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INTEGRATION OF QTL DETECTION OF GROWTH TRAITS AND ASSOCIATION STUDY FOR CHICKEN CHROMOSOME 4

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ABSTRACT: Quantitative trait locus (QTL) mapping opens a way for breeders to manipulate quantitative trait genes. The objective of this study is to detect the QTL related to growth performance in local breeds of chicken. A cross between three genetically different chicken' breeds was used to produce two generations populations. Total of 16 Saso cocks, and 32 hens (16 of each of Alexandia and Fayoumi) as parents were used to produce first generation (G_1). Data of 954 chicks produced during the auxiliary two generations of different crosses (S ♂ xF ♀, S ♂ xA ♀, SF ♂ xSF ♀, SA ♂ xSA ♀, SF ♂ xSA ♀ and SA ♂ xSF ♀) in such a way the genetic homogeneity from G0 to G2 recombinant populations has been considered. These populations were used for detection and localization of QTL related to the growth traits; body weight (BW), growth rate (GR), and average daily gain (ADG). A number of 25 microsatellite markers belong to chicken chromosome 4 (GGA4) have been genotyped, and the regression interval mapping approach was used to identify QTL. The results revealed that all selected markers were informative. There was a statistical evidence for QTL on GGA4 for BW at 8 and 12 weeks of age, whereas one QTL exceeded the significant threshold for the trait of BW at 8 weeks of age. The related trait, growth rate, reached the suggestive threshold. All of three QTL effects identified on GGA4 had their maximum test statistic in the region between 134-154 cM. In addition, most of significant markers (MCW0390, MCW0393, MCW0397, MCW0409, MCW410 and UMA0038) were associated with growth traits at all chicken ages. Although, the polymorphism information content (*PIC)* obtained over all microsatellite markers was 46%, that around 82% went to UMA0038 locus. Two private alleles were found for markers MCW0405 and MCW0409 with allele frequency around 0.025 in G_1 and G_2 respectively. Additionally, Chi-square test was used to investigate the deviation of loci from Hardy-Weinberg equilibrium individually, and four microsatellite markers (MCW0395, ADL0266, MCW0400 and UMA0038) were not in genetic equilibrium. In addition, analysis of molecular variance (AMOVA) revealed that 14% and 86% of variance were observed among and within individuals, respectively. The obtained small value of F_{ST} (ranged between 0.001 to 0.019) may reflect generous genetic differentiation. In conclusion, the recognized QTL, integrated with the association study, gave useful and practical information to distinguish molecular genetic factors that influence growth traits within the local populations of chicken.

Key words: Poultry, QTL, Microsatellite markers, Crossing, Growth, GGA4

INTRODUCTION

Among domestic animals, the chicken is ideal for genetic mapping and QTL analysis because of their high reproductive capacity, enabling several generations of large families to be generated in a reasonable timeframe. In addition, chicken is unique among agriculture species in that a number of selection lines are available. The diploid karyotype of chicken is comprised of eight pairs of macro-chromosomes, thirty pairs of micro-chromosomes, and two sex chromosomes. Micro-chromosomes are estimated to comprise 30% of genome, but they include 40% of the genes (Brown *et al*. 2003). The relatively small size of the haploid chicken genome (1.2x109 bp) as compared to that of mammals (3 x 109 bp) is a big advantage for subsequent research aiming for the identification and characterization of the genes underlying the QTL effects. In poultry, few genes with economical importance have been identified, e.g., the dwarf gene and sex-linked feathering genes. Many studies have reported an association between genetic markers and quantitative traits of economic importance (Liu *et al*., 2008; Zhang *et al*., 2008 and Sandercock *et al*., 2009; Nassar *et al*. 2015; Lyu *et al*. 2017). In the presence of known linked markers or genes affecting traits of interest, marker assisted selection (MAS) could support the traditional breeding system. Selection of the best animals using molecular information at DNA level can help to prevent alleging of high generation interval, low heritability of traits, and the nature of the traits to be sex limited. Primarily, microsatellite markers are highly polymorphic, meaning that there are many possible alleles at each locus that each animal can have. In chicken, numerous microsatellites have been mapped in reference populaces (Cheng *et al*. 1994, Crooijmans *et al*. 1993, 1996; Groenen *et al*. 2000; Rabie *et al*. 2005). These markers give an effective device to either quantitative genetics and fine mapping approaches (Rabie *et al*., 2005; Lyu *et al*., 2017), and have already been used effectively to focus on the hereditary relationships between and inside chicken populaces (Rosenberg *et al*. 2001; Vanhala *et al*. 1998; Zhou *et al*. 1999). The direct application of a molecular technique is a candidate gene approach that represents an effective approach to identify genetic markers associated with economically valuable production traits in livestock (Rothschild and Soller, 1997). Subsequently, a QTL on chromosome 4 (GGA4) influencing chicken growth performances until 20 weeks of age in F2 cross between the inbred New Hampshire (NHI) and White Leghorn (WL77) lines was detected with confidence in an 26.9 Mb interval between 61.5 and 88.4 Mb, in which 292 genes are residing (Nassar *et al*. 2015). The same QTL region was identified in other chicken populations, for instance in Silky Fowl x White Plymouth Rock cross (Gu *et al*. 2011) and Beijing-You chickens (Liu *et al*.2013). These QTLs were identified from phenotyping and genotyping of crosses between two breeds or two lines within the same breed. Moreover, many of the major genes and their variants reliable for growth traits in chicken have not been realized yet. Therefore, there is a subsistent need to conduct further studies for detecting part or all of genomic regions which clarify most of the genetic variations in desired traits. Despite of the great efforts made globally to reveal genetic loci affecting economically important traits related to growth in chicken, the goal of the current study is to identify QTL related to growth performance on chicken chromosome 4 that explain differences between indigenous lines that have been selected for many generations.

