

# Molecular identification

Species, individual, sex



# Identification of individuals

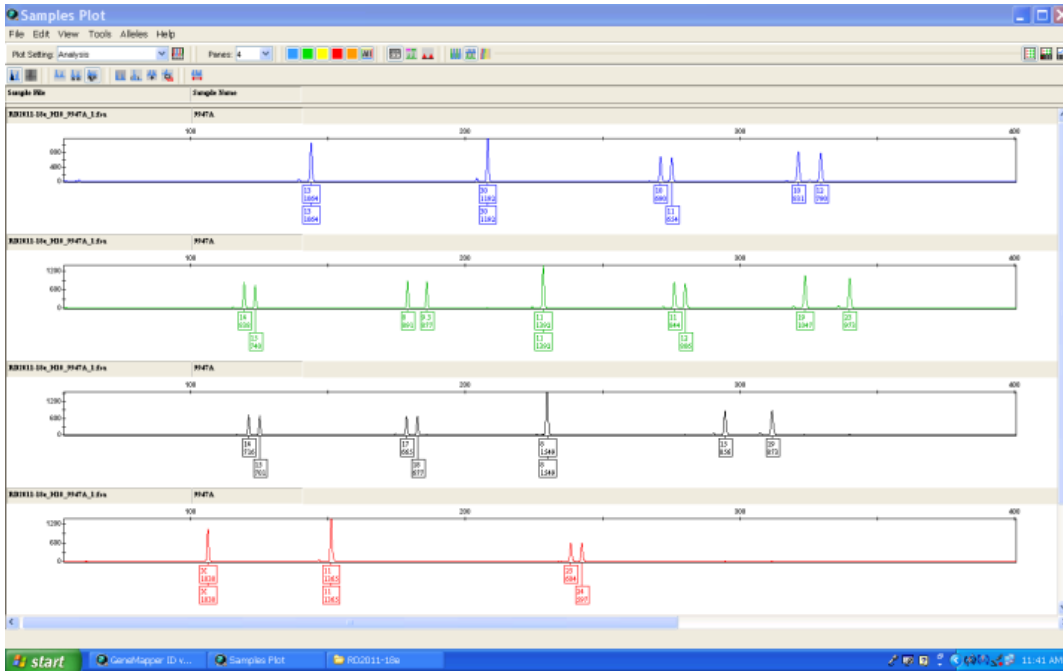
DNA fingerprinting  
(DNA profiling)

# Identification of individuals – why?

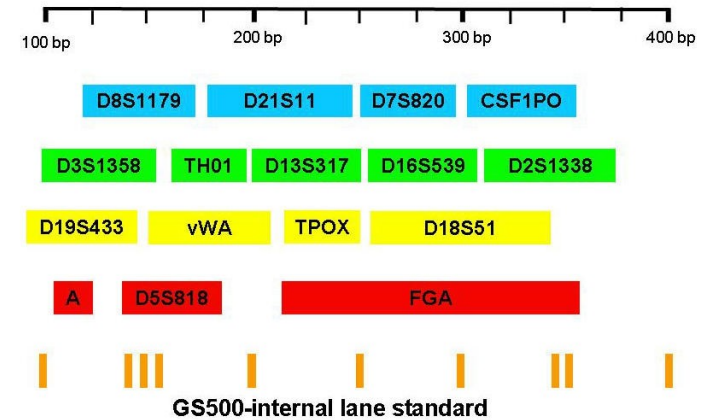
- if we do not see the individual
- non-invasive genetics – elusive animals, samples from faeces, urines, hairs – can be joined with individual variation of their diet
- forensic genetics – identification of DNA in animal products, poachers, etc.
- species conservation – e.g. in falconary (confirmation of parentage)



# Individual human identification



AmpFSTR® Identifiler™

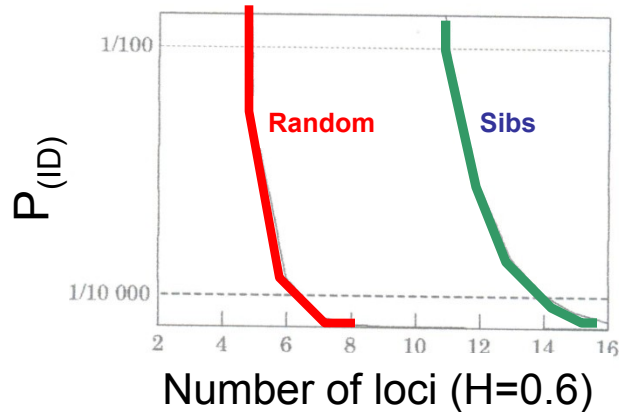


16 loci = reliable individual identification  
(Euro-American population)

