

Esempi e casi studio

Primo esempio

DNA e tracciabilità alimentare



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Food Research International

journal homepage: www.elsevier.com/locate/foodres



Review

DNA barcoding as a new tool for food traceability

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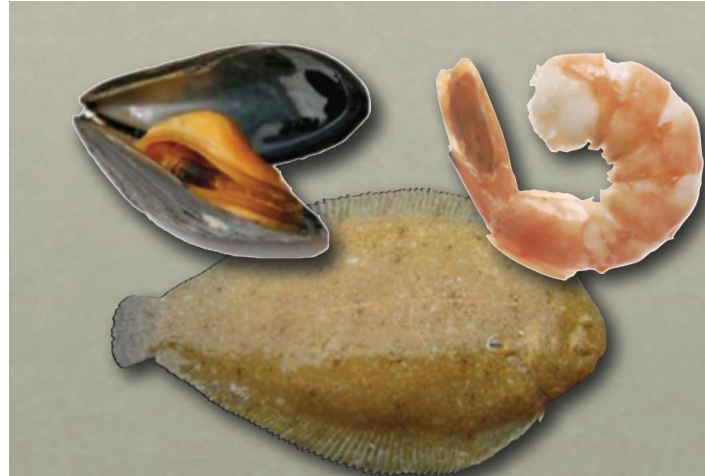
^b *Università degli Studi di Trieste, Dipartimento di Scienze della Vita, via L. Giorgieri 10, 34127 Trieste, Italy*

A B S T R A C T

Food safety and quality are nowadays a major concern. Any case of food alteration, especially when reported by the media, has a great impact on public opinion. There is an increasing demand for the improvement of quality controls, hence addressing scientific research towards the development of reliable molecular tools for food analysis. DNA barcoding is a widely used molecular-based system, which can identify biological specimens, and is used for the identification of both raw materials and processed food. In this review the results of several researches are critically analyzed, in order to exploit the effectiveness of DNA barcoding in food traceability, and to delineate some best practices in the application of DNA barcoding throughout the industrial pipeline. The use of DNA barcoding for food safety and in the identification of commercial fraud is also discussed.

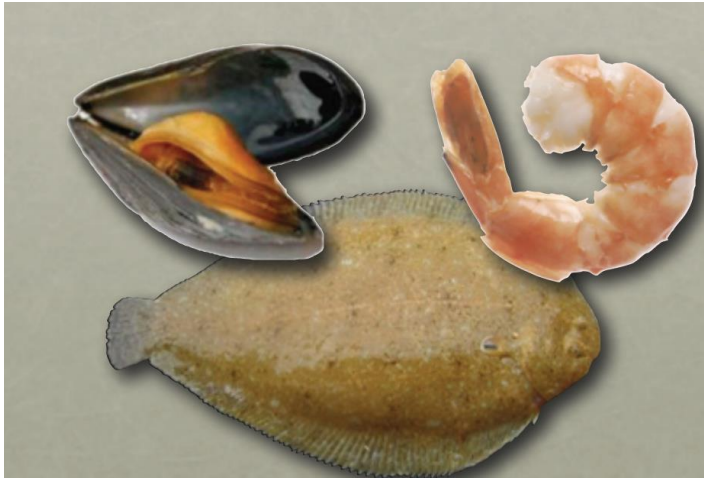
3.1. Seafood traceability and FISH BOL

“seafood”: termine usato per indicare forme di vita acquatica commestibile



Il DNA barcoding funziona per seafood perchè:

1. Alto numero di specie
2. Approcci di identificazione classica spesso non funzionano, soprattutto su cibo processato
3. Per questi organismi il livello di riconoscimento molecolare può andare oltre quello di specie, fino alle varietà permettendo un'ottima tracciabilità dei prodotti



cox1 buon potere di discriminazione per le specie di pesci (98% specie marine, 93% pesci di acqua dolce)

<http://www.fishbol.org>



iBOL WORKING GROUP | 1.1 VERTEBRATES

Fish barcode of life (FISH-BOL)

international
BARCODE
OF LIFE



Approcci di DNA barcoding basati sulla *cox1* non sono sempre di successo: non funzionano ad esempio per i gasteropodi



3.2. DNA barcoding and meat traceability: the problem of the lack of data

- Carne: lunga catena di produzione e distribuzione > necessità di un Sistema di tracciabilità
- Patologie (e.g. BSE, avian flu), e sofisticazioni illegali: consapevolezza nel consumatore riguardo alla qualità della carne



- Marcatore per barcoding: tante sequenze in banca dati per mtCytB come alternative alla Cox1

- Problemi:
- Presenza di sottotipi molecolari (esempio: cervo)



Cervo nobile (*Cervus elaphus* Linnaeus, 1758), noto anche come cervo reale, cervo rosso o cervo europeo



Il wapiti o vapiti (*Cervus elaphus canadensis* Erxleben, 1777) è una (sotto?)specie di cervo nobile diffusa in Nordamerica e nell'Asia nordorientale

- Problemi:
- Presenza di sottotipi molecolari (esempio: tacchino)



Meleagris gallopavo: molta variabilità interna alla specie



Rosso di
Borbone



Bianca
gigante



Nero
americano



Tacchino
Narragansett



Bronzato
d'America



Tacchino
bianco
d'Olanda



Bianca di
Beltsville

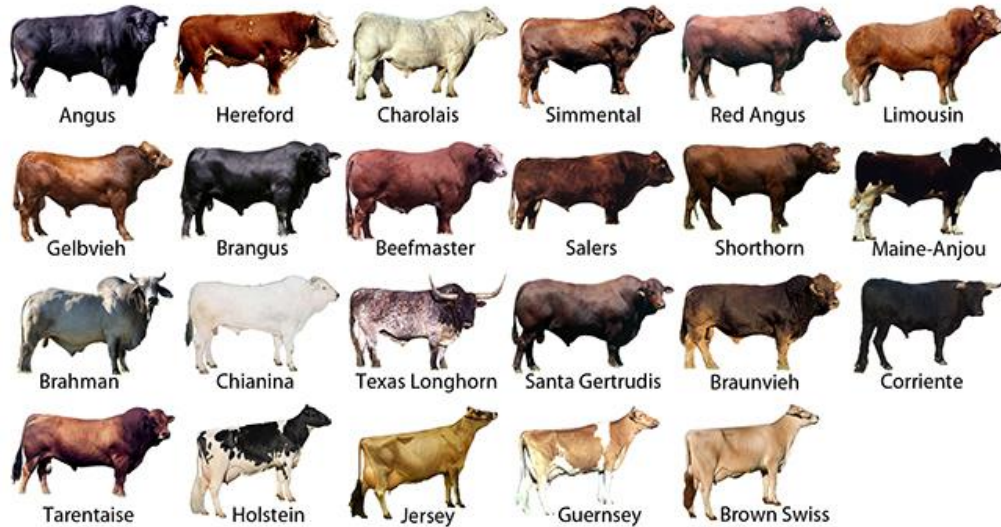


Slate turkey



Dindon rouge
des Ardennes

- Problemi:
- Specie diverse con lo stesso marcatore (poca variabilità interspecifica a causa di ibridazione)



The beef and milk cattle breeds

ibrido fra il bue domestico (*Bos taurus*) e il bisonte americano (*Bison bison*)



Bisonte americano



Beefalo

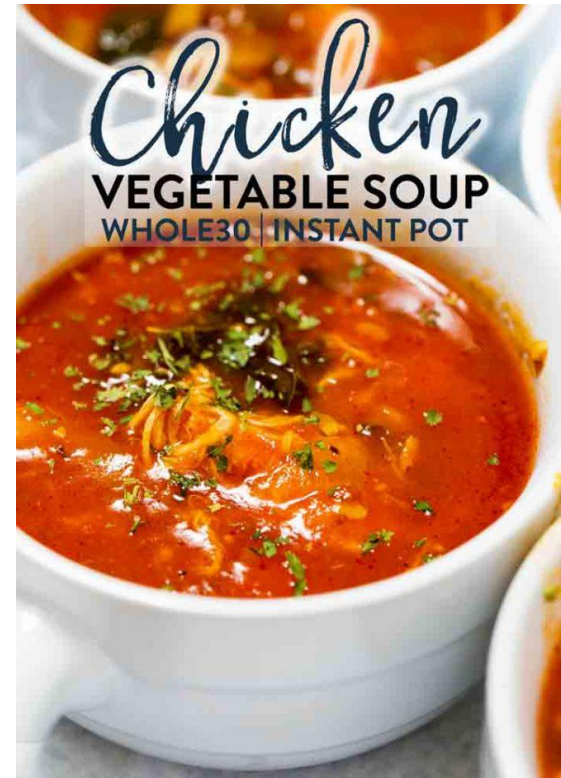
4. DNA barcoding as a traceability tool during food industrial processing

4.2. Characterization of mixed food products

Le regioni scelte per DNA barcoding e i primers usati per l'amplificazione sono universali (Hebert et al., 2003).

Amplificazioni (PCR) di campioni di DNA che derivano da matrici di cibo misto producono frammenti di diversa origine (diverse OTUs o specie).

Sanger-based DNA sequencing non è utilizzabile con campioni di cibo "misto" a meno di farlo precedere da un clonaggio, che comunque introdurrebbe errori

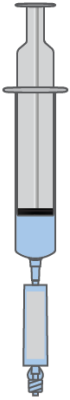


Applications

mixed food matrices

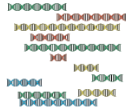
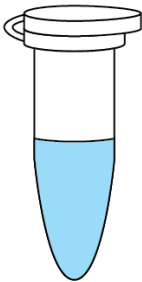
1

Collect an environmental sample



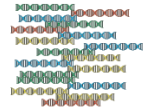
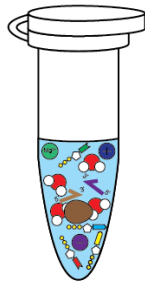
2

DNA extraction from environmental sample



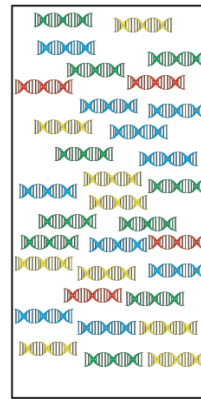
3

Amplify DNA markers



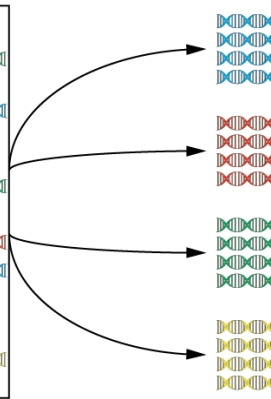
4

High-throughput sequencing



5

Bioinformatic processing



6

Species identification



5. Food safety and commercial frauds

Effetti negative sulla salute umana possono derivare da sostituzioni accidentali o deliberate di una specie con un'altra

Esempio1 : *Lates niloticus*, conosciuto comunemente come Persico del Nilo o Persico africano. Commercio soggetto a restrizioni, spesso usato al posto di altri persici.

