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Founder representation and effective population size in old vs. young breeds – genetic diversity of Finnish and Nordic Spitz

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Summary

Finnish Spitz is 130 years old breed and has been highly popular in Finland throughout its history. Nordic Spitz is very similar to Finnish Spitz by origin and use, but is a relatively recent breed with much smaller population size. In order to see how breed age and breeding history have influenced the current population, we performed comprehensive population genetic analysis using pedigree data of 28,119 Finnish and 9,009 Nordic Spitzes combined with genome-wide single nucleotide polymorphism (SNP) data from 135 Finnish and 110 Nordic Spitzes. We found that the Finnish Spitz has undergone repeated male bottlenecks resulting in dramatic loss of genetic diversity, reflected by 20 effective founders (f_a) and mean heterozygosity (Hz) of 0.313. The realized effective population size in the breed based on pedigree analysis ($\bar{N}_{ec}$) is 168, whereas the genetic effective population size (N_{eg}) computed the decay of linkage disequilibrium (r^2) is only 57 individuals. Nordic Spitz, although once been near extinction, has not been exposed to similar repeated bottlenecks than Finnish Spitz and had f_a of 27 individuals. However, due to the smaller total population size, the breed has also smaller effective population size than Finnish Spitz ($\bar{N}_{ec}$ = 98 and N_{eg} = 49). Interestingly, the r^2 data shows that the effective population size has contracted dramatically since the establishment of the breed, emphasizing the role of breed standards as constrains for the breeding population. Despite the small population size, Nordic Spitz still maintains SNP heterozygosity levels similar to mixed breed dogs (mean Hz = 0.409). Our study demonstrates that although pedigree analyses cannot provide estimates of the present diversity within a breed, the effective population sizes inferred from them correlate with the genotyping results. The genetic relationships of the northern Spitz breeds and the benefits of the open breed registry are discussed.

Introduction

Hunting with Spitz-type dogs can be seen as a continuation of thousands of years old cultural tradition in the Nordic countries (Mannermaa *et al.* 2014). Historically the medium sized Spitz dogs have been most important for hunting important fur game, such as squirrel and pine marten, but after fur trade became insignificant as a means of subsistence, the dogs have been

more commonly used for recreational hunting of capercaillie (*Tetrao urogallus* L.) and black grouse (*Tetrao tetrix* L.).

The cultural significance of native dog breeds was understood in Finland in the late 19th century. Together with Finnish hound, Finnish Spitz (Figure 1) was recognized as a breed in 1892. As a consequence, a subset of feral Spitz types (Figure 2), especially in northern Finland and Sweden, which did not fit to the Finnish Spitz breed standards were almost driven into extinction (Aarnio 2008). The first attempt to rescue the northern type was made in Sweden in 1910 by setting a breed standard for *Norrbottens skällande fågelhund*. Only few individuals were registered and the breed was thought to be extinct in 1947. In 1966 the breed was revived again as *Norbottenspets* or Nordic Spitz, using dogs collected from northern Sweden and Finland with the help of newspaper ads. The originally narrow pool of founders was further increased by dogs from the Finnish Lapland and Kuusamo region during 1973-1980. The studbook was closed in Sweden in the 1980s but has remained open in Finland for any unregistered Spitz fitting the breed standards. While Nordic Spitz has recently become popular in Finland with 382 puppies registered in 2016, the breed remains small in Sweden with only 108 registrations in 2016. Smaller populations exist also in other Nordic countries and Canada. Because of its status as a national dog, Finnish Spitz is among the most popular breeds in Finland with 737 registrations in 2016. The breed is also more popular than Nordic Spitz in Sweden with 147 registrations in 2016.

As Finnish and Nordic Spitz share the same functional niche and originate from the same geographical area, they represent a unique case to study the effects of artificial selection, discrete trait ideals and breed history on the genetic diversity of the breed. Finnish Spitz is an old breed, has a closed breed register and has quite likely been influenced by strong selective breeding for most of its history. Nordic Spitz is a much more recent breed, has a partly open registry with much more relaxed requirements for e.g. coat color and type than Finnish Spitz. Nordic Spitz, however, has always had a relatively small population size compared to Finnish Spitz, which likewise could have implications on the breed's genetic structure.

The genetic diversity of a species or a breed is ultimately connected to its evolutionary potential (Kristensen *et al.* 2015). Small populations suffer from inbreeding and the loss of genetic variation due to drift. Intentional inbreeding is commonly used to enrich favored phenotypes in domesticated animals and can have detrimental effects for breed health and reproduction

(Leroy *et al.* 2015). Even if mating of close relatives is avoided, strong selection for a few favored traits in dogs can unintentionally increase the amount of deleterious genetic variants (Marsden *et al.* 2016). An extreme example of a Spitz-type breed that was driven close to extinction due to a dramatic reduction in population size and extreme inbreeding is the Norwegian Lundehund, whose genetic rescue is attempted by crossing it with Nordic Spitz and possibly other suitable breeds (Kristensen *et al.* 2015).

In order to understand how breeding history and population size affects the genetic diversity of a breed, we have reconstructed the pedigrees for all known individuals of Finnish and Nordic Spitz in the Scandinavian countries, identified the population founders and their relative contributions to the present population, computed the levels of coancestry, as well as inbreeding, and their impact on effective population size, and compared the results with the effective population size and genetic diversity observed from SNP data from the breeds. The implications of the findings as well as the prospects of open breed registries and breed crosses are discussed.

Materials and Methods

Pedigree data and the evolution of pedigree knowledge

The pedigree data for 28,119 Finnish Spitz and 9,009 Nordic Spitz was obtained by complementing the Swedish and Finnish Kennel Club registries with data concerning dogs outside of these databases, collected by breed enthusiasts during the last four decades. The original datasets are much larger (> 60,000 for the Finnish Spitz), but contain a huge number of replicates under different registration numbers prior to the 1990s. The records have been manually curated but this has sometimes involved deleting dogs without offspring for the sake of overall clarity. Although all dogs born in the 2000s can now be traced down to the founder dogs, the offspring number of the early generations cannot be reliably calculated as a tradeoff.

A founder is defined as a member of a population with no known genetic relationship to any other member of the pedigree except for its own descendants. Unlike captive populations of animals in zoos, the founders of domestic animal populations are much harder to trace as this is dependent on the pedigree knowledge, which in the case of old breeds is virtually impossible. Also in our Finnish Spitz dataset, there are large numbers of “new” founders appearing in the 1940–1960s, which likely all are descendants of previously known founder dogs as the purpose

of the studbook was different from the modern practices. In fact, it is mentioned that only four dogs registered between 1920–1930s had unknown background (Simonlinna 1990) and the pedigree history of these dogs has likely been lost for good. The situation for the Nordic Spitz is much better due to its more recent history. Thanks to the volunteer work of breed enthusiasts, it has been possible to trace down pedigrees of some of the dogs taken into the studbook as new founders, but in reality these sometimes share several unregistered or registered ancestors.

