

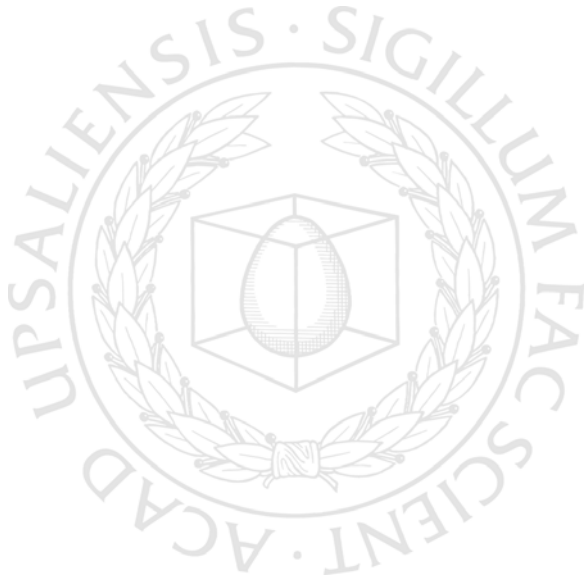


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Conservation Genetics of Wolves and their Relationship with Dogs

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Abstract

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Management of wolves is a complex issue, and molecular genetics is an important tool in this work. Molecular genetics can provide important information at the species, population and individual level, which can be essential for the development of management programs aiming at the long term survival of wolf populations.

In this thesis I developed new genetic markers on the canine Y chromosome to estimate the number of founders of the Scandinavian wolf population. This knowledge is important to reconstruct the history of the population and to design the most appropriate conservation strategies. Next, genetic markers with different pattern of inheritance have been used to identify hybrids between wolves and dogs. This allowed us to determine the direction of hybridization and to evaluate its possible impact on the gene pool of a wolf population. Furthermore, I also developed a method for a more reliable identification of the predator responsible of an attack by using saliva remains left on the prey. Since predation on livestock is perhaps the main reason for the negative opinions about the predator, the correct identification of the responsible for an attack (wolf, dog or hybrid) is essential.

Finally, this thesis has also been focusing on the domestication of dogs. By using Y chromosome markers (paternally inherited), it has been possible to complement previous studies based on mtDNA sequences (maternally inherited) and autosomal markers (inherited from both parents). In this way I have obtained a more complete picture of the domestication process and of the origin of breeds. This has shown that there has been a bias in the contribution of the two sexes in the origin of dog breeds (fewer males than females contributing to each breed) and that the origin of dogs was not marked by extensive backcrosses with male wolves over the entire species range.

Keywords: Canis lupus, Canis familiaris, mtDNA, Y chromosome, microsatellites, breed, hybridization, domestication, introgression, predation

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Abbreviations

| | |
|-------|--|
| AMOVA | Analysis of Molecular Variance |
| bp | Base pair |
| DNA | Deoxyribonucleic acid |
| FCA | Factorial Correspondence Analysis |
| FCI | Fédération Cynologique Internationale (World Canine Organization) |
| IUCN | World Conservation Union |
| MtDNA | Mitochondrial DNA |
| PCR | Polymerase Chain Reaction |
| SSCP | Single Strand Conformation Polymorphism |
| STRs | Single Tandem Repeat Polymorphisms |

Introduction

Conservation genetics

The management and conservation of wildlife is a complex issue in which biology interacts with human values (Nie 2003). Our planet has limited resources and the biodiversity is being rapidly depleted due to human activities. This loss of diversity takes place at all levels: ecosystems, species, populations and genetic diversity within species. Today many species need human actions to ensure their long-term survival (Frankham *et al.* 2002).

Conservation genetics can be defined as “the theory and practice of genetics in the preservation of species as dynamic entities capable of evolving to cope with environmental change to minimize their risk of extinction” (Frankham *et al.* 2002). This implies that the goals of conservation genetics go beyond the protection of small populations and aim at the preservation of evolutionary processes. Within conservation biology there are different topics for which genetics is central. These topics are the basis for the field of conservation genetics (Frankham *et al.* 2002).

- **Inbreeding.** The level of inbreeding increases through time in small populations. This often leads to inbreeding depression, which decreases individual fitness, as has been observed for Scandinavian wolves (Liberg *et al.* 2005, Räikkönen *et al.* 2006), and could lead to extinction (Frankham and Ralls 1998).
- **Loss of genetic diversity.** Populations need genetic diversity to be able to evolve. That is essential for the long-term survival of populations that they can cope with environmental changes (Lavergne and Molofsky 2007).
- **Population fragmentation and reduced gene flow.** Diversity in a population can only increase through mutation (a very slow process) or exchange of genes with neighbouring populations (Madsen *et al.* 1999, Vilà *et al.* 2003). However, habitat alteration by humans has led to fragmentation, increasing the level of threat.
- **Genetic drift.** In a small population, genetic drift will outcompete natural selection. Random genetic drift leads to loss of genetic diver-

sity and increases risk of extinction (Frankham *et al.* 1999, Saccheri *et al.* 1998).

- **Accumulation and loss (purging) of deleterious alleles.** All populations contain deleterious alleles. Many of these are recessive, but in case of inbreeding these alleles can be exposed and selection could remove them (Swindell and Bouzat 2006a, b). Purging may ameliorate inbreeding depression, although it unlikely to eliminate it (Frankham *et al.* 2002).
- **Genetic adaptation to captivity.** Captive breeding can be the only alternative for protecting species that can not survive in their natural habitat. Captive breeding programs aim at retaining high levels of genetic diversity. The long term goal for many breeding programs is reintroduction of the species into the wild. However, adaptation to live in captivity might reduce fitness when populations are returned to the wild (Frankham 2005).
- **Resolving taxonomic relationships.** Correct taxonomic status is important so that endangered species are not denied protection (for example, see Haig *et al.* 2001).
- **Defining management units.** Populations within species may require separate management due to differentiated adaptive characteristics or genetic composition. However it is not always clear how to identify these units (Mortiz 1994, Crandall *et al.* 2000).
- **Forensics.** Genetic markers can be used in cases of illegal hunting to identify species or stock of origin (Palumbi and Cipriano 1998).
- **Understand species biology.** Genetic methods can help answering questions about species biology that are important in conservation: estimate population size and effective population size (Roman and Palumbi 2003), detect selection (Fink *et al.* 2007), parental testing (Kimwele and Graves 2003), sex determination (Ellegren 1996), mating systems (Lebige *et al.* 2007), populations structure (Pilot *et al.* 2006), dispersal rates (Langergraber *et al.* 2007), diet (Kasper *et al.* 2004), disease (Wood *et al.* 2007), detect introgression and hybridization (Lecis *et al.* 2006).
- **Outbreeding depression.** Interbreeding between individuals originating from two separated populations can result in reduced reproductive fitness (Tymchuk *et al.* 2007). Also hybridization between different species can result in individuals with lower fitness than their parents (Veen *et al.* 2001).

