



ORIGINAL ARTICLE

The effects of dog breed development on genetic diversity and the relative influences of performance and conformation breeding

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Summary

Genetic diversity was compared among eight dog breeds selected primarily for conformation (Standard Poodle, Italian Greyhound and show English Setter), conformation and performance (Brittany), predominantly performance (German Shorthaired and Wirehaired Pointers) or solely performance (field English Setter and Red Setter). Modern village dogs, which better reflect ancestral genetic diversity, were used as the standard. Four to seven maternal and one to two Y haplotypes were found per breed, with one usually dominant. Diversity of maternal haplotypes was greatest in village dogs, intermediate in performance breeds and lowest in conformation breeds. Maternal haplotype sharing occurred across all breeds, while Y haplotypes were more breed specific. Almost all paternal haplotypes were identified among village dogs, with the exception of the dominant Y haplotype in Brittanys, which has not been identified heretofore. The highest heterozygosity based on 24 autosomal microsatellites was found in village dogs and the lowest in conformation (show) breeds. Principal coordinate analysis indicated that conformation-type breeds were distinct from breeds heavily used for performance, the latter clustering more closely with village dogs. The Brittany, a well-established dual show and field breed, was also genetically intermediate between the conformation and performance breeds. The number of DLA-DRB1 alleles varied from 3 to 10 per breed with extensive sharing. SNPs across the wider DLA region were more frequently homozygous in all pure breeds than in village dogs. Compared with their village dog relatives, all modern breed dogs exhibit reduced genetic diversity. Genetic diversity was even more reduced among breeds under selection for show/conformation.

Introduction

Studies of mtDNA indicate that dogs evolved from grey wolves as long as 16 300 years ago (Pang *et al.* 2009). Fossil evidence appears to place definitive dogs with people 12 000–14 000 years ago (reviewed Crockford & Kuzmin 2012). Dogs identical in appearance to modern village dogs were portrayed on

pottery from ancient Persia 7500 years ago, while distinct 'breeds' of dogs resembling Afghan Hounds or Salukis were first seen on pots from this same region around 6000 years ago (Hole & Wyllie 2007), suggesting that human selection of dogs began in earnest in the interim period. Dogs resembling modern sight-hounds, mastiffs and Basenjis were portrayed in a range of activities with humans in Egypt 4500 years

ago (Brewer *et al.* 2001). The oldest breeds include the Saluki and Afghan Hound from the Middle East, Siberian Husky and Samoyed from Russia, Akita Inu and Shiba Inu from Japan, Alaska Malamute from North America, Basenji from Africa, and the Chow Chow, Lhasa Apso, Pekingese, Shar Pei, Shi Tzu and Tibetan Terrier from China (Parker *et al.* 2004).

Although dogs have been exposed to human-related selection pressures for millennia, breeds in the modern sense began with the creation of the English Kennel Club in 1873 and the accurate documenting of pedigrees and registration of breeds. Pedigree or pure breeding involves the selection (registration) of a relatively closed pool of potential breeding stock that conforms to an idealized standard. Once the ideal traits become uniform among the breed, further refinements are subject mainly to how those standards were interpreted in the show ring or field.

Virtually, all modern dog breeds can trace their origins to performance of some type, whether it be pointing, retrieving, coursing, guarding, racing, the hunting of unique types of game or activities as obscure as bull-baiting. However, with the advent of pedigrees and dog showing, many breeds selected originally for performance are now bred mainly for conformation. Breeding practices for conformation and performance have much different goals. Performance breeding is less likely to lead to pronounced changes in basic form and function. Conformation breeding, by contrast, has led to significant changes in the outward appearance and performance skills of many breeds, sometimes to extremes. Indeed, the excesses of conformation breeding have been increasingly criticized (Harrison 2008). Nonetheless, conformation breeds enjoy great popularity because of their uniformity in appearance and temperament and most enjoy lives as long, and sometimes longer, than mixed-breed dogs (Proschowsky *et al.* 2003).

Although it is intuitively believed that breeding for conformation leads to excessive inbreeding, while breeding for performance is more apt to maintain diversity, the subject is more widely debated than researched. These debates have had limited influence on breed development, but have yielded some interesting compromises. Certain dog breeds are used equally or unequally for show and performance and share the same registry, while other breeds are independently registered for either show or performance. Still other breeds are recognized under the same name, but are registered separately in show or field registries. Some modern breeds have evolved from attempts to re-establish older breeds or to re-instil performance skills. Regardless of their origins or use, it is

generally conceded that dogs have lost genetic diversity at various points and rates in their evolution. The first loss occurred as dogs evolved from wolves. A second loss of diversity presumably occurred during the several millennia that followed the appearance of dogs that were phenotypically different from village dogs of their time. However, the third and greatest changes in canine form and function, and presumably genetic diversity, have been attributed to the post-Victorian era. Therefore, to compare the magnitude of genetic diversity loss among modern breeds, the genetic diversity that existed prior to the Victorian era must also be considered. Fortunately, the ancestral stock of present-day breeds, which also reflect the loss of diversity from their wolf ancestors, still exists in many regions of the world in the form of village dogs (Brown *et al.* 2011) or from modern breeds tracing their origins to random bred dogs of their same regions (Vilá *et al.* 1999; Savolainen *et al.* 2002; Pang *et al.* 2009).

This study tested the potential genetic influences of conformation versus performance breeding based on genetic information gleaned from eight recognized breeds and compared these findings with their randomly breeding indigenous village dog relatives. These breeds were selected for specific reasons. The Standard Poodle evolved from duck retrievers in Germany and France over 500 years ago, but has been extensively refined over the last century (reviewed Pedersen *et al.* 2012a). Likewise, the Italian Greyhound is a descendent of larger dogs used in ancient times for sighting and chasing game (reviewed by Pedersen *et al.* 2012b). The six performance breeds selected for the study evolved from a large number of regional breeds used for pointing and retrieving of fowl. However, these performance breeds have also been subject to intense human selection over the last century. The Brittany (L'Épagneul Breton) has been known for centuries but was not formally recognized as a breed until 1907 (Riddell 1997). The Brittanys' performance traits have been judiciously preserved, while still enjoying great popularity in the show ring, making it an excellent breed for studying the opposing effects of conformation and performance breeding. Show and field English Setters have been bred separately for each function for over 75 years and are now distinct in terms of both function and appearance, thus allowing another perspective of the two breeding goals. The German Shorthaired Pointer differs from the Wirehaired Pointer mainly in its coat. Both breeds have long been touted as outstanding all-purpose (field and water, pointing and retrieving) gun dogs, but are increasingly being shown (benched) in

the ring over the last few decades. The Red Setter originated after World War II, basically as a recreation of the performance Irish Setter, which has become predominantly a show breed and lost much of its hunting abilities (Sisley 2010).

