Transferrin Types in Indian Water Buffalo (*Bos bubalis*)

Taxonomically, Indian Water Buffalo (*Bos bubalis*) differs from the American and African buffaloes. Indian water buffalo was domesticated as early as 4000 years ago along with the Zebu Cattle[1]. Buffalo is an important dairy animal in India with a population of about 48 million[2] comprising 7–8 recognized breeds.

Transferrin polymorphism in the Indian buffaloes is not yet reported. However, the erythrocytic antigens cross reacting with the cattle blood typing reagents and hemoglobin polymorphism in them were reported[3-6]. Hemoglobin and transferrin in the American[7,8], African[9], and Thai[10] buffaloes were studied by different workers. In this communication, for the first time, transferrin polymorphism in the Indian buffaloes is reported.

Material and method. Random blood samples from 155 adult, healthy, Murrah buffaloes were collected from the local slaughter-house. The serum was separated and subjected to the horizontal starch-gel electrophoresis along with the known cattle Tf-AE and Tf-DD types (see Figure 1). The discountinuous buffer system described by KRISTJANSSON[11] was adopted. The samples were inserted by means of Whatman No. 3 filter paper strips and the runs were made horizontally for 2 h 15 min at 200–250 volts. The only modification adopted was to reduce the thickness of the gel to 3 mm and to stain it with Amido Black without slicing. The transferrin types were read on the reverse side of the gel after clearing.

Results and discussion. 3 transferrin phenotypes called Tf-AA, Tf-AB and Tf-BB, where AA had a faster mobility than BB type, were encountered in this study. The homozygous types had 3 bands while heterozygous had 4 bands (see Figures 1 and 2). Thus the homozygous phenotypes respectively simulated Tf-AA and Tf-DD types of cattle in their number of bands but their bands had slightly lower mobility when compared to those in cattle. The Table summarizes the percentage phenotype frequency, observed and expected numbers together with their χ^2 values and the gene frequency. The Tf-BB type was found to predominate (72.90%) over Tf-AA type which was rare (1.94%). 2 sire families with 4 matings each, when examined, showed that Tf-BB type was an inherited character. Attempts are being made to study the inheri-
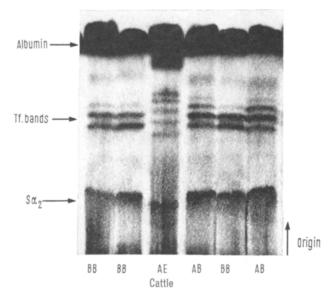
tance of Tf-AA type by screening animals in the dairy herds. It is likely that the transferrin types are determined by co-dominant allelic genes, Tf^A and Tf^B. Under this assumption the gene frequency of the 2 alleles was estimated to be $Tf^A = 0.145$ and $Tf^B = 0.855$. The frequency of expected and observed genotypes agreed well, giving the χ^2 value of 0.03004 for one degree of freedom.

The β-globulin responsible for the transferrin types in buffalo seems to be different but has a monomorphic form in American and African buffaloes[8,9]. The Thai

Buffalo transferrin phenotypes and their frequency observed and expected numbers in 155 samples examined

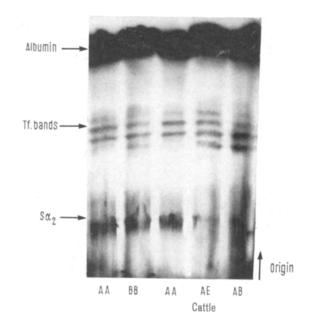| Phenotype	Observed	%	Expected	(d.f. $x^2 = 1$)
Tf-AA	3	1.94	3.26	0.03004
Tf-AB	39	25.16	38.42	
Tf-BB	113	72.90	113.30	
Gene Frequency	$Tf^A = 0.145$; $Tf^B = 0.855$			

Fig. 2. Starch gel electrophoretogram showing bufalo transferrin polymorphism.

[1] M. WHEELER, *The Indus Civilization* (Cambridge University Press, Cambridge 1953).

[2] M. S. RANDHAWA, *Agriculture and Animal Husbandry in India* (I.C.A.R., 1958), p. 287.

[3] S. P. DATTA and W. H. STONE, Nature *199*, 1208 (1963).

[4] CHET RAM, N. D. KHANNA and S. S. PRABHU, Ind. J. vet. Sci. *34*, 84 (1964).

[5] A. S. BALANI and J. BARNABAS, Nature *205*, 1019 (1965).

[6] S. N. NAIK and P. K. SUKUMARAN, Xth European Conference on Animal Blood Groups and Biochemical Polymorphism (Institut National de la Recherche Agronomique, Paris 1966), p. 401.

[7] M. BRAEND and C. STORMONT, Nature *197*, 910 (1963).

[8] C. STORMONT, Fedn Proc. USA *23*, 2 (1964).

[9] D. R. OSTERHOFF and E. YOUNG, Xth European Conference on Animal Blood Groups and Biochemical Polymorphism (Institut National de la Recherche Agronomique, Paris 1966).

