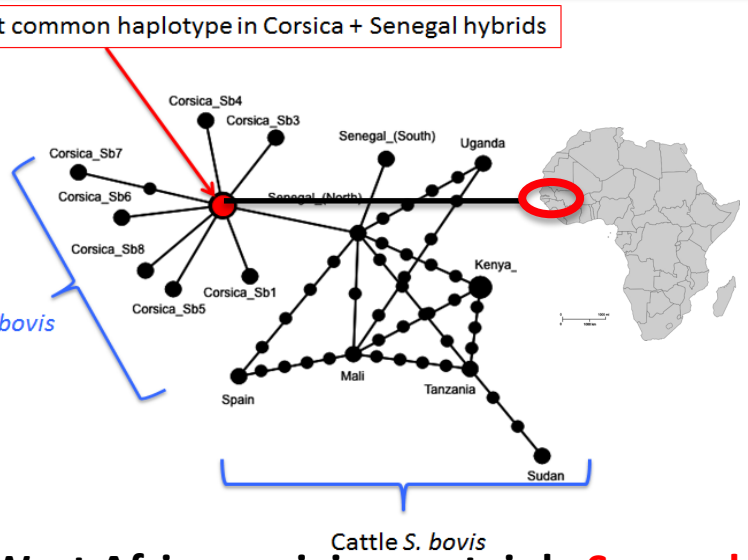
✓ **Genetic characterisation of the parasites (ITS & COI sequencing), 12 patients, 73 sequences:**

Pure *S. bovis*
(COI & ITS)

Pure
S. haematobium
(COI & ITS)



Hybrids
(COI *S. bovis*
X
ITS *S. haematobium*)



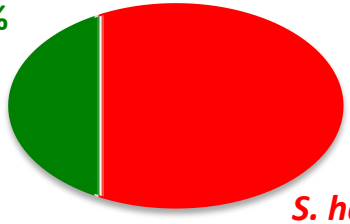
Boissier,…,Kincaid-Smith,… et *al*. 2016

West African origin : certainly Senegal

✓ **Genomic characterisation of the hybrids (WGS)**

Complex admixture of parental species

S. bovis
23%



S. haematobium
77%

Kincaid-Smith et *al. 2018, 2021*

Confirmation of ancient/imported hybrid parasites

Hybrid vigour (heterosis) / adaptive introgression

- Virulence - Host spectrum - Invasive capacity ?

Risk of animal reservoir & zoonotic transmission

Chronology of the outbreak

Year	Cases	
2013	2	*Berry et al. 2014* *Richter et al. 2014*
2014	River closed	?
2015	1	*Berry et al. 2016*
2016	2	*Ramalli et al. 2018* *Sampietro et al. 2018*
2017	5	
2018	2	*Ramalli et al. 2018*
2019	1	Rothe *et al. 2021*

National screening: **38,310** serological tests

106 cases

Brunet et al. 2015; Patard et al. 2015

Modeling estimations: **338** [166-510]^{IC95}

Noel et al. 2018

Maintenance: Snails ? Humans ?

Hybrids in animal reservoir = no

Oleaga,.., Kincaid-Smith et *al*. 2019 *(Plos NTD)*

3,519

2/34

June 2016: Autochthonous schistosomiasis becomes a **notifiable disease** in France

Developing endemicity : The Solenzara river

Other cases in 2020…

This biological invasion raised several important questions

1) What is the invasive capacity of the parasite in Europe ?

1) Is there a link between the “hybrid” form of the parasite and the outbreak in Corsica ?

1) Are *S. haematobium* x *S. bovis* hybrids more fit than parental species (heterosis or hybrid vigor)

HySWARM project

Identification of hybrid life history trait modifications and their molecular bases

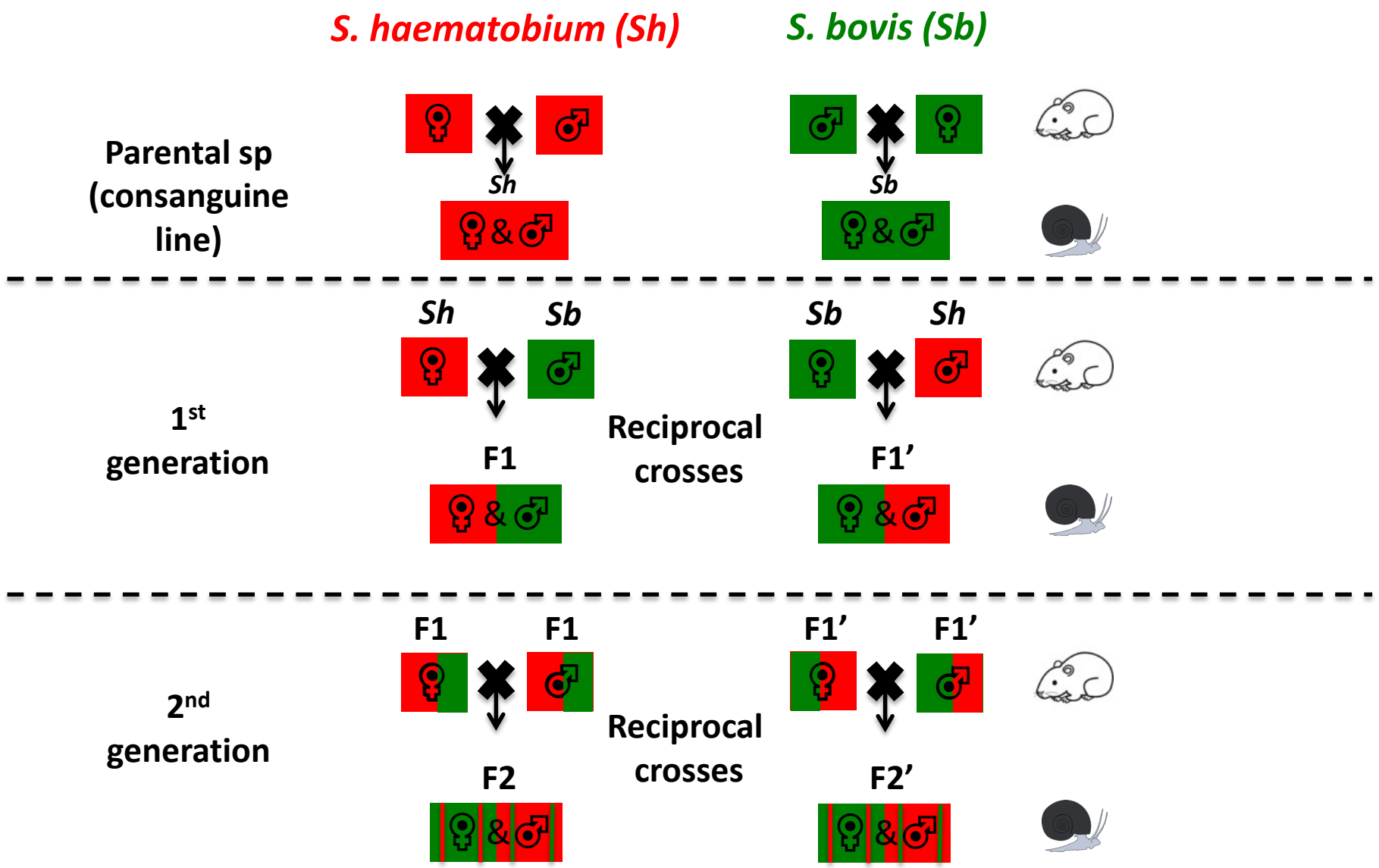
Kincaid-Smith 2018 (Thesis)

AGENCE NATIONALE DE LA RECHERCHE ANR

J. Boissier - IHPE

Experimental evolution protocol

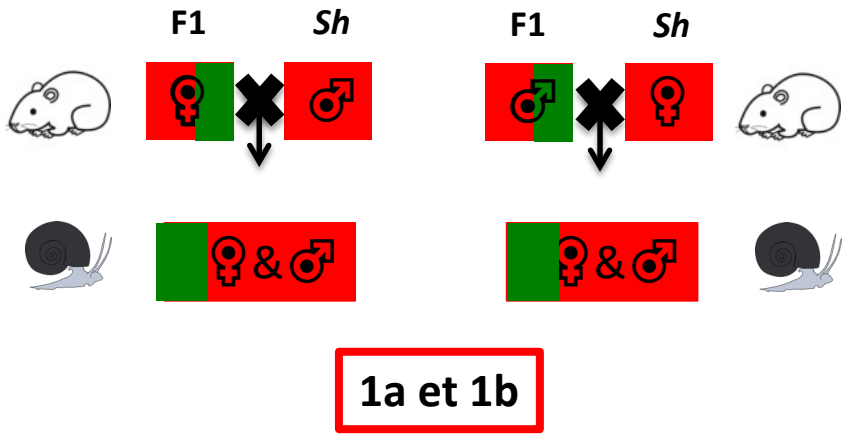
First step : recover parental species & identify molecular sex markers (Kincaid-Smith et al. 2016)



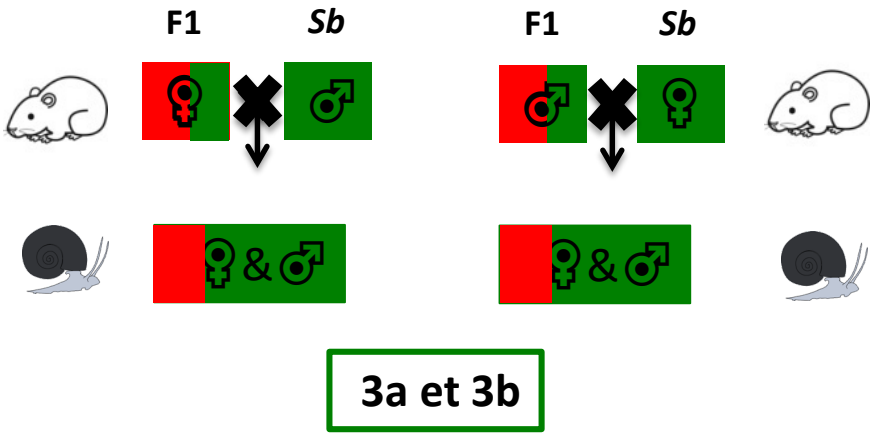
Backcrosses

1st generation with *S. haematobium*

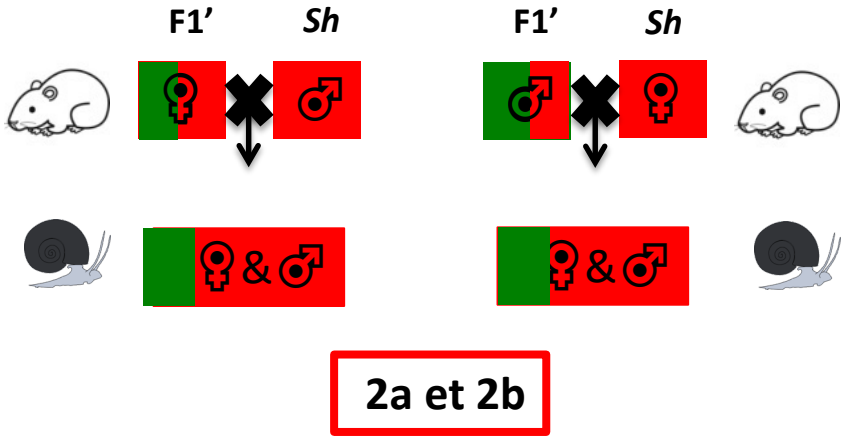
1st generation with *S. bovis*



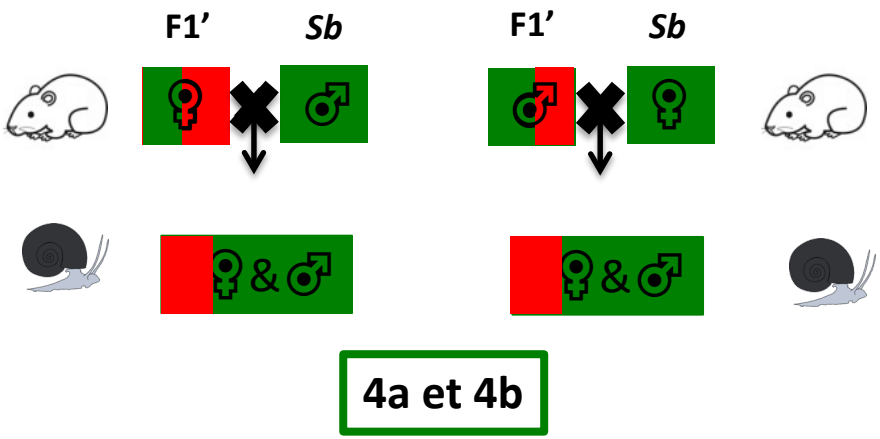
1a et 1b



3a et 3b



2a et 2b



4a et 4b

Lines obtained

15 parasite lines to characterize:

