TRANSFERRIN POLYMORPHISMS IN THE RING-NECKED PHEASANT

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As transferrins are polymorphic, they are useful in taxonomic studies. After using electrophoresis to compare the transferrin mobilities of 150 kinds of reptiles and amphibians, Dessauer et al. (1962) reported that in many cases transferrin patterns for closely related genera and species were quite similar and could be used to differentiate taxonomic categories.

Interspecific variations can be found in most components of avian serum, while intraspecific variations are restricted mainly to the transferrins, esterases, and prealbumins (Baker et al., 1963). The transferrin fractions of serum and conalbumin fractions of egg white, which are identical in the protein moiety (Williams, 1962), are considered important in taxonomic work with pheasants, because populations differ in the frequency of transferrin-conalbumin variants (Baker et al., 1966). Studies investigating transferrin and conalbumin polymorphism in serum and egg protein of pheasants also include those by Baker (1965), Baker et al. (1966), and Vohs and Carr (1969).

We studied transferrin polymorphisms in the blood serum of wild pheasants (*Phasianus colchicus*) in 1970-71 to determine (1) phenotypes and allelic frequencies, (2) inheritance patterns, and (3) the degree of polymorphism in widely separated and local populations.

METHODS AND MATERIALS

Collection of blood.—Blood was collected from wild pheasants in Brookings, Charles Mix, and Miner Counties, South Dakota, and Union and Montgomery Counties, Pennsylvania, using either the cardiac puncture or the jugular vein technique. Blood was held at room temperature for 3 to 4 hours to permit clotting and refrigerated overnight before centrifuging at 2,500 rpm for 15 minutes. Control serum, obtained by pooling sera from a large number of game farm pheasants, was used to check the consistency of protein migration in the electrophoresis chamber.

Electrophoresis.—Polyacrylamide gel (disc) electrophoresis was used throughout the study. The electrophoretic apparatus consisted of a 0- to 500-volt direct current 100-milliampere Thomas Model 21 power supply and a vertical electrophoresis chamber. All serum samples were run in duplicate as a quality control measure. Double-distilled water was used in all solutions.

Electrophoretic procedures were those described by Davis (1964), except that (1) the separation gel was the first to be prepared and 1.6 ml was delivered to each tube followed by 0.2 ml of spacer gel and 0.2 ml of sample gel (0.5 ml serum in 2.5 ml of spacer gel); (2) gels were placed in a 20 percent sulfosalicylic acid-protein fixative solution for about 30 minutes before staining; and (3)

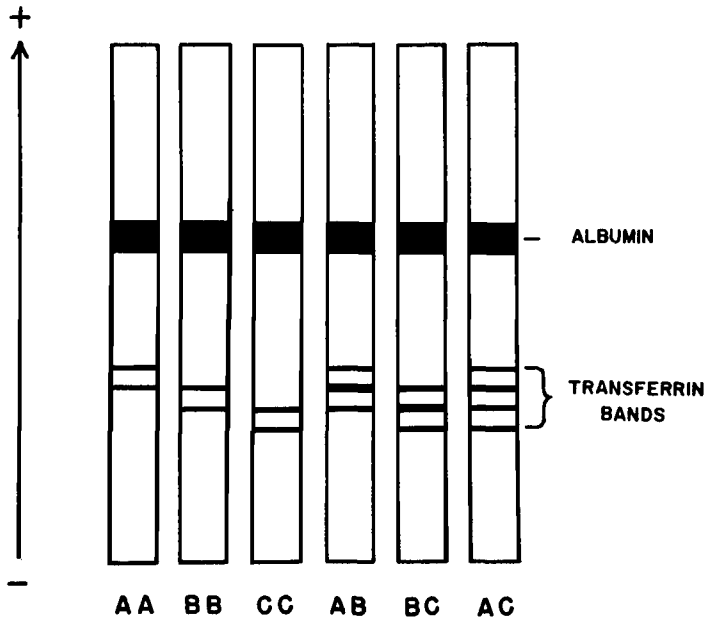

Figure 1. Transferrin patterns found in six phenotypes in South Dakota and Pennsylvania pheasants. Arrow indicates direction of migration.

Commassie Blue stain was used in place of Amido Black, and gels were destained with a solution of 10 parts methanol, 10 parts water, and 1 part acetic acid.

