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A model for the genetic employment of chickens local to warm Climate. 2. Genome scanning of two lines selected for growth

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ABSTRACT

The genome scanning was performed on four chicken populations to estimate the genomic changes associated with genetic selection. The populations were the local naked-neck line CE3 that has been selected for 6-wk BW for 6 generations with its genetic control line CE4, and a broiler dam line D with its genetic control line CD. In lines CE3 and D. The PCR was applied to pooled DNA samples of each line and sex using seven microsatellite markers. The average number of alleles per microsatellite locus was 2.14, denoting to the multi-allelic property of the detected loci. The total number of allelic bands and their frequencies differed among lines and sexes, demonstrating the genetic composition specificity of each line and the sexual dimorphism. The variability, heterozygosity and PIC averaged 0.54, 0.68 and 0.81 in line CE3 versus 0.46, 0.52 and 0.57 in line CE4. The moderate variability and moderately high heterozygosity estimates in line CE3 indicate that there is still much work can be done through selection. The distance between line CE3 and line CE4 averaged 2.92, and the distance between line D and its control line CD averaged 1.95. These distances reveal the progress of selection practiced in lines CE3 and D, that has been expressed in shifting the genetic composition of them from those of their control lines CE4 and CD. The longer distance between line CE3 and line CE4 than that between line D and line CD indicates the higher selection progress achieved in line CE3 compared to line D.

Key words: genetic distance, selection, heterozygosity, local breeds, polymorphism

INTRODUCTION

The environment is a limiting factor for the bird performance, which is reduced when environmentally stressed. In hot environments, heat stress can result in significant losses in poultry production. The most obvious loss is due to mortality. In addition, there are other losses in production efficiency, such as reduced growth rate, egg production, shell quality, egg size and hatchability. The limitations of exotic breeds to temperature and humidity stress under subtropical and tropical environments suggest the genetic improvement of local breeds that are genetically capable to withstand such environments (El-Gendy, 2009). An evaluation of the growth performances of three local chicken breeds (El-Gendy *et al.*, 1995 & 2007) and three strains of turkeys (Ilori, 2010) of hot environments introduced valuable information to justify the inclusion of their genes to the poultry industry. It was revealed that local breeds show much genetic variation which is unexploited yet and the massive information generated by genome scanning and collaborated with the phenotypic information has brought the local breeds into valuable research (El-Gendy, 2009).

Major genes in chickens are believed to confer not only adaptability to the tropical climate, but also resistance to diseases. The presence of Na gene in a single state or combined with F gene significantly increased body weight of chicks at hatch compared to the nanaff genotype (Mahrous *et al.*, 2008). The homozygous or heterozygous naked-neck chickens had heavier body weight compared to the normally-feathered sibs. El-Gendy (2009) reported significant and heavier body weight measurements of the

non-heated local chick genotyping for NaNa and Nana than those genotyping for na/na, denoting to a pleiotropic action of Na gene. Also, the naked neck crossbreds grew faster than the normally-feathered birds. Mérat (1990) reported the pleiotropic effect of Na gene on growth and heat tolerance.

Zhou and Lamont (1999) analyzed 42 microsatellites in 23 highly inbred chicken lines derived from White Leghorn, jungle fowl, Fayoumi and Spanish breeds. The phylogenetic consensus tree was constructed and four groups of chickens were clustered. The results were in accordance with the origin and breeding history of the inbred lines, and indicated that the microsatellites provide accurate and reliable information on genetic biodiversity. The more distant a breed or population is, the more likely it might carry unique genetic features (Weigend and Romanov, 2001). El-Gendy *et al.* (2006) reported an average polymorphism of 0.65 among the genomes of the Egyptian White Baladi, Fayoumi and Sinai Bedouin breeds. More variability and more heterozygosity were observed in Fayoumi. An average genetic distance of 0.42 was calculated between White Baladi and each of Fayoumi and Sinai Bedouin. The genetic distance between White Baladi and commercial broilers was 0.45. Fayoumi was genetically the furthest from Sinai Bedouin with an average genetic distance of 0.53. Beigi Nassiri *et al.* (2007a) investigated the genetic variation in an Iranian native breed, using 20 microsatellites. The number of alleles varied from 1 to 6. The averages of heterozygosity and PIC were 0.56 and 0.49, respectively. Beigi Nasiri *et al.* (2007b) evaluated polymorphism in Isfahan native chicken population using 10 microsatellite markers. Nine microsatellite loci were found to be polymorphic and PIC averaged 0.49. The average heterozygosity ranged from 0.50 to 0.74 per locus. It was concluded that Isfahan native chicken population has approximately low genetic diversity that could be attributed to a genetic improvement history.

