Doctoral thesis

A study on genetic diversity of Egyptian native livestock

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ABSTRACT

Genetic diversity is essential in optimizing both conservation and utilisation strategies for animal genetic resources. Local poultry breeds make up most of the world's poultry genetic diversity, and are still very important in developing countries where they represent up to 95 percent of the total poultry population. Local Egyptian poultry breeds are highly adapted to harsh environmental conditions and thought to constitute genetic reservoirs. For instance, Fayoumi chicken breed can be seen as a unique breed from the viewpoint of disease resistance and Sinai chicken strain possesses superiority in heat tolerance. The development and increased focus on more efficient selection programmes have accelerated genetic improvement in a number of breeds. As a result, highly productive breeds have replaced local ones across the world. Moreover, intensive selection caused narrowing of genetic base and inbreeding which is associated with declines in both population fitness and disease resistance. This development has led to growing concerns about the erosion of genetic resources, as the genetic diversity of low productive local breeds is likely to contribute to current or future traits of interest and they are considered essential for maintaining future breeding options. The first problem for conservation is the lack of information, so the identification and genetic characterization of all breeds particularly local ones has high priority in the FAO global strategy. Information of poultry genetic resources is considered a useful model for studying conservation of genetic diversity in wild animal species. Therefore in this study, I evaluated the genetic diversity of Egyptian chickens and pigeons in order to apply this information for conservation purpose by using two different strategies. The first strategy (in chapter 2) was maximization of genetic diversity based on neutral microsatellites genetic markers while the second strategy (in chapter 3) was genetic improvement by low selective pressure based on functional gene polymorphisms.

For the first strategy, I evaluated the genetic structure, breeds diversity and the breed contribution to aggregate genetic diversity as important criteria for their conservation by utilising three different prioritization methods in order to set the priorities for conservation of Egyptian chickens and pigeons based on neutral genetic markers (microsatellites). For chickens, the six studied Egyptian populations showed

a moderate level for both within-population (MN_A = 4.9; H_E = 0.595) and between-population (F_{ST} = 0.082) genetic diversity and were clustered into four clusters by STRUCTURE. Fayoumi, Dandarawy and El-Salam populations were assigned independently into their respective clusters while the remaining three populations (Baladi, Sinai and Golden Montazah) were clustered together forming admixed mosaic cluster. Regarding breed contribution to aggregate genetic diversity, Dandarawy breed contributed the most and ranked the first, while Fayoumi breed contributed negatively to aggregate genetic diversity and ranked the last. For pigeons, the six studied Egyptian populations showed moderate within-population (MN_A = 4.10; H_E = 0.584) and high between-population (F_{ST} = 0.211) genetic diversity. The Egyptian in addition to Japanese racing pigeon populations were clustered into six clusters. Krezly, Safi, Romani, Ablaq and Japanese racing populations were assigned independently into their respective clusters while the remaining two populations (Asfer Weraq and Zagel) appeared as a mosaic clusters. Zagel breed contributed the most and ranked the first, while Asfer Weraq ranked the last in the contribution for aggregated genetic diversity.

For the second strategy, I focused on the genes which might be related with effective utilisation of the poultry genetic resources. The polymorphisms of functional genes might be used as genetic markers for selection of high performance pigeons. For instance, the Lactate dehydrogenase gene family is involved in aerobic and anaerobic metabolism; therefore it determines muscle endurance, recovery and aerobic capacity. I found that the long allele (600bp) of Lactate dehydrogenase-*A* (*LDH-A*) gene showed significantly higher frequencies than short one (595bp) in the homing than non-homing in both Japanese and Egyptian pigeons and it might be useful for conservation and sustainable utilisation through improvement of local population's performance. Also for wildlife, basic understanding of *LDH-A* genotype and homing ability might be useful for studying of wild migrating birds. Consideration of neutral genetic diversity and functional genes diversity in addition to breed merits and threat status, enabled us to balance the trade-offs between conserving genetic diversity as insurance against future uncertainties and current sustainable utilisation.

1. GENERAL INTRODUCTION

Domestication of animals was an essential step in human demographic and cultural development. During the subsequent history of livestock, the main evolutionary forces of mutation, selective breeding, adaptation, isolation and genetic drift have created an enormous diversity of local populations. Local poultry breeds make up most of the world's poultry genetic diversity, and are still very important in developing countries where they represent up to 95 percent of the total poultry population (Besbes et al., 2007). During the last decades, development and increased focus on more efficient selection programmes have accelerated genetic improvement in a number of breeds. As a result, highly productive breeds have replaced local ones across the world. Moreover, intensive selection caused narrowing of genetic base and inbreeding which associated with declines in both population fitness and disease resistance (Spielman et al., 2004; FAO, 2007; Markert et al., 2010). This development has led to growing concerns about the erosion of genetic resources (FAO, 2007). As the genetic diversity of low productive local breeds is likely to contribute to current or future traits of interest (Notter, 1999; Bruford et al., 2003; Toro et al., 2009), they are considered essential for maintaining future breeding options.

Genetic diversity of poultry genetic resources allows for the sustained ability of a breed or population to respond to selection to increase productivity and for adaptation to changing environmental conditions, including not only those conditions associated with climate, but also to changes in markets, management and husbandry practices, and disease challenges. In turn, conservation of diversity of poultry genetic resources helps ensure long-term food security. In addition, conservation of specific poultry genetic resources may be necessary to preserve particular cultural and historical values (Hanotte et al., 2005). Not only conservation of poultry genetic resources, but also those of wild avian species are important, as 13 percent of known avian species are currently categorized as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN, 2012). Conservation of domestic poultry against the threat of infectious diseases like highly pathogenic avian influenza through efficient hygienic and management conditions can positively affect wild bird's conservation. For instance, infected poultry by-product and manure can pollute

wetland used by wild birds with highly pathogenic avian influenza virus causing infection of wild birds and passively affect conservation of endangered wild avian species (Bourouiba et al., 2011; Beato and Capua, 2011).

Domestic poultry can be used as a model for studying wild avian species as they are easily bred in the lab. Moreover, changes in the same genes, and even in some cases the same mutations, have recently been shown to underlie similar phenotypes in both wild and domesticated populations (Hoekstra, 2006). Because of the limited success in freezing semen of nondomestic species particularly wild bird species, the interspecies transplantation of primordial germ cells into developing embryo of domestic poultry is considered as an alternative tool to conserve germ plasm of endangered wild bird species, such as Pheasant-to-chicken and Houbara bustard-to-chicken germline chimeras (Kang et al., 2008; Wernery et al., 2010).

Considering archaeological records, the chicken origin is pertained likely to red jungle fowl (RJF) as early as 5400 BC (West and Zhou, 1989). The involved cockfighting behaviour in ancient pictorial assembling supported the historical bibliographies inferring the initial human concern over chickens was for religion, decoration and entertainment. There is no confirmed historical record as well as no molecular data concerning origins of Egyptian native chickens. However the historical records and ancient pictorial assembling referred that the Indo-Aryan culture carried ancestors (wild Jungle fowl) of the Egyptian Fayoumi fowl from India and Sri Lanka to the Middle East. These birds were valued as ceremonial birds, rather than for their economic value as food and they adapted to the Ancient Egyptian environment. Breeding as feral chickens in isolation for centuries, their unusual hybrid ancestry responded through natural selection to the harsh ecological realities of the Fayoum Basin's arid thorn forests. When Romans conquered Egypt 2,000 years ago, the refinement of the Fayoumi fowl as a purely domestic species began (Coltherd, 1966; Ekarius, 2007; Heinrichs, 2007). Fayoumi chickens are known worldwide and are exported to many countries where they are used in rural and backyard family poultry production systems. The first pure pedigree Fayoumi flock was established in Fayoum City in 1951 and has been closed ever since. The second pure Fayoumi flock was formed through buying birds that fulfilled the description of

the pure Fayoumi from local villages and buying day old chicks from Baladi hatcheries found in Fayoum City. A third pure pedigree flock was established in the Al-Azzab integrated poultry project in 1983 at Fayoum Governorate. El-Hossari (1970) was able to establish 2 strains from Fayoumi through selection; one strain was for egg production (PP) while the other strain was for fast growth (GG) and the original flock was designated (RR).

Egyptian local chickens are free scavenging birds. Most of them are more suited for egg production than meat production. They are known for their strong bones and egg shell and low egg-cholesterol. They are of high marketing prices owing to the lovely good flavour of their meat and eggs. In addition, they are characterized by high fertility and hatchability (Samia and Gafer, 2000). They are subdivided into three groups according to their external morphology (Hosny, 2006). The first group includes pure native breeds, i.e. Fayoumi and Dandarawy. The second group includes mongrel fowl, such as the Baladi and Sinai strains, which originated from hybridization among exotic and Egyptian native chickens during different times of old trade dispersal and colonization to Egypt. The third group includes improved local strains which originated from crossing between local and standardized exotic chicken strains accompanied by selection for fast growth, such as El-Salam strain (Abd El-Gawad et al., 1983) and for high egg production, such as Golden Montazah strain (Mahmoud et al., 1974). With regard to the commercial sector in Egypt, commercial broilers have contributed 63% of the total poultry production in 2005. This could reflect the substantial growing of commercial chicken industries in Egypt at the expense of native chicken resources, improvement and maintenance (Hosny, 2006). Egypt possesses versatile varieties of chickens including local types highly adapted to harsh conditions and thought to constitute genetic reservoirs. For instance, the Fayoumi breed has been demonstrated by several studies to possess increased resistance to coccidiosis (Pinard-Van Der Laan et al., 1998) and Marek's disease (Tixier-Boichard et al., 2009), and can thus be seen as a unique breed from the viewpoint of disease resistance (Tixier-Boichard et al., 2009). Similarly, there is evidence for superiority in heat tolerance, of Sinai strain over White Leghorn and broiler chicks (Arad et al., 1981).

Domestic pigeons were promoted by Darwin as a proxy for understanding natural selection in wild populations and species, and pigeons thus hold a unique station in the history of evolutionary biology (Baptista et al., 2009). Pigeons are also easily bred in the lab, and morphologically distinct breeds are interfertile. Therefore, hybrid crosses should be a fruitful method to map the genetic architecture of derived traits, many of which are known to have a relatively simple genetic basis (Sell, 1994). Domestic pigeons and wild bird species vary in many of the same traits, so domestic pigeons provide an entry point to the genetic basis of avian evolutionary diversity in general (Baptista et al., 2009).

Since the initial domestication of the rock pigeon in Neolithic times (Driscoll et al., 2009), breeders have selected striking differences in behavior, vocalizations, skeletal morphology, feather ornaments, colors, and color patterns to establish over 350 breeds (Price, 2002). Historical evidence shows that pigeons are among the first of any animals, and the first of all birds, to be domesticated. Records and carvings of doves have been found as early as 3000 B.C. (Glover and Beaumont, 1999), but some argue that domestication may have taken place as long as 10,000 years ago (Patent, 1997). The exact origins of the rock dove are unknown, but usually they are traced to North Africa and Middle East regions (Bodio, 1990). Pigeons are bred for many purposes like meat in the form of squabs, exhibition as fancy and ornamental, flying and sports like racing competition (Blechman, 2007; Jerolmack, 2007; Hiatt and Esposito, 2000) and finally for laboratory experiments of cognitive sciences (Watanabe et al., 1995). Ancient Egyptians used pigeons as food in the form of squab and used pigeon's nitrogen-rich guano or feaces as fertilizers (Jerolmack, 2007). They discovered the strong homing ability of pigeons and used them as a messenger (Glover and Beaumont, 1999). On the contrary, feral pigeons can cause some ecological and public health problems. Feral pigeons roost and breed in the manmade ledges causing damage to buildings and machinery. Pigeon's droppings create smell and cause accidents when walkways become slippery and dangerous and can carry diseases such as histoplasmosis, cryptococcosis and psittacosis which may be transmitted to humans (Krebs, 1974). Fortunately, pigeons are resistant to bird flu (Fang et al., 2006), but they can transmit the disease mechanically. There are some conflicts between racing

pigeon sport and raptors. Raptors attack racing pigeons and can cause significant financial losses to pigeon fanciers, so that some pigeon fanciers kill the raptors by shooting or by poisoning. Levi (1963) stated that good flying pigeon generally can outmaneuver hawks, unless the hawk dives from some height, or is working in partnership with another hawk. Therefore, improvement of pigeon flying and racing might help in solving this conflict.

Consideration of the conservation of genetic resources would start with complete information on all existing breeds, numbers, distribution and population structure, trends in numbers, productive performance and adaptive characters. Generally speaking, breeds sometimes are not distinguished in the developing world. Local populations may have different names, but without change in phenotype; a change in phenotype may occur without change in name; or all populations may have just one name and be phenotypically similar. Clearly the first problem for conservation is the lack of information, so the identification and genetic characterization of all breeds particularly local ones has high priority in the FAO global strategy (Barker, 1999). Conservation of all breeds is considered to be financially infeasible (Bennewitz et al., 2007). Also, conservation of all breeds may not be necessary or scientifically justifiable, depending on the goal of the conservation programme. Some breeds may be judged to have no particularly unique or valuable characteristics worth conserving, either for the immediate or long-term, and have little historical or cultural significance. In other cases, a group of breeds may be genetically similar, meaning that a sufficiently large proportion of the genetic diversity of the group can be captured by conserving only a subset of breeds. Therefore, prioritization is needed (Boettcher, 2010). Both genetic diversity and non-genetic criteria are important for prioritizing breeds for conservation (Gizaw et al., 2008). For genetic diversity, maximization of genetic diversity through evaluation of the breed contribution to aggregate genetic diversity (contributions to the between-breed and to the within-breed diversity components) is important criterion for prioritizing breeds for conservation. There are many methods used for evaluation of the breed contribution to aggregate genetic diversity such as; *D1* = Ollivier and Foulley (2005), *D2* = Petit et al (1998) and *GD* = Caballero and Toro (2002) methods. The non-genetic criteria include threat status and

breed merit. The threat status includes risk of extinction and efficiency of the breed utilisation, and breed merit includes economic or productive, ecological and socio-cultural values of the breeds (Ruane, 2000; Gizaw, 2008). Conservation is not only about endangered breeds but also about those that are not being utilised efficiently (Barker, 2001). In the global management of animal genetic resources, the fundamental distinction is not between those breeds that are endangered and those that are not, but between those that are perceived to have little or no current utility and those which do have current utility or seem likely to have in the immediate future. For each of these latter categories, the necessary action is conservation (Barker, 1999).

