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Molecular consequences of animal breeding

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The phenotypic diversity in domestic animals provides a unique opportunity to study genotype–phenotype relationships. The identification of causal mutations provides an insight into what types of mutations have contributed to phenotypic evolution in domestic animals. Whole genome sequencing has revealed that fixation of null alleles that inactivate genes, which are essential under natural conditions but disadvantageous on the farm, has not been a common mechanism for genetic adaptation in domestic animals. Numerous examples have been revealed where structural changes cause specific phenotypic effects by altering transcriptional regulation. An emerging feature is also the evolution of alleles by the accumulation of several consecutive mutations which affect gene function.

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Introduction

Domestic animals constitute a resource for biological research due to the remarkable phenotypic changes that have occurred since domestication. There are several mechanisms that have contributed to this evolution.

(i) *Directional selection for adaptive mutations*. These make the animals better adapted for human purposes. For a long time this was based on phenotypic selection, where humans kept animals with favourable phenotypes for breeding. After the development of the theory of quantitative genetics more and more sophisticated statistical procedures have been developed to select animals with outstanding estimated breeding values. This has led to a remarkable improvement in animal production during the last 50 years.

(ii) *Directional selection for phenotypic appearance — fancy breeding*. Not all the traits that have been selected in domestic animals are adaptive. Apparently humans have a strong preference for phenotypic diversity among our domestic animals. For all domestic animals, humans have selected mutants which cause appealing phenotypic appearance as long as this appearance does not interfere with the utility of the animals. This is an important reason why we have black pigs with white belts or dogs with dorsal hair ridges. For those domestic animals that are used as pets a broad range of mutations is tolerated. This is probably the main reason why dogs show such an extensive phenotypic diversity [1].

(iii) *Natural selection*. Throughout the history of animal domestication natural selection has been operating in parallel with human selection. Genetic variants which promote survival or reproductive output in the new environment created by humans have been favoured by natural selection.

(iv) *Genetic drift*. It is also anticipated that some of the traits have been altered simply by genetic drift due to relaxed purifying selection in the farm environment. It is possible that this has contributed to the rich diversity of coat and plumage colour in domestic animals, although selection for coat colour variants also can be adaptive by facilitating animal husbandry as well as being favoured by fancy breeding [2].

Mutations with large favourable effects have been under strong positive selection in domestic animals and the same exact mutation is often found in different breeds all over the world, in sharp contrast to the extreme allelic heterogeneity often underlying inherited disorders in humans. This is particularly common for novel gain-of-function or dominant-negative mutations, because such mutations often represent a rare event. Throughout the history of animal domestication, human trades have efficiently spread favourable mutations around the world. Examples of widespread mutations are a nonsense mutation in *DMRT3* causing the ability to perform alternate gaits in horses [3••] and an *FGF4* retrogene associated with short legs in dogs [4••]. In both these cases the same mutation on the same haplotypic background is present across many breeds. This situation facilitates the identification of causal mutations underlying phenotypic traits, because haplotype sharing across breeds can be used to fine map the mutation and the phenotypic effect of a mutation can be investigated on different genetic backgrounds. However, exceptions to this rule occur and then it is often when there is selection for a loss-of-function allele, as a gene can be inactivated in many

ways. A prominent example of this is that selection for muscle growth in beef cattle has resulted in an allelic series disrupting *myostatin* (*MSTN*) function; MSTN acts as a suppressor for muscle growth [5].

The aim of this paper is to review what we have learnt from the molecular characterization of loci underlying phenotypic variation in domestic animals. The main focus is on monogenic traits, since it is still challenging to reveal causal mutations which underlie multifactorial traits. The focus is on traits rather than inherited disorders, because what we can learn from deleterious mutations under purifying selection in domestic animals is not fundamentally different from what we can learn from the much more extensive literature on human disorders, whereas the rapid evolution of phenotypic traits in domestic animals provides a unique opportunity to gain insight into genotype–phenotype relationships.

Is less more?

