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The differences between three chicken generations by the change of DNA frequencies of random population

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Abstract

Random population studies are very important to estimate the environmental factors in nutritional, physiological and breeding experiments. Studying the quantitative traits, consisting of genetic, environmental factors and interaction between them is very important as well. In a random population study, the genetic variation for 50 chicken individuals (Mandarah strain) of three successive generations was examined using differential display technique. The difference between the generations was measured by the change of DNA frequencies for the random population. Age at sexual maturity was changed during the three generations, but these differences were not significant. The body weight at sexual maturity (BWSM) of the 2nd and the 3rd generations was decreased significantly compared with the 1st generation. Whenever this decrease was not frequent, although, the egg number (EN), egg mass (EM) and rate of laying (RL) were not constant over generations, differences between the generations were highly significant for EN and EM only. In addition, comparative analysis based on the molecular levels and genotype stability clarified the genetic variations between the examined groups (females or males). Eleven bands with different molecular weights were observed in case of females in comparison to only 9 bands in case of the males. Phylogenetic studies revealed that, there was a genetic variability between females of the 1st generation. Furthermore, low level of similarity was observed between the 2nd generation in respect to the 1st and 3rd generations. Genetic similarity between females and males of the 2nd and the 3rd generations was 55%. It could be concluded that the differences in egg production trait, which was found among generations, may be due to indirect or natural selection.

Keywords: Differential display, random mating, chicken and random population.

INTRODUCTION

Random sampling means that every member of the population has an equal chance of appearing in the sample, independently of the other members in the sample. The random population studies are very important to estimate environmental factors in the nutritional, physiological and breeding experiments. Meanwhile in selection experiments, the random population was used to estimate the response of selection (gain):

$$Rt = (St - St\text{-}1) - (Ct - Ct\text{-}1)$$

Where Rt represents the realized gain due to selection in the tth generation, S and C represent average performances of the selected and the control populations (Guill and Washburn, 1974).

In a large random population with no selection, no mutation or migration, the gene and genotype frequencies are constant from generation to generation (Falconer, 1989). Quantitative genetic methods effectively regard to the animal as a black box with many genes contributing to the expression of all traits under selection. Molecular genetics is now opening this black box by elucidating the effect of single gene on the phenotypic expression of traits. As breeding deals with identifying and exploiting the genetic basis of phenotypes, there is no doubt whatsoever that the use in breeding of knowledge of molecular genetics, that is molecular breeding, will totally change our current practices of selective

breeding in poultry breeding. In addition, it is likely to also affect the role of the poultry breeding industry in poultry production (Meuwissen et al., 2001).

This will affect the structure of breeding programmers and also impact the integration of breeding in the poultry production system. The new knowledge of the molecular basis of poultry phenotypes that is generated along the way will be used to engineer and re-design the poultry genome with novel technologies, and genetically engineered poultry breeds will be marketed within twenty years from today (http://www.thepoultrysite.com/ articles/752/the-future-of-molecular-genetics-in-poultry-breeding).

Many fingerprints can be displayed on a single gel, allowing the simultaneous comparison of the abundance for several hundred RNAs. In other studies, RNA fingerprinting has been used to identify transcripts that are aberrantly regulated in human tumors (Liang et al., 1992; Wong and McClelland, 1992), differentially expressed during mouse brain development (McClelland et al., 1993), differentially expressed during peroxide stress in Salmonella (Wong and McClelland, 1992), or differentially expressed due to diallyl disulphide (DADS) stress in *Sitophilus oryzae* and Callosobruchus chinensis (Seufi et al., 2007).

We predict that methods of assessing genetic variability at the genome level rather than at the phenotypic level (measuring traits in animals) will be the basis of selective breeding within ten years. The only relevant qualification of this prediction is on the time scale.

The main objectives of this study was to determine the differences between three chicken generations by the change of DNA frequencies of random population, and to demonstrate if the RAP technique is a good tool for examining the genetic variation in the same strain through a number of generations.

