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Analysis of Genetic Diversity of Egyptian Pigeon Breeds

Sherif Ramadan[1,2], Hideaki Abe[1], Azusa Hayano[1], Junichi Yamaura[3], Tomoaki Onoda[4], Takeshi Miyake[4] and Miho Inoue-Murayama[1]

[1] Wildlife Research Center, Kyoto University, Kyoto 606–8203, Japan
[2] Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Egypt
[3] Japan Racing Pigeon Association, Jonan Combine, Kashiwa 277–0085, Japan
[4] Graduate School of Agriculture, Kyoto University, Kyoto 606–8502, Japan

Ancient Egyptians used pigeons not only as food in the form of squab but also as a messenger by virtue of their strong homing ability. Pigeons are bred for many purposes like meat in the form of squabs, exhibition as fancy and ornamental, flying and sports like racing competition, and finally for laboratory experiments of cognitive sciences. In this study, a total of 133 pigeon samples of six Egyptian breeds ($n=110$) and Japanese racing pigeons ($n=23$) were surveyed. One sample from each breed was sequenced for mitochondrial *COI* gene and all samples were genotyped across 11 microsatellites loci. From *COI* sequence, all the seven studied populations were found to belong to same the species (*Columba livia*). By the analysis of 11 microsatellite loci a total of 89 alleles were observed with an average of 8.1 alleles per locus. The expected heterozygosities of the six Egyptian breeds and Japanese racing pigeons were 0.580 and 0.630, respectively. F_{ST} showed a relatively high mean of 0.203 which indicated that there is a great differentiation among the seven pigeon populations. Zagel breed and Japanese racing pigeons showed the lowest values for both pairwise F_{ST} (0.108) and Nei's genetic distance (0.154). 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 important species.

Key words: Egyptian breed, genetic diversity, microsatellite, mitochondrial *COI,* pigeon

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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). 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).

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 a messenger. 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 characterize 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) characterize by heavy body weight and used mainly for meat production in 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

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Correspondence: Dr. M. Inoue-Murayama, Wildlife Research Center of Kyoto University, Kyoto 606–8203, Japan.
(E-mail: mmurayama@wrc.kyoto-u.ac.jp)

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 genetic diversity and relationships of pigeon populations in Egypt. 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.

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-TTCTCCAACCAC-AAAGACATTGGCAC and BirdR1-ACGTGGGAGATA-ATTCCAAATCCTG (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℃ for 2 min, PCR amplification was performed for 35 cycles consisting of 95℃ for 30 sec, 50℃ for 30 sec, 74℃ for 60 sec, followed by a final extension of 74℃ 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 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℃ for 15 min, PCR amplification was performed for 35 cycles consisting of 94℃ for 30 sec, 55℃ for 90 sec, 72℃ for 60 sec, followed by a final extension of 60℃ for 30 min. Subsequently, the PCR products were electrophoresed on an ABI 3130xl DNA Sequencer (Applied Biosystems) and the sizes of the fragments were estimated based on 400 HD Rox size marker using the GENEMAPPER software (Applied Biosystems).

Data Analysis

The genetic diversity of each breed was assessed by calculating the observed number of alleles (N_A) and its mean (*MNA*), observed heterozygosity (H_O) and expected heterozygosity (H_E) by using the program package ARLEQUIN version 2.000 (Schneider *et al.,* 2000) software. *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 seven studied populations were calculated using the GENEPOP version 3.4 program (Raymond and Rousset, 1995). Genetic distances among the seven populations were evaluated by Nei's genetic distance (D_A; Nei *et al.,* 1983). A phylogenetic tree was constructed based on the D_A genetic distance 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/).

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 (Fig. 1) these seven populations clustered into the same clade with *Columba livia* sequence retrieved from GenBank (accession number GQ 481605). 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 1). 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 1). 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. 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. Table 2 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).

