휘 보

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Cytological and Cytochemical Studies of the Hybrids in Fresh Water Teleosts

Yoshio Ojima
Kwansei Gakuin University, Nishinomiya, Japan

Genetical characters and abilities will be appeared by the special gene or gene group on the chromosomes.

We have two different stand-points to this problem.

(I) If these genes become homozygotic, the genetic presentation will be emphasized and fixed as a pure line.

(II) The genetic presentation will be emphasized by the heterozygotic genes and, on the contrary, homozygotic genes only make no efficiency to emphasis in genetical characters.

The problem which is by far the better of the two standpoints, should be evaluated and determined by the purposes of human beings.

When the hybrids (F_1) induced the heterozygotic genes show much more excellent characters compared with the purity of parents, we recognize it as the" heterosis".

Either interspecific or intergeneric hybridization appear to occur rather rarely in nature. Most of the hybrids have been produced in captivity. Being prompted by the curiosity of amateur naturalists, a good deal of the earlier reports on hybridization in animals have been of an unsystematic nature, and therefore have not been directed toward the solution of any definite problem. With the development of evolutionary cytogenetics, however, experimental hybridization has increasingly been applied for studying the nature of species differences. Many species which never seem to produce hybrids can be induced to do so in the laboratory or in nature of in captivity, when the animals were kept under crowded condition, especially when there are no males of their own species. Some pairs of species which are regarded by taxonomists as very closely related species can be hybridzed. In other instances, it has possible to obtain hybrids which could never have arisen in nature, when the use of techniques of artificial insemination is made. Whenever, the isolating mechanism between two forms is incomplete or can be broken through by experimental means, some kinds of an F_1 generation are possible to obtain. In most such cases, there are produced either a few sickly embryos that never develop beyond a certain stage, or very vigorous individuals that become full adults. In many cases,

the F_1 progeny of an interspecific cross are sexually abnorml: in some cases the hybrids are all of one sex, or become intersexes due to the disturbance of the sexual development, while in other instances, their fertility becomes abnormal ranging widely from near normality to complete sterility.

Experimental hybridzation has been undertaken to reveal the extent and nature of the genetic systems or the taxonomic differences, between the animals under study and, when combined with a cytological analysis, the types of structural rearrangements which have taken place in their chromosomes may be made clear since they diverged in the course of evolution.

Already a considerable number of hybrid fish have, since long, been recorded by many ichthyologists. Most of them are of an unsystematic kinds, prompted by the curiosity of naturalists being not applied for the solution of any definite problem.

In spite of the fact that the study of hybridization in fishes is of special importance from the ecological standponts, no particular progress or attempt has been made in this direction during many centuries, owing probably to much difficulties of rearing up the progeny, though the crossing is rather easily done. Therefore, one knowledge on the cytology of bybrid fish has remained quite incomplete in comparison with birds and mammals in which economic importance likely occurs.

The author have undertaken during these some 25 years the crossing of the carp and funa in captivity in a hope to establish any strains favorable for food-fishes viewed from both economical and commercial standpoints.

The carp (*Cyprinus carpio*) belonging to the family Cyprinidae, is a common fresh water teleost having a worldwide distribution. In Japan the carp abundantly inhabits almost every river, lake and pond. It is highly valued as one of the food-fishes found in fresh water of our country. They easily breed in captivity, so that they are largely domesticated in small ponds for food, especially in mountainous regions far from the sea.

The funa (*Carassius aurarus*), a number of the Cyprinidae is of close relative to the carp. They inhabit very commonly every river, lake and pond together with the carp. The funa is more flavour in taste than the carp, but the growth of it less rapid than the carp in captivity, in addition to the technical difficulty of cultivation of the former.

It is very significant and desirable in Japan to breed a fish strain which has a rapid growth and flavour taste. In response to the above economic importance, the author has undertaken crossings between several varieties of carp and several local varieties of funa, with the purpose to establish favorable hybrid-strains between them which are valuable for food-fishes as a application of the genetic "heterosis".

When kept under confined condition, the carp and funa (gold-fish) mate and can produce hybrids. Reciprocal crosses are also possible with similar results. The hybrids grow rather rapidly, showing no observable abnormalities in the course of their development; the cleavage of fertilized eggs proceeds without mitotic abnormality. The

hybrid fishes are noted for their strength and resistance to disease. They rank intermediate between the parent species in several characters.

Dr. Makino (1939, 41), Hokkaido University, reported that the carp the funa are strikingly dissimilar in their chromosome patterns, not only in the number of chromosomes but also in their morphological aspect. The chromosomes of the carp are isomorphic in general appearance, every element being uniformly short and rod-shaped without any marked size difference, while in the funa, in the gold-fish as well, the individual chromosomes are of a slender long rod-type with a slight difference in size occurring among them.

The chromosome number of the carp is 104 in 2n and 52 in n, while funa shows 2n=94 and n, 47.

In the hybrids, every metaphase plate showed 99 chromosomes in 2n. This number just corresponds to the sum of the haploid sets of the parents. The chromosome complex consists of a combination of the haploid sets from the respective parents.

