Genetic diversity and population structure of indigenous chicken in Rwanda using microsatellite markers

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Abstract

Rwanda has about 4.5 million of indigenous chicken (IC) that are very low in productivity. To initiate any genetic improvement programme, IC needs to be accurately characterized. The key purpose of this study was to ascertain the genetic diversity of IC in Rwanda using microsatellite markers. Blood samples of IC sampled from 5 agro-ecological zones were collected from which DNA was extracted, amplified by PCR and genotyped using 28 microsatellite markers. A total of 325 (313 indigenous and 12 exotic) chicken were genotyped and revealed a total number of 305 alleles varying between 2 and 22 with a mean of 10.89 per locus. 186 distinct alleles and 60 private alleles were also observed. The frequency of private alleles was highest in samples from the Eastern region, whereas those from the North West had the lowest. The influx of genes was lower in the Eastern agro-ecological zone than the North West. The mean observed heterozygosity was 0.6155, whereas the average expected heterozygosity was 0.688. The overall inbreeding coefficient among the population was 0.040. Divergence from the Hardy-Weinberg equilibrium was significant in 90% of loci in all the populations. The analysis of molecular variance revealed that about 92% of the total variation originated from variation within populations. Additionally, the study demonstrated that IC in Rwanda could be clustered into four gene groups. In conclusion, there was considerable genetic diversity in IC in Rwanda, which represents a crucial genetic resource that can be conserved or optimized through genetic improvement.

Introduction

Poultry keeping is an agricultural enterprise with a high potential in Rwanda. More than 40% of households keep poultry out of which approximately 80% consists of indigenous chicken (IC). Raising IC is preferred to exotic breeds because of their small cost of production, scavenging capacity and adaptability to harsh environmental conditions. IC production serves a critical role in the source of revenue for resource-limited countryside families [1]. The productivity of IC in Rwanda, however, is low at an average of 40 to 100 eggs per hen per year and weight ranging from 0.8 to 1.8 kg per year, which is insufficient to meet the needs of the population [2]. This setback has restricted their potential to improve the livelihoods of smallholder farmers thus failing to contribute considerably to the mitigation of poverty in rural areas. To improve the genetic potential of IC in Rwanda, different crossbreeding programmes between IC and exotic chicken were initiated. These programmes, however, are not sustainable because of unpredictable stock and the prohibitive cost of buying and sustaining exotic cocks for breeding purposes in addition to decreased broodiness in the hybridized birds. Additionally, recent global efforts to preserve native genetic resources pose a threat to such programmes, [3]. There is, therefore, the need for an alternative approach to genetic improvement and conservation of IC. Genetic improvement through within-breed selection of IC in Rwanda could be a promising alternative strategy. Nonetheless, genetic enhancements need a resolute breeding objective, sustainable breeding plans, and an in-depth comprehension of the genetic diversity of prevailing genotypes and ecotypes [4]. Therefore, elucidating the genetic characteristics of the prevailing IC stock will not only augment genetic enhancement but will also expedite their preservation [3]. However, there is a scarcity of data on the genetic diversity of IC in Rwanda. The availability of such knowledge could give a clue of the origin and genetic variability in the population and guide selection decisions. As a result, it would be possible to develop apposite mating plans to uphold genetic variation and minimize inbreeding in the population, which would promote response to selection. The current study evaluated the

74 degree of genetic diversity and phylogenetic relationships between populations of IC in Rwanda
75 using simple sequence repeats (SSR) markers.

76 **Materials and methods**

77 **2.1 Collection of samples and DNA extraction**

78 In total, 313 distinct IC were sampled from five agro-ecological zones [51, 52, 53, 55, and 102
79 from Central South (CS), North West (NW), Central North (CN), South West (SW), and East (E),
80 respectively]. Twelve (12) exotic chicken (layers and broilers) were included as a reference for
81 comparison. Populations were reckoned according to agro-ecological zones [5]. A single blood
82 drop was drawn from veins in the wing of each bird and placed on Whatman FTA™ filter cards,
83 left to dry in a cool place for approximately one hour, and held in reserve in discrete envelopes at
84 room temperature awaiting further processing. The isolation of genomic DNA was done using
85 Smith and Burgoyne's boiling method [6]. The quality of genomic DNA was ascertained through
86 gel electrophoresis using 1% agarose. A NanoDrop Spectrophotometer (Thermo Scientific ™
87 Nanodrop 2000) was used to quantify the total DNA, which was adjusted to 10ng/µl before use in
88 the subsequent steps of polymerase chain reaction (PCR) and genotyping.

89 **2.2 PCR amplification and DNA polymorphism**

90 Twenty-eight fluorescently-labelled polymorphic SSR markers were chosen based on the extent of
91 polymorphism shown by a high polymorphism information content and the genome coverage
92 consistent across previous studies [7]. The PCR reactions had a total volume of 10µl consisting of
93 30ng target DNA, 5µl of One Taq 2MM and 0.2µl of each forward and reverse primer. The
94 amplifications were done in a thermocycler (Applied Biosystems 9700 Thermal Cycler Gene
95 Amp®) and entailed the first denaturation at 94°C for 3 minutes, 30 cycles of denaturation at 94ºC
96 for 30 seconds, the primer annealing at temperatures ranging between 58°C and 64°C based on the
97 primer components (Table 1) for 1 minute, and extension at 72°C for 2 minutes. The last extension
98 step was done at 72°C for 10 minutes. The PCR products of different fluorescent tags were

combined according to the exhibited colour and intensity of bands to create uniform signal strength. Hi-Di formimide was used to denature the combined amplicons at 95°C for 3 minutes, this step was followed by capillary electrophoresis separation in an ABI3730 DNA genetic analyzer by using GeneScan- 500 Internal LIZ and 1200 Internal LIZ Size Standards. The resultant fragment analysis data and sizes of alleles were counted using GENEMAPPER V 4.1 software (Applied Biosystems).

