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Immunogenetic and population genetic analyses of Iberian cattle

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Summary

Blood samples were collected from more than 100 animals in each of 2 Spanish cattle breeds (Retinto and De Lidia), 2 Portuguese breeds (Alentejana and Mertolenga), and American Longhorn cattle. All samples for the 4 Iberian breeds were tested for 20 polymorphic systems; American Longhorn were tested for 19 of the 20. For each breed an average inbreeding coefficient was estimated by a comparison of the observed and expected heterozygosity at 7 or 8 codominant systems tested. All breeds had positive values but only 3 breeds had estimates of inbreeding that were statistically significantly different from 0: De Lidia with $\bar{f} = 0.17$, Retinto with $\bar{f} = 0.08$ and Mertolenga with $f = 0.05$. The De Lidia breed especially may be suffering from inbreeding depression since this high value is greater than expected if all of the animals were progeny of half-sib matings. Genetic distances were calculated from the gene frequency data on these 5 breeds plus 9 other European breeds. Analyses of these distances show a closely related group of the 4 Iberian breeds and American Longhorn, confirming the close relationships among the Iberian breeds and the Iberian, probably Portuguese, origin of American Longhorn cattle.

Introduction

Studies of the genetic relationships among cattle breeds have aided the understanding of their evolutionary history (Kidd, 1969) and their potential use in crossbreeding programs

(Kidd et al., 1974). With immunogenetic parameters such as blood groups, allotypes and biochemical polymorphisms, a reasonably good, unbiased estimate of gene frequencies can be obtained (Rendel, 1967). Furthermore, it is possible to sample a large number of loci with these parameters, thus yielding data which are well suited for studies of population structure. The structure of the species is definable in terms of the relationships of the breeds as defined by gene frequency variation (Cavalli-Sforza & Edwards, 1967). The structure within an individual breed can be defined in terms of deviations from the expected Hardy-Weinberg proportions. These deviations for homozygotes and heterozygotes provide an estimate of the average inbreeding that reflects the effects of selection and of specific mating systems (Bodmer et al., 1972).

There have been 4 reports on Iberian cattle. Sotillo et al. (1968) sampled Spanish Friesian (dairy cattle) and the Rubia Gallega breed (beef). Only the blood groups were assayed. The Portuguese Alentejana breed (beef and draft) was studied by Bouquet et al. (1970) who concluded from data on blood groups and biochemical polymorphisms that the Afrikaner cattle could not have been derived from this breed. Vallejo (1978) studied several breeds including Retinto and showed that it was very closely related to the Criolla breeds of South America because Columbus brought Spanish cattle to America in 1493. Finally, Zarazaga et al. (1978) presented some limited gene frequency data on five polymorphic biochemical systems in the De Lidia breed.

The histories of the Iberian breeds are not well documented. All are believed to have descended from the prehistoric trunk of *Bos primigenius* native to the Iberian peninsula. The two Portuguese breeds are known to be closely related. The Retinto breed actually consists of three slightly different subbreeds; since our sample was taken in Andalucia, we probably have a sample largely representing Rubia Andaluza. The De Lidia breed may have origins partially different from that of the other three breeds; different herds within the breed may have quite different origins. However, the origins of modern De Lidia probably trace to early herds formed in A.D. 700-800. There are documents concerning the sale of De Lidia bulls in 1334 and 1508. The American Longhorn cattle are modern descendants of cattle brought by early Spanish settlers. As discussed by Miller (1966) considerable mixture of the original Spanish cattle and other European (primarily English) breeds occurred prior to the establishment of the current herds.

We wish to report the results of our immunogenetic analyses on two breeds of Spanish cattle (De Lidia and Retinto), two breeds of Portuguese cattle (Alentejana and Mertolenga), and American Longhorn cattle. In addition to the gene frequencies and phylogenetic relationships of these breeds to other breeds, we shall present evidence for an alarmingly high degree of inbreeding in the two Spanish breeds.

Materials and methods

Source of animals

To obtain as nearly a random sample of each breed as possible, we collected blood from over 100 animals of each breed, from no less than four geographically widely separated farms. On the average we obtained about 25 animals from each farm. We tried to sample as many unrelated animals as possible. Specifically, we sampled 124 De Lidia and 166 Retinto from Andalucia (the south of Spain) and 157 Alentejana and 149 Mertolenga from Evora (the south of Portugal). Most of the animals were females of various ages.

Our sample of American Longhorn cattle was obtained from the Wichita Wildlife Refuge, Oklahoma. This herd was previously studied by Miller (1966) who described its origins and history.

Blood types

Freshly collected whole blood of the 4 Iberian breeds was shipped on ice by air to the laboratory in Milan, Italy where they were blood typed for eleven of the cattle blood group systems (A, B, C, F, J, L, M, S, Z, R′, and T′) by the standard hemolytic test. Freshly collected whole blood of American Longhorn cattle was shipped to Madison, Wisconsin and typed there; typing was done for all of the above systems except R′ and T′. The data on the A, B, and C systems were abbreviated as described by Kidd et al. (1974) to simplify the analysis.

Allotypes

All serum samples were typed in Madison for immunoglobulin Al and A2 heavy-chain allotypes of IgG_2 and the B1 light-chain allotype by the double diffusion gel precipitation technique as described by Faber & Stone (1976a & b). The samples were also typed for the macroglobulin allotype Cl (Rapacz et al., 1968) and for the intracellular allotype Ec (Rapacz et al., 1975) using the single radial diffusion precipitation test. All serum samples were stored frozen (and shipped frozen from Milan to Madison) until typed.

