Biotecnia
Journal of biological and health sciences
http://biotecnia.unison.mx
Universidad de Sonora
ISSN: 1665-1456

Original Article

Whole-Genome Characterization and Analysis of Microsatellites in Domestic Turkey (*Meleagris gallopavo*) through in silico Approach

Caracterización del genoma completo y análisis de microsatélites mediante un enfoque in silico en pavos domésticos (*Meleagris gallopavo*)

Eman Gobily[1]✉, Shimaa Kandel[1]✉, Hadeer Auoda[1]✉, Radwa Ahmed[1]✉, Mostafa Helal[2]*✉

[1] Programa de Biotecnología, Facultad de Agricultura, Universidad de El Cairo, Giza, Egipto
[2]Departamento de Producción Animal, Facultad de Agricultura, Universidad de El Cairo, Giza, Egipto

ABSTRACT

The domestic Turkey (*Meleagris gallopavo*) is the second largest contributor to poultry meat production, following chickens. The study of microsatellite organization and distribution is highly important in genomics and evolutionary studies. The *in silico* mining for microsatellites leverages the power of computational biology to streamline, enhance the discovery of microsatellite markers and reduce the cost of microsatellite detection. The present study aimed to evaluate *in silico* mining for microsatellite loci in the genome of domestic turkey. Reference sequences of several chromosomes were obtained from NBCI and analyzed using Krait software. Chromosome 4 had the highest number of perfect microsatellites, while chromosome 18 had the lowest number. However, chromosome 27 had the highest relative abundance, followed by chromosome 13. Chromosome 18 again had the lowest relative abundance. Chromosome 4 had the most imperfect microsatellites and chromosome 18 had the least. A total of 121,248 microsatellite primers were designed. These microsatellite loci and markers will play important roles as instrumental in linkage mapping and will significantly enhance research on turkey genetics.

Keywords: *In silico*, *Meleagris gallopavo*, Microsatellites, Motifs, Turkeys

RESUMEN

El pavo doméstico (*Meleagris gallopavo*) es el segundo mayor contribuyente a la producción de carne de aves de corral, después de los pollos. El estudio de la organización y distribución de microsatélites es muy importante en los estudios genómicos y evolutivos. La minería *in silico* de microsatélites aprovecha el poder de la biología computacional para agilizar, mejorar el descubrimiento de marcadores de microsatélites y reducir el costo de la detección de microsatélites. El presente estudio tuvo como objetivo evaluar la minería *in silico* de loci de microsatélites en el genoma del pavo doméstico. Se obtuvieron secuencias de referencia de varios cromosomas de NBCI y se analizaron utilizando el software Krait. El cromosoma 4 tuvo el mayor número de microsatélites perfectos, mientras que el cromosoma 18 tuvo el menor número. Sin embargo, el cromosoma 27 tuvo la mayor abundancia relativa, seguido por el cromosoma 13. El cromosoma 18 nuevamente tuvo la menor abundancia relativa. El cromosoma 4 tuvo la mayor cantidad de microsatélites imperfectos y el cromosoma 18 tuvo la menor cantidad. Se diseñaron un total de 121.248 cebadores de microsatélites. Estos loci y marcadores microsatélites desempeñarán un papel importante en el mapeo de ligamiento y mejorarán significativamente la investigación sobre la genética del pavo.

Palabras clave: In silico, *Meleagris gallopavo,* microsatélites, motivos, pavos.

INTRODUCTION

Since the domestication of turkey (*Meleagris gallopavo*) in the Southwestern United States and Mexico (Thornton *et al*., 2012; Vergara *et al*., 2019), it has been considered one of the major important poultry species that contributes to meat production worldwide (Aslam *et al*., 2011). The United States is the leading country in turkey's meat intake, followed by Brazil and Germany, which accounted for 41, 8.1, and 8 % of the total intake of turkey meat (Hristakieva, 2021). Nevertheless, turkey meat still shares a small proportion of global meat demand. According to FAO, turkey meat production ranked second (5 %), after chicken (90 %), of the global poultry meat production. The environmental and ethical concerns surrounding industrial animal agriculture have become increasingly evident. Therefore, the intake pattern of meat-based proteins is projected to be reshaped significantly by 2030. While ruminants are a major contributor to greenhouse gas emissions (Giamouri *et al*., 2023), turkey production, once viewed as a promising alternative to traditional livestock, is also expected to decrease emissions in the coming decade (Kheiralipour *et al*., 2024; Clauss *et al*., 2020).

Generally, avian genomes are interesting because they tend to be compact, with less DNA overall, yet packed into more chromosomes compared to mammals (Axelsson *et al*., 2005). Turkey's genome is quite larger than chicken and consists of 1,115,474,681 bp, with 16,226 coding genes, and 30,708 gene transcripts (Dalloul *et al*., 2010). The genome of the turkey is not fully uncovered, and massive efforts are needed to be fully understood (Barros *et al*., 2023). For many decades, microsatellites (also called Short Sequence Repeats, SSRs) were the markers of choice for breeders and geneticists, as they were used for many purposes including the conservation of genetic resources (Olubunmi, 2019). Microsatellite loci are scattered throughout the genome in both coding

*Author for correspondence: Mostafa Helal
e-mail: mostafa.helal@agr.cu.edu.eg
Received: October 1, 2024
Accepted: February 4, 2025
Published: February 28, 2025

and non-coding regions. Certain repeats are preferred and are often predominant in certain genomic locations. However, the significance of this observation is unclear (Vieira *et al*., 2016). Microsatellite loci are among the variable types of DNA within the genome, and the changes in their polymorphisms derive mainly from changeability in length instead of within the essential arrangement (Abdul-Muneer, 2014). A more profound understanding of the developmental and mutational properties of microsatellites is in this manner required, not as it were to get it how the genome is organized, but moreover to accurately utilize microsatellites information in populace inheritance of important traits (Wöhrmann and Weising, 2011).

