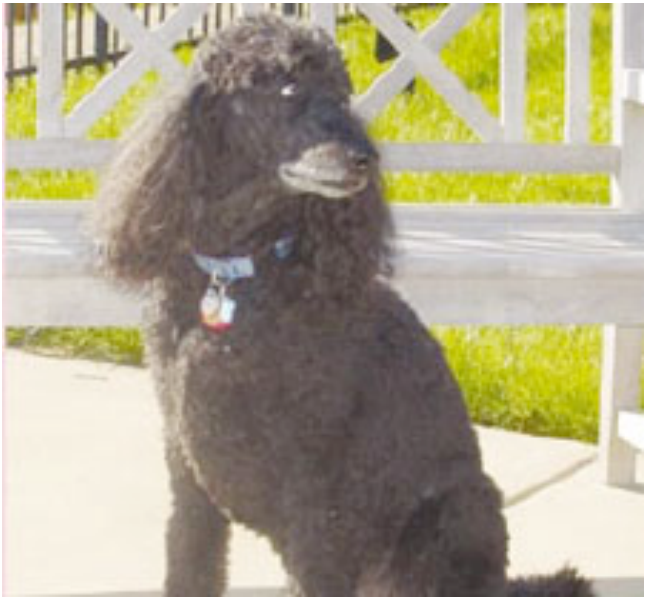
Engineering of the Dog and wild relatives

Tasha



Shadow

TALK OUTLINE

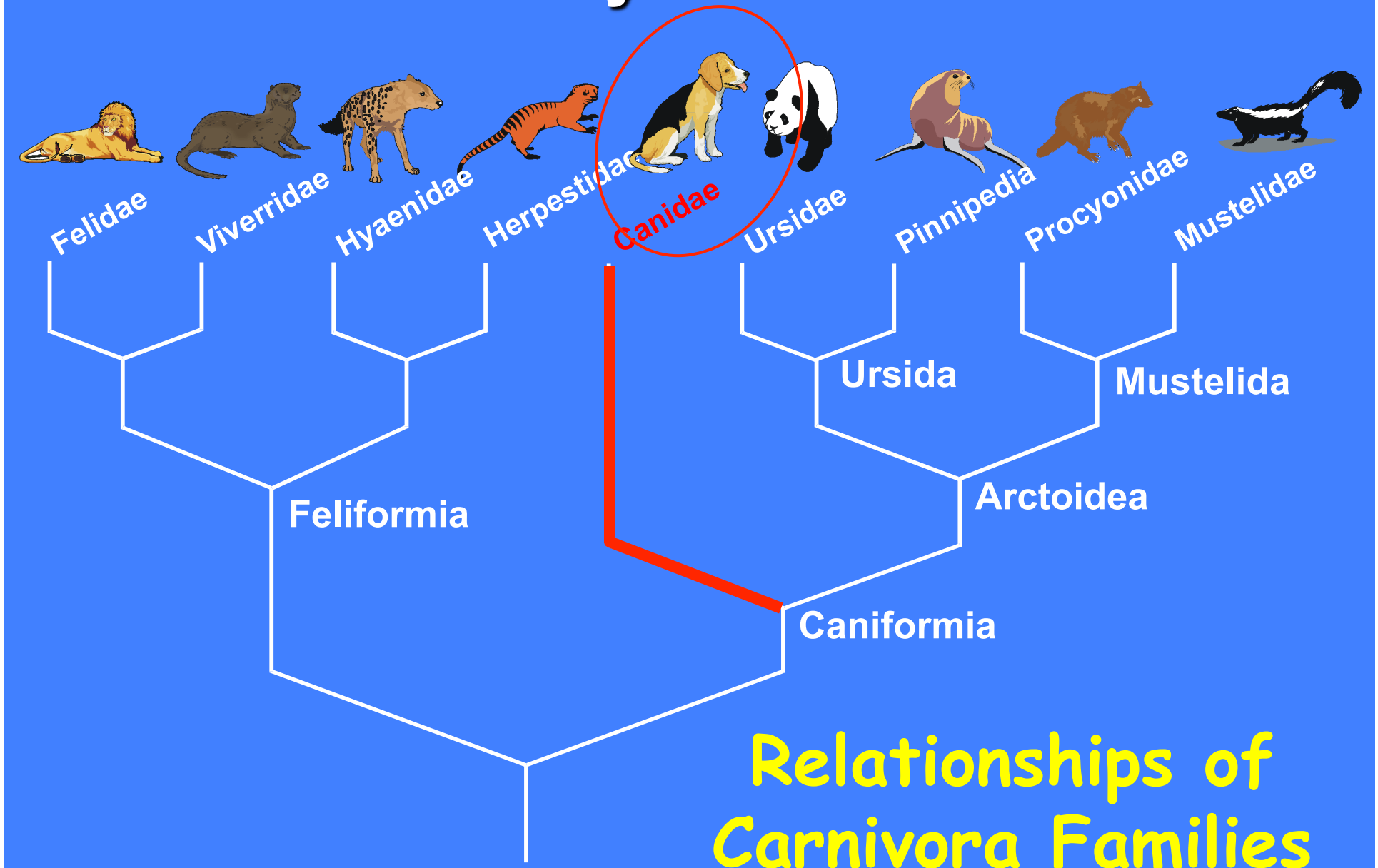
1) Introduction
   a) Evolutionary framework
   b) Origin and diversification
   c) The dog as a model species
   d) The canine genome and canmap projects

2) How to build and reverse engineer a dog
   a) Three ways to build a dog
   b) Association and selective sweep mapping
   c) IGF1,the body size gene
   d) Legs and fur
   e) The number of genes
   f) Evolving the dog

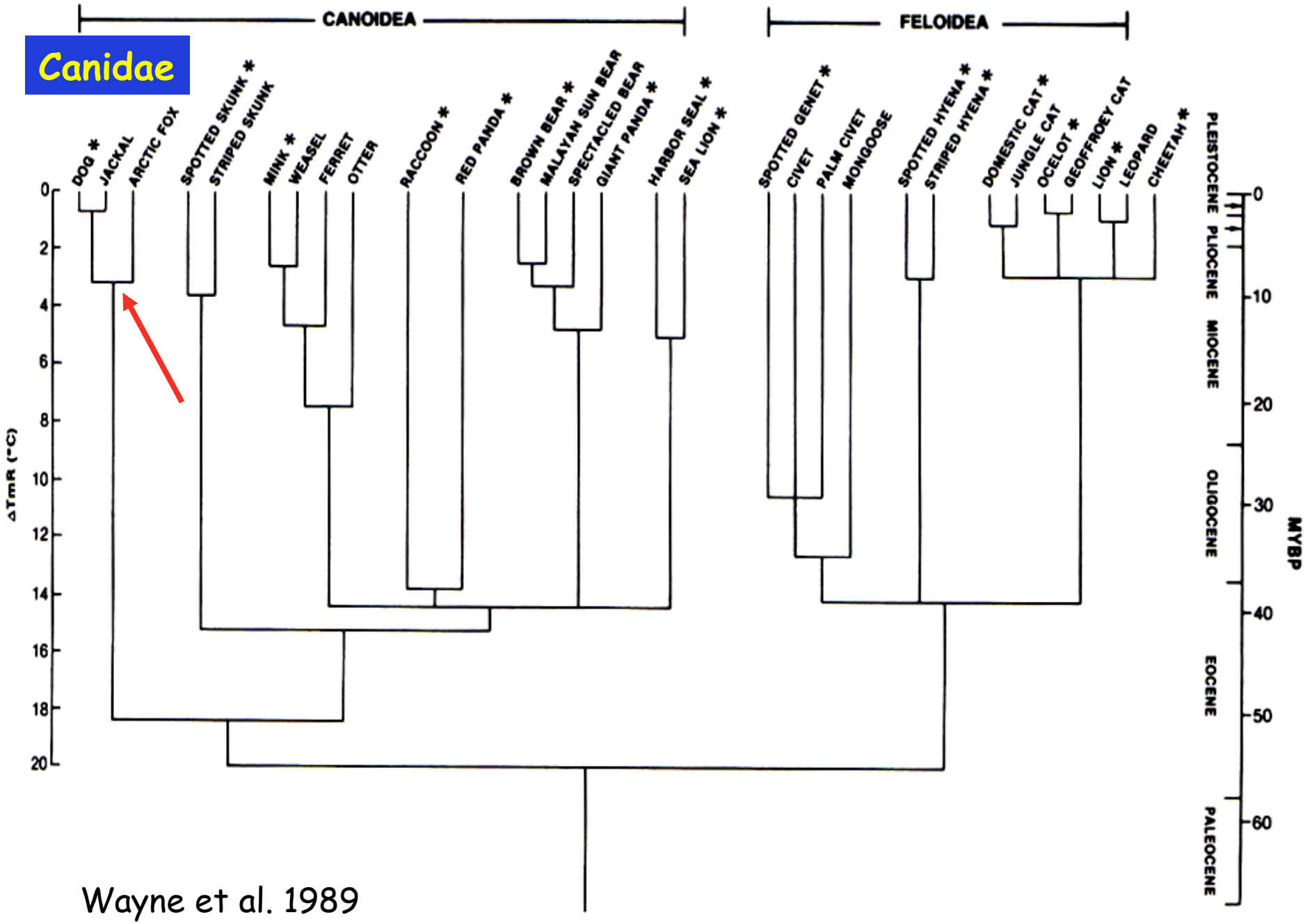
3) Engineering of the wild
   a) Black and gray coat colors of wolves

4) The future
   a) Exome capture and complete genome sequencing

a. The evolutionary framework



Relationships of Carnivora Families

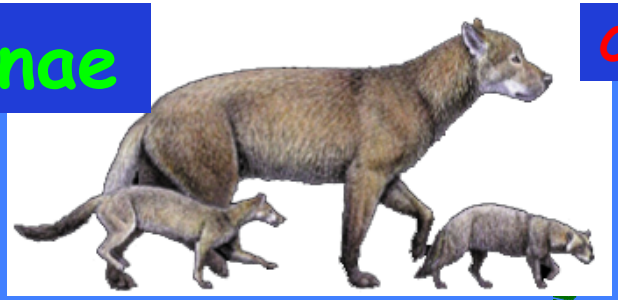


Wayne et al. 1989

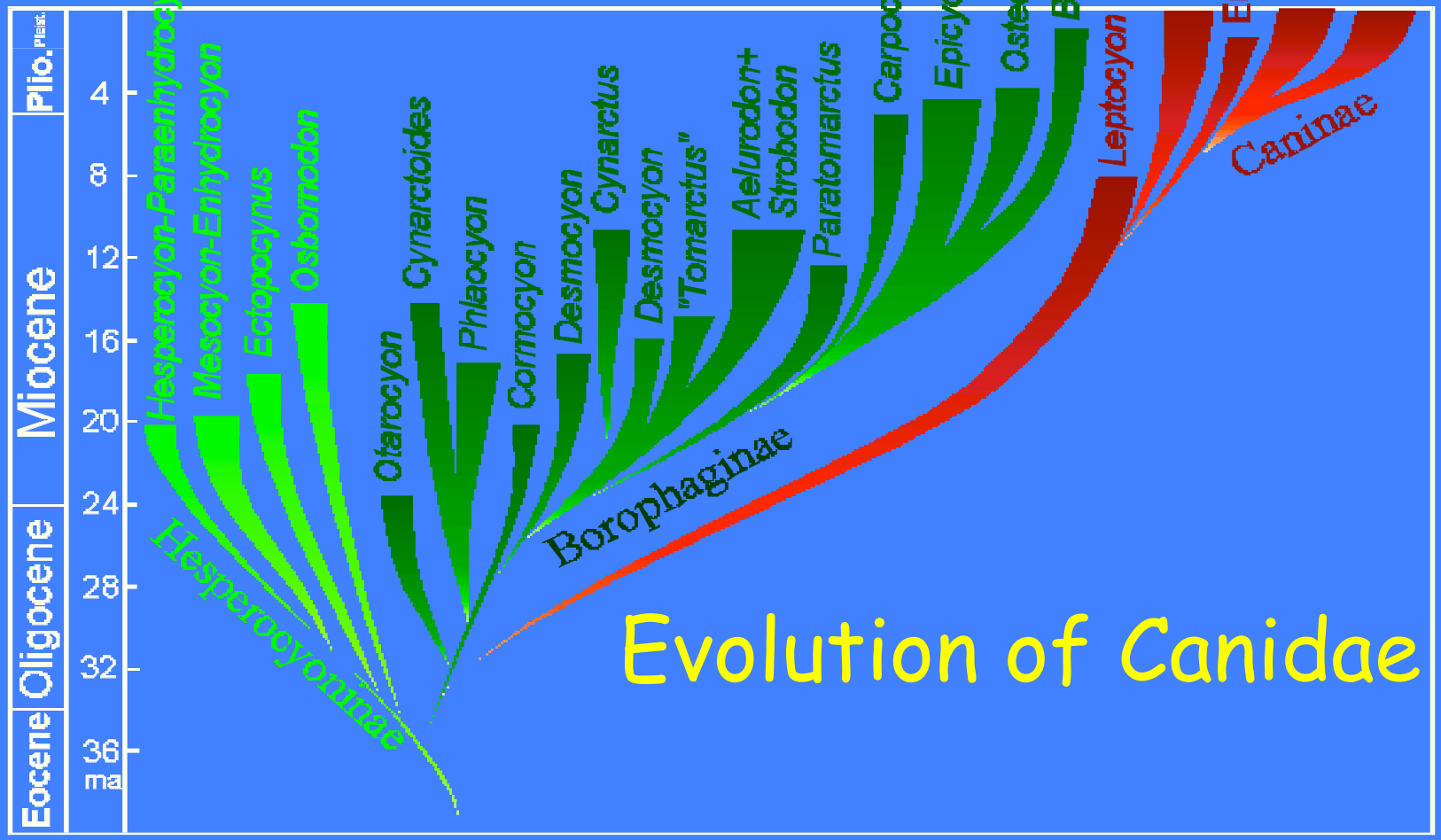
Evolution of Canidae

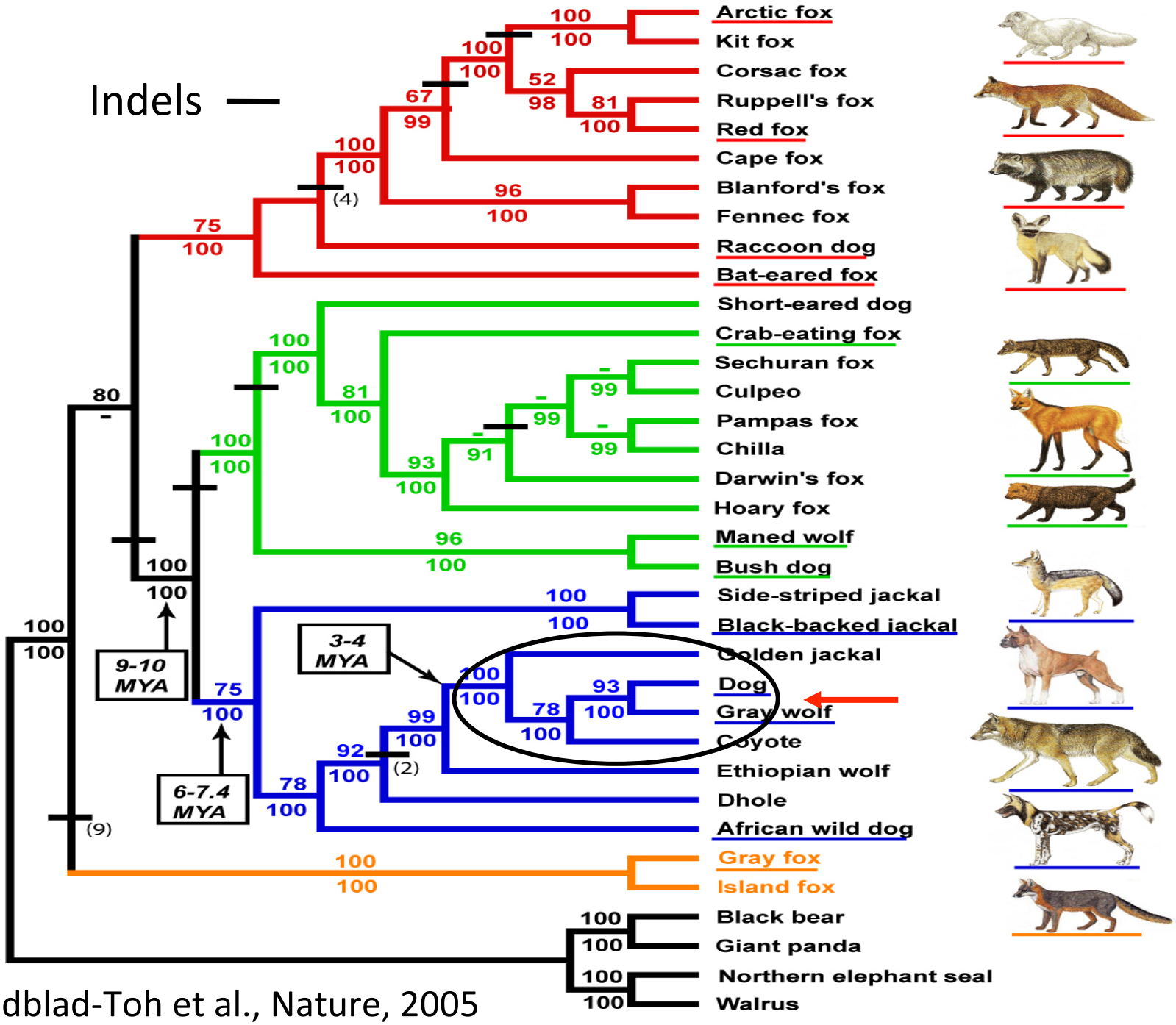
Hesperocyoninae

Borophaginae



Caninae





Lindblad-Toh et al., Nature, 2005

CONCLUSIONS

1) The dog has an ancient divergence from other carnivores dating to about 55 million years ago.

2) The extant Canidae has a relatively recent origin about 10 million years ago and is divided into three primary groupings. The dog is assigned to the wolf-like group.

3) The dog is extremely close to gray wolves and shows no evidence of origin from other wolf-like canids.

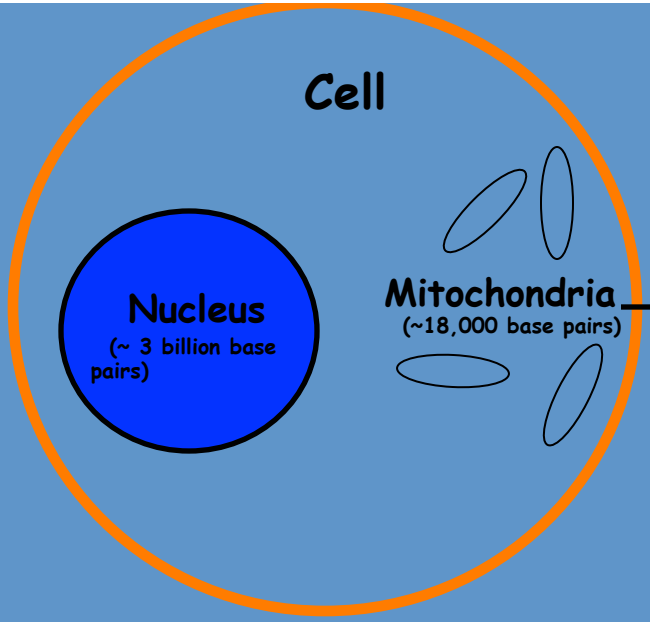
b. ORIGIN AND DIVERSIFICATION

QUESTIONS

1) When did the domestic dog originate?

2) Where did the dog originate
(from a single population & region)?

3) How have humans directed the evolution
of dogs?



Mitochondrial DNA (mtDNA)

- One linked array of genes
- One locus
- Control region sequence
- Maternally inherited

...ATCATCCC... **Specimen 1**

...ATCTTCCC... **Specimen 2**

140 dogs from 67 breeds

162 wolves from 27 populations

Vilà et al., Science, 1997

The sequence diversity within dogs is similar to that within wolves

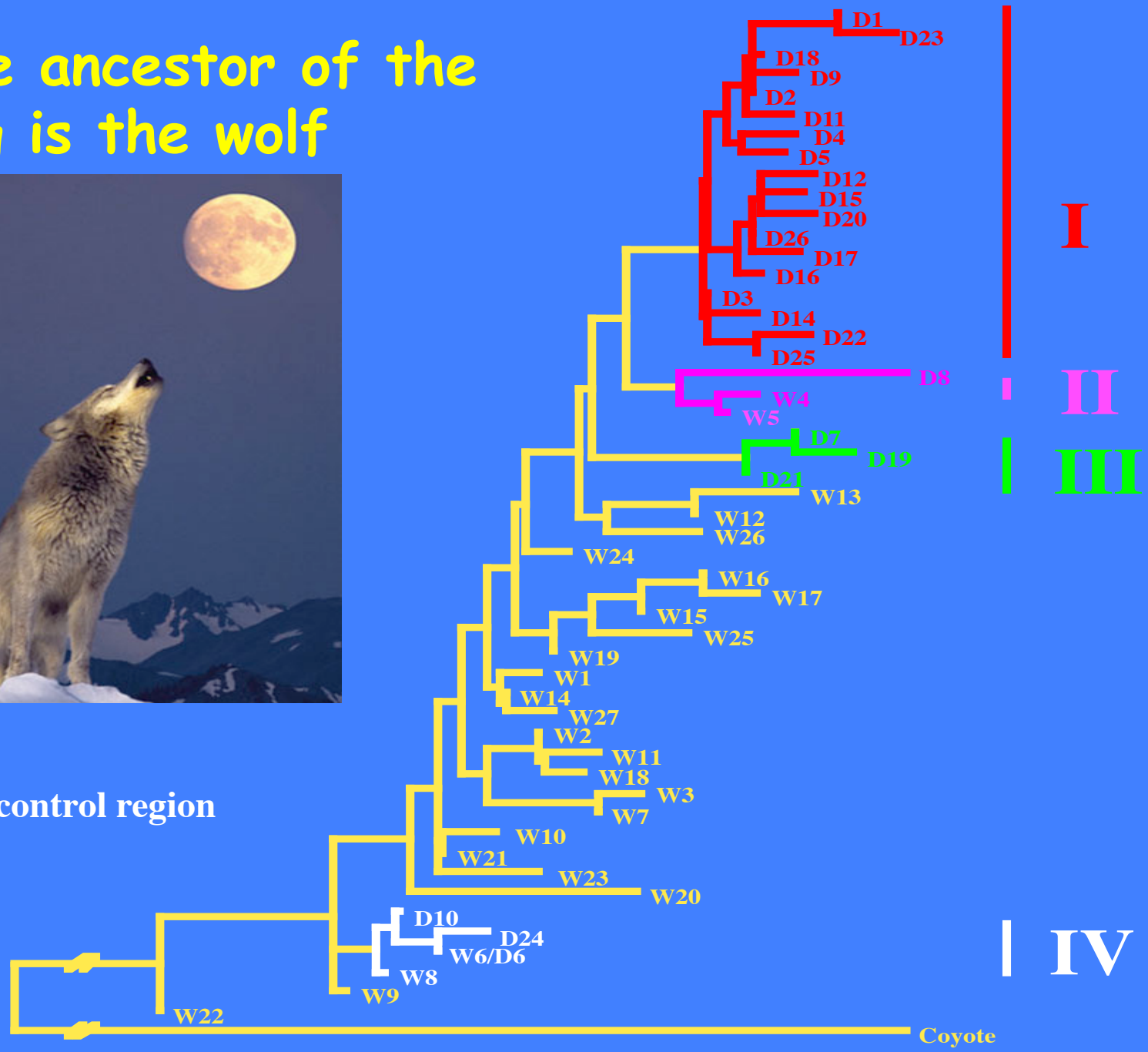
Average sequence divergence in dogs = 2.06% ± 0.07%
26 haplotypes in 140 dogs from 67 breeds

Average sequence divergence in wolves = 2. 1% ± 0.04%
27 haplotypes in 162 wolves from 27 populations

```
                    1  2  3  4   5   67
Dog 1        TT     AGCTGTCGATTCCA
Dog 2             TTAGCTATGGAATCGA
Gray Wolf 1       TTTGCTATGGATTCCT
Gray Wolf 2       TTTGGTATGGATTCCA
```

