INCOMPLETE FAMILY DATA, SELECTION AND POPULATION STUDIES OF TRANSFERRINS AND BLOOD GROUPS IN CATTLE.

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1. INTRODUCTION

APART from man, the blood groups of cattle are better known than those of any other organism. At least 12 different systems of red cell antigens are known and in the last decade 13 enzyme and other protein polymorphisms have been detected in blood and milk (see Cohen, 1962; Kiddy, 1964; Matousek, 1965; Grosclaude, 1967). The principal impetus for this effort has been utilitarian, the use of blood groups to resolve cases of doubtful parentage. The known variability of cattle red cell antigens is very great, greater than that of man; to encounter two animals with the same blood type is very unlikely and so many breed societies and artificial insemination organisations make blood typing a requirement of registration, particularly for the top strata of breeding bulls. The hope that something of importance might be discovered about the productive capacity of the animal has acted as an extra incentive (reviewed by Majjala, 1966). In the United States, laboratories for blood typing service and research have been in operation since about 1950 and in Scandinavia since 1954. Elsewhere the expansion has been very great in recent years.

In the course of their operation over a period of years, cattle blood group laboratories compile considerable genetic data. Such data have frequently been used to study the population genetics of cattle, which is becoming increasingly well described. But hitherto comparatively little effort has been made to use these segregation data to study the selective forces affecting the polymorphisms. One reason for this neglect is that much of the reliable family data obtained by blood group laboratories is not complete. The sire and offspring, or the dam and offspring, may be typed, but less commonly all three.

Geneticists are accustomed to dealing with complete family data (C.F.D.) and with population data (P.D.), but they have seldom used incomplete family data (I.F.D.). Consequently, cattle blood group workers, taking their genetical techniques from other fields, have tended to think in terms of C.F.D. and P.D. and overlook the use which can be made of I.F.D. This has meant that most workers have used P.D. only because C.F.D. are often not readily accessible.

Unfortunately P.D. are sensitive to heterogeneity of gene frequency between subpopulations as well as to departures from Mendelian segregation ratios. It has never been clear how much this matters in cattle. The small effective population size of some breeds or strains within breeds suggests that

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such heterogeneity may be sufficient to render the Hardy-Weinberg expectations invalid (Robertson, 1965) and so investigation of the question is necessary. In this paper, we will show that I.F.D. allow detection of heterogeneity of gene frequency and the effect of selection in such a way that the two can be distinguished.

Data from the FV and Z blood group systems and the transferrin system will be examined. We hope to provide an example which other blood group laboratories will follow by analysing their data. In order to assess our own results we have reviewed previously published family and population data. There exists a fairly large amount of data for the transferrin system. Our examination of these data has allowed us to make a critical re-appraisal of Ashton's (1965) argument that this polymorphism may be a balanced one.

2. MATERIAL

The study will be based on (1) information in numerous publications and (2) on data accumulated in our laboratory during the last decade. Our own data comprised 2897 registered bulls of the Swedish Red and White breed (SRB), which had been tested against 38-45 blood typing reagents detecting red cell antigens in 9 genetic systems. The sample included all SRB bulls used in artificial insemination or sold at the official breed sales during the period 1955-65. In addition it included stud bulls in a number of bull-breeding herds. The sample is representative of the top strata of the breed. The age at testing varied considerably. When the test for red cell antigens was first introduced in 1955, all available A.I. bulls were tested and so the sample includes 138 bulls born as early as in the period 1940-49. The bulls born after 1953 were usually tested when 12-24 months old, though in recent years there has been a tendency towards a decrease in age as the A.I. centres tend to buy their bulls as calves. The vast majority of the bulls have for some period of time been used for artificial or natural service.

As in most populations of breeding bulls this sample included animals of various degrees of relationship. The bulls were the progeny of a total of 707 sires, of which 330 were included in the red cell antigen tested sample. These 330 sires had a total of 1953 offspring in the sample. Their number of offspring varied from 1 to 67 with an average of 5.9 and a median of 13.

In 1959 transferrin testing was initiated. Transferrin type was available on 1685 animals, and of these 597 had also a transferrin tested sire. The larger part of the transferrin testing was carried out by Dr B. Gahne in our laboratory. The tests for red cell antigens were carried out as described by Rendel (1958) and the transferrin typing as given by Gahne (1961).

3. HETEROGENEITY OF GENE FREQUENCY IN CATTLE POPULATIONS: METHODS FOR ITS DETECTION

Heterogeneity of gene frequency arises because of the limited number of male parents in any one generation and the consequent rather large random changes in gene frequency which can occur from generation to generation. Most breeds tend to be broken up into subpopulations between which there is restricted genetic exchange and so rather rapid genetic drift occurs. Examples of this phenomenon have been given for blood group data for Swedish (Rendel, 1958, 1963), Danish (Neimann-Sørensen, 1958), and Dutch cattle (Bouw, 1960), for transferrins in British cattle (Jamieson, 1966)

and milk proteins in Swedish cattle (Sandberg, 1967). There may be two effects upon the Hardy-Weinberg expectations depending upon the mating practices within the breed:

1. *The Wahlund effect.* Wahlund (1928) first showed that if a population is subdivided into different subpopulations with differing gene frequencies, the expectations for the proportions of genotypes in the whole population are no longer given by the Hardy-Weinberg expectations. Instead, for the two-allele case, the two homozygous classes are each increased by a proportion equal to the variance in gene frequency between subpopulations and the heterozygous class is decreased by a proportion equal to twice that amount. Jamieson (1966) has found this effect in British Friesians.

