

Annotation manuelle d'une famille multigénique, les Cytochromes P450s chez des lépidoptères ravageurs de plantes

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Nocuidae: Spodoptera



S litura



S littoralis



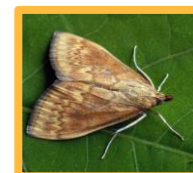
S frugiperda

<http://www.cbif.gc.ca>

Pyraustinae: Ostrinia



O scapularis



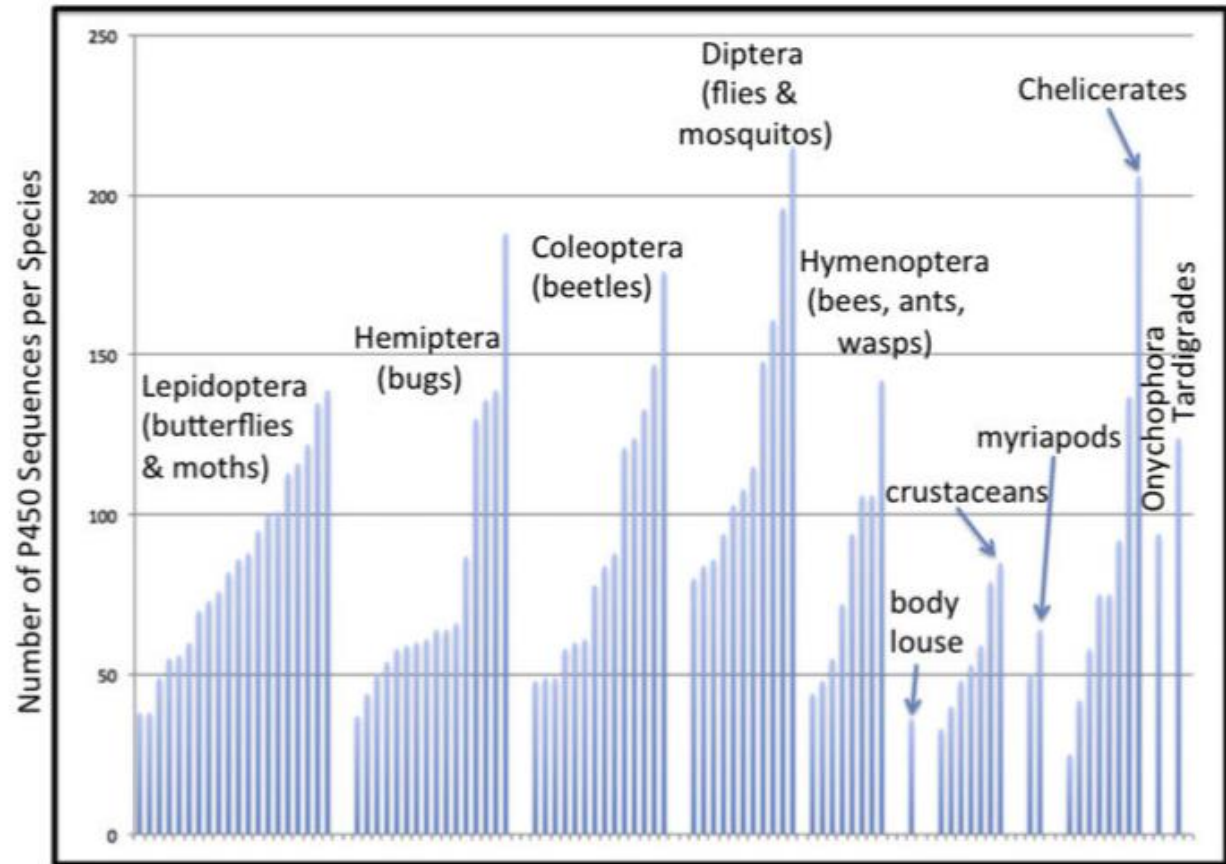
O nubilalis



O furnacalis

P450s: famille multigénique

<i>Homo sapiens</i>	57
<i>Rice</i>	435
<i>C. elegans</i>	82



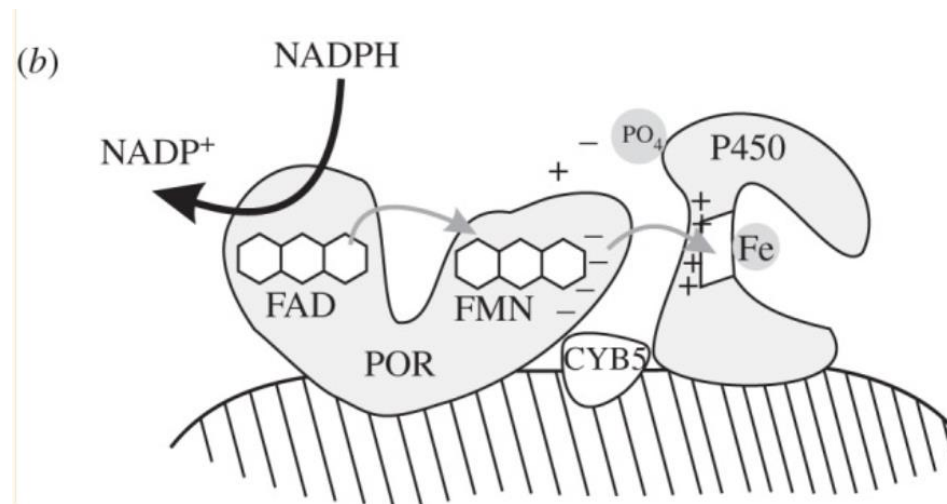
Distribution numbers of P450s in 87 panarthropods:

**27% des séquences de P450 sequences
contiennent des erreurs**

[Nelson 2018 Biochim Biophys Acta.](#)