Conservation of the species in focus for this thesis, the wolf, is especially difficult due to contrasting viewpoint about them (Fritts *et al.* 2003). In the conservation and management of wolves, genetics can be of great help. During recent years wolves have been extensively studied using genetic ap-

proaches to answer a very large number of questions. For example some studies have been done on the domestication process (Vilà *et al.* 1997, Savolainen *et al.* 2002, Leonard *et al.* 2002), genetic status and history of contemporary populations (Aspi *et al.* 2006, Fabbri *et al.* 2007, Ellegren *et al.* 1996, Valière *et al.* 2003), variability in extirpated populations (Flagstad *et al.* 2003, Leonard *et al.* 2005), phylogeography (Vilà *et al.* 1999), population structure (Pilot *et al.* 2006, Geffen *et al.* 2004), inbreeding (Bensch *et al.* 2006, Ellegren 1999, Liberg *et al.* 2005), inbreeding depression in captivity (Hedrick *et al.* 2001, Fredrickson *et al.* 2007, Laikre and Ryman 1991, Laikre *et al.* 1993) genetic results of reintroductions (Vonholdt *et al.* 2007, Ripple and Beschta 2007) and hybridization with dogs (Andersone *et al.* 2002, Randi and Lucchini 2002, Verardi *et al.* 2006) and with other canids (Fredrickson and Hedrick 2006, Wilson *et al.* 2000).

The wolf

The grey wolf (*Canis lupus*) is the land mammal with the largest natural distribution (Mech and Boitani 2003). The variation and adaptability found within this species is enormous; it can feed on large mammals or berries, it can live in the tundra, the desert or occasionally visit cities, it can vary in size from 13 kg up to 78 kg (Mech and Boitani 2003). The wolf's status for the World Conservation Union, IUCN, is "least concern". A taxon of Least Concern does not qualify as Critically Endangered, Endangered, Vulnerable or Near Threatened. Widespread and abundant taxa are included in this category (<http://www.iucn.org/>).

No other animal raises so many feelings among humans as the wolf. There are both people who love them, as well as people who hate them. These feelings can be really strong and sometimes it can even be hard to tell if stories about the wolves are truth or hearsay (af Klintberg 1994). Organized wolf hunting began already in the early Middle Ages and today it has been cut back from large parts of the originally range. About 200.000 wolves are estimated to be the current world population (Boitani 2003). During the last decades the population size has, for the first time in a long time, started to increase. The main reasons for this have been legal protection and ban on poison (Boitani 2003). However, in most of its current range, humans are still the major cause of wolf mortality. Persecution of wolves has always been out of proportion to the actual threat it can be to humans (Fritts *et al.* 2003).

Wolves in Europe

Until the early XIXth wolves were abundant in Europe but at that time extermination efforts begun (Wayne *et al.* 1991). In central Europe the wolf population was reduced during the XIXth century and, finally, the intense persecution led to extinction in this region in the early XXth century. The number of wolves in Eastern Europe was also reduced by the end of the XIXth century, but they managed to survive until today. Also in southern Europe, in Italy and the Iberian Peninsula, isolated populations of wolves survived although their numbers were seriously reduced. A rough estimate of the population size in Europe today is about 10.000 animals (Macdonald 2001, Sand *et al.* 2000). However, most populations are small and isolated from each other, making gene flow difficult (Wayne *et al.* 1991, Pilot *et al.* 2006).

Wolves in Scandinavia

Just like in many other parts of Europe, the wolves in Scandinavia were persecuted and hunted. The first wolf bounty was introduced in Sweden already in 1647 (Boitani 2003). In the beginning of the XIXth century there were probably about 1500 wolves in Sweden (Sand *et al.* 2000). This was followed by an intense hunt and in 1950 the population size was estimated to less then 35 individuals (Sand *et al.* 2000). Legal protection of wolves was declared 1966 in Sweden and 1972 in Norway (Wabakken *et al.* 2001). However, from 1964 no breeding took place and in the early 1970s the wolf was considered extinct from the Scandinavian Peninsula. In 1977 a few animals were seen in the northern most of Sweden and one breeding took place in 1978. None of these animals survived long, and again the wolves were considered extinct from Scandinavia.

In the early 1980s a few animals were seen in Värmland, in southern Sweden, and subsequently a litter was born in 1983 (Sand *et al.* 2000). This sudden appearance of wolves, about 1500 km away from the closest neighbouring population in Finland, caused intense speculation. Many people thought that the wolves could not have come to southern Sweden by themselves without being detected (Wabakken *et al.* 2001). One widespread idea was that the wolves had been released from Swedish zoos (af Klintberg 1994). However, this hypothesis could be ruled out on the basis of genetic analysis of both wild and captive wolves (Ellegren *et al.* 1996).

Like many other European wolf populations, the current Scandinavian population has increased in number during recent years. The latest population estimated from the winter of 2006/2007 is 109-117 individuals (Vilt-skade center 2006).

Wolf conservation and management

“Wolves can live almost anywhere in the Northern Hemisphere, and almost everywhere they do, they are an issue”

(Mech and Boitani 2003)

Wolf populations worldwide suffer several threats to their survival (Boitani 2003):

- **Human persecution.** Persecution by humans has been the dominant factor that led to the decline of wolf populations from many areas of the species' historical range. In many countries wolves are legally protected today. However, illegal hunting is still common and, due to the conflict with livestock, protective legislation is not enforced.
- **Wolf harvesting.** About 6000-7000 wolf skins are traded internationally every year. Although it is a big number, it is not a big threat to the wolves since the harvesting only occurs in areas where the population sizes are fairly big; Canada, former Soviet Union, Mongolia and China.
- **Habitat destruction.** Reduction and destruction of habitat suitable for wolves is the greatest long-term threat to wolves. Also, wolves that lack natural resources are likely to prey on domestic species.
- **Small population risk.** Fragmented and isolated populations are more likely to suffer from founder effects, bottlenecks, genetic drift and inbreeding. Small populations are also more likely to suffer from stochastic environmental and demographic events.
- **Hybridization.** Wolves are known to hybridize with both dogs and coyotes. These hybrids may compete with the wolves over different resources and, in case of backcrossing, affect the genetic composition of the pure species.
- **Diseases.** Rabies, canine distemper, sarcoptic mange and canine parvovirus are all possible mortality factors that can have drastic effects on wolf populations, as observed for other endangered canids (Sillero-Zubiri *et al.* 1996).

In this thesis the focus will be on two conservation issues: wolf predation on livestock –the main reason used to justify persecution- and hybridization.

Wolves and livestock

The main reason for wolf extermination efforts has been predation on livestock. This happened first in the Old World with the expansion of pastoralism about 1000 years ago, and later in the New World when European settlers and their livestock travelled to the west (Fritts *et al.* 2003). In every country where wolves and domestic species coexist, problems with wolf predation occur. In Europe, sheep is the most common domestic prey of wolves, since they are common and often vulnerable in wolf areas (Fritts *et al.* 2003). The economical losses due to livestock damages by wolves are significant. Many governments give economical compensation for livestock losses as well as support prevention measures like predator-safe fences (<http://www.naturvardsverket.se/sv/Arbete-med-naturvard/De-stora-rovdjuren/Ersattning-for-skador-av-rovdjur/>).

When wolf predation on domestic animals takes place, some form of wolf control and management is often inevitable. If governments do not act against the predation, livestock owners often try to solve the problem themselves. Control methods that are being used include both lethal and non-lethal (translocation and methods to prevent attacks from taking place), and these are often complemented with illegal persecution (Fritts *et al.* 2003).

