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Forensic Population Genetics—Short Communication

Genetic data from 15 STR loci for forensic individual identification and parentage analyses in UK domestic dogs (*Canis lupus familiaris*)

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ARTICLE INFO

ABSTRACT

Eighteen STR loci and one sex determination locus present in the Finnzymes Canine 2.1 STR Multiplex Reagent Kit were screened in the UK dog population providing allele frequencies and population genetic parameters necessary for the application of STRs to forensic genetic casework. A total of 375 dogs were genotyped, including representative samples from each of twelve breeds used to evaluate Hardy–Weinberg equilibrium and calculate inter-population pairwise F_{ST} values. Three loci were excluded from calculations of average random match probability due to deviations from Hardy–Weinberg Equilibrium or ambiguous genotyping. Random match probability based on fifteen STR loci and one sex locus was subsequently estimated to be 2.8×10^{-17} for unrelated individuals across breeds.

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1. Populations

United Kingdom (UK) dogs obtained from domestic dog collections held in Edinburgh (Scotland) and Cambridge (England). Samples included dogs from 12 major contributing breeds[1] ($n = 301$), 22 minor breeds[2] ($n = 47$) and 27 mixed breed individuals.

2. Extraction

DNA was extracted from whole frozen blood samples following standard QIAGEN DNeasy blood and tissue kit protocols.

3. PCR amplification

Amplification of eighteen STR loci and one sex determinant zinc-finger protein-linked locus was performed using the Finn-

zymes Canine 2.1 STR Multiplex Reagent Kit. This kit and its constituent markers have previously been validated for individual identification in dogs [1,2]. PCR conditions and thermocycling parameters for the multiple PCR kit followed the manufacturer's instructions. Samples were amplified on a calibrated MJ Research Dyad thermocycler.

4. Electrophoresis and genotyping

Genotypes were resolved under capillary electrophoresis on an Applied Biosystems, Inc 3730xl and subsequently analysed using proprietary GeneMapper v4.0 software. A standard system of allele nomenclature for these canine STRs has previously been developed [3] and was followed in this study.

5. Analysis of data

GENEPOP [4], POWER-STAT [5], and GENALEX [6] programs were used to calculate population genetic parameters and estimate discrimination power (Table 1).

6. Results

The mean allele frequencies per locus across breeds are shown in Table 1, together with locus specific Hardy–Weinburg test results, probabilities of discrimination and probabilities of exclusion.

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[1] Border Collie, Boxer, Cavalier King Charles Spaniel, English Springer Spaniel, German Shepherd , Golden Retriever. Jack Russell Terrier, Labrador, Rottweiler, Staffordshire Bull Terrier, West Highland Terrier, Yorkshire Terrier.
[2] Basset Griffon Vendeen, Border Terrier, Borzoi, Chow, Cocker Spaniel, Dachshund, Dobermann, Greyhound, Husky, Irish Setter, Lhasa Apso, Lurcher, Maltese Terrier, Miniature Poodle, Miniature Schnauzer, Patterdale, Pointer, Shih Tzu, Siberian Huskie, Standard Poodle, Tibetan Terrier, Visla.

Table 1
Allele frequencies for 15 loci in the Finnzymes Canine 2.1 STR panel genotyped across 375 individuals and 34 breeds of UK dog.

