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A total of 902 plasma samples from the Rambouillet, Targhee, Columbia, Lincoln, and Suffolk breeds of sheep were analyzed for transferrin type by disc electrophoresis. Six transferrin types, Tf^A, Tf^B, Tf^C, Tf^D, Tf^E, and Tf^P, were found in the Rambouillet, Targhee, and Columbia breeds while five, A, B, C, D, E, and four, A, B, C, D, types were observed in the Suffolk and Lincolns respectively. Mating data involving five of the six alleles confirmed previous reports that the transferrins are inherited as co-dominant autosomal allelic genes. No evidence was found to support a previous suggestion of maternal-fetal incompatibility expressed as a disturbed segregation ratio.

Gene frequencies were calculated for breeds and strains within breeds. It was shown that there were characteristic breed frequencies but they could not be used to predict the degree of

relationship between breeds. An excess of homozygotes was observed in the Rambouillet, Targhee, and Columbia breeds which was probably due to inbreeding and subdivision of the population. Characteristic frequencies were observed for inbreds, linecrosses, and control lines within breeds.

The frequency of the B allele was much lower in the Rambouillet, Targhee, and Columbia breeds of the U. S. Sheep Experiment Station at Dubois, Idaho, than in the Suffolk and Lincoln breeds of western Oregon. Since the Dubois Station differs considerably from western Oregon in severity of climate, altitude, management, plane of nutrition, and incidence of various diseases, it was suggested that the B allele might be at a disadvantage in higher altitudes with a more severe climate and a higher incidence of disease such as that present at the Dubois Station.

The effect of transferrin type on some pre-weaning and yearling performance traits was found to be significant for birth weight and average daily gain from birth to weaning. The effect of transferrin type on weaning weight and grease fleece weight, although not statistically significant, closely approached significance. Neither weaning nor yearling indices were significantly different among alleles. Since selection is by the index method in the sheep population at the Dubois Station, it was suggested that artificial selection has

little or no effect on frequency of transferrin alleles in these particular populations.

Genetics of Sheep Transferrins as Determined by Disc Electrophoresis

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GENETICS OF SHEEP TRANSFERRINS AS DETERMINED BY DISC ELECTROPHORESIS

INTRODUCTION

In a study of population dynamics there is a need for markers by which changes in the genetic variation or resemblance between different populations may be measured. The markers should show simple Mendelian inheritance, be fairly neutral with regard to production, viability, and reproduction, and in the case of domestic animals, be hidden from the breeder.

Developments in electrophoresis of proteins has stimulated the rapid discovery of genetic polymorphisms in the blood and other animal tissues and fluids. It is thus necessary to establish whether these markers are neutral or have some pleiotropic effect, large or small. If they are neutral or the pleiotropic effect is of minor importance, they should be of value in measuring the degree of relationship between breeds and the occurrence of strain differences within breeds.

The plasma transferrins can, because of their extensive polymorphism, serve as a model for testing the above statements. Consequently, the objectives of the present study were:

1. To determine the number and type of transferrin alleles present in American breeds and flocks of sheep. At the beginning of the present study, all the reported sheep transferrin typing had been

done in European and Australian breeds of sheep.

2. To determine the degree of relationship between breeds and the occurrence of strain differences within breeds by using blood markers, specifically the transferrins.

3. To determine if there is a natural adaptive value for different genotypes.

4. To determine if there is any association between transferrin type and some of the common productive traits in sheep.

Such a study is valuable for the knowledge it can have about the behavior of polymorphic systems in different populations as well as being of practical importance for animal breeders.

REVIEW OF LITERATURE

Physio-chemical Properties

The major part of the acid-soluble iron in plasma is reversibly bound to a specific metal-combining protein named transferrin (β-globulin, siderophilin) for its function of transporting iron to the bone marrow and tissue storage organs. This is an important function, since iron is a component of hemoglobin, myoglobin, cytochrome, catalase, succinic dehydrogenase, and xanthine oxidase. Transferrin also participates directly in the regulation and control of iron absorption and protects against iron intoxication.

The existence of a plasma iron binding protein was first demonstrated by Holmberg and Laurell (1945) and Schade and Caroline (1946). The physio-chemical properties of human transferrin have been extensively described in contrast to other mammals where these properties have not been described at all or in a very sketchy manner. Brief mention will be made of the physio-chemical properties of human transferrins and the assumption made that other animals show only minor differences from those of the human.

Ehrenberg and Laurell (1955) have shown that transferrin can combine with two atoms of ferric iron per molecule and the bond is ionic. The iron complex is stable in alkaline solutions, but

dissociates at neutral or acid pH (Laurell, 1960). Resistance to heat denaturation and hydrolysis by trypsin and chymotrypsin is dependent on the degree of iron saturation (Azaric and Feeney, 1958).

Crystalline transferrin is a glycoprotein, molecular weight 90, 000, with a carbohydrate portion of sialic acid, galactose, mannose, and N-acetylglucosamine in the molar ratio 4:4:8:8 (Jamieson, 1965). End group determinations indicate that transferrin C (human) has a single polypeptide chain with N-terminal valine (Putman, 1955). This is not conclusive evidence for a single polypeptide chain in that there are numerous examples in which N-terminal analysis has led to incorrect deductions as to the number and size of the polypeptide chains of proteins.

Smithies (1955) report in 1955 that electrophoresis of serum in starch gel separates the protein components much more effectively than other electrophoretic methods because the "sieving" effect of the gel is added to differentiation due to electrical charge, was probably the most important breakthrough in serum typing. Poulik and Smithies (1958), by use of the new technique, separated the β-globulin into several components, the major component being designated β-globulin C. Subsequently two more alleles, B and D, were described (Smithies, 1957; 1958). It was established by Smithies and Hiller (1959), on the basis of family studies, that the formation of the three β-globulins is determined by three allelic autosomal genes of equal

dominance. Smithies and Hiller (1959) also were able to show that the serum of heterozygotes has a normal amount of transferrin, each band being of about equal density. In a mixture of the sera of the two homozygotes, β-globulin C and D, the electrophoretic pattern of the β-globulin was indistinquishable from that of serum obtained from a heterozygote β-globulin CD. Smithies and Hiller (1959) cited evidence that the β-globulins were, in fact, transferrins and suggested the symbol Tf for the autosomal locus, with the superscript letters B, C, D. This notation will be observed throughout the remainder of this investigation.

Discovery of the polymorphism of transferrins in man stimulated interest in the possibility of various iron-binding proteins in other mammalian species. Investigation quickly led to the discovery of transferrin polymorphism in cattle (Ashton, 1957; Hickman and Smithies, 1957), sheep (Ashton, 1958a), goats (Ashton and McDougall, 1958; Millson and Pattison, 1961), horses (Ashton, 1958c), pigs (Ashton, 1960; Kristjanson, 1960), mice (Ashton and Braden, 1961; Cohen, 1961), chimpanzees (Boyer and Young, 1960), monkeys (Lai and Kirk, 1960; Blumberg, 1960; Goodman and Poulik, 1961), reindeer (Gahne and Rendel, 1961), and deer (Lowe and McDougall, 1961). In each species, where established, the inheritance has proved to be essentially the same as the human transferrins; namely, it is due to multiple autosomal alleles exhibiting co-dominance. These

investigations did reveal an unexpected difference in the pattern produced by a single allele. Each allele produced from one to four zones, the number of zones being characteristic of the species. The reasons for this multiplicity of zones from one allele has been discussed by various authors (Ashton and Braden, 1961; Cohen, 1961; Patras and Stone, 1961; Hines, Ludwick, and Rausch, 1967; Chen and Sutton, 1967).

