What's in a Mutt? An Intro to Dog DNA Analysis

> EN 601.147.13 Intersession 2019

Class Logistics

- Syllabus, assignments, and lectures in Piazza resources
- Attendance via sign in sheet; make sure you sign in each class!
- Class will be part lectures and part a data analysis project
- For the project you'll need:
 - A laptop with a Linux or Unix terminal/shell (Windows users can use a Virtual Machine)
 - A partner! Find a partner by Friday, or let me know if you'd prefer I assign you one

A bit about me

4th year graduate student in CS working on human genomics

Volunteer at the MD SPCA





Earliest documented genomic selection, 30k years ago!







Dog breeds, via Google Images

What even is a breed?

Over 300 dog breeds around the world

192 breeds are recognized by the American Kennel Club (AKC)

From the AKC:

"The simplest way to define a breed is to say it always "breeds true." That is, breeding a purebred Irish Setter to another purebred Irish Setter will always produce dogs instantly recognizable as Irish Setters."

AKC provides breed "standards" of recognizable, defining features for breeds



AKC registered Doberman Pinschers

"Ears normally cropped and carried erect. The upper attachment of the ear, when held erect, is on a level with the top of the skull."

"Tail docked at approximately second joint, appears to be a continuation of the spine."

AKC breed standard for a Doberman Pinscher



Do these dogs meet the AKC Doberman breed standard? Do dogs who do meet the standard (cropped ears, docked tail) breed true?

Defining breeds genetically instead of visually







Cladogram of 161 breeds. Parker et al , Cell Reports, 2017.

Why care about breed (or why not)?

Why care about breed (or why not)?

- Curiosity!
- Breed-specific medical concerns
 - Greyhounds have an extreme sensitivity to propofol, an anesthesia drug
 - **Herding breeds** more likely to have a mutation causing a Heartworm preventative drug, **ivermectin** to not metabolize and **build up to toxic levels**
 - Boxers more likely to have an adverse reaction to the sedative acepromazine
 - Likelihood of tumors being benign vs malignant correlates with breed
- Breed-specific legislation
- Shelter adoptions

Shelter Adoptions and Breed

Study of 120 shelter dogs (Olson *et al*, 2015):

"Whereas DNA breed signatures identified only 25 dogs (21%) as pit bull-type, shelter staff collectively identified 62 (52%) dogs as pit bull-type."

If shelters typically over identify dogs as pit bulls, might DNA testing increase adoption rates? Study labeling pairs of similar looking dogs as pit bull vs other breed labels (Gunter, Barber, and Wynne, 2016):



But what about mixed breeds?

- How easily can we look at a mutt and guess the breeds?
- When does a mix become its own breed?
 - What are Labradoodles? Yorkipoos? "Cross-breeds" as per the AKC
 - If two mutts of the same makeup breed true, is it a new breed?
 - How might you characterize whether a mix is or isn't a new breed genetically?
- Is knowing the breed makeup medically informative?



Commercial dog DNA sequencing



Commercial dog DNA sequencing

Breed makeup

Medical markers

Breed-specific kits

All commercial testing is currently unregulated

Read Atlantic article for discussion Wednesday

SCIENCE What Vets Think of '23andMe for Dogs'

More and more companies are selling DNA-test kits for pets.

SARAH ZHANG NOV 12, 2018



Sequencing for Research: Mutt Mix Project



Pup quiz! PollEv.com/rachelsherma442

Bella



PollEv.com/rachelsherma442



Bella's shoulders are knee high when standing next to a person of average height.



Other physical traits or features:

Double coat.

What is Bella's top breed?

