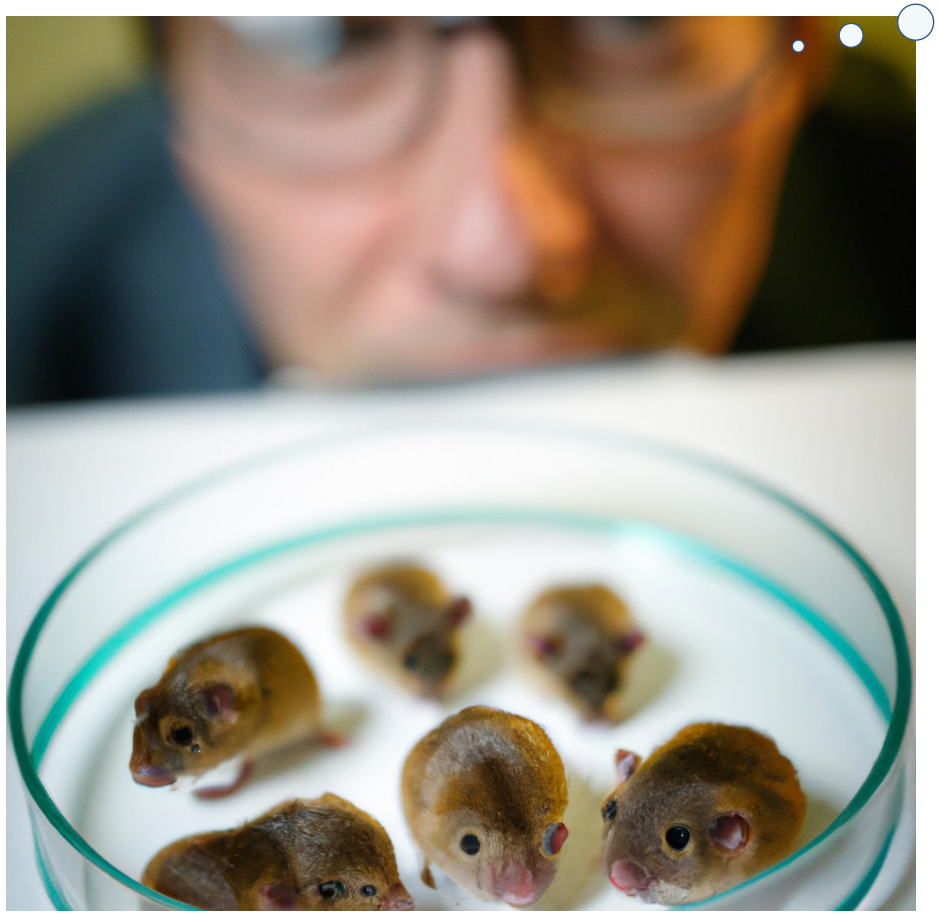
ESTIMATING THE SEXUAL MATURITY OF SMALL MAMMALS IN THE WILD

ROMAIN GALLET & ALOÏS BERARD



28 Mars 2023

Journées petits mammifères

THE BIORODDIS PROJECT

Main goal Understand the relationship between biodiversity and zoonotic infectious diseases

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How? By sampling rodents, detecting zoonotic agents and feeding epidemiological models with these data

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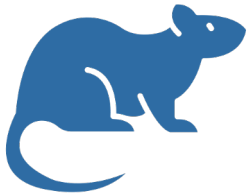
Sexual maturity



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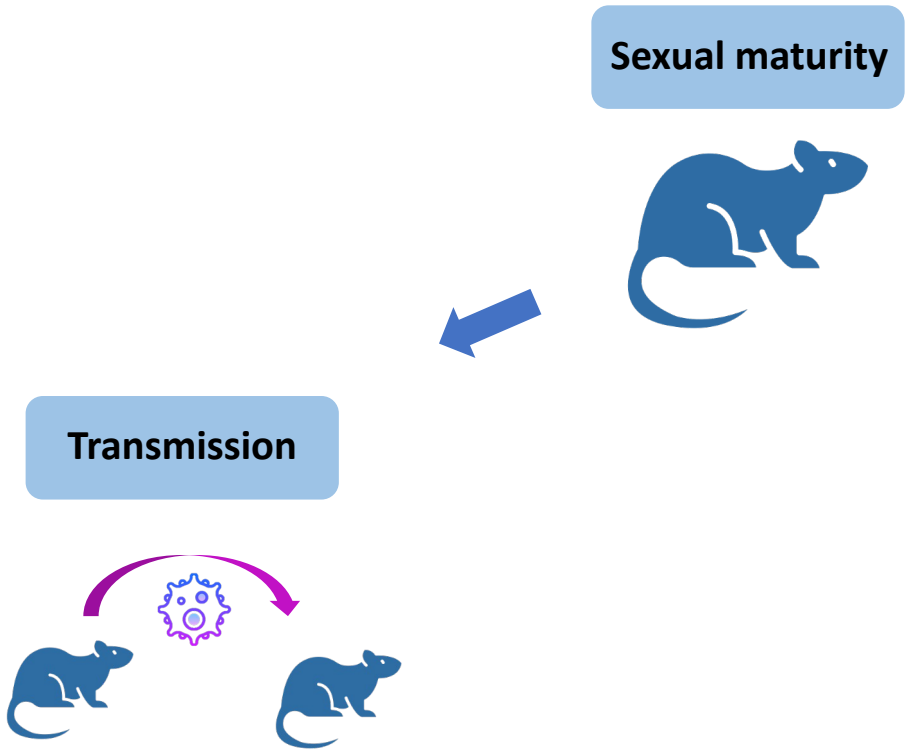

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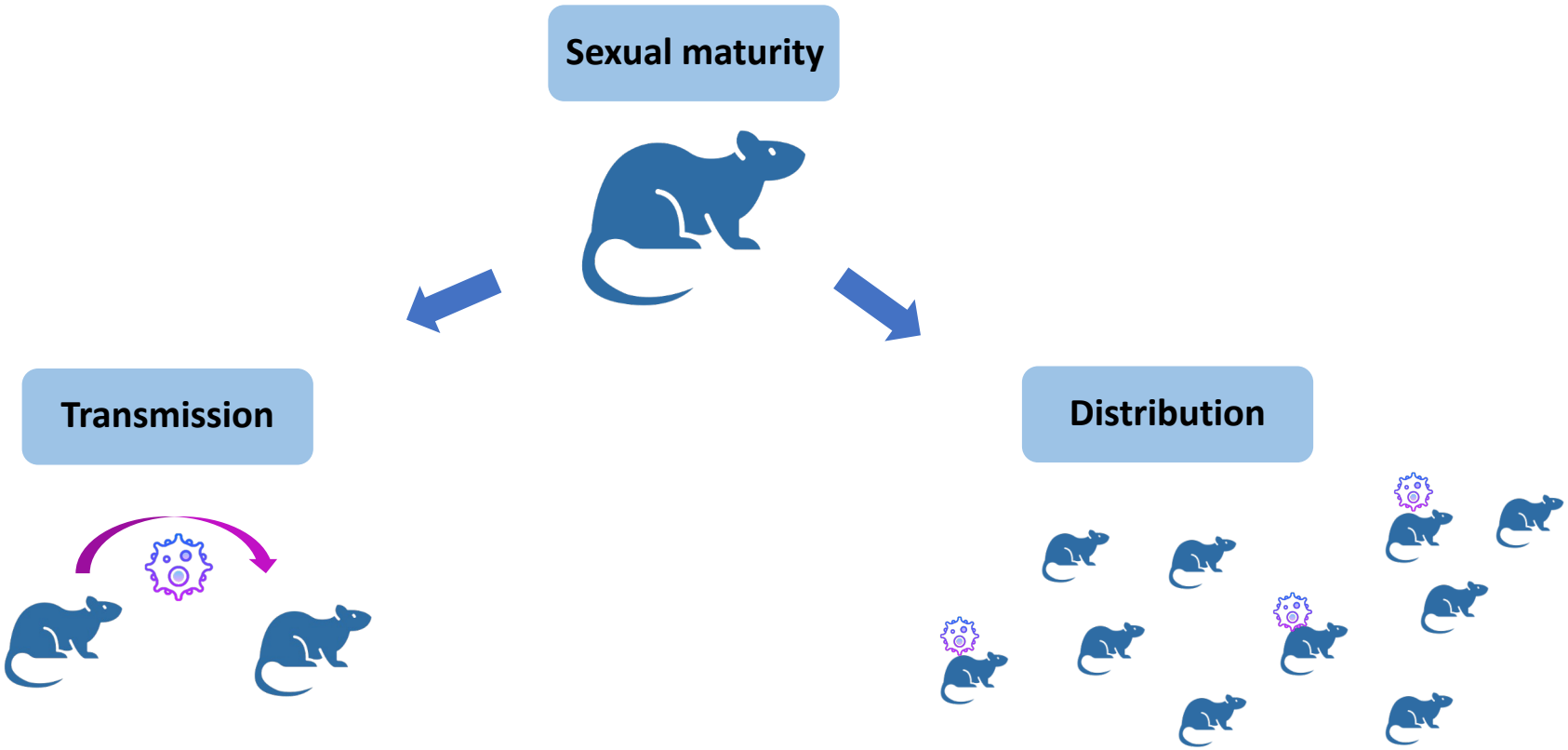

