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THE STATE OF POULTRY GENETIC RESOURCES AND GENETIC DIVERSITY OF HEN POPULATIONS

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Abstract

Poultry breeding has a very long history that dates back to 8000 BC in the case of hens. Selective breeding by humans has led to the creation of many breeds characterized by high productivity, leading to the displacement of local breeds and posing a threat to the survival of many native breeds. In 2010, the number of avian breeds classified at high risk of loss and under threat of extinction increased to 30%. Research into mammalian and avian genetic resources concentrates mainly on the application of biotechnological methods to *ex situ* conservation and on molecular analyses aimed at estimating genetic distance between and within populations. With advances in technology, a number of techniques for in-depth genome analysis and evaluation of genetic variation in different breeds were developed. The most important techniques include analysis of microsatellite sequences, mitochondrial DNA, SNP markers, or AFLP technique.

Key words: FAO, poultry, genetic resources, genetic diversity

Selection work focused on specific traits and advances in reproduction biotechnology have largely displaced local breeds of lower productivity, which posed a threat to the survival of many indigenous breeds. Concerns over erosion of genetic diversity provided the first impetus to undertake activities in the 1960s to preserve breeds threatened with extinction (Olivier, 1998). In 1995, the Member Governments of the Food and Agricultural Organisation (FAO) of the United Nations resolved to develop the Global Databank for Farm Animal Genetic Resources and the Domestic Animal Diversity Information System (DAD-IS). Information from this Global Databank was used to create a widely accessible inventory of genetic resources, known as the World Watch List for Domestic Animal Diversity (WWL-DAD) (Scherf, 2000).

Where relevant information on populations was known, breeds were classified according to risk status into four categories: extinct (1), critical (2), endangered (3) and not at risk (4). Categories 2 and 3 were further divided into: critical-maintained

and endangered-maintained, depending on whether active conservation programmes are in place for the breeds or not. This categorization was based on overall population size, number of breeding females, number of breeding males, percentage of females bred to males of the same breed, and the temporal trend in population size (Table 1) (Weigend and Romanov, 2002; FAO, 2007).

Table 1. FAO criteria for classification of farm animal populations into different risk categories

Category	Criteria		
	total population size	no. of males	no. of females
Extinct	-	-	-
Critical			≤100
		≤5	
	≤120 and decreasing		<80%*
Endangered			>100–1000
		>5–20	
	>80 and <100 and increasing		>80%*
	>1000–1200 and decreasing		<80%*
Not at risk		>20	>1000
	>1200 and increasing		

* percentage of females bred to males of the same breed.

1. State of the world's poultry genetic resources

The Global Databank for Farm Animal Genetic Resources currently contains data from 182 countries and 37 species (FAO, 2010). The total number of mammalian breeds recorded in October 2010 was 10 507 and decreased by 5 since 2006. This number for avian breed populations was 3 414 and decreased by 91 in the same period (FAO, 2010)

According to FAO data, a total of 1710 breeds (21%) are classified as being at risk (category 2 and 3) (FAO, 2010). These data show that the number of breeds of the main avian species (chicken, duck, goose, guinea fowl, Muscovy duck, ostrich, partridge, pheasant, pigeon, quail, turkey) classified into different risk categories increased from 610 breeds (20%) in 2006 (FAO, 2007) to 687 breeds (30%) in 2010 (FAO, 2010). Out of 1980 breeds of the five major poultry species (chicken, duck, goose, turkey, Muscovy duck) included in the Global Databank, 48 breeds (including 43 chicken breeds) are considered extinct. No population data are available for 786 breeds, and 522 breeds are classified as being not at risk. The other 624 breeds were divided into 4 categories: critical (201 breeds), endangered (285 breeds), critical-maintained (24 breeds) and endangered-maintained (114 breeds) (Figure 1) (FAO, 2010). Tables 2 and 3 present information, based on data from 2006 and 2010, concerning the breeds of major avian species.

