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Genetic Diversity of Indigenous Chicken (Gallus *Gallus domesticus*) from Ecozones of Egypt and Kingdom of Saudi Arabia

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Abstract

Genetic diversity of two chicken ecotypes from Ismailia-Egypt (ISM) and Taif-Saudi Arabia (TA) was evaluated using 39 microsatellites. DNA was extracted from blood of 25 chickens/ecotype. The number of alleles was 157 and 138, the number of alleles/locus averaged 4.2 ± 2.2 and 3.6 ± 1.6, and the highest number of private alleles was 9 and 5 for ISM and TA, respectively. Percentage of shared alleles between the two ecotypes was 45%. This panel of markers is reasonably informative as the mean polymorphic information content for ISM and TA was 0.47 ± 0.21, and 0.41 ± 0.2. Similar average of observed heterozygosity was attained for both ecotypes. Conversely, averages of expected heterozygosity differed between two ecotypes, 0.52 ± 0.23 vs. 0.45 ± 0.21 for ISM and TA. 8 and 12 loci have significantly deviated from HWE of ISM and TA. Estimate of genetic distance was 0.2 and F_{ST} index was 0.29. Results showed only 6% of genetic diversity is shared between these two ecotypes.

Keywords

Ecotypes, Genetic Diversity, Microsatellite, Polymorphic Information Content, Hardy Weinberg Expectation

1. Introduction

Chickens' ecotypes are being utilized in rural and backyard farming of developing countries all over the world. The significance of these ecotypes in the rustic economy is tremendous in various counties [1]-[7]. This significance is due to

affordable low production cost, scavenging competency as well as adaptability to harsh and stressful environmental conditions [8] [9]. Moreover, chicken ecotypes supply work security to the family as well as getting the accessibility of food. Jobless youth and ladies can likewise acquire pay through poultry cultivating [10]. Still, local ecotypes have low egg productivity and light mature body size [11] [12].

The poultry industry is globally dominated by commercial chicken breeds, where breeding strategy is based on few chicken genotypes. Such a strategy has the shortcoming of erosion of valuable genetic resources (ecotypes) [13]. Their low productivity does not meet the global demands of intensive production systems, as such ecotypes could barely compete with commercial breeds. Therefore, ecotypes are negatively selected regardless of their good quality of egg and meat, disease resistance as well as adaptation to the local environment [13]. The establishment of a framework for preserving these genetic resources is of need [8]. Investigating the genetic makeup of indigenous chicken breeds was carried out in several studies [11] [14] [15] [16] and has been used as a sampling guide in genetic diversity research [17].

At Taif governorate (~1.7 km above sea) local chickens are acclimated to the rough environment of high altitudes, such as low oxygen tension, as well as daily temperature fluctuations [18] [19] [20] [21].

Microsatellites (MS) markers are normally used because they are plentiful, codominant, randomly spread throughout the genome, and very polymorphic, also because of their reproducibility [22] [23]. This coherence has made MS markers a dependable tool for genetic diversity evaluation and assessment in many commercial chicken breeds and ecotypes [13] [18] [19] [20]. Quite a few MS markers are available in chickens and have been utilized to develop linkage maps in numerous chicken breeds [22] [24]. These markers introduced efficient machinery to QTL research and have additionally been effectively utilized to consider the hereditary associations among and within chicken populations [23].

Osaman *et al*., [25] utilized the complete sequence of mitochondrial DNA D-loop to explain the genetic descent of Egyptian indigenous chicken and Asian chicken. Results of this study revealed that both Egyptian native chicken and West and Central Asian chicken are sharing the same common ancestor as they branched together in the same clade. However, this work did not include native chicken strains from Saudi Arabia. Therefore, the current study was carried out as an attempt to introduce a better understanding of the genetic characterization of 2 ecotypes sampled from two different ecozones at Taif (Saudi Arabia) and Ismailia (Egypt). A dense microsatellites panel of 39 markers was genotyped for two ecotypes.

