

University of Veterinary Medicine Hannover

Institute for Animal Breeding & Genetics



## Longevity and histiocytic sarcomas

10th Bernese Mountain Dog Health Symposium  
Helsinki, Finland

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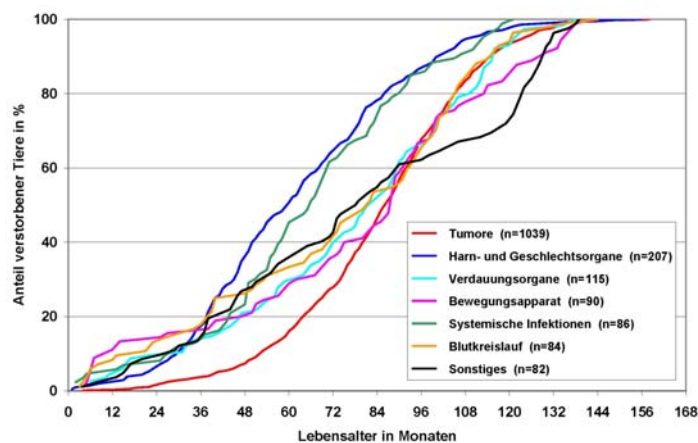
## Contents

- (1) **Data: phenotypes and genetic variants**
- (2) **Genomic breeding values**
- (3) **Inbreeding**
- (4) **Next Generation Sequences (NGS)**
- (5) **Validation study**
- (6) **Further developments**

## Data

- Phenotypes
- Heritability
- Breeding values
- Genomic data

## Longevity in Bernese Mountain Dogs



Hartmann et al. 2011

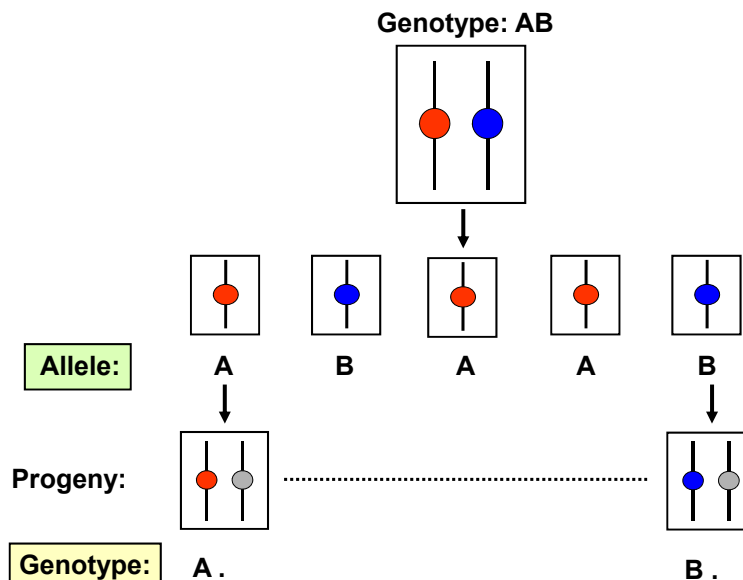
## Heritability for Longevity in Bernese Mountain Dogs

$h^2$ (1995-2007):	$0.318 \pm 0.038$
$h^2$ (1995-2012):	$0.294 \pm 0.035$

Data editing: SSV-Bernese Mountain Dogs  
Birth years: 1995-2007 and 1995-2012  
No. of animals: 8,390 und 10,987  
Pedigree data: 11,606 und 13,166  
Animal model for censored data

