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History and Genetic Parameters of Český Fousek

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Declaration

I declare I wrote this study, History and Genetic Parameters of Český Fousek, by myself just using cited literature.

In Prague, 19th of April 2013

……………………….
Signature

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Abstract

The thesis deals with the issue of genetic variability of Český Fousek (CF), unique Czech national dog breed. CF was carefully selected for the purpose of the thesis due to its exceptional history. It is one of the oldest pointers. Although CF was nearly extinct during the World War I., it was successfully revitalized afterwards. Nowadays, CF presents one of the very few dog breeds that maintain lineage breeding. That is important in order to sustain set of selected characteristic features. On the other hand, it may also lead toward to inbreeding. We decided to use CF as a model example of lineage breeding. Furthermore, our results should be applicable to other animal breeds, which are usually bred in a similar way. Our goal was to compare breeding data with various genetic criteria. In particular, CF was compared with German Wire-haired Pointer (GWP) as well as with German Short-haired Pointer (GSP). The reason for such choice was that both above-mentioned breeds were originated from CF and after that those breeds were used for regeneration of CF. In total, 206 samples were taken. 160 of them were taken from CF, 24 and 23 from GWP and GSP respectively. 19 microsatellite loci were used during the analysis. Results of heterozygosity were almost identical for all breeds. Inbreeding coefficient was higher for CF than for other breeds. Only CF breed was in disequilibrium according Hardy-Weinberg principle due to significant excess of homozygotes. Total outcome is not seriously alarming and CF is not threat of inbreeding depression. All breeds are genetically very close, share same alleles and there is visible gene flow effect. Especially strong similarity was found in CF with GWP breed. Also there was detected strong interior population structure in CF that surprisingly did not corresponded with each lineage. Nowadays some genetic lineages are crossbreeding individuals among themselves and thus maintain higher genetic variability.

Key words: microsatellites, genetic variability, inbreeding, Bohemian Wire-haired Griffon, Český fousek

Abstract

Tato práce se zabývá genetickou variabilitou českého národního plemene psa Český Fousek (CF). Plemeno CF bylo vybráno pro jeho výjimečnou historii. Jedná se o jednoho z nejstarších ohařů, avšak během první světové války plemeno téměř zaniklo a poté muselo být znovu obnoveno. Nyní jako jedno z mála psích plemen udržuje liniovou plemenitbu, která je výhodná pro udržení selektovaných znaků, avšak může vest ke zvýšenému příbuzenskému křížení. Toto plemeno jsme použili jako modelový příklad liniového chovu a našim cílem je vyvodit závěry, využitelné při chovu jiných plemen, které jsou šlechtěny stejným způsobem. Cílem výzkumu bylo porovnat šlechtitelská data s různými genetickými parametry. Plemeno CF bylo porovnáno s německým drátosrstým ohařem (NDO) a německým krátkosrstým ohařem (NKO), protože na jejich vzniku se v minulosti podílelo i plemeno CF a naopak jimi, bylo plemeno při revitalizaci chovu doplněno. Bylo odebráno celkem 206 vzorků z toho 160 CF, 24 GWP and 23 GSP. Pro analýzu bylo použito 19 mikrosatelitových lokusů. Hodnoty heterozygotnosti byly téměř shodné pro CF, GWP a GSP. Koeficient inbreedingu byl vyšší u CF než u dalších dvou plemen. Podle Hardy-Weinbergova pravidla pouze plemeno CF nebylo v rovnováze, byl zde nalezen signifikantně vyšší počet homozygotů. Ovšem výsledek není nikterak alarmující a plemeno CF není vážně ohroženo inbrední depresí. Dnes se některé genetické linie kříží mezi sebou a tak se udržuje vyšší genetická variabilita. Plemena a zvláště CF a GWP jsou si velmi geneticky blízká, sdílejí mnoho alel a je zde patrný genetický tok. Byla zjištěna silná vnitropopulační struktura u CF, která však překvapivě nekorespondovala s jednotlivými liniemi.

Klíčová slova: mikrosatelity, genetická variabilita, inbreeding, Český Fousek

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1 Introduction

Bohemian Wire-haired Pointing Griffon, mostly called Český Fousek or Cesky Fousek (CF) is one of seven Czech national breeds. This breed is important and has a long tradition in our country as a gundog for hunting. History of the breed is very interesting. First reference is dated to 1348 (Kuhn, 2005). CF is probably one of the oldest wire-haired pointers in Europe. CF was involved in formation of other European wire-haired breeds, but after WWI CF almost went extinct. The breed was regenerated mainly by German Wire-haired Pointers - due to low numbers of CF dogs which can be used for breeding. The population of CF might be interesting object for genetic research because of this huge bottleneck and crossbreeding with other pointers. Moreover population within CF has eight genetic lineages and strict breeding management. Nowadays, popularity of CF increases among gamekeepers in the Czech Republic. CF is ideal for our climatic and landscape conditions so this is the reason for his frequent using. He is able to work with hunter in cold weather in the field or in water because of rough coat and obedient character. CF is also popular abroad, for example in Germany, USA, Canada and New Zealand (Dostál, 1998; Kuhn, 2005).

1.1 Aim

This work aims to be an model research for investigation of various genetic parameters of specific group of breeds and their histories. CF breed was used as a model organism for a different type of studies about national breeds that are all over the world and need to have controlled breeding program. Important questions for all population of animals such as estimating of heterozygosity, inbreeding and possibility of bottleneck will be discussed in this work. The aim of the thesis is to: (1) confirm origin of CF, (2) evaluate degree of genetic variability and inbreeding depression of the breed and of each eight genetic lineages within CF, (3) measure gene flow between CF and wire-haired pointers. CF is compared with German Wire-haired Pointer (GWP) and German Short-haired Pointer (GSP) at various genetic parameters. After that their genetic analysis could be compared with historical data and phylogenetic documents.

2 Theoretical background

2.1 *Reason for examining genetic variability and inbreeding*

Animal breeding is a human activity with a long tradition. For scientific purposes, breeding begins to form in England in the 18th century (Parra et al., 2008). At that time, breeders began breeding of horses, cattle, pigs and sheep with known origin. Animals which are well-known till now were originated by these breeding methods. It is no wonder that the dog breeding was at a high level in England even higher than anywhere else in the world. First dog exhibition took place in England in 1859. Kennel Club, which is the breeder's organization of pure breed dogs, originated also in England in 1873 (Dostál, 2007).

Genetic variability may measure proportion of genotypes in a population. We can see how much one population is separated from another. Genetic variability within and among a populations is fundamental for biodiversity and evolution. Genetic variability is important for a population to adapt on environmental changes and save the population against extinction. Genetic variability is source of breeding progress for breeders. Nowadays genetic is getting to be the fastest developing biological science (Beebee and Rowe, 2008).

Many wild animal species suffer from lack informative genetic markers for analysing genetic variation and structure, which is essential for effective long term conservation and management.

Nowadays, examining of genetic variability of different species (livestock, pets or wildlife) is getting more available and it should be use more often for animal conservation (Eblate et al., 2011)

The modern dogs have a distinct population structure with hundreds of genetically isolated breeds, widely varying in morphological and behavioural characteristics (Lindblad-Toh et al., 2005). Selective breeding in domestic dogs is likely to have contributed to the large variety of phenotypic variation seen in various dog breeds. Mating among related individuals has often been used in dog breeding to establish new breeds or to fix desired characteristics (Lupke and Distl, 2005).

Most canine breeds were developed during the 19th century and therefore they are considered to be recent. In comparison to other domestic animals, strict selection in dogs has resulted in extremely diverse breeds. The strong selection pressure that was applied to develop the different breeds has led to increase of genetic variation. Dogs were breaded for specific use or visual aspect (Irion et al., 2003).

Since the development of genetical tools in population biology, the problem of inbreeding plays a special role in the theoretical value and important practical use in inbreeding. Inbreeding is mating of closely related individuals. Inbreeding is widely used for breeding because it allows fixing in the characteristics and quality of the best representatives of a breed (Shinkarenko et al., 2008).

Inbreeding can cause a loss of genetic variability or there is possibility of accumulation of harmful alleles. Subsequently fitness of individual may be reduced. On the other hand, when inbreeding is combined with selection, it may also reduce harmful alleles. In the population with low genetic variability may happen to select certain alleles (some alleles completely disappeared) especially when the population lives still in the same conditions for a long time therefore the genetic variability in that population decreasing. The population become uniform. This effect happened very often during wars (Lindblad-Toh et al., 2005).

Inbreeding also occurs naturally. A feral colony which is isolated from other population, by geographical or other limits, can become inbred, especially if a dominant male mates with his sisters, then with his daughters and grand-daughters. When he is deposed, his own son or grandson can comes on his place, therefore continues the inbreeding. The effect of harmful alleles may become visible in later generations as the majority of the offspring inherit these alleles (Menotti-Raymond and O'Brien, 1993).

The Mexican grey wolf appears to be extinct in the wild and exists now only in captivity and some reintroduced population in Arizona and New Mexico. Recent study did not find any inbreeding depression in captive animals. However new extensive research found that captive wolves with little or no known inbreeding had lower body size than wild-caught wolves. The captive population was descended from three founders until two other lineages, each descended from two founders, were recently added to the population. The differences in body size between these groups was evident (Fredrickson and Hedrick, 2001**)**

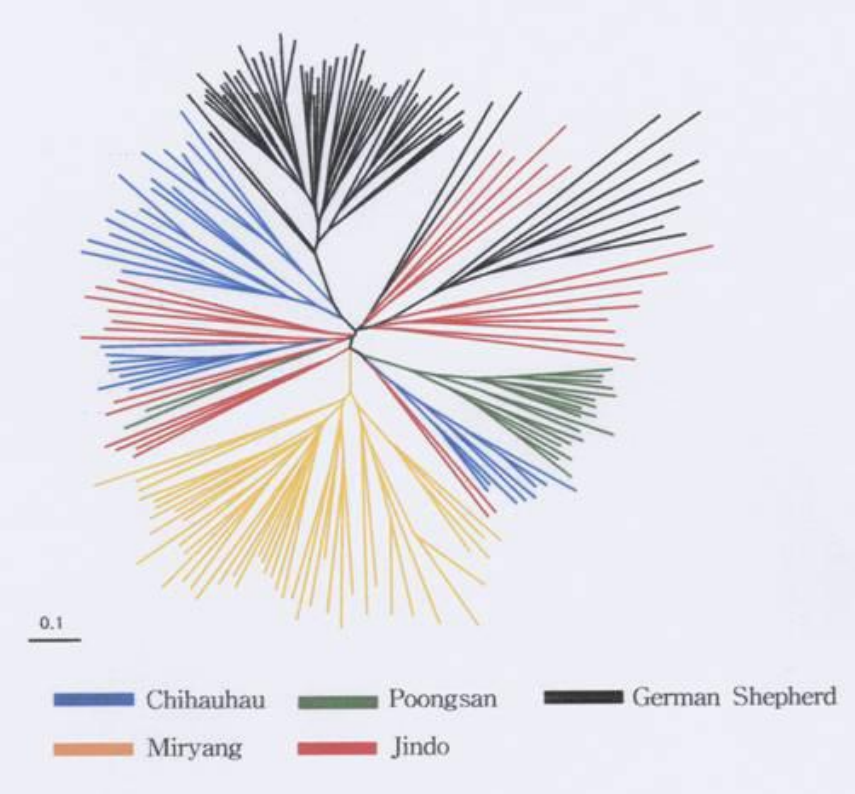
Nowadays more and more hereditary diseases have been identified in purebred dogs. Management of genetic variation has become a major concern for people involved in dog breeding. Also Kennel clubs are interested in parameters that evaluate the genetic variability in order to make decisions about selection, especially because inbreeding is sometimes considered as a selection method (Leroy et al., 2007).

It is reported that the dog has 20,439 genes, which is less than human has. These genes account only 5% of the entire DNA (Starkey et al., 2005). The greater part of DNA does not encode anything. The number of genes is not probably final. Further studies will certainly bring other discoveries. The dog is only the fifth mammal from 5500 species which scientists analysed. Only 0.2% of the DNA is a very conservative component which was found in all animal species and the component does not encode any protein structure. If it has been stated that the entire dog genome is known the, but the truth is that 99% of DNA was analysed. There are some sections where repetitive sequences are and it may be hard to distinguish the length of them. Currently it has been discovered and described the 2,5 million of different polymorphisms in dogs. Polymorphisms are changes (variations) in the composition of DNA, which may or may not be a cause of phenotypic variation. This could be the reason that we have more than 400 different breeds and the huge variety of dogs in exterior, behaviour, size and working ability. Also may be responsible for the development of hereditary diseases and defects (Starkey et al., 2005).

2.2 What is known about genetic variability of dogs?

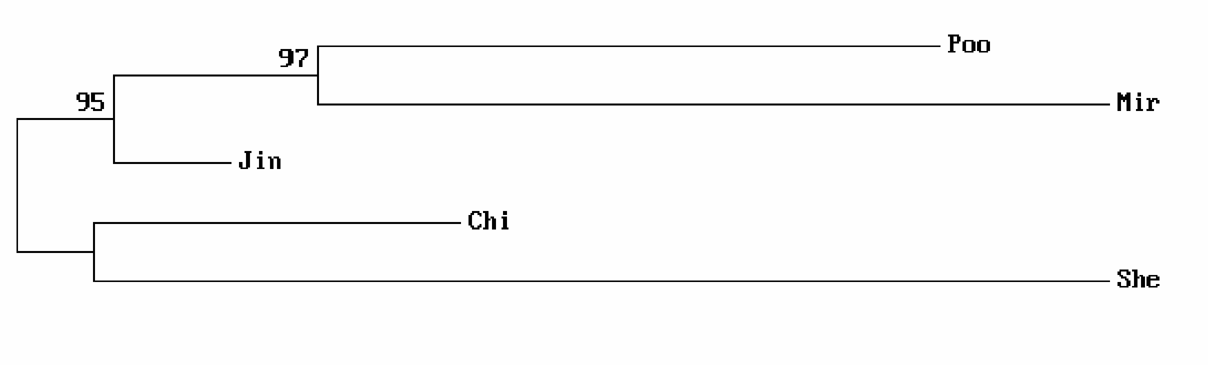
Lupke and Distl (2005) did a research on genetic variability of the Hanoverian Hounds in Europe. They have been bred in a small population. This breed suffered from decrease of individuals involved in a breeding program during both world wars. They analysed blood samples from 92 dogs using a set of 16 microsatellites. Observed heterozygosity (0,45-0,85) was slightly higher than the expected heterozygosity (0,47-0,87). Genetic variability was surprisingly higher than in previously published manuscript which was about dog breeds of similar population size. (Koskinen and Bredbacka, 2000). Another example of a small dog population with high heterozygosity was shown by Irion et al. (2003). It may be due to a large genetic variation of the founder animals of this breed (Lupke and Distl, 2005). Cho (2005) tested genetic relationship among 183 dogs of following breeds: native Korean Jindo dogs, Poongsan and Miryang dog, Chihauhau and GermaSheperd dog, Figure 1.

Figure 1 Radial dendrogram constructed from simple allele sharing statistics among 183 individuals representing five dog breeds (Cho, 2005)



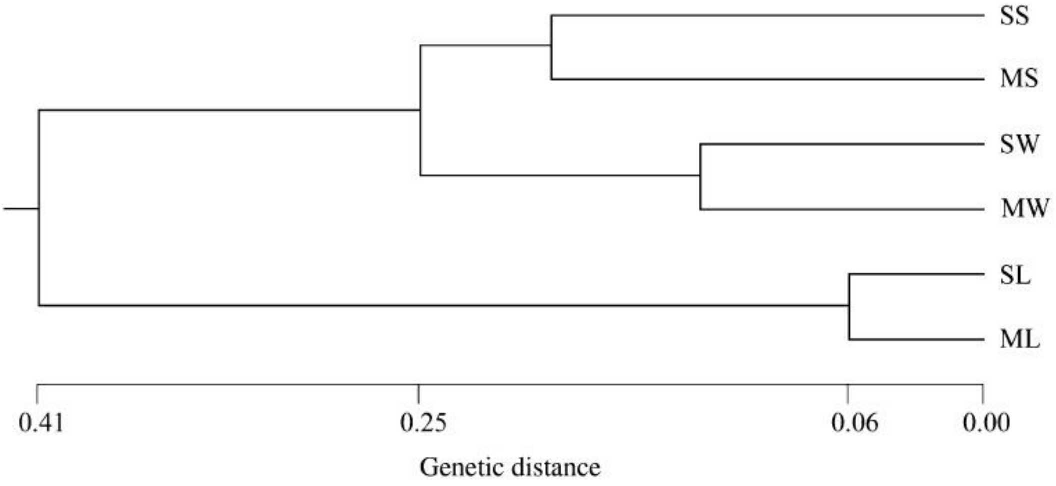
Cho (2005) used 11 microsatellite loci. Jindo dogs showed the highest expected heterozygosity (0,79). Phylogenetic tree showed two distinct clusters, Korean native dogs (Poongsan, Miryang, and Jindo) and other dog breeds (Chihauhau and German Shepherd). They show that Poongsan dog and Miryang dog are closely related to each other when compared with Jindo dog (Figure 2).

