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GENETIC STRUCTURE AND BOTTLENECK EXPLORING OF SINAI CHICKENS INDIGENOUS TO EGYPT

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ABSTRACT: Current research aimed to characterize Sinai chickens genetically by using nine microsatellite genetic marckers. Twenty-eight birds used in the study were randomly collected (12 males + 16 females). Sinai chickens are a mongrel fowl raised by dwellers and farmers in Sinai Peninsula desert areas and chiefly habituated and adapted to fluctuating circumstances in this region. This strain has a small body size, golden neck feather, brown or golden saddle and brown or black feathered tail for males, while females had golden feathered body with some black feathers in tail and neck, red single comb and they have variable plumage color. Nine microsatellites used produced 53 alleles with mean value of 5.88 allel/locus. Averages of observed and expected heterozygosity were 0.174 and 0.773, respectively. Informative content revealed was high overall loci ranging between 0.608 and 0.811 with average value of 0.718. Six out of 9 loci (66.66%) were not in genetic equaliprium. Bottleneck analysis revealed that, under graphical and SMM model analysis, Sinai chickens non-bottlenecked in recent past history. Results insured that, the utilized panel of markers showed their efficiency capturing genetic characteristics of Sinai chickens reflecting the valuable genetic variation in the studied population, enabling future genetic improvement for this strain avoiding inbreeding.

Keywords: Sinai chickens-genetic characterization-genetic bottleneck- microsatellites.

INTRODUCTION

Genetic improvement of animal genetic resources (poultry in the current research), specially, in the developing countries completely depend on their own genotypes (local/native and/or indigenous) which considered as a big wealth of such countries for there adaptability to poor management conditions, disease resistance and unique flavor of their products (Kolstad and Abdou, 2000). Sinai chickens represent one of these important genetic resources in Egypt which being mainly kept by bediouen people in Sinai peninsula for their meat and egg production. Recently, phenotypic as-well-as genetic (molecular) characterization of native poultry genetic resources completed eatch other, and they are both very important tools for construction of effective conservation plans for recovering and preserving indigenous, local and native breeds and/or strains versus future challenges like, environmental, agricultural ebidemic diseases, growing population and changes of consumer favors (Bianchi et al., 2011; Leroy et al., 2012). Genetic diversity is the basic material of breeding and sustainable improvement of all poultry breeds, and mainly affected by population size; artificial selection and/or inbreeding. Genetic characterization studies introduce a valuable information about how to manage and utilize chicken germplasm. Shrinking of population size (bottleneck) leads to decreasing of genetic diversity and suffering from inbreeding, reducing fitness, therefore, decreasing adaptation ability to future environmental changes (Jangjoo et al., 2016). Genetic bottleneck exploring playing a main role in succesfull conservation plans.

Microsatellite markers are the most effective ones for genetic studies in poultry species (Bianchi et al., 2011; Babar et al., 2012; Gruszczyńska and Michalska, 2013; Palacios et al., 2016). Recently, microsatellites marker analyses were involved in studies that aimed to assess genetic diversity and genetically discriminate within and between Egyptian local chicken strains (Farrag et al., 2013; Roushdy et al., 2013a; Roushdy et al., 2013b; Soltan et al., 2016; Soltan et al., 2018). The current study aimed to assess genetic structure and probability of genetic bottlenecks of indigenous Egyptian chicken strain "Sinai" utilizing microsatellite markers.

MATERIALS AND METHODS

Population: Sinai chickens originated in Sinai Peninsula desert areas and mainly used as a source of animal protein covering bediouen people needs of food. The used population was picked up from Sinai Peninsula in 1985 by Soltan et. Al., and kept as a closed flock for over 30 years at Poultry research farm at Faculty of Agriculture, Menoufia University (Soltan et al., 1985). Sinai layers reatch sexual maturity at 185 days and led average of 185 eggs yearly (45.1 g egg weight). Hens has a small body size with mature weights of about 1385 g. Sinai chicken has golden neck feather, brown or golden saddle and brown or black feathered tail for males, while females had golden feathered body with some black feathers in tail and neck, red single comb.

Sampling:

Twenty-eight birds (flock size about 500 birds) randomly sampled (12 males + 16 females) and 1 ml of blood was collected from wing vein using K_3-EDTA tubes (FL medical, Italy) and stored at -20 ^{0}C until DNA extration. Genomic DNA extracted by DNA Extraction Kit

(Biopolymer Isolation Technologies) and tested for purity on agarose 2%.

