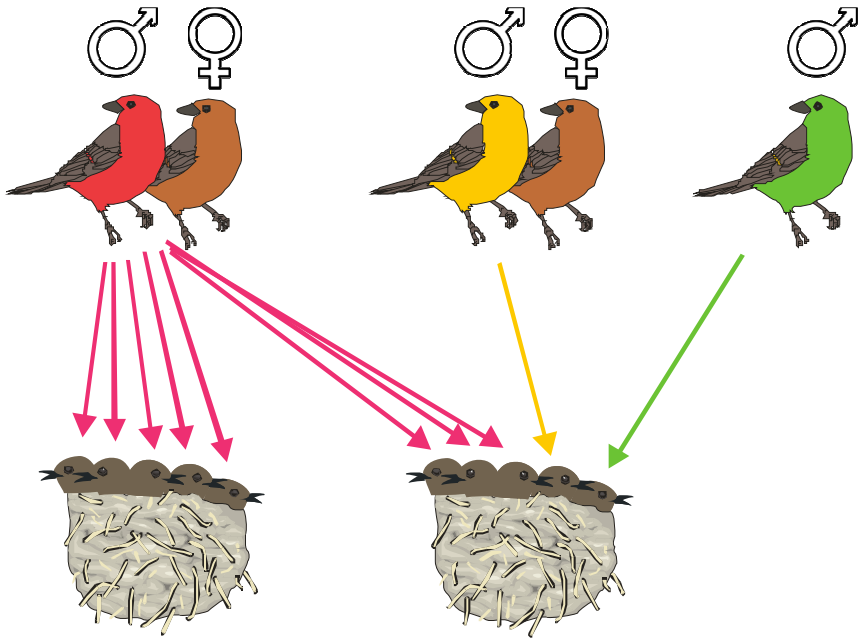
Molecular identification

Species, individual, sex



Identification of individuals

DNA fingerprinting
(DNA profiling)

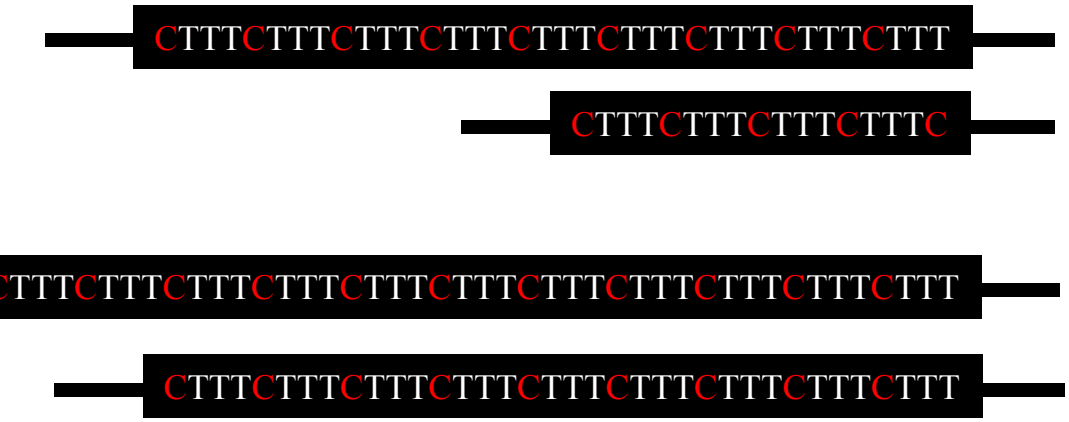
Identification of individuals – why?

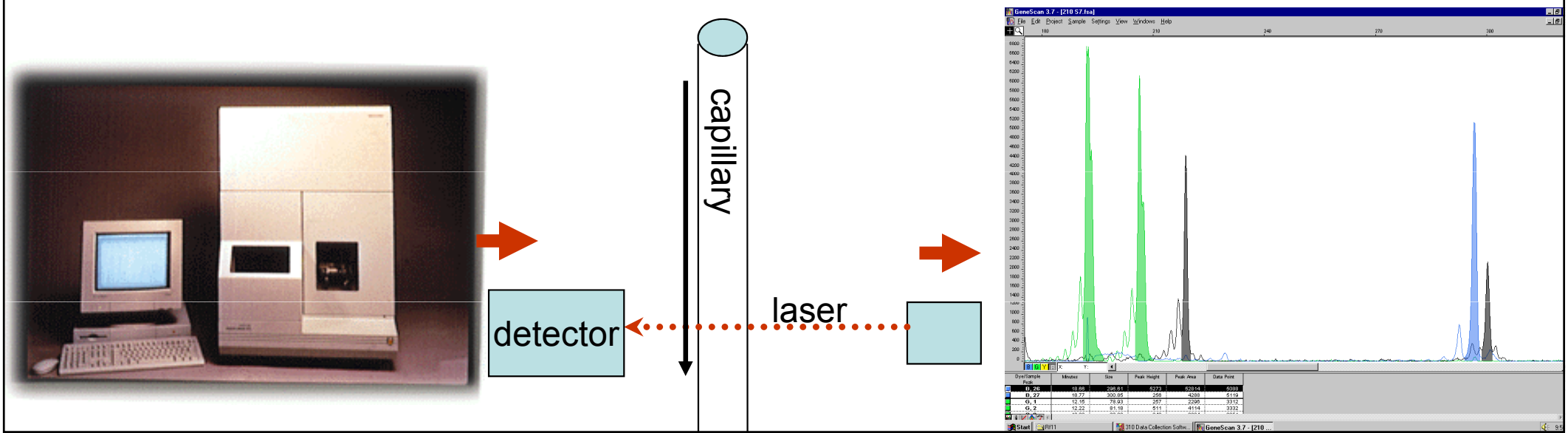
- if we do not see the individual
- non-invasive genetics – elusive animals, samples from faeces, urines, hairs – can be joined with individual variation of their diet
- forensic genetics – identification of DNA in animal products, poachers, etc.

