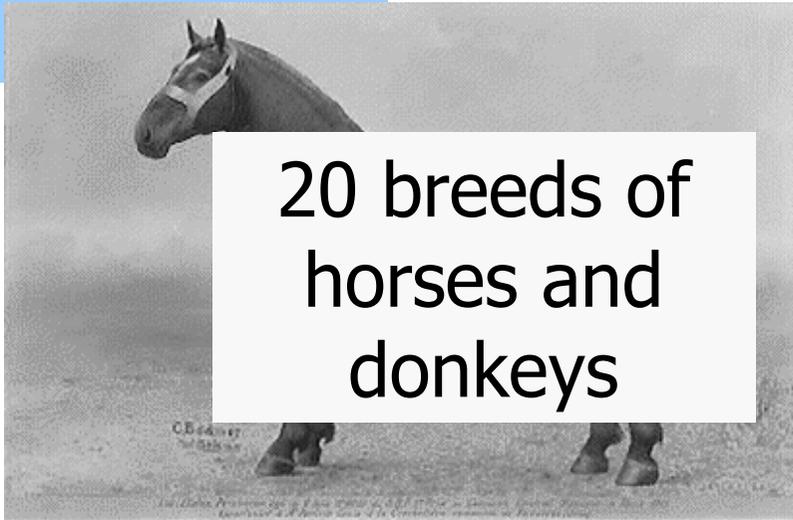


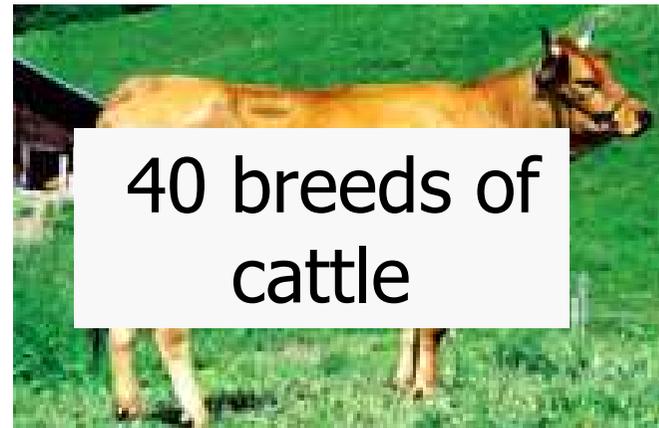
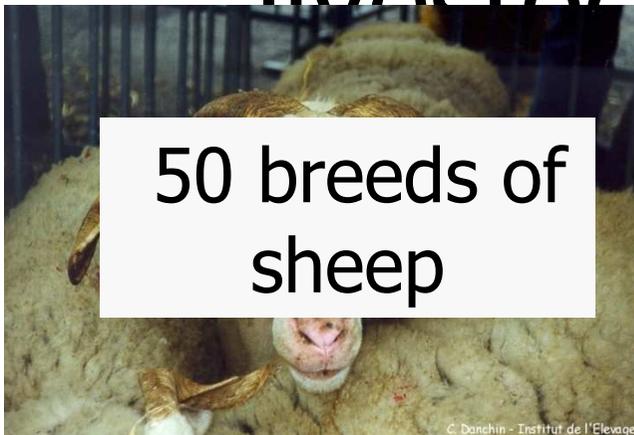
The French Cryobank of biological material as safe ex-situ conservation

C. Danchin-Burge
INRA - UMR GABI / Institut de l'Elevage
France





France is the land of a rich livestock



Etc.

... in theory !

=> Impoverishment of the breeds **biodiversity**

2 breeds represent **99%** of the goat population

3 breeds represent **75%** of the cattle population

... etc.

=> Impoverishment of the **within breed** genetic variability

Example : French Holstein cattle breed

2 millions cows => genetic diversity equivalent of **43 animals** !

With *in-situ* programs, **cryoconservation** is a complementary way to preserve farm animal diversity

