AARHUS
UNIVERSITY
INSTITUT FOR AGROECOLOGY

SMARTBEES

Diversity in European honey bees

How to conserve it?

Per Kryger

What do we have?

- Honey bees come in a range of natural subspecies
- Some artificial stock, Buckfast, Starline, hybrids
- Difficulties in determining what is pure
- Difficulties in maintaining pure bees
- Tools for conservation of diversity

Forskellige biracer. Kärntner- eller krainbien er meget udbredt p. gr. af sin flid og fredsommelighed. Den mørke race fra Lüneburger Heide går for at være angrebslysten, men er tålsom over for kulde. Den lyse italienske race, der er udbredt i Middelhavsområdet, regnes ikke for at være særlig arbejdsivrig.

Classical differentiation

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REVIEW ARTICLE

Standard methods for characterising subspecies and ecotypes of *Apis mellifera*

Marina D Meixner[1*], Maria Alice Pinto[2], Maria Bouga[3], Per Kryger[4], Evgeniya Ivanova[5] and Stefan Fuchs[6]

Table 5. Summary of characteristics of the different methods used for identification of honey bee subspecies.

Characteristic	Morphometrics	Allozymes	MtDNA	Microsatellites	SNPs
Number of individuals per colony	10-15	10	1	1 or more (depending on the goal)	1 or more (depending on the goal)
Characters/loci usually screened	Up to 41; wing venation	MDH, ME, EST, PGM, HK, ALP	COI-COII/*Dra*I, COI/ *Nco*I/ *Sty*I/ *Ssp*I, ND5/*Alu*I/ *Hind*II/*Fok*I, 16s rDNA/ *EcoR*I	Hundreds available. However, for most studies less than 20 screened (e.g. A7, A24, A28, A88, A113, B124, Ap43, A14, A107, A35, Ap55, Ap66)	Hundreds (1536 for Golden Gate Assay of Illumina) to thousands (with the Infinium Assay of Illumina)
Inheritance	Biparental	Biparental	Maternal	Biparental	Biparental
Dominance		Co-dominant	N/A	Co-dominant	Co-dominant
Polymorphism		Low	Very high in COI-COII intergenic region, otherwise medium to low	Very high	Can be high
Number of alleles		Multi-allelic	Multi-allelic	Multi-allelic	Biallelic
Abundance in the genome		Low		Medium	Very high
Cross-lab/study comparisons	Cross-checking recommended	Easy	Easy	Requires special preparation and cross calibration	Easy
Time to complete lab protocol	Depends on character suite typically 1 sample per day for full suite	1 day	Depends on assay, up to 2 days	1 locus or one multiplex up to 2 days	3 days
Main software packages	SPSS, Systat, Statistica, Morpheus, NTSYS, MORPHOJ	GenAlex, Genepop, and others	GenAlex, Genepop, Network, Structure, and others	Genepop, Arlequin, Structure, GenAlex, GeneClass, Adegenet, and other R packages	Plink, Structure, Admixture
Main Equipment	Microscope, camera, measuring software, computer	Centrifuge, Electrophoresis Unit, incubator	Thermal Cycler, Electrophoresis Unit, Centrifuge, Water Bath	Thermal Cycler, Electrophoresis Unit, Centrifuge, Automated Sequencer	Thermal Cycler, Analyst Plate Reader, Hybridisation Oven, Bead Array Reader
Cost of equipment	Low	Low	Medium	High	Very high
Cost of genotyping	Low	Low	Medium	High	Very High

Introgression

- Populations of honey bees have hybridised
- Genes of different subspecies have mixed
- Analog to invasive plant species, taking up space
- Chromosomes of introduced bees occupy native genome
- Selection process lead to depletion of rare variants

Norway

Sweden

Göteborg

Læsø, a rather isolated island
20 km to nearest coast
2000 inhabitants
20000 turists

Arhus

Denmark

Odense

Germany

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Image NASA
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162 km

Læsø case story

- Læsø island home to 500+ colonies and 25 beekeepers
- Last population of *Apis mellifera mellifera* in Denmark
- Also beekeepers with *A. m. ligustica* since 1970ies?
- Protection of the population in 1993
- Only *Apis mellifera mellifera* bees legal on Læsø

Protest!

- Commercial beekeepers on Læsø disagree
- They say all Læsø bees are introgressed/hybrids
- Not worthy of protection
- Also claim *A m mellifera* bees are unproductive
- They claim the law violates EU rule on free trade
- They disobey the law and keep their *A m ligustica* bees

Juul, a commercial beekeeper

He is happy with productive hybrid bees:
“I don’t want to become a custodian of poor bees”

Court case for 8 years

- Case went to EU court
- State wins and one beekeeper fined 150 EUR in 2001
- “Conservation is more important, than free trade”
- Negotiations with beekeepers finalised 2004
- New plan of co-existance on Læsø island

But how to co-exist?

- Cease fire on Læsø, history of sabotage of bees
- Mating behaviour of honey bees leads to hybrids
- Monitoring group of 6 University Professors
- Interest group of local beekeepers, for and against
- Science project of 400 000 EURO approved

