

To choose or not to choose a good mate: evolution of sex pheromone receptors in moths

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photography : Michel Renou (INRAE)

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Insect olfaction drives important behaviors

Photos:web



Sex pheromone communication in moths

Female: emission

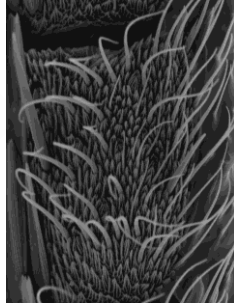
Male: detection



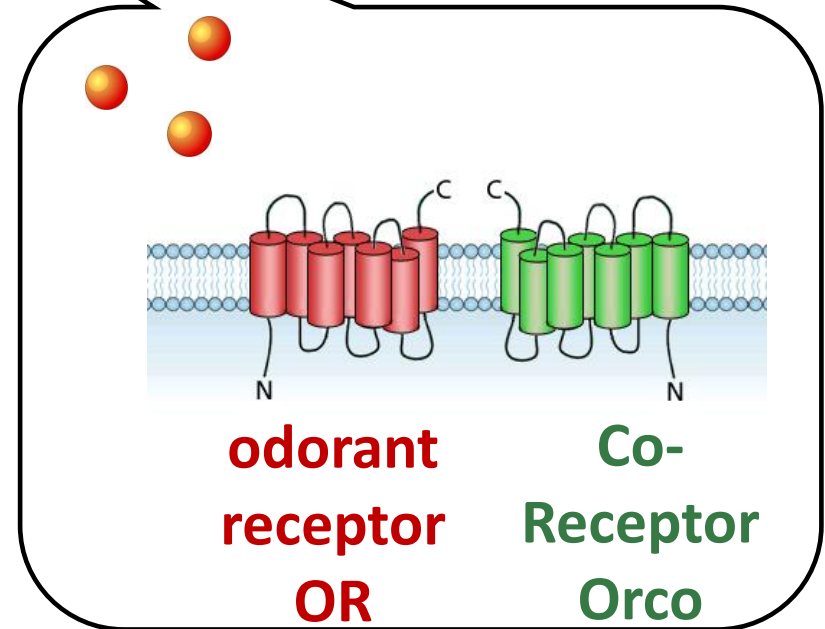
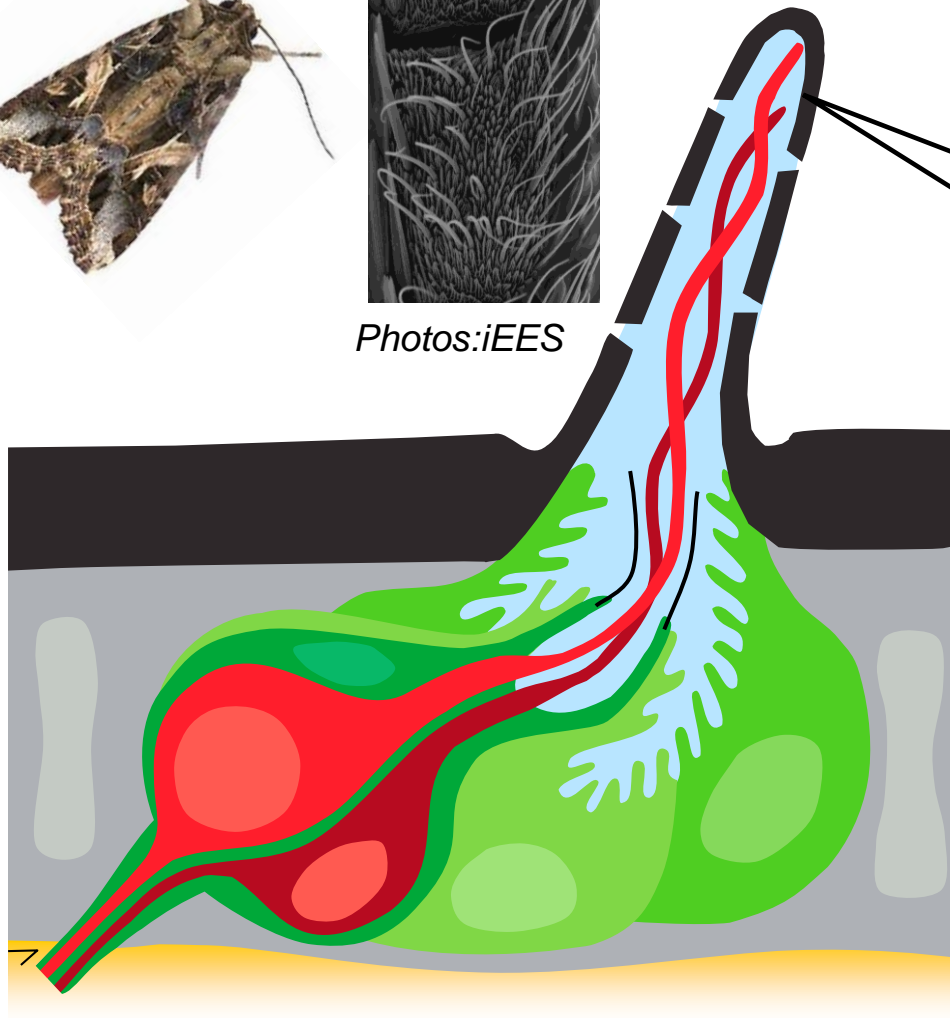
Photo by Samuel Woo, UC Davis

Even slight alterations in the pheromone communication can prevent mating and can be a starting point for **speciation**

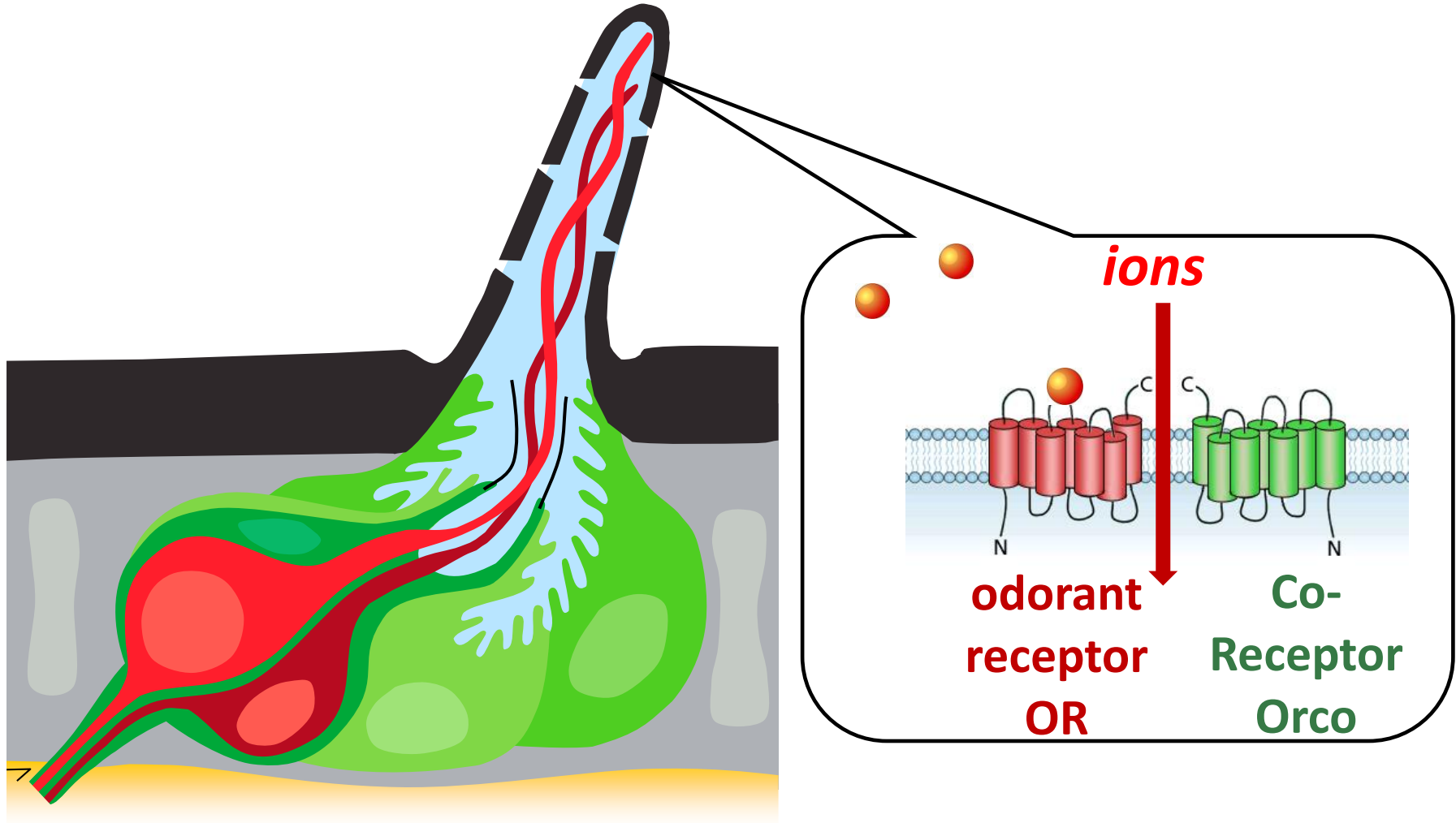
Pheromones are detected by dedicated Odorant Receptors (ORs): the Pheromone Receptors (PRs)