Biochemical polymorphisms

Aliquots of the serum samples were typed for transferrins in Madison and in Milan using the technique described by Fiorentini et al. (1968). Both laboratories obtained completely concordant results. The sera were typed for albumins, according to the technique of Kristjansson (1963). Haemoglobin types were determined on lysed erythrocyte samples by both laboratories using the technique of Fiorentini et al. (1977), again with concordant results. Finally, erythrocyte lysates were typed in Milan for amylase (Ashton et al., 1967; Schleger, 1971) and for carbonic anhydrase (Sartore et al., 1969).

Gene frequency estimation

Simple gene counting was used to estimate the allele frequencies for the co-dominant systems; the appropriate standard errors for those estimates are simple binomial standard errors. Maximum likelihood, using the program MAXLIK (written by T. E. Reed, personal communication), was used to calculate the allele frequency estimates and their standard errors for the simple dominant and the complex systems. The A and C systems were assumed to have three alleles with a linear dominance hierarchy. The B and S systems were assumed to have a more complex relationship of dominance and co-dominance; the specific dominance relationships are those given in Kidd (1969).

Breed structure and inbreeding

For a locus at which heterozygotes can be specifically identified it is possible to calculate an estimate of the inbreeding coefficient, f, as

$$f = (H_{\text{H-W}} - H_{\text{obs}})/H_{\text{H-W}} \qquad (1)$$

where $H_{\text{H-W}}$ is the expected proportion of heterozygotes assuming the genotypes are in Hardy-Weinberg proportions and H_{obs} is the observed proportion of heterozygotes in the sample. (Expected and observed numbers can also be used in formula 1.) A significant $f \neq 0$ is measured by the χ^2 test for deviation of all genotypes from Hardy-Weinberg expectations. The degrees of freedom for each χ^2 is calculated as 'number of phenotypic classes' minus 'number of alleles present', provided that the expected number in each genotype is greater than 5.

The deviation of observed from H-W expected heterozygosity for many loci can be combined into one estimate by weighting the measure of *f* at each locus by the reciprocal of its sampling variance.

The exact value of the sampling variance when there are 3 or more alleles is currently under investigation (A. Robertson & M. Curie-Cohen, personal communications), but can be approximated as

$$1/n\,(k-1)$$

where *k* is the number of alleles and *n* is the number of individuals tested for the locus (A. Robertson, personal communication). Thus,

$$f = \frac{\hat{f}_i n_i (k_i - 1)}{n_i (k_i - 1)},$$

where *i* represents the *i* locus.

Phylogenetic analyses

Genetic distances were calculated as the tau values of Kidd & Cavalli-Sforza (1974) or as the E values of Edwards as described in Kidd et al. (1974). Principal components analysis of a distance matrix was done using the same computer program described by Kidd (1974). Phylogenetic trees, which are a representation of the distance matrix, were produced and evaluated on an additive model using the least squares programs described in Kidd & Sgaramella-Zonta (1971).

Results

Phenotypes and allele frequencies

The phenotype distributions found for the four Iberian breeds and American Longhorn are given in Table 1-3. The allele frequency estimates and their standard errors are given in Table 4 for

Table 1. Genotype frequencies of five polymorphic biochemical systems in Iberian and in American Longhorn cattle.

Locus* (System)	Genotypes	Breeds					
		De Lidia	Retinto	Mertolenga	Alentejana	Longhorn	
Hb	*AA*	79	117	126	142	120	
	AB	37	33	20	13	41	
	BB	8	14	1	0	6	
	total	124	164	147	155	167	
Ca	*SS*	52	70	95	118	58	
	SF	21	28	22	12	16	
	FF	29	31	8	6	3	
	total	102	129	125	136	77	
Al	*SS*	0	0	1	0	1	
	SF	1	9	45	18	15	
	FF	123	155	102	139	61	
	total	124	164	148	157	77	
Am	*BB*	20	56	70	86	41	
	BC	36	67	62	60	26	
	CC	68	41	16	11	10	
	total	124	164	148	157	77	
Tf	*AA*	22	57	32	13	17	
	AD_1	1	13	19	25	6	} + 43
	AD_2	43	44	29	25	16	
	AE	12	31	17	19	7	
	D_1D_1	0	4	1	9	4	} +27
	D_1D_2	0	1	9	4	10	
	D_2D_2	18	6	25	23	27	
	D_1E	0	2	3	16	0	} + 7
	D_2E	13	4	10	22	2	
	EE	14	2	1	1	0	
	total***	123	164	146	157	89	+ 77**

* See 'Materials and methods'.

** D_1 + D_2 were not distinguished in these 77 Longhorns.

*** The exact number of animals tested for each system. (The exact numbers of each genotype observed are given in the table along with the total number of animals tested for each system. For a given breed the number of animals tested for each locus sometimes differed because all samples were not available for all of the tests. For American Longhorn, 77 animals were typed for transferrin at a time when the alleles D_1 and D_2 were not distinguished.)

Table 2. Numbers of Iberian and American Longhorn cattle with different allotypes.