MATERIALS AND METHODS

Experimental population and phenotyping

This study was conducted at the poultry research center, department of poultry production, faculty of agriculture, Alexandria University, and the biotechnology laboratory, department of animal Production, faculty of agriculture, Suez Canal University. The experimental population used to recognize the QTLs identified with growth performance i.e, body weight (BW), growth rate (GR), and average daily gain (ADG) was dependent on two generations (G_1, and G_2). Whereas, G0 was based on 16 males from Sasso line (S), and 16 females from each of Alexandia (A) and Fayoumi (F). Two females (One Alexandria and one Fayoumi) were randomly assigned to each Sasso male to produce the G_1. The G_2 was generated according to the mating system (SF ♂ x SF ♀, SA ♂ x SA ♀, SF ♂ x SA ♀ and SA ♂ x SF ♀). The trap nested eggs which were delivered from each exclusive breeding pen have been gathered and recorded daily for 7 consecutive days by genetic group. Eight hatches were taken biweekly in each generation. At hatching, the chicks were labeled with wing-banded, weighed, and brooded on floor, of 32°C gradually decreased 2-3°C weekly until reaching the normal temperature. At age of eight weeks, the chicks were sexed, weighed and moved to the rearing house. Individual chick BW was recorded to the nearest gram at hatching (day 0) then of 4, 8, and 12 weeks. Periodical GRs were estimated from day 0 to 4 wks (GR_{0-4}), from 4 to 8 wks (GR_{4-8}), from 8 to 12 wks (GR_{8-12}) and from day 0 to 12 wks (GR_{0-12}) of ages according to Brody (1945). In addition, the respective ADGs were calculated for each line (ADG_{0-4}, ADG_{4-8}, ADG_{8-12}, and ADG_{0-12}).

Blood samples, and DNA extraction

A total of 160 blood samples were collected from the wing vein (42 from G_0, and 118 samples from G_1, and G_2), and were collected in a tube treated with K3-EDTA (FL medical, Italy) and stored at -20°C until DNA extraction. Genomic DNA was extracted using PureLink Genomic DNA Mini; Microcentrifuge spin-column format (Invitrogen™ K182001, USA) to provide superior performance and high purity and yield of extracted DNA. The quality of extracted DNA was examined by NanoDrop® ND-1000 UV-Vis Spectrophotometer enabling highly accurate analyses with remarkable reproducibility.

Selection of markers and genotyping

Twenty-five microsatellite markers were selected along chromosome 4 (GGA4) according to their potential for detection, viability, and expected coverage of heterozygosity. Table 1 showed more information about the markers. The PCR reactions were performed in a 25µl final volume containing 6µl of 100 ng of DNA, 6 µl of the PCR Super Mix contained 1.1x buffer (Invitrogen, 10572-014), forward and reverse primers (0.2 – 1uM each), and nuclease-free dH2O to final volume of 25 ul. An Eppendorf thermal cycler was used along with the following PCR profile settings: 5 min at 95ºC followed by 35 cycles for 30 sec at 95ºC, 45 sec at 45ºC, 50ºC, or 55ºC annealing temperature, and 90 sec at 72ºC, followed by an elongation step at 72ºC for 10 min, and finally stop step at 4ºC. Subsequently, PCR products were electrophoresed on 1.5% agarose gel containing 0.5% ethidium bromide which viewed under UV light. Therefore, genotyping of the microsatellite markers was done using QIAxcel advanced system.

Statistical analysis

QTL Analysis

Total of 89 selected genotypes (22 G_0, 26 G_1, and 41 G_2) were used for QTL analysis combined with total of 25 informative markers mapped on GGA4. Linkage distance among loci was estimated by the Multilocus 1.3 (Agapow and Burt 2001) using the marker genotypes for all these markers and all individuals, the map distances given in centimorgans (cM) on haldane scale (Haldane 1919), was drawn using Mapchart 2.32 (Voorrips, 2002). The QTL examination was attempted with the regression interval mapping approach following the proposal of Van Kaam et al., (1998). This technique is an expansion of the strategy of Knott et al., (1996) for multi-marker regression method for outbred populaces with a half sib family structure. The significance thresholds for the test statistics were empirically derived using the permutation method outlined by Churchill and Doerge (1994). The 5% chromosomewise thresholds were determined by performing 1000 permutations at 5 cM interims. A test statistic was calculated at each cM in order to test the presence of QTL effects, against the null hypothesis of their absence.

Association between phenotypes and segregated alleles

From the observed codominant markers data, the deviations from Hardy-Weinberg equilibrium (HWE), the observed and effective () number of alleles (*No* and *Ne* respectively), the observed and the expected heterozygosity (*Ho* and *He* respectively), and polymorphism information content (*PIC*) were assessed using Cervus 3.0.7 software (Kalinowski *et al*., 2007). The F-statistics of pairwise genetic differentiation among the populations (F_{ST}), the decrease in heterozygosity because of inbreeding within a population (F_{IS}) were determined. Consequently, the association between microsatellite markers and growth traits at interval weeks were evaluated with the generalized linear model. The statistical model was based on that described by Ma *et al*. (2014) with amendment. Followed by multivariate analysis of variance analysis which was implemented by R (Core R Team, 2013) using "mvtnorm" package for the association between detected alleles and phenotype observations.