The ancestor of the dog is the wolf

261bp control region

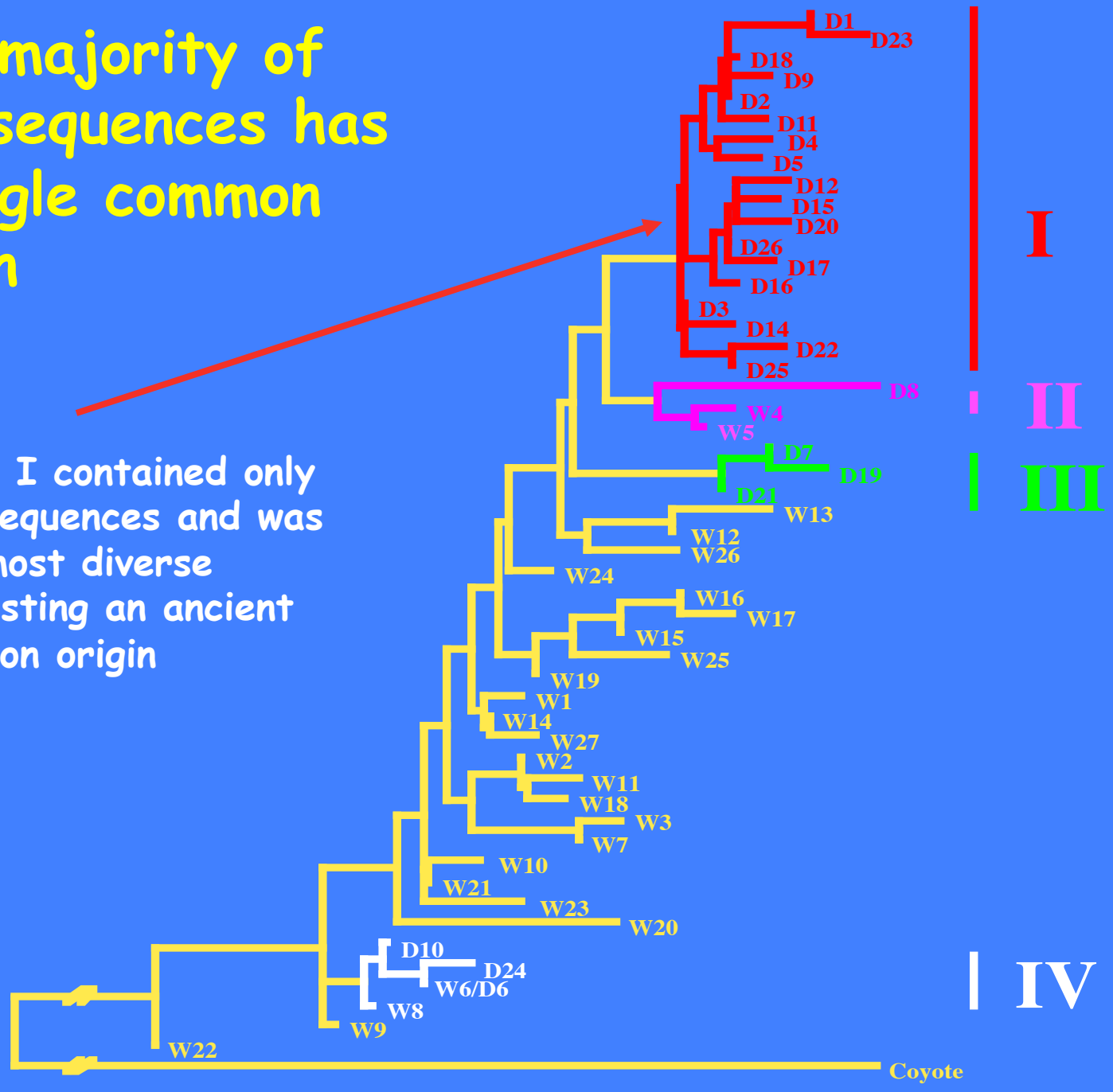


Dogs breeds were founded by multiple matralines or interbred

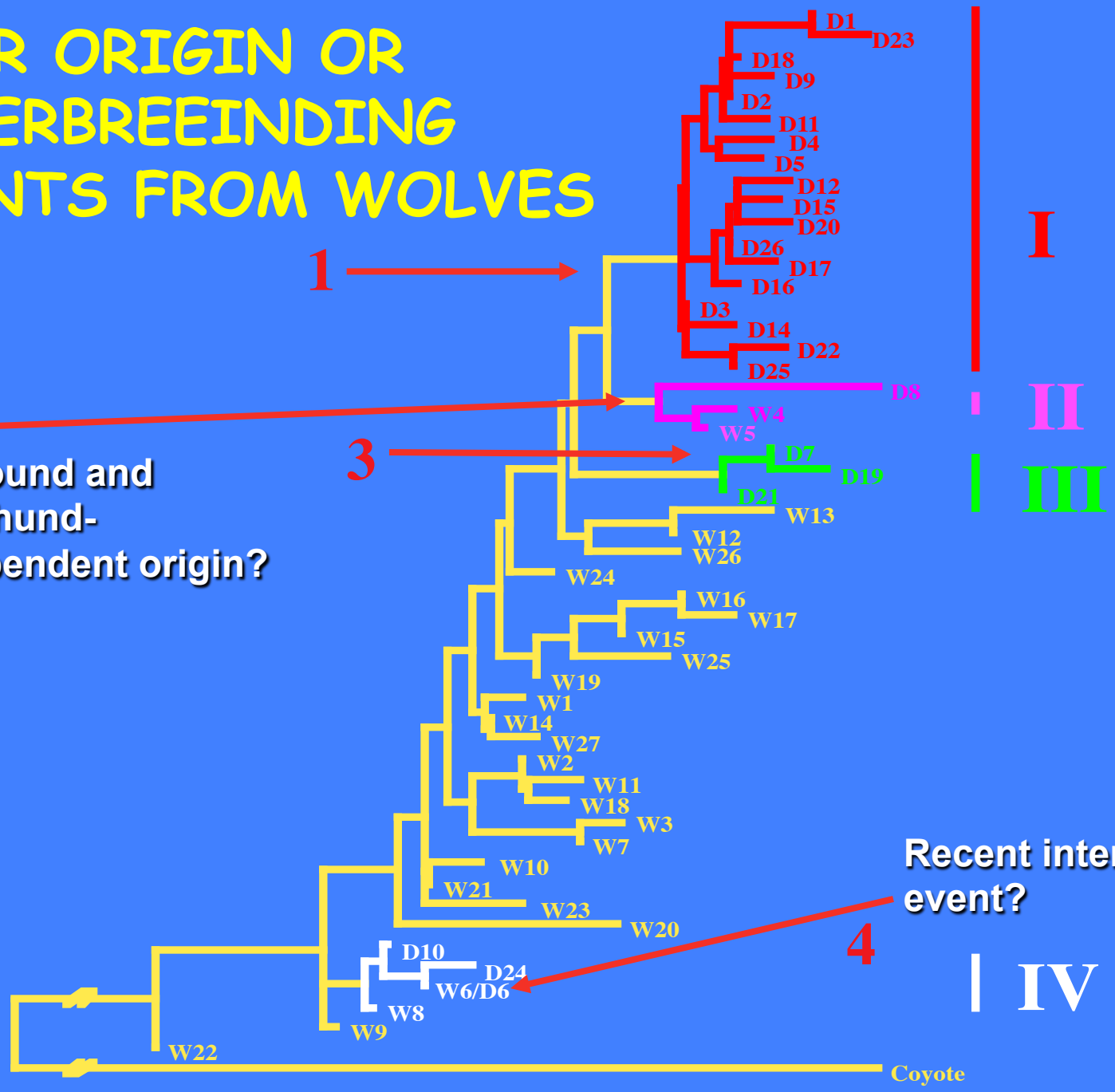
	I	II	III	IV
Chow Chow (n= 3)	D1, D2, D3			
Border Collie (n= 3)	D1, D5			
Wirehaired Dachshund (n= 3)	D5			D10
Australian Dingo (n= 4)	D18			
Norwegian Elkhound (n= 9)	D3	D8		
German Shepherd (n= 8)	D4, D5		D7, D19	D6
Afghan Hound (n= 3)				D6
Siberian Husky (n= 3)	D3, D18		D7	
Jämthund (n= 3)		D8	D7	
Flat-coated Retriever (n= 3)	D4			D10
Golden Retriever (n= 6)	D4, D15			D6, D24
Labrador Retriever (n= 6)	D4, D12			
Samoyed (n= 3)	D1, D4, D5			
Giant Schnauzer (n=3)	D4		D7	
English Setter (n= 4)	D3, D5			
Irish Setter (n= 3)	D1, D9			
Mexican Hairless (n= 19)	D1, D3, D4, D26		D7, D21	D6

The majority of dog sequences has a single common origin

Clade I contained only dog sequences and was the most diverse suggesting an ancient common origin



FOUR ORIGIN OR INTERBREEINDING EVENTS FROM WOLVES



Elkhound and Jämthund-Independent origin?

Recent interbreeding event?

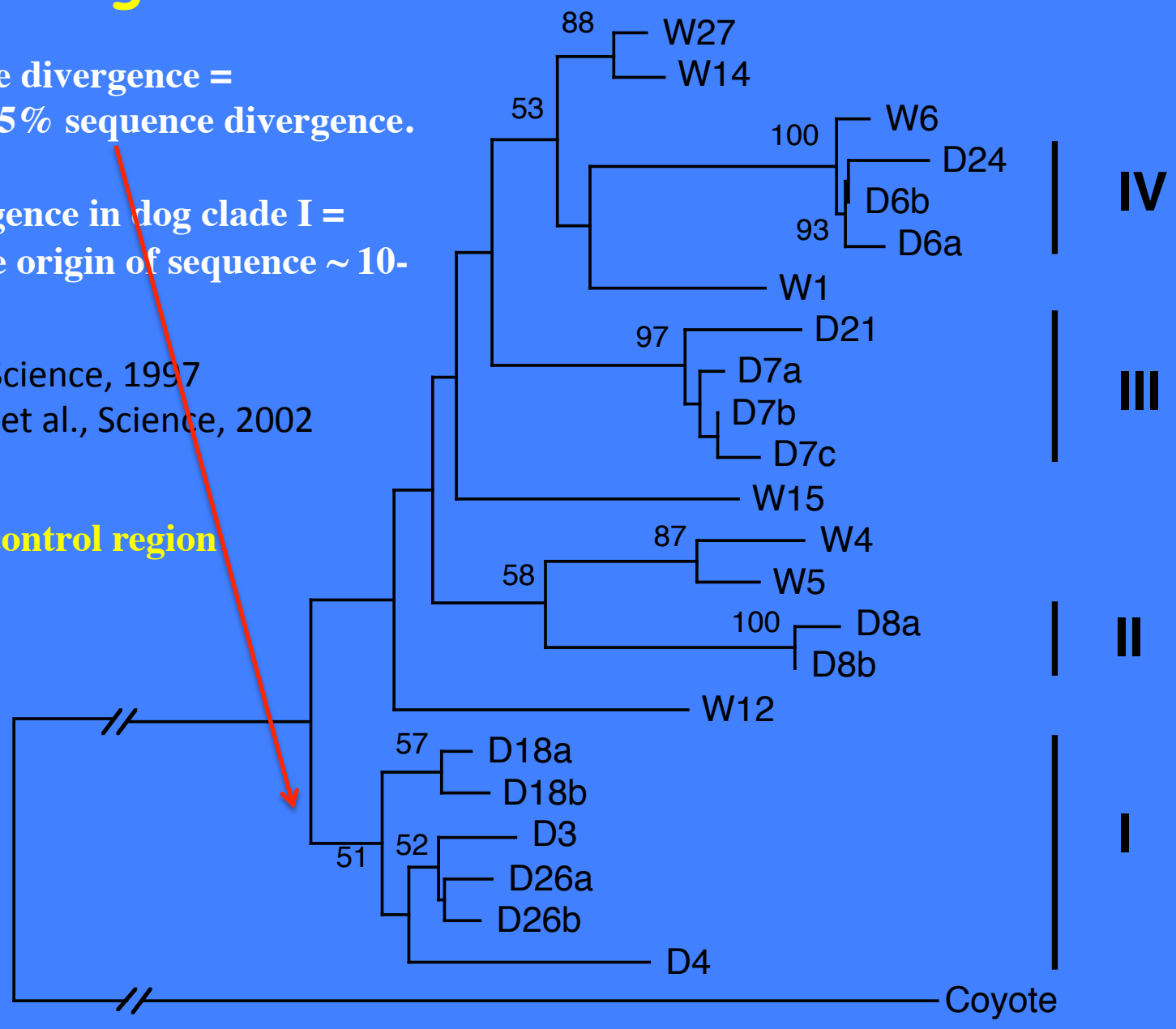
The Timing of Domestication

Wolf/coyote divergence =
1 mybp, 7.5% sequence divergence.

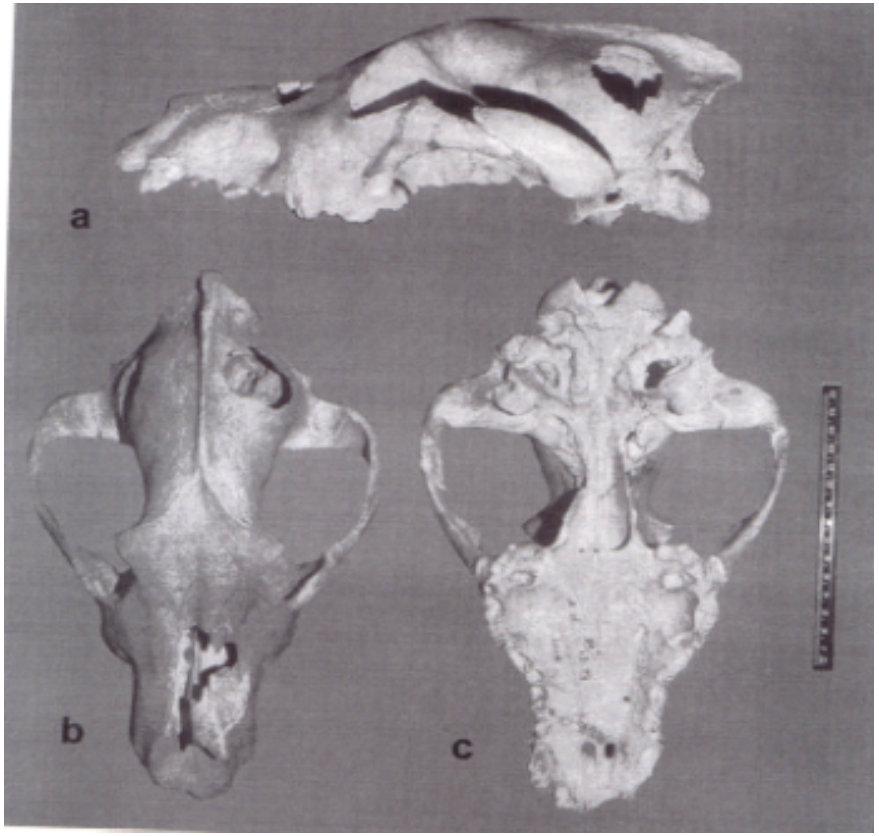
Max divergence in dog clade I =
1.0% hence origin of sequence ~ 10-100Kya

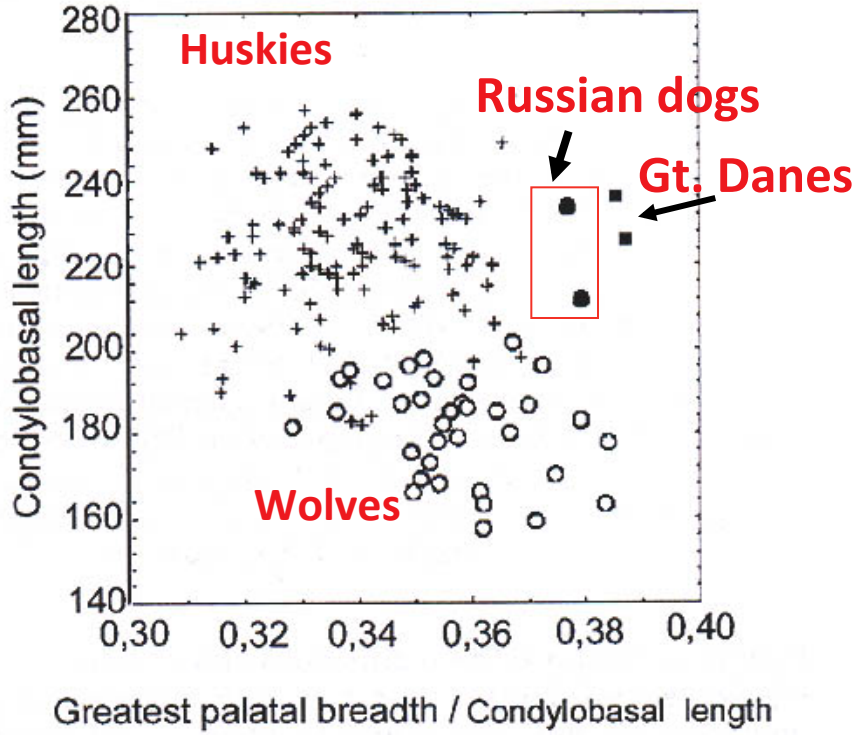
Vila et al., Science, 1997
Savolainen et al., Science, 2002

1030bp control region



The Oldest dogs: 15-31K years ago

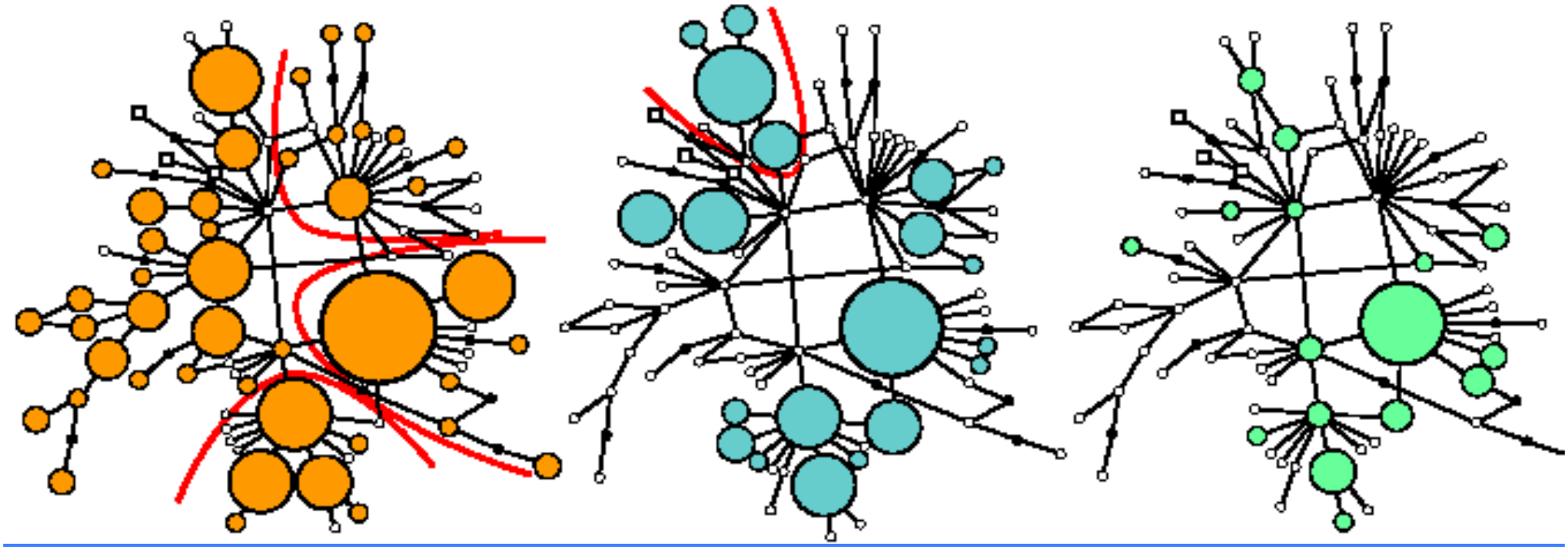




Origin in East Asia

1. High diversity in East Asia suggests an origin there.

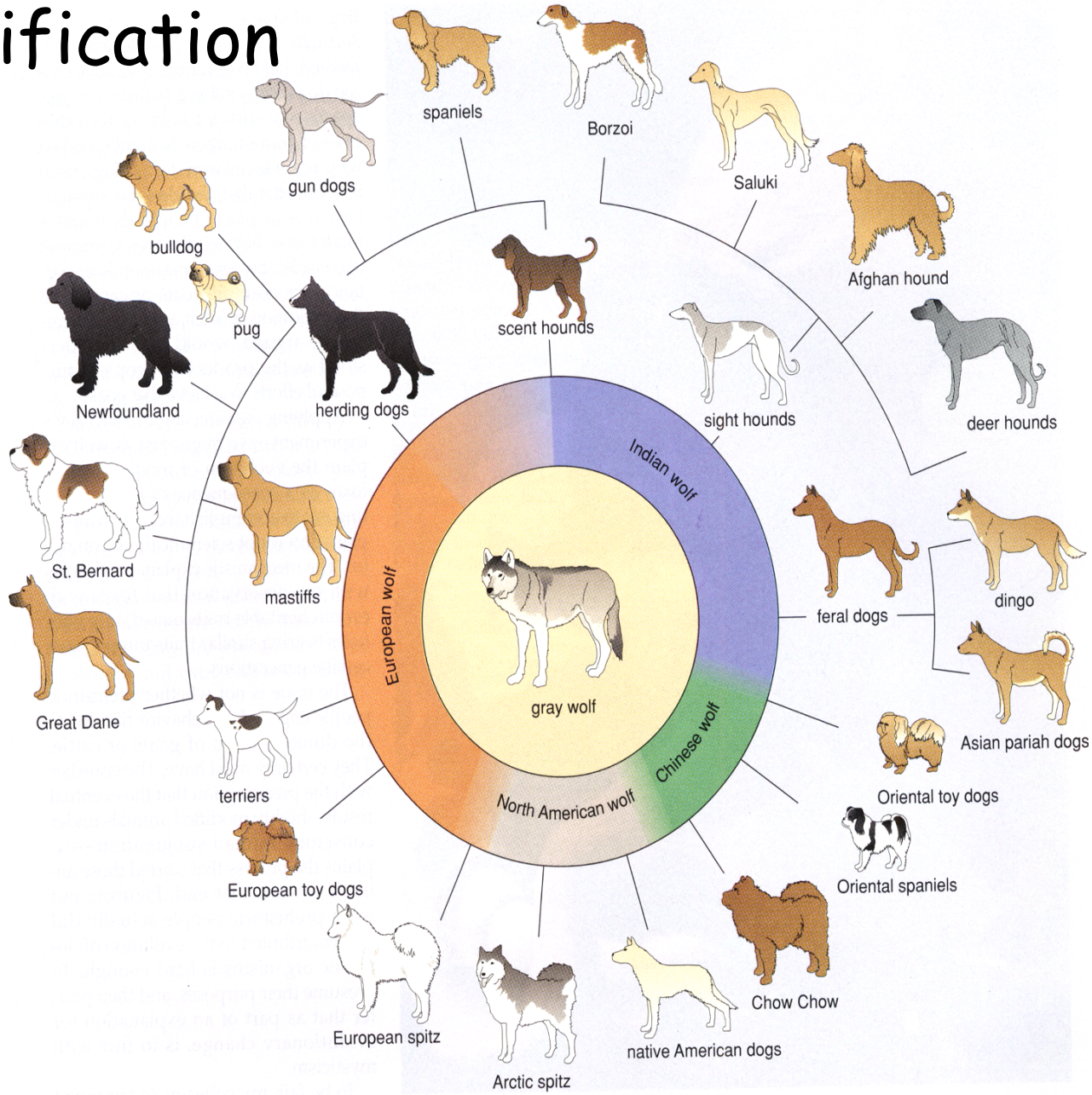
2. Founding by multiple matralines is consistent w/ origin 15 to 40k