Conseguenze economiche + sanitarie: il persico del Nilo che viene da fiumi africani è contaminato da metilmercurio ed altri contaminanti



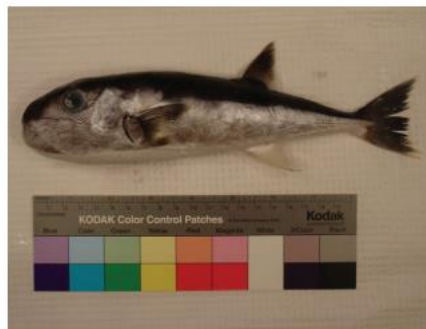
Journal of Food Protection, Vol. 72, No. 4, 2009, Pages 810-817

Public Health Response to Puffer Fish (Tetrodotoxin) Poisoning from Mislabeled Product

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Esempio2: il tossico pesce palla venduto come coda di rospo

Representative puffer fish (*Lagocephalus* sp.) from North American waters.



(A) Whole monkfish (authenticated *Lophius litulon*) and processed monkfish tail.

DNA barcoding reveals fraudulent substitutions in shark seafood products: The Italian case of “palombo” (*Mustelus spp.*)

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^b Mercato Ittico all'Ingrosso, ASL Città di Milano, Viale Molise, 20, 20137 Milan, Italy

^c Dipartimento di Biotecnologie e Bioscienze, Water Ecology Lab, Università di Milano Bicocca, P.zza della Scienza 2, 20126 Milano, Italy

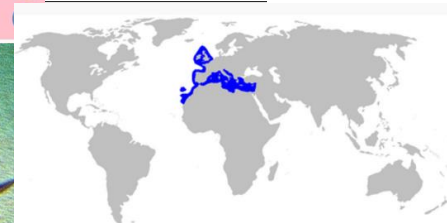
45 samples were isolated from slices or fillets (i.e. white muscular tissue) of sharks sold as “palombo” (we checked the name reported on the label) in different Italian fish markets and supermarkets.

DNA marker: COI

Only three (6.7%) can be unequivocally assigned to “palombo” and in particular to the species *M. mustelus*.

Further, six cases out of 45 (13.3%) refer to species belonging to the genus *Mustelus*. Altogether, we identified 35 cases of species substitution out of 45 (77.8%) collected samples.

Palombo



Mustelus mustelus

Spinarolo



Squalus acanthias

Squalo mako



Isurus oxyrinchus

Translating Omics to Food Microbiology

Aaron M. Walsh,^{1,2,3} Fiona Crispie,¹
Marcus J. Claesson,^{2,3} and Paul D. Cotter^{1,2}

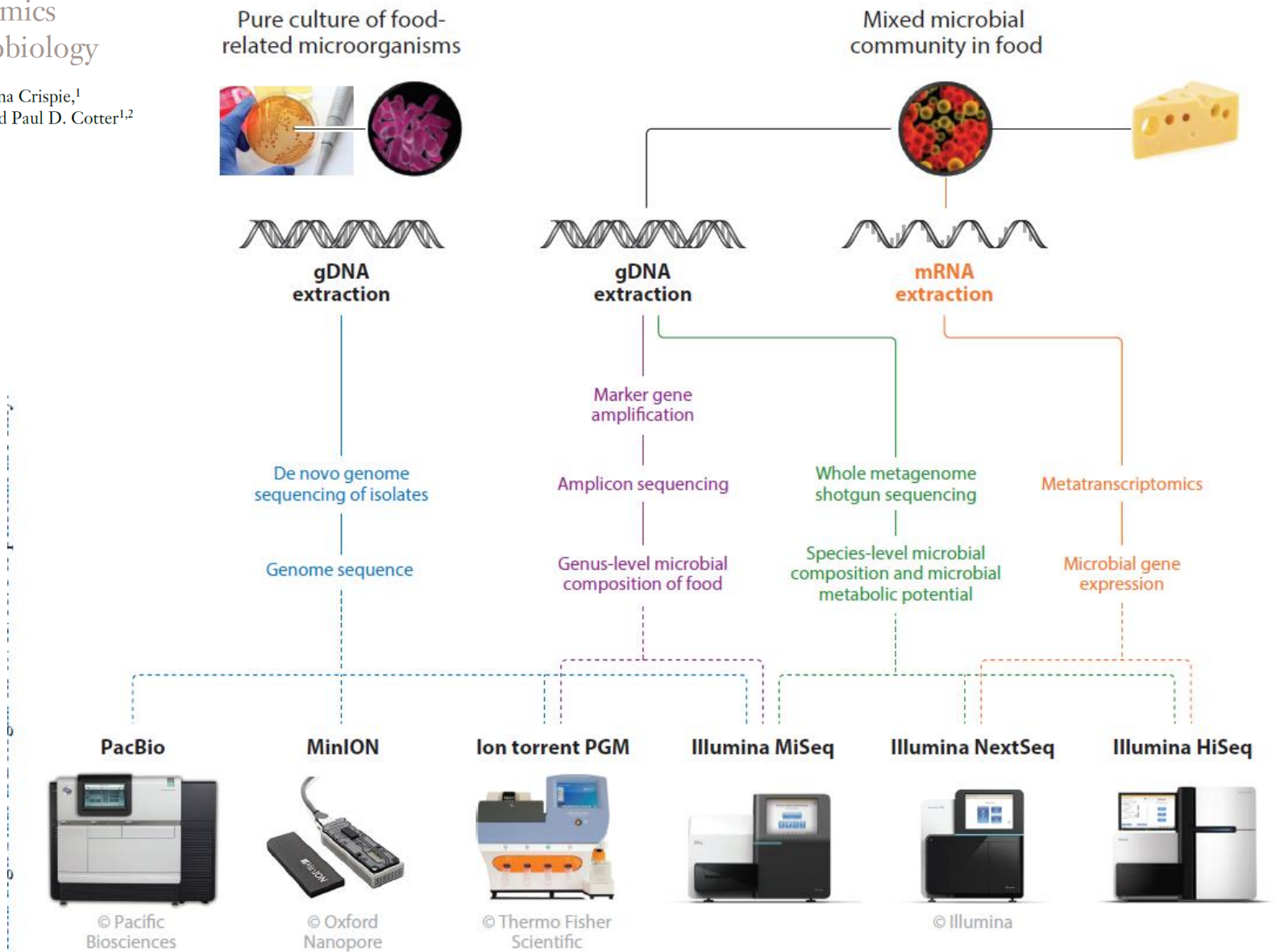


Figure 1

Schematic overview of the different high-throughput sequencing approaches applicable to food microbiology and suggestions for the sequencing platforms most suitable for each approach.

Secondo esempio

genetica della conservazione e
gestione delle specie



Università
degli Studi
di Ferrara

Ph.D. Course
in Evolutionary Biology and Ecology

CYCLE XXXI

DIRECTOR Prof. Guido Barbujani

Testudo hermanni:
conservation genetics and genomics



Candidate: **Roberto Biello**

Supervisor: **Prof. Giorgio Bertorelle**

Years 2015/2018

T. hermanni is included in the list of strictly **protected fauna species** by the Bern Convention on the Conservation of European Wildlife and Natural Habitat, and the western subspecies *T. h. hermanni* is classified as “**Endangered**” by the IUCN Red List

Testudo hermanni hermanni è la sottospecie con le dimensioni minori



Figura 6. Lunghezza delle suture pectorali e femorali nelle due sottospecie di *T. hermanni* (in alto). Presenza della caratteristica macchia a “buco della serratura” sul V scuto vertebrale in *T. h. hermanni* (in basso).

Testudo hermanni: aspetti di genetica e genomica di conservazione



Figura 1. Testuggine di Hermann (*Testudo hermanni*). (studbooks.eu)

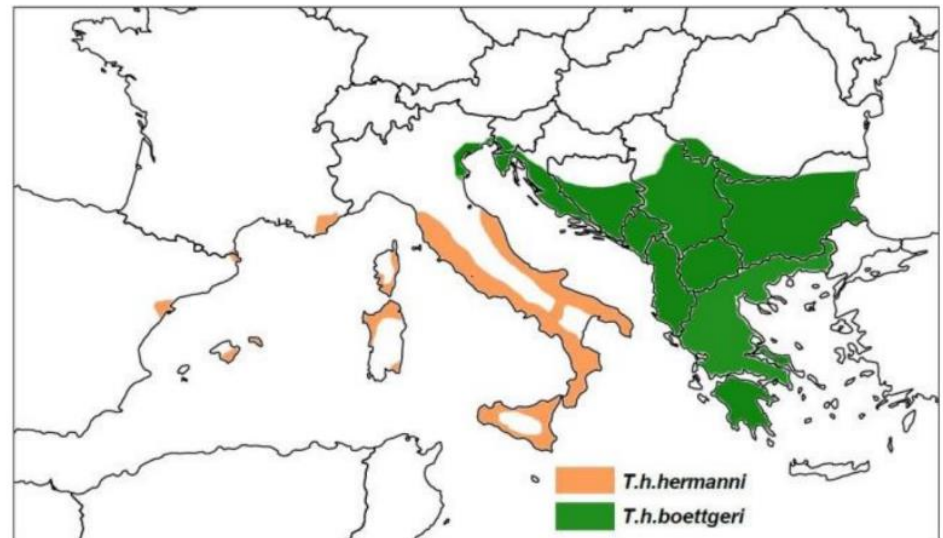


Figura 3. Areale di distribuzione delle due sottospecie di *T. hermanni*.

La distribuzione italiana di questa specie è frammentata e spesso le segnalazioni riguardano individui isolati dei quali non è accertata l'origine autoctona.

Popolazioni di *Testudo hermanni* fortemente in declino nella maggior parte del range di distribuzione.

Declino probabilmente iniziato in epoca antica (ultimo periodo del Pleistocene), specialmente in Francia e Spagna

Oggi in Spagna, Francia ed Italia: distribuzione fortemente discontinua e popolazioni in forte calo demografico.