Læsø

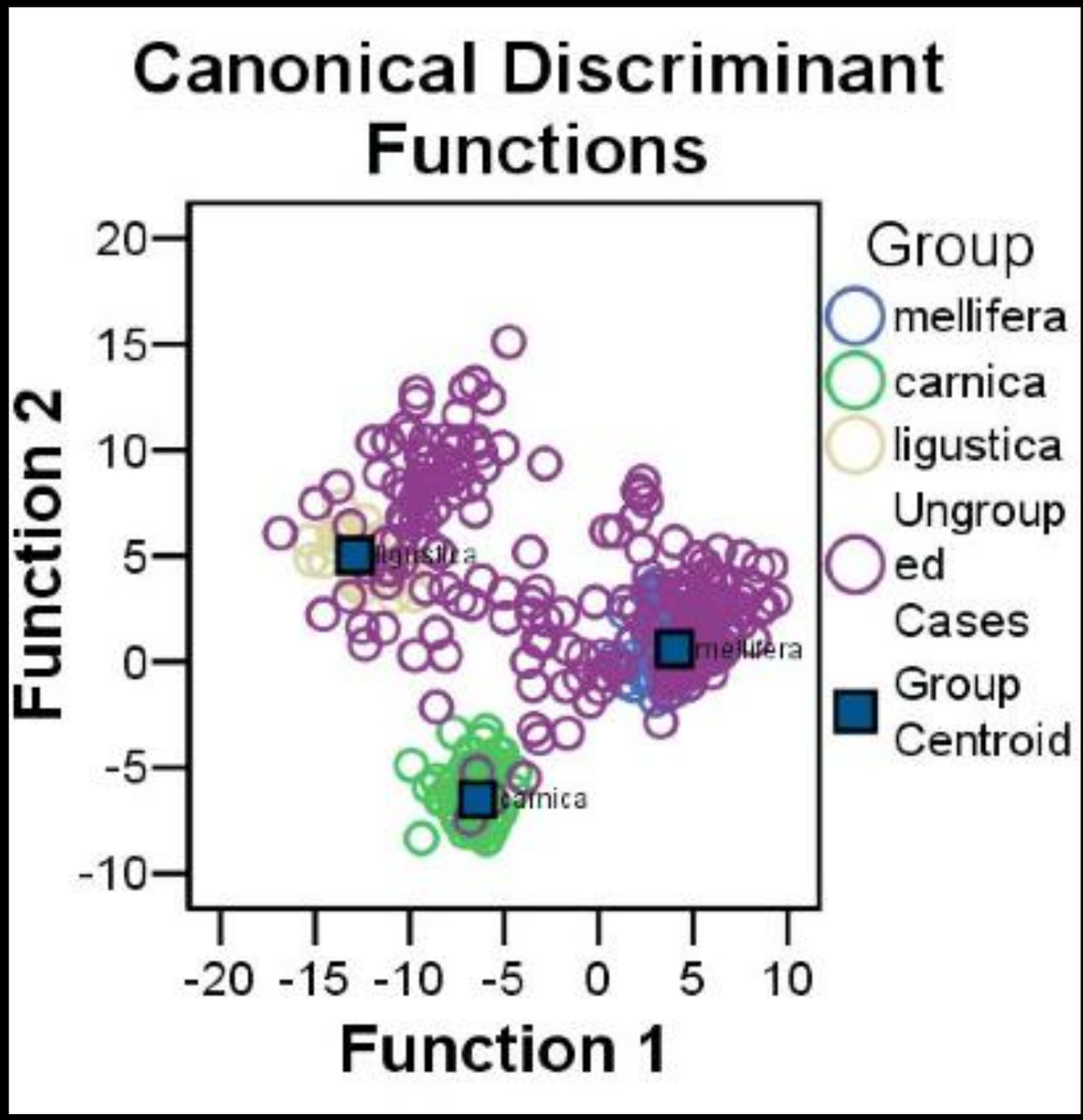

naTUR-kort Læsø
1:35.000
Ækvidistance 10 meter
Vesterø Havn
Østerby Havn
Kirkeplantage
Byrum Bugt
Sydbugt

Methods

- All bee yards on Læsø were sampled in 2005
- 8 bees from each hive
- 583 colonies (4664 bees) in 2005,
- 213 mellifera colonies (1704 bees) in 2006
- 283 mellifera colonies (2184 bees) in 2007
- 480 drones from 2007

