



Growth associated molecular markers and assessment of relatedness among four domestic quail genotypes in Egypt

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Received: 13-Jan-2022, Manuscript No. AASTGB-22-41646; **Editor assigned:** 17-Jan-2022, PreQC No. AASTGB-22-41646 (PQ); **Reviewed:** 01-Feb-2022, QC No. AASTGB-22-41646; **Revised:** 08-Feb-2022, Manuscript No. AASTGB-22-41646 (R); **Published:** 18-Feb-2022, DOI: 10.51268/2736-1810-22.10.054.

ABSTRACT

The DNA polymorphisms by microsatellites technique was applied to four genotypes of Japanese quail; Stino, White, Golden, and Japanese quail. The Stino quail had significantly the highest body weight at all ages followed by the White quail. The live body weight of both the Golden and Brown quail showed lower reciprocity order attitude across ages. The weight gain and growth rate of Stino and White quail were significantly the highest at almost periods. The mean number of alleles per locus (Na), effective allele (Ne), expected heterozygosity (He), Shannon's Information index (I) scored lower values for both Stino and the White quail (3.89, 2.97, 0.72, and 1.17), than the Golden and Brown quail. Stino quail had a higher Polymorphic information content value (0.81) than the White one (0.79) and they both scored lower values than the Golden and Brown quail (0.84 and 0.82), respectively. All genotypes were not under Hardy Wein-Berg equilibrium. Identity and genetic distance matrix had scores of 0.9267 and 0.0761 between the Stino and White quail, while 0.9275 and 0.0752 for the Golden and Brown quail, respectively. The phenotypic and molecular Neighbor joining procedures aggregated Stino and White quail in one cluster, Golden and Brown quail had joined in a different cluster. Unique microsatellite alleles were detected and recommended as marker aid selection in the Stino quail population as GUJ052, GUJ087 markers with live body weight at 21 day and GUJ054 for live body weight at 28 day. The GUJ059 is for carcass weight. Crossing between Golden and Japanese quail will have no benefit and is not recommended.

Keywords: Quail, Microsatellite markers, Growth parameters, Genetic variability, Marker aided selection.

INTRODUCTION

Japanese quail is useful for genetic studies due to its early sexual maturity, short generation

interval, low feed consumption, quicker rate of growth and its smaller body size (Devi KS et al., 2010; Jatoi AS et al., 2013). Live body weight stands out among the most noteworthy traits

since its general straight forwardness of estimation as well as for its direct relationship with other profitable meat production traits. Furthermore, it is known to be an intermediately heritable trait. This way, the choice of heavier individuals should result in the genetic improvement of the trait (Oke UK et al., 2004).

Three breeds of quail, Jidori, Shoukoku, and Oh-Shamo, (Oana H, 1951) were the original of most of today's Japanese quail genotypes. Microsatellite DNA markers have an abundance and codominant inheritance (Goldstein DB et al., 1997; Petit E et al., 1997). Genetic diversity is considered from the polymorphism of morphology, chromosome, blood protein and DNA. Microsatellite DNA markers are useful for kinship and paternity (Queller DC et al., 1993), genetic relationships among chickens, (Emara MG et al., 2002; Pandey AK et al., 2002; Zhang X et al., 2002; Zhang X et al., 2002) and for quail (Osman SA et al., 2004; Osman SM et al., 2005; Osman SA et al., 2006). Genetic mapping and linkage with quantitative traits (Tuiskula-Haavisto M et al., 2002), certain genes (McElroy JP et al., 2006) and identities among populations (Ya-Bo Y et al., 2006).

Recently, hundreds of microsatellite markers were developed for Japanese quail (Kayang BB et al., 2002; 19. Kayang BB et al., 2004). They used it to establish a linkage map (Kayang BB et al., 2004). Its polymorphism can clarify the phenotypic contrasts. Relations between molecular DNA markers and the quantitative trait loci had been stated by (Groenen MA et al., 2000; Yonash N et al., 2001), in broilers (Hardiman JW et al., 2010) and for chicken's genome (Gholizadeh M et al., 2007). The microsatellites linkage map in quail was studied (Kayang BB et al., 2004; Miwa M et al., 2005). The genetic diversity of populations and the evolutionary relationship in quail were previously studied (Amirinia C et al., 2007; Bai JY et al., 2013; Chang GB et al., 2007; Farrag S.A et al., 2011; Emrani H et al., 2011; Kim SH et al., 2007; Chazara O et al., 2010; Mukesh T et al., 2011; Rashid MA et al., 2020; Fathi M et al., 2018; Roh H et al., 2021).

The present study was conducted to distinguish between the growth performance capabilities of four Japanese quail populations through molecular application of nine particular microsatellite DNA markers. In addition, to find specific markers associated with growth traits and loci responsible for quail's meat production.

MATERIALS AND METHODS

Genetic background

This experiment was carried out in 2019 for a six-month period at the Stino Quail Farm, Al-Hoda cooperative association, Cairo-Alexandria

desert highway, 64 km, Egypt and the Poultry Experiment Station, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza. The molecular analysis was carried out at both the Molecular Biology Laboratory of the Genetic Engineering Research Center, Faculty of Agriculture, Cairo University, Giza, Egypt and the Animal Production Biotechnology Lab, Central Laboratory Network, National Research Centre, Dokky, Giza.

