GENETIC AND POPULATION STUDIES OF TRANSFERRIN POLYMORPHISM IN RING-NECKED PHEASANTS

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Techniques that allow the isolation, characterization, and analysis of genetically controlled molecular material within natural populations have been useful in population studies (Hubby and Lewontin 1966; Lewontin and Hubby 1966). Methods for detecting cellular antigens in Ring-necked Pheasants (*Phasianus colchicus* L.) and for using varying frequencies of the antigens to detect differences between populations were described by Vohs (1966). Identification of the transferrin or β-globulin portion of blood plasma of individual Ring-necked Pheasants using the Nitroso-R method was reported by Baker et al. (1966). Also, variation between samples obtained from widely separated populations was suggested. Baker et al. (1966) speculated that the transferrin variation was genetically controlled and suggested a hypothesis for its inheritance; however, they presented no genetic information to support the hypothesis.

Our study was initiated in 1964 to characterize further the transferrin bands in blood samples obtained from wild pheasants, to determine the mode of inheritance of the detected differences, and to explore the potential of the transferrin polymorphism for use in field studies of geographically isolated populations of wild pheasants.

MATERIALS AND METHODS

The methods of propagating families for genetic studies, capturing wild birds for population studies, and obtaining blood were similar to those reported by Vohs (1966). When plasma samples were desired, clotting was prevented by using potassium oxalate. Plasma and sera were stored frozen at –20°C until just before the samples were inserted into the gels.

Two types of electrophoresis were used: disc electrophoresis (7 per cent acrylamide gel, stacking at *p*H 8.9, running at *p*H 9.5; Canalco unit) and horizontal starch gel electrophoresis. Various buffers used in starch gel electrophoresis included: borate (*p*H 8.4; Smithies 1959), tris-EDTA-borate (*p*H 8.6), tris-HCl (*p*H 8.8; Kristjansson 1960), tris-citrate (*p*H 8.8; Kristjansson 1963), and LiOH-borate-tris-citrate (*p*H 8.0; Ferguson and Wallace 1961). Kristjansson's (1960) tris-HCl buffer gave the best resolution of the transferrin fractions.

Fourteen samples and a control were placed in each starch gel by the application of 16 μ liter of plasma or serum from each sample to a separate, 6×7 mm piece of filter paper. A drop of brom-thymol blue solution was placed in the control plasma sample in order to observe the advance of the protein front in the gel. Initial voltage of 165v was applied for 15 min, the filter paper inserts were removed, and the voltage increased to 280v for 60 min. Voltage was then raised to 350 or 400v. At the beginning of the 2–3 hr run the amperage was about 90–95 ma and gradually decreased to 35–50 ma. The run was terminated when the brom-thymol blue marker had progressed 9.1–9.2 cm. The gel was sliced, stained, and destained as described by Smithies (1959). Positive identification of transferrins was established by F. K. Kristjansson using autoradiography and Fe^{59} on samples provided by the authors.

RESULTS AND CONCLUSIONS

Serum samples from 320 wild birds captured at various locations in Iowa were studied initially. The patterns of bands that appeared in the transferrin region on the starch gels were separable into six types, and two additional types have been found among offspring of matings involving the original types. Four distinct patterns representing homozygous types have been identified. The patterns exhibited by homozygous individuals have been labeled as A, B, D, and C in decreasing order of mobility. The homozygous patterns have two major bands and either two or three associated minor bands. The minor bands are often difficult to distinguish on the starch gels, but the major bands are sufficiently distinct and separate so that the minor bands need not be considered in distinguishing between the patterns.

Transferrin A is the fastest in mobility among the four homozygous types (figs. 1, 2, 3). A minor band often trails the two major A bands. Transferrin B migrates as two major bands, with the faster band being slightly slower than the slower major A band. The faster of the two major bands of Transferrin

