

# Assessment of the population structure of five Finnish dog breeds with microsatellites

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## Summary

Genetic variabilities within and between Finnish populations of Golden Retrievers, German Shepherds, Wirehaired Dachshunds, Pembroke Welsh Corgis and Bedlington Terriers were quantified with microsatellite allele numbers, observed heterozygosities, expected heterozygosities,  $F_{IS}$  estimates,  $F_{ST}$  estimates and  $D_S$  distances. In a sample of 50 individuals from each breed and ten polymorphic loci, the highest genetic diversity was exhibited in the Wirehaired Dachshunds and lowest in the Bedlington Terriers. Although statistically significant deviations from the Hardy–Weinberg (H–W) equilibrium were observed, they occurred at an unexpectedly low frequency. Most strikingly, the extremely small Bedlington Terrier population displayed genotypes in H–W proportions in all investigated loci. The H–W deviations always occurred with positive  $F_{IS}$  estimates, which, on average, were not larger than values reported for free-living canids. Genetic differentiation between the breeds was very large. As a comparison, present estimates were, on average, over two times higher than previously observed between breeds of sheep, and over two times higher than the highest estimates reported between human populations. Moreover, the highest  $D_S$  distances were only slightly lower than the lowest values inferred between humans and chimpanzees. Severe bottlenecks in the recent past of the examined breeds were not statistically supported. The presented data imply genetic isolation and intense artificial selection in the history of these breeds of dogs.

**Keywords:** *Canis familiaris*, genetic distance, genetic diversity, microsatellite population structure

## Introduction

The extreme phenotypic variation between breeds of dogs (*Canis familiaris*) is likely to be

generated by their multiple origins, with an historical geneflow enriching the gene pool (e.g. Vilà *et al.* 1997) and heavy inbreeding pushing towards distinct morphological characters. Matings between close kin may increase the proportion of homozygotes in all loci, and if deleterious recessive alleles are abundant, enhance the risk of genetic disorders. In order to avoid hereditary defects, and to maintain the possibility to execute effective breeding programmes, kennel clubs are now showing increasing interest in conserving genetic variability within breeds of dogs.

In dog breeds, the traditional pedigree-based approach to estimate inbreeding (see Falconer & Mackay 1995) may result in erroneous estimates. First, as dog pedigrees are typically known over a small number of generations, the individuals set to have zero inbreeding coefficients may already exhibit non-zero inbreeding. Second, owing to falsely reported sibs, the pedigrees of dogs frequently contain errors (P. Bredbacka and M.T. Koskinen, unpublished data). In recent years, molecular genetics has experienced considerable advances, and polymerase chain reaction (PCR) amplified DNA markers now offer a convenient means for characterization of population structure. Microsatellites (e.g. Litt & Luty 1989) are increasingly becoming the markers of choice for population genetic studies (Bruford & Wayne 1993). However, despite their unquestionable advantages over, for example, protein polymorphisms, few investigations have used microsatellites for estimating genetic variability within pedigree dog breeds (Pihkanen *et al.* 1996; Zajc *et al.* 1997).

Studies of genetic differentiation between dog breeds have produced mixed results. For instance, serum proteins (Jordana *et al.* 1992) revealed markedly lower levels of inter-breed variation ( $F_{ST}$ ) than microsatellites (Pihkanen *et al.* 1996). This difference may be explained by the fact that microsatellites display, on average, higher levels of variation, and consequently, enable more efficient detection of population differentiation than allozymes. However, as Pihkanen *et al.* (1996) surveyed only three microsatellites, this is yet to be confirmed. Comparisons of genetic divergence between dog breeds and between populations within other

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**Table 1.** Numbers of alleles (All. no.), observed heterozygosities ( $H_O$ ) and expected heterozygosities ( $H_E$ ) for ten microsatellite loci within and across the investigated dog breeds ( $n = 50$  individuals per breed)