MATERIALS AND METHODS

This study was conducted at El- Sabahia Poultry Research Center, Alexandria, Animal production Research Institute, Agriculture Research Center. This work was carried out through three successive generations of random population of Mandarah chicken strain. Ten birds from each sex were randomly chosen from each generation to determine the kinship for three generation. Pen samples were randomly selected. Blood samples were withdrawn from the brachial vein from each bird under each sex in EDTA tube for DNA and mRNA isolation.

Breeding and management

The chicks were wing banded, weighed and brooded; sexes were separated at age of 8 weeks. The pullets were housed in individual laying cages at age of about 20 weeks and housed in breeding pens after the first 90 days of laying. Body weight at sexual maturity, age at first egg, egg number and egg weight during the first 90 days of laying were recorded. In addition, egg mass and rate of laying during the first 90 days were recorded.

Statistical analysis

Data were analyzed using fixed models of SAS institute (1988) Yijk = M + Gi + eik Where: Yijk is an observation, M is overall mean, Gi is the fixed effect of ith generation, and eik is the random error. Significant differences among means were tested by Duncan Test.

Reverse transcription of RNA

Reverse transcription reactions were performed using primer (5\-TTTTTTTTTTTTTTT-3\). Each 25 µl reaction mixture containing 2.5 µl 10 x buffer with MgCl2, 2.5 µl 2.5 mM dNTPs, 1 µl 10 pmol primer, 2.5 µl RNA and 0.2 µl reverse transcriptase. PCR amplification was performed in a thermal cycler programmed at 95oC for 5 min, 42oC for 1 h, 72oC for 10 min and reaction mixture were held at 4oC (Chen et al., 2005).

Differential display PCR

The reaction mixture for differential display PCR was carried out in a total volume 25 µl containing 2.5 µl 10 x buffer with MgCl2, 2 µl 2.5 mM dNTPs, 1 µl 10 pmol primer of 18S (5`GTAGTCATATGCTTGTCTC`3)(Wilson et al., 1996), 1.5 µl cDNA and 0.2 µl Taq DNA polymerase (5 units/µl). PCR amplification was performed in a thermal cycler programmed for one cycle at 95oC for 5 min. then 34 cycles were performed as follows: 30 s at 95oC for denaturation, 1 min at 45oC for annealing, 2 min at 72oC for elongation. 10 min at 72oC for final extension, reaction mixtures were held at 4oC. About 2 µl of loading dye was added prior to loading of 10 µl per gel pocket. Electrophoresis was performed at 100 Volt with 0.5 x TBE buffer in 12% polyacrylamide gel and then the gel was stained in 0.5 µg/ml (w/v) ethidium bromide solutions for only one minutes and destained in deionized water for about ½ h. Finally the gel was visualized and photographed by using gel documentation system (Chen et al., 2005).

Phylogenetic construction

Construction of phylogenetic for the data obtained by differential display was carried out by using Statica5 program. The construction bases on the presence/absence of the band patterns.

RESULTS

Table 1 show the results of age (ASM) and body weight (BWS) at sexual maturity, egg number (EN), egg weight (EW), egg mass (EM) and rate of laying (RL) during the first 90 days of laying by generation. Age at sexual maturity was changed during the three generations, which increased by 1.06 and 1.48 day at the second and the third generations, respectively, in respect to the first one. Statistical analysis revealed that the differences were not significant between generations as shown in Table 1. Body weights at sexual maturity (BWSM) of the 2nd and the 3rd generations of pullets were decreased significantly by 1364.15 and 1438.35 g, respectively, when compared with the 1st generation (1757.42 g), and this decrease was not frequent (Table 1). Egg number (EN), egg mass (EM) and rate of laying (RL) were not constant over generations, and the differences between

Table 1. L east square means and standard error of age (ASM) and body weight (BWS) at sexual maturity, egg number (EN) egg weight (EW) egg mass (EM) and rate of laying (RL) during the first 90 days of laying by generations.

