**ACADEMIA JOURNAL OF BIOLOGY** 2024, 46(2): 93–100
DOI: 10.15625/2615-9023/20446

# ANALYSIS OF GENETIC DIVERSITY OF VEN DOG BREED BASED ON MICROSATELLITE MARKERS

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Received 28 March 2024; accepted 21 June 2024

## ABSTRACT

Microsatellite (MS) is a genetic marker widely used in the studies of pedigree, individual identification, gene mapping, and genetic diversity within and between populations. The genetic diversity of the indigenous Ven dog breed, along with two imported dog breeds, the Berger and Poodle dogs raised in Vietnam, was analyzed based on seven microsatellite markers. A total of 32 alleles, an average number of alleles/loci of 4.6 were observed across 80 samples of the three dog breeds. The overall polymorphic information content (PIC) was 0.67 representing the quality of selected MS markers. Genetic diversity indices (Ho, He, Fit, Fst) showed the phenomenon of inbreeding between individuals in the Ven dog population. The genetic similarity level and phylogenetic tree also reasonably reflect the genetic relationship between the three analyzed dog breeds, in which the Ven dog showed a higher genetic distance compared to two imported dog breeds.

**Keywords:** Ven indigenous dogs, microsatellite marker, genetic diversity, inbreeding.

*Citation*: Nguyen Thi Dieu Thuy, Le Cong Trieu, Huynh Thi Phuong Loan, Bui Thi Tra Mi, Nguyen Huy Tuong, Do Vo Anh Khoa, 2024. Analysis of genetic diversity of Ven dog breed based on microsatellite markers. *Academia Journal of Biology*, 46(2): 93–100. https://doi.org/10.15625/2615-9023/20446

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## INTRODUCTION

Microsatellites are tandemly repeated sequences whose unit of repetition is between one and six base pairs (bp) and randomly distributed in an organism genome, for example (CA)n (Tautz & Renz, 1984). The length of a microsatellite (repeated sequence) is usually no larger than 100 bp. Microsatellites are highly polymorphic, their alleles are co-dominant and inherited in a Mendelian fashion (Buchanan et al., 1994). Microsatellite is one of the best genetic markers for analyzing pedigree, population structure, genome variation, evolution process, and fingerprinting (Zhou & Lamont, 1999).

In dogs, MS has also been widely used in parentage testing (Koskinen & Bredback, 1999; Kang et al., 2009), gene mapping (Mellersh et al., 2000), kinship analysis for dog breeding purposes in several countries (DeNise et al., 2004; Gentilini et al., 2004; Oishi et al., 2005), relationships between dogs for criminal investigation (Ciampolini et al., 2011), variability and genetic structure of dog populations (Irion et al., 2003; Lai et al., 2022). A panel of MS markers used in parentage testing and genetic diversity are recommended by the International Society for Animal Genetics (ISAG) (Halverson & Edwards, 2000; Halverson et al, 1995).

The Ven dog is a pet species with many rare characteristics such as identical color, good hunting abilities (housekeeping, good swimming), easy to raise and adapts well to different conditions, but its genetic properties have not been studied yet. Recently, the Ven dog has been included in the indigenous genetic resource conservation program of Ca Mau province (Khoa & Nghi, 2018). In particular, studies on physical characteristics, basic indicators in the blood formula and some measurement dimensions (Trieu et al., 2018, 2019, 2020), behavior traits (Trieu et al., 2024), as well as polymorphisms of the *HTR1D* candidate gene related to aggression (Khoa et al., 2024) of Ven dogs had also been done. In this study, the genetic diversity of the Vietnamese native Ven dog breed raised in the Mekong Delta was analyzed using seven MS markers to contribute to the picture of the characteristics of the Mekong Delta Ven dog. Two imported dog breeds, Berger and Poodle, raised in the same area were also included as references.

## MATERIALS AND METHODS

### Materials

The study was conducted on three populations including Ven indigenous dogs (n = 60), two imported Berger dogs (n = 10), and Poodle dogs (n = 10). Three dog breeds were raised on the farms in Mekong Delta provinces. Blood samples from individuals were collected and stored in tubes containing EDTA on ice for genomic DNA extraction.

