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Breeds Molecular Differentiation: Fine-Tuning of Microsatellite Marker Panels for Accurate Genetic Distinction in Autochthonous Balearic Dog Breeds

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SUMMARY

This study explores the genetic differentiation of autochthonous Balearic dog breeds through the fine-tuning of microsatellite marker panels. Microsatellite markers are utilized to unravel the genetic intricacies within populations, with each marker offering a unique molecular signature. The study investigates the efficacy of these markers in delineating distinct breeds within the Balearic canine population, providing insights into genetic diversity, informativeness, and deviations from Hardy-Weinberg equilibrium. The microsatellite markers examined form a molecular narrative, depicting the genetic tales of Balearic dog breeds. Results reveal specific markers with consistent values, indicating homogeneity within breeds, while others show variability, particularly in breeds with lower genetic diversity. The discussion emphasizes the selection of markers for a comprehensive panel, considering the nuances of genetic diversity in different breeds. The findings contribute valuable insights for breed management, conservation, and selection programs. Acknowledgments extend gratitude to breed associations for their support, enriching the study with essential data. Overall, this research refines our understanding of genetic dynamics in Balearic dog breeds, informing decisions for their sustainable management.

ADDITIONAL KEYWORDS
Balearic Islands.
Dog breeds diversity.
DNA microsatellites.
Genetic characterization.

Diferenciación Molecular de Razas: Ajuste Fino de Paneles de Marcadores Microsatélites para una Distinción Genética Precisa en Razas Autóctonas de Perros de Baleares

RESUMEN

Este estudio explora la diferenciación genética de las razas caninas autóctonas de las Islas Baleares a través del ajuste fino de paneles de marcadores microsatélites. Se utilizan marcadores microsatélites para desentrañar las complejidades genéticas dentro de las poblaciones, siendo cada marcador una firma molecular única. El estudio investiga la eficacia de estos marcadores en la delineación de razas distintas dentro de la población canina de las Islas Baleares, proporcionando información sobre diversidad genética, informatividad y desviaciones del equilibrio de Hardy-Weinberg. Los marcadores microsatélites examinados forman una narrativa molecular, representando las historias genéticas de las razas caninas baleares. Los resultados revelan marcadores específicos con valores consistentes, indicando homogeneidad dentro de las razas, mientras que otros muestran variabilidad, especialmente en razas con menor diversidad genética. La discusión enfatiza la selección de marcadores para un panel integral, considerando las sutilezas de la diversidad genética en diferentes razas. Los hallazgos contribuyen información valiosa para la gestión, conservación y programas de selección de razas. Los agradecimientos expresan gratitud a las asociaciones de razas por su apoyo, enriqueciendo el estudio con datos esenciales. En general, esta investigación perfecciona nuestra comprensión de las dinámicas genéticas en las razas caninas baleares, informando decisiones para su gestión sostenible.

PALABRAS CLAVE ADICIONALES
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Diversidad de razas caninas.
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INTRODUCTION

Microsatellite markers, also known as short tandem repeats (STRs), form a crucial component of genetic studies, offering unique insights into the diversity (González 2003) and relationships within populations (Martínez, Vega & Delgado 2019). In the realm of Balearic dog breeds, these microsatellite markers serve as genetic signposts, guiding researchers through the intricate landscape of canine genomes.

The list of microsatellite markers provided (Aguilera et al. 2022) encompasses a diverse set of loci, each with its own unique repetitive motif. These markers, such as AHT137, AHTh130, and others, are selected for their polymorphic nature (Piqueras 1993), where the number of repeated units varies among individuals. This polymorphism allows for the creation of distinct genetic profiles, turning each marker into a molecular signature for every dog.

Genetic diversity, a fundamental aspect of population health, is unveiled through the analysis of these markers. The average number of alleles and expected heterozygosity values provide a quantitative measure of the genetic richness within each breed (Levene 1949). The markers, acting as windows into the canine genome, reveal the unique genetic makeup of Balearic dog breeds.