Genotyping:

Nine microsatellite loci which represent a part of 30 microsattelite markers panel recommended by FAO (2011) were utilized. PCR singlplex reactions were done by Techne, 3primX thermocycler in 25 µl final volume containing 2.5 µl 10x reaction buffer (with 15 mM MgCl2), 200 µM dNTP mix, 0.25 µM of forward and reverse primer, 100 ng template DNA, and 1 U TaqDNA polymerase (GoTaq® Flexi DNA polymerase - Promega), as follow: initial hot start step at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 60 °C for 30 seconds (except MCW0014 and MCW0183 at 58°C) and 72 °C for 30 seconds. Finally, extension step at 72 °C for 5 minutes was done. Amplicons were electrophoresed in 6% agarose gel. Collected gel photos were analyzed using GelAnalyzer (v. 2010a) software (Lazer and Lazer, 2010) for detection of bands size. Data in Table 1 shows characterstics of the utilized set of markers.

Microsatellite DNA polymorphism and deviation from Hardy-Weinberg equilibrium:

Allele number, size and frequencies were used to evaluate genetic structure of studied population, additionally heterozygosity parameters, polymorphic information content (PIC), fixation index (F_{IS}) and number of observed genotypes per locus were detected (Tables 2 and 3). Program GenAlex 6.5 (Peakall and Smouse, 2012) was used to optain genetic parameters including: observedb(Na) and effective (Ne) number of alleles; allele frequencies; observed (Ho) and expected (He) heterozygosity and deviation from Hardy-Whinberg equaliprium (dHWE). Moreover, information content (PIC values) calculated by using program CERVUS 3.0.7 (Kalinowski et al., 2007).

Genetic Bottleneck exploring:

Bottleneck analyses was carried out utilizing program Bottleneck v. 1.2.02 (Cornuet and Luikart, 1997) looking for any genetic bottlenecks. For testing bottleneck hypothesis allele frequencies from 9 used polymorphic loci in a sample of 23 – 28 birds bsed on three aproches under infinite alleles model (IAM), two-phase model (TPM) and stepwise mutation model (SMM) using Sign test. Additionally, graphical method of Luikart et., al. (1998) to represent allele frequencies pattern was used to study possible genetic bottlenecks.

RESULTS AND DISCUSSION

Data in tables 2 and 3 shows the genetic parameters used in the current study. It have been reported that, all loci typed were polymorphic and produced a total of 53 alleles in 28 birds with mean Na per locus 5.889. Highest number of alleles assigned to LEI0094 locus and the lowest number of alleles revealed by both MCW0014 and MCW00248 loci (9 and 4 alleles, respectively). Number of effective alleles (Ne) ranged between 2.992 (MCW0014) and 6.939 (LEI0094) with an average of 4.391 (Table 3). Alleleic ritchness detected in the current study reflected the informativity of used panel of loci as well as future possibility to genetic improvement of Sinai chickes. In Egyptian local chickens average Na value located within 2.33 in Sinai chickens (Farrag et al., 2013) and 8.65 in Norfa chickens (Soltan et al., 2018). Lower values of Na per locus have been reported in Egyptian chickens by Roushdy et al., 2008 (5.2 and 5.6 alleles/locus for Fayoumi and Dandarawi chickens, respectively), while they recorded 4

alleles/locus in Brown Hy-line chickens utilizing a set of 5 loci. More recently, Soltan et. al. 2018 recorded mean number of 8.350 alleles/locus utilizing 20 microsatellite loci in two local Egyptian chicken strains (Norfa and Sinai). Chinese chicken breeds revealed mean observed number of alleles 5.00 and 5.44 in Chai (Zhao et al., 2010) and Guangxi Donglan Black-bone (Liao et. al., 2016a) chickens, respectively. Liao et al., 2016b noticed range of 3.27 and 5.94 alleles per locus per breed in 6 Guangxi chicken breeds and 2 commercial breeds from China; 4.65 alleles/locus were detected for Colombian Creole chickens (Palacios et al., 2016). In Europe, Sartore et al., 2016 recorded between 3.82 and 6.50 alleles/locus/breed in Italian chicken breeds. Higher values (7.067) in Chinese Guangxi Three-yellow (Jian-Min et al., 2010); 12.4 for Brazilian blue-egg Caipira chickens (Fonteque et al., 2014), and 13.1-13.3 in Brazilian chicken populations (Possamai et al., 2015). Therefore the current 5.889 alleles per locus in Sinai chickens are located in the range of Egyptian local chicken breeds (Table 2). The differences in mean number of alleles my be attributed to the variability of utilized number and nature of loci; sample size; location and variability of investigated populations.

