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# Key roles for chemical communication in animal biology

– insights from a comparative approach

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## Themes – chemical communication

- **a. Comparative approach**
  convergence across invertebrates and vertebrates

- **b. Biodiversity**
  variety of evolutionary solutions between and within taxa

## Chemical communication

**Key model systems in comparative physiology and biochemistry - for example:**

- Moth pheromones – signals, orientation
- Crustacea – signals, orientation
- Social insects – identity, gene expression
- *C. elegans* – dauer pheromone
- Locusts – gregarisation
- Goldfish – sex pheromones
- For pest / vector control? Moths, mosquitoes, lampreys, *Rhodnius,* barnacles

## Outline

1. What is a pheromone
2. Releaser & primer pheromones
3. Convergence in olfaction
4. Value of biodiversity in model systems
5. How pheromones evolve
6. Simple signals [= pheromones] vs. complex, variable odour signatures
7. Learning, memory and olfaction
8. Convergence in social breeding
9. Orientation to pheromone sources

## Outline

- **1. What is a pheromone**

## Pheromone

a chemical signal transmitted between members of the same species.

From 2 Greek words:

*pherein*, to transfer

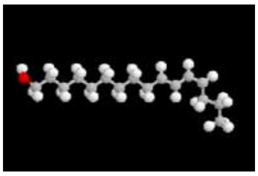
*hormon*, to excite.

Karlson & Lüscher 1959

## The first pheromone identified

Silk moth *Bombyx mori.* Female sex pheromone 'bombykol'

Butenandt *et al.* 1959



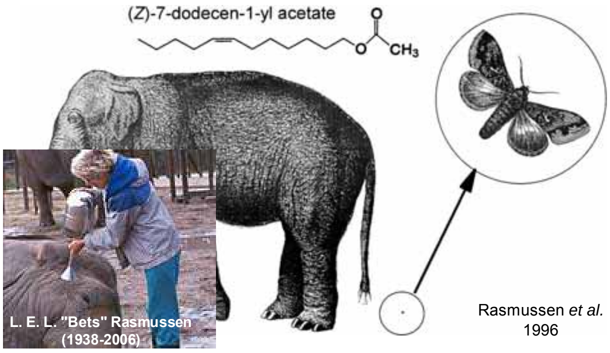
## Silk moth => early ideas about pheromones:

- "one unique compound per species"
- "kind of molecule (small, volatile)"
- "mammal ones won't be like this"
- "only long distance"
- "only affect behaviour"

**But the natural world turned out more interesting …**

## Moths & Asian elephants surprise:

**they share a pheromone**




Rasmussen *et al.* 1996

## Moths & elephants illustrate:

- **Pheromones used by (almost) all animals**
- **Unrelated species can share same pheromone**
- **Mammals use small molecule signals**
- **Pheromones can lead to physiological responses:**

  **An elephant Mae West might ask 'is that your trunk or are you pleased to see me?'**

## Why don't moths & elephants get confused

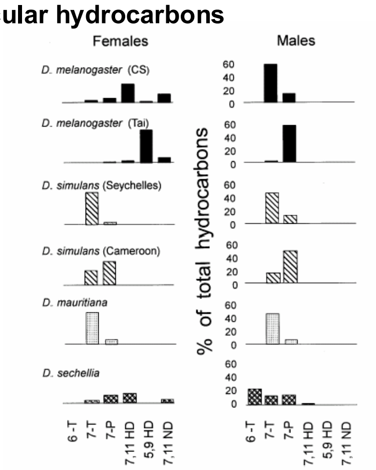
- Male elephant not attracted to moth ♀: tiny quantity of pheromone – picograms per hour from female moth
- Moth male not attracted to elephant ♀: (Z)-7-dodecen-1-yl acetate is just 1 of > 6 required chemicals of his species' pheromone blend.
  (each moth species has different combination)