The obvious resemblance to lactate dehydrogenase, where the pattern is produced by different combinations of two kinds of subunits into a tetramer, led Chen and Sutton (1967) to an investigation of bovine transferrin, the most complex pattern produced. Failure to dissociate the protein into subunits, led them to consider the carbohydrate moiety as a possible explanation. They suggested the most acceptable explanation yet presented; namely, the complex homozygous transferrin pattern can be explained in terms of the number of sialic acid residues per molecule. Those animals with a single protein (transferrin) product have four sialic acid residues per molecule. Those with two homozygous zones (sheep and goats) have a small percentage of transferrin molecules with only three sialic acid residues while the majority have four. Cattle would have a population of molecules with one, two, three, and four sialic acid residues per molecule. It would thus seem that some animals such as humans are more efficient in the attachment of sialic acid than other animals (Chen and

Sutton, 1967).

It would seem, however, that the electrophoretic differences of the major transferrin bands as seen in the heterozygote are due to amino acid changes in the polypeptide portion of the molecule. Wang, Howard, and Sutton (1967) isolated human transferrins C and D_{Chi} from a heterozygous $TfCD_{Chi}$ individual by a combination of rivanol percipitation and starch block electrophoresis. Peptide maps of the tryptic digests of the purified TfC and TfD_{Chi} showed the change of a single peptide. Chemical analysis indicated that a histidine residue in TfC was probably changed into an arginine residue in TfD_{Chi}.

Transferrin Type in Sheep

Type

Sheep transferrins show more marked diversity than those of other farm animals. Ashton (1958b) described a five allele system in British breeds and each allele produced two zones in starch gel, an intensely stained zone preceded by a less intense zone. He detected 14 out of the 15 possible types from Tf^A, Tf^B, Tf^C, Tf^D, and Tf^E. In a later study of Australian Merinos, Ashton and Ferguson (1963) described seven further transferrin alleles, Tf^F, Tf^G, Tf^H, Tf^J, Tf^N, Tf^K, and Tf^L. It was shown that there were marked breed differences in types of genes represented, and the frequency of these

genes, between the British breeds and the Australian Merino.

Khattab, Watson, and Axford (1963) examined the serum from a population of Welsh Mountain sheep and reported the discovery of a 13th allele, Tf^P. In addition to the new allele they found five of the previously reported alleles present in the population. Variation in frequency showed an apparent association between pairs of alleles as Tf^A and Tf^B; Tf^C and Tf^D; Tf^E and Tf^P, seemed to vary together. Alleles E and P maintained frequencies from 0.01 to 0.06 while the others varied between 0.2 and 0.3.

Efremov and Braend (1965) reported finding 15 transferrin phenotypes determined by six co-dominant alleles in five breeds of sheep, Old Norwegian, Dala, Steigar, Cheviots, and Oxford Down. All alleles were about the same frequency except Tf^P and Tf^S which were of very low frequency or were absent.

Nasrat and Oosterlee (1965) made a study of transferrin types in the serum of Texel sheep from the Netherlands, Osimi and Rahmani sheep from Egypt, and Suffolk sheep from Belgium. Four alleles were found in the Rahmani breed and five in the Texel, Osimi, and Suffolk breeds.

King and Fechter (1967) investigated the transferrin polymorphism in various South African breeds, South African Merino, German Merino, Letelle Merino, Dormer, Dorper, and Karakul. Data could not be found as to the number of alleles described, but

they did suggest that no characteristic breed differences were present in transferrin type distribution. It was also suggested that certain heterozygotes seemed to have a selective advantage. Fésüs (1967) reported the finding of a new allele, Tf^{I}, in a test of serum samples from Askanian Merinos.

Stormont (1968) investigated the transferrin types present in American flocks of sheep. He found five alleles, Tf^{A}, Tf^{B}, Tf^{C}, Tf^{D}, and Tf^{E}, in the Merino, Rambouillet, Corriedale, Dorset Horn, Suffolk, Southdown, and Navajo breeds. Only four alleles were found in the Targhee and Columbia breeds, Tf^{E} being absent. Frequencies of alleles within breeds were given and it was shown that breeds showed a characteristic frequency difference.

A total of 14 transferrin types have been reported from workers all over the world. Since these investigations have taken place in many different laboratories, misunderstandings concerning the symbols used for designation of types occurs. It was for this reason that a comparative test was established by various workers in the field of sheep transferrins (Oosterlee and Bouw, 1967). As a result of this study, it was recommended that nine transferrin types be recognized and that they be designated by the symbols P, E, D, M, C, B, G, A, and I in order of increasing mobility from cathode to anode. This nomenclature will be used in designation of transferrin type in the present study.

Association with Various Factors

Probably the first study associating transferrin type with another factor was conducted in 1958 when Ashton (1958b) reported that the frequency of Tf^E appeared to be greater in those breeds of cattle originating in parts of the British Isles with a more severe climate. The suggestion that transferrin polymorphism in cattle may be concerned with tolerance to climatic conditions is supported by some limited observations that the average heat adaptability coefficients tend to be higher in breeds with low Tf^E frequencies.

In 1960, Ashton (1960) associated transferrin type with milk production in dairy cattle. Ashton concluded that the transferrin locus is concerned in the genetic control of milk yield, the estimated mean genetic value of Tf^D or Tf^A being approximately +50 gallons per lactation. Rausch (1963) found a highly significant association between transferrin type and milk production. Animals which did not have Tf^E were much better producers than those which had Tf^E.

Concerning another interesting association, Ashton (1959) concluded from the results of matings between parents differing in transferrin type that the chances of survival of the bovine embryo are affected by the transferrin type of the dam. Ashton (1961) determined the transferrin type of 360 Jersey cows and 423 Australian Illawarra Shorthorn cows. In addition to the above, the transferrin type of 18

Jersey and nine Australian Illawarra Shorthorn bulls used for A. I. was tested. The results, expressed as breeding efficiencies of 1, 527 inseminations from the Jersey bulls and 1, 166 inseminations from the Australian Illawarra Shorthorn bulls were then examined with respect to the transferrin types of the bull and cow. He concluded that transferrin type had a highly significant effect on fertility in that homozygous matings were much more efficient than heterozygous matings (one or both parents heterozygous).

Ashton and Fallon (1962) sought to determine whether increased fertility among homozygous matings was due to differential mortality of the embryonic genotypes, or to differences in fertilization efficiency. They concluded that the transferrin locus affects fertility in cattle in both ways, at fertilization and in utero. In contrast, Moustgaard, Molles and Sorensen (1960) and Gahne (1961) were unable to associate transferrin type with lowered fertility in Danish and Swedish breeds of cattle.

In 1963, Khattab, Watson, and Axford (1963) were able to show a marked disturbance in segregation ratio at the transferrin locus in a population of Welsh Mountain sheep. It seems that the disturbance was more specific than that seen in cattle in that the disturbed segregation involved a particular allele; namely, when the dam possessed the Tf^{C} allele in the homozygous state, and the sire was heterozygous for the same gene.