Figure 2 Neighbor-joining trees by genetic distances (Cho, 2005)



The studied loci were more polymorphic in Jindo dogs than in other dog breeds and they proved that Jindo dogs (Cho, 2005) had the largest amount of genetic variation of all the populations studied. These results supports the hypothesis that Miryang dogs and Poongsan dogs are offspring from the ancestral populations of Jindo dog. Přibáňová et al. (2009) analysed genetic variation of 632 Dachshunds (DachshuStandard Smooth-haired, Standard Long-haired, Standard Wirehaired, Miniature Smooth-haired, Miniature Long-haired and Miniature Wire-haired). They used 10 microsatellite loci. Average expected heterozygosity on each loci varied between 0,58-0,70 and observed heterozygosity between 0,57-0,64. Other analyses showed that Standard Dachshunds shared allele frequencies most closely with their miniature equivalents, and smooth coat type dog is closer to Wire-haired than to the Long-haired dachshund. Relation among the dogs are visible in Figure 3 (Přibáňová, 2009).

Figure 3 Neighbour-joining dendrogram showing the genetic relationship among six Dachshund varieties based on genetic distances. Dachshund varieties: SS, Standard Smooth-haired; SL, Standard Long-haired; SW, Standard Wire-haired; MS, Miniature Smooth-haired; ML (Přibáňová et al.., 2009)



Similar study was performed by Leroy et al. (2009) on genetic variability of different dog’s breeds kept in France. A total of 1514 dogs (61 dog breeds) were genotyped using 21 microsatellite markers. The average coefficient of inbreeding ranged from 0,2% to 8,8%. The mean value of expected heterozygosity was 0,62 over all breeds. It was concluded that special attention should be concentrated on three breeds, especially Bull terriers with low average heterozygosity (0,37). It is generally stated that small population sizes suffer from high inbreeding and low heterozygosity values and allelic richness. On other hand Shinkarenko et al. (2008) did a research about genetic variability of 27 individuals of American Pit Bull Terriers. They used only 10 microsatellites loci. Dogs came from two genetic lineages that were separately breaded for 15 years. They found high inbreeding coefficient (43%). On the contrary the inbred dogs in this work are characterized by a high polymorphism and high heterozygosity level, which was really unexpected for such inbreeding coefficients and small population size (Shinkarenko et al., 2008). Pires et al. (2009) published study about native dogs breed in Portugal, Spain and Africa. He analysed non-cosmopolitan breeds and local populations. Portuguese native dog breed and other peripheral dog populations were analysed using 16 microsatellites. Expected the level of breed differentiation detected is below that of other dog breeds. Bayesian clustering methods showed an average of 73,1% of individuals were correctly assigned to the source populations.. Genetic diversity of stray dogs was also evaluated and there is no evidence that they are a threat to the preservation of the gene pool of native dog breeds (Pires et al., 2009).

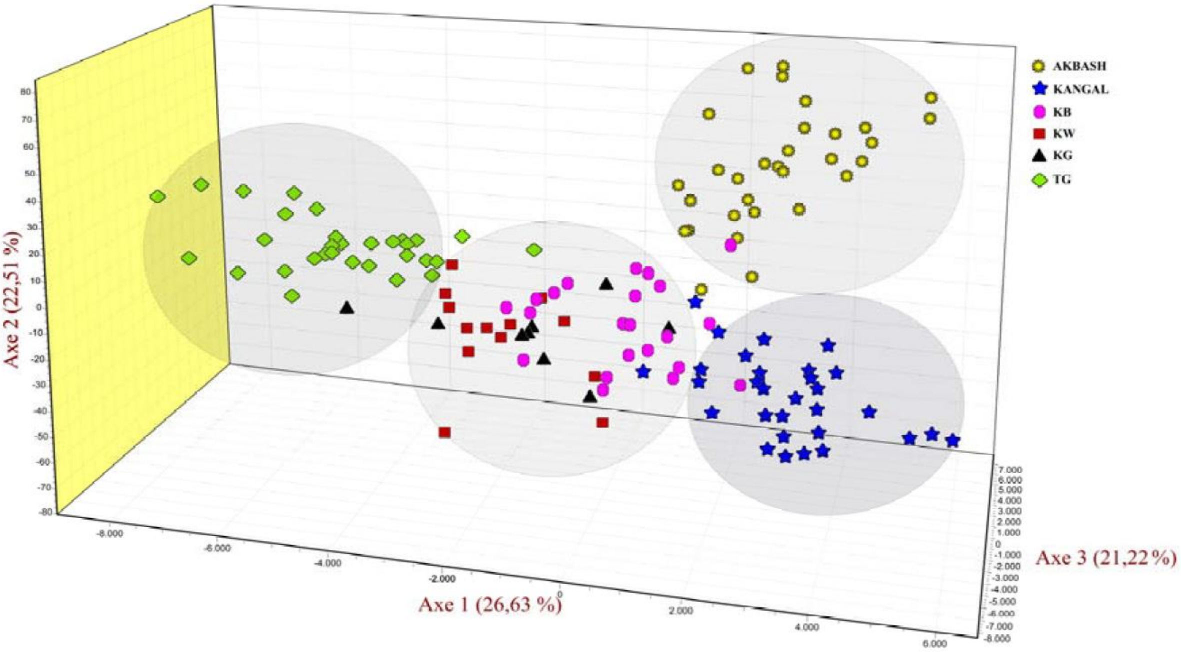
Really extensive research about genetic diversity of worldwide dog’s variability completed Mäki (2010). They collected 28 668 samples of Nova Scotia Duck Tolling Retriever (NS) and 4 782 of Lancashire Heeler dogs (LH) samples. The genetic diversity in the reference population was explained by two ancestors in the NS and five in the LH. Average inbreeding coefficients in the reference populations were 0,26 in retriever and 0,10 in heeler dog. Because of low genetic variability, crosses with other breeds are needed. Dogs with low kinship coefficients are valuable breeding dogs, because they may have new different alleles. In conclusion, both-breeds have very low genetic diversity. To increase genetic variation, a larger proportion of the dogs should be used in reproduction. Accurate breeding management is very important in this case (Mäki, 2010).

Campoliny et al. (2011) did a study about genetic variability of Bracco Italiano dog breed. This breed has similar historical background as CF. It is very old pointing breed used for hunting. The breed suffered from incorrect selection for hunting abilities and breeding management during 19th century and mainly during wars. Luckily, the breed was revitalized after the Second World War. Researchers collected 72 blood samples and used 21 microsatellites. Expected heterozygosity ranged from 0,44 to 0,81 (mean 0,64) and inbreeding coefficient was 0,406. There was a high genetic similarity within the whole population, it mean the high homogeneity of dogs. Same advice as Mäki (2010) gave in his research was declared in this publication. These results indicate the need for a careful genetic management of the population to avoid the risk of inbreeding depression and low genetic variation.

Thai Ridgebacks and Bangkaew dogs are probably only breeds originate in Thailand. Both breeds came from small population of founders. This can lead to possible inbreeding depression. They use 12 microsatellite markers in the research. Expected heterozygosity (He) was 0,72 and observed heterozygosity (Ho) was 0,77, in Bangkaew. In Ridgebacks He was

0,81 and Ho 0,78 which is much higher than the researchers expected. Also inbreeding coefficient was low. The final result showed that both breeds do not have inbreeding problems. Nowadays, breeders are better informed about inbreeding problems and most of them avoid mating with close relatives. However, with the very high kinship value, it will not be possible to maintain the inbreeding level steady in the long term (Phavaphutanon and Laopiem, 2011). Erdogan et al. (2013) studied native breeds in Turkey. They investigated 141 blood samples of Turkish Shepherd Dogs and Turkish Greyhounds (six breeds). Kangal Shepherd Dogs, Akbash Shepherd Dogs, White Kars Shepherd Dogs, Black Kars Shepherd Dogs, Grey Kars Shepherd Dogs and Turkish Greyhound. There were used 20 polymorphic loci (17 microsatellites and 3 proteins). Dogs were chosen as non-related to each other and as individuals, which are best representing their breed characteristics. The average observed heterozygosity values range between 0,64-0,77 Shepherd dogs breeds and Greyhound about 0,85. The mean heterozygosity values calculated for each breed were not statistically significant. The Fis, Fit and Fst values, calculated from all loci, are 0,085; 0,083 and 0,160. Multi-locus Fst values could be explained by breed differences and the remaining 98,08% by differences among individuals. All breeds were separated in clearly different clusters. These results show that Akbash and Kangal Shepherd dogs are different populations (Figure 4) Therefore, the generalized grouping of Turkish shepherd dogs into a single breed called Anatolian or Turkish shepherd dogs is incorrect (Erdogan et al., 2013).

Figure 4 Genetic relationships among indigenous dog breeds in Turkey.). Kangal Shepherd Dogs, Akbash Shepherd Dogs, White Kars Shepherd Dogs (KW), Black Kars Shepherd Dogs (KB), Grey Kars Shepherd Dogs (KG) and Turkish Greyhound (TG) (Erdogan et al., 2013)



Another study (Galiardi et al., 2011) of genetic variability of native breed was about Cimarron Uruguayo dogs. It is the only native breed of Uruguay used for hunting and working with cattle. They used 9 microsatellite markers to analyse the genetic polymorphism, heterozygosity and genetic variability of 30 dogs. The mean heterozygosity value was 0,649 which can be considered as high even that only nine microsatellites were analysed. Compared with data from other breeds, the results indicate that Cimarron Uruguayo dogs have high genetic diversity. According to the opinion of researchers, this breed is relatively new, it was not defeated by selective pressures as older breed (Galiardi et al., 2011).

Shariflou et al. (2011) analysed 32 registered dog breeds in Australia, containing breeds with very small population (120 Central Asian shepherd dogs) to very large population (252,521 German shepherd dogs). Data were provided by Australian National Kennel Council. The majority of registered breeds that were sampled did not use close breeding in their kennels. Inbreeding coefficient calculated from Australian pedigree was lower than 5%. Mean inbreeding coefficients of Australian breeds ranged from 0% (Central Asian shepherd dog) to 10,1% (Bichon). Breed effective population sizes ranged from 26 (Ibizan hound) to 1090 (Golden retriever), comparable with other breeds of domesticated animals. The low levels of inbreeding suggest that the hereditary diseases are caused by frequent using of popular males or close breeding in Australian registered dog breeds. Observed values for breed’s mean inbreeding coefficient were lower than in research made by Mäki (2010), who analysed international pedigrees of rare breeds. However similar as research from Calboli et al. (2008) and Leroy et al. (2009). It is possible that harmful allele fixation might be caused by founder effects, genetic drift or wrong selection methods, which were not evaluate in this analysis (Shariflou et al., 2011).

2.3 News about dog domestication and breeds origin

Archaeologists have favoured a date of 14,000-15,000 years before present (BP) for canine domestication (Lindblad-Toh et al., 2005). Von Holdt et al. (2010) traced back the origin of domestication of the dog (*Canis lupus familiaris*) from the grey wolf (*Canis lupus*) at least 15,000 years BP, but possibly as far back as 31,000 years BP (7,000-15,000 generations) and has been referred to as the first genetic bottleneck in dogs (Lindblad-Toh et al., 2005; Von Holdt et al., 2010).

However, the molecular biologists staunchly maintain that 14,000 BP date is wrong and that the dog has a more ancient historical beginning dated at 135,000 BP (Vila et al., 1997). Their assumption is based on mitochondrial DNA analyses of control regions . Geneticists have further hypothesized that dogs originated from wolf ancestors based upon the number of substitutions observed in dog and wolf haplotypes. However, the conflicting of theories of canine domestication have given rise to numerous questions. The dog family is sometimes considered as representative of the superfamily, but the Canidae is the most phylogenetically distinct family, diverging from other carnivores over 50 million years ago (Wayne and Ostrander, 1999).

There are two opinions about the dog origin. According to first one the dog comes only from a wolf, a second opinion is based on the fact that the dog comes from multiple ancestors, which may not only be a wolf, but dingo, jackal, coyote or other extinct canines. Dingoes show many wolf characteristics. Like the wolf and unlike the dog, dingoes do not bark. Great variability of the wolf leads the scientists to the idea of monophyletic origin of the domestic dog (Parker, 2007).

Charles Darwin believe in phenotypic diversity of dog breeds and more than one ancestor like Konrad Lorenz judged also that the dog may have come from the wolf and jackal by the observed behaviour (Lorenz, 1975). Molecular genetic data clearly support the origin of the dog from the wolf (Larson, 2012). Dogs have alleles of proteins in common with wolves, share highly polymorphic microsatellites and have similar or identical sequences of mitochondrial DNA. Much more controversial is the question of number, timing and location of the domestication process (Roy et al., 1994).

Middle East and Europe are the locations where were the first findings that are 14 000 years old. Several findings in North America were estimated to 8000 years old. Morphological comparisons show that dogs are closest to the Chinese wolf (Wayne and Ostrander, 1999). The examined dog’s mitochondrial DNA found four different lines, from which the most different varies from the wolf was 1%, while the jackal, which was separated 1 million years ago, varies from the wolf by 7,5%. The dog and wolf separation of populations happened more than 135,000 years ago (Wayne and Ostrander, 1999).

Humans began to select dogs for certain traits such as desired behaviours (hunting, herding, retrieving) and appealing morphology (coat structure and colour, body size). Dogs show more morphological diversity than any other domesticated animals. The scale in size and conformation is exemplified by little Chihuahua (0,5 kg) and the huge Great Dane (80 kg). There are more than 400 dogs breeds registered all over the world (Lindblad-Toh et al., 2005). In Parkers research dogs are divided into four distinct breed groupings representing separate “adaptive radiations” (Parker et al., 2004).

All species of the genus *Canis* are closely related and can be crossed. Genetic results indicated that most breeds are genetically diverse, but mutually not very different. It's expected fact because most of the breeds originated during the recent period and apparently originated from diversified and mixed gene pool. However, some breeds of the ancient origin, such as dingo, New Guinea dog, greyhounds and Molossoid breeds originated at the time when the human population was still isolated (Parker et al., 2004).

Variety of dog morphologies have existed for decade and the biggest reproductive isolation between them was formalized when breed clubs and breed standards began in the mid–19th century (Parker et al., 2004). There are registered 152 breeds at American Kennel Club (http://www.akc.org/). Investigated was also the Mexican Hairless Dog, created about a thousand years ago in Mexico. The research of 26 individuals showed that the found sequences are identical with other breeds. In addition, representatives of all four branches of the dog were found at the Hairless dog as well. The result shows that the population of dogs ancestors who migrated with humans to the New World for more than 10,000 years ago, was numerous and well-diversified. Any of genetic sequence which was found did not resemble wolves’ sequences of the New World. This means that there was not any independent domestication from the wolf (Wayne and Ostrander, 1999).

Vila et al. (1999) have deduced that given the broad phenotypic diversity of dogs, it has been suggested that domesticated dogs periodically mixed with wolves. Studies in molecular research rely upon using a selected control region within the mitochondrial DNA. According to Vila et al. (1999), this control region consisting of 261 base pairs (bp) was used for comparison in dogs, wolves, coyotes, Ethiopian wolves, and golden jackals. The dog and wolf sequence differed by 0-12 substitutions and dog always differed from coyotes, jackals and Ethiopian wolves by at least 20 substitutions. Within the dog sequences it was shown that the dog sequences clustered into 4 clades. Vila et al., (1999) concluded that either wolfs were domesticated in several places or that one domestication event was followed by several episodes of admixture between dogs and wolves (Vila et al., 1999).