Hybridization

Many existing wolf populations are small and isolated from other populations and they are thereby sensitive to inbreeding and genetic drift (Wayne *et al.* 1991). Furthermore, small isolated populations are also known to have a higher risk of hybridizing if there are opportunities for that to take place (Andersone *et al.* 2002). Since dogs were domesticated from wolves rather recently, from an evolutionary perspective, they are still very similar genetically. That means that a wolf and a dog can mate and produce fertile offspring. It can even be discussed if they really are two different species. In 1942, Mayr defined the biological species concept as “groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups” (Futuyma 2005). The strict usage of this definition would lead to consider wolves and dogs as members of the same species. However, wolves and dogs use different ecological niches and are therefore separated from each other, even when they both are found in sympatry.

Hybridization can take place both naturally or induced by humans. Some dog breeds have been recently formed as a direct result of hybridization induced by humans. For example, the Czechoslovakian wolfdog and Saarloos wolfdog were formed a few decades ago in Europe by crossing wolves and German shepherd dogs (Adlercreutz and Adlercreutz 1999).

When hybridization occurs in the wild it may be a threat to the wolf populations. The threat can be both direct, by hybrids competing with the wolves

over food, territory or other resources. But there is also an indirect, genetic threat, if the hybrids are successfully backcrossing into the wolf population. In this case dog genes will be transferred into the wolf population. Hybridization has been observed between wolves and dogs in the wild (Andersone *et al.* 2002, Randi and Lucchini 2002).

It is not only wolves and dogs that hybridize among the canids. There have been reports on hybridization in multiple canid species: dogs and coyotes (*Canis latrans*, Adams *et al.* 2003a), dogs and Ethiopian wolves (*Canis simensis*, Gotelli *et al.* 1994), coyotes and red wolves (*Canis rufus*, Adams *et al.* 2003b, Fredrickson and Hedrick 2006) and wolves, coyotes and Eastern wolves (*Canis lupus lycaon*, Kyle *et al.* 2006).

The dog

“For thousands of years wild wolves have competed with humans for game and killed farm animals, while the tame wolf has become man’s best friend- the domestic dog.”

(Macdonald 2001)

The tiny Chihuahua, the giant Great Dane, the slim greyhound and the massive mastiffs, they are all members of the same species, the dog (*Canis familiaris*). All dogs originate from one single wild ancestor, the grey wolf (Vilà *et al.* 1997). Today there are about 400 million dogs in the world (Coppinger and Coppinger 2002).

Domestication

Dogs were domesticated at least 14 000 years ago (Vilà *et al.* 1997, Savolainen *et al.* 2002). The earliest archaeological evidence were two dog craniums found in Russia and dated to 13 000-17 000 years old (Sablin and Khlopachev 2002). This was well before any other animal or plant species. In the early stage of the domestication process, dogs spread fast across continents; this suggests that dogs may have played an important role in primitive human societies (Clutton-Brock 1999). The domestication can be considered as a great biological success, since the dogs now outnumber their ancestor by thousand times (Coppinger and Coppinger 2002).

It is hard to know why the domestication process started at all. Today we use our dogs in many different ways: hunting, protection, herding, sled pulling, company and so on. However, we do not know the reason that led to their original domestication. It is even possible that the domestication took place on the wolves' initiative as they approached human settlements and developed a commensalistic association (Coppinger and Coppinger 2002).

Genetic studies using maternally inherited sequenced has suggested a limited number of domestication events, four (Vilà *et al.* 1997) and six (Savolainen *et al.* 2002), separated in time or space. However extensive backcrossing with wolves over time has probably been important for obtaining the genetic diversity seen in dogs today (Vilà *et al.* 2005).

Breeds

A breed is defined as “a group of animals that has been selected by man to possess appearance that is inheritable and distinguishes it from other groups of animals within the same species” (Sampson and Binns 2005). The goal of breeders has been to create dogs that are physically suited for specific purposes (Moody *et al.* 2005). The World Canine Organization (Fédération Cynologique Internationale, FCI) today recognizes 339 breeds of dogs. These breeds are divided into 10 groups, mainly based on their function (<http://www.fci.be>):

1. sheepdogs and cattle dogs (except Swiss cattle dogs)
2. pinscher and schnauzer, molossoid breeds, Swiss mountain and cattle dogs and other breeds
3. terriers
4. dachshunds
5. spitz and primitive types
6. scenthounds and related breeds
7. pointing dogs
8. retrievers, flushing dogs and water dogs
9. companion and toy dogs
10. sighthounds

The phenotypic diversity that is seen among dog breeds exceeds what is seen in any other mammal species and also what is seen in the entire Canidae family (Wayne 1986). Archaeological records suggest that already 4000 years ago in ancient Egypt there were different types of dogs (Clutton-Brock 1999). Furthermore, paintings from the XVIIth century depict dogs similar to

modern breeds such as spaniels (for example in “The Elevation of the Cross” by Peter Paul Rubens, 1611) and mastiffs (“Las Meninas” –“The Maids of Honour”- by Diego Velázquez, 1656).

Not all dogs in the world belong to recognizable breeds. On the contrary, the majority of dogs do not belong to a specific breed or have a registered pedigree, but are simply village and mongrel dogs (Coppinger and Coppinger 2002). Besides the 339 breeds recognized by FCI, there are numerous local types and varieties, as well as these village and mongrel dogs.

The modern breeds that we recognize today are all rather recent. The first dog show took place in 1843 and the first trial took place in 1865, both in Britain (Sampson and Binns 2005). It was also in England where the first kennel club was established, The Kennel Club, in 1873. Today this has spread all around the world, 84 countries are today members of the FCI. Additionally there are countries that not are member of FCI, but rather have their own kennel clubs, for example USA (American Kennel Club) and Great Britain (The Kennel Club).

Genetic markers

Wolves and dogs have the same genetic make up, 78 chromosomes: 38 pairs of autosomes and two sex chromosomes. The entire genome does not evolve in the same way. Different evolutionary forces are acting on different parts of the genome. Furthermore, different parts are transferred from one generation to the next in different ways: maternally (from mother to offspring), paternally (from father so son) or biparentally (from both parents to the offspring). By choosing the most appropriate kind of marker, or by combining different ones, many questions can be answered about the biology of a species.

Mitochondrial DNA

All cells in mammals contain mitochondrial organelles. These mitochondria have their own DNA, mitochondrial DNA (mtDNA). There are a number of characteristics of the mtDNA that make it an excellent tool for the study of natural populations. First, mammalian mitochondria are maternally inherited. Second, each mitochondria contains many copies of mtDNA, making them numerous (there are only two copies of each nuclear gene in each cell, but hundreds or thousands of copies of the mtDNA) and easy to use and amplify in genetic studies. Third, the rate of substitutions for mtDNA is about 5-10

times higher compared to nuclear DNA (Li 1997). Subsequently mtDNA accumulates information faster and can be used to characterize relatively recent evolutionary events (Kim *et al.* 1998). Last, mtDNA does not recombine, it is inherited from one generation to the next without exchanging bases (Brudford *et al.* 2003), and this makes phylogenetic analyses easy to interpret. A part of the mtDNA that is frequently used in intra-species studies is the so called control region. This is a non coding region and shows the highest level of variation within the mtDNA. Therefore this region can be used on a species level, to track geographic patterns of diversity, dispersal, gene flow, demographic expansions, genetic drift and hybridization (Brudford *et al.* 2003).