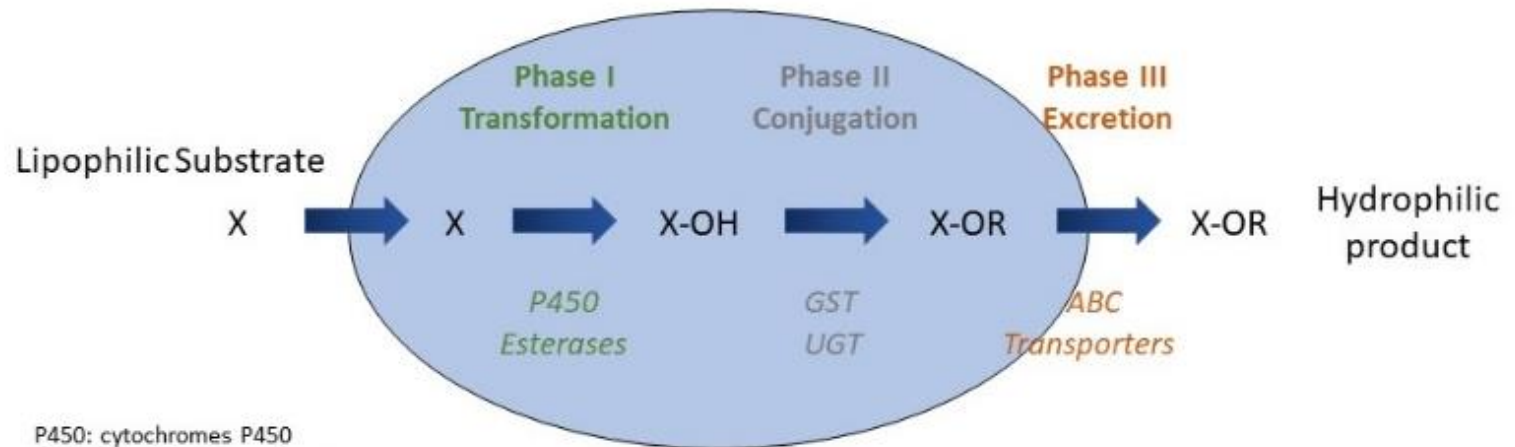
P450s: monooxygénases contenant un hème

- Protéines membranaires
- Protéines contenant un hème
- P450s ont besoin d'un partenaire redox [ex: Cytochrome P450 réductase (CPR or POR)]



P450 : enzymes de détoxication

Detoxification phases



P450: cytochromes P450
GST: glutathion S-transferases
UGT: UDP glycosyltransferases
ABC: ATP binding cassette transporters



CYP: Quelles Fonctions chez les insectes herbivores

Détoxication des métabolites secondaires de plantes

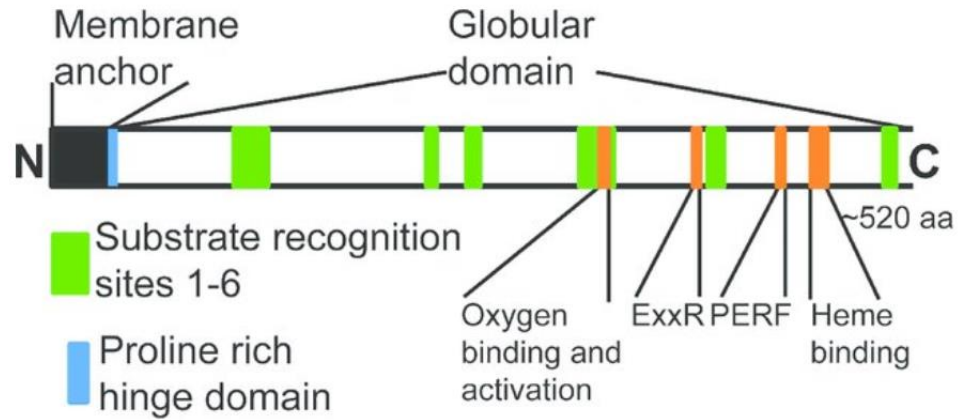
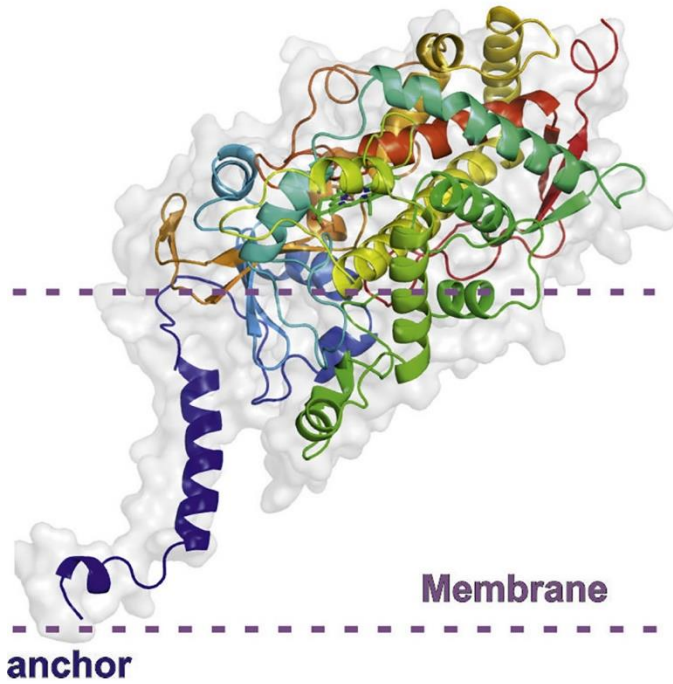
- Nicotine: Aphid CYP6CY3 (human CYP2A6),
- Flavonoïdes,
- Coumarines
- Méthyl-kénone: 2-Tridécanone, 2-undécanone
- Alkakoïdes cardiaques
- Pyrèthrines

Fonctions endogènes dans la physiologie des insectes

- Biosynthèse de l'hormone Juvénile: CYP15A
- Hormone de mue and biosynthèses de stérols: 18A, 302A, 306A, 307A, 314A, 315A
- Métabolisme des acides gras: CYP345G, CYP6K1
- Formation de la Cuticule (résistance à la dessiccation, communication chimique): CYP4G