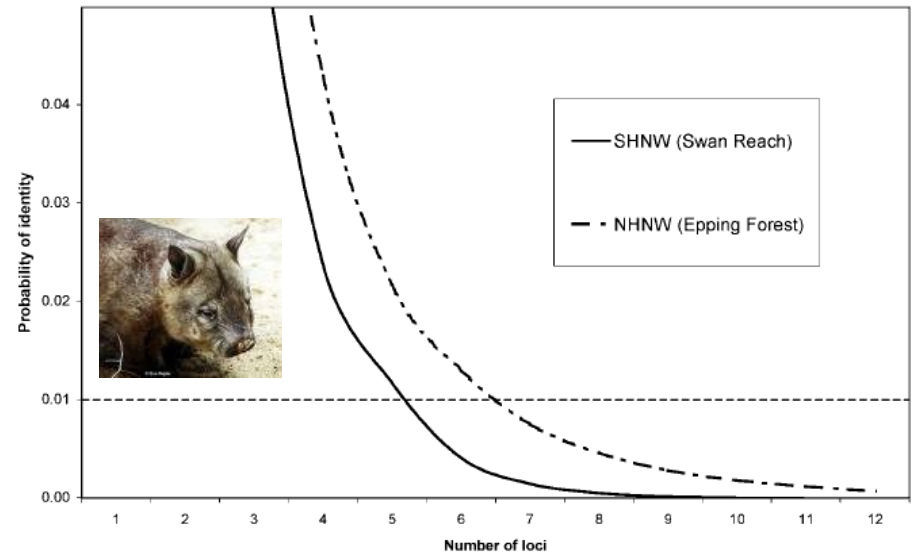


# Identification of individuals depends on level of polymorphism

- multilocus microsatellite fingerprinting – power estimated as „**probability of identity**“ ( $P_{(ID)}$ ) (Waits et al. 2001) – e.g. GenAlex program



- pilot studies with tissue samples are required to identify  $P_{(ID)}$  in a population studied by e.g. non-invasive methods





# Brown bears in Pyrenees

*Taberlet et al. 1997*



- Faeces and hairs
- 24 microsatellites
- 4 males and 1 female with unique multilocus genotypes (more than according footprints and photos)
- Multiple-tube approach

