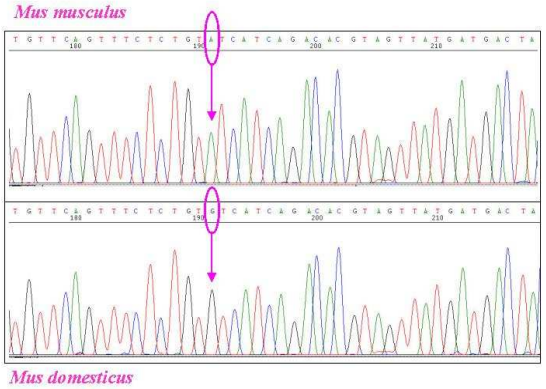
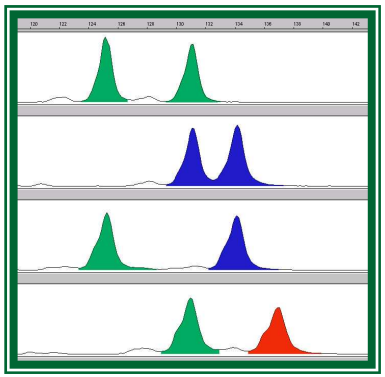
Ochranářská genetika

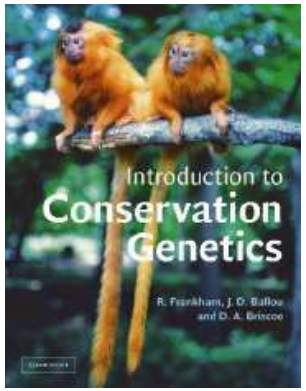
aneb co nám může molekulární ekologie napovědět
o životaschopnosti populací



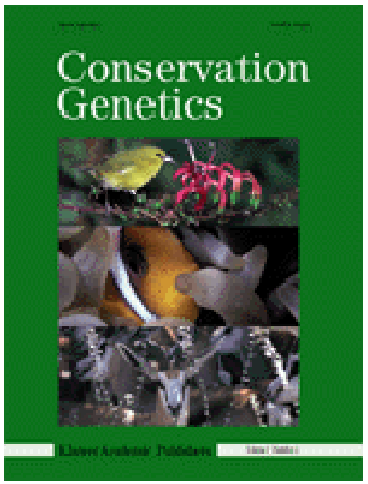


ATGCCGATTACCACACAGCTAATGCCGATTACCACA
CAGCTAATGCCGATTACCACACAGCTAATGCCGATT
ACCACACAGCTACTGATGGAAAGTCCTGCATC

Ochranářská genetika („conservation genetics")



- **Využití genetických metod v ochranářské biologii** – součást tzv. molekulární ekologie
- Pracuje na různých úrovních variability DNA – nejčastěji ale na úrovni populací
- PCR (90. léta) – počátek skutečné ochranářské genetiky (neinvazivní metody - již není potřeba destruktivního vzorkování)
- od r. 2000 - Conservation Genetics (IF = 1.784)
- recentní review a knihy
- European Science Foundation – program ConGen



Cíle přednášky

- Základní principy a metodické přístupy
- Praktické aplikace a problémy
- Budoucnost ochranářské genetiky

Metodické přístupy

1) Populační genetika – efektivní velikost populace, tok genů, „bottleneck", příbuznost, atd. ... neutrální variabilita (např. mikrosatelity)

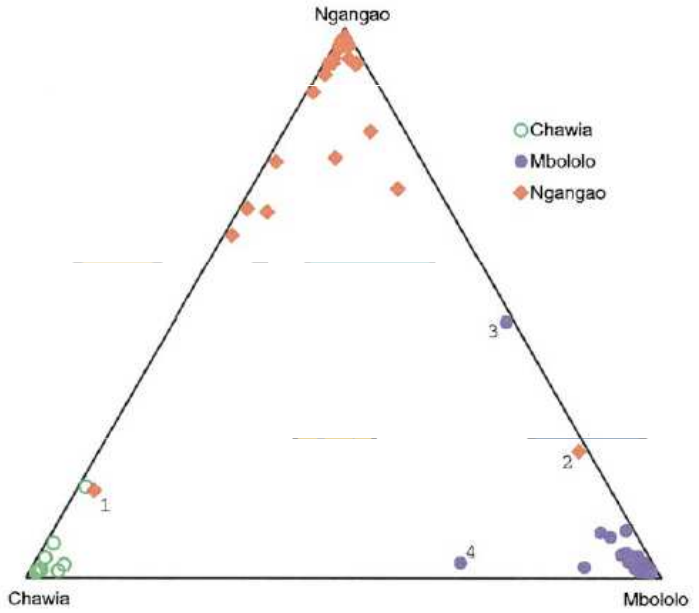
2) Fylogeografie – historický původ populací a jejich fylogenetické vztahy, ESU ... většinou neutrální variabilita

3) Speciální přístupy – neinvazivní genetické metody, vztah genetické diverzity a životaschopnosti populací, experimentální „conservation genetics", selektované znaky (adaptivní i škodlivé)

1) Populační genetika

- studium struktury populací
- nejčastěji neutrální znaky - mikrosatelity
- efektivní velikost populace N_e
- tok genů (sex-specific)
- „past bottleneck“
- původ jedinců („assignement tests“)
- příbuzenské křížení (inbreeding), atd.
- „founder contribution“

Bayesiánské analýzy (např. program STRUCTURE, GENELAND aj.)
- identifikace subpopulací („management units“)
- identifikace hybridů
- identifikace geografických bariér toku genů



Ne – effective population size

- velikost ideální populace (náhodné páření, rovnoměrný poměr pohlaví), která ztrácí genetickou diverzitu stejnou rychlostí jako aktuální populace
- ovlivněna genetickou a věkovou strukturou, poměrem pohlaví, intenzitou inbreedingu atd.

- vývoj genetické variability v malých populacích závisí na N_e více než na N

- $N_e/N \approx 0.11$ (Frankham 1995), ale velká variabilita

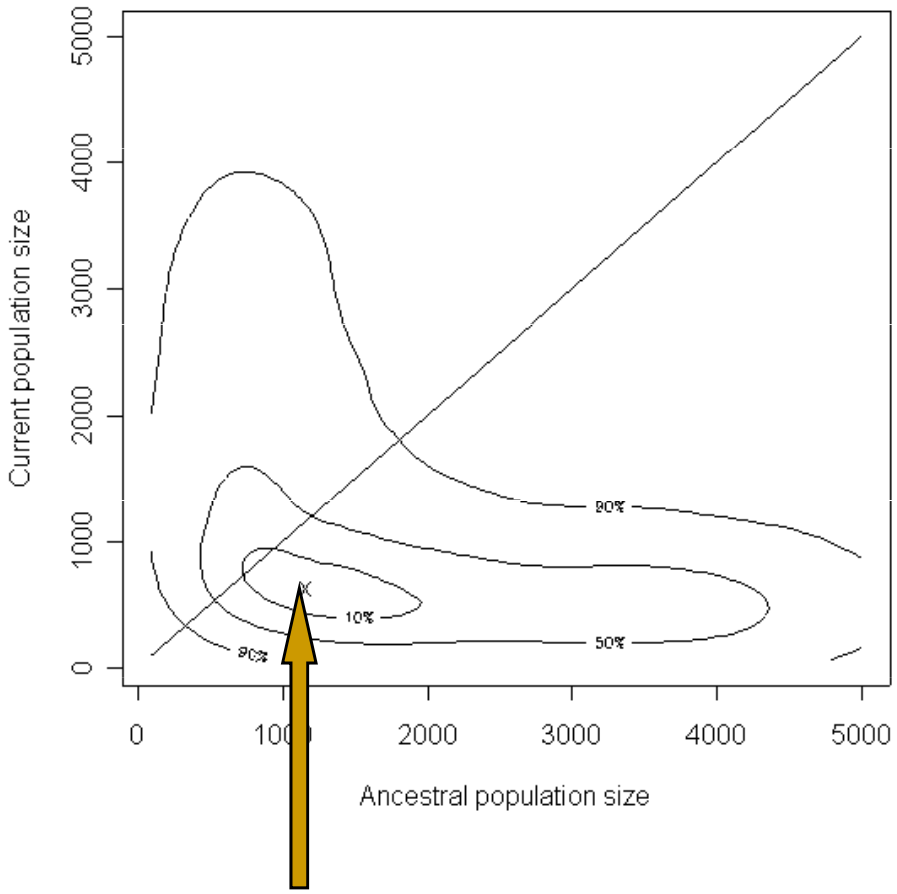
Odhady Ne

- $F_{ST} = 1/(4N_e.m + 1)$
- recentní přístup: coalescent theory methods
  - TMVP (Beaumont 2003)
  - CoNe (Anderson 2005)
  - MLNE (Wang and Whitlock 2003)
  - MSVAR (Beaumont 1999)

nejméně 2 časové vzorky populace

stačí 1 vzorek

TMVP



N_A vs N_0

N_A – ancestral N_e
N_0 – recent Ne

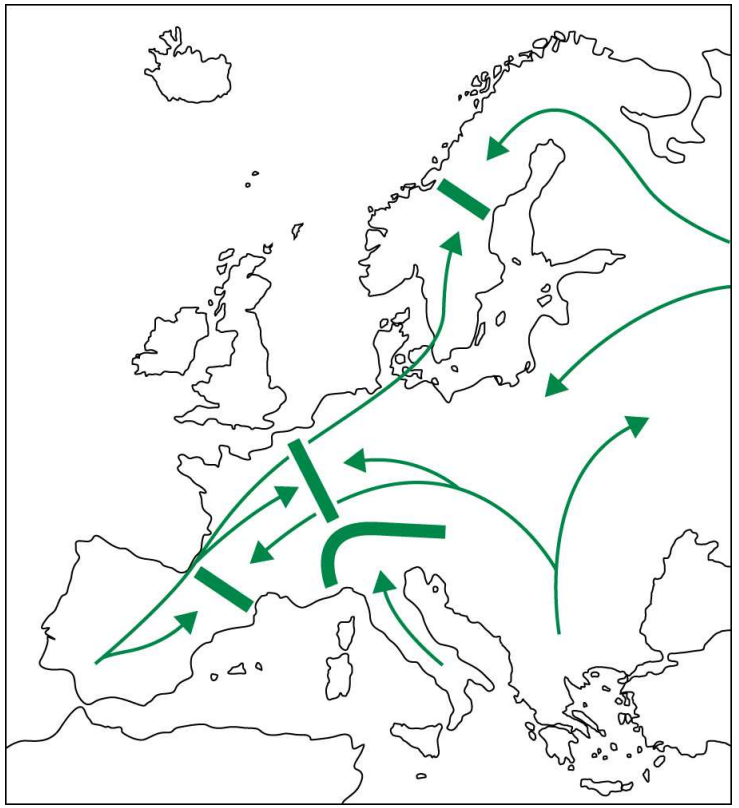
- testuje i rozdíly N_A a N_0
- přesnější odhady pokud je více časových vzorků, ale stačí jen dva