THE DATASET

4203 small mammals belonging to 18 different species collected in 5 European countries over 3 years

Species
Apodemus_agrarius
Apodemus_flavicollis
Apodemus_sylvaticus
Crocidura_leucodon
Crocidura_russula
Glis_glis
Microtus_agrestis
Microtus_arvalis
Microtus_minutus
Microtus_subterraneus
Mus_musculus
Myodes_glareolus
Neomys_fodiens
Rattus_norvegicus
Sciurus_vulgaris
Sorex_araneus
Sorex_coronatus
Sorex_minutus

HOW SHOULD I ESTIMATE SMALL MAMMALS SEXUAL MATURITY?

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Method 1 **Using databases**

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PanTHERIA

Myodes glareolus maturity = 20 g

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Myodes glareolus maturity = 20 g

Anova on the BioRodDis dataset

```
Analysis of Variance Table

Response: Weight
                 Df  Sum Sq Mean Sq F value    Pr(>F)
Gender            1   618.1  618.14 30.6848 3.662e-08 ***
Country           4  2942.3  735.58 36.5145 < 2.2e-16 ***
Period            8  3063.3  382.91 19.0077 < 2.2e-16 ***
Gender:Country    3   249.4   83.12  4.1261 0.0063489 **
Gender:Period     8   623.8   77.97  3.8705 0.0001605 ***
Country:Period    6   756.5  126.09  6.2592 1.695e-06 ***
Residuals      1314 26470.4   20.14
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---
```

Average weight of mature *M. glareolus*

	Species	Female	Male
Category 1	Myodes_glareolus	17.425	17.625
Category 2	Myodes_glareolus	18.666667	18.01905
Category 3	Myodes_glareolus	19.333333	18.38889
Category 4	Myodes_glareolus	19.464286	18.45455
Category 5	Myodes_glareolus	19.5	19.78
Category 6	Myodes_glareolus	20.31814	20
Category 7	Myodes_glareolus	21.04386	21.0375
Category 8	Myodes_glareolus	21.131579	21.125
Category 9	Myodes_glareolus	21.933333	21.60037
Category 10	Myodes_glareolus	22.117241	21.98333
Category 11	Myodes_glareolus	22.14	22.38889
Category 12	Myodes_glareolus	22.191667	22.53214
Category 13	Myodes_glareolus	23.011111	22.71429
Category 14	Myodes_glareolus	23.573913	23.57719
Category 15	Myodes_glareolus	23.73125	23.8381
Category 16	Myodes_glareolus	24.313929	24.33206
Category 17	Myodes_glareolus	24.84	25.1773
Category 18	Myodes_glareolus	25.531429	25.25
Category 19	Myodes_glareolus	25.541176	25.63077

HOW SHOULD I ESTIMATE SMALL MAMMALS SEXUAL MATURITY?

Method 1 Using databases

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Myodes glareolus maturity = 20 g