The utilisation of poultry genetic resources is the best means to ensure that they remain available for future generations. To be sustainable, this utilisation must efficiently meet current economic and social objectives (Besbes et al., 2007). Therefore, it is obvious, that the most promising strategy for the conservation of genetic resources should focus on utilisation and improvement of competitiveness of indigenous populations through selection (Wollny, 1995). It is often said that within the conservation of small populations no selection pressure should be imposed because it would reduce the levels of genetic diversity intrinsic within the population. In practice this is an impossible restriction to place on any conservation programme. Selection at some level will inevitably occur in all live conservation programmes and is essential in order to maintain the characteristics of the population. Thus a limited amount of selection should be an integral part of breed conservation. This selection should be targeted at maintaining the known characteristics and parameters of the breed. It should not be used to reduce the genetic diversity found within a breed being conserved in a small population (Henson, 1992). The wild rock pigeon has an innate homing ability meaning that it will generally return to its own nest and its own mate. This made it relatively easy to select the birds that repeatedly found their way home over long distances (Blechman, 2007). In pigeon, two SNPs of lactate dehydrogenase -A gene have been identified in intron 6 and showed significant difference for allele's frequencies between homing and non-homing groups. (Dybus et al., 2006).

In this thesis I focused on Egyptian poultry (chickens and pigeons) because poultry protein is the major source of animal protein in Egypt (Hosny, 2006), and also

because of the scanty information about genetic characterization of Egyptian poultry breeds. Microsatellites marker analyses were involved in some recent studies to assess genetic diversity within and between Egyptian local chicken breeds (Roushdy et al., 2008; Eltanany et al., 2011). However, as far as I know, no study has ever conducted genetic characterization of Egyptian pigeon breeds. In chapter 2 of this thesis, I evaluated the genetic diversity and the breed contribution to aggregate genetic diversity as important criteria for their conservation by utilising three different prioritization methods in order to set the priorities for conservation of different Egyptian poultry species (chickens and pigeons) based on microsatellite genetic markers. Finally, because the conservation of indigenous population genetic resources should focus on sustainable utilisation and improvement of their competitiveness, I investigated in chapter 3 the DNA polymorphisms within *LDH-A* gene which might be considered as a potential genetic marker for selection of high homing and racing ability pigeons. This finding is useful for basic understanding of the relationship between *LDH-A* genotypes and flying ability in different avian species especially wild migrating species. Moreover it will improve the performance and competitiveness of indigenous pigeon populations. This information provides a foundation for developing sustainable genetic improvement and conservation programs of these agriculturally and commercially important species.

2. GENETIC DIVERSITY AND ITS APPLICATION FOR CONSERVATION PRIORITY OF EGYPTIAN POULTRY

2.1 Evaluation of genetic diversity and conservation priorities for Egyptian chickens

2.1.1 Introduction

Conservation of genetic diversity is one of the main current issues in the conservation biology literature (Frankham, 1995). Conservation is not only about endangered breeds but also about those that are not being utilized efficiently (Barker, 2001). More than 7500 different breeds of livestock are recognized globally (FAO, 2007). Conservation of all livestock breeds is considered to be financially infeasible (Bennewitz et al., 2007) so that priorities need to be set on which population/breed is to be conserved. Both genetic diversity and non-genetic criteria are important for prioritizing breeds for conservation. In order to maximize genetic diversity, it is necessary to prioritize breeds through evaluation of aggregate genetic diversity. Aggregate genetic diversity includes two components; between-breed and within-breed diversity. Many methods exist in literature to evaluate aggregate genetic diversity. For instance there are methods by Ollivier and Foulley (2005), Petit et al (1998) and Caballero and Toro (2002). The non-genetic criteria include threat status and breed merit. The threat status includes risk of extinction and efficiency of the breed utilisation, and breed merit includes economic or productive, ecological and socio-cultural values of the breeds (Ruane, 2000). As a result of many years of domestication and breeding, a wide variety of chicken breeds exist today. However, an increasing number of local breeds are under threat of extinction and valuable genotypes and traits are at risk of being lost (Blackburn, 2006).The genetic erosion of these local breeds may lead to the loss of valuable genetic variability in specific characteristics that are momentarily unimportant in commercial breeding strategies (Weigend et al., 1995).

Egyptian local chickens are subdivided into three groups according to their external morphology (Hosny, 2006). The first group includes pure native breeds, as Fayoumi and Dandarawy. The second group includes mongrel fowl, such as the Baladi and

Sinai strains, which originated from hybridization among exotic and Egyptian native chickens continued along with different times of old trade dispersal and colonization to Egypt. The third group includes improved local strains which originated from crossing between local and standardized exotic chicken strains accompanied by selection for fast growth, such as El-Salam strain (Abd El-Gawad et al., 1983) and for high egg production, such as Golden Montazah strain (Mahmoud et al., 1974). With regard to the commercial sector in Egypt, commercial broilers have contributed 63% of the total poultry production in 2005. This could reflect the substantial growing of commercial chicken industries in Egypt at the expense of native chicken resources, improvement and maintenance (Hosny, 2006). Egypt possesses versatile varieties of chickens including local types highly adapted to harsh conditions and thought to constitute genetic reservoirs. For instance, the Fayoumi breed has been demonstrated by several studies to possess increased resistance to coccidiosis (Pinard-Van Der Laan et al., 1998) and Marek's disease (Tixier-Boichard et al., 2009) and can thus be seen as a unique breed from the viewpoint of disease resistance (Tixier-Boichard et al., 2009). Similarly, there is evidence for superiority in heat tolerance, of Sinai strain over White Leghorn and broiler chicks (Arad et al., 1981).

In Egypt, microsatellites marker analyses were involved in some recent studies to assess genetic diversity within and between local chicken strains (Roushdy et al., 2008; Eltanany et al., 2011) In this study, we evaluated the genetic diversity and the breed contribution to aggregate genetic diversity as important criteria for their conservation by utilising three different prioritization methods in order to set the priorities for conservation of Egyptian chickens based on microsatellite genetic markers.

2.1.2 Material and methods

Sample collection and DNA extraction

Feather samples were obtained from a total of 196 birds from six Egyptian local chicken strains: Fayoumi (n = 35), Dandarawy (n = 30), Baladi (n = 29), Sinai (n = 30), El-Salam (n = 36), and Golden Montazah (n = 36). For comparative purpose, samples were also obtained from two exotic pure chicken breeds: White Leghorn (WL,

n = 42) and Rhode Island Red (RIR, *n* = 43). The Egyptian samples were collected from Al-Azzab poultry farms belonging to the Poultry Integrated Project at the Fayoum governorate, Egypt. Flock sizes for each breed were about 5000 birds, with sex ratios of one rooster per ten hens. Samples of White Leghorn were collected from the National Institute of Livestock and Grassland Science, Tsukuba, Japan and those of Rhode Island Red from Gifu Prefectural Livestock Research Institute, Gifu, Japan. DNAs were extracted from feather samples using the QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA, USA).

Microsatellite genotyping

Molecular genotyping of the samples was carried out with a set of 21 autosomal (CA)n di-nucleotide microsatellite markers that are as uniformly distributed as possible throughout the chicken genome. These markers are from the revised set of microsatellites originally recommended by the FAO MoDAD project (http://www.fao.org/AG/AGAInfo/programmes/en/genetics/documents/ITWG3_Inf3.pdf) for diversity studies in chicken. These markers were used in multiplex PCR reactions employing the QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA, USA). PCR was carried out in 10 µl reactions containing 20 ng of DNA template, 0.2 µM of each primer, of which the forward ones were fluorescently labelled (6-FAM, NED, and HEX) and 2x QIAGEN Multiplex PCR Master Mix. After an initial incubation of 95°C for 15 min, PCR amplification was performed for 35 cycles consisting of 94°C for 30 sec, 60 - 63°C annealing for 90 sec, 72°C for 60 sec, followed by a final extension of 60°C for 30 min. Subsequently, the PCR products were electrophoresed on an ABI 3130xl DNA Sequencer (Applied Biosystems) and the size of fragments was estimated based on 400 HD Rox size marker using the GENEMAPPER software (Applied Biosystems).

Data analysis

Genetic diversity was assessed by calculating the observed and effective number of alleles (N_A and N_e), mean number of alleles (MN_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) by using GENALEX version 6.0 (Peakall and Smouse, 2006). Polymorphism information content (*PIC*) was calculated by using Molkin version 2.0 (Gutiérrez et al., 2005). *F-statistics* [fixation coefficient of an individual

within a subpopulation (F_{IS}), fixation coefficient of an individual within the total population (F_{IT}), and fixation coefficient of a subpopulation within the total population (F_{ST})] per locus, in addition to pairwise F_{ST} (Weir and Cockerham, 1984) across the eight studied populations were calculated using GENEPOP version 3.4 (Raymond and Rousset, 1995). Genetic distances among the eight populations were evaluated by Nei's genetic distance (Nei et al., 1983). A phylogenetic tree was constructed based on the Nei's genetic distance (D_A) by using the neighbor-joining (NJ) method (Saitou and Nei, 1987). The robustness of tree topologies was evaluated with a bootstrap test of 1,000 resampling across loci. These processes were conducted using POPULATIONS version 1.2.30 software (http: //bioinformatics-org/~tryphon/populations/).

We investigated the genetic structure of the sampled populations using a Bayesian clustering procedure implemented in STRUCTURE with the admixture method. We analyzed the clustering of the eight studied populations by using independent allele frequencies model (Pritchard et al., 2000). We did 50 runs for each different value of K ($2 \leq K \leq 8$) with 60,000 iterations following a burn-in period of 100,000. Pairwise comparisons of the 50 solutions of each K value were run along with 50 permutations using CLUMPP software (Jakobsson and Rosenberg, 2007). The software calculated the highest pairwise similarity index (H). CLUMPP software also outputs a mean of the permuted matrices across replicates after aligning the cluster membership coefficients of these replicate. Finally, the clustering pattern with the highest H value was graphically displayed for the selected K value using DISTRUCT software (Rosenberg, 2004). The most probable clustering numbers (best ΔK value) was assessed according to the equation: $\Delta K = m(|L''(K)|)/s[L(K)]$, (Evanno et al., 2005). STRUCTURE software identified the migrants and admixed individuals. If the membership coefficient of an individual was more than 0.80, it was assigned to the cluster completely. If the value was lower than 0.80, it indicated that the individual was admixed and assigned it to two or more population clusters. The membership coefficient could also identify the migrants who had infiltrated into other chicken population clusters (Li et al., 2009).

Different prioritization methods were utilized through measuring the breed contribution to aggregate genetic diversity (contributions to the between-breed and to the within-breed diversity) as in the following:

a) According to Ollivier and Foulley method (Ollivier and Foulley, 2005), the contribution to between-breed diversity (*CB*) was computed by estimation of Weitzman values (Weitzman, 1993) based on the Nei's genetic distance (Nei et al., 1983) with WEITZPRO (Derban et al., 2002). Within breed contributions to diversity (*CW*) were calculated using the average values of within-breed expected heterozygosity as in the formula: H_k= 1- $H(S/k)$ / $H(S)$, where H_k is the contribution to within-breed diversity (*CW*) of breed *k*, $H(S)$ is the average internal heterozygosity of the whole set *S* and $H(S/k)$ the average internal heterozygosity of the set excluding breed *k.* The aggregate diversity (*D1*) was obtained after weighting *CB* by F_{ST} and *CW* by 1-F_{ST} according to the following equation: $D1 = F_{ST}$CB + (1-F_{ST}) CW. Positive contributions to diversity from a given population using the Ollivier and Foulley method (Ollivier and Foulley, 2005) means that the remaining dataset decreases the overall diversity; consequently, the assessed population would be preferred for conservation.

b) According to Petit et al method (Petit et al., 1998), the rarefacted number of alleles per locus (*k*) was used to assess the contribution of the i^{th} population to the total allelic richness as $D2 = C_S+C_D$ where C_S is the contribution to the total allelic richness due to the allelic richness of the i^{th} population and C_D is the contribution due to its divergence. Positive contributions to diversity from i^{th} population using the Petit et al method mean that the remaining set has a lower number of alleles than the original set; consequently, the i^{th} population would be preferred for conservation. This procedure was computed using Molkin version2.0 (Gutiérrez et al., 2005).

c) According to Caballero and Toro method (Caballero and Toro, 2002), the partitions of the total gene diversity was calculated as in the following equation: (1-f) = (1-f) + D, where f is the average global coancestry; f is the average coancestry between populations; (1-f) = GD_T representing the total gene diversity; (1-f) = $GD_{w,}$ representing the within population component; and D is the Nei genetic distance between populations, representing the between population component. Positive

contributions to diversity from a given population using the Caballero and Toro method mean that the remaining dataset increases the overall diversity; consequently, the assessed population would not be preferred for conservation. This procedure was computed using Molkin version2.0 (Gutiérrez et al., 2005).

2.1.3 Results and discussion

Marker polymorphisms and population diversity

A total of 162 alleles were observed across all the eight populations, out of which 144 alleles (144/162, 88.9%), including 18 unique ones (18/144, 12.5%), were observed in the six Egyptian populations (Tables 2.1 and 2.2). In this study, across the six Egyptian populations the estimated means of N_A (6.9), N_e (3.0) and H_E (0.595) are relatively lower than those of Eltanany et al., (2011) who reported values of 7.34, 3.00 and 0.653, respectively, across ten Egyptian chicken strains using 29 microsatellites loci. The F_{ST} value across the 21 studied loci showed a moderate mean (0.082) indicating that there is genetic differentiation among the six Egyptian local strains. The estimated F_{ST} value was lower than that measured between pure-bred commercial chicken lines in a study in Zimbabwe which showed 0.357 of total genetic variation owing to line differences (Muchadeyi et al., 2007). However, it was slightly higher than the 0.068 previously reported across ten Egyptian chicken strains (Eltanany et al., 2011). In this study, F_{ST} recorded a high value (0.222) after adding the two exotic pure populations (WL and RIR) indicating that, there is high genetic differentiation between these two exotic pure breeds and Egyptian chicken populations (Table 2.1). The relatively low but positive F_{IS} average (0.051) might indicate non-random mating and also some loci might be under morphological or productive traits of selective interest. Moreover, F_{IS} is used to obtain a deeper insight to appraise the degree of inbreeding and endangerment potentiality and is considered as an important tool to judge the conservation priority (Simon and Buchenauer, 1993). Accordingly, when F_{IS} is less than 0.05, the breeds are not in danger; between 0.05-0.15, they are potentially endangered; between 0.15-0.25, they are minimally endangered; between 0.25-0.40, they are endangered; and more than 0.40, they are critically endangered. In this study, Fayoumi, El-Salam and RIR populations showed high levels of inbreeding

(0.110, 0.095 and 0.083, respectively) and were deviated from HWE, posing their potential endangerment (Simon and Buchenauer, 1993).