However, dogs differed from the coyotes and jackals by at least 20 substitutions, the study focused on a particular region of the mtDNA known as the control region which has been shown in mammals to have a high mutation rate and therefore might reveal differences between the dog and other *Canis* species support for the hypothesis that wolves were the ancestors of dogs. This particular genetic marker provides the basis for the study carried out by Parker et al. (2004). The microsatellite genotypes were used to demonstrate that dog breeds are predominantly closed breeding populations. As predicted from the existence of breed barriers, dogs from the same breed were more similar genetically than dogs from other breeds.

Another study of mtDNA by Savolainen et al. (2002), research on mitochondrial variation among 654 domestic dogs, confirm this premise, suggesting that the larger genetic variation in East Asia compared to other regions and the pattern of phylogeographic variation are indicative of an East Asian origin of the domestic dog approximately 15,000 years ago. Analysis of SNPs reveals long-range haplotypes across the entire dog genome, and defines the nature of genetic diversity within and across breeds. The current SNP map now makes it possible for genome-wide association studies to identify genes responsible for diseases and traits, with important consequences for human and companion animal health (Lindblad-Toh et al., 2005). VonHold et al. (2010) created genome-wide SNP and haplotype analyses. There was found that Middle Eastern wolves were a critical source of genome diversity. This study approves the Middle East could be a primary source of genetic variation for dog, with possible secondary sources of from Europe and East Asia. For example, the two ancient breeds with highest SNP haplotype diversity, saluki and Chinese Shar Pei, originated in widely different areas (the Middle East and China, respectively) (VonHoldt et al., 2010).

Dog domestication is the longest experiment in the human history. Dog is only fully domesticated animal. When, where and how was a dog truly domesticated is still not clear. Domestication is a continuous process, which is different by species, and genes and environment interact to produce selectable characters that can change according to circumstance (Driscoll, 2009).

2.4 History of CF

First information about hunting dogs can be found in the documents approximately 2400-2300 years old. The book is called "Kinegetikos" and it was written by Greek philosopher Xenofon.

Special dogs such as pointers probably originated during 768-814, they were bred to have good quality and speed for hunting. Bohuslav Balbín (Czech writer) wrote about Bohemian dogs which were famous in all Europe in his work in 1679.

Documents about CF (Canis Bohemicus) from 1348 were found in Karlštejn, According this documents Karel IV. gave those dogs to Ludvík Braniborský. A picture of dog which was very similar to CF was painted in 16th century (Figure 5)

Figure 5 Detail of „ Bohemian dog'' around year 1600 (Kuhn, 2005)

Association of gamekeeper had positive effect on CF breeding. The breed was quite popular for his calm attitude and work ability. Their aim was to improve hunt dogs and make a breed book.

In 1850 owners of arable land used hunt dogs very often, but they preferred smaller and thinner dogs than CF. Therefore CF was losing his popularity. The reason was that the CF is not good enough in finding small game. Pointers from foreign countries were more suitable. CF is more appropriate for foresters especially big game hunting is the best use for those dogs. First official exams for hunt dogs were organized in 1885.

Anyway Josef Zenker (one of founder of Czech hunting cynology) appealed on members of Czech forestry unit to keep original standard of CF. Many of CF were exported to Germany and registered as German wire-haired pointers.

1896 Czech pointers got the name “český fousek” but German club did not accepted that properly.

CF won exhibition champion title on world exhibition therefore breed getting be more popular and well-known for people.

However the WWI. reduced cynology at all. Consequences of that were tragic for the breed. At that time, CF almost extinct. After the war the situation was even worse because of difficult times when the breed books and documents about CF didn’t exist. Very strict bureaucracy rules made impossible to breed on dogs without right documents-especially the non-exist breed book. So these measures led to reduction of the breeding and almost disappearing of CF. Luckily the breed did not disappear completely thanks to a few enthusiasts who saved them. After the war those breeders had to start again from the beginning. New breeding program began quite soon after the war. Exterior, hairs (coat) and also work ability were evaluated more critically.

They established CF club. German people who lived near borders kept CF and registered them in their German breed books. Franišek Houska was very important person in regeneration of the breed. He founded the association for wire-haired pointers in 1924. After foundation of Czechoslovakian breed books, dogs were separated on Czech and German breeds. However CF individuals were registered in both of them, because of insufficient breeding ruler. Unfortunately they did not care about visual appearance only on work abilities. Official standard of CF was appeared in 1931 (Figure 6).

Figure 6 One of the oldest surviving pictures of CF (Kuhn, 2005)

Breed CF was accepted as the national breed in 1964 (Much later than many others Czech breeds). Breeders wanted faster progress in breeding so they used also different breeds for breeding.

However cynology did not improve during the protectorate so fast. Breeders started to use lineage breeding andpress issued news about the inbred lineages. There are official ten original lineages of evidence, based on significant individuals. CF dog called "Bojar z Bahňáku" is considered as the initial individual. Final consider about CF can belongs to FCI (federation cynologique international) in 1964 (Dostál, 1998; Kuhn, 2005).

CF was also exported to the foreign countries, even to USA in 1985. After the division of the Czech and Slovak republics, both club also separated to Czech and Slovak part in 1993.

Originally, 10 family lineages of CF existed in the past. Today, we have only 8 lineages as the lineages 6 and 8 already extinct (Dostál, 2009).

2.5 Genetic lineages of CF

Lineage I. – Jedlový vrch. It contains all the descendants of the dogs Avar from Ječnišť and Argo from Šebín forest Lineage I. This lineage has a fairly good perspective in the future because of quality of breeding males There was a reduction of interest about individuals of this lineage in the past, as a result of extensive hair loss (Kuhn, 2005). This lineage was numerous, it contain one third of all CF therefore even health problem did not destroyed this lineage. (Dostál, 1998).

Lineage II. – Považie. Individuals of this lineage achieved outstanding results at dog shows and on competitions. This lineage was greatly affected by hair loss in the past, which reduced the popularity and interest in the individual of this lineage (Dostál, 1998).

Lineage III. – Albrechtice. It is lineage that practically was not affected by hair loss, and is therefore very popular and promising line. Individuals from this lineage were used to pour blood into other lineages in the past and this lineage almost disappeared. Luckily, it was regenerated thanks to breeders of East and South Bohemia. Breeders paid attention on absence of teeth that has greatly expanded in this lineage. Today, breeders use method of add blood of individuals from lineage III to other all lineages (Kuhn, 2005). They use it when it is necessary to improve coat condition and reduce hair loss (Dostál, 1998).

Lineage IV. – Žampach. It is a lineage that has achieved significant reduction in recent years. Representatives of this lineage achieved outstanding results in examinations, competitions and exhibitions. It is a lineage that can be sustained to the future (Dostál, 1998).

Lineage V. – Soví doly. This lineage has come to a considerable reduction in number of individuals even though it was associated with lineage VI in the past. These dogs never find wider use, so we don´t have today better chance to maintain it. Dogs of this lineage can be used for stud service in lineage I., X. and IX., because this lineage is related to them and has many similar characteristics and properties. However, breeding between lineage V. with other lineages is not very appropriate (Dostál, 1998).

Lineage VII. – Zborov. It is widespread mainly in Moravia. A substantial reduction in the number of breeding dogs occurred in this lineage However, in the pats breeders use German Wire-haired Pointer for breeding with individuals in this lineage (Kuhn, 2005). These dogs have some percentage of pointers blood till today. A few years ago breeders had included a total of 15 breeding dogs and it was one of the largest lineages. It excels mainly in working results. It is a lineage suitable for beginners in cynology. These dogs demonstrated good attitude, obedience and working ability to their owners. This lineage is really important for all breeders in Moravia and they prevent it from extinction (Dostál, 1998).

Lineage IX. – ze Starého dolu. It is made up of descendants of the Old Dog Ajax mine. It cannot be substantially extended for several years. Its popularity starts to rise in recent years (Dostál, 1998).

Lineage X. – z Hložku. It was founded by brothers and half-brother of one of the most successful breeding dogs, Bor from Hložek, which was both international champion, as well as Res. CACIT or exterior and work top dog. This lineage was separated from the lineage I. very recently and it appears to be very promising for the future (Dostál, 1998; Kuhn 2005).

Lineages VI and VIII disappeared due to deficiency of individuals. Nowadays some breeding dogs are categorized in lineage called "other" (www.cesky-fousek.cz)

2.6 Lineage breeding

Lineage breeding or sometimes use line-breeding means all male offsprings from founder of the lineage to the last offspring. Genealogy of lineages helps to track kinship distance. Aim of the breeding is transferred genealogy lineage to breeding lineage. Each lineage is characterized by special qualities such as hunting ability, type of coat or attitude (Kuhn, 2005; Dostál 2009). This method of breeding is probably the most suitable for CF and other gun dogs. Nowadays, the lineage breeding is popular for Small Munsterlander dogs. They have 7 genetic lineages, however due to small number of dogs in each lineage; breeders could not realize clear lineage breeding. Sometimes they have to use dogs from different lineage for mating (from Germany and Austria) (www.ohari.eu/plemena-oharu/maly-munsterlandsky-ohar/historie). Lineage breeding use advantage of distant relative breeding so required character is slightly stabilized. These organization methods can lead to the fast and reliable selection. The crossbreeding with other breed makes the breed stronger, healthier and more balanced. Current population genetics results show that population size should not decrease under 200-500 individuals, otherwise there is risk of inbreeding and thus more identical homozygotes which may lead to higher mortality of pups and extinction of population (Dostál, 2009). The small population is when it's effective population size has approximately less than 400 individuals. The gene pool of the population is gradually decreasing and among individuals it is starting to show inbreeding depression (Pires et al., 2009). This state requires add blood of breed from which the breed originated, or relative of the breed. Unfortunately this process is repeated for several generations. Also breed is losing its characteristics. If there is no significant increase of individuals over the time, this breed fuses together with the breed which is genetically enriched (Dostál, 2009). Some heterozygosity must be maintained even in the most lineage breeding (inbreeding) populations. Such a small breed requires a large international collaboration of breeders and genetic experts and strict breeding management in order to maintain healthy and diminish the number of individuals (Pires et al., 2009).

Lineage breeding is more popular for livestock animal or laboratory animals that can be breed by lineage breeding method for various purposes. The medical study about rat's genetic lineages was used as a model of depression disease (Owerstreet, 2005). The breeding experiment with three lineages of Hereford cattle have been reported by Tallis et al. (1959). Montana Lineage I and Nebraska Lineage I bulls were selected on type and bred to randomly selected, outbred cows. The performance of the progeny of these inbred lineage bulls were compared with those of outbred bulls which had been selected on beef type. These data show that the Montana Lineage I and Nebraska Lineage I sired calves inclined to be more efficient in production. Similar study about genetic lineages of Hereford cattle was continuing for 75 years (MacNeil, 2009). The object of another study was about one lineage of pigs for two traits, select in another lineage for two other traits and then cross the two lineages. Each lineage was selected for five generations. The goal of the breeding plan was to improve the production in crosslineage pigs. The breeding plan proved to be effective in improving the performance of the crosslineage pigs (Kemp and Magee, 1970).

Lineage breeding was used in horse's breeding mainly in the past; nevertheless it is sometime use today as well. For example, Haflinger horse was divided into seven lineages. Lineage "A" is the biggest and the most famous. Czech Breeders cooperating together with Austrian's and Italian's breeders. Anyway this lineage breeding is not strict because the certain animal quality is more important for breeders (www.haflingove.cz/hafling.html). Czerneková et al. (2012) did analysis about the oldest Czech horse breed - Kladruber. They studied differences among paternal lines. They use 9 paternal lineages and the genetic variability is comparable to other horse breeds (Czerneková et al., 2010)

2.7 Standard of CF breed

CF is a strong hunting dog. The skull is quite rounded but looks square in profile. The nose is brown with wide open nostrils and the teeth meet in a scissors bite. The large, round eyes range from yellow to brown in colour. The medium sized ears are set at ear level hanging down and lying flat against the head. The body is slightly longer than it is tall and the back slopes slightly from the shoulders to the back part. The front legs are straight with round, webbed feet. The tail is usually docked by 1/3 to 1/2 its original length and carried straight or raised. The medium length, course, rough double coat has soft, thick, bushy eyebrows, beard and moustache. Coat colour is grey with brown markings (Figure 7). The brown can be shades of chestnut brown or roan. It can also come in white, solid brown, white and brown or white and orange (www.cmku.cz).

Figure 7 Exhibition of CF (Iveta Dočkalová, 2012)

- Height
  - Males: .. 60 - 66 cm
  - Females: 58 - 62 cm
- Weight
  - Males: .. 28 - 34 kg
  - Females: 22 - 28 kg
- Coat
  - CF has three different hair types: a thick, close fitting and water repellent undercoat (hair length 1,5 cm); an overcoat (hair length 3 - 4 cm) and finally a hard and wiry chest/shoulder/back/groin hair of 5 - 7 cm long.
- Health
  - The greatest problem of the breeders is the occurrence of baldness (massive loss of hair) in some breeding lineages . The breeding is very carefully

controlled. There is no problem of dysplasia, though it can occur (www.akc.org) (www.cmku.cz), (www.cesky-fousek.cz).

Temperament of CF is a loyal, skilled energetic field dog. They are intelligent and make distinguished gundogs and trackers. These excellent hunting companions can point and retrieve. The dog generally staying within the hunter's gun range. It is especially good for hunters on foot combining the ability to be directed by the hunter with the ability to think independently. They have very good sense of smell and a great passion for the hunt, especially for quail and hare. The dog works well in marshland and upland. Wirehaired coat protects him from dense brush and bad weather. The breed is also an excellent family pet and watchdog (www.akc.org).

2.8 Pointers

German wire-haired pointer (GWP) is very similar to CF (Figure 8). CF is the oldest wire-haired breed so it is consider to origin breed for GWP which origin in early 19th century. After that GWP was refine by Pudelpoiner, Griffon and German short-hair pointer (Dostál, 2009). When Germans wanted a dog that could be obedience and strong in hunting together, so they created the German Wire-haired pointer, a versatile, multi-purpose hunting dog. Strong and medium-sized, with a typical Pointer personality, the breed's most distinctive characteristic is its functional wiry coat. Weather resistant and water-repellent, the outer coat is straight, harsh, wiry and flat lying, which helps to protect the dog against rough cover while hunting. The coat must be liver and white in colour (www.akc.org).

Figure 8 German Wire-Haired Pointer (www.akc.org)

Most of the early wirehaired Pointers represented a combination of Griffon, Stichelhaar, Pudelpointer, and German Shorthair. The Pudelpointer was a cross between a Poodle dog and an English Pointer bitch, while the Griffon and the Stichelhaar were composed of Pointer, Foxhound, Pudelpointer, and a Polish Water dog (www.akc.org), (www.cmku.cz).

Another similar breed is German spine-haired pointer. The population is very small and it has been almost extinct for many years. It is impossible to meet this breed on dog shows. GWP is different from CF mainly by hard and brushy hair on the body. German breeders used corossbreding with CF (new blood) for many times to avoid close inbreeding. According to Dostál (2009) inbreeding value of GWP reach 37,4% in some litters, what can be quite serious genetic problem. The more frequently is the CF utilized for its regeneration, the more similar will GWP be to CF (Dostál, 2009).

German short-haired pointer (GSP) is versatile hunter and all-purpose gun dog (Figure 9). The German shorthaired pointer has good nature such as scenting power and high intelligence. The main source of basic foundation stock for the German Short-haired Pointer seems to have been the German Bird Dog. The breed is capable with many different types of game and sport, including trailing, retrieving, and pointing pheasant, quail, grouse, waterfowl, raccoons, possum, and even deer. A medium-sized dog, he has an aristocratic bearing and can be liver and white in colour.

Figure 9 German short-haired pointer (www.akc.org)

3 Materials and Methods

3.1 Sample collection

First step of the research was tissue sample collecting during the year 2012. All of the samples were collected by non-invasive cheek swab method mainly on dog shows (breed club shows and bonitations for young dogs). Dog shows were held in Jirkov 12.5.20012, Milovice u Hořic 2.6.2012, in Náměšť na Hané 9.5.2012 and in České Budějovice 14.10.2012. Samples were taken from dogs of different family and litter (only one male and one female). There were collected in total 206 dog samples, 160 CF, 24 GWP and 23 GSP (Appendix 1).

