Conceptual and methodological issues in studying and managing hybridization between wild and domestic species (**WDH**): implications for conservation policies

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or...There is more than genetics in hybridization..

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In this presentation:

- Categories of hybridization (H)
    - *Are they adequate to cover H with domestic forms ?*
- Detecting WDH: issues with sampling and lab techniques
    - *The case of the (Italian) wolves and dogs*
- Managing WDH: technical options
- H without introgression IS a problem: H is more than a genetic issue
- Implication for hybrid policies

Hybridization is broadly defined as

Mating between individuals from genetically distinct populations

(no need to have different taxonomic status)

It is a common and natural evolutionary process

Positive effects:

produce new gene combinations and new taxa, boost demographic dynamics, alleviate inbreeding effect, heterosis (hybrid vigor), increased fitness, adaptation to new environments, …

(e.g. Allendorf & Luikart 2007,…)

Negative effects of H on conservation:

loss of locally adapted genes, outbreeding depression, **modification of gene pools, extinction of taxa and ESU**

H is a conservation concern when, due to anthropogenic causes, a taxon is threatened with loss of unique characteristics and the acquired ones are heritable (Wayne & Brown 2001)

Its effects on management policy are obvious at

- Individual level → the hybrid is different from parents
- Population level → the population has a new genetic structure and identity

Many reviews: Rhymer & Simberloff 1996, Dowling & Secor 1997, Arnold 1997, Allendorf et al 2001, …

Matching H definition with conservation policies

Natural:

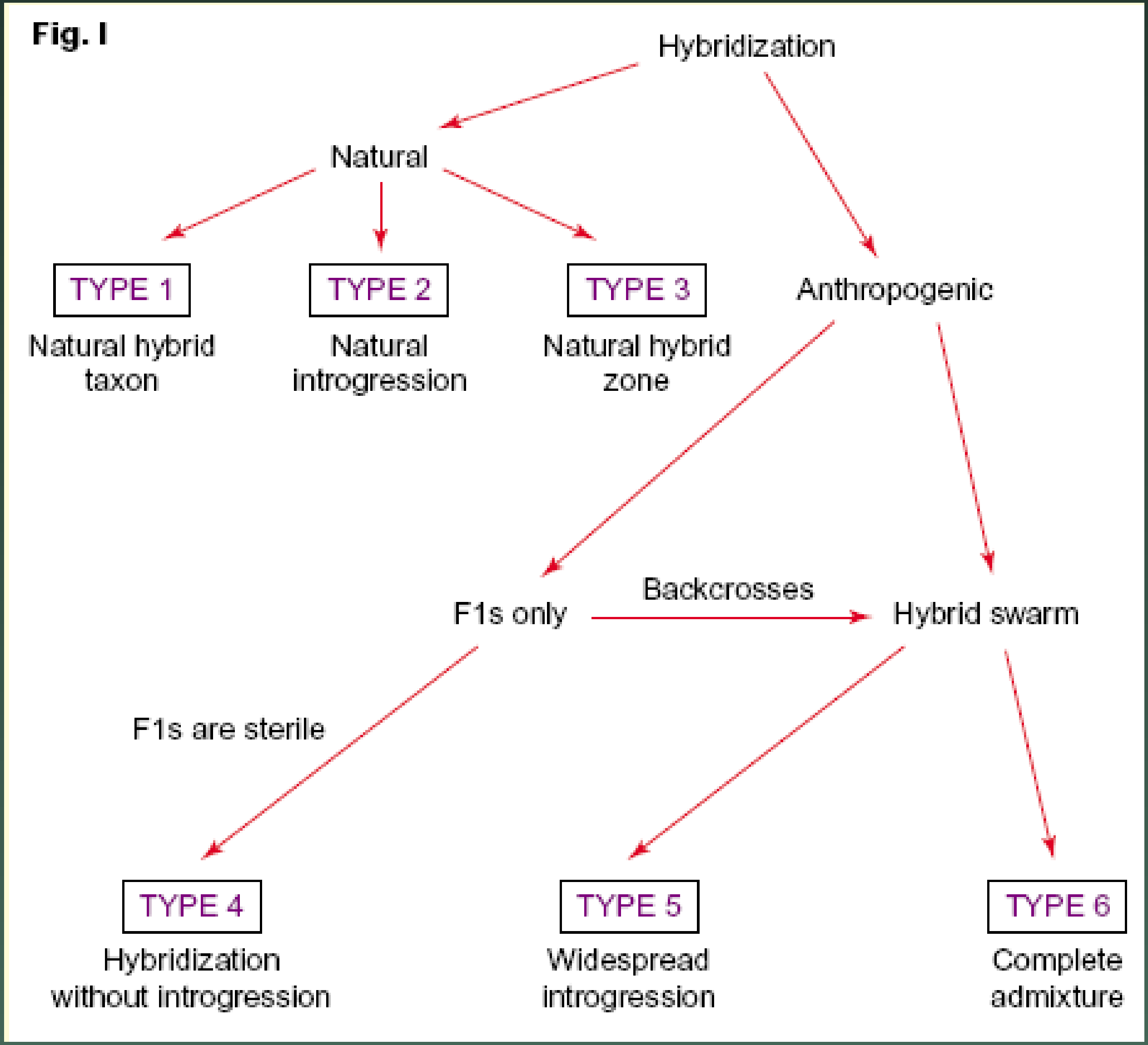
- occasional (e.g. bobcat/lynx)
- extensive (e.g. red wolf ?)

- Anthropogenic:
  - planned (e.g. Florida and Texas panthers)
  - accidental (e.g. northern spotted/barred owls, wolves/coyote ?)

Implications for management policy:

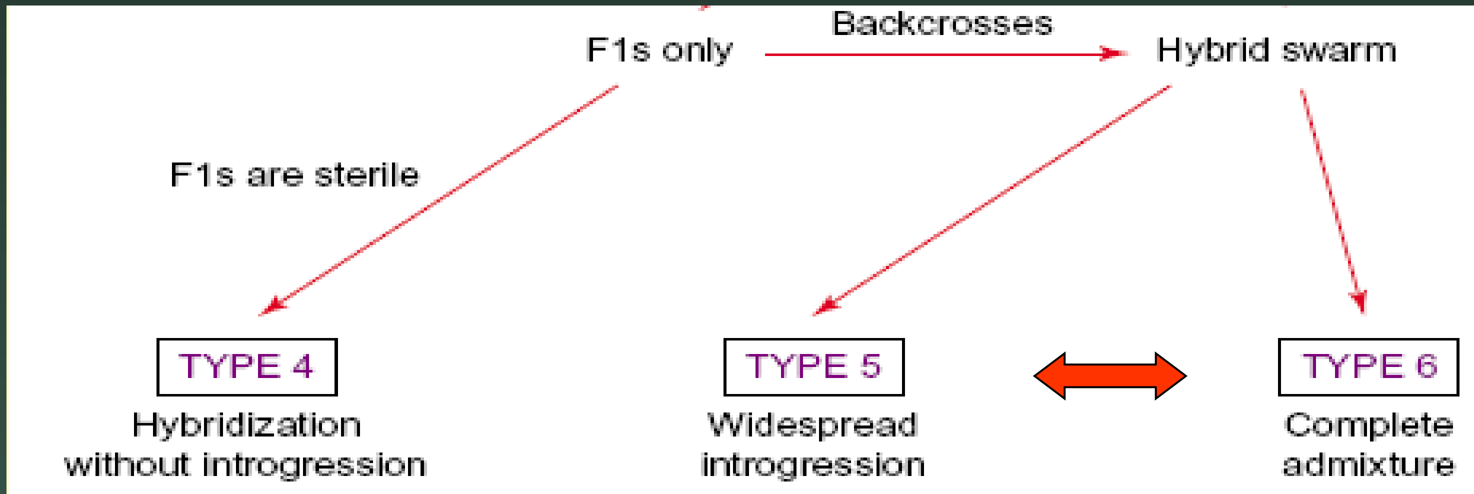
responsibilities can be identified, pressures can be counteracted

Categorization of hybridization



(Allendorf et al 2001)

Categories of anthropogenic hybridizations (AH)



Wolf-dog

(Vila &Wayne 1999, Randi & Lucchini 2002)

Red Wolf-coyote (Miller at al 2003, Fredrickson & Hedrick 2006)

Ethiopian wolf-dogs (Gottelli et al 1994)

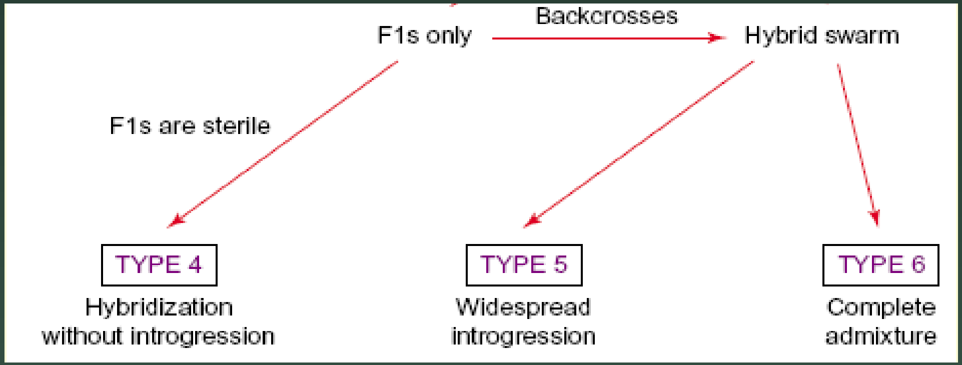
Scottish cats (Daniels et al 1998)

Algonquin wolf

(Grewal et al 2004)

Categories of anthropogenic hybridizations (AH)

This is a genetic perspective only, though a fundamental step to describe AH



On genetic basis:

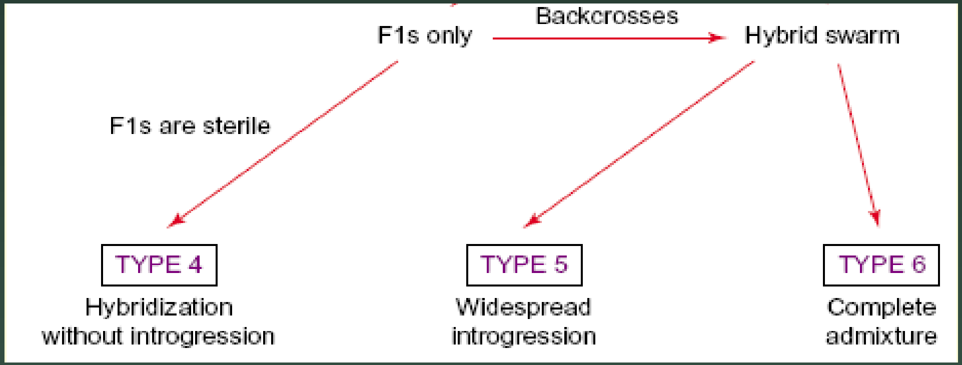
- Type 4 is a limited problem (but see Rhymer & Simberloff 1996)
- Type 6 is lost,
- Type 5 needs drastic (often immediate) action to counteract and reduce the damages

However, for management options to be considered,

the categories should be further qualified by ecological, ethological, economic, esthetic, epidemiological, etc. attributes

Especially when a wild species interbreed with its domestic relative (dogs, cats, pigs, ...and horses, goats, etc.)

Categories of anthropogenic hybridizations (AH)

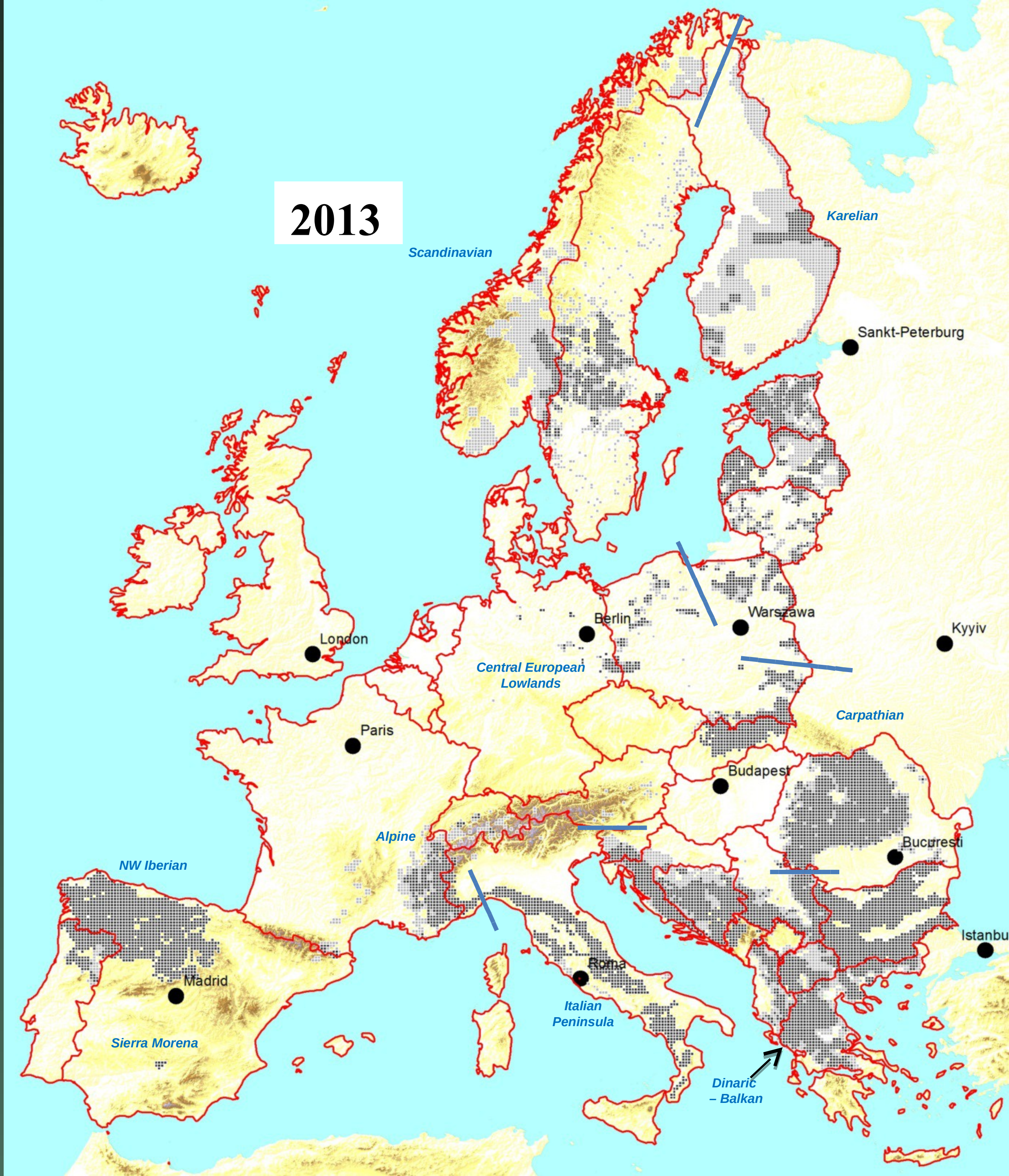


Is this framework still good to handle the Wild/Domestic Hybridization (WDH) ?

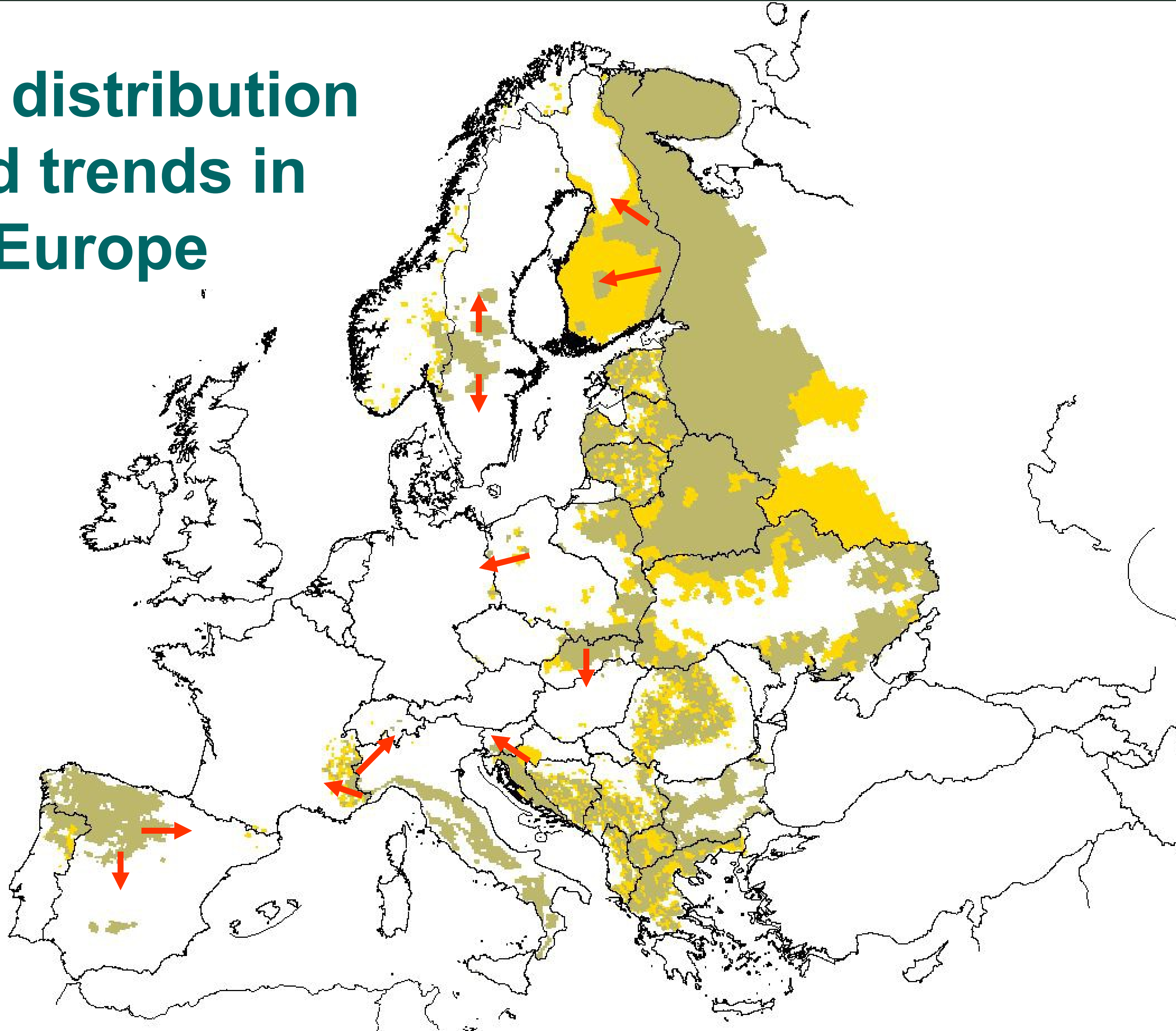
Dog, cat, pig, camel, cattle, horse, duck, etc.