In respect to the within population genetic diversity, the studied eight chicken populations could be categorized into a low diversity class (Fayoumi, WL, and RIR) and a high diversity class which includes the remaining five populations. This is in agreement with breed history and management. These populations (WL and RIR) had undergone selection for high growth rate (RIR) and high egg production (WL). Moreover, the Fayoumi strain recorded the highest value of F_{IS} (0.110) and complete allele fixation (monomorphic) of the *MCW014* locus (Table 2.2). This might be attributed to its narrow genetic base as it is an ancient native chicken bred as a closed population. The two Egyptian mongrel strains (Baladi and Sinai) recorded the highest genetic diversity (MN_A = 5.9; N_e = 3.4; H_O = 0.622, and H_E = 0.645 for Baladi and MN_A = 5.4; N_e = 3.4; H_O = 0.648, and H_E = 0.660 for Sinai) among the eight studied populations and this might be attributed to their wide genetic bases due to hybridization among exotic and Egyptian native chickens continued along with different times of old trade dispersal and colonization to Egypt (Hosny, 2006).

Genetic relationship

The Nei's genetic distance (D_A) and pairwise F_{ST} statistic were estimated for the eight studied chicken populations across the 21 microsatellite loci (Table 2.3). The closest pairwise Nei's genetic distance was recorded between the Sinai and Golden Montazah strains (0.038) and this was supported by clustering in the neighbor-joining phylogenetic tree (Figure 2.1). Similarly, the lowest pairwise F_{ST} value was recorded between the Baladi and Sinai strains (0.006). The close relation between Sinai and Golden Montazah and also between Baladi and Sinai can be attributed to the mongrel nature of Baladi and Sinai strains which originated from hybridization among exotic and Egyptian native chickens.

Population structure and individual's assignment

The most probable structure clustering of the eight studied chicken populations was at K = 6 (Figure 2.2). The pure breeds (WL, RIR, Fayoumi and Dandarawy) in addition to El-Salam were assigned independently into their respective clusters while the remaining three populations (Baladi, Sinai and Golden Montazah) were clustered

together forming admixed mosaic cluster. A probable explanation for the separation of El-Salam to form its own cluster is that it might have experienced high inbreeding and low gene flow from other strains under this study. This is in line with the relatively high F_{IS} (0.095) value within the El-Salam strain. The high genetic admixture and migrations between Baladi, Sinai, and Golden Montazah strains could contribute to gather them forming the admixed mosaic cluster.

The distribution of 35 admixed and five migrant individuals in the inferred six clusters according to their membership coefficients were evaluated (Table 2.4). All the studied populations recorded more than 0.80 membership coefficients in their inferred clusters except Baladi strain (0.69). Despite that Sinai and Golden Montazah strains had more than 0.80 membership coefficient, they were clustered together in the same cluster with Baladi strain. This might be explained by the genetically similar nature of these strains as confirmed by their pairwise genetic distances (Table 2.3) and the mosaic admixture in the STRUCTURE dendrogram (Figure 2.2). Thus, based on the preceding results, when the Baladi, Sinai, and Golden Montazah chicken strains are to be used for future research work it would be necessary to consider, in addition to random selection, the admixed individuals and migrants.

Conservation prioritization of the studied strains

In the current study different prioritization methods were utilized to measure the breed contribution to aggregate genetic diversity as an important criterion for its conservation (Table 2.5). All such methods revealed that Fayoumi strain contributed negatively to aggregate genetic diversity (*CW*= -4.20, *D1*= -1.15, *D2*= -1.89 and *GD*= 1.72). Therefore, Fayoumi according to such a determined criterion may be ranked last for conservation. On the contrary, Dandarawy contributed the most (*D2*= 2.49, *GD*= -1.40) to aggregate genetic diversity according to (Petit et al., 1998; Caballero and Toro, 2002). The two Egyptian mongrel strains ranked second (*D1*= 2.90, *GD*= -1.23 for Sinai and *D1*= 1.73, *D2*= 2.27 for Baladi), while Egyptian synthetic strains (Golden Montazah then El-Salam) came in the third position according to their contribution to aggregate genetic diversity. The preceding prioritization of the breeds for conservation is based only on molecular genetic marker information, but when we combine other non-genetic criteria the ranking may become different. Thus, according

to the preceding prioritization methods, Fayoumi ranked the last, but after considering its high level of inbreeding and breed merit in term of disease resistance ability (Marek's disease and coccidiosis), it may get advanced ranking. Similarly, Sinai strain ranked second, but after considering its breed merit (superiority in heat tolerance) it may get a different ranking.

In conclusion, the results from this study confirm the applicability and efficiency of this microsatellite panel for assessing genetic variation and setting the conservation priorities for Egyptian local chickens. Consideration of breed merits and threat status, in addition to genetic diversity, enabled us to balance the trade-offs between conserving diversity as insurance against future uncertainties and current sustainable utilisation. More detailed information about non-genetic aspect (threat status and breed merits) and a conceptual framework for a maximum utility through a weighted summation of measures of neutral diversity, breed merits and threat status of Egyptian chickens merits consideration.

Table 2.1 Observed (N_A) and effective (N_e) number of alleles, polymorphic information content (*PIC*), observed (H_O) and expected (H_E) heterozygosities, and *F*-statistics (F_{IS} , F_{ST}, and F_{IT}) across the six Egyptian strains

Locus	N_A±*SD*	N_e±*SD*	*PIC*±*SD*	*Ho*±*SD*	H_E±*SD*	F_{IS}±*SE*	F_{ST}±*SE*	F_{IT}±*SE*	dHWE [b]	n [c]
ADL268	6.0	4.0	0.767	0.686	0.751	0.093	0.070	0.156	*	2
ADL278	4.0	2.5	0.596	0.612	0.594	-0.034	0.132	0.102	n.s	0
ADL112	3.7	2.0	0.426	0.441	0.436	-0.014	0.115	0.102	n.s	1
MCW295	4.8	2.0	0.467	0.337	0.457	0.251	0.087	0.316	*	4
MCW216	3.2	2.1	0.465	0.408	0.518	0.217	0.044	0.251	*	2
MCW014	3.0	1.4	0.276	0.128	0.265	0.540	0.092	0.582	*	4
MCW098	2.0	1.3	0.192	0.224	0.216	-0.025	0.035	0.011	n.s	0
LEI234	10.0	5.7	0.851	0.638	0.817	0.220	0.069	0.273	*	3
MCW111	4.7	2.8	0.613	0.631	0.628	-0.004	0.055	0.052	n.s	0
MCW078	4.0	2.0	0.511	0.521	0.491	-0.053	0.201	0.159	n.s	0
MCW222	3.8	1.9	0.478	0.458	0.472	0.036	0.122	0.154	n.s	1
MCW183	8.0	5.3	0.843	0.712	0.811	0.121	0.068	0.180	*	2
LEI094	9.8	5.2	0.837	0.778	0.814	0.051	0.060	0.108	n.s	1
MCW069	5.5	3.4	0.700	0.659	0.698	0.047	0.067	0.111	*	2
MCW034	6.7	3.9	0.716	0.793	0.722	-0.098	0.050	-0.044	n.s	0
MCW037	3.0	2.6	0.555	0.622	0.613	-0.015	0.038	0.024	n.s	0
MCW067	3.2	2.6	0.572	0.601	0.615	0.027	0.059	0.085	n.s	0
MCW206	5.5	3.0	0.673	0.704	0.673	-0.042	0.088	0.050	n.s	0
MCW081	5.7	3.5	0.699	0.684	0.676	-0.010	0.092	0.083	n.s	0
LEI166	3.2	2.4	0.563	0.615	0.579	-0.067	0.114	0.055	n.s	0
MCW330	4.0	3.0	0.644	0.636	0.653	0.027	0.085	0.110	n.s	0
Mean	6.9±3.6	3.0±0.6	0.593±0.175	0.566±0.092	0.595±0.078	0.051±0.032	0.082±0.008	0.129±0.029		
Total mean [a]	7.7±4.2	2.8±0.5	0.649±0.137	0.536±0.078	0.564±0.069	0.051±0.018	0.222±0.023	0.261±0.026		

[a] Total mean includes WL and RIR in addition to the six Egyptian breeds

[b] = deviation from Hardy-Weinberg equilibrium. * = significant while n.s = not significant.

[c] = indicates the number of populations that deviated from Hardy-Weinberg equilibrium.

Table 2.2 Mean observed (MN_A) and effective (MN_e) number of alleles, unique alleles, observed (H_O) and expected (H_E) heterozygosities, and fixation coefficient of an individual within a subpopulation (F_{IS}) per breed.

Breed/strain	*n*	MN_A±SD	N_e±SD	Unique alleles	*Ho*±SD	H_E±SD	F_{IS}±SE	dHWE [a]	n [b]
Egyptian breeds mean	196	4.9±0.5	3.0±1.4	18	0.566±0.049	0.595±0.042	0.053±0.017		
Fayoumi	35	3.7	2.3	0	0.423±0.215	0.475±0.220	0.110	*	6
Dandarawy	30	4.4	2.7	4	0.560±0.197	0.591±0.147	0.050	n.s	3
Baladi	29	5.9	3.4	9	0.622±0.211	0.645±0.192	0.036	n.s	4
Sinai	30	5.4	3.4	2	0.648±0.179	0.660±0.142	0.020	*	5
El-Salam	36	4.8	2.9	0	0.527±0.243	0.582±0.211	0.095	*	3
Golden Montazah	36	5.4	3.2	3	0.616±0.224	0.618±0.202	0.003	n.s	2
WL	42	2.5	2.0	4	0.423±0.234	0.428±0.229	0.012	n.s	1
RIR	43	3.6	2.2	4	0.469±0.160	0.511±0.137	0.083	*	6
Total mean	281	4.5±0.5	2.8±1.2	26	0.536±0.048	0.564±0.042	0.052±0.014		

[a] = deviation from Hardy-Weinberg equilibrium.

[b] = indicates the number of loci that deviated from Hardy-Weinberg equilibrium.

* = significant while n.s = not significant.

Table 2.3 Nei's genetic distance (above diagonal) and pairwise F_{ST} (below diagonal) estimates for the 21 microsatellite loci between the eight studied chicken strains.

	Fayoumi	Dandarawy	Baladi	Sinai	El-Salam	Golden Montazah	WL	RIR
Fayoumi		0.185	0.104	0.143	0.165	0.155	0.506	0.501
Dandarawy	0.170		0.115	0.134	0.170	0.155	0.486	0.420
Baladi	0.079	0.062		0.040	0.081	0.059	0.469	0.419
Sinai	0.116	0.074	0.006		0.077	0.038	0.468	0.403
El-Salam	0.137	0.118	0.043	0.051		0.070	0.489	0.422
Golden Montazah	0.143	0.111	0.033	0.020	0.045		0.494	0.401
WL	0.399	0.354	0.318	0.311	0.351	0.352		0.326
RIR	0.392	0.316	0.285	0.268	0.299	0.279	0.302	

Table 2.4 Number of admixed and migrant individuals in the inferred clusters.

Cluster	Strain	Membership coefficient	Admixed individuals	Migrant individuals to another cluster
Cluster I	WL	0.99	0	-
Cluster II	RIR	0.97	0	-
Cluster III	Fayoumi	0.94	4	-
Cluster IV	Dandarawy	0.96	1	-
Cluster V	El-Salam	0.82	4	2 (Cluster VI)
Cluster VI	Baladi	0.69	10	2 (Cluster III and Cluster IV)
	Sinai	0.81	9	-
	Golden Montazah	0.86	7	1 (Cluster V)

Table 2.5 Contribution of each strain studied to aggregate genetic diversity.

Strain	*CW* [a]	*CB* [b]	*D1* [c]	*D2* [d]	*GD* [e]
Fayoumi	-4.202	33.970	-1.148	-1.892	1.716
Dandarawy	-0.168	34.920	2.639	2.490	-1.404
Baladi	0.840	11.950	1.729	2.273	-0.414
Sinai	2.521	7.240	2.899	0.989	-1.231
El-Salam	-0.504	17.430	0.931	0.196	0.194
Golden Montazah	0.840	10.930	1.648	0.791	-0.823

[a] *CW* = contribution to within-population genetic diversity; [b] *CB* = contribution to between-population genetic diversity (Weitzman, 1993); [c] *D1* = contribution to aggregate genetic diversity (Ollivier and Foulley, 2005); [d] *D2* = global diversity contribution (Petit et al., 1998); [e] *GD* = global diversity contribution (Caballero and Toro, 2002.

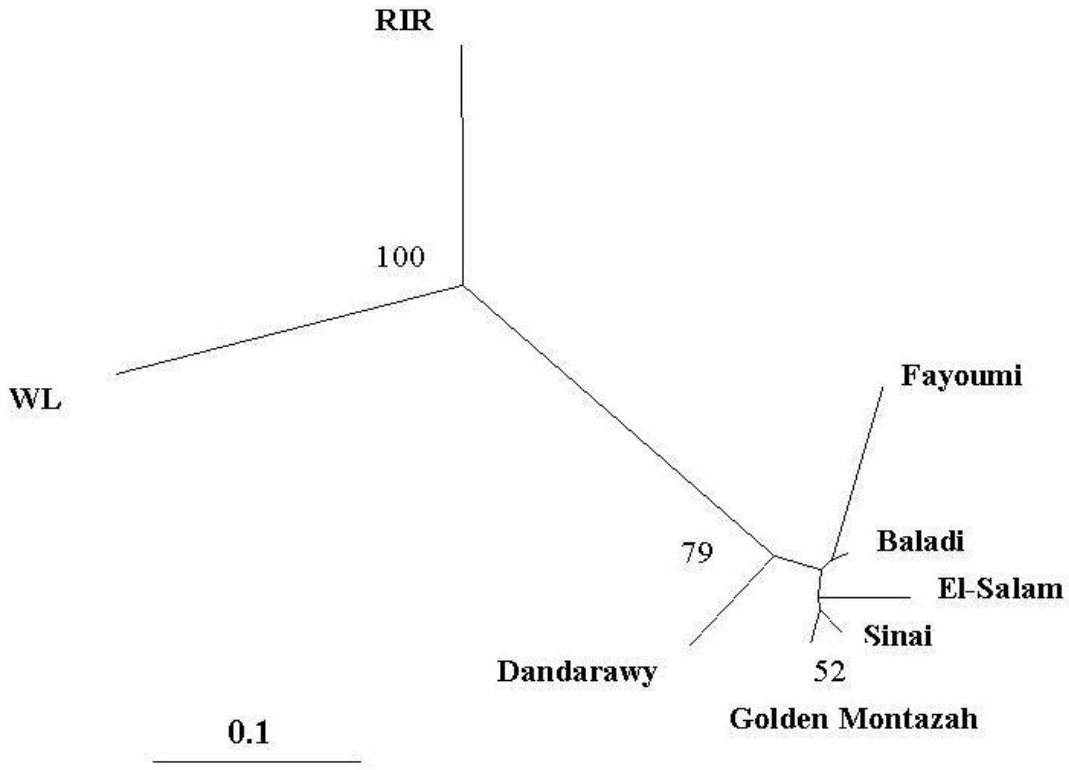

Figure 2.1 Neighbor-joining phylogenetic tree of the six Egyptian and the two exotic chicken populations based on Nei's genetic distance of 21 microsatellite loci. The consensus tree was generated with 1,000 bootstraps over loci and bootstrap values lower than 50 are not shown in the diagram.

