



**Defra Projects
FA0118 and FA0125**

TRACE
the wildlife forensics network

FERA/DEFRA Food Authenticity Conference 27th February 2014

Genetic tools for geographic traceability of fish and traditional breed identification

Dr Gill Murray-Dickson and Dr Rob Ogden

TRACE Wildlife Forensics Network at RZSS



Talk overview

- Introduction
- Populations genetics
- Defra projects FA0118 (Fish) and FA0125 (Traditional breeds of cattle and pig)
- Assignment analyses
 - Power
 - Interpretation
 - Considerations
 - Implementation
- Current status and future directions



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Introduction

- Mislabelling is one aspect of ‘food fraud’
- Mislabelling of:
 - Species
 - Breed
 - Variety
 - Geographic origin
- Has prompted EC regulation

e.g. **COUNCIL REGULATION (EC) No 1224/2009 Fisheries Compliance**

Article 58 Traceability (shall apply from 1 January 2011)

5. The minimum labeling and information requirements for all lots of fisheries and aquaculture products shall include:

(c) the FAO alpha-3 code of **each species**;

(g) the information to consumers provided for in Article 8 of Regulation (EC) No 2065/2001: the commercial designation, the **scientific name**, the **relevant geographical area** and the production method;

Common need for robust identification methods that are efficient and cost effective.



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

DNA applications

- What is it?
 - Processed or butchered form = Species ID difficult



- DNA methods for routine species identification are well established.

e.g. Large collaborative efforts such as FISHBOL, TRACEFISH, FISH and CHIPS and LABELFISH.

e.g. Commercial meat species testing.



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

DNA applications

- Where does it come from?
 - Complex supply chains and transshipments hard to monitor.
 - No physical characteristics for geographic origin.

Focus of DEFRA project FA0118 (Fish)

- Is it labelled correctly?
 - Substitution with a lower quality breed difficult to detect.

**Focus of DEFRA project FA0125
(Traditional breeds of cattle and pig)**



Defra Projects
FA0118 and FA0125



Aims

To produce efficient, reproducible, cost effective assays;
suitable for non-specialist use to authenticate...

Fish

Geographic origin

Target species:

Cod



Hake



Sole



Traditional breeds

Breed identification

Target breeds:

Oxford Sandy and Black
Gloucestershire Old Spot
Berkshire
Hampshire
British Lop



Target breeds:

Red Poll
Welsh Black
Aberdeen Angus
Trad. Hereford
Comm. Hereford



...using population genetic & assignment analyses.



Defra Projects
FA0118 and FA0125

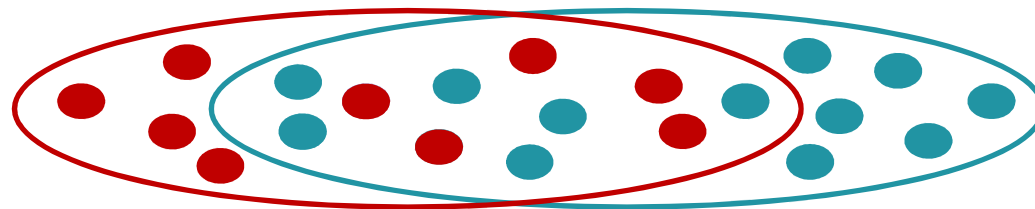
TRACE
the wildlife forensics network

Population genetics

- Populations are typically not genetically isolated (but there are exceptions).
- Genetic differences can arise among populations/groups when movement of individuals between populations is restricted.
- Populations/groups can become genetically distinct.

Population A

Population B





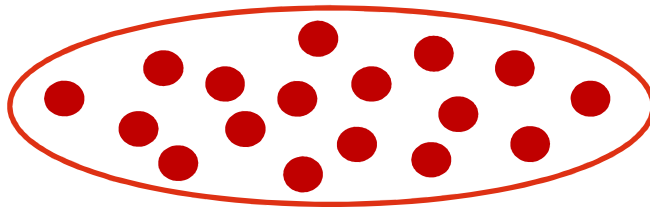
Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

