Genomics of sexual isolation and reinforcement in the house mouse

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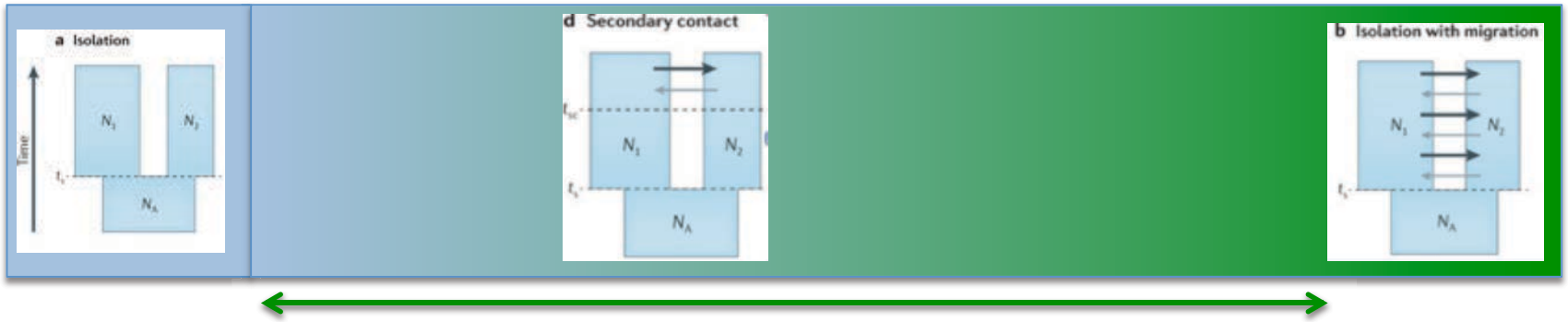
Secondary contacts and hybridisation are common

m (migration rate)

0 1/2

Allopatry | Recent secondary contact | Ancient secondary contact | Primary contact (sympatry)



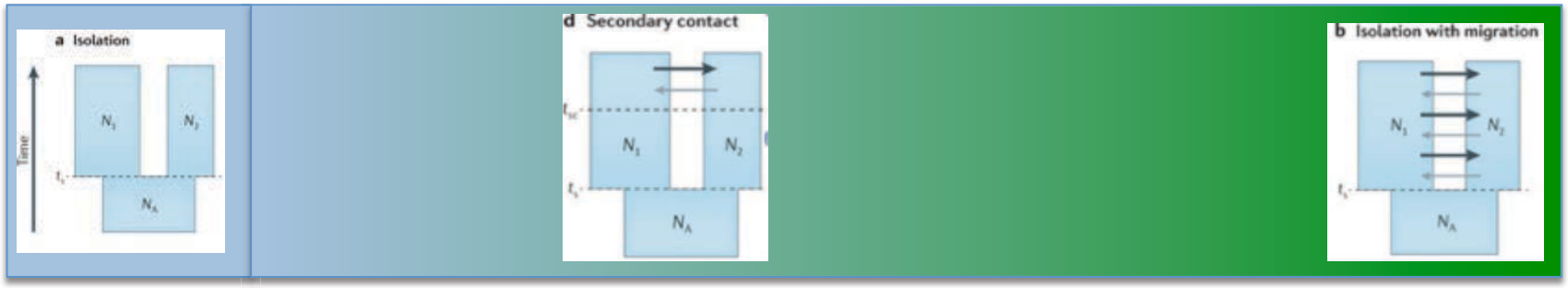
=> Detection of episodes of gene flow in many histories of speciation

Secondary contacts and hybridisation are common

m (migration rate)

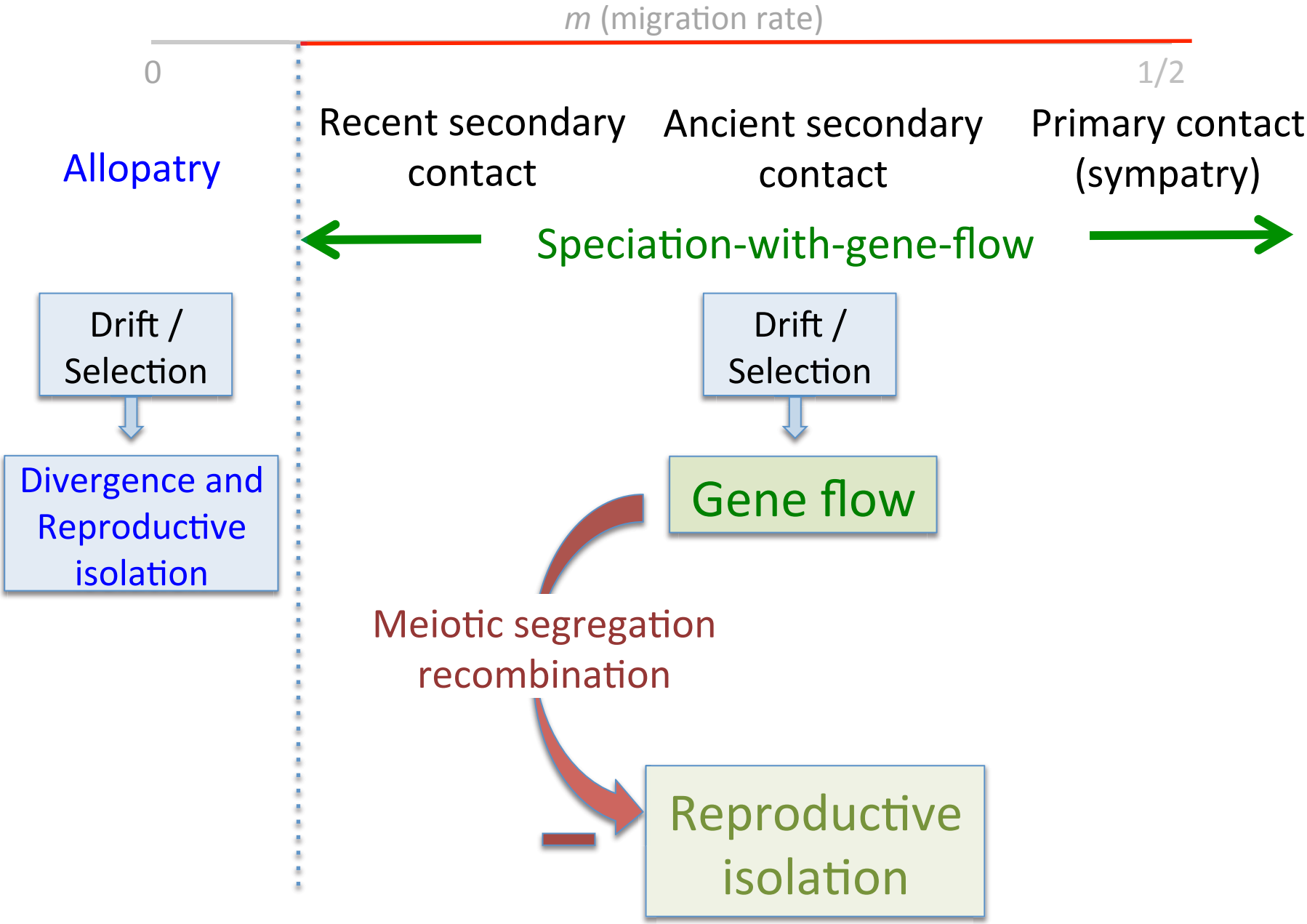
0 1/2

Allopatry | Recent secondary contact | Ancient secondary contact | Primary contact (sympatry)

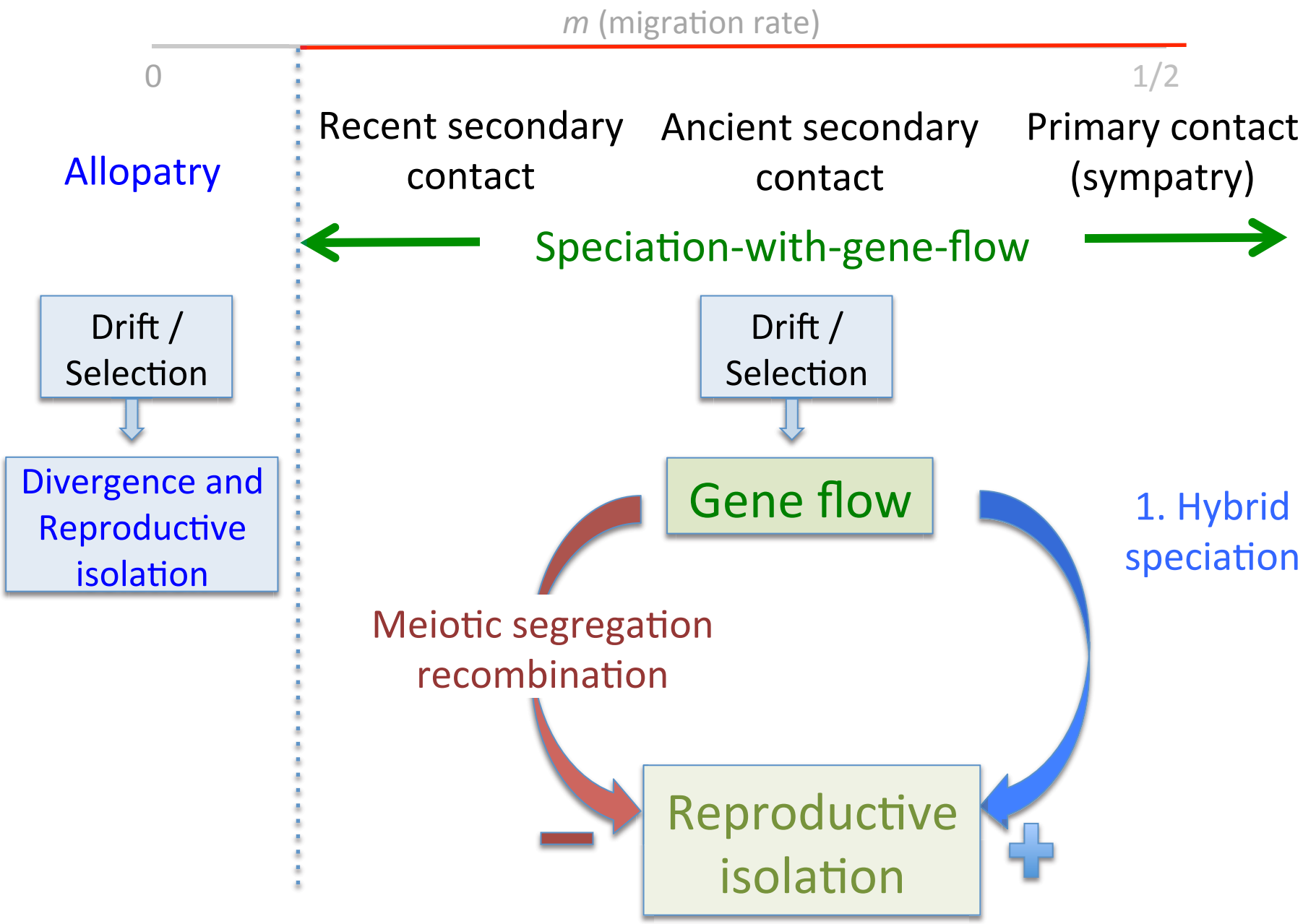


Speciation-with-gene-flow

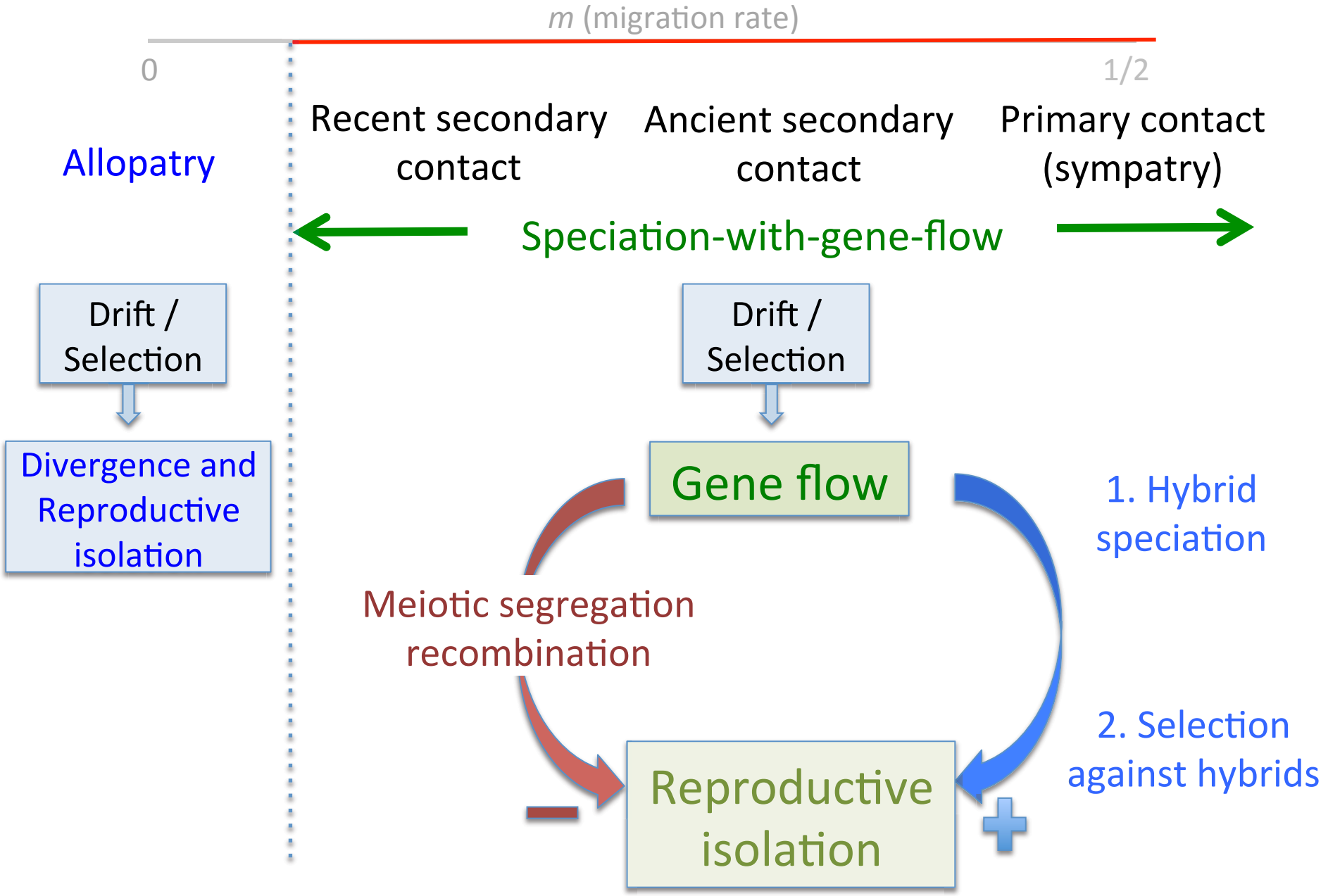
Hybridisation and speciation



Hybridisation and speciation



Hybridisation and speciation



Reinforcement

« Prezygotic isolating barriers are strenghtened
in contact zones
as a consequence of selection against hybridisation »
Dobzhansky, 1937

