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Article *in* Animal Genetics · September 2012

Impact Factor: 2.21 · DOI: 10.1111/age.12000 · Source: PubMed

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Low neutral genetic variability in a specialist puffin hunter: the Norwegian Lundehund

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Summary

The genetic variability of 125 Norwegian Lundehund and 27 Nova Scotia Duck Tolling Retriever was analysed using a set of 26 microsatellite markers. In Lundehund, the average number of alleles per locus was 1.73, and average observed (H_O) and expected (H_E) heterozygosity were 0.07. In Toller, all measures of genetic diversity were much higher than in Lundehund and similar to studies on other dog breeds. The cluster analysis correctly assigned individuals to their respective breed. The low genetic variability in Lundehund was not surprising, given the two strong bottlenecks in the 1940s and the 1960s. The relatedness of Lundehund to other Nordic small spitzes should be investigated in the view of possible outcrossing.

Keywords *Canis familiaris*, genetic bottleneck, inbreeding, Nova Scotia Duck Tolling Retriever, puffin dog

The Norwegian Lundehund is a small spitz, which since the 17th century was used to hunt puffins (*Fratercula arctica*, *lunde* in Norwegian) on steep mountainsides along the coast of northern Norway. This breed is characterized by a great flexibility of the joints and by polydactyly (i.e. the presence of extra toes). At the beginning of the 20th century, only a small population of Lundehund was left in the fishing village of Måstad (Værøy island, Lofoten archipelago). The breed went through two bottlenecks, one caused by canine distemper in the 1940s and one following the abandonment of Måstad in the 1960s. Today's Lundehund world population stems entirely from five surviving dogs, who shared a grandmother. Three of them also shared the mother (Frimann-Clausen & Laane 1968). A recent census (2010, Norwegian Lundehund Club) found that there are ca. 500 Lundehund in Norway and ca. 300 in other European countries.

The aim of this study was to evaluate Lundehund within breed genetic diversity; the same analyses were performed on a sample of Nova Scotia Duck Tolling Retriever (hereafter Toller) for comparison. A total of 125 Lundehund and 27 Toller were DNA sampled by buccal swabs at dog shows and meetings in Norway (2009–2011). DNA was extracted using the Isohelix DDK-50 isolation kit.

A total of 22 microsatellite markers from the ISAG panel (http://www.isag.us/Docs/consignmentforms/2005ISAGPanelDOG.pdf) and nine extra microsatellites (http://www.qiagen.com/literature/qiagennews/weeklyarticle/09_06/e10/default.aspx) were chosen to perform the molecular analyses. Amelogenin was used for sex identification. After exclusion of four markers that presented problems in amplification or scoring, 26 autosomal markers remained and were amplified by PCR in three multiplex panels (Table 1). All individuals were genotyped at the 27 loci in 10-μl reactions as described in Kekkonen *et al.* (2011), and alleles were scored using GENEMAPPER 4.0 software (Applied Biosystems).

For each locus, observed (H_O) and expected (H_E) heterozygosity and number of alleles were calculated by CERVUS 3.0.3 (Kalinowski *et al.* 2007). The inbreeding coefficient (F_{IS}) and allelic richness corrected for sample size (A_R) were calculated in FSTAT 2.9.3.2 (Goudet 1995). Within each breed, we tested for locus-specific departure from Hardy–Weinberg (HW) equilibrium using exact tests in GENEPOP 3.4 (Raymond & Rousset 1995). All markers were in HW equilibrium after Bonferroni correction except C01.246 in Lundehund (Table 1). We also tested for significant linkage disequilibrium (LD) with GENEPOP 3.4. In Lundehund, only 34 of 325 combinations could be tested owing to the presence of many monomorphic loci and very few alleles on variable loci, but significant LD was found in 1.84% of pairs

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Accepted for publication 07 August 2012

Table 1 Multiplex panel, locus name, CFA (*Canis familiaris* autosome), number of alleles, number of individuals sampled, observed (H_O) and expected (H_E) heterozygosity, level of significance (P) of test for deviation from Hardy–Weinberg equilibrium (HWE) and inbreeding coefficient (F_{IS}) of 26 microsatellite markers used in Norwegian Lundehund and Toller (2009–2011). The Bonferroni-corrected 5% level of significance in HWE tests was $P \leq 0.0019$.

