Szent István University

INVESTIGATION OF GENETIC DIVERSITY OF HUNGARIAN INDIGENOUS CHICKEN BREEDS BASED ON DIFFERENT MOLECULAR GENETIC MARKERS

Thesis of PhD dissertation

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Gödöllő

2012

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1. BACKGROUND AND OBJECTIVES

1. 1. Background

In animal husbandry the number of animal breeds is continually decreasing. Hence, it is assumed that the variability is declining. In 2000, over 6300 breeds of domesticated livestock had been reported to FAO. Of these, over 1300 are extinct or considered to be in danger of extinction. Many others have not been identified and may disappear before they become known. Europe records the highest percentage of extinct breeds or breeds at risk: 55% for mammalian and 69% for avian breeds (SCHERF, 2000). Reduced genetic variation lessens the possibilities to adapt breeding populations to changing requirements. Moreover, unique combinations of genes making up a specific genotype, which might constitute adaptation to local environment or other potentially beneficial traits are at risk of disappearing. Nowadays, few specialized chicken populations provide the basis for commercial breeding programs (CRAWFORD, 1990). Traditionally used breeds are excluded from competition in spite of their occasionally unique values as meat quality, disease resistance and adaptation to the local environment.

The major task of gene conservation is to preserve rare alleles, which is very difficult, since the gene pool is not expressed phenotypically. Molecular genetic markers are good tools for genetic comparisons between different chicken breeds, and help in selection of genebank stocks.

1. 2. Objectives

Aims of the study:

- to investigate the origin of 9 populations of 6 native Hungarian chicken breeds based on mitochondrial DNA D-loop information
- to characterize the genetic diversity of the Hungarian local chicken breeds and evaluate it relative to commercial and other European indigenous chicken breeds using microsatellite markers
- to confirm in molecular genetic way, that the same-coloured feathered and naked neck stocks are really distinct breeds and not just colour variants
- to analyze what extent of divergence developed in the subpopulations of the same breed since they were separated (for 30-40 years) in different Hungarian farms
- to study the genetic diversity of the Hungarian native chicken breeds based on SNPs

2. MATERIALS AND METHODS

In current study, nine populations of six Hungarian native chicken breeds were investigated: White, Yellow, Speckled Hungarian and Black, White and Speckled Transylvanian Naked Neck breeds. Stocks kept in Hódmezővásárhely and in Mosonmagyaróvár are subpopulations of the chicken populations from Gödöllő, where the main genebank of Hungarian indigenous chicken breeds is kept. The subpopulations of these Hungarian breeds (Yellow, Speckled Hungarian, and Speckled Transylvanian Naked Neck) derived from the same ancestral families but they have been kept at different locations as closed populations for 30-40 years; therefore they are now considered as distinct breeds.

2. 1. Origin of Hungarian native chicken breeds based on mitochondrial DNA

The partial D-loop segment was PCR amplified and sequenced for a total of 74 individuals, and the first 530 nucleotides of the sequences were used for the analysis. I determined the diversity measures, like the number of segregating sites (*S*), number of haplotypes (*Ht*), haplotype (*Hd*) and nucleotide (π) diversity. Median – Joining networks were drawn to illustrate the genetic diversity and relationship of Hungarian domestic chickens and to compare them with nine reference haplotypes (*LIUA1-LIUI1*) which represent well the different haplogroups of domesticated chicken. I used NCBI-BLAST for searches against chicken mtDNA records in the GenBank to assess how unique our Hungarian native chicken sequences are.

2. 2. Investigating the genetic diversity of the Hungarian indigenous chicken breeds using microsatellite markers

Thirty individual blood samples per population were taken from the wing vein; in total, 270 DNA samples were extracted. Individuals were genotyped at 29 microsatellite loci, chosen from the AVIANDIV project, a former European research project on chicken biodiversity, and DNA was amplified in seven multiplex reactions.