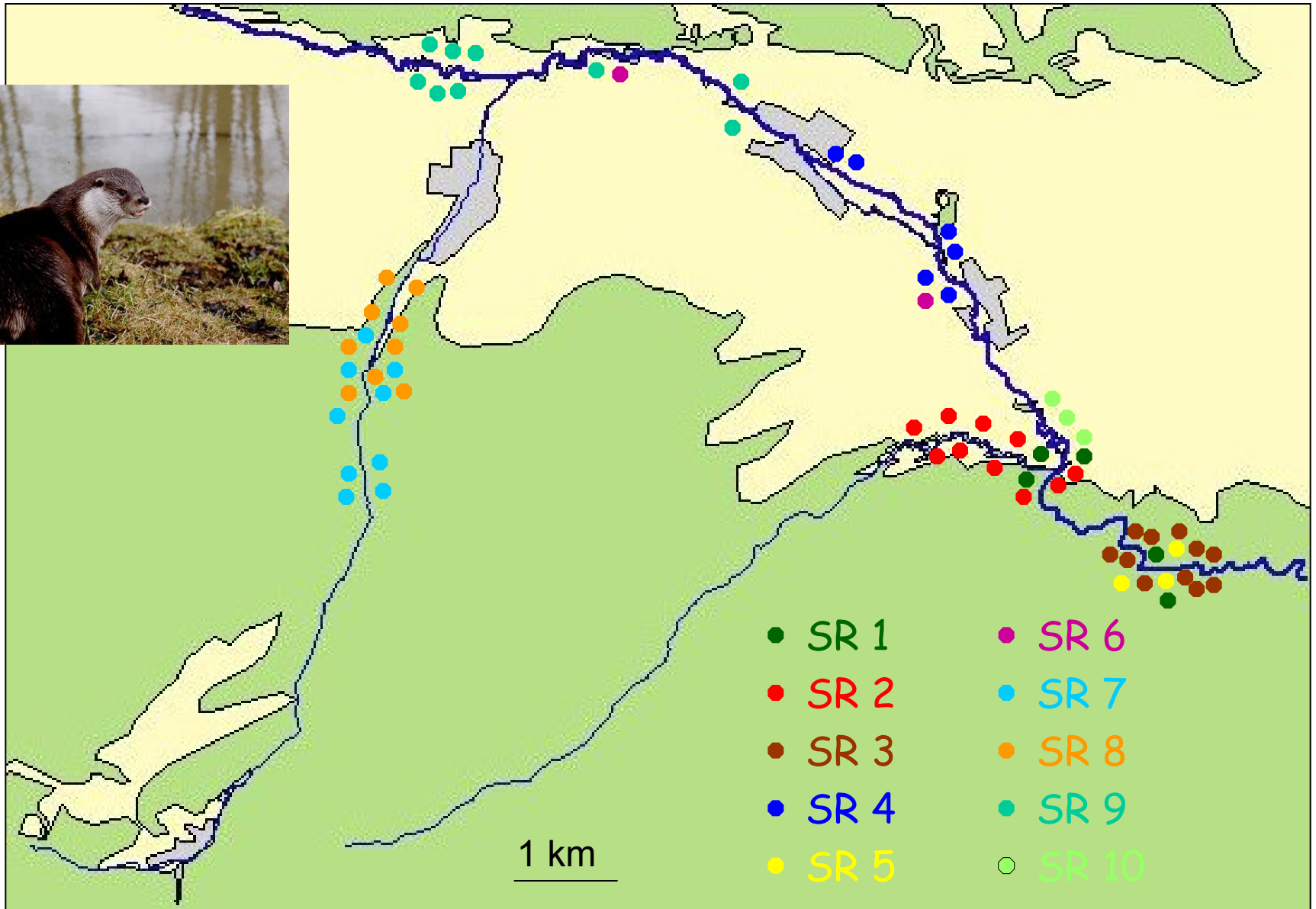
# Spatial activity of otters

- **P. Hájková – PhD thesis (2008)**



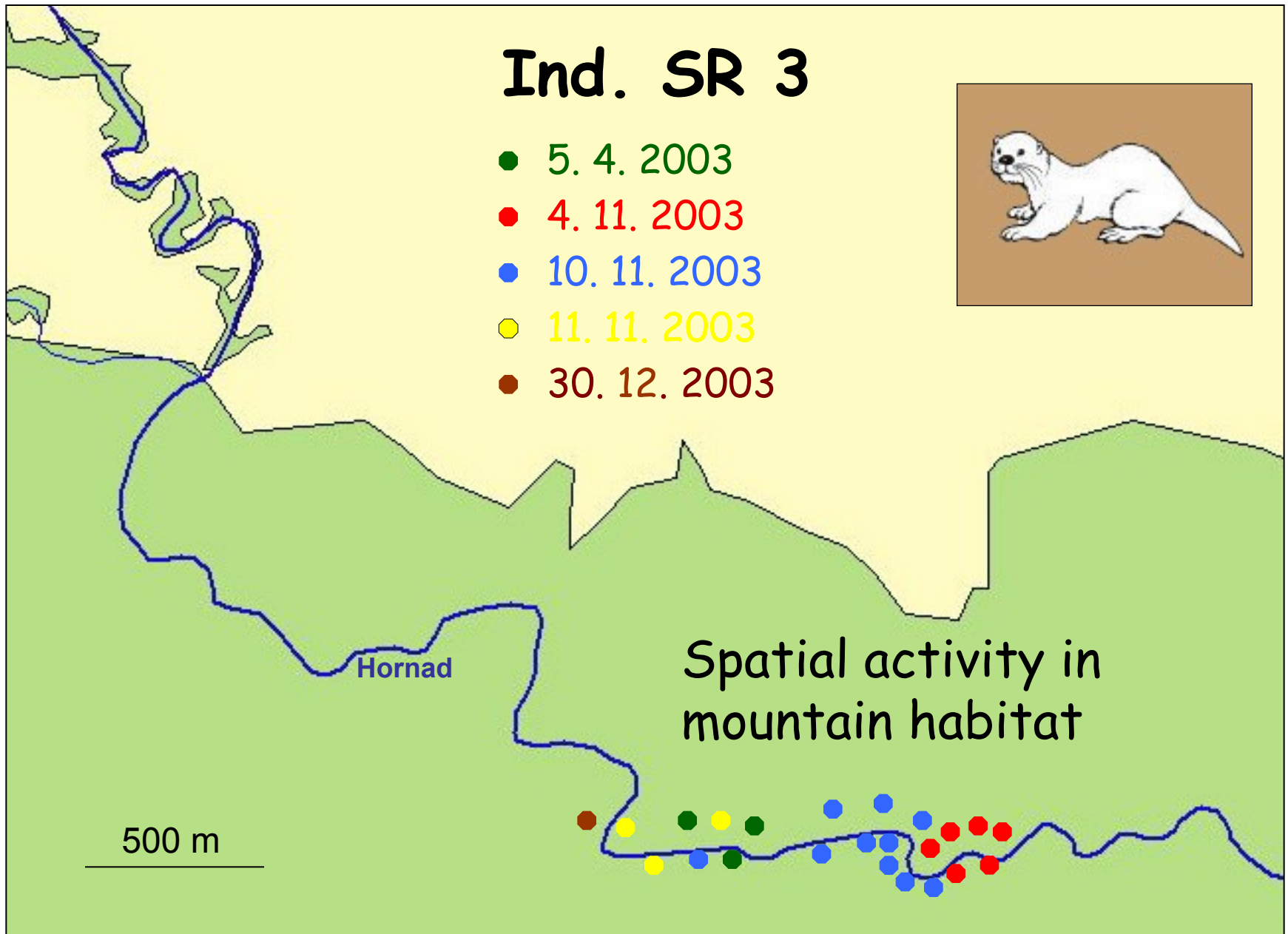


# Identified individuals - Hornád, NP Slovenský Raj



# Ind. SR 3

- 5. 4. 2003
- 4. 11. 2003
- 10. 11. 2003
- 11. 11. 2003
- 30. 12. 2003



# Human forensic genetics

- **Pozůstatky vojáků z války** Vietnam a Korea

Identifikace na základě mtDNA příbuzných osob  
(Ize jen někdy)

V současnosti: vzorek DNA (krve) při odvodu, jiné markery

