

Harnessing genomic information for livestock improvement

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Abstract | The world demand for animal-based food products is anticipated to increase by 70% by 2050. Meeting this demand in a way that has a minimal impact on the environment will require the implementation of advanced technologies, and methods to improve the genetic quality of livestock are expected to play a large part. Over the past 10 years, genomic selection has been introduced in several major livestock species and has more than doubled genetic progress in some. However, additional improvements are required. Genomic information of increasing complexity (including genomic, epigenomic, transcriptomic and microbiome data), combined with technological advances for its cost-effective collection and use, will make a major contribution.

Within-breed selection

A process by which sires and dams that have above average breeding values are selected as parents to produce the next generation of animals.

Genetic gains

Differences in the average breeding values of the population before and after selection. Genetic gain is a function of the amount of genetic variance, the accuracy of selection, the intensity of selection and the generation interval.

Since 1960, global livestock productivity (including carcass weight of meat-producing species, milk yield of dairy cows and egg production) has increased by 20–30% as a result of advances in nutrition, disease control and genetics¹. Genetic improvement has accrued through breed substitution, cross-breeding and within-breed selection. In contrast to the one-off measures of breed substitution and cross-breeding, within-breed selection drives sustained, cumulative genetic progress. It has increasingly relied on sophisticated statistical methods, including mixed model methodology, to provide ever more accurate individual estimated breeding values (EBVs)². Spectacular genetic improvements have been achieved in several species by combining within-breed selection with reproductive technologies (such as artificial insemination and embryo transfer) to more effectively disseminate elite genomes. For example, average annual milk yield per cow in the United States increased from 1,890 kg in 1924 to 9,682 kg in 2011, and more than 50% of this progress was attributed to improved genetics¹. Between 1957 and 2001, the time for broiler chickens to reach market weight decreased threefold despite a decrease in feed consumption^{3,4}. Typically, within-breed selection is expected to result in annual genetic gains of ~1–3%¹.

Currently, the most effective route to minimize the detrimental environmental impact of livestock is to increase productivity: the carbon footprint of 1 kg of milk produced in the United States in 2007 was 37% of that in 1944, and the carbon footprint of the total US dairy industry was reduced by 41% over the same period despite the 250% increase in total milk production⁵. However, exclusive emphasis on production has led to detrimental correlated responses in other traits, particularly those associated with fitness. For example,

although selection for milk production in dairy cattle was extremely successful, there was a substantial undesired decline in fertility over the same period⁶. Thus, selection schemes now increasingly attempt to balance animal health, fertility, production and environmental impact.

The emergence of genomics as a discipline in the 1980s led to the concept of marker-assisted selection (MAS), in which genetic variants and genes that influence agriculturally important traits would be identified and used to further increase genetic response. A global chase for quantitative trait loci (QTL) ensued in all livestock species. QTL with large effects on economically important traits were indeed mapped, first by linkage analyses and then by genome-wide association studies (GWAS), but these did not account for a large enough proportion of the heritability to render them useful selection tools on their own⁷. MAS met with limited enthusiasm from the breeding industry until a landmark paper proposed genomic selection (GS)⁸. In its simplest form, GS makes the same assumption as standard EBV selection: the genetic variance for the traits of interest reflects the additive effects of thousands of variants with very small (and, unlike QTL, unmappable) effects that are uniformly scattered throughout the genome^{2,9}. As soon as genome-wide single-nucleotide polymorphism arrays (SNP arrays) became an affordable reality, GS was tested and was soon widely adopted by the dairy cattle breeding industry as an effective and easily implemented alternative to the time-consuming and costly standard progeny testing (PT). Since 2008, more than 3 million dairy animals have been genotyped worldwide, and GS has become an essential tool for breeding companies that is expected to double genetic progress¹⁰.

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<https://doi.org/10.1038/s41576-018-0082-2>

Quantitative trait loci (QTL). Regions in the genome that encompass genetic variants with an effect on a quantitative trait of interest.

Genome-wide association studies

(GWAS). Scan of the entire genome to identify genetic variants for which variation in genotype is associated with variation for one or more phenotypes of interest.

Genomic selection

(GS). An ensemble of methods to estimate the breeding values of individual animals on the basis of genome-wide single-nucleotide polymorphism genotype information.

Single-nucleotide polymorphism arrays

(SNP arrays). Microarrays used to determine the genotype of individuals for hundreds to millions of SNPs at once.

Progeny testing

(PT). An approach by which the breeding value of an animal is estimated from phenotypic measures made on its progeny.

Genetic architecture

The description of the number, location and effects of the genetic variants that affect a phenotype of interest.

GS is increasingly being adopted by other livestock industries and in plant breeding^{11,12}. Similar methods are now also used in human genetics to study the genetic architecture of common complex diseases and to predict individual disease risk^{13–15}. Although GS as implemented today is expected to enable genetic progress of up to twofold in dairy cattle and layer hens, with more modest gains in other species, it is unlikely to be sufficient to meet the expected 70% increase in the world demand of animal products by 2050 (REF.¹⁶). Further improvements and additions to GS will be needed to meet this target.

In this Review, we examine the status of genomic resources available in the major livestock species (that is, cattle, sheep, goat, pig, poultry and salmon) and how these are being used to accelerate the discovery and management of defect-causing genes, to improve the accuracy and extend the scope of GS, to orient genome editing strategies and to develop new applications that take advantage of the genomic information that is becoming widely available.

Genomic resources for livestock species

New scaffolding methods have dramatically improved livestock reference genomes. Following the lead of the human and mouse genome projects, the animal genomics community generated draft reference genomes for the major livestock species (poultry¹⁷, cattle¹⁸, pig¹⁹, goat²⁰, sheep²¹ and salmon²²), first using Sanger sequencing with hybrid (clone-by-clone and whole genome) shotgun approaches²³, increasingly complemented with massively parallel generation of short reads. These efforts provided initial insights into the evolution of the gene repertoire underlying adaptive features (such as plumage and beak formation, rumination and lactation, wool growth, and smell and taste specification) and into changes resulting from whole-genome duplication in salmon. They also contributed to the identification of evolutionary conserved elements²⁴. However, the quality of most of these reference genomes has remained a source of concern. They were highly fragmented and littered with assembly errors, which affect positional cloning efforts and imputation accuracy, among other uses²⁵. Critical mass and funding have long been missing to upgrade their status from highly fragmented drafts to high-quality finished genomes. However, the development of new scaffolding approaches, including long-read sequences (such as PacBio), optical mapping (such as Bionano Genomics)

and chromatin conformation capture now provides an affordable path to high-quality reference genomes for all species²⁶. The integrated use of these methods has recently enabled spectacular improvements in the quality of reference genomes for goat²⁷ and other livestock species (TABLE 1) (genome assemblies are available through the [NCBI Genome database](#)).

Genome-wide SNP arrays are available for the main livestock species.

Draft reference genomes were typically accompanied by shallow (1–2-fold depth) sequencing of tens to hundreds of individuals representing distinct breeds and populations in order to characterize genetic variation and infer demographic history (see, for example, REFS^{19,28,29}). These efforts have uncovered millions of genetic variants for all the main livestock species and profoundly changed our understanding of the domestication process (BOX 1). Databases of available SNPs (such as [dbSNP](#)) have been used to develop a large number of arrays that allow cost-effective genotyping of tens of thousands to hundreds of thousands of variants in the major livestock species (Supplementary Table 1). These arrays are extensively used to conduct GWAS, as well as GS. It is estimated that at least 3 million cattle and possibly millions of pigs and poultry have been genotyped using genome-wide SNP arrays^{10,11}.

Population-based resequencing for imputation-based GWAS and GS.

As sequencing costs continue to decrease, livestock geneticists are resequencing the genomes of a growing number of animals. More than 2,500 cattle have had their whole genome resequenced, while the corresponding numbers are at least in the hundreds for pig, poultry, sheep and goats (M. Groenen, R. Hawken and G. Tosser-Klopp, personal communications). The best-known large-scale resequencing initiative in livestock genetics is the 1,000 Bull Genomes Project²⁵, but other large sequencing projects are being conducted by academic groups and breeding companies³⁰. These efforts are largely inspired by the human 1,000 Genomes Project³¹ and hope to achieve deep characterization of the genetic variation between and within populations. Importantly, sequencing the whole genomes of a reference population of hundreds of animals enables genotype imputation at millions of common variants in the much larger number of animals that have been genotyped with genome-wide SNP arrays. This approach can be

Table 1 | **Current status of the reference genomes for the most important livestock species**

Species	Assembly	Release date	Coverage	Number of contigs	Contig N50 (Mb)	Total (Gb)
Pig (<i>Sus scrofa</i>)	Sscrofa11.1	7 Feb 2017	65×	1,118	48.2	2.5
Goat (<i>Capra hircus</i>)	ARS1	24 Aug 2016	50×	30,399	26.2	2.9
Cattle (<i>Bos taurus</i>)	ARS-UCD1.2	11 Apr 2018	80×	2,597	25.9	2.7
Chicken (<i>Gallus gallus</i>)	GRCg6a	27 Mar 2018	82×	1,402	17.6	1
Sheep (<i>Ovis aries</i>)	Oar_rambouillet_V1.0	2 Nov 2017	126×	7,485	2.6	2.9
Zebu (<i>Bos indicus</i>)	AM293397v1	22 Feb 2018	100×	337,292	0.064	2.7
Salmon (<i>Salmo salar</i>)	ICSASG_v2	10 Jun 2015	206×	368,060	0.058	3

Box 1 | Genetic variation provides insight into the domestication process

Punctuated versus continuous domestication. One of the most striking insights gained from studying genetic variation in livestock species is the realization that the degree of genetic variation, measured, for instance, by the average heterozygosity per nucleotide site (π), is typically higher in livestock than in humans^{19,28,29}. This observation is against expectations. It is often assumed that animal domestication occurred through rare, isolated events involving a limited number of animals, which would have caused drastic genetic bottlenecks. Further reduction in effective population size would have accompanied more recent breed creation and been accentuated by intensifying selection schemes. However, domestic animal populations remain more variable than the people who domesticated them. This realization forces us to revisit our views of the domestication process. Domestication most likely involved continuous gene flow between domestic and wild individuals from the same species, as well as from inter-fertile sub-species, during most of agricultural history¹⁹¹. As a result, the genomes of the majority of domestic livestock species probably have a mosaic structure that is at least as pronounced as that of the laboratory mouse¹⁹² or human¹⁹³. Some haplotypes segregating within pig and cattle breeds have been shown to differ approximately every 100 bp, which is a similar sequence identity to humans and chimpanzees; thus, they possibly coalesced ~5 million years ago, which is before the creation of the studied species^{118,194,195}.

Hard sweeps, soft sweeps and polygenic adaptation during domestication. Comparisons between the genome sequences of domestic animals and their wild extant or extinct progenitors (that is, red jungle fowl¹⁹⁶, rabbit¹⁹⁷, wild boar¹⁹¹, bezaar¹⁹⁸ and auroch¹⁹⁹) have identified chromosome regions that may have undergone hard sweeps driven by the domestication process. These regions seem to be enriched in genes that control behaviour and stature. The most convincing 40 kb hard sweep signature encompasses the G558R missense mutation in the chicken thyroid stimulating hormone receptor (TSHR), known to have a key role in metabolic regulation and photoperiod control of reproduction¹⁹⁶. These genomic regions may correspond to islands of domestication that resist recurrent gene flow from wild progenitor species¹⁹¹. It is worth noting that the methods used to detect selective sweeps associated with domestication pick up only hard sweeps acting on very rare or de novo mutations. Soft sweeps acting on older and hence more common mutations (that is, standing variation in the wild progenitor) require alternative methods for their detection²⁰⁰. It is also noteworthy that evidence suggests that tame behaviour in rabbits and possibly other species evolved through shifts in allelic frequency at many loci (that is, polygenic adaptation) rather than critical changes at a few domestication loci¹⁹⁷.

