

## Multilocus genetic analysis of two-breed chicken hybrids

V. I. Tyshchenko<sup>1</sup>✉

<sup>1</sup> Russian Research Institute of Farm Animal Genetics and Breeding – Branch of the L. K. Ernst Federal Research Center for Animal Husbandry, Saint Petersburg, Pushkin, Russia

✉ E-mail: valeriter@mail.ru

**Abstract. Background.** Due to the fact that now many new forms of poultry are created by crossing existing breeds and populations, a comprehensive genetic analysis of hybrid chickens as a ground for further breeding work is of particular importance. **Aim.** To study the features of the population and genetic organization of the genomes of inter-breed chicken hybrids. **Materials and methodology.** The experimental work was based on the use of an oligonucleotide probe (GTG)<sub>5</sub>, which was labeled with digoxigenin. The probe was hybridized with genomic DNA on a nylon filter, and then labeled DNA fragments were visualized using a streptavidin-alkaline phosphatase conjugate chemistry. The number and distribution of DNA fragments was highly specific for each individual. These parameters under study include similarity coefficient (BS), genetic distances between groups (D) and average heterozygosity levels (H). **Scientific novelty.** For the first time, marker DNA fragments characterizing individual groups of birds have been identified; these fragments can be used in the certification of populations. The novelty of the work also lies in the determination of the main genetic characteristics in new groups of hybrid chickens, which will be used to consolidate the desired breeding traits. **Results.** Based on the data obtained, it can be concluded that there are relatively small genetic differences between various hybrid forms, which is the result of using the same source breeds. Brahma × Sussex Light and Uzbek Game × Amrock hybrids were relatively distant from each other (D = 0.070). Sussex Light × Amrock hybrids were the most genetically diverse according to the criterion of average heterozygosity (P = 0.66).

**Keywords:** multilocus analysis, DNA probe, heterozygosity, similarity coefficient, restriction endonucleases, hybridization.

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### Introduction

Scientists from around the world have long noticed that the biodiversity of commercially used poultry breeds is reduced in comparison with the wild predecessor forms living in nature. At the same time, the process of depleting the chicken gene pool is observed in parallel with the reduction in the number of bird species and breeds. The original forms of the bird are carriers of valuable genes that can be used in breeding work to improve the populations [1, pp. 34–35; 2, pp. 64–65]. Evolutionary processes in groups of wild forms of birds have led to the formation of complexes of interacting genes that ensure adaptability to living conditions, adaptive abilities to changing environment, resistance to diseases, etc. [3; 4, p. 1]. The wide distribution of highly productive industrial breeds of chickens has led to the displacement of local breeds of gene pool birds, reducing the number of their population size. The small number of bird groups, in turn, gives rise to an increase in homozygosity and the appearance of negative effects of inbreeding depression [5, p. 976], expressed as a decrease in the productive traits, reproductive qualities and viability of the livestock [6, p. 2]. This negative

phenomenon has long attracted the attention of scientists in order to understand the molecular mechanisms of the development of this depression phenomenon. It has been established that the appearance of CH3 methyl groups in nitrogenous bases in the genome plays a certain role in inbreeding depression [7, pp. 2678–2679]. Genetic drift due to the small number of gene pool populations also contributes to the accumulation of lethal and sublethal alleles in the genome [8, p. 7]. On the other hand, intensive selection in industrial breeds of chickens, when a limited number of cocks used in stock reproduction, also reduces the diversity of individuals in the population, ultimately leading to negative consequences [9, p. 756; 10, pp. 4–6].

Modern methods of genetics make it possible to simultaneously detect many mutant loci in chicken genomes. Especially informative is the analysis on chips, when tens and hundreds of thousands of loci are screened in one experimental run (single nucleotide polymorphism – SNP). This approach is widely used in identifying the population structure, determining homozygous regions (runs of homozygosity – ROH) as one of the indicators of the level of inbreeding, determining

genetic relationships and clarifying the origin of modern chicken breeds and populations [11, pp. 1–2]. Cases of gene introgression from industrial highly productive breeds to gene pool breeds are shown [12, pp. 618–619].

