MUTANT ANIMALS: IDENTIFICATION, BREEDING, ANALYSIS

ANIMAUX MUTANTS : IDENTIFICATION, ENTRETIEN, ANALYSES

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Master de Biothérapies Tissulaires, Cellulaires et Géniques

Module « Modèles Animaux »

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Lydia Danglot web page

Life Science & Imaging

Novembre 2, 2009

- Thème de recherche
- Publications
- Enseignement
- Liens favoris
- CONTACT

French English

Enseignement

Cours

- Master2 de Neurosciences - UE Synapse et synaptogenèse (code UE : MBIP5019) - Université Pierre et Marie Curie (Paris 6): Planning ***Neuritogenèse et polarité neuronale.***
- Master2 de Neurosciences - UE Communication Cellulaire (code UE : MBIP5003) - Université Pierre et Marie Curie (Paris 6): ***Les protéines SNARE et l'exocytose*** : classification des SNAREs, voie de recyclage des VS, comment mesurer l'exocytose, comment mesurer le recyclage, les protéines régulant l'assemblage des SNARE (Munc18, munc13, Syt, complexine), souris KO Syb2, souris mocha,...
- Master2 de Génétique - Université Paris Diderot (Paris 7), UE Neurobiologie cellulaire et développementale. ***Développement de l'hippocampe et synaptogenèse:*** Neuroanatomie générale, présentation du SNC, présentation du télencéphale et de l'hippocampe, développement de l'hippocampe, migration des neurones excitateurs et inhibiteurs, modèle des neurones dissociés d'hippocampe en culture, polarité neuronale, formation des synapses.
- Ecole doctorale Frontières du Vivant (Universités Paris V, VI, VII) Club Neurobiologie & Optique: ***Diversité et usage des protéines fluorescentes en Neurosciences.***

MANUEL de cours

Master2- Paris 6 Neuritogenèse et polarité neuronale.
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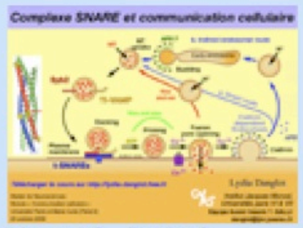

Master2- Paris 6 Complexe SNARE et communication cellulaire.
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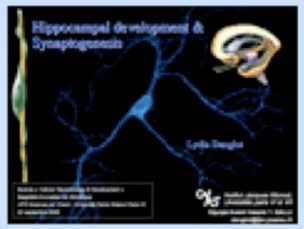

Master2- Paris 7 Développement de l'hippocampe et synaptogenèse
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Bibliography

✓ **Gerlai, R. (1996).**
Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype?
Trends Neurosci *19*, 177-81.

✓ **Lathe, R. (1996).**
Mice, gene targeting and behaviour: more than just genetic background.
Trends Neurosci *19*, 183-6; discussion 188-9.

✓ **Banbury Conference (Silva, A. J. and coll. 1997).**
Mutant mice and neuroscience: recommendations concerning genetic background. Banbury Conference on genetic background in mice.
Neuron *19*, 755-9.

✓ **Wolfer, D. P., Crusio, W. E., and Lipp, H. P. (2002).**
Knockout mice: simple solutions to the problems of genetic background and flanking genes.
Trends Neurosci *25*, 336-40.

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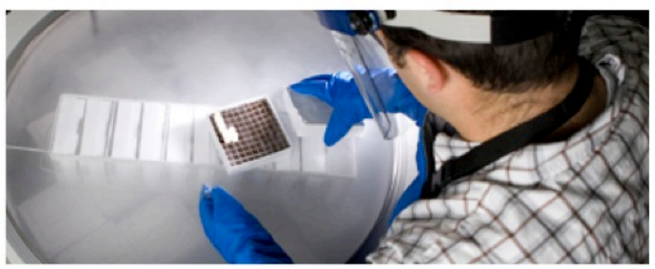


Total Revenue FY2002: $110.0 million

Public support, including program grants & contracts: $50.2 million

JAX Research Systems: $46.3 million

Contributions & Bequests - Operating: $4.3 million

Other: $3.0 million

Total Staff Size: 1,271 employees

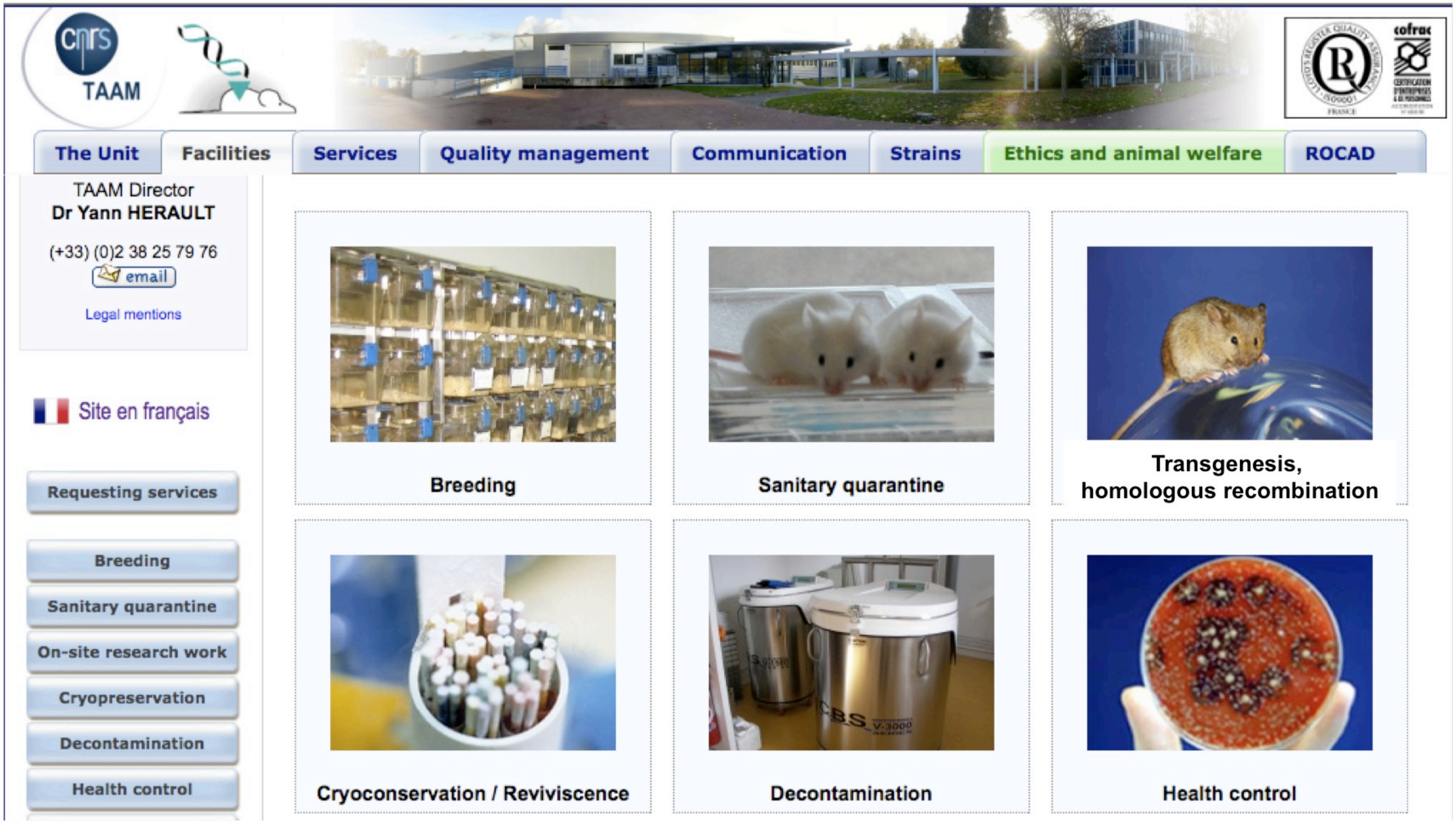
1.9 million JAX mice distributed

More than 2,256 varieties are available as breeding mice, Frozen embryos, or DNA samples.

Induced Mutant Resource:

More than 800 varieties of mice with targeted mutations, Including models for cancer, heart disease, Alzheimer's disease, ALS, Huntington's disease, and autoimmune diseases.