MLNE
- zároveň s N_e odhaduje i *m*

MSVAR
- stačí jeden vzorek mikrosatelitových dat

2) Fylogeografie

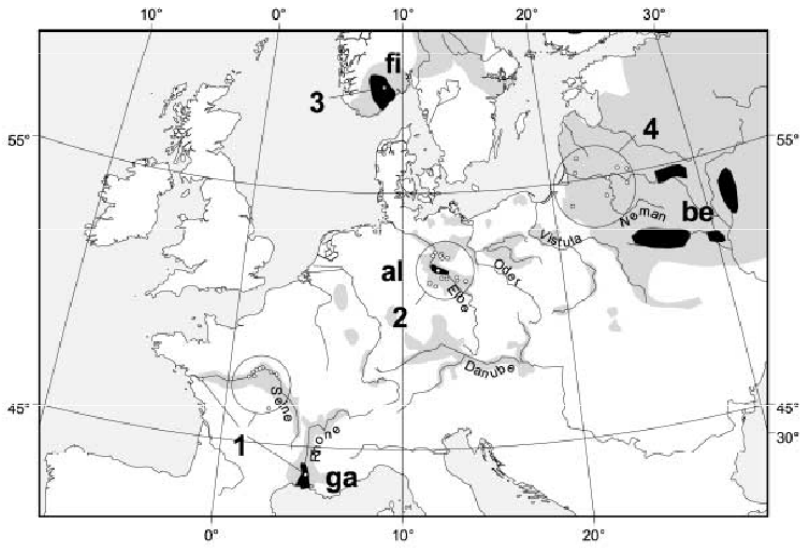
- použití fylogenetických metod na úrovni populací (nejčastěji sekvence mtDNA, jaderné markery jsou málo polymorfní)
- původ populací, jejich stáří a historické vazby
- detekce ESU („evolutionary significant units“) – lokální adaptace (mohou, ale nemusí)
- důležité pro reintrodukce

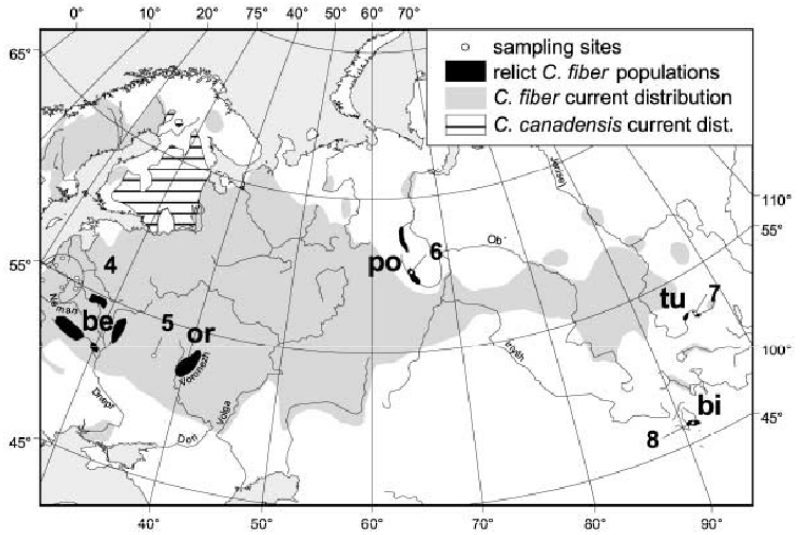


Př. Směry šíření z glaciálních refugií

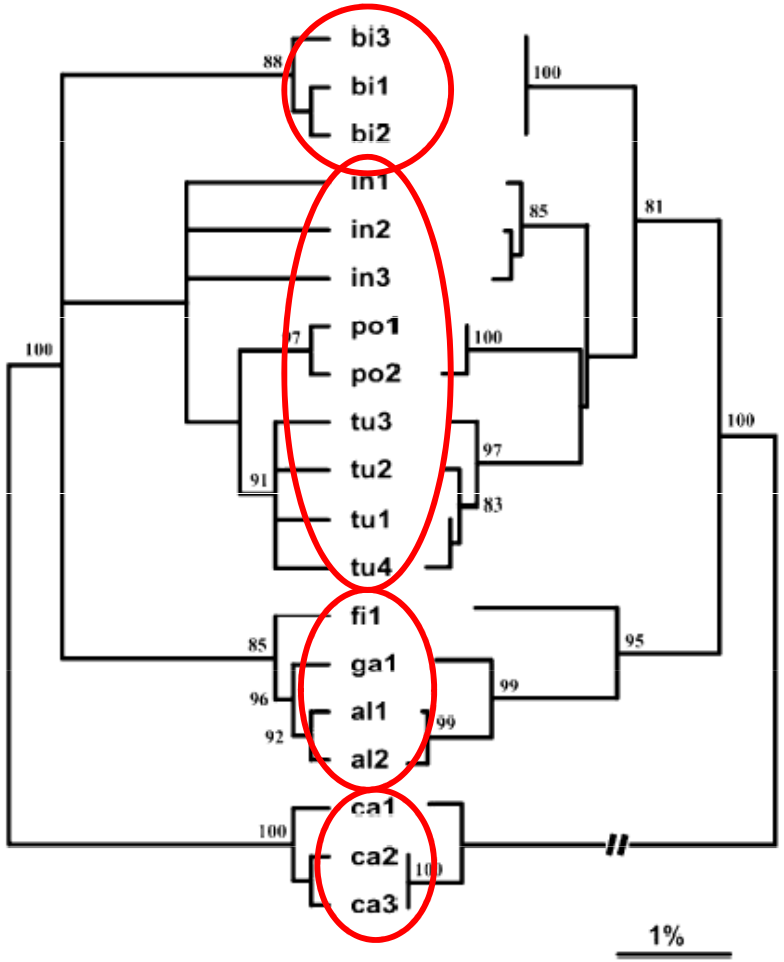
Příklad: Castor fiber , sekvence CR mtDNA

Durka et al., Mol.Ecol, 14: 3843-3856 (2005)





ESU, MU (spolu s populační genetikou)



Mongolsko

Polsko, Litva, Rusko

Norsko, Německo, Francie

C. canadensis

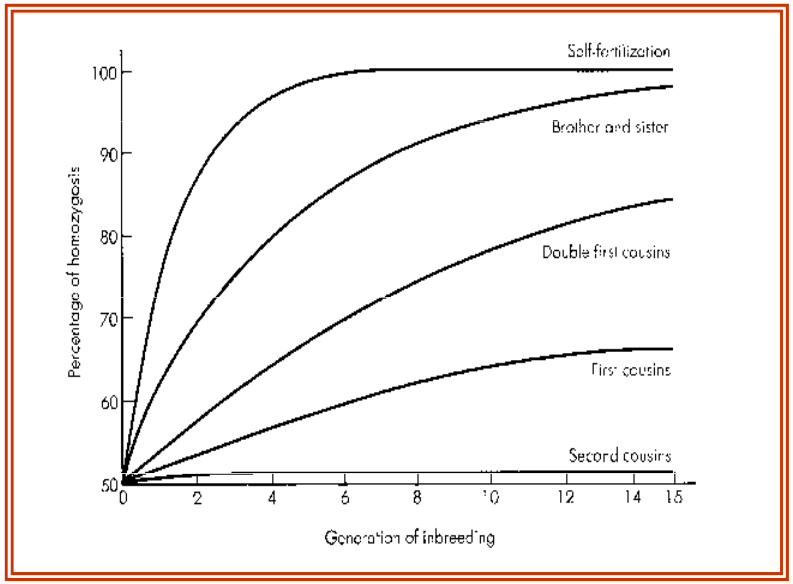
Fylogeografie

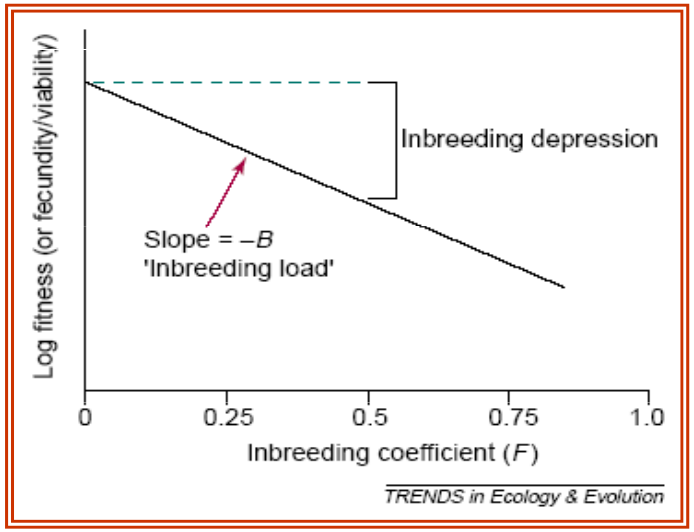
3) Speciální přístupy

- škodlivá (detrimental) variabilita – detekce inbrední deprese
- identifikace adaptivní variability – lokální adaptace
- experimentální ochranářská genetika (zejména hmyz a rostliny)
- neinvazivní genetické metody

Inbreeding a fitness

- Nárůst proporce homozygotů - efekt škodlivých recesivních alel
- Inbrední jedinci by měli mít nižší fitness (reprodukční úspěch nebo schopnost přežívat)
- Známo z laboratorního křížení (extrémní příklady)
- Studium v přírodě je obtížné - malé populace, nebo po projití hrdlem lahve

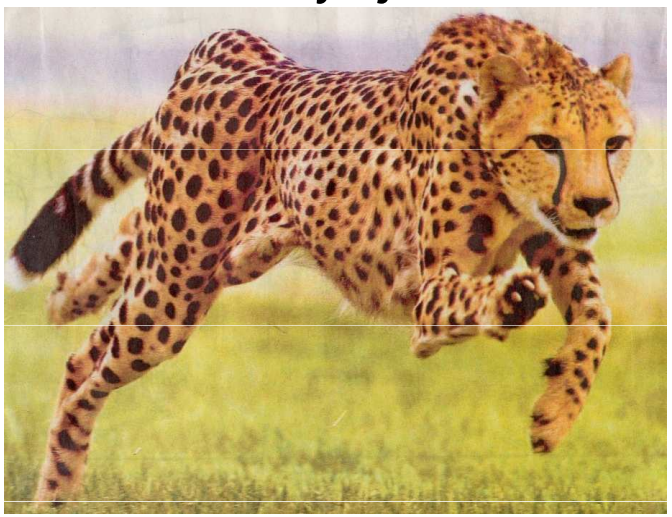




„Bottleneck“ efekt

- Druhy s výrazným snížení genetické variability - prošly hrdlem lahve
- Prokázané snížení variability (mtDNA, alozymy) – random drift
- gepardi - snížení o více než 90 %
- přesto se počty výrazně zvedly
- „**purging**“ – odstranění škodlivých alel v důsledku zvýšené selekce na homozygoty
- **fixace škodlivých alel** – Florida panther