2. Materials and Methods

2.1. Sample Collection

Chicken samples were collected from individual framers in local village either in

Ismailia (ISM) or Taif (TA) with same age as possible. From each location 25 chicken were collected and blood sample collected from chicken using EDTA contain collected tube.

2.2. DNA Extraction

Collected blood samples were used for DNA isolation using DNA extraction kit (QIAGEN) according to manufacture manual. Extracted DNA quality checked by electrophoresis in a minigel while the quantity checked spectrophotometrically using NanoDrop 2000C (Spectronic Genesys, Thermo Electron Corporation). DNA samples were stored at −20˚C until microsatellites analysis.

2.3. Microsatellite Genotyping

Thirty nine MS markers were utilized to assess DNA polymorphism of these 2 ecotypes. These MS markers were chosen based on reports by the International Society of Animal Genetics (ISAG)-FAO to think about the hereditary assorted variety of chickens [26] and additionally 150 microsatellites were investigated for the segregation of five thoroughbred KNC lines [20]. Names of each of the 39 MS markers, chromosome number and respective allelic size are appeared in **Table 1**.

Table 1. Marker's name, Chromosomal number (Chr) and allele size (bp) of 39 genotyped Microsatellites markers.

Marker	Chr	Allele size(bp)	Marker	Chr	Allele size(bp)
MCW248	1	205 - 225	MCW029	5	139 - 189
LEI141	2	220 - 242	MCW014	6	164 - 182
MCW087	2	267 - 283	MCW183	7	296 - 326
MCW063	2	132 - 150	ROS019	7	119 - 143
LEI234	2	217 - 315	ADL278	8	114 - 126
MCW206	2	226 - 240	GCT016	9	108 - 154
MCW288	2	108 - 122	ADL259	9	106 - 146
MCW264	2	224 - 240	MCW067	10	175 - 184
MCW127	3	227 - 241	MCW228	10	221 - 239
MCW016	3	134 - 146	MCW216	13	139 - 149
MCW037	3	152 - 156	MCW104	13	189 - 225
MCW222	3	221 - 225	MCW213	13	288 - 316
LEI166	3	354 - 370	MCW123	14	79 - 89
MCW098	4	261 - 265	ADL293	17	105 - 119
LEI094	4	254 - 280	ADL304	18	137 - 159
ADL317	4	178 - 204	MCW165	23	114 - 118
MCW295	4	88 - 106	LEI074	26	224 - 240
ROS013	5	220 - 242	MCW069	26	158 - 176
MCW078	5	135 - 147	LEI135	28	131 - 142
ADL292	5	110 - 138			

Polymerase Chain Reaction (PCR) amplifications were in absolute volume of 20 μL, 50 ng of genomic DNA, 10 pmol of fluorescent dye (FAM, VIC, NED, PET) labeled modified forward primer and normal reverse primer (Applied Biosystems, USA), 2.5 mM of each dNTPs (GeNet Bio, Korea), 10 X reaction buff (GeNet Bio, Korea), 2.5 unit of prime Taq DNA polymerase (GeNet Bio, Korea). The PCR was performed in an initial denaturation at 95C for 10 min followed by 35 cycles of 30 sec of denaturation at 95C, 30 sec of annealing at 60C, 30 sec of extension at 72C and final extension at 72C for 10 min using My-Genie 96 Thermal Cycler (Bioneer, Korea). The PCR products were initially electrophoresis on 3% agarose gel with ethidium bromide (EtBr) and confirmed whether they gave single PCR DNA band under the UV light. When the bands were clearly appeared, further genotyping will be performed. For the microsatellite genotyping, more than 20 times diluted PCR products will be used. The genotyping reaction contained 1 μL of diluted PCR products, 10 μl of Hi-Di Formamide (Applied Biosystems, USA) and 0.1 μL of GeneScan-500 LIZ size standard marker (Applied Biosystems, USA). After dilution, genotyping reaction mixture was denatured for 2 min at 95C and fragment analysis was performed using capillary array in Genetic analyzer 3130xl (Applied Biosystems, USA). The MS genotypes will be identified using GeneMapper ver.3.7 (Applied Biosystems, USA).

