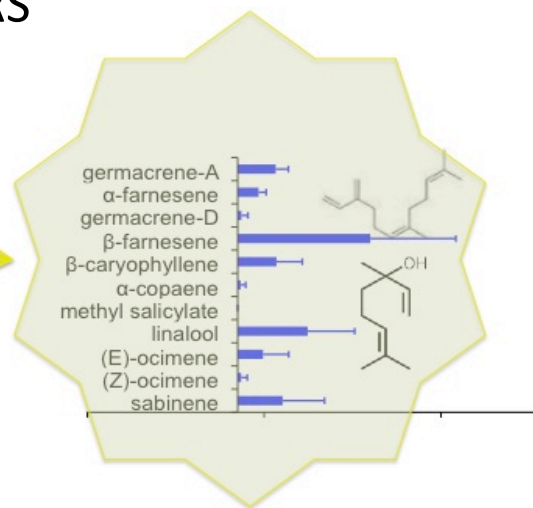


The ecological chemistry of the interactions between figs and fig-wasps: recent advances and future directions

Magali Proffit

CEFE - CNRS



Information in the environment



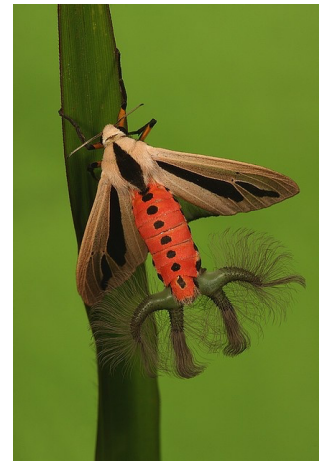
Visual



Vibrational



Olfactory



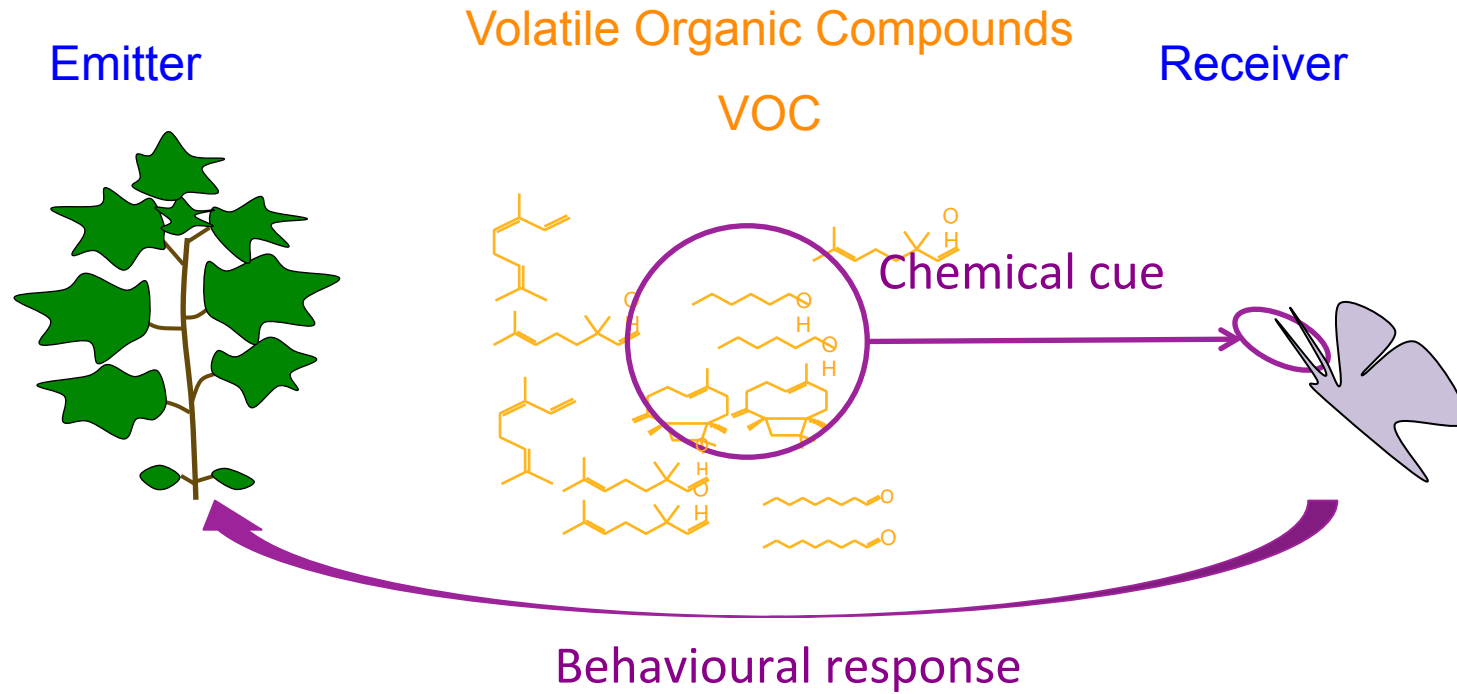
Tactile



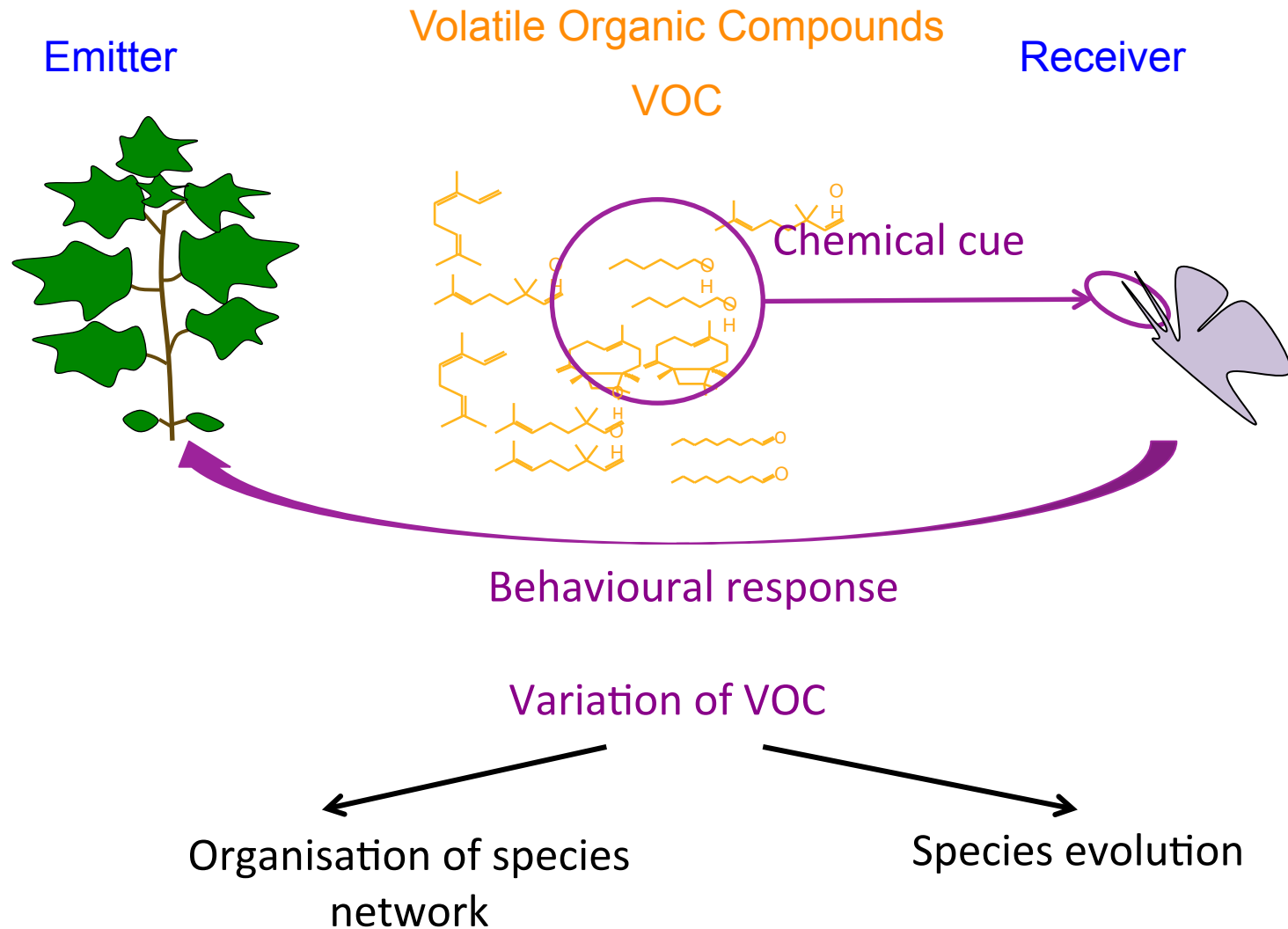
Infrared



Chemical signals in plant-insect interaction



Chemical signals in plant-insect interaction



Case of a complex network structured by chemical mediation

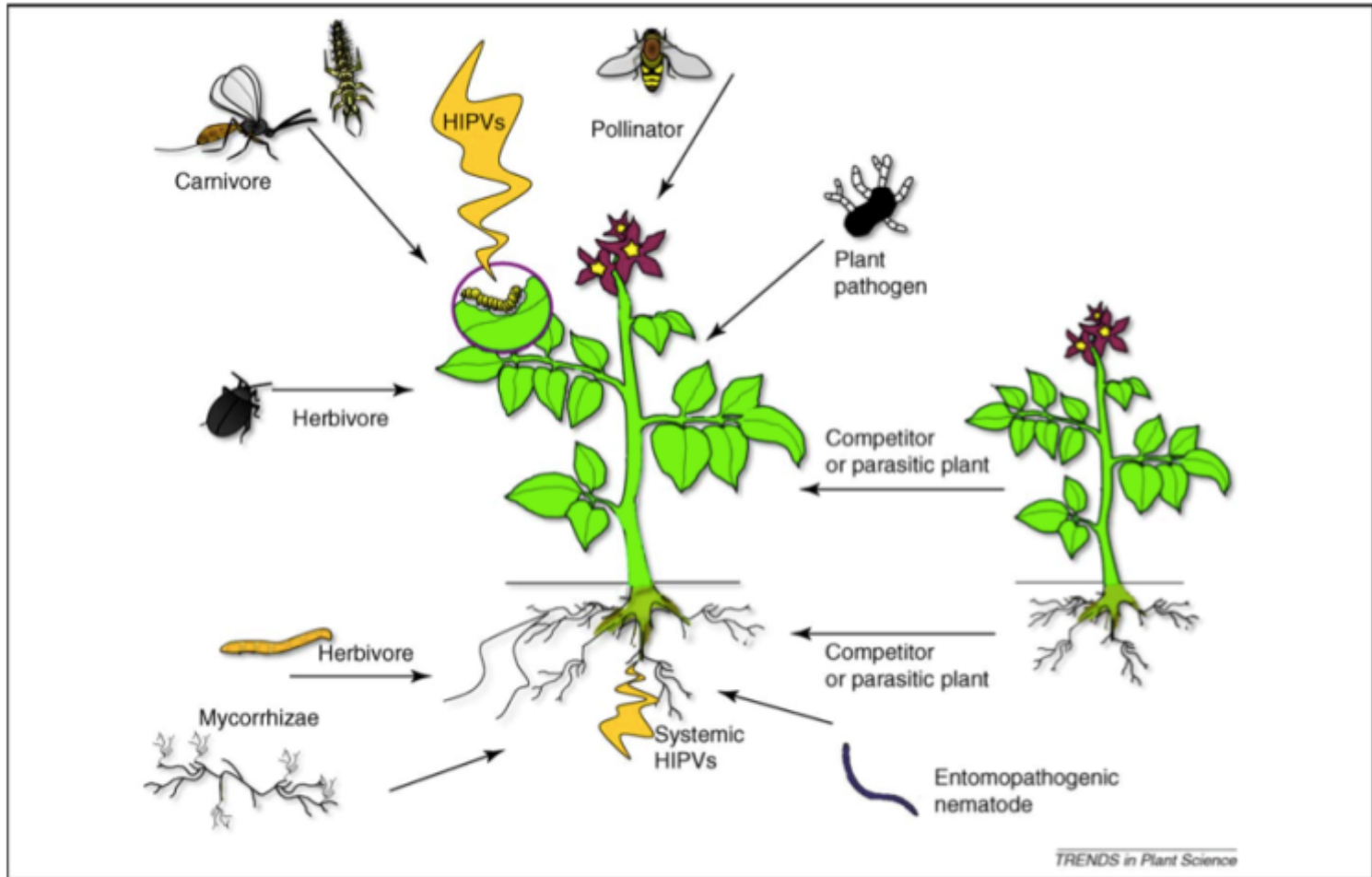


Figure 1. Plant damage and HIPVs. A plant that is locally damaged by a herbivore emits induced volatiles systemically, both above- and belowground. The HIPVs can affect various community members that each exert different selection pressures on the plant.

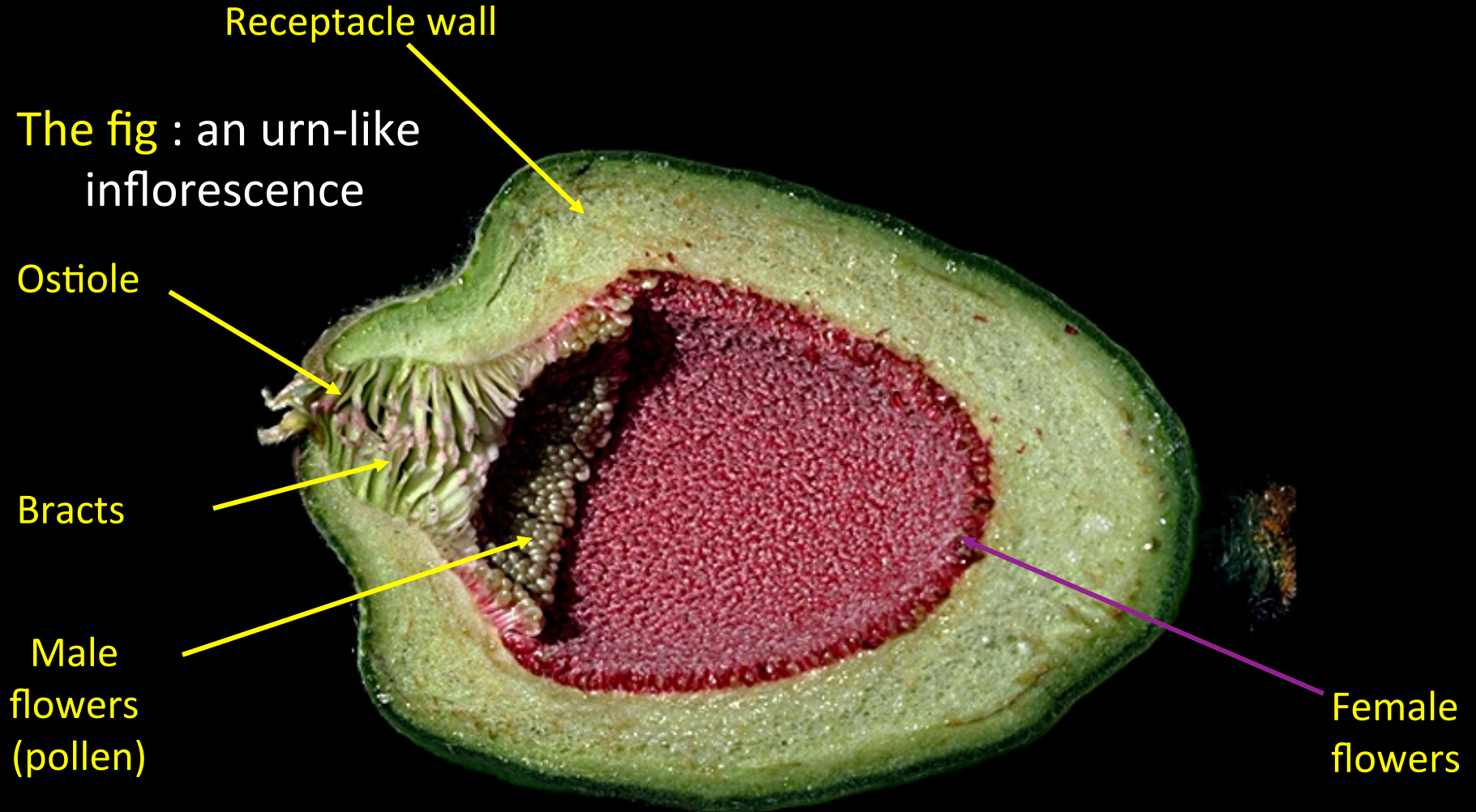
Case of a complex network structured
by chemical mediation:
communities of fig wasps associated with *Ficus* species