Armed Forces Repository of Specimen Samples for the Identification of Remains



- **Soudní pře**  
Clinton-Lewinská  
Pozůstatky ruského cara Nikolaje II



- **Kriminalistika**
- **Oběti tragických událostí**



# Clones

## Bambus *Sasa senanensis*

- Suyama et al. 2000
- Sampling at 10 hectares, AFLP genotyping
- 22 genetic clones
- 1 clone on the area with 300 m diameter



# Famous clonal invertebrates

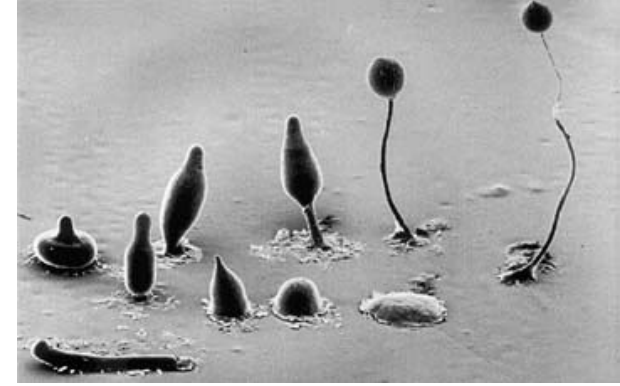
- Rotifera – Bdelloidea
- Ostracoda  
(*Darwinula*)
- Parthenogenetic clones of old age  
(millions of years)