- species conservation – e.g. in falconary (confirmation of parentage)

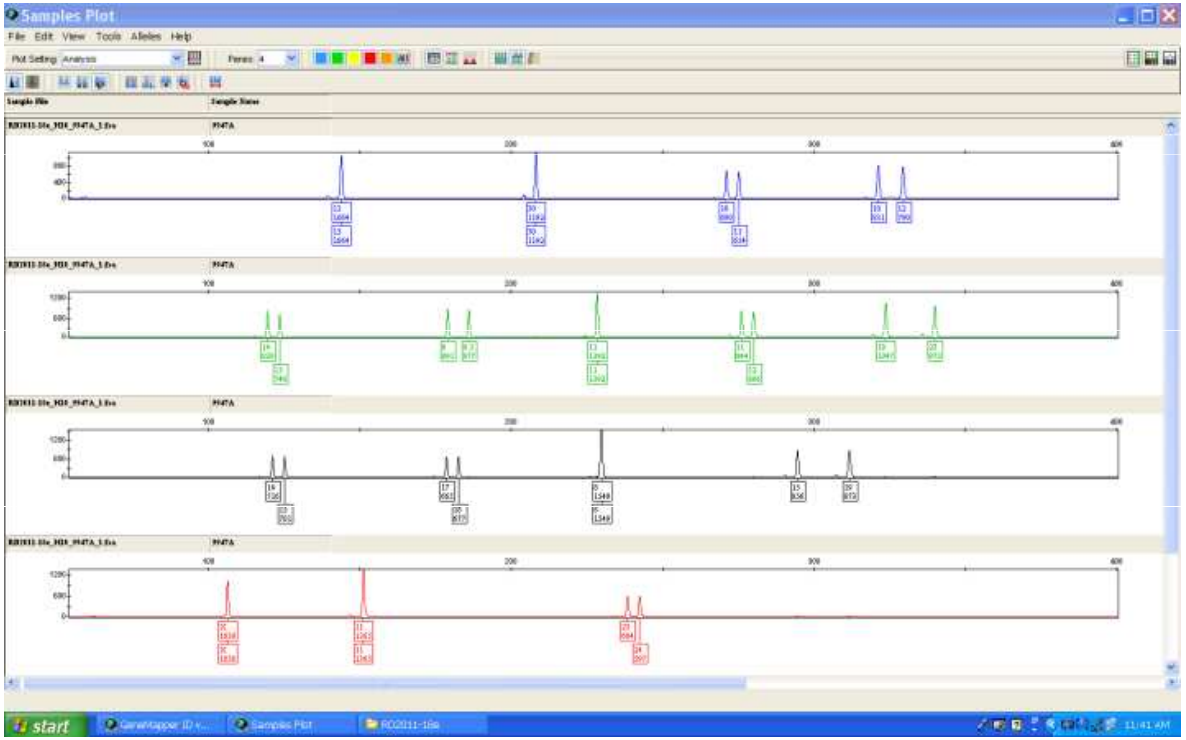
Microsatellites

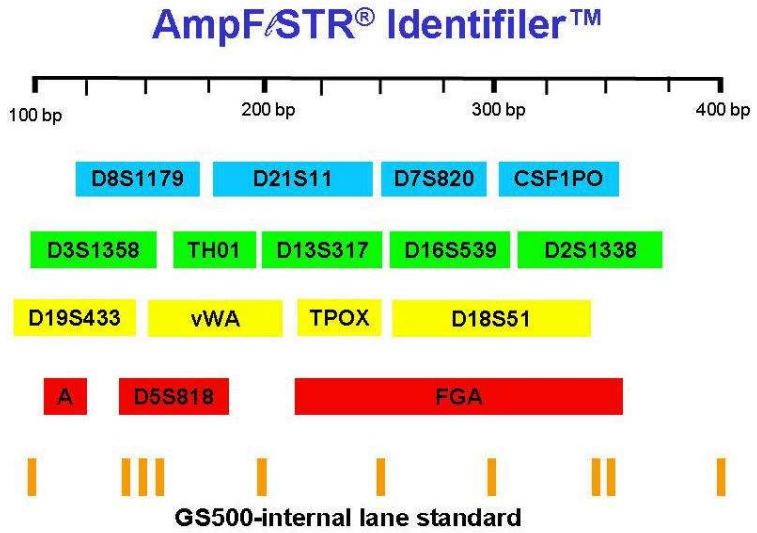
- Tandem repetitions of short motifs
- DNA extraction
- PCR
- Detection of alleles
  → sequencer, fragment analysis





Individual human identification



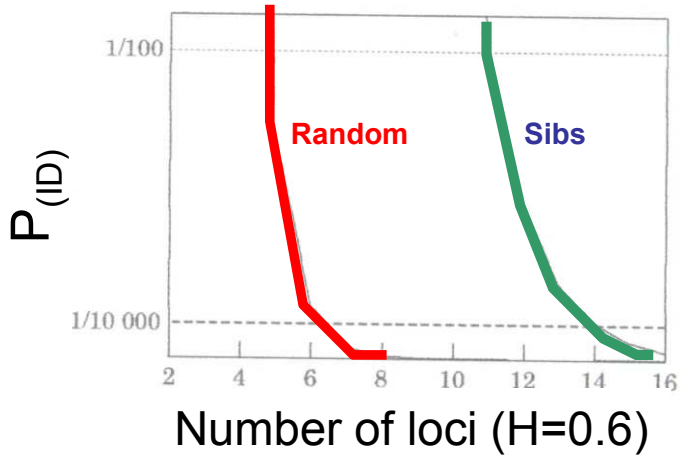


16 loci = reliable individual identification
(Euro-American population)