Utility of the markers was determined with calculating *PIC* (**P**olymorphic **I**nformation **C**ontent) values. Allele frequencies, mean number of alleles, expected and observed heterozygosity for all loci and populations were estimated to investigate the genetic diversity within stocks. Wright's fixation indices (F_{IT}, F_{ST} and F_{IS}) were calculated to define the genetic variance, the differentiation between populations. Furthermore, pair-wise F_{ST} values were estimated to calculate the REYNOLDS (1983) genetic distances and draw a phylogenetic tree for visualization.

Clustering of the Hungarian native chickens from multilocus genotypes was performed using the software STRUCTURE (PRITCHARD et al. 2000). The analysis involved an admixture

model with correlated allele frequencies. I used 20.000 iterations of burn-in followed by 50.000 iterations for each of a user defined number of clusters ranging from $2 \leq K \leq 9$. For each K value, 100 repeated runs were compared by calculating similarity coefficients. Solutions with similarity coefficients over 0.95 were considered as identical. The most likely clustering was determined using the EVANNO (2005) method, which means the calculation of delta K values based on the probability values generated by STRUCTURE.

Marker Estimated Kinship (MEK) values between and within populations were calculated according to EDING & MEUWISSEN (2001) and visualized on a contour plot and dendrogram to investigate the relationships between and within stocks. For comparison, data of nine commercial and nine European native chicken populations were taken from previous studies (GRANEVITZE et al. 2007). While the commercial set encompassed four broiler, three brown egg layer, and two white egg layer pure bred lines, the European breeds were taken from seven European countries representing a wide spectrum of diversity. Relationships between the 27 populations were visualized in a phylogenetic network.

The relative importance of each Hungarian breed was assessed by calculating the optimal contributions to a core set, given the MEK values of the nine Hungarian breeds. To assess the degree of uniqueness of Hungarian chickens relative to representatives of commercial lines and European local breeds respectively, I performed a safe set analysis using two different safe sets. The first safe set consisted of the nine commercial populations; the second safe set encompassed the European local populations in the data set. I evaluated which Hungarian populations contributed to either the set of commercial populations, or to the set of European local populations.

2. 3. Genetic diversity of the Hungarian native chicken breeds using SNPs

For searching SNPs, genes were chosen from the literature based on their polymorphism level and biological function, these are: HSP90, PIT54 and GHRL genes. Direct sequencing was used for the analysis. The panel consisted of 96 samples, including 8-8 individuals of nine Hungarian native, two white broiler and one brown egg layer populations. Primers were designed on the polymorph regions of the genes chosen, and the PCR was amplified with the correct temperature. After then, the samples were sequenced and the SNPs were identified.

In the course of analysis I determined the allele frequencies per locus, the basic diversity measures for populations, like the expected and observed heterozigosity, and inbreeding coefficient. Differentitation of populations were defined with Wright's fixation indices for the Hungarian and commercial breeds too. The REYNOLDS genetic distances were calculated and visualized as a cladogram. The allele frequencies were calculated per gene, while the basic diversity measures and other population genetics were performed for all three genes due to the small number of animals investigated and SNPs detected.

3. RESULTS

3. 1. Results of mitochondrial DNA analysis

Eleven haplotypes were identified (*HIC1-11, GQ258689-GQ258699*) from 17 polymorphic sites in the 74 sequences. All observed nucleotide changes were transitions but one C/A transversion.

Most haplotypes (n=5) and the highest haplotype diversity were found in the Speckled Transylvanian Naked Neck breed kept in Gödöllő. In contrast only one haplotype was identified in the White Transylvanian Naked Neck breed with all samples belonging to the most frequent haplotype among the Hungarian populations (*HIC1*). Haplotype *HIC8* was found in both Yellow Hungarian stocks, while haplotypes *HIC6* and *HIC7* were detected only in the Yellow Hungarian derived from Mosonmagyaróvár. Haplotype *HIC10* was identified only in one individual of the Black Transylvanian Naked Neck breed. Haplotype *HIC4* was found in all naked neck populations but the White Transylvanian Naked Neck.

Haplotypes identified in the Hungarian native chicken populations were compared to the D-loop chicken sequences annotated in the NCBI GenBank. Three sequences (*HIC3*, *HIC8* and *HIC9*) were unique to the Hungarian chicken populations. They are all closely related to reference haplotype *LIUE1,* the most commonly found chicken haplotype in Europe.

