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Consequences of the Domestication of Man's Best Friend, The Dog

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Abstract

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The dog was the first animal to be domesticated and the process started at least 15 000 years ago. Today it is the most morphologically diverse mammal, with a huge variation in size and shape. Dogs have always been useful to humans in several ways, from being a food source, hunting companion, guard, social companion and lately also a model for scientific research.

This thesis describes some of the changes that have occurred in the dog's genome, both during the domestication process and later through breed creation. To give a more comprehensive view, three genetic systems were studied: maternally inherited mitochondrial DNA, paternally inherited Y chromosome and biparental autosomal chromosomes. I also sequenced complete mitochondrial genomes to view the effect new living conditions might have had on dogs' genes after domestication. Finally, knowledge of the genetic structure in purebred dogs was used to test analytic methods usable in other species or in natural populations where little information is available.

The domestication process appears to have caused a relaxation of the selective constraint in the mitochondrial genome, leading to a faster rate of accumulation of nonsynonymous changes in the mitochondrial genes. Later, the process of breed creation resulted in genetically separated breed groups. Breeds are a result from an unequal contribution of males and females with only a few popular sires contributing and a larger amount of dams. However, modern breeder preferences might lead to disruptive selective forces within breeds, which can result in additional fragmentation of breeds. The increase in linkage disequilibrium that this represents increases the value of purebred dogs as model organisms for the identification and mapping of diseases and traits. Purebred dogs' potential for these kinds of studies will probably increase the more we know about the dog's genome.

Keywords: mitochondrial genome, Y chromosome, microsatellites, disruptive selection, breed structure, whole genome amplification, gene flow, linkage disequilibrium, *Canis familiaris*, *Canis lupus*

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*All knowledge, the totality of all questions and answers,
is contained in the dog.*

FRANZ KAFKA, *Investigations of the dog*

Cover photos by S Björnerfeldt

List of Papers

This thesis is based on the following papers, referred to by their Roman numerals throughout the text.

- I Björnerfeldt S*, Webster MT*, Vilà C (2006)
Relaxation of selective constraint on dog mitochondrial DNA following domestication. *Genome Research* **16**:990–994.

* Equal contribution.
- II Sundqvist A-K, Björnerfeldt S, Leonard JA, Hailer F, Hedhammar Å, Ellegren H, Vilà C (2006)
Unequal contribution of sexes in the origin of dog breeds. *Genetics* **172**:1121–1128.
- III Björnerfeldt S, Hailer F, Nord M, Vilà C
Disruptive selection within dog breeds. (Submitted)
- IV Björnerfeldt S, Hailer F, Vilà C
Estimation of recent gene flow in metapopulations: using poodles as a model organism. (Manuscript)
- V Björnerfeldt S, Vilà C (in press).
Evaluation of methods for single hair DNA amplification. *Conservation Genetics*, published online: 28 October 2006.

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Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of London. B: biological sciences* **270**:91–97.

Lindberg J*, Björnerfeldt S*, Saetre P, Svartberg K, Seehuus B, Bakken M, Vilà C, Jazin E (2005)
Selection for tameness has changed brain gene expression in silver foxes. *Current Biology* **15**:R915–R916.

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Seddon JM, Sundqvist A-K, Björnerfeldt S, Ellegren H (2006)
Genetic identification of immigrants to the Scandinavian wolf population. *Conservation Genetics* **7**:225–230.

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Abbreviations

AKC	American Kennel Club
AMOVA	Analysis of Molecular Variance
bp	Base pair(s)
d-loop	Displacement loop (mitochondrial control region)
d _N	Nonsynonymous substitution rate
DNA	Deoxyribonucleic acid
d _S	Synonymous substitution rate
FCI	Fédération Cynologique Internationale; World Canine Organisation
Gb	Gigabase (10 ⁹ base pairs)
kb	Kilobase (10 ³ base pairs)
LD	Linkage disequilibrium
Mb	Megabase (10 ⁶ base pairs)
MHC	Major histocompatibility complex
ML	Maximum-likelihood
mtDNA	Mitochondrial DNA
NCBI	National Center for Biotechnology Information
NJ	Neighbour-joining
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SNP	Single nucleotide polymorphism
tRNA	Transfer RNA
WGA	Whole Genome Amplification

Introduction

*He is your friend, your partner, your defender, your dog.
You are his life, his love, his leader.
He will be yours, faithful and true, to the last beat of his heart.
You owe it to him to be worthy of such devotion.*

UNKNOWN

Man's best friend

We are today surrounded by more than 400 million dogs worldwide; purebred, mongrel and feral dogs (Coppinger and Coppinger 2001), all of them descendents from the wolf (Vilà et al. 1997, Savolainen et al. 2002). These dogs have been subjected to strong selective pressures for a very long time, leading to their diversity in appearance and behaviour. They are today the most morphologically diverse mammalian species, with a huge variation in size and shape (Wayne 1986a, 1986b) divided into more than 400 recognized breeds (Clutton-Brock 1999).

The domestic dog has been man's best friend for at least 15 000 years. The strong bond that exists between dog and human cannot be compared to any other human-animal relationship and it is based on both practical reasons and affection. Dogs have been helping us for millennia, partly working as shepherds, guides, hunters and protectors and partly as model organisms for biomedical research. Nevertheless, the emotional side of the relation between humans and dogs is just as important, and the dog's most important role has been as devoted friend and companion (Moody et al. 2006).

Behaviour studies have shown that, as a result of this long coexistence with humans, dogs are exceptionally good at reading human signals, even better than chimpanzees and wolves (Hare et al. 2002, Miklósi et al. 2003). To be able to live with humans, this quality has probably been evolutionarily beneficial for the dog (Pennisi 2002) and also the first step in becoming man's best friend.

Dog domestication

Definition of domestication and domestic animals

Many authors have defined domestication as “a process whereby succeeding generations of tamed animals gradually became absorbed into human societies, were increasingly exploited, and eventually lost all contact with their wild ancestral species” (Clutton-Brock 1999). Accordingly, the definition of a domestic animal is an animal bred in captivity for purposes of benefit to humans who control its reproduction, food supply and territory organization and are thereby modified from its wild ancestors (Clutton-Brock 1999, Diamond 2002). Therefore, domestication is not the same as mere taming of wild-born animals and not all animals can be domesticated. In 1865 Francis Galton claimed that six conditions had to be fulfilled by a wild species before it could be domesticated. These were:

1. “*Hardiness*” – The animals should be able to adapt to new environments and not claim too much care.
2. “*Fondness for Man*” – They must feel connection to humans and always see him as the leader, despite demand for hard work.
3. “*Desire of Comfort*” – They are strongly attached to human habitations and do not have any flight tendency or panic in enclosures when faced with predators.
4. “*Usefulness to Man*” – The most obvious condition is that the animals had to be useful to humans. Otherwise, after growing up and losing the youthful ways, which had first attracted their captors, and was the reason why they kept them as pets, the animals would have been repelled. Undoubtedly, the most durable reason for maintaining the animals was as a store of future food.
5. “*Breeding freely*” – The domestic animals must be able to breed freely in captivity. This requirement is one of the most important of the six that have to be satisfied.
6. “*Easy to tend*” – The animals must be easily controlled by the humans. If the animals are gregarious, a large number of animals, as in a herd or flock, keep together and can therefore be controlled by only one or a few herdsman.

Considering that all of these six requirements mentioned above have to be fulfilled for an animal to be permanently domesticated, it is not so surprising that so few animals have been domesticated during the last thousands of years since the domestication process began.

Origin of domestic dogs

Charles Darwin (1868a,b) thought that domestication resembles the way evolution works with the exception that the changes are due to artificial selection rather than natural selection. Concerning the origin of dogs Darwin (1859) suggested, “that several wild species of Canidae have been tamed, and that their blood, in some cases mingled together, flows in the veins of our domestic breeds”. The same view was later supported by the Nobel laureate Konrad Lorenz (Lorenz 1953). Lorenz suggested that social dogs with a strong loyalty to their master derived mostly from wolves, while other dogs had a large influence of jackals. However, today we know that dogs derive only from wolves and that they were the first animals to be domesticated. Nevertheless, little is known about how the domestication process was initiated. Two main theories have been put forward to explain how the domestication could have taken place:

Theory 1: From pet keeping to domestic dogs

One theory, suggested by Galton (1865), was that primitive people kept tamed young wild animals [wolves] as pets, of which some remained docile even after reaching adulthood. Thereby domestication could have been a natural consequence of keeping pets. He also suggested that many animals have been tamed over and over again and therefore numerous opportunities could have arisen for the animals to be domesticated. This theory was later supported by Zeuner (1963), who also mentioned the apparent importance of food supply in the establishment of a close association between dog and man.

Theory 2: Human–wolf co-evolution, partnership or symbiosis

Schleidt and Shalter (2003) suggested an alternative view of dog domestication. They believe that due to co-evolution of wolves and humans occurring at different times and places, interactions between these two groups could have taken place at several occasions. Both were social species that hunted many of the same prey and wolves were also hanging around human camps looking for food. All these different events may have led to a closer relationship between humans and canids that gradually shaped their future interdependence.

Budiansky (1992, 1994) suggested that it might have been the wolves that initiated contact with humans, which later led to domestication due to willing partnership or symbiosis. These individuals, scavenging food around human settlements, were probably less fearful than other wolves, due to natural variation, and could thereby come close to humans and get comfortable with them. The association with humans gave these primitive dogs advantages such as food and warmth, but at the same time they might have lost some of the characteristics needed for survival in the wild.

The first domestication events – when, where and how many?

The fact that dogs derive from wolves is by now established, but where (location), when (timing) and the number of founders and/or domestication events is still controversial. The time for the initiation of dog domestication has been intensively debated and suggestions range from times around 13 000–17 000 years ago (Clutton-Brock 1999, Sablin and Khlopachev 2002) to 135 000 years ago (Vilà et al. 1997). However, to begin a taming and selection process, the humans had to be biologically and mentally capable before they could initiate this course of action. Behaviourally modern *Homo sapiens* emerged first around 55 000–80 000 years ago (Diamond 2002), which indicates that the domestication procedure probably started some time after this date.

Archaeological evidence of dog domestication

The earliest find of morphologically distinct domestic dogs were found from the Upper Paleolithic site Eliseevichi 1 (central Russia). The two complete dog craniums found are remains from adult dogs resembling Siberian huskies in shape and have been dated to 13 000–17 000 years ago, based on ^{14}C (Sablin and Khlopachev 2002). The earliest evidence of the close association between dog and human, on the other hand, is dated 10 000–12 000 years ago. The finding corresponds to a burial of an elderly person, with the left hand placed on the thorax of a 4–5 month old puppy (*Figure 1*). This was found in a limestone tomb in Ein Mallaha, Israel (Davis and Valla 1978).

Genetic evidence of dog domestication

By analyzing mitochondrial DNA (mtDNA) control region sequences from 140 dogs representing 67 breeds and 162 wolves representing 27 places around the world, Vilà et al. (1997) suggested that dogs originated more than 100 000 years ago. Four clades of dogs were found in the phylogenetic tree and the divergence time between dogs and wolves were based on a calculation of the time for the most recent common ancestor of clade I, the most diverse monophyletic group of dog sequences, and assuming that wolves and coyotes diverged at least one million years ago. Furthermore, indication of an episode of interbreeding between wolves and dogs was also found. By comparing the dog sequences with wolves and additionally samples from 5 coyotes and 8 jackals, they also found support for the hypothesis that wolves are the ancestors of dogs.

Savolainen et al. (2002) made a comparison of the mtDNA sequence variation in a sample set of 654 domestic dogs representing breeds all over the world. By using a revised molecular clock and assuming that several subclades were defined within clade I, representing different founding females, a calculation of the time to the common ancestor of each subclade



Photograph: Alain Dagand.

