INVESTIGATING THE GENETIC DIVERSITY OF SQUAB PIGEON BREEDS USING MITOCHONDRIAL DNA COI REGION

AUTHORS AND AFFILIATIONS

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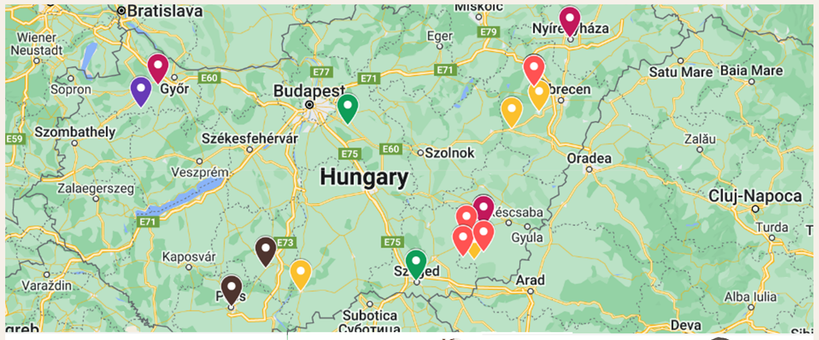
Supported by the ÚNKP-22-3-1 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.

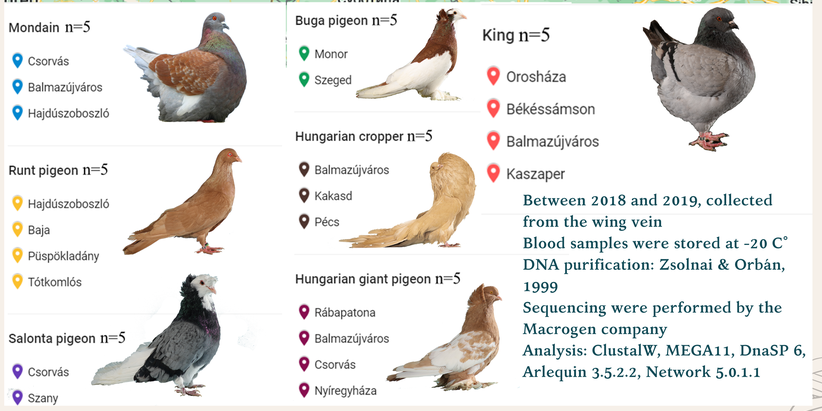
UNIVERSITY OF LIFE SCIENCES
"KING MIHAI I" FROM Timisoara
Multidisciplinary Conference on Sustainable Development
25-26 May 2023

1. Introduction

Pigeon breeding is a long-established activity, with archaeological and written evidence dating back thousands of years. Hungarian pigeon breeding has been influenced from several directions in the past, as several trade routes crossed the historical Hungary. Therefore, the ancestors of today's breeds probably originate partly from the East and partly from the West. The Turkish conquest left a large number of diverse pigeon breeds in Hungary, and pigeons from Russia also arrived in the Carpathian Basin through Polish mediation. Pigeons were introduced from the West thanks to Danube sailors. Seven species with one sample each of family Columbidae were collected via random sampling from different districts of Hungary to carry out this study.

2. Material and method





Between 2018 and 2019, collected from the wing vein
Blood samples were stored at -20 C°
DNA purification: Zsolnai & Orbán, 1999
Sequencing were performed by the Macrogen company
Analysis: ClustalW, MEGA11, DnaSP 6, Arlequin 3.5.2.2, Network 5.0.1.1

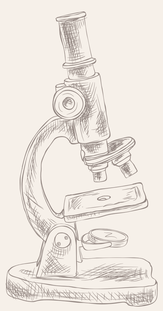
PCR reaction

PCR reaction mixture in 30 µl reaction medium:

- 7.2 µl distilled water
- 3 µl dNTP (2mM)
- 6 µl 10x DreamTaq Flexi Buffer 1.2 mM
- 6 µl MgCl 2 (25 mM)
- 0,6 µl 1 pmol/µl reverse primer 25 nmol
- 0,6 µl 1 pmol/µl forward primer 25 nmol
- 0,6 µl DreamTaq Flexi DNA Polymerase 1000 unit
- 6 µl 100 ng/µl isolated gDNA
- Used primer: Awan et al., 2013
- We used mtDNA COI region at 540 bp length

The PCR reaction conditions were as follows:

94°C 4:00 min
94°C 1:00min
56°C 1:00 min 35x
72°C 1:00 min
72°C 10:00 minutes
10°C ∞



Used NCBI IDs:
GU571830.1-31.
MG373562.1
MG271863.1-64.
KU722397.1
AY666494.1
JN793567.1
FJ808631.1
EF373367.1
KP975237.1-38.
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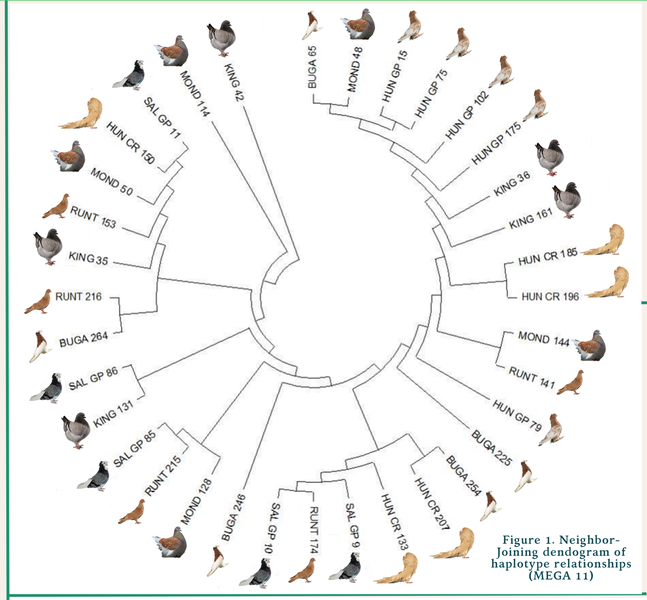


Figure 1. Neighbor-Joining dendogram of haplotype relationships (MEGA 11)

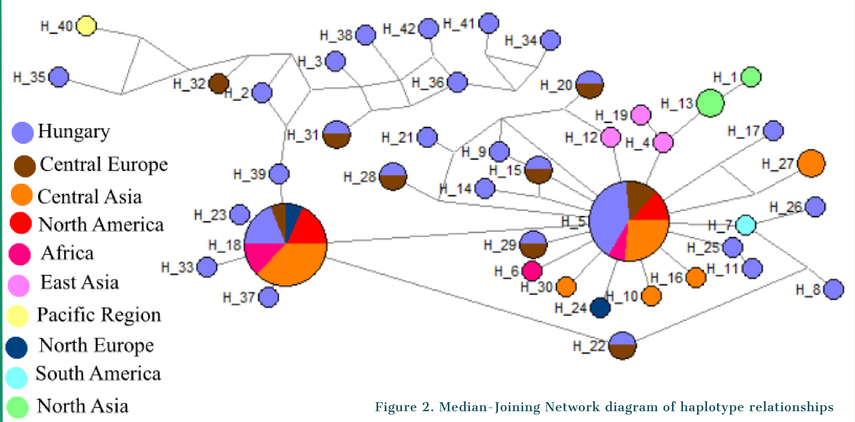


Figure 2. Median-Joining Network diagram of haplotype relationships

Table 3. The result of the AMOVA test for groupings based on different criteria

	Source of variation	df	Sum of squares	Variance components	Percentage of variation (%)
Breeds	Among populations	6	382.486	-4.5747	-5.58
	Within populations	28	2425.400	86.6214	105.58
Grouping by origin	Among populations	1	55.269	-1.6417	-2.01
	Within populations	33	2752.617	83.4126	102.01