P450 structure

CYP3A4



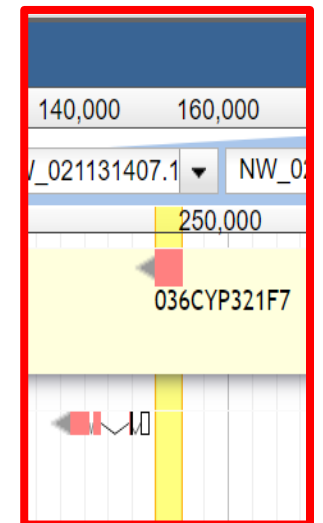
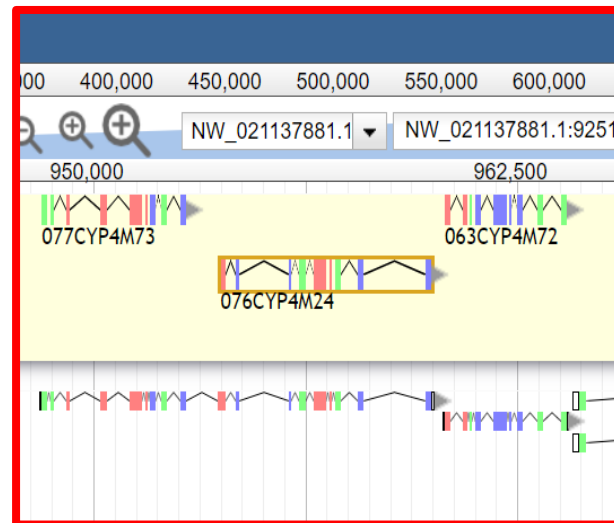
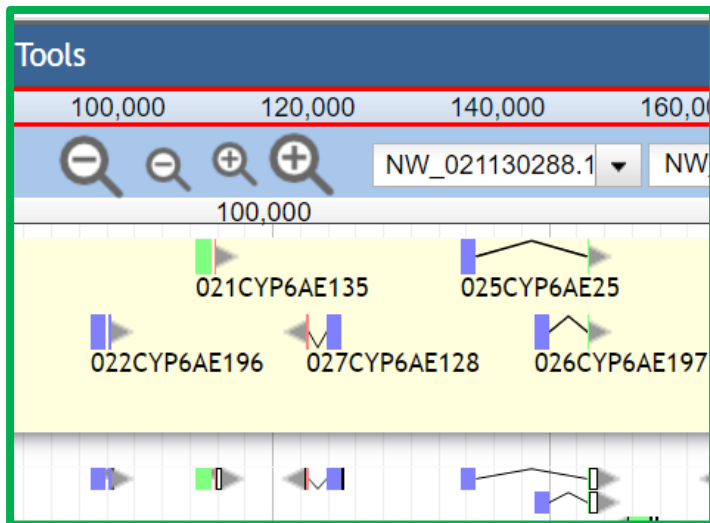
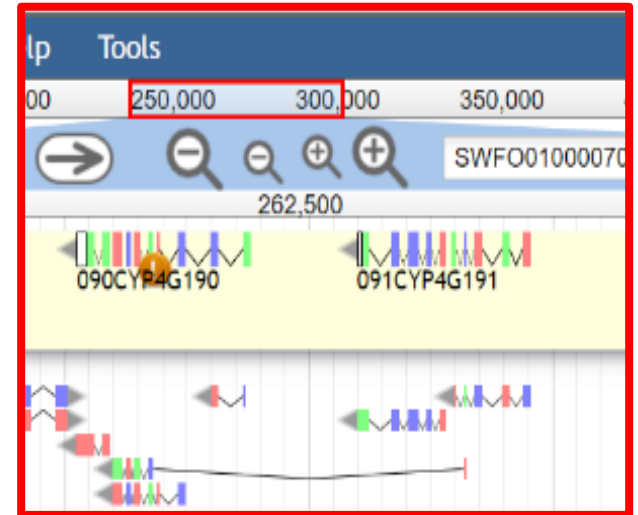
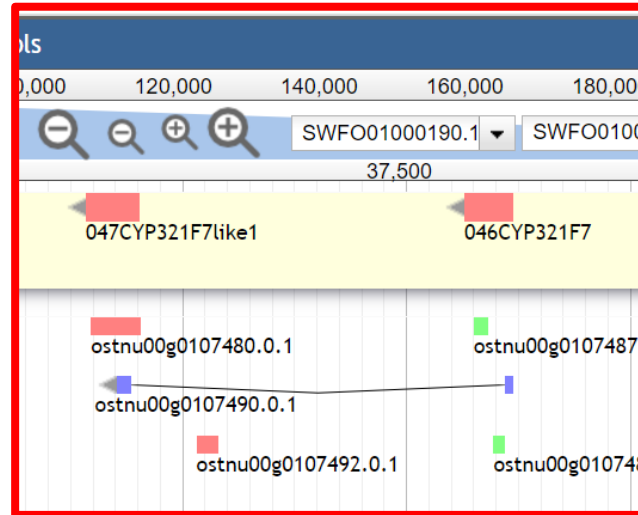
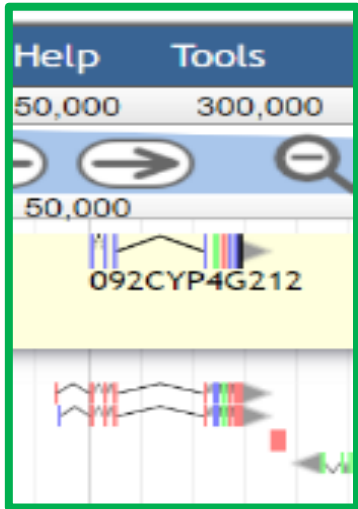
Srejber et al 2018 Journal of Inorganic Biochemistry

Prall et al 2016 PLOS ONE

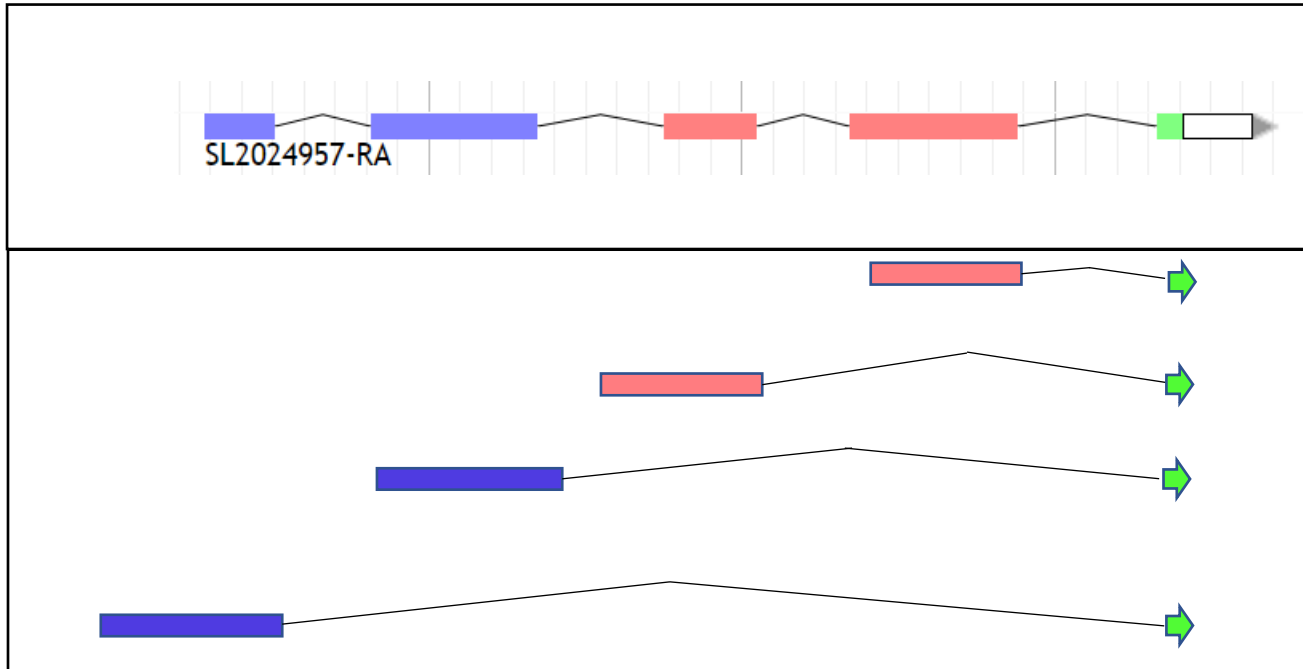
Annotation automatique des P450s dans les génomes

- **27% des séquences de P450 sequences contiennent des erreurs**
- **Faux modèles géniques dans les BD pourquoi?**
 - **Difficultés des prédictions exon/introns**
 - **Expressions tissu-spécifique et/ou inductible des P450s**
 - **Au moins 20-30% des P450s sont en tandem dans les génomes**

