

Conservation genetics and genomics of the Italian wolf population

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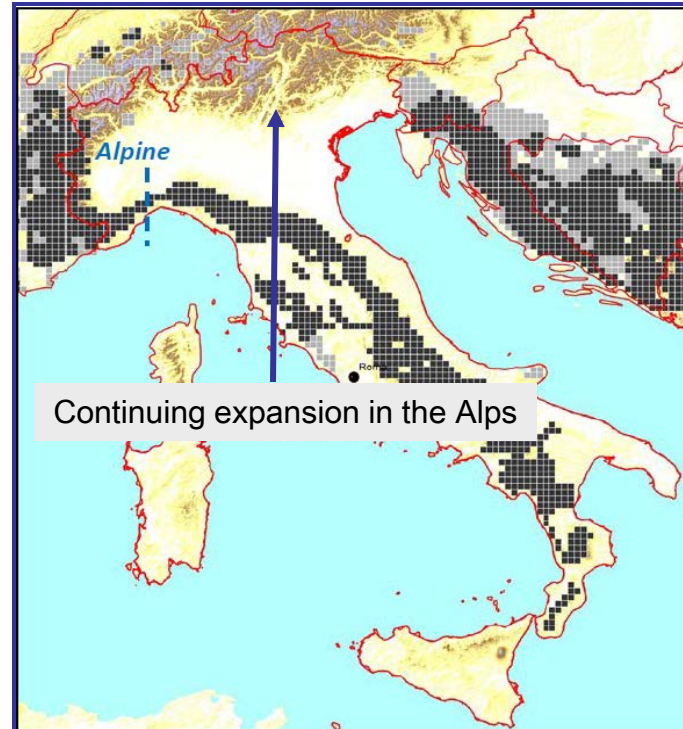
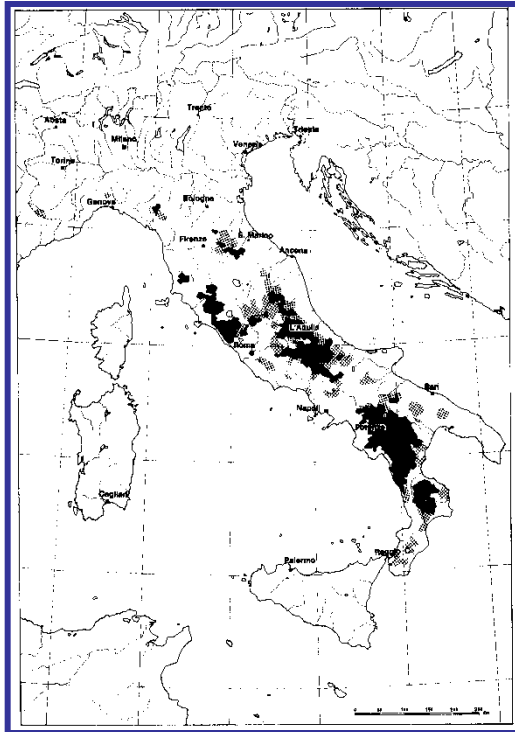
and students at the Conservation Genetics Laboratory, ISPRA

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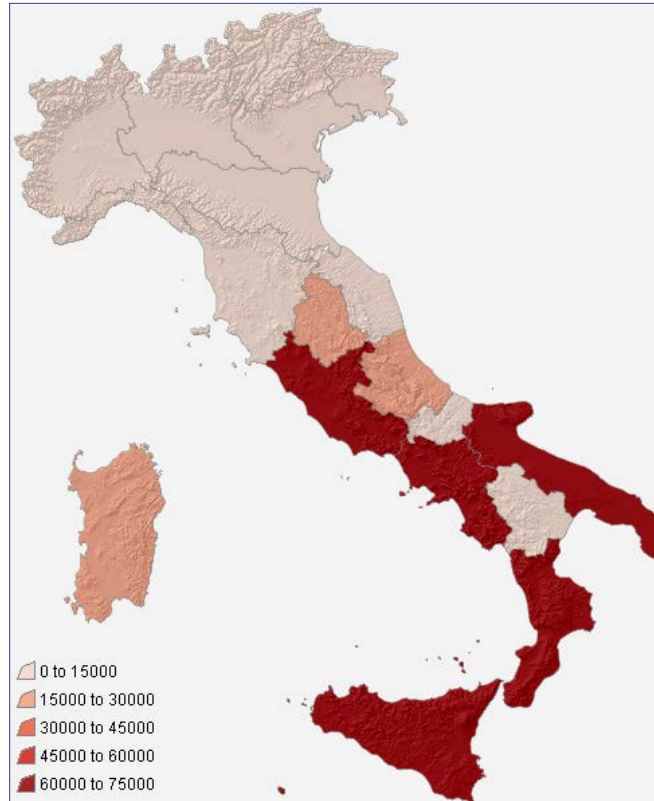
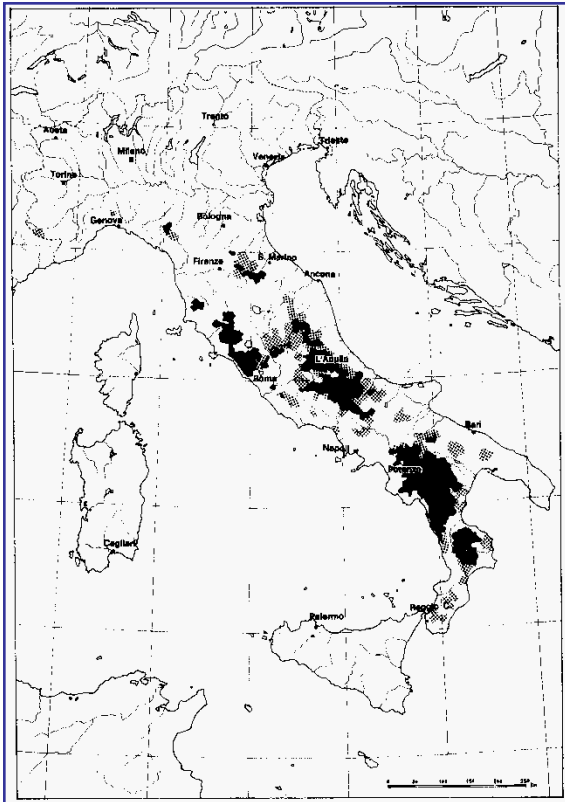
The bottleneck: < 100 wolves after WW II (Zimen & Boitani 1975)

The expansion: *c.* 800 – 900 wolves currently (IUCN/SSC LCIE 2012)