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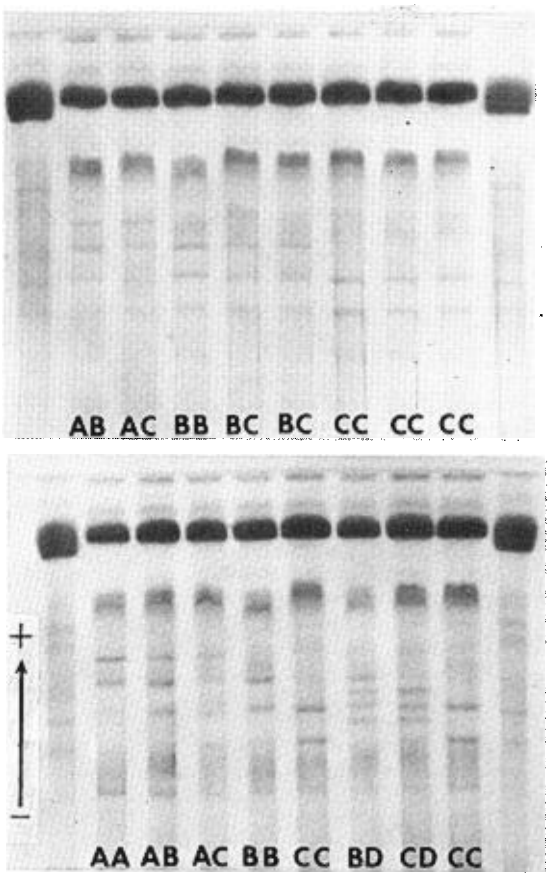

FIGURE 1. Top and bottom. Phenotypes identified from serum and/or plasma of Ring-necked Pheasants using starch gel electrophoresis. Outer samples are of Ruffed Grouse, all others are ring-necks. Arrow shows direction of migration.

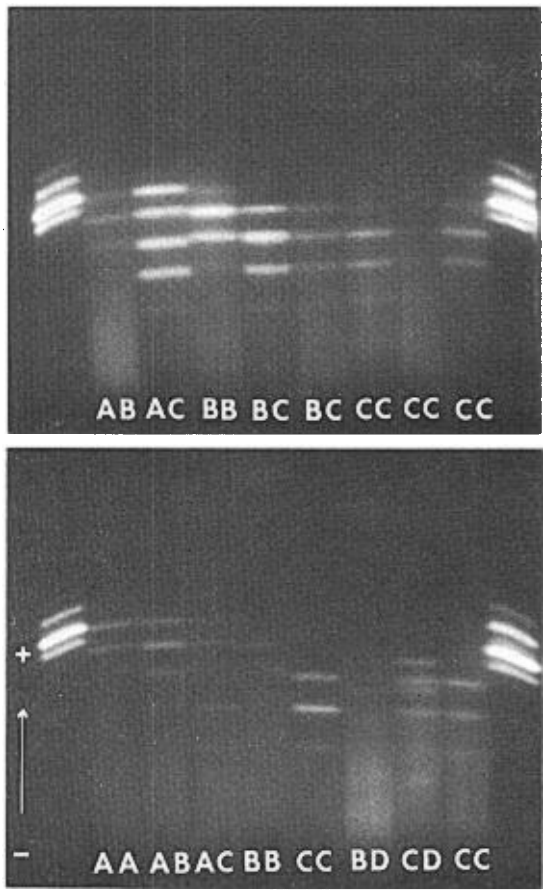

FIGURE 2. Top and bottom. Autoradiograph of serum of Ring-necked Pheasants using Fe^{59} to identify transferrins. Samples are arranged in order of figure 1. Outer samples are swine, all others are ring-necks. (Autoradiography courtesy of Dr. F. K. Kristjansson.)

C migrates more slowly than the slower major band of Transferrin B. Minor bands may precede and trail the major C bands. The faster major band of Transferrin D migrates between the two major B bands, and the slower major D band migrates between the two major C bands.

Combinations of the homozygous patterns, representing heterozygotes, have been identified. In combinations of patterns A and B and B and C the slower major band of the faster moving transferrin migrates very close to the faster major band of the slower transferrin (figs. 1, 2, 3). The two bands appear as a single, wider band between the leading and trailing bands of the combination. Therefore the pattern of these heterozygotes appears as three major bands, with the center band slightly wider than the leading and trailing bands. The combination of the A and C transferrins or A, B, or C with the D transferrin results in a pattern with four distinct major bands. The differences in density of the major bands on the autoradiograph (fig. 2) are probably due to differences in the amount of natural iron bound by transferrins in vivo.