## Simple blends & synergy?

- **Insects:**
  - bark beetles
  - moths – main basis of species specificity - defined ratio of 3 – 6 or so compounds for given species
  - *Drosophila* cuticular hydrocarbons – species specific blends (and regional variation)

## *Drosophila* cuticular hydrocarbons

**inter- and intra-species blend differences**



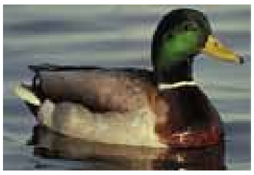

Cobb & Ferveur (1996)

## Simple blends & synergy cont.

- **Mammals**
  - mouse male aggression 2 cpds

3,4-dehydro-exo-brevicomin  2-*sec*-butyl-4,5-dihydrothiazole

## birds use pheromones too



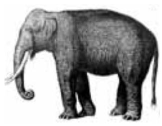
mallard duck
***Anas platyrhynchus***

**Uropygidial gland**

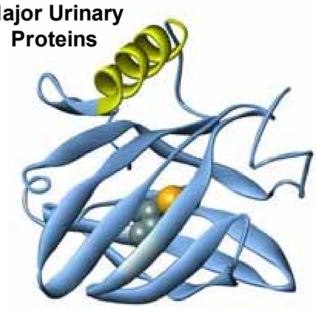
Balthazart & Schoffeniels 1979; Jacob *et al.* 1979

crested auklet
*Aethia cristatella*
Hagelin *et al* 2003
**cis-4-decenal + octanal**

## small molecule pheromones in terrestrial vertebrates often associated with proteins - lipocalins



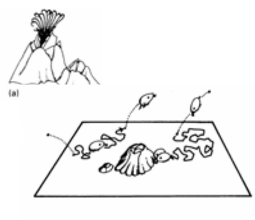
**Major Urinary Proteins**



**for signal activity, longevity and ? individuality**

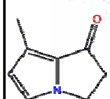
## Proteins (peptides) themselves as pheromones

magnificent tree frog
*Litoria splendida*
Wabnitz *et al* 1999
splendipherin **peptide pheromone**
GLVSSIGKALGGLLADVVKSKGQPA-OH
40 ng will attract female



barnacle larvae – intraspecific attachment signal - glycoproteins

## Communication distance – not necessarily far



Danaid male butterfly deposits pheromone on female's antennae

*Plethodon jordani*
**22 kDa & 7kDa glycoprotein pheromone transferred from his chin gland to nostrils (VNO) of female**

## Communication distance – bypassing the olfactory system

**Salamander *Desmognathus ochrophaeus***

directly 'injects' glycoprotein pheromone into female's capillary blood supply (Houck & Regan 1990)

**Male accessory gland proteins & hormones in semen: post-mating effects**

garter snakes

*Drosophila*

## Outline

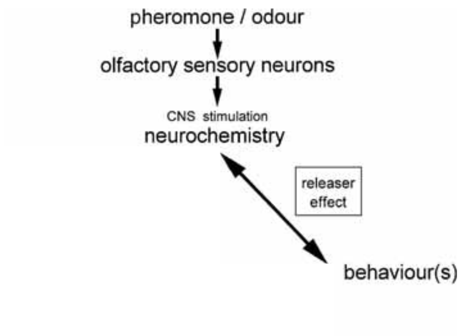
1. What is a pheromone
2. Releaser & primer pheromones

## Action of pheromones

- **Releaser**
  - **- on behaviour**
  - **- directly via sensory system to CNS - motor system**
- **Primer**
  - **- on behaviour and/or physiology**
  - **- indirectly via sensory system to CNS to endocrine system**