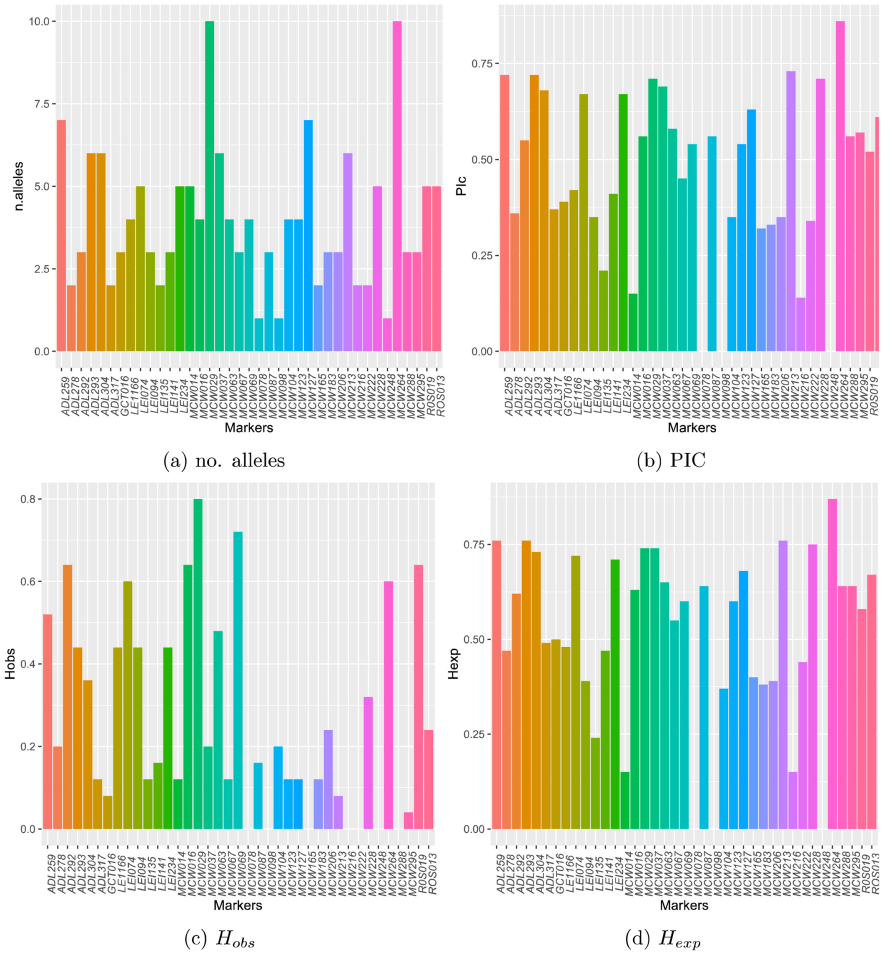
3. Data Analyses

Exploratory data analysis and graphical representations of the results were carried out under R statistical environment R Core Team [27]. Fundamental measures of genetic diversity, such as total number of alleles, allele frequencies, mean number of alleles, observed, and expected heterozygosity (Nei, 1987) were computed using adegenet R statistical package [28]. Deviation from Hardy-Weinberg equilibrium (HWE) using chi square test, and phylogenic analysis using Nei's distance [29] as well as population subdivision was examined using Weir and Cockerham [30] unbiased estimator of Wrights fixation indices (F_{IT}, F_{ST} and F_{IS}) [31] was carried out using "pegas" package R Population and Evolutionary Genetics Analysis System [32]. The null hypothesis was that the estimates were not significantly different from zero, and the level of significance ($P < 0.01$) was adjusted using Bonferroni correction. Allelic richness number was estimated using hierfstat package [33]. Phylogenetic analysis was carried out using APE package [34].