S. haematobium *S. bovis*

F1 F1’

F2 F2’

Comparison with the Corsican hybrids

S. haematobium **Backcrosses**

♀F1x♂sh ♂F1x♀sh

♀F1’x♂sh ♂F1’x♀sh

S. bovis **Backcrosses**

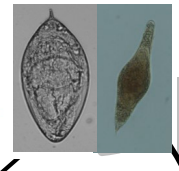
♀F1x♂sb ♂F1x♀sb

♀F1’x♂sb ♂F1’x♀sb

Life history traits analysis

Definitive host

Hamster containing adult worms

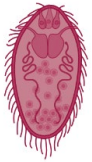


Eggs

- ✓ **Sexual interaction**
- ✓ ***Cercaria* infectivity**
- ✓ **Females prolificacy**
- ✓ **Induced pathology/morbidity**
- ✓ **Egg morphology**

Cercaria

Miracidium



- ✓ **Prevalence**
- ✓ **Host spectrum (*Bulinus*, *Planorbarius*)**

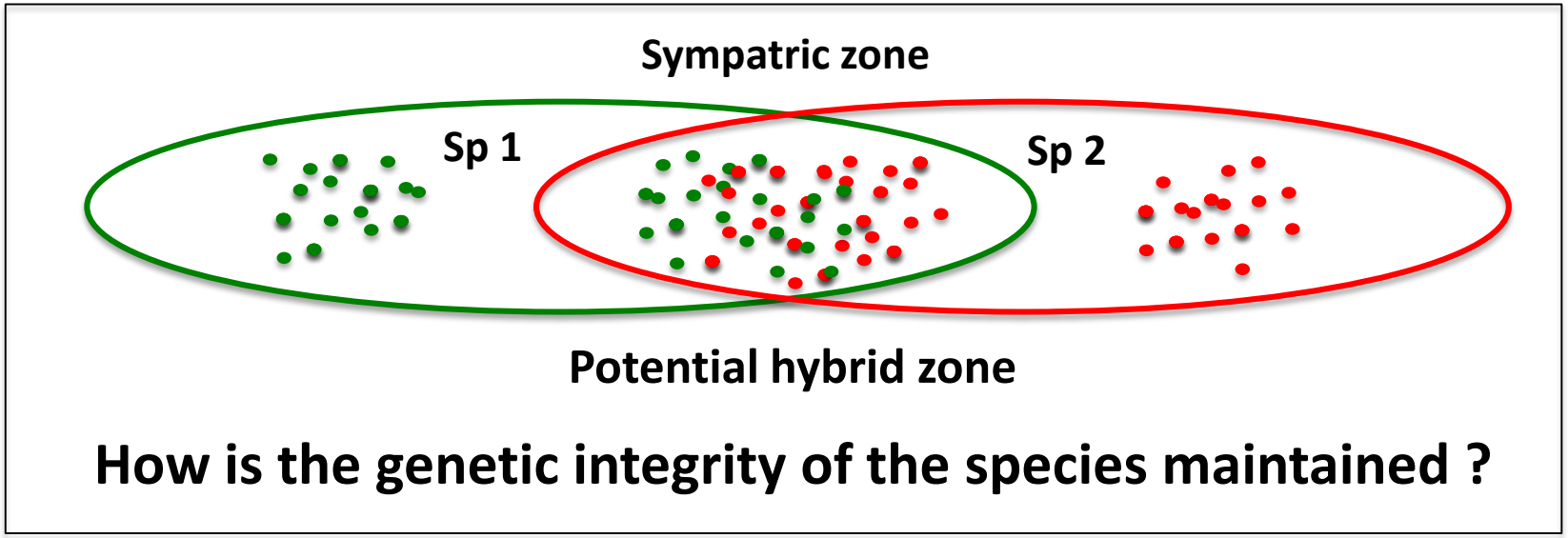
Molluscs

Intermediate hosts

B.t**: Bulinus (S. haematobium & S. bovis) ; Planorbarius : (S. bovis* **only)*

Are there any barriers to hybridization ?

Determine the potential occurrence of the phenomenon *in natura*



How is the genetic integrity of the species maintained ?

Reproductive isolation mechanisms:

Pre-zygotic barriers

- **Spatial dimension**
- **Host specificity / tropism**
- **Sexual choices and competition**

Post-zygotic barriers

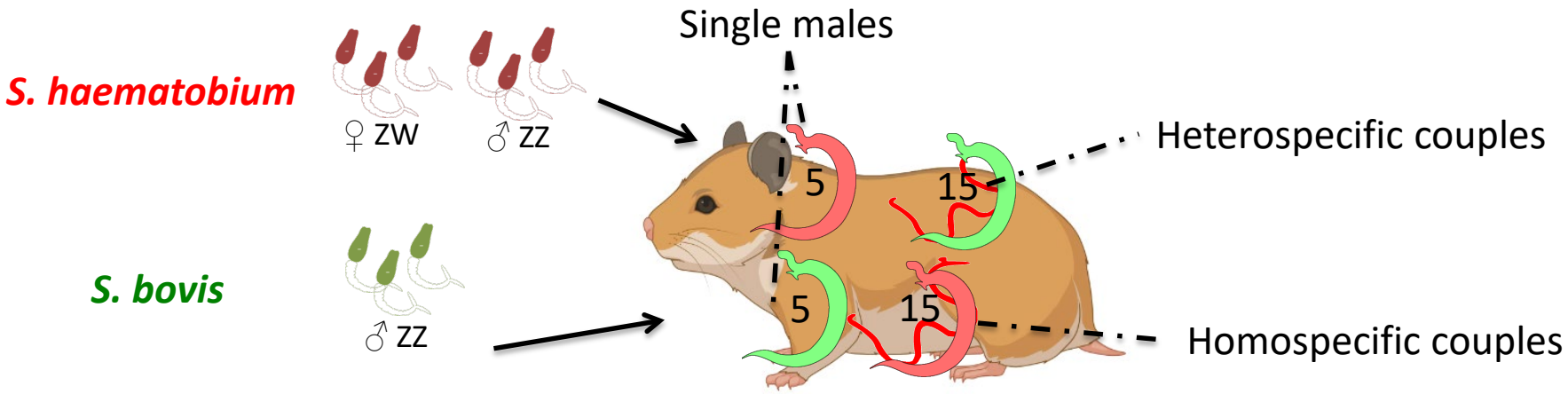
- **Non-viable**
- **Sterile**
- **Low performance**

Absence = Reproduction (Hybridization)

Are there any barriers to hybridization ?
1) Mating choices

Mate recognition system ? Pairing preference ?

- Exp 1 & 2: Male choice *
- Exp 3 & 4: Female choice *
- Exp 5: Full choice (all species and sex combined)+



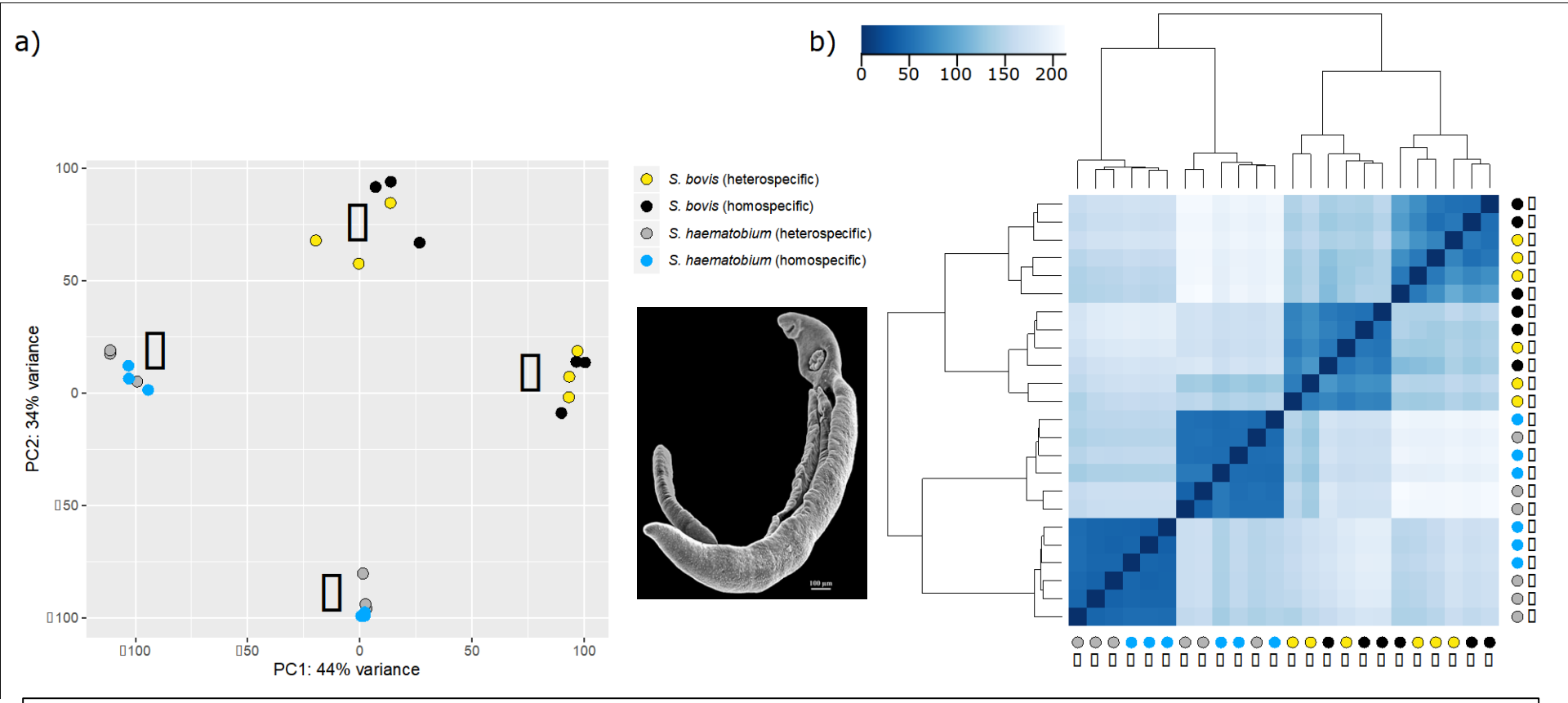
Example of *S. haematobium* female choice

ALL experiments show <u>random pairing between *S. haematobium* & *S. bovis*</u>

*null hypothesis of random pairing
+Chi-square tests.

Kincaid-smith et al. 2021

Are there any barriers to hybridization ?
2) Transcriptomic analyse of homo- vs. hetero-specific male and female