Figure 1. Burial in Ein Mallaha, Israel, showing a human skeleton buried together with a puppy. Reprinted by permission from Macmillan Publishers Ltd: *Nature* 276: 608–610 (Davis and Valla 1978) and Dr. Simon Davis.

could be performed and produced a range of possible origin dates. An average of these dates indicated a domestication time of around 15 000 years ago, more consistent with the archaeological evidence. An alternative calculation, assuming a single origin of clade I, provided a domestication time of around 40 000 years ago. Furthermore, the authors suggest an East Asian origin of dogs, based on the considerably higher diversity found in dogs from that area. However, the East Asian samples were based on mostly mongrels or local breeds not recognized by FCI (Fédération Cynologique Internationale; World Canine Organisation), whereas dogs representing other places in the world were mainly based on purebred dogs. Since dog breeds are often founded from a few individuals and are to a large degree inbred, they are expected to have lower genetic diversity than mongrels, and this could have influenced the results.

A study by Leonard et al. (2002), comparing mtDNA control region sequences from American dog remains pre-dating the arrival of Columbus in 1492, revealed that native American dogs had similar sequences to those found in modern Eurasian dogs. This was taken as an indication that dogs arrived to North America together with the humans colonizing the New World, and thereafter evolved in isolation. Furthermore, these results also imply that around 12 000 to 14 000 years ago, when humans arrived to the New World, they already had domesticated dogs, and also that dogs and humans at that time coexisted over three continents at least: Europe, Asia and America.

In a study by Lindblad-Toh et al. (2005), mathematical simulations performed on a dog population suggested a domestication time of 27 000 years ago. The different studies mentioned above all supports the view that the domestication of dogs is likely to be older than the existing archaeological remains indicate. However, the precise date of the dog domestication is not known yet.

Number of individuals involved in the domestication process

To solve the question about the actual number of founding individuals and/or domestication events, different genetic methods have been used. The huge genetic diversity found in dogs suggests multiple domestication events, possibly followed by occasional admixture with wolves. Vilà et al. (1997) suggest four separate domestication events based on the four distinct clades of dogs found in the neighbour-joining (NJ) tree built from mtDNA control region sequences. However, this only reflects the female part of contribution to domestication. Savolainen et al. (2002), however, suggest six or more founding events, based on a more extensive sample size, but also this reflecting only the maternal lines. A recent study on single nucleotide polymorphisms (SNPs) on the dog Y-chromosome, reflecting the paternal history of founding individuals during dog domestication, was estimating the possible number of male contributions (Natanaelsson et al. 2006). The

authors studied just ten dogs, but their results already indicated an origin from at least five different male wolf lineages. However, neither mtDNA nor Y chromosome studies can be used to infer the total number of wolves implicated in the domestication process, but they seem to indicate that multiple wolf populations may have been involved.

To study the contribution of both males and females Vilà et al. (2005) studied the diversity at the major histocompatibility complex (MHC) in different domestic mammals. The MHC is essential for the normal functioning of the immune system and typically shows a high level of genetic diversity. Balancing selection acts to maintain this MHC polymorphism over long time periods. Therefore, the MHC alleles present in the dog population are quite ancient, having been maintained for millions of years since the divergence from the wolf ancestors (Hughes and Yeager 1998). To explain this huge MHC diversity in dogs, Vilà et al. (2005) suggested at least 21 founders for the dog population. However, this is a minimum number and assumes that all founders are heterozygous for different alleles and is equally successful producing offspring and that no alleles are removed from the population by drift, which is highly unlikely. To obtain a more likely estimate of the number of founders, several simulations of the genetic diversity using models that varied in different demographic scenarios, led to the result that either one population with hundreds of wolves were involved in the domestication, or that hybridization between dogs and wolves has been frequent after the domestication contributing to the huge genetic diversity in several small populations. This last option seems much more realistic and is supported by recent studies showing hybridization between dogs and wolves (Andersone et al. 2002, Randi and Lucchini 2002, Vilà et al. 2003, Verardi et al. 2006).

Changes associated with domestication

Genetics

As soon as the first dogs became separated from their wild ancestors to live with humans, their genetic composition started to change. Since the founder group was likely small, a founder effect probably led to random genetic drift and a loss of genetic diversity. Thereafter domesticated animals changed in response to both natural and artificial selection over successive generations. A consequence following selection is selective sweeps, where loci tightly linked to the locus under selection, due to genetic hitchhiking, reduce the amount of variation in some areas of the genome and lead to changes in allele frequencies (Hartl and Clark 1997, Allendorf and Luikart 2007).

Young and Bannasch (2006) indicate that the fast changes in appearance in purebred dogs during the last 50–100 years may be explained by the occurrence of new mutations. However, Fondon and Garner (2004) believe

that this fast and continuous evolution on the morphology of dogs depends on length differences in gene-associated tandem repeats. Wayne, on the other hand, argues that since dogs undergo much more change in the shape of their skeleton postnatally than other canids (Wayne 1986a, 1986b), the action of developmental genes that prolong or truncate juvenile patterns of growth may be one of the reasons for the dramatic changes caused in adult dogs (Coppinger and Smith 1983, Wayne 2001).

Selection for tameness has resulted in gene expression changes in the brain. Comparison of expression differences for three different brain regions –frontal lobe, amygdala and hypothalamus– in wild (wolves and unselected silver foxes) and domesticated (dogs and tame silver foxes) animals, showed significant differences between the tamed and wild individuals (Saetre et al. 2004 and Lindberg et al. 2005). The hypothalamus (involved in many behavioural responses) showed an accelerated rate of divergence in gene expression for domestic dogs. Two of the neuropeptides in the hypothalamus showing this pattern, NPY and CALCB, have been implicated in energy control and feeding behaviour of mammals (Saetre et al. 2004), which is expected to have changed during the domestication process.

Morphology

The domestic dog varies remarkably in morphology. In fact, the huge diversity of sizes and proportions between the dog breeds is greater than that in the entire Family Canidae (Wayne 1986a, 1986b). Some studies have indicated that the morphological changes due to domestication did not appear quickly. Instead it might have taken at least 30 generations before changes were measurable (Bökönyi 1989).

Across domestic mammals, the main reason for the change in appearance that makes the domestic animal differ from its wild counterpart is the maintenance of juvenile characters in the adult animal (Morey 1994, Coppinger and Schneider 1995). Domestication has led to similar physical changes among different species (Clutton-Brock 1992, Morey 1994, Clutton-Brock 1999). Below I describe typical changes in early domesticated mammals.

Body size

The first morphological indication of domestication is a reduction of body size. This fact is generally true and therefore used as the main criterion to distinguish bone remains of domestic animals from their wild counterpart found at archaeological excavations (Zeuner 1963, Wayne 1986a, 1986b, Bökönyi 1989, Clutton-Brock 1992, Clutton-Brock 1999). The reason for this reduction of body size could be the alteration in the feeding regime. Alternatively, the farmers may have selected the smallest and most docile animals for breeding while the larger and more dominant males were killed for meat around the age of two, before breeding and thereby a selection for

smaller animals occurred naturally (Clutton-Brock 1992). During the later stages of domestication a selection for giant as well as dwarf forms of the animals has been performed and eventually breeds of very different sizes have been developed. This has resulted in a huge variation of body size, greater than that found under natural conditions (Zeuner 1963, Hemmer 1990, Clutton-Brock 1999).

Skull size

Another feature that changes during domestication is the facial region of the skull and the jaws, which both become shortened. This is one example of the maintenance of juvenile characteristics that shows in adult domestic dogs (Zeuner 1963, Wayne 1986a, 1986b, Morey 1994, Clutton-Brock 1999).

The fact that there is no corresponding reduction in size of the cheek teeth immediately after domestication causes a crowding or compaction of the premolars and molars in the jaw. The cheek teeth are genetically much more stable than the skull and therefore change more slowly. This characteristic of crowded teeth, together with the size reduction of skull and jaw, are used to separate early domestic animals from wild ancestors in ancient bone remains, as a sure proof of domestication (Zeuner 1963, Bökönyi 1989, Clutton-Brock 1999).

After some time, even the teeth are reduced in size, resulting in permanently smaller teeth in domestic dogs. For example, in a dog breed that is much larger than the wolf, such as the Great Dane, the teeth are still considerably smaller and have a less complicated cusp pattern compared to wolves (Zeuner 1963, Clutton-Brock 1999).

Another part of the skull, the tympanic bullae (the bony case of the ear drum), is also found to be considerably smaller in dogs compared to wolves as a result of domestication (Hemmer 1990, Clutton-Brock 1999).

Brain size

Most of the domestic animals in which brain size has been measured, have smaller brains relative to the body size compared to their wild progenitor (Zeuner 1963, Clutton-Brock 1999). They have also less perceptive senses than their wild ancestors. However, characteristics such as large brain size and good sharp eyes are crucial for survival in the wild but are not likely to be so important when living with humans (Diamond 2002).

Colour

A conspicuous characteristic of domestic animals is their diverse colouration, which is very different from the limited colouration patterns of their ancestors. For example, piebaldness, the white spots or areas on some animals' coat, is a result of the domestication process due to changes in the distribution of hair pigments. It can therefore be seen as evidence of domestication (Zeuner 1963, Hemmer 1990).

Behaviour

One of the reasons that facilitated the adaptation of wolves to life with humans could be, that the patterns of behaviour that are useful for a dog in a human society are the same as those that a wolf uses in wolf societies, such as the submissive behaviour of the individuals of lower rank towards the alpha male (Scott 1950). Some wolves were more adaptable to human society than others, accepting their submission towards man and could thereby be tolerated in human settlements. Those wolves that did not follow the rules were either driven away or killed (Morey 1994). However, to have the possibility of domesticating an animal, it must have some behavioural potential, such as being calm and submissive but not too fearful, even from the beginning (Budiansky 1992, Budiansky 1994, Clutton-Brock 1999). Although many dogs do not look like wolves (for example a chihuahua), their behaviour is still recognizably wolf-like to some extent. Therefore, to retain dominance over dogs, humans have selected for submissive behaviour, like that of a young animal towards its parent (Clutton-Brock 1999).

It is very likely that as soon as humans started to exert control over the first dogs, behavioural selection was initiated: only docile animals were allowed to reproduce regularly. Selection for tameness resulted in gene expression changes in the brain. Comparison of these gene expression differences showed specially marked differences between dogs and wolves at the hypothalamus (Saetre et al. 2004). Since this is involved in many central processes in the organism, the differences can have widespread effects on the phenotype of dogs.

During the process of domestication, dogs have also been selected for unique social-cognitive abilities that make it possible for them to communicate with humans in a special way. Already as a puppy these skills can be seen, compared with wolves raised by humans, who lack these communication skills. Dogs are even more skilful than chimpanzees in using these different kinds of cues (Hare et al. 2002). The same kind of skilful social-cognitive communicative abilities have been seen in experimentally domesticated silver foxes, despite the selection for tameness only (Belyaev 1979, Trut 1999, Hare et al. 2005).

Another behavioural change that has come up during domestication is the propensity to bark. This feature has never been well developed in any other wild living canids, even though both wolves and coyotes may bark occasionally in the wild (Scott 1950, Clutton-Brock 1999).

An effect of breed creation is the change of sexual maturity. Most modern dog breeds reach sexual maturity already at the age of 6–12 months, while wolves achieve maturity first around the age of 2 years (Morey 1994).

The dog (*Canis familiaris*) today

Species

Carl von Linné, better known as Linnaeus (1707–1778), published in 1758 the tenth edition of the *Systema Naturae*, which is today internationally accepted as the basis for zoological nomenclature. In this book Linné described, among more than 4 000 organisms, all the common domestic animals and named them (Linnaeus 1758, in Clutton-Brock J 1999).