105 **Table 1. Sequences and physical information of 28 SSR markers used for PCR amplification**

Name	Allele size (base-pairs)	Forward Primer 5'- 3'	Reverse primer 3'-5'	Annealing temperature (Tm: ^{o}C)
ADL0268	102-116	CTCCACCCCTCTCAGAACTA	CAACTTCCCATCTACCTACT	60
MCW0206	221-249	ACATCTAGAATTGACTGTTCAC	CTTGACAGTGATGCATTAAATG	60
LEI0166	354-370	CTCCTGCCCTTAGCTACGCA	TATCCCCTGGCTGGGAGTTT	60
MCW0295	88-106	ATCACTACAGAACACCCTCTC	TATGTATGCACGCAGATATCC	60
MCW0081	112-135	GTTGCTGAGAGCCTGGTGCAG	CCTGTATGTGGAATTACTTCTC	60
MCW0014	164-182	TATTGGCTCTAGGAACTGTC	GAAATGAAGGTAAGACTAGC	58
MCW0183	296-326	ATCCCAGTGTCGAGTATCCGA	TGAGATTTACTGGAGCCTGCC	58
ADL0278	114-126	CCAGCAGTCTACCTTCCTAT	TGTCATCCAAGAACAGTGTG	60
MCW0067	176-186	GCACTACTGTGTGCTGCAGTTT	GAGATGTAGTTGCCACATTCCGAC	60
MCW0104	190-234	TAGCACAACTCAAGCTGTGAG	AGACTTGCACAGCTGTGTACC	60
MCW0123	76-100	CCACTAGAAAAGAACATCCTC	GGCTGATGTAAGAAGGGATGA	60
MCW0330	256-300	TGGACCTCATCAGTCTGACAG	AATGTTCTCATAGAGTTCCTGC	60
MCW0165	114-118	CAGACATGCATGCCCAGATGA	GATCCAGTCCTGCAGGCTGC	60
MCW0069	158-176	GCACTCGAGAAAACTTCCTGCG	ATTGCTTCAGCAAGCATGGGAGGA	60
MCW0248	205-225	GTTGTTCAAAAGAAGATGCATG	TTGCATTAACTGGGCACTTTC	60
MCW0111	96-120	GCTCCATGTGAAGTGGTTTA	ATGTCCACTTGTCAATGATG	60
MCW0020	179-185	TCTTCTTTGACATGAATTGGCA	GCAAGGAAGATTTTGTACAAAATC	60
MCW0034	212-246	TGCACGCACTTACATACTTAGAGA	TGTCCTTCCAATTACATTCATGGG	60
LEI0234	216-364	ATGCATCAGATTGGTATTCAA	CGTGGCTGTGAACAAATATG	60
MCW0103	266-270	AACTGCGTTGAGAGTGAATGC	TTTCCTAACTGGATGCTTCTG	64
MCW0222	220-226	GCAGTTACATTGAAATGATTCC	TTCTCAAAACACCTAGAAGAC	60
MCW0016	162-206	ATGGCGCAGAAGGCAAAGCGATAT	TGGCTTCTGAAGCAGTTGCTATGG	60
MCW0037	154-160	ACCGGTGCCATCAATTACCTATTA	GAAAGCTCACATGACACTGCGAAA	64
MCW0098	261-265	GGCTGCTTTGTGCTCTTCTCG	CGATGGTCGTAATTCTCACGT	60
LEI0094	247-287	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	60
MCW0284	235-243	GCCTTAGGAAAAACTCCTAAGG	CAGAGCTGGATTGGTGTCAAG	60
MCW0078	135-147	CCACACGGAGAGGAGAAGGTCT	TAGCATATGAGTGTACTGAGCTTC	60
LEI0192	244-370	TGCCAGAGCTTCAGTCTGT	GTCATTACTGTTATGTTTATTGC	60
ADL0112	120-134	GGCTTAAGCTGACCCATTAT	ATCTCAAATGTAATGCGTGC	58
MCW0216	139-149	GGGTTTTACAGGATGGGACG	AGTTTCACTCCCAGGGCTCG	60

106 source: FAO [8]

2.3 Statistical analysis

Genetic diversity and relationship

The polymorphism information content (PIC) was estimated using Powermarker v.3.25 [9]. GenAlEx v.6.5 was used to estimate 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 28 microsatellite markers [10]. Jackknifing across populations using FSTAT produced standard deviation values that were used to obtain tests of significance per microsatellite locus by creating confidence intervals at 95% and 99% [11].

GENETIX 4.05.2 was used to estimate genetic variation per breed (He, Ho) and the average number of alleles [12]. Gene flow [13] was calculated using Powermarker v.3.25 [9]. Pairwise Fst values, which are indications of the fraction of genetic variation attributed to population sub-structuring, were calculated for various population pairs using GenAlEx v.6.5 [10]. Molecular analysis of variance (AMOVA) was computed using GenAlEx v.6.5 for within and among pre-grouped populations [10]. Powermarker v 3.25 was used to assess genotype frequencies for nonconformity with Hardy-Weinberg equilibrium (HWE) in addition to linkage disequilibrium. GenAlEx v.6.5 [10] was used to approximate Nei's standard genetic distances [14] among population pairs. The Neighbour-Joining (NJ) programme was used to develop an unrooted NJ cladogram using the Darwin software (v.6.0) according to pairwise kinship distance matrix between populations [15]. A consensus tree assessed by 1,000 bootstraps all through the group of loci was created.

Population structure

The possible sum of clusters was approximated using the Evanno method [16] as reported by Dent Earl and Bridgett [17]. A set of rules applied in STRUCTURE was used to group entities based on multi-locus genotypes [18]. The evaluation entailed an admixture model alongside

interrelated allele frequencies. During the STRUCTURE analysis, 5 replications of K (presumed sum of subpopulations), extending from 1 to 20 were used together with 100,000 reiterations of Markov Chain Monte Carlo (MCMC) and 50,000 burn-in period in the admixture model. Each estimation of K was redone 5 times to ensure the reproducibility of the outcomes. CLUMPAK (CLUMPAK server), which is a tool used to single out clustering types and bundle population structure deductions across K was used. The Factorial Correspondence Analysis (FCA), which is a multivariate model of analysis, was conducted to observe the associations between entities from unlike zones and to evaluate probable admixtures between the populations. The main variables were the frequencies of alleles at all loci in the populations. The FCA was computed using GENETIX programme [12].