Isolation south of the Alps for at least 100 years

Wolf expansion, free-ranging dogs and hybridization



Few hundred wolves

Free-ranging dogs = 1 000 000

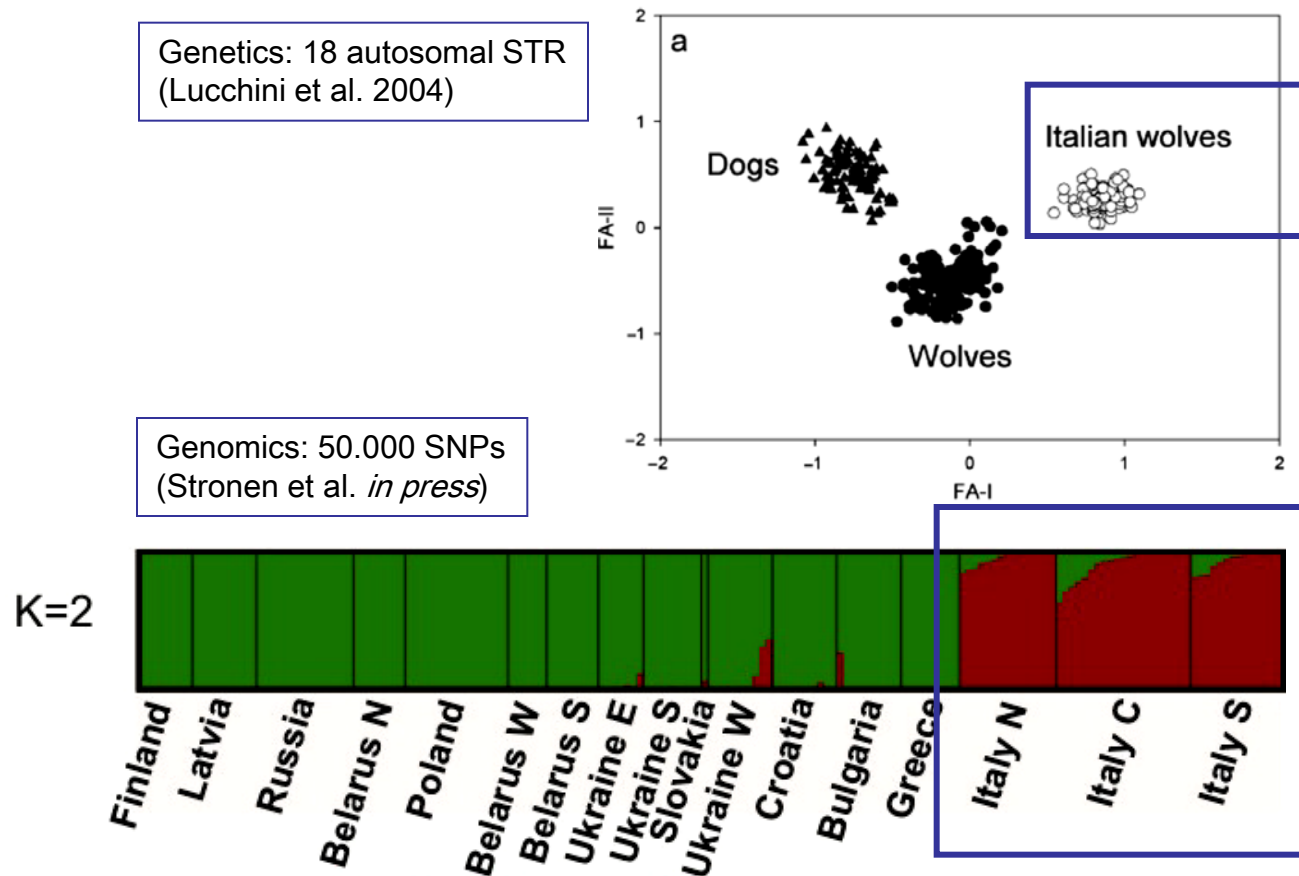
How many hybrids?

Conservation genetics of wolves in Italy

- ✓ ancient isolation south of the Alps: genetic divergence
- ✓ genetic consequences of the recent bottlenecks
- ✓ monitoring wolf expansion by molecular tools
- ✓ wolf x dog hybridization and gene introgression

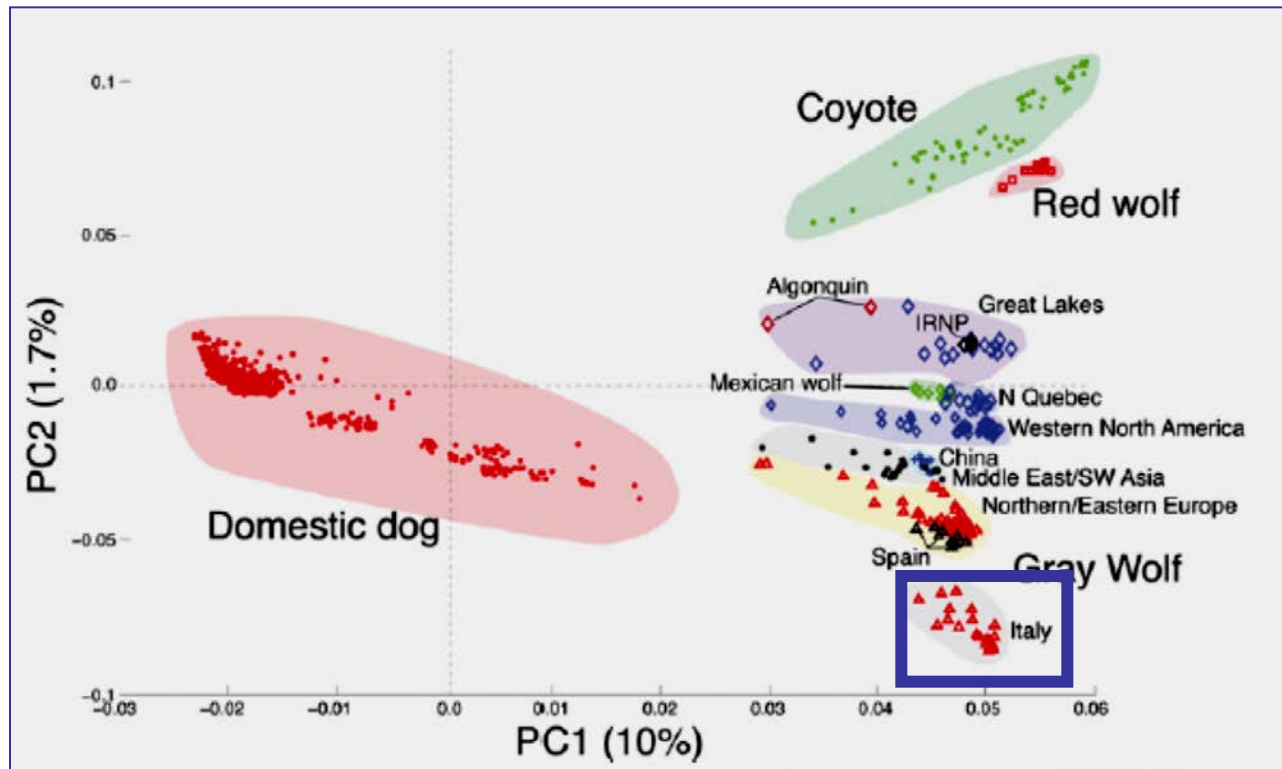
The consequences of isolation south of the Alps:

The Italian wolf is the genetically most distinct population in EU



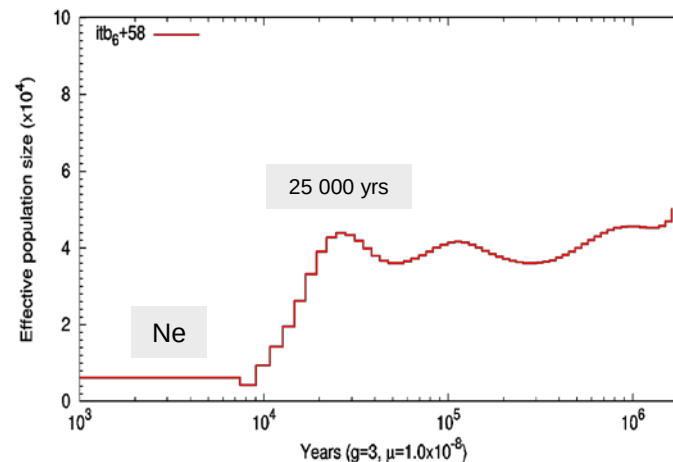
Genomics: the Italian wolf is a distinct population worldwide

50.000 SNPs
vonHolt et al. 2011



Long-term isolation and small Ne of wolves in the Apennine Peninsula

	Ne contraction	Years	Divergence Time Iberia / Italy
18 STRs (Lucchini <i>et al.</i> 2004)	100 – 1000x	2000 – 10 000	Holocene = climate change
50K SNPs (Pilot <i>et al.</i> in rev.)	100x	30 000	3000 – 6000 Neolithic = hunting
7 Whole genomes (including 2 IT wolves) (Freedman <i>et al.</i> in rev.)	10 – 20x	25 000	3000 – 6000 Neolithic = hunting



The consequences of long-term small effective pop size

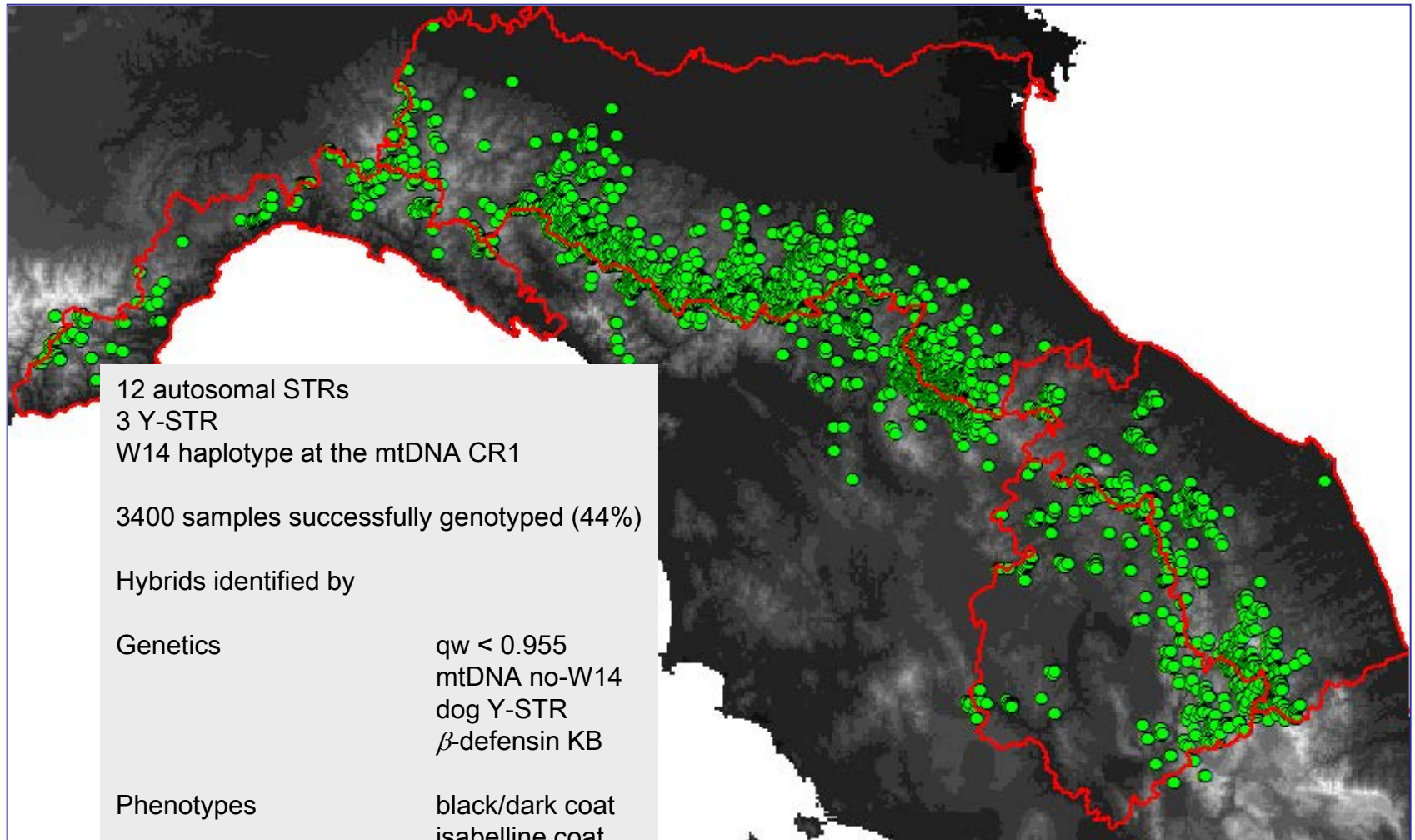
reduced genetic variability ...