4. Results and Discussion

Figure 1 & **Figure 2** show a descriptive overview of number of alleles per locus, Polymorphic Information Content (PIC) observed and expected Heterozygosity (H_{obs} and H_{exp}) for both ISM and TA ecotypes.

For the number of alleles per locus a considerable difference was observed in both ecotypes. The ISM ecotype has a sum of 157 alleles, number of alleles per



(a) no. alleles (b) PIC

(c) H_{obs} (d) H_{exp}

Figure 1. (a) Number of Alleles, (b) Polymorphic Information Content "PIC", (c) Observed heterozygosity "H_{obs}" and (d) Expected heterozygosity "H_{exp}" of ISM ecotype.

locus ranged from 1 to 10 alleles, averaged 4.2 ± 2.2. The TA ecotype exhibited a lower number of alleles sum 138, the number of alleles per locus ranged from 1 to 7 averaged 3.6 ± 1.6. These estimates are lower than the reported on other studies, e.g. Abebe *et al.*, [13] on Swedish native chicken (4.7), Muchadeyi *et al.*, [16] on Zimbabwe ecotypes (9.7), Van-Marle-Koster *et al.*, [35] on South Africa native chicken (6.1), and Ramadan *et al.*, [36] on native Egyptian and commercial chicken breeds (7.7). These lower estimates of the present study could be attributed to utilizing a larger number of loci. The number of alleles per locus could also be represented as "allelic richness". The number of observed alleles is basically a function of sample size. Allelic richness (R_t) was estimated across all loci (**Table 2**). One definition of R_t is the mean number of alleles per locus [37]. The significance of R_t is that decrease in R_t might result in reducing ability of the population to acclimate to future environmental changes, as such an assorted variety is the rough material for evolutionary forces, for example, common determination [38].

The mean of polymorphic information content (PIC) for ISM and TA ecotype were 0.47 ± 0.21, and 0.41 ± 0.2. This means that this penal of markers are