System[1] (locus)	Phenotypes	Breeds					
		De Lidia	Retinto	Mertolenga	Alentejana	Longhorn[2]	
Ig(g2)a	A1+A2–	65	119	107	93	59	} + 90
	A1+A2+	40	44	40	51	17	
	A1–A2+	18	3	2	11	1	
	total	123	166	149	155	167	
Ig(L)b	B1+	2	4	13	32	10	
	B1–	121	162	136	123	157	
	total	123	166	149	155	167	
Mc(*a*)c	C1+	30	40	9	33	11	
	C1–	93	126	140	124	109	
	total	123	166	149	157	120	
Ec	Ec+	10	53	59	68	12	
	Ec–	113	113	90	89	108	
	total	123	166	149	157	120	

[1] Ig(γ2)a = IgG_2; Ig(L)b = light chain allotype; Mc(*a*)c = macroglobulin allotype.
[2] Actual numbers of animals with each phenotype and the total number of animals typed for each breed and system are given. For the Alentejana and American Longhorn breeds not all samples were typed for all systems; for American Longhorn, 90 of the samples were not typed for A2.

those systems for which gene counting methods were used and in Table 5 for those systems for which maximum likelihood was used. For the transferrin and Ig(γ2)a loci of the American Longhorn, modified methods were used to allow gene counting estimates incorporating the data on animals not typed for all alleles. The incomplete nature of the data is reflected in the proportionately larger standard errors for those alleles in American Longhorn.

Table 3. Numbers of Iberian and American Longhorn cattle with different red blood cell types.

Locus	Genotypes or phenotypes	Breeds				
		De Lidia	Retinto	Mertolenga	Alentejana	Longhorn
Co-dominant[1]						
FV	FF	71	73	68	59	99
	FV	45	72	62	87	53
	VV	7	19	18	11	8
	total	123	164	148	157	160
R′S′	R′R′	0	0	1	0	NT[2]
	R′S′	7	14	17	31	NT
	S′S′	117	150	130	126	NT
	total	124	164	148	157	–

Table 3 (continued)

Locus	Genotypes or phenotypes	Breeds				
		De Lidia	Retinto	Mertolenga	Alentejana	Longhorn
Complex						
A	A_1	85	125	136	148	162
	A_2	22	21	8	3	4
	non-A	17	18	5	6	2
	total	124	164	149	157	168
B	B	72	61	28	58	42
	G	20	4	19	9	16
	B + G	14	29	21	11	18
	BGK	2	17	21	40	67
	null[3]	16	52	60	39	25
	total	124	163	149	157	168
C	C_1	51	82	78	107	119
	C_2	37	26	22	19	29
	non-C	36	56	49	31	19
	total	124	164	149	157	167
S	SH′	26	18	14	51	78
	SH′ U_1	0	2	5	6	10
	SH′ U_2	0	1	3	5	20
	H′	91	123	65	39	34
	H′ U_1	3	10	32	11	2
	H′ U_2	1	4	20	37	16
	H′ U_1U_2	1	0	6	4	1
	U_2	0	2	2	3	5
	null[4]	2	3	2	1	2
	total	124	163	149	157	168
Dominant						
J	J+	52	28	30	56	24
	J–	72	136	118	101'	144
	total	124	164	148	157	168
L	L+	19	5	31	8	66
	L–	105	159	117	149	101
	total	124	164	148	157	167
M	M+	0	1	2	0	0
	M–	124	163	146	157	167
	total	124	164	148	157	167
Z	Z+	115	150	122	119	126
	Z–	9	14	26	38	41
	total	124	164	148	157	167
T′	T′+	47	53	33	60	NT
	T′–	77	111	115	97	NT
	total	124	164	148	157	–

[1] There are no null alleles detected in these systems.
[2] Not tested.
[3] Null means no B, G, or K present.
[4] Null means no reactivity for any of the reagents at this locus.

Breed structure and inbreeding

For the simple dominant Systems there are not sufficient degrees of freedom to test for deviations from Hardy-Weinberg proportions. For the complex systems there are sufficient degrees of freedom to test agreement of the data with Hardy-Weinberg expectations, but it is not possible to determine observed heterozygosity because of dominance. Only the codominant systems allow a test of Hardy-Weinberg proportions and comparison of expected and observed homozygosity. For each of the 5 breeds Table 6 gives observed and expected homozygosity for the 8 codominant loci, the estimate of *f* (the inbreeding coefficient), and the χ^2 for deviation from Hardy-Weinberg proportions. The significance levels are adjusted for the number of loci tested in each breed. Each of the 4 Iberian breeds has some loci which show significant deviations; only one of these loci (FV in the Alentejana) has less than the expected amount of homozygosity and gives a negative estimate of the inbreeding coefficient. The average inbreeding coefficients, *f*, estimated for each of the five breeds are given in Table 7.

Genetic distances

Table 8 gives the genetic distances among the 4 Iberian breeds and American Longhorn calculated from the allele frequencies in Tables 4 and 5. The T´ locus was omitted because allele frequencies were not available for the American Longhorn. The R´ frequencies obtained by Miller (1966) were used for American Longhorn to allow that locus to be included in the calculations. To allow a comparison of these Iberian breeds

Table 4. Allele frequency estimates and their standard errors obtained by direct gene counting for eight immunogenetic systems in Iberian and American Longhorn cattle.