Populations and managements

Four lines of quail were used. Stino Quail, a selected line that is genetically improved for its higher body weight, white feathers and skin color by Prof. Farid Stino. He developed this Stino quail line from the Japanese quail line of Prof. Henery Marks (Tallahasse, FL) since 1971 (Marks HL, 1971). Dr. Stino started selecting this quail line since 1974 for its heavier 30-day old body weight. The original imported flock weighted about 150 gm at 4 weeks of age. The selection continued for 46 generations and it is still going on. About 25 generations ago, a recessive white mutation appeared in the population that was kept as a closed population since Dr. Stino had gotten it from the United States. This mutation affected the feather, skin and shank colors. The quail were almost completely white, with few brown feather patches. It also affected the color of the skin of the quail (white) and their shanks (white) instead of the slate color of the skin of the original Japanese population. This mutation was also associated with a slightly lower body weight than the original brown (Agoti) wild type. However, with the continuous phenotypic selection, the 46th generation of the Stino white quail weighs about 330 gm at 4 weeks of age. This weight is larger than any of the other commercial Japanese flocks worldwide.

The White quail line is a hybrid between males of Stino quail and a non-selected white line females. The Golden quail and the Brown quail (wild type) lines are two plumage-colors of the Japanese quail lines available at the Poultry Experiment Station of the Faculty of Agriculture, Cairo University.

Economic traits studied

1. Individual live body weight was measured to the nearest gram by a digital scale at hatch, 1, 2, 3, 4, and 5 weeks of age (Birds were fasted for 8 hours before weighing).
2. Body weight gain was calculated as $BWG = W_2 - W_1$, where both W_2 and W_1 represented the final and initial weight, respectively.
3. The Growth rate was calculated using the following formula (Brody S et al., 1946).

$$\text{Growth rate \%} = \frac{W_2 - W_1}{\frac{1}{2}(W_1 + W_2)} \times 100$$

Where W_1 =first weight; W_2 =second weight.

Slaughter performance traits

Slaughter traits were obtained at 5 weeks of age. A random sample of 30 birds from each line was chosen. Birds were weighed (LBW) and slaughtered after 8 hours of fasting (Papa CM et al., 1991). Birds were slaughtered by slitting the throat, cutting the carotid arteries, jugular veins, esophagus and trachea without severing the head (Sams A et al., 2001). After slaughtering each bird was hanged in a bleeding funnel for 3 minutes and weighed again to obtain the blood weight. Birds were then scalded in a 66°C water bath for 20 seconds, and then the feathers were removed by an automatic circular feather plucker. The birds were then weighed again to get the feathers weight. The shanks and head, without the neck, were then removed and the birds were eviscerated and chilled. Each empty chilled carcass was weighted to obtain the dressed weight. Dressing percentages were expressed as the percentage of dressed weight relative to live body weight. Edible organs such as the liver, heart, and gizzard were also weighed and calculated relative to carcass live body weights.

Statistical analysis

The least square means $\pm$ SE were generated by the general linear model using the x-stat, 2014 software for the statistical analysis of the phenotypic traits. The separation of means was done according to Duncan's multiple range test (Duncan DB, 1955) at a 5% significance level when differences between means exist. The following one-way ANOVA model was used:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

Y_{ij} =the observed value of the ij th observation.

μ =the overall mean.

G_i =the effect of the i th genotype

e_{ij} =Random error.

The phenotypic data for the four quail genotypes was clustered by clustering procedures used to calculate the nearest neighbor hierarchical method by SAS 9.4.

Blood DNA isolation

The random individual blood samples of 120 birds (30 per genotype) *via* heart puncture were collected at 30 days of age in coagulate buffer (0.20 ml blood per sample) in a treated tube of K3-EDTA (FL medical, Italy) and stored at 5°C until DNA extraction. A PureLink Genomic DNA Mini; Microcentrifuge spin-column format (Invitrogen™ K182001, USA) was applied in order to obtain a pure extracted DNA. A NanoDrop® ND-1000 UV-Vis Spectrophotometer procedure was used to examine the product (pure DNA) for purity.

Microsatellite markers statistics

Nine, of fifteen tested, microsatellite DNA markers (Table 1) were used. PCR reactions were performed in a final volume of 50 μ l reaction mixture, composed of 3 μ l DNA (40 ng/ μ l), 45 μ l of PCR SuperMix 1.1 $\times$ concentration (Invetrogen, USA), 1.5 μ l of each primer (10 pmol/ μ l). The amplification conditions, on a Genemate B960 gradient thermal cycling platform, were as follows: Initial denaturation step at 94°C for 3 mins, 30 cycles of amplification (45s of denaturation at 94°C, 60s of annealing at 55°C, 56°C or 60°C based on the optimal annealing temperature for the used primer, 60s of extension at 72°C), and this was followed by a final extension at 72°C for 12 min. PCR products were electrophoresed on 1.5% agarose gel containing 0.5% ethidium bromide then viewed under UV light, and documented using Uvp-BioDoc system.

Table 1. The microsatellite loci used herein, their accessions, flanking primers, annealing temperature (TA) and location on genome.