### Methods

Total DNA was extracted according to the basic method of Ausubel et al. (1995) with some modifications. DNA quality and concentration were assessed by electrophoresis on a 1% agarose gel and spectrophotometry at 260/280 nm wavelength on a Nanodrop device. DNA samples were diluted to a concentration of 50 ng/µL for MS analysis.

Seven MS markers were selected from 21 markers as recommended by ISAG (2005) and Cho (2005). Primer sequences and MS amplification PCR reaction conditions are presented in Table 1.

PCR amplifications were performed in 10 µL reactions containing 1X of PCR buffer, 2.5 mM of $Mg^{2+}$, 0.25 mM dNTPs, 0.25 pM of each forward or reverse primer, 0.5 U of *Taq polymerase* and 100 ng of genomic DNA. PCR thermal cycles were started at an initial denaturation at 95 $^{o}$C for 5 minutes followed by 35 cycles of 94 $^{o}$C for 30 seconds, annealing at optimum annealing temperature (Ta) for 45 seconds, extension at 72 $^{o}$C for 60 seconds with a final extension at 72 $^{o}$C for 10 minutes on the Thermal Cycler T9639 (BenchMark, USA).

PCR products were electrophoresed on a 2% agarose gel with a voltage of 110V for 15 minutes. The good ones were continuously run on a 10% polyacrylamide gel with a voltage of 70V for 16 hours. The gel panel

was observed under UV light using a Gel Logic 212 camera (Carestream Health, Inc, USA) and Kodak MISE software. DNA bands that differed in molecular size were analyzed by the estimation method in comparison to a length standard.

*Table 1.* Primer sequences and PCR reaction conditions

| MS | Forward primer (5'-3') | Reverse primer (5'-3') | Ta ($^oC$) | PCR size (bp) |
|---|---|---|---|---|
| *FHC2010* | AAATGGAACAGTTGAGCATGC | CCCCTTACAGCTTCATTTTCC | 57 | 208–260 |
| *FHC2079* | CAGCCGAGCACATGGTTT | ATTGATTCTGATATGCCCAGC | 61 | 266–294 |
| *PEZ6* | ATGAGCACTGGGTGTTATAC | ACACAATTGCATTGTCAAAC | 59 | 164–212 |
| *PEZ8* | TATCGACTTTATCACTGTGG | ATGGAGCCTCATGTCTCATC | 59 | 221–257 |
| *PEZ10* | CTTCATTGAAGTATCTATCC | CCTGCCTTTGTAAATGTAAG | 57 | 236–336 |
| *PEZ12* | GTAGATTAGATCTCAGGCAG | TAGGTCCTGGTAGGGTGTGG | 57 | 245–313 |
| *PEZ15* | CTGGGGCTTAACTCCAAGTTC | CAGTACAGAGTCTGCTTATC | 59 | 188–296 |

The number and frequency of alleles were determined by using the Genepop V4.2 software (Raymond & Rousset, 1995). Expected heterozygosity (He) was calculated according to the instructions of Wright (1965) while observed heterozygosity (Ho) and F-statistics (Fis, Fit and Fst) were calculated according to the method of Weir & Cockerham (1984) and the Fstat V2.9.3.2 software (Goudet, 2002). Polymorphic information content (PIC) was calculated according to Botstein et al. (1980). Genetic distances and similarity between populations were determined based on the number of analyzed markers and the number and frequency of alleles calculated on each locus, and a phylogenetic tree was also constructed by using UPGMA software (http://genom-es.urv.cat/UPGMA/).

## RESULTS AND DISCUSSION

### Genetic diversity

In this study, seven MS selected from the set of 21 MS were successfully amplified by PCR (Fig. 1).