Genetic diversity estimation by founder analysis

The effective population size (N_e) is regarded as one of the most important criteria for classifying the degree of endangerment for a given population as it will work as a proxy for the loss of genetic diversity in the preceding generations. N_e can be derived from the increase in inbreeding (ΔF) over generations (Wright 1931). However, in most domestic populations the size of the population as well as breeding strategies change dramatically over time and the inbreeding might not represent the cumulated effects of genetic drift (chance events) or the mating system (Boichard *et al.* 1997). An alternative is to model the behavior of alleles in a given pedigree to estimate the contribution of founders in the genetic make-up of the present generation. Here, in an approach called "gene dropping", two unique hypothetical alleles are assigned to each founder in the population and their segregation to each descendant is simulated by Monte Carlo methods (Maccluer *et al.* 1986). The simulation enables the estimation of inbreeding coefficients from identity by descent and assessment of the existing genetic variability. Because all gene dropping replicates are independent from each other, the simulation ignores linkage. The proportion of lost founder genomes is calculated from the proportion of replicates where the founder allele is not present in the living population. For clarity the values are given as mean proportion of retained alleles. It also gives an estimate of the genetic uniqueness (*GU*) of each founder, probability that the founder contains alleles not present in any other single animal in the current population. In reality, the actual proportion of lost genetic diversity will vary over a wide range and the estimates obtained by gene dropping represent a mean of this distribution.

Three founder indices can be obtained either from combining additive matrix analysis of relatedness or from gene drop simulation (Lacy 1989). 1) founder equivalent (f_e) is the number of equally contributing founders that would be expected to produce the same level of genetic diversity as in the current population. If all founders contribute equally to the next generation,

f_e would be equal to the number of founders. Although derived differently, f_e is conceptually related to the N_e and defined as

$$f_e = \frac{1}{\sum p_i^2}$$

where p_i represents the proportion of alleles in the descendent population contributed by the founder i. 2) f_g, founder genomic equivalent, which takes into account the genetic drift caused by limited number of offspring. When the alleles of a founder are passed on to the next generation, there is an above zero probability that an allele is not inherited by any of the descendants. f_g is similar to f_e but subtracts the proportion of the founder genome that likely has been lost by random drift.

$$f_g = \frac{1}{\sum(p_i^2/r_i)}$$

where r_i is the proportion of founder alleles that have been retained in the descendants determined from gene drop simulations. It should be noted that f_e overestimates the number of founders in mating systems under intensive selection, as for example in the case of extensive use of champion males, also known as matador breeding. While this is avoided by f_g by calculating the loss of alleles, a similar estimate can be derived from matrix analysis by taking into account redundancies caused by the same ancestor of an animal occurring more than one time in consecutive (overlapping) generations. 3) The so-called effective number of ancestors, or f_a, is calculated by recognizing such major ancestors and assigning them as "pseudo founders" in the descendant pedigree, eliminating collateral redundancies and the marginal contributions (Boichard *et al.* 1997). The concept is useful in detecting population bottlenecks when compared with other founder indexes and is defined as

$$f_a = \frac{1}{\sum p_k^2}$$

where p_k is the allelic contribution of a founder not yet explained by the other ancestors.

Further genealogical methods to estimate effective population size

The f_a should be least sensitive to the completeness of the pedigree information but to our knowledge the founder contribution indexes have never been tested against other methods of estimating N_e. One such highly effective estimate, not as sensitive to overlapping generations, population subdivision or other structures, is the realized effective population size ($\bar{N}_e$) (Cervantes *et al.* 2008, 2011).

$$\bar{N}_e = \frac{1}{2\Delta F}$$

where ΔF is the mean individual increase in inbreeding corrected for pedigree knowledge by using equivalent generations. Equivalent generations are the sum of all known ancestors in the pedigree of an individual to the term $(1/2)^n$, where n represents the number of generations separating the individual from the ancestors. A similar estimate can be also derived from the increase in coancestry (kinship) in the population over the generations ($\bar{N}_{ec}$).

$$\bar{N}_{ec} = \frac{1}{2\Delta c}$$

This is important as population substructures, such as the ones caused by geographical distance, increase inbreeding while keeping kinship values approximately stable (Cervantes *et al.* 2011). In our dataset this is particularly interesting as especially the majority of Nordic Spitz population is distributed between Finland and Sweden. Because of the differential breeding strategies and historical population sizes, $\bar{N}_{ec}$ is also much more useful in comparing Finnish Spitz with Nordic Spitz.

Software

The pedigree analyses for founder contributions were performed using the PedScope v.2.4.01ws software (Tenset Technologies LTD, Cambridge, UK) and equivalent generations as well as realized effective population sizes were computed using the ENDOG v.4.8 software (Gutierrez & Goyache 2005). The standard deviations for realized effective population sizes were calculated as in (Cervantes *et al.* 2011).

Molecular genetic analysis

Altogether 135 Finnish Spitzes and 110 Nordic Spitzes were genotyped using a commercial DNA testing service (MyDogDNA®) at Genoscoper Laboratories (Genoscoper Laboratories Oy, Helsinki, Finland). Of the Finnish Spitz samples, 73.3% were from Finland and 26.6% from the United States. Of Nordic Spitz samples, 93.6% were Finnish, 3.7% from Canada, 1.8% from Denmark and 0.9% from Sweden. The design and validation of the genotyping microarray has previously been described in detail (GenoscoperLaboratoriesOy 2016). For this study, data of 1,319 SNP markers representing each canine chromosome was available for each dog, with an average marker call rate of 99.6% (median 100%). All samples included in this

study reached a call rate of at least 95% of the analyzed markers ensuring high quality data. For the evaluation of the median heterozygosity in the breed, all analyzed individuals of the breed were included. The average genetic difference between individuals within the breed and between breed groups was calculated based on the SNP genotypes, with difference expressed as a ratio of all measured positions in the genome. Genetic relationships between individuals were illustrated by similarity matrix and multidimensional scaling (MDS) analysis, an eigen-decomposition principal component analysis transforming distances into similarities (Buja *et al.* 2008). As a part of the MyDogDNA® array, several known disease mutations and trait alleles were also tested for.