### Genotypes – Alleles

- Only alleles are transmitted to the progeny -



## Canine Illumina 170K High Density Bead Chip

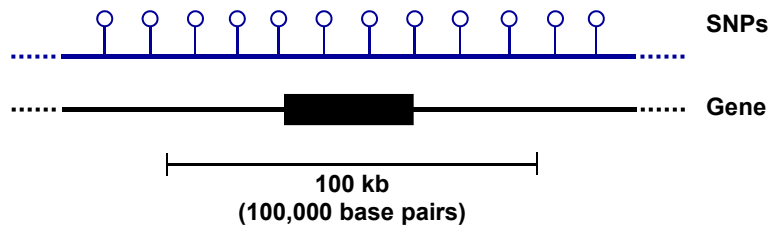


## Canine Illumina 170K High Density Bead Chip



- Use of >170,000 SNPs for calculation of Genomic Breeding Values
- Weighting of genetic variants according to their contribution to the trait
- Flexible tool for any genetic analysis
- No restriction to a specific trait
- Applicable for any trait with sufficient large reference samples
- Fast mapping of monogenic traits
- Extension to larger SNP sets through computational procedures well proven
- Incorporation of genetic variants without additional genotyping

## Genome-wide coverage through SNPs



No. of SNPs per 100 kb:	6.4
No. of genes per 100 kb:	~ 1

No. of SNPs per 1 Mb:	64
No. of genes per 1 Mb:	~ 10

## GWAS – Genome-Wide Association Study



Which genetic variants among a very dense set of SNPs determine the phenotype?



# GWAS - General analysis model

Target -Trait

Controls



## Genomic Breeding Values (GEBV) based on >170,000 SNPs



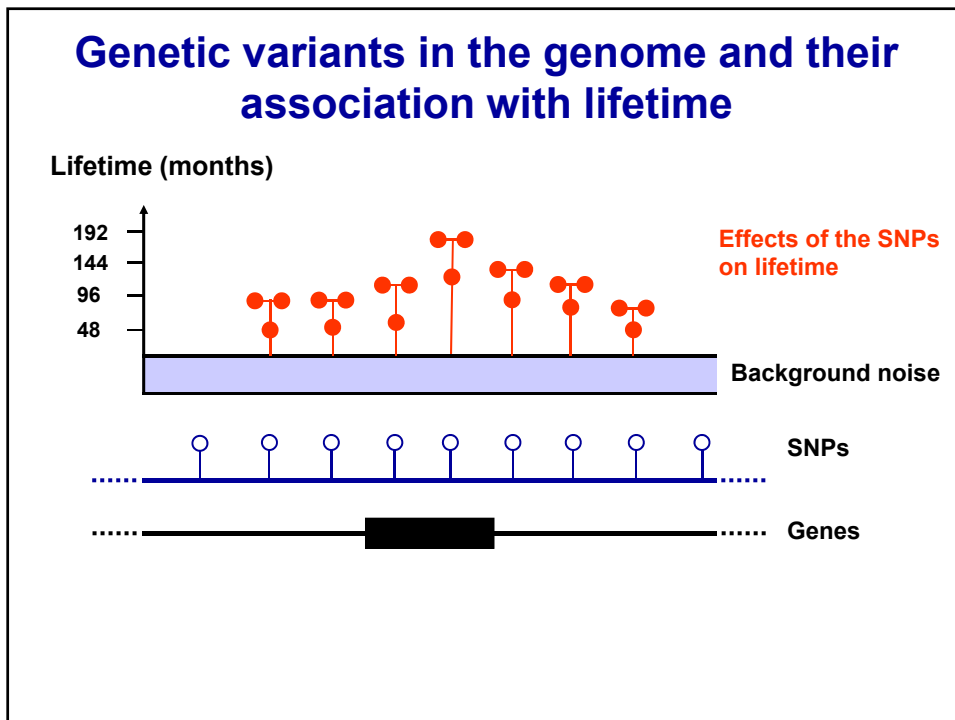
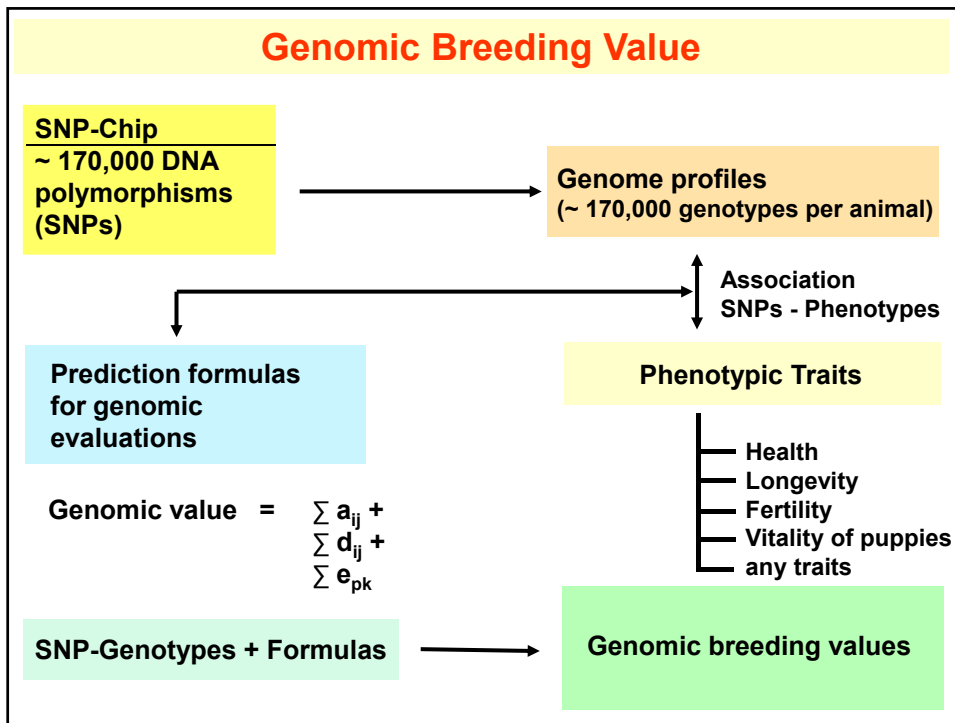
Phenotypes and genotypes