Acinonyx jubatus



Mirounga angustirostris

Florida panther

Detrimental variability

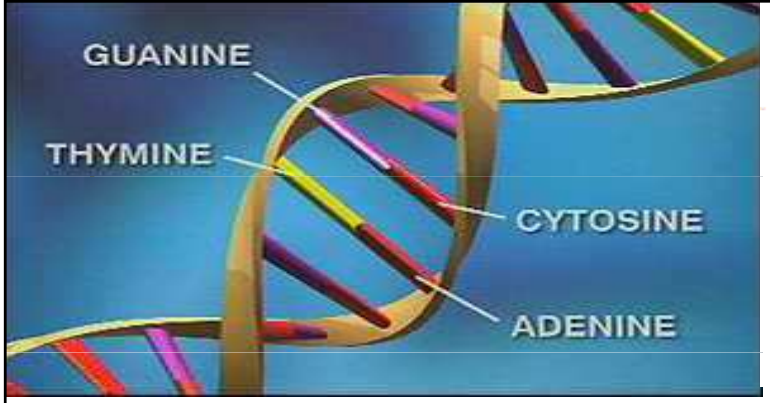
- cryptochordism, poruchy vývoje ocasních obratlů, srsti a spermií – téměř fixovány genetickým driftem
- pozitivní i negativní dopady introdukce teoreticky testovány (Hedrick 1995)
- introdukce osmi pum z Texasu – v následující generaci bylo 20 % genetické informace z Texasu
- ocas – 7 % vs. 88 %
- srst – 24 % vs. 93 %
- cryptochordism – 0 % vs. 68 %

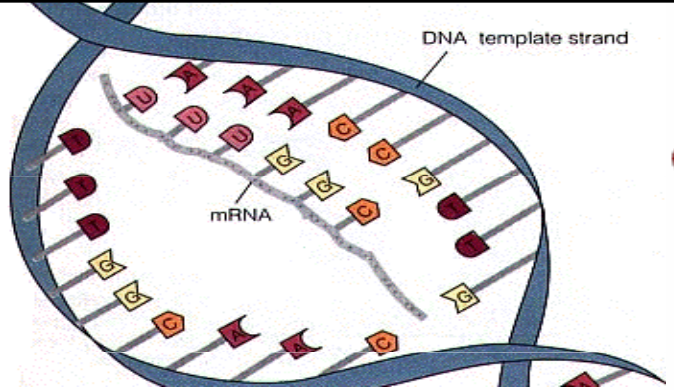
Puma concolor coryi

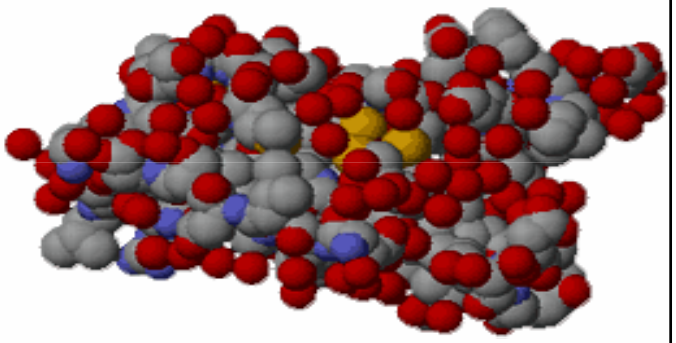
Puma concolor couguar

Adaptivní variabilita

- rozdílná prostředí → diverzifikující selekce → lokální adaptace
- outbrední deprese – narušení lokálních adaptací (důležitá při reintrodukcích)
- analýza funkční genetické variability – min. 9 přístupů (review in Vasemägi & Primmer 2005)
- genomika, proteomika, transkriptomika
- např. neutrality tests, genome scans, cDNA microarrays, QTL atd.







DNA → Transcription → mRNA → Translation → Proteins

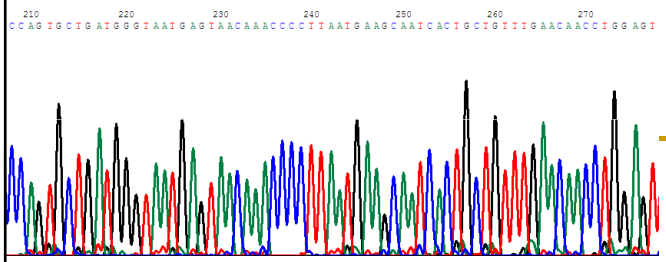
Genome — 3 billion bases

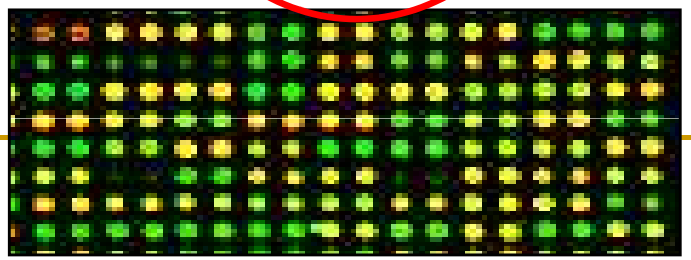
Transcriptome — 20-30,000 genes

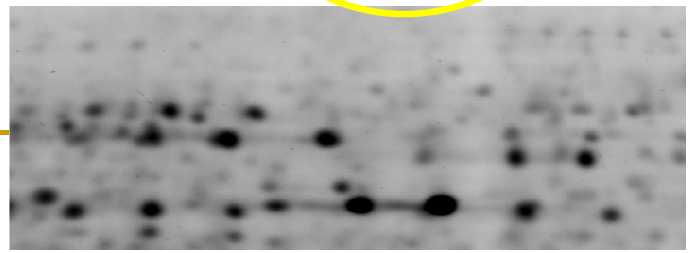
Proteome — ~100,000 proteins

Functional Genomics

Genomics — Transcriptomics — Proteomics







King and Wilson 1975

Science paper by King and Wilson 1975

- “all the biochemical methods agree that the genetic distance between humans and chimpanzees is probably too small to account for their substantial organism differences”
- “evolutionary changes in anatomy and way of life are more often based on changes in the mechanisms controlling the expression of genes than on the sequence changes in proteins”
- Dnes: člověk a šimpanz - 98.7% identita genomu, výrazné tkáňově-specifické rozdíly v expresi, zejména v mozku (Enard et al. 2002)

Study transcriptomics

- Study expression of single genes – quantitative real-time PCR

  - známý kandidátní gen (modelové druhy)

- Study expression of multiple genes (genomic scale) - microarray

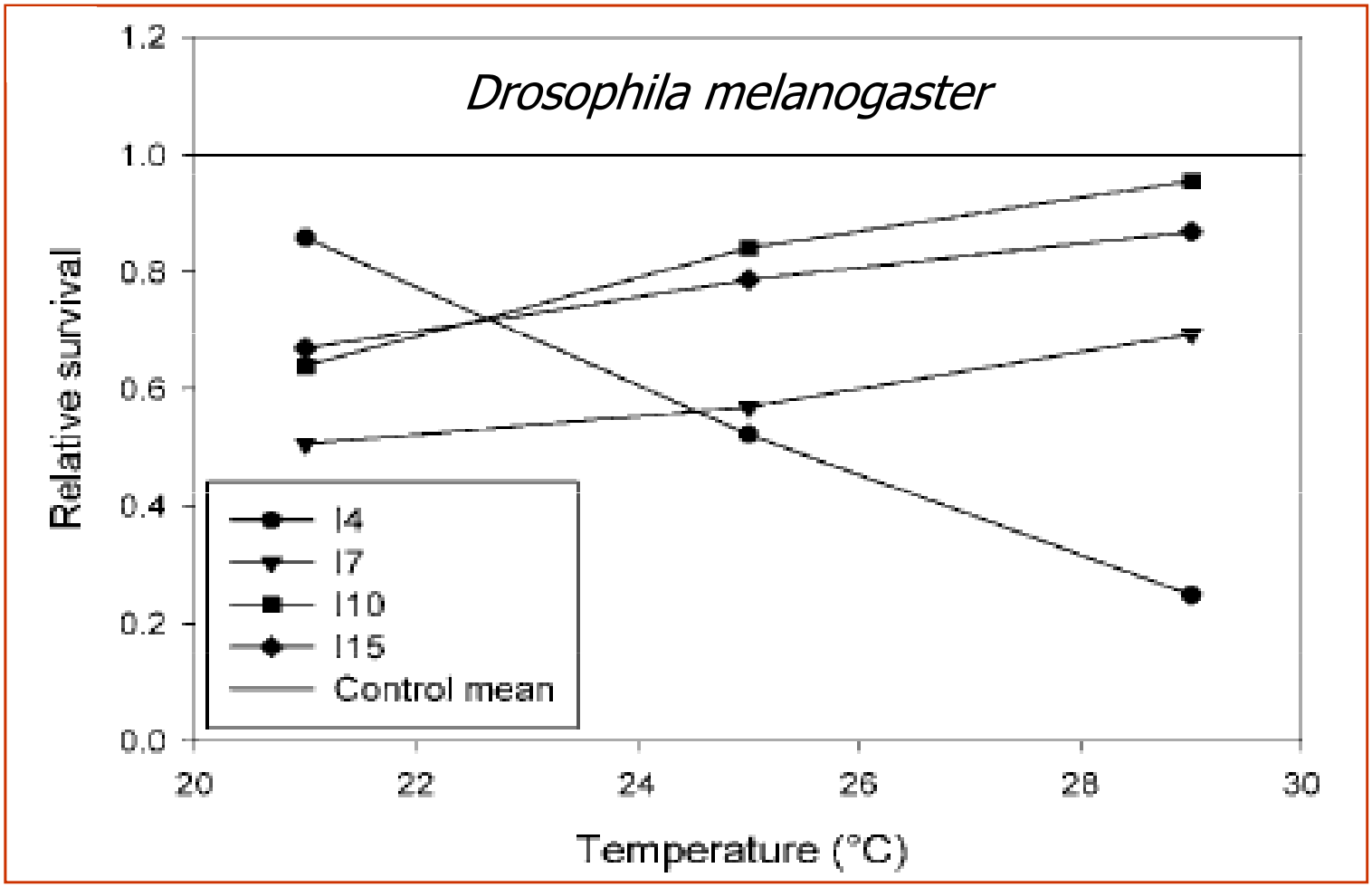
  - identifikace kandidátních genů – detekce jejich exprese může podat důležitou informaci o jeho funkci (různá v odlišném prostředí, vývojovém stadiu, stresu atd.)