Genetic comparisons in this study included the use of maternal, paternal and biparentally inherited markers (including 24 STRs and DLA class II gene sequences and SNPs). Genetic diversity parameters of these eight breeds were then compared against each other, and then to what might be considered a gold standard, Southeast Asian village dogs. All of these populations can trace their ancestry to randomly breeding indigenous dogs in this part of the world (Brown *et al.* 2011).

Materials and methods

Sample collection

Samples from 149 Standard Poodles, 77 Italian Greyhounds and 35 show English Setters were collected directly from shows, owners and breeders, while samples from 72 Brittany, 36 German Shorthaired Pointers and 11 German Wirehaired Pointers were collected both from shows and field trials. Samples from 13 field English Setters and 15 Red Setters were collected during field trials. Basic details such as breed, age, gender, health status, and registered name and/or number were obtained for each individual samples. Pedigrees for all breed dogs participating in this study were downloaded from the American Kennel Club (AKC) (Standard Poodle, Italian Greyhounds, Brittany, German Shorthaired and Wirehaired Pointers, and English Setters) or American Field Sporting Dog Association websites (Red Setter). Only breed dogs unrelated through grandparents were used in the study. Additionally, DNA was extracted from buccal swabs of village dog populations in Lebanon ($n = 54$), Iran ($n = 233$), Taiwan ($n = 63$), Thailand ($n = 49$), Philippines ($n = 18$), Brunei ($n = 24$), Cook Islands ($n = 20$) and Bali ($n = 72$) that were collected during past studies (Irion *et al.* 2005; Brown *et al.* 2011).

DNA extraction

DNA was extracted from whole EDTA blood or cytological brushes using Qiagen Gentra Puregene Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions or using a sodium hydroxide protocol (Irion *et al.* 2003; Brown *et al.* 2011).

Determination of maternal and paternal haplotypes

Mitochondrial DNA haplotypes were determined by sequencing 655 bp of the mitochondrial control region (nt 15452–16107) as described by Vilá *et al.* (1999). Primer sequences, conditions for PCR and sequencing were as described by Pedersen *et al.* (2012b). Sequencing was conducted in both directions for each sample and analysed using SEQMAN software (DNASTAR Lasergene 8; DNASTAR, Madison, WI, USA). Final sequences were compared with a large canine forensic database provided by Beth Wictum, Director of Veterinary Forensics, Veterinary Genetics Laboratory (VGL), UC Davis and the GenBank nucleotide database.

Y chromosome haplotypes were determined with a panel of seven Y-STR markers, including MS34A, MS34B, MS41A, MS41B, 990.35.4, 650.79.2 and 650.79.3 (Bannasch *et al.* 2005; Brown *et al.* 2011). Primer sequences and allele sizes have been previously reported (Brown *et al.* 2011; Pedersen *et al.* 2012b).

Data for village dog maternal mtDNA ($n = 421$ dogs) and Y haplotypes ($n = 300$ dogs) were determined as a part of a previously reported study (Brown *et al.* 2011). All other measurements were new to this study.

Genetic diversity using STR markers

Twenty-four STRs located on 20 different autosomes were used in the study. An additional marker for amelogenin (*AMELX* and *AMELY*) was added for the confirmation of gender. Primers, dye labels and multiplexes for 22 of these markers have been published as part of the 2005 International Society for Animal Genetics (ISAG) canine panel for parentage verification (accessed 23 May 2012 at www.isag.us/Docs/2005ISAGPanelDOG.pdf). This panel was augmented by three additional STRs, FH2001, FH2328 and LE1004. Repeat motif, chromosome assignment, known allele numbers and allele size range for this set of markers have been previously reported (Pedersen *et al.* 2012b). STR-based genotyping was conducted by the VGL, UC Davis, and data analyses conducted using GENEMAPPER v3.7 (Applied Biosystems, Carlsbad, CA, USA).

DLA class II genotyping

Alleles of the DLA class II DRB1 locus were determined by sequence-based typing using published locus-specific intronic primers (Kennedy *et al.*, 2007).

PCRs and sequencing procedures have been previously described (Pedersen *et al.* 2012b).

Vector NTI advanceTM software (Invitrogen, Carlsbad, CA, USA) was used for the alignment of sequence data. DLA class II alleles were determined by a subtractive approach (Kennedy *et al.*, 2007), wherein homozygous alleles were identified at each locus, and used alongside published allele sequences to identify heterozygous alleles. Allele nomenclature was determined from known sequences in GenBank and in the Immune Polymorphism Database (accessed 23 May 2012 at <http://www.ebi.ac.uk/ipd>).

DLA wide SNP typing

The DLA region was interrogated with a panel of 126 SNPs from across the entire DLA (nt 3 320 000–5 830 000) on CFA12 (Safra *et al.* 2011). SNPs were assayed using a Sequenom MassARRAY Compact 96 using iPLEX Gold technology (Sequenom, San Diego, CA, USA) by the Veterinary Genetics Laboratory, University of California, Davis.

Data analysis

Differences in genetic diversity across dog categories (village, performance and show breeds) were assessed using one-way analysis of variance (ANOVA) to compare heterozygosity (H_e) and inbreeding (F_{is}), which are relatively insensitive to sample size. Analysis of covariance (ANCOVA) was used to compare observed numbers of alleles, including sample size (ln-transformed) as a covariate. Fisher's least significant difference (LSD) tests were used to test significance of post hoc pairwise comparisons, while correcting for multiple tests.

As a blind genetic clustering approach, a Bayesian model-based method that utilizes genotype frequencies but no prior information on the population of origin was implemented in Structure v. 2.0, to assess substructure within the data set (Pritchard *et al.* 2000). The admixture model with correlated allele frequencies was employed. Runs of 20 000 MCMC cycles (first 10 000 discarded as burn-in) were conducted for numbers of clusters (K) ranging 1–6 to examine the occurrence of hierarchical (i.e. nested) substructure among and within breeds. Additionally, principle coordinates analysis (PCoA) was conducted using Genalex 6.