Locus	GenBank AN ^a	Repeat array	F/R	Primer sequence (5'-3')	Size rang (bp)	TA ^b (°C)
GUJ054	AB063122	(CA) ₇	F	GTGTTCTCTCACTCCCAAT	120-146	55
			R	ATGTGAGCAATTGGGACTG		
GUJ059	AB063127	(CA) ₁₀	F	GACAAAGTTACAGCTAGGAG	207-219	50
			R	TAGGTGCGAAAATCTCTGAC		
GUJ052	AB063120	(CA) ₁₂	F	AAACTACCGATGTAAGTAAG	96-108	55
			R	ATGAGATATATAAGGAACCC		
GUJ063	AB063131	(CA) ₇ CT(CA) ₂ CT(CA) ₇	F	GCTCAGGTTCTCAGCTGATG	242-250	55
			R	GGGAGAGATCAAGGGAACA G		
GUJ087	AB063155	(CT) ₁₂ AA(CA) ₁₁	F	CATGCCGGCTGCTATGACAG	151-155	55
			R	AAGTGCAGGGAGCGAGGAA G		

GUJ085	AB063153	(GT)14	F	ACAACCACTTCTCCAGCTAC	245-265	55
			R	GCTTGTGCTGCTGTTGCTAA		
GUJ071	AB063139	(CA)8	F	AGATCCTGCTCCTGGAATTG	160-178	54
			R	CAGCTGCACTTAATACAGGC		
GUJ010	AB035820	(CA)15	F	TTCCTTCTGGGTGCTGCTCA	154-158	62
			R	CATAGACACATCCCTCCCTC		
GUJ049	AB035859	(CA)11	F	GAAGCAGTGACAGCAGAATG	229-241	55
			R	CGGTAGCATTCTGACTCCA		
Note: The locus code GUJ stands for Gifu University Japanese quail ^a GenBank AN: GenBank accession number; ^b TA: Temperature of annealing. The information provided for GUJ010 and GUJ049 by (Kayang et al., 2004) and Others by (Kayang et al., 2002).						

Genotyping statistical analysis

The size of the alleles was estimated through comparison a with standard ladder DNA marker. Each allele size was estimated according to its repeated number for each microsatellite marker. The frequencies of different alleles were estimated in different genotype groups following gene-counting method by Nei index genetic diversity (Nei M, 1978). The input files for all genetic software using Convert version 1.3.1 (Glaubitz JC, 2004). POPGENE 3.2 software package, (Yeh FC, 1999) were used to calculate heterozygosity (H). The polymorphism information content (PIC) was calculated according to (Botstein D et al., 1980) by using CERVUS version 3 software (Kalinowski ST et al., 2007). The pair-wise alleles sharing were calculated manually from the row results. The Data in Table 1 summarizes effective microsatellite information as loci, accession numbers, flanking sequences, annealing temperatures and corresponding references.

Phylogenetic tree construction

A phylogenetic tree, from pairwise genome alignments, was constructed. The Sysat 7.0 software was used to draw the dendrogram presentations. The molecular phylogenic tree (unweighted pair group method with an arithmetic mean (UPGMA)) analysis was performed to establish phylogenetic dendrogram using 100 bootstrap replicates. The rooted tree was then constructed using the Neighbor-Joining method.

RESULTS

Growth performance

Table 2. The least square means for growth parameters, carcass and edible internal parts of four quail genotypes.

Live body weight				
Period	Stino quail	White quail	Golden quail	Brown quail
Hatch	12 ^a ± 0.12	11 ^b ± 0.12	9 ^c ± 0.12	9 ^c ± 0.12
7-Day	42 ^a ± 0.63	30 ^c ± 0.63	33 ^b ± 0.63	23 ^d ± 0.63

The quail growth performance that is presented in Table 2 revealed that the least square means of live body weights from hatch (BW0) up to the end of fifth's weeks of age (BW5) were significantly (P<0.05) the highest body weight was that of Stino quail and the lowest was that of the brown quail. Stino quail had also significantly (P<0.05) the higher body weight gain during all intervals. The Brown Japanese quail had significantly (P<0.05) the lowest weight gain of all genotypes during most of the periods. At the same time, the Golden Japanese quail (P<0.05) also had significantly lower body weight gain of all genotypes during late ages.

The carcass weight of Stino quail was significantly (P<0.05) the highest (250 gm) vs. 208, 180 and 151 gm for the White, Golden and Brown quail, respectively. The same trend was also observed for the for liver, heart and giblet weights, where Stino quail had a significantly (P<0.05) higher weight than the other genotypes, as shown in Table 2.

Genotypes allocation to clusters and cluster distance

As shown in Figure 1, phylogenetic tree for the phenotypic data aggregated the four quail genotype populations by the nearest neighbor method in three distinct clusters. The first one aggregates the golden and brown quail populations; they seem to be the most homologous groups. Their inter cluster distance was the lowest of all clusters (0.5348). The second cluster compresses Stino and its hybrid at 0.7422 distance points. Finally, the third clusters distance was obtained by the nearest-neighbor method for the four genotype populations was (1.1361).