Several definitions of “species” have been proposed: biological, evolutionary, phylogenetic, genealogical, recognition and cohesion species concepts are some among many concepts that have been suggested (Futuyma 2005). The purpose of having a common definition for species, is (1) to help us classify organisms in a systematic manner, (2) to be able to identify discrete groups seen in nature, (3) to help us understand how these groups arise, (4) to represent the evolutionary history of organisms and (5) to use the same criteria for as many organisms as possible. However, such definitions are not always useful, because no species concept can cover all these purposes (Coyne and Orr 2004). One of the most commonly advocated is the biological species concept, which was defined by Mayr (1942, cited in Futuyma 2005) as: “Species are groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups”. Based on this species definition, dogs and wolves cannot strictly be seen as two different species. Dogs can interbreed and produce fertile offspring with wolves. However, wolves and dogs tend to remain separate today even in areas of sympatry and can therefore be considered as reproductively isolated populations. Consequently, I will speak of them as two separate species.

The Family Canidae

The dog belongs to the family Canidae, which contains 34 species (*Figure 2*). Of these, the grey wolf (*Canis lupus*) is the dog’s closest relative, closely followed by coyote, golden jackal and Ethiopian wolf. All of these species can hybridize with dogs and produce fertile offspring. Next in the phylogenetic tree of the dog family come two species that have uniquely structured meat-slicing teeth: the dhole and the African wild dog. The two most basal members of the “dog” clade are the side-striped jackal and the black-backed jackal, supporting an African origin of the wolf-like canids. The three other clades found in the phylogenetic tree are the South American canids, the red fox-like canids and a small clade containing only the Grey fox and the Island fox, the most divergent of all canids (Lindblad-Toh et al. 2005).

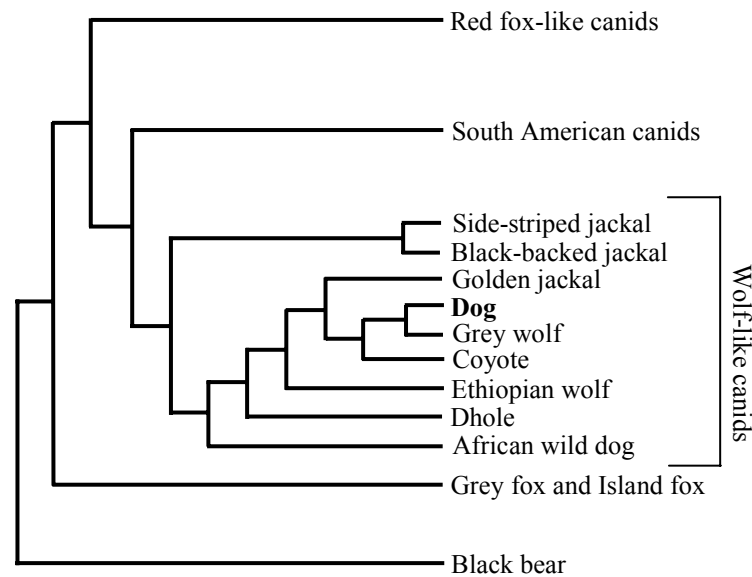


Figure 2. Phylogeny of canid species (modified from Lindblad-Toh et al. 2005).

The ancestor of the domestic dog: The Grey Wolf

The value of the dog as a model organism is especially high because its wild ancestor, the grey wolf, still exists in moderately large populations around Europe, Asia and America. This offers the possibility of comparing behaviour, physiology and genome in the domestic species and in the wild counterpart. Wolves represent the ancestral state against which dogs should be compared.

Wolves are social animals and live in packs with a strict hierarchy of dominant and submissive individuals who are constantly aware of their status in relation to each other (Mech 1970, Morey 1994, Clutton-Brock 1999, Mech 1999). This hierarchy is primarily a reflection of the age, sex and reproductive structure within a group. In nature, the wolf pack normally consists of one family group, including a breeding pair (an alpha male and an alpha female) and their offspring of the previous 1–3 years. In some cases the pack could consist of two or three such family groups (Mech 1999).

When the offspring begin to mature, they disperse from their natal pack, try to pair with other dispersed wolves to eventually occupy an empty territory, produce pups and establish their own pack (Mech 1999). Wolves use large areas and can travel more than 800 km from their natal territory (Fritts 1983, Merrill and Mech 2000). The dispersal distance is probably affected both by population density and by the probability of finding a mate

(Wabakken et al. 2001). The maturation and dispersal from the parental group could occur as early as around five months of age, but most of the offspring disperse when 1–2 years old and a few remain until 3 years or even older, even as late as up to 5 years of age (Mech 1999, Mech and Boitani 2003). The earliest ages at which free-living wolves are known to breed are 22 months, whereas some individuals are not sexually mature until they are 4 years old or later (Mech 1999, Grooms 1993). The breeding season occurs from late January through April and the gestation time lasts about 63 days (Mech 1970, Grooms 1993). Litters range in size from three to nine pups, but usually consist of four to six (Grooms 1993). Wolves have a high reproductive rate and thereby potential for rapid population growth (Pletscher et al. 1997, Wabakken et al. 2001).

The grey wolf was historically a widely distributed animal, living in most habitats containing large ungulates in the Northern Hemisphere (Young and Goldman 1944), but nowadays is it threatened with extinction in many places and exists only on limited areas around the world. They are effective predators and their hunt is often performed as a co-operation among members of the pack (Grooms 1993). The most important prey species are the large ungulates such as moose, deer, elk, sheep and bison (Mech 1970, Grooms 1993, Wabakken et al. 2001), but there is a very large geographic variation in diet.

The Dog Genome

The dog genome includes 78 chromosomes: 76 autosomes (38 pairs) and two sex chromosomes (Selden et al. 1975), *Figure 3*. The largest chromosome is the submetacentric X, estimated to be around 139 Mb in size and the smallest is the metacentric Y with its mere 27 Mb. The largest autosome is 137 Mb in size, with the remaining decreasing gradually in size. The size of the two smallest autosomes is 38 Mb (Langford 1995).

In 2003, the first domestic dog genome sequence was published (Kirkness et al. 2003). This sequence had a ~1.5-fold sequence redundancy and came from a male standard poodle. Two years later, Lindblad-Toh et al. (2005) published the second genome sequence of the domestic dog, this time from a female boxer. This high-quality draft sequence was covering about 99% of the euchromatic genome and had a ~7.5-fold sequence redundancy. The total dog genome assembly spanned a distance of 2.41 Gb and about 19 300 protein-coding genes were identified. The publication of these two complete genomes has triggered a lot of research on the evolution of the canine genome, and has enhanced the role of the dog as a model organism.

Also, as the result of the effort of multiple laboratories during many years, a well-resolved genetic map including a large number of markers is available (for example, at the Fred Hutchinson Cancer Research Center, <http://www.fhcrc.org>). In addition, a huge amount of sequence information

from dogs, wolves and coyotes deriving from many research groups is accessible from public databases, such as NCBI (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). This huge amount of available information about the dog's genome has been produced within the last years as a result, mainly, of a steadily growing interest from researches of using dogs as an adequate animal model for gene mapping of diseases.

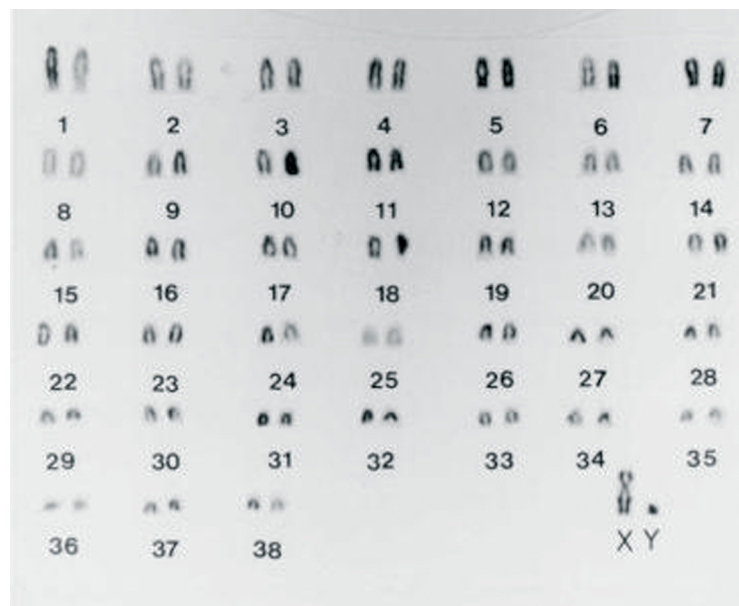


Figure 3. The 78 chromosomes of the dog. Modified from Rahal et al. 2004, <http://www.priory.com/vet/hypospadias.htm>. Published with permission of Dr Sheila C. Rahal and Vet On-Line™ (©Priory Lodge Education Ltd).

The diversity of dogs

The history of purebred dogs

Dogs have been highly variable in both size and shape for several thousands of years. The first evidence for potentially distinctive dog breeds has been found in ancient Egypt artistic representations, and is dated to around 5 500 years ago. Early Egyptian illustrations show two types of sight-hounds: one slender, erect-eared and with curly tail, and another shorter variant with a heavier muzzle, lop-eared and a sabre or curved tail. Both types were probably used for hunting. Art representations and skeletons also indicate another type of dog, a short-limbed hound, erect-eared and with curled, cocked or hanging tail. Furthermore, a limited number of skeletal remains

shows mastiff-type dogs that might have come to Egypt from Mesopotamia about 5 000 years ago (Brewer et al. 2001). However, the variations found between dogs at that time cannot be described as breeds as we know them today. Modern breeds represent populations that have been reproductively isolated since the establishment of Stud Books (in most cases less than 150 years ago) and the morphological similarity with ancient breeds does not imply genetic continuity (American Kennel Club 1998, Fogle 2000).

The first dog show ever held was at the Zoological Gardens of London in 1843. After this the enthusiasm for dog competitions increased and more dog shows followed. Between 1859 and 1873 around 50 different dog shows were held and in April 1873 the Kennel Club of England was established. In 1874 the first Stud Book was produced, covering the years of 1859 to 1874. A few years later, in September 1884, also the American Kennel Club (AKC) was established with their first Stud Book published in 1887. The World Canine Organization, FCI, was founded in May 1911 and works as an umbrella organization for the many other National Kennel Clubs that have been founded over the years. The founding countries of the FCI were Germany, Austria, Belgium, France and the Netherlands, but about 80 countries are included today (Sampson and Binns 2006).

Purebred

Breeds are defined as “intraspecies groups that have relatively uniform physical characteristics developed under controlled conditions by man” (Irion et al. 2003).

The founding of the concept “dog breed”, with narrowly defined morphologies, started around 1850, when dog shows became popular, different kennel clubs were established and the very first Stud Book was available (Sampson and Binns 2006). The “breed barrier rule” was also implemented at this time, meaning that no puppy could be registered as a specific breed unless both its dam and sire were members of the same breed (Parker et al. 2004, Sutter et al. 2004, Parker et al. 2006). This resulted in the interruption of gene flow between dogs from different breeds leading to reduced genetic variability within each breed and a high genetic differentiation between breeds. This has produced more than 400 recognized breeds of dogs (Clutton-Brock 1999). However, this number is probably an underestimation and more than 1000 breeds might exist today around the world (Morris 2001, in Ostrander et al. 2006, see below paper **III**). Apart from the reproductive isolation among breeds, founder effects, bottlenecks experienced during the time of breed creation, extreme selection and use of popular sires have also contributed to the decrease in genetic variability and to considerably inbred dog breeds (Zajc et al. 1997, Zajc and Sampson 1999, Koskinen and Bredbacka 2000, Ostrander and Kruglyak 2000, Sutter and Ostrander 2004, Ostrander and Wayne 2005).