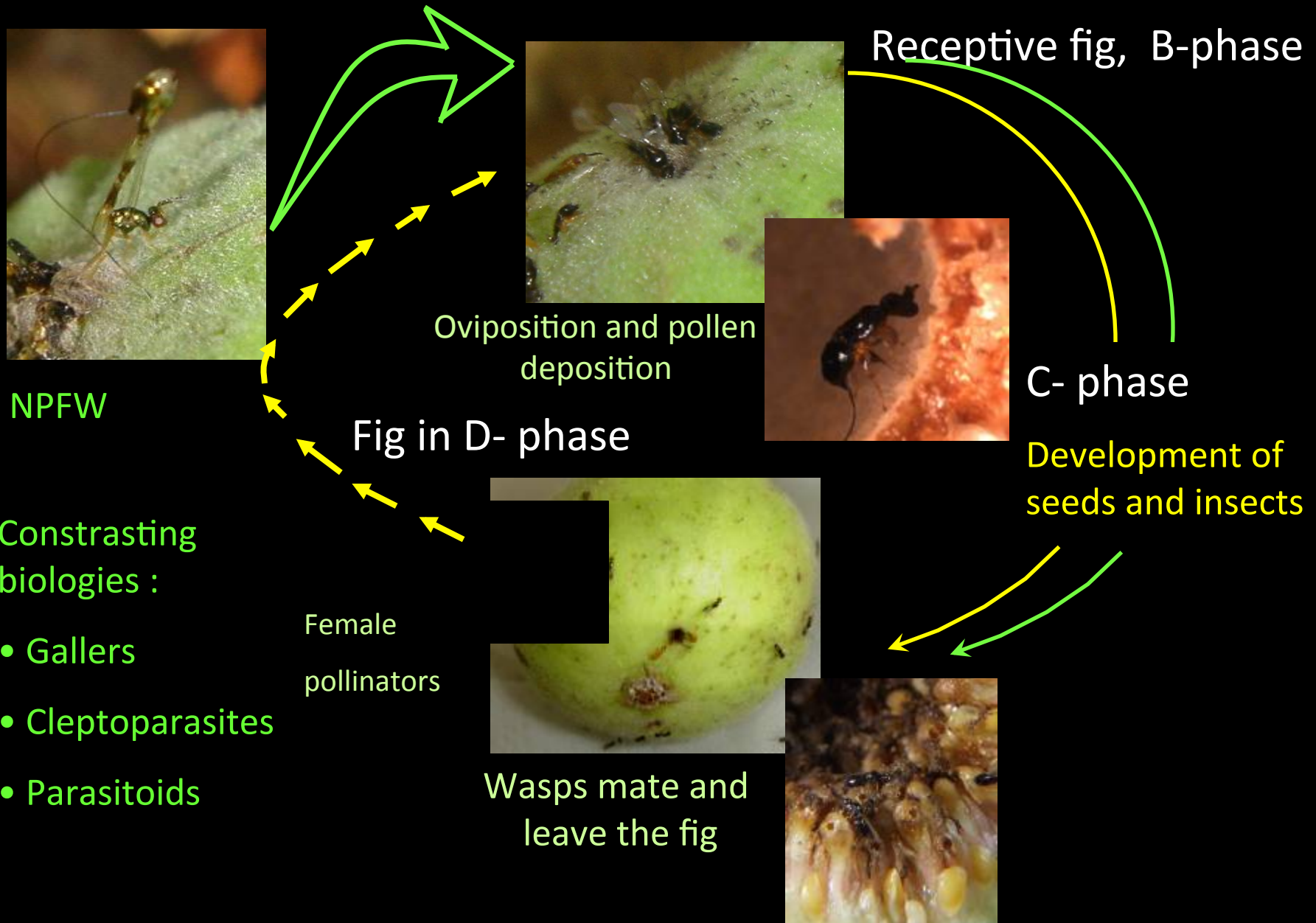
Over 750 distinct but comparable communities

- *Ficus* (Moracea) / pollinator (Agaonidae): nursery pollinator mutualism
- Usually one *Ficus* species-one pollinator species
- 1-30 species of Non-pollinating fig wasps (NPFW)/ community

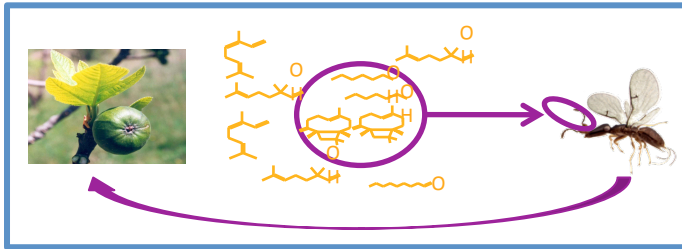
Ficus
(Moraceae)



Cycle fig/pollinator mutualism and NPFW

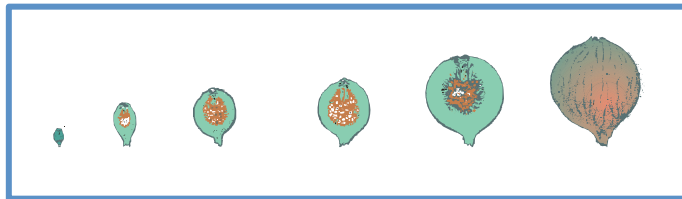


1. Importance of fig VOC in the organization of fig wasp communities



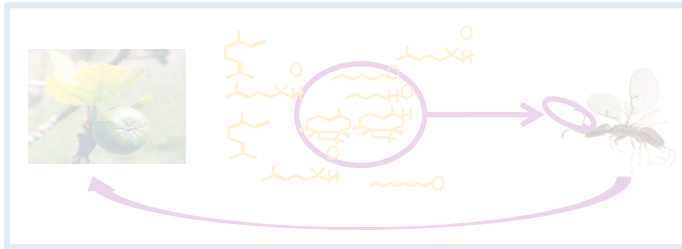
2. Mechanisms of fig and fig wasps chemical communication

3. Inter-specific variation of fig VOC and implication for fig wasp communities



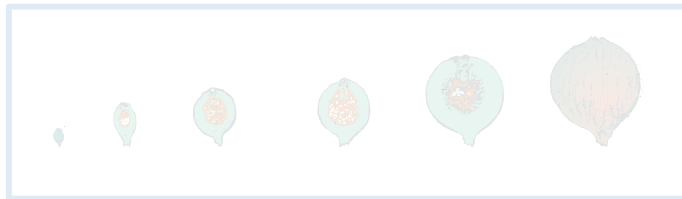
4. Intra-specific variation of fig VOC and implication for fig wasp communities

1. Importance of fig VOC in the organization of fig wasp communities



2. Mechanisms of fig and fig wasps chemical communication

3. Inter-specific variation of fig VOC and implication for fig wasp communities

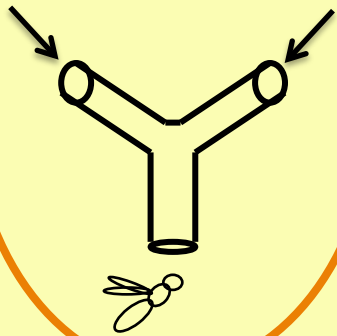


4. Intra-specific variation of fig VOC and implication for fig wasp communities

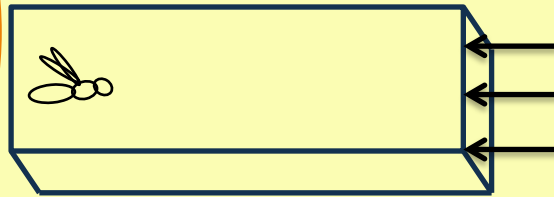
1. Importance of fig VOC in the organization of fig wasp communities

Attraction of fig wasps towards fig VOC

Y-tube olfactometer

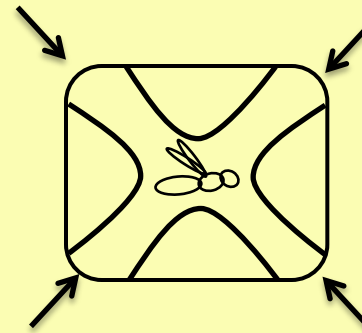


Wind tunnel

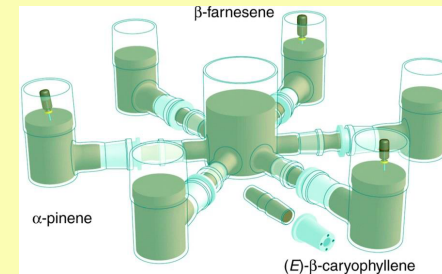


Methods

4-arms olfactometer



6-arms olfactometer



In the field

Sticky traps

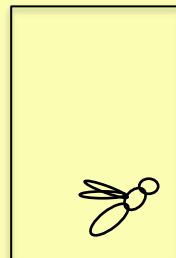
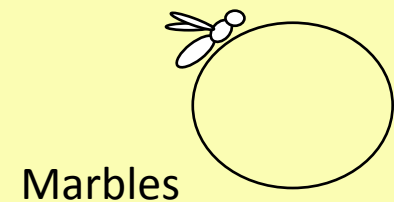


Fig VOC as contact stimulation



1. Importance of fig VOC in the organization of fig wasp communities

Pollinating fig wasps

Fig VOC are attractive for 11 pollinator spp.
(Europe, Asia, Africa)



Non-pollinating fig wasps (NPFW)

Fig VOC are attractive for 6 NPFW spp.
(Europe, Asia)



1. Importance of fig VOC in the organization of fig wasp communities

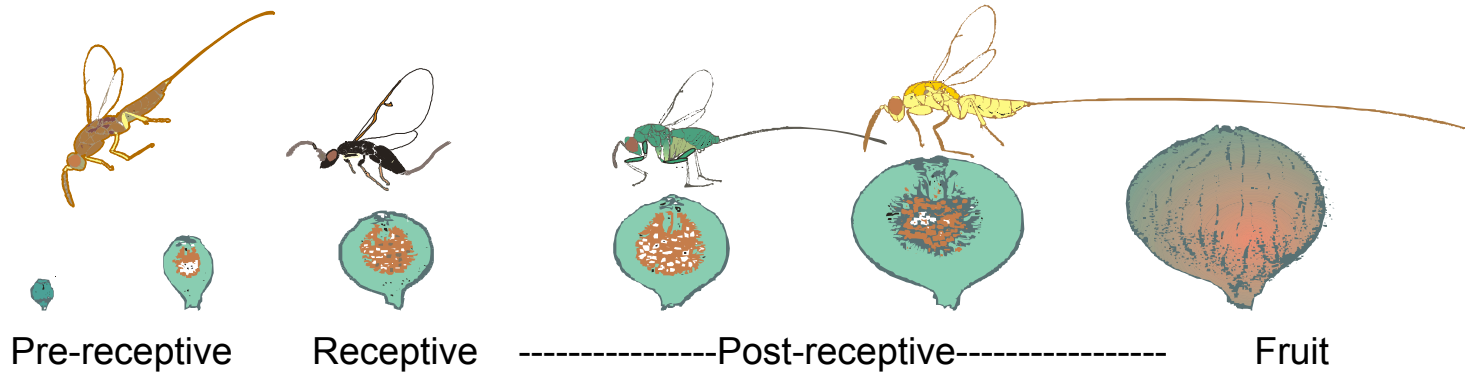
Pollinating fig wasps

Fig VOC are attractive for 11 pollinator spp.
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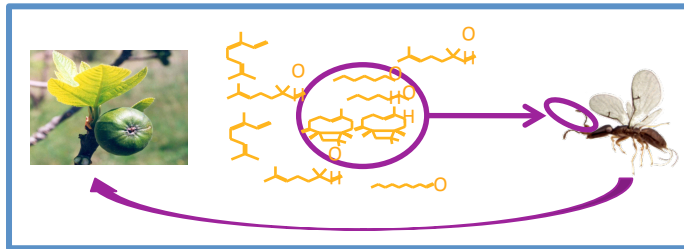
Non-pollinating fig wasps (NPFW)

Fig VOC are attractive for 6 NPFW spp.
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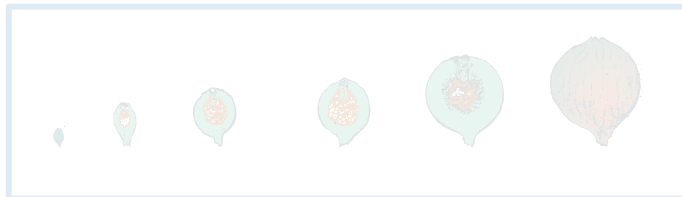
Chemical mediation facilitates resource partitioning of the fig wasp community

1. Importance of fig VOC in the organization of fig wasp communities



2. Mechanisms of fig and fig wasps chemical communication

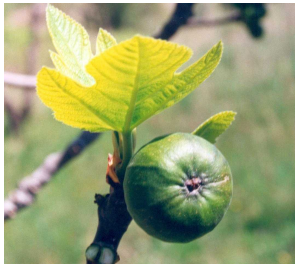
3. Inter-specific variation of fig VOC and implication for fig wasp communities



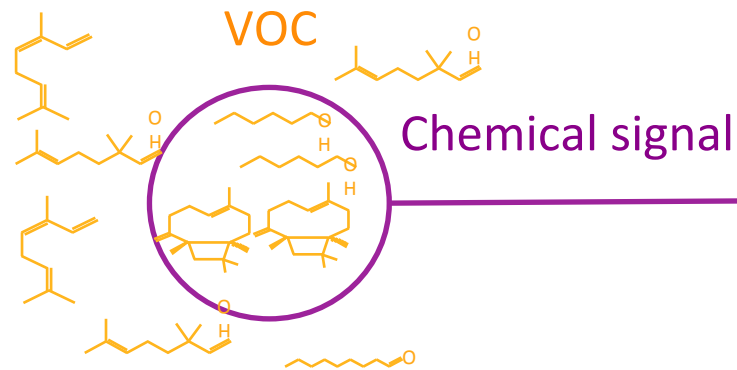
4. Intra-specific variation of fig VOC and implication for fig wasp communities

2. Mechanisms of fig and fig wasps chemical communication

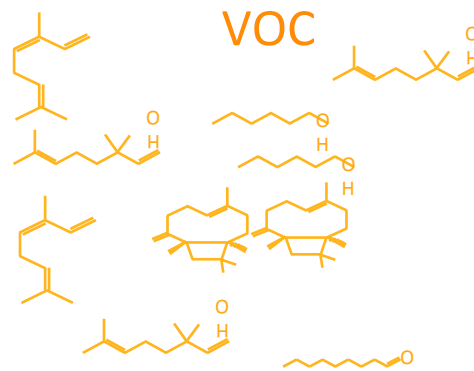
Emitter



Receiver



Behavioural response



2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOC emitted

Methods

- Collection of « headspace » by adsorption-desorption



Enclose figs in an inert plastic bag

VOC trapped on a cartridge with adsorbent



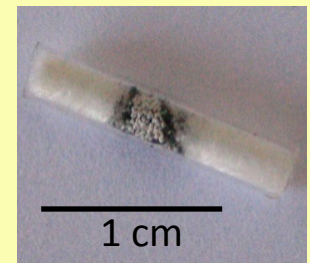
Air clean by charcoal filter



1 cm

Trap
(Porapak)

