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Accuracy in Pedigree Records in Hair Goats: New Microsatellite Based Paternity Test Panels[#]

ABSTRACT

Objective: The aim of the study was to establish microsatellite-based paternity test panels that can be used in paternity tests for Hair goat populations bred in Aydın and Denizli provinces, and to evaluate them in terms of paternity test parameters.

Material and Methods: The animal material of the study consisted of a total of 247 hair goats (42 bucks and 205 kids) in Hair goat farms in Aydın and Denizli provinces. The 18 microsatellites used in the study were evaluated in terms of molecular genetic parameters obtained from genotyping. After the evaluation, microsatellites were ranked from highest to lowest based on their individual exclusion probability values. Eighteen paternity test panels were created by sequentially adding a new microsatellite with a lower individual exclusion probability than the previous one to the microsatellite with the highest exclusion probability. Molecular genetic test statistics were obtained for the paternity test panels.

Results: In the study, 306 alleles were observed. The observed heterozygosity ratio (Ho) ranged from 0.69 to 0.95, while the expected heterozygosity ratio (He) ranged from 0.72 to 0.92. In the study, individual P-probability of exclusion (PE) values ranged from 0.316 to 0.719, while the combined probability of exclusion (CPE) values for the paternity test panels ranged from 0.7188 to 0.9999. Among the paternity test panels, Panel -7 and the following panels showed values above the threshold value reported in the literature in terms of the combined probability of exclusion.

Conclusion: According to the study findings, Panels 7 and 8, designed for paternity testing with fewer microsatellite markers, can be more cost-effective and practical for Hair goat populations compared to other panels. The findings obtained from the study make a significant contribution and provide a perspective for improving hand-mating practices. This is crucial within the framework of the "National Genetic Improvement Project for Small Ruminants at Breeders' Conditions" coordinated by the General Directorate of Agricultural Research and Policies.

Keywords: Microsatellite, probability of exclusion, goat, DNA

Kıl Keçilerinde Pedigree Kayıtlarında Doğruluk: Yeni Mikrosatellit Tabanlı Babalık Test Panelleri

ÖZ

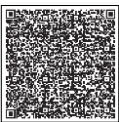
Amaç: Çalışmanın amacını, Aydın ve Denizli illerinde yetiştirilen Kıl keçi populasyonlarında babalık testlerinde kullanılabilen mikrosatellit temelli babalık test panellerinin oluşturulması ve bunların babalık test parametreleri açısından değerlendirilmesi oluşturmıştır.

Materyal ve Metot: Çalışmanın hayvan materyalini Aydın ve Denizli illerindeki Kıl keçi işletmelerinde bulunan 42 teke ve 205 oğlak olmak üzere toplam 247 baş Kıl keçi oluşturmuştur. Çalışmada kullanılan 18 mikrosatellit ilişkin genotipleme sonuçlarında elde edilen moleküler genetik parametreler bakımından değerlendirilmiştir. Değerlendirme sonucunda mikrosatellitler, bireysel dışlama olasılığı değerlerine göre büyükten küçüğe sıralanmış ve en yüksek dışlama olasılığı olan mikrosatellite bir öncekinden daha düşük bireysel dışlama olasılığı olan yeni bir mikrosatellit eklenerek on sekiz babalık test paneli oluşturulmuştur. Oluşturulan babalık test panellerine yönelik olarak moleküler genetik test istatistikleri elde edilmiştir.

Bulgular: Çalışmada 306 allel gözlemlenmiştir. Lokuslar bazında gözlenen heterozigotluk oranı (Ho) 0.69 ile 0.95 arasında, beklenen heterozigotluk oranı (He) ise 0.72 ile 0.92 arasında olmuştur. Çalışmada, bireysel dışlama olasılığı (PE) değerleri 0.316 ile 0.719 arasında değişim gösterirken oluşturulan babalık test panellerine ilişkin kombine dışlama olasılık değerleri (CPE) 0.7188 ile 0.9999 aralığında olmuştur. Oluşturulan babalık test panellerinden Panel -7 ve sonraki paneller literatür tarafından combine dışlama olasılığı bakımından bildirilen eşik değer üzerinde değerler almıştır.

