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Individual Identification and Assessment of Genetic Diversity Using Microsatellite Markers in Racing Pigeons Raised in Turkiye

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Abstract

The implementation of a swift and economical molecular genetic approach, ensuring both efficacy and cost-effectiveness and facilitating population certification, is of utmost significance for breeders and the conservation of Turkiye's native pigeon biodiversity. In this study, we aimed to examine the genetic structure of racing pigeons (*Columba livia domestica)* raised in Turkiye using a genetic marker panel consisting of eight short tandem repeat (STR) loci. For this purpose, DNA was isolated from the shed feathers of 216 pigeons. Genomic DNA was amplified using the multiplex allele-specific PCR and subsequent capillary electrophoresis with ABI PRISM 3130XL Genetic Analyzer. Next, PCR products were analyzed in the GeneMapper Software program (Applied Biosystems). For parent testing, paternity index (PI), combined paternity index (CPI), and cumulative probability of paternity (CPP) were calculated. Furthermore, population genetic diversity was evaluated using heterozygosity (He), polymorphism information content (PIC), and Hardy–Weinberg equilibrium (HWE) testing. Results revealed that the total number of alleles is 81 and the number of alleles per locus varies between 4 and 19. The similarity rate between parent and offspring was calculated as 99.99% and above. Since no pedigree information was given when the samples were analyzed, obtaining this similarity ratio demonstrates the reliability of the panel. He values range from 0.362 to 0.919, and the PIC values range from 0.295 to 0.909. Loci PG-1, PG-2, and PG-3 show significant genetic diversity, with moderate to high PIC values reflecting varied allele frequencies in the population. Consequently, the set of seven STR markers (+ one sex marker) can be applied to identify and confirm parentage on a regular basis, thereby facilitating efficient breeding programs and ensuring genetic diversity conservation. This panel enables efficient pedigree analysis and gender determination, optimizing cost-effectiveness. The methodology presented in this study is ideal for pedigree analysis and breed certification in the Turkish pigeon breeding industry. Consequently, we affirm that the study data carries considerable national importance.

Keywords: parentage testing, microsatellite markers, genetic diversity, population structure, *Columba livia domestica*

Introduction

The pigeon (*Columba livia*) is characterized by its remarkable diversity and extensive phenotypic variation, distinguishing it from other avian species. Furthermore, pigeons are globally recognized and appreciated for their widespread popularity (1). Enthusiasts and hobbyists worldwide, along with breeders spanning numerous countries, contribute to their widespread appeal (1, 2). Additionally, feral pigeon populations, often considered urban commensals, are prevalent in cities (3, 4). There are an estimated 350 species of pigeon (*Columba livia*), known as the first domesticated bird species (5, 6). Evidence for the domestication of the pigeon is found in hieroglyphs from ancient Egypt and dates back approximately 10,000 years (7). Pigeon, a popular bird species all over the world, is mostly bred for meat, ornamental, and racing purposes (2). There are different breeds of pigeons, some bred for different flying styles (6). Racing pigeons are bred for their ability to fly long distances and find their place (6). As racing pigeons become extremely expensive, there is a strong need for identity control and parentage verification (7).

Parental analysis has become an active enterprise spanning multiple research areas since the realization in the 1970s-1980s that genetic data could potentially diagnose

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parent-child relationships in nature (8). Sex determination and microsatellite variation analysis using molecular methods have proven to be valuable tools in wildlife conservation and for studies of sex allocation and behavioral ecology in birds (9, 10, 11). In both ordinary practice and forensic case studies, the use of short tandem repeats (STRs) as molecular markers for animal identification and parentage verification has shown to be an effective technique (12).

Lately, microsatellite markers have been used to characterize numerous pigeon breeds and populations. For instance, Podbielska and Radko, carried out the identity control and parentage determination of 519 racing pigeons raised in Poland with 17 STR markers (4). De Groot M and van Haeringen (12), 2017 characterized a total of 1421 domestic pigeons using 16 STR markers for parentage and identification analyses. Many countries have conducted such studies, utilizing STR marker profiles across various pigeon breeds (2, 13, 14). These analyses have proven instrumental in evaluating national biodiversity and genetically characterizing indigenous breeds. Comparable genetic studies are requisite within Turkiye. The establishment of a proficient database grounded in pedigree analysis holds paramount importance for discerning native pigeon breeds and certifying cultivated varieties.