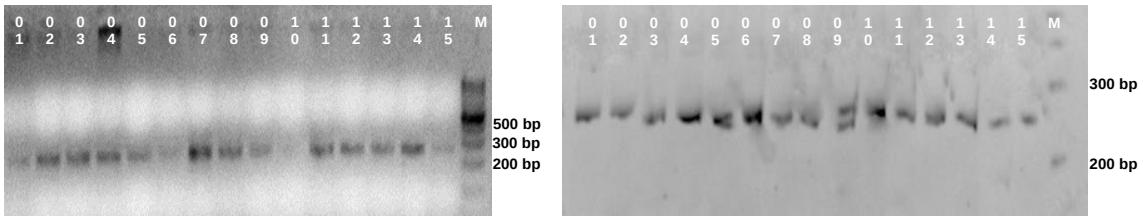


*Figure 1.* DNA amplification of *FHC2010* locus; 1-15: Samples; M: Length standard. (The left or right figure was respectively loaded on 2% agarose or 10% polyacrylamide gel)

The seven MS polymorphisms were all found in three dog breeds (except for the *PEZ15* marker which was monomorphic in the Berger dogs). The total number and the average number of alleles for each MS and each breed were calculated (Tables 2 & 3). The results showed that their number and distribution between loci and dog breeds ranged from 1–6 alleles per locus. The total number of alleles observed was 31 in the Ven and 18 in both the Berger and Poodle. Most markers had a high allele count in Ven dogs (3–6 alleles), followed by Poodles (2–4 alleles) and Berger dogs (1–5 alleles, including one monomorphic marker *PEZ15* responded to 5 and 4 alleles in Ven and Poodle dogs). The average number of alleles per locus was 4.6 with a range from 3 (*FHC2079*) to 6 (*PEZ10*). The highest average number of alleles per locus was found in the Ven Dog (4.4), followed by the Berger and Poodle (both 2.3). The results obtained in this study are lower than those in previous

studies. There, the number of alleles per locus varied from 7 (*FHC2079*) to 24 (*PEZ10*) with a mean of 12.73 and a total of 140 (Cho, 2005), from 7.7 to 15.4 (15.4 in Poongsans, 13.8 in Jindo, 9.72 in Beagles, 8.55 in Greyhounds and 7.7 in German Shepherds) (Kang et al., 2009).

*Table 2.* Number of alleles in each Microsatellite locus in the three dog populations

| Locus | Ven (n = 60) | Berger (n = 10) | Poodle (n = 10) | Total (n = 80) |
|---|---|---|---|---|
| *FHC2010* | 4 | 3 | 3 | 4 |
| *FHC2079* | 3 | 2 | 3 | 3 |
| *PEZ6* | 4 | 3 | 2 | 4 |
| *PEZ8* | 4 | 5 | 2 | 5 |
| *PEZ10* | 6 | 2 | 2 | 6 |
| *PEZ12* | 5 | 2 | 2 | 5 |
| *PEZ15* | 5 | 1 | 4 | 5 |
| Total | 31 | 18 | 18 | 32 |
| Average | 4.4 | 2.6 | 2.6 | 4.6 |

*Table 3.* Frequency of each Microsatellite allele in three dog populations

| Locus | Breed | Allele 1 | Allele 2 | Allele 3 | Allele 4 | Allele 5 | Allele 6 |
|---|---|---|---|---|---|---|---|
| *FHC2010* | Ven | 0.21 | 0.52 | 0.22 | 0.03 | - | - |
| | Berger | 0.16 | 0.38 | 0.44 | - | - | - |
| | Poodle | 0.58 | 0.25 | 0.16 | - | - | - |
| *FHC2079* | Ven | 0.22 | 0.72 | 0.05 | - | - | - |
| | Berger | - | 0.40 | 0.60 | - | - | - |
| | Poodle | 0.20 | 0.50 | 0.30 | - | - | - |
| *PEZ6* | Ven | 0.15 | 0.36 | 0.30 | 0.17 | - | - |
| | Berger | 0.12 | - | 0.08 | 0.79 | - | - |
| | Poodle | - | 0.07 | - | 0.92 | - | - |
| *PEZ8* | Ven | 0.22 | 0.36 | 0.35 | 0.06 | - | - |
| | Berger | 0.32 | 0.10 | 0.17 | 0.10 | 0.28 | - |
| | Poodle | 0.50 | - | 0.50 | - | - | - |
| *PEZ10* | Ven | 0.11 | 0.16 | 0.28 | 0.31 | 0.08 | 0.04 |
| | Berger | 0.40 | 0.60 | - | - | - | - |
| | Poodle | 0.10 | 0.90 | - | - | - | - |
| *PEZ12* | Ven | 0.18 | 0.26 | 0.43 | 0.05 | 0.06 | - |
| | Berger | 0.58 | 0.41 | - | - | - | - |
| | Poodle | 0.60 | 0.40 | - | - | - | - |
| *PEZ15* | Ven | 0.27 | 0.32 | 0.33 | 0.02 | 0.03 | - |
| | Berger | 1.00 | - | - | - | - | - |
| | Poodle | 0.16 | 0.16 | 0.22 | 0.44 | - | - |