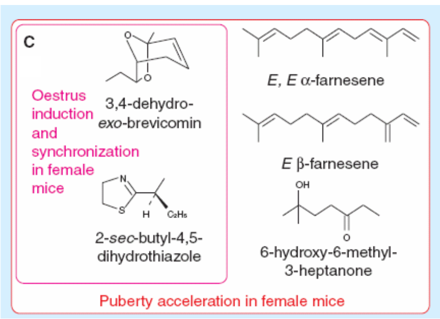
Wilson & Bossert 1963

## Primer vs. releaser?




Wyatt 2003 after Wilson & Bossert 1963 and Sachs 1999

## e.g. primer pheromones in mice

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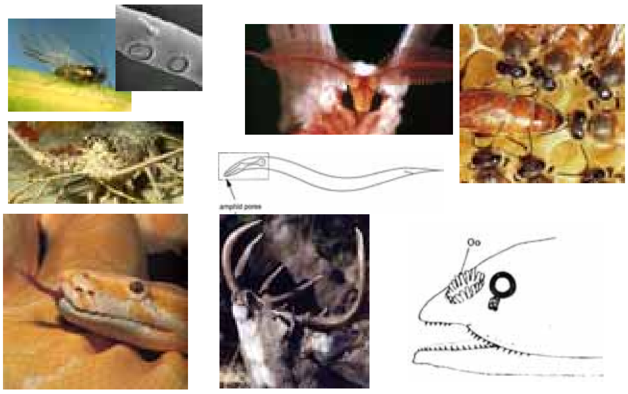
# 'Noses' to catch molecules



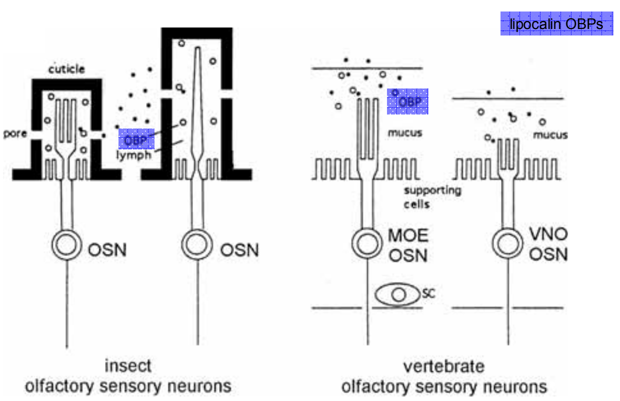
# Invertebrate & vertebrate olfaction:

**Superficial differences.**

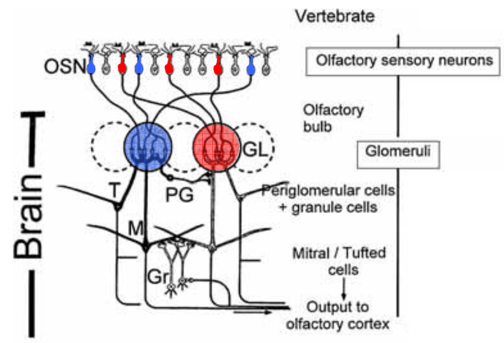
**Fundamental similarities:**

- **Olfactory receptor proteins**
- **Olfactory sensory neurons**
- **Functional (glomerular) organisation of sensory neurons & olfactory processing**

Insect | Vertebrate

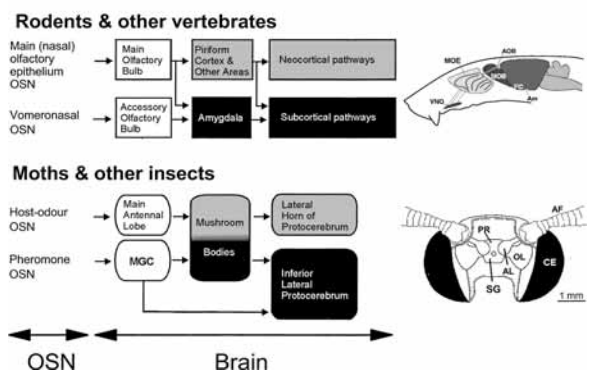



after Hildebrand & Shepherd (1997)




Wyatt 2003 after Mori *et al.* (1999)

# Olfactory Pathways in the Brain




Christensen & White 2000

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4. Value of biodiversity in model systems