A single threshold value will not work

Anova on the BioRodDis dataset

```
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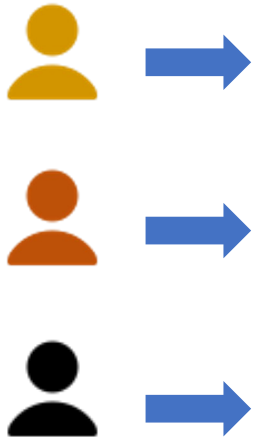

Method 1 **Using databases**

Method 2

Method 3

HOW SHOULD I ESTIMATE SMALL MAMMALS SEXUAL MATURITY?

Method 1 **Using databases**

Method 2

Method 3

… so I did the same… and made my own technique

WHAT I DID

Collected data

Country	sample name	Species	Locality	Gender	Weight	Body_length	Tail_length	Testes_position	Testes_length	Seminal_ves_dev	Vulva	Lactation	Placental_scars	Uterus_dev	Gestation
France	NCHA000797	Apodemus_sylvaticus	FRPLTO	F	8.3	6.5	6.1	NA	0	NA	0	0	0	1	1
France	NCHA000798	Apodemus_sylvaticus	FRPLTO	M	22.6	9.5	8.5	1	16	1	NA	NA	NA	NA	NA
France	NCHA000799	Apodemus_sylvaticus	FRPLTO	M	9.6	7.3	6.5	0	4	0	NA	NA	NA	NA	NA
France	NCHA000800	Apodemus_sylvaticus	FRPLTO	F	16.6	8.8	7.1	NA	0	NA	1	1	1	1	0
France	NCHA000801	Mus_musculus	FRPLTO	M	0	8.4	7.6	1	0	NA	NA	NA	NA	NA	NA
France	NCHA000802	Mus_musculus	FRPLTO	F	17.1	8	8.3	NA	0	NA	0	0	1	0	0
France	NCHA000803	Mus_musculus	FRPLTO	F	24.9	8.5	8.2	NA	0	NA	0	0	1	1	0

WHAT I DID

Collected data

Country	sample name	Species	Locality	Gender	Weight	Body_length	Tail_length	Testes_position	Testes_length	Seminal_ves_dev	Vulva	Lactation	Placental_scars	Uterus_dev	Gestation
France	NCHA000797	Apodemus_sylvaticus	FRPLTO	F	8.3	6.5	6.1	NA	0	NA	0	0	0	1	1
France	NCHA000798	Apodemus_sylvaticus	FRPLTO	M	22.6	9.5	8.5	1	16	1	NA	NA	NA	NA	NA
France	NCHA000799	Apodemus_sylvaticus	FRPLTO	M	9.6	7.3	6.5	0	4	0	NA	NA	NA	NA	NA
France	NCHA000800	Apodemus_sylvaticus	FRPLTO	F	16.6	8.8	7.1	NA	0	NA	1	1	1	1	0
France	NCHA000801	Mus_musculus	FRPLTO	M	0	8.4	7.6	1	0	NA	NA	NA	NA	NA	NA
France	NCHA000802	Mus_musculus	FRPLTO	F	17.1	8	8.3	NA	0	NA	0	0	1	0	0
France	NCHA000803	Mus_musculus	FRPLTO	F	24.9	8.5	8.2	NA	0	NA	0	0	1	1	0

Method

Rule 1 : if at least one sexual character is developed, the individual is considered mature

Rule 2 : if no sexual character is developed, the individual is considered immature

WHAT I DID

Collected data

Country	sample name	Species	Locality	Gender	Weight	Body_length	Tail_length	Testes_position	Testes_length	Seminal_ves_dev	Vulva	Lactation	Placental_scars	Uterus_dev	Gestation	
France	NCHA000797	Apodemus_sylvaticus	FRPLTO	F	8.3	6.5	6.1	NA	0	NA	0	0	0	1	1	Mature
France	NCHA000798	Apodemus_sylvaticus	FRPLTO	M	22.6	9.5	8.5	1	16	1	NA	NA	NA	NA	NA	Mature