3.2 DNA extraction

Second step was isolation of DNA. The Genomic DNA Mini Kit (Tissue)- Buccal Swab (www.geneaid.com) was used for the isolation. It is a genomic DNA Extraction Kit optimized for genomic DNA purification from a variety of animal tissue. This kit includes Proteinase K which is used to lyse cells and degrade proteins. In next step, DNA is easily bound by the glass fibber matrix of the genomic DNA spin column. Once any excess liquid have been removed, using a wash buffer (containing ethanol), the purified DNA was eluted by elution buffer and it was ready for use in PCR (www.geneaid.com). DNA of cheek swap samples were extracted by this special kit. All operating procedures were done according instructions enclosed in the kit. Laboratory work was performed at Czech University of Life Sciences in Prague.

After that, it was necessary to measure concentration of purified genomic DNA on Spectrophomter ND-1000 (Nanodrop®) in sequence laboratory of biological section on Faculty of Science of Charles University in Prague. Samples that had concentration higher than 10ng/µl were diluted by the water for optimal concentration for PCR. Samples were stored in freezer (-20°C).

3.3 PCR conditions

Next phase was Polymerase chain reaction (PCR). It was used 19 microsatellite loci that were developed in Finnzymes Diagnostics in Finland. The Canine Genotypes™ Panel 1.1 kit contains PCR master mix and the following 19 loci: AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU030, Amelogenin, FH2848, AHT121, FH2054, REN162C04 AHTh171 and REN247M23 (Table 1).

Table 1 List of primers: In the table are given loci names, position on chromosomes, repeat motif, range of sizes and fluorescent marking colour

Locus Name	Chromosome	Repeat Motif	Size Range (bp)	Dye Colour
AHTk211	26	di	79-101	blue
CXX279	22	di	109-133	blue
REN169O18	29	di	150-170	blue
INU055	10	di	190-216	blue
REN54P11	18	di	222-244	blue
INRA21	21	di	87-111	green
AHT137	11	di	126-156	green
REN169D01	14	di	199-221	green
AHTh260	16	di	230-254	green
AHTk253	23	di	277-297	green
INU005	33	di	102-136	black
INU030	12	di	139-157	black
Amelogenin	X	-	174-218	black
FH2848	2	di	222-244	black
AHT121	13	di	68-118	red
FH2054	12	tetra	135-179	red
REN162C04 7	7	di	192-212	red
AHTh171	6	di	215-239	red
REN247M23	15	di	258-282	red

These markers are included in the ‘core panel’ of loci. The Canine Genotypes™ Panel 1.1 kit allows co amplification of the above markers in a single multiplex PCR reaction. One primer from each primer pair is end-labeled with a fluorescent dye. The data represents a large selection of dog breeds. The list of primers, their size, number of chromosome and their fluorescent markings is shown in the Table 1. Locus Amelogenin is located on XY chromosome. It determines the individual´s gender. (Instruction Manual Canine genotypes Panel 1.1 F-860S/L).

The composition of a reacting mixture was: 3µl (c=10 µM) of fluorescently labeled forward primers and reverse primers, 3µl of PCR master mix and 0,7µl (c=1-10 ng/µl) genomic DNA of each sample. PCR run in Mastercycler Eppendorf Gradient thermocycler with temperature protocol according to producer.

Thermal cycling programs

1. 98 °C for 3 minutes
2. 98 °C for 15 seconds
3. 60 °C for75 seconds
4. 72 °C for75 seconds
5. go back to step 2. 29x
5. 72 °C for 5 minutes

The last laboratory step was the fragmentation analysis. The mixture for the fragmentation analysis contained 2 μl of PCR product, 7,5 μl of formamide and 0,5 μl of size standard (Gene ScanTM 500 LIZ Size Standard, Applied Biosystems). This mixture was denaturised for 5 minutes at temperature 95°C and then colded to 4°C and stored in the temperature of -20°C. Fragmentation analysis was made in sequencing laboratory centre of Faculty of Science of Charles University in Prague on sequencer ABI Prism 3100 Avant Genetic Analyser (Applied Biosystems) with polymer POP4 and standard DS-33.

3.4 Data analyses

Presence of null alleles and extremely large alleles was tested in programme Mikro-Checker which is using Monte Carlo simulations of expected difference in allele size (Van Oosterhout et al., 2004). Loci with high occurrence of null alleles displays apparently high number of homozygotes.

The length of each allele was manually scored in GeneMarker analysis software V2.2.0 (www.softgenetics.com) and recorded to Microsoft Excel table. All the data were rounded in the programme Autobin (www4.bordeaux-aquitaine.inra.fr/biogeco/Ressources/Logiciels/Autobin).

Program Genepop (Rousset, 2008) was used for calculation of basic descriptive genetic parameters which are following:

F-statistic: inbreeding coefficient (Fis) which measures decrease of heterozygosity due to non-random mating within the population. Fis shows the deficiency or excess of average heterozygotes in each population. Value can range from –1,0 (all individuals heterozygous) to +1,0 (no observed heterozygotes) Fixation index (Fst) shows decrease of heterozygosity of subpopulation in proportion to the total population because of genetic drift in the subpopulations. Fst is the degree of gene differentiation among populations in terms of allele frequencies. Scale value: 0 – 0,05 small differentiation, 0,05 – 0,15 middle differentiation, 0,15 – 0,25 significant differentiation, > 0,25 highly significant differentiation. It is about the differentiation ratio between populations. Either there was measured Hardy-Weinberg equilibrium (HWE) in the population which says that proportion of each allele stay constant in panmictic population from generation to generation. Heterozygosity deficiency or excess for whole population-global test were measured in Genepop program (Rousset, 2008). Markov chain parameters were: Dememorization number (1000) number of batches (100) and number of iterations per batch (1000).

Expected heterozygosity (He), observed heterozygozity (Ho) and number of alleles were also evaluated in Genepop.

Basic visualization of relationships between individuals and populations were proceeded in program Genetix (Belkhir et al., 2004) using 3D factorial correspondence analysis. Individuals were separated to three groups (populations) according to the breed. Also we compared data of Czechoslovakian wolfdogs, German Shephers and wild wolf population with our populations (data were used from the previous study of Smetanová, 2012). These results are shown only in Genetix program as 3D visualization.

To assign individuals into populations and to detect population structure it was used Bayesian clustering method implemented in the program Structure. The program Structure version 2.3 implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. The method was introduced in a paper by Pritchard et al. (2000). Applications of the method include demonstrating the presence of population structure, identifying distinct genetic populations, assigning individuals to populations, and identifying migrants and admixed individuals (Pritchard et al., 2010). Admixture model without *a priori* information on population membership with correlated allele frequencies was used for the analysis (Falush et al., 2003). Five independent runs for each number of clusters (K) (from one to fifteen) were performed. Each run consisted of a burn-in period of 100,000 iterations followed by 900,000 iterations of Marcov Chain Monte Carlo (MCMC). The selection of the best K, based on the widely used method of Evanno et al. (2005), was performed in STRUCTURE HARVESTER (Earl et al., 2012).

3.5 Microsatellites

Microsatellites are popular molecular markers for population genetics and evolution (Bee and Rowe, 2008). Observed microsatellite alleles are DNA fragments of different sizes detected by early amplification using polymerase chain reaction (PCR) and visualization by capillary electrophoresis. Size polymorphism reflects variation in the number of repeats of a simple DNA sequence (2–6 bases long). Microsatellite loci are highly polymorphic and easy to survey therefore offer the hope of greater understanding of population structure (Goldstein et al., 1995).

Microsatellites have wide usage for identification of individuals called fingerprints which can be used in forensic analysis or determining paternity and parentity. This method is suitable for population genetic researches about relation between populations and evolution studies (Chapuis and Estoup, 2006).

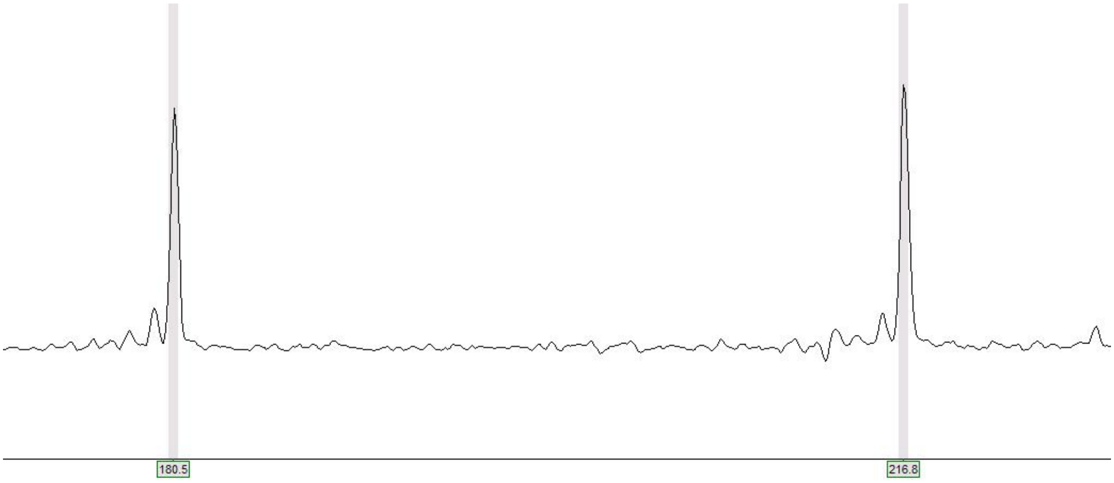
However, they correspond to neutral variability as they don’t code any proteins or don’t regulate anything and they are not under the selection pressures. Information value is than limited by the fact, that the mutation rate is very high and some historical aspect may not be detected by the microsatellites.

4 Results

We were able to obtain genotypes of 151 individuals: 114 samples of CF breed, 18 samples of GWP and 19 samples of GSP. All 19 loci were polymorphic, which means that there was more than one allele at each locus. This population is possibly in Hardy Weinberg disequilibrium with loci REN54P11, REN169D01, AHTk253, FH2848, AHTh171, showing signs of a null allele. Other loci show no evidence for scoring error due to stuttering. No evidence for large allele or null alleles.

Locus Amelogenin was not used in further analysis however help us to check correctness of samples. Locus Amelogenin is located on XY chromosome. It determined gender of single individuals. Females are homozygotes with size 160 (as they have XX chromosomes) and males are heterozygotes with alleles 180 and 216 (as they have XY chromosomes). For picture how do the microsatellites look like in in GeneMarker program, we visualized it in Figure 10.

Figure 10 Male individual displayed in GeneMarker program at locus Amelogenin



Average inbreeding coefficients (Fis) of CF population was 0,1377. Inbreeding coefficient (Fis) was 0,0831 for GWP and 0,0944 for GWP. Our positive value may indicate that there is an increased number of homozygotes in the whole CF breed. Average fixation index (Fst) when CF population was measured against the GWP population was 0,0597 and between CF population and GSP was 0,0759. Both results mean that differentiation between CF and other population is small. Populations of GWP and GSP are confidently close to CF.

This result is also connected to Hardy-Weinberg equilibrium (HWE). CF shows significant departures from HWE (p<0,05). Test in Genepop proved significant excess of homozygotes in the breed (p<0,05). Contrary GWP and GSP populations were in HWE as there was no significant presence of excess of homozygotes and excess of heterozygotes.

Overall observed heterozygosity (Ho) of CF population was 0,6626 and average expected heterozygosity (He) was 0,7645. Heterozygosity observed was 0,6890 for GWP and 0,6441 for GSP. Heterozygosity expected was 0,7256 and 0,6913 for GSP. Detailed results for each locus are given in the Table 2.

Table 2 In the table there are given: loci names, F_{st} (fixation index) for comparison of Cesky Fousek (CF) with German Wire-haired Pointer (GWP) and German Short-haired Pointer (GSP) population and CF with GSP population, F_{is} (coefficient of inbreeding), number of allele and observed (Ho) and expected heterozygosity (He) of CF population

Locus name	**F_{st} CF:GWP**	**F_{st} CF:GSP**	**F_{is}**	**Number of allele**	**H expected**	**H observed**
AHTk211	0,0158	0,0216	0,0682	11	0,6616	0,6195
CXX279	0,1588	0,1709	0,0470	15	0,7487	0,7168
REN169O18	0,0834	0,1142	0,0273	13	0,8618	0,8421
INU055	0,1544	0,0176	0,2908	7	0,6395	0,4561
REN54P11	-0,0135	-0,0049	0,1319	14	0,8345	0,7281
INRA21	0,0451	0,0052	0,0402	8	0,7642	0,7368
AHT137	0,0365	0,1057	-0,0029	15	0,8273	0,8333
REN169D01	-0,0034	0,0766	0,3259	13	0,7504	0,5088
AHTh260	0,0075	0,0126	0,0819	14	0,6427	0,5929
AHTk253	0,1201	0,1211	0,3907	11	0,6726	0,4123
INU005	0,0114	0,0165	0,0505	13	0,7793	0,7434
INU030	0,1122	0,1077	0,1703	8	0,7153	0,5965
Amelogenin	-	-	-	-	-	-
FH2848	-0,0079	0,0626	0,2452	21	0,8116	0,6161
AHT121	0,1108	0,0554	0,0791	18	0,8129	0,7522
FH2054	0,1108	0,0554	0,0656	15	0,8316	0,7807
REN162C04	0,0270	0,1154	0,2874	12	0,7589	0,5439
AHTh171	0,0153	0,0139	0,1459	21	0,7971	0,6842
REN247M23	0,0926	0,1410	0,1078	11	0,8512	0,7632
Total	**0,0597**	**0,0759**	**0,1377**	**13,3**	**0,7645**	**0,6626**

Table 3 Fis (coefficient of inbreeding), observed (Ho) and expected heterozygosity (He) of German Wire-haired Pointer (GWP), German Short-haired pointer (GSP) populations and Cesky Fousek (CF) population

Population	**F_{is} (total number)**	**He (total number)**	**Ho (total number)**
Population GWP	0,0831	0,7286	0,6890
Population GSP	0,0944	0,6913	0,6441
Population CF	0,1377	0,7645	0,6626

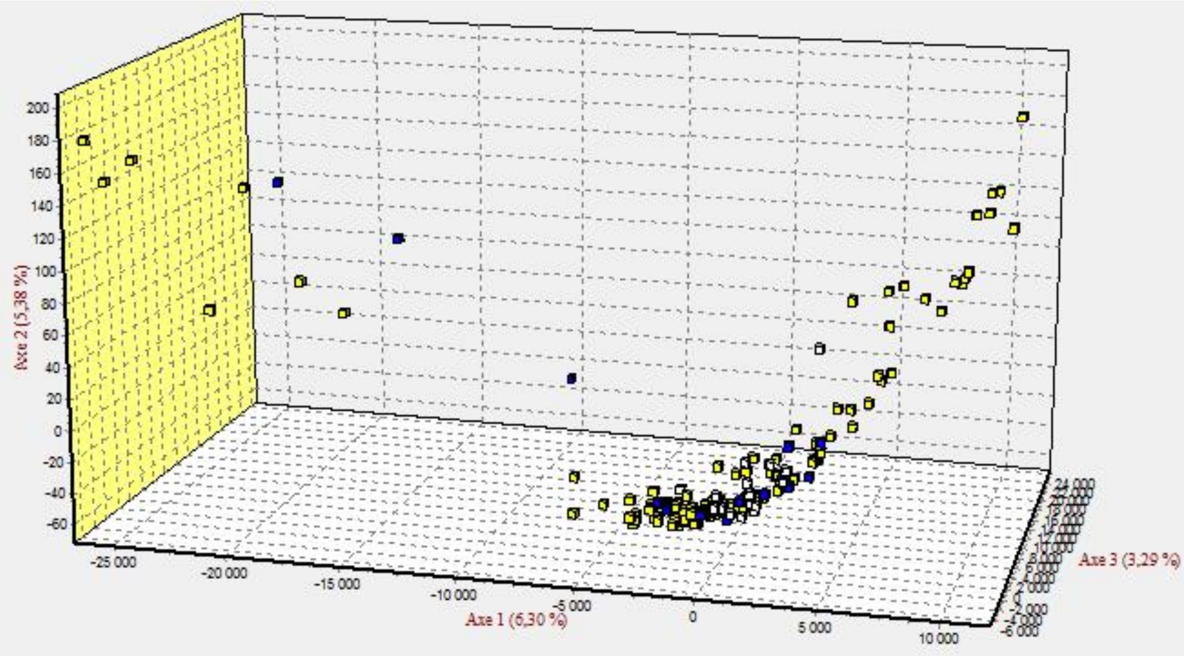
Fis is higher in CF population compare with other two populations. Comparing genetic lineages of CF showed that Fis is even higher in each lineage separately. Interesting point is that Fis for lineage number 7 is the lowest probably due to crossbreeding with GWP in the past (according breeding documents) (Kuhn, 2005).