			N alleles		*N* individuals		H_O		H_E		HWE		F_{IS}	
Panel	Locus	CFA/Linkage group	Lundehund	Toller	Lundehund	Toller	Lundehund	Toller	Lundehund	Toller	Lundehund	Toller	Lundehund	Toller
1	AHT121	CFA13	2	4	125	27	0.016	0.778	0.016	0.589	1.000	0.311	−0.004	−0.328
	AHTh171	CFA06	2	5	105	27	0.000	0.667	0.019	0.747	0.005	0.562	1.000	0.109
	AHTh260	CFA16	1	5	102	12	0.000	0.500	0.000	0.736	–	0.008	–	0.330
	AHTk211	CFA26	1	3	114	27	0.000	0.444	0.000	0.492	–	0.357	–	0.098
	AHTk253	CFA23	1	3	119	27	0.000	0.593	0.000	0.551	–	1.000	–	−0.076
	C22.279	CFA22	1	5	125	27	0.000	0.778	0.000	0.679	–	0.906	–	−0.148
	FH2054	CFA12	3	7	125	27	0.040	0.667	0.040	0.772	1.000	0.017	−0.011	0.139
	INRA21	CFA21	1	3	121	27	0.000	0.407	0.000	0.343	–	0.706	–	−0.192
	REN162C04	CFA07	1	4	114	27	0.000	0.741	0.000	0.635	–	0.574	–	−0.171
	REN54P11	CFA18	3	5	124	27	0.016	0.741	0.016	0.784	1.000	0.539	−0.002	0.056
2	AHT137	CFA11	2	8	125	26	0.008	0.923	0.008	0.815	–	0.339	0.000	−0.135
	FH2848	CFA02	2	3	125	26	0.504	0.538	0.501	0.627	1.000	0.747	−0.013	0.143
	INU005	CFA33	2	4	117	27	0.077	0.407	0.074	0.563	1.000	0.213	−0.035	0.280
	INU030	CFA12	1	5	125	26	0.000	0.692	0.000	0.674	–	0.468	–	−0.027
	INU055	CFA10	2	2	125	26	0.008	0.346	0.008	0.340	–	1.000	0.000	−0.018
	REN105L03	CFA11	2	4	125	26	0.408	0.462	0.370	0.544	0.331	0.033	−0.099	0.154
	REN169D01	CFA14	1	5	125	26	0.000	0.731	0.000	0.729	–	0.726	–	−0.002
	REN169O18	CFA29	1	3	124	26	0.000	0.500	0.000	0.595	–	0.402	–	0.162
	REN247M23	CFA15	2	3	124	26	0.008	0.115	0.008	0.112	–	1.000	0.000	−0.027
3	C01.246	CFA01	3	2	118	27	0.017	0.444	0.066	0.391	0.000	0.645	0.745	−0.139
	C08.410	CFA08	1	8	120	27	0.000	0.741	0.000	0.692	–	0.843	–	−0.104
	C11.873	CFA11	2	4	121	27	0.463	0.630	0.469	0.603	0.848	0.729	0.018	−0.045
	C26.733	CFA26	2	5	122	27	0.008	0.556	0.008	0.611	–	0.774	0.000	0.092
	CXX.672	Unlinked	1	3	123	27	0.000	0.296	0.000	0.296	–	0.550	–	−0.002
	FH2016	CFA01	4	6	107	27	0.374	0.593	0.349	0.682	0.986	0.234	−0.070	0.133
	FH2516	L23	1	2	122	27	0.000	0.111	0.000	0.171	–	0.181	–	0.355
		Average	1.731	4.269			0.075	0.554	0.075	0.568				

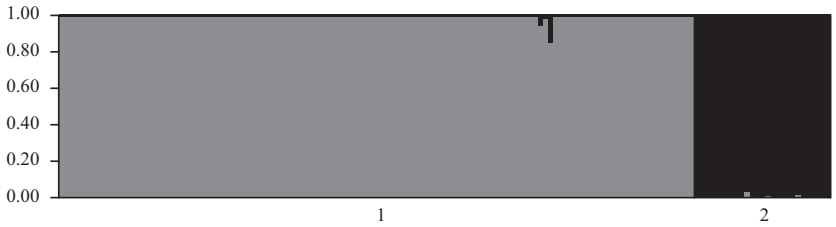

Figure 1 Results of Bayesian clustering of 152 dogs from two breeds (125 Norwegian Lundehund and 27 Toller sampled in 2009–2011) with the program STRUCTURE. Population assignment of individuals to two clusters is represented as the coloured areas. Numbers refer to the breeds: 1 = Lundehund, 2 = Toller.

of loci. In Toller, all loci combinations could be tested, and significant LD was found in 2.15% of cases.