  - geny zahrnuté do stejného procesu mohou mít stejnou expresi (co-regulated genes)

Experimentální ochranářská genetika

- rekonstrukce historických procesů v laboratoři
- testování hypotéz
  - ztráta genetické diverzity v malých populacích
  - síla selekce a genetického driftu v malých populacích
  - význam environmentálního stresu na expresi funkčních genů
  - efekt inbreedingu na přežívání
  - apod.
- modelové organismy – převážně hmyz a rostliny

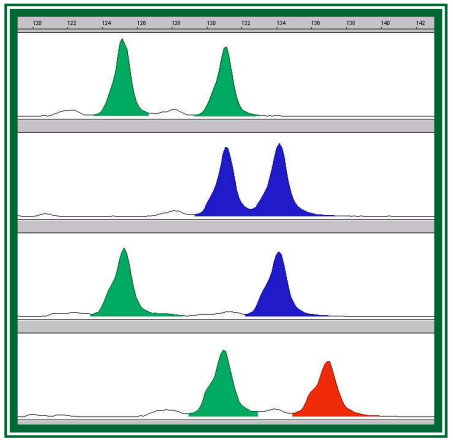
Př.: Vztah inbreedingu a teploty



Vermeulen and Bijlsma, Heredity 2004

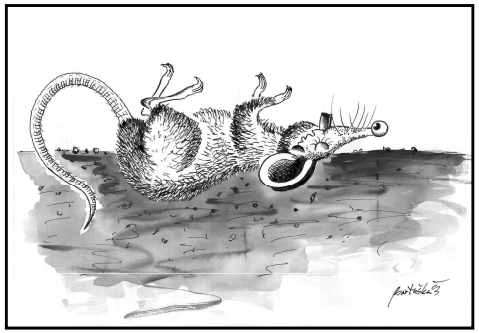
Non-invasive genetic methods in conservation genetics

- by definition „*conservation of rare and endangered animals*“ – not possible to kill or even disturb them
- need of methods allowing collection of genetic data without direct contact
- **non-invasive genetic methods**



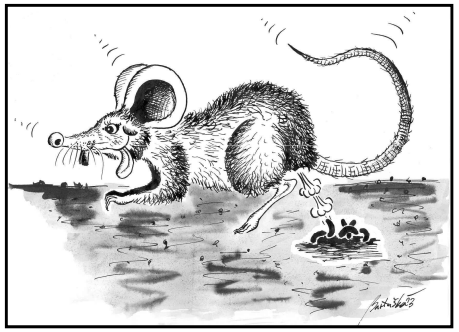
Three different DNA sampling methods

1. **Destructive** – animal is killed to obtain the tissues necessary for genetic analysis



2. **Non-destructive (invasive)** – animal is often captured and a biopsy or blood sample is taken invasively

3. **Non-invasive** – source of the DNA is left behind by the animal and can be collected without having to catch or disturb the animal



Sources of DNA - faeces

faeces (cells shed from intestinal lining)
→ „**molecular scatology**“

otters

chamois

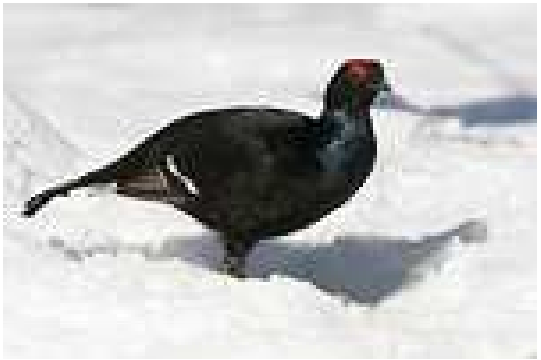
Sources of DNA – hairs

- follicles
- hair traps with glue patches
- more hairs –risk of **mixed samples**

marmots
bears
lynx
roe deer

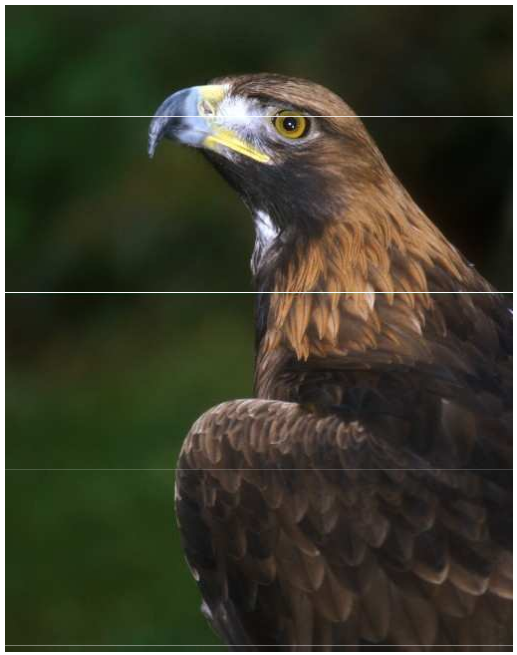
Sources of DNA – feather samples

- root end of individual feathers
- better plucked feathers (traps) than moulted feathers (often very old)



black grouse
(*Tetrao tetrix*)

capercaillie (*Tetrao urogallus*)



imperial eagles
(*Aquila heliaca*)

Sources of DNA - others

- **urine** – rarely used (Hausknecht et al. 2006 and references therein)
- more material available than faeces (urination rates – six time higher than defecation frequencies
- wolves – 33 samples with positive DNA concentration – 14 (42%) congruent results for all loci

- **shed skin** – reptiles
- **eggs shells**
- **buccal cells from pellets**

... artificial „bug-eggs“

- blood-sucking bugs Triatominae (Heteroptera)
- measures of stress level
- usefull also as „less invasive“ method in conservation genetics

Becker et al. 2006

Tracking of the endangered Pyrenean brown bear population

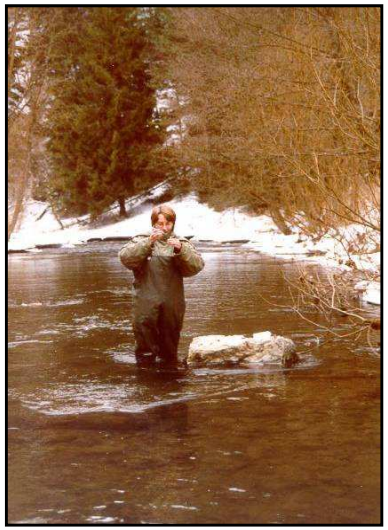
- hair and faeces
- 24 microsatellite loci
- one yearling, three adult males, one adult female
- spatial activity
- suggestions for conservation management

Taberlet et al. 1997, Molecular Ecology

Use of non-invasive methods

- **elusive animals** - easily obtained samples (faeces, hairs ...)
- **rare, endangered and protected species** - faeces, urine – no CITES restrictions
- **no disturbing effect** on animals – behavioural studies

Workflow in analysis of DNA from faeces



sampling of fresh (≤ 18 hod.) material

(into 96% ethanol, SilicaGel etc.)

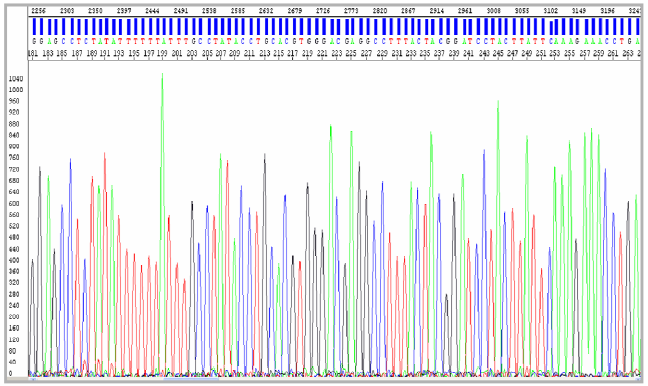
↓

DNA extraction

(commercial kits for rare DNA)

↓

PCR with high-quality polymerases (Hot-start)



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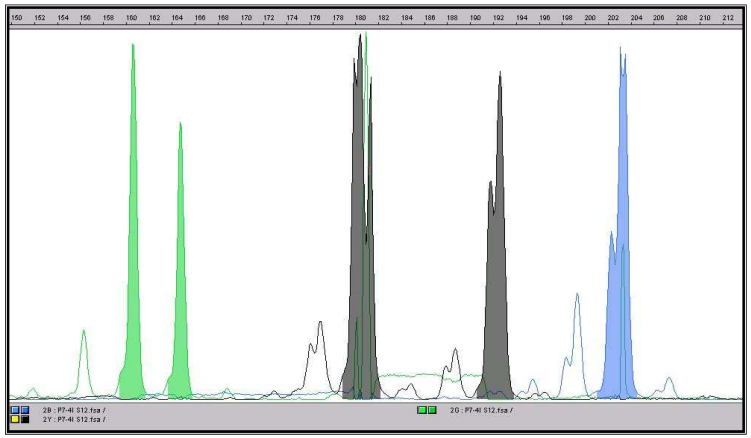
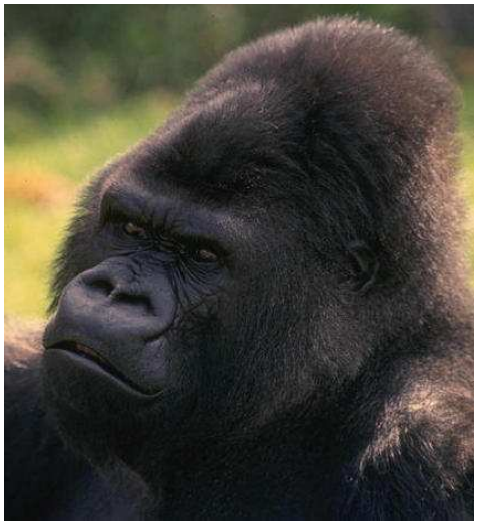
analysis of PCR products



Identification of species

- mtDNA – many copies in one cell
- *species-specific primers* (foxes – Dalén et al. 2004)
- *PCR-RFLP* (mustelids – Riddle et al. 2003, Gómez-Moliner et al. 2004)
- *sequencing* (felids -Farrell et al. 2000)
- distribution, phylogeography