1940-2000: highly debated

2000+: increasing empirical and theoretical support

Reinforcement

« Prezygotic isolating barriers are strenghtened
in contact zones
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1940-2000: highly debated → 2000+: increasing empirical and theoretical support

Theoretical models

Conditions

Gene flow

Selection

Initial divergence

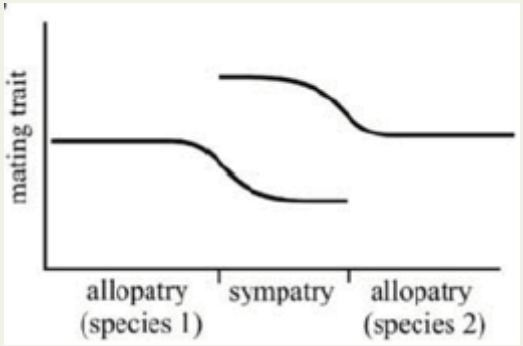
Factors promoting reinforcement

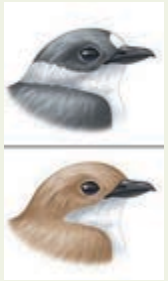
Phenotypic architecture

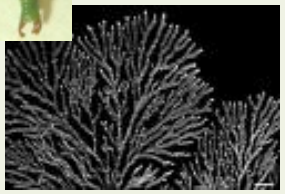
Genetic architecture

Testing for reinforcement

Reproductive character displacement



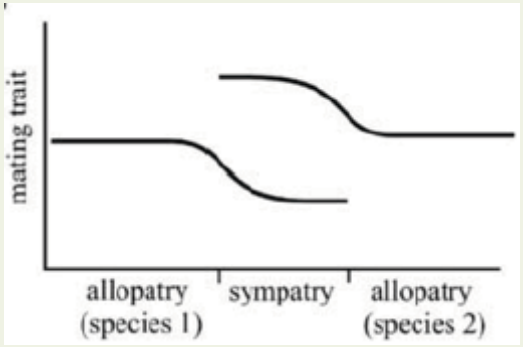


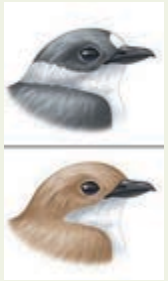


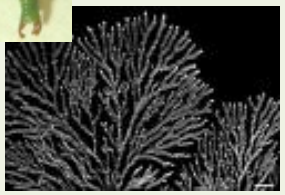
Indirect evidence

Testing for reinforcement

Reproductive character displacement







Indirect evidence

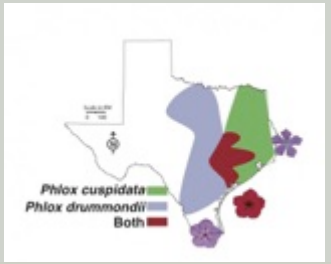
Toward more direct evidence

Experimental evolution

Drosophila serrata & *birchii*

Combining evidence at the phenotypic and genomic levels

Phlox

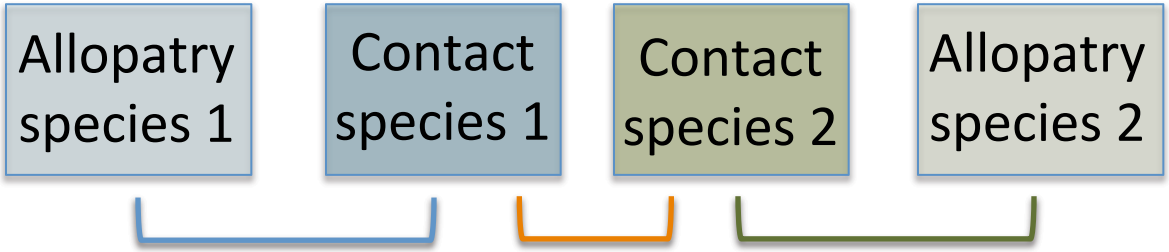


Studying reinforcement

Current challenges

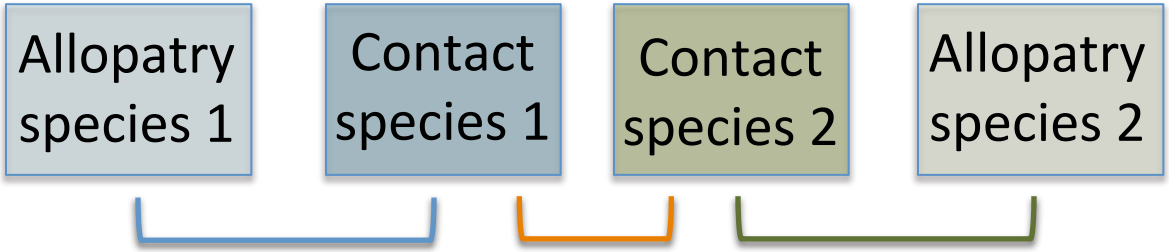
- Characterise genetic changes underlying prezygotic divergence
- Evidence the signature of reinforcing selection in natural systems
- Identify genetic factors favouring reinforcement

Genomics of prezygotic isolation and reinforcement



Genomic divergence between allopatric and contact populations

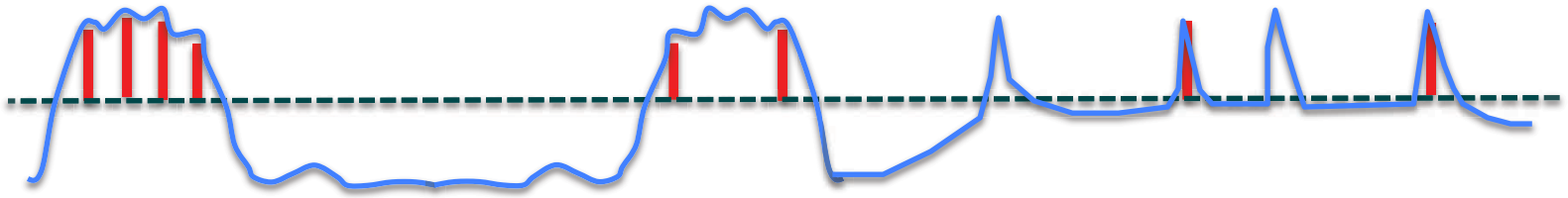
Genomics of prezygotic isolation and reinforcement



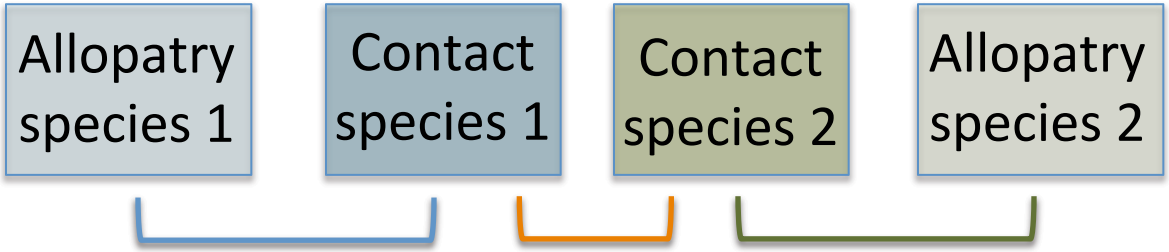
Genomic divergence between allopatric and contact populations

Genetic changes underlying prezygotic divergence

Identity and distribution of barrier loci



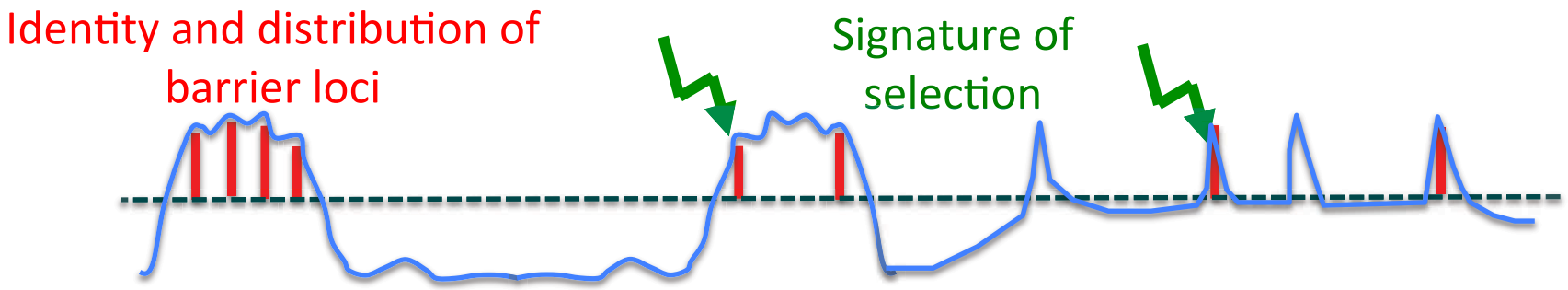
Genomics of prezygotic isolation and reinforcement



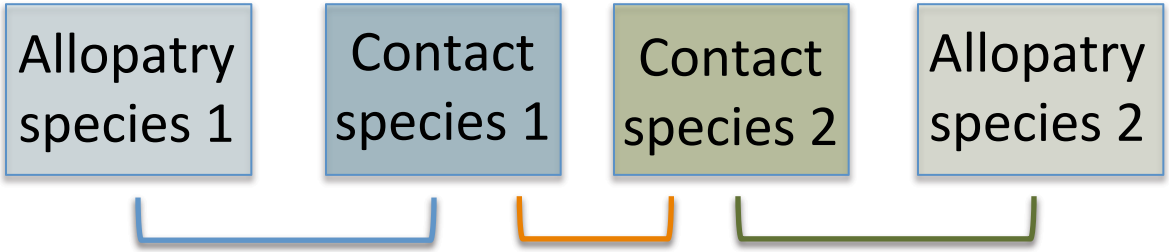
Genomic divergence between allopatric and contact populations

Genetic changes underlying prezygotic divergence

Signature of selection specific to contact populations?

