

Host specialization in two sister species of moths: life history traits and differentially expressed genes

Marion Orsucci

Philippe Audiot

Louise Brousseau

Denis Bourguet

Franck Dorkeld

Bernhard Gschloessl

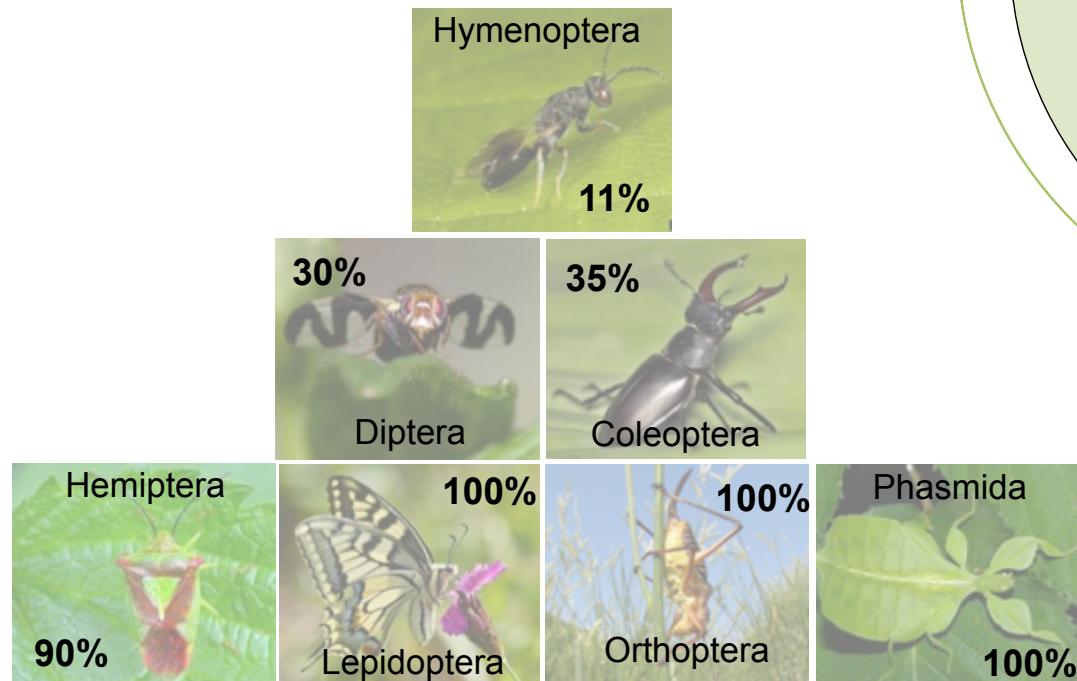
Sabine Nidelet

Réjane Streiff

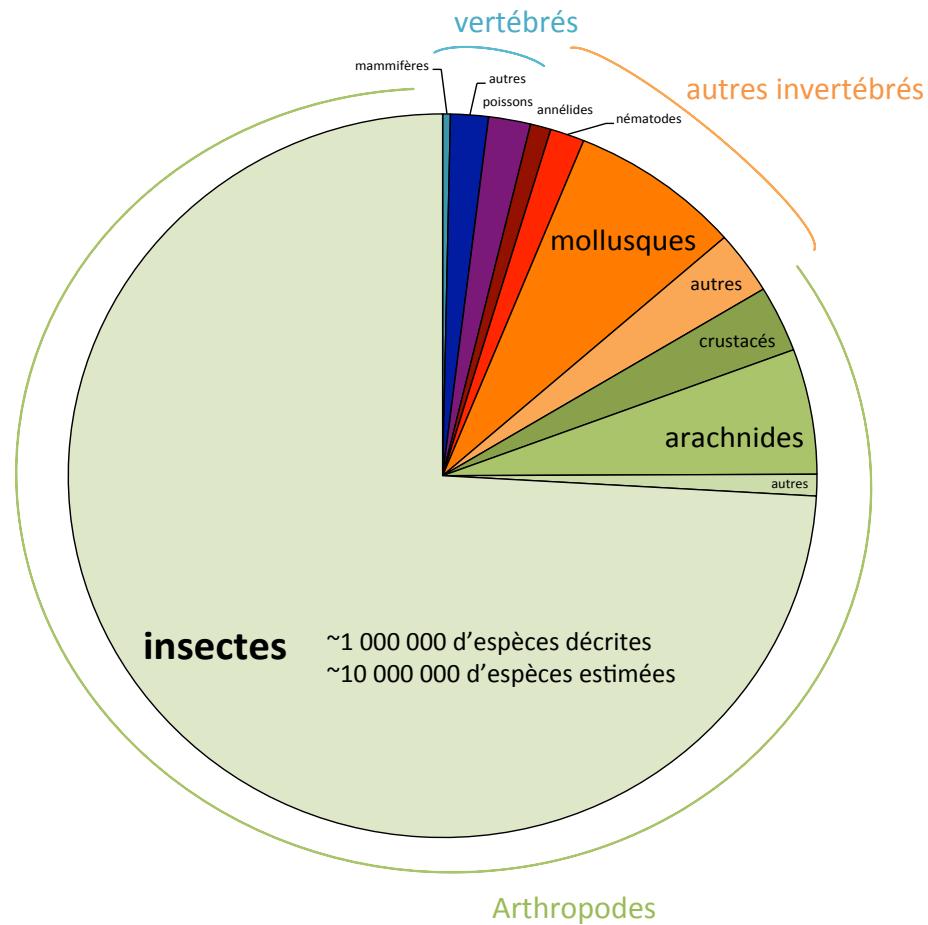


Centre de Biologie pour la Gestion des Populations

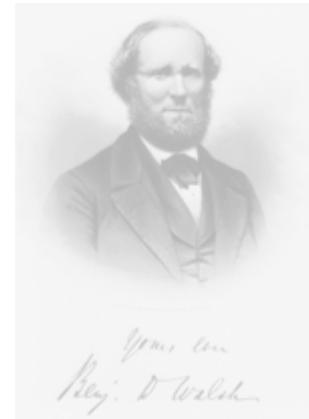
- **70% of animal species are insects**
- **A majority of insects is herbivorous**
- **A majority of the herbivorous insects are specialized to a limited number of host plants**



Herbivory level per insect family



The diversification of the phytophagous insects



372

Review

TRENDS in Ecology & Evolution Vol.16 No.7 July 2001

Ecology and the origin of species

Dolph Schlüter

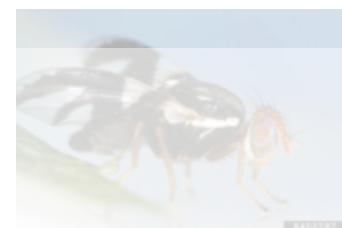
The ecological hypothesis of speciation is that reproductive isolation evolves ultimately as a consequence of divergent selection in different environments. Ecological speciation, which may occur in sympatry, involve many agents of natural selection, and result from a combination of adaptive processes. The ecological hypothesis has been the scarcity of empirical cases. Several new cases have recently emerged. I review the mechanisms that give rise to new species by divergent selection, compare them with other mechanisms of speciation, summarize recent tests of the ecological hypothesis, and discuss their research.

- **Coevolution** direct selection on premating isolation (REINFORCEMENT). Distinguishing the ways in which divergent selection has led to reproductive isolation is among the greatest challenges of the empirical study of ecological speciation.
- **Host shift** some species shifts to build an allogeny as a response to unique aspects of their environment.
- **Local adaptation** some species adapt to unique aspects of their environment.
- **Ecological speciation** some species adapt to unique aspects of their environment.

Abstract

Ecological speciation 116: 251–267, 2002
© 2002 Kluwer Academic Publishers. Printed in the Netherlands

doi: 10.1111/j.1461-0248.2004.00715.x



251

Howard D. Rundle^{1*} and Patrik Nosil²

¹Department of Zoology and Entomology, University of Queensland, Brisbane,

Herbivorous insects: model systems for the comparative study of speciation ecology

Daniel J. Funk¹, Kenneth E. Filchak² & Jeffrey L. Feder²

¹Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, USA (Phone: (615) 322-2214; Fax: (615) 343-0336; E-mail: daniel.funk@vanderbilt.edu); ²Department of Biological Sciences, University of Notre Dame, South Bend, IN 36556, USA (Phone: (219) 631-4160; Fax: (219) 631-7413; E-mails: filchak.1@nd.edu, jeffrey.l.feder.2@nd.edu)

DOI: 10.1111/j.1570-7458.2009.00916.x

Received 12 May 2002 Accepted 18 June 2002

MINIREVIEW

Ecological speciation in phytophagous insects

Kei W. Matsubayashi¹, Issei Ohshima² & Patrik Nosil^{3,4*}

¹60-0810, Japan, ²Department of Evolutionary Biology, ³Department of Ecology and Evolutionary Biology, ⁴Institute for Advanced Study, Berlin, 14193,



apples



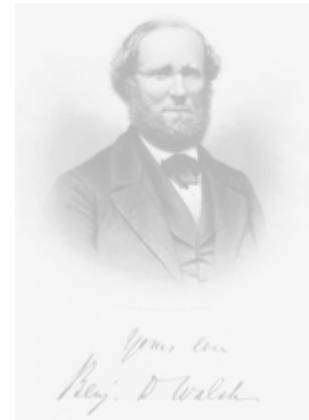
hawthorns



Germany



The diversification of the phytophagous insects



372 Review *TRENDS in Ecology & Evolution* Vol.16 No.7 July 2001

Ecology and the origin of species

Dolph Schlüter

The ecological hypothesis of speciation is that reproductive isolation evolves ultimately as a consequence of divergent selection in different environments. Ecological speciation, which may occur in sympatry, involve many agents of natural selection, and result from a combination of adaptive processes.

- **Coevolution** direct selection on premating isolation (ENFORCEMENT). Distinguishing the ways in which divergent selection has led to reproductive isolation is among the greatest challenges of the empirical study of ecological speciation.
- **Host shift** host shifts often serve to build an analogy as species by divergent selection, compare the two mechanisms to unique aspects
- **Local adaptation** research
- **Ecological speciation**

Abstract

Ecological speciation is the process by which formation of new species when barriers to gene flow are overcome by environmental gradients. This review highlights the diversity of mechanisms that can lead to ecological speciation and the need for comparative studies of speciation in phytophagous insects.