50K SNP	H _{obs}	H _{exp}	P%
Italy (n = 50)	0.17	0.18	83.8
E. Europe (n = 127)	0.26	0.28	99.9

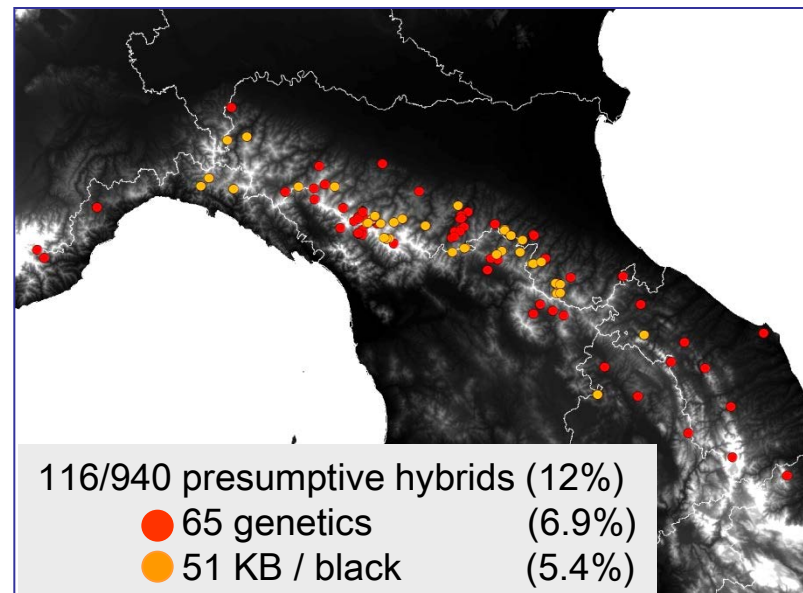
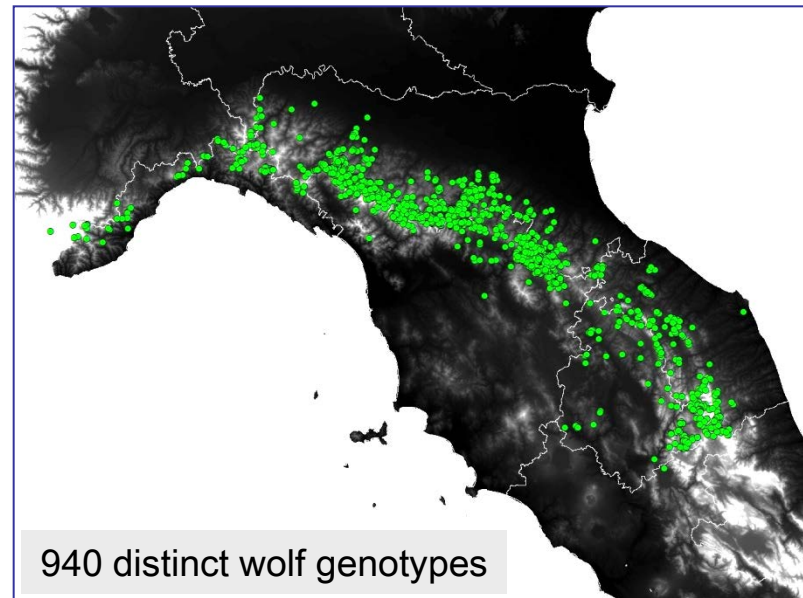
... and a number of diagnostic molecular markers

- 1 private mtDNA CR haplotype (W14)
- 2 private Y-STR haplotypes
- 5 private MHC haplotypes at DRB1, DQA1, DQB1 genes
- A high-frequency β -defensin melanistic deletion
- A number of population-specific diagnostic SNP (*in progress*)

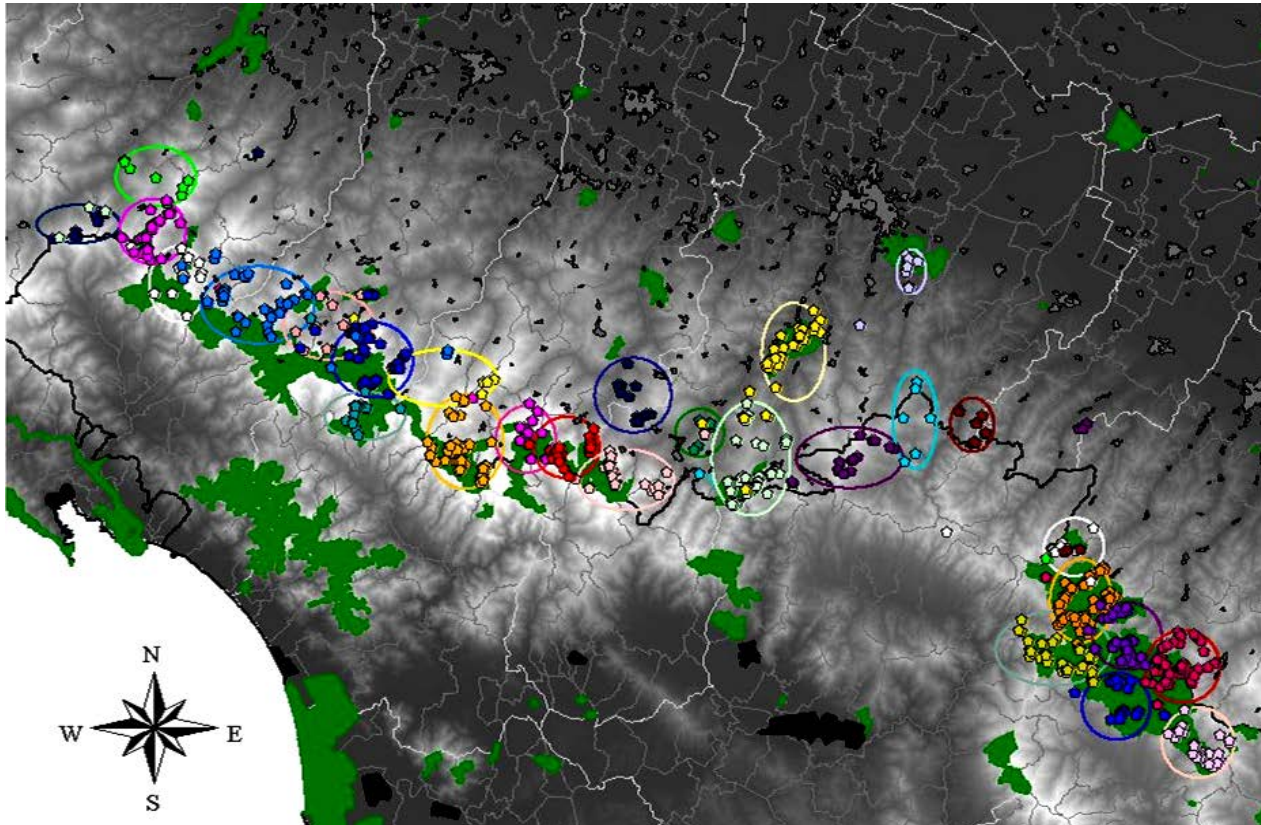
Non-invasive genetic sampling (NGS) 2002 – 2012 ($n = 7575$)