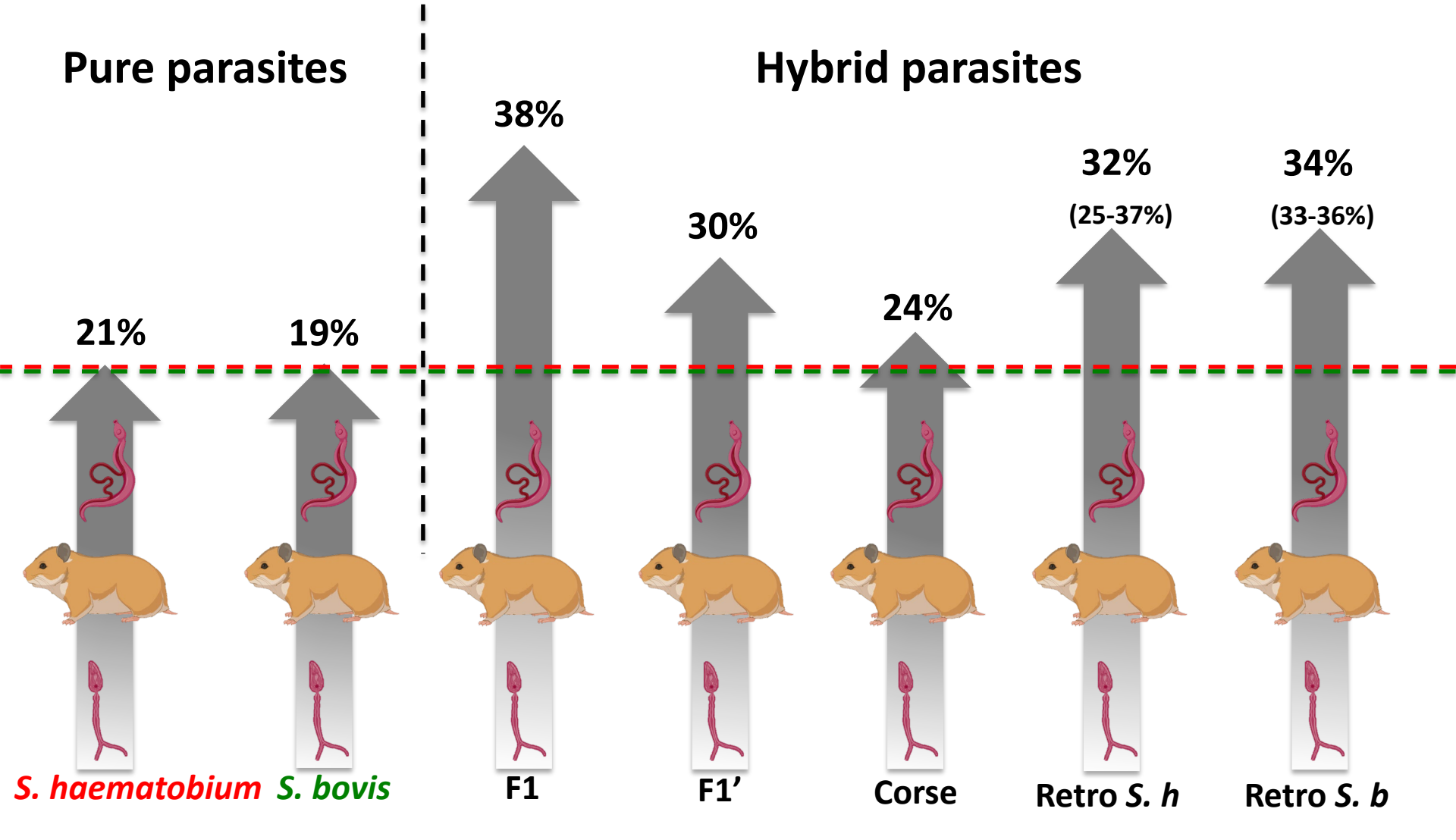
Few genes affected (DGE) - Log2 ratio low : some genes known in male-female interactions

Conclusion

- No pre-zygotic barrier (sexual choice + DGE) = Importance of host specificity
- Hybridization may be frequent (**which host ?**)

Cercaria infectivity

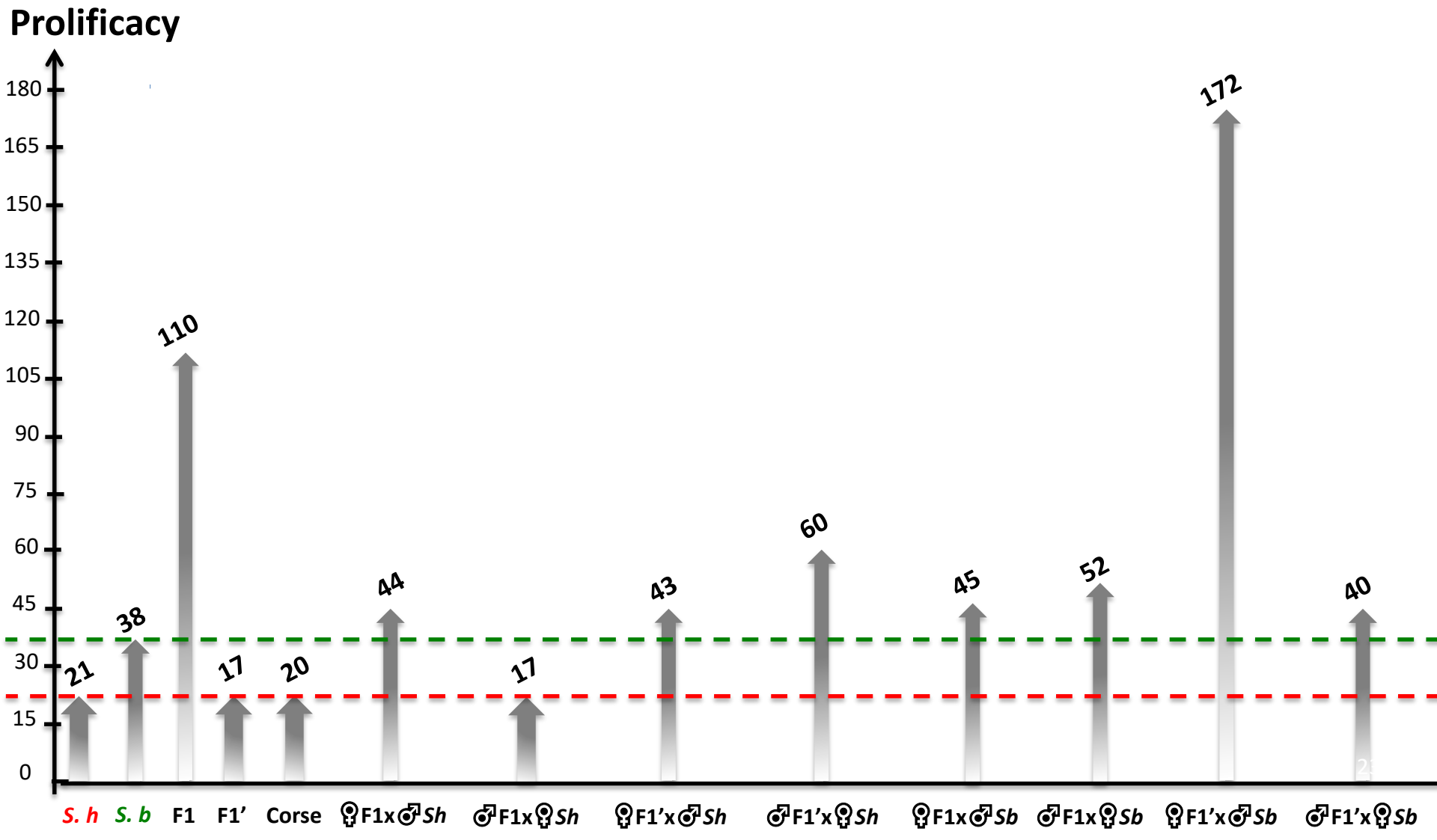
Significant (KW = 30,08: p= 0,003) but pair comparison NS (premature death / high variance / low sampling)



Female prolificacy

Number of eggs per day per female.

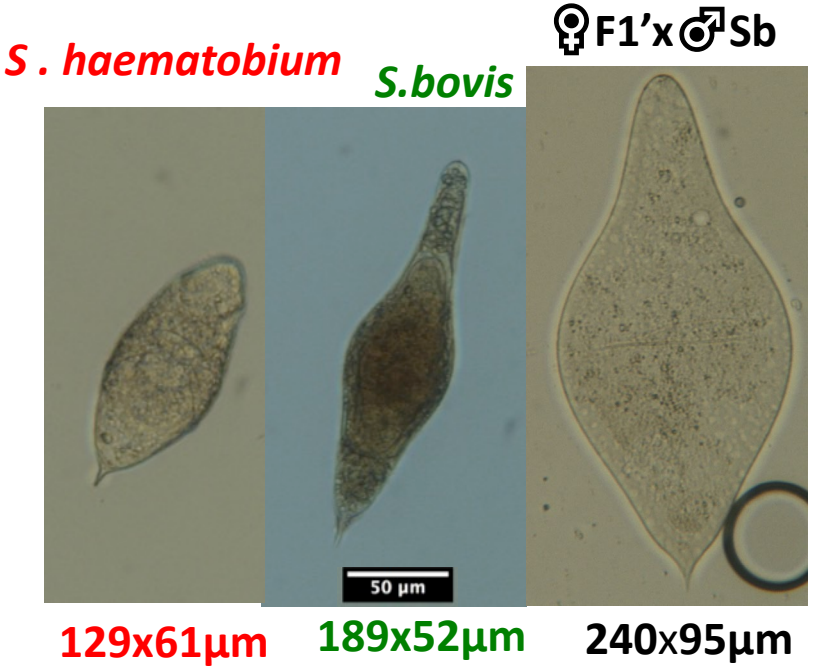
Significant difference (KW: 41.348; p <0.001) but pair comparison NS



Egg morphology

- **Parasitological diagnostic gold standard**
- **Responsible for pathology**

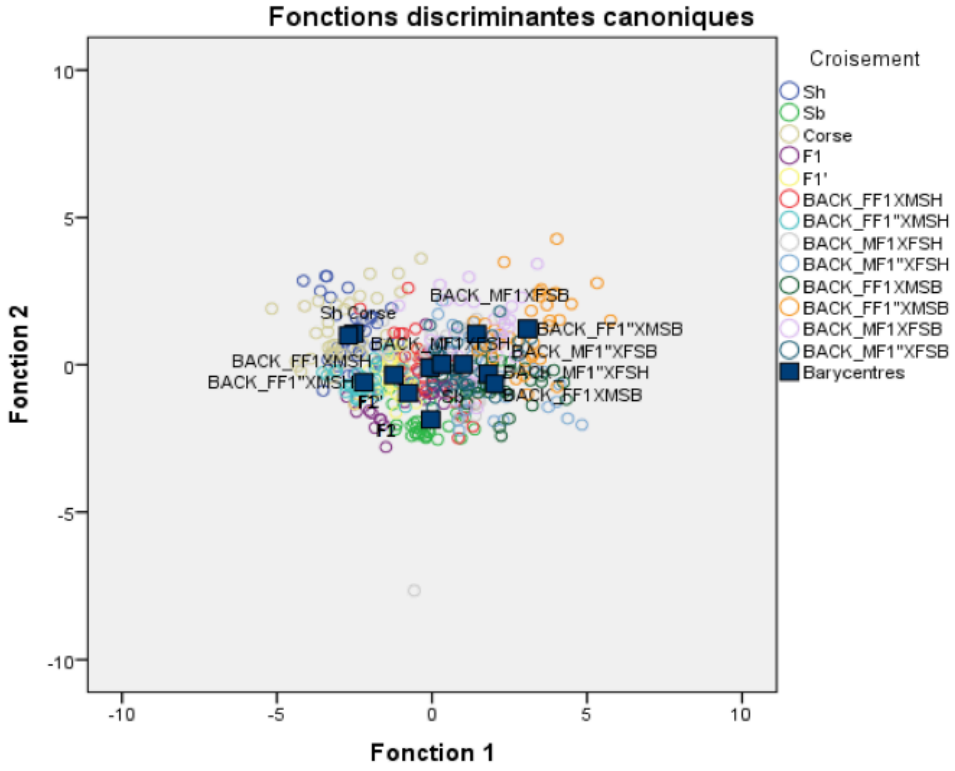
Length / Width / Size of the spur / Area (μm^2)



Significant increase in egg size

Together with prolificacy = increased pathology

⇒ Evolutionary dead end ?

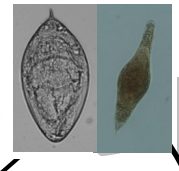


Egg morphology is not an indicator of hybridization

Life history traits analysis

Definitive host

Hamster containing adult worms



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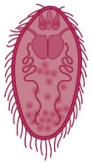