Sequence relations among haplotypes were investigated using Median-Joining Network. Hungarian domestic chicken mtDNA sequences can be assigned or related to three previously identified reference haplotypes: *LIUA1*, *LIUB1* and *LIUE1*. Reference sequence *LIUE1* is identical to our major haplotype *HIC1*. Reference sequence *LIUA1* is immediately linked (1 bp mutation) to haplotype *HIC10*, which is only found in one Black Transylvanian Naked Neck chicken. Haplotype *HIC11* presents in 9 birds is very close to reference sequence *LIUB1* (1 bp difference). *HIC10* and *HIC11* are clearly separated by several mutations from the major *HIC1* haplotype, which is surrounded in a star-like formation by 8 one based pair different other haplotypes. The two haplotypes *HIC10* and *HIC11* were blasted against NCBI GenBank and showed 100 % identity with sequences from South East Asia.

3. 2. Results of analyzing the Hungarian native chicken breeds with microsatellite markers

In total, 168 alleles (including 6 private alleles on 5 loci) were found in Hungarian breeds across all 29 microsatellite loci. The mean number of alleles per population ranged from 2.9 to 4.2. Observed frequencies of heterozygotes did not differ significantly from the frequencies expected if populations were in Hardy-Weinberg equilibrium as indicated by F_{IS} values. Wright's F-statistics for all loci and all populations showed that many heterozygote indiviuals are in the Hungarian chicken stocks, the genetic differentiation (F_{ST}) was 21%, which is high in chicken.

Pair-wise F_{ST} estimates and REYNOLDS genetic distances were lowest between the two Speckled Hungarian populations, and highest between the Yellow Hungarian from Mosonmagyaróvár and the Black Transylvanian Naked Neck. These estimates between subpopulations were lower than between non-related Hungarian populations, but in some cases two distinct Hungarian breeds were closer to each other than subpopulations of the same breed. The similarity of parallel breeds and the differentiation between the feathered and naked neck populations can be seen on the phylogenetic tree; however, the White Hungarian and White Transylvanian Naked Neck populations were close to each other.

Within breed kinship estimates of the nine Hungarian native chickens breeds were higher compared to the estimates between populations. The contour plot and the dendrogram drawn from kinship estimates also show that the most inbred stocks are the Black Transylvanian Naked Neck and White, and there is a high similarity between the feathered and naked neck white populations.

Results of STRUCTURE analysis indicated that at the lowest *K* value the two Yellow Hungarian populations split from others and remained together until the highest value (*K=9*). It suggests that the two Yellow Hungarian populations are indistinguishable. The most probable clustering is *K=5* (*N=75*) based on the EVANNO method (2005). At *K=5* the Black Transylvanian Naked Neck breed appeared as a discrete population and the parallel breeds clustered together.

Diversity of the nine Hungarian chicken populations was analysed in relation to two sets of reference consisting of nine commercial pure bred lines and nine European local chicken populations, respectively. The inbreeding coefficient at the total sample level (F_{IT}) was highest in the European set followed by commercial lines, and least in the Hungarian populations. The genetic differentiation (F_{ST}) of the European set was slightly lower than the corresponding estimate of the commercial lines. Both estimates of within (F_{IS}) and between (F_{ST}) population variation were lowest in the Hungarian breeds.

The relationships between the nine Hungarian, nine commercial and nine European native breeds are presented as a network tree derived from kinship distances of the 27 populations. The Hungarian stocks clustered away from all European native breeds except for the Polish breed, and

are closer to the commercial lines. Considering only the Hungarian populations, the feathered and naked neck stocks formed different clusters; however, the White Transylvanian Naked Neck clustered close to the feathered white stock.

Contributions of Hungarian populations to total diversity of the two different sets of reference considered as safe showed that the total diversity of both safe sets was 0.881. Since diversity (Div) is expressed as $(1–f)$, these results suggest that 88% of the genetic variance in the founder population is conserved in the commercial lines and the European indigenous populations, respectively. Now, both the total genetic diversity of safe set plus one Hungarian breed Div(S+i), and the diversity added by a single Hungarian breed not in the safe set d(i) are given. In both safe sets the diversity added by Black Transylvanian Naked Neck was lowest. There was not much difference in priority setting of Hungarian chicken breeds using either the European or the commercial safe set.