Olson [6] proposed that loss of gene function may be an important mechanism for rapid genetic adaptation to a new environment in natural populations as well as in domesticated plants and animals. The argument is that genetic mechanisms which control for instance behaviour, reproduction or growth that are of crucial importance for adaptation under natural conditions may be disadvantageous in the farm environment. One example when less is more is homozygosity for null alleles at the *myostatin* locus in beef cattle that releases repression of muscle growth [5], and another is the disruption of a repressor binding site in intron 3 of *IGF2* that leads to increased muscle growth in pigs [7••]. We have carefully searched for the presence of such inactivating mutations in coding sequences by using whole genome resequencing of pooled samples which represent different populations of chickens [8•] and pigs [9•]. These screens did not reveal a single example of a null allele in a well-conserved, single copy gene which occurs at a high frequency in any of the populations studied. False negatives may occur in these screens because both the chicken and pig genome assemblies are not finished assemblies, which means that we may have failed to detect inactivating mutations because the gene model was incorrect or incomplete. Nevertheless, we conclude that fixation of null alleles has not been a common mechanism for phenotypic evolution in domestic animals.

Structural changes mediate phenotypic changes by altering transcriptional regulation

Structural changes have played a prominent and important role for phenotypic evolution in domestic animals (Table 1). Duplications appear as the most common structural variant associated with phenotypes followed by deletions, inversions and translocations, and there is one example of an expressed *FGF4* retrogene causing chondrodysplasia in dogs [4••]. A common theme for these structural changes is that they lead to an altered

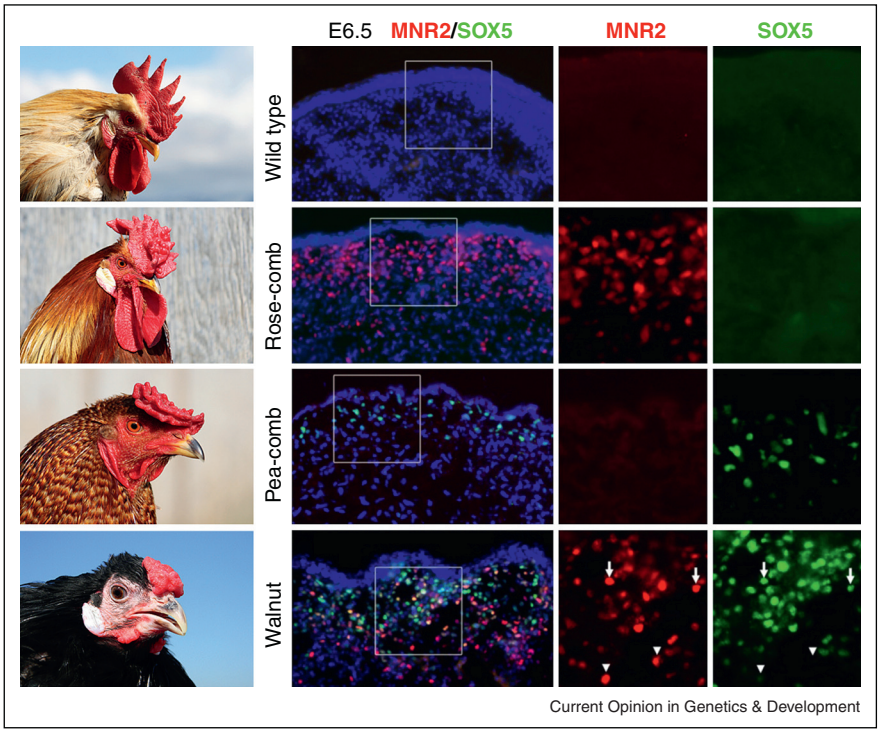
Table 1

Examples of structural variants associated with phenotypic traits in domestic animals

Species	Trait	Mutation	Gene(s)
Cattle	Colour sidedness	492 kb translocation[a]	*KIT* [31••]
Chicken	Pea-comb	Copy number expansion[b]	*SOX5* [14]
	Rose-comb	7.4 Mb inversion[a]	*MNR2* [15•]
	Dark brown colour	8.3 kb deletion	*SOX10* [32]
	Naked neck	~70 kb translocation	*BMP12* [33••]
	Fibromelanosis	Complex[c]	*EDN3* [34]
Dog	Hair ridge	133 kb duplication	*FGF3*, *FGF4*, *FGF18*, *ORAOV1* [35]
	Chondrodysplasia	Retrogene insertion	*FGF4* [4••]
	Wrinkles[d]	16.1 kb duplication[d]	*HAS2* [36]
	Amylase activity	~8 kb duplication	*AMY2B* [37•]
Goat	Polled[e]	11.7 kb deletion	*PISRT1*, *FOXL2* [38]
Horse	Greying with age[f]	4.6 kb duplication	*STX17*, *NR4A3* [39,40•,41]
	Tobiano white spotting	~40 Mb inversion	*KIT* [42]
Pig	Dominant white colour	Several duplications	*KIT* [9•,43,44]
Sheep	White colour	190 kb duplication	*ASIP*, *AHCY* [45]

