Forensic Application Evaluation of a Novel Canine STR System in Pembroke Welsh Corgi and Shiba Inu Groups

Yating Fang,1,2 Jinlong Yang,3 Yajun Deng4, and Bofeng Zhu1,2,4

1Guangzhou Key Laboratory of Forensic Multi-Omics for Precision Identification, School of Forensic Medicine, Southern Medical University, Guangzhou 510515, China
2Multi-Omics Innovative Research Center of Forensic Identification, Department of Forensic Genetics, School of Forensic Medicine, Southern Medical University, Guangzhou 510515, China
3Beijing Zhongzheng DNA Evidence Institute, Beijing 101318, China
4Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi’an Jiaotong University, Xi’an 710004, China

Correspondence should be addressed to Yajun Deng; 18601285369@163.com and Bofeng Zhu; zhubofeng7372@126.com

Received 15 April 2021; Revised 19 October 2021; Accepted 5 November 2021; Published 25 November 2021

Aim. To evaluate the forensic application values of 19 autosomal short tandem repeat (STR) loci in canines.

Methods. The 19 STR loci in two canine groups (Pembroke Welsh Corgis, n = 200; Shiba Inus, n = 175) were analysed by the capillary electrophoresis platform. The allele frequencies and forensic parameters were calculated, and the genetic relationships between these two canine groups and a previously reported Labrador group were analysed.

Results. These two canine groups conformed to the Hardy-Weinberg equilibrium at all STRs except for locus VGL3438 in the Pembroke Welsh Corgi group, and there was no linkage disequilibrium among pairwise loci at the 19 STRs. All STRs were polymorphic in the Pembroke Welsh Corgi and Shiba Inu groups, of which the locus C38 had the highest polymorphism. And it was found that the genetic relationship between the Pembroke Welsh Corgi and Labrador groups were closer in the three canine groups (Pembroke Welsh Corgi, Shiba Inu and Labrador).

Conclusion. The 19 STR loci had high genetic polymorphisms and forensic application values in Pembroke Welsh Corgi and Shiba Inu groups.

1. Introduction

In recent years, it has been found that the study of non-human DNA genetic polymorphism has great significance for case investigation in the forensic practice, especially for canine which is closely related to human. Canine is the most frequently kept pet in the world today. Meanwhile, canine-related cases are increasing rapidly, such as cruelty to animal, attack on people or animal, involvement in crime scene, property damage, and the identification of lost pet. In 1999, Schneider et al. used mitochondrial DNA as case evidence that were extracted from canine hair [1]. In 2002, Padar et al. analysed short tandem repeats (STR) loci to detect a case involving a Hungarian canine which attacked a child to death [2]. In 2004, Wang et al. successfully extracted the canine DNA from the victim’s mixed stain and genotyped by using the canine STR multiplex amplification technology in a vicious rape case involving domestic canine, and identified that the mixed stain contained the canine sperm. In 2016, Barrientos et al. reported a robbery and homicide case in which highly degraded DNA was extracted from canine stool samples [3]. In 2017, a fatal attack case was solved with canine genetic markers by Ciampolini et al. [4].

The analyses of DNA genetic polymorphisms are helpful for the identifications and pedigree controls of canine individuals. In 2011, the International Society for Forensic Genetics (ISFG) clarified that the non-human DNA analysis was similar to human DNA testing [5]. STRs are widely existing tandem repeat sequences with fragment length polymorphisms in the biological genome. It has been widely used in forensic identification and paternity testing. The STR analysis is not only low-cost and easy to operate in the
capillary electrophoresis platform but also a widely popularized and highly utilized method in primary forensic DNA laboratories.

The molecular genetic markers in canine DNA also need to be evaluated in different groups like the genetic markers in human DNA. However, there are still relatively few studies on genetic polymorphisms and forensic application values of molecular genetic markers in different canine groups. Pembroke Welsh Corgis, the long-bodied and short-legged canines with erect ear and fox-like head, are the small canines originated from Wales of England. Shiba Inu is a kind of canine which has ancient origin from Japan. In this study, 200 purebred Pembroke Welsh Corgis and 175 purebred Shiba Inus were used as research objects to evaluate the genetic polymorphisms of 19 STR loci. It can not only assist in breeding pure Pembroke Welsh Corgis and Shiba Inus but also provide scientific evidences for the case investigations involving Pembroke Welsh Corgis or Shiba Inus in forensic caseworks.