In the wild, phenotypic variations typically arise through natural selection in response to environmental changes. However, in domesticated animals, breeders select for desirable traits based on economic importance or preferred appearance. This selective breeding for specific visual characteristics reduces variation, resulting in pigeons with morphological traits favored by pigeon enthusiasts. Different breeds exhibit significant diversity in craniofacial structures, plumage color and patterning, feather placement and structure, as well as flight behaviors (15, 16). An important aspect of ornamental bird breeding, similar to other domestic animals, is the presence of individuals with superior traits compared to others within the population. These superior features may include desirable flight characteristics in racing and tumbler pigeons, as well as preferred morphological traits. Thus, the selection of these superior birds for breeding plays a critical role in driving genetic improvement within the population (1, 15, 16, 17). Adults of many avian species lack sexual dimorphism (structural differences) and sexual dichromatism (color differences), making gender determination challenging (18, 19). While sexual dimorphism does exist between breeds and traits (20), often favoring male pigeons, there remains a necessity for a reliable method of gender discrimination. In the realm of pigeon breeding, the utilization of efficient molecular genetic techniques holds significance, serving both for accurate pedigree analysis and precise gender determination. Furthermore, pigeon breeding occupies a prominent and significant position in Turkiye. Hence, the introduction of a rapid and cost-effective molecular genetic technique that ensures both effectiveness and affordability, and enables the certification of populations, holds paramount importance for breeders and the preservation of Turkiye's indigenous genetic biodiversity. This study aimed to assess individual identification and parental analysis through the utilization of a panel comprising nine STR markers and to investigate the population structure and genetic diversity of certain racing pigeon breeds raised in Turkiye.

Materials and Methods

Sample collection and genetic marker selection

The pigeon samples selected for this study (n=216) consist of archived samples taken for parental verification, identity control, and sex determination. The microsatellit markers [6-FAM (PG4, PG1, PG2, PG3), VIC (PG5, PG6, PG7), NED (PG9, PG8)] were selected based on the panel provided by the International Society for Animal Genetics (ISAG) (https://www.isag.us/). A gender marker (PG-8) has been added for extra control. Unlike mammals, ZZ corresponds to the male gender, and heterogametic ZW corresponds to the female gender in avian species. Positive control samples, containing known parental verification, identity control, and sex determination data, were analyzed to validate the test's reliability.

DNA isolation and quantification

In this study, due to the challenges and potential harm associated with collecting blood and tissue samples from pigeons, feathers extracted from the wings and tail regions were used. Before the isolation stage, the calamus and superior umbilicus parts of the bird's feathers (approximately four feathers), containing DNA were cut into small pieces under sterile environmental conditions (21). To obtain DNA from shredded bird feathers; DNA isolation was performed with the NucleoGene Nucleic Acid Extraction Kit according (NucleoGene, Turkey) to the Mammalian Tissue and Rodent Tail Genomic DNA Purification Protocol. Figure 1 depicts a schematic overview of the isolation stages.

Figure 1. Steps of DNA Isolation.

Genotyping

Genomic DNA was amplified using the seven markers recommended by the ISAG as a core panel (+ sex marker) with multiplex PCR. An informative overview of the marker loci was provided in Table 1. The PCR conditions for all reactions consisted of an initial denaturation of 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension step of 72 °C for 30 min. PCR products were then mixed with 8.5 µl Applied Biosystems Hi-Di FormAmide (Ref: 4311320) and 0.5 µl GeneScan 600 LIZ Size Standard v2.0 (Ref: A25794) and added to the MicroAmp Optical 96-well Reaction Plate (Ref: N8010560). The mixture (9 µl) was distributed into each well. 1 µl of PCR products was added to the mixture distributed into the wells and mixed by pipetting. The plate was covered with Plate Septa 96-well (Ref: 4315933). The plate was spun and denatured at 95 °C for 3 minutes and placed on an ice block. The plate was loaded onto the ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems). Analysis was performed in the FragmentAnalysis36_POP7_1 run module and the results were analyzed in GeneMapper Software (v3.2.1, Applied Biosystems).

Table 1. Brief information on the marker loci.