14-Day	95 ^a ± 1.67	77 ^b ± 1.67	60 ^c ± 1.67	41 ^d ± 1.67
21-Day	192 ^a ± 2.41	146 ^b ± 2.41	103 ^c ± 2.41	102 ^c ± 2.41
28-Day	282 ^a ± 2.22	249 ^b ± 2.22	161 ^d ± 2.22	171 ^c ± 2.22
35-Day	385 ^a ± 2.96	274 ^b ± 2.96	209 ^c ± 2.96	156 ^d ± 2.96
Body weight gain				
0:14 Day	82.7 ^a ± 1.66	66.1 ^b ± 1.66	50.72 ^c ± 1.66	31.19 ^d ± 1.66
0:21 Day	179.9 ^a ± 2.39	134.9 ^b ± 2.39	92.97 ^c ± 2.39	91.84 ^c ± 2.39
0:35 Day	336.6 ^a ± 2.70	262.8 ^b ± 2.70	207.70 ^c ± 2.70	199.25 ^c ± 2.70
21:35 Day	156.7 ^a ± 3.38	127.9 ^b ± 3.38	106.28 ^d ± 3.38	115.85 ^c ± 3.38
28:35 Day	69.6 ^a ± 3.38	24.2 ^c ± 3.38	48.18 ^b ± 3.38	46.12 ^b ± 3.38
Growth rate				
0:14 Day	152.9 ^a ± 2.14	147.9 ^b ± 2.14	144 ^b ± 2.14	109.9 ^c ± 2.14
0:21 Day	176.1 ^a ± 0.65	169.3 ^b ± 0.65	164.8 ^c ± 0.65	164.9 ^c ± 0.65
0:35 Day	186.6 ^a ± 0.22	184.0 ^b ± 0.22	181.9 ^d ± 0.22	182.8 ^c ± 0.22
21:35 Day	58.2 ^b ± 1.77	62.9 ^b ± 1.77	67.8 ^a ± 1.77	72.3 ^a ± 1.77
28:35 Day	5.8 ^a ± 1.77	2.3 ^b ± 1.77	6.6 ^a ± 1.77	5.8 ^a ± 1.77
Edible inner organs				
Organ	Stino quail	White quail	Golden quail	Brown quail
Carcass	250 ^a ± 1.83	208 ^b ± 1.83	180 ^c ± 1.83	151 ^d ± 1.83
Liver	9.8 ^a ± 0.11	7.4 ^b ± 0.111	5.5 ^c ± 0.11	5.7 ^c ± 0.11
Gizzard	4.1 ^c ± 1.42	5.9 ^a ± 1.42	3.1 ^d ± 1.42	4.5 ^b ± 1.42
Heart	5.8 ^a ± 0.33	3.6 ^b ± 0.333	1.1 ^d ± 0.33	2.2 ^c ± 0.33
Giblet	19.7 ^a ± 0.41	16.9 ^b ± 0.412	9.9 ^d ± 0.41	12.7 ^c ± 0.41
Probability	0.0001	0.0001	0.0001	0.0001
Edible inner organs %				
Carcass	72 ^c ± 1.01	77 ^b ± 1.01	87 ^a ± 1.01	70 ^c ± 1.01
Liver	2.8 ^a ± 0.05	2.8 ^{a,b} ± 0.05	2.7 ^b ± 0.05	2.7 ^b ± 0.05
Gizzard	1.2 ^c ± 0.48	2.2 ^a ± 0.48	1.7 ^b ± 0.48	2.2 ^a ± 0.48
Heart	1.7 ^a ± 1.10	1.3 ^b ± 1.10	0.5 ^d ± 1.10	0.9 ^c ± 1.10
Giblet	5.8 ^b ± 1.35	6.9 ^a ± 1.35	4.9 ^c ± 1.35	5.8 ^b ± 1.35
Note: ^{a,b,c} Values within rows between quail lines followed by different superscript, different (P<0.05).				

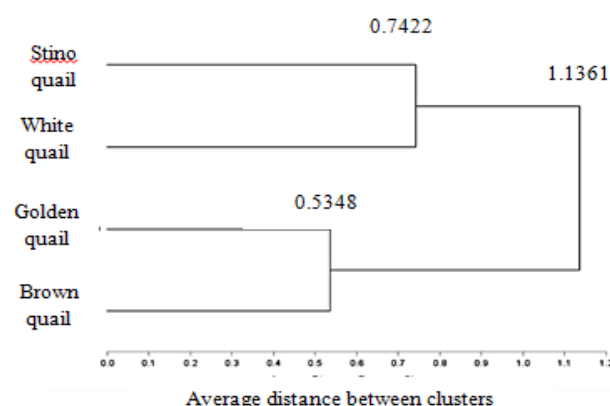


Figure 1. Phenotypic Dendrogram trees between four quail genotypes using the nearest neighbor hierarchical cluster method. **Note:** Number at the node indicates the average distance of a cluster.

Microsatellite profile

The data of the nine microsatellites that was analyzed is presented in Table 3. The average number of alleles were 3.89, 4.56, and 4.67 for Stino quail, White quail, Golden and Brown quail,

respectively. Stino and White quail had a lower average number of effective alleles (2.97) compared to 3.42, and 3.48 for the Golden and Brown quail. The mean observed heterozygosity was 0.53, 0.56, 0.57, and 0.60 for Stino, White, Golden and Brown quail, respectively. The

expected heterozygosity for Stino and White quail recorded the lowest (0.72) vs. 0.78 and 0.79 for the Golden and Brown quail populations, respectively. The average heterozygosity was the lowest for Stino quail (0.61) on the contrary, they were 0.65, 0.70, and 0.82 for White, Golden and Brown quail groups. The fixation index values (I) followed the same trend for Stino and White quail

1.17 vs. 1.34 and 1.36 for Golden and Brown quail, respectively. The polymorphic information content was higher for Stino quail (0.81) as compared to the white cross (0.79). At the same time, they were still lower than the Golden and Brown quail, 0.84 and 0.82, respectively.

Table 3. The statistics for a Microsatellites profile, the observed (Ho) and expected (He) heterozygosity and Hardy-Weinberg equilibrium (HWE) for each genotype.