Deviations from Hardy-Weinberg equilibrium (Raymond 1995) observed in some markers hint at the complex interplay of evolutionary forces within these populations. These deviations, whether due to selective pressures, genetic drift, or other factors, add layers of nuance to our understanding of the genetic dynamics at play.

Informativeness, measured through parameters like Polymorphic Information Content (PIC), underscores the utility of these markers in distinguishing between individuals (Serrote et al. 2020). Highly informative markers contribute significantly to the precision of genetic analyses, aiding in the delineation of genetic relationships and the identification of unique genetic traits within each breed.

Beyond the borders of individual breeds, these microsatellite markers weave a genetic tapestry that connects Balearic dog populations. They facilitate the examination of genetic relationships between breeds (Martínez, Vega & Delgado 2019), offering glimpses into shared ancestry and aiding conservation efforts for these distinct canine lineages.

In essence, the microsatellite markers listed stand as molecular storytellers (García et al. 2022), narrating the genetic tales of Balearic dog breeds. Their polymorphic melodies echo the diversity within each breed, their allelic variations paint portraits of individuality, and their deviations from equilibrium unveil the evolutionary dances shaping the genetic landscapes of these remarkable canine populations.

The primary objective of this study is to assess the efficacy of the employed microsatellite marker panel for the differentiation of distinct breeds within the Balearic canine population. This evaluation aims to rigorously scrutinize the selected panel's capacity to discern genetic variations and delineate breed-specific profiles. Through meticulous analysis, we seek to determine the discriminatory power, informativeness, and overall suitability of the microsatellite markers in elucidating breed-specific genetic characteristics. The findings of this assessment will contribute valuable insights into the reliability and precision of the utilized genetic panel for the purpose of breed differentiation within the Balearic dog breeds.

MATERIAL AND METHODS

GENETIC PROFILING OF BALEARIC DOG BREEDS: MICROSATELLITE MARKER INSIGHTS

The studies and reports performed by the Laboratory of Molecular Genetics of the AGR218 group of the University of Córdoba were used to summarize the information in regards the microsatellite marker panels used to evaluate genetic diversity in dog breeds. This set of Microsatellites is recommended by the International Society of Animal Genetics (ISAG). Table 1 reports information for the set of microsatellites used for the five breeds and breed agroupation autochthonous to the Balearic Archipelago.

UNIDIMENSIONAL TEST OF EQUALITY OF THE MEANS OF THE CLASSES:

A unidimensional test of equality of means was performed to evaluate whether there were significant differences in the averages of a set of specific microsatellite markers among distinct breeds—Ca de Bestiar, Ca de Bou, Podenco Ibicenco or Ibicean Hound, Ca Rater, Ca Mè, and Ca de Conills—. Unidimensional test of equality of means within the ANOVA package of the Modelling data Menu of XLSTAT Version 2014.5.03.

RESULTS

Table I presents a summary of the information about microsatellite markers available accross Balearic dog breeds and breed agroupation.

Table II presents the results of unidimensional test of equality of means. The table presents the results of a microsatellite marker analysis, with Lambda (λ), F-statistic (F), and Rank values for each marker. The first group of markers, including AHT137, AHTh130, and others, demonstrates consisten (Goleman et al. 2019)t Lambda and F-statistic values of 0.9664 and 0.9183, respectively, resulting in a top rank of 1. This suggests a high degree of similarity or uniformity in these markers. In the second group, markers like AHTK211 and ATH121 exhibit slightly higher Lambda values (0.9735 to 0.9768) and lower F-statistic values (0.7174 to 0.6277), earning them a rank of 2. The third group, led by AHTK211 with a Lambda of 0.9794, shows a gradual decrease in Lambda and F-statistic values, earning a rank of 4. The last group, characterized by markers such as AHT137 and AHTh130, demonstrates the highest Lambda values (0.9853) and the lowest F-statistic values (0.3947), resulting in a collective rank of 5. These findings suggest potential variations in genetic markers across different groups, providing valuable information for genetic diversity studies.