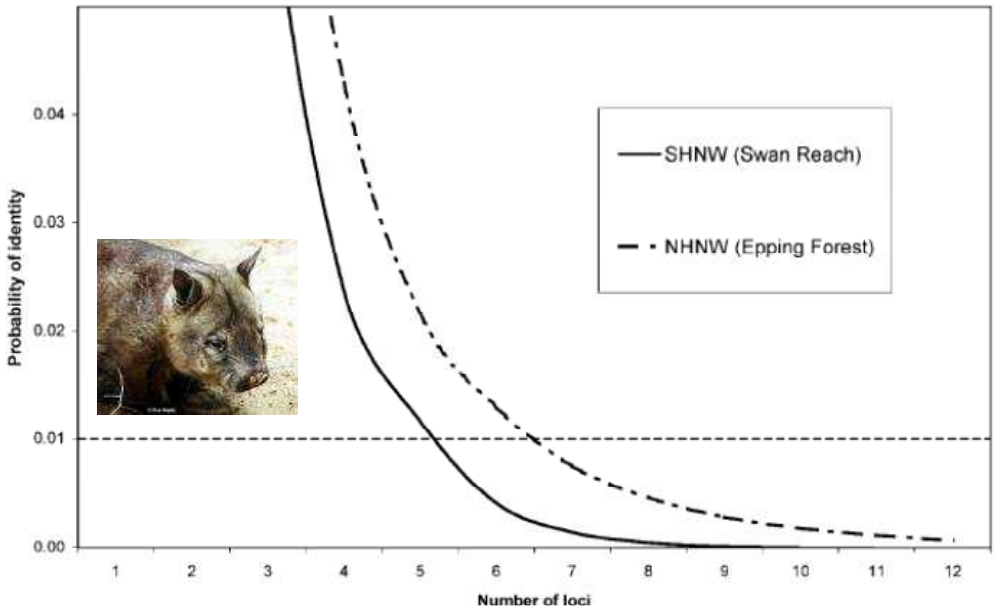
Identification of individuals depends on level of polymorphism

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

$$P_{(ID)} = \sum p_i^4 + \sum\sum (2 p_i p_j)^2$$



$$P_{(ID)sib} = 0.25 + (0.5\sum p_i^2) + \left[0.5(\sum p_i^2)^2\right] - (0.25\sum p_i^4)$$



- 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

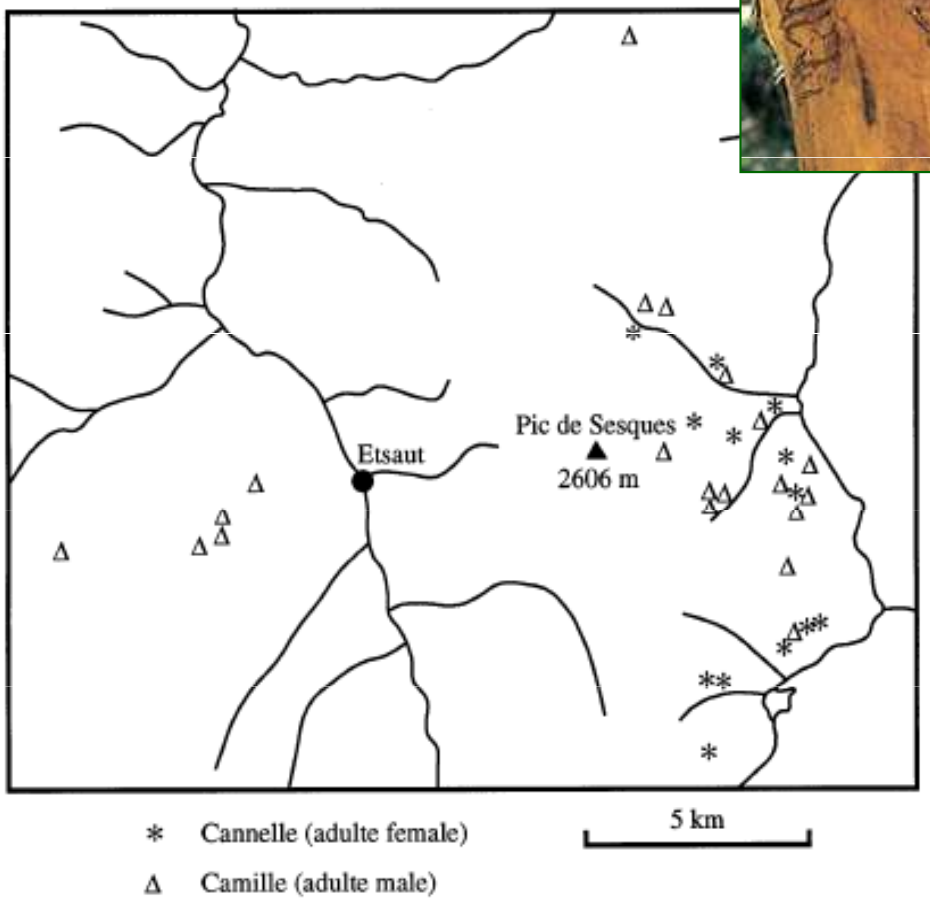


Fig. 3 Home range of two Pyrenean brown bears obtained by noninvasive genetic sampling and genotyping.

