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Use of Eelctrophoresis to Determine Populations of Pheasants in South Dakota

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USE OF ELECTROPHORESIS TO DETERMINE POPULATIONS OF PHEASANTS IN SOUTH DAKOTA

BY

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USE OF ELECTROPHORESIS TO DETERMINE POPULATIONS

OF PHEASANTS IN SOUTH DAKOTA

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

The ring-necked pheasant (Phasianus colchicus) is an important game bird, and various management techniques have been implemented in its behalf. One of the most common practices has been transplanting or stocking wherein pheasants are introduced into an area with a depleted population in hopes of increasing pheasant production.

The effects of such programs are difficult to evaluate since there is no way of knowing if the introduced birds are in any way responsible for production changes which subsequently occur. If the taxonomic characteristics of the native and the introduced populations could be determined, the effect that introduced birds have on the local gene pool could more readily be determined. The objectives of this study were to (1) determine taxonomic characteristics of South Dakota pheasants using protein electrophoresis, (2) attempt to identify populations within the state, and (3) determine taxonomic characteristics of pheasants stocked into South Dakota from Pennsylvania.

LITERATURE REVIEW

Subspecies Differentiation and Hybridization

Electrophoresis has been used as a biochemical technique in studies involving the taxonomic classification of animal populations. Using starch gel electrophoresis, Dessauer et al. (1962a) found that the zone of hybridization and introgression between two sub-species of whip-tailed lizard (Cnemidophorus tigris) extended farther than was indicated by purely morphological analyses. Manwell and Baker (1963) used much the same technique to identify a "sibling species" of sea cucumber (Thyonella gemmata). They found differences in the hemoglobins and esterases of two morphologically similar types called "stouts" and "thins", indicating that two separate populations existed. Cowan and Johnston (1962) used paper electrophoresis to identify differences between three subspecies of black-tailed deer (Odocoileus hemionus hemionus, O. h. sitkensis, O. h. columbianus,) and between those subspecies and the white-tailed deer (O. virginianus ochrourus).

Working on avian species, Brandt et al. (1952) found that certain protein components present in hybrid serum were closely related electrophoretically to components found in one or both parent birds. Greater differences were present in the pheasant-chicken hybrid than in the case of pheasant hybrids. Fox et al. (1961) observed that the hybrid offspring from two species of toad (Bufo valliceps and B.

fowleri) inherited one transferrin and one hemoglobin protein band from each of their parents. Manwell et al. (1963) found starch gel electrophoresis to be sufficiently accurate to detect hybridization. In studying hemoglobins from various bird and fish hybrids, they found that generally no new proteins resulted but that the progeny possessed the proteins of both parents. They described this phenomenon as "simple additivity." Some exceptions were observed, particularly in the centrarchid fishes where hybrid hemoglobins appeared which differed from those of the parent species. Dessauer and Fox (1964) reported that electrophoresis was a useful tool for studies at the specific and intraspecific taxonomic levels if developmental and physiological variables were recognized. Working with water snakes (Natrix sipedon) they found that plasma proteins of several subspecies from different geographical locations were more variable than those of individuals from a single population. Marsh et al. (1969) were able to hybridize two subspecies of ground squirrel (Spermophilus beecheyi beecheyi and S. b. douglasii). Electrophoresis of the serum from five hybrid offspring revealed that they all inherited the slower transferrin protein band from their dam and the faster transferrin protein band from their sire.

Sibley and Johnsgard (1959) reported that serum proteins of various avian species vary with age, sex, and reproductive condition. They recommended that a large sample size be used and that birds be

separated into age and sex classes if serum protein is to be utilized in taxonomic studies. Amin (1961) found that serum protein patterns of day-old and week-old chicks were different from those of pooled sera of adult roosters. The differences involved the mobility and number of various protein fractions, globulins in particular.

Transferrin Polymorphism

Transferrins are the most heterogeneous of the blood proteins and, therefore, are well adapted to taxonomic studies. After using electrophoresis to compare the transferrin mobilities of 150 kinds of reptiles and amphibians, Dessauer et al. (1962b) reported that in many cases transferrin patterns of 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 fraction of serum and the closely related conalbumin fraction of egg-white are considered very important in taxonomic work with pheasants, as populations differ in the frequency of transferrin-conalbumin variants (Baker et al. 1966). Several taxonomic studies investigating avian egg protein using electrophoresis have been conducted (Baker and Manwell 1962; Baker, 1964, 1965; and Baker

et al. 1966). After comparing transferrin and conalbumin, Williams (1962) reported that the two appeared to be identical in their main protein components and differed only in their carbohydrate prosthetic groups.