Order	Locus	Dye	Size range (bp)	Primer Concentration F+R (µM)
PG-1	Cli_T13	FAM	198–240	2.0
PG-2	PIGN10	FAM	271–325	2.4
PG-3	PIGN26	FAM	364–494	0.6
PG-4	Cli_D01	FAM	75–130	1.4
PG-5	PIGN57	VIC (HEX)	153–189	1.8
PG-6	PIGN12	VIC (HEX)	241–371	4.0
PG-8*	CHD	NED (ATTO)	266–290	1.0
PG-9	Cli_T43	NED (ATTO)	191–229	1.0

*Sex marker

Statistical analysis

Genetic diversity parameter estimation and HWE testing were performed using Cervus v3.0 software. The following equations were used to analyze genotypic data for determining potential parents of the respective young pigeons as suggested by Nei (22), Botstein et al. (23) and Lee et al. (14)

I- For the probability of a match (Pm), power of discrimination (Pd), and cumulative power of discrimination (CPd):

$$Pm = \sum_{k=1}^{n} (Pk)^2$$

where Pk is the genotype frequency.

Power of discrimination (Pd)=1-Pm

$$CPd = 1 - \prod_{i=1}^{n} Pm_i$$

II- For paternity testing, the Paternity Index (PI) was calculated as follows:

$$PI = \frac{Probability\ X\ (\ Alleged\ pigeons\ contributed\ the\ obligate\ allele)}{Probability\ Y\ (Random\ pigeons\ contributed\ correct\ allele)}$$

In this context, X denotes the likelihood that the putative parents are the true biological parents, determined by the matching alleles. Meanwhile, Y represents the probability that random pigeons serve as the biological parents and share the alleles coincidentally.

III- The Combined Paternity Index (CPI) was determined as the product of each PI. Subsequently, the Cumulative Probability of Paternity was computed.

$$Cumulative\ Probability\ of\ Paternity\ (CPP) = \frac{CPI}{(CPI\ +\ 1)}$$

IV- Expected heterozygosity (He), and polymorphism information content (PIC) were calculated as follows:

$$He = 1 - \sum_{i=1}^{n} P_i^2$$

$$PIC = 1 - (\sum_{i=1}^{n} P_i^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2\, P_j^2$$

Results

The findings from the present investigation of racing pigeons in Turkey revealed a notable degree of genetic diversity. A total of 81 different alleles were observed. The average number of alleles per locus was 10.125, ranging from 4 alleles in PG-4 to 19 alleles in PG-6 (Table 2). At locus PG-4, allele 3 had the highest frequency, accounting for 42.86% of the alleles observed, followed by allele 32 (PG-1) with a frequency of 32.85%. Conversely, at locus PG-3, allele 13 was the most prevalent, representing 20.83% of the alleles, while allele 3 had the lowest frequency at 0.42%. This variability in allele frequencies across different loci highlighted the genetic heterogeneity present in the population, which was crucial for accurate paternity determination. At locus PG-2, alleles 5 and 8 exhibited lower frequencies, each ac-

counting for only 0.0083 of the observed alleles. Allele 32 had also remarkably lower frequency compared to alternative alleles in PG-9. This suggests a lower prevalence of these alleles within the population, as shown in Table 2.

Table 2. Allele frequencies of each STR locus.

Allele	Locus							
	PG-1	PG-2	PG-3	PG-4	PG-5	PG-6	PG-8*	PG-9
2						0.0455		
3		0.0165	0.0042	0.4286		0.0682		
4			0.0500	0.1349		0.1174		
5		0.0083	0.0625	0.2262	0.0072	0.0644		
6		0.0165	0.0125	0.2103	0.0145	0.0758		
7		0.0165			0.4094	0.1402		
8		0.0083	0.0250		0.2464	0.0985		
9		0.0331	0.0458		0.3225	0.0644	0.2358	
10		0.0331						
11		0.0620	0.0292					
12		0.0165	0.0542			0.0189		
13		0.0124	0.2083			0.0038		
14		0.0124	0.1208			0.0189	0.7642	
15		0.2149	0.1250			0.0038		
16		0.3140	0.1000			0.0189		
17		0.0165	0.0333					
18		0.0413	0.0292			0.0152		
19		0.1157	0.0583			0.0227		
20		0.0620	0.0333			0.0682		
21			0.0083			0.1174		0.1022
22						0.0227		0.3248
23						0.0152		0.1058
24								0.0511
25								0.0438
26								0.1569
27								0.1496
28	0.0551							0.0474
29	0.1287							0.0146
30	0.0662							
31	0.2243							
32	0.3285							0.0036
33	0.1581							
34	0.0441							
Na	7	17	17	4	5	19	2	10

Na: number of different alleles.