2. Materials and Methods

2.1. Sampling and DNA Extraction. Saliva samples were taken from 200 purebred healthy Pembroke Welsh Corgis and 175 purebred healthy Shiba Inus based on the recommendations of the ISFG on non-human DNA analysis and the ethics committees of the Xi’an Jiaotong University Health Science and Southern Medical University. All canines were registered with the National General Kennel Club. Informed consents had been obtained from the owners of the canines before the study began. Genomic DNA was extracted by using the Chelex-100 method [6].

2.2. Amplification. The 19 autosomal STR loci (PEZ02, PEZ20, FH2010, FH2054, FH2001, vWF.X, FH2088, PEZ21, PEZ17, FH2328, FH2361, VGL2136, VGL3235, PEZ01, VGL3438, FH2004, C38, FH2611, and FH2137) of the commercial PBG Canine Genotype STR system (Beijing Protect Baby Gene Technology, China) were amplified in a table.
25 μl system, which included 1 μl DNA template, 5 μl PCR buffer, 5 μl Primer Mix, and 14 μl sterile water. The amplification was performed on a GeneAmp® 9700 PCR thermocycler. The reaction conditions were as follows: initial denaturation at 95°C for 2 min, followed by 29 cycles at 94°C for 5 s and at 60°C for 1 min, and final incubation at 60°C for 10 min. And then, amplified products were held at 10°C.

2.3. Genotyping. After amplification, 1 μl of the product or 1 μl of ladder were combined with 9 μl Hi-Di formamide and 0.3 μl internal size standard. The 19 STR genotypes were conducted by capillary electrophoresis on Applied Biosystems™ 3500XL genetic analyser with default instrument settings. Subsequently, the raw data and the allelic ladder were analysed by GeneMapper ID-X software. All sizes were calculated by using internal size standards on the 70, 80, 100, 125, 150, 175, 200, 233, 266, 300, 333, 366, 400, 445, 490, and 500 bp.

2.4. Data Analyses. The Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested by the Arlequin software v3.5 [7] and the SHEsis online software [8], respectively. The allele frequencies and forensic parameters of STRs were calculated by the online software STRAF [9]. The inbreeding coefficient (FIS) was evaluated by gene-pop software v4 [10]. The principal component analysis (PCA) was performed by the R software v3.6.2 (https://cran.r-project.org/bin/windows/base/old/3.6.2/). The phylogenetic tree was conducted by MEGA X software [11]. The genetic structure was analysed by the STRUCTURE software v 2.3.4 [12], and the optimal K value was calculated by the online software Structure Harvester [13].

