doi:10.1111/j.1365-2052.2007.01658.x

Genetic differentiation in pointing dog breeds inferred from microsatellites and mitochondrial DNA sequence

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Summary

Recent studies presenting genetic analysis of dog breeds do not focus specifically on genetic relationships among pointing dog breeds, although hunting was among the first traits of interest when dogs were domesticated. This report compares histories with genetic relationships among five modern breeds of pointing dogs (English Setter, English Pointer, Epagneul Breton, Deutsch Drahthaar and German Shorthaired Pointer) collected in Spain using mitochondrial, autosomal and Y-chromosome information. We identified 236 alleles in autosomal microsatellites, four Y-chromosome haplotypes and 18 mitochondrial haplotypes. Average F_{ST} values were 11.2, 14.4 and 13.1 for autosomal, Y-chromosome microsatellite markers and mtDNA sequence respectively, reflecting relatively high genetic differentiation among breeds. The high gene diversity observed in the pointing breeds (61.7–68.2) suggests contributions from genetically different individuals, but that these individuals originated from the same ancestors. The modern English Setter, thought to have arisen from the Old Spanish Pointer, was the first breed to cluster independently when using autosomal markers and seems to share a common maternal origin with the English Pointer and German Shorthaired Pointer, either via common domestic breed females in the British Isles or through the Old Spanish Pointer females taken to the British Isles in the 14th and 16th centuries. Analysis of mitochondrial DNA sequence indicates the isolation of the Epagneul Breton, which has been formally documented, and shows Deutsch Drahthaar as the result of crossing the German Shorthaired Pointer with other breeds. Our molecular data are consistent with historical documents.

Keywords dog breeds, genetic diversity, microsatellites, mitochondrial DNA, population structure, Y chromosome.

Introduction

The domestic dog, *Canis familiaris* L., is an extraordinary example of phenotypic variability, showing traits that range from coat colour to complex diseases, different morphologies (Parker & Ostrander 2005) and behaviour (Sundqvist *et al.* 2006). These traits characterize breeds and are the cause and consequence of the dog being the first domesticated animal (Olsen 1985; Clutton-Brock 1987). Despite this dramatic diversity in phenotype, dogs diverged very recently from their wild ancestor, the grey wolf (*Canis lupus*) (Wayne & Ostrander 1999). The genetic structure of the domestic dog has been investigated using mitochondrial DNA (Tsuda *et al.* 1997; Vilà *et al.* 1997; 1999) or microsatellite markers (Koskinen & Bredbacka 2000; Irion *et al.* 2003; Parker *et al.* 2004). The variation observed in mtDNA indicates that all dogs originate from a common gene pool. Parker *et al.* (2004) deconstructed the relationships among 414 dogs representing 85 breeds using a Bayesian model-based clustering algorithm (Pritchard *et al.* 2000) to identify genetically distinct subpopulations based on microsatellite marker allelic frequency patterns. This algorithm assigns most dogs to unique breed-specific clusters. However, these studies have not focused on pointing breeds, although hunting is one of the first traits of interest when dogs were domesticated, for which some breeds were deliberately bred 4000 years ago (Clutton-Brock 1987) in the Middle East and North Africa.

The ancestors of today's pointing breeds were those animals that showed the 'defective' behavioural feature of

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Accepted for publication 3 August 2007

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stopping in front of their prey. In the 17th century, when hunters took up the use of fire arms, pointing dogs were required to both point at the prey and not be startled by the hunting rifle, and the breeding of pointing dogs was enhanced. The Fédération Cynologique Internationale (http://www.fci.be) assigns pointing dogs to Group VII, comprising 38 breeds from 12 countries, with one recognized ancestor, the Old Spanish Pointer, which is practically extinct (Arkwright 1902). This common ancestor was probably established in 100–250 BC and shows two types: shorthaired (distributed throughout the Iberian Peninsula) and longhaired (distributed across the north of the Iberian Peninsula, possibly on both sides of the Pyrenees).