Cooper (1967) in an examination of the transferrin type in Australian Merino sheep concluded that heterzygotes may have a marked selective advantage in inbred lines. He was unable, however, to find any support for the previous suggestion of Khattab, Watson, and Axford of maternal-fetal incompatibility based on transferrin type.

MATERIALS AND METHODS

Sample Sources

Five breeds of sheep, Columbia, Lincoln, Rambouillet, Suffolk, and Targhee, served as sample sources for this study. Columbia, Rambouillet, and Targhee samples came from the flock at the U. S. Sheep Experiment Station at Dubois, Idaho. The Suffolks sampled are part of the flock at Oregon State University. Mr. Don Kessi of Harlan, Oregon provided the flock from which the Lincoln samples were taken.

Some explanation should be given concerning the breeding system used at the U. S. Sheep Experiment Station as this involves a major part of the study. The Columbia and Targhee breeds were developed by the U. S. Sheep Experiment Station from crossing Lincoln and Rambouillet, after which breeding and selection were done in closed flocks. Three major breeds, Columbia, Rambouillet, and Targhee, are bred as lines, tester stock, outbreds, controls, and linecrosses. Only the inbreds, linecrosses and controls are of interest here.

Several inbred lines were established in each breed and subsequently, a diallel mating system was set up in order to obtain as many linecrosses as possible without reducing the existing lines.

Four lines and their respective linecrosses in each breed were selected to serve as a source of blood samples. All possible samples were taken in the 4 x 4 diallel system but sample size was unequal due to infertility and death. The control line in each breed was also sampled. The production data such as birth weight, average daily gain from birth to weaning, weaning type, weaning condition, weaning weight, average daily gain from weaning to yearling, yearling type, yearling condition, yearling weight, yearling grease fleece weight, and yearling fleece grade were furnished by the U. S. Sheep Experiment Station where available.

The Suffolks sampled came from a closed 3 sire, 45 dam line established at Oregon State University in 1952. The Lincolns are maintained as an open flock. No production data were collected on the Suffolk and Lincoln breeds as they were included in the study only for the purpose of the determination of transferrin types present and frequencies of these types in breeds of sheep other than those at the U. S. Sheep Experiment Station.

Method of Collection

The standard method of blood collection from mature animals was used; namely, a 14 guage by 3 inch teflon coated veterinary bleeding needle was inserted into the jugular vein and the blood collected in a test tube containing 2-3 drops of heparin (ammonium

sulfate, 1000 USP units/ml). A sodium citrate solution was used during the early stage of collection but later was discontinued in favor of heparin. Some lambs were bled using the same procedure.

Processing and Storage

Following collection, the samples were immediately processed or, if it was inconvenient to do so, they were stored for a few hours at 3-8° C until processing could be completed. All samples were processed within 16 hours after collection.

Processing was begun by centrifuging the samples at approximately 1800 R.P.M. for 20 minutes in order to separate the plasma from the cellular material. The above speed and time of centrifugation was found to give the most satisfactory results. Following centrifugation, about 4 ml of plasma from each sample was withdrawn with a 10 ml syringe and transferred to a 5 ml plastic semen vial which was sealed with a plastic cap.

The plasma samples were then stored in the freezer compartment of a refrigerator or large walk-in cooler (about 15-20° C) until they could be analyzed electrophoretically. Where it was necessary to transport the samples for long distances, as from the U. S. Sheep Experiment Station, an ordinary ice-chest packed with dry ice was used.

The length of storage had no apparent adverse effects on the

plasma transferrin type as some samples were thawed and refrozen many times with no change in type. This agrees with the report of Ashton and Ferguson (1963) that English reference sera which had been stored at -17° C for four years showed no change in transferrin type.

Electrophoresis

Most of the transferrin typing has been done with starch-gel but this method has a distinct disadvantage in being relatively slow. Where large numbers of samples have to be analyzed, a more rapid method is advantageous. With the development of disc electrophoresis by Ornstein and Davis (1962) came the faster method but it also raised the question as to whether the transferrin types revealed by the disc method are the same as those resolved by starch-gel. Rausch (1963) compared both methods with regard to determination of bovine transferrin type and found no difference in the two methods in terms of resolution of different types. With the above in mind, disc electrophoresis was used for the present study because of the obvious advantage of speed of analysis. The apparatus and procedures used in the present study are patterned from those suggested by Davis (1964) for use in determining serum types in humans.

A polyacrylamide gel column, formed in a small glass tube 75 mm in length, 7.0 mm outside diameter and 5.0 mm inside

diameter, was composed of three layers: (1) A large-pore gel (sample gel) containing the sample ions, 3 μl (micro liters) of plasma, in which the electrophoretic concentration of these ions was initiated; (2) A large-pore gel (spacer gel) in which electrophoretic concentration of the sample ions was completed; (3) A small-pore gel in which electrophoretic separation took place. The large-pore gels were designed to serve primarily an anticonvection media, while the small-pore gel served as a sieving as well as an anticonvection medium. After complete polymerization of all the gels, electrophoresis was performed in a vertical position, the gel containers attached to an upper buffer reservior and the lower ends immersed in the buffer solution of a lower reservior. The upper buffer chamber was filled with buffer, 5 ml of 0.001 percent bromophenol blue, tracker dye, added and the power supply connected, cathode to the upper reservior. A constant amperage of 3.5 milliamps per tube was applied until the light blue albumin band had migrated approximately 5 cm ($\pm$0.3 mm) from the origin, the origin being the junction of the small-pore gel and spacer gel. Since within a given run, the proteins in different gels migrated at slightly varying rates, it was often necessary to remove the gels which had migrated 5 cm, plug the reservior, reconnect the power supply, and continue the run until the albumin in all gels had migrated about 5 cm. The actual electrophoretic process usually took about one and one-half hours.

After electrophoresis, the gels were removed from the tubes and placed in 13 x 100 mm test tubes containing about 5 ml of fixative stain solution (one percent amido schwartz in seven percent acetic acid). The gels were removed from the fixative stain solution after about 45 minutes, washed in tap water, and placed in 15 x 125 mm test tubes containing seven percent acetic acid for destaining. Destaining was accomplished by repeated washings in seven percent acetic acid and usually was completed in 24 to 96 hours depending on the frequency with which the wash was changed and the intensity of the stain. Following destaining, the gels were placed in 10 x 75 mm test tubes, filled with seven percent acetic acid, and sealed with cork stoppers. They were stored in this manner until they could be analyzed for transferrin type.

Identification of Transferrin Bands

For determining transferrin type it was first necessary to establish which of the many bands present in the gel are transferrins. Visual examination of density and location of bands was the method used but confirmation of this method by more specific tests was desired. Sutton and Karp (1965) described a method of removing most of the proteins other than transferrin. For 0.1 ml of plasma, 0.1 ml of 0.15 percent ferric chloride solution (in 5 mM tris) was added; then 0.4 ml of 0.6 percent rivanol (2-ethoxy-6, 9-diaminoacridine

lactate), also in 5 mM tris, was added. The mixture was well shaken and the precipitate then centrifuged down. The clear yellow supernatant was used for electrophoresis. Electrophoresis of samples prepared in this manner gave upon staining with amido schwartz, a slight albumin band and the transferrin band(s) about midway between the albumin and the origin.