The SNP data was also used to estimate the effective population size, based on the decay in linkage disequilibrium (r^2) between linked markers. For convenience, we abbreviate this genetic effective population size as N_{eg} to differentiate it from the other approximations of N_e presented earlier. r^2 was computed using the PLINK! software (Purcell *et al.* 2007). After frequency (minor allele frequency < 0.05) and genotyping (missingness < 0.1) pruning in PLINK!, 1,116 SNPs were included in the analysis and the results grouped into distance frames of 0.1 Mb (Pfahler & Distl 2015). For each frame, the mean $\overline{r^2}$ values were calculated and the effective population size was obtained from

$$N_{eg} = \frac{1 - \overline{r^2}}{4c\overline{r^2}}$$

where c is the recombination rate in Morgan units (M) (Sved 1971), assuming that 100 Mb = 1 M (Pfahler & Distl 2015). For the historical analysis, the relationship between generation t and recombination rate is calculated as

$$t = \frac{1}{2c}$$

Results

Reconstructing the breed history

The pedigree analysis revealed 345 known and 73 unknown founders for the current Finnish Spitz population and 112 and 47 for the Nordic Spitz, contributing to 10.88 and 6.54 equivalent generations, respectively (Table 1). In cases where only one parent is known, the other is accounted for as an unknown founder. While the mean inbreeding coefficient and coancestry

for the two breeds was modest, the founder representation was dramatically small compared to the actual number of founder individuals. For example, the founder equivalent (f_e) reveals that the number of equally contributing founders that would be expected to produce the same level of genetic diversity as in the current population is as low as 30 for the Finnish Spitz and 42 for the Nordic Spitz (Table 1). If the random loss of alleles (f_g) and detected population bottlenecks (f_a) are taken into account (Boichard *et al.* 1997), the same genetic diversity can be generated in both breeds by random mating of 20–26 individuals. Whereas f_g is highly similar for the two breeds, the effective number of ancestors (f_a) is 35% higher for the Nordic Spitz population, despite the breed having three-fold fewer founders than the Finnish Spitz. The realized effective population size ($\bar{N}_e$), based on the increase in inbreeding ($\Delta\bar{F}$) over equivalent generations, gives a lower estimate for the Finnish Spitz than the one ($\bar{N}_{ec}$) based on increase in coancestry ($\Delta\bar{C}$). Interestingly, the opposite is true for the Nordic Spitz.

Retention of founder alleles

In an ideal case, all founder alleles are sampled to the next generations, resulting in 100% retention of the founder genotypes. If the founder has only one descendant that passes on the alleles, only 50% of founder genotypes will be sampled. If this bottleneck is repeated in the next generation, the founder allelic retention will be only 25% and so on. When examining the mean allelic retention for the Finnish Spitz founders by decade, it becomes evident that 39–50% of founder genomic variation has been lost because of such bottlenecks (Table 2). The genome uniqueness (*GU*) of founders, or the probability that the founder contains alleles not present in any other single animal in the current population, indicates that especially the 1981–2010 founders have suffered from the bottlenecks (Table 2). The situation is not as dramatic for the Nordic Spitz, although similar bottlenecks exist and some 14–40% of founder alleles can be estimated to have been lost. Due to the smaller population size and shorter time scale, the mean contribution of Nordic Spitz founders to an average dog in the population is considerably larger than in the Finnish Spitz.

Decay of linkage disequilibrium and the effective population size

Considering their limited quantity, the 1,116 SNPs used for computing the $\overline{r^2}$ were rather equally distributed on the 39 canine chromosomes and 21–59 r^2 values were obtained for each 0.1 Mb frame spanning 0.05 to 35 Mb. $\overline{r^2}$ decreases almost exponentially as the function of marker distance, being noticeably similar for both breeds at 8 – 10 Mb before plateauing around

15 Mb (Figure 3A). Similarly, the historical N_{eg} based on the SNP data, increases dramatically over the past generations, being around 800–1000 individuals 500 generations ago (Figure 3B). Probably due to low marker coverage with potential bias on certain common variants, some 0.1 Mb frames show higher $\overline{r^2}$ values than their adjacent ones. As this causes generation to generation variation for the nearest N_{eg} values (Figure 3C), we estimate the current N_{eg} for Finnish Spitz to be 57 and 49 for the Nordic Spitz as an average of the nearest five generations.

Genetic diversity

The median heterozygosity (*Hz*) level for the Finnish Spitz is 31.3%, and 40.9% for the Nordic Spitz based on the SNP analysis (Table 3). As a comparison, the median heterozygosity of all dogs in the MyDogDNA database is 34.6%, and that of all mixed breed dogs is 43.4%. These estimates are intercomparable for this study, noting on a more general level that absolute heterozygosity is influenced by the specific set of SNPs studied. The average SNP minor allele frequency in the Spitz dataset was 29.8%, but considerably higher for the Nordic Spitz compared to the Finnish Spitz (32.2% vs. 23.8%, respectively). In the examined variants, the Nordic Spitz also showed a higher degree of polymorphism, and had less variants with a rare minor allele (MAF<0.05; Table 3) compared to the Finnish Spitz. It should be noted that a genome-wide SNP analysis gives a much more comprehensive view on genetic diversity than the traditionally used few microsatellite markers (Balloux *et al.* 2004; Chapman *et al.* 2009; Marras *et al.* 2015).

Matador breeding as the culprit of genetic bottlenecks

The analysis of the population structures of Finnish and Nordic Spitz revealed a dramatic male bias in the breeding population (Figure 4). The female to male ratio for the breeding population has been 1.4–2.1 for the Finnish Spitz and 1.4–2.2 for the Nordic Spitz throughout the population history. When genetic bottlenecks are considered, more important than the female to male ratio are the relative contributions of individual males to offspring of each generation. Matador breeding or popular sire phenomenon, the dominant use of champion males for fathering offspring, has been an unfortunately widespread kennel practice also for hunting dogs. The effects of matador breeding become obvious when looking at the relative breeding male contribution to the offspring in the two breeds (Table 4). The mean contribution of a breeding male to the next generation has been normalized to 1.0 and the closer to mean the median contribution is, the more equal the males are in terms of offspring number. The male

contributions show a remarkable skew, which is also evident in the quartile values. Until recently, less than 25% of all breeding males produced more than half of the all offspring. In dogs registered in 1951-60 the highest ranking male fathered almost 30 times more pups than an average breeding male and half of the pups in this time period are descendants of 16 males, representing only 6.7% of all males with offspring. Notably, despite the increase in the population (Figure 3A), 50% of the offspring born in the 1980s and 1990s have also been conceived by only 8-10% of the breeding males. When compared to the overall population, the actual figure is even smaller as only about 20% of all males registered in the same time period were used for breeding.

