UNIVERSITY OF OXFORD

Frontiers in Olfaction, Trieste, IT
24-28 July 2017

ICTP

What is a pheromone?

Implications for evolution, perception, and processing

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just email me if you'd like my slides ppt

Outline

1. **What are pheromones?**
2. **How pheromones evolve**
3. **Operational definition**

What are pheromones?

Pheromone:

a chemical signal transmitted between individuals of the *same* species.

From 2 Greek words:

pherein, to transfer *hormōn*, to excite

Invisible signals

- Ancient Greeks: female dog
- 17th C bee-keeping manual
- 19th C J-H Fabre: moths

Charles Darwin (1871) *The descent of man and selection in relation to sex*

photo: P Nijenhuis

"During the season of love, a musky odour is emitted by … glands of the crocodile, and pervades their haunts." p29

Also: smelly male elephants, goats, pythons, birds …

Gradual evolution of male scent glands by female choice

Success of the smelliest

***Surely* these smells were chemical signals …**

but the quantities were far too small to identify
(pico / micro gram per individual)

First pheromone identification: 1959
female sex pheromone of silk moth *Bombyx mori*
by Adolf Butenandt & team

silk moth *Bombyx mori*

Wing fluttering by male gives test that pheromone is present
= **bioassay** (= reliable test)

The first pheromone identification: 1959
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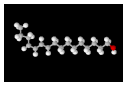


'bombykol'
(*E*10, *Z*12)-hexadeca-10,12-dien-1-ol

Established gold standard:
1. bioassay (wing flutter)
2. isolation (500,000 ♀)
3. identification
4. synthesis
5. bioassay confirms activity

Mobo et al (2003) PNAS

Every kind of animal, not just sex

- Aggregation pheromones
  – both sexes attractive e.g. bark beetles
- Nematode dauer (development)
- Mammary pheromones – rabbit

Social insects (ants, bees, wasps)
- Alarm pheromones
- Trail pheromones e.g. ants, termites
- Primer effects e.g. queen pheromones

A pheromone (in any animal):

- A chemical signal between members of same species
- Behavioural and/or physiol response
- **Same mol(s)** in e.g. all males of the species (but poss different amounts)
- Usually a **combination** of molecules
- Detected by sense of smell (usually)

Karlson & Lüscher (1959) *Nature* 183: 55-56
Wyatt (2014) *Pheromones and animal behavior.* 2 edn. CUP

Pheromones are …

"molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, which are emitted by an individual and received by a second individual of the same species, in which they cause a specific reaction, for example, a stereotyped behavior or a developmental process"

Wyatt (2010) *J Comp Physiol A*, modified after Karlson & Lüscher (1959) *Nature*.

Evolved signal versus **cue**

- Signals alter the behavior of other organisms, have evolved [in signaller] because of that effect, and work because the receiver's response has also evolved
  (Maynard Smith & Harper 2003, p. 3)
- Responses to cues are only evolved in the receiver

  e.g. Noni fruit odour cues attract *Drosophila sechellia* (fly has highly evolved receptors and circuits to detect) Hansson, Stensmyr, et al

Moth pheromones: evidence of evolution

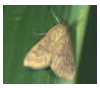
Female: signaller

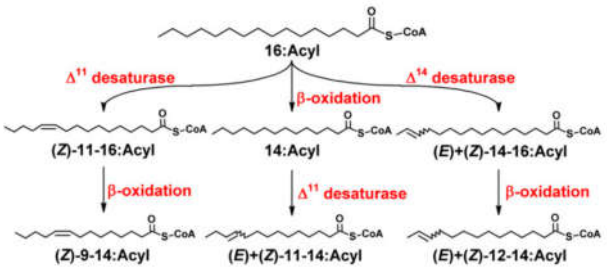
- **Production**
  - Enzyme pathways
  - Structures (glands)
  - Hormone controls
- **Behaviours** ('calling')

Male: receiver

- **ORs**
- **MGC**
- **Neural circuits**
- **Behaviours** (flight up plume)

Ostrinia **moth ♀ pheromones, enzyme pathways**





Lassance *et al* (2013) *PNAS*

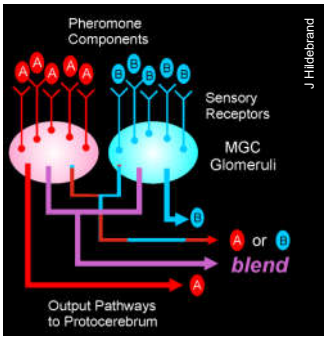
Moths - be the first male

- female sex pheromone = species, sex, & receptivity in one message
- selection on males for sensitivity, flight, tracking accuracy →
  sexual dimorphism in antennae & brain