Another method described by Ornstein (1965) sought to identify transferrin uniquely by a color reaction for the bound Fe^{+++}. The iron stain is made as follows: 0.5 ml of 0.25 percent solution of 2, 4-Dinitroso-1, 3-naphthalenediol in absolute ethanol; 0.5 ml of a 10 percent solution of hydroquinone in absolute ethanol; and 10 ml of an acetate buffer (7 ml of glacial acetic acid and 16 gms of sodium acetate trihydrate, made up to 100 ml water), are mixed and the solution is filtered if cloudy.

Duplicate gels were run; one gel was placed in the iron stain and the other in the amido schwartz stain. A blue-green disc appeared about midway between the site of the albumin band and the origin in the gel placed in the iron stain. Comparison with the amido schwartz stained gel revealed that the transferrin band corresponded to the fairly dense band midway between the heavy albumin band and the origin.

Transferrin Type Determination

In order to determine the transferrin type of a sample, the position of the transferrin bands must be determined. There are several ways in which this can be accomplished. Three methods were used in the present study and, unless at least two of the methods gave identical results, the sample was run again. The first was by visual comparison of the unknown with a gel containing a sample of known transferrin type. The second was by placing the gel in a plexiglass block, 10 cm long, which had a groove 5 mm wide and 2 mm deep in the center and measuring the distance of migration of the transferrin band(s) and albumin band from the origin by means of lines which had been milled into the block (50 lines per inch). An R_A value was calculated according to the following formula:

$$R_A = \frac{\text{distance of transferrin from origin}}{\text{distance of albumin from origin}}$$

The R_A value obtained for the unknowns was then compared to a R_A value calculated for six reference samples, Tf^A, Tf^B, Tf^C, Tf^D, Tf^E, and Tf^P. The third method involved constructing a reference chart by making several runs of the reference samples, 200 total gels, measuring each one, and recording the distance of migration of each transferrin band at a particular albumin migration distance. When the unknown was measured, referral was made to the chart to find the

corresponding albumin migration distance and the correct transferrin type could then be read from the chart.

Statistical Analysis

All gene frequencies were calculated according to the following formula:

$$\text{Frequency of A} = \frac{2\,(\text{number AA}) + \text{AB} + \ldots + \text{AP}}{2\,(\text{total number of animals})}$$

The Chi-square method was used to test for significant differences in the frequency between breeds and lines.

The genotypic distribution was tested for agreement with the Hardy-Weinberg ($p^2 + 2pq + q^2 = 1$) expectation. The Chi-square method was used to test for significant deviations from expectation. In order to test for possible associations between transferrin type and certain production traits the least-square method of analysis was used for adjusting the production traits for effects of year of birth, breed, age of dam, type of rearing (single or twin) and sex prior to calculating the means of the traits for each genotype.

RESULTS AND DISCUSSION

Transferrin Type

As shown in Table 1, a total of 902 plasma samples were collected from sheep representing five genetic backgrounds and two different environments.

Table 1. Populations studied and their sources.

Populations	Sources	Number of Animals by Sources
1. Rambouillet	USDA Sheep Experiment, Dubois, Idaho	151
2. Targhee	as above	271
3. Columbia	as above	329
4. Suffolk	Oregon State University, Corvallis, Oregon	94
5. Lincoln	Mr. Don Kessi, Harlan, Oregon	57
Total		902

Each sample was analyzed electrophoretically and classified according to transferrin type. Six types, Tf^A, Tf^B, Tf^C, Tf^D, Tf^E, and Tf^P, each represented by a zone pair, were found in the Rambouillet, Targhee, and Columbia breeds (Table 2). Stormont (1968) found only five types in these three breeds, Tf^P being absent. The

frequency of Tf^P is very low and as will be shown later, Tf^P is even absent in certain strains within breeds. Thus, Tf^P could be easily overlooked due to sampling error and sampling within a particular strain.

Table 2. Transferrin types found in five populations of sheep.

	Populations				
Alleles	Rambouillet	Targhee	Columbia	Suffolk	Lincoln
Tf^A	x	x	x	x	x
Tf^B	x	x	x	x	x
Tf^C	x	x	x	x	x
Tf^D	x	x	x	x	x
Tf^E	x	x	x	x	-
Tf^P	x	x	x	-	-

Five and four transferrin types were observed in the Suffolk and Lincoln flocks, respectively. From Table 2 it can be seen that Tf^E was found in the Suffolks but not in the Lincolns while Tf^P was absent in both breeds.

No plasma sample contained more than two zone pairs and many contained only one. When two zone pairs were present, each was of about equal density and approximately one-half that of the one zone pair sample. Assuming that each zone pair is produced by the action of a single allele, the maximum number of phenotypic combinations can be calculated according to the formula

$$\frac{k(k+1)}{2},$$

where k represents the total number of alleles. Twenty-one combinations were found in the Rambouillet, Targhee, and Columbia breeds although all combinations were not found in each breed. Ten combinations were observed in the Lincolns and 14 in the Suffolks. Some examples of the various combinations are given in Figure 1.

The ratios of the phenotypes of the offspring of 151 matings agree with the previously reported findings that the polymorphism is controlled by a series of co-dominant autosomal allelic genes. Data on matings involving five of the six alleles, Tf^A, Tf^B, Tf^C, Tf^D, and Tf^E are presented in Tables 3 and 4.

No offspring has a transferrin type incompatible with the assumption of a single locus model. In Table 4 deviations from expectation were calculated for each mating and none were found to be significant. No evidence was found to support the findings of Khattab, Watson, and Axford (1963) of a maternal-fetal incompatibility, although this does not mean that such an association does not exist. Such small numbers were involved in the present study as to make the negative results inconclusive. Also Khattab, Watson, and Axford were investigating a breed of sheep, Merinos, different from those used in the present study.

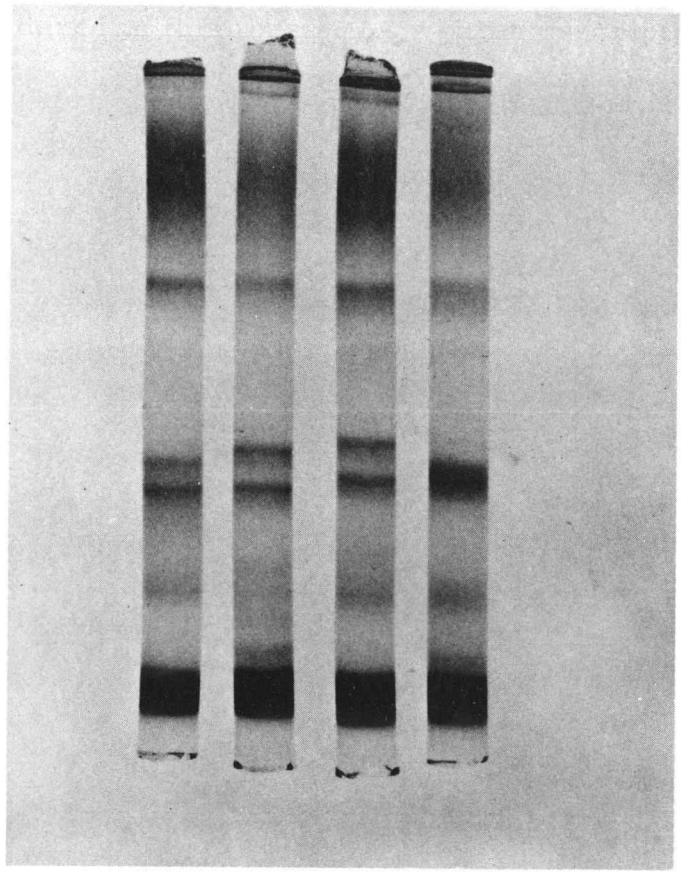

Figure 1. Polyacrylamide gels stained with amido schwartz. The origin is at the top (the dense black ring) and the broad band at the bottom is the albumin. The dense bands at about the middle of the gels are the transferrins. The transferrin types are from left to right, AD, AE, BP, and BB. The transferrins are labelled according to relative speed of mobility with A being the most rapid and P the slowest.