Although some Nordic Spitz males have also been used excessively, especially in the early days until 1980s, the male contribution has been much more equal in this breed compared to the Finnish Spitz (Table 4). It should be noted that the values for the dogs born after 2011 are biased because of the small number of males having offspring.

The evolutionary relationship of the Finnish and Nordic Spitz

In agreement with their geographical origin and similar functional niche, the Finnish and Nordic Spitz are genetically closely related (Figure 5A). The mean SNP difference between these two breeds is 32.0%, which makes them most similar to each other among the northern Spitz breeds. The second closest breed to both Finnish Spitz and Nordic Spitz among the Nordic Hunting Dogs is West-Siberian Laika with mean SNP difference to Finnish Spitz is 33.9% and to Nordic Spitz is 33.3%. The higher heterozygosity retained in Nordic Spitz when compared to Finnish Spitz also has an effect in lowering the number of SNP differences between related breeds.

Within the breed, the mean difference of individuals is 23.1% for Finnish Spitz and 28.5%, for Nordic Spitz. While the Finnish Spitz population shows partly divergent genetic lineages between geographical origin of the dogs, the Nordic Spitz population seems to be unstructured (Figure 5B–C). However, only few dogs outside of Finland were available for the analysis.

Discussion

The histories of the Finnish and Nordic Spitz are dramatically different but simultaneously intimately interconnected. The recognition of the red Spitz as a breed and giving it a status as

the national dog of Finland almost drove its patched cousin to extinction. We performed founder analysis using large pedigree information to estimate genetic indicators such as inbreeding acknowledging the potential unreliability especially towards the older data. However, we argue that using the whole available pedigree information is useful as it enables to expose the evolution of individual breeding success. For example, a strong bias in the male reproductive success, the predominant use of few champion dogs, generates genetic bottlenecks that might not be evident in simple effective population size calculations. For example, if two male dogs contribute equally to a large number of puppies in the descendent generation, the random loss of alleles is minimal. If the representation would be unequal, the risk of losing alleles from less contributing founder increases, resulting in the loss of genetic diversity in terms of heterozygosity and allelic variants.

In our analysis, the bottlenecks become evident from the pedigree data as small effective number of ancestors (f_a) (Table 1). While the effective number of founders (f_e) predicts, based on probabilities of allele origin, how the contribution of founders to genetic diversity is maintained across generations under selection (probability of parenting offspring) and variation in family size, founder genome equivalents (f_g) and f_a also account for genetic drift and bottlenecks in the pedigree. As f_a in Finnish Spitz is smaller than the predicted founder genomic equivalent, this implies that redundancies in the pedigree (overlapping generations, repeated use of champion males) are stronger determinants of genetic diversity in this breed than random drift alone. In contrast in Nordic Spitz the two indicators are almost identical. Because f_a has emphasis on major founders it is also considerably robust to lack of pedigree information (Boichard *et al.* 1997). While the founder representation analyses are based on estimation of genetic contribution of founders, the effective population size (N_e) estimates the variance in allele frequency as a function of relative increase in inbreeding or coancestry. In fact, f_g is related to inbreeding as in a randomly mating population the expected loss of genetic diversity (F) will be $1/(2f_g)$ (Lacy 1989). As N_e is function of increase of inbreeding, when N_e remains the same across the generations in a population with a constant structure (Cervantes *et al.* 2011), f_e, f_g and f_a decrease over time in all finite populations without an influx of new founders (Boichard *et al.* 1997). Although $\overline{N}_{ec}$ of 168 for Finnish Spitz and 98 for Nordic Spitz are small considering the total population size, they are comparable to figures published from other breeds (Wijnrocx *et al.* 2016).

Although derived differently, both the founder equivalents and effective population size estimates account for the unbalanced contributions of the parents to the next generation. For example, small N_e will also account for higher decrease in f_g over time. In our study we have compared these parameters to test them as predictors of observed genetic diversity but they also provide similar message from the pedigree structures of Finnish and Nordic Spitz. $\bar{N}_e$ gives a lower estimate for the Finnish Spitz than $\bar{N}_{ec}$, while the opposite is true for the Nordic Spitz, although the difference between the two parameters falls within the variation in Nordic Spitz (Table 1). The difference seen in Finnish Spitz is interesting as populations split in subpopulations, $\bar{N}_e$ has smaller values than $\bar{N}_{ec}$ (Cervantes *et al.* 2011). With the Finnish Spitz this is likely caused by the fact that because of its popularity the overall population size has always been large and there has been tendency for isolation for geographical reasons or because of breeder preference for local males. Multiple selection policies can also cause bias in favor of $\bar{N}_{ec}$ (Cervantes *et al.* 2011), although this is unlikely for a hunting breed. The Nordic Spitz population has been considerably smaller, as reflected by $\bar{N}_{ec}$, but also because of this the breeders have probably been more aware of avoiding direct inbreeding. In the breeding advisory for the Nordic Spitz, *F* calculated for seven generations should not exceed 3.0% and moreover, the offspring of combinations where seven generation *F* exceeds 6.25% are not registered by the FKC. Finnish Spitz has the same advisory for keeping the seven generation F under 3.0% but does not have restriction for registering litters exceeding this.

Because of the available SNP data, we had the opportunity to compare the performance of the pedigree analyses against the N_e calculated based on the observed decay of linkage disequilibrium ($\overline{r^2}$) between alleles. Measuring $\overline{r^2}$ over marker distances has been successfully used as a proxy of N_{eg} (Neibergs et al. 2010; Pfahler & Distl 2015). Similarly to these studies from different breeds, we also find that the historical effective population size of both Spitz breeds has been considerably larger than the present (Figure 3B). Interestingly, while the N_{eg} of Finnish Spitz shows only moderate reduction in the N_{eg} for the last 40 generations, the effective population size of Nordic Spitz has decreased dramatically (Figure 3C). The N_{eg} for Nordic Spitz was consistently above 100 individuals 15 generations ago, coinciding strikingly with the timing of the breed's establishment. The decline might be explained by the small total population, compared to the probably broad original genetic pool of the geographically separated founders from Sweden and Finland. As many dog breeds were widespread and common also in the rural areas of Sweden and Finland in the 1970–80s, it is likely that the feral

founders of the Nordic Spitz have had more or less mixed background. This assumption is backed by the fact that the mutation that causes ataxia in the Finnish Hound (Kyostila *et al.* 2012) is also present in a subset of Nordic Spitzes originating from the same founder (Donner *et al.* 2016), indicating recent breed hybridization. Because of the rather recent decline in N_{eg}, the *Hz* has not yet had time to erode in Nordic Spitz, being still comparable with the mixed breed dogs (Table 3). In the case of Finnish Spitz, although much reduced from the historical values, is reassuring that N_{eg} seems to be rather stably maintained over the generations if the overall population is large enough (Figure 3C). As of note, both examples demonstrate how breed establishment will irreversibly shrink the effective population size over a short period of time.