RESULTS AND DISCUSSION

Genetic assortment based on microsatellite markers:

Genetic assortment based on microsatellite markers: Twenty-five microsatellite markers have been used, and successfully tested on chicken genomic DNA. All analyzed markers were informative, Therefore, allele frequencies for all loci were analyzed and the markers' characteristics such as *No*, *PIC*, private alleles (PA), *Ho* and *He* across all generations are given in Table (2). Although, the obtained *PIC* over all positions was 46%, that for UMA0038 marker was around 82%. This in agreement with Rabie *et al*., (2005) who reported that the average *PIC* in chickens in the first generation ranged from 53 to 83%. In addition, Hillel *et al*. (2003) obtained 69% of *PIC* when 22 microsatellite markers were used for a diversity study. The obtained average of number of alleles per locus was 3.52±0.28, with a mean proportion of typed loci 0.94 similar to those obtained by both Roushdy *et al*., (2013a,b), and Soltan *et al*. (2018) when they studied local Egyptian's chicken populations, it ranged from 4.2 to 13.8. A total of two PA was found and distributed in G_1 for MCW0405 with allele frequency 0.024, and for MCW0409 in G_2 with allele frequency 0.025 (Figure 2). The percentage of PA was around 2.27% which was lower than those recently detailed in Egyptian local chicken breeds the scope of 13.18 to 45.28% of total alleles (Soltan *et al*. 2018; and Roushdy *et al*., 2012a). This was

probably due to crossing between divergent chickens, and the modest number of samples analyzed per generation. that utilized for the analysis. Mean of the detected number of alleles per locus (Na) was 3.52±0.28 (Table 2). Allelic patterns for co-dominant data are shown in Figure (1). In general, F_{ST} value lower than 0.05 may reflect substantial genetic differentiation. Surprisingly, in this study F_{ST} value averaged 0.007±0.001, despite the fact that, this value estimates the level of genetic differentiation, it reflected a slight genetic contrast with the utilized locus (Balloux & Lugon-Moulin, 2002). In addition, to measure the degree of molecular diversion, locus-by-locus investigation on molecular variance (AMOVA) was executed and the outcomes illustrated that the differentiation between generations was null for the total genetic variance, while 14 and 86% of change was observed among, and within individuals respectively (Table 3) whereas, these results were far from the findings of El-Sayed *et al*. (2011), Eltanany *et al*. (2011), and Soltan *et al*. (2018) for local Egyptian strains. Additionally, to investigate the deviation of loci from Hardy-Weinberg equilibrium (HWE) individually, Chi-square test was used, and the results are shown in Table 2. Across all used loci, four microsatellite markers MCW0395, ADL0266, UMA0038 ($P \leq 0.05$), and MCW400 ($P \leq 0.001$) were not in genetic equilibrium. In addition, the inter-population genetic differentiation coefficient (G_{ST}) values were mostly negative. Despite of the distributional range, the estimation of inter-population examination supports low degree of genetic separation between these generations. The F_{ST} comparisons of the entirely unexpected components of the genome will offer bits of knowledge into the statistical history of populations (Holsinger and Weir, 2009). The current low F_{ST} value (0.001 to 0.019) formulated slight hereditary contrasts as indicated by the utilized microsatellite markers (Balloux and Lugon-Moulin, 2002) and low values reflected generous genetic differentiation. Also, the low non-significant F_{ST} values suggested that the genotype of the individuals of the studied generations were closely related to each other.

QTL analysis

Eleven traits related growth genotypes of 89 individuals were used in this study. The acquired QTL with significant and suggestive linkages for each trait are summarized in Figure 2. There was significant statistical evidence for two growth-related traits on GGA4. A significant linkage with BW8 situated on GGA4 in the middle of 134-154 cM (MCW400 -MCW395) was observed. Suggestive linkage was noted for BW12 (UMA0038 -MCW0404) at position 145 cM. In addition, suggestive QTL for GR0-4 was detected at position 154 cM (LEI0094-MCW0395). Details of the markers flanking each QTL, and their position on the chromosome are presented in Figure 2. Similarly, Nassar *et al*. (2015) reported that most elevated QTL impacts for the phenotypic F2 variance (from 4.6 to 25.6%) were found on GGA4 somewhere in the range of 142 and 170 cM. Furthermore, the confidence interval for the QTL region on GGA4 is located between 61.5 and 88.4 Mb in the chicken genome and harbours 292 genes (http://www.ensembl.org/Gallus_gallus/). Similar patterns of QTL were found by Cahyadi *et al*. (2016) through improving the Korean native chicken, twelve microsatellite markers have been used on GGA4, where the QTL was discovered to influence BW4 and BW8. The QTL was situated between 23 and 37 cM on GGA4, while the QTL peak was the nearest to the ADL0203 marker. In addition, Cahyadi *et al*. (2016) found a positional candidate gene located in the QTL region on GGA4 for

growth- related traits at 6 to 8 weeks of age , moreover, Nassar *et al*. (2015) declared the QTL that essentially influence growth from 5 to 20 weeks on the genomic region somewhere in the range of 153 and 159 cM on GGA4 a peak exist in the range of 75.24 and 79.39 Mb. Table 4 briefs the detected QTLs situated on chicken chromosomes at different ages from several different genetic resources and crosses. Moreover, Wang *et al*. (2012) using F2 population cited that there were three significant QTLs and 10 others at suggestive level on chromosome 3 and 4. Strikingly, a QTL for BW at 12 weeks of age situated on GGA4 had a significant additive substance impact which clarified 13.8% of the phenotypic variation, although, the biggest dominance effect for QTL represented 6.5% of the phenotypic variation (Khalil *et al*. 2016). Moreover, the low heterozygosity between the studied generations (Figure 3) might indicate the absence or not reaching the significance threshold of QTL for GR and ADG. In addition, the means of the expected and observed heterozygosity for the markers across the generations in Table 2 being about 0.54, may be due to the minute inspected samples and genotypes.