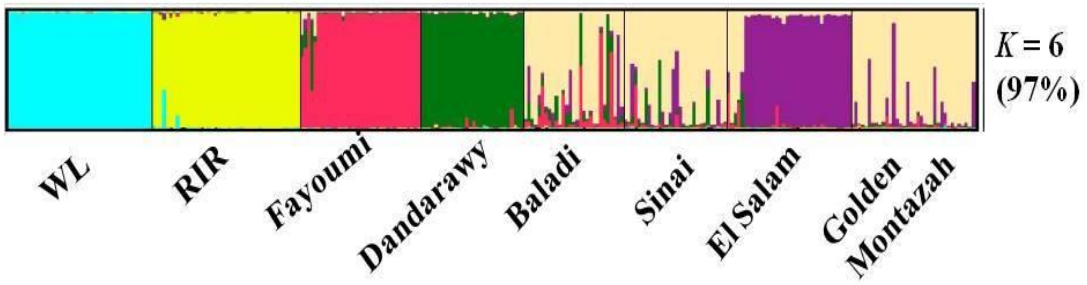

Figure 2.2 Structure clustering of the six Egyptian and the two exotic pure chicken populations obtained for K = 6. The percentage inside the parenthesis is the average pairwise similarity index (H) of the individuals Q matrix, while K is the cluster number. WL, White Leghorn; RIR, Rhode Island Red.

2.2 Analysis of genetic diversity of Egyptian pigeon breeds

2.2.1 Introduction

The importance of maintaining genetic diversity in domestic livestock is advocated worldwide by the Food and Agriculture Organization (FAO). Therefore, conservation of native breeds as a genetic resource is important to fill unanticipated breeding demands in the future (Tadano et al., 2007b). Among these species, pigeons are believed to be domesticated as early as 3000 B.C. (Glover and Beaumont, 1999) and today there are over 300 breeds of domestic pigeons, all originating from one wild source, the rock dove (Bodio, 1990). Today there are over 300 breeds of domestic pigeons, all originating from one wild source, the rock doves (Bodio, 1990). Pigeons are bred for many purposes like meat in the form of squabs, exhibition as fancy and ornamental, flying sports like racing competition (Hiatt and Esposito, 2000; Blechman, 2007; Jerolmack, 2007) and finally for laboratory experiments of cognitive sciences (Watanabe et al., 1995). Origins of the rock dove are often traced to North Africa and Middle East regions even though it is not exactly known (Bodio, 1990). Pigeons appeared on Egyptian bas-reliefs from at least 2700 B.C. Ancient Egyptians used pigeons as food in the form of squab and used pigeon's nitrogen-rich guano or feaces as fertilizers (Jerolmack, 2007). They discovered the strong homing ability of pigeons and used them as messengers. An Egyptian bas-relief from around 1350 B.C. depicts a flock of doves being released from their cages to fly and then return (Glover and Beaumont, 1999).

The six Egyptian indigenous pigeon breeds used in this study don't belong to feral pigeons. Five of these breeds: Ablaq (Levi, 1996), Krezly, Zagel, Safi and Asfer Weraq characterized by strong homing and flying abilities and mainly used for certain kind of a very popular flying game in Egypt, whereas the last one (Romani breed) characterized by heavy body weight and used mainly for meat production in the form of squabs. During the flying game, pigeons stock may often be seen flying in circles over rooftops. The breeder trains his birds to fly to nearby rooftops where they meet another's stock. Birds may become disoriented when their stock meets unknown others. If the stock is well trained, the pigeons would return with or steal strays from

other stock (Jerolmack, 2007). In Egypt, despite the importance of this species, little is known about its genetic diversity regarding the different types of uses and local population size. Research work on the genetic variation of this species is important to characterize the genetic structure of local populations. This serves as an important first step to identify the valuable genetic characters and resources of the domestic pigeon for conservation against future needs. In the face of daunting global challenges such as climate change, emerging diseases, population growth, and rising consumer demands, it is likely that maintaining genetic variation is quite important for the future (Kayang et al., 2010).

Mitochondrial DNA (mtDNA) has been widely employed in phylogenetic studies of various animals because it evolves much more rapidly than nuclear DNA, resulting in the accumulation of differences between closely related species. In fact, the rapid pace of sequence changes in mtDNA results in differences between populations that have only been separated for brief periods of time (Hebert et al., 2004). Pigeon mtDNA sequence was used to construct a phylogeny for *Streptopelia*, *Columba* and related taxa (Johnson et al., 2001). Because of their high degree of polymorphism, being numerous and ubiquitous throughout the genome and codominant inheritance, microsatellite markers are valuable tools for the studying of genetic diversity between populations and assessing the relationships among closely related livestock breeds (Tadano et al., 2007a). Indeed, pigeon DNA microsatellites were used to clarify the origin and genetic relationship between different pigeon lines (Traxler et al., 2000) and to provide a rapid identification to resolve identification and paternity disputes arising from racing pigeons (Lee et al., 2007).

In this study, we applied the previous markers for analysis of the genetic diversity and the breed contribution to aggregate genetic diversity as we did with chickens in 2.1 Such information would provide a foundation for developing sustainable genetic improvement and conservation programs aimed at enhancing the flying ability, meat quality, as well as growth and reproduction traits of this agriculturally and commercially valuable species.

2.2.2 Materials and methods

Sample collection and DNA extraction

A total of 133 pigeon samples were obtained from six Egyptian indigenous breeds: Krezly (*n* = 26), Zagel (*n* = 21), Safi (*n* = 21), Romani (*n* = 21), Asfer Weraq (*n* = 12), and Ablaq (*n* = 10) together with Japanese racing pigeons (*n* = 23; 8 from Japanese Imanishi line and others from Belgian lines). Mitochondrial cytochrome c oxidase subunit I gene (*COI*) sequence was analyzed also for one individual each from some wild pigeon species, oriental turtle-dove (*Streptopelia orientalis*), emerald dove (*Chalcophaps indica),* white-bellied green pigeon (*Treron sieboldii*) and whistling green pigeon (*Treron formosae)* for comparison. Egyptian samples were collected from eight breeders in four provinces (Cairo, Giza, Kaliobia and Zagazig) located in the Nile river delta in the northern part of Egypt, whereas, samples of Japanese racing pigeons were collected from one breeder in Kashiwa city, Chiba, Japan. Samples of wild pigeons were obtained from Osaka Museum of Natural History, Osaka, Japan. DNA was extracted from feather samples using the QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA, USA).

Mitochondrial COI analysis

A determined region near the 5´ terminus of the *COI* was amplified using primers BirdF1-TTCTCCAACCACAAAGACATTGGCACand BirdR1-ACGTGGGAGAT-AATTCCAAATCCTG (Hebert et al., 2004). The PCR was performed on a 15 µl reaction mixes including 20 ng of genomic DNA, 2x PCR buffer, each dNTP at 400 µM, each primer at 0.4 µM and 0.75 U of *LA-Taq* DNA polymerase (TaKaRa, Shiga, Japan). After an initial incubation at 95°C for 2 min, PCR amplification was performed for 35 cycles consisting of 95°C for 30 sec, 50°C for 30 sec, 74°C for 60 sec, followed by a final extension of 74°C for 10 min. The amplified products were purified using PCR purification kit (Roche, Mannheim, Germany) and the resultant products were sequenced by using the same primers and the Big Dye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the standard protocol, and electrophoresed on an ABI PRISM 3130xl sequencer (Applied Biosystems). The MEGA 5 Software (Kumar et al., 2008) was used for sequences alignment and to infer the phylogenetic relationships based on neighbor-joining (NJ) methods (Saitou and Nei, 1987).

Microsatellite analysis

Eleven microsatellite markers (*CliμD17, CliμT17, CliμD16, CliμD32, CliμT13* and *CliμD01* from Traxler et al., 2000; *PG1, PG2, PG4, PG6* and *PG7* from Lee et al., 2007) were used in multiplex PCR reactions employing the QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA, USA). PCR details are as described in case of chickens in 2.1 except for annealing temperature which is 55°C.

Data analysis

The genetic diversity of each breed was assessed by calculating the observed number of alleles (N_A) and its mean (MN_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) by using the program package ARLEQUIN version 2.000 (Schneider et al., 2000) software. The Mode-shift indicator test was utilised as a method to detect bottlenecks by using Bottleneck ver. 1.2.02 software (Cornuet and Luikart, 1996). The non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency and by using graphical representation utilising allelic class and proportion of alleles they show a normal 'L' shaped distribution. In contrast, the bottlenecked population causes a characteristic mode-shift distortion in the distribution of allele frequencies at selectively neutral loci. Bottleneck causes alleles at low frequency (< 0.1) to become less abundant than alleles in one or more intermediate allele frequency classes. The low and high allele frequency classes were between 0.0 - 0.1 and 0.9 -1.0 respectively (Luikart et al., 1998; Cornuet and Luikart, 1996). *F*-statistics (F_{IS}, F_{IT}, F_{ST}), Nei's genetic distance (D_A), neighbor-joining phylogenetic tree were assessed as described in case of chickens in 2.1. The genetic structure of the seven studied populations was investigated using a Bayesian clustering procedure implemented in STRUCTURE with the admixture method for each different value of K ($2 \leq K \leq 7$) as described in case of chickens in 2.1. We utilised the same three prioritization methods (Ollivier and Foulley, 2005; Petit et al., 1998; Caballero and Toro, 2005) which were used for measuring the breed contribution to aggregate genetic diversity for chicken breeds.

2.2.3 Results and discussion

Mitochondrial COI analysis

We obtained 693 base pairs of sequence for one sample each of the six Egyptian breeds, the Japanese racing pigeons and the four wild pigeon species. After alignment, there were only two substitution sites among the six studied Egyptian breeds and the Japanese racing pigeons. From the NJ phylogenetic tree (Figure 2.3) these seven populations clustered into the same clade with *Columba livia* sequence retrieved from GenBank (accession number GQ481605). The branching pattern of other species reflected their phylogeny. The low sequence divergence within Egyptian breeds together with the Japanese racing pigeons can be explained as all these breeds belong to same species (*Columba livia*) and the mtDNA *COI* sequence divergence is more suited for the analysis of among species divergence than within species divergence (Hebert et al., 2004).

Microsatellite analysis

Genetic diversity

A total of 89 alleles were detected in the six Egyptian breeds together with Japanese racing pigeons by 11 microsatellite markers. Across all the populations, the number of alleles per locus ranged from 3 (*PG6*) to 14 (*CLiμD16*) and the average number of alleles observed was 8.1 (Table 2.6). Across populations, locus *PG6* had the lowest values for both H_O (0.429) and H_E (0.426), whereas locus *CLiμT17* and locus *CLiμD01* had the highest H_O (0.680) and H_E (0.710), respectively (Table 2.6). The average numbers of alleles, expected and observed heterozygosity in addition to F_{IS} for each population across 11 loci are shown in Table 2.7. Across 11 loci, the lowest value of expected heterozygosity (0.423) was obtained for the Ablaq breed, and the highest value (0.732) was found for Romani breed. The relatively high diversity obtained for Romani breed may be explained as Romani breed is kept by fanciers as meat breed in a large area of Egypt, which leads to breeding from many individuals. This condition seems to result in higher degree of diversity than other breeds which are mainly kept for racing and flying. The overall expected heterozygosity of Egyptian indigenous pigeons together with Japanese racing pigeons was 0.584. As a measure of deviation from Hardy-Weinberg equilibrium, the F_{IS} value was calculated and found to range from -0.200 (Asfer Weraq) to 0.073 (Japanese racing) with mean -0.017. Moreover, F_{IS} is used to obtain a deeper insight

to appraise the degree of inbreeding and endangerment potentiality and is considered as an important tool to judge the conservation priority (Simon and Buchenauer, 1993). In this study, Japanese racing population showed high levels of inbreeding (0.073), indicating its potential endangerment. We recommend out-breeding of Japanese racing population to overcome inbreeding effect.

Table 2.7 shows that 12 breed-specific alleles were detected among the seven populations. The number of breed-specific alleles per breed ranged from 0 (Zagel and Asfer Weraq) to 4 (Krezly and Romani). The qualitative graphical method was used for identifying bottlenecked population from distributions of allele frequencies. Asfer Weraq population might recently suffer from genetic bottleneck because; high proportion of alleles is located in the intermediate allele frequency classes (0.3 and 0.6 classes) as shown in (Figure 2.4a). However, we should be cautious about the interpretation of Mode-shift indicator test because of the small sample size of Asfer Weraq breed. Small samples are likely to miss alleles at low frequency and thus cause allele frequency distributions to resemble those from a bottlenecked population. Consequently we recommend sampling more than 25 individuals to avoid misinterpretation (Luikart et al., 1998; Cornuet and Luikart, 1996). The other six populations (Krezly, Zagel, Safi, Romani, Ablaq and Japanese racing) might not recently suffer from genetic bottleneck effect as their distributions of allele frequencies follow normal (L) shape distribution (Figure 2.4a – 2.4g).

Genetic differentiation among populations

Genetic differentiation was examined by fixation indices F_{IS}, F_{ST}, F_{IT} for each locus (Table 2.6). The fixation coefficients of subpopulations within the total population, measured as F_{ST} value, for the 11 loci varied from 0.154 (*CLiμD17*) to 0.313 (*PG4*), with a relatively high mean 0.203 which indicated that there is a great differentiation among the seven pigeon populations. It is clear that about 20.3% of the total genetic variation corresponds to differences of populations. This F_{ST} value is higher than in some other poultry species. For instance, Shahbazi et al., (2007) reported a F_{ST} value of 0.15 from five Iranian native chicken populations, and Bao et al., (2008) reported a F_{ST} value of 0.167 from Chinese domestic fowls. The global deficit of heterozygote across populations (F_{IT}) amounted to 20.9% (Table 2.6). For

the coefficient F_{IS}, which shows the degree of departure from random mating, positive F_{IS} values indicate deficit of heterozygote, while the negative F_{IS} values indicate an excess of heterozygous genotypes with respect to the expected value. In this study, the relatively high average F_{IS} (0.008) in addition to five loci (*CLiμD16, CLiμD01, PG1, PG6,* and *PG7*) showing a deficit of heterozygote might indicate that these loci are under selection (genetic hitchhiking effect) with some morphological or productive traits of selective interest.