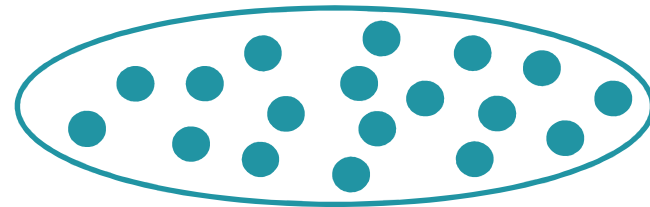
Population genetics

- Populations are typically not genetically isolated (but there are exceptions).
- Genetic differences can arise among populations/groups when movement of individuals between populations is restricted.
- **Populations/groups can become genetically distinct.**

Population A



Population B





Defra Projects
FA0118 and FA0125



Population markers

- **Informative markers**

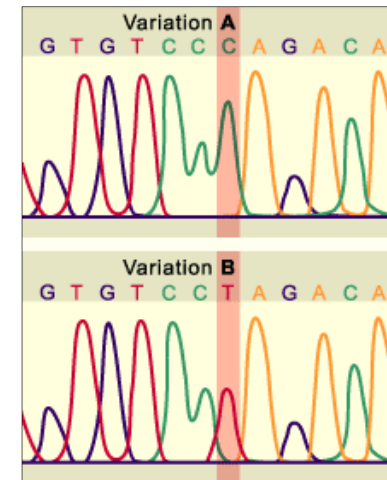
Genetic regions that display variation among populations/breeds, within a species.

- **SNP**

Single Nucleotide Polymorphism

- **Identified SNPs**

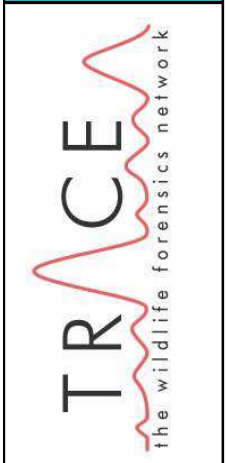
- 54K Bovine
- 60K Porcine
- 400-1250 Fish



- Genome-wide SNP data can be mined for **population or breed-informative markers.**



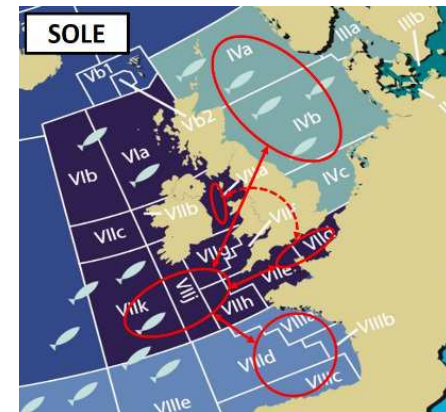
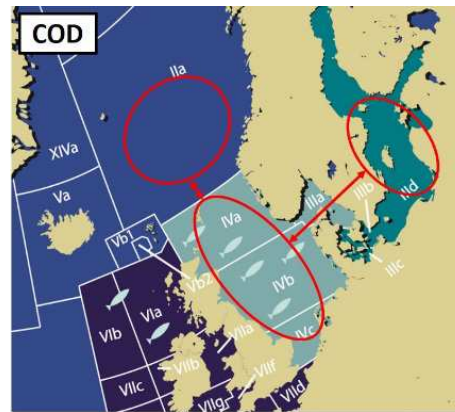
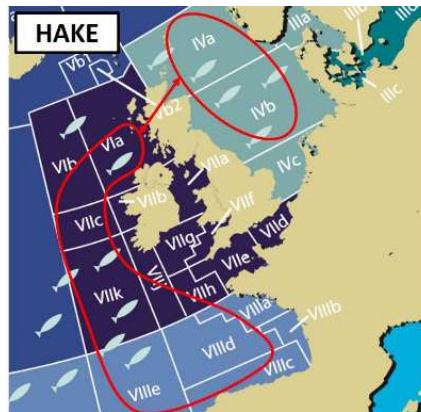
Defra Projects
FA0118 and FA0125



FA0118: Fish

- Target species:

Species	HAKE	COD	SOLE
Identified FPT* SNPs	> 1000	~ 400	> 1000
Panel size	13	9 (x 2 panels)	35
Scenarios	1	2	4



- Panels are used to assign individuals between a claimed origin and alternative.