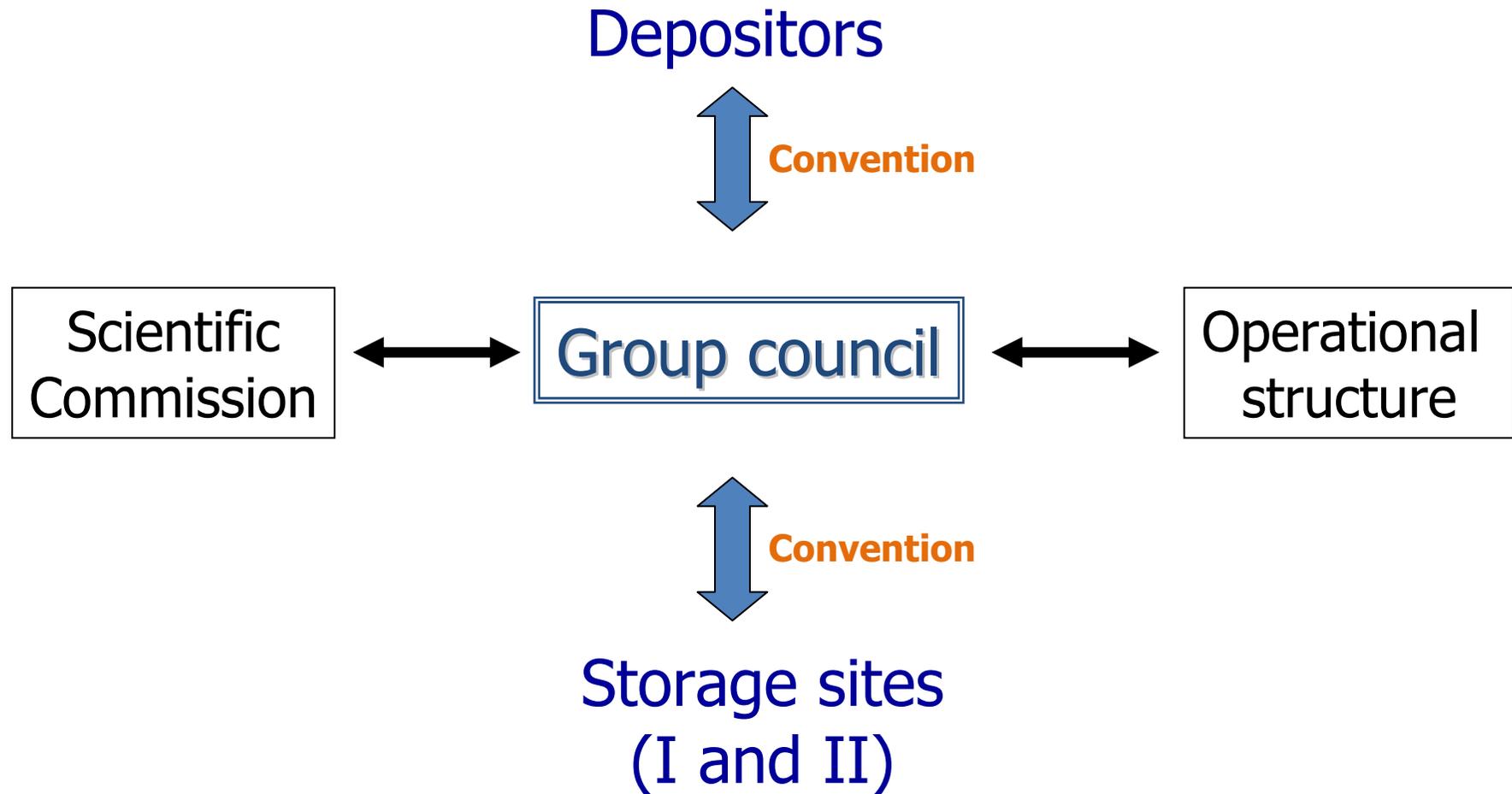
The French national Cryobank

12 partners

CRYOBANQUE NATIONALE



Organization of the French Cryobank



What can you find in the collection ?

- **Goal = 9 livestock species**
- **Type of Biological Material:**
 - ✓ Semen (++++)
 - ✓ Embryos (++)
 - ✓ Cells (+)
 - ✓ DNA (0)
 - ✓ Serum (0)
- **Improved Animal Populations (breeds, strains...)**
- **Categorized in three types of genetic variability**



Rules of Management

- Depositor / Cryobank convention
- Group Council decides on what biological material can be put in or taken out of the Cryobank
- Depositor keeps its ownership but leaves the collection management to the Group Council
- To use a sample, the depositor's agreement is mandatory

Sampling strategies

Rare breeds



Type I

Exceptional animals
not used as breeders



Type II

Improved
breeds

Selected
breeding stock



Type III

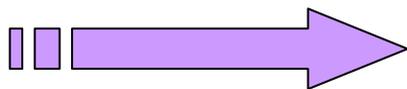
"Type I" material

- **Aim :**

To reconstruct a breed (extinction or loss of a substantial number of animals)

- **Constraints :**

- Technical : success rate of AI / ET
- Effective size: minimum number of animals to reconstruct



25 unrelated males

Example **cattle**: 800 doses / male

Or 600 embryos

"Type II" material

Animal **exceptional** by one or several traits but not used as a breeder

- Exceptional by a production / functional trait
- Exceptional by its pedigree
- Exceptional by its genotype

"Type III" material

- A breed "snapshot"
 - Sampled on its average generation interval
 - Allows a "live" measure of a breed genetic improvement
 - Allows a "live" measure of correlated traits that are selected (*against or positively*) with an improved trait

What animals are sampled, by type ?