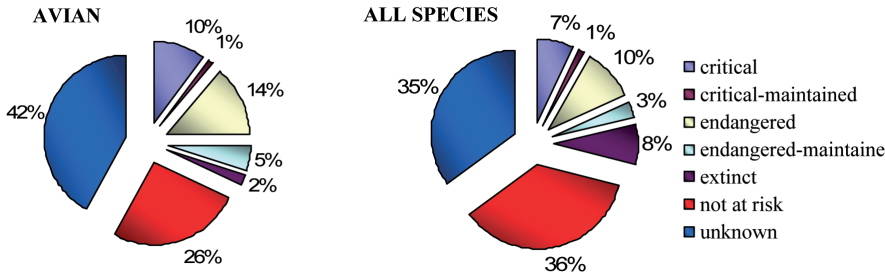

Figure 1. Proportion of the world's breeds by risk status category (FAO, 2010)

Table 2. Risk status of the world's avian breeds in January 2006 (FAO, 2007)

Risk status/Avian species	Chicken	Duck	Goose	Muscovy duck	Turkey	Total
Critical	156	32	22	1	20	231
Critical-maintained	9	5	4	1	1	20
Endangered	212	12	20	3	14	261
Endangered-maintained	42	2	10	0	0	54
Extinct	40	3	0	0	2	45
Not at risk	321	65	65	5	25	481
Unknown	493	96	60	14	41	704
TOTAL	1273	215	181	24	103	1796

Table 3. Risk status of the world's avian breeds in October 2010 (FAO, 2010)

Risk status/Avian species	Chicken	Duck	Goose	Muscovy duck	Turkey	Total
Critical	130	27	25	1	18	201
Critical-maintained	16	4	1	2	1	24
Endangered	226	18	24	3	14	285
Endangered-maintained	83	16	14	0	1	114
Extinct	43	3	0	0	2	48
Not at risk	357	72	61	5	27	522
Unknown	563	104	62	14	43	786
TOTAL	1418	244	187	25	106	1980

According to these data (Table 4) Europe and the Caucasus have the largest number of extinct poultry species. The year of extinction has been reported for only 27% of extinct mammalian and avian breeds. Eight breeds are reported to have be-

come extinct before the year 1900, 105 between 1900 and 1999, and 58 after 1999, of which two became extinct during the last two years (Figure 2) (FAO, 2010).

Table 4. Number of extinct avian breeds (FAO, 2010)

Species/Region	Africa	Asia	Europe and the Caucasus	North America	World
Chicken	0	5	37	1	43
Duck	0	0	3	0	3
Turkey	0	0	2	0	2
Total	0	5	42	1	48

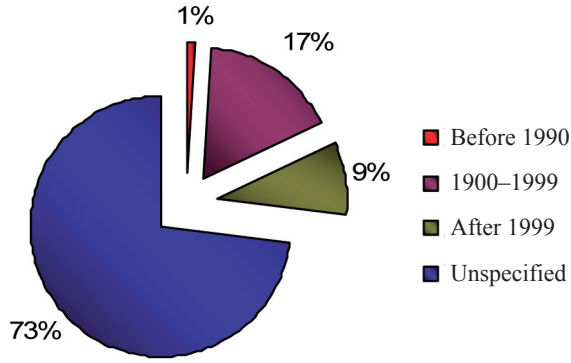

Figure 2. Proportion of the world's breeds classified as extinct (FAO, 2010)

2. Genetic diversity of animals – molecular analysis

Genetic variation is of crucial importance to all organisms living on Earth. The greater the adaptability of a population to varying environmental conditions, the larger the gene pool of this population. Targeted and long-term selection, especially within small populations, may considerably reduce the gene pool, which may result in lower adaptability. It is therefore recommended to monitor changes in the genetic structure of animals. One of the ways to determine differences between populations is to estimate the genetic distance of these populations based on class I and II genetic marker polymorphism. Polymorphic proteins and blood groups were the first markers used in genetic study of farm animals. However, microsatellite sequences are more often used to analyse genetic diversity because of the low polymorphism of class I markers. With advances in technology, several new techniques were developed for in-depth genome analysis and evaluation of genetic variation in different species. The most important of these include Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNP) markers, and mitochondrial DNA analysis.