A brief history of wolves in Italy

Wolf distribution in Europe



Wolf distribution and trends in Europe



A brief history of wolves in Italy

- Common all over Italy until mid XIX century
- Eradicated from the Alps in 1920s’, from Sicily during 1940s’
- Strongly reduced in the Apennines during 40s’ – 60s’

Causes of decline in recent times

- Habitat loss and fragmentation
- Reduction of wild prey densities
- Direct persecution

Natural recovery of the wolf

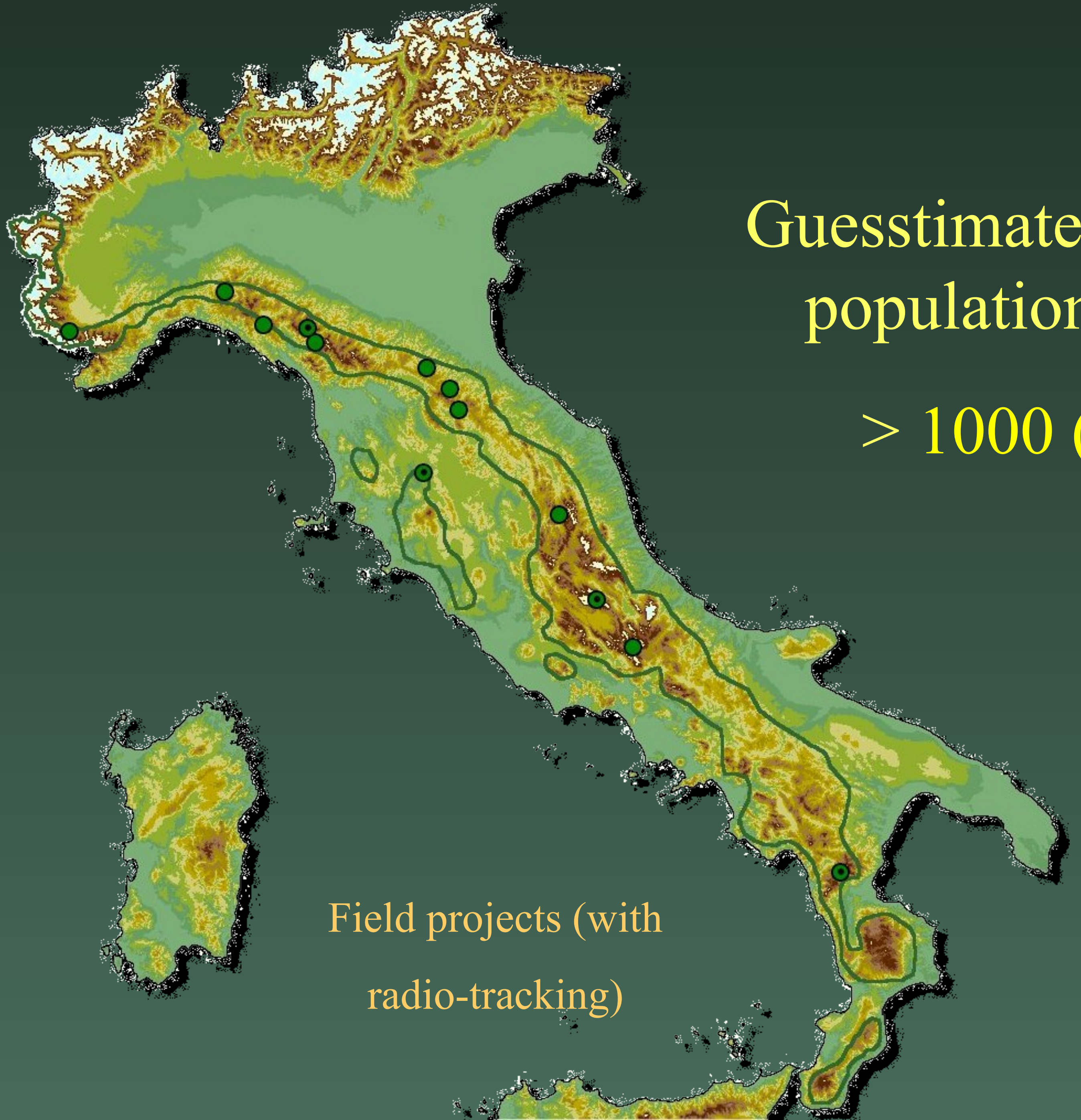
- *Legal* aspects
- *Environmental* factors
  - Protection of critical habitats
  - Recovery and protection of wild preys
- *Historical-economic* factors
- *Biology of the species*
  - high productivity
  - high ‘dispersal’
  - (..breeding with dogs ?)

Wolf population in Italy: 1973 - today

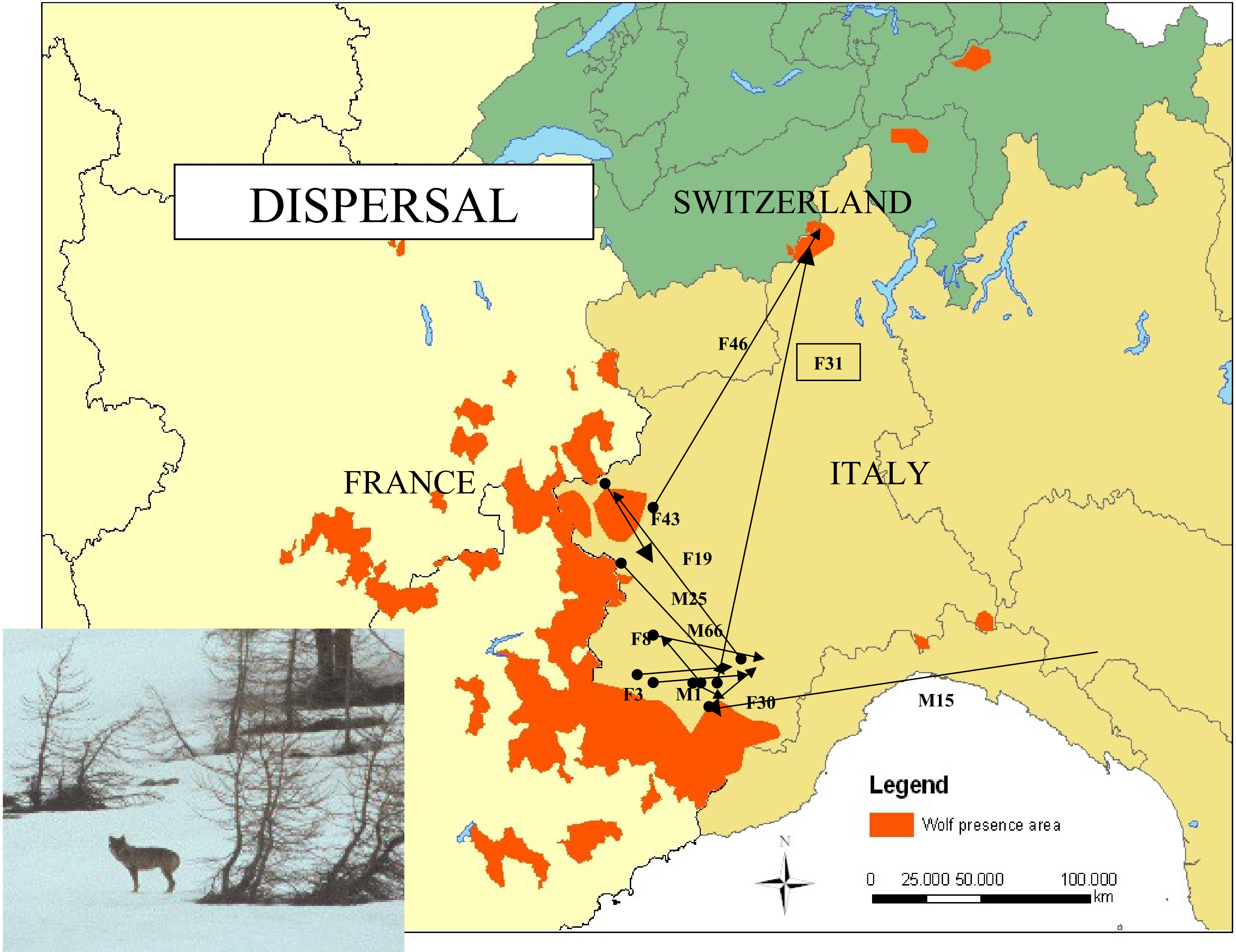
- positive trends: about 5% per year
- local eradication and recolonisation processes
- local densities fluctuate widely

Guesstimate of the wolf population in 2014:

> 1000 (1500?)



DISPERSAL


SWITZERLAND
FRANCE
ITALY
F46
F31
F43
F19
M25
M66
F8
F3
M1
F30
M15
Legend
Wolf presence area
N
0
25.000 50.000
100.000
km

Dispersal di M15

- March 2004 – February 2005
- 317 days
- 1243 km

Imagine if M15 were a hybrid

Imagine its founder effect

Dog and wolf densities in Italy

2-4 wolves/100 km^2

vs.