- **Pattern is not process. Ex: is specialization the cause or a consequence of species divergence?**



251

Daniel J. Funk¹, Kenneth E. Filchak² & Jeffrey L. Feder²

¹Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, USA (Phone: (615) 322-2214; Fax: (615) 343-0336; E-mail: kufunk@vanderbilt.edu); ²Department of Biological Sciences, University of Notre Dame, South Bend, IN 36556, USA (Phone: (219) 631-4160; Fax: (219) 631-7413; E-mails: filchak.1@nd.edu, jeffrey.l.feder.2@nd.edu)

DOI: 10.1111/j.1570-7458.2009.00916.x

Received 12 May 2002 Accepted 18 June 2002

MINIREVIEW

Ecological speciation in phytophagous insects

Kei W. Matsubayashi¹, Issei Ohshima² & Patrik Nosil^{3,4*}

¹60-0810, Japan, ²Department of Evolutionary Biology, ³Department of Ecology and Evolutionary Biology, ⁴Institute for Advanced Study, Berlin, 14193, Germany



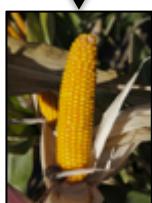
- The case of insect pests



- The case of insect pests

Domestication

teosinte



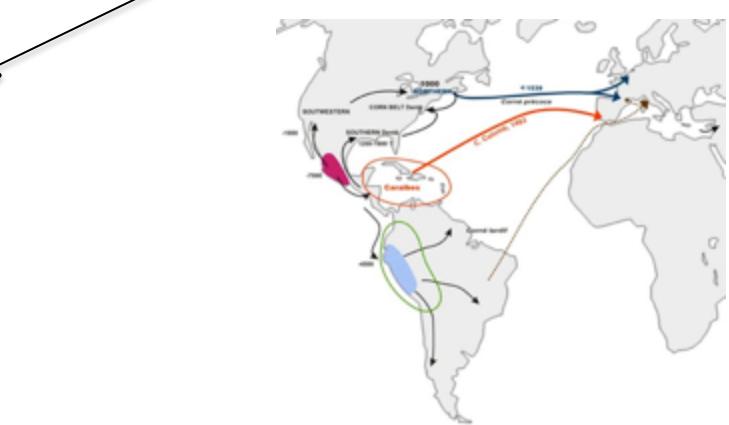
corn

Plant introduction

New niches

+

Crop intensification



Specialization to new habitats





A) Domestication



B) Host shifts



C) Introduction



Diatraea grandiosella : from the teosinte to maize



A) Domestication



B) Host shifts



C) Introduction

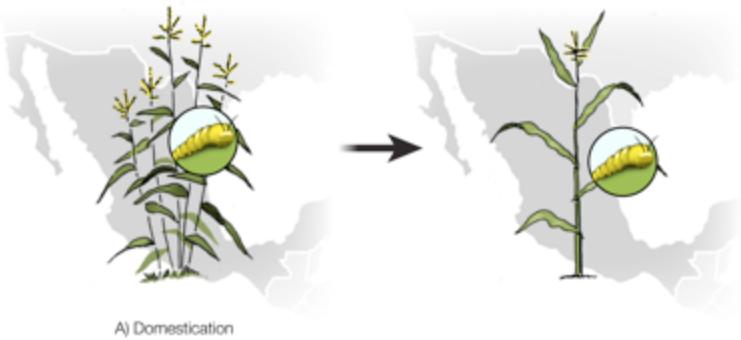


Diatraea grandiosella : from the teosinte to maize

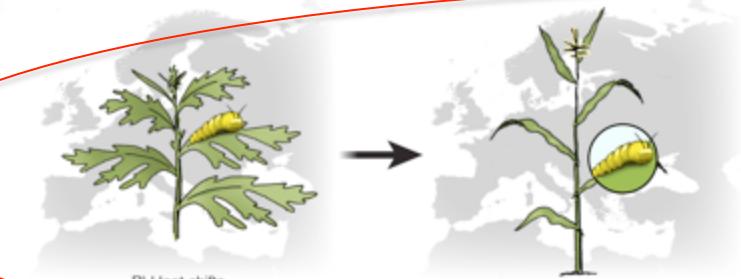


Helicoverpa armigera and *zea*: from south america to worldwide

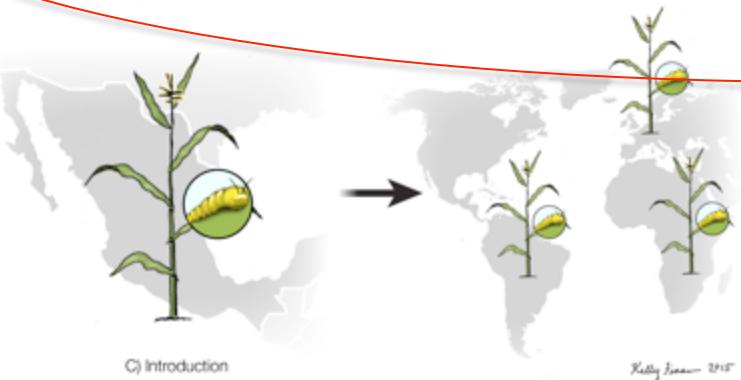
From Chen, Y. H. (2016). Journal elementa



A) Domestication



B) Host shifts



C) Introduction



Diatraea grandiosella : from the teosinte to maize



Ostrinia : from native host to introduced maize



Helicoverpa armigera and *zea*: from south america to worldwide

➤ Context:
Ostrinia genus, an eurasian origin and an exotic plant



- Context:
Ostrinia genus, an eurasian origin and an exotic plant



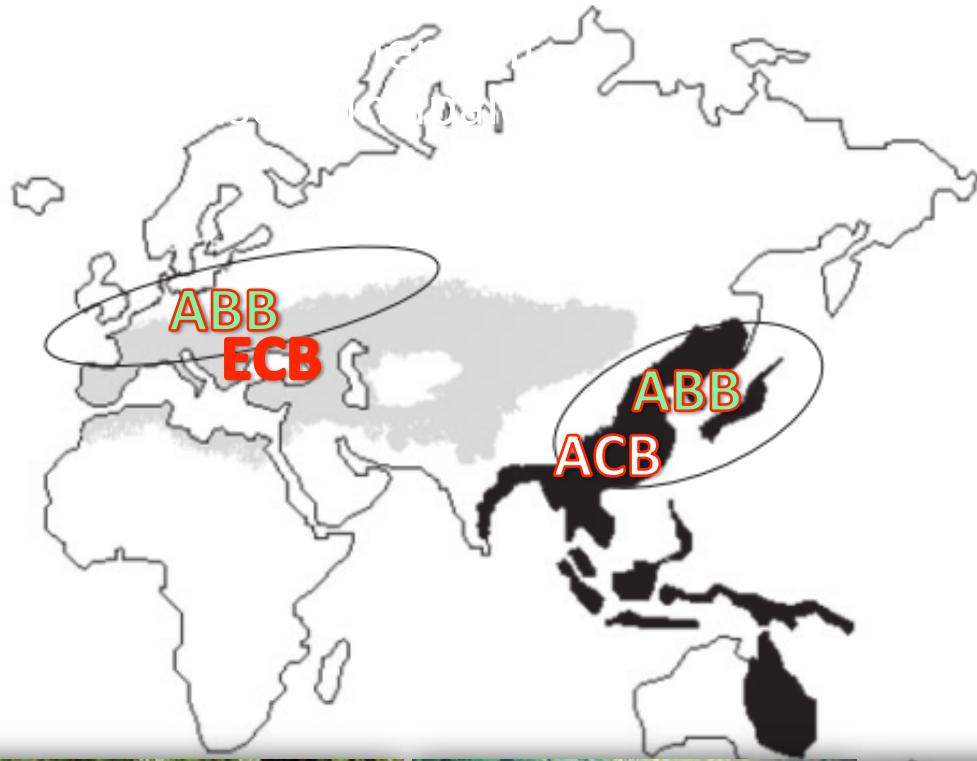
○ *O. scapulalis* (sensu nov.)



ECB

ACB

- Context:
Ostrinia genus, an eurasian origin and an exotic plant



- Context:
Ostrinia genus, an eurasian origin and an exotic plant

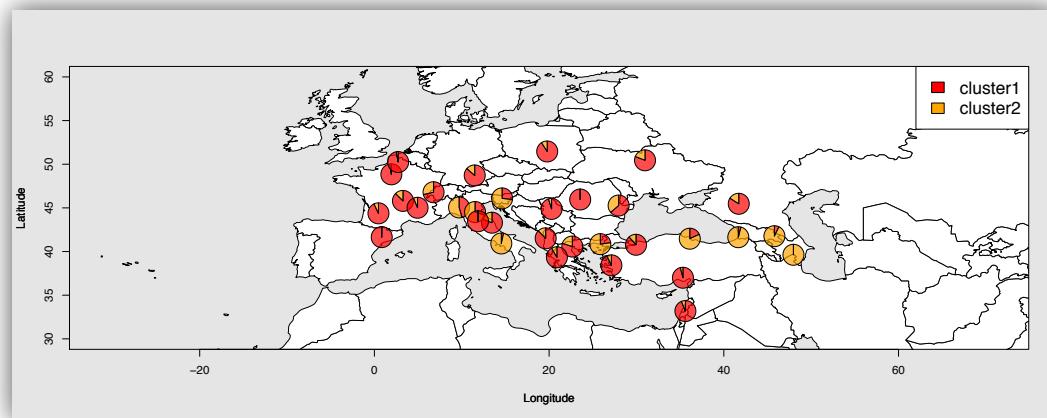


- Context:
Ostrinia genus, an eurasian origin and an exotic plant



Objectives:

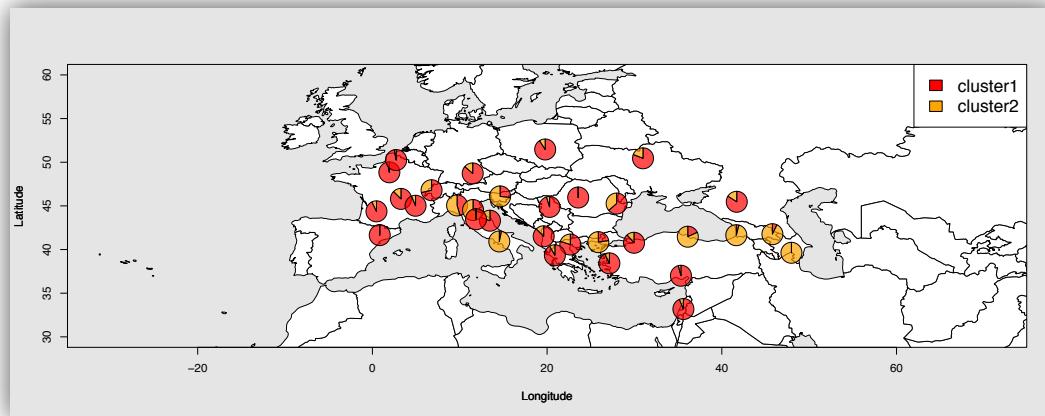
- * Evolutionary history of the populations and species of the *Ostrinia* genus