2. *The Robertson effect.* Robertson (1965) has called attention to another possibility. In a single herd, the limited number of bulls make it likely that large random differences in gene frequency between bulls and cows can occur. Robertson shows that the number of heterozygotes will be increased over Hardy-Weinberg expectations by a proportion equal to $1/8 M$, where M is the number of sires. Thus, if an attempt is made to circumvent Wahlund's effect by analysing data within herds, Robertson's effect may be encountered. Robertson himself only considered the possibility of sex differences within herds. The same effect could be obtained when the bulls used in a breed come from one particular section of the breed, whose gene frequency differs from that of most other parts of the breed.

The existence of these two effects places the cattle geneticist in a predicament. Usually there will not be enough ancillary information to judge the importance of the two effects. The P.D. themselves do not have enough degrees of freedom to test for the effects of population structure and selection separately. However, if I.F.D. are available one can detect Wahlund's effect and circumvent Robertson's effect. Essentially, this method is an expansion of a suggestion by Cooper (1966).

Consider a population (e.g. a breed of cattle) subdivided into a series of subpopulations (e.g. herds). There are two non-dominant alleles A_1 and A_2 and the frequency of A_1 in the i th population is p_i . The overall frequency in the whole population, i.e. the mean of the p_i s is p . Suppose that I.F.D. for the whole population is available. It is a relatively easy matter to show that the proportions of the various parental genotypes and parental-offspring combinations are as in table 1.

Here V is the variance in gene frequency between any subpopulations and X is the third moment of p_i about p . Note that both V and X are characteristics of the population and not of any sample from it; V is not the sampling variance but it is a measure of the degree of heterogeneity of the gene frequency. The observed numbers are indicated by O and an appropriate subscript, for example $O_{12 \cdot 11}$ designates the observed number A_1A_1 progeny from A_1A_2 parents.

Assuming that $V = X = 0$, it is possible to obtain four observed ratios from table 1 for which there are simple expectations. These are:

$$\left. \begin{array}{l} A_1A_1:A_1A_2 \text{ progeny from } A_1A_1 \text{ parents} \\ A_1A_1:A_2A_2 \text{ progeny from } A_1A_2 \text{ parents} \\ A_1A_2:A_2A_2 \text{ progeny from } A_2A_2 \text{ parents} \end{array} \right\} = p:(1-p)$$

and

$$(A_1A_1 + A_2A_2):A_1A_2 \text{ progeny from } A_1A_2 \text{ parents} = 1:1.$$

The first three ratios will be called the gametic gene ratios. These expectations are shown in table 2 together with the possible causes of significant departure from them.

TABLE 1

Expected proportions and observed numbers amongst incomplete family data (I.F.D.) taken from a genetically subdivided population

Available parent		Progeny. Expected proportions and observed numbers of genotype		
Genotype	Expected	A_1A_1	A_1A_2	A_2A_2
A_1A_1	$p^2 + V$	$p^2 + 3pV + X$ $O_{11.11}$	$p^2(1-p) + (1-3p)V - X$ $O_{11.12}$	—
A_1A_2	$2p(1-p) - 2V$	$p^2(1-p) + (1-3p)V - X$ $O_{12.11}$	$p(1-p) - V$ $O_{12.12}$	$p(1-p)^2 + (2-3p)V + X$ $O_{12.22}$
A_2A_2	$(1-p)^2 + V$	—	$p(1-p)^2 - (2-3p)V + X$ $O_{22.12}$	$(1-p)^2 + 3(1-p)V - X$ $O_{22.22}$

V = Variance in gene frequency between subpopulations.

X = Third moment of the distribution of the subpopulation gene frequencies about their mean, p .

p = Gene frequency for the entire population.

TABLE 2

Expectations and possible causes of significant departures for χ^2 test on incomplete family data (I.F.D.)

Description of the test	Observed	Expected	Expectations affected (+) and unaffected (−) by		
			Viability	Wahlund's effect	Robertson's effect
Viability ratio	$(O_{12.11} + O_{12.22}) : O_{12.22}$	1:1	+	−	−
Heterogeneity of gametic gene ratios	$O_{11.11} : O_{11.12}$ $O_{12.11} : O_{12.22}$ $O_{22.12} : O_{22.22}$	$p : (1-p)^*$	+	+	−
Hardy-Weinberg equilibrium in parent	Parental genotypic ratios	$p^2 : 2p(1-p) : (1-p)^2$	+	+	+

* Assumes $V = X = 0$, table 1.

From table 2 it can be seen that the above tests will show if there are:

- (1) differences in viability between genotypes;
- (2) heterogeneity of gene frequency in the population (Wahlund's effect);
- (3) sex differences in gene frequency (Robertson's effect).

In general, the action of the three causes can be separately detected by the pattern of results obtained by testing with χ^2 and the direction of any significant departures from expectation.

For a locus with more than two alleles, separate analyses can be made such that the presence versus absence of each allele is considered.

Also, if A_j represents any other alleles besides A_1 and A_2 , the frequencies of $A_1A_1: A_1A_2: A_2A_2: A_1A_j: A_2A_j$ amongst the progeny of A_1A_2 parents are $\frac{1}{2}p_1: \frac{1}{2}(p_1+p_2): \frac{1}{2}p_2: \frac{1}{2}p_j: \frac{1}{2}p_j$ respectively. Hence $(A_1A_1+A_2A_2):A_1A_2$ and $A_1A_j:A_2A_j$ are both equal to 1:1.