In older studies in cytogenetics, there were many difficulties in precise chromosome investigations; the obstacles involving in most part the difficulty of obtaining favorable materials provided with dividing cells, in addition to technical difficulty. The chromosomes of fish are a subject of particular difficulty for study because of their small size and considerably high number.

In recent years, surprising advances in cytological techniques, simple and reliable, have rendered facile much more precise chromosome analysis than was possible with older classical methods. These techniques can be combined with colcimide treatment to accumulate cells at metaphase and facilitate spreading of the chromosomes; particularly a current air-drying method has made possible the accurate delineation of the chromosomes in somatic cells.

Based on the above view, the author has undertaken reinvestigations of the chromosomes of the carp, funa, gold-fish and carp-funa hybrids.

Chromosome observations were exclusively made on excellent and reliable metaphasic cells. The results of chromosome counting of the carp showed that both germ cells and somatic cells derived from males contained 100 chromosomes at metaphase. The somatic cells from females also had 100 chromsomes in a diploid complement.

Whereas the hybrid males generally have testes of an usual size in the breeding season, the spermatogenetic activity is disturbed, except at the course of spermatogonial proliferation proceeds in a normal fashion. Every metaphase plate of the spermatogonia under study showed 100 chromosomes in 2n as mentioned already.

Meiosis in the hybrid testes is highly disturbed. Most spermatogonia pass through the early stages of the meiotic prophase. But the greater part of them undergo degeneration during the period from late leptotene to pachytene of the growing period. The nuclei of the degenerating cells are at first marked by pycnosis into an aggregation, or aggregation of the chromatic elements. They show a strong affinity to the Feulgen reaction. They are referred

to as cells of pycnosis I. With the passage of time, pycnosis is proceeds further and the nuclei become compact in appearance with an increase of affinity to the Feulgen reaction. They are referred to as cells of pycnosis II.

The nuclei become much more compact and you can not identify the chromatin threads in each nucleus. It is then evident that the degeneration of the germ cells sets in during a rather early stage of the meiotic prophase. There is consequently no first metaphase, and all the cells undergo degeneration without going through a first meiotic division. Thus, gametogenesis in the hybrid testes is arrested before the first meiotic division, and consequently no spermatozoa are formed.

The results of the experiment by exposing testis-pieces to Ringer's solution have shown that the germ-cells likely undergo degeneration in the stages of meiotic prophase ranging from leptotene to pachytene.

This seems to imply that the cells at meiotic prophase are in a state quite unstable in respect to their physiological conditions of meiotic cells, the chromosomal disharmony occurring in hybrid nuclei may join to disturb the physiological activity of cells, and the degeneration of the cells may be ultimately induced.

White (1954) distinguished the types of meiotic conditions occurring in species hybrids as follows:

(1) complete pairing of the two sets of chromosmes,

(2) incomplete pairing, meiotic divisions being otherwise normal,

(3) complete pairing, but physiology of meiosis is abnormal,

(4) incomplete pairing with abnormal spindle mechanism,

(5) no pairing but meiosis is normal,

(6) and no pairing, spindle mechanism being abnormal.

The carp-funa hybrids seem to furnish and example to be included in the category of type 4.

The cause of hybrid sterility is a matter which involves a very complex mechanism, many problems being still unsolved. Yamashina (1943) working on the cytology of bird hybrids, has expressed the view that the cause of sterlity in hybrids may be the disharmonious relation existing between the maternal cytoplasm and the paternal chromosomes.

But, in carp-funa hybrids, female can produce the eggs without any disturbances through the course of oogenesis.

So this is not true of the material of carp-funa hybrids.

According to Dobzhansky (1951), the lack or disturbances in chromosome pairing in the gametogenesis of the F_1, which in turn lead to sterility of hybrids, may be due either to the dissimilarities in the gene arrangements between the chromosomes of the parent species (that is chromosomal sterility) or to the effect of complementary genetic factors (that is genic sterility).

White (1954) is of opinion that although the distinction of chromosomal and genic sterility may be theoretically important, it is difficult to apply it in practive and that many cases of hybrids sterility probably depends on a combination of mechanical and physiological impediments of pairing of

the chromosomes.

A consideration is probable that the synaptic failure of chromosomes and the degenerative change of the nucleus are matters different in sequence, since there is no positive evidence to show that the degeneration of the nucleus was induced by the failure of chromosome synapsis.

It is well accepted that deoxyribonucleic acid (DNA) is the most important constituent of living cell nuclei. The DNA measurements in individual nuclei of liver cells were made in the carp, funa, carp-funa hybrids F_1 and back-cross hybrids (R_2). There is no meaning difference of DNA amount between the hybrids F_1, and their parent species. In the back-cross hybrids R_2, however, the value of DNA showed triploidy. It is very clear that this results depend or the triploid chromosome number 150 showing in the dack-cross hybrids R_2 as mentioned already.

As the author explained before, all the germ cells undergo degeneration during the early prophase of meiosis, showing pycnosis of nuclei.

On the basis of the histograms, the following statements may be made; the DNA amount in pachytene nuclei here obtained is approximately twice that obtained in liver nuclei in both the carp and funa. But the DNA amount in pachytene nuclei of hybrid germ-cells is less than the 4n value.