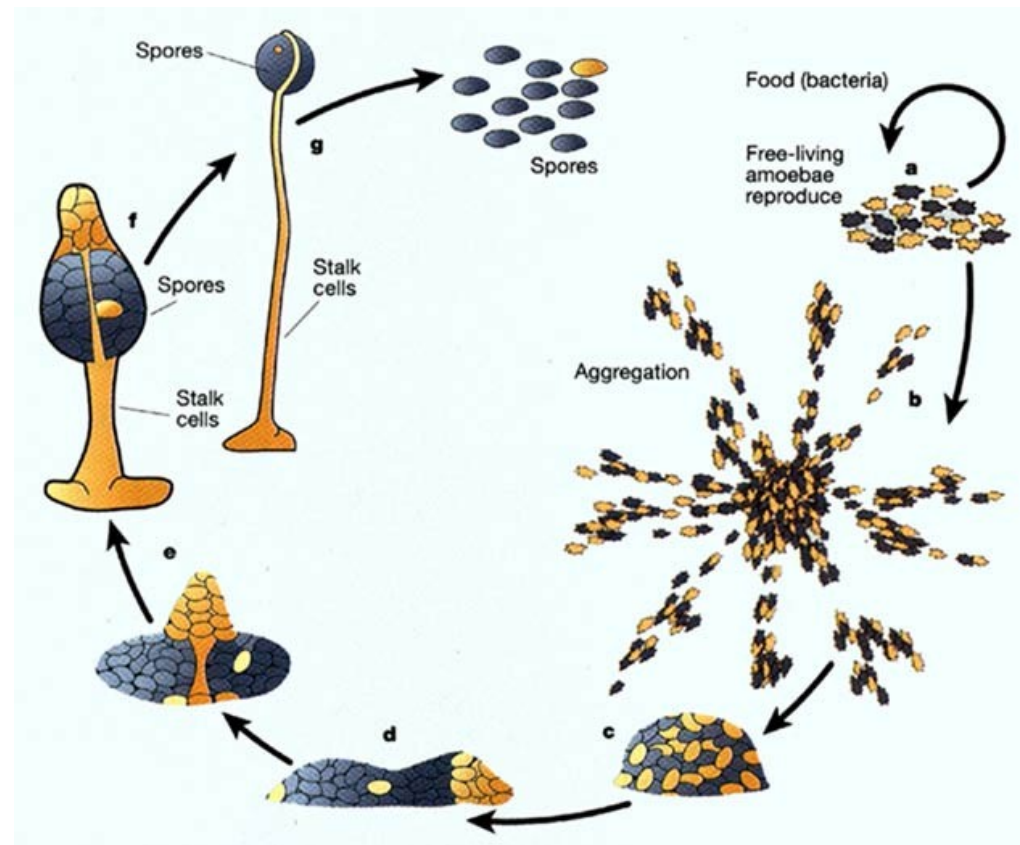
*Darwinula stevensoni*



# Genetic chimeras



- organisms composed from cells with different genotypes
- *Dictyostelium discoideum* chimerism is a regular part of life cycle



# Genetic chimeras

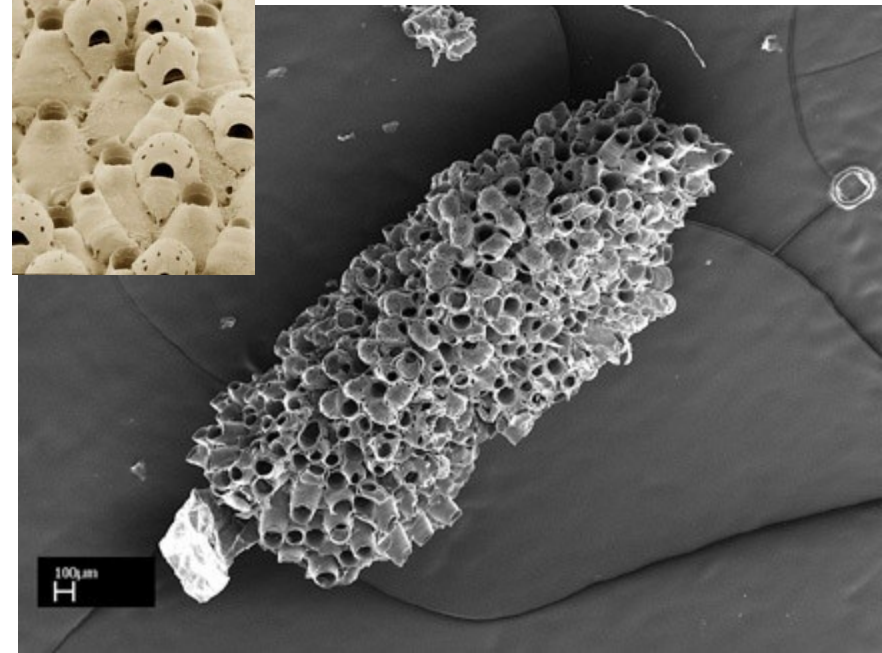
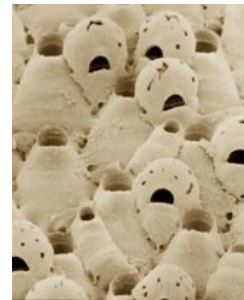
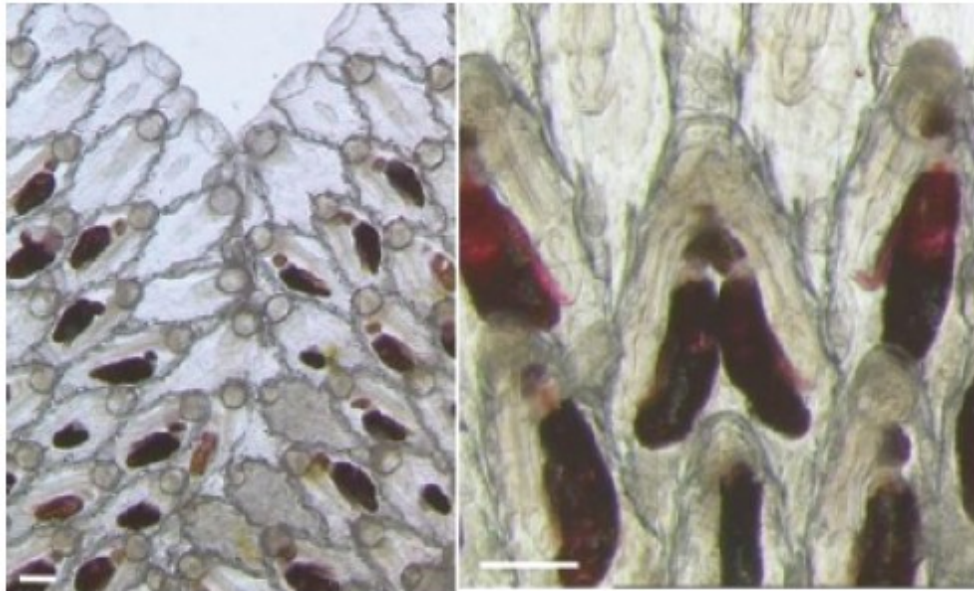
- *Ficus* – already roots from several individuals
- ascidians *Botryllus schlosseri* chimeric colonies from related individuals
- *Diplosoma listerianum* (even unrelated)