Genotype imputation

The *in silico* prediction of the genotype of an individual for ungenotyped variants on the basis of known genotypes at neighbouring variants and a reference population with genotype information for all variants. Imputation exploits the nonrandom association of alleles at neighbouring variants, referred to as linkage disequilibrium.

Soft sweeps

The process by which the frequency of a favourable old variant rapidly increases in the population by positive selection until eventual fixation. Soft sweeps are not associated with the concomitant fixation of one predominant haplotype, as the variant has been distributed over multiple haplotypes by recombination before selection. Old variants that are substrates for new selection constitute the standing variation in the population.

implemented using pyramidal schemes in which a top layer of a few (possibly hundreds) highly influential animals are sequenced, an intermediate layer of ‘multiplier’ animals are genotyped with high-density SNP arrays and the most populated bottom layer of animals are genotyped with low-density SNP arrays. Sequence information is then projected from the upper two layers onto the animals of the bottom layer using a two-step imputation strategy³². Livestock represent a unique opportunity to implement this approach because samples from key ancestors of the population are often available in the form of semen straws or ampules. In the 1,000 Bull Genomes Project, bulls born in the 1960s are included in the set of sequenced animals. The availability of whole-genome sequence information for tens to hundreds of animals from specific breeds has proved extremely useful to pinpoint the causative mutations underlying monogenic defects^{25,33}. Imputation of sequence information on large cohorts of phenotyped animals greatly accelerates fine-mapping and identification of causative variants for QTL detected by GWAS^{25,34}. It is also anticipated that imputed sequence information could increase the accuracy of GS (see Increasing the accuracy of GS using whole-genome sequence imputation).

Epigenome maps and eQTL data sets enable functional follow-up of GWAS hits. It is increasingly recognized that regulatory (rather than coding) variants account for the majority of the genetic variation underlying complex traits, such as common complex diseases in humans or economically important traits in plants and animals^{35,36}. Most of these regulatory variants are expected to affect components of gene switches, that is, proximal promoters and more distant enhancers and silencers. To aid in the identification of such regulatory variants and the genes whose expression they affect, the animal genomics community has begun to generate epigenome maps, mainly using ChIP-Seq (chromatin immunoprecipitation followed by sequencing), DNase-Seq (DNase I hypersensitive site sequencing) and ATAC-Seq (assay for transposase-accessible chromatin using sequencing), which will provide exhaustive catalogues of gene regulatory elements in livestock. Most of these efforts are coordinated through the international Functional Annotation of Animal Genomes (FAANG) project^{37,38}. Liver-specific comparative enhancer maps based on histone modification data have already been generated for 20 mammals, including cow, pig and rabbit³⁹. In addition, bovine DNA methylation maps have been generated for ten somatic tissues using reduced representation bisulfite sequencing⁴⁰.

Epigenome maps are complemented by multi-tissue transcriptome data sets for the analysis of expression quantitative trait loci (eQTL). In cattle, such data sets have been generated for mammary gland, liver, blood and adrenal gland and have been used to identify causative genes underlying GWAS-identified QTL^{41–45}. In pigs, eQTL studies have been conducted in skeletal muscle, lung, adipose tissue and liver^{46–57}. In poultry, genome-wide eQTL analyses have been reported for liver, bone, adrenal gland and hypothalamus^{58–61}. The time seems right for the animal genomics community to take advantage of working with livestock species to collaboratively generate large, multi-omic, multi-tissue data sets similar to the human Genotype-Tissue Expression (GTEx) data set⁶². This approach would provide invaluable comparative information about genome function and greatly facilitate follow-up studies of GWAS and GS hits in these species.

Important Mendelian traits in livestock

The early 20th century saw a heated debate between Mendelists and Galtonists, with Galtonists claiming that Mendelian genes accounted for only a small proportion of inherited features. The debate was settled when it was realized that quantitative traits derive their continuous distribution from the combined effects of many segregating Mendelian genes (that is, they are polygenic traits). It remains true, however, that Mendelian traits — that is, phenotypes that are fully determined by one gene (monogenic) or a small number of genes (oligogenic) — are the exception rather than the rule. In humans, Mendelian traits are largely limited to blood groups and an admittedly long list of severe genetic defects that are compiled in the [Online Mendelian Inheritance in Man](#) (OMIM) database and that include the ‘inborn errors of metabolism’. In addition to blood

Epigenome

The combination of chemical modifications of the DNA sequence (such as cytosine methylation) or nucleosomes (such as methylation of Lys 27 of histone H3) that mark functionally distinct segments of the genome (such as active enhancers) and are inherited mitotically and/or meiotically.

ChIP-Seq

A combination of chromatin immunoprecipitation and next-generation sequencing for genome-wide mapping of binding sites occupied by specific DNA-binding proteins or chromatin regions enriched in specific histone modifications.

DNase-Seq

A method based on next-generation sequencing for genome-wide detection of gene-switch components on the basis of their open chromatin conformation and resulting hypersensitivity to digestion by DNase I.

ATAC-Seq

An assay based on next-generation sequencing for genome-wide detection of gene-switch components on the basis of their open chromatin conformation and resulting increased accessibility to transposase Tn5.

Expression quantitative trait loci

(eQTL). Quantitative trait loci that influence the transcript levels of specific genes. *Cis*-eQTL are due to regulatory variants that control the levels of RNA molecules transcribed from gene copies located on the same DNA molecule as the variant. *Trans*-eQTL are due to regulatory variants that can also control the levels of RNA molecules transcribed from gene copies located on different DNA molecules to the variant (homologous or other chromosomes).

Pleiotropy

The ability of a genetic variant to affect more than one phenotype.

Hypomorphic

Pertaining to an allele with partial loss of function when compared with the wild-type allele.

groups and a similar list of severe genetic defects compiled in the [Online Mendelian Inheritance in Animals](#) (OMIA) database, Mendelian traits in domestic animals also include an extended list of breed-defining characteristics, such as coat colour, tegument variation, polledness, double-muscling and hyper-prolificacy.

Most breed-defining traits have been molecularly characterized. For millennia, animal breeders have performed what amounts to a mega-scale phenotype-driven mutagenesis screen. In the process, they have identified a series of mutations with large phenotypic effects that — when desirable — were selected, often becoming trademarks and breed-defining features. In many instances, mutant variants were valued because of their aesthetic effects on the animals, such as patterns of coat and plumage colour; shape of ears, horns, wattles or combs; and tonality of songs. The long-standing interest of breeders for ‘fancy’ animals is well illustrated by 7,000-year-old rock paintings in the Sahara⁶³. In other instances, the value of the mutant variants reflects their utility. For instance, mutations with major beneficial effects on hair (such as quality of angora or cashmere) and skin texture (such as heat tolerance of slick cattle), fertility (such as twinning) and muscularity (for example, double-muscling) are all highly desired. Although some mutant phenotypes are easily recognized, others are subtler and may require human–animal proximity for their detection. An example of such a phenotype is pacing in horses, which was shown recently to result from a premature stop codon in *DMRT3*, a gene that controls spinal circuitry⁶⁴. It is hard to imagine that such a phenotype could be detected in the systematic phenotype-driven screens that are currently being conducted in the mouse.

Over the past 10 years, as genomic resources and methods improved, the causative genes and mutations underlying most of these breed-defining characteristics have been identified, and a number of dominant themes have emerged⁶⁵ (TABLE 2). Most (75%) of the corresponding mutations are at least partially dominant, that is, heterozygotes express a phenotype; such mutations would have been easier to detect and maintain in the population than recessive ones. A large proportion of mutations affect gene regulation (43%), resulting in gain-of-function phenotypes through ectopic gene expression. Regulatory mutations are often structural (64%) and involve duplications, insertions (including of retroelements), inversions or combinations thereof. The same phenotype is often determined by mutations in the same gene in different species and by allelic series within species, which indicates that mutations of only that gene can generate the corresponding phenotype without major deleterious pleiotropy. Different phenotypes are sometimes caused by allelic series that have evolved one from the other by serial accumulation of multiple mutations.

The molecular dissection of breed-defining traits has revealed some remarkable biology, including the demonstration of serial translocation by circular intermediates, which is likely to be an ancient exon shuffling mechanism⁶⁶, the identification of a hypomorphic *MSN* mutation resulting from the acquisition of an illegitimate microRNA target site⁶⁷ and the interplay between

cis-effects and microRNA-mediated *trans*-effects underlying polar overdominance of the callipyge phenotype in sheep⁶⁸. However, it is noteworthy that the molecular underpinnings of the cashmere and mohair wool types in goat and the very widespread recessive piebald phenotype in cattle remain unknown.

About the number of defect-causing recessive mutations carried per individual. Diploidy has enabled an increase in genome size while ensuring that most individuals in the population have at least one functional copy of each gene. Concomitantly, most individuals are expected to be heterozygous for loss-of-function (LoF) alleles in a number of haplosufficient genes. The analysis of whole-genome sequences from large numbers of individuals indicates that this number is ~100 in humans⁶⁹. It appears to be very similar in livestock species, including the cow³⁰. This estimate is much higher than expected from epidemiological studies, which suggest that humans carry on average ~0.5–1 allele that is lethal when homozygous⁷⁰. This apparent conundrum can be explained by the observation that for the majority of genes (~75%), homozygosity for LoF mutations is viable but confers a modest selective disadvantage that is sufficient to preclude fixation of the mutations, which explains the evolutionary conservation of the corresponding gene. Indeed, data from the [International Mouse Phenotype Consortium](#) indicate that only ~25% of mammalian genes are essential in the sense that at least one functional allele is needed for survival until reproductive age. Homozygosity (or compound heterozygosity) for LoF mutations in the corresponding genes are lethal, either before (embryonic lethal (EL)) or after birth. The number of such recessive lethal alleles that are carried, on average, by healthy individuals has been of considerable interest for a long time⁷¹. Indeed, this number determines, for instance, the increased morbidity endured by offspring of consanguineous marriages or matings. Simulations for mammalian genomes³⁰ suggest that this number increases with effective population size (N_e), from ~0.5 for $N_e = 100$ (which is the N_e for many livestock populations) to ~5 for $N_e = 10,000$ (which is the N_e of the human population). Approximately 1% (independent of N_e) of conceptuses succumb from homozygosity (or compound heterozygosity) for at least one of around ten common EL mutations (frequency >0.02) in livestock compared with at least one among thousands of rare EL mutations in humans³⁰. This observation suggests that managing severe genetic defects in livestock populations (including EL mutations) is a tractable problem that requires the identification and tracking of around ten such common mutations per population. It is noteworthy that in humans (and probably in other mammals), an estimated 3,000 genes are haploinsufficient and hence LoF-intolerant⁷².