Desorption by solvent



1 cm

Micro trap
(Tenax + Carbotrap)

Thermal desorption



2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOC emitted

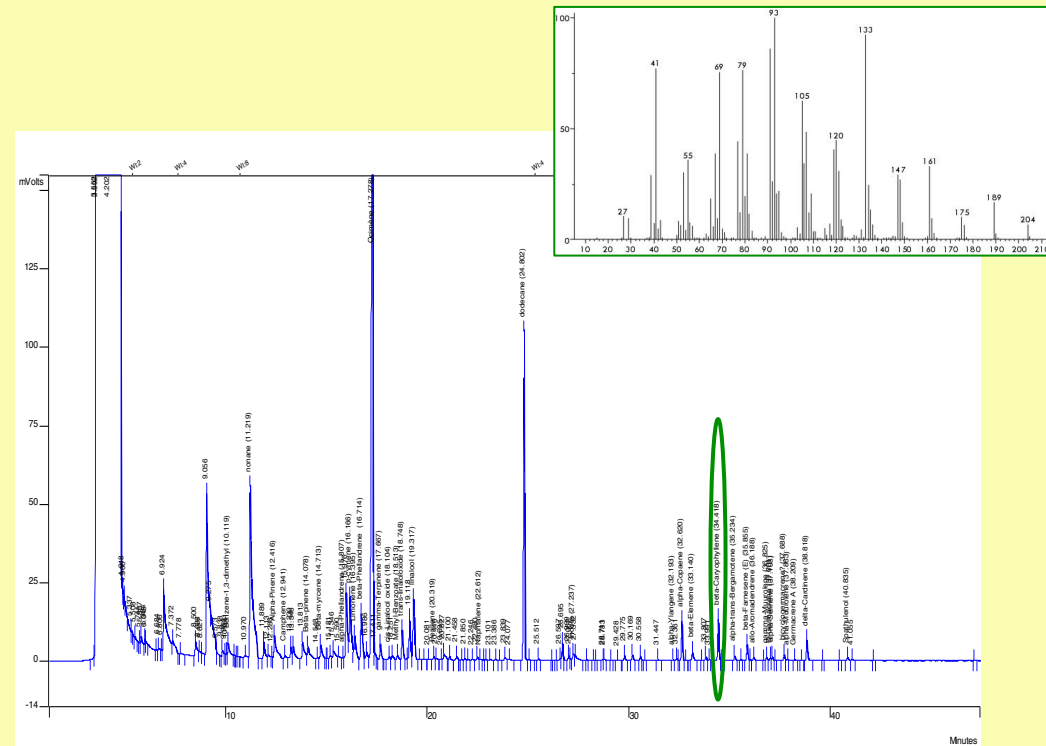
Methods

- Analyze of the samples by Gas – Chromatography and Mass Spectrometry (GCMS)



Plateforme d'Analyse Chimique en Ecologie (PACE)

Chemical message emitted by receptive figs of *Ficus hispida*



2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOC emitted

Headspace of 30 *Ficus* spp.

- in total < 360 VOC
- Mean VOC / individual (Mean $\pm$ SD) : 24 ± 12
- More than 50% of the samples contained :
 - Monoterpenes
 - (E)- β -ocimene
 - linalool
 - Sesquiterpenes
 - α -copaene
 - α -humulene
 - germacrene D

2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOC emitted

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- More than 50% of the samples contained :

Monoterpenes

(E)- β -ocimene
linalool



Occur in more than 50 % of plant families
(Knudsen et al., 2006, Bot. Rev.)

Sesquiterpenes

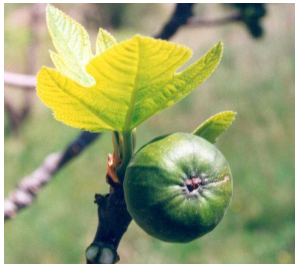
α -copaene
 α -humulene
germacrene D



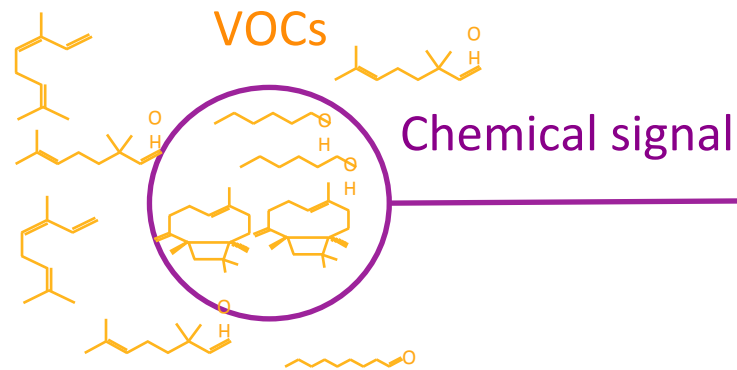
Fig volatile profile is composed of common floral VOC

2. Mechanisms of fig and fig wasps chemical communication

Emitter



Receiver



Behavioural response

2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOC used by fig wasps

In insects specific attraction to host plant is mediated by :

Raguso, 2008, Annu. Rev. Ecol. Evol. Syst

- **Specific ratio of common compounds**
- **One unique compound**

2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOC used by fig wasps

In insects specific attraction to host plant is mediated by :

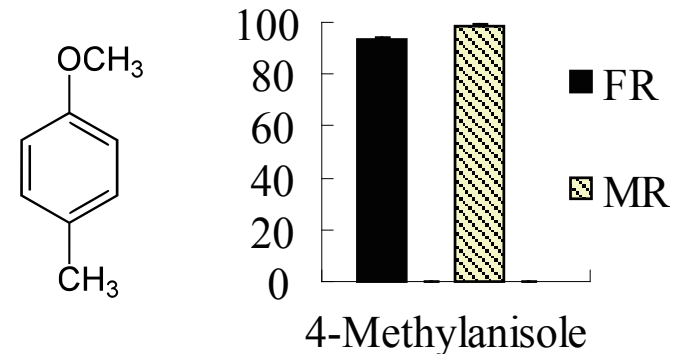
Raguso, 2008, Annu. Rev. Ecol. Evol. Syst

- **Specific ratio of common compounds**

- For most fig / pollinating fig wasp

- **One unique compound** ➡ Private channel

- *C. gravelyi* is attracted by one compound emitted by *F. semicordata*



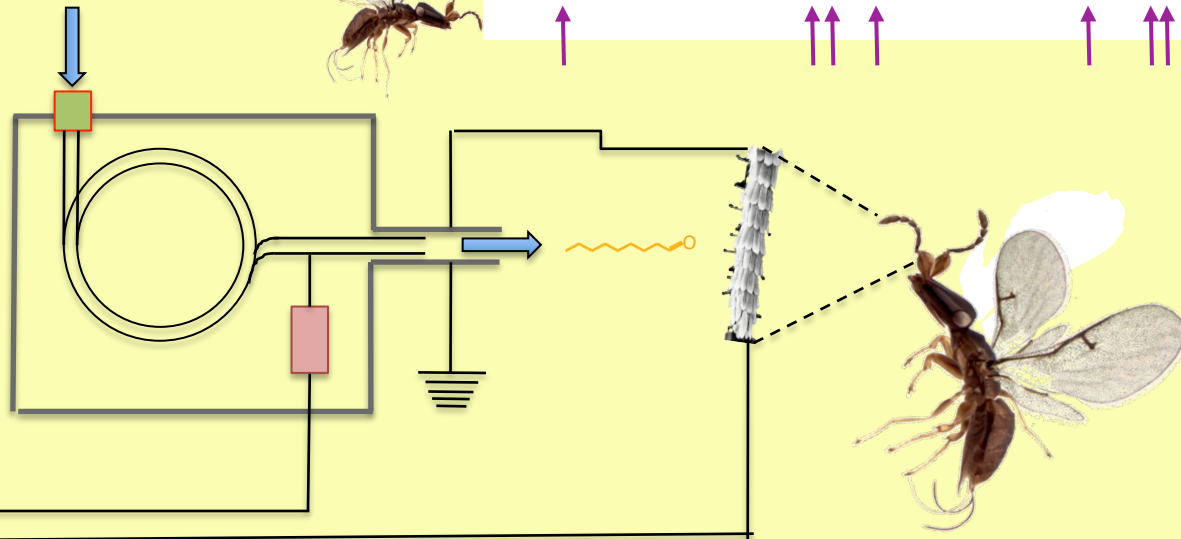
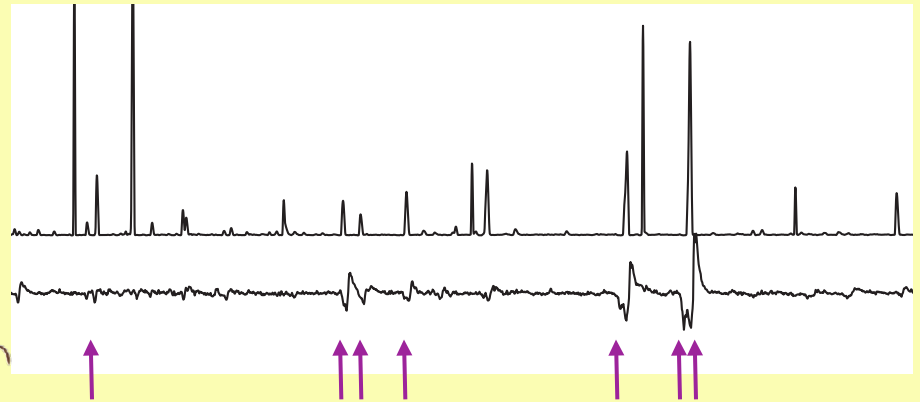
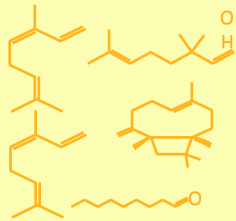
Chen et al. 2009, Func. Ecol.

2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOCs detected by fig wasps

Methods

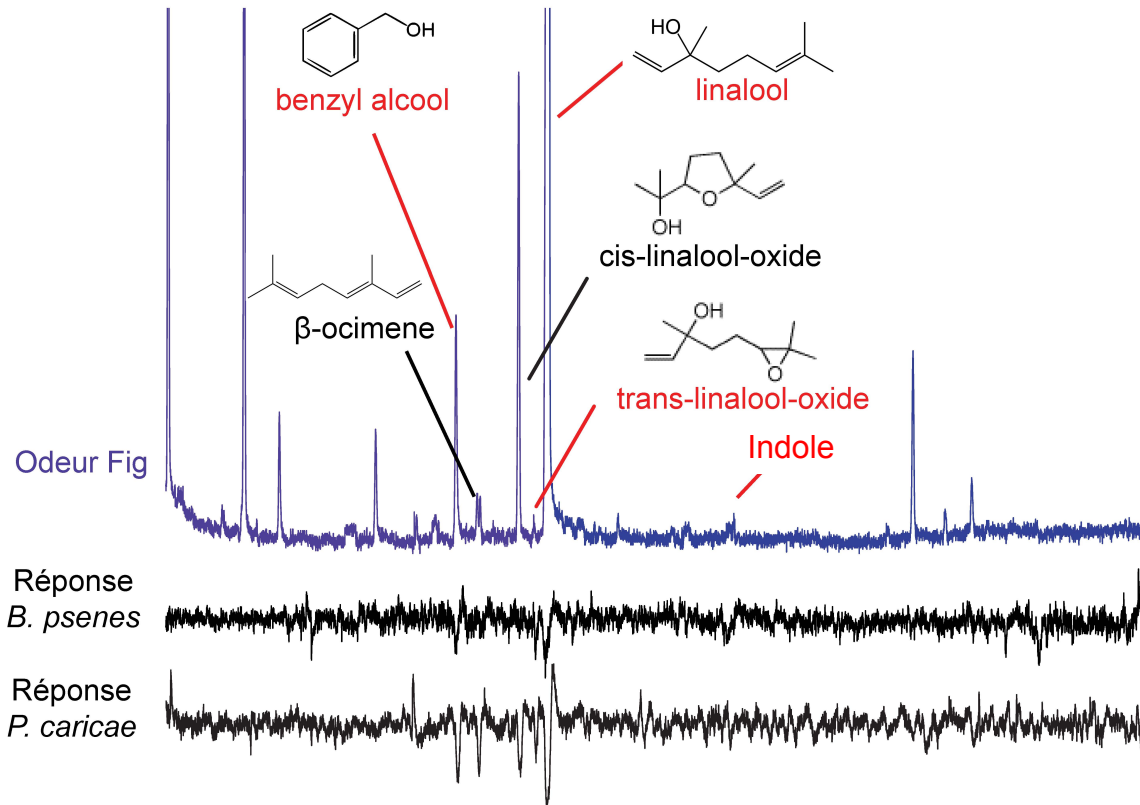
Gas chromatography coupled with Electro-antennographic recordings



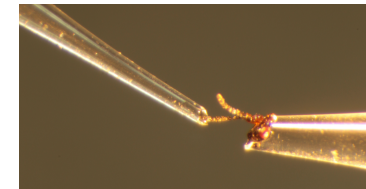
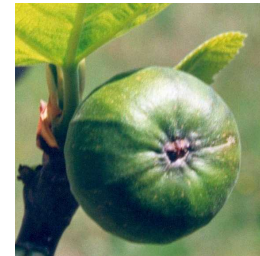
2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOCs detected by fig wasps

F. carica / *B. psenes*



Study site Montpellier

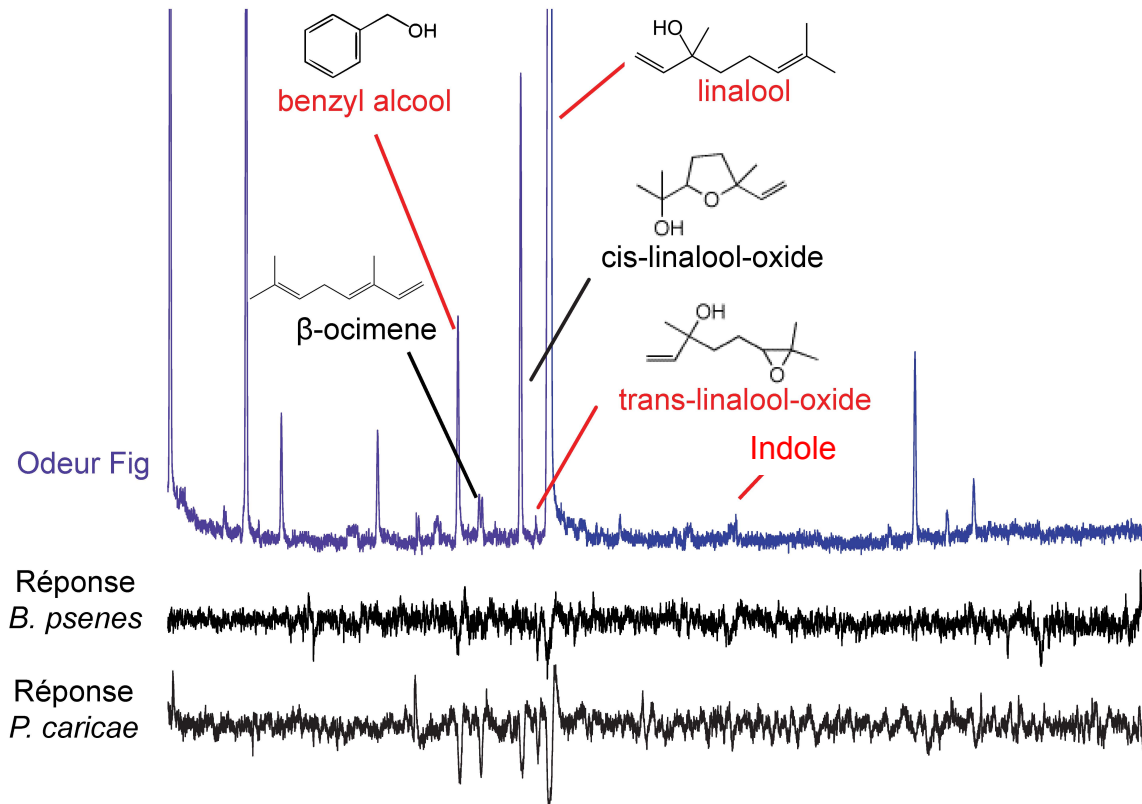


N = 7 / sp

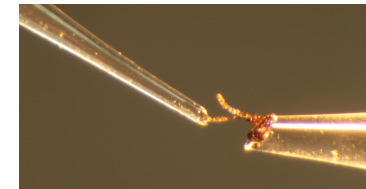
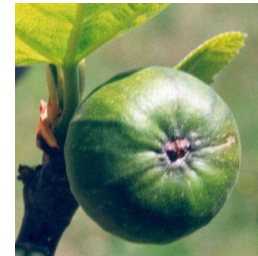
2. Mechanisms of fig and fig wasps chemical communication

Characterization of the VOCs detected by fig wasps

F. carica / *B. psenes*



Study site Montpellier



N = 7 / sp

- ➔ ✓ *B. psenes* detects only 4 of the 40 VOC emitted by receptive figs
- ✓ *P. caricae* detects 2 VOC extra

Proffit et al., in prep.