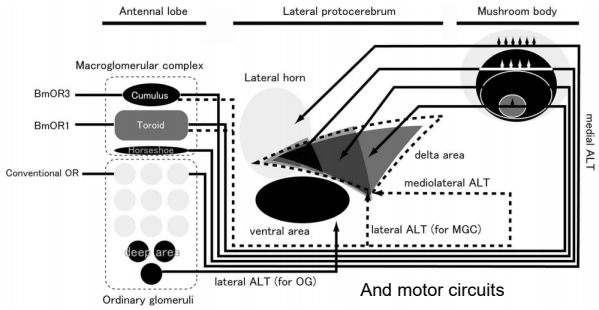
Photo Bob Harrison

Moths - male perception

- Combinatorial processing of blend in antennal lobe
- Molecules from wrong species, stop



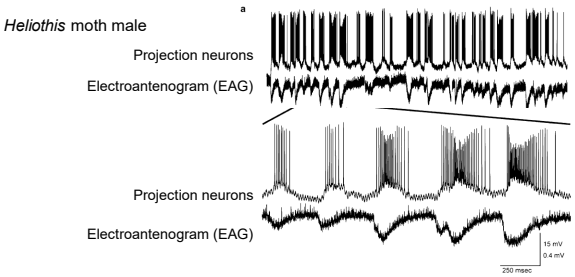
Moths - male neural circuits



B mori

Sakurai et al 2014, *Frontiers Physiol* – modified from Namiki et al., 2013

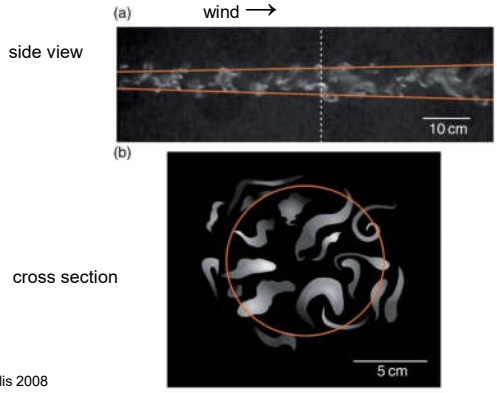
Moths - male neural circuits



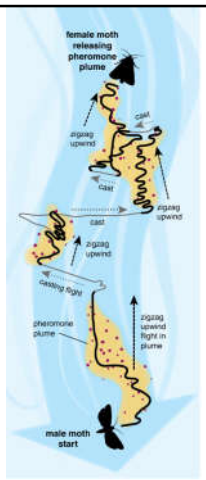
And then can also record from descending neurons, ultimately stimulating flight muscles

Vickers et al (2001) *Nature* 410:466-470

Moth males: finding a pheromone source in a turbulent plume



Willis 2008

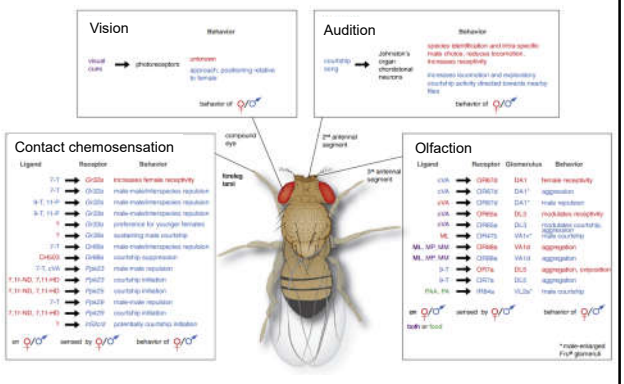


Male moths search for female

- 'Surge' upwind if detect pheromone
- 'Cast' side to side if lose it
- = 2 neural programmes

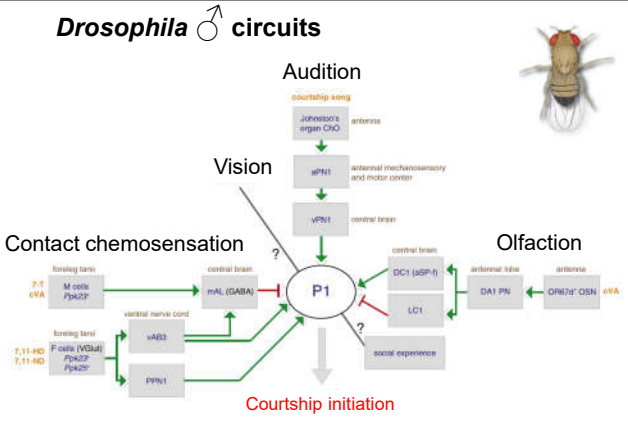
After Baker, TC (1990).

