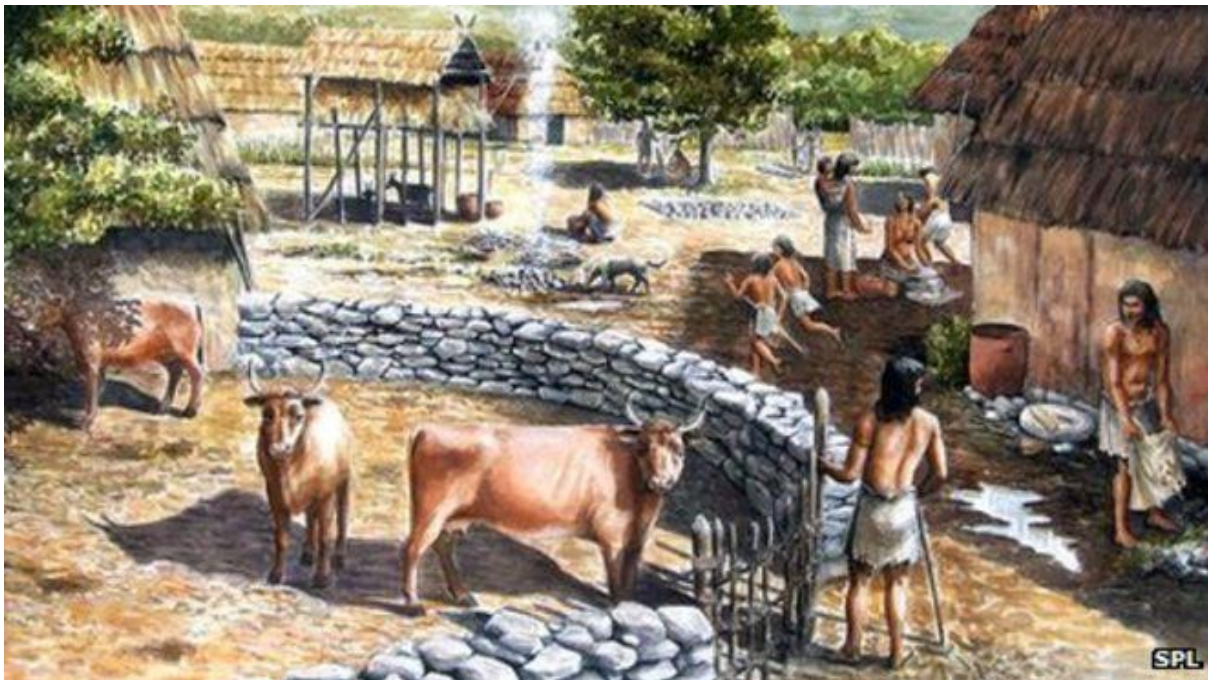# The role of humans in the adaptation of domesticated animals to local environments

What happened to the genomes of domesticated animals after migration?

Artist impression of a Neolithic farm

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Bachelor thesis

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1. Abstract

The domestication of plants and animals was a turning point in human history. After the emergence of cities, early Neolithic farmers started to migrate out of the initial place of domestication for many animals, the Fertile Crescent. What happened to the genomes of domesticated animals after the migration to novel environments, where there had been no domesticated animals before, has been a point of debate in the domestication research field for a long time. Early evidence suggests that farmers kept their animals separated from wild local populations, suggesting a small role for humans in the shaping of the genome of domesticated animals. With the emergence of Next Generation Sequencing, it became clear that the role of humans might be more complicated. Farmers intentionally initiated gene flow between wild populations and their domesticated stock, and there is evidence that farmers also selected for economically interesting traits in their livestock. This paper reviews genetic evidence on this matter, in order to shed a light on the role of humans in shaping the domesticated genome.

Key words

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The role of humans in the adaptation of domesticated animals to local environments

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2. The domestication of animals

Plant and animal domestication has changed the course of human evolution during the Holocene, causing a large cultural shift (Larson & Fuller 2014). Starting with the domestication of the dog, at least 11-16k years ago (Freedman *et al.* 2014), humans went on to domesticate animals who changed the hunter-gatherer's way of life. This change of life gave way for an exponential population increase of humans (Vigne 2011; Larson & Burger 2013). In order to understand the emergence of the modern human societies, it is essential to know the processes of domestication (Larson & Burger 2013). Research on domestication is often carried out by multiple disciplines: zooarchaeologists, anthropologists, zoologists, ecologists and, most recently, geneticists have been studying the origins of domestication. The questions surrounding the starting time and starting place of domestication are nowadays better understood (Zeder 2012). Using ancient DNA extraction methods and next generation sequencing tools, researchers have narrowed down the possible places of initial domestication, see Larson and Burger (2013) or Larson and Fuller (2014) for detailed reviews on this topic. However a lot is still not understood about post-domestication processes, such as the management and breeding of animals after domestication. The emergence of cities led to migration to other parts of the world where hunter-gatherers still roamed the fields and woods. The aim of this review is to look at what this migration of domesticated animals has done to their genomes. To understand the post migratory genomic adaptations better, it is first necessary to learn about the initial process of domestication, as this provides the basis upon which later farmers had to build further to shape the genomes of their domesticated animals. Humans had learned from the initial domestication of animals, so it is highly likely that the post-migratory management of animals involved a great deal of human intent, which may be evidenced by selection on traits interesting for humans.

Plant domestication is unrelated to animal domestication in many ways, as the main traits for animal domestication lie in the behaviour of an animal that were unconsciously selected by the commensal relationship of humans and animals, whereas plants do not display any behaviour. Defining domestication is highly problematic, because it is studied by multiple disciplines for a long time (Zeder 2012). In this review, **domestication** is defined as a process in which "humans assume control over the domesticate's movement, feeding, protection, distribution and, above all, its breeding" (Zeder 2012, p. 162). Currently available evidence from bone morphology and DNA sequencing, suggests that there were multiple pathways in the creation of domesticated animals from wild progenitors. For a long time, the general assumption was that all animals were domesticated via the **commensal pathway**. Today, at least three pathways to animal domestication are recognized, put forward by Melinda Zeder in her influential work *The Domestication of Animals* (2012).The commensal pathway entails the domestication of animals who are attracted by human populations, especially human refuse, or animals who prey upon the scavenging animals killed by humans (Zeder 2012). In this pathway, the largest role is played by the animals themselves in the initial phase, as they have to go near humans, but subsequent stages required direction by humans (Larson & Burger 2013). Animals and humans had to get accustomed to each other, which meant that, over several generations, there was

unconscious selection for animals that displayed docile behaviour. The first domesticated animals (dogs & pigs) where domesticated via the commensal pathway (Zeder 2012).