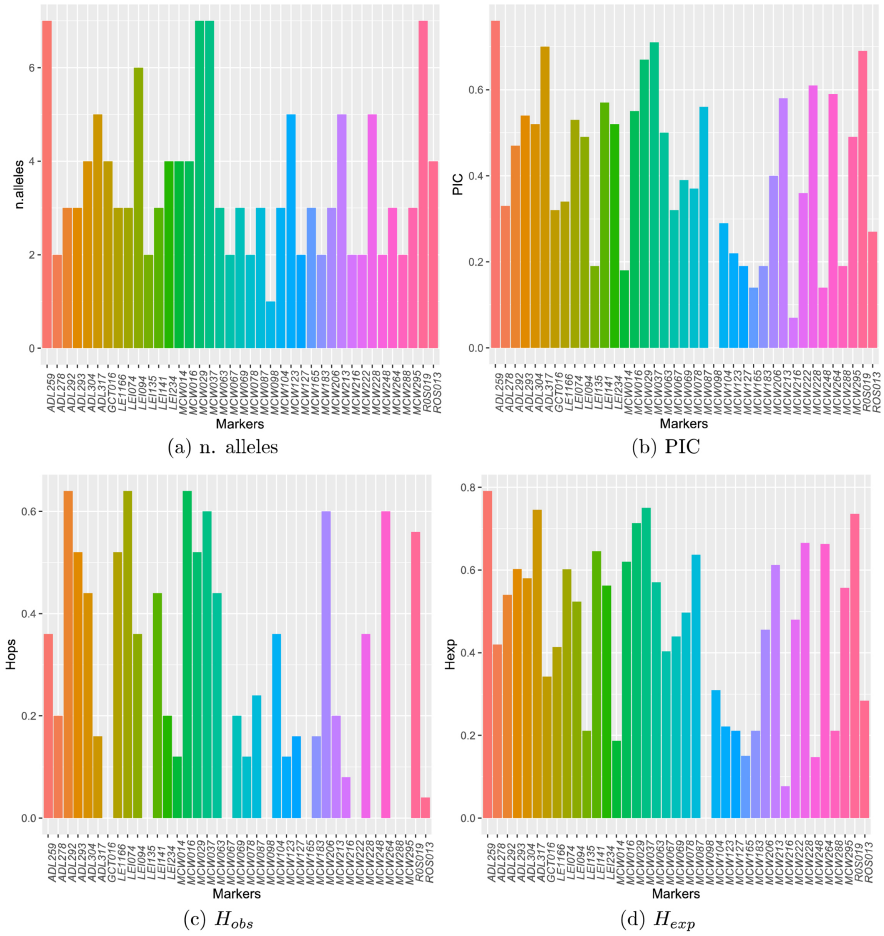


Figure 2. (a) Number of Alleles, (b) Polymorphic Information Content "PIC", (c) observed heterozygosity "H_{obs}" and (d) Expected heterozygosity "H_{exp}" of TA ecotype.

reasonably informative as PIC exceeds 0.25 Botstein *et al.*, (1980). For ISM ecotype 54% of markers are highly informative as PIC was higher than 50%, where only 38% of TA's markers were highly informative. The mean value of PIC in this study was smaller than the estimates of other studies on different native breeds e.g., Seo *et al.*, [20] estimate of PIC was 0.77 on five native Korean lines, Ramadan *et al.*, [36] reported 0.65 on six Egyptian native breeds, and Choi *et al.*, [39] on commercial Korean native breed reported mean PIC value of 0.68, and Soltan *et al.*, [40] estimation of PIC was 0.84 on Sinai and Norfa Egyptian native chicken. However, lower estimate of 0.55 was reported by Abebe *et al.*, [13] on five local Swedish breeds. Again, these differences in mean value of PIC could be ascribed to the dense panel of markers were used in the present study.

The presence of private alleles was also investigated (**Table 3**). Only 4 markers did show private alleles between the two ecotypes, namely ADL278, MCW087, MCW222 and MCW295. For ISM ecotype the highest number of private alleles was 9, where the highest number of private alleles for TA ecotype was only 5. Existence of private alleles might be indicating of diversifying selection of these specific alleles. In general, the percentage of shared alleles between the two

Table 2. Allelic Richness (R_t) of genotyped Microsatellites markers across ISM and TA ecotypes.

Marker	ISM	TA	Marker	ISM	TA
MCW222	2	2	MCW295	6	5
MCW165	2	3	ADL293	6	3
ADL304	6	4	MCW123	8	5
ROS013	5	4	MCW014	5	4
MCW127	7	2	GCT016	3	4
LEI141	3	3	MCW104	4	3
MCW078	1	2	ADL259	7	7
LEI234	5	4	MCW098	1	1
MCW216	2	2	ADL292	3	3
MCW016	4	4	MCW288	3	2
MCW069	4	3	MCW037	6	7
MCW087	3	3	MCW228	5	5
MCW063	4	3	R0S019	5	7
MCW248	1	2	LEI074	5	3
MCW067	3	2	LEI135	2	2
ADL278	2	2	LEI094	3	6
MCW183	3	2	ADL317	2	5
LE1166	4	3	MCW213	6	5
MCW206	3	3	MCW264	10	3
MCW029	10	7			

Table 3. Number of private alleles of genotyped Microsatellites markers across ISM and TA ecotypes.

Markers	ISM	TA	Markers	ISM	TA
MCW165	0	1	MCW123	1	2
ADL304	4	2	MCW014	3	2
ROS013	3	2	GCT016	3	4
MCW127	5	0	MCW104	3	2
LEI141	2	2	ADL259	3	3
MCW078	1	2	MCW098	1	1
LEI234	2	1	ADL292	1	1
MCW216	1	1	MCW288	2	1
MCW016	4	4	MCW037	2	3
MCW069	2	1	MCW228	3	3
MCW063	2	1	R0S019	3	5
MCW248	0	1	LEI074	2	0
MCW067	1	0	LEI135	1	1
MCW183	2	1	LEI094	1	4
LE1166	3	2	ADL317	0	3
MCW206	1	1	MCW213	5	4
MCW029	5	2	MCW264	9	2
ADL293	4	1			

