CATTLE SERUM TRANSFERRINS: A BALANCED POLYMORPHISM?

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FISHER (1930) defined the most likely condition for a stable polymorphism involving two alleles as one in which the heterozygote showed superior fitness relative to both homozygotes. The conditions for stability of a polymorphism in which more than two alleles occur have been examined by several authors (for review see SMITH 1961). There is general agreement that overdominance is a prerequisite for maintaining stability. A number of polymorphisms are known in invertebrates in which heterozygote advantage has been demonstrated experimentally (FORD 1964; SHEPPARD 1958). In vertebrates heterozygote advantage has been established with certainty as a balancing mechanism only in the case of the sickle cell hemoglobin heterozygote (ALLISON 1956), although there is mounting evidence (MORTON and CHUNG 1959) that the human MN blood group polymorphism may also be maintained by heterozygote advantage.

So far heterozygote advantage has not been demonstrated for any of the serum protein polymorphisms now known in vertebrates. ASHTON (1958) reported that differential fertility might be occurring between matings of various cattle serum transferrin genotypes. Matings between parents from which equal numbers of offspring like and unlike the mother (with respect to transferrin genotype) were expected showed a significant excess of like-mother progeny. Other workers were not able to confirm this result (BRUMMERSTEDT-HANSEN, MOUSTGAARD and MØLLER 1963), but the results of the first segregation analysis prompted a direct investigation of the breeding efficiency of matings between parents of known transferrin genotype (ASHTON 1961). Pooled data for 2544 inseminations are shown in Table 1. The most successful matings were from homozygous parents of unlike genotype (61.6%), followed by matings between homozygous parents of like genotype (55.6%). Matings involving heterozygotes were less efficient, homozygotes $\times$ heterozygotes giving a mean breeding efficiency of 49.2%, and matings between two heterozygotes giving 45.6%. Because Tf^E was absent from the Jersey cattle examined, and at low frequency in the Australian Illawarra Shorthorns, the data in Table 1 refer only to matings involving the alleles Tf^A and Tf^D . Although these results seemed to show heterozygotes at a disadvantage, subsequent analysis of the returns-to-service data (ASHTON and FALLON 1962) indicated that heterozygotes were more viable *in utero* and therefore at an advantage (see below).

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TABLE 1

*Breeding efficiencies (percentage of inseminations resulting in a live or stillborn calf)
for matings between various transferrin genotypes*

Cow transferrin genotype	Bull transferrin genotype		
	A/A	A/D	D/D
A/A	55.9	45.3	59.5
A/D	51.0	45.6	46.9
D/D	61.9	50.3	55.3

Data from ASHTON and FALLON, (1962).

Consideration of data from several sources suggests that four phenomena affecting reproductive performance are associated with transferrin polymorphism in cattle. These are (1) superiority of heterozygotes *in utero*, (2) mother-fetus incompatibility, (3) differential fertility of heterozygous and homozygous bulls, and (4) differential fertility of heterozygous and homozygous cows. Interaction between these factors produces the observed distribution of breeding efficiencies. Notwithstanding the relatively poor breeding efficiency of matings involving heterozygous parents it can be shown that the advantage of the heterozygote *in utero* may be sufficient to produce a balanced polymorphism.

Heterozygote superiority: Because Tf^E is relatively infrequent, or even absent, in European breeds of cattle the data available refer mainly to Tf^A and Tf^D , and only these alleles are considered in the main treatment in this paper. It is now known that " Tf^D " is really two alleles Tf^{D1} and Tf^{D2} (KRISTJANSSON 1962; ASHTON 1965). Nearly all the published segregation and fertility data treat these two alleles as a single Tf^D , and it is necessary to remember that "TfAD" can be either TfAD1 or TfAD2, and "TfDD" can be TfD1D1, TfD1D2, or TfD2D2.

Evidence for superiority of the transferrin heterozygote TfAD comes from three sources:

(a) *Returns-to-service data.* Table 2 shows the distribution of unsuccessful inseminations as evidenced by the reappearance of oestrus ("return-to-service") for

TABLE 2

Returns-to-service in artificially inseminated cows from two sources

Source	Mating	Total inseminations	Returns					
			0-24 days			25 days or more		
			No.	Percent total	χ^2	No.	Percent total	χ^2
ASHTON and FALLON (1962)	Like homozygotes	426	131	30.8	...	58	13.6	...
	Unlike homozygotes	273	82	30.0	0.04	23	8.4	4.37*
New Zealand D. P. M. B. (1963)	Like homozygotes	101	28	27.7	...	10	9.9	...
	Unlike homozygotes	119	29	24.4	0.32	5	4.2	2.79

* $P < 0.05$.

the two time periods 0–24 days, and 25 days or longer, after insemination. Most of the inseminations which do not result in conception tend to occur in the first period. Returns in the second period (“long returns”) are partly or mainly composed of inseminations which caused conception but from which the products of conception did not remain viable. There is of course considerable overlap between the two categories (ASHTON and FALLON 1962). Two sets of data only have been published which report returns-to-service. These are shown in Table 2. Each set shows that long returns are least frequent from matings between unlike homozygotes i.e., TfAA male $\times$ TfDD female and TfDD male $\times$ TfAA female. Analysis by χ^2 (SNEDECOR 1956) shows that the effect is significant in the Australian data ($P < 0.05$) but not in the New Zealand data. However the two sets of data are not significantly heterogeneous with respect to the distribution of long returns ($\chi^2 = 0.57$, 2 d.f., $P > 0.7$) and the difference in the pooled data is significant ($\chi^2 = 5.88$, 1 d.f., $P < 0.02$).