In the past few decades, there has been significant effort focused on the development of microsatellites in the genome of turkeys. In 1999, several turkey genomic libraries were constructed, and 50 microsatellite loci were characterized (Huang *et al*., 1999), followed by the construction of a linkage map contacting 74 markers (Burt *et al*., 2003), and arranging 314 microsatellite loci in 29 linkage groups. The latter resulted in the identified of ~800 microsatellite markers (Reed *et al*., 2007). Recently, a set of 34 microsatellites was identified (Canales Vergara *et al*., 2020), and successfully used to estimate genetic diversity parameters in 10 domestic turkey populations.

Given the high cost, labor-intensive nature, and limited scalability of developing microsatellite markers, *in silico* approaches present a valuable alternative, offering faster and more comprehensible insights into target genomes. Therefore, this study aimed to perform an *in silico* analysis of the whole-genome sequence of the turkey (*Meleagris gallopavo*) mining it to identify a panel of microsatellite loci, and explore the distribution and density of microsatellites within the turkey genomes.

MATHEMATICAL MODEL

Data source. Sequence data of the domestic turkey chromosomes were obtained from the National Center for Biotechnology Information (NCBI). The analyzed reference sequences were uploaded to NCBI in 2019, with a reference of Turkey_5.1 (GCA_000146605.4).

***In silico* mining of whole-genome-wide SSRs.** The sequence data were downloaded in FASTA format. The Krait software v.1.1.0 (Du *et al*., 2018) was used for microsatellite mining. Krait software is based on several data mining algorithms for microsatellite detection. It uses pattern recognition to identify repeat sequences within genomic data, sequence alignment to compare these sequences against databases for accuracy, and also conducts statistical analysis to calculate frequency and distribution of microsatellites across chromosomes. The authors selected to use Krait for the analysis because it is an ultrafast tool with a user-friendly graphical interface, making it ideal for genome-wide microsatellite analysis. Additionally, Krait is a powerful tool that not only detects various types of microsatellites (both perfect and imperfect) but also assists in designing primers for them. This makes it ideal for efficiently identifying and defining valuable microsatellite markers. The analysis was carried out based on to the following criteria: mono-nucleotide repeat motifs were required to have at least of 10 repeats, di-nucleotide repeat motifs were at least 7 repeats, tri-nucleotide repeat motifs at least 5 repeats, and tetra-, penta-, and hexa-nucleotide repeat motifs at least 4 repeats.

The Primer3 tool (Rozen and Skaletsky, 1999) integrated within the Krait software package was used to design primers for the identified microsatellite markers. Primer3 uses empirical formulas to calculate the melting temperature of potential primers to select suitable Tm ranges. Additionally, Primer3 checks the primer specificities by aligning the primers against target sequences in order to minimize non-specific binding. The program also assesses the primer lengths and GC contents regarding optimal annealing and stability (Untergasser *et al*., 2012).

The total numbers obtained were normalized either as a percentage or as the number of SSRs per megabase (Mb) of sequence, enabling comparison across genome sequences of different sizes such as relative abundance. The estimated repeat density (base pairs per Mb) was obtained by dividing the total number of base pairs occupied by SSRs by the total genome size. Correlation coefficients between different SSR-related parameters were estimated using the software SPSS (Morgan *et al*., 2019).

RESULTS

The domestic Turkey is one of the most important poultry species, with a large genome consisting of 1,061,817,103 base pairs. A total of 30 autosomal and two sexual chromosomes were analyzed. The total sequence length was 1,115,474,681, and the total unmapped length was 1,080,180,254, with scaffolds of 187,695. As shown in Figure 1, the largest chromosomes of the turkey genome are chromosomes 1, 2, and 3, followed by the Z chromosome. In contrast, chromosome 18 is the smallest autosomal chromosome measuring 244,177 bp.

Perfect microsatellites. Table 1 presents the number of perfect microsatellites detected in different chromosomes. Interestingly, the number of perfect SSRs did not correlate with chromosome size. The highest number of perfect microsatellites (16743) was found in chromosome 4, despite not being a large chromosome (74,864,452 bp, and it ranked 5th in size within the turkey genome). The second highest numbers were detected on chromosomes 8, 1, 15, and Z, respectively. However, significant positive correlation coefficients were observed between chromosome size and both total number (0.44) and total length (0.457) of perfect microsatellites as shown in Table 2. Conversely, the lowest number of perfect microsatellite was detected in chromosome 18, where only one microsatellite was found. No microsatellites were detected in chromosome W nor mitochondrial DNA.

Table 1 also shows the total length of perfect SSRs with the highest value for chromosome 4, due to the large number of microsatellites detected. This was followed by