In addition to carrying out these tests based upon I.F.D., heterogeneity of the gene frequencies between years was also tested in our data, and so was the Hardy-Weinberg equilibrium within years.

4. RESULTS

Analyses of published segregation and population data

The FV and Z systems. Stormont (1952) and Neimann-Sørensen (1958) presented segregation data for 341 and 225 matings respectively in the FV system and 722 and 357 matings respectively in the Z system, where at least one of the parents was of the heterozygous type (F/V or Z/-). The distribution of the offspring was very close to expectation. The ratio between homozygotes and heterozygotes was 280:286 for the FV and 532:547 for the Z system. No details of sex of parent were given for the matings. The effects, if any, of heterozygous superiority and maternal-foetal antigenic incompatibility tending to eliminate heterozygotes could not therefore be separated.

A relatively large number of breeds have now been tested for the agreement with the Hardy-Weinberg expectations with regard to the distribution of the genotypes in the FV and Z systems.

The results from studies of breed samples in which more than 250 cattle were typed are summarised in table 3. Two of the 16 populations deviated significantly from equilibrium with regard to the FV system. However, the sum of χ^2 for all the samples was not significant and there was no trend towards an excess or deficiency of heterozygotes. The results for the Z system were somewhat different. Agreement with Hardy-Weinberg expectation was tested for both the FV and Z systems in 7 samples and in all but one of these χ^2_Z was higher than χ^2_{FV} . In 9 of the total of 11 Z comparisons there was an excess of heterozygotes, even though only one was so large that the distribution deviated significantly from equilibrium. The sum of χ^2 for all the samples was highly significant. The overall result of the population studies for the Z system indicates the possibility of extra fitness of the heterozygote or a systematic misclassification of a certain proportion of the Z/Z homozygotes as Z/-.

The transferrins. The amount of published segregation data for the cattle transferrins is remarkably large. Ashton (1959) presented results which indicated that the survival of the calf foetus might be influenced by the transferrin types of the dam and its foetus. These results have evidently stimulated compilation of large bodies of segregation data. Ashton (1965) summarised the results of studies published before 1965 and postulated the following effects of transferrins on fitness: (1) superiority of heterozygotes *in utero*, (2) maternal-foetal incompatibility, (3) differential fertility of heterozygous and homozygous bulls and (4) differential fertility of heterozygous and homozygous cows. It was further suggested that the superiority of the heterozygotes *in utero* may be sufficient to produce a balanced

TABLE 3

The observed numbers of heterozygotes in population data as compared to the expected numbers at Hardy-Weinberg equilibrium

Type of data	Source	Total No.	FV system, No. of heterozygotes		χ^2 for agreement with equilibrium, 1 d.f.	Z system, No. of heterozygotes		χ^2 for agreement with equilibrium, 1 d.f.
			Obs.	Exp.		Obs.	Exp.	
<i>Breed</i>								
Red Danish, A.I. bulls	Neimann-Sørensen, 1958							
< 1 year		289	23	23.9	0.50	66	61.5	1.7
> 1 year		793	57	54.9	1.10	214	202.3	2.6
elite cows		289	33	32.9	0.01	77	78.0	0.1
German Fleckvieh, bulls	Buschmann, 1962	1000	351	354.7	0.10	—	—	—
Braunvieh, bulls	Buschmann, 1962	620	220	221.4	0.02	—	—	—
Frankenvieh, bulls	Buschmann, 1962	480	127	130.0	0.25	—	—	—
Montbeliarde, herd data	Grosclaude and Millot, 1962	400	52	58.9	5.47*	—	—	—
Friesian, bulls	Bouw, 1960	1200	284	283.8	0.00	—	—	—
M.R.Y., bulls	Bouw, 1960	538	140	128.7	3.86*	—	—	—
Ayrshire, bulls	Maijala and Lindström, 1966	1220	365	370.2	0.24	—	—	—
Finncattle, bulls	Maijala and Lindström, 1966	540	170	174.0	0.28	—	—	—
Swedish Red and White, bulls	Rendel, 1958	630	218	224.3	0.50	241	235.2	0.38
Friesian, A.I. bulls	Tolle, 1960	682	160	169.3	2.00	—	—	—
Icelandic cattle, herd data	Braend <i>et al.</i> , 1962	922	9	11.0	—	427	411.9	0.66
South West Polled, herd data	Braend <i>et al.</i> , 1964	1010	255	256.1	0.02	228	223.4	2.68
Trönder, herd data	Braend <i>et al.</i> , 1964	1012	56	57.0	—	—	—	—
Telemark, herd data	Braend, 1959	1000	33	32.6	—	526	499.8	2.75
Döla, herd data	Braend, 1959	1000	142	138.8	0.53	440	426.2	1.05
American Friesian, herd data	Stormont, 1952	1482	—	—	—	630	645.0	1.15
American Guernsey, herd data	Stormont, 1952	2047	—	—	—	1043	1021.9	0.87
American Longhorn, herd data	Miller, 1966	303	77	77.3	0.00	179	146.4	15.10***
		17457	2772	2799.9	14.88 (16 d.f.)	4071	3951.6	29.04 (11 d.f.)

* $P < 0.05$; *** $P < 0.001$.