Photos:iEES



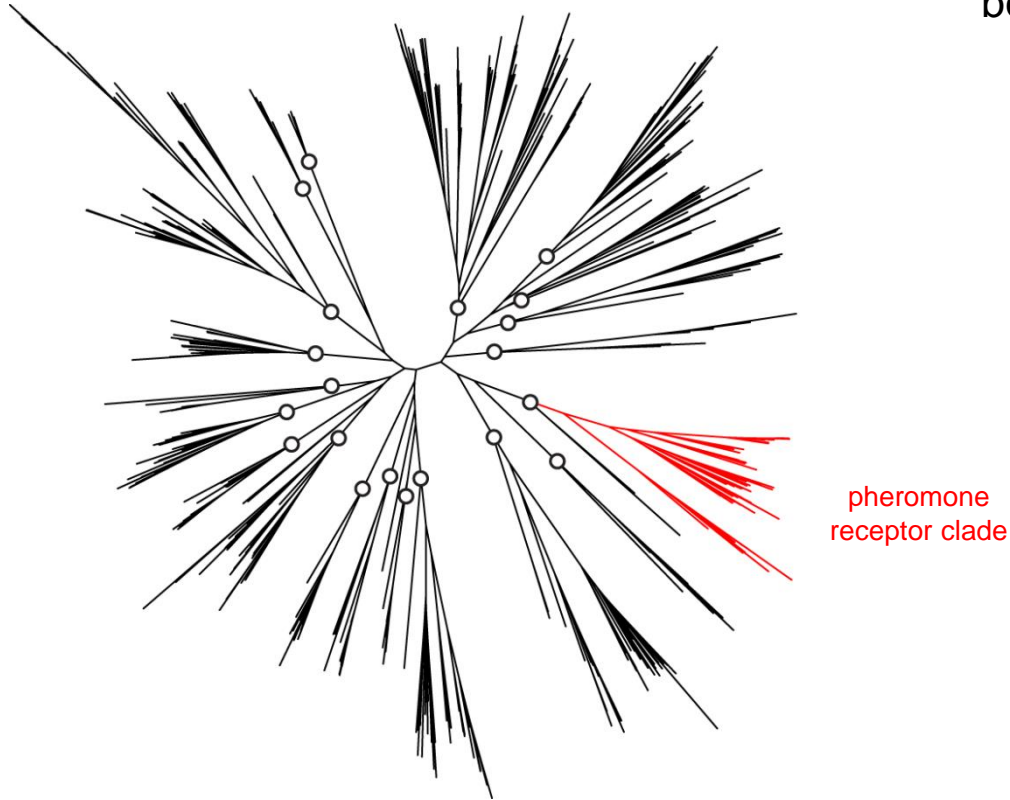
Pheromones are detected by dedicated Odorant Receptors (ORs): the Pheromone Receptors (PRs)



ORs are responsible for the transduction of a chemical signal into an electrical signal

Moth sex pheromone receptors (PRs): a subclass of ORs

Lepidopteran OR phylogeny



The function of **more than 50 PRs** have been characterized, in ~20 moth species

- ✓ All PRs tuned to type I pheromones **belong to the same clade** of the phylogeny
- ✓ They are **more expressed in male** than in female antennae

→ Good criteria to identify candidate PRs

Our main model: the noctuid moth *Spodoptera littoralis*

- ✓ Crop pest in Africa, Middle East and all the mediterranean basin
- ✓ Caterpillars are highly polyphagous



>80 host plants



Photos:iEES



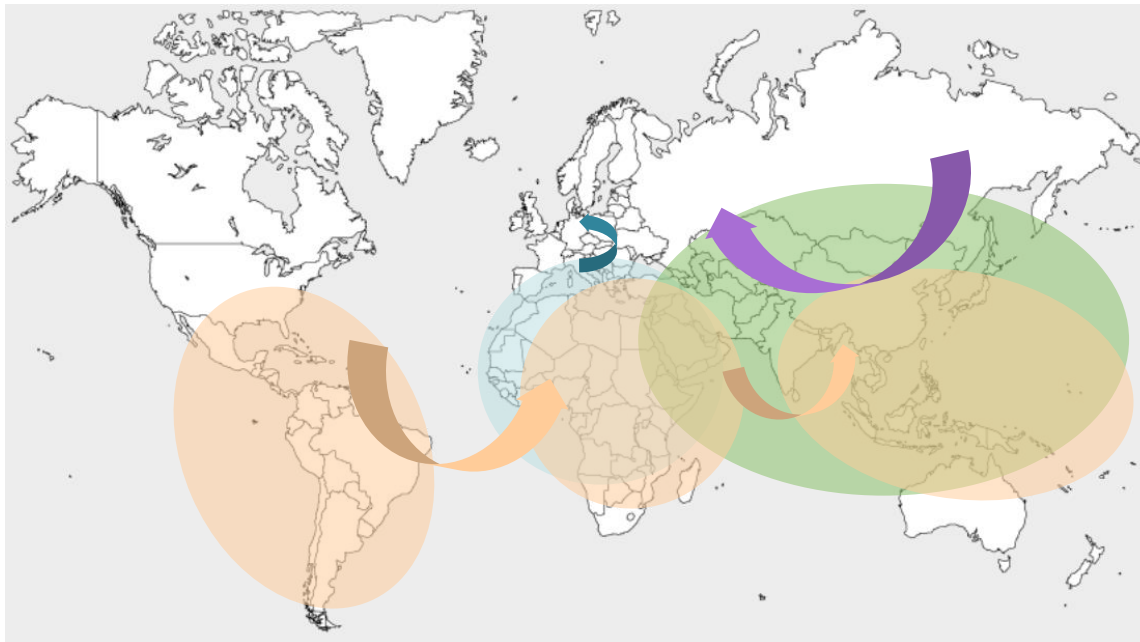
● *S. frugiperda*

● *S. littoralis*

● *S. litura*

Our main model: the noctuid moth *Spodoptera littoralis*

- ✓ Crop pest in mediterranean countries
- ✓ Caterpillars are highly polyphagous
- ✓ belongs to the *Spodoptera* complex