Allele	De Lidia	Retinto	Mertolenga	Alentejana	Longhorn
Hb^A	0.786±0.026	0.814±0.022	0.925±0.015	0.958±0.011	0.841±0.020
Hb^B	0.214±0.026	0.186±0.022	0.075±0.015	0.042±0.011	0.159±0.020
Ca^S	0.613±0.034	0.651±0.030	0.848±0.023	0.912±0.017	0.857±0.028
Ca^F	0.387±0.034	0.349±0.030	0.152±0.023	0.088±0.017	0.143±0.028
Al^S	0.004±0.004	0.027±0.009	0.159±0.021	0.057±0.013	0.110±0.025
Al^F	0.996±0.004	0.973±0.009	0.841±0.021	0.943±0.013	0.890±0.025
Am^B	0.306±0.029	0.546±0.028	0.682±0.027	0.739±0.025	0.701±0.037
Am^C	0.694±0.029	0.454±0.028	0.318±0.027	0.261±0.025	0.299±0.037
Tf^A	0.407±0.031	0.616±0.027	0.442±0.029	0.303±0.026	0.319±0.026
Tf^{D1}	0.004±0.004	0.073±0.014	0.113±0.019	0.201±0.023	0.143±0.027
Tf^{D2}	0.374±0.031	0.186±0.022	0.336±0.028	0.309±0.026	0.489±0.034
Tf^E	0.215±0.026	0.125±0.018	0.110±0.018	0.188±0.022	0.048±0.012
$Ig(\gamma 2)\ a^1$	0.691±0.030	0.849±0.020	0.852±0.021	0.765±0.024	0.877±0.027
$Ig(\gamma 2)\ a^2$	0.309±0.030	0.151±0.020	0.148±0.021	0.235±0.024	0.123±0.027
FV^F	0.760±0.027	0.665±0.026	0.669±0.027	0.653±0.027	0.784±0.023
FV^V	0.240±0.027	0.335±0.026	0.331±0.027	0.347±0.027	0.216±0.023
$R'S'^{R'}$	0.028±0.011	0.043±0.011	0.064±0.014	0.099±0.017	NT
$R'S'^{S'}$	0.972±0.011	0.957±0.011	0.936±0.014	0.901±0.017	NT

Table 5. Allele frequencies and their standard errors estimated by maximum likelihood for 12 immunogenetic systems in Iberian and American Longhorn cattle.

Allele[1]	De Lidia	Retinto	Mertolenga	Alentejana	Longhorn
Ig(L)b[1]	0.008±0.006	0.012±0.006	0.045±0.012	0.109±0.018	0.030±0.009
Ig(L)b	0.992±0.006	0.988±0.006	0.955±0.012	0.891±0.018	0.970±0.009
Mc(a)c[1]	0.130±0.022	0.129±0.019	0.031±0.010	0.111±0.018	0.047±0.014
Mc(a)c	0.870±0.022	0.871±0.019	0.969±0.010	0.889±0.018	0.953±0.014
Ec[1]	0.041±0.013	0.175±0.022	0.223±0.026	0.247±0.026	0.051±0.014
Ec	0.959±0.013	0.825±0.022	0.777±0.026	0.753±0.026	0.949±0.014
J^J	0.238±0.029	0.089±0.016	0.107±0.019	0.198±0.024	0.074±0.015
J^j	0.762±0.029	0.911±0.016	0.893±0.019	0.802±0.024	0.926±0.015
L^L	0.080±0.018	0.015±0.007	0.111±0.019	0.026±0.009	0.222±0.024
L^l	0.920±0.018	0.985±0.007	0.889±0.019	0.974±0.009	0.778±0.024
M^M	0	0.003±0.003	0.007±0.005	0	0
M^m	1	0.997±0.003	0.993±0.005	1	1
Z^Z	0.731±0.043	0.708±0.037	0.581±0.037	0.508±0.035	0.505±0.034
Z^z	0.269±0.043	0.292±0.037	0.419±0.037	0.492±0.035	0.495±0.034
T′T′	0.212±0.028	0.177±0.022	0.119±0.019	0.214±0.025	NT
T′t′	0.788±0.028	0.823±0.022	0.881±0.019	0.786±0.025	NT
A^{A1}	0.439±0.037	0.512±0.034	0.705±0.039	0.761±0.039	0.811±0.038
A^{A2}	0.191±0.036	0.156±0.031	0.112±0.035	0.044±0.024	0.080±0.035
A^a	0.370±0.042	0.331±0.037	0.183±0.040	0.195±0.039	0.109±0.038
B^{BGK}(	0.008±0.006	0.054±0.013	0.073±0.015	0.137±0.020	0.225±0.024
B^G	0.152±0.024	0.108±0.018	0.153±0.023	0.076±0.016	0.141±0.023
B^B	0.460±0.038	0.342±0.030	0.192±0.025	0.306±0.031	0.275±0.031
B^b	0.380±0.038	0.496±0.032	0.582±0.032	0.481±0.033	0.360±0.033
S^{U2}	0.008±0.006	0.022±0.008	0.107±0.018	0.163±0.021	0.131±0.019
$S^{U1H'}$	0.016±0.008	0.037±0.011	0.156±0.022	0.069±0.015	0.039±0.011
$S^{SH'}$	0.111±0.021	0.066±0.014	0.076±0.017	0.225±0.025	0.394±0.030
$S^{H'}$	0.740±0.048	0.728±0.040	0.575±0.041	0.507±0.038	0.349±0.038
S^8	0.124±0.044	0.146±0.037	0.086±0.034	0.036±0.030	0.087±0.031
C^{C1}	0.233±0.029	0.293±0.028	0.310±0.030	0.436±0.033	0.464±0.033
C^{C2}	0.228±0.033	0.123±0.023	0.117±0.023	0.120±0.026	0.199±0.032
C^c	0.539±0.038	0.584±0.032	0.573±0.034	0.444±0.036	0.337±0.036

[1] The 'alleles' for the *A*, *B*, *S*, and *C* loci were defined by considering only a subset of the antigens determined at each of these complex factor union systems. Thus, each allele represents a collection

with other European breeds, allele frequency data on 14 of these loci for 9 additional breeds were used to calculate the genetic distances in Table 9. The unpublished allele frequencies for these 9 additional breeds are based on the data in Kidd (1969) supplemented with data on additional animals typed since that study (Stone et al., unpublished; see also Kidd, 1974).