Nécessité d'une Curation manuelle des prédictions automatiques des gènes



CYP avec épissage alternatif



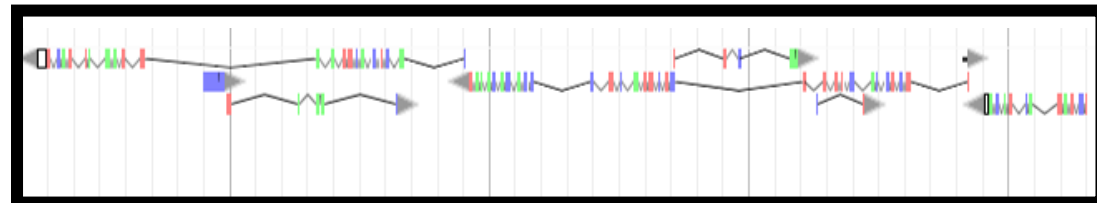
SL2024957-RA

Automatic annotation

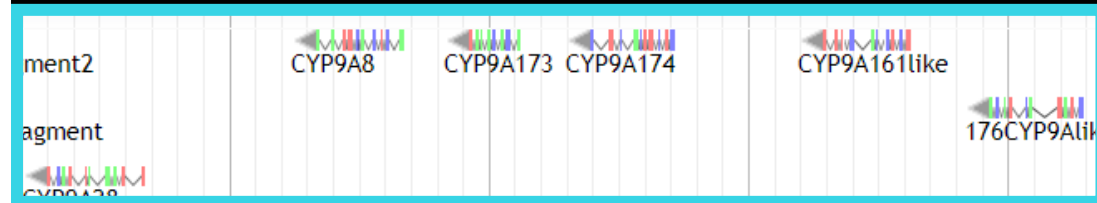
Manual curation of the
annotation

Nécessité d'une Curation manuelle après transfert d'annotation manuelle

Automatic predictions
V2



Manual
curation



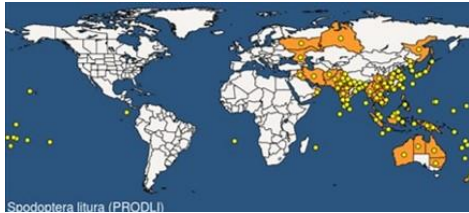
Outils pour accélérer la curation manuelle

- Dans le génome
 - Recherche avec HMMER : profil P450
 - Définition de nouveaux HMMER spécifiques (espèces d'intérêt, famille ou clan) et nouvelle recherche de P450
 - Utilisation d'exonerate et de P450 curés
 - Utilisation des reads de RNAseq mappés sur le génome

Invasive species

Tobacco cutworm

Spodoptera litura



EPPO database

Fall armyworm

Spodoptera frugiperda



EPPO database

Egyptian cotton leafworm

Spodoptera littoralis



EPPO database

Polyphagous



Cotton



Gossypol

Polyphenol



Tobacco



Nicotine

Alkaloid



Sorghum



Dhurrin

Cyanogenic
glycoside



Maize



DIMBOA

Benzoxazinoid



Rice

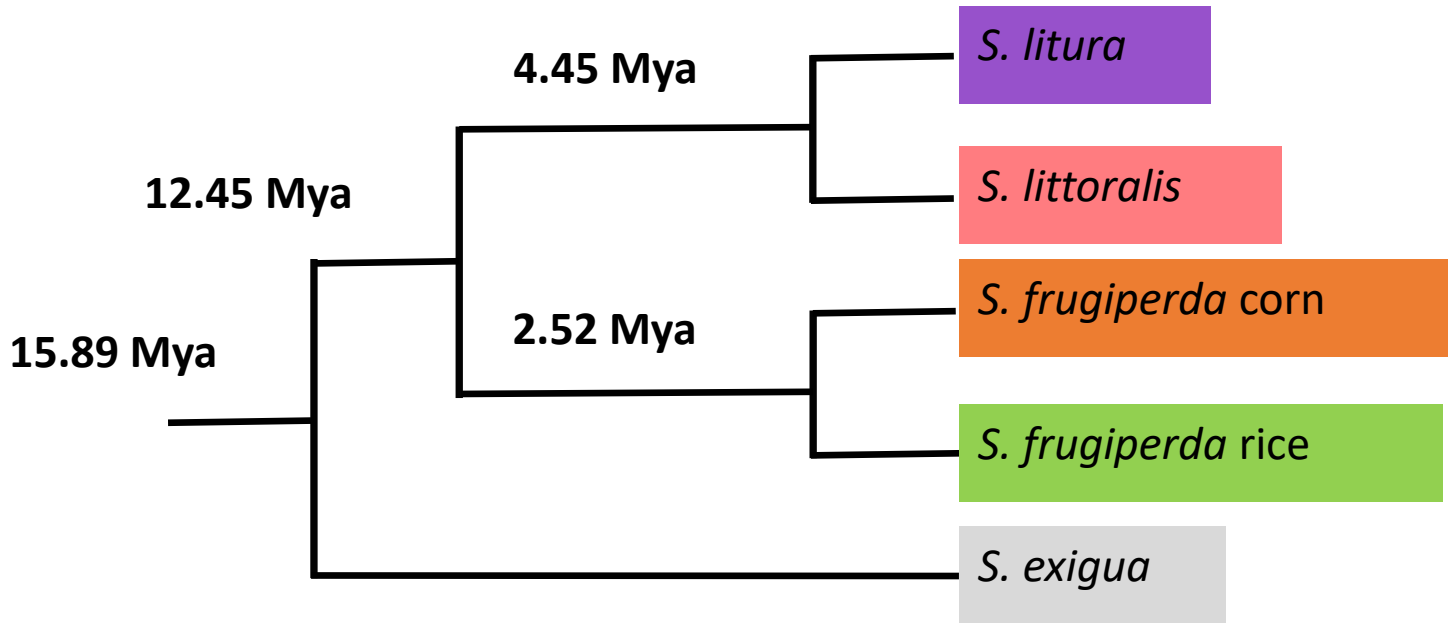


Momilactone

Diterpenoid

Poaceae

Phylogeny of Spodoptera species



CYP repertoires from four Spodoptera

□ Behind Gene family numbers, wide disparity hidden

	Species	clan2	clanmito	clan3	clan4	Total
Lepidoptera	<i>Spodoptera frugiperda</i> <i>corn</i>	8	11	59	39	117
	<i>Spodoptera frugiperda</i> <i>rice</i>	8	11	61	55	136
	<i>Spodoptera littoralis</i>	8	10	54	56	128
	<i>Spodoptera litura</i>	7	11	61	54	131
	<i>Bomby mori</i>	7	12	30	34	83