Australian Cattle Dog	A
Australian Shepherd	B
Basenii	C
Basset Hound	D
Beagle	Ē
Bornoro Mountain Dog	E
Dishas Files	c
Bichon Frise	5
Bloodhound	P
Boston lerner	1
Boxer	7
Bull Terrier	K
Buildog	L.
Cavalier King Charles Spaniel	м
Chihuahua	N
Chow Chow	0
Cocker Spaniel	P
Collie/Border Collie	Q
Corgi	R
Dachshund	S
Dalmation	т
Doberman Pinscher	U
English Springer Spaniel	v
English Springer Spanler	W
Garman Shanhard	x
Gelden Betreiver	v
Golden Redeiver	7
Great Dane	20
Great Pyrenees	AA
Greyhound	AB
Havanese	AC
Irish Setter	AD
Irish Wolfhound	AE
Italian Greyhound	AF
Jack Russell Terrier	AG
Labrador Retriever	AH
Lhaso Apso	AI
Maltese	AJ
Mastiff	AK
Newfoundland	AL
Old English Sheepdog	AM
Pomeranian	AN
Poodle	AO
Pur	ΔP
Pothusilor	40
Rottweiter	AD
Saint Bernard	AC
Shiba Inu	AS
Shih Tzu	AI
Siberian Husky	AU
Staffordshire Bull Terrier (Pit bull)	AV
West Highland Terrier	AW
Yorkshire Terrier	AX
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What is Bella's second best breed?

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B Basset H B Bernese Mountai Bichor Bloodt Boston [*] Built Built Built Built Built Cavalier King Charles S Collie/Border! Dach Collie/Border! Dach Darh Doberman Pis Collie/Border! Dach Darh Doberman Pis Collie/Border! Dach Darh Dach Basset Collie/Border! Dach Dach Darh Dach Darh Dach Dach Darh Collie/Border! Dach Darh Dach Collie/Border! Dach Dach Dach Dach Dach Dach Collie/Border! Dach Collie/Border! Dach Basset Golden Ret Golden Ret Golden Ret Great Dach Coreat Coreat Coreat Dach Coreat Coreat Dach Coreat Coreat Dach Coreat Coreat Dach Coreat Coreat Dach Coreat Dach Coreat Dach Coreat Dach Coreat	Australian Shepherd
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Staffordshire Bull Terrier (Pit West Highland T	Siberian Husky
West Highland 1	Staffordshire Bull Terrier (Pit bull)
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rorkshile i	Yorkshire Terrier



32% of participants guessed at least one breed correctly, 2% guessed 2 breeds correctly, and 1 participant could guess all three breeds.

Rosie

PollEv.com/rachelsherma442





Rosie's shoulders are knee high when standing next to a person of average height.



Other physical traits or features:

Stripe of wavy fur down center of back. Black spots on tongue.



Australian Cattle Dog
Australian Shenherd
Rasenii
Passet Hound
Basset Hound
Bornoro Mountain Dor
Demese Mountain Dog
Bichon Prise
Brodunia Rester Terrier
Boston lener
Boxer D. II Train
Buil lemer
Buildog
Cavalier King Charles Spaniel
Chihuahua
Chow Chow
Cocker Spaniel
Collie/BorderCollie
Corgi
Dachshund
Dalmation
Doberman Pinscher
English Springer Spaniel
French Bulldog
German Shepherd
Golden Retreiver
Great Dane
Great Pyrenees
Greyhound
Havanese
Irish Setter
Irish Wolfbound
Italian Groubound
Instantion oreyhound
Jack Russell lemer
Labrador Retriever
Lhaso Apso
Maltese
Mastiff
Newfoundland
Old English Sheepdog
Pomeranian
Poodle
Pug
Rottweiler
Saint Bernard
Shiba Inu
Shih Tzu
Siberian Husky
Staffordshire Bull Terrier (Pit bull)
West Highland Terrier
Yorkshire Terrier



What is Rosie's second best breed?

Australian Cattle Dog
Australian Shepherd
Basenji
Basset Hound
Beagle
Bernese Mountain Dog
Bichon Frise
Bloodhound
Boston Terrier
Boxer
Bull Terrier
Bulldog
Cavalier King Charles Spaniel
Chihuahua
Chow Chow
Cocker Spaniel
Collie/Border Collie
Corei
Dachshund
Dalmation
Doberman Pinscher
English Springer Spanial
English Springer Spanier
Garman Shonhard
Golden Retreiver
Great Dana
Great Dane
Great Pyrenees
Greyhound
Havanese
Irish Setter
Irish Wolfhound
Italian Greyhound
Jack Russell Terrier
Labrador Retriever
Lhaso Apso
Maltese
Mastiff
Newfoundland
Old English Sheepdog
Pomeranian
Poodle
Pug
Rottweiler
Saint Bernard
Shiba Inu
Shih Tzu
Siberian Husky
Stafford shire Bull Terrier (Pit bull)
West Highland Terrier
Yorkshire Terrier



45% of participants guessed at least one breed correctly, 6% guessed 2 breeds correctly, and 2 participants could guess all three breeds.