2.1. Microsatellites

Microsatellite markers are short DNA stretches consisting of a repeat motif of usually a two- or four-nucleotide sequence, also known as simple tandem repeats (STR) (Kwiatkowska and Słomski, 1996).

Today, microsatellites are the most popular markers used for genetic analysis of farm animals. The codominant inheritance of these markers makes it possible to estimate genetic diversity within and between breeds, and to determine genetic admixtures among breeds even if they are closely related (Sunnucks, 2001). They are also commonly used to evaluate genetic relatedness between populations and animals. Nei's genetic distance (DS) is the most commonly used measure of genetic differentiation among populations (Nei, 1972). However, Cavalli-Sforza chord distance (DA) is recommended for closely related populations in which genetic drift is the main factor determining genetic differentiation (Nei et al., 1983). A dendrogram that shows genetic relationships among breeds is created to identify the mechanisms underlying genetic differentiation.

In 1995, an international group of FAO experts developed the Global Project for the Maintenance of Domestic Animal Genetic Diversity (MoDAD; FAO, 1995). MoDAD was designed to quantify the genetic diversity amongst breeds of the 14 major species of domestic animals throughout the world including four poultry species, using analysis of microsatellite DNA sequences. The first set for chickens contained 25 markers (FAO, 1997). In 2004, FAO presented a new group of markers recommended for study of genetic diversity and analysis of genetic distance. For hens, the set was extended to 30 microsatellite sequences: ADL0268, MCW0206, LEI0166, MCW0295, MCW0081, MCW0014, MCW0183, ADL0278, MCW0067, MCW0104, MCW0123, MCW0330, MCW0165, MCW0069, MCW0248, MCW0111, MCW0020, MCW0034, LEI0234, MCW0103, MCW0222, MCW0016, MCW0037, MCW0098, LEI0094, MCW0284, MCW0078, LEI0192, ADL0112 and MCW0216 (FAO, 2004).

The year 2000 saw the conclusion of an international project to determine biodiversity in 50 chicken breeds using a set of 25 microsatellite markers. The results obtained were used to create a widely accessible database for breeds included in the AVIANDIV project (http://aviandiv.tzv.fal.de/). Based on analysis of 22 microsatellite sequences, Hillel et al. (2003) evaluated the biodiversity of 52 chicken populations, including two subspecies of the Red Junglefowl (*Gallus gallus gallus* and *G. g. spadiceus*). The degree of polymorphism and the genetic distance among the analysed populations were estimated based on specific gene pools. The results obtained also supported the hypothesis that the Red Junglefowl is the main progenitor of the domesticated chicken. Research involving analysis of genetic diversity in poultry was also conducted by Tadano et al. (2007 a), who evaluated the commercial chicken lines of 5 breeds (Leghorn, Plymouth Rock, Rhode Island Red, Cornish, New Hampshire Red) based on analysis of 40 microsatellite markers. They found high genetic differences between the White Leghorn (WL) breed and the other breeds as well as high genetic similarity among WL lines despite their different breeding histories. Berthouly et al. (2008) used data from the AVIANDIV project to assess the proportion of European and Asian chicken breeds in aggregate genetic diversity of