France	NCHA000799	Apodemus_sylvaticus	FRPLTO	M	9.6	7.3	6.5	0	4	0	NA	NA	NA	NA	NA	Immature
France	NCHA000800	Apodemus_sylvaticus	FRPLTO	F	16.6	8.8	7.1	NA	0	NA	1	1	1	1	0	Mature
France	NCHA000801	Mus_musculus	FRPLTO	M	0	8.4	7.6	1	0	NA	NA	NA	NA	NA	NA	Mature
France	NCHA000802	Mus_musculus	FRPLTO	F	17.1	8	8.3	NA	0	NA	0	0	1	0	0	Mature
France	NCHA000803	Mus_musculus	FRPLTO	F	24.9	8.5	8.2	NA	0	NA	0	0	1	1	0	Mature

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France	NCHA000797	Apodemus_sylvaticus	FRPLTO	F	8.3	6.5	6.1	NA	0	NA	0	0	0	1	1	Mature
France	NCHA000798	Apodemus_sylvaticus	FRPLTO	M	22.6	9.5	8.5	1	16	1	NA	NA	NA	NA	NA	Mature
France	NCHA000799	Apodemus_sylvaticus	FRPLTO	M	9.6	7.3	6.5	0	4	0	NA	NA	NA	NA	NA	Immature
France	NCHA000800	Apodemus_sylvaticus	FRPLTO	F	16.6	8.8	7.1	NA	0	NA	1	1	1	1	0	Mature
France	NCHA000801	Mus_musculus	FRPLTO	M	0	8.4	7.6	1	0	NA	NA	NA	NA	NA	NA	Mature
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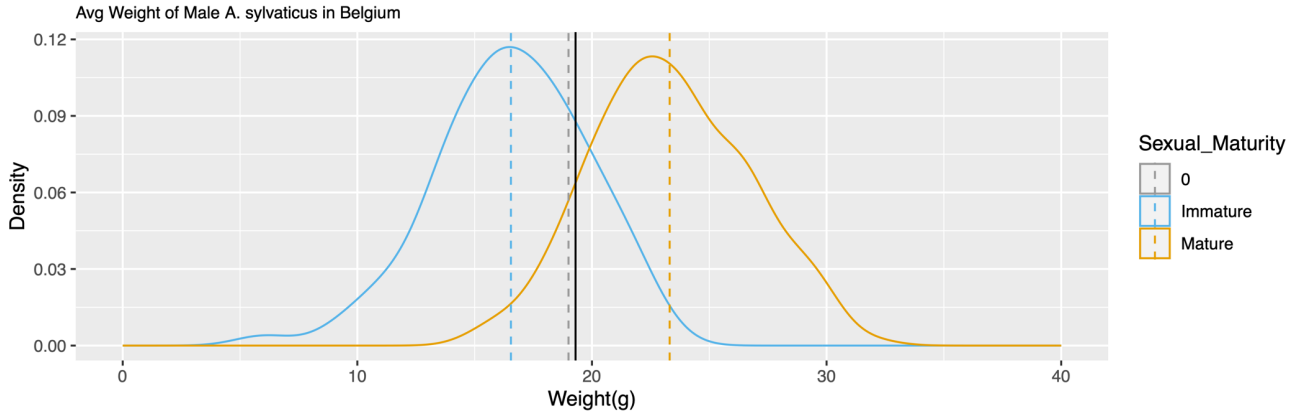

PROBLEM

Not all data were collected in some countries

Country	sample name	Species	Locality	Gender	Weight	Body_length	Tail_length	Testes_position	Testes_length	Seminal_ves_dev	Vulva	Lactation	Placental_scars	Uterus_dev	Gestation
Belgium	BRDb0001	Myodes_glareolus	BEPFO6	M	10	7.2	3.7	0	5.5	0	NA	NA	NA	NA	NA
Belgium	BRDb0002	Apodemus_sylvaticus	BEPFO6	F	20	9	7.6	NA	NA	NA	0	1	NA	1	1
Belgium	BRDb0003	Apodemus_sylvaticus	BEPFO6	M	16.5	7.9	7.9	0	NA	0	NA	NA	NA	NA	NA
Belgium	BRDb0004	Myodes_glareolus	BEPFO6	F	12	7	3.5	NA	NA	NA	1	0	NA	0	0
Belgium	BRDb0005	Myodes_glareolus	BEPFO6	F	15.5	7.5	4	NA	NA	NA	0	0	NA	0	0
Belgium	BRDb0006	Myodes_glareolus	BEPFO6	F	20	6.5	4	NA	NA	NA	0	1	NA	0	0
Belgium	BRDb0007	Myodes_glareolus	BEPFO6	F	18	8.5	5.2	NA	NA	NA	0	0	NA	1	1
France	NCHA000797	Apodemus_sylvaticus	FRPLTO	F	8.3	6.5	6.1	NA	0	NA	0	0	0	1	1
France	NCHA000798	Apodemus_sylvaticus	FRPLTO	M	22.6	9.5	8.5	1	16	1	NA	NA	NA	NA	NA
France	NCHA000799	Apodemus_sylvaticus	FRPLTO	M	9.6	7.3	6.5	0	4	0	NA	NA	NA	NA	NA
France	NCHA000800	Apodemus_sylvaticus	FRPLTO	F	16.6	8.8	7.1	NA	0	NA	1	1	1	1	0
France	NCHA000801	Mus_musculus	FRPLTO	M	0	8.4	7.6	1	0	NA	NA	NA	NA	NA	NA
France	NCHA000802	Mus_musculus	FRPLTO	F	17.1	8	8.3	NA	0	NA	0	0	1	0	0
France	NCHA000803	Mus_musculus	FRPLTO	F	24.9	8.5	8.2	NA	0	NA	0	0	1	1	0
Germany	GEFKUE2104002	Apodemus_flavicollis	GEFKUE	M	46.8	12.9	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104003	Apodemus_flavicollis	GEFKUE	F	31.3	11.6	NA	NA	NA	NA	0	1	NA	NA	0
