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Characterization of the genetic diversity, structure, and admixture of 7 Chilean chicken breeds

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ABSTRACT The Mapuche fowl is an autochthonous breed raised in Chile and represents an important zoogenetic resource for the local economy. This study aimed at investigating the genetic diversity, relationship and population structure of 96 local Chilean chickens derived from 3 ecotype of Mapuche fowl (Kollonka, Ketro, and Kollonka de aretes), 2 ecotype Chilean (Trintre, Cogote pelado) and 2 breeds (Light Brahma and Barred Plymouth Rock) using 12 microsatellite markers. In total, 113 alleles were detected in all populations, with a mean of 7.6 alleles per population. In all population chicken breeds, the observed and expected heterozygosity ranged from 0.91 to 0.98 and from 0.69 to 0.79. Furthermore, all populations showed significant deviations from Hardy-Weinberg expectations. Across each population, the global heterozygosity deficit (FIT) was −0.174, population differentiation index (FST) was 0.073, and the global inbreeding of individuals within breed (FIS) was −0.267. The phylogenetic relationships of chickens were examined using neighbor-joining trees constructed at the level of population. The highest Nei's standard genetic distance value of 0.559 was observed between Barred Plymouth Rock and Light Brahma, whereas the minimum value (0.099) was found between Kollonka and Trintre. The neighbor-joining tree constructed at population level revealed 2 main clusters, with Light Brahma, Barred Plymouth Rock, Ketro and Kollonka de aretes in 1 cluster, and Kollonka, Trintre and Cogote pelado breeds in the second cluster. Based on the results of the STRUCTURE analysis, the most likely number of clustering of the population evaluated was at $K = 3$, with Light Brahma and Barred Plymouth Rock breeds forming their own distinct clusters, while Kollonka, Ketro, Kollonka de aretes, Trintre and Cogote pelado breeds clustered together. This study represents the first report of genetic diversity in these populations in Chile. These results can be used as baseline genetic information for genetic conservation program, for instance, to control inbreeding and to implement further genetic studies in local Chilean chickens.

Key words: Mapuche fowl, genetic diversity, chicken breed, zoogenetic resource

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INTRODUCTION

The domestic chicken is widely recognized as the most popular and extensively distributed poultry species, serving as a vital source of meat and eggs, rich in proteins, for human consumption (Food and Agriculture Organization of the United Nations (FAO), 2007; Sykes, 2012). In many developing countries, village poultry plays a crucial role in alleviating poverty and ensuring household food security (Alders and Pym, 2009; Mahammi et al., 2021, 2014, 2016; Berrezoug et al., 2019; Al-Jumaili et al., 2020; Ameur et al., 2020; Boudali et al., 2022). Over the years, extensive domestication and breeding efforts have resulted in a diverse array of chicken breeds (Romanov and Weigend, 2001; Food and Agriculture Organization of the United Nations (FAO), 2007). However, numerous local chicken breeds are currently facing the risk of extinction, putting valuable genotypes and traits in jeopardy (Hillel et al., 2003;

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Blackburn, 2006; Chen et al., 2008; Mahammi et al., 2014, 2016; Al-Jumaili et al., 2020; Ameur et al., 2020; Boudali et al., 2022). These local chicken populations represent significant genetic reservoirs that have evolved over thousands of years, adapted to extreme and challenging environments with minimal veterinary and management intervention (Hall and Bradley, 1995; Kaya and Yıldız, 2008; Mahammi et al., 2014, 2016; Al-Jumaili et al., 2020; Ameur et al., 2020; Boudali et al., 2022). Autochthonous chickens are considered to make a significant contribution to food security and the economical sustainability of rural households (Guèye, 2002; Aboe et al., 2006; Faustin et al., 2010; Mahammi et al., 2014). The Mapuche fowl is the designation of the native Chilean fowl associated with the Mapuche people (Punnett, 1933; Alacalde, 2016). These fowl include the rumpless blue/green egg-laying "Kollonka" (Dunn, 1934) and the tailed ear-tufted "Ketro" which lays mostly brown eggs (Bustos, 1922; Castello, 1924; Somes, 1978; Pabilonia and Somes, 1983). Crossing of the Kollonka and Ketro gave origin to the tufted rumpless standard of the Araucana breed from North America and Europe during the 20th century (Castello, 1924). The Trintre and Cogote pelado breeds are recognized as part of the Mapuche fowl, as they coexist in Chilean backyard systems and share distinctive characteristics such as the green color of the eggshell and the absence of a tail (Wilhelm, 1953, 1963; Moya and Montero, 2007; Alacalde, 2016). A In the second half of the 20th century, chicken breeds such as Barred Plymouth Rock, Rhode Island Red, Black Menorca, and Catalanas were introduced to Chile. These breeds became widely popular in the Chilean countryside and were used for crossbreeding with the Mapuche fowl (Wilhelm, 1953, 1963; Alacalde, 2016).