○ *S. frugiperda*

○ *S. littoralis*

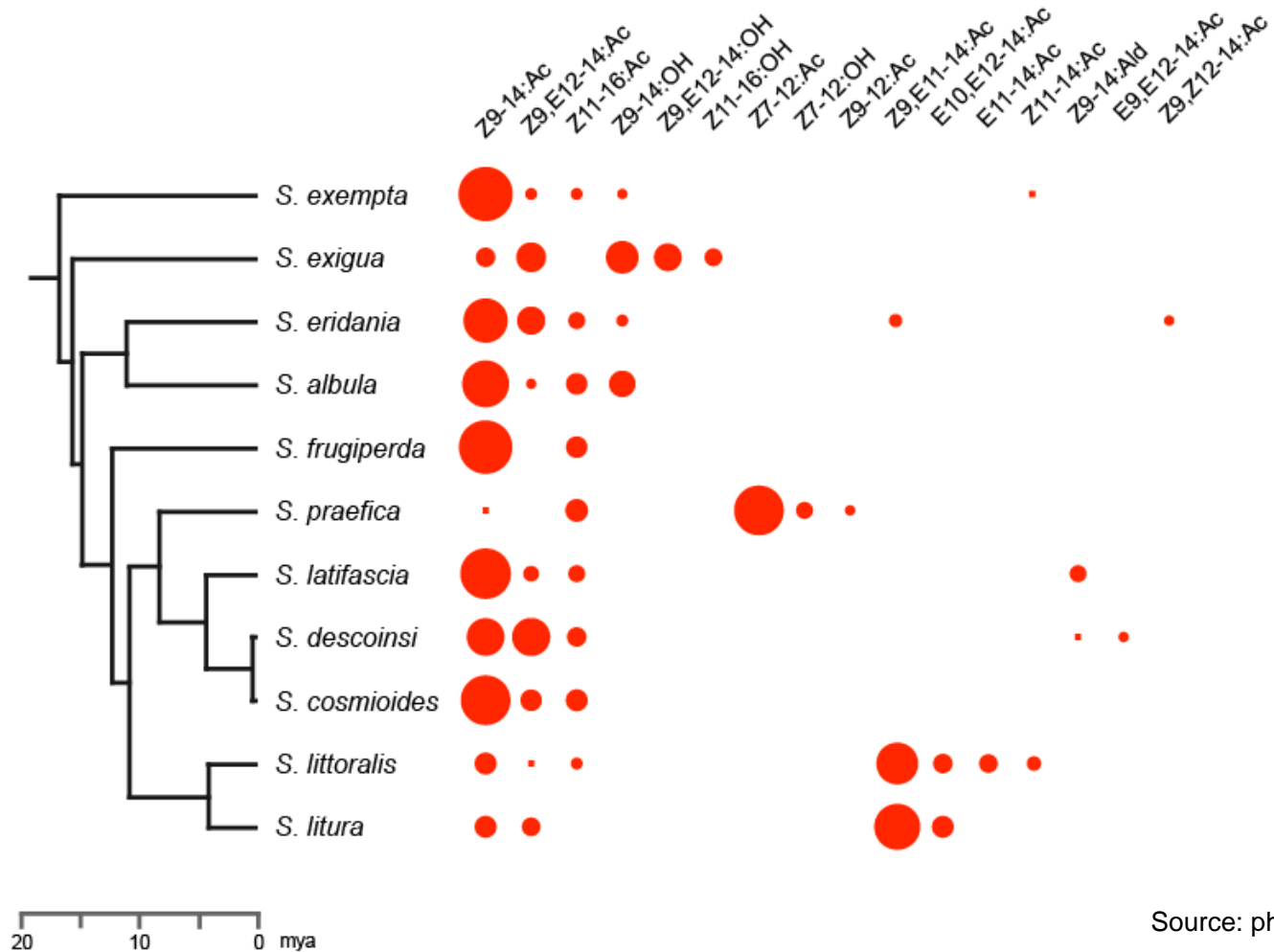
○ *S. litura*



Photos:iEES



Diversity and conservation of sex pheromone components in the genus *Spodoptera*

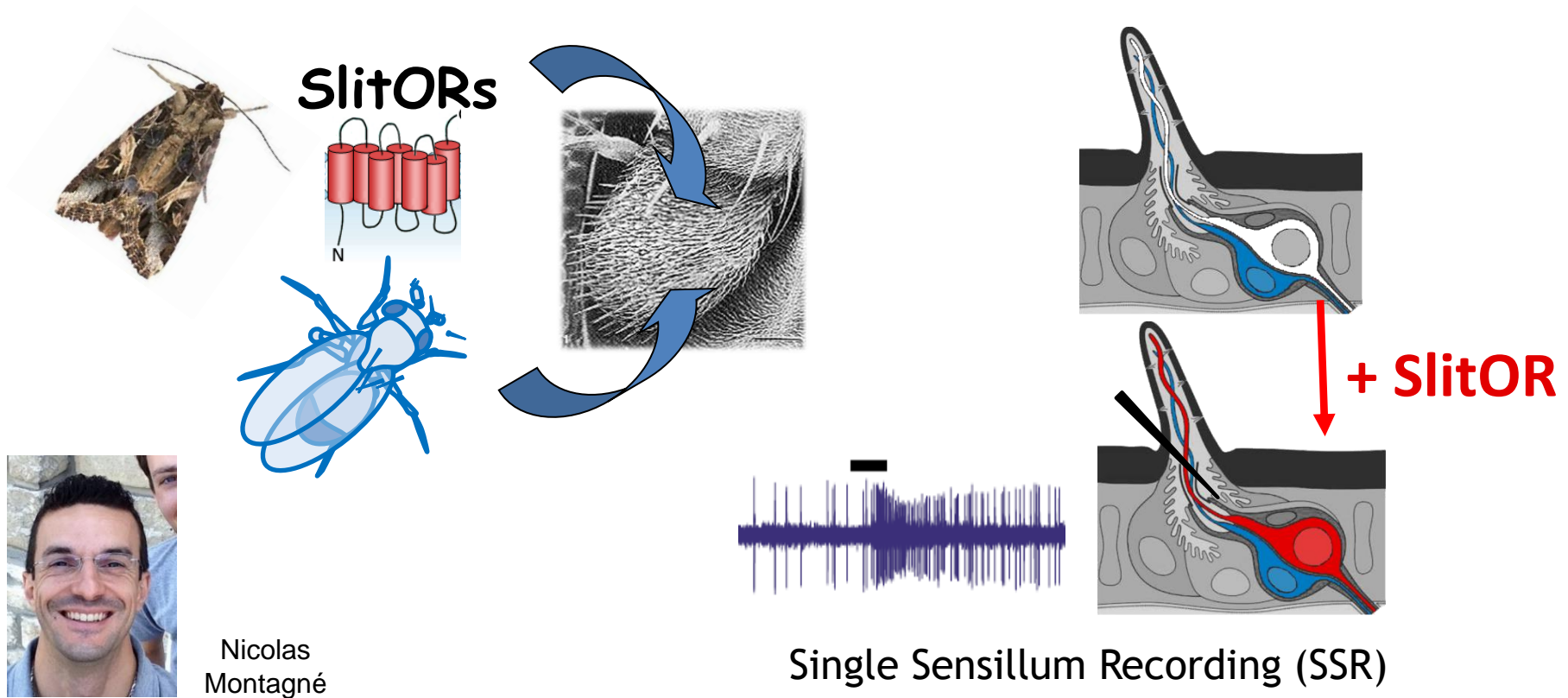


Source: pherobase

Identifying pheromone receptors in *S. littoralis*

antennal transcriptome and genome sequencing identify PR candidates

➔ Functional characterization via expression in *Drosophila* neurons coupled to electrophysiology (Kurtovik et al 2007 Nature)



Nicolas Montagné

Legai et al 2011 BMC genomics

Meslin et al 2022 G3.