Type I	—	Animals as diverse as possible <i>Goal: preserve the maximum genetic variability possible of a breed</i>
Type II	—	Extreme and/or original genotypes <i>Goal: preserve rare genotypes within/between breeds</i>
Type III	—	Representative of the population <i>Goal: preserve an accurate “picture” of a breed for a given period of time</i>

What are the potential uses of the material?

Type I



- 1. Help for in situ management**
2. Restoration of an extinct population

Type II



- 1. Change of genetic goals**
2. Research

Type III



1. “In situ” analysis of the genetic improvement of a population
2. Change of genetic goals
3. Research

Focus: sampling procedure in dairy cattle

TYPE II: bulls semen

- progeny tested
- non commercialized

Yearly sampling / breed

(between 10 to 650 males tested/year/breed)

200 doses / male, on 2 sites

TYPE III: bulls semen

- progeny tested
- commercialized

Yearly sampling / breed

100 doses / male, on 2 sites

TYPE II.1

Original / Extreme EBVs

Based on **Estimated Breeding Value**

Reproduction and functional traits

Longevity
Fertility
Cells counts
Morphologic Synthesis

EBV > + 2 s

Production

Milk, Protein, Fat

EBV > + 3 s or < - 3 s

TYPE II.2 : Rare pedigrees

For a given year t :

1 son of each bull sire without commercialized son

A male stored in the Cryobank for a given year t for a rare pedigree is eliminated if at least one of his half-sib is commercialized the following years

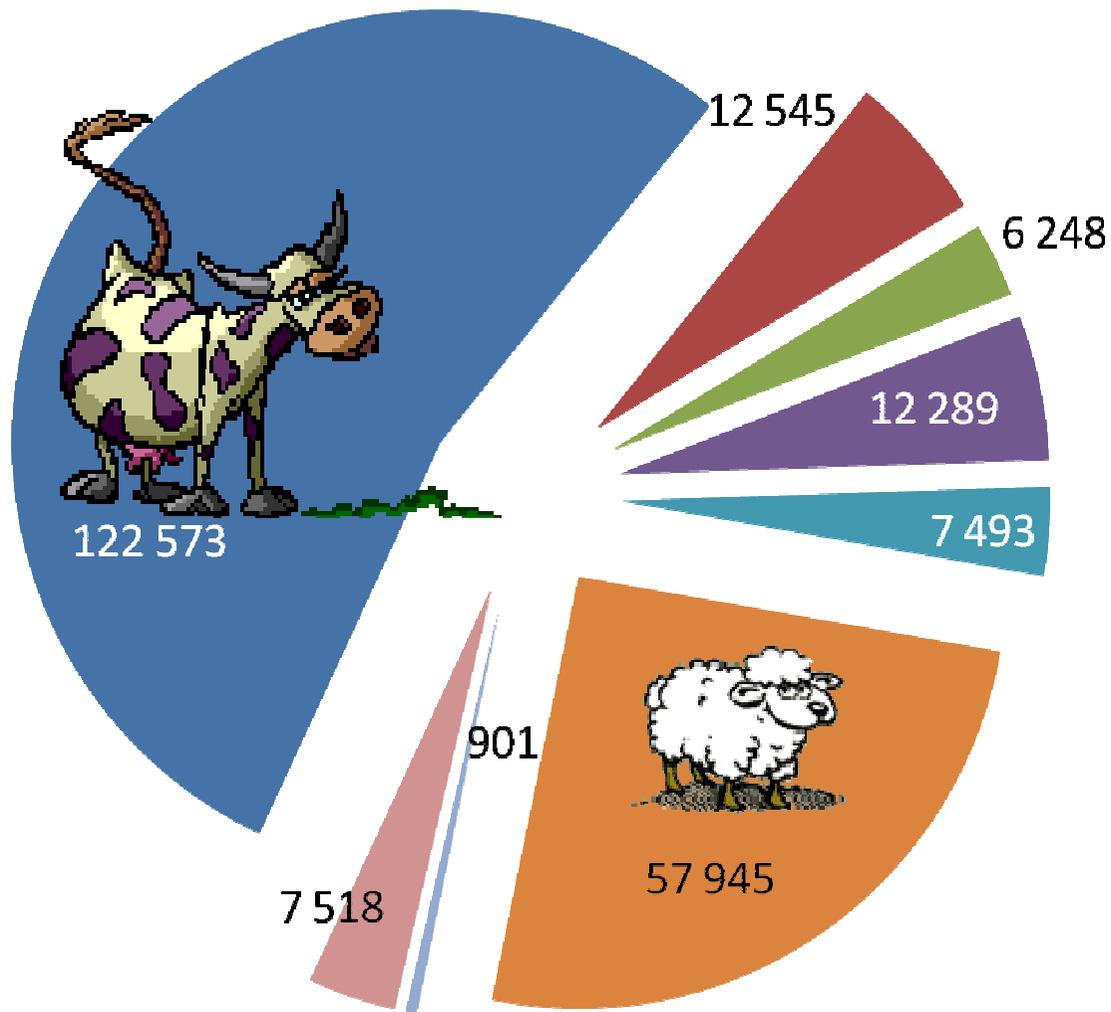
Nowadays, it is more about saving paternal line than saving original strains... it is already too late !