TABLE 4

The number of homo- and heterozygous offspring in matings where the unlike-homozygous-mother-effect should not operate, i.e. where the dam is one of the three heterozygous types

Mating		Offspring		Total	Source
♂♂	♀♀	Homozygous	Heterozygous		
A/D × A/D		63	44	107	Krummen, 1965
		88	75	163	Buschmann, 1965
		34	23	57	Datta <i>et al.</i> , 1965
		11	13	24	Jamieson, 1965
		196	155	351	Sum
A/A × A/D		15	14	29	Krummen, 1965
		3	14	17	Buschmann, 1965
		16	6	22	Datta <i>et al.</i> , 1965
		34	34	68	Sum
D/D × A/D		33	27	60	Krummen, 1965
		84	74	158	Buschmann, 1965
		19	19	38	Datta <i>et al.</i> , 1965
		136	120	256	Sum
A/E × A/E		1	1	2	Jamieson, 1965
		10	7	17	Gahne, 1961
		4	9	13	Brummerstedt-Hansen <i>et al.</i> , 1963
		15	17	32	Sum
A/A × A/E		1	0	1	Buschmann, 1965
		2	2	4	Gahne, 1961
		25	23	48	Brummerstedt-Hansen <i>et al.</i> , 1963
		28	25	53	Sum
E/E × A/E		6	2	8	Gahne, 1961
E/E × D/E		5	2	7	Gahne, 1961
D/E × D/E		0	1	1	Jamieson, 1965
		5	5	10	Gahne, 1961
		4	4	8	Brummerstedt-Hansen <i>et al.</i> , 1963
		9	10	19	Sum
D/D × D/E		0	2	2	Krummen, 1965
		9	12	21	Buschmann, 1965
		11	7	18	Brummerstedt-Hansen <i>et al.</i> , 1963
		20	21	41	Sum
Total		449	386	835	
Ashton, 1965		637	717	1354	
Grand total		1086	1103	2189	

χ^2 Total = 4.7; 1 d.f.; $P = 0.02-0.05$.

χ^2 Ashton = 4.7; 1 d.f.; $P = 0.02-0.05$.

χ^2 Grand total = 0.1; 1 d.f.; $P = 0.7-0.8$.

χ^2 Heterogeneity, Ashton—Total = 9.4; 1 d.f.; $P = 0.001-0.01$.

polymorphism for the transferrins. The postulates (1) and (2) will be dealt with in this section.

TABLE 5

The unlike-homozygous-mother-effect. Summary of segregating matings involving dams homozygous for transferrins; equal number of homo- and heterozygous offspring to be expected

Mating		No. of homozygous	No. of heterozygous	Total	Source
♂♂	♀♀				
A/D × D/D		82	94	176	Krummen, 1965
		125	93	218	Buschmann, 1965
		16	11	27	Datta <i>et al.</i> , 1965
		223	198	421	Sum
		210	168	378	Ashton, 1965
A/D × A/A		16	12	28	Krummen, 1965
		28	23	51	Buschmann, 1965
		8	6	14	Datta <i>et al.</i> , 1965
		52	41	93	Sum
		268	241	509	Ashton, 1965
A/E × E/E		2	1	3	Gahne, 1961
		0	3	3	Brummerstedt-Hansen <i>et al.</i> , 1963
		2	4	6	Sum
A/E × A/A		3	1	4	Buschmann, 1965
		19	18	37	Gahne, 1961
		17	18	35	Brummerstedt-Hansen <i>et al.</i> , 1963
		39	37	76	Sum
D/E × D/D		2	3	5	Krummen, 1965
		9	5	14	Buschmann, 1965
		12	10	22	Brummerstedt-Hansen <i>et al.</i> , 1963
		23	18	41	Sum
D/E × E/E		3	2	5	Gahne, 1961
		0	1	1	Brummerstedt-Hansen <i>et al.</i> , 1963
		3	3	6	Sum
Total this study		342	301	643	
Ashton, 1965		478	409	887	
Grand total		820	710	1530	

χ^2 Total = 2.6; 1 d.f.; P = 0.1-0.2.

χ^2 Ashton = 5.4; 1 d.f.; P = 0.02-0.05.

χ^2 Grand total = 7.9; 1 d.f.; P = 0.001-0.01.

χ^2 Heterogeneity, Ashton Total = 0.1; 1 d.f.; P = 0.7-0.8.

Ashton found that in matings between heterozygous sires and homozygous dams (*e.g.* A/D ♂♂ × D/D ♀♀) there was a significant excess of offspring like the mother while in the reciprocal matings there was an excess of heterozygotes and also in the matings between A/D heterozygotes. The

latter was presumed to be due to superior viability of the heterozygote *in utero* and the former was ascribed by Ashton to maternal-foetal incompatibility. Such interaction is well documented for some red cell antigens in

TABLE 6

A comparison of the segregation ratios in D/D ♂♂ × A/D ♀♀ and A/D ♂♂ × D/D ♀♀ matings tested in the same laboratories

Mating		Offspring type, No.			Laboratory
♂♂	♀♀	DD	AD	Total	
D/D × A/D (1)		33	27	60	Krummen, 1965
A/D × D/D (2)		82	94	176	
Sum		115	121	236	
	(1)	84	74	158	
	(2)	125	93	218	
Sum		209	167	376	Buschmann, 1965
	(1)	19	19	38	
	(2)	16	11	27	
Sum		35	30	65	Datta <i>et al.</i> , 1965
	(1)	76	82	158	
	(2)	129	96	225	
Sum		205	178	383	
	(1)	7	6	13	
	(2)	13	12	25	Brummerstedt-Hansen <i>et al.</i> , 1963
Sum		20	18	38	
	(1)	13	22	35	
	(2)	41	29	70	
Sum		54	51	105	Ashton, 1958
	(1)	10	15	25	
	(2)	22	27	49	
Sum		32	42	74	Ashton and Fallon, 1962
Total	(1)	242	245	487	
	(2)	428	362	790	
Grand total		670	607	1277	