Identification of sex



- sexual structure of population
- genetically determined sex
- markers: mammals – *SRY*, amelogenin; birds – CHD
- species-specific markers must be used (otherwise cross-amplification with species in the diet)

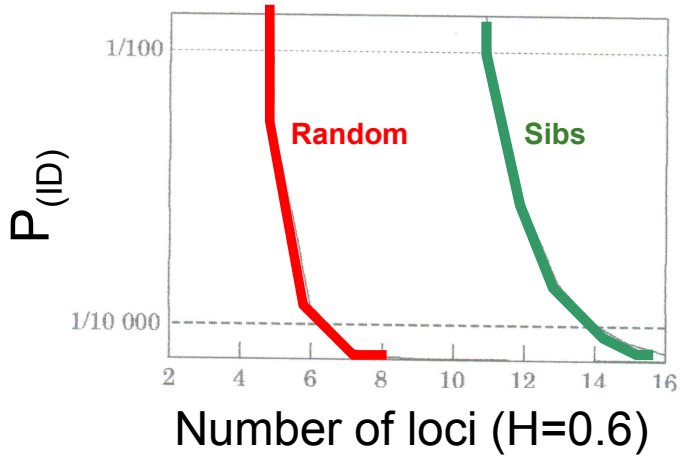
x

Murphy et al. 2003

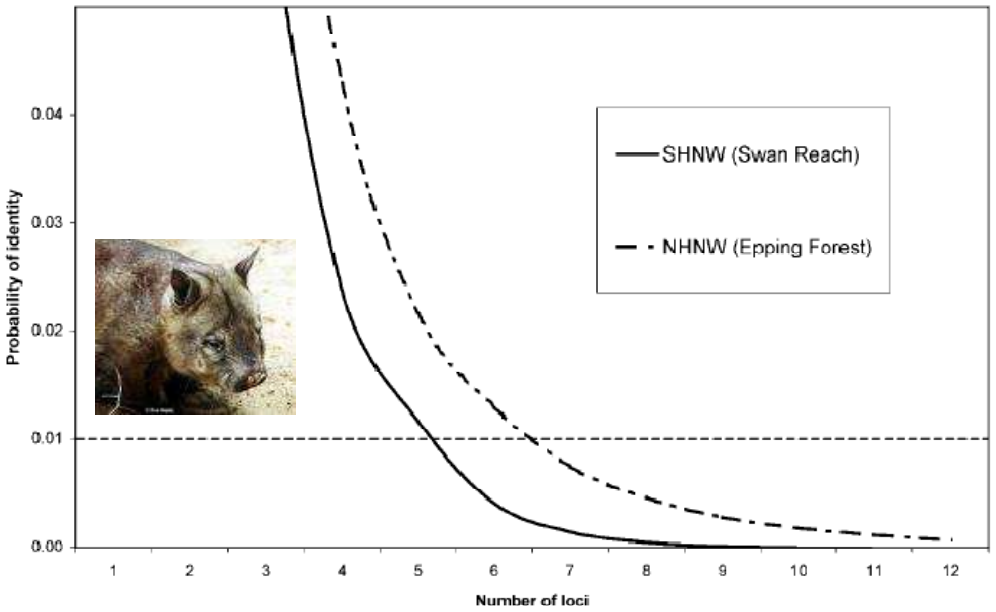
Identification of individuals

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

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



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



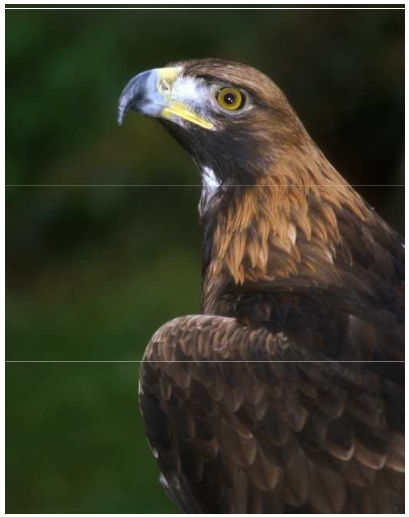
- pilot studies with tissue samples are required to identify $P_{(ID)}$ in a studied population

Identification of individuals

- **spatial activity**
- relatedness (**parentage**)

Constable et al. 2001 –chimpanzees

Rudnick et al. 2005 - eagles



- faeces – links to **individual diet** in coyotes (Fedriani & Kohn 2001)

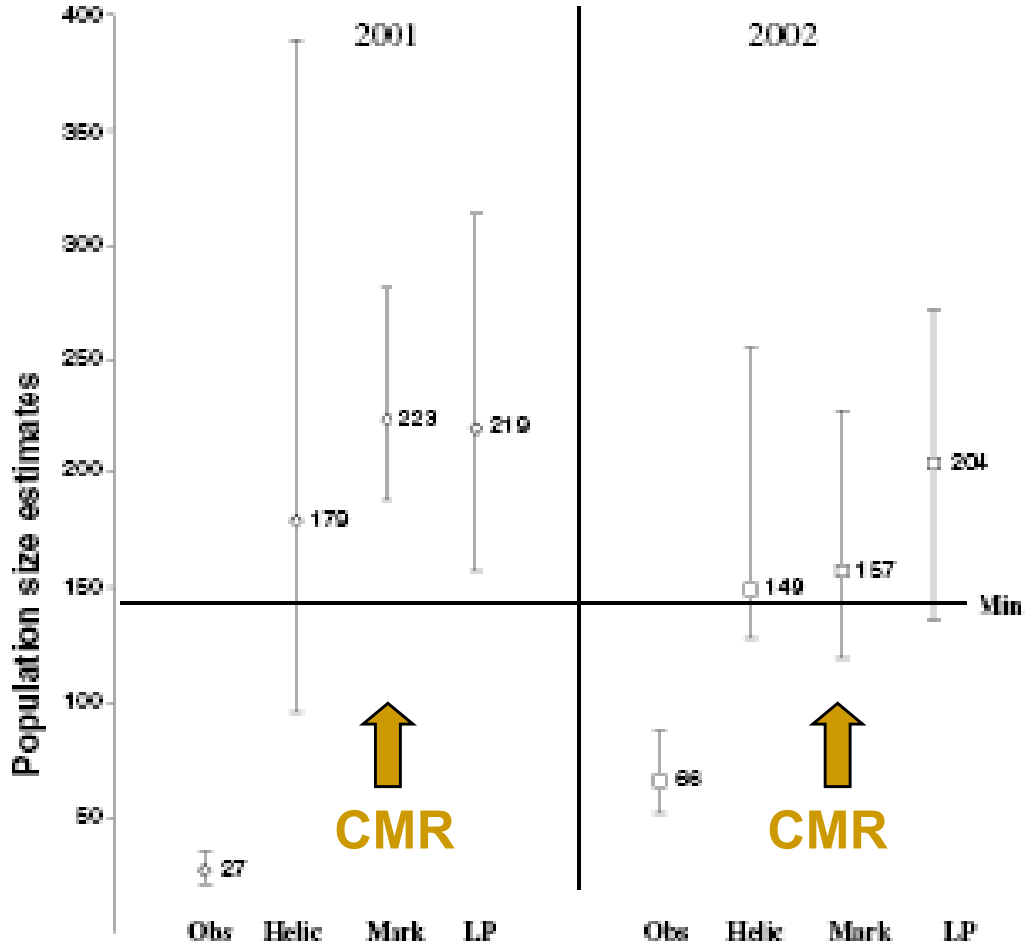
Non-invasive CMR studies

- **population size**
- „capture-mark-recapture“ - review in Lukacs & Burnham (2005)
- repeated sampling of the same animal
- survival rate, capture rate (amplification success), recruitment etc.
- closed population models, open population models, Robust design models
- corrections for genotyping errors

Population dynamics of coyotes (Prugh et al. 2005)

Brown bears (*Ursus arctos*) in Scandinavia

(Hakon Solberg et al. 2006, Biological Conservation)

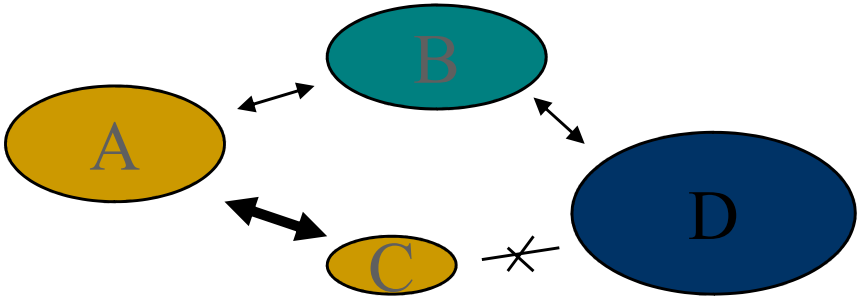


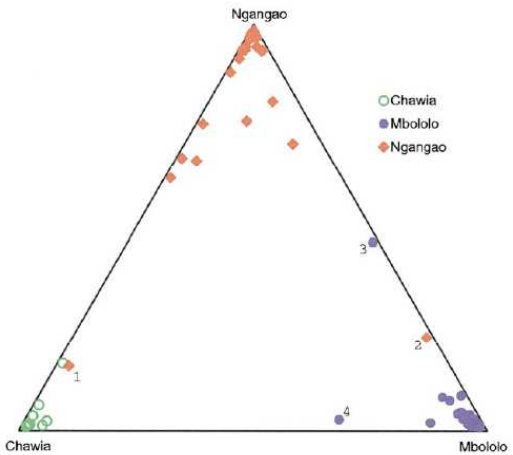
- observations – underestimate numbers
- non-invasive CMR is cheaper and more precise than helicoptere census

Population genetics

- **genetic structure of populations**

- effective population size N_e
- gen flow (often sex-specific)
- identification of past bottlenecks
- origin of migrants („assignment tests“)
- detection of inbreeding
- founder contribution
- „management units“
- hybrids
- gene flow barriers