Molluscs

- ✓ **Prevalence**
- ✓ **Host spectrum (*Bulinus*, *Planorbarius*)**

Intermediate hosts

B.t**: Bulinus (S. haematobium & S. bovis) ; Planorbarius : (S. bovis* **only)*

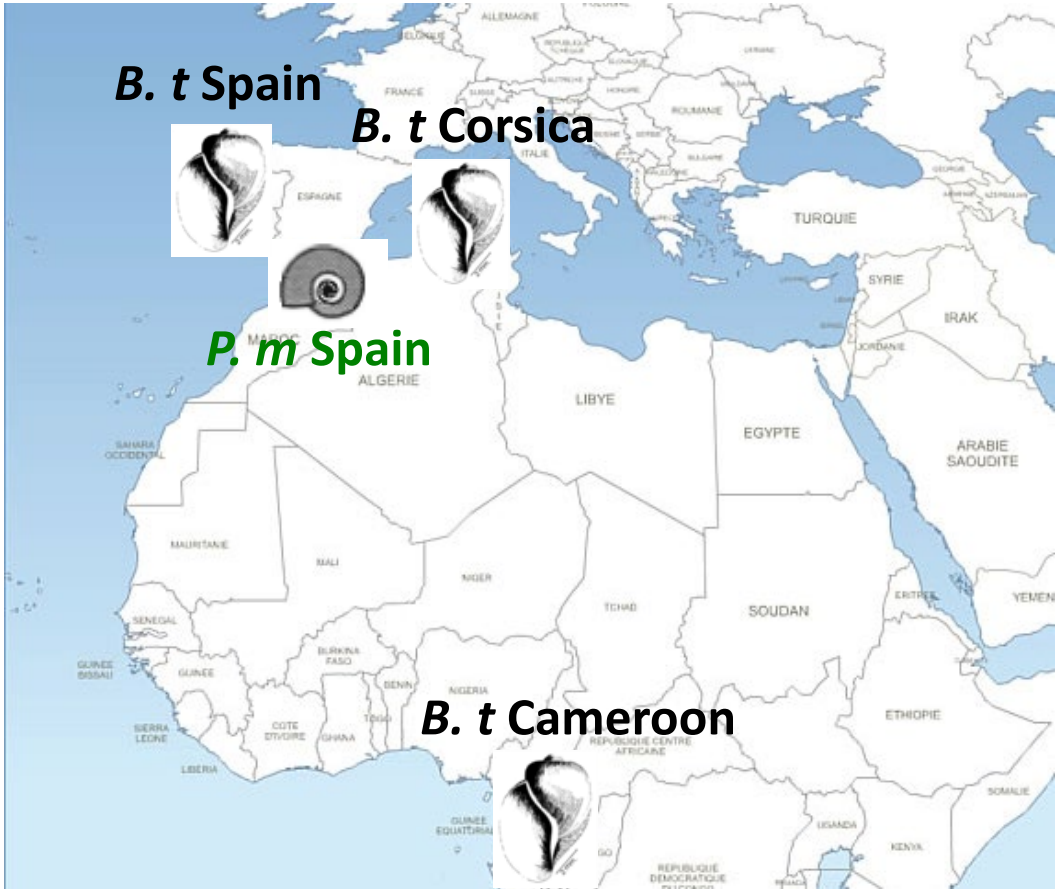
Parasites-molluscs interaction

15 Parasite lines

4 molluscs strains

Prevalence

Host spectrum

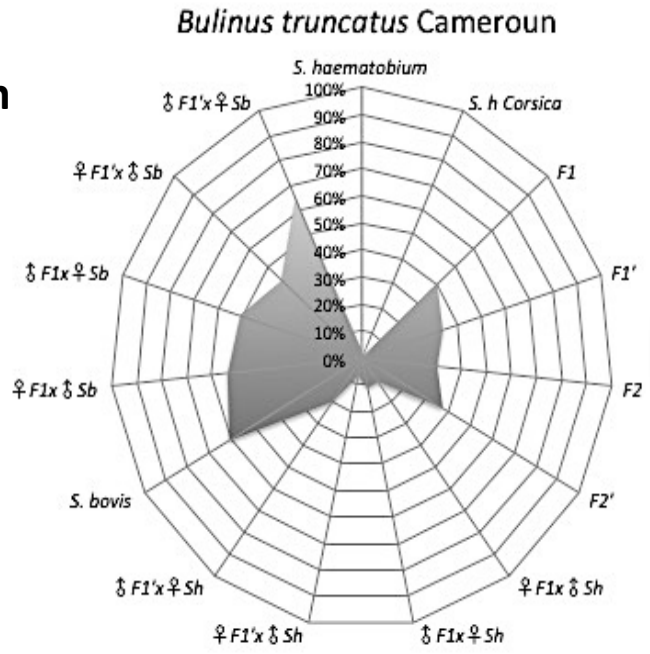


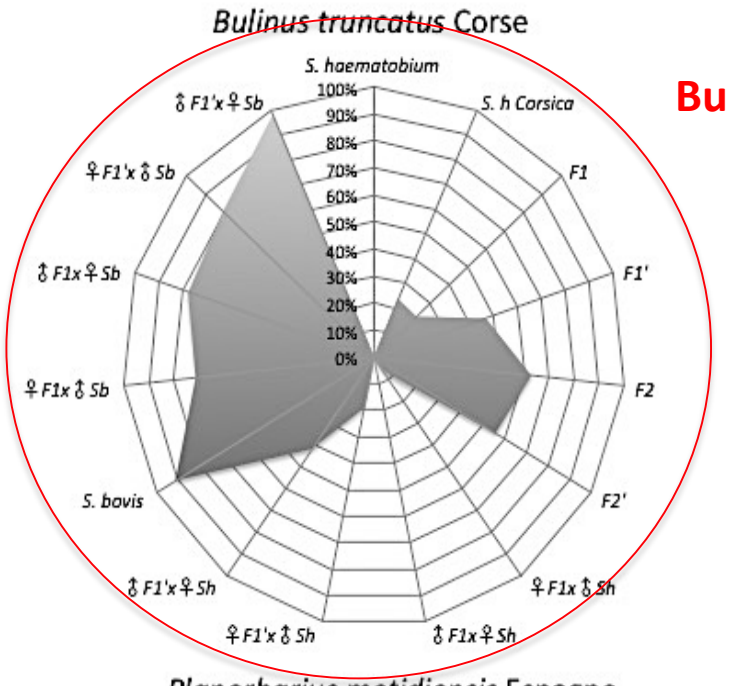
***B.t**: Bulinus truncatus (S. haematobium & S. bovis) ; **P.m**: Planorbarius metidjensis (S. bovis **only**)*