Table 3. Segregation of five transferrin alleles.

Sire	Dam	Offspring
AA	BC	AB
AA	BC	AC
CD	AA	AD
CD	AA	AC
CD	AD	AD
CD	AD	AC
CD	AD	DD
AE	CE	EE
AE	CE	AC
AE	DE	AD
AE	DE	AE
AB	DD	AD
AB	DD	BD
AD	AB	AD
AD	AB	AD
AD	AB	BD
AD	CD	AD
AD	CD	AC
AD	CD	CD

Table 4. Matings involving five transferrin alleles.

Parents Type (♂ x ♀)	No. of Matings	Observed Progeny Ratios			Chi-square
		TfAA :	TfA+ :	Tf++	
TfAA x TfA+[1]	8	6	2	0	2.000
TfA+ x Tf++	31	-	18	13	0.806
Tf++ x TfA+	8	-	4	4	0.000
TfA+ x TfA+	19	6	7	6	1.320
		TfBB :	TfB+ :	Tf++	
TfB+ x Tf++	3	-	1	2	0.400
Tf++ x TfB+	11	-	6	5	0.090
		TfCC :	TfC+ :	Tf++	
TfC+ x TfCC	3	1	2	-	0.400
TfC+ x TfC+	12	6	5	1	3.830
TfC+ x Tf++	8	-	4	4	0.000
Tf++ x TfC+	18	-	9	9	0.000
		TfDD :	TfD+ :	Tf++	
TfD+ x TfD+	14	1	10	3	3.150
		TfEE :	TfE+ :	Tf++	
TfE+ x Tf++	9	-	1	8	5.440
Tf++ x TfE+	7	-	3	4	0.140

[1] + is any allele not involved in the particular mating.

Frequencies

Between Breeds

As shown in Table 5 the frequencies of the alleles were calculated for each of the five breeds. The Chi-square test was then used to test for significant breed differences. It was found that each breed was significantly different from every other breed.

Table 5. Frequencies of transferrin alleles in five breeds of sheep. [1]

	Breeds				
Alleles	Rambouillet	Targhee	Columbia	Lincoln	Suffolk
Tf^A	0.268	0.384	0.281	0.228	0.340
Tf^B	0.076	0.066	0.061	0.298	0.266
Tf^C	0.202	0.220	0.307	0.289	0.170
Tf^D	0.315	0.221	0.284	0.184	0.192
Tf^E	0.126	0.089	0.062	0.000	0.032
Tf^P	0.013	0.020	0.003	0.000	0.000

[1] The frequency of each allele is given as a percentage of the total alleles in each of the populations sampled.

The Columbia sheep were developed by crossing Lincoln rams on Rambouillet ewes while the Targhee breed was created by crossing Rambouillet rams to Lincoln-Rambouillet and Lincoln-Rambouillet-Corriedale ewes. From this type of origin one would predict the gene frequencies for Targhees to be generally

intermediate between those for Rambouillet and Columbia. This appears to be true for alleles B, C, and E, but not for the others (Table 5). The Columbia breed does not exhibit any predictable relationship to the Rambouillet or Lincoln. The Lincolns sampled in the present study, however, are not necessarily a representative sample of those that were used in the original development of the Columbia and Targhee breeds. The Lincoln population sampled was relatively small; thus the gene frequency observed could be drastically different from other Lincoln populations as a result of genetic drift.

From these results it can be concluded that the transferrin locus can show definite breed differences in frequencies, but as a model for predicting breed relationships it is inadequate. In order to show breed relationships, several loci would be needed.

The transferrin locus was tested for genetic equilibrium in all five breeds. Chi-square tests showed that there was agreement with the expected Hardy-Weinberg frequencies in the Suffolk and Lincoln breeds but not in the Rambouillet, Targhee, or Columbia. The observed values compared with those expected under random mating are presented in Table 6.

This apparent disagreement with expectation in the Rambouillet, Targhee, and Columbia breeds could be a result of several factors. It could mean that a representative sample was not obtained

Table 6. Frequencies of transferrin phenotypes observed in 902 sheep compared with those expected under random mating.

	Targhee			Rambouillet			Columbia			Lincoln			Suffolk		
Phenotype	Obs	Exp.	O-E	Obs.	Exp.	O-E	Obs.	Exp.	O-E	Obs.	Exp.	O-E	Obs.	Exp.	O-E
AA	48	39.96	8.04	12	10.86	1.14	35	25.98	9.02	4	2.96	1.04	9	11.58	-2.58
BB	3	1.18	1.82	2	0.87	1.13	9	1.22	7.78	3	5.06	-2.06	9	6.1	2.90
CC	14	13.12	0.88	7	6.16	0.84	48	31.01	16.99	3	4.76	-1.76	3	2.41	0.59
DD	15	13.12	1.88	21	14.98	6.02	48	26.54	21.46	2	1.93	0.07	2	3.47	-1.47
EE	3	2.15	0.85	10	2.40	7.60	9	1.26	7.74	-	---	---	0	0.10	-0.10
PP	1	0.11	-0.89	1	0.03	0.97	0	0	0	-	---	---	-	---	---
AB	1	13.74	-12.74	4	6.15	-2.55	1	11.28	-10.28	7	7.74	-0.74	15	16.83	-1.83
AC	51	45.79	5.21	23	16.35	6.65	65	56.76	8.24	8	7.51	0.49	12	10.56	1.44
AD	47	46.00	1.00	27	25.49	1.51	45	52.51	-7.51	3	4.79	-1.79	18	12.67	5.33
AE	13	18.52	-5.52	3	10.20	-7.20	4	11.46	-7.46	-	---	---	1	2.11	-1.11
AP	1	4.14	-3.14	0.	1.05	-1.05	0	0.56	-0.56	-	---	---	-	---	---
BC	1	7.86	-6.86	0	4.64	-4.64	1	12.32	-11.32	13	9.82	3.18	7	7.67	-0.67
BD	5	7.91	-2.91	3	7.23	-4.23	6	11.40	-5.40	8	6.25	1.75	9	9.21	-0.21
BE	18	3.18	14.82	12	2.89	9.11	14	2.49	11.51	-	---	---	1	1.54	-0.54
BP	4	0.72	3.28	0	0.30	-0.30	0	0.12	-0.12	-	---	---	-	---	---
CD	33	26.35	6.65	22	19.22	2.78	37	57.37	-20.37	6	6.06	-0.06	4	5.77	-1.77
CE	6	10.61	-4.61	2	7.69	-5.69	3	12.52	-9.52	-	---	---	3	0.96	2.04
CP	1	2.38	-1.38	0	0.79	-0.79	1	0.61	0.39	-	---	---	-	---	---
DE	3	10.66	-7.66	0	11.99	-11.99	2	11.59	-9.59	-	---	---	1	1.15	-0.15
DP	2	2.38	-0.38	1	1.24	-0.24	1	0.56	0.44	-	---	---	-	---	---
EP	1	0.96	0.04	1	0.50	0.50	0	0.12	-0.12	-	---	---	-	---	---
Total	271			151			329			57			94		