Locus <sup>1</sup>	Breed												Mean across breeds					
	Golden Retriever			German Shepherd			Wirehaired Dachshund			Pembroke Welsh Corgi			Bedlington Terrier			All. no.	$H_O$	$H_E$
	All. no.	$H_O$	$H_E$	All. no.	$H_O$	$H_E$	All. no.	$H_O$	$H_E$	All. no.	$H_O$	$H_E$	All. no.	$H_O$	$H_E$			
<i>C04107B</i>	4	0.62	0.70	4	0.26	0.33	5	0.56	0.54	7	0.50	0.72	3	0.48	0.62	0.48	0.58	
<i>CXX.2054</i>	4	0.60	0.60	6	0.66	0.70	7	0.80	0.82	4	0.62	0.67	3	0.62	0.66	0.66	0.69	
<i>CXX.2004</i>	5	0.58	0.60	4	0.58	0.53	6	0.48	0.62	4	0.16	0.15	2	0.36	0.35	0.43	0.45	
<i>CXX.2088</i>	4	0.58	0.60	4	0.42	0.48	5	0.52	0.57	4	0.44	0.59	3	0.20	0.19	0.43	0.49	
<i>CXX.2137</i>	7	0.80	0.75	6	0.62	0.65	8	0.70	0.77	6	0.70	0.74	6	0.28	0.32	0.62	0.65	
<i>CXX.2146</i>	11	0.64	0.63	12	0.78	0.87	15	0.84	0.88	10	0.66	0.71	9	0.74	0.75	0.73	0.77	
<i>CXX.2175</i>	5	0.16	0.21	8	0.74	0.77	6	0.70	0.72	5	0.78	0.73	4	0.72	0.63	0.62	0.61	
<i>CXX.2132</i>	5	0.62	0.61	9	0.74	0.73	14	0.70	0.88	8	0.54	0.78	14	0.84	0.89	0.69	0.78	
<i>CXX.2001</i>	7	0.76	0.78	6	0.70	0.69	8	0.78	0.82	5	0.58	0.59	4	0.66	0.61	0.70	0.70	
<i>CXX.2168</i>	4	0.76	0.72	5	0.74	0.70	6	0.64	0.60	6	0.50	0.70	4	0.58	0.61	0.64	0.67	
Average	5.6	0.61	0.62	6.4	0.62	0.64	8.0	0.67	0.72	5.9	0.55	0.64	5.2	0.55	0.56	—	—	

<sup>1</sup> All markers except *C04107B* (Yuzbasiyan-Gurkan, personal communication. Primer sequences available in Koskinen and Bredbacka (1999)) are described in Francisco *et al.* (1996).

**Table 2.**  $F_{IS}$  values,  $P$  values ( $P$  val.) for a null hypothesis of random union of gametes and their standard errors (SE) for ten microsatellite loci within the investigated dog breeds ( $n = 50$  individuals per breed)

Locus	Golden Retriever			German Shepherd			Wirehaired Dachshund			Pembroke Welsh Corgi			Bedlington Terrier		
	$F_{IS}^1$	$P$ val. <sup>2</sup>	SE <sup>3</sup>	$F_{IS}^1$	$P$ val. <sup>2</sup>	SE <sup>3</sup>	$F_{IS}^1$	$P$ val. <sup>2</sup>	SE <sup>3</sup>	$F_{IS}^1$	$P$ val. <sup>2</sup>	SE <sup>3</sup>	$F_{IS}^1$	$P$ val. <sup>2</sup>	SE <sup>3</sup>
<i>G04107B</i>	0.11	0.28	–	0.21	0.19	–	–0.03	0.51	0.02	0.31	<0.01	<0.01	0.23	0.09	–
<i>CXX.2054</i>	0.01	0.23	–	0.05	0.15	0.01	0.02	0.72	0.02	0.08	0.06	–	0.07	0.40	–
<i>CXX.2004</i>	0.03	0.52	0.02	–0.10	0.66	–	0.22	0.12	0.01	–0.05	1.00	–	–0.04	1.00	–
<i>CXX.2088</i>	0.03	0.82	–	0.13	0.01	–	0.09	0.71	0.02	0.25	0.11	–	–0.08	1.00	–
<i>CXX.2137</i>	–0.06	0.47	0.02	0.04	0.89	0.01	0.09	0.34	0.02	0.05	0.65	0.02	0.13	0.40	0.03
<i>CXX.2146</i>	–0.12	0.31	0.04	0.11	0.02	0.01	0.05	0.76	0.04	0.07	0.01	0.01	0.02	0.16	0.02
<i>CXX.2175</i>	0.22	<0.01	<0.01	0.04	0.71	0.02	0.03	0.48	0.02	–0.07	0.09	0.01	–0.14	0.83	–
<i>CXX.2132</i>	–0.14	0.17	0.01	–0.02	0.15	0.02	0.20	0.04	0.02	0.31	<0.01	<0.01	0.05	0.11	0.02
<i>CXX.2001</i>	0.03	0.70	0.02	–0.02	0.07	0.01	0.04	0.22	0.02	0.02	0.37	0.01	–0.08	0.43	–
<i>CXX.2168</i>	–0.06	0.45	–	–0.05	0.29	0.01	–0.07	0.40	0.02	0.29	<0.01	<0.01	0.05	0.95	–
All loci <sup>4</sup>	0.01	0.11	–	0.04	<0.01	–	0.07	0.33	–	0.13	<0.01	–	0.02	0.53	–