*Note:* (-): no allele.

Considering each MS locus, the expected heterozygosity frequency (He) was always higher than the observed heterozygosity frequency (Ho). Specifically, Ho values in Ven dogs ranged from 0.04–0.25 (highest at locus *PEZ12* - 0.25 and lowest at locus *FHC2010* - 0.04) and He ranged from 0.37–0.69 (highest at locus *PEZ6* - 0.69) and lowest at locus *FHC2010* - 0.37). In contrast to this study, the results of Kang et al. (2009) showed that He values (0.71 in German Shepherds and 0.85 in Jindos) were higher

than Ho values (0.65 in German Shepherds and 0.78 in Beagles). Low Ho values indicate a lack of heterozygosity at most of the loci examined. Therefore, the average Fis value of 0.690 (range 0.50-0.89) in the Ven dog population was quite high (Table 3). Theoretically, the Fis value represents the inbreeding coefficient between individuals in the population, fluctuating between -1 and +1. If the Fis value is < 0, then the individuals in the population have an overwhelming heterozygosity level. On the contrary, if Fis >0, the population tends to be more homozygous. That means that with the relatively high Fis value obtained (close to the highest value +1), inbreeding may be occurring in the Ven dog population. It should be noted and considered in the strategy for the conservation of the Ven dog genetic resource. Phavaphutanon & Laopiem (2011) used 12 MS to evaluate the genetic diversity of Thai dog breeds and found that Ho, He, PIC, and Fis values were 0.72, 0.77, 0.73, and 0.072 in the Bangkaew dog and 0.81, 0.78, 0.75 and -0.019 in the Ridgeback breed, respectively. This result showed that the mating in these two dog breeds was in control.

In addition, according to Nei (1978), the difference of Fst is considered small, medium, and high corresponding to the values Fst < 0.05, 0.05 < Fst< 0.15 and > 0.15, respectively. Thus, the genetic difference in the Ven dog population is at an average level (0.14). There, loci with high genetic variance include *FHC2010* (0.44), *PEZ10* (0.20), and *PEZ8* (0.15). The loci with moderate genetic differences are *PEZ6* and *PEZ5*. The remaining loci (*FHC2079* and *PEZ12*) show small genetic variation. The results in Table 4 also show that the selected MSs carry high polymorphic information (PIC ≥ 0.6), except *FHC2079*. The highest and lowest polymorphisms were found at the *PEZ10* (0.77) and *FHC208* (0.42) loci, respectively. The relatively high PIC value in the Ven population demonstrated the polymorphic quality of the selected markers.

*Table 3.* Frequencies of He, Ho, Fis, PIC in Ven dogs

| Locus | Number of alleles | Ho | He | Fis | Fst | PIC |
|---|---|---|---|---|---|---|
| *FHC2010* | 4 | 0.04 | 0.37 | 0.89 | 0.44 | 0.62 |
| *FHC2079* | 3 | 0.20 | 0.41 | 0.50 | 0.00 | 0.42 |
| *PEZ6* | 4 | 0.18 | 0.69 | 0.74 | 0.05 | 0.71 |
| *PEZ8* | 4 | 0.11 | 0.59 | 0.80 | 0.15 | 0.69 |
| *PEZ10* | 6 | 0.23 | 0.60 | 0.61 | 0.20 | 0.77 |
| *PEZ12* | 5 | 0.25 | 0.68 | 0.63 | 0.02 | 0.69 |
| *PEZ15* | 5 | 0.22 | 0.66 | 0.66 | 0.05 | 0.70 |
| Average | 4.6 | 0.17 | 0.57 | 0.69 | 0.14 | 0.67 |