Generation	Number	ASM	BWSM	EN	EW	EM	RL
1	121	174.77+1.39	1757.42+43.92^{a}	39.69+0.91	44.02+0.26^{b}	1752.72+43.50^{ab}	0.44+0.01
2	71	175.83+1.59	1364.15+16.43^{b}	38.24+0.98	44.51+0.28^{b}	1699.59+43.53^{b}	0.42+0.01
3	117	176.25+0.81	1438.35+13.36^{b}	40.24+0.95	46.21+0.18^{a}	1855.09+43.32^{a}	0.45+0.01
Significance		ns	**	ns	**	*	ns

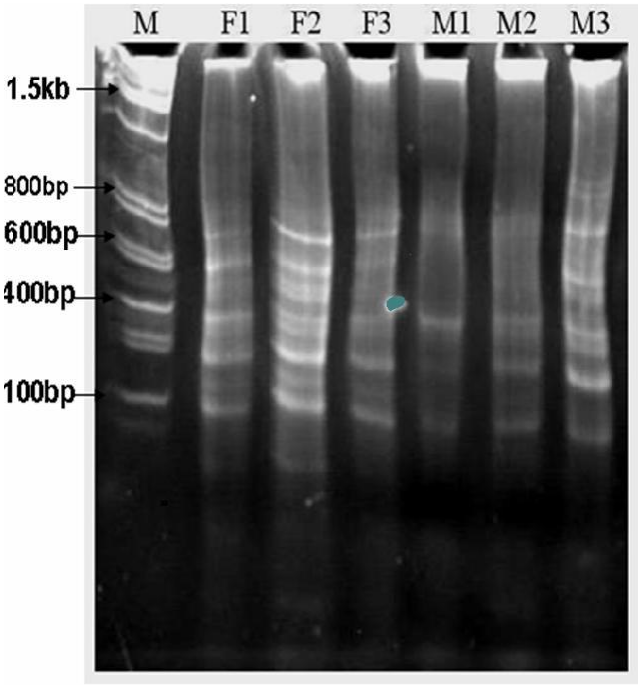
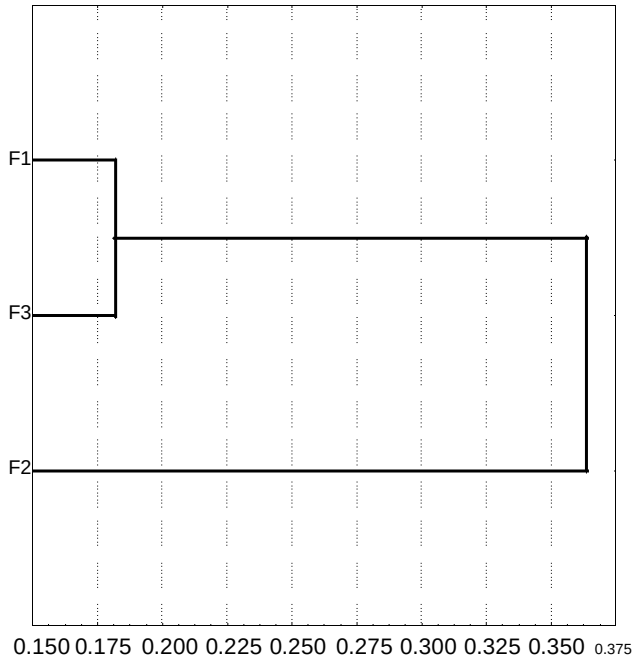


Figure 1. 12% polyacryalmide gel of differential display for 10 chicken offspring using 18S as arbitrary primer. Lane M: DNA marker, lanes from F1-F3 the G1 to G3 of the females chicken. Lanes: M1-M3 the G1 to G3 of the males chicken.