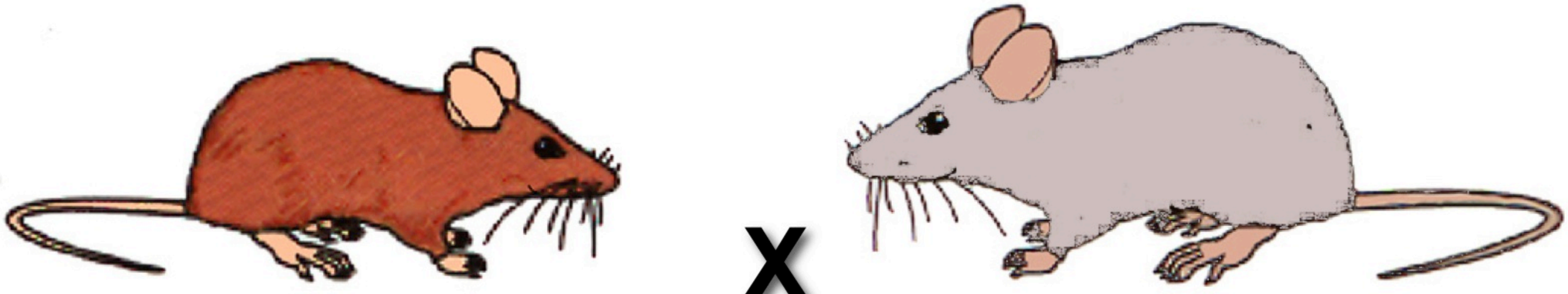
Centre de Développement des Techniques Avancées en Expérimentation Animale
CDTA
CNRS - Institut de Transgénose à Orléans-La-Source
http://transgenose.cnrs-orleans.fr/cdta



EMMA (European Mouse Mutant Archive)
http://www.emmanet.org

Definitions & Reminder

Mating (accouplement)



Female X Male

Allele

One of the variant forms of a gene or locus. They differ in their nucleotide sequence by:
as little as a single base
or by the complete absence of a sequence.

Ex: for one gene A, you can have two alleles: a1 & a2.

Homozygote

An animal with two identical alleles at a particular locus under analysis.
Genotype : a1/a1 or a2/a2

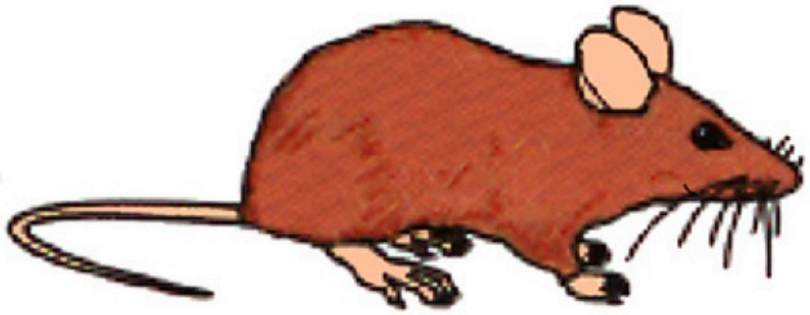
Heterozygote

An animal with two different alleles at a particular locus under analysis.
Usually one is normal and the other is abnormal (mutant).
Genotype : a1/a2

F_1

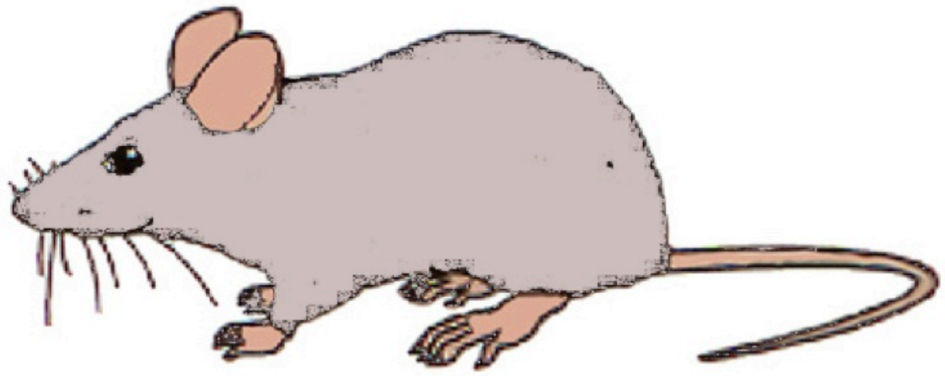
The first filial generation; the offspring of an outcross between two inbred strains.

Mating / accouplement



Female

X



Male

Gestation (21 jours)

Pregnancy (21 days)

Litter-mate (portée)

Sources of laboratory mice

Advantages to working with mice:

- availability of **standard strains** (= souche) such as C57BL/6 (abbreviated B6), BALB/c
- **eliminate genetic variability** which allows :
  - **reproducibility in space**: comparable results in Japan, Canada, Germany, or any other country in the world.
  - **reproducibility in time**: results obtained in 1992 can be directly compared to results obtained in 1962 or any other year.

What is a standard strain ?

- refers to a group of mice that are bred (élevé) within a closed colony in order to maintain certain defining characteristics.
- strains can be :
  - **inbred** (=consanguine):
    result from at least 20 sequential generations of brother-sister matings (accouplement). This process is called **inbreeding**.
    The strain is essentially homozygous at all loci.
    ex: C57BL/6J, BALB/c,DBA/2
  - or **non-inbred**:

Sources of laboratory mice

Coisogenic strains:

Identical except for a difference at a single genetic locus;

can presumably arise only as a result of mutation in an established inbred strain (Flaherty, 1981).

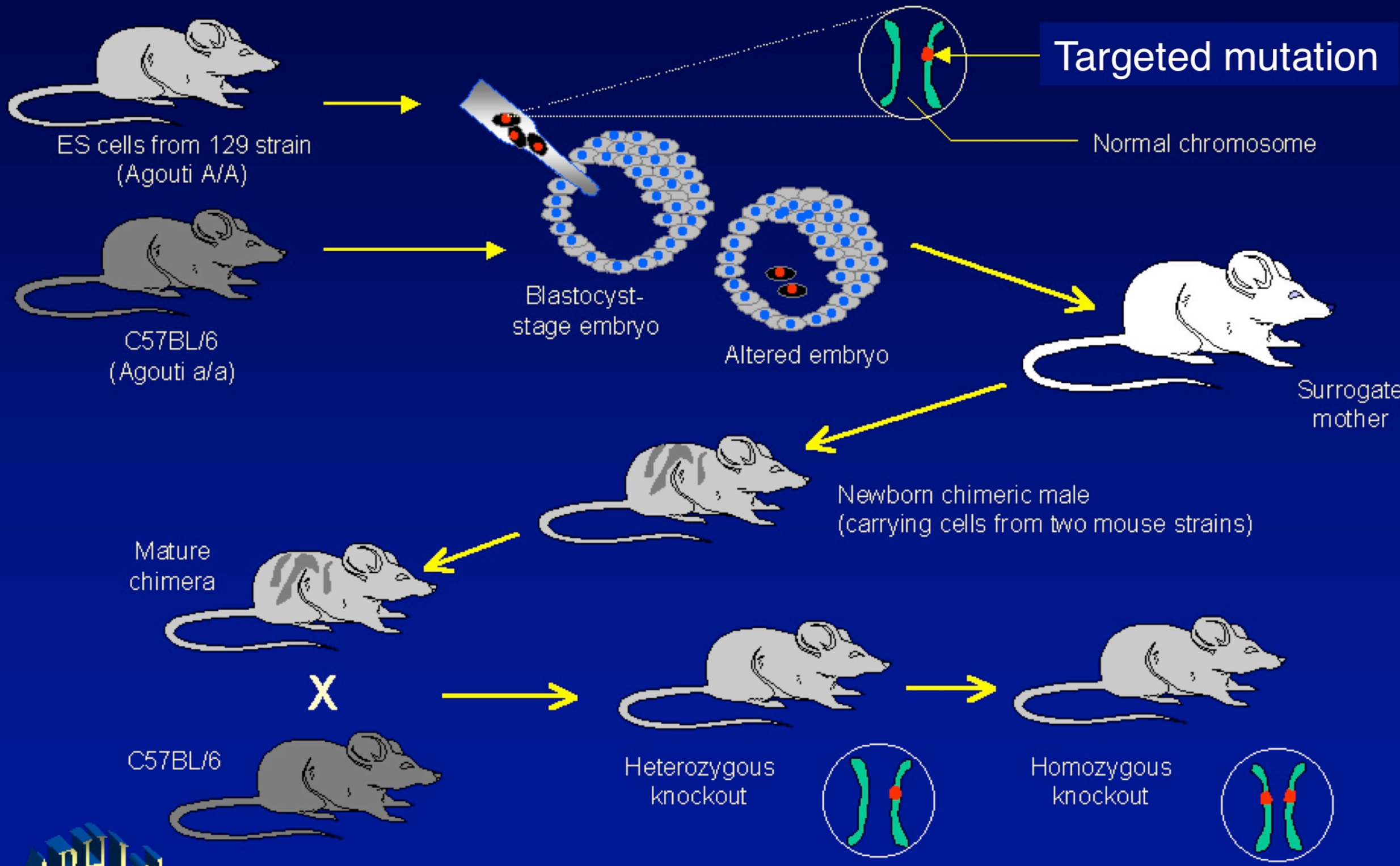
Congenic strains:

Identical except for a short chromosomal segment (ex H-2 = Snell, 1948);