Table 4 Inbreeding coeficient (Fis) and heterozygosity observed (Ho) and expected (He) for genetic lineages of Cesky Fousek (CF) population

Genetic lineages CF population	**F_{IS}**	**He**	**Ho**
1	0,1549	0,7430	0,6466
2	0,1189	0,7399	0,6869
3	0,1387	0,7358	0,6508
7	0,0256	0,6913	0,7389
9	0,11731	0,7229	0,6313
10	0,1928	0,6780	0,6111

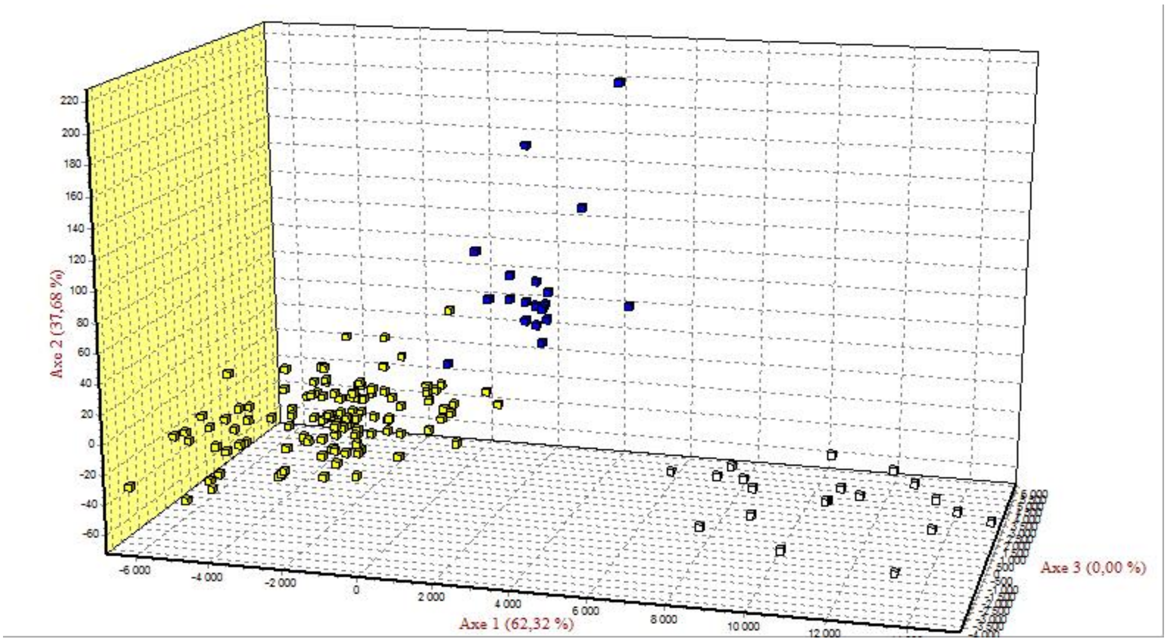
Factorial corresponds analysis was completed in the program Genetix. This program provided visualization of relation among populations. There is could be seen different size and location of populations. In our case the visualization is not so clear because there are genetically very close populations.

Figure 11 Three-dimensional factorial correspondence analysis (3D FCA Individual distance) Individuals of each population are classified according colour of the population. Cesky Fousek (CF)– yellow, German Wire-haired Pointer (GWP) – blue and German Shord-haired Pointer (GSP) – white



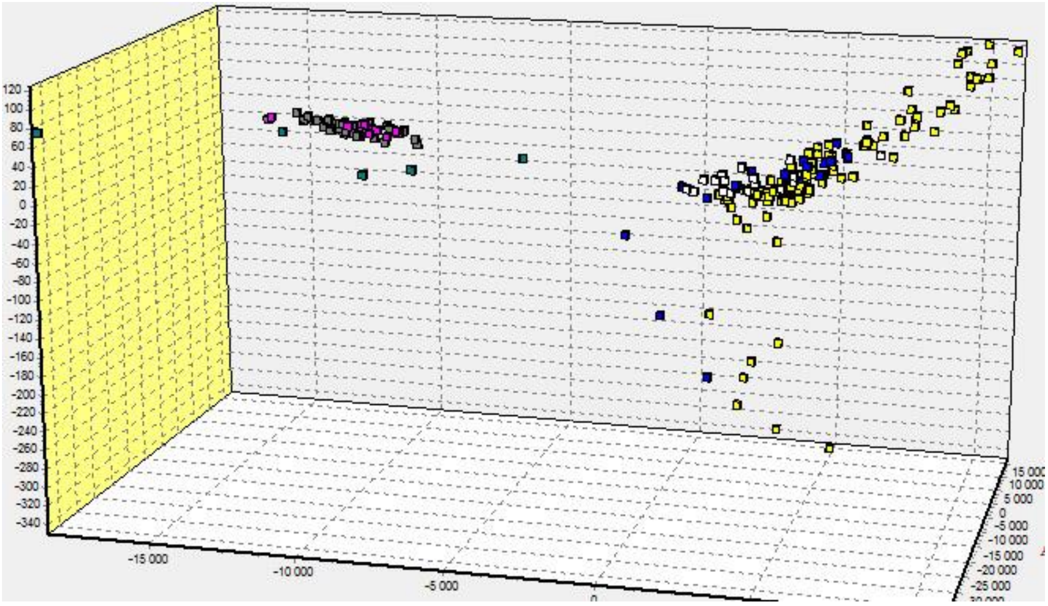
In the Figure 11 is not visible significant distance between GSP and CF with GWP. CF population is genetically really closer with GWP than with GSP, which was expected due to historical genetic background and current similar visual appearance. Figure 12 displayed only distance among populations. The program measured the result according populations setup.

Figure 12 Three-dimensional factorial correspondence analysis (3D FCA Population distance). Populations are identifiable by different colours. Cesky Fousek (CF)– yellow, German Wire-haired Pointer (GWP) – blue and German Short-haired Pointer (GSP) – white



There is comparison of genetic closeness with German Shepherd (GS) population, Czechoslovakian Wolfdogs (CSW) and wolfs population. There is clearly visible a big genetic difference between our study populations (CF, GWP, GSP) and other populations (CSW, GS, wolfs). Also wolfs are genetically really closer to GSW and GS than our study populations.

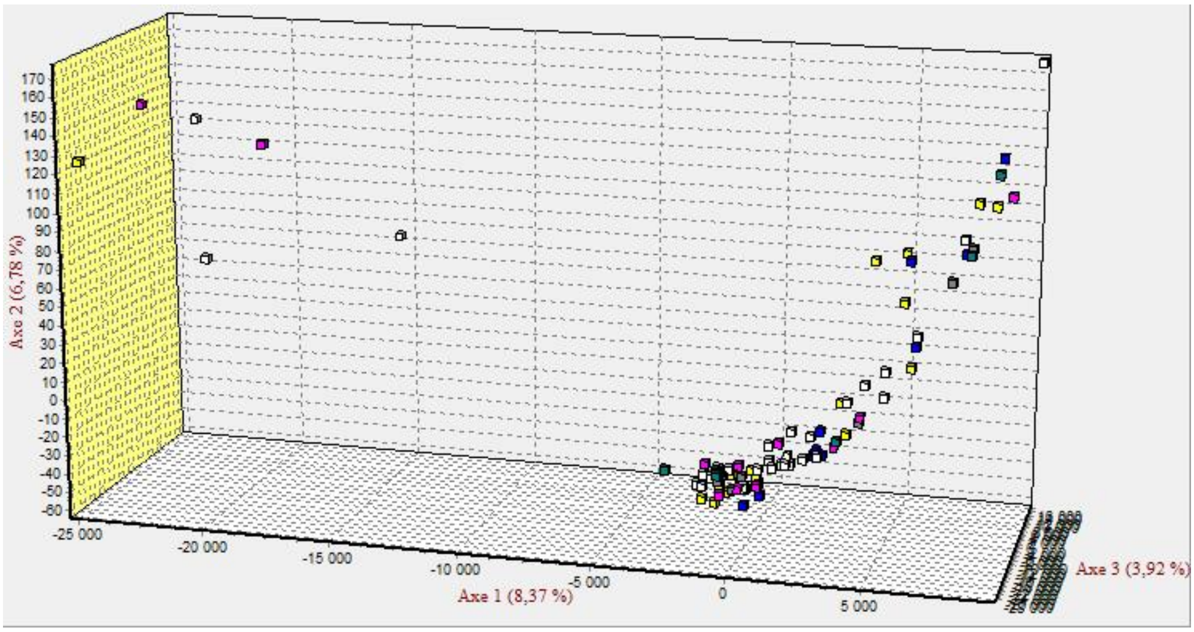
Figure 13 (3D FCA Individual distance) Individuals of each population are classified according colour of the population. Cesky Fousek (CF)– yellow, German Wire-haired Pointer (GWP) – blue, German Short-haired Pointer (GSP) – white, Czechoslovakian Wolfdog (CSW)-grey, German Shepherd (GS)-pink, wolf-green (Smetanová, 2012)



It is also possible to display relations among individuals within the one population. CF population is interesting because of lineage breeding system. Individuals are separated to different group according their genetic lineage. There were chosen only individuals with clear categorization to the certain lineage (Appendix 2).

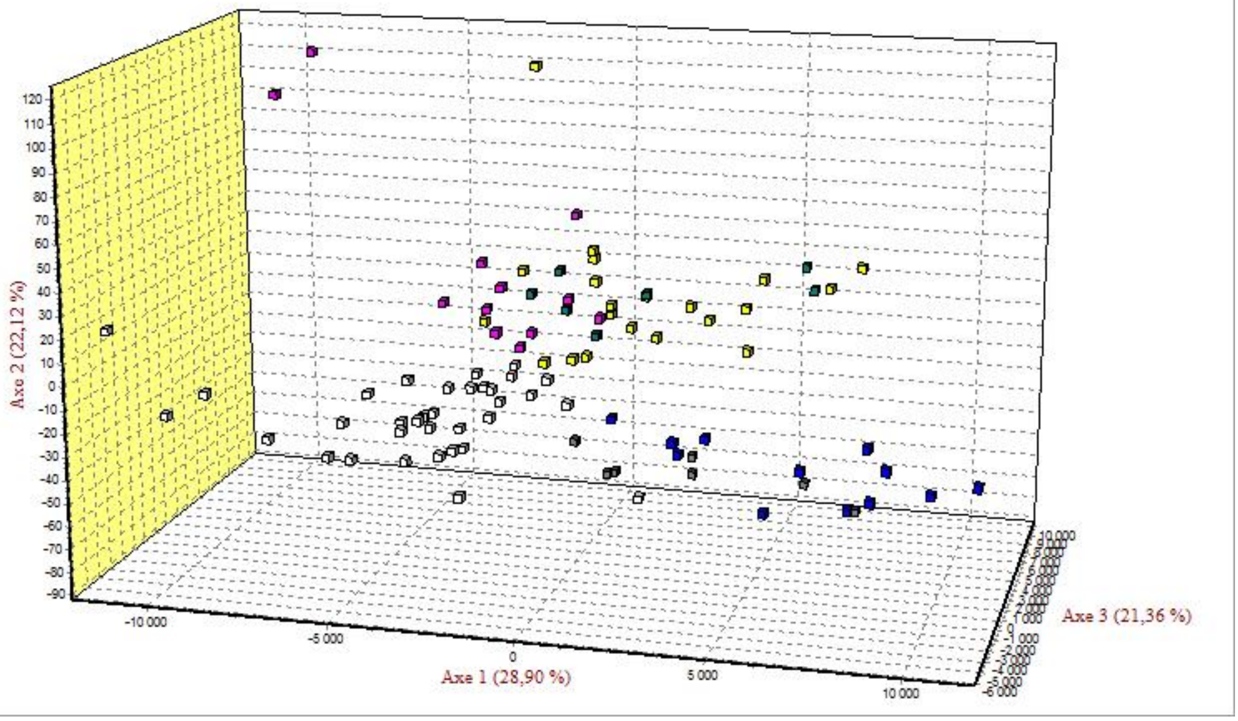
The difference among the lineages is not clearly visible due to mixing of some lineages together in the past and closed relation among individuals. Unfortunately there is not balanced number of individual in each lineage. Some lineages have only one or two individuals which were appropriated for this research (Figure 14).

Figure 14 (3D FCA Individual distance) Genetic lineages of Cesky Fousek (CF). Lineage 1-yellow, lineage 2-blue, lineage 3- white, lineage 7-grey, lineage 9-pink, lineage 10-green



In Figure 15 is displayed only distance among lineages. The program measured the result according lineages setup. All individuals form lineages are genetically close to each other. There is not much visible distance among each lineage. However lineage 2 and 7; those are tending to be separated from others. Also lineage 3 seems to be more separated however it could be caused by higher number of individuals in this lineage.

Figure 15 (3D FCA Population distance) Distance of Genetic lineages of Cesky Fousek (CF). Lineage 1-yellow, lineage 2-blue, lineage 3- white, lineage 7-grey, lineage 9-pink, lineage 10-green



Program Structure could be used for separating each individual to certain cluster (K). For this analysis was used 138 individuals which were appropriate. According to method of Evanno et al. (2005), the most appropriate seems the highest likelihood for K=6 (Figure 16). There were separated GWP and GSP from four parts of CF population. Evident divergence of GWP and GSP was visible also for likelihood K=4 (Figure 17 and Figure 18).

It can be seen that GWP and GSP are more pure or close relative compare to the CF in a structure test analysis (Figure 17 and Figure 18). The GSP is the most uniform which is also visible at triangle visualization (Figure 19).

Another analyses was among genetic lineages of CF population. There was chosen only clear one-lineage individuals. The highest likelihood was for K=4 in that case (Figure 20). Also the result did not show clear clusters. The outcome was unexpected due to accurate lineage breeding in this breed. There was detected strong interior population structure conversely it did not corresponded with each lineage (Figure 21).