The second pathway, involving more human direction from the start, is the **prey pathway**. The major livestock species of today (cattle, goat and sheep) were domesticated this way (Zeder 2012). Wild progenitors of these animals were hunted by humans, who began to experiment with hunting strategies due to pressures on food supply resulting from environmental changes. Prey animals began to disappear and humans adapted their way of hunting by killing only old males to ensure the reproduction of populations, for example (Zeder 2012). Later, the amount of management increased, until slowly the prey species began to turn into tamed animals and later domesticated herds. The latter process involved unconscious selection on common behavioural and morphological traits, such as docility and acceptance of human presence. Darwin already noted the traits many domesticated animals have in common in his 1868 work *The Variation of Plants and Animals under Domestication*. The combination of these traits is now being referred to as the "Domestication Syndrome" (Wilkins *et al.* 2014). Thus, hunting management slowly turned into herding management, which meant that humans had gained multigenerational control over the animals' reproduction and movements (Zeder 2012).

The last pathway, the **directed pathway**, involves human intent and human directional selection. This pathway required sufficient knowledge gained from the two other domestication pathways, which means it was initiated at a later stage (Zeder 2012). Animals that were domesticated this way did not show any of the "Domestication Syndrome" traits (morphological traits such as reduced head size, curly tails and also juvenile behaviour), meaning that their domestication process had to be heavily dominated by human intent. Humans had to know what they wanted to end up with, because unconscious selection on neural crest cells (which creates the traits of the "Domestication Syndrome" of the other two pathways) would not have sufficed to create domesticated animals (Wilkins *et al.* 2014). Not only knowledge, but also technological developments to aid in the catching of animals helped this process of heavy selection by humans (Zeder 2012). The most exemplary animals domesticated by the directed pathway are horses and donkeys. For an overview of the different pathways to domestication see figure 1.
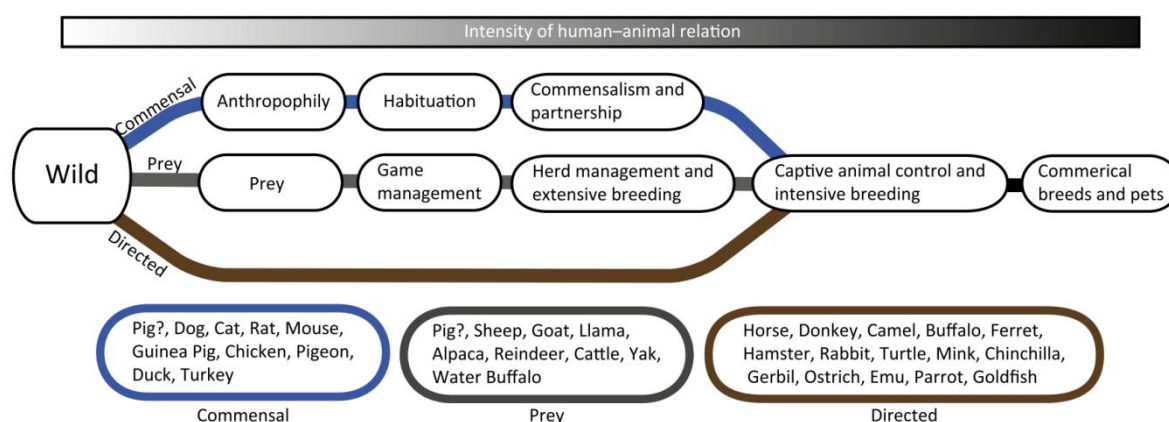

Figure 1. The three different pathways and some of the animals domesticated via each pathway. Pigs are now put into the commensal pathway. Source: Larson and Burger 2013.

After the initial process of domestication, a lot is still unknown about the spread of domesticated animals all around the globe (Larson *et al.* 2014). Many methods are used to answer the questions surrounding migration, such as biometry (the measurements of bones related to breeds of animals). More recently advances in the methods of DNA sequencing, with the introduction of Next Generation Sequencing, have proven to be very useful for the study of domestication and may shed more light on this blind spot of knowledge. The genomics approach paints a clearer picture of past domesticates than ancient mitochondrial DNA studies alone (Larson & Burger 2013). Understanding the process of domestication, and the subsequent spread of domesticates around the world, is essential to the understanding of modern human society, but also for the continuation of breeding processes with modern animals in order to create healthy a livestock (Larson & Burger 2013).

Because the initial process of domestication is better understood and mostly resolved of its mysteries, the focus of this paper is on the subsequent spread of farmers who took their animals with them and adaptation to new specific environments by these domesticated animals. After migration, lots of processes acting on the genome, such as natural selection and admixture with local wild progenitors, shaped the genome of local populations of domesticated animals (Librado *et al.* 2015; Park *et al.* 2015). These processes may have happened naturally and randomly, but it is also possible that humans influenced these processes to a certain degree and selected for certain phenotypes or chose to mix local wild progenitors with their own domesticates. Therefore, the main question of this paper is: to what degree have humans directed post-migratory genome changes in domesticated animals, and if so, how did they do this?

To answer these questions, studies of the genomes of domesticated animals compared to those of local, wild progenitors are reviewed. The processes that underlie the genomic differences between wild and domesticated animals include: admixture, artificial selection, natural selection, undirected selection. These processes will be explained at the start of the following chapter. Only economically important animals will be discussed, as they have shaped the society of today and are still important, for example, in terms of dietary contribution (cows, pigs, and horses). Economically important animals are defined in this paper as those animals that are found in archaeological sites in context of use as dietary contributions as primary products (meat) or secondary products (milk, silk, and fibres) and labour (traction animals, and beasts of burden). All evidence is based on the study of the DNA (either modern, or ancient DNA), other types of evidence will not be considered, except if it is relevant in combination with DNA-related evidence.

3. The use of ancient DNA in domestication research

Ancient DNA is simply normal DNA that has been preserved over a long period of time, sometimes more than 10000 years. It is useful to use ancient DNA of both domesticated animals and wild progenitors, because there will be detectable changes in the genomes of these animals, that may reveal something about breeding processes or management strategies in the past (Zeder 2015). Archaeologically interesting specimens lay in the ground for periods of sometimes over 10000 years. This means that the environment in which the

bones or teeth are found have a great impact on how well the DNA is preserved (Linderholm 2016). For example in warm and humid environment DNA degrades more easily than in permafrost regions. The genomic structure of the DNA gets fragmented and the overall degradation by other organisms in the soil make it hard to get correct ancient DNA sequences (Shved *et al.* 2014). The most damaging to the correct analysis if DNA fragments, however, are miscoding lesions, a type of modification that causes DNA polymerase to misread the sequence (Gilbert *et al.* 2007). Post-mortem modification of nucleotides is an important factor to keep in mind as well: there is a high amount of cytosine deamination to uracil in ancient DNA too (Park *et al.* 2015).