The nine loci MCW0067, MCW0014, MCW0183, ADL0268, MCW0206, LEI0166, MCW0248, LEI0094 and MCW0216 produced 6, 5, 12, 5, 5, 11, 4, 12 and 4 genotypes in Sinai chicken population, respectively. Depending on allele frequencies, studied loci has one dominant gene with few exceptions that have more than one dominant gene (Table 2). Results of recent study are in a good agreement with those previously detected in Egyptian Norfa chicken strain (Soltan et. al., 2016).

Gene diversity parameters were presented in Table 3, Ho and He were 0.174 (range of 0.00 – 0.731) and 0.773 (range of 0.678 – 0.851), respectively. Heterozygosity is an effective indicator of genetic variability in a population. Expected heterozygosity in Sinai chickens in the current research were greater than Egyptian (Fayoumi 0.56; Dandarawi 0.65) as indicated by Roushdy et al., 2008; Chinese (Guangxi Donglan Black-bone chicken 0.596) as recorded by Liao et al., 2016a. Greater expected than observed heterozygosity in recent study has been recorded. On the other hand higher values of Ho than He in Chinese (Zhao et al., 2010), Iranian (Nassiri et al., 2007; Esfahani et al., 2012) chicken breeds. This may be caused by negative overall F_{IS} (-0.1212) in their study (Liu et al., 2008). Results in the current study agreed with those reported by Soltan et. al., 2016 and Soltan et. al., 2018 in Egyptian Norfa and Sinai chickens; by Fonteque et al., 2014 for Brazilian chickens and by Palacios et al., 2016 in Colombian local chickens. Sartore et al., 2016 observed heterozygosity values of 0.547 to 0.613 (observed) and 0.541to 0.654 (Expected) for seven breeds from Italy. Additionally, higher values of Ho and He were reported in two Brazilian chicken lines 0.650-0.671 and 0.820-0.804 respectively (Possamai et al., 2015). The highest values of heterozygosity (0.910) and (0.734) for Ho and He, respectively were noticed in Chinese Guangxi Three-yellow chickens by Jian-Min et al., 2010. The range of Ho in Egyptian local chicken breeds ranged between 0.220 in Fayoumi chickens (Roushdy et al., 2009) and 0.668 in Golden Montazah chickens (El-Tanany et

al., 2011). Furthermore, expected heterozygosity values located within range of 0.415 (Farrag et al., 2013 for Sinai chickens) and 0.828 (Soltan et al., 2018 for Norfa strain) in Egyptian chickens. On the other hand, the lowest heterozygosity values ranged from 0.0 to 0.1 have been recorded by Zhou and Lamont (1999) within twenty-three highly inbred lines. There are many factors affect heterozygosity in chicken breeds including: the number and nature of used markers, differed locations, number of birds in the sample, how many microsatellite markers were used and the effect of null alleles and/or other factors. Results of the current research showed possible inbreeding, selection against heterozygotes and/or Wahlund effect.

Studied panel of markers were highly informative (PIC > 0.5), the lowest PIC value was 0.608 (MCW0014), while the highest one was 0.811 with an average of 0.718 as shown in Table 3 reflecting the efficiency of utilized set of markers in Sinai chickens for studying genetic diversity. PIC or polymorphic information content is one of the best indicators of allele polymorphism. It has been early reported that, highly, reasonable and slightly informative loci had PIC values of > 0.50, 0.25 < PIC < 0.50 and < 0.25, respectively (Botstein et al., 1980). Which mean that, the higher PIC value for locus is the higher genetic information introduced by this locus. Different PIC values have been recorded previously for different chicken breeds and populations; 0.5108-0.5199 in Chai, 0.538 in Guangxi Black-bone and 0.4766-0.6154 in 6 native and 2 commercial Chinese chicken breeds (Zhao et al., 2010; Liao et al., 2016a; Liao et al., 2016b); 0.4939 and 0.5291 in Iranian local Mazandaran and Isfahan chicken populations (Nassiri et al., 2007; Esfahani et al., 2012), and 0.580 in Colombian Creole chickens (Palacios et al., 2016). Results from current study agreed with those previously recorded by Jian-Min et al., 2010 and Fonteque et al., 2014. While larger PIC values (0.794-0.765) have been detected in Brazilian local chickens (Possamai et al., 2015). In Egyptian local chicken populations PIC values ranged between 0.327 (Farrag et al., 2013) and 0.807 (Soltan et al., 2018) for Sinai and Norfa chicken populations, respectively.