<sup>1</sup> As in Weir and Cockerham (1984).

<sup>2</sup> According to the exact probability test of GENEPOP v.3.1b applying a Markov chain method always when numbers of alleles exceed four.

<sup>3</sup> Estimated whenever a Markov chain method was applied.

<sup>4</sup>  $F_{IS}$  values average across loci,  $P$  values using Fisher's method implemented by GENEPOP v.3.1b. Statistically highly significant deviations given in bold.

canids, or within other mammalian species are surprisingly rare.

In this study, we characterize population structures within and between five dog breeds. The main objectives were to reveal possible genetic depletion and inbreeding within the included smaller breeds relative to the larger ones, and to compare genetic differentiation between the breeds with estimates previously reported among some other mammalian populations.

## Materials and methods

### *Sampling strategy of the animals and characterization of the populations*

In order to reduce sampling bias, exclusion of first order relatives from analyses is commonly performed when assigning population structures of domestic animals (e.g. Buchanan *et al.* 1994). For many dog breeds finding an adequate number of non-sibling individuals for a population genetic study would be extremely laborious. Therefore, we proceeded as follows: a number, of approximately 60–80 animals, (officially registered by the Finnish Kennel Club) from each breed were randomly sampled across Finland. From these, 25 non-siblings from each breed were taken in a random order. An additional 25 dogs from each breed were randomly sampled, thus resulting in a total of 50 individuals per breed.

Of the examined breeds (Tables 1–3), the Golden Retrievers and the German Shepherds comprise large population sizes, with well over 18000 registered individuals over the previous 10-year period. The Wirehaired Dachshunds and the Pembroke Welsh Corgis are intermediate in their sizes, with approximately 4000 registrations, whereas the Bedlington Terriers

exemplify an extremely small breed, with only 138 registered individuals during the past decade in Finland. Although some of the breeds can essentially be divided into varying selection lines (e.g. appearance versus performance), such division is subjective and, therefore, was not considered when sampling the animals.

### *Microsatellite analyses*

Details on the DNA extractions, multiplex PCR amplifications of the ten microsatellites (Tables 1 and 2), and the semiautomated electrophoresis can be found in Koskinen & Bredbacka (1999).

GENEPOP v.3.1b program (Raymond & Rousset 1995) was employed in calculation of allele numbers, observed heterozygosities ( $H_O$ ) and expected heterozygosities ( $H_E$ ). GENEPOP v.3.1b was further used to test for deviations from the Hardy–Weinberg (H–W) equilibrium, and to assign  $F_{IS}$  and  $F_{ST}$  estimates (Weir & Cockerham 1984). For the H–W equilibrium estimation, we followed the probability test approach (Guo & Thompson 1992). Corrections for multiple significance tests were performed using Fisher's method and by applying a sequential Bonferroni type correction (Rice 1989).  $F_{IS}$  estimates were calculated across all populations and loci (global  $F_{IS}$ ), and for populations and loci individually. Similarly, the  $F_{ST}$  calculations were performed over all populations (global  $F_{ST}$ ), plus all combinations formed by two populations were addressed individually (pairwise  $F_{ST}$ ).