METHODS AND MATERIALS

Collecting and Sampling Techniques

Wild pheasants were collected from three locations in eastern South Dakota (Fig. 1), which were thought likely to have genetically different populations. Areas recently stocked with introduced or game-farm birds were not sampled.

Pheasants were collected by personnel of the South Dakota Department of Game, Fish and Parks in Miner County using a nightlighting technique described by Labisky (1959). These birds were kept at the South Dakota State University wildlife research area. Blood samples were taken from all live birds using the jugular technique described by McClure and Cedeno (1955). The brachial vein technique was used initially but it was found difficult to apply. Blood samples from birds collected in Charles Mix and Brookings Counties were taken using the cardiac puncture technique as described by Baker et al. (1966).

Live hens were obtained through the cooperation of the Pennsylvania Game Commission; these birds were captured in two locations (Fig. 1). Upon arrival, blood samples were taken using the jugular technique (Fig. 2) and the birds were later used in a breeding experiment.

The blood was held at room temperature for 3 to 4 hours to facilitate clotting and then refrigerated overnight. The next day it

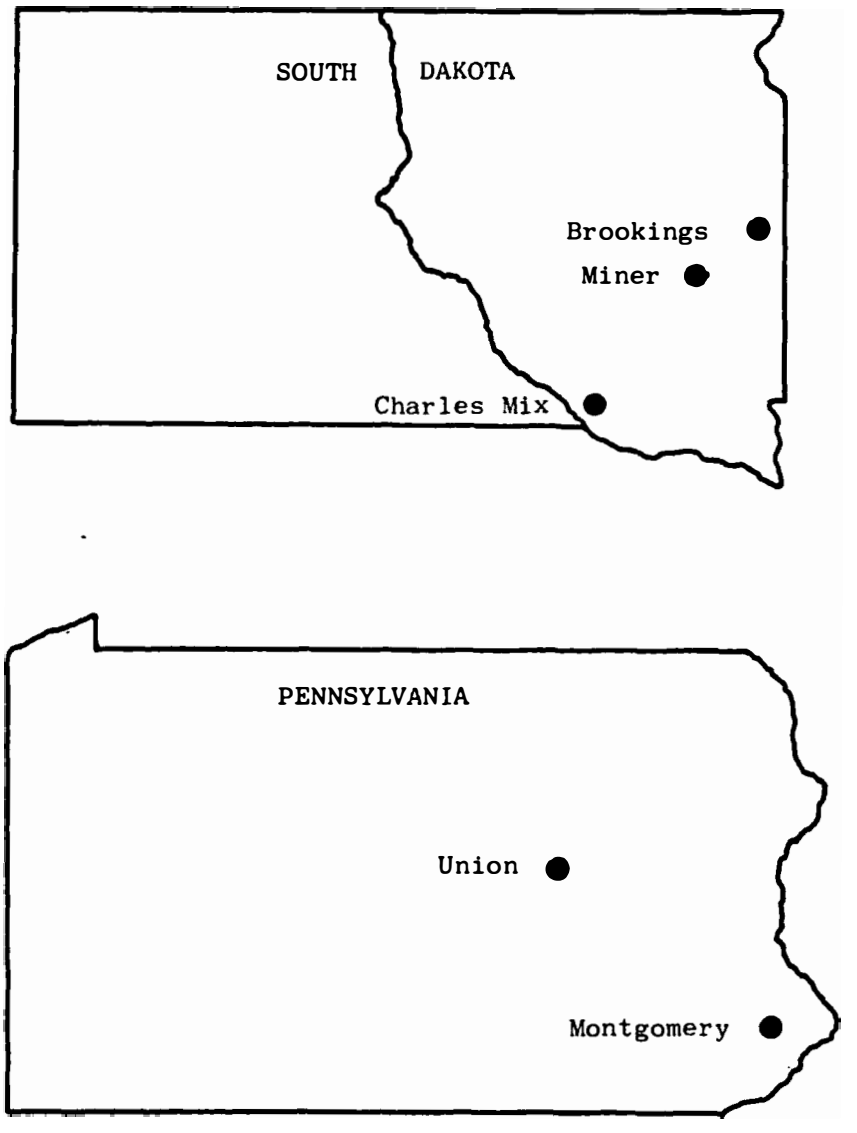

Fig. 1. Locations in South Dakota and Pennsylvania where pheasants were collected.