# *Celleporella hyalina* (Bryozoa)

Hughes et al. 2004

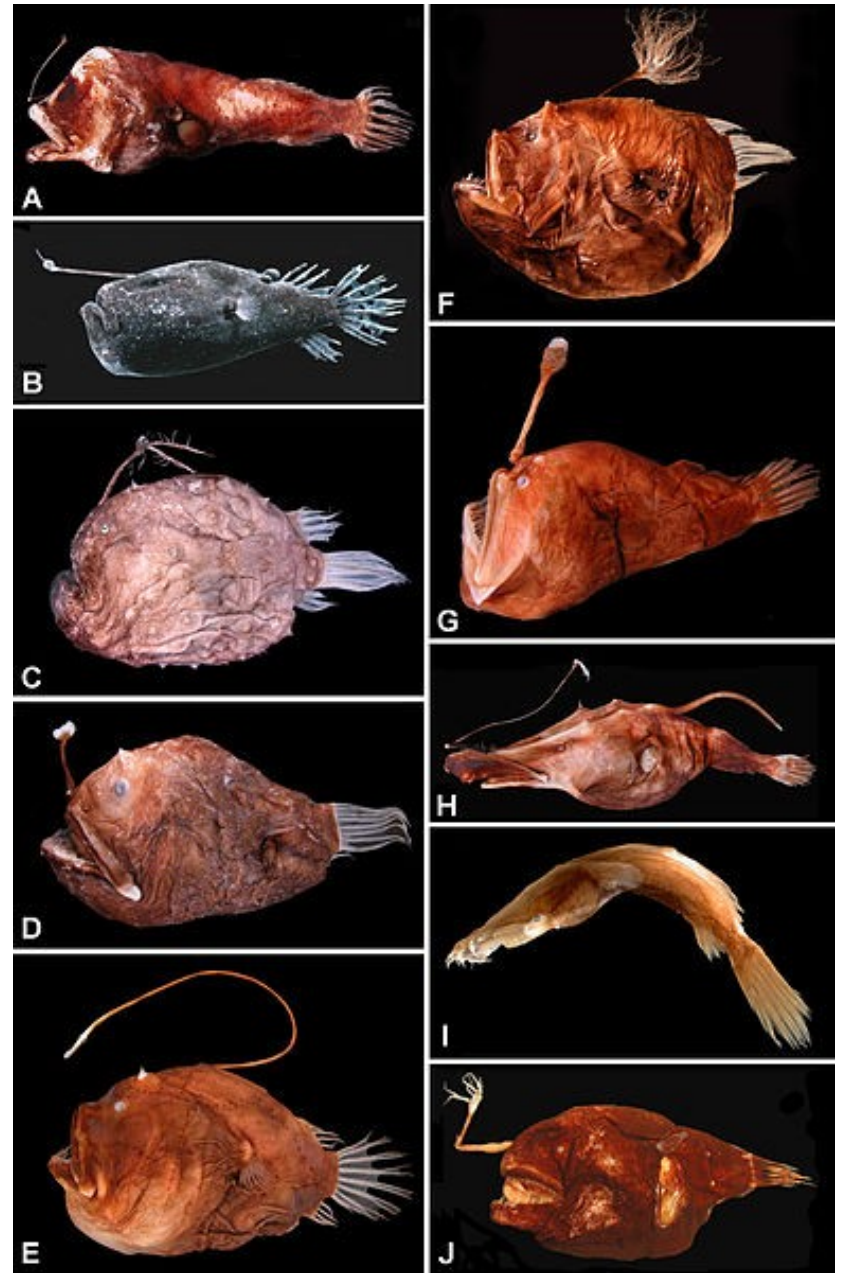
- Probability of fusion correlates with relatedness
- „Histocompatibility“
- Better recognition in more developed ontogenetic phases  
→ maturation of immunocompetence
- Special proteins (spongicans...)