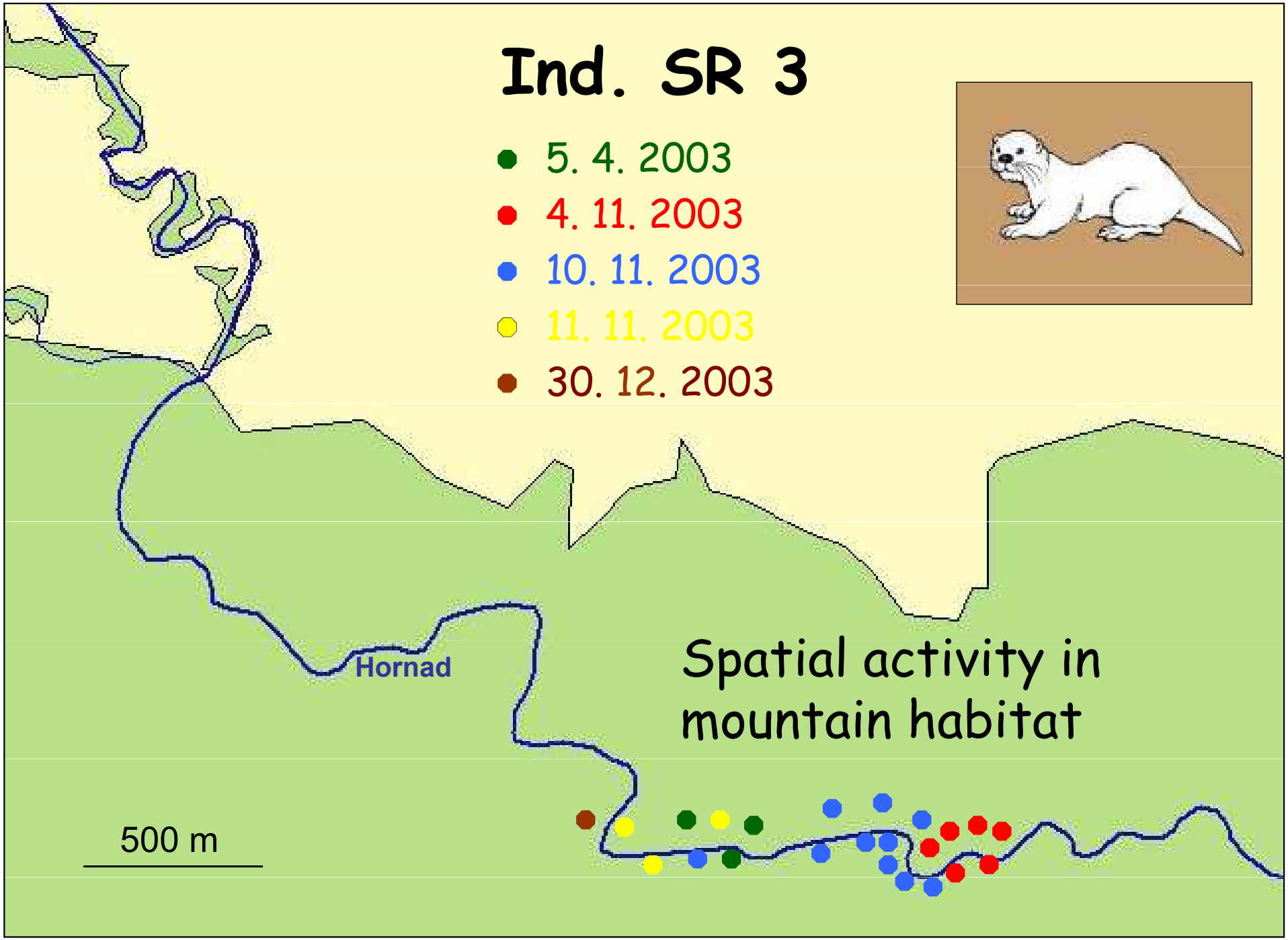
Spatial activity of otters

- **P. Hájková – PhD thesis (2008)**

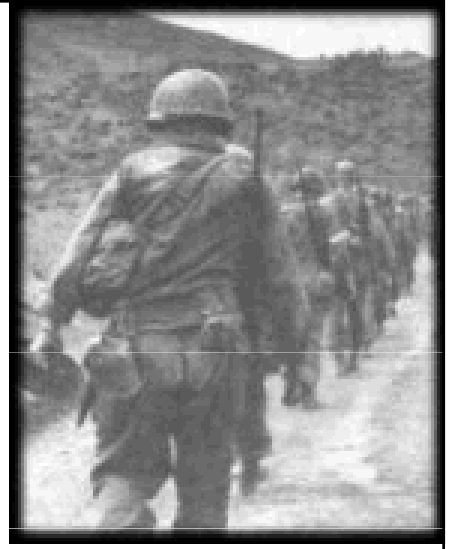
Identified individuals - Hornád, NP Slovenský Raj

SR 1
SR 2
SR 3
SR 4
SR 5
SR 6
SR 7
SR 8
SR 9
SR 10

1 km


Ind. SR 3
5. 4. 2003
4. 11. 2003
10. 11. 2003
11. 11. 2003
30. 12. 2003
Hornad
Spatial activity in mountain habitat
500 m

Human forensic genetics



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

  Identifikace na základě mtDNA příbuzných osob
  (lze 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**

Klony

Bambus *Sasa senanensis*

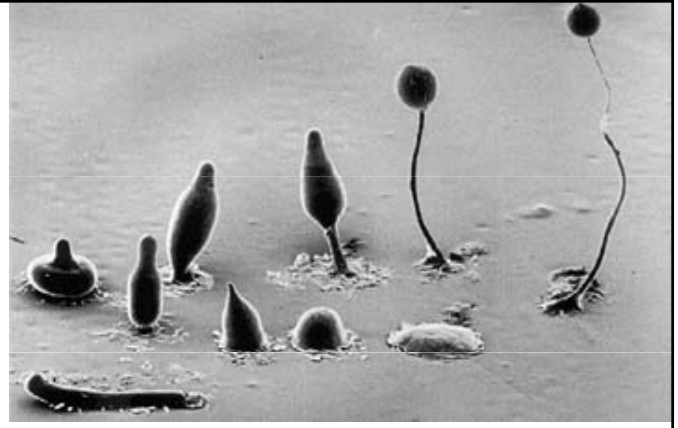
- Suyama et al. 2000
- Plocha 10 hektarů
- AFLP
- 22 klonů
- Klon na ploše 300 m v průměru