Association between the locus and growth traits.

Chicken BW isa polygenic inheritable trait, which takes a long time to be improved. Integration of the molecular marker technology and its relatedness to growth will contribute to more practical selection for growth traits in broilers (Deeb and Lamont, 2002; Sazanov *et al*., 2010). In the current study, the performed association analysis between the 25 loci and the growth-related traits indicated that most of the associated markers were located within the detected QTL region, whereas 13 markers were in the flanked area between MCW400 and MCW395. While the percentage of the associated markers 26.67% for chicken GR, and 33.3% for ADG to 53.3% for BW at different ages. The significant and highly significant association between markers and traits are shown in figure 1. Interestingly, most of the significant associations for GR and ADG were in between markers MCW0390, MCW0393, MCW0397, MCW0409, and MCW410 while UMA0038 was associated with BW at all ages studied. (0, 4, 8, and 12 weeks of age). This locus located on GGA4 at 48 Mb, in relation to the two genes *PPA1K*, *ADGRL3* which might also be related to growth-traits (https://www.ensembl.org/Gallus_gallus/). The positional candidate gene (s) on GGA4 within the QTL region is also related to growth traits in local chicken. Cahyadi *et al*. (2016) found a potential candidate gene, the insulin receptor substrate 4 gene (*IRS4*) that plays a notable responsibility not only in growth, but also in reproduction and glucose homeostasis (Sadagurski *et al*., 2014). Somewhere, it acts as an interface between multiple growth factor receptors such as insulin-like growth factor 1 receptor (*IGF1R*), and fibroblast growth factor receptor 1 (*FGFR1*) which showed an important role in cell development, in addition, metabolic homeostasis, growth, and reproduction (Hinsby *et al*., 2004).

Furthermore, the detected QTL in this study (42.5 - 50.5 Mb), the candidate genes such as *PDGFRA* (platelet-derived growth factor receptor, alpha polypeptide; Locus: 4q12), also *IGFBP7* (insulin-like growth factor binding protein 7; Locus: 4q12) was found (https://www.ensembl.org/Gallus_gallus/Location/). Nevertheless, the region harbours several functional candidate genes, so that fine mapping is important to physically decrease the chromosomal interim and along these distance the potential candidate genes that demonstrate either individually or in interactions could be detected.

IN CONCLUSION,

for the effective usage of QTL data into particular breeding programs, segregation of QTL should be approved within the population in interest. Along these lines, the recognized QTL areas in this study utilizing useful and practical information to distinguish molecular genetic factors that influence growth traits within the local populations. In addition, the data from this investigation will add to endeavors to improve the body weight in local chicken breeds.

Table (1): Molecular characteristics, information and annealing temperature for microsatellite loci belong to chicken chromosome 4.

Locus name	forward primer (5'-3')	reverse primer (5'-3')	Mb[1]	Tm[2]
ADL0246	GCAGGCTGATAGAAAAATGC	CTGCAAGCTGCTCTGGTATT	37.66	55
MCW0408	GGTGCTACACGAAGGTACTG	TTTCTGAGCTGCTTGTCCTC	38.27	55
MCW0085	GTGCAGTTATATGAAGTCTCTC	GGTATCAGGGCTTCTGAAACA	38.79	50
MCW0410	CACGAAGAAGAGAACCTTCC	CACCTCCTGTGTTGGTCCAG	39.84	55
MCW0398	GTTCTTCCATCAGAGCACAG	GGAGCGTAGACTGTATCAGG	40.56	55
LEI0122	AATCCCTATAGAACTTTGTGC	GATCTTACTGGATTACCATTC	40.91	55
MCW0396	CTCACTTTCTGCAGTTACCC	CTGGTGACACCTTCAAACTG	42.14	50
MCW0400	GGATTTATCCCATGCCTCAG	GGGACAGAGAGAAGCAGTGG	42.50	55
MCW0397	TGAGTCAGGCTTGATTCTGC	ACCACCCCTCACATGGATTC	43.07	50
MCW0401	GAGTGGAATTACCGGAGAAC	CTAGCTACTGTTAGGTGGAG	44.31	50
MCW0390	TACTACACAACCCCCTCTAC	GACTAATTCAGGGTGCTCTC	44.52	50
MCW0402	ACTGTGCCTAGGACTAGCTG	CCTAAGTCTGGGCTCTTCTG	44.72	55
ADL0266	GTGGCATTCAGGCAGAGCAG	AATGCATTGCAGGATGTATG	45.52	55
MCW0391	AGGATTACCAGCTCCCAGAC	CTTTTCACTGCTCCGTAGAC	46.20	50
MCW0405	GGAGCTGAGATTTGTTGAGC	GCTGCAAGGTGAAGGAAAAC	46.71	55
UMA4.038	CATTTGCAAGTGCCATACAG	GCCCTGGTAAACTGGTGTCC	46.71	45
MCW0404	GCACAGACTAAACCTTGCTC	GTTAGTAAGCAGGGGGTCTG	47.69	55
MCW0403	GGTACGGAAGAACTGATAGG	GACATGGTAGAACTGCAAGG	47.83	55
MCW0393	GGGAGAGGTGAGACAGATAG	TCTAGAGGAGGCTTTGCTAC	48.24	50
MCW0394	ATCAAGTCCTCCGATACTGC	GAACAACTGGCTAGGCTAAG	50.15	50*
LEI0094	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC	50.33	55
MCW0395	TGCTTGTGCAGAGATGAAGC	AGTAAGTACAGAGCCACTGC	50.50	50
MCW0411	GAAGGTCTCCCAGCTATAAG	TTTGGTGTGGGTAGAAGGTG	50.89	50*
MCW0409	GCACACTGAGCTACCTTTAG	GTTCTGGAGAAGACTGCTTG	51.05	50
MCW0284	CAGAGCTGGATTGGTGTCAAG	GCCTTAGGAAAAACTCCTAAGG	53.60	50