These results imply that heterozygotes, produced from matings between unlike homozygous parents, are more likely to survive *in utero* than homozygotes, produced from matings between like-type homozygous parents.

It would be anticipated that matings producing homozygotes and heterozygotes in equal numbers would show an intermediate frequency of long returns. However, as shown later some of these matings are subject to maternal-fetal incompatibility and it is not possible to separate the loss due to the two causes by an examination of long returns data.

(b) *Matings between like heterozygotes.* Direct evidence of heterozygote superiority comes from analysis of segregation data from matings expected to produce homozygotes and heterozygotes in equal numbers i.e., A/D male $\times$ A/D female, A/A male $\times$ A/D female, and D/D male $\times$ D/D female. In these matings the mother will possess both alleles present in the fetus, and incompatibility will not be a complicating factor. Several workers have now reported segregation data, and their results are shown in Table 3 for the three relevant matings. The data were analyzed by the method given in SNEDECOR (1956). Overall, 717 heterozygotes and 637 homozygotes were produced, which differs significantly from equality ($\chi^2 = 4.73$, 1 d.f., $P < 0.05$). There was no significant heterogeneity between matings with regard to the distribution of homozygotes and heterozygotes ($\chi^2 = 0.65$, 2 d.f., $P > 0.7$). Further data are necessary before the extent of the effect can be established with certainty, but the weighted data in Table 3 imply that only about 89% of the homozygotes expected were produced by these matings.

(c) *Heterozygote excess in cattle populations.* A third type of evidence which would suggest heterozygote advantage would be observation of more heterozygotes in random samples of cattle populations than expected from the gene frequencies. ASHTON and FALLON (1962) summarized data showing a significant excess ($P < 0.02$) of about 6% of heterozygotes in several cattle populations. It is not easy to assess data of this sort, because cattle are not usually randomly bred, and moreover not every survey shows such an excess. An artefactual excess of heterozygotes will be obtained by the usual method of computing expected dis-

TABLE 3

Distribution of homozygotes and heterozygotes from matings in which maternal fetal incompatibility is not possible

♂ Mating ♀	Source*	Progeny		
		Homozygotes	Heterozygotes	Total
$A/D \times A/D$	A	198	231	429
	B	62	72	134
	C	23	26	49
	D	0	2	2
	E	26	31	57
	F	36	35	71
	Total	366	420	786
$A/A \times A/D$	A	105	98	203
	B	26	17	43
	C	6	6	12
	E	25	42	67
	F	0	6	6
	Total	162	169	331
$D/D \times A/D$	A	61	66	127
	B	15	16	31
	C	7	6	13
	D	3	3	6
	E	13	22	35
	F	10	15	25
	Total	109	128	237
TOTALS		637	717	1354

* Source key: A, BRUMMERSTEDT-HANSEN *et al.* (1963). Progeny test data from 2079 matings (their Table 4). B, BRUMMERSTEDT-HANSEN *et al.* (1963). Data relating to bull calves (their Table 6). C, GAHNE (1960). D, GALL and BERG (1964). E, ASHTON (1958b). F, ASHTON and FALLON (1962).

tributions of genotypes, owing to the restricted use of sires (ROBERTSON 1965) in dairy and beef cattle herds. This difficulty could be resolved by working within sire groups, but in most cases the relevant information necessary to extract sire groups is not presented.

An alternative approach is to compare within the same population the distribution of transferrin homozygotes and heterozygotes with another genetic system which does not show significant deviation from a Hardy-Weinberg distribution. In this way the extent of heterozygote excess due to limited use of sires should be expressed in both systems. If a consistent extra excess of heterozygotes is found for the transferrin system and not for other systems this would imply a true excess of transferrin heterozygotes in cattle populations. Relevant data are shown in Table 4 contrasting the transferrin and post-albumin (ASHTON 1963) genotype distributions for several populations of cattle of different breeds. There was a significant excess of transferrin heterozygotes ($P < 0.01$) but not of post-albumin heterozygotes ($P > 0.5$). There were on average 5.5% more transferrin heterozygotes than expected, and 2.5% more post-albumin heterozygotes. The

TABLE 4

Expected and observed distributions of transferrin and post-albumin heterozygotes from surveys of several cattle populations

Source*	Population	Transferrin heterozygotes		Post-albumin heterozygotes	
		Observed	Expected	Observed	Expected
G	Sahiwal	176	168	106	113
	Nganda	113	101	34	30
	Boran	192	193	123	114
	Tanganyika zebu	87	84	29	30
	Teso	55	53	29	27
	Ankole	207	198	121	117
H	Steers	43	36	30	27
	Breeders (Classified)	79	72	46	46
	Breeders (Unclassified)	119	113	74	72
	Brisbane	100	92	61	61
		1171	1110	653	637

* Source Note: G from ASHTON and LAMPKIN (1965); H from ASHTON, FRANCIS and RITSON, unpublished data.

value of 5.5% for transferrin heterozygote excess for these herds is close to the 6.2% excess found in other surveys (ASHTON and FALLON 1962).