Figure 16 Determination of the likelihood -Structure harvester (CF, GWP, GSP populations) behalf of K, by Evanno et al. (2005)

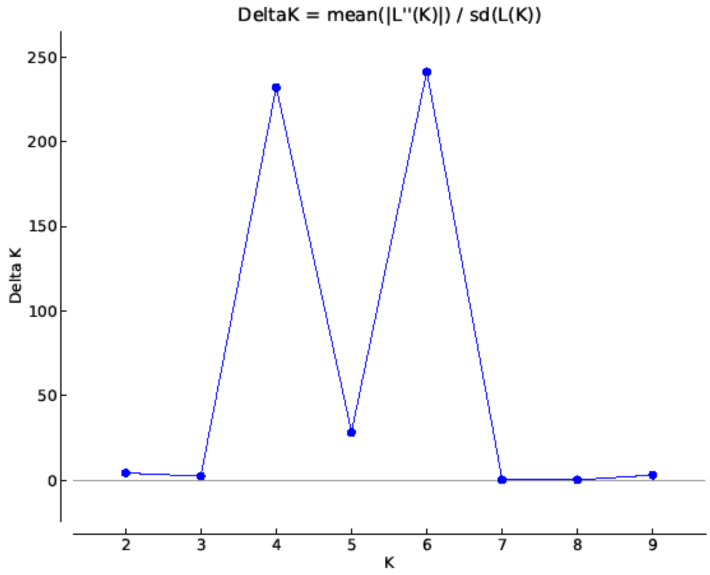


Figure 17 The structure analysis of all three breeds for the highest likelihood, K=6. Number 1-Cesky Fousek (CF), number 2-German Wire-haired Pointer (GWP), and number 3-German Short-haired Pointer (GSP)

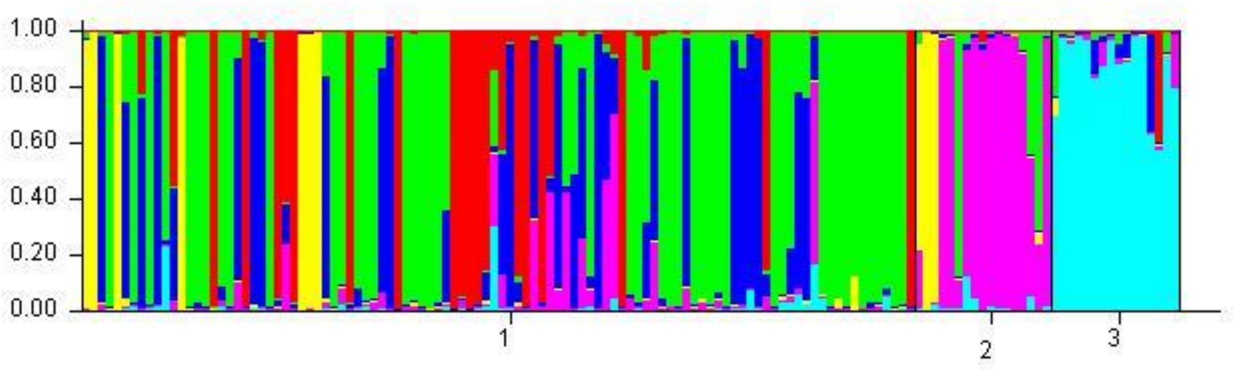


Figure 18 The structure analysis of all three breeds for the second higher likelihood, K=4. Number 1-Cesky Fousek (CF), number 2-German Wire-haired Pointer (GWP), and number 3-German Short-haired Pointer GSP

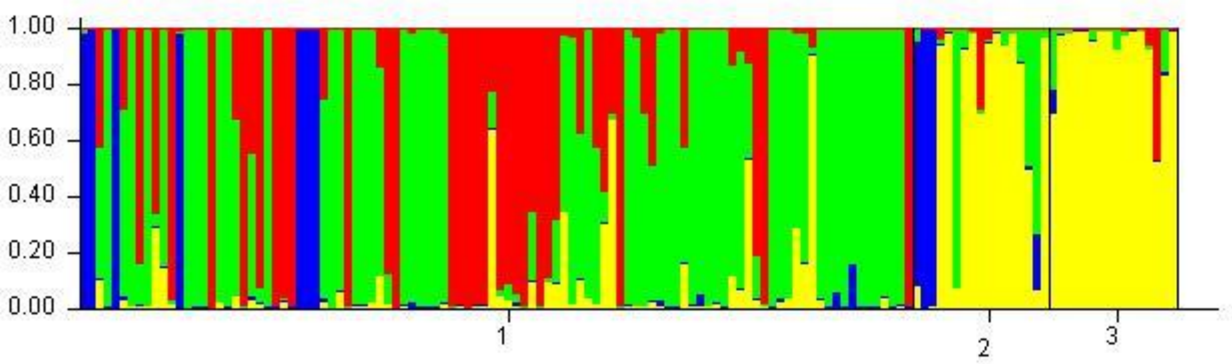


Figure 19 Triangle plot-Structure. Population 1 Cesky Fousek (CF)-red colour, population 2 German Wire-haired Pointers (GWP)-green colour and population 3 German Short-haired Pointer(GSP)-blue colour

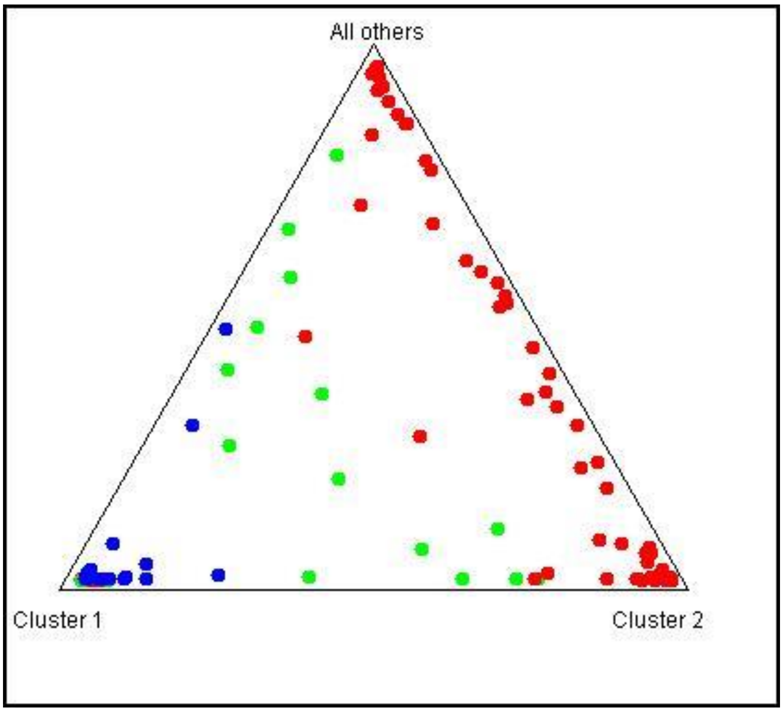


Figure 20 Determination of the likelihood -Structure harvester (CF-lineages) behalf of K, by Evanno et al. (2005)

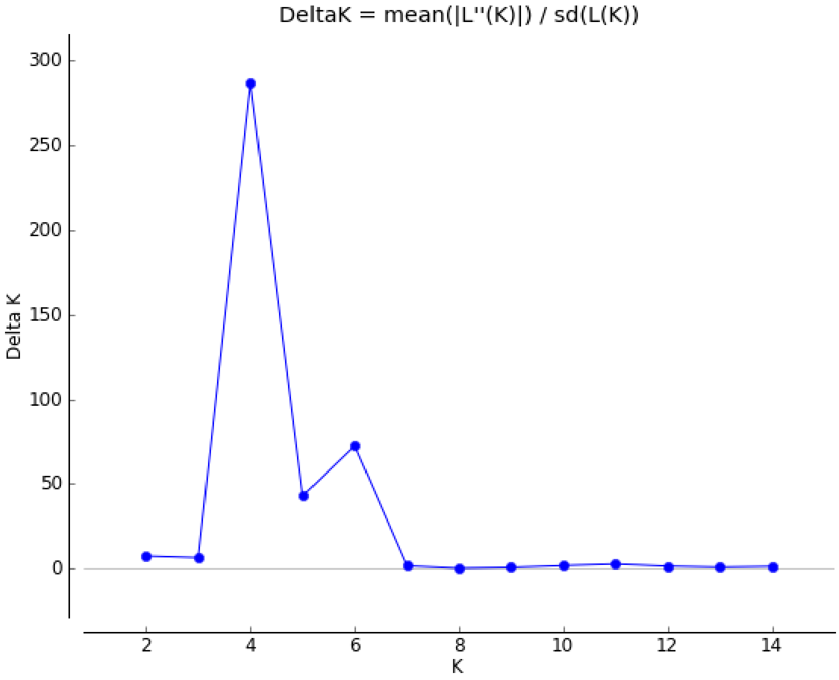
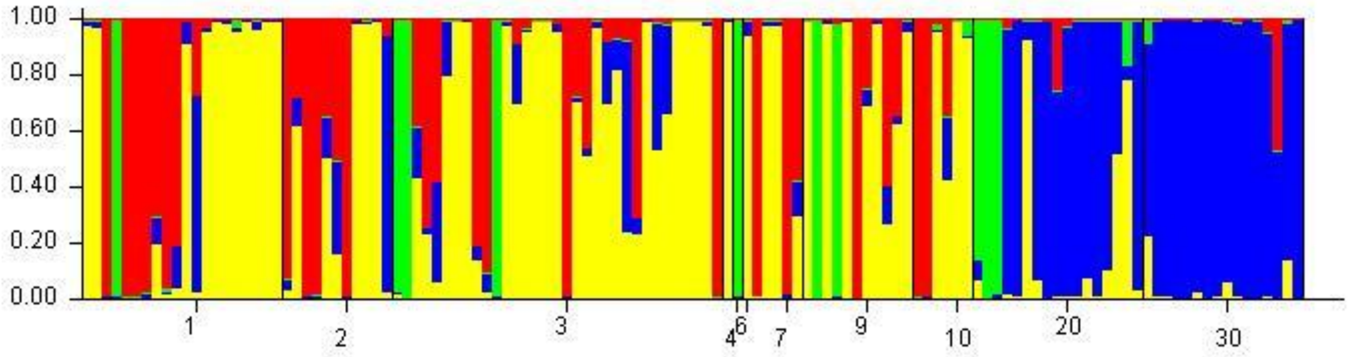


Figure 21 The structure analysis of all genetic lineages of Cesky Fousek (CF) and other two breeds. The highest likelihood, K=4. Lineage of CF: numbers 1; 2; 3;4, 6; 7; 9; 10; number 20-German Wire-haired Pointer (GWP) and number 30-German Short-haired Pointer (GSP)



5 Discussions

CF breed has controlled breeding program. Breeding advisor of Club of breeders picks for each individual the most suitable mating partner. GWP and GSP don’t have so strict breeding rulers however dog owner can follow advisor recommendation about breeding couple. CF population is relatively small and also influenced by historical events - inbreeding, bottleneck during WWI. or founder effect, all these can influence our result.

Loss of genetic variation is expected to occur whenever a population goes through a bottleneck, especially when post-bottleneck recovery is slow. This problem may affect any kind of animals. For instance genetic diversity of The Kruger NP’s elephants population is very low in comparison to East African elephants. Scientist supposed that there was some bottleneck caused by disease in the past (Whitehouse and Harely, 2001) like for example rinderpest pandemic (Vanhoofed et. al., 2000).

Our samples were taken mostly from animals of similar age. We tried not to take more than one specimen of the same gender from the one litter. All 19 loci were polymorphic in our research.

F-statistic results were calculated for each breed in our study. Average inbreeding coefficient (Fis) of CF population was 0,1377. The result is higher than for also native breeds like Bangkaew (0,072) and even Thai Ridgeback dogs (-0,019) (Phavaphutanon and Leopiem, 2011). The origin of the Thai Ridgeback dog is undocumented, but it is known that Bangkaew dog is an old breed so there could be wide gene pool and many different alleles. High value of Fis was also for Lancashire Heeler (0,04-0,1) (Mäki, 2010), but lower than in 5 pointing dog breeds (0,33-0,37) (English Setter, English Pointer, Epagneul Breton, Deutsch Drahthaar and German Shorthaired Pointer), that originate from the same ancestors (Parra et al., 2008). Nova Scotia Duck Tolling Retriever (0,24 - 0,32) (Mäki, 2010) which is considered as an endangered breed because of the small number of dogs (Mäki, 2010). Our result of inbreeding coefficients were 0,0831 for GWP and 0,0944 for GWP that is slightly higher than in reports about GSP by Leroy et al. (2009) or by Erdogan et al., (2013) with the Turkish native dogs. Even Czechoslovakian Wolfdog population show lower Fis (0,02) despite of the origin of CSW breed is based just on four wolf individuals and the breed is very young (Smetanová, 2012)

The result shows that there is not enough heterozygotes in the population. Even worst was outcome when we calculated values of Fis in each genetic lineage of CF separately. Inbreeding coefficient is even higher in each lineage. Interesting point is that Fis for genetic lineage number 7 is the lowest. This can be probably caused by the crossbreeding with GWP in the past. This crossbreeding method was necessary for the breed regeneration. Information about crossbreeding were found in historical documents about breeding management and CF pedigree (Kuhn, 2005).

Average fixation index (Fst) when CF population was measured against the GWP population was 0,0597 and between CF population and GSP, it was 0,0759. Both results mean that differentiation between CF and other population is small. CF is obviously more similar to GWP (also according to result of FCA). Our observed populations are definitely very close. CF population is genetically really closer with GWP than with GSP, which was expected due to historical genetic background and current similar visual appearance. There is also not visible difference among lineages due to mixing some lineages together and closed relation among individuals. Similar low genetic distance was observed among Dachshunds (0,0634) (Přibáňová et al., 2009).

The oldest variety was Standard Smooth-haired Dachshund. Then cross-breeding with Spaniels was used to develop Long-haired (SL), and by mating with Terriers developed breed Wire-haired Dachshunds. A miniature Pincher was an ancestor of smaller varieties (Přibáňová et al., 2009). That historical background reminds CF crossbreeding with GWP.

Many breeders tend to prefer a few breeding males that proved to be successful at shows or working tests. Then, the variability of population could be influenced by genetic drift and can vary substantially generation by generation. F-statistic showed it in study about Dachshunds where dogs shared some alleles (Přibáňová et al., 2009; Mäki, 2010) and they also have common ancestor and crossbreed together in the past because of cross-breeding of Standard Dachshunds with both smaller varieties and different coat type varieties are strictly forbidden since 1970 (Přibáňová et al., 2009).

Population of CF suffers from departures from HWE, the analysis proved significant excess of homozygotes ($p<0,05$). CF population has to struggle against deficiency of heterozygote in the population. In the non-random mating population is difficult to find absolute HWE. Furthermore the difference of He and Ho is related with Hardy-Weinberg principle. There is no balance in our case. Large Fis is either related with deviation from Hardy-Weinberg equilibrium due to heterozygote deficiency (Lupke and Distl, 2005). GWP and GSP populations are in HW equilibrium, there is not excess nor deficiency of heterozygotes.

Overall observed heterozygosity (Ho) of CF population was 0,6626. Heterozygosity ranged between 0,4123 on locus ATHk253 to 0,8707 on locus FH2054. Average expected heterozygosity (He) was 0,7645, ranged between 0,6395 on locus INU055 to 0,8618 on locus REN169O18. Average observed heterozygosity was 0,6890 for GWP and 0,6441 for GSP breed. Average expected heterozygosity was 0,7256 for GWP and 0,6913 for GSP. The result of heterozygosity is comparable with other studies about genetic variability of Dachshunds (Koskiner and Bredbacka, 2000; Přibaňová et al. 2009) or Bracco italiano pointin dog (Ciampolini et al. 2011). The original type of Italian pointing dog almost disappeared due to wrong breeding management. This breed was revitalized after WWII (Ciampolini et al., 2011). Also here is some historical parallel. Furthermore the result is similar to another hunting dogs breeds (Table 5) (Leroy et al., 2009). Detailed comparison of heterozygosity is displayed in Table 5.

Finally, in our result there is not significant sign of inbreeding depression in this population.

Example of a small dog population with high heterozygosity was shown in comparison of the genetic variability of miniature bull terriers with bull terriers in the USA (Iron et al., 2003). It has been reported that the Ho and He values for Akbash, Kangal and Turkish Greyhound are 0,715; 0,701; 0,710 and 0,620; 0,701; 0,705 even those are quite small populations as CF (Erdogan et al., 2013). Expected heterozygosity of the Bracco Italiano is also similar and comparable of other European dog breeds (0,56 Bedlington Terrier; 0,62 Golden Retriever; 0,64 Pembroke Welsh Corgi; 0,64 German Shepherd; and 0,72 Wirehaired Dachshund) (Koskiner and Bredbacka, 2000).

However, different number of microsatellites and different number of individuals which were used in those studies cause it less comparable (Koskiner and Bredbacka, 2000).

Many breed clubs in Finland have (with the Finnish Kennel Club), decided to set a restriction to the number of offspring for a single dog to equalize the contributions of reproducing animals. For the Nova Scotia Duck Tolling Retriever (NS), this limit has been set to 60 from the beginning of the year 2009 (Mäki, 2010). Retriever got about 8 puppies in one litter. Which means about 7 litters that is still a lot. Lancashire heeler is limited by 20 offspring only. This breed is smaller so there could be about 4 litters during the live.

The high average kinship coefficients indicate that the retriever dogs are more closely related to each other than full-siblings. This situation makes it very hard to find pairs of potential breeding dogs that are not closely related to each other (Mäki, 2010) Compared to the findings of Leroy et al. (2009) where breeders did not have any problems to keep low kinship coefficient.

Factorial corresponds analysis (FCA) was completed by the program Genetix (Belkhir et al., 2004). In the graph, a distance between CF, GWP and GSP was not very well detectable. In our study the visualization is not so clear because populations are genetically very close.