Stino quail population							
Locus	Na	Ne	Ho	He	Ave-Het	PIC	I
GUJ054	4	2.94	0.6	0.73	0.6	0.77	1.19
GUJ059	4	2.94	0.6	0.73	0.6	0.84	1.19
GUJ052	4	2.94	0.6	0.73	0.6	0.84	1.19
GUJ063	4	2.94	0.6	0.73	0.6	0.84	1.19
GUJ087	4	2.94	0.6	0.73	0.6	0.82	1.19
GUJ085	6	4.51	0.6	0.87	0.78	0.84	1.64
GUJ071	3	2.38	0.4	0.64	0.58	0.82	0.94
GUJ010	3	2.17	0.4	0.6	0.45	0.79	0.89
GUJ049	3	2.94	0.4	0.73	0.66	0.77	1.088
Mean ± SE	3.89 ± 0.93	2.97 ± 0.65	0.53 ± 0.10	0.72 ± 0.07	0.61 ± 0.09	0.81 ± 0.03	1.17 ± 0.21
White quail population							
GUJ054	4	2.94	0.6	0.73	0.66	0.77	1.19
GUJ059	4	2.94	0.6	0.73	0.66	0.84	1.19
GUJ052	4	2.94	0.6	0.73	0.66	0.84	1.19
GUJ063	4	2.94	0.6	0.73	0.66	0.84	1.19
GUJ087	4	3.57	0.6	0.73	0.66	0.77	1.19
GUJ085	4	3.57	0.6	0.8	0.72	0.79	1.41
GUJ071	4	2.77	0.6	0.71	0.64	0.82	1.17
GUJ010	3	2.17	0.4	0.6	0.54	0.79	0.89
GUJ049	3	2.94	0.4	0.73	0.66	0.77	1.09
Mean ± SE	3.89 ± 0.60	2.97 ± 0.2	0.56 ± 0.09	0.72 ± 0.05	0.65 ± 0.05	0.79 ± 0.03	1.17 ± 0.01
Golden quail population							
GUJ054	6	4.16	0.6	0.84	0.76	0.87	1.61
GUJ059	4	2.94	0.6	0.73	0.66	0.84	1.19
GUJ052	4	2.94	0.6	0.73	0.66	0.84	1.19
GUJ063	4	2.94	0.6	0.73	0.66	0.82	1.19
GUJ087	4	2.94	0.6	0.73	0.66	0.82	1.19
GUJ085	5	3.57	0.6	0.8	0.72	0.84	1.41
GUJ071	5	4.16	0.6	0.84	0.76	0.84	1.5
GUJ010	4	3.33	0.4	0.8	0.7	0.82	1.28
GUJ049	5	3.84	0.4	0.82	0.74	0.84	1.47
Mean ± SE	4.56 ± 0.73	3.42 ± 0.53	0.57 ± 0.09	0.78 ± 0.05	0.70 ± 0.04	0.84 ± 0.02	1.34 ± 0.16
Brown quail population							
GUJ054	5	3.57	0.6	0.8	0.72	0.64	1.42
GUJ059	4	2.94	0.6	0.73	0.66	0.88	1.19
GUJ052	5	3.57	0.6	0.8	0.72	0.87	1.42
GUJ063	4	2.94	0.6	0.73	0.66	0.82	1.19
GUJ087	4	2.94	0.6	0.73	0.66	0.82	1.19

GUJ085	5	3.84	0.8	0.82	0.74	0.87	1.47
GUJ071	5	3.57	0.4	0.8	0.72	0.84	1.42
GUJ010	5	4.1	0.6	0.84	0.76	0.82	1.5
GUJ049	5	3.84	0.6	0.82	0.74	0.84	1.47
Mean $\pm$ SE	4.67 $\pm$ 0.500	3.48 $\pm$ 0.44	0.60 $\pm$ 0.10	0.79 $\pm$ 0.04	0.82 $\pm$ 0.04	0.82 $\pm$ 0.07	1.36 $\pm$ 0.13
Note: Na=Number of different alleles; Ne=Effective number of alleles; Ho=Observed heterozygosity; He=Expected heterozygosity; Ave-Het=Average heterozygosity; PIC=Polymorphic information content; I=Shannon's Information index.							

Genetic variation and breeds diversity

The genetic differentiation among different quail populations was analyzed by the molecular procedure based on the identity and distance matrix (Table 4). The closest genetic makeup

was observed between Stino and its hybrid the White quail. They had an identity score of 0.9267 and a genetic distance of 0.0761. The same trend was observed between the Golden and Brown quail, where they had identity score of 0.9275 and genetic distance of 0.0752 (Nei M, 1978).

Table 4. Nei's unbiased measures of genetic identity and genetic distance.

Population	Stino quails	White quails	Golden quails	Brown quails
Stino Quails	****	0.9267	0	0
White Quails	0.0761	****	0	0
Golden Quails	0	0	****	0.9275
Brown Quails	0	0	0.0752	****
Note: Genetic identity (above diagonal) and genetic distance (below diagonal).				