NGS 2002 – 2012: distributions of wolf and hybrid genotypes



Pedigree reconstructions and pack identification

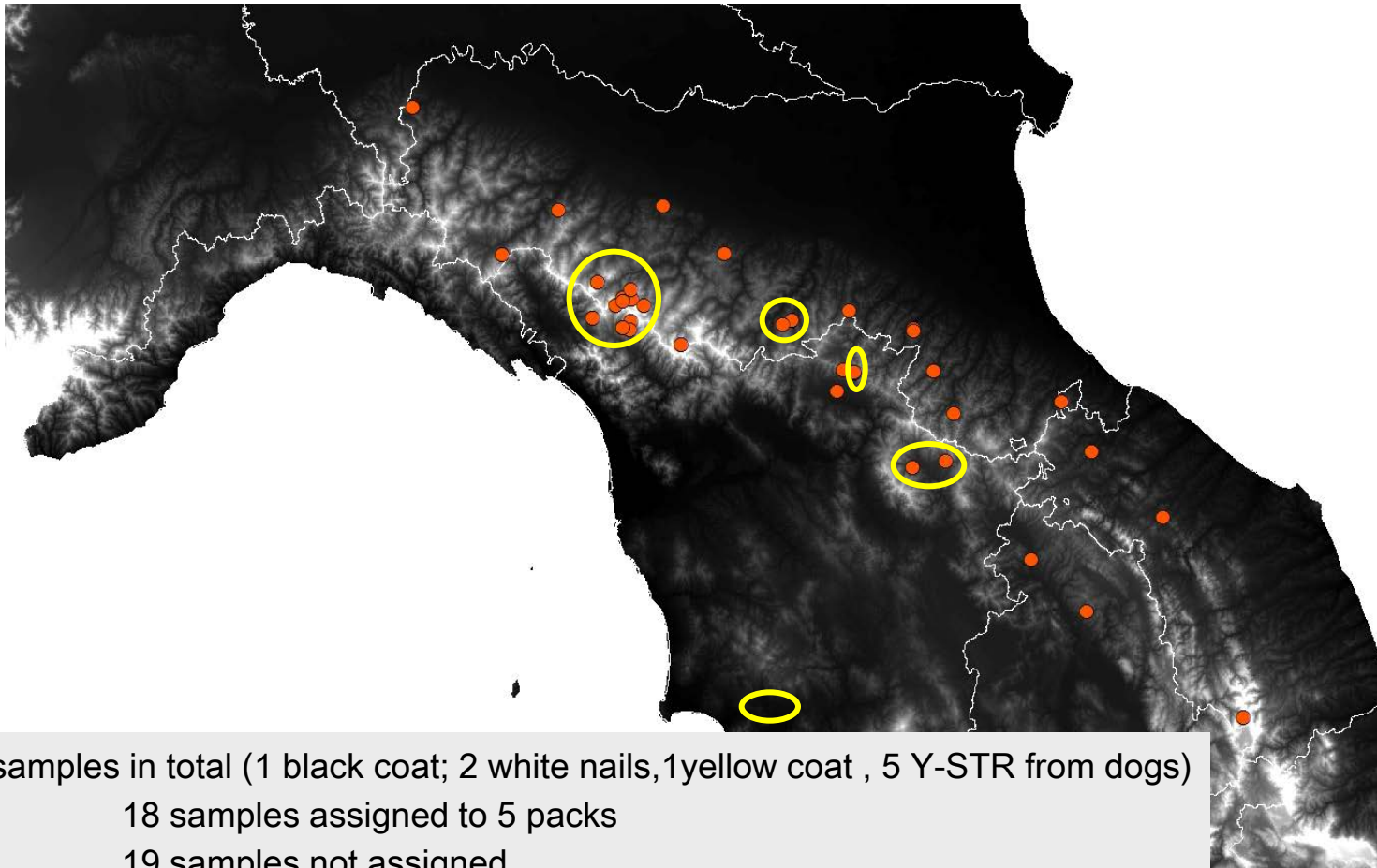


48 packs / 26 genealogies reconstructed
Turnovers of breeding pairs in 19% of the packs
No inbreeding

Average pack size	= 5.6 ± 2.5 wolves (min 3 - max 13)
Pop size estimated from packs	= 48 packs x 6 wolves x 0.7 = 202 wolves in winter
Pop size from CMR (2009)	= 187 wolves (95%CI = 116 - 334) in winter
Minimum pack core range	= 74 ± 52 km ² (Caniglia et al. <i>in rev</i>)