The genetic diversity also causes the Nordic Spitz to differ on average by almost as much as they differ from a Finnish Spitz (Figure 5A). Moreover, a geographical differentiation was evident in the Finnish Spitz, where dogs from United States were partly divergent from Finnish dogs (Figure 5B–C). Since ~94% of the genotyped Nordic Spitz were from Finland, no conclusion can be drawn regarding the possible differentiation between Finnish and Swedish populations. Because of the closed studbook and smaller population size, further analysis of the Swedish population would be desirable.

Although the different founder indices and effective population size estimates are not comparable, they reveal a similar pattern in the studied breeds. Despite the large Finnish Spitz population, its genetic diversity has reduced considerably during the generations. Based on the founder analysis, some 40–50% of the allelic variants present in the founders are predicted to be lost, possibly explaining the low SNP diversity in the breed (Table 2, Table 3). Our analysis of the relative male contributions indicates that although the Nordic Spitz has also witnessed breeding male bottlenecks, they have not been as extreme, have not persisted as long as with the Finnish Spitz (Table 4). On one hand, low diversity makes a breed more uniform and predictable in its qualities. The Finnish Spitz has been bred to excellence in its hunting skills, demonstrated by the fact that they outperform Nordic Spitzes in the hunting tests. For example, the Swedish Skallkungen bird barking competition has been won only once by a Nordic Spitz during 2000–2015 (http://ssf-riks.se/skallkungen-sm/).

Albeit the genetic diversity of Finnish Spitz is lower than in Nordic Spitz, it is at the same level as in another large nordic hunting breed Grey Norwegian Elkhound and close to the average

heterozygousity of dogs (www.mydogdna.com). Finnish Spitz can be considered an healthy breed, unlike many other breeds with a similar history with bottlenecks (Farrell *et al.* 2015). This is noteworthy as overrepresentation of a popular sires in the pedigree can result in the spread of recessive disorders as the allele frequency of recessive deleterious variants carried by the champion sire increases in the population and results in increased probability of having alleles identical by descent in the following generations (Farrell *et al.* 2015). As the Finnish Spitz has always been used as an active hunting dog, it might have been protected from the enrichment of monogenic disorders due to strong selection against unfit phenotypes, purging deleterious alleles from the population. The reduced genetic diversity is nevertheless worrying as this could make the breed vulnerable to sudden changes in the environment. The genetic diversity of immunity related genes can protect populations against pathogens and has been shown to be important factor influencing survival of endangered species (Osborne *et al.* 2015). High levels of heterozygosity also seem to be beneficial for the cognitive skills, learning and memory as well as reproduction in various animals, although the underlying mechanism of the phenomenon is unclear (Nepoux *et al.* 2010; Gandin *et al.* 2015; Gokcek-Sarac *et al.* 2015). Low variation could also prove catastrophic if the population shrinks in the future and large number of the remaining individuals are affected by genetic disorders, as has been the case with the Norwegian Lundehund (Kristensen *et al.* 2015). High genetic diversity should therefore be primarily regarded as a buffer against the adverse effects caused by the fluctuations of the breed popularity (Farrell *et al.* 2015). Although genetic diversity should not be seen as value *per se*, as also mixed breed dogs can be predisposed to several types of genetic disease (Bellumori *et al.* 2013; Oberbauer *et al.* 2015), it needs be valued in dog breeding for the benefits of evolutionary potential and the heterosis-associated buffer preventing the manifestation of recessive monogenic disorders.

While an open breed registry can be seen as an asset for breed development, introducing new individuals to the present population should be done judiciously to avoid the introduction of undesired traits. It is also striking that despite the steady influx of recent founders, these have not hindered the decline of N_{eg} in Nordic Spitz, indicating that many of the newcomers might not have been true founders but descendants of registered dogs (Figure 3C). SNP genotyping could therefore provide a useful tool to estimate how an unregistered dog could benefit the genetic makeup of the breed. Alternatively, crosses of Nordic Spitz with related breeds with known background and controlled risk of deleterious alleles could be considered. Especially the mixes between Finnish and Nordic Spitz would prove to be interesting as the two breeds

are clearly closely related. Besides helping to improve the hunting performance of the Nordic Spitz, the crossbreds could be seen as reconstruction of the ancestral feral founders, whose restricted introduction into the population will help to maintain a healthy, generic hunting Spitz for the future generations.

As a conclusion, both the founder analysis and SNP heterogeneity measure are effective in detecting genetic bottlenecks and structural differentiation in a population. In our study, the lower founder indices and $\bar{N}_{ec}$ in Finnish Spitz, compared to the total population, correlates with the low SNP heterozygosity, although the differences to Nordic Spitz are not proportional. SNP heterozygosity analysis could therefore offer an easy measure for the genetic diversity within a dog breed without a need for a detailed pedigree analysis. The estimation of historical N_e based on decay of linkage disequilibrium suggests that once the breed is established, the effective population size is likely to decrease, emphasizing the importance of maintaining a large total population and restricting the relative contribution of single individuals to the gene pool of coming generations.

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Conflict of Interest

HA and JD represent Genoscoper, a private company providing dog genotyping services. However, they do not financially benefit from marketing the product(s) that is/are related to this research.

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Tables

Table 1. Overview of the pedigree data showing the number of individuals (*N*), founders, equivalent generations, degree of inbreeding (F), mean increase in inbreeding over equivalent generations ($\Delta\bar{F}$), coancestry coefficient (*C*, or kinship), mean increase in coancestry over equivalent generations ($\Delta\bar{C}$), founder equivalent (f_e), founder genomic equivalent (f_g), the effective number of ancestors (f_a), realized effective population size based on $\Delta\bar{F}$ ($\bar{N}_e$) and $\Delta\bar{C}$ ($\bar{N}_{ec}$).

		Finnish	**Nordic**
N		28,119	9,009
Founders	known	345	112
	unknown	73	47
Mean equivalent generations		10.88	6.54
F	mean	6.33 %	4.36 %
	range	0-55.0 %	0-38.0 %
	$\Delta\bar{F}$	0.68 %	0.46 %
C	mean	2.90 %	2.80 %
	$\Delta\bar{C}$	0.30 %	0.51 %
f_e		30.71	42.35
f_g		26.49	26.62
f_a		20.18	27.91
$\bar{N}_e$		73.53±8.57	108.70±10.43
$\bar{N}_{ec}$		168.11±13.08	97.79±9.86

Table 2. Founder contribution per decade in the two Spitz breeds, including the number of founders (*N*), their mean contribution to the current population, allelic retention (1.0 = all alleles retained in the population, 0.0 = one founder allele remains in the population) and genomic uniqueness (*GU*).