## Importance of diversity and comparative approaches

Model systems for pheromone research - in insects (male moths) & mammals (mice) …

- essential
- powerful
- best if combined with diverse models

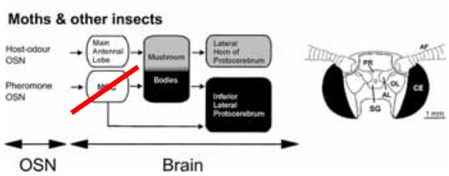
## Pheromone model system 1
## moth pheromone system
**highly sensitive sensory system in males**

- **narrowly tuned OR proteins**
- **specialised OSN**
- **specialised glomeruli – MGC the Macro Glomerular Complex**

**=> *universal* model of insect pheromone sensory systems?**

**Possibly not …**

### → most insects do NOT have MGC




For example:

• honey bee workers – alarm pheromone (processed by glomeruli also responding to non-pherom (Galizia et al 1999)

• ant workers – responding to alarm pheromone, processed in specific group of ~5 glomeruli
Yagamata et al 2006

## Pheromone model system 2
## mouse pheromone system

**=> Idea among some molecular biologists was:**

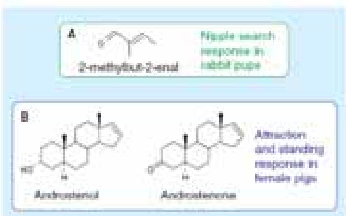
**a) "VNO only detects pheromones"**

**b) vice versa – "if there are pheromones then they will act through the VNO"**

But the evidence from a comparative approach across vertebrates indicates overlapping roles of vomeronasal (VNS) & main olfactory systems (MOS)

## Evidence from diversity of vertebrates

- **Yes, VNO mediates responses to *some* pheromone signals – mice, snakes, hamsters**
- **VNO detects food odours e.g. snakes**
- **Plus, interactions VNO & MOS systems (hamsters)**
- **Also some pheromone signals via main olfactory system (MOS), not the VNO**



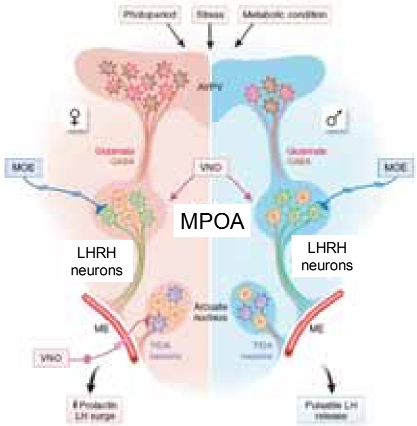

## Views changed with 3 neat papers on mice at end of 2005

**Yoon et al (2005) *Cell* – tracing afferent pathways to LHRN neurons in hypothalamus = from main olfactory epithelium NOT VNO**

**Boehm et al (2005) *Cell* – inputs to LHRN from both olfactory and pheromone relays – feedback loops**

**Mandiyan et al (2005) *Nat Neurosci* – male aggression and mating needs functioning main olfactory epithelium, but also inputs from VNO.**

## rodent hypothalamus integration




Keverne 2005

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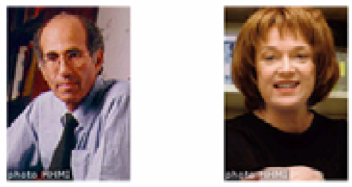
## How pheromones evolve

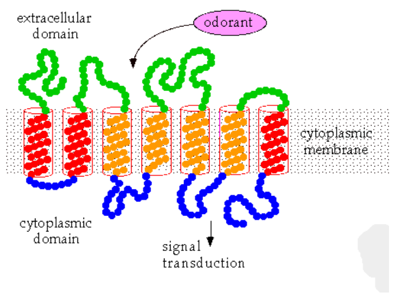
- **As a direct consequence of the organisation of olfaction and natural selection**
- **Starts with odour receptor proteins – the clue comes from their variety**