Autosomal microsatellites

Short tandem repeat polymorphisms (STRs) are short DNA sequences that are repeated many times in tandem at a particular locus in the genome (Hartl and Jones 2000). When the repeat units in the STRs are 2-9 bases, they are often called microsatellites. Autosomal microsatellites are simply microsatellites that are located on the autosomes (not on the sex chromosomes). Autosomal microsatellites are biparentally inherited; each individual inherits one copy from its mother and one copy from its father. During cell division, microsatellites are exposed to replication slippage which may alter the number of repeats (Ellegren 2004). The polymorphism in microsatellites therefore derives from differences in length resulting in different alleles. The genetic variation within microsatellites is often estimated by the level of heterozygosity (proportion of loci found in heterozygous state; an individual is heterozygote when there are two different alleles at one locus). The fact that microsatellites are the most variable sequences in the genome, makes them invaluable as genetic tools. For example, they are used in linkage mapping, paternity testing, forensics and inference of demographic processes (Ellegren 2004).

Y chromosome

In mammals, males are the heterogametic sex, having one X chromosome and one Y chromosome (females being the homogametic sex, having two X chromosomes). The Y chromosome is therefore paternally inherited, all males having essentially the same Y chromosome sequence as their father. The Y chromosome is the smallest chromosome in the canine karyotype (Mayers-Wallen 2005). During meiosis only a limited part of the Y chromo-

some pairs with the X chromosome. That means that the Y chromosome does not recombine over most of its length (Lahn *et al.* 2001). Loci located on this non recombining part of the Y chromosome will be permanently linked (Hurles and Jobling 2001) and can therefore be treated as haplotypes. Haplotypes are defined as unitary heritable packages that incorporate multiple variable sites (Bradley 2006). So far only a limited number of studies have been focused on the canine Y chromosome (Bannasch *et al.* 2005, Natanaelson *et al.* 2006). One explanation to this is that the Y chromosome tend to contain less polymorphic sites compared to the rest of the genome (Hellborg and Ellegren 2004, Lindgren *et al.* 2004, Shen *et al.* 2000, Wallner *et al.* 2003) making genetic studies difficult. Also, the scarcity of genes in this chromosome has led researchers to select a female for whole-genome sequencing (Lindblad-Toh *et al.* 2005), allowing for a better coverage of the X chromosome.

Research aims

The aim of this thesis has been to study different aspects of wolf conservation genetics and the process by which dog breeds were formed. More specifically, the aims have been:

1. Develop markers located on the canid Y chromosome, to be used in studies of natural populations.
2. Use genetic tools for identification of hybrids between wolves and dogs and assess the importance of genetic introgression into a natural wolf population.
3. Use genetic information on the structure of wolf populations and dog breeds to understand the process how breeds have been formed.
4. Develop methods for the identification of canid predators by using saliva remains left by the predator on the prey.

Present investigations

Paper I. Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers

The maternally inherited mitochondrial DNA has commonly been used as a tool for population genetic studies. Since this marker only considers female lineages, it can give a biased picture of population histories. Here we developed four microsatellites on the canid Y chromosome. These markers were also used to characterize the genetic diversity in the Scandinavian wolf population. This population was thought to be extinct in the 1970s and the current population originates from only a few individuals that reappeared in southern Sweden in the 1980s.

Material and methods

Two microsatellite sequences on the canid Y chromosome were identified by Olivier *et al.* (1999). However PCR amplification of these sequences revealed two fragments in male dogs and none in female dogs. To test for the possibility of sequence duplication, which is common on the Y chromosome (Jobling *et al.* 1996, Lahn & Page 1997, Tilford *et al.* 2001), the fragments obtained by PCR were cloned. The clones were screened using single-strand conformation polymorphism, SSCP. Clones identified as containing different inserts on the SSCP gel were sequenced. From the sequences we discovered two copies of duplicated microsatellites on the canine Y chromosome. New specific forward primers were designed to allow independent amplification of the duplicated fragments, resulting in four microsatellites: *MS34A*, *MS34B*, *MS41A* and *MS41B*. These four markers yielded single fragments in amplification of male wolf DNA.

These four microsatellites were typed in 14 Scandinavian male wolves, 13 wolves from Swedish zoos and 73 male wolves from other north European populations. Since these markers are located on the nonrecombining region of the canid Y chromosome, they were combined into haplotypes.

Result and discussion

Three Y chromosome haplotypes were obtained from the 14 Scandinavian male wolves (A, B and C; Table 1). However, haplotype C was only found in one individual killed in northern Sweden in 1977, prior to the wolf reappearance in southern Sweden. Another haplotype, D, was found in all zoo wolves typed. In addition, 14 haplotypes were found in other north European wolf populations. Haplotype A and B were not found outside Scandinavia or in the Swedish zoo population. However, many of the haplotypes found in north Europe were seen in very low frequency, indicating that some haplotypes could have been missed in this sample set and that it was possible that the haplotypes found in Scandinavia were also present elsewhere.

Table 1. Temporal distribution of haplotypes A, B and C in the Scandinavian wolf population. Each year correspond to the date when the individual male wolves were killed.

| Haplotype A | Haplotype B | Haplotype C |
|-------------|-------------|-------------|
| | | 1977 |
| | 1984 | |
| | 1986 | |
| | 1986 | |
| | 1989 | |
| | 1992 | |
| | 1992 | |
| 1993 | | |
| 1996 | | |
| | 1997 | |
| | 1998 | |
| 1999 | | |
| | 2000 | |
| | 2000 | |

The two haplotypes found in the current Scandinavian population, A and B, indicate that at least two male wolves were involved in the founding of the contemporary population. Haplotype B was present in the population as early as 1984, and haplotype A appeared in 1993 for the first time and could represent a male wolf arriving later to the population (Table 1). These results agree with those of Ellegren et al. (1996) in suggesting that the Scandinavian wolf population might be founded by as few as 3 individuals, based on their observation of one fixed mtDNA type and a maximum of 5 alleles at any of

the studied autosomal microsatellite loci. We can conclude that there might have been only one female and two males involved in the founding of the current Scandinavian wolf population. These results have more recently been confirmed in a separate study (Vilà et al. 2003).

Paper II. Combined use of maternal, paternal and biparental genetic markers for the identification of wolf-dog hybrids

When two closely related species (like dogs and wolves) hybridize, it can be difficult to identify the hybrids both by phenotypic aspect and by genetic composition. However, by combining markers with different patterns of inheritance, hybrids can be more reliably identified, and also the direction of hybridization can be determined. In this study we used three different kinds of genetic markers to identify a possible hybrid.

Material and methods

In October 1999 a suspected juvenile hybrid was killed by a car in Østfold, southern Norway (sample A). From the same area another sample (drops of blood in snow) had been collected during the previous winter (in March 1999, sample B). The individual from which sample B originated was thought to be the mother of A. These two samples were analyzed together with 25 Scandinavian wolves, 78 wolves from north East Europe (Finland, Russia, Latvia and Estonia) and 44 purebred dogs (additionally, 38 male dogs were also typed for the Y chromosome marker).

