

## ALLOTYPES OF CHICKEN IMMUNOGLOBULIN G (IgG)

J. DERKA

Department of Epizootiology and Microbiology, University of Veterinary Science, 612 42 Brno

*Received November 25, 1978***Abstract**Derka J.: *Allotypes of Chicken Immunoglobulin G (IgG)*. Acta vet. Brno, 48, 1979: 25–33.

Ten allotypic specificities of chicken IgG designated K4, K5, K6, K7, K8, K9, K10, K20, K21 and K30 were detected with the alloantisera prepared. The progenies of selected parent crossings were used for their genetic analysis which showed that each of the first seven specificities, i. e. K4 to K10, characterizes one of the seven codominant alleles, alternating in that part of autosomal chromosome for which the designation locus *a* was chosen. It was further found that these specificities determining the individual alleles can be accompanied by the remaining three specificities to form phenogroups. K20 segregated into them with either specificity K4, K6, K7 or K10, whereas K21 was closely associated with K5 or K9. It was observed that K30 did not segregate in the birds analyzed. A possible mechanism of genetic control of allotypic specificities forming phenogroups is discussed in this study.

*Chicken, immunoglobulins, IgG, allotypes, allotypic specificities.*

Since the existence of allotypes of serum proteins in the domestic fowl was proved by Skalba (1964) investigations have been directed at studies of allotypes of immunoglobulins; these studies were aimed at the extension of the spectrum of detectable allotypic specificities, at knowledge of their molecular localization and genetic associations. Accepting the phenomenon of the allotype also for chicken IgG was already derived from the occurrence of allotypes in serum proteins with an electrophoretic mobility of gamma-globulins (Skalba 1966; McDerimid et al. 1969). David et al. (1969) were more convincing in showing the localization of allotypic specificities on molecules corresponding to IgG; besides electrophoresis they used column chromatography and ultracentrifugation. They also studied the way of genetic control of the allotypes proved. Ivanyi (1975) also furnished evidence of alloantigenic polymorphism of the chicken IgG. Wakeland and Benedict (1975, 1975a, 1976) and Wakeland et al. (1977, 1977a) recently aimed at structural and genetic studies of allotypes of chicken 7S immunoglobulin. They detected 6 allotypic specificities and found that on the same IgG heavy chain they can be represented either individually or, more frequently, in various combinations and these combinations are inherited together in the form of phenogroups. Each detected phenogroup corresponded to the allele of the locus which they designated *CS-1*. There is a minimum number of 10 of these alleles.

Investigations of immunoglobulin allotypes of chickens were performed also in our laboratory and proved allotypic polymorphism of both chicken IgM and IgG (Derka 1975). This study is a survey of our knowledge on allotypic specificities of chicken IgG.

**Materials and Methods****Experimental animals**

For alloimmunization we used outbred chickens of various breeds and inter-breed hybrids, mostly cockerels, of the White Leghorn breed of at least 6 months of age. For genetic analyses we obtained chickens from parental combinations selected according to the representation of the respective allotypes.

**Preparation of anti-allotypic alloantisera**

Antibacterial sera obtained from chicken donors given 3–5 times, in weekly intervals, intravenous injections of 4 mg of the heat-killed bacteria *Proteus vulgaris* OX 19 in 1 ml of saline were used as source of immunoglobulin alloantigens for evoking the production of antiallotypic antio-

dies. A week after the last injection the donors were exsanguinated. The antisera obtained (more frequently, for higher yields, plasma was used separated from blood sampled into a 5 % solution of sodium citrate of 4:1) were divided à 1 ml and kept at  $-20^{\circ}\text{C}$  until used for the preparation of the immunization dose for the immunization proper. The dose was prepared in such a way that the agglutinate produced during the reaction of 1 ml of the anti-Proteus serum (plasma) with the added bacterial suspension (4 mg of bacteria in 0.1 ml of saline) was washed 3 times in a cooled saline. After the last washing it was resuspended in 2 ml of saline. Immunization was performed by intravenous applications once a week for several months (mostly 3–4). The immunity response was followed in the recipients in monthly intervals. The antisera obtained were stored at  $-20^{\circ}\text{C}$ .

### Testing of allotypes

A double radial immunodiffusion (Ouchterlony) was used in 1 % Noble agar Difco modified to an 8 % content of NaCl and 1:10 000 of Merthiolate. Plates were read within a period of 18–36 hours depending on the distance of the starting wells used after incubation in a damp chamber under room temperature. Immunoelectrophoresis has been described previously (Derka 1972).