Number of orthologs between insect orders	4-5	4-5	0	1-3	8-13
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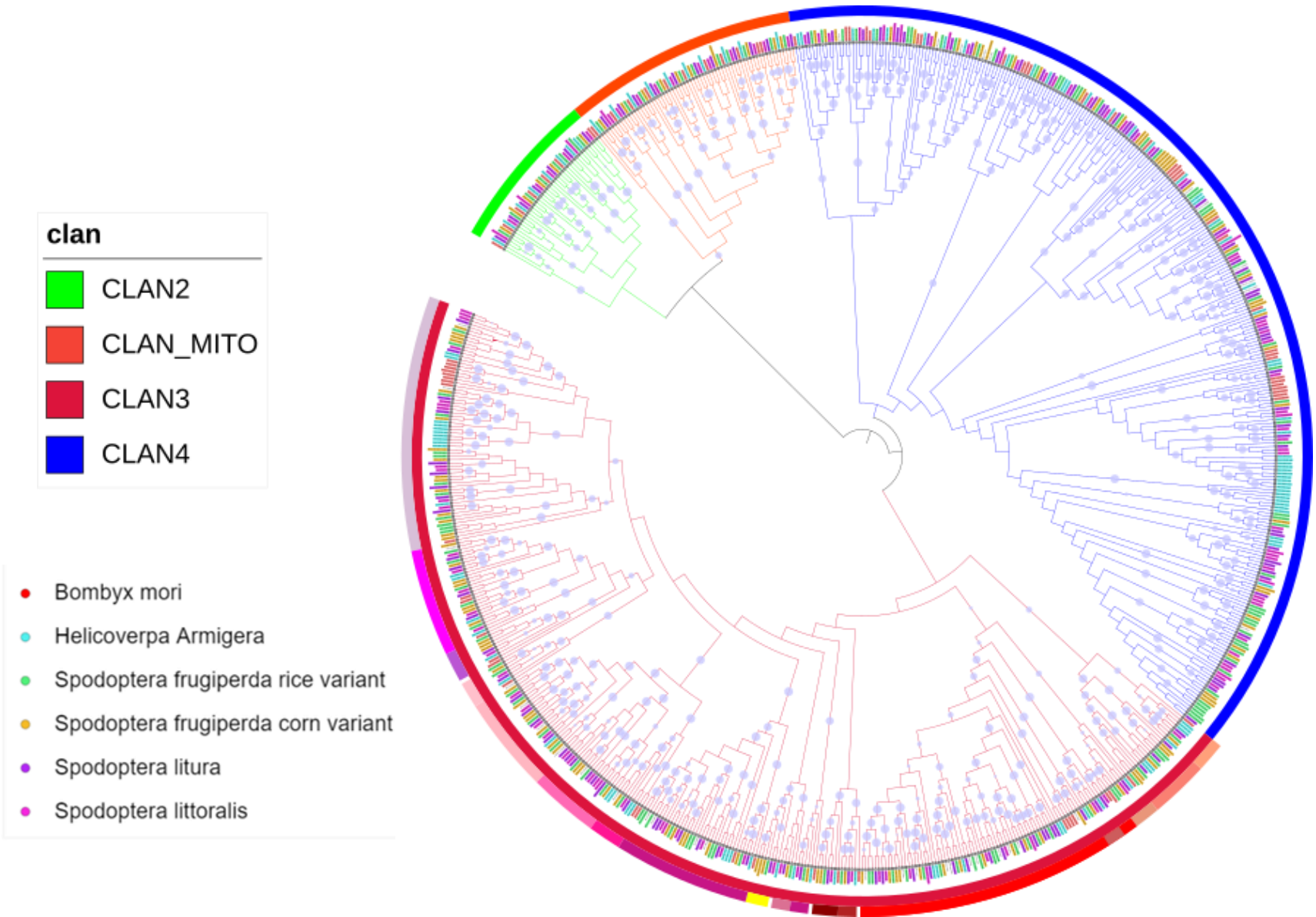
Gene numbers high variability for clans CYP3 and CYP4:

Numerous independent expansion (BLOOMING) leading to P450 diversity

Few 1:1 orthologs

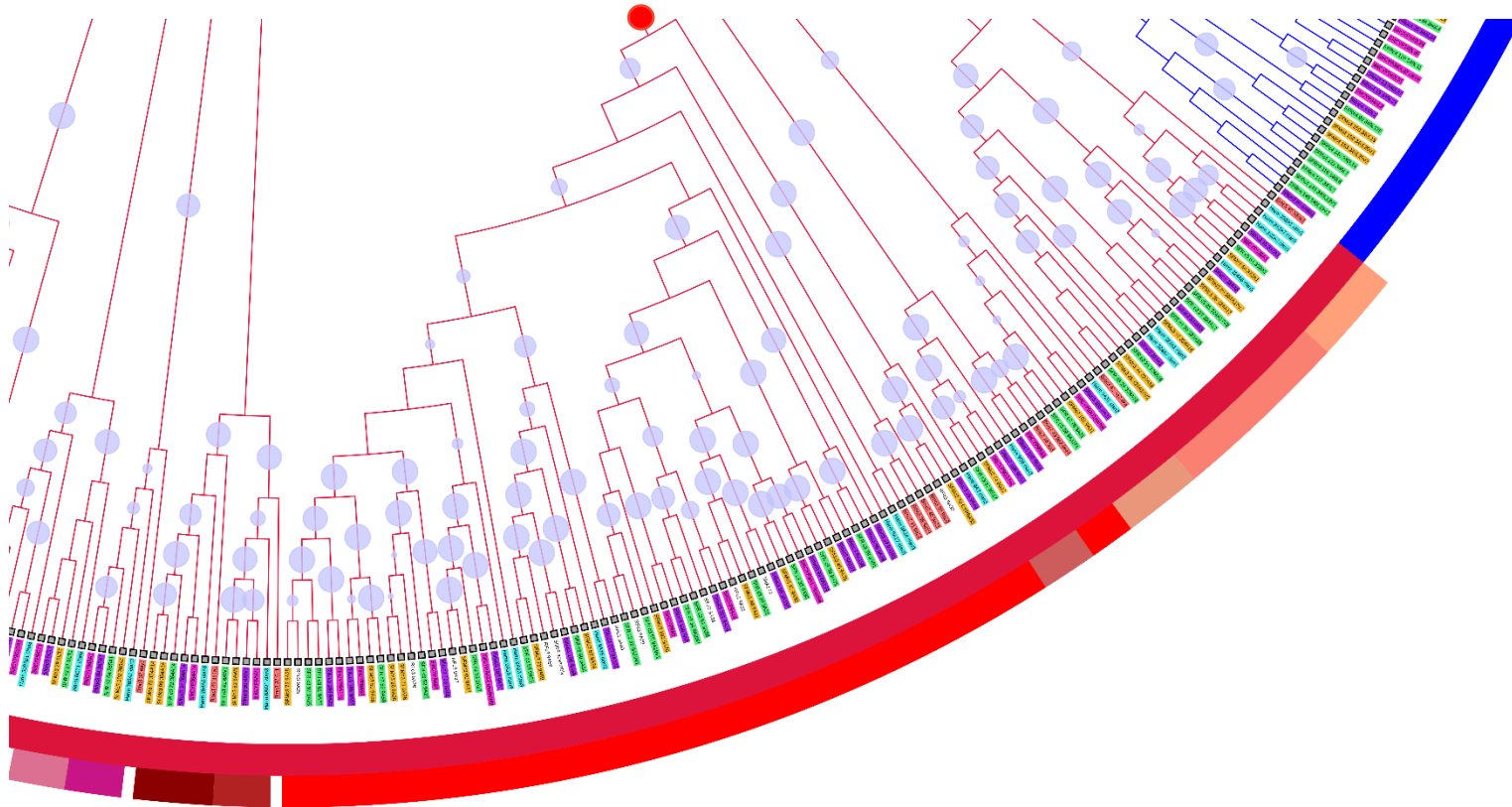
Gouin et al., 2017; Cheng et al. 2017, Hilliou et al., 2021