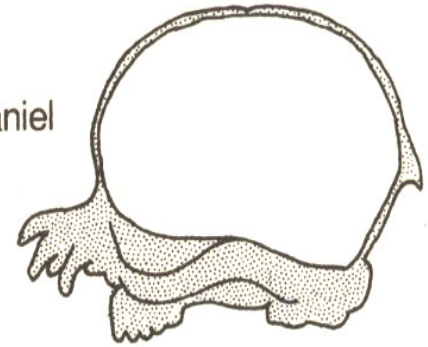
East Asia | Europe | Southwest Asia

Savolainen et al., Science, 2002

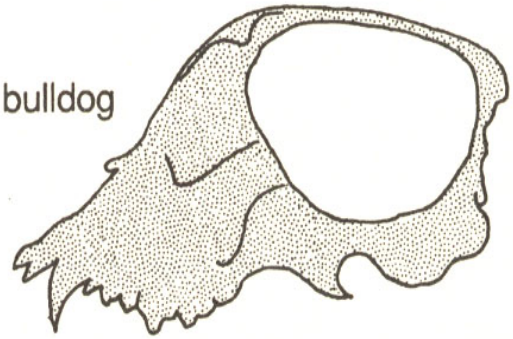
Diversification



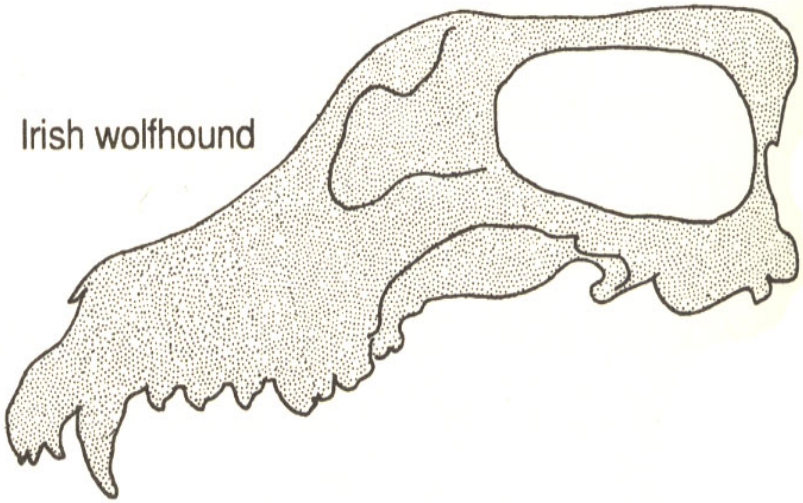
King Charles spaniel



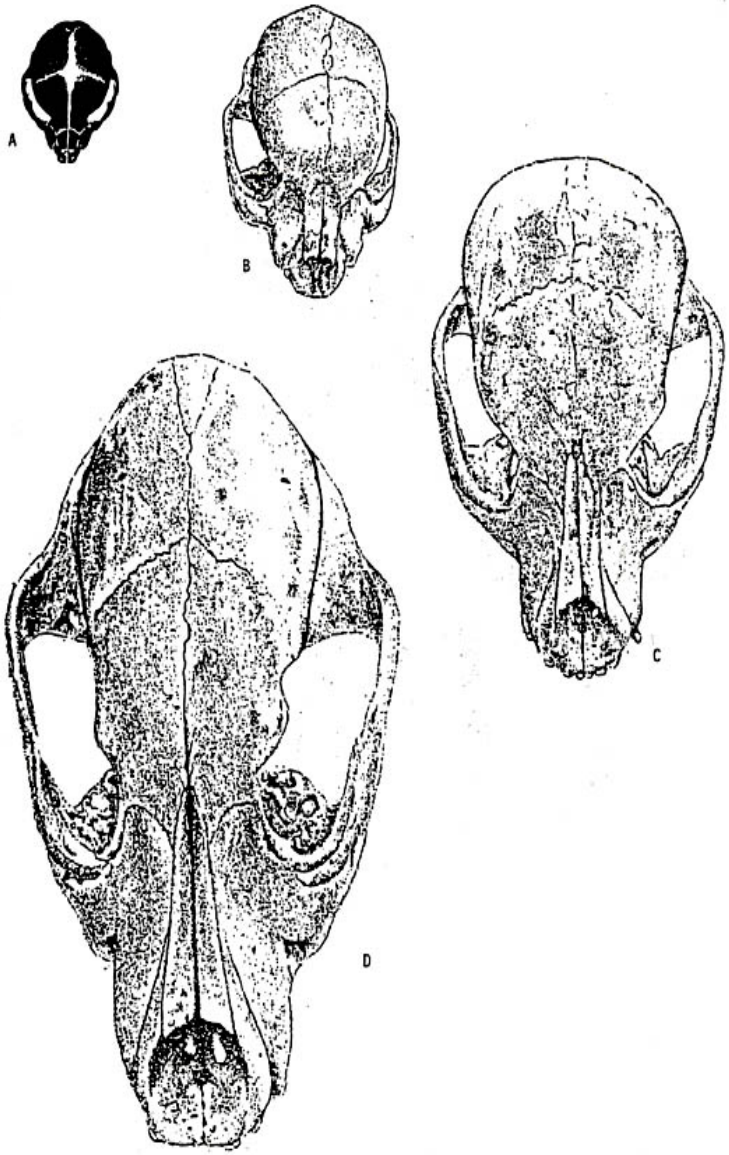
English bulldog



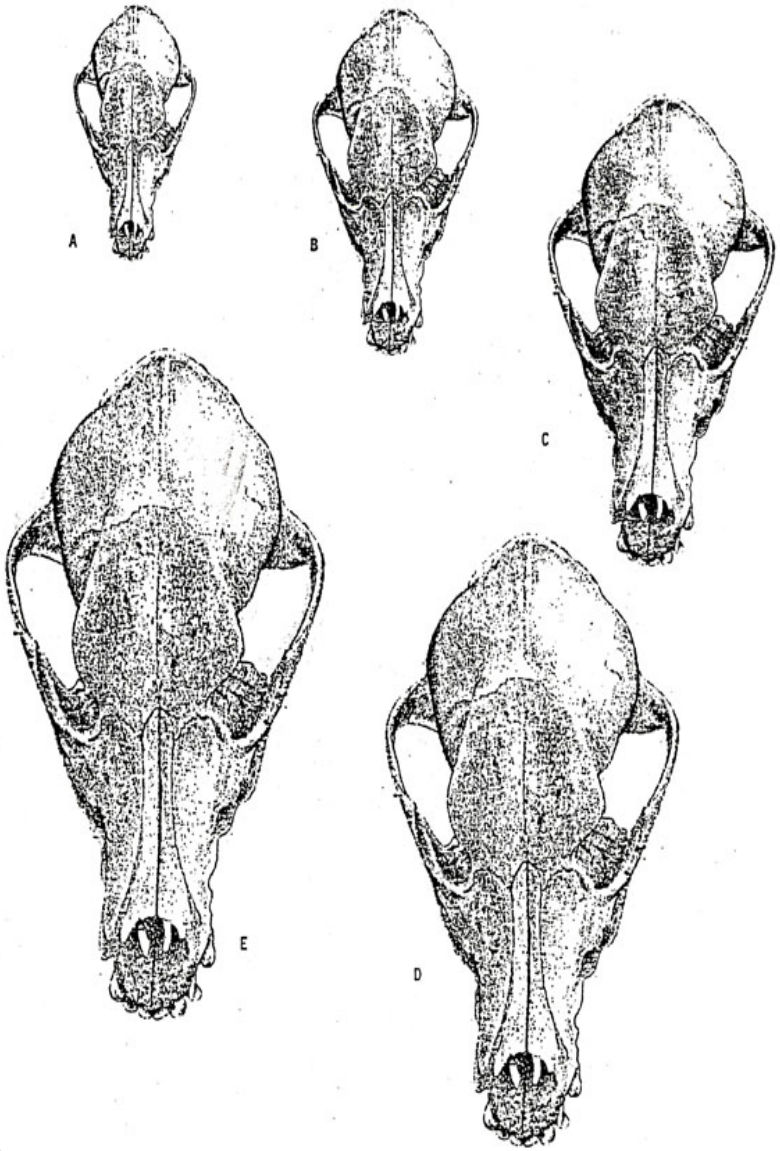
Irish wolfhound

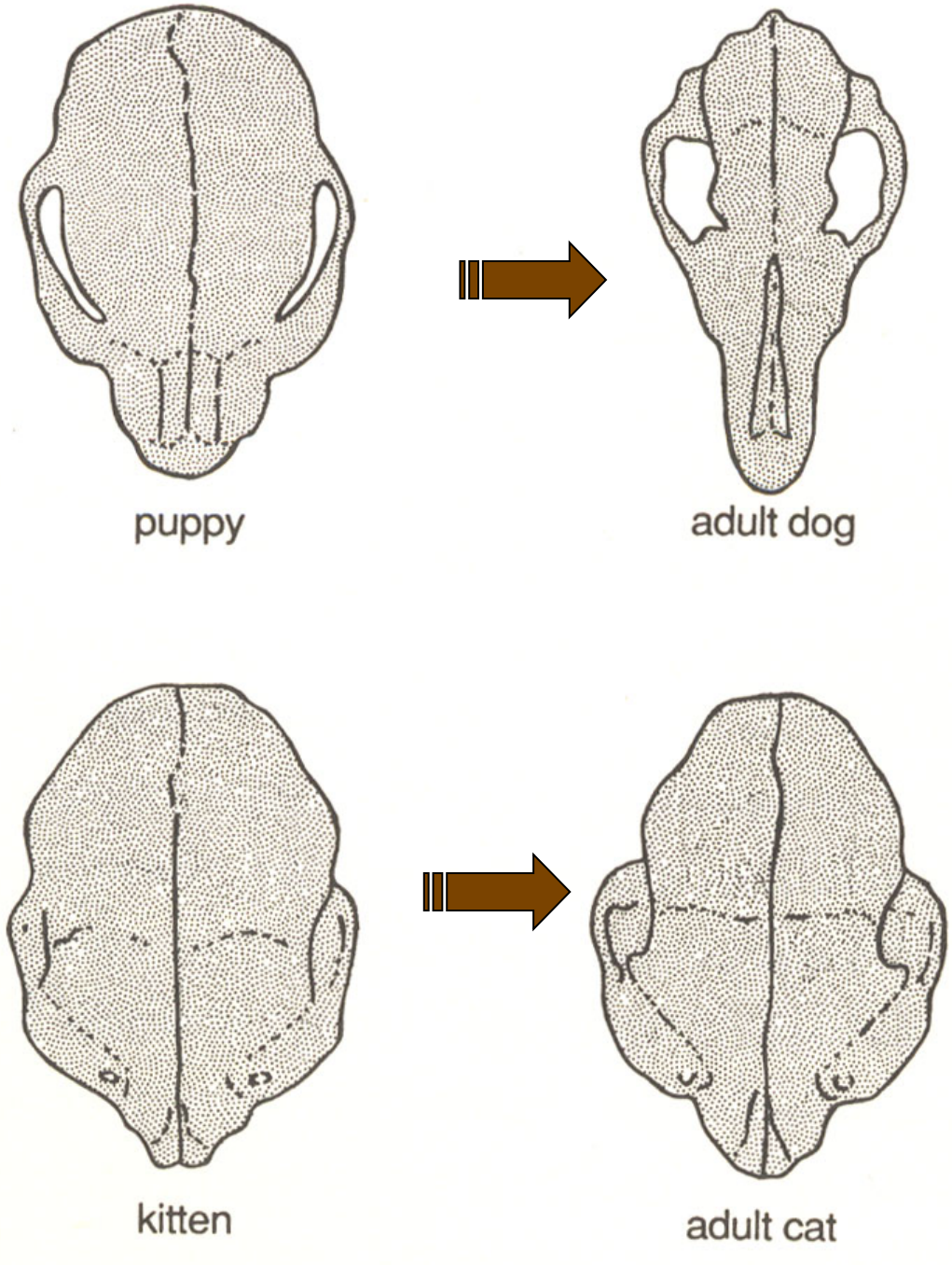


Allometric Growth

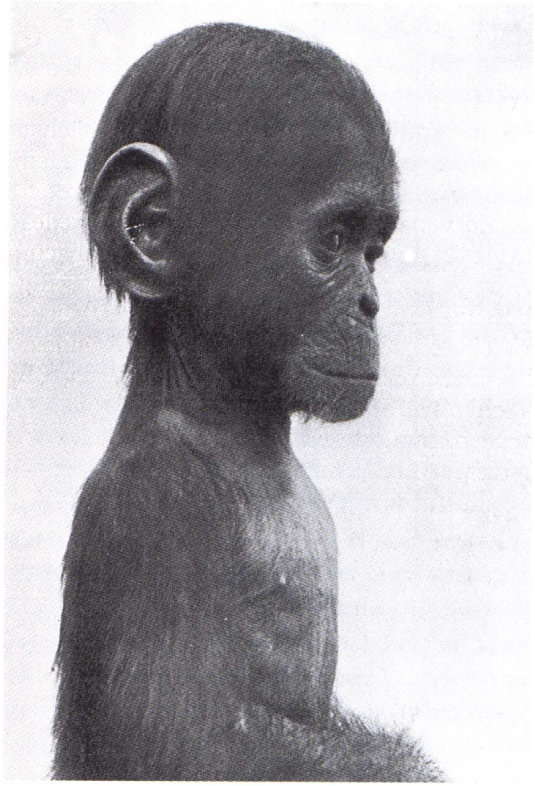


Proportionate Growth





Wayne, Evolution, 1986



Neoteny (juvenilization)

CONCLUSIONS

1) The dog has high mitochondrial DNA sequence diversity suggesting a diverse origin from multiple wolf matralines from a wide region.

2) However, the higher diversity of dog mtDNA in east Asia suggest that dogs may have had a long history there.

3) Dogs are the oldest domesticated animals, originating 10-100,000 years ago.

4) Neoteny has been a common mode of change, affecting behavior and morphology.

Questions

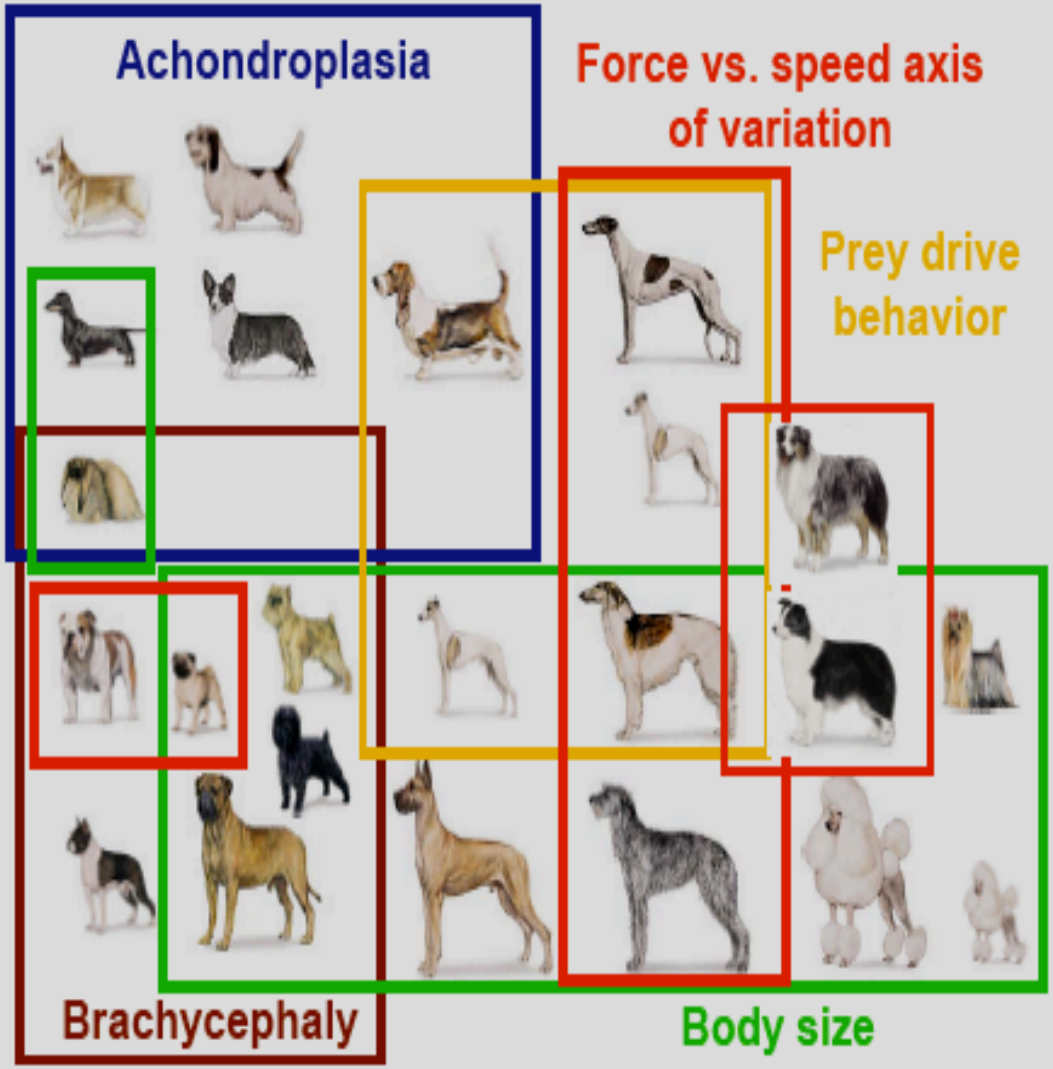
1) The reason dogs appear to have evolved in East Asia is based on:
   a. The fossil record
   b. Historical record
   c. Morphological studies
   d. Higher mtDNA diversity
   e. Lower mtDNA diversity

2) The apparent phenotypic diversity in skull shape in dogs as compared to cats reflects what general difference between the two species:
   a. Selection for bite strength
   b. Selection for bigger brain size
   c. Occlusal and breathing limitations
   d. Differences in developmental gradients

3) The dog is derived from:
   a. an extinct species
   b. a diversity of wolf-like species
   c. gray wolves
   d. none of the above

C. The Dog as a Novel Model Species

- Each breed fixed for specific traits
- Creation of breeds recent and rapid
  - Closed breeding
  - Popular sires
  - Population bottlenecks
- Small number of genes responsible for many complex traits



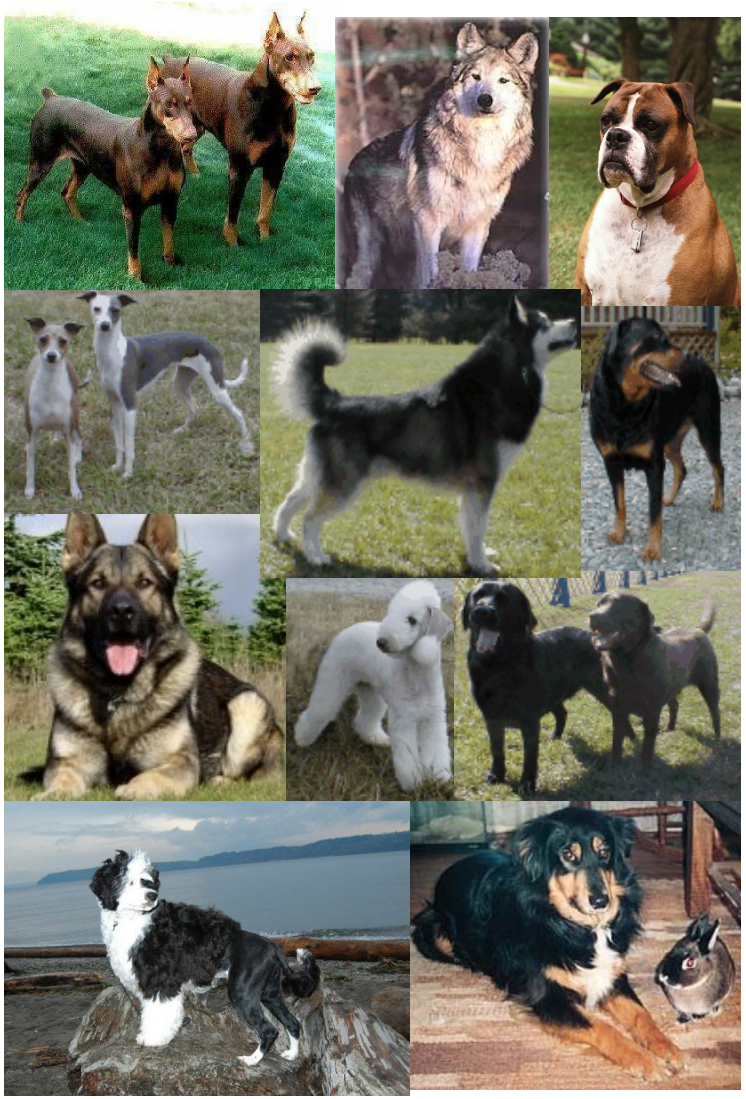
D. The Sequencing of the Dog

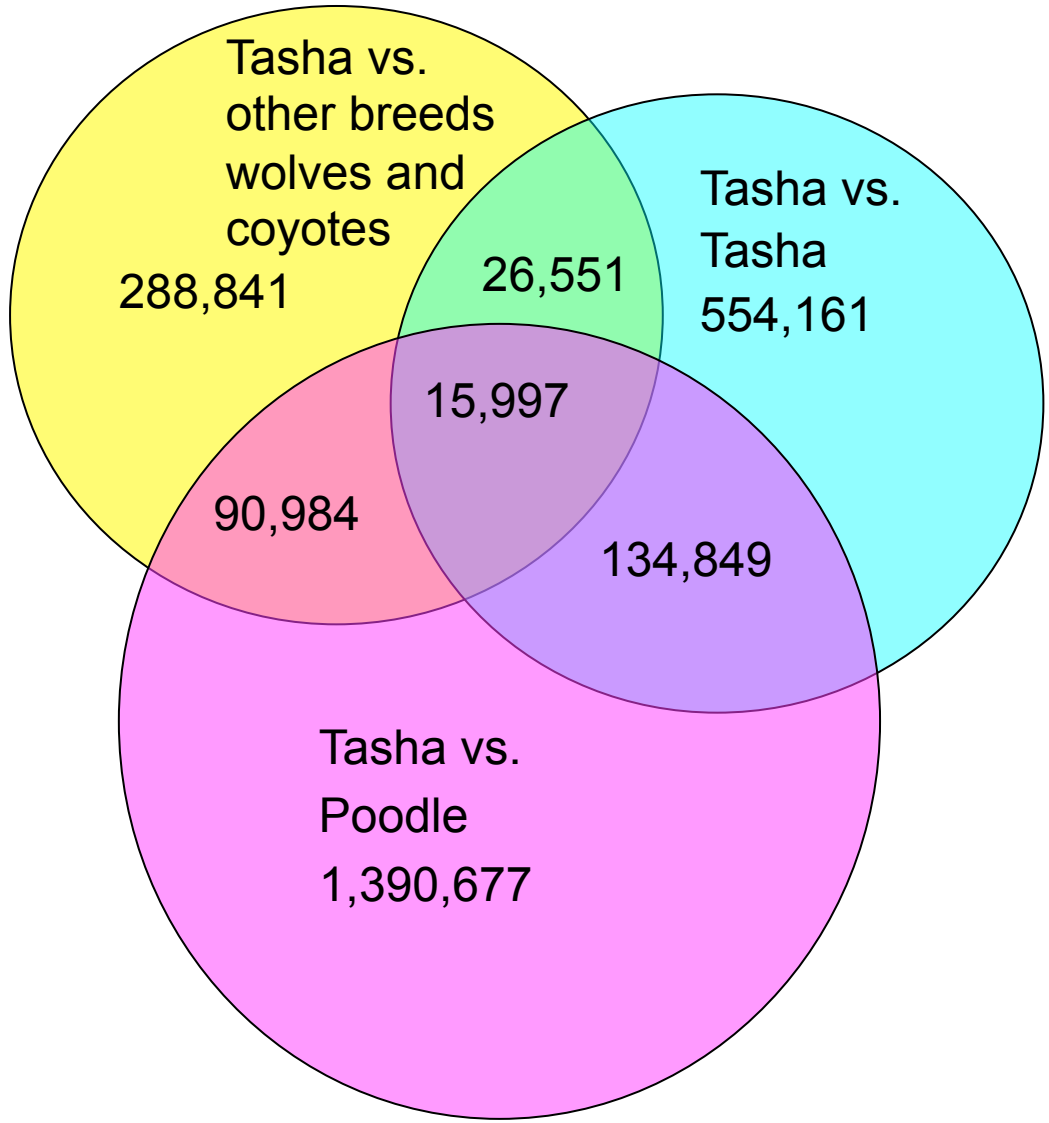
<u>Dog Genome Resources</u>

- Complete genome sequenced - 2.4 Gb
- Boxer – 7.5X coverage (Linblad-Toh et al., Nature, 2005)
- Poodle – 1.5X coverage (Kirkness et al., Science 2004)
- 19,120 Dog genes predicted, nearly all with human homologs
- 2.1-2.6 Million SNPs generated from Boxer and Poodle complete genomes, 10 other dog breeds (100,000 sequence reads each), and 4 wolves and one coyote (20,000 sequence reads each)
- 127,000 Dog SNP microarray chip
- Searchable annotated genome (e.g. UCSC Genome Browser, Ensembl) e.g. allows identification of new microsatellite or SNP loci in regions of interest
- Syntenic map of Dog, Human, Mouse, Rat
- Cost: 46 million dollars

The Dog Genome Project

2.6 Million SNPs Total-SNP Every 5 Kb





E. The CanMap Project

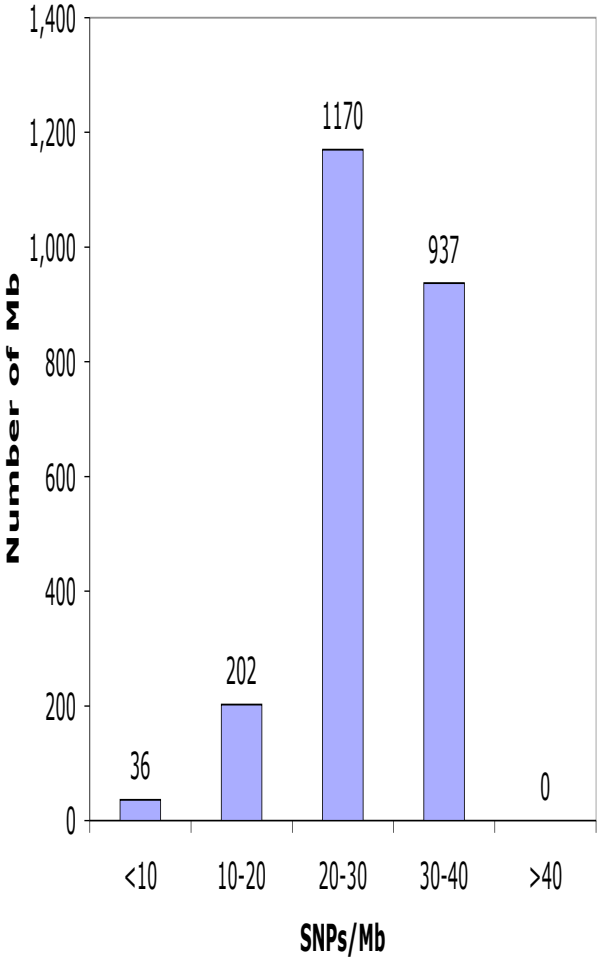
(like the human hapmap project aims to characterize genomic variation)

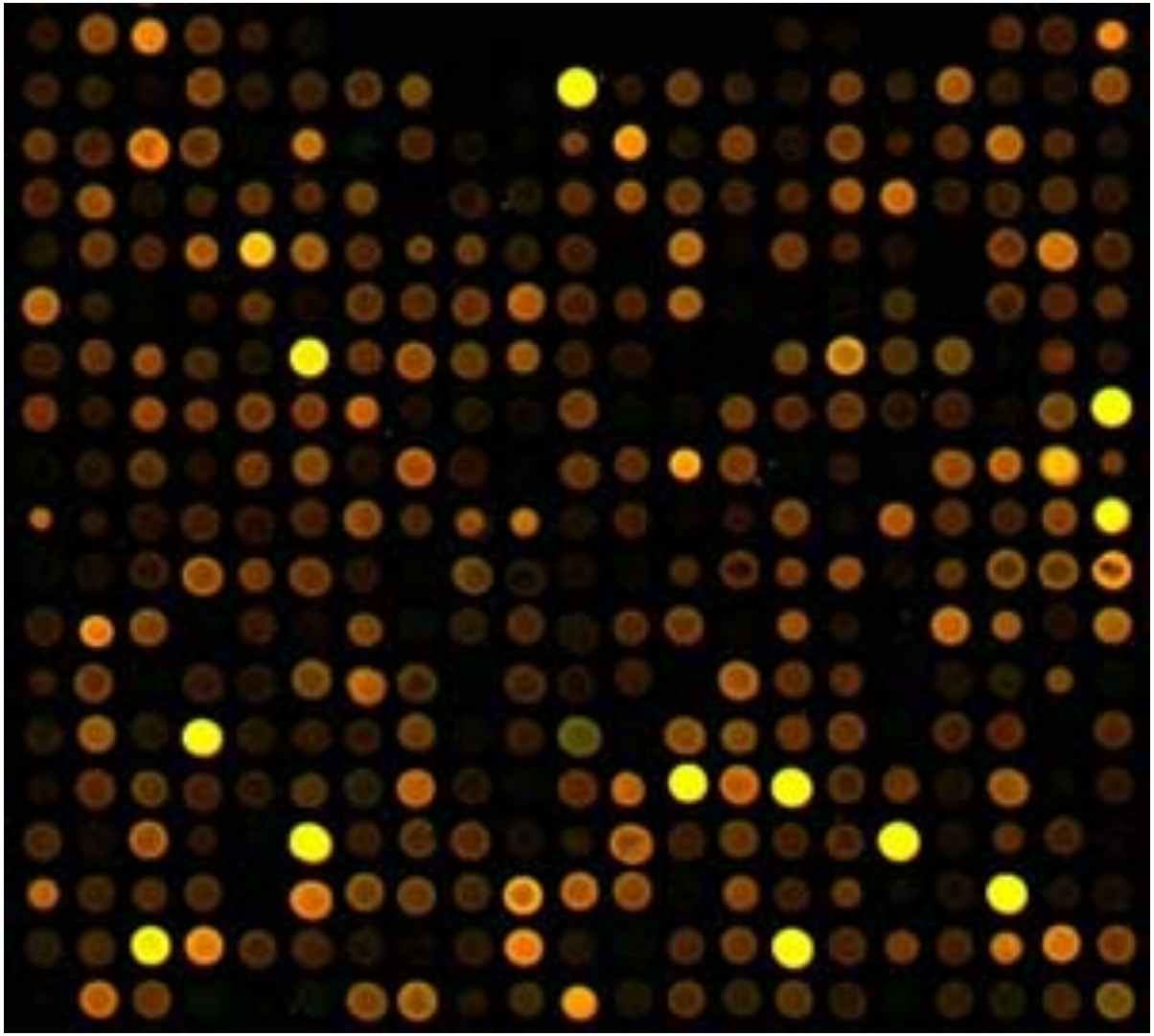
- CanMap Project:
  - Dr. Elaine Ostrander (NHGRI/NIH - CGB)
  - Dr. Robert Wayne (UCLA EEB)
  - Dr. Carlos Bustamante (Cornell/Stanford)
  - Affymetrix and Michele Cargill
- 127,000 SNPs (all Perfect Match oligos)
  - Magic genotyping algorithm- 50-70K
- Quality assessment for standardizing across batches
- Excluding SNPs:
  - When sample call rate is <95%
  - When SNP MAF < 1%
  - When SNP heterozygosity > 60

Samples

Collect DNA from 5-10 unrelated dogs from 85 breeds and gray wolves (the ancestor of dogs)

The Canine SNP genotyping microarray





About

file:///Users/carlosb/CanMap/index.html

Google

The CanMap Project

Mapping Genes Underlying Phenotypic Diversity among Domestic Dog Breeds

- About
- Genetic Diversity
- Breed History
- Phenotypes
- Software
- Upload data for Analysis

The domestic dog is a phenotypically diverse species in which discrete phenotypes segregate along breed boundaries. We aim to characterize genetic variation in the domestic dog and identify genomic regions associated with skeletal and coat color differences among dog breeds. Our research design consists of three distinct components:

(1) A genome-wide survey of SNP variation.

(2) Screening the dog genome for footprints of selection.

(3) Genome-wide association mapping of breed-specific traits.