Today, there are various local Chilean chicken breeds present in the backyard systems of peasant family agriculture (Moya and Montero, 2007). Most of the local breeds became threatened by extinction when the commercially international breeds became more common (Cerdán, 2001; Moya et al., 2009). Typically, only 1 or few populations with a small number of chickens remained when the Chilean association for local poultry ASOGICH (www.asogich.cl) rescued them. The association is still working on maintaining the local chickens in the form of live gene bank. Studies on autochthonous Chilean chickens are limited. In various parts of the world, the genetic diversity of local Chicken has been assessed using molecular markers including microsatellites (Muchadeyi et al., 2007; Chen et al., 2008; Kaya and Yıldız, 2008; Cuc et al., 2010; Clementino et al., 2011; Wilkinson et al., 2012; Mahammi et al., 2016; Al-Jumaili et al., 2020; Habimana et al., 2020). Currently, microsatellite loci are the method of choice to study the genetic diversities within and between populations because they are highly polymorphic, show codominant inheritance, found to be abundant and evenly distributed throughout the genome (Lode, 1993; Hillel et al., 2003; Rajkumar et al., 2007; Granevitze et al., 2007; Anmarkrud et al., 2008). So far, many studies have been conducted to assess chickens genetic diversity using microsatellite markers and the reported results are clear evidence of the usefulness of these panels for biodiversity studies (Romanov and Weigend, 2001; Hillel et al., 2003; Chen et al., 2008; Kaya and Yıldız, 2008; Wilkinson et al., 2012; Abebe et al., 2015; Habimana et al., 2020; Sartika et al., 2023). Using microsatellites on our samples from Chilean ecotypes also allow comparison with published studies of breeds in other countries. Thirty microsatellite markers have been suggested by the Food and Agriculture Organization to be used in the evaluation of genetic diversity in chicken (Food and Agriculture Organization of the United Nations, 1998; FAO, 2011). The aim of this study was to determine the levels of genetic diversity within Chilean chicken ecotypes and 2 breeds, the genetic structure of breeds and the levels of admixture in these populations, using the microsatellite marker.

MATERIALS AND METHODS

Study Area

The study areas selected were the Loncoche, Pitrufquén, and Villarrica communes in Southern Chile. These communes belong to the Province of Cautín, in the La Araucanía Region. Loncoche (39° 22′ 0″ South, 72° 37′ 60″ West), Pitrufquén (38° 58′ 60″ South, 72° 39′ 0″ West) and Villarrica (39° 16′ 0″ South, 72° 13′ 0″ West).

Mapuche Fowl Production System

Backyard systems (**BS**), also known as rural, traditional, domestic, nonspecialized, or indigenous poultry farming, constitute a traditional livestock production system carried out by rural families in their households' backyard or surrounding areas. It involves raising a small group of nonspecialized birds that are fed with inputs produced by the farmers themselves, including what they forage in the field and household scraps (Juárez and Ortiz, 2001). The Mapuche fowl, known for its production of blue eggs, is bred by the Mapuche people (Moya and Montero, 2007; Alacalde, 2016). However, over time, a wide range of morphological characteristics has emerged among the birds in rural chicken coops as a result of crossbreeding with different introduced breeds (Moya et al., 2007, 2009). Currently, there is a significant morphological diversity (high heterogeneity) among the birds within the same coop due to the crossbreeding of the original Mapuche chicken with various breeds introduced during Spanish colonization, subsequent settlement, and the development of industrial poultry farming (Moya et al., 2007, 2009). Nevertheless, in some instances, original traits associated with the Mapuche fowl are still manifest (Cerdán, 2001).

Sampling and Microsatellite Analysis

The blood samples were obtained from Mapuche fowl, Kollonka ($n = 38$), Ketro ($n = 11$), Kollonka de aretes

Table 1. Location, breed, and number of individuals among the studied populations.

Location	Populations	Number of individuals
Loncoche	Light Brahma	17
	Barred Plymouth Rock	7
	Kollonka	10
	Ketro	1
	Kollonka de aretes	2
Pitrufquén	Ketro	10
	Kollonka de aretes	4
Villarrica	Kollonka	28
	Trintre	11
	Cogote pelado	6
Total		96

(Araucana) ($n = 17$), Trintre ($n = 11$), Cogote pelado ($n = 6$). In addition, Light Brahma ($n = 17$) and Barred Plymouth Rock ($n = 7$) were included as outgroups for phylogenetic analysis (Table 1).

Collection of Samples and DNA Extraction

Material was collected from each animal with the Vacutainer system and stored at −20°C until analysis. DNA extraction was carried out with the ZYMO RESEARCH Quick-DNA Miniprep Plus Kit. To verify the presence and quality of the DNA, a 1% agarose gel electrophoresis was performed. Additionally, DNA quantification was carried out by readings on a NanoQuant Infinite 200 PRO spectrophotometer (Tecan Trading AG, Switzerland). A ratio of absorbance (260/280) greater than 1.8 was considered acceptable to proceed with the microsatellite amplification process. Likewise, the stock DNA was diluted to a final concentration of 10 ng/μL. The final DNA dilutions were stored at −20°C for future use. A selection of 12 chicken microsatellites was used in each of the flocks, as suggested by the FAO-ISAG group for biodiversity studies (Commission on Genetic Resources for Food and Agriculture, and FAO 2011). The name of each marker and genetic variability measures at the 12 different microsatellite loci analyzed across studied chicken populations are presented in Table 2.

PCR Amplification and DNA Polymorphism

PCR were optimized to amplify all microsatellites carried out in an Heal Force Thermal Cycler Thermal T960 (Heal Force, Shanghai, China). Each PCR amplification was performed in a total volume of 25 μl containing 0.5 unit of Taq DNA polymerase and the PCR program was: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 60°C for 40 s, annealing temperature, and 72°C for 30 s, and a final extension at 72°C for 15 min. The PCR products were identified and classified by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Foster City, SA) and GeneScan 500 LIZ size standard (Thermo Fisher Scientific). Raw capillary electrophoresis data were analyzed using the Geneious v 8.0.5 software (Biomatters, http://www.geneious.com/).

Statistical Analysis

The polymorphism information content (**PIC**) and frequencies of null alleles were estimated using Cervus v3.0.7 (Kalinowski et al., 2007). GenAlEx v 6.5 was used to determine the following parameters: the allele frequencies, total alleles, expected heterozygosity (**HE**), observed heterozygosity (**HO**), and Wright's *F*-statistics as well as other parameters such as inbreeding coefficient over all populations (FIS), among populations (FIT), and within populations (FST) for 12 microsatellite markers (Peakall and Smouse, 2012). Micro-checker v.2.2.3 (Van Oosterhout et al., 2004) was used to detect genotyping errors due to allelic dropout, stuttering and null alleles (null allele estimates as per the method of Brookfield, 1996). To estimate the phylogenetic distances between the flocks, Nei's standard genetic distance was calculated (Nei, 1978) and the neighbor-joining (**NJ**) tree was developed with The PHYLIP software v. 3.62 (Felsentein, 2005). A consensus

Table 2. Genetic variability measures at the 12 different microsatellites loci analyzed across studied chicken populations. Polymorphic information content (PIC), Wright's *F*-statistics (FIT, FST and FIS), observed heterozygosity (HO), expected heterozygosity (HE), coefficient of gene differentiation (GST) and Hardy-Weinberg equilibrium (HWE).