2. Mechanisms of fig and fig wasps chemical communication

VOCs used by fig wasps

Pollinating fig wasps

- Specific ratio of common compounds

Or

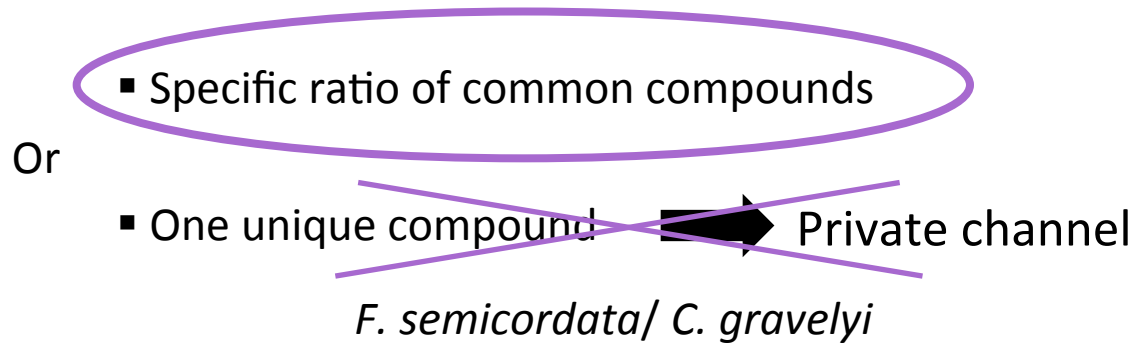
- One unique compound  Private channel

F. semicordata/ *C. graveleyi*

2. Mechanisms of fig and fig wasps chemical communication

VOCs used by fig wasps

Pollinating fig wasps

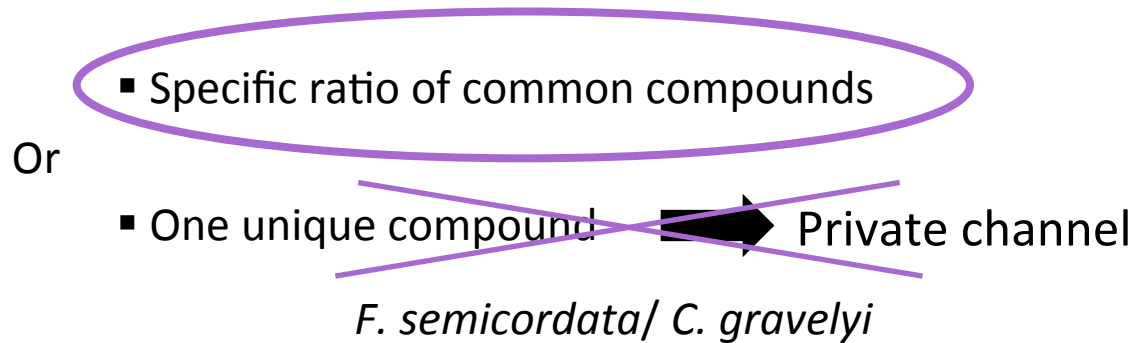


- ✓ Minor compounds have a major role in the attraction
 - ✓ 4-Methylanisole is emitted in small amount by *F. curtipes*
- (Gu et al., 2012, J. chem. ecol.)

2. Mechanisms of fig and fig wasps chemical communication

VOCs used by fig wasps

Pollinating fig wasps

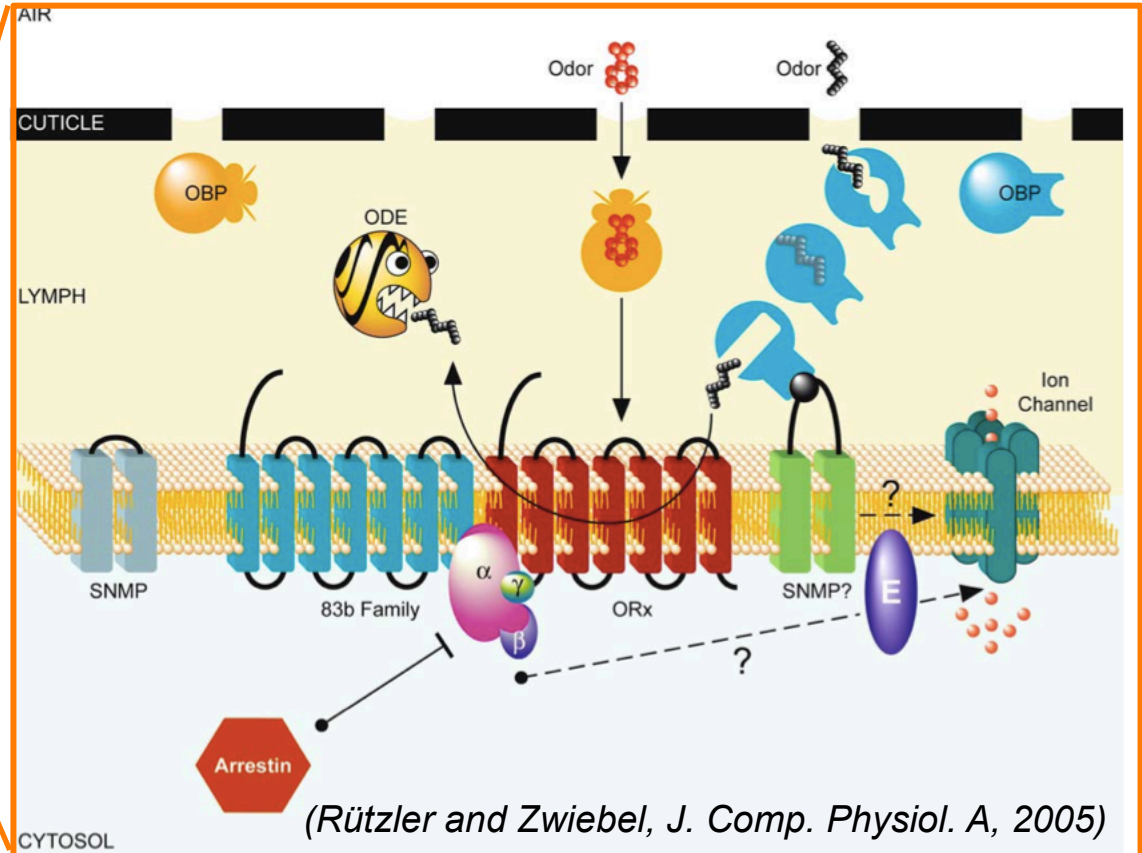
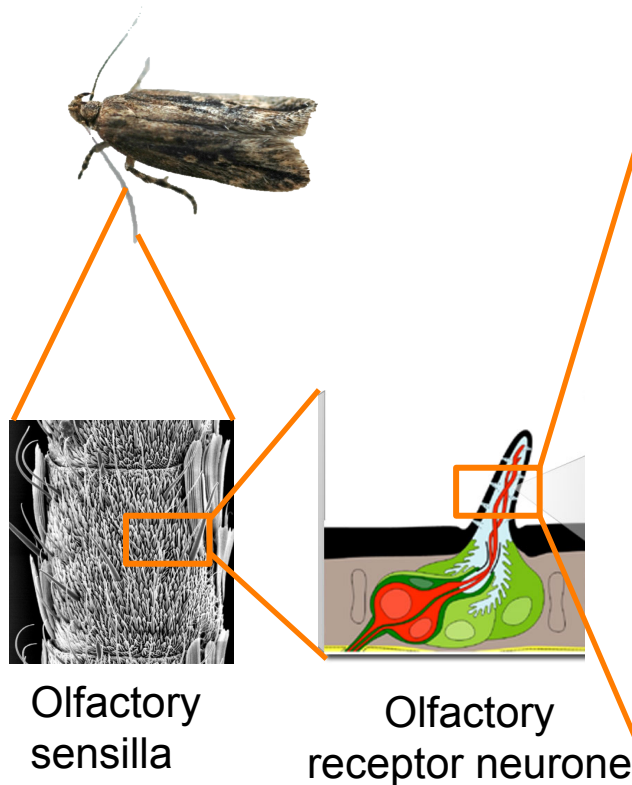


- ➡ ✓ Minor compounds have a major role in the attraction
- ✓ 4-Methylanisole is emitted in small amount by *F. curtipes*
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NPFW

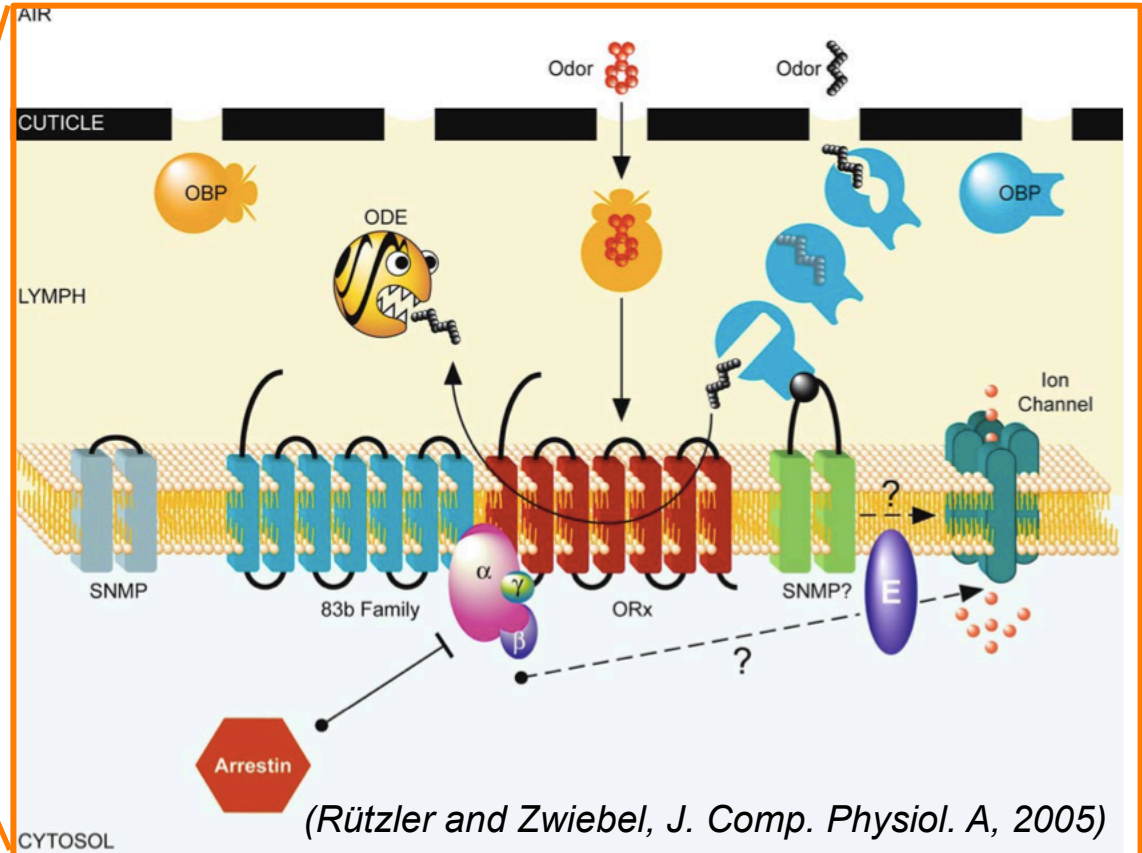
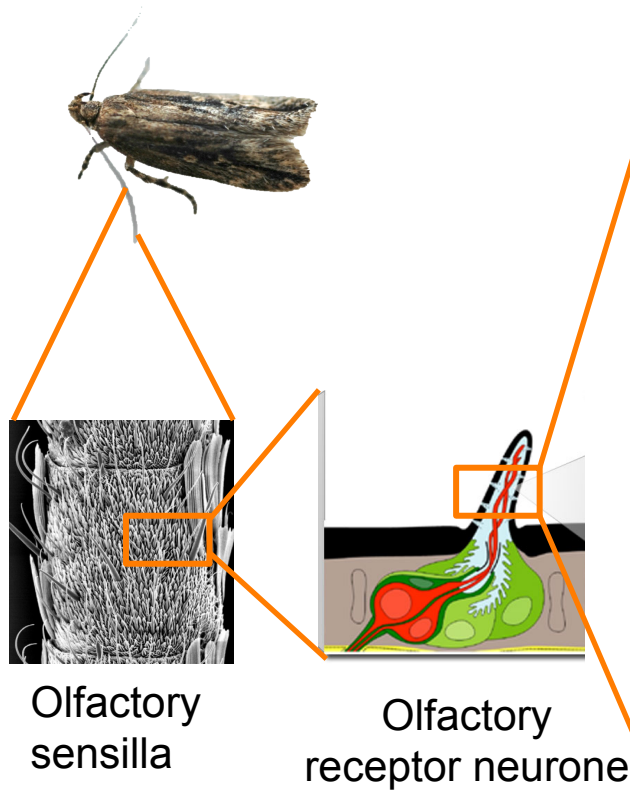
?

2. Mechanisms of fig and fig wasps chemical communication



- ✓ Many proteins are involved in the molecular process of VOC detection in insect
 - odorant-binding proteins (**OBPs**), chemosensory proteins (**CSPs**)
 - odorant receptors (**ORs**), gustatory receptors (**GRs**), ionotropic receptors (**IRs**), sensory neuron membrane proteins (**SNMPs**)
 - odorant-degrading enzymes (**ODEs**)

2. Mechanisms of fig and fig wasps chemical communication



- ✓ Many proteins are involved in the molecular process of VOC detection in insect
- ✓ Several multigenic families code for these proteins

2. Mechanisms of fig and fig wasps chemical communication

■ Characterization of the genes involved in the olfaction

Methods

Antennal transcriptome compared with body transcriptome

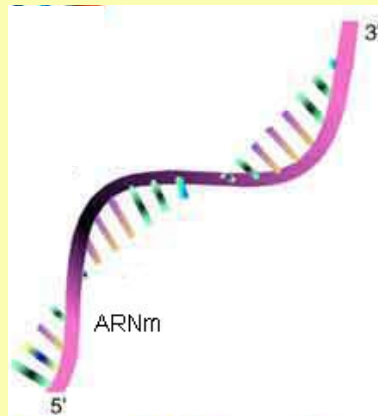
Dissection of different parts
of the body

ARN extraction

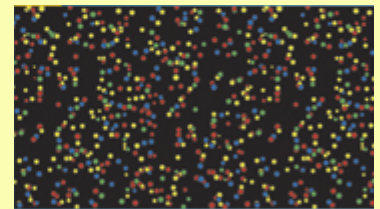
cDNA bank construction



2 500 females !!!!!