2. How to build a dog

2a. How to build a dog

A. Fixing mutations of large effect

X

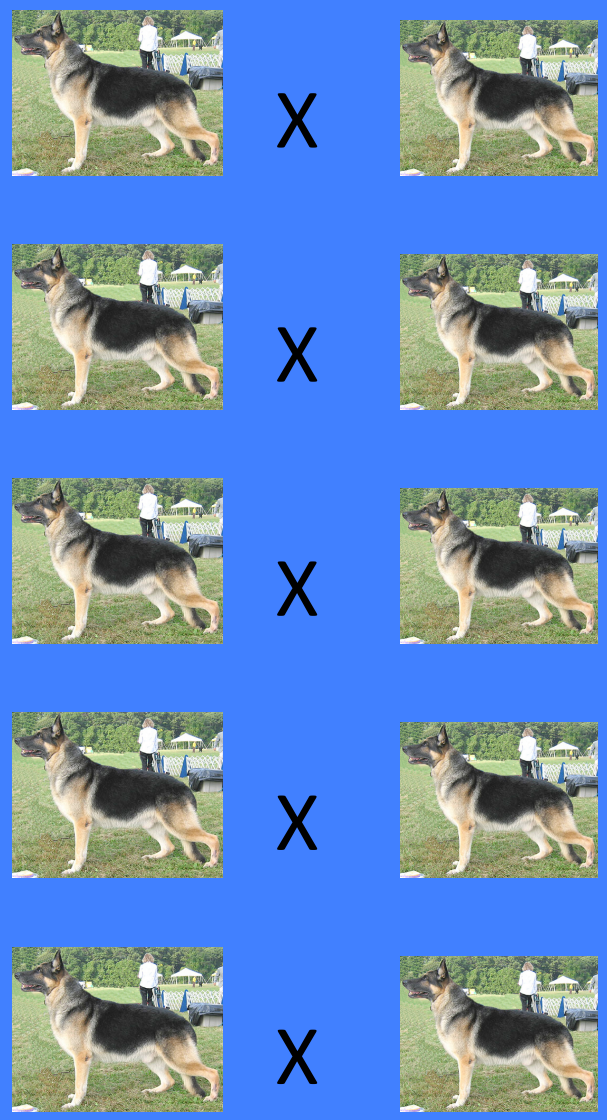
2. How to build a dog

B. Crossing distinct breeds

X

2. How to build a dog

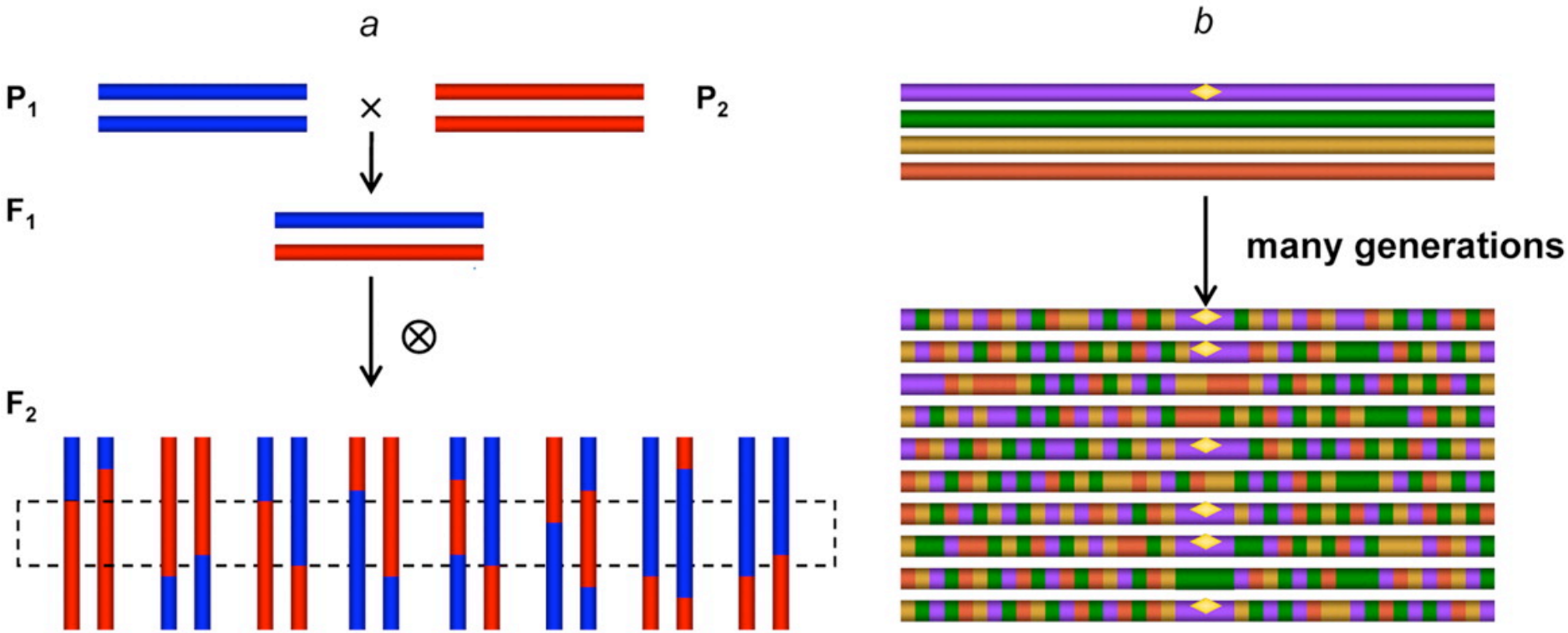
C. Progressive “idea” driven selection



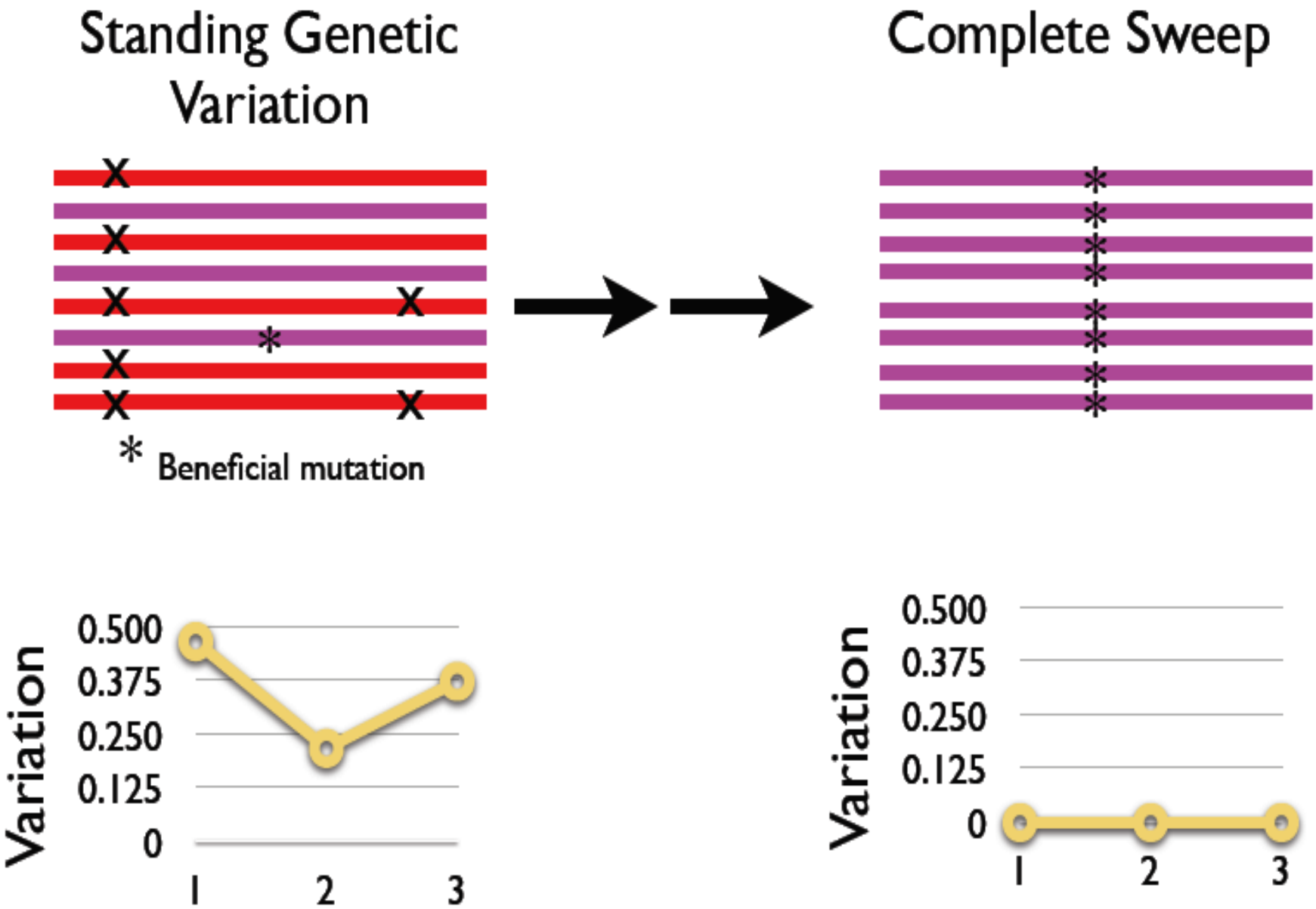
Shorter legs

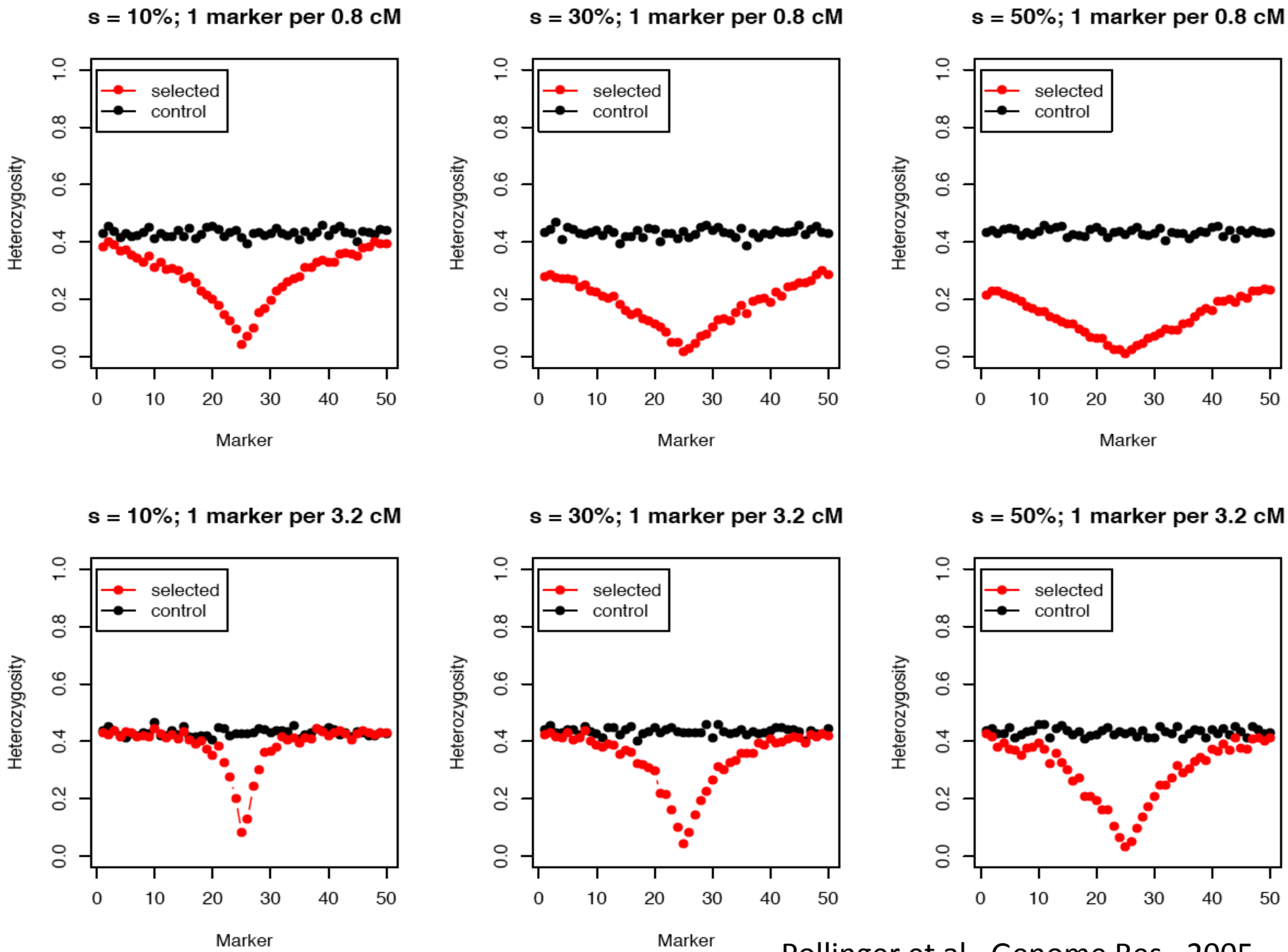
b. Reverse engineering the dog

Linkage and Association Mapping



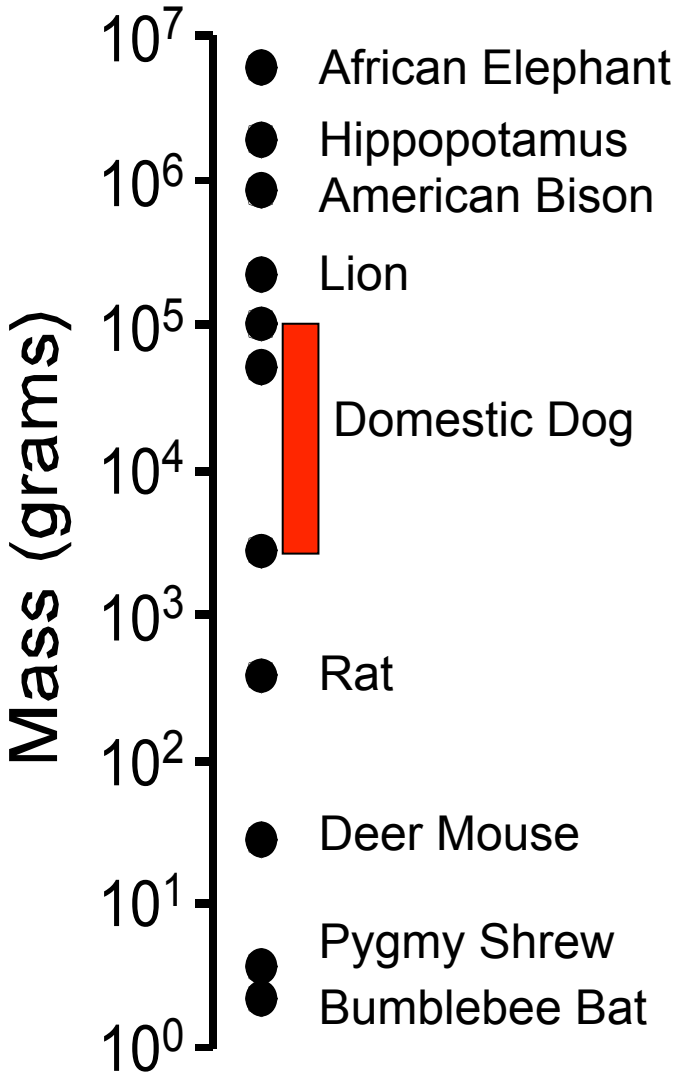
b. Selective Sweep Mapping





Pollinger et al., Genome Res., 2005

B. IGF1, the body size gene



Sutter et al., Science, 2007

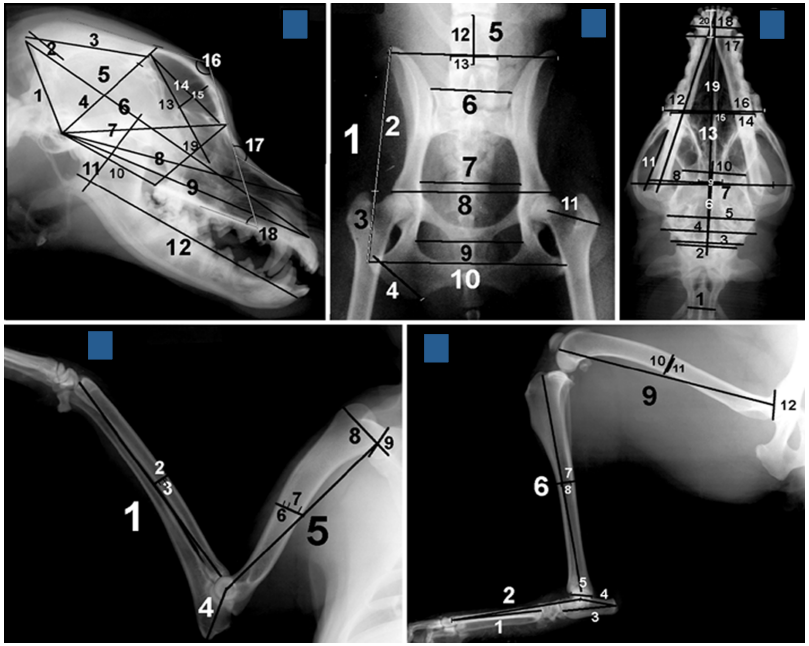
A General Approach For Finding Genes Under Selection (such as body size)

- First, genome-wide association study in a single breed (Portuguese Water Dogs) that segregates size morphs to find markers associated with size
- Second, selective sweep mapping in candidate regions in large and small dogs to find regions of reduced variation and high divergence.
- Third, association mapping, find haplotypes that correspond with small size, narrow genomic interval

Step 1: Genome-wide scan

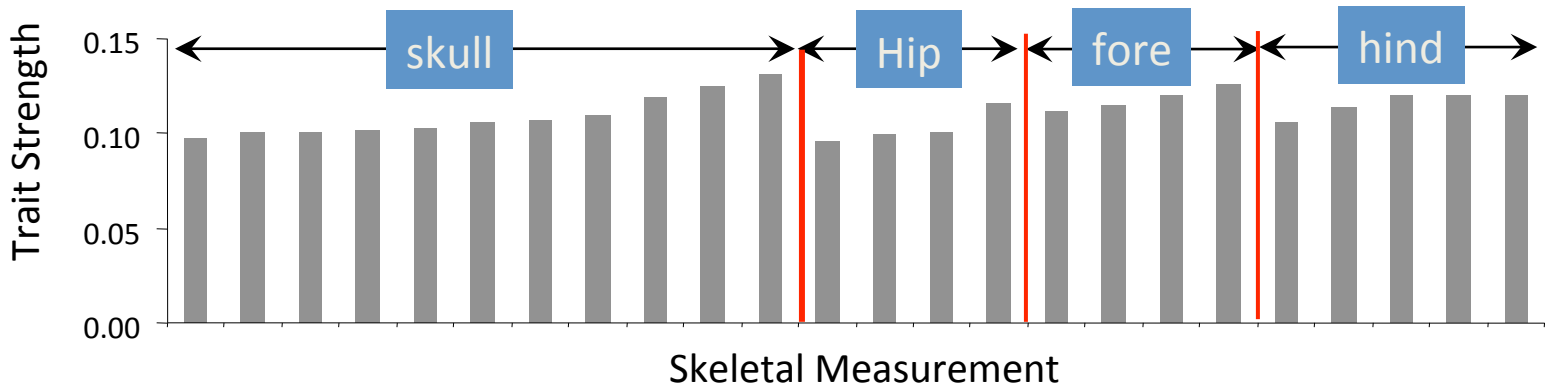
Genotype, Measure Skeletal Phenotypes, and Do Principal Component Analysis

- Focus on the Portuguese water dogs that have small and large morphs
- Genotype 500+ pedigreed dogs at 500 microsatellite markers
- Collect 91 skeletal measurements from x-ray images



- Do principal component analysis, which classifies phenotypic variation into independent systems of correlated traits
- Principal components are phenotypes so we can search for markers associated with them

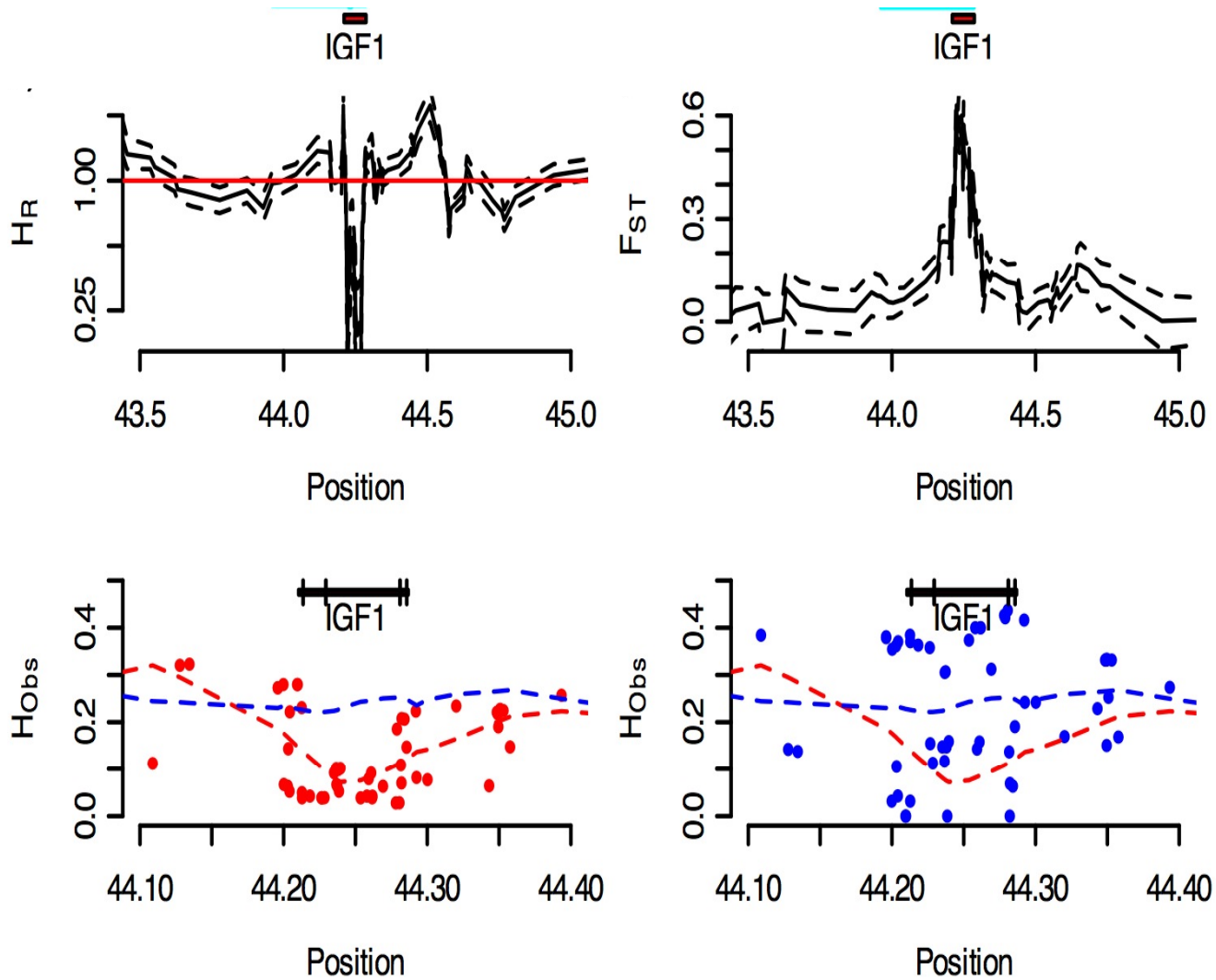
Principal Component 1 (Skeletal Size)



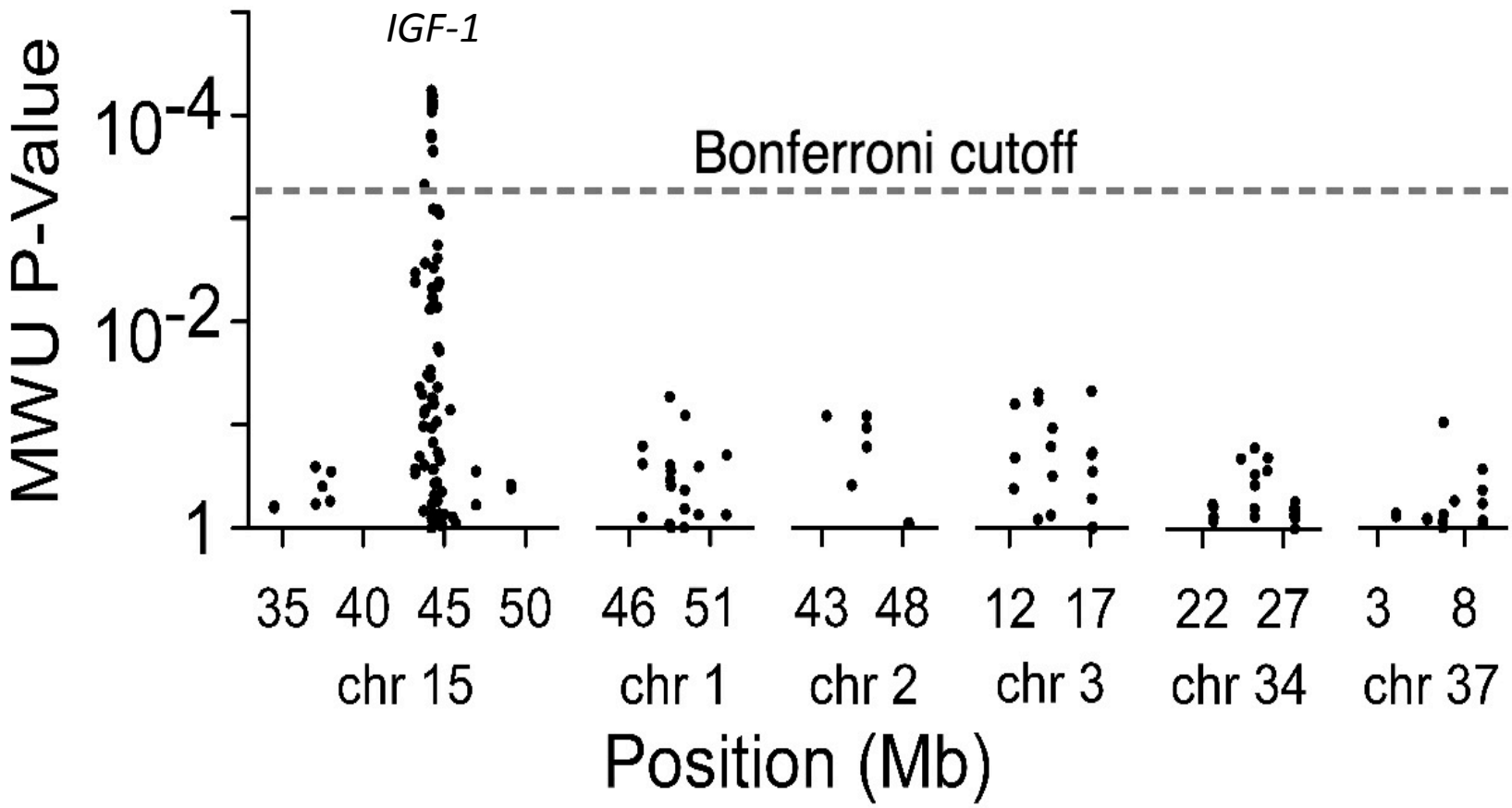
- PC1 is based on 42 metrics from pelvis, fore and hind limbs
- Highly heritable (h^2 0.45)
- 7 QTLs affect size
- A locus of interest: **FH2017 on CFA15 is linked to *IGF1!***
- Extensive re-sequencing near *IGF1* led to SNP discovery

Step 2: Selective sweep mapping using a dog panel

(kg)	Breed
2	Chihuahua
2	Toy Fox Terrier
2	Pomeranian
3	Yorkshire Terrier
3	Japanese Chin
3	Chinese Crested
4	Italian Greyhound
4	Pekingese
5	Shih Tzu
6	Cav. King Charles Spar
6	Border Terrier
7	Miniature Schnauzer
7	Jack Russell Terrier
8	Boston Terrier
32	Giant Schnauzer
44	Akita
45	Bernese Mountain Dog
49	Great Pyrenees
54	Bullmastiff
54	Irish Wolfhound
59	Saint Bernard
	Great Dane
	Mastiff
<9	All Small Dogs
>31	All Giant Dogs