3. 3. Results of detecting SNPs

Considering that in most populations, especially in case of GHRL gene the number of valuable samples decreased, the population genetic analyses were done for all three genes together. In total, 22 SNP loci were identified, which resulted 17 variable loci considering the linkage determined between them. Notably, that increasing the samples size investigated can provide a clearer picture of the Hungarian populations.

Three private alleles were identified in the 12 populations, two only in the Hungarian stocks: one in White Hungarian, other in Yellow Hungarian from Mosonmagyaróvár.

There was not significant difference between observed and expected heterozygosity, populations were in Hardy-Weinberg equilibrium as indicated by F_{IS} values. Wright's F-statistics of the Hungarian stocks showed the same as microsatellite analysis; the genetic differentiation (F_{ST}) was 21%, while in case of commercial lines this estimate was much lower, 6%.

The cladogram drawn from REYNOLDS genetic distances indicated that the two Yellow Hungarian populations formed a cluster separate from the others. The same is in case of Black Transylvanian Naked Neck itself, and there is a close relationship between the feathered and naked neck white stocks. In general the feathered and naked neck populations were separated from each other except for the two white stocks mentioned before, and the feathered and naked neck speckled populations from Hódmezővásárhely showed higher similarity. The white broilers and the brown egg layer clustered with the feathered and naked neck speckled populations.

4. CONCLUSIONS AND SUGGESTIONS

4. 1. Conclusions on mitochondrial DNA analysis

I investigated the phylogenetic origin of Hungarian indigenous chicken breeds. Eight populations were polymorphic out of 9. The White Transylvanian Naked Neck stock was monomorphic at the mitochondrial DNA level with a single haplotype *HIC1* observed in all individuals (n=9). This is in agreement with the low variation observed at microsatellite loci in the same population and it is a likely consequence of lost of haplotypes variation through inbreeding and genetic drift.

Nucleotide diversity (π) ranged from zero to 0.00906 and was quite similar to that estimated by LIU et al. (2006) for chickens sampled in Europe, Middle East, South East and East Asia.

Considering that the Genbank database does not contain many native chicken breeds and mostly sequences from the Far Eastern and African breeds are available in the literature, we cannot exclude that haplotypes found uniquely in our Hungarian (*HIC3*, *HIC8* and *HIC9*) chicken might be present in other European and more particularly East European populations.

To address the question of the possible multiple origin of Hungarian chickens, I included reference sequences published previously by LIU et al. (2006). The majority of the sequences grouped with the *LIUE1* haplotype which may originate from the Indian subcontinent, while other sequences (*HIC10* and *HIC11*) grouped with the reference sequences *LIUA1* and *LIUB1* respectively, found in South East Asia, China and Japan.

In conclusion, my results indicate that the majority of the today mitochondrial DNA haplotypes (86%, n=64) found in indigenous Hungarian chicken belongs to a haplogroup which likely originated from the Indian subcontinent (LIU et al. 2006). It supposedly reached Hungary through the Middle East, the Mediterranean and the Black Sea (WINKLER 1921; BAKOSS 1931). A genetic influence from the East, brought into the Carpathian basin from Asia by Hungarian conquerors at the end of the ninth century, remains however possible, with the detection of two maternal lineages of South East Asian origin. Alternatively, the eastern haplotypes could have been brought earlier around the sixth century by the Avars whose burials contain many chicken remains (MATOLCSI 1975). However, haplotypes *HIC10* and *HIC11* are also found in commercial chicken lines and more evidences are therefore required. Further analysis of East European native chickens as well as of ancient remains may provide further insight on the origin and history of Hungarian chicken.

4. 2. Conclusions on microsatellite analysis

Observed frequencies of heterozygotes were very similar to the expected one, and consequently F_{IS} estimates were not significantly different from zero suggesting that populations are close to Hardy-Weinberg equilibrium state. This is in agreement with breed management reported (SZALAY 2002) where the genetic variance were maximized based on phenotypic traits and sire rotation was used between families.

