The Journal of Animal & Plant Sciences, 25(6): 2015, Page: 1741-1745
ISSN: 1018-7081

GENETIC DIVERSITY IN EIGHT PURE BREEDS AND URBAN FORM OF DOMESTIC PIGEON (COLUMBA LIVIA VAR. DOMESTICA) BASED ON SEVEN MICROSATELLITE LOCI

A. Biała[1,2], A. Dybus[3], E. Pawlina[4] and W. S. Proskura[3,*]

[1]visionyourvision.com, Scientific and Commercial Illustrating Company, Gdańsk, Poland
[2]Department of Genetics, University of Gdańsk, Gdańsk, Poland
[3]Laboratory of Molecular Cytogenetics, Department of Ruminants Science, West Pomeranian University of Technology, Szczecin, Poland
[4]Department of Genetics, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland
*Corresponding author: witold.proskura@zut.edu.pl

ABSTRACT

The aim of this study was to examine if there was a gene flow between different breeds and forms of domestic pigeon. Seven pigeon microsatellite markers were used to examine the genetic variability and relationships among eight pure breeds and urban form of domestic pigeon. In total, 7 microsatellite loci were genotyped in 364 individuals giving a total of 122 alleles. This study is the first to report microsatellite analysis in Wroclaw Meat pigeons. The inbreeding coefficient showed a departure from random mating, which is expected for a breeding flock and the artificial selection of breeding pairs. Interestingly, the German Nun breed showed a mixed genetic heritage derived from the Fantails, Kassel Tumblers and Vienna highflier breeds. Urban pigeons appeared to form a separate population with completely different features than the rest of those analysed and should probably be considered as a crossbreed population.In conclusion all breeds and diverse morphological forms of *Columbalivia* should be considered as a result of extraordinary phenotypic plasticity of this species rather than as outcome of total isolationof subpopulations.

Keywords:*Columba livia*; microsatellite DNA; pigeon breeds; population structure.

INTRODUCTION

More than one thousand well-established pigeon breeds have been reported by breeders and scientists throughout the world (Nowicki*et al.*, 2007). Many of these breeds are exclusively maintained for their extraordinary phenotype, sporting activities or for food (Nowicki and Pawlina, 2002; Nowicki*et al.*, 2007). In the opinion of almost all breeders, very expensive breeds are kept in total isolation and under very strict reproductive methods, depending on the breed and breeder. It is also widely believed that there is no gene flow among breeds, or that this gene flow is highly suppressed to maintain the particular breed characteristics.

Various molecular markers such as mitochondrial cytochrome oxidase subunit I (COI) and numerous microsatellites were tested for their utility as species or breed discriminators in birds. The COI region has been used to distinguish between different populations for some invertebrates (Sonoda*et al.*, 2011), and also is a reliable marker for differentiation of bird species (Hebert*et al.*, 2004). Ramadan*et al.* (2011) tested the COI sequences for use as an intraspecific genetic marker among populations of some pigeon breeds, but the marker was too conserved for population studies. Therefore we decided to focus only on microsatellite DNA. A range of microsatellite markers is available for *C. livia* and many display a cross-species application (Traxler*et al.*, 2000; Lee*et al.*, 2007; Ramadan*et al.*, 2011; Stringham*et al.*, 2012). In this study, seven pigeon microsatellite markers were used to examine the genetic variability and relationships among eight purebreeds and urban form of domestic pigeon.

MATERIALS AND METHODS

Samples were collected from 364 individuals of 9 breeds including: Urban pigeon, English Fantail, German Beard, German Nun, Indian Fantail, Kassel Tumbler, King, Strasser, Wroclaw Meat and Vienna Highflier. The samples came from different localities across Poland: Chojnice, Gdańsk, Gdynia, Iława, Lublin, Malbork, Rabka, Sopot, Szczecin, Toruń, Warszawa, Wejherowo and Wrocław.

Genomic DNA was isolated from blood-filled growing feathers using a standard phenol–chloroform method (Green and Sambrook, 2012) and from Wroclaw Meat using a method described by Bello*et al.* (2001).