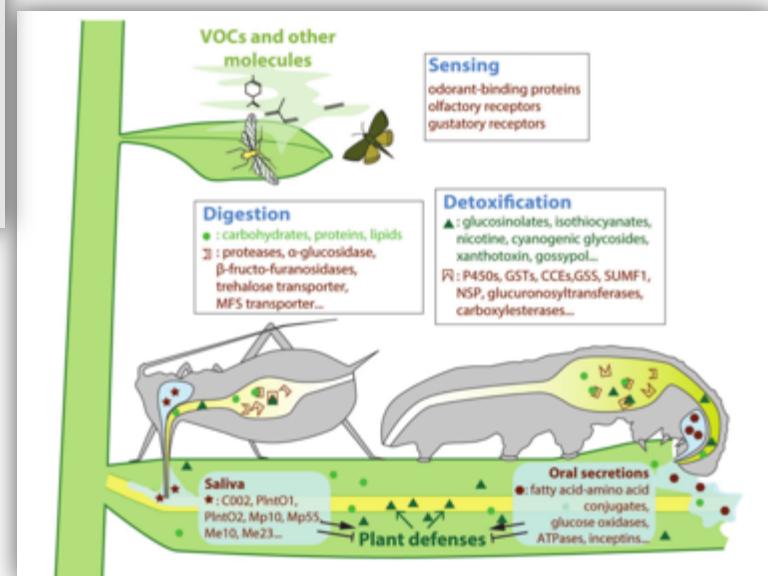
Genetic differentiation and association to the host plant

Objectives:

- * Evolutionary history of the populations and species of the *Ostrinia* genus
- * Mechanism involved in the specialization to the host plant (phenotypic, molecular, genomic)

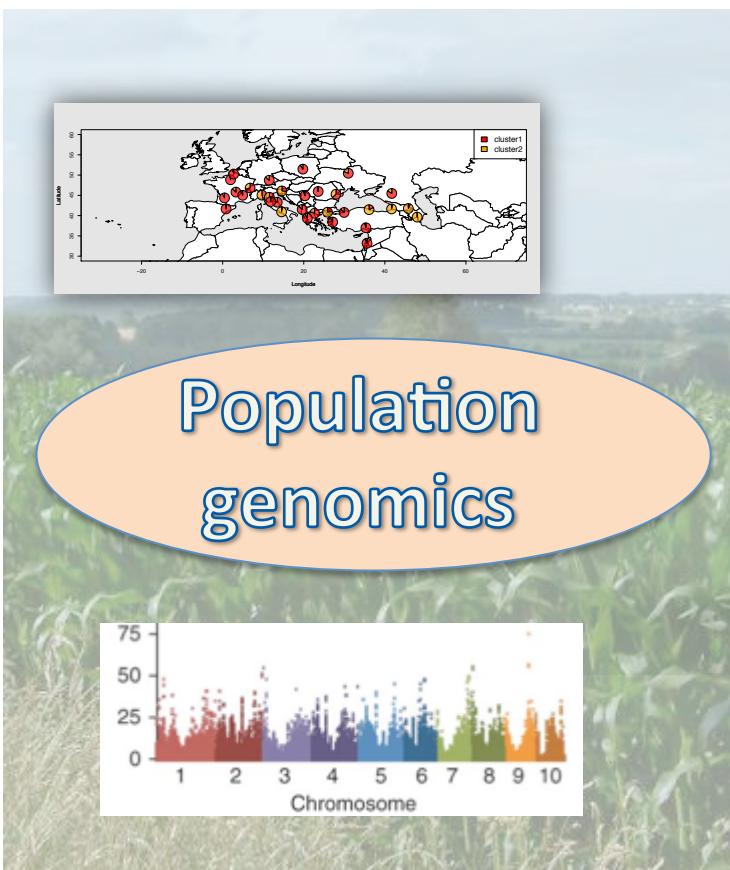


Genetic differentiation and association to the host plant



Mechanisms: recognition, digestion, detoxification (Simon et al 2015)

- methods:
complementary approaches in natural and experimental populations



THV



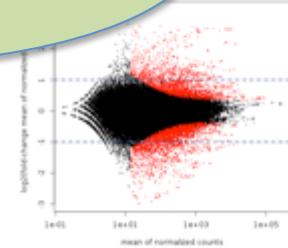
Transcriptomics

➤ Specialization and gene expression

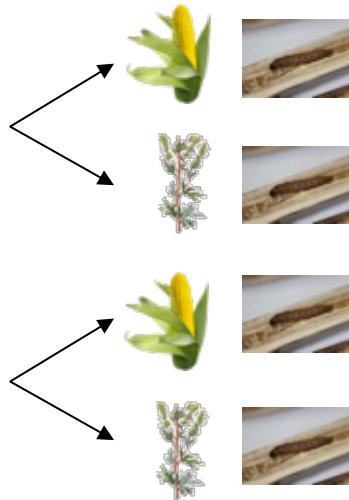
THV



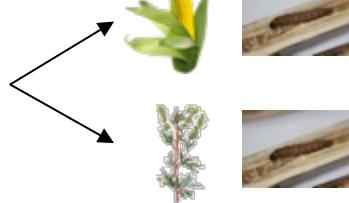
Transcriptomics



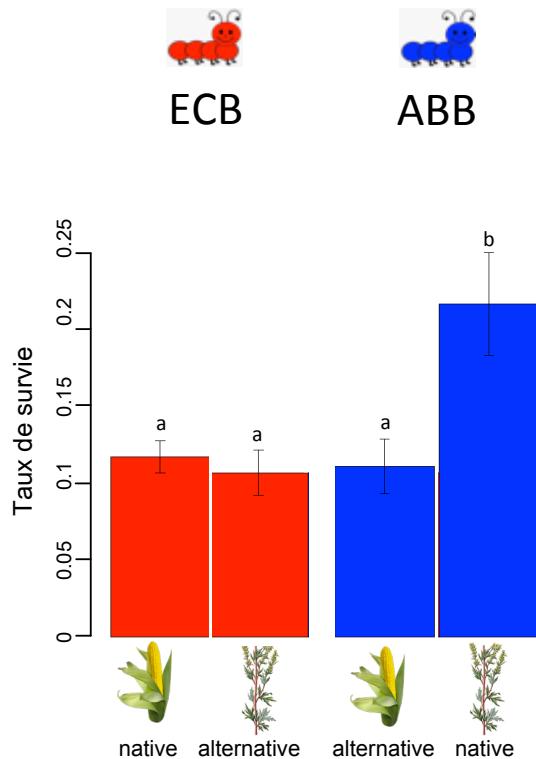
Specialization at the larval stage



- Count (~ survival)
- Weight
- Developmental time



Specialization at the larval stage



Survival

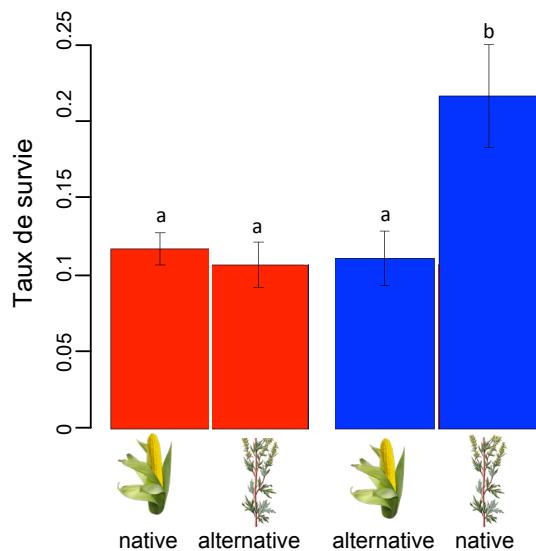
Specialization at the larval stage



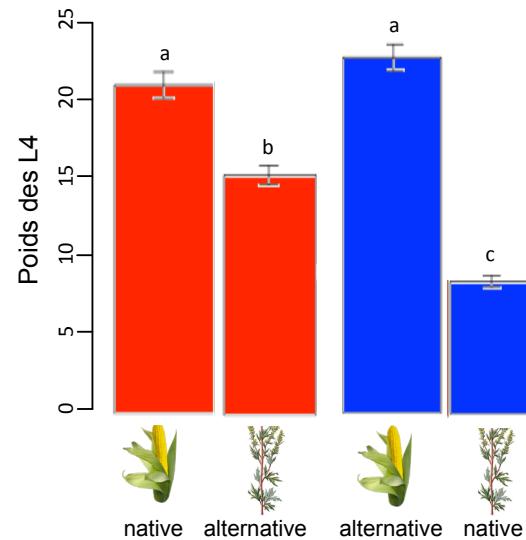
ECB



ABB



Survival



Weight

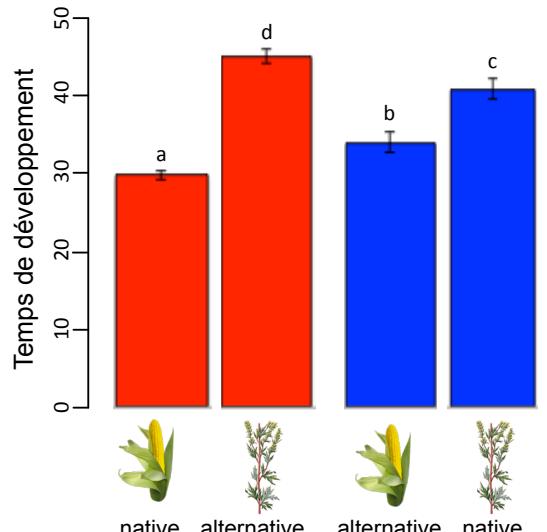
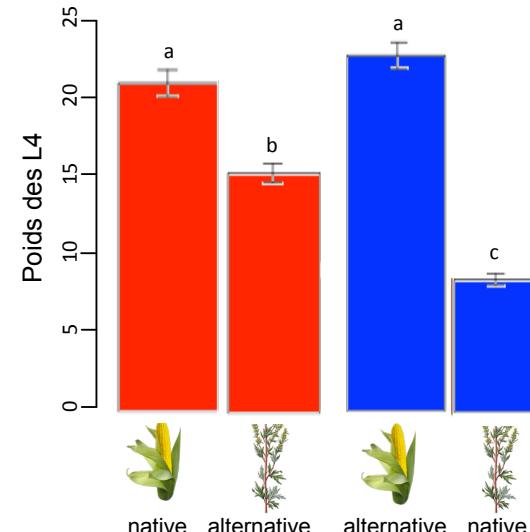
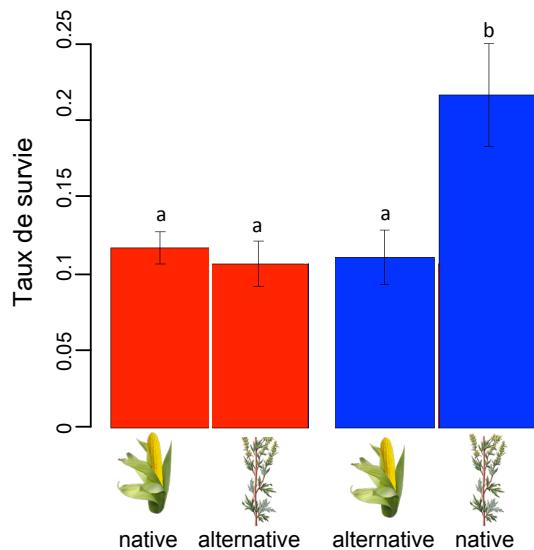
Specialization at the larval stage