or it might suggest that some selective force natural or artifical was disturbing the equilibrium. If selective forces were acting on the transferrin locus, one might expect it to favor the heterozygote, although heterozygote advantage is not the only selective mechanism possible. Examination of Table 6 reveals a consistent excess of homozygotes in the Rambouillet, Targhee, and Columbia breeds while the data for the Lincoln and Suffolk breeds fit the expected values rather closely. There are some exceptions in that the BE heterozygote shows a large excess in the Rambouillet, Targhee, and Columbia and the AC heterozygote is also in excess but not to the same extent as BE. These will be discussed later. The excess of homozygotes in those breeds with a disturbed equilibrium suggests another alternative. A subdivision of a large population into strains would be expected to give an excess of homozygotes in the entire population. Likewise, a population that is inbred should show a deficiency of heterozygotes that is proportional to the degree of inbreeding. In the five populations sampled in the present study only the Rambouillet, Targhee, and Columbia breeds were subdivided into smaller populations. The Rambouillet, Targhee and Columbia sheep populations also show various levels of inbreeding while the Suffolk and Lincoln populations have essentially no or low levels of inbreeding. Thus the Rambouillet, Targhee, and Columbia populations exhibit an excess of homozygotes due to subdivision and inbreeding. Where subdivision

and inbreeding are not present as in the Suffolk and Lincolns the agreement with the Hardy-Weinberg expectation is seen.

This is somewhat in disagreement with most reports where breed samples have been tested for agreement with Hardy-Weinberg expectation. Surprisingly most seem to agree closely with the expected values. Chance deviations might easily occur in gene frequency between sexes in small breeding units, and as shown by Robertson (1965) this will yield an excess of heterozygotes. Thus in a large random sample of a breed the excess of homozygotes due to subdivision may be partly cancelled by the excess of heterozygotes due to the differential sex frequency.

The frequency by sex in the Rambouillet, Targhee, and Columbia breeds is given in Table 7. There is no difference in frequency by sex over the entire population. The frequency was not broken down into smaller breeding groups because of insufficient numbers. It might be noted that Tf^{B} and Tf^{E} have consistently higher frequencies in the males, although the differences are small.

Within Breeds

As mentioned previously sampling in the Rambouillet, Targhee, and Columbia breeds of sheep was done in a 4 x 4 diallel system over a three year period. An unselected control line was also sampled in 1967. The frequencies of the transferrin alleles in the

inbreds, linecrosses, and control lines within breeds are presented in Table 8. All inbreds and all linecrosses were grouped within a breed because of small sample sizes.

Table 7. Frequencies by sex in three breeds of sheep.

	Rambouillet		Targhee		Columbia	
Alleles	Male	Female	Male	Female	Male	Female
Tf^A	.244	.310	.367	.398	.298	.268
Tf^B	.083	.063	.071	.061	.066	.057
Tf^C	.202	.190	.217	.223	.268	.336
Tf^D	.315	.324	.226	.217	.287	.286
Tf^E	.131	.113	.102	.080	.081	.047
Tf^P	.024	.000	.018	.022	.000	.005

Table 8. Frequencies of transferrin alleles in inbreds, linecrosses, and controls in three breeds of sheep.

Breeds	No. of Animals	Alleles					
		Tf^A	Tf^B	Tf^C	Tf^D	Tf^E	Tf^P
Rambouillet							
Inbreds	47	0.31	0.12	0.11	0.34	0.12	0.00
Linecrosses	55	0.32	0.06	0.26	0.27	0.08	0.02
Controls	52	0.16	0.09	0.21	0.34	0.18	0.02
Targhee							
Inbreds	72	0.53	0.07	0.14	0.21	0.06	0.00
Linecrosses	119	0.36	0.06	0.26	0.22	0.08	0.03
Controls	43	0.29	0.11	0.22	0.23	0.12	0.04
Columbia							
Inbreds	95	0.19	0.10	0.35	0.26	0.09	0.007
Linecrosses	144	0.29	0.04	0.32	0.29	0.05	0.003
Controls	45	0.30	0.03	0.29	0.32	0.06	0.00

It can be seen that there are characteristic differences among strains within breeds. The Columbia sheep show less stratification than the other two breeds. Results from the Chi-square test for fit to the Hardy-Weinberg expectation, data from hemoglobin studies by Templeton (1968), and data furnished by Stormont (1968) suggest that the Columbia control sample of the present study was not a representative sample. The Rambouillet and Targhee control lines fit the Hardy-Weinberg expectation and are thus considered to be good samples.

The A allele is relatively high in the inbreds in comparison to the control line in the Rambouillet and Targhee breeds (Table 8). Since the Columbia control is not representative, little can be said about the difference in the frequency of the A allele for inbreds and linecrosses in this breed. Also it can be seen from Table 8 that the C allele is lower in the inbreds than in the other groups in the Rambouillet and Targhee breeds.

Table 9, in which the inbreds are broken down into individual lines, reveals that A and C are high in some lines and relatively low in others. It would thus seem that there is little or no selection for or against these particular alleles but rather a chance fixation of alleles in some lines. When small lines are created the frequency of the genes will be determined largely by chance. In brief, the factor of chance variation dominates the change in gene frequencies of small

groups, and the selection force may be completely ineffective.

Table 9. Frequency of transferrin alleles by inbred line in three breeds of sheep.

Breeds	Line	No. of Animals	Alleles					
			Tf^A	Tf^B	Tf^C	Tf^D	Tf^E	Tf^P
Rambouillet	39	15	0.17	0.13	0.13	0.47	0.10	0.00
	45	12	0.46	0.04	0.08	0.29	0.08	0.00
	49	10	0.10	0.10	0.20	0.45	0.15	0.00
	50	10	0.50	0.20	0.05	0.20	0.05	0.00
Targhee	3	22	0.61	0.11	0.07	0.16	0.05	0.00
	11	8	0.25	0.00	0.19	0.44	0.13	0.00
	14	18	0.53	0.14	0.14	0.11	0.08	0.00
	8	12	0.92	0.00	0.00	0.04	0.04	0.00
Columbia	6	15	0.00	0.20	0.43	0.37	0.00	0.00
	7	19	0.11	0.13	0.47	0.21	0.08	0.00
	9	24	0.29	0.06	0.33	0.19	0.10	0.02
	10	29	0.37	0.10	0.14	0.28	0.10	0.00

In Table 10 the number of homozygotes and heterozygotes observed is compared to those expected under random mating. All inbred lines behave as predicted in that a significant excess of homozygotes is observed. The Columbia control line probably represents sampling error as already mentioned while the excess of homogygotes in the Columbia linecross may be due to sampling error or population subdivision.