Lo status delle popolazioni Balcaniche sembra leggermente migliore

Major issue

Major Threats:

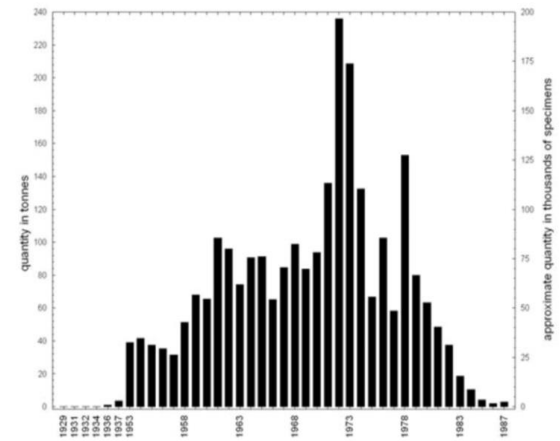
- Habitat reduction
- Intensive agricultural practices
- Forest fires
- Intensive harvesting for pet trade

Illegal Reintroductions

- Thousands of animals released illegally, principally from Balkans to Italy

Captive Animals

- Confiscated by Carabinieri Corps or unrecognized animals holders: many enclosures with animals of unknown origin and difficult to manage



Quantity of animals (in tonnes and number of individuals per year) imported from the former Yugoslavia to Western Europe (Ljubisavljević et al., 2011)

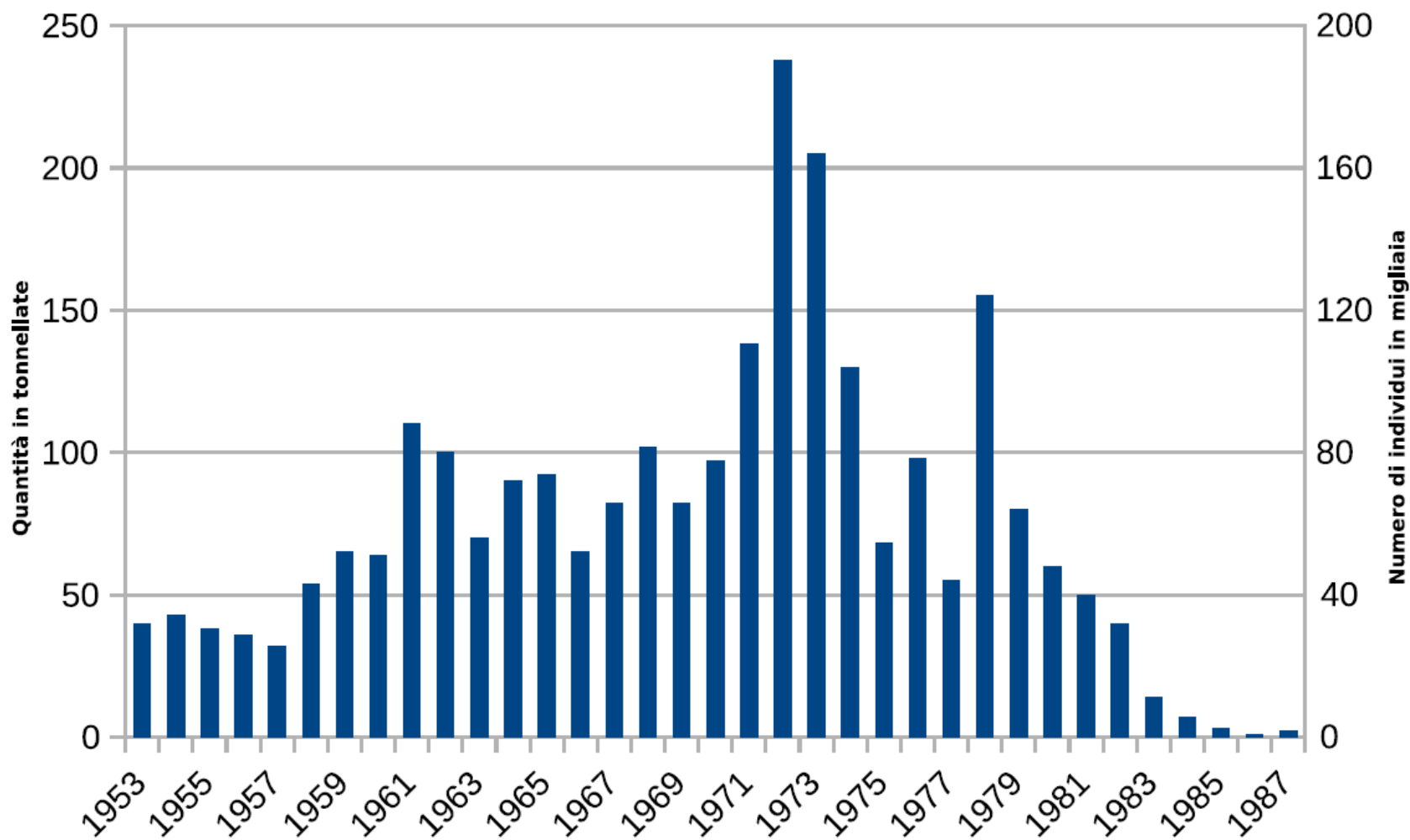


Figura 7. Grafico delle esportazioni annuali di testuggini (*Testudo* spp) dall'ex-Jugoslavia verso l'estero (in tonnellate e in migliaia di esemplari) (riprodotto da Ljubicavljevic et al. 2011).

La genetica può fornire potenti strumenti per aiutare i progetti di reintroduzione e l'aumento delle popolazioni selvatiche.

- Fase 1: precedente al rilasciato in natura.
 - (i) capire la struttura delle popolazioni naturali della specie target per selezionare la giusta popolazione sorgente
 - (ii) verificare l'effettiva estinzione locale per evitare ibridazioni in seguito a reintroduzione
 - (iii) assistere e migliorare gli sforzi di riproduzione in cattività.

- Fase 2: post-rilascio degli individui.
 - seguire gli spostamenti degli individui rilasciati nel tempo (es. analisi delle feci)
 - valutare la dimensione complessiva della popolazione reintrodotta dopo il rilascio
 - stimare il numero di animali che si riproducono e che caratteristiche hanno (identificando geneticamente le parentele)

Obiettivo principale



**Aumentare le conoscenze sulla variabilità genetica e
genomica intra ed interpopolazionistica**

E

**Tradurre queste conoscenze in strumenti di
conservazione e gestione**



Animali in cattività



Campionamento di sangue e variabili morfometriche



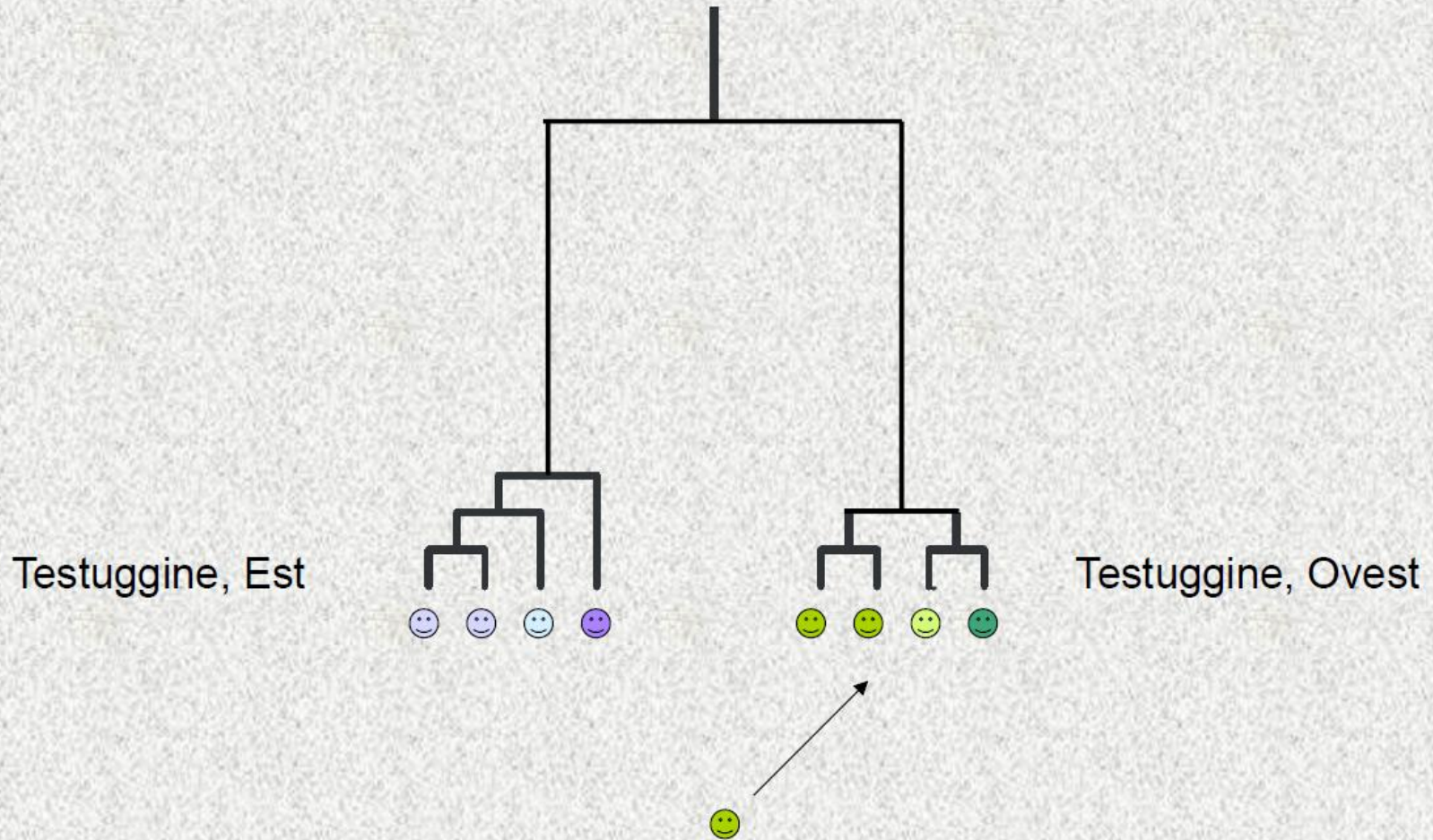
IDENTIFICAZIONE DI VARIETA' : IL CASO DELLA TESTUGGINE



Bisogna fare uno studio preliminare per avere
il database delle varietà !

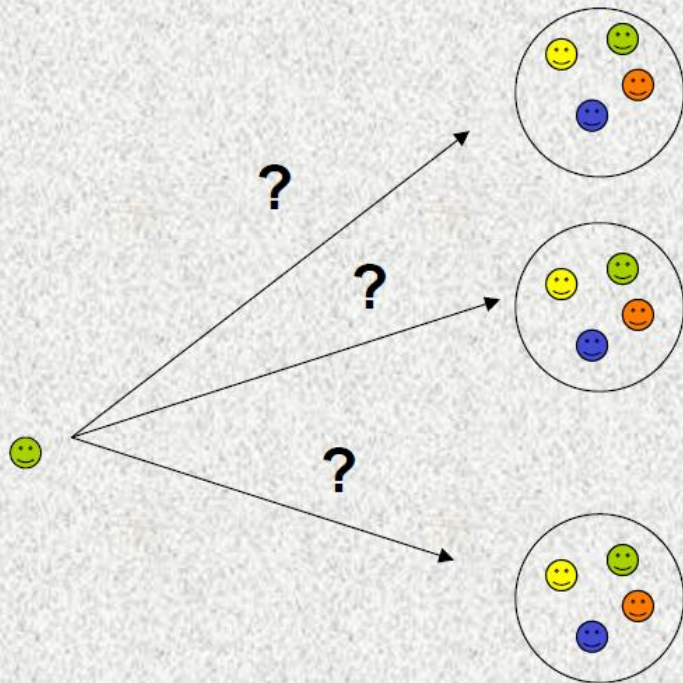
(POCHISSIMI DATI IN GENE BANK)