Genetic relationship

As shown in Table 2.8, the pairwise Nei's genetic distance between the seven studied pigeon populations ranged from 0.154 (Zagel-Japanese racing) to 0.518 (Zagel-Ablaq). The closest pair was thus Zagel and Japanese racing pigeons. Similarly, the genetic differentiation (pairwise F_{ST}) values were lowest in Zagel-Japanese racing pair (0.108). These results are supported by clustering in the neighbor-joining phylogenetic tree (Figure 2.5) where the tree topology showed close relation between Zagel breed and Japanese racing pigeons. The close relation between Zagel breed and Japanese racing pigeons may be explained as they may have a common ancestor. The origin of Japanese racing pigeons was said to be military messenger pigeons imported from European countries since early 1900s, and the old ancestors of the Japanese samples used in this study would be from France, Netherlands and Belgium (Komahara, 1980). The early use of pigeons as messengers led to their value as a commodity and to its further global proliferation. As far as conquerors and traders moved, they brought their pigeons with them. Even as the invaders left, descendents of their pigeons stayed behind to be bred for future wars with new enemies. By the middle of the nineteenth century, Belgians had established the modern messenger and racing pigeon now used throughout the world through the continual crossbreeding of several types of pigeons (Jerolmack, 2007).

Population structure

The most probable structure clustering of the seven studied pigeon populations was at $K = 6$ (Figure 2.6). Five pigeon populations (Krezly, Safi, Romani, Ablaq and Japanese racing) were assigned independently into their respective clusters while the remaining two populations (Asfer Weraq and Zagel) appeared as a mosaic clusters.

Asfer Weraq population appeared as mosaic structure which was made from Safi and Ablaq populations and this may be due to gene flow between these populations. The mosaic admixture between Zagel and Japanese racing might be explained by the genetically similar nature of these two populations as they may have a common ancestor and this was confirmed by their low pairwise F_{ST} and genetic distances (Table 2.8).

Conservation prioritization of the studied breeds

In the current study different prioritization methods were utilized to measure the breed contribution to aggregate genetic diversity as an important criterion for its conservation (Table 2.9). All such methods revealed that Asfer Weraq population contributed negatively to aggregate genetic diversity (*D1*= -0.257, *D2*= -2.741 and *GD*= 1.439). Therefore, Asfer Weraq according to such a determined criterion may be ranked last for conservation. On the contrary, Zagel population contributed the most (*D1*= 8.169, *GD*= -4.260) to aggregate genetic diversity according to (Ollivier and Foulley, 2005 & Caballero and Toro, 2002). The preceding prioritization of the breeds for conservation is based only on molecular genetic marker information, but when we combine other non-genetic criteria like breed merit and threat status the ranking may be different.

In conclusion, we confirm the applicability and efficiency of microsatellites for assessing genetic variation and divergence in Egyptian native pigeon breeds and populations. Relatively reliable results can be obtained even with a small number of microsatellites, as shown in this and other similar studies (e.g., Vanhala *et al*., 1998). The information from this study would be useful for genetic characterization and provide a foundation for developing sustainable genetic improvement and conservation programs of this agriculturally and commercially valuable species. We suggest that an increase in the effective number of populations for breed reproduction will assist in preventing both a decline in genetic diversity and an increase of inbreeding. The further cataloging and genetic characterization of Egyptian pigeon breeds and populations based on highly variable mitochondrial DNA markers (mtDNA control region) together with more microsatellite loci are eagerly anticipated.

Table 2.6 Observed (N_A) number of alleles, observed (H_O) and expected (H_E) heterozygosities, and *F* statistics [fixation coefficient of an individual within a subpopulation (F_{IS}), within the total population (F_{IT}) and fixation coefficient of a subpopulation within the total population (F_{ST})] across 7 studied populations.

Locus	N_A	H_O	H_E	F_{IS}	F_{ST}	F_{IT}	dHWE [c]	n [d]
CLiμD17	7	0.646	0.619	-0.012	0.154	0.144	n.s	2
CLiμT17	9	0.680	0.677	-0.008	0.160	0.154	n.s	1
CLiμD16	14	0.529	0.614	0.075	0.202	0.261	n.s	1
CLiμD32	11	0.494	0.481	-0.050	0.246	0.208	n.s	0
CLiμT13	7	0.644	0.660	-0.032	0.210	0.185	n.s	1
CLiμD01	12	0.676	0.710	0.067	0.177	0.232	n.s	2
PG1	5	0.514	0.573	0.080	0.182	0.247	n.s	1
PG2	9	0.667	0.641	-0.005	0.193	0.189	n.s	1
PG4	6	0.523	0.511	-0.072	0.313	0.264	n.s	0
PG6	3	0.429	0.426	0.004	0.177	0.180	n.s	0
PG7	6	0.480	0.514	0.018	0.222	0.236	n.s	1
Mean [a]	8.1±3.3	0.571±0.092	0.584±0.090	0.008±0.015	0.203±0.014	0.209±0.013		
Mean [b]	7.7±2.7	0.570±0.028	0.559±0.025	-0.007±0.013	0.211±0.012	0.206±0.120		

The means are given ± SD for N_A, H_O and H_E and ±*SE* for F_{IS}, F_{ST} and F_I

[a] = mean of the six studied Egyptian in addition to Japanese racing pigeon populations, Mean [b] = mean of the six studied Egyptian populations only.

[c] = deviation from Hardy-Weinberg equilibrium.

[d] = indicates the number of populations that deviated from Hardy-Weinberg equilibrium.

* = significant while n.s = not significant.

,

Tables 2.7 Observed (N_A) and mean (MN_A) number of alleles, unique alleles, observed (H_O) and expected (H_E) heterozygosities, and fixation coefficient of an individual within a subpopulation (F_{IS}) per breed.

	n	N_A±SD	MN_A±*SD*	Unique alleles	H_O±SD	H_E±SD	F_{IS}±SE	dHWE [a]	n [b]
Egyptian pigeon	110	44.5±14.1	4.1±1.3	10	0.570±0.112	0.580±0.125	0.032±0.036		
Krezly	26	57	5.2	4	0.600±0.152	0.620±0.159	0.027	n.s	1
Zagel	21	53	4.8	0	0.668±0.107	0.686±0.097	0.016	n.s	0
Safi	21	44	4	1	0.558±0.203	0.548±0.161	-0.052	n.s	0
Romani	20	58	5.3	4	0.695±0.115	0.732±0.112	0.032	*	3
Asfer Weraq	12	29	2.6	0	0.507±0.339	0.450±0.265	-0.200	n.s	0
Ablaq	10	26	2.4	1	0.390±0.239	0.423±0.169	-0.014	n.s	1
Japanese racing	23	52	4.7	2	0.581±0.169	0.630±0.099	0.073	*	4
Total average		45.6±13.2	4.1±1.2	1.7	0.571±0.102	0.584±0.116	-0.017±0.034		

[a] = deviation from Hardy-Weinberg equilibrium.
[b] = indicates the number of loci that deviated from Hardy-Weinberg equilibrium.
* = significant while n.s = not significant.

Table 2.8 Nei's genetic distance (above diagonal) and pairwise F_{ST} (below diagonal) estimates for 11 loci between seven pigeon populations.

	Krezly	Zagel	Safi	Romani	Asfer Weraq	Ablaq	Japanese racing
Krezly		0.226	0.249	0.247	0.298	0.355	0.229
Zagel	0.160		0.375	0.270	0.392	0.518	0.154
Safi	0.201	0.223		0.301	0.245	0.353	0.371
Romani	0.135	0.132	0.179		0.365	0.405	0.296
Asfer	0.228	0.264	0.240	0.204		0.321	0.389
Ablaq	0.255	0.324	0.342	0.233	0.326		0.493
Japanese racing	0.140	0.108	0.236	0.143	0.261	0.330	

Table 2.9 Contribution of each breed to aggregate genetic diversity

breed	*CW* [a]	*CB* [b]	*D1* [c]	*D2* [d]	*GD* [e]
Krezly	1.509	14.020	4.149	-0.630	-0.049
Zagel	3.799	24.510	8.169	7.305	-4.260
Safi	-0.989	17.190	2.847	-0.785	0.575
Romani	5.395	16.740	7.788	8.915	-3.123
Asfer	-4.389	15.190	-0.257	-2.741	1.439
Ablaq	-5.325	29.760	2.078	-0.397	0.298

[a] *CW* = contribution to within-population genetic diversity; [b] *CB* = contribution to between-population genetic diversity (Weitzman, 1993); [c] *D1* = contribution to aggregate genetic diversity (Ollivier and Foulley, 2005); [d] *D2* = global diversity contribution (Petit et al., 1998); [e] *GD* = global diversity contribution (Caballero and Toro, 2002).

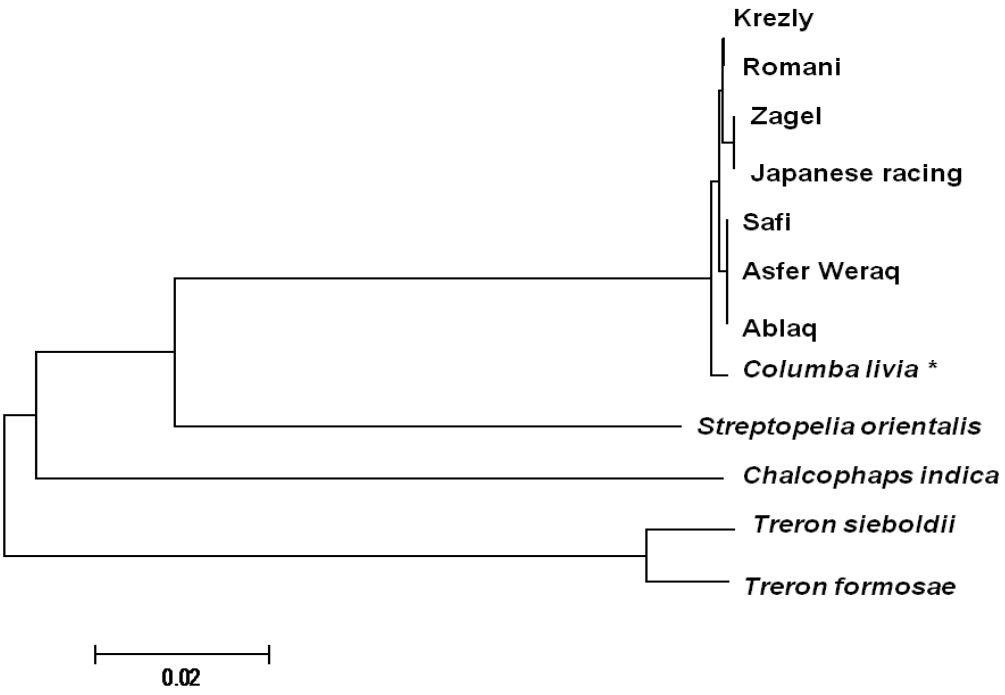

Figure 2.3 Neighbor-joining tree of mitochondrial *COI* gene sequence of six Egyptian breeds, Japanese racing pigeons, and four wild pigeon species. The sequence for *Columba livia* with asterisk was retrieved from GenBank (accession number GQ481605). The four wild pigeon species act as outgroup.

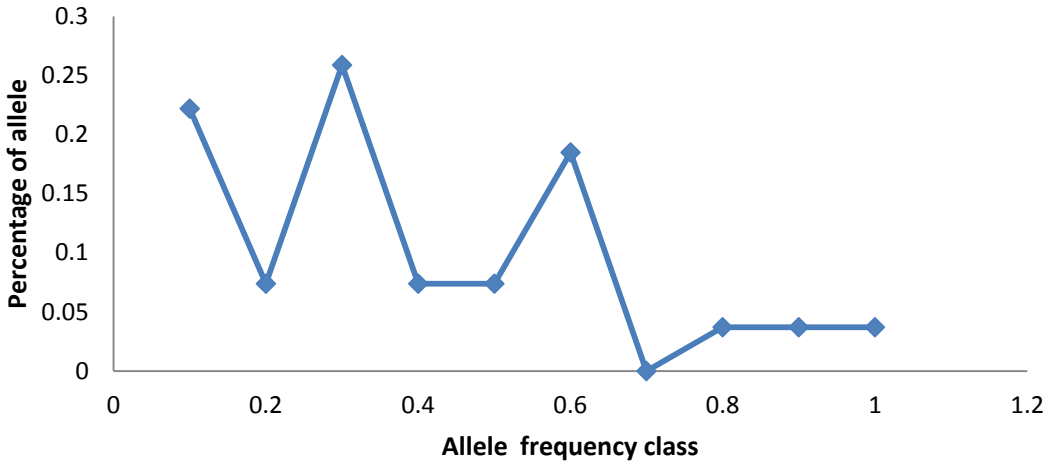

Figure 2.4a Graphical representation of percentage of alleles and their frequencies in Asfer Weraq pigeon population.

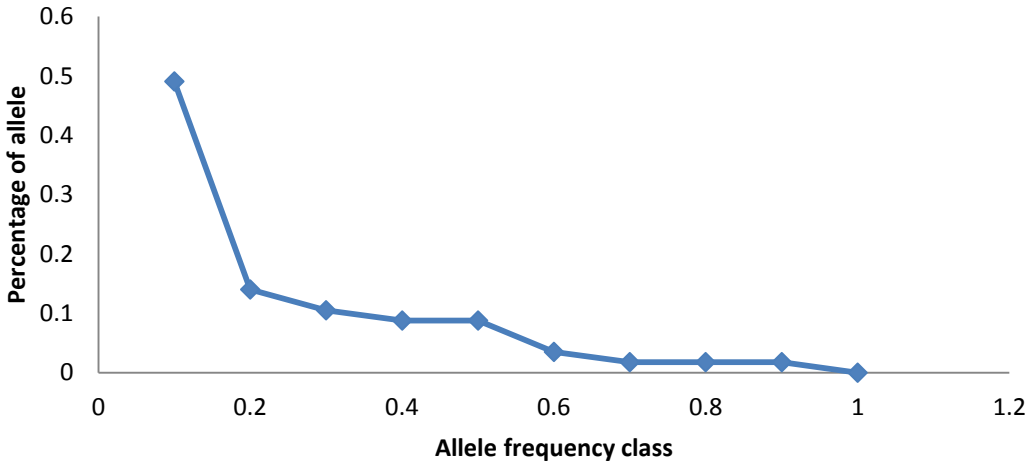

Figure 2.4b Graphical representation of percentage of alleles and their frequencies in Krezly pigeon population.

