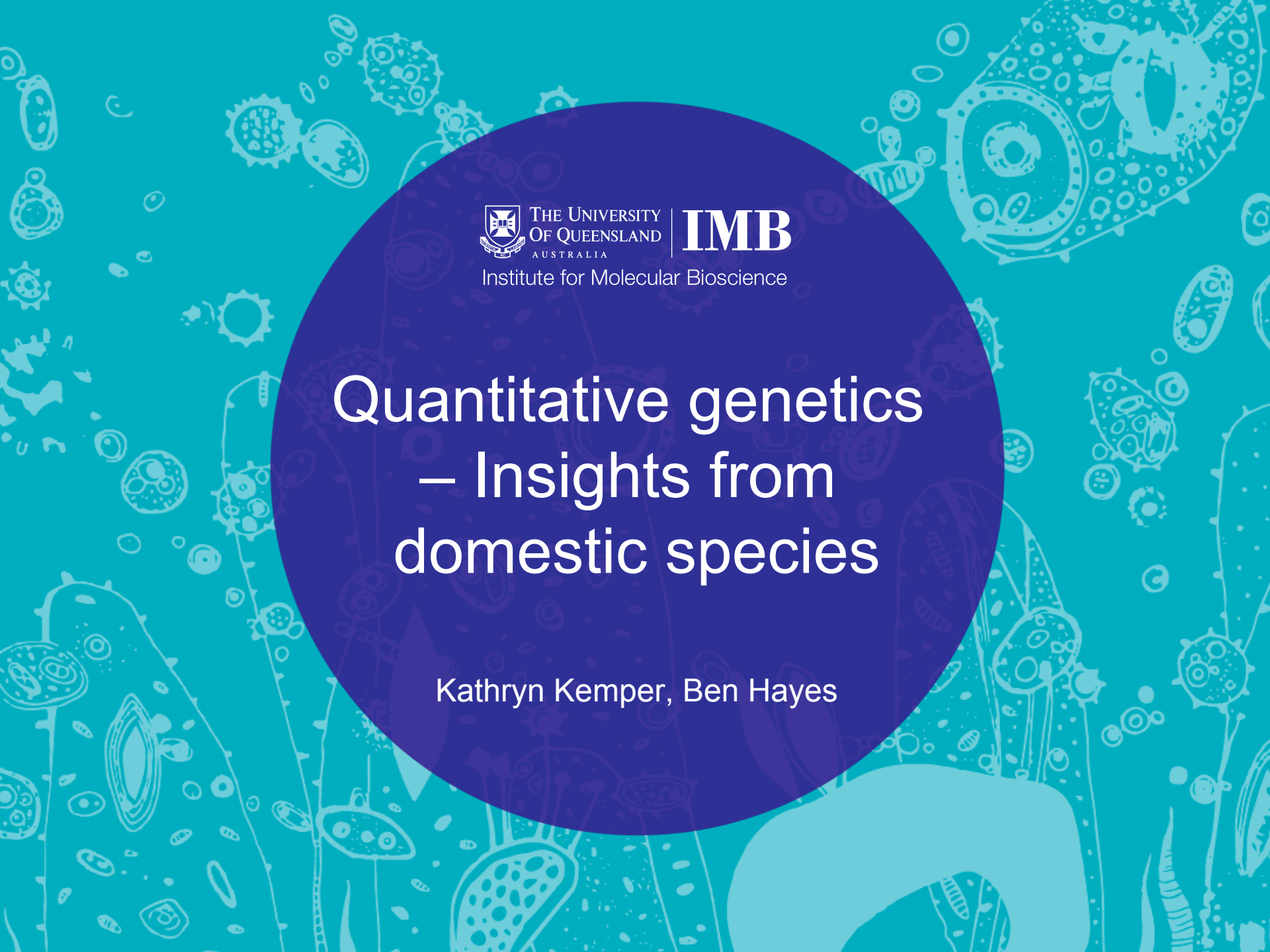

The University Of Queensland
AUSTRALIA
IMB
Institute for Molecular Bioscience
Quantitative genetics – Insights from domestic species
Kathryn Kemper, Ben Hayes

Aims of presentation

1. Compare and contrast genetics/genomics research in domestic species and human populations.
    - Understanding biology; genetic architecture
    - Genomic prediction; estimating genetic merit [or BV] of individuals

2. ‘Big issues’ people are currently addressing in livestock

Genetic research in humans and livestock

Genetic research in Livestock	Genetic research in Humans
Motivation is to increase production through changed genotype. Accurate prediction of genetic merit is the main aim. Commercial interests, large multinational companies readily apply techniques. Some interest in phenotype prediction. Lower importance as less impact. Quantitative genetics widely applied since 80's (AI); some molecular work on mendelian traits	Motivation is to improved outcomes in human health. Prediction of phenotype main goal. No predictions available for quantitative traits (?). Historically more emphasis on mendelian traits, family studies

Level of polymorphism, cattle vs. humans

Daetwyler et al. (2016)

Average number of heterozygous sites:

Cattle	1.44/kb
Yoruba	1.03/kb
European	0.68/kb

ARTICLES

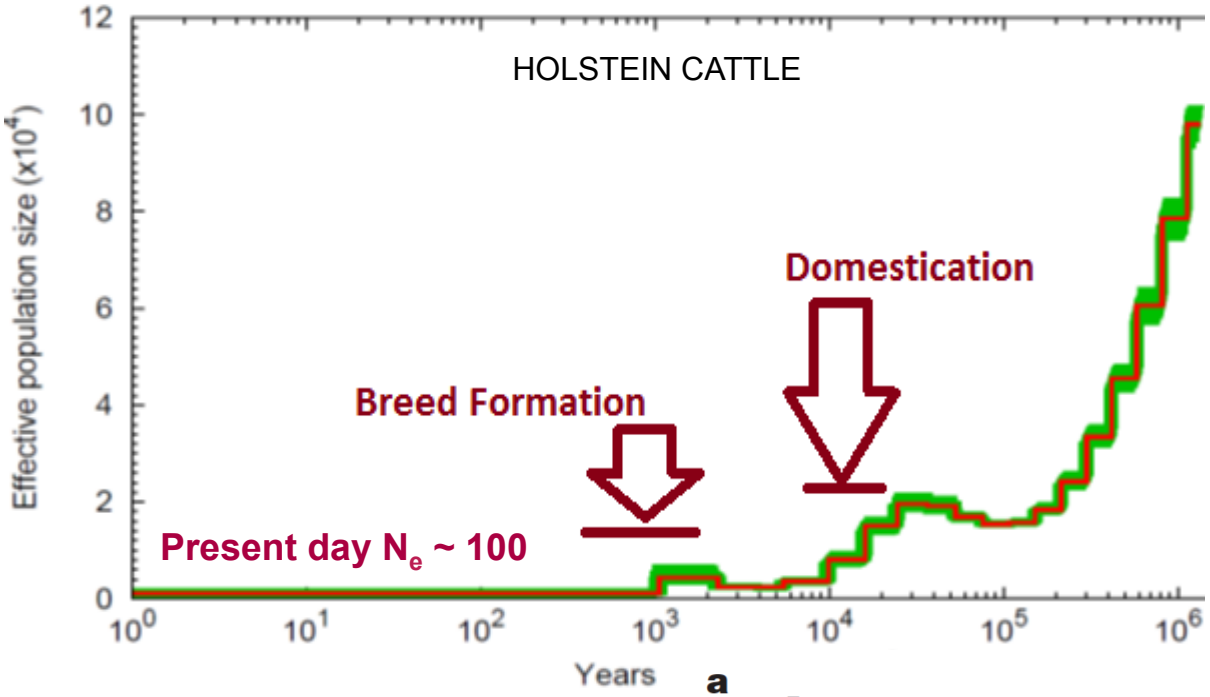
nature genetics

Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle

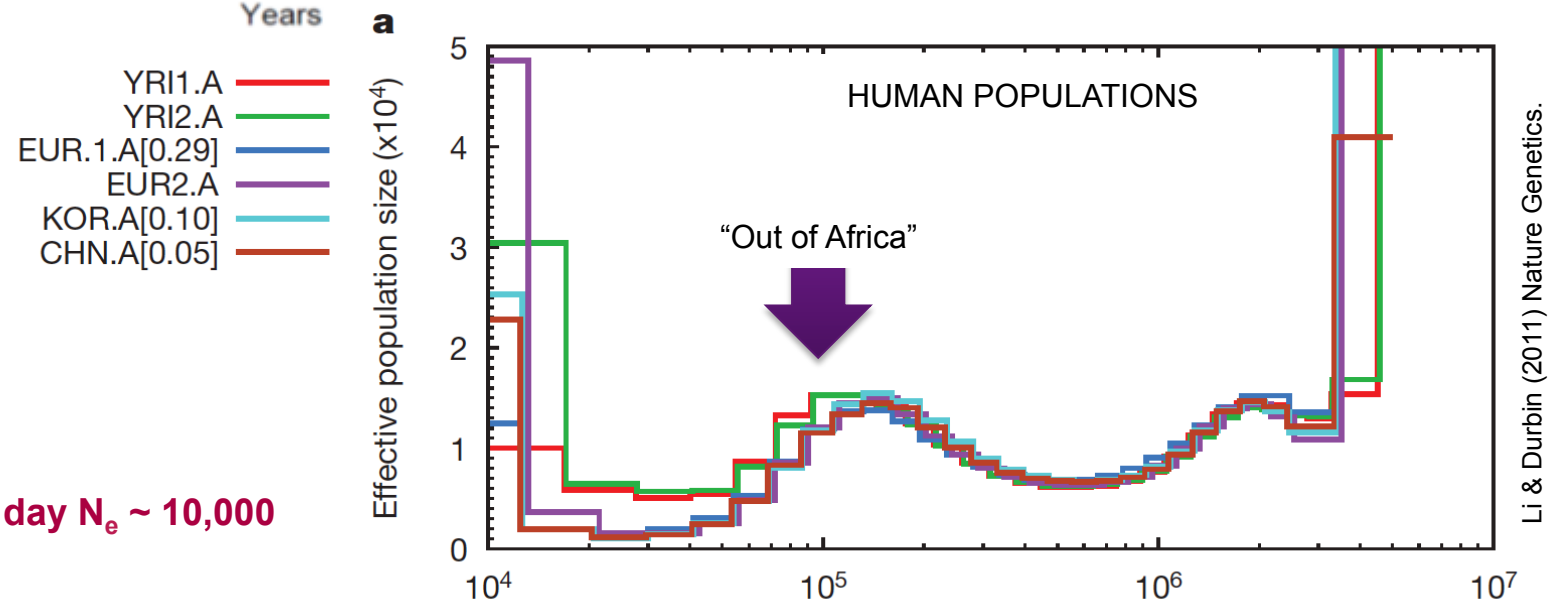
Hans D Daetwyler[1–3], Aurélien Capitan[4,5], Hubert Pausch[6], Paul Stothard[7], Rianne van Binsbergen[8], Rasmus F Brøndum[9], Xiaoping Liao[7], Anis Djari[10], Sabrina C Rodriguez[4], Cécile Grohs[4], Diane Esquerré[11], Olivier Bouchez[11], Marie-Noëlle Rossignol[12], Christophe Klopp[10], Dominique Rocha[4], Sébastien Fritz[5], André Eggen[4], Phil J Bowman[1,3], David Coote[1,3], Amanda J Chamberlain[1,3], Charlotte Anderson[1], Curt P VanTassell[13], Ina Hulsegge[8], Mike E Goddard[1,3,14], Bernt Guldbrandtsen[9], Mogens S Lund[9], Roel F Veerkamp[8], Didier A Boichard[4], Ruedi Fries[6] & Ben J Hayes[1–3]