*Sex marker

Table 3 presents an in-depth analysis of microsatellite marker diversity characteristics crucial for understanding the genetic landscape of the studied population. Key parameters such as observed heterozygosity, He, and PIC provide insights into the extent of genetic variation at each locus. He ranged from 0.362 to 0.919 while PIC ranged from 0.295 to 0.909. Loci PG-1, PG-2, and PG-3 exhibited notable levels of genetic diversity, reflected in their moderate to high PIC values, indicative of diverse allele frequencies within the population. Conversely, loci PG-5 and PG-81 demonstrated relatively lower genetic diversity, with PIC values of 0.599 and 0.295, respectively. Loci PG-6 and PG-9 displayed relatively high levels of observed heterozygosity, suggesting a higher degree of genetic variation within these loci. Deviation from HWE was observed for the PG-1, PG-5, PG-6, and PG-9 (Table 3).

Table 3. Genetic diversity parameters across 8 microsatellite markers pigeon samples.

Locus	K	Ho	He	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	*F* (Null)	HWE
PG-1	7	0.809	0.797	0.766	0.575	0.397	0.212	0.070	0.371	-0.000	NS
PG-2	17	0.587	0.832	0.811	0.491	0.323	0.142	0.047	0.347	0.1746	***
PG-3	17	0.625	0.900	0.889	0.340	0.205	0.065	0.019	0.306	0.1796	***
PG-4	4	0.619	0.706	0.653	0.720	0.547	0.370	0.138	0.433	0.0540	*
PG-5	5	0.717	0.670	0.599	0.768	0.615	0.457	0.179	0.461	-0.000	NS
PG-6	19	0.386	0.919	0.909	0.293	0.172	0.048	0.013	0.296	0.4068	NS
PG-8[1]	2	0.472	0.362	0.295	0.935	0.852	0.767	0.474	0.688	-0.000	*
PG-9	10	0.796	0.822	0.798	0.520	0.346	0.164	0.053	0.354	0.0173	NS

K: number of unique alleles; HO: observed heterozygosity; HE: expected heterozygosity; PIC: polymorphic information content; NE-1P: non-exclusion first parent; NE-2P: non-exclusion second parent; NE-PP: non-exclusion parent pair; NE-I: non-exclusion identity; NE-SI: non-exclusion sibling; F(null): estimated frequency of null allele; HWE: Hardy–Weinberg equilibrium.

1Sex marker

*P<0.05; ***P<0.001

Figure 2. Pigeon specimens underwent certification, identity verification, and identification processes utilizing microsatellite markers in this study.

The precision of the test results was assessed through the CPP, which is ideally set at a minimum of 99%. This signifies that there is only a <1% chance that a male chosen randomly from the general population is the father, while there is a >99% probability that the purported father is indeed the biological father of young individual. The CPI for each offspring examined, as claimed by the mother and father, was calculated to be 99.99%. Analysis of the Applied Biosystems GeneMapper Software revealed that all samp-

les corresponded with the sex provided by the breeder, as determined by the sex determination marker. The analysis of individuals designated as positive controls, verifying both paternity and gender, further validated the accuracy of the findings.

Matches between mothers, fathers, and offspring were determined using microsatellite marker profiles for parentage determination. An illustration of the families established based on the genetic analysis results is depicted in Figure 3. The marker profiles of these individuals are shown in Figure 4. In this family, the microsatellite profiles of the mother (Figure 4A) and father (Figure 4B) corresponded to that of the target offspring (Figure 4C). Furthermore, based on the sex marker (PG-8), it was discerned that the offspring was male.

Figure 3. An example of a Homing Pigeon family selected for STR analysis.