Identifying pheromone receptors in *S. littoralis*

An antennal transcriptome identifies PRs candidates

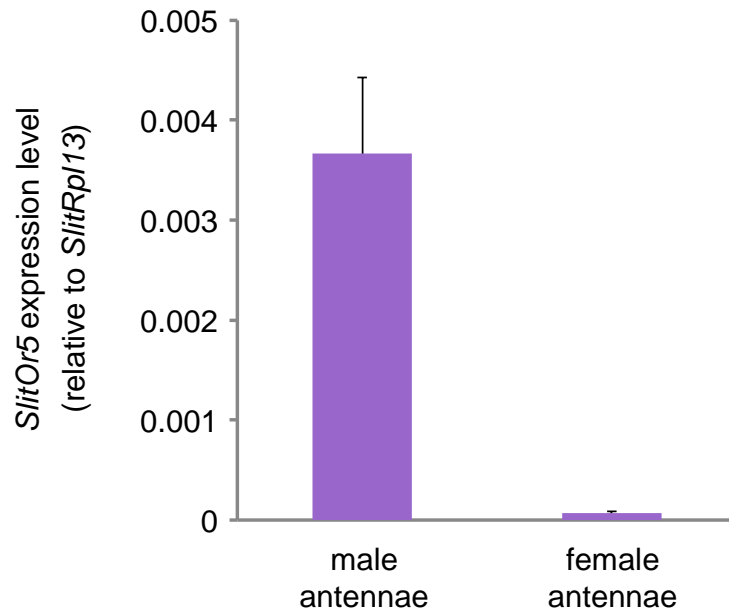
➔ Functional characterization via expression in *Drosophila* neurons coupled to electrophysiology

	<i>S. littoralis</i> pheromone component	Odorant receptor
Major component	(Z,E)-9,11-14:OAc	???
Minor components	(Z,E)-9,12-14:OAc	SlitOR6 + SlitOR13
	(Z)9-14:OAc	SlitOR13
	(Z)11-14:OAc	
	(E)11-14:OAc	
	(E,E)-10,12-14:OAc	

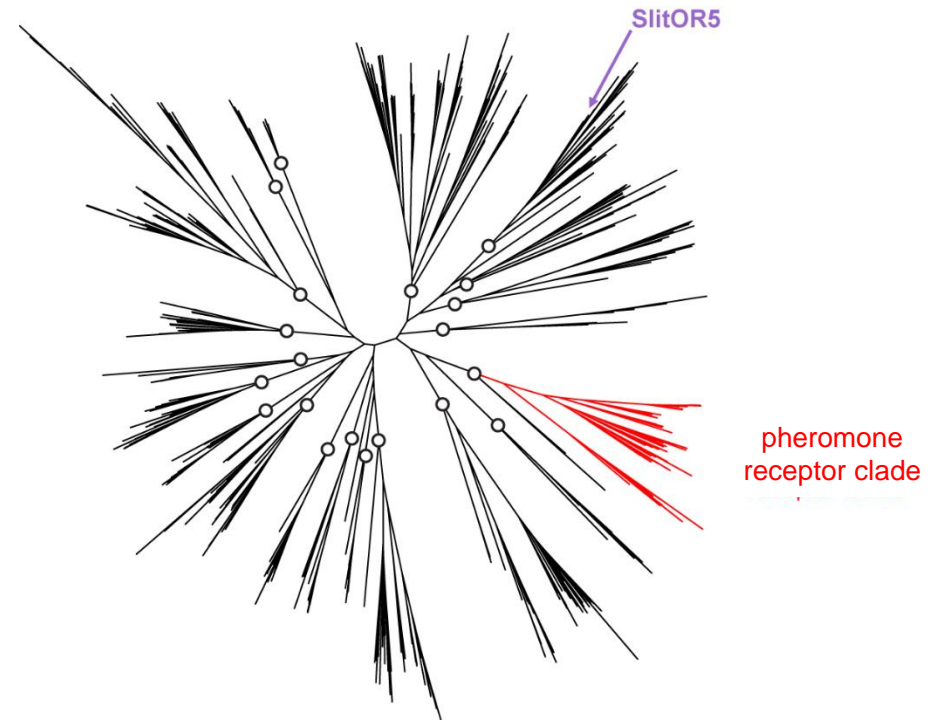
➔ The receptor detecting the major pheromone component of *S. littoralis* remained unknown

A novel lineage of moth pheromone receptors

qRT-PCR on *S. littoralis* adult antennae



Lepidopteran OR phylogeny



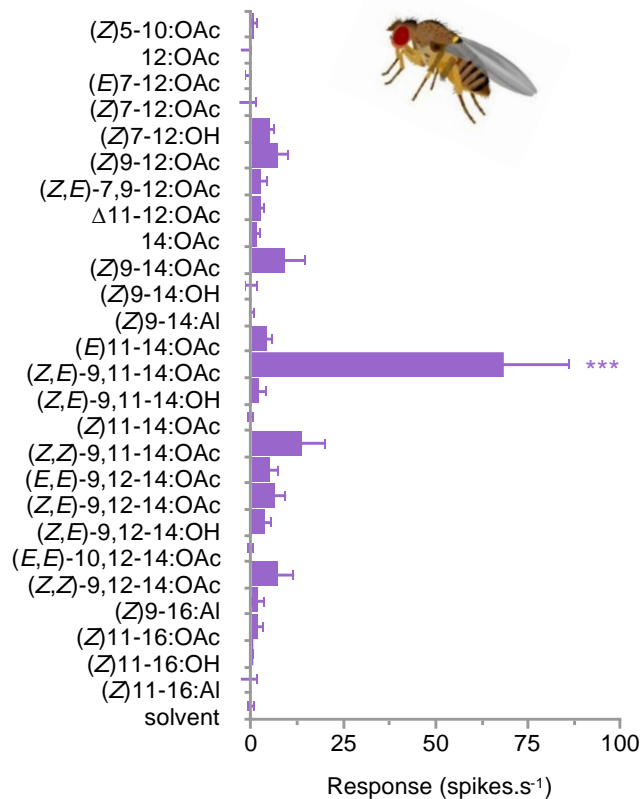
SlitOR5 is strongly male-biased, but does not belong to the classical PR clade