Genetic Differentiation Among Populations: Genetic differentiation was examined by fixation indices F_{IS}, F_{ST}, F_{IT} for each locus (Table 1). 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

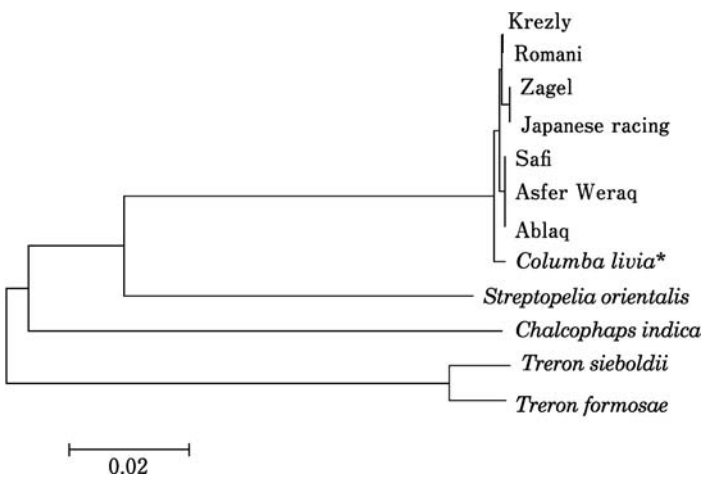

Fig. 1. **Neighbor-joining tree of mitochondrial *COI* gene sequence of six Egyptian breeds, Japanese racing pigeons, and four wild pigeon species.** The sequence for *Columba livia* with asterisk was retrieved from GenBank (accession number GQ481605). The four wild pigeon species act as outgroup.

Table 1. **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}
CLiμD17	7	0.646	0.619	−0.012	0.154	0.144
CLiμT17	9	0.680	0.677	−0.008	0.160	0.154
CLiμD16	14	0.529	0.614	0.075	0.202	0.261
CLiμD32	11	0.494	0.481	−0.050	0.246	0.208
CLiμT13	7	0.644	0.660	−0.032	0.210	0.185
CLiμD01	12	0.676	0.710	0.067	0.177	0.232
PG1	5	0.514	0.573	0.080	0.182	0.247
PG2	9	0.667	0.641	−0.005	0.193	0.189
PG4	6	0.523	0.511	−0.072	0.313	0.264
PG6	3	0.429	0.426	0.004	0.177	0.180
PG7	6	0.480	0.514	0.018	0.222	0.236
Mean*	8.1±3.3	0.571±0.092	0.584±0.090	0.008±0.015	0.203±0.014	0.209±0.013

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

Table 2. **Observed (N_A) and mean (*MNA*) 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	*MNA*±*SD*	Unique alleles	H_O±SD	H_E±SD	F_{IS}±SE
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
Zagel	21	53	4.8	0	0.668±0.107	0.686±0.097	0.016
Safi	21	44	4	1	0.558±0.203	0.548±0.161	−0.052
Romani	20	58	5.3	4	0.695±0.115	0.732±0.112	0.032
Asfer Weraq	12	29	2.6	0	0.507±0.339	0.450±0.265	−0.200
Ablaq	10	26	2.4	1	0.390±0.239	0.423±0.169	−0.014
Japanese racing	23	52	4.7	2	0.581±0.169	0.630±0.099	0.073
Total average		45.6±13.2	4.1±1.2	1.7	0.571±0.102	0.584±0.116	−0.017±0.034

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 1). 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 3, 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 (Fig. 2) 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 a messenger 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

Table 3. **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	

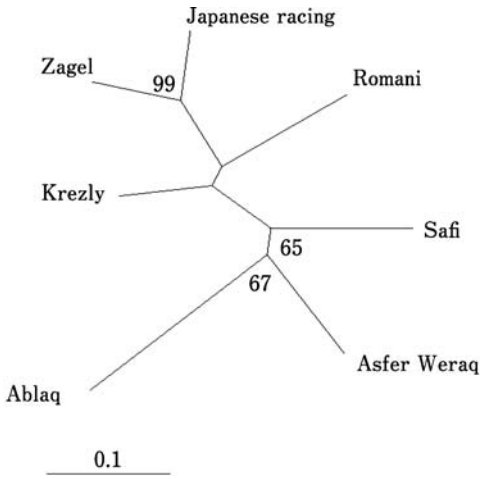

Fig. 2. **Neighbor-joining tree of six Egyptian breeds and Japanese racing pigeons by 11 microsatellites.** The consensus tree was generated with 1000 bootstraps over loci and bootstrap values lower than 50 are not shown in the diagram.

racing pigeon now used throughout the world through the continual crossbreeding of several types of pigeons (Jerolmack, 2007).

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.

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