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DETERMINING GENETIC SIMILARITY AND GENETIC DIVERSITY IN SELECTED ALEXANDRIA CHICKENS USING RAPD TECHNIQUE

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ABSTRACT: The Alexandria chicken considered as one of our Egyptian local strain. A lot of attempts were done to improve productive traits. A control line (or a base line) and another selected one are derived from this local strain. The selected line subjected to a long term selection program lasted for 16 generations from 1995 to 2011 in order to improve some egg production traits and also to reach earlier sexual maturity. This long term selection program expected to reduce most of the variations in this closed population which normally reflected on the response of selection. So, the main target was to estimate the genetic similarities by calculating the genetic improvement occurred during different generations. RAPD analysis technique used to estimate the degree of diversity and stability for this selected line. The mean average percentage differences (MAPD), genetic similarity, genetic distance based on band frequencies or based on band sharing which had been estimated for Alexandria selected and control lines. The mean average percentage difference (MAPD) into Alexandria selected and control lines recorded 0.415 and 0.667 respectively. While, genetic similarity had recorded into selected line (0.635) and into control line (0.351). The genetic distance had been calculated by two methods, the first method calculated the genetic distance on the basis of band frequency recorded 0.46. This result explained the genetic identity index of selected and control Alexandria lines. While the second method calculated the genetic distance on the basis of band sharing recorded 0.41. The phylogenetic relationships within 12 samples randomly taken from Alexandria selected line cleared that the degree of similarity reached 80% within line from primer 1 ,70% from primer 2 ; 65% from primer 3,50% from primer 4; 55% from primer 5 and 60% from primer 6. While; this similarity when calculated within samples into line each primers recorded approximately %63. The results of this present research concluded that, the Alexandria selected line did not consume some of the variability present in this studied population and did not yet reach genetic stability. So, it can to continue the selection program.

Key Words: RAPD-PCR, polymorphism, selection.

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INTRODUCTION

The Egyptian local strains have some useful genetic attributes such as adaptability to our local environment, besides its resistance to some diseases. These attributes can be harnessed in a cross breeding program, especially for the development of meat and egg type chickens (Nwosu, 1987; Nwosu et al., 1985). Local strains in developing countries are still out of competition with commercial ones which benefit from the technology advantages and economic of scale (Hoffmann, 2005). We are in a great need to establish breeding programs to improve the productive performance of our local strains which distinguished with better meat quality, resistance to some diseases and its better adaptation to our local environmental conditions. Most of our developed local strains originated from cross breeding programs (Kosba and Abd El-Halim, 2008). So, crossbreeding can be used as an effective tool that allows manipulating of genetic variation to change the productive performance of these local strains in a fashion that attempts to optimize the desired phenotype and also to obtain crosses enjoyed with improved fitness and fertility traits (Willham and Pollak, 1985; Hanafi and Iraqi, 2001; Mekky et al., 2008 and Nimbkar, et al., 2008). A lot of researches do their best to develop this selected line (Kosba, 1966). They estimated the heritability (h^2) of age sexual maturity and selection response (R) for the selected line during different generation; h^2estimated 0.28 and R was -13.8(Ghanem, 1995); h^2 estimated 0.03, 0.06, 0.05 and R was -3.9 (Abd El-Halim 1999); h^2 estimated 0.28 and R was -5.6 (El-Tahawy 2000); h^2 estimated 0.32 (Ghanem 2003); h^2 estimated 0.34 and R was +6.3 (El-Delbshany 2004) and h^2 estimated 0.26 and R was -4.2 (Khalil 2010).

The aim of this present study was to estimate the genetic similarities by calculating the genetic improvement occurred during different generations by using RAPD analysis technique trying to answer the question does the selected line reaches Plateau stage or not.

MATERIAL AND METHODS

Experimental stocks:

The Alexandria line had long term selection program lasted for 16 generation. The criteria of selection had earlier age at sexual maturity (Zatter, 1994 and Ghanem, 1995). The comparison held between this selected line and another one taken randomly from the base population.