[a] Two alleles identified (see Table 2).
[b] Massive expansion of a duplicated sequence.
[c] The mutation is a complex rearrangement where two fragments, 129 kb and 172 kb in size and located 417 kb apart on the wild-type version of chicken chromosome 20, are both duplicated. In addition, the duplicated copy of the 172 kb fragment is inserted between the two copies of the 129 kb fragment but in an inverted orientation!
[d] This mutation also predisposes to Familial Shar-Pei Fever — a periodic fever syndrome. The duplication shows a copy number expansion.
[e] Lack of horn, also associated with intersexuality in males.
[f] This mutation also predisposes to melanoma development.

Figure 1



Four comb phenotypes in chickens, wild type (or single-comb), Rose-comb, Pea-comb and Walnut-comb, and immunohistochemical labelling of MNR2 and SOX5 in comb tissue sections from embryonic day (E) 6.5. Nuclei are visualized by DAPI. Boxed regions are shown magnified as single colour. Arrows in the Walnut-comb tissue sections indicate double-labelled cells whereas arrowheads indicate single-labelled cells.
Photos by Freyja Imsland (wild type, Rose-comb and Pea-comb) and David Gourichon (Walnut-comb). On the basis of Imsland *et al.* [15].

configuration of regulatory elements by duplicating, deleting or translocating regulatory elements in relation to coding sequences and thereby alter gene expression. The regulatory sequences which are affected by these events are usually present as a single copy sequence in the actual species as well as in other species.

The genetic basis of comb shapes in chicken provides an illustrative example (Figure 1). The genetics of comb shapes are of historical interest, since the Rose-comb and Pea-comb phenotypes were included in Bateson's [10] seminal paper in which Mendelian inheritance in animals was first demonstrated. A few years later Bateson and Punnet [11] showed that the Walnut-comb is determined by the combined effect of *Rose-comb* and *Pea-comb*, the first example of genetic interaction between genes or digenic inheritance. Interestingly, an increasing number of human disorders which show digenic inheritance have been documented [12,13]. First, we had demonstrated that the Pea-comb phenotype is caused by a copy number expansion of a duplicated sequence (from 2 copies to about 30 copies) located in intron 1 of *SOX5* [14]. This leads to ectopic expression of SOX5 during a few days of development in a layer of mesenchymal cells in the area where the comb develops (Figure 1). Why the copy number expansion leads to ectopic SOX5 expression is not known, but there are several possibilities including: first, the large copy number expansion may transform a weak regulatory element to a strong constitutively active element; second, the repeated organization may attract epigenetic silencing; or third, the copy number expansion may alter the interaction of regulatory elements in the region. Similarly, a mutation underlying the Rose-comb phenotype constitutes a 7.4 Mb inversion that translocates the gene for the MNR2 homeodomain protein from a distal position to a more proximal position on chromosome 7, which in turn leads to ectopic expression of MNR2 in the same area of the skull as SOX5 in Pea-comb birds (Figure 1) [15•]. The interpretation that the translocation of *MNR2* is causing the altered comb phenotype was strongly supported by the identification of a second *Rose-comb* allele, named *Rose2*, that must have originated by a recombination event between *Rose1* and a wild-type chromosome which restored most of the inversion but left a small fragment of the inversion in the translocated position, and this fragment included the translocated *MNR2* gene that is located very close to the inversion break-point. The genetic interaction between *Rose-comb* and *Pea-comb* can now be explained by ectopic expression of SOX5 and MNR2 in the same layer

of cells during comb development. *Pea-comb* and *Rose-comb* are both regulatory mutations, and provide beautiful illustrations of the importance of studying the right tissue at the right time of development to reveal an altered gene regulation. Furthermore, immunohistochemistry has provided the spatial resolution to exactly pinpoint which cells show altered regulation (Figure 1), in a standard qPCR experiment using RNA from a tissue sample the specific signal from the affected cells would be severely diluted.