Identifying causative mutations for recessive defects has become trivial. Livestock populations are characterized by recurrent outbursts of genetic defects. This is particularly true for species such as cattle in which artificial insemination allows elite sires to have tens

Table 2 | Breed-defining traits that have been characterized at the molecular level

Gene	Species	Transmission	Phenotype	Mutations	
				Coding	Regulatory
Coat or feather colour: melanocyte development					
KIT	Bovine	dom	Colour-sided	–	DUPC6 ^{a,b} , DUPC29 ^{a,b}
	Bovine	1/2 dom	Degree of white spotting	–	Unknown
	Pig	dom	White	(SS + DUP1 ^a + DUP2–4 ^a) ^b
	Pig	dom	Patch	–	DUP1 ^{a,b}
	Pig	dom	Belt	–	(DUP2–4) ^{a,b}
	Pig	codom	Roan	SS	–
KITLG	Bovine	codom	Roan	MS	–
	Goat	codom	Roan	Unknown	–
MIFT	Bovine	dom	White, blue eyes, hearing loss	MS	3bpDEL
	Bovine	1/2 dom	Degree of white spotting	–	Unknown
SOX10	Chicken	rec	Dark brown	–	DEL ^a
TWIST2	Bovine	dom	White belt	–	QUAD ^a
CDKN2A	Chicken	Z-linked dom	Extreme dilution	–	(SNP1–2) ^b
	Chicken	Z-linked 1/2 dom	Dilution	(MS1 + SNP1–2) ^b
	Chicken	Z-linked dom	Barring	(MS2 + SNP1–2) ^b
EDNRA	Goat	1/2 dom	Degree of white spotting	(MS + CNV ^a)
EDNRB2	Chicken	rec	Mottled	MS	–
	Chicken	rec	White	MS	–
Coat or feather colour: melanin synthesis					
MC1R	Bovine	dom	Black	MS	–
	Bovine	rec	Red	FS	–
	Bovine	rec	Telstar	–	Unknown
	Pig	dom	Black	MS1, MS2	–
	Pig	rec	Red	MS3 + MS4	–
	Pig	–	Coat-colour diversity	MS1–8, FS1	–
	Pig	som	Black spotting	(FS + MS2)	–
	Sheep	dom	Black	MS1, MS2	–
	Goat	dom	Black	MS	–
	Goat	rec	Red	SG	–
	Chicken	dom	Extended black	MS1 ^b	–
	Chicken	rec	Buttercup	(MS1 + MS2) ^b	–
ASIP	Bovine	1/2 dom	Brindle	–	INS (LINE) ^a
	Sheep	dom	White or tan	–	DUP ^a
	Sheep	rec	Self-colour black	FS, 9bpDEL, MS	–
TYR	Bovine	rec	Albino	FS	–
	Chicken	rec	Albino	6bpDEL	–
	Chicken	rec	White	–	INS (ERV) ^a
TYRP1	Bovine	rec	Dun	MS	–
	Pig	1/2 dom	Brown or blond	6bpDEL	–
	Sheep	rec	Light coat	MS	–
	Goat	dom	Brown	MS	–
Coat or feather colour: melanin transport					
PMEL	Bovine	rec	Dilution	MS, 3bpDEL	–
	Chicken	dom	White	9bpINS ^b	–
	Chicken	dom	Smokey	(9bpINS + 12bpDEL) ^b	–
	Chicken	dom	Dun	15bpDEL	–

Table 2 (cont.) | **Breed-defining traits that have been characterized at the molecular level**

Gene	Species	Transmission	Phenotype	Mutations	
				Coding	Regulatory
Coat or feather colour: melanin transport (cont.)					
MLPH	Bovine	rec	Cool grey	FS	–
	Chicken	rec	Lavender	MS1	–
	Chicken	rec	Grey dilution	MS2	–
COPA	Bovine	dom	Red	MS	–
SLC45A2	Bovine	rec	Oculocutaneous albinism	MS1, MS2	–
	Chicken	Z-linked rec	Silver	MS1, MS2	–
Muscle mass					
MSTN	Bovine	1/2 dom	Double-muscling	MS1–3, FS1–2, SG1–3, SS1	–
	Sheep	1/2 dom	Double-muscling	FS1, FS2	3′ UTR SNP
DLK1 + PEG11	Sheep	Polar overdominance	Callipyge	–	IG SNP
Dermal features: horns					
BTA1 (1.7–1.9 Mb)	Bovine	dom	Polled	–	INDEL1 ^a , INDEL2 ^a , DUP1 ^a
TWIST1	Bovine	dom	Scurs type 2	FS	–
RXFP2	Sheep	dom	Polled	–	3′ UTR INS (PG) ^a
	Sheep	1/2 dom, sex-dep	Horn size	–	3′ UTR SNP
FOXL2	Goat	dom	Polled	–	DEL ^a
Dermal features: hair, feather or shell structure					
KRT27	Bovine	dom	Curly coat	MS	–
PRLR	Bovine	dom	Slick coat	SG	–
	Chicken	Z-linked dom	Slow feathering	–	DUP ^a + INS ^a (ERV)
IRFBP2	Sheep	rec	Short and woolly fleece	–	3′ UTR INS (PG) ^a
FMN1/GREM1	Goat	dom	Wattles	Unknown	–
PPARD	Pig	1/2 dom	Large and floppy ears	MS	–
SLCO1B3	Chicken	1/2 dom	Blue eggshell	–	INS1 (ERV) ^a , INS2 (ERV) ^a
KRT75	Chicken	1/2 dom	Frizzled feathering	69bpDEL	–
CYP19A1	Chicken	dom, sex-dep	Henny feathering	–	INS (ERV) ^a
PDSS2	Chicken	rec	Silk feathering	–	SNP
HOXB8	Chicken	1/2 dom	Muffs and beard	–	CNV ^a
GDF7	Chicken	1/2 dom	Naked neck	–	INS ^a
FGF20	Chicken	rec	Featherless or scaleless	SG	–
Dermal features: comb					
SOX5	Chicken	dom	Pea comb	–	DUP ^a
MNR2	Chicken	dom	Rose comb	–	Complex CNV ^a
SOX5 + MNR2	Chicken	epistasis	Walnut comb	–	(SOX5 DUP ^a + MNR2 CNV ^a)
EOMES	Chicken	1/2 dom	Duplex comb	–	DUP ^a
Dermal features: pigmentation					
BCO2	Sheep	rec	Yellow fat	SG	–
	Chicken	rec	Yellow skin and shank colour	SG	–
EDN3	Chicken	dom	Silkie and dermal hyperpigmentation	–	Complex CNV ^a

Table 2 (cont.) | Breed-defining traits that have been characterized at the molecular level

Gene	Species	Transmission	Phenotype	Mutations	
				Coding	Regulatory
Fertility					
BMP15	Sheep	X-linked 1/2 dom	Prolificity	MS1–5, SG1–2, FS1–2	–
BMPR1B	Sheep	1/2 dom	Prolificity	MS	–
GDF9	Sheep	1/2 dom	Prolificity	MS1–5	–
B4GALNT2	Sheep	1/2 dom	Prolificity	–	(SNP1 + SNP2)

Multiple mutations constituting one allele are bracketed. CNV, copy number variant; codom, co-dominant; DEL, deletion; dom, dominant; DUP, duplication; ERV, endogenous retrovirus; FS, frameshift; IG, intergenic; INDEL, insertion and/or deletion; INS, insertion; LINE, long interspersed nuclear element; MS, missense; PG, pseudogene; QUAD, quadruplication; rec, recessive; sex-dep, sex-dependent; SG, stop gain; SNP, single-nucleotide polymorphism; som, somatic; SS, splice site; UTR, untranslated region; xbpINS, x bp insertion; ybpDEL, y bp deletion. ^aStructural variants. ^bAllelic series involving the serial accumulation of multiple mutations.

Overdominance

The phenotypic superiority (for example, on a quantitative scale) of heterozygotes ('Aa') over both homozygous classes ('AA' and 'aa').

Haplosufficient

Pertaining to genes for which one functional copy is sufficient to ensure normal development and function.

Compound heterozygosity

Pertaining to the inheritance of two distinct mutations in different alleles of the same gene, one from each parent.

Autozygosity mapping

Mapping of a recessive mutation on the basis that all affected individuals will be homozygous for the same (autozygous) haplotype. Typically applied in genetically isolated populations in which the hypothesis of allelic homogeneity is reasonable.

Modifier locus

A locus with variants that may (depending on the genotype of the individual) affect the phenotypic expression conferred by specific variants at another locus. The effects of modifier loci include suppression and epistasis.

Reverse genetic screens

Process aimed at completing the phenotype–genotype map by sorting individuals according to their genotype at a variant with unknown function and searching for shared phenotypes, as opposed to forward genetics, which consists of sorting individuals according to a phenotype and searching for shared variants.

of thousands to hundreds of thousands of offspring, thereby disseminating their deleterious mutations in the general population and leading to the emergence of affected individuals in subsequent generations. Before the advent of genomics, determining whether an elite sire carried such a common defect required time-consuming and expensive PT, that is, mating the sire with affected dams when possible (such as for mule-foot)⁷³ or to known carriers or daughters and verifying the occurrence of affected offspring. In the 1980s, it became possible to positionally clone the responsible gene, with the aim to develop a diagnostic marker. However, this was a very tedious multi-year effort that often required the generation of informative pedigrees to prove the genetic nature of the condition (which was not always obvious otherwise) and allow linkage mapping. This situation changed dramatically when genome-wide SNP arrays became available, which enabled effective autozygosity mapping using only a few affected individuals⁷⁴. More recently, whole-genome resequencing of small numbers of affected individuals has enabled autozygosity mapping and, in approximately half of cases, simultaneous identification of the causative mutations in a matter of days, especially when taking advantage of sequenced reference populations such as the 1,000 Bull Genomes Project²⁵ (FIG. 1a). An increasingly common approach for the management of recessive defects in livestock is to systematically collect samples from animals with severe defects and to sequence the whole genome of small sets of animals with similar symptoms as soon as they become available. Identifying an autozygous genomic segment — or better, a causative mutation — shared by all affected individuals confirms the genetic origin of the defect and readily provides a diagnostic test. As a result of these advances, the list of characterized defects in livestock in OMIA has markedly increased in recent years (FIG. 1b).

Germline mosaicism for deleterious dominant mutations is common. A number of genetic defects that were assumed to be recessive because of their rarity — only a small number of affected animals were detected among thousands of offspring from a healthy sire — could not be mapped by autozygosity mapping. In several cases, these defects were demonstrated to be caused by dominant de novo mutations (DNMs) for which the sire

was germline mosaic^{25,33,75,76}. Unlike in humans, who typically produce only one affected offspring, samples were available from a number of affected animals, which greatly facilitated the identification of the causative mutation. In at least one instance, neurocristopathy, it also enabled a modifier locus that affected disease severity to be mapped³³.

Preliminary studies of the bovine germ line have shown that sires are mosaic for ~30% of DNM present in a sperm cell and that dams are mosaic for ~50% of DNM present in an oocyte⁷⁷. These numbers may be considerably higher than those in humans. Our preliminary data suggest that early embryonic cleavage is 20-fold more mutation-prone than cell divisions occurring later in development⁷⁷ and that this increased rate of DNM might be due, in part, to reproductive technologies routinely used in livestock, such as the combination of oocyte pick-up, in vitro oocyte maturation and in vitro oocyte fertilization (C.C., unpublished observations).