IDENTIFICAZIONE DI VARIETA' : IL CASO DELLA TESTUGGINE



IDENTIFICAZIONE DI VARIETA' : IL CASO DELLA TESTUGGINE

Spesso più difficile a scala geografica più ridotta



Testuggine, Centro-Italia



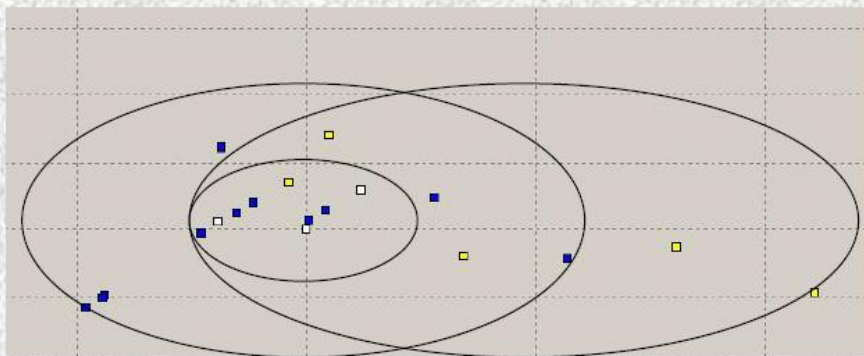
Testuggine, Francia



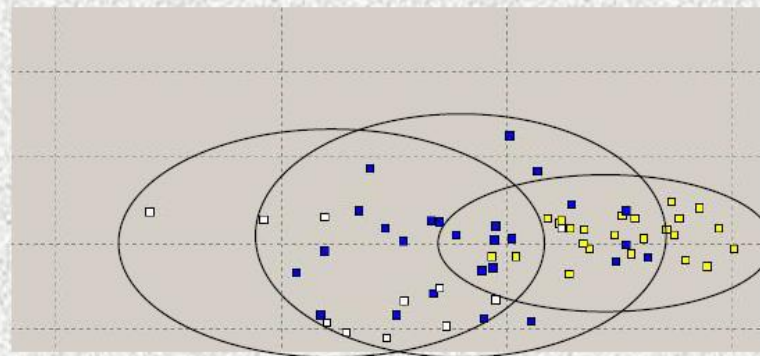
Testuggine, Sicilia

Bisogna ricorrere ai tanti marcatori molto variabili, anche se non sempre è sufficiente: conta la differenza tra varietà

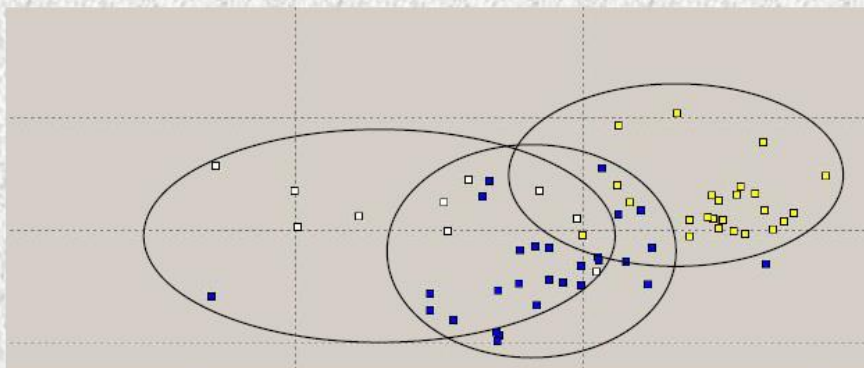
IDENTIFICAZIONE DI VARIETA' : IL CASO DELLA TESTUGGINE



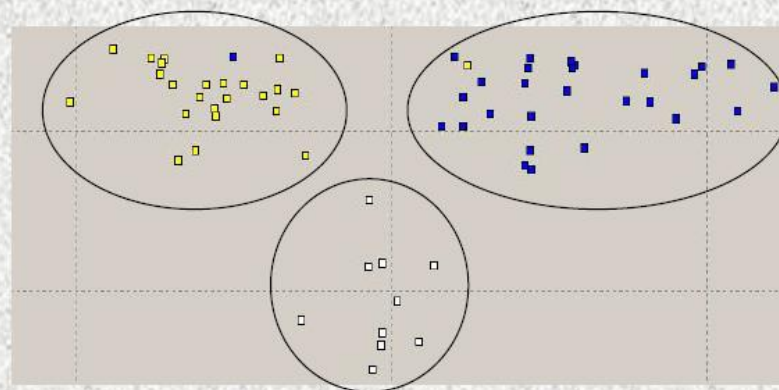
1 marcatore



3 marcatori



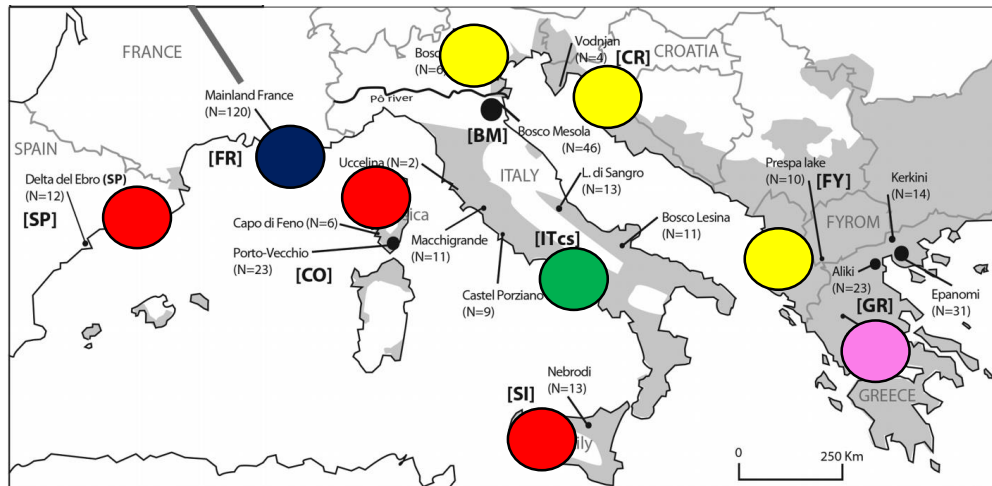
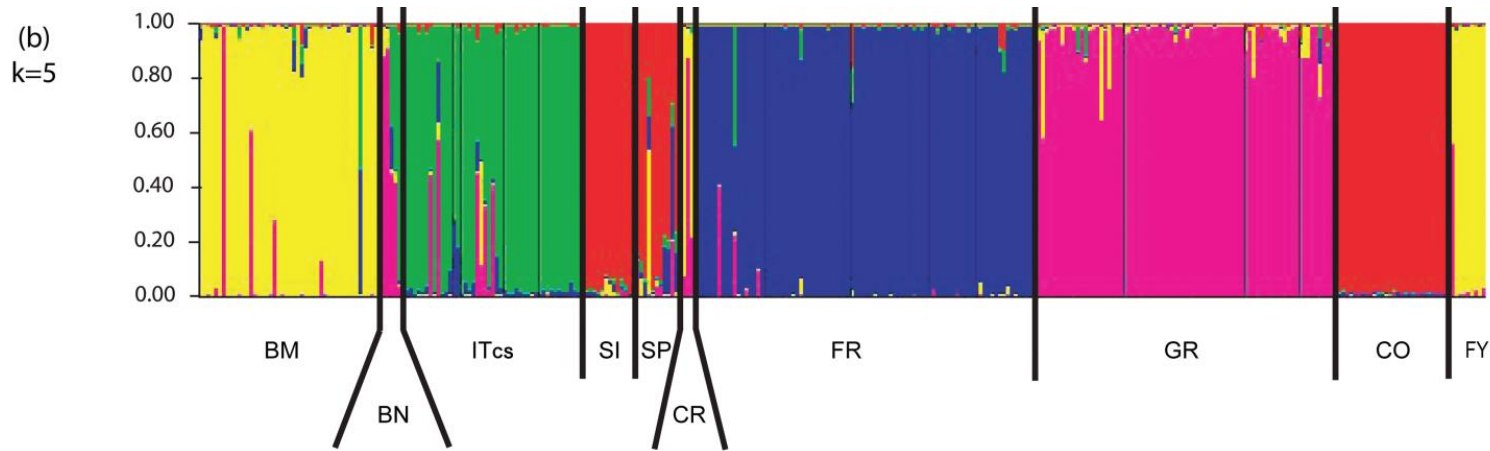
6 marcatori



9 marcatori

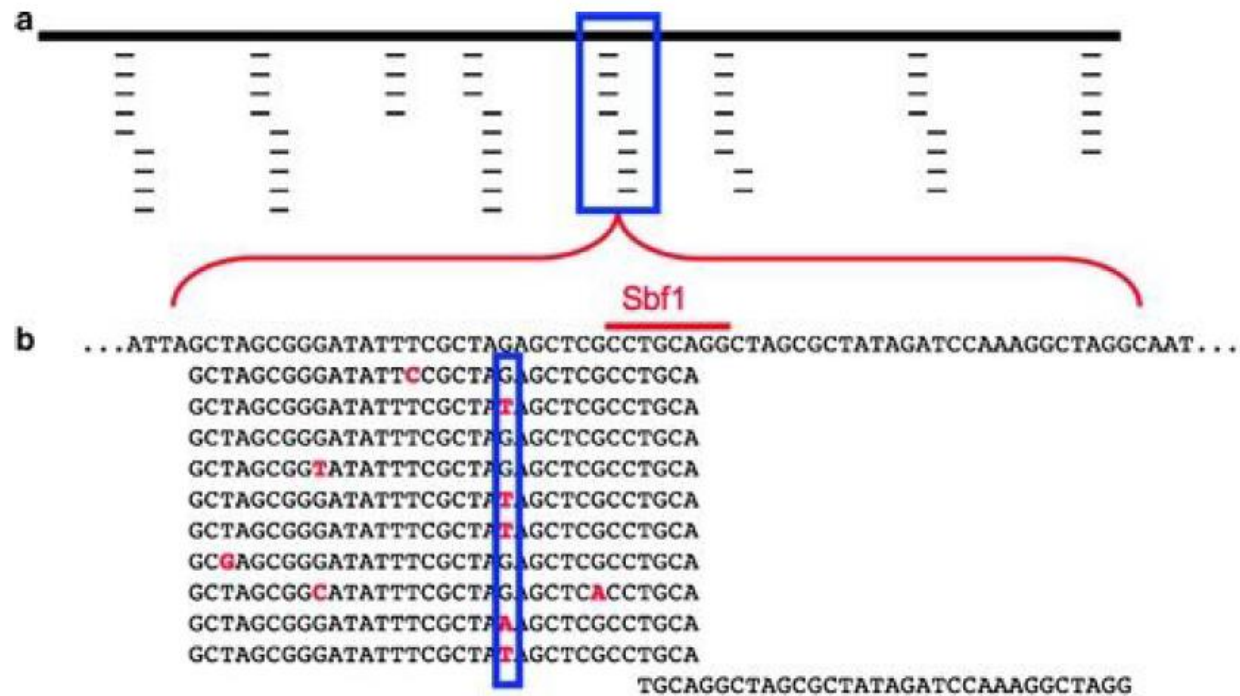
Studi precedenti: Microsatellite Data (STR)

Structure -> identifies populations from the data and assigns individuals to that population representing the best fit for the variation patterns found