DISCUSSION

The consistent Lambda (λ) and F-statistic (F) values in the first group of markers suggest a uniform genetic profile among breeds like Ca de Bestiar (CB). The top rank of 1 indicates that these markers can be considered homogenous across this breed (Kardos et al. 2016), offering a potential set of stable markers for genetic characterization. This homogeneity is particularly valuable when aiming to assess high genetic diversity within the Ca de Bestiar breed (Méndez et al. 2011). The low intraracial genetic differentiation (FIS) of 0.021 suggests minimal deviation from Hardy-Weinberg Equilibrium (HWE), further supporting the reliability of these markers for genetic studies (Garrido et al. 1999).

Table I. Microsatellite markers summary of information accross Balearic dog breeds and breed agroupation.

Breed/Breed Agroupation	Microsatellites	Alleles (Average)	Allelic Range	Highest Heterozygosity	Lowest Heterozygosity	Observed Heterozygosity Range	PIC Informativeness	Deviations from HWE	Intraracial Genetic Differentiation (FIS)	Remarks
Ca de Bestiar (CB)	21	7.05	4 to 11	AHTh130 (0.855)	FH2848 (0.559)	0.563 to 0.915	All markers highly informative, except REN247M23 (moderate, PIC = 0.481)	5 markers	Average FIS = 0.021	High genetic diversity
Ca de Bou (CBOU)	21	5.05	2.5 to 10	CXX279 (0.797)	REN162C04 (0.091)	0.0094 to 0.810	All markers highly informative, except AHTH253, INU030, REN247M23, and REN54P11	-	Average FIS = 0.081	Low genetic diversity
Podenco Ibicenco (PI)	21	5.75	4 to 11	AHTh260 (0.829)	FH2848 (0.348)	0.177 to 0.864	All markers highly informative, except FH2848 and REN54P11	-	Average FIS = 0.200	Moderate genetic diversity
Ca Rater (CR)	33	6.61	2 to 15	0669RD (0.866)	1055RD (0.255)	0.161 to 0.964	All markers highly informative, except INU055	Four markers	Average FIS = 0.044	Moderate genetic diversity
Ca Mè (CM)	21	6.5	4 to 11	AHTh171 (0.859)	INU030 (0.428)	0.372 to 0.824	All markers highly informative, except INU005 and INU030	None	Average FIS = 0.038	Moderate genetic diversity
Ca de Conills (CC)	21	6.60	4 to 10	AHTh121 (0.857)	REN247M23 (0.477)	0.415 to 0.806	All markers highly informative, except REN247M23	Ten markers	Average FIS = 0.048	Moderate genetic diversity

Table II. Summary of results of unidimensional test of equality of means for the muicrosatellite markers used to assess genetic diversity in dog breeds.

Microsatellite Marker	Lambda	F	Rank
AHT137	0,9664	0,9183	1
AHTh130	0,9664	0,9183	1
AHTh171	0,9664	0,9183	1
AHTh260	0,9664	0,9183	1
FH2848	0,9664	0,9183	1
INRA21	0,9664	0,9183	1
INU030	0,9664	0,9183	1
INU005	0,9664	0,9183	1
ATH121	0,9664	0,9183	1
FH2054	0,9664	0,9183	1
INU055	0,9664	0,9183	1
AHTK253	0,9664	0,9183	1
REN54P11	0,9664	0,9183	1
REN64E19	0,9664	0,9183	1
CXX279	0,9664	0,9183	1
REN105L03	0,9664	0,9183	1
REN162C04	0,9664	0,9183	1
REN169D01	0,9664	0,9183	1
REN169O18	0,9664	0,9183	1
REN247M23	0,9664	0,9183	1
AHTK211	0,9735	0,7174	2
ATH121	0,9735	0,7174	2
AHT121	0,9768	0,6277	3
0669RD	0,9768	0,6277	3
0123RD	0,9768	0,6277	3
0176RD	0,9768	0,6277	3
0323RD	0,9768	0,6277	3
0914RD	0,9768	0,6277	3
0959RD	0,9768	0,6277	3
1055RD	0,9768	0,6277	3
1257RD	0,9768	0,6277	3
1404RD	0,9768	0,6277	3
1878RD	0,9768	0,6277	3
2469RD	0,9768	0,6277	3
2642RD	0,9768	0,6277	3
AHTK211	0,9794	0,5540	4
AHT137	0,9853	0,3947	5
AHTh130	0,9853	0,3947	5
AHTh171	0,9853	0,3947	5
AHTh260	0,9853	0,3947	5
FH2848	0,9853	0,3947	5
INRA21	0,9853	0,3947	5
INU030	0,9853	0,3947	5
FH2054	0,9853	0,3947	5
INU005	0,9853	0,3947	5
INU055	0,9853	0,3947	5
REN54P11	0,9853	0,3947	5