CYP phylogeny of 4 Spodoptera species



CYP9A gene expansion in a gene cluster

CYP9A

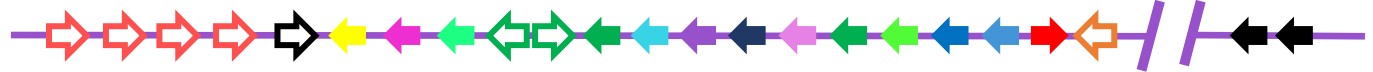


CYP9A cluster organisation

70 to 100 kb



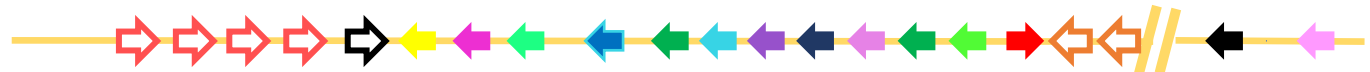
S. litura



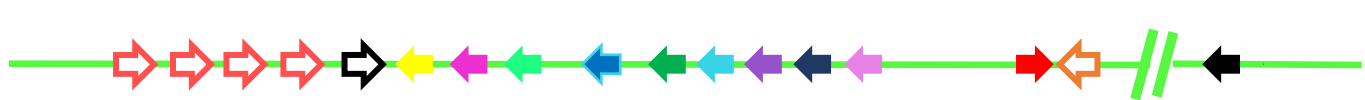
S. littoralis



S. frugiperda
Corn variant



S. frugiperda
rice variant



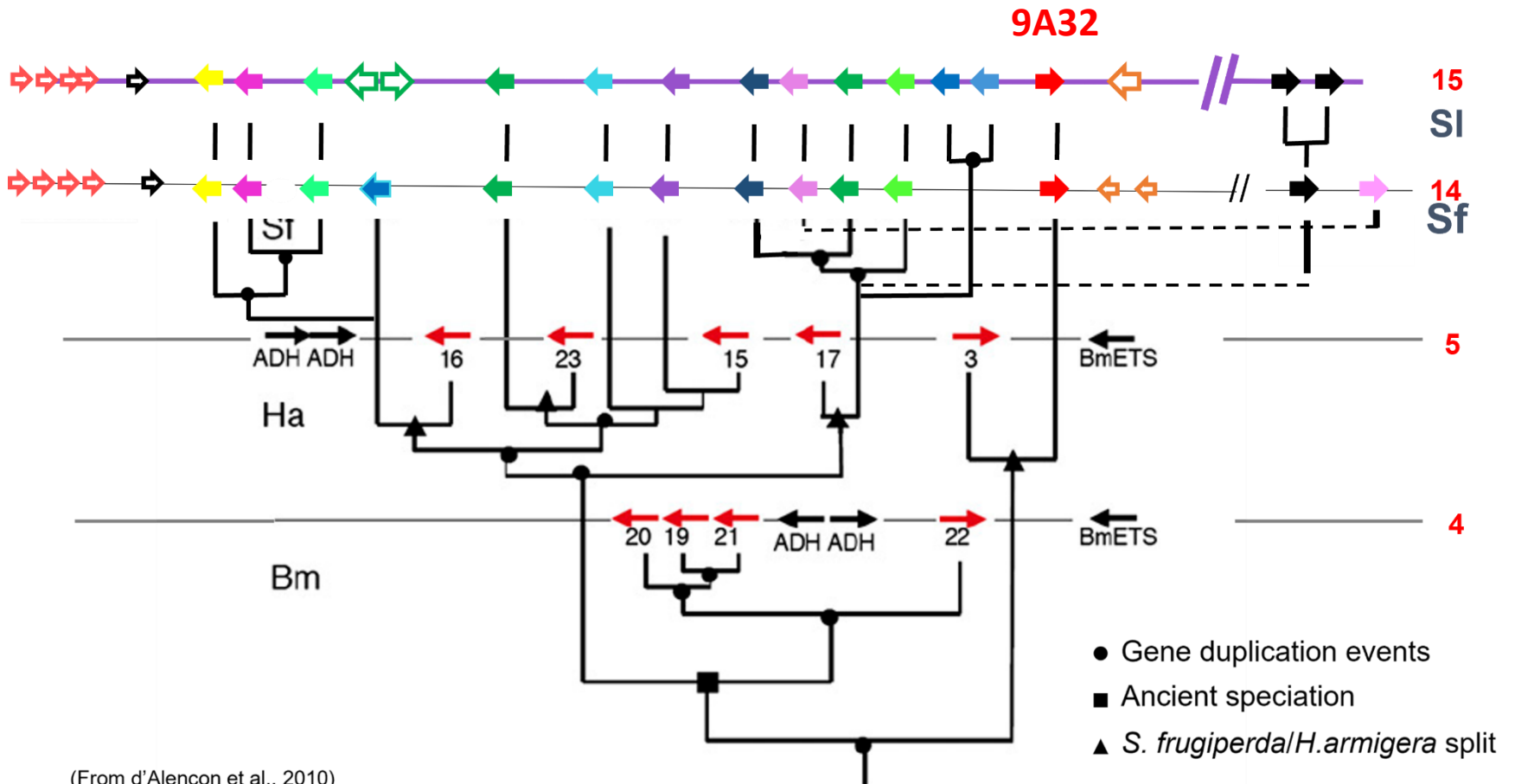
← CYP9A

⇨ ETS-TF

⇨ ADH