- 300 campioni
- 5 clusters genetici
- Pochi siti di campionamento in Italia

RADseq: analyzing the data



With or without a reference genome

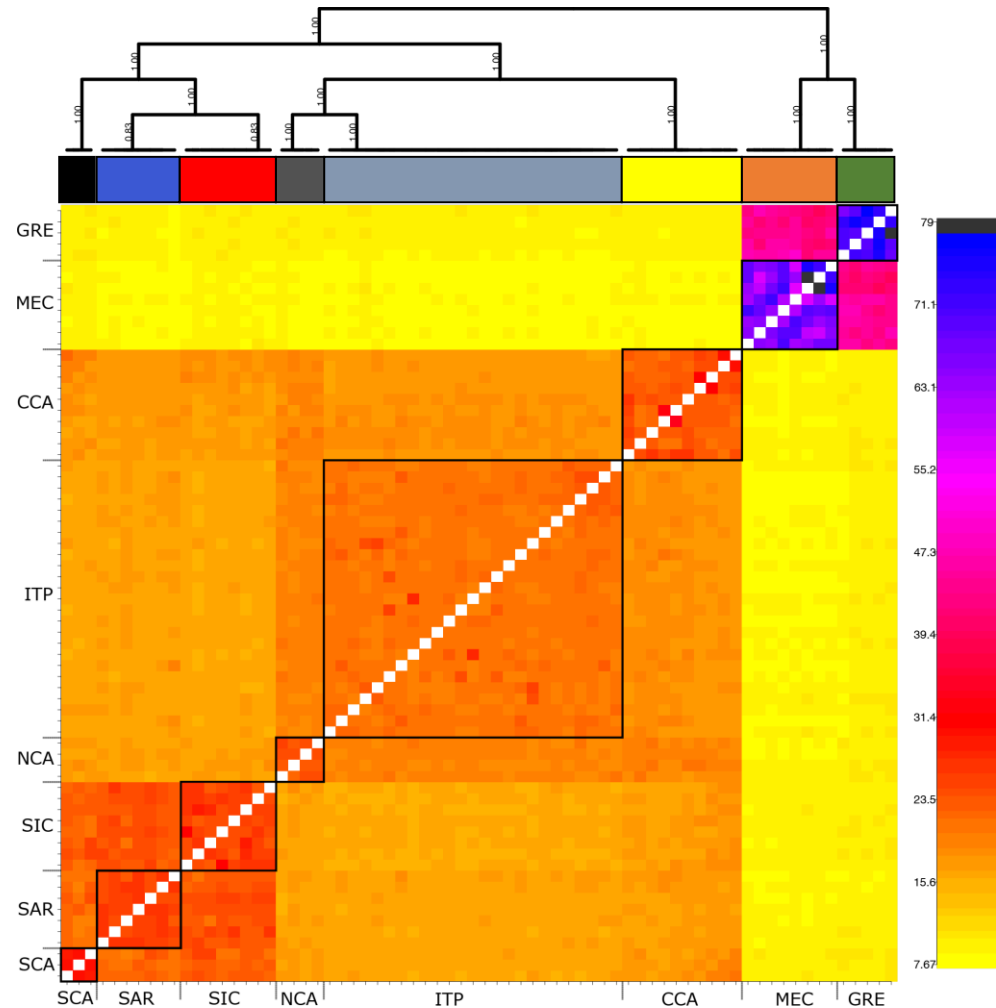
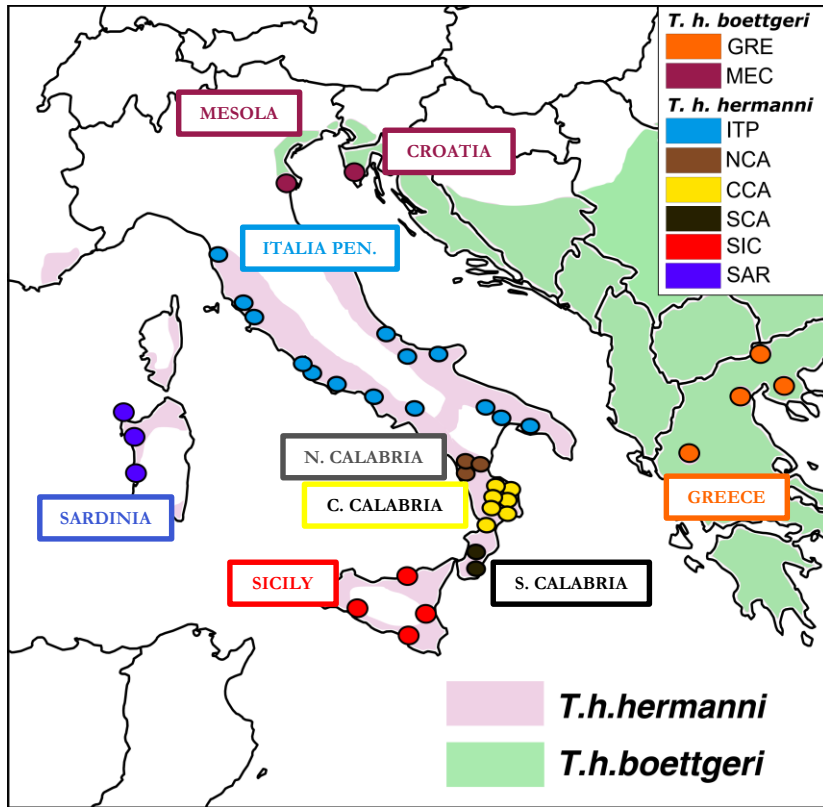
Stacks, PyRAD, ...

Only with a reference genome

Mapping + GATK, ANGSD, ...

Wild populations: fineRADstructure

Co-ancestry matrix -> most recent coalescence (common ancestry) among individuals



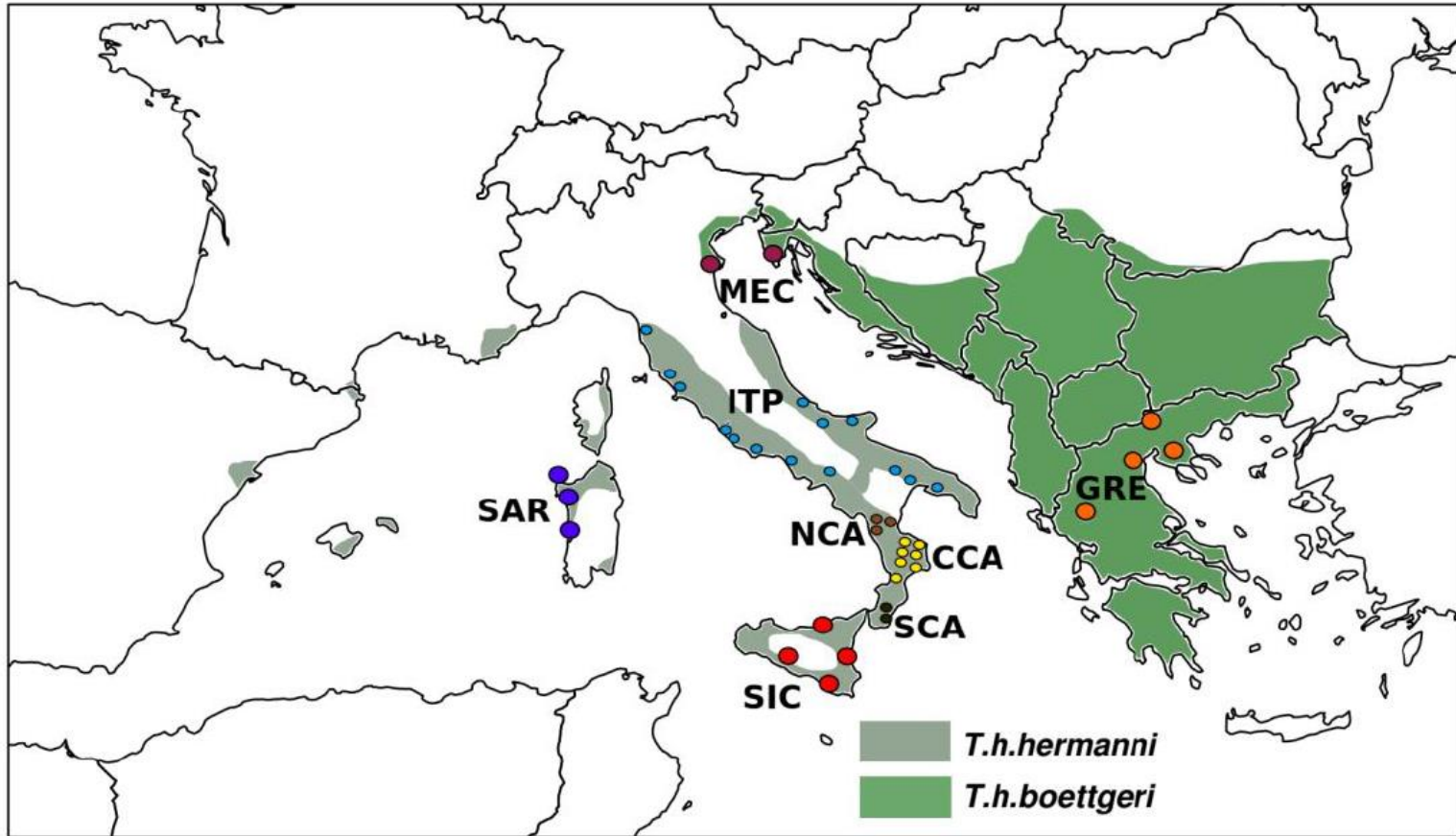
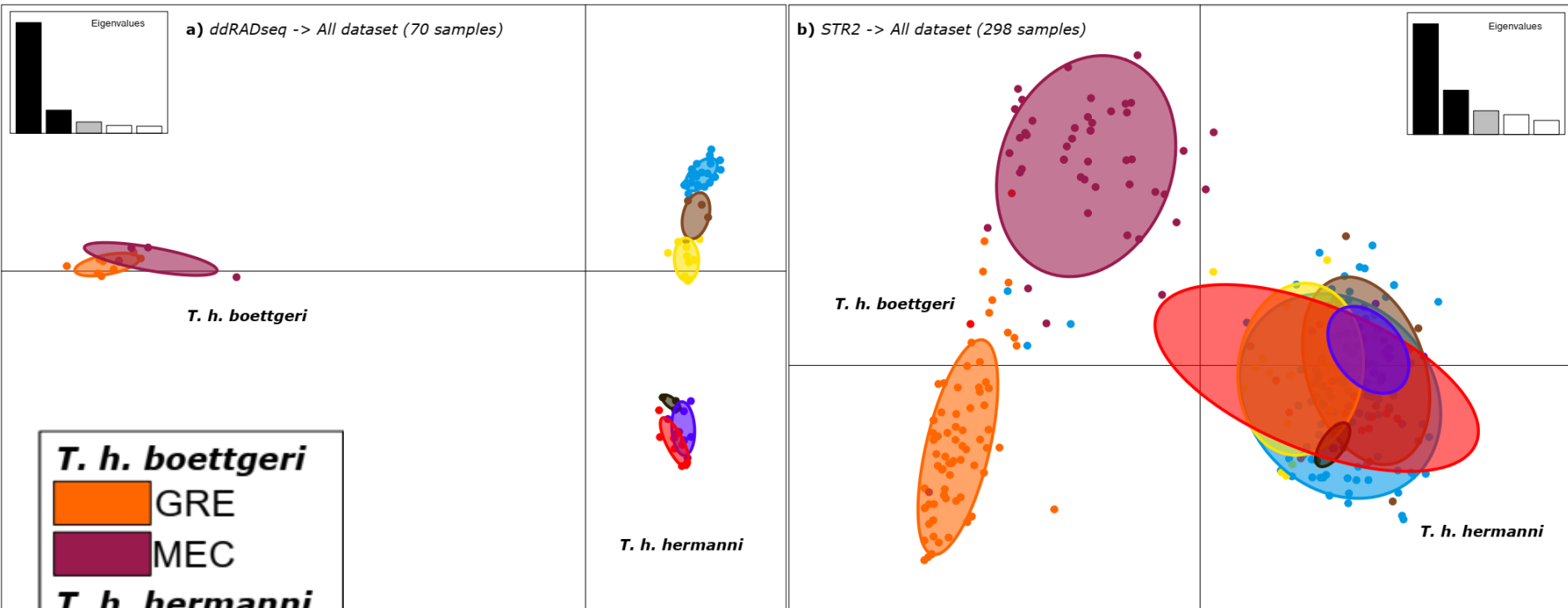


Figura 4. Località di campionamento degli individui utilizzati per il ddRADseq e assegnazione al cluster basata su analisi di co-ancestry svolta con fineRADstructure. GRE = Grecia; MEC = Mesola e Croazia; ITP = Italia Peninsulare; NCA = Nord Calabria; CCA = Centro Calabria; SCA = Sud Calabria; SIC = Sicilia; SAR = Sardegna.