Step 3: Association mapping

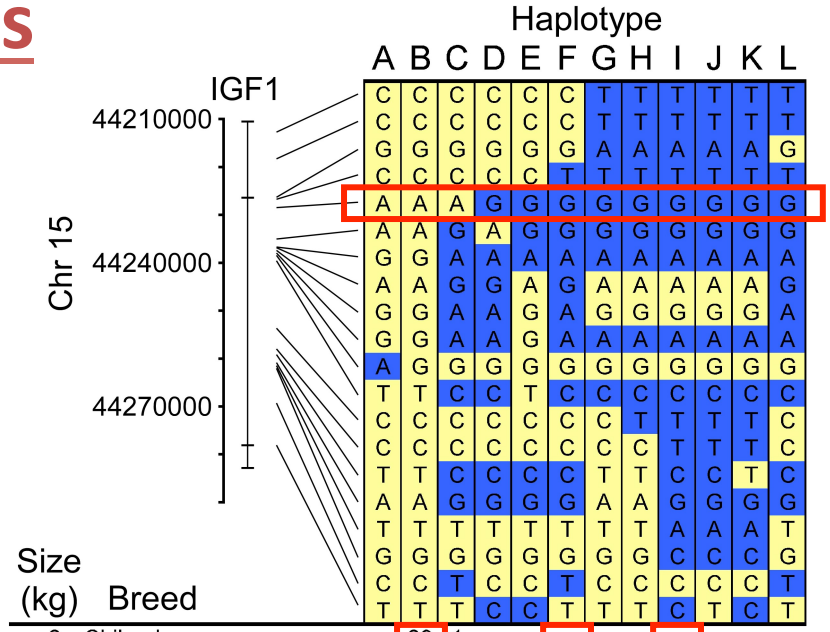


Step 3: Haplotype analysis

A single unique haplotype is associated with small size

- …Suggests a single ancient mutation for small size

- Giant breeds carry two haplotypes rare in small breeds

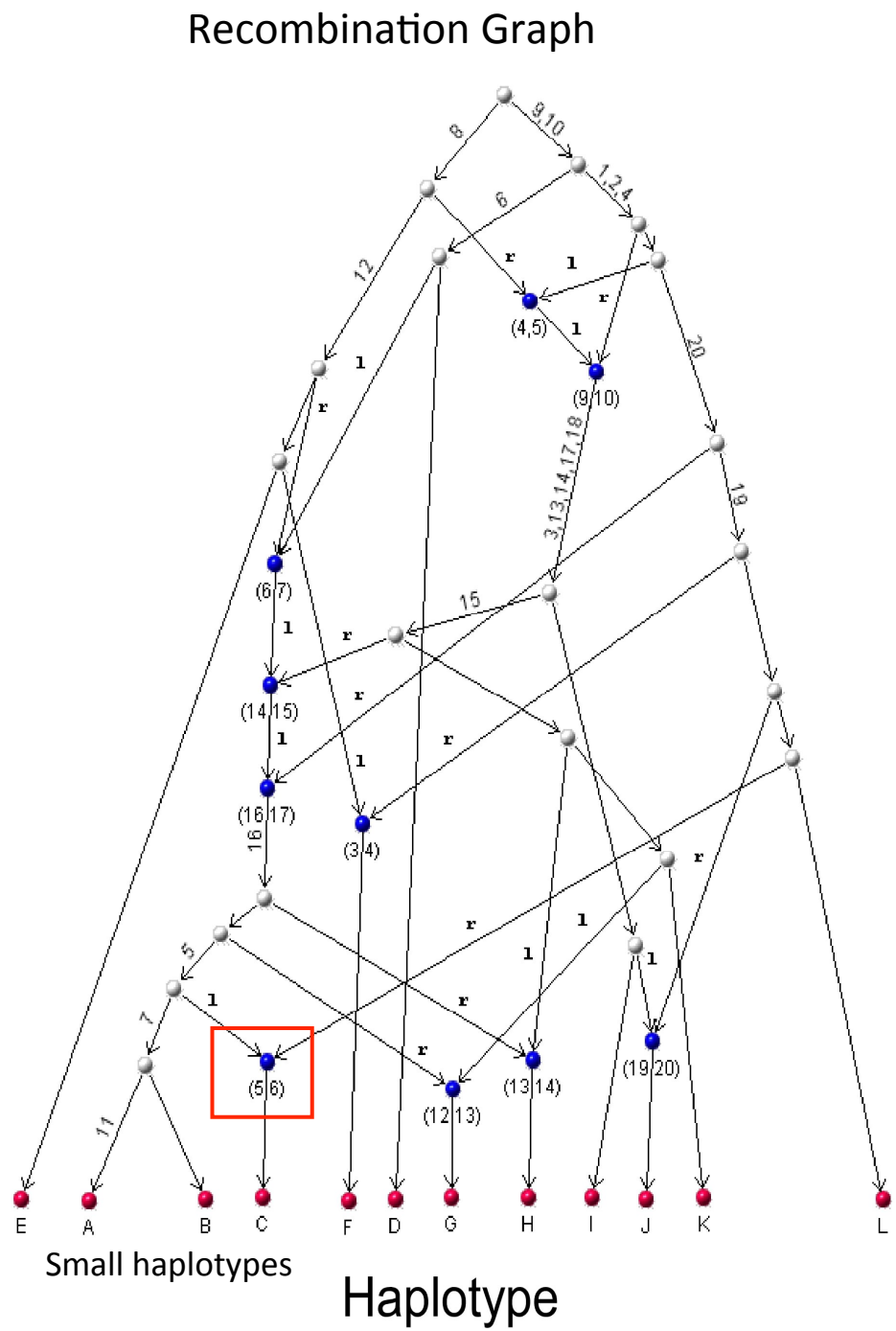


Size (kg)	Breed	A	B	C	D	E	F	G	H	I	J	K	L
2	Chihuahua	-	29	1	-	-	-	-	-	-	-	-	-
2	Toy Fox Terrier	2	26	-	-	-	-	-	-	-	-	-	-
2	Pomeranian	4	66	-	-	-	-	-	-	-	-	-	-
3	Yorkshire Terrier	-	30	3	-	-	-	-	-	-	-	-	-
3	Japanese Chin	-	12	-	-	-	-	-	-	-	-	-	-
3	Chinese Crested	-	12	-	-	-	-	-	-	-	-	-	-
4	Italian Greyhound	-	12	7	-	-	-	-	-	-	-	-	-
4	Pekingese	-	22	-	-	-	-	-	-	-	-	-	-
5	Shih Tzu	3	73	-	-	-	-	-	-	2	-	-	-
6	Cav. King Charles Spaniel	-	47	-	-	-	-	-	-	2	-	-	-
6	Border Terrier	-	76	2	-	-	-	-	-	-	-	-	-
7	Miniature Schnauzer	-	49	-	-	-	-	-	-	3	-	-	-
7	Jack Russell Terrier	3	22	-	-	-	-	-	-	-	-	-	-
8	Boston Terrier	-	34	-	-	-	-	-	-	3	3	1	-
32	Giant Schnauzer	-	2	-	-	-	-	-	9	37	-	-	-
44	Akita	-	-	-	9	8	4	-	-	3	-	-	-
45	Bernese Mountain Dog	-	-	-	-	-	-	9	-	5	-	-	-
49	Great Pyrenees	-	-	-	-	-	7	-	-	47	-	-	-
54	Bullmastiff	-	8	-	-	-	-	-	-	6	-	-	5
54	Irish Wolfhound	-	-	-	-	-	3	-	-	17	-	-	-
59	Saint Bernard	-	-	-	-	-	15	-	-	21	-	-	-
73	Great Dane	-	-	-	-	-	39	-	-	25	-	-	-
82	Mastiff	-	19	-	-	-	5	-	-	58	-	4	3
<9	All Small Dogs		510				0			10			
>31	All Giant Dogs		29				73			219			

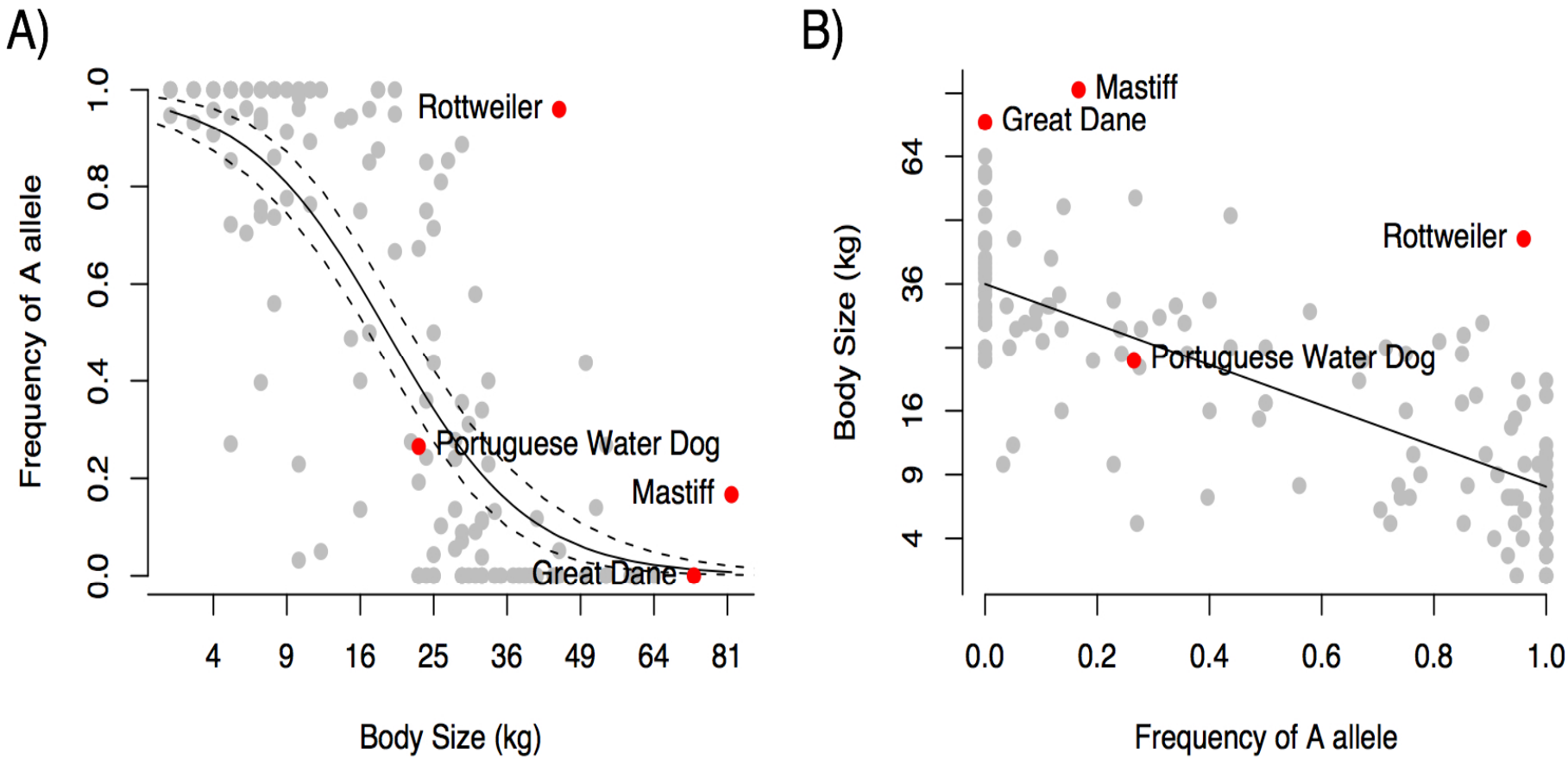
Step 3: Association mapping

Core region of 8.7 kb contains only two possible causative mutations

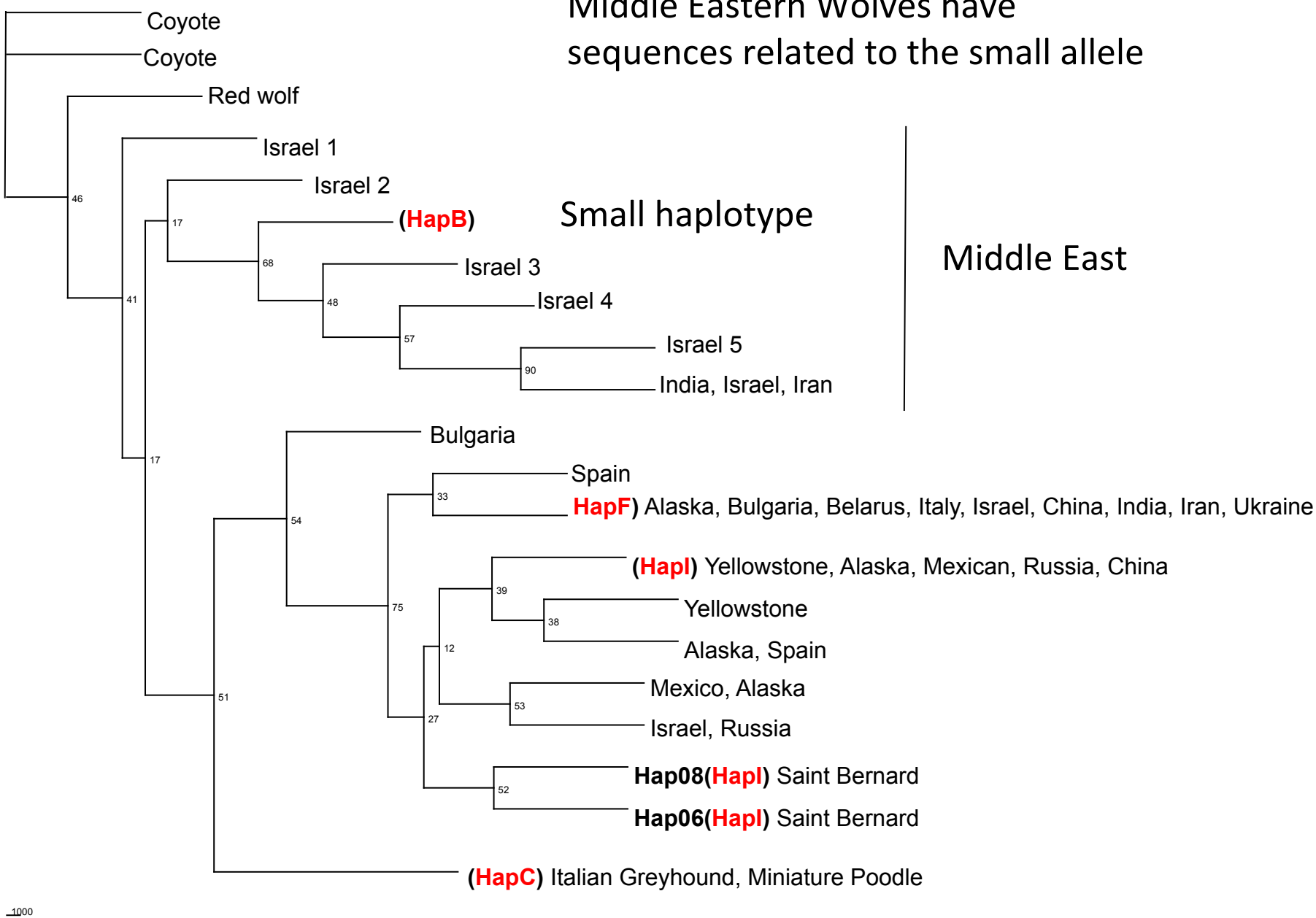
1. SINE element
2. CA_n mutation in *IGF1* promoter



IGF-1 explains more than 50% of the variation across 143 breeds

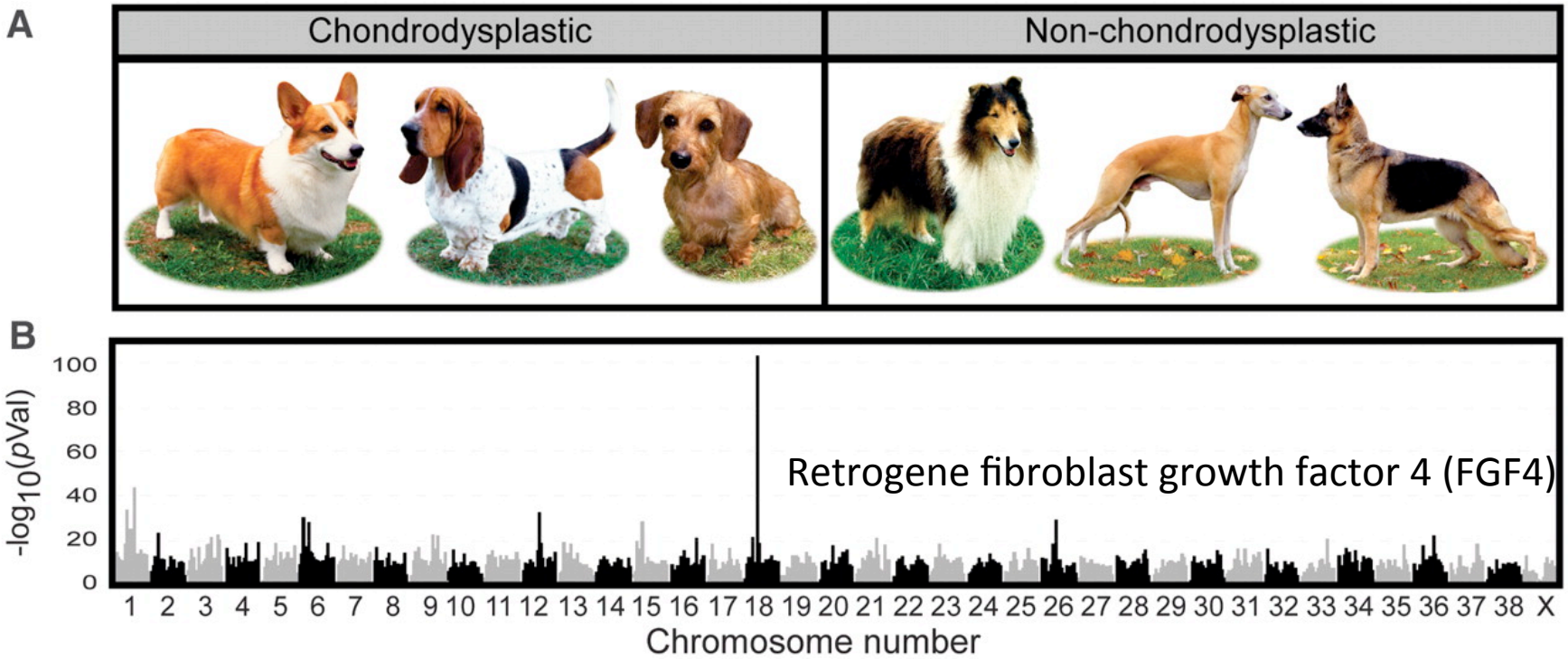


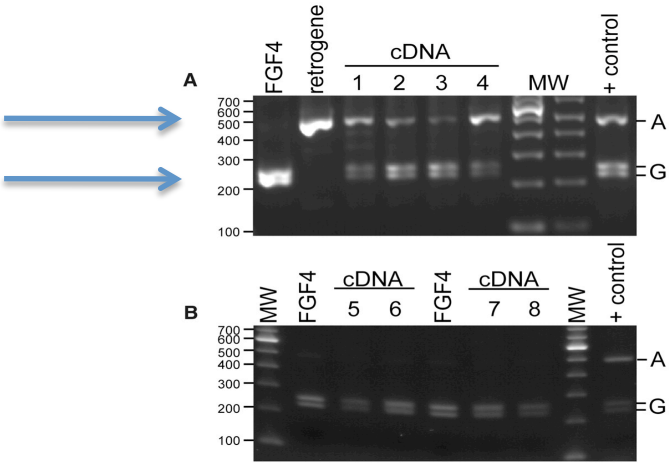
Middle Eastern Wolves have sequences related to the small allele



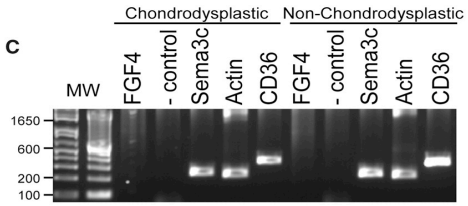
Gray et al., in press

c. The short legs gene: association mapping



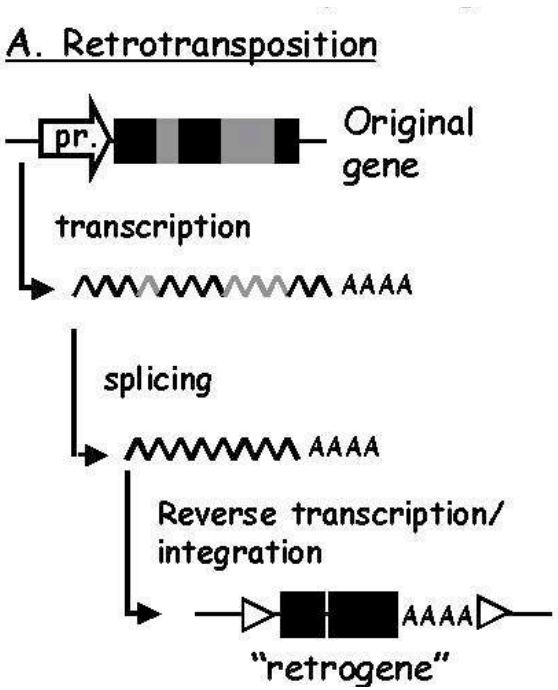


Juveniles



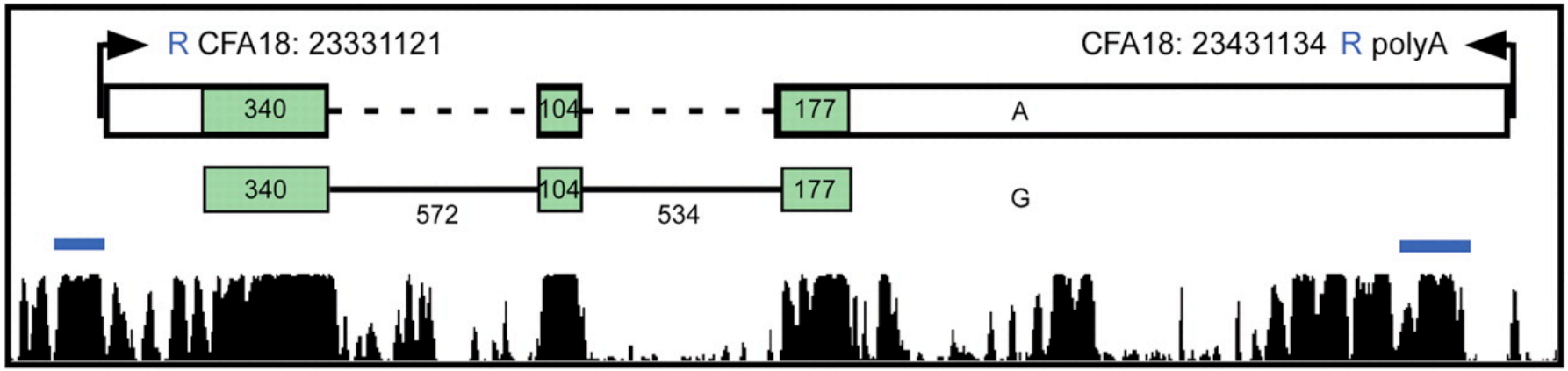
Adults

Parker et al.
Science, 2009



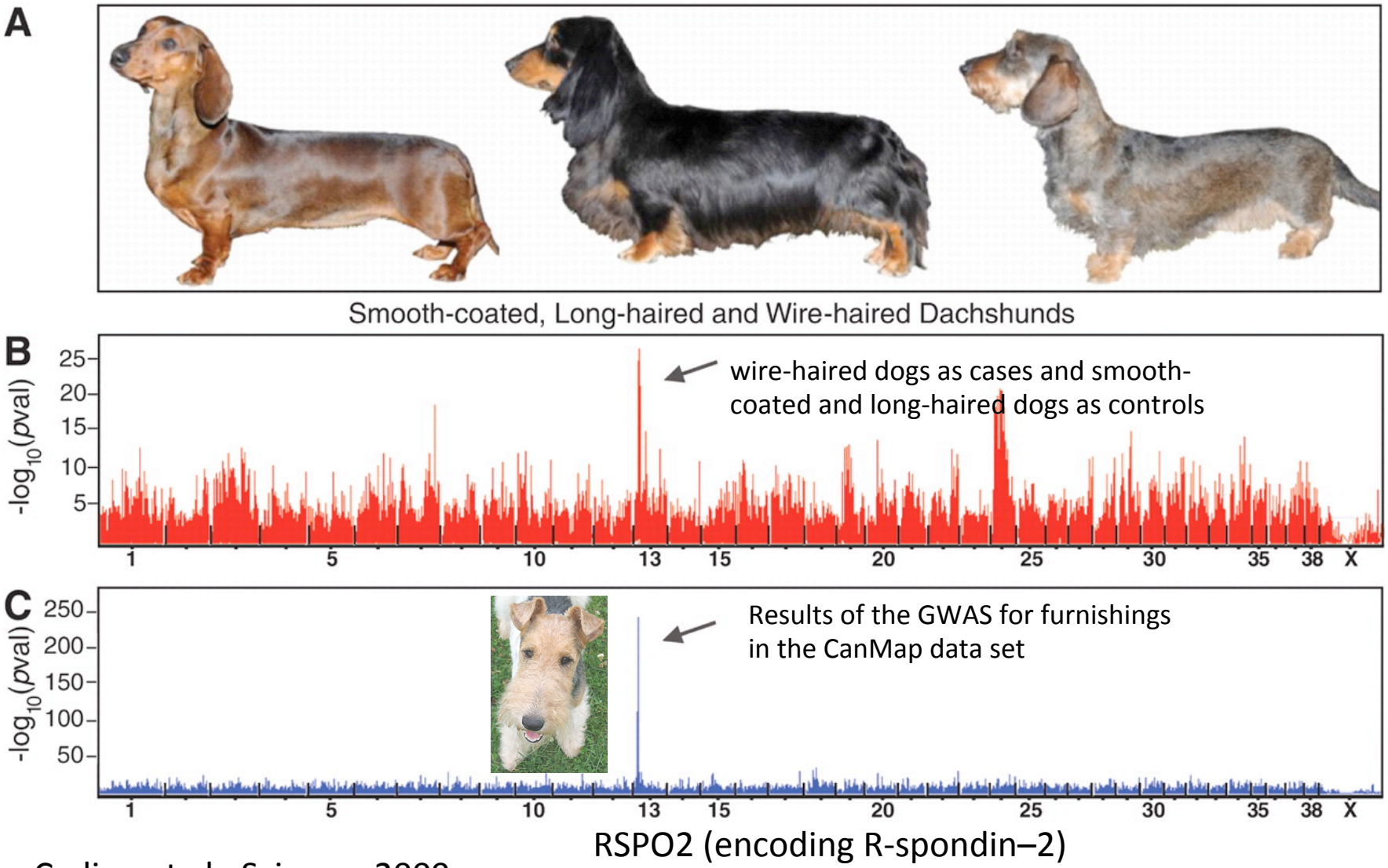
Pseudogene features

- Absence of introns and promoter sequences
- flanked by direct repeats
- presence of poly-A tract
- randomly integrated anywhere in the genome



Parker et al.
Science, 2009

d. Fur Types



RSPO2 (encoding R-spondin–2)

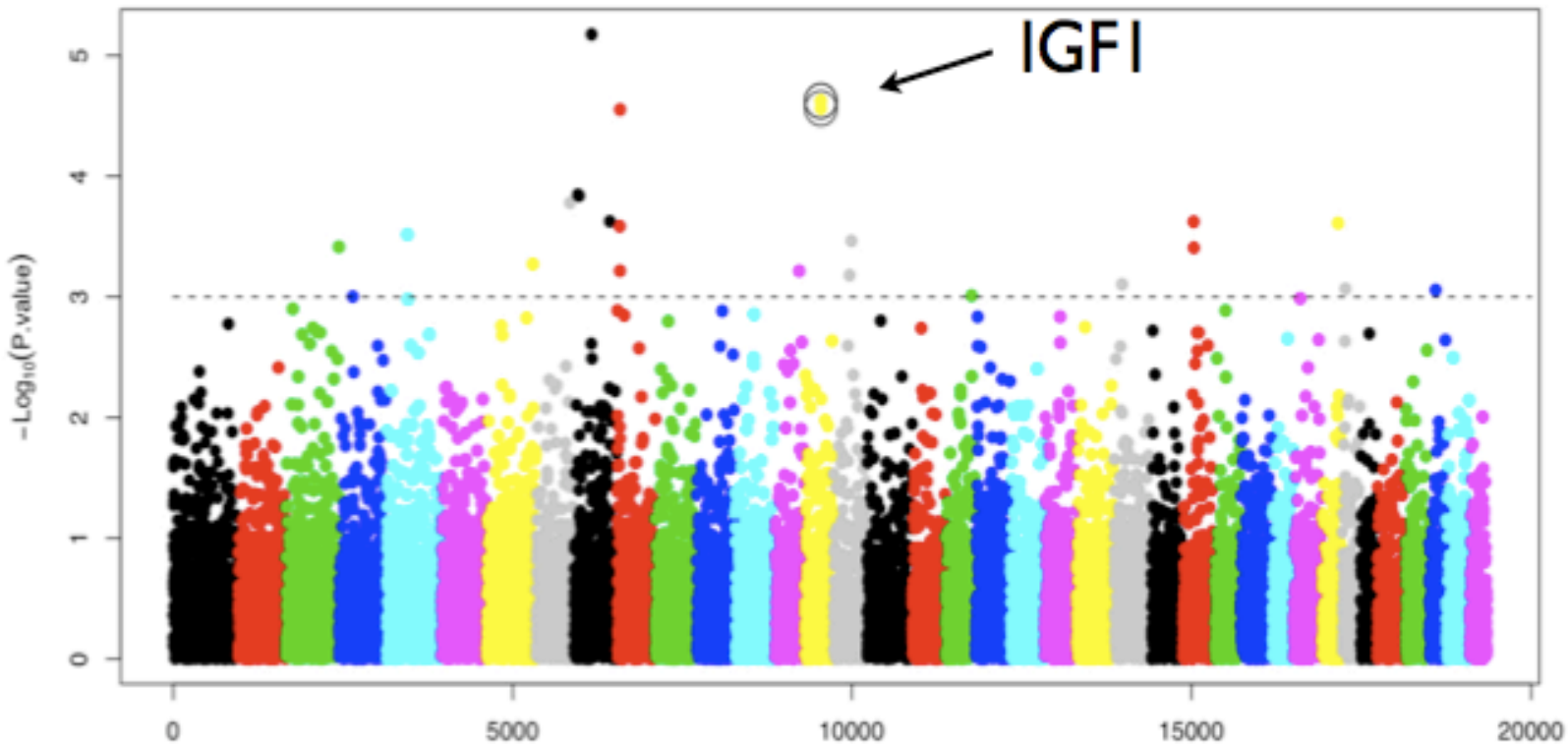
Cadieu et al., Science, 2009

Three mutations in various combinations explain the observed pelage phenotype of 95% of dogs sampled