Genetic divergence between the breeds was further quantified with  $D_S$  distances (Nei 1972).  $D_S$  distances were attained using the program DISPAN (T. Ota, Pennsylvania State University), which also produced a neighbour-joining (N–J) dendrogram and bootstrap support estimates to indicate statistical reliability for each formed

**Table 3.** Mean pairwise  $F_{ST}$  estimates (above diagonal) and Nei's (1972)  $D_S$  distances with their associated standard errors (below diagonal) across ten microsatellite loci between the investigated dog breeds ( $n = 50$  individuals per breed)

	Golden Retriever	German Shepherd	Wirehaired Dachshund	Pembroke Welsh Corgi	Bedlington Terrier
Golden Retriever		0.266	0.184	0.240	0.282
German Shepherd	0.975 ± 0.229		0.182	0.201	0.280
Wirehaired Dachshund	0.604 ± 0.153	0.650 ± 0.235		0.190	0.219
Pembroke Welsh Corgi	0.774 ± 0.202	0.602 ± 0.215	0.684 ± 0.188		0.291
Bedlington Terrier	0.839 ± 0.214	0.891 ± 0.263	0.678 ± 0.167	0.963 ± 0.273	

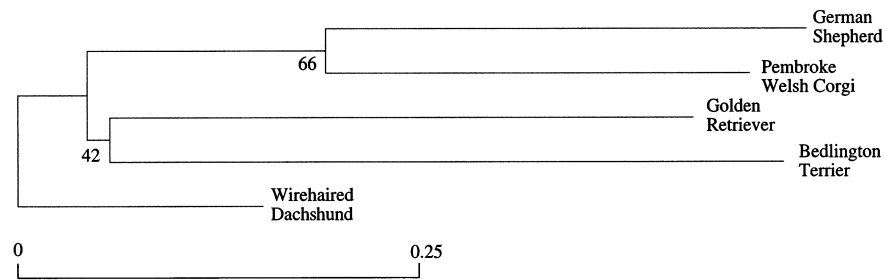


Fig. 1. N-J dendrogram showing the genetic relationships among the investigated dog breeds, based on Nei's (1972)  $D_S$  distances calculated using ten microsatellite loci. Values along branches represent bootstrap support in percent over 1000 replications of resampled loci. The scale indicates genetic distance.

group of populations (given as percentages over 1000 replicates).

Severe reductions in effective sizes of the breeds (i.e. bottlenecks) were tested using the program BOTTLENECK (Cornuet & Luikart 1996). In a recently bottlenecked population, the observed  $H_E$  (heterozygosity expected under the H-W equilibrium) exceeds the heterozygosity expected in a population at a mutation-drift equilibrium ( $H_{EQ}$ ) (Luikart *et al.* 1998). BOTTLENECK determines whether a given population exhibits a significant number of loci with such heterozygosity excess. Following the recommendations of Luikart *et al.* (1998), we assumed a two-phase microsatellite mutation model (TPM), with 95% of one-step mutations and 5% of multi-step mutations. BOTTLENECK implements three tests for the detection of  $H_E$  excess, namely a 'sign test', a 'Wilcoxon sign-rank test', and a 'standardized differences test'. As the 'standardized differences test' requires at least 20 polymorphic loci (Cornuet & Luikart 1996), only the first two tests were applied.

## Results

Allele distributions and frequencies varied largely between the breeds (details available upon request). For the ten microsatellites, the observed mean heterozygosities ( $H_O$ ) ranged from 0.55 (Pembroke Welsh Corgis and Bedlington Terriers) to 0.67 (Wirehaired Dachshunds), whereas the expected mean heterozygosities ( $H_E$ ) varied between 0.56 (Bedlington Terriers) and 0.72 (Wirehaired Dachshunds), with 2–15 alleles segregating at each locus within the populations (Table 1). Over all populations, from six (CXX.2088) to 24 (CXX.2146) alleles per locus were observed (data not shown).