Heterogeneity between laboratories, segregation for mating (1) $\chi^2 = 3.9$; 6 d.f.; $P = 0.5-0.7$.
Heterogeneity between laboratories, segregation for mating (2) $\chi^2 = 8.4$; 6 d.f.; $P = 0.2-0.3$.
Heterogeneity between total (1) and total (2) $\chi^2 = 2.4$; 1 d.f.; $P = 0.1-0.2$.
Deviation from 1:1 in total (2) $\chi^2 = 5.5$; 1 d.f.; $P = 0.01-0.02$.

several species. The term incompatibility implies some immunological mechanism for which there is so far no evidence with regard to the transferrins. Therefore the more neutral term unlike-homozygous-mother or "UHM effect" will be used here.

Segregation ratios for transferrins are now available in a number of publications in addition to those reviewed by Ashton (1965). The results of these additional matings involving heterozygous dams are presented in table 4. The matings for which an UHM effect is possible are summarised in table 5. As seen from table 4, Ashton's postulate of a superiority of the heterozygote *in utero* could not be verified here. There was a significant

TABLE 7

The expected and observed frequencies of heterozygosity for transferrins in population studies

Breed and type of sample	Total No.	No. of heterozygotes		χ^2 for agreement with Hardy-Weinberg distribution	D.F.	Source
		Obs.	Exp.			
Swedish Red and White, bulls	404	236	243.9	1.8	3	Gahne, 1961
Finnish Ayrshire, herd data	4040	2154	2170.0	9.5*	3	Vasenius, 1965
Red Danish, cows from random herds	1128	681	679.5	0.4	3	Brummerstedt-Hansen <i>et al.</i> , 1963
Danish Friesian, cows from random herds	576	300	303.8	1.5	2	Brummerstedt-Hansen <i>et al.</i> , 1963
Jersey, cows from random herds	425	185	175.4	1.2	1	Brummerstedt-Hansen <i>et al.</i> , 1963
Jersey, A.I. cows	375	199	186.1	1.8	1	Ashton and Fallon, 1962
Illawarra Shorthorn, herd data	622	356	325.2	16.4***	3	Ashton and Fallon, 1962
Hungarian Spotted, herd data	631	262	264.7	1.5	2	Márkus <i>et al.</i> , 1964
German Fleckvieh, parentage data	3347	950	941.7	0.3	2	Buschmann, 1965
German Braunvieh, parentage data	731	312	319.5	0.7	2	Buschmann, 1965
German Friesian, parentage data	675	319	365.9	13.9**	2	Buschmann, 1965
Simmentaler, parentage data	1020	337	337.7	0.1	1	Krummen, 1965
Friburger, parentage data	397	152	158.2	0.6	1	Krummen, 1965
Icelandic cattle, herd data	941	437	437.5	0.3	2	Braend <i>et al.</i> , 1965
American Friesian, one herd	332	168	166.7	0.0	1	Datta <i>et al.</i> , 1965
British Friesian, A.I. bulls	414	236	275.0	25.5**	9	Jamieson, 1966
British Ayrshire, cows	491	322	342.0	6.4	9	Jamieson, 1966
	16550	7606	7692.8	82.0	47	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

deviation from the expected 1:1 ratio though this was in the opposite direction to that of Ashton. If the two sets of data are pooled an almost perfect 1:1 ratio is obtained. The ratios for the segregating matings where the UHM effect is possible go in the same direction in both Ashton's and the present data. There is a significant excess of homozygotes like the mother. The pooling of data from many laboratories would be hazardous if systematic typing errors occurred. The A/D and D/D types are sometimes difficult to distinguish from one another. Segregation ratios based on the A/D and D/D types may therefore be affected by systematic differences between laboratories. In order to minimise this possible source of error the results of the compatible mating (D/D ♂♂ × A/D ♀♀, mating type 1 and the

corresponding incompatible one A/D ♂♂ × D/D ♀♀, mating 2) were compared within laboratories. As seen from table 6, there was no significant heterogeneity between laboratories for either mating. However, there was a significant excess of homozygotes (D/D) and this was entirely due to the segregation in the matings where an UHM effect is possible.

The agreement of the transferrin type distribution with the Hardy-Weinberg expectations has been investigated in numerous populations. Table 7 summarises the results obtained in 17 breed samples with at least 300 tested animals. The deviation from genetic equilibrium was significant in 4 of the samples. In one there was a significant excess of heterozygotes and in the remaining there was a surplus of homozygotes. The total number of homozygotes was slightly higher than the expected number summed over all breeds.

Ashton and Fallon (1962), who compared the observed and expected frequency of transferrin heterozygotes in several populations, two of which are included in our table 7, found an excess of about 6 per cent. of heterozygotes. Ashton (1965) compared the number of observed and expected heterozygotes for transferrins and postalbumins in 6 small African breed samples and in 4 small Australian samples. There was an overall excess of 5.5 per cent. heterozygotes for transferrins and 2.5 per cent. heterozygotes for the postalbumins. Ashton apparently regards the slightly higher excess of heterozygotes in the transferrin system as evidence for a selective advantage of transferrin heterozygotes. The strength of such evidence is difficult to assess because no statistical test was provided to measure the significance of the difference.