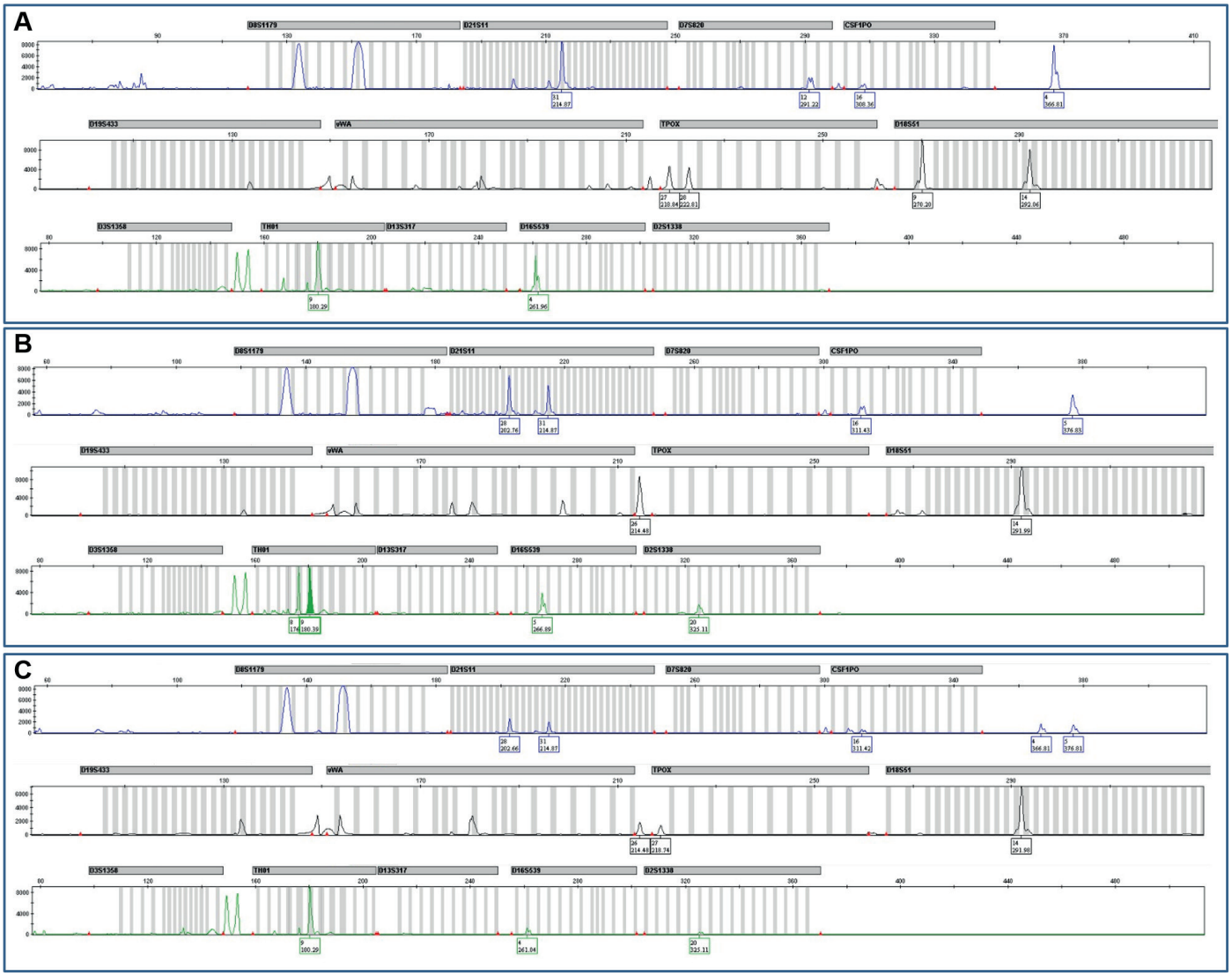

Figure 4. Microsatellite marker profiles of the multiplex used for paternity testing. At each of the eight loci, there is a matching allele shared by the offspring (C) in comparison to both the mother (A) and father (B). The suspicious offspring (C) is male based on the sex marker. D21S11: PG-1, D7S820- CSFIP0: PG-2, vWA: PG-3, D8S1179: PG-4, TH01: PG-5, D16S539 – D2S1338: PG-6, D18S51: PG-8, TPOX: PG-9. The analysis was conducted using the Applied Biosystems 3130xl Genetic Analyzer, employing the FragmentAnalysis36_POP7_1 run module, and subsequently, the results were analyzed using the Applied Biosystems GeneMapper Software. Through the examination of microsatellite profiles of both parents and offspring, parentage was established, and pedigree data was compiled. Certification was completed through individual identification, and pedigree information was duly recorded. The microsatellite markers [6-FAM (PG4, PG1, PG2, PG3), VIC (PG5, PG6, PG7), NED (PG9, PG8)] were chosen from the panel provided by the International Society for Animal Genetics (ISAG), accessible at https://www.isag.us/.

Discussion

Developing genetic markers for parental analysis in wild animal populations such as birds has enabled a more precise examination of genetic structure in and between populations of free-living organisms. Microsatellites emerge as one of the powerful tools for identification and parentage analysis not only in humans but also in animals (12). Developments in the field of molecular ecology in the 1990s laid the foundations for the use of microsatellite markers in the era of modern parenting analysis. Microsatellites directly meet the need for convenient and highly sensitive genotyping for parental verification and identity control for domestic pigeons in both routine laboratory applications and forensic case studies. Microsatellites have been investigated in several avian species (24, 25, 26, 27, 28). These markers hold significant importance in pigeon breeding, a branch of considerable economic and social significance. In addition, preserving genetic diversity is vital for species to retain their evolutionary capacity, making the conservation of genetic diversity paramount in species preservation. Microsatellite markers are extensively utilized in molecular ecology and population genetics research of endangered and indigenous animals owing to their widespread presence in the genome, abundant polymorphic information, and co-dominant inheritance pattern (28).

The effectiveness of microsatellite markers in paternity tests and control of pedigree records is related to whether they contain high-level information. The informativeness of these markers is contingent upon the number of alleles they yield and their frequencies within the population under study (29). For instance, a study examining parental analysis in pigeons investigated seven STR loci using a sample of 96 pigeons from Taiwan racing pigeons, revealing a total of 37 alleles, with