Values of F_{IS} ranged from 0.126 to 1.000 with mean value of 0.778 (as presented in Table, 3) indicated significant deficit of heterozygosity in Sinai chickens ($P<0.01$) and reflecting the higher level of inbreeding that should be avoided in the future planes for genetic improvement of Sinai chickens. Lower values (0.369 and 0.451) were detected previously for Egyptian Norfa and Sinai chicken populations (Soltan et al., 2018). In cotrast, Das et al., 2015 reported lower F_{IS} 0.0909-0.1159 for 2 selected lines of RIR (Rhode Island Red) and control line chickens. Negative F_{IS} value (-1.0094) has been detected in native to Iran Isfahan chickens (Esfahani et al., 2012). Moreover, Liao et al., 2016b found a compine of low negative and positive values for F_{IS} (-0.0139 to -0.3391 and 0.0141 to 0.0632) in 8 local Chinese chicken breeds. In Italy, Sartore et al., 2016 recorded F_{IS} values within the range of -0.055 and 0.136 for local chicken populations. Lower and fluctiuated F_{IS} values than that detected in the current study were reported previously in Egyptian local chicken populations, sometimes, negative -0.04 to -0.07 (Roushdy et al., 2013a) and positive in some cases 0.003 (Golden Montazah

chickens) (Ramadan et al., 2012). The largest previously recorded value of F_{IS} (0.592) has been detected in Norfa chicken strain (Soltan et al., 2016) and was completely agreed with the current obtained value (0.592) in studied Sinai chicken population.

Sinai chicken population under investigation showed that it was in a case of genetic disequilibrium because six out of nine loci were not in HWE ($P<0.01$), this may be due to artificial selection that current population subjected to. Results of the recent work were in a good agreement with those reported in Egyptian chicken strains (Norfa and Sinai) as detected by Soltan et. al., 2016 and Soltan et. al., 2018; in Brazilian chicken lines (Possamai et al., 2015) and in Chinese local breeds (Liao et al., 2016b), which also showed deviation from HWE. It has been reported previously that, 25 test could be enough to obtain accurate results in such studies (Barker, 1994). Therefore, the twenty-eight samples utilized in the current report are adequate for the sampling requirement, and argued that, detected HW disequilibrium should be caused by other factors such as selection and/or inbreeding.

Bottlneck analysis revealed that, the studied Sinai chicken population non-bottlnecked. Since the minimum number of loci that recommended for exploring recent bottlenecks using standerdized differences test is 20 polymorphic loci (Cornuet and Luikart, 1997), only quantitative Sign test were utilized in the current study. Additionally, graphical method for testing shift-mode of mutation (Luikart et. al., 1998) was used to study the possibility of recent bottlenecks in studied population (Fig. 1). Results in Table 4 represent bottleneck analysis in Sinai chickens. Results revealed that, utilizing Sign test, although, the test under IAM model indicated that Sinai chicken population undergone recent bottleneck. Bottleneck analysis under TPM and SMM models showed that, no bottlenecks were detected in recent past history for studied population, this result was assured by the graphical distribution of allele frequencies optained by Mode-shift test.

Sinai chickens showed normal L-shaped distribution (Fig. 1) revealing no current or recent bottlenecks and thereby no risk of extinction. Genetic bottleneck exploring has been applied in genetic studies on Indian (Pandy et. al., 2005) and Chinese (Hui-Fang et. al., 2009) chicken breeds. It have been reported previously that TPM and SMM models is the most powerful tests for microsatellite analysis (Vij et. al., 2006; Radha et. al., 2011), so, it can be concluded that the studied population didn't undergone bottleneck in the nearest past.

CONCLUSION

Results from the current study showed the potential possibility for improving Sinai chickens genetically due to the significant genetic polymorphism revealed by SSR markers utilized, as well as, the priority of construction of a conservation plan to reserve such genetic resources in Egypt. But, inbreeding should be avoided (full-sibs and half-sibs mating should be prevented). Information presented in the recent study introduce a scientific basis for the evaluation of Sinai chickens and indicated that, Sinai chicken didn't undergone any bottelnecks in the recent past.