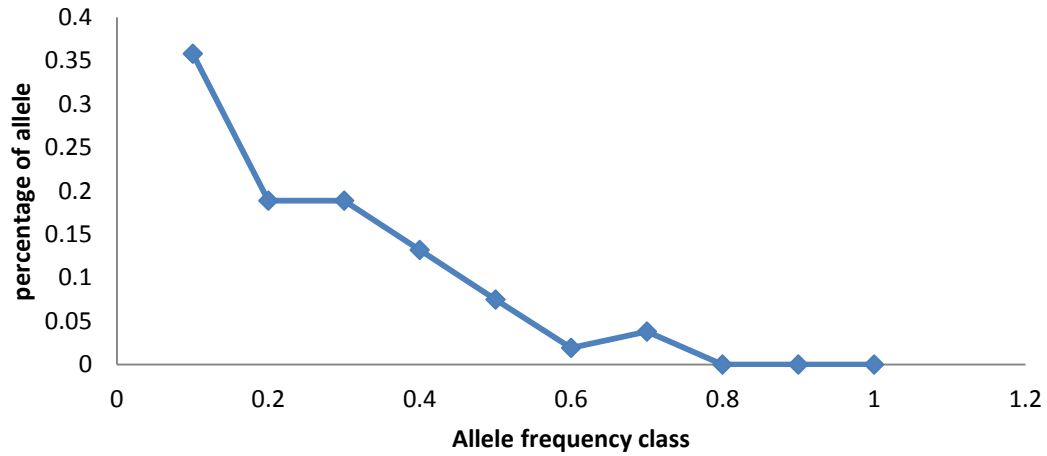

Figure 2.4c Graphical representation of percentage of alleles and their frequencies in Zagel pigeon population.

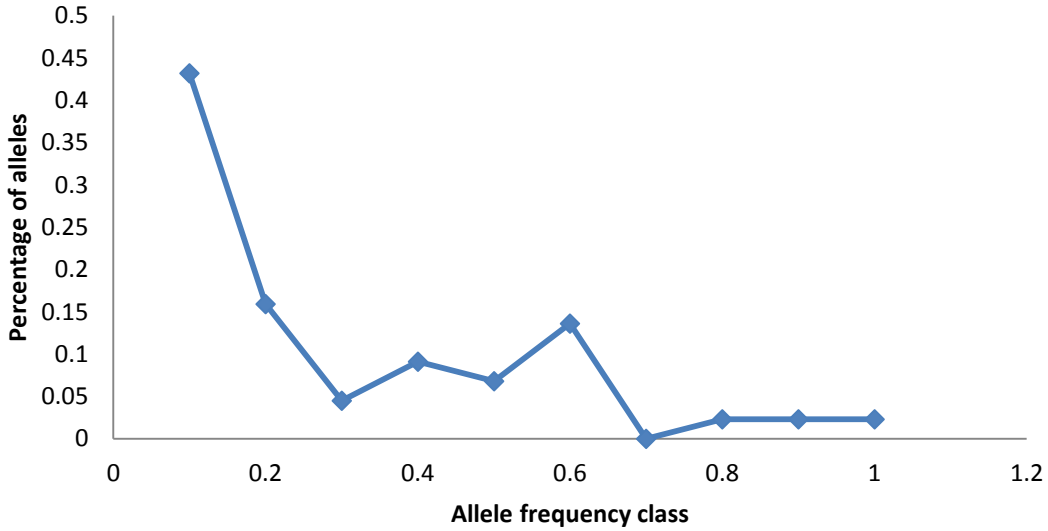

Figure 2.4d Graphical representation of percentage of alleles and their frequencies in Safi pigeon population.

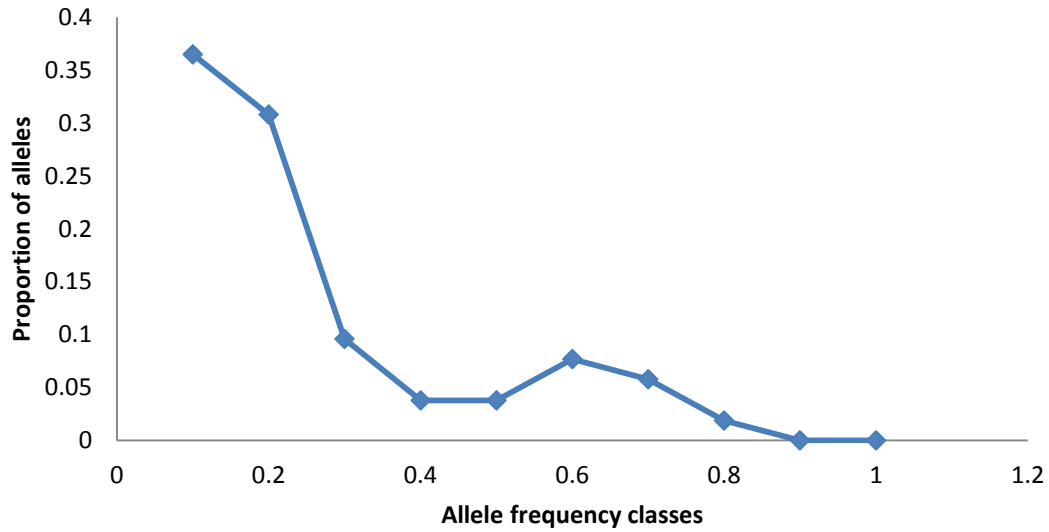

Figure 2.4e Graphical representation of percentage of alleles and their frequencies in Romani pigeon population

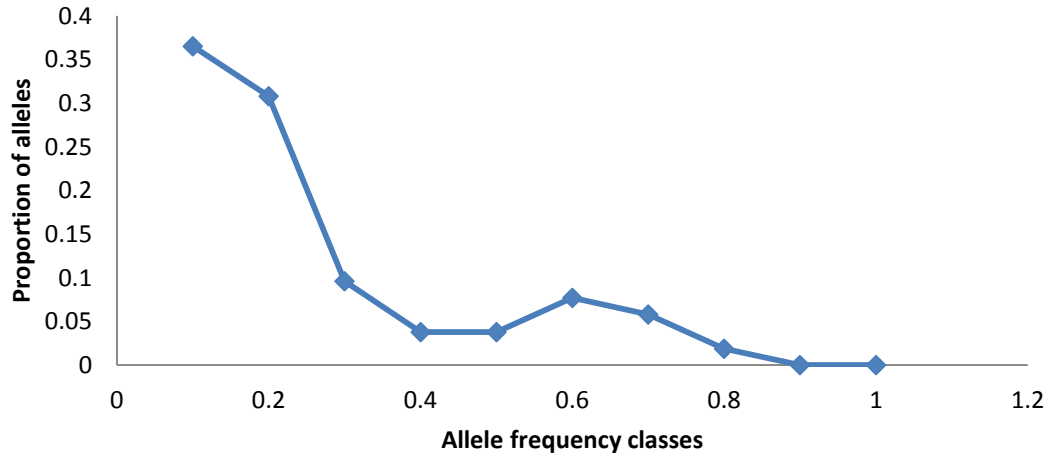

Figure 2.4f Graphical representation of percentage of alleles and their frequencies in Ablaq pigeon population.

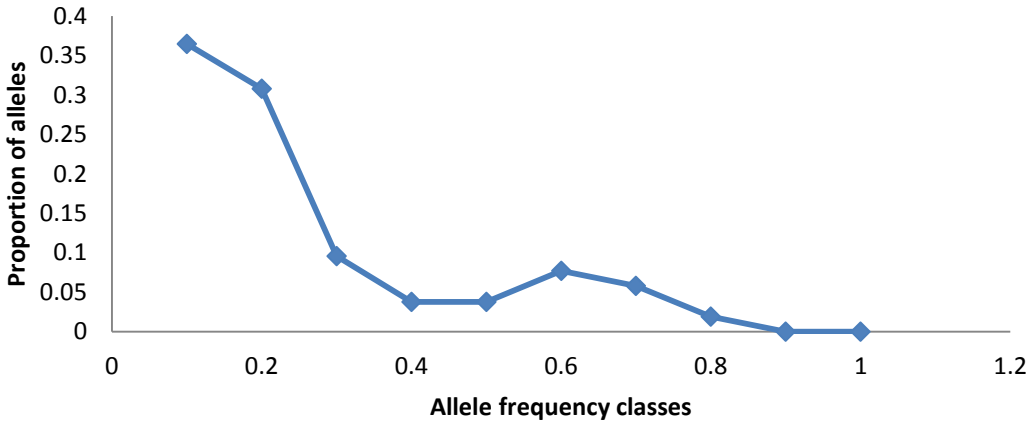

Figure 2.4g Graphical representation of proportion of alleles and their frequencies in racing pigeon population.

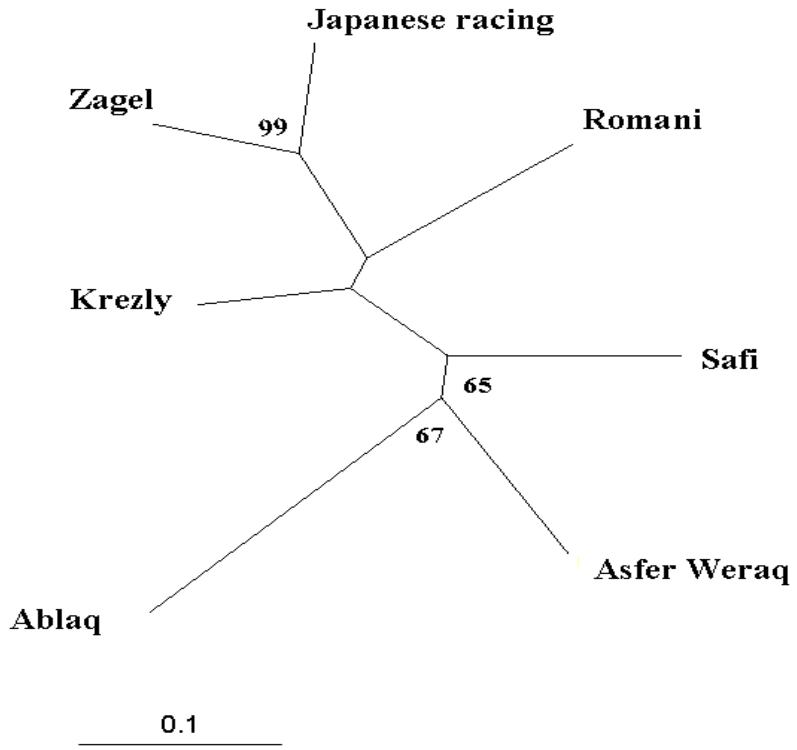

Figure 2.5 Neighbor-joining phylogenetic tree of six Egyptian breeds and Japanese racing pigeons based on Nei's genetic distance of 11 microsatellites. The consensus tree was generated with 1000 bootstraps over loci and bootstrap values lower than 50 are not shown in the diagram.

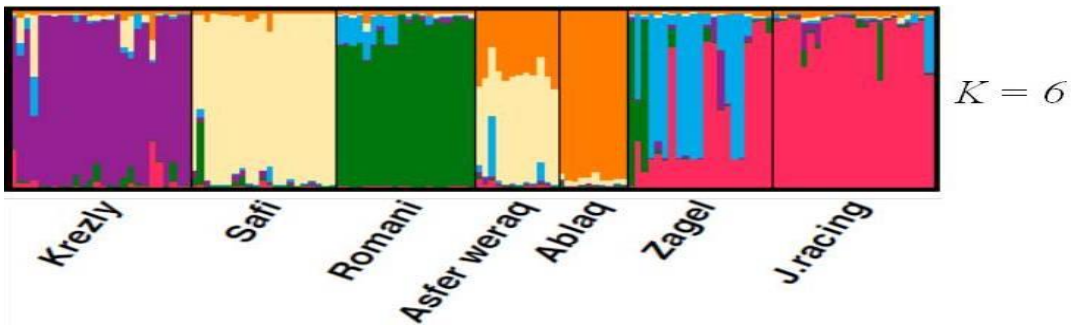

Figure 2.6 Structure clustering of the six Egyptian and Japanese racing pigeon populations obtained for $K = 6$. K is the cluster number

3. GENETIC DIVERSITY AND POLYMORPHISMS OF FUNCTIONAL GENE

3. *LDH-A* gene polymorphism in pigeon (*Columba livia*)

3.1 Introduction

Since the domestication of the wild rock pigeon (*Columba livia*) several hundred different domestic pigeon breeds have been established, most breeds are kept exclusively because of their appearance, flying capabilities or for the sport of pigeon racing (Traxler et al., 2000). The homing pigeon is a variety of domestic pigeon derived from the wild Rock Pigeon (*Columba livia domestica*) selectively bred to find its way home over extremely long distance (Levi, 1963). The wild rock pigeon has an innate homing ability meaning that it will generally return to its own nest and its own mate. This made it relatively easy to breed from the birds that repeatedly found their way home over long distances (Blechman, 2007). During pigeon racing, the competing birds are taken from their lofts to the racing start gate and then must race home. The time taken and distance are recorded and the fastest bird is declared the winner. Races are generally between 100 and 1800 km in distance (Walcott, 1996).

The L-lactate dehydrogenase (LDH, EC1.1.1.27) isozymes are encoded by three different genes: *LDH-A* (muscle), *LDH-B* (heart) and *LDH-C* (testis). The expression of these genes is developmentally regulated and tissue specific (Mannen et al., 1997). The *LDH* gene family is involved in aerobic and anaerobic metabolism; therefore it determines muscle endurance, recovery and aerobic capacity (Li, 1998). Skeletal muscle not only plays an important role in lactate production, but also in lactate removal from the circulation. Lactate is not an end product of skeletal muscle carbohydrate metabolism but instead seems to be an important fuel source for mitochondrial respiration in skeletal muscle. *LDH- A* catalyses the interconversion of pyruvate and lactate with nicotinamide adenine dinucleotide as a coenzyme (Van Hall et al., 1999; Van Hall, 2000). In human, mutation of *LDH-A* exon 6 greatly affects muscle causing painful muscle stiffness, cramps and easy fatigability after strenuous exercise (Kanno et al., 1988). In pigeon, two SNPs of *LDH-A* gene have been identified in intron 6 and showed significant difference for allele's frequencies between homing and non-homing groups. (Dybus et al., 2006).

Introns are non-coding sequences in a gene that are transcribed but spliced out of the precursor mRNA (Berget et al., 1977). Generally speaking, introns have little functional significance, although in some cases, introns polymorphism may influence the level of gene expression and consequently affect the phenotype (Wang et al., 2002; Kersting et al., 2008; Hejjas et al., 2009). Moreover, variations in introns have potential usefulness as genetic markers; it is thus possible to identify genotypes of certain interest to breeders prior to obtaining information on the performance (Dybus and Kmiec, 2002). Lessa (1992) introduced intron-targeted PCR, in which a non-coding intron was amplified using primers designed from highly conserved exon sequences; this approach is called Exon-Primed Intron-Crossing (EPIC)-PCR. This technique has been shown to yield substantial variability, mainly from intron length polymorphism, and was successfully used in several population genetic surveys (Hassan et al., 2003). In this study we reported the detection of DNA polymorphisms in pigeon's *LDH-A* gene intron 5 and analysed the genotypes/alleles frequency in homing pigeons and a collection of other breeds summarized as non-homing pigeons.