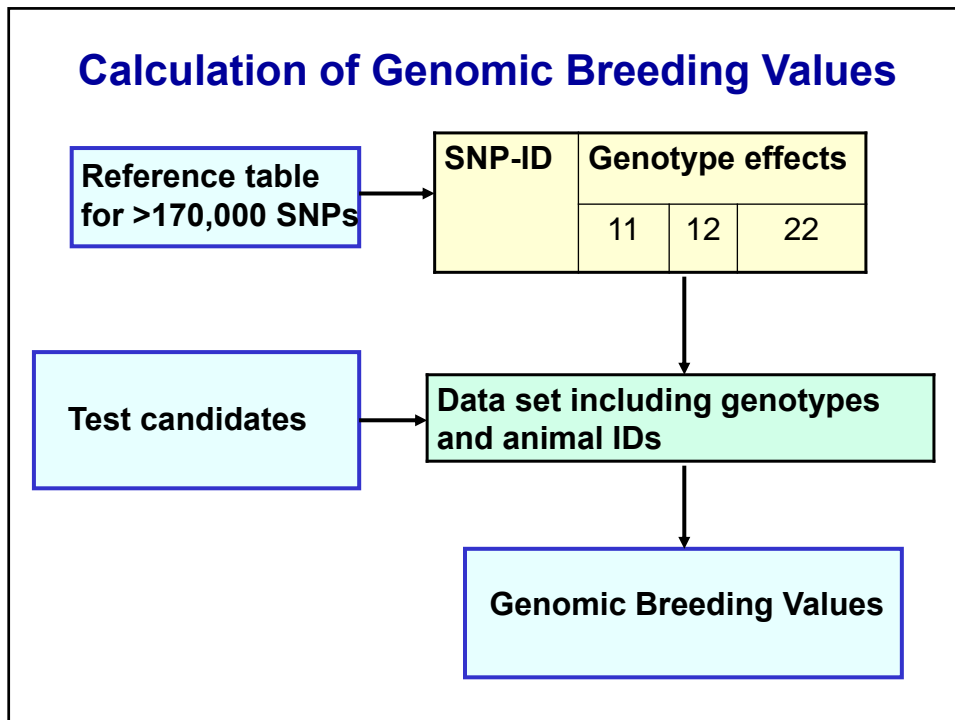
Genotypes

$$\text{GEBV} = \text{SNP-Allele}_1 * a_1 + \text{SNP-Allele}_2 * a_2 + \dots \text{SNP-Allele}_n * a_n$$