Parasites-molluscs interaction

Bulinus from Cameroon

32%



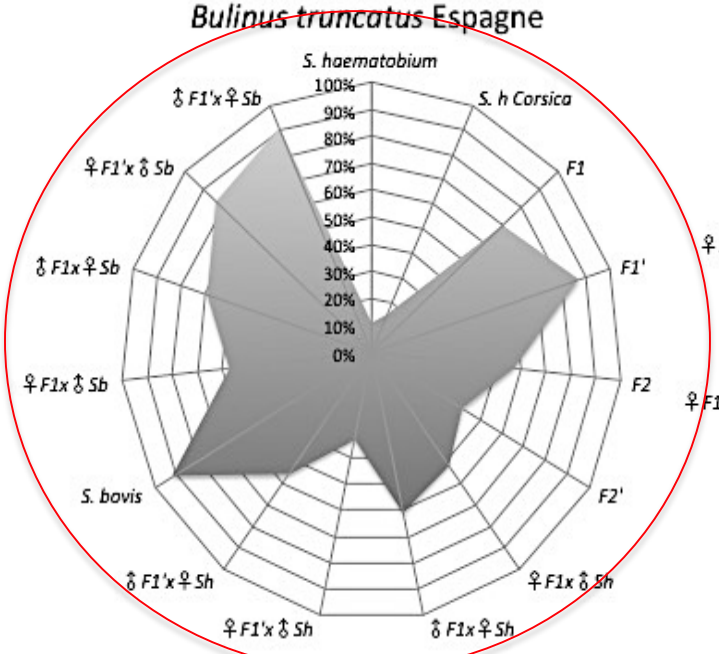


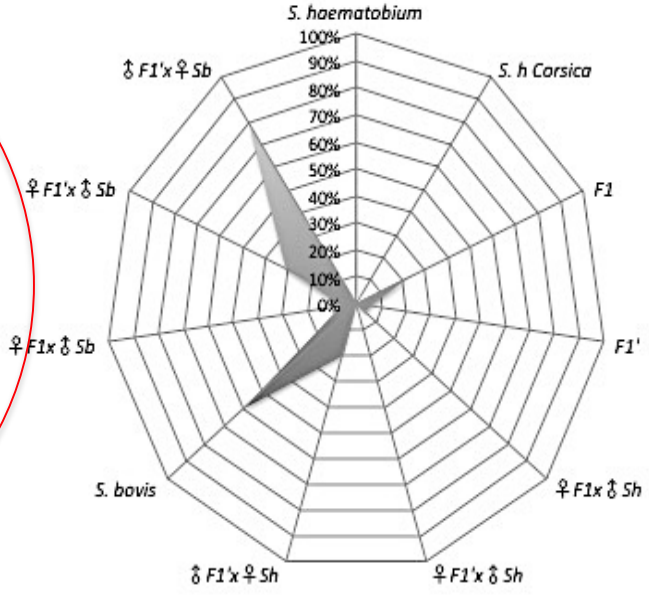
Bulinus from Corsica

45%

Bulinus from Spain

48%

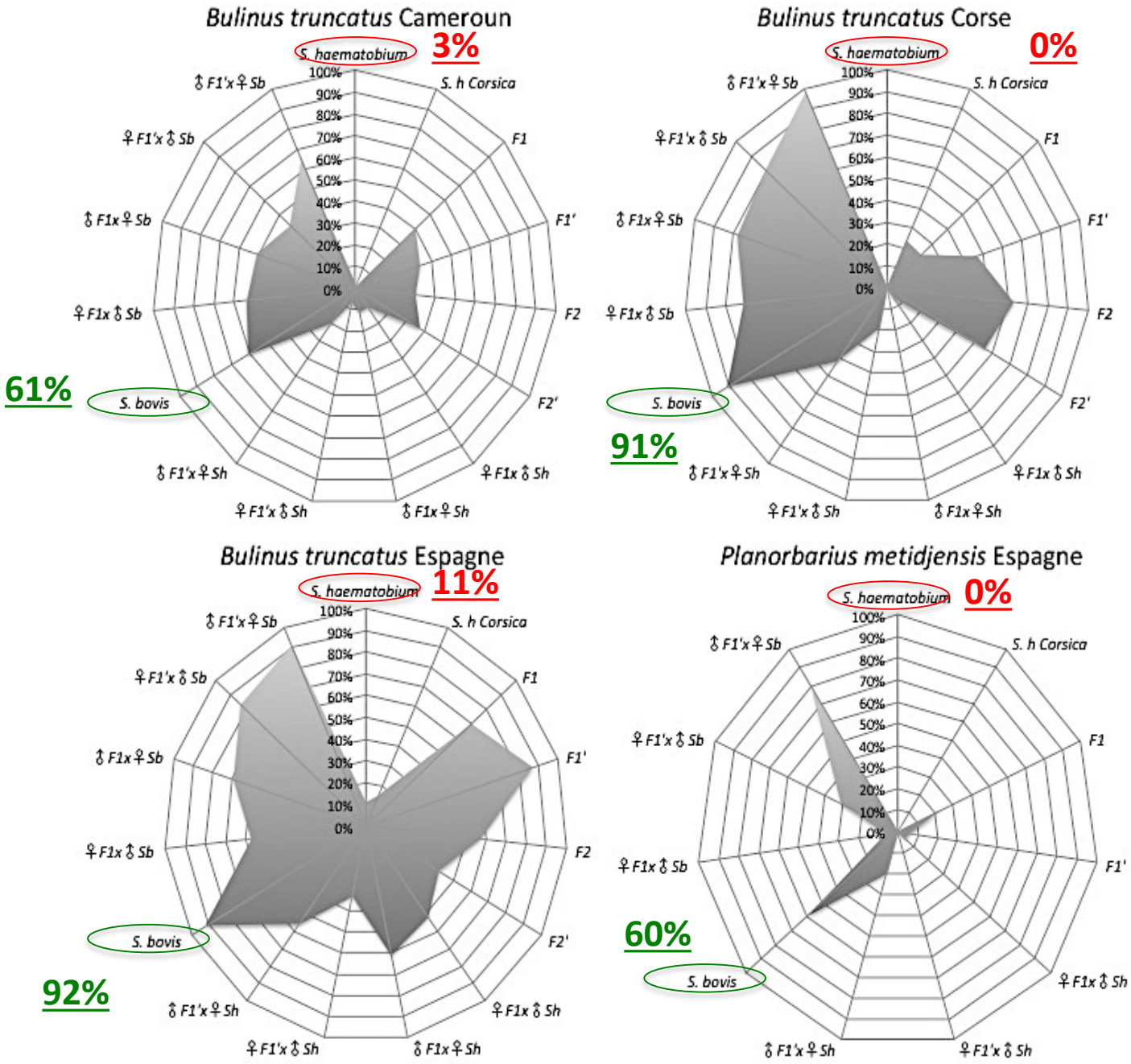




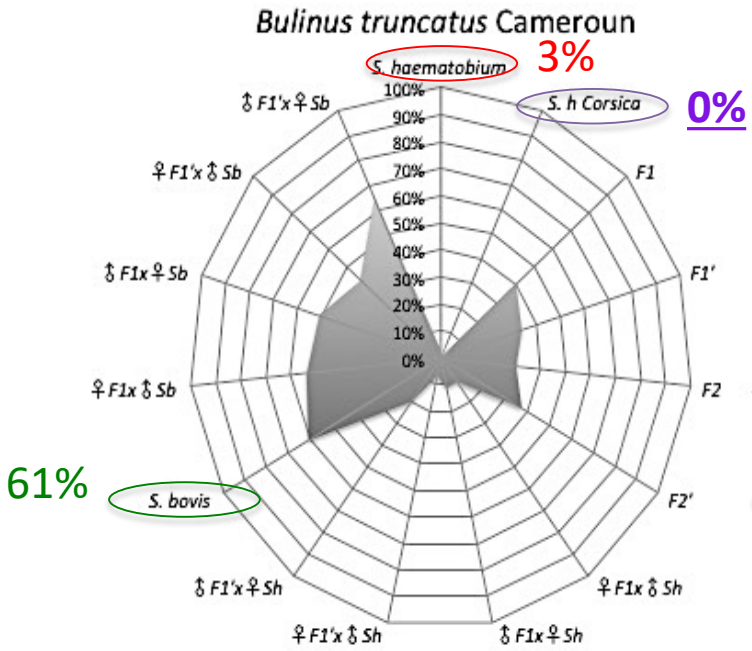
20%

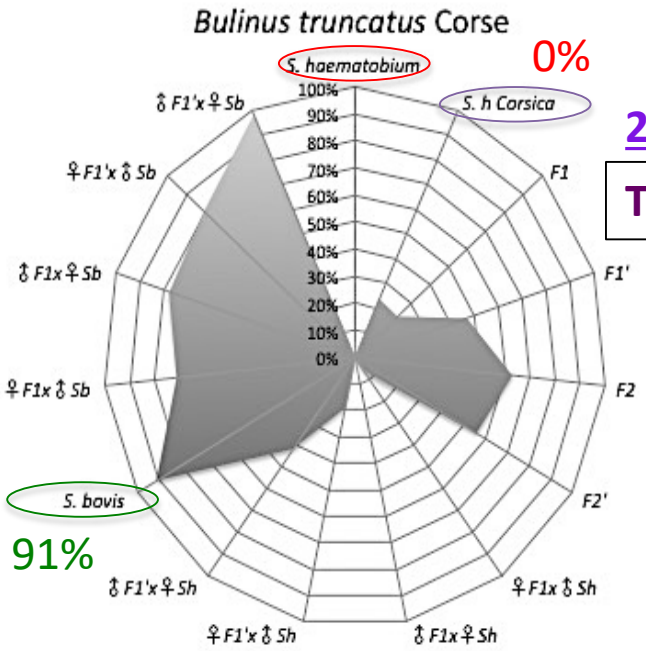
Planorbarius from Spain

Parasites-molluscs prevalence and host spectrum

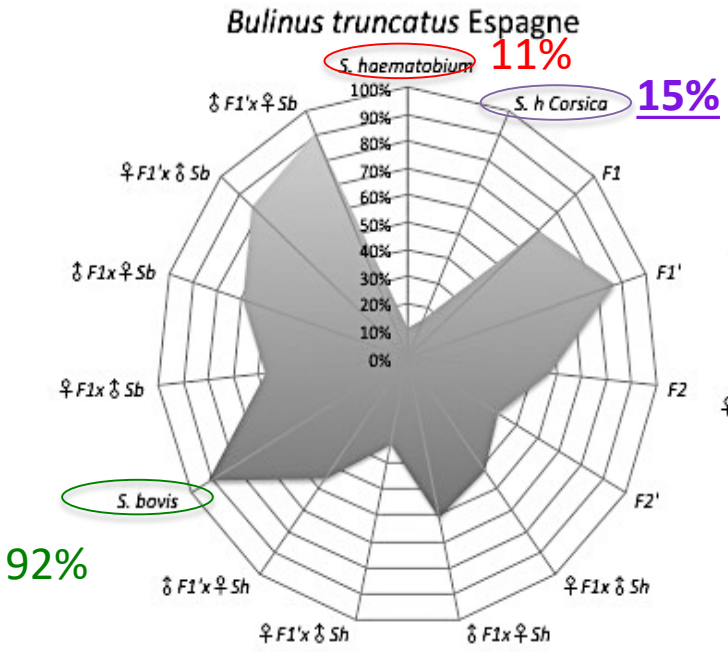


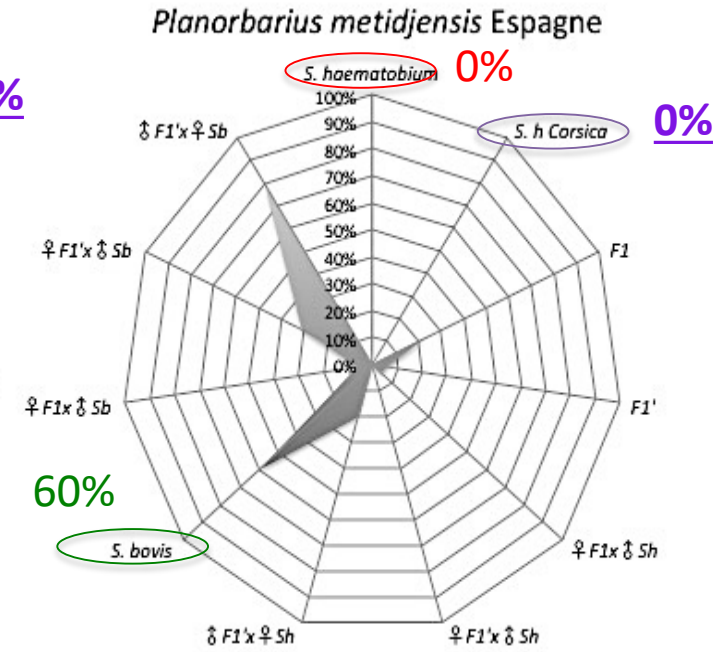
Parasites-molluscs prevalence and host spectrum