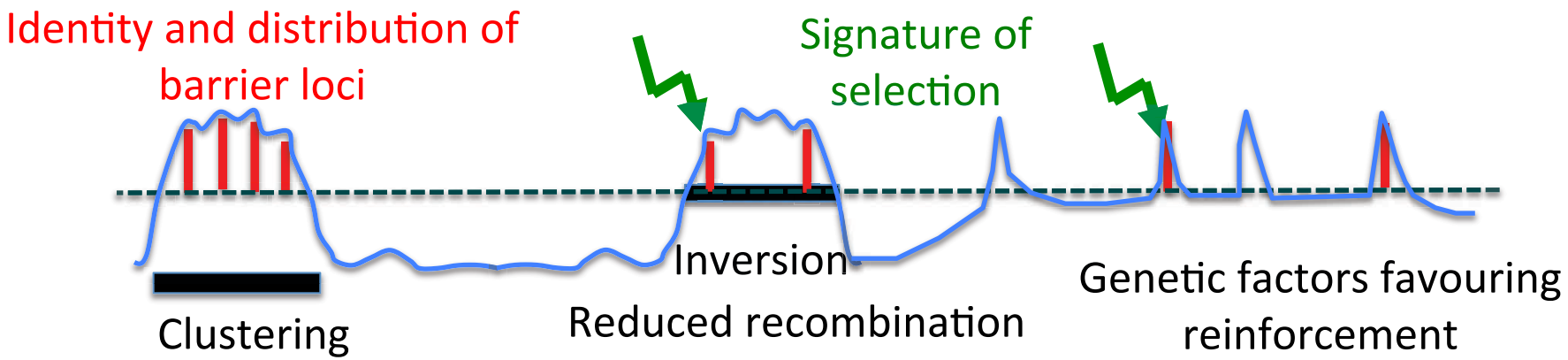


Genomics of prezygotic isolation and reinforcement



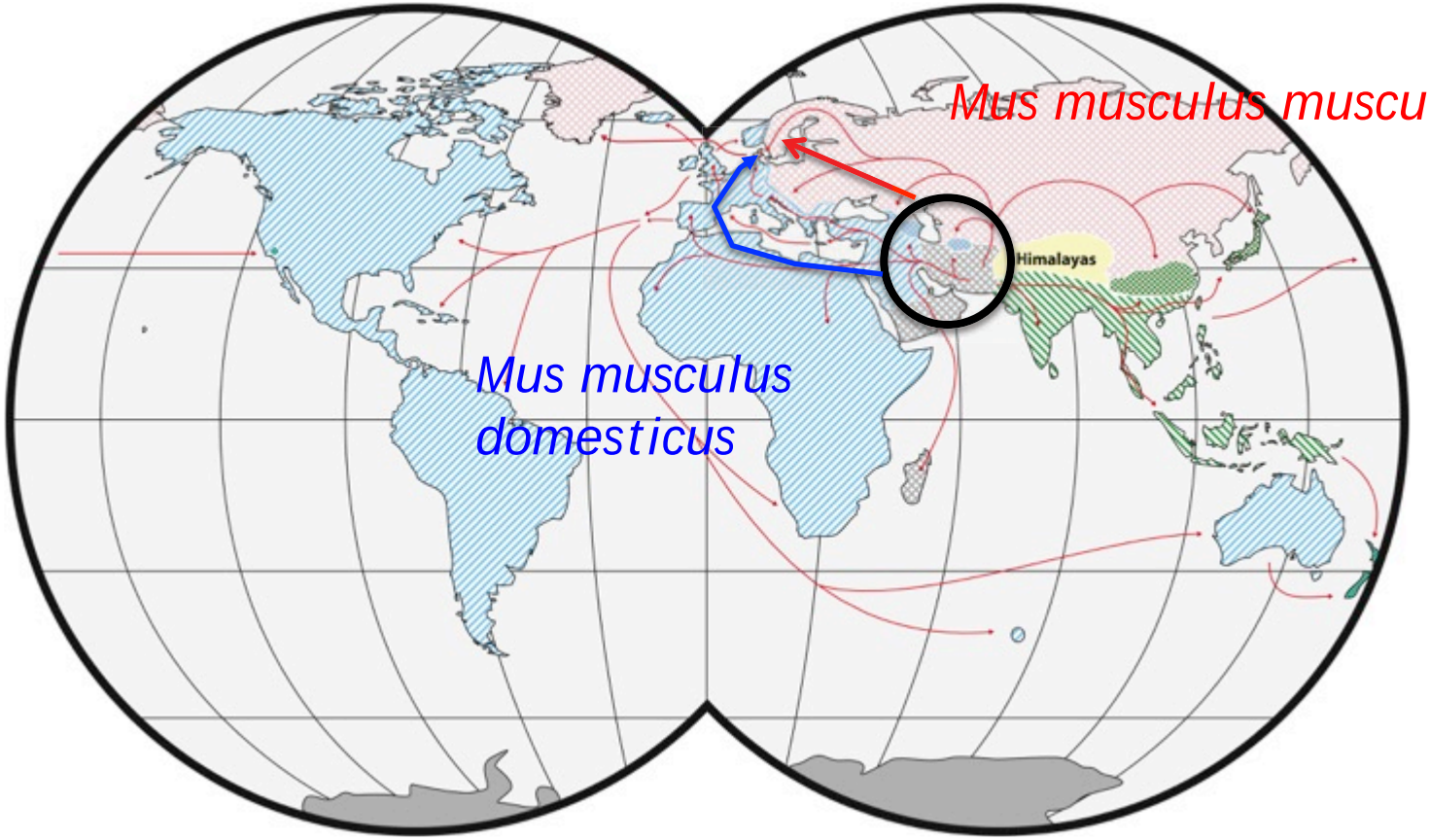
Genomic divergence between allopatric and contact populations

Genetic changes underlying prezygotic divergence	Signature of selection specific to contact populations?	Genetic factors favouring divergence



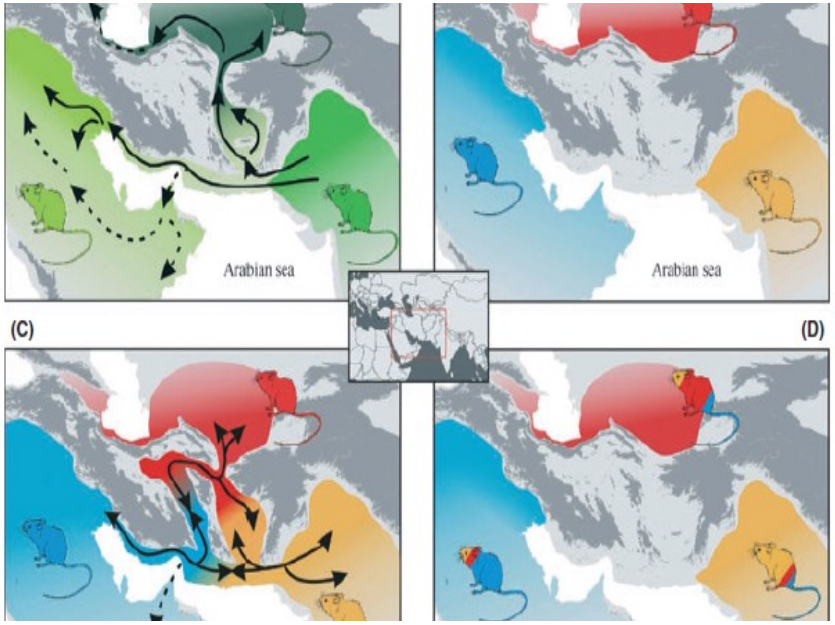
Genomics of sexual isolation and reinforcement in the house mouse

History of divergence between the two European subspecies of house mice



Divergence in allopatry for 0.5 million years
Recent secondary contact in Europe

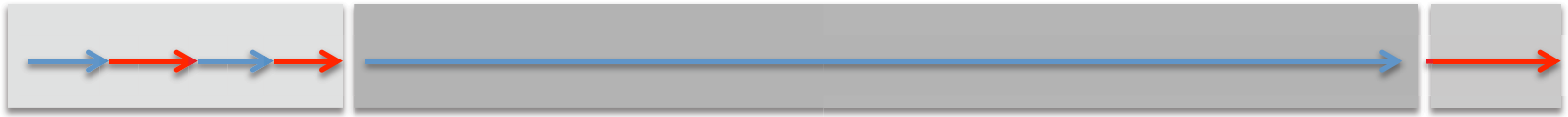
History of divergence between the two European subspecies of house mice



Initial divergence in central Asia: alterning periods of geographic isolation and gene flow

Long phase of allopatry

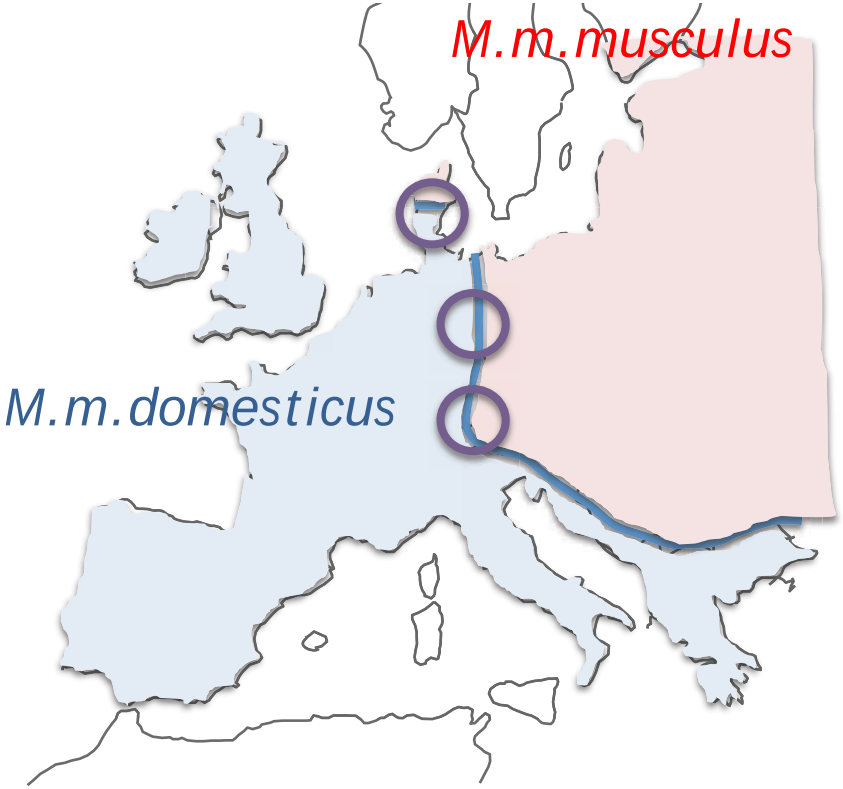
Recent secondary contact in Europe



Multiple episodes of allopatric divergence and secondary contacts

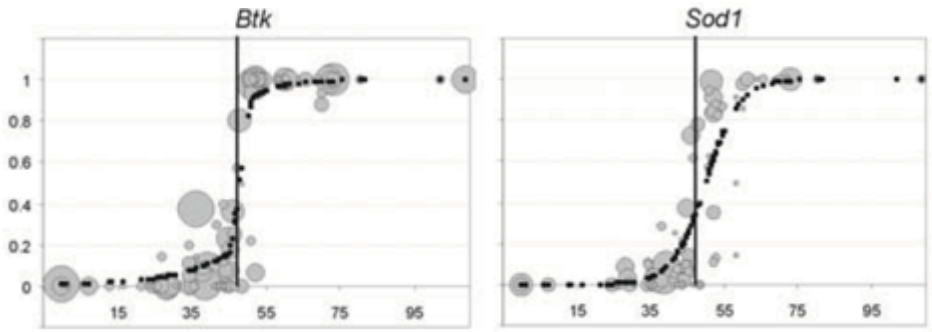
Strong genetic differentiation ($F_{ST\ autosomes}$ = 0.38; $F_{ST\ chrX}$ = 0.46)

Secondary contact and hybridisation in Europe



- Recent secondary contact (5,000 and 3,000 years ago)

- Tension zone



Reproductive isolation

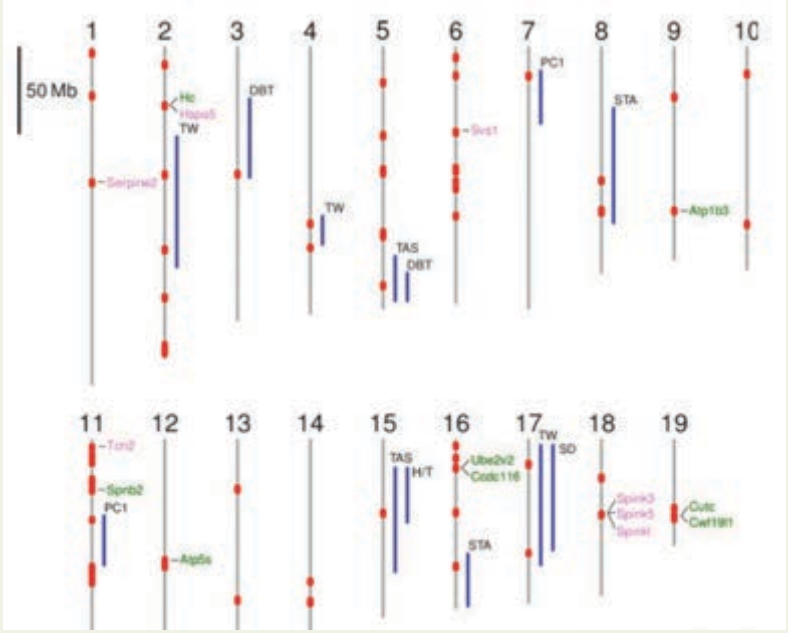
1. Selection against hybrids

Phenotypes

- **Hybrid male and female sterility**
- Hybrid microbiome dysfunction (Wang et al. 2015)
- Sexual selection against hybrids (Latour et al. 2015)