| Alleles | Alleles C38 | Alleles FH2004 | Alleles FH2137 | Alleles FH2361 | Alleles PEZ02 | Alleles PEZ20 | Alleles VGL2136 |
|---------|-------------|---------------|---------------|---------------|--------------|--------------|---------------|
| 10      | 0.0029      | 0.0029        | 0.0029        | 0.0029        | 0.1229       | 0.0086       | 8             |
| 11      | 0.0114      | 0.0129        | 0.0600        | 0.0242        | 0.4400       | 0.2343       |               |
| 14.1    | 0.0171      | 0.1771        | 0.0057        | 0.0343        | 0.0857       | 10           |               |
| 15.2    | 0.1143      | 0.5429        | 0.0457        | 0.1714        | 0.2686       | 12           |               |
| 16      | 0.0029      | 0.0571        | 0.0543        | 0.0029        | 0.1143       | 13           |               |
| 16.2    | 0.0771      | 0.0057        | 0.0286        | 0.0829        | 0.0057       | 14           |               |
| 17.2    | 0.0914      | 0.0943        | 0.0029        | 0.0029        | 0.0771       | 15           |               |
| 18.2    | 0.4086      | 0.0086        | 0.0143        | 0.2057        | 0.3343       |               |               |
| 19.2    | 0.0257      | 0.0057        | 0.0143        | 0.0686        | 0.1514       | 17           |               |
| 20.2    | 0.0200      | 0.0029        | 0.0029        | 0.0086        | 0.2629       | 18           |               |
| 26.1    | 0.0029      | Alleles FH2010 | 0.0143        | 0.0057        | 0.2343       | Alleles VGL3235 |
| 27.1    | 0.0029      | 0.1143        | 0.5743        | 0.0057        | 0.3057       | 12           |               |
| 29.1    | 0.0229      | 0.2371        | 0.0114        | 0.0031        | 0.2343       |               |               |
| 30.1    | 0.0800      | 0.0094        | 0.0686        | 0.0011        | 0.0029        | 14           |               |
| 31.1    | 0.0914      | 0.5543        | 0.0029        | 0.0029        | 0.0086       | 15           |               |
| 32.1    | 0.0257      | Alleles FH2054 | 0.0714        | Alleles FH2611 | Alleles PEZ20 | 16           | 0.0257       |
| 35.1    | 0.0029      | 0.0314        | 0.0057        | 0.0086        | 0.1514       | 17           | 0.1743       |
| Alleles | Alleles FH2001 | 0.2000       | 0.0171        | 0.192         | 0.0543       |               |               |
| 6       | 0.0171      | 0.3514        | 24.2          | 0.0029        | 0.5857       | 19           | 0.0771       |
| 8       | 0.1486      | 0.1714        | Alleles FH2328 | 0.0029        | 0.1714       | 20           | 0.0057       |
| 9       | 0.0114      | 0.0943        | 0.0114        | 0.0029        | 0.1143       |               |               |
| 10      | 0.0171      | 0.0117        | 0.0029        | 0.0029        | 0.0011       | 11           | 0.1800       |
| 11      | 0.3143      | 0.0014        | 0.1543        | 0.0309        | 0.0029        | 12           | 0.3143       |
| 12      | 0.0314      | 0.0200        | 0.1657        | 0.0226        | 0.0057       | 13           | 0.1771       |
| 13      | 0.1086      | Alleles FH2088 | 0.0600        | 0.0600        | 0.0057       | 14           | 0.0200       |
| 13.2    | 0.3486      | 0.0029        | 0.1000        | 0.202         | 0.0029       | 18           | 0.0057       |
| 14      | 0.0029      | 0.2314        | 0.0343        | 0.0029        | 0.0057       | 16           | 0.0057       |
| Alleles | PEZ201      | 0.3771        | 0.2629        | 0.0057        | 0.2714       | 17           | 0.0086       |
| 9       | 0.0029      | 0.2543        | 0.0100        | 0.242         | 0.0029       | 0.0057       | 18           | 0.0057       |
| 11      | 0.6571      | 0.1286        | 0.0143        | 0.0020        | 0.1743       |               |               |
| 12      | 0.0143      | 0.0571        | 0.0057        | 0.0029        | 0.3286       | 10           |               |
| 13      | 0.2400      | 0.1514        | 0.0057        | 0.3400        |               | 11           |               |
| 14      | 0.1000      | 0.0343        | 0.0057        | 0.3400        |               |               |               |
| 15      | 0.0057      | 0.0057        | 0.0143        | 0.1571        |               |               |               |
3. Results

3.1. Allele Frequency Distributions. A total of 165 alleles were observed at 19 STR loci in 200 purebred Pembroke Welsh Corgis with the allele frequency distributions from 0.0025 to 0.8325 (Table 1), and 180 alleles were in the Shiba Inu group with the allele frequency distributions from 0.0029 to 0.6371 (Table 2). In both the two groups, the allele distributions of locus C38 were the most extensive with a total of 18 alleles in the Shiba Inu group and 19 in the Corgi group (numbers of allelic repeat units were ranged from 10 to 37). In the 19 loci, locus FH2137 had the largest allele numbers with a total of 19 and 20 alleles in the Shiba Inu and the Pembroke Welsh Corgi groups, respectively. PEZ21 and vWF.X were the lowest number of alleles in the Pembroke Welsh Corgi group (only a total of 4), so were FH2010 and vWF.X in the Shiba Inu group.

3.2. HWE, LD, and FIS Tests. In HWE tests, the correction level was adjusted to 0.0026 (0.05/19) after the Bonferroni correction.
correction. The estimated $P$ values of all 19 loci were greater than 0.0026, suggesting that the two canine groups in this study were consistent with HWE except for locus VGL3438 in the Pembroke Welsh Corgi group.