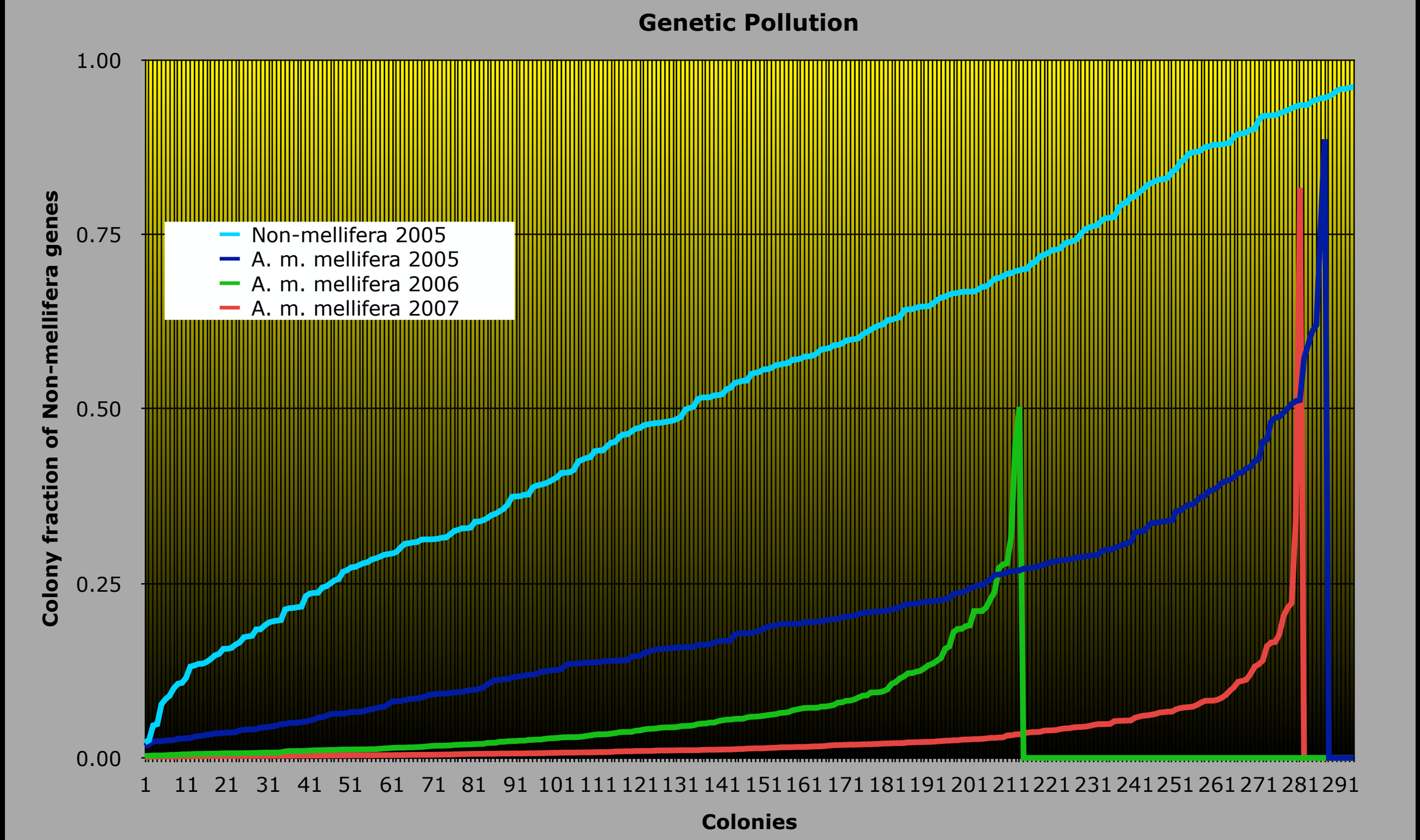
Morphometric control

- Bees from Læsø analysed by Stefan Fuchs, Oberursel
- Bees of conservation group are *Apis mellifera mellifera*
- Commercial beekeepers instead have *Apis mellifera ligustica*



Bjørn, a traditional beekeeper

He prefers the local bees *Apis mellifera mellifera*
“Father always said, these bees are fine”

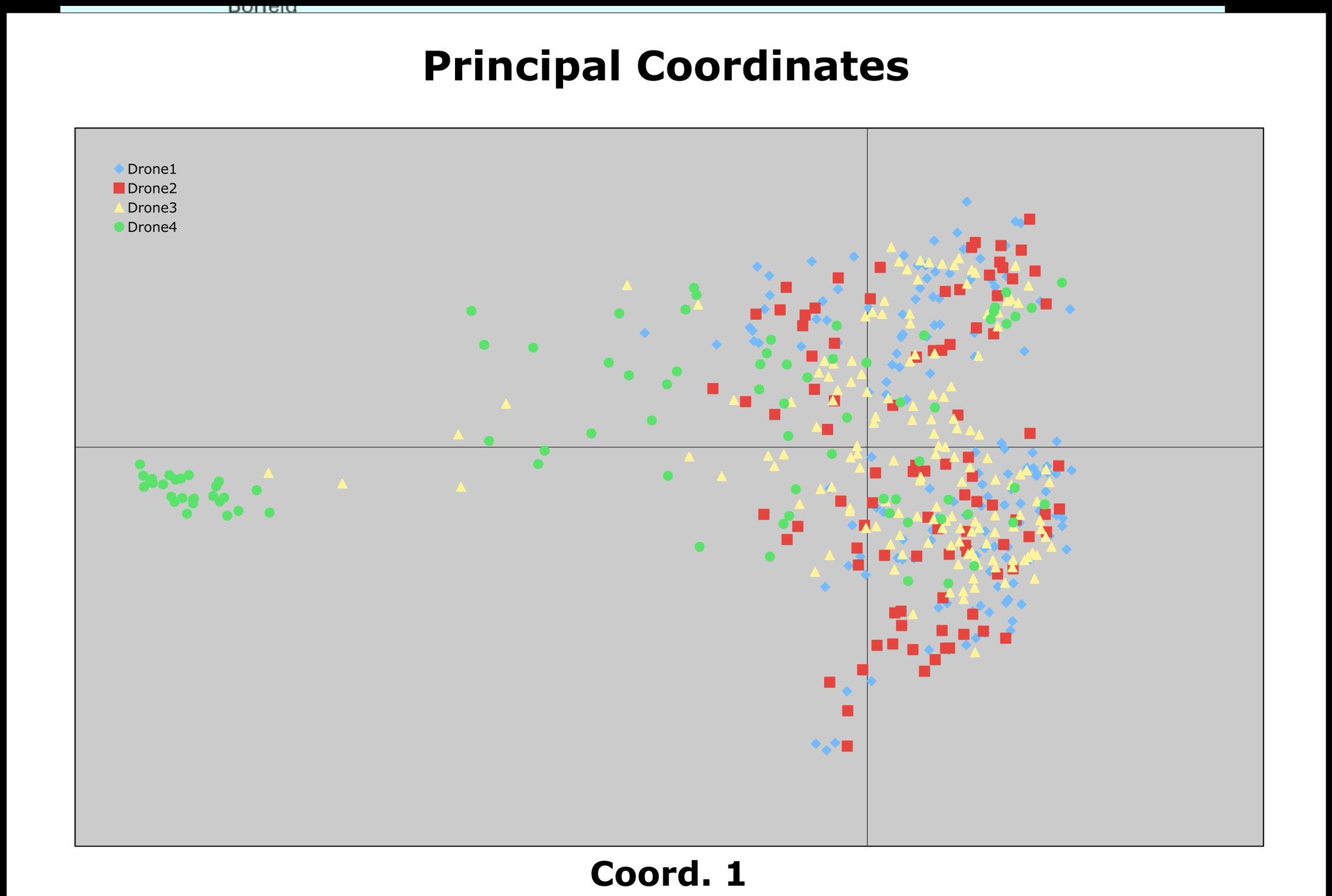


Results of a Three-Year study

Mating station

- How to protect the local population against introgression?
- Honey bee queens fly far, drones fly far and they mate!
- We introduced apartheid on Læsø for the bees
- Eastern end of Læsø reserved for *Apis mellifera mellifera*
- But would 6 km be sufficient?