A novel lineage of moth pheromone receptors

Single-sensillum recordings on fly neurons expressing SlitOR5



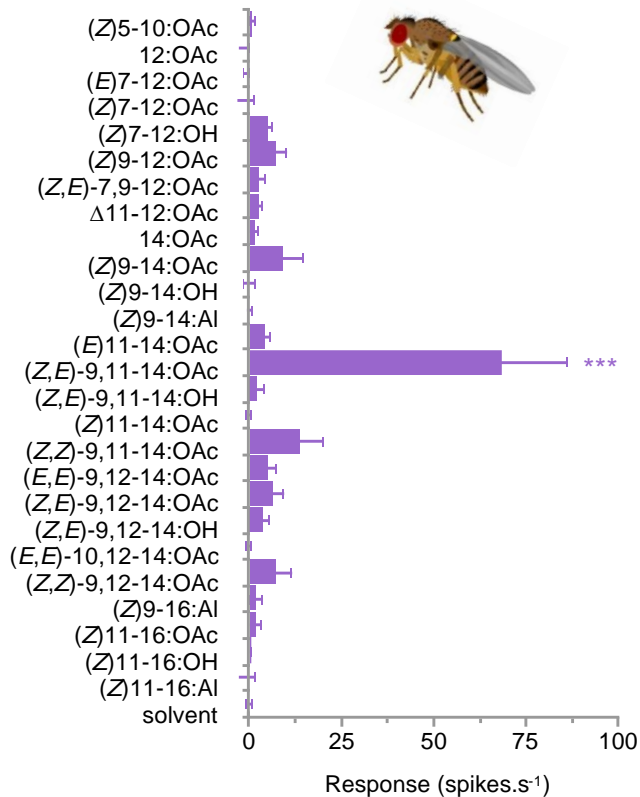
Lucie Bastin-Héline



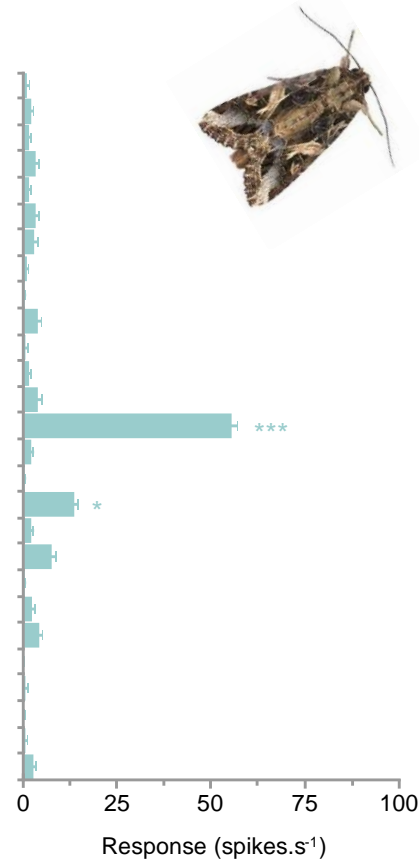
SlitOR5 is tuned to the major pheromone component of *S. littoralis*

A novel lineage of moth pheromone receptors

Single-sensillum recordings on fly neurons expressing SlitOR5



Single-sensillum recordings on *S. littoralis* adult antennae



Lucie Bastin-Héline



Christelle Monsempe

SlitOR5 is tuned to the major pheromone component of *S. littoralis*

A novel lineage of moth pheromone receptors



Fotini Koutroumpa



Marie-Christine François



Christelle Monsempes



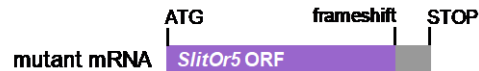
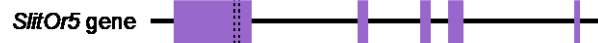
Lucie Bastin-Héline

Deletion in the *SlitOr5* gene via CRISPR-Cas9

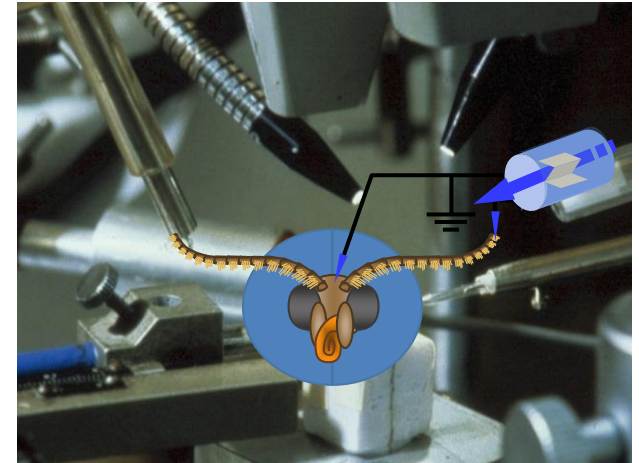


gRNA/Cas9 injection

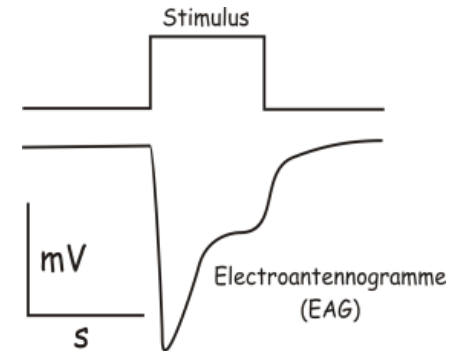
PAM target sequence for gRNA
 wild-type 5' TACGTGCCACTGGGTCCCAGTATTATGCTGGCATGC 3'
 mutant 5' TACGT-----CCAGTATTATGCTGGCATGC 3'



Phenotyping via electro-antennography in *S. littoralis* males



Photos:iEES



A novel lineage of moth pheromone receptors

Electro-antennography in *S. littoralis* males

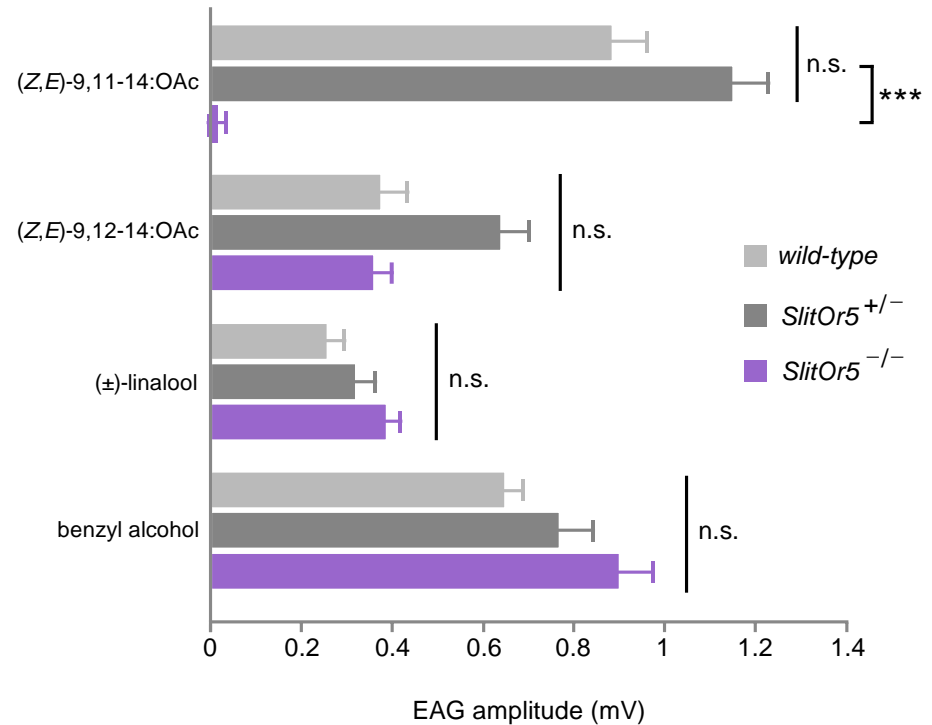
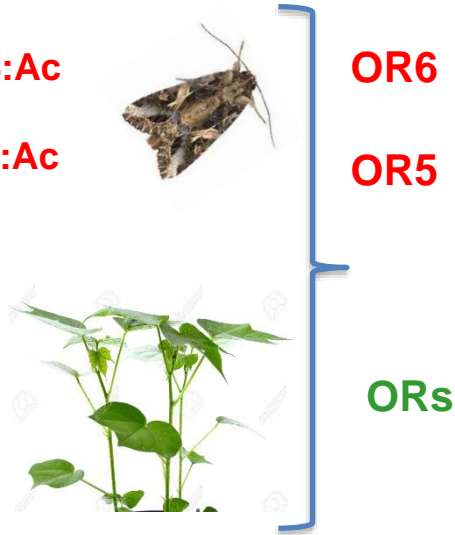
Odorant tested:

Z9, E12-14:Ac

Z9, E11-14:Ac

Linalool

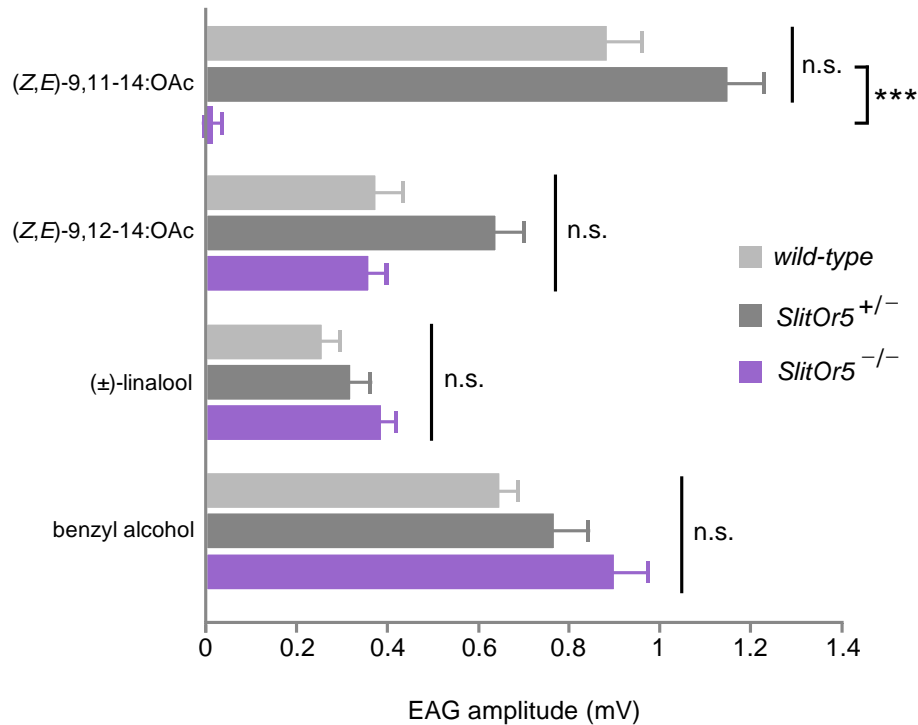
benzyl OH



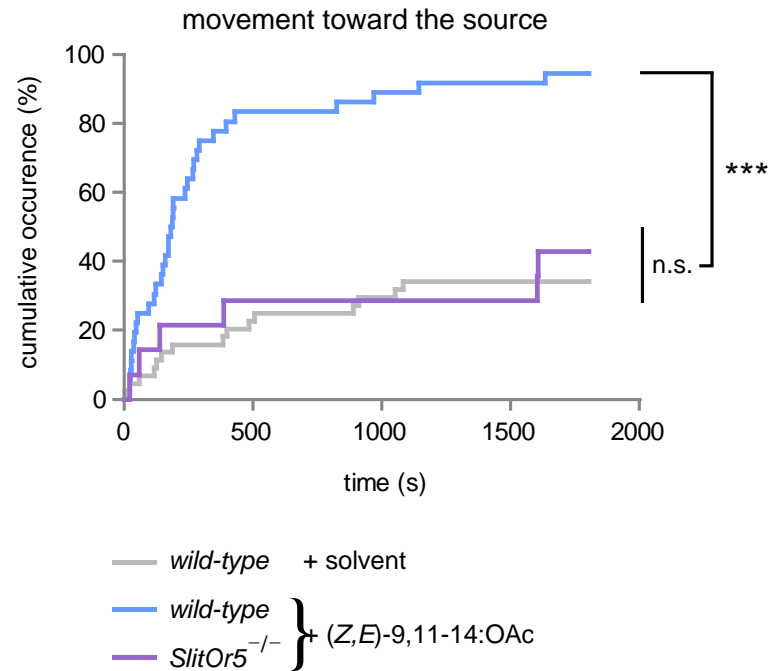
Inactivation of the *SlitOr5* gene by CRISPR-Cas9 impairs detection of (Z,E)-9,11-14:OAc