Results

3.1 Genetic diversity

Marker Polymorphism across the studied IC populations

The parameters of the variability of the investigated loci are shown in Table 2. Overall, 305 alleles were noted at 28 microsatellite loci with an average of 10.89 alleles per microsatellite marker. The total sum of alleles ranged from 2 (MCW0037) to 22 (LEI0192). The effective number of alleles (NE) ranged between 1.6504 (MCW0078) and 8.901 (LEI0234), with an overall mean of 3.8194. The PIC ranged from 0.3488 (MCW0103) to 0.8775 (LEI0234). Out of the total number of alleles, 20% were private alleles (60), whereas ADL0112 revealed the maximum sum of private alleles (6). The within-population insufficiency in heterozygosity as determined by F_{IS} factor, extended between −1.00 (MCW0037) and 0.338 (LEI0234) with a mean of 0.041 for all loci. The inbreeding coefficient among populations (F_{IT}) values ranged from -1.00 (MCW0037) to 0.354 (LEI0234), with a mean of 0.089. Global population differentiation evaluated by F_{ST} was estimated at 0.054. The contribution of 28 microsatellites for population segregation was determined by F_{ST} statistics. F_{ST} values varied from 0.000 (MCW0037) to 0.158

157 (ADL0268). The overall F-statistics differed significantly from zero ($p < 0.05$). This
158 differentiation had a significant contribution from all loci. The values for Ho ranged from 0.3015
159 (MCW0165) to 1 (MCW0037), with an overall mean of 0.6155, while the values of He ranged
160 from 0.394 (MCW0078) to 0.8877 (LEI0234), with a general mean of 0.688. The average
161 number of migrants per generation (Nm) in the whole population and across all the loci was
162 found to be 6.06. About 10% of the loci in all IC populations, did not differ considerably from
163 the HWE.

164 **Table 2. Marker Polymorphism and diversity parameters across studied IC populations in Rwanda**

Loci	MAF	NG	NA	NE	NPA	He	Ho	PIC	I	Fis	Fit	Fst	Nm	HWE pV
ADL0112	0.499	27	16	2.720	6	0.632	0.594	0.572	1.318	0.097	0.128	0.034	7.006	0.000
ADL0268	0.245	39	14	6.241	3	0.840	0.582	0.820	2.022	0.176	0.306	0.158	1.332	0.000
ADL0278	0.300	39	12	5.349	4	0.813	0.548	0.789	1.885	0.252	0.283	0.041	5.869	0.000
LEI0094	0.392	45	17	4.360	3	0.771	0.714	0.744	1.867	0.017	0.034	0.017	14.344	0.000
LEI0192	0.317	66	22	5.699	4	0.825	0.775	0.806	2.149	-0.005	0.036	0.041	5.829	0.000
LEI0234	0.177	77	17	8.902	2	0.888	0.569	0.878	2.393	0.338	0.354	0.024	10.202	0.000
MCW0014	0.512	29	10	3.107	1	0.678	0.486	0.645	1.493	0.142	0.263	0.142	1.517	0.000
MCW0016	0.317	39	15	4.699	4	0.787	0.772	0.759	1.841	0.002	0.023	0.021	11.392	0.000
MCW0020	0.305	29	8	4.661	0	0.785	0.720	0.753	1.676	0.050	0.095	0.047	5.027	0.000
MCW0034	0.351	46	14	5.211	5	0.808	0.775	0.788	1.927	-0.003	0.032	0.035	6.965	0.191
MCW0037	0.500	1	2	2.000	0	0.500	1.000	0.375	0.693	-1.000	-1.000	0.000		0.000
MCW0067	0.395	31	11	3.573	1	0.720	0.680	0.679	1.622	0.038	0.137	0.103	2.181	0.000
MCW0069	0.339	26	10	3.671	0	0.728	0.739	0.680	1.503	-0.011	0.028	0.038	6.309	0.104
MCW0078	0.766	11	5	1.650	0	0.394	0.369	0.372	0.820	-0.006	0.006	0.011	21.491	0.015
MCW0081	0.494	42	11	3.001	1	0.667	0.560	0.622	1.483	0.126	0.156	0.034	7.140	0.000
MCW0098	0.465	27	9	2.571	1	0.611	0.523	0.535	1.176	0.105	0.170	0.072	3.212	0.000
MCW0103	0.708	9	6	1.736	2	0.424	0.375	0.349	0.693	0.131	0.160	0.033	7.343	0.000
MCW0104	0.489	43	18	3.271	4	0.694	0.649	0.662	1.701	0.066	0.096	0.033	7.385	0.000
MCW0111	0.595	21	8	2.440	0	0.590	0.483	0.550	1.226	0.110	0.141	0.035	6.800	0.000
MCW0123	0.523	38	14	3.103	3	0.678	0.640	0.650	1.568	0.015	0.031	0.016	15.002	0.000
MCW0165	0.635	7	4	1.924	0	0.480	0.302	0.386	0.755	0.325	0.341	0.024	10.050	0.000
MCW0183	0.292	34	11	5.516	3	0.819	0.659	0.796	1.873	0.119	0.189	0.080	2.885	0.000
MCW0206	0.394	24	9	3.992	2	0.750	0.699	0.714	1.583	-0.004	0.044	0.048	5.000	0.000
MCW0222	0.400	11	6	2.972	2	0.664	0.646	0.600	1.210	-0.030	0.023	0.051	4.641	0.000
MCW0248	0.679	6	4	1.816	1	0.449	0.492	0.366	0.713	-0.236	-0.185	0.041	5.864	0.344
MCW0284	0.368	29	8	3.900	0	0.744	0.689	0.706	1.620	0.050	0.117	0.070	3.321	0.000
MCW0295	0.465	34	13	3.482	3	0.713	0.579	0.680	1.632	0.131	0.214	0.096	2.341	0.000
MCW0330	0.302	26	11	5.376	5	0.814	0.615	0.790	1.827	0.147	0.281	0.157	1.339	0.000
Mean	0.437	30.571	10.893	3.819	2.140	0.688	0.616	0.645	1.510	0.041	0.089	0.054	6.060	
Total			305		60									

MAF, major allele frequency; NG, number of genotypes; NA, number of alleles; NPA, number of private allele; Ne, number of effective alleles; I, Shannon's information index; He, expected heterozygosity; Ho, observed heterozygosity; PIC, polymorphic information content, Nm: number of migrants, F, inbreeding coefficient over all populations (F_{IS}), among populations (F_{IT}) and within populations (F_{ST}), HWE pV, Hardy-Weinberg equilibrium p-value

166 **Genetic diversity indices for IC populations from each agro-ecological zone**

167 Genetic diversity indices for IC from each zone is summarized in Table 3. All the loci were
168 polymorphic. The observed frequencies of heterozygote were statistically similar to the expected
169 one ($p>0.05$), hence, the inbreeding coefficient (F) estimates observed were not substantially
170 different from zero. The mean sum of alleles varied from 5.143 to 8.25. The highest count of
171 alleles (8.2) was found in the Eastern IC population. The highest count of private alleles (21) was
172 observed in the Eastern population, while the NW population did not harbor any private allele.
173 The effective sum of alleles ranged from 3.311 to 3.62. The Shannon Index (I), which is an
174 expression of population diversity in a particular habitat, was high in the SW (1.458) and low in
175 exotic chicken (1.305). Furthermore, the lowest observed heterozygosity was in the CS (0.598)
176 while the highest was recorded in exotic chicken (control) population (0.667). The expected
177 heterozygosity in the populations ranged from0.644 (CN) to 0.680 (SW).