Finnish Spitz	***N***	**Mean Contribution**	**Mean alleles retained**	**Mean *GU***
-1920	23	0.00	0.56	0.06
1921-30	4	0.00	0.56	0.17
1931-40	8	0.00	0.61	0.03
1941-50	138	0.00	0.58	0.07
1951-60	100	0.00	0.57	0.06
1961-70	9	0.00	0.54	0.08
1971-80	4	0.00	0.56	0.06
1981-90	28	0.00	0.50	0.21
1991-2000	28	0.00	0.51	0.25

2001-10	3	0.00	0.50	0.48

Nordic Spitz	N	Mean Contribution	Mean alleles retained	Mean GU
-1970	40	0.001	0.61	0.01
1971-80	29	0.001	0.60	0.12
1981-90	10	0.007	0.77	0.15
1991-2000	30	0.003	0.86	0.10
2001-10	3	0.001	0.67	0.28

Table 3. Minor allele frequencies (MAF), SNPs fewer than 5% MAF, frequency of polymorphic SNPs and the median heterozygosity (Hz) in Finnish and Nordic Spitz. Heterozygosity is significantly higher in Nordic Spitz (p<0.001, two-tailed t-test with two sample equal variance).

	Average MAF (%)	SNPs with MAF < 5% (%)	Polymorphic SNPs (%)	Median Hz (range) (%)
Finnish Spitz	23.8	15.1	96.4	31.3 (21.4-39.2)
Nordic Spitz	32.2	2.5	99.5	40.9 (34.3-44.9)

Table 4. Relative breeding male contribution to the next generation per decade, taking only into the account individuals with offspring (mean contribution = 1.0). The lower than average contribution of males in the upper quartile for some decades demonstrates the fact that only a handful of males have been fathering the majority of the population.

Finnish Spitz	-1920	1921-1930	1931-1940	1941-1950	1951-1960	1961-1970	1971-1980	1981-1990	1991-2000	2001-2010	2010-2013
Median	0.38	0.79	0.47	0.37	0.28	0.29	0.22	0.37	0.39	0.53	0.88
Range	0.38-4.14	0.40-3.17	0.47-4.73	0.37-15.58	0.28-29.54	0.29-10.01	0.22-21.32	0.15-22.43	0.08-14.24	0.09-6.86	0.18-3.68
Lower/Upper quartile	0.38-1.13	0.40-1.19	0.47-0.98	0.37-0.75	0.28-0.84	0.29-0.86	0.22-0.88	0.15-0.90	0.23-0.93	0.35-1.23	0.70-1.05

Nordic Spitz	-1970	1971-1980	1981-1990	1991-2000	2001-2010	2011-2013
Median	0.39	0.36	0.60	0.61	0.81	0.88
Range	0.19-10.04	0.18-15.04	0.15-6.65	0.10-4.77	0.09-3.35	0.17-3.65
Upper/lower quartile	0.19-0.87	0.18-0.90	0.30-1.21	0.41-1.35	0.45-1.54	0.52-1.35

Figure legends

Figure 1. The defining characteristics of Finnish Spitz have early on been a relatively square trunk and fully red coat without additional markings. (A) A Finnish Spitz from early 1920s, A.E. Järvinen collection, The Hunting Museum of Finland, used with permission. (B) Modern Finnish Spitz, national dog and an excellent hunting companion with a long history. Photo by Thommy Svevar.

Figure 2. Compared to the Finnish Spitz, the Nordic Spitzes generally have generally a longer trunk with varying coat pattern on white background. (A) Feral Spitz from Sompio, Finnish Lapland, 1930s. Photo by Samuli Paulaharju, used with the permission of Finland's National Board of Antiquities. (B) The modern Nordic Spitz resembles, if not fully, the early feral Spitz dogs. Photo by Jaakko Pohjoismäki.

Figure 3. Population genetics of Finnish and Nordic Spitz based on SNP genotyping. (A) Decay of linkage disequilibrium (r^2) at marker distances of 0.05–50Mb. (B) Ancestral effective population sizes of Finnish and Nordic Spitz spanning 500 generations as computed from r^2. (C) A closer look at the effective population sizes during the last 40 generations. Notice the relatively stable N_e levels in Finnish Spitz and the rapid decline in Nordic Spitz during the last 15 generations, corresponding to the founding of the breed in the 1960s. Trend curves drawn to intersect the estimated current N_e and are given for illustrative purposes only.

Figure 4. The proportion of breeding individuals per total population per decade in Finnish and Nordic Spitz. The quantities of breeding males (black bars) and females (gray bars) compared to the total population (white bars) per registration decade. Notice the scale.

Figure 5. Genetic similarity of northern Spitz breeds. (A) SNP similarity matrix, showing the average genetic difference (%) between individuals of different Nordic Spitz -type dogs. Breeds included in the analysis were (*N*): Finnish Spitz (135), Nordic Spitz (110), West-Siberian Laika (2), Karelian Bear Dog (91), Finnish Lapphund (238), Russian-European Laika (9), East-Siberian Laika (32), Lapponian Herder (22), Swedish Elkhound (11), Norwegian Elkhound, Grey (410), Swedish Vallhund (211) and Norwegian Lundehund (17). Few closely related individuals could bias the values in breeds with less than 30 analyzed individuals (*). As reported earlier (Pfahler & Distl 2015), variation between Lundehund individuals is