All samples were analyzed using three different kinds of markers: maternally inherited mtDNA sequences, one paternally inherited Y chromosome microsatellite and 18 biparentally inherited autosomal microsatellites.

The obtained genotypes for samples A and B were compared to the different reference populations using an assignment test (Paetkau *et al.* 1995, Paetkau *et al.* 1998, Waser and Strobeck 1998). This test gives the likelihood for the samples to originate from each of the reference populations. Synthetic genotypes were generated to simulate the diversity that could be found in dogs and wolves. These genotypes were subjected to the same assignment analyses. Since sample B showed to be likely to derive from the mother of sample A, the paternal genotype could be partially reconstructed. This partial genotype was then used to assess the origin of the father.

Result and discussion

The same mtDNA type was found in samples A and B. This was the same haplotype that is fixed in the current Scandinavian wolf population. From this we can conclude that both sample A and B derive from individuals that are either pure wolves or hybrids with wolf maternal origin. The amplification of the Y chromosome microsatellite failed for sample B, confirming that this sample probably originated from a female. The Y chromosome allele amplified in sample A had not been seen in the Scandinavian wolf population, but was present both in other north European wolf populations as well as in dogs. This suggested that the father of sample A might have an origin other than the Scandinavian wolf population.

The genotypes based on the autosomal microsatellites generated from sample A and B were compared to Scandinavian wolves and dogs in an assignment test (Figure 1). From this we concluded, first; that sample A derived from a hybrid, with Scandinavian wolf maternal origin and dog paternal origin. Second, sample B derived from an individual belonging to the Scandinavian wolf population.

The partial genotype reconstructed for the father of sample A was assigned to the dog population, confirming our previous results. The comparison with synthetic genotypes shows that sample A and B fall within the range of genotypes that could be expected for hybrids between Scandinavian wolves and dogs, and for Scandinavian wolves respectively.

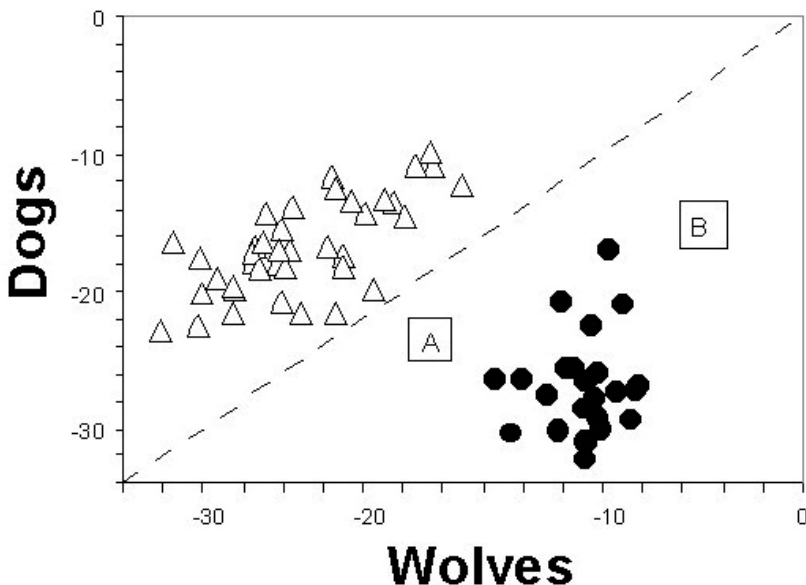


Figure 1. Log likelihood of assignment for dogs (open triangles) and wolves (black circles). The log likelihoods for the two target samples, A and B, are also indicated.

Paper III. Hybridization between wolves and dogs: impact on a wolf population

Gray wolves and dogs can hybridize and this has been observed in several wolf populations. Hybridization could affect the wolf populations in two ways: by some individuals missing the chance of mating with a member of their own species or by the introgression of maladaptive genes into the wolf population. While the first process would be important mainly in very small populations, the second one could be relevant in populations of any size. However, the existence of hybridization does not necessarily imply the existence of introgression. If the hybrids do not survive, fail to integrate themselves into the wolf population, or fail to reproduce, no introgression of dog genes into the wolf population will occur and the hybridization will have a lower impact on the population.

In this study we used three different kinds of markers (mitochondrial DNA sequences, Y chromosome microsatellites and autosomal microsatellites) with different patterns of inheritance and mutation rates, to assess introgression of dog genes into a wolf population.

Material and methods

The Spanish wolf population was selected for this study. This population represents a unique opportunity to evaluate to what degree introgression of dog genes can affect a relatively stable wolf population, in an area with a high human population density and a large number of feral and uncontrolled dogs. Our samples included wolves (170), purebred dogs (70), feral and mixed dogs (32) and canids of uncertain species affiliation, which could also include hybrids (13).

The samples were sequenced for mtDNA and genotyped 6 for Y chromosome microsatellites (only the males) and 27 autosomal microsatellites. The degree of differentiation between populations was visualized using a Factorial Correspondence Analysis (FCA) and quantified by calculating pairwise F_{ST} values (Weir and Cockerham 1984) using GENETIX 4.05 (Belkhir et al. 1996-2004). Individual assignments were performed using two different Bayesian-based methods, STRUCTURE v.2.2 (Pritchard *et al.* 2000, Falush *et al.* 2003, 2007) and NEWHYBRIDS v. 1.1 beta (Anderson and Thompson 2002). The first program provides an estimate of the proportion of the genome of each individual that comes from each species. The second program calculates the probability that an individual genotype corresponds to one of the following classes: pure wolf, pure dog, F1 hybrid, backcross to wolf or backcross to dog. To evaluate the power of NEWHYBRIDS to separate hybrids, backcrosses and pure individuals, 1500 synthetic hybrids were simulated (500 F1s, 500 backcrosses of F1s to wolves and 500 backcrosses to

dogs) and 50 additional runs was performed in NEWHYBRIDS including these simulated genotypes for which the correct class was known..

Result and discussion

The markers used in this represent a powerful tool to discriminate between wolves and dogs and their hybrids. The differentiation between wolves and dogs was large for autosomal microsatellites ($F_{ST}= 0.19$) and both mtDNA and Y chromosome had haplotypes that were species-specific.

We detected six F1 hybrids (3.4% of the wolf sample). All of these were the result of a female wolf mating with a male dog. This biased direction of hybridization could be the result of physiological differences between dogs and wolves. While wolves have a well defined mating season and are sexually inactive during most of the time, dogs can often reproduce twice in the same year, can produce pups during any month, and males show high testosterone levels during the entire year. This makes male dogs able to fertilize all female wolves while male wolves are unlikely to be sexually active at the time most female dogs are receptive (and these wolves will then have to compete with many male dogs).

The results of our simulations combined with field data indicates that only one backcross to wolf was reliable identifiable in our sample. This low number of backcrosses suggests that the fitness of hybrids is lower than that of pure wolves, or that hybridization occurs in regions were those hybrids are more likely to find dogs than wolves as mating partners. This results in a limited introgression of dog alleles into the wolf population. This suggests that, although the management plans for wolves in most European countries specify plans for the eradication of hybrids, the threat of hybridization for wolves may have been overestimated in the past..