178 **Table 3. Common genetic diversity indices as revealed among IC populations in Rwanda**

Populations	N	%PL	NA	PA	Ne	Ho	He	uHe	F	I
Central North	51	100	6.929	6	3.354	0.623	0.644	0.650	0.021	1.322
Central South	55	100	7.286	15	3.359	0.598	0.661	0.668	0.077	1.372
Exotic chicken	12	100	5.143	4	3.386	0.667	0.665	0.669	-0.019	1.305
East	102	100	8.250	21	3.367	0.611	0.654	0.657	0.056	1.358
North West	52	100	6.500	0	3.311	0.613	0.645	0.651	0.042	1.306
South West	53	100	7.964	14	3.620	0.626	0.680	0.686	0.063	1.458
Total	325	100	7.011	60	3.400	0.623	0.658	0.668	0.040	1.353

179 NA, number of alleles; PA, number of private allele; Ne, number of effective alleles He, expected
180 heterozygosity Ho, observed heterozygosity uHe: unbiased expected heterozygosity F, inbreeding
181 coefficient I, Shannon's information index.
182

183 The p-values of HWE are summarized in Table 4 and confirm that Ho and He do not differ
184 significantly. Thus, taking all the loci into account none of the IC populations diverged from the
185 HWE law.

Table 4: Tests for the Hardy-Weinberg equilibrium probability of loci in the IC population in Rwanda

Locus	North West	Central North	Central South	East	North-south	Exotic chicken
ADL0112	0.551 ns	0.000***	0.000***	0.003**	0.000***	0.028*
ADL0268	0.000***	0.000***	0.163ns	0.000***	0.000***	0.330ns
ADL0278	0.000***	0.000***	0.000***	0.000***	0.003**	0.349ns
LEI0094	0.001**	0.976ns	0.000***	0.051ns	0.001***	0.812ns
LEI0192	0.000***	0.000***	0.000***	0.002**	0.024*	0.913ns
LEI0234	0.000***	0.000***	0.000***	0.099ns	0.000***	0.720ns
MCW0014	0.000***	0.000***	0.000***	0.000***	0.000***	0.634ns
MCW0016	0.012*	0.000***	0.000***	0.239ns	0.108ns	0.200ns
MCW0020	0.048*	0.586ns	0.190ns	0.620ns	0.000***	0.980ns
MCW0034	0.050*	0.735 ns	0.316ns	0.000***	0.816ns	0.412ns
MCW0037	0.000***	0.000***	0.000***	0.000***	0.000***	0.001***
MCW0067	0.000***	0.000***	0.870ns	0.000***	0.000***	0.095ns
MCW0069	0.965ns	0.529ns	0.971ns	0.967ns	0.295ns	0.279ns
MCW0078	0.911ns	0.251ns	0.985ns	0.232ns	0.003**	0.916ns
MCW0081	0.739ns	0.000***	0.000***	0.000***	0.000***	0.004**
MCW0098	0.681ns	0.000***	0.000***	0.000***	0.000***	0.005**
MCW0103	0.012*	0.752ns	0.000***	0.913ns	0.000***	0.574ns
MCW0104	0.001**	1.000ns	0.355ns	0.000***	0.755ns	0.213ns
MCW0111	0.046*	0.189ns	0.127ns	0.003**	0.687ns	0.545ns
MCW0123	0.503ns	0.909ns	0.000***	0.002**	0.000***	0.003**
MCW0165	0.540ns	0.000***	0.004**	0.000***	0.018*	0.327ns
MCW0183	0.000***	0.010*	0.000***	0.000***	0.012*	0.001**
MCW0206	0.590ns	0.020*	0.009**	0.908ns	0.000***	0.658ns
MCW0222	0.000***	0.096ns	0.000***	0.783ns	0.968ns	0.283ns
MCW0248	0.429ns	0.922ns	0.057ns	0.991ns	0.247ns	0.035*
MCW0284	0.121ns	0.021*	0.846ns	0.000***	0.000***	0.437ns
MCW0295	0.279ns	0.000***	0.017*	0.000***	0.046*	0.015*
MCW0330	0.633ns	0.992ns	0.000***	0.000***	0.150ns	0.001***

ns, not significant, * P<0.05, ** P<0.01, *** P<0.001

188 Analysis of molecular variance (AMOVA) revealed that ninety-two percent (92%) of the total
189 variation originated from variation within populations (Table 5).

190 **Table 5. Analysis of molecular variance of all loci for the IC population in Rwanda**

Source	Degree of freedom	Sum square	Mean square	Estimated variances	% of estimated variances
Among Populations	5	574.201	114.840	1.838	8%
Within Populations	319	6346.643	19.895	19.895	92%
Total	324	6920.843		21.733	100%

191

192 **3.2 Genetic relationship**

193 The matrix of pairwise genetic distances between populations (Table 6 and Fig 1) showed a
194 low genetic distance (0.029) between NW and CN populations. A similar trend was observed
195 in SW and CS (0.048). On the other hand, by considering only the IC populations, the highest
196 genetic distance was observed between E and SW populations (0.125). The genetic distance
197 between the IC population in Rwanda and exotic chicken was relatively high (0.231).

198 **Table 6. Genetic distance among the IC population in Rwanda**

Populations	North West	Central North	Central South	Exotic chicken	East
Central North	0.029				
Central South	0.094	0.077			
Exotic chicken	0.199	0.213	0.231		
East	0.112	0.097	0.117	0.196	
South West	0.104	0.092	0.048	0.118	0.125

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200

201

202 The extent of genetic distinction among the population with regard to allele frequencies (F_{ST})
203 and gene flow (Nm) are presented in Table 7. The results revealed a low genetic differentiation

and a high gene flow between CN and NW, and likewise between SW and CS. A relatively high gene differentiation, however, was found between the E population and other populations.