Allele	FH2001	FH2004	FH2010	FH2054	FH2088	FH2107	FH2309	FH2328	FH3377	PEZ02	PEZ05	PEZ16	PEZ17	PEZ21	VWF.X
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.007
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.468
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.347
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.075
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.051
6	0.010	–	–	–	–	–	–	–	–	–	0.018	–	–	–	0.041
7	0.044	–	–	–	–	–	–	–	–	–	0.003	–	–	–	0.012
8	0.190	–	–	0.001	–	–	–	–	–	0.003	0.436	–	–	0.017	–
8.2	–	–	–	–	–	–	–	–	0.104	–	–	–	–	–	–
9	0.017	–	0.105	0.008	–	–	–	–	–	–	0.321	–	–	0.346	–
9.2	–	–	–	–	–	–	–	–	0.059	–	–	–	–	–	–
10	0.060	–	0.536	0.086	–	–	–	0.100	–	0.039	0.180	–	–	0.149	–
10.2	–	–	–	–	–	–	–	–	0.111	–	–	–	–	–	–
11	0.389	0.221	0.181	0.282	–	–	–	0.004	–	0.060	0.041	–	–	0.461	–
11.2	–	–	–	–	–	–	–	–	0.288	–	–	–	–	–	–
12	0.195	0.266	0.172	0.253	0.003	–	0.007	0.151	–	0.213	–	–	0.022	0.026	–
12.2	–	–	–	–	–	–	–	–	0.059	–	–	–	–	–	–
13	–	0.234	0.005	0.070	–	–	0.077	0.120	–	0.270	–	–	0.218	–	–
13.1	0.083	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13.2	–	–	–	–	–	–	–	–	0.027	–	–	–	–	–	–
14	–	0.145	–	0.085	0.129	–	0.003	0.186	–	0.307	–	–	0.144	–	–
14.2	0.004	–	–	–	–	–	–	–	−0.025	–	–	–	–	–	–
15	0.007	0.031	–	0.113	0.143	–	0.004	0.204	–	0.023	–	0.023	0.263	–	–
15.2	–	–	–	–	–	–	–	–	0.001	–	–	–	–	–	–
16	0.001	0.001	–	0.067	0.421	–	0.013	0.135	–	0.053	–	0.129	0.299	–	–
17	–	–	–	0.034	0.189	–	0.001	0.082	–	0.033	–	0.140	0.036	–	–
17.2	–	–	–	–	–	–	–	–	0.010	–	–	–	–	–	–
18	–	–	–	0.001	0.113	–	0.020	0.014	–	–	–	0.184	0.015	–	–
18.2	–	–	–	–	–	–	0.025	–	0.064	–	–	0.001	–	–	–
19	–	–	–	–	0.001	–	0.082	0.003	–	–	–	0.263	0.001	–	–
19.2	–	–	–	–	–	–	0.024	–	0.091	–	–	0.185	–	–	–
20	–	–	–	–	–	–	–	–	–	–	–	–	0.001	–	–
20.2	–	–	–	–	–	–	0.003	–	0.105	–	–	0.001	–	–	–
21	–	–	–	–	–	–	–	–	–	–	–	0.051	–	–	–
21.2	–	–	–	–	–	–	0.011	–	0.045	–	–	–	–	–	–
22	–	–	–	–	–	–	–	–	–	–	–	0.013	–	–	–
22.2	–	–	–	–	–	–	0.152	–	0.006	–	–	–	–	–	–
23	–	0.001	–	–	–	–	–	–	–	–	–	0.005	–	–	–
23.2	–	–	–	–	–	–	0.080	–	–	–	–	–	–	–	–
24	–	0.001	–	–	–	–	–	–	–	–	–	0.003	–	–	–
24.2	–	–	–	–	–	–	0.096	–	–	–	–	–	–	–	–
25	–	0.001	–	–	–	–	–	–	–	–	–	0.001	–	–	–
25.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26	–	0.003	–	–	–	–	–	–	–	–	–	0.001	–	–	–
26.2	–	–	–	–	–	–	0.201	–	0.004	–	–	–	–	–	–
27	–	0.003	–	–	–	–	–	–	–	–	–	–	–	–	–
27.2	–	–	–	–	–	–	0.017	–	–	–	–	–	–	–	–
28	–	0.023	–	–	–	–	–	–	–	–	–	–	–	–	–
28.2	–	–	–	–	–	–	0.021	–	–	–	–	–	–	–	–
29	–	0.030	–	–	–	–	–	–	–	–	–	–	–	–	–
29.2	–	–	–	–	–	–	0.044	–	–	–	–	–	–	–	–
30	–	0.026	–	–	–	–	–	–	–	–	–	–	–	–	–
30.2	–	–	–	–	–	–	0.004	–	–	–	–	–	–	–	–
31	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–	–
32	–	0.007	–	–	–	0.001	–	–	–	–	–	–	–	–	–
33	–	–	–	–	–	0.007	–	–	–	–	–	–	–	–	–
33.2	–	–	–	–	–	–	0.001	–	–	–	–	–	–	–	–
34	–	–	–	–	–	0.023	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	0.045	–	–	–	–	–	–	–	–	–
36	–	–	–	–	–	0.030	–	–	–	–	–	–	–	–	–
37	–	–	–	–	–	0.173	–	–	–	–	–	–	–	–	–
38	–	–	–	–	–	0.173	–	–	–	–	–	–	–	–	–
39	–	–	–	–	–	0.236	–	–	–	–	–	–	–	–	–
39.2	–	–	–	–	–	0.003	–	–	–	–	–	–	–	–	–
40	–	–	–	–	–	0.133	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	0.117	–	–	–	–	–	–	–	–	–
42	–	–	–	–	–	0.028	–	–	–	–	–	–	–	–	–
43	–	–	–	–	–	0.020	–	–	–	–	–	–	–	–	–
43.2	–	–	–	–	–	0.003	–	–	–	–	–	–	–	–	–
44	–	–	–	–	–	0.005	–	–	–	–	–	–	–	–	–
44.2	–	–	–	–	–	0.001	–	–	–	–	–	–	–	–	–
48.2	–	–	–	–	–	0.001	–	–	–	–	–	–	–	–	–
Obs. H	0.677	0.601	0.471	0.693	0.604	0.744	0.680	0.662	0.659	0.623	0.421	0.617	0.620	0.499	0.538
Exp. H	0.662	0.586	0.488	0.723	0.603	0.778	0.708	0.702	0.693	0.609	0.510	0.647	0.595	0.523	0.542
P	0.804	0.281	0.906	0.119	0.412	0.548	0.527	0.717	0.022	0.356	0.006	0.001	0.987	0.481	0.084