Drosophila inputs



Auer & Benton (2016) *Curr Op Neurobiol* 38: 18-26

Drosophila ♂ circuits



Auer & Benton (2016) *Curr Op Neurobiol* 38: 18-26

Mammals

Mammals do have pheromones

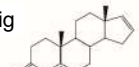
(small and large molecules)

detected by both VNO & main olfactory system

Rabbit

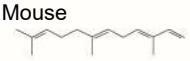
2-Methylbut-2-enal

Pig

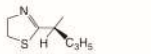


Androstenone

Mouse

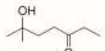


E, Eα-farnesene



2-*sec*-butyl-4, 5-dihydrothiazole

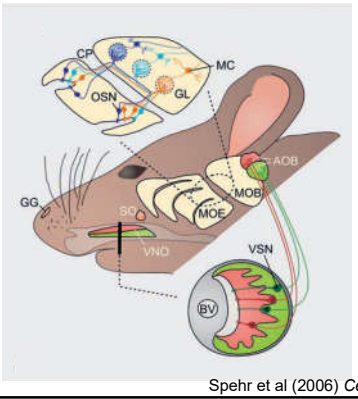
Methylthio methanethiol



6-Hydroxy-6-methyl-3-heptanone

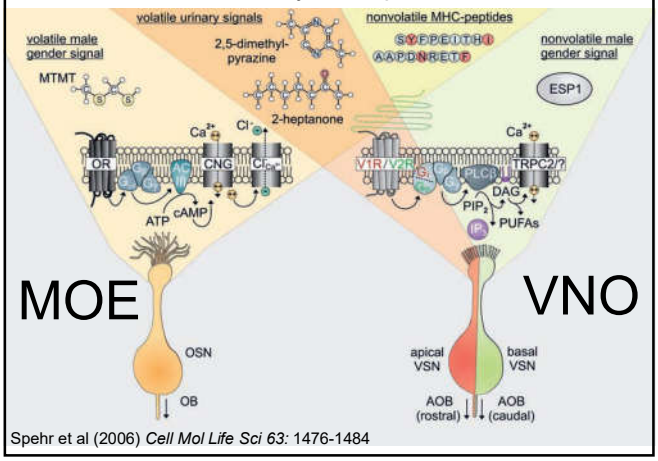
Plus peptide pheromones: ESP1, Darcin

VNO and MOE both involved in pheromone detection



Spehr et al (2006) *Cell Mol Life Sci* 63

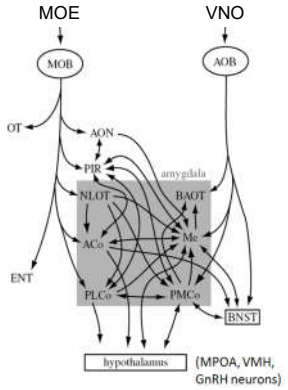
Pheromones - sensory overlap of MOE and VNO



Spehr et al (2006) *Cell Mol Life Sci 63:* 1476-1484

MOE & VNO inputs converge in amygdala,

for integrated behavioural, endocrine, & autonomic responses



sensory neurons

olfactory bulbs

amygdala

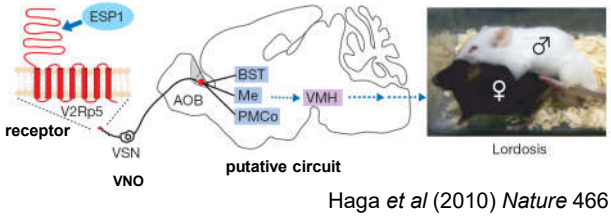
hypothalamus

Brennan & Kendrick (2006) *Phil Trans R Soc B* 361: 2061-2078

Mouse short range (tear secretion of males to female nose) [via VNO]

peptide pheromone

ESP1 = Exocrine gland-secreting peptide 1

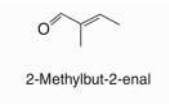


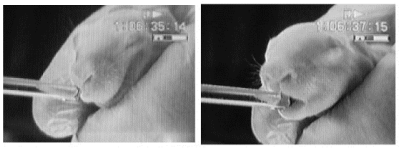
Haga *et al* (2010) *Nature* 466

Rabbit pups

[via main olfactory system – NOT VNO]

- **respond to mammary pheromone**
  - same for every rabbit mother





Schaal *et al* (2003) *Nature* 424: 68-72

What molecules have evolved to be pheromones?