Another informative approach to the analysis of farm animal populations is the use of polymorphism in microsatellite DNA repeats. In this case, individuals differ not by one nucleotide per locus, but by the number of repeating units consisting of two or three nucleotides. In some cases, it was possible to detect high heterogeneity of populations without dividing them into separate subpopulations [13]. In addition to microsatellite DNA, the population structure of chicken gene pool breeds is studied using data on the nucleotide sequence in the D-loop of mitochondrial DNA (mitochondrial haplotypes) [14, pp. 830–832]. Sufficient attention is paid to the search for associations of polymorphic regions in individual genes and the manifestation of productive traits in birds [15, pp. 4–6].

In many cases, the creation of new breeds of poultry occurs by crossing between existing breeds and populations, followed by selection for the consolidation of desirable traits [16, p. 24–26]. Thus, “traces” of the original breeds can be detected using modern methods of genetic analysis [17, pp. 82–84].

**Methods**

**1. Object of study.** Chicken hybrids from two-breed crossing were used as the object of the experiments. In particular, hybrids Brahma Light × Sussex Light, Uzbek Game × Amrock, Sussex Light × Amrock, Tsarskoye Selo × Sussex Light were studied. Blood was taken from the axillary vein, in each group there were 15 individuals. After collection, the samples were stored in a freezer until use. These hybrids were selected taking into account breeding schemes accepted in the Bioresource Collection of our research institute (table 1). The weight of hens, egg production and autosexity, which makes it possible to separate hens and cocks at an early age of their development, were chosen as ultimate breeding goals when working with these hybrids.

**2. DNA isolation and digestion with a restriction endonuclease.** Genomic DNA was extracted using water-saturated phenol and proteinase K. This approach allowed us to obtain high quality DNA samples suit-

able for further work ( $A_{260}/A_{280} \geq 1.8$ ). *Bsu*RI digestion (Thermo Scientific™) was performed according to the product manufacturer’s recommendations. 10 µg of DNA and 50 u of enzyme were used in each reaction tube. The mixture was incubated in a thermostat for three hours at a temperature of 37 °C.

**3. Electrophoresis and hybridization with a DNA probe.** The DNA fragments obtained after digestion with restriction endonuclease were separated by size using electrophoresis in 0.8 % agarose gel. At the end of the process, the DNA fragments from the gel were transferred to a nylon membrane in a vacuum apparatus; the DNA was fixed under an ultraviolet lamp, pre-hybridized in buffer containing 5xSSC – 0.1 % SDS – 5x Denhardt’s solution, hybridized in the same solution with the addition of an oligonucleotide (GTG)<sub>5</sub> labeled at the 5’-end with digoxigenin, washed out of the non-included digoxigenated label in buffer 5xSSC – 0.1 % SDS.

**4. Signal detection.** After washing, the filters were incubated in a buffer with maleic acid, then in a buffer with a blocking solution (Roche™), which contained an antibody to digoxigenin conjugated with the alkaline phosphatase enzyme. The binding sites of the latter were determined by color reaction with chromogenic substrates NBT and BCIP (Thermo Scientific™). The reaction appeared as blue colored bands on the filter, corresponding to the binding sites of the conjugate with the digoxigenin-tagged DNA fragments.