GEBV for test candidates



## Calculation of Genomic Breeding Values



## Reference scale of Genomic Breeding Values for Longevity ( $100 \pm 10$ )

Genomic BV	No. of Dogs	Expectation for length of lifetime		
		Category	Deviation	Years
75-80	5	Lower	- 70 ± 18	2.6
81-85	16	Lower	- 55 ± 18	4.0
86-90	36	Lower	- 34 ± 18	5.7
91-95	42	Lower	- 20 ± 18	6.8
<b>96-100</b>	<b>39</b>	<b>Mean</b>	<b>100 ± 18 months</b>	<b>8.3 years</b>
101-105	49	Higher	+ 20 ± 18	10.2
106-110	41	Higher	+ 34 ± 18	11.2
111-115	22	Higher	+ 44 ± 18	12.0
116-120	13	Higher	+ 54 ± 18	12.8
>121	3	Higher	+ 68 ± 18	14.0



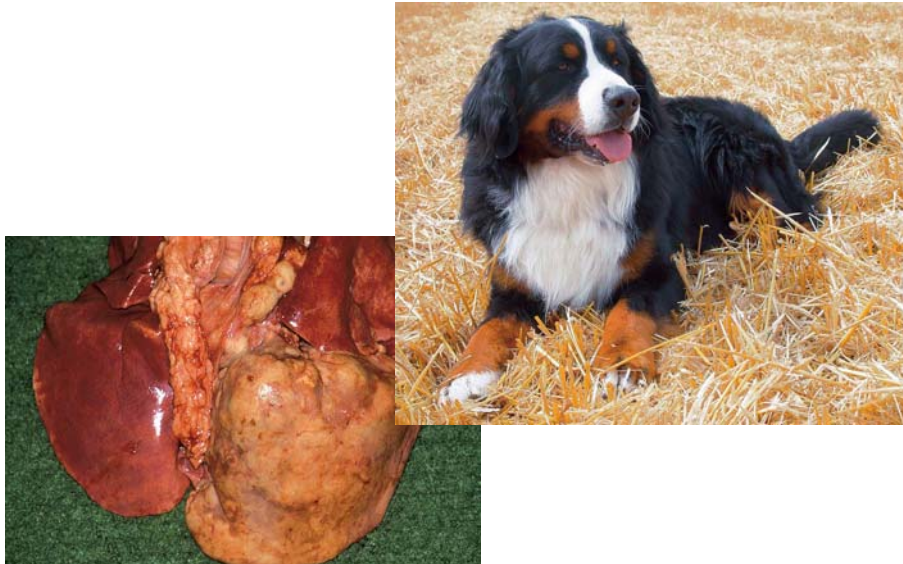
### Distribution of Genomic Breeding Values for hip dysplasia ( $85 \pm 10$ )

Genomic BV	No. of dogs	Hip dysplasia (%)				
		A	B	C	D	E
60-75	75	100				
76-80	60	100				
81-85	116	100				
86-90	100	100				
91-95	70	92	7	1		
96-105	38	76	21	3		
106-110	30	39	46	15		
111-120	49	3	31	66		
>120	61	0	16	61	13	10

### Distribution of Genomic Breeding Values for elbow dysplasia ( $85 \pm 10$ )

Genomic BV	No. of dogs	Elbow dysplasia (%)			
		Free	ED-I	ED-II	ED-III
60-75	90	100			
76-80	95	100			
81-85	114	100			
86-90	106	100			
91-95	68	97	3		
96-100	46	87	13		
101-105	33	64	36		
106-115	28	18	82		
116-140	23	17	44	30	9
>141	24	0	0	0	100

## Histiocytic Sarcoma (CHS)



### CHS-risk, genomic breeding values for CHS and longevity and observed length of lifetime in 730 Bernese Mountain Dogs

CHS-risk	No. of dogs	GEBV-CHS	GEBV-Longevity	Age (yrs) (n=269)
Very low	367	90	102	9.9
Low	246	104	97	7.1
Moderate	60	114	95	6.9
High	57	145	94	7.1