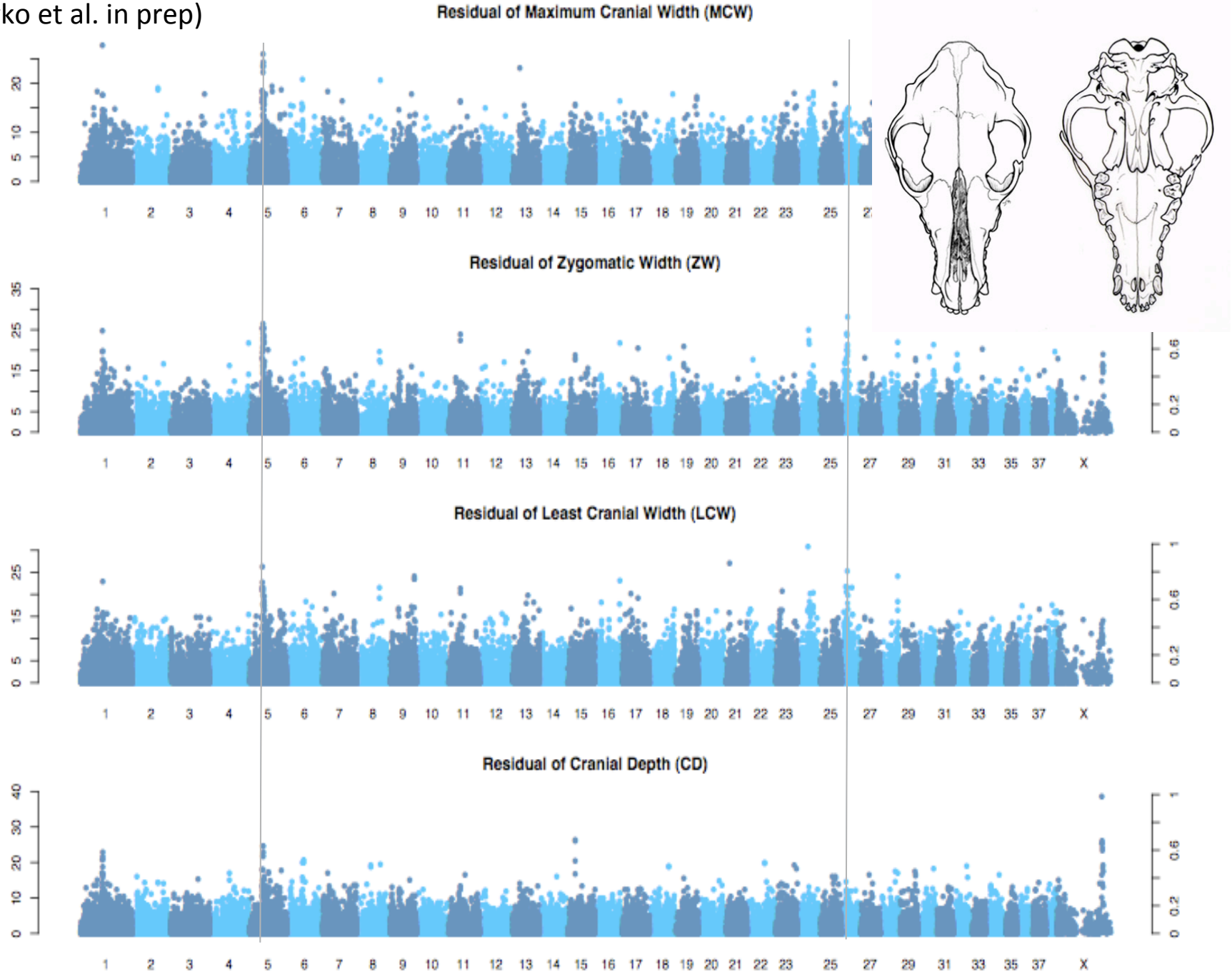
	PHENOTYPE	*FGF5*	*RSPO2*	*KRT71*
A	Short	-	-	-
B	Wire	-	+	-
C	Wire and Curly	-	+	+
D	Long	+	-	-
E	Long with Furnishings	+	+	-
F	Curly	+	-	+
G	Curly with Furnishings	+	+	+

(encoding R-spondin–2, fibroblast growth factor–5, and keratin-71)

e. The number of genes

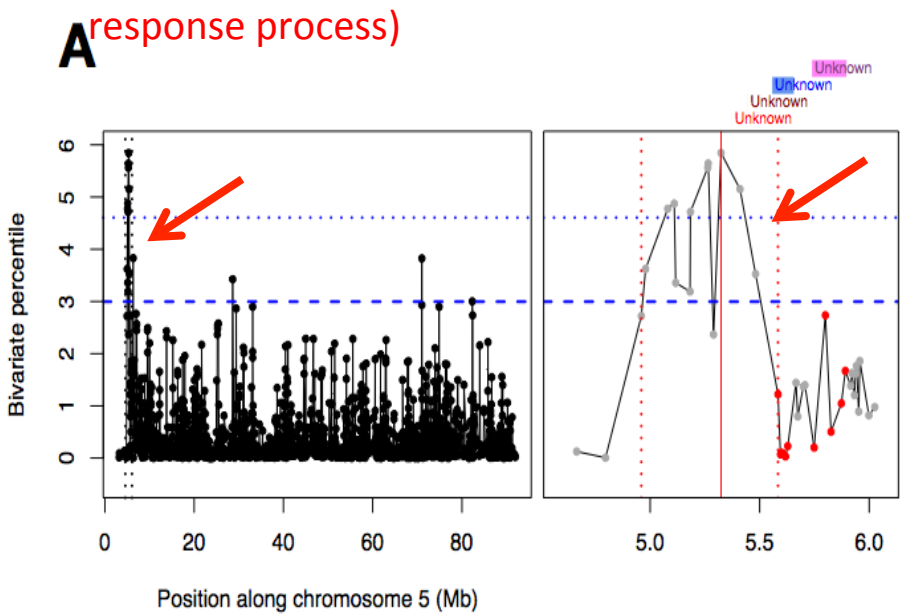


(Boyko et al. in prep)

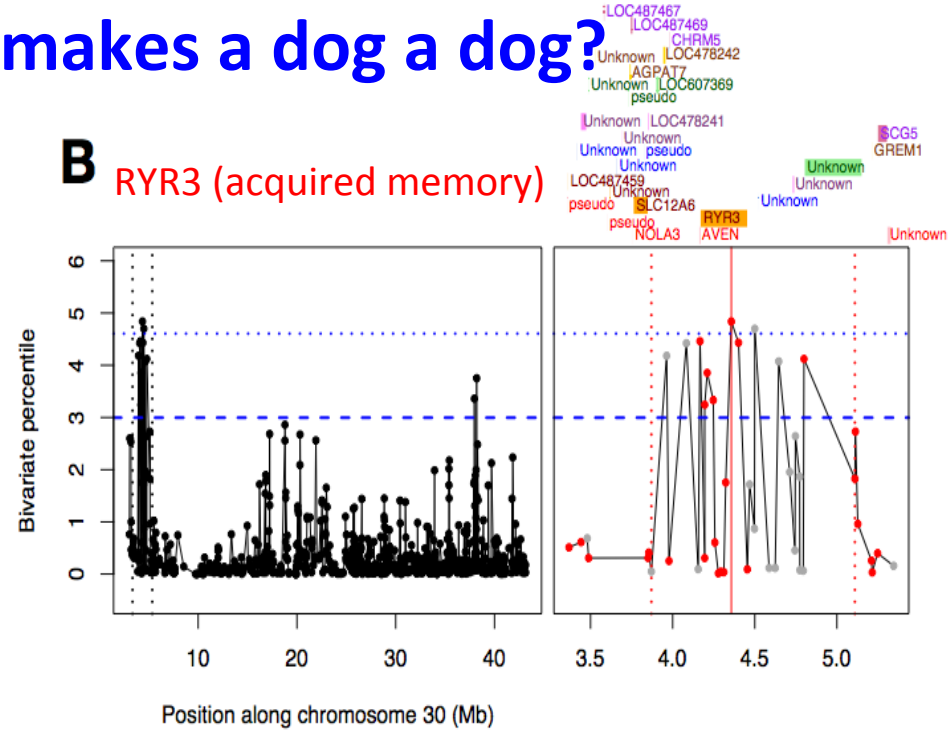


What makes a dog a dog?

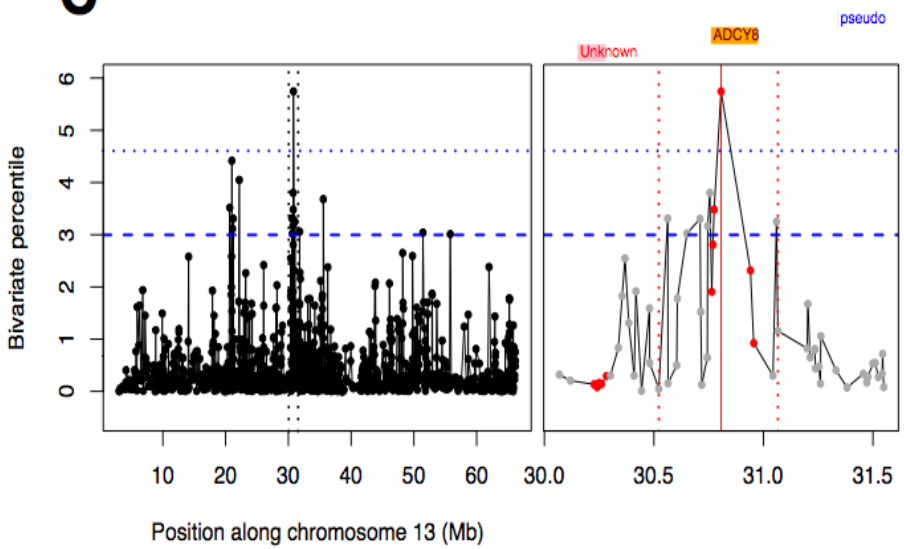
AA similarity to neurotrimin an an opioid receptor (involved in the stress response process)



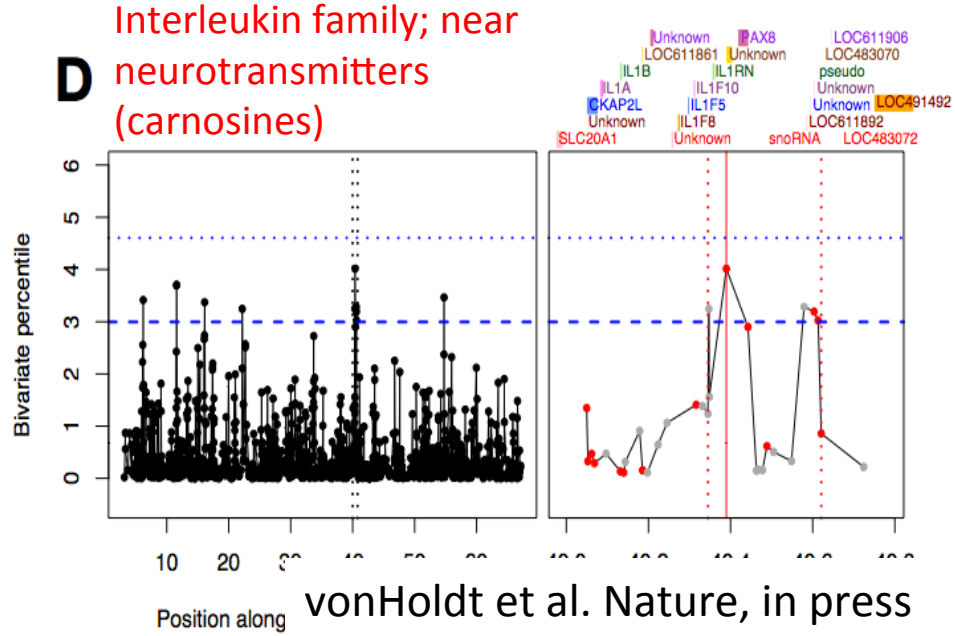
RYR3 (acquired memory)



ADCY8 (memory formation)



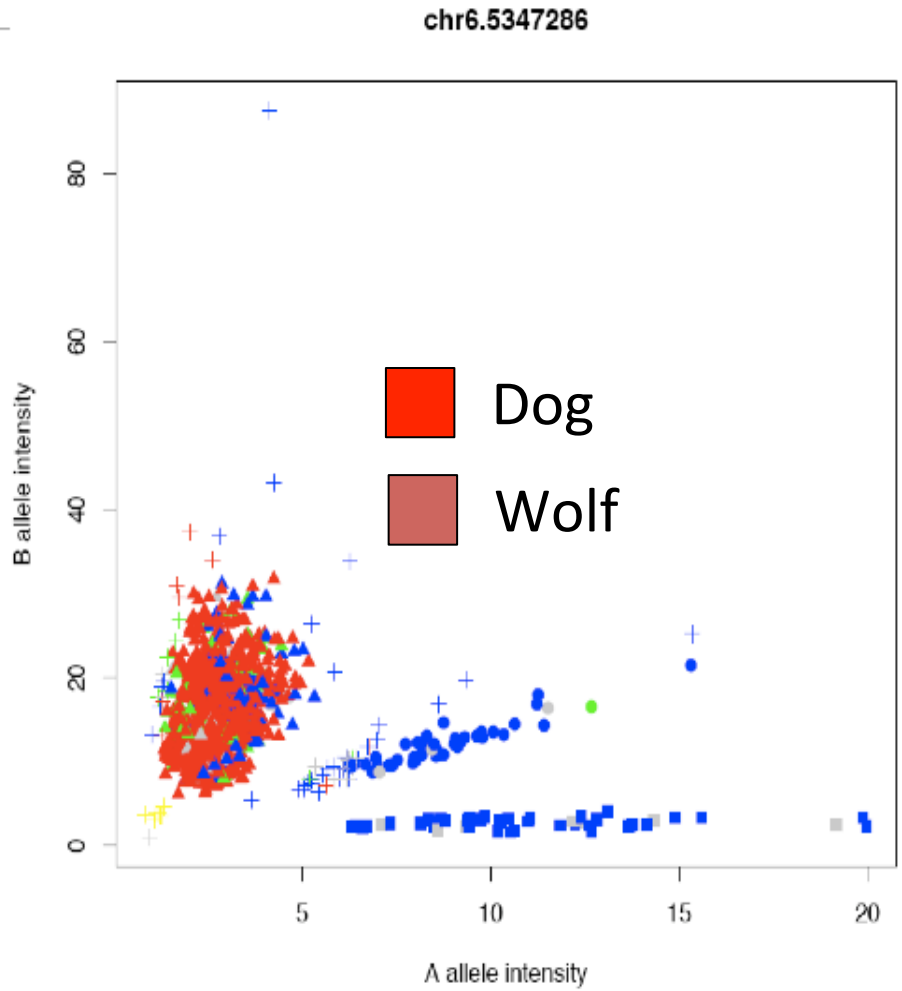
Interleukin family; near neurotransmitters (carnosines)



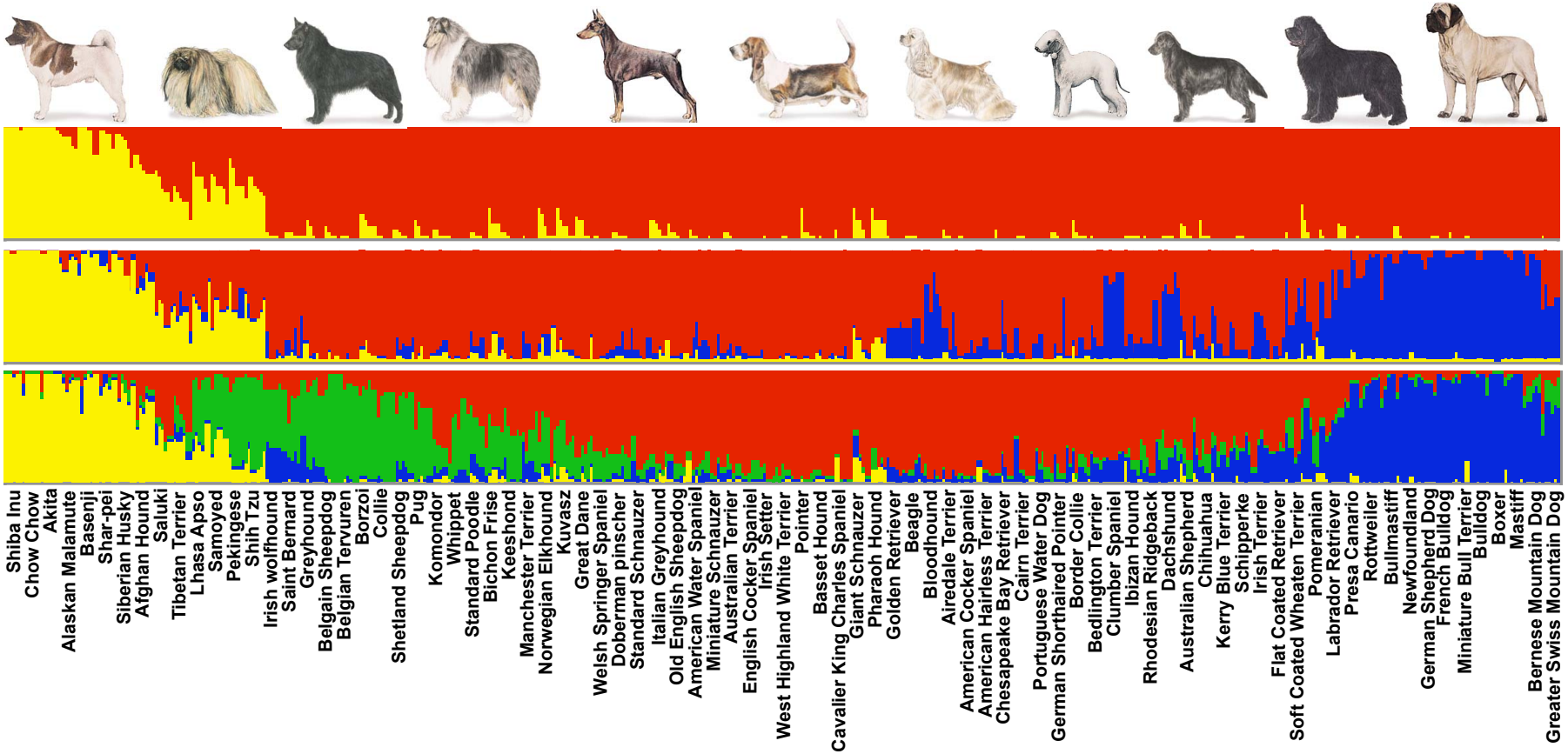
vonHoldt et al. Nature, in press

Alluring story-telling II...

- WBSCR17: Williams syndrome (WS; also Williams-Beuren syndrome or WBS) is a rare neurodevelopmental disorder caused by a deletion of about 26 genes from the long arm of chromosome 7. It is characterized by a distinctive, "elfin" facial appearance, along with a low nasal bridge; an unusually cheerful demeanor and ease with strangers, coupled with unpredictably occurring negative outbursts; a predisposition to violent outbursts; mental retardation coupled with unusual (for persons who are diagnosed as mentally retarded) language skills; a love for music; and cardiovascular problems, such as supravalvular aortic stenosis and transient hypercalcaemia.



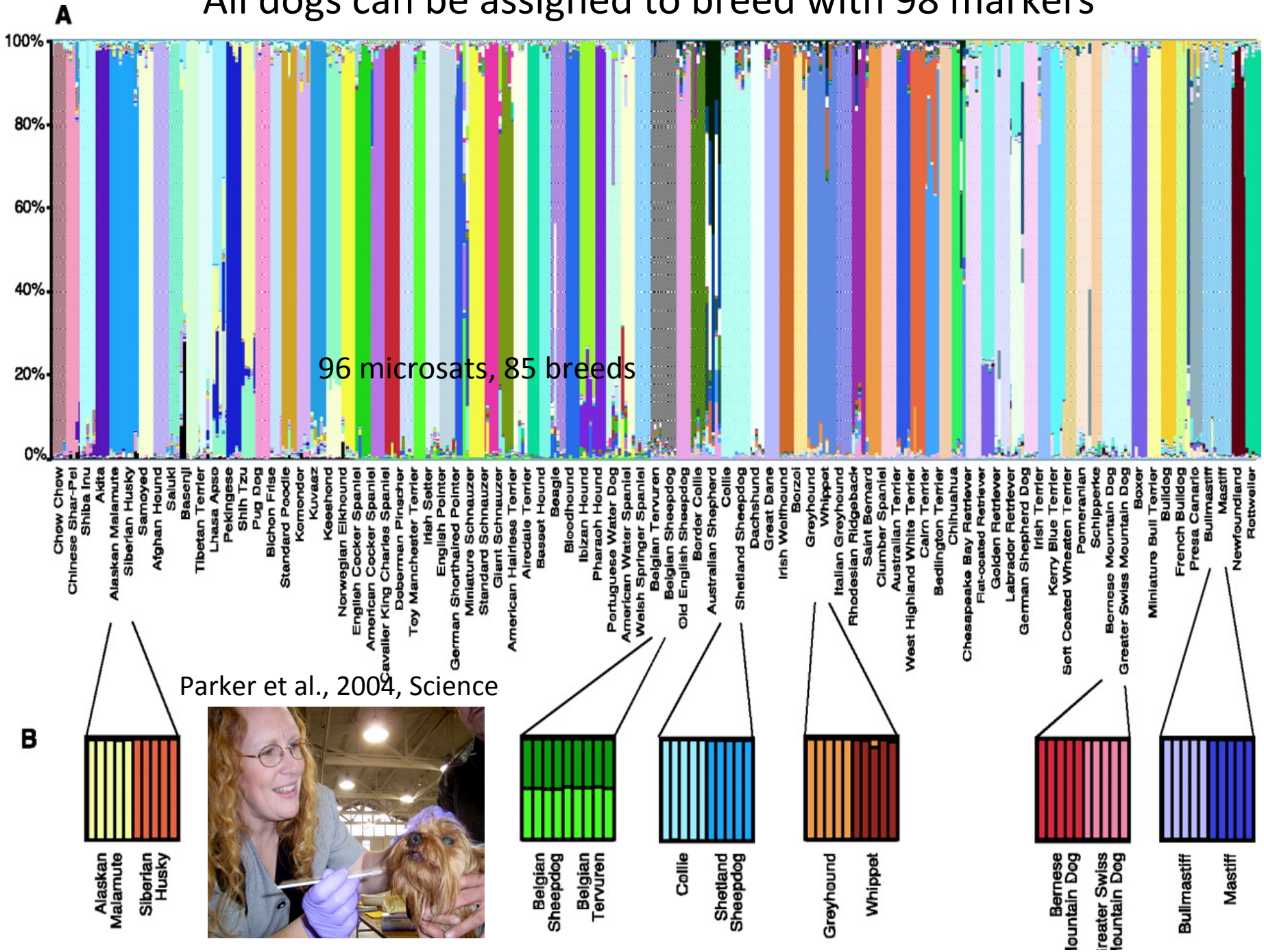
f. Evolving the Dog



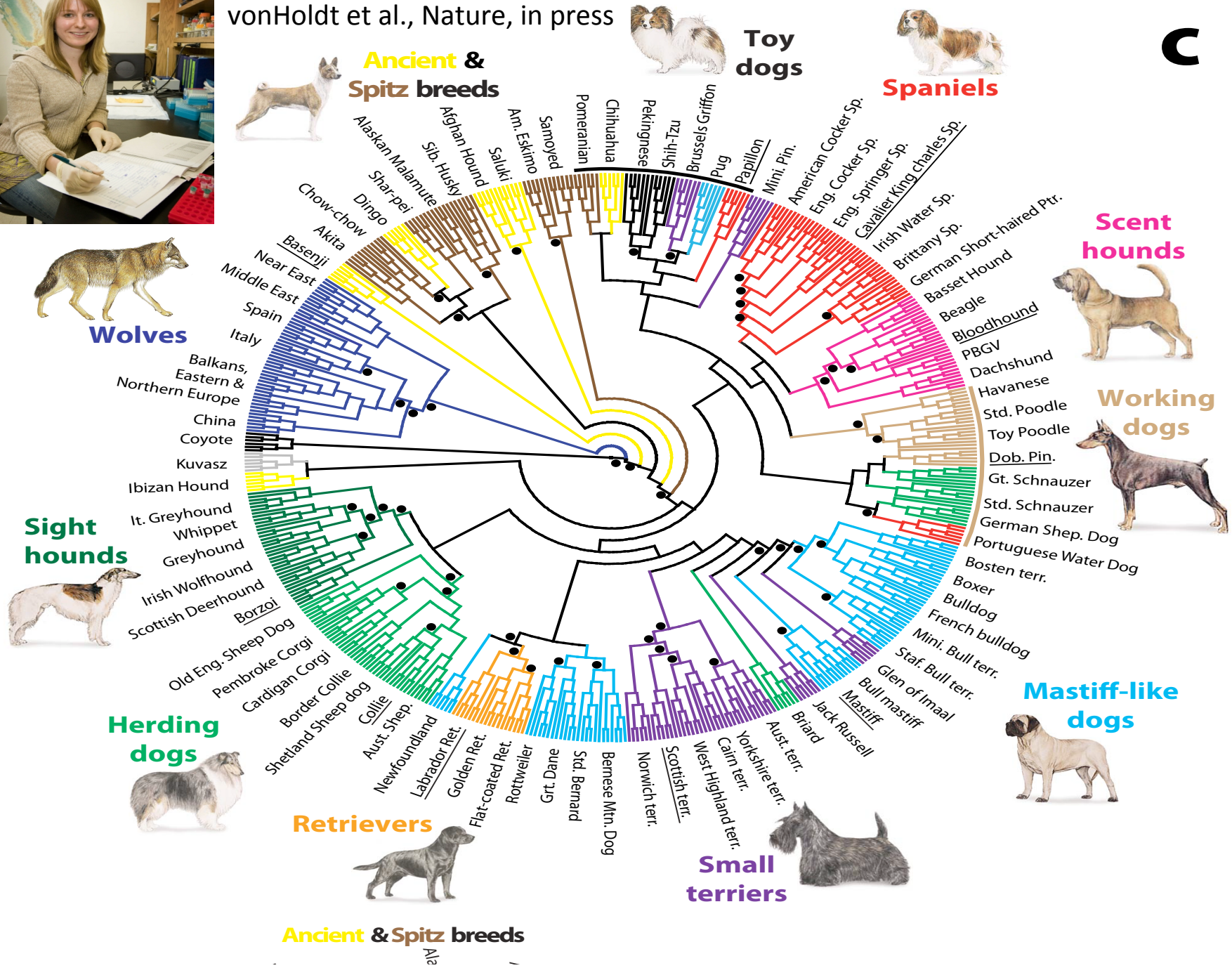
Population Structure of 85 Domestic Dog Breeds

Parker et al. Science, 2004

All dogs can be assigned to breed with 98 markers



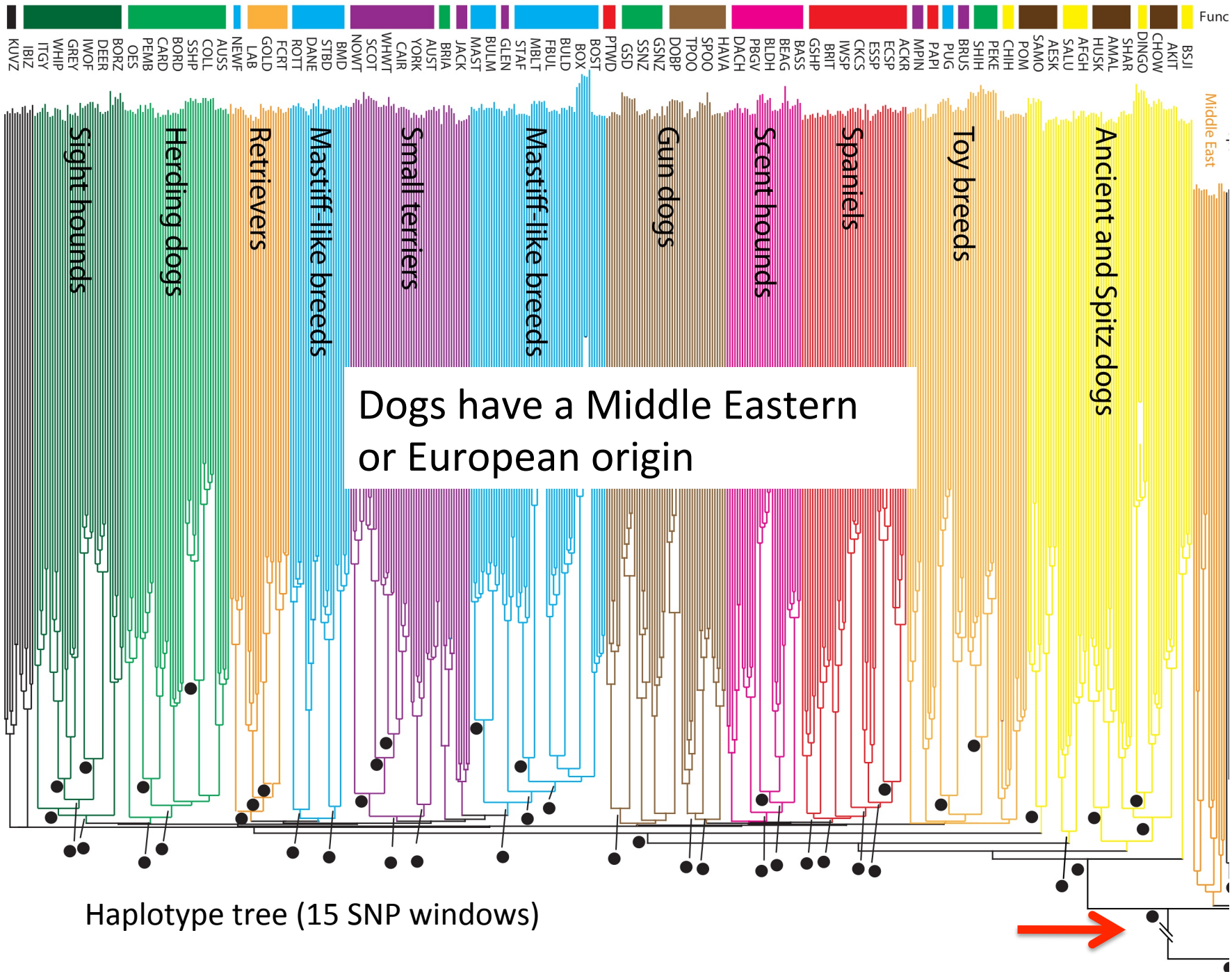
vonHoldt et al., Nature, in press



Supplemental Table 4. History of breeds with discordant phenotypic/functional and genetic group assignments[2,3].