Phylogenetic analysis

Fig. 1 is the best least squares tree for the distances given in Table 8. Fig. 2 is the best least squares tree of the cattle breeds whose distances are given in Table 9. The

Table 6. Estimates of the inbreeding coefficient, *f*, calculated from data on electrophoretic and other closed immunogenetic systems. Each estimate off is calculated as the proportionate deviation from expected heterozygosity at the locus. The χ^2 measures the significance of the deviation from Hardy-Weinberg proportions for all genotypes in a given system.

System	De Lidia					Retinto				
(locus)	homozygosity		f	χ^2	d.f.	homozygosity		f	χ^2	d.f.
	obs.	exp.				obs.	exp.			
Hb	0.7	0.66	0.112	1.56	1	0.8	0.7	0.335	18.5*	1
Ca	0.79	0.53	0.566	32.7*	1	0.78	0.55	0.552	35.2*	1
Al	0.99	0.99	++	++	–	0.95	0.95	–0.028	++	–
Am	0.71	0.57	0.317	12.5*	1	0.59	0.5	0.176	4.71	1
Tf+	0.44	0.35	0.135	19.6 *	2	0.42	0.43	–0.02	5.14	3
Ig(γ2)a	0.67	0.57	0.238	6.99*	1	0.73	0.74	–0.04	++	–
R′S′	0.94	0.95	–0.03	++	–	0.91	0.92	–0.05	++	–
FV	0.63	0.63	–0.003	0.02	1	0.56	0.55	0.015	0.04	1

System	Mertolenga					Alentejana				
(locus)	homozygosity		f	χ^2	d.f.	homozygosity		f	χ^2	d.f.
	obs.	exp.				obs.	exp.			
Hb	0.86	0.86	0.017	++	–	0.92	0.92	-0.044	++	–
Ca	0.82	0.74	0.317	12.58*	1	0.91	0.84	0.452	27.74*	1
Al	0.7	0.73	-0.138	2.83	1	0.89	0.89	-0.061	++	–
Am	0.58	0.57	0.033	0.17	1	0.62	0.55	0.01	0.01	?
Tf+	0.4	0.33	0.107	12.65*	4	0.29	0.26	0.041	26.38*	6
Ig(γ2)a	0.73	0.75	–0.067	++	–	0.67	0.64	0.086	1.15	?
R′S′	0.89	0.88	0.044	++	–	0.8	0.82	–0.110	++	–
FV	0.58	0.56	0.054	0.44	1	0.45	0.55	–0.223	7.78*	1

System	Longhorn				
(locus)	homozygosity		f	χ^2	d. f.
	obs.	exp.			
Hb	0.75	0.73	0.081	1.08	1
Ca	0.79	0.76	0.152	1.77	1
Al	0.81	0.8	0.008	++	–
Am	0.66	0.58	0.194	2.9	1
Tf+	0.51	0.5	0.016	0.93	2
Ig(γ2)a	0.78	0.78	–0.021	0.033	1
R′S′	–	–	–	–	–
FV	0.67	0.66	0.02	0.07	1

* $P < 0.05$.

\+ Does not consider D_1 and D_2 as separate alleles in Longhorn only.

++ Insufficient data to calculate value usually because one or more classes had an expected value < 1.0.

qualitative relationships among the Iberian breeds are the same in both figures but there are some differences in the proportions of the segments in the tree. Such small differences are not unusual when matrices based on different numbers of loci are used. Fig. 3 shows a different graphical representation of the distances in Table 9.

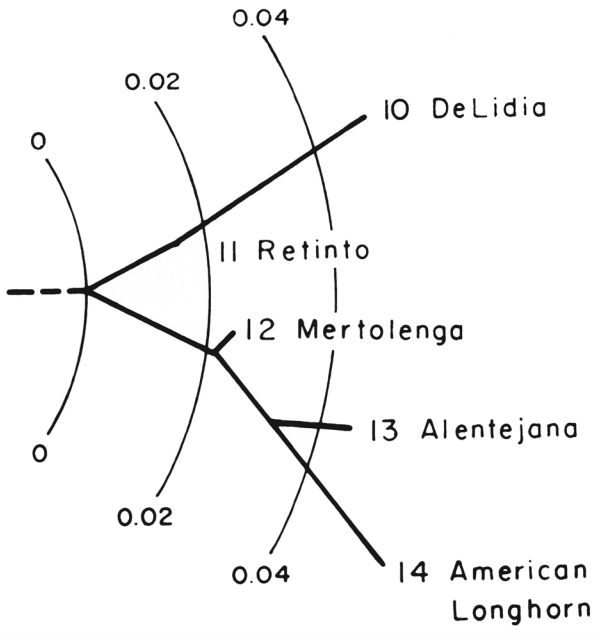

Fig. 1. Evolutionary relationships among Iberian cattle. This is the best phylogenetic tree for the genetic distances in Table 8; all possible trees for these five populations were evaluated (Kidd & Sgaramella-Zonta, 1971). The tree is drawn on polar coordinates; actual lengths, as estimated by least squares, are the radial lengths of each segment.