Primer sequences and dyes for amplification of seven microsatellite loci were used as described by Traxler*et al.* (2000). The PCR conditions were as follows: each primer 0.5 µM, 0.25 mM of each dNTP, 2 mM MgCl2, 0.75 U Taq polymerase (Invitrogen, Life Technologies),1x PCR buffer and approximately 100 ng

DNA, filled up with waterto 15 µl final volume. Microsatellites were amplified on a Biometra TProfessional thermal cycler for 5 min at 94°C, followed by 30–32 cycles of 30 sec at 95°C, 30 sec at 58°C and 45 sec at 72°C, and finally 5 min at 72°C. PCR products were separated on the ABI PRISM 3100 Genetic Analyser using GeneScan 2.0 (Perkin-Elmer Applied Biosystems), applying standard conditions described by Wenz*et al.* (1998). Alleles were sized against an internal size standard RoxGS500. A mixture of HiDiFormamide, PCR products and a size standard was denatured and loaded onto a POP4 acrylamide gel and later automatically analysed on a PeakScanner1.0 (Applied Biosystems).

For data analysis, allele number (N_A), observed heterozygosity (H_O) and the inbreeding coefficient (F_{IS}) were calculated for all breeds and at each locus using GENEPOP 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008). The genetic diversity was summarised for each population using the mean number of alleles per locus and mean observed heterozygosity. We tested for a deficiency of heterozygotes under the assumption of a Hardy-Weinberg (HW) equilibrium for all loci, using randomisation tests implemented in GENEPOP 4.0.10. The genetic population structure among 9 breeds of pigeons was investigated by various methods. Initially, allele frequency distributions in populations were compared using exact probability tests in GENEPOP 4.0.10, where the explicit assumption was that significant differences in allele frequency distributions were indicative of reproductively isolated populations. Secondly, a pair-wise estimate of the fixation index (F_{ST}) (Weir and Cockerham, 1984) was calculated, as a measure of the genetic differentiation over subpopulations (Hedrick, 1999). Finally, using the software STRUCTURE 2.3.4 (Pritchard*et al.*, 2000; Falush*et al.*, 2003; Evanno*et al.*, 2005; Falush*et al.*, 2007; Hubisz*et al.*, 2009) and STRUCTRE Harvester (Earl and Vonholdt, 2012), the optimal K (from 1 to 9 potential groups) was estimated, from the ΔK likelihood evaluation using the admixture model with 10,000 steps conducted as "burn-in", followed by 100,000 steps Markov Chains with five interactions per K.

RESULTS

In total, seven microsatellite loci were genotyped in 364 individuals from 9 breeds of pigeon giving a total of 122 alleles (Table 1). Table 2 presents parameters of genetic diversity estimated for different breeds. Although the observed heterozygosity was lowest (0.492) in English Fantail, the expected value was significantly higher (0.604), which might be the result of a strong selection against heterozygotes. The inbreeding coefficient (F_{IS}) was positive for all investigated groups (Table 2). The inbreeding coefficient showed a departure from random mating, which is expected for a breeding flock and the artificial selection of breeding pairs.

Analysing the population structure using STRUCTURE and STRUCTURE Harvester, and adopting the Bayesian clustering analysis of the whole data set provided a best-fit model with four clusters (K = 4) (Fig. 1), with no additional subdivision for the investigated data set (Fig. 1A). The estimated number of pigeon subpopulations might be explained by their history and origin. Wroclaw Meat was created as a new breed from a mixture of Racing Pigeon, Polish Lynx, King and Strasser(Nowicki*et al.*, 2007). This investigation did not include the first two breeds, but Strasser, King and Wroclaw Meat cluster together in STRUCTURE, which is expected. Interestingly, the German Nun breed showed a mixed genetic heritage derived from the Fantails, Kassel Tumblers, and Vienna highflier breeds. Urban pigeons appear to form a separate population with completely different features than the rest of those analysed and should probably be considered as a crossbreed population. The genotype of this subpopulation accidentally occurs in Kassel Tumbler, which supports the hypothesis concerning a weak reproductive barrier.