Comparison of STR and ddRADseq data: PCA

ddRADseq

STR



T. h. boettgeri

GRE

MEC

T. h. hermanni

ITP

NCA

CCA

SCA

SIC

SAR

Development of a SNPs panel

Scopo -> Ridurre il numero di SNPs per:

- Mantenere il Massimo dell'informazione -> possibilità di evidenziare la struttura genetica
- Ridurre il costo della tipizzazione

Panel_PCA

Panel_Fst

Panel_Fst_PCA

Panel_PCA_Fst

Panel_RF

48 SNPs

96 SNPs

5 Methods

x

2 Sizes

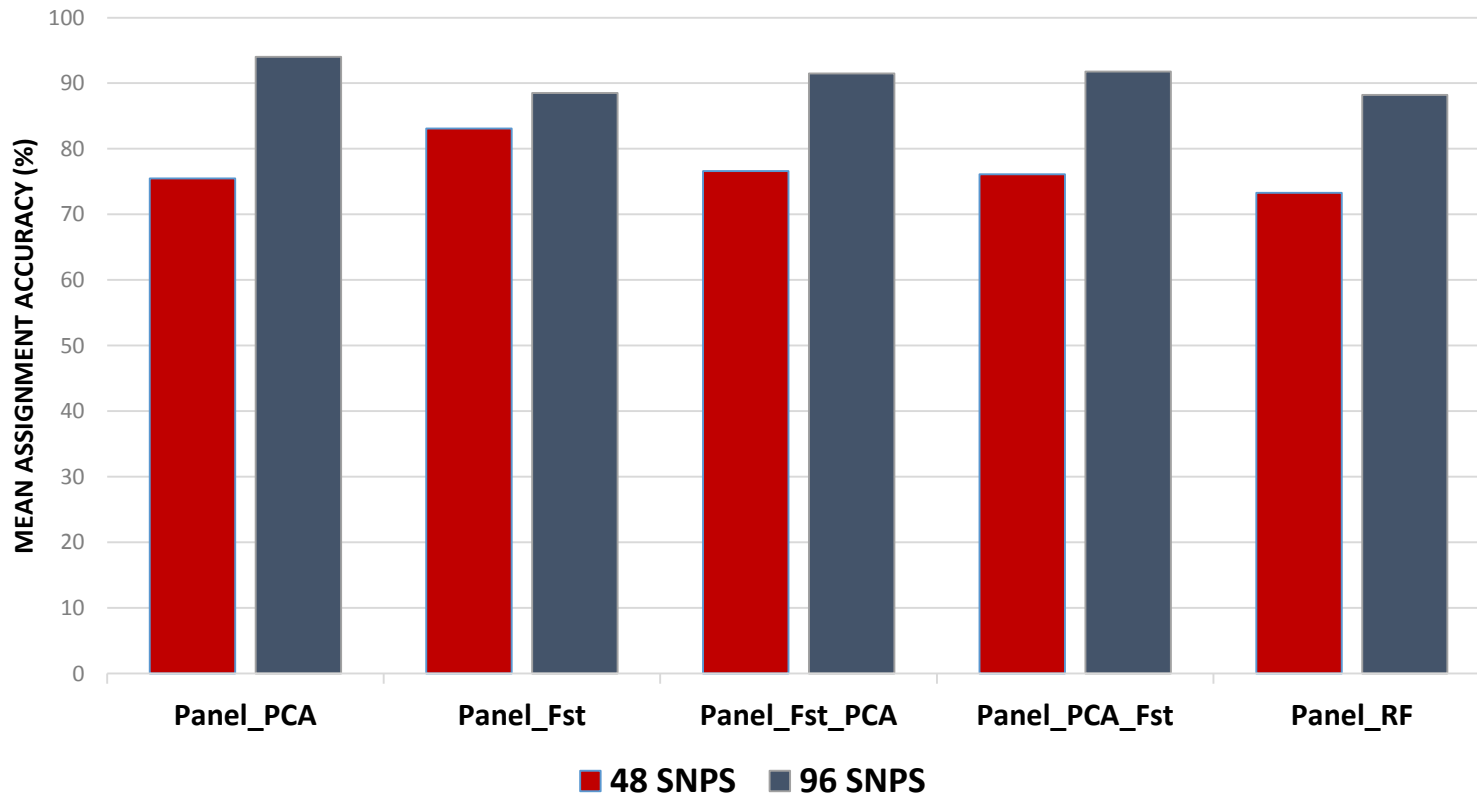
=

10 panels

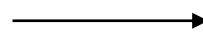
Development of a SNPs panel

Scopo -> Ridurre il numero di SNPs per:

- Mantenere il Massimo dell'informazione -> possibilità di evidenziare la struttura genetica
- Ridurre il costo della tipizzazione



Kompetitive Allele Specific PCR



48 SNPs -> ~11€
96 SNPs -> ~22€

Progetto di reintroduzione

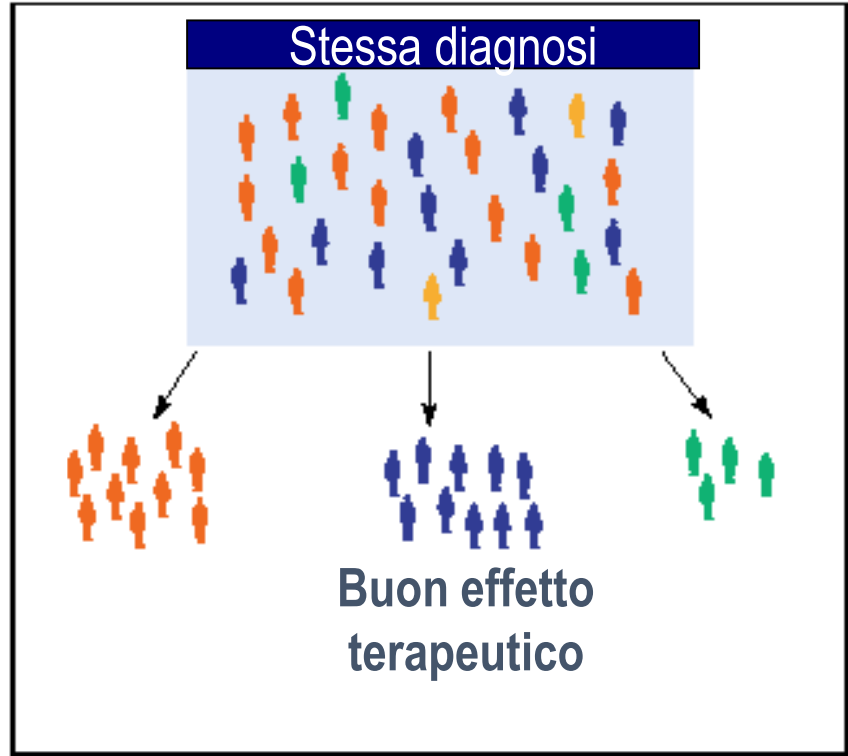
Procedere con un piano di reintroduzione usando campioni confiscati assegnati sulla base di marcatori genetici in un'area dove la specie è attualmente estinta (Parco del Conero, Marche)



Terzo esempio

farmacogenetica

Pharmacogenetics



Fallimento
terapeutico

Buon effetto
terapeutico

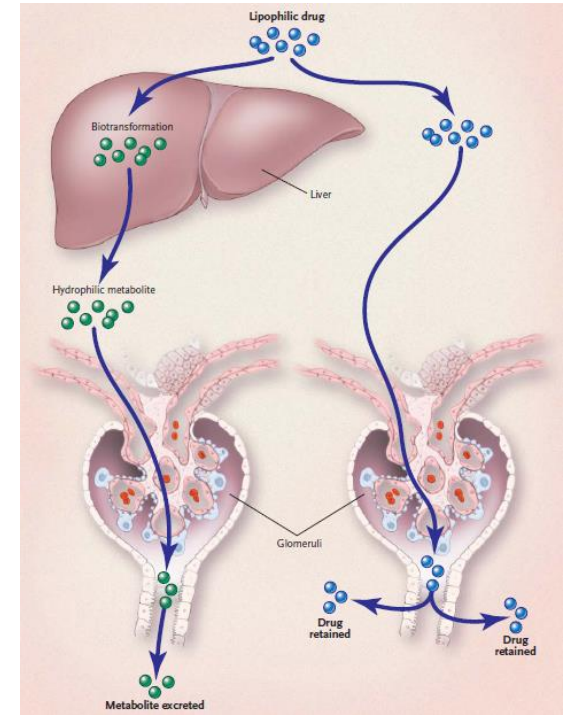
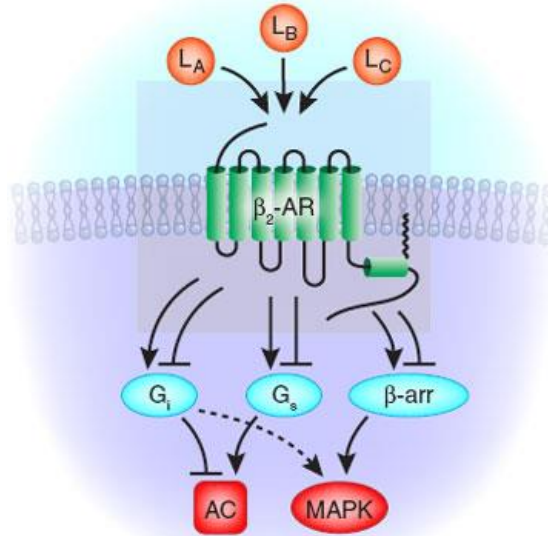
verse
dAdrug
reaction
(ADR)



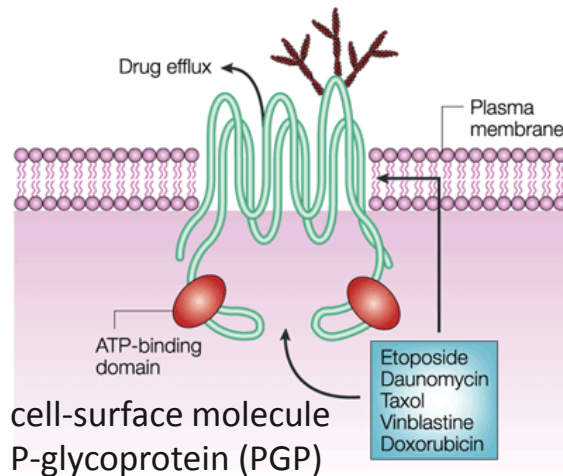
?