3.2 Materials and methods

Sample collection and DNA extraction

A total of 221 (123+36 for Japanese and 31+31for Egyptian) feather and blood samples of two different groups of pigeons; 123 samples of Japanese racing pigeons representing Japanese homing group and 36 samples of free living wild rock dove, for simplicity termed non-homing group were genotyped. To avoid individuals with consanguinity and over-representation of popular sires and/or dams within the pedigree, a set (n = 40) of Japanese racing pigeons (where the selected birds are expected independent of each other for five generations) was selected from the genotyped racing pigeons (n = 123). Japanese racing pigeon samples were collected from five different breeders, Chiba, Japan, whereas, Japanese wild Rock dove was collected from Rescue Center of Kyoto Municipal Zoo, Kyoto, Japan

***Replication samples*:** To validate the finding, we genotyped a total of 62 pigeon feather samples from two groups. Thirty-one samples were collected from nine Egyptian local breeds bred mainly for flying game (Ramadan et al., 2011) and racing

purposes: Zagel (*n* = 7), Safi (*n* = 4), Rehani (*n* = 3), Ablaq (*n* = 3), Otatti (*n* = 4), Morasla (*n* = 4), Keshk (*n* = 4), Messawed (*n* = 1), and Karakandy (*n* = 1) representing Egyptian homing group. Another thirty-one samples were collected from three Egyptian local breeds for ornamental and fancy purposes as Nemthawy (*n* = 24) and Egyptian exhibition Tumbler (*n* = 5) and for meat production in form of squabs as Romani (*n* = 2), where these breeds termed for simplicity non-homing group. Egyptian samples were collected from seven breeders in four provinces (Cairo, Giza, Kaliobia and Zagazig) located in the Nile river delta in the northern part of Egypt. DNA was extracted from feather and blood samples using the QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA, USA).

Genotyping and data analysis

The direct sequence method was used for detecting polymorphism in the *LDH-A* gene. The cDNA sequence of the *LDH-A* gene of *Columba livia* was already known (Mannen et al., 1997; GenBank L76362). From this sequence, PCR primers were designed in exons by using Primer3 software to amplify part of exon 5 and 6 with an intervening intron. These primers were: LDH-A56F 5′-CCTGAAGGCTCTTCATCC-AG-3′ and LDH-A56R 5′-TTGGGTGCACTCTTCTCAAA-3′.The PCR was performed on a 15 µl reaction mixes including 20 ng of genomic DNA, 2x PCR buffer, each dNTP at 400 µM, each primer at 0.3 µM and 0.5 U of *LA-Taq* DNA polymerase (TaKaRa, Shiga, Japan). After an initial incubation at 95°C for 2 min, PCR amplification was performed for 35 cycles consisting of 95°C for 30 sec, 55°C for 45 sec, 74°C for 30 sec, followed by a final extension of 74°C for 10 min. The amplified products were purified using PCR purification kit (Roche, Mannheim, Germany) and the resultant products were sequenced by using the same primers and the Big Dye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the standard protocol, and electrophoresed on an ABI PRISM 3130xl sequencer (Applied Biosystems). BLAST software (http://www.ncbi.nlm.nih.gov/) was used for sequence identification and confirmation. The MEGA 5 (Kumar et al., 2008), Finch TV 1.4.0 (http://www.geospiza.com/finchtv/) and Bioedit 7.0.5.3 (Hall, 1999) Softwares were used for sequences alignment and polymorphism detection. Genotypes and alleles

frequencies in homing and non-homing pigeons were statistically tested by Fisher's Exact Test. The pairwise linkage disequilibrium comparison (LD) of the four polymorphic sites (indel, T182C, G249A and T297G) was tested within the studied pigeon groups (Japanese homing and non-homing & Egyptian homing and non-homing). The remaining two SNPs (C442T and A443G) were excluded from the analysis because they showed non-significant difference between homing and non-homing for both Japanese and Egyptian pigeons. The pairwise comparison of linkage disequilibrium (LD) was estimated by using LD function from Genetics package while haplotype frequency was estimated by Haplo.EM function from Haplo.stats package. All analyses were conducted using the software R 2.14.0 (R Development Core Team).

3.3 Results

Six polymorphic sites have been identified in *LDH-A* gene intron 5: one indel and five SNPs (Table 3.1 and 3.2). The indel polymorphism was either 5 bp or 10 bp insertion/deletion resulting in three different allele's length; 605bp, 600bp and 595bp (Gen Bank: AB744076; AB744077 and AB744078) as shown in Figure (3.1) The 605bp allele mutation appeared with low allele and genotype frequencies in the all studied pigeon groups and was excluded from statistical analysis. High statistical significant differences in allele and genotype frequencies were observed in four (Indel, T182C, G249A, T297G) out of the six loci between Japanese homing and non-homing pigeons, whereas only one locus (Indel) showed significant differences in allele and genotype frequencies between Egyptian homing and non-homing pigeons (Tables 3.1 and 3.2). The frequency of 600bp allele was higher in homing (nearly two-fold in Japanese and more than three-fold in Egyptian) than in non-homing pigeons ($P < 0.0001$). In contrast, the *T* allele of T182C, *G* allele of G249A and *T* allele of T297G loci showed significant higher frequencies in Japanese non-homing than in homing pigeons, while it recorded an insignificant trend towards a higher frequency in Egyptian non-homing pigeons (Table 3.1).

The frequency of 600/600bp genotype was higher in homing (both Japanese and Egyptian) than in non-homing pigeons ($P < 0.0001$). The *T/T* of T182C, *G/G* of

G249A and *T/T* of T297G loci showed significant higher frequency in Japanese non-homing than in homing pigeons, while it recorded a non-significant trend towards a higher frequency in Egyptian non-homing pigeons (Table 3.2). When all the genotyped samples of Japanese racing pigeons (n = 123) were considered, the four loci (Indel, T182C, G249A, T297G) showed the same trend as the result from selected samples (data not shown). The two loci (C442T and A443G) showed no significant differences in allele or genotype frequencies in both Japanese and Egyptian pigeons.

There was a significant linkage for the all pairwise comparisons only in Japanese homing group (Table 3.3 a, b). There were eight haplotypes in the studied groups (Table 3.4). Haplotype *b* showed very low frequency and completely absent in Egyptian non-homing pigeons, while haplotype *f* and *h* appeared only with low frequency in Japanese non-homing and Egyptian homing groups respectively. Egyptian pigeons (homing and non-homing) have haplotype *g* while it was disappeared from Japanese pigeons.

3.4 Discussion

SNP and intron length polymorphisms have gained widespread use as effective markers in studying the genetic polymorphism of various animal populations (Vignal et al., 2002; Hassan et al., 2003). In this study, we investigated the DNA polymorphism within *LDH-A* gene intron 5 between homing and non-homing pigeons. During strong exercises, when oxygen is absent or in short supply and the rate of demand for energy is high; *LDH-A* enzyme converts pyruvate, the final product of glycolysis, to lactate with nicotinamide adenine dinucleotide as a coenzyme (Van Hall et al., 1999; Van Hall, 2000). Lactate is considered as an important fuel source of energy for muscular activity, and homing pigeons have been strongly selected for rapid return from distant released places in their breeding history.

In this study, the allele and genotype frequencies of six polymorphic loci in *LDH-A* gene were compared between homing and non-homing pigeons in both Japanese and Egyptian populations. The frequency of 600bp allele was higher in both Japanese and Egyptian homing than in non-homing pigeons ($P < 0.0001$). For our interest, this result is also corresponding to the previous knowledge studied by Dybus et al. (2006)

using Polish, Chinese and Taiwanese homing pigeons. The allele frequency difference of the *LDH-A* locus may reflect the history of the selection for speed and endurance of homing pigeons. The significant linkage for the all pairwise comparisons of the four polymorphic sites only in Japanese homing group can be attributed to the higher selective pressure exerted on Japanese homing than other pigeon groups. The inconsistent trend for the pairwise LD comparisons within different pigeon groups suggests that these four sites might not be completely linked. Further studies like functional genomics, protein survey and linkage analysis are necessary for confirming the relationship between homing ability and these genetic variants.

These findings have the potential not only to empower racing pigeon breeders, owners and trainers to make decisions that will maximize a pigeon's genetic ability, but also to understand genetic background of stamina for flying ability for migrating bird species.

Table 3.1 allele frequencies of Japanese and Egyptian homing and non-homing pigeons

Polymorphism	Allele	Japanese			Egyptian		
		homing (n = 40)	non-homing (n = 36)	*P value*	homing (n = 31)	non-homing (n = 31)	*P value*
Indel	*595*	0.213	0.583	1.30E-06[a]	0.435	0.823	2.58E-05
	600	0.712	0.334		0.533	0.177	
	605	0.075	0.083		0.032	0.000	
T182C	*T*	0.575	0.847	0.0003	0.855	0.887	N.S.[b]
	C	0.425	0.153		0.145	0.113	
G249A	*G*	0.775	0.972	0.0002	0.919	0.984	N.S.
	A	0.225	0.028		0.081	0.016	
T297G	*T*	0.775	0.944	0.005	0.903	0.952	N.S.
	G	0.225	0.056		0.097	0.048	
C442T	*C*	0.888	0.875	N.S.	0.952	0.984	N.S.
	T	0.112	0.125		0.048	0.016	
A443G	*A*	0.888	0.819	N.S.	0.952	0.984	N.S.
	G	0.112	0.181		0.048	0.016	

Positions of the SNPs were estimated according to 605 bp allele (Gen Bank: AB744076)
[a] 605 bp allele was excluded from statistical analysis
[b] N.S. means not significant

Table 3.2 genotype frequencies of Japanese and Egyptian homing and non-homing pigeons

Polymorphism	Genotype	Japanese			Egyptian		
		homing (n = 40)	non-homing (n =36)	*P value*	homing (n = 31)	non-homing (n = 31)	*P value*
Indel	*595/595*	0.050	0.417	2.99E-05[a]	0.194	0.710	0.0001
	595/600	0.325	0.333		0.484	0.226	
	600/600	0.475	0.111		0.290	0.064	
	600/605	0.150	0.111		0.000	0.000	
	605/605	0.000	0.028		0.032	0.000	
T182C	*T/T*	0.325	0.750	0.001	0.742	0.806	N.S.
	C/T	0.500	0.194		0.226	0.162	
	C/C	0.175	0.056		0.032	0.032	
G249A	*G/G*	0.550	0.944	7.68E-05	0.839	0.968	N.S.
	G/A	0.450	0.056		0.161	0.032	
	A/A	0.000	0.000		0.000	0.000	
T297G[b]	*T/T*	0.550	0.917	0.0001	0.839	0.936	N.S.
	G/T	0.450	0.056		0.129	0.032	
	G/G	0.000	0.027		0.032	0.032	
C442T[c]	*C/C*	0.775	0.778	N.S.	0.934	0.968	N.S.
	C/T	0.225	0.194		0.033	0.032	
	T/T	0.000	0.028		0.033	0.000	
A443G[d]	*A/A*	0.775	0.750	N.S.	0.934	0.968	N.S.
	A/G	0.225	0.139		0.033	0.032	
	G/G	0.000	0.111		0.033	0.000	

[a] 600/605 and 605/605genotypes were excluded from statistical analysis.
All the loci follow HWE except the following:
[b] T297G locus deviated from HWE in Japanese (P = 0.022) and Egyptian (P = 0.001) non-homing groups.
[c] C442T locus deviated from HWE in Egyptian homing group (P = 0.002) .
[d] A443G locus deviated from HWE in Japanese non-homing (P = 0.006) and Egyptian homing (P = 0.002) groups.

Table 3.3a *P*-values for pairwise comparison of linkage disequilibrium for Japanese homing (above diagonal) and Japanese non-homing (below diagonal) pigeons

	Indel	T182C	G249A	T297G
Indel [a]	-	2.363e-05 *	0.006 *	0.006 *
T182C	4.848e-07 *	-	2.363e-05 *	2.363e-05 *
G249A	0.048*	0.436	-	2.220e-16 *
T297G	0.849	0.462	2.789e-08 *	-

[a] indel includes *L* (600bp) and *S* (595bp) alleles while 605bp allele was excluded from analysis
***=** significant

Table 3.3b *P*-values for pairwise comparison of linkage disequilibrium for Egyptian homing (above diagonal) and Egyptian non-homing (below diagonal) pigeons

	Indel	T182C	G249A	T297G
Indel [a]	-	0.003*	0.035 *	0.020 *
T182C	1.486e-09*	-	0.347	0.280
G249A	0.031*	0.731	-	2.540e-12*
T297G	0.000*	0.530	8.695e-06*	-

[a] indel includes *L* (600bp) and *S* (595bp) alleles while 605bp allele was excluded from analysis
***=** significant

Table 3.4 haplotypes frequency for the different pigeon groups

Haplotype / Group	*a* [a]	*b*	*c*	d	e	*f*	*g*	*h*
	LCGT [b]	LTAT	SCGT	STAT	SCGG	SCAG	SCAT	STAG
Japanese homing	0.44	0.00	0.06	0.25	0.25	-	-	-
Japanese non-homing	0.17	0.00	0.14	0.03	0.63	0.03	-	-
Egyptian homing	0.15	0.00	0.30	0.08	0.45	-	0.02	0.00
Egyptian non-homing	0.11	- [c]	0.02	0.02	0.82	-	0.03	-

[a] are the haplotype names

[b] are the haplotype sequences produced from indel, T182C, G249A and T297G respectively. While, L and S are the long (600bp) and short (595bp) alleles of the indel.

[c] indicates that this haplotype not found in this pigeon group.