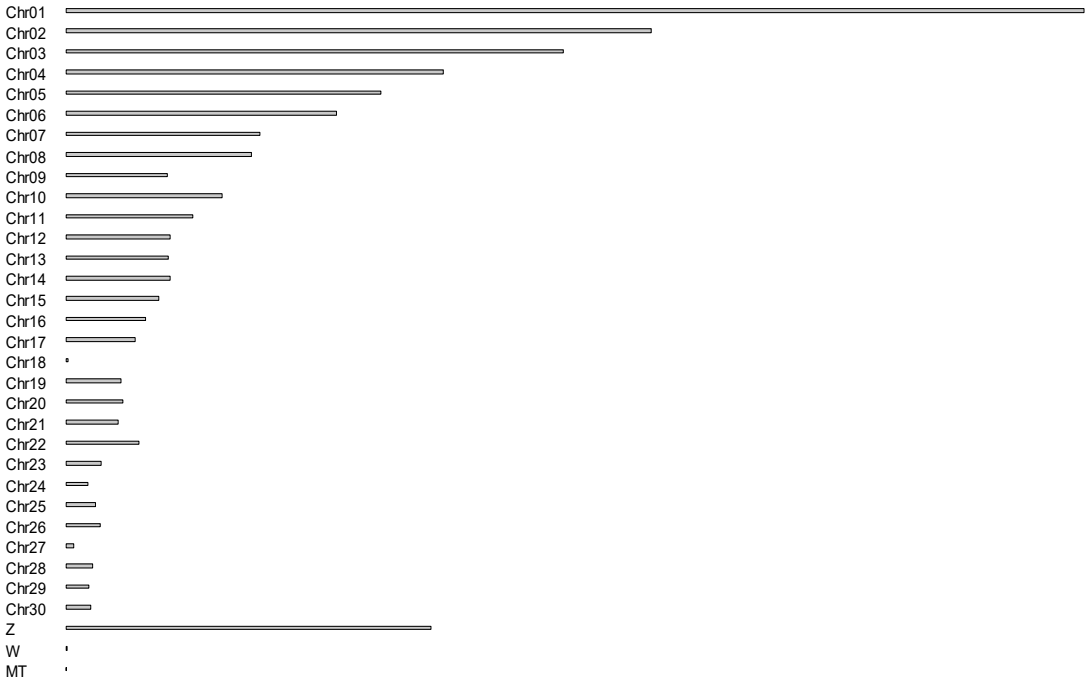

Fig. 1. Ideogram of the turkey genome
Fig. 1. Ideograma del genoma del pavo.

chromosomes 8 and 1, respectively. Figure 2 illustrate the relative abundance of perfect microsatellites across all chromosomes. Chromosome 27 exhibited the highest relative abundance with a value of 285.5, followed by chromosome 13 at 262.4. In contrast, chromsome 18 showed the lowest relative abundance, primarily due to the low number of microsatellites detected on that chromosome. This was followed by chromosome 24 with a relative abundance of 102.68.

The estimated repeat density values (bp/Mb) in each chromosome are shown in Figure 3. The repeat density pattern closely mirrors the pattern of relative abundance, with chromosome 27 and 13 exhibiting the highest repeat densities. In contrast, chromosome 18 had the lowest repeat density.

The overall distribution of SSR repeat types is presented in Figure 4, mononucleotide repeats were highly frequent, accounting for 63% of the total detected SRRs, followed by tetranucleotide repeats (14%), and dinucleotide (11%). Trinucleotide, pentanucleotide, and hexanucleotide repeats were less common, each making up less than 10% of the total. The number of imperfect microsatellite repeats detected per chromosome is presented in Table (3). Notably, only one mononucleotide microsatellite was detected in chromosome 18. Excluding chromosome 18, the highest number of mononucleotide microsatellite was detected in chromosomes 4, while the lowest was detected on chromosome 27. Chromosome 4 also had the highest dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats. In contrast, chromosomes 28, 29, and 27 had the lowest numbers of dinucleotide, trinucleotide, and tetranucleotide repeats, respectively.

Imperfect microsatellites. Table 1 presents the number of imperfect microsatellites detected across different chromosomes. The highest number (68202) was detected in chromosome 4 (Figure 2). The second highest counts were detected on chromosomes 8, 1, 15, and Z, respectively. On the other hand, chromosome 18 had the lowest count with only 15 imperfect microsatellites detected. These results have a similar trend to those obtained for perfect microsatellites.

Table 1 also presents the total length of imperfect SSRs with the highest value observed on chromosome 4, due to the large number of microsatellites detected. This was followed by chromosomes 8 and 1, respectively. Figure 2 depicts the relative abundance of imperfect microsatellites across all chromosomes. Chromosome 27 exhibited the highest relative abundance reaching 1,113.54, followed by chromosome 13 at 1,030.26. In contrast, chromosome 29 had the lowest relative density. Significant positive correlation coefficients were obtained between chromosome size and both total number (0.432) and total length (0.441) of imperfect microsatellites as shown in Table 2. Similar to perfect microsatellites, no imperfect microsatellites were obtained in chromosome W or in the mtDNA.

The estimated repeat density values (bp/Mb) of each chromosome are shown in Figure 3. The pattern of repeat density closely mirrors that of relative abundance, with chromosomes 27 and 13 exhibiting the highest repeat densities. In contrast, chromosome 18 had the lowest repeat density.

The overall distribution of the type of detected imperfect SSR repeats is presented in Figure 4. Mononucleotide repeats were the most frequent and accounting for 42 % of the total SSRs detected, followed by trinucleotide repeat (29

Table 1. The summary information of different microsatellite types.
Tabla 1. Resumen de información de diferentes tipos de microsatélites.