loci involved in metabolism of exogenous molecules

- Phasel and PhaselI metabolizing enzymes
- Targets
- Receptors
- Transporters



Weinshilboun 2003



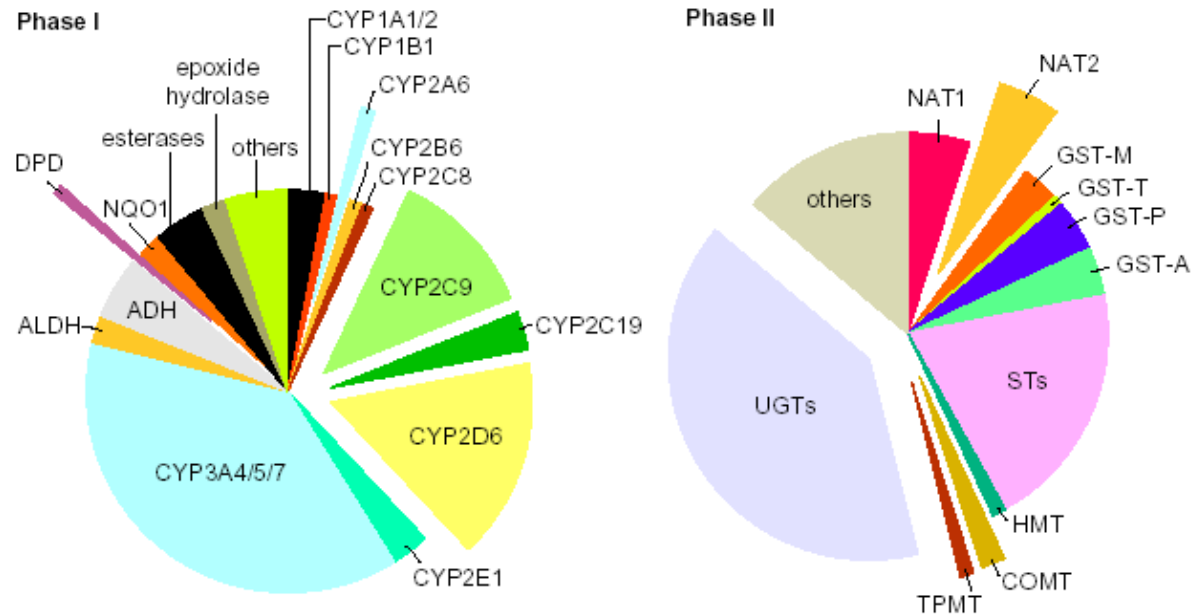
Nature Reviews | Cancer

Mediators between the organisms and their environment

Extreme levels of genetic variation!

Contributo di ogni enzima al metabolismo dei farmaci comuni

Drug-metabolizing enzymes (DMEs)



...G G **T** A A C T T G
...G G **C** A A C T T G

Usare gli SNPs per predire la risposta ai farmaci

Genotype - SNP profile

Pazienti con effetto clinico



Pazienti **senza effetto** clinic
(o con effetto avverso)



Alleli che predicono efficacia



Alleli che predicono la non
efficacia (o l'effetto avverso)



CYP2C9

Anticoagulant Warfarin

129 populations



CYP2C19

Proton pump inhibitors

146 populations



CYP2D6

Cancer treatment: Tamoxifen

Psychiatric drugs

138 populations



Cause of death (CoD)

- (a) disease or trauma, which initiated the train of morbid events leading directly to death or
- (b) The circumstances of the accident of violence which produced the fatal injury.

Manner of death (MoD)

the way or circumstances that led to the underlying medical CoD **natural, occupational, accidental, suicide, homicide or war** (in some counties medical treatment is an additional class).

Sudden unexpected death (SUD)

No evident pathology or trauma

Uninformative laboratory screening (e.g. toxicology and biochemistry)

Ambiguous autopsy results

typically sudden cardiac deaths (SCDs), sudden death in epilepsy (SUDEP), sudden infant death syndrome (SIDS) or drug-related deaths

Sudden unexpected death (SUD)

No evident pathology or trauma

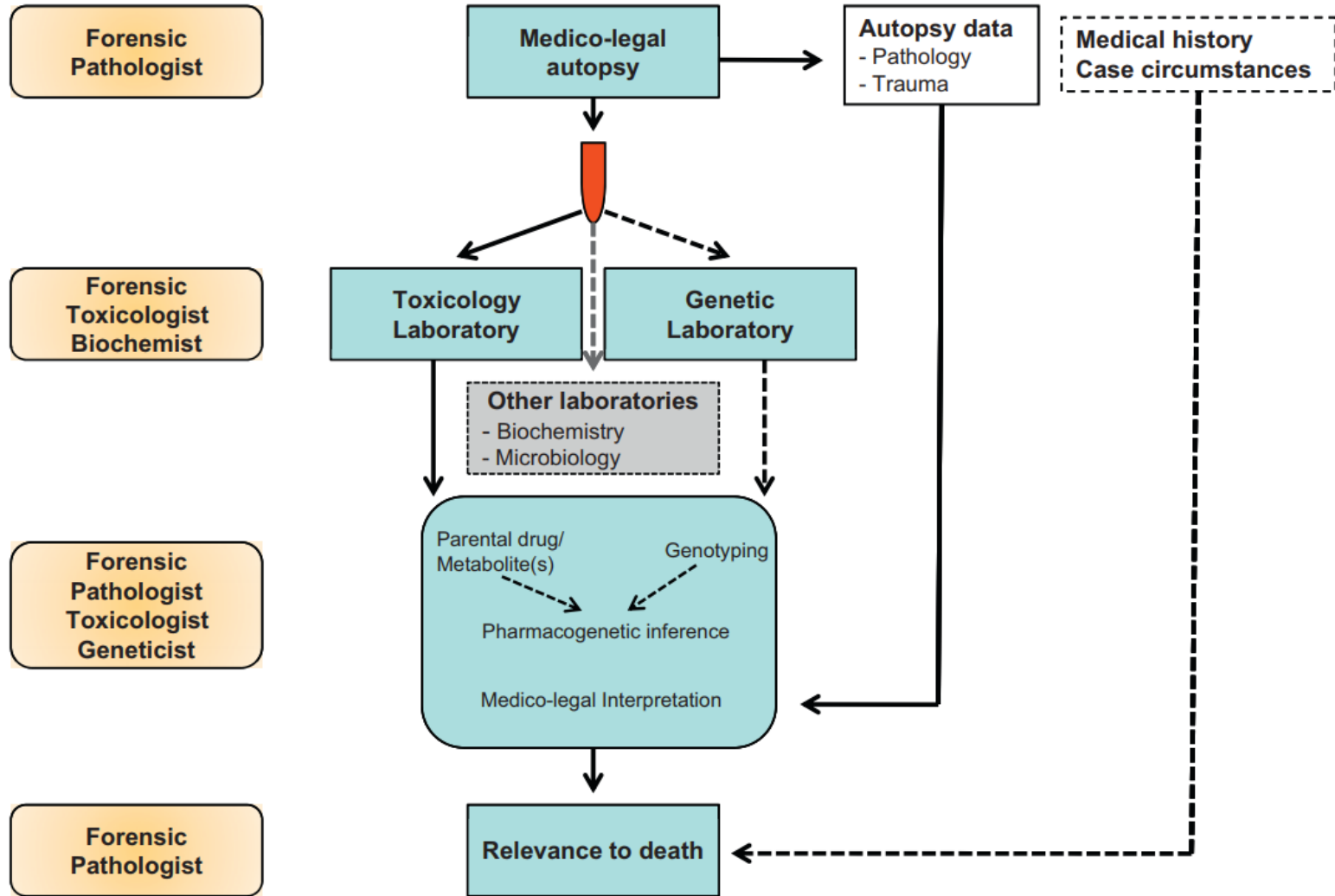
Uninformative laboratory screening (e.g. toxicology and biochemistry)

Ambiguous autopsy results

“Molecular autopsies”: genetic tests

Medico-legal genetics:

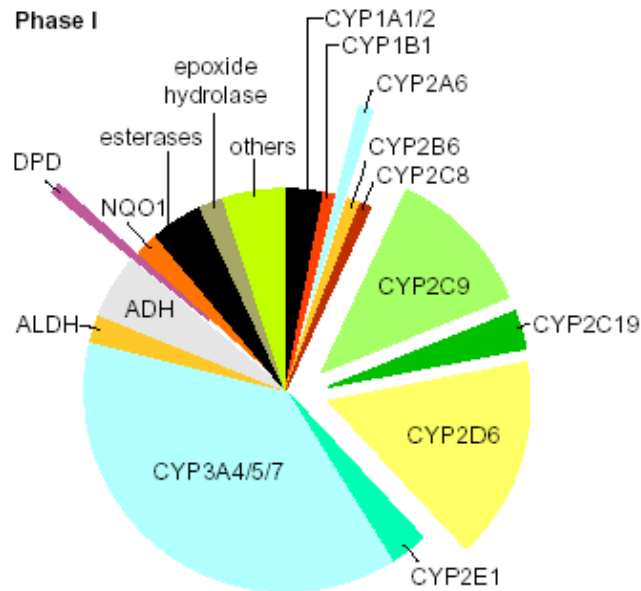
- Pathology
- Toxicology
- Genetics



3. Framework for medico-legal genetics and interaction between forensic pathologists, toxicologists and geneticists in post mortem pharmacogenetic investigations.

Post mortem pharmacogenetics

Soprattutto basata sull'analisi dei polimorfismi dei geni codificanti i CYP450 (drug metabolizing enzymes)



Disordini psichiatrici e suicidio rappresentano una larga percentuale di autopsie medico-legali nel mondo occidentale

Caso 1

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Fluoxetine-Related Death in a Child with Cytochrome P-450 2D6 Genetic Deficiency