Given the historical significance of hunting dogs, we compared the histories of pointing breeds with molecular data related to gender contributions of the breeds and their genetic backgrounds. This study examines genetic relationships among the five most popular modern breeds of pointing dogs collected in Spain, based on three types of molecular markers.

Materials and methods

Sample collection

The study population comprised 173 individuals representing the five main breeds of pointing dogs in Spain: German Shorthaired Pointer (GSP; $n = 31$), Deutsch Drahthaar or German Wire-haired Pointing Dog (DD; $n = 10$), Epagneul Breton (EB; $n = 16$), English Pointer (EP; $n = 50$) and English Setter (ES; $n = 66$). Samples for analysis were collected as blood stains on filter paper from animals participating in hunting competitions organized by the Spanish Hunting Federation.

Microsatellites

Twenty-one autosomal microsatellites and four microsatellites located on the Y chromosome were selected on the basis of their predicted genetic variability and listed in Table S1. The Y-chromosome microsatellites (*MS34A, MS34B, MS41A* and *MS41B*) were analysed using only two pairs of primers, MS34 and MS41 (Olivier *et al.* 1999), as each amplified fragment contained two different microsatellite loci (Sundqvist *et al.* 2001). The Y-chromosome microsatellites were typed in 90 males (GSP = 10, DD = 4, EB = 7, EP = 27 and ES = 42).

Mitochondrial DNA

Two fragments of the canine mitochondrial D-loop sequence (U96639; Kim *et al.* 1998) were amplified using the following primers: a 458-bp fragment spanning nucleotides 15402–15860 (primers MITFOR: 5′-GCTCTTGCTCCACCATCAGC-3′ and MITREV: 5′-ATCGAGATGTCCCATTTGCG-3′) and a 379-bp fragment spanning nucleotides 15745–16124 (primers L16452: 5′-GGGCCCATACTAACGTGGGG-3′ and H222: 5′-AACTATATGTCCTGAAACC-3′; Vilà *et al.* 1997). MtDNA sequences were analysed in 52 male and female animals (GSP = 10, DD = 10, EB = 10, EP = 10 and ES = 12). After amplification and sequencing using standard techniques, the sequences were assembled using the overlapping region and a mtDNA sequence of ~649 bp, from positions 15458–16105, was obtained. Individual sequences were aligned using CLUSTALW 1.82 software (Thompson *et al.* 1994). These sequences were aligned with others representing the six canine mitochondrial groups (Savolainen *et al.* 2002).

Statistical analysis

For microsatellites, the polymorphisms per locus were analysed as numbers of alleles, effective number of alleles (Kimura & Crow 1964), and observed (H_o) and expected (H_e) heterozygosity. The classic Wright indices (F_{IT}, F_{IS} and F_{ST}) were also calculated (Wright 1965; Weir & Cockerham 1984). These values were obtained using the GENETIX 4.03 program (Belkhir *et al.* 2001). Hardy–Weinberg equilibrium (HWE) was tested (i) using an exact test based on Guo & Thompson's (1992) Markov chain Monte Carlo algorithm combined across locus/population; and (ii) using a Chi-squared test for each pair of locus/population by the Fisher's method implemented in the GENEPOP 3.33 package (Raymond & Rousset 1995). Haplotypes were constructed based on the alleles present for the four Y-chromosome microsatellites and allele and haplotype diversity were calculated. The concept of molecular coancestry (Caballero & Toro 2002) was also used to calculate genetic diversity within breeds.

Several diversity measures were computed from the mtDNA sequences using ARLEQUIN 2.00 software (Schneider *et al.* 2000) including nucleotide and mtDNA diversity, measures of polymorphism as number of haplotypes, exclusive haplotypes, number of polymorphic sites, nucleotide differences per site, haplotype (gene) diversity and nucleotide diversity. Relationships among the breeds were analysed using F_{ST} distances calculated for individuals, haplotypes and breeds. Events of insertion/deletion were not taken into account. Standard errors were estimated by bootstrapping 1000 iterations.