Reverse genetic screens for EL mutations that compromise carrier fertility. It is likely that for the majority of LoF variants in essential genes, homozygosity will result in embryonic or fetal lethality rather than a genetic defect manifesting at or after birth. The economic effect of such mutations when carried by influential sires, in terms of lost pregnancies, can run into hundreds of millions of dollars^{78,79}. Embryonic lethality is a complex phenotype that is not observed directly and hence is difficult to study. Daughters of sires that carry EL mutations are expected to be less fertile (as measured, for instance, by the probability to return to heat after insemination), but the effect on the sire's EBV is detectable only for EL mutations that have reached high frequencies in the population⁸⁰. Reverse genetic screens have been devised to overcome this issue. First, scientists have mined large genome-wide SNP data sets for haplotypes with considerable autozygous depletion, that is, haplotypes for which no individuals are found to be homozygous despite some being expected (FIG. 1c). At least 17 such haplotypes have been identified in cattle^{78,81–83} and 4 in pig^{84,85} (Supplementary Table 2). In a number of cases, mining of population-based resequencing data sets in the corresponding genomic regions has identified the causative mutations^{25,79,86–88}. However, to be effective, this approach requires that the haplotype and the causative

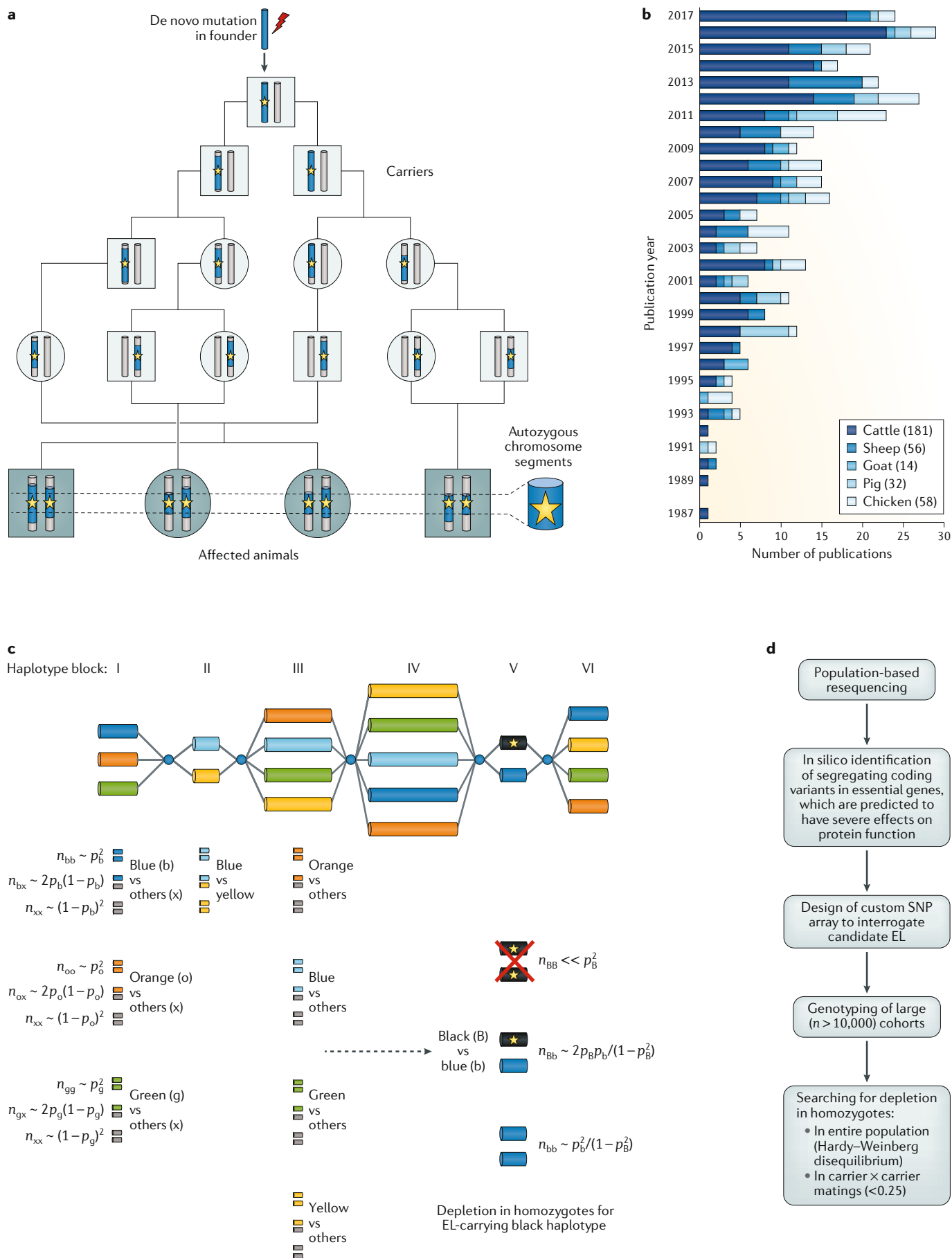


Fig. 1 | Identification of mutations and genes causing monogenic defects in livestock. **a** | Autozygosity mapping: a defect-causing mutation (yellow star) in a haplosufficient gene appears by de novo mutation on a specific haplotype (labelled in blue) in a founder individual. The mutation spreads in the population as it is transmitted to descendants of the founder. In subsequent matings between healthy carriers, 25% of individuals are affected and are homozygous for the mutation and at least part of the blue haplotype of the founder. **b** | Number of monogenic traits for which the causative mutation was discovered by publication year and livestock species (data compiled from Online Mendelian Inheritance in Animals). **c** | Identification of haplotypes carrying embryonic lethal (EL) mutations: haplotypes are identified within blocks, and a Hardy–Weinberg-based test for their depletion in homozygotes in the population is performed for each haplotype. As the limits between haplotype blocks are poorly defined in livestock, window-based or hidden Markov model-based approaches are used. **d** | Flow chart of the steps involved in a sequence-based reverse genetic screen for EL mutations in livestock. SNP, single-nucleotide polymorphism.

mutation are in near perfect linkage disequilibrium (LD), and this is certainly not always the case. An alternative approach, therefore, is to mine the sequence data for candidate EL mutations (that is, LoF variants in essential genes) and to genotype these directly in large cohorts. The absence of homozygous individuals, particularly in the offspring of carrier-by-carrier matings, strongly supports the EL nature of the corresponding variants. Using this approach, nine EL were uncovered in cattle that would not have been detected using haplotype-based approaches³⁰ (FIG. 1d; Supplementary Table 2).

Culling carriers of deleterious recessive variants is not the right approach. Once information became available about EL mutations, the spontaneous reaction of breeding companies was to cull breeding animals, particularly sires, that carry known deleterious mutations in order to assure customers that their animals will not have a known recessive defect. However, as the number of identified mutations increases, the proportion of ‘mutation-free’ animals becomes vanishingly small. Furthermore, excessive use of such mutation-free sires is bound to cause the emergence of novel defects in subsequent generations and hampers genetic progress for other traits. Instead, the costs of the defect should be properly modelled and weighted against the economic value of the other traits in a selection index. Alternatively, the dam population could be genotyped to avoid matings between parents carrying the same deleterious variants (see From selecting animals to selective matings using genomic information), or cross-breeding could be used, as it is less likely that the same EL defects segregate in different breeds.

GS for complex agricultural traits

With the exception of the breed-defining characteristics, inherited defects and EL mutations discussed above, nearly all economically important traits in livestock are complex polygenic traits. They include milk yield and composition (that is, milk protein and fat yield), carcass yield, composition and quality (for example, marbling and tenderness), egg yield, growth rate, feed efficiency, fertility and disease resistance. Although some of these traits are categorical, most are continuously distributed quantitative traits. The phenotypic variation of these traits is assumed to reflect the combined effects of developmental noise, differences in environmental

exposure and genotypic differences at a large number of loci scattered throughout the genome. The heritability (that is, the proportion of the trait variance that is caused by genetic factors) of these economically important traits in livestock typically ranges from ~5% to 50%. For a handful of traits, QTL mapping studies and GWAS analyses have identified loci with relatively large effects on the considered trait. However, the joint effects of these detectable loci typically explain only a limited fraction of the overall heritability. The most convincing evidence indicates that the remainder of the heritability is highly polygenic, corresponding to hundreds if not thousands of genetic variants that each has a very small effect on the trait of interest⁸⁹. The contribution of each variant to overall heritability is assumed to be mainly additive, that is, dominance and epistasis account for only a modest fraction of the variance⁹.

Before genomic tools made it possible to identify specific loci by mapping experiments, quantitative geneticists made the simplifying assumption that heritability of quantitative traits was determined by an infinitely large number of variants of infinitely small effect that were spread uniformly throughout the genome. This model is known as Fisher’s infinitesimal model and is one of the pillars of quantitative genetics theory². The infinitesimal model makes predictions about the expected phenotypic similarity of relatives as a function of their relatedness, underpins the methods used to estimate heritabilities and breeding values (BVs) of individuals in pedigreed populations and guides the design of selection schemes in plant and animal breeding. The proven efficacy of plant and animal breeding and the good fit of genetic progress to predictions based on the infinitesimal model⁸⁹ strongly suggest that a sizeable proportion of the genetic architecture of most quantitative traits can be approximated reasonably well by the infinitesimal model.

GS under the infinitesimal model. One of the key factors that determines the rate of genetic progress in a breeding programme is the accuracy of selection, that is, the accuracy with which animals with the best BVs are chosen as parents of the next generation. Among the methodological breakthroughs that have contributed most to improving the accuracy of selection is the development and use of mixed models to estimate BVs⁹⁰. In these linear models, known environmental factors are fitted together with the BVs of individual animals, which are fitted as random effects. In attempting to find the best solutions for the random BVs, prior knowledge of their joint distribution is used. For instance, because full-sibs or parents and their offspring are closely related, their EBVs are expected to be more similar than those of unrelated animals. The infinitesimal model makes precise predictions about the shape of this joint distribution: BVs are normally distributed, and the covariance between the BVs of two animals is a simple function of their relatedness (that is, their kinship coefficient)^{91,92} and the genetic variance of the trait. BVs for millions of animals can be simultaneously estimated from corresponding sets of linear equations, yielding so-called best linear unbiased predictors (BLUPs) of the EBVs (BOX 2).

Haplotypes

A combination of alleles at multiple variant positions transmitted by a gamete. The term is often used to describe variants that are located close to each other in the genome.

Linkage disequilibrium

(LD). The nonrandom association of alleles at two or more loci, which is manifest by the over-representation of specific haplotypes and the concomitant under-representation of others.

Selection index

A weighted sum of breeding values for several traits, each weighted by economic or perceived relevance.

Kinship coefficient

A measure of genetic relatedness between two individuals. The kinship coefficient corresponds to the probability that two alleles (one from each individual) drawn at random from the two possible alleles (maternal and paternal) for each individual for a randomly selected locus in the genome are identical by descent. The kinship coefficient between two individuals corresponds to the expected inbreeding coefficient of their putative offspring.

Box 2 | **BLUP versus GBLUP**

BLUP uses pedigree information to estimate BVs. Livestock selection has relied for decades on the use of mixed model methodology to estimate breeding values (BVs). The animals' phenotypes are assumed to reflect differential exposure to known environmental factors (such as herd-year-season effects), as well as differences in intrinsic genetic ability (that is, the animals' BVs). BVs are modelled as random effects with multivariate normal distribution. Following standard quantitative genetics theory, the covariance structure of the BVs is assumed to reflect genetic relatedness:

$$\sigma_{ij} = 2\Theta_{ij}\sigma_A^2 \quad (1)$$

where Θ_{ij} is the kinship coefficient of individuals i and j and σ_A^2 is the additive genetic variance, where the matrix of $2\Theta_{ij}$ values is known as the additive relationship matrix A . Note that this model implicitly assumes the infinitesimal model. Kinship coefficients are computed from pedigree information and used to constrain the BV solutions, yielding so-called best linear unbiased predictors (BLUPs)^{2,90}. BLUP-type approaches are extremely efficient and are able to extract information from millions of equations. An interesting feature of BLUP is that it allows estimation of BVs for animals without phenotypic records, including BVs for number of eggs or quantity of milk for males and BVs for unborn animals. Thus, BLUP can be seen as a method of statistical learning, in which the model is trained on a reference population that has both information on phenotypes and genetic relatedness and can then be used to predict the phenotype (including individual BVs) for animals for which only relatedness information is available. For a non-inbred offspring with no record of its own but with perfect information for the parents, the maximum accuracy of BLUP is 0.707 (the square root of 0.5, as half the genetic variance is between families and half is within).

GBLUP uses genome-wide SNP information to predict Mendelian sampling. In genomic BLUP (GBLUP), the kinship coefficient, Θ_{ij} , between two individuals is typically estimated as the correlation between standardized (that is, standard deviations from the mean) allelic dosages across all single-nucleotide polymorphisms (SNPs)^{201,202}. The matrix of $2\Theta_{ij}$ values computed from SNP data is known as the genomic relationship matrix G . The accuracy of GBLUP is a function of the size of the training population (N), the trait heritability (h^2) and the number of loci affecting the trait (M_e)^{203,204}. A simple equation that incorporates these parameters is:

$$M_e = \sqrt{Nh^2 / (Nh^2 + M_e)} \quad (2)$$

Under the infinitesimal model, M_e corresponds to the effective number of independent chromosome segments in the population. Various estimates for M_e are available, with the simplest being:

$$M_e = 2N_eL \quad (3)$$

where N_e is the effective population size and L is the length of the genome in Morgans²⁰⁴. M_e is approximately 6,000 in Holstein-Friesian cattle and 45,000 in Merino sheep. Assuming the same-sized reference population, genomic estimated BV (GEBV) will be more accurate in cattle^{205–207}. Note that this formula for the accuracy of GBLUP is population-based — M_e is the number of independent chromosome segments in the population. If there are large full-sib or half-sib families in the population, accuracy is increased in some cases by taking advantage of the fact that within these families, M_e is much smaller²⁰⁴. Estimates of M_e can also take into account the effect of family structure between the reference population and selection candidates, as well as the covariance among chromosome segment effects, when N_e is low²⁰⁸. The maximum accuracy of GBLUP approaches 1 for progeny with no records of their own, given an extremely large reference population. GBLUP can be seen as estimating SNP effects but also as using an estimator (based on SNPs) of realized instead of expected (based on pedigree) relationships²⁰⁹. The difference between expected and realized relationships results in the improved accuracy²⁰⁴.