3 antennae
3 heads
3 bodies



Body Head Antennae

2. Mechanisms of fig and fig wasps chemical communication

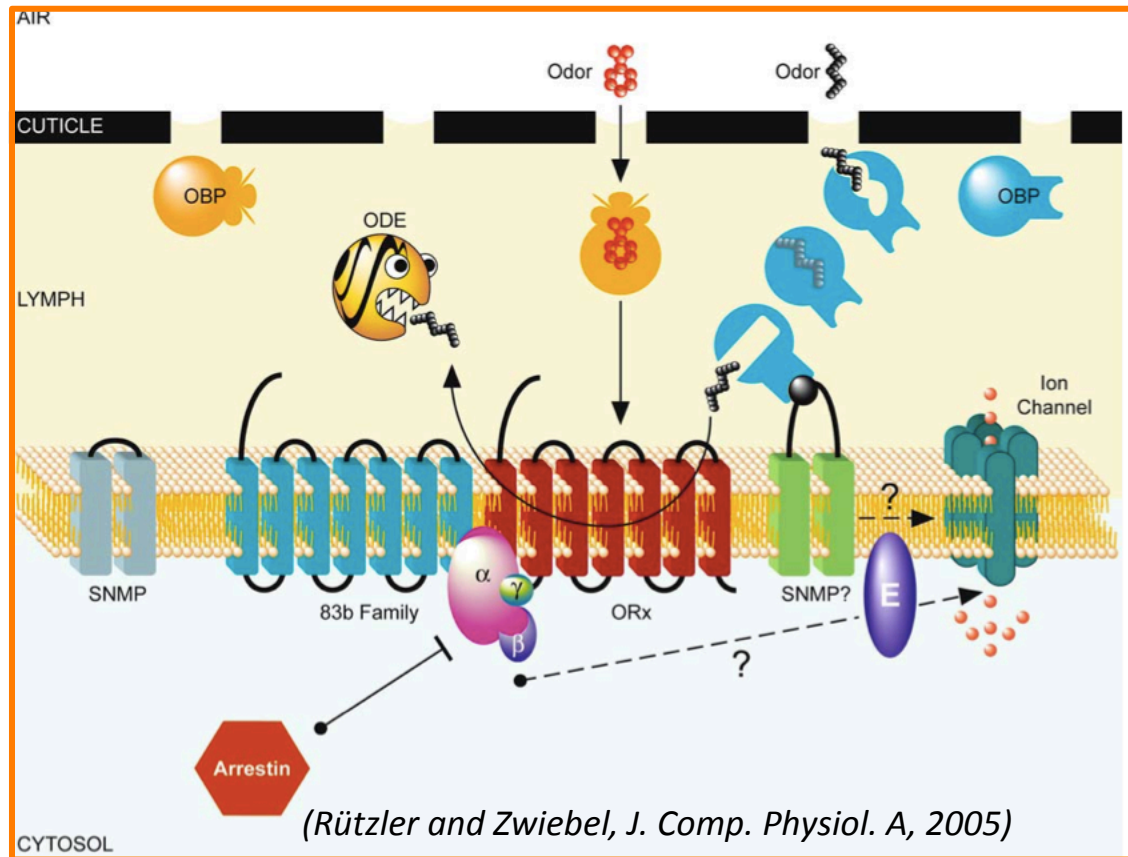
■ Characterization of the genes involved in the olfaction

✓ Putative chemosensory genes expressed in the antennae of

B. psenes:

- 16 ORs (incl. Orco)
- 7 IRs
- 2 SNMPs
- Plus de 30 OBPs et ODEs

Proffit et al., in prep.



2. Mechanisms of fig and fig wasps chemical communication

■ Characterization of the genes involved in the olfaction

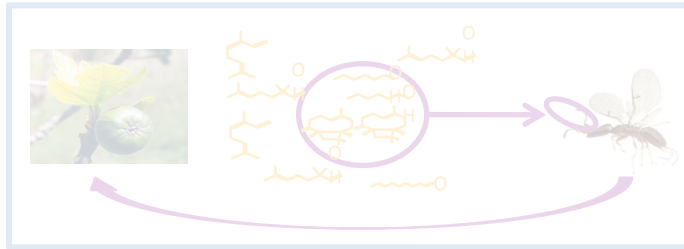
✓ Putative chemosensory genes expressed in the antennae of

<i>B. psenes:</i>	<i>C. solmsi</i>	<i>Nasonia vitripennis</i>
- 16 ORs (incl. Orco)	46	301
- 7 IRs	11	10
- 2 SNMPs	NA	?
- Plus de 30 OBPs et ODEs	7 + NA	90 + ?

Xiao et al., 2013, Genome Biology

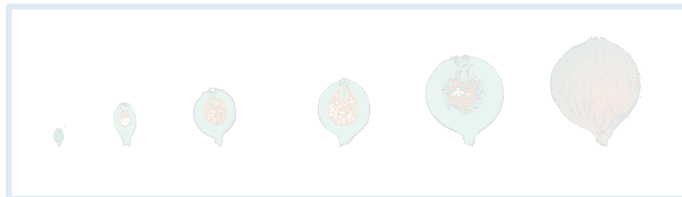
➡ Is specificity “coded” by a reduced number of chemosensory genes and proteins ?

1. Importance of fig VOC in the organization of fig wasp communities



2. Mechanisms of fig and fig wasps chemical communication

3. Inter-specific variation of fig VOC and implication for fig wasp communities

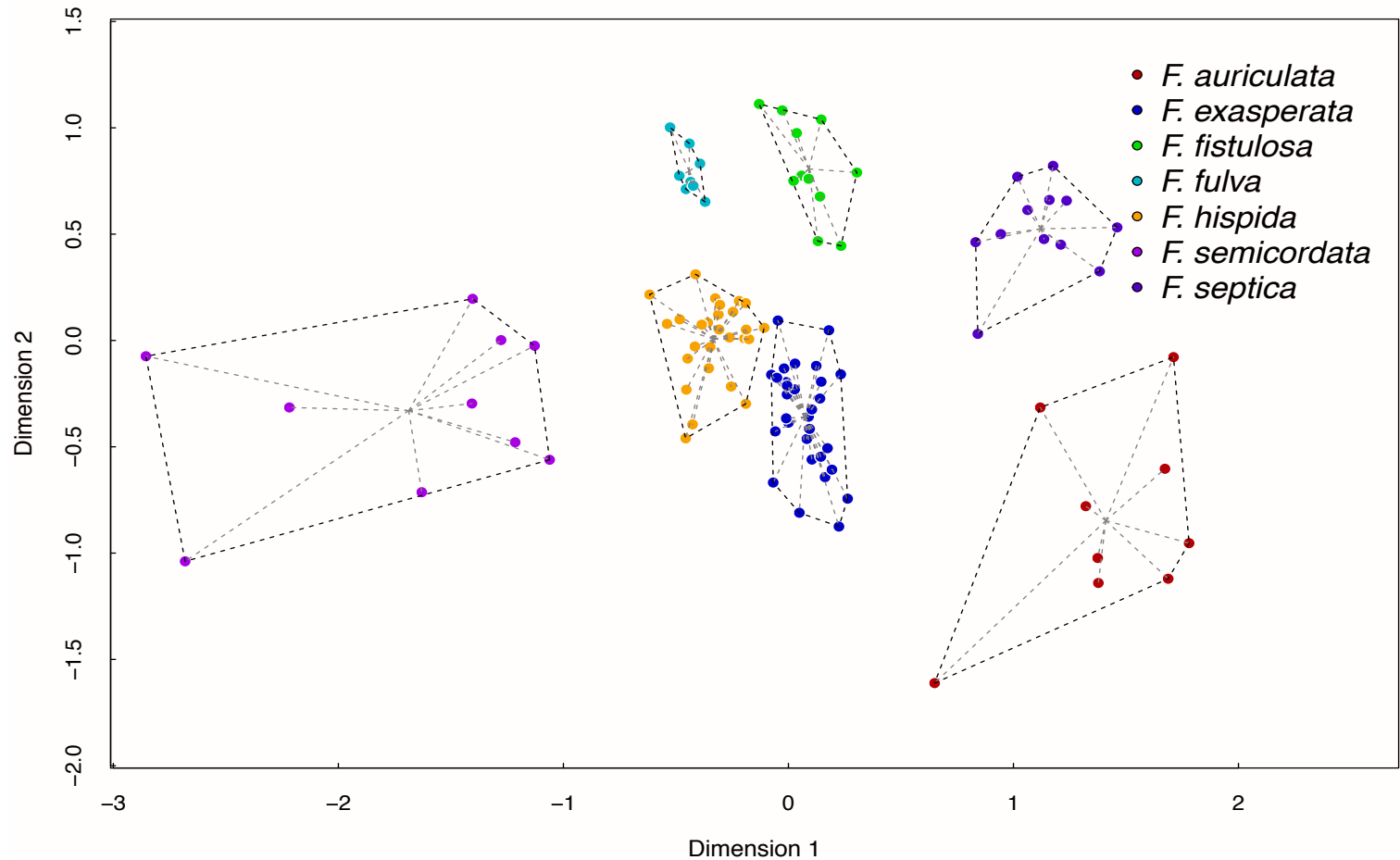


4. Intra-specific variation of fig VOC and implication for fig wasp communities

3. Inter-specific variation of fig VOC

Comparison of VOC profiles among 7 fig species (3 subgenera)

NMDS originated from relative amount of VOCs



Inter-specific variation of the total bouquet of VOC emitted by figs

3. Inter-specific variation of fig VOC

➡ Chemical mediation maintains host specificity
of fig wasps

Grisson-Pigé et al., 2001; *Chen et al., Funct. Ecol.*,
2009; Proffitt & Johnson, *S. Afr. J. Bot.* 2009;
Proffitt et al., *Ento. Exp. Appl.*, 2009



➡ Chemical mediation can represent a barrier to host shift
and pre-zygotic barrier for *Ficus spp.*

3. Inter-specific variation of fig VOC

Breakdowns of strict species-specificity exist

Intra - community



Two or more agaonid wasps
pollinate the same host

Inter - communities



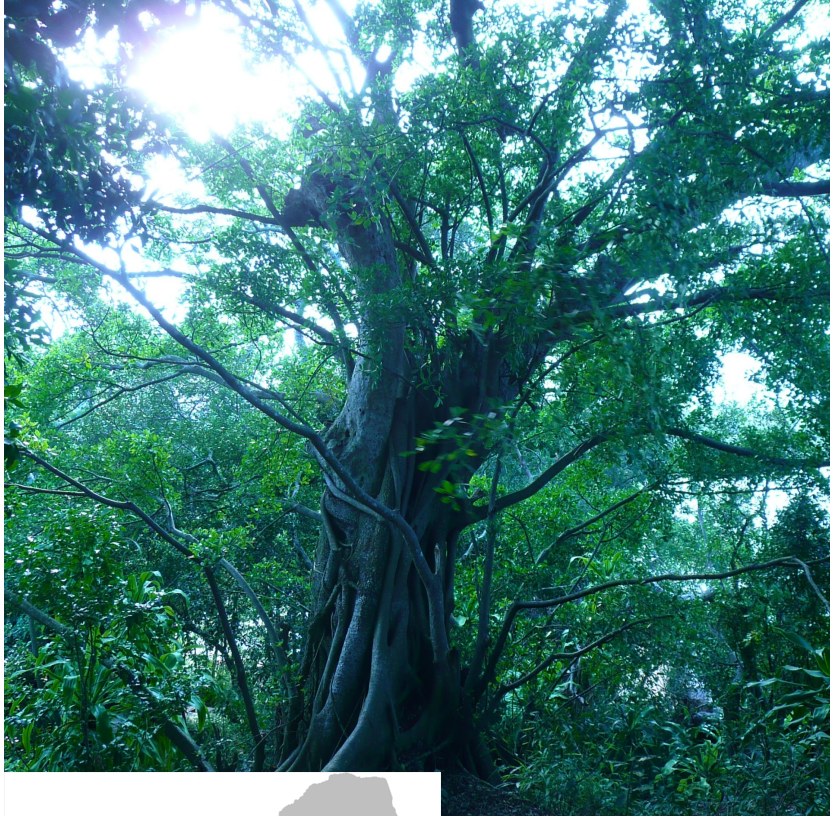
One agaonid wasp pollinates
several species

For NPFW

What are the biological traits allowing this breakdown of specificity?

3. Inter-specific variation of fig VOC

Breakdowns of strict species-specificity exist



F. n. natalensis

KwaZulu-Natal, South Africa



Amandine Cornille



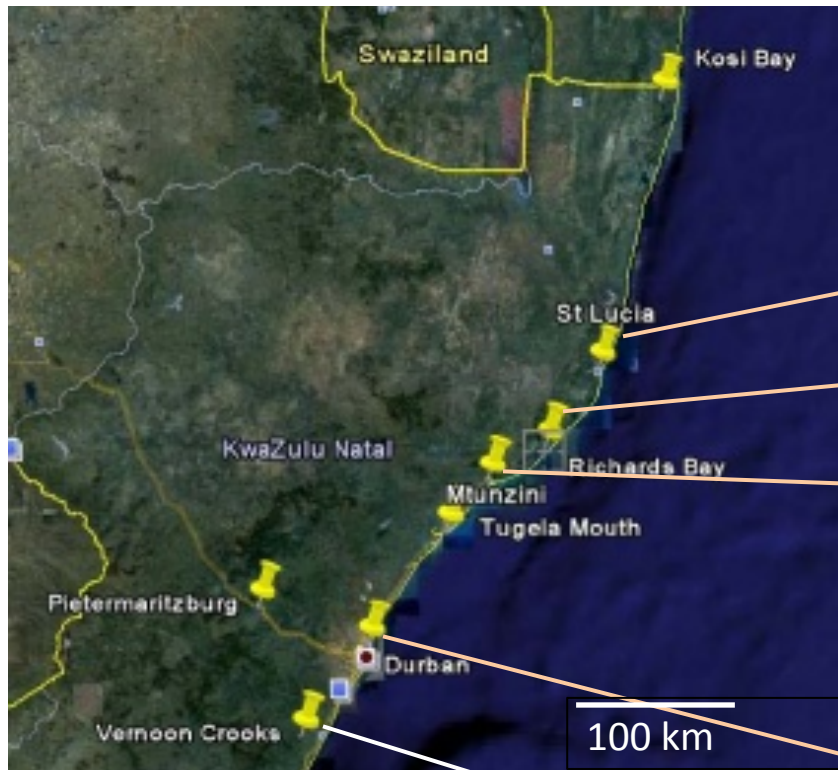
*Elisabethiella
socotrensis*



*Elisabethiella
stuckenbergi*

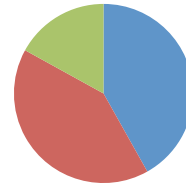
3. Inter-specific variation of fig VOC

Breakdowns of strict species-specificity exist

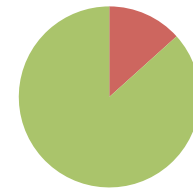


Proportion of figs / tree visited by :

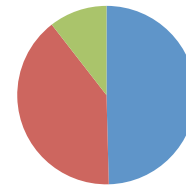
St Lucia n = 5



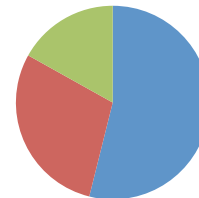
Richard's bay n = 1



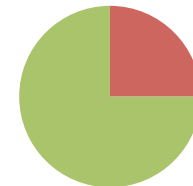
Mtunzini n = 8



Durban n = 8



Vernon Crooks n = 1



■ *E. socotrensis*

■ *E. stuckenbergi*

■ both species

➡ Relative frequency of the two pollinators homogeneous in KZN

(Cornille et al., Proc. R. Soc. Lond. B, 2012)