### Isolation of IgG

Isolation was performed according to Benedict (1967). From the delipidated serum the IgG were purified by  $\text{Na}_2\text{SO}_4$  precipitation followed by filtration on a G-200 Sephadex (column  $2.5 \times 100$  cm) using borate buffer of a  $\text{pH} = 8.2 - \Gamma/2 = 0.16$ .

Table 1

Alloantisera against allotypic specificities of chicken IgG prepared in our laboratory

Code designation of		Allotypic specificity IgG determined by them
referential antiserum	duplicate antisera	
a/17	a/14	K10
a/19	a/12	K9
a/22	a/20, a/23	K8
a/37		K7
a/56	a/53, a/55	K6
a/71		K5
a/68		K4
a/27	a/1	K20
a/21	p/37, p/38	K21
a/77		K30

## Results

### Allotypic antisera and detection of allotypic specificities

Using alloimmunization antisera were obtained reacting in a double diffusion on agar with alloantigens (allotypes) of serum proteins of the respective recipients and other hens and forming one or more precipitation lines. Presuming that they contained antibody against the IgG allotype, such antisera could be chosen from them that formed an expressive precipitation line already within the first 24 hours localized closer to the well with the applied antiserum and in immunoelectrophoresis determined the serum protein with the mobility of gamma globulins. This presupposition was proved when these antisera precipitated the isolated IgG carrying the respective allotype. Ten allotypic specificities of the chicken IgG were then detected with these antisera designated K4 to K10, K20, K21 and K30. Table I gives a list of alloantisera detecting these specificities. Fig. 1A–K shows the results of precipitation reactions of referential antisera with the sera of chickens

of which some have the corresponding allotype. The precipitation reaction of the isolated IgG carrying five allotypic specificities with the respective anti-allotypic sera is given in Fig. 1L.