However there was found interior structure in CF population. Probably this structure is due to lineage breeding management in this population. There is noticeable big difference among individuals which is not correspondent to genetic lineage at all. Compare to Smetanová (2012) where was not found any interior structure of the Czechoslovakian wolfdog (CSW). Individuals are in all area unstructured and the population is genetically uniform (Smetanová, 2012). CSW breed has got a controlled breeding program which is quite similarly as CF, but there is not separation to lineages (Smetanová, 2012).

Our investigated breeds did not show a clear genetic divergence. Relations among individuals within the one population show very close breeding despite of lineage breeding process. Nevertheless, there was not visible distance among each lineage. The unbalanced quantity of individuals in all lineages may cause the problem as well. In this case, Visser et al. (2004) supposed very close inbreeding of three commercial and three indigenous goat populations from South Africa but the result was finally different. Fst indicated clear differentiation between populations, Fst was about 0,1-0,3 which is surprisingly higher than in our study (0,07-0,1). Likewise their result from Genetix program show clear genetic differentiation and no interior structure (Visser et al., 2004) same as study about CSW (Smetanová, 2012).
The result of unclear separation of population to cluster can be explained by recent gene flow among populations. Recent history of CF breed show crossbreeding with GWP that correspond with the microsatellite result. Program structure doesn’t tests hybridization but it shows measure of shared alleles. The reason of sharing same alleles could be caused by crossbreeding or moreover due to the same origin and common ancestor of CF and GWP. GWP breed was developed from CF breed according historical documents (Kuhn, 2005). CF and GWP are really close so the separation is not very clear in the Structure program.

Result of our research about the genetic variability inside the CF population is not easily identifiable especially in graphic demonstrations. This was surprising outcome because I expected separation to cluster according lineages. Microsatellites show more recent history than ancient times. Nowadays genetic lineages are not strictly kept by breeders as in the past. There is crossbreeding among lineages for healthier dogs. Very often we can find CF individuals which are offspring of parents from lineage 1 and 7 or 3 and 9 respectively. There is either big gene flow among lineages, that covers differentiation of lineages when we use microsatellite data or it can be a bias of used methods. In the future, we will analyze selected individuals one more, to be sure, that no errors were done in laboratory proceedings.

Table 5 Comparing of heterozygosity for different breeds, number of individuals and microsatellites. Expected heterozygosity (He), observed heterozygosity (Ho)

Breed	**Number of individuals**	**Number of microsatellites**	**Ho**	**He**	**Reference**
Cesky Fousek	114	19	0,66	0,77	This paper
German Wire-haired Pointer	18	19	0,69	0,73	This paper
German Short-haired Pointer	19	19	0,64	0,69	This paper
German Short-haired Pointer	8 392	21	0,69	0,70	Leroy et al., 2009
Griffon Korthals	6 711	21	0,71	0,69	Leroy et al., 2009
Griffon Gascone	4 234	21	0,70	0,74	Leroy et al., 2009
Bull Terier	3 378	21	0,41	0,44	Leroy et al., 2009
Bull Terier	27	10	0,65	0,55	Shinkarenko et al., 2008
Czech Dachshund	632	10	0,67	0,74	Přibáňová et al., 2009
Hanoverian Hound	92	16	0,52	0,66	Lupke and Distl, 2005
Cimarron Uruguayo	30	9	0,65	0,63	Galiardi et al., 2011
Bracco italiano	72	21	0,64	0,60	Ciampolini et al., 2011
Jindo	30	11	0,76	0,80	Cho, 2005
Poongsan	20	11	0,50	0,55	Cho, 2005
Thai Ridgeback	39	12	0,81	0,78	Mäki, 2010
Bankew	82	12	0,72	0,77	Mäki, 2010
Turkish Greyhound	31	17	0,71	0,70	Erdogan et al., 2013
Greyhounds	50	19	0,33	0,36	Zajc et al., 1997
Golden Retriever	25	10	0,62	0,62	Koskinen and Bredbacka 2000
Pembroke Welsh Corgi	25	10	0,55	0,64	Koskinen and Bredbacka 2000

6 Conclusion

The analysis proved that populations of CF, GWP and GSP are genetically very close to each other. Purebred animals display a increased degree of homozygosity compared to mixed breeds and random-bred animals.

Inbreeding coefficient was highest for CF breed, which can be caused by rapid decrease of individual in population during the war or in recent. Positive values of Fis indicate that there is an increased number of homozygotes. Also inbreeding coefficient was higher for each lineage of CF than for all population CF. However the degree of inbreeding coefficient is not disturbing. There is only limited threat of inbreeding depression, but for the future, breeders should avoid of interbreeding between closely related individuals. The situation is not alarming because there are several breeding stations of CF in Czech Republic or in abroad. Moreover, breeders don't keep strict mating in one genetic lineage; they combine breeding couple from different lineages, which we find positive, because in the most mixed lineage 7, the Fis was lowest from the whole breed.

Equal number of individual in each population should be recommended for the next analysis. Also another wire-haired breed, such as pudlpointer, French wire-haired Korthals Griffon, Hungarian wirehaired vizsla or Slovak wirehaired pointer which was crossbreed with CF or GWP in the past could be included to the next research. For more exact outcome would be belter to use different markers such as single-nucleotide polymorphism (SNP) which are more suitable for detaching ancient historical events of the population and compile phylogenetic tree. This is real difference between SNP and microsatellites. Microsatellites show mainly recent history of population so we could not evaluate all procedures which happened during the population existence. Also the difference in processing during PCR and consequent correlation and correcting of results could generate difference in result. I had to stay with this outcomes due to time press and impossibility to recount all samples and rewrite almost 8000 of numbers.

Information about effective population size, heterozygosity levels and inbreeding coefficient for individuals, can be used to design breeding programs which can improve genetic variation.

CF is important model organism which can be used in researches focused on genetic diversity within and between populations or as a line-breeding management model.

History of CF is very difficult and complex and some genomic methods with more markers may be better to reveal these old and not easily traceable events.

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Appendix 1: There are all collected samples. ČF-Cesky Fousek, NDO-German Wire-haired Poiner, NKO-German Short-haired Pointer. It shows number of samples, breed, name of individuals, gender and age

Číslo vzorku	Plemeno	Jméno psa	Pohlaví	Věk
1	ČF	Dora z putimy	F	1
2	NDO	Mola	F	1
3	NKO	Erik z Břízké cihelny	M	1
4	ČF	Gáby z ventova dvora	F	1
5	ČF	Barra Pedrix Bohemia	F	2
6	ČF	Glen z Těšínovských buků	M	1
7	ČF	Cip z Novoveského lesa	M	1
8	ČF	Chyt od Pitné vody	M	1
9	ČF	Ed od pitné vody	M	10
10	ČF	Blesk z Lovčických tarasů	M	3
11	ČF	Mora z Aufrizu	F	2
12	ČF	Bak z Oldřichovského Buku	M	3
13	ČF	Arny z Údolí potoka	M	2
14	ČF	Gina od Frimlů	F	3
15	ČF	Blachie od Ječinských dubů	F	4
16	ČF	Greisy z Ventova dvora	F	1
17	ČF	Eysha z Růžičkova dvora	F	1
18	ČF	Fida z Ventova dvora	F	2
19	ČF	Connie de Jean Baptiste	F	3
20	ČF	Alu z Údolí potoka	F	2
21	ČF	Chlup od Pitné vody	M	1
22	ČF	Eldorádo z Těšínovských buků	M	2
23	ČF	Art ze Zelených domků	M	2
24	ČF	Cindy z Novoveského lesa	F	1
25	ČF	Ara z Šerosvitu	F	1
26	ČF	Dona z Bohušovické tůně	F	1
27	ČF	Coudy z Křenkovských luk	M	2
28	ČF	Adar z Žaboklik	M	10
29	ČF	Endy z Ventova dvora	F	8
30	ČF	Brenda z Novoveského lesa	F	2
31	ČF	Polly z hložku	F	2
32	ČF	Iris z Ventova dvora	F	1
33	ČF	Winner z Těšínovských buků	M	4
34	ČF	Orka z Radějovicka	F	1
35	ČF	Clea od Růvra	F	3
36	ČF	Sir z langova dvora	M	1
37	ČF	Aida ze Senického dvora	F	2
38	ČF	Dar z Huťské osady	M	5
39	ČF	Jerry Akcezal	M	8
40	ČF	Aran z Rohacka	M	1
41	ČF	Chán od Frimlů	M	1
42	ČF	Archie zpod Velinské stráně	M	1
43	ČF	Agáta z pod Velinské stráně	F	1
44	ČF	Hassy od Vavřineckého rybníka	F	1
45	ČF	Fela ze Staropleských luk	F	2
46	ČF	Fido ze Staropleských luk	M	2
47	ČF	Bad z Bernartických hájů	M	1

48	ČF	Bona Gala Bouček	F	2
49	ČF	Any z Honsova sadu	F	4
50	ČF	Dina Rybnov	F	1
51	ČF	Heda Krupá	F	2
52	ČF	Dora z Lívy	F	2
53	ČF	Cira od Lejnarů	F	2
54	ČF	Aida z Městečka na dlani	F	2
55	ČF	Alma od Suché Vody	F	3
56	ČF	Mirka z Koblova	F	1
57	ČF	Argo z Vrzavky	M	2
58	ČF	Lesan z Koblova	M	2
59	ČF	Gordy z Podhoránku	F	2
60	ČF	Ina z Ventova dvora	F	1
61	ČF	Ajša z Řáholečku	F	3
62	ČF	Nyka od Krále z Brusnice	F	8
63	ČF	Ben z Vrzavky	M	1
64	ČF	Zara z Fešandy	F	3 měs.
65	ČF	Ax z Řáholečku	M	3
66	ČF	Dixi z Křenovských luk	F	1
67	ČF	Nessi z Aufrízu	F	1
68	ČF	Cip z Honsova sadu	M	3
69	ČF	Fatima ze Staropleských luk	F	2
70	ČF	Arina z Městečka na dlani	F	2
71	ČF	Andy od Šibené hory	F	2
72	ČF	Dona z Kablaně	F	2
73	ČF	Asta od Šibené hory	F	2
74	ČF	Čelsi z Debce	F	1
75	ČF	Bard Gala Bouček	M	2
76	ČF	Cita z Otmického polesí	F	1
77	ČF	Baron z Cmolova pole	M	2
78	ČF	Akim Fadra	M	1
79	ČF	Emír od Olší	M	2
80	ČF	Gir z Křepických strání	M	3
81	ČF	Flor z Citoňských luk	M	2
82	ČF	Cvik z Debce	M	3
83	ČF	Bára z Blatin	F	2
84	NDO	Dora od Robotného mostu	F	4
85	NDO	Uran z Těšnice	M	3
86	NDO	Car od Robotného mostu	M	5
87	NDO	Fiete z Rafajova revíru	M	1
88	NDO	Asta z Koldínského lesa	F	3
89	NDO	Brita z Malého a velkého vrchu	F	1
90	ČF	Ura Klape z Brna	F	2
91	NDO	Cora od Verunky	F	2
92	NDO	Cira z Koldínského lesa	F	1
93	ČF	Ajla od Žalkovických polí	F	2
94	NDO	Cir od Velké lípy	M	2
95	ČF	Gloria z těšínovských buků	F	1
96	NDO	Bert z Koldínského lesa	M	3
97	NDO	Gastor od Oskavy	M	3
98	ČF	Dora z Včelínského lesa	F	3
99	NDO	Jack z Adamovských revírů	M	2
100	ČF	Alma z kamenného kopce	F	2

101	ČF	Borka z Křenovských luk	F	3
102	ČF	Monča z Koblova	F	1
103	ČF	Dita od panského rzbníka	F	2
104	ČF	Bára z Mandátu	F	3
105	NDO	Ikar od letu	M	6
106	ČF	Asta z Rakodavských bran	F	2
107	ČF	Era z Krajčovickej farmy	F	2
108	ČF	Pondy z Belečských strání	F	2
109	NDO	Gim od Oskavy	M	3
110	NDO	Ema z Myslivcova dvora	F	2
111	NDO	Tor z Těšince	M	5
112	ČF	Besy z Ucháče	F	2
113	NDO	Men od Bačurina	M	2
114	NDO	Erna od Olší	F	
115	ČF	Brixa z Lubiechowa	F	2
116	ČF	Arka z Kamenného kopce	F	2
117	ČF	Bora z Rohozné	F	2
118	ČF	Jeny ze Staré vinice	F	2
119	ČF	Abi Farní dvůr	F	1
120	ČF	Cira z Ucháče	F	1
121	ČF	Connie z Pískové zahrady	F	1
122	ČF	Heda z Přerovska	F	3
123	ČF	Alan z Podveselských lesů	M	1
124	ČF	Gama z Nolkopu	F	3
125	ČF	Dona Gala bouček	F	1
126	ČF	Asta Jarpol	F	1
127	ČF	Fira z Citoňských luk	F	2
128	ČF	Kazan ze Strážného kopce	M	3
129	ČF	Alma Jarpol	F	1
130	ČF	Borek z Cmolova pole	M	2
131	ČF	Andy z Rakodavských bran	F	2
132	ČF	Hart z Nolkopu	M	2
133	ČF	Arka z Kudrnova dvora	F	2
134	ČF	Arra z Neřádova stavení	F	2
135	ČF	Brit z Volánských louží	M	2
136	ČF	Bessy z lesní	F	1
137	ČF	Hexa z Přerovska	F	3
138	NDO	Aska Nitranské Janíkovice	F	4
139	ČF	Grika z Včelínského lesa	F	
140	ČF	sestra 139	F	
141	ČF	Argo od Jenčinských dubů	M	
142	NDO	Grand Žitňan	M	
143	NDO	Kid od Letu	M	3
144	NDO	Blek od Zábořského soudku	M	1
145	ČF	Aida z Bohušovické tůně	F	6
146	ČF	Don Hrabické remízky	M	8
147	ČF	Akina z Kazimburských strání	F	2
148	ČF	Besina od Půvra _?	F	5
149	ČF	Fany z Ventova dvora	F	
150	ČF	Bessy z Rohatecké slatiny	F	
151	ČF	Andy z Debce	M	7
152	ČF	Alan z Huťské osady	M	8
153	NKO	Lena z Lavičné	F	1

154	NKO	Alfa z Tomešova dvora	F	6
155	NKO	Beauty AnukkJa-Sta Czech	F	2
156	NKO	Axa z Budiměřických polí	F	4
157	NKO	Norma od Řeky Lomnice	F	1
158	NKO	Alma od Mutěnických rybníků	F	3
159	NKO	Euforie z Chlumína	F	2
160	NKO	Absolut pod Doubravským vrachem	M	2
161	NKO	Fabby Ja-Sta Czech	F	1
162	NKO	Argo od Bojanovského smrku	M	3
163	NKO	Besy z Nezdin	F	4
164	NKO	Bailey pod Doubravskýcm vrchem	F	1
165	NKO	Every Step Ja-Sta Czech	F	1
166	NKO	Ela ze Slavonic	F	2
167	NKO	Bax z Krzáku	M	1
168	NKO	Afrodíta od Čertovi studánk	F	1
169	NKO	Aira z Rumzí	F	2
170	ČF	Garo z Čepelu	M	1
771	ČF	Hankybelge	M	
172	ČF	Borek z Chatrniosti	M	
173	ČF	Hexa od Líbalky	F	
174	ČF	Salto z Kyjovic		
175	ČF	Besi z Blatin	F	
176	ČF	Bary z Honzova Sadu		
177	ČF	Alka z Cmolova Pole	F	1
178	ČF	Lex z Drňovek	M	2
179	ČF	Katy u Klobásné	F	1
180	ČF	Hera z Křepických strání	F	1
181	ČF	Cedra z Vltavského Luhu	F	1
182	ČF	Backy ze Zemanského dvora	F	1
183	ČF	Bessi z Bernatických hájů	F	1
184	ČF	Quanta z Hložku	F	
185	ČF	Dafy Cerohaz	M	3
186	ČF	Daf Z Chladné stráně	F	1
187	ČF	Ira ze Zvíkovské bašty	F	1
188	ČF	Cedra Z Mutických vršků	F	1
189	ČF	Borka z Otmického polesí	F	3
190	ČF	Hasta z Podhoránku	F	1
191	ČF	Geny z Křepických strání	F	3
192	ČF	Herkules z Těšínovských buků	M	1
193	ČF	Ben od Jemčinských dubů	M	4
194	ČF	Borek z Duškova dvora	M	10
195	ČF	Cira ze Zemanského dvora	F	1
196	ČF	Kira u Klobásné	F	1
197	ČF	Bora z Duškova dvora	F	10
198	ČF	Adar z Havlovky	M	2
199	ČF	Bára od Lejnarů	F	
200	NKO	Car z Nehybova dvora	M	7 měs
201	NKO	Damar z Nehybova dvora	M	3 měs
202	NKO	Dorka z Nehybova dvora	F	3 měs
203	NDO	Frigo Orlovská Holubina	M	5
204	NKO	Dona Afoli	F	
205	NKO	Eby z Jesenky	F	4