Until approximately 10 years ago, kinship coefficients (needed to estimate BVs) were computed using genealogical information. Once genome-wide SNP arrays became available for livestock species, it became possible to estimate kinship coefficients from SNP genotypes

rather than pedigree data^{93–95}. Estimating EBVs with mixed models using kinship coefficients deduced from SNP information is referred to as genomic BLUP (GBLUP) as opposed to standard pedigree-based BLUP, the corresponding estimates are known as GEBVs instead of EBVs, and the corresponding approach to breeding is called genomic selection (GS) (BOX 2).

One advantage of GBLUP over BLUP is that it is not reliant on pedigree information, which may not always be available or accurate. However, its main advantage is that SNP information better tracks and captures Mendelian sampling than does pedigree information. For example, on the basis of pedigree information only, full-sibs all have the same EBV. By contrast, SNP information enables allelic transmission to be tracked at positions where the parents are heterozygous and can therefore differentiate sibs. This information can have a major effect on the accuracy and utility of EBVs. Before GS, candidate elite dairy sires that had identical EBVs based on pedigree information (for instance, because they were full-sibs) required expensive and time-consuming PT to expose differences in the BVs: their individual EBVs were estimated from the performances of tens to hundreds (depending on the country) of daughters, and PT took at least 5 years at a cost of ~US\$50,000 per bull¹⁰. By contrast, as long as the reference population is large enough, GBLUP provides information of a similar accuracy to PT but at birth or earlier if SNP genotyping is performed on blastocyst biopsies (FIG. 2). For traits with high heritability (such as milk yield, $h^2 \approx 0.3$), the accuracy of GBLUP is typically somewhat lower than that of a combination of PT and BLUP. However, this modest penalty is more than compensated for by the fact that the information becomes available 5 years earlier. For low heritability traits (such as fertility, $h^2 \approx 0.05$), GBLUP information is not only available 5 years earlier but can even be more accurate than results obtained with PT combined with BLUP, depending on the size of the daughter groups used for PT. It is, therefore, no surprise that GBLUP was eagerly adopted worldwide by the dairy cattle breeding industry. As large-scale SNP genotyping rapidly became an outsourced commodity, the implementation of within-breed GBLUP in place of BLUP initially required only modest methodological adjustments, which also greatly facilitated the transition¹⁰.

The use of phenotypic records of hundreds of thousands to millions of pedigreed animals across many generations made a substantial contribution to the accuracy of BLUP. Most of these animals could obviously not be retrospectively SNP genotyped. However, statistical methods have been devised to combine valuable pedigree information with SNP data when available. These single-step approaches are now routinely used in practice^{96–101}.

An evaluation of the impact of 7 years of GS in US dairy cattle¹⁰² shows that the rates of genetic gain per year increased 50–100% for high heritability traits, such as milk yield, and 300–400% for low heritability traits, such as somatic cell counts (SCCs, a measure of udder health) and daughter pregnancy rate (a measure of female fertility). Thus, GS enabled genetic gain that

Hard sweeps

The process by which the frequency of a favourable new variant rapidly increases in the population by positive selection until eventual fixation of the variant and the haplotype upon which it occurred.

Balancing selection

A selective force on a locus that leads to a steady state whereby multiple alleles are simultaneously maintained in the population, rather than one allele becoming fixed at the expense of the others.

was not dominated by production but was instead balanced between traits. As expected, the generation interval dramatically shortened, from 7 years to 2.5 years in the 'sire of bull' path (that is, elite bulls used to breed the next generation of bulls). The rate of inbreeding did not seem to be affected. Although the gains are less than those in dairy cattle, GS has also been applied and deemed profitable in defined contexts in beef cattle^{103,104}, pigs¹⁰⁵, sheep^{106–108} and layer chickens¹⁰⁹.

Another potential advantage of GS is that new traits can be included in selection indices to maximize genetic gain for profit. Provided a suitable reference population for these new traits can be constructed, genotyped selection candidates will have a GEBV for these traits, and selection decisions can be made accordingly. For example, including GEBV for feed efficiency in the selection index for dairy cattle is expected to improve genetic gain for profitability by at least 3%¹¹⁰.

Accommodating non-normally distributed gene effects with Bayesian approaches.

As with BLUP, GBLUP assumes that all segments of the genome contribute equally to the heritability of the trait, in accordance with the infinitesimal model. It can be shown that this is mathematically equivalent to a BLUP model that fits the effect of individual SNPs¹¹¹. However, GWAS indicate that this assumption (that the effect of each SNP comes from a normal distribution, with the same variance across all SNP, such that all SNP effects are small) may not always be appropriate. Indeed, effects of a magnitude that is virtually impossible under this model have been identified and with GBLUP, their effects will be over-conservatively regressed downwards in genomic predictions. Examples of such major gene effects segregating within breeds include, among others, variants in *MSTN* in cattle^{112–115} and sheep⁶⁷ and *RYR1*, *PRKAG3* and *IGF2* in pig^{116–118}, which all affect muscularity; *DGAT1*, *GHR* and *ABCG2*, which affect milk yield and composition in cattle^{119–121}; and *PLAG1*, *HMGA2* and *LCORL*, which affect stature in cattle^{122,123}.

To better accommodate such large effects in GS, alternative prior distributions have been considered, mostly in a Bayesian framework. For instance, BayesA assumes a Student's *t* distribution of effects, which makes somewhat larger effects more likely⁸; BayesB assumes a mixture distribution with a large spike of zero effects and a Student's *t* distribution for the remaining effects⁸; BayesC π assumes many effects at zero and the rest following a normal distribution¹²⁴; and BayesR assumes SNP effects follow a multi-normal distribution¹²⁵. The accuracy of GEBV will be highest when the prior distribution best matches the true distribution of SNP effects¹¹¹. For example, for milk production traits (particularly fat percentage) affected by the *DGAT1* mutation in cattle, accuracies of GS are higher with BayesA and BayesB than with GBLUP¹²⁴.

The same methods can be used to study the genetic architecture of traits of interest. For example, a Bayesian method and a large number of dairy cattle with imputed whole-genome sequence data and milk production phenotypes were used to estimate the number of loci affecting these traits³². The study estimated that for milk yield, 4,330 SNPs had a non-zero effect, with only 7 SNPs

explaining 1% or more of the genetic variation. Similar numbers were obtained for milk fat and protein yield.

Balancing selection for variants with large effects is common in livestock. The occurrence of variants with large effects on complex quantitative traits seems to be much more common in livestock than in humans. This may be due, in part, to the strong directional selection to which nearly all livestock populations are subjected. DNMs with large effects on the selected traits sequentially undergo hard sweeps, causing large effects detectable by GWAS until the corresponding variants reach fixation¹²⁶. Furthermore, genomic studies have provided ample evidence that several variants with large effects are maintained in livestock populations by balancing selection through a variety of mechanisms¹²⁷.

For instance, a growing list of variants is known to improve performance in heterozygotes but cause a defect in homozygotes, and these variants are hence subject to balancing selection. Classic examples include a *RYR1* variant in pigs that increases carcass yield in heterozygotes but causes porcine stress syndrome and related syndromes in homozygotes¹¹² and bovine *MSTN* LoF variants that increase muscle mass in heterozygotes but cause birthing difficulties for mothers of homozygous calves. Accordingly, the double-muscling phenotype, which is caused by homozygosity or compound heterozygosity for *MSTN* LoF variants, is avoided in most cattle breeds. In Belgian Blue cattle, disruptive variants in at least four genes (*MRC2*, *RNF11*, *WWP1* and *ATP2A1*) are known to increase muscularity in heterozygotes but affect viability or fitness in homozygotes^{30,128–130}. Mutations in the ovine *BMP15* and *GDF9* genes increase litter size in heterozygotes but cause sterility in homozygotes^{131,132}. In Scandinavian dairy cattle, a specific haplotype increases milk production in heterozygotes but is lethal in homozygous embryos⁸⁰.

Balancing selection also affects pleiotropic variants that have a positive effect on a desired trait but a negative effect on another trait. For example, a missense mutation in the SH2 domain of *SOC2* in sheep increases stature and milk yield but also increases susceptibility to mastitis¹³³. Similarly, an allele of the relaxin-like receptor 2 (*RFXP2*) locus in Soay rams increases horn size (and hence reproductive success) but reduces survival¹³⁴.

The *CLPG* mutation, which causes callipyge muscular hypertrophy in sheep, is an example of polar overdominance, by which only heterozygous animals inheriting the mutation from a particular parent (the sire in the case of *CLPG*) express the phenotype^{68,135,136}. Salmon provide a remarkable example of balancing selection of *VGLL3* variants, in which antagonistic selection for age at maturation in males versus females creates sexual conflict that is partially resolved by sex-dependent dominance^{137,138}.

Other examples of balancing selection may reflect breeding objectives that change over time or differ between countries. For example, the K232A *DGAT1* variant in cattle results in increased fat yield but decreased protein yield. As breeding objectives evolved, this allele went from being favoured to penalized to neutral¹¹⁹. The segregation of *PLAG1* variants that have a major