- A very wide range, covering every chemical dimension of structure, functional group, size & combination
- Limited only by the range of molecules organisms can produce or obtain.
- Potentially *any* molecule can become a pheromone

How pheromones evolve

- As a direct consequence of the organisation of olfaction
  + natural and sexual selection
- Starts with chemosensory receptor proteins: hugely varied & co-opted from different families

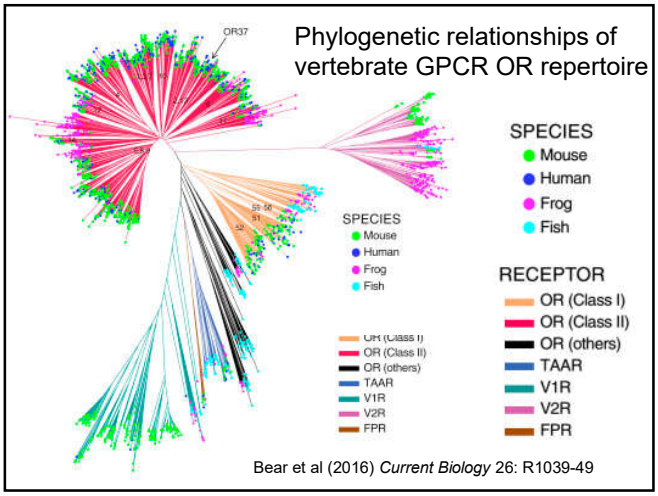
Chemoreceptors are co-opted from receptors with other functions

Birth and death of chemoreceptor genes

- repeated gene duplication and deletion or inactivation as pseudogenes
  = **birth-and-death evolution**
- vertebrate OR, VR1, & VR2 gene families represent extreme cases - dramatic gene duplication and diversification
- enormous expansion of OR genes in terrestrial vertebrates when came onto land

Nei et al (2008) *Nat Rev Genet* 9:951-963

Phylogenetic relationships of vertebrate GPCR OR repertoire

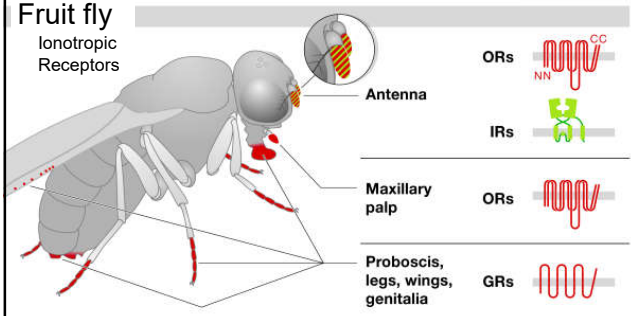


Bear et al (2016) *Current Biology* 26: R1039-49

Insect chemoreceptors are completely unrelated to vertebrate ones

Fruit fly

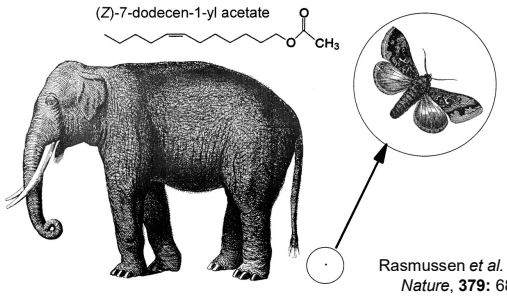
Ionotropic Receptors



Silbering & Benton (2010) *EMBO Rep*

140 spp of moths & Asian elephants share a ♀ pheromone molecule

but the ORs detecting it in male moths and elephants will be completely different



Wyatt

Rasmussen *et al.* 1996 *Nature*, **379:** 684

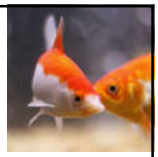
Two main routes for pheromone evolution:

1. **Sender precursors**
2. **Receiver sensory bias**

Bradbury & Vehrencamp (2011) *Animal communication.* Sinuaer.