3. Inter-specific variation of fig VOC

Breakdowns of strict species-specificity exist

F. natalensis

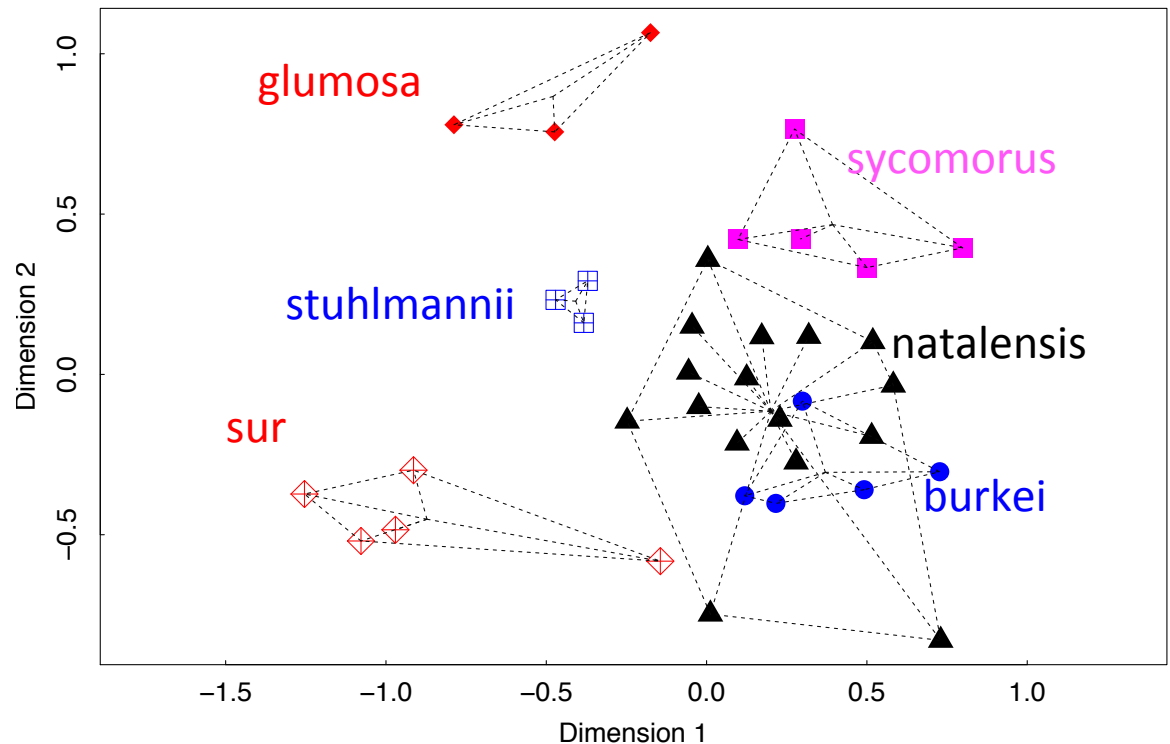
F. burkei

F. glumosa

F. stuhlmannii

F. sur

F. sycomorus



➡ Volatile profiles of *F. natalensis* and *F. burkei* overlap

3. Inter-specific variation of fig VOC

Phylogenetic inertia

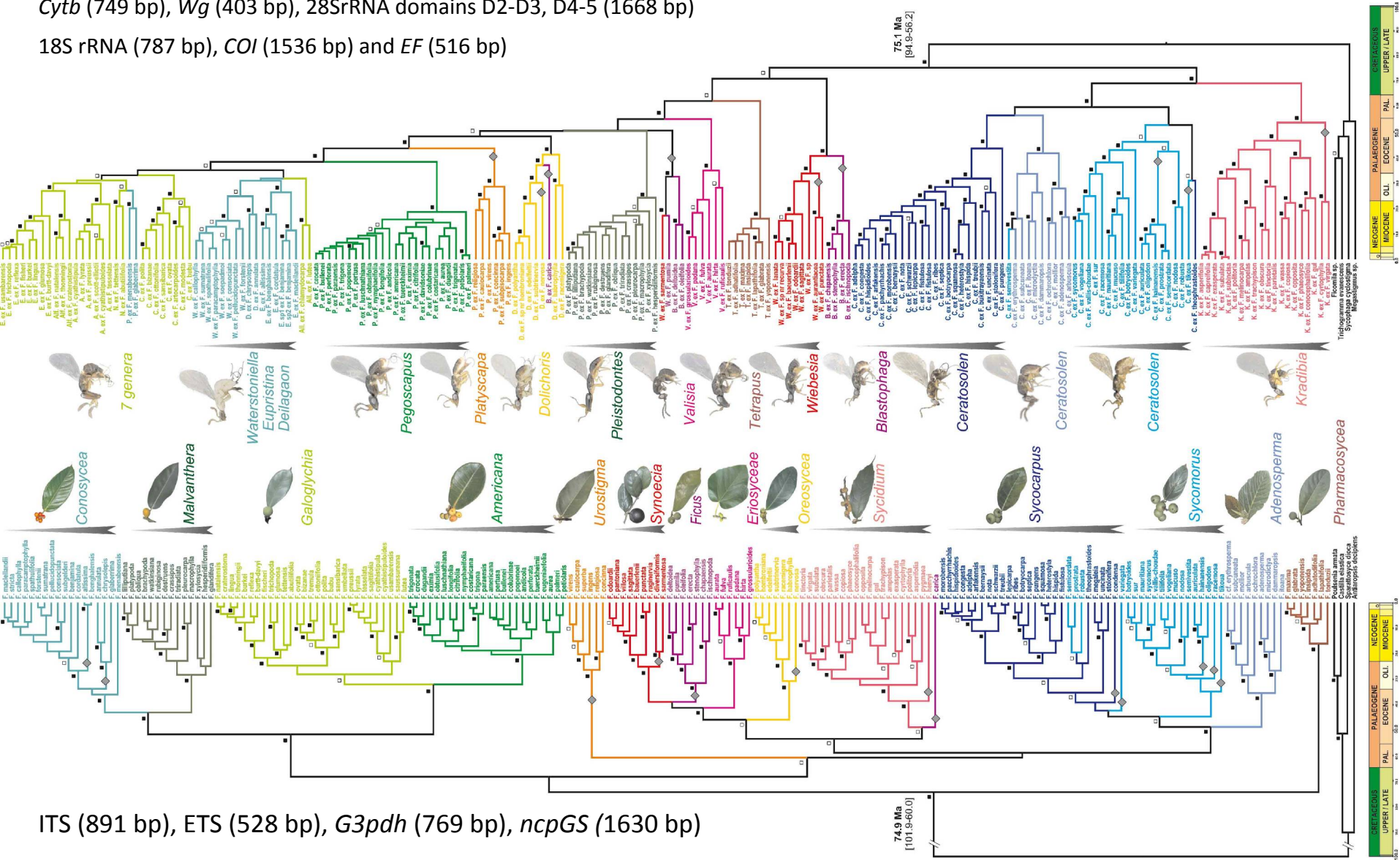
Related figs usually have related pollinators

a story about co-diversification

from Cruaud et al., *syst.biol.*, 2012

Cytb (749 bp), Wg (403 bp), 28SrRNA domains D2-D3, D4-5 (1668 bp)

18S rRNA (787 bp), COI (1536 bp) and EF (516 bp)



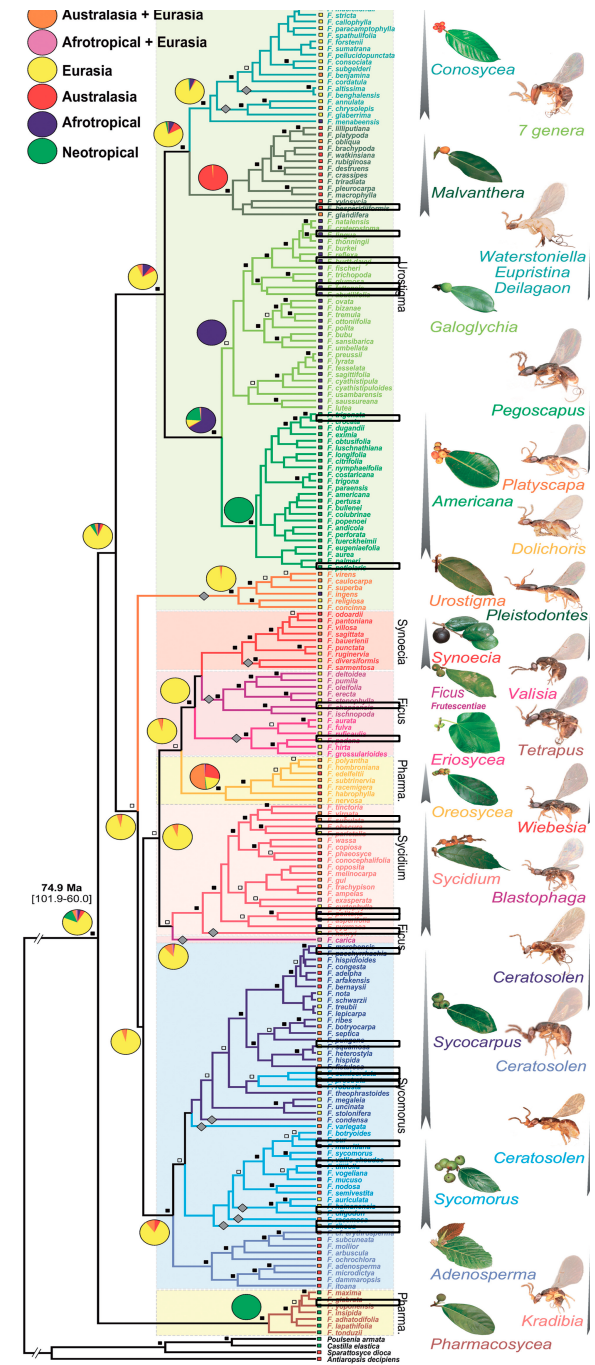
3. Inter-specific variation of fig VOC

Phylogenetic inertia

Catherine Soler



Soler et al., in prep.



3. Inter-specific variation of fig VOC

Phylogenetic inertia

25 *Ficus* spp.
(9/14 sections)

3 < n < 30 par sp

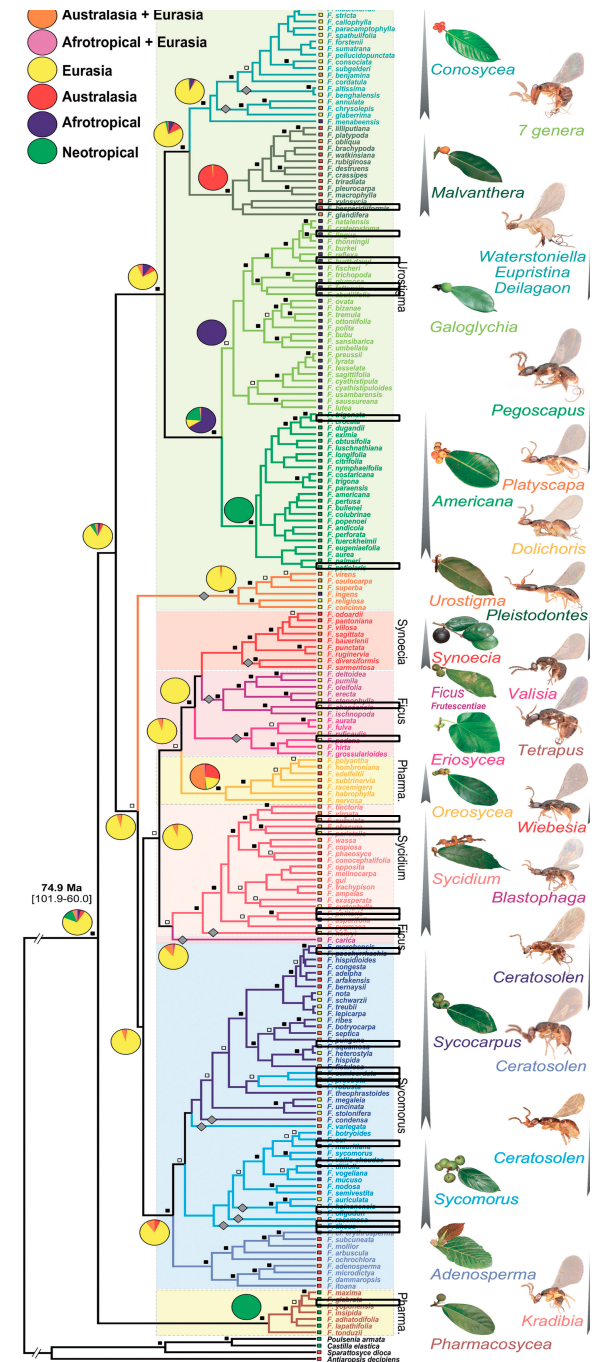
360 VOCs

Scent matrix (0/1 and %)

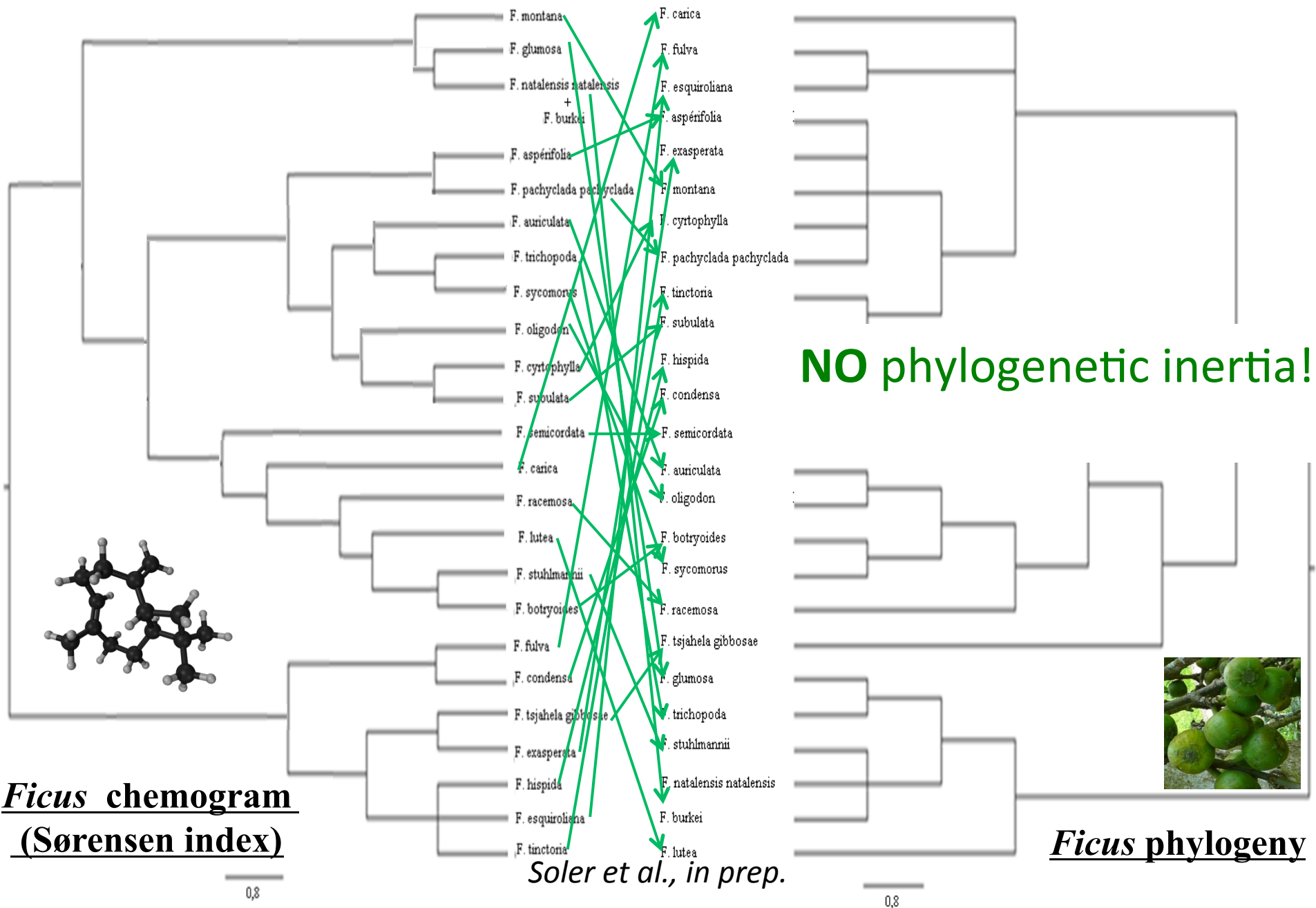
build chemograms (=“scent phylogenies”)
&

Compare them with Phylogenies

Soler et al., in prep.