Table (1): Some properties of the used microsatellite markers in the current study

Primer	Chr.	Motif	Primer sequence (5' -> 3')		TA (°C)	Genbank AN
MCW0067	10	(AT)6(GT)11	F	GCACTACTGTGTGCTGCAGTTT	60	G31945
			R	GAGATGTAGTTGCCACATTCCGAC		
MCW0014	6	(AC)9	F	TATTGGCTCTAGGAACTGTC	58	...
			R	GAAATGAAGGTAAGACTAGC		
MCW0183	7	(CA)14	F	ATCCCAGTGTCGAGTATCCGA	58	G31974
			R	TGAGATTTACTGGAGCCTGCC		
ADL0268	1	(GT)12	F	CTCCACCCCTCTCAGAACTA	60	G01688
			R	CAACTTCCCATCTACCTACT		
MCW0206	2	(GT)9	F	CTTGACAGTGATGCATTAAATG	60	AF030579
			R	ACATCTAGAATTGACTGTTCAC		
LEI0166	3	(CA)18(TA)1	F	CTCCTGCCCTTAGCTACGCA	60	X85531
			R	TATCCCCTGGCTGGGAGTTT		
MCW0248	1	(CA)9	F	GTTGTTCAAAAGAAGATGCATG	60	G32016
			R	TTGCATTAACTGGGCACTTTC		
LEI0094	4	(CA)16	F	GATCTCACCAGTATGAGCTGC	60	X83246
			R	TCTCACACTGTAACACAGTGC		
MCW0216	13	(GT)9	F	GGGTTTTACAGGATGGGACG	60	AF030586
			R	AGTTTCACTCCCAGGGCTCG		

Chr. = chromosome number, Genebank AN = Genbank accession number

Table (2): Observed alleles (size, frequencies and size range of alleles per locus) and number of genotypes in Sinai chickens utilizing 9 microsatellite loci

Locus	N	Observed Alleles										Size range	OGC
MCW0067	25	Allele	155	160	165	168	176	186				155-186	6
		Freq.	0.320	0.240	0.160	0.040	0.120	0.120					
MCW0014	28	Allele	154	162	164	180						154-180	5
		Freq.	0.071	0.179	0.464	0.286							
MCW0183	23	Allele	295	306	319	330	343	371	384			295-384	12
		Freq.	0.043	0.109	0.109	0.196	0.152	0.152	0.239				
ADL0268	25	Allele	104	109	111	114	116					104-116	5
		Freq.	0.120	0.280	0.040	0.360	0.200						
MCW0206	26	Allele	233	242	249	265	277					233-277	5
		Freq.	0.038	0.269	0.077	0.346	0.269						
LEI0166	26	Allele	354	396	403	422	431	447	470	492		354-492	11
		Freq.	0.308	0.115	0.135	0.192	0.096	0.077	0.058	0.019			
MCW0248	25	Allele	201	206	212	219						201-219	4
		Freq.	0.200	0.280	0.200	0.320							
LEI0094	28	Allele	236	245	254	260	266	283	293	299	313	236-313	12
		Freq.	0.018	0.054	0.036	0.018	0.214	0.232	0.107	0.179	0.143		
MCW0216	28	Allele	120	127	130	136	148					120-148	4
		Freq.	0.071	0.143	0.321	0.429	0.036						

N = sample size; Freq. = frequencies of alleles per locus; OGC = observed genotype count