Sonuç: Gerçekleştirilen çalışma sonuçlarına göre daha az mikrosatellit işaretleyici ile babalık testleri için oluşturulan Panel 7 ve 8'in diğer panellere göre Kıl keçi populasyonlarında daha ucuz ve pratik olarak kullanılabilen ortaya konmuştur. Ayrıca gerçekleştirilen araştırmadan elde edilen bulgular Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü'nün koordinasyonunda gerçekleştirilen "Halk Elinde Hayvan Islahı Ülkesele Projeleri" kapsamında önemli bir sorun olan elde aşım uygulamalarının kontrolüne yönelik önemli bir katkı ve bakış açısı sağlamıştır.

Anahtar Kelimeler: Mikrosatellit, dışlama olasılığı, keçi, DNA



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INTRODUCTION

Goat breeding traditionally holds a special place in the Turkish economy. This importance stems from the goat's ability to utilize generally short and infertile pastures, fallow, stubble, and areas unsuitable for crop production and transform them into products such as meat, milk, fleece, hair, and leather (Koyuncu and Taşkın, 2016; Tolunay et al., 2016; Ceyhan et al., 2017; Günlü and Mat, 2021).

Hair goats constitute the majority of our goat population (FAOSTAT, 2022). In this context, the livelihoods, especially the food requirements, of the rural population living in mountain villages in and near forests are largely dependent on Hair goats. The reason why many people stay in rural areas is for goat or sheep breeding. Small ruminant breeders, especially hair goat breeders, utilize natural resources to produce their goods at almost zero cost, except for their labor (Cedden et al., 2020; Ergün and Bayram, 2021; Günlü and Mat, 2021).

Determining the yields of hair goats, increasing the existing yields by considering breeder and consumer demands, and ensuring the sustainability of the breeding structure can be achieved through a genetic breeding program tailored to breeder conditions and specific to local circumstances. An effective breeding program can be achieved through pedigree breeding (Özsoy and Yıldız, 2019).

As with other livestock, it is crucial to maintain mating and birth records in goat breeding to gather information on the production potential of herds in a healthy manner (Çelikyürek et al., 2019). In addition, it is important to emphasize the formation of the possibility of maintaining special yield records and yield records based on pedigree records. However, the challenge of verifying the accuracy of the results reported in hand-mating practices implemented in numerous breeding programs conducted in the field, as well as the precise identification of the parents, remains relevant (Yılmaz and Karaca, 2012; Yılmaz et al., 2018; Keskin et al., 2019).

Especially in farm animals such as sheep and goats, which have multiple births and are raised together in flocks, many mistakes can occur in recording parental information due to various reasons. Incorrect data can be entered into pedigree records. In such suspicious cases, accurate information can be revealed by implementing parental control methods (Yılmaz, 2016; McClure et al., 2018; Flanagan and Jones, 2019; Cui et al., 2020).

Parental errors can lead to a negative direct maternal genetic correlation taking a positive value. They can also result in a decrease in the level of direct maternal heritability, genetic progress, and the accuracy of the estimated breeding value (Badzioch et al., 2003; Harder et al., 2005; Hinrichs and Suarez, 2005). In addition, a pedigree error of 10% per year results in a 3-4% decrease in genetic progress (Israel and Weller, 2000; Banos et al., 2001; Vandeputte et al., 2006; Nwogwugwu et al., 2020).

The most reliable tests used to determine paternity are performed by molecular genetic methods (Anuniação and Filho, 2000; Ma et al., 2006). The most commonly used DNA-based genetic analysis methods in parentage testing are SNP and STR methods (Yılmaz and Karaca, 2012; Yılmaz, 2016; Kaiser et al., 2017; Yılmaz et al., 2018; Flanagan and Jones, 2019; Keskin et al., 2019; Ossowski et al., 2022).