3. Results

The haplotype diversity values were high in all cases, while the nucleotide diversity values were generally considered low. For nucleotide diversity, the International group shows higher values (π=0.3364 ± 0.0793) compared to the Hungarian group (π=0.2887 ± 0.0699) (Table 1). The Hungarian Giant Pigeon showed the lowest nucleotide diversity (0.1305 ± 0.0187) and the King showed the highest nucleotide diversity (0.3785 ± 0.1382). Table 2. shows a pairwise comparison of the Fst values of the pigeon breeds tested. Overall, the values obtained are very low, which may indicate that there are not that many differences between the populations. However, it can be clearly seen that the Hungarian Cropper is quite distinct from the Salonta pigeon (-0.14801), the Mondain (-0.12267), and the Runt pigeon (-0.12240). It can also be seen that the Salonta pigeon is distinct from the Mondain (-0.12033) and the Runt pigeon (-0.13916), which is used as an outgroup. In this comparison, it is clear to see that besides King, Mondain is also relatively distinct from the native Hungarian breeds. Table 3., shows the results of the molecular analysis of variance (AMOVA). In both clustering criteria, we obtained extremely high values within populations (breeds: 105.58; grouping by origin type: 102.01) but not between populations, which means that genetic differentiation is typical between breeds. In both cases, negative values were obtained between populations, suggesting that there is no genetic structure between the populations.

Table 1. Diversity indices of the studied groups

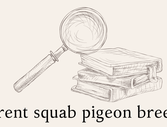
Groups \ Indices	Number of elements (n)	Number of polymorphisms	Number of haplotypes	Haplotype diversity (H_d) ± SD	Nucleotide diversity (π) ± SD
Hungarian breeds	20	504	20	1.000 ± 0.016	0.2887 ± 0.0699
Buga Pigeon	5	396	5	1.000 ± 0.126	0.3472 ± 0.1305
Hungarian Cropper	5	411	5	1.000 ± 0.126	0.3526 ± 0.1485
Hungarian Giant Pigeon	5	113	5	1.000 ± 0.126	0.1305 ± 0.0187
Salonta Pigeon	5	408	5	1.000 ± 0.126	0.3545 ± 0.1438
International breeds	15	505	15	1.000 ± 0.024	0.3364 ± 0.0793
King	5	420	5	1.000 ± 0.126	0.3785 ± 0.1382
Mondain	5	411	5	1.000 ± 0.126	0.3707 ± 0.1334
Runt Pigeon	5	354	5	1.000 ± 0.126	0.3120 ± 0.1175

Table 2. Pairwise comparison based on Fst values for all breeds

Breeds	Buga Pigeon	Hungarian Cropper	Hungarian Giant Pigeon	Salonta Pigeon	King	Mondain	Runt Pigeon
Buga Pigeon		-0.061	0.025	-0.066	-0.054	-0.065	-0.041
Hungarian Cropper			0.028	-0.148	-0.070	-0.123	-0.122
Hungarian Giant Pigeon				0.070	0.032	-0.012	0.093
Salonta Pigeon					-0.063	-0.120	-0.133
King						-0.074	-0.050
Mondain							-0.099
Runt Pigeon							

Figure 2. shows the relationships between the haplotypes of the varieties studied and the haplotypes of other geographical regions. It can be seen that the haplotypes of Hungarian varieties are mixed with those of Central European haplotypes and the Central Asian region, which is considered to be the centre of domestication.

4. Conclusion



In the study, we assessed the genetic diversity and genetic structure of Hungarian populations of different squab pigeon breeds, and the extent to which international breeds influence the genetic stock of Hungarian breeds. Although there is no significant difference between Hungarian and foreign breeds based on the source of variance, King, Mondain and Hungarian Cropper showed a greater distance from the other breeds based on the FST value per pair and Nei genetic distances. In conclusion, our mtDNA COI region can be effectively used to assess the genetic diversity and genetic structure of Hungarian pigeon breeds and other domestic pigeon populations.

References:

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2. Awan, A. R., Umar, E., Ul Haq, M. Z., Firyal, S., Molecular classification of Pakistani collared dove through DNA barcoding. 2013. MolecularBiology Reports. 40. 11, pp. 6329-6331