⇨ FT

⇨ GR

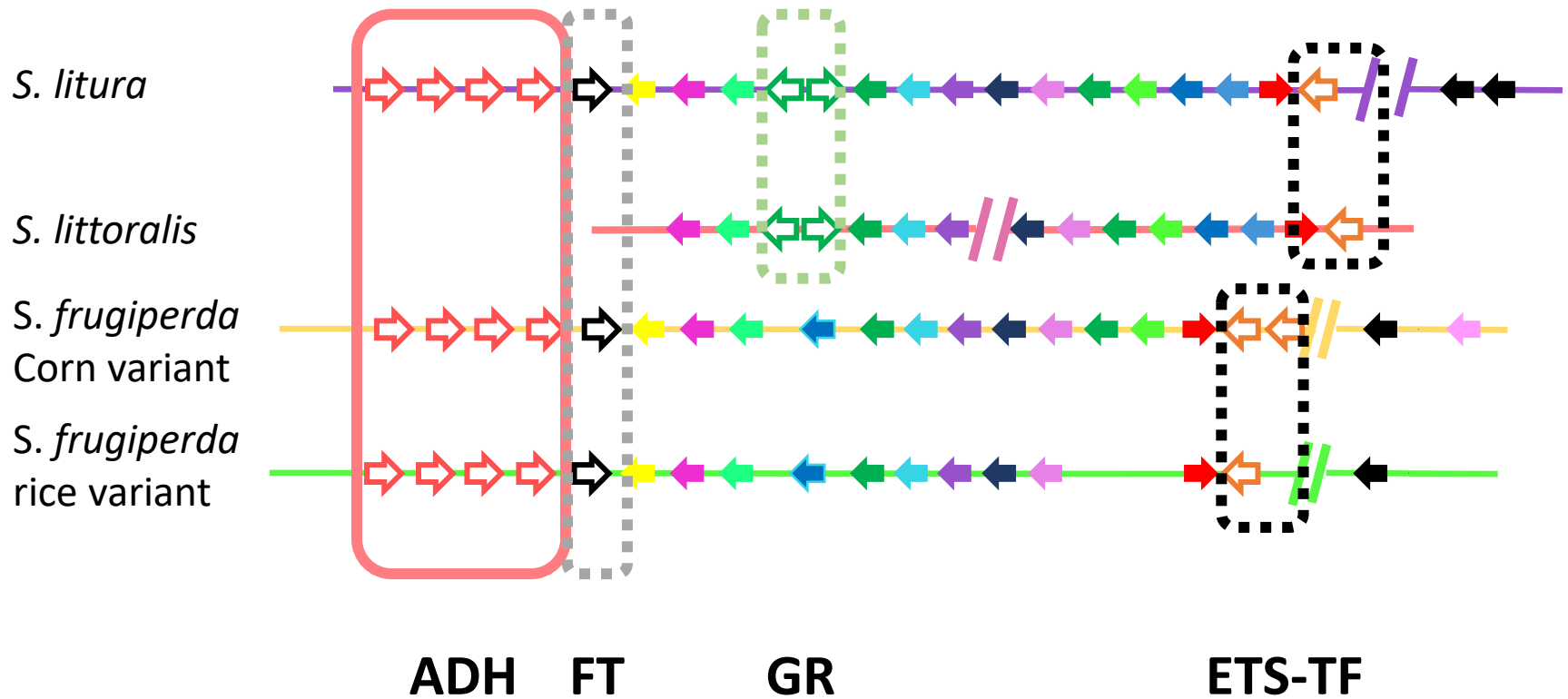


(From d'Alençon et al., 2010)

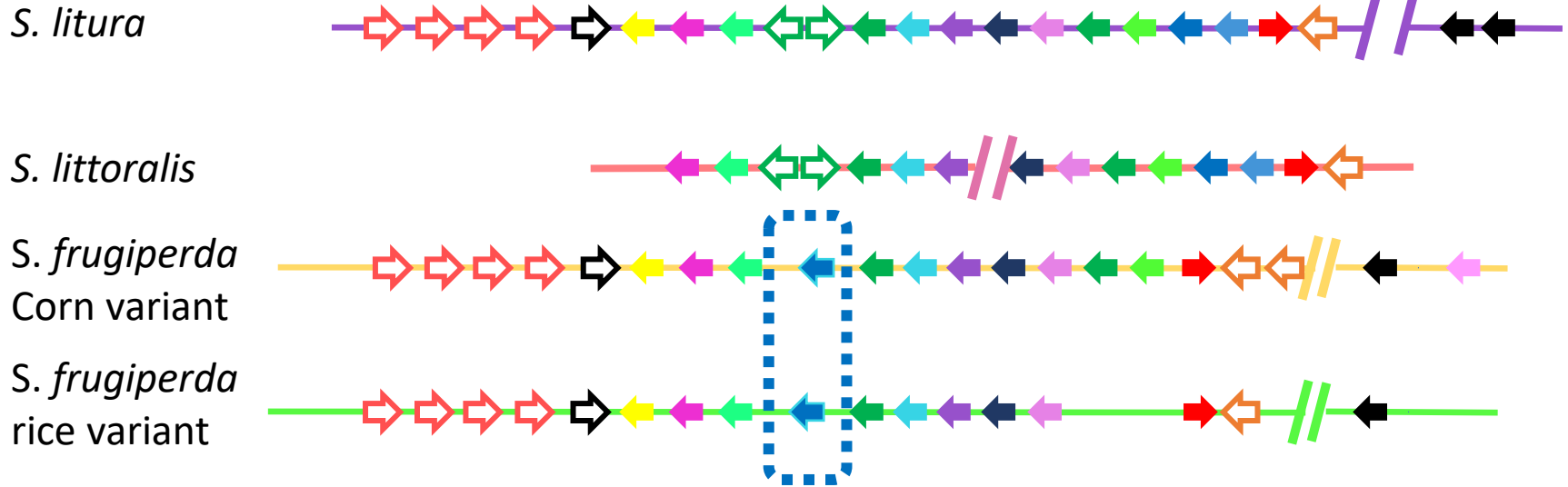
(From Gouin et al., 2017, Cheng et al., 2017)

- **CYP9A32** ancestor was at the origin of CYP9A cluster in Spodoptera
- **CYP9A32** mRNA : induced by xanthotoxin and ARE (antioxydant response element) found in its promoter

Conserved CYP9A cluster organisation: biological significance?