Chr	Perfect SSR				Imperfect SSR			
	Total number of perfect SSRs	Total length of perfect SSRs	Relative abundance	Relative density	Total number of imperfect SSRs	Total length of imperfect SSRs	Relative abundance	Relative density
1	4610	80986	246	4320	18445	546084	983.9	29128
2	2131	34285	196	3156	9669	261983	889.9	24113
3	2216	38855	240	4216	9061	269405	983.2	29234
4	16743	271480	240	3896	68202	1939960	978.7	27837
5	1120	17626	192	3017	5264	144004	901	24649
6	1129	18470	226	3690	4817	135581	962.4	27090
7	320	5459	180	3079	1482	41222	835.9	23249
8	6927	109015	203	3195	31039	847657	909.6	24839
9	184	3597	148	2891	1103	31471	886.5	25294
10	642	10265	223	3570	2733	77480	950.5	26945
11	177	3167	153	2740	934	25310	808.1	21898
12	174	2969	148	2531	1134	31925	966.6	27211
13	249	4202	263	4436	976	27770	1030	29314
14	164	2877	172	3017	755	21227	791.7	22260
15	2960	48817	173	2850	15055	409929	878.8	23929
16	225	3465	152	2339	1247	32134	841.9	21695
17	190	3166	210	3506	792	21741	877	24075
18	1	12	61.9	742.4	15	498	928.1	30811
19	89	1393	178	2790	448	12460	897.1	24952
20	66	1034	125	1954	370	9806	699.3	18534
21	86	1622	178	3360	470	13679	973.5	28332
22	81	1386	121	2075	545	14215	815.9	21279
23	128	1880	193	2831	619	15498	932.1	23338
24	371	7135	103	1975	3300	90183	913.3	24959
25	102	1876	183	3363	549	17922	984.2	32130
26	101	1639	158	2567	547	14707	856.6	23031
27	20	337	286	4811	78	1907	1114	27225
28	31	530	125	2138	204	5655	822.8	22807
29	36	634	179	3156	128	3587	637.2	17856
30	42	655	189	2941	224	6056	1006	27193
Z	1190	20969	187	3299	5814	168594	914.6	26521

Table 2. Correlation coefficient of chromosome size with total number and total length of perfect and imperfect SSRs
Tabla 2. Coeficiente de correlación del tamaño de los cromosomas con el número total y longitud de SSRs perfectos e imperfectos.

Variable 1	Variable 1	Correlation coefficient	
		Perfect SSR	Imperfect SSR
Total number of SSRs	Chromosome Size	0.440	432
P <		0.013	0.010
Total length of SSRs	Chromosome Size	0.475	0.441
P <		0.010	0.013

%), dinucleotide (15 %), and tetranucleotide (10 %). Pentanucleotide and hexanucleotide repeats were less frequent, each accounting for less than 5 %. The number of imperfect microsatellite repeats detected per chromosome is shown in Table 3. The highest numbers of mononucleotide microsatellites was detected on chromosomes 8, while the lowest was found on chromosome. Similar to the results obtained for perfect microsatellites, the highest numbers of dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats were observed in chromosome 4. However,

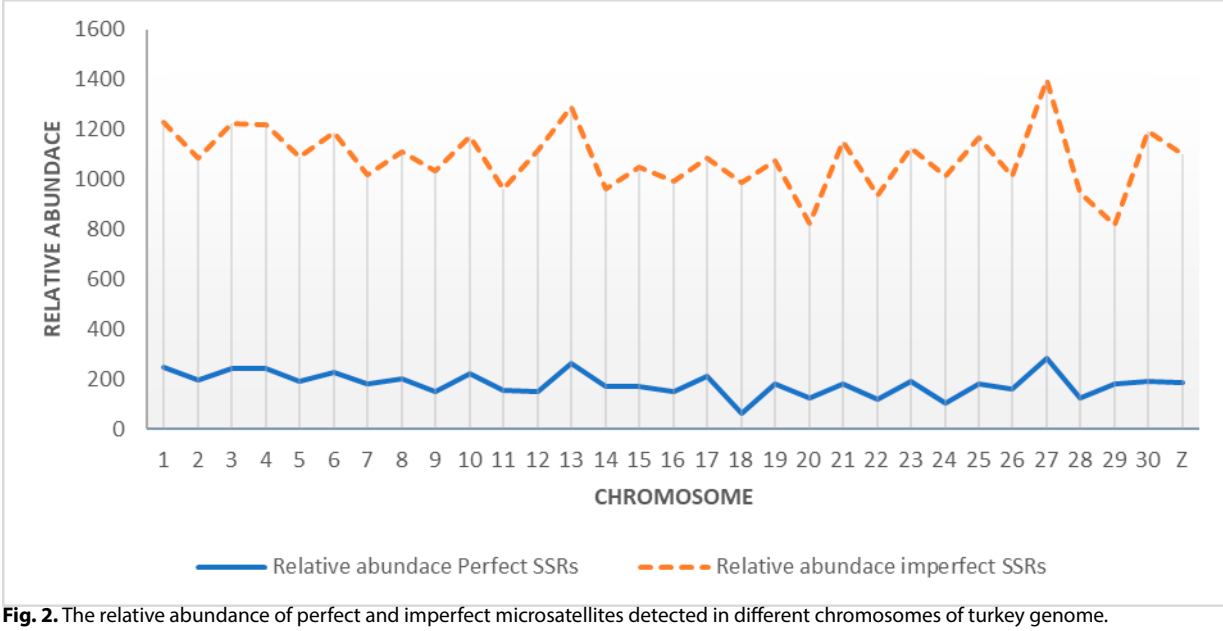

Fig. 2. The relative abundance of perfect and imperfect microsatellites detected in different chromosomes of turkey genome.
Fig. 2. Abundancia relativa de los microsatélites perfectos e imperfetos detectados en diferentes cromosomas del genoma del pavo.