Spectrophotometry and transferrin ratios.—After gels were destained, they were scanned on a Gilford Model 2400 automatic recording spectrophotometer to facilitate analysis. Because of the variation in migration rates within electrophoretic runs, it was not feasible to make direct comparisons of transferrin patterns. Therefore a ratio was derived in which the distance between the origin and the slowest transferrin band was divided by the distance between the origin and albumin band. To check the reliability of this ratio, an electrophoretic run was allowed to continue until the albumin fraction reached the end of the gels. Although the transferrin bands migrated much farther than normal, the transferrin to albumin ratio remained similar. Ratios for the slower transferrin band of the three homozygous types found in this study ranged from 0.416 to 0.476 for phenotype CC, 0.488 to 0.502 for phenotype BB, and 0.518 to 0.524 for phenotype AA. Phenotypes were determined by the ratio values and the number of bands present.

Serum samples exhibiting transferrin ratios that were near the extremities of the above ranges were checked by mixing them with an equal amount of serum that had a ratio known to fall near the middle of the range. If electrophoresis of the serum mixture produced no new bands, the transferrin type was confirmed. Serum samples provided by Paul A. Vohs, Jr., Oregon State University, were also used to help confirm transferrin types; these samples represented transferrin types identified by him in wild pheasants in Iowa using starch gel electrophoresis.

Breeding experiment.—Cock pheasants from South Dakota were mated with

TABLE 1
PHENOTYPES AND ALLELIC FREQUENCIES OF SOUTH DAKOTA AND PENNSYLVANIA PHEASANTS IN 1970-71

Source	No. of birds tested	Transferrin phenotype						Allelic frequency		
		AA	AB	AC	BB	BC	CC	Tf^A	Tf^B	Tf^C
South Dakota										
Brookings Co.	26	3	1	3	2	2	15	0.19	0.14	0.67
Miner Co.	50	0	1	3	0	19	27	0.04	0.20	0.76
Charles Mix Co.	25	0	0	1	0	2	22	0.02	0.04	0.94
TOTALS	101	3	2	7	2	23	64	0.07	0.15	0.78
Pennsylvania										
Montgomery Co.	22	0	1	3	0	7	11	0.09	0.18	0.73
Union Co.	24	0	0	1	1	8	14	0.02	0.21	0.77
TOTALS	46	0	1	4	1	15	25	0.05	0.20	0.75

hens from Pennsylvania. Hens were held in individual laying cages and bred to cocks that were kept singly in larger breeding cages. Eggs were collected daily and numbered. Once weekly they were placed in forced-draft incubators. Newly-hatched chicks were wingbanded and placed in brooders. When chicks were 4 to 6 weeks of age, blood was drawn from the jugular vein.

RESULTS AND DISCUSSION

Transferrin patterns of wild pheasants.—Transferrin patterns found in wild pheasants constituted three homozygous and three heterozygous types. The two transferrin bands in phenotype AA were the fastest in mobility of the three homozygous types (Figure 1). Transferrin in phenotype BB migrated as two bands, with the faster band moving at nearly the same rate as the slower band in phenotype AA. The faster of the two transferrin bands of phenotype CC migrated at about the same rate as the slower band in phenotype BB.

Transferrin in phenotype AB consisted of three bands representing a combination of the bands AA and BB. The slower band of pattern AA migrated very nearly in the same position as the faster band of pattern BB, resulting in a wider and heavier middle band. Similarly, the combination of patterns in phenotype BC had three bands, the slower band of pattern BB and the faster band of pattern CC migrating as a heavier middle band. Transferrin in phenotype AC migrated as four separate bands.

These results indicate that transferrin inheritance in South Dakota and Pennsylvania pheasants could be genetically controlled by three codominant alleles at a single locus. The alleles were designated Tf^A, Tf^B, and Tf^C, using nomenclature described by Ashton et al. (1966). Vohs and Carr (1969) hypothesized that transferrin was controlled by

TABLE 2
TRANSFERRIN PHENOTYPES RESULTING FROM MATINGS OF SOUTH DAKOTA COCKS AND PENNSYLVANIA HENS

Type of mating	No. of matings	No. of offspring with phenotype				Total offspring
		AB	AC	BC	CC	
CC × CC	4	—	—	—	56	56
CC × BC	5	—	—	15	10	25
CC × AC	1	—	0	—	4	4
BC × AC	1	0	2	0	2	4
TOTALS	11	0	2	15	72	89

four codominant alleles at a single locus in Iowa pheasants. They found that one bird out of 869 sampled carried the Tf^{D} allele. It is possible that if pheasants in South Dakota and Pennsylvania were sampled more extensively the Tf^{D} allele would also be found.

Phenotype CC was found to be most common in both South Dakota (63 percent) and Pennsylvania (54 percent) pheasants (Table 1). Phenotype AA was found in three birds from Brookings County in South Dakota. This homozygous phenotype was not found in Iowa pheasants (Vohs and Carr, 1969).