*Data generated by FishPopTrace - <https://fishpoptrace.jrc.ec.europa.eu/>



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

FA0125: Traditional breeds

- Breeds generally selected for specific traits.
- Breed treated as populations.
- Previously:
 - ~120,000 SNPs  192 SNPs
 - Capable of distinguishing 12 breeds.
- Now aiming for:
 - 192 SNPs  ~10-SNP panels
 - Breed specific panels.
- Application is one of **breed exclusion**, whereby breeds are excluded but not identified.



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Assignment

SNP Panels are selected to achieve maximum power with the minimum number of markers

- Reference population/breed genetic profiles produced by combining informative SNP markers.
- For populations:
 - If a population boundary can be defined geographically, DNA population profiles can be used to describe geographic origins.
 - Requires correspondence between genetic population and regulatory spatial structure.
- Compare **individual** 'unknown' to reference population/breed profiles and generate a likelihood ratio.

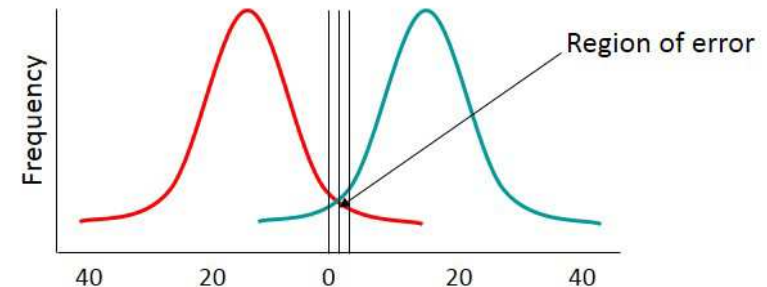
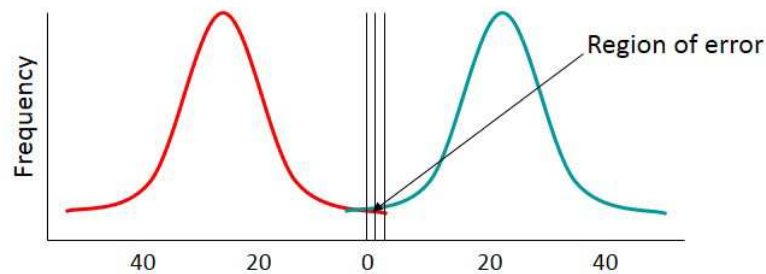


Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Assignment Power

- Software e.g. GeneClass is used to generate assignment likelihoods and exclusion probabilities.



Different likelihood
distributions

=

↓ Assignment error

Similar likelihood
distributions

=

↑ Assignment error

- Chance of incorrect assignment is reduced by ensuring that comprehensive, reference populations are used.



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Considerations

- Population/breed specific markers relatively easy to identify for:
 - Geographically distinct populations
 - 100% pure-breds
- Assignment can be problematic using small panels when:
 - High level of movement between populations
 - Geographic population \neq Genetic population
 - Cross breeding

Important to clearly define the application of a specific panel and use it appropriately



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Interpretation of results

- **Possible outcomes:**

(1) Individuals **are** assigned to their claimed origin.

(2) Individuals **are not** assigned to their claimed origin.

There are several explanations:

- The claim was fraudulent
- The test is ineffective
- The fish has migrated or dispersed beyond what is expected.