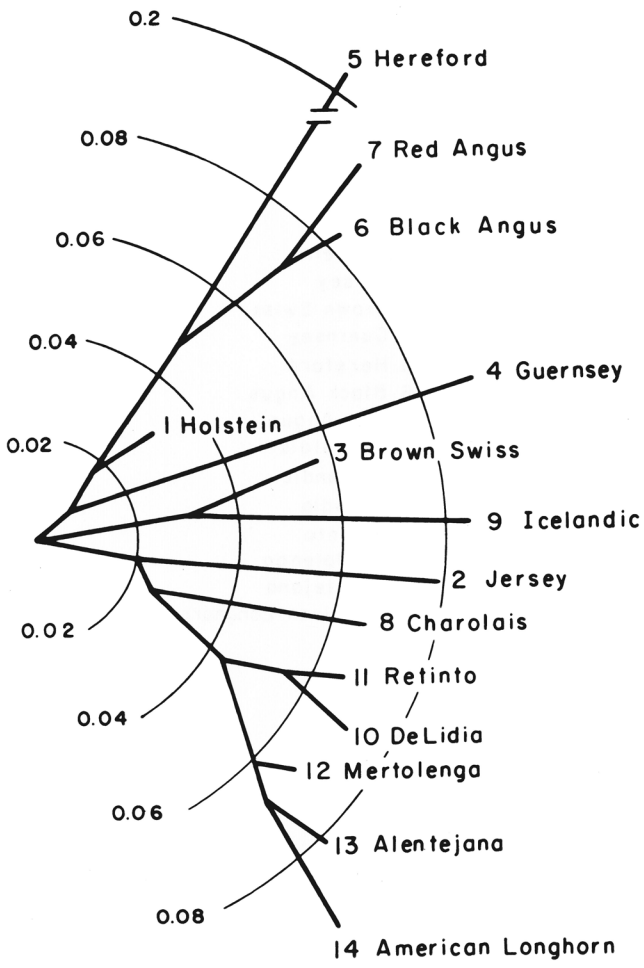

Fig. 2. Evolutionary relationships among 14 cattle breeds. From among the many examined, this is the phylogenetic tree that gives the best additive representation of the genetic distances in Table 9. The tree is drawn on polar coordinates; actual lengths, as estimated by least squares, are the radial lengths of each segment.

Table 7. Average inbreeding of Iberian and American Longhorn cattle.

Breed	Number of loci used	$\overline{f}$ ± S.E.[1]
De Lidia	7	0.171 ± 0.030 **
Retinto	8	0.080 ± 0.025 **
Mertolenga	8	0.054 ± 0.026 *
Alentejana	8	0.018 ± 0.025 NS
Longhorn	7	0.049 ± 0.034 NS

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

[1] Each $\overline{f}$ is the estimate of the average inbreeding coefficient for the breed based on the *f* values for several loci given in Table 6. The method for averaging the *f* values is referred to in the text.

Table 8. Genetic distances[1] among Iberian and American Longhorn cattle.

		1	2	3	4	5
De Lidia	1	0.0	0.0077	0.0134	0.0190	0.0162
Retinto	2	0.0308	0.0	0.0067	0.0106	0.0122
Mertolenga	3	0.0753	0.0334	0.0	0.0033	0.0079
Alentejana	4	0.0960	0.0548	0.0248	0.0	0.0089
Longhorn	5	0.1005	0.0737	0.0378	0.0394	0.0

[1] The distances given are the tau (mixed) values (lower half of matrix) with their standard errors (upper half of matrix). The distances were calculated from the allele frequencies at the following 19 loci: *A, B, C, F, J, L, M, S, Z, R′, Hb, Ca, Al, Am, Tf, Iga, Igb, Mc, Ic.*