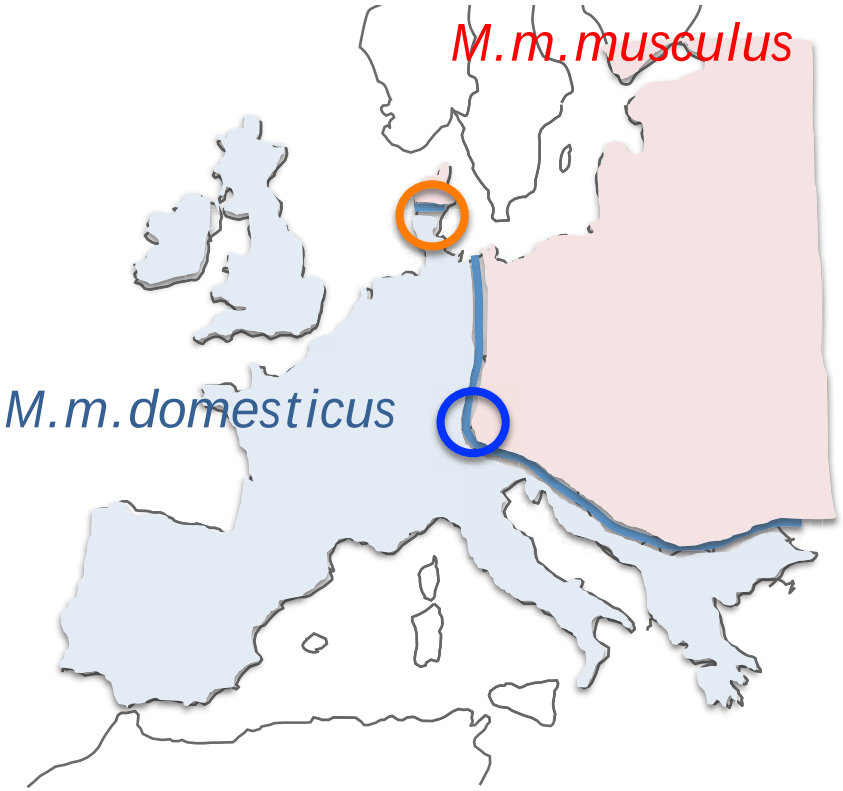
Genetic basis of hybrid sterility

Dobzhansky-Muller incompatibilities

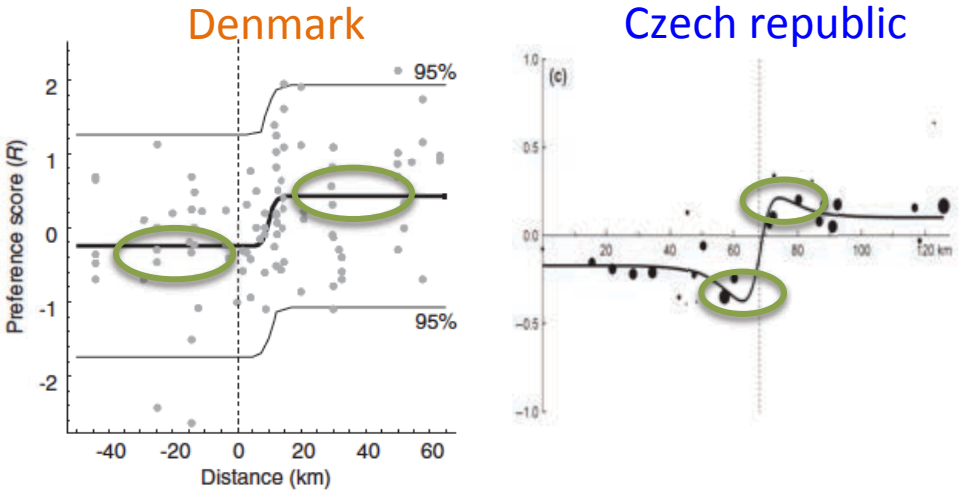


Reproductive isolation

2. Sexual isolation



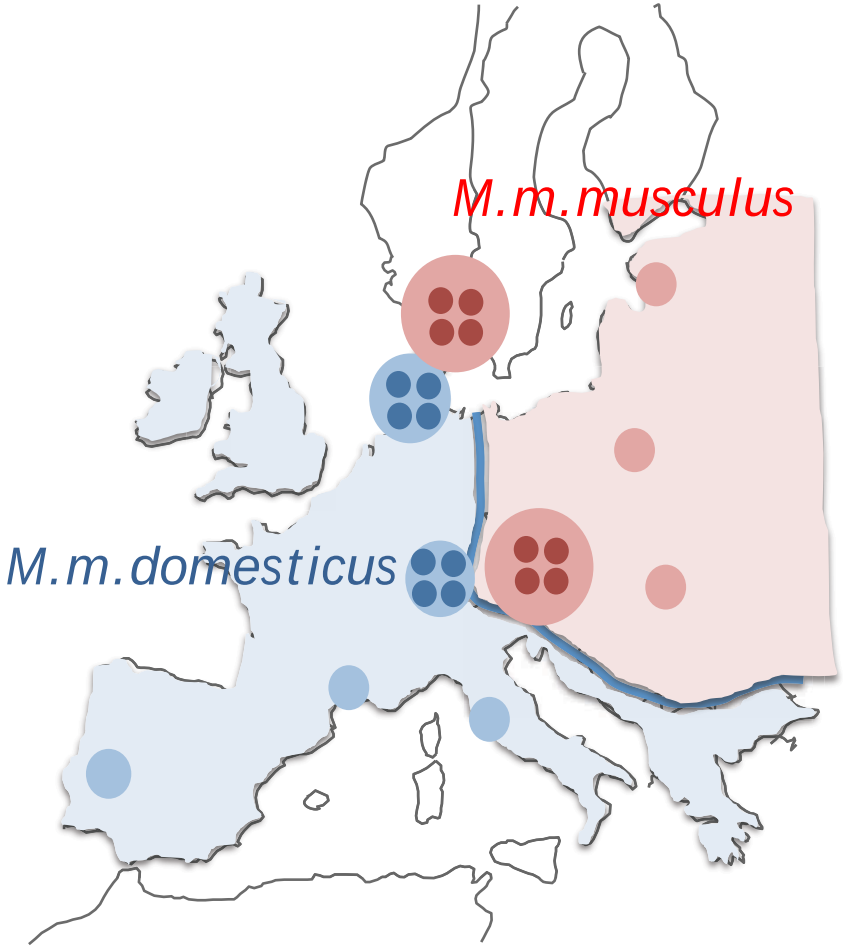
Cline of mate preferences across the hybrid zone



Assortative mate preference in populations from the border of the hybrid zone (2 to 10% introgressed)

Reproductive isolation

2. Sexual isolation



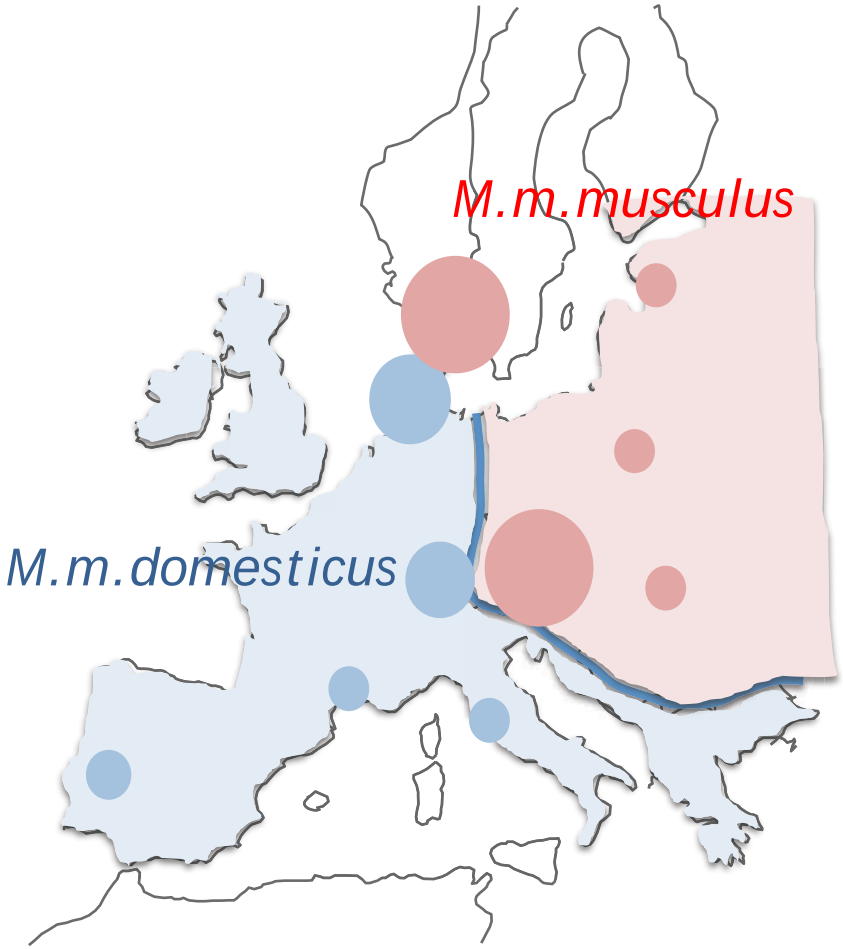
- Consistent assortative mate preference in border populations
- Both males and females
- Stronger on the *musculus* side
- Absent in allopatric populations

⇩

Reproductive character displacement

Reproductive isolation

Reinforcement?



Selection against hybrids

Sexual isolation:

assortative mate preferences in the hybrid zone

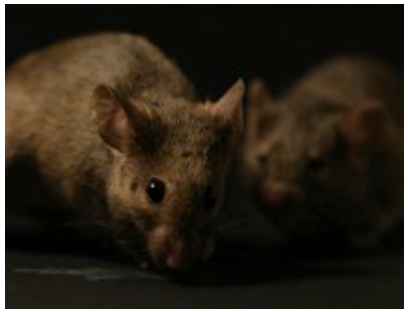
Reproductive character displacement

Arguments in favour of reinforcement

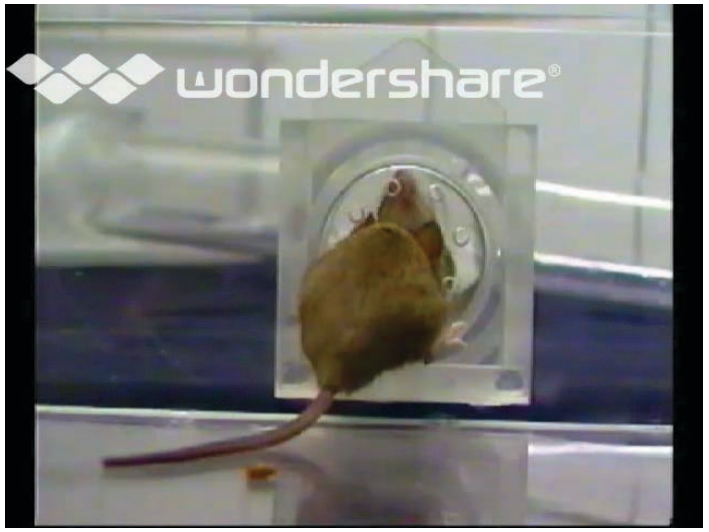
Genomics of sexual isolation

Assortative mate choice

Olfaction



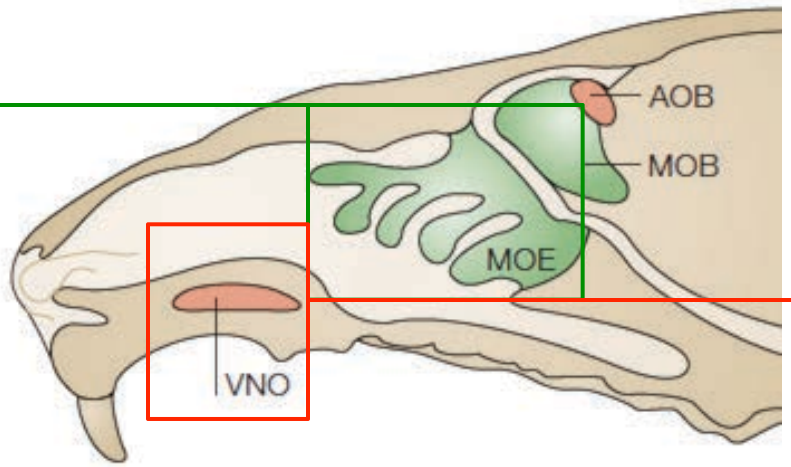
Key step: olfactory recognition of mating signals present in urine



Genes involved in the recognition of olfactory cues as good candidates

Olfactory systems in mice

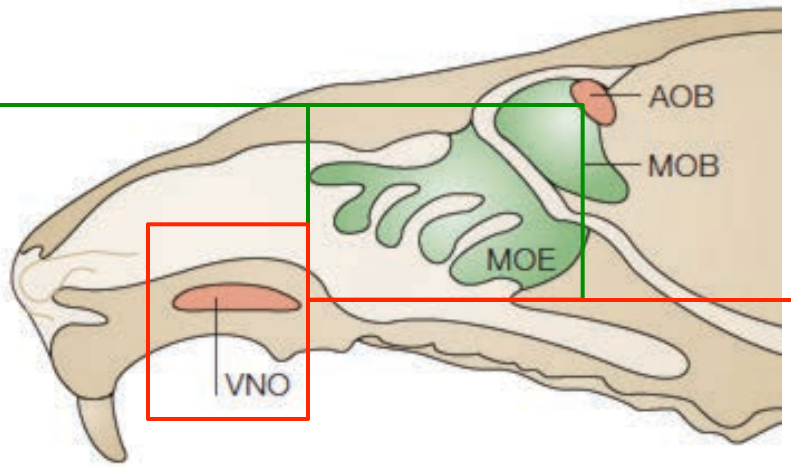
Main olfactory epithelium (MOE)



Vomeronasal organ (VNO)

Olfactory systems in mice

Main olfactory epithelium (MOE)

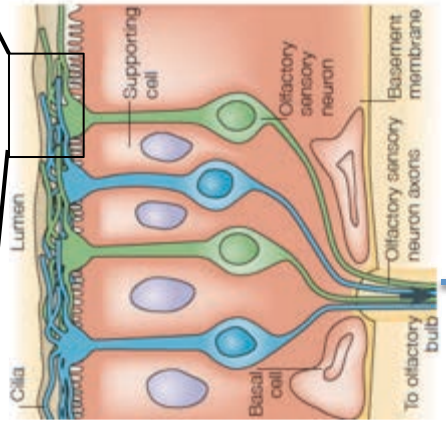


Vomeronasal organ (VNO)

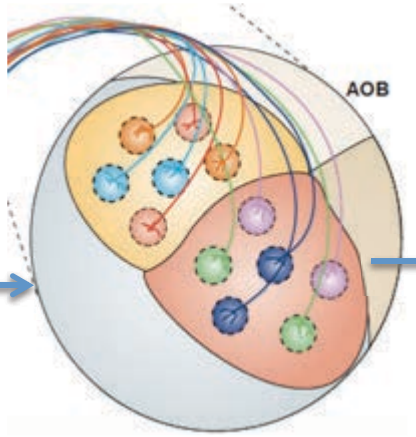
Olfactory receptors

Binding proteins

Olfactory signals



Olfactory sensory neurons



brain

Glomeruli in olfactory bulb

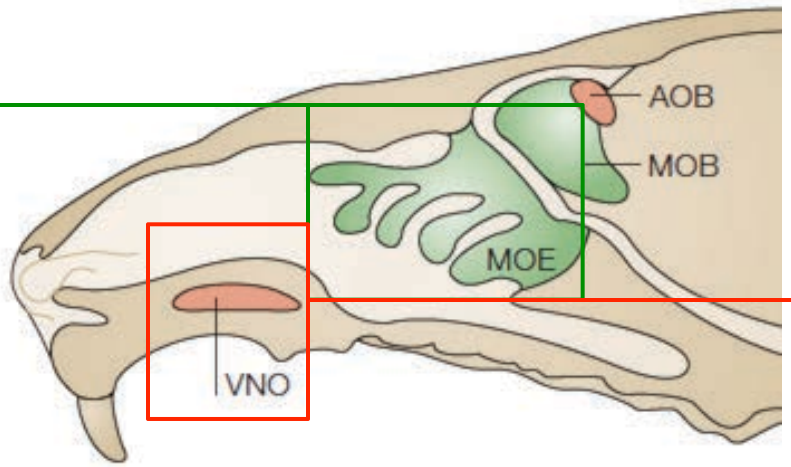
Functional organisation

Main olfactory epithelium (MOE)

Olfactory receptors (ORs)

Airborne signals

Recognition of signals from the environment



Vomeronasal organ (VNO)

Vomeronasal receptors (VRs)