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

Dogs were domesticated from the grey wolf at least 14000 years ago. Although morphologically differentiated types of dogs existed already 4000 years ago (Clutton-Brock 1999), modern dog breeds were probably not established until about 200 years ago. Previous studies have shown that breeds are genetically differentiable using autosomal microsatellite markers (Irion *et al.* 2003, Kim *et al.* 2001, Koskinen 2003, Parker *et al.* 2004, Zajc and Sampson 1999). However, when using mtDNA no differentiation between breeds has been found (Savolainen *et al.* 2002, Vilà *et al.* 1997). In this

study we investigated the origin of dog breeds using three different kinds of markers: maternally inherited mtDNA sequences, paternally inherited Y chromosome microsatellites and biparentally inherited autosomal microsatellites. For comparison, we also typed the same markers in wolf populations.

Material and methods

One hundred male dogs from 20 different breeds and 112 male wolves from 6 different populations were analyzed in this study. They were sequenced for a fragment of the mtDNA control region, and genotyped for four Y chromosome and 18 autosomal microsatellites. A neighbor joining tree for the dog mtDNA sequences was constructed using PAUP* 4.0b10 (Swofford 1998). The four Y chromosome microsatellites were combined into haplotypes and a network was constructed using the program TCS 1.8 (Clement *et al.* 2000). The autosomal genotype data were used to construct neighbor joining tree based on pairwise distances with PAUP*.

Furthermore, additionally mtDNA data from 430 dogs from Savolainen *et al.* (2002) and Y chromosome microsatellite data from 214 male dogs representing 89 breeds were used in an analysis of molecular variance, AMOVA, approach, as implemented in the program Arlequin 2.001 (Excoffier *et al.* 1992).

Result and discussion

A phylogenetic tree representing the similarity between the 100 male dogs, based on autosomal microsatellites markers, showed that the breeds were differentiable from each other. However, neither mtDNA nor Y chromosome haplotypes showed clear differences between breeds. Rather, the opposite pattern was observed for both markers: individuals belonging to the same breed could have very different haplotypes and haplotypes were shared between very different breeds. This pattern can be explained by the recent origin of breeds that have not yet allowed fixed differences.

Within breeds more mtDNA types than Y chromosome types were generally found. This contrasts with the situation within wolf populations, where the opposite relation was seen. Since wolves live in packs with only one breeding pair (Mech and Boitani 2003), approximately equal number of males and females are contributing genetically to the next generation. The pattern seen within dog breeds can therefore be explained by a bias in the contribution of the two sexes in the origin of dog breeds, more females than males contributing genetically to each breed.

The mtDNA and Y chromosome haplotype diversity was also compared across the breed groups recognized by the FCI (<http://www.fci.be>). This analysis showed that the groups were more differentiated from each other on the Y chromosome haplotype frequencies than they were regarding mtDNA.

This can be explained by selection strategies based on choosing individuals as breed founders: male founders are more likely to derive from a similar breed than female founders.

Paper V. Wolf or dog? Genetic identification of predator from saliva collected around bite wounds on prey

Wolf predation on livestock is a management problem in many areas. Predation can lead to control measures to limit wolf populations and it can also promote a negative public attitude toward wolves (Ericsson and Heberlein 2003, Chavez *et al.* 2005). However, wolves coexist with dogs in many areas and dogs could therefore be responsible for some attacks blamed on wolves. Since the predator is rarely seen, the identification of the predator often has to rely on traces left on the prey site, for example tracks, hair, blood and the condition of the surroundings. Although these traces usually differ from wolves and dogs (wolves being more skilful hunters) the identification of the predator is not always clear. Also for economical reasons the correct identification of the predator is important since in many areas farmers get economical compensation if their livestock was killed by a wolf, but not if killed by a dog. In this study we evaluated the possibility of genetically identifying the predator by analyzing saliva left on prey.

Material and methods

A total of eight samples were collected from two sheep that had been seriously injured in one canid attack. Also blood samples from the two sheep were taken as well as from the two shepherd dogs living in the same farm where the attack had occurred. Eight autosomal microsatellites were genotyped in all samples. For each sample and marker, three replicates were run since allelic dropout is a common problem when working with low quality DNA (Taberlet *et al.* 1996). For genotypes to be considered reliable we wanted to see heterozygote genotypes two times and homozygote genotypes three times (Hedmark and Ellegren 2006). The genotypes obtained were compared to those from Scandinavian wolves and dogs available at our Department.

A visual representation of the similarity between genotypes was generated using a factorial correspondence analysis (FCA) in GENETIX 4.05 (Belkhir *et al.* 1996-2004). The likelihood of finding other individuals with the same

genotype was estimated using the probability of identity (Paetkau and Strobeck 1994).

Result and discussion

Two of the microsatellite markers led to PCR amplification also in the sheep samples. Although the amplifications in the sheep were slightly different from those in canids, these markers were excluded from further analysis to avoid any misinterpretation.

The amplification success varied between the samples from 0% to 83%. This suggests that it is advisable to take many samples from an attack, since some of them will not contain any predator DNA.

As expected, cases of allelic dropout were seen in all markers, varying from 27% up to 69%. Since the aim of this study was to assess the degree of success using standard protocol, we did not do re-runs of the genotypes, which is advisable in forensic cases.

In none of the markers more than two alleles were seen. Furthermore, the low probability of identity for the studied loci suggests that it would be extremely uncommon to have several dogs with the same genotype over these six loci. We therefore assume that a single individual was responsible for the attack. A genotype was constructed combining data from all samples. In a FCA analysis (Figure 2) it is seen that the saliva sample clearly originates from a dog. The two dogs from the farm were clearly different from this one and could be excluded as responsible for the attack.

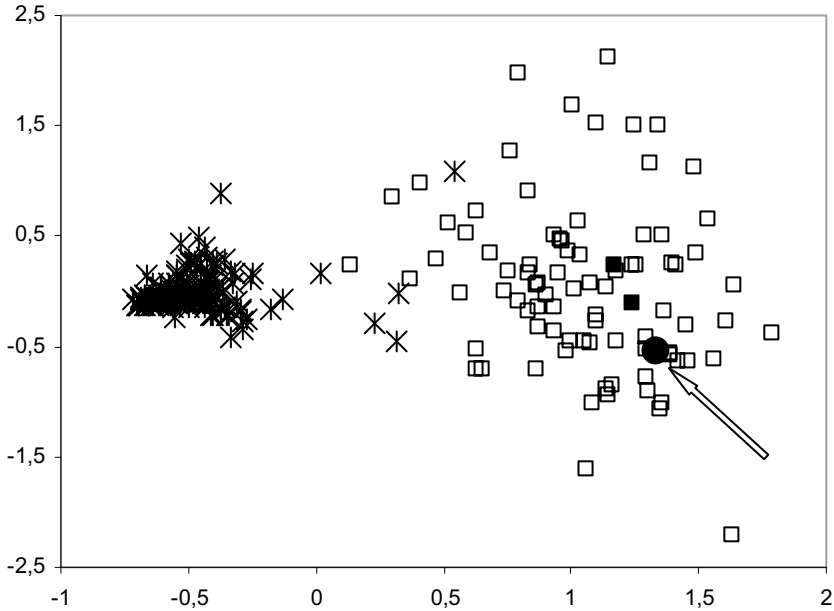


Figure 2. Factorial correspondence analysis of wolves (stars), dogs (open squares), two farm dogs (filled squares) and the saliva sample (filled circle, marked with an arrow).