[1]The position of marker in Mb according to chicken genome sequence data. [2] The optimal annealing temperature in the PCR reaction, the temperature that marked with asterisk needed more elongation time compared to other markers.

Table (2): Genetic characterizations for the studied 25 microsatellite markers

Microsatellite marker (Locus)	Mean N	Mean *Na*	Mean *Ne*	Mean *Ho*	Mean *He*	H_t	F_{IS}	F_{ST}	G_{ST}	*PIC*	*HWE*
MCW390	88	4.000	3.235	0.781	0.687	0.695	-0.137	0.011	0.001	0.639	NS
MCW391	88	5.000	2.003	0.546	0.498	0.500	-0.097	0.005	-0.007	0.461	NS
MCW393	87	4.000	2.325	0.593	0.566	0.571	-0.048	0.009	-0.003	0.524	NS
MCW394	83	3.000	1.898	0.560	0.472	0.474	-0.185	0.003	-0.008	0.375	NS
MCW395	88	3.000	2.632	0.525	0.618	0.623	0.151	0.008	-0.006	0.546	*
MCW396	84	2.000	1.289	0.223	0.224	0.224	0.004	0.001	-0.013	0.202	NS
MCW397	88	3.000	1.986	0.468	0.489	0.495	0.043	0.012	-0.001	0.431	NS
MCW398	89	3.000	1.655	0.355	0.391	0.394	0.092	0.007	-0.006	0.349	NS
MCW400	67	4.000	3.114	0.595	0.678	0.690	0.122	0.017	-0.002	0.634	***
MCW401	73	3.000	1.409	0.246	0.290	0.290	0.152	0.001	-0.018	0.272	NS
MCW402	88	4.667	3.230	0.759	0.689	0.694	-0.101	0.008	-0.004	0.633	NS
MCW403	89	2.000	1.957	0.605	0.489	0.490	-0.238	0.002	-0.008	0.37	NS
MCW404	88	3.000	1.320	0.246	0.241	0.242	-0.019	0.003	-0.010	0.22	NS
MCW405	88	6.333	3.222	0.742	0.689	0.691	-0.077	0.003	-0.008	0.646	NS
MCW408	86	2.000	1.801	0.417	0.445	0.445	0.063	0.001	-0.013	0.347	NS
MCW409	87	6.333	4.421	0.835	0.774	0.777	-0.079	0.005	-0.007	0.745	NS
MCW410	68	2.000	1.986	0.458	0.496	0.498	0.078	0.004	-0.013	0.374	NS
MCW411	77	2.667	1.803	0.476	0.442	0.447	-0.075	0.011	-0.003	0.352	NS
ADL0266	79	4.000	3.063	0.712	0.673	0.677	-0.058	0.007	-0.007	0.628	*
LEI0122	77	3.000	2.487	0.644	0.598	0.609	-0.078	0.019	0.006	0.532	NS
MCW0085	86	3.000	2.108	0.578	0.524	0.526	-0.104	0.004	-0.008	0.444	NS
UMA0038	79	7.000	6.017	0.848	0.833	0.838	-0.017	0.006	-0.008	0.816	*
ADL0246	88	3.000	1.840	0.388	0.455	0.458	0.147	0.006	-0.008	0.379	NS
LEI0094	86	3.000	1.578	0.436	0.359	0.363	-0.213	0.011	0.001	0.305	NS
MCW0284	80	2.000	1.798	0.473	0.443	0.446	-0.068	0.006	-0.007	0.345	NS

N= number of sampled individuals, Mean Na = Mean number of different alleles over generations, Mean Ne = Mean number of effective alleles over generations, Mean Ne = Mean number of effective alleles over generations. Mean *Ho* = Mean observed heterozygosity over k generations, Mean *He* = Mean expected heterozygosity over k generations, H_t = Total expected heterozygosity, F_{IS}: heterozygosis deficit, F_{st} = Inbreeding coefficient within generations, relative to total = genetic differentiation among generations. G_{ST} = Genetic differentiation coefficient, *PIC*= The polymorphic information content, and HWE = Hardy-Weinberg equilibrium.

Table(3): Analysis of molecular variance (AMOVA) in studied generations.