Contamination of the ancient DNA sample is another issue as well. Almost always, the DNA of interest is contaminated by the DNA of other organisms, often by a coating of alien DNA from Bacteria, which makes it difficult to read the sequence correctly. Furthermore, starting at the excavation and ending at the sequencing process, the ancient DNA runs the risk of being contaminated by the researchers themselves (Linderholm 2016). To battle contamination, the ancient DNA is radiated with UV-light and treated with bleach (Burger *et al.* 2004). Despite all the drawbacks of ancient DNA research, the field has evolved to a field of study that can compete with the more traditional fields of zooarchaeology. In the past, it was a secondary field of study, often relying on traditional zooarchaeology (bone morphology and the study of bone wear and pathology) to confirm research questions. But, because of technical developments (better separation of contaminated and DNA of interest, amplification of short ancient DNA strands, and the advances in sequencing whole genomes) combined with the establishment of standardized protocols in handling the ancient DNA, this field of study has become a new primary research field as well (Linderholm 2016). For example, domestication was first evidenced by changes in bone morphology, bones got smaller immediately after domestication, and later slowly increased in size again. Nowadays, mostly (mitochondrial) DNA evidence is used as this provides us with the most detailed history of domestication. One example that the study of ancient DNA has brought forward is the domestication history of pigs. Before the use of whole genome sequencing, it was thought that pigs were domesticated in Europe and South East Asia (as evidenced by mtDNA), whereas pigs are now recognized to have been domesticated in the Fertile Crescent and South East Asia (Mignon-Grasteau *et al.* 2005).

4. What happened to the genomes of domesticated animals?

A positive outcome of the domestication of plants and animals was increased food production, which paved the way for the emergence of towns and cities as people became more sedentary. However, domestication has also led to increased mortality, health decreases and an increased workload for humans (Dusseldorp & Amkreutz 2015). The increased population is believed to have triggered an exodus of both people and animals from the original places of domestication to other parts of the world (Larson *et al.* 2010).

Along the way, farmers encountered wild populations of progenitors, for example, when farmers brought their cattle from the Fertile Crescent to elsewhere, they encountered local populations of wild aurochs. When the farmers settled down, gene flow between the domesticated species and the wild species may have happened, a process called **admixture**

(Larson & Burger 2013). Admixture leads to the introduction of other genetic lineages into the population. Many scientists use admixture interchangeably with **introgression**, but in this paper only the term **admixture** will be used.

Humans who brought their domesticated animals along their migratory route already had some knowledge about how domestic animals were created, or how the domestication traits could be maintained by management of the animals. Therefore, a**rtificial selection**, selection by humans based on the phenotype of the animal, is a likely process that has happened. In each location in the world, the development of breeding knowledge could have been different. Not only humans were selecting individuals for reproduction, but the environment too has to be taken into account. The animals had to adapt to new environments, and some animals were more equipped to survival in certain environments than others. This is an example of **natural selection**. Lastly, the commensal pathway of domestication involved **unconscious selection**, animals with docility traits were selected for, although this was not done with human intent or direction, but rather a coincidental process different from pure natural selection.

4.1 Admixture: a story of escaping animals?

Evidence for admixture is found in several places of the world, but it is mostly researched in Europe. Large part of the research focuses on the major livestock species of today: pigs and cattle. Many of the studies reviewed here focused on ancient DNA obtained from archaeological remains, whereas some researchers try to infer the history of domesticated animals by analysing genetic material of modern populations.
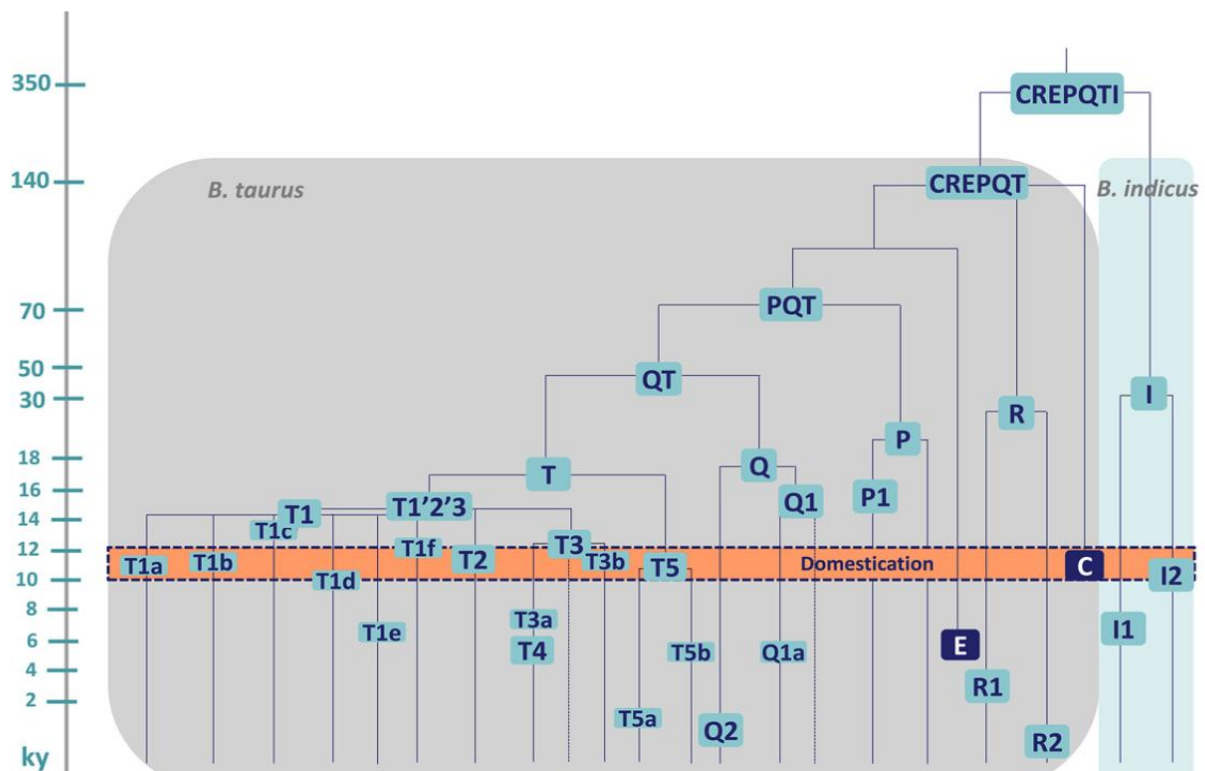

Figure 2. Schematic representation of mtDNA phylogeny in cattle. Most domesticates have one of the T-haplotypes. P haplotype is found in wild European aurochs, Q in wild European aurochs who lived south of the Alps. Taurine cattle (*Bos taurus taurus*) and Zebu cattle (*B. t. indicus*) are both domesticated from auroch populations, although separately from each other and Zebu cattle came 2000 years after the initial Taurine cattle domestication. The two progenitor-auroch populations diverted around 350 kya from each other. Source: Olivieri *et al.* 2015.

Research on the genomes of Neolithic (~10200 to 4500 years BC) cattle sheds a light on the similarities between populations in different parts of Europe (Bollongino *et al.* 2006). The researchers looked at the ancient mitochondrial DNA (mtDNA) of cattle from the Middle East, the Balkan and Mainland Europe. The sequencing was successful in 48% of the time (39 out if 81 samples). Surprisingly, most of the specimens had a haplotype (haplotype T3, see figure 2) that originated in the Fertile Crescent, suggesting that the maternal lineage did not hybridise with local auroch bulls. Only one domesticated cow was found with auroch mtDNA: the researchers concluded that this was likely due to a sequencing error, rather than it being evidence for admixture (Bollongino *et al.* 2006). In 2008, Bollongino and colleagues tested their previous results again, but this time with samples obtained from other archaeological bones. They looked at mtDNA, but also at Y-SNPs (Single Nucleotide Polymorphisms on the Y-chromosome, to include the maternal lineage (mtDNA) as well as the paternal lineage (Y-SNPs). Y-SNPs results yielded that many specimens were supposedly hybrids, which suggests admixture with wild female aurochs. However, the lack of auroch haplotypes (P) for the mtDNA contradicts this. Their final conclusion was that there was no conclusive evidence for admixture (Bollongino *et al.* 2008).