A consideration may be entered into the DNA content in germ cells. Theoretically considered, the following relationship of the DNA content may occur in germ cells of a pure line. The nuclei of spermatogonia of the carp-funa hybrid contain a basic mean DNA value which is referred to as a 2n value, but those of the pachytene stage show a value less than 4n value, though the cells of the pure lines have the 4n value at the same stage. This seems to be an important and significant feature of hybrid cells in association with their physiological properties.

Probable consideration may be allowable that, if DNA should not normally increase in amount in the nucleus, cellular activity would be disturbed, and subsequent physiological abnormalities or death of cells may result.

In this connection Leuchtenberger *et al.* (1953, '55) reported that a remarkable constant amount of DNA in spermatocytes and spermatozoa of fertile men was found, while infertile man had significantly lower DNA values in their spermatocytes and spermatozoa.

Recently, by subjecting extracts of liver, blood, kidney, muscle and spleen tissue fragments from the carp, funa and their hybrids F_1 to electrophoresis, distinct migratory patterns of the esterase, total protein and LDH activity were investigated.

Hybrid F_1 showed roughly an intermediate new pattern between the parents. The correlation between new enzymatic pattern and the phenotypic heterosis should be solved in the future.

Recent Studies on the Serological Breeding of Farm Animals

S. Suzuki
Laboratory of Animal Breeding, Tokyo University of Agriculture

In 1900 Landsteiner made his fundamental discovery of the A, B and O groups of human blood. Studies of animal blood groups were also initiated in 1900, when Fhrlich and Morgenroth demonstrated individual differences in the blood of goats. Unfortunately the early studies on animal blood groups were not promoted owing to the use of naturally occurring antibodies only, as reagents. However, the advance of the experimental studies on animal blood groups was caused by the immunisation technique adopted by many researchers.

Since 1940 the studies on animal blood groups have remarkably developed in the fields of animal serology and breeding. In the period of 1950, especially, the studies on the relationship between blood groups and production characters in farm animals were performed. In 1962 the constitution of the International Immunogenetics Society was proposed. The aims of the Society are

TABLE 1 A summary of the genetic systems controlling blood antigens in cattle (*J. RENDEL*)

System	Antigenic factors	Number of alleles	Comments
A	A_1, A_2, H, D_1, D_2, Z′	10	
B	B, G, K, I_1, I_2, O_1, O_2, O_3, P, Q, T_1, T_2, X_1, X_2, A′, B′, D′, E'_1, E'_2, E'_3, F′, G′, I′, J′, K′, O′, Y′ and others	>300	Genotypic classification directly from the phenotype often possible
C	C_1, C_2, E, R_1, R_2, X_2, L′.W, and others	>35	Genotypic classification sometimes possible
FV	F_1, F_2, V_1, V_2	4	Genotypic classification generally possible
J	J	>3	J is a soluble plasma constituent. An J presents in genotyoe j/j, which lacks J
L	L	2	...
M	M_1, M_2	3	...
N	N	2	...
SU	S_1, H′, U_1, U_2, U, and others	about 20	Genotypic classification often possible
Z	Z_1, Z_2	3	Genotypic classification generally possible
R′-S′	R′, S′	2	Genotypic classification possible
T′	T′	2	

to encourage the study of immunogenetics and to make the closer association of workers in the fields of immunogenetics.

July of last year the 12th International Conference on Blood Groups and Biochemical Polymorphisms in Animals was held in Budapest of Hungary.

(A) Blood Groups and Biochemical Polymorphisms

After the introduction of isoimmune-hemolysins for the study on cattle blood groups by Ferguson(1941) and Ferguson *et al.*(1942) a large number of strongly reactive and highly specific reagents could be produced in cattle blood sera. The number of cattle blood antigenic factors exceeds 60, and 12 systems of cattle blood groups are available for the international ones.

Recent discoveries of various inherited biochemical polymorphisms such as transferrins, hemoglobins, post-albumin, acid-phosphatase, amylase, albumin, α-lactoalbumin, β-lactoalbumin, $\alpha_1$casein, β-casein and κ-casein have provided additional markers for linkage investigations.

TABLE 2 Genetic variation of blood and milk proteins in cattle (ASHTON et al)

Protein	Locus symbol	No. of alleles
Haemoglobin	Hb	5
Prealbumin	Pr	···
Albumin	Alb	3
Postalbumin	Pa	2
Transferrin	Tf	8
Ceruloplasmin	Cp	···
Haptoglobin	Hp	···
α_2-globulin	S	2
Alkaline phosphatase	F	2
Amylase	Am	3
Carbonic anhydrase	Ca	2
Serum esterase	···	···
Red cell esterase	···	···
β-lactoglobulin	Lg	3
α-lactoalbumin	La	2
αS_1-casein	αS_1-Cn	4
β-casein	β-Cn	5
κ-casein	κ-Cn	2

Stone (1962) studied on the relation of human and cattle blood groups. The J substance of cattle and the A substance of human are serologically and chemically related. On the relation of Rh system of human and B system of cattle, the B system of cattle contains over 200 phenogroups, detected with one or more of 20-add reagents and the Rh system of human also contains over 200 phenogroups, detected with ten to 12 standard reagents. The former more nearly parallels the latter. Hemolytic disease does not occur in cattle either naturally or by active or passive immunisation. Erythrocyte chimerism is much more frequent in cattle than in humans.