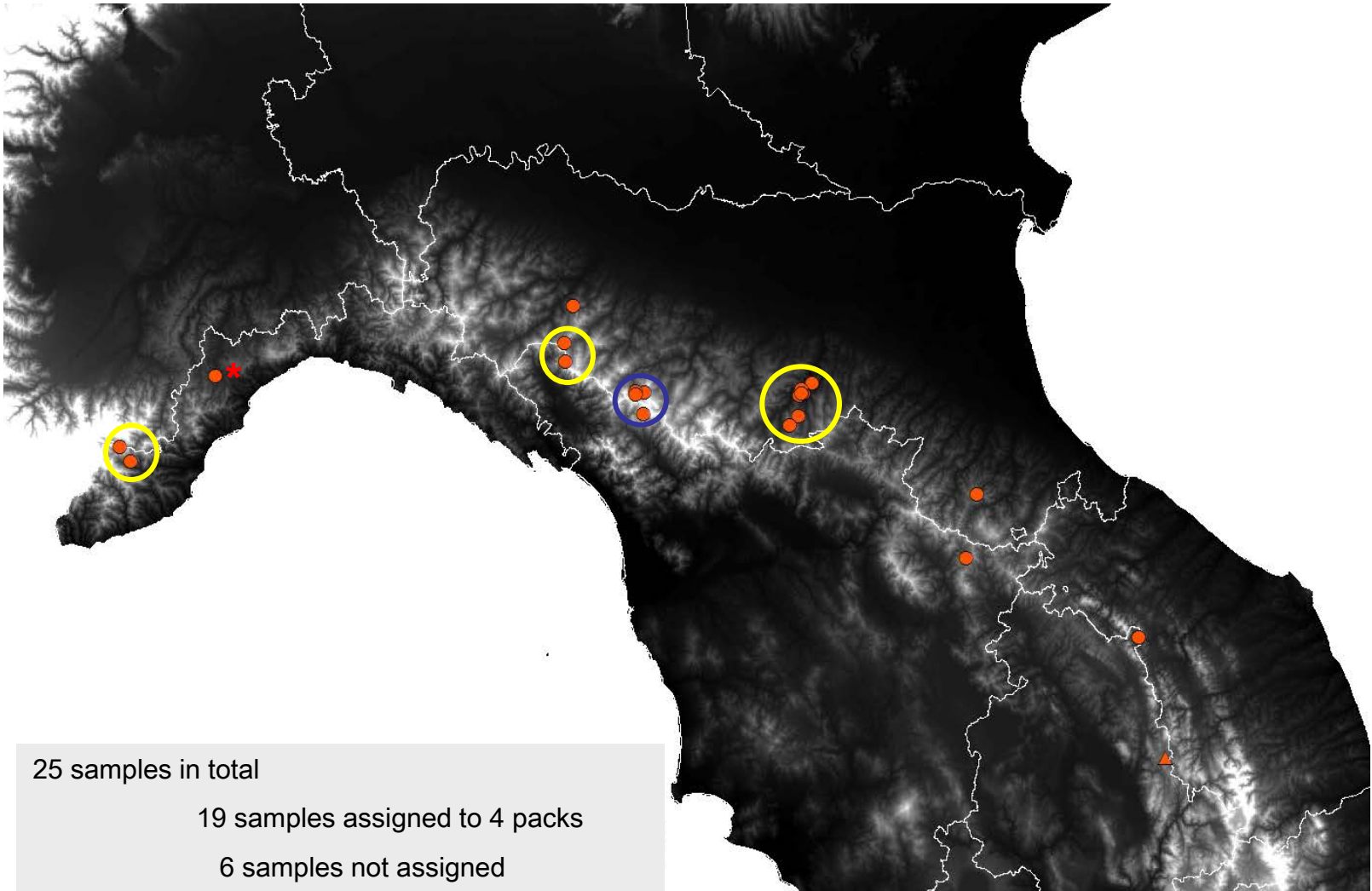
Assignment of hybrids to the packs

12 STR - structure $q_w < 0.955$

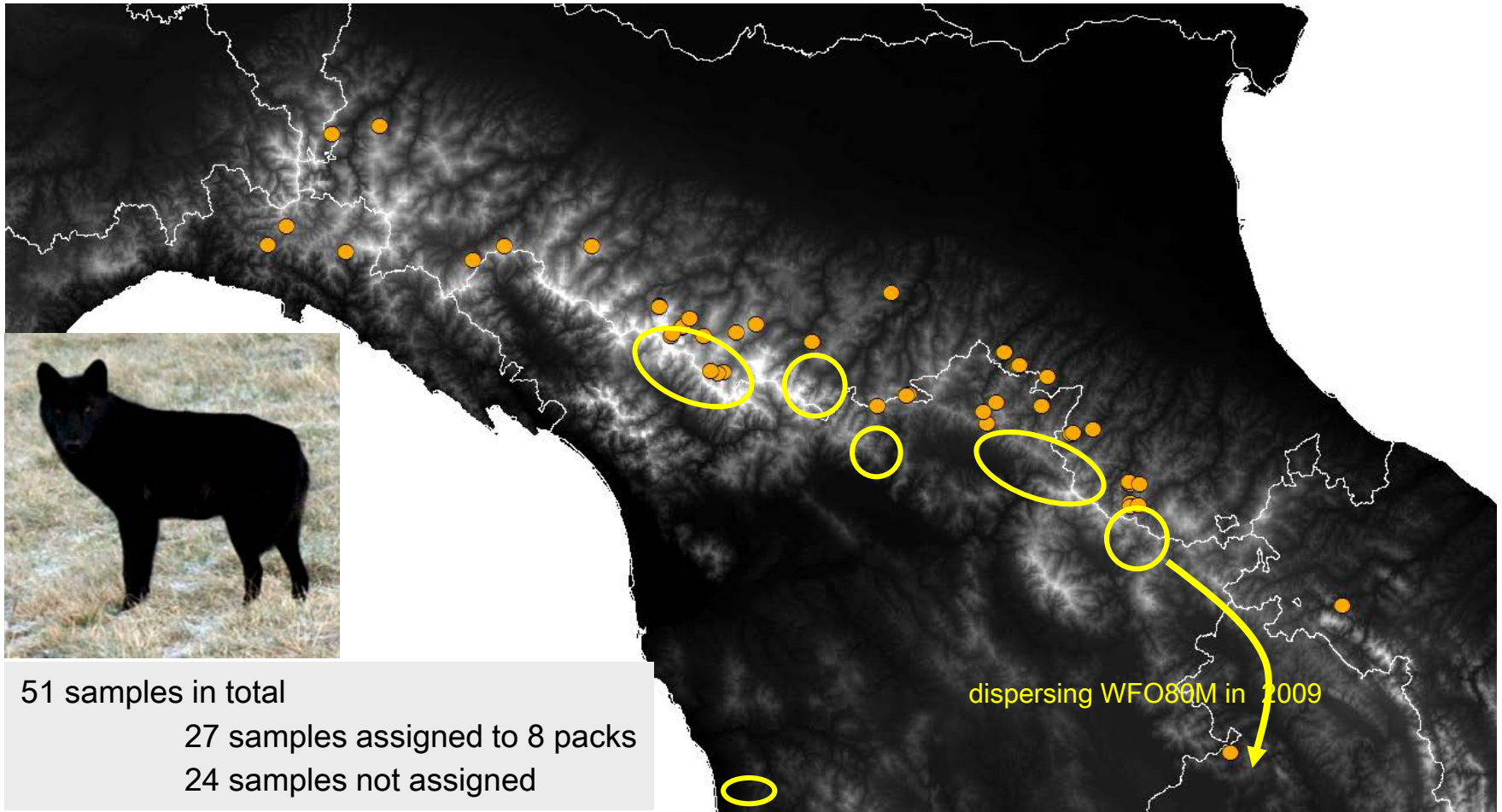


Assignment of hybrids to the packs

Y-STR from dogs



Assignment of KB and black coats to the packs



Origin of the melanistic KB deletion

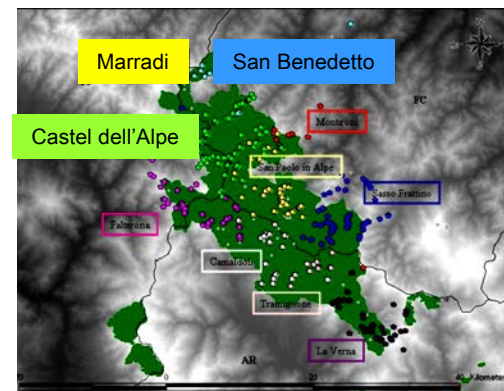
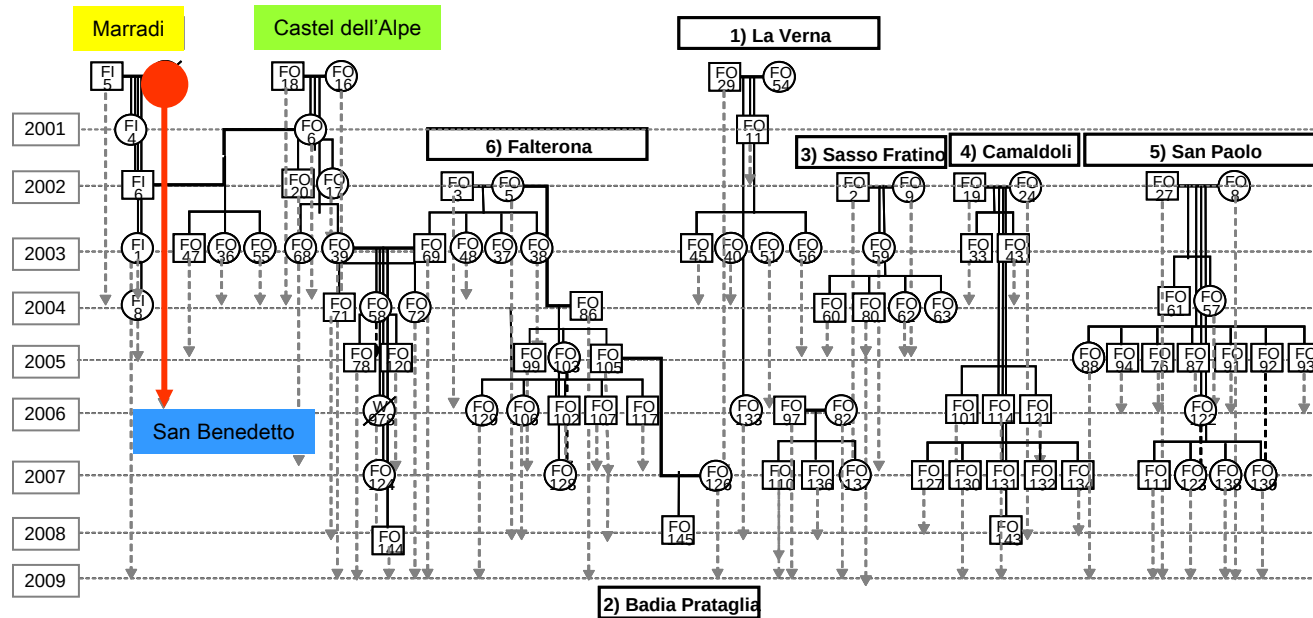
mutation in the IT wolf population ?
introgression from dogs via hybridization ?

NGS 2002 – 2012: frequency of hybrids in the Apennine wolf population

	including KB		excluding KB	
Hybrids	116	(12%)	65	(7%)
Hybrids assigned to packs	68		40	
Packs with hybrids	14		7	



Pedigree reconstructions and identification of hybrid packs



In the ISPRA wolf database (> 9000 samples) = 1151 distinct “wolf” genotypes

Haplotypes at the mtDNA CR1 ($n = 582$ IT “wolves”)

2 D haplotypes from dogs