100-300 free-ranging dogs/100 km^2

(24-82 feral dogs/100 km^2)

(Boitani 1984, Genovesi & Dupré 2000, Ciucci et al 2001)

The first evidence of hybridization in Italy, 1975

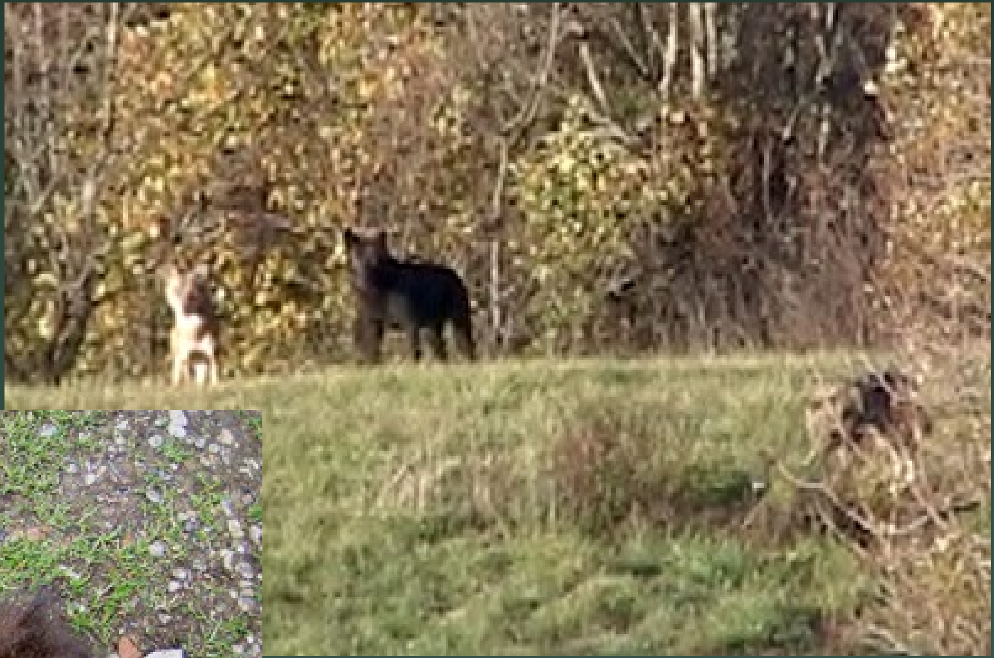
(Boitani 1982, 1986; Zimen 1997)

More evidence of hybridization……

Black wolves 1978-2014

(Randi & Lucchini 2002; Apollonio et al. 2004; Randi 2006, Verardi et al. 2006)

More black “wolves”…, 2002



The recent case of the Maremma Natural Park (Tuscany)

Caniglia et al (2013)

First signs in 2000

6-7 hybrids until 2010

Litters 2004-2009

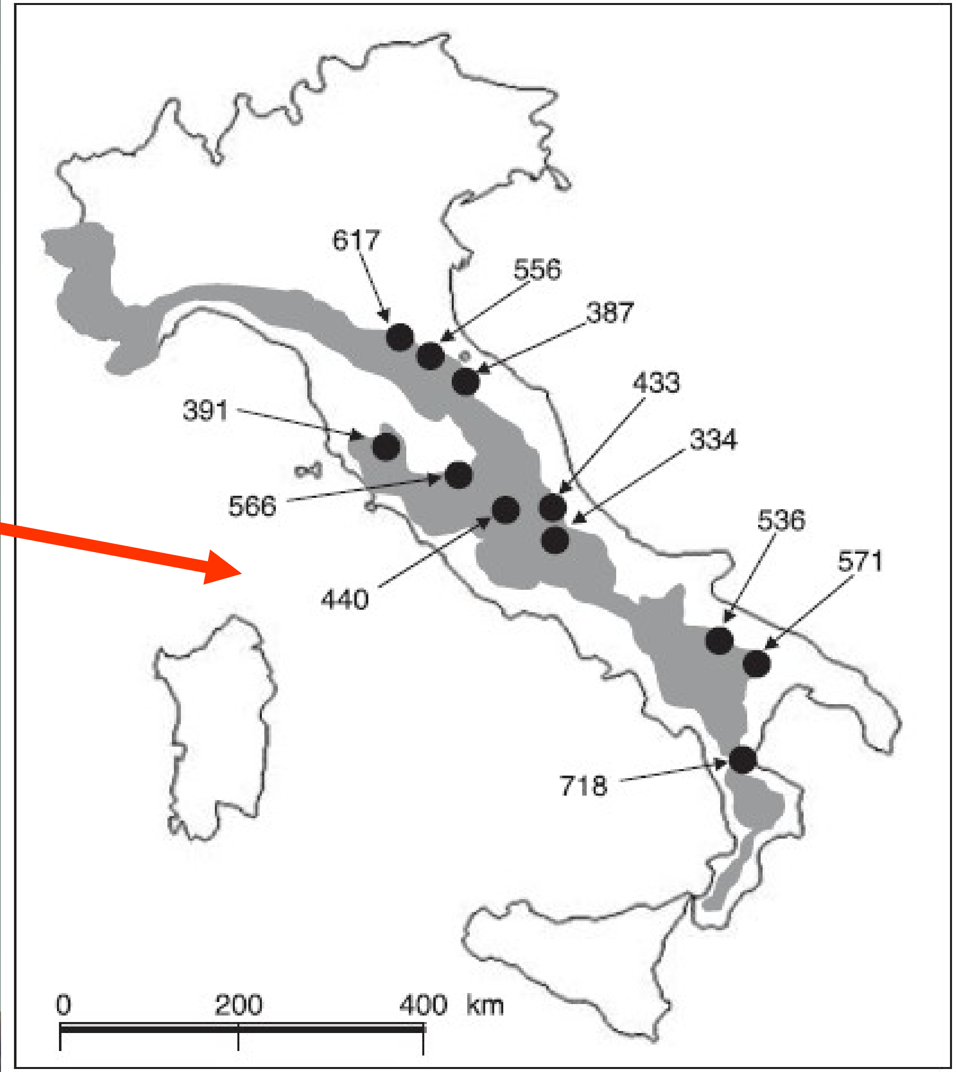


Fig. 1 Approximate wolf distribution range in Italy and locations of putative hybrid samples (see Table 3).

Main genetic studies on wolves in Italy (before 2000)

Author/year	Sample size wolves/dogs	Sampling time frame	Genetic marker (no)	Main results concerning hybridization
Randi 1993	30	5 years	mtDNA	No introgression
Randi et al 1993	30	5 years	Allozyme variation	No introgression
Lorenzini & Fico 1995	46/58 (mostly from Central Italy	3 years	Allozyme variation (41)	No introgression **

** literally: " The patterns of genetic variability … do not suggest substantial interbreeding between wolf and dog".

Author/year	Sample size wolves/dogs	Sampling time frame	Genetic marker (no.)	Main results concerning hybridization
Randi et al. 2000	101/50 (all Italy)	1984-1999	mtDNA CR (546 bp)	**No introgression** from maternal lineage
Dolf et al. 2000	70/90 (all Italy)	?	unlinked microsat. (7)	No hybrids, **no introgression**
Randi & Lucchini 2002	104+3/95 (all Italy)	1984-1999	unlinked microsat. (18)	1-2 admixed ancestry (0.9-1.9%), **no introgression**
Lucchini et al. 2002	14(+100)/100 (Western Alps)	1999-2000	unlinked microsat. (6-9)	No hybrids, **no introgression**
Ciucci et al 2003	3(+101)/95 (Siena Prov.)	1993	unlinked microsat. (18)	Dew-claws as sign of admixed ancestry
Scandura 2004	52(+22)/19 (Arezzo Prov.)	1998-2003	unlinked microsat. (10)	No hybrids, **no introgression**
Scandura 2005	14(+22)/20 (Arezzo Prov.)	1998-2003	unlinked microsat. (10)	1 admixed ancestry (7.1%), **no introgression**
Randi 2006	193/95 (Emilia Romagna)	2002-2005	unlinked microsat. (6)	4 admixed ancestry (2.1%), **no introgression**
Verardi et al. 2006 *	220*/85 (all Italy)	1987-2002	unlinked (4) and linked (16) microsat.	11 admixed ancestry (5%), introgressive hybridization recurrent but **negligible introgression**
Randi et al 2014	271 wolves, 69 dogs, 103 hybrids	………	39 microsats	Extensive introgression (87% backcrosses)
Next ?	XX ?		48 + microsat, SNPs, …	A hybrid swarm ?

* 6 confirmed hybrids from southern Tuscany (Randi, pers. com.) and 3 confirmed hybrids from Siena and Grosseto provinces (Ciucci et al 2004) have not been included in the analysis.

Examples of technique-dependent results: W535 e WRE10

W535 – 'wolf' with 6-10 loci, but backcross with 18 loci
(Ciucci et al. 2003)

WRE10 – 'wolf' with 6 loci and >15 scat samples (2002-2007), + 1 tissue sample, but backcross with 12 loci
(M. Andreani. com. pers.)

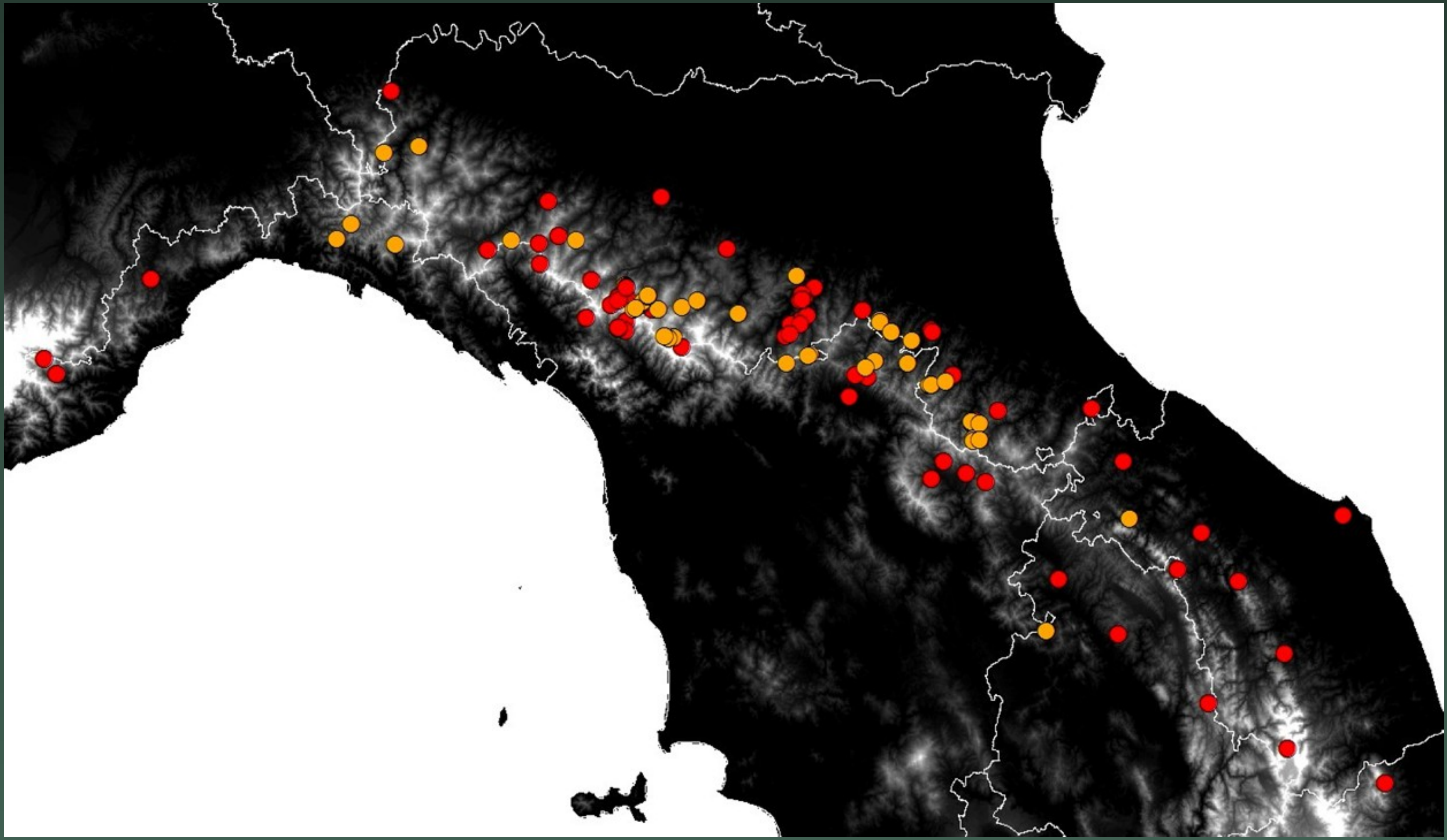
Many papers on wolf/dog hybrids in Italy have dismissed the importance of hybridization by concluding that:

1. Introgression is limited by behavioral separation, i.e. wolves and dogs do not form social bonds
2. Hybridization is marginal as it occur mostly in the periphery of the range
3. Hybridization is irrelevant to wolf conservation because it has been ongoing for centuries

These statements are not supported by robust evidence and represent a good case of non application of the precautionary principle, accepting the risk of a Type II error.

Distribution of hybrid genotypes (red) and black coats (yellow)

Northern Apennines, Randi et al 2012



Distribuzione dei genotipi ibridi (*n* = 65 ●) e neri (*n* = 51 ●)
(identificazioni genetiche e fenotipiche; *n* = 116)

Wolf-dog hybridization in Europe

Russia	Ryabov 1985, Bibikov 1988
Norway	Vila et al 2003
Latvia	Andersone et al 2002
Germany	I. Reinhart, pers. com.
Italy	Boitani 1984, Verardi et al 2006
Spain	Blanco et al 1992, Vila & Llaneza pers. com.
Serbia, Croatia,…	D. Huber pers. com.
Bulgaria,	Randi et al 2000,
Israel	Mendelsohn, pers. com.
etc…	

Wolf-dog hybrids have been documented in Europe and Middle East

Hybridisation between wolves and dogs in **Latvia** as documented using mitochondrial and microsatellite DNA markers. *Andersone et al. 2002 Mamm. Biol.*

Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf-dog hybrids. (in **Sweden**) *Vila et al. 2003 Heredity*

Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the **Iberian** Peninsula. *Godinho et al. 2011, Mol. Ecol.*

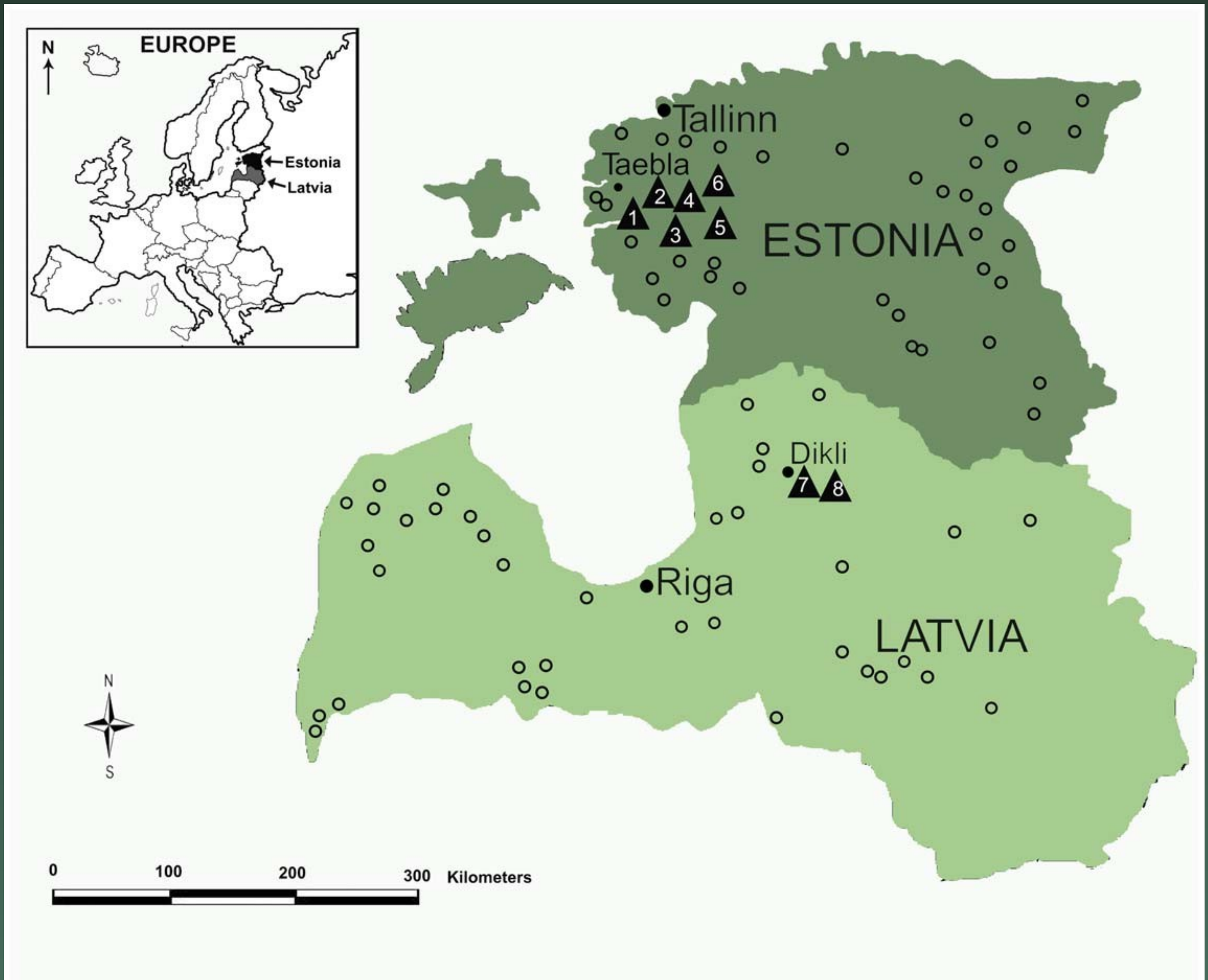
Bucking the trend in wolf-dog hybridization: first evidence from Europe of hybridization between female dogs and male wolves. (in **Estonia**) *Hindrikson et al. 2012, PlosOne*

Detecting hybridization between **Iranian** wild wolf (Canis lupus pallipes) and free-ranging domestic dog. *Khosravi et al. 2013 Zool. Science*

Gene flow between wolf and shepherd dog populations in **Georgia** (Caucasus). *Kopaliani et al 2014 J. Heredity*

Multilocus detection of wolf x dog hybridization in **Italy**, and guidelines for marker selection. *Randi et al. 2014 Plos ONE*

Sampling locations of wolves (small open circles) and wolf-dog hybrids (black triangles 1–8) in Estonia (Taebla) and Latvia (Dikli), hunted during the winter period of 2008–2009.



Number of shot wolves and stray dogs per year in Latvia
2500
2000
1500
1000
500
0
1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013
Wolves
Dogs

Wolf-dog hybrid in Germany 2003

Wolf dog hybrids hunted in Serbia, 2005

All genetic results (with the exception of *Hindrikson et al. 2012 in Estonia)* confirm the previously described directionality of wolf–dog (and wolf–coyote) hybridization:

(i.e., female wolves mating with male dogs or coyotes)

Boitani 1983; Lehman et al. 1991; Gottelli et al. 1994; Roy et al. 1994; Vilà and Wayne 1999; Randi et al. 2000; Randi and Lucchini 2002; Vilà et al. 2003).