Locus	PIC	FIT	FST	FIS	HO	HE	GST	HWE
MCW0111	0.78	−0.23	0.05	−0.30	0.97	0.81	0.02	*
ADL0268	0.81	−0.17	0.05	−0.24	0.96	0.84	0.01	**
MCW0037	0.76	−0.25	0.07	−0.35	0.97	0.79	0.04	***
MCW0067	0.87	−0.05	0.05	−0.12	0.89	0.89	0.01	NS
LEI0166	0.71	−0.30	0.08	−0.43	0.97	0.75	0.06	***
MCW0222	0.69	−0.14	0.07	−0.24	0.86	0.71	0.04	***
MCW0206	0.68	0.00	0.13	−0.15	0.68	0.72	0.09	NS
MCW0034	0.83	−0.16	0.06	−0.24	0.99	0.85	0.02	*
MCW0123	0.82	−0.20	0.05	−0.27	0.99	0.84	0.02	*
MCW0183	0.91	−0.09	0.05	−0.15	1	0.92	0.01	NS
ADL0112	0.82	−0.13	0.11	−0.28	0.97	0.84	0.08	*
MCW0078	0.75	−0.31	0.05	−0.39	1	0.78	0.02	***
Average	**0.79**	**−0.174**	**0.073**	**−0.267**	**0.94**	**0.81**	**0.03**	

Significant *P* values means deviations from Hardy-Weinberg equilibrium per locus; NS: No significant.
In bold, the average of each parameter evaluated in the table is highlighted.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

tree assessed by 1,000 bootstraps all through the group of loci was created. The genetic structure of the populations was analyzed using Bayesian clustering algorithms implemented in the software STRUCTURE v.2.3.4 (Pritchard et al., 2000). A set of rules applied in STRUCTURE v.2.3.4 was used to group entities based on multilocus genotypes (Falush et al., 2007). The evaluation entailed an admixture model alongside interrelated allele frequency. During the STRUCTURE analysis, were used together with 100,000 reiterations of Markov chain Monte Carlo (**MCMC**) and 10,000 burning period in the admixture model. Evanno's ΔK statistic was calculated. The most likely K value in the dataset was calculated, 2 to 7 inferred clusters with prior information on the breed of origin were performed with 100 independent runs for each K according to Evanno (Evanno et al., 2005) using the STRUCTURE HARVESTER web server (http://taylor0.biology.ucla.edu/structureHarvester/). CLUMPP v 1.1.2 (Jakobsson and Rosenberg, 2007), which is a tool used to single out clustering types and bundle population structure deductions across K was used. To assess whether the populations of Chilean chicken breeds have undergone recent reductions in effective population size or genetic bottlenecks, various approaches were employed (Piry et al., 1999). In the first approach, 3 distinct tests were utilized: a "sign test," a "standardized differences test," and a "Wilcoxon sign-rank test," all under different microsatellite evolution models such as the Infinite Allele Model (**IAM**) (Kimura and Crow, 1964), the Stepwise Mutation Model (**SMM**) (Ohta and Kimura, 1973), and Two-Phase Models (**TPM**) (Di Rienzo et al., 1994). These methods examine deviations from mutation-drift equilibrium based on heterozygosity excess or deficiency. The probability distribution was established through 1,000 simulations under 3 models. The analysis was conducted using BOTTLENECK v1.2.02 software (https://www1.montpellier.inrae.fr/CBGP/software/Bottleneck/bottleneck.html).

The second approach employed a qualitative graphical method to visualize allele frequency spectra (Luikart et al., 1998). Microsatellite alleles were categorized into 10 frequency classes, allowing verification of whether the distribution followed the expected normal L-shaped pattern, with the lowest-frequency alleles (0.01−0.1) being the most abundant. This analysis was also performed using Bottleneck v1.2.02 software.

RESULTS

All the microsatellite loci were amplified in the 7 populations analyzed. In total, 218 different alleles were identified from the 12 microsatellite loci in the entire populations. Table 2 shows the measures of genetic variability corresponding to these 12 loci. The estimated PIC values considering all markers were highly informative (0.79). MCW0206 was found to be the least informative marker, whereas MCW0183 was the most informative (Table 2). The average number of observed alleles per locus was 18.16, with a variation in the number of alleles across different loci. The average effective number of alleles across all breeds was 4.63. In the present study, the average observed heterozygosity of the loci was 0.94, whereas expected heterozygosity was 0.81. No null alleles were found in any of the 12 microsatellite loci used, as these result from point mutations in the flanking regions, where primers align, which reduces their recognition ability during PCR fragment amplification (Callen et al., 1993; Reece et al., 2004). The presence of null alleles in population genetics studies leads to a departure from Hardy-Weinberg equilibrium due to a heterozygote deficit (Callen et al., 1993; Reece et al., 2004). In Table 3, the average expected and the observed heterozygosity and inbreeding coefficient per populations are presented. The maximum number of observed alleles per breed was found in Kollonka (159), and the minimum value was observed in Barred Plymouth Rock (61) (Table 3). Furthermore, Barred Plymouth Rock had the highest gene diversity value (HE = 0.98), while Cogote pelado had the lowest value (HE = 0.91) (Table 3). Considering the whole population, 9 of the 12 loci analyzed showed significant deviation ($P < 0.05$) from HWE (Table 2). Table 4 presents the Nei's genetic distance assessed across between the studied chicken populations. The lowest value of Nei's genetic distance was observed between Kollonka and Trintre populations (0.166), while the highest value was between Light Brahma and Barred Plymouth Rock breeds (0.55). Figure 1 presents the phylogenetic relationship among populations constructed by a neighbor joining tree using a genetic distance matrix. The 7 populations were clustered into 2 separate groups: 1 group were Light Brahma, Barred Plymouth Rock, Ketro and Kollonka de aretes and the rest of the populations

Table 3. Populations studied, simple size of each population (N), number of alleles per populations (NA), mean number of alleles per population (Na), number of effective alleles (Ne), mean observed (HO) and expected heterozygosity (HE), inbreeding coefficient (FIS) per population, and Shannon's index (I).