The accuracy of parental information in the pedigree register is crucial for establishing a reliable pedigree register for breeding studies in small ruminants breeding. Small ruminants breeding is one of the significant branches of livestock breeding in our country. An important infrastructure has been established for performance recording under breeder conditions in many provinces through the sub-projects within the scope of the "National Genetic Improvement Project for Small Ruminants at Breeders' Conditions" coordinated by the General Directorate of Agricultural Research and Policies. However, there are challenges that need to be addressed in ensuring the accuracy of the parentage information of the offspring resulting from the hand-mating activities that are being implemented or attempted in a limited segment of the population with significant efforts in these sub-projects.

In this study, the aim was to determine the molecular genetic characteristics of Hair goats raised in Aydın and Denizli provinces using specific microsatellite markers and to explore the feasibility of developing paternity test panels with suitable markers.



MATERIAL and METHODS

Animal Material

The animal material for the study comprised a total of 247 hair goats, including 205 kids born on the farms where hand-mating was implemented as part of the "Hair Goat Breeding" project initiated by the General Directorate of Agricultural Research and Policies in Aydın and Denizli provinces, and 42 goats born on farms where hand mating was practiced. The distribution of animal material is provided in Table 1.

Table 1. Animal material

Tablo 1. Hayvan materyali

Province	District	Number of Farms	Number of Samples		Total
			Kids	Bucks	
Aydın	Bozdoğan	3	96	20	116
	Karacasu				
	Kuyucak				
Denizli	Babadag	4	109	22	131
	Çal				
	Honaz				
Total		7	205	42	247

DNA Isolation Method

DNA was isolated from blood samples taken from the jugular vein into vacuum tubes containing K3-EDTA using a commercial isolation kit (Applied Biological Materials Column-Pure Blood Genomic DNA Kit, Canada). The quantity and quality of the DNA samples obtained were controlled using NanoDrop 2000 (Thermo Scientific, USA).

PCR and Genotyping

In the study, 18 microsatellite markers recommended by FAO (2011) were used. Information about the multiplex groups formed with the microsatellites used is provided in Table 2.

Table 2. Multiplex groups formed with microsatellites

Tablo 2. Çalışmada oluşturulan multipleks gruplar

Multiplex-1 (M1)	Multiplex-2 (M2)	Multiplex-3 (M3)
INRA0023	CSR0247	INRA063
INRA0005	McM0527	MAF0065
OarFCB20	SRCSR0005	SRCSR0008
ILST0019	ILST0087	SRCSR0024
BM1818	SRCSR0023	BM1258
INRA0132	HSC (OLADRB)	
	BM1329	

A PCR mixture containing 10X PCR Buffer, MgCl₂, dNTP mixture (dATP, dTTP, dGTP, dCTP), 18 fluorescently labeled microsatellite markers (Sigma, Interlab, İzmir), Taq DNA Polymerase Enzyme, ~100 ng of genomic DNA and sterile ddH₂O were prepared in tubes with a total volume of 25 µl. The Touch-Down PCR method (Hecker and Roux, 1996) was utilized to conduct DNA amplification more efficiently and rapidly (Table 3).



Table 3. Touchdown PCR conditions

Tablo 3. Touchdown PCR koşulları

Multiplex Group	First Denat.	Denat.	Annealing	Extension	Cycle	Final Extension
M1	95 °C (5 min)	95 °C (40 s)	60-50 °C (40 s)	72 °C (1 min)	30	72 °C (10 min)
M2	95 °C (5 min)	95 °C (40 s)	60-50 °C (40 s)	72 °C (1 min)	30	72 °C (10 min)
M3	95 °C (5 min)	95 °C (40 s)	63-50 °C (40 s)	72 °C (60 s)	30	72 °C (10 min)

Fragment analyses of fluorescently labeled microsatellites were performed on a Beckman Coulter GeXP genetic analyzer according to the manufacturer's recommendations.

Design of Paternity Test Panels

Firstly, the microsatellites used in the study were ranked from largest to smallest based on their individual exclusion probability values. Secondly, a new microsatellite with a lower individual exclusion probability than the previous one was added to the microsatellite with the highest exclusion probability to form eighteen paternity test panels (Table 4).