### Genetic analysis

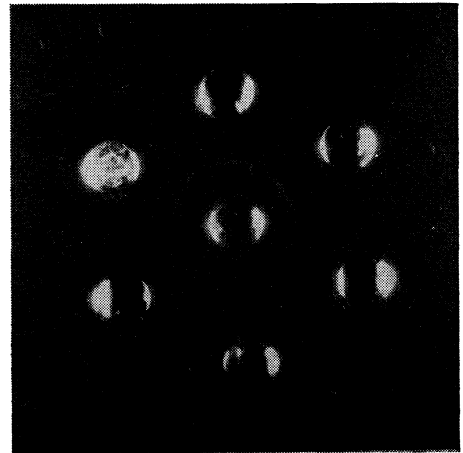
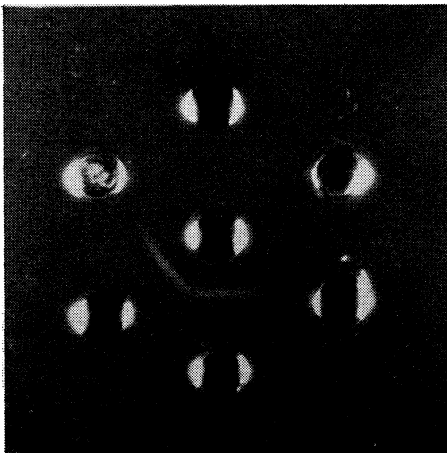
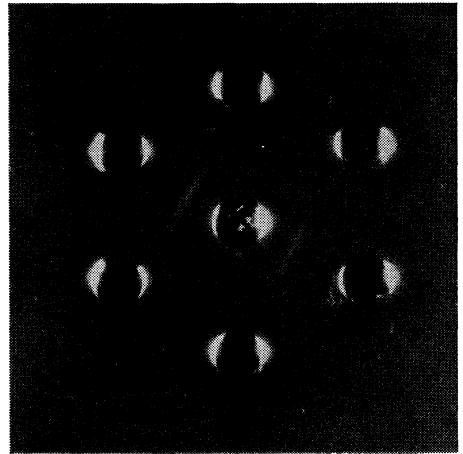
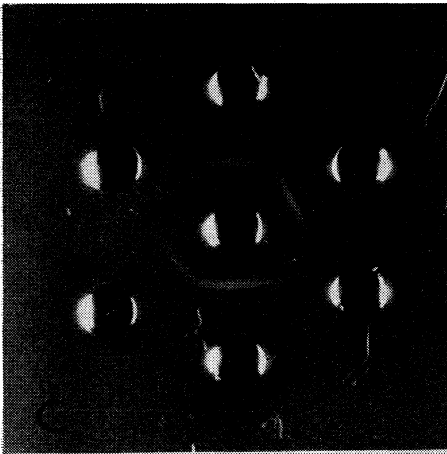
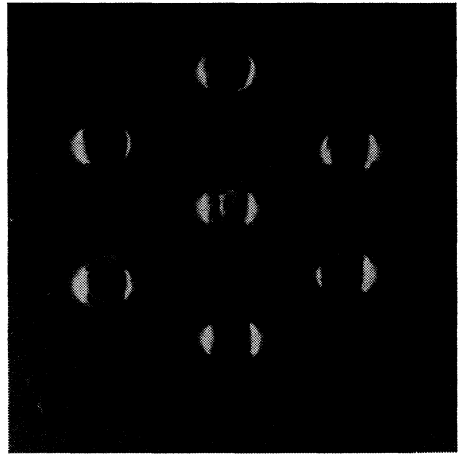
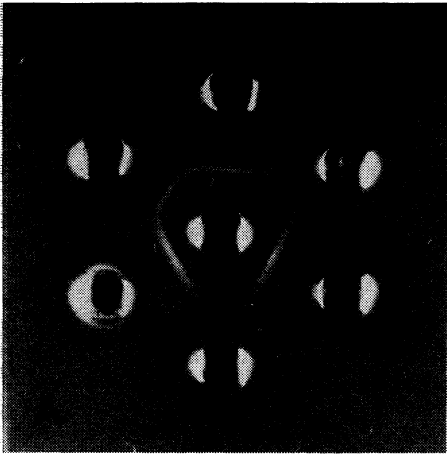
This analysis was based on the knowledge that responsible for the allotypic specificities K9 and K10 are two codominant alleles ( $Ka^9$  and  $Ka^{10}$ ) of one autosomal locus designated  $a$  (Derka 1972). That is why gradually the relationship of every further discovered allotypic specificity IgG to specificities significantly controlled from this locus was tested by analyzing the progeny of selected parental crossings. On the basis of segregation ratios K8, K7, K6, K5 and K4 could be indicated as markers of further alleles of the locus  $a$ . We do not give the results of all partial analyses as this fact was proved also in results of the genetic analysis of parents and progeny given in Table 2. As concerns the further allotypic specificities, K20 was found to occur in all chickens which had any of K4, K6, K7 or K10, whereas K21 was assigned to K5 or K9. K30 did not segregate and was represented in all the birds analyzed in Table 2.

Table 2  
Genotypes of progeny of selected parental crossings

Parent genotype		Possible genotypes of progenies	Expected number	Number observed	$\chi^2$	P
cockeral	hen					
$a^4a^9$	$a^5a^7$	$a^4a^5$ $a^7a^9$ $a^4a^7$ $a^5a^9$	16 16 16 16	19 15 17 13	1.248	> 0.70
$a^4a^9$	$a^5a^8$	$a^4a^5$ $a^4a^8$ $a^5a^9$ $a^8a^9$	16 16 16 16	17 16 14 17	0.374	> 0.90
$a^4a^9$	$a^5a^{10}$	$a^4a^5$ $a^4a^{10}$ $a^5a^9$ $a^9a^{10}$	15.75 15.75 15.75 15.75	15 18 15 15	0.428	> 0.90
$a^4a^9$	$a^6a^7$	$a^4a^6$ $a^4a^7$ $a^6a^9$ $a^7a^9$	8 8 8 8	9 7 12 4	4.250	> 0.10
$a^4a^9$	$a^7a^8$	$a^4a^7$ $a^4a^8$ $a^7a^9$ $a^8a^9$	6 6 6 6	6 4 7 7	0.998	> 0.80

### Discussion

The results presented showed that our studies on the allotypes of immunoglobulins in the domestic fowl (Derka 1975) proved extensive alloantigenic polymorphism of the chicken IgG. The ten allotypic specificities which can be determined in the immunoglobulin of this class may be considered as the most frequent of all the groups of genetic markers of chicken IgG previously described by e. g.