Crossbred and feral dogs

Combinations between dogs of different breeds can be deliberately mated or a result from breeding without the supervision or planning by humans. Planned crosses can result in either a *crossbreed*, which are a mixture between two known breeds, or *mixed breed* dogs, which are a mix among more than two breeds, e.g. if crossbred dogs are mated to each other. Dogs that are interbreeding freely without human control for several generations are called *random-bred* dogs and might be descendant of feral dog populations. These dogs probably represent the majority of the about 400 million dogs that live nowadays (Coppinger and Coppinger 2001), and they are not related to any of the officially recognized breeds. *Random-bred* dogs are often stray dogs without owners, which feed on urban garbage on the streets. Several generations of indiscriminate mixing might lead to a more standardized appearance between the dogs, where the differences between the dogs are to some extent limited. These dogs are typically yellow to light brown or black and of medium weigh and medium height (normally between 38 and 57 cm tall at the withers). This intermediate appearance may represent the exterior of the modern dog's ancestor (Fogle 2000, Cunliffe 2004).

An advantage of mating over the breed barriers is that mixed breed dogs tend to be healthier due to their higher genetic variation compared to purebred dogs (Fogle 2000, Cunliffe 2004).

The definition of feral dogs is: "those that live in a self-sustained population after a history of domestication" (Clutton-Brock 1999). These domesticated animals that return to live in the wild and become feral, usually change back to a physical form similar to that of their wild ancestors as a consequence of natural selection and their anew independence of humans. Still, the decrease in brain size that arose during domestication, measured as the cranial capacity, do not change back when becoming feral, instead it will remain small compared to their wild ancestors (Clutton-Brock 1999). One example of this is the dingo, the feral dog that has been living in the wild for thousands of years. Their brain size is still similar to that of domestic dogs and much smaller compared to the wild wolves around the world (Hemmer 1990).

Dogs as a model organism for the study of human diseases

The availability of two complete dog genome sequences as well as extensive sequence information for a larger number of dogs (Kirkness et al. 2003, Lindblad-Toh et al. 2005, Wang and Kirkness 2005) has facilitated the development of new markers and the identification of genes, which have increased the value of the dog as a model organism for the study of human diseases.

Dogs are considered as a good model organism for several reasons. First, purebred dogs are considered as phenotypically uniform groups indicating a high degree of genetic homogeneity (Ostrander and Giniger 1997). This has also led to a large genetic differentiation between breed groups and a large extent of linkage disequilibrium (LD) within breeds. The amount of LD, which is about 10–100 times more extensive than that found in humans, decreases the number of markers needed for association mapping (Sutter et al. 2004, Sutter and Ostrander 2004, Lindblad-Toh et al. 2005, Ostrander and Wayne 2005). Second, the few founders of most dog breeds have resulted in a large degree of inbreeding that is leading to the expression of a great number of genetic diseases. Many of these diseases have very high breed specificity. Moreover, some diseases found in dogs also frequently occur in humans as well, such as cancer, heart problems, deafness, blindness and joint diseases (Zajc et al. 1997, Wayne and Ostrander 1999, Ostrander et al. 2000, Ostrander and Kruglyak 2000, Sutter et al. 2004, Sutter and Ostrander 2004). As many as 360 different genetic diseases are found both dogs and humans, of which many disorders also have similar physiology, disease presentation and clinical response (Ostrander and Kruglyak 2000, Ostrander and Wayne 2005, Parker and Ostrander 2005). Third, dogs live in the same environment as humans and are exposed to the same substances and allergens as us (Ostrander et al. 2000, Ostrander and Kruglyak 2000, Parker et al. 2004), compared to laboratory rats living in a restricted environment. Due to all these reasons, association mapping in dogs can facilitate the discovery of genes involved in human diseases.

Dogs as a model organism for the study of biodiversity

Just by observing the domestic animals, Darwin (1868a,b) learned how species respond to (artificial) selection. This knowledge set the stage for the development of his theory on the origin of species by means of natural selection (Darwin 1859).

Today a huge diversity is found within the dog population (Vilà et al. 1999, Wayne and Ostrander 1999, Sutter and Ostrander 2004), which makes dogs useful as animal models for the study of the origin of biodiversity. The selection for different phenotypically characteristics, representing breed creation, could be seen as a process analogous to adaptation in response to natural selection and speciation. However, in the creation of breeds, the evolutionary changes are faster due to repeated founder effects, genetic drift and the extreme selective forces applied. To study these changes might help us to understand the underlying mechanisms how the phenotypic diversity has developed.

Furthermore, dogs can also be used when testing new analysis methods, before applying them on natural animal populations. The information about population structure in natural populations is very limited. Consequently, it

is impossible to know if estimates derived from genetic data accurately reflect the processes that have affected the population during recent times. However, the fact that extensive information about the relationship between dogs within a breed is available, in the form of pedigrees and breed registries, allows the use of purebred dogs as a controlled scenario upon which different analytical methods can be evaluated.

Genetic markers used in domestication studies

There are some genetic markers that are commonly used in domestication studies. The markers described below have different modes of inheritance and therefore contribute different information about the domestication process.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) is the most widely used molecular tool in domestication studies today. This marker has several characteristics that have facilitated evolutionary studies of domestic animals (Bruford et al. 2003).

In mammals, each cell contains from few to hundreds of mitochondrial organelles, depending on cell type (Robin and Wong 1988). Each mitochondrion encloses about 0–11 copies of the mitochondrial genome, leading to a large copy number of the mitochondrial genome in every cell (Cavelier et al. 2000). The mitochondrial genome is a circular and double stranded plasmid (Chinnery and Schon 2003) and the size of a complete dog mitochondrion genome is about 16.7 kb. The control region, a noncoding fragment of the genome, covers about 7% (Kim et al. 1998), whereas the remaining 93% consists of 37 genes encoding for 13 respiratory chain polypeptides and also two ribosomal RNAs (rRNA) genes and 22 transfer RNAs (tRNAs) genes necessary for the transcription and translation of the genome (Chinnery and Schon 2003), *Figure 4*.

MtDNA has an almost exclusively uniparental inheritance in animals, which results in that only the history of females can be traced in a population. The genome also has a lack of recombination (with some rare exceptions; Ujvari et al. 2007). The 5–10 times faster substitution rate of mtDNA compared to nuclear sequences (Brown et al. 1979, 1982, Kim et al. 1998) allows good phylogenetic resolution when studying closely related populations (Bruford et al. 2003). Furthermore, the control region has been estimated to evolve at a rate of 5–20 times faster (Sigurðardóttir et al. 2000) than the coding region, according to a study in humans.

The fact that mtDNA is haploid, maternally inherited, does not undergo recombination (Giles et al. 1980), has a high copy number (Bogenhagen and Clayton 1974), and a high mutation rate (Brown et al. 1979, Brown et al.

1982), makes it useful for studies of dog domestication history. However, most studies are based solely on the control region of the mtDNA, due to its high variability (Vilà et al. 1997, Leonard et al. 2002, Savolainen et al. 2002).

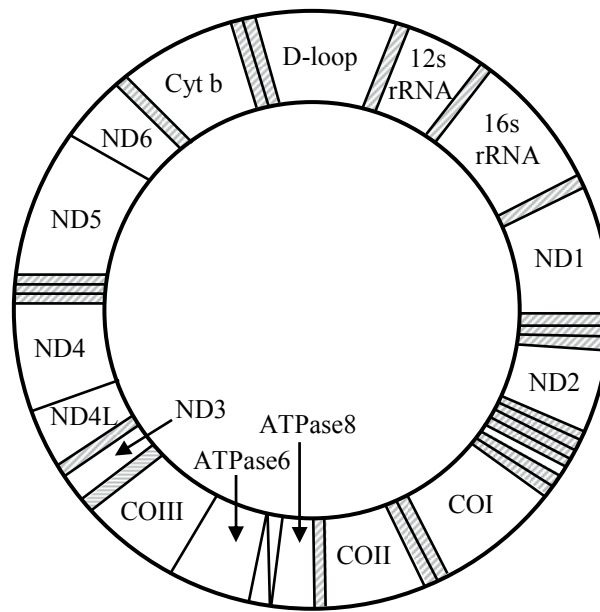


Figure 4. Schematic overview of the mammalian mitochondrial genome. The figure shows the control region (d-loop), the genetic arrangement of 13 protein coding genes, 22 tRNA (indicated by grey stripes) and 2 rRNA.

Y chromosome

The Y is a small, heterochromatic, gene-poor chromosome that consists largely of highly repetitive sequences (Marshall Graves 1998, Lahn et al. 2001). Natural selection, both positive and negative, has been shown to affect the Y chromosome, influencing haplotype distribution in populations (Jobling et al. 1998, Jobling and Tyler-Smith 2000, Quintana-Murci et al. 2001). Nevertheless, this chromosome is useful in population studies due to its haploid state and absence from females (Jobling and Tyler-Smith 2000). Furthermore, the absence of recombination makes the interpretation of results more straightforward (Jobling and Tyler-Smith 1995, Jobling and Tyler-Smith 2000, Jobling and Tyler-Smith 2003).

Since the Y chromosome is paternally inherited, it represents the perfect complement to studies using mitochondrial DNA. However, the chromosome variation within species is quite low compared to most other

genomic sequences, which complicates phylogenetic studies (Bruford et al. 2003), and it is also poorly conserved between species (Marshall Graves 1998). Still, the fact that ~95% of the Y chromosome is non-recombining and that the inheritance is uniparental make it useful for evolutionary studies.

Autosomal microsatellites

Autosomal microsatellites are biparentally inherited, short (1–6bp) repetitive nuclear sequences with a variable number of repeat units, spread throughout the genome (Bruford and Wayne 1993, Bruford et al. 2003, Ellegren 2004). The mutation rate of microsatellites, where new length alleles are generated by polymerase slippage mutations during replication (Levinson and Gutman 1987, Schlötterer and Tautz 1992), is estimated to be within the range of 2×10^{-3} – 5×10^{-6} , increasing as the number of repeat units increases (Bruford and Wayne 1993, Ellegren 2000, Ellegren 2004). Microsatellites are very easy to study (normally only size variation is considered) and offer the possibility of tracking biparental inheritance. These markers are highly polymorphic and therefore useful in domestication studies for intra-species comparisons (Bruford and Wayne 1993). Microsatellites have also been used for studies of natural populations, to measure genetic variation within (diversity, relatedness, substructuring) and between (population differentiation) populations and estimate admixture (hybridization, gene flow). However, the premier usage of microsatellite markers has been to construct genome maps that allows mapping of genes (Jacob et al. 1995, Breen et al. 1997, Womack et al. 1997, Yerle et al. 1998, Mellersh et al. 2000).

Research aims

The aim of this thesis was first to investigate the genetic changes occurred in dogs due to domestication and breed creation. Secondly, using dogs as a model to evaluate methods for the study of natural populations, by taking advantage of breed structure and the genomic information available.

The main objectives were the following:

1. Use mitochondrial DNA to investigate how the change in lifestyle, resulting from the domestication, has affected the canine genome.
2. Investigate how breeds were created, using markers separating maternal and paternal contribution in addition to biparentally inherited markers.
3. Evaluate different genetic approaches, used to estimate differentiation and gene flow between natural populations, by comparing them with more accurate estimates derived from pedigree information. Furthermore, to evaluate noninvasive genotyping methods by using dog samples.

Present Investigations

Paper I. Relaxation of selective constraint on dog mitochondrial DNA following domestication

The domestication process of dogs probably caused a dramatic change in living conditions compared with the lifestyle of their ancestor, the grey wolf. We hypothesize that these changes of lifestyle also led to a relaxation of the selective forces that acted upon the species, which in turn might have an effect on the dog's genome.

To study this hypothesis, we focused on complete mitochondrial DNA (mtDNA) sequences from a number of dogs, wolves and coyotes. The mitochondrial genome is involved in heat and energy production and mutations here are likely to affect individual fitness.