GEBV-CHS explain ~ 29% of the variance for longevity  
Correlation GEBV-CHS with GEBV-Longevity: 0.56

## Degenerative Myelopathy (DM)

- Mutations in the superoxiddismutase1 gene (SOD1)
  - ✓ Exon1 c.52 A>T, p.T18S  
Bernese Mountain Dog
  - ✓ Exon2 c.118G>A, p.E40K  
Several breeds, mixed breeds
- Inheritance: autosomale recessive
- Incomplete penetrance
- Degree of risk for BMDs undetermined

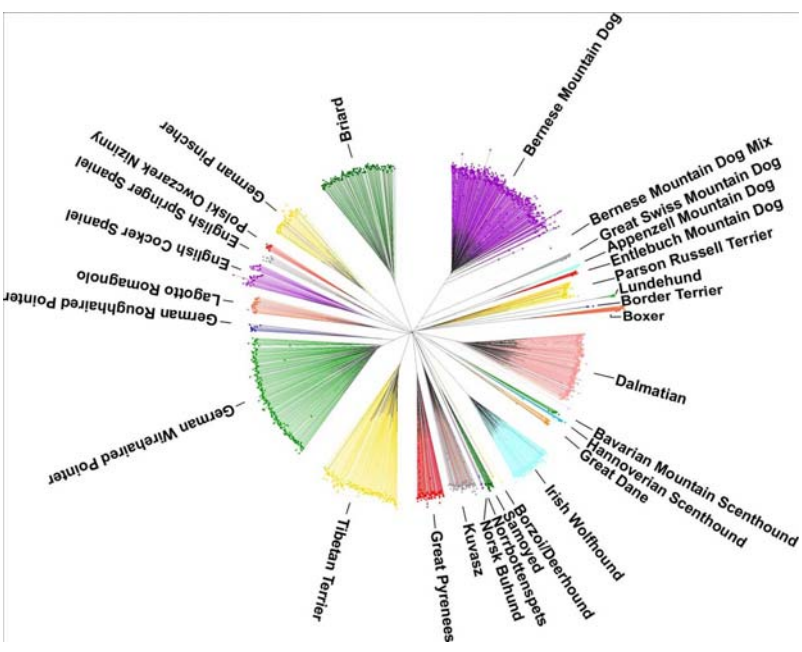
Awano et al. 2010  
 Winger et al. 2011  
 Pfahler et al. 2014  
 Zeng et al. 2014

### SOD1-genotyping results - Bernese Mountain Dogs -

Exon 1	Exon 2			Total
	100/100	100/200	200/200	
100/100	273	279	44	596
100/200	81	47	0	128
200/200	4	0	0	3
Total	358	326	44	728

# Genomic data – Further analyses

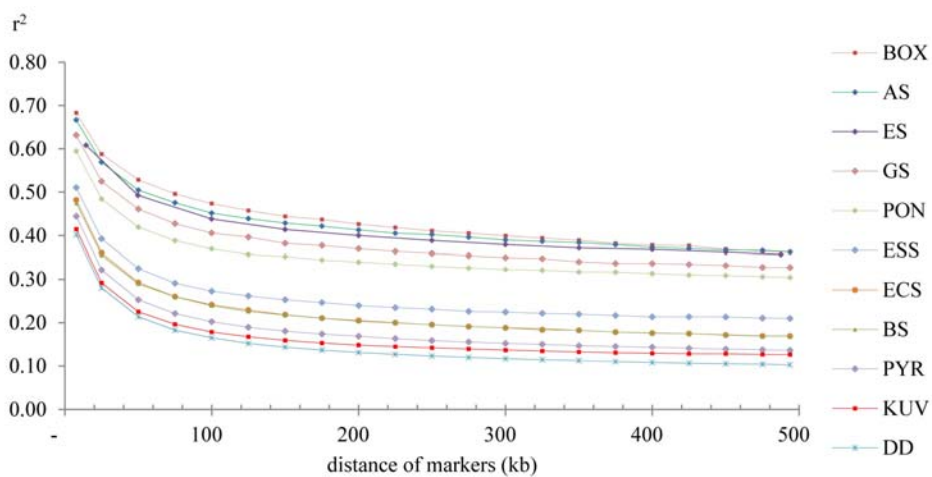
## Breed diversity using high density SNP data