The field work was done at the Poultry Research Center, Faculty of Agriculture, Alexandria University, while, the lab work, including RAPD analysis was fulfilled at the Dept. of Poultry Production, Faculty of Agriculture, Ain Shams University.

DNA preparation:

A sample of 1-2 ml blood was taken via the brachial vein at 16 wk of age from each of 16 samples (6 samples from each line) randomly chosen from each of the fore lines. Each blood sample was mixed with 50 µl of 0.5 M EDTA, and frozen at -70°C until analyzed. The DNA was isolated from blood using AXYGEN kit Axyprep TM). Isolated genomic DNA was purified using spin column. Both products from Axygen Scientific, inc. USA Cat. No. AP-MN-BL-GDNA-50.

PCR cycling parameter:

Amplification was performed in a thermo cycler (LongGene - MG96G/china) with the following temperature profiles: initial denaturation 95 °C for 4 min, 37 cycles (denaturatiom 95°C 1 min/annealing temp. depending on the primer (°C) 1 min/extension 72°C 1 min and Final extention 72°C 5 min. The reaction was hold at 4°C.

Amplified DNA Analysis:

The PCR products (15 µl) were resolved by electrophoresis by using 1.5% agarose gel (horizontal) or in 10% PAG (vertical) in 1X Tris–Borate–EDTA (TBE) buffer at 70 V for 90 min. and 100 bp DNA ladder (Larova GmbH/Germany) were used as DNA size markers. Gels were stained with ethidium bromide (EtBr) 10 ug/ml for 15 min followed by water washing for 15 min. Gels were visualized with UV light and and photographed by a Sony digital camera.

Genetic improvement and data analysis:

The improvement in the last fourth generation calculated by this equation (Zein El-Dein 1977)

$$= \partial p_{13}/\partial p_{16} * h^2_{13}/h^2_{16}$$

Where,

∂p_{13}= square root of the total phenotypic variance of 13th generation,

∂p_{16}= square root of the total phenotypic variance of 16th generation,

h^2_{13}= heritability estimated of 13th generation,

h^2_{16}= heritability estimate of 16th generation.

Phylogenetic tree was constructed on the basis of similarity matrix obtained with neighbor joining (NJ) method using Jaccard formula $d_{jk} = M/(M+N)$. The relationships between genotypes were displayed as dendrogram using the NTSYSpc 2.01 software package (Rohlf, 1998).

As for, percent polymorphism it was calculated by using the formula,

$$\text{Percent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \text{ X } 100$$

Genetic similarity among chicken groups was estimated by scoring the presence and absence of bands produced by primers. The presence (1) or absence (0) of a band of a particular molecular weight were scored as two alleles at a single locus.

Molecular size of SSR and RAPD – PCR bands separated on gels were calculated by analyzing gel images with GelAnalyzer software package version 2010a (free wear).

Table (1): the name and Sequence of primer

Primer name	Primer sequence
OPA-01	5\ CAGGCCCTTC 3\
OPB-01	5\ GTTTCGCTCC 3\
OPB-03	5\CATCCCCCTG 3\
OPB-14	5\ TCCGCTCTGG 3\
OPG-01	5\ CTACGGAGGA 3\
OPZ-01	5\ TCTGTGCCAC 3\

Mean Average Percentage Difference (MAPD):

Mean Average Percentage Difference (MAPD) was calculated as a measure of inter lines genetic divergence and was expressed in the form of mean described by Gwakisa et al. (1994) by using the formula:
Percentage Difference (PD):
PD = (Nab/Na+Nb)×100
Average Percentage Difference (APD): APD = 1/C G Pdi.
Mean Average Percentage Difference (MAPD): MAPD = 1/R G Pdi
Where: Nab = The number of fragments that differ., Na and Nb = The number of fragments in pool a and b. , C = The number of inter-lines pair-wise comparisons.
R = The number of random primers used.
Genetic similarity based on band frequency: The within lines genetic similarity (WF_i) was estimated using the equation given by Singh and Sharma and Dhama (2007).
$WF_{ij} = 1/N\sum V_i$
Where: Vi = The proportion of individuals possessing the I th band across all the individuals. , N = The total number of bands amplified.
The genetic similarity between two lines known as genetic identity index (Yu and Pauls 1993; Zhang et al., 1995) was obtained from the following formula;
$BF_{ij} = \sum 1/N [2(V_i^{(1)} V_i^{(2)}) / V_i^{(1)})^2 + (V_i^{(2)})^2]$
Where: $V_i^{(1)}$ and $V_i^{(2)}$ = The frequency of occurrence of the i th band in Line 1 and 2, respectively., N = The total number of bands scored.