Germany	GEFKUE2104004	Myodes_glareolus	GEFKUE	F	21.1	9.3	NA	NA	NA	NA	0	0	NA	NA	1
Germany	GEFKUE2104005	Apodemus_flavicollis	GEFKUE	M	51.9	13.1	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104006	Myodes_glareolus	GEFKUE	M	21.5	9.1	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104007	Myodes_glareolus	GEFKUE	M	24.1	9.5	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104008	Myodes_glareolus	GEFKUE	M	23.2	10.2	NA	1	NA	NA	NA	NA	NA	NA	NA
Ireland	IRFF3L21B120	Myodes_glareolus	IRFF3L	F	22.7	9.26	4.81	NA	NA	NA	1	1	NA	0	0
Ireland	IRFF3L21B121	Myodes_glareolus	IRFF3L	M	18.9	9.11	4.32	0	6.3	0	NA	NA	NA	NA	NA
Ireland	IRFF3L21B122	Myodes_glareolus	IRFF3L	F	21.5	9.82	4.65	NA	NA	NA	1	0	6	0	0
Ireland	IRFF3L21B123	Myodes_glareolus	IRFF3L	M	20	9.09	4.02	0	9.6	0	NA	NA	NA	NA	NA
Ireland	IRFF3L21B124	Myodes_glareolus	IRFF3L	M	18.9	8.73	4.56	0	7.75	0	NA	NA	NA	NA	NA
Ireland	IRFF3L21B125	Myodes_glareolus	IRFF3L	M	21.7	9.21	4.47	1	8.35	1	NA	NA	NA	NA	NA
Ireland	IRFF3L21B126	Myodes_glareolus	IRFF3L	F	21.3	9.26	3.74	NA	NA	NA	0	0	0	0	0
Poland	POFMLD177	Myodes_glareolus	POFMLD	F	16	8.2	4.1	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD178	Apodemus_agrarius	POFMLD	M	19	8.5	7.8	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD179	Apodemus_agrarius	POFMLD	F	16.5	8.6	9.5	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD180	Apodemus_agrarius	POFMLD	M	25.1	10.8	10.7	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD181	Apodemus_agrarius	POFMLD	F	25.5	10.2	10.2	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD182	Apodemus_agrarius	POFMLD	M	21.5	9.5	10	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD183	Myodes_glareolus	POFMLD	F	15.8	8.7	3.7	NA	NA	NA	NA	NA	NA	NA	NA

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Belgium	BRDb0001	Myodes_glareolus	BEPFO6	M	10	7.2	3.7	0	5.5	0	NA	NA	NA	NA	NA
Belgium	BRDb0002	Apodemus_sylvaticus	BEPFO6	F	20	9	7.6	NA	NA	NA	0	1	NA	1	1
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Belgium	BRDb0004	Myodes_glareolus	BEPFO6	F	12	7	3.5	NA	NA	NA	1	0	NA	0	0
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Belgium	BRDb0006	Myodes_glareolus	BEPFO6	F	20	6.5	4	NA	NA	NA	0	1	NA	0	0
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France	NCHA000798	Apodemus_sylvaticus	FRPLTO	M	22.6	9.5	8.5	1	16	1	NA	NA	NA	NA	NA
France	NCHA000799	Apodemus_sylvaticus	FRPLTO	M	9.6	7.3	6.5	0	4	0	NA	NA	NA	NA	NA
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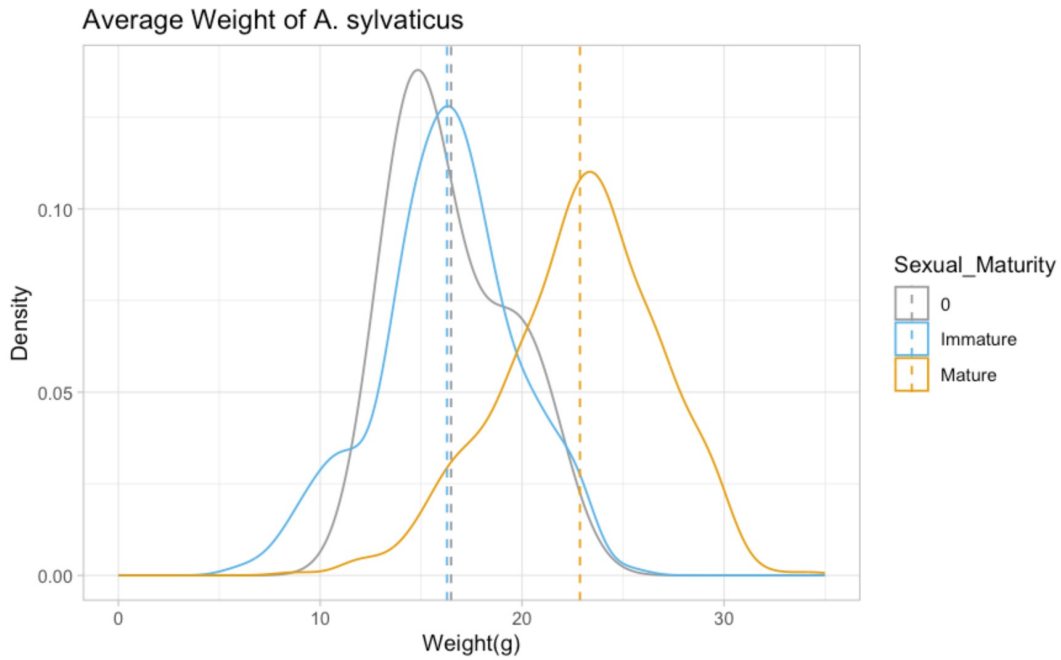