Genetic relationships among breeds were established using three methods. First, the pair-wise F_{ST} was used to establish genetic distances among breeds under the hypothesis that genetic drift is the main factor promoting genetic differentiation between breeds. Prior to the cluster analysis, distance matrices were converted to measures with ultrametric properties (Weitzman 1992), rendering unique trees independently of the algorithm that was used. Dendrograms based on genetic distances between breeds were obtained by the UPGMA clustering procedure (Sneath

& Sokal 1973) implemented in MEGA 2.0. Second, the STRUCTURE program (Pritchard *et al.* 2000) was used to identify genetically distinct subpopulations based on patterns of allele frequencies for autosomal microsatellites. This method estimates the proportion of the individual genome derived from each inferred ancestral population and the number of ancestral populations represented in the samples. The genetic cluster analysis was performed assuming an admixture model (Pritchard *et al.* 2000). Third, a network analysis was used to visualize the spatial distribution of the sequence variation among the different mtDNA haplotypes. This procedure reveals possible evolution pathways (Bandelt *et al.* 1995) and is based on a maximum parsimony procedure (Templeton *et al.* 1992) according to the homonymous algorithm and the analysis undertaken using TCS software (Clement *et al.* 2000).

Results

Genetic diversity

For the 21 autosomal microsatellites, 236 alleles (mean 137.2 per breed) and 58 private alleles were identified, resulting in a mean value of 6.5 alleles per breed per marker. The main characteristics and diversity parameter values from these microsatellites are presented in Table S1. The markers for the entire population showed a very high number of alleles per marker (mean = 11.2), ranging from 3 to 29, which contrasts with the low mean effective number of alleles (ENA = 4.3). No significant deviations from HWE were detected with the exception of the marker *UCMCF96* for the English Setter. Allele frequencies are listed in Table S2.

As expected, polymorphisms for the Y-chromosome microsatellites were fewer than for autosomal markers, and no polymorphism in the Y-chromosome markers was observed in Deutsch Drahthaar. Only four Y-chromosome haplotypes or patrilineages were observed, with Haplotypes 1 and 2 being the most frequent (Table S3) across all animals at 48% and 48% frequency each.

Eighteen mtDNA haplotypes were detected (4.7 per breed). Twenty-eight per cent of the haplotypes found in 53% of the individuals were not specific to any breed, indicating high genetic maternal diversity within and among breeds (Table S4). The mean diversity over all haplotypes was 1.45%. Eight of the haplotypes belong to canine groups A, B and C (Savolainen *et al.* 2002) and the 10 remaining haplotypes are newly described, some of which are exclusive of one breed, as is the case for *MIT8* in German Shorthaired Pointer. The new haplotypes have been deposited in GenBank (EF380216–EF380225).

Levels of breed subdivision were measured using the F_{IS} parameter and ranged from 1.5% to 4.4% for Deutsch Drahthaar and English Setter respectively (Table 1). Coancestry for each breed, computed as the Malecot's kinship coefficient, the inbreeding, the distribution of the genetic variability within each breed (G_i) and the genetic diversity computed for the different molecular information sources are shown in Table 1. The negative value of F_{IS} for the Deutsch Drahthaar breed indicates an excess of heterozygous genotypes with respect to the expected value under HWE. The English Setter shows the highest values for coancestry and inbreeding. It should be noted that when the proportion of genetic diversity between individuals (G_i) is 50%, random mating occurs in the population. Therefore, greater values of G_i indicate that variability is preferentially distributed between individuals, as occurs for the English Setter.

The mean values of F_{ST} were 11.2, 14.4 and 13.1 for autosomal microsatellite, Y-chromosome microsatellites and mtDNA information respectively, reflecting the differentiation level present among breeds (Table 2). A high correlation ($r = 0.64$) was observed between the results obtained for autosomal loci and Y-chromosome loci, contrasting with low correlations between mtDNA and autosomal markers ($r = 0.22$) and Y-chromosome markers ($r = 0.14$).