Existence of two blood types in red cells of the Japanese black and the brown breed of cattles were clarified by *F. chumbinho* seed extract (Lectin) (Ikemoto *et al.* 1968). It was apparent that the frequencies for positive type in the Japanese black, brown breed of cattles and Australian breed of cattles were 40.57%, 36.67%, and 17.50%, respectively.

A number of cases of abnormal transmission of blood groups of the B system were reported (Bouw *et al.* 1964). It is proposed that most of these irregularities could be explained on the assumption of linked genes in which each gene corresponed to a part of the complex blood group.

Also, the irregularities are considered to be a result of crossing over within the chromosomal region which controls this blood system.

Several mutation and recombination-like events have been recorded by the studies on the inheritance of the B phenogroups in cattle (Stormont, 1963; Larsen, 1966).

On cattle serum albumin phenotypes, observations have been made by starch gel electrophoresis (Ashton, 1964), and it was clarified that albumin polymorphism in cattle is controlled by two autosomal codominant alleles Alb^A and Alb^B, and albumin phenotypes are three, AlbA, AlbAB and AlbB. While Alb^B is frequent in Zebu beef and dairy cattle, it is apparently absent in European cattle. The appearance of the phenotypes changes on storage and at acid pH.

On amylase polymorphism in cattle, Ashton (1965) demonstrated three autosomal codominant alleles Am^A, Am^B and Am^C in Danish breeds of dairy cattle.

Gasparski *et al.* (1968) described that amylase polymorphism in common breeds of dairy and beef cattle in Canada was controlled by three autosomal codominant alleles; and the gene frequencies of amylase iosozymes in French Charolais were significantly different from Holstein-Fricsian and Aberdeen Angus. Serum amylase types may be added to blood groups for parentage tests.

Three new reagents, anti I_0, -G_0, -R_0 were produced by Duniec *et al.* (1968); and moreover a new allele, F in the cattle FV blood group system was found by Osterhoff *et al.* (1968). In accordance with the international nomenclature, the allele was named F^f, the small letter indicating that the allele is responsible for the absence of antigenic factors F and V in the FV system.

By means of immunoelectrophoresis, Veselsky (1968) has observed the formation of antibodies which can be produced by spermatozoa from the tail of the epididymis, ampulla and ejaculated spermatozoa of bulls. Furthermore, Veselsky *et al.* (1970) found considerable individual differences in the formation of precipitates with esterase activity in the seminal vesicle fluid and the ampulla or fluid.

Genetically determined heterogeneity of ceruloplasmin in cattle was demonstrated (Schröffel *et al.*, 1966). The intensity of colouring of the two slower fractions, designated B and C, is weaker than that of fraction A, and their mobility is subjected to a certain variability.

Kovács (1968) clarified the occurre nce of a new transferrin allele (D_1. D_2) in two Hungarian cattle breeds.

In U. S. S. R., the investigation of the genetic polymorphism of blood serum and milk proteins in cattle was started in 1965. Belayev *et al.* (1968) studied on the biochemical polymorphism of albumins, post-albumins, transferrins and β-lactoglobulins in Estonian Black-spotted and Yakutian cattle of Siberia.

Makaveyev (1968) also studied on albumins, transferrins, serum amylase and blood groups in 150 Bulgarian water buffalo, Indian auffalo and crosses between them by starch gel electrophoresis.

Using electrophoresis on starch gel with urea and mercaptoethanol added, four polymorphic systems of milk proteins, α-, β- and κ-caseins and β-lactoglobulin in Bohemian spotted, Slovakian spotted, Pinzgau, Ayshire cattle breeds were demonstrated by Macha *et al.* (1968).

Matousek (1970) reported that seminal vesicle fluid of bulls contains a hemolytic

TABLE 3 Inheritance of the individual blood factors (Stormont et al.)

Blood factors	Mating types of parents					
	+ × +		+ × −		− × −	
	Number of offspring with (+) and without (−) blood factors					
	+	−	+	−	+	−
A_1	294	30	154	88	0	73
A_2	504	13	105	15	0	2
A′	148	28	120	83	0	260
C	461	39	106	26	0	7
D	16	4	98	69	1*	451
H	14	2	80	79	2*	462
J	55	7	123	122	0	332
K	63	16	111	94	1*	354
P_1	207	48	157	119	0	108
P_2	250	50	158	119	0	62
P′	2	6	72	85	0	479
Q	183	41	197	92	0	126
R+	168	11	75	33	1*	23
S+	83	18	65	61	0	84
T	332	45	125	92	0	45
U	98	20	165	110	0	228

*Exceptions to the genetic theory (see text)

+Necessity to perform absorptions accounts for reduced on R and S.

TABLE 4 Frequencies of 16 blood factors in Shetland Ponies and Thoroughbreds

(Stormont et al.)