Maternal-fetal incompatibility: ASHTON (1958a) described an excess of like-mother offspring from matings involving the alleles Tf^A and Tf^D suggesting mother-fetus incompatibility. Other workers failed to confirm this effect, but it is nevertheless clear from their results that incompatibility exists. Segregation from matings which have an equal chance of producing progeny compatible or incompatible with the mother are shown in Table 5 for all the available data. Overall, these matings produced 478 compatible and 409 incompatible progeny, equal numbers of each being expected ($\chi^2 = 5.36$, $P < 0.05$). The distribution of compatible and incompatible progeny was not significantly heterogenous ($\chi^2 = 0.74$, 1 d.f., $P > 0.3$) between mating groups.

The ratio between incompatible and compatible progeny is 0.86 which suggests that only 86% of incompatible progeny survive. However, it is impossible to derive a true estimate for incompatibility because all matings of this type produce heterozygotes which may be at an advantage *in utero*, and this will confound the incompatibility effect.

Heterozygote fertility: Apart from the two effects of transferrin type on fitness considered above, there is evidence that the fertility of heterozygous bulls is less than that of homozygous bulls, irrespective of cow genotype. Also there is less substantial evidence of a similar effect with respect to homozygous and heterozygous cows.

Data relevant to bull fertility are shown in Table 6. These relate to Tf^{AD} bulls compared with pooled data for Tf^{AA} and Tf^{DD} bulls, bulls with Tf^E being relatively infrequent. In assessing the relative fertility of bulls of different genotypes

TABLE 5

Distribution of compatible and incompatible progeny from matings where equal numbers of both types are expected

Mating ♂ × ♀	Source*	Progeny		
		Compatible	Incompatible	Total
$A/D \times D/D$	A	99 D/D	78 A/D	177
	B	30	18	48
	C	13	12	25
	D	5	4	9
	E	41	29	70
	F	22	27	49
	Total	210	168	378
$A/D \times A/A$	A	159 A/A	134 A/D	293
	B	55	59	104
	C	12	14	26
	D	7	4	11
	E	20	12	32
	F	15	28	43
	Total	268	241	509
TOTAL		478	409	887

* Source key: See Table 3.

TABLE 6

Relative fertility of homozygous (mainly $TfAA$ and $TfDD$) and heterozygous (mainly $TfAD$) bulls

Source	Fertility parameter	Bull fertility		Ratio (a)/(b)
		Homo- zygotes (a)	Hetero- zygotes (b)	
ASHTON (1961)				
Jersey bulls	Live and stillbirths	54.8	43.6	1.257
A.I.S. bulls	Live and stillbirths	54.0	47.1	1.146
ASHTON and HEWETSON (unpublished data)				
Jersey × zebu bulls	Live and stillbirths	62.2	57.5	1.082
DATTA (1963)				
Blaine herd	Live births	57.5	53.8	1.069
D.C.R.C. herd	Live births	45.0	33.7	1.335
HICKMAN and DUNN (1961)				
Holstein bulls	Non-returns (period unstated)	74.3	74.4	0.999
Ayreshire bulls	Non-returns (period unstated)	76.9	73.8	1.042
Jersey bulls	Non-returns (period unstated)	75.5	75.5	1.000
Guernsey bulls	Non-returns (period unstated)	69.7	64.8	1.076
Angus bulls	Non-returns (period unstated)	78.2	79.0	0.989
New Zealand D.P.M.B. (1963)	49-day non-returns	65.0	64.5	1.008

the major problem is the small number of bulls usually available for comparison. Large numbers of inseminations for a single bull will increase the precision of the estimate of that bull's fertility, but will not contribute to the estimate of the mean fertility for bulls of a given genotype, which is the main interest. Table 6 shows that in the data so far published, TfAD bulls are mostly less fertile than TfAA and TfDD bulls. The extent of the effect varies, and it is not possible to get an accurate estimate of the difference in fertility of the two types of bulls from Table 6. It is necessary to weight the ratios for homozygote:heterozygote fertility by the number of bulls of each type used in deriving the ratios, and this information is not available for all these data.