3 W16 (unknown origin; unrelated)
577 W14

dog mtDNA introgression in IT wolves

Haplotypes at the Y chromosome ($n = 280$ IT “wolves”)

1 Y1 Czechoslovakian wolfdogs
7 Y5 (5 brothers) from dogs
1 Y6 from dogs
1 Y34 from dogs
53 Y26
217 Y17

dog Y introgression in IT wolves

Haplotypes at 3 MHC loci ($n = 112$ IT “wolves”)

23 in total
3 from dogs
4 “private” in hybrids
5 “private” in wolves

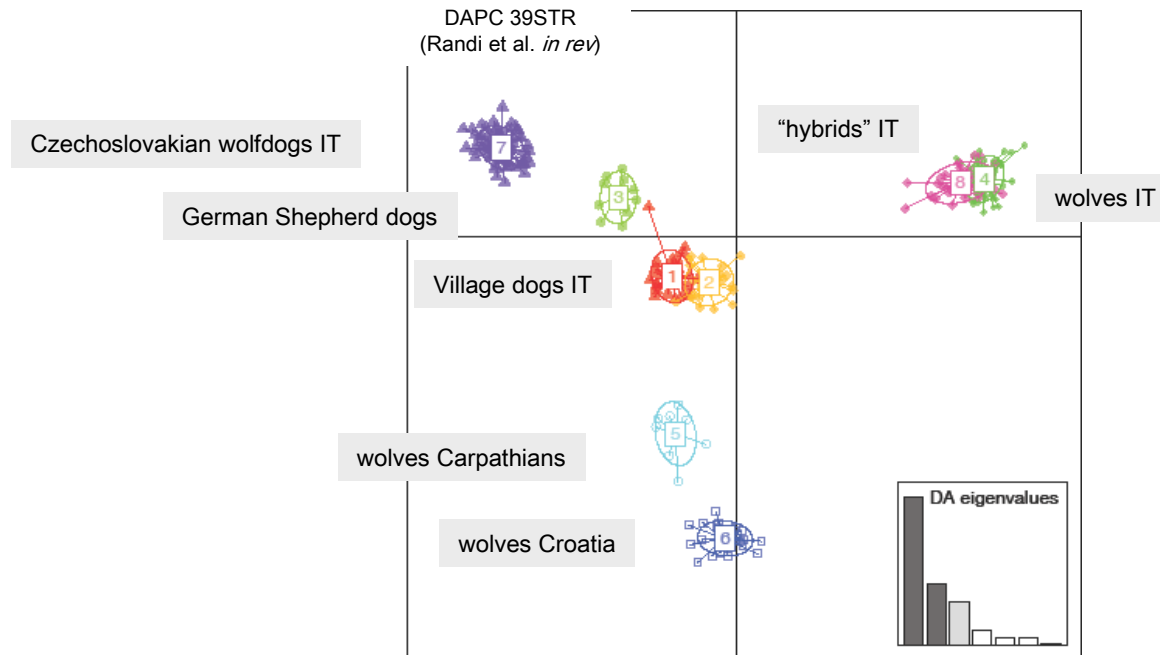
dog MHC introgression in IT wolves

KB frequency ($n = 360$)

26% in 273 IT “wolves”
36% in 87 dogs sampled in IT

dog KB introgression in IT wolves

Conclusions: 3% - 9% of the IT “wolf” samples show signals of hybridization



$qw < 0.990 = 104/1151 = 9.0\%$
 $qw < 0.950 = 43 = 3.7\%$
 $qw < 0.900 = 33 = 2.9\%$

7% F1 or F2 - 93% backcrosses

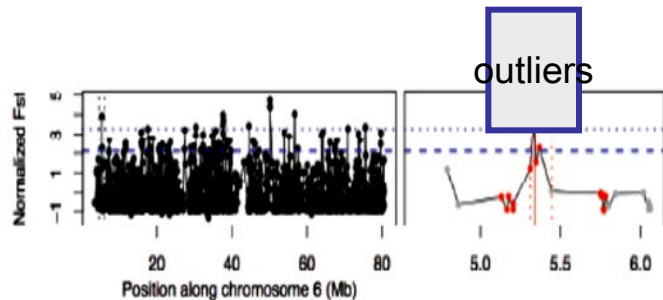
Most hybridization events map in predigrees before 2001