Blood factors	Shetland Ponies		Thoroughbreds	
	Number typed	Frequency	Number typed	Frequency
A_1	391	0.534	276	0.927
A_2	391	0.869	276	0.927
A′	391	0.583	276	0.057
C	391	0.879	276	0.927
D	391	0.250	276	0.000
H	391	0.181	276	0·007
J	391	0.227	276	0.278
K	391	0.327	276	0.123
P_1	391	0.597	276	0.369
P_2	391	0.621	276	0.503
P′	391	0.094	276	0.173
Q	391	0.519	276	0.797
R	316	0.911	185	0.611
S	316	0.354	185	0.632
T	391	0.698	276	0.884
U	391	0.534	276	0.275

factor towards which cattle red cells react with different sensitivities. According to the degree of sensitivity, cattles were divided into three groups. The first and most numerous groups (R) comprised animals with resistant red cells, in the second, least numerous groups there were animals (S) with very sensitive red cells and finally, the third group of animals (RS) was between the very resistant and very sensitive groups.

The study on horse blood groups began shortly after the discovery of ABO system of human blood groups and numerous reports have been established.

Stormont *et al.* (1964) presented on the inheriatnce and distribution of 16 horse blood factors.

On the other hand, Podliachouk (1968) found 18 horse blood factors which are defined by anti-A_1, C, D_1, D_2, E, F, G, H_1, H_2, I, J_1, J_2, K, O, F_{T2}, F_{T3}, F_{T4}, and F_{T5}, belong to at least 8 genetic systems.

Also, 17 horse blood typing reagents have been prepared by Sandberg (1968). The inheritance of the correspondng factors, A_1, A′, C, D_1, E, H_1, J_2, K, P_1, P′, Q_1, S_{w12}, U_1, U_2, S_{w9}, S_{w10} and S_{w14} was investigated on family material from two native Swedish horse breeds. The factors were assigned to 8 blood group systems,one of which appeared to be "new".

In Poland, Siudzinski *et al.* (1968) also succeeded in obtaining immune sera, anti-A, anti-D, anti-H, anti-k and anti-PO_3=S_{w10}.

By starch gel electrophoresis a genetic polymorphism in the enzyme carbonic anhydrase from horse blood cells was studied by Sandberg (1968), and the existence of 10 carbonic anhydrase phenotypes controlled by 5 codominant autosomal alleles, CA^F, CA^I, CA^L, CA^O CA^S was revealed. F, I, L, O and S were the different bands named in order of decreasing electrophoretic mobility.

Serum types, transferrin, hemoglobin and esterase types were researched in Basuto ponies which are the only equine in Southern Africa, by Osterhoff *et al.* (1968), Podliachouk *et al.* (1968) were unable to establish the presence of 17 horse blood factors on the spermatozoa of 13 Bulgarian stallions, using inhibition, elution and mixed agglutination test.

By Kaminski *et al.* (1968) frequecies of 5 esterases occurring in horse sera were compared.

The researches on transferrin polymorphism in horses carried out by Braend *et al.* (1964), Gahne(1966), Hesselholh(1966) and Podliachouk *et al.*, (1966) showed their great variety enabling distinction of breeds or types of horses.

11 transferrin phenotypes and 3 albumin phenotypes were clarified by Tomaszewska *et al.* (1968) in Polish "Tarpan" breed. It was found that similar to other breeds of horse 3 albumin phenotypes were present: FF, FS and SS. The frequencies of the alleles were q_F=0.4589 and q_S=0.5410.

Serum transferrin and hemoglobin polymorphism were researched in Lipizzaner horses from Austria, Hungary and Czechoslovakia by Schleger *et al.* (1968). On the other hand, Braend *et al.*(1964) observed 16 transferrin phenotypes in horses, these types were attributed to the action of six, codominant, autosomal alleles designated Tf^D, Tf^F, Tf^H, Tf^M, Tf^O, and Tf^R, each of which controls two bands on starch gels. But they clarified no individual variation in hemoglobin and haptoglobin types.

Serum transferrin types and serum albumin types of horses in Japan were examined by means of starch gel electrophoresis (Mogi *et al.*, 1970). By using a modified discontinuous buffer system, both serum transferrin types and serum albumin types were determined in the same running starch gel. Seventeen serum transferrin phenotypes were found in 848 horses examined. Genetic studies in Thoroughbreds and Anglo-Arabs breeds comfirmed that serum transferrin types were controlled by 5 codominant autosomal alleles which were named Tf^A, Tf^B, Tf^C, Tf^D, and Tf^F. However, in Kiso native breed Tf^F gene was found. The gene frequencies were Tf^B=0.42~0.48, Tf^A=0.19

$\sim$0.32, Tf^C=0.018 $\sim$0.129, Tf^D=0.024$\sim$0.115, Tf^E=0.044$\sim$0.215 and Tf^F=0.007. Three albumin phenotypes were determined by 2 codominant autosomal alleles which were called Alb^A and Alb^B. Gene frequencies were Alb^A=0.482 and Alb^B=0.518 in Kiso native breed, while in other breeds Alb^A =0.251$\sim$0.329 and Alb^B=0.675$\sim$0.749.

In sheep, seven blood group systems are recognized, R-O, X-Z, A, D, M, C,B systems. Generally speaking, studies on blood groups in sheep are less advanced than in other animals.

An association between the potassium types and the antigen M on the red cells of adult sheep was studied by Rasmusen *et al.* (1966). All high potassium red cells (HK type)were M-positive,whereas low potassium red cells (LK type) were either M-positive or M-negative. Sheep red cells which were M-negative were always LK type. Tucker (1968) also studied on the relation between M antigen and potassium types in lambs.