Table 1.Genetic polymorphism of seven microsatellite markers in 364 domestic pigeons of nine breeds.

Locus	N_A	H_O	H_E	F_{IS}	F_{IT}	F_{ST}
CliµD01	14	0.752	0.715	–0.052	0.104	0.148
CliµD16	22	0.586	0.701	0.164	0.230	0.079
CliµD17	10	0.378	0.491	0.230	0.320	0.117
CliµD19	21	0.301	0.659	0.542	0.648	0.231
CliµD32	21	0.564	0.663	0.149	0.327	0.210
CliµT13	8	0.643	0.612	–0.050	0.045	0.091
CliµT17	26	0.639	0.742	0.140	0.273	0.155

N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS},fixation coefficient of an individual within a subpopulation; F_{IT}, fixation coefficient of an individual within the total population; F_{ST}, fixation coefficient of a subpopulation within the total population across nine studied populations.

Table 2. Genetic diversity of seven microsatellite markers in different domestic pigeon breeds.

Breed	n	N_A**±SE**	U_A	H_O**±SE**	H_E**±SE**	F_{IS}**±SE**
Wrocław Meat	44	42.000±1.363	0.143	0.507±0.044	0.683±0.027	0.251±0.070
Vienna Highflier	59	54.714±1.554	0.000	0.518±0.052	0.623±0.029	0.168±0.073
Indian Fantail	42	40.429±0.869	0.000	0.545±0.108	0.601±0.045	0.115±0.137
English Fantail	53	51.857±0.857	0.571	0.492±0.076	0.604±0.044	0.183±0.111
King	26	24.286±1.286	0.143	0.640±0.096	0.603±0.054	0.062±0.129
Feral Pigeon	38	36.857±0.459	3.143	0.646±0.114	0.791±0.027	0.195±0.128
Strasser	41	38.714±1.491	0.000	0.548±0.052	0.681±0.031	0.178±0.092
Kassel Tumbler	28	27.143±0.261	0.000	0.576±0.087	0.617±0.024	0.081±0.123
German Nun	33	31.143±0.962	0.000	0.496±0.040	0.688±0.060	0.264±0.049

n, number of individuals; N_A, number of alleles; U_A, unique of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS},fixation coefficient of an individual within a subpopulation per breed.

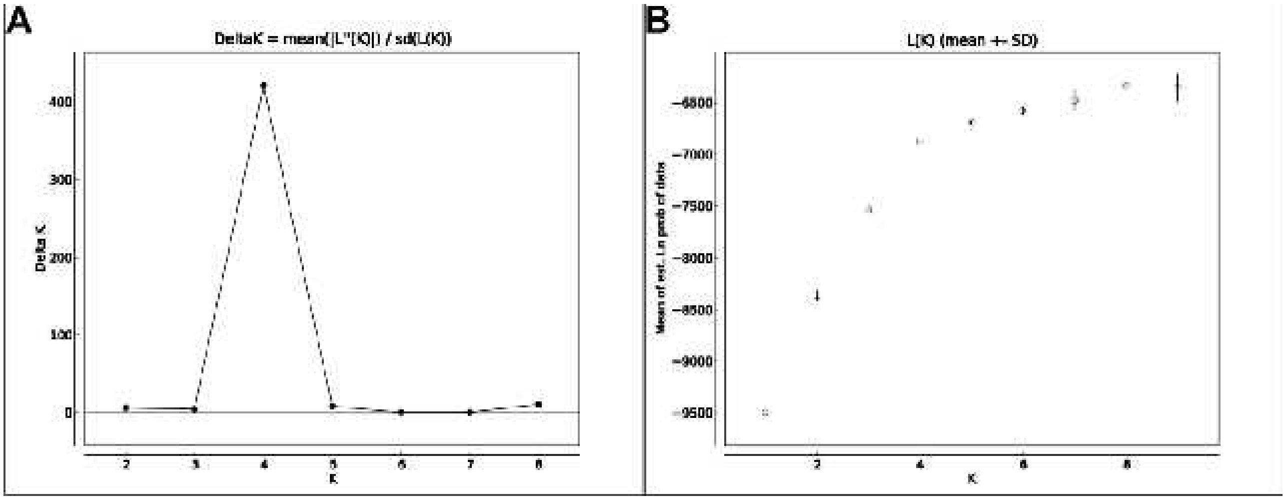

Figure 1.Determination of optimal K (A) and ΔK likelihood evaluations (B).
Illustrations created with the software STRUCTURE and STRUCTURE Harvester shows the results of Bayesian population assignment test based on seven microsatellite loci.