Correlation with growth performance

Six of the studied Microsatellites had a significantly ($P<0.05$) positive association with the live body weight and carcass traits. Their molecular weight ranged from 126-451 bp (Table 5). Four of them were correlated with Stino quail growth at specific ages. The GUJ052 and GUJ087 microsatellites had a high correlation coefficient of 0.7620 with a body weight at 21-days of age. The live body weight at 28-days of age was correlated (0.6984) with locus GUJ054. A correlation coefficient of 0.6944 between GUJ059

marker and carcass weight was also observed. One allele, GUJ085 was highly correlated with live body weight at 21 days of age (0.810) of the golden quail population. Finally, GUJ010 and GUJ087 were highly correlated with hatch weight in brown quail with a correlation coefficient of 0.8018 and 0.6547, respectively. However, the negative correlations were observed between the allelic numbers for the GUJ049, GUJ052, GUJ063, GUJ071, GUJ085, GUJ087 markers and most studied traits were significant ($P<0.05$).

Table 5: Microsatellite alleles specific to each genotype, their size and phenotypic correlation coefficients with live body weights at different ages and edible organs weights.

Stino quail												
Locus	Alleles	Alleles size (bp)	Days						Carcass	Liver	Heart	Gizzard
			Hatch	7	14	21	28	35				
GUJ054	1	126	0.2217	-0.0437	0.0437	-0.0437	0.6984	-0.3492	0	0.5388	-0.133	-0.4658
GUJ059	1	176	0.387	0.5715	-0.3429	0	0.5715	0.0381	0.6944	0.5487	0	0.0813
GUJ052	1	140	-0.2322	-0.4572	0.3048	0.762	0.2667	-0.0762	-0.7329	0.2744	0.0387	-0.4066
GUJ063	1	223	-0.1548	0.5715	0.0762	0.3048	0.0762	0.2286	-0.0772	0	-0.4644	0.0813
GUJ087	1	159	-0.2322	-0.4572	0.3048	0.762	0.2667	-0.0762	-0.7329	0.2744	0.0387	-0.4066
GUJ085	1	238	-0.1548	-0.3048	-0.6858	-0.1524	0.2667	-0.1143	0.1157	0	0.2709	-0.1627
GUJ071	2	149, 116	-0.0362	-0.0356	0.1426	0.0356	0.0713	-0.1782	0.2887	0.33	0.5068	-0.2282
GUJ010	2	130, 351	0.0362	0.0356	-0.1426	-0.0356	-0.0713	0.1782	-0.2887	-0.33	-0.5068	0.2282
GUJ049	1	264	-0.0387	-0.2667	0.3048	-0.2667	0.3048	-0.3429	-0.1157	0.3527	0.0774	0.1627
White quail												
GUJ054	1	126	-0.0904	-0.1356	-0.2619	-0.3065	-0.2627	0.0439	-0.1347	-0.0443	-0.1768	0.3273
GUJ059	1	176	-0.0904	-0.1356	0	0	-0.3503	0.1318	-0.3592	0.1773	0	-0.3273

GUJ052	1	140	-0.0904	0	-0.1746	0.4816	0.3503	0.3514	0.5837	0.532	-0.1768	0.3273
GUJ063	1	223	0.4218	0.3616	0	-0.2919	-0.3503	0.527	-0.2395	0.1773	-0.4714	-0.2182
GUJ087	1	159	-0.1183	-0.355	-0.2667	-0.1529	0.1147	0.1533	-0.8231	-0.8126	-0.1543	-0.0476
GUJ085	1	238	0.4519	-0.4067	-0.6111	-0.7006	-0.3503	0.1757	-0.1347	0.0887	0.3536	0.3273
GUJ071	1	149	-0.3616	0	0.6111	0.3941	-0.2189	0.0878	-0.5388	-0.2217	-0.5303	-0.2182
GUJ010	2	130, 351	0.1183	-0.2367	-0.0381	0.344	0.3058	0.0383	0.0392	0.0387	0	-0.5238
GUJ049	1	264	-0.4218	0.3616	0.1746	0.1168	-0.0584	-0.0586	0.2395	0.3547	-0.2357	0.2182
Golden quail												
GUJ054	1	131	-0.374	-0.4054	-0.1318	0	-0.0439	-0.0901	-0.3113	-0.1402	-0.0445	-0.1808
		208,198, 140,150, 169										
GUJ059	5		-0.0467	-0.4054	0	0.3094	0.527	-0.3604	0.0889	0.187	0.4892	-0.0452
GUJ052	1	115	-0.2337	-0.2703	-0.6588	-0.1768	-0.0439	0.1802	-0.3113	-0.1402	-0.0445	-0.1808
GUJ063	1	248	0.1145	0.0368	0.5021	0.5774	0.2152	-0.7356	0.1816	0.0763	0.1089	-0.2214
GUJ087	1	96	0	0.1966	0.3834	0.27	0.115	0.1966	-0.0388	-0.2856	-0.3494	-0.0789
GUJ085	1	238	-0.1224	-0.1966	0.3067	0.8101	0.5367	-0.7078	0.2329	0.2856	0.1941	-0.1578
GUJ071	1	270	0.0935	0.1351	0.4392	0.4419	0.1757	-0.3153	0.0445	-0.1402	-0.2668	-0.6779
GUJ010	2	140, 451	0.408	0.4718	0.115	-0.3086	-0.3067	-0.1573	0.1165	0	0.2717	0.2761
GUJ049	2	257, 333	-0.1632	-0.0393	0.115	-0.4243	-0.3834	0	0.2717	-0.2856	-0.5435	-0.5128
Brown quail												
GUJ054	1	131	-0.2182	-0.1044	0.5937	0.0348	-0.2437	0.4904	0.2811	0.4472	-0.0354	0.4568
GUJ059	4	133,119, 104,90	-0.0891	0.3553	0.5346	-0.1421	0.1421	-0.143	0.1793	0.0000	0.433	-0.4662
GUJ052	1	115	0.0891	-0.0711	0	0.0711	-0.2132	-0.572	-0.6096	0.0000	-0.1083	-0.7172
GUJ063	3	248, 387, 547	-0.8018	0.3553	0.3208	0.2843	-0.2132	-0.1788	-0.2869	0.0000	-0.2165	0.5021
GUJ087	1	96	0.6547	-0.0348	-0.1397	-0.5919	0.3133	0.1051	0.246	0.0000	0.4596	-0.773
GUJ085	1	238	-0.3273	0.5222	0.3056	0	0	-0.1751	-0.4392	0.0000	0.0884	-0.1318
GUJ071	1	270	0.3563	0.2843	-0.0356	-0.4264	0.3553	0	0.1793	0.0000	0.5052	-0.6455
GUJ010	2	142, 451	0.8018	-0.3553	-0.3208	-0.2843	0.2132	0.1788	0.2869	0.0000	0.2165	-0.5021
GUJ049	3	115,227, 33	0.3273	-0.1741	-0.6984	0.087	-0.2611	-0.5254	-0.571	0.0000	-0.3536	-0.7027
Note: **Values in bold are different from 0 with a significance level alpha=0.05.												