Boone

PollEv.com/rachelsherma442





Boone's shoulders are knee high when standing next to a person of average height.



What is Boone's top breed?

Australian Cattle Dog Great Pyrenees Greyhound Havanese Irish Setter Irish Wolfhound Italian Greyhound Jack Russell Terrier Labrador Retriever Lhaso Apso Maltese Mastiff Newfoundland Old English Sheepdog Pomeranian Poodle Pug Rottweiler Saint Bernard Shiba Inu Shih Tzu Siberian Husky Staffordshire Bull Terrier (Pit bull) West Highland Terrier Yorkshire Terrier Australian Shepherd Basenji Basset Hound Beagle Bernese Mountain Dog **Bichon Frise** Bloodhound **Boston Terrier** Boxer Bull Terrier Buildog Cavalier King Charles Spaniel Chihuahua Chow Chow Cocker Spaniel Collie/Border Collie Corgi Dachshund Dalmation Doberman Pinscher English Springer Spaniel French Bulldog German Shepherd Golden Retreiver Great Dane

What is Boone's second best breed?

Australian Shepha Base Baset Hou Beinese Mountain C Bichon Fr Bichon Fr Bichon Fr Bichon Fr Bichon Fr Builter Builter Cavalier King Charles Span Chihuah Cavalier King Charles Span Chihuah Cocker Span Collie/Border Col Cocker Span Collie/Border Col Cocker Span Cocker Spa	Australian Cattle Dog
Bass Basset Hou Beag Bernser Mountain D Bichon Fr Bicoon Ten Built Terre Built Cavalier King Charles Span Chihuah Chih	Australian Shepherd
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Bernese Mountain C Bichon Fr Biochou Botton Ten Bot Cavalier King Charles Span Cohinah Cavalier King Charles Span Cohinah Coker Span Colie/Border Coi Coc Dachshu Dalmat Doberman Pinsci English Springer Span French Bull German Shephe Golden Retreis Greyhou Havane Irish Set Greyhou Havane Irish Stet Irish Wolftou Jack Russell Ter Labrador Retries Labrador Retries Labrador Retries Chaso Ap Matit Shiba I Shiba I Shiba I Shiba I Shiba I Shiba I	Beagle
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Jack Russell Ten Labrador Retrie Lhaso Ay Maite Mas Oid Engish Sheepd Oid Engish Sheepd Oid Engish Sheepd Pomeran Pomeran Pomeran Pomeran Pomeran Shiha I Sibelia Shih Sibelia Hus Staffordshire Bull Terrier (Pithan Hus Staffordshire Bull Terrier (Pithan Terrier West Highland Terr	Italian Greyhound
Labrador Retrie Lhaso Ar Mas Mas Old English Sheepd Old English Sheepd Porodo P Porodo Saint Berna Shiba I Shiba I Shi	Jack Russell Terrier
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Staffordshire Bull Terrier (Pit bu West Highland Terri Vorkshire Torr	Sibarian Humby
West Highland Terr	Staffordshire Bull Terrier (Pit bull)
Verkehire Terr	West Lighland Torrier
TORKShille Tell	Yorkshire Terrier



78% of participants guessed at least one breed correctly, 11% guessed 2 breeds correctly, and 11 participants could guess all three breeds.

Maxine





Maxine's shoulders are thigh high when standing next to a person of average height.



Other physical traits or features:

Hair not fur

PollEv.com/rachelsherma442

What is Maxine's top breed?