The 1000 bull genomes project supports the goal of accelerating the rates of genetic gain in domestic cattle while at the same time considering animal health and welfare by providing the annotated sequence variants and genotypes of key ancestor bulls. In the first phase of the 1000 bull genomes project, we sequenced the whole genomes of 234 cattle to an average of 8.3-fold coverage. This sequencing includes data for 129 individuals from the global Holstein-Friesian population, 43 individuals from the Fleckvieh breed and 15 individuals from the Jersey breed. We identified a total of 28.3 million variants, with an average of 1.44

…One likely explanation for the high rate of heterozygous sites per individual observed in our analysis is a large past effective population size. This would have allowed the accumulation of large amounts of variation, which has been retained between breeds or even between and within individuals of the same breed….



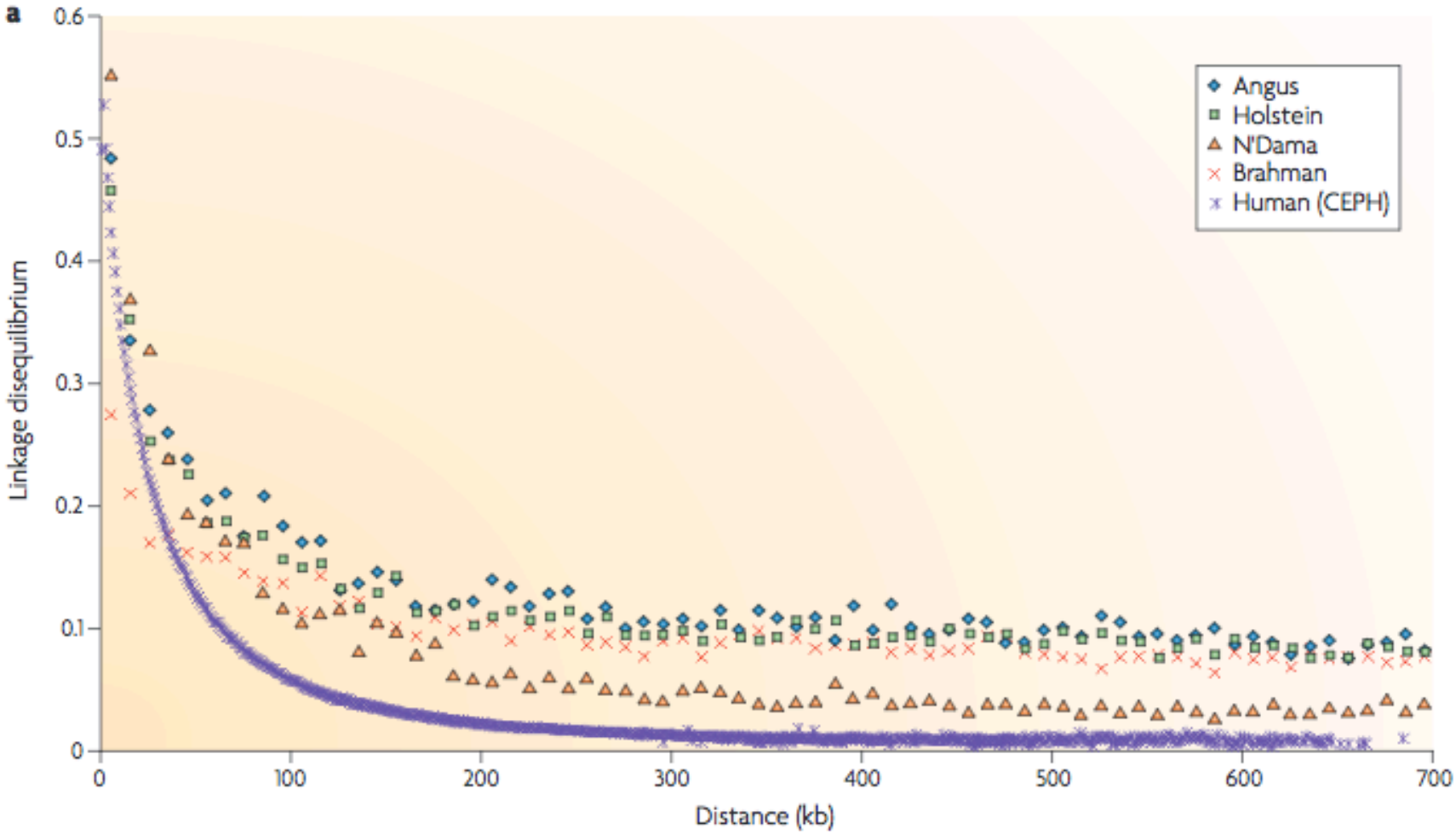
MacLeod et al. (2013) *Molecular Biology and Evolution.*



Li & Durbin (2011) Nature Genetics.

Present day N_e ~ 10,000

Level of LD – cattle vs. human



Goddard & Hayes (2009) Nature Reviews Genetics.

Understanding complex traits

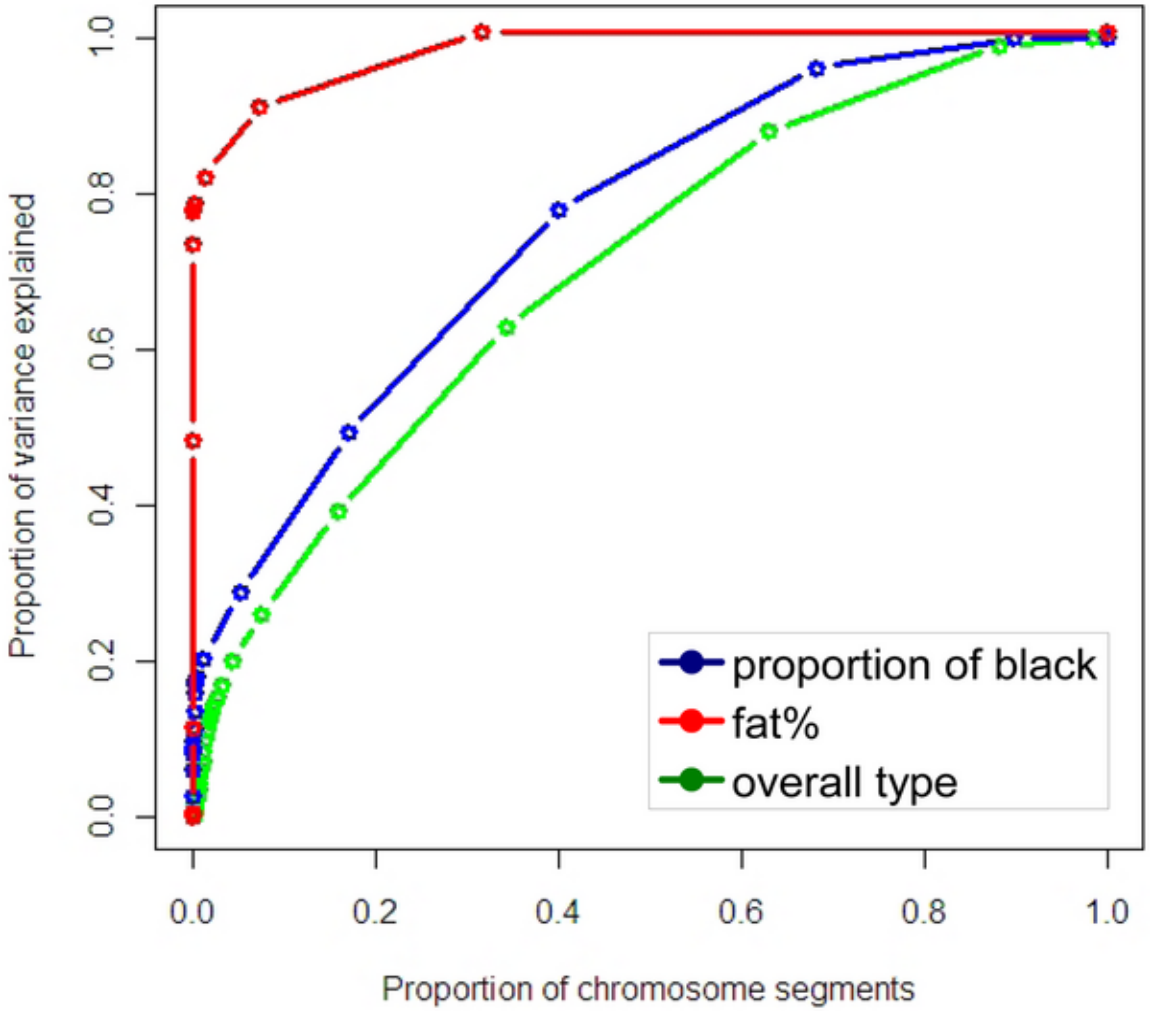
All traits are complex traits, but sometimes large effect mutations also segregate

Domestic species often have large pedigreed populations (with inbreeding) & numerous phenotypes / animal

What observations can we make about complex traits in domestic species?

- Alleles can reach high frequencies due to inbreeding (small N_e), this maybe deliberate (artificial selection) or by change (genetic drift)
- Large-effect mutations usually the targets of selection
- Admixture & introgression common
- Large-effect mutations often have pleiotropic effects. Sometimes unexpected.

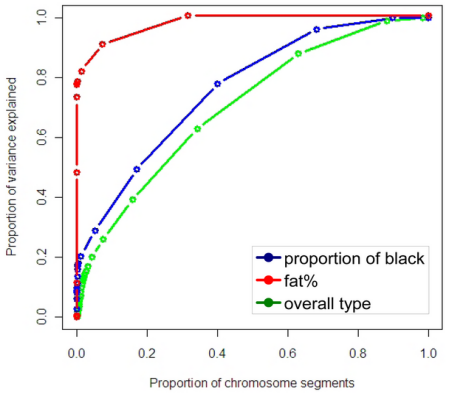
Even ‘simple’ traits are influence by many loci



Hayes BJ, et al. (2010) PLOS Genetics 6(9): e1001139.