From 50 instances (5 populations, 10 loci), eight deviations significant at the 5% level from the H-W equilibrium were detected (only 2–3 are expected owing to chance events). Interestingly, all locus specific genotype proportions of

the Bedlington Terrier population were congruent with the H-W equilibrium expectations (Table 2), despite the breed's small population size. Observed H-W equilibrium deviations were not consistent over loci, but generally occurred with different microsatellites in different populations. After corrections for multiple significance tests, deviations over all loci remained highly significant in the German Shepherd ( $P = 0.009$ ) and the Pembroke Welsh Corgi ( $P < 0.001$ ) populations (Table 2). Over all populations and loci, H-W disequilibrium was statistically highly significant.

At individual loci,  $F_{IS}$  estimates ranged between  $-0.137$  (the locus CXX.2175, Bedlington Terriers) and  $0.311$  (the locus C04107B, Pembroke Welsh Corgis). Mean  $F_{IS}$  estimates for the ten microsatellites were between 0.020 (Bedlington Terriers) and 0.127 (Pembroke Welsh Corgis) (Table 2), the global  $F_{IS}$  being 0.058. Statistically significant departures from the H-W equilibrium occurred consistently with positive  $F_{IS}$  estimates, reflecting the deviations to be a result of heterozygote deficiencies (Table 2).

Across the ten markers, the mean genetic differentiation ( $F_{ST}$ ) of the pairwise comparisons ranged from 0.182 (German Shepherds and Wirehaired Dachshunds) to 0.291 (Pembroke Welsh Corgis and Bedlington Terriers) (Table 3), with a global  $F_{ST}$  of 0.233. The largest  $D_S$  distances ( $0.975 \pm 0.229$ ) were observed between the Golden Retrievers and the German Shepherds, and the smallest ( $0.602 \pm 0.215$ ) between the German Shepherds and the Pembroke Welsh Corgis (Table 3). Accordingly, in the N-J dendrogram, the German Shepherds and the Pembroke Welsh Corgis grouped together with moderate bootstrap support (Fig. 1).

None of the studied breeds exhibited statistically significant excess of  $H_E$  over  $H_{EQ}$  across the ten microsatellites under a TPM model for mutations. This was the case regardless of the testing method applied. Therefore, recent bot-

tlenecks in the history of the breeds were not statistically supported.

## Discussion

### *Genetic structures within breeds*

In terms of numbers of alleles,  $H_O$  and  $H_E$ , microsatellites revealed differences between the breeds (Table 1). Partly, the within-population variation was higher in breeds with larger numbers of registered individuals. Notwithstanding, the Pembroke Welsh Corgis and the Bedlington Terriers exhibited identical mean observed heterozygosities, regardless of the greater than 25-fold difference in their long-term registries. All diversity indices were clearly highest in the Wirehaired Dachshunds (Table 1), implying they harbour a more variable gene pool than the other breeds (differences, e.g. in the allele numbers between Wirehaired Dachshunds and all other breeds were statistically significant:  $P < 0.05$ ; details available upon request). Repeated arrival of dogs from diverged populations could have affected the genetic diversity of the studied breeds. However, the Finnish populations have been relatively isolated and, therefore, contribution of imported breeding individuals to the seen genetic variation has probably been small.

In general, the average numbers of alleles and levels of  $H_O$  and  $H_E$  exceeded those observed in previous microsatellite studies of the domestic dog (Pihkanen *et al.* 1996; Zajc *et al.* 1997), and were similar to values calculated for wolves, coyotes and jackals (Roy *et al.* 1994), or Australian dingos (Wilton *et al.* 1999). Comparison of the observed microsatellite allele numbers, mean  $H_O$  and mean  $H_E$  (Table 1) to estimates reported for Spanish sheep (mean  $H_O = 0.71-0.75$ , mean  $H_E = 0.66-0.81$ ; Arranz *et al.* 1998) or cattle breeds (mean  $H_O = 0.56$ ; Georges *et al.* 1995) suggests similar levels of microsatellite variation in pedigree dogs and in livestock populations.