Australian Cattle Dog Great Pyrenees Greyhound Havanese Irish Setter Irish Wolfhound Italian Greyhound Jack Russell Terrier Labrador Retriever Lhaso Apso Maltese Mastiff Newfoundland Old English Sheepdog Pomeranian Poodle Pug Rottweiler Saint Bernard Shiba Inu Shih Tzu Siberian Husky Staffordshire Bull Terrier (Pit bull) West Highland Terrier Yorkshire Terrier Australian Shepherd Basenji Basset Hound Beagle Bernese Mountain Dog **Bichon Frise** Bloodhound **Boston Terrier** Boxer Bull Terrier Buildog Cavalier King Charles Spaniel Chihuahua Chow Chow Cocker Spaniel Collie/Border Collie Corgi Dachshund Dalmation Doberman Pinscher English Springer Spaniel French Bulldog German Shepherd Golden Retreiver Great Dane



What is Maxine's second best breed?

Australian Cattle Dog
Australian Shepherd
Basenji
Basset Hound
Beagle
Bernese Mountain Dog
Bichon Frise
Bloodhound
Boston Terrier
Boxer
Bull Terrier
Bulldog
Cavalier King Charles Spaniel
Chihuahua
Chow Chow
Cocker Spaniel
Collie/Border Collie
Corgi
Dachshund
Dalmation
Doberman Pinscher
English Springer Spaniel
French Bulldog
German Shepherd
Golden Retreiver
Great Dane
Great Pyrenees
Greyhound
Havanese
Irish Setter
Irish Wolfbound
Italian Greybound
Jack Russell Terrier
Labrador Retriever
I haso Aneo
Linaso Apso
Maltese
Mastin
Newfoundland
Old English Sheepdog
Pomeranian
Poodle
Pug
Rottweiler
Saint Bernard
Shiba Inu
Shih Tzu
Siberian Husky
Stattordshire Bull Terrier (Pit bull)
West Highland Terrier
Yorkshire Terrier



29% of participants guessed at least one breed correctly, 6% guessed 2 breeds correctly, and 39 participants could guess all three breeds.

Mutt Mix Project Results

- 26,639 participants and 432,743 guesses for 31 dogs
- Number of breeds detected doesn't correlate with guessing accuracy
 - Four of the mutts had over 19 breeds detected but two of those weren't as tough to guess as some of the dogs with far fewer breeds
- Average of 25% of guesses correct, 28% for the 4,804 participants in dog professional fields
- Most guessed breeds: Labrador Retriever, German Shepherd Dog, Golden Retriever, Beagle, and Border Collie
- Most commonly detected breeds: Chow Chow, German Shepherd Dog, Staffordshire Bull Terrier, Golden Retriever, and Labrador Retriever

Meet Our Mutts



Reilly

Clarence

Finch
Take a break and then we'll talk about how we sequence DNA!

Meet Tasha!

Tasha is a boxer and she's the basis of the dog reference genome, released in 2005

- 39 chromosomes
- 2.8 billion base pairs (A, C, G, T)
- 19,000 genes (approximately)

A *reference genome* is a genome we have put together to be representative of a species. We'll talk more about their creation later.



DNA



Dog reference genome



Human K B E 9 20 61 20 21 X 22 なえる 37 Х 36 38

(33.9 Mb)

(26.5 Mb)

(126.9 Mb)

Genotypes and Genomes



The "genome" is all DNA on all chromosomes for an individual

Actually pairs of chromosomes, so dogs have 78 total; 76 autosomes and 2 sex chromosomes

At each base pair (2.8 billion of them in dogs), we have a *genotype* of the two bases present

Genotypes and Genomes

Genotype is a description of both copies, the possibilities for each copy are *alleles*

Interpretations are likelihoods; often the *phenotype* is affected by other factors

C			
n of	Who	Genotype	What It Means
ilities s		AA	In Europeans, 85% chance of brown eyes; 14% chance of green eyes; 1% chance of blue eyes.
		AG	In Europeans, 56% chance of brown eyes; 37% chance of green eyes; 7% chance of blue eyes.
y	Benjamin Langmead	GG	In Europeans, 72% chance of blue eyes; 27% chance of green eyes; 1% chance of brown eyes.
		\ Sc	urces: 23andme.com, Ben's genome
G From mother	G From father	A and	d G are <i>alleles</i>
Chromosom	e		Slide from Ben Langr

Tasha ... ATCGGAATAGCGAGTA ... dog of interest (one copy) ATGGGAATAGCTAGTA

But SNPs don't occur this often - on average, we see 1 SNP every ~1,000 bases between dogs.