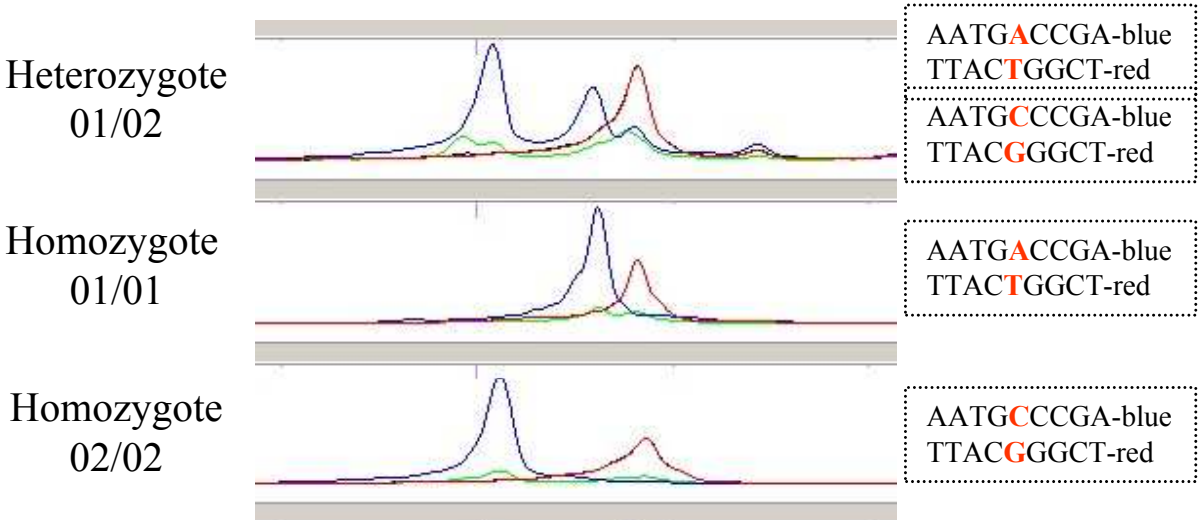




Bayesian approach
STRUCTURE

Adaptive variation of populations

- MHC („major histocompatibility complex“) variation – PCR and SSCP
- comparison of MHC and neutral (microsatellites) variation – detection of contemporary selection
- faeces – parasitological examination – correlation between MHC and parasite prevalence



• simple screening by fluorescent PCR
•capillary electrophoresis (CE)-SSCP
• both strands are fluorescently labeled

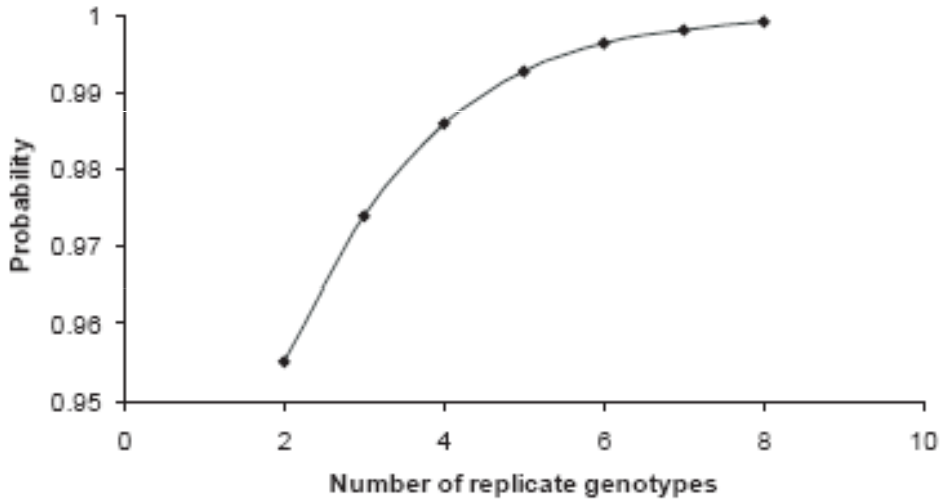
Disadvantages and their solutions

- low quality and/or quantity of DNA – low success rate of genotyping and high contamination risk

- identification of crucial factors of succesfull analysis
- avoiding of genotyping errors and contamination

Increase of genotyping success rate

- multi-samples, multi-extracts (Goosens et al. 2000)
- PCR - multiple-tubes approach (Taberlet et al. 1996)
- cost and time-consuming
- pilot studies are reasonable



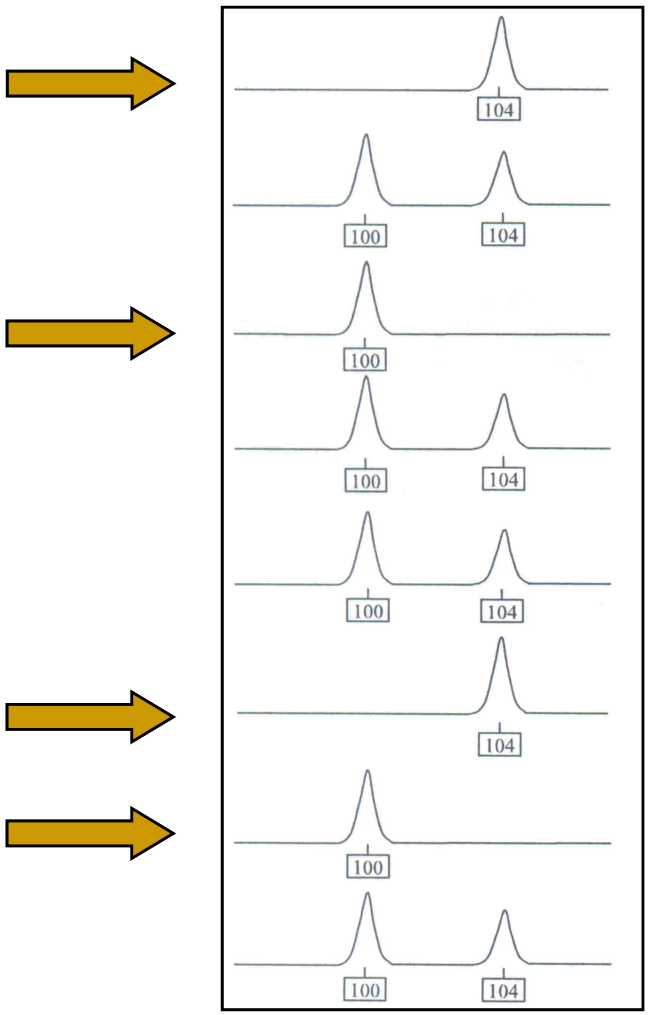
(Parsons 2001)

Dolphins - tissues vs. fresh faeces

100% probability of obtaining at least two correct genotypes when analysing 8 samples

Genotyping errors I.

- **allelic drop-out**
- very low concentration of DNA in samples - only one allele in heterozygotes is amplified
- **multiple tube approach**
- **statistical correction**



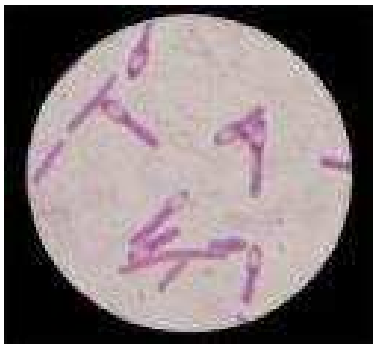
Heterozygote 100/104
(8 different PCRs)

Genotyping errors II.

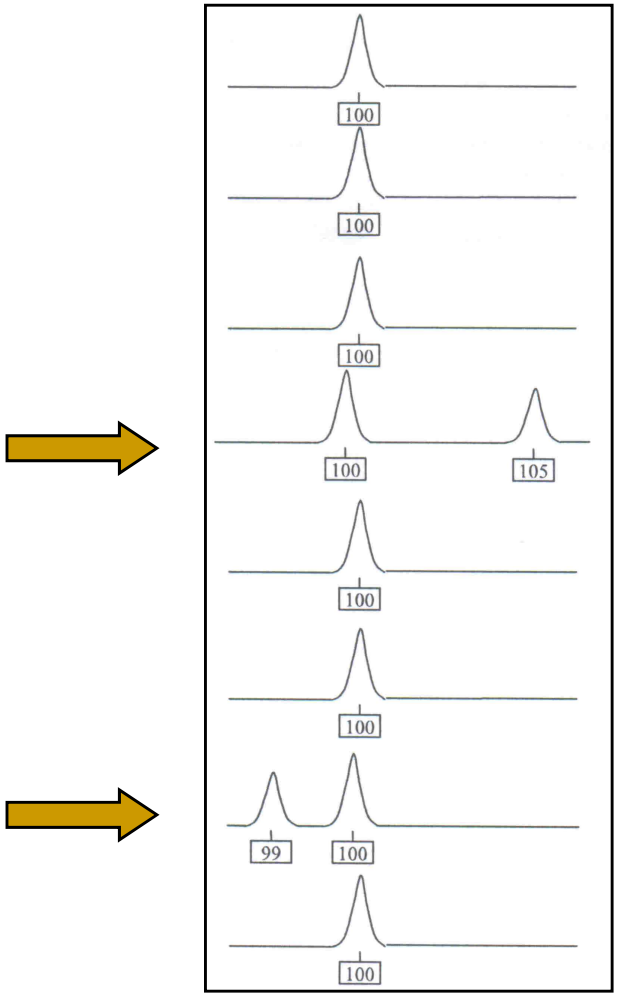
- **false alleles**
- PCR artefacts – rarely replicated when using „multiple-tubes“ approach
- co-amplification of microbial DNA from faeces (Bradley & Vigilant 2002) – confusions with „real“ alleles

gorilla

x



Clostridium



Homozygote 100/100
(8 different PCRs)

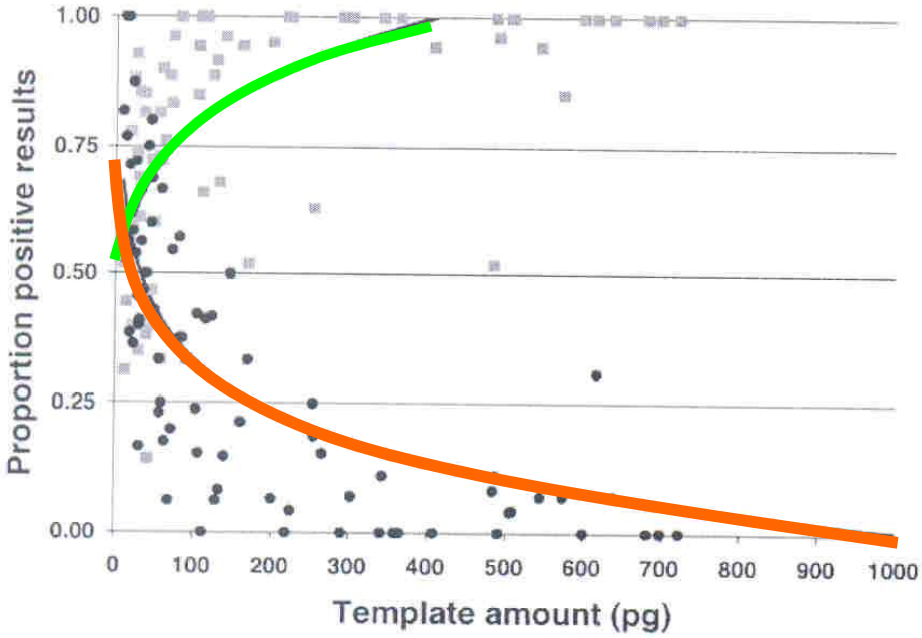
Increase of DNA concentration

- pre-amplification (Bellemain & Taberlet 2004)