Linkage correlation coefficient could be used to not only test the existence of LD but also evaluate the strength of LD. The correlation coefficient between the two loci was reflected by $r^2$ values in this study. Figures 1(a) and 1(b) showed the $r^2$ values of the linkage disequilibrium tests, in which 0 represented the value less than 0.01, 1 represented the value was from 0.01 to 0.02, and 2 represented the value was from 0.02 to 0.03. The results showed that all $r^2$ values were less than 0.02, indicating that there was no linkage disequilibrium at the 19 STR loci in these two canine groups.

The $F_{IS}$ was calculated to evaluate the level of inbreeding in these two canine groups. The $F_{IS}$ value was 0.0619 in the Pembroke Welsh Corgi group, while the $F_{IS}$ of the Shiba Inu group was 0.0865.

3.3. Forensic Parameters. As shown in Figure 2, the forensic application values of 19 STR loci in two canine groups were explored by calculating forensic parameters including matching probability (MP), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), power of discrimination (PD), and power of exclusion (PE).

In the corgi group, the Ho values of 19 STR loci ranged from 0.3000 to 0.8250, and the He values were from 0.2965 to 0.8426. The heterozygosity values (Ho and He) of loci PEZ01 and FH2004 were less than 0.5, and there were 7 loci (C38, VGL2136, FH2328, FH2137, PEZ02, FH2611, and FH2001) which were greater than 0.7. All PIC values were
greater than 0.25, and 16 loci were greater than 0.5. The MP values ranged from 0.0450 to 0.5101, and the combined random match probability (CMP) was $3 \times 10^{-17}$. The PD and PE values ranges from 0.4899 to 0.9551 and 0.0635 to 0.6462, respectively. The combined power of discrimination (CPD) and the combined power of exclusion (CPE) were $0.99999999999999999910$, and 0.999911, respectively.

In the Shiba Inu group, the He values were a range from 0.5277 to 0.8428 with the average of 0.7166. The Ho values of all loci were greater than 0.5 (except for PEZ01 and PEZ21), so were the PIC values. The MP, PD, and PE values were in the range from 0.0475 to 0.2837, 0.7163 to 0.9525, and 0.1443 to 0.5471, respectively. The CMP, CPD, and CPE were $8.98 \times 10^{-19}$, $0.99999999999999999910$, and 0.999911, respectively.

3.4. Interpopulation Genetic Analyses. As shown in Figure 3, the differences of allele numbers at 19 STRs were compared among these two studied canine groups and a Labrador group ($n = 214$) [14]. The allele numbers of locus FH2137 were the largest in three canine groups, while the loci FH2010, FH2088, PEZ01, PEZ21, and vWF.X were relatively small.

The PCA and genetic structure analyses were performed to reveal the genetic structure of three canine groups. As shown in Figure 4(a), the clustering diagram of PCA divided the Pembroke Welsh Corgis, Shiba Inus, and Labradors into three clusters, and the small parts of three clusters overlapped with each other: Shiba Inus fell on the upper left of the axis, Labradors fell on the upper right, and Pembroke Welsh Corgis located on the lower. The numbers of assumed populations ($K$) were set to 2-7 in the structure analysis, and the optimal $K$ value was determined to be 3 after calculations. As shown in Figure 4(c), the three canine groups had different ancestral components when $K = 3$. In Figure 4(b), a phylogenetic tree was conducted to understand the genetic relationships among these groups, and the results showed that the genetic relationship between the Pembroke Welsh Corgi group and the Labrador group was closer in all three groups.

4. Discussion

In this study, we investigated the genetic polymorphisms of 19 STR loci in 200 Pembroke Welsh Corgis and 175 Shiba Inus. Canine samples were collected, stored, and conducted in the manner that were similar with corresponding human forensic DNA analysis process, and the allele frequencies and forensic genetic parameters of the 19 loci in these two canine groups were evaluated, respectively, according to the recommendations of ISFG on non-human DNA analysis in forensic cases [5].

The present results showed that there was no linkage disequilibrium among the 19 STR loci, and only one locus
have high potential in the applications of individual identity, PIC, and PD values was high genetic diversities in the Pembroke Welsh Corgi and Shiba Inu groups, which may be the reason that the genetic relationship between the Pembroke Welsh Corgi and Labrador groups was closer in three canine groups. By genetic relationship analysis among the two studied groups and the previously reported canine group, the Pembroke Welsh Corgi, Shiba Inu, and Labrador groups had different genetic backgrounds and structures, and the genetic relationship between the Pembroke Welsh Corgi and Labrador groups was relatively closer in three kinds of canine groups.