Comparison between *Ficus* phylogeny and chemogram



3. Inter-specific variation of fig VOC

Phylogenetic inertia

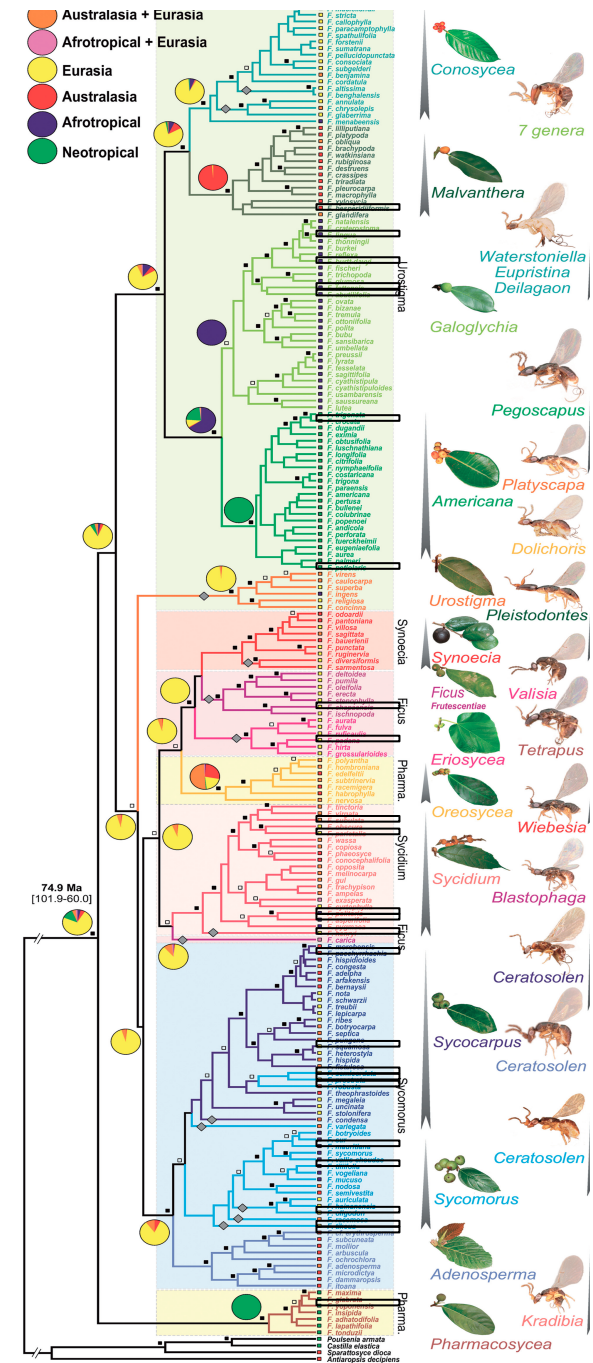


Phylogenetic inertia on scent seems negligible

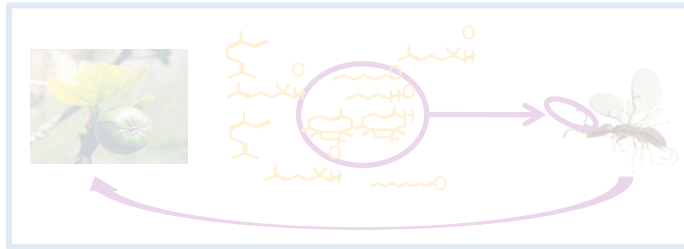
Confirms previous studies

(e.g., *Levin et al., 2003, Syst. Biol.*)

Soler et al., in prep.

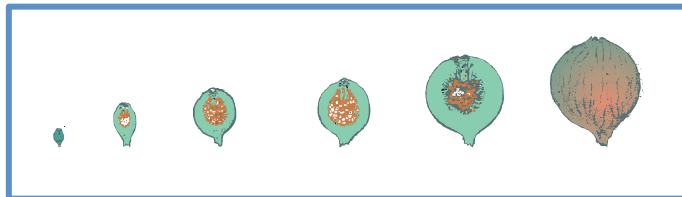


1. Importance of fig VOC in the organization of fig wasp communities



2. Mechanisms of fig and fig wasps chemical communication

3. Inter-specific variation of fig VOC and implication for fig wasp communities



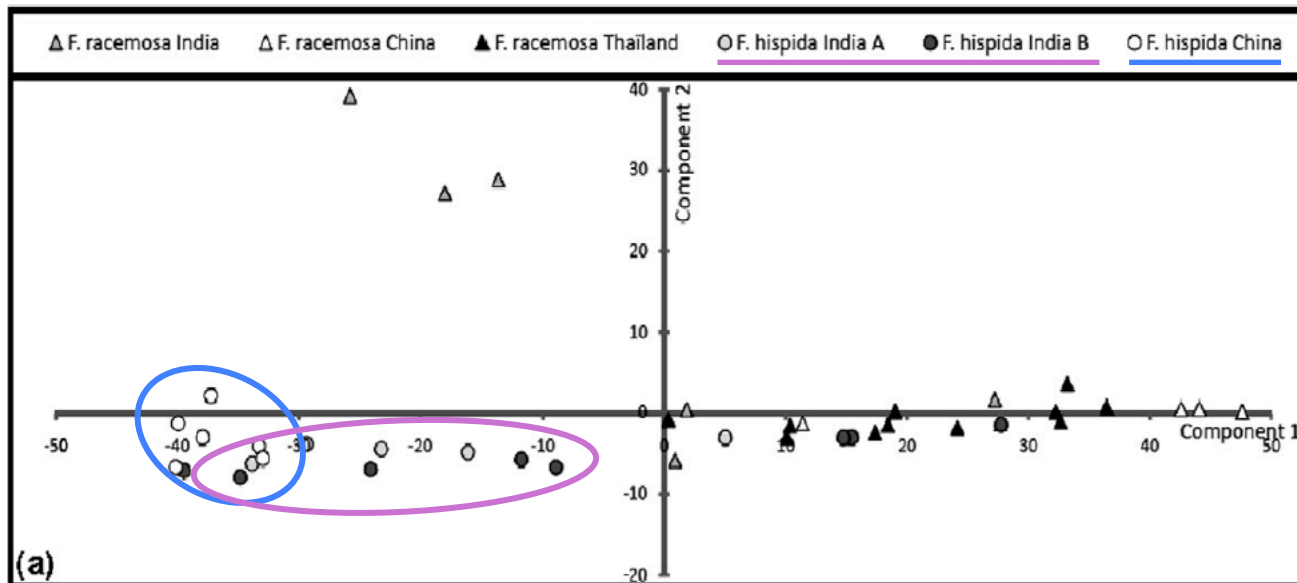
4. Intra-specific variation of fig VOC and implication for fig wasp communities

4. Intra-specific variation of VOC

4. Intra-specific variation of VOC

-> Spatial variation

➡ Two species with large geographic ranges (*F. hispida* and *F. racemosa*):
scent is different in India but constant within East Asia



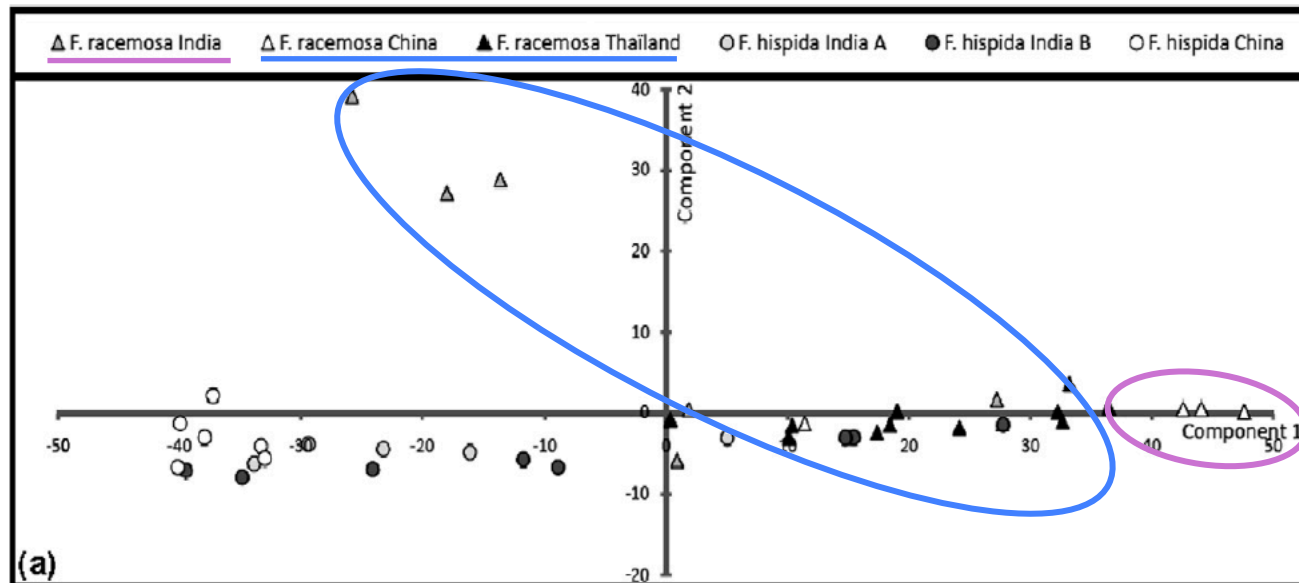
(Soler et al., 2011)

4. Intra-specific variation of VOC

-> Spatial variation

➔ Two species with large geographic ranges (*F. hispida* and *F. racemosa*):
scent is different in India but constant within East Asia

Molecular data show that population in China and India-Thailand form
distinct groups (*Bain et al., 2016, Evol. Ecol.*)



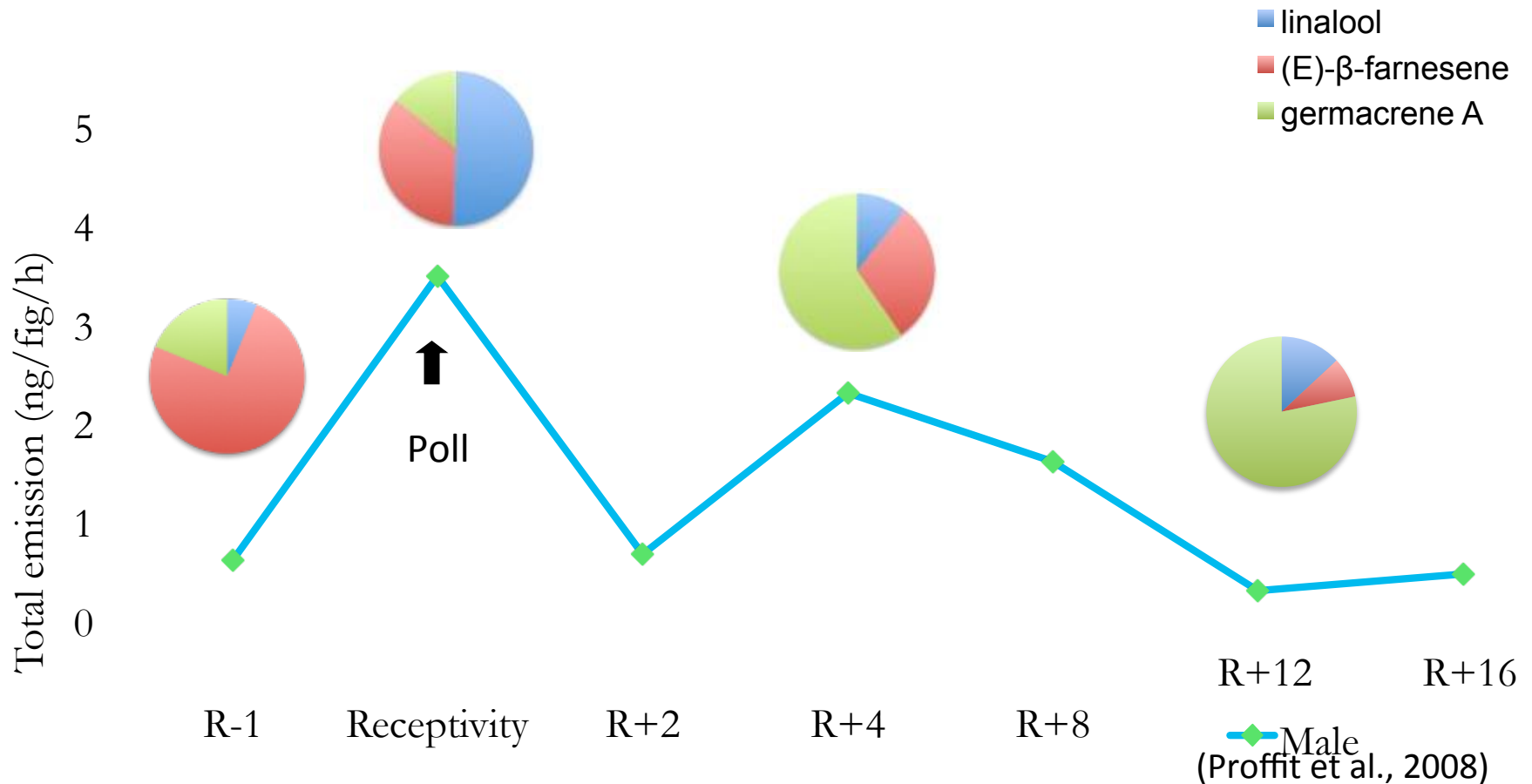
(Soler et al., 2011)

4. Intra-specific variation of VOC

-> Temporal variation

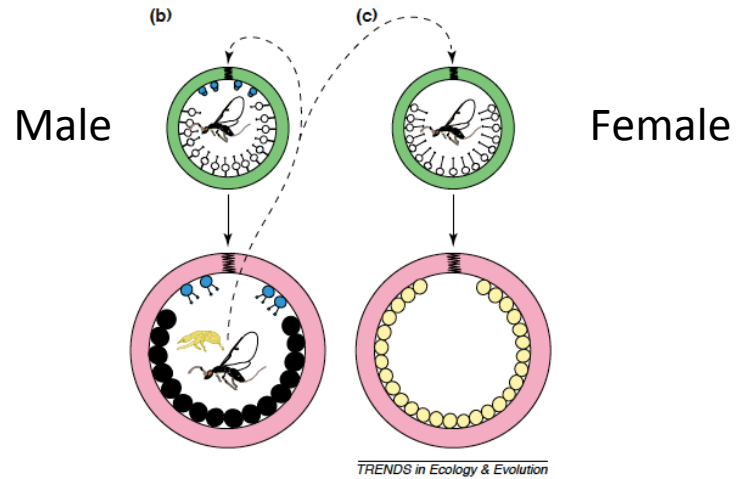
Floral ontogeny / Post-pollination

➔ Qualitative and quantitative differences of floral scent over the development
Pic of emission during receptivity



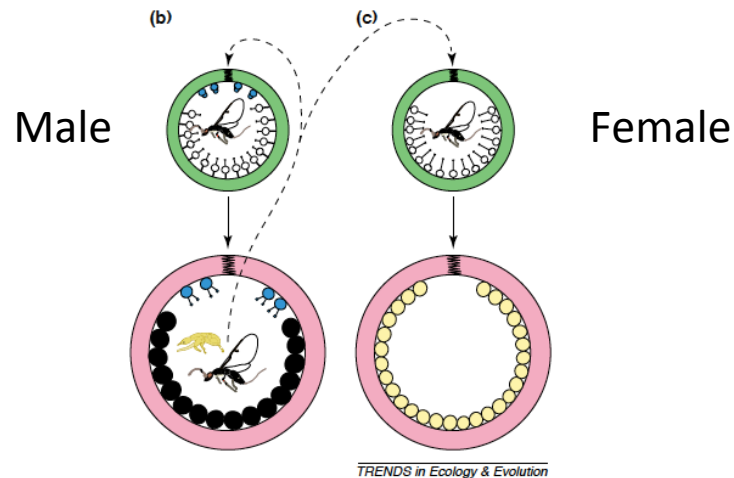
4. Intra-specific variation of VOC

Inter-sexual variation



4. Intra-specific variation of VOC

Inter-sexual variation



Why don't wasps avoid female figs?