Ivanyi (1975) or Wakeland et al. (1977a). Therefore, some of these specificities (as the results of comparative typization between the laboratories have indeed proved) are new specificities which can contribute to the general classification of chicken IgG allotypes. All the specificities were proved by immunodiffusion on agar. It shows that the immunization process chosen was very suitable for the production of strongly precipitating antiallotypic antisera. The detection method used could not, of course, detect the antiallotypic antibodies in those sera which are incapable of precipitation in agar gel but can be obtained from the recipients as shown by Wakeland and Benedict (1975) who succeeded in detecting allotypic specificity with such an antiserum using radioimmunoassay. It is, therefore, possible that using this more sensitive method the number of detected allotypic IgG specificities could be even larger and it is also necessary to bear in mind that some antisera which are considered to be mono-specific to one allotypic specificity could show their possible polyspecific character. It should be added that in a similar way as shown by Ivanyi (1975) and as mentioned by Wakeland and Benedict (1975a), after immunization of the recipients with the rinsed agglutinate, we also obtained many antisera which contained antibodies detecting allotypes of other serum proteins along with immunoglobulin allotypes. The majority of these antisera, however, could be used for the detection of IgG allotypic specificities without antibody absorption, as monospecific to the IgG allotypic specificity because the precipitation line corresponding to this specificity could be well distinguished from the lines of IgM allotypes and other proteins according to the period necessary for its formation.

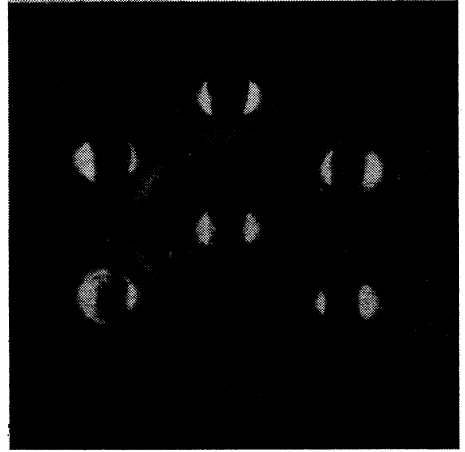
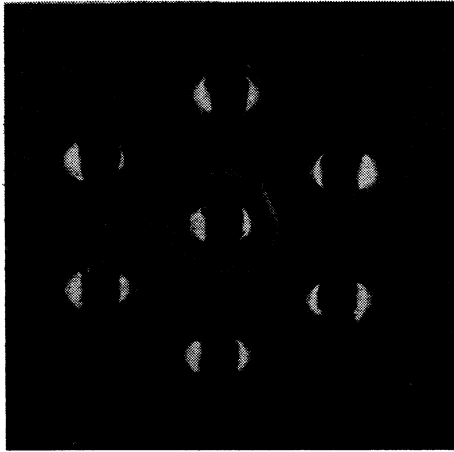
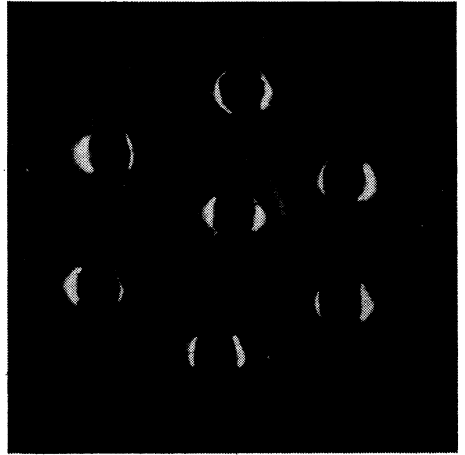
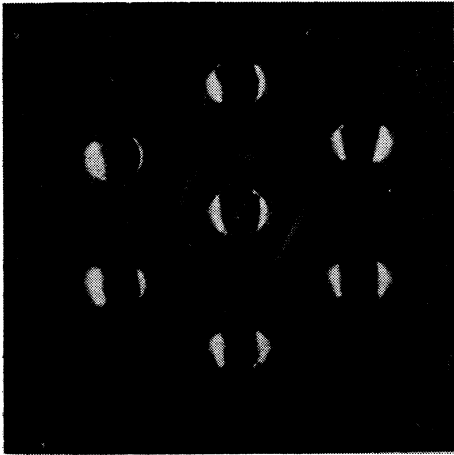
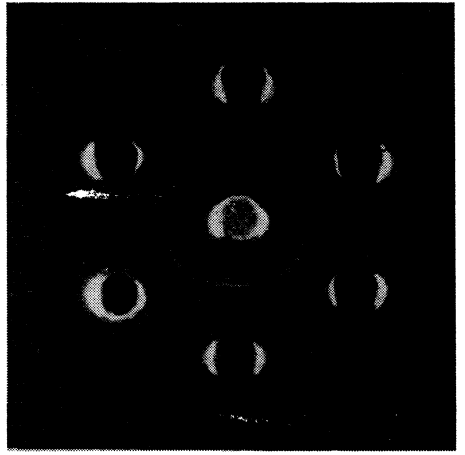
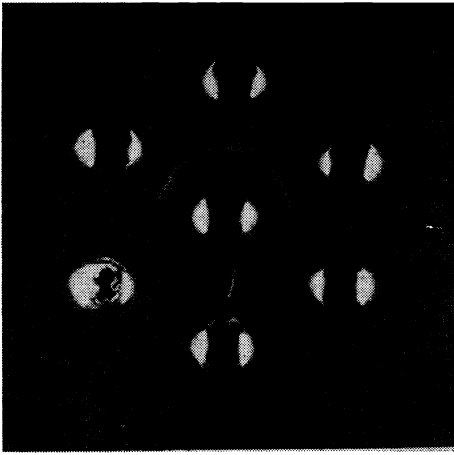
Evidence of the presence of allotypic specificities on chicken IgG formerly derived from the electrophoretic mobility (Derka 1972) also supported by results acquired in studies of the occurrence of allotypic specificities K9 and K10 in the egg yolk and in chicken in the first three weeks of life (Derka 1972a), was now founded on results of immunodiffusion of the isolated IgG of chicks with anti-allotypic antisera. Even though direct experiments directed at structural localization of allotypic specificities in the IgG molecule were not performed, the fact that they occurred in the IgG class only could prove that the structures responsible for these specificities were localized on the heavy (H) chain of this class, thus concluding that they are related to the allotypic specificities detected by Ivanyi (1975) or Wakeland et al. (1977a).