Source	*df*	*SS*	*MS*	Est. Var.	%	F-Statistics	P
Among generations	2	11.038	5.519	0.000	0%	-0.007	0.991
Among Individuals	86	705.844	8.207	1.014	14%	0.141	0.001
Within Individuals	89	550.000	6.180	6.180	86%	0.135	0.001
Total	177	1266.882		7.194	100%		

df: degrees of freedom, *SS:* sum of squares, *MS:* mean square, Est. Var: Estimated variance.

Table (4): Summary of genomic region with QTL for body weight of the chicken.

GGA	Position	Age	Cross[1]	G[2]	References
1	137-152	12 wks.	BL x WL	F_2	*Podisi et al., 2013*
	402	9 wks.	SS x WR	F_2	*Uemoto et al., 2009*
	598	4-5 wks.	BS x LD	F_2	*Liu et al., 2008*
	590	6-12 wks.			
	553	4 wks.	BS x LD	F_2	*Liu et al., 2007*
	195-555	5 wks.			
	548	6 wks.			
	551	7 wks.			
	351	8 wks.			
	528	9 wks.			
	394	7 wks.	BS x BD	BC_1- F_2	*Atzmon et al., 2006*
	--	5 wks.	BS x LD	F_2	*Nones et al., 2006*
		6 wks.			
		8 wks.			
	80-100	35 d	TT x CC	F_2	*Nones et al., 2005*
	184-200	35 d			
	125-139	42 d			
	72-122	8, 46, 112, 200 d	RJ x WL	F_2	*Carlborg et al., 2003; Kerje et al., 2003*
	70	8 wks.	BS x F &WL	F_1	*Deeb and Lamont 2003*
	550	10 wks.			
	534	11,12 wks.			
	151-169	3 wks.	WL x CB	F_2	*Sewalem et al., 2002*
	169-205	6 wks.			
	426-527	9 wks.			
	240	7 wks.	BD x BD	F_2	*Van Kaam et al., 1998*
	240	7 wks.	BD x BD	F_3	*Van Kaam et al., 1999*
	179-205	5 wks.	WPR x WPR		*Jennen et al., 2005*
	386	1 d	RJ x WL	F_2	*Carlborg et al., 2003*
2	60-119	13 wks.	S x WPR	F_2	*Tatsuda & Fujinaka 2001*
	2-60	16 wks.			
	292-302	6, 9 wks.	WL x CB	F_2	*Sewalem et al., 2002*
	384-452	200 d	RJ x WL	F_2	*Carlborg et al., 2003 ; Kerje et al., 2003*
3	40-216	48 wks.	BL x WL	F_2	*Podisi et al., 2013*
	220	9 wks.	SS x WR	F_2	*Uemoto et al., 2009*
	102	35, 41d	BS x WL	F_2	*Ruy et al., 2007*

Continue table (4):

GGA	Position	Age	Cross[1]	G[2]	References
4	384-452	8 wks.	BS x WL	F_2	*Zhou et al., 2006*
	0-161	6 wks.			
	0-177	12 wks.	BL x WL	F_2	*Podisi et al., 2013*
	37			F_3	*De Koning et al., 2003*
	23	8 wks.	KNC x KNC	F_1	*Cahyadi et al., 2016*
	37	4 wks.			
	200-220	35, 55 d	BSD x WL	F_2	*Schreiweis et al., 2005*
	112-120	3 wks.	WPR x WPR	F_9	*Rabie et al., 2004*
	130-140	5 wks.			
	142-170	10 , 15, 20 wks.	NHI x WL77	F_2	*Nassar et al., 2015*
	194-216	40 wks.	RIR x WL	F_2	*Tuiskula-Haavisto et al., 2002*
	120	7 wks.	BD x BD	F_2	*Van Kaam et al., 1998*
8	24	5 wks.	WPR x WPR	F_8	*Pakdel et al., 2004*
	25-94	3, 6, 9 wks.	WL x CB	F_2	*Sewalem et al., 2002*
	46	8 d	RJ x WL	F_2	*Kerje et al., 2003*
	91	3 wks.	WPR x WPR	F_9	*Rabie et al., 2005*
10	34	2 wks.	WPR x WPR	F_8	*Pakdel et al., 2004*
	88	3 wks.	WPR x WPR	F_8	*Rabie et al., 2005*
20	6	46, 112 d	RJ x WL	F_2	*Carlborg et al., 2003*
Z	118-165	3 wks.	WL x CB	F_2	*Sewalem et al., 2002*

[1]RIR=Rhode Island Red layer; RJ=Red Junglefowl. CB=commercial broiler; KNC= Korean native chicken; h-/l-AFC=high/low abdominal fat content; S=Satsumadori; WL=White Leghorn layer; WPR=White Plymouth Rock broiler; NHI= inbred New Hampshire; WL77= White Leghorn 77; BS = broiler breeder sire line. [2]G=Generation.