Can be produced by several ways: backcross, cross-intercross

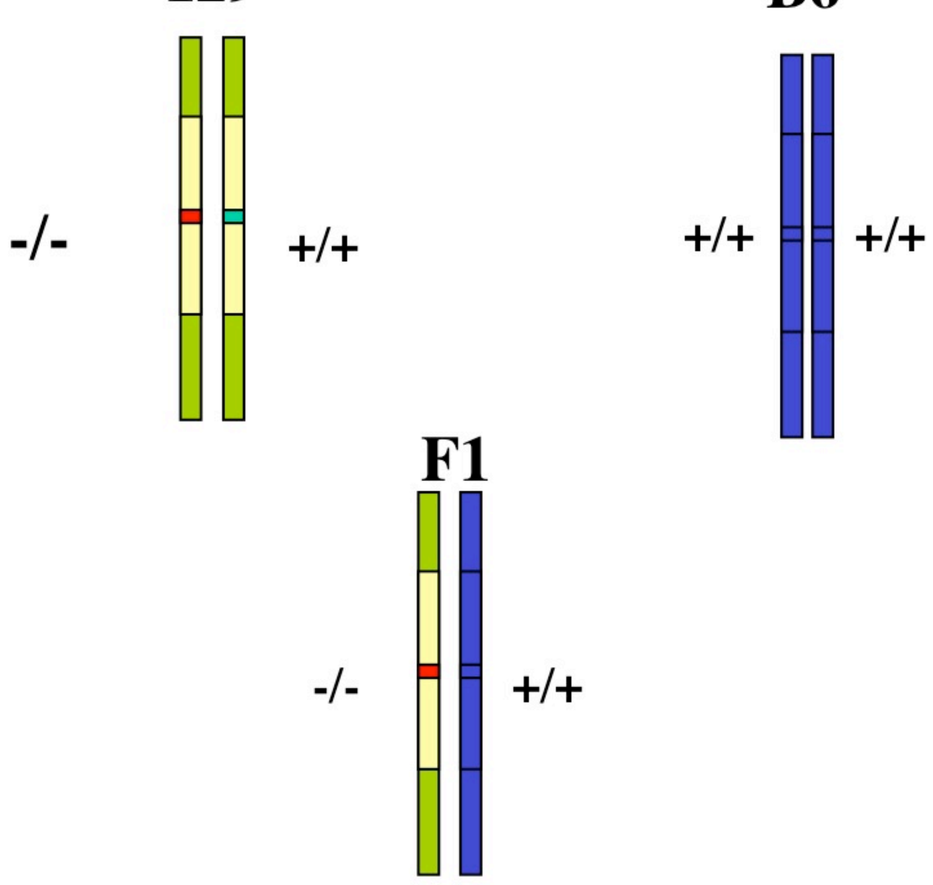
(depending on the nature of the differential locus; Lyon et Searle, 1989).

TARGETED GENE REPLACEMENT IN MICE



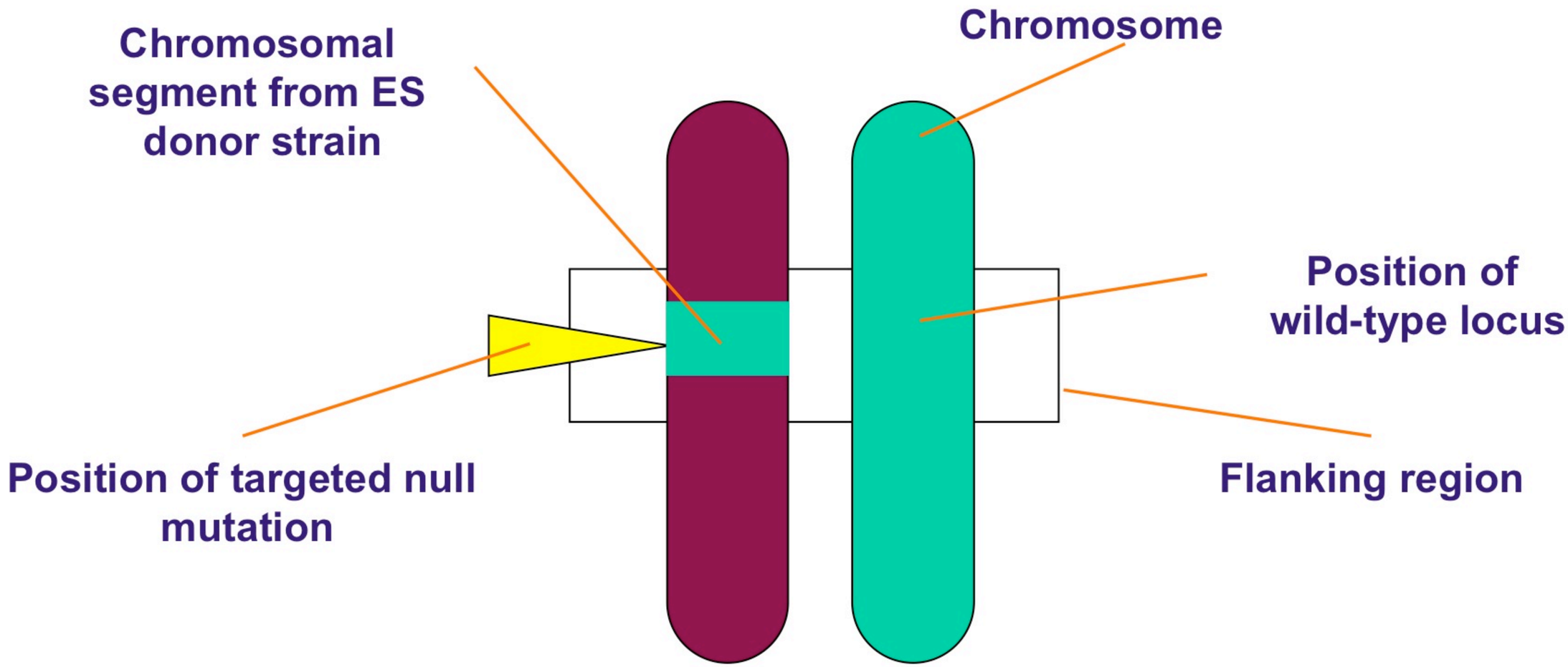
Mutations induced by homologueous recombinaison

Gerlai, TINS 19, 177-181 (1996) Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype?