Table 1 (*Continued*)

Allele	FH2001	FH2004	FH2010	FH2054	FH2088	FH2107	FH2309	FH2328	FH3377	PEZ02	PEZ05	PEZ16	PEZ17	PEZ21	VWF.X
PD	0.904	0.926	0.815	0.936	0.891	0.957	0.970	0.957	0.957	0.917	0.835	0.943	0.902	0.821	0.819
PE	0.320	0.276	0.164	0.402	0.239	0.483	0.380	0.368	0.405	0.308	0.160	0.322	0.333	0.196	0.201

Obs. H and Exp. H = observed and expected heterozygosities averaged across breed, *P*-value = Hardy–Weinberg equilibrium exact test, PD = power of discrimination, PE = power of exclusion.

7. Other remarks

Canine DNA is being increasingly used as evidence in a wide range of crimes. Domestic dogs may be involved as the perpetrator or victim of crime, or as a contributor to trace DNA evidence that may link suspects, evidence items and crime scenes [7]. Within the UK, canine DNA profiling has been successfully used to investigate crimes including murder, dog-fighting and animal abuse for over five years. However until now, the application of profile matching has been limited by the lack of available population data for UK dogs, necessitating the use of a US database with highly conservative match probability statistics to estimate the probability of a random DNA profile match. The data provided in this study can be used to calculate more accurate estimates of forensic genetic parameters associated with canine DNA profiling in the UK.

The Finnzymes Canine 2.1 STR Multiplex Reagent Kit includes eighteen STR markers and a sex determinant zinc-finger protein-linked locus. Tests for deviation from Hardy–Weinberg equilibrium (HWE) showed highly significant ($P < 0.001$) differences between observed and expected genotype frequencies at one locus (FH2017) across multiple breeds, suggesting the presence of null alleles. This locus was therefore excluded from subsequent analysis. Significant deviations at loci PEZ05 and PEZ16 (Table 1) were driven by results within single breeds (Staffordshire Bull Terriers and Yorkshire Terriers respectively) and are not considered to be problematic. Loci FH2361 and FH3313 were excluded due to the presence of microvariants that complicated allele calling. The future development of an allelic ladder should resolve this issue.