Since the Rambouillet, Targhee, and Columbia breeds were sampled in three different years, the frequency was calculated by breed within year in order to check sampling. It was found that the

Table 10. Frequency of homozygotes vs. heterozygotes in the inbreds, linecrosses, and controls in three breeds of sheep.

Breeds	Homozygotes Obs.	Exp.	O-E	Heterozygotes Obs.	Exp.	O-E	Chi-square (1 d.f.)
Rambouillet							
(47)[1] Inbreds	23	11.72	11.28	24	35.28	-11.28	14.463**
(55) Linecrosses	12	13.78	-1.78	43	41.22	1.78	0.307
(52) Controls	16	11.65	4.35	36	40.35	-4.35	2.09
Targhee							
(72) Inbreds	34	25.15	8.85	38	46.85	-8.85	4.786*
(119) Linecrosses	32	30.38	1.62	87	88.62	-1.62	0.116
(43) Controls	7	9.22	-2.22	36	33.78	2.22	0.680
Columbia							
(95) Inbreds	49	23.68	25.32	46	71.32	-25.32	36.060**
(144) Linecrosses	58	40.13	17.87	86	103.87	-17.87	11.031**
(45) Controls	26	12.65	13.35	19	32.35	-13.35	19.598**

[1]Total number of animals. *P < .05 **P < .005

variation in frequency by years was very low for those alleles of low frequency but higher where the allele frequency was higher. None of the yearly variations were extremely large, however. The variations observed agree closely with sampling theory which predicts that variations due to small sample size will be lower for those genes of low frequency than for genes of higher frequency.

Adaptive Advantage

As noted previously it can be seen from Table 5 that the B allele is very low in frequency in the sheep at the Dubois station while it is relatively high in sheep of western Oregon. The two environments are considerably different with respect to climate, topography, season of lambing, planes of nutrition and levels of animal care. There are no significant differences among inbreds, linecrosses, and controls for the B allele in the Rambouillet, Targhee, and Columbia breeds (Table 8). This would suggest that the low frequency of B at the Dubois station is not associated with artifical selection.

From Turnbull and Giblett's (1960) work it does not appear that human transferrins differ in their iron-binding capacity. There is the possibility, however, that they differ in the facility with which they transfer it to the tissues, thus endowing some genotypes with an advantage that others lack. The B allele might be at a disadvantage in a severe climate and at a high altitude such as that of the Dubois

station. Where the climate is mild and the altitude is relatively low such as in western Oregon the allele might be neutral. Thus at Dubois the B allele would be selected against and its frequency would be relatively low while in western Oregon the frequency of the B allele would be determined entirely by chance. It is possible, however, that the high frequency of the B allele in flocks of sheep under western Oregon conditions is the result of genetic drift. The populations studied in western Oregon are small. Since the Suffolk flock was established in 1952 one would expect the frequency of the B allele to be lower if there was selection against it in western Oregon. Thus even if a high frequency of the B allele due to genetic drift had been originally present, one would expect the frequency to go down if selection was working against it. This does not appear to have happened in the Suffolk flock at Oregon State University.

Another possible explanation for the low frequency of the B allele at the Dubois station is that the transferrin variants may confer some advantage under appropriate conditions associated with disease. Martin and Jandl (1960) presented evidence that the transferrins are able to suppress viral multiplication in vitro. There is not sufficient evidence that transferrins also possess this function in vivo but the possibility exists that they may be able to do so. It is also possible that the transferrin variants might confer some resistance to bacterial infections either directly or by resistance to viral

disease which would prevent the lethal effect of secondary bacterial invaders.

The station at Dubois had an outbreak of contagious vibrionic abortion in 1962. They have also had a more or less endemic occurrence of epididymitis. As far as the author knows neither of these have appeared in the western Oregon flocks during the past few years.

If there is a differential resistance to these diseases, or others, among the transferrin alleles, one would expect those alleles that exhibited the least resistance to decrease in frequency in the population. Thus those animals with the B allele might be particularly susceptible to disease with accompanying death or greatly reduced vigor. Periodic outbreaks of diseases would serve to keep the frequency of the B allele at a very low level.

There appears to be an inconsistency relative to the B and E allele in the Rambouillet, Targhee, and Columbia breeds. As has already been shown the homozygotes are in excess at the expense of heterozygotes in these three breeds. There is one heterozygote, BE, which is in considerable excess. One might argue that the BE heterozygote was incorrectly identified; however, this seems highly unlikely in that the excess was consistent in the Rambouillet, Targhee, and Columbia breeds and not present in the Suffolks. Another plausible explanation might be that there is a heterozygous advantage for the BE combination. There is no evidence to allow one to speculate either for

or against this explanation.

Another explanation and the one that appears to be the most logical is that there is a sex difference as to the frequency of the B and E alleles in small breeding units. This would be expected to give an excess of heterozygotes (Robertson, 1965). It has already been shown that the only appreciable sex difference in allelic frequency involves the B and E alleles. Thus in small breeding units where often the number of sires is one, the observed number of heterozygotes should be greater than the Hardy-Weinberg expectation when the gene frequency shows a sex difference. This is apparently what happened in the case of the BE heterozygote in the present study.

Association of Transferrin Type with Production Traits

The performance of sheep with a given transferrin allelle was compared with those having a different allele by the least squares analysis of variance. The grouping of genotypes was done as follows:

2AA	2BB	2CC	2DD	2EE	2PP
1AB	1BA	1CA	1DA	1EA	1PA
1AC	1BC	1CB	1DB	1EB	1PB
1AD	1BD	1CD	1DC	1EC	1PC
1AE	1BE	1CE	1DE	1ED	1PD
1AP	1BP	1CP	1DP	1EP	1PE

Each homozygote was weighted by a factor of two and the heterozygotes by one. This method gives the general effect of each allele on the various production traits. Estimates of the effect of each

genotype would be better than estimates of the general allelic effect but the number of observations in the present study were not adequate to give a reliable estimate of the effect of some genotypes. The analysis was separated into two parts, traits measured during the pre-weaning period and traits measured at the yearling stage, because yearling data were not available on some of the animals. The effects of age of dam, sex, type of rearing, breed, and year of birth were either significant or highly significant for most of the production traits used in the present study (Tables 11 and 12). Transferrin type had a significant effect on birth weight and average daily gain from birth to weaning. The effect of the transferrin locus on weaning weight and grease fleece weight closely approached statistical significance (Tables 11 and 12).

It is rather surprising that the effect due to transferrin type was found to be significant for any of the pre-weaning production traits since the performance of the young is highly influenced by the maternal effect in most domestic animals. If transferrin type had an effect on milk production in sheep, it should be manifest in the dam. The experimental procedure of the present study was such that it was not possible to correlate the transferrin type of the dam with the performance of her offspring. What is seen in the present study is the general allelic effect and this is probably an indirect reflection of the effect due to transferrin type of the dam. It is also possible that the

Table 11. Mean squares for pre-weaning performance traits in the Rambouillet, Targhee, and Columbia breeds of sheep.