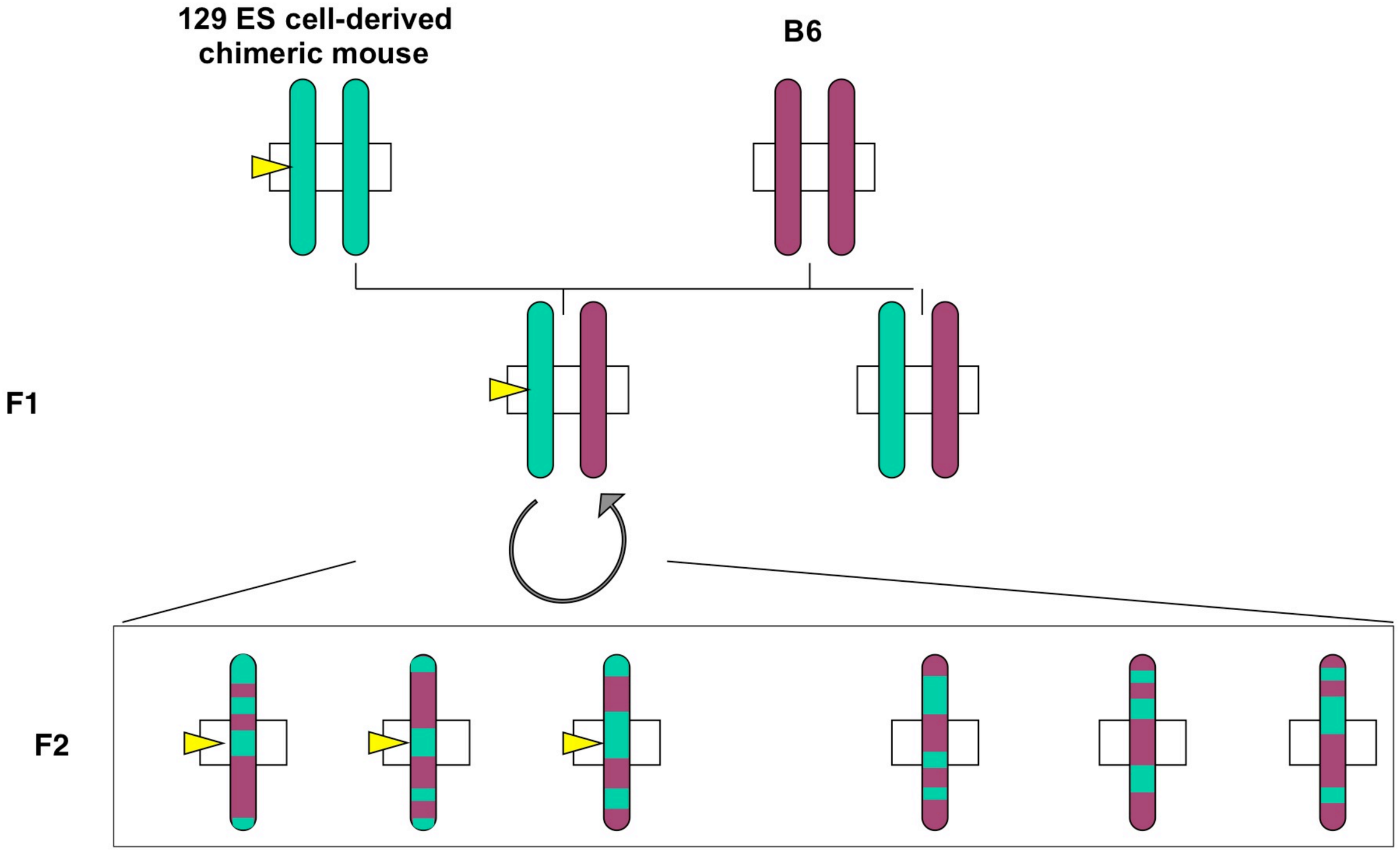


1- Flanking region

2 - Genetic background



TINS, 2002, vol 25

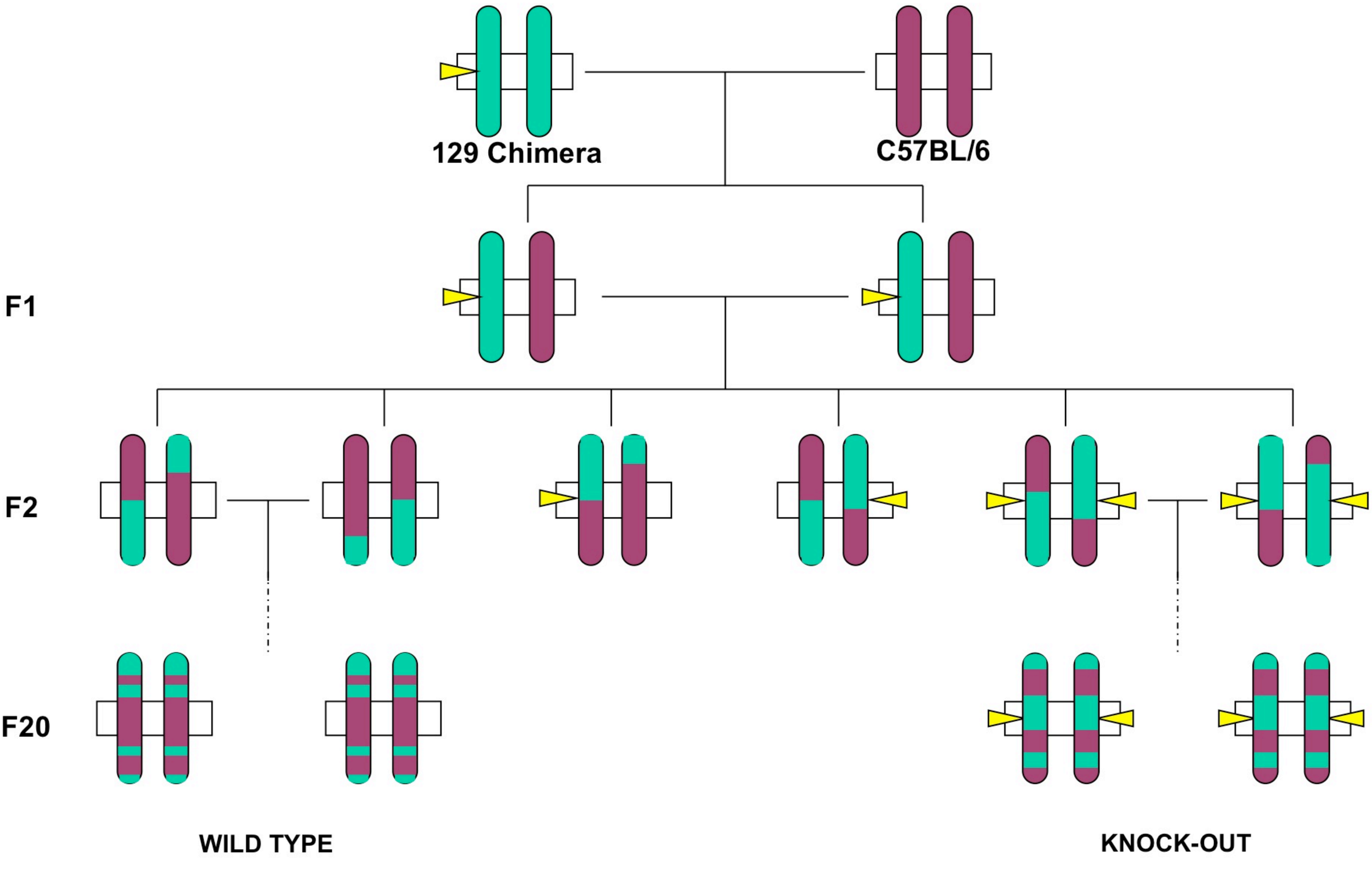


1- Flanking region

2- Genetic background

Breeding strategy

Inbreeding homozygous mice



Breeding strategy
Inbreeding homozygous mice

Violation of both principles of the Banbury conference:

1- Unknown genetic background

2- Unreproducible “ “

Over consecutive generations:

- Random segregation events
- Random fixation of alleles

No appropriate control

Systematic differences between wt and ko

Genetics of mouse behavior: interactions with laboratory environment

J. C. Crabbe, D. Wahlsten and B. C. Dudek

Science 284, 1670-2 (1999)

Multi-Center Trial of a Standardized Battery of Tests of Mouse Behavior

NIH Office of Behavioral and Social Science Research via NIAAA and NIDA

Web site: http://www.albany.edu/psy/obssr

Experimental factors

Genotype (Stock): (8)

A/J
C57BL/6J
BALB/cByJ
DBA/2J
129/SvEvTac
5HT1B++
5HT1B--
B6D2F2

Sites : (3)

Albany
Edmonton
Portland

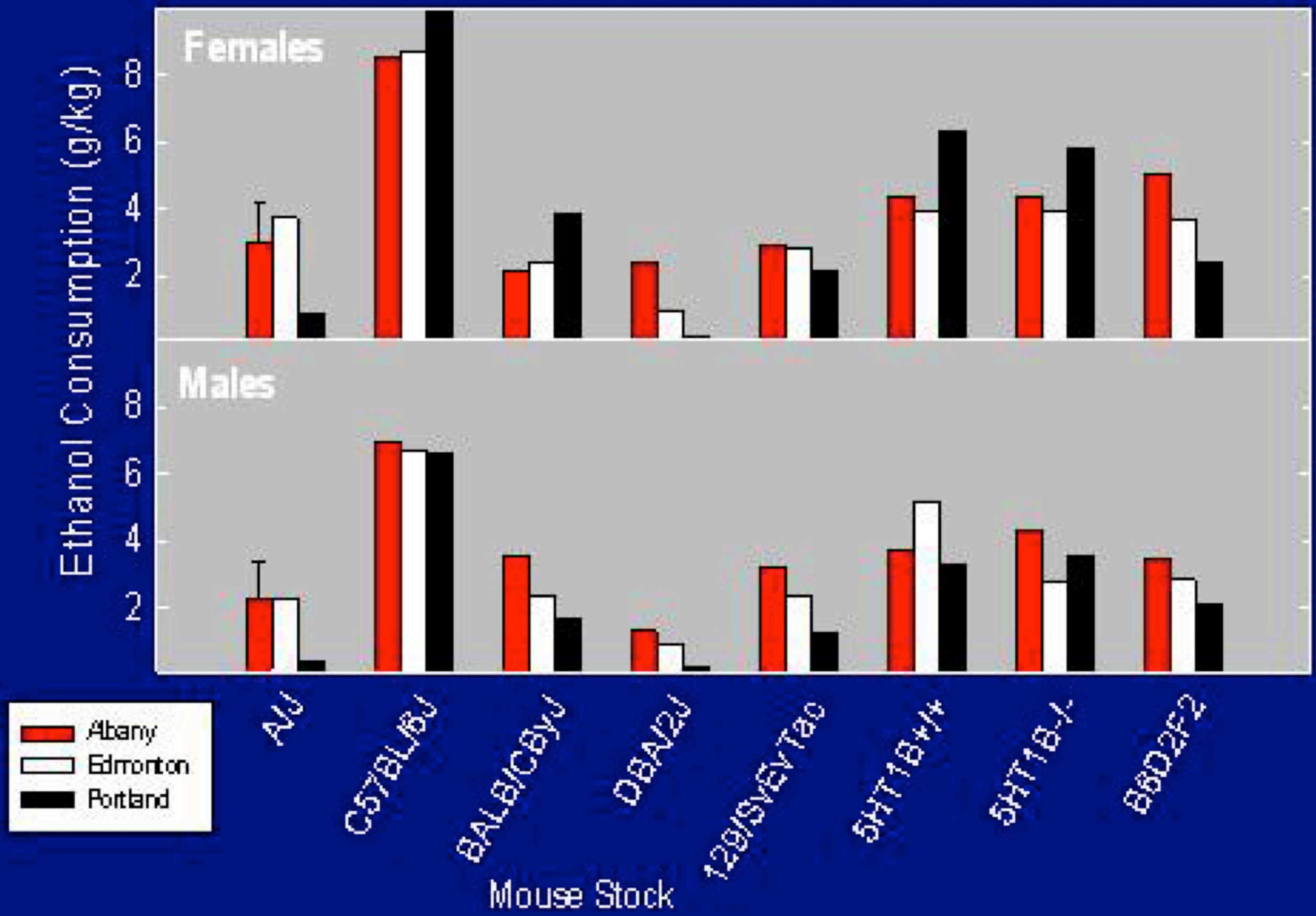
Sexes : (2)