With more recent developments in sequencing techniques, better evidence was found that the results of Bollongino *et al.* 2006 and Bollongino *et al.* 2008 were actually false. Stock *et al.* (2009) focused on cytochrome b analysis of the mtDNA in Mesolithic and Neolithic aurochs and domesticated cattle, obtained from sites in Britain, Germany and Slovakia. They found the P haplotype in domesticated cattle, which means that there has been matrilineal admixture, but only to a small degree (Stock *et al.* 2009). Many of the past studies have focused on mtDNA or the Y-chromosome, mainly due to technical limitations , but recent studies tell an even more diverse
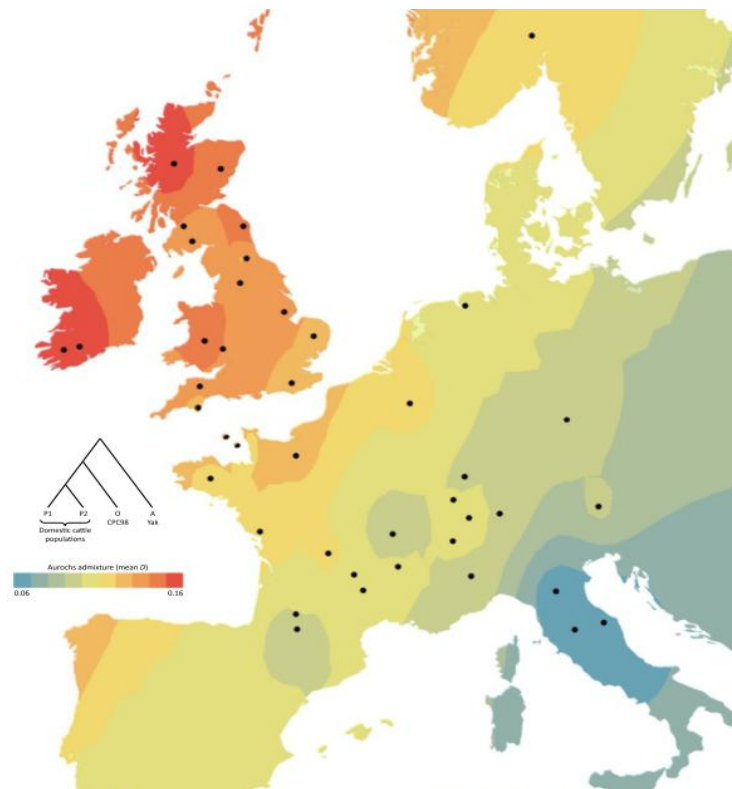

Figure 3. The correlation of geograpic distance to the Middle East and the degree of admixture. Value of D=0 means no shared alleles between aurochs and domesticated cattle, D=1 means all alleles are shared. Values for D range from 0.06 (blue) to 0.16 (red). Source: Park *et al.* 2015.

history of cattle management (Park *et al.* 2015). High-throughput DNA sequencing techniques have made it possible to look at whole genomes. This opportunity has led domestication researchers to focus on studying high-density SNPs on autosomes, especially genes associated with neurobiology, metabolism and growth, and immunology (Park *et al.* 2015). This team has found compelling evidence that there is a link between the geographic

distance to the Fertile Crescent (the initial point of domestication) and the degree of admixture. By using ABBA/BABA analysis (Martin *et al.* 2015) and Patterson's D statistics specifically designed to study the significance of admixture in modern populations (Durand *et al.* 2011), the researchers proved that in all places of Europe admixture with local wild aurochs has occurred (Park *et al.* 2015). In Great Britain, the degree of admixture (Patterson's D=0.16) is relatively high compared to the values found in the south of Europe (south of Italy, Patteron's D=0.06) (Park *et al.* 2015), see figure 3. The explanation of this may be that management of herds was different in the north of Europe, for example because of a decreased amount of trade due to the large distance to other farmers. This research shows that just looking at the mtDNA and Y-chromosomes can give entirely wrong conclusions, just because these regions do not possess enough power to show the true relations accurately enough.

In Africa, the history of cattle is different. Zebu cattle is morphologically different than Taurine cattle, and both are present in Africa. It has long been wrongly thought that African cattle had a different origin, and that Zebu cattle was independently domesticated from a different ancestor than the auroch (Stock & Gifford-Gonzalez 2013). Present genetic evidence sheds a better light on the history of cattle after migration out of the Fertile Crescent. Two different cattle species now live in Africa, Taurine and Zebu cattle, whereas in Europe only Taurine cattle is present. Presence of Zebu cattle in Africa, which was originally domesticated in the Indus valley, was likely a result of trade (Olivieri *et al.* 2015). After migration to Africa, the genomes of Taurine and Zebu cattle changed differently from each other. A study based on fully sequencing 17 genes in modern cattle breeds from Europe and Africa found that there was greater diversity in Zebu cattle, which they attributed to a higher amount of admixture with local wild African aurochs (Murray *et al.* 2010). Their suggestion was that the gene flow between wild and domestic cattle was directed by the farmers themselves to enhance hybrid vigour (increasing the biological quality of hybrid individuals by outbreeding) and locally adaptive traits. This process was repeated several times in Zebu cattle. Evidence for admixture in Taurine cattle was found too, but this was less present, which led the researchers to believe that the subspecies had entirely different post-domestication histories (Murray *et al.* 2010). Other evidence from Africa, Egypt to be precisely, supports these findings. Olivieri and colleagues (2015) worked on 31 mtDNA samples of two modern breeds. They used Illumina sequencing to obtain information on variation on the mitogenome (mitochondrial genome) in Egypt. They found that all specimens had the expected haplotype (T1: 58.0%, T2: 19.4%, T3: 16.1%, and Q1: 6.5%). However, due to the large haplotype diversity and the large differences between the Egyptian mitogenomes and the Fertile Crescent mitogenomes, the researchers concluded that during original stages admixture had taken place in Egypt, be it in a less universal way as the genetic evidence would suggest. This was explained by the fact that the founder populations already showed a relatively large diversity (Olivieri *et al.* 2015). Thus, there is ample evidence for admixture of domesticated cattle with wild aurochs in Africa.