Populations	N	NA	Na	Ne	HO	HE	FIS	I
Light Brahma	17	105	8.75	5.00	0.95	0.76	−0.24	1.75
Barred Plymouth Rock	7	61	5.08	3.67	0.98	0.69	−0.45	1.37
Kollonka	38	159	13.25	5.61	0.92	0.79	−0.18	1.94
Ketro	10	86	7.16	4.74	0.93	0.75	−0.24	1.63
Kollonka de aretes	7	77	6.41	4.73	0.94	0.76	−0.23	1.63
Trintre	11	82	6.83	4.27	0.97	0.75	−0.30	1.60
Cogote pelado	6	69	5.75	4.44	0.91	0.72	−0.26	1.52
Average	**13.71**	**91.28**	**7.60**	**4.63**	**0.94**	**0.75**	**−0.27**	**1.63**

In bold, the average of each parameter evaluated in the table is highlighted.

Table 4. Matrix of Nei's standard genetic distances between the studied populations.

Populations	BR	PR	K	Q	KdA	T	CP
BR (17)	0.000	-	-	-	-	-	-
PR (7)	0.559	0.000	-	-	-	-	-
K (38)	0.360	0.450	0.000	-	-	-	-
Q (10)	0.365	0.486	0.212	0.000	-	-	-
KdA (7)	0.364	0.363	0.235	0.189	0.000	-	-
T (11)	0.417	0.449	0.099	0.262	0.214	0.000	-
CP (6)	0.358	0.414	0.149	0.263	0.270	0.185	0.000

BR = Light Brahma; PR = Barred Plymouth Rock; K = Kollonka; Q = Ketro; KdA = Kollonka de aretes; T = Trintre; CP = Cogote pelado.

(Kollonka, Trintre, and Cogote pelado) were placed in the second group. The structure of the populations was analyzed using a Bayesian approach that inferred the number of clusters (K) present in the population, permitting detection of differences among populations and hidden structures within populations. Results of Structure analyzed are shown in Figure 2 for K ranging from 2 to 7 and the most probable solutions are reported per each K. The most likely number of clustering of the 7 populations was at $K = 3$ (Table 5 and Figure 3), with Light Brahma and Barred Plymouth Rock breeds forming their own distinct clusters, while Kollonka, Ketro, Kollonka de aretes, Trintre and Cogote pelado populations clustered together.

F-statistics of overall loci were FIS = −0.267, FST = 0.073, and FIT = −0.174. Homozygote deficiency (FIS) showed highest slightly lower heterozygosity values in loci (Table 2). In addition, the highest inbreeding level was observed in the Kollonka population (FIS = −0.18), and lowest in the Barred Plymouth Rock (FIS = −0.45); however, the FIS value is particularly excessive (Table 2). The FST values among the 7 populations studied ranged from 0.05 (MCW0111, ADL0268, MCW0067, MCW0123, MCW0183 and MCW0078) to 0.13 (MCW0206) with an overall 7.3% of genetic differentiation level among populations. The average GST value relative to the diversity of the whole population (GST = 0.03) indicates that 3% of the total genetic variation corresponded to the differences between populations, whereas 97% was explained by differences among individuals (Table 2). However, all the loci provide a reasonable indicator of genetic differentiation, confirming the remarkable level of genetic variability within these populations.

The results of the bottleneck analysis in Chilean chicken breeds are presented in Table 6. In the case of the Light Brahma breed, when assessing its genetic history under different models and tests, diverse outcomes are observed. IAM no significant signs of recent bottleneck events were found ($P = 0.445$). However, when using the TPM, significance ($P = 0.015$) was detected in the sign test, suggesting the possibility of a recent bottleneck under this model. Similarly, under the SMM, significant results were observed ($P = 0.016$) in the sign test, and further significance was found in the standardized differences test ($P = 0.00001$) and the Wilcoxon rank test ($P = 0.034$). No change in allele distribution was observed in the Mode-shift test (Figure 4A). For the Barred Plymouth Rock breed, the results indicate no significant signs of recent bottleneck events in its genetic history under any of the considered models or tests. Both IAM, TPM, and SMM revealed no evidence of significant reductions in effective population size. No change in allele distribution was observed in the Mode-shift test (Figure 4B). The Kollonka breed reveals an intriguing genetic dynamic. Under IAM, no significant signs of a recent bottleneck were found, suggesting relative stability in its effective population size. However, both TPM and SMM show significant evidence of a recent bottleneck, with very low P values ($P = 0.00002$) in all tests. This indicates that at some point in its