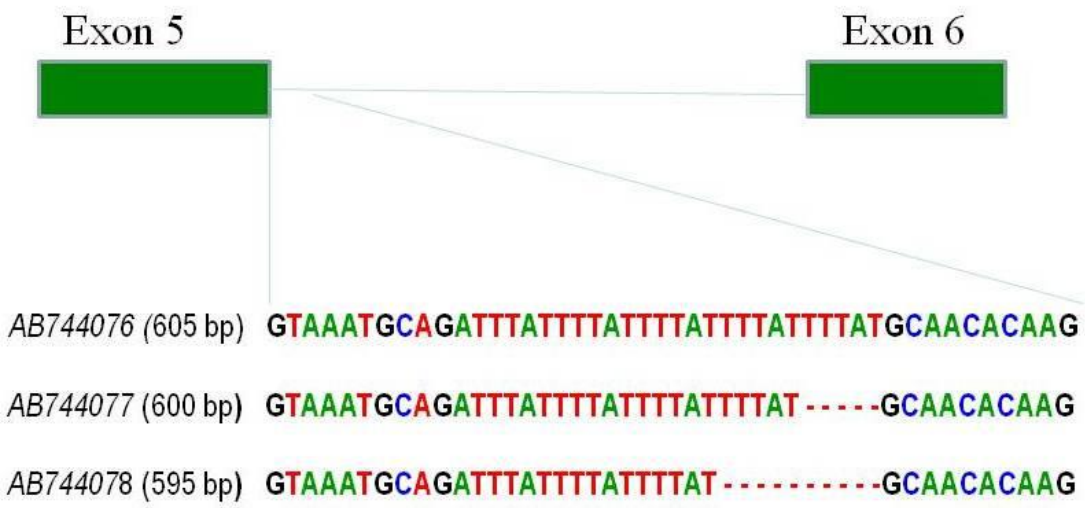

Figure 3.1 Part of the three *LDH-A* alleles resulting from indel polymorphism

4. GENERAL DISCUSSION AND FINAL REMARKS

Genetic diversity is essential in optimizing both conservation and utilisation strategies for poultry genetic resources. Genetic diversity is required for populations to evolve in response to environmental changes. In addition, low genetic diversity is linked directly to reduced population fitness via inbreeding depression (Markert et al., 2010). As resources for conservation are limited, prioritization is often necessary.

In this thesis, I evaluated the genetic diversity and polymorphisms of Egyptian chickens and pigeons in order to apply this information for conservation purpose. In chapter 2, I evaluated the genetic diversity and the breed contribution to aggregate genetic diversity as important criteria for their conservation by utilising three different prioritization methods in order to set the priorities for conservation of Egyptian chickens and pigeons based on neutral genetic markers (microsatellites). The three methods used in this study for evaluation of the breed contribution to aggregate genetic diversity were; *D1* = Ollivier and Foulley (2005) & *D2* = Petit et al (1998) and *GD* = Caballero and Toro (2002) methods. As far as I know, no study has ever conducted genetic characterization of Egyptian pigeon breeds even though there were few studies about Egyptian chicken breeds (Roushdy et al., 2008; Eltanany et al., 2011).

In the current study, the six studied Egyptian chicken populations showed a moderate level for both within-population (MN_A = 4.9; H_E = 0.595) and between-population (F_{ST} = 0.082) genetic diversity. For instance, the measured MN_A and H_E values in this study exceeds the estimated mean values produced by different studies on a wide range of chicken breeds including local European chicken breeds such as Finnish (MN_A = 3.75; H_E = 0.501), French (MN_A = 3.57; H_E = 0.508), Hungarian (MN_A = 3.64; H_E = 0.535) chicken breeds (Vanhala et al., 1998; Berthouly et al., 2008; Bodzsar et al., 2009). This might be attributed to the high selective pressure exerted over these European breeds. However, these averages were lower than those reported for native Zimbabwe chicken ecotypes (MN_A = 6.32; H_E = 0.651), native Vietnamese (MN_A = 6.72; H_E = 0.76) chicken breeds (Muchadeyi et al., 2007; Granevitze et al., 2007). The F_{ST} of Egyptian chickens showed a moderate mean value (0.082) indicating that there is genetic differentiation among the six Egyptian local

strains. The estimated F_{ST} value was slightly higher than the 0.068 previously reported across ten Egyptian chicken strains (Eltanany et al., 2011). However, it was lower than that measured between pure-bred commercial chicken lines of a study in Zimbabwe which showed 0.357 of total genetic variation owing to line differences (Muchadeyi et al., 2007).

For contribution to aggregate genetic diversity, Dandarawy chicken breed contributed the most according to Petit et al (1998) & Caballero and Toro (2005) methods (*D2*= 2.49; *GD*= -1.40) and ranked the first, while Fayoumi breed contributed negatively to aggregate genetic diversity and ranked the last according to the estimated three methods (*D1*= -1.15; *D2*= -1.89 and *GD*= 1.72). Although both of Fayoumi and Dandarawy are native pure local chicken breeds and were originated as closed populations (for Fayoumi originated in Fayoum oasis while for Dandarawy in Dandara province in Upper Egypt), Dandarawy contributed the most while Fayoumi contributed the least. This might be attributed to the narrow genetic base and the non-random mating (inbreeding) of Fayoumi population. This was supported by the lowest number of alleles (3.7), and the highest F_{IS} (0.110) values recorded by Fayoumi breed in this study. For pigeons, the six studied Egyptian populations showed moderate within-population (MN_A = 4.10; H_E = 0.584) but high between-population (F_{ST} = 0.211) genetic diversity. The Egyptian in addition to Japanese racing pigeon populations were clustered into six clusters. Krezly, Safi, Romani, Ablaq and Japanese racing populations were assigned independently into their respective clusters while Asfer Weraq appeared as mixture from Ablaq and Safi breeds and finally Zagel appeared as a mosaic clusters with Japanese racing. Zagel breed ranked the first, while Asfer Weraq ranked the last in the contribution for aggregate genetic diversity. Zagel breed is the most widely distributed breed all over the country, the breeders usually use them for racing competitions. Zagel has wide genetic base so that it contributed the most to aggregate genetic diversity. The low contribution from Asfer Weraq to aggregate genetic diversity might be attributed to the bottleneck effect and to the non-random mating. Also, small sample size used in this study might be a reason for low genetic contribution from Asfer Weraq.

By comparing the intra- and inter-population genetic diversity and the breed contribution to aggregate genetic diversity between Egyptian chickens and pigeons, we found that Egyptian chickens showed higher within-populations and lower between-populations genetic diversity than Egyptian pigeons. Also the breed contribution to aggregate genetic diversity of Egyptian chickens was lower than that of pigeon breeds. These findings might be attributed to two reasons; first, in the current study, the surveyed chicken populations include mongrel and synthetic strains, which usually show high within but low between population genetic diversity. Second, the pigeons usually are bred by fancier or breeders in smaller populations than chickens and this may cause lower genetic diversity within population.

In chapter 3, another strategy for conservation was used; conservation through utilisation strategy. The polymorphisms of functional genes might be used as genetic markers for selection of high performance pigeons. We found that the long allele (600bp) of *LDH-A* gene showed significant higher frequencies in the homing than non-homing in both Japanese and Egyptian pigeons and it might act as a genetic marker for selection of pigeons with high homing and racing ability. Improvement of local pigeon's performance will improve their competitiveness with foreign breeds. This is an encouragement to the local fancier for keeping and utilisation of these local breeds and consequently conservation through utilisation strategy.

In this thesis, we used neutral genetic marker information as a first step for prioritization of the local breeds for conservation. As a next step, we need to consider the non-genetic criteria like threat status and breed merit of Egyptian local poultry breeds in the prioritization procedure. For threat status, we need to evaluate risk of extinction and efficiency of the breed utilisation, and for breed merit we need to evaluate economic or productive, ecological and socio-cultural values of all local breeds. Another important criterion we should consider for conservation priority is molecular functional diversity. One breed can harbor genetic variation that is not found in other breeds, and it might be important to conserve this variation within the breed. The characterization of functional diversity to identify causal mutations for morphological traits by investigating the polymorphism of a candidate gene across various populations provide us with the information to prioritize conservation choices

on the basis of functional molecular diversity (Engelsma, 2012). Because of their high phenotypic variation within one species (*Columba livia*), pigeons are considered good model for studying functional gene diversity in both wild and domesticated species. In this thesis, I used one example of pigeons functional gene diversity (*LDH-A*) which might be useful for conservation and sustainable utilisation through improvement of local population's performance. Also for wildlife, this result might be useful for basic understanding of *LDH-A* genotype and flying and homing abilities in wild migrating birds.

One of the most promising ways to characterize molecular functional diversity is the use of recent biotechnologies such as next-generation sequencing and high-resolution whole genome arrays. Such technologies make it possible to deal with molecular mass information and a growing number of genetic data enable us to broadly compare genetic diversity and polymorphisms in functionally important genes in different populations. Another promising approach to estimate genetic diversity in more detail, and to improve prioritization of animals for conservation of genetic diversity is the availability of large numbers of SNP markers by using SNP chip. With SNP markers, differences in genetic diversity at specific regions of the genome could be identified, and conserving a single allele and or unique alleles at a specific frequency and maximize the overall genetic diversity is possible (Engelsma, 2012).Genotyping costs have decreased since the introduction of SNP chips, and in the future these costs will further decrease. Therefore we expect that the use of SNP markers for conservation purposes will become more popular in the future.

In conclusion, the results from this study confirm the applicability and efficiency of microsatellites as neutral genetic markers for assessing genetic variation and setting the conservation priorities for Egyptian local populations. Consideration of breed merits and threat status, in addition to genetic diversity and polymorphisms, enabled us to balance the trade-offs between conserving diversity as insurance against future uncertainties and current sustainable utilisation. More detailed information about non-genetic aspect and a conceptual framework for a maximum utility through a weighted summation of measures of neutral diversity, functional gene diversity in addition to breed merits and threat status of Egyptian poultry is eagerly anticipated.

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6. APPENDICES

Appendix A
List of primers used in this study

chapter	name	sequence	Tm(°C)	References
2.1.2	ADL268-F ADL268-R	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	60	(FAO,2004)
2.1.2	ADL278-F ADL278-R	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	60	(FAO,2004)
2.1.2	ADL112-F ADL112-R	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC	58	(FAO,2004)
2.1.2	MCW295-F MCW295-R	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	60	(FAO,2004)
2.1.2	MCW216-F MCW216-R	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	60	(FAO,2004)
2.1.2	MCW014-F MCW014-R	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	58	(FAO,2004)
2.1.2	MCW098-F MCW098-R	GGCTGCTTTGTGCTCTTCTCG CGATGGTCGTAATTCTCACGT	60	(FAO,2004)
2.1.2	LEI234-F LEI234-R	ATGCATCAGATTGGTATTCAA CGTGGCTGTGAACAAATATG	60	(FAO,2004)
2.1.2	MCW111-F MCW111-R	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	60	(FAO,2004)
2.1.2	MCW078-F MCW078-R	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	60	(FAO,2004)
2.1.2	MCW222-F MCW222-R	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	60	(FAO,2004)
2.1.2	MCW183-F MCW183-R	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	58	(FAO,2004)
2.1.2	LEI094-F LEI094-R	GATCTCACCAGTATGAGCTGC TCTCACACTGTAACACAGTGC	60	(FAO,2004)
2.1.2	MCW069-F MCW069-R	GCACTCGAGAAAACTTCCTGCG ATTGCTTCAGCAAGCATGGGAGGA	60	(FAO,2004)
2.1.2	MCW034-F MCW034-R	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG	60	(FAO,2004)
2.1.2	MCW037-F MCW037-R	ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA	64	(FAO,2004)
2.1.2	MCW067-F MCW067-R	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	60	(FAO,2004)
2.1.2	MCW206-F MCW206-R	ACATCTAGAATTGACTGTTCAC CTTGACAGTGATGCATTAAATG	60	(FAO,2004)
2.1.2	MCW081-F MCW081-R	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	60	(FAO,2004)
2.1.2	LEI166-F LEI166-R	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	60	(FAO,2004)
2.1.2	MCW330-F MCW330-R	TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC	60	(FAO,2004)
2.2.2	ClimD17-F ClimD17-R	TCTTACACACTCTCGACAAG GTTTCCACCCAAATGAGCAAG	54	(Traxler et al., 2000)

List of primers used in this study (continue)

chapter	name	sequence	Tm(°C)	References
2.2.2	ClimT17-F ClimT17-R	ATGGGTTTGGAGATGTTTTG GTTTGATGGAGTTGCTATTTTGCT	53	(Traxler et al., 2000)
2.2.2	ClimD16-F ClimD16-R	GCAGTGATAAAGTTCTGGAACA GTTTGCCTCACCGTGACATCA	54	(Traxler et al., 2000)
2.2.2	ClimD32-F ClimD32-R	GAGCCATTTCAGTGAGTGACA GTTTGCAGGAGCGTGTAGAGAAGT	57	(Traxler et al., 2000)
2.2.2	ClimT13-F ClimT13-R	TCCAGAAGACACAGGCTAGT GTTTGCAAGCCCTGGTTATCTCA	56	(Traxler et al., 2000)
2.2.2	ClimD01-F ClimD01-R	GATTTCTCAAGCTGTAGGACT GTTTGATTTGGTTGGGCCATC	54	(Traxler et al., 2000)
2.2.2	PG1-F PG1-R	ATGTGTGTTTGTGCATGAAG ATGAAAGCCTGTTAGTGGAA	53	(Lee et al., 2007)
2.2.2	PG2-F PG2-R	CCTTCCAACCCACATTATT CCAGCCTAAGTGAAACTGTC	53	(Lee et al. 2007)
2.2.2	PG4-F PG4-R	CCCATCTCCTTGCCTGATGC CACAGCAGGATGCTGCCTGC	60	(Lee et al. 2007)
2.2.2	PG6-F PG6-R	AAGCAATCAGAACAGTGCTTCG GTCCCTATGTTGCCTTCCCTC	57	(Lee et al. 2007)
2.2.2	PG7-F PG7-R	CATTGGTCAGGAGGTGGTGGG TCTGCCACTCACTCGCCCTC	60	(Lee et al. 2007)
3.1.2	LDH-A56-F LDH-A56-R	CCTGAAGGCTCTTCATCCAG TTGGGTGCACTCTTCTCAAA	55	(This study)

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8. PUBLICATIONS ASSOCIATED WITH THIS THESIS

This thesis consists of three papers that have already been published in peer-reviewed scientific journals. Although I was assisted by co-authors, I was the coordinator and main contributor to all parts of this scientific research, including laboratory works, data analysis, and manuscript writing.

The publications associated with this thesis are the following:

Chapter 2

Ramadan, S., Kayang, B.B., Inoue, E., Nirasawa, K., Hayakawa , H., Ito, S., Inoue-Murayama, M. 2012. Evaluation of genetic diversity and conservation priorities for Egyptian chickens. Open Journal of Animal Sciences. 2, 183–190.

Ramadan, S., Abe, A., Hayano, A., Yamaura, J., Onoda, T., Miyake, T., Inoue-Murayama, M. 2011. Analysis of genetic diversity of Egyptian pigeon breeds. Journal of Poultry Science. 48, 79–84.

Chapter 3

Ramadan, S., Yamaura, J., Miyake, T., Inoue-Murayama, M. 2013. DNA polymorphism within *LDH-A* gene in pigeon (*Columba livia*). Journal of Poultry Science. 50, 194–197.

9. LIST OF FIGURES

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