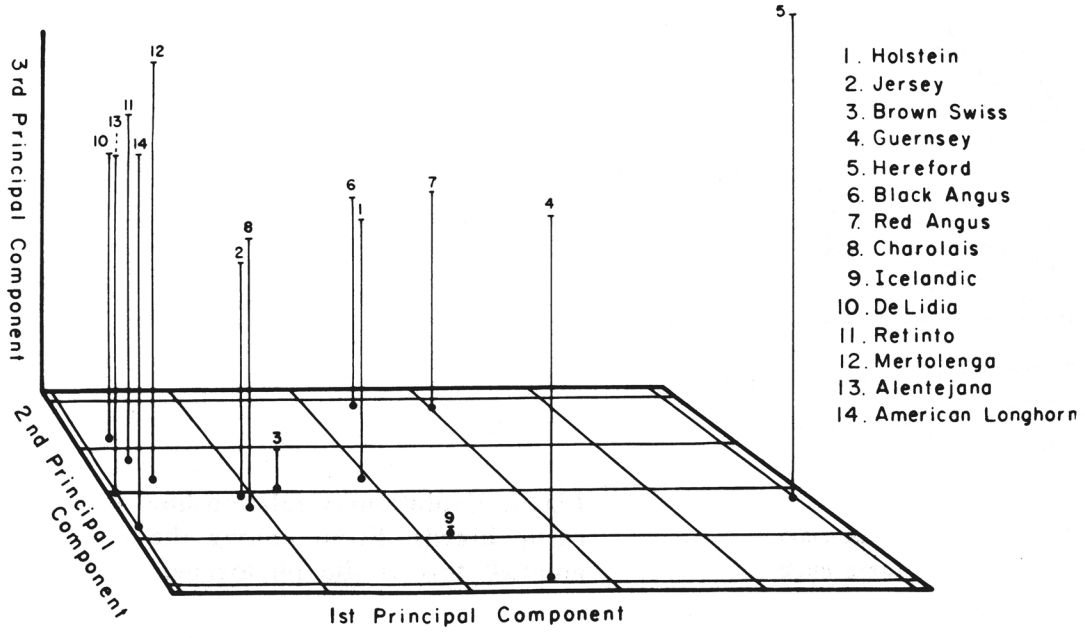

Fig. 3. Evolutionary distances among 14 cattle breeds. The positions of 14 cattle breeds in this genetic space are defined by the first three principal components of the distance matrix in Table 9.

Table 9. Genetic distances among 14 breeds of cattle. The distances given are the tau (mixed) values calculated from allele frequencies at the following 14 loci: *A, B, C, F, J, L, M, S, Z, Hb, Tf, Iga, Mc, Ic.* The distances among the Iberian breeds differ slightly from those given in Table 8 because only a subset of the loci used for Table 8 were used for calculating these distances; the qualitative relationships among Iberian breeds are essentially identical in the two tables.

Holstein	1	0.0													
Jersey	2	0.1015	0.0												
Brown Swiss	3	0.1090	0.1300	0.0											
Guernsey	4	0.1088	0.1631	0.1817	0.0										
Hereford	5	0.2056	0.2908	0.2892	0.1803	0.0									
Black Angus	6	0.0820	0.1515	0.1368	0.2184	0.2204	0.0								
Red Angus	7	0.0910	0.1963	0.1452	0.1971	0.1795	0.0393	0.0							
Charolais	8	0.1077	0.1123	0.1150	0.1326	0.2779	0.1520	0.1688	0.0						
Icelandic	9	0.1218	0.1648	0.0804	0.1329	0.2575	0.1724	0.1688	0.1434	0.0					
De Lidia	10	0.0931	0.0840	0.1138	0.2075	0.2958	0.1107	0.1437	0.0918	0.1793	0.0				
Retinto	11	0.0896	0.0937	0.1056	0.1937	0.2703	0.1225	0.1667	0.0973	0.1752	0.0267	0.0			
Mertolenga	12	0.0838	0.1240	0.1377	0.1595	0.2657	0.1289	0.1574	0.0799	0.1872	0.0590	0.0300	0.0		
Alentejana	13	0.1161	0.1514	0.1158	0.1653	0.3265	0.1498	0.1678	0.0909	0.1675	0.0694	0.0506	0.0251	0.0	
Longhorn	14	0.1186	0.1221	0.1371	0.1395	0.3282	0.2032	0.2101	0.1125	0.1679	0.0926	0.0833	0.0472	0.0431	0.0
		1	2	3	4	5	6	7	8	9	10	11	12	13	14

Discussion

Inbreeding

With the exception of the M^M allele, all of the alleles studied reached an appreciable frequency in at least one of the 5 breeds. A large amount of genetic drift or a large founder effect in the ancestry of any of these breeds would be manifest as a loss of genetic variability and a reduction in the number of polymorphic alleles. There is no evidence for such an effect at the level of the gene frequency. However, the data in Table 6 show that in each of the 4 Iberian breeds at least 25 % of the loci studied show a significant deviation from Hardy-Weinberg proportions. Ten of the 11 significant deviations are associated with a positive estimate for the in-breeding coefficient ($P < 0.01$ using a one-tailed sign test). More importantly, the combined estimates of inbreeding (Table 7) show that 3 of the 5 breeds have in-breeding coefficients significantly greater than zero. The inbreeding coefficient of the Spanish De Lidia breed is exceedingly high (0.171); in fact it is greater than that expected if all of the animals were progeny of half-sib matings. Also, the inbreeding coefficient of the other Spanish breed (Retinto) is greater than that expected from matings of first cousins.

An *f* value based upon the difference between observed and expected heterozygosity can have two different explanations. It could reflect actual inbreeding of individual animals in the sample. It could also be due to the Wahlund effect which occurs with subdividion of a population. If random mating existed only within the subdivisions of the breed and the sample consisted of some individuals from two or more of those subdivisions, any deficiency in heterozygotes in the 'pooled' sample would be a measure of the degree to which allele frequencies differed among the subdivisions. Almost certainly for De Lidia and possibly also for Retinto both explanations contribute to the large *f* values observed. Our sample of each breed clearly consisted of a pool drawn from separate herds. Artificial insemination programs for these breeds are just beginning and individual breeders still tend to rely on their own or neighborhood bulls. Such breeding practices give ample chance for gene frequency variation among herds as well as actual inbreeding in the ancestry of any particular animal. Interestingly, the same breeding patterns exist for the Portuguese breeds and the same sampling technique was used, but these breeds do not show a markedly elevated *f*. A very small rate of migration between the geographic populations would be sufficient to prevent the development of a large *f*. Hence, a likely conclusion is that the amount of geographic isolation among herds is greater for these Spanish breeds than for the Portuguese breeds.