5. RESULTS FROM INCOMPLETE FAMILY DATA (I.F.D.) AND OUR OWN POPULATION STUDIES

In tables 8 and 9 the I.F.D. for the FV, Z and transferrin systems are given. All our own data relate to male parents and their male offspring. The transferrin tested sample from this laboratory is rather small. Table 9 also includes a larger body of data published by Brummerstedt-Hansen *et al.* (1963) but not analysed by them. These data were derived from male parents and their female offspring. The offspring were all pure bred; 63 per cent. belonged to the Red Danish (RDM), 24 per cent. to the Danish Friesian (SDM) and 13 per cent. to the Danish Jersey breed.

In table 10 the data in tables 8 and 9 are tested for agreement with the expected 1:1 ratios. There are no significant departures and so no indication of any differences in viability.

In table 11 data are presented on the genotypic numbers for the total population of bulls born in 1940-66 and tested in this laboratory. The distributions are in good agreement with the Hardy-Weinberg expectations.

The female gametic gene ratios (*cf.* table 2) are tested for heterogeneity in table 12. For the FV and Z systems there are no significant departures, so there is no indication of viability differences between genotypic classes or any suggestion of heterogeneity of gene frequencies in the population. However, when one turns to the figures in table 13 there is an apparent contradiction. There is significant temporal heterogeneity of gene frequency both for the FV system and the Tf^D and Tf^E alleles in the transferrin system.

For the FV system this heterogeneity is partly ascribable to the trend in gene frequency. The difference between the average gene frequencies for 1940-58 and 1959-66 is highly significant. For Tf^E the difference between these two periods is just significant but for Tf^D most of the heterogeneity is not due to

TABLE 8
Incomplete family data for the FV and Z systems

Sires' type	Sons' type		
	V/V	F/V	F/F
V/V	10	31	—
F/V	75	297	268
F/F	—	276	996
	-/-	Z/-	Z/Z
-/-	768	265	—
Z/-	326	417	103
Z/Z	—	56	18

TABLE 9
Incomplete family data for the transferrin system

Sires' types	Types of progeny					
	A/A	A/D	A/E	D/D	D/E	E/E
	(1) This investigation					
A/A	125	39	51	0	0	0
A/D	33	33	17	9	20	0
A/E	53	16	66	0	14	29
D/D	0	8	0	0	4	0
D/E	0	10	14	2	10	2
E/E	0	0	21	0	4	17
	211	106	169	11	52	48
	(2) Brummerstedt-Hansen <i>et al.</i> , 1963					
A/A	241	206	41	(1)†	(1)†	0
A/D	260	469	41	190	45	0
A/E	27	37	38	0	32	11
D/D	(4)†	179	(1)†	145	23	0
D/E	0	19	31	11	21	3
E/E	0	0	0	0	0	0
	532	910	152	347	122	144

† Offspring's type not in accord with genetic theory.

any trend. The heterogeneity of gene frequency in the transferrin system is reflected by a highly significant heterogeneity of female gametic gene ratios for Tf^D ($\chi^2 = 27.5$; table 12). The sires which carried the Tf^D gene were mated to females where the gametic output of the Tf^D and ($Tf^A + Tf^E$) occurred in a ratio of approximately 1:2 while the corresponding ratio for the mates of the sires lacking the Tf^D gene was 1:5. There was evidently a stratification of the breed with regard to the occurrence of the Tf^D gene.

Despite the time trend and heterogeneity of gene frequency the genotypic distributions of all three systems showed good agreement with Hardy-Weinberg expectations for the total frequencies between 1940 and 1966. Tests were also performed for each single year or in case of small numbers on pooled data from adjacent years. In one single case only a significant

TABLE 10

χ^2 tests on expected 1:1 ratios in incomplete family data for cattle transferrins and the FV and Z systems; data derived from tables 8 and 9

Sires' type	Type of progeny	Observed numbers		Total	χ^2_1
		This investigation	Brummerstedt-Hansen <i>et al.</i> , 1963		
<i>Transferrin</i>					
A/D	A/D:(A/A+D/D)	33:42	469:450	502:492	0.10
	A/E:D/E	17:20	41:45	58:65	0.40
A/E	A/E:(A/A+E/E)	66:82	38:38	104:120	1.14
	A/D:D/E	16:14	37:32	53:46	0.49
D/E	D/E:(D/D+E/E)	10:4	21:14	31:18	3.45
	A/D:A/E	10:10	19:31	29:41	2.06
<i>F/V system</i>					
F/V	F/V:(F/F+V/V)	297:343	—	—	3.31
<i>Z system</i>					
Z/-	Z/-:(Z/Z+--/-)	417:429	—	—	0.17

TABLE 11

The genotypic distributions in the FV, Z and transferrin systems and the respective Hardy-Weinberg expectations

	Total No.	FV system				Z system			
		FF	FV	VV	χ^2_1	-/-	Z/-	Z/Z	χ^2_1
Obs.	2897	1872	904	121	} 0.8; P>0.3	1600	1127	170	} 2.38; P>0.1
Exp.	2897	1864.3	919.3	113.3		1615.7	1095.6	185.7	
	Total No.	Transferrin system							χ^2_3
		AA	EE	DD	AE	AD	DE		
Obs.	1685	556	131	49	517	279	153	} 4.1; P>0.2	
Exp.	1685	540.2	128.9	41.6	527.8	300	146.5		

deviation was indicated (*Tf*-system in 1953 $\chi^2_1 = 3.94$). However, total χ^2 for agreement within years was non-significant (table 13).