Slavní klonální bezobratlí

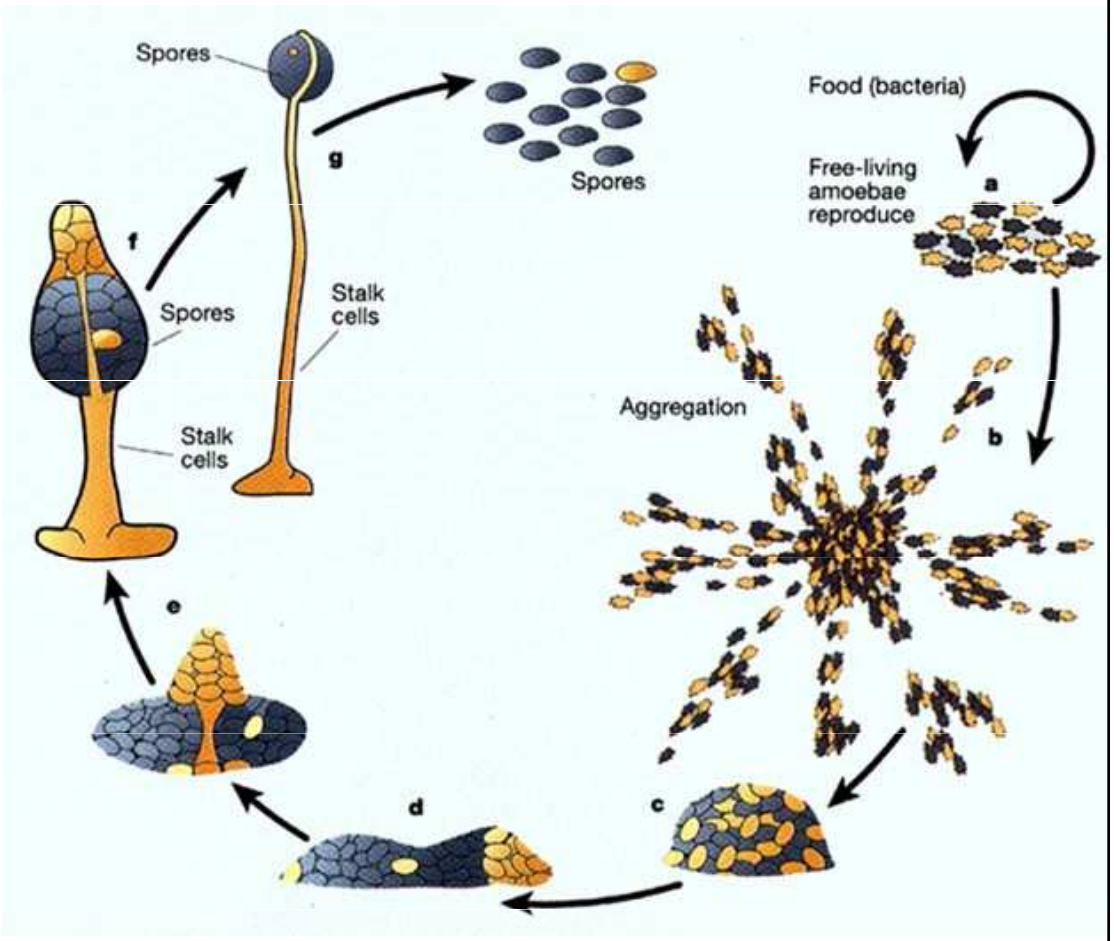
- Rotifera – Bdelloidea
- Ostracoda (*Darwinula*)
- Partenogenetické klony vysokého stáří (milióny let)

Darwinula stevensoni

Genetické chiméry



- organismy složené z buněk s různými genotypy
- *Dictyostelium discoideum*
  chimérismus je pravidelná součást života



Genetické chiméry

- *Ficus* srůst kořenů různých jedinců

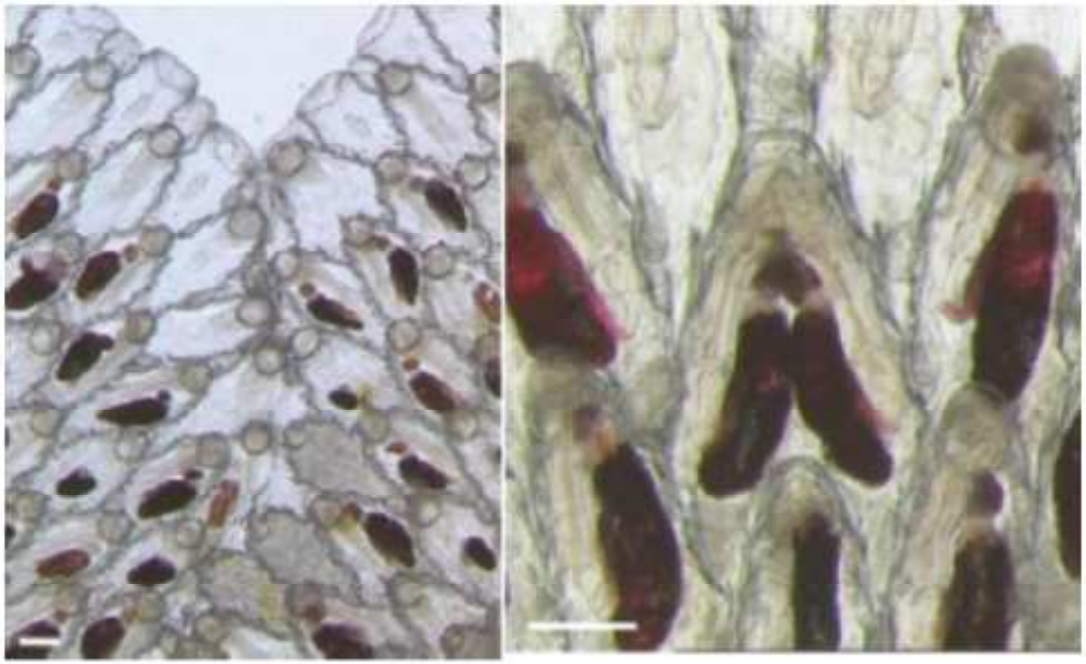
- sumky *Botryllus schlosseri* chimérické kolonie příbuzní jedinci