Drone catching on Læsø




JEGENS BUGT
Østerby Havn
NORDMARKEN
Gammel Østerby
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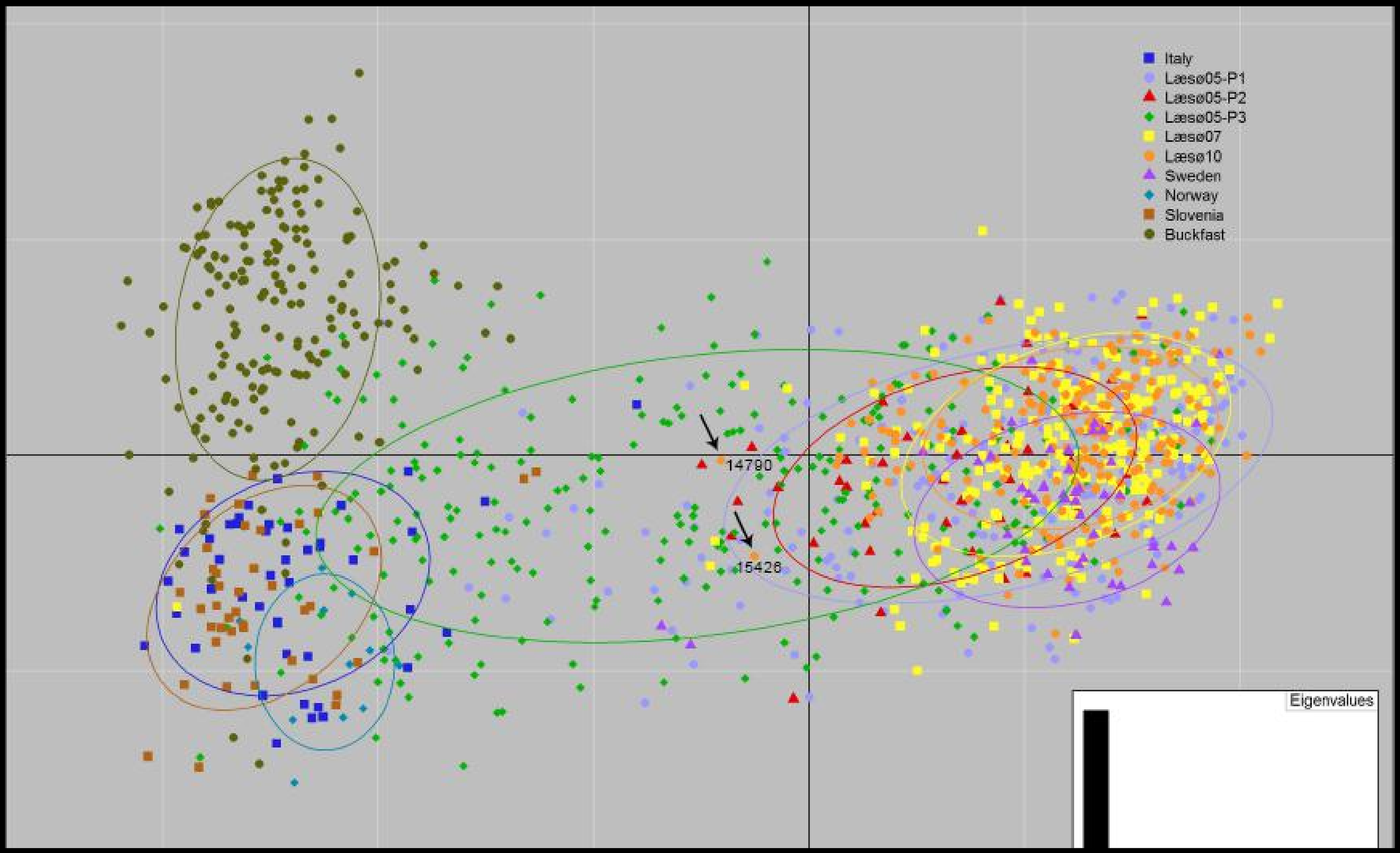
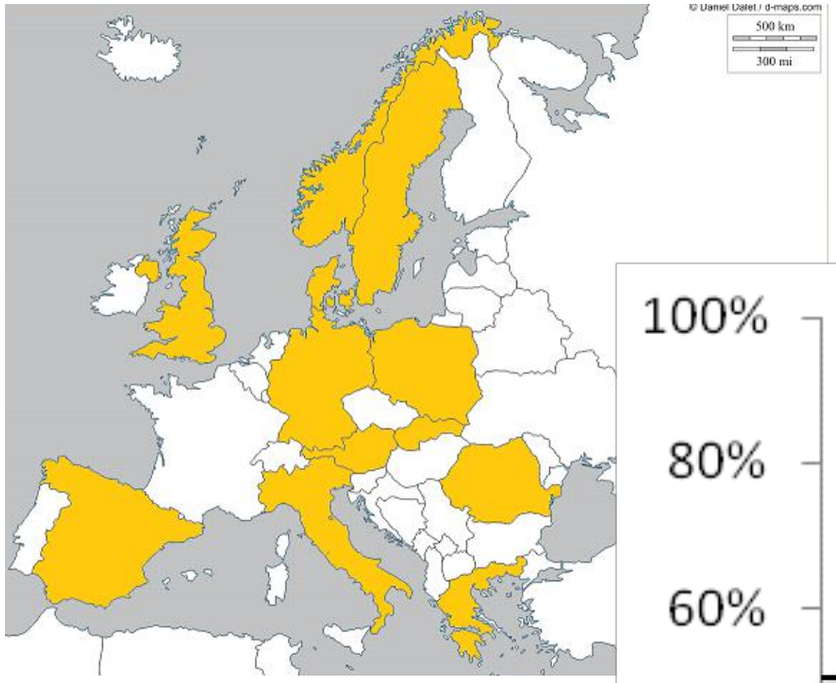
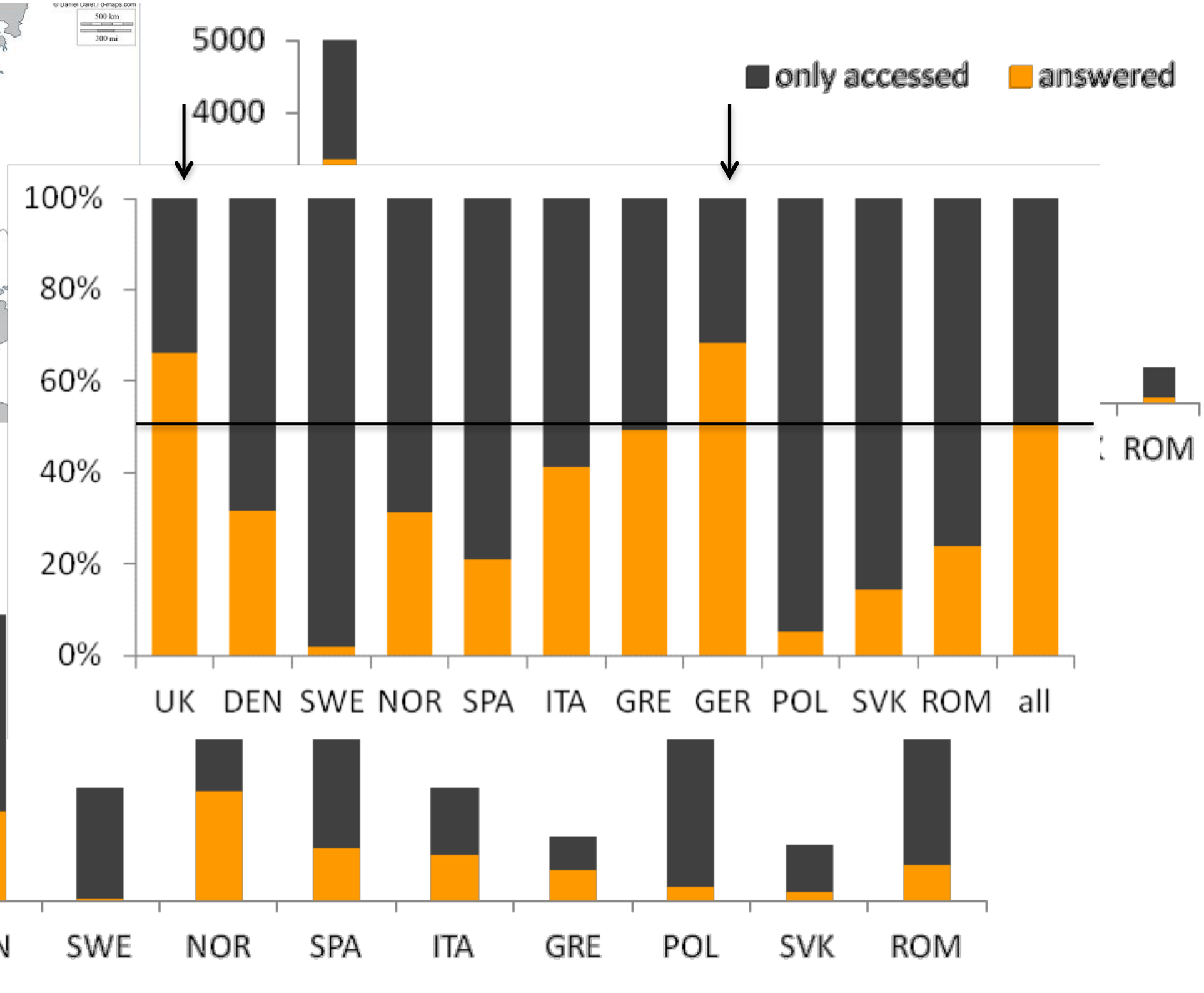