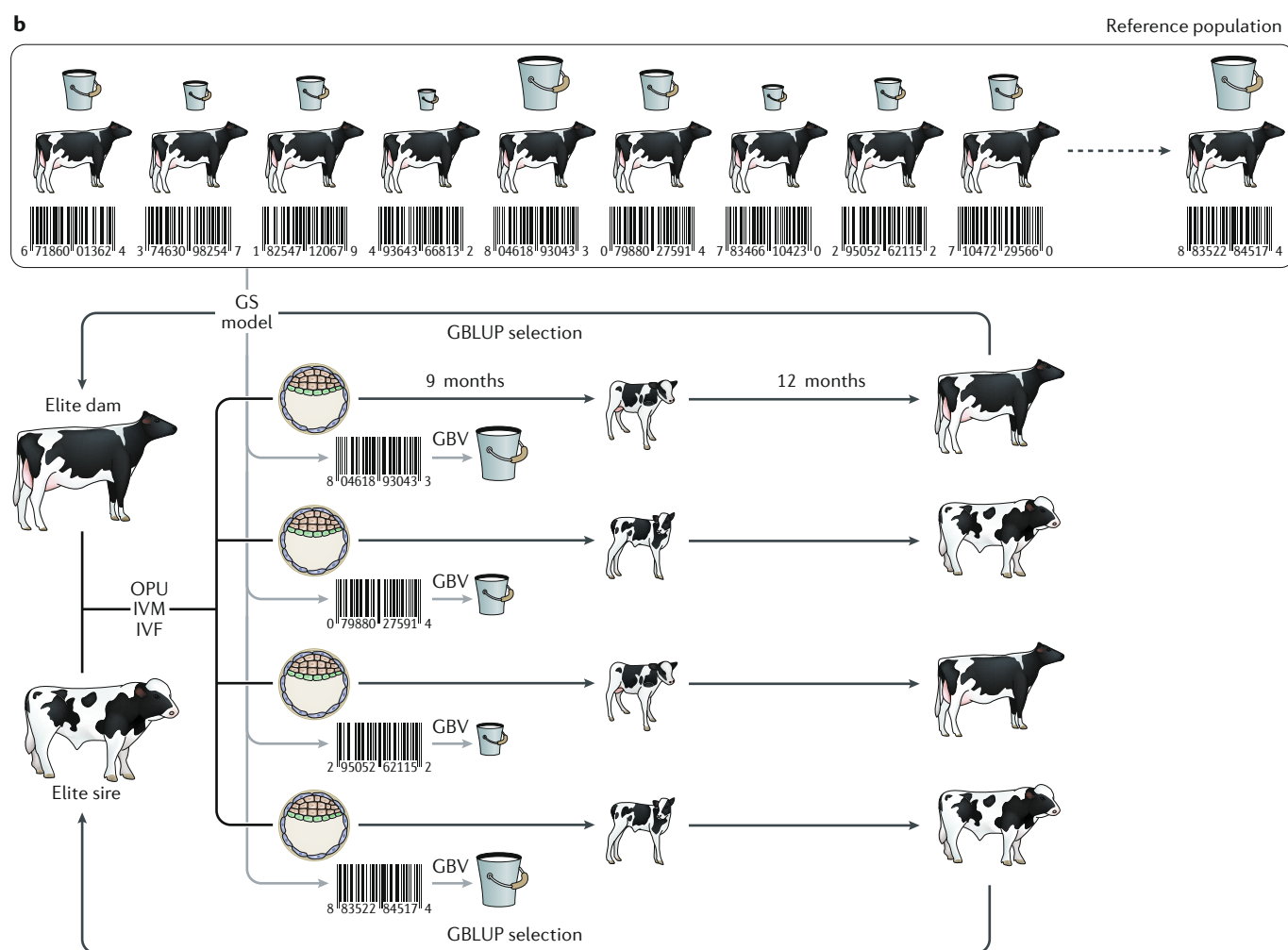
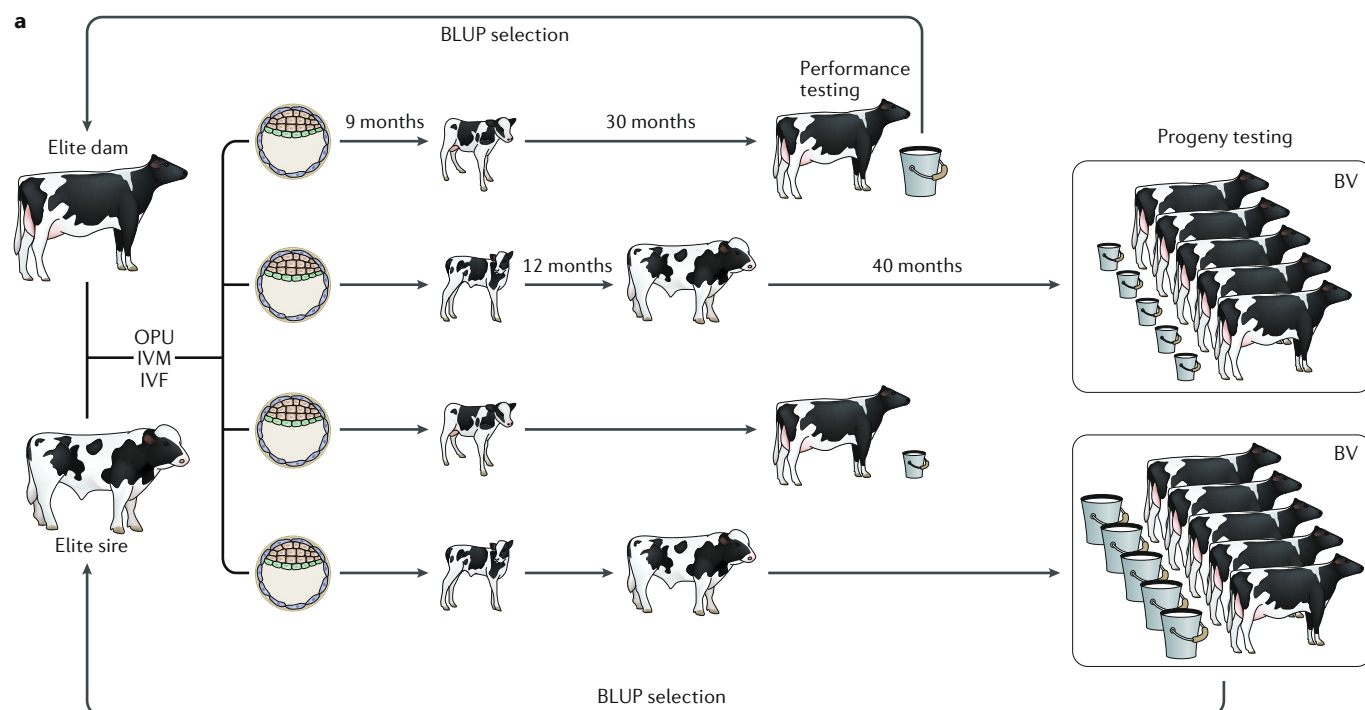


Fig. 2 | Selection procedure of elite dairy sires and cows. a | Before genomic selection (GS), elite sires and cows with the highest estimated breeding values (EBVs) were mated, often using reproductive technologies such as oocyte pick-up (OPU), in vitro maturation (IVM) and in vitro fertilization (IVF) to generate multiple embryos. Female calves (born after 9 months) would produce milk after ≥ 30 months, providing direct information about their value (in a performance test). Male calves would produce semen after ≥ 12 months and, ≥ 40 months later, milking daughters providing information about their breeding values (EBVs) (in a progeny test). Best linear unbiased predictors (BLUPs) of the EBVs of the new generation of cows and sires were computed using mixed models fitting environmental effects and using pedigree information to constrain the solutions of the EBVs. **b** | With GS, elite cows and sires are mated (often using OPU, IVM and IVF), and genomic DNA is extracted from offspring at birth or even as embryos before implantation and genotyped with genome-wide single-nucleotide polymorphism (SNP) arrays. Genomic BLUPs (GBLUPs) of the offspring EBVs are then computed using DNA information to constrain the solutions of the genomic EBVs (GBVs). The statistical models for GS are trained on a reference population of animals that have both SNP genotypes and phenotypic information.

effect on stature may occur because, after an extended period of selection for smaller cattle (in comparison to the ancestral auroch), larger cows are now preferred for some breeds in some countries but not others^{112,139}. Moreover, the *PLAG1* variants that increase stature may negatively affect fertility, particularly age at puberty¹⁴⁰.

A more complete understanding of balancing selection operating at specific loci could be exploited to prioritize or avoid specific matings in breeding programmes (see From selecting animals to selective matings using genomic information).

Increasing the accuracy of GS using whole-genome sequence imputation. To control costs, GS is typically conducted using low-density or, at best, medium-density SNP arrays that interrogate 10,000–50,000 SNPs. With the exception of the handful of thoroughly studied major genes described above, causative variants remain unknown and have, therefore, seldom been included on the arrays. GS presumably works through indirect association, that is, because causative variants are in LD with one or several of the genotyped variants. Directly interrogating the causative variants would most certainly be better. Indeed, LD between causative and tagging variants is likely not perfect (that is, $r^2 < 1$) most of the time. LD will further decay — and the accuracy of GS will decrease — as the number of generations separating the reference population from the selection candidates increases¹⁴¹. Reproductive technologies, such as in vitro fertilization (IVF) of oocytes from prepubertal heifers, will increasingly enable selection based on SNPs only over multiple shortened generations, further exacerbating this issue.

One way to compensate for the fact that most causative SNPs are not directly interrogated on the arrays is to impute full sequence information on genotyped animals. Population-based resequencing efforts have been undertaken to generate reference populations needed for accurate imputation of at least the common single-nucleotide variants; rare variants, structural variants (including copy number variants (CNVs)) and DNMs are typically poorly imputed or ignored in this process. However, as the number of common variants to consider increases from tens of thousands to millions without a concomitant increase in phenotypic data points, so too does the curse of dimensionality; that is, it becomes more difficult

to accurately estimate the effects of the growing number of SNPs. Although numerical methods have been developed to handle millions of variants^{101,142–145}, indiscriminate use of whole sequence information has only modestly increased ($\leq 5\%$) the accuracy of genomic predictions^{146–148}. Strategies to further improve the accuracy of whole-genome-sequence-based GS currently involve either selecting or assigning more weight to a subset of imputed variants that are more likely to be causative.

A first approach consists of ranking the variants based on their strength of association with the trait of interest. Variant effects should ideally be estimated by fitting them all simultaneously in the model. Increases in GS accuracy of up to 6% have been obtained by adding 1,623 top variants detected by sequence-based GWAS to the default 50,000 genome-wide SNPs in European dairy cattle¹⁴⁶.

A second approach aims to exploit prior biological information. Causative variants are either coding or regulatory. Coding variants can be readily identified by mining the sequence data. For example, more than 100,000 non-synonymous variants, which are expected to be enriched in causative variants, have been identified in cattle, and these can be ranked according to the predicted severity of the amino acid substitution on protein function^{25,30}. Regulatory variants are more difficult to identify. Nearly 1 million evolutionarily constrained elements that overlap potential promoters, enhancers and insulators have been identified²⁴. SNPs in these elements are likely to be enriched in regulatory variants. However, growing evidence suggests that enhancers, in particular, are rapidly evolving^{39,149}. Thus, the establishment of exhaustive catalogues of regulatory elements in livestock will likely require the generation of species-specific and tissue-specific epigenome maps, and this is the aim of the FAANG project³⁷. Much remains to be learned about how variants perturb regulatory elements, including whether they need to be within the element or can influence regulatory function from a distance. Transposable elements (TEs) are also a possible source of causative regulatory variants. TEs are still active in the genome of livestock species and generate numerous polymorphic insertion sites, of which several are likely to influence the expression levels of neighbouring genes¹⁵⁰.

Finally, it is possible that QTL information for intermediate phenotypes, including eQTL, will help pinpoint causative regulatory variants¹²². The contribution of regulatory variants to intermediate phenotype variation is likely to be higher than that for the agricultural trait that they influence, which could facilitate fine-mapping¹⁵¹. Intermediate phenotypes of particular interest in livestock are fatty acid profiles, as these affect the health and monetary values of animal food products. Across cattle, pigs and sheep, mutations of moderate to large effect have been mapped to or close to a small number of key genes involved in fatty acid synthesis^{152–154}. eQTL information can certainly help to identify the target genes whose expression is perturbed by these regulatory variants^{41,42,155}.

Methods are also being developed to better take advantage of prior association or biological information for GS. Variants that are more likely to be

Non-synonymous

Variants that cause a change in the amino acid sequence of a protein. By contrast, synonymous variants are variants in the open reading frame of a protein-coding gene that do not change the amino acid sequence. Most non-synonymous variants affect the first and second codon positions, while most synonymous variants affect the third codon position.

Intermediate phenotypes

Phenotypes that mediate the link between a causative variant and the end-point disease or agricultural phenotype of interest — includes transcript, protein and metabolite levels.

functional based on biological information, including gene expression, can receive a higher prior probability in the model¹⁵⁶ or be assigned a distinct probability distribution of effect classes in BayesRC³². An alternative approach is to give additional weight to preselected variants (for example, in proportion to the amount of genetic variation they explain in GWAS) in the genetic relationship matrix (G matrix) (BOX 2) of the single-step method widely used for routine genetic evaluations¹⁵⁷.

Within-breed GS versus across-breed GS. To be effective, GS requires reference populations of tens of thousands of individuals. Many livestock breeds are numerically too small to make this a realistic scenario. Across-breed GS has been proposed as an alternative approach for smaller populations, in which GS models trained in a large breed are used to make predictions in a small breed. However, this method has proved to be ineffective when using data from low-density or even medium-density SNP arrays¹⁵⁸. Several factors may explain this failure.