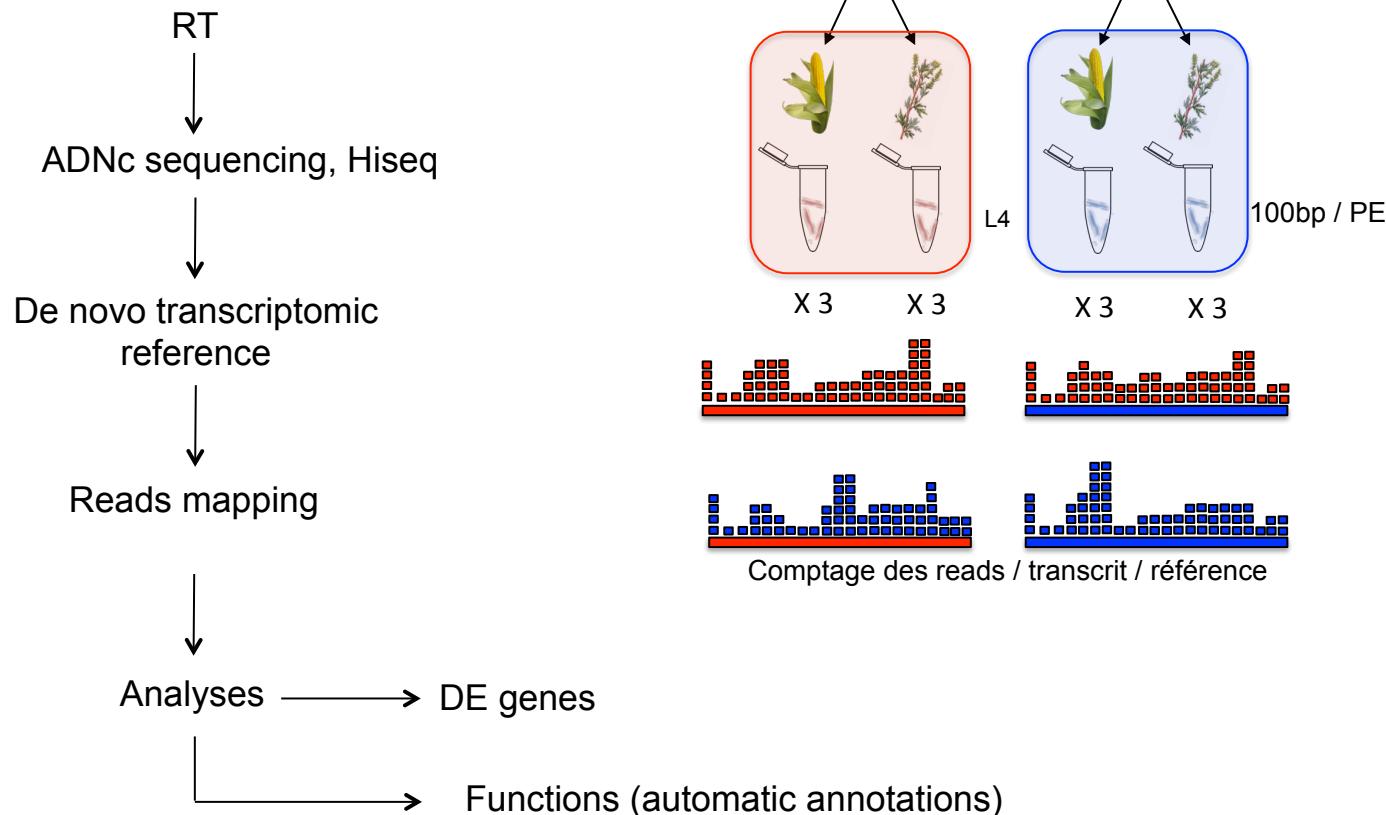
ECB



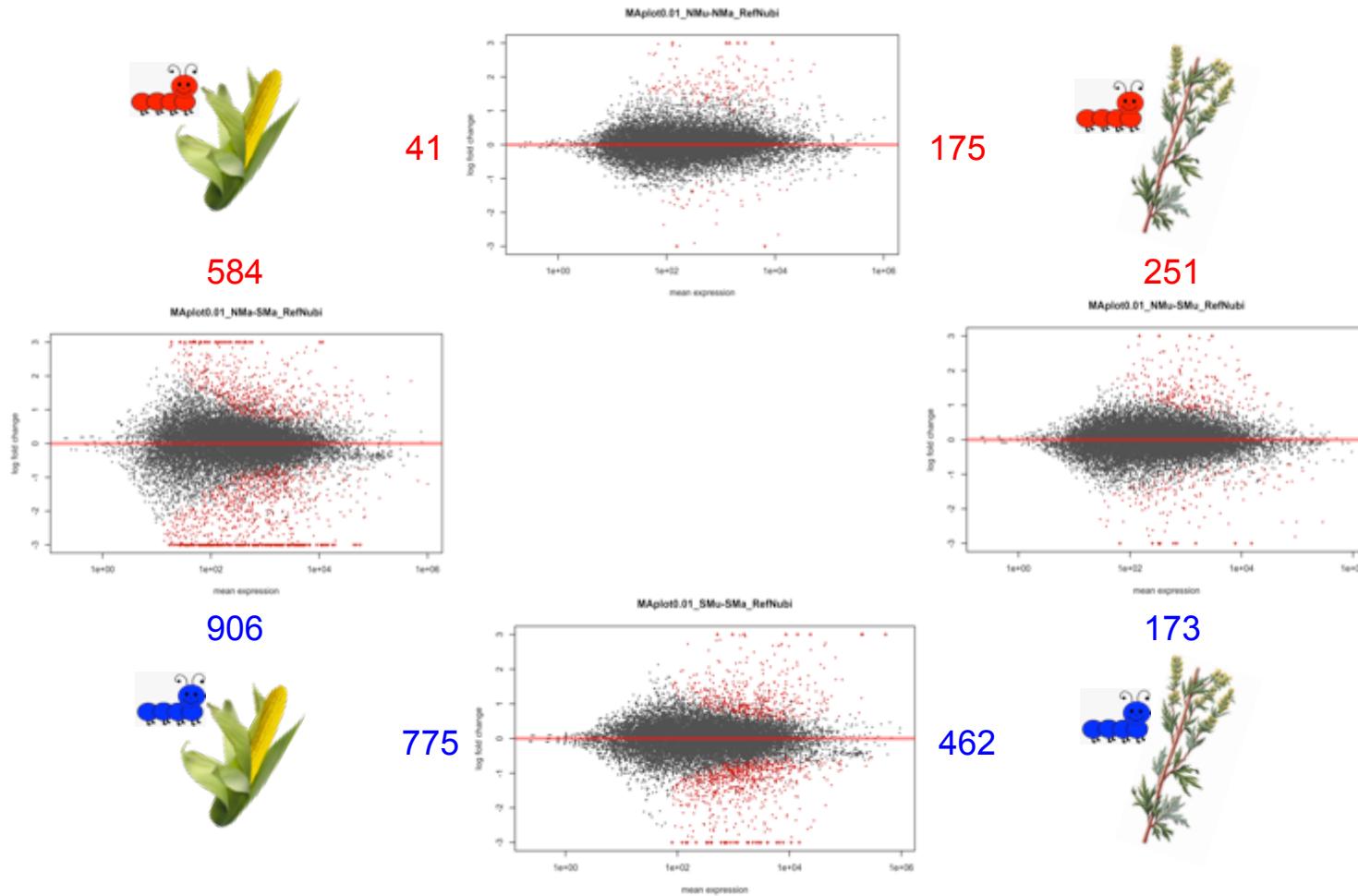
ABB



Gene expression at the larval stage



Gene expression at the larval stage



Gene expression at the larval stage

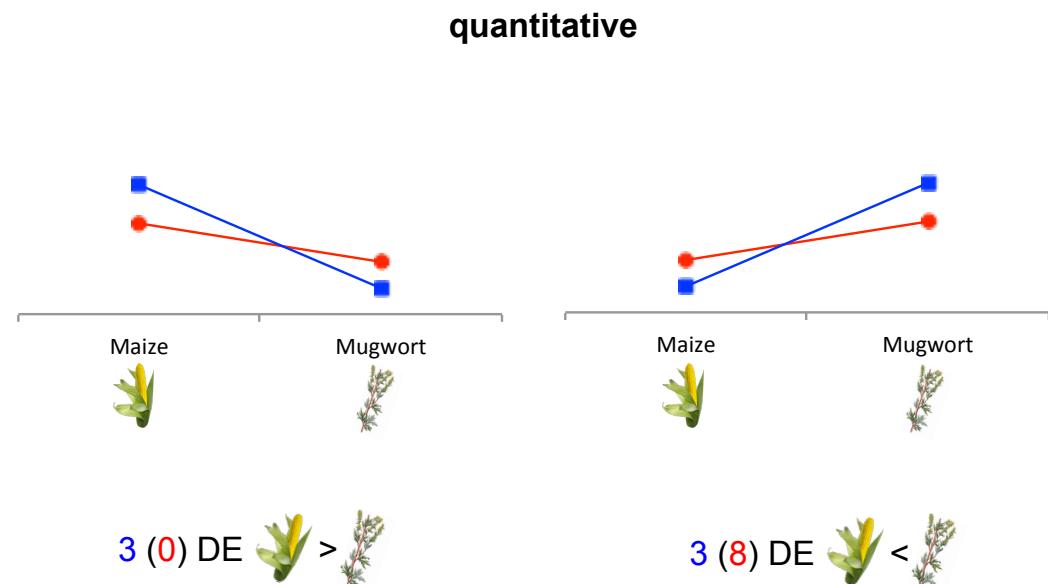
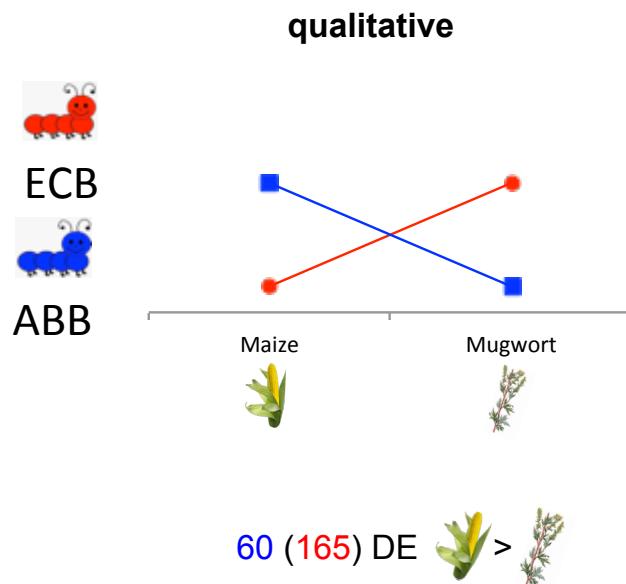
- General mixed model:

Counts gene i = Species + Plant + Species x Plant

Gene expression at the larval stage

- General mixed model:

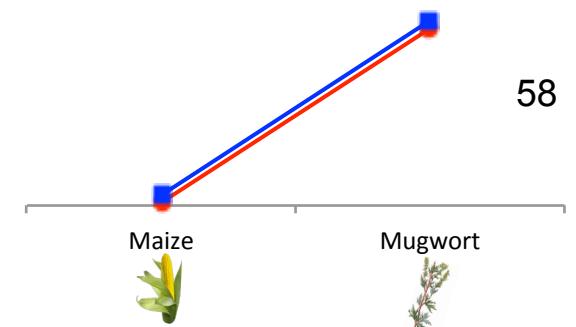
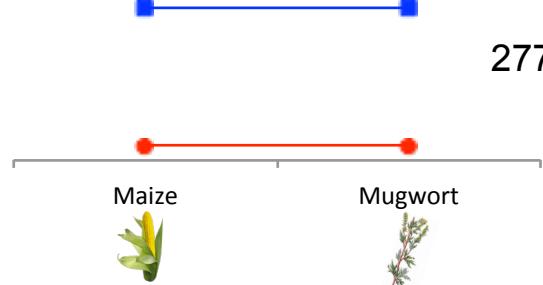
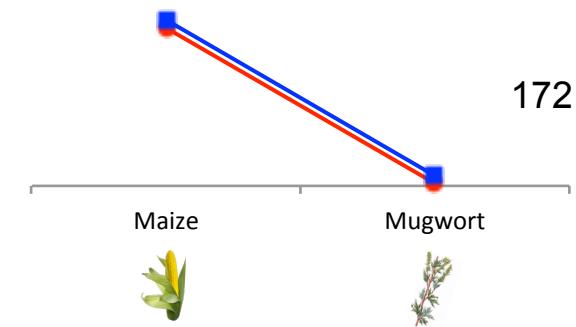
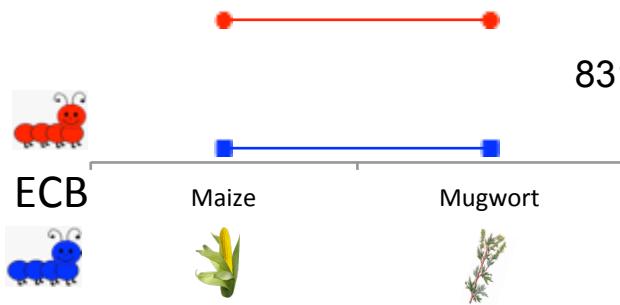
Counts gene $i = \text{Species} + \text{Plant} + \text{Species} \times \text{Plant}$



Gene expression at the larval stage

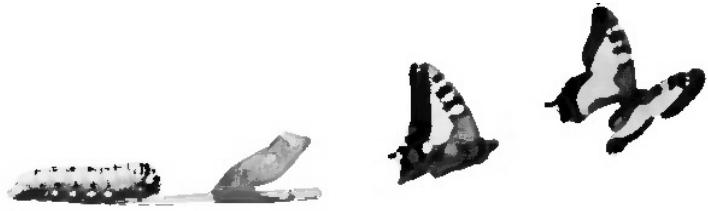
- General mixed model:

Counts gene $i = \text{Species} + \text{Plant}$



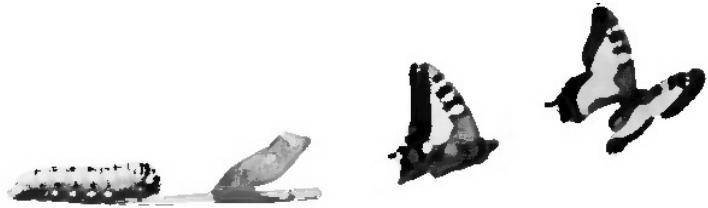
Over-represented functions in DE genes

- Development (**ECB** et **ABB**)
- Digestion (**ECB** et **ABB**) : serine endopeptidases, alpha-amylase, fatty acyl-CoA reductase
- Olfaction (**ECB** et **ABB**) : ORfur12 et 19 ; ORfur44; OnubOR5ag
- Detoxification (**ABB**) : Glutathione S-transferase, P450



Over-represented functions in DE genes

- Development (**ECB** et **ABB**)
- Digestion (**ECB** et **ABB**) : serine endopeptidases, alpha-amylase, fatty acyl-CoA reductase
- Olfaction (**ECB** et **ABB**) : ORfur12 et 19 ; ORfur44; OnubOR5ag
- Detoxification (**ABB**) : Glutathione S-transferase, P450
- Immunity (**ABB**) : PGRP, CTL, Serine Protease, Serine protease inhibitor, immunlectin, chitinase





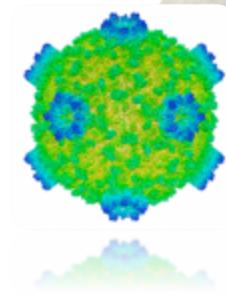
Over-represented functions in DE genes

→ plant

Over-represented functions in DE genes

→ plant

→ virus

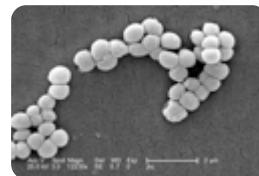
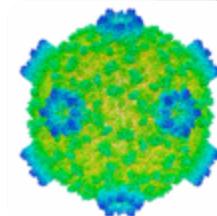


Over-represented functions in DE genes

→ plant

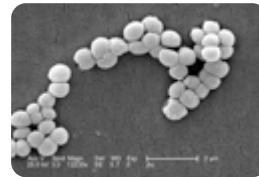
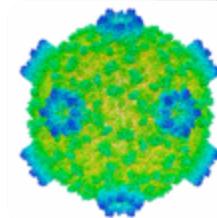
→ virus

→ bactéries



Over-represented functions in DE genes

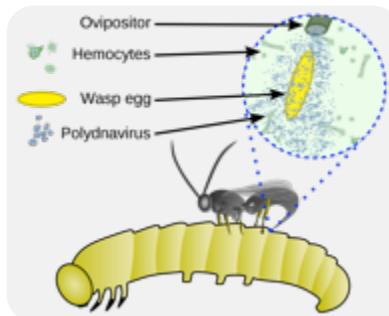
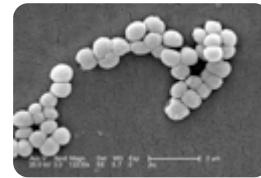
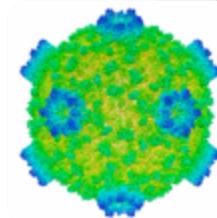
- plant
- virus
- bactéries
- levures





Over-represented functions in DE genes

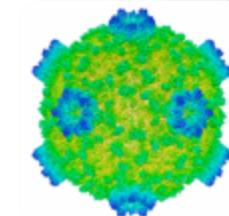
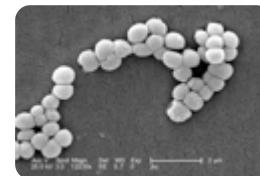
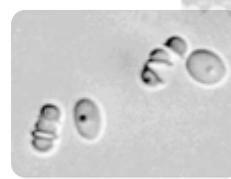
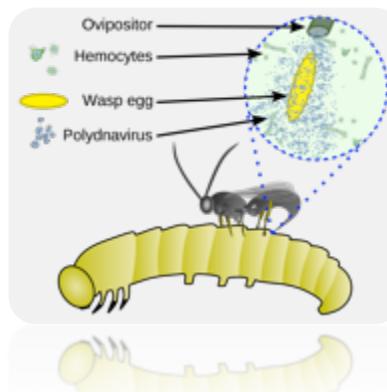
- plant
- virus
- bactéries
- levures
- parasitoïdes et virus associés





Over-represented functions in DE genes

- plant
- virus
- bactéries
- levures
- parasitoïdes et virus associés

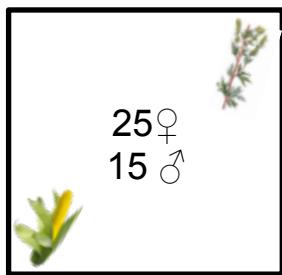


ECB		ABB	
maize	mugwort	maize	mugwort
cypoV +	cypoV +	cypoV+	--
Symbiont +	--	Symbiont +	--

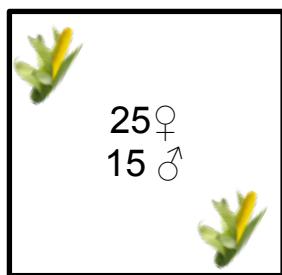
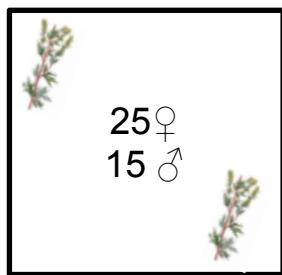
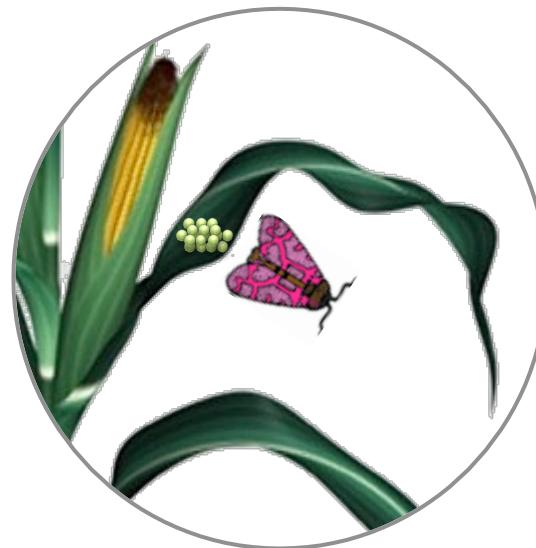