Probably 90% of the hybridization events were before 2001 = 3-4 past generations

Genomic data (recombination among haplotype blocks) are needed

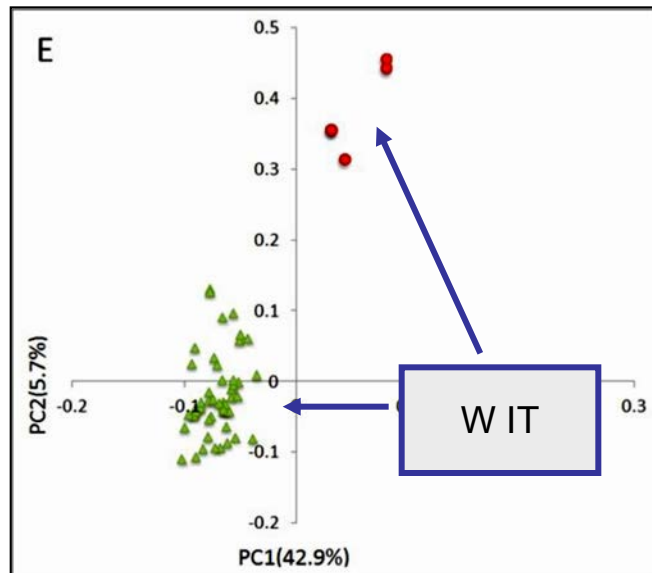
Perspectives from wolf genomics

Population structuring, selection, local adaptations and outlier SNPs



1) Maximizing wolf/dog F_{st} and hybrid detection likelihood

Domestication genes



2) Maximizing wolf population differentiation (F_{st})

Divergent selection

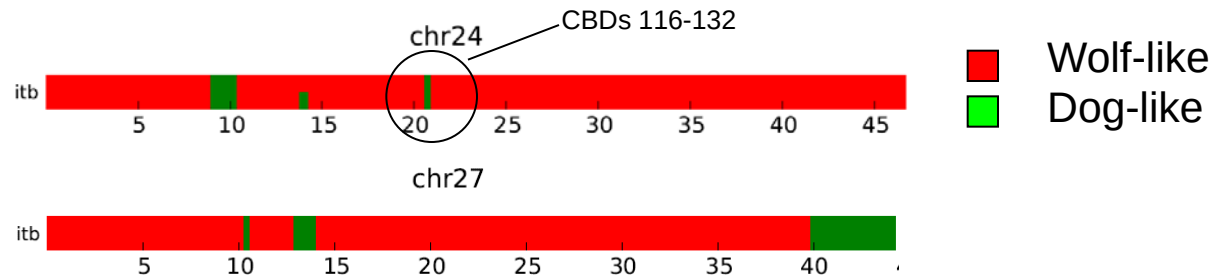
F_{st} between IT & EU wolves

at 40K SNPs = 0.20 – 0.30

at 35 outlier SNPs = 0.80 – 0.90

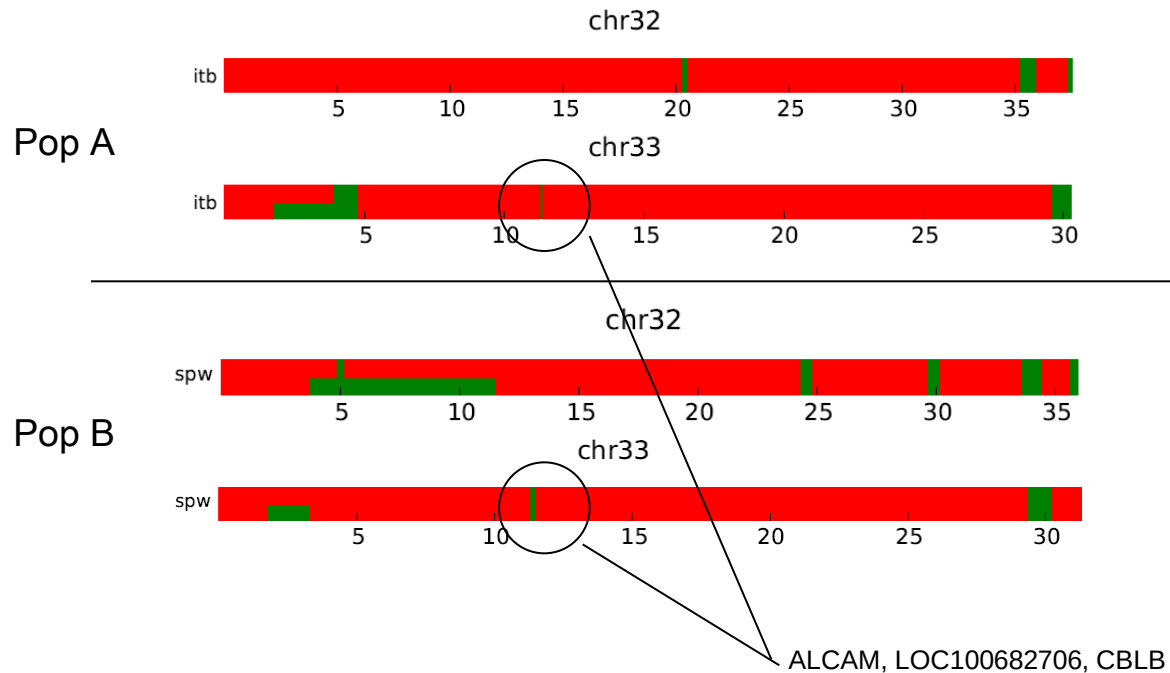
Perspectives from wolf genomics

Admixture analyses and detection of dog x wolf introgression



Perspectives from wolf genomics

Admixture analyses and detection of dog x wolf introgression



low-cost efficient non-invasive genomics

Collaborations

➤ RK Wayne, E Ostrander, P Silva et al. (USA; Portugal):

wolf genomics (whole genome sequencing) & transcriptomics (RNAseq)

➤ M Pilot, B Jędrzejewska et al. (Poland):

population genetics/genomics of wolves in EU

➤ U Saarma (Estonia); A Galov (Croatia); P Hulva (Czech Republic); R Godinho (Portugal):

diagnostic SNP markers by 170K Illumina Canine Microarray (a shared EU wolf dataset)

➤ D Huber, A Galov, J Kusak (Croatia):

wolf expansion in the Balkan-Dinaric-Alpine regions

➤ C Nowak (Germany), C Pertoldi, L Andersen and A Stronen (Denmark), A Speksnijder (The Netherlands):

wolf expansion in central Europe

Ask us for laboratory details, materials & methods, and for a list of papers published by the wolf group at the Conservation Genetics Lab - ISPRA