Table 7: Gene flow (upper diagonal) and Gene differentiation (lower diagonal)

Populations	Central North	Central South	Exotic chicken	East	North West	South West
Central North		2.304	1.412	2.051	6.274	2.040
Central South	0.022		0.925	1.471	1.533	3.847
Exotic chicken	0.052	0.058		3.432	1.188	2.791
East	0.025	0.027	0.050		1.783	1.560
North West	0.012	0.026	0.053	0.028		1.471
South West	0.026	0.014	0.036	0.028	0.027	

The phylogenetic relationship by the Neighbour-Joining tree showed four (4) IC genetic clusters, namely I, II, III and IV (Fig. 2). The eastern population stands alone unlike the other populations: IC populations from the NW clustered together with those from the CN. Few individuals from the SW population clustered together with the exotic chicken in group III, and finally the rest of SW individuals clustered with those from the CS in group II (Fig 2).

3.3 Population structure

Data from the Bayesian cluster analysis showed the existence of four (4) main gene pools in the whole IC population in Rwanda. The highest value for ΔK was obtained for K = 4 (Table 8 and Fig 3). The first gene pool (I) was composed of CN and NW populations. The second gene pool (II) was made of the Eastern population only. The third (III) included individual from SW and CS and the fourth gene pool (IV) was composed of the remaining individuals of SW and exotic chicken. A high proportion of the admixture was observed in the gene pool III.

Table 8: Determination of the number of clusters (K) based on the progression of the average estimate of Ln likelihood of data in IC populations in Rwanda

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	5	-27680.120000	0.192354	—	—	—
2	5	-26645.700000	81.765916	1034.420000	301.520000	3.687600
3	5	-25912.800000	30.968694	732.900000	82.920000	2.677543
4	5	-25262.820000	3.056469	649.980000	558.300000	182.661785
5	5	-25171.140000	37.017671	91.680000	21.920000	0.592150
6	5	-25057.540000	46.761341	113.600000	19.200000	0.410596
7	5	-24963.140000	9.161496	94.400000	81.200000	8.863182
8	5	-24949.940000	63.605566	13.200000	55.340000	0.870050
9	5	-24881.400000	42.680968	68.540000	29.880000	0.700078
10	5	-24842.740000	77.738491	38.660000	87.640000	1.127369
11	5	-24891.720000	114.353824	-48.980000	14.060000	0.122952
12	5	-24954.760000	210.975195	-63.040000	330.240000	1.565302
13	5	-24687.560000	104.370245	267.200000	510.500000	4.891241
14	5	-24930.860000	402.389690	-243.300000	41.440000	0.102985
15	5	-25132.720000	914.525050	-201.860000	542.960000	0.593707
16	5	-24791.620000	296.572178	341.100000	183.320000	0.618129
17	5	-24633.840000	54.568333	157.780000	129.560000	2.374271
18	5	-24605.620000	64.775126	28.220000	204.760000	3.161090
19	5	-24782.160000	498.369745	-176.540000	100.700000	0.202059
20	5	-24858.000000	559.214181	-75.840000	—	—

The results of the Factorial Correspondence Analysis (FCA) are shown in Fig 4. It showed tree clusters whereby the Eastern region was still standing alone. NW and CN populations clustered together. Finally, the majority of individuals from the CS, SW and exotic chicken were in the same group.

Discussion

4.1 Genetic diversity

The average PIC was the best index to estimate the polymorphism of alleles [19]. It showed that more information could be obtained from the loci when PIC>0.5. On the other hand, 0.25<PIC<0.5 was an indication of a moderately instructive locus, whereas PIC<0.25 indicated a vaguely informative locus [20]. In this study, 82.3% of all loci were highly informative, which verified that they were suitable for estimating the genetic diversity of IC populations in Rwanda. The highest value of PIC (0.87) was that of LEI0234 and the mean PIC was 0.6451. The PIC values found in this study exceeded those (0.29-080) of Cameroon's IC [21], and (0.31-0.49) of Chinese IC [21,22], but lower than those obtained by Tang for black-bone IC breeds (0.67) [24]. The mean frequency of alleles per marker found in this study (10.89) exceeded those recorded in previous reports in Cameroon (9.04) [21],in Ghana (7.8) [25], in Iran (5.4) [26], in China (3.8) [27], in Egypt (7.3) [28], in Pakistan (9.1) [29] and in Vietnam (6.41) [30]. The values obtained in this study were, however, lower than those from Brazilian (13.3) [31] and in the same range as from Ethiopian chicken ecotypes (10.6) [32].

The mean number of effective alleles (3.81) obtained in the current study was higher than 3,13 observed in Cameroon [21] and Indian chicken [33]. Heterozygosity can also be considered in genetic diversity. The degree of mean population heterozygosity is an indication of the level of population constancy. Low population heterozygosity informs high population genetic constancy [34]. The present study indicated that Ho of the different IC population varied from 0.3015 to 1 with an overall mean value of 0.6155, while He ranged from 0.394 to 0.887 with an overall average of 0.688.

This study also discovered that the values of Ho and He were similar. As a result, there was no significant difference between zero and the resultant F estimates (0.040), which suggested that the IC populations were in HWE. An implication of this supposition is that the population is under artificial selection, which is indicative of population stability. However, the little variation observed between Ho and He could be attributed to discrepancies in sample size, location, population composition, and the origin of microsatellite markers [35].

The IC populations in Rwanda had a similar level of diversity as their Ethiopian [36], Egyptian [28] and Cameroonian [22] counterparts, but had lower and higher diversity than those observed in southern China [23] and European and Asian IC breeds [25], respectively.

Among Rwanda IC, all populations showed a significantly high degree of inbreeding, which could have an impact on trait fixation in the populations. This degree of inbreeding exceeded that observed for Yunnan IC breeds (0.25) [22] and Turkish IC (0.301) with 10 SSR loci [35].

F_{ST} value (0.054) revealing the diversity between IC populations in Rwanda was higher than 0.048 for Ethiopian IC ecotypes [37] and (0.003-0.040) for Kenyan IC [38] and lower than 0.080 found in Cameroonian IC [21].

4.2 Genetic relationships

Wright's F-statistics strictures showing the inbreeding coefficient in this study was 0.041, which was higher than 0.03 found in Cameroon [21], but was similar to values obtained in many Chinese IC [24, 27]. The F_{ST} permits the approximation of migratory entities in a population per generation (Nm) based on loci. In IC populations in Rwanda, Nm varied from 1.332 to 21.491, with an average of 6.060. This value was higher than that obtained in Cameroun [21].