Female
Male

Shipping Status : (2)

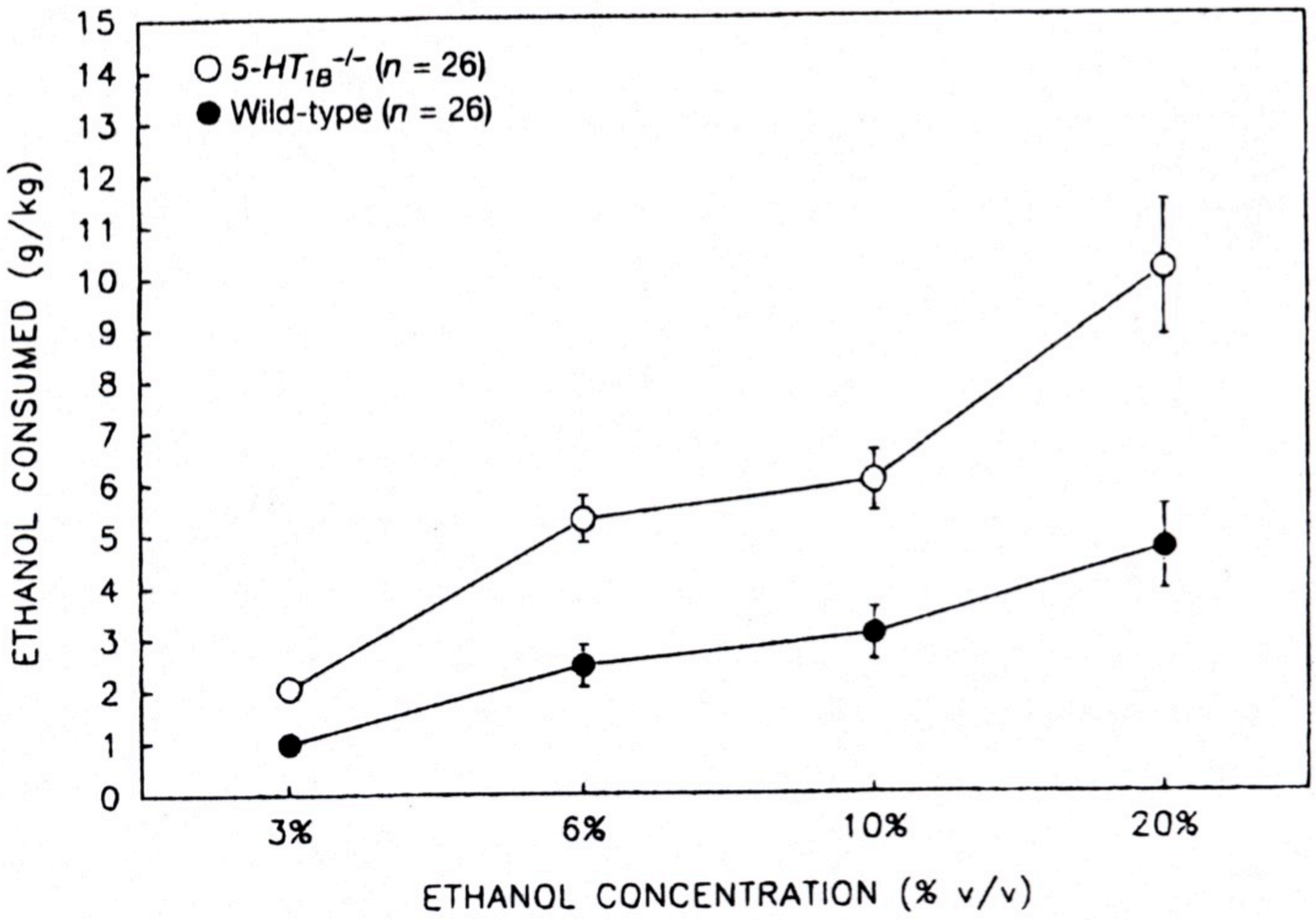
Bred In House
Shipped

Average Four Day EtOH Consumption (g/kg)



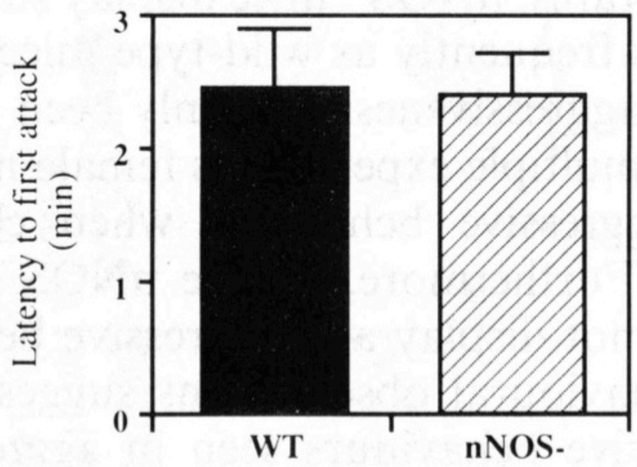
Elevated alcohol consumption in null Mutant mice lacking 5-HT1B serotonin receptors