France	NCHA000801	Mus_musculus	FRPLTO	M	0	8.4	7.6	1	0	NA	NA	NA	NA	NA	NA
France	NCHA000802	Mus_musculus	FRPLTO	F	17.1	8	8.3	NA	0	NA	0	0	1	0	0
France	NCHA000803	Mus_musculus	FRPLTO	F	24.9	8.5	8.2	NA	0	NA	0	0	1	1	0
Germany	GEFKUE2104002	Apodemus_flavicollis	GEFKUE	M	46.8	12.9	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104003	Apodemus_flavicollis	GEFKUE	F	31.3	11.6	NA	NA	NA	NA	0	1	NA	NA	0
Germany	GEFKUE2104004	Myodes_glareolus	GEFKUE	F	21.1	9.3	NA	NA	NA	NA	0	0	NA	NA	1
Germany	GEFKUE2104005	Apodemus_flavicollis	GEFKUE	M	51.9	13.1	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104006	Myodes_glareolus	GEFKUE	M	21.5	9.1	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104007	Myodes_glareolus	GEFKUE	M	24.1	9.5	NA	1	NA	NA	NA	NA	NA	NA	NA
Germany	GEFKUE2104008	Myodes_glareolus	GEFKUE	M	23.2	10.2	NA	1	NA	NA	NA	NA	NA	NA	NA
Ireland	IRFF3L21B120	Myodes_glareolus	IRFF3L	F	22.7	9.26	4.81	NA	NA	NA	1	1	NA	0	0
Ireland	IRFF3L21B121	Myodes_glareolus	IRFF3L	M	18.9	9.11	4.32	0	6.3	0	NA	NA	NA	NA	NA
Ireland	IRFF3L21B122	Myodes_glareolus	IRFF3L	F	21.5	9.82	4.65	NA	NA	NA	1	0	6	0	0
Ireland	IRFF3L21B123	Myodes_glareolus	IRFF3L	M	20	9.09	4.02	0	9.6	0	NA	NA	NA	NA	NA
Ireland	IRFF3L21B124	Myodes_glareolus	IRFF3L	M	18.9	8.73	4.56	0	7.75	0	NA	NA	NA	NA	NA
Ireland	IRFF3L21B125	Myodes_glareolus	IRFF3L	M	21.7	9.21	4.47	1	8.35	1	NA	NA	NA	NA	NA
Ireland	IRFF3L21B126	Myodes_glareolus	IRFF3L	F	21.3	9.26	3.74	NA	NA	NA	0	0	0	0	0
Poland	POFMLD177	Myodes_glareolus	POFMLD	F	16	8.2	4.1	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD178	Apodemus_agrarius	POFMLD	M	19	8.5	7.8	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD179	Apodemus_agrarius	POFMLD	F	16.5	8.6	9.5	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD180	Apodemus_agrarius	POFMLD	M	25.1	10.8	10.7	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD181	Apodemus_agrarius	POFMLD	F	25.5	10.2	10.2	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD182	Apodemus_agrarius	POFMLD	M	21.5	9.5	10	NA	NA	NA	NA	NA	NA	NA	NA
Poland	POFMLD183	Myodes_glareolus	POFMLD	F	15.8	8.7	3.7	NA	NA	NA	NA	NA	NA	NA	NA

How should we deal with missing values ?

THE SOLUTION

Since Gender, Country, Season affects small mammal weights

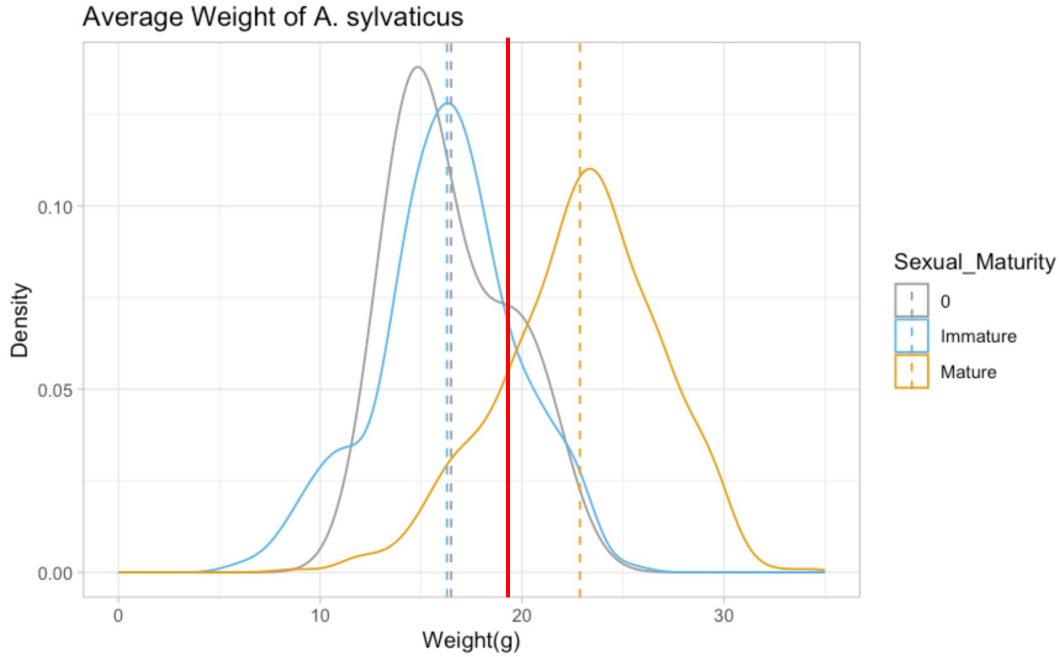
➡ 1. Calculation of the average weights of mature and immature individuals for each Gender/Country/Season



THE SOLUTION

Since Gender, Country, Season affects small mammal weights