The number of private alleles (PA) distributed all through the ecotypes showed that there was genetic diversity between populations. In this study, the number of PA was higher in the East

(21) followed by CS (15) and SW (14). The NW population, however, did not exhibit any private allele (0). Despite, the number of private alleles being a good indicator of population relationship and structure, further studies need to be carried out to identify possible traits that may be controlled by these private alleles. The total number of private alleles in this study (60) was higher than that found in Cameroun [21].

Findings from AMOVA showed the largest portion of the genetic variation in IC populations in Rwanda existed in individuals within the population (92%). A comparable trend was noted in the Ethiopian [31] and Cameroonian [21] IC ecotypes. The quality of the product, cultural uses of chicken, and the ease with which chicken adapts to the environment are the factors that motivate small-scale farmers to rear IC. These factors highlight the importance of within-population diversity as a key incentive in the rearing of IC [39].

Genetic distance within a population is a useful indicator of separation between various sub-populations. The key assumption of Nei's standard genetic distance is that hereditary dissimilarities are caused by mutations and genetic drift, whereas Reynolds distance assumes that the increase of genetic differences is due to genetic drift only. The genetic distance between IC populations in SW and CS as well as between NW and CN were not significantly different ($P>0.05$). It was noted that these regions border each other, thereby implying that there is a high likelihood of sharing genetic materials. Another possible explanation is that these regions could be highly favorable to the IC population or IC populations in these regions could be big enough to prevent mutation and genetic drift. The genetic distances reported in this study fluctuated from 0.029 to 0.213. These values are in the range of those found in Egyptian IC [28] and in Chinese IC populations [40]. They are, however, higher than those observed in Chinese Bian chicken [23].

When estimating genetic differentiation using allele frequency in such scenarios, the genetic variance between populations can be explained by four major forces, namely, selection, mutation, migration, and genetic drift-[35]. Even though mutation plays a critical role in the long term, short-term evolution is mainly influenced by genetic drift in cases where populations segregated by reproduction[41]. IC populations showed segregation by distance and appeared to be at equipoise under the influence of dispersal and genetic drift. There is a high likelihood that these chickens arrived at their current locations earlier than it had been assumed because there was insufficient time for segregation through distance to come into operation. Furthermore, long-distance gene dispersion is not satisfactorily evident to deter genetic deviation. For this, further investigations need to be conducted using more markers, for example, high-density SNP arrays and mitochondrial DNA.

4.3 Population structure

The genetic similarity in a collection of breeds with high diversity can be resolved efficiently by cluster analysis, which facilitates the identification of individuals with similar or diverse multi-locus genotypes [42]. In our study, the cluster based on the neighbour-joining approach revealed grouping arrays of association and genetic relationships among individuals. These individuals were grouped in four clusters formed by ecotypes from distinct collection sites (NW and CN; SW1 and CS; SW2 and control and finally, East stands alone). This was confirmed by the STRUCTURE analysis which revealed four gene pools across IC in Rwanda. These gene pools are distributed exactly according to the different clusters as shown by the neighbour-joining method. The observed gene pools could be accounted for by the sum of private alleles recorded in the population besides the genetic distance between populations. For example, the Eastern region recorded the highest frequency of private alleles, whereas the NW had the lowest number.

319 This observation could be attributed to the large population of IC in the Eastern region out of all
320 the study sites, which minimized gene inflow in this area. Conversely, the lowest number of IC
321 was noted in the NW region, which could be interpreted to mean that the majority of people in
322 this area either buy chicken or exchange cocks from the neighbouring areas such as CN.
323 Consequently, there is a high influx of genes in these regions This is not surprising since these
324 areas border each other geographically. These findings corroborated the observations of a study
325 conducted in Kenya where the Mantel test had uncovered a positive association between
326 hereditary and geographic distances [43]. Our study also confirmed that geographic distances
327 affected the population's genetic structure [43]. The portion of SW chicken populations that
328 clustered with the exotic chicken (control) could be attributed to the fact that different crossing
329 programmes between IC and improved chicken breeds have been introduced in that region to
330 improve the genetic potential of IC in Rwanda [44].
331
332 **Conclusion**
333 The results portrayed by this study are the first to recount the genetic diversity and constitution
334 of IC from Rwanda. Overall, the IC populations in Rwanda had high levels of significant genetic
335 variability as per different genetic diversity parameters applied in this study. Therefore, data on
336 genetic diversity estimated by assimilating within and between population variances may inform
337 preservation strategies and the better establishment of priorities. In addition, this study found that
338 IC in Rwanda belongs to four major gene pools that could be preserved independently to uphold
339 their genetic diversity. Generally, these findings provide the fundamental step in the direction of
340 judicious decision-making before the development of genetic enhancement and preservation
341 programmes without interfering with the uniqueness of IC in Rwanda.

342

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355

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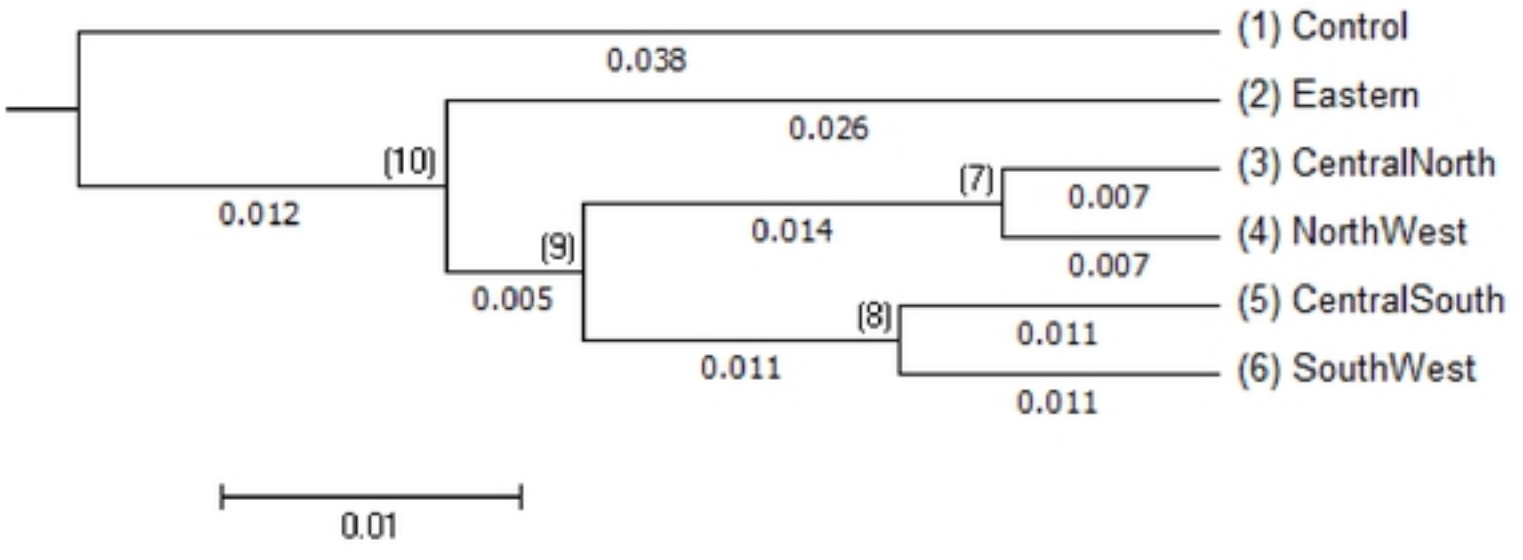