The French cryocollection

www.cryobanque.org

Collection - 2010 update

Number of doses



Number of doses:

≈ 228 000

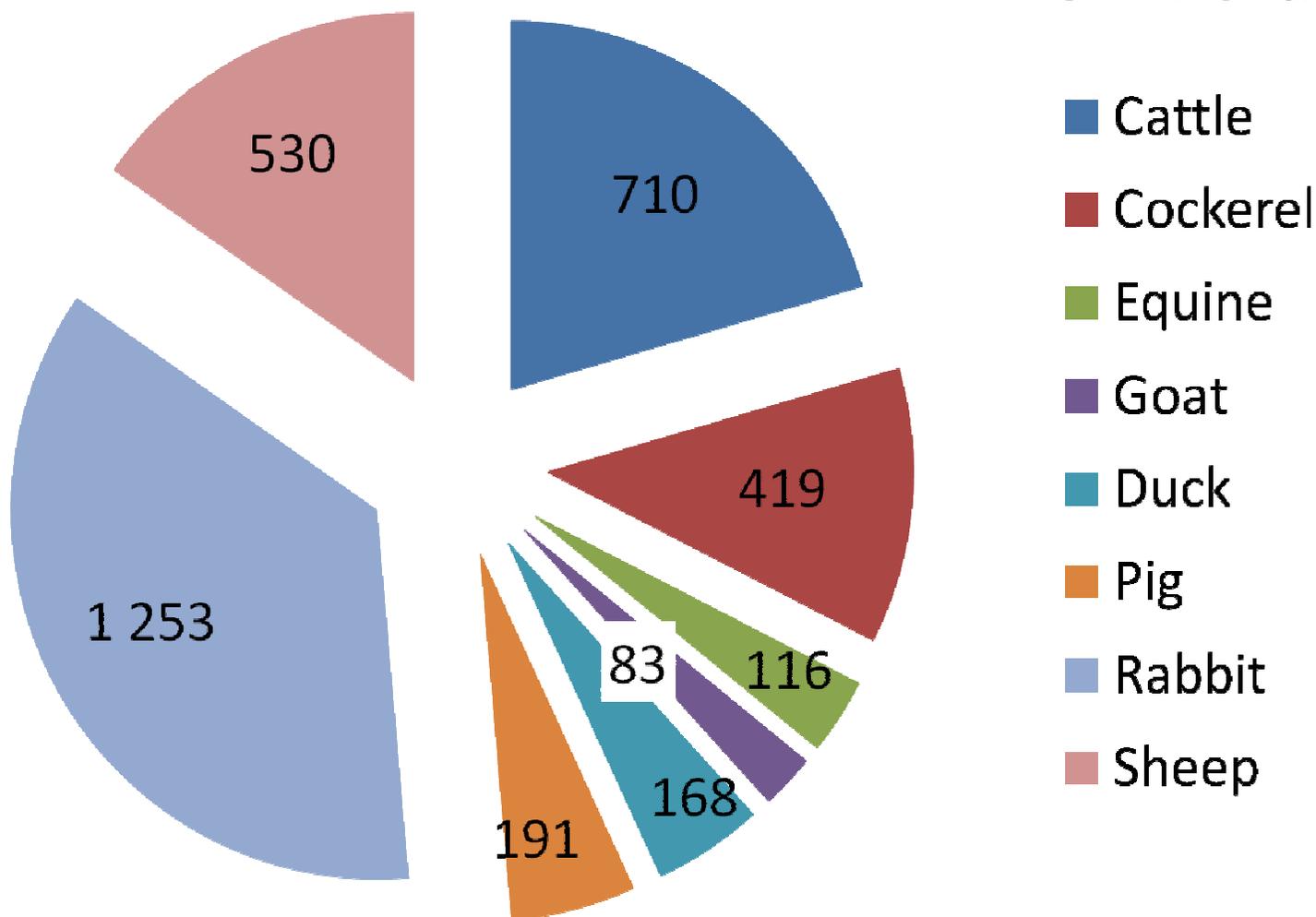
- Cattle
- Cockerel
- Goat
- Rabbit
- Pig
- Sheep
- Duck
- Equine