**The Nobel Prize in Physiology or Medicine 2004**

“for their discoveries of odorant receptors and the organisation of the olfactory system”




## Olfactory receptor (OR) proteins



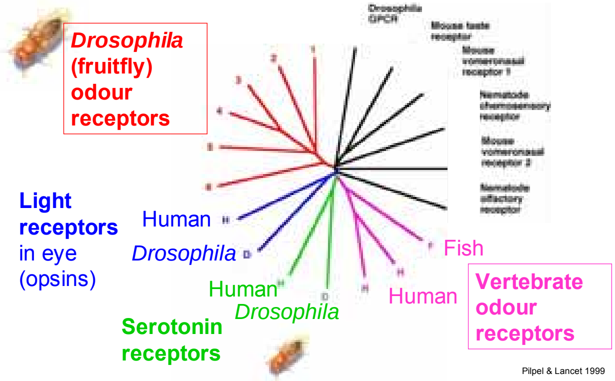

seven-transmembrane domain G-protein coupled proteins

Lancet

With ~1000 different types of receptor proteins (vertebrates) [~100 (insects)]:

- Each receptor protein sensitive to a different shape and other characteristics of odorant molecules, but ~**broadly tuned**
- System is pre-adapted (ready) for any new chemical in environment
- Any chemical is likely to stimulate ***some*** receptors

## Independent evolution of odour receptor proteins (GPCRs)

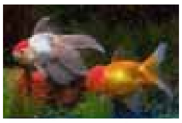


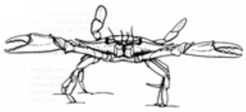

Pilpel & Lancet 1999

## How pheromones evolve 1

### Evolution of response.

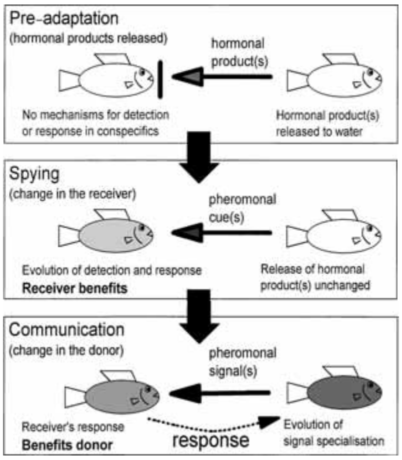
Odours with selective significance (e.g. odour released by mature females)→ males will be selected to respond to the odour(s).





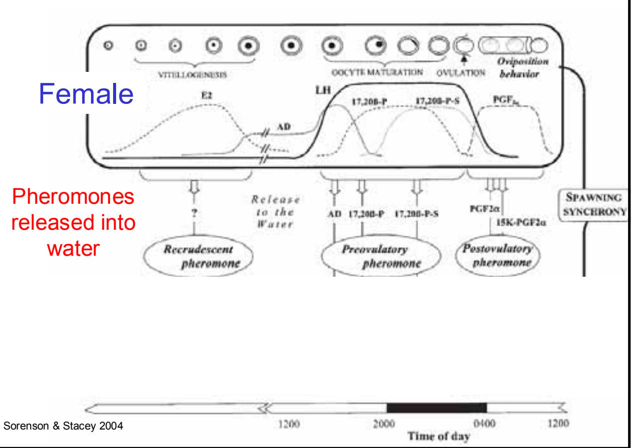
Goldfish & crabs – female pheromones similar to hormones.

Males started out with a few receptors for hormones …




Sorensen & Stacey 1999

## primer & releaser pheromones in goldfish




Sorenson & Stacey 2004

How pheromones evolve 2 – another effect

## Sensory drive

**~ 'pre-existing bias'**
**'sensory exploitation'**

Exploits pre-existing sensitivity to stimuli

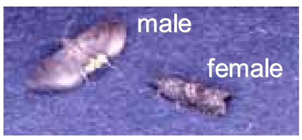
How pheromones evolve 2

## Sensory drive

**Oriental Fruit Moth** *Grapholitha molesta*
Pre-existing female sensitivity to plant odours for host-plant finding – for egg laying

When males find a female they wing fan and release a male pheromone

The ***male*** pheromone is ethyl-*trans*-cinnamate (derived from plants).