Table 4. Paternity test panels based on individual exclusion probabilities of microsatellites

Tablo 4. Mikrosatellitlerin bireysel dışlama olasılıklarına göre oluşturulan babalık testi panelleri

Locus	Panels																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
HSC	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
BM1258		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
INRA0023			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SRCRSP005				*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CSRD0247					*	*	*	*	*	*	*	*	*	*	*	*	*	*
MAF0065						*	*	*	*	*	*	*	*	*	*	*	*	*
BM1818							*	*	*	*	*	*	*	*	*	*	*	*
OARFCB20								*	*	*	*	*	*	*	*	*	*	*
SRCRSP0023									*	*	*	*	*	*	*	*	*	*
SRCRSP0008										*	*	*	*	*	*	*	*	*
BM1329											*	*	*	*	*	*	*	*
INRA0132												*	*	*	*	*	*	*
McM0527													*	*	*	*	*	*
ILTS0087														*	*	*	*	*
SRCRSP0024															*	*	*	*
ILTS0019																*	*	*
INRA005																	*	*
INRA063																		*

Statistical Analysis

The genotyping rate of the study material using the microsatellites was calculated. Some molecular genetic polymorphism parameters, such as allele number (Na), effective allele number (Ne), observed (Ho), and expected (He) heterozygosity, were calculated using the GenAlEx genetic analysis program (Peakall and Smouse, 2012). The CERVUS 3.0 program (Slate et al., 2000; Marshall, 2006; Kalinowski et al., 2010) was used to obtain parameters such as polymorphic information content (PIC), probability of exclusion (PE), probability of identity (PI), combined probability of exclusion (CPE), combined probability of identity (CPI), and frequency of null allele (F(Null)).



RESULTS

Statistical values for microsatellite-based genetic polymorphism and paternity tests for Hair goats bred in Aydın and Denizli provinces, which constitute the animal material of the study, are presented in Table 4.

Table 5. Molecular genetic polymorphism statistics of microsatellite loci used in the study

Tablo 5. Çalışmada kullanılan mikrosatellit lokuslarına ait moleküler genetik polimorfizm istatistikleri

Loci	GR (%)	Na	Ne	Ho	He	PIC	PE	PI	F(Null)
INRA005	94.33	19	3.56	0.70	0.72	0.69	0.333	0.109	0.0022
INRA0023	95.95	16	9.28	0.77	0.89	0.88	0.641	0.021	0.0761
OARFCB20	97.57	23	7.20	0.90	0.86	0.85	0.574	0.033	-0.0240
ILTS0019	98.38	10	4.48	0.67	0.78	0.75	0.413	0.073	0.0047
INRA0132	100.00	17	6.26	0.76	0.84	0.82	0.519	0.044	0.0556
BM1818	95.14	12	7.80	0.80	0.87	0.86	0.591	0.029	0.0433
BM1329	97.57	17	6.09	0.70	0.84	0.82	0.529	0.040	0.0877
HSC	95.95	21	12.32	0.85	0.92	0.91	0.719	0.012	0.0406
CSRD0247	88.66	21	8.85	0.91	0.89	0.88	0.631	0.023	-0.0168
McM0527	99.19	14	6.08	0.77	0.84	0.82	0.514	0.045	0.0408
SRCSRPO023	96.36	23	6.32	0.95	0.84	0.83	0.542	0.038	-0.0725
ILTS0087	98.79	14	6.21	0.78	0.84	0.82	0.513	0.045	0.0369
SRCSRPO05	98.79	15	8.97	0.82	0.89	0.88	0.633	0.023	0.0419
BM1258	97.98	18	9.85	0.72	0.90	0.89	0.662	0.019	0.1095
SRCSRPO024	90.28	18	5.04	0.74	0.80	0.78	0.456	0.061	0.0409
SRCSRPO008	99.60	21	6.42	0.71	0.84	0.83	0.537	0.041	0.0885
INRA063	95.14	8	3.68	0.69	0.73	0.68	0.316	0.119	0.0074
MAF0065	100.00	19	7.84	0.75	0.87	0.86	0.603	0.027	0.0787
Mean	96.65	17	7.01	0.78	0.84	0.83			