Multiplex preamplification of all loci – increase of microsatellite DNA

Semi-nested PCR for 1-3 loci with one internal and one external primer

- quantitative PCR (Morin et al. 2000)

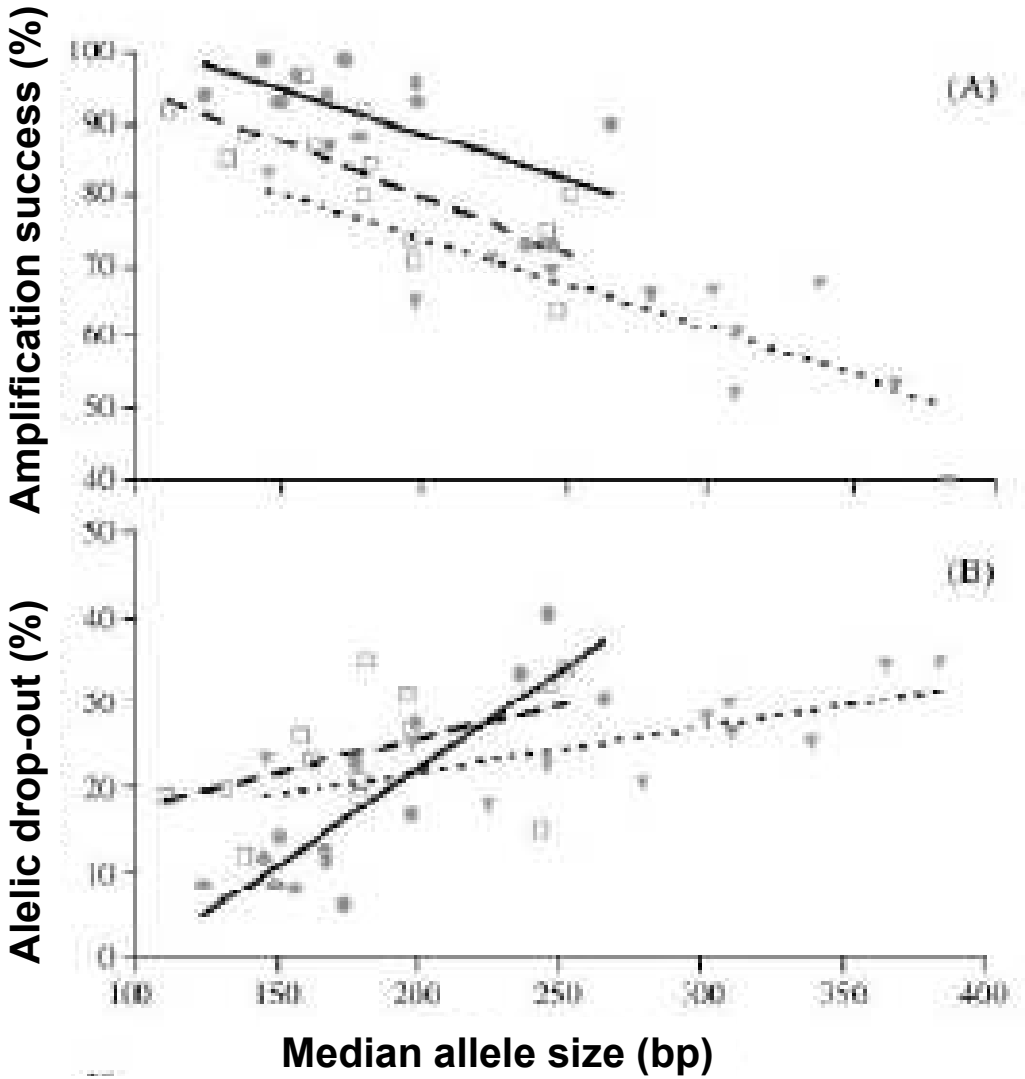


Positive PCR Allelic dropout

Genotyping of only „good“ samples

Disadvantages and their solution

Effect of locus



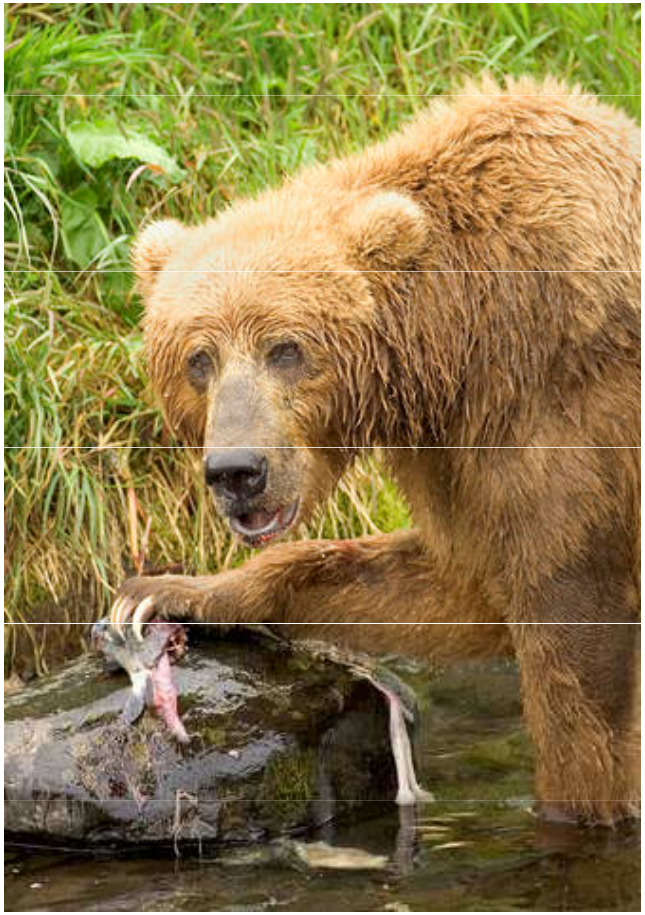
(Buchan et al. 2005)

Degraded DNA → amplification of **short fragments** is preferred

Disadvantages and their solution

Influence of diet on faecal DNA amplification

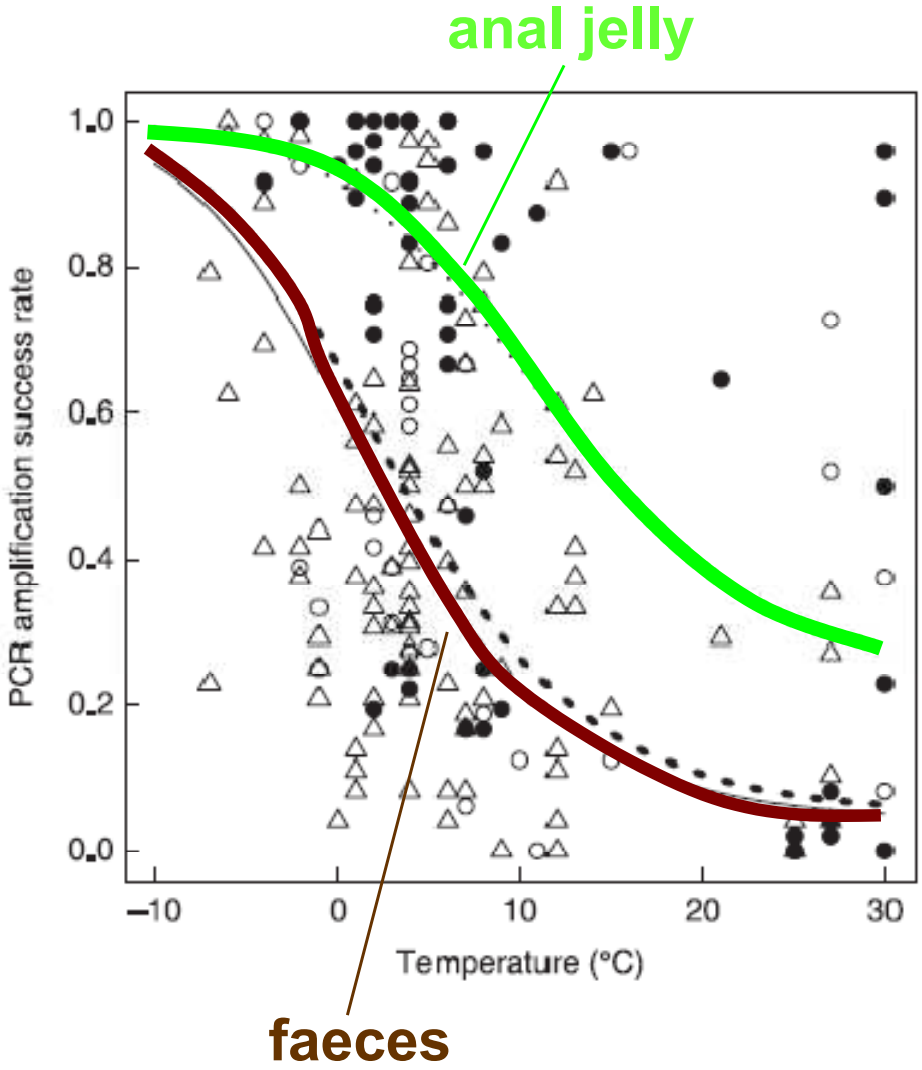
- poorly known
- Murphy et al. 2003 – brown bears
- salmons in the diet – significant decrease of amplification success
- herbivores – better results than carnivores