Figure 1. Proportion of black phenotype.

Bull with 95% black (A) and bull with 5% black (B).



A

B

Hayes BJ, Pryce J, Chamberlain AJ, Bowman PJ, Goddard ME (2010) Genetic Architecture of Complex Traits and Accuracy of Genomic Prediction: Coat Colour, Milk-Fat Percentage, and Type in Holstein Cattle as Contrasting Model Traits. PLOS Genetics 6(9): e1001139. doi:10.1371/journal.pgen.1001139
http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1001139

PLOS | GENETICS

Large-effect mutations in domestic spp.

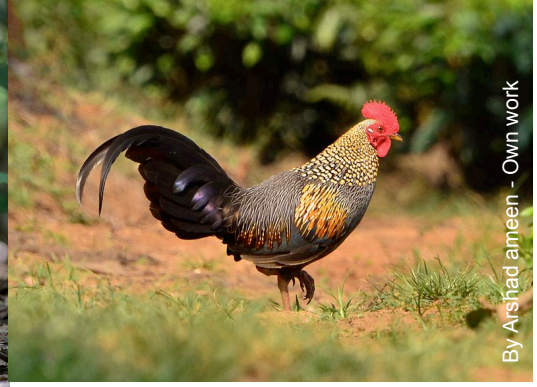
	dog	cattle	cat	pig	sheep	horse
Mendelian trait/disorder	285	231	94	66	100	51
Mendelian trait/disorder; key mutation known	211	132	62	31	47	37

http://omia.angis.org.au/home/

Examples include:

- 'Breed defining' characteristics
  - e.g. wattle type in poultry; horn/poll locus in cattle, sheep and goats; double muscling in cattle and sheep; coat colour and coat characteristics (e.g. hair type); tail length
- Lethal or severely debilitating diseases
  - e.g. embryonic lethal

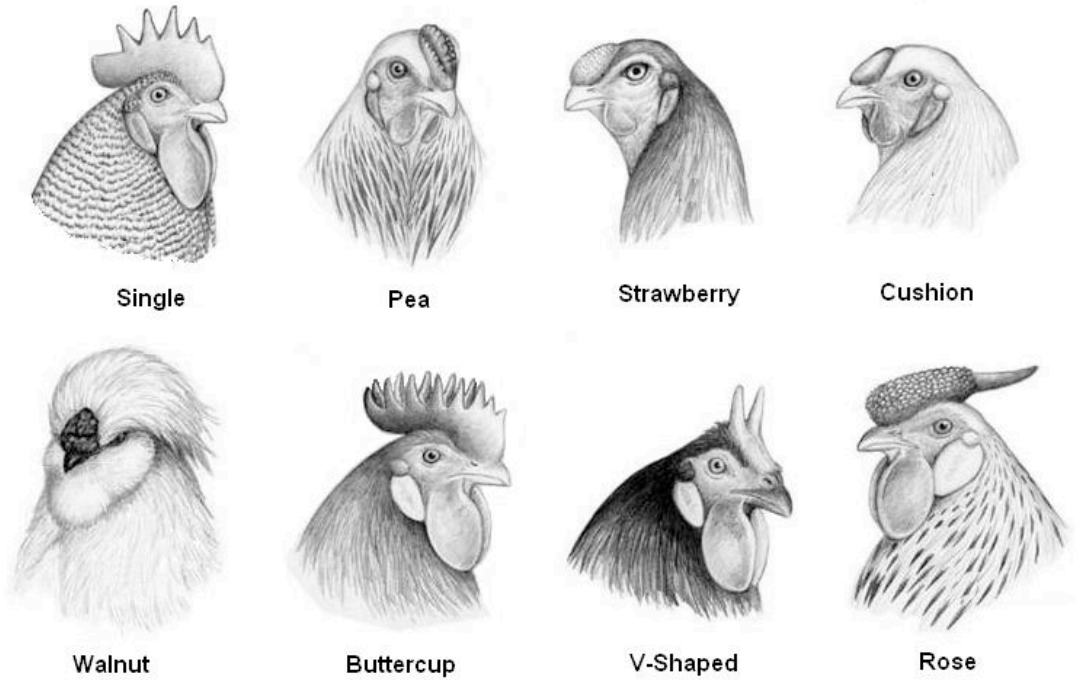
RED JUNGLE FOWL



GREYJUNGLE FOWL

Bateson (1902) – pea comb, rose comb, shank colour, polydactyly, white plumage

Bateson & Punnett (1905) – 'epistastic' walnut comb
F1: Pea x Rose = Walnut
F2: Walnut x Walnut =
9 Walnut : 3 Pea : 3 Rose : 1 single



https://tbnranch.files.wordpress.com/2012/01/comb_types.jpg

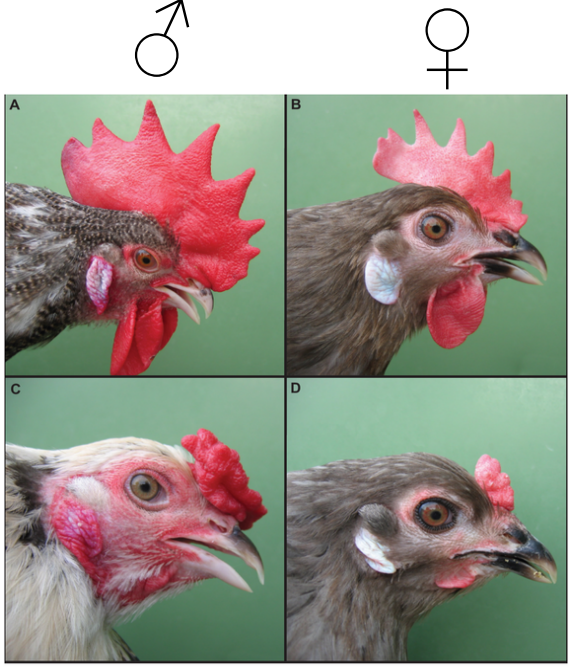
Dorshorst et al (2015) –

“These findings complete our characterization of the genetic basis of the three major comb loci in the chicken,

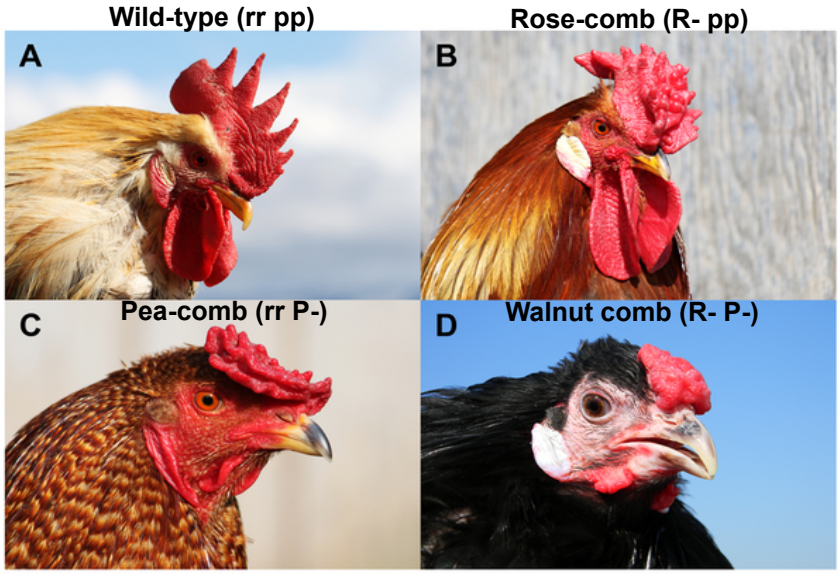
…all of which are caused by large-scale structural genomic variants…

that drive ectopic expression of transcription factors in the comb region during chicken embryo development"

Dorshorst B et al. (2015) PLoS Genet 11(3): e1004947.



Wright D et al. (2009) PLoS Genet 5(6): e1000512.



Imsland F, et al (2012) PLoS Genet 8(6): e1002775.

Bateson & Saunders (1902) – dominant POLLED in cattle

Georges et al. (1993) – mapped POLLED to BTA1

Medugorac et al. (2012) – Allelic heterogeneity at POLLED. “Celtic” and “Frisian” alleles. Both complex indels located in a region with no known function.

Horn and poll phenotypes in Simmental cattle.

Wiedemar N, et al. (2014). PLoS ONE 9(3): e93435. doi:10.1371/journal.pone.0093435

OPEN ACCESS Freely available online

PLoS one

Bovine Polledness – An Autosomal Dominant Trait with Allelic Heterogeneity

Ivica Medugorac[1]*, Doris Seichter[2], Alexander Graf[3], Ingolf Russ[2], Helmut Blum[3], Karl Heinrich Göpel[4], Sophie Rothammer[1], Martin Förster[1], Stefan Krebs[3]

1 Ludwig-Maximilians-University Munich, Munich, Germany, 2 Tierzuchtforschung e.V. München, Grub, Germany, 3 Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-University Munich, Munich, Germany, 4 Göpel Genetik GmbH, Herleshausen, Germany

Abstract

The persistent horns are an important trait of speciation for the family *Bovidae* with complex morphogenesis taking place briefly after birth. The polledness is highly favourable in modern cattle breeding systems but serious animal welfare issues urge for a solution in the production of hornless cattle other than dehorning. Although the dominant inhibition of horn morphogenesis was discovered more than 70 years ago, and the causative mutation was mapped almost 20 years ago, its molecular nature remained unknown. Here, we report allelic heterogeneity of the *POLLED* locus. First, we mapped the *POLLED* locus to a ~381-kb interval in a multi-breed case-control design. Targeted re-sequencing of an enlarged candidate

Carlson et al. (2016) –

To our knowledge this is the first empirical validation of a putative causative allele in livestock, and this report provides evidence that P_C, a sequence variant duplication of

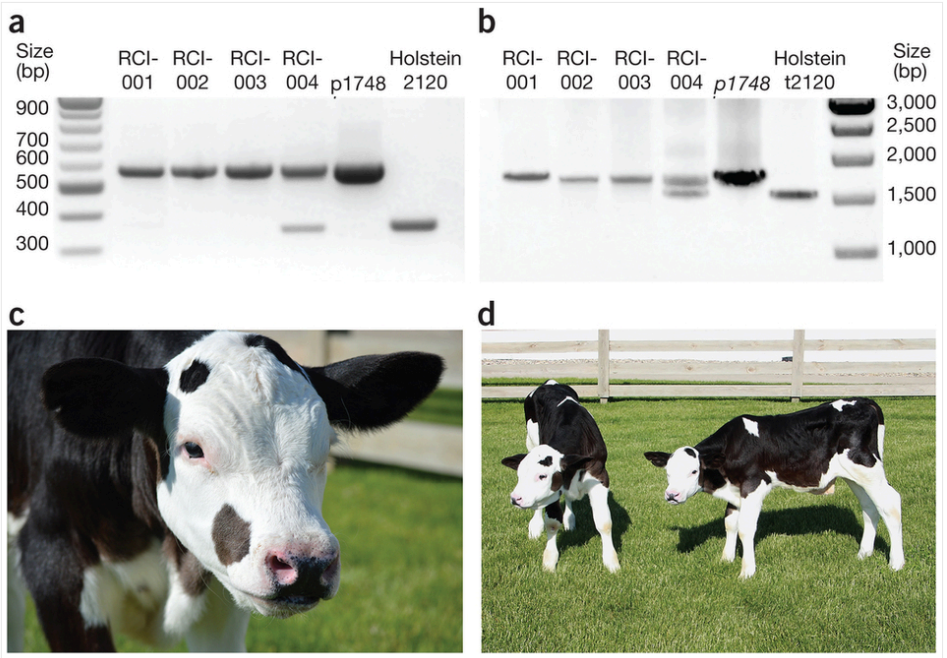
…. unknown function in a genomic region with no known or predicted coding or noncoding genes….

is causative for polled.