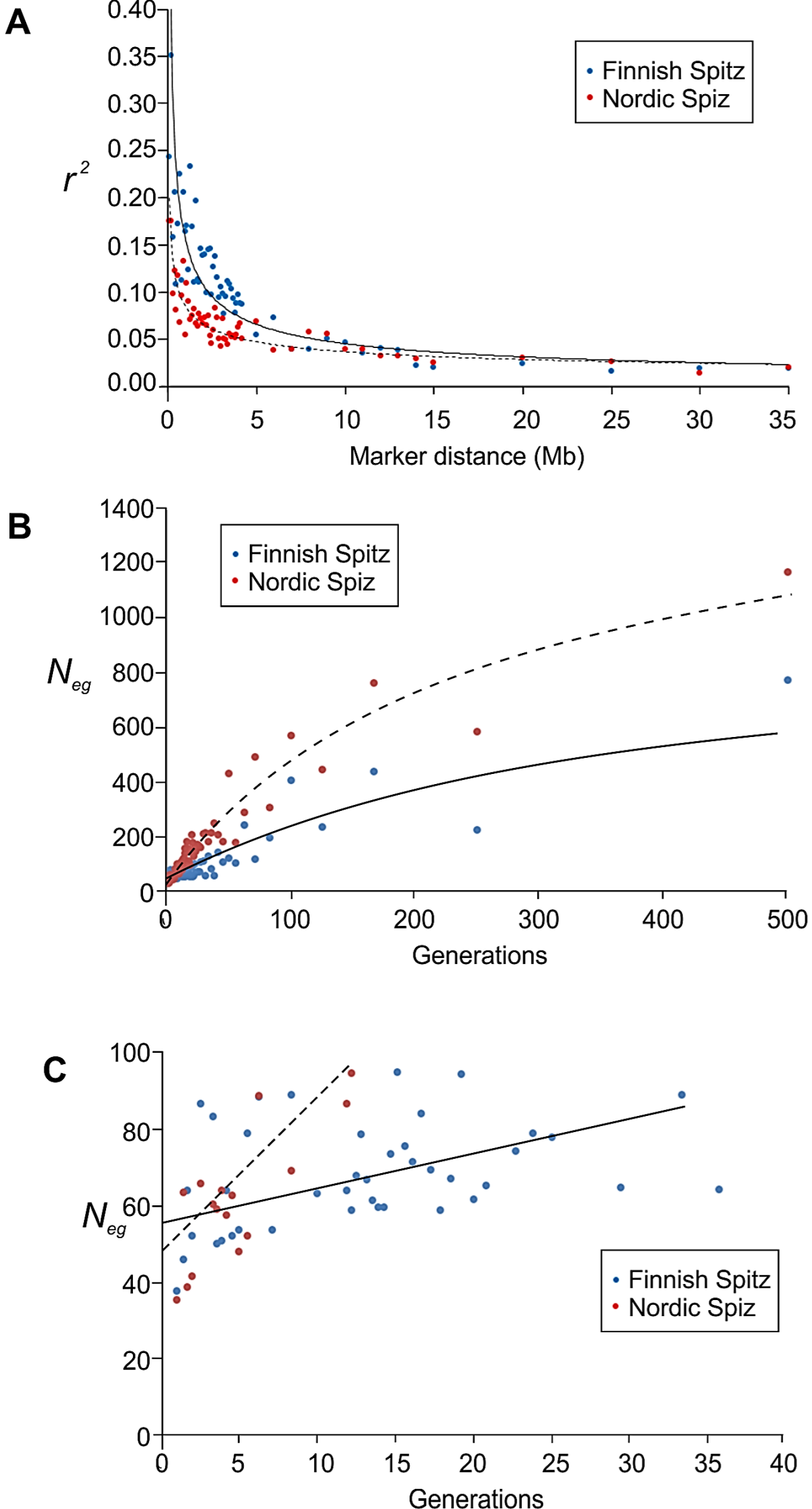
extremely small. (B) Multidimensional scale (MDS) plot of genetic similarities within the Finnish Spitz breed. The different populations of Finnish Spitz cluster tightly together and show differentiation between the geographical regions. (C) Contrary to the Finnish Spitz, the MDS of the Nordic Spitz shows loose clustering of individuals, embedding the samples from different geographical locations.