Results

Fourteen maternal mtDNA haplotypes were identified among the 8 breeds studied, and major haplotype

sharing was extensive among breeds, with some minor haplotypes being breed specific (Table 1). Individuals within the Standard Poodle and Italian Greyhound breeds were predominantly from one specific maternal lineage and therefore had the lowest haplotype diversity (0.316 and 0.351, respectively). In contrast, each of the breeds used for performance evolved from several major and minor maternal haplotypes, which were widely shared and showed much greater haplotype diversity (0.617–0.724).

All breeds exhibited very low Y haplotype diversity compared with that observed in SW and SE Asian village dogs, where the Y chromosome diversity was significantly higher than mtDNA diversity (Brown *et al.* 2011). Five Y-STR haplotypes were observed in one or more of the eight breeds (Table 2). Male dogs of every breed possessed one, or one major and one minor, Y haplotypes, while Red Setters had two haplotypes in similar frequency. German Shorthaired, Wirehaired Pointers, Red Setters, field English Setters and Standard Poodles shared the DKEGICH haplotype (Table 2). The second paternal haplotype present in Red Setters was unique within this study but has been found in other breeds and village dogs (Bannasch *et al.* 2005; Brown *et al.* 2011). The minor Y haplotype (DKEGICF) of Brittany and Italian Greyhound is shared with show English Setters. A single male Brittany shared the same paternal haplotype found in all show English Setters, while 31 of 32 Brittany exhibited a paternal haplotype (DLFGHCH) not previously observed in dogs (Bannasch *et al.* 2005; Brown *et al.* 2011). The Italian Greyhound haplotype (DJEGICF), although unique within this study, was previously found in 7 other small or toy breeds and in a Southeast Asian village dog (Brown *et al.* 2011).

Allelic diversity data from the 24 autosomal STRs were used to analyse the genetic diversity and population structure of the eight breeds selected for this study and of village dogs (Table 3). Heterozygosity differed among village dog populations, performance breeds and conformation breeds ($F_{2,11} = 17.0$, $p < 0.001$), and all pairwise differences were significant (Fisher's LSD $p < 0.016$; Table 3), with diversity highest for village dogs, intermediate for breeds used for performance and lowest for conformation breeds. The number of alleles per locus also differed significantly ($F_{2,10} = 70.1$, $p < 0.001$), as did all pairwise comparisons ($p < 0.001$), and followed a similar trend as heterozygosity. As expected, sample size was also a highly significant covariate ($F_{1,10} = 82.5$, $p < 0.001$; Figure 1). There was no significant difference among the three dog categories in inbreeding coefficient ($F_{2,11} = 3.27$, $p = 0.077$).

Table 1 The frequency of mitochondrial DNA (mtDNA) haplotypes among eight registered breeds

mtDNA type	Equivalent haplotypes ^a	Known breed distribution	Brittany n = 69	English Setter show n = 32	English Setter field n = 10	German SH pointer n = 27	German WH pointer n = 9	Red Setter n = 14	Italian Greyhound n = 111	St. Poodle n = 100
A	B1, B2, B3, B5, B6, B13	UT ^b	0	0	1 (10.0)	6 (22.2)	2 (22.2)	0	15 (13.5)	82 (82.0)
B	A18, A20	UT	1 (1.5)	15 (46.7)	5 (50.0)	6 (22.2)	0	1 (7.1)	0	7 (7.0)
C	A11, A13	UT	8 (11.6)	8 (25.0)	2 (20.0)	13 (48.2)	1 (11.1)	0	3 (2.7)	8 (8.0)
D	A24, A25	Great Pyrenees, Irish wolf hound	23 (33.3)	0	0	0	0	5 (35.7)	0	2 (2.0)
F	D5	Turkey (Kangal)	0	0	0	0	0	0	0	1 (1.0)
H	A1, A2	Widespread in Eurasia	3 (4.4)	0	0	0	1 (11.1)	1 (7.1)	0	0
J	A16, A17, A33	UT	28 (40.6)	0	0	1 (3.7)	5 (55.6)	5 (35.7)	88 (79.3)	0
K	C3	UT	2 (2.9)	7 (21.9)	2 (20.0)	0	0	1 (7.1)	0	0
L	1 nt different C3	Novel	4 (5.8)	0	0	0	0	0	0	0
M	1 nt different A11, A13	Novel	0	2 (6.3)	0	0	0	0	0	0
O	A3, A5, A7, A8, A9	UT	0	0	0	1 (3.7)	0	0	0	0
P	1 nt different A11, A13	Novel	0	0	0	0	0	1 (7.1)	0	0
Q	B11	China, Chinese crested dog	0	0	0	0	0	0	4 (3.6)	0
R	C1, C2	UT	0	0	0	0	0	0	1 (0.9)	0
		Haplotype diversity	0.713	0.666	0.660	0.667	0.617	0.724	0.351	0.316

^aHaplotype names used in Savolainen *et al.* 2002.^bUT = universal type, meaning that these haplotypes have been found in dogs all over the world and in many breeds.

The population structure analysis of performance-related dogs, plus one show breed (show English Setter), revealed a hierarchical (nested) pattern, whereby increasing levels of *K* (numbers of genetic clusters) led to splitting of clusters at lower levels of *K* (Figure 2). In particular, the first breed to be distinguished was the show variety of English Setters ($K = 2$). Brittanys were distinguishable from the show English setters and all other breeds at $K = 3$. At $K = 4$, both German Wirehairs and Shorthairs clustered out, followed by Red Setters and field English Setters (clustering together) at $K = 5$, which also included some subdivision within German Shorthairs, in common with three Red Setters.

A PCoA plot was constructed for the eight breeds based on data from the 24 autosomal STRs (Figure 3a). Standard Poodles and Italian Greyhounds formed distinct and well-differentiated populations, while the remaining show and performance breeds tended to cluster together, although with some degree of separation. When the data shown in Figure 3a

were plotted in a different dimension, C2 versus C3 (Figure 3b), the show English Setter formed a well-differentiated breed like the Standard Poodle and Italian Greyhound. In an attempt to define the five performance breeds, the Standard Poodles, Italian Greyhounds and show English Setters were removed from the analysis and the genetic differentiation among the five performance breeds became more evident (Figure 3c). German Wirehaired Pointers clustered within the German Shorthaired Pointers reflecting a common ancestry (Figure 3c). The Red Setters formed a separate population, while the field English Setters overlapped both the Red Setters and the two German Pointer breeds. These relationships support the use of field English Setters in the creation of the Red Setter and the possible use of German Pointers to enhance the performance of the field English Setter. The Brittany formed a distinct population among the five breeds, supporting its long history as a distinct and closed breed.