Geographic isolation alone seems unlikely to account for such extremely large *f* values as found in the Spanish breeds. Actual inbreeding from the mating of closely related animals probably exists for both breeds. Unfortunately, breeding records for the Retinto were not available in sufficient detail to reconstruct pedigrees. The breeding records for the De Lidia cattle are probably quite detailed but are jealously guarded. The

individual breeders take great pride in their particular lineage and almost certainly practice inbreeding.

One caveat to the significance of the high levels of inbreeding comes from an inspection of the *f* values in Table 6. One locus, *Ca*, gives the highest estimate of *f* in 4 of the 5 breeds and is second highest in the fifth. A priori, that distribution is very unlikely if the 8 loci provide truly independent and unbiased estimates of *f*. We are unsure of the proper correction for the evaluation of its statistical significance given that it was observed to be extreme because we do not know at what level we would have noted a 'systematically' deviant locus. Thus, the possibility exists that the deficiency of heterozygotes at the *Ca* locus represents a systematic factor other than inbreeding. Two possible explanations are: a problem in detection of heterozygotes or a viability problem for heterozygotes.

If the *Ca* locus estimates of *f* are inflated, the *f* estimates would be lower than we have calculated but would still be appreciable for the two Spanish breeds.

Genetic relationships

The genetic relationships among the Iberian breeds (Table 8 and Fig. 1) present no surprises. The small genetic distances among these breeds relative to those between this group (including American Longhorn) and the other breeds confirm that these are closely related breeds. Fig. 2 and 3 show relatively tight clustering of Iberian breeds in two different graphical representations. The major separation within the Iberian breeds reflects the geographic distinction between Spanish and Portuguese breeds. Fig. 1 suggests that De Lidia is a more highly derived breed originating from a breed not genetically distinguishable from modern Retinto. This agrees with the historical data. Fig. 2 does not give the same impression, but is based on many fewer loci.

The clustering of these breeds with Charolais and Jersey suggests a common Western European origin in agreement with their present location. There is no evidence that any of these breeds represents a 'recent' immigration into the area. Historical records of De Lidia sales in the 14th century further support the conclusion that these native Iberian breeds have been native to the Iberian Peninsula for a long time.

Though descended from cattle brought by the Spaniards, the American Longhorn cattle were considerably hybridized with other cattle breeds, notably Hereford and Shorthorn (Miller, 1966). In establishing the herds at the Wichita Wildlife Refuge in Oklahoma, an attempt was made to select those animals which phenotypically were closest to the pure Longhorn type. In his study of these Longhorns, Miller (1966) found some *B* locus phenogroups typical of English beef cattle breeds, but in general, noted considerable differences between Longhorn and most other American cattle breeds. He concluded that much of the 'Spanish' ancestry had been preserved in the remnants of the American Longhorn breed. Our results agree with this conclusion and show by direct comparison with existing Iberian breeds that the American Longhorn breed does not

differ from the two Portuguese breeds any more than the Portuguese breeds differ from the Spanish breeds (see Table 8 and Fig. 1).

Fig. 2 and 3 are also informative on the genetic relationships of several economically important breeds. The most noticeable aspects of Fig. 2 are the extreme length of the branch to Hereford and the very short length of the branch to Holstein. According to the evolutionary model used to calculate the distances and reconstruct the tree (Kidd & Cavalli-Sforza, 1974) these lengths are proportional to time since divergence (in generations) divided by twice the effective population size. These results would suggest that Hereford has a much smaller effective population size, averaged over time, than other breeds studied. Holstein has evidently had a much larger effective population size. Both conclusions agree with the histories of these breeds.

Economic significance

Inbreeding, per se, serves only to change the distribution of genotypes in the population such that the proportion of homozygotes for all alleles is increased. In most mammalian species an increase in the proportion of homozygous loci results in an average decrease in fitness because of the greater chance of a rare deleterious recessive trait being expressed. This has been confirmed for cattle in a large study of inbreeding in dairy cattle (Young et al., 1969). Height, girth, body length and width, body weight, milk yield, fat yield, and fat percentage all showed significant decreases with inbreeding. In that large collaborative study inbreeding coefficients, by pedigree evaluation, were rarely above 0.25-0.30. Individual animals with these higher levels of inbreeding showed very large and highly significant decreases in almost all economically relevant traits, including mortality. Indeed, though in-breeding studies summarized by Young et al. (1969) were undertaken as a means of establishing improved strains of dairy cattle, the results indicated that inbreeding would have only limited application 'due to its many detrimental effects'. Assuming that the increased homozygosity at these polymorphic loci provides accurate estimates of the inbreeding coefficients and, hence, of increased probabilities of homozygosity at all loci, the De Lidia and Retinto breeds likely show a large amount of inbreeding depression.

Though the De Lidia breed is not of agricultural importance, the high inbreeding coefficient found suggests that breeding practices are not optimal. The Retinto is a breed of agricultural importance and our findings suggest that by altering breeding practices the productivity of the breed could probably be improved. The non-random inbreeding could be reduced to near zero almost immediately by a simple distribution of bulls. An increase in viability, health, and productivity should result from the decrease in the degree of inbreeding.

As can be seen from Fig. 3 the American Longhorn breed shows a close genetic relationship to the Spanish and Portuguese breeds in our study. Thus, there is available for breeding within the United States a genetic resource representative of Iberian cattle.

The distinctive genetic heritage of American Longhorns coupled with the selection this breed has experienced during its history in North America qualifies it as an important germ plasm resource for preservation and possible inclusion in future breeding programs.

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