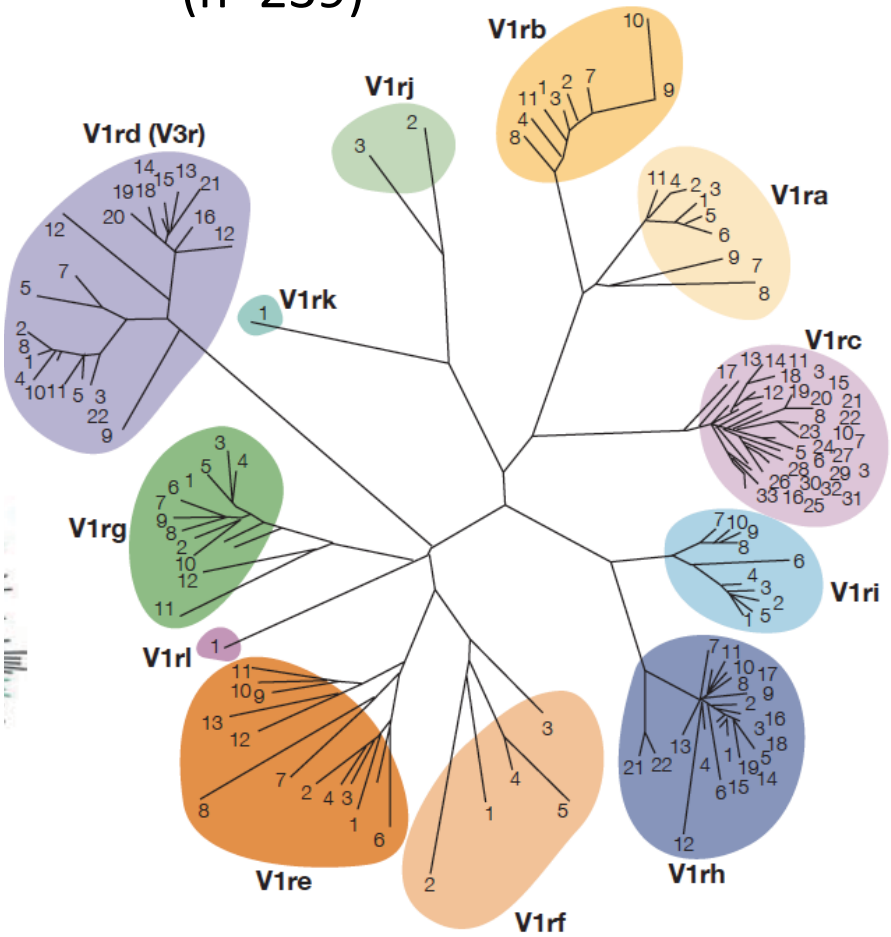
Airborne signals
Peptide signals

Kairomone and pheromone recognition

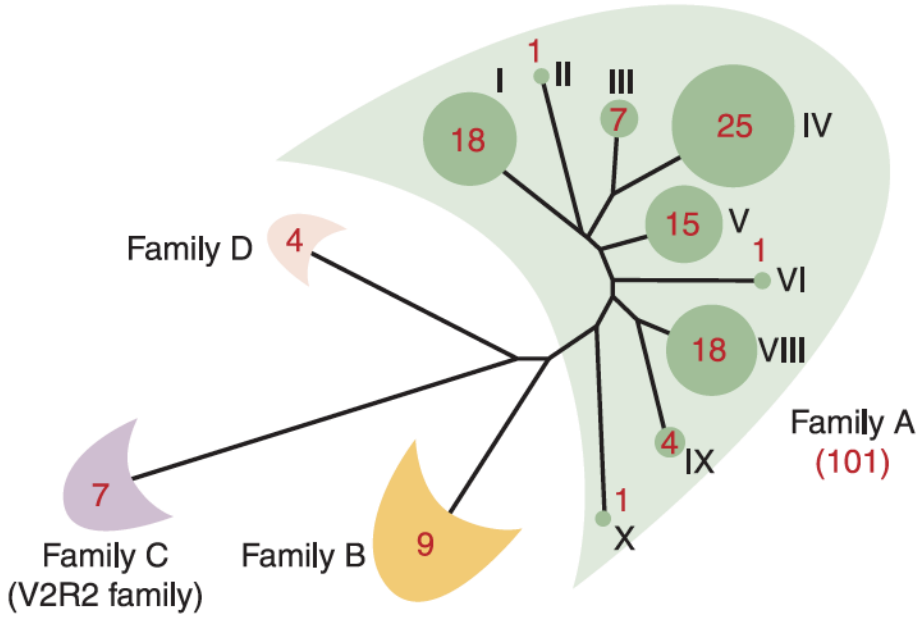
Organisation in the mouse genome

- Two large multigene families

Vomeronasal receptor type 1 (V1R) (n=239)

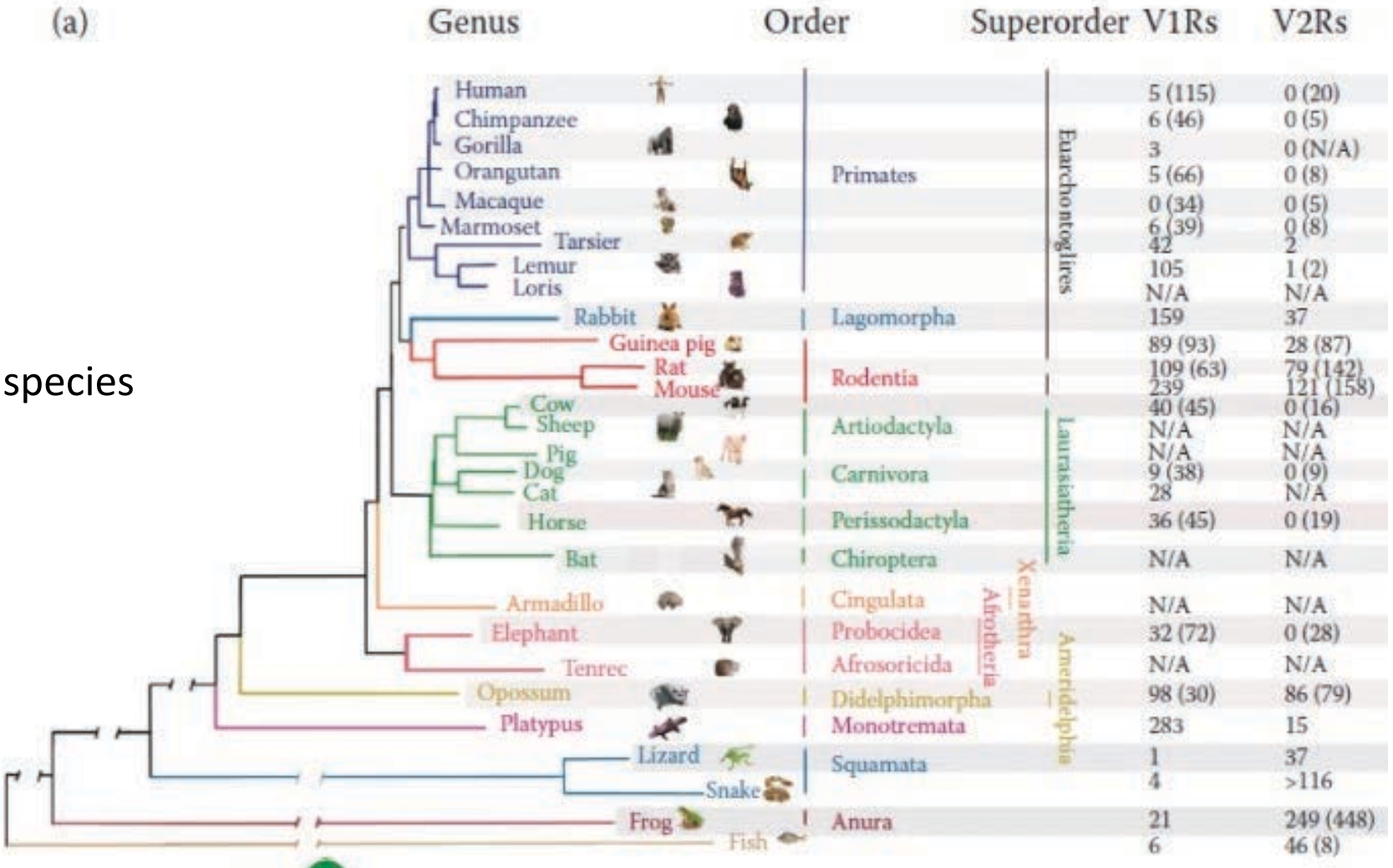


Vomeronasal receptor type 2 (V2R) (n=121)



Evolution of vomeronasal receptor gene families

- Structural variation: duplications, deletions, pseudogeneisation



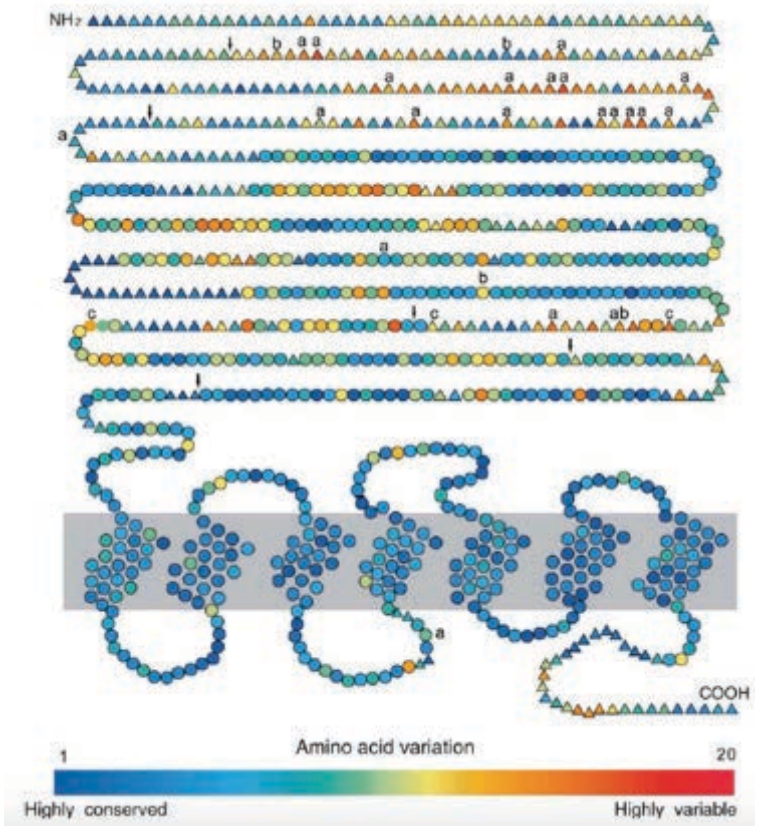
Mammal species

Evolution of vomeronasal receptor gene families

- Positive selection

Rodents V1Rs & V2Rs

Primates V1Rs & V2Rs



Evolution of vomeronasal receptor gene families

- Expression

Changes in expression patterns at some VRs

(*e.g. Broad & Keverne 2012*)

Changes in mate preference behaviour (between laboratory strains)

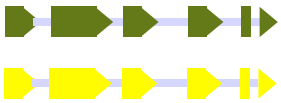
Vomeronasal receptors as candidates for sexual isolation

Rapid evolution

Divergence among populations

Allopatry species 1 — Contact species 1 — Contact species 2 — Allopatry species 2

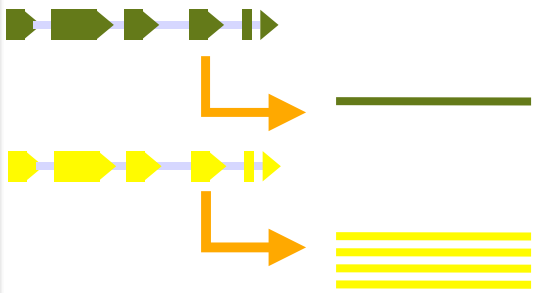
Sequence



Copy Number Variation (CNV)

	V1R	V2R
pop1	119	164
pop2	115	163
pop3	117	163
pop4	118	170

Expression



Research objectives

Identify genomic regions underlying behavioural divergence between allopatric and contact populations

Test for the role of vomeronasal receptors in sexual isolation

Genomics of sexual isolation and reinforcement in the house mouse

Divergence at candidate genes?

Vomeronasal receptors (VRs)

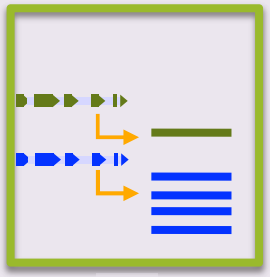
Whole genome (3Gb)

Genomics of sexual isolation and reinforcement in the house mouse

Divergence at candidate genes?

Vomeronasal receptors (VRs)

Expression



RNA-seq on VNOs

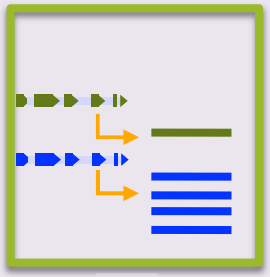
Whole genome (3Gb)

Genomics of sexual isolation and reinforcement in the house mouse

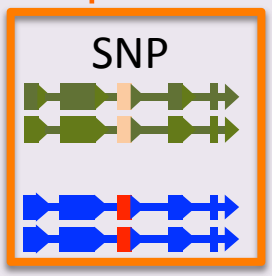
Divergence at candidate genes?

Vomeronasal receptors (VRs)

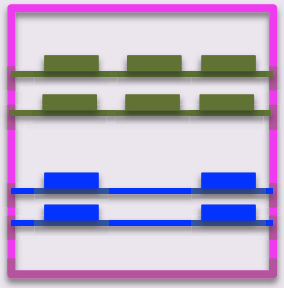
Expression



Sequence



Copy Number Variation (CNV)



RNA-seq on VNOs

Whole genome pool-seq

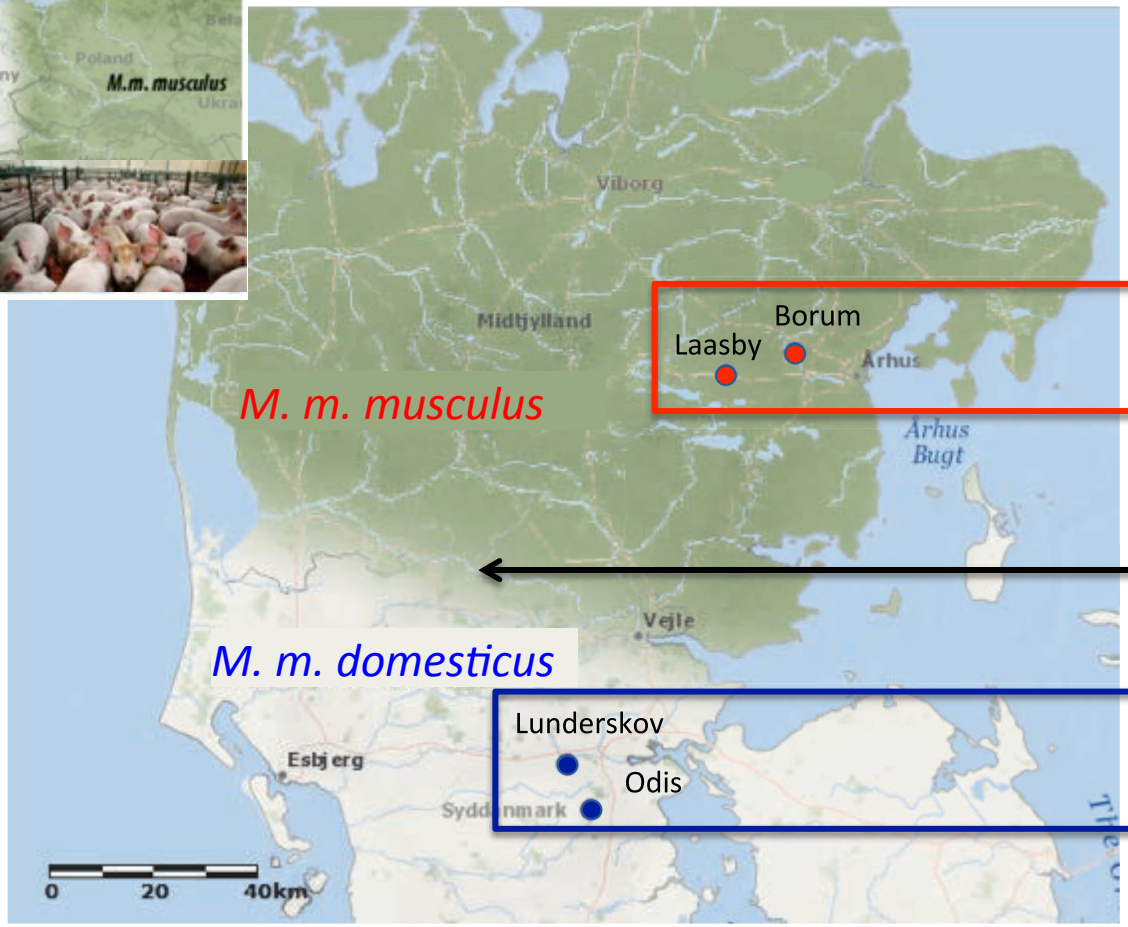
Whole genome (3Gb)