Table 2 Y-STR haplotypes of show and performance breeds

Y haplotype alleles ^a	Equivalent haplotypes ^b	Comments ^c	Brittany	English Setter (show)	English Setter (field)	German SH Pointer	German WH Pointer	Red Setter	Italian Greyhound	Standard Poodle
DJEGICF	8d, n4	SE Asian village dogs, Several breeds	0	0	0	0	0	0	42	0
DKEGICF	12d, 6m, 6q	Dingo, Several breeds	1	16	0	0	0	0	2	0
DKEGICH	6p, 6t, 6za, 9x	SE Asian village dogs, Several breeds	0	0	8	19	8	5	0	91
DLEGICG	6zi	SE Asian village dogs, Several breeds	0	0	0	0	0	6	0	0
DLFHGCH	Novel	1-off 11f, 6ze in Cavalier king charles Spaniel	31	0	0	0	0	0	0	0
Haplotype diversity		0	0.061	0	0	0	0	0.496	0.087	0

^aLettered alleles correspond to successive numbers of dinucleotide repeats corresponding to the following loci (and amplicon lengths) as follows: 650.79.2a (A = 114 bp), 650.79.2b (A = 114 bp), 990.35.4 (A = 118 bp), MS34CA (A = 160 bp), MS34TT (A = 160 bp), MS41A (A = 202 bp), MS41B (A = 204 bp), respectively.

^bNames correspond to haplotypes in Brown *et al.* 2011; those haplotypes include an additional locus, 79.3, not used here, resulting in multiple haplotype equivalents where haplotypes differ only at the 79.3 locus.

^cIndicates where haplotype equivalents were previously reported in several breeds (Bannasch *et al.* 2005) and in Southeast Asian village dogs (Brown *et al.* 2011).

Table 3 Expected (H_e) and observed (H_o) heterozygosity sorted from greatest to least H_e , and average number of alleles per locus based on the 24 autosomal markers, for village dogs (v) and registered breeds used for performance (p) and/or conformation (c)

Population	Type	n	H_e	H_o	No. alleles
Taiwan	v	63	0.806	0.799	9.1
Iran	v	233	0.798	0.778	10.8
Cook Islands	v	65	0.792	0.743	9.0
Thailand	v	49	0.792	0.729	8.9
Brunei	v	24	0.784	0.769	7.5
Philippines	v	18	0.777	0.777	6.8
Bali	v	72	0.761	0.716	9.2
Ger. WH. Pointer	p, c	11	0.730	0.716	5.1
Ger. SH. Pointer	p, c	36	0.720	0.707	6.2
Eng. Setter (field)	p	13	0.655	0.685	4.7
Brittany	p, c	72	0.654	0.627	6.5
Red setter	p	17	0.625	0.620	4.8
Std. poodles	c	149	0.615	0.576	6.8
Italian greyhound	c	77	0.597	0.570	5.1
Eng. Setter (show)	c	36	0.463	0.432	3.7

The genetic diversity of the eight recognized breeds was compared with a pool of village dogs from Iran, Taiwan, Thailand, Bali, Philippines, Brunei and Cook Island using PCoA (Figure 4a). The village dogs tended to cluster together with little apparent differentiation by geographical origin. Italian Greyhounds,

Standard Poodles and show English Setters were clearly differentiated from all other breeds and village dogs. Three of the performance-type breeds (German Shorthaired and Wirehaired Pointers, field English Setter) clustered among the indigenous dogs, indicating that they had differentiated the least from ancestral type. The Red Setter and Brittany populations also appeared to be evolving as distinct breeds, but to a lesser extent than the three predominantly conformation-type breeds. This evolution became more apparent when the three conformation breeds were removed from the analysis, with the Brittany emerging as a separate population that was distinct from the other performance-type breeds and indigenous dogs (Figure 4b). Bali dogs were also beginning to differentiate as a separate population from village dogs of other regions. The unique status of Bali dogs compared with other village dogs of the Middle East and SE Asia has been ascribed to their island isolation over several thousands of years (Brown *et al.* 2011). Cook Island dogs formed two separate populations within the cluster of indigenous dogs, one related to village Bali dogs and one aligned with village dogs from Iran.

The genetic relationship among the eight breeds was also investigated using data from the STRs by first constructing a matrix of genetic distances (Nei's DA) and then using a neighbour joining tree (Figure 5).

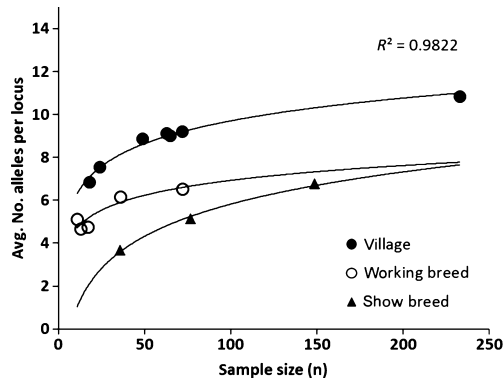


Figure 1 The number of microsatellite alleles (averaged across 24 loci) observed as a function of sample size in Asian village dogs, performance (working) breeds, and conformation (show) breeds, illustrating differences in genetic diversity corresponding to breeding history. Village dog populations (and sample sizes) include Lebanon (54), Iran (211), Taiwan (34), Thailand (47), Philippines (14), and Brunei (24); working breeds include Brittany (72), English setter, field variety (13), German shorthaired (36), German wirehaired (11), and red setter (15); show breeds include English setter, show variety (35), Italian greyhound (77), and standard poodle (149).

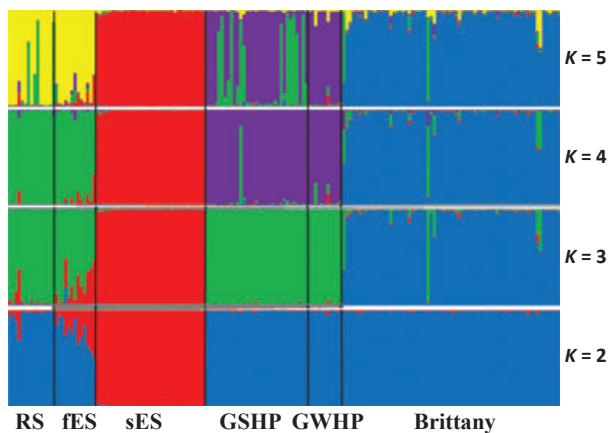


Figure 2 Bayesian cluster analysis from program Structure of 5 dog breeds used in performance trials indicating hierarchical structure (RS, Red Setter; fES, field English Setter; sES, show English Setter; GSHP, German Shorthaired Pointer; GWHP, German Wirehaired Pointer).