poultry. They determined genetic variation for 14 local breeds of French chickens, 2 breeds from China and Japan, and 6 breeds conserved in Taiwan based on analysis of the sequences of 22 microsatellites, and concluded that the Coucou de Rennes and Hua-Tung breeds contributed the most to the global diversity of each subset. Based on analysis of 29 microsatellite loci, Bodzsar et al. (2009) showed that Hungarian chicken breeds contributed more to genetic diversity of the European chicken breeds than to variation of commercial lines. Research to date on native chicken breeds, using microsatellites, showed the high genetic diversity within varieties of chicken breeds from Iran, China, Turkey, Korea, Sudan and countries of south-eastern Africa (Muchadeyi et al., 2007; Shahbazi et al., 2007; Bao et al., 2008; Kaya and Yildiz, 2008), in contrast to native Japanese breeds which show low genetic similarity to other types of chickens (Tadano et al., 2007 b; 2008). Nevertheless, the Japanese breed of Toumaru chickens showed a characteristic gene pool containing a large number of unique alleles (45.7%) with a frequency above 20% (Tadano et al., 2007 b). By way of example, 70% of the unique alleles detected in Zimbabwean chickens had a frequency below 1% (Muchadeyi et al., 2007).

2.2. AFLP

Amplified Fragment Length Polymorphism, a method developed in 1995 by Vos et al., combines the advantages of two techniques: Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP). AFLPs are reproducible biallelic markers (Jones et al., 1997) that are distributed through the genome as restriction sites, and they allow estimates of genetic differentiation between breeds and related species (Ajmone-Marsan et al., 2002). This method evaluates the presence or absence of amplification of specific fragments, which corresponds to insertion, deletion between restriction sites, or single nucleotide polymorphism at a site recognized by restriction enzymes. De Marchi et al. (2006) used AFLP markers to determine genetic diversity of local chicken breeds in north-eastern Italy: Golden Comet broilers (15 birds) and four endangered breeds: Ermellinata (17), Padovana (22), Pépoi (25) and Robusta (20). Cluster analysis based on Nei's genetic distance levels indicated that the Padovana and Pépoi breeds were closely related (low Ds value) and revealed the greatest genetic distance between Padovano and Robusta breeds.

2.3. SNP markers

SNP markers are fixed DNA point mutations resulting from transition or transversion. They occur throughout the nuclear DNA genome and the mitochondrial genome, in both coding and non-coding regions. They are characterized by binary polymorphism and small size of their amplicons, as a result of which they are easier to use than STR markers (Sobrino et al., 2005).

Single nucleotide polymorphism markers are used alternatively to microsatellites in analysis of genetic diversity. Several techniques are available for detection and determination of SNP sequences. Due to the low polymorphism of these markers, it is necessary to analyse a larger number of sequences to reach the level of information obtained using a standard panel of 30 microsatellite loci. Riztyan et al. (2010) evaluated genetic variation in 4 local populations of Indonesian chickens: Black Kedu

(BK), Kedu, Kampung and Arab (a total of 192 birds), using 73 SNP markers, of which 63 were polymorphic. The analysis confirmed that Kedu and Kampung breeds are closely related and distinguished the distance between BK and Arab in relation to the other two breeds. SNP markers were also used to assess genetic relatedness between 10 populations of chickens (including Ingie) and 4 populations of their probable ancestor: the Red Junglefowl, the Gray Junglefowl, the Green Junglefowl and the Ceylon Junglefowl, by analysing 77 markers. The Red Junglefowl cluster was found to be located closely to the clusters of the analysed chicken breeds unlike the Gray Junglefowl, the Green Junglefowl and the Ceylon Junglefowl (Shimogiri et al., 2010).