MtDNA is used most often in the study of admixture, but has some drawbacks. The most obvious drawback is that mtDNA is maternally inherited (see Ballard & Whitlock 2004 for a complete overview of this). It has been suggested that loci on the Y-chromosome give

a better representation, especially when compared with mtDNA studies (Bollongino *et al.* 2008; Pérez-Pardal *et al.* 2010). Some researchers have therefore introduced the study of interspersed multilocus microsatellites on the Y-chromosome, loci that yield several amplified small, but different, bands from one individual male (Pérez-Pardal *et al.* 2010). They were interested in looking into the phylogenetic history of cattle, and comparing their results with more traditional ways. More geographically isolated places were found to have higher levels of admixture between wild male aurochs and the domestic populations. Most surprisingly, according to Pérez-Pardal *et al.* (2010), North and Central European cattle may have had admixture with wild aurochs as well, providing a counterargument for others who focused on other loci (Bollongino *et al.* 2006, 2008). It has long been stated that management strategies differed from north Europe to south Europe (Beja-Pereira *et al.* 2003, quoted in Pérez-Pardal *et al.* 2010), and this claim is supported by the fact that south European herders have mixed their cattle with wild aurochs more often than northern European farmers (Pérez-Pardal *et al.* 2010). This is a strange finding, as it contradicts the more recent conclusion of Park *et al.* 2015. The conclusion of Park *et al.* (2015) in their study of autosomal polymorphisms is more likely to be true than the conclusion reached by Pérez-Pardal *et al*. 2010, as the technical developments since 2010 have provided researchers with better tools to recreate past animal management practices.

The domestication of cattle is seen as central to the development of sedentary societies, and ultimately, the first urban civilizations (Park *et al.* 2015). The focus of most researchers is on the development of cattle farming in relation to human cultural development. However, others also focus on other domesticated species, such as pigs. Pigs (*Sus domesticus*) were independently domesticated in Anatolia but also the Mekong delta (South East Asia) (11kya), and 1500 years later migrated with humans into Europe (Frantz *et al.* 2015). Previously postulated hypotheses stated that early farmers isolated their domesticated pigs from the wild boars, but this has been overturned by Franz *et al*. (2015). By looking at autosomal DNA obtained from the blood of modern domesticated pigs (European and Asian) and wild boars, the researchers showed that significant admixture with wild boars had occurred shortly after farmers migrated to Europe, but this was not done on purpose. Early European farmers focused on mobility and let their pigs roam free in the woodlands. Wild boars could therefore easily interbreed with the domesticated pigs, a process that was unintentionally started by humans (Frantz *et al.* 2015).

Most studies focus on a comparison between different areas, rather than a comparison over time within one area. In Romania, pigs were introduced by migrating farmers from Anatolia. MtDNA extracted from pig molars (ranging from Mesolithic to Iron Age) were used to see changes in the haplogroups to which domesticated pigs belong (Evin *et al.* 2015). Their results show that shortly after humans had supposedly migrated to Romania, pigs shared the mtDNA haplogroup with Anatolian pigs. However, there was a gradual turnover and the haplotype changed to the one shared with local wild boars. This means that admixture had taken place between the Romanian wild boars and the domesticated pigs. Although the study proves the existence of admixture, it does not comment on the implications of this for management of the pigs. Another drawback of this study is that Early and Middle Neolithic finds are rare, and the finds increase during later

times. This means that the pig has likely become more important for people in later times, but also that the sample size for the extraction of DNA is smaller (Evin *et al.* 2015). In Europe, there is no consensus whether humans initiated admixture on purpose. Some research suggests they did, others state that it was undeliberate, but completely due to the management practices.

In Asia, similar events of admixture have occurred, making this a more worldwide phenomenon. Research in Japan, focusing on both mtDNA and microsatellites on autosomes, has shown that founding populations displayed low genetic variation (Murakami *et al.* 2014). They showed that after the farmers had migrated there and brought pigs from south-east Asia, the populations of domestic pigs interbred with local wild boars on several occasions to increase the genetic diversity of domesticated pigs. Similar results were yielded when domestic pig breeds from different locations in China were investigated using whole genome sequencing techniques and selecting high-quality SNPs (Ai *et al.* 2015). The researchers found a high modern diversity on regions with reduced recombination rates and attributed this to the admixture with local wild boars in prehistoric times. The pigs were interbred so that they could become adapted to the local environmental conditions, and the researchers noted that it was probably initiated by humans (Ai *et al.* 2015). Also in Asia, where the pig was independently domesticated, evidence for admixture is found, which was deliberately started by humans.

How and where horses have been initially domesticated has been a longstanding debate for decades (Warmuth *et al.* 2012). Recent research suggests that horses have been domesticated on the Eurasian steppes, somewhere in Ukraine or Kazakhstan (Ludwig *et al.* 2009; Warmuth *et al.* 2012). Warmuth *et al.* (2012) investigated the spread of domesticated horses over the rest of Europe and Asia by using modern DNA of 322 non-breed horses (horses used solely for work, and who do not belong to a particular breed). 26 microsatellite loci were amplified to tell something about the admixture that had happened in the past. To maintain viable populations in other parts of Eurasia, capturing wild horses for breeding purposes was essential. Farmers almost exclusively caught wild mares, which might seem counterintuitive because admixture in other domesticated animals is done with wild males. The explanation for this is that stallions were likely too hard to handle (Warmuth *et al.* 2012). Thus, the domestication and subsequent spread of domesticated horses throughout Eurasia involved a great deal of human intent.

4.2 Selection: did early farmers know what they were doing, or was it natural selection?

The study of admixture in prehistory shows that humans were able to direct breeding processes in their populations to a certain extent. Purposefully interbreeding with wild local populations is, however, less directed than selecting certain animals for the traits a farmer finds desirable. Evidence that humans have tried to actively improve their stock will prove that prehistoric farmers possessed knowledge of breeding, next to just management of their livestock. Other types of evidence for selection of desirable traits (such as coat quality, and milk and meat yield) are present in the archaeological record in the form of biometric

differences, which is extrapolated from bone lengths or weights (Zeder *et al.* 2006). Using DNA sequencing techniques it becomes possible to precisely locate the specific genomic information that lies at the basis of these selection processes. Research in this area mainly focuses on horses and pigs, though surprisingly, insects have received a great deal of attention as well.

Domesticated pigs are morphologically very different from the wild boar, their wild progenitor (see figure 4). This led researchers to suggest that these morphological differences were the basis for selection on the basis of phenotypic traits (Frantz *et al.* 2015). Genetic evidence confirms this idea: artificial selection for certain traits led to slightly different morphologies, even though admixture with wild boars still happened, which are now shown in the DNA as 'islands of domestication'. These traits show only in the domestic pigs and are unaffected by admixture with wild boars (Frantz *et al.* 2015). The creation of morphological diversity, while at the same time allowing gene flow to happen, requires a great deal of intent from the human perspective, as well as a great amount of knowledge of breeding and inheritance of desirable traits.
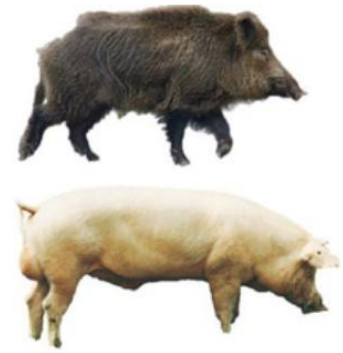

Figure 4. Wild boar (above) and domesticated pig (below). Source: Rubin *et al.* 2012.