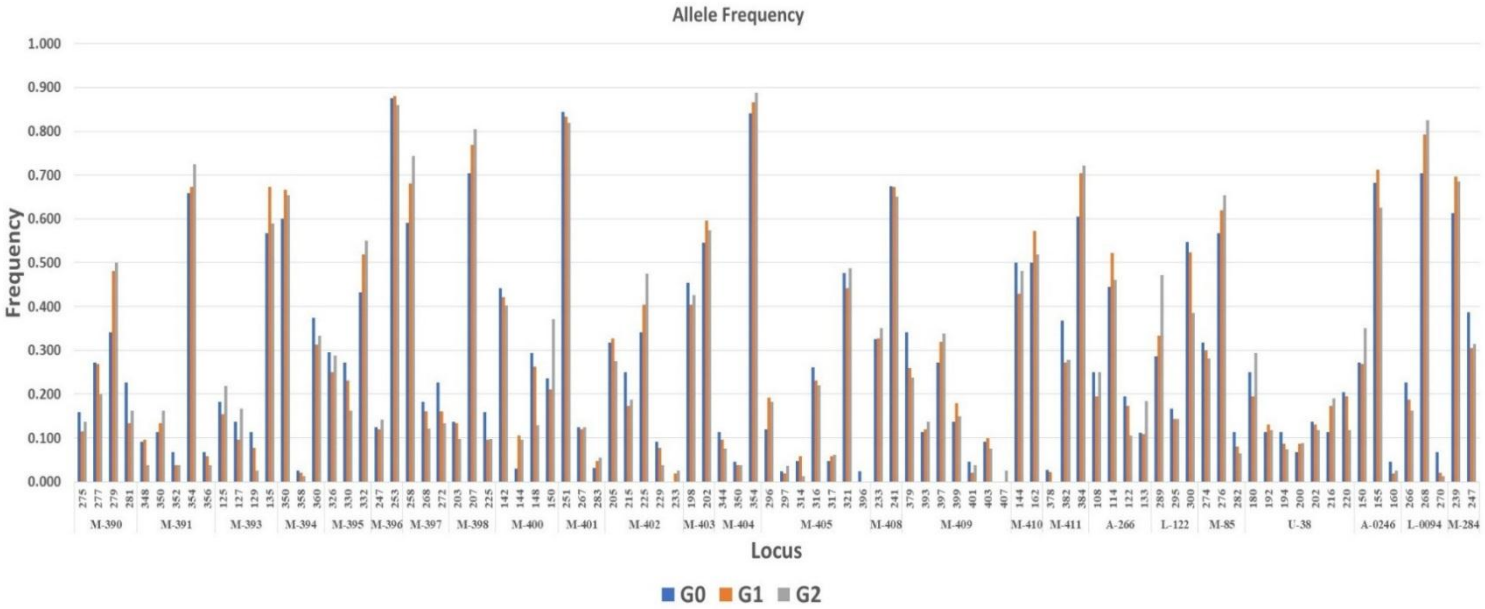

Figure (1): The observed allelic size and frequency per locus in each generation. The allele size with asterisk is a private allele for each generation. M- is MCW locus, L- is LEI locus, A- is ADL locus, and U is UMA locus. G_0, G_1, and G_2 are the successive generations.

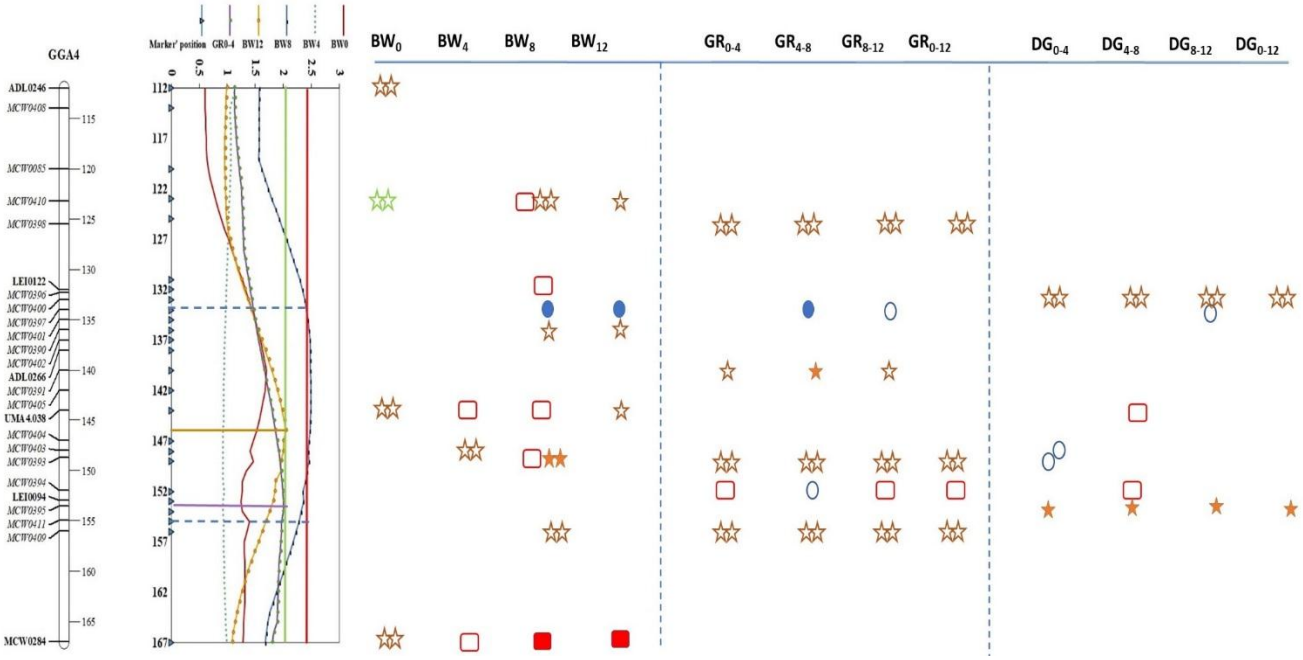

Figure (2): The test statistic values from the QTL analysis on chromosome 4. 10% chromosomewise significance thresholds are included. The association between the phenotypic traits and each microsatellite marker are integrated. While the trait associated with the specific marker has a significant effect with the sex (Male , and Female , at P≤0.05, and 0.01, respectively), related to generation 1 (G1) ☆, ★, and G2 ☆☆ , ★★ at P≤0.05, and 0.01, respectively), Finally the ★ referred to the significant effect at P≤0.001.DG= ADG