The following years no more introgression!

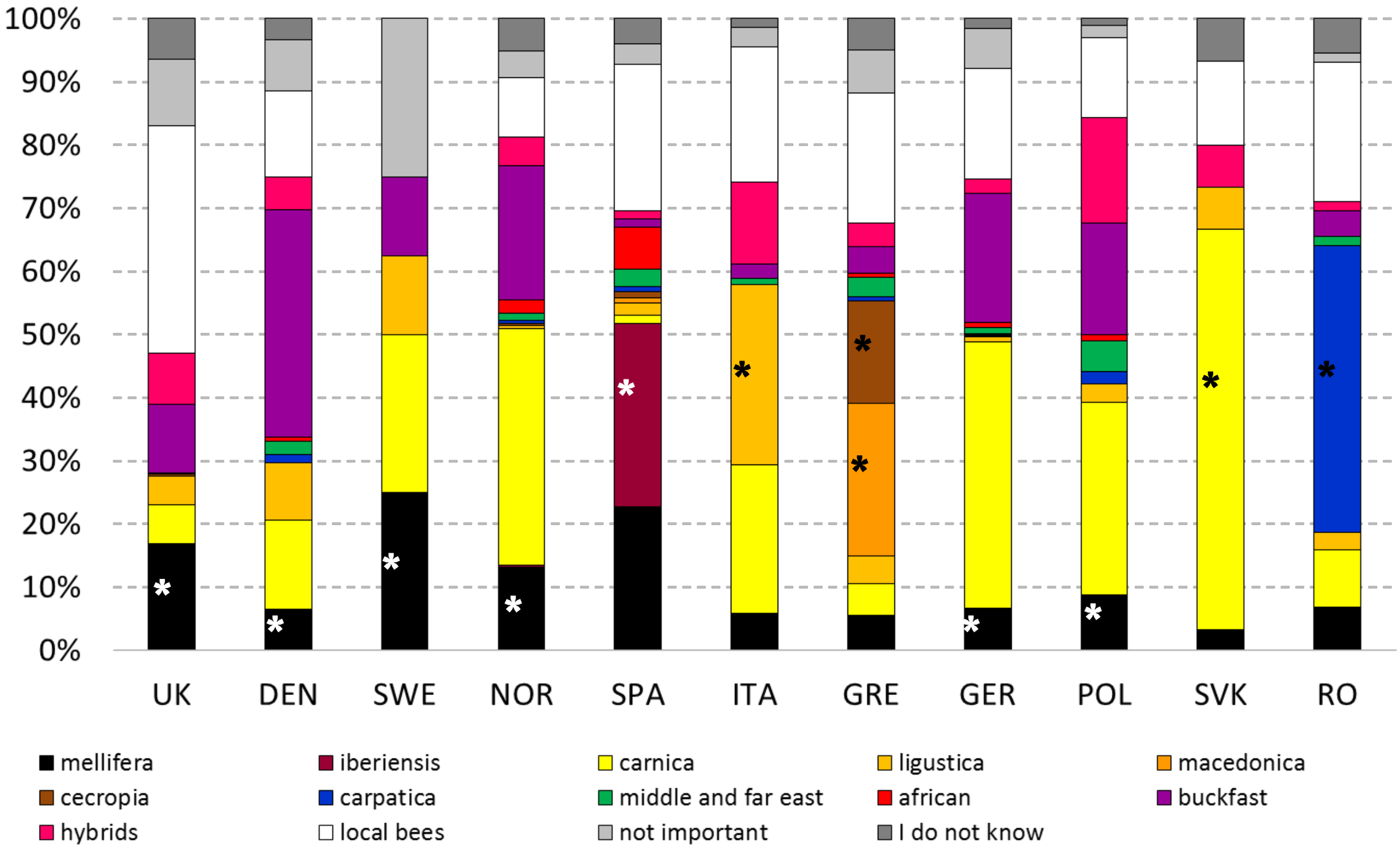
Replies SmartBees questionnaire





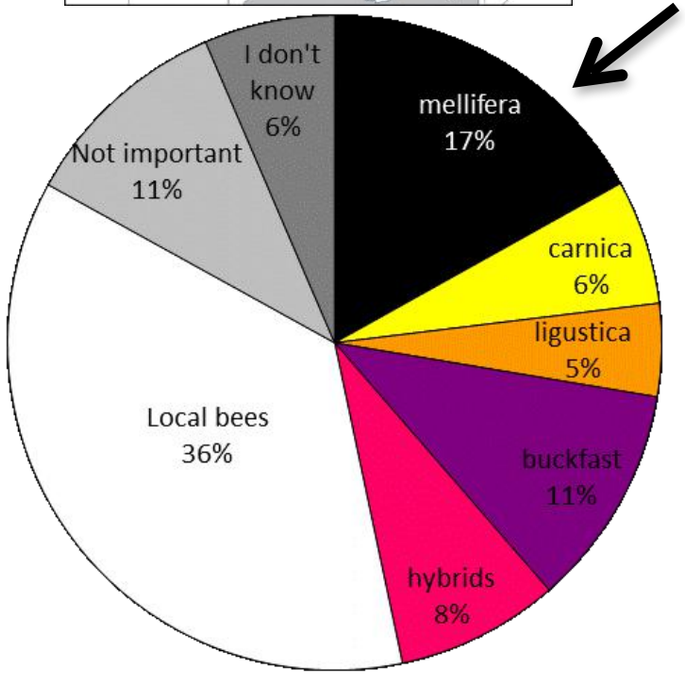
Prefered subspecies

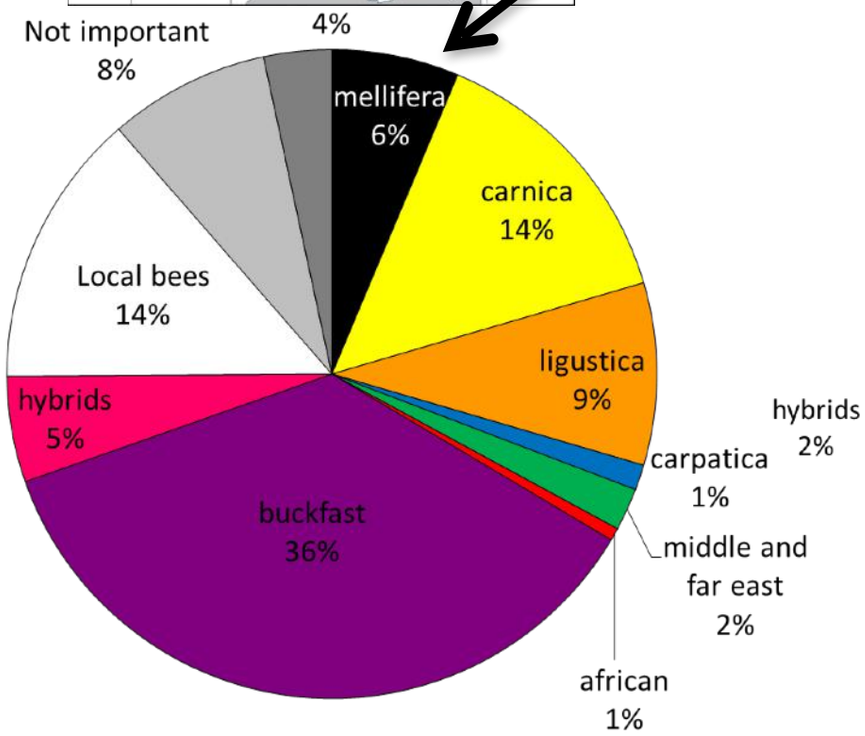
Which subspecies do you keep?