Material and methods

The complete mitochondrial genome was sequenced for three coyotes, six wolves and fourteen dogs from 13 breeds representing the four clades of dogs described by Vilà et al. (1997). A phylogenetic tree was built based on the mtDNA sequences excluding the d-loop and the four dog clades appeared clearly separated from the wolves (*Figure 5*).

To distinguish between mutations along the different branches of the gene tree, wolf and dog branches were classified as wolf internal, wolf external, dog internal and dog external. Branches leading to each of the four dog clades could not conclusively be assigned to either dogs or wolves and were therefore excluded from the analysis.

Maximum-likelihood (ML) estimates for the ratio d_N/d_S (nonsynonymous substitution rate / synonymous substitution rate) were calculated for each individual branch. We also reconstructed the ancestral sequences at each node and estimated the actual number of synonymous (S) and nonsynonymous changes (NS) along each branch. Finally, the nonsynonymous changes were characterized upon their potential severity and phenotypic effect based on both polarity and charge and the changes were classified as radical or conservative.

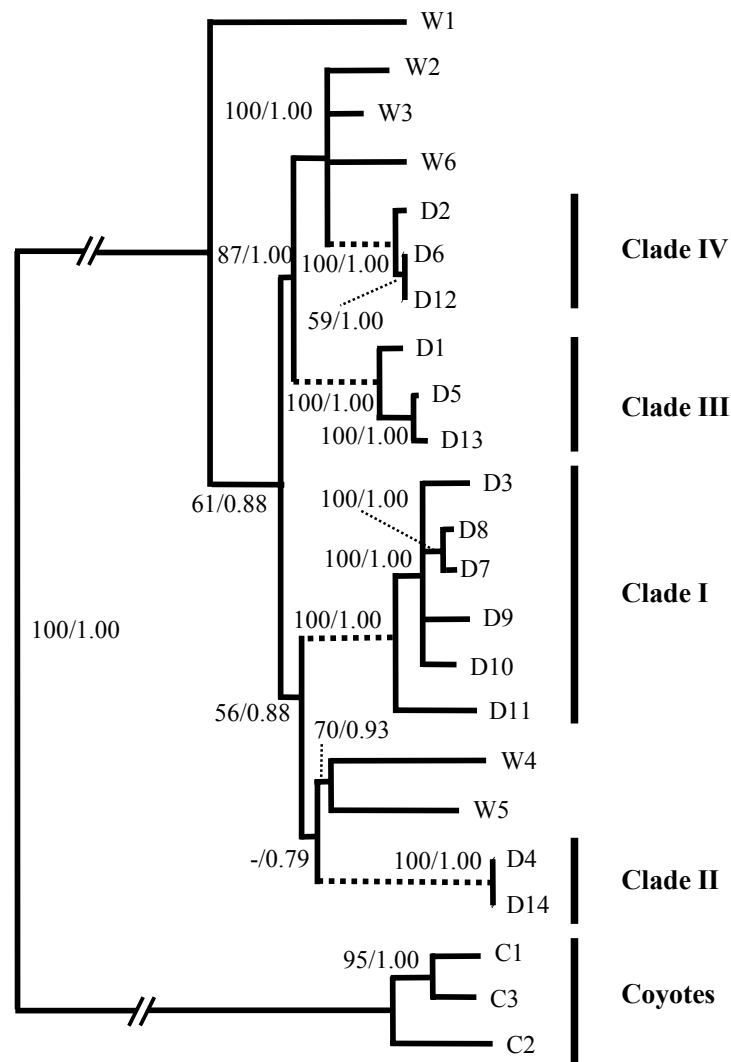


Figure 5. Phylogenetic tree of complete mitochondrial DNA sequences representing the different dog clades in relation to the wolves.

Results and discussion

There was no significant difference in substitution rate between wolves and dogs that could indicate that the mutation rate is higher in dogs. However, weakly deleterious mutations are expected to be more common in intra-specific variation than in the divergence between species because of the shorter period of time for purifying selection to act (Akashi 1999, Piganeau

and Eyre-Walker 2003, Kivisild et al. 2006). This was shown to be the case when a comparison of ML estimates of d_N/d_S ratios were made for the wolves and coyotes. Significantly lower d_N/d_S was found along the branches separating coyotes and wolves than for the average values estimated along the wolf branches in the gene tree, which suggests that many weakly deleterious mutations are segregating within the wolf population. When the average d_N/d_S ratio was estimated for the dog branches, it showed a significantly higher value than found among the wolves. Because selection increases the probability of losing deleterious alleles when populations are growing, this is a surprising result, considering the large population growth of dogs since the time of domestication. This result could be explained by a relaxation of the selective constraint acting on the dog mtDNA genome but not on wolves. Another explanation could be that dog branches reflect a shorter evolutionary time than those in wolves (shorter time for selection to remove deleterious mutations).

Deleterious alleles are expected to be removed from a population as a result of purifying selection over time. To investigate that the difference in d_N/d_S ratio between wolves and dogs are not due to the higher proportion of terminal branches for the dogs, where selection might not have had time to act, d_N/d_S ratio comparisons were estimated for the internal and external branches separately and showed that the result were consistent within dog and wolf branches and therefore represent true differences: the ratio was higher for dogs than for wolves. The differences in d_N/d_S between wolves and dogs could not be attributed to any particular gene or gene class.

An analysis of the potential phenotypic effects of mutations showed no difference in the proportion of conservative or damaging changes between dog branches, wolf branches or along the coyote/wolf divergence. This indicates that dogs do not accumulate (or remove) a larger proportion of radical or damaging changes than wolves. However, radical or damaging changes are often strongly deleterious and would probably not reach detectable amounts. Weakly deleterious alleles on the other hand can, according to our hypothesis, accumulate in dogs due to a relaxation of the selective constraint.

The accumulation of deleterious mutations in today's dogs is probably due to two possibilities. First, dogs have a smaller effective population size compared to wolves, which results from the limited number of wolves involved in the domestication process. Second, man has after the initial domestication, operated selection for preferable traits on the dog (for example tameness) and has controlled their breeding and also living conditions. We hypothesize that this change in lifestyle has also led to increased survival and chances of reproduction for individuals carrying weakly deleterious mutations. This resulted in a relaxation of constraints leading to more nonsynonymous mutations on the mitochondrial genome, which could even affect the entire dog genome. The relaxation of selective

constraints could therefore have contributed to the huge phenotypic diversity found in dogs, but may have also contributed to the large number of diseases that affect our dogs today.

Paper II. Unequal contribution of sexes in the origin of dog breeds

The domestication of dogs started at least 15 000 years ago and archaeological evidence suggests that dogs with similar phenotypes as modern breeds existed already about 5 000 years ago. However, today's dog breeds have a much more recent origin, probably less than 200 years.

The aim of this study was to examine the origin of contemporary dog breeds by combining the analysis of three genetic marker systems with different modes of inheritance. The patterns of variation of these markers across breeds and in grey wolf populations, the ancestor of the domestic dog, can illustrate how breeds were formed.

Material and methods

Eighteen biparentally inherited autosomal microsatellites, 4 paternally inherited Y chromosome microsatellites and the maternally inherited mtDNA control region sequence were used as genetic markers in this study.

A sample size of 100 male dogs from 20 different breeds, with 5 dogs representing each breed, were analyzed to compare the patterns of variability within breeds for each marker. To study the degree of differentiation among and within the group of dog breeds recognized by the FCI (World Canine Organization) based on mtDNA and Y chromosome, additional samples were tested. For the Y chromosome study, 214 male dogs from 89 breeds were analyzed in addition to the previously genotyped 100 male dogs and for the mtDNA analysis, an already published data set of 430 haplotypes from purebred dogs was used (Savolainen et al. 2002). In addition to the dog samples, six different populations of male grey wolves from across North America and Eurasia were typed for the Y chromosome and sequenced for the mtDNA, to compare the patterns of variability with those observed for the dog breeds.

A neighbour-joining phylogenetic tree was used to characterize the relationship between dog mtDNA haplotypes. Haplotype divergence for the Y chromosome was represented by a network based on mutational differences. Patterns of diversity between groups of breeds for the two marker systems were assessed by an Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992), based on haplotype frequencies and on the distance between haplotypes. Finally, to check for individual similarity

within breeds, microsatellite genotypes were analysed using clustering methods.

Results and discussion

Breeds are expected to be genetically differentiable. This was confirmed by three clustering methods investigating the structure of 20 breeds using 18 autosomal microsatellites: individuals from a breed were most similar to each other when their autosomal microsatellite genotypes were compared. Almost all dogs were thus assigned to their correct breed. In contrast, neither mtDNA nor Y chromosome haplotypes show any kind of structure between breeds. For mtDNA, several haplotypes originating from different clades can be found in one single breed as well as phenotypically different breeds can share the same mtDNA haplotype. For Y chromosome markers, 12 out of 20 breeds had only one single haplotype in all five male dogs and, as for the mtDNA, the same Y chromosome haplotype could be shared by several morphologically different breeds.

The lack of structure for Y chromosome and mtDNA haplotypes can be an indication of a recent origin of the breeds, suggesting that they have not been isolated for a very long time. Comparing the number of haplotypes found per breed for these two different markers showed a higher number of mtDNA than Y chromosome haplotypes for most of the breeds investigated. A comparative test with the same markers performed on wolves showed the opposite situation, with more Y chromosome than mtDNA haplotypes per population (Table 1). The slightly higher number of Y chromosome than mtDNA haplotypes seen in wolves can be a consequence of higher mutation rate in the Y chromosome or a higher dispersal rate among male wolves. The differences seen between wolves and dogs might be an indication of different breeding strategies: while wolves live in packs with only one breeding pair, popular dog sires can be bred to several females and thereby give an imbalanced contribution.

The diversity of mtDNA and Y chromosome haplotypes was also compared within and between commonly recognized groups of dog breeds. The result showed that groups are more differentiated from each other for Y chromosome than mtDNA haplotypes. The relative differentiation was about three times larger for Y chromosome haplotypes than for mtDNA haplotypes. This might indicate that during the formation of breeds, male founders were more likely than females to derive from a similar breed from the same group.

Table 1. The number of mtDNA and Y chromosome haplotypes observed in dog breeds and wolf populations.

Dog breed/wolf population	mtDNA haplotypes	Y chromosome haplotypes
Dogs (5 males per breed)		
Airedale terrier	3	2
Beagle	1	1
Bernese mountain dog	2	1
Border terrier	2	1
Boxer	3	1
Cairn terrier	3	1
Cavalier King Charles spaniel	2	1
Collie, rough/smooth	3	1
Dalmatian	3	2
Flatcoated retriever	2	1
German pointer	3	1
German shepherd	3	1
Golden retriever	2	1
Greyhound	3	2
Irish soft-coated wheaten terrier	4	2
Newfoundland	1	1
Poodle, miniature/standard	4	2
Rottweiler	1	2
Shetland sheepdog	3	3
West Highland white terrier	3	2
Wolves		
Alaska (n = 12)	6	6
Russia (n = 12)	4	6
Inuvik (n = 13)	4	7
Finland (n = 31)	3	7
Spain (n = 20)	2	5
Baltic States (n=24)	2	9

Paper III. Disruptive selection within dog breeds

Breeds are considered to be well-defined groups with similar physical characteristics. Furthermore, always breeding with dogs from the same breed is leading to breed barriers, large genetic difference between the breeds and large linkage disequilibrium within breeds. However, mating is not random within breeds. Certain phenotypes may be preferred over others by the breeders. For example, some colour or size variants can be preferentially

mated, while other are not allowed. Similarly, some members of a breed can be selected for working purposes while others simply for their appearance. We hypothesize that these preferences result in disruptive selection within breeds, leading to fragmentation and violating the assumption of breed uniformity (*Figure 6*).