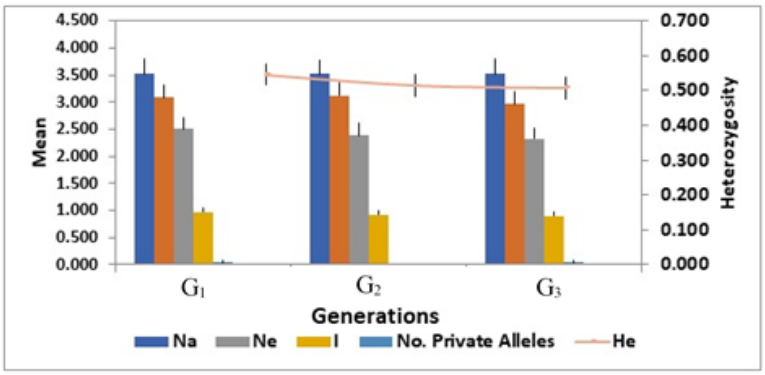

Figure (3): Allelic patterns across generations (G_1, G_2, and G_3). Where Na = Number of different alleles, Ne = Number of effective alleles, I = Shannon's Information Index, He = Expected heterozygosity.

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الملخص العربى

تكامل مواقع الصفات الكمية ودراسة الارتباط لصفات النمو الواقع على الكروموسوم الرابع للدجاج

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تفتح خرائط مواقع الصفات الكمية (QTL) الطريق أمام المربين للتعامل مع الجينات المتحكمة في الصفات الكمية. الهدف من هذه الدراسة هو اكتشاف الـ QTL المتعلقة بأداء النمو في سلالات الدجاج المحلية. تم استخدام الخلط بين ثلاثة سلالات مختلفة وراثيا لإنتاج جيلين متتالين. حيث تم استخدام عدد 16 من الديوك ساسو (S) ، و 32 دجاجة [16 من كل من الإسكندراني (A) والفيومي (F)] كآباء لإنتاج الجيل الأول (G_1) .بيانات 954 فرد تم إنتاجها خلال جيلين من خلطات مختلفة كالتالي (♂SxF♀ و♂SxA♀ و♂SFxSF♀ و♂SAxSA♀ و ♂SAxSF♀و♂SFxSA♀) وقد تم النظر في تجانس العشيرة من G_0 إلى G_2 المؤتلف. تم استخدام هذه العشائر لإكتشاف وتوطين الـ QTL المتعلقة بصفات النمو ؛ وزن الجسم (BW) ، معدل النمو (GR) ، ومتوسط النمو اليومي (ADG) .

تم استخدام عدد 25 من الواسمات الوراثية (مايكروستلايت) و التي تنتمي إلى الكروموسوم الرابع للدجاج (GGA4) ، وتم استخدام نهج تعيين معدل فاصل الانحدار لتحديد مواقع الصفات الكمية و كشفت النتائج أن جميع الواسمات المختارة كانت ذات معلوماتية مفيدة. كان هناك دليل إحصائي على وجود الـ QTL على GGA4 لـ BW عند عمر 8 و 12 أسبوعًا ، وتجاوز منحنى الـQTL لصفة الـ BW عند عمر 8 أسابيع الحد الإحصائي. الصفات ذات الصلة مثل معدل النمو ، وصلت إلى العتبة الإحصائية التي توحي بوجود تأثير. جميع تأثيرات الـ QTL الثلاثة المتحصل عليها على GGA4 كان لها تأثير إحصائي معنوي و تقع بين cM154-134 . بالإضافة إلى ذلك ، ارتبطت معظم الواسمات (MCW0390،MCW0393،MCW0397،MCW0409، MCW410 و UMA0038) بصفات النمو في جميع أعمار الدجاج. على الرغم من أن محتوى معلومات تعدد الأشكال (*PIC*) الذي تم الحصول عليه عبر جميع الواسمات (مايكروستلايت) كان 46٪ ، وكانت النسبة حوالي 82٪ بالنسبة لـ UMA0038 . تم العثور على أليلين خاصين للواسمات MCW0405 و MCW0409 مع تردد أليل حوالي 0.025 في G_1 و G_2 على التوالي. بالإضافة إلى ذلك ، تم استخدام اختبار Chi-square للتحقيق في انحراف كل موقع عن توازن هاردي فاينبرج بشكل فردي ، ولم تكن أربعة واسمات (MCW0395 ، ADL0266 ، MCW0400و UMA0038) في حالة توازن وراثي. بالإضافة إلى ذلك ، كشف تحليل التباين الجزيئي (AMOVA) أن 14 ٪ و 86 ٪ من التباين لوحظت بين وداخل الأفراد ، على التوالي. قد تعكس القيمة الصغيرة التي تم الحصول عليها للـ FST (تراوحت بين 0.001 إلى 0.019) التمايز الوراثي الكبير جدا. و نستخلص من هذه الدراسة أن الـ QTL المتحصل عليها ، والمتكاملة مع دراسة الارتباط ، قد قدمت معلومات مفيدة وعملية لتمييز العوامل الوراثية الجزيئية التي تؤثر على صفات النمو داخل عشائر الدجاج المحلي.