Baker et al. (1966) stated a hypothesis for genetic control of the transferrins, involving a single chromosomal locus and two codominant alleles. The researchers labeled the slower migrating pair as "slow" transferrin (C bands in this study) and designated the allele postulated as controlling the appearance of the slow bands as Tf^s. The faster migrating pair was called "fast" transferrin (B bands in

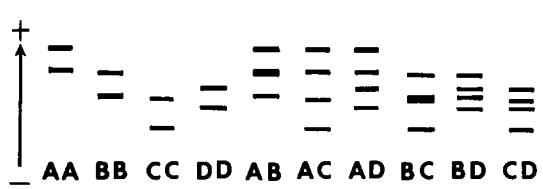

FIGURE 3. Diagram of the relationship of major transferrin patterns of the Ring-necked Pheasant.

TABLE 1. Designation of the 10 allelic combinations possible under the single-locus, four-allele hypothesis.

Genotype	Phenotype
Tf^A Tf^A	Tf AA
Tf^A Tf^B	Tf AB
Tf^A Tf^C	Tf AC
Tf^A Tf^D	Tf AD
Tf^B Tf^B	Tf BB
Tf^B Tf^C	Tf BC
Tf^B Tf^D	Tf BD
Tf^C Tf^C	Tf CC
Tf^C Tf^D	Tf CD
Tf^D Tf^D	Tf DD

this study), and the respective allele was designated Tf^f. Under this hypothesis, only three patterns were possible, ff, fs, and ss. To explain the appearance of three- and four-band phenotypes, Baker et al. (1966) speculated that different electrophoretic conditions changed the relative migration rates of the band pairs so that, when overlapped, the pattern appeared as three bands with a diffuse middle band and, when separated, as four distinct bands. Our efforts to change the relative migration rate using acrylamide gels, disc electrophoresis, and starch gels with tris-EDTA-borate buffer (pH 8.6) or the buffer of Ferguson and Wallace (1961), resulted in patterns identical to those observed when the method just outlined was used. Changes in starch concentration, pH, and duration and distance of protein migration failed to separate the three-banded pattern to four bands as suggested by Baker et al. (1966). The identification of a fifth pattern representing a combination of the A and B bands and the discovery of the D bands eliminated the possibility of genetic control by postulating only two alleles. The simplest genetic explanation consistent with the patterns observed in this study was a hypothesis of genetic control by a single locus and four codominant alleles. Therefore, the postulated alleles were designated Tf^A, Tf^B, Tf^C, and Tf^D in order of their discovery. Genotype and phenotype designations are indicated in table 1. The AD and DD phenotypes have not been observed, but their occurrence must be postulated under our hypothesis.

The nomenclature proposed by Baker et al. (1966) for transferrins in pheasants was not followed during this study because the "fast" and "slow" designation suggested left no suitable alternative designations for the A and D bands. In addition, investigators (Ashton et al. 1966) working with domestic animals have adopted the convention of labeling transferrins with capital letters.

GENETIC ANALYSIS

Reciprocal matings of all available genotypes were made to test the null hypothesis of a single-locus, four-allele mode of inheritance of the transferrin proteins (table 2). Thirty-eight matings were made and 963 offspring were tested. Pheasants exhibiting phenotypes Tf AA, Tf AD, Tf BD, and Tf DD were not available for mating.

Segregation data for the F_1 and F_2 generations from two matings involving Tf^C and Tf^B are presented in figure 4. The pattern of inheritance shown for the F_1 and F_2 generations is consistent with expected Mendelian ratios of alleles exhibiting codominance at the single locus.

No difference in inheritance due to sex was noted and results of each type of mating were pooled (table 2). The distribution of offspring from the matings closely approximates the expected with the exception of a single mating of Tf AB × Tf AB where the number of Tf^A alleles, of both the heterozygous and homozygous types, were significantly below the expected number. The Tf AA phenotype has never been found among wild birds and Tf AB is rare (table 3). It seems that a higher

TABLE 2. Segregation of transferrin phenotypes of 963 offspring from matings of Ring-necked Pheasants.