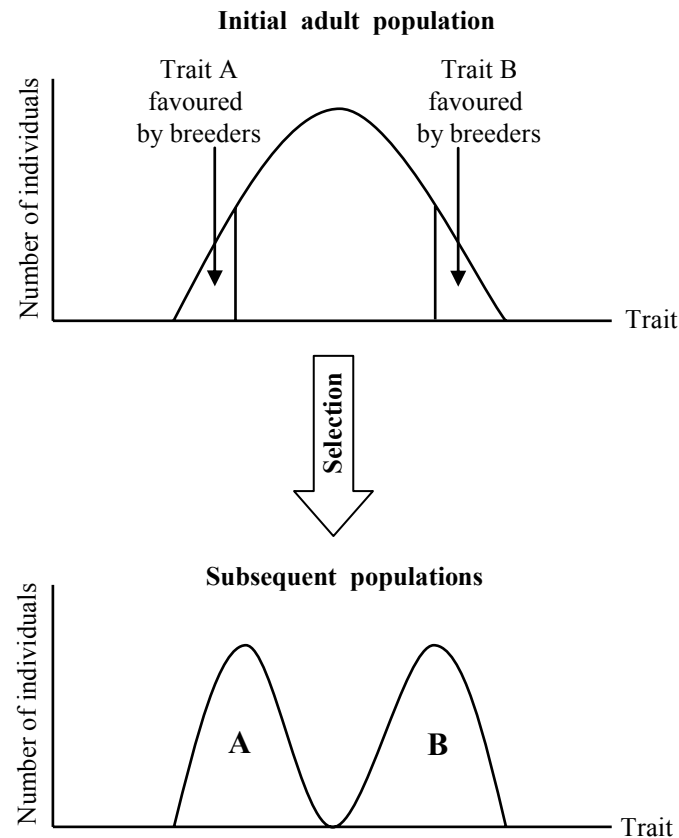


Figure 6. Schematic drawing describing the effect of disruptive selection.

Material and methods

For this study we focused on a phenotypically diverse breed, the poodle. This breed has four recognized sizes (standard, medium sized, miniature and toy) and five colours (black, brown, white, silver and apricot). The diversity found within this breed was also compared to that between breeds. For this reason we also studied dogs from eight other breeds: bull terrier, fox terrier

(smooth and wire), German shepherd, Labrador retriever, giant schnauzer, miniature schnauzer and Siberian husky.

All dogs were typed for a panel of 27 autosomal microsatellites. We used the program STRUCTURE to study the uniformity within poodles. Registry information at the Swedish Kennel Club was later used to define new groups, which better fit the genetic structure.

The genetic differentiation between the breeds in this study was measured with F_{ST} and an assignment test. The assignment test was used to calculate how many individuals assigned to their correct breed based on their multi-locus genotype.

Estimates of F_{IS} (inbreeding coefficient) and observed and expected heterozygosity were measured to get an indication of the uniformity or possible structure within the separate breeds.

Results and discussion

The results showed a clear sub-structure within poodles. The dogs were divided into five groups based on a combination of size and coat-colour, not by the four size classes as expected. This was shown by the result from the program STRUCTURE based on the individuals' genotypes (*Figure 7*), but was also confirmed by pedigree information about the mating for the last three generations. The strongest separation was between standard poodles and all other groups of smaller poodles.

As suggested by many other studies, all breeds were clearly differentiated, indicated by F_{ST} and assignment tests. Furthermore, our results indicated that, within poodles, standard poodles were as different from the smaller sized poodles as breeds are from each other.

High inbreeding coefficient (F_{IS}) values and high heterozygote deficit in the other breeds suggest that a substructure similar to that found in poodles is likely to be present also in other breeds. The occurrence of substructure within breeds might be a consequence of disruptive selection, where breeders select for different characteristics within the same breed (e.g. either show dogs or working dogs), leading to genetically different subgroups of the same breed.

Reproductive barriers separating breeds into discrete groups have made dogs useful in association mapping studies aimed at locating genes responsible for certain phenotypic effects and also for the identification of candidate genes for diseases. However, uniformity within breeds is assumed in this kind of studies. The existence of a genetic substructure within breeds may confound these association studies if ignored. On the other hand, if these subgroups are recognized, a larger amount of genetically differentiated groups of dogs becomes available for these studies and linkage disequilibrium within these subgroups are also expected to be larger. This could facilitate association mapping studies.

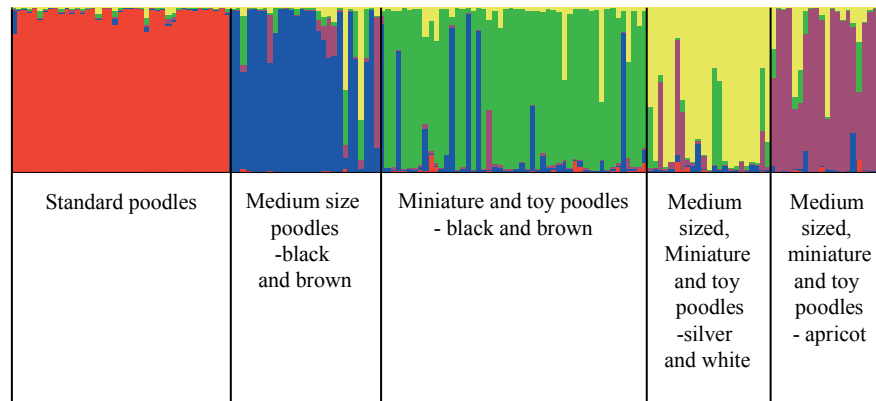


Figure 7. Clustering assignment of 164 purebred poodles, sorted according to colour and size combinations, using STRUCTURE 2.1. Each individual is represented as a bar and all poodles are divided into 5 groups.

Paper IV. Estimation of recent gene flow in metapopulations: using poodles as a model organism

Most species in natural environments have some degree of population structure due to recent or historical events. Knowledge about genetic differentiation and gene flow between these populations is important to take appropriate conservation and management decisions, as well as to understand the biological background of species.

Direct and indirect approaches can be used to estimate inter-population gene flow and both of these methods have their pros and cons. The aim of this study was to test if indirect estimates based on genetic data could reflect true recent gene flow. This was done by comparing indirect gene flow estimates to the level of genetic exchange between five groups of poodles calculated from registry information. The poodle population structure is analogous to a metapopulation, with subpopulations connected by limited gene flow.

Material and methods

Genetic data for 18 smooth fox terriers and 164 poodles, divided into five distinct classes defined by a combination of colour and size (Paper III), were obtained for 27 microsatellites. Registry information at the Swedish Kennel Club was used to calculate gene flow, as the proportion of ancestors originating from each one of the poodle classes (*Figure 8*).

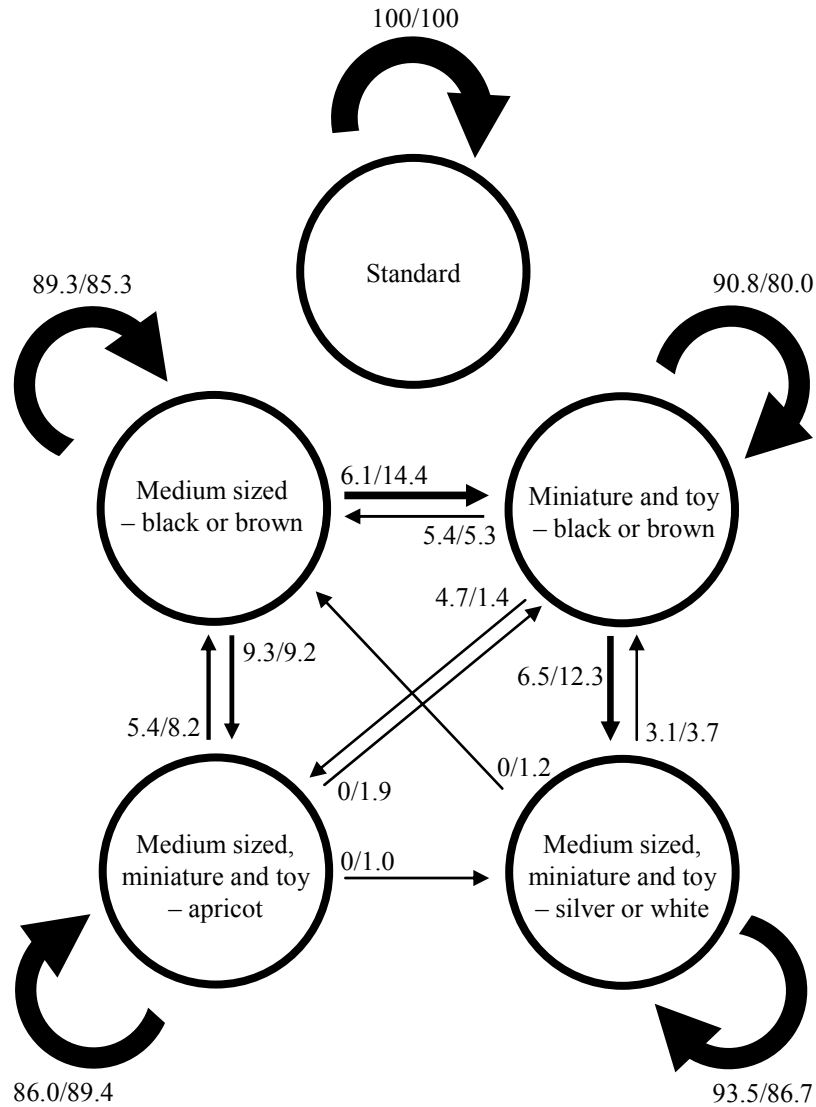


Figure 8. Proportion (%) of ancestry contribution for five groups of poodles. Numbers indicate percentage of contribution (left number, parents; right number great-grandparents).

Five indirect methods using genetic data to estimate gene exchange between populations, measured as the number of migrants per generation (N_m) or as proportion of ancestry, were assessed in this study: N_m estimated from F_{ST} , and the software programs STRUCTURE, ADMIX, BayesAss and MIGRATE. Estimates obtained were compared to the direct estimate of gene flow based on dog registry information. We used a Mantel correlation test to

evaluate the consistency of estimates obtained from indirect and direct methods.

Results and discussion

Registry information for the five groups of poodles indicated that genetic differentiation between the groups corresponded to real mating patterns. The results also revealed complete isolation between standard poodles and the smaller poodles during the last generations. Among the other poodle groups some degree of genetic exchange was found.

The estimates obtained from registry data were not correlated to the estimates obtained from the indirect methods represented by N_m from F_{ST} , STRUCTURE and MIGRATE. The program BayesAss provided results that were not consistent across runs and failed to converge. Finally, the last program, ADMIX, also failed to provide reliable estimates.

The reason for the inconsistency between pedigree data and indirect measurements might be the assumptions made for the indirect estimates. Additionally, the complexity of gene exchange across the poodle groups might also affect the result. Estimates of gene flow performed with the computationally demanding program MIGRATE might have been affected by too short run lengths, since it usually needs extremely long runs to achieve reliable estimates. The reason why ADMIX failed could be that all populations were at the same time “hybrid” and parental for the others and the program was, therefore, unable to portray this complex relationship between the populations. Finally, more markers and samples might also help to improve the estimates obtained.

Our results indicate that indirect measures of gene flow may not be reliable when populations deviate from equilibrium or when the (meta)population structure is very complex, with large biases and differences in the gene flow between the subpopulations. This situation occurs in dog breeds and is also likely to frequently occur in natural populations. Under those circumstances caution is needed to interpret indirect gene flow estimates based on genetic data.