STRUCTURE 2.3.3 (Pritchard *et al.* 2000; Pritchard & Wen 2003) was used to determine the structure of the genetic data. In STRUCTURE, we ran five iterations for each $K = 1–10$ (with 100 000 burn-in period length, 500 000 Monte Carlo repetitions) using the admixture model and correlated allele frequency. Furthermore, we followed the procedure of Evanno *et al.* (2005) to identify the principal hierarchical level of structure in our data. Clustering was performed, and individuals were assigned to groups using *q*-values. The clustering analysis produced two distinct clusters and correctly assigned each individual to its respective breed (Fig. 1).

In Lundehund, 12 markers were homozygous and 14 were heterozygous, whereas all markers were heterozygous in Toller (Table 1). There was a large difference in number of alleles per locus in Lundehund (average = 1.731) and Toller (average = 4.269). A_R was 1.313 in Lundehund and 3.828 in Toller. Mean H_O was 0.075 in Lundehund (range 0–0.504) and 0.554 in Toller (range 0.111–0.923). Similarly, mean H_E was 0.075 in Lundehund (range 0–0.501) and 0.568 in Toller (range 0.112–0.815). F_{IS} across all loci was 0.003 in Lundehund and 0.025 in Toller. None of the F_{IS} at each locus differed significantly from zero after Bonferroni correction ($P = 0.001$).

The genetic variability in our sample of Norwegian Lundehund is, to our knowledge, the lowest reported in any study on dog breeds. This was not unexpected, however, given the history of the breed. Yet, a study on 92 Hanoverian Hounds (Lüpke & Distl 2005) found that this breed still retained a rather high genetic variability with an average H_E of 0.66, despite the breed's two genetic bottlenecks (11 dogs born during World War I and 27 during World War II). The authors suggested that the founders of this breed had a high genetic variability. This was obviously not the case in Lundehund, probably because the population size was relatively low also before, between and after the two bottlenecks.

Leroy *et al.* (2009) used 21 microsatellite markers to study 61 dog breeds in France and found a H_E of 0.62 (range 0.37–0.77), similar to the value we observed in Toller. In Lundehund, H_E was almost 10 times smaller. Similarly, in Leroy *et al.* (2009), average A_R was 4.60 (range 2.30–6.90).

Lundehund show some signs of inbreeding depression, for example, through reduced litter size (Gautun 2012) and a high occurrence of a syndrome suspected to be an inherited polygenic disorder affected by environmental conditions and possibly partly due to pleiotropic effects of mutation for polydactyly (Galis *et al.* 2001). Other inherited disorders that are common in many dog breeds have not been described in the Lundehund. This might suggest that the bottlenecks the Lundehund went through 'purged' the breed for some (recessive) deleterious alleles. Purging has however not been investigated in dogs (Leroy 2011).

Since the 1970s, the Norwegian Lundehund Club has used a breeding programme based on the recommendations: (i) use as many individuals as possible in reproduction; (ii) avoid mating parents who share one or more grandparents; (iii) limit the number of puppies produced per male (to 5% of the total born in the last 5 years) and (iv) use reproductive males throughout their whole life and not only in the first years, to slow down genetic drift. A potentially useful tool to increase diversity in an established breed is outcrossing (Leroy 2011). Possible outcrossing breeds, which are believed to be close relatives to Lundehund, might be the Norwegian Buhund or the Iceland sheepdog. However, the relatedness between potential outcrossing breeds should be further investigated by means of molecular methods, e.g. to avoid introducing new inherited diseases to the breed through introgression (Leroy 2011).

Acknowledgements

We are grateful to the Norwegian Lundehund Club and all the people who helped us with data collection. Annelen Sandø helped with the laboratory work. The project was financially supported by the Norwegian Genetic Resource Center.

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 doi: 10.1111/age.12000