Fig. 2. The jugular vein technique for sampling wild pheasants.

was centrifuged at 2500 rpm for 15 minutes. The resultant serum was labeled and frozen for future use. A portable centrifuge was used in the field and serum was kept frozen with dry ice. Control serum was obtained by pooling sera from a large number of game farm pheasants.

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 (Fig. 3) with capacity for eight gels. All serum samples were run in duplicate as a quality control measure. This made it possible to run three samples and a control simultaneously. To eliminate problems with pH, double-distilled water was used in all solutions.

The following electrophoretic procedures were adopted. They are similar to those described by Davis (1964) with minor modifications.

(1) Stock solutions and the small-pore solution No. 2 (Table 1) were removed from the refrigerator and allowed to warm to room temperature.

(2) Glass tubes, which served as gel containers, were thoroughly washed in detergent solution and then rinsed, first in distilled water and then in distilled water containing 1 part in 200 of

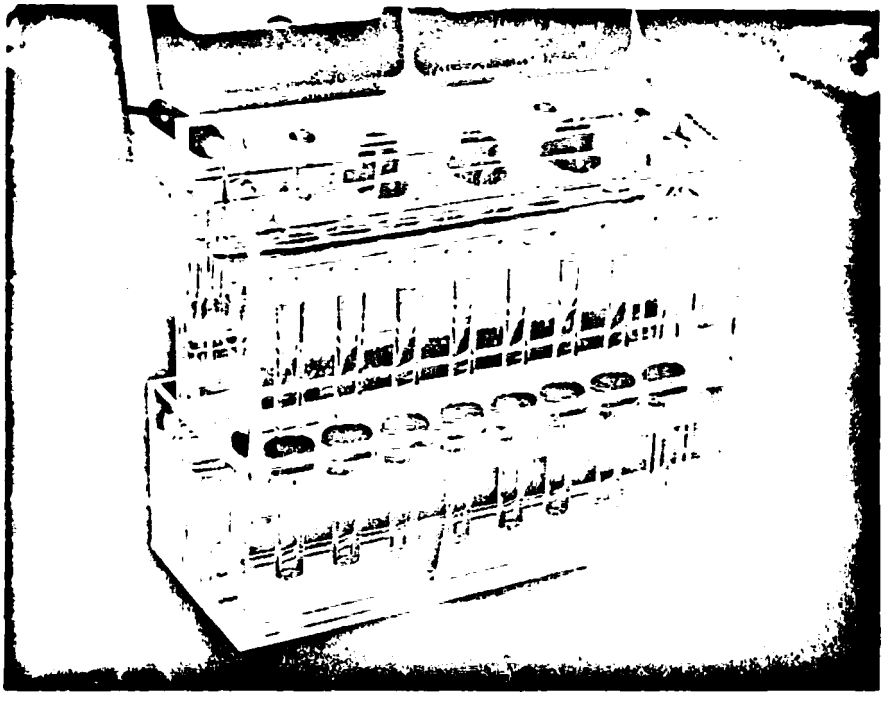

Fig. 3. Electrophoresis chamber in operation.

Table 1. Stock and working solutions used in preparing the various gels. (after Davis 1964)

Stock Solutions			
(A)		(B)	
1N HCl	48 ml	1N HCl	approximately 48 ml[a]
TRIS[b]	36.6 g	TRIS	5.98 g
TEMED[c]	0.23 ml	TEMED	0.46 ml
water	to 100 ml	water	to 100 ml
(pH 8.9)		(pH 6.7)	
(C)		(D)	
Acrylamide	28.0 g	Acrylamide	10.0 g
BIS[d]	0.735 g	BIS	2.5 g
water	to 100 ml	water	to 100 ml
(E)		(F)	
Riboflavin	4 mg	Sucrose	40 g
water	to 100 ml	water	to 100 ml

Working Solutions			
Small-pore Solution No. 1	Small-pore Solution No. 2	Large-pore Solution	Stock Buffer Solution for Reservoirs
1 part A 2 parts C 1 part water pH 8.9 (8.8-9.0)	Ammonium persulfate 0.14 g water to 100 ml	1 part B 2 parts D 1 part E 4 parts F pH 6.7 (6.6-6.8)	TRIS 6.0 g Glycine 28.8 g water to 1 liter pH 8.3

[a]pH adjusted by titrating with 1N HCl.