Bootstrap support was strong pairing the two German breeds and the two English Setter breeds. These patterns mirrored the principle coordinate analyses (Figures 3 and 4).

The use of SNP markers in the DLA region to construct zygosity maps provided a graphical overview of heterozygosity for alternative SNP alleles (Figures 6 and 7). Zygosity maps of the eight breeds were then

compared with that from a similar sized group of Southeast Asian village dogs. The DLA region of indigenous dogs was much more heterozygous than that of any of the eight breeds, and there was more balanced use of alternative alleles. The show English Setter was the most homozygous across the DLA region of all eight breeds, while all of the other seven breeds appeared similar in heterozygosity to each other. The main difference between breed and indigenous dogs was in the relative number of individuals within each population that were homozygous across most of the DLA region. Over one-half of show English Setters, one-third of Standard Poodles, one-fourth of Brittanys, one-fifth of German Shorthaired Pointers, one-half of German Wirehaired Pointers, one-fourth of field English Setters and one-eighth of Italian Greyhounds were homozygous across most of the DLA region for a major or minor allele (Figures 6 and 7). Only three indigenous dogs were more homozygous, but to a much lesser extent than that of breed dogs, which had large regions of homozygosity.

DRB1 is the most polymorphic of the three DLA class II loci and was therefore used to compare class II

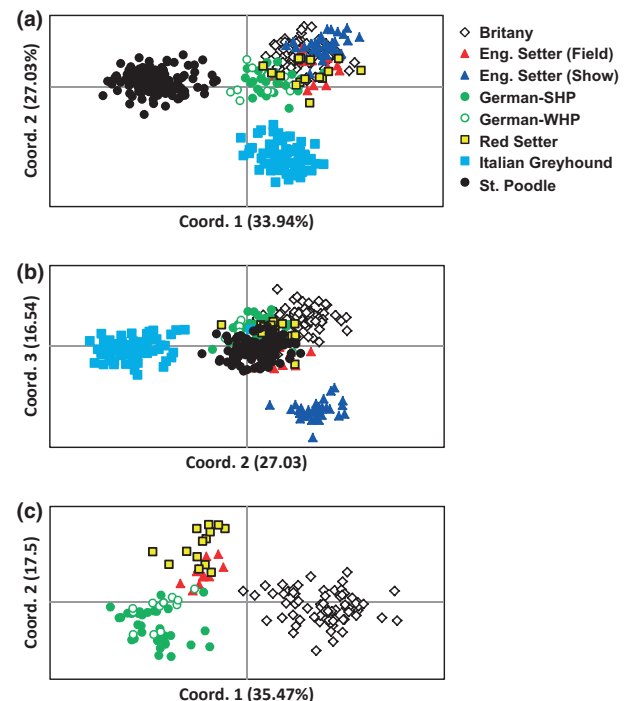


Figure 3 (a) PCoA plot showing genetic relationships of eight conformation and performance-type breeds. (b) The same samples as in (a) were plotted using dimension coordinates 2 versus 3, demonstrating that English Setters are a distinct population like Standard Poodles and Italian Greyhounds. (c) PCoA plot showing genetic relationships of the five breeds used predominantly for performance.

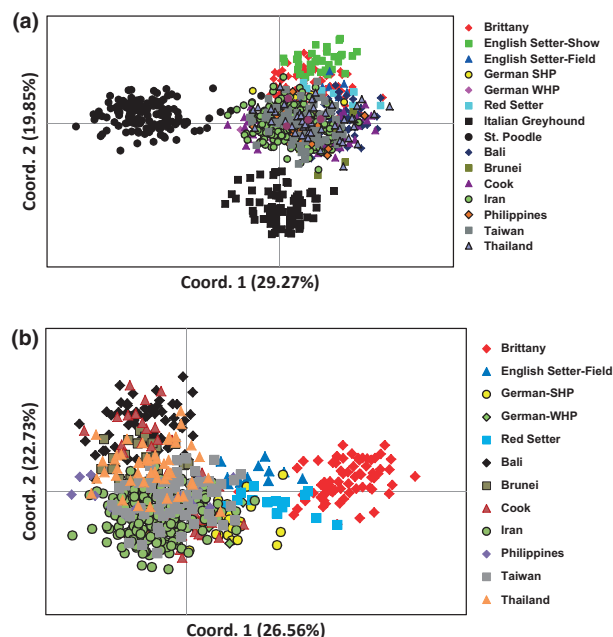


Figure 4 (a) PCoA of conformation (show English Setter, Italian Greyhound, Standard Poodle), performance breeds (Brittany, field English Setter, German Shorthaired and Wirehaired Pointers, and Red Setter) and various village dog populations based on the genetic diversity of autosomal STR data. (b) PCoA of the five performance breeds and Village dog populations.

diversity across breeds in this study. Nineteen different alleles were detected among the eight breeds (Table 4). All but one of these alleles (DRB1*00106) in Standard Poodles had been previously recognized among other breeds. DRB1*00101 was found in all eight breeds, but was the dominant allele in show and field English Setters. DRB1*01501 was found in all breeds except the Italian Greyhound and was either the dominant or one of the dominant alleles in the Standard Poodles, Brittany, Red Setter and field English Setter. There was extensive sharing of alleles among the seven breeds that have been historically pointers and retrievers and have a long common history. The DRB1 alleles of the Italian Greyhound were clearly different from these seven breeds, indicating a distinct origin. The Italian Greyhound evolved from coursers of game and not pointers and retrievers (reviewed Pedersen *et al.* 2012b). The diversity (equivalent to expected heterozygosity) ranged from 0.397 in the Standard Poodle to 0.847 in the German Wirehaired Pointer (Table 4). Based on the results from Standard Poodles, it was apparent that the more individuals sampled, the more alleles (especially minor alleles) would be found. Nonetheless, enough dogs were sampled in each breed to identify clear trends. The Italian Greyhound exhibited eight differ-

ent DRB1 alleles in somewhat comparable proportions. The German Shorthaired Pointer used eight DRB1 alleles in a similar manner as the Italian Greyhound. Therefore, these two breeds were the most genetically diverse in terms of the DLA-DRB1 locus. German Wirehaired Pointers, Red Setter and field English Setter, while demonstrating fewer alleles, proportioned their available alleles between individuals in a balanced fashion. In comparison, the show English Setter, Brittany and Standard Poodle, which were three of the four breeds heavily involved in showing, used one DRB1 allele considerably out of proportion to other alleles. The DRB1 alleles recognized in each breed, and their frequencies mirrored most breed relationships shown previously for PCA plot and unrooted neighbour joining tree (Figures 2–4).