The relative genetic distances among breeds were examined by calculating pairwise F_{ST} values among all breeds. All pairwise comparisons among true breeds showed significant F_{ST} values ($F_{ST} > 0$, $P = 0.01$) confirming the expected reduction in gene flow among breeds due to separation of breeding lines. The mean F_{ST} estimate across all breeds was 0.186, which is the value used to represent theta in the match probability equation [8]. As previously discussed by Dawnay et al. [9], there is currently no consensus about how best to account for individual inbreeding within breeds, *f*, commonly estimated as F_{IS}. Inbreeding within UK breeds is high [10] and will bias match probability estimates in favour of the prosecution. Existing published methods to mitigate this [11] are not always applicable [9]. The only current alternative is to use sibling match probabilities; the PI_{sibs} estimate for this current study is 2.49×10^{-7}.

8. Quality control

All data were generated in a GLP certified laboratory operating in compliance with ISO17025 testing standards. ISFG recommendations on the analysis of the DNA polymorphisms [12] and non-human DNA [13] were followed throughout. This publication follows ISFG guidelines for the publication of population genetic data [14].

References

[1] S. Kanthaswamy, B.K. Tom, A.-M. Mattila, E. Johnston, M. Dayton, J. Kinaga, B. Joy-Alise Erickson, J. Halverson, D. Fantin, S. DeNise, A. Kou, V. Malladi, J. Satkoski, B. Budowle, D. Glenn Smith, M.T. Koskinen, Canine population data generated from a multiplex STR kit for use in forensic casework, J. Forensic Sci. 54 (2009) 829–840. , doi:10.1111/j.1556-4029.2009.01080.
[2] M. Dayton, M.T. Koskinen, B.K. Tom, A.M. Mattila, E. Johnston, J. Halverson, D. Fantin, S. DeNise, B. Budowle, D.G. Smith, S. Kanthaswamy, Developmental validation of short tandem repeat reagent kit for forensic DNA profiling of canine biological material, Croat. Med. J. 50 (2009) 268–285.
[3] B.K. Tom, M.T. Koskinen, M. Dayton, A.M. Mattila, E. Johnston, D. Fantin, S. Denise, T. Spear, D.G. Smith, J. Satkoski, B. Budowle, S. Kanthaswamy, Development of a nomenclature system for a canine STR multiplex reagent kit, J. Forensic Sci. 55 (2010) 597–604.
[4] M. Raymond, F. Rousset, GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism, J. Heredity 86 (1995) 248–249.
[5] A. Tereba, Tools for Analysis of Population Statistics, Profiles in DNA 3, Promega Corporation, 1999 (www.promega.com/geneticidtools/powerstats/).
[6] R. Peakall, P.E. Smouse, GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research, Mol. Ecol. Notes 6 (2006) 288–295.
[7] B. van Asch, F. Pereira, State-of-the-art and future prospects of canine STR-based genotyping, Open Forensic Sci. J. 3 (2010) 45–52, 45.
[8] D.J. Balding, R.A. Nichols, DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands, Forensic Sci. Int. Genet. 64 (1994) 125–140.
[9] N. Dawnay, R. Ogden, R.S. Thorpe, L.C. Pope, D.A. Dawson, R. McEwing, A forensic STR profiling system for the Eurasian badger: a framework for developing profiling systems for wildlife species, Forensic Sci. Int. Genet. 2 (2008) 47–53.
[10] F.C.F. Calboli, J. Sampson, N. Fretwell, D.J. Balding, Population structure and inbreeding from pedigree analysis of purebred dogs, Genetics 179 (2008) 593–601.
[11] K.L. Ayres, A.D.J. Overall, Allowing for within-subpopulation inbreeding in forensic match probabilities, Forensic Sci. Int. Genet. 103 (1999) 207–216.
[12] P.M. Schneider, Scientific standards for studies in forensic genetics, Forensic Sci. Int. Genet. 165 (2007) 238–243.
[13] A. Linacre, L. Gusmão, W. Hecht, A.P. Hellmann, W.R. Mayr, W. Parson, M. Prinz, P.M. Schneider, N. Morling, ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations, Forensic Sci. Int. Genet. (2010), doi:10.1016/j.fsigen.2010.10.017.
[14] A. Carracedo, J.M. Butler, L. Gusmão, W. Parson, L. Roewer, P.M. Schneider, Publication of population data for forensic purposes, Forensic Sci. Int. Genet. 4 (2010) 145–147.