Genetic distances and clustering

Genetic distances among breeds, determined by UPGMA clustering of the F_{ST} distance matrix for autosomal microsatellites, are depicted at the top of Fig. 1. In the lower part of this figure, we provide a phylogeny based on information obtained through the Bayesian model-based procedure and

Table 1 Coancestry, inbreeding, F_{IS} value, proportion of genetic variability between individuals (G_i) and expected heterozygosity of five dog breeds.

					Expected heterozygosity (H_e)		
Breed	Coancestry	Inbreeding	F_{IS}*	G_i	Autosomal	Y chromosome	mtDNA
GSP	0.36	0.37	3.0	50.9	64.9	6.3	77.8
DD	0.38	0.35	−1.5	47.6	62.2	–	64.4
EB	0.36	0.37	3.7	50.2	65.7	23.4	88.9
EP	0.32	0.33	2.2	50.6	68.3	24.0	77.2
ES	0.38	0.41	4.4	52.2	61.7	15.9	75.6

GSP, German Shorthaired Pointer; DD, Deutsch Drahthaar; EB, Epagneul Breton; EP, English Pointer; ES, English Setter.

*All values are significantly different from 0 ($P < 0.05$).

Table 2 Pair-wise F_{ST} values (in %) estimated using the autosomal microsatellites[1], Y-chromosome microsatellite[2] and mtDNA[3] information among five dog breeds.

Breed	GSP	DD	EB	EP	ES
GSP		NS	44.9	15.7	35.9
		NS	*NS*	*10.5*	*2.9*
DD	11.2		45.9	NS	41.7
			12.9	*5.7*	*10.9*
EB	14.1	15.4		NS	NS
				4.4	*NS*
EP	6.3	11.8	10.9		NS
					1.8
ES	12.9	14.8	11.9	10.9	

GSP, German Shorthaired Pointer; DD, Deutsch Drahthaar; EB, Epagneul Breton; EP, English Pointer; ES, English Setter.
NS, values not significantly different from 0 at $P \leq 0.05$.
[1]Autosomal microsatellites below the diagonal.
[2]Y-chromosome microsatellites above the diagonal.
[3]mtDNA in italic above the diagonal.

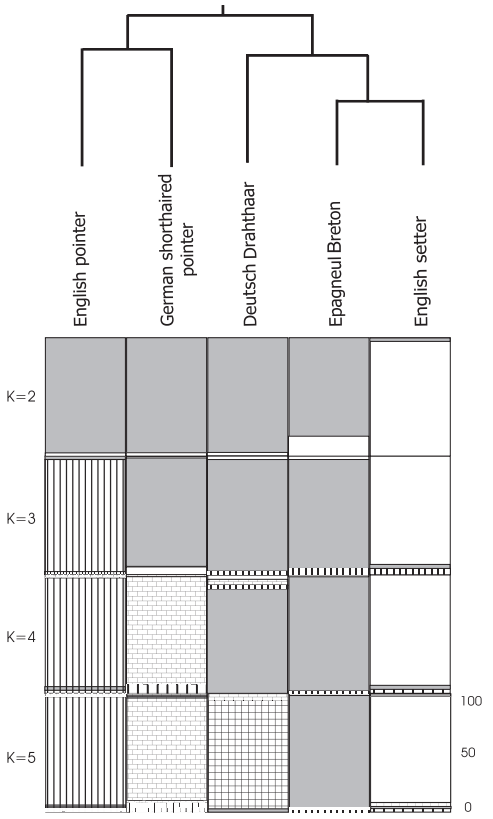

Figure 1 (Top) UPGMA clustering of the F_{ST} distance matrix. (Bottom) Estimated membership fractions of dog breeds for each of the *K* inferred clusters ($K = 2$–5) using the STRUCTURE program (Pritchard *et al.* 2000). Each breed is represented by a stacked column divided into *K* colour or grid segments, indicating the proportion of membership of each breed to the *K* clusters.