Comparing the results to an earlier report of a wide range of chicken populations, the average number of alleles per population as well as the observed heterozygosity and F_{IS} estimates, were in the same range as those of 65 chicken populations (GRANEVITZE et al. 2007).

Core set contributions of the nine Hungarian breeds gave a different ranking than the estimates of heterozygosity and F_{IS}. Whereas the Speckled Hungarian from Hódmezővásárhely scored highest for *MNA* and H_E, it was the second lowest in core set contributions. This indicates that this population appears to be diverse, but shows considerable overlap (high kinship) with the other eight breeds. In contrast, the Speckled Transylvanian Naked Neck from Gödöllő scored the highest core set contribution, but achieved only moderate scores for heterozygosity and F_{IS} estimates. The high core set contribution may be attributed to the low kinship estimates with other breeds. These results indicate that taking account of the genetic overlap between breeds is an important aspect of genetic diversity assessment.

Pair-wise F_{ST} values and REYNOLDS genetic distances observed between subpopulations of the same breed were generally lower than between independent breeds indicating moderate to high genetic similarity between subpopulations. However, the observed similarity was not as much as expected based on the bald fact that subpopulations came from the same breeds. This agreed well with MEK and STRUCTURE analyses, respectively. Subpopulations showed relatively high kinship but some exceptions can be found where two distinct breeds showed higher MEK.

STRUCTURE analysis showed a clear clustering of the Hungarian local chickens and agreed well with the clustering observed in the phylogenetic network analysis based on kinship distances. Results suggest that both yellow populations are indistinguishable although they were kept separately for more (20) generations. It might be that there was some gene flow between them in the past. Other reason can be that in 1991-92 the Yellow Hungarian in Gödöllő was found again with crossbreeding the Yellow Hungarian animals deriving back from Canada and those kept in Mosonmagyaróvár.

Furthermore, clustering demonstrated that feathered and naked neck populations appeared as separated suggesting that they make up distinct clusters and are not just colour variants of the same Hungarian or Transylvanian chicken with frequent gene flow.

The only exceptions were the two white stocks. They clustered together and showed the highest between populations kinship. This may correspond to the fact that these two populations were presumably kept as one breed in the sixties - when Hungarian breeders thought these are the same - causing exchange of genetic material. Other approach, that the same commercial line was used for improving both white populations (HREBLAY 1900, SZALAY 2002). This is in agreement with the results of mtDNA analysis, and with the comparison of Hungarian populations and commercial breeds.

Individuals of the Black Transylvanian Naked Neck breed clustered with both Speckled Transylvanian Naked Neck populations at low *K* value in STRUCTURE analysis, but made up their own group at the most probable clustering, *K=5*. Similar phylogenetic relationships are displayed in network analysis. The two Speckled Transylvanian Naked Neck populations as well as the two Speckled Hungarian populations stay together up to moderate level of resolution in STRUCTURE analysis, but appeared as clearly distinguishable subpopulations at higher levels.

I evaluated genetic diversity of Hungarian chicken populations relative to commercial and European local chickens. Although these sets of breeds are not exhaustive, my main interest was to achieve a first insight into the degree of uniqueness of the Hungarian native chicken breeds. By adding data of two sets of chicken populations from earlier studies I aimed at evaluating the conservation potential (i.e. the contribution to genetic diversity) of Hungarian breeds in a wider context across European countries and commercial chicken lines.

Analysis of the F_{IT} showed that heterozygote deficiency was lowest in Hungarian local breeds and almost fully explained by between population differentiation (F_{ST}). Similar as for European breeds and commercial lines which can be classified as separate populations, the high F_{ST} estimates indicated a clear sub-structuring of Hungarian populations. This is in agreement with STRUCTURE results. In contrast, in previous studies we found no clear sub-structuring of the Zimbabwean populations along distant agro-ecological zones (MUCHADEYI et al. 2007). Compared to European chickens and commercial lines, Hungarian populations showed lowest degree of inbreeding (F_{IS}) indicating that they are well managed populations in terms of maintaining genetic variation within these flocks.