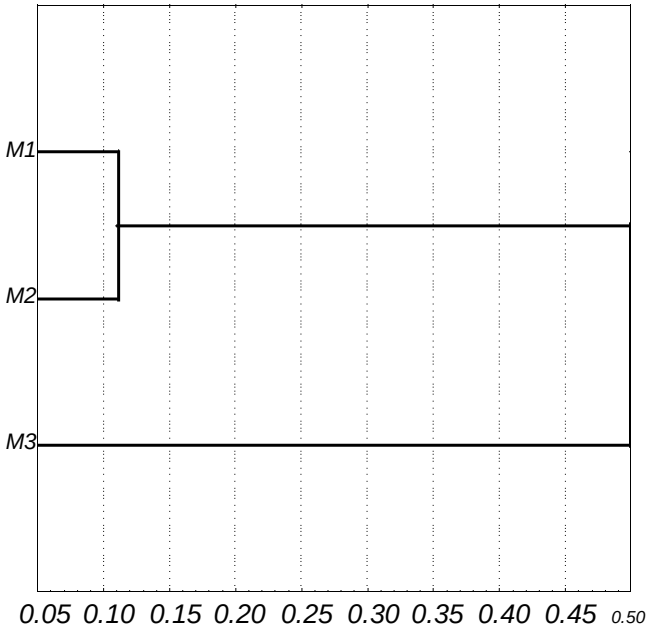
generations were highly significant for EN and EM but it was not significant for RL. Pullets of the 3rd generation laid the highest number of eggs (40.24 eggs) and those of the 2nd generation laid 38.24 eggs while pullets of the 1st one laid 39.69 eggs. The rate of laying (RL) and egg mass (EM) gave a similar pattern to egg number. So, the pullets of the third generation had the maximum EM and RL (1855.59g and 0.45 egg/ day), respectively, but the 2nd generation had the lowest means (1699.59 g and 42.0 egg/ day, respectively). Eggs produced from pullets at the 3rd generation were significantly ($p< 0.01$) heavier (46.21 g) than those produced from both the 1st and 2nd generations (44.02 and 44.51 g, respectively). Body weight at sexual maturity, egg number, egg mass and rate of laying were not constant over generations. These results indicated that the natural selection was affected in these traits.

On the other hand, when comparing the three generations of the random population at molecular level for studying genotype stability and genetic differences between them. The 18S primer as house keeping gene was used for studying the genetic variation, which may occur in the genome of the chicken strains during three successive generations (each one consists of 10 birds) . The total RNA was extracted from the blood samples and cDNA was synthesized using oligo dT primers. Genetic pool was constructed by gathering total cDNA for all individuals in each generation and then differentially displayed. The results presented in Figure 1 revealed that there are genetic variations between the examined groups (females or males) . The total number of cDNA bands (genes) resolved in 12% polyacryalmide gel for both females and males in here successive generations was 11 and 9 bands in case of females and males, respectively (molecular size ranged from 900 to 50 bp). Only two bands were differentially expressed through the three generations of female and male chickens. Several common bands (molecular size ranged from 600 to 100 bp (seven bands) in case of females and from 800 to 100 bp (five bands) in case of males) were observed through the three generations (genes were turned on). On the other hand, 4 bands of 700, 500, 350 and 50 bp and 3 bands of 900, 350 and 200 bp were recorded in 2nd and 3rd generations in case of females and males, respectively, these bands exhibited irregular patterns of expression without an obvious relation to the generation. Generally the results of differential display revealed that many up-regulated (turned on) and down-regulated genes (turned off) were observed in case of female and male chickens through the three generations.