**5. Calculations of population-genetic parameters.** The genetic relationship of the compared populations was determined on the basis of genetic distance parameter (D) and the similarity coefficient (BS), which reflects the proportion of common DNA fragments within groups as well as between groups of the total number of detected fragments in all pairwise comparisons. BS values and other parameters were calculated using the Gelstats™ program, which is based on the formula:

$$BS = \frac{2 B_{xy}}{B_x + B_y}$$

where  $B_{xy}$  is the number of matching fragments in the compared electrophoretic lanes;

$B_x$  and  $B_y$  are the total number of fragments on tracks  $x$  and  $y$ , respectively. Average heterozygosity was determined by the formula of Stephens:

*Table 1*  
**Initial breeds of chickens and their main characteristics used for obtaining the analyzed hybrids**

Chicken breeds	Initial chicken breeds	Egg production, pcs/year	Egg mass, g	Live mass, kg
Sussex Light	Dorking, Cornish, Cochin, Orpington, Brahma	155–170	59–61	♂3.0-4.0 ♀2.5-3.1
Uzbek Game	Local chicken, Central Asian chicken	100–120	59–61	♂4.0-6.0 ♀2.8-3.5
Brahma Light	Malay chicken and Cochin	130–150	57–59	♂3.5-5.0 ♀3.0-4.5
Amrock	Javanese chicken and Cochin	160–180	59–60	♂3.0-4.5 ♀2.5-3.0
Tsarskoye Selo	Poltava clay chicken, New Hampshire, fawn-striped 4-lines cocks of «Broiler-6» cross	145–170	59–62	♂2.8-3.2 ♀2.2-2.5

$$H = \frac{2n}{2n-1} \times \left[ \frac{\sum_{k=1}^A S_k}{A} - 1 \right]$$

where  $S_k$  is the occurrence of the k-th fragment in the samples;

$A$  is the observed number of all fragments;

$n$  is the number of samples.

### Results

The work was carried out in two stages. On the first one, three groups of hybrids were used – Brahma Light × Sussex Light, Sussex Light × Amrock, and Uzbek Game × Amrock. At the second stage, using the second filter – Tsarskoye Selo × Sussex Light, Uzbek Game × Amrock (repeatedly) and Sussex Light × Amrock (repeatedly). The absolute values of the parameters for the same hybrids observed in two experiments differed somewhat, however, within each experiment, conclusions were identical. The highest value of the intragroup similarity coefficient was noted in the Uzbek Game × Amrock hybrid ( $BS = 0.56$ ), the lowest value was in the Sussex Light × Amrock hybrid ( $BS = 0.48$ ). It should be noted that between the two-breed hybrids, the values of the genetic distance were not large. Certain differences were observed between Brahma Light × Sussex Light and Uzbek Game × Amrock hybrids ( $D = 0.070$ ). Brahma Light × Sussex Light and Uzbek Game × Amrock hybrids were somewhat distant from each other ( $D = 0.070$ ) (table 2).

Marker DNA fragments characterizing certain groups in the first experiment were observed in Brah-

ma Light × Sussex Light hybrids (fragment No. 10, frequency of occurrence 0.87). In the remaining two groups of hybrids, the frequency was only 0.27 (table 3). Interestingly, in the Uzbek Game × Amrock hybrids, a monomorphic fragment (No. 43) was detected, which was observed in all 15 individuals (frequency of occurrence 1.00).

Intrapopulation genetic diversity can be calculated from the level of mean heterozygosity ( $H$ ) using the Gelstats™ program (table 4).

In general, the hybrid bird has a fairly high level of genetic diversity, with the maximum value in the Sussex Light × Amrock group ( $H = 0.65$ ). The lowest indicator was found in Uzbek Game × Amrock hybrids ( $H = 0.54$ ). Thus, there are no large differences between hybrids in terms of their heterozygosity. It should be noted that our earlier studies demonstrated significant differences in the level of intragroup diversity between different industrial chicken breeds. In second experiment the lowest value of genetic distance was found between Uzbek Game × Amrock and Sussex Light × Amrock ( $D = 0.020$ ) (table 5).

Fragment 54 is found in Uzbek Game × Amrock hybrids with a frequency of 0.80 (marker fragment), while it was rare in Tsarskoye Selo × Sussex Light (0.13). Fragment 69 in Tsarskoye Selo × Sussex Light hybrids occurs with a frequency of 0.87, i. e. is a marker for these hybrids. The same fragment is rare in Uzbek Game × Amrock hybrids with a frequency of 0.13 (table 6).

