USING MOLECULAR GENETICS TO STUDY THE MOST IMPORTANT GENETIC DIFFERENCES IN TWO LINES OF JAPANESE QUAIL

By

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B. Sc. Agric. Sc. (Agric. production), Alexandria University, 2003

M. Sc. Agric. Sc. (Poultry Breeding), Ain Shams University, 2009

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ABSTRACT

Heba Abd-Elwahab Mahmoud Assi: Using of Molecular Genetics to Study the Most Important Genetic Differences in The two Lines Japanese Quail. Unpublished Ph.D. Thesis, Department of Poultry Production, Faculty of Agriculture, Ain Shams University, 2015.

This study was carried out at Poultry Breeding Farm, Poultry Production Department, Faculty of Agriculture, Cairo University and at National Gene Bank, Agricultural Research Center. The goal of this experiment was to detect the genetic differences between two lines of Japanese Quail (Beige and Grey color) and also to determine the molecular description for these two lines by using means of molecular genetics under environmental Egyptian conditions. The experiment was started on parent stock (males and females) from two quail lines and distributed into batteries under the same environmental conditions. Fertilized eggs were collected during two weeks to be hatched. During the process of hatching, egg production has been recorded for four weeks. The feed and water were provided ad libitum. All chicks (offspring) were reared under similar environmental, managerial and hygienic conditions. They were divided into two groups, first group (100 quails each line) housed in two batteries to calculated feed consumption and feed conversion ratio , the second group were brooded in electrical brooding batteries up to two weeks of age. At the end of the second week of age, birds were housed on floor until they reach the marketing age (6 weeks of age).The feed and water were provided *ad libitum*. To estimate variability between quail lines, six microsatellite markers were used. The markers constructed for 6 autosomal 1, 3, 4, 6, CJA 06 and QL08 chromosomes.

Main results could be summarized as follows:

- The significantly (P>0.05) of higher mean egg production was recorded to Grey line quail compared to Beige one.
- The results showed that body weight of Grey line was significantly higher than that of Beige line at 3, 4, 5 and 6 weeks.
- There was a significant difference in carcass, liver, giblets and Edible meat parts weights between Grey and Beige quail lines. However, non-significant differences occurred for gizzard and heart weights between quail lines.

Genetic diversity of Beige and Grey lines showed that the mean number of alleles per line varied from 6.00 in Beige to 6.50 in Grey line.

Total numbers of alleles were 54 based on six microsatellite loci. The number of alleles per locus overall lines ranged from 5 (UBC005) to14 (UBC001) with a mean of 9.00.The genetic diversity within the two lines (Beige and Grey) analyzed were described by the number of alleles per locus. These results showed that, the genetic diversity was highest in Beige line as compared to Grey line and reflected the efficiency of used microsatellite markers set in studying the genetic variation within and between closely related Japanese quail lines. The mean values of F_{IS} obtained were 0.82 and 0.80 for Beige and Grey lines, respectively, indicating the high level of inbreeding in lines studied confirming by IC which equal to 0.80 and 0.82, respectively. The mean values of F_{IS}, F_{IT} and F_{ST} obtained were 0.80, 0.82 and 0.12 per locus, respectively. Wright's fixation index (Fis) values among loci ranged from -0.158 (for UBC0005) to 1.000 (for UBC0001, UBC0002 and UBC0004). The mean Fis for 6 microsatellite loci was 0.80. The majority of the genetic diversity obtained in the current study is presented by within individuals (15.24%) rather than others. Fixation indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. Population fixation indices traced a 0.85 of variation referring to differences among individuals versus total variance (F_{IT}). Percent of molecular variance among lines varied from 2% with the UBC0005 locus to 25% with the GUJ0028. While, the Percent of molecular variance within lines varied from 75% with the GUJ0028 locus to 98% with the UBC0005 locus. These results revealed that, the variance within lines higher than among lines per locus. The highest allele frequency overall loci was 0.68 of allele 060 at locus UBC0005 in the case of Grey line. Whereas, the lowest one was 0.02 associated with Grey and Beige lines at two locus GUJ0028 (for alleles, 099, 108 and 126) and GUJ0029 for allele 116 with Beige line. The highest average of allele frequency estimated was (0.33) for Beige line at locus UBC005. On the other hand, the lowest one was 0.10 in the case of locus UBC001 for Beige line. The average PIC of six markers was 0.66 and 0.71 with Beige and Grey line, respectively which indicated that the six microsatellite markers contained highly polymorphic loci in both lines of Japanese Quail. Finally, these results could be attribute to find marker assisted selection (MAS) for the traits to improve the performance of Japanese quail under environmental Egyptian conditions. It can be concluded that the high polymorphism of microsatellite loci observed in this study suggested that these markers can be utilized in future efforts to asses genetic diversity of Japanese quail.