The female gametic gene ratios for the transferrin system in the data of Brummerstedt-Hansen *et al.* are also tested for heterogeneity in table 12. The ratios for *Tf^F* show highly significant heterogeneity, *Tf^D* is not significantly heterogenous, but the value of χ^2 is quite large ($\chi^2_2 = 5.42$; $P = 0.05-0.10$) and *Tf^A* is not heterogenous at all. The results are rather to be expected from the breed composition of the sample. The RDM,

TABLE 12

 χ^2 tests for heterogeneity of the female gametic gene ratios for the transferrin, FV and Z systems

System and ratio	Source of data	Total observed gametic ratio	Heterogeneity χ^2
Tf^A versus its absence, i.e. Tf^A : ($Tf^E + Tf^D$) in the offspring from A/A, A/+ and +/+ sires	This paper	264:201	0.54
Tf^E versus its absence, etc.	This paper	136:353	3.75
Tf^D versus its absence, etc.	This paper	114:440	27.53**
Tf^A versus its absence, etc.	Brummerstedt-hansen <i>et al.</i> , 1963	757:728	1.52
Tf^D versus its absence, etc.	Brummerstedt-hansen <i>et al.</i> , 1963	621:895	5.42
Tf^E versus its absence, etc.	Brummerstedt-hansen <i>et al.</i> , 1963	227:1784	112.62***†
FV (F^F versus F^V)	This paper	361:1295	0.20
Z (Z versus z)	This paper	1150:386	0.55

† One degree of freedom only.

** $P < 0.01$. *** $P < 0.0001$.

TABLE 13

Heterogeneity of gene frequency with time (measured per year† between 1940-66) and its effect upon Hardy-Weinberg expectations; degrees of freedom in brackets

χ^2 test and gene frequency	FV	Z	Transferrin		
	F^F	Z	Tf^A	Tf^D	Tf^E
Heterogeneity of gene frequency between years for bulls born 1940-66	63.26*** (17)	14.50 (17)	15.59 (15)	25.83* (15)	31.31** (15)
χ^2 for difference between gene frequencies in 1940-58 and 1959-60	21.82** (1)	0.00 (1)	3.25 (1)	0.00 (1)	3.90* (1)
Gene frequency 1940-66	0.802	0.253	0.566	0.157	0.277
Gene frequency 1940-58	0.784	0.251	0.544	0.158	0.299
Gene frequency 1959-66	0.827	0.256	0.577	0.157	0.266
Agreement of total numbers for 1940-66 with Hardy-Weinberg expectations, χ^2	0.81 (1)	2.38 (1)	3.729 (3)		
Sum of χ^2 for agreement with Hardy-Weinberg for numbers within each year from 1940 to 1966	11.50 (18)	14.41 (18)	70.39 (54)		
Total numbers	2897		1685		

† Except when numbers were too small, then pooled.

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

SDM and Jersey breeds have the following gene frequencies. Tf^A 0.472, 0.452 and 0.761; Tf^D 0.395, 0.502 and 0.286; and Tf^E 0.132, 0.030 and 0.002 respectively. Despite the fact that the sample of offspring was composed of a mixture of three breeds, the pooled distribution of genotypes did not deviate significantly from the Hardy-Weinberg expectation ($\chi^2_3 = 2.3$; $P = 0.5$). However, the heterogeneity was easily revealed by the I.F.D. technique described here.

6. DISCUSSION

The family data of both kinds (I.F.D. and C.F.D.) and the population data for the two red cell antigenic loci are essentially in agreement with their respective expectations. The only exception is the population data for the American Longhorn, where there is an excess of heterozygotes (Miller, 1966). These data stem from a small closed herd and so the most probable explanation is the Robertson effect. Selection for heterozygotes or misclassification of Z/Z as Z/- are possible, since Z/Z is recognised by a dosage reagent. The latter seems unlikely but the possibility of selection for heterozygotes should be entertained a little further. Of the 11 samples for Z, 9 show an excess of heterozygotes. However, the I.F.D. 1:1 ratio does not.

Despite the fact that the FV and Z systems have been known for a decade longer than the transferrin polymorphism there exists a great deal more published family data for the latter. Much data for FV and Z probably remain unexamined in blood group laboratories. As a result of Ashton's proposals (see Ashton, 1965, for a summary of his viewpoint) a number of workers have gathered and published segregation data for transferrins. Here we have summarised what we believe is all larger bodies of published material. The unlike-homozygous mother effect for A/A and D/D mothers in matings with A/D sires seems to exist. The tendency is consistently present in nearly all the data. No trivial explanation in terms of typing error seems possible. The underlying biological mechanism is unknown. The reciprocal matings give the expected Mendelian ratios.

There is less support for the notion of a selective advantage of heterozygotes through differential viability either *in utero* or at any other time during the life history of the animal. Ashton (1965) summarised a number of studies and found that in segregating matings where the unlike-homozygous mother or UHM effect cannot occur, *i.e.* in A/D ♀♀ by A/A, A/D or D/D ♂♂ a significant excess of heterozygotes existed. In our more complete survey this is not so. There is close agreement with expectation. This is also mainly true of the population data except for one population where there is a clear excess of heterozygotes and another two where there are deficiencies. These latter are probably due to variance in gene frequency between subpopulation, Wahlund's effect. This effect will cause an apparent deficiency of the number of heterozygotes, tending to nullify the effect of any selective advantage for heterozygotes, so that the population data are not very critical. Above we have recommended the use of I.F.D. to separate Wahlund's effect and the effect of selection. However, in this case the combination of the existence of the UHM effect and the fact that cattle I.F.D. are in the form of sire groups makes the interpretation of the results for I.F.D. difficult. For the expected 1:1 ratio of heterozygotes to corresponding homozygotes, the UHM effect will produce a deficiency of heterozygotes from male parents. That it does not do so in this case (table 10)