Genome wide phylogenies

Phylogenies using the neighbor joining procedure, using genetic distance DA were constructed as shown in Figure 2. The multiple

alignments of concatenated nine allele loci aggregated the Stino and white quail closely, in one cluster, whereas golden and brown quails had joined in a different closely cluster branch. Both were supported by 100% bootstrap confidence (Nei M, 1978).

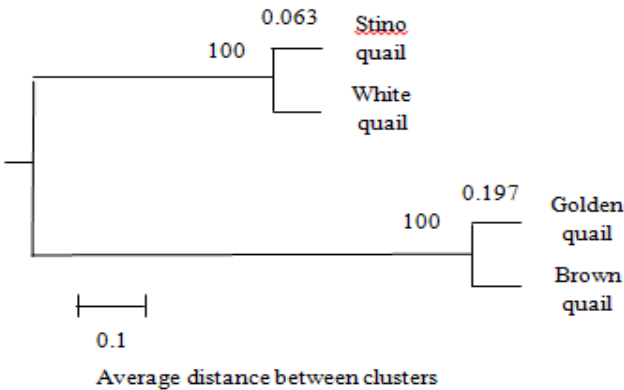


Figure 2. Rooted neighbor-joining tree of four quail genotypes produced by UPGMA clustering based on Nei’s genetic distance using 9 micro-satellite loci. **Note:** The scale bar represents the DA genetic distance calculated according to Sysat 7.0 software. Bootstrap values are 100%.

DISCUSSION

Growth performance

The phenotypic data for growth performance (Table 2) revealed that the least square means of live body weight from hatch (BW0) up to the end of fifth's weeks of age (BW5) was significantly ($P<0.05$) higher for Stino quail followed by its hybrid white one. Stino quail and its hybrid white had significantly ($P<0.05$) the highest two ranks of body weight gain during the four age intervals. The Brown quail significantly had ($P<0.05$) the lowest weight gain (31 gm) of all genotypes during the early ages (0-14 days) compared to 83, 66 and 51 gm for Stino, White and Golden quail, respectively. Similarly, the Golden quail significantly ($P<0.05$) had the lowest weight gain (106 gm) of all genotypes during its late ages, (21-35 d) compared to 157, 128 and 116 gm for Stino, White and Golden quail, respectively. Stino quail and its hybrid White quail significantly had ($P<0.05$) the highest two ranks of growth rates during the three intervals (0-14, 0-21, 0-35). Stino quail significantly had ($P<0.05$) a higher carcass weight than all the other groups (250 gm) vs. 208, 180 and 151 gm for White, Golden and Brown quail, respectively. The highest ($P<0.05$) absolute liver weight, heart and gilet weights and percentage was that of the Stino quail (Table 2). This is due to their higher body weight as a result of their metabolic rate efficiency as a response to the successive 46 generations of selection for high 30-day live body weight.

Genotypes allocation to clusters and cluster distance

As shown in Figure 1, the phylogenetic tree for the phenotypic performance aggregated the four quail genotype populations by the nearest neighbor method in three distinct clusters. The first one aggregates the Golden and brown quail populations, they seem to be the most homologous groups. Their inter cluster distance was (0.5348). They represent two plumage-colors of Japanese quail; Golden quail has been thought to be derived from the Brown quail by simple mutation. The second cluster compresses Stino and White quail at a 0.7422 point of distance. The White quail is the hybrid of male Stino quail and a non-selected white female line. These findings are in consistent with these obtained by (Ugbo YS, 2010) who stated that similar genotypes possess the existence of a high gene flow. Finally, the third cluster distances obtained by the nearest-neighbor method aggregated the four genotype populations with a total distance of (1.1361).