GR: genotyping rate Na: number of allele, Ne: Number of effective allele, Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphic information content, PE: probability of exclusion, PI: probability of identity, F(Null): null allele frequency

A total of 306 alleles were observed at 18 microsatellite loci. Allele numbers (Na) varied between 8 (INRA063) and 22 (OarFCB20 and SRCSRPO05) and the mean allele number (MNa) of 17.00. The mean value of the number of effective alleles (Ne) was 7.01. The polymorphic information content (PIC) value, which plays a crucial role in paternity testing by measuring the informativeness of genetic markers, was notably high (0.83) in the present study. The overall average of expected (Ho) and observed heterozygosity (He) values for all loci studied was 0.78 and 0.84, respectively.

When the individual exclusion probability belonging to microsatellite loci, a crucial parameter for paternity tests, was assessed, the lowest value was observed at the INRA063 locus (0.316), while the highest value was observed at the BM1258 locus (0.662). The PI value, also known as the probability of encounter, facilitates the determination of the number of individuals sharing the same DNA profile. In other words, it represents the likelihood of unrelated individuals having the same genotype in populations with random mating. In the study, the PI value ranged from 0.012 to 0.119.

When the presence of a null allele, which results in one allele not being amplified by Polymerase Chain Reaction (PCR) in heterozygous individuals, causing only one allele to peak as homozygous and leading to misinterpretation, was investigated, it was found that all microsatellites utilized in the study exhibited a null allele frequency of less than 20%.

In the study, microsatellite loci were combined based on their individual exclusion probability values to design various paternity test panels. This approach aimed to facilitate more practical and cost-effective paternity tests. Statistical findings for the paternity test panels are presented in Table 5.



Table 6. Paternity test panels based on individual exclusion probability values of microsatellites

Tablo 6. Mikrosatellitlerin bireysel dışlama olasılığı değerlerine göre oluşturulan babalık testi

Panel	NMP	MNa	MHe	MPIC	CPE	CPI
1	1	21.00	0.92	0.91	0.7188069	1.2E-02
2	2	19.50	0.91	0.90	0.9049682	2.3E-04
3	3	18.33	0.91	0.90	0.9658640	4.9E-06
4	4	17.50	0.90	0.89	0.9957674	9.4E-09
5	5	18.20	0.90	0.89	0.9953717	2.5E-09
6	6	18.33	0.89	0.88	0.9981643	6.8E-11
7	7	17.43	0.89	0.88	0.9992501	2.0E-12
8	8	18.13	0.89	0.88	0.9996808	6.4E-14
9	9	18.67	0.88	0.87	0.9998538	2.4E-15
10	10	18.90	0.88	0.87	0.9999322	9.9E-17
11	11	18.73	0.88	0.86	0.9999681	4.0E-18
12	12	18.58	0.87	0.86	0.9999846	1.8E-19
13	13	18.23	0.87	0.86	0.9999925	7.9E-21
14	14	17.93	0.87	0.85	0.9999964	3.5E-22
15	15	17.93	0.86	0.85	0.9999980	2.2E-23
16	16	17.44	0.86	0.84	0.9999988	1.6E-24
17	17	17.53	0.85	0.83	0.9999992	1.7E-25
18	18	17.00	0.84	0.83	0.9999995	2.1E-26

NMP: Number of microsatellites in the panel, MNa: mean number of alleles, MHe: mean expected heterozygosity, MPIC: mean polymorphic information content, CPE: combined probability of exclusion, CPI: combined probability of identity.

Among the panels formed based on the individual exclusion probabilities, Panel-1 exhibited the highest average number of alleles, while Panel-18 had the lowest. The highest He value was observed in Panel 1. It is observed that the PIC values are quite high in all microsatellite panels. In terms of combined probabilities of exclusion (CPE), the lowest value was obtained in Panel-1 (0.7188069), while the highest value was obtained in Panel-4 (0.9999964), as expected. Table 5 shows that the CPI value varies between 2.00×10^{-26} and 1.20×10^{-2} .