Genetic distance based on band frequency:

An index of genetic distance Dij between two lines was calculated by the following equation.
Dij = - ln (Bfij). Where: (BFij) = The genetic identity index of two lines.

Genetic distance based on band sharing:

The genetic distance between the lines were also calculated based on band sharing between the pooled samples RAPD profiles. The genetic distance (Dab) between the selected line (A) and control line (B) was calculated as: Dab = 1/N G1-{Nab/ (Na+ N b -Nab)}
where: Nab = No. of common bands between A and B., Na = No. of bands in A. N b = No. of bands in B., N = No. of primers used.

STATISTICAL ANALYSIS

All scored bands of DND data was firstly corrected to estimate each allele size according to its number of repeats for each marker GelAnalyzer software package was adopted for this purpose. Then, a spread sheet program (Microsoft Excel) was used to arrange the included data for each line regarding each locus. All possible extracted population figures were carried out employing a GENEPOP software package after data conversion using CON.

RESULTS AND DISCUSSION

A long term selection program lasted from 16 generations in order to reach early age sexual maturity and also to improve some egg production traits, was practice in a selected line proved from Alexandria strain. Accordingly different genetic responses and genetic improvements were attended from generation to another, specially in the last four generation (Table 2). The attend results indicated that we are still in need to continue these selection program for another several generations to attain more and more selection responses in order to improve these studies traits.

Table (2): Heritability estimates and cumulative selection response of age at sexual maturity for Alexandria selected egg line.

Author	Hertability	Selection Response (days)
Ghanem, 1995	0.28	- 13.8
Abd El-Halim, 1999	0.03, 0.06, 0.05	-3.9
El-Tahawy, 2000	0.28	-5.6
Ghanem, 2003	0.32	-
El-Dlebshany, 2004	0.34	+ 6.3
Khalil, 2010	0.26	-4.2

Genetic improvement in the last fourth generation = $\partial p_{13}/\partial p_{16} * h^2_{13}/h^2_{16}$ = 438/317 * 0.34/0.26=1.81

Table 3 showed the mean average percentage difference (MAPD) into the selected line and also into the control. The corresponding estimates were 0.415, 0.667 respectively. Moreover, genetic similarity which recorded for the selected line was 0.635, while it was 0.351 only in the control one. . Chatterjee et al., 2006 and 2007 estimated the MAPD between different inbred (HS and FS) the non inbred White Leghorn (WL) population. There results demonstrated the MAPD was lower for the non inbred populations when compared with those of pure ones.

The genetic distance had been calculated by two methods, the first calculated genetic distance one the based of band frequency. Accordingly, the genetic distance recorded 0.46, this results indicates the genetic identity of these two line (the selected line and the control one). The second methods calculated the genetic distance on the base on the base of band sharing. According to this last methods, the genetic distance recorded 0.41.

Table (3): Mean average percentage difference, genetic similarity and genetic distance from selected and control lines.