[b]2-Amino-2-(hydroxymethyl)-1,3-propanediol.

[c]N,N,N',N' - Tetramethylethylenediamine.

[d]N,N' - Methylenebisacrylamide.

Kodak Photo-Flo solution. Once dry, the glass tubes were inserted into rubber stoppers and placed in a stand.

(3) Separation gel was prepared by mixing small-pore solution No. 1 and small-pore solution No. 2 (Table 1) and 1.6 ml was delivered to each tube with a syringe. Water was then carefully layered on the surface of each gel to prevent a miniscus from forming. Gels were left standing at room temperature until polymerization occurred, approximately 30 minutes.

(4) Once the gel polymerized, the water layer was poured off and absorbent paper was used to remove any water remaining in the gel tubes.

(5) Spacer gel was prepared by layering 0.2 ml of large-pore solution (Table 1) on the separation gel. Water was then layered on top of the large-pore solution and the gel tubes were placed in front of a fluorescent light source for 20 to 30 minutes to allow photopolymerization to take place. Once the gels polymerized, step No. 4 was repeated.

(6) A sample gel consisting of 0.05 ml serum in 2.5 ml of spacer gel was made up and 0.2 ml was layered on the spacer gel. The same procedure was then followed for the sample gel as was used for the spacer gel (step No. 5).

(7) Following polymerization, the gel tubes were removed from their rubber stoppers and inserted into the rubber grommets of the upper buffer reservoir.

(8) The stock buffer solution (Table 1) was diluted to 1/10 strength with distilled water and 500 ml was added to the lower reservoir. Another 500 ml of 1/10 strength buffer was mixed with 1 drop of Bromphenol Blue dye and added to the upper reservoir.

(9) The reservoirs were connected to the power supply, the upper reservoir to the negative terminal and the lower reservoir to the positive terminal. The current was set at 3 milliamperes per tube for a total of 24 milliamperes for eight tubes. An electrophoretic run took approximately 1.5 hours.

(10) Once the free Bromphenol Blue dye migrated to the end of the gels, the tubes were removed from the upper reservoir. Gels were removed from the glass tubes by rimming with a 26-gauge needle attached by plastic hose to a water outlet.

(11) The gels were placed in a 20 percent sulfosalicylic acid-protein fixative solution for 30 minutes and then in Commassie Blue stain overnight.

(12) Gels were removed from the Commassie Blue stain and placed in a solution of 10 parts methanol, 10 parts water, and 1 part acetic acid for destaining. The destaining solution was changed periodically until all excess stain was removed.

(13) Following destaining, the gels were stored in a 7 percent acetic acid solution until needed.

Each electrophoretic run was terminated when the first Bromphenol Blue tracer band came to the end of the gel. Because of variations in migration rates, this did not always allow the tracer bands to reach the end in every gel. In future work of this type, it might be better to allow the tracer band to reach the end in every gel. This can be accomplished by the following steps:

(1) Turn off current when first band(s) reaches the end of gel(s).

(2) Pour off buffer and remove appropriate gel tube(s) from chamber.

(3) Replace gel tube(s) with empty tube(s) and refill chamber with buffer (replacement tubes should be high enough to prevent buffer from running through).

(4) Apply current (3 milliamperes per tube) until the next tracer(s) reaches the end.

(5) Repeat the above steps until all tubes have been removed. This procedure should help eliminate much of the variation due to differences in migration rates from gel to gel.

Spectrophotometry and Transferrin Ratios

After gels were completely destained, they were run through a Gilford Model 2400 Automatic Recording Spectrophotometer to facilitate quantitative analysis. The various protein bands within the gel column, which retain the Commassie Blue stain, cause peaks or "blips" to occur on the resulting graph (Fig. 4). The model 2400 utilizes a photometer which provides direct linear readings of absorbence. Gilford settings used include: slit width - 0.104 mm, wave length - 570 nm, filter slide - blank, aperture plate - 0.20 x 2.36 mm, and graph speed - 2 inches per minute.