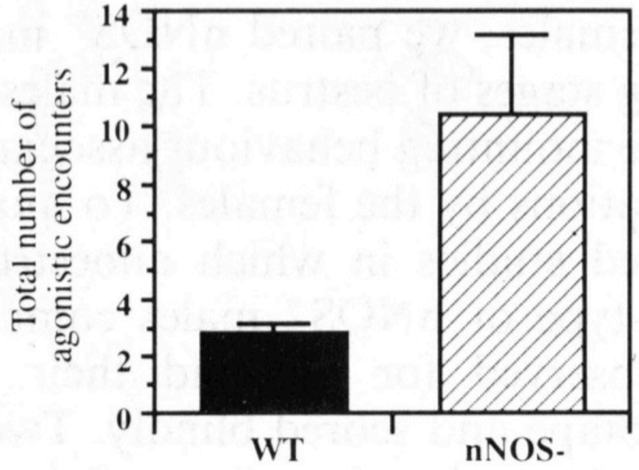
Crabbe et al., Nature Genetics 14, 1996

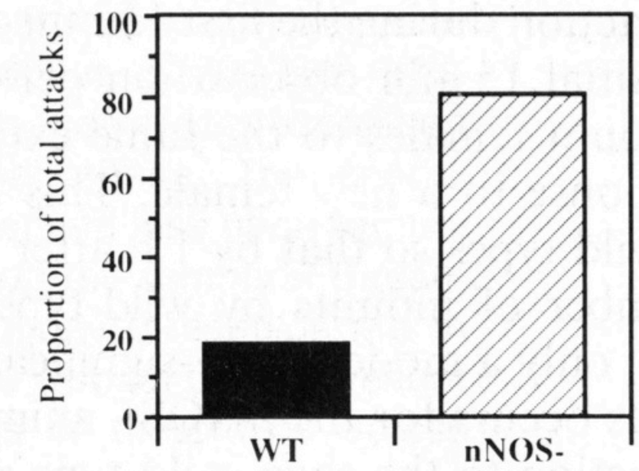


Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase

Nelson et al., Nature 378, 1995





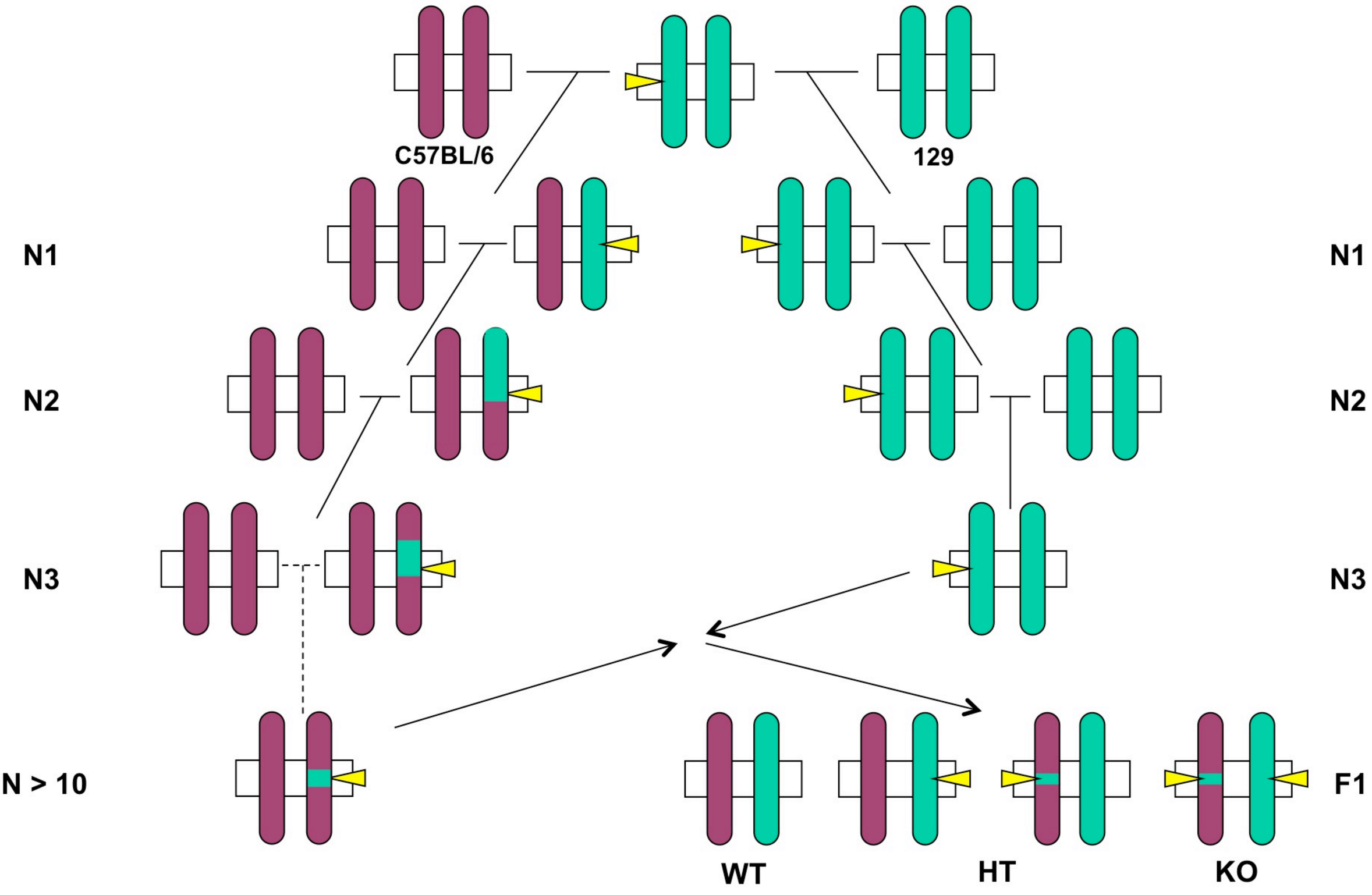


1/ Increased in aggressive behaviour
2/ Excess and inappropriate sexual behaviour

✓ nNOS^{-} KO mice

✓ WT = age-matched C57B6/J and 129 SvEv

Breeding strategy
Simultaneous derivation of two congenics



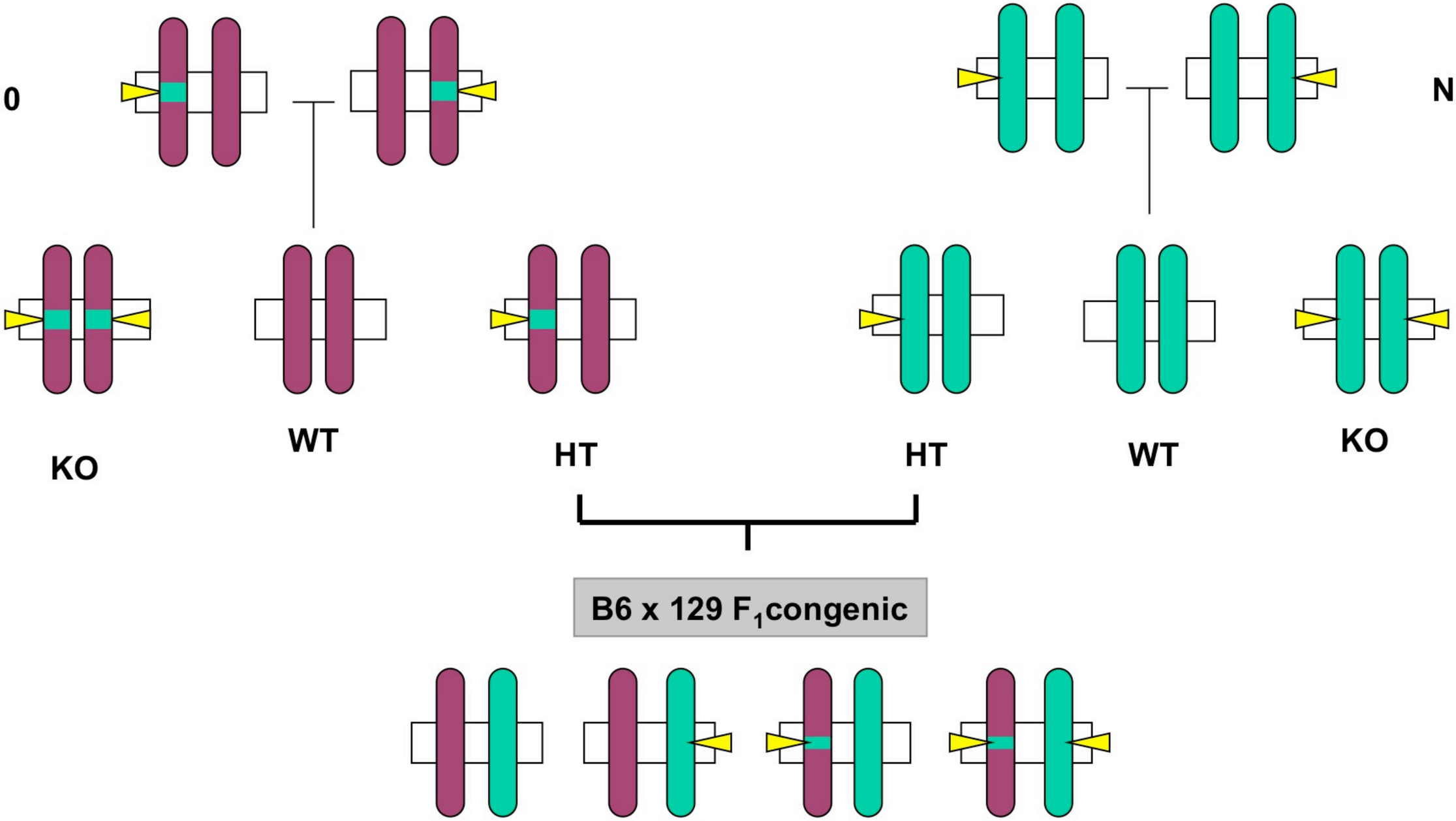
Breeding strategy
Simultaneous derivation of two congenic lines

B6 congenic

129 coisogenic

N>10

N3



KO

WT

HT

HT

WT

KO

B6 x 129 F_1congenic

Breeding strategy
Simultaneous derivation of two congenics

Respect of both principles of the Banbury conference :

1- Known genetic background

2- reproducible “ “

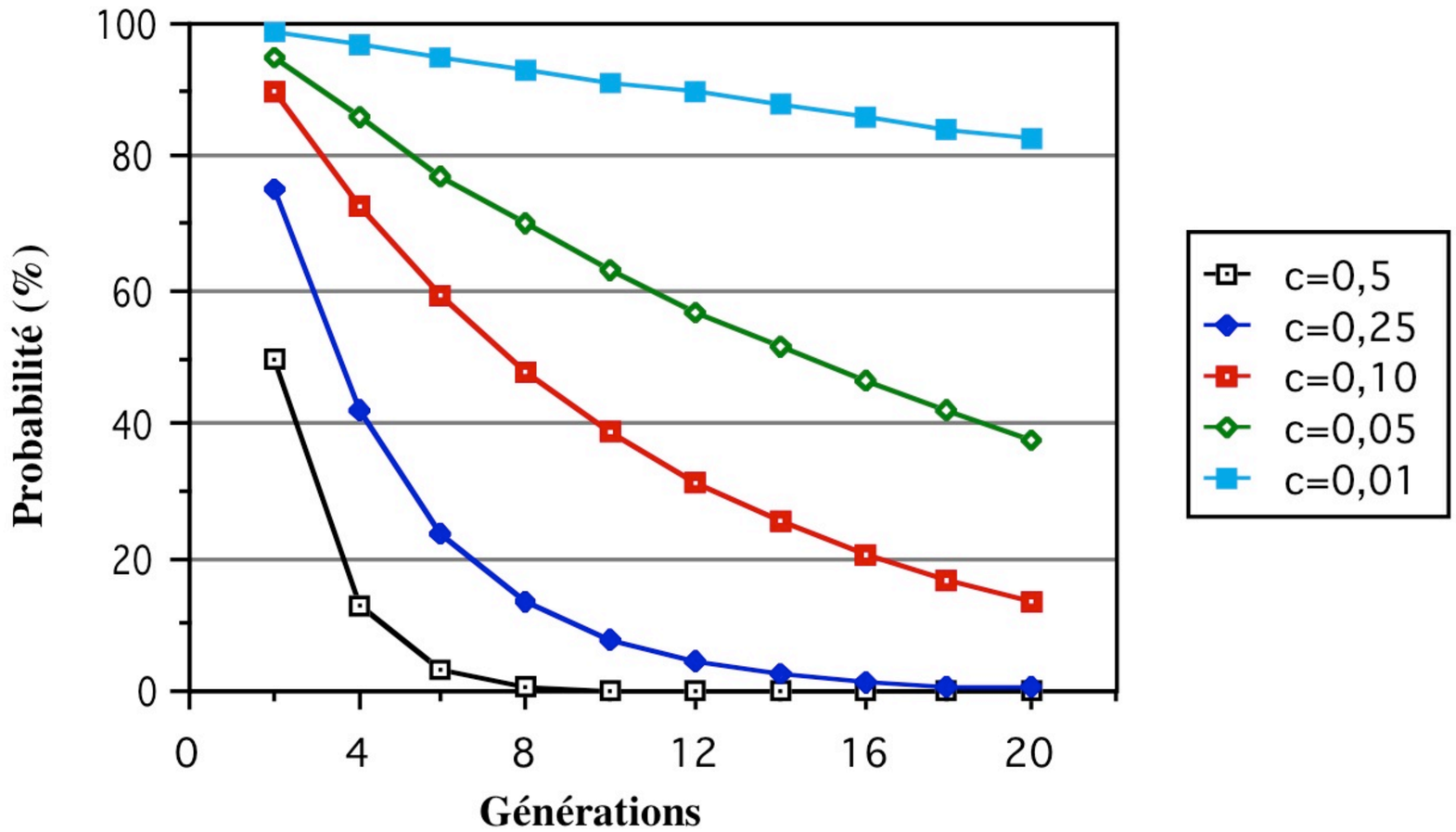
Continuous backcrossing:

✓Reduces the risk of genetic drift

✓Reduce the size of the differential segment

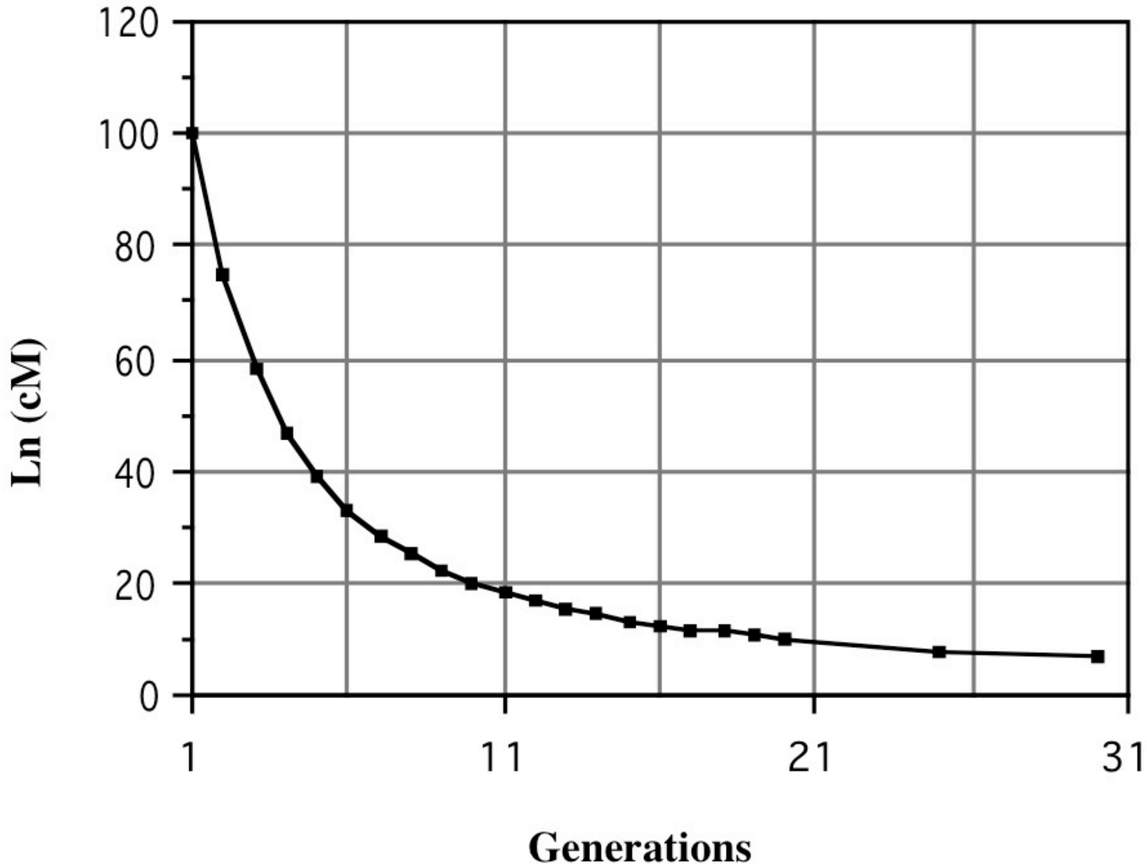
- Differential segment
- The choice of a genetic background: B6 x 129 F1

Probabilité de garder un gène de la lignée donneuse



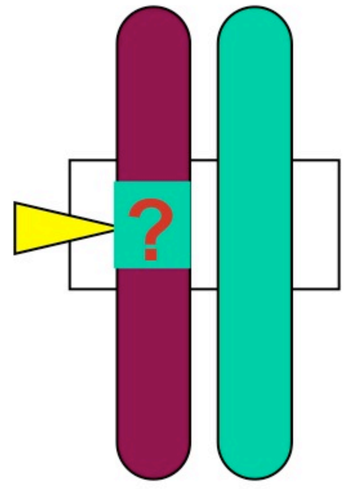
At n backcross generations,
where c is the frequency of recombination between the two loci
(From Flaherty, 1981)

Length of chromosomal segment Ln containing the differential locus as a function to the backcross generation



At G12, the length is about 17cM (congenic for a region - Flaherty, 1981).
n=number of backcross; Ln=mean length of differential chromosomal segment