ecotypes was 45%. The incidence of heterozygous individuals in population is measured by the observed proportion of heterozygosity (H_{obs}). A similar average values of (H_{obs}) for both ecotypes were attained although narrower range of values were found for TA ecotype. For the ISM ecotype the H_{obs} ranged from 0.0 to 0.8 with average of 0.27 ± 0.24. For the TA ecotype the H_{obs} ranged from 0.0 to 0.64 with average of 0.27 ± 0.23. The expected heterozygosity (H_{exp}) is an estimate of the likelihood that a pair of gametes arbitrary picked from genetic stock are of various alleles. In the contrary to H_{obs} the averages of H_{exp} differed between the two ecotypes, 0.52 ± 0.23 vs. 0.45 ± 0.21 for ISM and TA. This variability of the two parameters might be result of variation in evolutionary process on each of the two ecotypes.

Test for deviation from Hardy Weinberg Equilibrium was also carried out. For the ISM ecotypes only 8 loci were significantly deviated from Hardy Weinberg expectation, where 12 loci of TA ecotype.

Breaking up the genetic diversity within and among populations is best described through Wright's F-statistics (Wright, 1951). Table 4 shows Wright's fixation indices (F_{ST}, F_{it}, and F_{IS}) across ISM and TA ecotypes for the genotyped microsatellites markers. Figure 3 shows a boxplot for the estimates of Wright's

Table 4. Wright's fixation indices (F_{ST}, F_{it}, and F_{IS}) across ISM and TA ecotypes for the genotyped Microsatellites markers.

Marker	F_{ST}	F_{it}	F_{is}	Marker	F_{ST}	F_{it}	F_{is}
MCW222	0.01	0.01	1	MCW295	−0.01	−0.03	0.98
MCW165	0	0	1	ADL293	0.05	0.1	0.42
ADL304	0.07	0.12	0.53	MCW123	0.02	0.03	0.84
ROS013	0.12	0.22	0.82	MCW014	−0.02	−0.03	0.8
MCW127	0.03	0.06	0.81	GCT016	0.15	0.26	0.95
LEI141	0.04	0.07	0.62	MCW104	0.01	0.01	0.59
MCW078	0.21	0.35	0.91	ADL259	0.01	0.03	0.51
LEI234	0.06	0.11	0.62	MCW098	0.32	0.48	1
MCW216	−0.02	−0.03	0.93	ADL292	0.09	0.17	0.14
MCW016	0.13	0.23	0.17	MCW288	0.05	0.1	1
MCW069	0.15	0.26	0.37	MCW037	0.04	0.07	0.55
MCW087	0	−0.01	0.76	MCW228	0.05	0.1	0.61
MCW063	0.08	0.15	0.41	R0S019	0.08	0.15	0.25
MCW248	−0.02	−0.04	1	LEI074	0.02	0.03	0.22
MCW067	−0.01	−0.01	0.92	LEI135	0.19	0.32	0.91
ADL278	−0.02	−0.03	0.73	LEI094	0.11	0.19	0.45
MCW183	0	−0.01	0.79	ADL317	0.02	0.04	0.83
LE1166	0.15	0.27	0.3	MCW213	0.06	0.12	0.84
MCW206	0	0	0.39	MCW264	0.05	0.1	0.32
MCW029	0.01	0.01	0.23				