The poorer fertility of heterozygous bulls is substantiated in another way. Fertility is an important factor in bulls standing at Artificial Breeding Centers, and it might be anticipated that an excess of homozygous bulls would be found at such centers as a result of bull selection. The distribution of 766 bulls of seven breeds, standing at more than 20 centers in the United Kingdom in the period 1956-57, is shown in Table 7. The only other data relating to bulls at Artificial Breeding Centers were published by GAHNE (1961), for 404 SRB bulls in Sweden, and these show the same effect. Analysis of the data in Table 7 by χ^2 (SNEDECOR 1956) showed a significant difference between the expected and observed distribution of homozygous and heterozygous bulls ($\chi^2 = 8.61$, 1 d.f., $P < 0.01$) and gave no evidence of heterogeneity between breeds ($\chi^2 = 8.60$, 7 d.f., $P > 0.2$). There were 18.9% more homozygous than heterozygous bulls based on expected distributions. This contrasts with the excess of heterozygotes found in cow populations (Table 4) in which selection for milk yield rather than fertility is usual.

Data showing the relative fertility of homozygous and heterozygous cows are given in Table 8 and relate largely to TfAA, TfAD and TfDD cows. They support the conclusion that TfAD cows are less fertile than the homozygous cows, but the effect is not significant. Analysis by WOOLF's (1955) method gave χ^2 for

TABLE 7

Observed and expected distributions of homozygous (TfAA, TfDD and TfEE) and heterozygous (TfAD, TfAE and TfDE) bulls standing at Artificial Breeding Centers

Source	Breed	Bull genotype			
		Homozygotes		Heterozygotes	
		Observed	Expected	Observed	Expected
ASHTON, 1958b (United Kingdom)	Jersey	29	28	20	21
	Guernsey	32	31	30	31
	Angus	29	26	23	26
	Ayresshire	59	61	65	63
	Hereford	52	39	25	38
	Shorthorn	82	72	59	69
	Friesian	135	119	126	142
GAHNE (1961) (Sweden)	S.R.B.	168	160	236	244
Totals		586	536	584	634

TABLE 8

Relative fertility of heterozygous (mainly $TfAD$) and homozygous (mainly $TfAA$ and $TfDD$) cows

Source	Fertility parameter	Cow fertility			
		Homozygotes		Heterozygotes	
		No. of inseminations	Percent successful	No. of inseminations	Percent successful
ASHTON (1961)					
Jersey cows	Live and stillbirths	746	52.6	781	49.6
A.I.S. cows	Live and stillbirths	476	56.3	690	45.7
New Zealand D.P.M.B. (1963)	49-day non-returns	443	66.1	393	63.1
DATTA (1963)					
Blaine cows	Live births	225	51.6	206	60.2
D.C.R.C. cows	Live births	112	40.2	143	41.3
ASHTON and HEWETSON (unpublished data)	Live and stillbirths	477	60.0	387	59.4

deviation of incidence ratio from unity = 1.58, which is not significant, and χ^2 for heterogeneity = 4.52, 5 d.f., $P > 0.5$. The analysis showed that homozygous cows were about 6.2% more fertile than heterozygous cows on average.

The proposed model: A model which fits the order of breeding efficiencies obtained by ASHTON and FALLON (1962) is shown in Table 9. This model utilizes the four demonstrated effects of transferrin type on fertility and is developed for the two alleles Tf^A and " Tf^D ", little being known about Tf^E , or the two individual D alleles.

(1) *Heterozygote superiority.* Matings between unlike homozygotes which

TABLE 9

Model proposed to explain the observed distribution of breeding efficiencies from the pooled data of Ashton and Fallon (1962)

Mating ♂ × ♀	Observed		Model				
	Observed breeding efficiency (percent)	Ratio obs.*	Weighting factors				Ratio expected†
			Heterozygote superiority	Incom-patibility	Bull fertility	Cow fertility	
AA × AA	55.9	0.90	0.85	1.00	1.0	1.0	0.94
AA × AD	51.0	0.82	0.92	1.00	1.0	0.9	0.91
AA × DD	61.9	1.00	1.00	0.90	1.0	1.0	1.00
AD × AA	45.3	0.73	0.92	0.90	0.9	1.0	0.83
AD × AD	45.6	0.74	0.92	0.93	0.9	0.9	0.77
AD × DD	50.3	0.81	0.92	0.90	0.9	1.0	0.83
DD × AA	59.5	0.96	1.00	0.90	1.0	1.0	1.00
DD × AD	46.9	0.76	0.92	0.95	1.0	0.9	0.87
DD × DD	55.3	0.89	0.85	0.98	1.0	1.0	0.92

* Ratio observed taking 61.9% for AA male × DD female as 1.00.

† Ratio expected taking product of weighting factors for AA male × DD female mating and equating to 1.00 by multiplying product by 1.11.

produce only heterozygotes will suffer no disadvantage from this effect and are weighted by the factor 1.00. Matings between like homozygotes, which produce only homozygotes, are at a disadvantage and are weighted by the factor 0.85. This factor comes from analysis of the data presented in Table 3, where it is seen that approximately 87% as many homozygotes as heterozygotes are produced. Matings between homozygote and heterozygote are weighted by the factor 0.92, as only half their zygotes will be homozygotes.