Source of Variation	Average Daily Gain	Birth Weight	Type	Condition	Weaning Weight	Weaning Index	Degrees of Freedom
Age of Dam	0.096**	25.69**	5.31*	12.01**	1,569.32**	96.45	3
Sex	0.509**	84.71**	2.45	45.87**	8,036.68**	822.41*	1
Type of Rearing	0.730**	168.50**	102.22**	168.58**	11,838.16**	107.43	2
Breed-year	0.069**	6.91*	68.44**	33.78	1,867.77**	15,248.77**	8
Transferrin Type	0.018*	5.16*	2.67	3.09	238.70+	155.33	5
Error	0.0059	2.27	1.89	1.87	109.99	133.43	619

*P < .05 **P < .01 +.10 > P > .05

Table 12. Mean squares for yearling performance traits in the Rambouillet, Targhee, and Columbia breeds of sheep.

Source of Variation	ADG to Yearling	Type	Condition	Yearling Body Weight	Fleece Grease	Grease Fleece Weight	Yearling Index	Degrees of Freedom
Age of Dam	0.003	6.03*	6.64*	910.73*	0.536	23.66**	79.13	3
Sex	0.044**	32.85**	89.89**	22,551.14**	2.66	412.40**	1,413,157.17**	1
Type of Rearing	0.021**	6.92*	14.85**	3,044.96**	0.273	68.98**	416.54	2
Breed-year	0.011**	81.83**	77.51**	3,071.59**	87.49**	69.51**	408,082.92**	5
Transferrin Type	0.0008	0.609	1.19	265.88	0.547	7.97+	200.33	5
Error	0.0016	1.99	2.14	198.35	0.992	3.72	574.01	410

*P < .05 **P < .01 +.10 > P > .05

effect on average daily gain from birth to weaning is a reflection of a differential effect of transferrin type on general vigor. Animals with a particular transferrin type might be more vigorous than animals with a different type and would thus be better able to forage for themselves under range conditions. This increased vigor would be reflected in a slightly higher average daily gain. It would be difficult from the results of the present study to distinguish between the two alternatives. It is worth mentioning that the differences among allelic combinations in average daily gain from weaning to yearling was not significant (Table 12). The effect of dam was no longer present and the animals were in feedlots where better foraging ability would be less important.

The significant effect of transferrin type on birth weight might be due to a slight maternal-fetal incompatibility. The incompatibility would not be lethal, which would result in a disturbed segregation ratio. Rather the incompatibility, if present, would be slight and could possibly account for differences in pre-natal growth. A more detailed study of specific genotypic comparisons would be necessary to substantiate claims of sub-lethal maternal-fetal incompatibility.

The least-squares means for the pre-weaning and yearling performance traits are presented in Tables 13 and 14. The means give little indication which of the alleles is responsible for the significant effect for average daily gain from birth to weaning and

Table 13. Least squares means by alleles for pre-weaning performance traits in the Rambouillet, Targhee, and Columbia breeds of sheep.[1]

Alleles	Average Daily Gain	Birth Weight	Type	Condition	Weaning Weight	Weaning Index
P	0.538	10.93	8.56	7.95	75.29	144.25
E	0.550	10.42	8.23	7.72	75.47	143.44
D	0.547	10.70	8.43	7.81	75.06	144.24
C	0.554	10.83	8.35	7.75	76.13	144.90
B	0.548	10.60	8.51	7.97	75.20	145.45
A	0.545	10.67	8.44	7.87	75.13	144.23

[1]Values given for type, condition, and weaning index are relative.

Table 14. Least squares means by alleles for yearling performance traits in the Rambouillet, Targhee, and Columbia breeds of sheep.[1]

Alleles	ADG Weaning to Yearling	Type	Condition	Yearling Body Weight	Fleece Grade	Grease Fleece Weight	Yearling Index
P	0.106	8.49	8.26	103.55	3.69	10.51	219.19
E	0.098	8.33	8.01	101.31	3.41	10.51	220.73
D	0.104	8.35	7.98	103.69	3.45	10.67	223.16
C	0.102	8.29	8.03	104.34	3.49	11.10	224.33
B	0.109	8.28	7.94	104.60	3.37	10.57	223.58
A	0.104	8.36	8.06	103.61	3.47	10.69	222.33

[1]Values given for type, condition, fleece grade, and yearling index are relative.

birth weight. The mean for the P allele is lower for ADG but higher for birth weight in comparison with the other means . Since selection is practiced by the index method at yearling age, the pre-weaning significant effects of transferrin type are lost unless they are included in the index. Even if they are included they may be weighed such that the significant effect is lost. In other words if the effect due to transferrin type is small, as apparently it is, and its effect is not reflected in the index (Tables 11 and 12), artificial selection will be completely ineffective in establishing frequencies within breeds. This point is rather important if one proposes to use marker genes in breed comparisons and in studies of genetic variability. If marker genes had a pleiotropic effect on production traits, disease resistance, and climatic tolerance, slow but directional changes in gene frequency would then be added over hundreds of generations, and similarity between breeds for marker genes would indicate a common environment rather than a common origin. The results of the present study indicate that pleiotropic effects are indeed present, but they are small and probably of minor importance at least for the production traits.

SUMMARY AND CONCLUSIONS

A total of 902 plasma samples from the Rambouillet, Targhee, Columbia, Lincoln, and Suffolk breeds of sheep were analyzed for transferrin type by disc electrophoresis. Six types, Tf^A, Tf^B, Tf^C, Tf^D, Tf^E, and Tf^P, were found in the Rambouillet, Targhee, and Columbia breeds. The different types were classified according to the nomenclature recommended by Osterlee and Bouw (1967). Five types were observed in the Suffolks, Tf^P being absent, while the Lincolns lacked both Tf^P and Tf^E. Available mating data confirmed previous reports that the transferrins are inherited as autsomal co-dominant alleles. No disturbance in segregation ratios as tested by Chi-square was found.

The frequency of the alleles by breed was calculated and significant breed differences were observed. It was impossible from the results presented in this study to ascertain the degree of relationship between breeds. Frequencies of the various alleles by inbred lines, linecrosses and controls were calculated within breeds in the Rambouillet, Targhee, and Columbia breeds. It was found that there were significant differences among the inbreds, linecrosses, and controls in all three breeds. The inbred lines behaved as predicted by inbreeding theory; namely, a significant excess of homozygotes was observed in the inbreds. The Columbia breed was somewhat

peculiar in that homozygotes were also in excess in the linecrosses and controls. The excess of homozygotes in the linecrosses was suggested to be a result of sub-division while the excess of homozygotes in the control line probably represented inadequate sampling.

The frequency of the B allele was found to be much lower in the Rambouillet, Targhee, and Columbia breeds than in the Lincoln and Suffolk breeds. It was suggested that this might have resulted from differences in climate and altitude. Animals with the B allele could be at a disadvantage at higher altitudes and in more severe weather such as that at the Dubois station. The suggestion was also made that the transferrins might confer differential resistance to disease. Some instances of heterozygous excess were observed. The excess of the BE heterozygote was the most pronounced. It was suggested, however, that this excess was due to a differential sex frequency in small breeding groups.

The least-squares analysis of variance was used to test for possible associations of pre-weaning and yearling performance traits with transferrin type. The data were such that only the general effect of each allele could be tested. It was found that the effect due to transferrin type was statistically significant for birth weight and average daily gain from birth to weaning. The association of transferrin alleles with weaning weight and grease fleece weight closely approached statistical significance. The least-squares means were

given for all production traits used in the present study. It was suggested that artificial selection has little or no effect on allelic frequency at the transferrin locus in those populations examined in the present study.

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