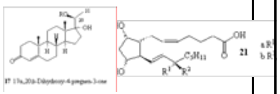
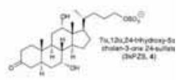

Exploiting pre-existing female sensitivity?

Löfstedt *et al* 1989

## Which compounds are pheromones?

- **Great variety of compounds used as pheromones**
- **Why**
  - organisms use the compounds available
  - organisms have a shared biochemistry
  - different small molecules limited in number

## Clues to evolution of pheromones

| animal | original function | pheromone |
|---|---|---|
| goldfish | hormones | Sex e.g.  |
| lamprey | bile | sex  |
| ants | defence | Alarm  e.g. *Formica rufa* formic acid |

## Pheromones – selection to match message

- Small volatile molecules for short lived messages e.g. ant alarm pheromones

- Large involatile molecules for long lasting messages e.g. hyena territorial marker pheromones

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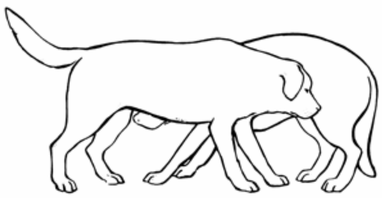
**Simple (anonymous) signals [= pheromones]**

**vs.**

**Complex variable odour signatures**



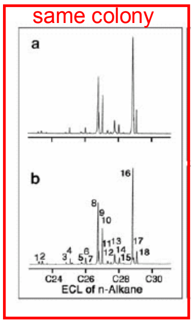
- **Species**
- **Sex (m/f)**
- **Maturity**
- **Oestrus**
- **Individual**

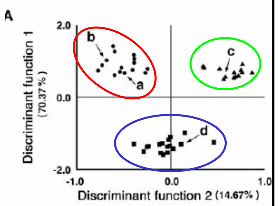
- **Species**
- **Age**
- **Sex**
- **Ovarian stage**
- **Caste**
- **Colony**

- **Anonymous [category information]**
  - Uniform throughout a group or level (eg species, worker, queen, male, female) – identifies as member of that category but not distinguish from other members
- **Variable**
  - Vary, identifier signaller as an individual or member of particular subgroup (clan, colony)

after Hölldobler & Carlin (1987)

Gas chromatography of cuticular hydrocarbons from ants from same and different colonies

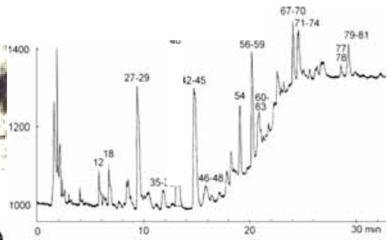



*Camponotus japonicus*

Ozaki et al 2005, *Science* (and Symposium 17, tomorrow)

**e.g. variable** signal(s) &
**anonymous** overlaid



SPME-GC trace of cuticular hydrocarbons

'queenless' ant, *Dinoponera quadriceps*

Peeters *et al*

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## Societies organised by smell

**Recognition –
who's a member**

**all involve learning**

## Pheromone-receiver systems

| | Pheromone or odour | Receiver system / perception |
|---|---|---|
| Anonymous | | |
| Variable | | |

## Kin recognition cues

- **Kin recognition cues may be any aspect of the phenotype that reliably signifies kinship**
- **Chemical cues are widely used**
  - Ubiquity of receptors – from earliest life forms
  - Enormous variety of compounds – unlimited no. of combinations
  - us. low metabolic cost (not us. produced for recognition)

Sherman *et al.* (1997)

## Imprinting

**Konrad Lorenz & graylag goslings**

**'Biologically relevant learning during a sensitive period defined by particular developmental stage or physiological state.'**