Babierz *et al.* (1968) worked to determine the percentage distribution of individual transferrin types in Polish Merino sheep, and reported that most sheep carried transferrins in heterozygous combination and the transferrins which occurred in homozygous state were A, G, C, M, D and E.

The M-L blood group system and its influence on red cell potassium levels in sheep were investigated by Tucker *et al.* (1970).

On inherited variations in the prealbumins of sheep serum. two protein systems, *Pr* and X in the prealbumin region were found by Efremov *et al.* (1968). *Pr* and X system showed six different phenotypes, respectively. The faster migrating system designated *Pr* and the slower migrating system designated X.

Suzuki *et al.* (1968) studied serelogical constitution of goats. Four antigenic factors, Ch_1, Ch_2, Ch_3, and Ch_4, classified by isoimmune antisera were shown to be genetically controlled by independent dominant genes. The serum transferrin was classified into three phenotypes genetically controlled. Also, the serum albumin of goats divided into three phenotypes, AlAA, AlBB and AlAB, was shown to be genetically controlled. Two isozymes, Apf and Aps of alkaline phosphatase were recognized in sera of goats. Large differences in frequencies of cellar antigens, serum trans-

TABLE 5 Blood group systems in the pig (DINKLAGE)

Blood group systems	Blood group factors	Alleles
A		A^A, a^a O
S	A(Ac, Ap)	S, s
B	Ba, Bb	B^a, B^b
C	Ca	Ca,C^-
D	Da	D^a,D^-
E	Ea,Eb,Ed,Ee,Ef,Eg, Eh,Ei	E^{bdg},E^{edgh}, E^{efdh}, E^{bdf}, E^{bef},E^{eg},E^{aegf}
F	Fa,(Fb)	F_a,(F^b)
G	Ga,Gb	G^a,G^b
H	Ha,Hb,Hc	H^a,H^b,H^{ab},H^c,H^-
I	Ia,Ib	I^a,I^b
J	Ja	J^a,J^-
K	Ka,Kb,Kc,Kd,Ke	K^{ace},K^{ade},K^{ac},K^b,K^-
L	La,Lb,Lc,Ld,Lf,Lg,Lh, Li,Lj,Lk,Ll	L^{adhi},L^{dcgi},L^{bdfi},L^{adhjk}, $L^{adhj}{}_{l}$,L^{agi}
M	Ma,Mb,Mc, Md	M^a, M^b, M^c, M^d, M^{aB}, M^{Bc},M^{cd},M^-
N	Na,Nb,Nc,	N^a,N^B,N^{Bc}
O	Oa,Ob	O^aO^b

In addition, more than 70 non-systematized blood group reagents are recognized.

ferrin types, serum aloumin types and alkaline phosphatase types were observed among breeds of goats.

Also, Salerno *et al.* (1968) studied on the distribution of the transferrins and albumins in goats of South Italy.

Serum amylase types in Angora goats were researched by Fechter *et al.*(1970). Three amylase types were found which were denoted A for the fast moving type, S for the slow-moving type and AS for the combination of the two types. The frequencies in 85 Angora goats were 81 A-type, 2 AS-type and 2 S-type.

Pig blood group systems, factors and alleles are listed in Table 5 ; and systems detectable by means of starch gel electrophoresis are as Table 6.

TABLE 6 Systems detectable by means of starch gel electrophoresis (DINKLAGE)

System name	Locus symbol	Gene symbols
Transferrin	Tf	A,B,C,D,
Prealbumin	Pa(Pra)	A,B
Albumin	Alb_1	A,B,O,
Hemopexin hematin-binding β-globuiin, haptoglobin)	Hp (Hpx, Hx, Hg,)	O,1,1F(4),2,3,3F
Amylase	Am	1,2,2F(2)3;or A, B,BF(B),C
Ceruloplasmin	Cp	1,2;orA,B
$s\alpha_2$-globulin	$S\alpha_2$	A,B,C
6-phosphogluconic dehydrogenase	6-PGD	A,B
Alkaline phosphatase	Akp	

Andresen *et al.* (1964) found that the genetic locus in pigs for the C system is linked to the locus for the J system. They estimated a recombination value between the two loci of 5.3%+1.1%. Resmusen (1965) also reported that the estimated crossover percentage of 7.0%+3.4% agrees quite closely with their figure of 5.3%.

Hemolytic disease of pigs caused by anti-Ba was studied by Andresen *et al.* (1965). The serological and hematological findings confirmed the clinical evidence of accelerated red blood cell destruction caused by Ba-anti-Ba interaction in pigs. The Ba-negative pigs were unaffected and healthy.

The experiment was carried out to clarify whether the interaction between blood group factor Ea and anti-Ea of pigs induced the disease or not. (Abe *et al.*, 1970). Anti-Ea in the serum was found to be fairly common among the sow population. However, when the titer in the colostrum was less than 256, no acute hemolytic anemia nor any clinical symtom was observed in the newborn, except some changes in the osmotic fragility of red blood cells.