The Tf^{C} allele was the most prevalent, with a frequency of 78 percent in South Dakota birds and 75 percent in Pennsylvania birds (Table 1). Allelic frequencies differed significantly between South Dakota populations ($P < 0.01$). The Tf^{C} allele varied from 67 percent in Brookings County to 94 percent in Charles Mix County, while the Tf^{B} allele varied from 4 percent in Charles Mix County to 20 percent in Miner County. Brookings County had a Tf^{A} frequency of 19 percent, while Charles Mix and Miner Counties had 2 and 4 percent, respectively.

Pheasants taken from Union and Montgomery Counties in Pennsylvania (Table 1) did not differ in allelic frequencies ($P > 0.05$). Overall allelic frequencies in South Dakota and Pennsylvania pheasants were quite similar. The allelic frequencies for Tf^{C} of four populations of wild pheasants in Iowa were not found to differ significantly, averaging 88 percent for the state (Vohs and Carr, 1969).

Apparently there are factors selecting for pheasants with the Tf^{C} allele in wild populations. The large variation in allelic frequencies for South Dakota populations indicated that selection factors varied from one part of the state to another. For instance the Charles Mix population might have been influenced by an environment that favored birds with the Tf^{C} allele, while the environment in Brookings County was less selective for that allele. Vohs and Carr (1969) hypothesized that rapid population increases may result in diverse phenotypes and decreased selec-

tion pressure toward birds homozygous for Tf^C. They also indicated that, because of the high frequency of the Tf^C allele, it should be possible to determine the effect of introducing less frequent alleles into the existing gene pool.

Transferrin inheritance.—Native South Dakota cocks were crossed with Pennsylvania hens in 11 matings to observe the inheritance mechanism for transferrin (Table 2). Pheasants with CC, BC, AB, and AC phenotypes were available for mating and 89 offspring were produced. Because of low production and poor survival, offspring from matings involving Tf AB phenotype were not represented. Phenotypes of offspring from matings of Tf CC X Tf CC and Tf CC X Tf BC were not different ($P > 0.05$) than expected ratios in the F_1 generation for codominant alleles at a single locus; this agrees with the findings of Vohs and Carr (1969). The offspring numbers from the remaining two matings were too low to be of importance in checking expected ratios.

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SUMMARY

Six different transferrin phenotypes were found in pheasants from South Dakota and Pennsylvania. Transferrin phenotype CC was most common and occurred in 63 percent of the pheasants from South Dakota and 54 percent of the pheasants from Pennsylvania. Transferrin inheritance could be controlled genetically by three codominant alleles (Tf^A, Tf^B, and Tf^C) at a single locus. The Tf^C allele occurred most frequently in all birds sampled and environmental factors may have selected for that allele.

Allelic frequencies for three populations of pheasants within South Dakota differed significantly ($P < 0.01$), while those for two populations in Pennsylvania were not different ($P > 0.05$).

LITERATURE CITED

ASHTON, G. C., D. G. GILMOUR, C. A. KIDDY, AND F. K. KRISTJANSSON. 1966. Proposal on nomenclature of protein polymorphism in farm livestock. Immunogenetics Letter, 4: 160–167.

BAKER, C. M. A. 1965. Molecular genetics of avian proteins. 4. The egg white

proteins of the Golden Pheasant, *Chrysolophus pictus* L., and Lady Amherst's Pheasant, *C. amherstiae* Leadbeater and their possible evolutionary significance. Comp. Biochem. Physiol., 16: 93–101.

BAKER, C. M. A., W. L. ANDERSON, J. A. ELLIS, H. C. HANSON, J. A. HARPER, AND R. LABISKY. 1963. Polymorphism of avian proteins. Federation Proc., 22(2, Part I): 611. Abstr. 2694.

BAKER, C. M. A., C. MANWELL, R. F. LABISKY, AND J. A. HARPER. 1966. Molecular genetics of avian proteins 5. Egg, blood and tissue proteins of the Ring-necked Pheasant, *Phasianus colchicus* L. Comp. Biochem. Physiol., 17: 467–499.

DAVIS, B. J. 1964. Disc electrophoresis-II. Method and application to human serum proteins. Ann. New York Acad. Sci., 121: 404–427.

DESSAUER, H. C., W. FOX, AND F. H. POUGH. 1962. Starch gel electrophoresis of transferrins, esterases, and other plasma proteins of hybrids between two subspecies of whiptail lizard (Genus *Cnemidophorus*). Copeia, 4: 767–774.

VOHS, P. A., JR., AND L. R. CARR. 1969. Genetic and population studies of transferrin polymorphism in Ring-necked Pheasants. Condor, 71: 413–417.

WILLIAMS, J. 1962. A comparison of conalbumin and transferrin in the domestic fowl. Biochem. J., 83: 355–364.

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