Production of hornless dairy cattle from genome-edited cell lines

Daniel F Carlson, Cheryl A Lancto, Bin Zang, Eui-Soo Kim, Mark Walton, David Oldeschulte, Christopher Seabury, Tad S Sonstegard & Scott C Fahrenkrug

Nature Biotechnology **34**, 479–481 (2016) | doi:10.1038/nbt.3560
Published online 06 May 2016



(**a**,**b**) Diagnostic PCRs for the Pc allele using primer pairs btHP-F1 + btHP-R2 (**a**) and HP1748-F1 + HP1594_ 1748-R1 (**b**) (Supplementary Methods), respectively, confirmed homozygous introgression in RCI-001, Spotigy (RCI-002) and Buri (RCI-003), and heterozygous introgression in RCI-004 relative to the donor cell line that is negative. The identity of PCR products was confirmed by Sanger sequencing. The positive control (p1748) was a plasmid containing the P_C allele[10]. (**c**) Photograph of Spotigy at 2 months of age, so named after the black spots where horn buds would have developed. (**d**) Photograph of Buri (left) and Spotigy at 2 months of age.

Genetic screening for deleterious alleles

'Forward genetics' = phenotype -> genotype
'Reverse genetics' = genotype -> phenotype

Forward genetics limited to high freq alleles & those that cause obvious phenotypes (i.e. still birth)

Strategy:

1. Screen exome of ~ 600 animals for LOF & missense mutations
2. Genotype ~ 40,000 individuals for ~ 3,800 candidates and screen for depletion of homozygotes
3. Confirm by sequencing carrier x carrier trios

Found 15 % of LoF and 6 % of missense suggestive of EL. Confirmed 9 EL mutations via sequencing of 200 carrier x carrier matings

9 identified variants account for loss of ~ 0.6% of conceptuses

Research

NGS-based reverse genetic screen for common embryonic lethal mutations compromising fertility in livestock

Carole Charlier,[1,5] Wanbo Li,[2,5] Chad Harland,[1,3] Mathew Littlejohn,[3] Wouter Coppieters,[1,4] Frances Creagh,[3] Steve Davis,[3] Tom Druet,[1] Pierre Faux,[1] François Guillaume,[1,6] Latifa Karim,[1,4] Mike Keehan,[3] Naveen Kumar Kadri,[1] Nico Tamma,[1] Richard Spelman,[3] and Michel Georges[1]

[1]Unit of Animal Genomics, GIGA-R & Faculty of Veterinary Medicine, University of Liège (B34), 4000-Liège, Belgium; [2]State Key Laboratory for Pig Genetic Improvement and Production Technology, Jiangxi Agricultural University, Nanchang, 330045, Jiangxi Province, P.R. China; [3]Livestock Improvement Corporation, Newstead, Hamilton 3240, New Zealand; [4]Genomics Platform, GIGA, University of Liège (B34), 4000-Liège, Belgium

We herein report the result of a large-scale, next generation sequencing (NGS)-based screen for embryonic lethal (EL) mutations in Belgian beef and New Zealand dairy cattle. We estimated by simulation that cattle might carry, on average, ~0.5 recessive EL mutations. We mined exome sequence data from >600 animals, and identified 1377 stop-gain, 3139 frame-shift, 1341 splice-site, 22,939 disruptive missense, 62,399 benign missense, and 92,163 synonymous variants. We show that cattle have a comparable load of loss-of-function (LoF) variants (defined as stop-gain, frame-shift, or splice-site variants) as humans despite having a more variable exome. We genotyped >40,000 animals for up to 296 LoF and 3483 disruptive missense, breed-specific variants. We identified candidate EL mutations based on the observation of a significant depletion in homozygotes. We estimated the proportion of EL mutations at 15% of tested LoF and 6% of tested disruptive missense variants. We confirmed the EL nature of nine candidate variants by genotyping 200 carrier × carrier trios, and demonstrating the

Genetic screening for deleterious alleles

Table 1. Estimation, by simulation (≥2000 generations), about lethal mutations as a function of the effective population size (N_e; range: 50–10,000) and the rate of recessive lethal mutations per gamete (MU; 0.01 or 0.015)

N_e	MU	NR SEGR SITES[a]	NR MUT/IND[b]	MUT FREQ[c]	% DEATH[d]	% > 0.02[e]
50	0.01	4.84 (2.30)	0.37 (0.22)	3.74 (1.64)	1.05 (1.82)	1
	0.015	7.36 (2.85)	0.58 (0.31)	3.96 (1.44)	1.87 (2.38)	0.98
100	**0.01**	**11.01 (3.34)**	**0.53 (0.21)**	**2.41 (0.69)**	**1.01 (1.18)**	**0.94**
	0.015	**17.19 (4.60)**	**0.85 (0.30)**	**2.49 (0.58)**	**1.73 (1.60)**	**0.98**
500	0.01	68.42 (8.86)	1.14 (0.22)	0.84 (0.11)	1.02 (0.60)	0.7
	0.015	104.77 (10.83)	1.78 (0.28)	0.85 (0.09)	1.69 (0.75)	0.69
1000	0.01	151.58 (13.51)	1.65 (0.21)	0.54 (0.05)	1.07 (0.40)	0.48
	0.015	220.54 (15.18)	2.29 (0.22)	0.52 (0.04)	1.39 (0.45)	0.43
5000	0.01	899.31 (27.71)	3.53 (0.19)	0.2 (0.01)	0.99 (0.18)	0.02
	0.015	1366.06 (41.38)	5.37 (0.22)	0.2 (0.01)	1.5 (0.19)	0.02
10,000	**0.01**	**1925.4 (43.46)**	**4.95 (0.16)**	**0.13 (0.01)**	**0.99 (0.12)**	**0.0006**
	0.015	**2936.68 (55.92)**	**7.7 (0.19)**	**0.13 (0.00)**	**1.54 (0.14)**	**0.0001**

Simulations were conducted assuming complete selection against homozygotes. Numbers in parentheses correspond to standard deviations. Values for $N_e = 100$ and $N_e = 10{,}000$, corresponding to the effective population size of cattle and human, respectively, are in bold.
[a]Number of segregating recessive lethal mutations.
[b]Number of recessive lethals carried, on average, per individual.
[c]Average frequency of the corresponding recessive lethals in the population.
[d]Percentage (total) of conceptuses dying as a result of homozygosity for a recessive lethal mutation.
[e]Percentage of these deaths (cf. footnote d) that are due to homozygosity for common recessive lethal mutations (defined as MAF ≥ 0.02).

(1) Human's carry more recessive lethal mutations than cattle
(2) Recessive lethals at higher frequency in cattle
(3) 'genetic load' is about the same

Genetic screening for deleterious alleles

Donner et al. (2016)

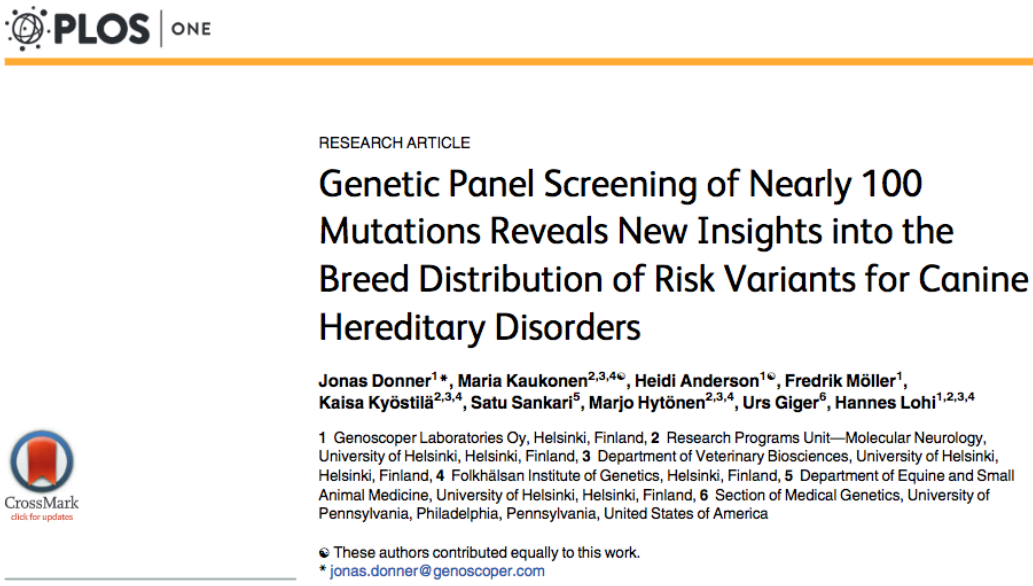
….DNA panel screening test for nearly 100 disorders on a cohort of almost 7000 dogs, we found a….

high overall frequency of mutation carriers (17.8%)

….while discovering a…

sixth (15/93) of the tested disease-causing variants in additional breeds.

…They often–but not always–cause the same condition in the additional breeds.