Paper V. Evaluation of methods for single hair DNA amplification

Hair samples are commonly used as a source of DNA in wildlife monitoring as well as in forensic work. A problem that can occur when hair samples are collected from free-ranging animals using e.g. a barbed wire, is that it is not always known if the bunch of hairs are from one single animal or a few different individuals. Analyzing each hair root separately can solve this

problem. For forensic studies, the problem might be the opposite, e.g. only one single hair is found as evidence. For both situations, an effective and reliable method is needed to achieve the correct genotype, despite the very low amount of DNA found in a single hair.

The aim with this study was to compare different DNA amplification methods to evaluate which one gives the most reliable genotyping result based on one single hair root.

Material and methods

To compare the different methods, hairs from two dogs with known genotypes were used. The methods compared were a standard hair DNA extraction using Chelex[®] 100Resin (BioRad Laboratories, Hercules, CA, USA; *extraction alone*), a whole genome amplification using GenomiPhi DNA Amplification Kit (Amersham Biosciences, NJ, USA) applied directly on the hair root (*WGA alone*) and finally a combination of those two methods (*extraction + WGA*) where extracted DNA from the Chelex method was used for WGA amplification.

For the two methods *extraction alone* and *WGA alone*, 16 freshly plucked large guard hairs with a visible follicle (8 from each dog) were subjected to DNA extraction (*extraction alone*) or to whole genome amplification (*WGA alone*) previous to microsatellite PCR amplification. For the third test, *extraction + WGA*, one microliter of the DNA obtained from each of the 16 Chelex extractions was subjected to whole genome amplification before microsatellite amplification.

The final comparison between these methods was then made by typing 3 replicates per hair sample for eight nuclear microsatellite markers, using identical PCR protocols. The microsatellites used were biparentally inherited and distributed across the canine genome. A consensus genotype was then built from those three amplifications, using criteria common to many non-invasive genotyping studies (Taberlet et al. 1996, Gagneux et al. 1997, Flagstad et al. 1999, Bayes et al. 2000, Hedmark et al. 2005): for a locus to be considered homozygous, all three replicates had to show the same genotype; for heterozygous loci, at least two of the three replicates had to show the same genotype.

Results and discussion

Our results showed that *WGA alone* produced consistently better results through all the tests compared to the other methods (Table 2). However, the differences compared to *extraction alone* were only significant for one test; the number of failed PCR amplifications. All these tests were based on locus-by-locus comparisons. We also compared the different methods based on multilocus genotypes obtained for each hair, where the criterion was to

achieve the correct genotype for 7 or 8 loci. *WGA alone* provided again the best results. A third comparison was performed, based on a theoretical calculation using the results from the locus-by-locus test. In many studies a minimum number of successfully genotyped loci are requested for a genotype to be included in the data analysis. If, for example, 8 successfully genotyped loci out of a total of 10 are requested in a certain study, samples typed using *WGA alone* would have around three times higher probability to provide a usable genotype than *extraction alone*. For all these tests and comparisons, *extraction + WGA* was the least successful method. This method was extremely prone to allelic dropouts.

Even though *WGA alone* performed the best overall, time and cost of the methods should be taken into account when deciding what method to use. The difference in price for using the GenomiPhi DNA Amplification Kit compared to Chelex is significant and it is probably not economically defendable to use GenomiPhi when working with large-scale genotyping projects. Therefore the recommendation would be that if only a limited amount of material is available for a study or if the sample is especially valuable, *WGA alone* could be used to increase the chance of obtaining a usable multilocus genotype. However, due to its low cost and simplicity, *extraction alone* would be preferable when larger sample sizes are involved.

Table 2. Genotyping success for single hair samples of dogs, following three different protocols (numbers presented as percentages, %).

	<i>WGA alone</i>	<i>Extraction alone</i>	<i>Extraction + WGA</i>
# failed amplifications ¹	25.0	34.9	29.2
# successful homozygote replicates ²	75.0	61.9	71.4
# successful heterozygote replicates ³	69.9	60.2	40.7
# allelic dropouts ⁴	5.1	7.4	29.6
# successful homozygote genotypes ⁵	71.4	51.8	67.9
# successful heterozygote genotypes ⁶	72.2	63.9	41.7
Correct genotype (for homozygote and heterozygote samples in total) ⁷	71.9	58.6	53.1

¹ Failed amplifications = no alleles were amplified by PCR.

² Successful homozygote replicate = positive PCR amplification providing the correct homozygote genotype.

³ Successful heterozygote replicate = positive PCR amplification providing the correct heterozygote genotype.

⁴ Allelic dropout = one allele in a heterozygote loci is not obtained.

⁵ Successful homozygote genotype = 3 positive PCR replicates providing the correct homozygote genotype.

⁶ Successful heterozygote genotype = 2 or 3 positive PCR replicates providing the correct heterozygote genotype.

⁷ Correct genotype (in total) = correct genotype obtained after three replicates (weighted arithmetic mean of # successful homozygote genotypes and # successful heterozygote genotypes).

Concluding remarks

Dog domestication has been an important milestone in human history. Since then dogs have helped humans in many different ways and still do, not just as working companions and friends, but also by solving important questions in research.

This thesis demonstrates some of the alterations that have affected the dog's genome due to selective forces during the domestication process and also later by breed creation. The formation of genetically differentiated breed groups and the easily available pedigree information has also made the dog an important tool in scientific research.

The sequence of the complete mitochondrial genome has given a first view of the changes appearing within the genes of the dog's mtDNA after the initiation of domestication. It is possible that these changes have been caused by a change in living conditions and effective population size compared to the wolf and they could have affected the nuclear genome. However, this is still to be investigated.

The choice of informative genetic markers can be of great importance in domestication studies. A powerful tool for investigating the creation of breeds has been the combination of several markers with different patterns of inheritance. Analysing these markers separately would give a one-sided or weak explanation about breed origin. However, together they give a powerful explanation of the genetic contribution from both maternal and paternal point of view.

Strong selection during breed creation has led to genetically differentiated dog breeds, higher homozygosity within breeds and large extent of linkage disequilibrium (LD). These characteristics have made dogs an advantageous model for scientific research. The genetic analysis in this thesis shows that breeders are imposing additional selective forces upon breeds, which can result in further fragmentation of the existing breeds. This can have important consequences in future research aiming at, for example, using dogs as model organisms for gene mapping.

Furthermore, the importance of the dog as a model organism goes beyond medical and pharmaceutical research: as shown in this thesis, it has allowed me to evaluate the performance of genetic methods to assess recent gene flow and methods used in non-invasive and forensic research.

Future prospects

Dogs are today, as they will be in the future: an extremely useful tool in scientific research. This is due to the fact that dogs have strictly controlled pedigrees, are organized in genetically differentiated breed groups, and last but not least, often have the same kind of diseases as humans.

This thesis has shown the importance of comprehensive knowledge about breeding practices within dog breeds. This knowledge could improve the advantages of using dogs as study models in future science. Subgroups within breeds have a higher amount of linkage disequilibrium, which is useful in association studies.

The sequence of the complete mitochondrial genome in wolves, dogs and coyotes has open new possibilities for further investigations. Until now most calculations on domestication time have been calculated based on changes on the control region of the mtDNA. However, this part of the genome does not evolve at a constant evolutionary rate and might therefore be less suited to estimate evolutionary events. Using the complete mitochondrial genome, excluding the d-loop, would probably give a better estimate of the divergence time between wolves and dogs.

Furthermore, if the same relaxation of selective constraints is found to affect also the complete nuclear genome, it could contribute to explain the huge number of phenotypically different breeds of dogs today. By studying the mitochondrial genome and additional genes from the nuclear genome from both dogs and wolves, we can learn more about the mechanism causing the fast differentiation of dog breeds and get an insight in the mechanisms used by nature to generate genetic and phenotypic diversity.

Another interesting question is why dogs were the first animals to be domesticated? What was the reason for humans to allow wolves close to their human society, and finally, how did dogs contribute to the life of humans and vice versa?

Svensk sammanfattning

Bakgrund

Hunden var det första djur att domesticeras och processen startade för mer än 15 000 år sedan. Sedan dess har hunden levt tillsammans med människan som en trogen vän och arbetspartner. Hunden var troligtvis från början sedd främst som en matreserv men har genom åren använts av människan som bland annat vakthund, vallhund, sällskapshund, i jakt och på senare tid även som djurmodell inom forskningen.

Det finns idag ungefär 400 000 000 hundar runt om i världen, allt från renrasiga till blandraser av olika slag. De renrasiga hundarna är uppdelade i minst 400 olika raser med varierande storlek, färg och form. Morfologiskt är hunden det mest variationsrika däggdjuret som finns. Trots den enorma variationsrikedomen så härstammar alla hundar från vargen.

Hundens utveckling från domesticering till rasbildning

Domesticering är den process då tamdjur under flera generationer avlas av människor under kontrollerade former och där människan har fullständig kontroll över deras fodertillgång och rörelsefrihet, allt för att tillgodose människornas egna behov. Domesticeringen är en mer avancerad process jämfört med att enbart tämja ett djur. Det finns sex kriterier som ett djur måste uppfylla för att det överhuvudtaget ska kunna domesticeras, dessa är att: anpassa sig lätt till nya miljöer, se människan som en ledare, leva inhägnat utan att drabbas av panik i hotfulla situationer, vara användbar för människan, kunna föroka sig i fångenskap samt vara lätthanterliga. Alla djur är därför inte möjliga att domesticera, även om det går att tämja dem.

Hur domesticeringsprocessen initierades är fortfarande oklart, men det finns många olika teorier om hur det kan ha gått till. En teori baserar sig på idén att vargvalpar hittades, tämjdes och behölls som sällskap. Om de var hanterbara som vuxna fick de stanna hos människorna och från där har domesticeringen utvecklats. Andra menar att det var vargen som tog det första steget, genom att driva runt människornas boningar i sökandet efter föda. De vargar som var minst skygga kunde involvera sig i människornas samhälle och där dra nytta av de fördelar människorna hade att ge dem, så som mat och värme.

När och var domesticeringen initierades samt hur många individer som var involverade är fortfarande oklart. De tidigaste arkeologiska fynden av domesticerade hundar är daterade till kring 13 000–17 000 år sedan. Genetiska studier har dock visat på en separation mellan hund och varg på upp till 135 000 år. Var domesticeringen verkligen startade är det inte heller någon som vet helt säkert ännu. Genetiska studier har indikerat att domesticeringen kan ha startat i östra Asien. Antalet domesticeringstillfällen är beräknat att vara fyra (baserat på minsta antalet tikar) eller fem (baserat på minsta antalet hanar), då man har studerat mitokondrie DNA (som nedärvs från moder till avkommor) respektive Y kromosomen (som nedärvs från far till söner). Det kan dock ha skett på fler platser eller med fler individer.

Som ett resultat av domesticeringsprocessen får ofta domesticerade arter en förändring i storlek, utseende och beteende. Till en början brukar djuren få en mindre kroppsstorlek, en egenskap som ofta karakteriserar domesticering. Senare i domesticeringsprocessen kan större och mindre varianter av djuret selekteras fram. Pälsfärgen förändras också och hunden uttrycker fler varianter av färger jämfört med deras vilda släkting. Beteendet förändras och medför att hundarna är undergivna människan även i vuxen ålder. Det kan sägas att hunden bibehåller en del av valpens egenskaper även som vuxen. En annan viktig egenskap som hundar har utvecklat under domesticeringen är att de kan läsa och förstå människornas signaler, en egenskap som däremot saknas hos vargen. Hundens benägenhet att skälla har också uppkommit under domesticeringen.

Olika typer av hundar har funnits i tusentals år, men olika hundraser, så som vi ser dem idag, har bara funnits sedan mitten av 1800-talet. Den första hundutställningen hölls 1843 i England och strax därefter bildades den första kennelklubben. Avelsböcker producerades, vilka medförde att strikt avel inom hundraser var ett krav för att hunden skulle kallas renrasig. Det resulterade i genetiska barriärer mellan raserna och att den genetiska variationen inom varje ras minskade.