By Wiatroszak (1968) studies on the blood cell antigen were carried out on 488 wild boars, and very distinct differences in blood cell antigen structure were found between the wild boar and the domestic pig, especially in blood group system A, F and G.

In the reproduction of pigs, use of blood groups for double mating was discussed by Matousek (1968) and Linhart (1968).

Immunogenetic study of the polymorphism of serum proteins in pigs was carried out using anti-allotypic isoimmune sera by Tikhonov *et al.* (1968).

Bromelin, papain, trypsin and ficin have been used with several different antibodies in pig red cell typing by Hardy (1968).

By Dinklage (1968) the alkaline phos-

phatase polymorphism in the pig was clarified to be 6 phenotypes being controlled by 5 alleles, AKP^A, AKP^B, AKP^C, AKP^D, and AKP^E.

On serum amylase of pigs (Hesselholt, 1968) at least 4 zones of amylase activity were distinguished; and esterase in the blood serum of pigs was clarified to be three phenotpes A, B and AB controlled by two codominant alleles Es^A and Es^B (Kubek, 1968).

The first individual differences in the antigens of chicken red blood cells were discovered in 1924 by Landsteiner and Miller. During the last decade, a number of workers have studied on the blood groups of chickens. Chicken blood group systems and antigenic factors are listed in Table 7.

TABLE 7 Blood group systems in chicken

System	No. of alleles
A	14
B	21
C	7
D	5
E	11
H	2
I	2
J	2
K	2
L	2
P	3
Hi	2
Th	2

Complex antigens of the A and B system in inbred lines of chickens were analysed by Hala *et al.* (1968).

By Schmid *et al.* (1968) specific antileukocyte sera have been obtained by immunisation of chickens with spleen, bone marrow and blood from other chickens. By means of these specific antileukocyte reagent specific leukocyte antigens were detected.

Comparison tests of chicken blood group reagents were conducted between McDermid, England and Okada, Japan (1970). The blood group antigens A_F and A_3; A_H and A_2; A_P and A_b; B_1 and B_{13}; and B_G and B_9 are comparable. Some similarities exist between the atigens B_{EM} and B_2; B_c and B_b; B_E and B_{14}; B_G and B_7, B_{33}; B_K and B_{15}; B_L and B_1, B_{11}; B_M and B_{12}, B_{19}; and B_{MO} and B_1, B_2, B_8.

Br1, Br2, Br3 and Br4 agglutinins of bromelin were recognized by Iwase (1970) as the type specific agglutinins of chicken blood groups in antisera of rabbit immunized with chicken blood cells. By these agglutinins the blood groups of chicken were classified into 12 groups as follows; Br_1 group, Br_2, Br_3, Br_4 Br_1 Br_3, Br_1 Br_4, Br_2 Br_3, Br_2 Br_4, Br_3 Br_4, Br_1 Br_3 Br_4, Br_2 Br_3 Br_4 ond O.

Proteins of the seminal fluid from the vas deferens of cocks were studied by starch gel electrophoresis and immunoelectrophoresis (Stratil, 1968). The presence of "transferrins" (Tf_{sp}) and "albumins" (Alb_{sp}) was proved. Both proteins are immunologically related to the corresponding proteins of the blood serum.

By Vyshinsky *et al.* (1968), transferrin variations of five types were found; A, B, AB, BC and AC.

Csuka *et al.* (1968) reported that polymorphic plasma esterase fractions in chickens are influenced by physiological processes. In the period before reaching sexual maturity active esterase phenotypes (EsA, EsB and EsAB) change into the EsO type.

In cocks such a change does not occur. In younger populations and in hens with a high level of egg production the active phenotypes disappear earlier than in less productive populations.

Genetic linkage of six loci for polymorphic proteins in chickens (Alb, Tf, Pa, OV, G_1 and G_2) and three loci for somatic markers (W/w, P/P and I/i) were investigated by Stratil(1970). The existence of close linkage was poved between Ov and G_1.

Okada *et al.* (1968) reported on liver acid phosphatases of chickens. Two regions of acid phosphatase activity were resolved from liver extracts of chickens by starch gel electrophoresis. With respect to Region II, three phonotypes have been observed. These phenotypes were shown to be controlled by a pair of autosomal codominant alleles; $Acp\text{-}2^A$ producing a fast moving band and $Acp\text{-}2^B$ producing a slow band. Heterozygotes have, in addition to the fast and slow enzymes, a hybrid enzyme of intermediate electrophoretic mobility.

Alkaline phosphatase of chicken plasma was separated by polyacrylamide gel electrophoresis (Tamaki *et al.*, 1970). Zymograms of single plasma samples revealed either a fast or a slow type of the enzyme. Both the fast type and the slow type had two bands. when the samples of plasma of both types were mixed in vitro, three bands were observed. The fast and the slow types were found to be controlled by a completely dominant (Akp^F) and a completely recessive (Akp^S) allele, respectively.

(B) Practical Utilization of Blood Groups and Serum Polymorphisms

1) Determination of parentage

Recently as a result of the very rapid spread of artificial insemination in animals the method of parentage test has been necessary. Blood grouping makes possible a check of the accuracy of the breeding records in regard to parentage.