Collection - 2010 update

Number of donors

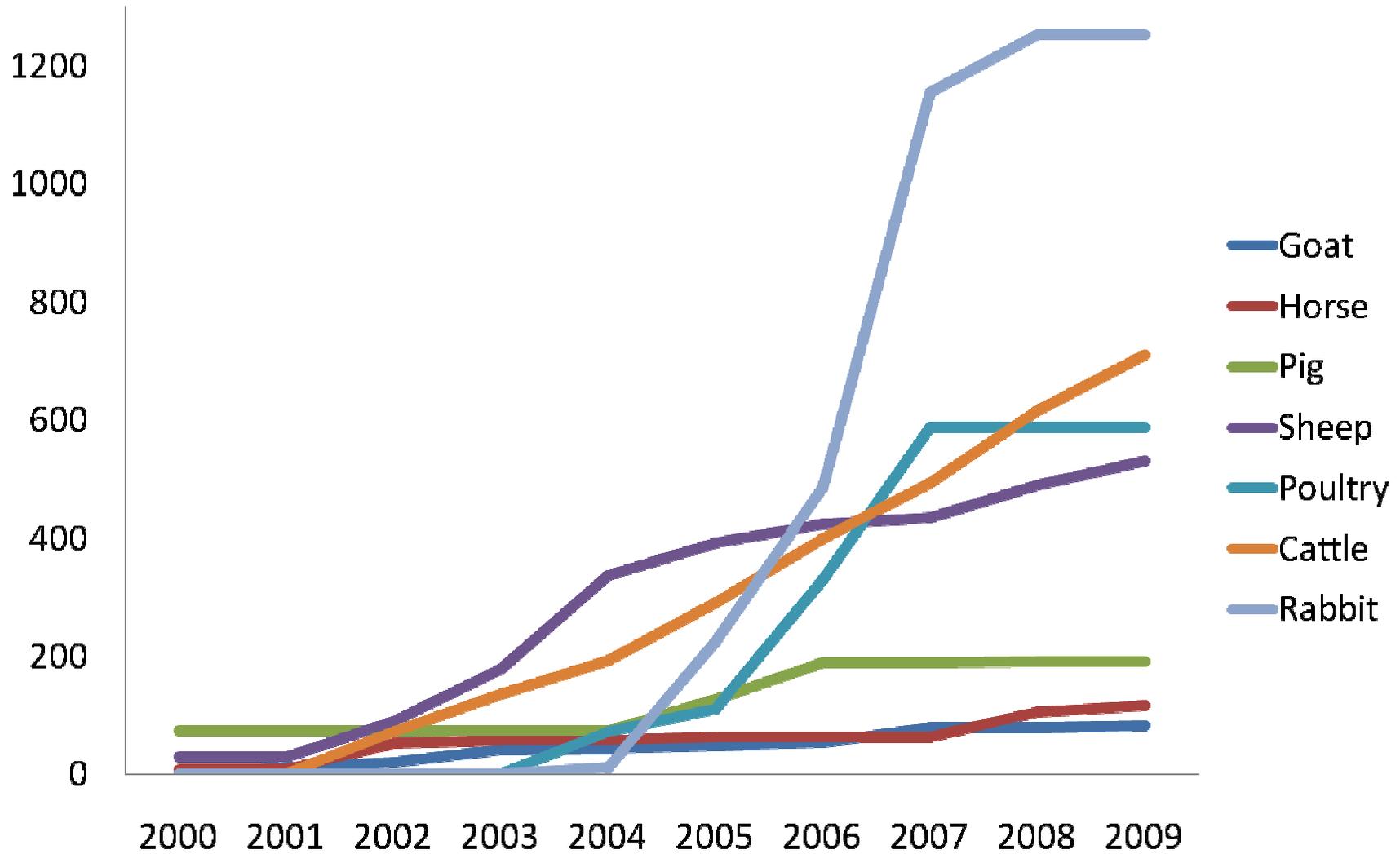
CRYOBANQUE NATIONALE

3 470 donors



Evolution of the number of donors per year

CRYOBANQUE NATIONALE



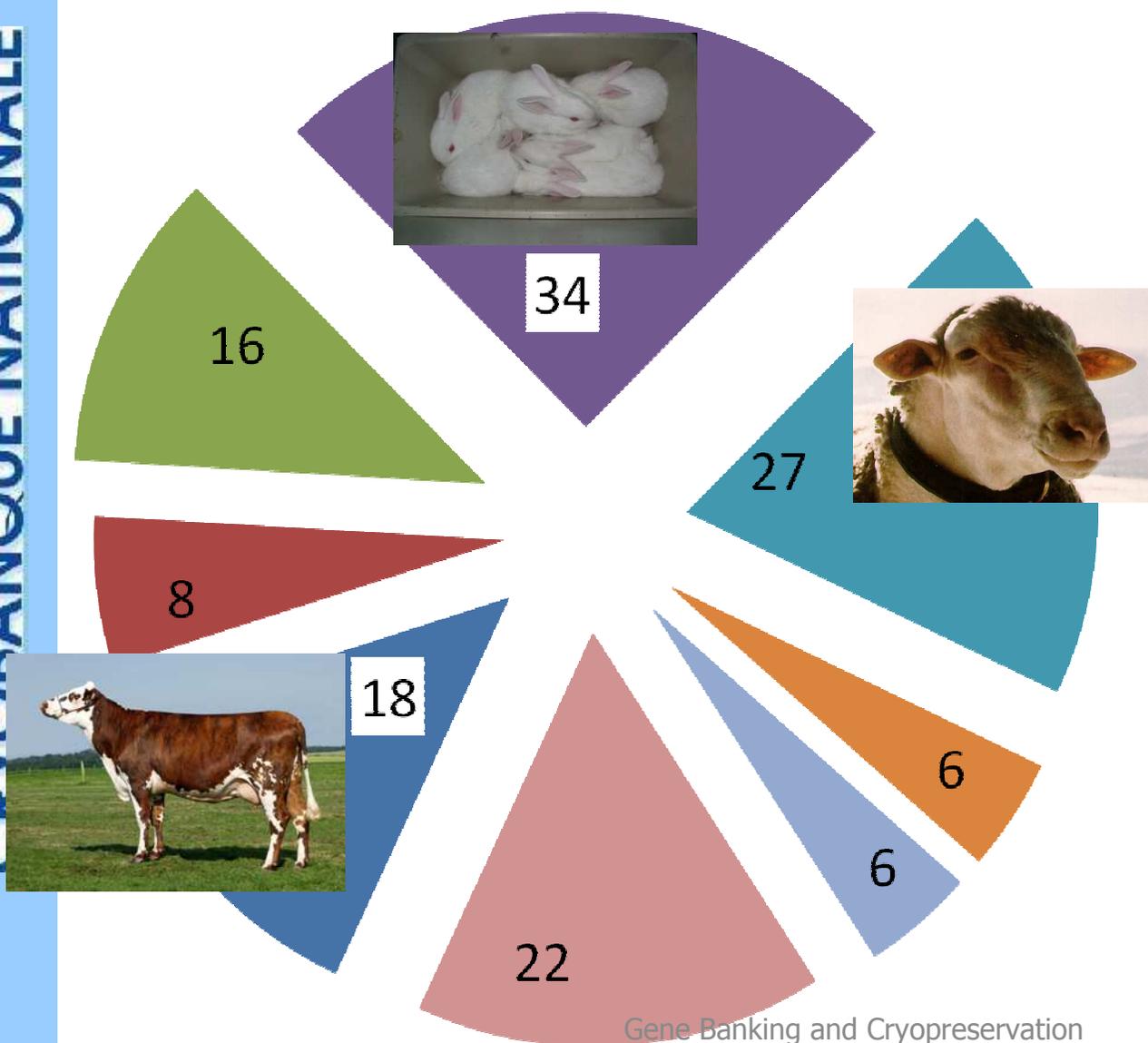
Collection - 2010 update

Number of breeds

CRYOBANQUE NATIONALE

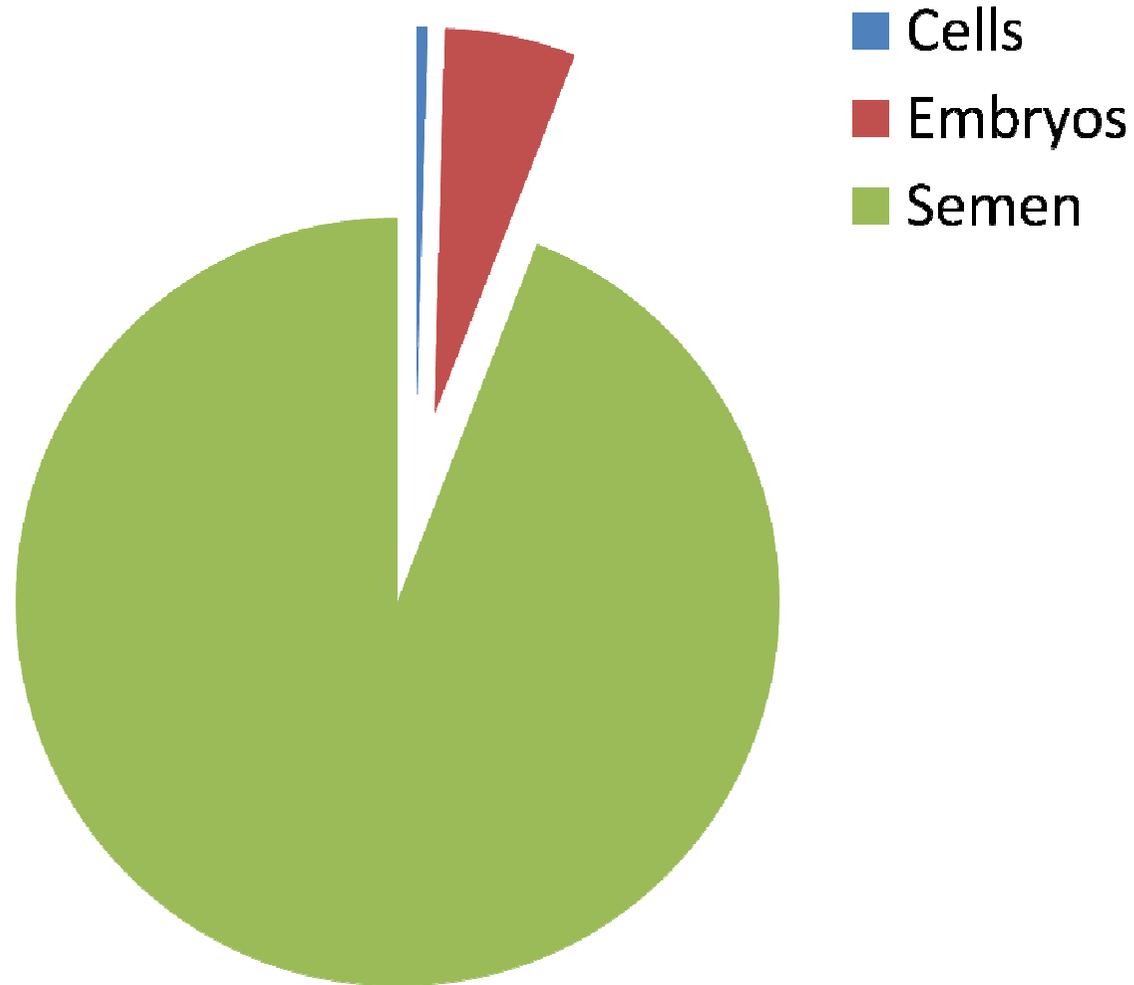
137 breeds

- Cattle
- Goat
- Horse
- Rabbit
- Sheep
- Duck
- Pig
- Cockerel



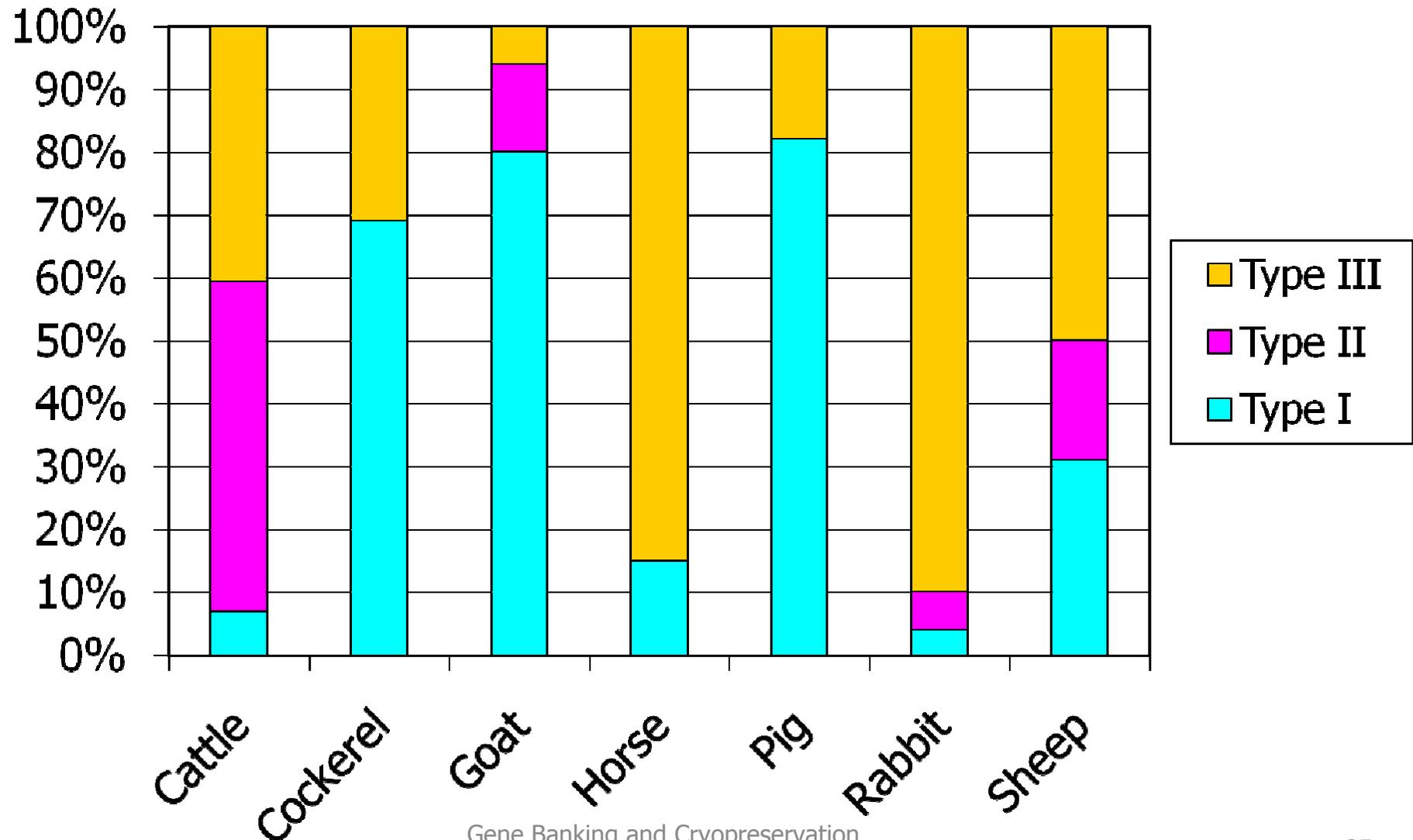
Collection - 2010 update

Type of biological material



Collection - 2010 update

By genetic type





Case study: Example of the local pig breeds



Gene Banking and Cryopreservation
Training Workshop

How many doses should we collect ?

$$D = dN (rn - 1) / (r - 1)$$