(2) *Mother-fetus incompatibility*. Matings producing only compatible fetuses are given the weighting 1.00 while matings producing equal numbers of compatible and incompatible fetuses are given the weighting factor 0.90. This is an arbitrary value close to the value of 0.87 derived from the data in Table 5. For reasons discussed above it is impossible to get an unbiased estimate of the effect due to incompatibility. Also the model requires the effect of incompatibility to be somewhat less than the effect of heterozygote advantage to meet the observed distribution of breeding efficiencies.

Although the model is developed for the two alleles Tf^A and " Tf^D ", the observed distribution of breeding efficiencies was obtained from herds in which Tf^{D1} and Tf^{D2} were segregating. It is necessary to make provision for this in the model. In the mating AD male $\times$ AD female there is the possibility of incompatibility between TfAD1 embryos and TfAD2 mothers, TfAD2 embryos and TfAD1 mothers, and TfD1D2 embryos and TfAD1 or TfAD2 mothers. If Tf^{D1} and Tf^{D2} were equally frequent in these populations the effect of incompatibility would be less than that from TfAA male $\times$ TfDD female (weighted at 0.90), but more than that from TfDD male $\times$ TfAD female weighted at 0.95. Thus the mating TfAD $\times$ TfAD is given the weighting 0.93. Similarly the mating TfDD male $\times$ TfDD female will give rise to a proportion of incompatible zygotes, and is weighted by the factor 0.98.

(3) *Heterozygote fertility*. Both heterozygous bulls and cows are less fertile than their corresponding homozygotes. The exact values for their relative fertility is not clear. The mean unweighted ratio of homozygote:heterozygote bull fertility (Table 6) is 1.091, and the mean unweighted ratio for cow fertility is 1.062 (Table 8). However, cows and bulls coded TfDD will include a proportion of heterozygous TfD1D2 individuals, and it is likely that the ratios for homozygote:heterozygote fertility are underestimates. Homozygote fertility for both cows and bulls is given the weighting factor 1.0, and heterozygote fertility 0.9.

Theoretical values for the relative breeding efficiencies were derived by multiplying the weighting factors for the four effects (heterozygote superiority, incompatibility, bull fertility, cow fertility) and equating the ratio for the best mating, AA male $\times$ DD female to 1.00 (Table 9). The ratio of the observed breeding efficiencies (from Table 1) was calculated taking the breeding efficiency of AA male $\times$ DD female as 1.00. Comparison of the ratios obtained from the observed breeding efficiencies with those produced by the model showed a good fit.

The model assumes that the effects postulated are independent, which is unlikely to be true. However, it provides a working hypothesis which can be used

as a guide for interpretation of more stringent data as these become available.

Polymorphic equilibrium: The proposed model can be considered satisfactory if in addition to meeting the observed distribution of breeding efficiencies it also results in a balanced polymorphism. This may be examined mathematically, and I am indebted to DR. Y. HIRAIZUMI for discussion and assistance with the relevant calculations. The mathematical analysis was limited to consideration of two alleles, Tf^A and a single Tf^D . It consists of a comparison between the distribution of genotypes in any parental generation with that in the first filial generation, using the parameters of the model. From this comparison it is possible to determine whether the model would lead to a balanced polymorphism.

The frequency of each mating in the parental generation is readily calculated from the frequency of each parental genotype. From each mating the expected distribution of progeny after applying weighting factors for heterozygote superiority, incompatibility and male and female heterozygote fertility can be derived (Table 10). The expected distribution of each genotype in the progeny can be determined by summation and is equivalent to:

Frequency of AA in progeny at generation $t = p_t = \Sigma AA/\bar{w}$

Frequency of AD in progeny at generation $t = q_t = \Sigma AD/\bar{w}$

Frequency of DD in progeny at generation $t = 1 - p_t - q_t = \Sigma DD/\bar{w}$

where $\bar{w} = \Sigma AA + \Sigma AD + \Sigma DD$.

Putting: V_{AA} = relative viability of TfAA progeny = 0.85

V_{DD} = relative viability of TfDD progeny = 0.85

V_{AD}^c = relative viability of TfAD progeny in compatible (TfAD) mothers = 1.00

TABLE 10

Expected genotype and mating frequencies in parents at generation t and progeny in generation t + 1