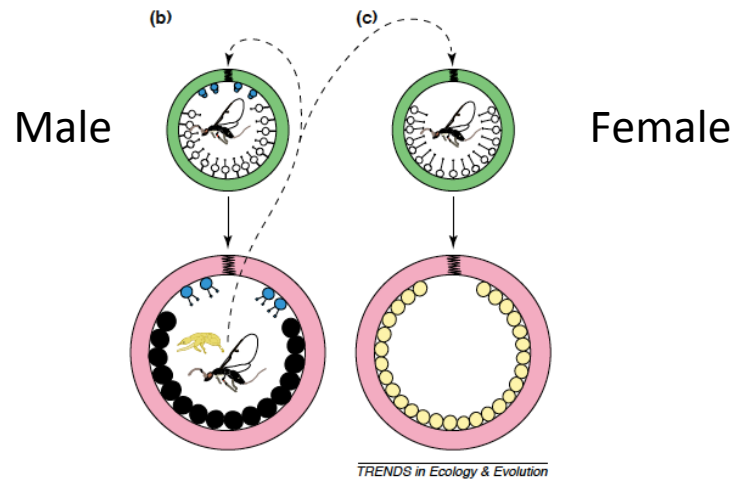
➔ Chemical mimicry hypothesis

(Grafen & Godfray 1991)

(Hossaert-McKey et al., 2016, Sc. Report)

4. Intra-specific variation of VOC

Inter-sexual variation



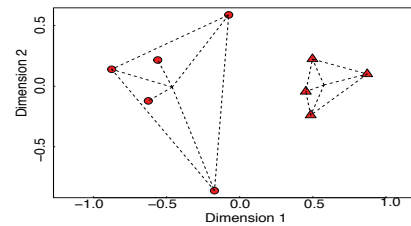
Why don't wasps avoid female figs?

➔ Chemical mimicry hypothesis

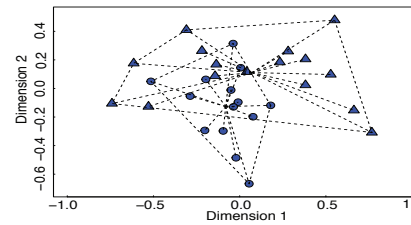
(Grafen & Godfray 1991)

Asynchronous species

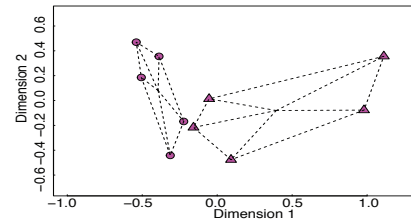
F. auriculata



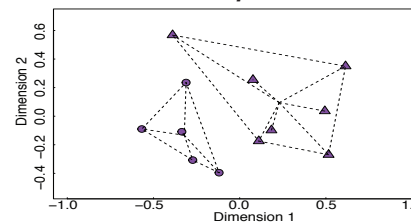
F. exasperata



F. semicordata

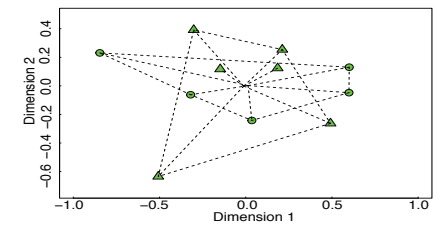


F. septica

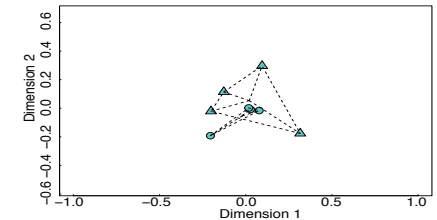


Synchronous species

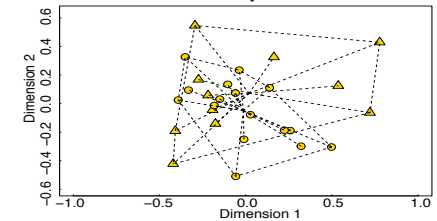
F. fistulosa



F. fulva



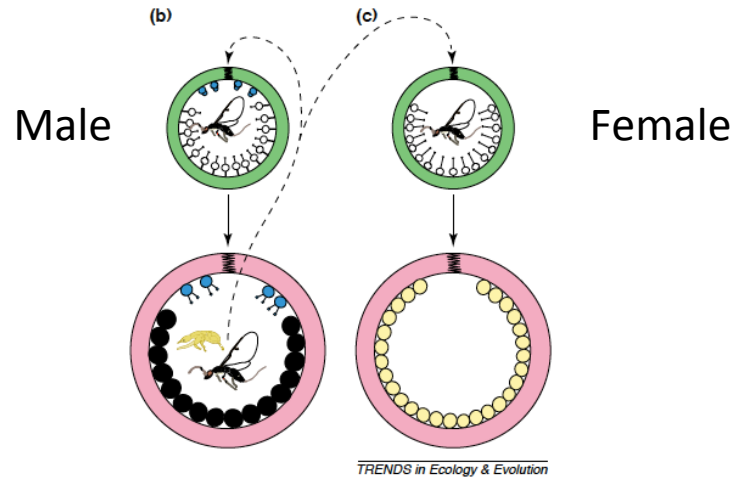
F. hispida



(Hossaert-McKey et al., 2016, Sc. Report)

4. Intra-specific variation of VOC

Inter-sexual variation

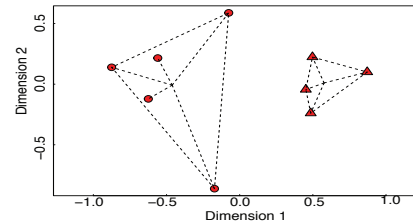


Why don't wasps avoid female figs?

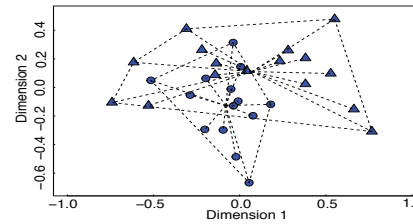
➡ Chemical mimicry

~~Asynchronous species~~

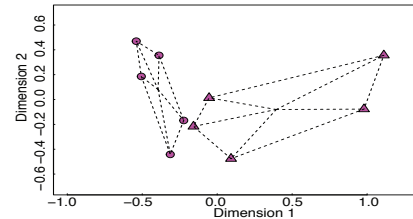
F. auriculata



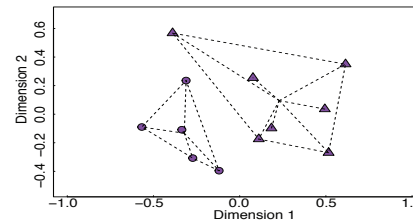
F. exasperata



F. semicordata

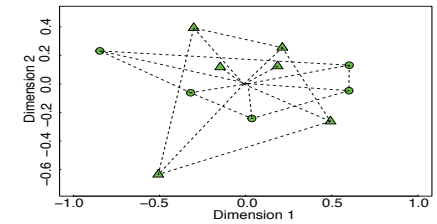


F. septica

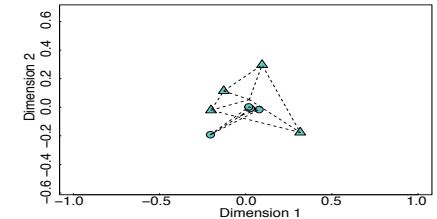


Synchronous species

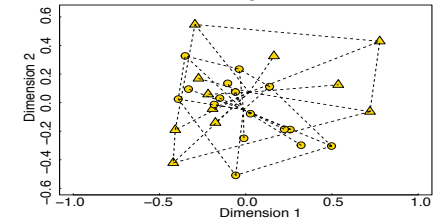
F. fistulosa



F. fulva



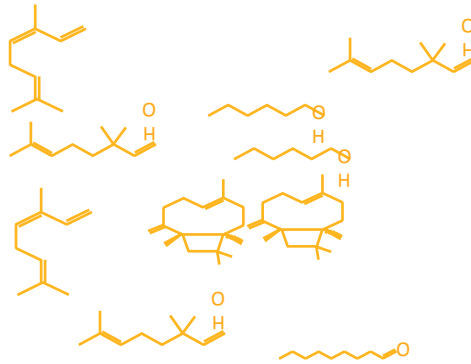
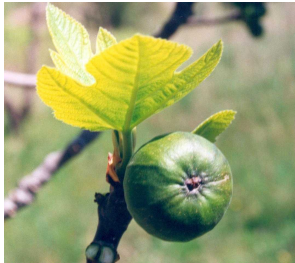
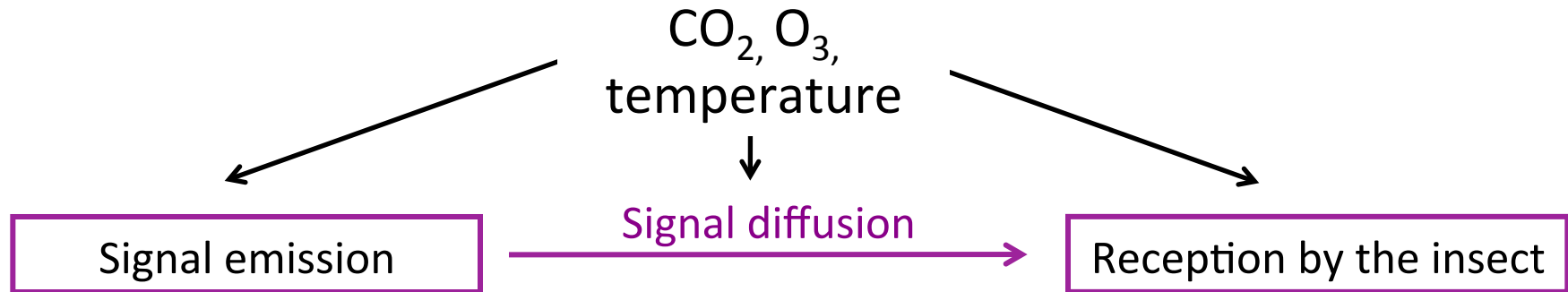
F. hispida



(Hossaert-McKey et al., 2016, Sc. Report)

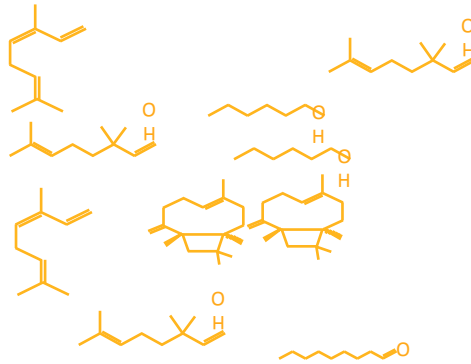
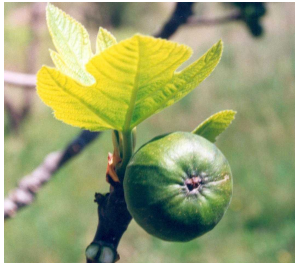
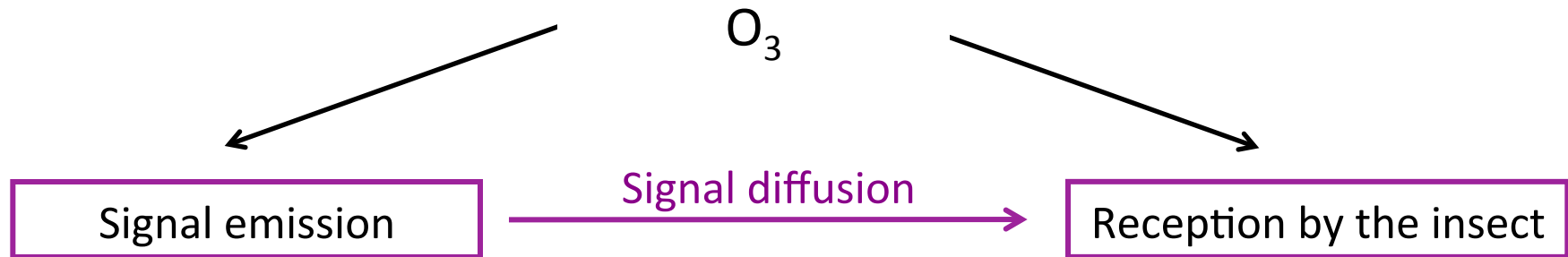
4. Intra-specific variation of VOC

Impacts of abiotic factors



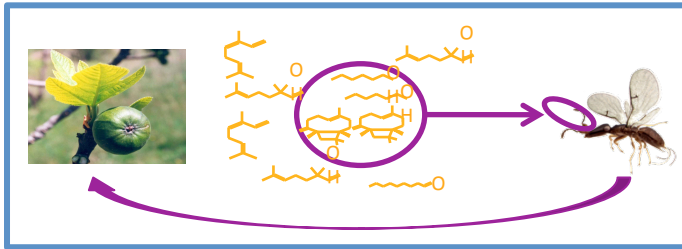
4. Intra-specific variation of VOC

Impacts of abiotic factors



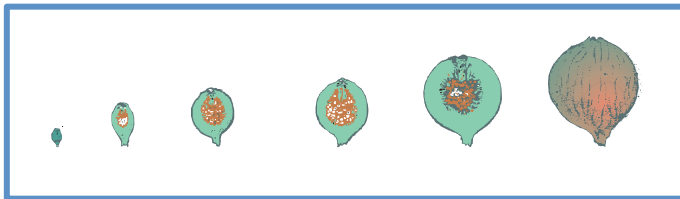
What to do next ?

1. Importance of fig VOC in the organization of fig wasp communities



2. Mechanisms of fig and fig wasps chemical communication

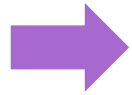
3. Inter-specific variation of fig VOC and implication for fig wasp communities



4. Intra-specific variation of fig VOC and implication for fig wasp communities

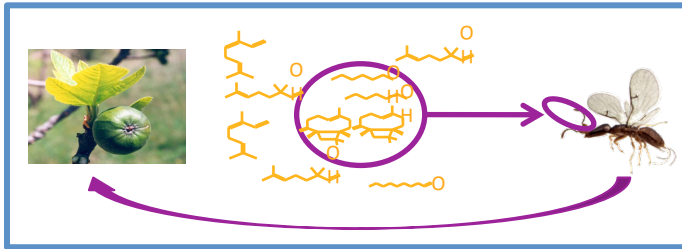
Perspectives

1. Importance of fig VOC in the organization of fig wasp communities



Increase the number of species, specially NPFW

Perspectives



2. Mechanisms of fig and fig wasps chemical communication

- ➡ - Compare the VOC used by the pollinator and NPFW
- Compare the genes coding for olfactory proteins

Perspectives

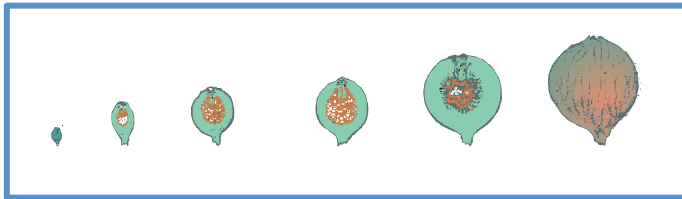
➡ Compare the VOC detected and used by wasps sharing the same host

3. Inter-specific variation of fig VOC and implication for fig wasp communities



Perspectives

➡ Quantify the variation within VOC ratio still eliciting a behavioral response



4. Intra-specific variation of fig VOC and implication for fig wasp communities

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- Chinese academy of sciences

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