Fig 1: Neighbour-Joining Pair-wise of the IC population in Rwanda

fig1

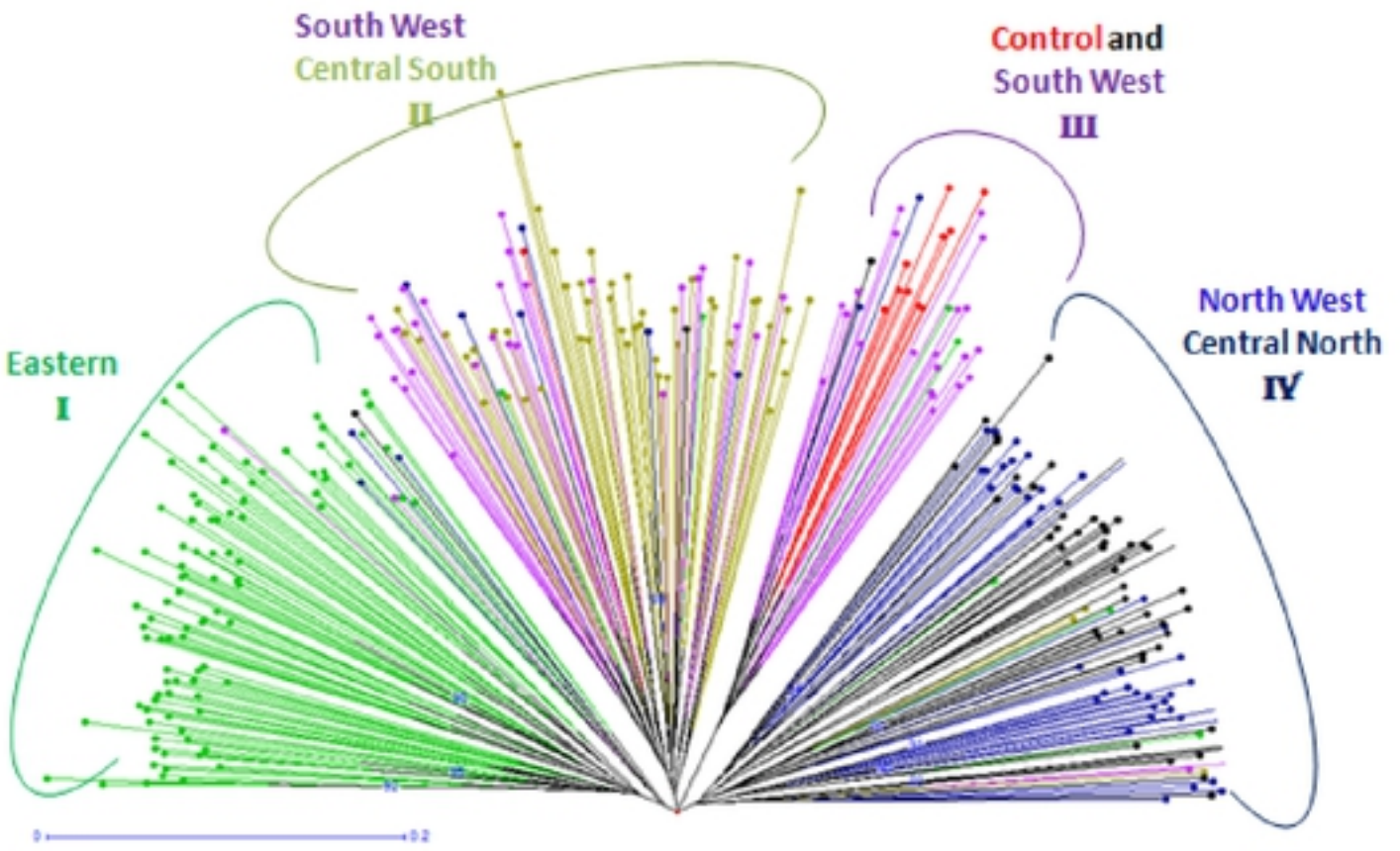

Fig. 2: Neighbour-Joining tree of the clustering pattern among IC populations in Rwanda

