GENETICS OF TRANSFERRINS IN BURROS (*EQUUS ASINUS*)¹

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AFTER the development of the starch gel electrophoresis technic, reports of serum protein variations within and between species became numerous. With few exceptions, genetic analyses of these variations showed that they were determined by a series of codominant alleles (see GIBLETT [1962] and JAMIESON [1966] for extensive references). Variations in β -globulin components, transferrins, have been studied extensively because they are easily identified using radioactive iron and autoradiography (GIBLETT *et al.* 1959). The literature describing transferrin polymorphism is now fairly voluminous in terms of the number of species studied, the number of individuals studied within a species, and the genetic analyses performed (GIBLETT 1962; JAMIESON 1965, 1966). The only study of transferrin variation in burros (*Equus asinus*) that we know of was reported briefly by PODLIACHOUK, KAMINSKI and DABCZEWSKI (1965). They described six phenotypes of fast β -globulins and four phenotypes of slow β -globulins. Each of the six fast β -globulin phenotypes consisted of one to four bands, and each of the slow phenotypes consisted of 0, 2 or 4 bands. Autoradiography with Fe^{59} showed that all slow β -globulin bands bound iron, but one of the fast bands did not. It was not specified which fast band did not bind iron, consequently close comparison with the results reported here is not possible.

MATERIALS AND METHODS

Over a period of three years, 222 burros (136 females, 86 males), maintained by the University of Tennessee, AEC Agricultural Research Laboratory, at Oak Ridge, Tennessee, were sampled. Husbandry practices with the burros varied. Sometimes females were isolated with a single male, but at other times up to 20 burros (males and females) were maintained in a pen or pasture. However, females were isolated before delivery and the foal was ear-tagged a day or two after birth and branded when older. Because of these husbandry practices, the only family data considered reliable were jenny-foal pairs.

Clotted bloods were shipped on ice to Madison; the sera were collected and stored below -20°C until used. Vertical starch gel electrophoresis (SMITHIES 1959) was employed. Electrophoresis was begun within 24 hr after the gel was prepared and was run overnight in the cold (about 4°C) at 5 to 8 v/cm. Recently, platinum wire replaced the Ag/AgCl electrodes. Immediately before insertion into the gel, each sample received about $\frac{1}{5}$ volume of Fe^{59} (as either $\text{FeC}_6\text{H}_6\text{O}_7$ or FeCl_3) with a specific activity of 10 to 20 mc/mg Fe and a concentration of about 0.002 mg Fe/ml.

After electrophoresis, the gel was sliced longitudinally. The top slice was stained in amido black 10B, washed with methanol:water:glacial acetic acid (5:5:1), and photographed with

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FIGURE 1.—Starch gel stained with amido black showing burro transferrin patterns. Phenotypes and genotypes are indicated. Dots mark bands that bind iron as determined from autoradiograms.

Polaroid type 55 P/N film. The bottom slice was wrapped in "Saran Wrap" plastic sheeting for autoradiography. Kodak "No-screen" X-ray film was exposed for one day to two weeks either in a refrigerator or freezer, after which the autoradiographs were developed with Kodak X-ray film developer.

Any animal whose serum gave equivocal results was re-bled and the serum assayed again.

RESULTS AND DISCUSSION

Among the 222 serum samples assayed, ten transferrin phenotypes were clearly distinguishable and reproducible as autoradiograms. The patterns could not be

TABLE 1

Gene frequencies of burro transferrins

Allele	Males	Females	Males + females
<i>Tf^A</i>	0.52	0.51	0.51
<i>Tf^B</i>	0.17	0.15	0.16
<i>Tf^C</i>	0.19	0.17	0.18
<i>Tf^D</i>	0.12	0.17	0.15
N	86	136	222

determined from stained gels alone because they overlapped with other β -globulins. These phenotypes and their proposed genotypes are shown in Figure 1. The notation follows the recommendations described by ASHTON *et al.* (1967). Four of the patterns had two bands and six of them had four bands. The six patterns showing four bands resembled combinations of the other four patterns taken two at a time. This suggested that the two-band patterns represented homozygous types and that the four-band patterns represented heterozygous types. This is analogous with the patterns in other animals in which the transferrins are determined by codominant autosomal alleles.

The frequencies of the four proposed transferrin alleles for the 222 burros are listed in Table 1. The genotype frequencies for a random breeding diploid population did not differ significantly from equilibrium frequencies ($P > 0.995$). Autosomal inheritance is assumed because both sexes exhibited the phenotypes of presumed heterozygous genotypes.

The nature of the breeding practices allows use of only jenny-foal incomplete family data to test the hypothesis of autosomal inheritance with four alleles. The original data are presented in Table 2.

The calculations in Table 3A are based on the method described by COOPER and RENDEL (1967). The method is briefly as follows: When the genotype of one parent is known the ratio of genotypes among the offspring will equal the ratio of gene frequencies among the other parental sex. For example, where X

TABLE 2

Transferrin phenotypes of 98 jenny-foal pairs

[illegible]

TABLE 3

Transferrin types among foals of jenny-foal pairs.