## Genomic architecture causes correlation among alleles



## Correlation among alleles by breed



## Genomic effective population size (Ne) and increase of inbreeding (%) per generation ( $\Delta F$ )

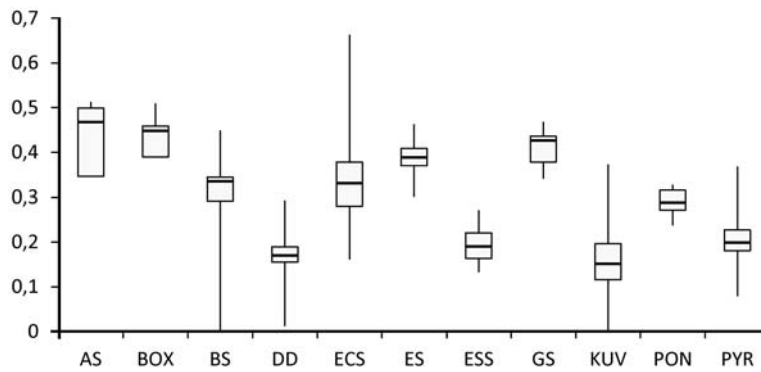
Breed	Ne	$\Delta F$	Ne <sub>100 generations ago</sub>
Appenzell	30	1.67	88
Bernese	99	0.51	247
Entlebuch	30	1.67	90
Great Swiss	35	1.43	103

$$Ne = (1-r^2) / (4cr^2)$$

## Genomic Inbreeding Coefficient

- Calculation of the degree of inbreeding using genomic data
  - Homozygous genomic segments > 65 SNPs (IBD)
  - Total autosomal genome size
- $F_{ROH}$  = Size of IBD-genomic segments/total genome size

## Genomic Inbreeding ( $F_{ROH}$ )



**BS: Bernese Mountain Dog**

## Next-Generation-Sequencing of Bernese Mountain Dogs

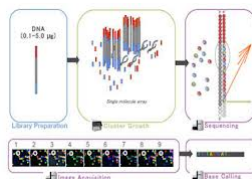


### Next-Generation-Sequencing (NGS)



Illumina HiSeq-2500

6 Mammalian genomes 30X in 12 days – bioinformatics ~ 4 weeks



- Reference genomic data
  - complete DNA-sequences from 6 BMDs
  - Private BMD-variants
  - Private BMD-regions of homozygosity
  - Substitution of genotyped BMDs with ref genome
  - Inherited defects and lethal mutations
  - Longevity without major diseases

### Next-Generation-Sequencing



- Number of Bernese Mountain Dogs: 6
- Number of dogs from other breeds: 30
- Number of autosomal SNPs: 14,937,775





## NGS of Bernese Mountain Dogs

- Number of autosomal SNPs: 14,937,775
- BMD- specific SNPs: 235,964
- SNPs with effects on protein: 2,301
- CHS-associated SNPs: 154
- Longevity-associated SNPs: 138
- Number of SNPs for validation: 29



## NGS of Bernese Mountain Dogs

First results seem promising

- 10 CHS-associated mutations: significant results
- 1 Longevity-associated mutation: significant result
- Complex genetics of CHS and longevity



## Summary

- **Reference samples are important**
- **BMD-breeders can use the GEBV-service**
- **Younger BMDs with promising GEBVs**
- **Supplementation with NGS-data**
  - Increasing power for association studies
  - Increasing power for GEBVs
  - Increasing genetic diversity



## Summary

### **Service for BMD breeders**

- **Validation samples can be shipped to TiHo (Europe)**
- **BMD with CHS or lifetime >12 yrs are tested for free (Europe) at TiHo**
- **Validation with the newly developed genetic variants from next-generation sequencing data of CHS-BMD is open for all BMDs and without fees for the tests**
- **Testing and validation of BMDs in US/Can > please contact us for specific arrangements**



**Thank you!**