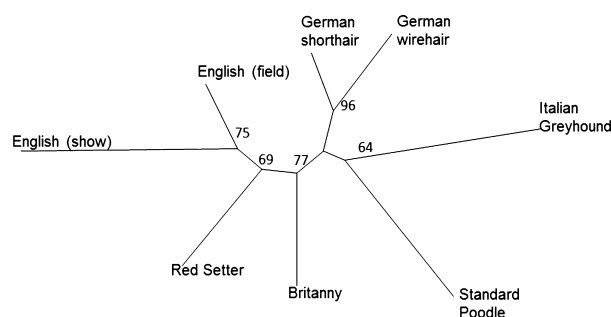
Discussion

The present study compared genetic diversity among eight breeds that have been increasingly selected solely for conformation (show and aesthetics), performance (work and field) or varying degrees of both activities. The first and most basic comparisons were between maternal and paternal haplotypes. In this study, only five Y-STR haplotypes were observed in the eight breeds ($n = 230$) compared with 95 Y-STR haplotypes among 300 village dogs from Southeast Asia and Island South Pacific (Brown *et al.* 2011). No significant differences were observed in Y haplotype diversity between conformation and performance-related breeds, and the number of Y haplotypes represented only 5% of those found in the village dogs. This confirmed what is known about Y haplotypes in modern breeds, that is, most exhibit one or two dominant Y haplotypes with extensive haplotype sharing (Bannasch *et al.* 2005). A unique finding in this study was the identification of a Y haplotype in 31 of 32 Brittanys dogs not identified heretofore in any other dogs, but one mutation distant from a haplotype in the Cavalier King Charles Spaniel.

Fourteen maternal haplotypes were observed across all eight breeds ($n = 383$), while 54 maternal haplotypes were identified using the same mtDNA sequence in 421 village dogs from the Middle East and Southeast Asia (Brown *et al.* 2011). The relative numbers and maternal haplotypes among the eight breeds in this study were consistent with what has been reported for 27 breeds by Kropatsch *et al.* (2011). Even though the latter sequences covered a larger region of the mtDNA, they identified only 1–5 matriline (usually 2–4) per breed tested. The Standard Poodle and Italian Greyhound were

Table 4 DLA-DRB1 alleles and their relative frequencies in eight different breeds

DRB1	GSHP n = 24	GWHP n = 12	RS n = 16	fES n = 12	Br n = 70	sES n = 34	SP n = 168	IG n = 142
*00106							2 (1.19)	
*00101	4 (16.67)	1 (8.33)	6 (37.5)	6 (50.0)	8 (11.43)	25 (73.53)	1 (0.60)	10 (7.04)
*01501	3 (12.5)	1 (8.33)	8 (50.0)	4 (33.33)	42 (60.0)	1 (2.94)	129 (76.8)	
*01502	2 (8.33)						16 (9.52)	
*01503							4 (2.38)	
*00901	6 (25.0)	6 (50.0)			8 (11.43)		3 (1.79)	
*02001	4 (16.67)	2 (16.67)					10 (5.95)	
*01201	1 (4.17)	2 (16.67)						
*00102	1 (4.17)				12 (17.14)			
*00102:01			2 (12.5)					
*01801	1 (4.17)							
*00601	2 (8.33)			2 (16.67)				23 (16.2)
*00603								35 (24.65)
*01301						8 (23.53)		10 (7.04)
*00201							1 (0.60)	9 (6.34)
*00203								18 (12.68)
*010011							1 (0.60)	
*01101							1 (0.60)	26 (18.31)
*02901	0.847	0.681	0.594	0.611	0.584	0.403	0.397	11 (7.75)
Diversity								0.843

**Figure 5** Unrooted neighbor-joining tree based on pairwise genetic distances among 8 dog breeds. Numerals indicate bootstrap support for nodes on the basis of resampling across the 24 STR loci.

each derived from one specific maternal lineage and also had the lowest haplotype diversity (0.316 and 0.351, respectively). The six performance or performance-related breeds shared several major and minor maternal haplotypes and had a greater maternal haplotype diversity (0.617–0.724). The present findings on paternal and maternal haplotypes supports those of Sundqvist *et al.* (2006) that there is a sex bias in the origin of dog breeds, with fewer males than females contributing genetically. This clearly differs from the random breeding patterns in village dogs reported herein and wild grey wolf populations (Sundqvist *et al.* 2006), where both sexes have similar contributions.

The loss of genetic diversity within the canine autosomes resulting from breed development is well recognized and has been associated with specific regions of the dog genome that have undergone intense human-directed selective pressures for fixing desired phenotypic traits (Akey *et al.* 2010; Vaysse *et al.* 2011). However, broader genome-wide losses of genetic diversity may also occur from inbreeding associated with the selection process. This type of loss was reflected by allelic diversity data from the 24 autosomal STRs. As expected, genetic diversity was highest for indigenous dogs and lowest for show breeds, with performance breeds being intermediate. Similar findings have been previously reported by Irion *et al.* (2003).

Loss of genetic diversity among breed versus indigenous dogs was also evident across the entire DLA region. The MHC is under balancing selection, which acts to systematically resist loss of rarer alleles. Therefore, loss of alleles in this region is one of the earliest indicators of loss of genetic diversity because of inbreeding. The DLA region of indigenous dogs was more heterozygous than in breed dogs, blocks of homozygosity were comparatively small, and there was a more balanced use of alleles. A number of individuals within each breed had lost heterozygosity across most of the DLA. Such highly inbred individuals were recognized by Calboli *et al.* (2008), who chose 10 representative breeds from the UK and analysed their pedigrees back as many as eight genera-