The highest heterozygosity was found in Sussex Light × Amrock ( $H = 0.66$ ), the lowest in hybrids Uzbek Game × Amrock ( $H = 0.59$ ) (table 7).

Table 2  
Population and genetic parameters in 3 groups of two-breed chicken hybrids: Brahma Light × Sussex Light, Uzbek Game × Amrock, Sussex Light × Amrock

Two-breed chicken hybrids	$n$	Bands per lane $X \pm m$	$P$	$BS^1$	$BS^2$	$D$
Brahma Light × Sussex Light	15	32.00 ± 2.57	$1.07 \times 10^{-9}$	0.52		
Uzbek Game × Amrock	15	35.47 ± 2.10	$1.44 \times 10^{-9}$	0.56	0.47	0.070
Brahma Light × Sussex Light	15	32.00 ± 2.57	$1.07 \times 10^{-9}$	0.52		
Sussex Light × Amrock	15	33.53 ± 2.77	$1.49 \times 10^{-11}$	0.48	0.44	0.060
Uzbek Game × Amrock	15	35.47 ± 2.10	$1.44 \times 10^{-9}$	0.56		
Sussex Light × Amrock	15	33.53 ± 2.77	$1.49 \times 10^{-11}$	0.48	0.47	0.045

Note.  $P$  is the occurrence of two individuals with an identical set of all DNA fragments;  $BS^1$  is the coefficient of similarity within groups;  $BS^2$  is the coefficient of similarity between groups;  $D$  is the genetic distance.

Table 3  
Specific DNA fragments and alleles with different frequency of occurrence in 3 groups of two-breed chicken hybrids: Brahma × Sussex Light (I), Uzbek Game × Amrock (II), Sussex Light × Amrock (III)

DNA fragment	Frequency of DNA fragment			Allele frequency $q = 1 - \sqrt{1-p}$		
	I	II	III	I	II	III
10	0.87	0.27	0.27	0.64	0.15	0.15
43	0.47	1.00	0.60	0.27	1.00	0.37
52	0.87	0.33	0.27	0.64	0.18	0.15
80	0.87	0.33	0.40	0.64	0.18	0.23

Table 4  
Heterozygosity ( $H$ ) in two-breed chicken hybrids

Two-breed chicken hybrids	$n$	Number of loci	Number of alleles	Number of polymorphic loci	$H$
Brahma Light × Sussex Light	15	20.24	3.66	0.95	0.58
Uzbek Game × Amrock	15	23.00	3.26	0.91	0.54
Sussex Light × Amrock	15	20.31	3.74	1.00	0.65

Table 5  
Population-genetic parameters in 3 groups of two-breed chicken hybrids: Tsarskoye Selo × Sussex Light, Uzbek Game × Amrock, Sussex Light × Amrock

Two-breed chicken hybrids	n	Bands per lane $X \pm m$	P	BS <sup>1</sup>	BS <sup>2</sup>	D
Tsarskoye Selo × Sussex Light	15	33.67 ± 2.19	2.73 × 10 <sup>-10</sup>	0.52	0.49	0.040
Uzbek Game × Amrock	15	33.87 ± 1.62	4.07 × 10 <sup>-10</sup>	0.53		
Tsarskoye Selo × Sussex Light	15	33.67 ± 2.19	2.73 × 10 <sup>-10</sup>	0.52	0.48	0.030
Sussex Light × Amrock	15	33.13 ± 1.54	6.67 × 10 <sup>-11</sup>	0.49		
Uzbek Game × Amrock	15	33.87 ± 1.62	4.07 × 10 <sup>-10</sup>	0.53	0.49	0.020
Sussex Light × Amrock	15	33.13 ± 1.54	6.67 × 10 <sup>-11</sup>	0.49		