Bao *et al.* (2009) studied the genetic diversity in chickens in relation to the ecology, using the red jungle fowl and 14 Chinese indigenous chicken breeds and 29 microsatellite loci. The number of allelic bands per locus was 2 to 5 alleles. The average expected heterozygosity and polymorphism information content of all loci were 0.67 and 0.50, respectively. The overall expected heterozygosity in Chinese chicken breeds was 0.67. Msoffe *et al.* (2005) evaluated the genetic structure of nine Tanzanian local chicken ecotypes and a White Leghorn population using 20 microsatellite markers. There was a high genetic relationship within the chicken ecotypes than between different ecotypes. Also, The F_1 Barazilian broiler and layer chickens have been evaluated by Rosário *et al.* (2009), using 34 microsatellites on chromosomes 1, 3 and 4. The allele frequencies ranged from 0.03 to 0.82. The observed heterozygosity was 0.68–0.71 in both F_1 generations. The results have been reported to be used for family comparison and selection of families with highly informative microsatellite markers for QTL studies.

The objective of this study was to evaluate the information generated from the genome scanning of two local lines selected for increased 6-week body weight in comparison to their genetic control lines.

MATERIALS AND METHODS

1. Populations and Breeding Scheme

The genomes of four chicken populations with different genetic backgrounds were extracted and used in this study. The populations were the local naked-neck line (CE3) that has been

selected for 6-wk BW for 6 generations (El-Gendy, 2009) with its genetic control line (CE4), and a broiler dam line (D) with its genetic control line (CD).

2. Genome Extraction and Scanning

Blood samples were collected from brachial veins in sterilized tubes containing ethylene diamine tetra acetic acid (EDTA). Individual blood samples were collected from the males and females of line CE3 and line D that shared in constructing the families in each line (10 families, one male and 3-4 females in each). In each of lines CE4 and CD, the individual blood samples were randomly collected from 10 males and 10 females.

The genomic DNA was extracted from each blood sample according to Manual ArchivePure DNA Purification kit (5 PRIME Inc., Gaithersburg, Germany). For this, 300 µl of the blood sample were put in a sterilized 1.5 ml microcentrifuge tube and 900 µl of RBC lysis solution were added to the blood, and the contents were mixed thoroughly. Then, the mixture was incubated for one minute at room temperature during which it was inverted gently, and centrifuged at -4ºC and 13,000-16,000 x g for 20 seconds. The supernatant was then removed without disturbing the visible white pellet. The pellet was shacked by a vortex to resuspend the white blood cells in the residual liquid for 10 seconds. Then, 300 µl of cell lysis solution were added to the suspended cells and mixed by pipetting, and then cooled on ice for 1 minute. Then, 100 µl of protein precipitation solution was added to the cell lysate, vigorously shacked by vortex for 20 seconds, and centrifuged at -4ºC and 13,000-16,000 x g for 1 minute, where a tight, dark brown pellet became visible. The supernatant was transferred to a 1.5 ml micro-centrifuge tube containing 300 µl of 100% isopropanol. The solution was mixed gently by inversion until the white thread-like strands of DNA formed visible mass. The supernatant was centrifuged at 13,000-16,000 x g for 1 minute at -4ºC, and DNA had become visible as a small white pellet. The supernatant was removed and one sample volume of ethanol (70%), at room temperature, was added to DNA. The mixture was gently inverted several times to wash the DNA pellet, and then centrifuged at 13,000-16,000 x g for 1 minute at -4ºC. The ethanol was then aspirated using a sequencing pipette tip. The DNA pellet was let to air-dry for 10-15 minutes. One hundred µl of DNA rehydration solution was added to the tube and incubated at 65ºC for 2 hours. DNA concentrations in the samples were determined by the spectrophotometer (PG Instruments, England).