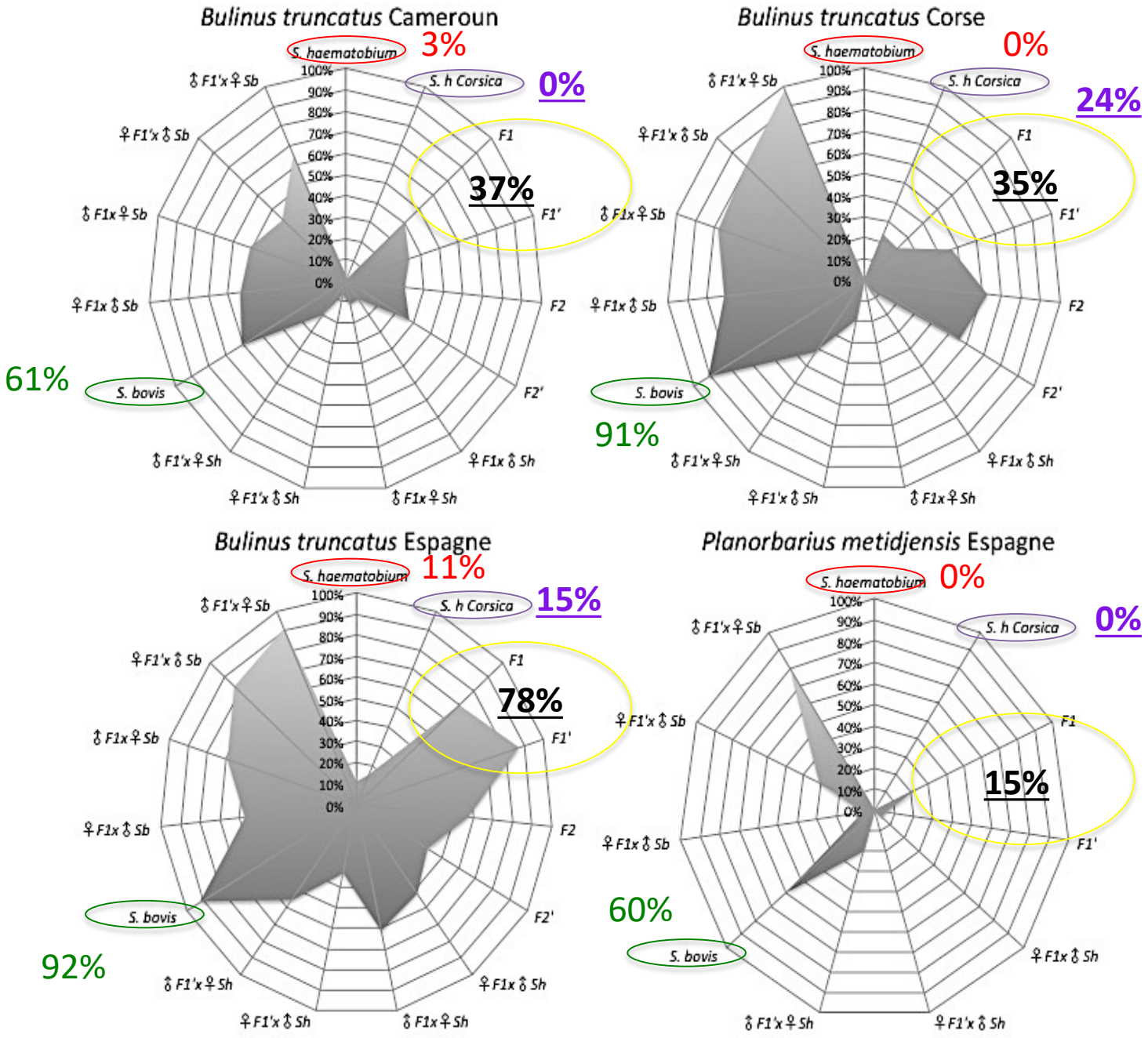


The exception

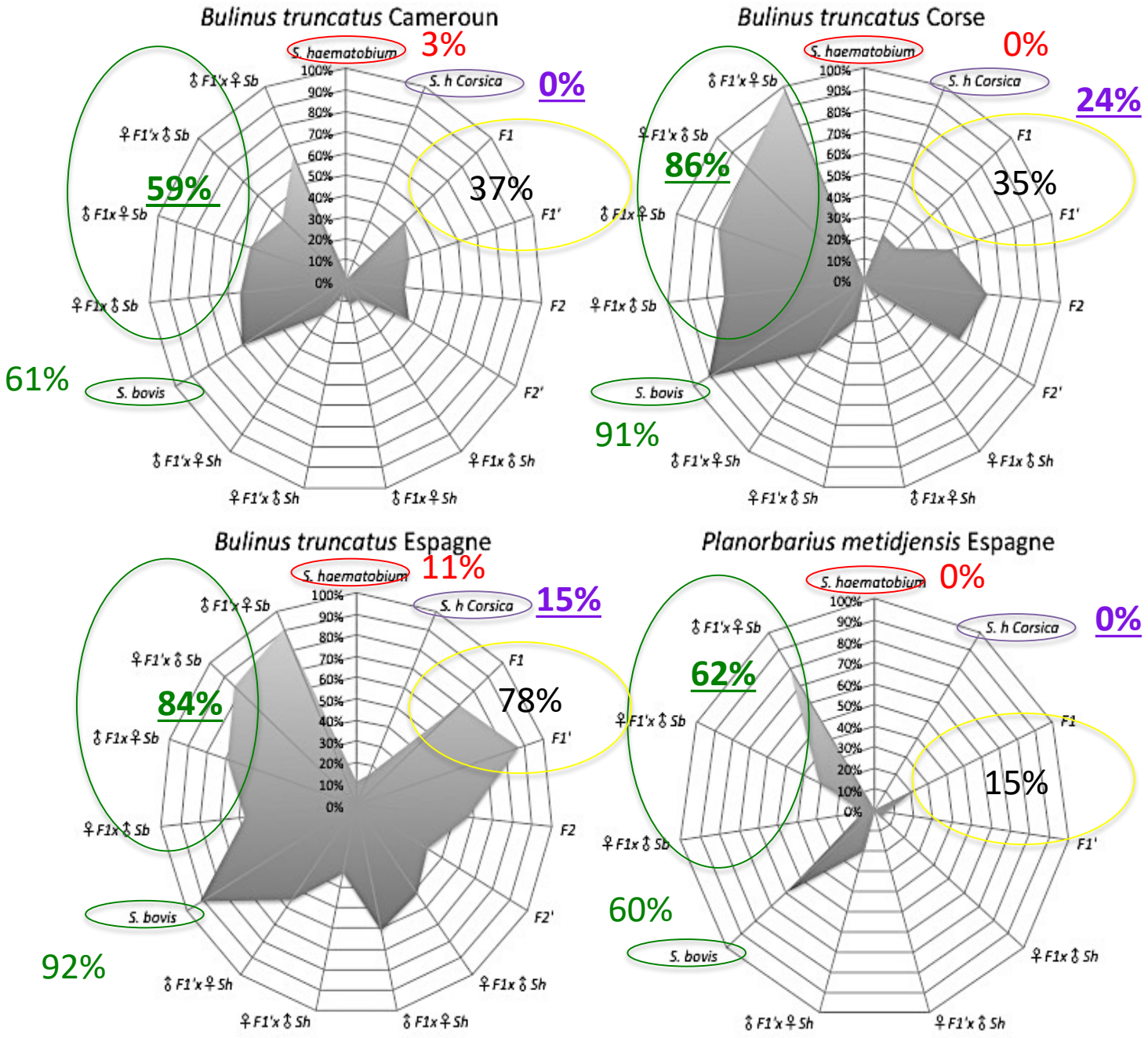




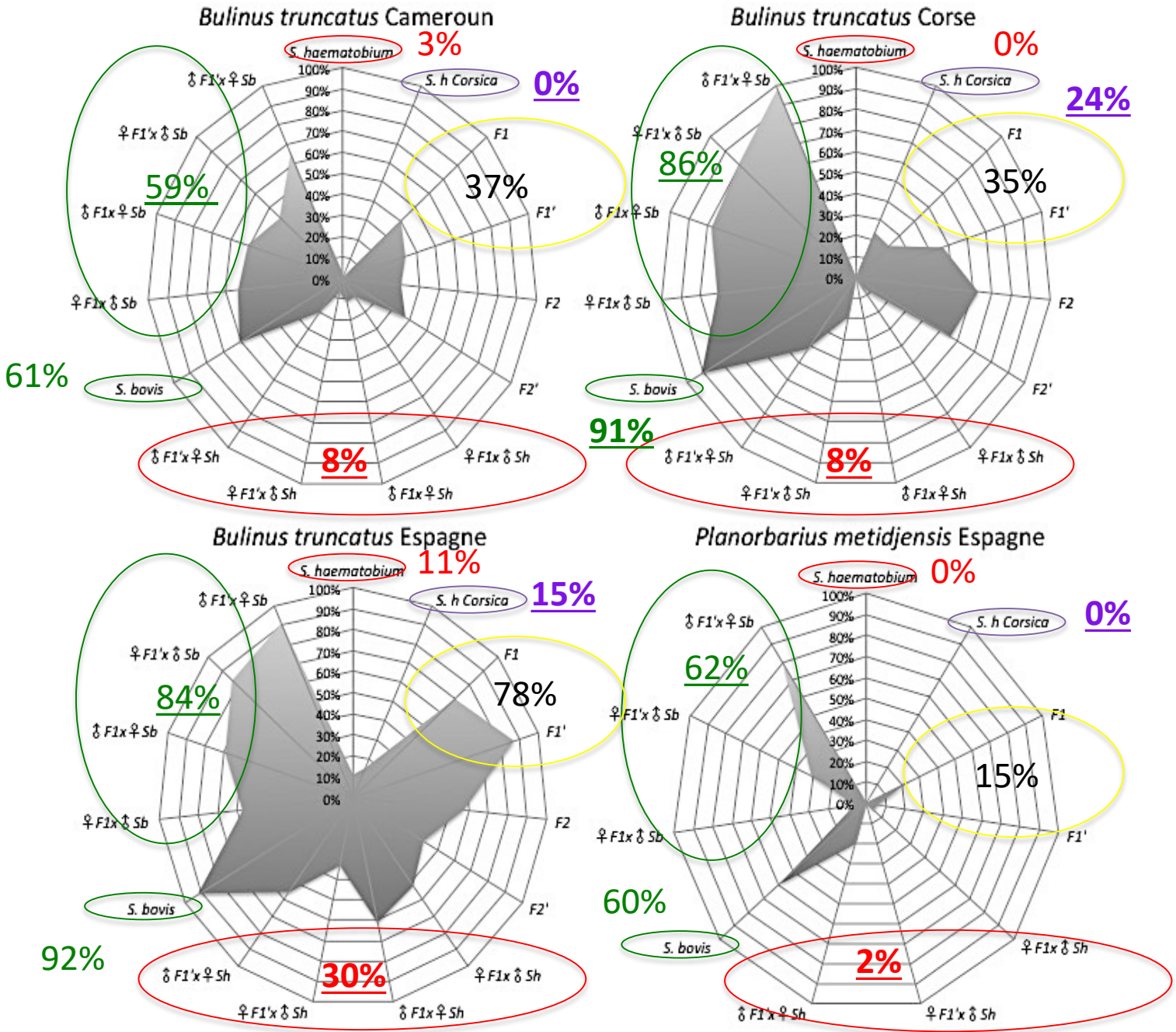
Parasites-molluscs prevalence and host spectrum



Parasites-molluscs prevalence and host spectrum



Parasites-molluscs prevalence and host spectrum



Conclusions:

Schistosomiasis is no longer restricted to tropical and sub-tropical areas

<u>The emergence in Corsica is not a coincidence</u>

✓ **European snails compatible with African parasites (Corsica, Spain at risk)**

In which hosts are the parasites maintained over the years ??

Mouflon

Hedgehogs

Local Humans

Molluscs that overwinter

✓ **Importance to consider hosts/vectors current and future distribution (<u>Africa</u>, <u>Europe</u>)**

Trends in Parasitology
Volume 33, Issue 8, August 2017, Pages 600-609
CellPress

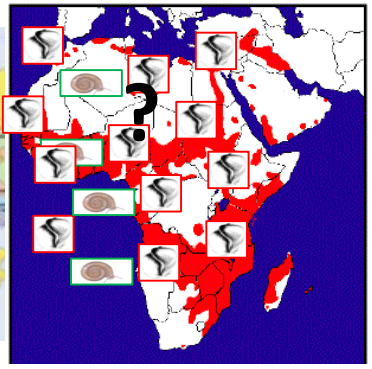
Opinion

Emerging Schistosomiasis in Europe: A Need to Quantify the Risks

Julien Kincaid-Smith [1,†], Olivier Rey [1,†], Eve Toulza [1], Antoine Berry [2], Jérôme Boissier [1]

Monitoring & Surveillance

Kincaid-Smith et al. 2017



✓ **Host-parasite compatibility : anticipate risks of emergence, in and out of endemic areas**

Conclusions: what role for hybridization?

The hybrids isolated in Corsica are similar *to S. haematobium* (phenotypes and genome)
but compatibility with the Corsican *Bulinus* (fortuitous or genes of *S. bovis ?*)

Experimental hybridization *S. haematobium* x *S. bovis* = <u>hybrid vigour</u>

Regarding the vertebrate :

- ✓ Sexual choices : not a barrier (post-zygotique ?)
- ✓ Higher infectivity
- ✓ Females more prolific
- ✓ Extreme phenotypes (eggs)
  - ✓ Increased morbidity and mortality

↗ **Occurrence of hybridization**

↘ **Transmission**

Regarding the molluscs :

- ✓ Broader hosts spectrum (both parental snails)
- ✓ Increased prevalence

↗ **Transmission & Invasive capacities**

<u>Complex parental effects: phenotypes depend on direction of the cross & introgression levels</u>

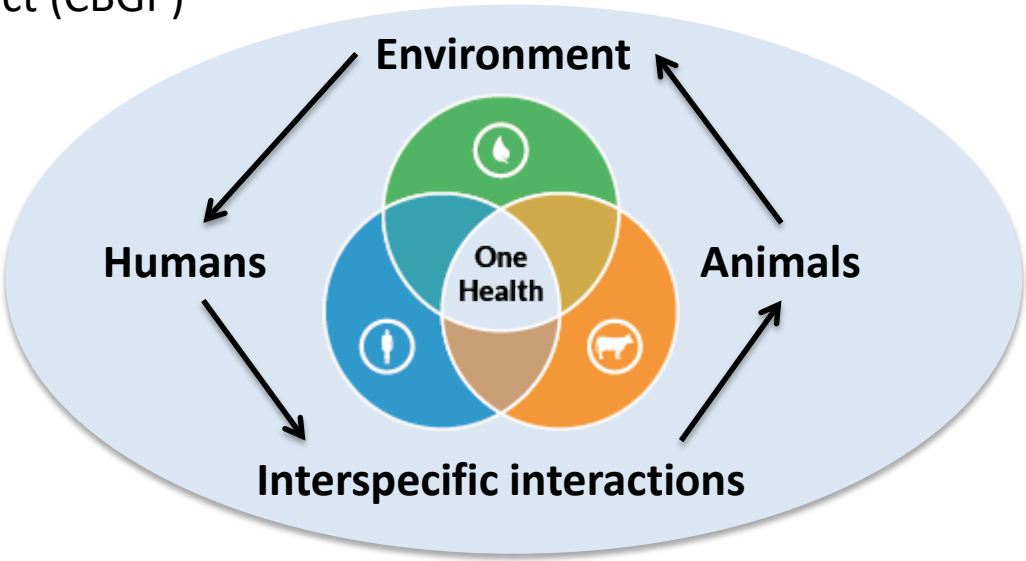
Many other life history traits to explore

- ✓ More field data required (distribution & hybridization dynamics)
- ✓ Assess if hybridization is affecting transmission and morbidity
- ✓ Diagnostics and treatment with PZQ (unique drug available)
- ✓ Zoonotic transmission & animal reservoir

Perspectives for a sustainable disease management and control

Projects that aim for an integrative view of the pathosystem

- Postdoctoral project (CBGP)
- IRD proposal



Postdoctoral project

Implication des rongeurs dans la transmission et l'hybridation des schistosomes au Sénégal : des marqueurs du risque sanitaire ?

CBGP : J. Kincaid-Smith L. Granjon, M. Kane, Y. Niang, C. Tatard, P. Gauthier, C. Brouat

IHPE-UPVD – J. Boissier

JEAI ESBILH-SEN – B. Senghor

Institut de Recherche pour le Développement FRANCE

CBGP Centre de Biologie pour la Gestion des Populations

Postdoctoral Project CBGP

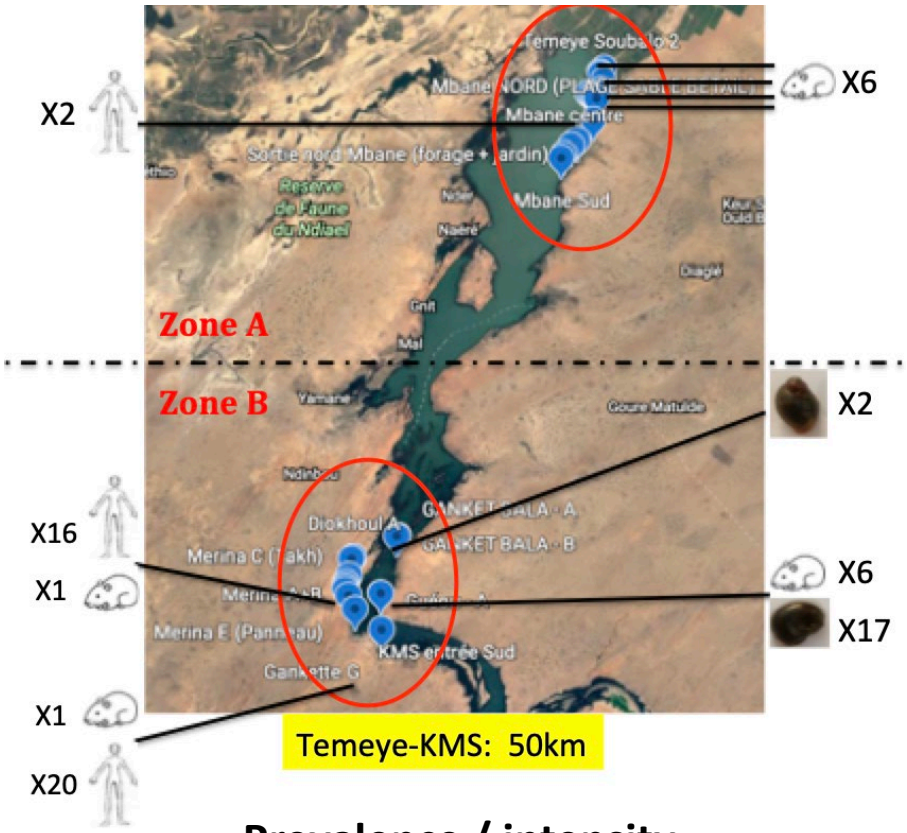
From populations (field)