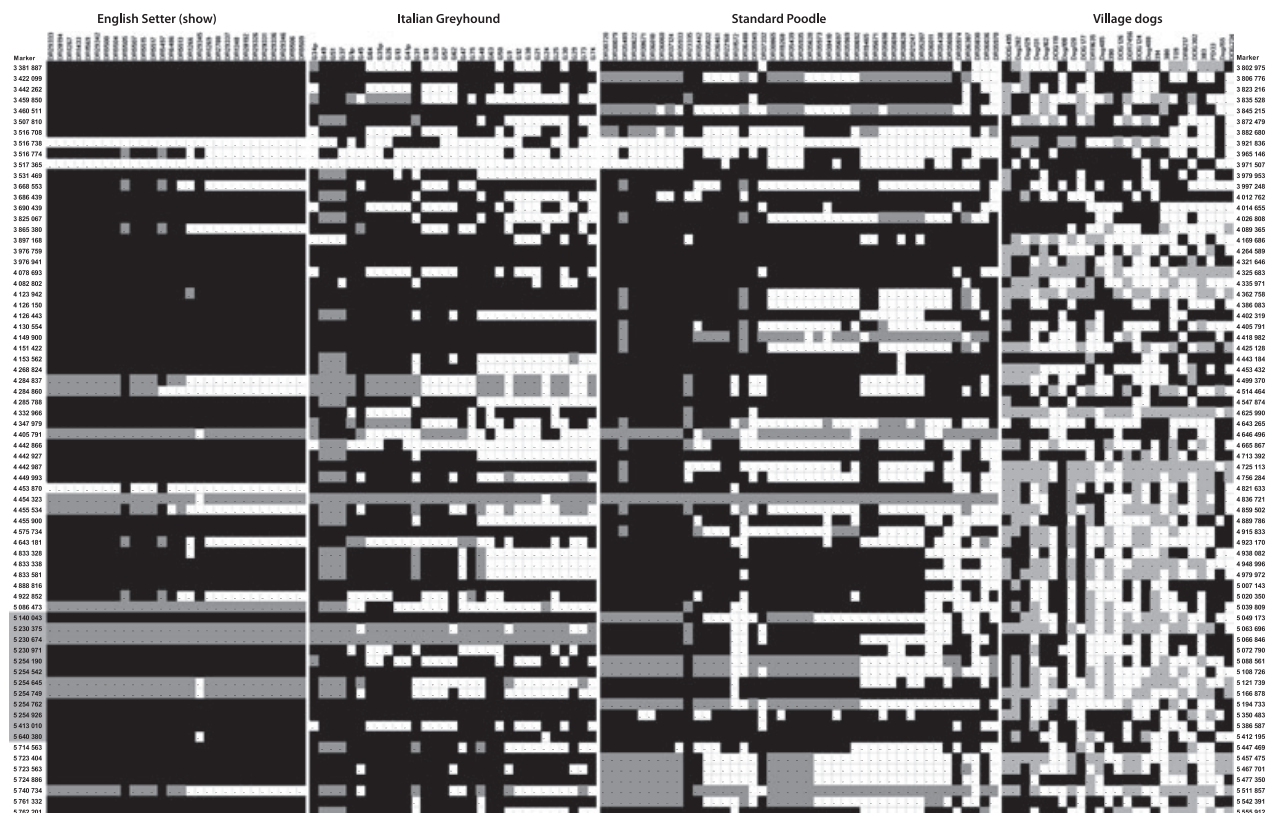


Figure 6 Comparative zygosity mapping of DLA region based on DLA wide SNP scan of three pure conformation breeds as compared to a group of village dogs. White represents regions of heterozygosity, black represents regions of homozygosity for major allele, and grey represents regions of homozygosity for minor alleles. SNPs spanning the DLA class II regions are shaded (left hand column).

tions. They found extremely inbred individuals within 9 of 10 breeds and estimated that >90% of unique genetic variants were lost in seven of these 10 breeds over six generations.

A differential loss of genetic diversity in conformation versus performance breeds was also apparent in the DLA class II region of dog breeds in the present study. Breeds that were selected mainly for performance (German Shorthaired and Wirehaired Pointers, Red Setters and field English Setters) exhibited higher diversity than breeds more heavily selected for show (Brittany, show English Setter and Standard Poodle). This supports the selective use of certain dogs and bloodlines over others in conformation compared with performance breeds. The specific DLA-DRB1 alleles identified in each breed also indicated possible common ancestry. Seven of the breeds shared a number of alleles, and all of these breeds either are currently used for retrieving or were notable retrievers in the past (Standard Poodle and show English Setter). The Italian Greyhound, which evolved from coursers over several 1000 years, was markedly different from the seven other breeds.

The time period over which genetic diversity has been parcelled out between and within the eight breeds within this study was not determined. However, gross estimates can be made from historical records as well as genetic structure. Modern conformation breeds such as the Standard Poodle are hundreds of years old, while the Italian Greyhound has existed in its present form for several 1000 years. Nonetheless, there is ample evidence that both breeds have been extensively inbred over the last century or less (Reviewed by Pedersen *et al.*, 2012a,b). Their genetic structure was distinct from each other, from village dogs and from the other six breeds on PCA analyses. Although an unrooted neighbour joining tree demonstrated all eight breeds to be genetically distinguishable, the 6 breeds with long or recent ties to performance breeds still maintained deeper genetic relationships on both PCA and Bayesian cluster analysis. This is in support of genetic studies of various pointing breeds by Parra *et al.* (2008).

Although it was not a primary goal of the study, structure analyses also provided useful information on the relationship between the eight breeds, and