DNA samples, within line and sex were used to prepare pooled (mixed) samples, by mixing equal concentrations of the different individual DNA samples to bring the total concentration up to 25 ng/µl. The pooled samples were screened by microsatellite markers (Metabion international AG, D-82152 Martinsried, Deutschland), by applying the microsatellite-PCR analysis. Seven microsatellite markers (Table 1) were used for genotyping the pooled DNA samples. A total volume of 25 µl was prepared for the polymerase chain reaction (PCR) as shown (Table 2), where each PCR tube contained 75 ng of genomic DNA, 25 pmol of each of the forward and reverse primers, 12.5 µl Master mix (Bio Basic Inc., Canada), and 4.5 µl PCR-grade water. The PCR products were obtained using a thermal cycler (Techne, TC3000, Barloworld scientific Ltd, Beacon Road Stone, UK), according to the PCR program. The amplification required initial denaturation

at 95°C for five minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing for 45 seconds (the annealing temperature varied according to the microsatellite markers and ranged 53-56°C), extension at 72°C for one minute, and then the final extension at 72°C for 10 minutes.

Allele separation was on 2% agrose gel (2gm agarose gel + 100 ml TAE 1%). The PCR products were mixed with sequencing loading dye (10 µl from PCR product + 2 µl loading dye). The mixture was loaded on the horizontal electrophoresis. A DNA marker (GeneDireX, 50-100 bp DNA Ladder RTU, cat. No. DM012-R500) was also loaded. The run was at 80 v in 1x TAE buffer and lasted for 75 minutes. The agarose gel was stained with 2 µl ethidium bromide. The DNA was visualized and photos were captured using a gel documentation system.

3. Measurements:

The photos were analyzed to generate molecular data about allele size and frequency. The data were then used to estimate the polymorphic information content (PIC), the variability using the formula of Kuhnlein *et al*. (1989), the expected heterozygosity according to Ott (1992) and the indices of genetic distances between different lines according to Kuhnlein *et al*. (1989) and Haymer and McInnis (1994).

RESULTS AND DISCUSSION

The primers detected a total of 83 DNA bands of 15 different microsatellite alleles in all genetic groups (Table 3). The molecular weight of all allelic bands ranged 52-350 bp. The number of alleles varied among different primers from one to three alleles, with an average of 2.14 alleles per microsatellite locus. This reveals the multi-allelic property of the microsatellite loci. Also, the number of allelic bands and their frequencies varied between different genetic groups as well as between sexes within each group. The total number of allelic bands detected among line and sex ranged 6-11 bands. The differences in number of alleles and their frequencies among lines and sexes demonstrate the genetic composition specificity of each line and the sexual dimorphism.

Table (4) presents the variability, heterozygosity and polymorphic information content (PIC) in each line. The genomic variability in line CE3 varied according to the alleles detected by each primer and ranged between 0.00 and 0.83. In comparison, the control line CE4 showed variability for up to 0.75. The heterozygosity was for up to 0.94 in line CE3 versus 0.88 in line CE4. The averages, overall primers, of variability and heterozygosity were 0.54 and 0.68 in line CE3 and 0.46 and 0.52 in line CE4. The results reflect the breeding scheme being practiced in each line. Line CE3 has undergone 6 generations of selection for increased 6-wk body weight; hence the moderate variability and moderately high heterozygosity estimates suggest that there is still more progress might be expected by continuing selection. The lower variability and heterozygosity estimates in line CE4 is eventually attributed to the random mating practiced as well as the small population size which both allow for increasing homozygosity and in turn lowering variability. The polymorphic information content obtained for line CE3 ranged 0.00-1.00, with an average of 0.81 and averaged 0.57 in line CE4. The results express the high polymorphism in both lines and reveal the multi-allelic loci in both lines. The monomorphism was only shown in the microsatellite locus detected by the primer MCW0217, by which no variability and heterozygosity were appeared. Also, the available data did not

denote to the existence of line-specific alleles. The averages, overall primers, of genomic variability and heterozygosity were 0.30 and 0.36 in line D versus higher averages of 0.49 and 0.58 in line CD. The PIC averaged 0.45 in line D versus 0.62 in line CD, revealing the polymorphism of the microsatellite loci. However, the locus detected by the primer MCW0217 was also monomorphic in both lines D and CD. These results also express the genetic background of each line, where line D is selected as a dam line for high 6-wk body weight and line CD is maintained as the genetic control.