Another scales of wolf-dog H: *deliberate crossbreeding*

First mentions from Aristotle (±2,400 BP) and Pliny (±1900 BP)

Widespread in 17th and 18th century to improve dog breeds

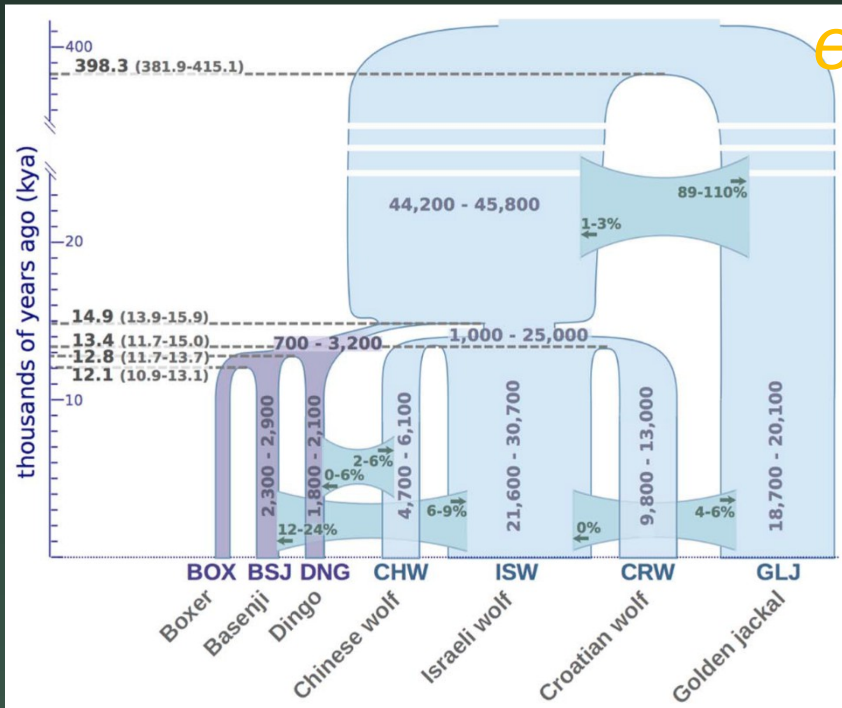
Several new wolf - dog breeds :

Sarloo dog

Lupo italiano

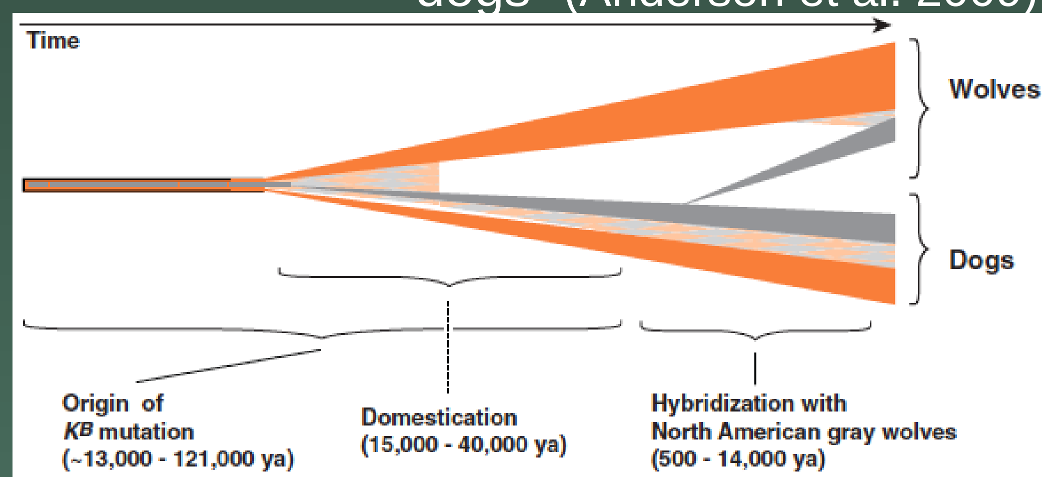
Czechoslovakian wolf-dog

Another scales of wolf-dog H: *ancient events*

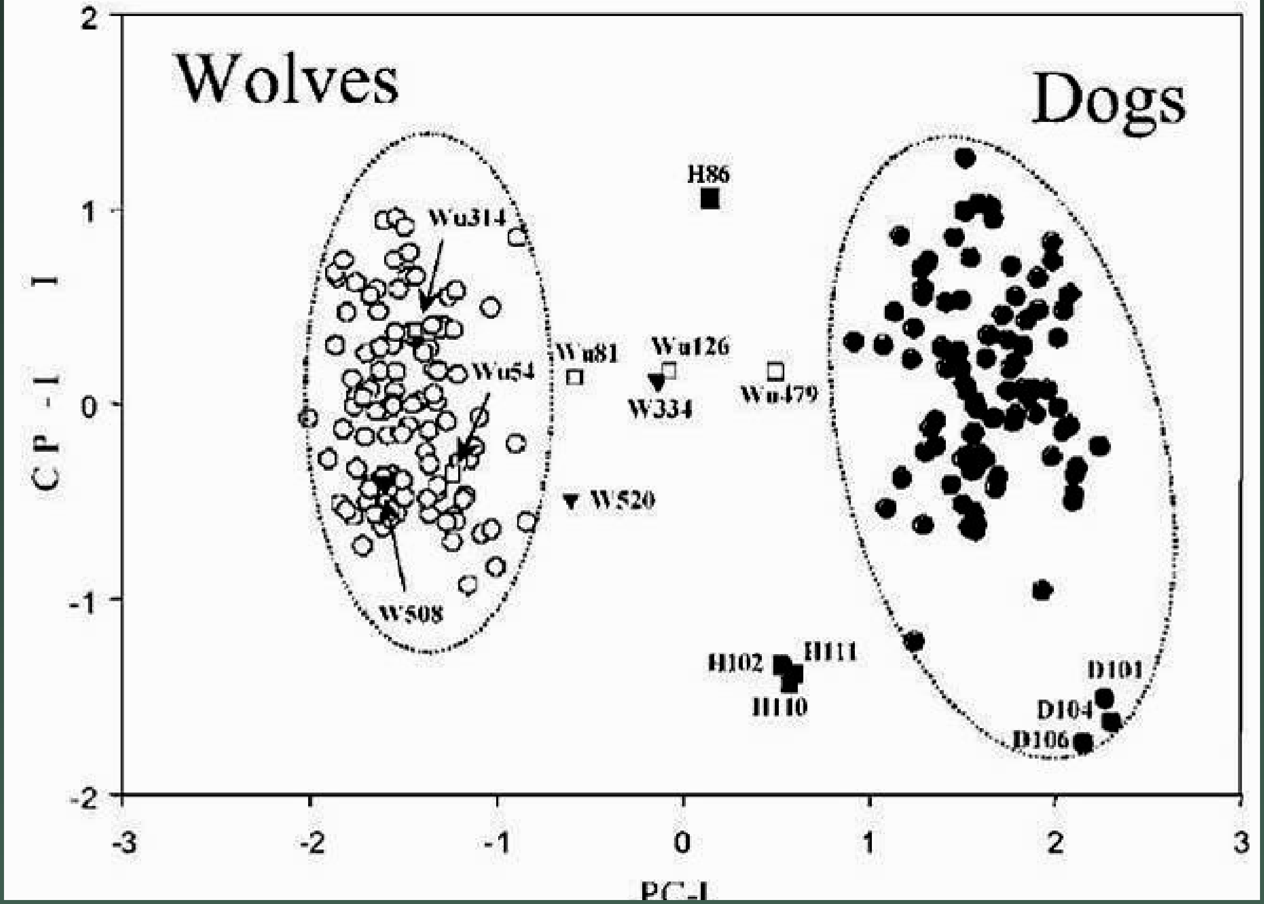


Freedman et al. (2014)'s model suggesting considerable gene flow between some wolf and dog populations.

"The melanistic K locus mutation in North American wolves derives from past hybridization with domestic dogs" (Anderson et al. 2009)

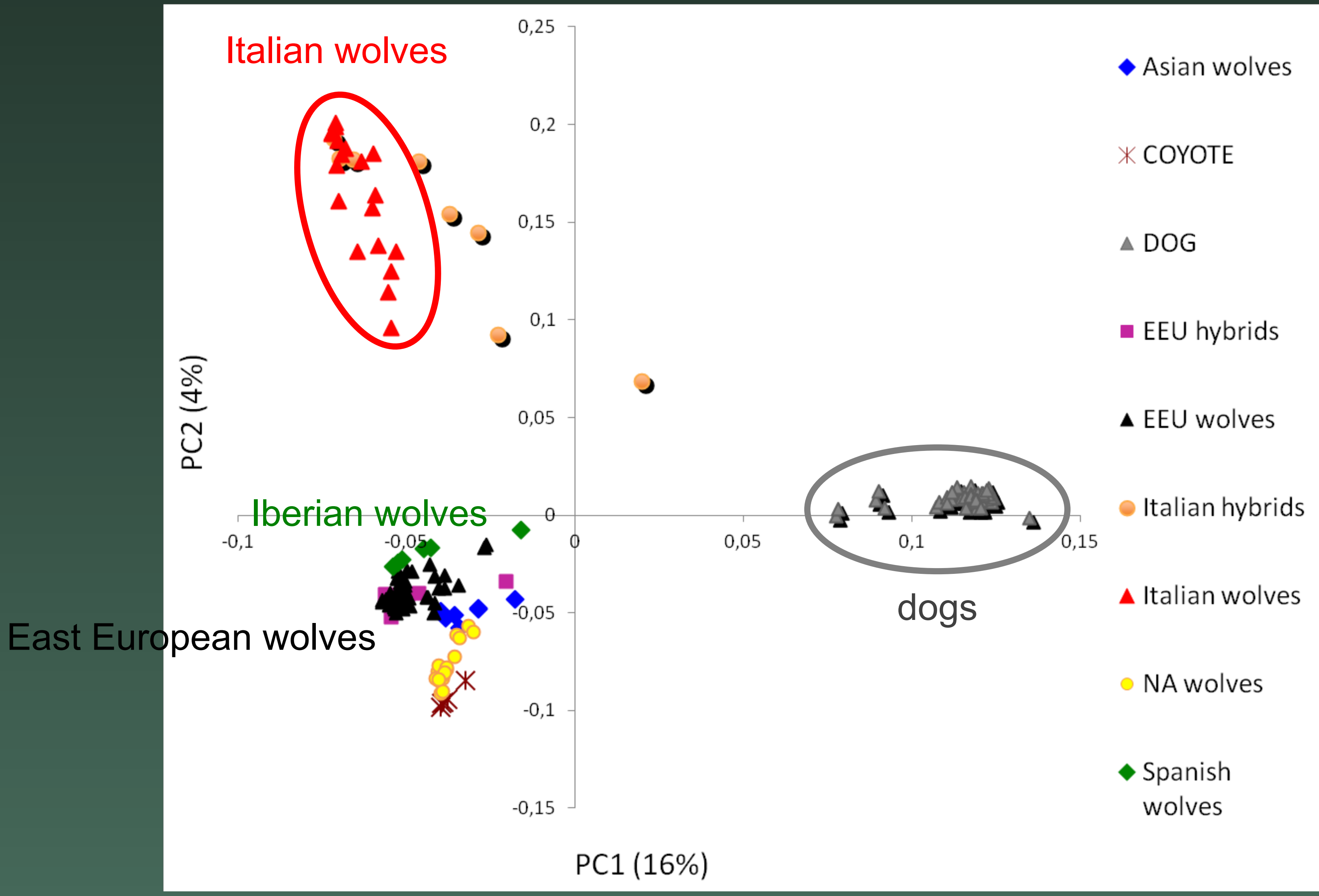


Identifying the hybrids (Randi & Lucchini 2002)



Scores of individual wolf and dog microsatellite genotypes plotted on the first two axes (PC-I, PC-II) of a principal coordinate analysis performed using Pcagen. H = known captive-reared hybrid wolves; Wu = captive-reared wolves of unknown origin; W334 and W508 = "black wolves"; W520 = "fifth finger wolf".