1. Calculation of the average weights of mature and immature individuals for each Gender/Country/Season
2. Calculation of an optimal cutpoint (cutpointr package)



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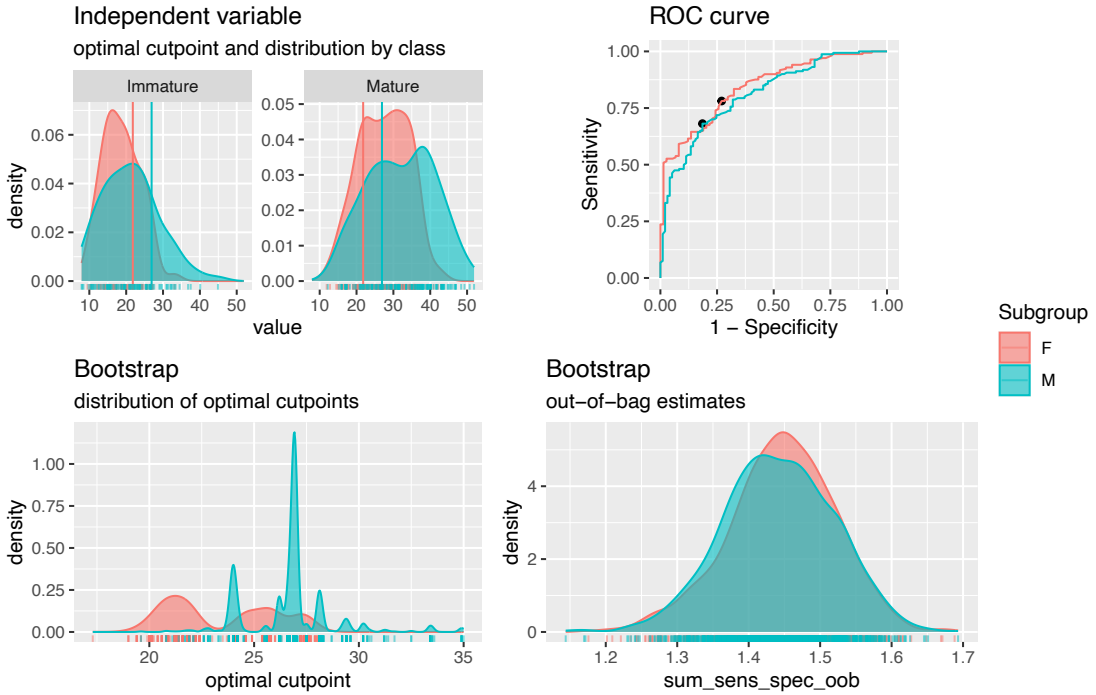

CutpointR uses ROC curve to identify cutpoint values minimizing errors

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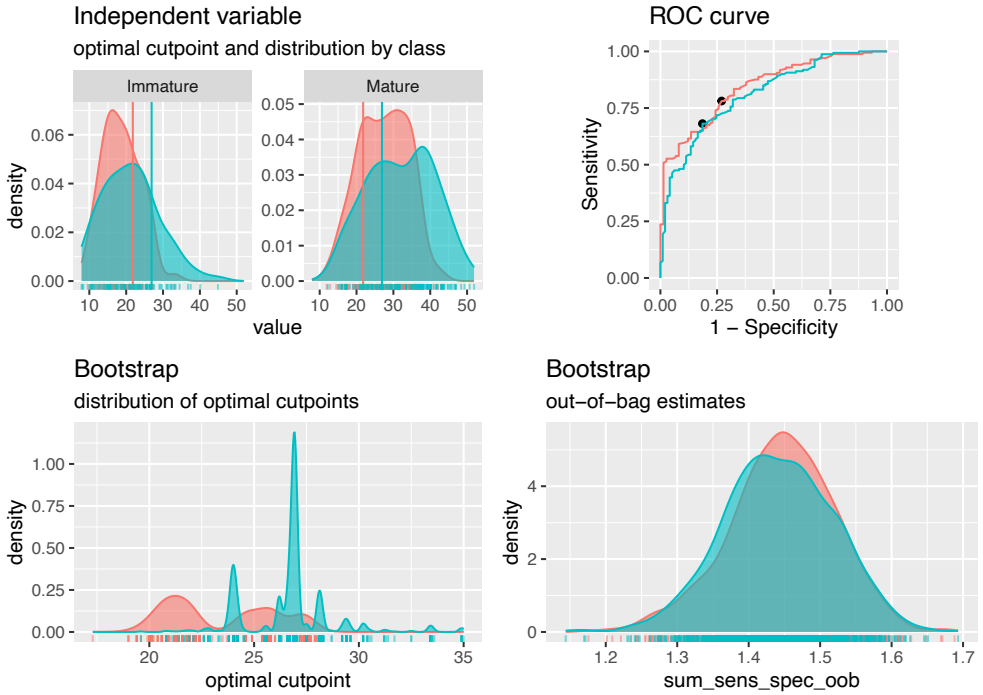

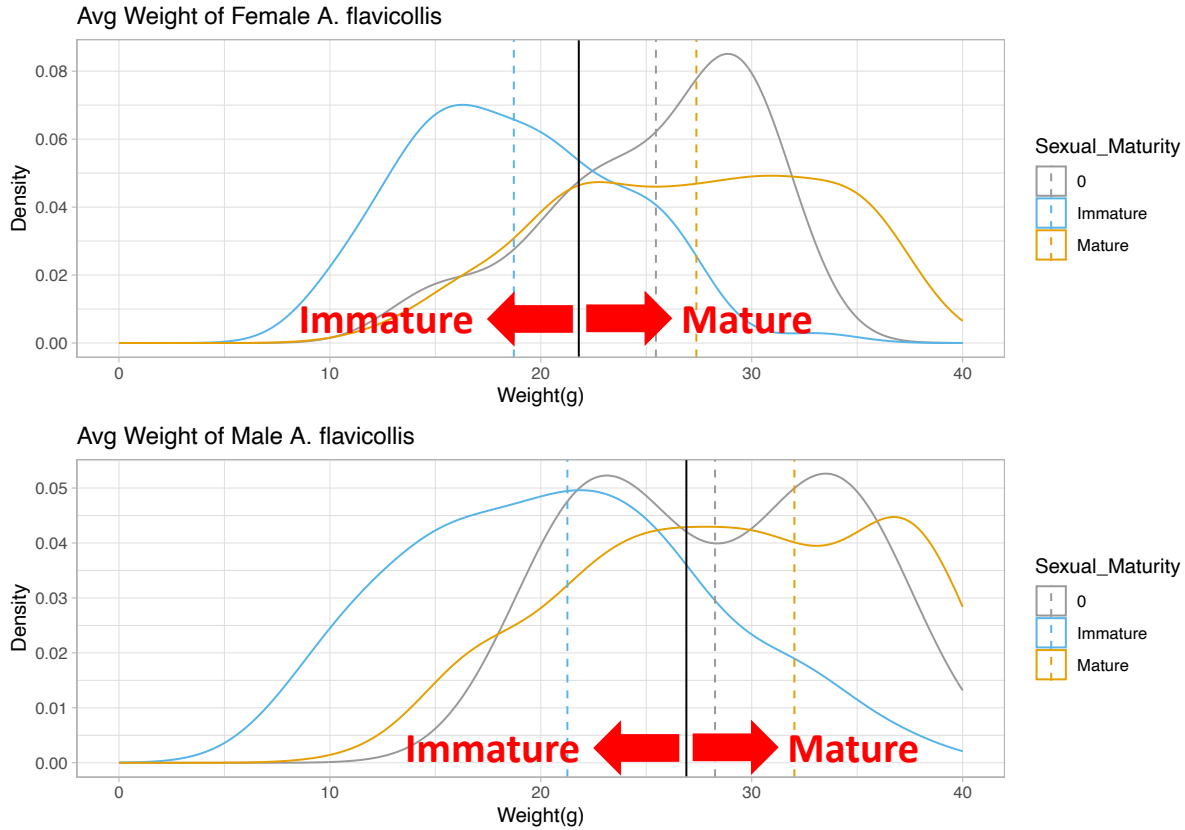

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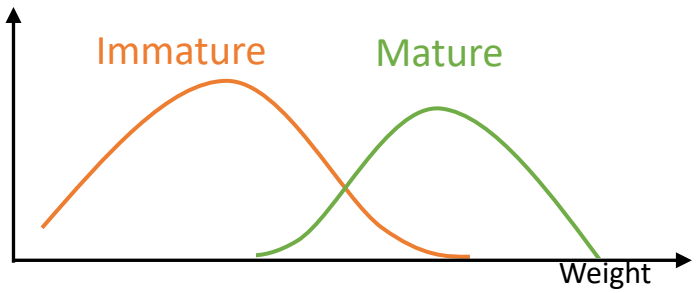
- Weight distributions of Immature and Matures seem to confirm the quality of the discrimination process



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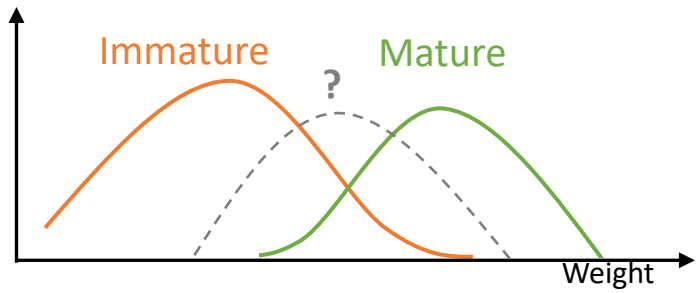

For individuals with missing values (15-20% of the dataset) ➡ calculation of the average weight of mature and immature indivs per Gender/Country/Season

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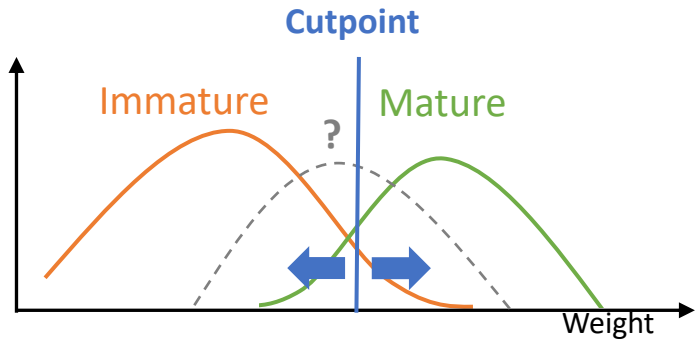


For individuals with missing values (15-20% of the dataset) → calculation of the average weight of mature and immature indivs per Gender/Country/Season

→ calculation of the cutpoint value minimizing errors

Aloïs BERARD's internship

Analysis of co-infection patterns between orthohantaviruses and rodent gastrointestinal helminths

The need to distinguish immature individuals from mature ones

(Meta)population of interest

- *Rattus norvegicus* trapped in Bamako → South-Sahelian urban context
- 350 individuals : 149 ♂ and 201 ♀ *(SR 0,74)*
- Morphological data set :

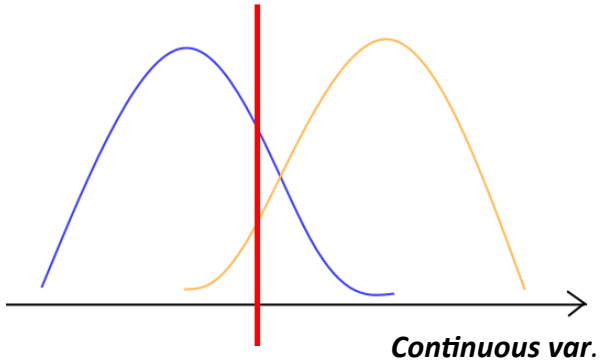
		Binary var.		*Continuous var.*		
	Sex	**Development of seminal vesicles**	**Development of the breast**	**Testicular length**	**Length of the uterus**	**Non-sexual morphological character**
Individual 1	M	0	NA	10	NA	100
Individual 2	M	1	NA	NA	NA	150
Individual 3	F	NA	NA	NA	30	200

NA red: missing values not measured (NA unrelated to sex)

Methods considered

- **Approach presented previously (by Romain):**

1. Maturity according to the development of sexual characteristics *(var. 0/1)*
2. Threshold segregating the distributions *(continuous var.)* for the undetermined



- **Approach proposed by Laurent**

1. Threshold values *(continuous var.)* from the percentage of individuals with the developed trait *(var. 0/1)* by class

Romain’s method

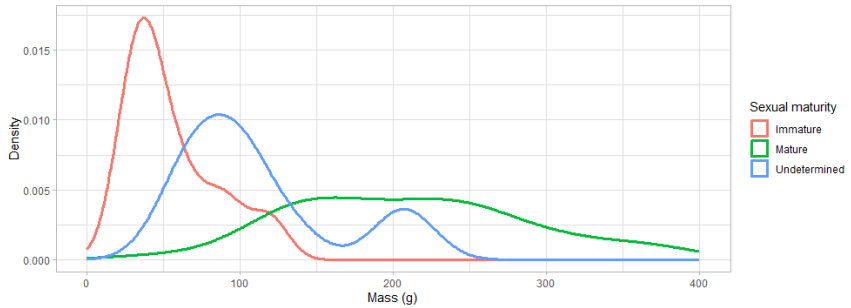