In this study, we found that the 19 STR loci have high genetic polymorphisms and forensic application values in the Pembroke Welsh Corgi and Shiba Inu groups. By genetic analysis among the two studied groups and the previously reported canine group, the Pembroke Welsh Corgi, Shiba Inu, and Labrador groups had different genetic backgrounds and structures, and the genetic relationship between the Pembroke Welsh Corgi and Labrador groups was closer than the genetic relationships between these two groups and the Shiba Inu group.

5. Conclusion

In this study, we found that the 19 STR loci have high genetic polymorphisms and forensic application values in the Pembroke Welsh Corgi and Shiba Inu groups. By genetic analysis among the two studied groups and the previously reported canine group, the Pembroke Welsh Corgi, Shiba Inu, and Labrador groups had different genetic backgrounds and structures, and the genetic relationship between the Pembroke Welsh Corgi and Labrador groups was relatively closer in three kinds of canine groups.

Data Availability

The data underlying the findings of the study can be obtained by contacting the corresponding authors.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

Yating Fang and Jinlong Yang contributed equally to this work and were co-first authors.

References

[1] P. M. Schneider, Y. Seo, and C. Rittner, “Forensic mtDNA hair analysis excludes a dog from having caused a traffic accident,” *International Journal of Legal Medicine*, vol. 112, no. 5, pp. 315-316, 1999.
[2] Z. Pádár, B. Egyed, K. Kontadakis et al., “Canine STR analyses in forensic practice. Observation of a possible mutation in a dog hair,” *International Journal of Legal Medicine*, vol. 116, no. 5, pp. 286-288, 2002.
[3] L. S. Barrientos, J. A. Crespi, A. Fameli et al., “DNA profile of dog feces as evidence to solve a homicide,” *Legal Medicine*, vol. 22, pp. 54-57, 2016.
[4] R. Ciampolini, F. Cecchi, L. Spinetti, A. Rocchi, and F. Biscarini, “The use of genetic markers to estimate relationships between dogs in the course of criminal investigations,” *BMC Research Notes*, vol. 10, no. 1, p. 414, 2017.
[5] A. Linacre, L. Gusmão, W. Hecht et al., “ISFG: recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations,” *Forensic Science International. Genetics*, vol. 5, no. 5, p. 501, 2011.
[6] P. S. Walsh, D. A. Metzger, and R. Higuchi, “Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material,” *BioTechniques*, vol. 10, no. 4, pp. 506-513, 1991.
[7] L. Excoffier and H. E. Lischer, “Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows,” *Molecular Ecology Resources*, vol. 10, no. 3, pp. 564-567, 2010.
[8] Y. Y. Shi and L. He, “SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci,” *Cell Research*, vol. 15, no. 2, pp. 97-98, 2005.
[9] A. Gouy and M. Zieger, “STRAF: A convenient online tool for STR data evaluation in forensic genetics,” *Forensic Science International. Genetics*, vol. 30, pp. 148–151, 2017.
[10] F. Rousset, “genepop’007: a complete re-implementation of the genepop software for Windows and Linux,” *Molecular Ecology Resources*, vol. 8, no. 1, pp. 103–106, 2008.
[11] S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, “MEGA X: molecular evolutionary genetics analysis across computing platforms,” *Molecular Biology and Evolution*, vol. 35, no. 6, pp. 1547–1549, 2018.
[12] L. Porras-Hurtado, Y. Ruiz, C. Santos, C. Phillips, Á. Carracedo, and M. V. Lareu, “An overview of STRUCTURE: applications, parameter settings, and supporting software,” *Frontiers in Genetics*, vol. 4, p. 98, 2013.
[13] G. Evanno, S. Regnaut, and J. Goudet, “Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study,” *Molecular Ecology*, vol. 14, no. 8, pp. 2611–2620, 2005.
[14] M. L. Wang, X. Y. Jin, X. Xiong et al., “Polymorphism analyses of 19 STRs in Labrador retriever population from China and its heterozygosity comparisons with other retriever breeds,” *Molecular Biology Reports*, vol. 46, no. 2, pp. 1577–1584, 2019.