assuming different K values (i.e. the number of clusters). It should be noted that methods based on genetic distance matrices lose information by collapsing all genotype data for pairs of breeds into a single number and this value differs from the phylogenetic figure given by the clustering model-based method. Likelihood of the data was maximised when $K = 5$. A high degree of clustering was found, with the exception of the German Shorthaired Pointer, and 90% or more of the genomes of the sampled breeds were inferred to have arisen from a single cluster.

The network analysis of the mitochondrial haplotypes suggests that the maternal populations arose from four groups of genetically distinct lineages. However, no clear haplotype distribution pattern appears between the breeds (Fig. 2).

Discussion

The heterozygosity observed in the pointing breeds included in this study (61.7–68.3%) indicates a strong contribution of different individuals, although these individuals descended from the same ancestors (Arkwright 1902). This heterozygosity is higher than that reported in other studies, e.g. 31% in canids (Kim *et al.* 2001) and 53% in wild populations of wolves (Roy *et al.* 1994; García-Moreno *et al.* 1996). Although canine breeds emerged from a limited genetic pool, and non-random mating and inbreeding constitutes the basis for most breeding practices in this species (Fredholm & Winterö 1995), the pointing dogs show low F_{IS} values and genetic variability between individuals close to the variability that would be expected for random mating ($G_i = 0.5$). The most likely explanation for these results are that these breeds originated from animals of wide genetic diversity, that there was high genetic flow among populations, and/or that canine microsatellites show a high mutation rate (Irion *et al.* 2003). The English Setter ($G_i = 0.52$) and Deutsch Drahthaar ($G_i = 0.48$) are the two extremes, the latter showing a negative F_{IS} probably as the consequence of a Wahlund effect discussed below.

Relatively high genetic divergence of autosomal microsatellite markers ($F_{ST} = 11.2\%$) was found in the pointing dogs. These values are similar to Asian dogs ($F_{ST} = 15.4\%$; Kim *et al.* 2001) and Spanish dogs based on allozymes ($F_{ST} = 10\%$; Jordana *et al.* 1992), but lower than those reported by Parker *et al.* 2004 ($F_{ST} = 27\%$) who analysed 85 breeds of dogs.

Both the English Setter and the English Pointer primitive population nuclei were produced by individuals of Old Spanish Pointer during trade relationships with the British Isles in the 14th to 17th centuries. Also, at the start of the 18th (after the Treaty of Utrecht) and 19th centuries, shorthaired Old Spanish Pointer dogs were taken abroad to the British Isles and crossed with other pointers and hounds, to give rise to the modern English Pointer, which spread into Europe at the end of the 19th century and beginning of the 20th century (Arkwright 1902; Contera 1982; Sanz