Genome-wide differentiation of European wolf populations



Based on data from vonHoldt et al. 2010

In the absence of diagnostic alleles, as might happen in complexes of closely related taxa, intermediate allele frequencies can provide evidence for hybridization <u>if the genetic variability of the parental species is well characterized and a sufficiently large number of loci is analyzed</u>

(the allele frequencies method assumes HWE or require ways for correcting deviation. Wolves often don't show HWE)

However, in the case of dogs (a highly variable and artificially maintained “taxon”),

an appropriate sampling scheme (in time and space) is essential for ruling out the possibility that the two samples being compared are effectively potentially interbreeding.

Assessing dingos, dogs and hybrids. (Elledge et al 2006)

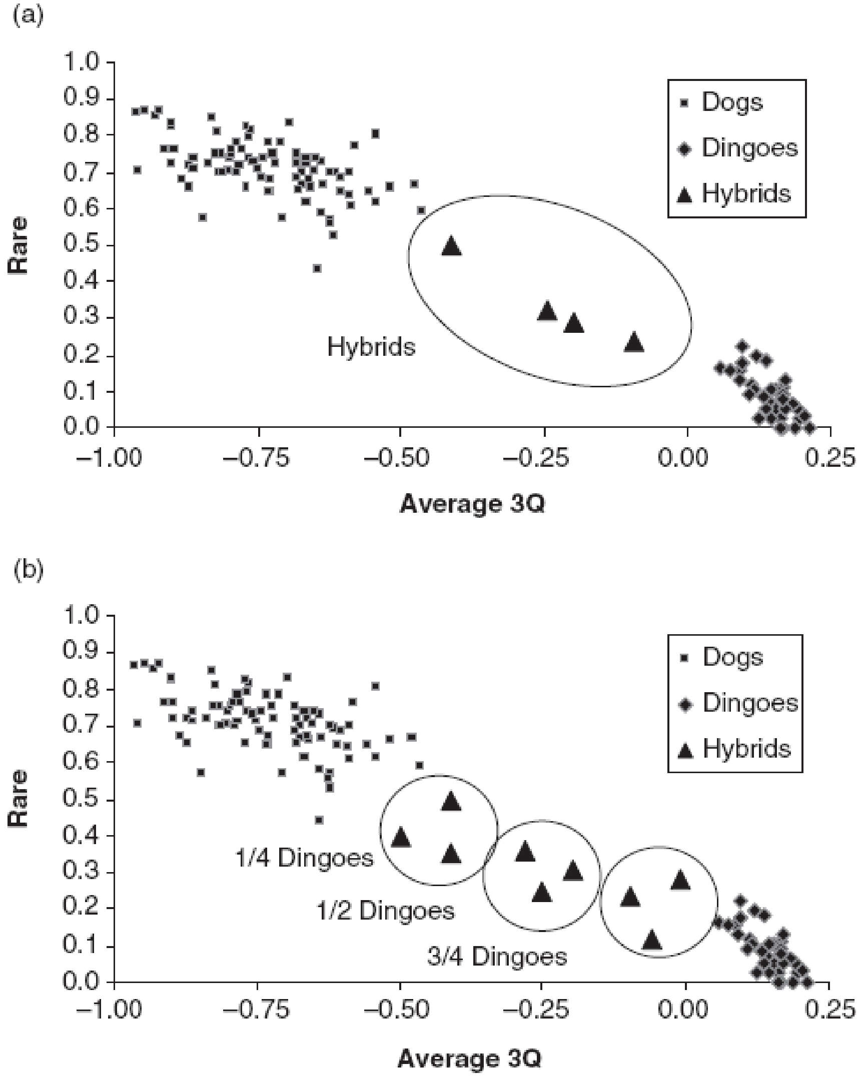


Fig. 1. Clustering methods can be used to visually determine the approximate status of test animals using results from the analysis of genetic variation. The discriminatory power of this method can be improved by using hybrids with known proportions of dingo ancestry (b) as a reference rather than the pooled use of hybrids (a). Data from Wilton (unpublished).

Detecting WDH: broad conclusions

- Results have been largely technique dependent:
    - -mtDNA, unlinked microsat, linkage groups, SNPs, etc.
    - -Sampling strategies: local, global, time-frames, etc.
- Genetic analyses provide an (imperfect) diagnostic tool
- Genetic analyses are unable to identify hybrids after F2 (and B1)
- Still unclear the performance of the techniques in distinguishing between natural polymorphism and hybridization
- Genetic, morphological and ecological analyses should complement genetics to detect WDH

Detecting H: broad implication for policy

- Genetic results ARE important but NOT sufficient to draw conclusions on a very complex phenomenon, especially if these conclusions dictate management policies

An example of morphology complementing genetics in expanding ways to detect and define hybrids

Pelage patterns of a sample of wild-living cats from the north-east of Scotland

(Daniels et al 2001)

Diagnostic characters for the Scottish wildcat

(Kitchener et al 2005)

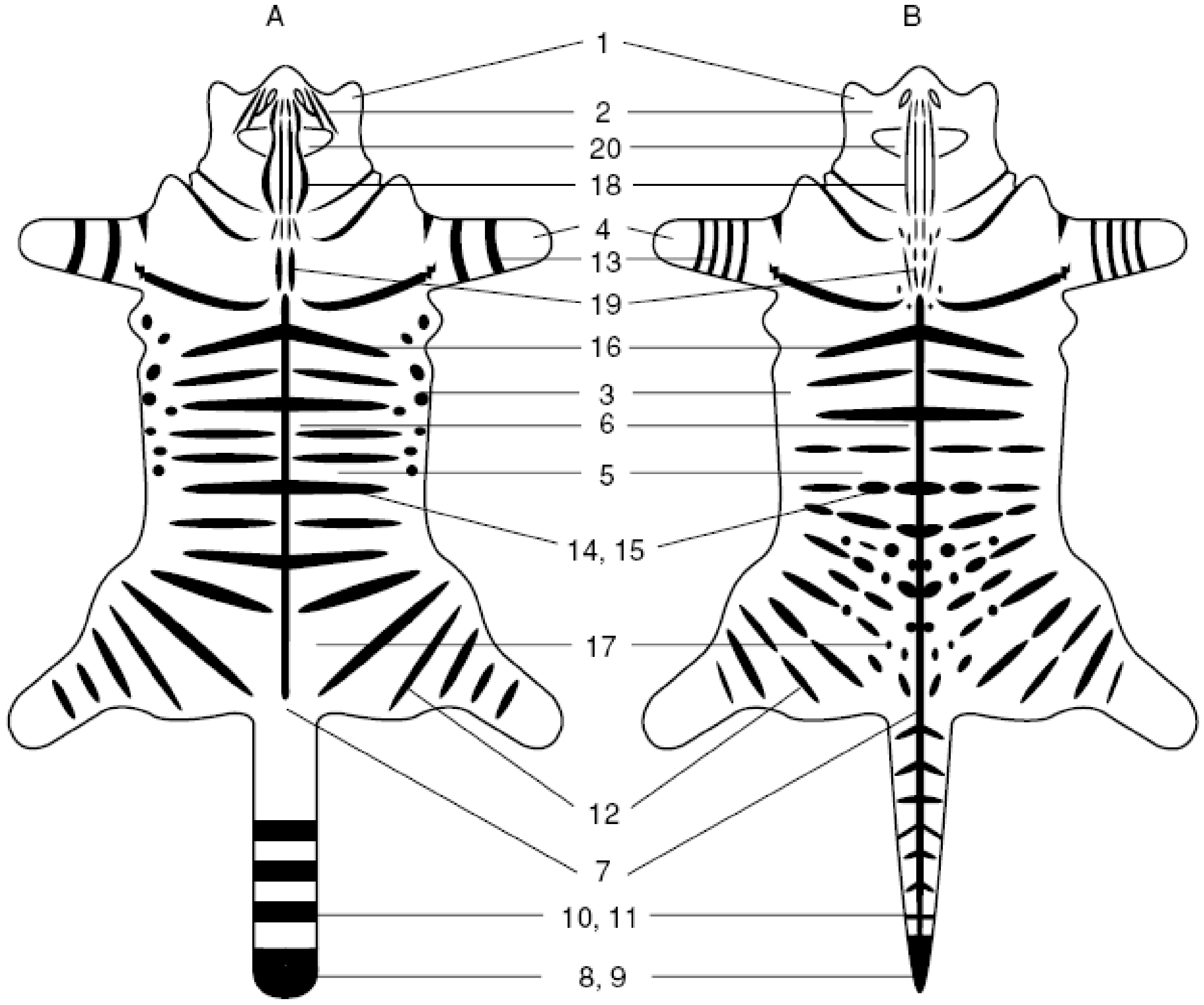
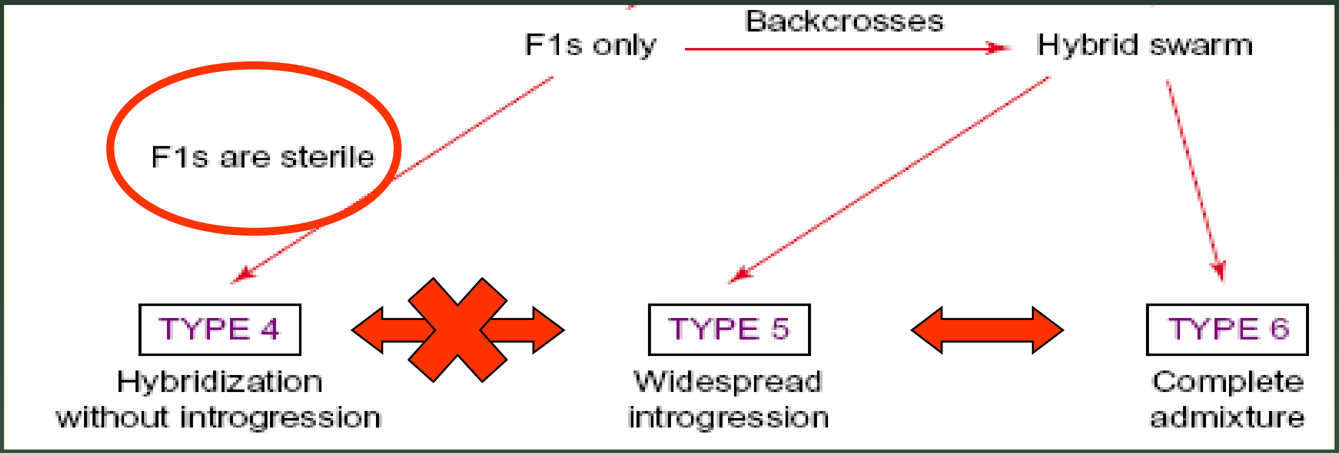