A novel lineage of moth pheromone receptors

Electro-antennography in *S. littoralis* males

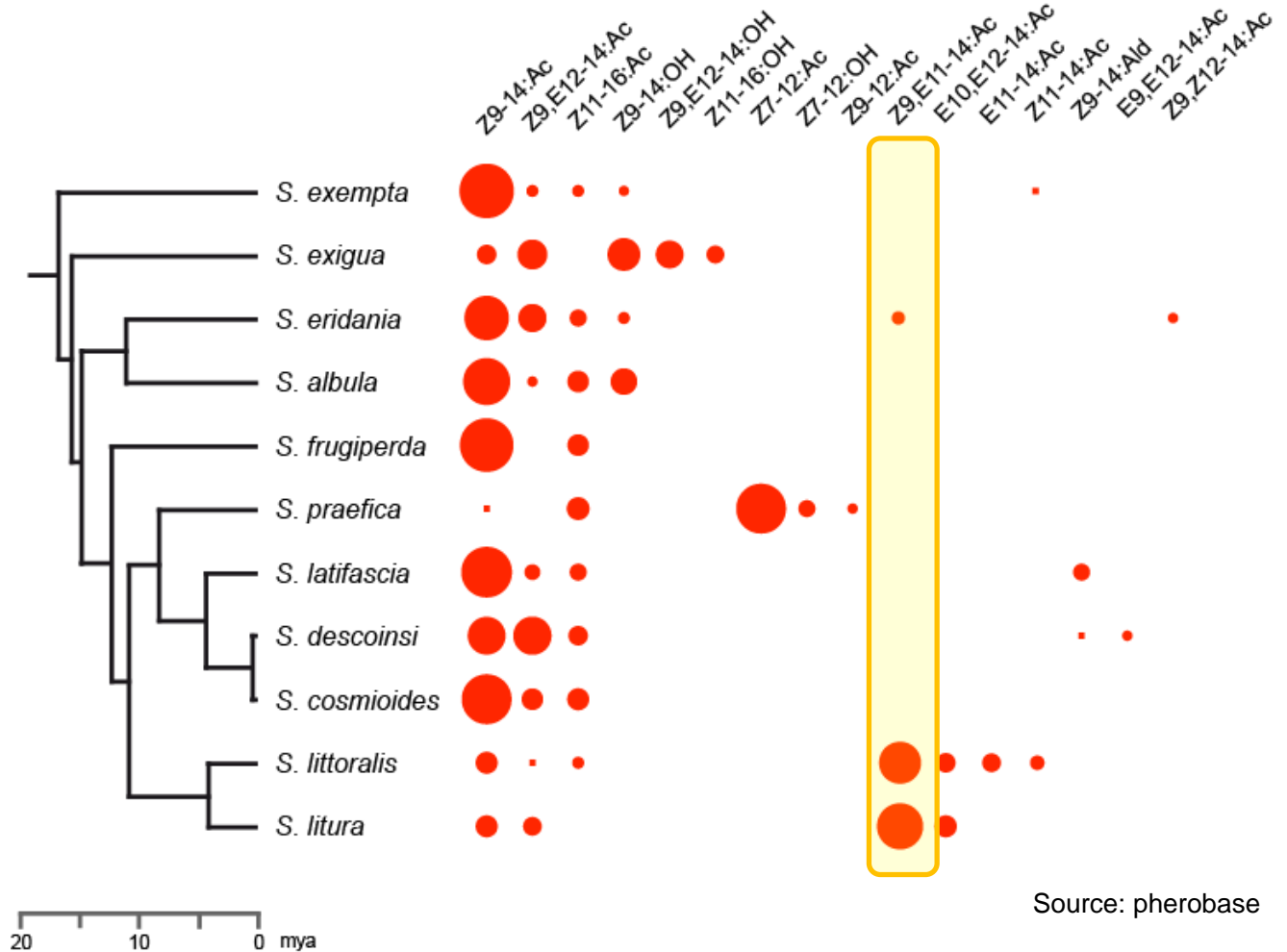


Male response (behavior)



Inactivation of the *SlitOr5* gene by CRISPR-Cas9 impairs detection of (Z,E)-9,11-14:OAc

Evolution of sex pheromones in the genus *Spodoptera*



How did the capacity to detect (Z,E)-9,11-14:OAc evolve ?