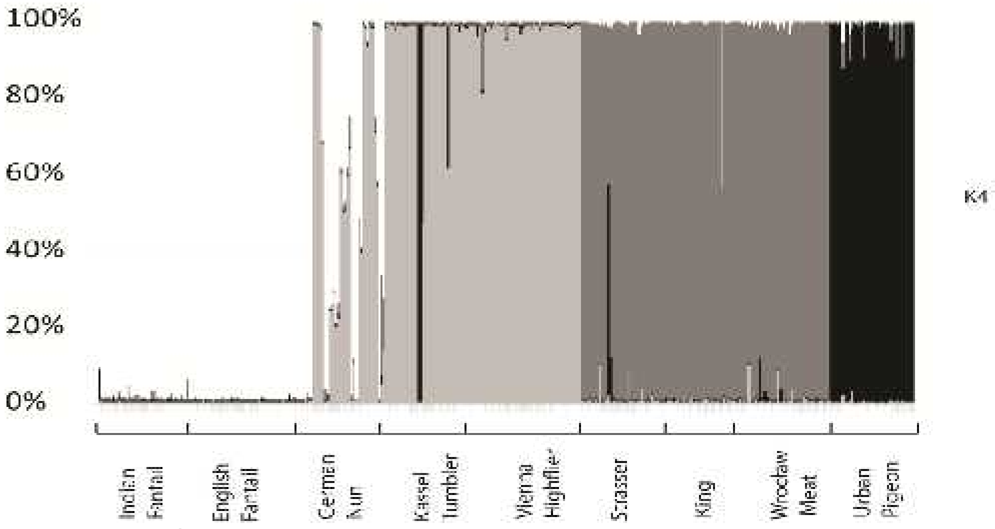

Figure 2.Data visualisation for the Bayesian population assignment test based on seven microsatellite loci.
Created with STRUCTURE (Harvester) for K = 4; each inividual represented as a single, vertical line is partitioned into K coloured segments. Pigeon breeds are in chronological order of their origin according to Nowicki*et al.* (2007).

DISCUSSION

The STRUCTURE results appear to support the hypothesis concerning total breed isolation or major group isolation. However, *C. livia* is a common species in many countries throughout the world and is very easy to breed for meat or pleasure purposes and it is therefore unlikely for a species to have such a complete breed isolation as breeders would like to imagine. Fig. 2 clearly shows that subpopulations that are separated by a long stretch of time, cluster together and the barriers among them are quite strong, but young populations such as King, Strasser and Wroclaw Meat appear to represent the same genetic pool. However, if data for one thousand well-established breeds were collated, this conclusion (Fig. 1 and 2) would probably be less clear and the barriers among subpopulations would be vague. This speculations might be supported by data from Shapiro*et al.* (2013), who found the best-fit model K = 1 for all investigated breeds. Although their investigations were based on SNPs markers, many of those markers have the same co-dominant type of inheritance as microsatellites, so in some sense could be helpful in this discussion. A direct comparison of microsatellite variation between studies is problematic, because different microsatellite loci might generate different levels of variation. However, in this study, breeds were used that are considered to be the oldest (Fantail, German Nun) and the youngest (Wroclaw Meat), with breeds created somewhere between the 15th and 20th centuries, and a comparison of our results with those from Ramadan*et al.* (2011) shows an extremely high degree of similarity. Possibly, the historical bottlenecks occurred several times in the history of the species, especially during war or periods of starvation, but in general, the breeds appear to be subject to constant genetic interbreeding. This process might depend on human activity, either consciously or unconsciously. This has led to the conclusion that time is the population determinant and might reflect many events from the history of each subpopulation (including short term isolation).

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