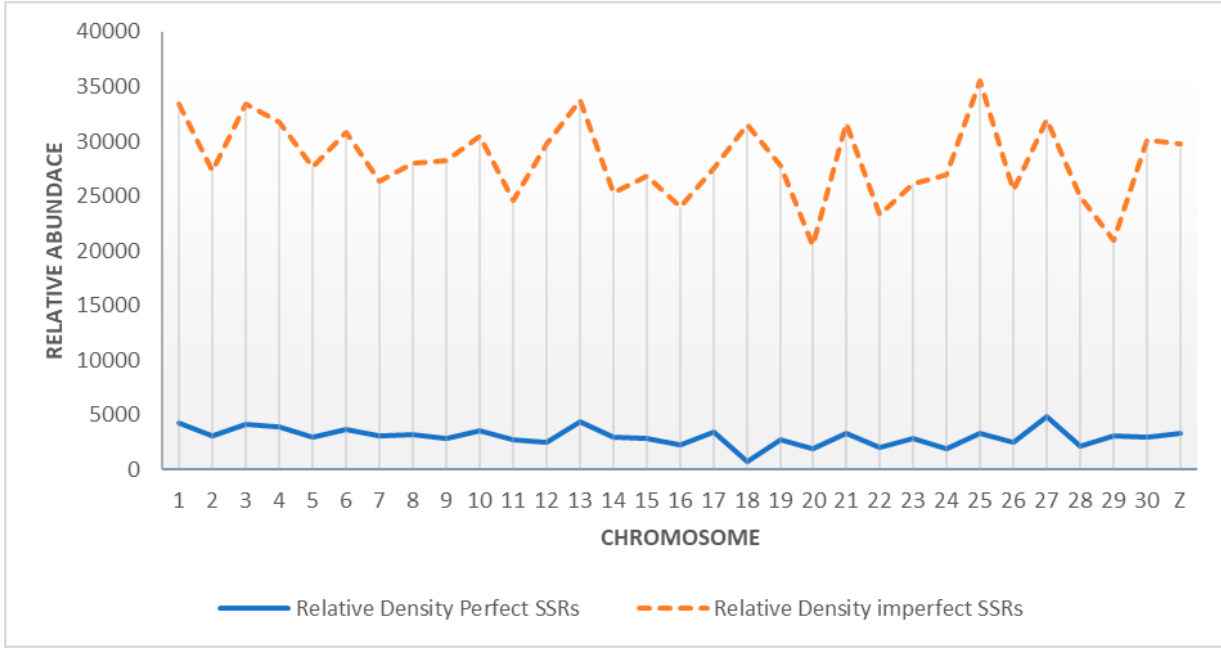

Fig. 3. The repeat density of perfect and imperfect microsatellites detected in different chromosomes of turkey genome.
Fig. 3. Densidad de repetidos perfectos e imperfectos de microsatélites detectados en diferentes cromosomas del genoma del pavo.

chromosome 18 had the lowest numbers for dinucleotide, trinucleotide, and tetranucleotide repeats.

Designed Primers. A total of 121248 SSR primers were designed. A list of these primers has been deposited in a public repository and can be accessed via the following link: (https://github.com/mosthamed/SSR-primers-Meleagris-gallopavo-.git).

DISCUSSION

Genome-wide studies offer valuable insights into the evolutionary forces that shape the distribution and diversity of microsatellites (Pannebakker *et al*., 2010), enhancing our understanding of genome architecture. Microsatellites are a significant component of the genome in all organisms, which their abundance closely correlating to genome size (Akemi *et al*., 2012). However, the biological significance of this genomic regions remains poorly understood . A thorough analysis of microsatellites is essential for uncovering their functional roles (Gochi *et al*., 2023). Variations in their abundance, variation and repeat types are key factors that contribute to their functions. This study presents a genome-wide analysis of microsatellite distribution in the turkey genome.

Table 3. The distribution of microsatellite repeats on the different chromosomes in turkey genome.
Tabla 3. Distribución de repetidos de microsatélites en los diferentes cromosomas del genoma del pavo.

	Motif											
	Perfect SSR						*Imperfect SSR*					
Chr	Mono	Di	Tri	Tetra	Penta	Hexa	Mono	Di	Tri	Tetra	Penta	Hexa
1	2640	452	370	820	256	72	7776	2804	4995	2150	570	150
2	1444	225	161	233	63	5	3954	1428	3083	934	225	45
3	1249	233	185	380	133	36	3860	1361	2404	1048	302	86
4	10960	1707	1184	2252	555	85	31205	9709	18009	7148	1713	418
5	764	110	72	141	31	2	2173	759	1648	539	124	21
6	746	126	71	132	49	5	2159	745	1302	442	128	41
7	193	38	37	25	22	5	508	208	553	142	55	6
8	4661	731	498	833	182	22	13208	4551	9348	3094	680	158
9	101	22	18	26	16	1	299	152	512	102	35	3
10	425	65	43	88	19	2	1130	392	831	292	78	10
11	109	19	7	31	7	4	341	129	339	94	25	6
12	93	28	18	20	13	2	279	169	553	74	51	8
13	155	24	22	34	12	2	445	116	270	116	23	6
14	90	15	19	27	10	3	237	116	284	79	27	12
15	1926	315	242	355	118	24	5526	2172	5476	1429	365	87
16	157	23	18	20	6	1	492	168	435	119	29	4
17	108	33	16	25	8	0	277	143	279	71	19	3
18	1	0	0	0	0	0	4	0	8	2	1	0
19	64	6	7	8	4	0	177	67	148	41	13	2
20	41	8	6	9	1	1	107	71	139	44	7	2
21	45	8	13	14	4	2	126	61	215	45	21	2
22	47	15	3	10	4	2	119	100	265	45	13	3
23	93	15	7	12	1	0	261	90	208	51	9	0
24	17	197	64	57	28	8	1130	452	1304	297	94	23
25	43	9	17	17	13	3	139	76	242	48	32	12
26	63	15	9	11	3	0	174	93	206	52	17	5
27	4	9	4	2	1	0	15	23	30	6	4	0
28	17	4	3	4	2	1	48	34	95	19	7	1
29	22	5	1	7	1	0	46	23	38	19	2	0
30	27	4	4	6	1	0	96	31	62	27	8	0
Z	607	137	101	236	97	12	2314	905	1670	676	201	48

Compared to traditional methods of microsatellite identification, *in silico* genome mining offers several advantages, making it a preferred approach in modern genomics research. The *in silico* approach is highly efficient and cost-effective, allowing for large genomes to be scanned for potential microsatellite regions without the need for extensive wet lab experiments. By leveraging computational tools and databases, vast amounts of data can be generated quickly. This approach is particularly valuable in fields such as biodiversity studies, conservation genetics, and breeding programs. Additionally, the precision of computational algorithms ensures high accuracy in marker identification, reducing the risk of errors that can occur with manual methods (Safaa *et al*., 2023).