# Ceratioid anglerfish

- miniature male searches females
- skin of a female produces hydrolytic enzymes and a male permanently joins a female
- -> hermaphroditic chimera



# Genetic chimeras – „microchimerism“



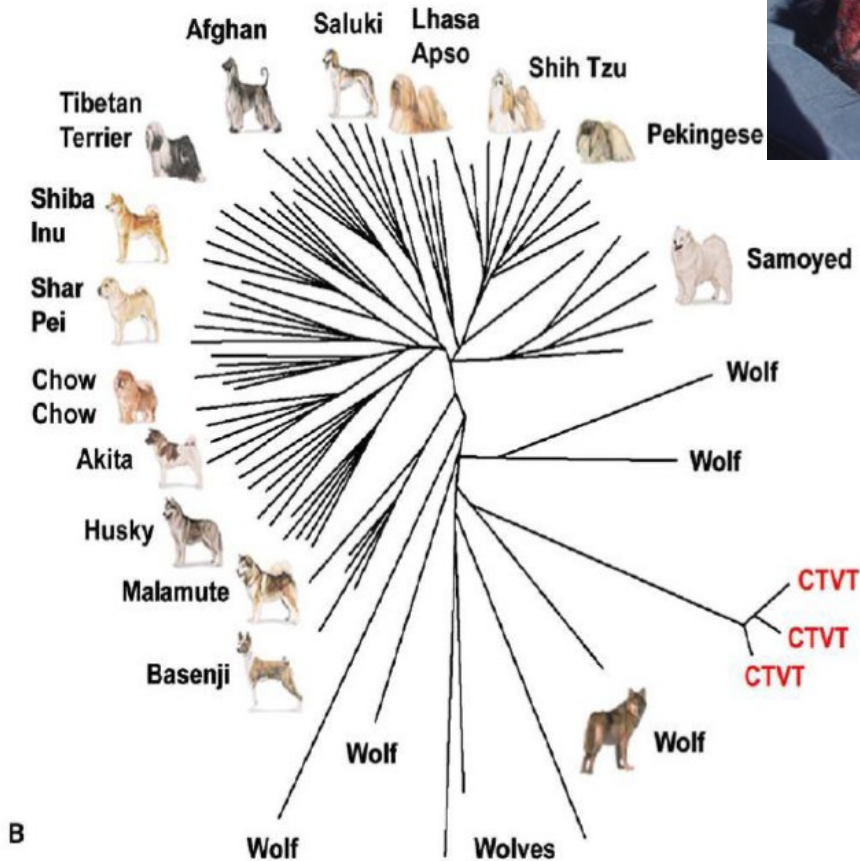
- common marmoset *Callithrix jacchus* (probably also genus *Saguinus*)
- dizygotic twins
- DNA fingerprinting from blood - hematopoietic chimeras
- reciprocal exchange of bone marrow cells during embryonic development
- probably it concerns only blood (because the results from non-invasive genotyping, i.e. from hairs and faeces, produced only a single genotype)
- embryonic erythroblasts can penetrate placenta also in humans (genetic sex identification of embryo from peripheral blood of mother)

# Canine transmissible venereal tumor (CTVT)



## Devil facial tumour disease

- parasitic cancer
- „single cell parasitic wolf“



# Famous „human chimeras“



## Foekje Dillema

46XX/46XY woman

- Dutch athlete, champion in 100 and 200 m run
- rejected sex identification test
- mozaic (chiméra) found postmortem (in 2007)

## Lydia Kay Fairchild The Twin Inside Me - Chimera

### The Twin DNA

Lydia Fairchild was twenty one when she had her first baby. Despite being separated from the baby's father, Jamie Townsend, she and Jamie had a second baby a year later. Another year on and she became pregnant for the third time after which she and Jamie split up again. With no steady work and unable to support herself and the children she applied for state benefit.

Her world was about to be turned upside down.

The State Prosecutor's Office required DNA tests from Jamie to prove that he was the father of the children and, as a matter of course, Lydia was also tested.

In December 2002 she received a phone call from the prosecutor's office asking her to come in for the results. This was unusual and it soon became apparent why. The results confirmed that Jamie was the father but they also revealed that Lydia was **not** the mother. A normal DNA test proving a mother-child link would show a 50% match between their DNA patterns. Yet Lydia's DNA showed no match at all.