DISCUSSION and CONCLUSIONS

The MNa, Na, and Ne values obtained were higher than those reported in some related studies (Siwek and Knol, 2010; Al-Atiyat et al., 2015; Awobajo et al., 2015) and lower than the values reported in other studies (Agaoglu and Ertugrul, 2012; Murital et al., 2015; Gül et al., 2020; Demiray et al., 2024). It is thought that this difference may be attributed to variations in the number of samples, breeds, and microsatellites studied. In addition, these differences are believed to be the result of evolutionary processes such as geographical isolation, selection, and genetic drift in the breeds used in other studies. When the Na, Ne, MNa, and PIC values obtained in the study are analyzed, it is noteworthy that the microsatellites used exhibit a very high level of polymorphism. The polymorphic information content (PIC) values obtained in the study, which is an important criterion in the selection of microsatellites for paternity tests, are significantly higher than those reported in similar studies (Siwek and Knol, 2010; Guang-Xin et al., 2019; Whannou et al., 2023). This demonstrates that the microsatellites utilized in this study can be effectively employed in paternity tests. The He values obtained were higher than those reported in some studies (Awobajo et al., 2015; Guang-Xin et al., 2019; Whannou et al., 2023) and lower than in others (Gül et al., 2020; Demiray et al., 2024) across different goat breeds.

Individual probability of exclusion (PE) values, a crucial parameter in paternity tests, were found to be comparable to those reported in previous studies (Bolormaa et al., 2008; de Araújo et al., 2010; Siwek and Knol, 2010). It is known that microsatellites with high individual exclusion probability values identify father candidates more accurately. In this context, the findings indicate that the microsatellites utilized in this study can be reliably used in paternity tests. In contrast to the individual exclusion probability value, a high PI value indicates that there is more genetic similarity between the individuals examined, making it challenging to exclude non-father



candidates in paternity tests. Considering this situation, it can be said that PE and PI values are negatively correlated. When the findings are analyzed, this relationship becomes evident.

Dakin and Avise (2004) reported that null allele frequencies below 0.20 had no significant effect on paternity tests. When the null allele frequencies obtained for the microsatellites used in the study were analyzed, frequency values below the threshold value reported in the literature were found. This indicates that the loci utilized in this study can be safely used in paternity tests.

Considering the paternity test panels formed based on the individual exclusion probability values of microsatellites, it is noteworthy that all panels between Panel 7 and Panel 18 reach the minimum CPE value recommended in the literature (Luikart et al., 1999; Sherman et al., 2004; Van Eenennaam et al., 2007) for accurately identifying the true father. Increasing the number of microsatellites used in the panels naturally increases the combined exclusion probability values. However, working with fewer microsatellite loci will save time and costs. In this context, it has been revealed that paternity tests can be carried out in Hair goat populations at a lower cost, faster, and safer way using Panel-7 and Panel-8. These panels contain fewer microsatellites compared to others and provide sufficient exclusion probability values. If it is necessary to choose between these two panels, it is clear that Panel-8, which has a higher level of combined exclusion probability (CPE=0.9997), is the most suitable panel for paternity testing in Hair goat populations.

The National Genetic Improvement Project for Small Ruminants at Breeders' Conditions, initiated by the General Directorate of Agricultural Research and Policies for livestock breeding in Turkey, has taken an important step forward. It has been possible to initiate record-keeping habits in breeders' conditions and to make them widespread over time. Within the scope of the studies conducted in field conditions, the issue of verifying the accuracy of the results reported in hand-mating applications and correctly identifying the parents remains relevant.

In this study, affordable, quick, and dependable paternity test panels were introduced to assess the reliability of hand-mating in Hair goat populations with a high level of accuracy. The findings obtained from the study make a significant contribution and offer a perspective on controlling hand-mating practices. This issue is crucial within the National Genetic Improvement Project for Small Ruminants under Breeders' Conditions, coordinated by the General Directorate of Agricultural Research and Policies.

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Author contributions: All authors contributed equally to the preparation of the article.

Competing interests.: There is no conflict of interest between the authors in this study

Ethical statement: The Animal Experiments Local Ethics Committee of Aydın Adnan Menderes of Applied Sciences approved all the procedures performed in these studies; Approval no: 050-04/2012/103.

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