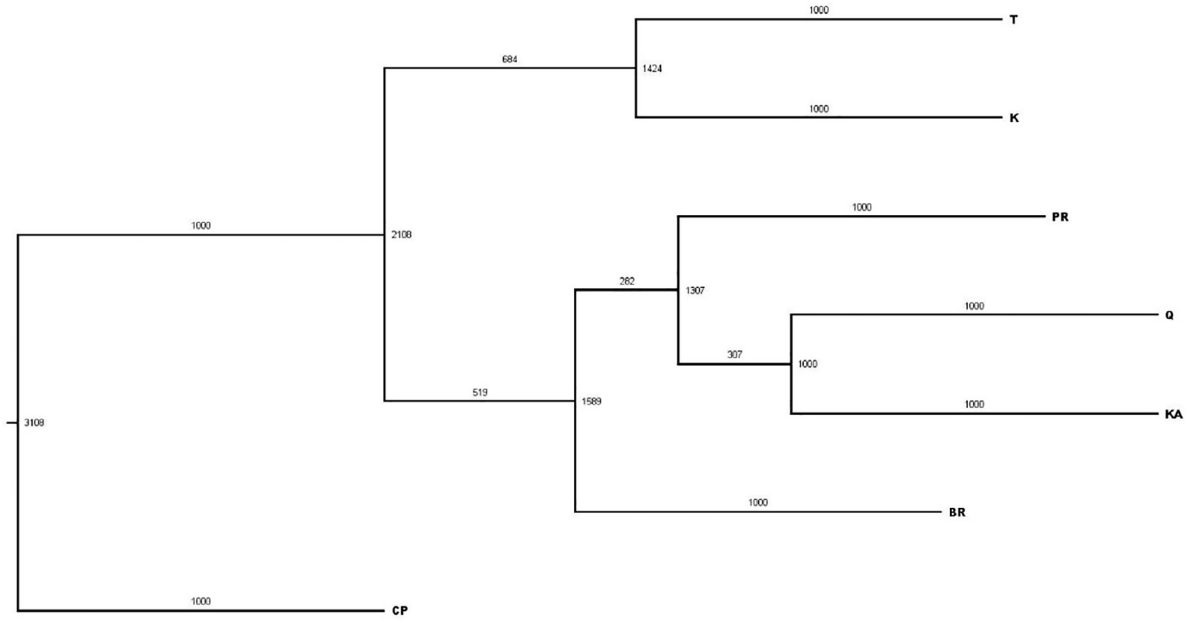

Figure 1. Neighbor-joining network constructed using the distance of Nei's (1978) among the studied populations. BR = Light Brahma; PR = Barred Plymouth Rock; K = Kollonca; Q = Ketro; KA = Kollonca de aretes; T = Trintre; CP = Cogote pelado.

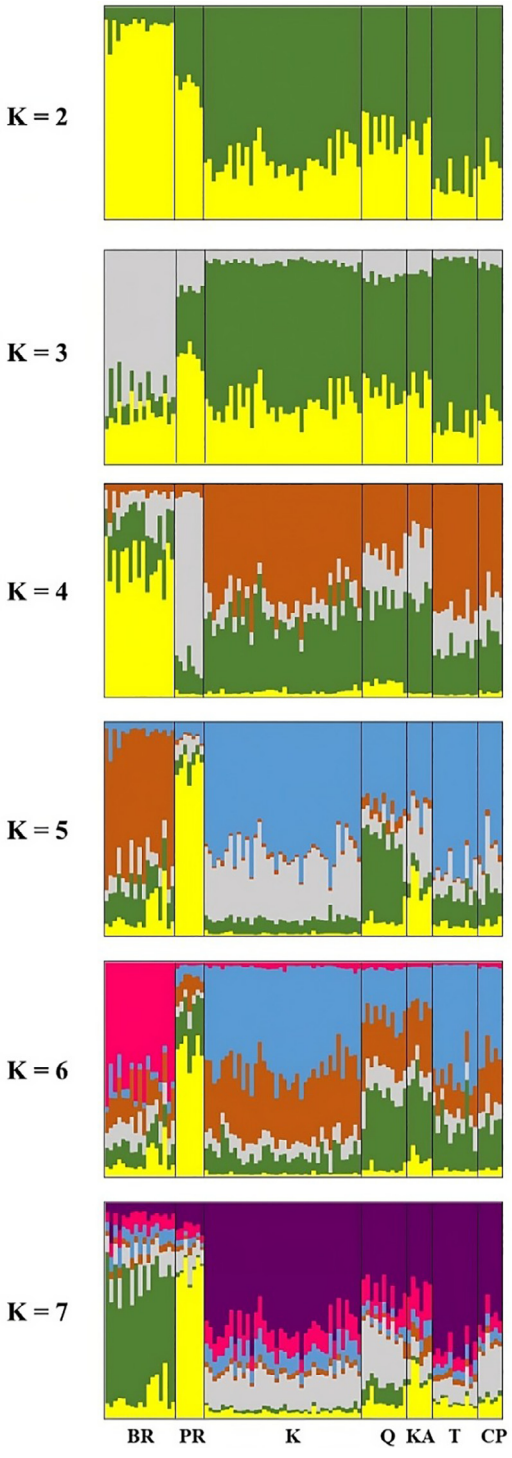

Figure 2. Graphical representation of the estimated membership fractions of individuals of the populations analyzed in each of the *K*-inferred clusters, for $K = 2$ to $K = 7$. BR = Light Brahma; PR = Barred Plymouth Rock; K = Kollonca; Q = Ketro; KA = Kollonca de aretes; T = Trintre; CP = Cogote pelado.