Hudson 1993, *Curr Op Neurobiol 3*:548-552

[*not* genomic imprinting ]

- **Two main periods of olfactory imprinting:**

**When young**

- learn species & colony – ants, bees, wasps
- learn parents, siblings*
  e.g. mice, ground squirrels, humans
  *affects later adult mate choice

**When adult**

- learn mates – crickets (Coolidge effect), mice (Bruce effect)
- learn offspring – sheep, mice, humans

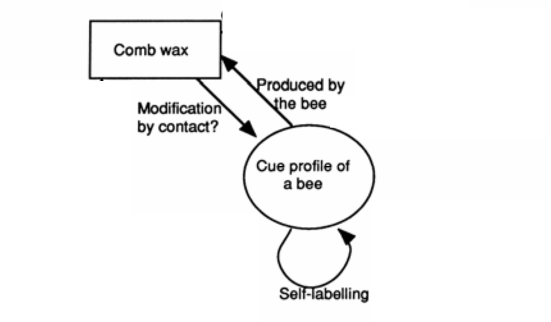
## Honeybees

Guard bees

Guard bee fighting intruder

M. Breed

## Honey bees – comb wax as intermediary for recognition





Breed 1998, *Bioscience 48*

## Odour signature passport for entry to colony ➡ cheating

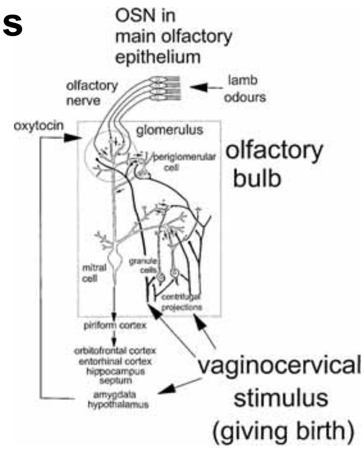
**Camouflage by smell:** beetle stealing food from ants

## Neural basis of learning

**Olfactory imprinting in adults**
**some model systems include:**

- **Mice – Bruce effect**
  - Critical period 0-4 h after mating, long-term memory formed by female for male's individual odour [in AOB, sensed by VNO]
- **Sheep – maternal recognition**
  - Long lasting bond between ewe and lamb established 0-2 h after birth [in Main olfactory bulb, sensed by main olfactory epithelium]

## Sheep & lambs




Kendrick *et al.* (1997).

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8. Convergence in social breeding animals

## Societies organised by smell

**Who reproduces**

**- queens & workers**

**- α-female & subordinates**

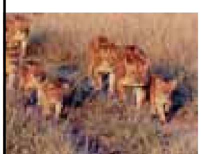
## Who reproduces?

Eusociality continuum

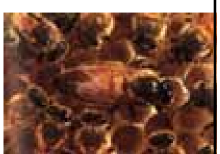
index of female reproductive skew

0 — **Plural breeding:** all females in group reproduce equally

1 — **Singular breeding:** only one female in group reproduces



Lion



Honey bee

## Queen + worker honey bees


Retinue of messengers surround her

## Honey bee queen pheromone QMP

- QMP constantly produced by the queen.
- If QMP present, workers do not lay eggs.
- Workers start to respond within 30 min of queen's absence – rearing new queen, laying their own eggs
- BUT is this – **control or cooperative signal?**
- Honest cooperative signal by queen – "I'm alive & well and laying eggs"
- If queen is signalling, workers do better supporting her than laying their own eggs (kin selection).