Plante hôte → **Plante + faune associée**

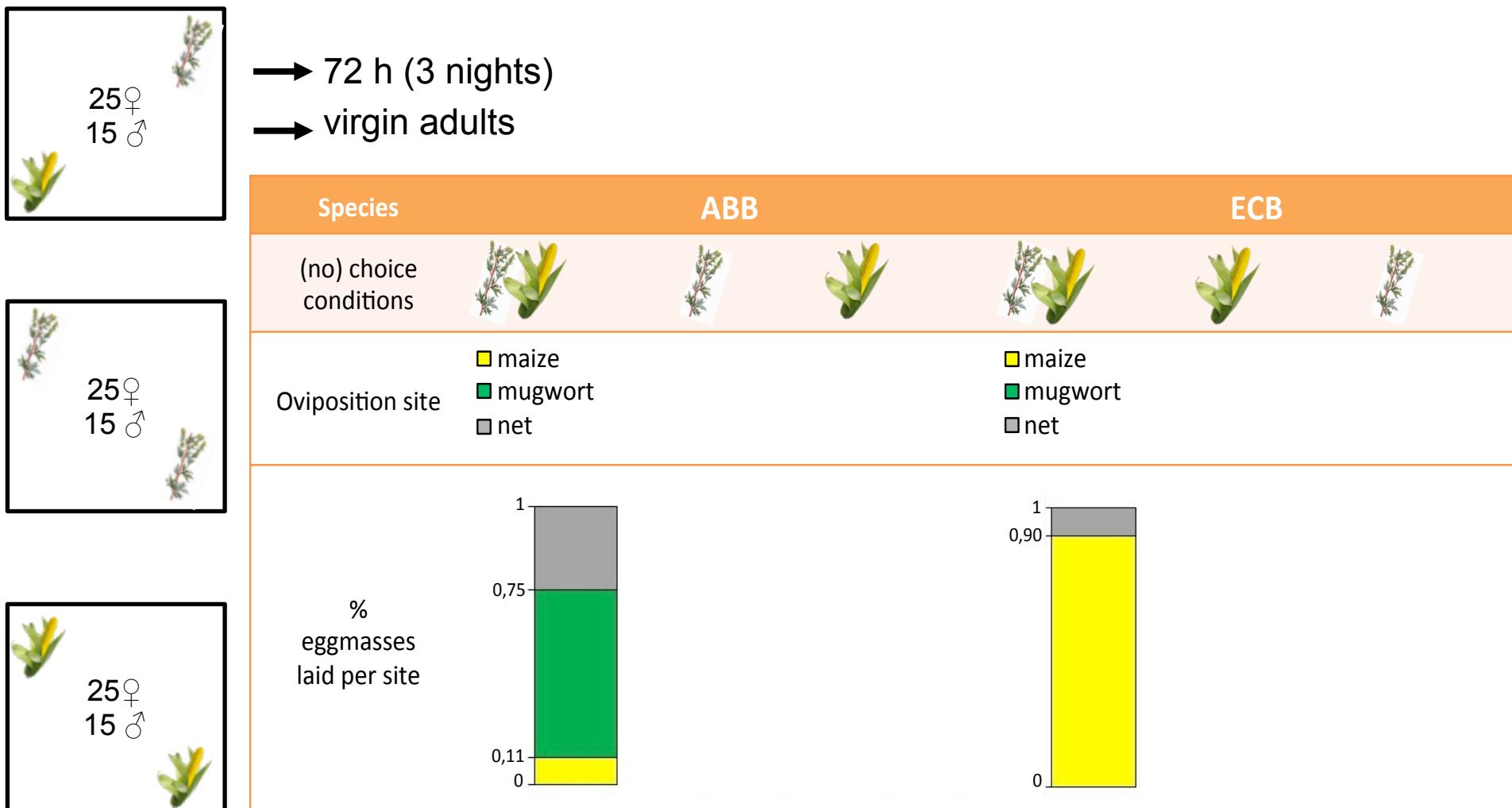
Specialization at the adult stage



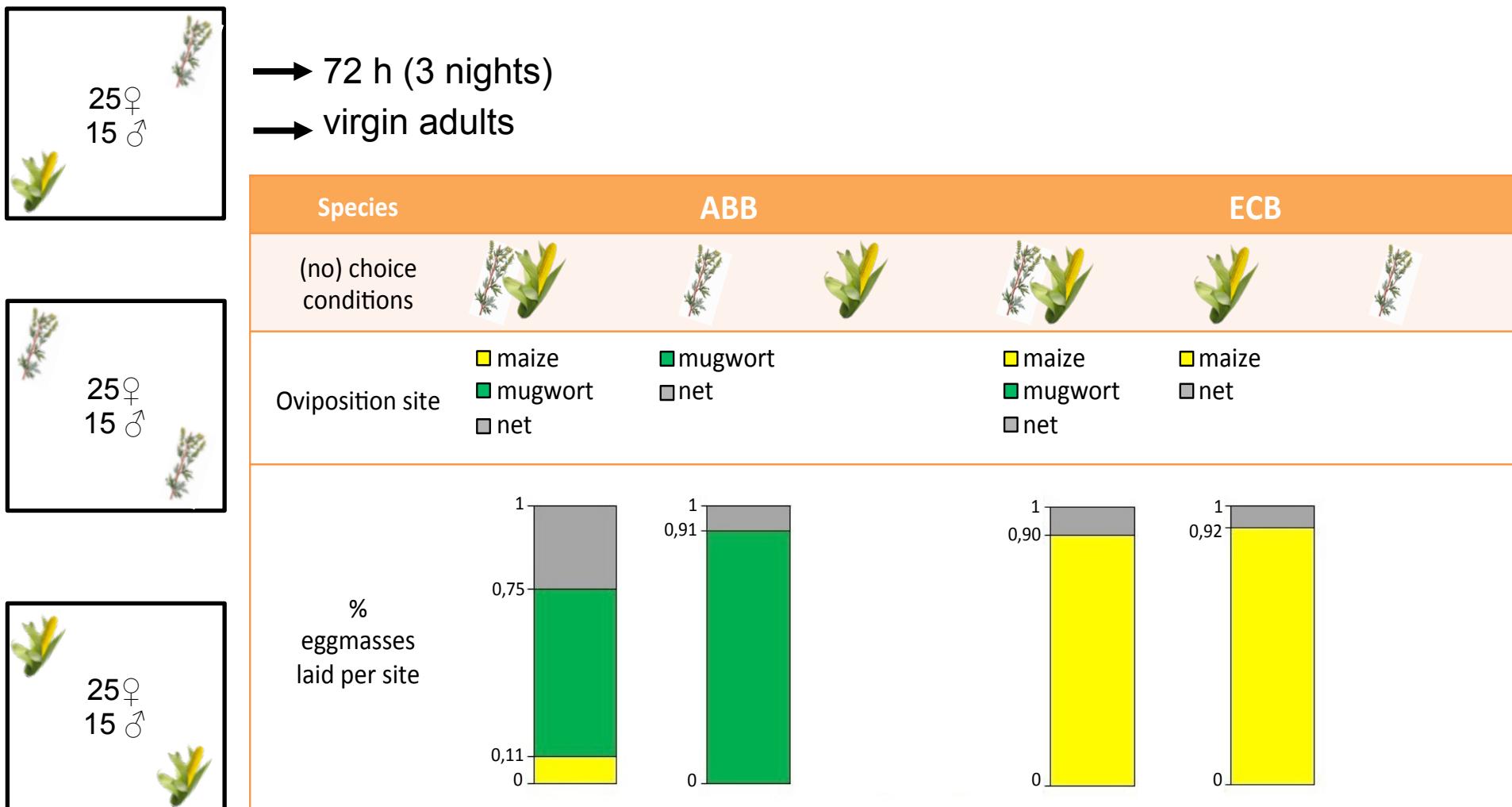
→ 72 h (3 nights)
→ virgin adults



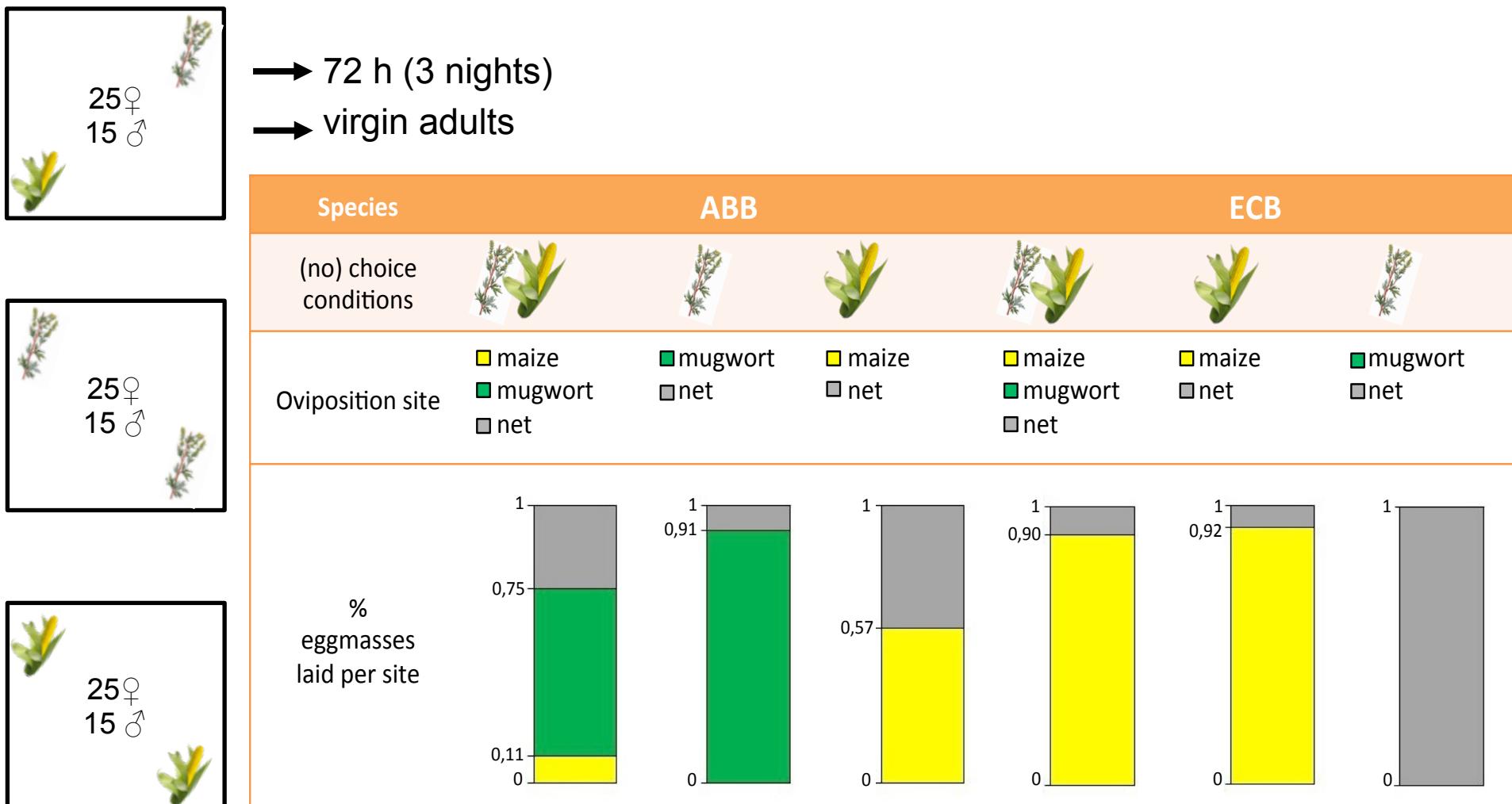
Specialization at the adult stage



Specialization at the adult stage



Specialization at the adult stage



Gene expression at the adult stage

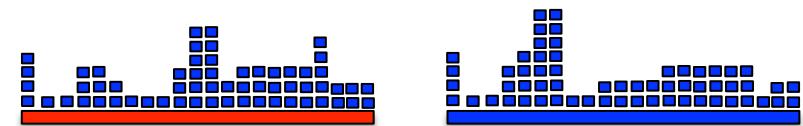
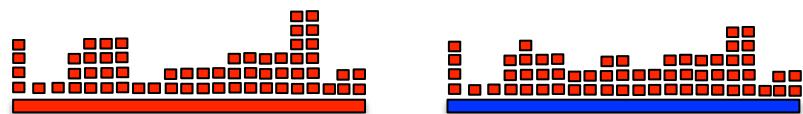
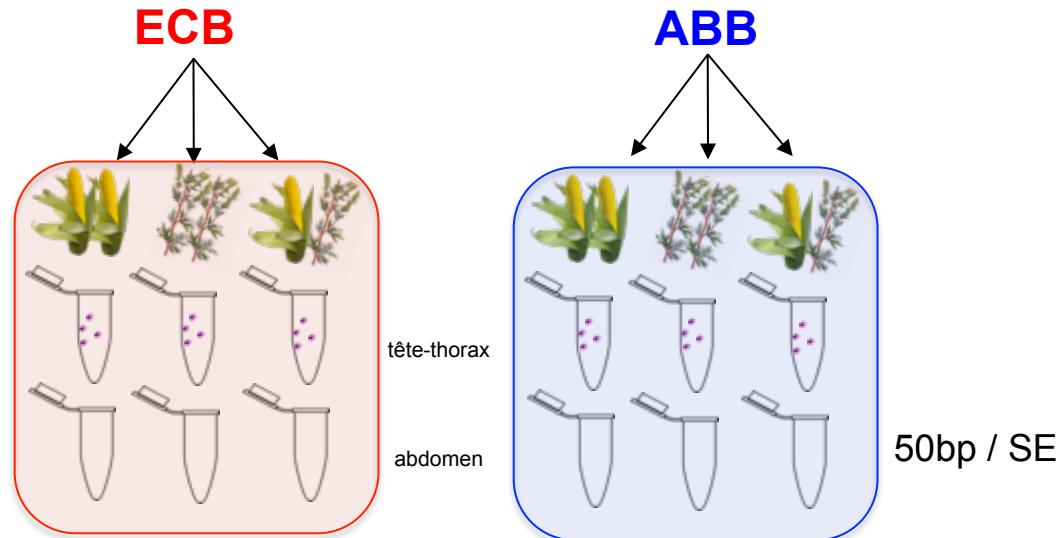


Ostrinia spp.

Expérience
↓
Séquençage ADNc

Construction de références
de novo (~12 000)

Alignement des reads
sur transcriptomes *de novo*
(~70%)



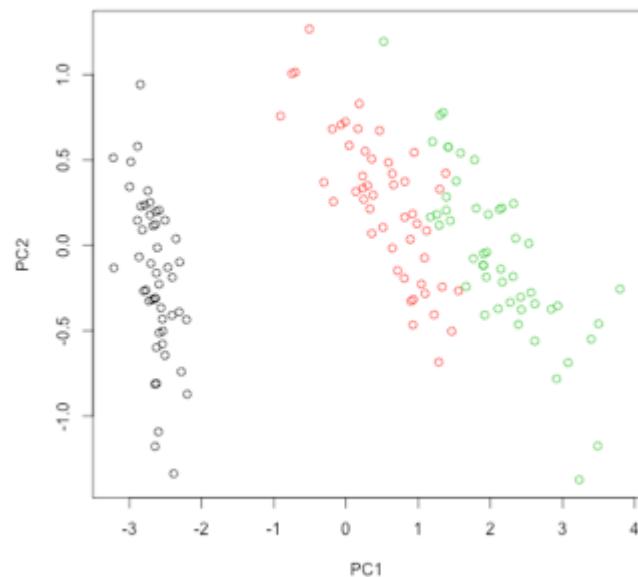
Comptage des reads / transcript / référence

Gene expression at the adult stage