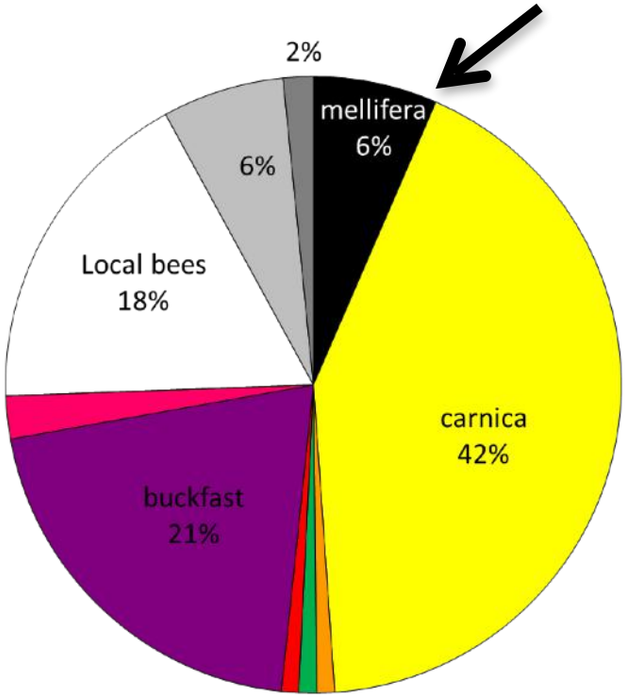


Abandoned their original bees

Prefered subspecies





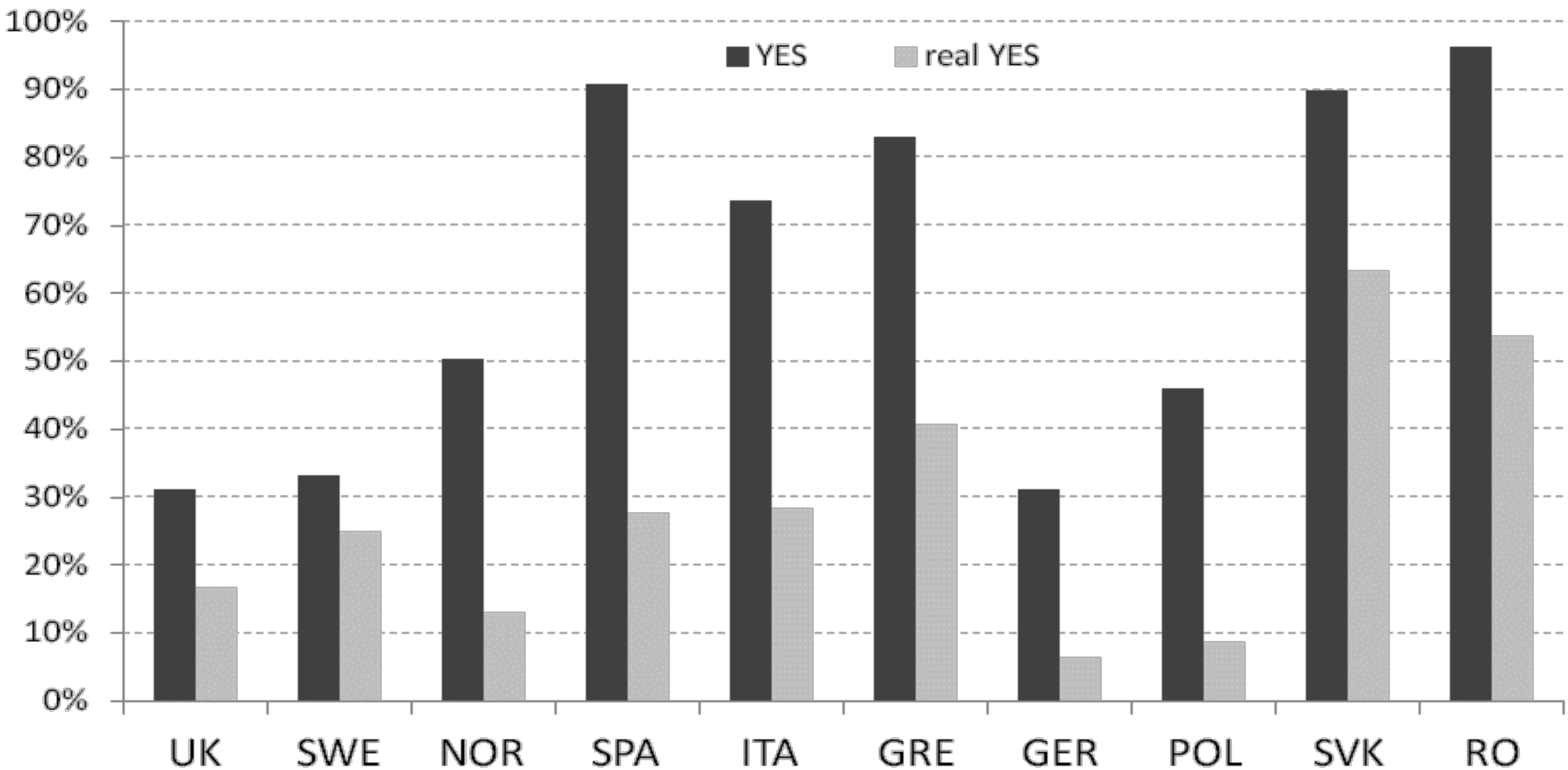


Original subspecies exchanged or hybridised

Introduced bees (*carnica*, *ligustica)* and hybrids (Buckfast) spread widely

Prefered subspecies

Do you keep the original bee?



Overestimation or poor knowledge of taxonomy?