All detected deviations from the H-W equilibrium were a result of deficiencies of heterozygotes (global  $F_{IS} = 0.058$ ). The most noteworthy outcome of the H-W analysis, however, was the observation that the Bedlington Terrier population was in equilibrium in all investigated loci (Table 2). This implies that, despite the depleted levels of genetic diversity (see Table 1), the use of inbreeding in the small Bedlington Terrier population has remained at a low level over recent generations and, in that sense, their breeding strategy has been optimal. In contrast,  $F_{IS}$  estimates of the Welsh Corgis were, on average, two times higher than in the

other breeds, and at the upper level when compared with values calculated for pedigree dogs in previous studies (e.g. Jordana *et al.* 1992). Excluding the Bedlington Terriers, the numbers of grandparents were similar in all breeds (data not shown), suggesting that the higher deficit of heterozygotes in the Welsh Corgi population results from stronger linebreeding. Given that, for the ten microsatellites, there have been no indications of non-amplifying alleles when performing parentage testing (P. Bredbacka and M.T. Koskinen, unpublished data), it is unlikely that they are misleading our  $F_{IS}$  estimate based conclusions on inbreeding.

$F_{IS}$  estimates in this study (Table 2) were similar to those calculated for free-living populations of wolves, coyotes and jackals (Roy *et al.* 1994). This observation, together with the comparisons of the allele numbers and heterozygosities from other canid species (see above), interestingly imply that recent artificial selection has not affected the within-breed genetic composition of the studied dog populations more than the natural selection occurring among wild canids.

### *Genetic differentiation and genetic affinities among breeds*

The present  $F_{ST}$  estimates were markedly higher than those calculated between Spanish dog breeds from protein polymorphisms (Jordana *et al.* 1992), but supported previous microsatellite based observations of large variation between breeds (Pihkanen *et al.* 1996). The apparent differences between the results of these two marker classes are likely owing to the lower power of allozymes in detection of between-population variation in dogs. Based on the  $F_{ST}$  data, the inter-breed genetic structuring seems to be similar to or higher than that occurring within genera, and among species of wolflike canids, which are known to form breeding packs showing very little or no geneflow between them (Roy *et al.* 1994).  $D_S$  distances of this study (0.602–0.975; Table 3) were high and pointed to much deeper divergence between breeds of dogs than revealed, for instance, between sheep breeds ( $D_S = 0.079-0.502$ ; Buchanan *et al.* 1994) or human populations ( $D_S = 0.005-0.355$ ; Deka *et al.* 1995). Exemplifying the extent of the present inter-breed differentiation, the highest  $D_S$  distances were not very much smaller than the lowest estimates inferred between humans and chimpanzees ( $D_S = 1.334-1.901$ ; Deka *et al.* 1995). Such large variation between breeds of dogs has, perhaps, been induced by genetic isolation related random

drift and strong artificial selection. Significant reductions in the effective sizes of the breeds, which could have contributed to the deep interbreed divergence, appeared unlikely (see above). However, the current available testing methods can detect only events that have occurred in the very recent past; after a certain number of generations, a new equilibrium is established (Cornuet & Luikart 1996). Therefore, it can not be determined whether bottlenecks have occurred in the more distant history of the breeds.

The Bedlington Terriers generally showed the highest levels of between-population divergence with the other breeds ( $F_{ST} = 0.219-0.291$ ,  $D_S = 0.678-0.963$ ). From the remaining comparisons, estimates between the Golden Retrievers and the German Shepherds and between the Golden Retrievers and the Pembroke Welsh Corgis were larger than among the other population pairs (Table 3). The varying levels of genetic divergence between the breeds (Table 3) may reflect differences in the historical gene flow among them. Also, recent events, such as strong selection may have induced for instance the relatively deeper divergence of the Bedlington Terriers. The moderate bootstrap support for grouping of the German Shepherd and the Pembroke Welsh Corgi populations (Fig. 1) suggests that these breeds share common ancestors in their recent history. This is indeed possible, given that the Welsh Corgi has been considered one of the founders of a lineage from which most shepherd dogs have perhaps later diverged (Geary 1978).

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