In the current study, we examined the distribution of perfect microsatellites across different chromosomes. The

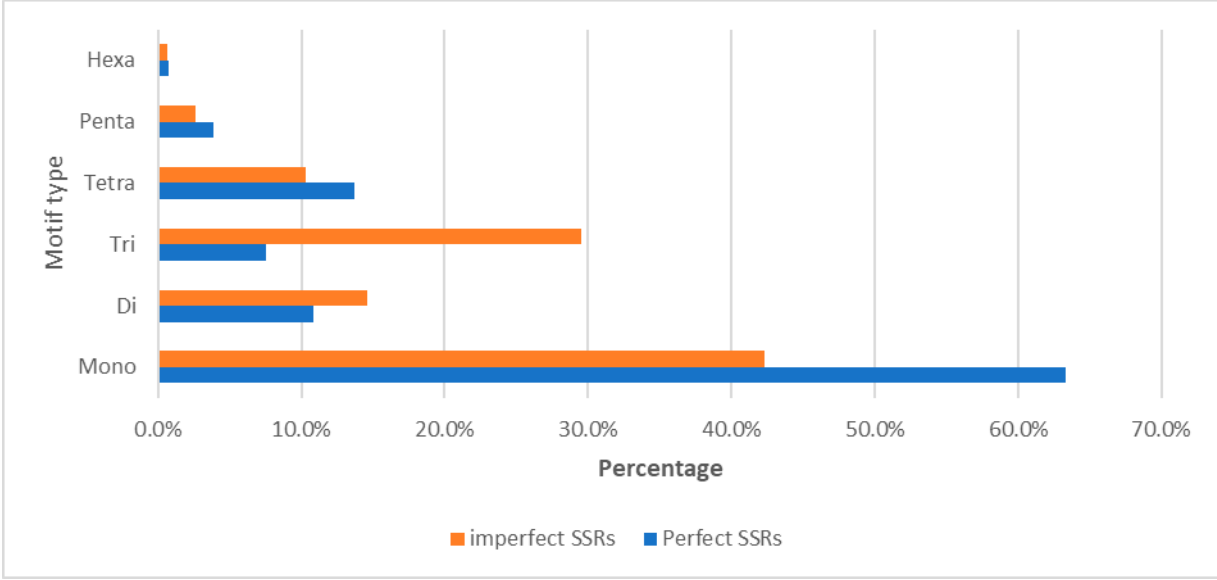

Fig. 4. The frequency of the detected perfect and imperfect SSRs in turkey genome.
Fig. 4. Frecuencia detectada de los SSRs perfectos e imperfectos en el genoma del pavo.

data revealed no correlation between the number of perfect SSRs and chromosome size. Notably, chromosome 4, which ranks fifth in size in the turkey genome, has the highest number of perfect microsatellites. Chromosomes 8, 1, 15, and Z also showed high numbers of SSRs. Similarly, chromosome 4 also exhibited the highest number of imperfect microsatellites. Previous studies (Zhao *et al*., 2011; Duhan *et al*., 2023) have generally found that microsatellite density increases with genome size.

To better understand this trend, we calculated the correlation coefficients between chromosome size and both the total number and total length of detected microsatellites for the two types. Moderate positive correlations were observed, suggesting that the abundance of SSRs can vary widely across animal species, of which mammals tend to have more SSRs than avian species due to the differences in chromosome size. However, further studies should investigate the relationships between the number of chromosomes and the SSR number.

Previous research has reported different levels of correlations between genome size and the number of detected SSRs. For example in insects, the number of SSRs is positively correlated with genome size, with a correlation coefficient of 0.499, similar to our findings. However, the correlation between SSR density and genome size in insects was negative at −0.228 (Ding *et al*., 2017). In contrast, bovid species show a very high positive correlation (0.980) between SSR number and chromosome size (Qi *et al*., 2015). Similarly, in macaque species, the correlation between chromosome size and SSR number was positive, while the correlation with SSR density was negative (Liu *et al*., 2017).

In most vertebrates, mono- and di-nucleotide motifs are the most abundant microsatellite motifs (Zhao *et al*., 2011; Wattanadilokchatkun *et al*., 2022; Kumpatla and Mukhopadhyay, 2005). In the present study, mono-nucleotide motifs were the most prevalent for both perfect and imperfect SSRs. However, the di-nucleotide motifs ranked 3rd, following tetra-nucleotide and trinucleotide motifs for perfect and imperfect SSRs, respectively. In ducks, dinucleotide motifs were found to be the most abundant, accounting for over 50% of the total SSR motifs.

This finding contradicts the previously observed positive relationship between microsatellite density and genome size. The results of the current study suggest that the factors influencing microsatellite distribution may be more complex than a simple linear correlation with genome size. Further research is required to fully elucidate the evolutionary processes shaping microsatellite characteristics in avian species and across boarder range of taxa.