Evolution of alleles

Another emerging feature in domestic animals is the evolution of alleles. The evolutionary history of domestic animals is sufficiently long to allow the accumulation of several consecutive mutations affecting the function of a single gene. Five convincing examples for alleles with two or more consecutive mutations in the same gene are compiled in Table 2. In the previous section, this concept was illustrated by *Rose-comb* where an inversion event was followed by a recombination event generating a second *Rose-comb* allele [15•]. Perhaps the most extreme example for evolution of alleles concerns the *KIT* locus in pigs which harbours three different white spotting alleles *Patch*, *Belt* and *Dominant white* where the latter shows the most extreme phenotype. *Dominant white* differs from the *wild-type* allele by at least five mutations, four duplications named DUP1–DUP4 and a splice mutation leading to exon skipping [9•]. Interestingly, DUP1 alone, a 450 kb duplication including the entire *KIT* and flanking sequences, is underlying the *Patch* allele and DUP2–DUP4 are all associated with the *Belt* allele. Thus, the combined effect of these five mutations, where at least three are assumed to affect function, is a *KIT* allele that is fully viable but despite this has the most dominant effect on pigmentation described in any species [9•].

Another particularly interesting example of evolution of alleles concerns *PMEL* in chickens (Table 2). *PMEL* encodes the premelanosome protein that shows melanocyte-specific expression and forms an amyloid structure in eumelanosomes (melanosomes containing black eumelanin). Dominant white colour is caused by a 9 bp insertion in *PMEL* that introduces three amino acids (WAP) in the PMEL transmembrane region [16]. Dominant white is a very common colour variant in chicken and billions of chickens are homozygous for *Dominant white*. In such a flock of white chickens a few birds appeared where the plumage pigmentation had been partially restored. This new colour variant was named Smoky and molecular characterization revealed that the causative mutation was a 12 bp deletion that affected a highly conserved part of PMEL and the mutation had occurred on the *Dominant white* allele [16]. This suggested a possible scenario where the 9 bp insertion (causing Dominant white colour) constitutes a dominant negative, whereas the 12 bp insertion (causing Smoky) is a loss-of-function mutation that inactivates the dominant negative effect. This hypothesis has been confirmed by two recent studies. Firstly, Hellström *et al.* [17] developed a knockout mouse and demonstrated that a *PMEL* null allele produces a much milder pigmentation defect than the *Dominant white* allele in chicken. Secondly, Watt *et al.* [18] showed that the *Dominant white* allele generates a PMEL protein producing aberrant fibrils and that the *Smoky* mutation prevents the formation of these aberrant fibrils, which explains why it rescues pigment production.

The history of animal domestication is sufficiently old to allow evolution of alleles but sufficiently young to reveal the phenomenon, because the intermediate forms can often be found as illustrated here for the *KIT* locus in pigs and the *PMEL* locus in chickens. It would be exceedingly difficult to unravel that a genetic difference at one locus between two species, say human and chimpanzee, are in

Table 2

Examples of evolution of alleles in domestic animals

Species	Phenotype	Gene	Allele	Mutation	Ref.
Pig	Patch	*KIT*	*Patch*	a: 450 kb duplication	[43]
	Belt		*Belt*	b: three duplications[a]	[9•]
	Dominant white		*Dominant white*	a + b + c: splice mutation	[43,46]
Pig	Dominant black	*MC1R*	E^D	a: missense mutation	[47]
	Black spotting		e^p	a + b: 2 bp insertion	[48]
Chicken	Dominant white	*PMEL*	*Dominant white*	a: 9 bp insertion	[16]
	Smoky		*Smoky*	a: + b: 12 bp deletion	[16]
Chicken	Rose-comb	*MNR2 + CCDC108*	*Rose1*	a: 7.2 Mb inversion	[15•]
	Rose-comb	*MNR2*	*Rose2*[b]	b: non-homologous recombination	[15•]
Cattle	Colour sidedness	*KIT*	Cs_{29}	a: 492 kb translocation	[31••]
	Colour sidedness		Cs_6[c]	b: 575 kb translocation	[31••]

[a] Not all three duplications have to be functionally important, but all three are exclusively found in domestic pigs showing white spotting.
[b] Originating from a recombination event between *Rose1* and a wild-type chromosome.
[c] Originating from a recombination event between Cs_{29} and a wild-type chromosome.

fact due to several functionally important mutations. The observation of evolution of alleles in domestic animals has important implications for assessing the association between genetic and phenotypic diversity in natural as well as human populations. The simple one-to-one relationship between one mutation and one phenotypic effect that occurs for deleterious mutations which underlie mutants in experimental organisms or some inherited disorders in humans may not apply for most of the diversity we observe in nature. For instance, it is generally assumed that significant genome-wide association studies (GWAS) SNPs 'are in high LD with a causal mutation' [19], it may be more appropriate to assume that such a SNP is unequally represented among haplotypes formed by a set of causal mutations which affect the trait/disease under consideration. Haplotype effects like those exemplified in Table 2 may be the rule rather than the exception. This notion is consistent with the view that a larger part of the mammalian genome is functionally important than was previously thought, due to the many regulatory regions in the genome [20].