First, the linkage phase between causative variants and distant genotyped SNPs may differ between breeds. Indeed, predictions for across-breed GS improved using data from higher density arrays containing 600,000 SNPs, which are bound to include SNPs closer to the causative variants^{154,159–163}. The improvement was highest (+8.7%) when using imputed sequence-based selection with Bayesian models³². Note that for this imputation-based strategy to be effective, whole-genome sequence data from a sufficient number (>100) of animals representing the small breed need to be available.

Second, the effectiveness of across-breed GS may be limited by the degree to which segregating causative variants are shared across breeds, which will be determined by the proportion of causative variants that existed before breed formation and how much gene flow there has been between the breeds. It is reasonable to assume that the degree of sharing will be inversely proportional to the effect size. Indeed, the allelic frequency of small-effect variants will change more slowly under selection (that is, polygenic adaptation) than will large-effect variants, which are expected to undergo selective sweeps that lead to rapid fixation. Moreover, the sharing of large-effect variants between breeds is highly suggestive of balancing selection, which may reveal associated deleterious effects. GWAS conducted in multiple dairy cattle breeds indicate that ~50% of QTL might be shared across breeds and might predate breed formation if not domestication^{161,164}. Some sharing is also the result of more recent between-breed introgression. For example, European breeders reportedly imported Asian pigs to improve local stock at the end of the 19th century, and this has resulted in the near fixation of Asian haplotypes conferring desired features at, for instance, the *IGF2*, *FASN*, *ME1* and *KIT* loci^{118,165}.

Finally, it is important to remember that the effects of genetic variants are neither constant across populations nor across generations. Indeed, in the case of dominance, the effects of allelic substitution are a function of allelic frequency and may be affected by gene-by-gene (GxG) and gene-by-environment (GxE) interactions².

From selecting animals to selective matings using genomic information. The prevailing paradigm in present-day animal breeding is to identify the animals that have the highest EBVs within their generation as genitors of the next. However, at some point, genetic progress may be limited by the rate at which favourable alleles combine by recombination. It is conceivable that the fastest route towards the ideal allelic combination does not involve selecting the best animals from each generation but instead requires an approach that uses temporarily suboptimal individuals. Thus, a new paradigm may be to find the fastest mating scheme to concentrate a maximum of favourable alleles in one individual. In its simplest version, such an approach may consist of identifying matings that have the potential to produce the best offspring, that is, genomic mate selection. For example, a corresponding sire and dam may not have the highest EBVs, but their genotypes might complement each other in a way that produces superior offspring. Furthermore, the availability of genomic information makes it possible to control inbreeding at the genome level while simultaneously making rapid gains from GS^{166–168}. However, it is important to estimate EBVs and inbreeding using the same source of information (that is, either based on pedigree or genomic information) when controlling inbreeding using the approach of optimum contribution selection¹⁶⁶.

Editing livestock genomes

Programmable nucleases have revived interest in editing livestock genomes. Early reports describing the generation of transgenic animals^{169,170} led to expectations of a revolution in animal breeding. Slow-pace selection would make way for the engineering of plant and animal genomes. Genetic improvement would no longer require the variants to pre-exist in the breed of interest. Beneficial mutations could be transferred between populations, transferred between species or even designed at will. However, the anticipated revolution has yet to occur. Generating transgenic animals remained extremely arduous and expensive, demonstrably useful transgene constructs few, and western consumers apprehensive of genetically modified organisms. The initial pronuclear microinjection technique was not only inefficient but carried with it the risk of insertional inactivation of endogenous genes. Retroviral vectors provided limited cargo capacity, were subject to epigenetic inactivation and could also perturb endogenous genes upon insertion. The inability to derive embryonic stem cells prevented homologous recombination-based techniques until the development of somatic cell nuclear transfer (SCNT)¹⁷¹, which enabled refined gene replacement by homologous recombination in cultured fetal fibroblasts followed by nuclear transfer to enucleated oocytes. However, the exceedingly low rate of spontaneous homologous recombination severely limited its applicability (reviewed elsewhere¹⁷²).

The development of programmable nucleases, including FokI-based zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), and, more recently, of the CRISPR–Cas9-based nucleases profoundly changed the picture¹⁷³. These nucleases

Gene flow

The passage of alleles between populations as a result of migration or interbreeding.

Polygenic adaptation

The process by which a phenotype caused by many genes evolves in a population under selection, not by massive changes in the frequency of a few variants with major effects on the phenotype (hard and soft sweeps) but by very small changes in the frequency of many variants with minor effects on the phenotype.

induce double-stranded breaks in DNA with high efficiency, which facilitate the generation of LoF mutations in any gene of interest by inducing error-prone non-homologous end-joining (NHEJ) and result in a ~100-fold increase in the rate of gene replacement by homology-dependent repair (HDR) compared with spontaneous homologous recombination. The ease of development of target-specific CRISPR–Cas9 reagents and the rapidly expanding toolbox of CRISPR–Cas9-derived methods¹⁷⁴ make them the preferred option over ZFNs and TALENs. Since 2011, a growing number of genome-edited domestic animals have been produced through the combined use of SCNT and programmable nucleases (reviewed elsewhere^{172,175}). Indeed, the rate of double-stranded break-induced NHEJ is now high enough that, despite mosaicism commonly reducing germline transmission, it has become more effective (in terms of the number of embryos required to obtain an edited offspring) to circumvent SCNT and inject the programmable nucleases directly into the zygote when aiming to generate LoF mutations¹⁷². Although the efficiency of HDR may still be too low for direct zygotic injection to be an attractive proposition for gene replacement, the development of effective CRISPR–Cas9-dependent knock-in methods, such as precise integration into target chromosomes (PITCh)¹⁷⁶ and homology-independent targeted integration (HITI)¹⁷⁷, expands the number of applications of direct zygotic injection.

Genomic advances are uncovering a growing list of target genes and mutations. Thus far, efforts in editing the genome of livestock have mostly concentrated on largely uncontroversial human health applications, such as generating animal models of human genetic diseases, producing biopharmaceuticals and xenotransplantation. However, genomic and other advances are uncovering a growing list of putative target genes and mutations for which editing has been or is being pursued in order to improve aspects of livestock production^{175,178,179}, such as growth rate, muscle mass, meat fat content, milk composition, wool growth, resistance to bacterial, viral and parasitic diseases, temperature tolerance, animal welfare and environmental impact (TABLE 3). A specific application that is worth mentioning is the generation of male-specific double-muscling by inserting a *trans*-acting inhibitor of *MSTN* on the Y chromosome, which could have a major economic impact by improving the carcass yield and thereby the value of male dairy calves¹⁸⁰.

Combining GS with genome editing. As mentioned above, nearly all economically important traits in livestock are highly polygenic. As the number of animals with both phenotypic records and SNP genotypes increases into the millions and as fine-mapping methods continue to improve, a growing number of causative variants (particularly those with the largest effects) is bound to be identified, as has been shown to occur for common complex diseases in humans³⁶. The efficiency of CRISPR-based genome editing has increased to the point that multiple edits can be introduced simultaneously in cells with relative ease^{181,182}. A strategy termed 'promotion of alleles by genome editing' (PAGE) has

been proposed, which combines genome editing with GS¹⁸³. In PAGE, sires would first be selected based on their GEBV using GS. Before dissemination, the sire's genome would be edited for a number of causative variants to render them homozygous for the favourable allele. Under optimal conditions, it is estimated that PAGE could increase genetic response 2–4-fold over GS alone. Although far from trivial, schemes for the practical implementation of PAGE have been proposed^{175,184} that involve establishing, SNP genotyping and editing fetal fibroblast cell lines followed by SCNT. Although it will initially be limited by the number of known causative variants and technical hurdles, the feasibility of PAGE is likely to be explored by a number of breeding organizations in the near future.

New applications of genomic technology

Detecting cows with subclinical mastitis by bulk genotyping of tank milk. As growing numbers of animals are genotyped with genome-wide SNP arrays as part of GS programmes, new applications emerge, such as the early detection of cows with subclinical mastitis by bulk genotyping of tank milk¹⁸⁵ (FIG. 3). Dairy farms typically comprise tens to hundreds of cows whose milk is stored in large tanks before daily collection by milk processing factories. One of the major health issues on dairy farms is mastitis¹⁸⁶. Milk from affected cows is characterized by an increase in SCCs as immune cells migrate into the udder and milk. The normal range for SCCs is <100,000 cells per ml, but it may reach millions in cows suffering clinical mastitis, and even before overt symptoms appear, cows with subclinical mastitis may already have >200,000 cells per ml. At that stage, their milk yield is typically decreased, and the milk quality of the entire tank is affected. Early detection of cows with clinical mastitis is therefore paramount. A new cost-effective way to achieve this is to SNP genotype the tank milk, which yields estimates of the allelic ratio (the B-allele frequency) in the milk for tens of thousands to hundreds of thousands of markers. This ensemble of allelic ratios reflects the combination of the cows' known SNP genotypes, and the unknown proportion of DNA contributed by each cow to the tank milk. These proportions can be accurately estimated by solving a corresponding set of linear equations and converted to SCCs for individual cows. The number of SNPs needed to achieve adequate accuracy depends on the number of cows on the farm: tens of thousands of SNPs are sufficient for farms with tens of cows, but hundreds of thousands of SNPs are needed for farms with several hundred cows. However, low-density SNP arrays, comprising 10,000 SNPs, are most commonly used to genotype cows. Nonetheless, whole-genome sequence information can be imputed for the cows, and the tank milk can be genotyped with high-density SNP arrays or by shallow sequencing (M.G., unpublished observations). Sequencing also enables simultaneous microbiome analysis to identify bacterial contaminants.

Importantly, the ability to determine the relative contribution of genotyped individuals or their genotyped ancestors to samples of bulk animal products provides novel opportunities to trace and authenticate animal food products¹⁸⁷.

Mosaicism

The occurrence of mutations in some but not all cells of an organism that is entirely derived from a single zygote.

Table 3 | Target genes and mutations for editing livestock genomes

Phenotype	Gene	Editing type	Effect	Species	Refs	
					Gene editing	Naturally occurring variation
Carcass yield and composition						
Increased muscle mass	GH	RKI	Ectopic expression in all tissues	Pig, sheep, salmon	210–214	–
Increased muscle mass	GHRF	RKI	Ectopic expression in all tissues	Pig, sheep	211,212	–
Increased muscle mass	IGF1	RKI	Ectopic expression in all tissues	Pig	211,215	–
Increased muscle mass	cSKI	RKI	Ectopic expression in all tissues	Mouse	216	–
Increased muscle mass	MSTN	KO or KD	Constitutive or conditional KD	Cattle, sheep, goat	217–222	67,113–116
Increased muscle mass	IGF2	PAGE	–	–	–	118
Increased muscle mass	FST	RKI	Ectopic expression in muscle	Mouse	223	–
Increased muscle mass	FLRG	RKI	Ectopic expression in muscle	Mouse	223	–
Increased linoleic and alpha-linoleic content	FAD2	RKI	Ectopic expression in adipose	Pig	224	–
Increased omega-3 fatty acids content	FAT1	RKI	Ectopic expression in all tissues	Pig, cattle, sheep	225–227	–
Decreased fat content	UCP1	HRKI	Constitutive expression	Pig	228	229
Milk yield and composition						
Increased milk volume	GHR	PAGE	–	–	–	120
Increased casein content	CSN (ABK)	RKI	Increased (mammary gland) expression	Cattle	230	231
Humanized milk	BLG	KO or KD	Constitutive or conditional KD	Cattle, goat	232–235	–
Increased nutritional quality	LALBA	RKI	Ectopic expression in mammary gland	Pig	236	–
Humanized milk	LALBA	RKI	Ectopic expression in mammary gland	Cattle	237	–
Low-lactose milk	LPH	RKI	Ectopic expression in mammary gland	Mouse	238	–
Fatty acid altered composition	SCD	RKI	Ectopic expression in mammary gland	Goat	239	–
Increased omega-3 fatty acids content	FAT1	RKI	Ectopic expression in all tissues	Cattle	226	–
Wool yield and composition						
Increased fleece growth rate	GH	RKI	Ectopic expression in all tissues	Sheep	214	–
Increased fleece growth rate	IGF1	RKI	Ectopic expression in wool follicle	Sheep	240	–
Altered fleece quality	K2.10	RKI	Ectopic expression in wool follicle	Sheep	241	–
Increased fleece growth rate	CYS (E, M, K)	RKI	Ectopic expression in all tissues	Sheep	242	–
Resistance to infectious diseases						
Mastitis resistance	HLZ	RKI or HRKI	Ectopic expression in mammary gland	Goat, cattle	243,244	–
Mastitis resistance	LSS	RKI or HRKI	Ectopic expression in mammary gland	Cattle	245,246	–
Resistance to <i>Flavobacterium columnare</i> and <i>Edwardsiella ictaluri</i>	CecB	RKI	Ectopic expression in all tissues	Catfish	247	–
Resistance to tuberculosis	HBD3	RKI	Ectopic expression in lung	Cattle	248	–
Resistance to <i>Actinobacillus pleuropneumoniae</i>	PBD2	RKI	Ectopic expression in all tissues	Pig	249	–
Resistance to tuberculosis	SP110	HRKI	Ectopic expression in all tissues	Cattle	250	251–253
Resistance to foot-and-mouth disease	Anti-FMDV shRNA	–	–	Pigs	254	–
Resistance to influenza and classical swine fever	MX1	RKI	Ectopic expression in all tissues	Pigs	255,256	–