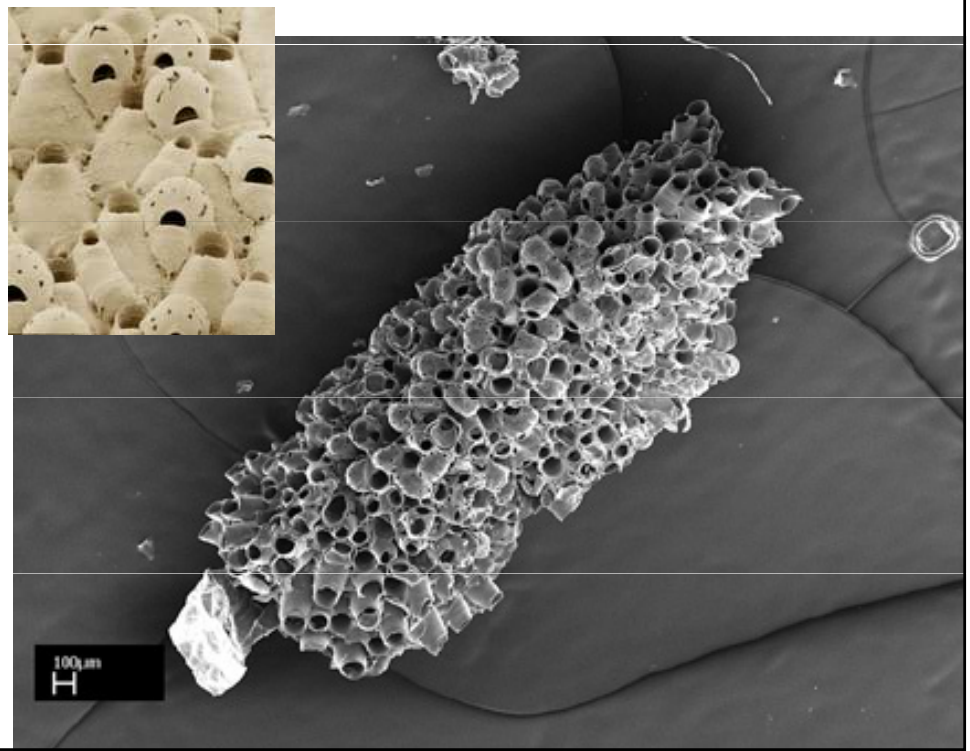
- *Diplosoma listerianum* i nepříbuzní

Celleporella hyalina (Bryozoa)

Hughes et al. 2004

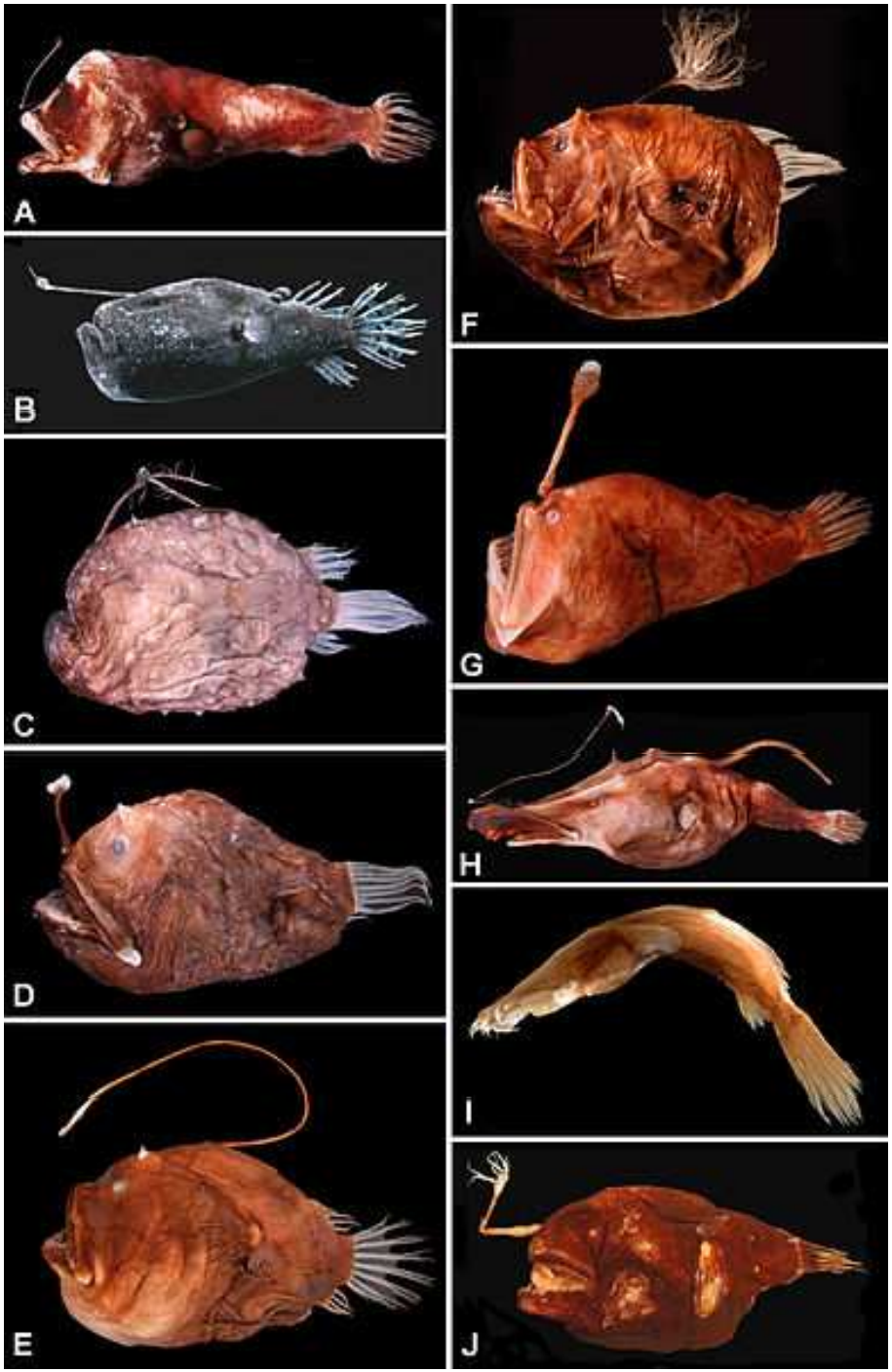
- Pravděpodobnost fůze koreluje s příbuzností
- Histokompatibilita
- Lepší rozpoznávání v pokročilejších fázích
  → dozrávání imunokompetence
- Speciální proteiny (spongikany...)