Epidemiology &
Spatio-temporal dynamics

To molecules

Molecular characterization of
***S. mansoni* populations**

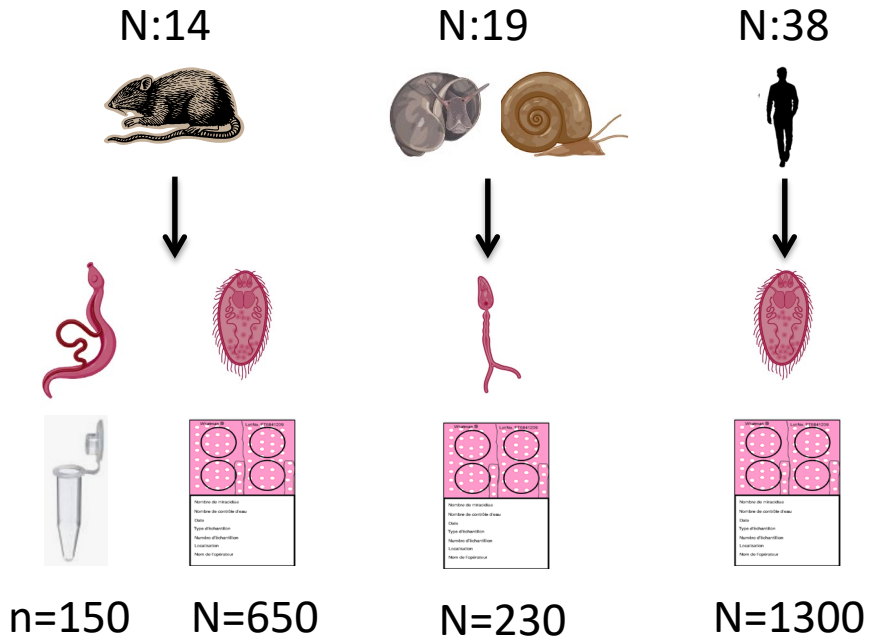
Lac de Guiers - Senegal



- Prevalence / intensity

Sh, S.h x S.b hybrids and *S.m* in humans

Only *S.m* in rodents



- **Genetics:** (Cox-ITS) – **Species ID**
- **Genomics** (RADseq) - **Structure**

- **Experimental procedure**

(RAD optimisation & amplification bias)

Collab IHPE -**JEAI ESBILH-SEN**

IRD proposal

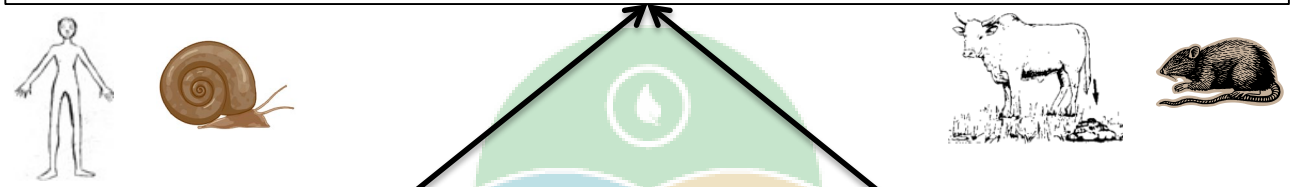
One Health approach of zoonotic schistosomiasis in Senegal : a study from populations to molecules

WHO roadmap for schistosomiasis elimination as a public health problem (2030)

Objectives: Assess the role of schistosomiasis zoonotic transmission in the persistence of the health risk and its consequences in terms of control.

Articulation of the proposal

WP1: Determine at a local scale the spatio-temporal dynamics of infection and parasitic gene flows at the human-animal-environment interface

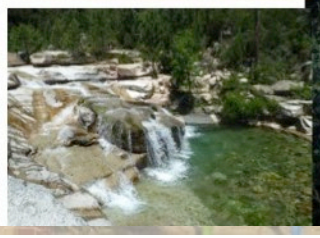


WP3: Assess relationships between mass treatment of human pops, host changes and the risks of reduced parasite susceptibility to treatment

WP2: Identify the eco-evolutionary factors linked to schistosomiasis zoonotic transmission to anticipate and prevent health risks locally

IMPACTS: reduce the risks & provide decision-makers / local actors information for sustainable control and elimination of schistosomiasis

kincaid-smith.julien@ird.fr



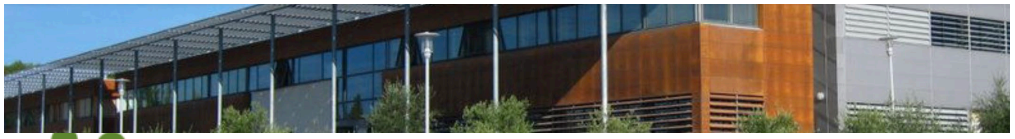
J. Boissier E. Toulza

IHPE
Intéractions-Hôtes-Pathogènes-Environnements

Thanks for your attention !

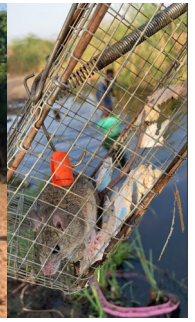
C. Brouat

L. Granjon



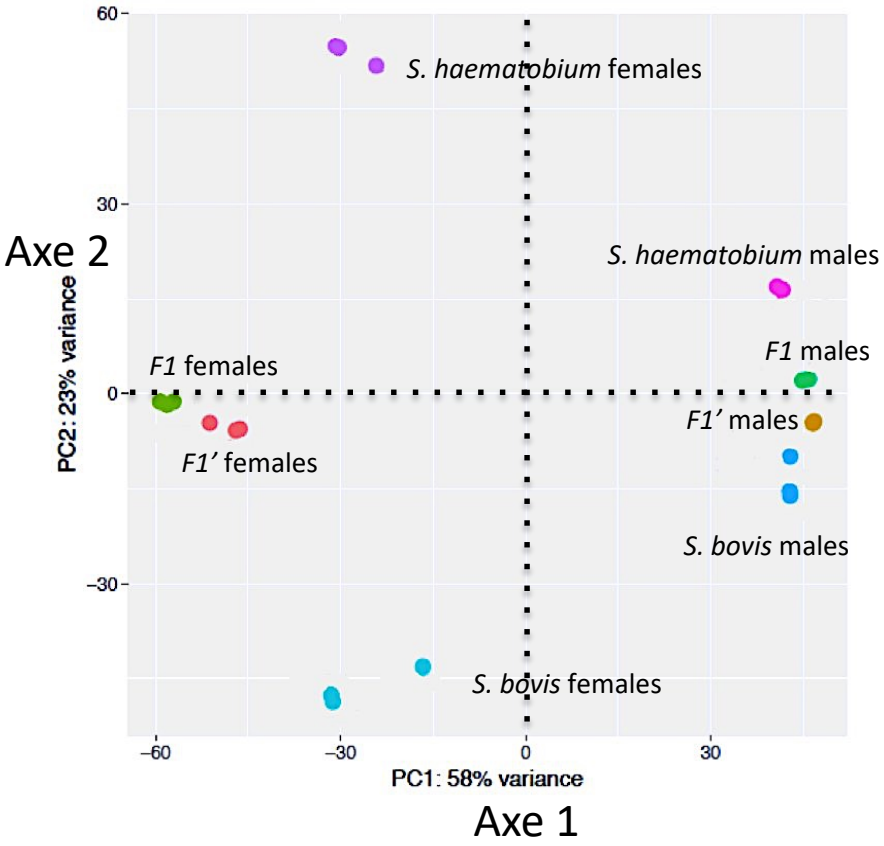
CBGP
Centre de Biologie pour la Gestion des Populations

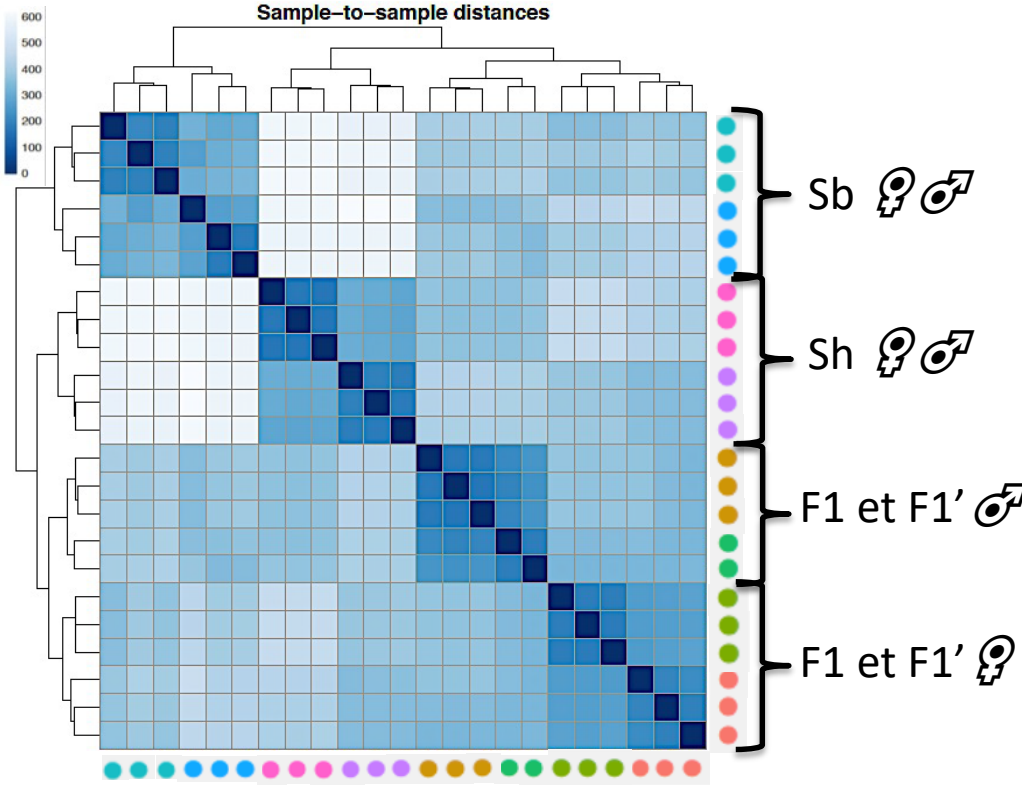
Institut de Recherche pour le Développement
FRANCE



Transcriptomic analysis between F1 and parents

- ***De novo*** **assembly (common ref to all comparisons)**
- **Differential gene expression (perspectives : allele specific expression)**



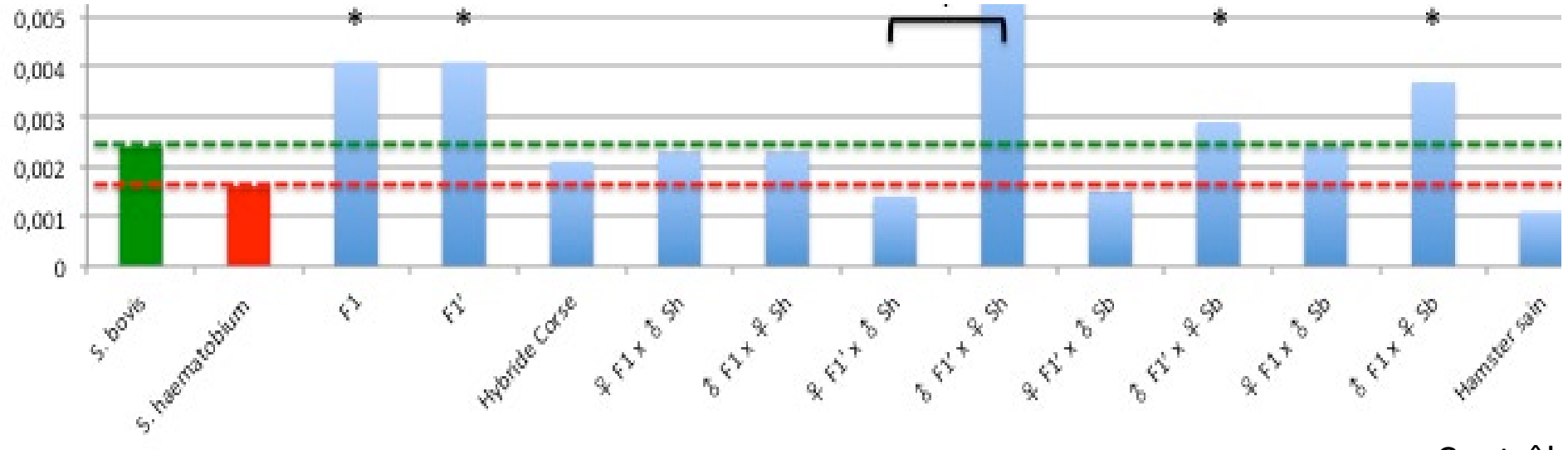


F1 intermediate = half of each parent's genes

Significant reprogramming of transcriptome (paper upcoming) « Genomic shock » (McClintock 1984)