Conservation areas

- Establishment of a conservation network
- Data collection in relation to current conservation areas for honey bees across Europe
- Webpage available to report conservation efforts
- Direct contact from SmartBees to organisations
- Laetitia Papoutsis is preparing a publication

Thus far we have

Apis mellifera	**Country**
mellifera	Switzerland, Poland, France, Belgium, United Kingdom, Norway, Russia, Denmark, and Ireland
iberiensis	Spain and Portugal
carnica	Slovenia and Croatia
ligustica	Italy
siciliana	Italy
anatoliaca	Turkey
caucasica	Turkey
ruttneri	Malta

United Kingdom

- In Isle of Man a beekeepers Federation (www.iombeekeepers.com) attempts to unify the conservation activities of three local associations
  In the island there is no Varroa mite, so importation of bees and used beekeeping equipment is prohibited by law

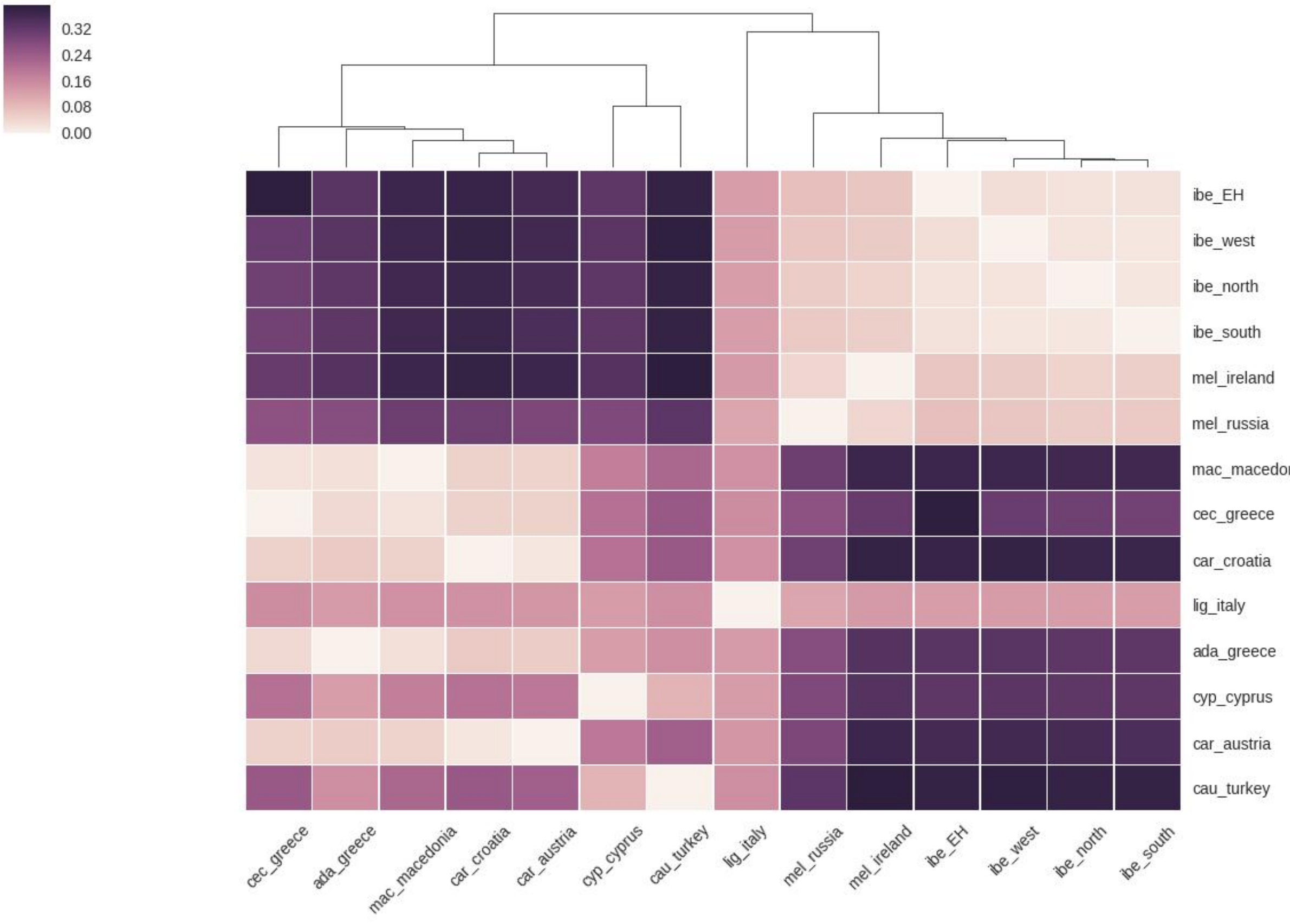
- Colonsay Island is also Varroa free

- In Cornwall a project aims at surveying the local population (both feral and kept colonies) in order to investigate the quality of the population and to further conserve the Cornish bee population.


Colonsay
Isle of Man
Cornwall
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image Landsat / Copernicus
Google Earth

Genetic monitoring

- We in SmartBees are developing new tools
- Sequencing the whole genome of all subspecies
- Single nucleotide polymorphism (SNP) discovered
- SNPs for subspecies differentiation, 25 EUR/sample
- SNPs for varroa tolerance and inbreeding too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cec_greece
car_croatia
lig_italy
ada_greece
cyp_cyprus
car_austria
cau_turkey
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car_austria
cyp_cyprus
cau_turkey
lig_italy
mel_russia
mel_ireland
ibe_EH
ibe_west
ibe_north
ibe_south

Open for business

- We accept samples!
- Please get in touch for validation study samples
- We are testing the developed SNPs
- Three panels are combined:
  Subspecies differentiation, Varroa tolerance, and Complementary Sex Determining locus

Conservation needs:

1. Beekeepers with a keen interest in conservation
2. Mating control - islands and valleys
3. Breeding plan for improvement, but avoiding inbreeding
4. Scienctific evaluation of genetic background
5. Education and cooperation

SMARTBEES

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