Once the protein bands (peaks on graph) were identified, the distance from the origin to each peak was measured and recorded. Because the transferrin fraction reflects genetic characteristics, it was used in the analyses of pheasant populations.

Due to 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

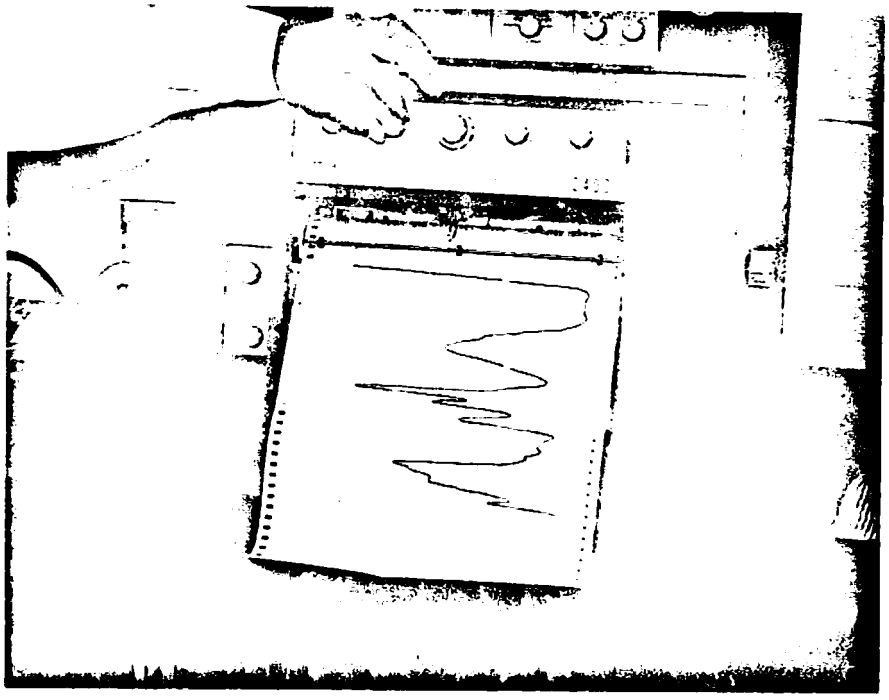

Fig. 4. Spectrophotometer and graph showing protein fractions as peaks.

transferrin to albumin ratio remained nearly the same. Similar ratios were calculated for all the serum samples. Ratios for the slower transferrin band of the three homozygous types found in this study ranged from 0.4160 to 0.4757 for Transferrin C, 0.4884 to 0.5022 for Transferrin B, and 0.5179 to 0.5235 for Transferrin A. Phenotypes were determined by the ratio values and the number of bands present.

Serum samples exhibiting transferrin ratios which were near the extremities of the above ranges were checked by mixing them with an equal amount of a serum 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., were also used to help confirm transferrin types. These samples represented the transferrin patterns identified in wild pheasants in Iowa using starch gel electrophoresis.

Breeding Experiment

A breeding experiment was conducted in which South Dakota cocks were crossed with Pennsylvania hens to observe transferrin inheritance in pheasants. The hens were held in individual laying cages while the cocks were kept in large individual wire cages. Various mating combinations were derived and matings were accomplished by placing the hens with designated cocks overnight. Hens were brought into production early in the spring using a 16-hour day with fluorescent lights.

Eggs were collected daily and marked with the date and hen number. Once weekly, they were placed in forced-draft incubators. Newly-hatched chicks were tagged with No. 3 aluminum patagial wingbands and placed in brooders. At the age of 4 to 6 weeks, chicks were blood sampled using the jugular technique.

RESULTS AND DISCUSSION

Transferrin Patterns of Wild Pheasants

Transferrin patterns found in wild pheasants from South Dakota and Pennsylvania were separated into three homozygous and three heterozygous types. Homozygous types were labeled Transferrin A, B, and C in decreasing order of mobility (Vohs and Carr 1969). Transferrin A (phenotype AA), with two bands, was the fastest in mobility of the three homozygous types (Fig. 5). Transferrin B (phenotype BB) migrated as two bands, with the faster band moving at nearly the same rate as the slower A band. Likewise, the faster of the two bands of Transferrin C (phenotype CC) migrated at about the same rate as the slower band of Transferrin B. Transferrin D, as reported by Vohs and Carr (1969) in Iowa, was not found in the South Dakota or Pennsylvania pheasants sampled.