Tasha ... ATCGGAATAGCGAGTA...

dog of interest (two copies)

ATGGGAATAGCTAGTA ATGGGAATAGCGAGTA

TashaATCGGAATAGCGAGTA...dog of interest
(two copies)GT
GGGG

When we look at SNPs, especially commercially, we use SNP chips aka genotyping arrays. These **only** look at predetermined sites.

Tasha ... ATCGGAATAGCGAGTA... dog of interest (two copies) G

So if we don't look at a site with a SNP, we won't know it's there.



















Different nose color!







Dog breeds, via Google Images, all black noses!







We can look for associations between SNPs or patterns of SNPs and:

Breeds

Traits





First Two Traits Mapped With SNP Chip in Dogs. Two traits were mapped as a proof of principle test of the canine SNP chip. (a) White coat color in boxers was mapped to the gene MITF, a developmental gene involved in pigmentary and auditory disorders, using 10 white dogs and 9 pigmented dogs. (b) The dorsal hair ridge in the Rhodesian ridgeback breed was mapped to a duplication of 4 genes, including three fibroblast growth factor genes, using 12 ridged dogs and 9 ridgeless dogs

...

Dog:	1	2	3	4	5	•••
SNP1	AG	AA	GG	AA	AA	
SNP2	CC	CA	CC	CC	CC	
SNP3	AA	AA	TT	AA	AT	
SNP4	AT	TT	TT	ΤT	TT	
SNP5	GG	GG	GG	GG	GG	
SNP6	GG	CC	GG	GG	GG	

We'll only consider **biallelic** SNPs; this means there are 3 possibilities per SNP



Say we want to differentiate 100 dog breeds. How many SNPs do we need?

...

Dog:	1	2	3	4	5	•••
SNP1	AG	AA	GG	AA	AA	
SNP2	CC	CA	CC	CC	CC	
SNP3	AA	AA	TT	AA	AT	
SNP4	AT	TT	TT	TT	TT	
SNP5	GG	GG	GG	GG	GG	
SNP6	GG	CC	GG	GG	GG	

We'll only consider biallelic SNPs; this means there are 3 possibilities per SNP

AA, Aa, aa

Say we want to differentiate 100 dog breeds. How many SNPs do we need?

...

3 [×] ≥ 100	×	•••	5	4	3	2	1	Dog:
x = 5			AA	AA	GG	AA	AG	SNP1
X J	genotype		CC	CC	CC	CA	CC	SNP2
	possibilities		AT	AA	TT	AA	AA	SNP3
What's the			ΤT	TT	TT	TT	AT	SNP4
		i	GG	GG	GG	GG	GG	SNP5
oblem here?	рі	1	GG	GG	GG	CC	GG	SNP6

Say we want to differentiate 100 dog breeds. How many SNPs do we need?

...

Dog:	1	2	3	4	5	•••
SNP1	AG	AA	GG	AA	AA	
SNP2	CC	CA	CC	CC	CC	
SNP3	AA	AA	TT	AA	AT	
SNP4	AT	TT	ΤT	ΤT	TT	
SNP5	GG	GG	GG	GG	GG	
SNP6	GG	CC	GG	GG	GG	

This would require us to see every combo of possible SNP patterns!

Related breeds likely to have similar patterns/differ in fewer SNPs

A given SNP might only be informative for some (or no) breeds





2.5 million documented dog SNPs (~10% of the genome)

Data we'll be working with is 148,737 SNPs (~0.005% of the genome)

How were the ~150k sites chosen?

How do we actually look at (genotype) these SNP sites?

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SNP genotype: AA



SNP genotype: AA











AG








Illumina Bead SNP Array Recap



Before Wednesday

- Make sure you're on Piazza: <u>piazza.com/jhu/spring2019/en60114713</u>
- Fill out a (short!) survey on your background, and if this lecture was the right speed/level, posted on Piazza
- Read Atlantic article: *What Vets Think of '23andMe for Dogs'*
- Start looking for a partner!