**Genetic similarity and phylogeny**

Over the past several decades, MS has been an effective tool in studies to determine bloodlines and evaluate genetic structure, and genetic diversity of dog breeds/populations (Bigi et al., 2015; Arata et al., 2016; Radko et al., 2018; Goleman et al., 2019). Selection and cross-breeding can lead to similarities in certain traits in individuals. If the number of parent individuals is limited, it will lead to a low level of genetic diversity (Mellanby et al., 2013; Lampi et al., 2020). In this study, among the three populations, the genetic similarities between Ven and the Berger/Poodle were 64.2% and 67.9%, respectively, while the highest value was observed (72.2%) between the Berger and the Poodle (Table 5).

Their genetic relationship was represented in the phylogenetic tree in which the Ven dogs were placed in a distinct branch, while the Berger and Poodle dogs were grouped in the same branch (Fig. 2). The above results also reflect the differences in the genetic nature of

the three dog populations in which the Ven dog is a local breed, and the Berger and Poodle are imported breeds; therefore they are more genetically closely related.

*Table 5.* Genetic similarity among dog populations

| | Ven | Berger | Poodle |
|---|---|---|---|
| Ven | - | 64.2 | 67.9 |
| Berger | - | - | 72.2 |
| Poodle | - | - | - |

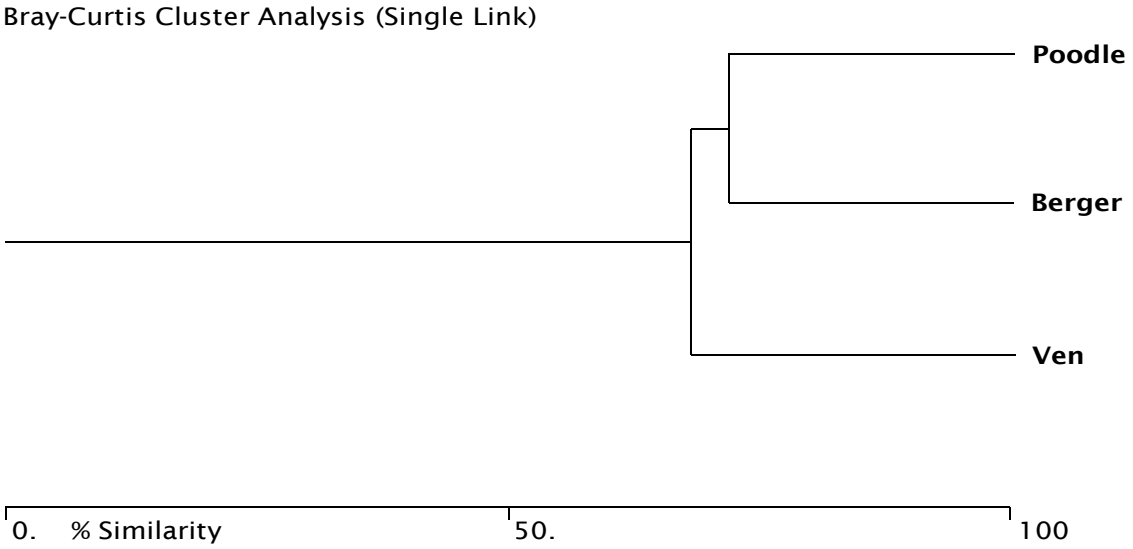


*Figure 2.* Phylogenic tree constructed by UPGMA software based on the genetic similarity among the three dog populations (Ven, Berger, and Poodle)

## CONCLUSION

The genetic diversity of the native Ven dog breed and two imported foreign dog breeds like the Berger and Poodle raised in the Mekong Delta analyzed by seven MS showed that the number of alleles and PIC values were relatively high, demonstrating the quality of the selected MS markers. The genetic diversity indices (Ho, He, Fit, Fst) suggest that there is inbreeding in the Ven dog population. The genetic similarity and phylogenetic tree also reflected the genetic relationships among the three dog breeds.

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