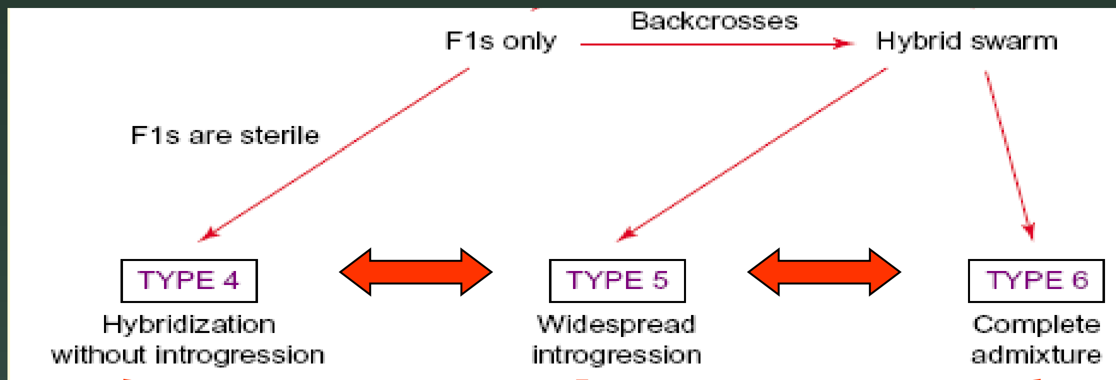
Fig. 1. Pelage characters and their character states for non-colour characters. All characters scored 3 each in A and all characters in B scored 1 each except character-14 (tabby pattern).

How should we categorize wolf-dog hybridization in Europe (Italy) ?



a) F1 are not sterile
b) No data for lower hybrid fitness, differences in reproductive periods, litter survival, etc.
c) Backcrossing not limited by ethological barrier
d) Backcrossing may be partially limited by structured spatial dynamics (territories, prey densities, human disturbance, etc.), and vice versa
e) Introgression may be acting on large scale and long term before being detected.
f) In ITALY: Huge disparity in relative wolf and dog numbers. Introgression is a fact. It is now widespread across most of the Apennines.

Managing H (Allendorf 2001)



Removal of F1 and the threatening species

Focus on maintaining remaining pure populations

Consider conservation of hybrids

a) …but this a genetic only perspective!

b) Rank of importance implicit (4 to 6)

Managing WDH-type 4: the issues

Hybridization without introgression may be a conservation concern **as serious as** introgression: *the wolf-dog case*

- Adaptive value of hybrids' phenotype characters (e.g. color)

  → increase survival, facilitate predation on livestock, etc.

- Hybrids' territorial behaviour

  → reduce empty areas suitable for recolonization by wolves

- Predation on livestock and wild prey blamed to wolves

- Competition for mate and prey

- Economic loss (when wolves are game species)

- Social and political conflict

- But… also the positive functional role of hybrids ?!

Managing WDH-type 4: the issues

(continued)

WDH-4 is a conservation concern when, due to anthropogenic causes, a species is threatened with loss of unique characteristics and the acquired ones are heritable even when hybridization may have not affected substantially the genetic characteristics of the species

(adapted from Wayne & Brown 2001)

Managing WDH-type 5 : the issues

Different **spatial patterns**, **extent** and **processes** of introgression require very different management approaches

- Localized vs. widespread introgression

→ locally intense hybrid control more feasible than geographically dispersed interventions. Intervention urgent before it becomes an hybrid swarm (type 6).

- What level of introgression warrant action

→ no scientifically based prescriptions, but a politically relevant decision → need to build an educated consensus ?

- Relative numbers and dynamics of hybridizing taxa

- Functional role of hybrids may be crucial to ecosystem health

- Conservation vs. animalist ethics

Managing WDH-type 6 : the issues

Complete genetic admixture may not correspond to complete phenotypic variation: *the Scottish cats case*

- Long term artificial selection of certain phenotypes may help re-building a new hybrid population (at least phenotypically similar to the original)

→ The ESA's Similarity-of appearance clause as a guideline ?

Managing WDH- all types: the issues

Operational and legal issues

- What happens if one kills an hybrid of a protected species ?
- Who pays the compensation for damage done by an hybrid ?
- Who decides what level of hybridization is the limit between protected/non-protected ?
- What status for hybrids in a protected vs. non-protected areas ?
- Which legislation cover hunting of hybrids
- ….

Managing WDH: the actions

What to do (a strategy) depends on:

- naming the hybrid; name determines the legal status
- defining operational thresholds for:
    - the spatial and temporal extent of the problem
    - the level of acceptable introgression
    - the possibility to identify the hybrids in the field
    - the level of acceptable generations/backcrossing
- evaluating all potential costs and benefits of hybrids
- functional integration of science and managers
- conservative vs. post-hoc approach

Compare mngmt of wolf-dog hybrids in Norway and Latvia vs. Germany and Italy

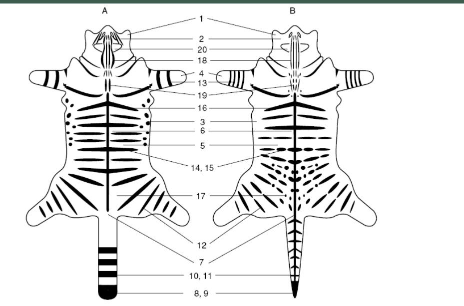


Fig. 1. Pelage characters and their character states for non-colour characters. All characters scored 3 each in A and all characters in B scored 1 each except character-14 (tabby pattern).

Managing WDH: what and how to do it

What to do is case specific: there is no one solution for each category of WDH

What to do is logistically difficult, often 'politically incorrect' and socially unacceptable (removal of animals)

Science must guide management, through a combination of perspectives, techniques, disciplines (genetics, ecology, morphology, ethology, ..), and should fully apply the *precautionary principle*

Politics must take the responsibility of decisions, through a participatory process, a clear legal framework, a coherent and long term management plan

Managing WDH: what and how to do it

Allendorf et al. 2004 proposed 3 main criteria for decision on type 5:

- Amount of evolutionary divergence between hybridizing taxa
- Geographic extent of introgression
- How many pure populations of the taxon remain

Is this approach still useful on WDH ??

What about the option of phenotype conservation ?

Although phenotypic variation is not an indicator of the amount of hybridization, it could be another criterion to decide on the feasibility of human intervention (a proxy for genetic recovery)

Managing WDH: the legal frameworks

Deciding and implementing a management policy depends on the legal status of parent species and hybrids:

Given the many uncertainties and context-dependent variations in defining, detecting, managing hybrids, no national or international legislation provide clear guidelines and policies on management of hybridization:

- ESA:
    - Original Hybrid Policy (no protection) withdrawn in 1990
    - Proposed policy 1996, never finalized
    - Now, case-specific plans
    - similarity-of appearance clause (section 4[e])

- Habitat Directive: hybrid control implied, but not explicit

- Optimal policies: freedom within frames ? or open-ended approaches ?

Summary of issues with WDH & introgression

hybridization = great challenge for conservation & management

1. Identification of hybrids remains problematic
2. Difficult to assess whether hybridization is recent or ancient
3. Level of introgression difficult to assess
4. What is an acceptable level of introgression?
5. What status for hybrids (cf. CITES!) and which practices?
6. How to manage hybrids? (lethal control, captivity, animal welfare problems, etc.)
7. Various legal status of feral dogs in Europe

Final recommendations to scientists

a. Geneticists have great responsibilities: key conservation issues are based on the reliability of their diagnostic tools
b. H assessment should not be a by-side product of other studies, but must be prioritized and planned independently
c. Plan appropriate (spatial, temporal) sampling designs to address the H problem with adequate power and resolution
d. Declare ALL uncertainties, weaknesses, pitfalls, precariousness, etc. of the scientific data
e. Integrate perspectives from a variety of disciplines and approaches
f. Be aware of the implications of your advices