Other loci, non-coding regions

Genetic architecture

Samples

♂ and ♀ behaviour:



Choosy +++ (stronger assortative mate preferences)

Centre of the hybrid zone

Choosy + (weaker assortative mate preferences)

Samples

♂ and ♀ behaviour:

Non-Choosy
(no directional mate preferences)

Choosy +++
(stronger assortative mate preference)

Centre of the hybrid zone

Choosy + (weaker assortative mate preference)

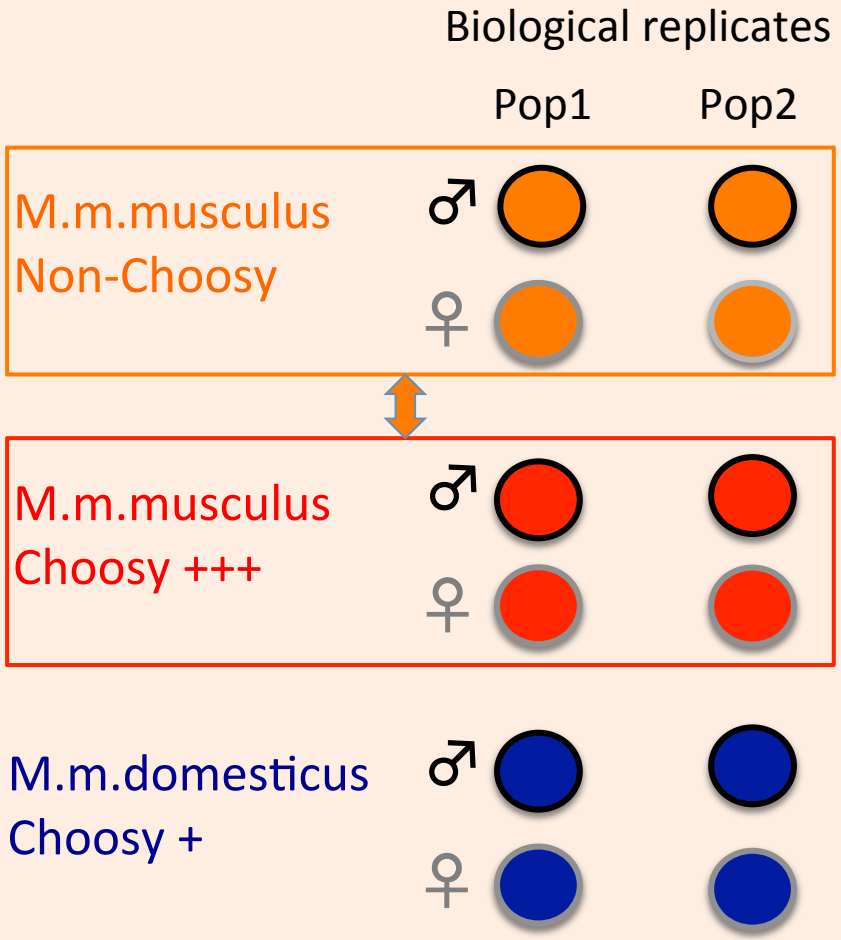
Non-Choosy

Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Samples



Hypothesis:

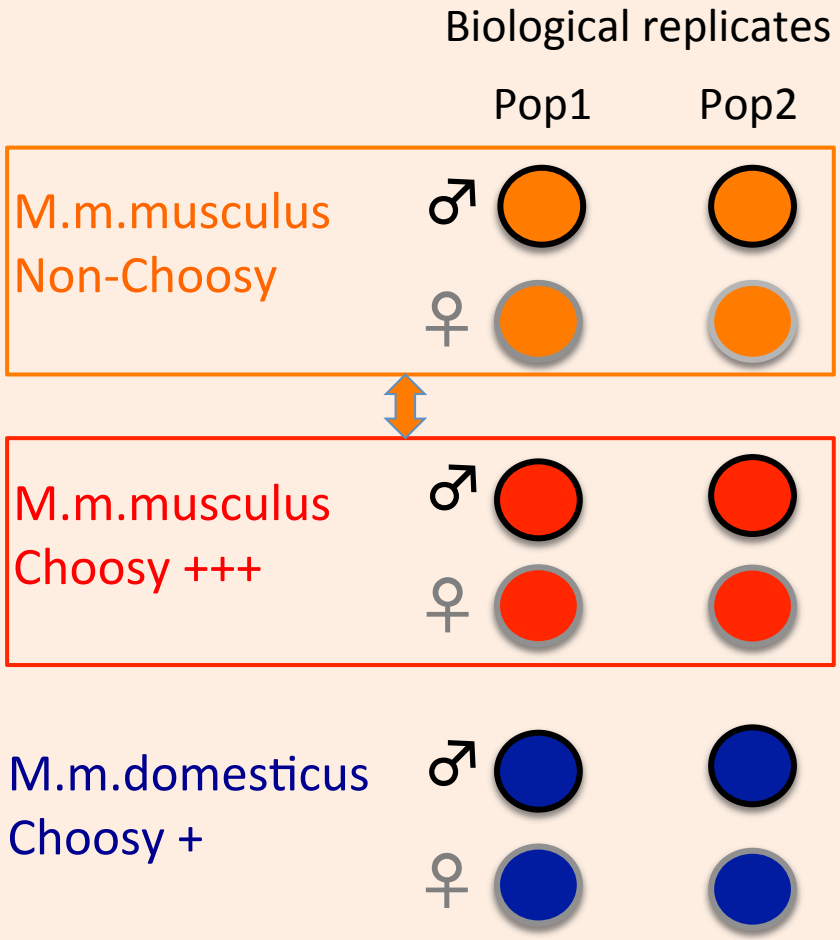
Expression divergence at VRs between Choosy and Non-Choosy samples could explain the shift in behaviour between these populations

Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Samples



Methods

- Wild-caught adult male and female mice
- Maintenance in the laboratory under controlled conditions (diet, olfactory environment)
- Vomeronasal organ dissection
- Pooling (8 individuals / sample)
- Deep PE Illumina RNA-sequencing (60M reads / sample)

Differential expression

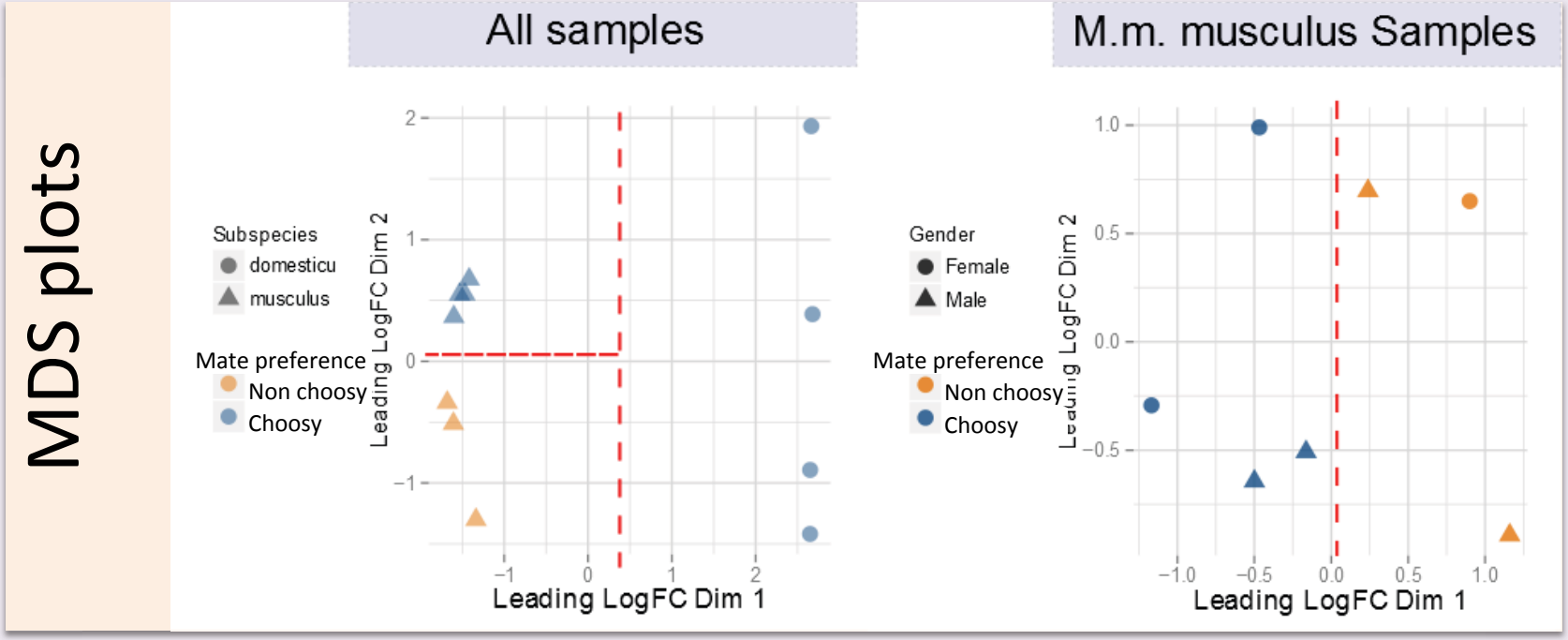
(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Expression variation among samples

MDS plots



perMANOVA :

All samples:

$P_{subspecies} = 0.004$

$P_{sex} = 0.547$

M. m. musculus samples:

$P_{choosiness} = 0.062$

$P_{sex} = 0.547$

Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Differential expression analyses

Musculus vs. domesticus	Musculus choosy vs. non choosy

Differential expression

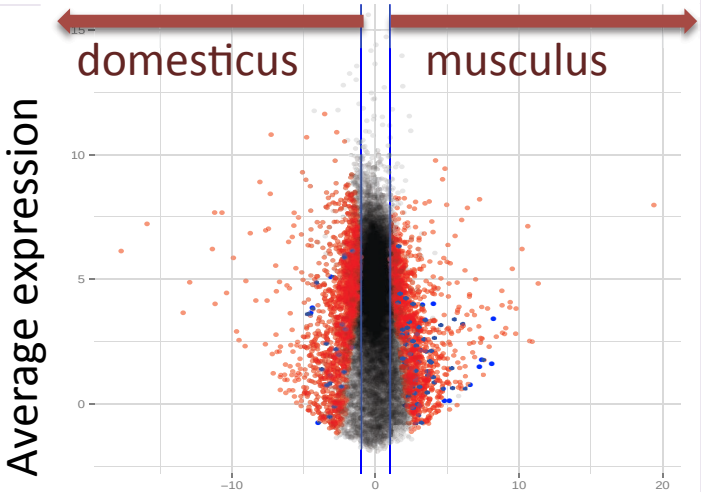
(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Differential expression analyses

Musculus vs. domesticus



DE genes: 2624

DE VR genes: 90

Musculus
choosy vs. non choosy

Differential expression

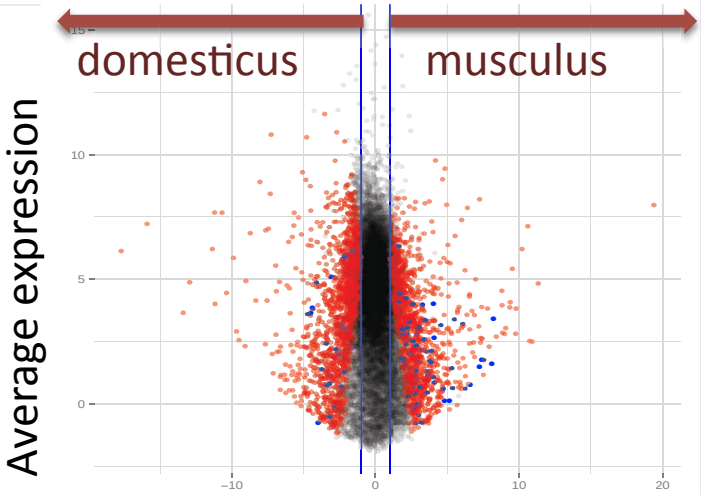
(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