Att använda hunden som djurmodell i forskning har visat sig ge många fördelar i olika aspekter. En stor fördel är att hunden oftast lever i samma miljö som människan och utsätts för samma potentiella allergener, jämfört med laboratiemöss som lever i en strikt och kontrollerad laboratiemiljö. Dessutom har många frekvent återkommande sjukdomar hos människan, så som cancer, hjärtsjukdomar och höftproblem, också visat sig drabba hundar. Den låga genetiska variation som existerar hos renrasiga hundar kan underlätta genetiska studier genom att färre markörer behövs för att lokalisera bland annat sjukdomsgener.

Syftet med min studie var dels att studera de genetiska förändringar som skett under domesticeringen och bildandet av hundraserna och dels att använda hunden som djurmodell för att utvärdera metoder som används i studier på naturliga populationer. Nedan följer en sammanfattning av mina studier.

Artikel I. Ett försvagat selektivt tryck på mitokondriens DNA som ett led av domesticeringen

När hunden domesticerades förändrades dess livssituation markant. För att studera hur denna förändring har påverkat hundens genom sekvenserades hela mitokondrie DNA genom för 14 hundar, 6 vargar och 3 prärievargar.

Släktskapsträd baserat på de 23 mitokondrie DNA sekvenserna skapades för att därefter beräkna antalet synonyma (S, aminosyran förblir densamma) respektive icke-synonyma (NS, leder till en ny aminosyra) förändringar som skett på respektive gren hos både hundar och vargar samt beräkna förhållandet mellan icke-synonyma och synonyma förändringshastigheter (d_N/d_S). Resultatet visade att det generellt sett inte var någon skillnad mellan antalet förändringar som skett i hundens genom jämfört med vargens. Däremot var förhållandet av hur snabbt icke-synonyma/synonyma förändringar (d_N/d_S) uppkommit mycket högre hos hundar än hos vargar. Det resultatet tyder på att hundarnas naturliga selektionstryck förmodligen har blivit försvagat och därmed inte rensar bort skadliga varianter i samma utsträckning som hos vargarna.

Genom att dagens avel styrs av uppfödarnas preferenser, så kommer hundar med något mindre fördelaktiga genvarianter få avla, så länge de har tillräckligt attraktiva egenskaper. Därmed selekteras inte varianter med negativa egenskaper bort i samma utsträckning utan bibehålls i genom. Om detta har skett i hela hundens genom, så kan det ha medverkat till den stora variation som man kan se bland dagens hundar. Men det kan likväl vara en orsak till den stora mängd sjukdomar som påverkar hundarna idag.

Dessa kompletta mitokondrieDNA-sekvenser kan användas i fortsatta studier, exempelvis för att ta reda på mekanismen och orsaken bakom uppkomsten av förändringarna och därmed få svar på vilka mekanismer som i naturen genererar genetisk och utseendemässig variation.

Artikel II. Ojämn könsfördelning vid bildandet av hundraser

Syftet med den här studien var att, med tre typer av genetiska markörer som representerar olika nedärvningsmönster, undersöka bildandet av dagens hundraser.

Markörerna som användes i studien var mitokondrie DNA (nedärvs från modern), Y kromosom (nedärvs från far till son) och mikrosatelliter (nedärvs från båda föräldrarna). Till studien användes 100 hanhundar representerande 20 raser som analyserades för att undersöka variationen inom raserna för varje separat markör. För att därefter ta reda på hur separerade hundraser är i

förhållande till varandra studerades enbart Y kromosomen och mitokondrie DNA för ett större antal hundar samt sex vargpopulationer.

Hundraserna idag förväntas vara genetiskt skiljda åt p g a strikt avel inom varje separat ras. Resultatet från mikrosatellitdata indikerar att så verkligen är fallet, då nästan alla 100 hundar grupperande sig till rätt ras. Däremot fanns ingen struktur mellan raser baserat på vare sig Y-kromosomdata eller mitokondrieDNA-data. Det resultatet indikerar att raserna bildades relativt nyligen och att de inte har varit separerade under så lång tid.

Analysen av det större antalet hundar samt vargarna, med Y-kromosom och mitokondrieDNA-markörerna, visade att fler mtDNAhaplotyper än Y-kromosomhaplotyper fanns hos hundarna, medan förhållandet var det motsatta hos vargarna. Skillnaderna i förhållandet mellan vargar och hundar kan bero på olika parningsförhållanden. Hos vargarna lever de i en flock där enbart alfaparet para sig, medan i hundaveln används ett fåtal hanhundar till ett flertal tikar och därmed blir den genetiska fördelningen mellan tikar och hanar skev.

Studien bidrog till en ökad förståelse om hur hundens genetiska variation har påverkats genom rasbildning. Dessutom gav studien inblick i hur olika genetiska markörer påverkas av ett kort tidsperspektiv, som rasbildning innebär. Denna kunskap kan man ha nytta av i framtida studier när man ska välja markörer för studier av olika tidsperspektiv.

Artikel III. Riktad selektion leder till struktur inom hundraser

En hundras sägs vara en enhetlig grupp där individerna inom gruppen ska ha gemensamma egenskaper. Trots det finns tillåtna varianter av olika storlekar och färger inom vissa raser, som t ex hos pudeln.

Eftersom renrasiga hundar används inom forskningen just p g a att de anses vara en homogen grupp, så var syftet med den här studien att ta reda på om så verkligen var fallet. För studien användes pudlar representerande de inom rasen tillåtna fyra storlekarna (stor, mellan, dvärg och toy) och fem färgerna (svart, brun, vit, silver och aprikos). För jämförande studier mellan raser användes ytterligare 8 raser. Tester för att undersöka hur genetiskt separerade raser är från varandra samt om man kunde se någon struktur inom ras utfördes baserat på 27 mikrosatellitmarkörer.

Resultatet visade att den genetiska skillnaden mellan raser är tydlig, vilket troligtvis beror på de genetiska barriärer som bildats p g a förbudet att avla över rasgränserna sedan 1800-talets mitt. Inom pudlar fanns en tydlig struktur, där storpudel, oavsett färg, skiljde sig markant från de mindre storlekarna av pudel. Den genetiska skillnaden mellan storpudel och de mindre storlekarna är minst lika stor som den genetiska skillnaden som

existerar mellan raser generellt. Mellan de tre mindre storlekarna av pudel kunde man också hitta en struktur, som baserades på en kombination av storlek och färg. De genetiskt lika grupperna var; svart eller brun mellanpudel, svart eller brun dvärg- respektive toypudel, silverfärgade pudlar samt aprikosfärgade pudlar. Pudlar har därmed en gruppering av fem genetiskt enhetliga grupper inom rasen. Resultat från de övriga raserna indikerar att en liknande struktur som observerats hos pudlarna, troligtvis även existerar inom andra raser. Denna struktur inom ras har förmodligen uppkommit genom att hunduppfödare avlar för olika ändamål inom en och samma ras, t ex avlar för fina utställningsexemplar eller bra arbetshundar. Det leder så småningom till genetiskt separerade individer inom samma ras. För att på bästa sätt utnyttja hundars homogenitet inom forskningen bör detta fenomen beaktas för att resultaten inte i slutändan ska bli missvisande.

Artikel IV. Uppskattning av genflöde inom en metapopulation, genom att använda pudel som modell

Inom de flesta arter finns en viss grad av populationsstruktur. Den kan vara svår att upptäcka och kan därför påverka t ex bevarandet av utrotningshotade djur. Det kan göras en uppskattning hur separerade olika populationer är i förhållande till varandra genom att studera mängden genetiskt utbyte mellan grupperna. Antingen används direkta metoder, som att studera antal djur som byter population och förökar sig. Eller alternativt används indirekta metoder, genom att studera den genetiska sammansättningen av individer i olika populationer. Genetiska data för indirekta metoder analyseras ofta med olika beräkningsmetoder och dataprogram. Syftet med den här studien var att jämföra hur väl indirekta beräkningsmetoder återspeglar verkligheten genom att jämföra resultaten med verkliga värden på genflöde. Fem genetiskt skilda grupper av pudel användes som testpopulation. Pudelpopulationen kan liknas vid en metapopulation, där ett antal små grupper/populationer hålls samman genom en viss grad av genutbyte. Det verkliga genetiska utbytet mellan grupperna beräknades som den procentuella andelen äldre släktingar (tre generationer bakåt i tiden) som ”på sin tid” tillhörde en annan grupp inom pudelpopulationen. Dessa släktingar har därmed bidragit med gener från andra pudelgrupper till dagens population/grupp.

Det visade sig att ingen av de fem testade metoderna återspeglade den verkliga mängden av genetiskt utbyte som beräknats existera mellan pudelpopulationerna. Detta kan bero på ett komplext släktskapsmönster hos pudel som inte representerar naturliga populationer. Alternativt att indirekta metoder har höga krav på att populationen måste följa vissa kriterier, som i många fall är omöjligt för populationer i naturligt tillstånd. Slutsatsen är att man inte ska lita för mycket på enbart indirekta beräkningar, utan helst kombinera med direkta observationer för att få ett tillförlitligt resultat.

Artikel V. Utvärdering av metoder för att amplifiera DNA från enstaka hårstrån

DNA analys från hårrötter används ofta för övervakning och inventering av vilda djurpopulationer samt i kriminalfall. I de fall enbart ett enda hårstrå är tillgängligt för analys är det viktigt att man använder sig av en tillförlitlig amplifieringsmetod, så att korrekt resultat kan erfordras i slutändan trots en liten mängd DNA. Målsättningen med den här studien var därför att utvärdera olika amplifieringsmetoder under förutsättningen att enbart ett hårstrå var tillgängligt för analys.

De metoder som utvärderades var extrahering med Chelex[®], amplifiering med GenomiPhi applicerat direkt på hårroten samt GenomiPhi amplifiering baserat på ett startmaterial från DNA extraherat med Chelex[®]. Metoderna jämfördes därefter med hjälp av mikrosatellitdata.

Resultatet visade att amplifiering med GenomiPhi applicerat direkt på hårroten gav genomgående bättre resultat för alla testen. Chelex extraktionen var dock bara signifikant sämre på ett test. GenomiPhi baserat på redan extraherat DNA (för att amplifiera upp större mängd) gav väldigt dåligt resultat, då ett förekommande fel var att en av de två varianterna hos en heterozygot individ föll bort och därmed gav missvisande resultat.

Rekommendationen vilken metod som man bör använda för att amplifiera DNA från enstaka hårrötter beror på situationen. I de fall mängden hår är begränsat, eller om provet är speciellt värdefullt och viktigt, bör GenomiPhi direkt applicerat på hårroten användas för att vara säker på att få ett korrekt resultat från en liten mängd DNA. Däremot, om provmängden är stor (flera hårstrån från samma individ finns tillgängligt), bör Chelex[®] extraktionen användas, eftersom det är en mycket billigare metod och väldigt enkel att använda.

Slutsats

Ända sedan domesticeringens början har hunden varit till stor hjälp för människan, inte bara som vän och jaktpartner, utan på senare tid även som djurmodell i forskningen.

I min avhandling har jag visat hur hundens DNA har förändrats genom domesticeringsprocessen och rasbildningen. Detta resultat kan ligga till grund för fortsatta studier, t ex gällande mekanismer som i naturen orsakar stora genetiska och morfologiska skillnader, något som man även kan se mellan dagens hundraser. Dessutom har jag genom mina studier givit en inblick i hundens genetiska sammansättning och hur detta kan vara till en fördel i framtida studier inom t ex sjukdomsforskning (underlätta sökandet av sjukdomsrelaterade gener) eller för studier av naturliga djurpopulationer (där hunden används som modell för att efterlikna verkliga populationer).

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