Table 3 (cont.) | Target genes and mutations for editing livestock genomes

Phenotype	Gene	Editing type	Effect	Species	Refs	
					Gene editing	Naturally occurring variation
Resistance to infectious diseases (cont.)						
Resistance to influenza	shRNA decoy	RKI	Ectopic expression in all tissues	Chicken	257	–
Resistance to visna	Envelope protein of visna	–	–	Sheep	258	–
Resistance to avian leukosis virus	ALV6	RKI	Ectopic expression in all tissues	Chicken	259	–
Resistance to PRRS	CD163	KO/HRKO	Constitutive KO	Pig	260,261	–
Resistance to African swine fever	RELA	KO or HRKI	Constitutive expression	Pig	262,263	–
Resistance to BSE	PRP	RKI or KO	Constitutive KO or KD	Cattle, goat, sheep	264–268	269
Resistance to trypanosomiasis	APOL1	RKI	Ectopic (haematocyte) expression	Cattle	270	271
Animal welfare and environmental impact						
Heat tolerance	PRLR	PAGE	–	Cattle	–	75
Cold tolerance	UCP1	HRKI	Constitutive expression	Pig	228	229
Cold tolerance	wfiAFP6	RKI	Ectopic expression in all tissues	Salmon	272	273
Hornless	POLL	HRK or PAGE	–	Cattle	274	275–277
Reduced faecal phosphorus output	APPA	RKI	Ectopic expression in salivary gland	Pig	278	–

BSE, bovine spongiform encephalopathy; HRKI, knock-in by homologous recombination; HRKO, knockout by homologous recombination; KD, knockdown; KO, knockout; PAGE, promotion of alleles by genome editing; PRRS, porcine reproductive and respiratory syndrome; RKI, random knock-in; shRNA, short hairpin RNA.

Combined genomic and metagenomic predictions of high-value traits.

In addition to an animal's own genome, the species composition and abundance of the gut microbiome have been shown to be associated with some traits. For example, two studies have demonstrated that rumen microbiome profiles are associated with methane emission levels in cattle and sheep^{188,189}. It is worth noting that BLUP models can be used to evaluate the effect of the microbiome on phenotypes of interest by replacing the genetic relationship matrix with a microbiome similarity matrix.

It is not yet clear whether predictions of future phenotypes for high-value traits, such as methane emissions and feed efficiency, can be improved by integrating genomic predictions from SNP genotypes with gut (or rumen for cattle) microbiome profiles. Although high-throughput sequencing enables rapid and cost-effective profiling of gut microflora, the number of animals with high-value traits measured, gut microflora profiled and SNPs genotyped is still quite small. However, a very preliminary study tested the concept in a small sample of 28 Holstein–Friesian dairy cattle for which 30,000 SNP genomic predictions for feed efficiency and rumen microbiome profiles were available¹⁹⁰. The genomic and microbiome profile predictions were combined using a linear regression model, and although the results must be interpreted with caution because of the small data set, the prediction accuracy in cross validation was maximized when both SNP

and rumen microbiome profiles were used ($r_{\text{SNP}} = 0.33$; $r_{\text{SNP+M}} = 0.57$). These results are encouraging, and larger scale studies may lead to future phenotype predictions that will enable selection of animals that will perform well over their lifetimes.

Conclusions and future perspectives

The field of animal breeding just completed a prototypical, once-in-a-lifetime Gartner hype cycle. The innovation trigger was the emergence of genomics as a new scientific discipline at the end of the 1980s. The peak of inflated expectations coincided with the initial investment of the public and private sectors in efforts to map QTL that would enable transformative MAS in the 1990s. A trough of disillusionment followed the realization that mapped QTLs explained insufficient variation to be of practical use. A slope of enlightenment followed the landmark paper that introduced GS⁸ and the development of cost-effective SNP genotyping arrays. It turned remarkably rapidly into widespread adoption of the technology, with millions of animals being enlisted in GS programmes over the past 10 years. GS has revolutionized animal breeding and allowed an estimated doubling of genetic gains in some species. The success of the underlying methodology and the ensuing support for the infinitesimal model have influenced other disciplines, including plant breeding and medical genomics.

Understandably, attention to and investments in research and development faded somewhat as breeding

Gartner hype cycle

A model first proposed by the Gartner firm to explain the phases of maturation, adoption and social application of new technologies.

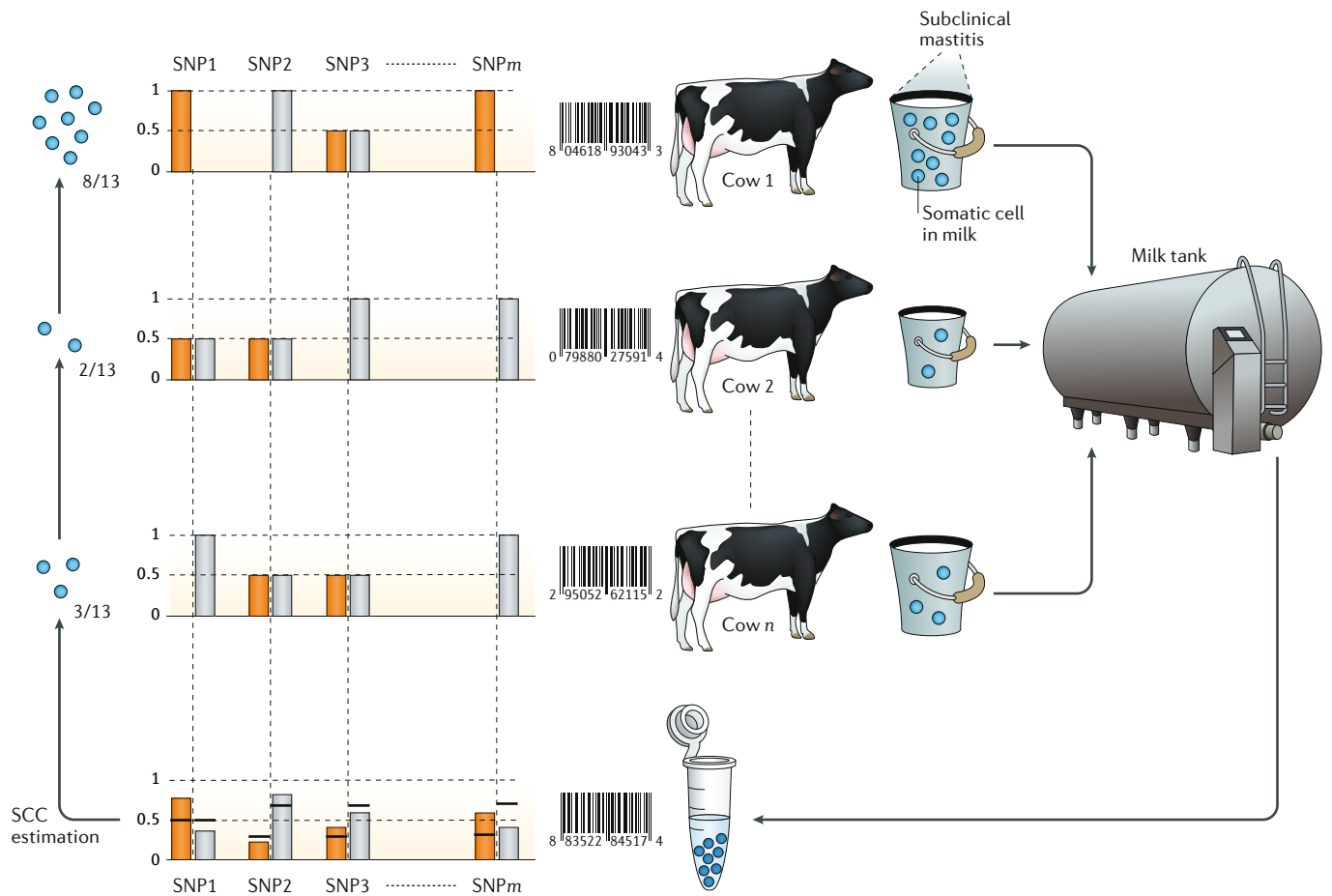


Fig. 3 | Identifying cows with subclinical mastitis by bulk genotyping of tank milk. Assuming that all cows on the farm that contributed milk to the tank have been genotyped with whole-genome single-nucleotide polymorphism (SNP) arrays, it is possible to determine what proportion of DNA each cow contributed to the tank by SNP genotyping the tank milk and measuring the allelic ratio (B-allele frequency) for each SNP. If the milk volume contributed by each cow is known and if the somatic cell counts

(SCCs) in the tank are known, the SCC for each individual cow can be determined. The bar graphs represent the allelic dosages for allele A (orange) and allele B (grey) for m SNPs in individual cows (upper three graphs) and tank milk (bottom graph). The horizontal lines in the bottom graph correspond to the expected allelic dosage, assuming that all cows contributed equal amounts of DNA to the tank milk. The deviation from expectation allows the contribution of each cow to be estimated.

organizations focused on the implementation and consolidation of GS. Now that these goals have been largely accomplished and GS has been widely adopted, its limitations as implemented today are recognized and better understood. Logically, there is, therefore, renewed interest and investment in the next round of innovation. Identified objectives include more effective forward and reverse genetic screens to identify genes with major effects, efforts to systematically identify and incorporate

causative variants into improved models of GS, renewed attention to the CRISPR–Cas9-based editing of livestock genomes combined with GS and the development of novel uses of the growing body of genomic data. Thus, genomic information will be a critical component of the global response to the world's pressing nutritional requirements.

Published online: 04 December 2018

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This paper illustrates the engineering of a desirable phenotype in livestock by TALEN-mediated allele swapping and SCNT.

Acknowledgements

C.C. is Senior Research Associate of the Fonds de la Recherche Scientifique (FRS-FNRS). Research in animal genomics conducted by the authors is funded by the European Research Council (ERC) Advanced DAMONA, H2020 GpE and WALLInnov CAUSEL grants to M.G. and the DGRNE Rilouke and ULiège RetroBlue grants to C.C. The authors are grateful to T. Druet for fruitful discussions and comments on the manuscript and to M. Goddard for excellent discussions over the years.

Author contributions

All authors researched data for the article, made substantial contributions to discussions of the content and reviewed and/or edited the manuscript before submission. M.G. and B.H. wrote the article.

Competing interests

The authors declare no competing interests.

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Reviewer information

Nature Reviews Genetics thanks D. Garrick and A. Legarra for their contribution to the peer review of this work.

Supplementary information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41576-018-0082-2>.

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