A


B


A


B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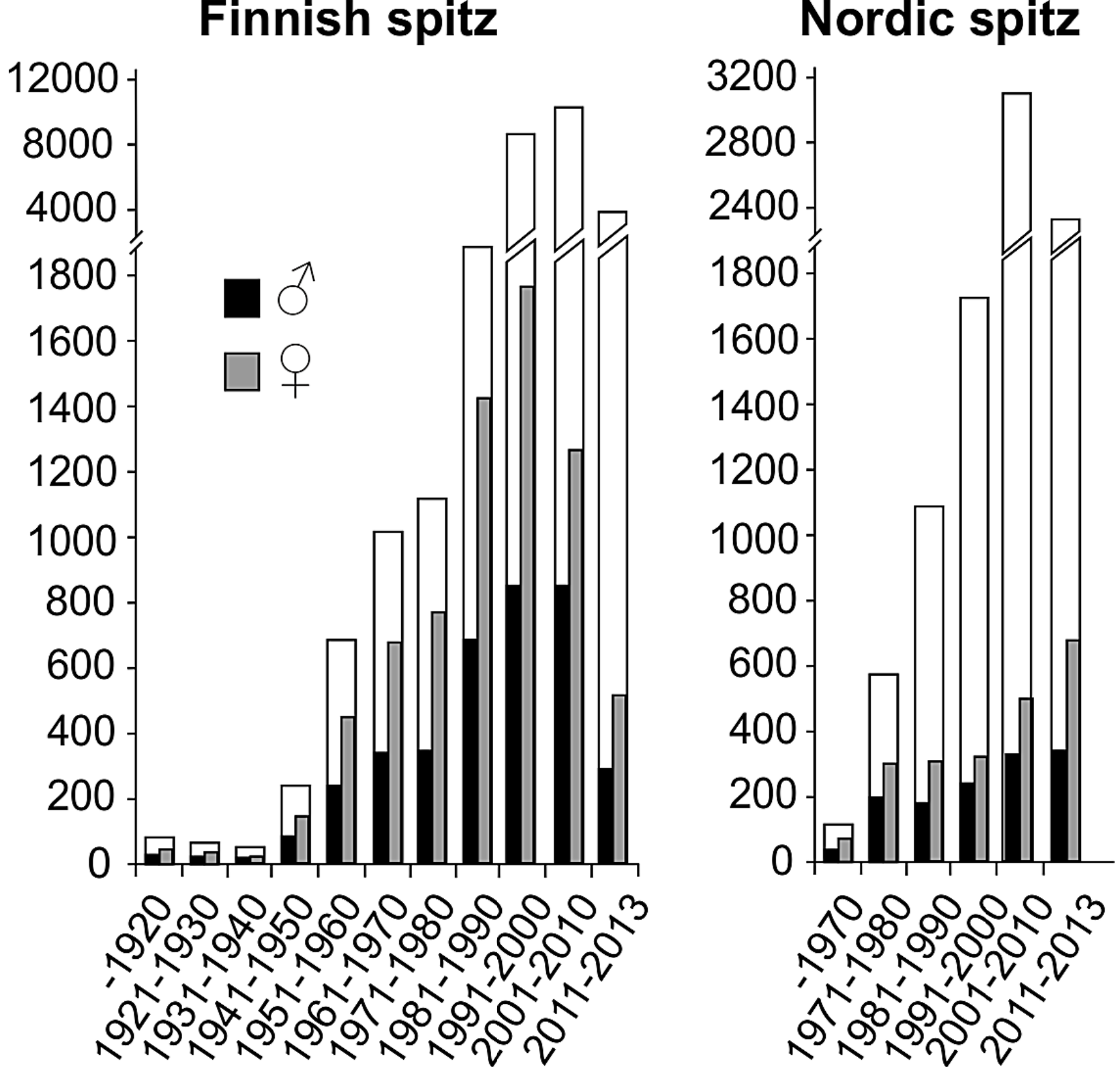
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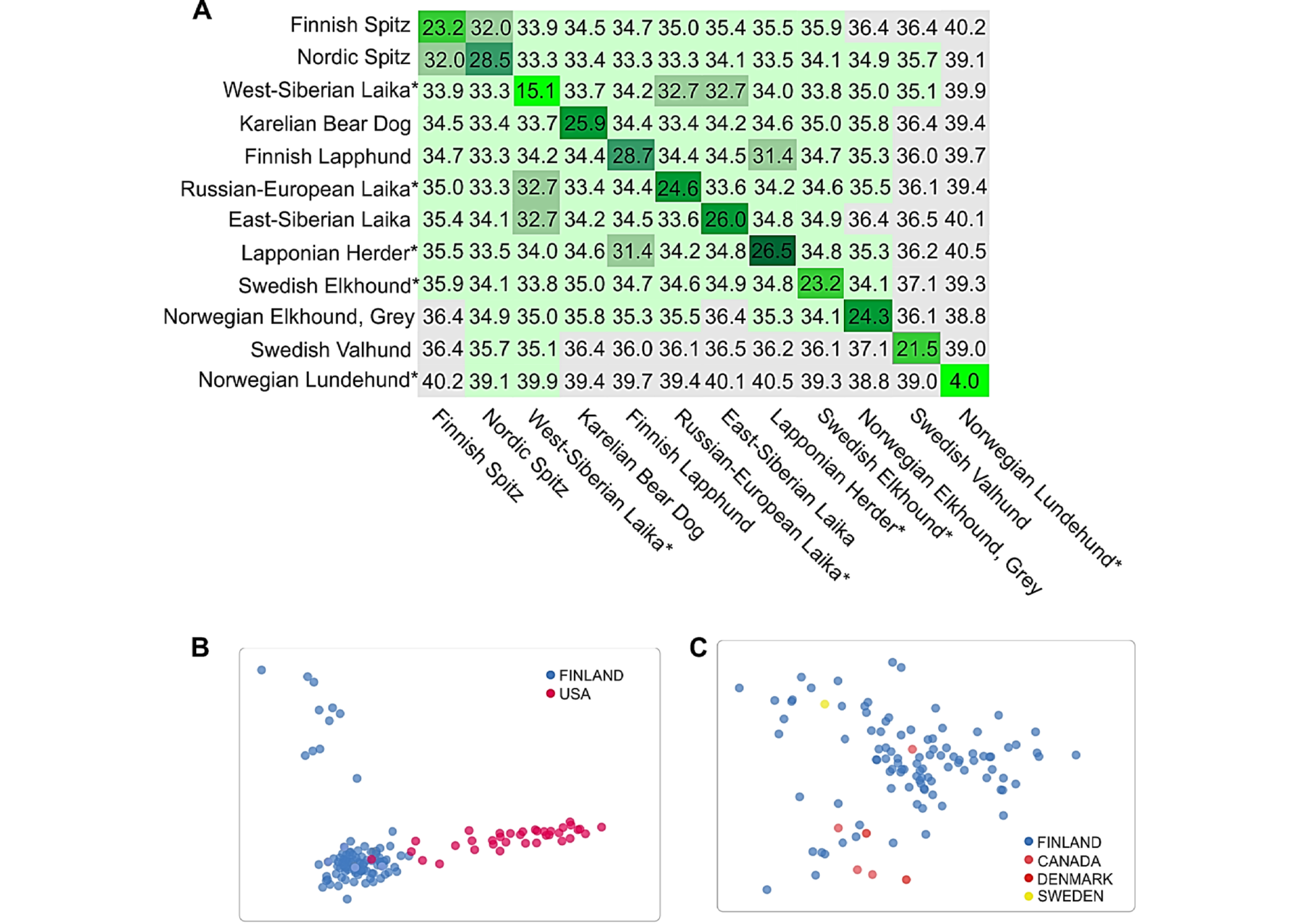
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Finnish Spitz 23.2 32.0 33.9 34.5 34.7 35.0 35.4 35.5 35.9 36.4 36.4 40.2
Nordic Spitz 32.0 28.5 33.3 33.4 33.3 33.3 34.1 33.5 34.1 34.9 35.7 39.1
West-Siberian Laika* 33.9 33.3 15.1 33.7 34.2 32.7 32.7 34.0 33.8 35.0 35.1 39.9
Karelian Bear Dog 34.5 33.4 33.7 25.9 34.4 33.4 34.2 34.6 35.0 35.8 36.4 39.4
Finnish Lapphund 34.7 33.3 34.2 34.4 28.7 34.4 34.5 31.4 34.7 35.3 36.0 39.7
Russian-European Laika* 35.0 33.3 32.7 33.4 34.4 24.6 33.6 34.2 34.6 35.5 36.1 39.4
East-Siberian Laika 35.4 34.1 32.7 34.2 34.5 33.6 26.0 34.8 34.9 36.4 36.5 40.1
Lapponian Herder* 35.5 33.5 34.0 34.6 31.4 34.2 34.8 26.5 34.8 35.3 36.2 40.5
Swedish Elkhound* 35.9 34.1 33.8 35.0 34.7 34.6 34.9 34.8 23.2 34.1 37.1 39.3
Norwegian Elkhound, Grey 36.4 34.9 35.0 35.8 35.3 35.5 36.4 35.3 34.1 24.3 36.1 38.8
Swedish Valhund 36.4 35.7 35.1 36.4 36.0 36.1 36.5 36.2 36.1 37.1 21.5 39.0
Norwegian Lundehund* 40.2 39.1 39.9 39.4 39.7 39.4 40.1 40.5 39.3 38.8 39.0 4.0
Finnish Spitz
Nordic Spitz
West-Siberian Laika*
Karelian Bear Dog
Finnish Lapphund
Russian-European Laika*
East-Siberian Laika
Lapponian Herder*
Swedish Elkhound*
Norwegian Elkhound, Grey
Swedish Valhund
Norwegian Lundehund*
B
FINLAND
USA
C
FINLAND
CANADA
DENMARK
SWEDEN