fig2

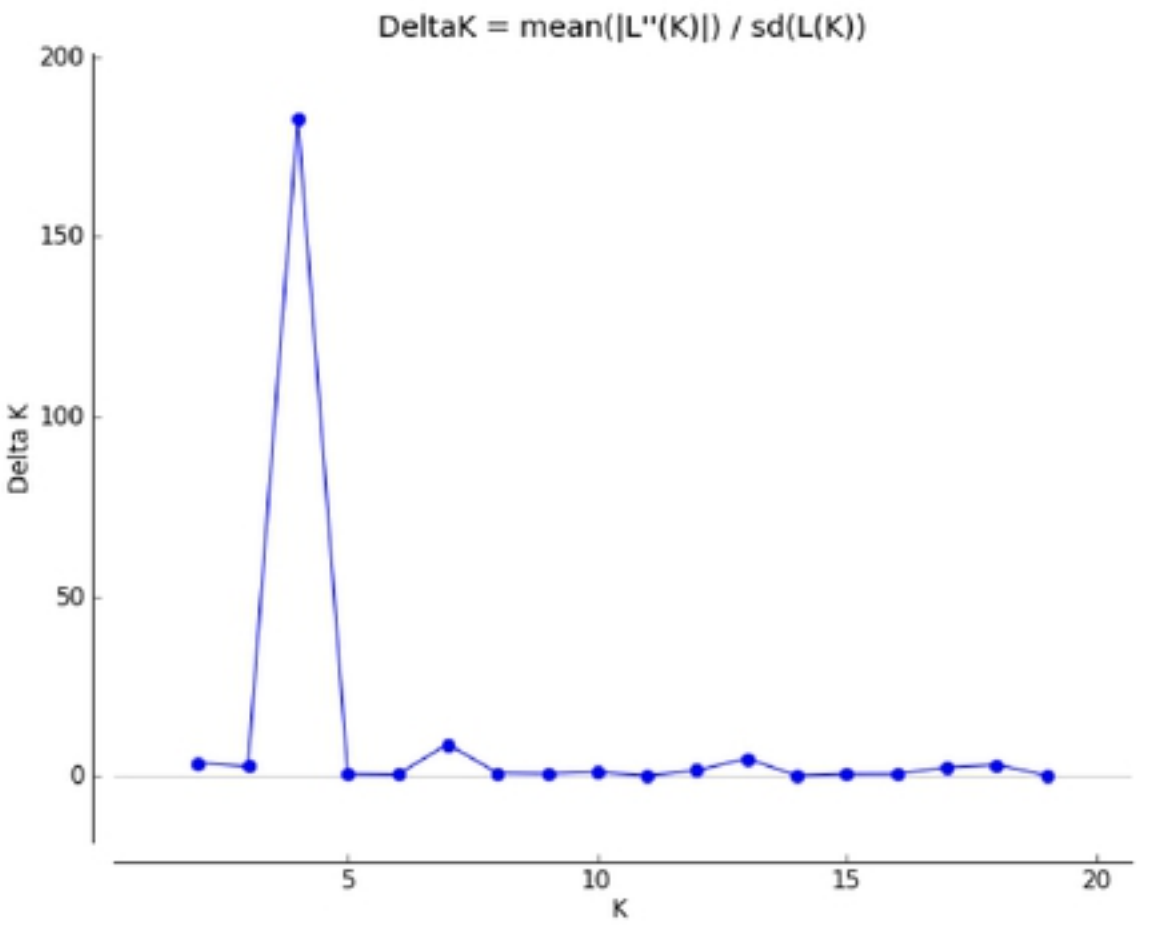

Fig 3: Determination of ΔK approximating the more possible number of clusters in IC populations in Rwanda

Fig3

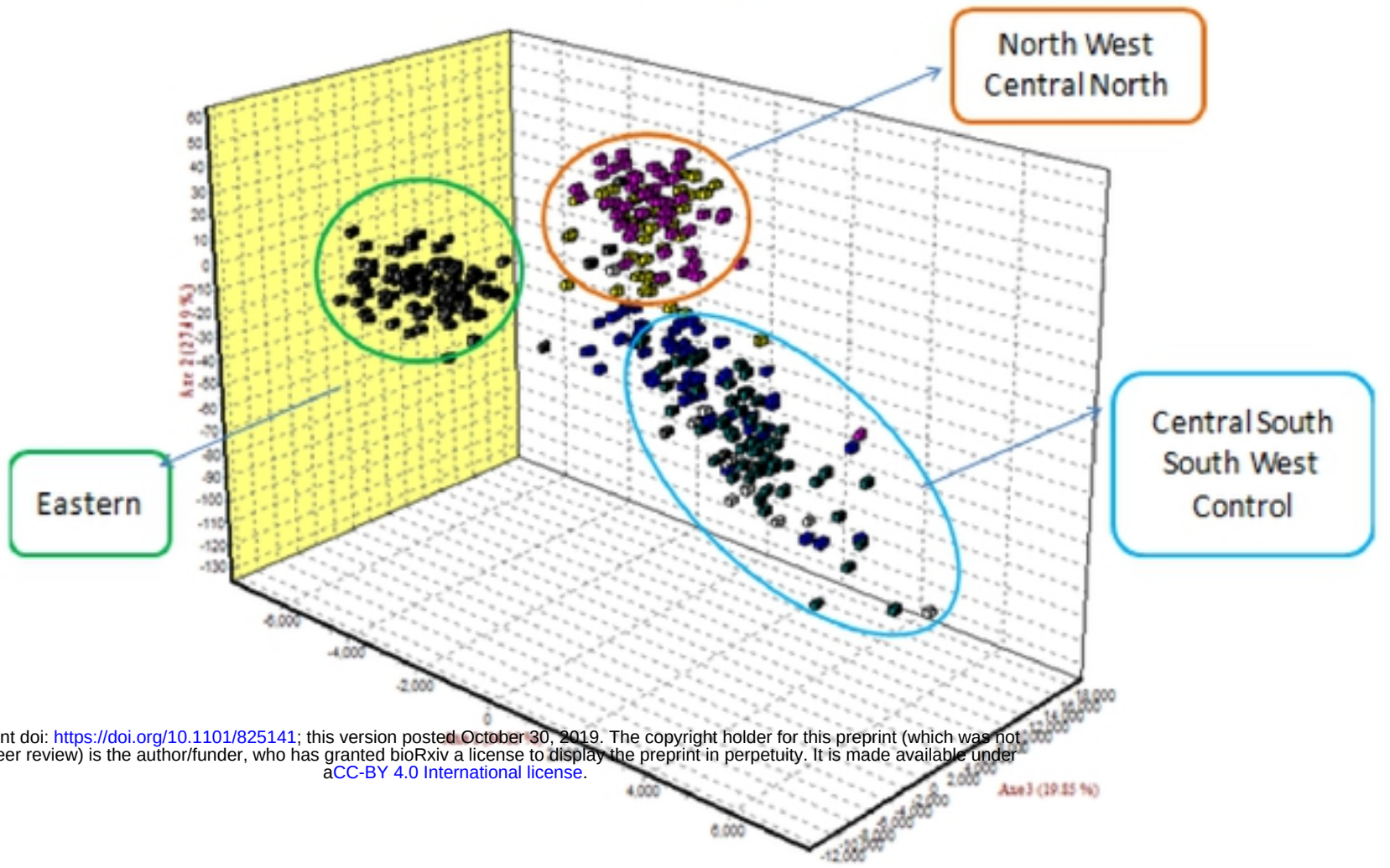

Fig 4. Factorial correspondence analysis

Fig4