Selection of humans on preferential phenotypes of pigs only started to happen on a large scale during the Industrial Revolution, when pigs became more central to the human food supply (Bosse *et al.* 2014). However, it is still possible to detect selection regions from earlier times. Modern domesticated pig DNA was used to assess which regions of the genome, and to what degree, farmers have selected for in prehistoric times (Bosse *et al.* 2015). The researchers found that regions associated with production of fat tissues were under high selection. The farmers chose the preferred phenotypes (positive selection) and removed the other phenotypes from the breeding pool (purifying selection) (Bosse *et al.* 2015). This research shows that farmers had the knowledge of inheritance, because they selected for traits they found interesting, in this case the production of meat.

Other researchers have found similar results. Larson *et al.* (2007) showed that after humans migrated to Europe in the Neolithic, the amount of domesticated pigs with A-side haplogroups (mtDNA) greatly increased at the cost of the C-side haplogroups. To investigate if humans had been actively selecting for haplotype A, researchers looked at the mtDNA if haplotype A is morphologically different from haplotype C (Lega *et al.* 2016). Haplotype A and C have been introduced initially by admixture with wild boars, therefore modern wild boar DNA was used to assess this hypothesis. Haplotype A boars were significantly larger (7.63 kg larger), longer (10 cm longer) and had a wider chest (6.3 cm wider). Furthermore, ear size was larger in A haplotype boars as well, a trait that is still retained in domesticated pigs, but this is more likely to have happened as unintentional selection (Lega *et al.* 2016). Neolithic farmers indeed selected for pigs that had larger sizes, to increase the meat yield.

Coat variability is significantly larger in domesticated pig when compared to that of wild boars (Fang *et al.* 2009). This has been used as a basis to study the relationship

between molecular and morphological differences in the study of selection by early farmers. The *MC1R* gene is the master regulator for melanogenesis, causing a camouflaging coat in wild boars. By sequencing 80 DNA samples (68 domesticated pigs and 12 wild boars) the researchers showed that non-camouflaging coats were indeed under heavy selection by early farmers. *MC1R* is only involved in melanogenesis and does not cause any pleiotropic effects on other traits, which explains why there is still a lot of diversity. Early farmers herded their pigs in forests and it is therefore understandable that highly visible coats would be preferred. Not only to make the pigs more easily visible, but also to distinguish them from wild boars (Fang *et al.* 2009). More recent research has looked into selection on coat colour as well. Focusing on modern DNA samples of wild boar and domesticated pigs (37 pigs, 11 wild boars), Rubin *et al.* (2012) revealed as well that the *MC1R* gene was under high selection pressure. They found four other loci under selection (*NR6A1G*, *PLAG1*, *LCORL*, and *KIT*), three of which are found in pathways unrelated to any traits that might be interesting to farmers (*NR6A1G*, *PLAG1*, and *LCORL*). The *KIT* locus (responsible for coat pigmentation) in pigs is an example of evolution of alleles in domesticates under strong selection. The dominant allele in domesticated pigs (*Dominant white*) is only viable in homozygous form, whereas other alleles are lethal or sub-lethal. This is because of heavy selection, combined with mutations on the allele that made less preferential alleles lethal (Rubin *et al.* 2012). Coat colour too, was under selection by heavy farmers, primarily to make them distinguishable from wild boars.

Evidence in pigs suggests that humans were directly selecting for economically interesting traits and non-camouflaging traits. In other animals, like horses, there is less clarity. Horses have aided humans greatly and had a great impact on warfare, transportation and communication capabilities, which resulted in the fact that great value was always attributed to them (Ludwig *et al.* 2009). This led to the hypothesis that, because of their importance, humans have always selected heavily on preferential traits, such as strength. To investigate this, Ludwig *et al.* (2009) looked at DNA sequence polymorphisms in loci responsible for coat colour variation. Before domestication happened in horses, only two coat colours were found, namely bay and black. After domestication, coat colours radiated and newly mutated alleles were found and selected for by humans. There was indeed directed selection by humans on coat colour after the domestication of horses, evidenced by this radiation (Ludwig *et al.* 2009). Although later research proved that coat colour variation had a slightly more complicated history, today there is no doubt that farmers have selected certain coat colours in their domesticated horses that were not camouflaging (Imsland *et al.* 2016). The researchers noted that some coat colour alleles (*non-dun1*) had a pre-domestication origin. Their preliminary conclusion was that the radiation of coat colours therefore might not have been caused by selection after domestication. However, the *non-dun1* allele proved to be the basis allele from which other alleles arose after domestication that were possessed by domesticated horses. Through deletion of several nucleotides, the *non-dun2* allele arose, which is only possessed by domesticated horses, and it explains the colour variation of coats (Imsland *et al.* 2016).

Pleiotropic effects are associated with coat colour as well. Leopard spotting (*LP*) has been a regularly occurring phenotype visible in the coats of domesticated horses since the

beginning of the domestication process (Ludwig *et al.* 2015). Several SNPs in the *TRPM1* (*transient receptor potential cation channel subfamily M, member 1*) gene are the cause for *LP*, and homozygosity for *LP* is also associated with congenital night blindness. By analysing ancient DNA from 96 horse bone and tooth specimens (ranging from the Late Pleistocene to the Middle Ages) at the site of the SNPs associated with both *LP* and night blindness, the researchers tried to get an answer on selection preferences of early farmers. In wild horses the *LP* phenotype was more frequent than in later domesticated populations. During the Bronze Age the frequency of the *LP* allele (selection coefficient -0.5176) plummeted, which could not be explained by genetic drift alone. However, during the Iron Age, there was a shift in preference and the allele frequency of *LP* rose again. This means that preferences with regard to phenotype changed over time (see figure 5). These changing selection regimes, when holding truth for other loci too, might explain why there is a relatively large genetic diversity despite extensive selection (Ludwig *et al.* 2015).
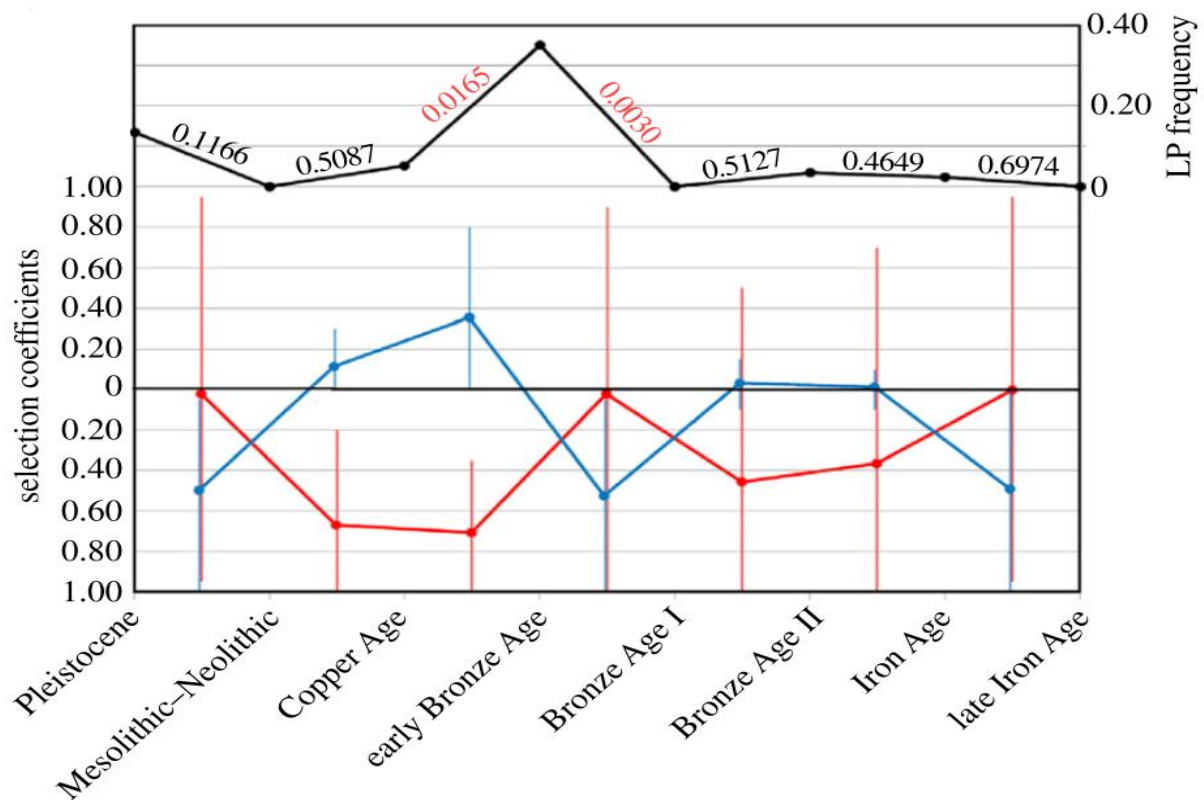