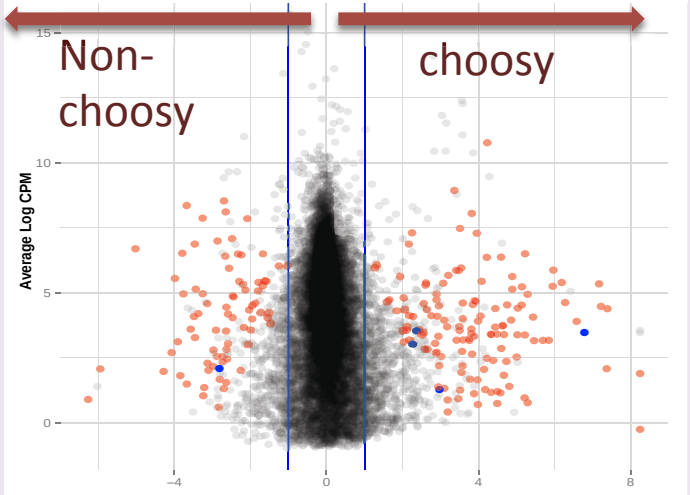
Main results

Differential expression analyses

Musculus vs. domesticus



Musculus choosy vs. non choosy



	Musculus vs. domesticus	Musculus choosy vs. non choosy
DE genes: ●	2624	236
DE VR genes: ●	90	**5**

Significant enrichment $P < 0.01$

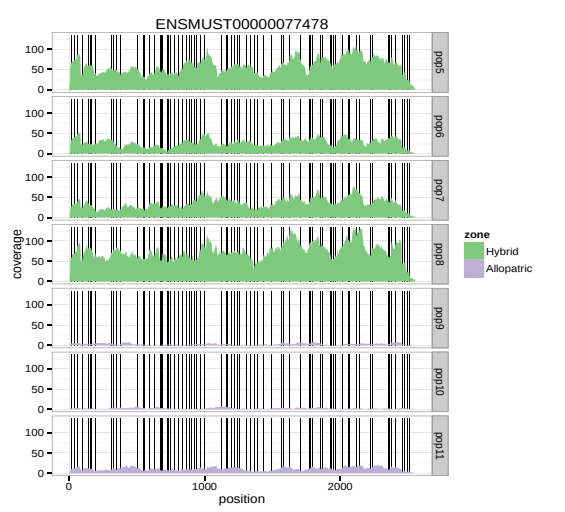
Differential expression

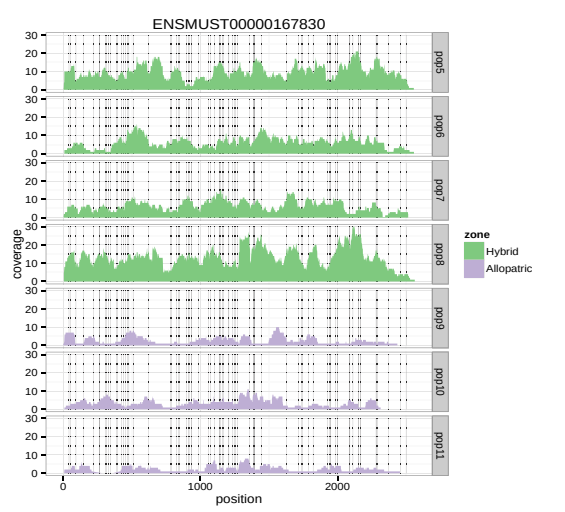
(Loire et al. 2017 Mol Ecol)

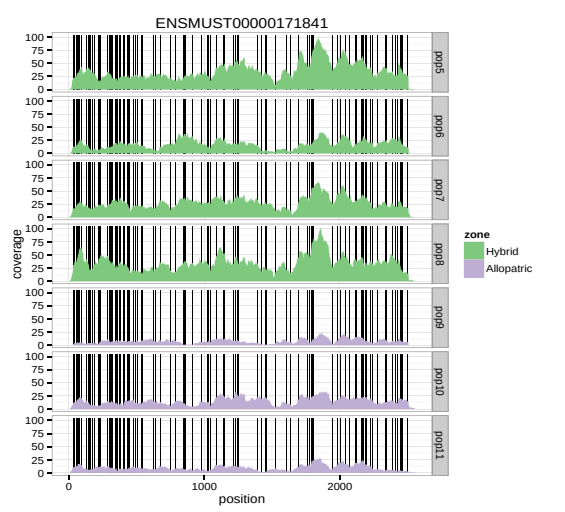
Main results

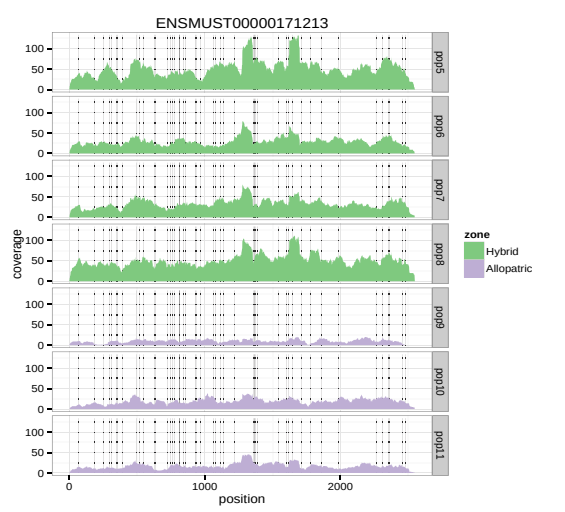
RNA-seq experiment

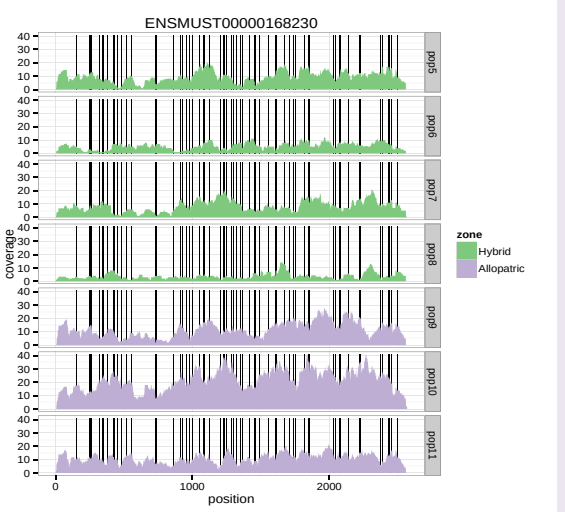
Coverage along DE genes











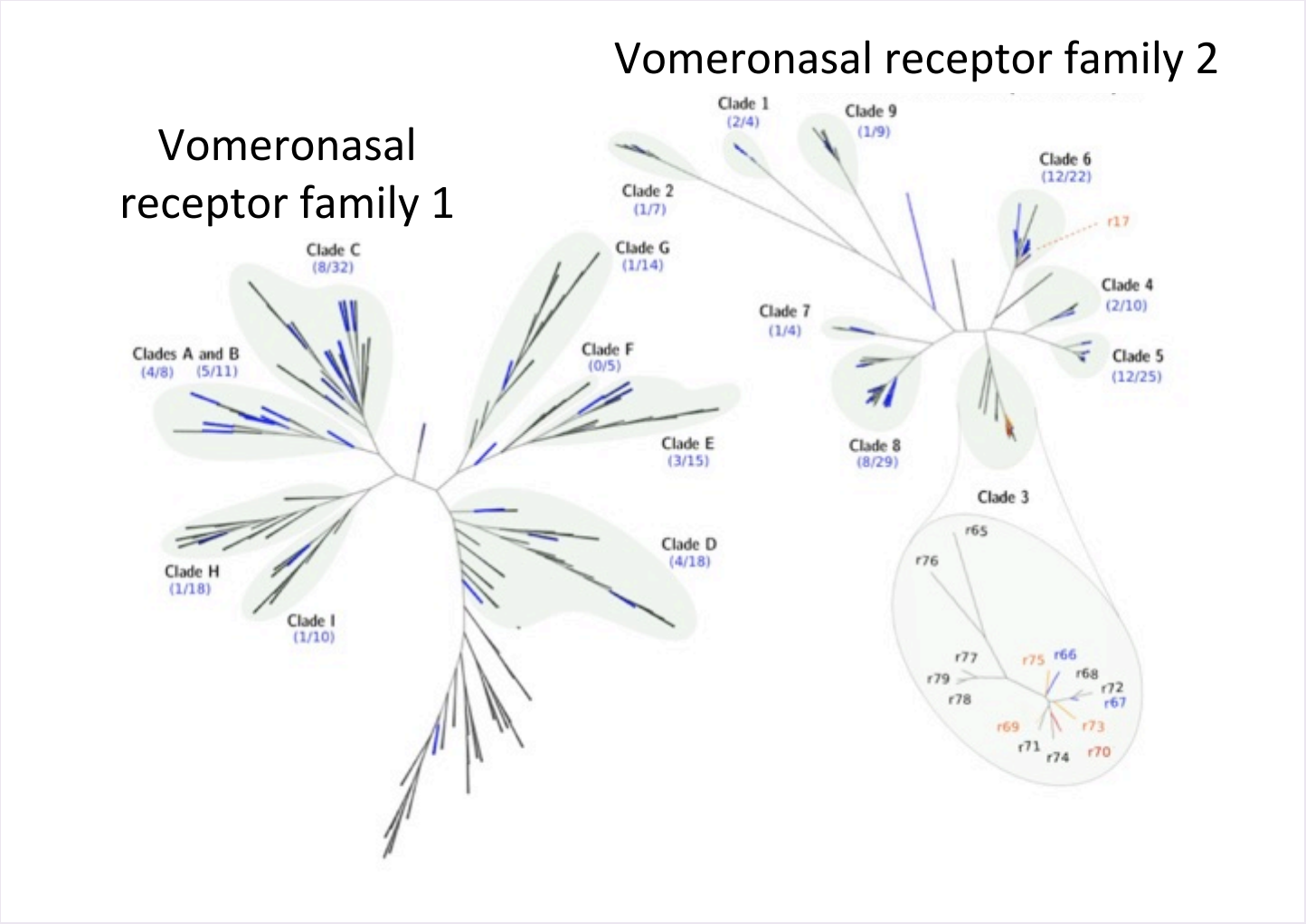
Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Over-representation of vomerosasal receptors of type 2



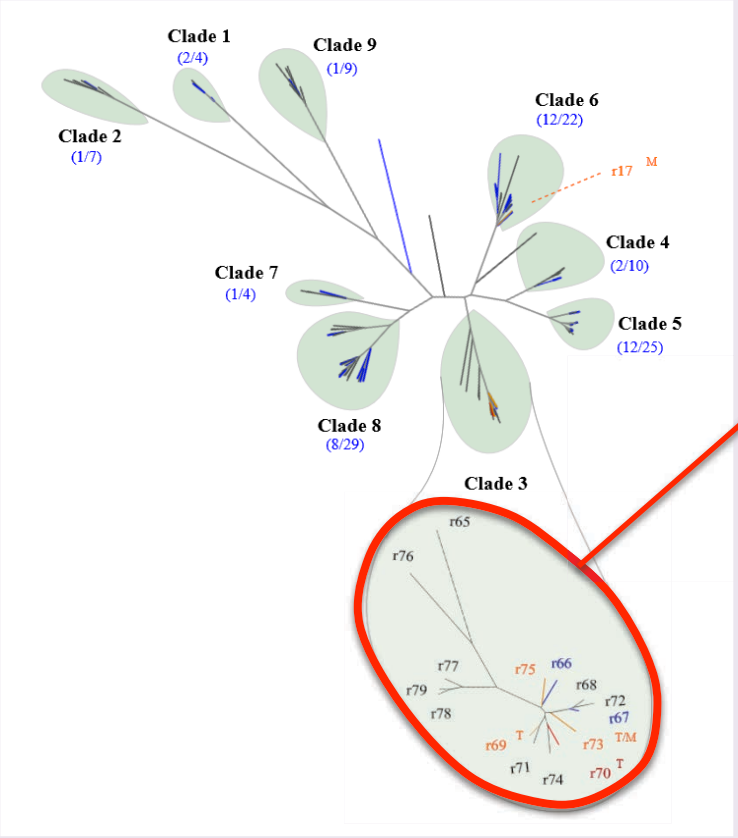
Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Phylogenetic clustering of differentially expressed VRs



4 out of 5 belong to subclade 3

Phylogenetic distance smaller than expected by chance ($p < 0.004$)

Specialisation of subclade 3 in subspecies recognition and assortative mate preference?

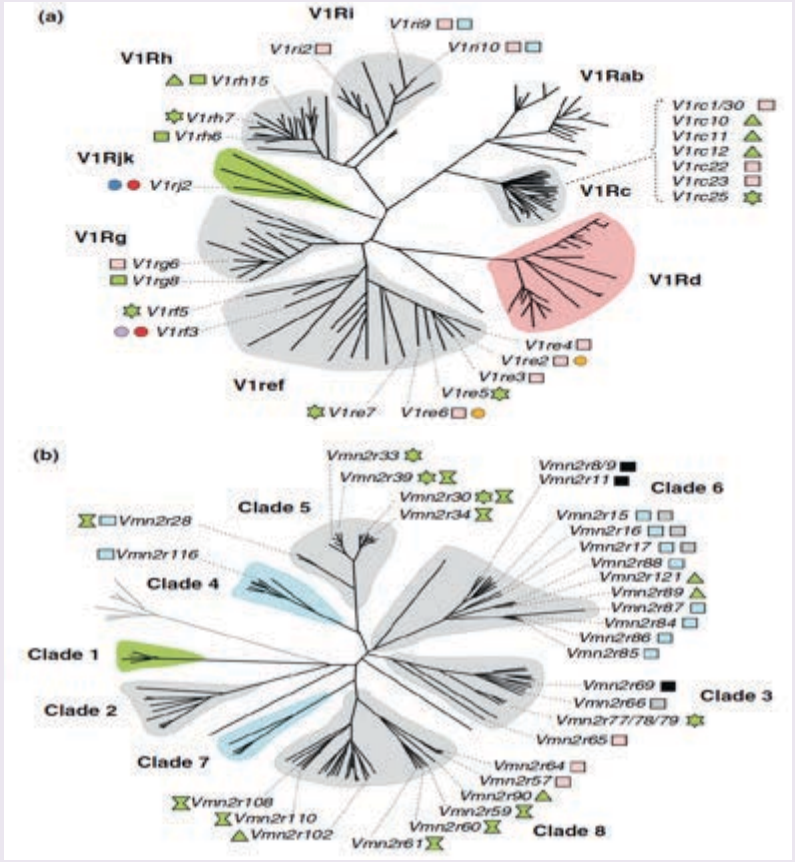
Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Specialised phylogenetic VR clusters (Isogai et al 2011 Nature)



"*M. m. domesticus*" VNOs:

Heterospecific signals (predators, closely related species)