allele counts ranging from 3 to 18 per locus (14). Similarly, an investigation on Egyptian pigeons analyzed 11 STR loci in 133 pigeons from Egyptian and Japanese racing breeds, yielding a total of 89 alleles, with allele counts varying between 3 and 14 per locus and an average of 8.1 alleles per locus (2). Another study explored genetic diversity and inter-breed relationships across nine pigeon breeds (Urban pigeon, English Fantail, German Beard, German Nun, Indian Fantail, Kassel Tumbler, King, Strasser, Wroclaw Meat and Vienna Highflier) using seven STR loci, genotyping a total of 364 individuals and identifying 122 alleles, with allele counts ranging from 8 to 26 per locus (13). In the study by de Groot and van Hannigan (12) pursued parental analysis and individual identification utilizing 16 STR loci recommended by ISAG. These researchers reported the results for the detection of 179 distinct alleles, with the number of alleles per locus ranging from 4 to 32 (12). Podbielska and Radko (4), focused on 16 STR loci in their investigation of the genetic composition of pigeons reared in Poland, encompassing a total of 519 specimens. They identified 146 distinct alleles, with the number of alleles per locus ranging from 4 to 19. The average count of alleles per locus was reported as 9.125 (4). In our research focusing on racing pigeons bred in Turkiye, we observed a total of 81 alleles across the seven examined STR loci (excluding sex marker). The allelic count per locus ranged from 4 to 18. The methodology outlined in our study has been deemed suitable for application in pigeons bred in Turkiye.

In genetic research, population genetic indices such as He and PIC are pivotal for evaluating population structure based on genetic variability within specific genes or gene sets. The levels of He can provide insights into breeding characteristics within a population; for instance, a decrease in He may indicate the occurrence of inbreeding over time, thereby influencing the genetic diversity and overall health of the population (30). PIC values serve as prevalent indices for assessing the polymorphism level of a marker. The degree of polymorphism directly influences the utility of a marker in segregation analysis. PIC values are categorized into three classes: PIC > 0.50 denotes high informative marker, 0.25 < PIC < 0.50 indicates moderate informative marker, and PIC < 0.25 reflects low informative marker, as proposed by Botstein et al. (23) These classifications offer a framework for evaluating the efficacy of markers in genetic analyses and provide valuable insights into their suitability for various applications in genetic research and breeding (30, 31). Microsatellites are dispersed throughout the genome and are highly polymorphic. Our analysis further validates this interpretation. We observed high values of He and PIC for all markers in the panel. With the exception of the sex marker, all microsatellite markers were deemed highly informative based on PIC levels (Table 3).