Phylogenetic relationship between the examined females (Figure 2A and Table 2) and the males (Figure 2B and Table 2) was constructed based on the differrences appeared in band patterns. In case of the females there was genetic difference between females of the 2nd generation and the other two generations. Females of the 2nd generation showed less similarity with the 1st (73%) and 3rd (55%) generations. The percentage similarity between the females of the 1st and 3rd generations was 81% (Figure 2A and Table 2). These results were in agreement with the results presented in Table 1, which indicated that the females of 2nd generation produced less number of eggs than the other two generations. Phylogenetic analysis of the three generations of the males revealed that there was genetic difference bet-



A



B

Figure 2. Dendogram for the examined the genetic variation for chicken based on the differential display band pattern. A: The female birds B: Male birds.

ween males of the 3rd generation and the other two generations. Males of the 3rd generation showed less similarity with the 1st (71%) and 2nd (55%) generations. The percentage similarity between the males of the 1st and 2nd generations was 73% (Figure 2B and Table 2). Furthermore, it can be observed that the genetic similarity between the 2nd and 3rd generations was very low (55%) in case of both female and male.

Table 2. The agreement percent between the examined based on the statistical analysis.

Females	F1	F2	F3	Males	M1	M2	M3
F1	100	73	81	M1	100	73	71
F2	73	100	55	M2	73	100	55
F3	81	55	100	M3	71	55	100

DISCUSSION

Genetic analyses of experimental data and commercial populations can be carried out to obtain insight into the impact of genetic and environmental factors on production characteristics. The contribution of genetic factors is measured as genetic variation and genetic correlations between traits. Development in statistics and computing researches continue to enable more realistic modeling and analysis of the traits (Meuwissen et al., 2001). In this study we observed some of the genetic variation in a random population along three generations. To demonstrate the genetic variation in the chicken genome, a genetic pool (for 10 individual birds) from each generation was analyzed by differential display technique. Emara and Kim (2003) used other techniques like RAPD-PCR, RFLP and SNPs for DNA fingerprinting.

Differential display technique was found to be the most suitable technique to study the genetic variability in chicken genome especially when one of the house keeping genes was used as indicator for RNAs changes (18S r RNA gene) (Basak et al., 2006). The same idea was carried out by Poel and Veenendaal (1998) and Delany (2000) but their studies was carried out on the DNA not on RNA. Actually, differential display technique was able to differentiate between the examined strains in the same species (female or males) based on the differences of the type and the numbers of RNAs.

The results of age at sexual maturity which presented in Table 1 were in the same trend with that reported by Mohamed (1997) and Kosba et al. (2002). On the other hand, the body weight at the sexual maturity of the pullets during the 2nd and the 3rd generations was not comparable to the results reported by Ghanem (1995) and Abd-Alla (1997). The results of the rate of laying and egg mass which reported herein were agreeable to those found by Abd-Alla (1997) and Kosba et al. (1997). The differences in egg produced by control population were in the same trend to that reported by El-Tahawy (2000) and El-Sayed et al. (2001). The results presented in Table 1 provided that the production parameters which estimated during three successive generations on the control popu-

lation changed from generation to generation. These differences were due to environmental factors such as light, diet, management and small population or genetic factors such as indirect selection or natural selection, mutation, migration and the gene frequencies which not under control or due to the interaction between them. Also, when the genetic profit for selection in the population was estimated, the authors removed the effect of the environmental factors by using control population as a reference and they neglected the effect of uncontrolled genetic factors because it is very difficult to estimate by a quantitative method.

On the other hand, the results presented in Figures 1 and 2 A and 2 B and Table 2 clarified that there is inter- and intra- genetic variation in successive generations (males or females) for the same control population. These results were consistent with the results obtained by Delany (2000). Genome analysis of the females showed that a change was carried out in the 2nd and 3rd generations. This result revealed that there were differences between the first and the second generations (22%) but it was (19%) in case of the third generation. These differences may be due to the genetic factors, environmental factors and/or the interaction between them (Himly et al., 1998). On the other hand, the genetic variation between the second and the third generations was in agreement with the quantitative traits in Table 1.

In conclusion, the differences in egg production traits which were found among generations in the control population may be due to indirect selection or natural selection.

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