Key words: Productive traits, microsatellite markers, Japanese quail, characterization, gene and marker assisted selection.

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LIST OF ABBREVIATIONS

PCR	:	Polymerase Chain Reaction.
DNA	:	Deoxyribonucleic acid.
HBW	:	High body weight.
EN	:	Egg number.
BW	:	Body weight.
GR	:	Growth rate.
EW	:	Egg weight.
FCR	:	Feed conversion ratio.
FI	:	Feed intake.
N_a	:	Number of alleles per locus.
N_e	:	Effective number of alleles per locus.
H_e	:	Expected heterozygosity.
H_o	:	Observed heterozygosity.
PIC	:	Polymorphism information content.
F	:	Fixation index.
F_{st}	:	Is the effect of subpopulations (s) compared to the total population (t).
F_{it}	:	Is the inbreeding coefficient of an individual (i) relative to the total (t) population
F_{is}	:	Is the inbreeding coefficient of an individual (i) relative to the Subpopulation (s).
HWE	:	Hardy-Weinberg Equilibrium.
gm	:	Gram.
kg	:	Kilogram.
SPSS	:	Statistical Package for Social Science.
µl	:	Micro liter.
ng	:	Nanogram.
µM	:	Micromole.
mM	:	Mille mole.
min	:	Minute.

s	:	Second of time.
SSR	:	Simple sequence repeats.
AMOVA	:	Analysis of molecular variance.
SMM	:	Stepwise mutation model.
TA	:	Temperature of annealing.
Gen Bank Ac	:	Gen Bank accession number.
BWG	:	Body weight gain.
ENA	:	Effective number of alleles

1. INTRODUCTION

Quail are an economically important avian species and provide an alternative egg and meat sources to the more commonly used chicken. They require less space and low initial investment and have good export potential. Quail are in the genus *Coturnix,* family *Phasianidae* and order *Galliformes* **(Sharma *et al.,* 2000)**. Gaining in popularity as an experimental animal in both research and education, the Japanese quail (*coturnix japonica*) is a small, early maturing, highly efficient egg and meat producer **(Pisenti *et al.,* 1999)**.The Japanese quail, originally domesticated around the 11th century as a pet song bird (**Howes, 1964** and **Crawford, 1990).** Several aspects account for the utility of this bird. First, it has attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavor. Second, the low maintenance cost associated with its small body size (80–300 g) coupled with its short generation interval, resistance to diseases and high egg production; render it an excellent laboratory animal. Third, Japanese quail is phylogenetically closely related to the chicken **(Stock** and **Bunch 1982).** Production performance of the Japanese quail can be improved by increasing their genetic potential and favorable management conditions. It demands to produce more quail meat in shorter period. This can be achieved by a specific selection program for higher body weight at specific age **(Malarmathi *et al.,* 2010).** Countries having shortage in animal protein supply may consider using all available protein resources for human nutrition **(Singh *et al.,* 1981).**Genetic progress in body weight for Japanese quail is accomplished by continuous selection for the trait (**Marks, 1971** and **Anthony *et al.,* 1986)**.

Characterization of the genetic diversity of indigenous animal populations is a prerequisite for providing needed information for the conservation of useful genotypes against future uncertainties in the face of daunting global challenges such as climate change, emerging diseases, population growth, and rising consumer demands (**Kayang *et al.,* 2010).**

INTRODUCTION

Continued genetic improvement of livestock is dependent on the fact that substantial genetic variation exists within individual breeds allowing them to respond to selection for different traits **(Sruoga *et al.*, 2007).** Recent advances in molecular technology have opened up new horizons for estimating genetic relatedness between and within animal populations, and molecular markers may serve as an important initial guide to develop conservation strategies (**Davila *et al.*, 2009)**. Genetic diversity measures using microsatellites yield reliable estimations of variability within and genetic relationships among chicken populations and was extensively investigated **(Kayang *et al.*, 2010** and **Roushdy *et al.*, 2008a,b).**

The present study was done using 6 microsatellite markers for genotyping two lines of Japanese quail, Beige and Grey color.

The goal of this experiment was to detect the genetic differences between two lines of Japanese Quail (Beige and Grey color) and also to determine the molecular description for these two lines by using means of molecular genetics under environmental Egyptian conditions.