Lydia Fairchild

- maternity not confirmed genetically
- mother was a genetic chimera

# Identification of sex

DNA sexing

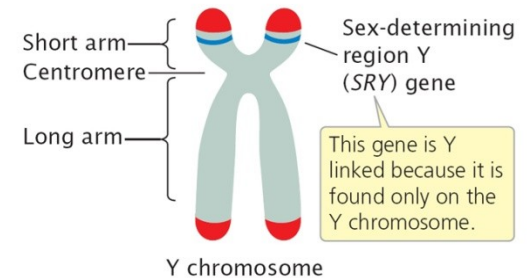


# Why?

- 1) species without sex dimorphism (birds, but also many mammals)
- 2) embryos, larvae
- 3) non-invasive methods

# Genetic sex identification

- genetic sex determination
- birds ( ♂ =ZZ, ♀ =ZW)
- mammals ( ♂ =XY, ♀ =XX)
- DNA amplification of W/Y chromosome
- W, Y – small chromosomes



# Sex identification – birds

*Griffith et al. 1998*

- *CHD1W* and *CHD1Z*, genes at sex chromosomes (chromobox-helicase-DNA-binding gene (CHD) – Griffiths & Tiwari 1995)
- Primers amplifying introns of both genes
- Introns differ by their length
- Up to three primer combinations
- Problematic species, e.g. Struthioniformes





# *Manorina melanocephala*

(Meliphagidae) *Arnold et al. 2001*

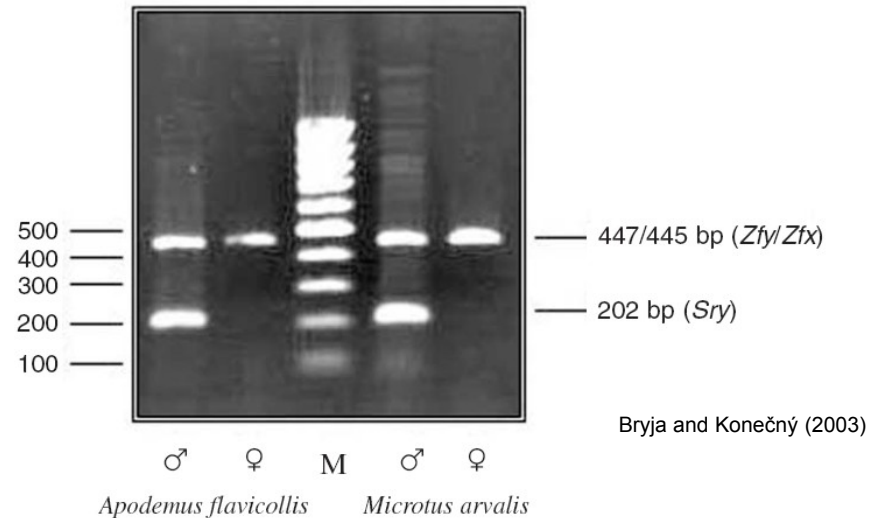
- Sons = „helpers“
- In adults  
2,31 males vs. 1 female
- Offspring in nests  
sex ratio 1:1 (57:57)
- Males are hatching first  
(in 17 out of 18 nests)  
they are bigger and heavier when leaving  
the nest



medosavka hlučná

# Sex identification - mammals

- Amplification of a gene at Y-chromosome (*Sry*)  
(in duplex PCR with X-linked or autosomal fragment)



- Microtus cabrerae*  
Sry at Chr X  
*Ellobius*, *Tokudaia*  
Sry completely missing
- Nannomys*  
Large variability

*M. cabrerae*



*Nannomys*

*Ellobius*



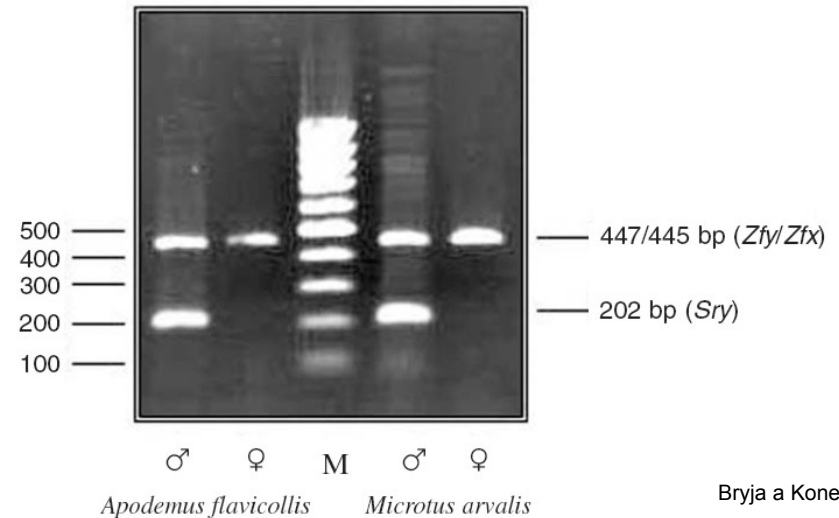
*Tokudaia osimensis*



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# Sex identification - mammals

- Amplification of a gene at Y-chromosome (*Sry*)  
(in duplex PCR with X-linked or autosomal fragment)



- Faecal analyses: species-specific primers are required to avoid a cross amplification with species in the diet



**X**



Murphy et al. 2003