The variability and heterozygosity estimates were remarkably higher in line CE3 than in line D. This may be attributed to the genetic background from which each line has been derived. Line CE3 has been derived from local chickens carrying Na gene with a wide unspecified gene pool, however line D has been derived from a local breed with specified genetic characters. Therefore the genetic variability and heterozygosity in line CE3 is still considered higher although the selection is practiced. In the study of El-Gendy *et al.* (2006) on Egyptian local breeds (White Baladi, Fayoumi and Sinai Bedouin), the polymorphism averaged 0.65 with more variability and heterozygosity in Fayoumi than in the other breeds. Beigi Nassiri *et al.* (2007a,b) investigated the genetic variation in an Iranian native breed, using 20 microsatellites. The heterozygosity ranged from 0.50 to 0.74 per locus with an average of 0.56. Polymorphism information content ranged between 0.38 and 0.70 per locus and the average PIC was 0.49. Olowofeso *et al.* (2005) measured the genetic relationships among 4 Chinese chicken populations, using 15 microsatellite markers. The mean allele number, across populations, for all loci ranged 5.73 to 6.00, with an average of 5.88. Heterozygosity ranged 0.65 to 0.70, with an average of 0.68. The PIC ranged 0.61 to 0.65.

Table (5) presents the genetic distances between lines CE3 and its genetic control line CE4 and between line D and its genetic control line CD. The distance between line CE3 and line CE4 averaged, overall primers, 2.92, versus a genetic distance of 1.95 between line D and line CD. These distances reveal the progress of selection practiced in lines CE3 and D, which has been expressed in shifting the genetic composition of them from those of their control lines CE4 and CD. The longer distance between line CE3 and line CE4 indicates the higher selection progress achieved in line CE3 compared to line D. Thus the results obtained from the genomic data were reliable to withdraw a conclusion about the breeding scheme. Romanov and Weigend (2001) constructed three genetic branches for different genetic stocks, where the red jungle fowl was in a branch, the commercial layer strains formed the second branch and the German populations formed the third branch. Olowofeso *et al.* (2005) measured the genetic relationships among four Chinese chicken populations, using 15 microsatellite markers. The genetic distances ranged from 0.17 to 0.34. Also, El-Gendy *et al.* (2006) showed different genetic distances among Egyptian breeds, and the farthest distance of 0.53 was between Fayoumi and Sinai Bedouin. Zhou and Lamont (1999) constructed the phylogenetic tree for 23 highly inbred chicken lines derived from White Leghorn, red jungle fowl, Fayoumi and Spanish breeds. The results were in accordance with the origin and breeding history.

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Table 1. The molecular information of microsatellite primers

Markers	Ch.#	Lenghth, bp	Repeat	Reverse primer	Forward primer	# Bases		GC, %	
						F	R	F	R
ADL0183	1	102	(TG)16	TTG TGA AGT GGA TAA GAT GA	ACA GAA ATG GAA AGC GAG AC	20	20	35.0	45.0
ADL0299	28	159	(TG)24	GTC TAG GCC CCT TGC CAA AC	CCA CCC CCA TGT TCA GGT CA	20	20	60.0	60.0
LEI0075	Z	226-259		CTATGCTATCATTGAAACACAGC	ATCCAGTGCGTGTCTGGTCAG	23	21	39.1	57.6
LEI0082	5	253-280		TATCCATACAGTACCCTCCTG	CCTTAGCTGGCTCAGTGGATG	21	21	57.1	52.4
LEI0094	4	253-285	X83246	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	21	21	52.4	47.6
LEI0106	1	289-300	X82854	AAACCTTCAAATGGTTAAAATGC	GTCAGCATGACAGCAGCTGAG	22	22	50.0	40.9
LEI0120	15	278-316		CGTAACACATGCAACTCAATG	TTAGAATGAAAAGGCTGTTCC	21	21	42.9	38.1
MCW0193	5	302-317		TATTCAATAGAGTTACGCTGTC	ATTACGTCTGCACCAGTACAG	21	22	47.6	36.4
MCW0217	18	153-174		GATCTTTCTGGAACAGATTTC	CTGCACTTGGTTCAGGTTCTG	21	21	38.1	52.4

ADL = Avian Disease and Oncology Laboratory, Michigan State University, East Lansing, Michigan, USA.
LEI = University of Leicester, Leicester, UK.
MCW = Microsatellite Chicken Wageningen, The Netherlands.