history, the Kollonka population experienced a substantial reduction in genetic diversity. No change in allele distribution was observed in the Mode-shift test (Figure 4C). The results for the Ketro breed suggest an interesting genetic history. Under IAM, no significant signs of a recent bottleneck were found ($P = 0.080$), possibly indicating some recent stability in its effective population size. However, TPM revealed significant evidence of a recent bottleneck in the sign test ($P = 0.041$), though not in the other tests. Additionally, SMM yielded mixed results, with significance in the sign test ($P = 0.015$) and the standardized differences test ($P = 0.029$), but not in the Wilcoxon rank test. No change in allele distribution was observed in the Mode-shift test (Figure 4D). For the Kollonca de aretes breed, the bottleneck analysis results indicate no significant signs of recent bottlenecks under any of the 3 mutation models considered. In both IAM and TPM, as well as SMM, the tests did not provide evidence of excess or deficit of heterozygotes, suggesting that this bird population may have maintained relative stability in its effective population size in the recent past. No change in allele distribution was observed in the Mode-shift test (Figure 4E). Regarding the Trintre breed, the bottleneck analysis results suggest no significant signs of recent bottlenecks under IAM or TPM, as these tests did not reveal evidence of excess or deficit of heterozygotes. However, SMM showed significant signs of a bottleneck in the standardized differences test ($P = 0.014$). This indicates that although the Trintre population does not exhibit bottleneck signs under the IAM and TPM models, the SMM model suggests it may have experienced a significant reduction in effective population size in the recent past. No change in allele distribution was observed in the Mode-shift test (Figure 4F). For the Cogote pelado breed, the bottleneck analysis results indicate no significant signs of recent bottlenecks under any of the 3 mutation models evaluated: IAM, TPM and SMM. The tests did not provide evidence of excess or deficit of heterozygotes in any of the populations. No change in allele distribution was observed in the Mode-shift test (Figure 4G).

DISCUSSION

The present study provides the first survey on the genetic variability of Mapuche fowl based on the analysis of microsatellite markers. In this study, 100% of all loci were highly informative, which confirmed that they are suitable for estimating the genetic diversity of autochthonous chicken populations in Chile. The highest value of PIC (0.91) was that of MCW0183 and the mean PIC was 0.79. The PIC values found in this study did not exceed those reported for Turkish chickens (0.29 −0.80) (Kaya and Yıldız, 2008), and of African chickens (0.348−0.877) (Zhu et al., 2014). The number of alleles per population found in this study (13.71) exceeded those recorded in previous reports in Cameroon (Tiambo et al., 2014), Ghana (Berthouly et al., 2009), Spain (Dávila et al., 2009), Iran (Mohammadabadi et al., 2010), China (Chen et al., 2008), Egypt (Eltanany et al., 2011), Pakistan (Ellahi et al., 2012), Vietnam (Cuc et al., 2006), and Rwanda (Habimana et al., 2020).

The fixation indices (FIT, FST, and FIS) per locus across the 7 populations are depicted in Table 2. The measures of population subdivision (FST) revealed the presence of a moderate level of genetic differentiation

Table 5. Values of K, the number of repetitions for each K, the mean log-likelihood and standard deviations of the data (LnP(K)), the mean difference between consecutive likelihood values of K (Ln′(K)), the absolute values of the second-order rate of change of the likelihood |Ln″(K)|, and the most likely number of clusters (Delta K) of 7 Chilean local chicken breeds calculated following Evanno et al. (2005).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln′(K)	|Ln″(K)|	Delta K
2	100	−4752.04	12.179972	—	—	—
3	100	−4704.486	16.163083	47.554	53.096	3.285017
4	100	−4710.028	38.027048	−5.542	48.738	1.281667
5	100	−4764.308	26.780951	−54.28	14.556	0.543521
6	100	−4804.032	40.611579	−39.724	13.393	0.329783
7	100	−4830.363	42.029008	−26.331	—	—

(FST) among populations, implying that 92.7% of the genetic variability can be attributed to the variation among individuals within the populations and 7.3% to unique allelic differences. The mean FST in the present study was approximately equivalent to mean FST values of 0.07 and 0.08 reported by Eltanany (Eltanany et al., 2011) for 3 Egyptian chicken breeds and Liao (Liao et al., 2016) for 6 Chinese native chicken breeds, respectively. The average inbreeding coefficient of individuals within the subpopulations, measured as FIS value, across the 12 loci was −0.267. The Mapuche fowl was characterized by a significant number of observed alleles. The inbreeding level observed across all populations and loci was consistently low, where Barred Plymouth Rock has the lowest FIS value. The low FIS value (−0.27) implied that mating is random between individuals and suggested that all of the studied loci in the breeds were heterozygous (Bodzsar et al., 2009; Osei-Amponsah et al., 2010; Mahammi et al., 2016).

The analysis of heterozygote excess revealed significant deviations from Hardy-Weinberg equilibrium ($P <$ 0.05) at several loci in the Chilean chicken populations (Table 2). The basis for this deviation is associated with the negative values of FIS (estimation of inbreeding within the population) obtained at several loci (Table 2), as reflected in the high average estimation of inbreeding within the population (FIS = −0.27). In general, the heterozygote excess observed in all populations may be related to management conditions of the flocks as in the case of different local chicken breeds. In general, the excess of heterozygotes observed in all populations might be related to the management conditions of the flocks, as is the case with different local chicken breeds. Moreover, genealogical data are not available for this breed in the different backyard systems where they are raised (Moya and Montero, 2007; Moya et al., 2009).

The neighbor-joining tree constructed at breed level revealed 2 main clusters, with Light Brahma, Barred Plymouth Rock, Ketro and Kollonka de aretes populations in 1 cluster, and Kollonka, Trintre and Cogote pelado populations in the second cluster. The consensus tree among the different populations based on Nei's genetic distance confirmed the existence of moderate genetic differentiation in the populations, which was evidenced by the short genetic distance between some groups. As expected, Kollonka and Trintre appeared in close neighborhood in the tree due to historical crossbreeding practices, but most likely due to past gene flow among them (Moya, 2004; Moya and Montero, 2007; Moya et al., 2009).