Ceratioid anglerfish

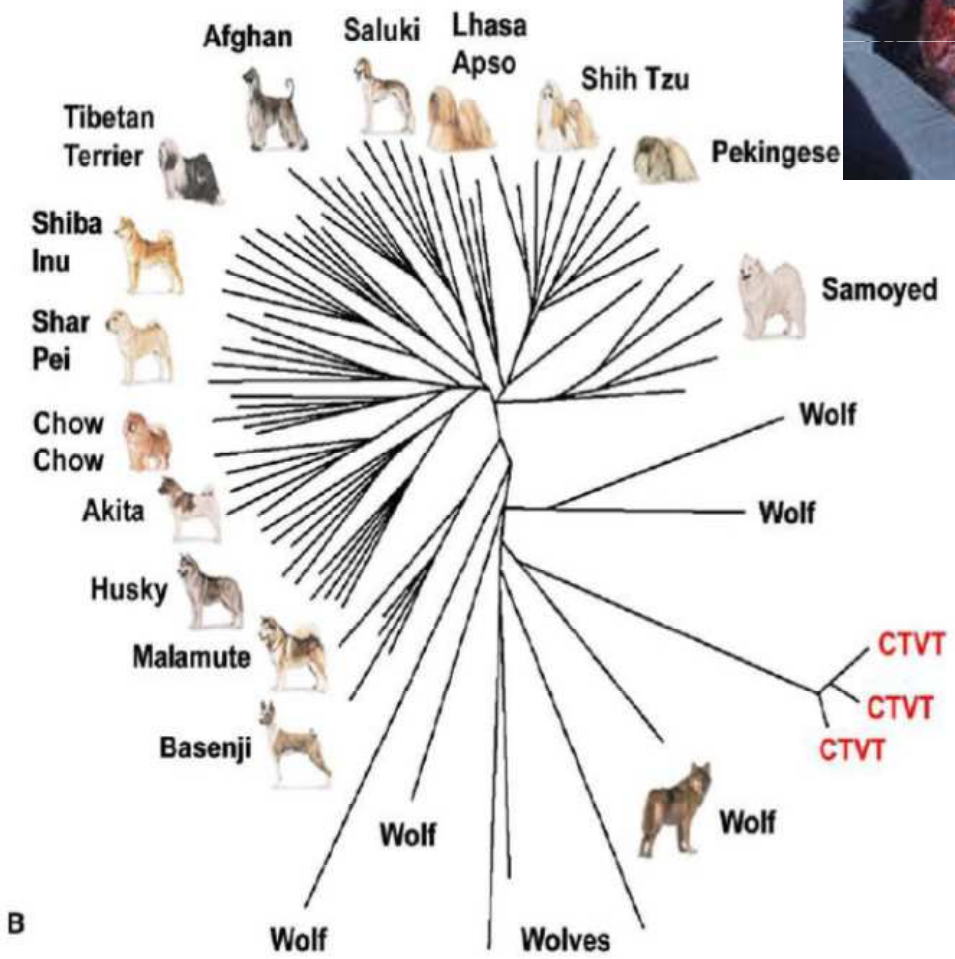
- miniaturní samec po narození vyhledá samici, její kůže vyloučí hydrolytický enzym a samec přiroste
- vzniká hermafroditická chiméra



Genetické chiméry – „microchimerism“

- kosman bělovousý *Callithrix jacchus* (asi i rod *Saguinus*)
- dizygotická dvojčata
- DNA fingerprinting krve - hematopoietické chiméry
- během embryonálního vývoje vzájemná výměna buněk kostní dřeně
- týká se to asi jen krve (neinvazivní metody – chlupy, trus → jeden genotyp)
- průnik embryonálních erytroblastů a volné DNA přes placentu i u člověka
- (pohlaví dítěte před narozením lze určit i pomocí PCR sekvencí typických pro Chr Y, jako templát je periferní krev matky)

Canine transmissible venereal tumor (CTVT)



Devil facial tumour disease

- **parasitic cancer**
- **„single cell parasitic wolf“**

Známé „lidské chiméry“

Foekje Dillema

46XX/46XY woman

- holandská atletka, mistryně na 100 a 200 m
- odmítla test na pohlaví
- mozaika zjištěna až posmrtně (v r. 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 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.