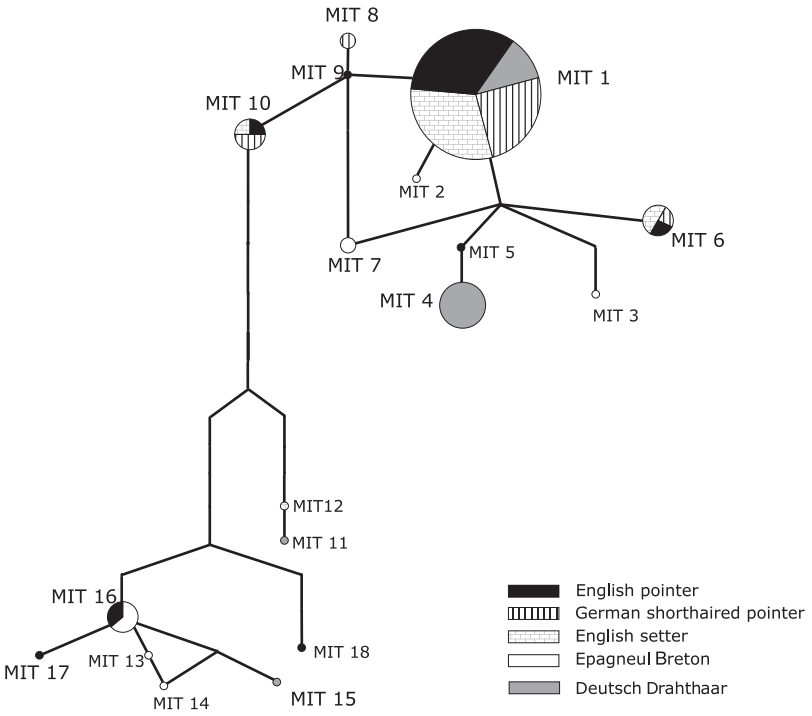

Figure 2 Network analysis of eighteen mtDNA haplotypes found in this study. The size of the circle is proportional to the observed frequency in the whole population, and the frequency observed for each mtDNA haplotype for each breed is represented by different sectors in the circle.

Timón 1982). The modern English Setter was the first breed to cluster independently when using autosomal markers (Fig. 1). That breed, as well as English Pointers and German Shorthaired Pointers, seem to share a common maternal origin either via domestic breed females in the British Isles or through Old Spanish Pointer females taken abroad as shown by their maternal genetic proximity in Table 2. In addition, the limited Y-chromosome genetic differentiation observed in English Setters and English Pointers may reflect the same paternal origin, although results of the Bayesian model-based procedure indicate a divergent evolution for the two populations with no gene exchange.

Few mtDNA haplotypes were shared between the Epagneul Bretons and the English Pointers and English Setters, reflecting the isolation of this breed respective to the others. The spread of the Longhaired Old Spanish Pointer from the Pyrenees to French Brittany was brought about mainly by male individuals that were crossed with local females. This gave rise to the spaniel nucleus (representing the origins of today's 22 spaniel breeds) as ancestors of the Epagneul Breton. These dogs were crossed at the end of the 19th century with the English Setter, English Pointer and other English Spaniels, shaping today's Epagneul Breton (De Benito 1998). This common paternal origin is reflected by the absence of significant paternal diversity between this breed and the English Pointer.

The lack of genetic divergence detected between the German Shorthaired Pointer and Deutsch Drahthaar breeds shown with Y-chromosome (paternal path) or mitochondrial DNA (maternal path) data strengthens the hypothesis of a common origin. Historical information indicates that Spanish Shorthaired animals taken to central Europe in 1467 (Eggert 1984) were mixed with local dogs, creating the Antique German Pointer breed. Also in Spain, the Burgos Pointing dog breed appeared in the 18th century from a mix of the Spanish hound and Old Spanish Pointer dogs. This new breed was exported massively to Germany at the beginning of the 19th and 20th centuries, where they were crossed with the Old German Pointer, and possibly with the English Pointer to establish the present-day German Shorthaired Pointer (Sanz Timón 1982). On the other hand, Deutsch Drahthaar was affected by a possible Wahlund effect reflected by a heterozygote excess. This breed was created at the end of the 19th/beginning of the 20th century by crossing the German Shorthaired Pointer, Griffon Khortals, Deutsch Stichelhaar and Pudelpointer (this last breed originated from the English Pointer and Standard Poodle) (Giulliani 2004). Its genetic relationship with German Shorthaired Pointer is through both local females or females arising from the Old Spanish Pointer bitches (maternal path) and the use of German Shorthaired Pointer males to avoid intense inbreeding (paternal path).

In conclusion, the use of genomic (autosomal and Y chromosome) and mitochondrial markers in five breeds which were domesticated early due to their hunting capabilities, reveals historically recognized genetic relationships and helps the understanding of the genetic background of these breeds.

Acknowledgement

The authors thank the Spanish Hunting Federation for providing the blood samples and partially funding this research.