- d = number of doses by AI
- n = number of generations of inseminated females
- N = number of females to obtain at the n generation
- f = expected number of fertile females after AI
- $r = 1/f$
- For pig ($n=5$) we obtain $D = 53$

History of the cryopreservation programs

- In the eighties:

Large number of males are collected

However number of doses / male is small

Cryoconservation technique = pellets

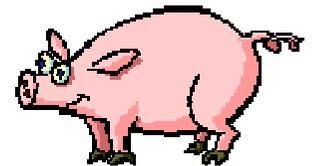
Funding is very variable according to time / breed

- In the nineties

Specific funding (EC)

Larger number of doses collected by male

Cryoconservation technique = straws

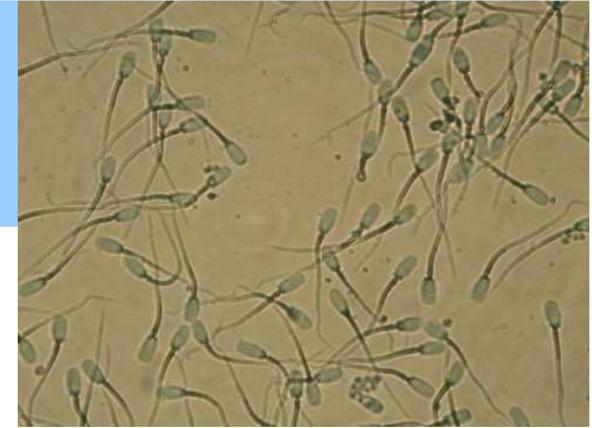


First use of doses from the French Cryobank