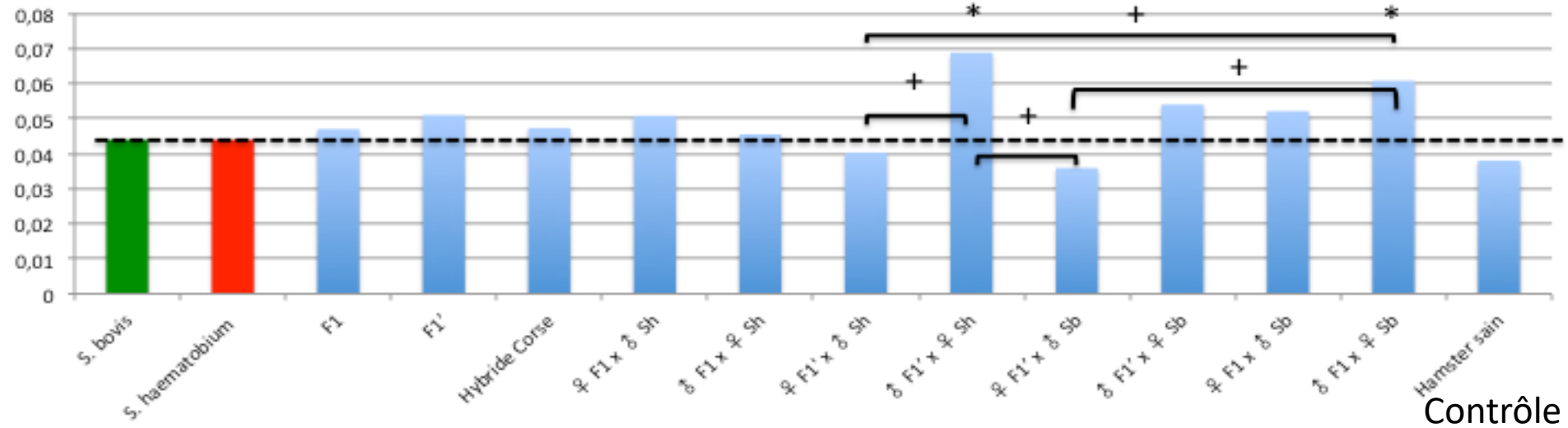
Pathologie induite

Mesures de splénomégalie



Contrôle

Mesures de l'hépatomégalie



Contrôle

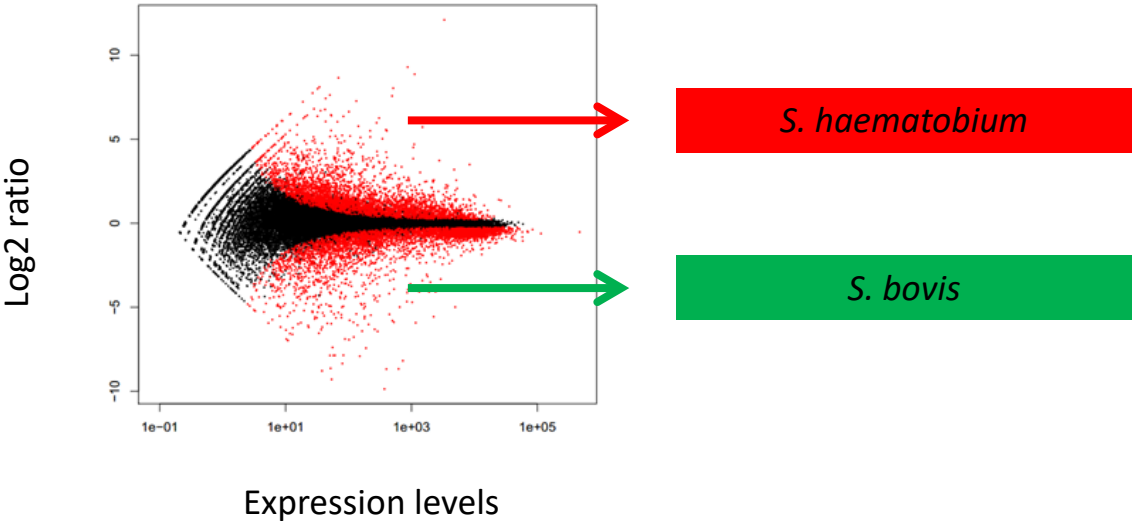
(*) Différences significatives avec les hamsters sains, (+) Différences significatives entre hybrides

Table 8: Host Induced Mortality. The lines are classified according to the host-induced mortality. Hamsters with an early perfusion date are animals for which we were able to measure all life traits in contrast to dead animals.

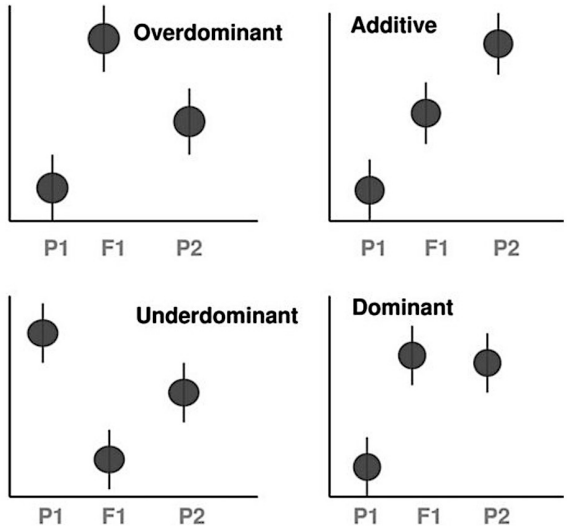
Lines	Number of infested animals	Number of dead hamsters	Hamsters perfused early (Nb. days after infestation)
♀ F1' x ♂ Sb	6	4	2 (63-67 days)
♂ F1' x ♀ Sh	6	3	1 (85 days)
♂ F1 x ♀ Sb	6	2	1 (82 days)
F1	7	2	1 (67 days)
F1'	7	2	1 (81 days)
♂ F1' x ♀ Sb	6	1	1 (80 days)
♀ F1 x ♂ Sh	6	1	0
♀ F1' x ♂ Sh	6	0	1 (85 days)

Mécanismes de régulation de l'expression génique

- Analyse du différentiel d'expression

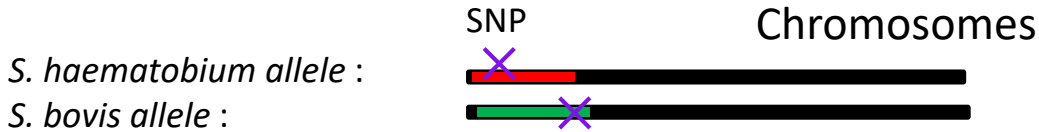


Modes d'actions des gènes

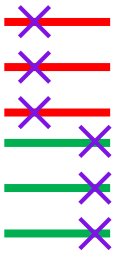


Autres mécanismes:

- **Expression génique allèle spécifique et conflit allélique**
- Gènes à expression espèce-spécifique: ***S. haematobium*** ou ***S. bovis***

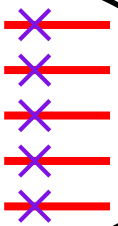


Expression biallélic 50/50



or

Expression monoallélique



Expression de l'allèle *S. haematobium*

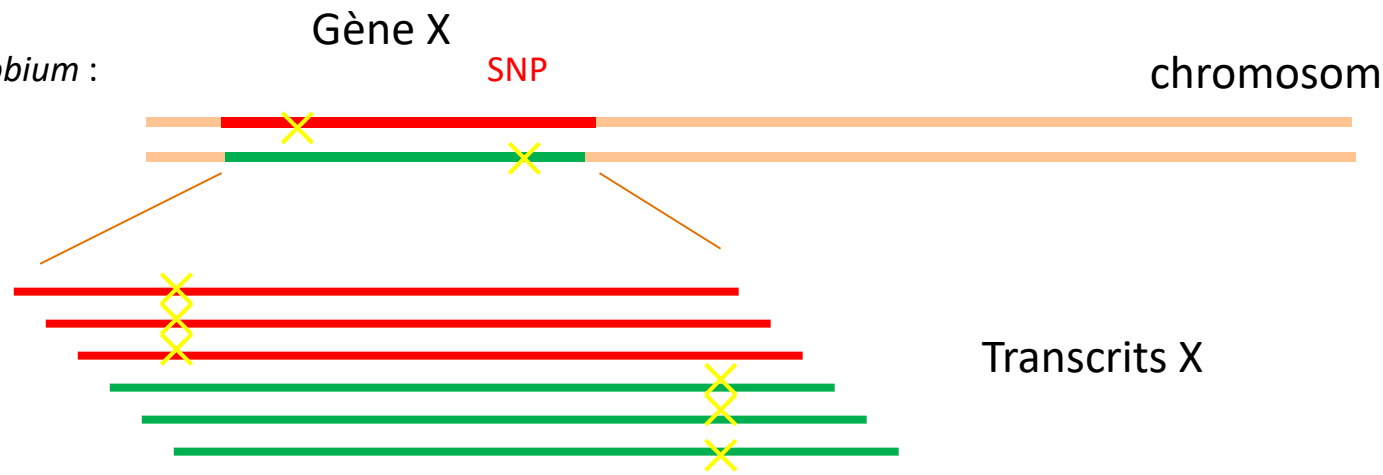
Gène de virulence ?

Analyse transcriptomique

Quel génome impose son allèle dans la descendance ?

allèle *S. haematobium* :

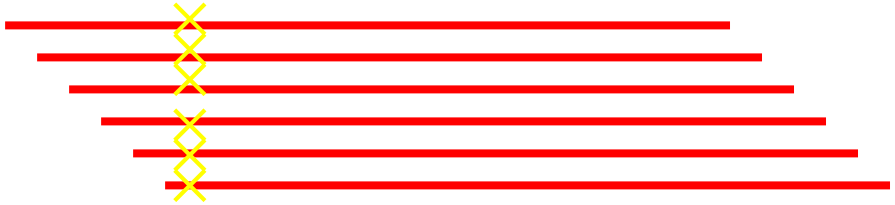
allèle *S. bovis* :



Expression Biallélique 50/50

Ou

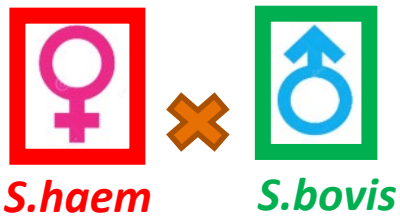
Expression Monoallèlique

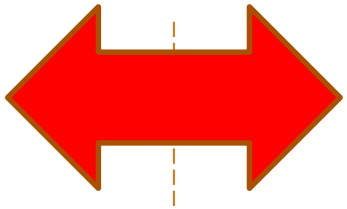


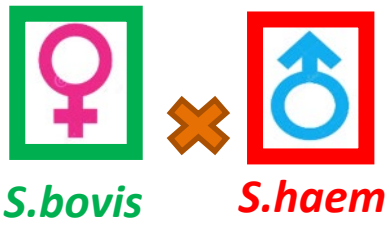
Effet de l'espèce *S. haematobium*

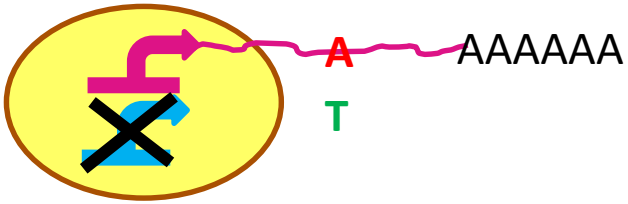
Gène de virulence?

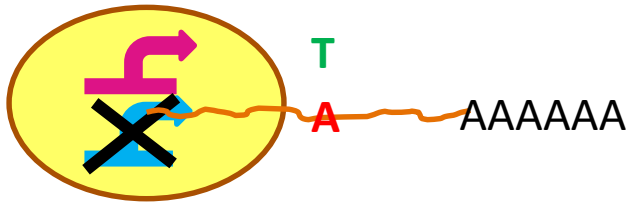
Comparaison entre croisements réciproques





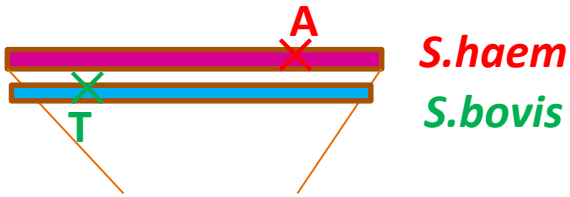


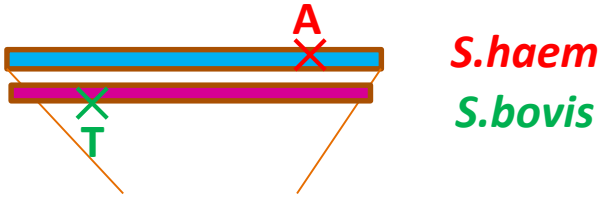




Expression de l'allèle ***S.haem*** / Maternel

Expression de l'allèle ***S.haem*** / Paternel





Effet de l'espèce *S. haematobium*