How many mutations might the flanking DNA from strain 129 contain?
Richard LATHE



Genetic distance: 129 and B6: 1 mutation per kb

Mouse genome: 1600 cM = 100 000 genes

1 gene: 30 kb = 1,1 kb of coding region

Silent mutations (codon redundancy): 2/3

Neutral mutation: 50 %

Backcross:
N = 3 generations
60 cM = 3750 genes

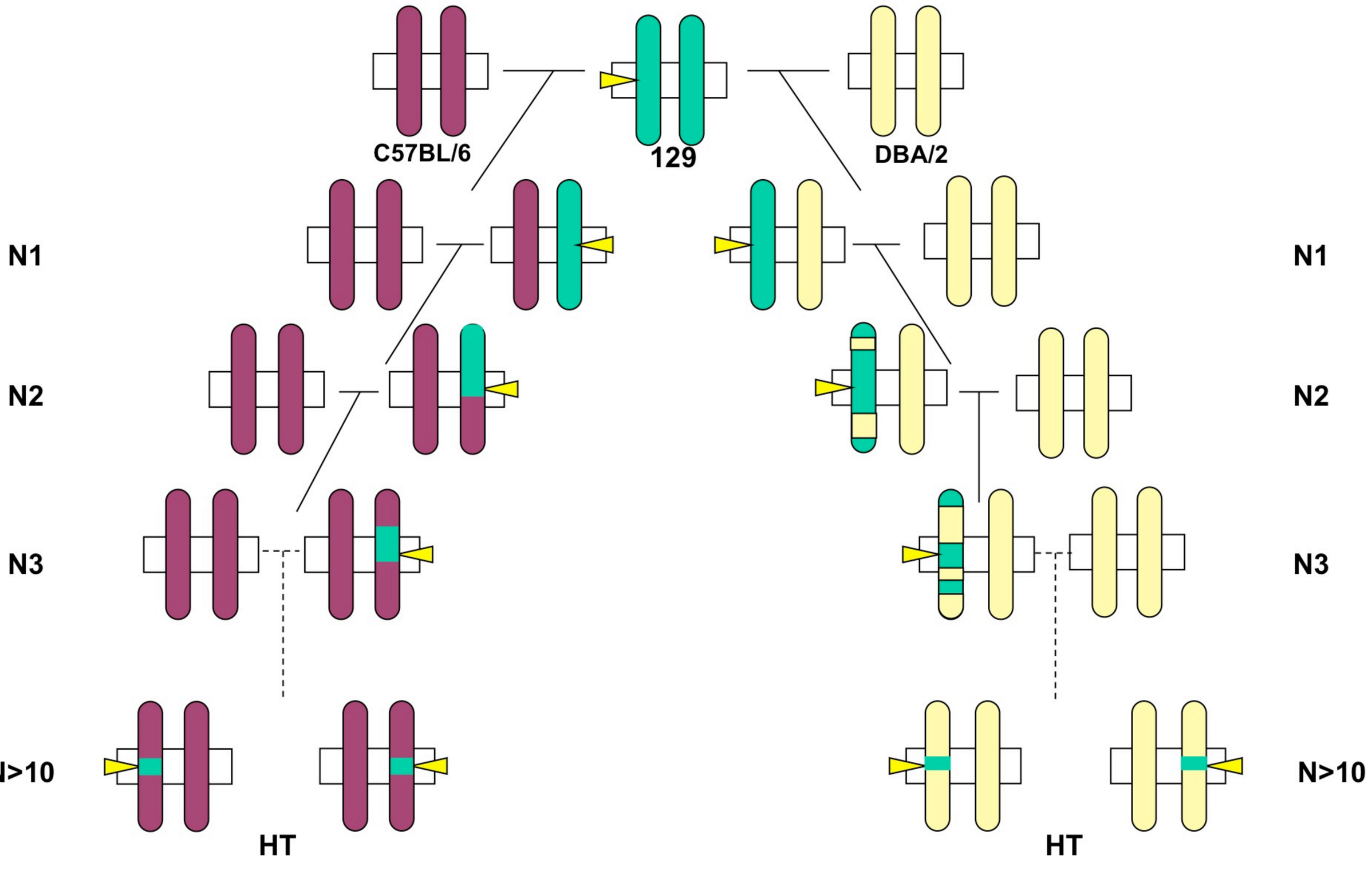
➢ **375 linked-genes from 129**

Backcross:
N = 12 generations
17 cM = 1000 genes

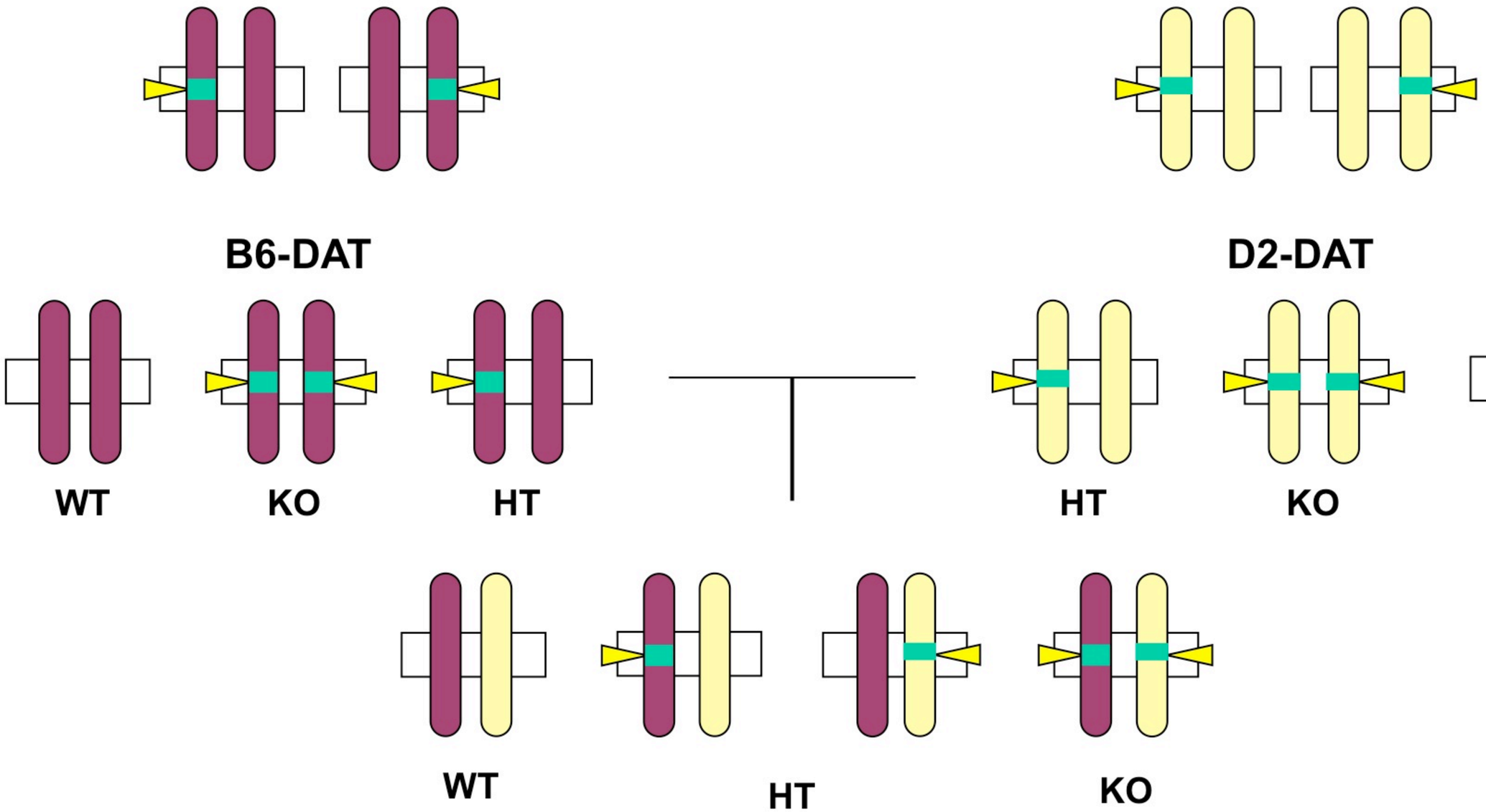
- ≈ 360 genes with a significant mutation
- Genes expressed in the CNS: 30 %:

➢ **100 linked-genes from 129**

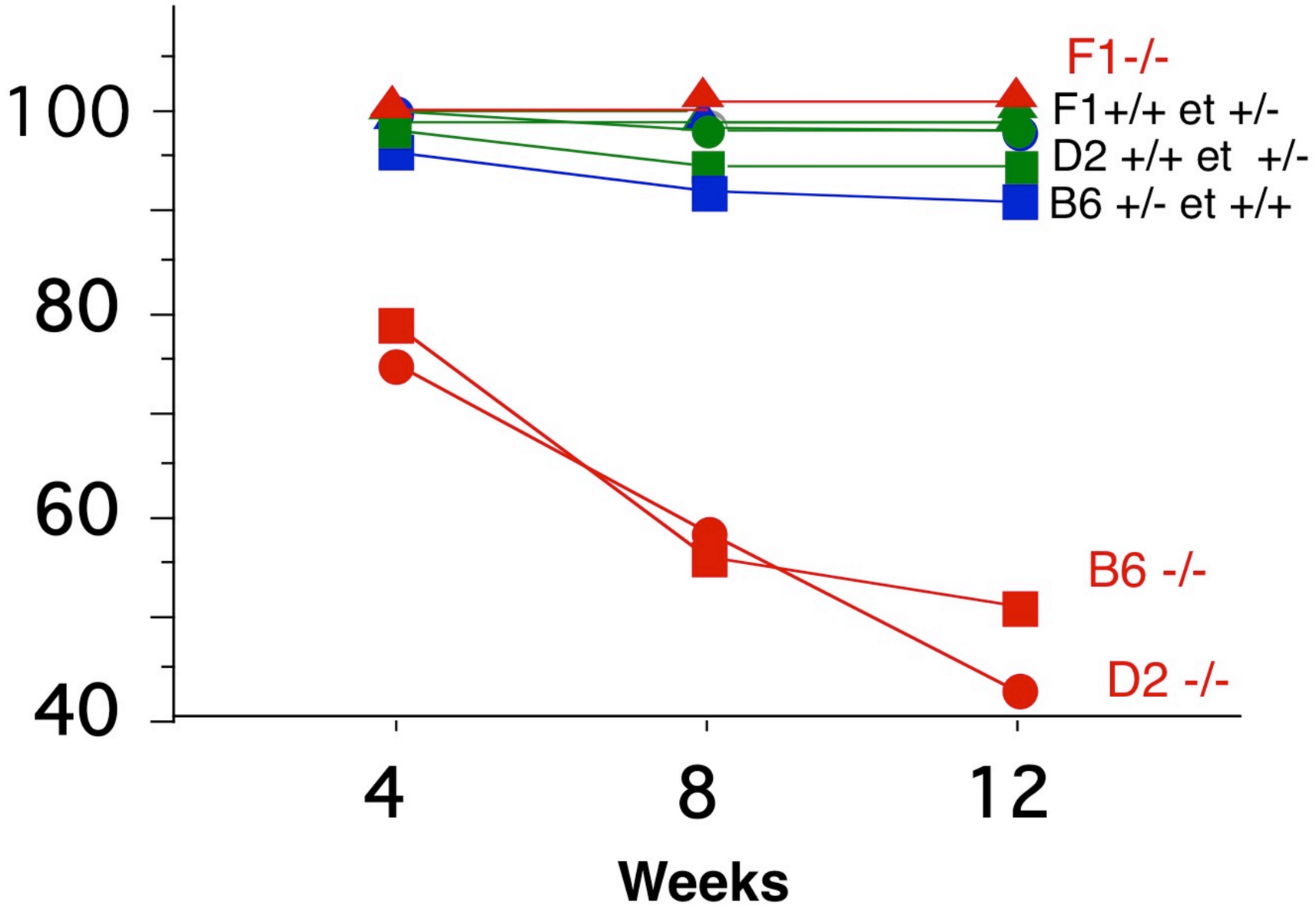
Breeding strategy
The choice of the genetic backgrounds
Example of the DAT-KO mice



Breeding strategy
The choice of the genetic backgrounds
Example of the DAT-KO mice



DAT-KO, genetic backgrounds and survival (%)



Morice et al., 2004

CONCLUSIONS

Banbury conference Recommendations

✓ Any report/publication: detailed description of the genetic background

✓ genetic background chosen for:

- reproducibility
- facilitate the comparison across experiments
- facilitate the comparison among laboratories

✓ Mutants maintained in congenic lines

✓ Mutants analysed in hybrid F1 genetic background

✓ Mutations should be derived simultaneously in different ES cells

✓ Mutations should be analysed simultaneously in different backgrounds

✓ Genetic backgrounds can be used as a tool to analyse a mutation (Quantitative trait loci analysis, Identification of modifier genes)