TABLE 8 Examples of parentage cases in cattle solved after blood grouping (a) (J. RENDEL)

Calf	280	A/	$BO_3YB'E'_2$/	C_1W	V/V —/— —/— —/— S/	Z/—H'/
Dam	107	—/—	$BO_3YA'E'_2$/	C_1W	F/VJ/—/— M/	S/ —/— H'/
Bull	833	A/	GXE'_1/	C_1W	F/V—/— —/— M/ S/	Z/—H'/
Bull	48	A/	$BO_1YD'/BO_2YA'E'3\ C_2$		F/FJ/—/— —/— —/— —/—	Z/Z —/—

2) Diagnosis of zygosity

Blood grouping has been used in the diagnosis of zygosity in cattle twins. For diagnosing zygosity, both transferrin blood types were more efficient than either alone.

Frythrocyte mosaicism for hemoglobin types is demonstrated in a pair of dizygotic cattle twins by Stormont *et al.* (1964).

3) Dagnosis of freemartins

The majority of twin heifers from pairs of opposite sex will be sterile. Stone *et al.* demonstrated that blood grouping furnished a simple method of predicting which heifers will be fertile and which will be sterile.

4) Blood groups and hemolytic disease of the newborn

Hemolytic disease of the newborn is caused by the incompatibility of the blood groups between mother and fetus.

5) Blood groups in the registration of

pedigree.

Pedigree registration is very important and necessary in animal breeding. Determination of blood groups is the most important method being adopted in the registration of pedigree of animals.

6) Blood groups and animal breed structure

Frequencies of appearance of the blood antigenic factors in animals have been found to vary between different breeds. Blood groups limit the structrue of animal breeds.

7) Blood groups and production characters

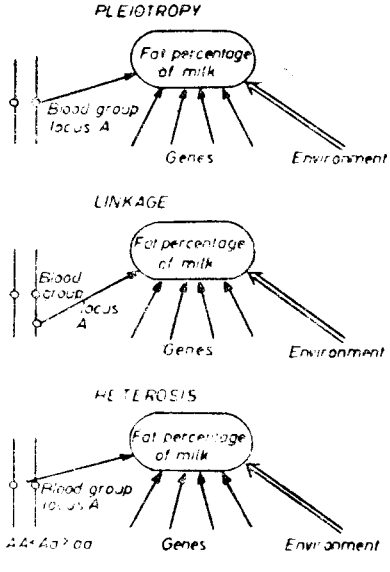
Relationships between blood group genes and production characters in domestic animals may principally arise from the following three different sources (by Rendel):

a. Pleiotropic effects of the genes.

b. Linkage between two loci.

One of which determines a blood character, the other one influences production ability.

c. Heterotic effects of blood group genes.



In the case of chicken:

Considerable data has now been accumulated relative to the association of blood group diffences with various performance traits of economic importance.

1. Hatchability was the first trait shown to be associated with B blood group genotypes.

2. Association between the blood types and the weight of inbred chicks at 9 weeks of age was also apparent in the early work at the Texas Station.

3. It has now been well established that the B system genes may be associated with economic traits in birds resulting from crossing inbred lines. The practice of crossing inbred lines or straing in producing commercial chickens is quite compatible with the efficient utilization of blood groups.

In the case of cattle:

1. Transferrin type Tf^D/ Tf^D cows have a slightly higher average milk yield than cows of the other genotypes, and the transferrin locus does not affect fertility (Datta *et al.* 1965).

2. Association between a blood group and butter fat production in dairy cattle:

A significant effect of the B phenogroup BO_1 Y_2 D′ on butter fat percentage was found. The percentage butter fat of cows with this allele was 0.33 higher than in those lacking it.

3. On linkage among the cattle A, B, C, FV, J, L, M, S and Z blood group loci and the milk protein loci β-lactoglobulin, α_{S1}-casein, β-casein and κ-casein, significant evidence of linkage was found by Hines *et al.* (1968) between the J and Lg loci, the α_{s1}-Cn and β-Cn loci, the α_{s1}-Cn and κ-Cn loci, and the β-Cn and κ-Cn loci.

TABLE 9 Transferrin locus and production traits

(DATTA et al.)

Herd[a]	Genotype	Cows	Butterfat yield		Milk yield	
			Mean	S.D.	Mean	S.D.
		No.	%	%	lb.	lb.
B	A/A	25	3.70	0.27	13,454	2,606
	A/D	72	3.85	0.29	12,627	2,655
	D/D	42	3.78	0.24	13,730	2,961
M	A/A	16	3.66	0.20	13,588	2,484
	A/D	40	3.68	0.28	14,073	1,811
	D/D	20	3.65	0.27	14,390	2,320

a. B=Blaine Farm; M=Madison.

TABLE 10 Partial regression coefficients of economic factors on blood group genes

(CONNEALLY et al.)

Blood group genes	Total milk yield (lb.) b±Sb	Butterfat %, b±Sb	Total butterfat (lb). b±Sb
$B^{BO}{}_1{}^{Y}{}_2{}^{D'}$	—967.18±1.430.4	0.332±0.136	—12.28±35.68
$B^{GY}{}_2{}^{E}{}_1$	251.0.8±1,019.9	0.058±0.114	1.85±29.83

*Significant at the 5 percent level.

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