The phylogenetic network based on 27 populations' MEK distances showed a clear distinction between the three sets (Hungarian, commercial and European). Not only the clustering but the relatively short branches of Hungarian breeds indicated that Hungarian populations are different from the Europeans which have longer branches indicating a higher degree of inbreeding.

Furthermore, Hungarian breeds – especially the two yellow populations – clustered very close to the broiler lines. This agreed well with safe set analysis where Hungarian stocks contribute

slightly more to European than to commercial safe set indicating a higher similarity to the commercial lines.

In both core set and the two safe set analyses the Speckled Transylvanian Naked Neck from Gödöllő scored consistently high. However, ranking in other breeds differed from core set to safe set analysis. This was most notable in the White Hungarian population. The differences in ranking in core set and both safe sets seem to indicate that while White Hungarian adds considerable genetic diversity to the safe sets, it shares this with other Hungarian breeds. This results points to the care that must be given in taking account of the context in which genetic diversity is evaluated.

Black Transylvanian Naked Neck was found to contribute least to both safe sets (European and commercial) and core set, while it formed an own cluster at low *K* value in STRUCTURE analysis. This may be explained as follows: this population showed the highest within population MEK indicating that this population is of small effective size and has experienced more inbreeding than any other Hungarian population. This can be seen in the network tree from the relatively long branch. Thus, high inbreeding, causing a large "distance" will result in an early separation of an inbred population, when analyzed with STRUCTURE. Notable, that inbreeding level is only relatively high; in general, the state of the black naked neck population is also satisfying.

In summary, the Hungarian local chicken breeds are genetically distinct from other chicken genetic resources, and effort should be made to conserve them, and in parallel, to study their genetic features in detail.

4. 3. Conclusions on SNP analysis

The results of SNP analysis are in agreement with the results received at the microsatellite level. In two of the three genes investigated (*HSP90, GHRL*) I assumed linkage between certain loci based on the same allele frequencies and the difference of haplotypes. In case of *HSP90* it is interesting that one of the linked loci (more than 100 nucleotides long) is in intron, may play a role in regulator function.

Therefore, I performed the detection of mutations with 17 loci for the Hungarian populations, which were previously included in the microsatellite analysis. In case of Hungarian chickens, the difference between expected and observed heterozigozity was not significant, and the estimates of inbreeding coefficient (F_{IS}) were also low, which means the populations are very close to the Hardy-Weinberg Equilibrium. The average heterozigosities measured in the Hungarian populations were in the same range as written by TWITO et al. 2007, just like the values of the three commercial lines.

Fixation indices calculated from SNP loci were the same as microsatellite results, indicating big differentitation of Hungarian stocks. The inbreeding level is acceptable, the rate of heterozigotes in most Hungarian populations is high, which is represented by the low F_{IT} and F_{IS} values, indicating that the breeding program is appropriate. However, the F_{IT} and F_{IS} values of the three commercial lines did not differ much from the estimates of Hungarian chickens, the genetic differentiation is very low.

The cladogram drawn from kinship coefficients (calculated from allele frequencies of SNP loci) indicates a clear clustering of Hungarian populations. The Black Transylvanian Naked Neck separated from the others soon, and the same breeds from different regions of Hungary were close together, but still distinguishable based on 17 SNP loci. These are confirmed by statistical analyses of microsatellites, like the cluster analysis or the Marker Estimated Kinship method. Close relationship was observed between the feathered and naked neck white populations, reasons were already mentioned before. If we compare the Hungarian populations to commercial lines, especially the feathered and naked neck speckled stocks were close to them, while at microsatellite level the two Yellow Hungarian populations did the same.

In summary, the results of 17 SNP loci were quite similar to those from the 29 microsatellite markers, and it took less time. The number of genes and individuals investigated should be increased to make certain conclusions.