Lydia Fairchild

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.

- geneticky nepotvrzené mateřství
- chiméra matky

Identification of sex

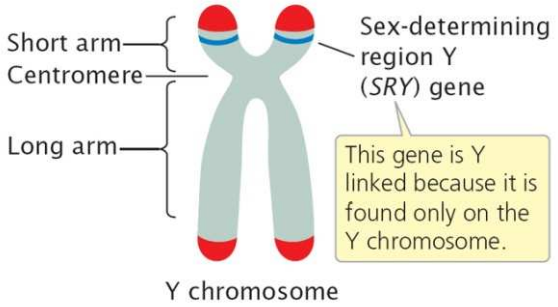
DNA sexing

Why?

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

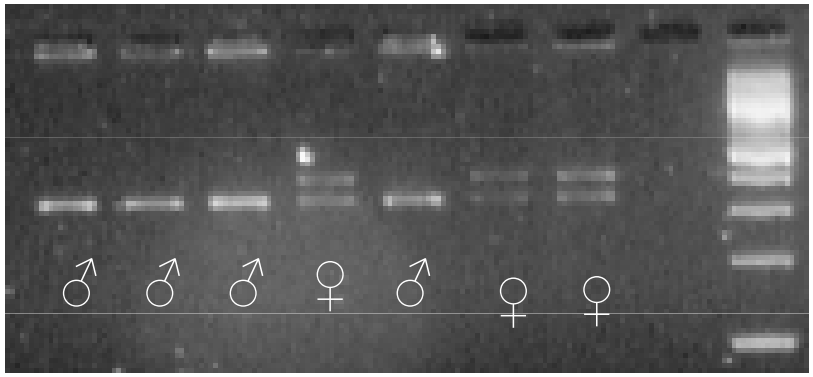
Genetic sex identification

- 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

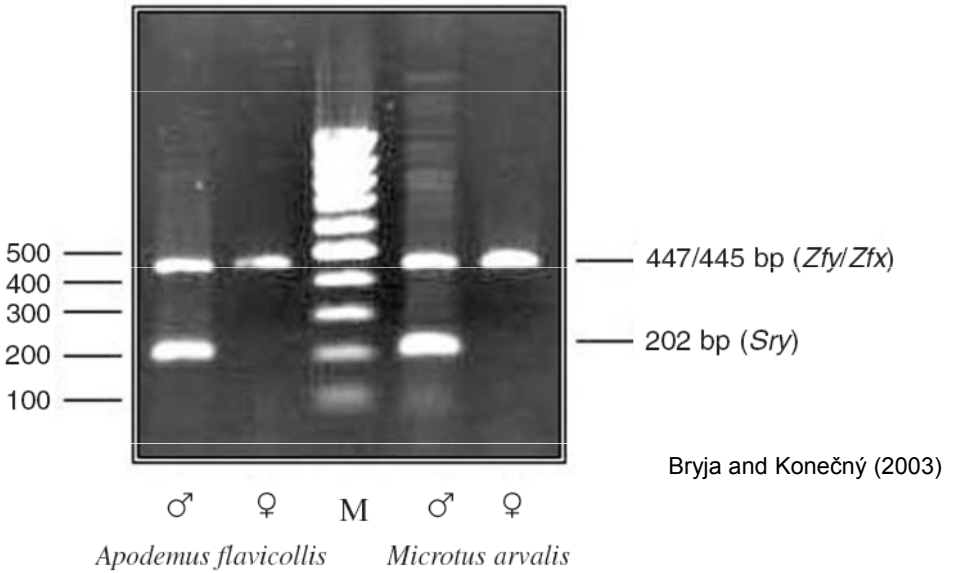
(Meliphagidae) *Arnold et al. 2001*

medosavka hlučná

- 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

Sex identification - mammals

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



Bryja and Konečný (2003)

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

M. cabrerae

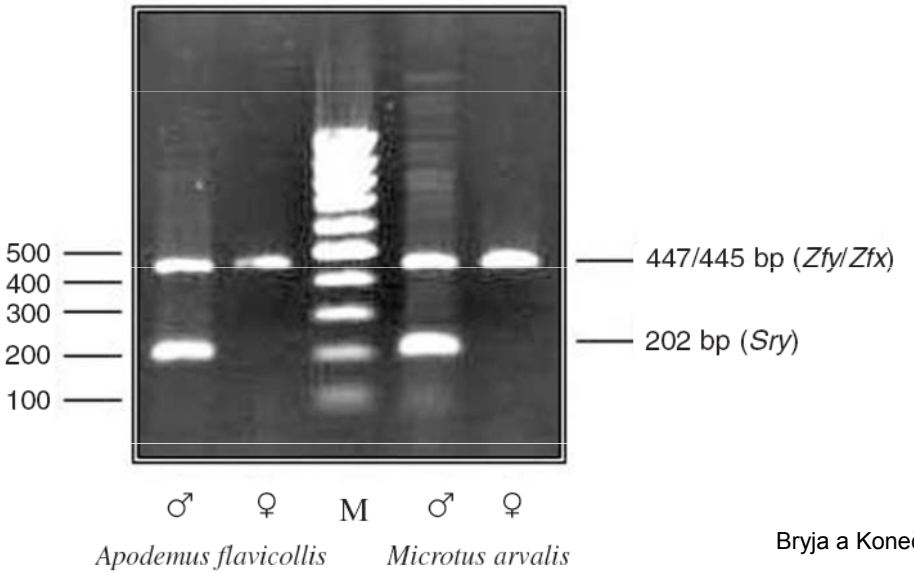
Ellobius

Nannomys

Tokudaia osimensis

Sex identification - mammals

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



Bryja a Konečný 2003

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

x

Murphy et al. 2003