Wyatt, TD (2014) *Pheromones and animal behavior:* 2 edn. CUP.

1. Sender precursors



linasmith

Some goldfish female pheromones are hormones.

Hormones leaking from mature females

→ any males smell sensitive to cue molecules get to female first

→ males selected for greater smell sensitivity & receptor specificity

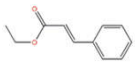
→ females selected to release more, *as a **signal*** (pheromone)

Stacey & Sorensen (2006)
Wyatt (2014)

2. Receiver sensory bias

e.g. Pre-existing female sensitivity to host plant odours

Oriental fruit moth *Grapholitha molesta*



A host plant odour ethyl-*trans*-cinnamate has become the male pheromone, released from 'hair pencils'

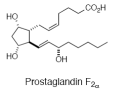
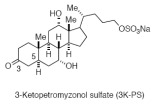
Females prefer males with the most cinnamate.

Sensilla type that detects the male pheromone is type associated with plant odour perception

Tom Baker

Löfstedt *et al* (1989) *Oikos*

Clues to evolution of pheromones

animal	original function	pheromone
goldfish	hormones	♀ sex  Prostaglandin $F_{2\alpha}$
lamprey	bile acid	♂ sex  3-Ketopetromyzonol sulfate (3K-PS)
ants	defence	alarm e.g. ***Formica rufa*** **formic acid** 

Wyatt

Wyatt (2014)

Most pheromones are perceived by combinatorial glomerular olfaction *not* gustation – why?

Why by combinatorial glomerular olfactory system?

- Combinatorial systems have wide range of receptors – more likely to detect new molecules
- Combinatorial processing of smell – mutations need not have dramatic effects (damping of circuits)
- Combinatorial systems have more flexibility and subtlety

Pheromone changes with speciation

Peptide pheromones – change a.a.

- **Newt *Cynops pyrrhogaster* decapeptide pheromone, sodefrin**
  Ser-Ile-Pro-Ser-Lys-Asp-Ala-Leu-Leu-Lys(OH)
- Incipient speciation: local population produces & responds to [Val^8] substituted sodefrin

Nakada et al (2007) *Peptides*

Newt *Cynops ensicauda*
(silefrin, 2 aa ▲)

Yamamoto et al (2000) *FEBS Lett*

Most sex pheromones seem to be multicomponent, NOT a single molecule

With speciation

- Species1: pheromone molecule A
- Sibling sp. 2: A & A-OH etc

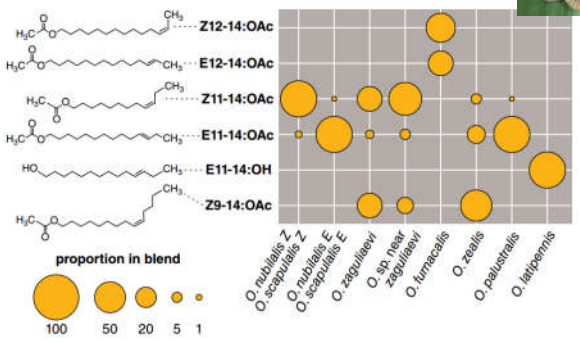
Leading to "variations on a chemical theme" in related species

Multicomponent pheromones

- many ways to vary – no. & position of double bonds, *E*/*Z*, functional groups, ratios of existing molecules
- Many, many examples in moths – *Ostrinia* spp, *Heliothis* spp

[likely that more multicomponent pheromones will be found in mammals & other vertebrates]

Ostrinia moth ♀ pheromones



Lassance *et al* (2013) *PNAS*

Sibling moth species: corn borers *Ostrinia*

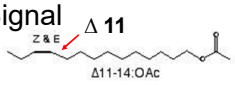
AsianCB diverged ~ 1 MYA

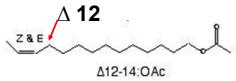
Roelofs et al (2002) PNAS

European Corn Borer (ECB)
O. nubilalis

Asian Corn Borer (ACB)
O. furnacalis

♀ Signal





♂ Receiver

One aa (SNP) in OR3 pheromone receptor switches ♂ ACB sensitivity

Leary *et al* (2012) *PNAS*

Slide after Löfstedt

Pheromone changes with speciation

but not if specificity is not selected for

e.g. Aphids

- Sex pheromones, species specific, multicomponent, much variation between species
- Alarm pheromones, NOT species specific (most use (*E*)-β-farnesene)

(predators will eat all species)

Operational definition of pheromone

Operational definition

- For many pheromones, not known how production and/or reception may have evolved. So, need *operational definition* of pheromone:
- e.g. rabbit mammary pheromone: "fully identified molecule(s), in all lactating mature females, which when synthesized elicit the same characteristic response in the conspecific receiver [pup] as the natural stimulus."