Paper VI. A paternal view on the domestication of dogs

The domestication of dogs has been studied using genetic markers with different patterns of inheritance. Studies based on maternally inherited mtDNA have suggested a limited number of domestication events. On the contrary, a study based on the diversity of MHC alleles suggested extensive back-crossing between wolves and dogs. The authors hypothesized that this could be the result of male-biased gene flow. If this was the case we would expect to find a large diversity of Y chromosome lineages in dogs originating from different wolf populations.

In this study we investigate these two alternatives by studying the patterns of variation on the Y chromosome in dogs and wolves.

Material and methods

We used a panel of 10 samples (five wolves, four dogs and one coyote) to screen for polymorphism in eight Y chromosome sequence fragments pub-

lished by Natanaelsson *et al.* (2006). We found polymorphic sites (single nucleotide polymorphisms, SNPs) in four of these fragments.

A total of 463 male wolves and 362 male dogs were genotyped for four Y chromosome microsatellite markers (Sundqvist *et al.* 2001). Among these, we selected a subset of 45 male wolves and 46 male dogs containing all the haplotypes discovered with the four microsatellites. These samples were then typed for the polymorphic sites identified above. Additionally two more Y chromosome microsatellite markers (Bannasch *et al.* 2005) were also typed.

The SNP data were used to construct a network of haplogroups. Microsatellite haplotypes were then used to construct networks within each one of the haplogroups using the program TCS 1.21 (Clement *et al.* 2000). These networks occasionally had loops due to the existence of alternative evolutionary paths. To be able to determine which evolutionary path was more likely we considered the number of alleles observed at each marker. We assumed that markers with few alleles were less likely to mutate multiple times leading to homoplasy. This allowed us to select the evolutionary paths containing the smallest number of mutations in markers with low variability.

Result and discussion

Using a first group of four Y chromosome microsatellites we selected 91 samples out of a total of 825 individuals. This pre selection proved to be a good strategy to reduce the number of samples to analyze: in most of the cases where more than one sample had been genotyped for a specific haplotype, the samples still contained the same Y chromosome haplotype after including the data for the new SNPs and microsatellite markers. However, when one four-microsatellite-haplotype haplotype was shared between wolves and dogs, the addition of new genetic markers often resulted in separation of wolf and dog haplotypes.

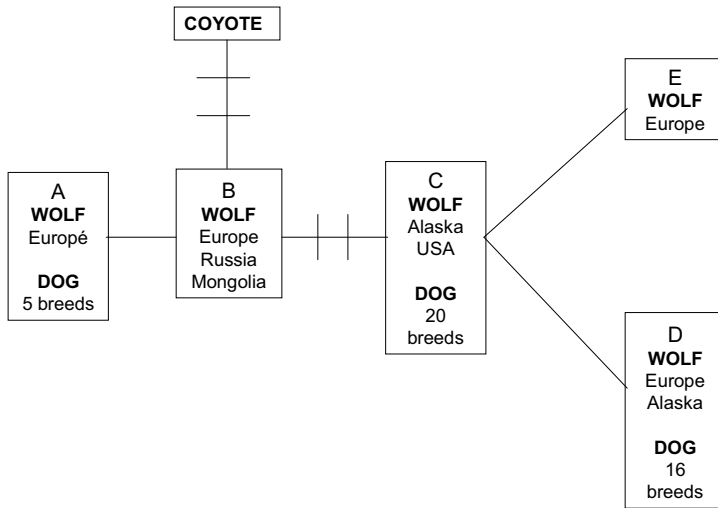


Figure 3. Network of the five SNP haplogroups (A-E) found in wolves and dogs. One coyote was also typed as outgroup.

A total of six SNPs were found in the fragments screened, which defined five haplogroups (Figure 3). Dogs were found in three of these haplogroups. This suggests that dogs have been domesticated at least three times separated in time and/or space. However, when looking at the networks based on microsatellites for each of the haplogroups, traces of two more domestication events could be detected.

Therefore, our results suggested three to five domestication events, supporting the view provided by previous mtDNA studies indicating a limited number of domestication events (Vilà et al. 1997, Savolainen et al. 2002). Introgression and backcrossing from the wild has probably not been as extensive as suggested by MHC studies (Vilà et al. 2005). Alternatively, this backcrossing has been limited in space and only has involved a few populations of wolves.

Concluding remarks

Wolf management is a complex issue since humans worldwide tend to have strong feelings about wolves. These feelings and attitudes can be positive as well as negative. Molecular genetics is an important tool in management and conservation in cases like this, since it aims at gathering objective facts about the wolves, both at the species and population levels, and also on an individual level. This information can then be used in management programs that include promoting the long term survival of the wolf. Wolves are dealing with a number of threats to their long-term survival.

This thesis has been focusing on the genetic aspect for some of these threats. We have used genetic tools to estimate the number of male founders in the Scandinavian wolf population. This population was founded by a very limited number of individuals in the early 1980s. Establish the exact number of founders and their origin is important to allow the correct management of this population avoiding the harmful effects associated with myths and hearsays.

We have also used genetic markers with different pattern of inheritance for a more reliable identification of hybrids. By using these set of markers we were also able to address more specific questions regarding hybridization: direction and effects of hybridization in wolf populations.

We have also developed a method for a more reliable identification of predator by using saliva remains left on prey. By using this method it is not only possible to distinguish wolves from dogs as predator, but it should also allow identification of their hybrids. This is an important issue in management of wolves since wolves can be blamed for attacks caused by dogs.

Finally we have been studying the domestication of Man's best friend, the dog. This process started at least 14 000 years ago and has resulted in all the dog breeds that we see today. By looking at this event using Y chromosome markers (that are paternally inherited), we have been able to complement previous studies of the domestication process that were based on mtDNA sequences (that are maternally inherited). In this way a more accurate picture of the domestication has been obtained. Finally, we have looked into the genetic origin of dog breeds. This was done by combining genetic markers with different pattern of inheritance. This has shown that there has been a bias in the contribution of the two sexes in the origin of dog breeds (fewer males than females contributing to each breed).

Svensk sammanfattning

Bakgrund

Bevarande och skötsel av vår natur är ett komplicerat område som inte alltid går hand i hand med människans intressen och resurserna som vi har tillgängliga på vår jord förbrukas snabbt. Det har bland annat fått som följd att många olika arter behöver hjälp för att kunna överleva. Bevarande genetik kan definieras som ”teori och praktik av genetik för bevarande av arter som dynamiska enheter, som kan utvecklas i samspel med miljön för att minimera risken för utrotning” (Frankham *et al.* 2002). Det innebär att det inte bara är viktigt att några få individer från en viss art ska kunna överleva, utan att det även finns tillräcklig genetisk variation inom arten, för att den ska kunna utvecklas i förhållande till sin miljö, som ständigt är föränderlig.

Det finns sannolikt inget annat djur som väcker så mycket känslor som vargen, både positiva och negativa. Vargen har en stor anpassningsförmåga vilket gör att den är det däggdjur som har störst naturlig utbredning. Redan på medeltiden började vargen jagas och förföljas, och den har i dag blivit utrotad från stora delar av dess ursprungliga områden. I dag uppskattar man att det finns ungefär 200 000 vargar i världen.