4. 4. Suggestions

- to investigate the origin of native chicken breeds kept in the Carpathian basin using mitochondrial DNA to make the picture much more clear where those breeds came from;
- to characterize the native chicken breeds of the Carpathian basin with molecular genetic markers to get information, which can be applied for preserving genetic diversity and avoid inbreeding;
- to test more breeding programs (considering gene conservation) using molecular genetic markers;
- to study more genes related to important traits (production, physiology) not only for investigating genetic diversity, but detecting genetic variants causing functional changes

5. NEW SCIENTIFIC RESULTS

1. I investigated firstly the maternal origin of the Hungarian indigenous chicken breeds based on mitochondrial DNA. The majority of the Hungarian sequences originates on the Indian subcontinent, while the other two haplogroups likely originate from South-East Asia, China and Japan

2. I confirmed in a molecular genetic way (SSR, SNP) that the same-cloloured feathered and naked neck populations are not just colour variants but really distinct breeds, in spite of the high relationship between the White Transylvanian Naked Neck and White Hungarian chicken

3. I established the effect of selection and/or genetic drift in parallel breeds, which came from the same ancestral family but were kept separately, as closed populations for more than 30 generations. However the similarity was high between these populations, they were distinguishable with different kind of statistical methods used in population genetics

4. Hungarian native chicken breeds showed higher similarity to commercial lines than to most European local chicken breeds investigated in this study using microsatellite markers, mtDNA and SNP loci

5. I tested the breeding program applied for the Hungarian native chicken breeds based on Wright's fixation indices (inbreeding coefficients). The current state of the populations is satisfying; maximize the genetic variance based on phenotypic traits and use sire rotation between families is a good breed management, relative to other European local chickens

6. PUBLICATIONS RELATED TO THE DISSERTATION

International scientific papers (impact factor)

N. Bodzsár, T. Révay, H. Eding, A. Hidas, S. Weigend (2009) Genetic diversity of Hungarian indigenous chicken breeds based on microsatellite markers. *Animal Genetics*, 40: 516-523.

T. Révay, **N. Bodzsár**, V. E. Mobegi, O. Hanotte, A. Hidas (2010) Origin of Hungarian indigenous chicken breeds inferred from mitochondrial DNA D-loop sequences. *Animal Genetics*, 41: 548-550.

International posters

N. Bodzsár, K. Szentes, T. Révay, Zs. Kotsis, A. Hidas: Investigation of Hungarian indigenous chicken breeds with molecular genetic markers
IV. European Poultry Genetics Symposium, Dubrovnik, 7-8 October, 2005.

N. Bodzsár, K. Szentes, T. Révay, A. Hidas: Genetic analysis of Hungarian indigenous chicken breeds with molecular genetic markers
XII. European Poultry Conference, Verona, 10-14 September, 2006.

International proceedings

N. Bodzsár, T. Révay, H. Eding, A. Hidas, S. Weigend: Genetic diversity of Hungarian indigenous chicken breeds based on microsatellite markers
V. European Poultry Genetics Symposium, Denmark, 25-28 September, 2007.

N. Bodzsár, H. Eding, S. Weigend, V. Mobegi, O. Hanotte, T. Révay, A. Hidas: The origin and genetic diversity of Hungarian indigenous chicken breeds based on molecular markers
6th Hungarian-Vietnamese International Conference, Gödöllő, 2 July, 2009.

N. Bodzsár, T. Révay, V. Mobegi, O. Hanotte, A. Hidas: Origin of Hungarian native chicken breeds based on mitochondrial DNA D-loop information
VI. European Poultry Genetics Symposium, Bedlewo-Poznan, 30 September-2 October, 2009.

Hungarian poster

Bodzsár N., Révay T., H. Eding, S. Weigend, Hidas A.: The origin and genetic diversity of Hungarian native chicken breeds based on molecular markers

Young Researchers for a Livable Earth, FVM, Budapest, Hungary, 24 November, 2008.

Hungarian proceedings

Bodzsár N., Révay T., S. Weigend, H. Eding, Hidas A.: Molecular genetic characterization of the Hungarian indigenous chicken breeds with microsatellite markers
VII. Hungarian Genetic Congress, Balatonfüred, 15-17 April, 2007.

Bodzsár N., Révay T., H. Eding, S. Weigend, O. Hanotte and Hidas A.: Hungarian indigenous chicken breeds – from a molecular point of view
Genetic Workshops in Hungary, VII. Miniconference, Szeged, 12 September, 2008.