- Two local breeds (**Bayeux, Blanc de l'Ouest**) have tremendously reduced their genetic variability since the 80's
- Breeding associations contacted the French National cryobank to use pellets from old boars (4/breed)
- Setting up of an experiment in a research facility
- Some of the piglets obtained by AI will be collected in the future
- Quality of the semen
 - “Blanc Ouest” = poor
 - “Bayeux” = good



Results



- Porc Blanc de l'Ouest

Four sows inseminated => 6 to 13 live piglets/ S
2 males were collected for AI in 2009

- Bayeux

Four sows inseminated => only 2 females born !
“Multifactorial problem”

=> Sows behavior when in flock (very aggressive)

=> Sanitary problems (breeders= small holders)

=> Breed physiology very different from industrial lines

Conclusions / case study



- “Local breeds”
 - ⇒ Farmers less likely to be used to AI protocols (or even they won't agree to do it !)
 - ⇒ Sanitary statute might be lower than average
 - ⇒ Experiment on cryopreservation, AI protocols etc. made with improved lines
- **Discrepancy** between theory (D= number of doses to collect / breed) and practice
 - ⇒ Wiser to increase D for local breeds

Conclusion



- Cryobanks: in fashion and promoted worldwide ...
- ... but there are difficult to set up ! (*sampling, ownership, sanitary rules...*)
- Long term purpose =>
 - ✓ Need to be funded steadily
 - ✓ Permanent staff also needed
 - ✓ Questions raised by the collections supply
collections not increasing **VS** constant raise