Figure 5. Selection coefficients for *LP* and night blindness. The black line shows the frequency of the *LP* allele. The blue line shows the selection coefficient for *LP* and the red line for night blindness. Different time periods had different selection preferences. Source: Ludwig *et al.* 2015.

Some domesticated horse populations however have not been subjected to selection by humans. Horses form the Yakut people, located in northern Siberia, have been gradually adapting to sub-arctic conditions since the 13th century AD (Librado *et al.* 2015). The Yakut people migrated from Turkey, bringing their horses with them. Librado *et al.* (2015) used ancient Pleistocene DNA of wild horses found in Siberia and modern DNA from Yakutian horses to shed light on the origin of these horse populations. They found no evidence of admixture with local wild horses after the Yakut people had arrived. The successful adaptation of the horses of the Yakut people to the sub-arctic conditions of

Siberia were ascribed to natural selection alone. The horses developed several physiological and morphological adaptations, for example adaptations that had to do with the avoidance of ice-formation in the blood vessels, but the researchers were unable to find a human directed selection on loci for these adaptations (Librado *et al.* 2015). So, despite all the previous evidence, other natural selection might have also been at play.

Some of the less likely domestication processes that have occurred are those where insects have been domesticated. These processes did not involve the migration of the animal to novel environments. Today, the iconic model organism *Drosophila melanogaster* is used to study the effects of unconscious selection by humans on the genes associated with the "Domestication Syndrome": genes that cause tameness and docility (Stanley & Kulathinal 2016). In prehistoric times, the domestication and subsequent spread of insects was not always as easy as today. People have managed honey bees (*Apis mellifera*) since the Neolithic, not only for honey, but also beeswax (Roffet-Salque *et al.* 2015). This has not led to the domestication of honey bees however, although the introduction of hives has made honey bees more reliant upon humans (Oldroyd 2012). To investigate what happened to the genomes of honey bees by human management, researchers have analysed the DNA of different colonies all over the world (Harpur *et al.* 2012). They found that genetic diversity in honey bees had increased since people started to manage them, which is completely opposite to what happened to genetic diversity in domesticated animals. This can be ascribed to how colonies are managed: there is a high level of importation of other stocks, which means lots of admixture, but not really any directed selection by humans (Harpur *et al.* 2012). Larger animals were more easily domesticated, which may mean that knowledge or intent missed for the domestication of honey bees, although they can be seen as tamed animals.

One success story in the domestication of insects is the domesticated silk moth (*Bombyx mori*), which was domesticated in China almost 5000 years ago (Goldsmith *et al.* 2005). Silk moths were an important economic product, which makes it likely that people have selected certain characteristics to increase economic gain. In order to prove selection in the silk moth by humans, Fang *et al.* (2015) identified differently expressed genes in domesticated versus wild silk moths. Cocoons of wild silk moths are much smaller than the domesticated ones, and wild silk moths produce only one-third of the silk that domesticated silk moths produce. They found a genetic basis for this: genes associated with biosynthesis and secretion of silk were highly upregulated in the domesticated silk moths, which was caused by human selection (Fang *et al.* 2015). Other research also shows evidence for selection in silk moths, in the form of purifying selection after domestication (Hughes 2013). By using mtDNA from the silk moth, Hughes investigated the amount of purifying selection after domestication. He found that the ratio nonsynonymous SNPs to synonymous SNPs was higher in domesticated silk moths than in wild silk moths. This larger diversity suggests there was purifying selection in silk moths after they were domesticated (Hughes 2013). Even traits of insects were thus selected on, in this case to increase silk production.

Evidence for selection in domesticated animals has been reviewed here, but only for animals that have received the most attention in research: pigs, horses, and even silk moths.

For other domesticated species there is less evidence of selection by humans, mainly because of a lack of research.

5. Synthesis

This paper has reviewed multiple studies that focused on the genomic alterations in domesticated animals in the time period after the domestication up to now, specifically pigs, cattle, horses, silk moths and honey bees. Many processes of alterations in the genome involved migration to novel environments where there had been only wild animals before and admixture (in the case of pigs, cattle, and horses), or selection for specific economic traits (silk moths and in some cases pigs). Many alterations in the genome were due to selection on traits that were economically interesting (fat production in pigs, silk production in silk moths). Because of the wide variety in types of evidence, it is useful to compile all research papers into one table, so that it might be possible to see a general trend in the processes that shaped the genome of the domesticated animals. In 10 out of 12 papers, evidence of admixture with local wild populations was found. In case of admixture, most evidence suggests that if there was admixture, this was mostly directed and initiated on purpose by humans (7 out of 10 papers). For selection it goes that human directed selection in prehistory was more likely than natural selection on certain traits, or at least more easily detectable: 9 out of 12 papers found evidence for human directed selection, and only 2 find evidence for natural selection, and one is inconclusive. This makes it evident that there is no consensus in the practices of humans in the shaping of domesticated genomes, but it suggests that in many regions, humans have influenced evolutionary processes in the genomes of domesticated animals.

Table 1. The synthesised research papers used in this review. The studied animal, the main conclusion, and the method of research is listed per paper, and when possible, if it was an intentional process directed by humans. The last column shows the traits that had been selected for, or unrelated noteworthy results.