Parents at generation t		Progeny at generation (t + 1)		
♂	♀	AA	AD	DD
AA × AA		$p_t^2 V_{AA} F_{AA} f_{AA}$		
AA × AD		$\frac{1}{2} p_t q_t V_{AA} F_{AD} f_{AA}$	$\frac{1}{2} p_t q_t V_{AD}^c F_{AD} f_{AA}$	
AA × DD			$p_t (1 - p_t - q_t) V_{AD}^i F_{DD} f_{AA}$	
AD × AA		$\frac{1}{2} p_t q_t V_{AA} F_{AA} f_{AD}$	$\frac{1}{2} p_t q_t V_{AD}^i F_{AA} f_{AD}$	
AD × AD		$\frac{1}{4} q_t^2 V_{AA} F_{AD} f_{AD}$	$\frac{1}{2} q_t^2 V_{AD}^c F_{AD} f_{AD}$	$\frac{1}{4} q_t^2 V_{DD} F_{AD} f_{AD}$
AD × DD			$\frac{1}{2} q_t (1 - p_t - q_t) V_{AD}^i F_{DD} f_{AD}$	$\frac{1}{2} q_t (1 - p_t - q_t) V_{DD} F_{DD} f_{AD}$
DD × AA			$p_t (1 - p_t - q_t) V_{AD}^i F_{AA} f_{DD}$	
DD × AD			$\frac{1}{2} q_t (1 - p_t - q_t) V_{AD}^c F_{AD} f_{DD}$	$\frac{1}{2} q_t (1 - p_t - q_t) V_{DD} F_{AD} f_{DD}$
DD × DD				$(1 - p_t - q_t)^2 V_{DD} F_{DD} f_{DD}$
		ΣAA	ΣAD	ΣDD

Frequency of parental genotypes TfAA = p_t , TfAD = q_t , and TfDD = $(1 - p_t - q_t)$. For other symbols see text.

V_{AD}^i = relative viability of TfAD progeny in incompatible (TfAA or TfDD) mothers = 0.90

and: Fertility of TfAA cows = $F_{AA} = 1.0$
 Fertility of TfDD cows = $F_{DD} = 1.0$
 Fertility of TfAD cows = $F_{AD} = 0.9$
 Fertility of TfAA bulls = $f_{AA} = 1.0$
 Fertility of TfDD bulls = $f_{DD} = 1.0$
 Fertility of TfAD bulls = $f_{AD} = 0.9$

Then:

$$\frac{dp_t}{dt} = \frac{1}{\bar{w}} [q_t^2(0.1721 + 0.0208p_t) + p_t q_t(0.845 + 0.1p_t) + p_t(0.1p_t^2 + 0.75p_t - 0.85)]$$

$$\frac{dq_t}{dt} = \frac{1}{\bar{w}} [0.0208q_t^3 + q_t^2(0.1p_t - 0.37) + q_t(0.1p_t^2 - 1.9p_t + 0.005) + 1.8p_t(1 - p_t)]$$

and $\bar{w} = 0.85 + 0.1p_t - 0.08q_t - 0.1p_t^2 - 0.0208q_t^2 - 0.1p_t q_t$.

If selection operates on Tf^A and Tf^D it is assumed to be equal. Then at equilibrium the following relationship will hold:

$$\hat{p} + 0.5\hat{q} = 0.5$$

where $\hat{p}$ and $\hat{q}$ are the estimated equilibrium frequencies of p and q .

When $dp_t/dt = 0$, then $0.89q^3 - 26q^2 + 184q - 90 = 0$.

Of the three roots to this equation two are imaginary, and the meaningful root gives:

$$\hat{q} = 0.5278 = \text{equilibrium frequency of TfAD}$$

and hence

$$\hat{p} = 0.2361 = \text{equilibrium frequency of TfAA}$$

$$1 - \hat{p} - \hat{q} = 0.2361 = \text{equilibrium frequency of TfDD.}$$

This implies that a population at equilibrium will show an excess of heterozygotes of 5.6% which is in good agreement with the values actually found (Table 4; and ASHTON and FALLON 1962).

The stability of this equilibrium, maintained by heterozygote advantage, can be tested by applying the "characteristic equation" (ЛОТКА 1956). Let $p_t = \hat{p} + \Delta p_t$, and $q_t = \hat{q} + \Delta q_t$, where Δp_t and Δq_t are slight deviations from the equilibrium values of $\hat{p}$ and $\hat{q}$. Then

$$\frac{d\Delta p_t}{dt} = -0.0024\Delta p_t + 0.3020\Delta q_t + \dots \text{ (higher order values of } \Delta p_t \text{ and } \Delta q_t \text{)}$$

$$\frac{d\Delta q_t}{dt} = 0.00\Delta p_t - 0.7863\Delta q_t + \dots \text{ (higher order values of } \Delta p_t \text{ and } \Delta q_t \text{)}.$$

and the characteristic equation for this system is

$$\begin{vmatrix} -0.0024\Delta p_t - \lambda & 0.3920 \\ 0.00 & -0.7863 - \lambda \end{vmatrix} = 0$$

giving $\lambda = -0.0024$ or -0.7863 .

Since both values of λ are real and negative the equilibrium very probably is stable, for under these conditions Δp_t and Δq_t approach zero with elapsing time.

It should be noted that the differential equations dp_i/dt and dq_i/dt are more strictly written as finite difference equations, for example, $\Delta p_i + 1$. The arithmetic involved in the solution of such equations is complex, and the approximate but more readily soluble differential equations have been used here to obtain a general solution. A computer program for examining stability of equilibria is currently being written in this department (YASUDA, personal communication). This will permit checking of the equilibrium for other values of p and q .