CONCLUSIONS

In the current study, we conducted a genome-wide analysis of the distribution and density of microsatellites in the turkey genome. While the findings provides a foundation for future studies into the role of microsatellites in gene regulation, further investigation is needed to understand how these SSRs are distributed across different regions of the genome, including both coding and non-coding areas. A large set of SSR markers was identified across the entire genome, which will be instrumental for linkage mapping and will significantly improve research in turkey genetics. This extensive characterization of SSR markers not only enhances our understanding of turkey genetics but also creates a foundation for further investigations into their functional role in genomic regulation.

ETHICS APPROVAL

This work was approved (CU/I/F/32/23) by the Institutional Animal Care and Use Committee at Cairo University (CU-IACUC).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abdul-Muneer, P.M. 2014. Application of microsatellite markers in conservation genetics and fisheries management: Recent advances in population structure analysis and conservation strategies: Genetics Research International: 1-11: 10.1155/2014/691759.

Akemi, A., Pereira, J., Macedo, P. and Alessandra, K. 2012. Microsatellites as tools for genetic diversity analysis: Genetic Diversity in Microorganisms: 2: 64: 10.5772/35363.

Aslam, M.L., Bastiaansen, J.W.M., Crooijmans, R.P.M.A., Vereijken, A. and Groenen, M.A.M. 2011. Whole genome QTL mapping for growth, meat quality and breast meat yield traits in turkey: BMC Genetics: 12: 1-10: 10.1186/1471-2156-12-61.

Axelsson, E., Webster, M.T., Smith, N.G.C., Burt, D.W. and Ellegren, H. 2005. Comparison of the chicken and turkey genomes reveals a higher rate of nucleotide divergence on microchromosomes than macrochromosomes: Genome Research: 15: 120-125: 10.1101/gr.3021305.

Barros, C.P., Derks, M.F.L., Mohr, J., Wood, B.J., Crooijmans, R.P.M.A., Megens, H.J., Bink, M.C.A.M. and Groenen, M.A.M. 2023. A new haplotype-resolved turkey genome to enable turkey genetics and genomics research: GigaScience: 12: giad051: 10.1093/gigascience/giad051.

Burt, D.W., Morrice, D.R., Sewalem, A., Smith, J., Paton, I.R., Smith, E.J., Bentley, J. and Hocking, P.M. 2003. Preliminary linkage map of the Turkey (*Meleagris gallopavo*) based on microsatellite markers: Animal Genetics: 34: 399-409: 10.1046/j.1365-2052.2003.01033.x.

Canales Vergara, A.M., Landi, V., Delgado Bermejo, J.V., Martínez, A.M., Cervantes Acosta, P., Pons Barros, A., Bigi, D., Sponenberg, P., Helal, M., Banabazi, M.H. and Camacho Vallejo, M.E. 2020. Design and development of a multiplex microsatellite panel for the genetic characterisation and diversity assessment of domestic turkey (*Meleagris gallopavo* gallopavo): Italian Journal of Animal Science: 19: 392-398: 10.1080/1828051X.2020.1745695.

Clauss, M., Frei, S., Hatt, J.M. and Kreuzer, M. 2020. Methane emissions of geese (*Anser anser*) and turkeys (*Meleagris gallopavo*) fed pelleted lucerne: Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology: 242: 110651: 10.1016/j.cbpa.2020.110651.

Dalloul, R.A., Long, J.A., Zimin, A. V., Aslam, L., Beal, K., Blomberg, L.A., Bouffard, P., Burt, D.W., Crasta, O., Crooijmans, R.P.M.A., Cooper, K., Coulombe, R.A., De, S., Delany, M.E., Dodgson, J.B., Dong, J.J., Evans, C., Frederickson, K.M., Flicek, P., Florea, L., Folkerts, O., Groenen, M.A.M., Harkins, T.T., Herrero, J., Hoffmann, S., Megens, H.J., Jiang, A., de Jong, P., Kaiser, P., Kim, H., Kim, K.W., Kim, S., Langenberger, D., Lee, M.K., Lee, T., Mane, S., Marcais, G., Marz, M., McElroy, A.P., Modise, T., Nefedov, M., Notredame, C., Paton, I.R., Payne, W.S., Pertea, G., Prickett, D., Puiu, D., Qioa, D., Raineri, E., Ruffier, M., Salzberg, S.L., Schatz, M.C., Scheuring, C., Schmidt, C.J., Schroeder, S., Searle, S.M.J., Smith, E.J., Smith, J., Sonstegard, T.S., Stadler, P.F., Tafer, H., Tu, Z., van Tassell, C.P., Vilella, A.J., Williams, K.P., Yorke, J.A., Zhang, L., Zhang, H. Bin, Zhang, X., Zhang, Y. and Reed, K.M. 2010. Multi-platform next-generation sequencing of the domestic Turkey (*Meleagris gallopavo*): Genome assembly and analysis: PLoS Biology: 8: e1000475: 10.1371/journal.pbio.1000475.

Ding, S., Wang, S., He, K., Jiang, M. and Li, F. 2017. Large-scale analysis reveals that the genome features of simple sequence repeats are generally conserved at the family level in insects: BMC Genomics: 18: 1-10: 10.1186/s12864-017-4234-0.

Du, L., Zhang, C., Liu, Q., Zhang, X. and Yue, B. 2018. Krait: An ultrafast tool for genome-wide survey of microsatellites and primer design: Bioinformatics: 34: 681-683: 10.1093/bioinformatics/btx665.

Duhan, N., Kaur, S. and Kaundal, R. 2023. ranchSATdb: A genome-wide Simple Sequence Repeat (SSR) markers database of livestock species for mutant germplasm characterization and improving farm animal health: Genes: 14: 1481: 10.3390/genes14071481.