Female signals

Male signals

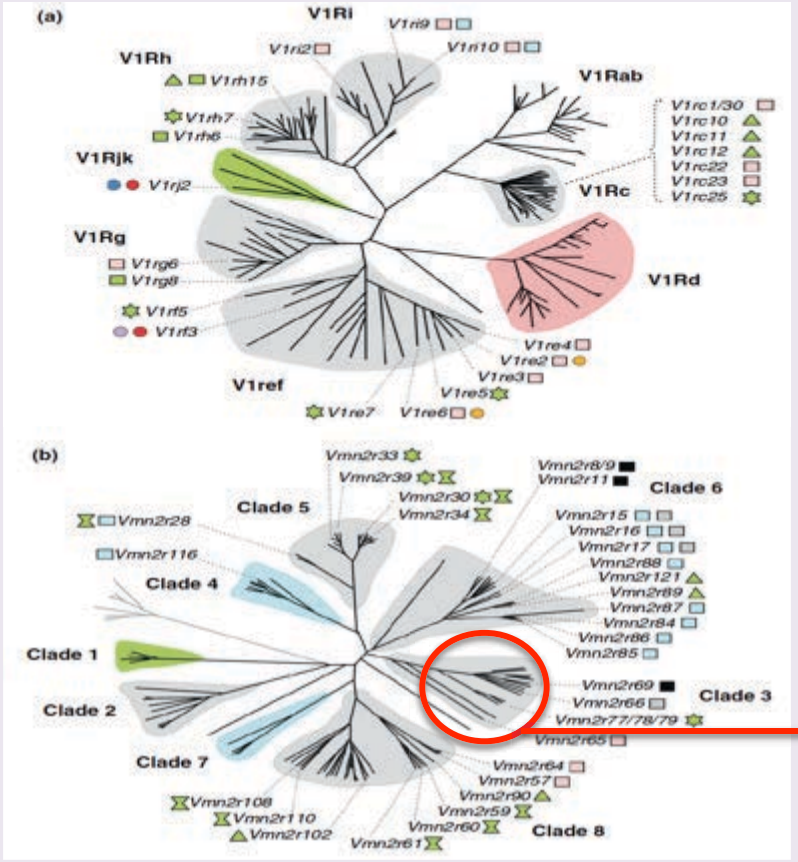
Differential expression

(Loire et al. 2017 Mol Ecol)

RNA-seq experiment

Main results

Specialised phylogenetic VR clusters (Isogai et al 2011 Nature)



"*M. m. domesticus*" VNOs:

Heterospecific signals (predators, closely related species)

Female signals

Male signals

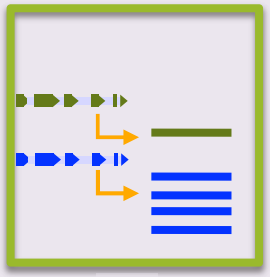
Subclade 3 = role in recognition of *M. m. musculus* scents

Genomics of sexual isolation and reinforcement in the house mouse

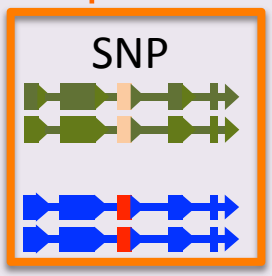
Divergence at candidate genes?

Vomeronasal receptors (VRs)

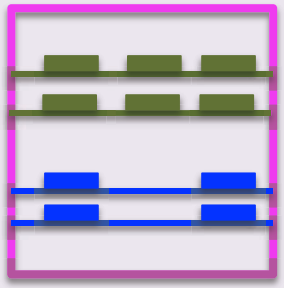
Expression



Sequence



Copy Number Variation (CNV)



RNA-seq on VNOs

Whole genome pool-seq

Whole genome (3Gb)

Other loci, non-coding regions

Genetic architecture

Genome-wide patterns of divergence

(Loire et al. in prep)

Pool sequencing

1. To be done

- More detailed sequence analysis in these candidate regions
- Analysis of patterns of recombination and linkage disequilibrium (LDx program, Feder et al. 2012)

2. First conclusions

- ORs and VRs as the most differentiated genes
- Regulatory regions particularly differentiated

Genomics of reinforcement in the house mouse

Conclusions and perspectives

RNA-seq

Expression

Pool-seq

Sequence

CNV ?

Genomics of reinforcement in the house mouse

Conclusions and perspectives

RNA-seq

Expression

Pool-seq

Sequence

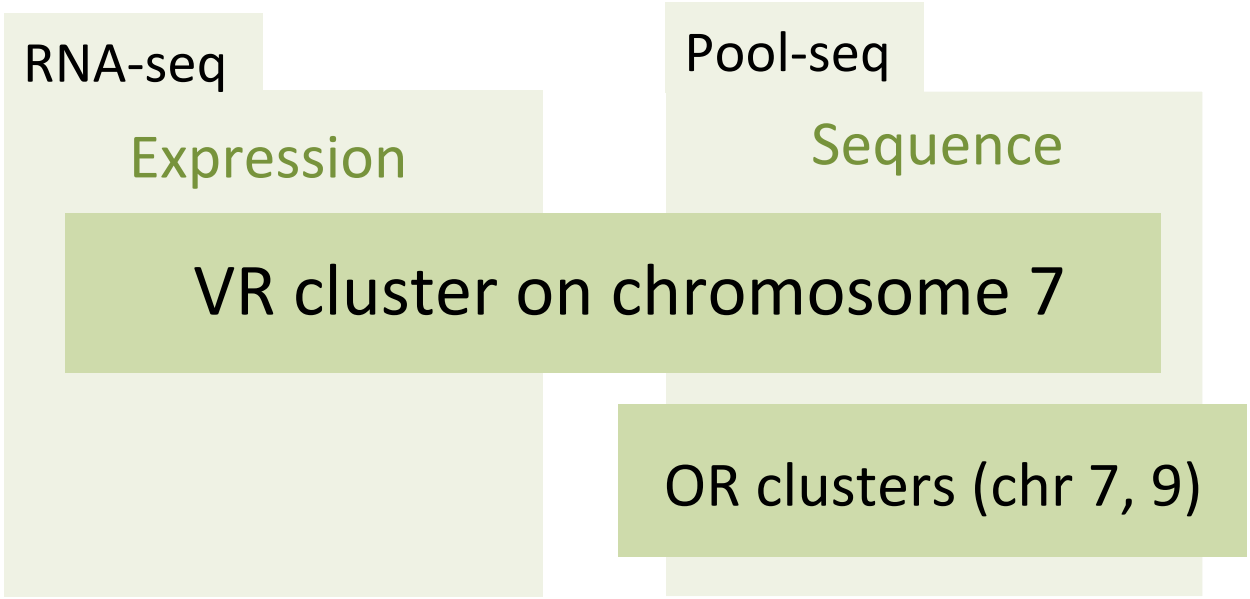
VR candidate cluster on chromosome 7

Promising candidate for the genetic basis of behavioural divergence

Specialised cluster?

Genomics of reinforcement in the house mouse

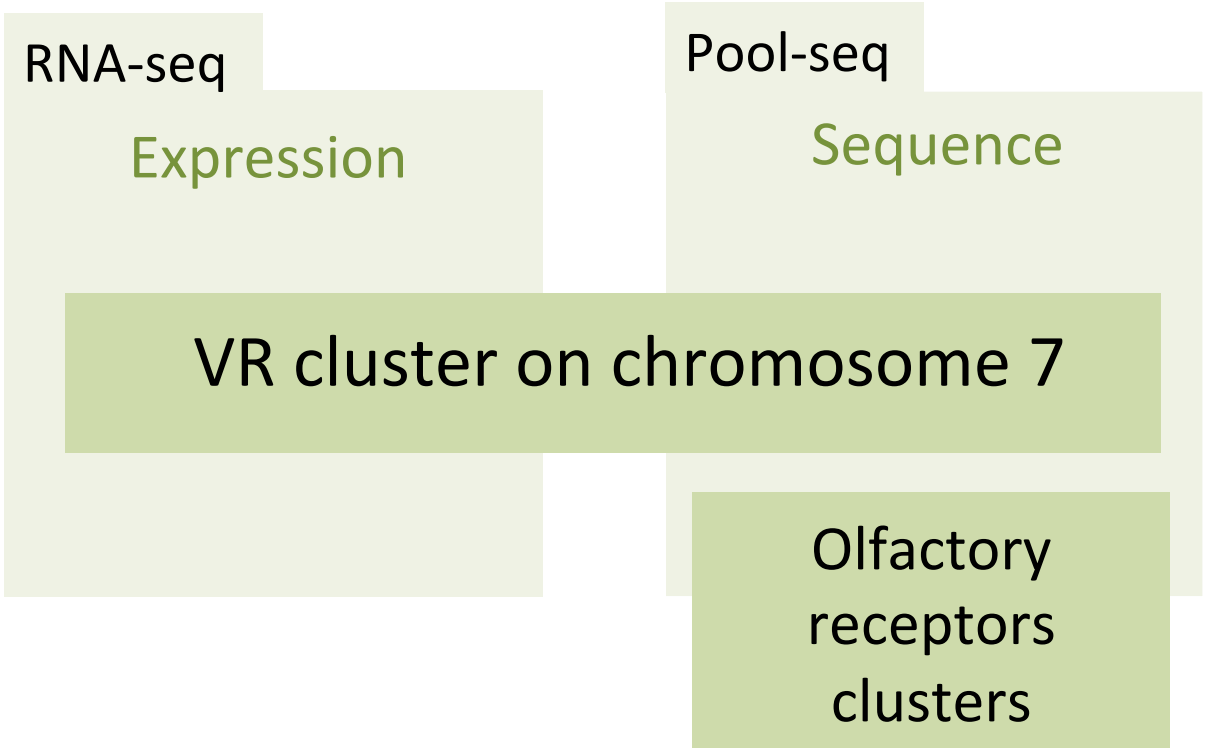
Conclusions and perspectives



In line with theory (Yeaman & Whitlock 2011, Yeaman 2013)
predicting tight linkage among barrier loci
- due to tandem gene duplications -
=> should favour coupling and divergence with gene flow
=> could be selected for in populations experiencing gene flow

Genomics of reinforcement in the house mouse

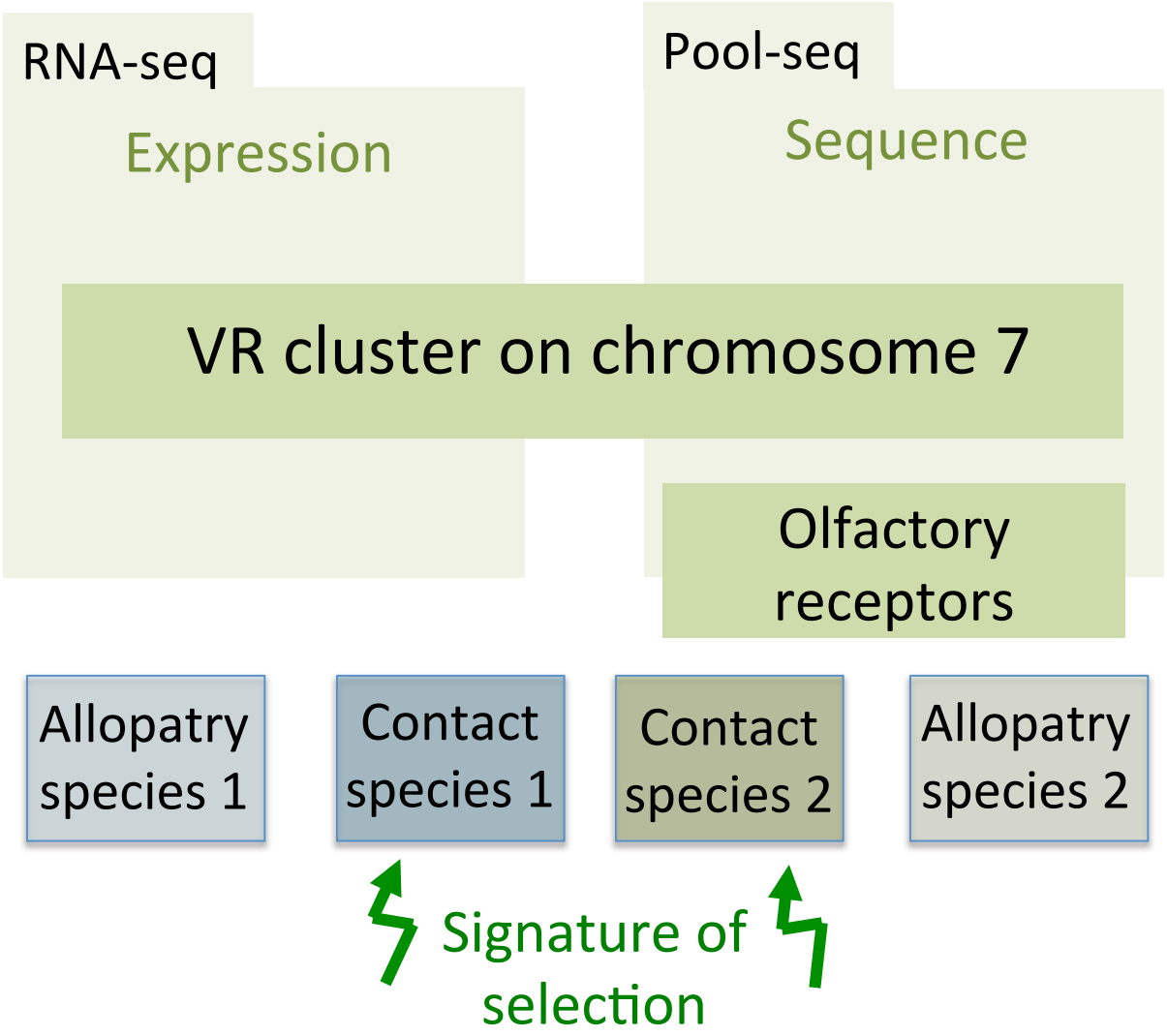
Conclusions and perspectives



- Role of both Vomeronasal and Olfactory Receptors?
- Role of learning and imprinting ?

Genomics of reinforcement in the house mouse

Conclusions and perspectives



Acknowledgments

Danish farmers

E. LOIRE
(Postdoc)

S. TUSSO
(Master student)

G. DUMONT
(Master student)

P. CAMINADE
(technician)

P. BOURSOT

G. GANEM

M. GAUTIER