Selection: A polymorphism maintained by heterosis is usually subject to selective pressures, evidenced by inequality in the frequencies of the genes comprising the polymorphism. The nature of the selective forces acting on cattle transferrins in the feral state will most likely remain conjectural. However, evidence of powerful man-made selection is available from several sources, favors the transferrin D alleles, and is associated with selection for milk yield.

OSTERHOFF (1964) has examined the distribution of transferrin genotypes of female dairy cattle in different age groups. Up to 12 months of age the frequencies of Tf^A and Tf^D were about the same, approximately 0.48, and the frequency of Tf^E about 0.04. Tf^D then showed a progressive rise and Tf^A a progressive fall in frequency with advancing age of the group examined, Tf^D approaching 0.65 and Tf^A 0.25. The frequency of Tf^E also increased slightly approximating 0.1. OSTERHOFF points out that selection for higher milk yield is a continuous process, with the result that the older cows usually are the best producers. Other evidence of the effect of selection for milk yield on transferrin gene frequency comes from the data presented by LARSEN (1961). He examined the transferrin types of the three highest producing and three lowest producing heifers in each of 18 groups in the Danish progeny testing stations in 1959–60. The transferrin gene frequencies in the lowest yielding group were Tf^A 0.731, Tf^D 0.241 and Tf^E 0.028, and in the highest yielding group Tf^A 0.630, Tf^D 0.333 and Tf^E 0.037. Although the difference in gene frequencies was not significant, Tf^D is more frequent in the high producing group.

Selection for Tf^D is also apparent in the available segregation data from the six published sources examined in Tables 3 and 5. Six matings are available for assessing this effect, uncomplicated by heterozygote advantage or differential maternal-fetal incompatibility (Table 11). The relative "survival" of the progeny from these matings can be ranked in the order $D > E > A$. While it is true that

TABLE 11

Distribution of progeny from certain matings pooled from the six sources quoted in Tables 3 and 5, showing selection favoring $Tf^D > Tf^E > Tf^A$

Mating		Progeny genotypes		
♂	♀	With Tf^A	With Tf^D	With Tf^E
$AD \times EE$		1 AE	13 DE	..
$AD \times AD$		154 AA	191 DD	..
$DE \times AA$		..	12 AD	8 AE
$DE \times DE$		..	10 DD	7 EE
$AE \times DD$		11 AD	..	15 DE
$AE \times AE$		7 AA	..	9 EE

those results could also be explained by postulating prezygotic selection of D sperm in preference to E or A sperm it seems much more likely that postnatal selection for TfDD cows is the real cause. It must be borne in mind that the progeny which are available for segregation analysis come mainly from dairy herds, will be mainly females, and will be from different age groups. Such considerations also make it more difficult to measure the extent of the effects shown in Tables 3 and 5.

Direct evidence that milk yield is influenced by transferrin type was obtained by ASHTON, FALLON, and SUTHERLAND (1964) who found that TfDD cows produced an average 45 imperial gallons of milk more than TfAA cows, TfAD cows being intermediate. These results confirmed earlier observations of ASHTON (1960) who examined the contemporary comparisons of bulls, found significant differences and forecast that TfDD cows would yield on average about 50 gallons more milk per lactation than TfAA cows.

While strong man-made selection is affecting the frequency of Tf^D in dairy herds the frequency of Tf^A is still high, and in some cases higher than Tf^D (ASHTON 1958b; MARKUS, KOVACS, and FÉSZIS 1964). The reasons for this are not known, but it seems an inescapable conclusion that Tf^A is at a considerable advantage, as yet undetermined.

Tf^E is usually present at low frequency in most European breeds. Its frequency is always higher in these breeds when they are located in the tropics. Tf^E is also frequent in zebu breeds (ASHTON and LAMPKIN 1965), and it seems likely that the selective advantage maintaining this allele is somehow associated with tolerance to hotter climates. Unpublished observations revealed that sweating rate of TfAE and TfDE beef cattle is greater than of animals lacking Tf^E . Sweat and milk glands have a common embryological origin, and a common mechanism may link the advantages of Tf^D and Tf^E . If Tf^E also is maintained by heterozygote advantage, the necessary information will only come from studies of populations with a high frequency of this allele. So far the information available about fertility of Tf^E cows and bulls and segregation of Tf^E genotypes is too scanty to warrant consideration. Similar comments apply to the transferrin alleles Tf^B , Tf^F , and Tf^G found in zebu cattle.

Conclusion: The data summarized here show significant effects for the four aspects of fertility somehow affected by transferrin type. While maternal-fetal incompatibility is reasonably well established for other antigenic systems, particularly the Rh and ABO blood groups in man, it is not at all clear why heterozygous fetuses should be more viable *in utero* than homozygotes, and quite conjectural as to how heterozygous parents can be overall less fertile than homozygotes. Because of these gaps in our knowledge the title to this paper requires a question mark. Unequivocal demonstration of a polymorphism balanced in the complex manner described will require more stringent data than are now available.