2.4. Mitochondrial DNA markers

Mitochondrial DNA (mtDNA) polymorphism is widely used in phylogenetic and genetic analysis of diversity. Haploid mtDNA is characterized by maternal inheritance, a high mutation rate compared to nuclear DNA, and lack of recombination (Coble et al., 2004; Vallone et al., 2004). These characteristics make it an ideal marker for reconstruction of evolutionary relationships between and within species. MtDNA may also enable rapid detection of hybridization between species and subspecies of farm animals (Nijman et al., 2003). The polymorphism of the sequences of mtDNA D-loop hypervariable regions was analysed to identify the wild ancestors of domesticated animals, the geographic patterns of genetic diversity and the process of farm animal domestication (Bruford et al., 2003). Hoque et al. (2010) used the mtDNA D-loop sequence in addition to MHC (major histocompatibility complex) region polymorphism to evaluate genetic diversity and differentiation of Korean chicken breeds. The experiment used 693 chickens and 336 mtDNA sequences were obtained. The sequencing results showed that most of the analysed animals had 19 haplotypes, which were used to construct a phylogenetic tree showing genetic diversity and relatedness of the breeds. Polymorphism of the mtDNA D-loop region was also employed to assess genetic relationships between the populations of Asian (17 populations), south-eastern European (20 populations) and north-central European chickens (25 populations), with analysis made on a total of 640 birds. The analysis of mtDNA polymorphism of the chicken populations studied revealed that most European populations belong to a single mtDNA group, which may have originated from India (Weigend et al., 2010). Analysis of sequences of mtDNA control regions revealed that native chicken breeds show variable levels of genetic diversity, which is high in Sri Lankan chickens (Silva et al., 2009), intermediate in Zimbabwean and Indian chickens (Pirany et al., 2007; Muchadeyi et al., 2008) and low in Chinese, Japanese and several native African chickens (Niu et al., 2002; Oka et al., 2007; Muchadeyi et al., 2008).

Studies that include analysis of genetic distance and population diversity were also carried out in Poland. However, the results obtained for the native Polish breeds of chickens are inconsistent. Using Random Amplification of Polymorphic DNA (RAPD), Wężyk et al. (2000) found the largest genetic distance between the Leghorn (H22) and Greenleg Partridge (Z11) and Yellowleg Partridge breeds (Ż33), and the smallest distance between the Rhode Island Red (R11) and Sussex breeds (S66). No

close relationship between the G99 and H22 lines of Leghorn hens was noted. These data differ from the findings of Brodacki et al. (2001, 2003) who found the smallest genetic distance between G99 and H22 lines, as well as high similarity between flocks derived from Greenleg Partridge hens. The highest similarity was also observed between the Greenleg Partridge (ZK) and Ż33 lines and between the ZK and Z11 lines, and the smallest between RD2 (Rhode Island Red) and Z11 lines. These results show that further in-depth analysis is needed.

Molecular characterization may play a major role in estimating diversity and in describing and evaluating the population structure. It may also prove useful in the "genetic management" of small populations to limit the excessive effect of inbreeding. In the future, the rapid development of marker-assisted molecular techniques will probably cause microsatellites to be replaced with SNP markers. They have a large potential due to their considerable number in the genome and suitability for automated procedures and evaluations. Information on genetic diversity and distance between different populations is essential to the conservation programmes for endangered breeds. Development of a global database containing detailed information on the genetic diversity of farm animals may prove a useful tool in the conservation and restoration of endangered species in the future.

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EWELINA SEMIK, JÓZEFA KRAWCZYK

Stan zasobów genetycznych drobiu i zróżnicowanie genetyczne populacji kur

STRESZCZENIE

Hodowla drobiu ma bardzo długą historię sięgającą w przypadku kur 8000 lat p.n.e. Efektem prowadzonej przez człowieka pracy hodowlanej jest wykształcenie się wielu ras charakteryzujących się wyso-

ką wydajnością. Przyczyniło się to do wypierania lokalnych ras i stworzyło zagrożenie dla przetrwania licznych ras rodzimych. W 2010 roku liczba ras ptaków zakwalifikowanych do grupy ryzyka utraty i zagrożenia wyginięciem wzrosła do 30%. Badania naukowe dotyczace zasobów genetycznych ssaków i ptaków koncentrują się głównie na zastosowaniu metod biotechnologicznych w ochronie *ex situ* oraz na analizach molekularnych, zmierzających do szacowania dystansu genetycznego pomiędzy i w obrębie populacji. Wraz z rozwojem technologii opracowano szereg technik umożliwiających dogłębną analizę genomu oraz ocenę zmienności genetycznej poszczególnych gatunków. Do najważniejszych z nich należą analiza sekwencji mikrosatelitarnych, mitochondrialnego DNA, markerów SNP czy też technika AFLP.