Breed Name	Genetic Cluster (see Fig. 1)	Phenotypic/ Functional Group	Breed Information[2,3]	Concordance with Historical Evidence
Briard	Small Terrier	Herding	Possible East Asian origin from crosses with local dogs to create a new breed used for flock guarding	No historical evidence for breed admixture between small terriers and herding dogs
Brussels	Toy	Terriers	European origins from crosses with Affenpinscher (terrier) and Toy breeds (i.e. English Toy Spaniels, Yorkshire Terriers, Pekingese, or Pug) to miniaturize the breed	Evidence for breed admixture between toy and terrier breeds
Chihuahua	Toy	Ancient	Probable Chinese origins with introduction to Mexico from Spanish traders returning from East Asia	Evidence for breed admixture between East Asian Ancient and toy breeds
German Shep. Dog	Gun	Herding	European breed with recent origins	Inconclusive
Gt. Schnauzer	Gun	Herding	European origins likely from crosses with smooth-haired dogs and possibly Great Danes	Inconclusive
Glen of Imaal	Mastiff-like	Terriers	European origins from crosses of Bullterriers, Staffordshire terriers (Mastiff-like breeds) and other fighting dogs; Glen of Imaal is an aggressive hunter (e.g. badgers, rats)	Evidence for admixture between Mastiff-like and terrier breeds
Mini. Pin.	Toy	Terriers	European origins from crosses of German Pinscher (terrier) and Dachshunds or Italian greyhounds	Evidence for admixture between toy and terrier breeds
Newfoundland	Retrievers	Mastiff-like	North American origins with possible crosses to Mastiff or Portuguese Water dog; considered an ancestor of the modern Labrador Retriever	Evidence for Retriever and Mastiff-like breed admixture
Papillon	Toy	Spaniels	European origins from crosses of Spaniels and Bichon-type (toy) breeds	Evidence for admixture of toy and spaniel breeds
Pekingnese	Toy	Herding	Chinese origins; considered a dwarfed Tibetan terrier or Pug (toy)	Evidence of admixture of toy and other breeds
Pomeranian	Toy	Spitz	European origins from crossing European herding and spitz-type breeds	Inconclusive
Portuguese Water Dog	Gun	Spaniels	European origins; bred to be a water dog	Inconclusive
Pug	Toy	Mastiff-like	Chinese origins; considered a “mini-mastiff”, likely from miniaturizing the Affenpinscher (Terrier) or the English Bulldog and crossing with the Tibetan Mastiff (Mastiff-like breeds)	Evidence for breed admixture of Mastiff-like and toy breeds
Shih Tzu	Toy	Herding	Tibet/Chinese origins; considered a dwarf of Tibetan terriers or Lhasa Apsos (herding breeds)	Evidence for admixture of Toy and herding breeds
Std. Schnauzer	Gun	Herding	European origins from crossing the Standard Pinscher, Poodles, “Wolfspitzs", or Shepherds	Inconclusive

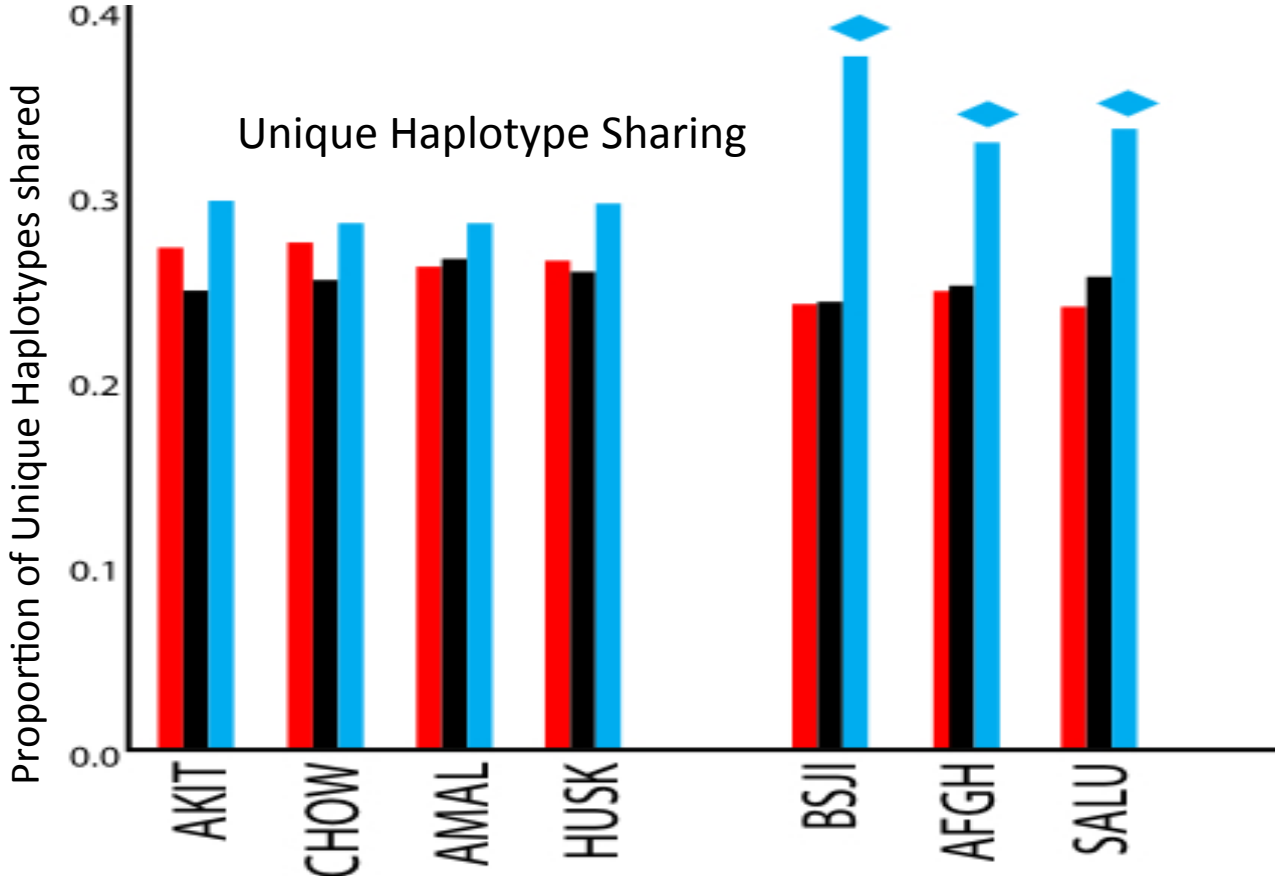
vonHoldt et al., Nature, in press



Dogs have a Middle Eastern or European origin

Haplotype tree (15 SNP windows)

Dogs share more unique haplotypes with European and Middle Eastern Wolves

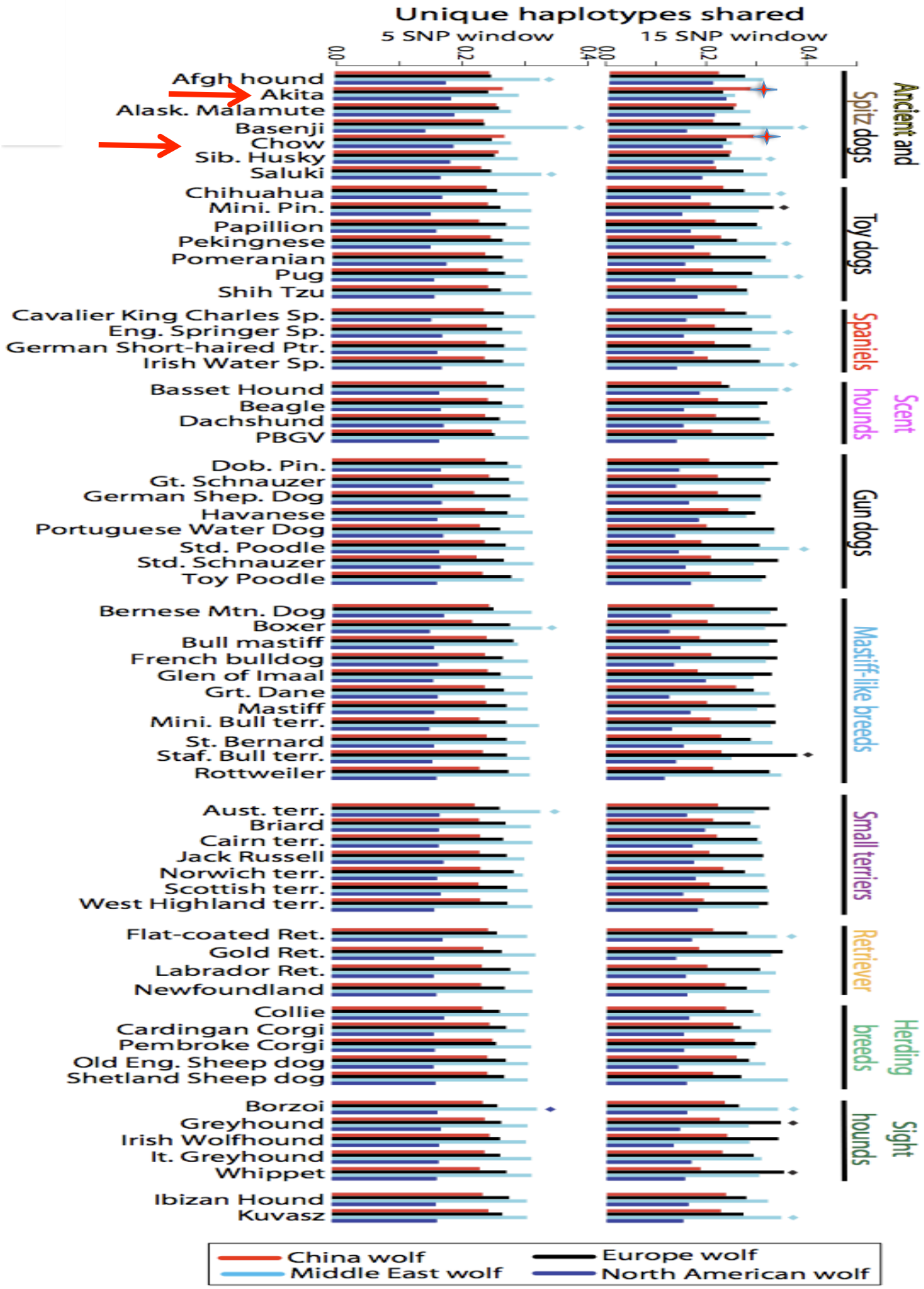


Kirk Loemueller
(Cornell/Stanford)

Shared with Chinese wolves

Shared with European wolves

Shared with Middle Eastern wolves


Unique haplotypes shared
5 SNP window
15 SNP window
0.0
0.2
0.4
Afgh hound
Akita
Alask. Malamute
Basenji
Chow
Sib. Husky
Saluki
Chihuahua
Mini. Pin.
Papillion
Pekingnese
Pomeranian
Pug
Shih Tzu
Cavalier King Charles Sp.
Eng. Springer Sp.
German Short-haired Ptr.
Irish Water Sp.
Basset Hound
Beagle
Dachshund
PBGV
Dob. Pin.
Gt. Schnauzer
German Shep. Dog
Havanese
Portuguese Water Dog
Std. Poodle
Std. Schnauzer
Toy Poodle
Bernese Mtn. Dog
Boxer
Bull mastiff
French bulldog
Glen of Imaal
Grt. Dane
Mastiff
Mini. Bull terr.
St. Bernard
Staf. Bull terr.
Rottweiler
Aust. terr.
Briard
Cairn terr.
Jack Russell
Norwich terr.
Scottish terr.
West Highland terr.
Flat-coated Ret.
Gold Ret.
Labrador Ret.
Newfoundland
Collie
Cardingan Corgi
Pembroke Corgi
Old Eng. Sheep dog
Shetland Sheep dog
Borzoi
Greyhound
Irish Wolfhound
It. Greyhound
Whippet
Ibizan Hound
Kuvasz
Ancient and
Spitz dogs
Toy dogs
Spaniels
Scent
hounds
Gun dogs
Mastiff-like breeds
Small terriers
Retriever
Herding
breeds
Sight
hounds
China wolf
Middle East wolf
Europe wolf
North American wolf

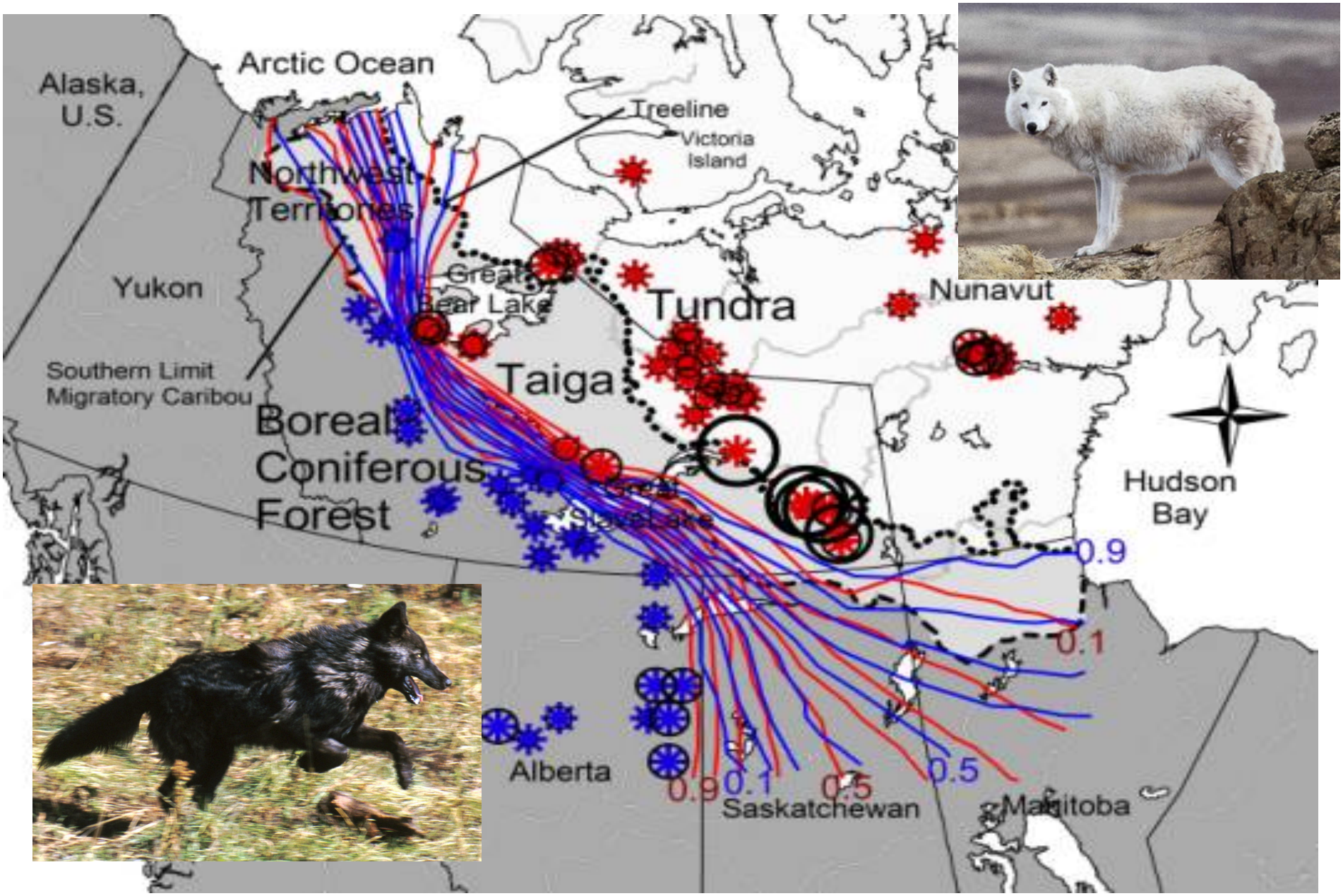
Questions

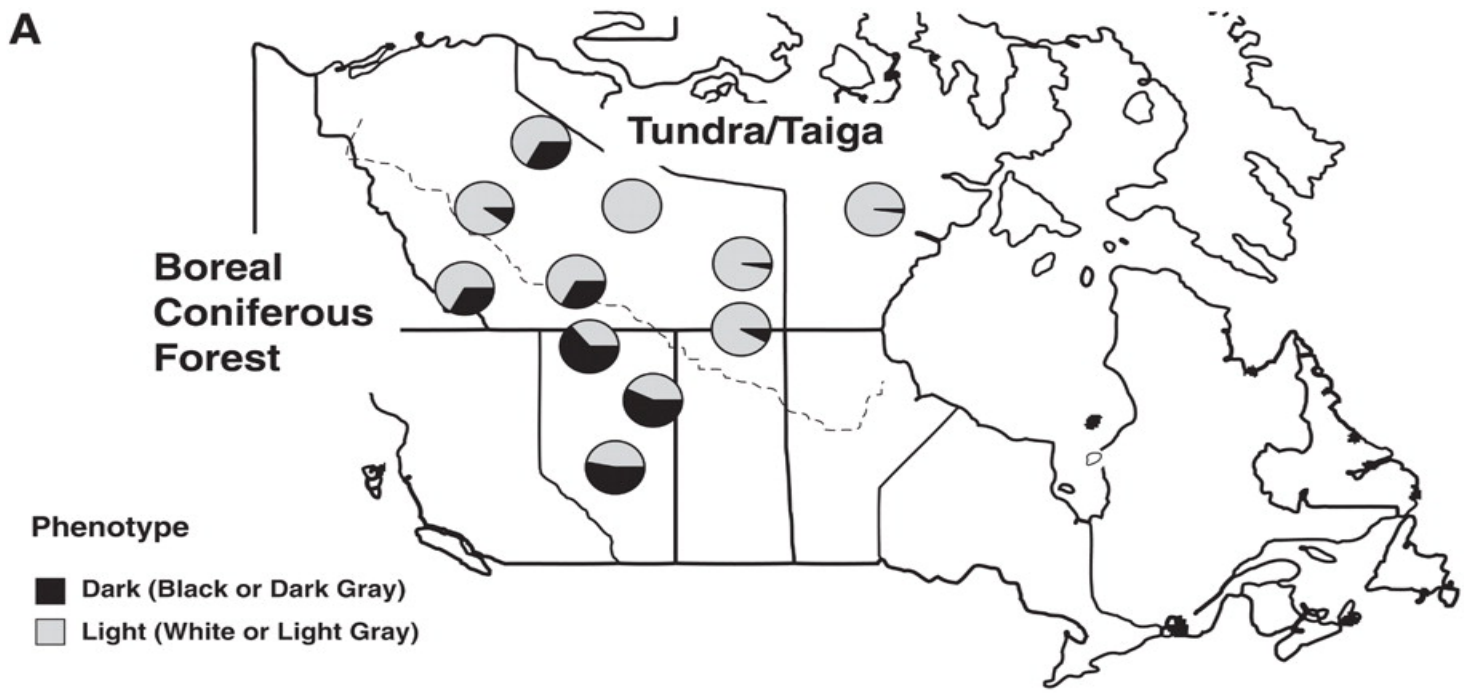
1) The primary reason body size differs among dogs breeds is:
   a. A single gene of large effect
   b. Multiple genes of small effect
   c. Nutritional differences
   d. Exercise differences
   e. None of the above

2) Unlike other domesticated species, dogs phenotypes are determined by
   a. Multiple genes of small effect
   b. A few genes of large effect
   c. One master gene
   d. Transposable elements

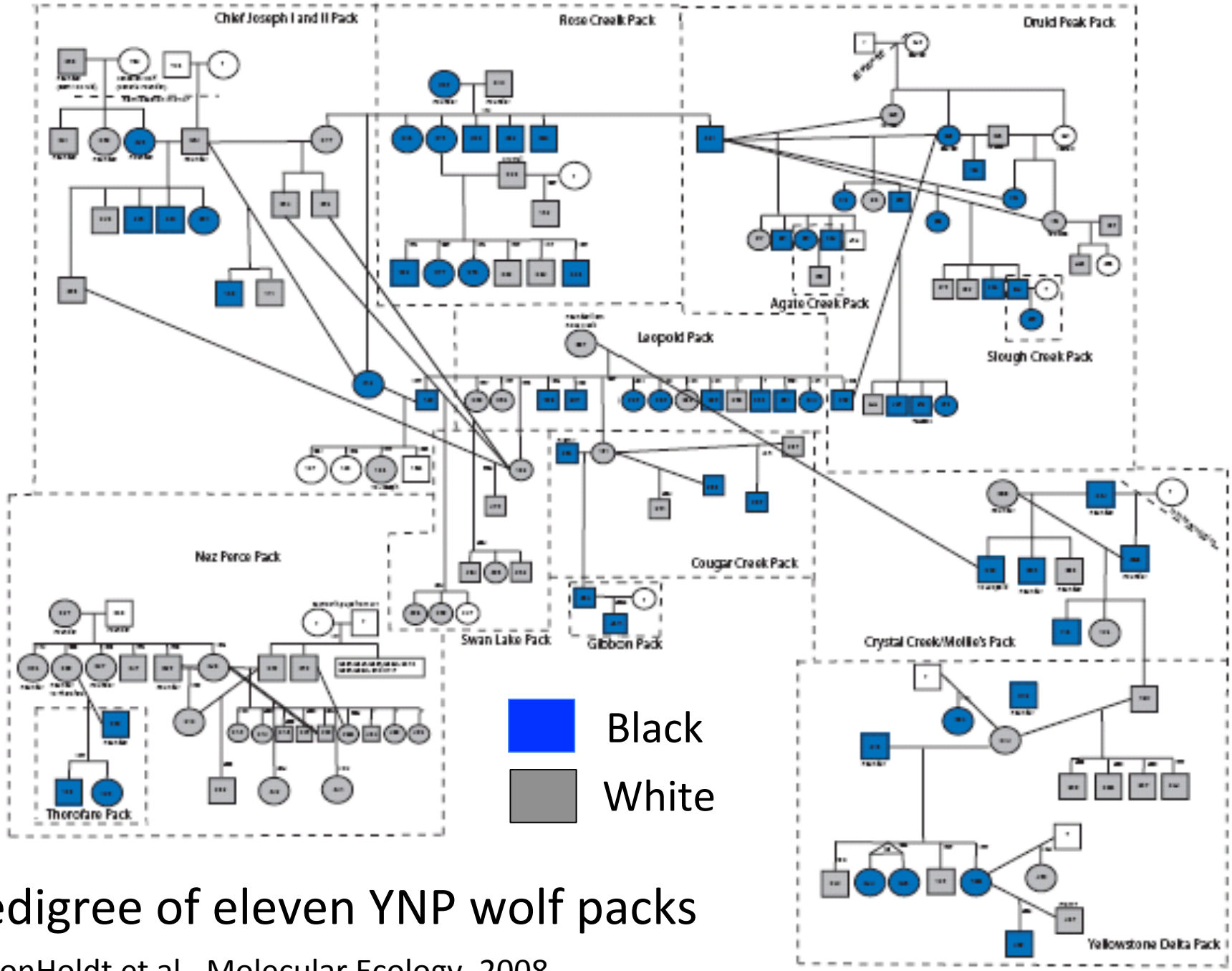
3) Diversity in dogs is limited in part by:
   a. Human creativity
   b. Expense of breeding
   c. Longevity
   d. The nature of mutations

3. Engineering of the wild: Black and Gray coat colors of wolves




A
Tundra/Taiga
Boreal
Coniferous
Forest
Phenotype
Dark (Black or Dark Gray)
Light (White or Light Gray)


B
10 mo
10 yrs
10 mo
10 yrs



Pedigree of eleven YNP wolf packs

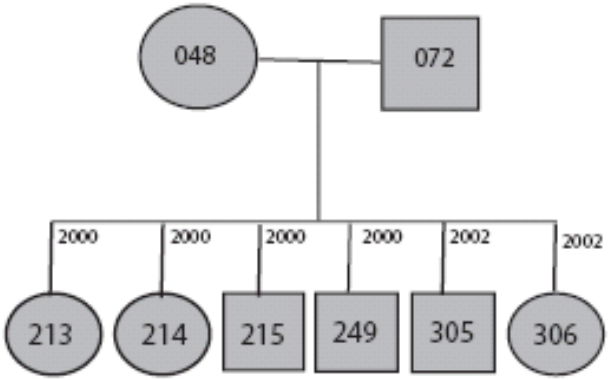
vonHoldt et al., Molecular Ecology, 2008

Coat Color and Dominant Inheritance

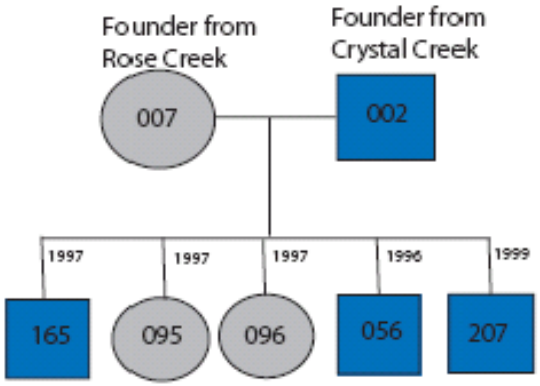
Model	Grand mu	1-1	1-2	2-2	Scale	Freq1	Freq2	LOD	Free Par.	SIC
Sporadic	1.336	----	----	----	0.6301	0.5	0.5	-233.789	5	471.1795
Genotypic	-0.7631	1.658	2.731	-4.389	0.2924	0.8038	0.1962	-199.246	5	402.0933
Dominant	1.426	-0.5353	0.5353		0.2921	0.7871	0.2129	-193.736	4	390.9771
Recessive	2.047	0.5353		-0.5353	0.2921	0.2129	0.7871	-193.736	4	390.9771
Allelic	1.336	0.5	----	0.5	0.6031	0.5	0.5	-411.234	4	825.9739