Type of mating	No. matings	Phenotype of offspring										Total offspring	χ^2 for expected ratio and probability of a larger value
		AA	AB	AC	AD	BB	BC	BD	CC	CD	DD		
CC × CC	10	–	–	–	–	–	–	–	296	–	–	296	–
CC × BC	8	–	–	–	–	–	112	–	123	–	–	235	0.51, $p = 0.49$
CC × AC	1	–	–	6	–	–	–	–	4	–	–	10	–
BC × BC	7	–	–	–	–	46	109	–	42	–	–	197	2.41, $p = 0.30$
BB × CC	3	–	–	–	–	–	62	–	–	–	–	62	–
BC × AC	1	–	–	1	–	–	2	–	2	–	–	5	–
BB × BC	1	–	–	–	–	8	13	–	–	–	–	21	1.19, $p = 0.27$
BB × BB	4	–	–	–	–	84	–	–	–	–	–	84	–
BB × AB	1	–	1	–	–	6	–	–	–	–	–	7	–
AB × AB	1	2	15	–	–	13	–	–	–	–	–	30	8.08, $p = 0.017$
BC × CD	1	–	–	–	–	–	2	3	5	3	–	13	–

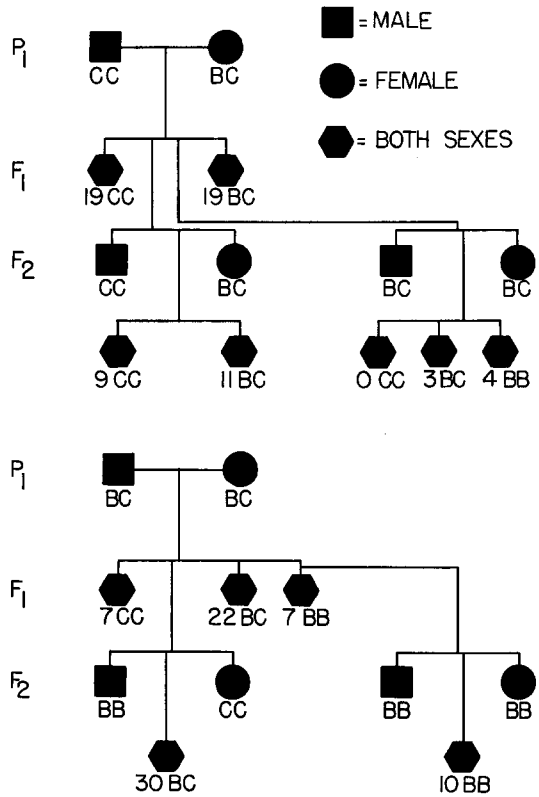

FIGURE 4. Segregation of transferrin phenotypes among F_1 and F_2 generations of Ring-necked Pheasants.

mortality may occur among Iowa birds carrying the Tf^A allele.

WILD POPULATIONS

Plasma or serum from 869 wild Ring-necked Pheasants captured in Iowa were analyzed to determine the frequencies of the phenotypes in various populations (table 3). The most common phenotype, Tf CC, occurred in 76.8 per cent of the birds tested. The other phenotypes identified in the wild populations occurred in the following percentages: BC, 19.3; BB, 1.4; AC, 2.0; AB, 0.5; and CD, trace. The greatest diversity of phenotypes was found among the Union-Adair County population (table 3, fig. 5). The greatest uniformity occurred on the Hamilton County area where no BB, AC, AB, or CD phenotypes were observed in the sample. Only two Tf BB birds were observed in the Story County population.

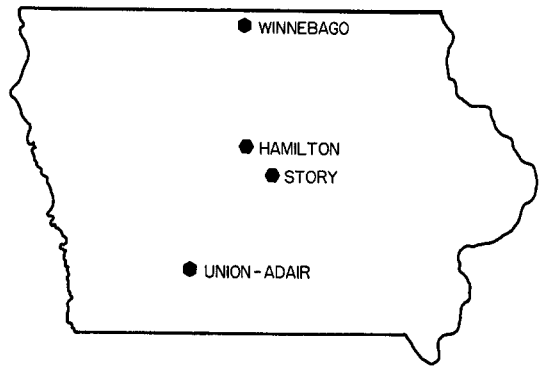

FIGURE 5. Geographic location of populations of wild pheasants sampled for electrophoretic study.