Heterozygous types represented by combinations of the homozygous types were identified (Fig. 5). The combination of patterns A and B (phenotype AB) consisted of three bands. The slower band of pattern A migrated very nearly in the same position as the faster band of pattern B, resulting in a wider and heavier middle band. Similarily, the combination of patterns B and C (phenotype BC) had three bands, the slower band of pattern B and the faster band of pattern C migrating as a heavier middle band. The combination of patterns A and C (phenotype AC) migrated as four separate bands.

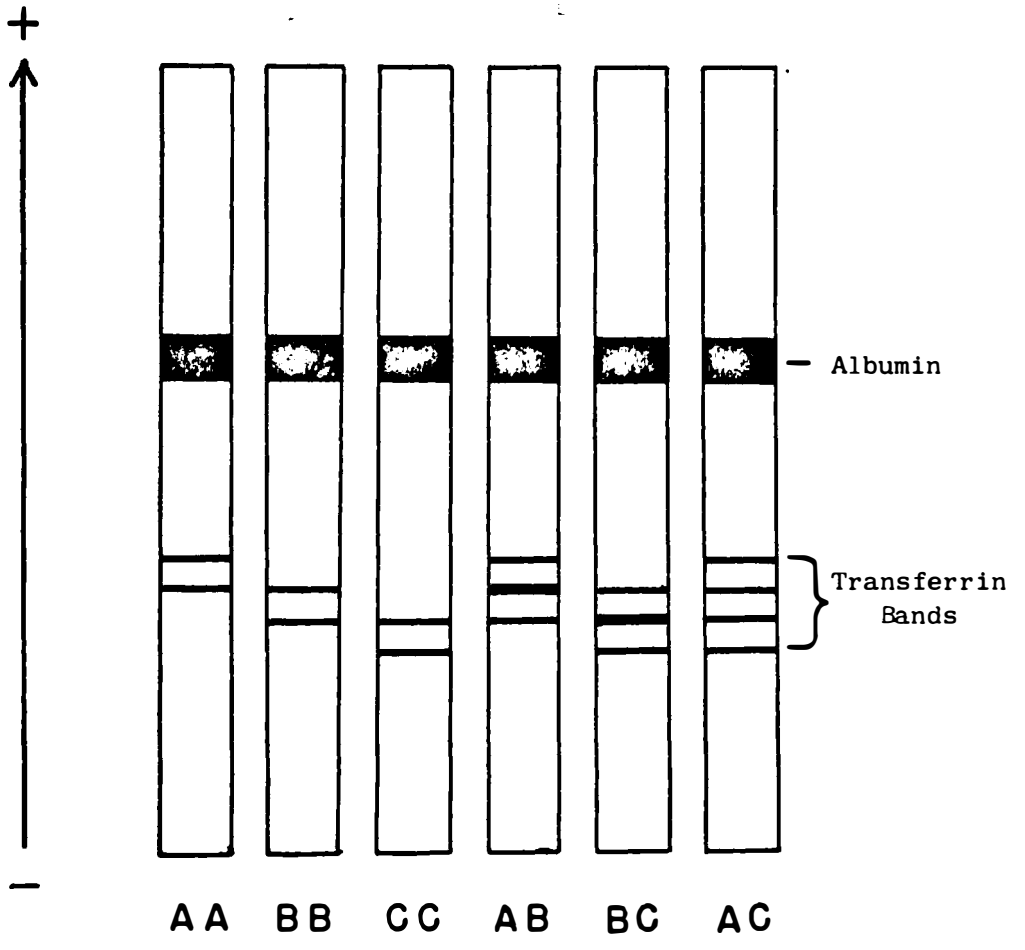

Fig. 5. Transferrin patterns found in six phenotypes in South Dakota and Pennsylvania pheasants. Arrow indicates direction of migration.