Microsatellite profile

Summarized information is shown in Table 3 using the effective nine microsatellites for the

four quail populations studied. The average number of alleles (N_a) for all loci within each genotype was 3.89 for both Stino and white quail. However, they were 4.56 and 4.67 to the Golden and Brown quail, respectively. Stino and White quail had lower average number of (2.79) of effective alleles (N_e). The lower allelic number and effective alleles of Stino quail points out how they have a homogeneous aggregation for growth alleles as a result of consecutive selection for body higher body weight at 30-days of age. Consequently, White quail had received a copy of them owing to the additive heritable nature of growth traits (Oke UK et al., 2004). The higher allelic number, (3.42 and 3.48) for Golden and Brown quail is due to their heterogeneous genetic makeup. The observed (H_o) heterozygous confirm the previous in which where lower values were scored for Stino and White quail, 0.53, 0.56 as compared to Golden and Brown quail, 0.57 and 0.60, respectively. The average heterozygosity was the lowest for Stino quail (0.61), which had undergone selection for high live body weight at 30 days of age for 46 generations, compared to 0.65, 0.70, and 0.82 for the White, Golden and Brown quail. Heterozygosity reflects the degree of genetic variation within group (Bai JY et al., 2016; Hata A et al., 2020).

Other allelic statistics, Fixation index was the same for Stino and White quail 1.17 vs. 1.34 and 1.36 for the Golden and Brown quail, respectively.

The polymorphic information content results reveal that all microsatellite alleles fragments are highly polymorphic due to the obtained $PIC > 0.5$ (Bai JY et al., 2016; Bai JY et al., 2018). For all quail populations Stino quail had scored a higher PIC value (0.81) than the White quail (0.79). This may be due to possessing different allelic copies (unique) than the White quail genotypes. This finding is similar to those stated by (Parmar SN; Ramadan GS et al., 2014; Ramadan GS et al., 2018; Vanhala T et al., 1998) in chicken. The selection for high body weight increases the number of alleles, some of them is responsible for high polymorphism. The same finding was obtained by (Bai JY et al., 2018, Daquan MQ et al., 2007) in quail. The higher values of PIC for Golden quail (0.81) and Brown quail (0.84) may reflect their heterozygosity. A higher value of expected heterozygosity was obtained in Stino and white quail (0.72) vs. 0.78 and 0.79 for the simplifies that these populations are not under the Hardy-Weinberg equilibrium but under a disequilibrium case due to an external force. Stino quail is known to be undergoing selection for 46 generations as one of the disturbance forces.

The study of (Rahim A et al., 2017) stated a disequilibrium the Hardy-Weinberg state for all microsatellites used under selection force for egg

production in chickens. The other quail populations may be under different disequilibrium factors. Disturbed or inappropriate random mating may not be only one of them but may also be the principle one. The different numbers of selected microsatellite DNA markers let the comparative of previous studies hard.

Genetic variation and diversity

Many studies have referenced the application of microsatellites for genetic identity and distance, (Delany ME 2003, Okumu ON et al., 2017) Our results were calculated from nine microsatellite among different quail populations analyzed by molecular variance (Table 4). Stino and White quail had an identity and genetic distance score of 0.9267 and 0.0761. This finding indicates that they possess a close genetic makeup between them which resulted from crossing Stino male quail once with a white female line four generations ago. The same trend was observed between the Golden and Brown quail. They scored 0.9275 and 0.0752 for identity and genetic distance, respectively. They are thought to have common ancestors, where the Golden quail has been derived from the Brown ones by a simple mutation. Crossing between golden and brown quail is not recommended because a very low heterosis benefit could be achieved.

Correlation with growth performance

The results presented in Table 5 indicated a significant ($P < 0.05$) association between specific microsatellites and the growth at specific ages for the Stino quail. The GUJ052 and GUJ087 microsatellites had a high correlation coefficient of 0.7620 with a body weight at 21 days of age. Live body weight at 28-days of age was significantly ($P < 0.05$) correlated (0.6984) with locus GUJ054. A significant ($P < 0.05$) correlation of 0.6944 was observed between GUJ059 marker and carcass weight as presented. (Ramadan GS et al., 2014; Ramadan GS et al., 2018; Uemoto Y et al., 2009) observed the existence of a positive correlation coefficients between DNA microsatellite markers, body weight, and other carcass characteristics.

Genome wide phylogenies

The phylogenies by neighbor joining procedure, using genetic distance DA had categorized various genetic populations into specific clusters and branches (Avisé JC et al., 2004). The obtained relationships as shown in Figure 2, were obtained from the genetic study was in accordance with the results obtained through the phenotypic clusters tree described in Figure 1. The multiple alignments of concatenated nine allele loci had aggregated Stino and white quail closely in one cluster, where they are homologous groups. White quail is the hybrid of male Stino quail with a non-selected white

females' line. Stino and White quail had not been thought to be genetically close to Golden or Brown quail. Golden and Brown quail had joined in a different close cluster branch. Similar genotypes possess the existence of a high gene flow (Ugbo YS, 2010). Such relatedness reveals a possibility of having common ancestors. It has been thought that golden quail had been derived from Brown ones by a simple mutation. Crossing between Golden and Brown quail is not recommended because of the very low heterosis benefit for growth that could be achieved.

CONCLUSION

The microsatellite DNA markers were successfully reliable in indicating a relation and variability among four quail populations. Specific markers had high positive correlations with body weight at 21 and 28-days of age and carcass weight for the Stino quail population. Therefore, these markers are recommended in a breeding program (Marker Aid Selection). It is not recommended to cross the Golden and the Brown quail to enhance growth between because of their high genetic identity score.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENT

I would like to express my sincere gratitude and appreciation to prof., Ahmed El-sharkawy, who provides his Molecular Biology Laboratory of the Genetic Engineering Research Center, Faculty of Agriculture, Cairo University, Giza, Egypt for our molecular analysis.

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