Parameters	Lines	
	Selected	Control
Mean Average Percentage Difference	0.415	0.667
Genetic similarity	0.635	0.351
Genetic distance based on band frequency	0.46	
Genetic distance based on band sharing	0.41	

Phylogenetic relation:

Cluster analysis of the RAPD pattern (Fig. 1) of the selected line show difference and similarity between samples of selected line. Within-population genetic variation may reflect the different sources of origin of the lines and their subsequent propagation. Using Statistica software, Unweighted Pair Group Average Method (UPGAM) of analysis was performed based on RAPD data, and a dendrogram was constructed to show the phylogenetic relationships within 12 samples randomly chosen from Alexandria selected lines of consideration (Figure 2). The degree of similarity reach 80% within line from primer 1,70% from primer 2 ; 65% from primer 3,50% from primer 4; 55% from primer 5 and 60% from primer 6. While; when calculating similarity within samples into line, each primer reaches approximately %63. The Alexandria selected line had originated from different sources (Alexandria, Norfa and Matrouh.), were crossed and subjected to similar selection regimes but their response to selection could vary Hence, some diversity between them is expected. due to their differential reproductive and productive potentials. The cluster analysis in other chicken populations was also observed by earlier workers (Chatterjee et al., 2006; Ahlawat et al., 2004).

Khosravinia et.al, 2005 studied genetic distance and genetic similarity within lines HC, BPB2, CPB2, PB2 and UM1 lines used randomly amplified polymorphic DNA techniques (RAPD techniques) and reached the same observation and conclusion. Within-line genetic similarity/distance estimates derived from band sharing from Alexandria selected lines concerned are presented in fig 1and 2. The genetic similarity values based on band frequency were utterly of same trend as those derived from band sharing.

The control line used this experiment due to vindicate the degree of differentiations of selected lines. RAPD Markers have many important and useful applications in poultry improvement. Some of the many applications will be briefly discussed: Establishing genetic relationships; predicting heterosis; Genomic selection; and Marker Assisted Selection (MAS). A more comprehensive discussion of some of these areas can be found in Muir (1994; 1996, 1997), Francesch et al., (1997), and Ahlawat et al., (2004).

The selected line not reach Plateau stage and the additive variance not consumed due to the selection programs which avoided danger inbreeding. In the present study the focus was on determining the genetic similarity and variability between the selected line and control line, since the lines belong to the same breed, characterization at the molecular level was necessary to unravel the genetic variation between them as a result of recurrent inbreeding.

CONCLUSION

The Alexandria line which subjected to a long term selection program lasted for 16 generations in order to reach earlier sexual maturity had not yet reaches stability stage.

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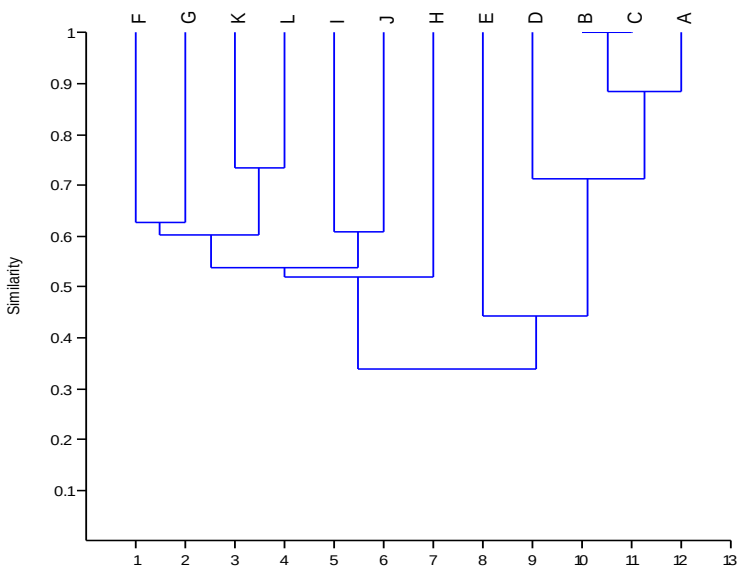

Fig. (1): dendrogram genetic similarity into Alesendria selected line according to RAPD analysis