Appendix 2: Only Cesky Fousek (CF) breed. There are information about individuals number of samples, genetic lineages, blood-crossbreeding , name, breeding station, gender, date of birth and colour (made by Sobolík personally, 2013)

No.	Breed	Pedigreed	Blood	Lineage	Name	Breeding station	Sex	Birth	Colour
1	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Dora	z Putimi	F	17.5.2011	bělouš
4	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Gáby	z Ventova dvora	F	8.2.2011	hnědák
5	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Barra	Perdix Bohemia	F	4.1.2010	hnědák
6	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Glen	z Těšínovských buků	P	4.4.2011	bělouš
7	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Cip	z Novoveského lesa	P	21.7.2011	bělouš
8	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Chyt	od Pitné vody	P	4.6.2011	bělouš
9	ČF	VÍCELINIOVÝ HYBRID	CF	***4***7***	Ed	od Pitné vody	P	6.5.2002	bělouš
10	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Blesk	z Lovčických tarasů	P	7.4.2009	bělouš
11	ČF	VÍCELINIOVÝ HYBRID	CF	1********9*	Mora	z Aufrízu	F	18.6.2010	bělouš
12	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Bak	z Oldřichovského Buku	P	31.12.2008	bělouš
13	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Arny	z Údolí potoka	P	18.6.2010	hnědák
14	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Gina	od Frimlů	F	25.1.2009	bělouš
15	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Blackie	od Jemčinských dubů	F	22.11.2008	hnědák
16	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Greisy	z Ventova dvora	F	8.2.2011	hnědák
17	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Eysha	z Růžičkova dvora	F	18.3.2011	hnědák
18	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Fida	z Ventova dvora	F	19.2.2010	bělouš
19	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Connie	de Jean Baptiste	F	18.4.2009	bělouš
20	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Alu	z Údolí potoka	F	18.6.2010	bělouš
21	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Chlup	od Pitné vody	P	4.6.2011	bělouš
22	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Eldorádo	z Těšínovských buků	P	10.8.2010	bělouš
23	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Art	ze Zelených domků	P	23.6.2010	bělouš
24	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Cindy	z Novoveského lesa	F	21.7.2011	bělouš
25	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Ara	z Šerosvitu	F	14.1.2011	bělouš
26	ČF	VÍCELINIOVÝ HYBRID	CF	**3******9*	Dona	z Bohušovické tůně	F	21.1.2011	hnědák
27	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Coudy	z Křenovských luk	P	28.5.2010	hnědák
28	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Adar	z Žaboklik	P	6.5.2002	hnědák
29	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Endy	z Ventova dvora	F	28.6.2006	hnědák
30	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Brenda	z Novoveského lesa	F	9.6.2005	bělouš
31	ČF	VÍCELINIOVÝ HYBRID	CF	**3******9*	Polly	z Hložku	F	30.7.2010	bělouš
32	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Iris	z Ventova dvora	F	13.6.2011	bělouš
33	ČF	VÍCELINIOVÝ HYBRID	CF	**3******9*	Winner	z Těšínovských buků	P	7.3.2008	bělouš

34	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Orka	z Radějovicka	F	14.4.2011	bělouš
35	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Clea	od Růvra	F	12.1.2009	hnědák
36	ČF	KŘÍŽENEC_KO	CF/KO	*2*3*******	Sir	z Langrova dvora	P	4.8.2011	hnědák
37	ČF	VÍCELINIOVÝ HYBRID	CF	*****6****	Aida	ze Senického dvora	F	22.4.2010	bělouš
38	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Dar	z Huťské osady	P	2.6.2007	bělouš
39	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Jerry	Akcezal	P	1.1.2004	bělouš
40	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Aran	z Rohacka	P	19.9.2011	bělouš
41	ČF	VÍCELINIOVÝ HYBRID	CF	***4******	Chán	od Frimlů	P	15.3.2011	bělouš
42	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Archie	zpod Velinské stráně	P	24.4.2011	bělouš
43	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Agáta	zpod Velinské stráně	F	24.4.2011	bělouš
44	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Hassy	od Vavřineckého rybníka	F	22.1.2011	bělouš
45	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Fela	ze Staropleských luk	F	25.12.2010	hnědák
46	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Fido	ze Staropleských luk	P	25.12.2010	hnědák
47	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Bad	z Bernartických hájů	P	28.3.2011	bělouš
48	ČF	VÍCELINIOVÝ HYBRID	CF	**3*4******	Bona	Gala Bouček	F	19.8.2010	bělouš
49	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Any	z Honsova sadu	F	5.9.2008	bělouš
50	ČF	JEDNOLINIOVÝ HYBRID	CF	********9*	Dina	Rybnov	F	23.2.2011	hnědák
51	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Heda	Krupá	F	20.8.2010	hnědák
52	ČF	VÍCELINIOVÝ HYBRID	CF	1******7***	Dora	z Lívy	F	14.9.2010	hnědák
53	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Cira	od Lejnarů	F	5.5.2010	hnědák
54	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Aida	z Městečka na dlani	F	19.5.2010	hnědák
55	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Alma	od Suché Vody	F	18.7.2009	hnědák
56	ČF	JEDNOLINIOVÝ HYBRID	CF	1*********	Mirka	z Koblova	F	8.2.2011	hnědák
57	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Argo	z Vrzavky	P	10.2.2010	bělouš
58	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Lesan	z Koblova	P	15.2.2010	hnědák
59	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Gordy	z Podhoránku	F	17.5.2010	hnědák
60	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Ina	z Ventova dvora	F	13.6.2011	bělouš
61	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Ajša	z Řáholečku	F	12.6.2009	hnědák
62	ČF	JEDNOLINIOVÝ HYBRID	CF	1*********	Nyka	od Krále z Brusnice	F	3.3.2004	hnědák
63	ČF	KŘÍŽENEC_NDO	CF/NDO	**3****7***	Ben	z Vrzavky	P	4.6.2011	hnědák
64	ČF								
65	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Ax	z Řáholečku	P	12.6.2009	hnědák
66	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Dixi	z Křenovských luk	F	20.4.2011	bělouš
67	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Nessi	z Aufrízu	F	1.1.2011	bělouš

68	ČF	JEDNOLINIOVÝ HYBRID	CF	*********10	Cip	z Honsova sadu	P	5.5.2010	bělouš
69	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Fatima	ze Staropleských luk	F	25.12.2010	hnědák
70	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Arina	z Městečka na dlani	F	19.5.2010	bělouš
71	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Andy	od Šibené hory	F	1.7.2010	hnědák
72	ČF	KŘÍŽENEC_NDO	CF/NDO	********9*	Dona	z Kablaně	F	20.2.2010	bělouš
73	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Asta	od Šibené hory	F	1.7.2010	bělouš
74	ČF	VÍCELINIOVÝ HYBRID	CF	1********9*	Čelsi	z Debce	F	3.4.2011	hnědák
75	ČF	VÍCELINIOVÝ HYBRID	CF	**3*4******	Bard	Gala Bouček	P	19.8.2010	bělouš
76	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Cita	z Otmického polesí	F	13.3.2011	hnědák
77	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Baron	z Cmolova pole	P	9.3.2010	bělouš
78	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Akim	Fadpa	P	15.4.2011	hnědák
79	ČF	KŘÍŽENEC_KO	CF/KO	*2********	Emír	od Olší	P	3.1.2010	bělouš
80	ČF	KŘÍŽENEC_KO	CF/KO	1*********	Gir	z Křepických strání	P	7.2.2009	bělouš
81	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Flor	z Citoňských luk	P	1.12.2010	hnědák
82	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Cvik	z Debce	P	13.3.2009	hnědák
83	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Bára	z Blatin	F	15.2.2010	hnědák
90	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Ura	Klape z Brna	F	15.3.2010	bělouš
93	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Ajla	od Žalkovických polí	F	14.10.2010	bělouš
95	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Gloria	z Těšínovských buků	F	4.4.2011	bělouš
98	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Dora	z Včelínského lesa	F	11.5.2009	bělouš
100	ČF	KŘÍŽENEC_KO	CF/KO	**3*******	Alma	z Kamenného kopce	F	22.6.2010	hnědák
101	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Borka	z Křenovských luk	F	15.2.2009	hnědák
102	ČF	JEDNOLINIOVÝ HYBRID	CF	1*********	Monča	z Koblova	F	8.2.2011	hnědák
103	ČF	VÍCELINIOVÝ HYBRID	CF	1*********10	Dita	od Panského rybníka	F	19.7.2010	bělouš
104	ČF	KŘÍŽENEC_NDO	CF/NDO	1*********	Bára	z Mandátu	F	22.4.2009	bělouš
106	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Asta	z Rakodavských bran	F	28.11.2010	bělouš
107	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Era	z Krajčovičovej farmy	F	7.6.2010	bělouš
108	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Pondy	z Bělečských strání	F	26.4.2010	bělouš
112	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Besy	z Ucháče	F	8.9.2010	bělouš
115	ČF	KŘÍŽENEC_KO	CF/KO	**3*******	Brixa	z Lubiechowa	F	28.7.2010	hnědák
116	ČF	KŘÍŽENEC_KO	CF/KO	**3*******	Arka	z Kamenného kopce	F	22.6.2010	bělouš
117	ČF	KŘÍŽENEC_KO	CF/KO	*2********	Bora	z Rohozné	F	18.6.2010	bělouš
118	ČF	VÍCELINIOVÝ HYBRID	CF	1********9*10	Jeni	ze Staré vinice	F	28.11.2010	bělouš
119	ČF	KŘÍŽENEC_KO	CF/KO	********9*	Abi	Farní dvůr	F	19.4.2011	bělouš

120	ČF	KŘÍŽENEC_NDO	CF/NDO	*********10	Cira	z Ucháče	F	3.5.2011	bělouš
121	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Connie	z Pískové zahrady	F	10.4.2011	bělouš
122	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Heda	z Přerovska	F	1.6.2009	bělouš
123	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Alan	z Podveselských lesů	P	8.3.2011	hnědák
124	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Gama	z Nolkopu	F	18.1.2009	bělouš
125	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Dona	Gala Bouček	F	7.7.2011	bělouš
126	ČF	KŘÍŽENEC_NDO	CF/NDO	1******7***	Asta	JARPOL	F	1.5.2011	bělouš
127	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Fira	z Citoňských luk	F	1.12.2010	bělouš
128	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Kazan	ze Strážného kopce	P	8.4.2009	bělouš
129	ČF	KŘÍŽENEC_NDO	CF/NDO	1******7***	Alma	JARPOL	F	1.5.2011	bělouš
130	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Borek	z Cmolova pole	P	9.3.2010	bělouš
131	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Andy	z Rakodavských bran	F	28.11.2010	bělouš
132	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Hart	z Nolkopu	P	16.5.2010	bělouš
133	ČF	VÍCELINIOVÝ HYBRID	CF	1********9*	Arka	z Kudrnova dvora	F	18.5.2010	bělouš
134	ČF	KŘÍŽENEC_NDO	CF/NDO	******7***	Arra	z Neřádova stavení	F	15.5.2010	bělouš
135	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Brit	z Volánských louží	P	14.4.2010	hnědák
136	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Bessy	z Lesní	F	27.5.2011	hnědák
137	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Hexa	z Přerovska	F	1.6.2009	hnědák
139	ČF								
140	ČF								
141	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Argo	od Jemčinských dubů	P	3.2.2007	bělouš
145	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Aida	z Bohušovické tůně	F	27.9.2006	bělouš
146	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Don	Hrabicské remízky	P	6.10.2004	hnědák
147	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Aki	z Hazmburských strání	F	15.7.2010	hnědák
148	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Besina	od Růvra	F	31.3.2007	hnědák
149	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Fany	z Ventova dvora	F	19.2.2010	hnědák
150	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Bessy	z Rohatecké slatiny	F	22.4.2008	hnědák
151	ČF	VÍCELINIOVÝ HYBRID	CF	1********9*	Andy	z Debce	P	11.5.2005	bělouš
152	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Alan	z Huťské osady	P	22.6.2004	bělouš
170	ČF								
172	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Borek	z Chatrnosti	P	17.5.2006	bělouš
173	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Hexa	od Líbalky	F	3.1.2010	hnědák
174	ČF	KŘÍŽENEC_KO	CF/KO	*2********	Salto	z Kyjovic	P	13.1.2009	hnědák
175	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Bessi	z Blatin	F	15.2.2010	hnědák
176	ČF	VÍCELINIOVÝ	CF	*******	Bary	z Honsova sadu	P	11.9.2009	bělouš

		HYBRID		**10					
177	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Alka	z Cmolova pole	F	10.6.2011	bělouš
178	ČF	JEDNOLINIOVÝ HYBRID	CF	1*********	Lex	z Drňovek	P	25.5.2010	bělouš
179	ČF	VÍCELINIOVÝ HYBRID	CF	1**3******9*	Katy	u Klobásné	F	13.2.2011	bělouš
180	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Hera	z Křepických strání	F	20.1.2011	bělouš
181	ČF	VÍCELINIOVÝ HYBRID	CF	1*********10	Cedra	z Vltavského luhu	F	12.5.2011	bělouš
182	ČF	KŘÍŽENEC_KO	CF/KO	**3*******	Backy	ze Zemanského dvora	F	8.2.2011	hnědák
183	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Bessi	z Bernartických hájů	F	28.3.2011	bělouš
184	ČF	VÍCELINIOVÝ HYBRID	CF	********9*	Quanta	z Hložku	F	17.3.2011	bělouš
185	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Dafy	Cerohaz-A+P	P	9.10.2009	hnědák
186	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Dafy	z Chladné stráně	F	7.3.2011	bělouš
187	ČF	VÍCELINIOVÝ HYBRID	CF	**3*******	Ira	ze Zvíkovské bašty	F	30.4.2011	bělouš
188	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Cedra	z Mutických vršků	F	5.1.2011	hnědák
189	ČF	VÍCELINIOVÝ HYBRID	CF	*2********	Borka	z Otmického polesí	F	20.11.2009	hnědák
190	ČF	JEDNOLINIOVÝ HYBRID	CF	*2********	Hasta	z Podhoránku	F	7.9.2011	hnědák
191	ČF	KŘÍŽENEC_KO	CF/KO	1*********	Geny	z Křepických strání	F	7.2.2009	bělouš
192	ČF	VÍCELINIOVÝ HYBRID	CF	*********10	Herkules	z Těšínovských buků	P	8.12.2011	bělouš
193	ČF	VÍCELINIOVÝ HYBRID	CF	1*********	Ben	od Jemčinských dubů	P	22.11.2008	bělouš
194	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Borek	z Duškova dvora	P	5.7.2002	bělouš
195	ČF	KŘÍŽENEC_KO	CF/KO	**3******9*	Cira	ze Zemanského dvora	F	25.9.2011	hnědák
196	ČF	VÍCELINIOVÝ HYBRID	CF	1**3******9*	Kira	u Klobásné	F	13.2.2011	bělouš
197	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Bora	z Duškova dvora	F	5.7.2002	bělouš
198	ČF	KŘÍŽENEC_NDO	CF/NDO	********9*	Adar	z Havlovky	P	30.5.2010	bělouš
199	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Bára	od Lejnarů	F	16.4.2004	hnědák
206	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Caddy	z Rohatecké slatiny	F	28.4.2009	hnědák
771	ČF	JEDNOLINIOVÝ HYBRID	CF	**3*******	Hankybelge		P	28.4.2008	bělouš