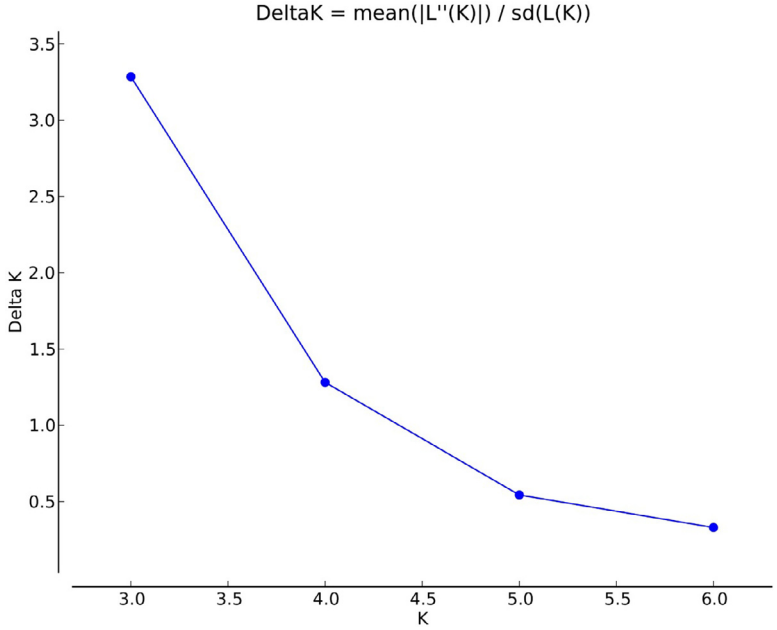

Figure 3. A scatter plot of the different values of K vs. Delta K, and the highest peak shows the most likely number of clusters of local Chilean Chickens included in the present study.

Table 6. Bottleneck analysis for Chilean chicken populations using the sign test, standardized differences test, and Wilcoxon rank test under the Infinite Allele Model (IAM), Stepwise Mutation Model (TPM), and Two-Phase Model (SMM).

Breeds	Model	*P* value sign test	*P* value standardized differences test	*P* value Wilcoxon rank test	Mode-shift test
Light Brahma	IAM	0.445	0.233	0.266	Normal L distribution
	TPM	0.015*	0.001*	0.052	
	SMM	0.016*	0.00001*	0.034*	
Barred Plymouth Rock	IAM	0.176	0.057	0.092	Normal L distribution
	TPM	0.465	0.468	0.733	
	SMM	0.512	0.388	0.969	
Kollonka	IAM	0.458	0.475	0.677	Normal L distribution
	TPM	0.00002*	0*	0.0002*	
	SMM	0.00002*	0*	0.0002*	
Ketro	IAM	0.080	0.165	0.092	Normal L distribution
	TPM	0.041*	0.120	0.176	
	SMM	0.015*	0.029*	0.077	
Kollonca de aretes	IAM	0.224	0.085	0.042*	Normal L distribution
	TPM	0.527	0.457	0.850	
	SMM	0.535	0.315	0.791	
Trintre	IAM	0.416	0.125	0.129	Normal L distribution
	TPM	0.345	0.076	0.301	
	SMM	0.150	0.014*	0.176	
Cogote pelado	IAM	0.148	0.201	0.423	Normal L distribution
	TPM	0.558	0.429	0.969	
	SMM	0.545	0.336	0.969	

*$P < 0.05$.

We also investigated population structure by varying K from 2 to 7. STRUCTURE-based clustering further supports the low among ecotype differentiation of the Chilean chickens (Figure 2). The lack of observed substructuring among autochthonous Chickens at values of $K = 3$ (Table 5 and Figure 3) suggest that Chilean autochthonous chickens essentially form 1 population. This finding agrees with observed Wright's (1951) fixation indices (Table 2). Substructuring according to geographic location (ecotype) could not be observed. Furthermore, clustering of the Chilean chickens was not related to phenotypic classes. The separation of the purebred lines (Light Brahma and Barred Plymouth Rock) at $K = 3$ emphasizes the distinctiveness of the Chilean population. This observation could be due to either a very large effective population size or relatively strong and continuous gene flow between populations (Moya et al., 2009; Mahammi et al., 2016). Gene flow among populations would result in equal allele frequencies across all populations and give no cause of the inferred substructures. The portion of chicken populations that clustered with the exotic chicken could be attributed to the fact that different crossing programs between autochthonous Chicken populations and improved chicken breeds have been introduced in that region to improve the genetic potential of autochthonous Chickens in Chile (Moya et al., 2009).

This study reveals interesting patterns in the genetic history of the Chilean chicken breeds studied compared to previous findings in other bird populations. For the Light Brahma breed, the analysis under IAM did not show significant signs of recent bottlenecks, which could suggest some stability in its effective population size. However, both the TPM and SMM models indicated the possibility of a recent bottleneck, highlighting the importance of considering multiple models in bottleneck analysis. These results are consistent with previous studies in other Indian chicken populations (Chaudhary et al., 2023)that have also used the TPM and SMM models as the most powerful tests for microsatellite analysis. In the case of the Barred Plymouth Rock breed, the results did not show significant signs of recent bottlenecks under any of the considered models or tests. This suggests that this bird population may have maintained relative stability in its effective population size in the recent past, which is consistent with other studies in different African breeds (Abou-Elewa and Farrag, 2018). The Kollonka breed exhibits an interesting genetic dynamic, as IAM did not show significant signs of recent bottlenecks, but both TPM and SMM indicated significant evidence of a recent bottleneck. This suggests that at some point in its history, the Kollonka population experienced a substantial reduction in its genetic diversity, as described by Chaudhary et al. (2023). For the Ketro breed, results under IAM suggest some stability in its effective population size in recent times. However, TPM reveals significant evidence of a recent bottleneck in the sign test. Additionally, SMM yields mixed results, with significance in the sign test and the standardized differences test, but not in the Wilcoxon rank test. These findings emphasize the importance of considering multiple tests to obtain a comprehensive picture of a population's genetic history (Abou-Elewa and Farrag, 2018; Chaudhary et al., 2023). The Kollonca de aretes breed did not show significant signs of recent bottlenecks under any of the 3 considered mutation models, suggesting that this bird population may have maintained relative stability in its effective population size in the recent past. Similar results were obtained by Vij et al. (2006) with a normal L-shaped distribution, concluding that the Punjab Brown breed had not experienced any recent genetic bottleneck.