References

Arkwright W. (1902) *The Pointer and His Predecessors*. Arthur Humphreys, London, UK.

Bandelt H.J., Forster P., Sykes C. & Richards M.B. (1995) Mitochondrial portraits of human populations using median networks. *Genetics* **141,** 743–53.

Belkhir K., Borsa P., Chikhi L., Raufaste N. & Bonhomme F. (2001) *Genetix, Logiciel Sous Windows™ Pour la Génétique des Populations, Laboratoire Génome, Populations, Interactions, CNRS UPR 9060*. Université de Montpellier II, Montpellier, France.

Caballero A. & Toro M.A. (2002) Analysis of genetic diversity for the management of conserved subdivided populations. *Conservation Genetics* **3,** 289–99.

Clement M., Posada D. & Crandall K.A. (2000) TCS: a computer program to estimate genealogies. *Molecular Ecology* **9,** 1657–9.

Clutton-Brock J. (1987) *A Natural History of Domesticated Animals*. Cambridge University Press, Cambridge, UK.

Contera C. (1982) Ensayos históricos de recuperación del pachón Navarro en España. *I Simposium Nacional de las Razas Caninas Españolas*, pp. 389–401.

De Benito E. (1998) *Libros Prácticos de los Perros de Caza. El Epagneul Bretón*. Editorial Grupo V, Madrid, Spain.

Eggert B. (1984) *Die Jagd in Bayern: von der Vorzeit bis zur Gegenwart*. Rosenheim Verlagshaus Alfred Förg GmbH, Rosenheim, Germany.

Fredholm M. & Winterö A.K. (1995) Variation of short tandem repeats within and between species belonging to the *Canidae* family. *Mammalian Genome* **6,** 11–8.

García-Moreno J., Matocq M.D., Toy M.S., Geffen E. & Wayne R.K. (1996) Relationship and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. *Conservation Biology* **10,** 376–89.

Giulliani C. (2004) *Gran Libro del Cazador y de los Perros de Caza*. Editorial de Vecchi, Spain.

Guo S.W. & Thompson E.A. (1992). Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics* **48,** 361–72.

Irion D.N., Schaffer A.L., Famula T.R., Eggleston M.L., Hughes S.S. & Pedersen N.C. (2003) Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. *Journal of Heredity* **94,** 81–7.

Jordana J., Piedrafita J., Sanchez A. & Puig P. (1992) Comparative F statistics analysis of the genetic structure of ten Spanish dog breeds. *Journal of Heredity* **83,** 367–74.

Kim K.S., Lee S.E., Jeong H.W. & Ha J.H. (1998). The complete nucleotide sequence of the domestic dog (*Canis familiaris*) mitochondrial genome. *Molecular Phylogenetics and Evolution* **10,** 210–20.

Kim K.S., Tanabe Y., Park C.K. & Ha J.H. (2001) Genetic variability in East Asian dogs using microsatellite loci analysis. *Journal of Heredity* **92,** 398–403.

Kimura M. & Crow J.F. (1964) The number of alleles that can be maintained in a finite population. *Genetics* **49,** 725–38.

Koskinen M.T. & Bredbacka P. (2000) Assessment of the population structure of five Finnish dog breed with microsatellites. *Animal Genetics* **31,** 310–7.

Olivier M., Breen M., Binns M.M. & Lust G. (1999) Localization and characterization of nucleotide sequences from the canine Y chromosome. *Chromosome Research* **7,** 223–33.

Olsen S.J. (1985) *Origins of the Domestic Dogs*. University Arizona Press, Tucson, AZ, USA.

Parker H.G. & Ostrander E.A. (2005) Canine genomics and genetics: running with the pack. *PLoS Genetics* **1,** e58.

Parker H.G., Kim L.V., Sutter N.B., Carlson S., Lorentzen T.D., Malek T.B., Johnson G.S., DeFrance H.B., Ostrander E.A. & Kruglyak L. (2004) Genetic structure of the purebred domestic dog. *Science* **304,** 1160–4.