Our study's findings demonstrate the effectiveness of the panel endorsed by ISAG (https://www.isag.us/) for pigeon genetics of the breeds raised in Turkiye. This panel has proven to be reliable not only within our study but also across diverse geographical locations, underscoring its utility for individual identification, quality assurance, and parental analysis in pigeon breeding programs worldwide. Furthermore, the inclusion of the sex marker enabled the identification of two variants, the W and Z alleles, in pigeons, aligning accurately with the sexes reported by breeders. Notably, the genetic similarity between offspring and parents exceeded 99.99% across all families, indicating a high degree of accuracy in our analysis, even in the absence

of pedigree information provided with the samples. Thus, an effective parent and gender determination was achieved with the microsatellite marker-based panel in this study, as shown in Figures 3 and 4.

The racing pigeon population in Turkiye is diverse, with breeders engaging in buying, selling, and occasionally exchanging birds through various auctions. Owners continually seek superior pigeons to enhance their performance in competitive events. However, pigeon racing, like any sport, demands rigorous dedication. Breeding racing pigeons entails meticulous selection, breeding efforts, training, proper feeding, and ensuring the birds' overall well-being and fitness (4).

Native pigeon breeds represent essential national genetic assets. Preserving and characterizing these breeds is crucial for Turkiye's biodiversity. In recent years, extensive research has focused on identifying and characterizing native pigeon breeds of Turkiye (15, 32, 33, 34, 35, 36). To effectively conduct certification studies at the population level and enhance breeding practices, the initial step is to perform individual identification and parentage determination using a rapid, cost-effective, and reliable genetic method.

Various analyses involve the simultaneous use of numerous markers, a common strategy aimed at enhancing efficiency and accuracy. However, the economic conditions in Turkiye, coupled with fluctuating exchange rates, significantly escalate the cost of molecular genetic analyses. Consequently, there arises a pressing need to develop the most effective panel at minimal expense. While comprehensive analyses employing diverse genetic markers have been conducted extensively in the literature (4, 14), adopting pragmatic approaches tailored to Turkiye's context would prove advantageous in pigeon breeding, as well as in animal husbandry at large. In this study, guided by the outcomes of testing different panels, we devised a restricted yet practical panel in terms of molecular genetics. This panel facilitates effective pedigree analysis and gender determination while maximizing efficiency at a relatively reduced cost. The methodology outlined in this study is well-suited for pedigree analysis and breed certification within the Turkish pigeon breeding sector. Hence, we assert that the data generated from this study holds significant national value.

Conclusion

This article presents the investigation of the population structure and genetic diversity of racing pigeons raised in Turkiye using the STR markers. Eight loci, including seven STR markers and the sex marker, were successfully amplified and analyzed by multiplex PCR amplification. The findings showed that the similarity between offspring and parents was 99.9999% for each family of known origin. This demonstrates that the method is entirely effective and can be applied to cases of uncertain parentage and sex determination. This panel enables cost-effective pedigree analysis and gender determination, optimizing efficiency. The methodology described in this study is ideal for pedigree analysis and breed certification in the Turkish pigeon breeding sector. Furthermore, these molecular genetic investigations may also prove beneficial for individual identification and parentage testing of other avian species bred for hobby purposes, including parrots, budgies, and goldfinches, commonly bred in Turkiye. Pigeon breeding holds considerable influence throughout Turkiye. Moving forward, such genetic studies will be essential for enhancing racing pigeon breeding programs in Turkiye.

Conflict of Interest

Sena Ardicli serves as an editor for the Journal of Research in Veterinary Medicine, while the remaining authors state that they do not have any pertinent competing interests.

Ethical Considerations

Approval from an ethics committee was not necessary for this study, as it did not involve any invasive research on live animals or humans. DNA isolation was exclusively conducted using fallen feathers, with no collection of blood or tissue samples.

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