1. Prior determination by the development of sexual characteristics (0/1)

350 individuals → 223 matures, 109 immatures, 18 undetermined

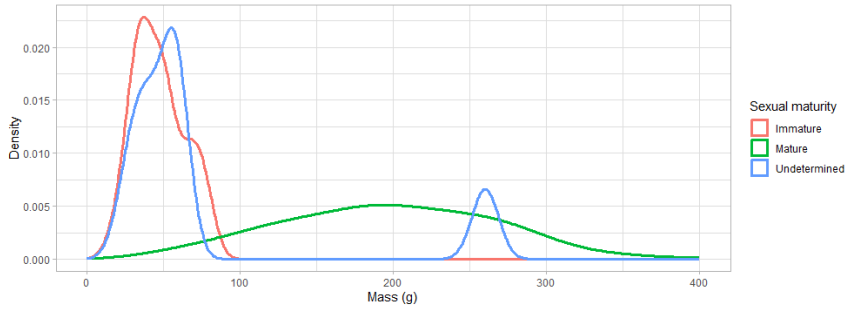
Resulting distributions :

Bodyweight (g)

♂



♀



Segregation of consistent body weight distributions using sex characteristics

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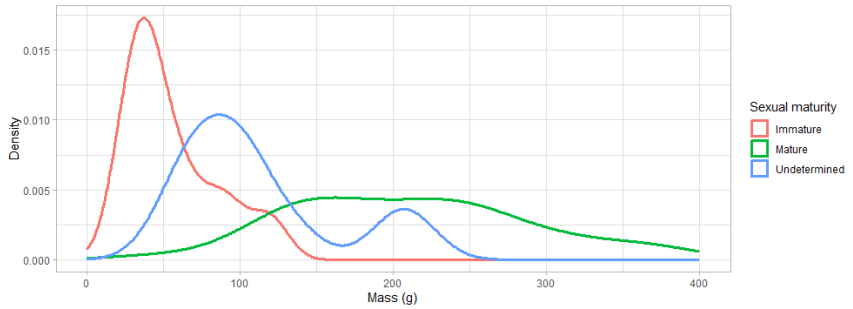

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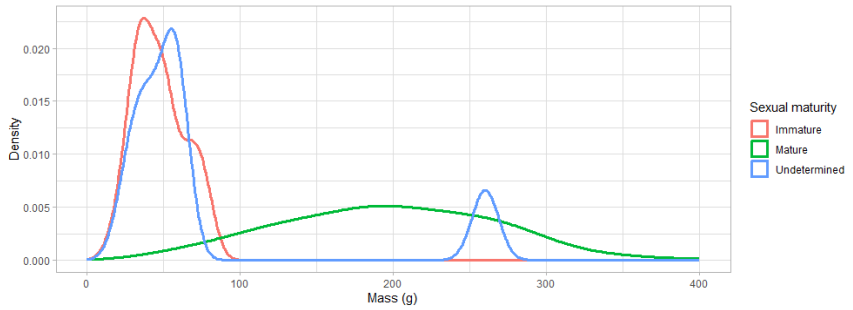
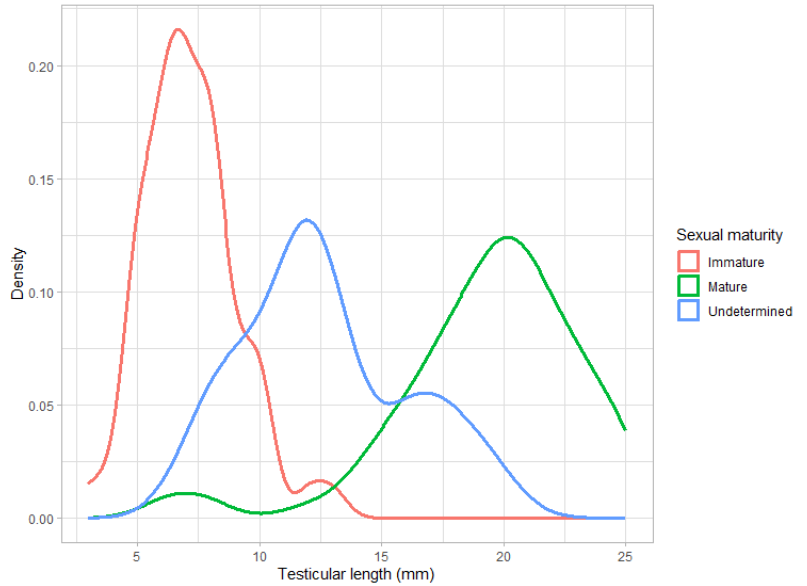


Testicular length (mm)

♂



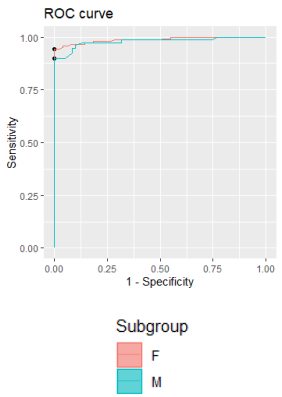
Segregation of consistent testicular length distributions using sex characteristics

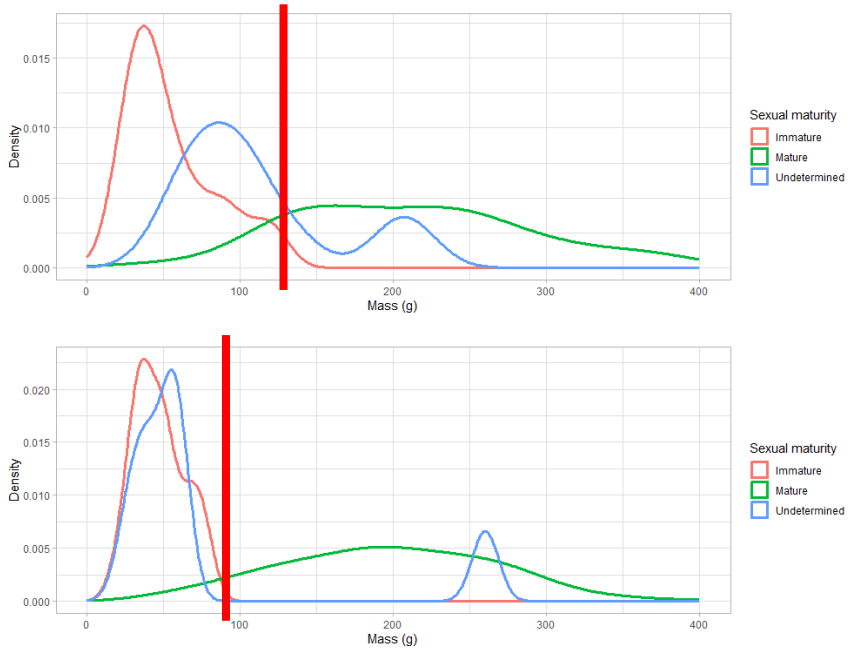
Romain’s method

2. Determination of threshold values segregating distributions

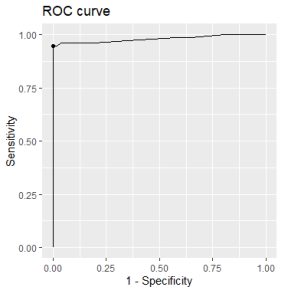
Resulting distributions and threshold values :

Bodyweight (g)

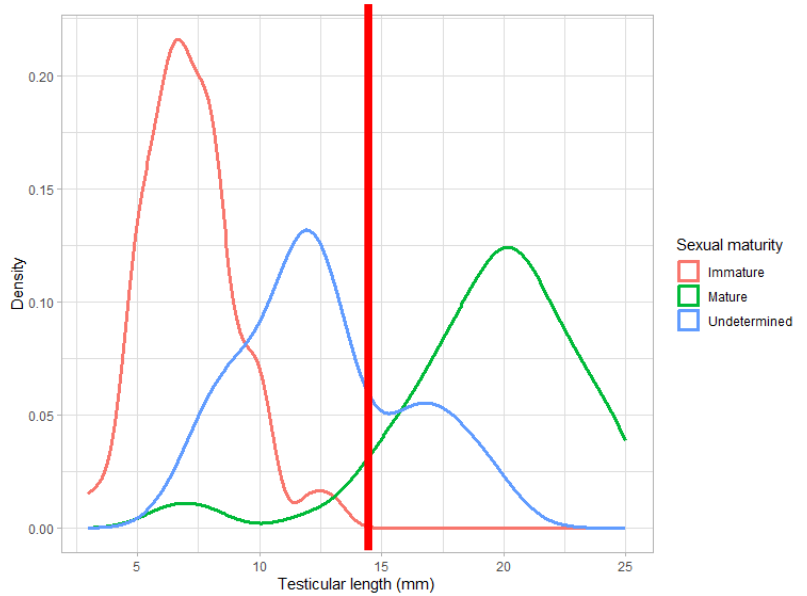




♂ : 127 g *(AUC : 0,9796)*
♀ : 88 g *(AUC : 0.9875)*



Testicular length (mm)



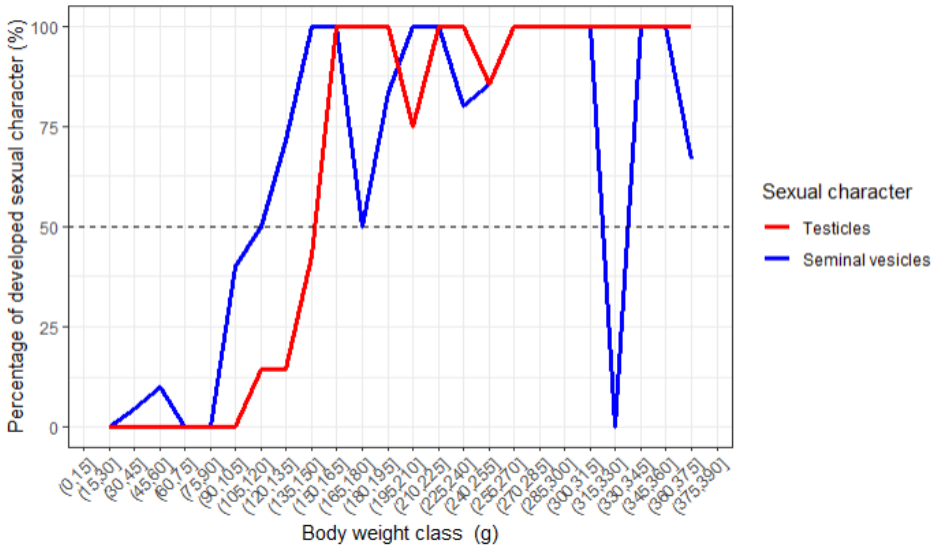
♂ : 14 mm *(AUC : 0.9794)*

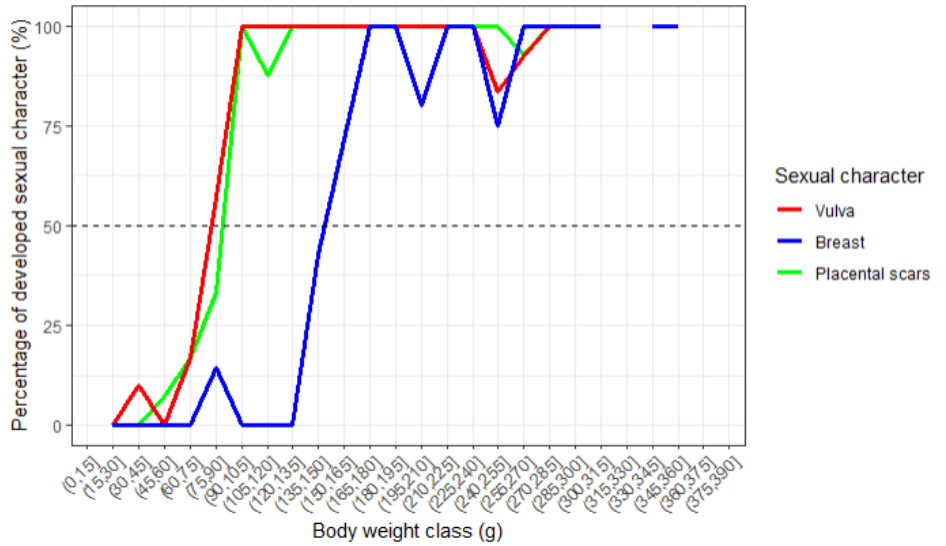
Laurent's method

1. Determination of threshold values when the percentage per class ≥ 50%

When based on 15 g <u>bodyweight</u> classes :

♂





♀

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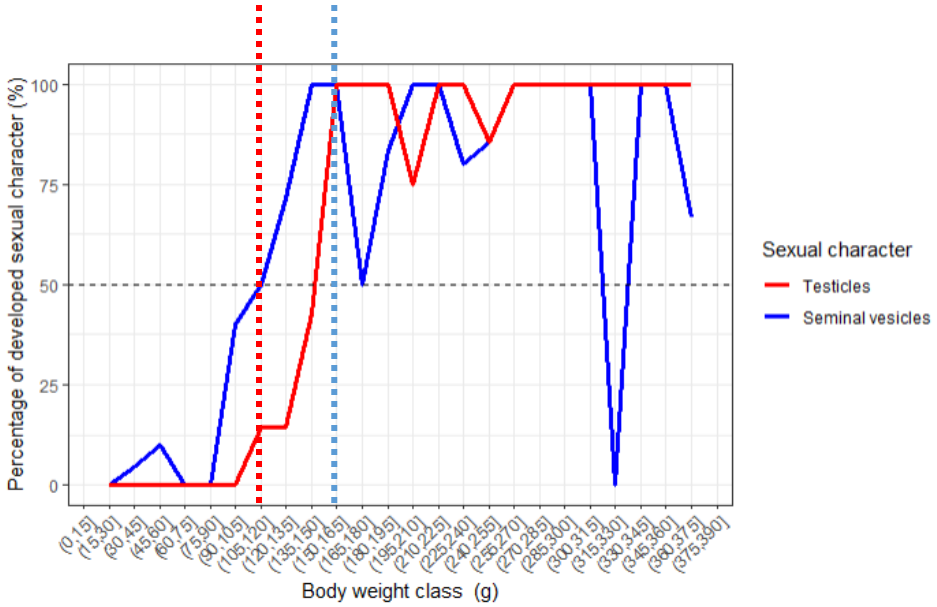
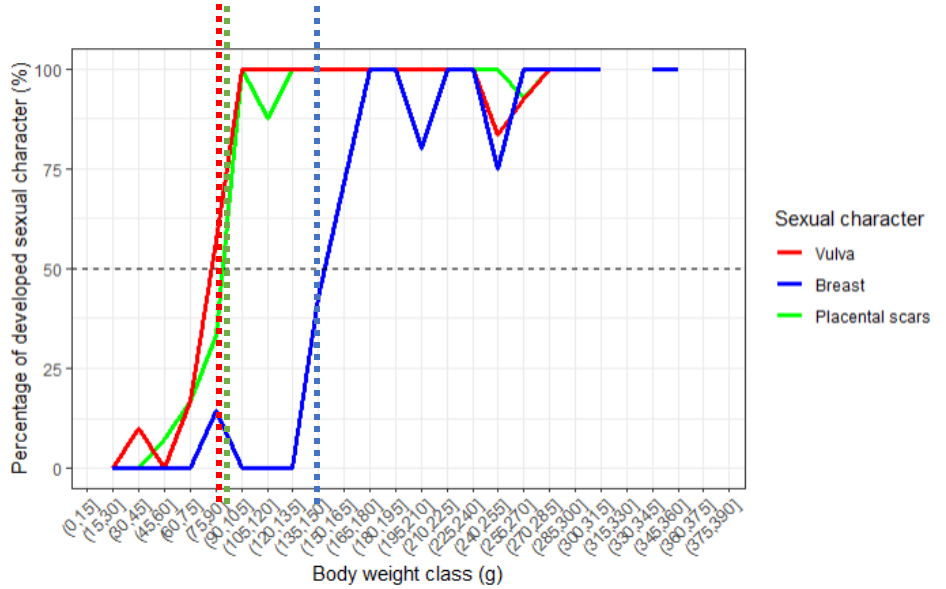




♀

	Testicles	Seminal vesicles
Threshold class	]105;120]	]150; 165]
Class's middle	112,5	157,5
Mean	**135**	

	Vulva	Breast	Placental scares
Threshold class	]90;105]	]75;90]	]75;90]
Class's middle	97,5	82,5	82,5
Mean		**87,5**	

Threshold comparison and congruence

With the continuous variable: Bodyweight :

Romain‘s method: 227 matures and 123 immatures
♂ : 127 g and ♀ : 88 g

Laurent’s method: 205 matures and 145 immatures
♂ : 135 g and ♀ : 87,5 g

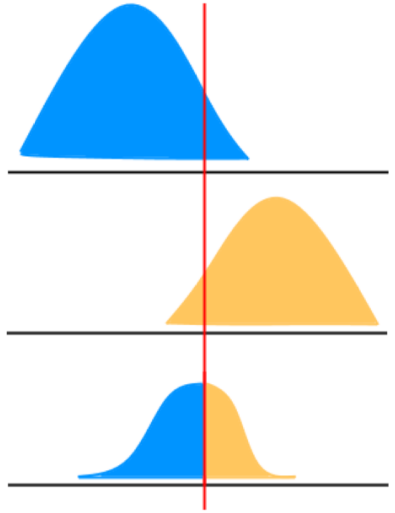
93% of the individuals are characterized identically

Different philosophies

- **Approach presented previously (by Romain):**

Strong influence of the 0/1 status of the set of traits considered

Depends less on the threshold chosen for the continuous variable



Threshold only applied to a certain subgroup

- **Approach proposed by Laurent**

Strong influence of the determined threshold value

Less influenced by the 0/1 status of the traits

Threshold applied to everyone