Moving to the second group, markers like AHTK211 and ATH121 exhibit slightly higher Lambda values and lower F-statistic values, resulting in a rank of 2. These markers may serve as indicators of variability within breeds like Ca de Bou (CBOU), characterized by low genetic diversity (Hauser, Athrey & Leberg 2021). The deviation from HWE (average FIS = 0.081) in this breed emphasizes the importance of selecting informative markers that capture specific genetic characteristics, especially when dealing with populations showing lower diversity.

The third and fourth groups, showing a gradual decrease in Lambda and F-statistic values, may be suitable for breeds such as Ca Mè (CM) and Ca de Conills (CC). These breeds exhibit moderate genetic diversity, and the informative markers identified can be instrumental in understanding the underlying genetic structure. The average FIS values of 0.038 and 0.048 for Ca Mè and Ca de Conills, respectively, suggest a moderate level of intraracial genetic differentiation.

The last group, with the highest Lambda values and lowest F-statistic values, is associated with breeds like Podenco Ibicenco (PI), where moderate genetic diversity is observed. The markers in this group may play a crucial role in capturing the unique genetic variations within this breed, as indicated by the observed heterozygosity range (0.177 to 0.864) and average FIS of 0.200 (Koskinen & Bredbacka 2000) .

Combining the information from both tables (Tables I and II) reveals insights into selecting markers for a comprehensive panel to assess genetic diversity in dog breeds. In the microsatellite marker analysis, the first group of markers, including AHT137, AHTh130, and others, consistently demonstrated Lambda and F-statistic values, making them promising candidates for inclusion in a genetic diversity panel (Scientific & Haeringen 2019). These markers, with a rank of 1, indicate a high degree of uniformity and similarity across breeds like Ca de Bestiar. In contrast, markers in the second group, such as AHTK211 and ATH121, exhibited slightly higher Lambda values and lower F-statistic values, earning them a rank of 2. These markers may capture subtle variations within breeds like Ca de Bou, emphasizing the need for their inclusion in a panel designed for breeds with lower genetic diversity (Sams et al. 2020). However, caution should be exercised in considering markers from the third and fourth groups, characterized by a gradual decrease in Lambda and F-statistic values, as well as the highest Lambda values and lowest F-statistic values. While these markers may be less informative for assessing genetic diversity, they could still offer insights into breed-specific characteristics. Careful selection and combination of markers from different groups would contribute to the creation of a tailored panel (Radko, Rubiś & Szumiec 2018), ensuring a nuanced evaluation of genetic diversity in various dog breeds.

CONCLUSIONS

In conclusion, the microsatellite marker analysis provides a nuanced understanding of genetic diversity across different breeds. Tailoring panels based on the specific characteristics of each breed allows for more accurate and targeted genetic studies, providing valuable insights for breed management, conservation, and selection programs. The information derived from such analyses contributes significantly to the field of animal genetics and aids in making informed decisions for the sustainable management of diverse breeds.

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