Table 2. PCR reaction components

Component	Amount
Genomic DNA (75 ng)	3.0 µl
Forward primers (25 Pmol)	2.5 µl
Reverse primers (25 Pmol)	2.5 µl
Master mix	12.5 µl
PCR grade water	4.5 µl
Total volume	25.0 µl

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Table 3: The number and molecular weights of alleles detected among different lines CE3 is the naked-neck selected line, CE4 is the naked-neck control line, D is the broiler dam line and CD is the broiler dam control line.

Primer	Total	Mw range, bp	CE3		CE4		Parents		D		CD	
			?	?	?	?	CE3, ?	D, ?	?	?	?	?
ADL0299	3	54-157	2	2	2	1	2	2	2	1	1	1
MCW0217	1	155	1	1	1	1	1	1	1	1	1	1
LE10094	2	310-350	1	2	1	1	-	1	1	1	1	-
LE10082	3	52-280	-	1	1	1	1	3	3	1	1	3
LE10075	2	58-270	-	1	-	1	1	2	2	2	1	2
LE10120	2	320-350	-	1	1	1	-	1	1	1	-	1
ADL0183	2	55-118	2	2	2	1	1	1	1	-	1	1
Total	15		6	10	8	7	6	11	11	9	6	9
Average	2.14		0.86	1.43	1.14	1.00	0.86	1.57	1.57	1.29	0.86	1.29

CE3 is the naked-neck selected line, CE4 is the naked-neck control line, D is the broiler dam line and CD is the broiler dam control line.

Table 4: The genomic variability, expected heterozygosity and polymorphic information content (PIC) estimates in different lines

Primer	# alleles	CE3				CE4				D				CD			
		# alleles	Var.	Hetero	PIC	# alleles	Var.	Hetero.	PIC	# alleles	Var.	Hetero.	PIC	# alleles	Var.	Hetero	PIC
ADL0299	3	3	0.33	0.48	0.67	2	0.33	0.33	0.33	2	0.44	0.52	0.67	1	0.83	0.92	0.67
ICW0217	1	1	0.00	0.00	0.00	1	0.00	0.00	0.00	1	0.00	0.00	0.00	1	0.00	0.00	0.00
LEI0094	2	1	0.83	0.94	1.00	1	0.50	0.50	0.50	2	0.50	0.72	1.00	1	0.75	0.88	1.00
LEI0082	3	2	0.78	0.93	1.00	1	0.67	0.67	0.67	3	0.00	0.00	0.00	3	0.33	0.50	0.67
LEI0075	2	2	0.67	0.89	1.00	1	0.75	0.88	1.00	2	0.00	0.00	0.00	2	0.25	0.38	0.50
LEI0120	2	1	0.83	0.94	1.00	1	0.75	0.88	1.00	1	0.50	0.50	0.50	1	0.75	0.88	1.00
ADL0183	2	2	0.33	0.56	1.00	2	0.25	0.38	0.50	1	0.67	0.78	1.00	1	0.50	0.50	0.50

CE3 is the naked-neck selected line, CE4 is the naked-neck control line, D is the broiler dam line and CD is the broiler dam control line.

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Table 5: The genetic distance indices between the selected lines and their genetic control lines.

Microsatellite Marker	CE3 ↔ CE4	D ↔ CD
ADL0299	1.620	2.109
MCW0217	1.000	1.000
LEI0094	3.303	3.564
LEI0082	3.830	1.761
LEI0075	3.564	1.357
LEI0120	3.564	1.693
ADL0183	3.564	2.178
Average	2.921	1.952

CE3 is the naked-neck selected line, CE4 is the naked-neck control line, D is the broiler dam line and CD is the broiler dam control line.

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