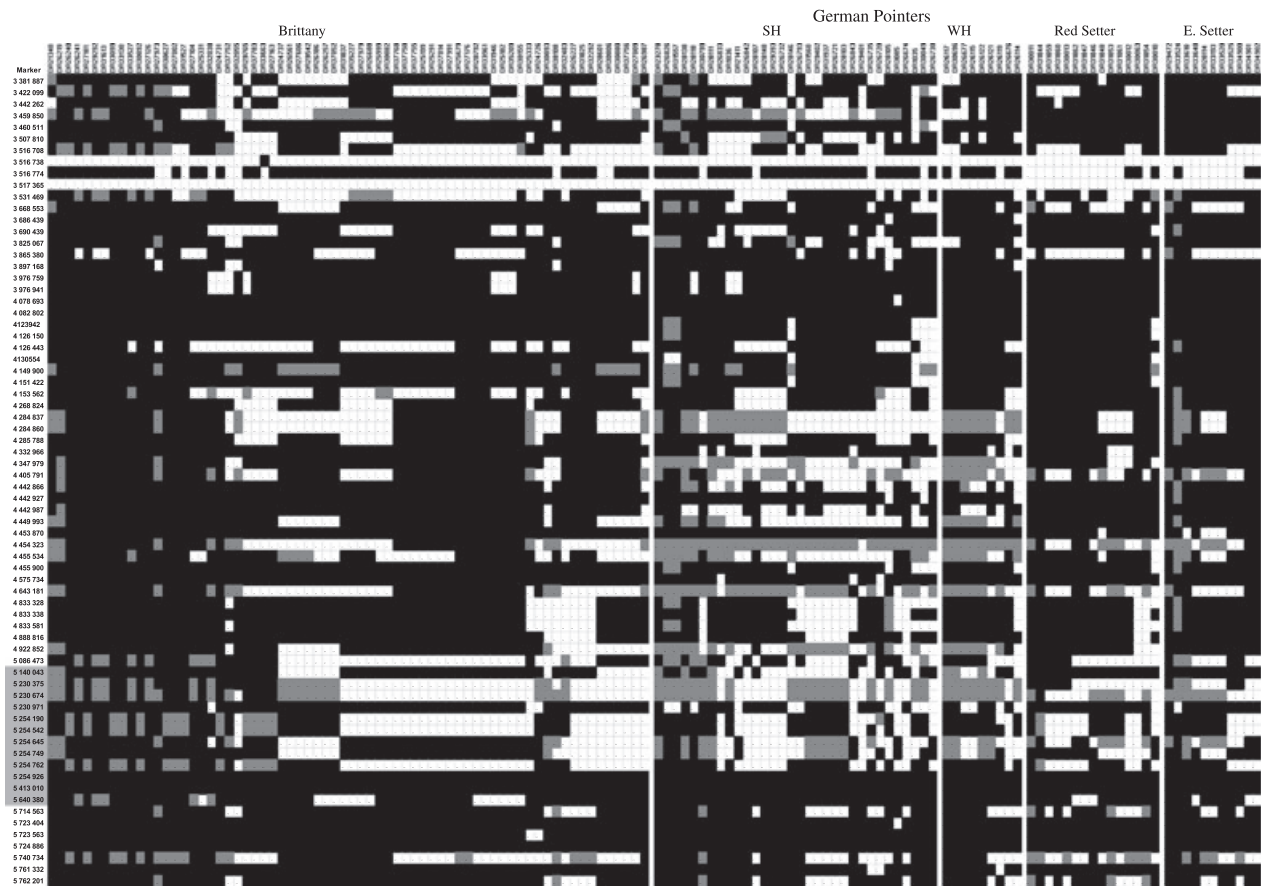


Figure 7 Comparative zygosity mapping of DLA region based on DLA wide SNP scan of five breeds used for performance and show (Brittany, German Shorthaired and Wirehaired Pointers) or primarily for field work (Red Setter and field English Setter). SNPs spanning the DLA class II regions are shaded (left hand column).

especially among the six performance or performance-related breeds. PCA comparisons of these breeds showed clear evolution of the Brittany from other breeds and partial separation of the show English Setter and Red Setter. By comparison, the field English Setter and German Shorthaired and Wirehaired Pointers are much more deeply rooted together, and in a separate PCA, with village dogs. This reflects the comparative historical timelines and evolution of the more performance-related breeds. The Brittany was accepted as a breed in France in 1907, but has a much longer history, with images of orange and white hunting dogs from the 17th century and histories of hunts with bobtailed dogs from 1850 (Riddell 1997). The genetic distinctness of the Brittany from other pointing breeds was first shown by Parra *et al.* (2008). The Brittany was rumoured to have an influx of English Setter blood around this same period (Riddell 1997). This could not be confirmed by the present PCA or Bayesian Cluster analysis, but there was sharing of two major DRB1 alleles and 1 of 32 male Britta-

nys shared the same paternal haplotype found in show English Setters. The uniqueness of the breed was most evident from the heretofore unique paternal haplotype shared by almost all Brittans. There is strong genetic and historical support for the recent development and breed isolation of the show English Setter and the Red Setter. The English Setter is unique in that show, and field English Setters have been bred separately for each function for over 75 years, have become distinct breeds in terms of both function and appearance and have differentiated from each other genetically over this same period. The Red Setter was created around 60 years ago, purportedly by crossing field English Setters with Red Irish Setters (Sisley 2010). The Red Setter was situated between the Brittany and the English Setters on an unrooted neighbour joining tree of all eight breeds and shares one major maternal haplotype with the Brittany.

It is apparent from the present studies that considerable genetic diversity has been lost as breeds have evolved. This conclusion is in agreement with the

extensive genomic changes that have been occurred during breed development (Akey *et al.* 2010; Vaysse *et al.* 2011). However, without summing genetic diversity among all known breeds, and comparing it to the diversity that existed prior to extensive human selection, the magnitude of this loss cannot be determined. Nonetheless, the present study provides a window into how such a determination might be approached and reconfirms the origin of modern breeds from village dogs in the Middle East and SE Asia. Randomly breeding village dogs with genetic links with most modern breeds have existed in the Middle East and SE for as long as 10 000 years (Brown *et al.* 2011), with isolated populations in islands such as Bali and Australia dating back 3000–5000 years ago (Brown *et al.* 2011; Oskarsson *et al.* 2011). Therefore, these relatively isolated village dog populations are probably the best existing reservoir of original genetic diversity. Studies should then extend to random bred dogs that have long existed in more distant and less isolated regions. However, it must be remembered that the direct ancestral stock of many breeds may no longer exist in the regions from which they originated or have spawned closely related populations in quite distant regions. Such a study must ultimately include modern breeds themselves, as evidenced by the Brittany, which has a Y haplotype different from all other dogs studied to date.

The present findings, although limited to eight select breeds, supports the hypothesis that selection for conformation has resulted in more inbreeding than selection for performance. There are several explanations for this conclusion. Performance dogs have retained much of the outward physical appearance of their village ancestors, whereas conformation breeds are often bred to the extremes of their breed standards, and outward morphology has often been drastically altered. The lure of the show ring and championships is also a strong incentive to selectively overuse certain individuals or bloodlines. Based on pedigree analyses, 42 of 149 (28%) of randomly collected Standard Poodles and 70 of 128 (55%) of Italian Greyhounds from the US listed the same individual more than once within three generations (Pedersen *et al.*, 2012a,b). Conformation traits affecting size, coat colour, hair characteristics and body conformation (e.g. brachycephaly and achondroplasia) usually involve single or small sets of genes (Ostrander & Ruvinsky 2012) and are therefore easier to fix by inbreeding. In contrast, the genetics of performance traits are complex, and heritability is extremely variable depending on the particular trait and how it is measured (Täubert *et al.* 2007; Willis 1995).

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