A. Calculation of expected frequencies*

Jenny genotype	Foal genotype		
	Tf^X/Tf^X	Tf^X/Tf^Y	Tf^Y/Tf^Y
Tf^X/Tf^X	$P_X N_1$	$P_Y N_1$	
Tf^X/Tf^Y	$\frac{1}{2} P_X N_2$	$\frac{1}{2} (P_X + P_Y) N_2$	$\frac{1}{2} P_Y N_2$
Tf^Y/Tf^Y		$P_X N_3$	$P_Y N_3$

 Tf^X is any transferrin allele. Tf^Y is all transferrin alleles except Tf^X . P_X = frequency of Tf^X allele among males. P_Y = sum of frequency of all alleles except Tf^X . N_1 = number of foals of Tf^X/Tf^X jennies. N_2 = number of foals of Tf^X/Tf^Y jennies. N_3 = number of foals of Tf^Y/Tf^Y jennies.

* Based on the method of COOPER and RENDEL (1967).

B. Observed frequencies and χ^2 values

Tf^X		$P_X N_1$	$P_Y N_1$	$\frac{1}{2} P_X N_2$	$\frac{1}{2} (P_X + P_Y) N_2$	$\frac{1}{2} P_Y N_2$	$P_X N_3$	$P_Y N_3$	χ^2
Tf^A	Obs.	12	10	14	22	14	12	14	1.00
	Exp.	11	11	12.5	25	12.5	13	13	
Tf^B	Obs.	0	0	3	18	12	13	52	0.48
	Exp.	0	0	3	16.5	13.5	11.7	53.3	
Tf^C	Obs.	2	4	2	10	11	10	59	1.09
	Exp.	3	3	1.7	11.5	9.8	10.4	58.6	
Tf^D	Obs.	0	1	2	15	15	10	55	0.87
	Exp.	0.5	0.5	2.6	16.0	13.4	10.4	54.6	

represents Tf^X and $Y Tf^Y$, among progeny of jennies of the genotype X/X the ratio of $X/X:X/Y$ foals will be the ratio $P_X:P_Y$ where P_X is the gene frequency of X and P_Y is the frequency of Y . Likewise, among progeny of X/Y jennies the ratio $X/X:Y/Y$ is $P_X:P_Y$. As can be seen in Table 3B, the analysis of these data indicates that the distribution of transferrin types among the foals did not differ significantly from expected ($P > 0.25$).

As shown in Table 4, the data were analyzed further for segregation by a similar method (COOPER 1966). This method utilizes unique genotypes of heterozygous jennies. Simply, it is based on the expectation that among the progeny of heterozygous jennies the ratio of homozygotes:heterozygotes is 1:1. From Table 4 it is obvious that these ratios did not differ significantly from expected based on the autosomal codominant hypothesis.

If the maternal and offspring transferrin types had been a random sample from the general population of types, one would have expected that there would have been foal and jenny pairs with no transferrin allele in common. This was

TABLE 4

*Genotypic ratios among foals of heterozygous jennies**

Jenny genotype Tf^X/Tf^Y	Foal genotype				
	Tf^X/Tf^X	Tf^Y/Tf^Y	Tf^X/Tf^Y	Tf^X/Tf^Z	Tf^Y/Tf^Z
Tf^A/Tf^B	5	2	6	4	3
Tf^A/Tf^C	2	0	4	1	2
Tf^A/Tf^D	7	2	4	3	5
Tf^B/Tf^C	1	1	3	3	0
Tf^B/Tf^D	0	0	0	3	2
Tf^C/Tf^D	1	0	0	1	4
	16	5	17	15	16

$(Tf^X/Tf^X + Tf^Y/Tf^Y) : Tf^X/Tf^Y = 21:17$, $\chi^2 = 0.42$, $P > 0.5$.

* Based on the method of COOPER (1967).

never observed. The probability of randomly drawing a foal for each of the 98 jennies so that every foal would have at least one transferrin allele in common with the jenny is less than 10^{-14} .

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SUMMARY

The transferrins of 136 female and 86 male burros were typed by starch gel electrophoresis. Autoradiography was required to clearly identify ten different phenotypes. Jenny-foal family data were consistent with the hypothesis that transferrins are controlled by four codominant autosomal alleles each determining two bands.

LITERATURE CITED

- ASHTON, G. C., D. C. GILMOUR, C. A. KIDDY, and F. K. KRISTJANSSON, 1967 Proposals on the nomenclature of protein polymorphisms in farm livestock. *Genetics* **57**: 353-362.
- COOPER, D. W., 1966 A note on the estimation of genotypic ratios in domestic animals using incomplete family data. *Anim. Prod.* **8**: 511-513.
- COOPER, D. W., and J. RENDEL, 1967 The utilization of incomplete family data in selection and population studies of transferrins and blood groups in cattle. *Heredity* (In press).
- GIBLETT, E. R., 1962 The plasma transferrins. *Prog. Med. Genet.* **2**: 34-63.
- GIBLETT, E. R., C. G. HICKMAN, and O. SMITHIES, 1959 Serum transferrins. *Nature* **183**: 1589-1590.
- JAMIESON, A., 1965 The genetics of transferrins in cattle. *Heredity* **20**: 419-441. — 1966 The distribution of transferrin genes in cattle. *Heredity* **21**: 191-218.
- PODLIACHOUK, L., M. KAMINSKI, and Z. DABCZEWSKI, 1965 Groupes sanguins et seriques des anes. *Ann. Biol. Anim., Bioch., Biophys.* **5**: 445-450.
- SMITHIES, O., 1959 An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal humans. *Biochem. J.* **71**: 585-587.