PLOS ONE

RESEARCH ARTICLE

Genetic Panel Screening of Nearly 100 Mutations Reveals New Insights into the Breed Distribution of Risk Variants for Canine Hereditary Disorders

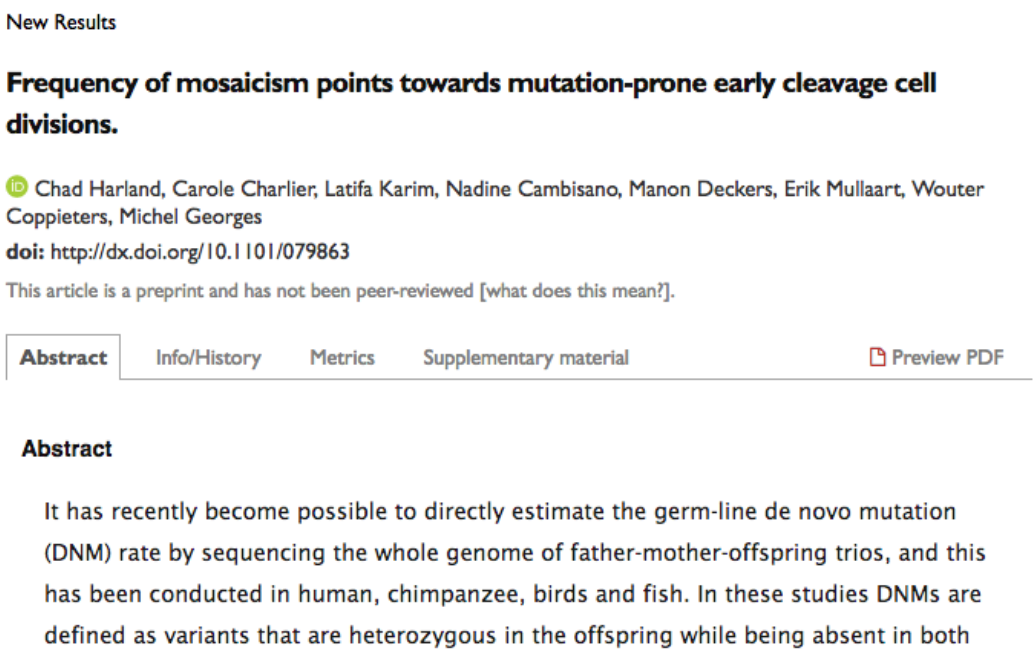
Jonas Donner[1]*, Maria Kaukonen[2,3,4☯], Heidi Anderson[1☯], Fredrik Möller[1], Kaisa Kyöstilä[2,3,4], Satu Sankari[5], Marjo Hytönen[2,3,4], Urs Giger[6], Hannes Lohi[1,2,3,4]

1 Genoscoper Laboratories Oy, Helsinki, Finland, 2 Research Programs Unit—Molecular Neurology, University of Helsinki, Helsinki, Finland, 3 Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland, 4 Folkhälsan Institute of Genetics, Helsinki, Finland, 5 Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland, 6 Section of Medical Genetics, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

☯ These authors contributed equally to this work.
* jonas.donner@genoscoper.com

Origin of *de novo* mutations

"As much as 30% and 50% of de novo mutations may occur during the early cleavage cell divisions in males and females, respectively, causing frequent mosaicism and a high sibling recurrence risk of DNM-dependent diseases"



New Results

Frequency of mosaicism points towards mutation-prone early cleavage cell divisions.

Chad Harland, Carole Charlier, Latifa Karim, Nadine Cambisano, Manon Deckers, Erik Mullaart, Wouter Coppieters, Michel Georges

doi: http://dx.doi.org/10.1101/079863

This article is a preprint and has not been peer-reviewed [what does this mean?].

Abstract | Info/History | Metrics | Supplementary material | Preview PDF

Abstract

It has recently become possible to directly estimate the germ-line de novo mutation (DNM) rate by sequencing the whole genome of father-mother-offspring trios, and this has been conducted in human, chimpanzee, birds and fish. In these studies DNMs are defined as variants that are heterozygous in the offspring while being absent in both

Variation between breeds

breed-defining loci (generally) under selection

- Mutations in dogs
  - *IGF1* mutation in small dogs
  - *FGF4* mutation causing disproportionate short stature
  - ~ 6 loci explain most σ^2_P

Disproportionate short stature

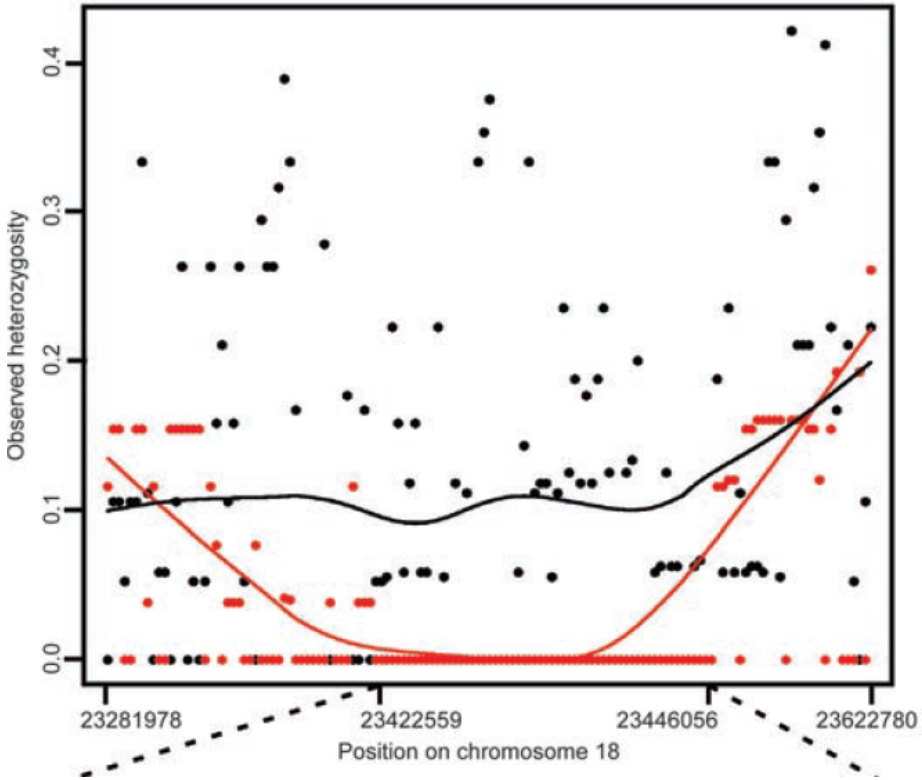
Parker et al. (2009) *Science* **325**(5943)**:** 995-998

short-legged dogs are homozygous (the same) for a 24 kb haplotype

Short-legged dogs have been selected for the same mutation

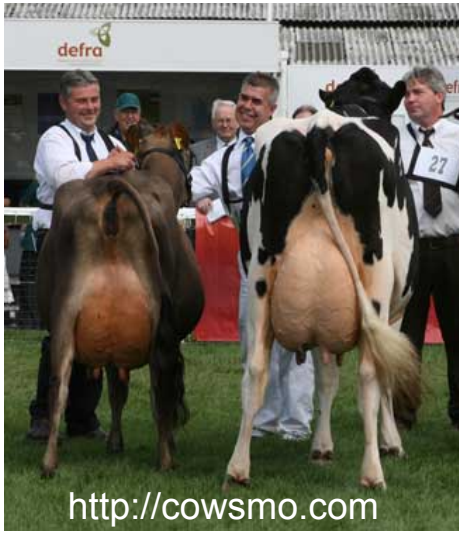
Gene is *FGF4* retrogene

(FGF = fibroblast growth factor)



Variation between breeds

breed-defining loci (generally) under selection

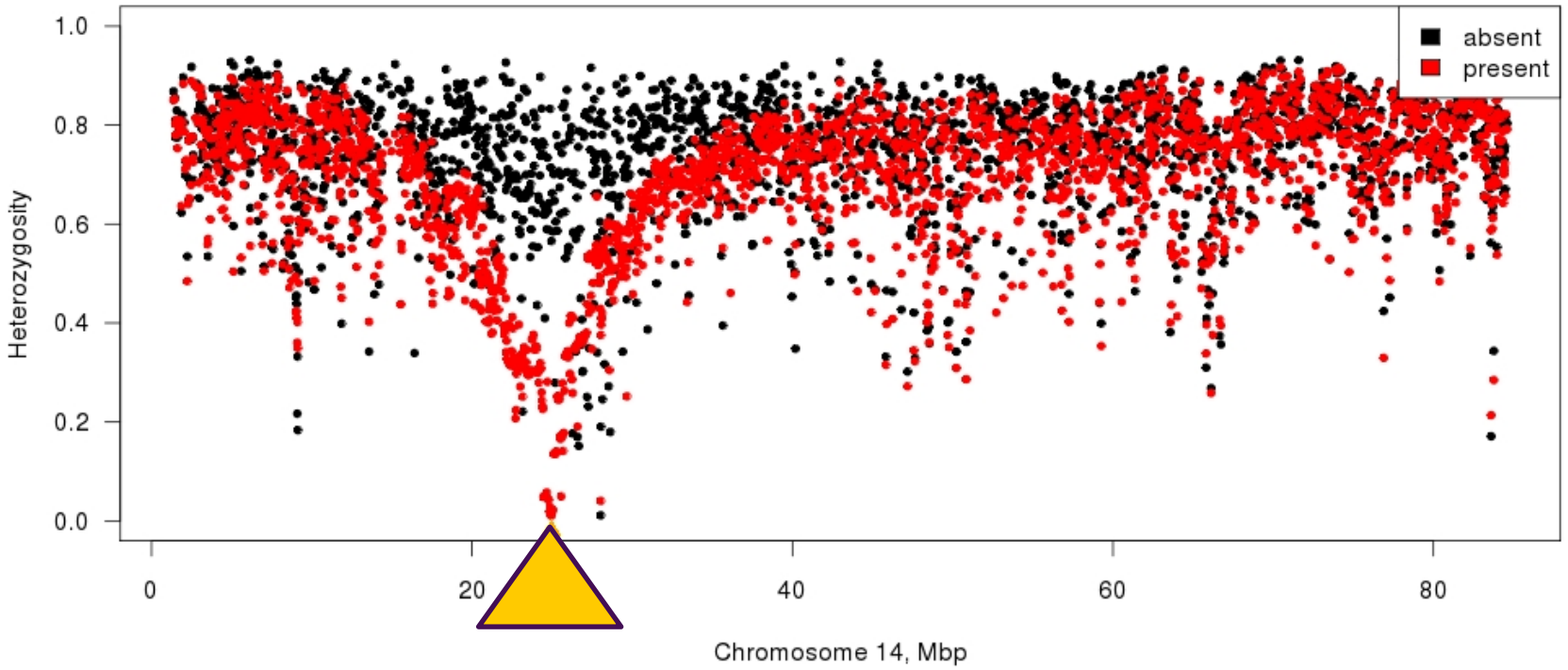


- *PLAG1-CHCHD7* mutation in Jersey x Holstein dairy cattle
  - effect ± 0.4 SD

Karim et al. (2011) Nature Genetics. 43(5):.405-413.