Genetic analysis of the allotypic specificities K4 to K10 showed that each of them can be considered to demonstrate one codominant allele alternating in that

Fig. 1.

Photographs A—K demonstrate the results of double radial immunodiffusion between referential allotypic antisera and sera of chickens whose IgG either carries or lacks the respective allotypic specificity.

- A. Central well was filled with antiserum a/68 (anti-K4). The peripheral wells were filled with sera of 6 chicks, progenies of cockerel 1709, carrier of allotypic specificities K4, K9, K20, K21, K30 and hen with allotypic specificities K5, K7, K20, K21, K30.
- B. Central well was filled with antiserum a/19 (anti-K9). The peripheral wells were filled as in A.
- C. Central well was filled with antiserum a/71 (anti-K5). The peripheral wells were filled as in A.
- D. Central well was filled with antiserum a/37 (anti-K7). The peripheral wells were filled as in A.
- E. Central well was filled with antiserum a/56 (anti-K6). The peripheral wells were filled with sera of 6 chicks, progenies of cockerel 1709 and hen with allotypic specificities K6, K8, K20, K30.
- F. Central well was filled with antiserum a/22 (anti-K8). The peripheral wells were filled as in E.



part of somatic chromosome designated as locus *a* (Derka 1972). This part of the chromosome will most probably be identical with the one which was designated by Ivanyi (1975) as locus G1, or locus CS (Wakeland and Benedict 1975). It follows, for example, from the evidence of the existence of a close linkage between locus *a* and locus *d* determining the IgM allotypes (Derka 1974, 1975) which corresponds with data on the relationship between the loci G1 and M1 described by Pink and Ivanyi (1975). In accordance with the fact that each of the allotypic specificities K4 to K10 is considered to demonstrate one allele of locus *a* was also the occurrence of at most one pair of these seven specificities in any of the chickens of various outbred populations as yet tested (Derka 1975). It is, however, necessary to evaluate this knowledge with regard to the detection method used because Foppoli et al. (1978), using radioimmunoassay, proved the presence of allotypic specificity in two inbred lines of hens formerly defined as negative for this allotype.