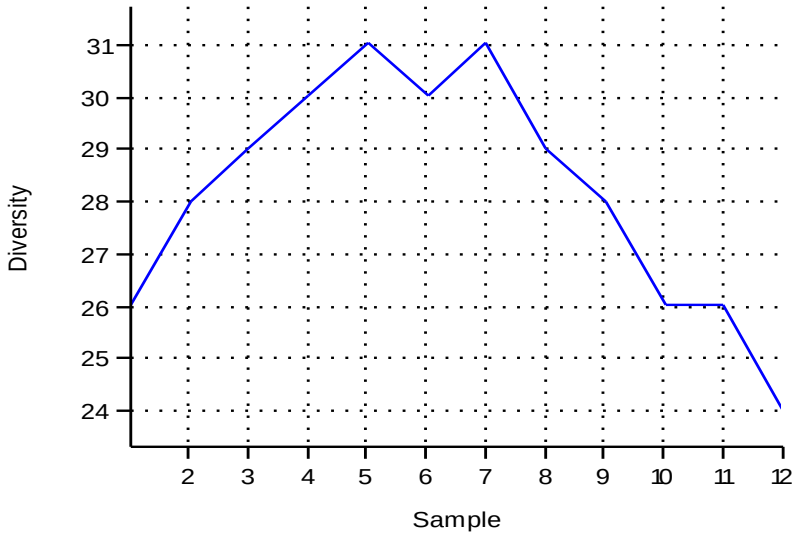

Fig. (2): Cluster analyses of genetic diversity within Alexandria selected line according to RAPD analysis.

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الملخص العربي

تقدير التشابه الوراثى والتباين الوراثى فى دجاج الاسكندرية المنتخب باستخدام تقنيةRAPD

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يعتبر دجاج اسكندرية من إحدى السلالات المحلية المحسنة والذى اجرى علية كثير من الأبحاث لتحسين صفاته الأنتاجية. تم الانتخاب على المدى الطويل لتحسين صفات انتاج البيض والبلوغ الجنسى المبكر وذلك لمدة ١٦ جيل أعتبارا من موسم ١٩٩٥ إلى ٢٠١١ وهذا النوع من برامج الأنتخاب على المدى الطويل فى العشائر المغلقة يؤدى إلى تقليل الأختلافات وينعكس ذلك على الأستجابة للإنتخاب.

الهدف الأساسى من هذه الدراسة تقدير التشابه الوراثى وذلك بحساب التحسين الوراثى خلال الأجيال المختلفة. والأكثر من ذلك استخدام تكنيك RAPD لتقدير درجة الأختلاف و الثبات فى الخط المنتخب. متوسط نسبة الأختلافات MAPD داخل الخط المنتخب و الخط المقارن بلغ ٠.٤١٥ و ٠.٦٦٧ على الترتيب بينما التشابه الوراثى بلغ ٠.٦٣٥ فى الخط المنتخب و ٠.٣٥١ فى الخط المقارن.

المسافة الوراثية تم حسابها بطريقتين: الطريقة الأولى تم حساب المسافة الوراثية على اساس تكرار الحزم و بلغت ٠.٤٦ . هذه النتيجة تفسر دليل التعرف الوراثى فى الخط المنتخب و الخط المقارن. بينما الطريقة الأخرى لحساب المسافة الوراثية على اساس الحزم المشتركة بلغت ٠.٤١ .

اوضح التشابة الوراثى داخل الـ١٢ عينة عشوائية التى تم اخذها من خط اسكندرية المنتخب ان درجة التشابة وصلت إلى ٨٠% داخل الخط المنتخب من البادىء ١ (primer1)و ٧٠% من البادئ ٢(primer 2) و ٦٥% من البادئ ٣ (primer3)و ٥٠% من البادئ ٤ و ٥٥% من البادئ ٥ (primer 5)و ٦٠% من البادئ ٦(primer 6) . بينما عندما تم حساب هذا التشابة للعينات داخل الخط لكل البوادئ (primers) سجلت تقريبا ٦٣% .

تتلخص نتائج هذه الدراسة فى أن خط الأسكندرانى المنتخب لم يستهلك بعض الأختلافات التى ظهرت فى العشيرة المدروسة و لم تصل بعد للثبات الوراثى لذلك يمكن ان يستمر برنامج الأنتخاب.