- General Mixed Model: Counts gene $i =$ Species + (no) choice set-up + Species x Set-up
- PCA analysis on normalized counts: another way of classifying Species/Choice/ No choice effects

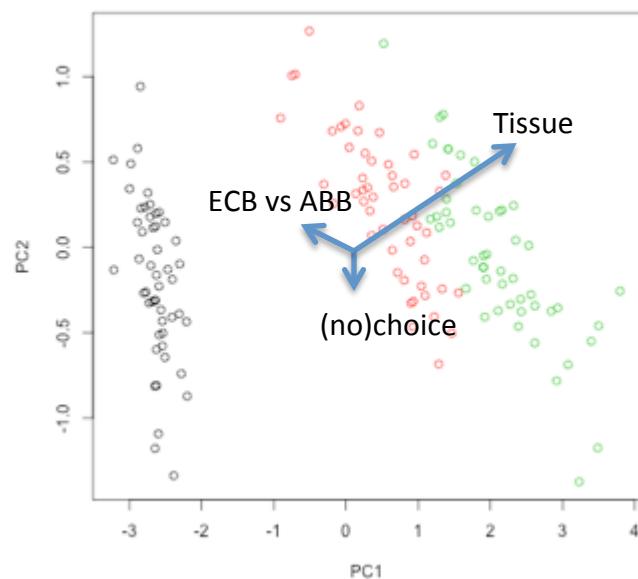
Gene expression at the adult stage

- General Mixed Model: Counts gene $i = \text{Species} + (\text{no}) \text{ choice set-up} + \text{Species} \times \text{Set-up}$
- PCA analysis on normalized counts: another way of classifying Species/Choice/No choice effects

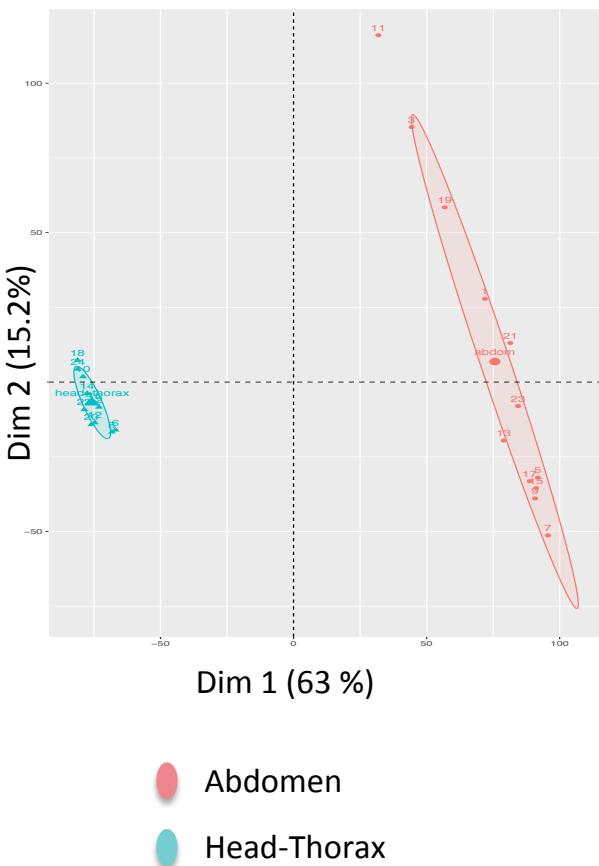


Gene expression at the adult stage

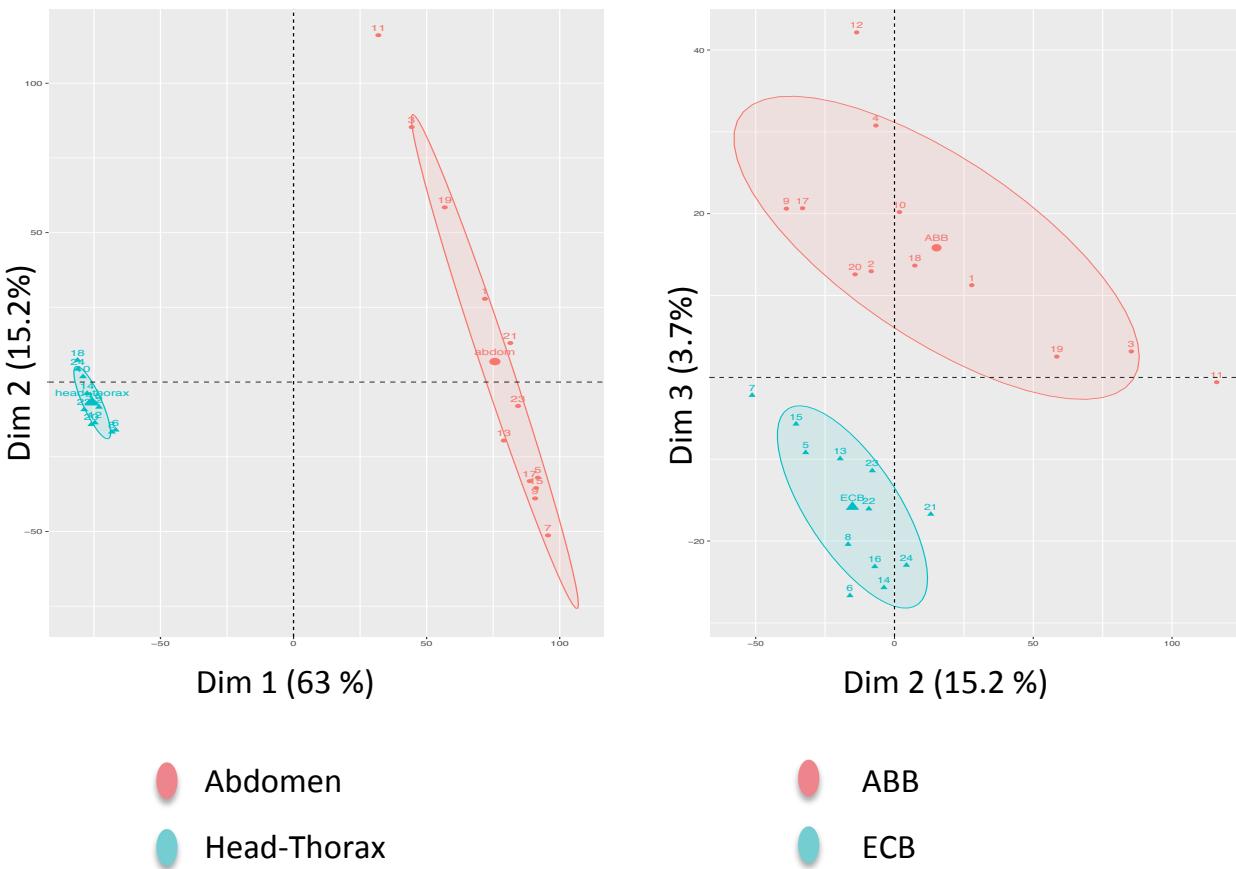
- General Mixed Model: Counts gene $i = \text{Species} + (\text{no})\text{choice set-up} + \text{Species} \times \text{Set-up}$
- PCA analysis on normalized counts: another way of classifying Species/Choice/No choice effects