Source	Animal	Conclusion	Method	Human directed	Notes
Bollongino *et al.* 2006	Cattle	No admixture	ancient mtDNA	-	
Bollongino *et al.* 2008	Cattle	No admixture	ancient mtDNA	-	
Stock *et al.* 2009	Cattle	Admixture	ancient mtDNA	Yes	Admixture via the maternal DNA
Park *et al.* 2015	Cattle	Admixture	ancient autosomal DNA	Yes	Positive correlation between geogrpahic distance to the Middle East and the degree of admixture
Murray *et al.* 2010	Cattle	Admixture	modern DNA	Yes	
Olivieri *et al.* 2015	Cattle	Admixture	modern mtDNA	Yes	Admixture only during the fist stages of migration
Pérez-Pardal *et al.* 2010	Cattle	Admixture	modern DNA	Yes	Different strategies in North and South Europe
Frantz *et al.* 2015	Pig	Admixture	modern autosomal DNA	No	
Evin *et al.* 2015	Pig	Admixture	ancient mtDNA	n/a	
Murakami *et al.* 2014	Pig	Admixture	mtDNA, autosomal DNA	n/a	
Ai *et al.* 2015	Pig	Admixture	modern DNA	Yes	
Warmuth *et al.* 2012	Horse	Admixture	modern DNA	Yes	Wild mares were captured
Frantz et al. 2015	Pig	Selection	modern DNA	Yes	
Bosse *et al.* 2015	Pig	Selection	modern DNA	Yes	Positive selection and purifying selection
Lega *et al.* 2016	Pig	Selection	ancient mtDNA	Yes	Positive selection for size, unconscious selection for ear size
Fang *et al.* 2009	Pig	Selection	modern DNA	Yes	Positive selection on coat colour
Rubin *et al.* 2012	Pig	Selection	modern DNA	Yes	Positive selection on coat colour
Ludwig *et al.* 2009	Horse	Selection	modern DNA	Yes	Positive selection on coat colour
Imsland *et al.* 2016	Horse	Selection	modern DNA	Yes	Positive selection on coat colour
Ludwig *et al.* 2015	Horse	Selection	ancient DNA	Yes	Selection preferences changed over time
Librado *et al.* 2015	Horse	Selection	ancient DNA, modern DNA	No	Natural selection
Harpur *et al.* 2012	Honey bee	Admixture, no selection	modern DNA	No	Honey bees were never really domesticated
Fang *et al.* 2015	Silk worm	Selection	modern DNA	Yes	Silk secretion upregulated
Hughes 2013	Silk worm	Selection	modern mtDNA	n/a	Purifying selection

6. Humans had a great influence in shaping the genomes of domesticates

Drawing from the evidence reviewed in this paper it becomes clear that humans played a major role in the way the genomes of domesticated animals are shaped. In several locations in the world, and independently from each other, early farmers have facilitated gene flow between domesticates and their wild progenitors. Almost everywhere, this was done on purpose. The intent behind admixture might not have been entirely focused on producing economically interesting animals, but this is likely the case for human directed selection. A lot of evidence for selection is found in domesticated animals: this involved a great deal of knowledge from the farmers. They had to know what they were looking for, what they were interested in, but more importantly, they had to find out that breeding certain animals could yield more meat or milk. The domestication process in the Fertile Crescent took very little with regard to human intent, and the evidence for admixture and selection suggests that farmers learned from the initial stages to innovate their breeds.

The papers reviewed here show that there is a large overlap of evidence for the involvement of humans in the genome of the domesticated animal. In all parts of the world, evidence for admixture is found in cattle and pigs, and even in horses. The same goes for selection of coat colour in pigs and horses, which seems to be global too. This suggests that knowledge was created in the Fertile Crescent and subsequently spread during migration, or that there were limited possibilities and it is coincidental that all areas saw comparable processes happening, or the communication between farmers post-migration was very good. The time period between domestication and the mass migration to other areas of the world is rather long, so it is highly likely that the knowledge was created in one place (the Fertile Crescent, or in the case of the silk moth and the pig also in Asia) and was spread by migrants to other parts of the world.

One problem that arises from the evidence reviewed is the use of mtDNA. The fact that matrilineal inheritance can only be investigated obscures a lot of the truth from the record. Furthermore, mtDNA is under high selection pressures and follows unusual evolutionary rules (Ballard & Whitlock 2004). Researchers have tried to overcome this by incorporating Y-chromosome SNPs in their research as well. This does not mean that the problem is solved: now we have evidence for matrilineal and patrilineal inheritance, but not for inheritance that is not linked to the sex of an animal. Luckily, with the improvements being made in the sequencing of DNA and the recovery of ancient DNA samples, the evidence will become more applicable to the domestication research. This does not mean however that mtDNA will be disregarded from now on, as there is still recent research being performed on the ancient mtDNA of animals. The likely cause for this is the costs involved, mtDNA research is cheaper than whole genome sequencing.

Selection for certain traits interesting to farmers seems to be proven for pigs, horses, and even silk moths. For other animals this has been less well-researched. This may either mean that selection did not happen in other animals, or that it only happened on a small scale, or even that it is just more common practice of researchers to focus on the study of certain species. The latter seems to be the most likely, as there is some evidence for the

selection in the early stages after migration for certain traits in chickens (Rubin *et al.* 2010), dogs (Cagan & Blass 2016), and cattle (Zhang *et al.* 2015).

The detection of natural selection within the genome can be difficult as well. It is hard to distinguish between traits that were under pure natural selection or under human directed selection. In the case of admixture for example: is this a form of natural selection (local wild mtDNA is more fit than domesticated mtDNA) or is this a form of human management? The evidence from the research papers tells that several loci were under human selection, but overall, the domesticate genome has not truly been investigated, making it hard to talk about the proportion of human selection against natural selection.

The findings of admixture and selection on a large scale raise some important questions. How common were these processes really? Is the archaeological record sufficient enough to draw conclusions about the universality of these processes? In the case of selection, how and why did the selection preferences change over history? If admixture was common practice until recent history, how did farmers maintain the phenotypic and behavioural domestication traits? In order to answer these questions, genetic evidence is not enough, but there is large need for sociocultural context as well as the landscapes in which the management happened was different for a lot of places (Marshall *et al.* 2014). A good way to investigate the change of selection preferences would be to investigate changes on a large timescale within one site, following Ludwig *et al.* (2015) for example. A problem that may arise, however, is that only a small number of sites show a large enough sample size over time.

Understanding the human side of the equation is essential as well, as humans were the main actors in the admixture of wild and domesticated animals, but especially in the selection process. Furthermore, the domestication of animals has led to the emergence of large cities and human society as a whole and has transformed large parts of the earth into agricultural fields (Smith 2012). This is another reason for the incorporation of multiple fields of study, as domestication shaped human life and society too. Although the evidence reviewed in this paper suggest a large amount of human intent, a better understanding with regard to the intentions of farmers may be found when this evidence is combined with other archaeological or ethno-historical evidence. Evidence on societies as a whole may reveal certain preferences for traits that exist in one place but are absent in another.

All in all, this review shows that early farmers have had a big influence on the genomes of their domesticated animals. Farmers gathered a lot of knowledge in the Fertile Crescent since the initial domestication, and these farmers brought their knowledge to other parts of the world when they migrated. Similar results are found in many regions of the world, suggesting that either the knowledge with regard to farming was global, or the possibilities with regard to management were limited. Looking at the limited possibilities to communicate, the latter seems to be the likeliest, but future research has to answer this.

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