Pheromone: 'Operational definition'

1. Synthetic elicits same response as natural stimulus in bioassay
2. **All** compound(s) necessary & sufficient
3. Only this/these (NOT similar molecules that animal would encounter)
4. At realistic / natural concentrations
5. Credible pathway for evolution

Wyatt, TD (2017 [07 August]). Pheromones. *Current Biology,* 27, R1-R5.

1. Synthetic elicits same response

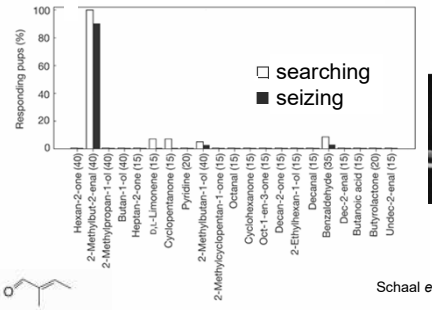
e.g. mouse

- Male preputial gland produces (*Z*)-5-tetradecen-1-ol, into urine.
- Bioassay: attracts female
- Androgen dependent secretion
- Found via receptor (heterologous expression of OR288 in *Xenopus* oocytes)
- Synthetic molecule equally attractive

Yoshikawa et al (2013) *Nature Chem Biol* 9: 160-162

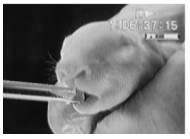
2. All compd(s) necessary & sufficient
3. Only this/these

Rabbit mammary pheromone



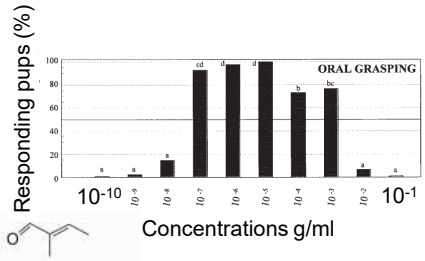
2-Methylbut-2-enal

Rabbit pup responses



Schaal *et al* (2003) *Nature* 424: 68-72

4. At natural concentrations



2-Methylbut-2-enal

Rabbit pup responses

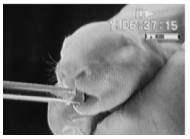
Coureaud *et al* (2004) *Chem Senses*

In moths, some pheromone receptors respond to non-pheromones if concentration is high enough

5. Credible path for evolving

Strongest possible selection pressure on newborn mammals to find nipple & suckle quickly.

Ditto on mammal mothers for young to suckle & survive.



Schaal & Al Aïn (2014) *Anim Behav* 97: 289-299

A. Operational definition for pheromones
   1. Synthetic elicits same response
   2. All compound(s) necessary & sufficient
   3. Only this/these molecules
   4. At realistic / natural concentrations
   5. Credible pathway for evolution

B. Robust bioassay is essential

C. Published data essential

human pheromones:

How a corporation hijacked the science

(see poster at this meeting)

Wyatt TD (2015) The search for human pheromones. *Proceedings Royal Society B* 282 [open access]

Summary

1. Pheromones inevitable consequence of olfactory organization – they will evolve
2. Any kind of molecule can become a pheromone (if 'useful')
3. Most pheromones are multicomponent
4. Operational definition of pheromone is needed as often evolution missing

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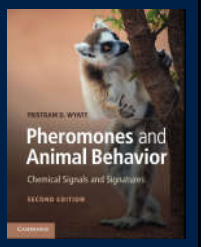
Thanks for listening
email me if you'd like the slides
tristram.wyatt@zoo.ox.ac.uk
@pheromoneEvo

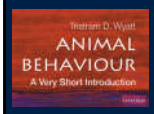


Wyatt TD (2015) The search for human pheromones. *Proc Roy Soc B* 282 (open access)

Wyatt TD (2017) Pheromones. *Curr Biol* 27:R1-R5 [out 07 August]

Winner, Royal Society of Biology Postgrad Textbook Award 2014

2 edn. fully updated & rewritten.



Wyatt TD (2017) *Animal Behaviour: A Very Short Introduction.* OUP