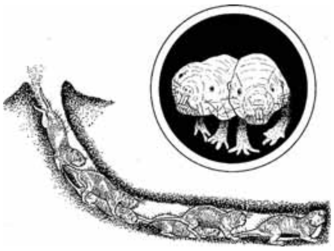
Keller & Nonacs 1993

## Mammals
### Common marmoset
### *Callithrix jacchus*
(South American primate)

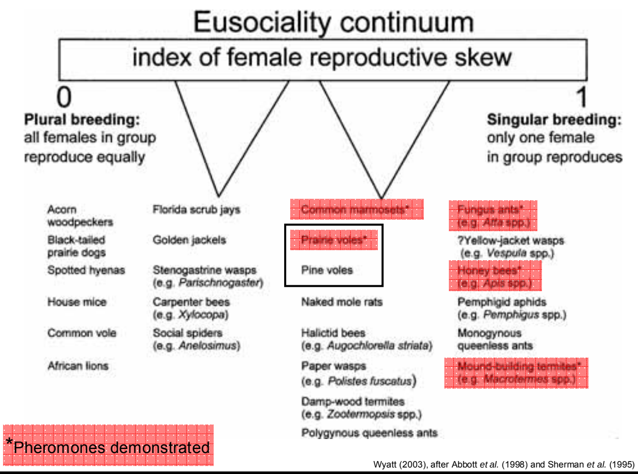
- **Only 1 female reproduces**
- **Her daughters = 'helpers at the nest'**
- **Suppression daughter reproduction by combination of visual, behavioural, and smell**
- **Suppression maintained by smell alone**
- **Dominance (or is it signal?)**

## naked mole-rats
*Heterocephalus glaber*

- **The most eusocial mammal**
- **Queen + colony of ≤ 300 non-breeding workers**
- **But, suppression of worker reproduction NOT by pheromone**
- **Physical 'shoving' by queen**



Faulkes & Abbott 1993,
Bennett *et al.* 1999




Wyatt (2003), after Abbott *et al.* (1998) and Sherman *et al.* (1995)

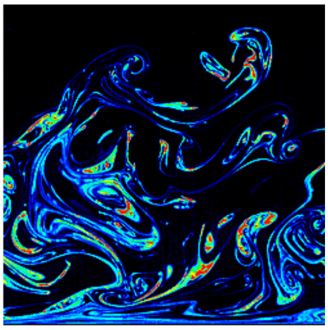
## Contrasts in close relatives

| **Uses smell in 'dominance'** | **Does NOT use smell in 'dominance'** |
| --- | --- |
| Common marmoset *Callithrix jacchus* | Golden lion tamarin *Leontopithecus rosalia* |

## Outline

1. What is a pheromone
2. Releaser & primer pheromones
3. Convergence in olfaction
4. Value of biodiversity in model systems
5. How pheromones evolve
6. Simple signals [= pheromones] vs. complex, variable odour signatures
7. Learning, memory and olfaction
8. Convergence in social breeding
9. Orientation to pheromone sources

# Finding odour sources is challenging



**odour filaments arrive in a turbulent flow of air or water**

Odour plumes visualised with lasers

red = highest concentration

Crimaldi lab http://bechtel.colorado.edu/~crimaldi/

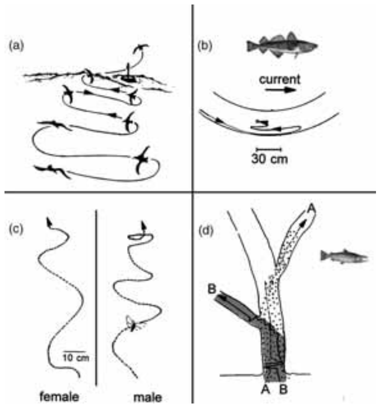
# Zigzag movement up current

albatross

cod

moths

salmon




Arbas *et al.* 1993

# Zigzag movement up current

Convergence and/or similar underlying mechanisms?

Or – superficial similarity and widely different mechanisms?

# Summary

1. **Chemical communication is universal**
2. **Evolves from existing molecules & sensory systems**
3. **Convergence in olfaction**
4. **Simple signals [= pheromones] vs. complex variable odour signatures**
5. **Learning, memory and olfaction**
6. **Social controls – pheromones in some spp.**
7. **Orientation behaviour**
   **– underlying mechanisms?**

Thanks for listening

www.online.ox.ac.uk/pheromones

Do get in contact

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