Table (3): Microsatellite polymorphism parameters of Sinai chicken population

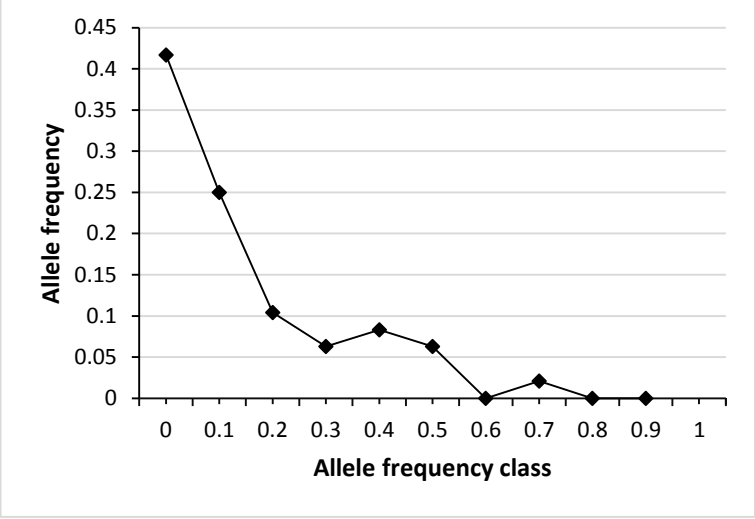
Locus	N	Na	Ne	Ho	He	PIC	F_{IS}	HWE
MCW0067	25	6	4.630	0.000	0.800	0.752	1.000	***
MCW0014	28	4	2.992	0.071	0.678	0.608	0.896	***
MCW0183	23	7	5.997	0.478	0.851	0.811	0.444	ND
ADL0268	25	5	3.788	0.000	0.751	0.691	1.000	***
MCW0206	26	5	3.674	0.000	0.742	0.679	1.000	***
LEI0166	26	8	5.496	0.731	0.834	0.796	0.126	NS
MCW0248	25	4	3.834	0.000	0.754	0.691	1.000	***
LEI0094	28	9	6.939	0.286	0.847	0.810	0.667	ND
MCW0216	28	5	3.187	0.000	0.699	0.633	1.000	***
Overall mean		5.889	4.391	0.174	0.773	0.718	0.778	
SE		0.588	0.387	0.090	0.021		0.100	

N = Sample Size, Na = Number of observed alleles, Ne = number of effective Alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, F_{IS} = Fixation Index, HWE = Hardy-Weinberg equilibrium, SE = standard error, ND = not detectable.

Table (4): Bottleneck analysis of Sinai chickens under different mutation models with Sign test.

Model		IAM	TPM	SMM
Sign rank test (number of loci with heterozygosity excess)	Expected	5.09	5.22	5.27
	Observed	8	8	5
	Probability	0.045*	0.055	0.554

Figure (1): Graphical representation for allele frequencies distripution in the studied Sinai chicken population.



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الملخص العربى

التركيب الوراثى واستكشاف عنق الزجاجة الوراثية فى دجاج سيناء المصرى

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هدفت الدراسة الحالية إلى توصيف دجاج سيناء وراثياً باستخدام تسع واسمات جزيئية وراثية (ميكروساتلايت) . تم اختبار عدد 28 طائر (12 ذكر + 16 أنثى) اختيرت بصورة عشوائية . دجاج سيناء هي طيور ذات أصول خليطة تمت تربيتها من قبل السكان والمزارعين في المناطق الصحراوية لشبه جزيرة سيناء، وهى طيور مهيأة ومتكيفة مع الظروف المتقلبة في هذه المنطقة . تتصف هذه الطيور بحجم الجسم الصغير ، تمتلك الذكور ريش ذهبى على الرقبة ولون السرج البنى أو الذهبى مع تلون ريش الذيل باللون البنى أو الأسود ، أما الإناث فلون ريشها ذهبى مع وجود بعض الريشات السوداء على الرقبة والذيل ، العرف مفرد وتختلف فى ألوان ريشها . أنتجت التسع واسمات المستخدمة فى الدراسة 53 أليل بمتوسط 5.88 أليل لكل موقع وراثى . متوسط الاختلاف الزيجوتى المشاهد والمتوقع كان 0.147 و 0.773 على الترتيب . المحتوى المعلوماتى الوراثى كان مرتفع بصفة عامة وتراوحت قيمته مابين 0.608 إلى 0.811 بقيمة متوسطة قدرت بـ 0.718 . وأظهرت ستة مواقع وراثية من أصل تسع مواقع عدم اتزانها وراثياً بنسبة 66.66 % من المواقع الوراثية المدروسة . وقد أظهرت نتائج استكشاف عنق الزجاجة الوراثية باستخدام طريقة SMM والطريقة الرسومية أن دجاج سيناء لم يتعرض لأى عنق زجاجة وراثية فى الماضى القريب . وقد دللت النتائج على كفاءة المجموعة المستخدمة من الواسمات الجزيئية فى رسم صورة جيدة للتركيب الوراثى لدجاج سيناء ، وأظهرت التباينات الوراثية القيمة الموجودة فى تلك السلالة تحت الدراسة والتى لها دور فعال فى التحسين الوراثى المستقبلى لدجاج سيناء بشرط تجنب حدوث التربية الداخلية .