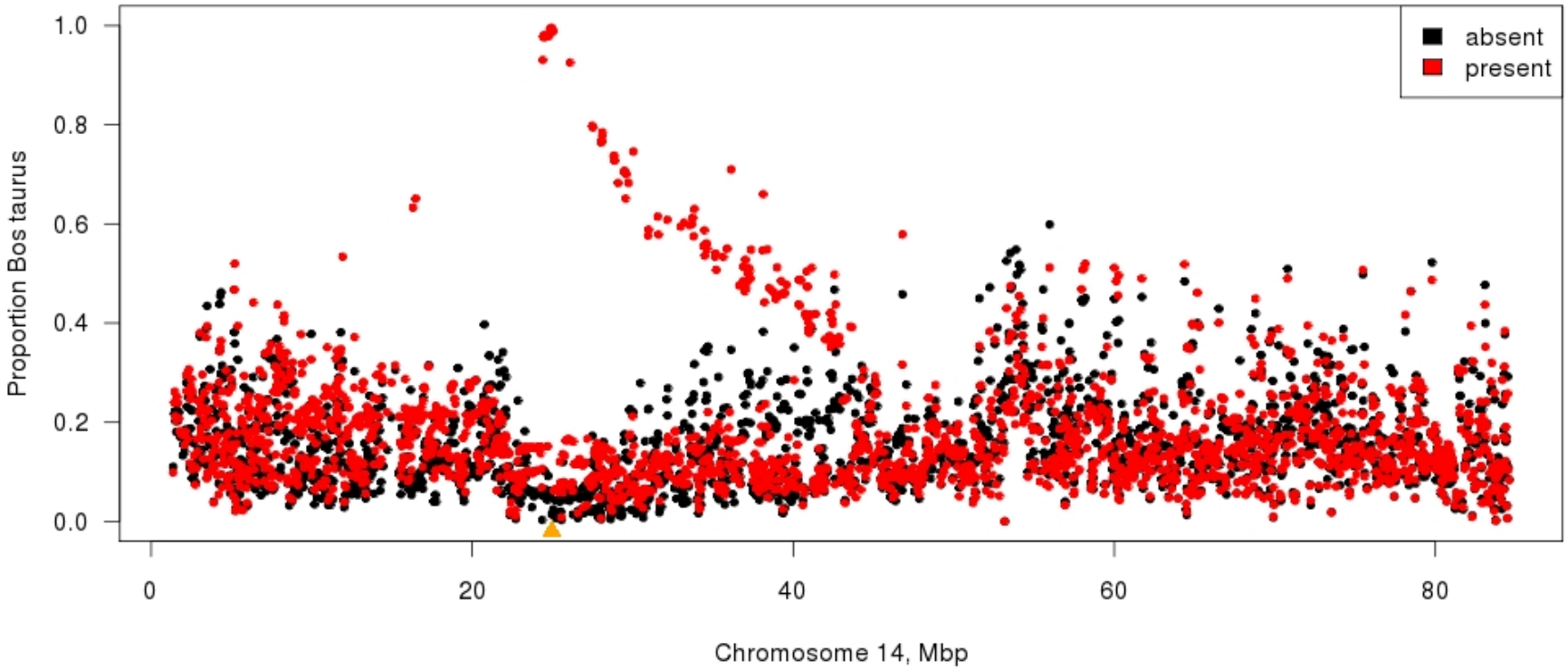
Example: *B. taurus* PLAG1 mutation in *B. indicius*

e.g. heterozygosity is reduced for chromosomes with and without a putative causative mutation, suggestive of selection for this locus in Brahman cattle

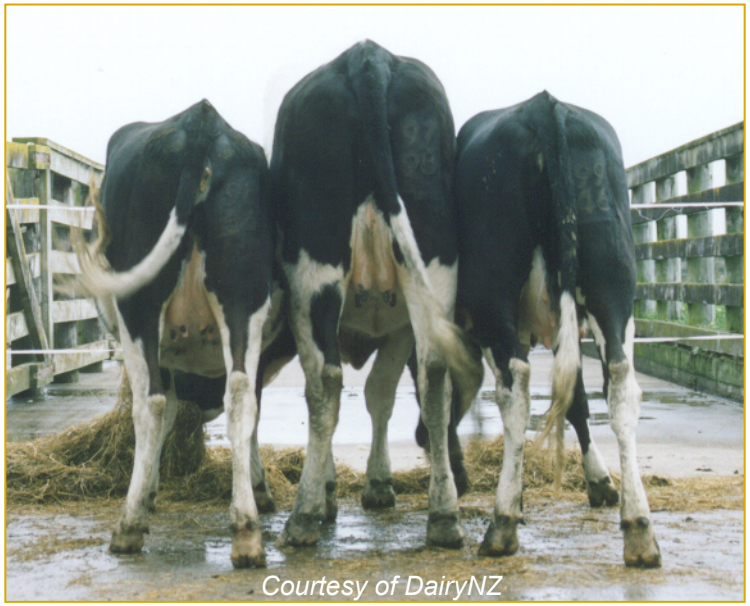


Example: scan of heterozygosity on BTA14

e.g. heterozygosity is reduced for chromosomes with and without a putative causative mutation, suggestive of selection for this locus in Brahman cattle



Genetic architecture of stature in cattle



Pryce *et al.* validated human genes from GWAS in cattle

mutation segregating in the same genes across species

How old are QTL?

(1) About half the QTL segregate across breeds

(2) QTL segregating within Holstein are a mix of 'young' and 'old' QTL while across-breed QTL are always 'old'

(3) Few QTL segregate across *B. taurus* & *B. indicus*

Kemper et al. (2015) Animal Genetics / Bolormaa et al. (2013) Genetic Selection Evolution

Based on haplotype length, we estimated the QTL to be:

- Younger than the *B. taurus* & *B. indicus* split (45,000 – 125,000 generations)
- Older than when breeds were developed (~ 400 generations)

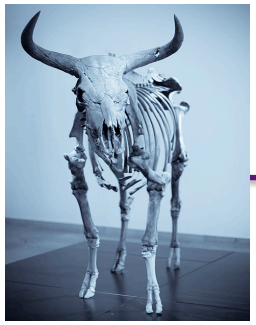
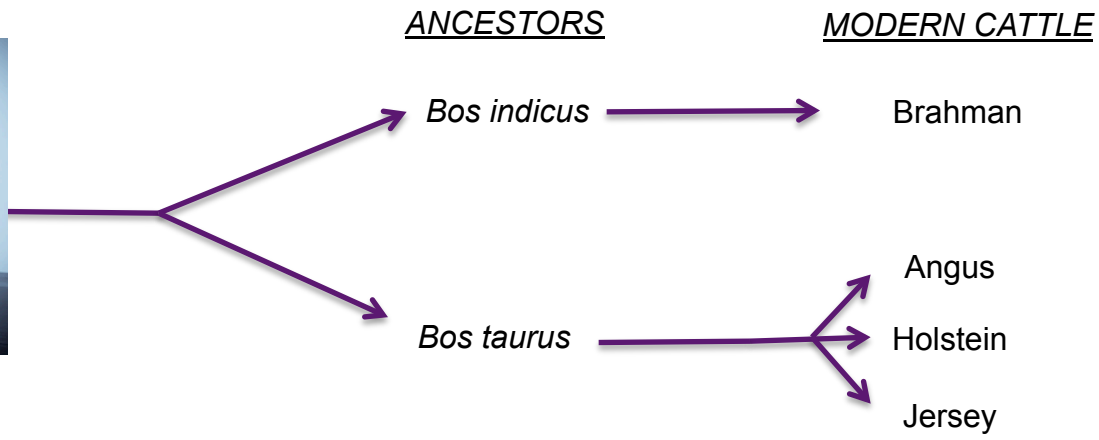
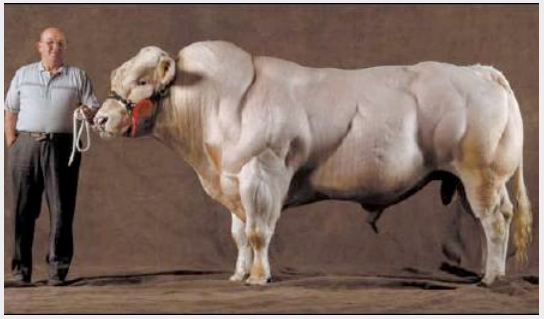


Photo: Marcus Sümnick

Auroch
(extinct)



Moderate-large effect mutations often have pleiotropic effects

locus	trait	Notes
PLAG1	stature 	Discovered in F1 Holstein x Jersey population (*B. taurus*). Introgressed into Brahmans (*B. indicus*). Affects fertility in Brahman.
MSTN	double muscling 	Six different LOF mutations in numerous breeds. Pleiotropic effects on meat quality, carcass dressing % & calving ease.
DGAT1	milk composition & yield	Mutant allele decreases milk fat, increases protein yield & milk volume. Intermediate frequency in numerous breeds

Kemper and Goddard (2012) *Human Molecular Genetics*.

Understanding complex traits

All traits are complex traits, but sometimes large effect mutations also segregate

Domestic species often have large pedigreed populations (with inbreeding) & numerous phenotypes / animal

What observations can we make about complex traits in domestic species?

- Alleles can reach high frequencies due to inbreeding (small N_e), this maybe deliberate (artificial selection) or by change (genetic drift)
- Large-effect mutations usually the targets of selection
- Admixture & introgression common
- Large-effect mutations often have pleiotropic effects. Sometimes unexpected.

Predicting complex traits

Strategy: Use a ‘reference’ population of phenotyped and genoytped animals to predict a ‘validation’ population (who were no included in the predictions).