SUMMARY

Fertility in cattle is influenced by four effects of the serum transferrin locus. These are (1) heterozygote advantage *in utero*, (2) maternal-fetal incompati-

bility, (3) poorer fertility of heterozygous bulls, and (4) poorer fertility of heterozygous cows. Estimates of these effects were derived from published data. A model with these parameters produced the observed distribution of breeding efficiencies. The model leads to an apparently stable equilibrium for the two alleles considered, with an estimated population excess of heterozygotes very close to that observed.

LITERATURE CITED

- ALLISON, A. C., 1956 Population genetics of abnormal human haemoglobins. *Acta Genet. Statist. Med.* **6**: 430-434.
- ASHTON, G. C., 1958a β -globulin polymorphism and early foetal mortality in cattle. *Nature* **183**: 404-405. — 1958b Polymorphism in the serum proteins of mammals, with particular reference to cattle. Thesis, University of Liverpool. — 1960 β -globulin polymorphism and economic factors in dairy cattle. *J. Agric. Sci.* **54**: 321-328. — 1961 β -globulin type and fertility in artificially bred dairy cattle. *J. Reprod. Fertil.* **2**: 117-129. — 1963 Polymorphism in the serum post-albumins of cattle. *Nature* **198**: 1117-1118. — 1965 Serum transferrin-D alleles in Australian cattle. *Aust. J. Biol. Sci.* **18**: 665-670.
- ASHTON, G. C., and G. R. FALLON, 1962 β -globulin type, fertility and embryonic mortality in cattle. *J. Reprod. Fertil.* **3**: 93-104.
- ASHTON, G. C., G. R. FALLON, and D. N. SUTHERLAND, 1964 Transferrin (β -globulin) type and milk and butterfat production in dairy cows. *J. Agric. Sci.* **62**: 27-34.
- ASHTON, G. C., and G. H. LAMPKIN, 1965 Transferrin and post-albumin polymorphism in East African cattle. *Genet. Res.* **6**: 209-215.
- BRUMMERSTEDT-HANSEN, E., J. MOUSTGAARD, and I. MØLLER, 1963 Serum transferrin polymorphism in Danish cattle breeds. *Roy. Vet. Agric. Coll. Copenhagen, Yearbook*, 13-26.
- DATTA, S. P., 1963 Immunogenetic studies of cattle transferrins and blood groups. Thesis, University of Wisconsin.
- FISHER, R. A., 1930 *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- FORD, E. B., 1964 *Ecological Genetics*. Wiley, New York.
- GAHNE, B., 1961 Studies of transferrins in serum and milk of Swedish cattle. *Anim. Prod.* **3**: 135-144.
- GALL, G. A. E., and R. T. BERG, 1964 Studies of the inheritance of bovine serum transferrins. *Anim. Prod.* **6**: 107-117.
- HICKMAN, C. G., and H. O. DUNN, 1961 Differences in percentage of non-returns to service between transferrin types of bulls. *Can. J. Genet. Cytol.* **3**: 391-395.
- KRISTJANSSON, F. K., 1962 Recent research in serum protein polymorphisms of livestock. *Proc. 8th Annual Blood Group Conference, Ljubljana, Yugoslavia*. Mimeographed proceedings.
- LARSEN, H., 1961 Investigations concerning the possible influence of serum β -globulins, haemoglobins and β -lactoglobulins upon yielding ability of cattle. *Inst. F. Sterilitetsforskning, Aarsberetning*. pp. 125-134.
- LOTKA, A. J., 1956 *Elements of Mathematical Biology*, p. 60. Dover, New York.
- MÁRKUS, J., G. KOVÁCS, and L. FÉSZ, 1964 The frequency of genes controlling serum β -globulin types in Hungarian spotted cattle. *Acta Vet.* **14**: 437-442.
- MORTON, N. E., and C. S. CHUNG, 1959 Are the MN blood groups maintained by selection? *Amer. J. Human Genet.* **11**: 237-251.
- New Zealand Dairy Production and Marketing Board, 1963 Farm Production Report No. 39, p. 54.

- OSTERHOFF, D. R., 1964 Recent research on biochemical polymorphism in livestock. J. S. Afr. Vet. Med. Ass. **35**: 363-380.
- ROBERTSON, A., 1965 The interpretation of segregation ratios in domestic animal populations. Anim. Prod. **7**: (In press).
- SHEPPARD, P. M., 1958 *Natural Selection and Heredity*. Hutchison, London.
- SMITH, C. A. B., 1961 Statistical methods and theory. p. 140. *Recent Advances in Human Genetics*. Edited by L. S. PENROSE. Little, Brown, Boston.
- SNEDECOR, G. W., 1956 *Statistical Methods*. 5th ed., p. 213. Iowa State College Press., Ames.
- WOOLF, B., 1955 On estimating the relation between blood group and disease. Eugenics **19**: 251-253.