The Tf^C allele was most frequent in every population for every year that samples were available (table 3). The frequency varied from 0.82 in the Story population sampled in 1967 to 0.93 in the Hamilton population sampled in 1965. Frequency of Tf^B varied from 0.07 in the 1966 Winnebago and 1965 Hamilton samples to 0.18 in the 1967 Story population sample. The 95 per cent confidence intervals for Tf^C overlap in all instances, indicating that no significant differences in the frequency of the allele occurred among the samples from the various populations. Since Tf^A, Tf^B, and Tf^D were repre-

TABLE 3. Phenotypes observed and gene frequency of transferrin alleles in Iowa populations of Ring-necked Pheasants.

Source and Year	No. birds tested	Phenotype										Gene frequency				SD	95% confidence interval
		AA	AB	AC	BB	BC	CC	AD	BD	CD	DD	Tf^A	Tf^B	Tf^C	Tf^D	Tf^C	Tf^C
Union-Adair 1967	172	0	4	7	1	36	123	0	0	1	0	.03	.12	.84	.01	.02	.80–.88
Union-Adair 1966	335	0	1	7	7	65	255	0	0	0	0	.01	.12	.87	.00	.02	.85–.91
Union-Adair 1964-65	96	0	0	1	2	20	73	0	0	0	0	.01	.11	.88	.00	.03	.82–.94
Union-Adair Sub-total	603	0	5	15	10	121	451	0	0	1	0	.01	.12	.86	.01	.02	.82–.90
Winnebago 1966	35	0	0	2	0	5	28	0	0	0	0	.03	.07	.90	.00	.05	.80–1.00
Hamilton 1965	58	0	0	0	0	1	57	0	0	0	0	.00	.07	.93	.00	.03	.87–.99
Hamilton 1966	55	0	0	0	0	9	46	0	0	0	0	.00	.08	.92	.00	.04	.84–1.00
Hamilton Sub-total	113	0	0	0	0	10	103	0	0	0	0	.00	.07	.93	.00	.02	.89–.97
Story 1966	33	0	0	0	0	61	27	0	0	0	0	.00	.09	.91	.00	.05	.80–1.00
Story 1967	85	0	0	0	2	26	57	0	0	0	0	.00	.18	.82	.00	.04	.74–.90
Story Sub-total	118	0	0	0	2	32	84	0	0	0	0	.00	.15	.85	.00	.03	.79–.91
Total	869	0	5	17	12	168	666	0	0	1	0	.01	.11	.88	.01	.01	.86–.90

sented by such small numbers, confidence limits were not computed for these alleles.

The frequency of the Tf^C allele within the entire Iowa population appears relatively stable. The frequencies of Tf^A and Tf^B appear less fixed among the various populations sampled. The Tf^D allele is extremely rare in Iowa pheasant populations, and its significance can not be ascertained from the present samples. Further sampling of larger groups of birds may indicate the value of the Tf^A, Tf^B, and Tf^D alleles in separating the birds into breeding populations, or demes, and allow evidence to be gathered concerning the mechanism responsible for relatively low frequencies of Tf^A, Tf^B, and Tf^D. The diversity of phenotypes in the Union-Adair population may reflect the rapid population increase that has occurred during the past decade and concomitantly decreased selective pressure toward birds homozygous for Tf^C, or it could reflect differences in sample size.

The relative stability and high frequency of the Tf^C allele in Iowa pheasant populations provide a base for determining the rate of flow of genetic material into an existing population and should make it possible to analyze the effect of introducing Tf^A, Tf^B and Tf^D into the pre-existing gene pool.

SUMMARY

Eight distinct transferrin phenotypes were found by using starch gel electrophoresis to separate blood proteins of Ring-necked Pheasants. The genetic polymorphism is best explained by the occurrence of four codominant alleles at one autosomal locus. Offspring totaling 963 from 38 matings were examined to test the proposed mode of inheritance. The distribution pattern of observed and expected phenotypes among the families supported the proposed hypothesis.

The Tf^C allele occurred most frequently (0.88) among 869 wild birds obtained from four geographic locations in Iowa. Frequencies of Tf^A, Tf^B, and Tf^D were 0.01, 0.11, and trace, respectively. The frequency of Tf^C appeared to be stable among Iowa populations. Larger sample sizes from wild birds are needed to determine the relative stability of Tf^A, Tf^B, and Tf^D.

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