Methods:

- GWAS – NEVER used; too much LD, winners curse
- GBLUP
- Non-linear (e.g. BayesR), best when large-effect QTL segregate

Two strategies to evaluate:

- Cross-validation (subsample population, excluding sire groups, beef/sheep)
- Next generation (birth-year cut off, typically in dairy)

Predicting Complex traits

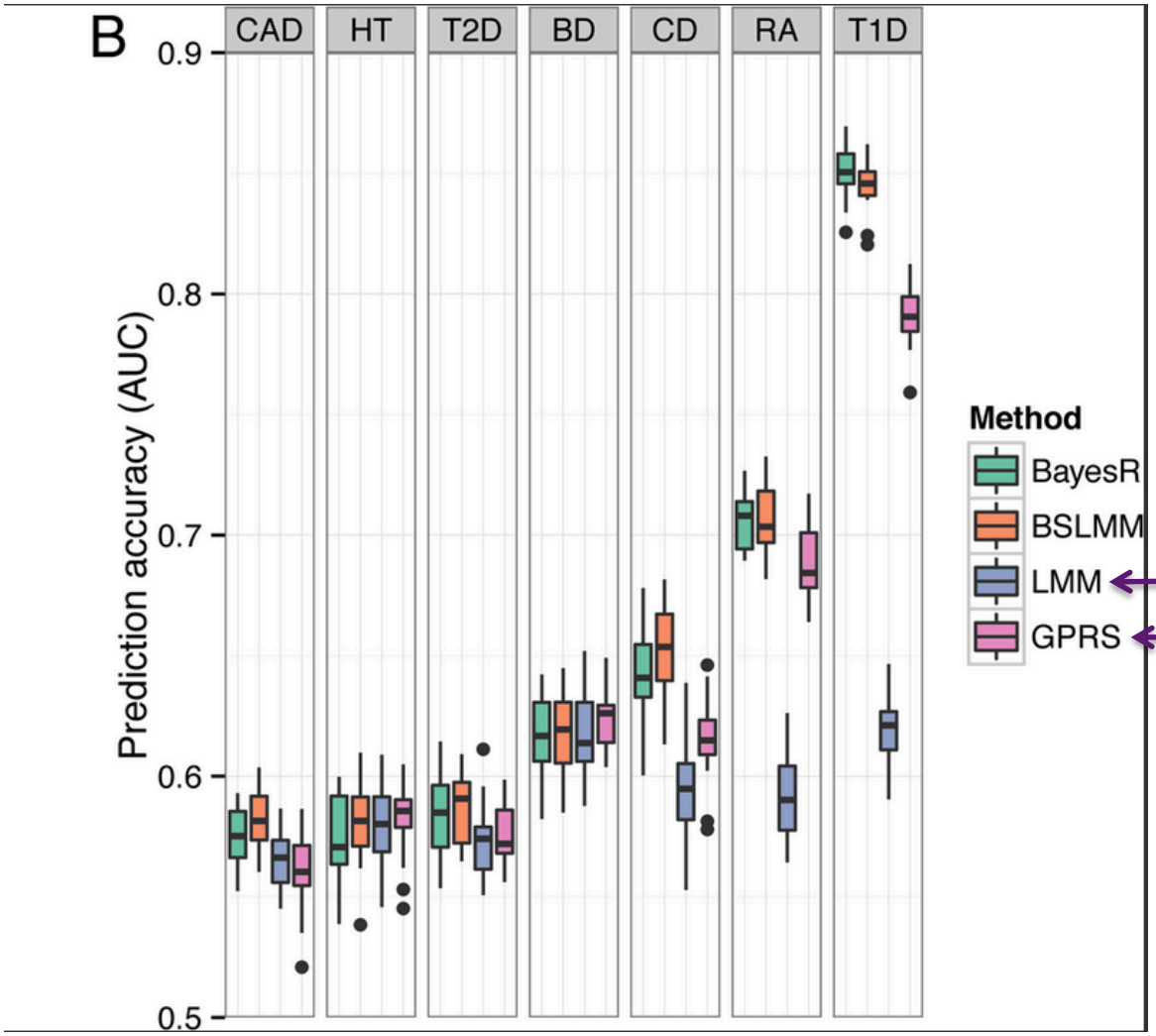
Predicting milk traits in Holstein:

Reference Set	Method	Milk volume (L)	Fat %
Holstein	GBLUP	0.58	0.71
Holstein	BayesR	0.62	0.81
Holstein & Jersey	GBLUP	0.59	0.72
Holstein & Jersey	BayesR	0.63	0.81
Jersey	GBLUP	0.10	0.15
Jersey	BayesR	0.21	0.48

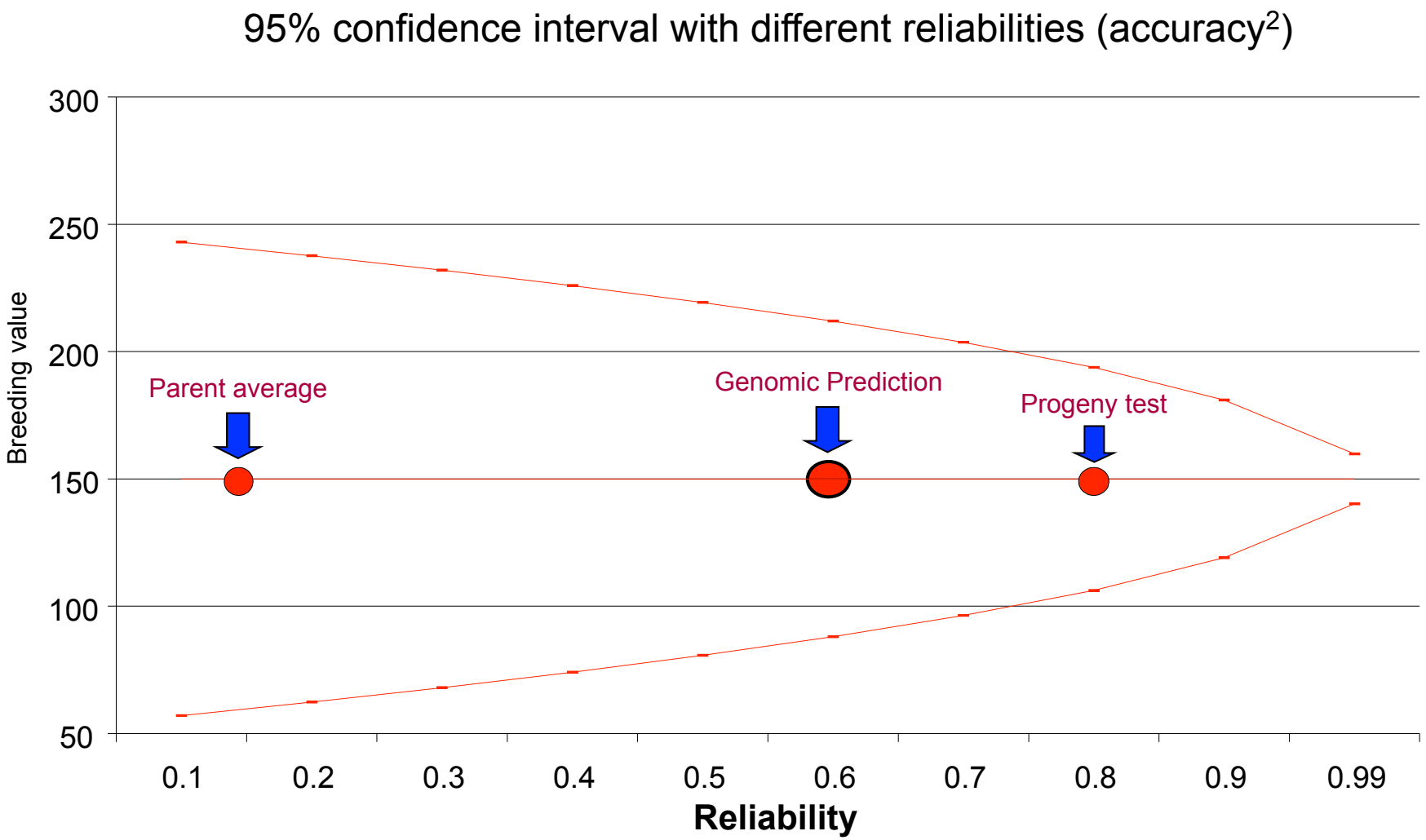
**differences in LD between SNP & QTL

**some QTL only segregate within one breed

Prediction, WTCCC



Predicting complex traits, PEV

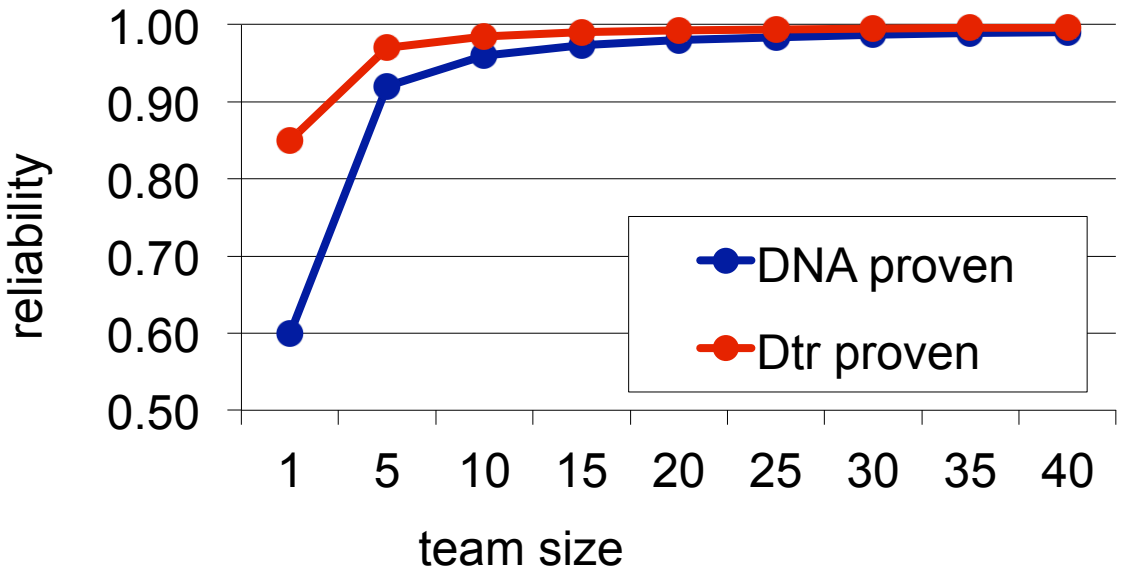


Reliabilities for an individual less important

Use ‘teams’ of genomically tested bulls

genomically tested bulls have lower reliability than proven bulls but using a team of bulls reduces risk

i.e. EBV of some bulls in the team might go down but others will go up



Dtr proven = daughter proven, Australian daughters

‘DNA’ proven = genomically tested (only), no daughters

Predicting Complex traits, why all the excitement?

Industry	Potential increase
Dairy Cattle	60-120% (Pryce et al. 2011)
Meat sheep	21% (van der Werf 2011)
Wool sheep	38% (van der Werf 2011)
Beef cattle	29-158% (Van Eenennaam 2011)
Layers	40% (Dekkers et al 2009)
Broilers	20% (Dekkers et al. 2009)

Advantageous when:

*sex-limited traits, i.e. milk yield from bulls or egg production from roosters!