Emerging findings for multifactorial traits

GWAS have demonstrated that most of the multifactorial traits and disorders in humans are highly polygenic, that is, they are controlled by a large number of loci, each with a small effect. Human height is an excellent example where a combined analysis of several studies comprising a total of 183 727 individuals revealed 180 associated loci that in aggregate explained only 12% of the variation [21]. The general finding from domestic animals is that in addition to many loci with small effects, it is common to find some loci with moderate to large effects affecting multifactorial traits. This was already indicated in the first family-based QTL experiments carried out in domestic animals. For instance, QTL mapping by using an intercross between wild boars and domestic pigs revealed one locus on chromosome 4 which explained about 20% of the F_2 variance for abdominal fat deposition [22] and a paternally expressed locus on chromosome 2 which explained 20–30% of the variance for muscle mass [23,24]. GWAS across dog breeds have revealed a small number of loci, including *IGF1*, *HMGA2* and *LCORL*, which appear to explain a considerable portion of the genetic variance for body size in dogs [25•,26]. Similarly, a recent screen for selective sweeps in pigs has revealed three regions of the genome, including *NR6A1*, *PLAG1* and *LCORL*, associated with increased body length [9•]. The *PLAG1* and *LCORL* regions only, explained about 20% of the residual variance in body length in a wild boar intercross [9•]. This is almost twice as much as explained by the 180 most significant human loci affecting stature! Alleles with moderate to large effects have been enriched in domestic animals due to the strong directional selection. Such alleles must exist in the human population as well, but most of them have occurred fairly recently, are rare and therefore each single variant explains only a tiny portion of the population variance for common disorders. However, they may have a large impact on the individual risk to develop a common disorder [27].

Thousands of QTLs affecting various traits are listed in the Animal QTL Database [28]. However, there are still few examples where conclusive evidence for causative mutations which underlie QTLs has been revealed. This is explained by the notoriously poor resolution in QTL mapping and because many QTLs will be due to haplotype effects caused by several linked polymorphisms. But there are some success stories that show that a major QTL can be as simple as a single base change creating an illegitimate site for a microRNA in a 3′UTR region, as observed for the *myostatin* gene and muscle growth in sheep [29••], or a single base change which disrupts the binding site for a repressor as observed for *IGF2* and muscle growth in pigs [7••,30•]. Furthermore, the control of locomotion must be considered as a highly complex trait where a large number of genes may affect phenotypic diversity. Nevertheless, a recent GWAS study based on only 70 Icelandic horses led to the identification of a nonsense mutation in *DMRT3*, encoding the doublesex and mab-3 related transcription factor 3, with a large effect (approaching monogenic inheritance) on the control of gait [3••]. The mutation occurs at a high frequency in all the tested gaited breeds, where these breeds show ambling gaits or pace, as well as in horses bred for harness racing. The DMRT3 protein is expressed in a specific subset of neurons in the spinal cord, and functional studies in mice revealed that these neurons are interneurons with inhibitory character that make synaptic connections to motor neurons and *Dmrt3* null mice show an altered gait control [3••]. This illustrates how mutations with large effects on multifactorial traits have been selected during the course of animal domestication and how such mutations create an opportunity to better understand important biological mechanisms, in this case the control of limb movements.

Conclusion

In summary, genetic studies of domestic animals constitute an invaluable complement to genetic studies of humans and experimental organisms. Current human genetics focuses on the identification of mutations under strong purifying selection that cause monogenic disorders and GWA studies of the standing genetic variation contributing to multifactorial traits and disease. Domestic animals provide an opportunity to study those genetic changes that have occurred due to strong positive selection during a rapid evolutionary process. This approach has provided fresh insight into genotype–phenotype relationships. These studies have revealed how structural changes have contributed to phenotypic variation, primarily by altering transcriptional regulation, how alleles may differ by multiple substitutions affecting gene function and that mutations with moderate to large effects on

multifactorial traits have often been enriched during the course of evolution of domestic animals.

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The study used high-resolution proteomics to fish out the protein that binds the mutated site in *IGF2* intron 3 which underlies a major QTL for muscle mass in pigs [7]. The gene, *ZBED6*, is unique to placental mammals and has evolved from a domesticated DNA transposon.

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