Pritchard J.K., Stephens M. & Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155,** 945–59.

Raymond M. & Rousset F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86,** 248–9.

Roy M.S., Geffen E., Smith D., Ostrander E.A. & Wayne R.K. (1994) Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. *Molecular Biology and Evolution* **11,** 553–70.

Sanz Timón J.M. (1982) El Perro perdiguero burgalés. *I Simposium Nacional de las Razas Caninas Españolas*, pp. 359–86.

Savolainen P., Zhang Y.P., Luo J., Lundeberg J. & Leitner T. (2002) Genetic evidence for an East Asian origin of domestic dogs. *Science* **298,** 1610–3.

Schneider S., Roessli D. & Excoffier L. (2000) *Arlequin: A Software for Population Genetics Data Analysis*, Ver 2.0. Genetics and Biometry Lab, Department of Anthropology, University of Geneva, Geneva, Switzerland.

Sneath P.H. & Sokal R.R. (1973) *Numerical Taxonomy*. Freeman, San Francisco, CA, USA.

Sundqvist A.K., Ellegren H., Olivier M. & Vilà C. (2001) Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers. *Molecular Ecology* **10,** 1559–66.

Sundqvist A.K., Bjornerfeldt S., Leonard J., Hailer F., Hedhammar A., Ellegren H. & Vilá C. (2006) Unequal contribution of sexes in the origin of dog breeds. *Genetics* **172,** 1121–8.

Templeton A.R., Crandall K.A. & Sing C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132,** 619–33.

Thompson J.D., Higgins D.G. & Gibson T.J. (1994) CLUSTAL-W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22,** 4673–80.

Tsuda K., Kikkawa Y., Yonekawa H. & Tanabe Y. (1997) Extensive interbreeding occurred among multiple matriarchal ancestors during the domestication of dogs: evidence from inter and intraspecies polymorphisms in the D-loop region of mitochondrial DNA between dogs and wolves. *Genes and Genetic Systems* **72,** 229–38.

Vilà C., Savolainen P., Maldonado J.E., Amorim I.R., Rice J.E., Honeycutt R.L., Crandall K.A., Lundeberg J. & Wayne R.K. (1997) Multiple and ancient origins of the domestic dog. *Science* **276,** 1687–9.

Vilà C., Maldonado J.E. & Wayne R.K. (1999) Phylogenetic relationships, evolution and genetic diversity of the domestic dog. *Journal of Heredity* **90,** 71–7.

Wayne R.K. & Ostrander E.A. (1999) Origin, genetic diversity and genome structure of the domestic dog. *BioEssays* **21,** 247–57.

Weir B.S. & Cockerham C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution* **38,** 1358–70.

Weitzman M.L. (1992) On diversity. *The Quarterly Journal of Economics* **107,** 363–405.

Wright S. (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* **19,** 395–420.

Supplementary material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01658.x

Table S1 Number of alleles (N) and effective number of alleles (ENA), observed (H_o) and expected (H_e) heterozygosity and Wright's F-statistics, F_{IS}, F_{ST}, F_{IT}, for each of the 21 microsatellite markers found in five European pointing dog breeds: English Pointer, English Setter, Epagneul Breton, Deutsch Drahthaar and German Shorthaired Pointer.

Table S2 Microsatellite name, allele size (first row) and allele frequency (second row) for each of the 21 autosomal microsatellite markers identified in five European pointing dog breeds: German Shorthaired Pointer, Deutsch Drahthaar, Epagneul Breton, English Pointer and English Setter.

Table S3 Haplotype distribution of the Y-chromosome microsatellites examined in five European pointing dog breeds.

Table S4 Different mtDNA haplotypes defined in five pointing dog breeds, indicating the polymorphic sites numbered according to GenBank accession number U96639 and their distribution per breed and frequency per haplotype.

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