Evolution of sex pheromones in the genus *Spodoptera*

Photos:web



S. exigua



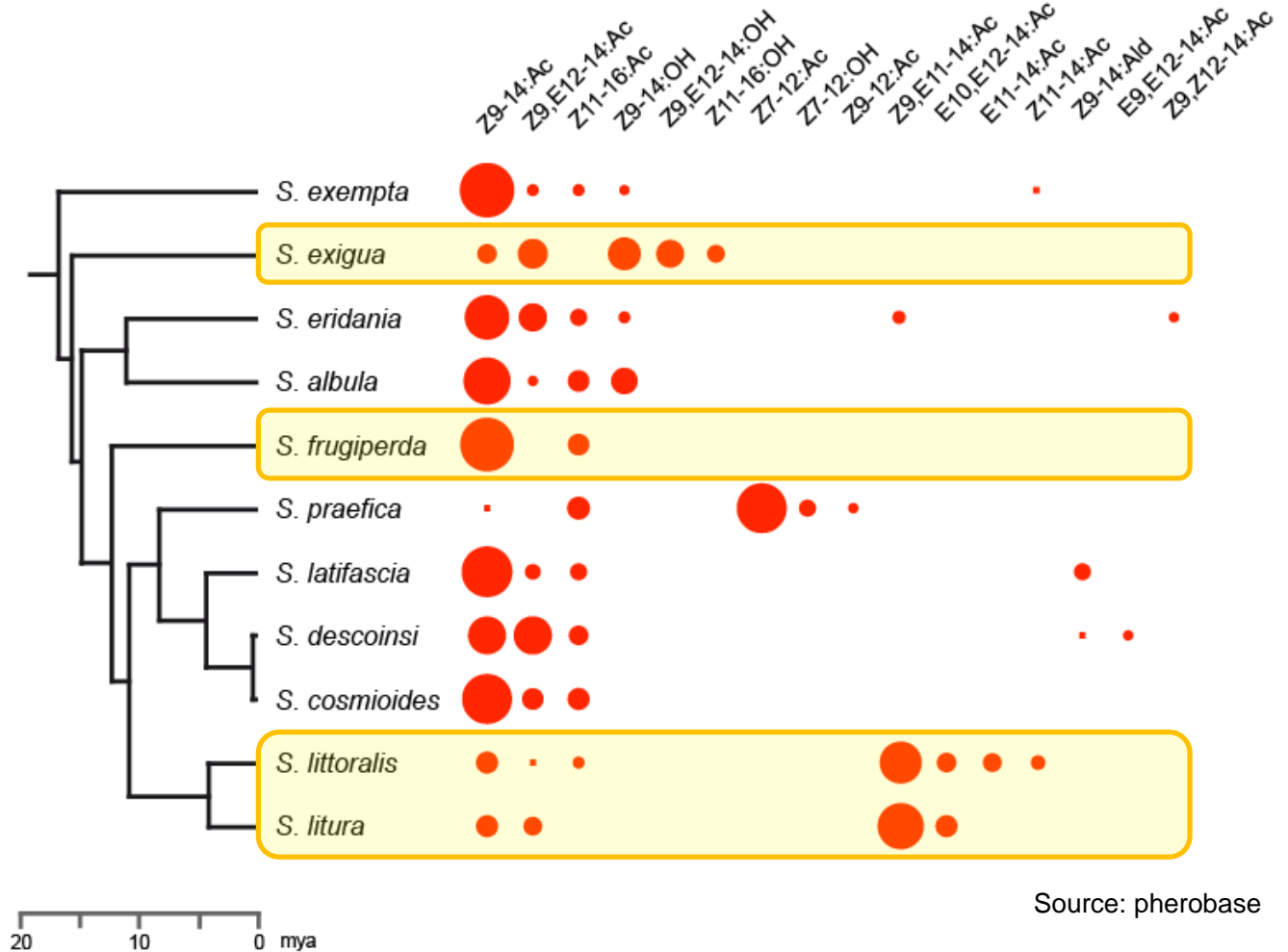
S. frugiperda



S. littoralis



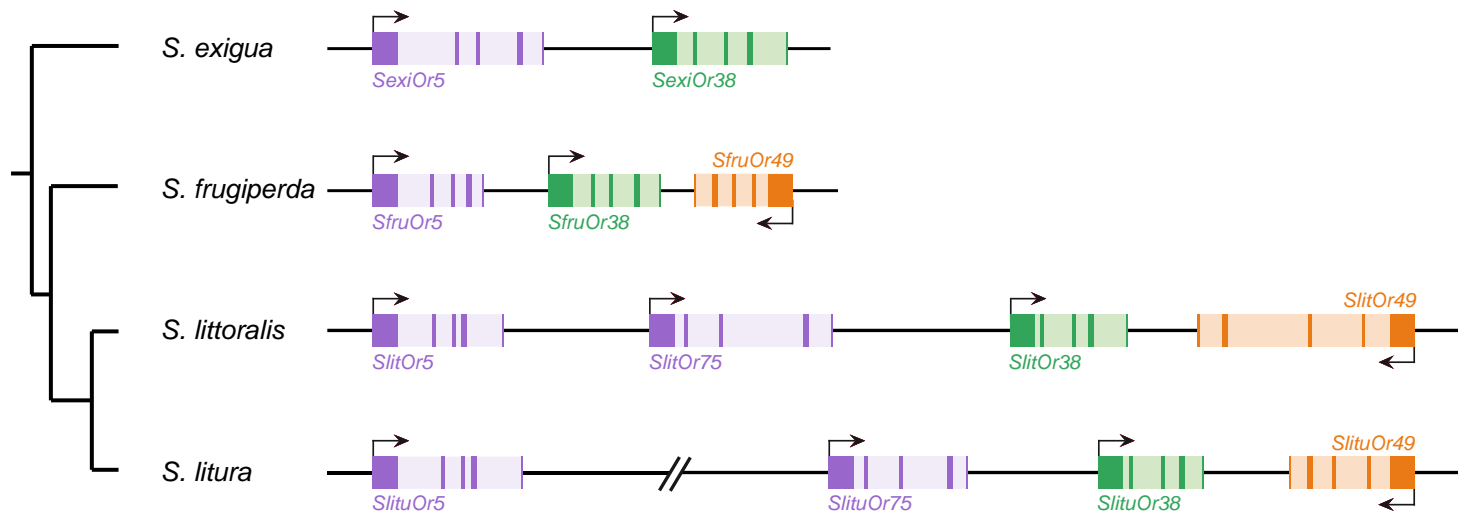
S. litura



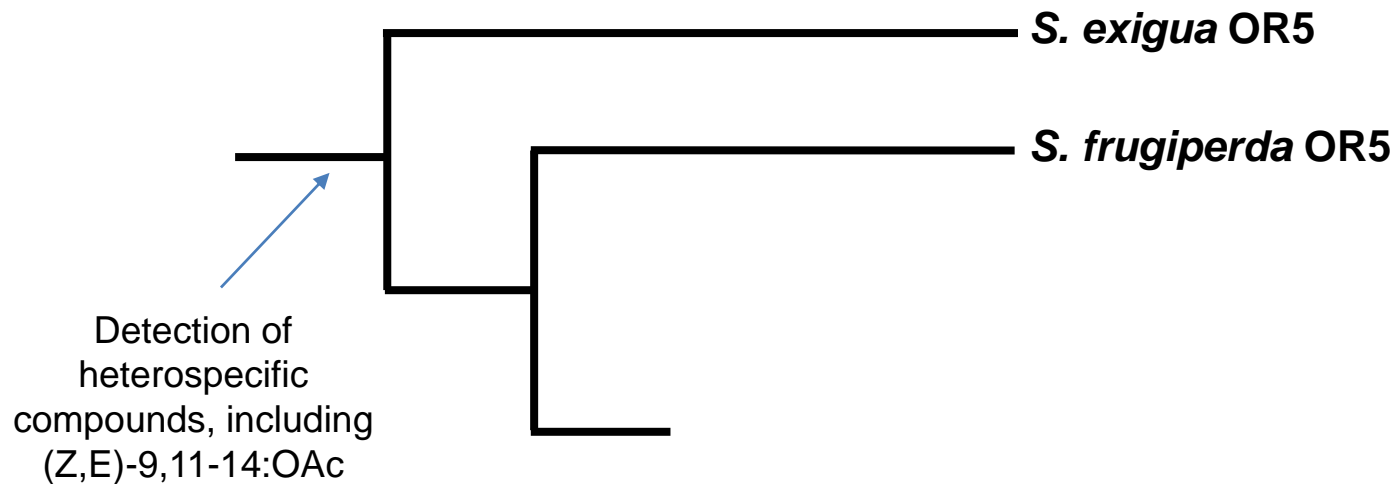
How did the capacity to detect (Z,E)-9,11-14:OAc evolve ?

Identification of SlitOR5 orthologues and paralogues functional characterization

Genome analyses

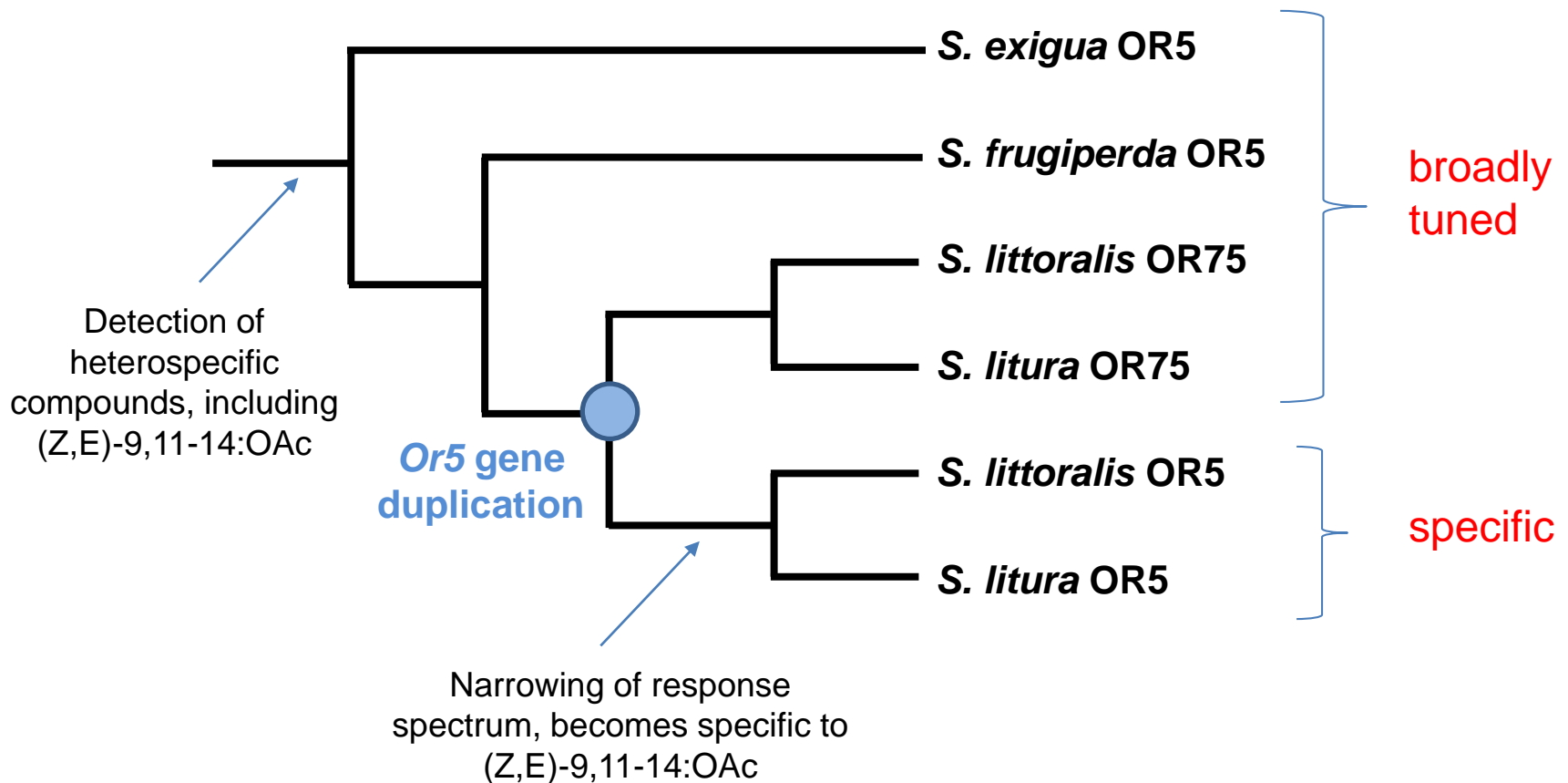


The functional characterization of SlitOR5 orthologues and paralogues (unpublished) led us to propose an evolutionary scenario for the Or5 gene in *Spodoptera*



unpublished

Evolutionary scenario for the Or5 gene in *Spodoptera*



unpublished

Conclusion and perspectives

- ✓ an atypical pheromone receptor identified, defining a new clade of PRs
-> *PR evolved at least time in Lepidoptera*
- ✓ Identification of mutations in insect ORs responsible for shifts in function
-> *links between structure and function*
- ✓ Structure-based virtual screening for the identification of OR agonists
-> *development of novel products for pest management*
- ✓ Evolution of sex pheromone biosynthesis

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Marie-Christine François

Thomas Chertemps

Annick Maria



Sai Zhang

Song Cao

Yang Liu

Guirong Wang



SLU Sweden

William Walker et al