The results further showed that the allotypic specificity determined as a marker for some of the alleles of locus *a* was accompanied by further allotypic specificities (K20 or K21) which can be determined in the IgG of chicks; the stability of these complexes (phenogroups) was proved in genetic analyses. It thus became evident that it was possible either to consider the specificities forming the phenogroup as a demonstration of closely linked genes or as a manifestation of the allele of one locus (Derka 1975). Of these two possibilities it is most probably the second one which corresponds more to the real situation as can be assumed on the basis of findings of Wakeland et al. (1977). When two allotypic specificities occurred in the phenogroups, as described by Wakeland and Benedict (1975), these specificities were proved on the same H chain which indicated that each phenogroup was probably the product of one allele of the structural gene *CS-1*. As the specificity K30 did not segregate in the birds analyzed, the present results do not enable to decide unequivocally whether this specificity is really a component of the phenogroup genetically directed by one allele. The individual alleles of locus *a* can be designated in such a way that the determining numbers of allotypic specificities which we presume to be controlled by the respective allele are written above the symbol of the locus, similarly as was used in one of the nomenclatures of allotypes of mice immunoglobulins (see Mage et al. 1973), i. e.  $a^{5,21}$ , or  $a^{7,20}$ , etc.

The results given also enable to use similar divisions for the allotypic specificities of chicken IgG as those used in mice H-2 antigens (Klein 1971). As each of the specificities from K4 to K10 was limited to one allele of locus *a* only, they could be indicated as private specificities. Thus, K20 or K21 could be

Fig. 1.

- G. Central well was filled with antiserum a/71 (anti-K5). The peripheral wells were filled with sera of 6 chicks, progenies of cockerel 1709 and hen with allotypic specificities K5, K10, K20, K21, K30.
- H. Central well was filled with antiserum a/17 (anti-K10). The peripheral wells were filled as in G.
- I. Central well was filled with antiserum a/27 (anti-K20). The peripheral wells were filled as in A.
- J. Central well was filled with antiserum a/21 (anti-K21). The peripheral wells as in A.
- K. Central well was filled with antiserum a/77 (anti-K30). The peripheral wells as in A.

Photographs L demonstrates the result of double immunodiffusion between IgG isolated from the serum of cockerel 1709 (central well) and allotypic antisera a/27 (1), a/68 (2), a/77 (3), a/19 (4), and a/21 (5).

public specificities due to their representation in several alleles of the given locus. Only future studies of allotypic specificities of IgG in different populations of the domestic fowl will prove whether this manifestation will be generally accepted.

### Alotypy kuřecího imunoglobulinu G (IgG)

Přípravenými aloantiséry bylo detekováno 10 alotypických specificit kuřecího IgG, označených K4, K5, K6, K7, K8, K9, K10, K20, K21 a K30. Potomstva vybraných rodičovských křížení bylo použito k jejich genetické analýze. Tato ukázala, že každá z prvních sedmi specificit, tj. K4 až K10, charakterizuje jednu ze sedmi kodominantních alel, alternujících v té části autosomálního chromosomu, pro niž bylo zvoleno označení lokus *a*. Dále se ukázalo, že tyto určující specificity pro jednotlivé alely, mohou být doprovázeny zbývajícími třemi specificitami za vzniku fenoskupin. Do nich se sdružovala specificita K20 s některou ze specificit K4, K6, K7 nebo K10, kdežto specificita K21 se vyskytovala v těsném spojení se specificitou K5 nebo K9. Specificita K30 u analyzovaných ptáků nesegregovala. Je diskutován možný mechanismus genetické kontroly alotypických specificit tvořících fenoskupinu.

### Аллотипы иммуноглобулина G (IgG) цыплят

С помощью подготовленных аллоантисывороток проводилось проявление 10 аллотипичных особенностей иммуноглобулина цыплят, обозначенных K4, K5, K6, K7, K8, K9, K10, K20, K21 и K30. Потомства избранных родительских гибридаций были использованы для их генетического анализа, выявившего, что каждая из первых семи особенностей, т. е. K4—K10, характеризует одну из семи кодоминантных аллел, чередующихся в той части автосоматической хромосомы, для которой было определено обозначение локус *a*. Далее было выявлено, что данные определяющие особенности для отдельных аллел могут сопровождаться остающимися тремя особенностями при возникновении фенотипов. В них объединялась особенность K20 с некоторой из особенностей K4, K6, K7 или K10, однако, особенность K21 имела место в тесной связи с особенностью K5 или K9. Особенность K30 в случае анализированных цыплят не сегрегировала. Обсуждается возможный механизм генетического контроля аллотипичных особенностей, образующих фенотипы.

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