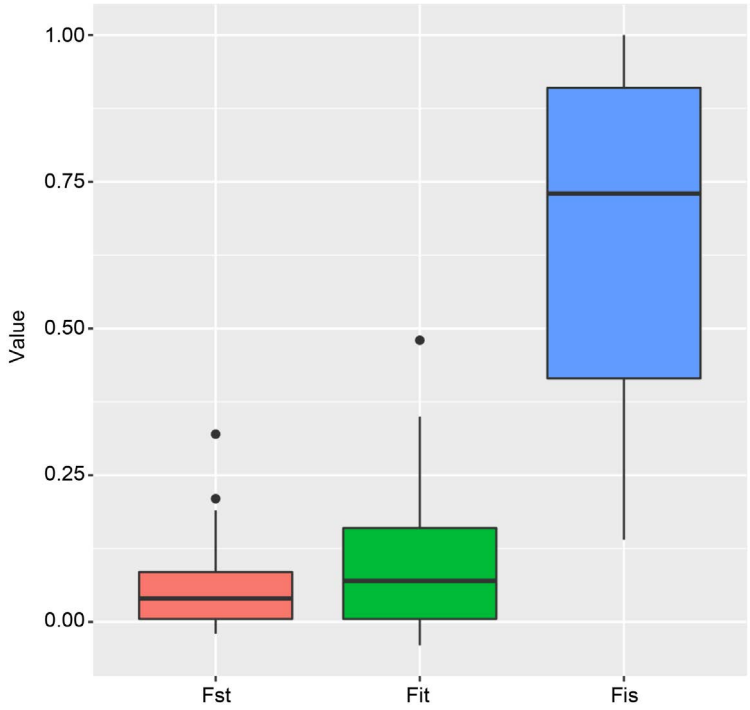


Figure 3. Wright's fixation indices (F_{ST}, F_{IT}, and F_{IS}) across ISM and TA ecotypes across 39 microsatellites loci.

F_{ST}, F_{IT} and F_{IS}. The average values of the three indices were all positive. The average of F_{ST} was small (0.06) which indicates similarity of allele frequencies within each breed, which in turn indicate that genetic variation among ecotypes accounted for only 6%, which shows homology of allele frequencies of both ecotypes [41], thus indicate that genetic variation between ecotypes represents 6% of entire genetic variation. This small estimate of F_{ST} could be considered as indicative that none of the 39 MS loci were under selection [42] [43]. The estimate of F_{ST} ranged from −0.02 to 0.32. This estimate was lower than the estimates of Abebe *et al.* [13] on Swedish local breed, Seo *et al.* [20] on Korean chicken as well as Ramadan *et al.*, [36], Soltan *et al.*, [40] on Egyptian native breeds.

Average value of global heterozygosity index (F_{IT}) was 0.102, ranged from −0.04 to 0.48. This index could be considered as correlation between alleles within individuals with respect to combined sample [41]. The inbreeding coefficient (F_{IS}) across the 39 MS loci averaged 0.65 ranged from 0.14 to 1.00. This estimate is higher than the other studies on indigenous chicken breeds e.g. 0.187 on Swedish breeds [13], 0.0093 on Korean indigenous breeds [20] and 0.018 on Egyptian indigenous breeds [36], but Soltan *et al.*, [40] reported somewhat closer estimate on Sinai and Norfa indigenous Egyptian chicken breeds. F_{IS} is also taken as an indicative of endangerment potentiality and devised to assess the conservation priorities [44]. Thus, when F_{IS} is lower than 0.05, breed is not in danger; the range from 0.05 to 0.15, breed is potentially endangered; between 0.15 - 0.25, they are slightly endangered; between 0.25 - 0.40, they are endangered; and more than 0.40, breed is seriously endangered [36] [40].

Diversity assessment between population is normally quantified by estimation of genetic distance. Both Nei's genetic distance [29] as well as Nei's pairwise F_{ST} index between all pairs of populations [45] was estimated for the two ecotypes over the 39 MS loci. The estimate of Nei's genetic distance was 0.2 where Nei's pairwise F_{ST} was 0.29. This small value is comfortable with the percentage of shared alleles of the two ecotypes 45%.

5. Conclusion

The results of the present study are, presumably, the first to recount the genetic diversity between two ecozones Egyptian and Saudi Arabia ecotypes. To be concluded, a small proportion of genetic diversity due to allele frequency differences was between these two ecotypes was only 6%. This result is also supported by small estimate of Nei's genetic distance (0.2) as well as small Nei's pairwise F_{ST} (0.29). The percentage of shared alleles of the two ecotypes was 45%.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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