Människans bästa vän, hunden, har domesticerats från vargen. Hunden var det första djur som domesticerades och det skedde för minst 14 000 år sedan. Den processen har givit upphov till alla de olika hundraser som finns idag. Den enorma variation, både i utseende- och beteende, som finns bland dagens hundar, kan man inte återfinna hos någon annan djurart.

Målet med denna avhandling har varit att:

- Utveckla genetiska markörer på vargens och hundens Y-kromosom.
- Använda genetiska tekniker för att identifiera hybrider, samt studera effekten av hybridisering hos vargpopulationer.
- Använda genetisk information från vargpopulationer för att i sin tur förstå hur bildandet av hundraser gått till.
- Utveckla en metod där man genom salivrester på bytesdjur kan se om rovdjuret var en hund, varg eller hybrid.

Nedan följer en sammanfattning av mina studier.

Artikel I. Y-kromosomvarianter hos skandinaviska vargar (*Canis lupus*), baserat på mikrosatelliter.

Mitokondriella DNASEkvenser har traditionellt använts till många olika genetiska studier. Eftersom detta DNA enbart nedärvs från modern till avkomman kan man få en skev bild av historien. Genom att komplettera tidigare studier baserade på mtDNA med Y-kromosom markörer (som nedärvs från far till son) kan man få en bättre bild av verkligheten. I den här studien utvecklade vi fyra mikrosatelliter på vargens Y-kromosom. Dessa använde vi sedan för att studera den genetiska statusen hos den skandinaviska vargpopulationen. Vargen ansågs vara utrotad från Skandinavien på 1970-talet. I början av 80-talet dök det upp ett fåtal individer i Värmland, vilka alla dagens vargar i Skandinavien härstammar ifrån.

Hos de skandinaviska hanvargarna hittades två olika Y-kromosomvarianter. Detta tyder på att minst två hanvargar har deltagit i grundandet av dagens skandinaviska population. Ytterligare genetiska studier (Vilà *et al.* 2003) har senare bekräftat detta och även fastslagit att det totalt var enbart tre individer som grundat den skandinaviska vargstammen, två hanar och en hona. Dessa individer hade sitt ursprung i den finska vargpopulationen.

Artikel II. Identifiering av varg/hund hybrider baserat på genetiska markörer med olika nedärvning.

När två närbesläktade arter som varg och hund hybridiserar så kan det vara svårt att, baserat på utseende men även med hjälp av genetiska metoder, fastslå om en viss individ är varg, hund eller hybrid. I den här studien använde vi oss därför av tre olika delar av genomet, som nedärvs på olika sätt: mitokondriellt DNA (nedärvs från mor till avkomma), autosomala mikrosatelliter (nedärvs från både mor och far till avkomma) samt en mikrosatellit på Y-kromosomen (nedärvs från far till son). Genom att använda oss av dessa tre olika markörer så kunde vi fastslå att en misstänkt hybrid som omkommit i samband med en trafikolycka, verkligen var en hybrid. Vi kunde även fastställa att mamman till denna individ var en varg tillhörande den Skandinaviska vargstammen samt att pappan var en hund.

Artikel III. Hybridisering mellan vargar och hundar: genetiska konsekvenser hos vargarna.

Vargar och hundar kan hybridisera och detta har observerats i ett flertal vargpopulationer. Detta kan påverka vargar på åtminstone två olika sätt: några individer missar tillfället att para sig med rätt art och gener från hundar kan spridas inom vargpopulationer. Om hybridisering sker, men hybriderna inte lyckas beblanda sig med vargarna och i sin tur få någon avkomma, så kommer heller inte några gener från hundar att kunna komma in i varg populationen. I denna studie använde vi oss av tre olika typer av genetiska markörer med olika nedärvning och mutationshastighet för att kunna bedöma i vilken utsträckning gener från hund har spritts i den spanska vargpopulationen. Den spanska varg populationen finns i ett tätbefolkat område med många lösspringande och boskapsvaktande hundar, således borde det finnas gott om tillfällen för hybridisering att äga rum.

Våra resultat visade att 3.4% av den spanska vargpopulationen utgörs av hybrider. Med tanke på att vargar i detta område lever nära inpå hundar är det en förhållandevis liten andel. Vi kunde bara se något enstaka fall där en hybrid i sin tur parat sig med en varg och fått egen avkomma. I den här relativt stabila vargpopulationen verkar således hybridisering med hund inte vara ett stort genetiskt problem.

Artikel IV. Ojämn könsfördelning vid bildandet av hundraser.

Hundar domesticerades från vargar för minst 14 000 år sedan, antagligen ännu tidigare. Även om det fanns hundar av olika typer redan för ca 4000 år sedan, så är de hundraser vi ser idag ett väldigt modernt påfund. De flesta raser bildades för ca 200 år sedan. För att studera hur rasbildningen gått till analyserade vi både hundar och vargar. Återigen använde vi oss av markörer med olika nedärvning: mitokondriellt DNA, autosomala mikrosatelliter, samt mikrosatelliter på Y-kromosomen.

Resultaten visade att färre tikar än hanar använts vid bildandet av varje ras. Detta skiljer sig således från vad man ser hos vargarna, där lika många tikar och hanar bidrar till nästa generation. Vidare kunde vi se att hanar inom rasgrupper är mer lika än tikar. Förklaringen kan vara att vid bildandet av nya raser har högre selektion lags på hanar, som har valts från raser med liknande användning.

Artikel V. Varg eller hund? Identifiering av rovdjur genom genetisk analys av saliv från bitskador på bytesdjur.

Vargattacker på tamdjur är ett stort problem i många områden. Eftersom det även finns hundar i de flesta områdena, så kan en del attacker som vargarna beskylls för, i själva verket vara orsakade av hundar. I den här studien utvärderar vi en metod för att utvinna DNA från saliv, lämnat av hunden eller vargen invid bitsår på bytesdjuret, för att genom genetiska studier fastslå om förövaren var en varg, hund eller hybrid.

Från salivrester på två allvarligt skadade får lyckades vi utvinna DNA av tillräckligt bra kvalitet för att genomföra en genetisk analys. Den genotyp vi fick fram jämfördes med data från Skandinaviska vargar och hundar. Från denna jämförelse kunde vi se att förövaren i detta fall var en hund.

Artikel VI. Hundens domesticering baserad på hanarnas historia.

Domesticeringen av hundar från vargar har tidigare studerats med hjälp av mitokondriellt DNA (mtDNA), som nedärvs på mödernet, och dessa studier har föreslagit att domesticeringen skett vid endast ett fåtal tillfällen. Motsatsen till detta har dock indikerats vid analyser av nukleära markörer, då istället ett utbrett genetiskt flöde mellan arterna föreslagits. Detta stora genetiska utbyte skulle i så fall ha kunnat skett genom att hanvargar återkommande parat sig med hundtikar. För att kunna få en bättre bild av domesticeringsprocessen studerade vi den genetiska variationen på Y kromosomen hos vargar och hundar.

Våra resultat visar på tre till fem domesticeringstillfällen. Detta tyder på endast ett fåtal vargpopulationer har legat till grund vid domesticeringen, så som föreslagits tidigare baserat på mtDNA. Vi kunde inte heller se några spår av ett genetiskt utbyte mellan hanvargar och hundar, efter att domesticeringen ägt rum.

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