In the case of the Trintre breed, bottleneck analysis results suggest that no significant signs of recent bottlenecks were found under the IAM or TPM models, as the tests did not provide evidence of heterozygote excess or

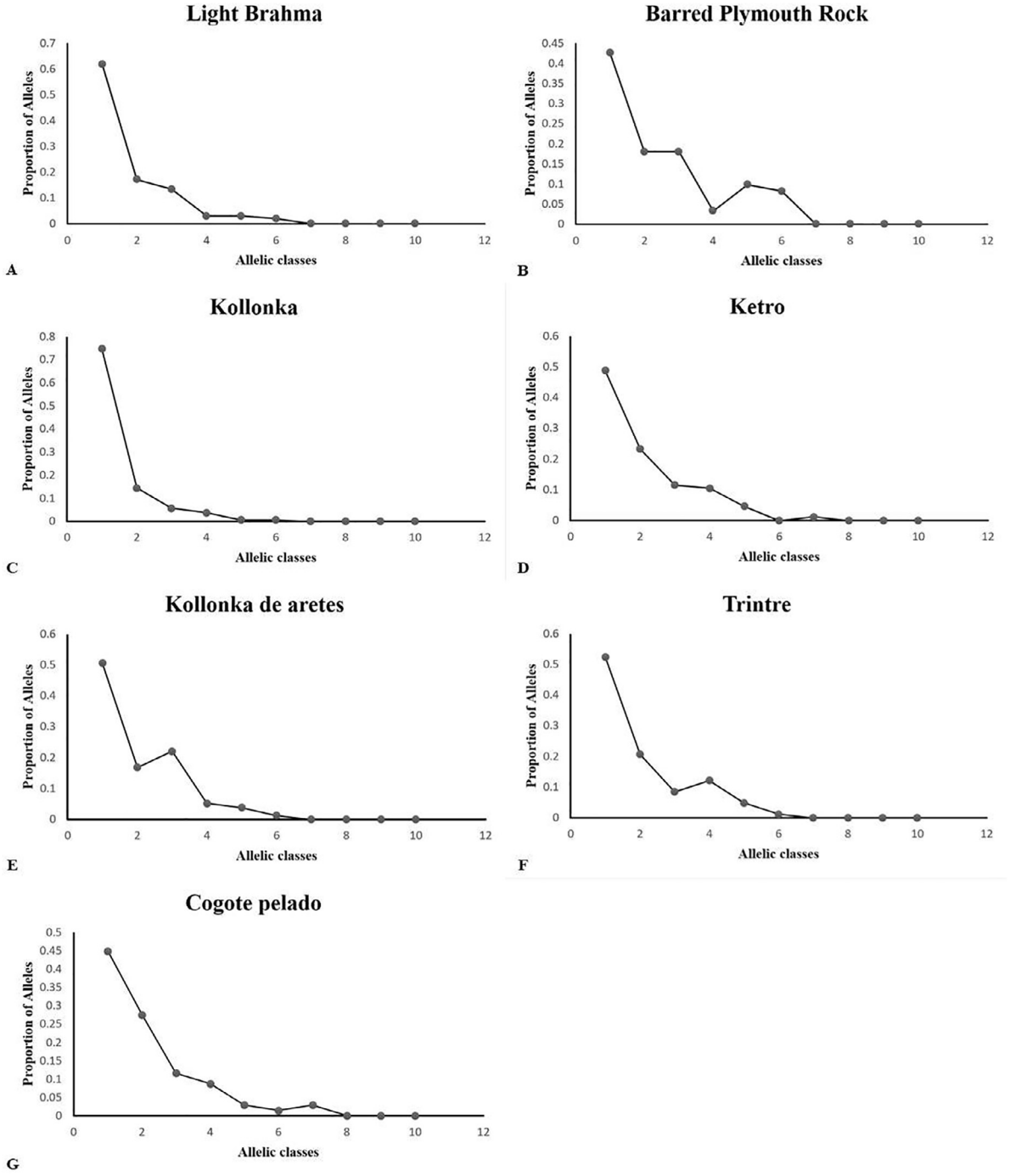

Figure 4. The mode-shift test using graphic representation in local Chilean Chickens populations. (A) L-distribution graph for the Light Brahma breed. (B) L-distribution graph for the Barred Plymouth Rock breed. (C) L-distribution graph for the Kollonka breed. (D) L-distribution graph for the Ketro breed. (E) L-distribution graph for the Kollonka de aretes breed. (F) L-distribution graph for the Trintre breed. (G) L-distribution graph for the Cogote pelado breed.

deficiency. However, the SMM revealed significant signs of a bottleneck in the standardized differences test. This indicates that although the Trintre population does not show signs of bottlenecks under the IAM and TPM models, the SMM model suggests that it may have experienced a significant reduction in its effective population size in the recent past. Lastly, for the Cogote pelado breed, bottleneck analysis results indicate that no significant signs of recent bottlenecks were found under any of the 3 considered mutation models. The tests did not provide evidence of heterozygote excess or deficiency in any of the populations, suggesting that this bird population may have maintained relative stability in its effective population size in the recent past, similar to the results obtained by Vij et al. (2006) and Mtileni et al. (2016).

CONCLUSIONS

This study represents the first genetic characterization of Mapuche fowl in Chile, revealing that the native chicken populations and the internationally evaluated breeds

exhibited high levels of genetic variability. This information can be used to support and implement conservation and/or genetic improvement programs. The genetic heritage of these evaluated breeds is not only essential for the conservation of local breeds but also contributes to the global genetic diversity of the specie, playing a crucial role in meeting the current demands for food security and the sustainability of poultry farming worldwide.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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