[10] P. LOUPETJRA, Veternaerog Landbohojskole, Copenhagen, 221 (1962). Quoted by J. RENDEL, Bulletin on Blood Groups and Protein Polymorphism in the Domestic Buffalo (1967).

[11] F. K. KRISTJANSSON, Genetics, *46*, 907 (1961).

Fig. 1. Starch gel electrophoretogram showing buffalo transferrin types in comparison with cattle Tf-AE.

buffaloes, on the other hand, showed Tf-AA, Tf-DD and Tf-AD phenotypes, which were indistinguishable from the respective types in cattle[10]. However, the frequency of the gene Tf^A was only 0.27 while that of Tf^D was 0.73. The gene frequency for the 2 transferrin types Tf^A and Tf^B were found to be respectively 0.145 and 0.855 in Indian buffaloes. This shows a similar situation to that encountered in the Thai buffaloes, except that the transferrin types in Indian buffaloes differed from those of cattle Tf-AA and Tf-DD types in their mobility. Probably this difference is brought about by the use of KIRSTJANSSON's buffer as observed by STORMONT[8]. The exact relationship between the transferrin types of the African and the Thai buffaloes and the cattle will be clear if the study is repeated following the method of KRIST-JANSSON[11]. In general, transferrin polymorphism in buffaloes is less pronounced than in cattle. The exclusive prevalence of Tf-AA type in African buffaloes[9], and its significantly low frequency in the Thai[10] and Indian buffaloes, shows a differential geographical distribution of a genetical character from East to West provided the transferrin phenotypes in buffaloes are proved identical. The high frequency of Tf-BB (0.855) and very low frequency of Tf-AA type in the Indian water buffalo, in relation to natural selection and adaptability is a problem for future study[12].

Zusammenfassung. Bei 150 indischen Wasserbüffeln (*Bos bubalis*) wurden für das Transferrin 3 Phänotypen gefunden, die durch 2 codominante Gene determiniert werden.

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Some Aspects of Complementation with Carotenogenic *al* Loci in *Neurospora crassa*

The effect of conidial input ratios in heterokaryons on complementation of albino mutants has been previously noted[1]. A likely explanation is that a hybrid protein is formed similar to the situation in some glutamate dehydrogenase mutant pair heterokaryons[2] such that conformationally distorted polypeptide monomers with intact active sites may be structurally re-oriented by conformationally normal polypeptide monomers with non-functional active sites producing a functional multimeric protein. Previous complementation studies[3,4] have noted some positive complementation between the 2 genetically distinguishable subunits of the region[5] but never any positive complementation within either subunit. The discovery of new phenotypes[6] and the synthesis of new albino strains with linked nutritional markers (FM strains) necessitated a re-examination of the locus. Genetic studies[5] have localized rose and white 'albinos' in the first subunit and cream, lemon yellow, pale yellow and white 'albinos' in the second larger subunit. These complementation studies were begun in order to determine the number and extent of the cistrons present and to relate this information to the possible mode of gene function.

Inoculations were made with a concentrated (>10^7 conidia/ml) conidial suspension in a salt solution to prevent osmotic bursting. A small quantity (0.1 ml) of each parental strain was applied to a small tube then incubated at 25°C for 10 days under intense fluorescent lighting before scoring. Media, culture and classification methods have been described elsewhere[5]. In all cases a control heterokaryon was made with a wild type strain (74A-OR23-1A) to examine the possibility of suppressor activity. The original screening (Figure 1) was performed with the available mutant strains[7] in a simple mixed conidial inoculation in all pairwise combinations without the aid of forcing nutritional markers. Each test was repeated 4 times and any incidence of complementation was scored as positive complementation for that pair. Strains representing each phenotype and each complementary group (see '†' in Figure 1) were selected and forcing marker strains were synthetized.

The forcing markers used were either arginineless (arg-6 No. 2997) or lysineless (lys-3 No. 4545). Heterokaryons with forcing markers often produced poor or no

growth at all, possibly because of various heterokaryon incompatibility gene[8] combinations, arising from the heterogeneous backgrounds of the strains used.

Further examples of positive complementation were discovered during the analysis of successive conidial isolates of apparent wild type prototrophs in a concurrent high resolution genetic study[5]. The apparent wild type prototrophs proved to be complementing heterokaryons either as a result of non-disjunction (pseudowild types[9]) or the hyphal inosculations of germling ascospores. The complementation results from the
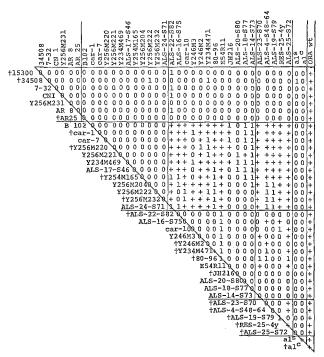

Fig. 1. Mixed culture complementation results. +, positive complementation (pmk); ○, complementation not detected; 1, partial complementation (yellow); †, strain selected for high resolution studies. Failure to complement cannot be ascribed to '0' cultures as there is no proof of heterokaryon formation.