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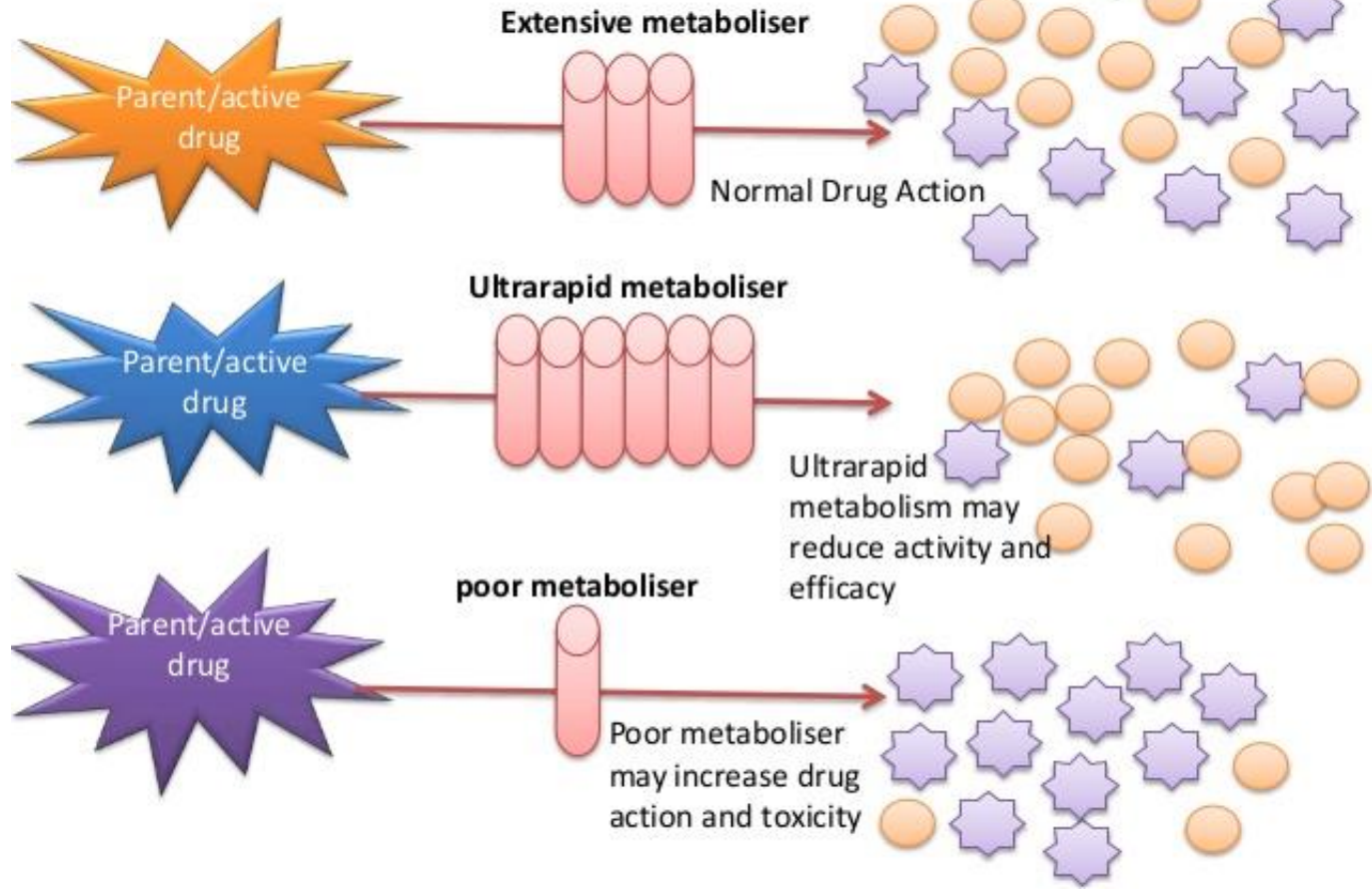
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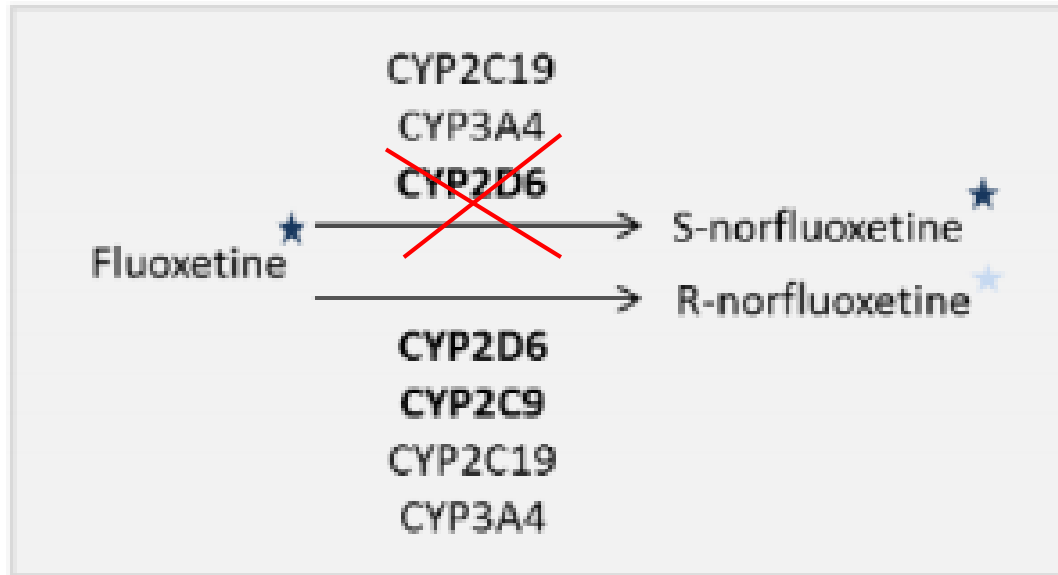
Abstract

The clinical course of a 9-year-old diagnosed with attention-deficit hyperactivity disorder, obsessive-compulsive disorder, and Tourette's disorder and treated with a combination of methylphenidate, clonidine, and fluoxetine is described. The patient experienced over a 10-month period, signs and symptoms suggestive of metabolic toxicity marked by bouts of gastrointestinal distress, low-grade fever, incoordination, and disorientation. Generalized seizures were observed, and the patient lapsed into status epilepticus followed by cardiac arrest and subsequently expired. At autopsy, blood, brain, and other tissue concentrations of fluoxetine and norfluoxetine were several-fold higher than expected based on literature reports for overdose situations. The medical examiner's report indicated death caused by fluoxetine toxicity. As the child's adoptive parents controlled medication access, they were investigated by social welfare agencies. Further genetic testing of autopsy tissue revealed the presence of a gene defect at the cytochrome P450 CYP2D locus, which results in poor metabolism of fluoxetine. As a result of this and other evidence, the investigation of the adoptive parents was terminated. This is the first report of a fluoxetine-related death in a child with a confirmed genetic polymorphism of the *CYP2D6* gene that results in impaired drug metabolism. Issues relevant to child and adolescent psychopharmacology arising from this case are discussed.

CYP metaboliser status



CYP2D6 Slow metabolizers: adverse drug reaction



★ Active ★ Less active metabolite

Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother

Gideon Koren, James Cairns, David Chitayat, Andrea Gaedigk, Steven J Leeder

April 2005: a full-term healthy male infant, delivered vaginally, showed intermittent periods of difficulty in breastfeeding and lethargy starting on day 7.

Day 11: the paediatrician noted that the baby had regained his birthweight.

Day 12: grey skin and his milk intake had fallen.

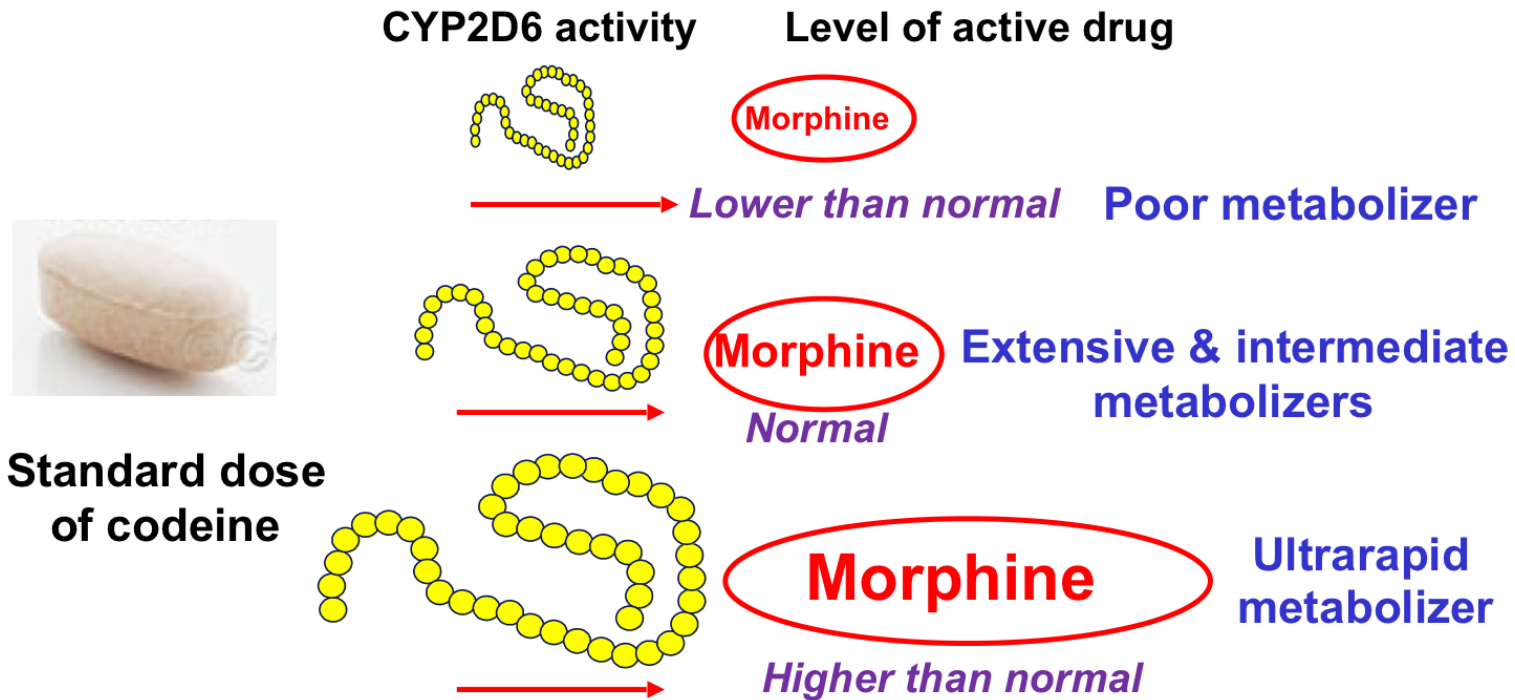
Day 13: he was found dead

Postmortem analysis showed no anatomical anomalies.

Blood concentration of **morphine (the active metabolite of codeine) was 70 ng/mL** (neonates breastfed by mothers receiving codeine typically have morphine serum concentrations of 0–2.2 ng/mL)

CYP2D6 Polymorphisms & Drug Metabolism

Codeine is a pro-drug & is metabolized to morphine (active) by CYP2D6



The mother assumed a combination preparation of codeine 30 mg and paracetamol 500 mg.

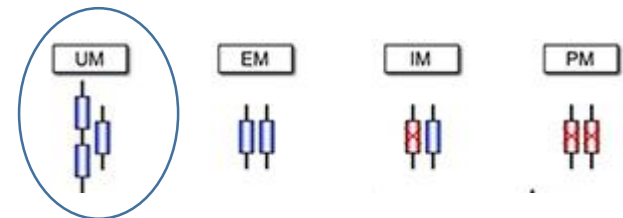
She continued the tablets for 2 weeks. Because of poor neonatal feeding, she stored milk on day 10, which was later assayed for morphine by GC-MS.

A **morphine** concentration of **87 ng/mL** was found—the **typical range** of milk concentrations after repeated maternal codeine is 2–20 ng/mL at doses of 60 mg every 6 h

Genotype analysis was done for cytochrome P450 2D6 (CYP2D6), the enzyme catalysing the O-demethylation of codeine to morphine.

The mother was heterozygous for a CYP2D6*2A allele with CYP2D6*2×2 gene duplication, classified as an ultra-rapid metaboliser.

This genotype leads to increased formation of morphine from codeine, consistent with the somnolence and constipation she experienced



Neonates invariably have impaired capacity to metabolise and eliminate morphine.