could be taken as evidence of selective advantage for the heterozygotes but it is more likely to be due to the relatively small magnitude of the UHM effect and the small numbers contributing to the observed ratio. Considering the entirety of the data, it is clear that any viability advantage for the heterozygotes must be a small one, if it exists at all. As already pointed out, Ashton has postulated some rather complicated associations between transferrin type and fertility. Ashton's argument for the existence of these associations must be discussed since they make the assumption that there is no heterogeneity of gene frequency in his populations. Ashton found a slight excess of transferrin homozygotes among bulls standing at A.I. centres, which was taken as evidence for higher fertility of homozygotes. However, the possibility should not be overlooked that the departures from Hardy-Weinberg equilibrium might have been due to Wahlund's effect, particularly as no data were given for bulls which were rejected by the A.I. service. In at least one of the breeds concerned, the British Friesian, Wahlund's effect has been found to operate (Jamieson, 1966). Similar observations apply to Ashton's data associating various parameters of fertility and transferrin type (table 6; Ashton, 1965). The data do not allow spurious associations between fertility and transferrin type to be excluded. In a recent study Gahne (1968) found no indication whatsoever of an effect of transferrin type on fertility. Ashton placed considerable weight upon differences which are quite non-significant. For example, his assertion that homozygous cows are more fertile appears to be based upon a difference giving a χ^2 of 1.58. The only effect of the transferrins for which the evidence is good is the unlike homozygous mother effect in A/A and D/D mothers and this makes the transferrin polymorphism seem more like an unstable one. The analogy of the Rh system is obvious.

In our own data there was temporal heterogeneity of the gene frequency for the FV and transferrin systems. For FV this was partly the result of a trend in gene frequency with time but for transferrin no strong trend was evident. A great deal of this heterogeneity would disappear if allowance were made for the degree of relatedness in the population. Pooling data from different years with differing gene frequencies is equivalent to pooling data from different populations and so should lead to Wahlund's effect. In fact, it did not. For the FV system which had the largest heterogeneity, χ^2 , the variance in gene frequency between years was 0.0017. (This estimate was based on the gene frequencies for the single years except for 1940-44, 1945-49 and 1965-66 where poolings were made. The difference in numbers per year was ignored.) Thus the Hardy-Weinberg expectations for the FV system (table 11) need only be modified by adding $0.0017 \times 2897 = 4.91$ to the two homozygous classes and subtracting twice that from the heterozygous class, a modification which is insignificant. In both our own data and those of Brummerstedt-Hansen *et al.* there was a significant heterogeneity of the female gametic gene ratios for transferrins. In the latter sample heterogeneity would be expected on the basis of the gene frequencies in the three breeds of which the sample was composed. The heterogeneity in our own data suggests a stratification of the SRB breed with regard to the distribution of transferrin genes. It is interesting that despite the large values of χ^2 for heterogeneity of female gametic transferrin gene ratios in the two samples, there were no significant departures from the Hardy-Weinberg expectation in either case. The customary test for agreement with the Hardy-Weinberg

distribution is evidently rather inefficient in detecting stratification of breeding populations.

Finally we may note that in our analysis the rarer genes (Tf^D in the SRB and Tf^E in the Danish cattle) showed heterogeneity in the I.F.D. whereas the other more common ones did not. The reason for this can be found in table 1. Heterogeneity of gene frequency causes a much greater relative alteration to the expected values of the less frequent phenotypic classes than to those of the more frequent. That is, the effect of heterogeneity of gene frequency upon blood type frequencies in cattle populations is very similar to the effect of inbreeding upon the frequency of rare recessive phenotypes in man.

7. SUMMARY

1. Cattle blood group laboratories tend to accumulate data on the blood types of one parent and a number of its progeny, *e.g.* when a sire and his sons are typed for registration purposes. Such data are referred to as incomplete family data (I.F.D.), as distinct from complete family data (C.F.D.), and population data (P.D.).

2. For P.D., two sorts of gene frequency heterogeneity (Wahlund's effect and Robertson's effect) can lead to departure from Hardy-Weinberg expectations in cattle populations without there being any selective differences between genotypes.

3. A simple analysis of I.F.D. allows the effects of selection and gene frequency heterogeneity to be separated. This is not so for P.D., which are much more often analysed and published for cattle blood groups.

4. Analyses of I.F.D. and P.D. for the FV and Z red cell antigen groups and the serum transferrin (Tf) locus for data from the Swedish Red and White Breed (SRB) are interpreted in association with other published P.D., I.F.D. and C.F.D. for these systems.

5. There is no evidence for selection at the FV and Z loci.

6. Published C.F.D. for the Tf locus agree with a previous suggestion that $Tf^A Tf^D$ ($\sigma\sigma$) $\times$ $Tf^D Tf^D$ ($\phi\phi$) matings tend to give more $Tf^D Tf^D$ offspring than expected. There is, however, no convincing evidence for a superior selective advantage for heterozygotes at the cattle Tf locus.

7. Heterogeneity of the frequency of Tf^D was found in the I.F.D. for the SRB and for Tf^E in data from a mixture of Danish breeds. This heterogeneity was not detected in the P.D., which suggests that I.F.D. are more sensitive to such heterogeneity.

8. Examination of the gene frequencies in bulls in different years showed significant heterogeneity for gene frequency between years for the FV and Tf systems. For FV there was a gene frequency trend with time.

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