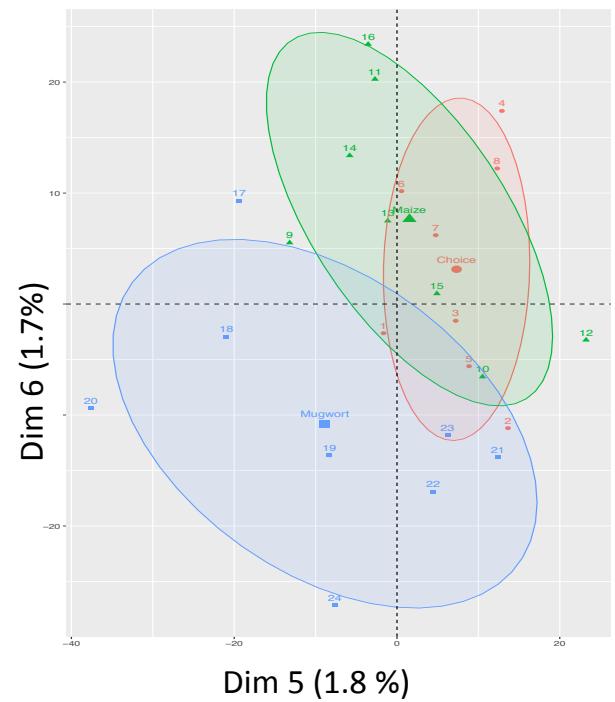
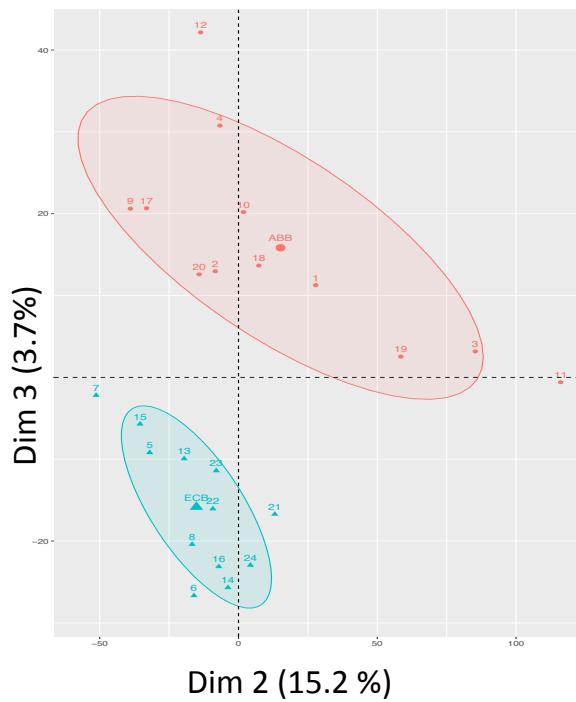
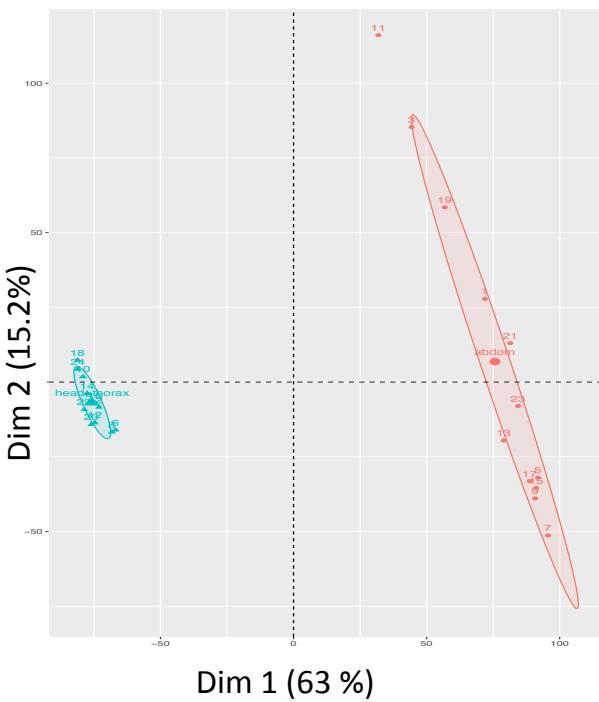
Gene expression at the adult stage



Gene expression at the adult stage

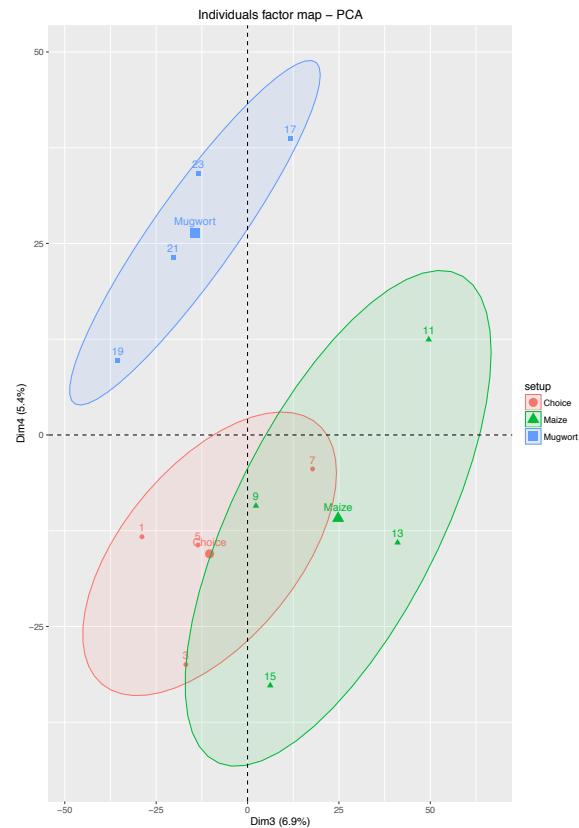


Gene expression at the adult stage



Gene expression at the adult stage

- Abdomen only



```
isotig01215 -0.7462918 5.307489e-03
isotig04501 -0.7612812 4.023504e-03
isotig05508 -0.7614681 4.009152e-03
isotig08305 -0.7621112 3.960054e-03
isotig08789 -0.7652629 3.726008e-03
isotig03495 -0.7664987 3.637162e-03
isotig04815 -0.7668057 3.615342e-03
isotig03046 -0.7803249 2.748908e-03
isotig04748 -0.7857541 2.449615e-03
isotig04964 -0.7933184 2.074869e-03
isotig04001 -0.8066352 1.523328e-03
isotig05092 -0.8174815 1.163832e-03
isotig10481 -0.8178808 1.151979e-03
isotig11212 -0.8182348 1.141548e-03
```

\$quali

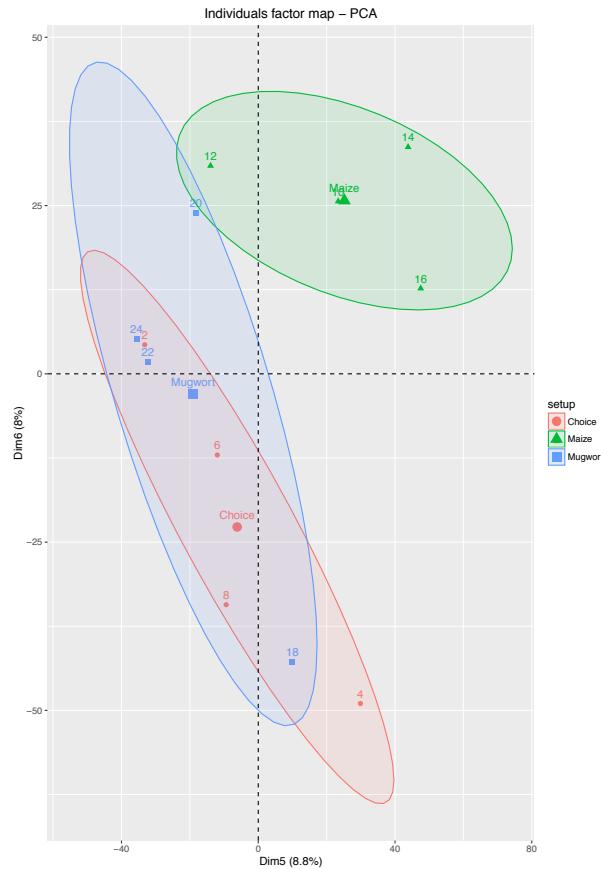
R2	p.value
setup	0.6930558
	0.004917763

\$category

Estimate	p.value
Mugwort	26.40022
	0.0008750745

Gene expression at the adult stage

- Head-Thorax only



Axes 5-6: maize effect

```
isotig01427 -0.7799042 0.0027732078
isotig07806 -0.7839070 0.0025484930
isotig05820 -0.7856138 0.0024570182
isotig03971 -0.7881675 0.0023248625
isotig07757 -0.7963768 0.0019364964
isotig10392 -0.8013399 0.0017271133
isotig03368 -0.8088253 0.0014446649
isotig09939 -0.8143705 0.0012594186
isotig08136 -0.8236766 0.0009901817
isotig01928 -0.8279819 0.0008818027
isotig01183 -0.8352600 0.0007196502
isotig07846 -0.8385420 0.0006545692
isotig00683 -0.8399198 0.0006286475
isotig01679 -0.8401764 0.0006239101
isotig02186 -0.8445209 0.0005477992
isotig08910 -0.8601840 0.0003311712
```

```
$squali
  R2  p.value
setup 0.4184365 0.08723425
```

```
$category
  Estimate  p.value
Maize 25.19092 0.03132546
```

Annotated functions and DE genes at the adult stage

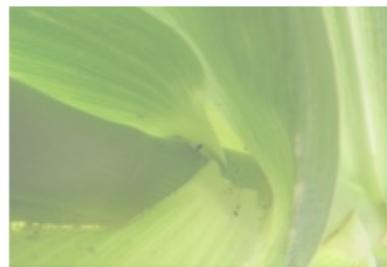
For the plant effect (choice versus no choice effect):

- Development (**muscle development, oogenesis, neurones**)
- Digestion/ detoxification
- Immunity
- Chemosensory??

For the species effect (choice versus no choice effect) : to be done

Conclusions

- Specialization at the larval stage and adult stage for ECB: survival, preference, and avoidance
- Specialization at the adult stage for ABB: preference
- Host specialization higher in ECB than in ABB



Conclusions

- DE genes at the larval stage:
 - Species >> interaction >> plant effect
 - Candidates genes to host specialization and their relative pattern in ECB and ABB: evolutionary history
 - Host specialization uncovers various biological functions: development, digestion, detoxification, sensing and IMMUNITY

Conclusions

- DE genes at the adult stage:
 - Tissue >> Species >> plant effect
 - Candidates genes to host specialization and their relative pattern in ECB and ABB: evolutionary history
 - Host specialization uncovers various biological functions: development, digestion, detoxification, and **IMMUNITY**

Perspectives

- Validation
qPCR, functional genomics
- Cross validation
Population genomics
- Generalization
THV in various European populations
- Evolutionary history
Phylogeography with neutral markers and candidate genes