- **Confidence in data comes from:**

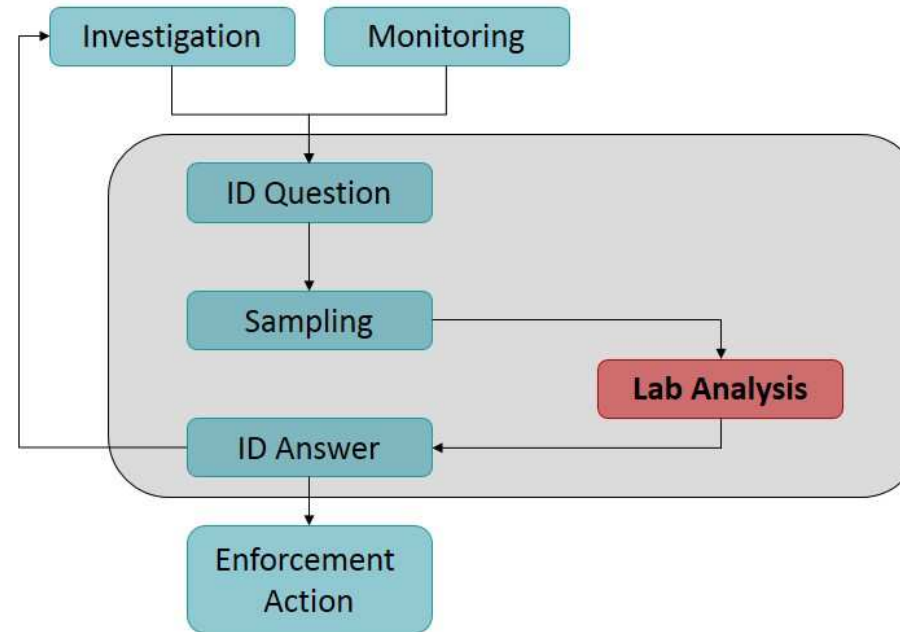
- Internal and independent external validation.
- Good reference populations.
- Adherence to validated SOP.



**Defra Projects
FA0118 and FA0125**



Implementation



- Potential for incorporation to routine monitoring of the supply chain.
- Need to formulate statistics in an appropriate format for legal presentation.
- Likelihood ratios used as human forensic evidence.



**Defra Projects
FA0118 and FA0125**



Current status

- Fish SNP assay SOP
 - Independent, external validation by LGC
- Assay performance assessed for:
 - Different types of sample
 - Sensitivity analysis
 - Geographic assignment
- SNP Panels for cattle and pig
 - Internal validation
- External validation this summer
 - By Minton, Treharn & Davies Ltd

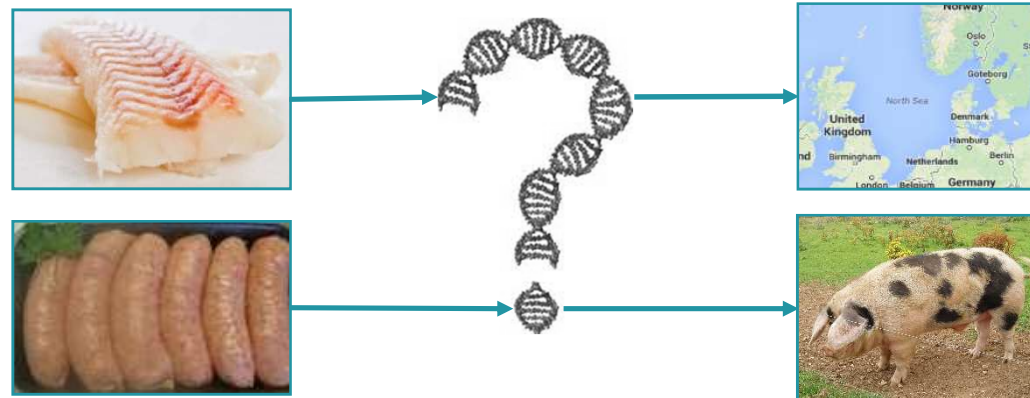


Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Future directions

- Knowledge transfer events scheduled for this Autumn.
- Discussions transfer to public analyst labs and commercial service providers.
- Demonstrates potential use of genetic assignment for authentication aspects of food traceability.





**Defra Projects
FA0118 and FA0125**

TRACE
the wildlife forensics network

Acknowledgements

Funded by:



Department
for Environment
Food & Rural Affairs

Validation:



Thanks to:

Dr Ross McEwing
Jenny Kaden
Dr Helen Senn
Caroline Whitson



**ROYAL ZOOLOGICAL
SOCIETY OF SCOTLAND**

Professor Alan Archibald
Dr Pam Weiner
Dr Sam Wilkinson



Dr Hendrik-Jan Megens (Wageningen University)
Marcus Bates (British Pig Association)
Peter Talbot (Traditional Hereford Breeders Association)
All contributing breed societies



Defra Projects
FA0118 and FA0125

TRACE
the wildlife forensics network

Any questions?



...AGATCTGAACGTCAGATCTGACTGACCTGACACTGCATCGTCACTGCAT...
...AGATCTGAACGTCAGATCTGACTGTCCTGACACTGCATCGTCACTGCAT...



gmdickson@rzss.org.uk