Table 6  
Specific DNA fragments and alleles with different frequency of occurrence in 3 groups of two-breed chicken hybrids: Tsarskoye Selo × Sussex Light (I), Uzbek Game × Amrock (II), Sussex Light × Amrock (III)

DNA fragment	Frequency of DNA fragment			Allele frequency $q = 1 - \sqrt{1-p}$		
	I	II	III	I	II	III
13	0.93	0.67	0.53	0.16	0.43	0.31
16	0.67	1.00	0.73	0.43	1.00	0.48
54	0.13	0.80	0.73	0.07	0.55	0.48
69	0.87	0.13	0.33	0.64	0.07	0.18

Table 7  
Heterozygosity (H) in two-breed chicken hybrids

Two-breed chicken hybrids	n	Number of loci	Number of alleles	Number of polymorphic loci	H
Tsarskoye Selo × Sussex Light	15	19.98	3.46	1.00	0.62
Uzbek Game × Amrock	15	21.35	3.51	0.91	0.59
Sussex Light × Amrock	15	19.95	3.71	1.00	0.66

### Discussion and Conclusion

Carrying out two experiments on different filters showed that the absolute values of the population genetic parameters may differ slightly, however, the general conclusions on the relationships in the groups are close. When taking into account all DNA fragments on the filters, individual subjective errors may occur, which leads to slight differences in the calculations. However, taking into account the large number of detected DNA fragments, individual inaccuracies are leveled. Based on the results of the analysis of the first filter, it was found that the intrapopulation similarity according to the similarity coefficient criterion for Sussex Light × Amrock hybrids was 0.48, the second filter in the same group showed a close value of 0.49. According to both experiments, the lowest genetic diversity within the groups was found in Uzbek Game × Amrock hybrids, which can be explained by the origin of Uzbek Game chickens, which were bred on the basis of local populations and historically never were not crossed with other breeds, ensuring the relative homogeneity of this population (table 1). When creating the Sussex Light and Amrock breeds, Cochin chickens were used, therefore, in both experiments, the genetic distances between the groups of Uzbek Game × Amrock and Sussex Light × Amrock hybrids was the smallest. Surely, genetic relationships in populations are determined not only by the breed, but also by the history of the creation and breeding of a particular population. In many cases, gene pool

breeds are represented by small groups of birds with a specific genetic structure, which can be identified using multilocus analysis. More detailed characteristics of chicken populations can be obtained using chip technology with simultaneous analysis of multiple loci, which is envisaged by plans for further studies using these populations. In general, the data obtained allow us to formulate the following conclusions:

1. It was found that Sussex Light × Amrock hybrids were characterized by the highest diversity within the group with similarity coefficients of 0.48–0.49 (first and second experiment, respectively).
2. When considering the genetic differences between two-breed hybrids, it was found that according to this criterion, Brahma Light × Sussex Light and Uzbek Game × Amrock were the most different (D = 0.070), and the hybrids Uzbek Game × Amrock and Sussex Light × Amrock, as well as Tsarskoye Selo × Sussex Light and Sussex Light × Amrock turned out to be relatively close (D = 0.030).
3. Two-breed hybrids of chickens Sussex Light × Amrock had the highest heterozygosity (0.66 and 0.65 in two experiments), the lowest – in Uzbek Game × Amrock hybrids.
4. A specific DNA fragment No. 10 was detected with a frequency of 0.87 in hybrids of chickens Brahma Light × Sussex Light, and in hybrids of chickens Uzbek Game × Amrock and Sussex Light × Amrock its frequency was only 0.27.

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### Author's information:

Valentina I. Tyshchenko<sup>1</sup>, candidate of biological sciences, senior researcher, ORCID 0000-0003-4964-9938, AuthorID 155224; +7 921 558-78-24, [valeriter@mail.ru](mailto:valeriter@mail.ru)

<sup>1</sup> Russian Research Institute of Farm Animal Genetics and Breeding – Branch of the L. K. Ernst Federal Research Center for Animal Husbandry, Saint Petersburg, Pushkin, Russia