- Examples to support dominant inheritance

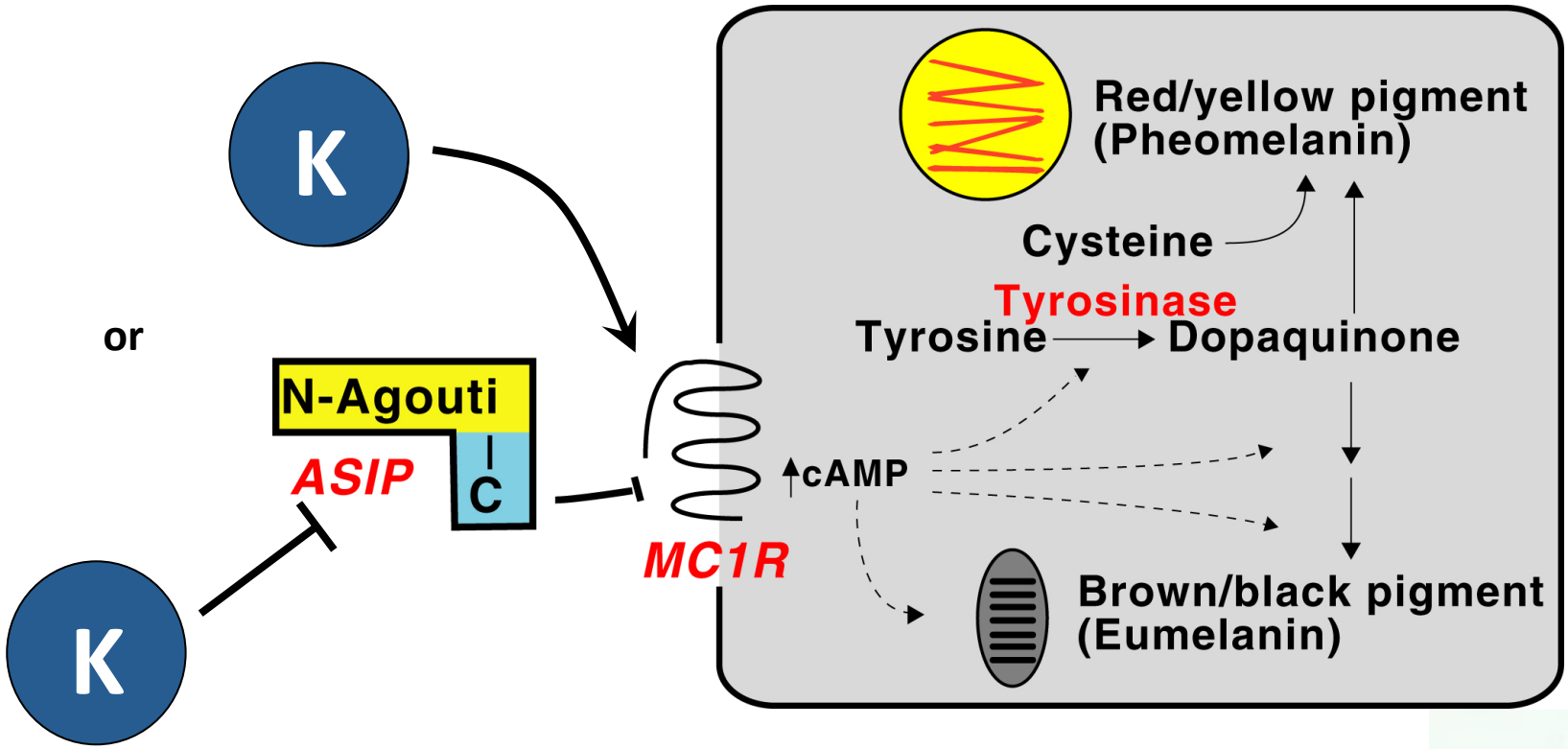
Nez Perce Pack



Leopold Pack



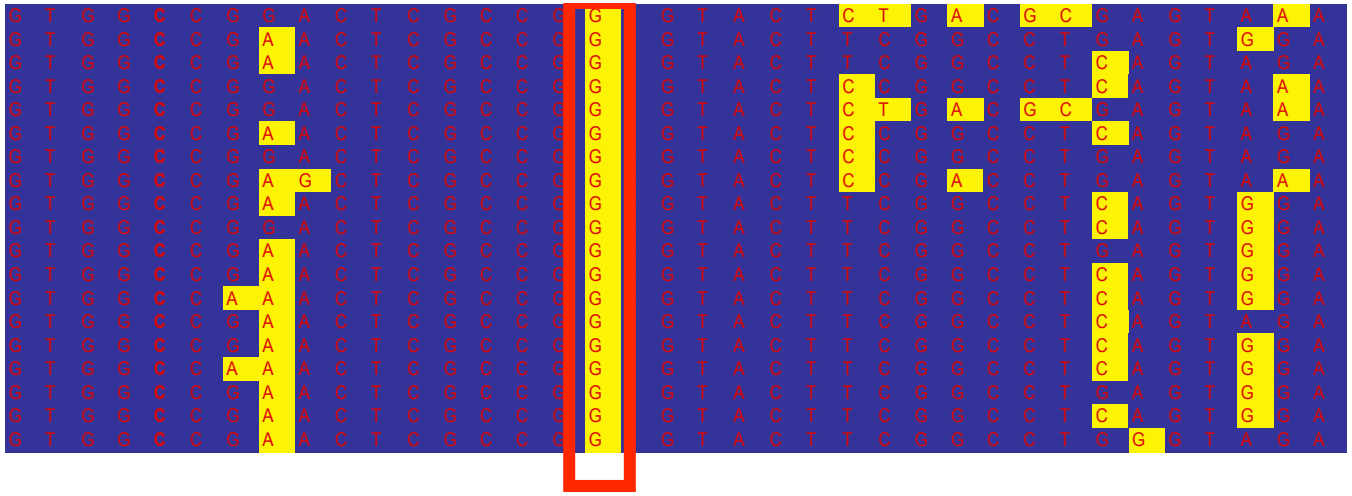
The K locus encodes a secreted protein that affects the Agouti-Mc1r system



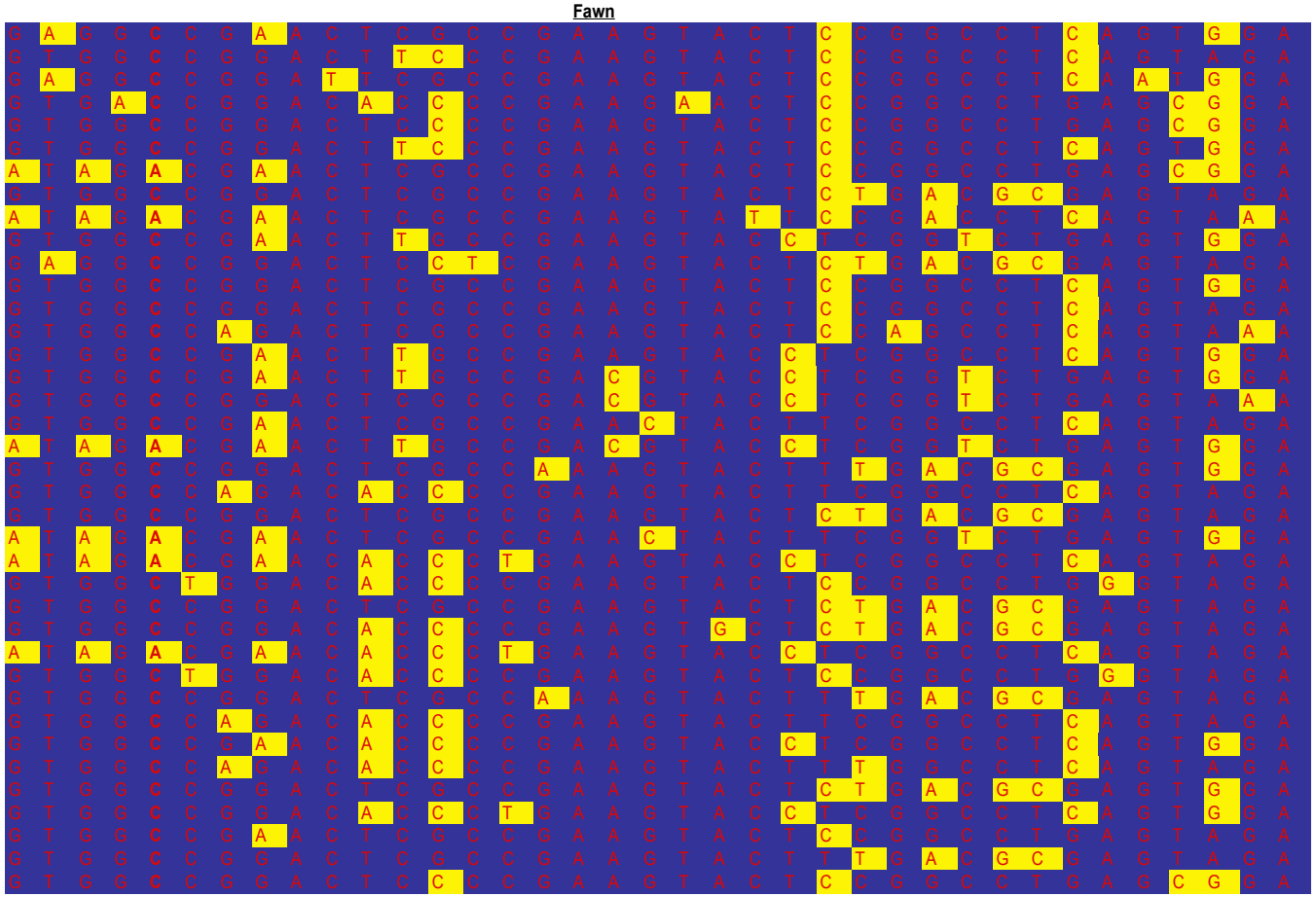
In a sample of 24 gray and 28 black wolves, there was perfect association of the K allele with black color.

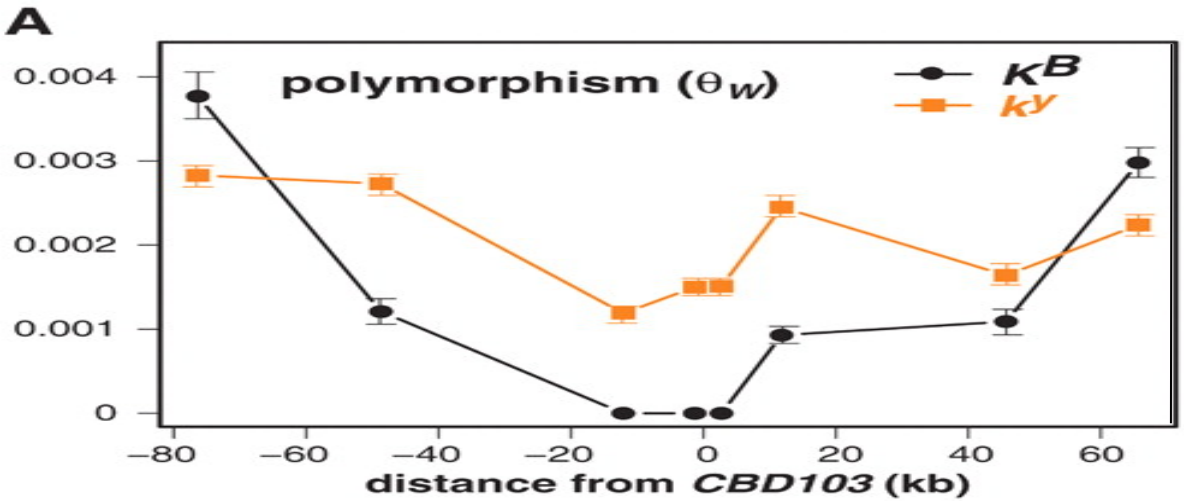
Anderson et al., Science, 2009

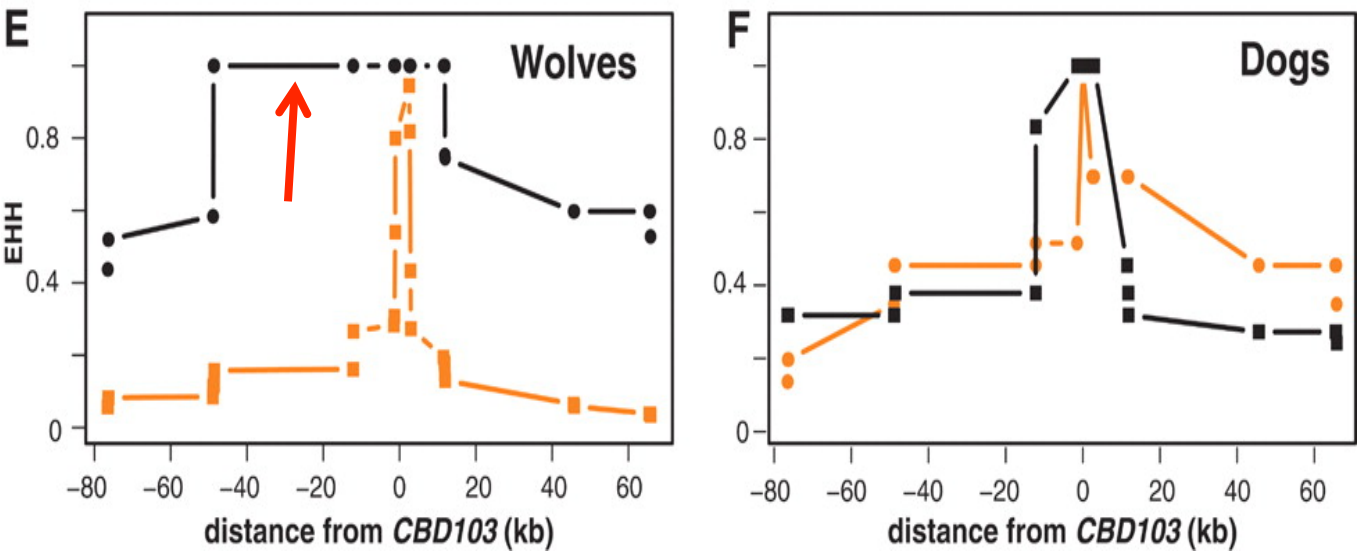
Tovi Anderson



SNP variation near the K locus evidence for selective sweep

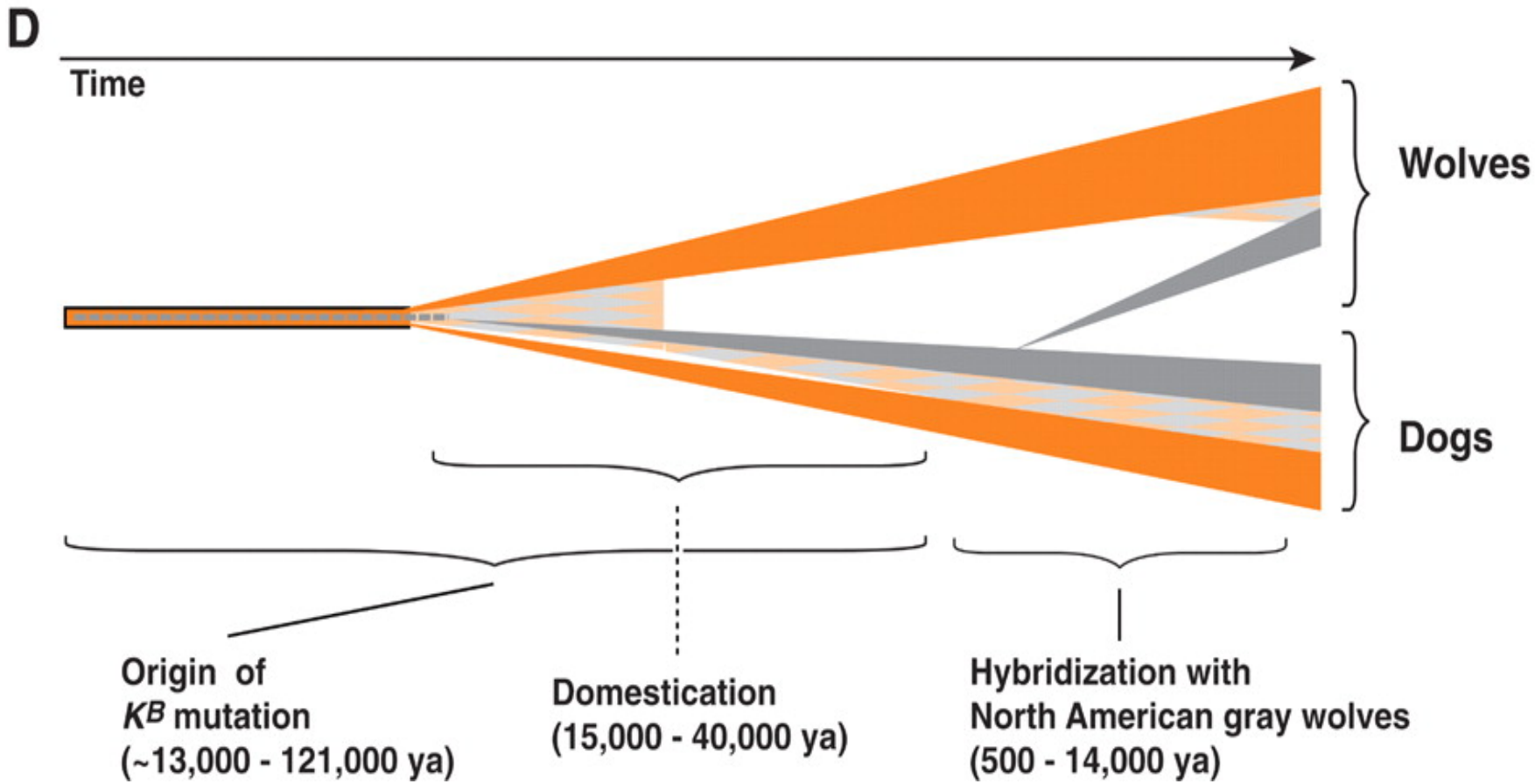






Anderson et al., 2009, Science

Dogs have enriched the genetic heritage of wolves



Genetic variants that are selected under domestication can transform wild progenitor populations

CONCLUSIONS

1) The engineering of the dogs has involved a relatively small number of genes of large effect.

2) These genetic variants have been transferred to established breeds by crossing. The results is the expression on different genetic backgrounds and enhanced phenotypic diversity.

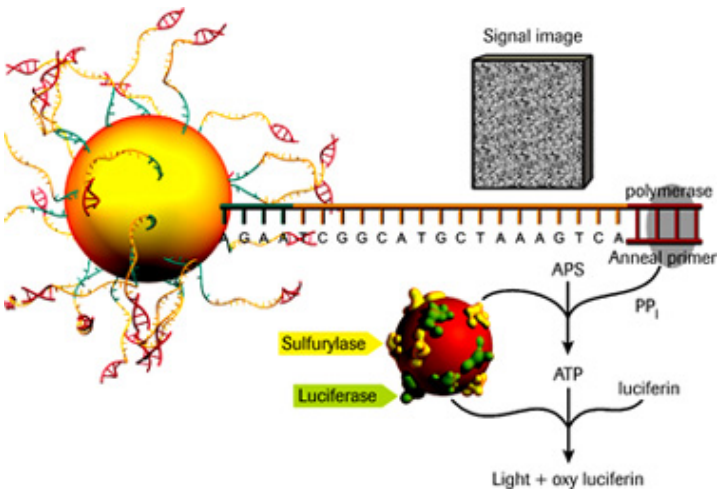
3) Mutation, as well as goal driven selection, limits diversity in dogs.

4. Variants selected under domestication can be transferred to wild kin.

4. The future, Genomic and expression studies

Mass sequencing technology has become a practical tool

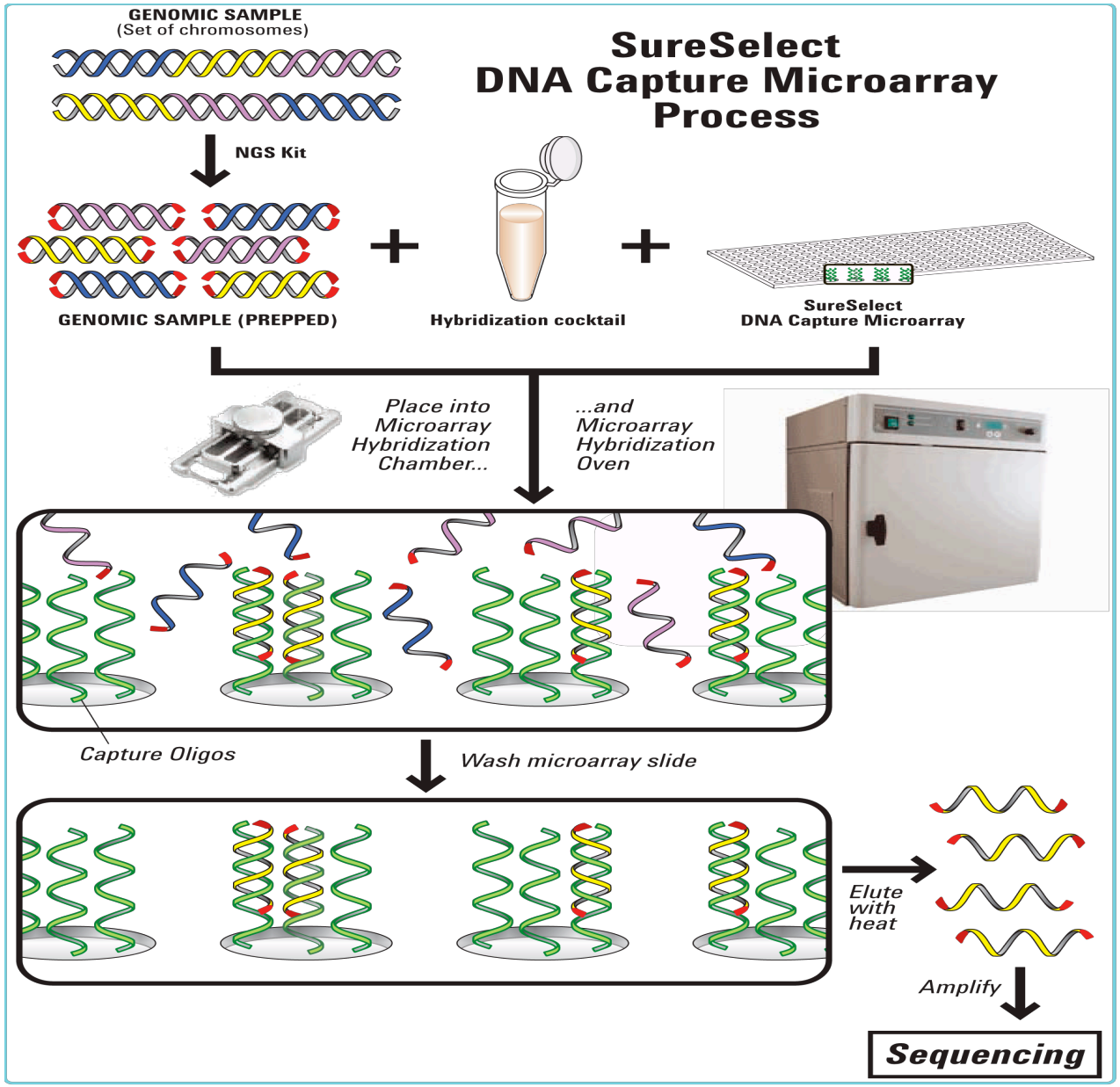
Roche GS FLX 454 Genome Sequencer



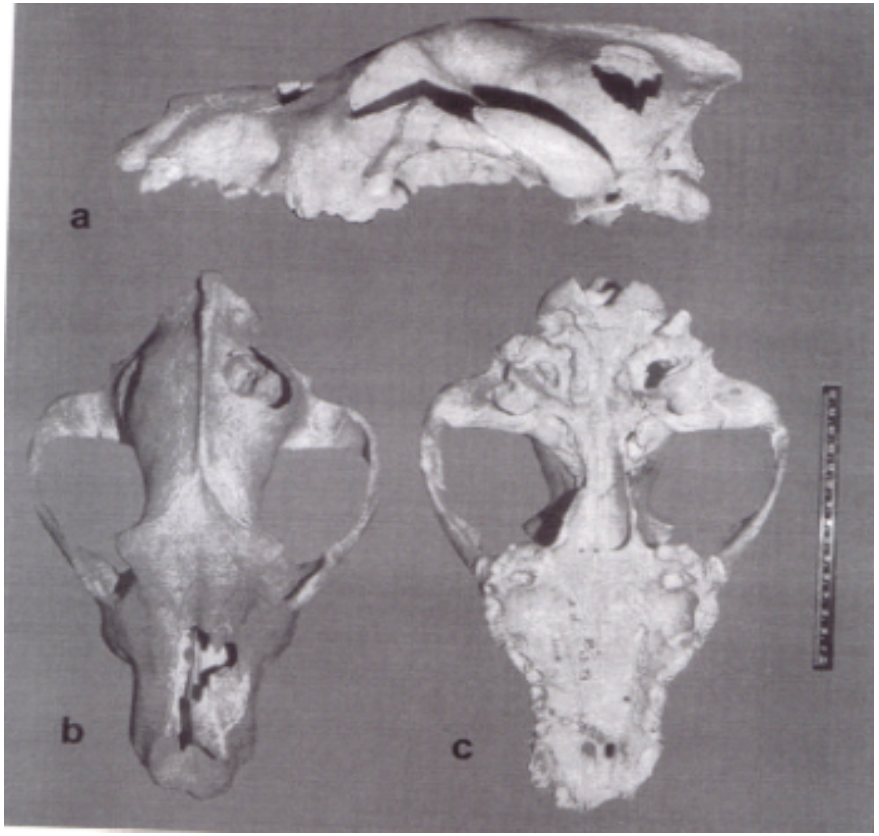
Sequencing reaction of the Genome Sequencer System. Millions of copies of a single clonal fragment are contained on each DNA Capture Bead.

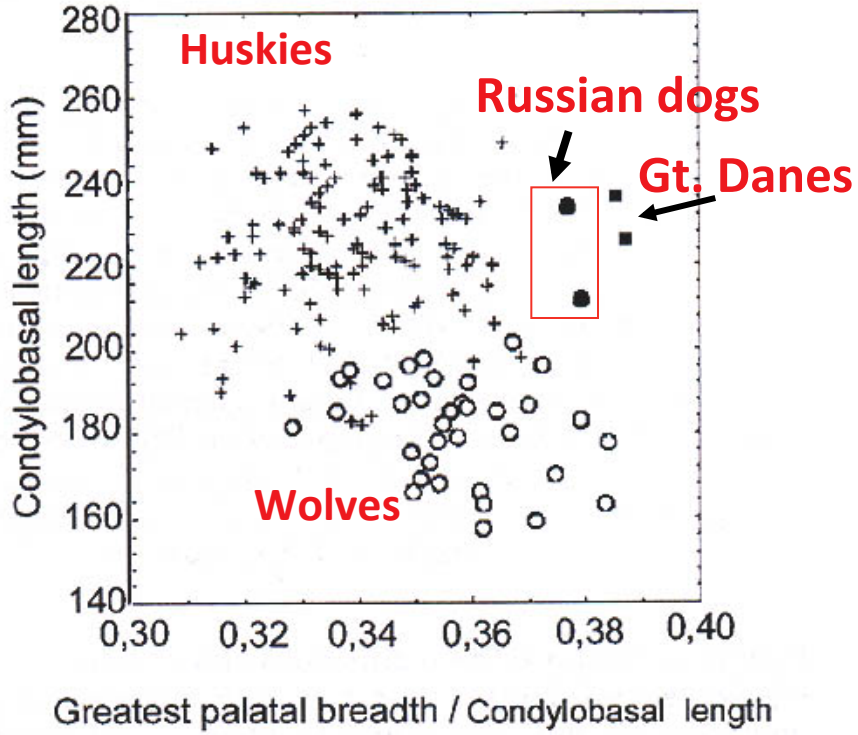
- Pyrosequencing technology
- Clonal amplification of starting material
- **500 million bp per run (1 million reads at average 400-500bp length)**
- 10 hour run
- Ability to run 2, 4, 8 & 16 separate lanes (physical separation)
- 10bp tags built into 454 adaptors allow further sample pooling (160 unique tags available)
- Cost ~$15/1000 reads

Population Genomics



The Oldest dogs: Sabin and Khlopachev, 2002





4. future studies

Domestication of the red fox

THE FUTURE OF DOG EVOLUTION