Giamouri, E., Zisis, F., Mitsiopoulou, C., Christodoulou, C., Pappas, A.C., Simitzis, P.E., Kamilaris, C., Galliou, F., Manios, T., Mavrommatis, A. and Tsiplakou, E. 2023. Sustainable strategies for greenhouse gas emission reduction in small ruminants farming: Sustainability (Switzerland): 15: 4118: 10.3390/su15054118.

Gochi, L., Kawai, Y. and Fujimoto, A. 2023. Comprehensive analysis of microsatellite polymorphisms in human populations: Human Genetics: 142: 45-57: 10.1007/s00439-022-02484-3.

Hristakieva, P. 2021. Current state of turkey meat production in the world and in Bulgaria.: Bulgarian Journal of Animal Husbandry/Životnov Dni Nauki: 58: 42-49.

Huang, H.B., Song, Y.Q., Hsei, M., Zahorchak, R., Chiu, J., Teuscher, C. and Smith, E.J. 1999. Development and characterization of genetic mapping resources for the turkey (*Meleagris gallopavo*): Journal of Heredity: 90: 240-242: 10.1093/jhered/90.1.240.

Kheiralipour, K., Rafiee, S., Karimi, M., Nadimi, M. and Paliwal, J. 2024. The environmental impacts of commercial poultry production systems using life cycle assessment: a review: World's Poultry Science Journal: 80: 33-54: 10.1080/00439339.2023.2250326.

Kumpatla, S.P. and Mukhopadhyay, S. 2005. Mining and survey of simple sequence repeats in expressed sequence tags of dicotyledonous species: Genome: 48: 985-998: 10.1139/g05-060.

Liu, S., Hou, W., Sun, T., Xu, Y., Li, P., Yue, B., Fan, Z. and Li, J. 2017. Genome-wide mining and comparative analysis of microsatellites in three macaque species: Molecular Genetics and Genomics: 292: 537-550: 10.1007/s00438-017-1289-1.

Morgan, G.A., Barrett, K.C., Leech, N.L. and Gloeckner, G.W. 2019. IBM SPSS for introductory statistics: Use and interpretation: Routledge: 10.4324/9780429287657.

Olubunmi, O.O. 2019. Application of microsatellite in fish biotechnology: Prospects and drawback-review: Olagunju Oluwatosin Olubunmi. Application of Microsatellite in Fish Biotechnology: Prospects and Drawback-Review. International Journal of Bioengineering & Biotechnology: 4: 37-43.

Pannebakker, B.A., Niehuis, O., Hedley, A., Gadau, J. and Shuker, D.M. 2010. The distribution of microsatellites in the Nasonia parasitoid wasp genome: Insect Molecular Biology: 19: 91-98.

Qi, W.H., Jiang, X.M., Du, L.M., Xiao, G.S., Hu, T.Z., Yue, B.S. and Quan, Q.M. 2015. Genome-wide survey and analysis of microsatellite sequences in bovid species: PLoS ONE: 10: e0133667: 10.1371/journal.pone.0133667.

Reed, K.M., Chaves, L.D. and Mendoza, K.M. 2007. An integrated and comparative genetic map of the turkey genome: Cytogenetic and Genome Research: 119: 113-126: 10.1159/000109627.

Rozen, S. and Skaletsky, H. 1999. Primer3 on the WWW for general users and for biologist programmers: Bioinformatics methods and protocols: 365-386.

Safaa, H., Khaled, R., Isaac, S., Mostafa, R., Ragab, M., Elsayed, D.A.A. and Helal, M. 2023. Genome-wide in silico characterization, validation, and cross-species transferability of microsatellite markers in Mallard and Muscovy ducks: Journal of Genetic Engineering and Biotechnology: 21: 105: 10.1186/s43141-023-00556-z.

Thornton, E.K., Emery, K.F., Steadman, D.W., Speller, C., Matheny, R. and Yang, D. 2012. Earliest mexican turkeys (*Meleagris gallopavo*) in the Maya region: Implications for pre-hispanic animal trade and the timing of Turkey domestication: PLoS ONE: 7: e42630: 10.1371/journal.pone.0042630.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. and Rozen, S.G. 2012. Primer3—new capabilities and interfaces: Nucleic acids research: 40: e115-e115.

Vergara, A.M.C., Landi, V., Bermejo, J.V.D., Martínez, A., Acosta, P.C., Barro, Á.P., Bigi, D., Sponenberg, P., Helal, M., Banabazi, M.H. and Vallejo, M.E.C. 2019. Tracing worldwide Turkey genetic diversity using D-loop sequence mitochondrial DNA analysis: Animals: 9: 10.3390/ani9110897.

Vieira, M.L.C., Santini, L., Diniz, A.L. and Munhoz, C. de F. 2016. Microsatellite markers: What they mean and why they are so useful: Genetics and Molecular Biology: 39: 312-328: 10.1590/1678-4685-GMB-2016-0027.

Wattanadilokchatkun, P., Panthum, T., Jaisamut, K., Ahmad, S.F., Dokkaew, S., Muangmai, N., Duengkae, P., Singchat, W. and Srikulnath, K. 2022. Characterization of microsatellite distribution in siamese fighting fish genome to promote conservation and genetic diversity: Fishes: 7: 251: 10.3390/fishes7050251.

Wöhrmann, T. and Weising, K. 2011. In silico mining for simple sequence repeat loci in a pineapple expressed sequence tag database and cross-species amplification of EST-SSR markers across Bromeliaceae: Theoretical and Applied Genetics: 123: 635-647: 10.1007/s00122-011-1613-9.

Zhao, X., Tan, Z., Feng, H., Yang, R., Li, M., Jiang, J., Shen, G. and Yu, R. 2011. Microsatellites in different Potyvirus genomes: Survey and analysis: Gene: 488: 52-56: 10.1016/j.gene.2011.08.016.