Effect of sample type and temperature

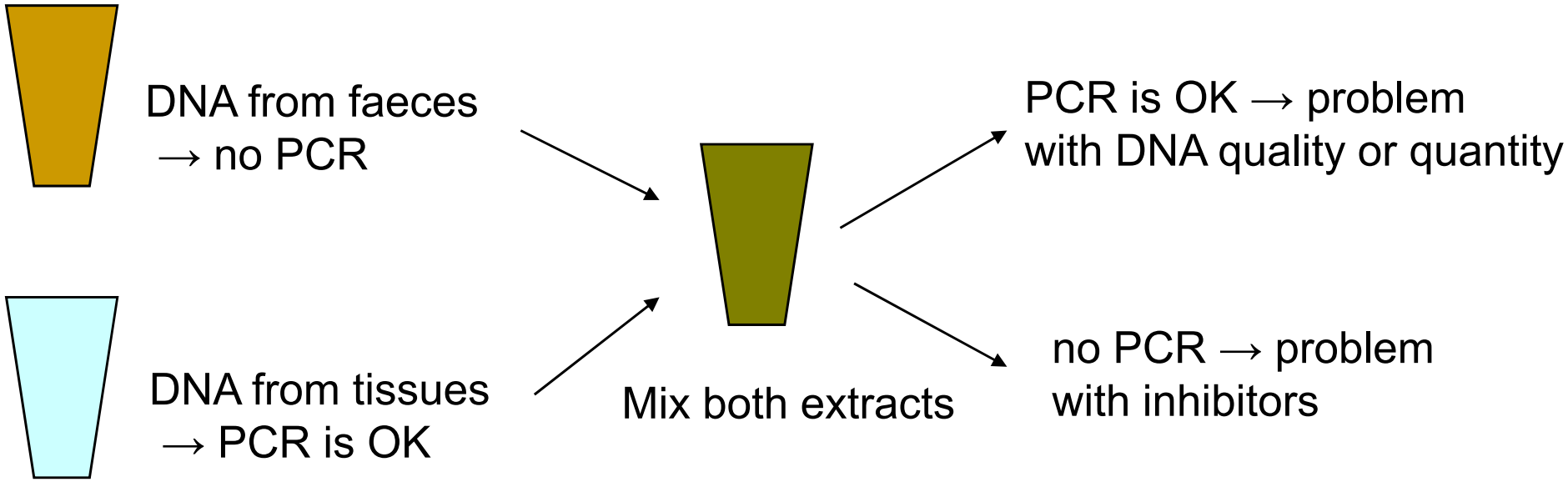
- otters – fish eating carnivores
- effect of sample type: anal gelly (82%) vs. faeces (34%) in frozen faeces of otters
- effect of temperature
- very quick degradation of DNA

Hájková et al. 2006



Effect of PCR inhibitors (faeces)

- many inhibitors in faeces (products of digestion, chemicals in plants) – addition of special reagents (BSA), hot-start etc., dilution of template etc.



High contamination risk

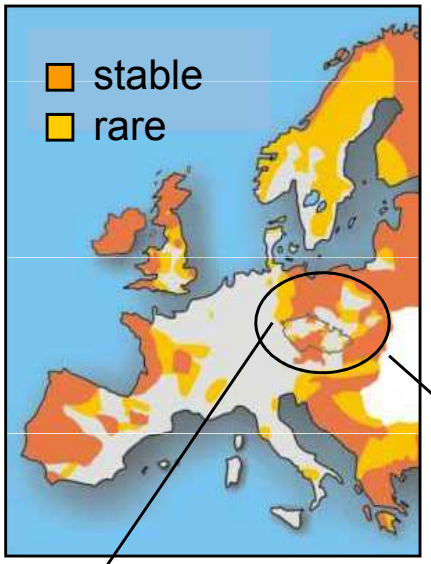
- avoiding of „laboratory“ contamination (tips with filters, separated pre- and post-PCR laboratories, UV sterilisation, etc.)
- „mixed samples“ – problems in social species (communal latrines, marking in fixed sites) or in sampling at broad intervals („hair traps“) – usually identified by 3 or more alleles/sample; problem in species with low genetic variability
- primates – contamination with human DNA

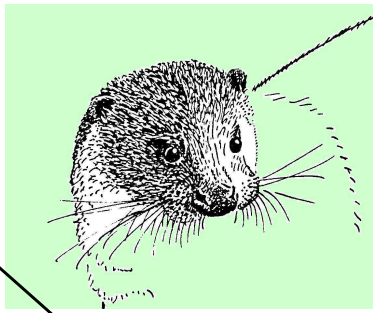
Otters in central Europe

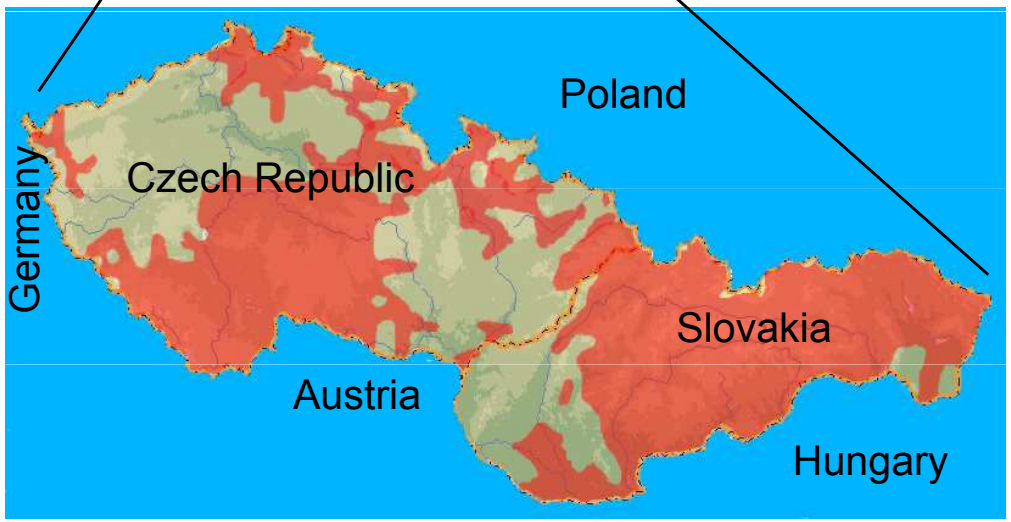
- strong decline of population numbers in last century
- fragmentation of distribution area

Aims:

- estimate population numbers from faeces
- population genetic analysis – identification of gene flow barriers, Ne, bottlenecks etc.
- spatial activity in different habitat types and relatedness of individuals







(P. Hájková – Ph.D. thesis, B. Zemanová – M.Sc. thesis)

Methods and results

- succesful identification of otter faeces by multiple-tube approach (more than 20000 PCRs - Hájková et al. 2006) – sample type and temperature are most important factors
- individual identification – abundance is much higher in fishpond areas than in mountains
- Czech and Slovak populations are genetically separated (F_{st} = 0.15)
- evidence for recent bottleneck 15-30 years ago (Bottleneck, MSVAR) (Hájková et al. 2007)
- analyses of spatial activity and relatedness (Zemanová 2006)

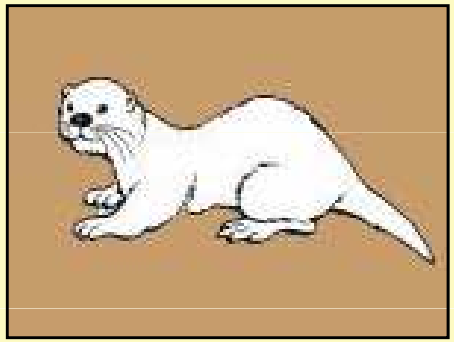
Map of study area with identified otter individuals

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



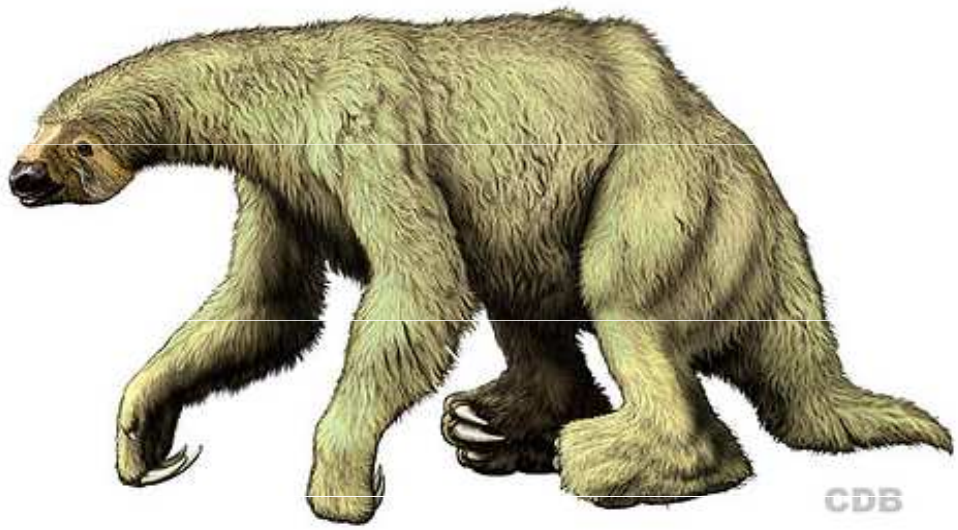
Hornad

Spatial activity
in mountains

500 m

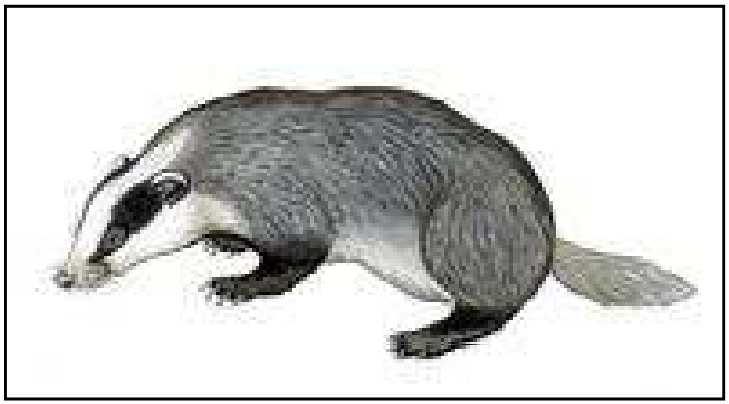
Diet of the extinct Ground Sloth (*Nothrotheriops shastensis*)

- Poinar et al. (1998) – Science
- 20 000 years ago
- chemical modification of DNA in ancient faeces before PCR
- identification of species and phylogeny to modern mammals
- cpDNA – diet of the Ground Sloth



Badger hair in luxury shaving brushes

- Domingo-Roura et al., Biological Conservation (2006)



Eurasian badger (*Meles meles*)

x

hog badger (*Arctonyx collaris*)

- 4 brushes from 8 came from the Eurasian badgers
- 3 of them from the Netherlands where it is illegal to possess, sell, transport or use for commercial purposes dead European badgers or products derived from them

Praktické problémy ochranářské genetiky

- mladé odvětví = mnoho problémů
- význam genetické variability pro životaschopnost populací
- extrémní neznalost adaptivní variability u volně žijících druhů (MHC apod.)
- identifikace ochranářských jednotek (ESU, MU apod.) na základě genetických dat → praktická ochrana
- neinvazivní metody – nutno snížit ceny a zvýšit přesnost – optimalizace počtu analyzovaných znaků pomocí počítačových simulací