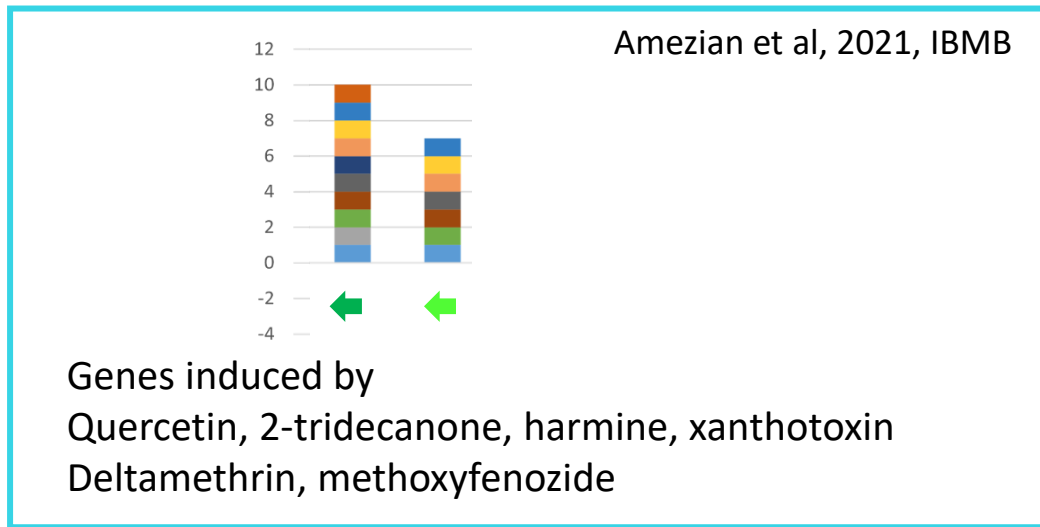
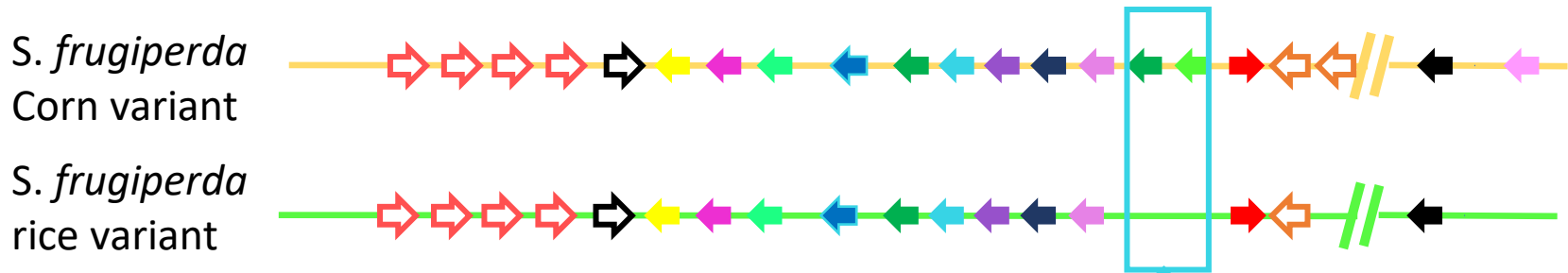


CYP9A cluster organisation: a CYP specific to *S. frugiperda*



- Induced in a dose-dependent-way by Methoprene (JH analog) and I3C (degradation compound of glucosinolates)
- Induced by 2 Tridecanone, Harmine, and methoxyfenozide (ecdysone receptor agonist)

2 CYPs are specific to *S. frugiperda* corn variant



← : involved metabolon linked to DIMBOA detoxification (Israni et al 2022)

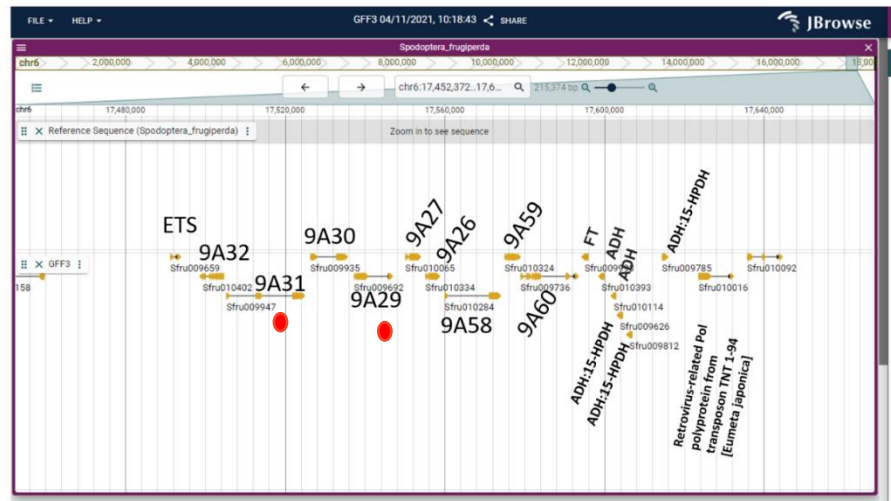
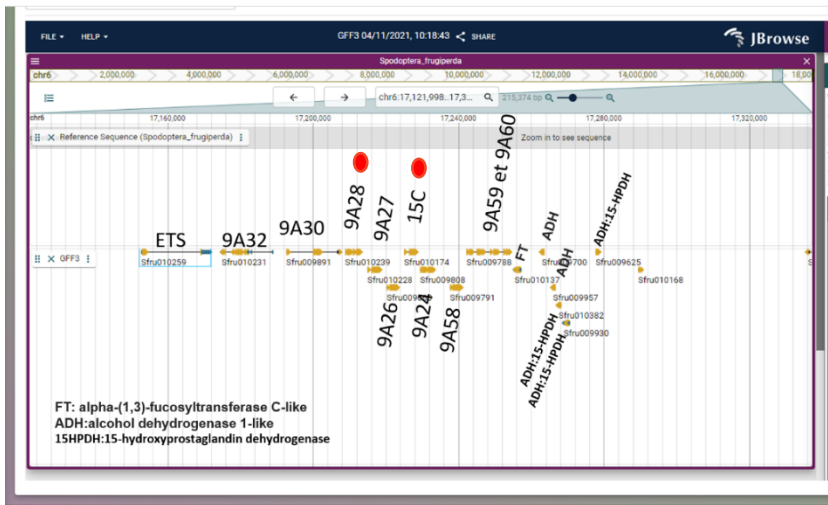
Plasticity of CYP9A cluster in *S. frugiperda*

- CNV of CYP9A cluster in Puerto Rico geographical populations with strong selective pressure show resistance to deltamethrin (Gimenez et al., 2020)

- CNV of CYP9A cluster in *S. frugiperda* hybrid collected from chinese corn field

<http://v2.insect-genome.com/JBrowse>, individual sequenced where collected in China on corn field

<http://v2.insect-genome.com/JBrowse>, individual sequenced where collected in China on corn field



InsectBase 2.0

CYP15C1: involved in JH biosynthesis

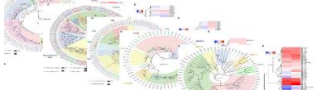
Mei et al., *Nucleic Acids Research*, 2022
Xiao et al., *Mol Ecol Resour* 2020

Function(s) of the cluster?

- **CYP9A specific to lepidoptera:**
 - **Role in insect development as some members are induced:**
by JH analog,
ecdysone receptor agonist
but also direct interaction with JH analog (Amezian et al 2022)
 - **Role in detoxification of plant compounds, as some members are induced**
xanthotoxin, indole, 2-tridecanone, harmine, quercetin
 - **Involved in the metabolon link to DIMBOA detoxification** (Israni et al, 2022)
 - **resistance to pesticide (deltamethrin, Gimenez et al. ,2020)**
- **Alcohol DeHydrogenase : redox sensing, HaADH5 binds to CYP6B6 promoter and regulates the metamorphosis and development of *H. armigera***
- ***In B. mori, ETS from CYP9A cluster is regulating the embryonic diapause***

Cluster: catalytic diversity and potentially enhanced detoxification capacities
Role in Insect adaptative evolution?

Detox annot group



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Fabrice Legeai
Stéphanie Robin

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