*traits that are hard to measure, e.g. feed efficiency

*traits expressed later in life, e.g. fertility or adult wool production

Genomic prediction in US dairy cattle

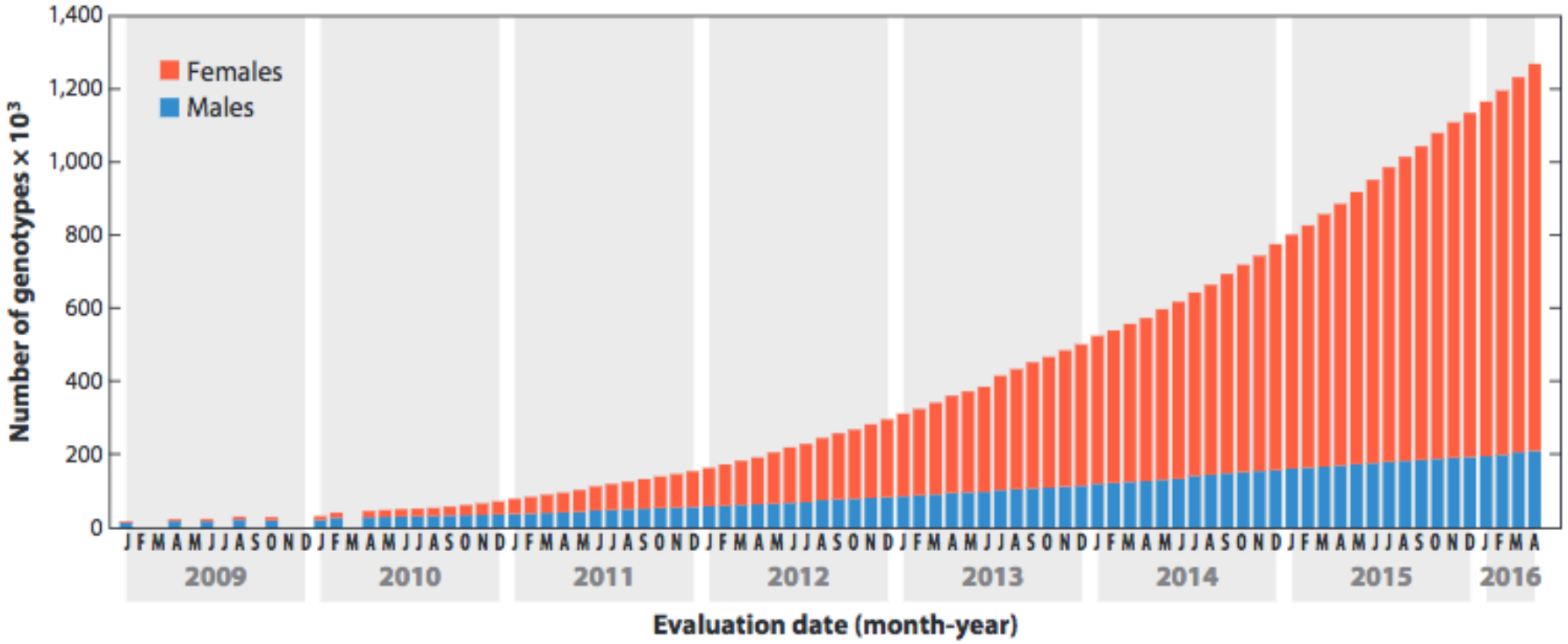
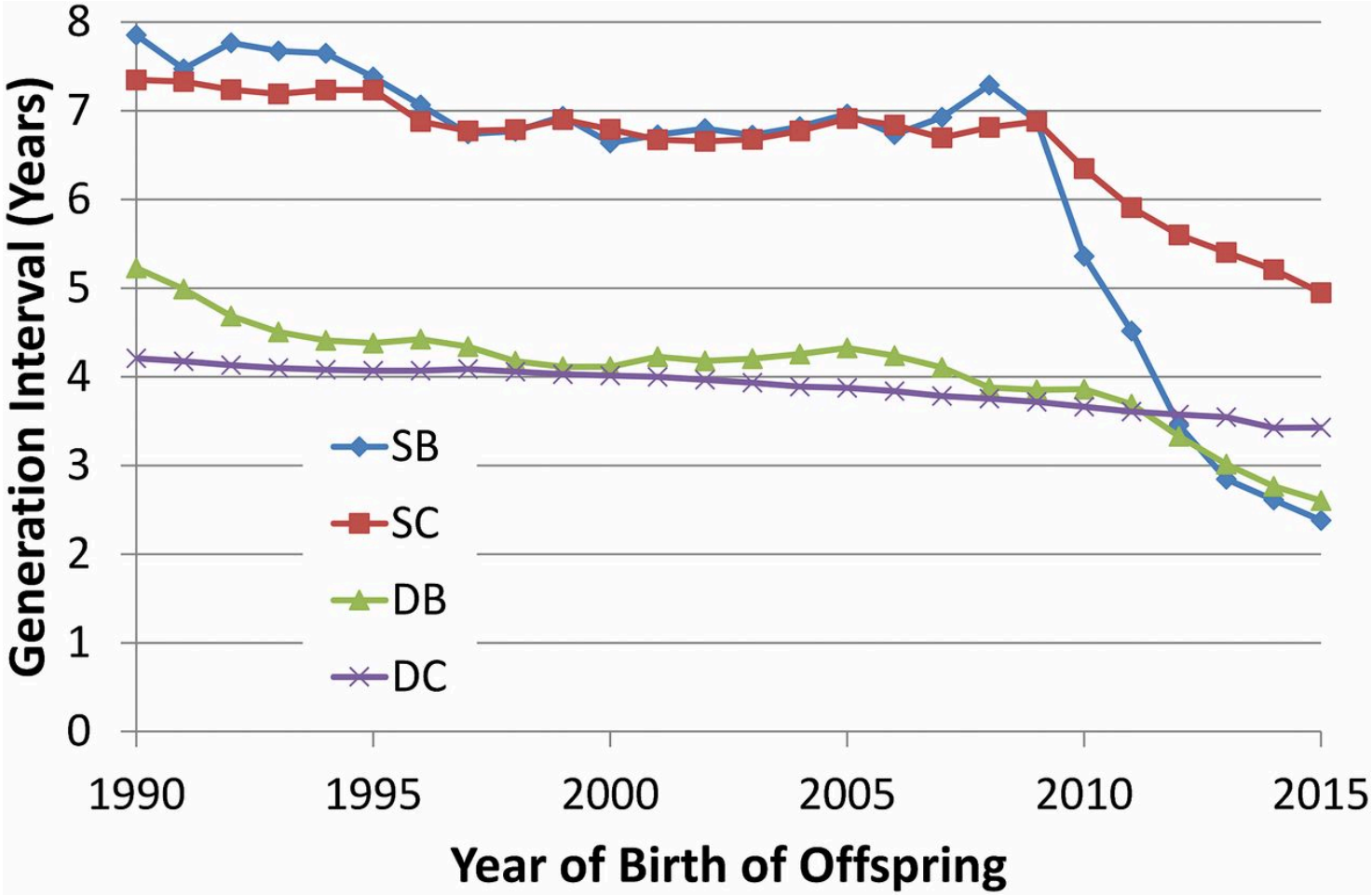


Figure 1

Number of genotyped animals included in US genomic evaluations for dairy cattle since January 2009. Official US genomic evaluations were first released to the dairy industry in January 2009 for Holsteins and Jerseys, in August 2009 for Brown Swiss, in April 2013 for Ayrshires, and in April 2016 for Guernseys. Data for figure generation were reported by the Council on Dairy Cattle Breeding (27). Months without data represent months in which official evaluations were not released.

Wiggans et al. (2016) Annual Review of Animal Biosciences

Genomic prediction in US dairy cattle



GI for four paths of selection (SB, SC, DB, and DC) by birth year of offspring for Holsteins.

Adriana García-Ruiz et al. PNAS 2016;113:E3995-E4004

Genomic prediction in US dairy cattle

Realised improvements in the rate of genetic gain

Reduction in generation interval has been primary driver

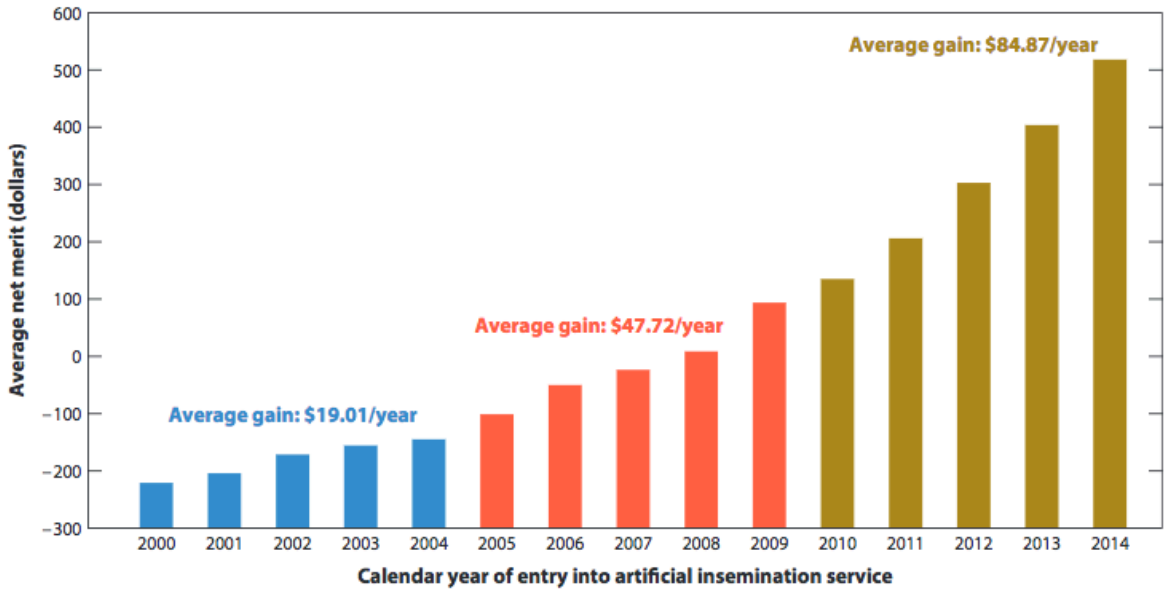


Figure 5

Net merit in April 2015 of marketed US Holstein bulls that entered artificial-insemination service in 2000 and later. Net merit is a genetic-economic index that was developed as a lifetime profit function that uses actual incomes and expenses for traits of economic importance (37). The economic values and traits included in the net merit index are updated as needed to reflect changes in the dairy industry, and the latest revisions were made in 2014 (38). The data for figure generation were provided by the Council on Dairy Cattle Breeding (**https://www.cdcb.us**).

Wiggans et al. (2016) Annual Review of Animal Biosciences

In the pipeline…

1. Understanding complex traits:
    - genome structure and function, regulatory regions
    - ‘phenomics’
    - human genetics leads the way in understanding biology

2. Predicting complex traits:
    - Prediction of BV, incorporation of pedigree and genomic information, i.e. ‘single-step’ methodologies
    - Prediction of future phenotypes
    - Across breed predictions
    - More important for livestock to achieve good results in prediction

Thank you