These results indicate that transferrin in South Dakota and Pennsylvania pheasants is genetically controlled by three codominant alleles at a single locus. The alleles were designated $\underline{Tf^A}$, $\underline{Tf^B}$, and $\underline{Tf^C}$ using nomenclature described by Ashton et al. (1966). The various genotypes and phenotypes are given in Table 2. Vohs and Carr (1969) hypothesized that transferrin was controlled by four codominant alleles at a single locus in Iowa pheasants. They found that one bird out of 869 sampled carried the $\underline{Tf^D}$ allele. It is possible that if pheasants in South Dakota and Pennsylvania were sampled more extensively the $\underline{Tf^D}$ allele would also be found in those populations.

Serum samples from 101 South Dakota birds and 46 Pennsylvania birds (Appendix A-E) were analyzed to determine their transferrin phenotypes (Table 3). Phenotype Tf CC was most common in all populations with a 63 and 54 percent frequency in South Dakota and Pennsylvania pheasants, respectively. The Tf AA phenotype was found in three birds from Brookings County in South Dakota. This rare phenotype was not found in Iowa pheasants (Vohs and Carr 1969).

The $\underline{Tf^C}$ allele was the most prevalent, with a frequency of 78 percent in South Dakota birds and 75 percent in Pennsylvania birds (Table 3). Allele frequencies differed significantly between South Dakota populations ($P < 0.01$). The $\underline{Tf^C}$ allele varied from 67 percent in Brookings County to 94 percent in Charles Mix County while the $\underline{Tf^B}$ allele varied from 4 percent in Charles Mix County to 20 percent in

Table 2. Allelic combinations possible under the single-locus, three-allele hypothesis.

Genotype	Transferrin Phenotype
$\underline{Tf^A\ Tf^A}$	AA
$\underline{Tf^A\ Tf^B}$	AB
$\underline{Tf^A\ Tf^C}$	AC
$\underline{Tf^B\ Tf^B}$	BB
$\underline{Tf^B\ Tf^C}$	BC
$\underline{Tf^C\ Tf^C}$	CC

Table 3. Phenotypes and allele frequencies of South Dakota and Pennsylvania pheasants in 1970-71.

Source	No. Birds Tested	Transferrin Phenotype						Allele 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

Miner County. Brookings County had a 19 percent $\underline{Tf^A}$ frequency while Charles Mix and Miner Counties had 2 and 4 percent, respectively.

Pheasants taken from Union and Montgomery Counties in Pennsylvania did not differ in allele frequencies ($P > 0.05$). Because of the large variation in allele frequencies for South Dakota populations, no comparison was made between South Dakota and Pennsylvania birds. However, when the overall allele frequencies (Table 3) were compared, they were quite similar for the two states. The $\underline{Tf^C}$ allele frequencies of four populations of wild pheasants in Iowa were not found to differ significantly, averaging 88 percent for the state (Vohs and Carr 1969).

It was apparent from the above results that there were factors selecting for the $\underline{Tf^C}$ allele in wild pheasant populations. The large variation in allele frequencies for South Dakota populations indicated that selection factors varied from one area of the state to another. For instance, the Charles Mix population might have been influenced by an environment which caused increased selection pressure toward birds with the $\underline{Tf^C}$ allele while the environments in Brookings and Miner Counties were less selective for that allele. Vohs and Carr (1969) hypothesized that rapid population increases may result in diverse phenotypes and decreased selection pressure toward birds homozygous for $\underline{Tf^C}$. They also indicated that because of the high frequency of the $\underline{Tf^C}$ allele it should be possible to determine the effect of introducing less frequent alleles into the pre-existing gene-pool.

Transferrin Inheritance

Native South Dakota cocks were crossed with Pennsylvania hens in 11 matings and 89 offspring were produced (Table 4). Pheasants with CC, BC, AB, and AC phenotypes were available for mating. Because of low production and poor survival, offspring from matings involving the 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. The offspring numbers from the remaining two matings were too low to be of importance in checking expected ratios.

Table 4. Transferrin phenotypes of 89 offspring from matings of South Dakota cocks and Pennsylvania hens.

Type of Mating	No. of Matings	Phenotype of Offspring						Total Offspring
		AA	AB	AC	BB	BC	CC	
CCxCC	4						56	56
CCxBC	5					15	10	25
CCxAC	1						4	4
BCxAC	1			2			2	4
Totals	11			2		15	72	89

CONCLUSIONS

(1) Polyacrylamide gel electrophoresis was found to be an effective technique for determining taxonomic characteristics of pheasant populations.

(2) South Dakota pheasants differ in genetic make-up ($P < 0.01$) from one area to another indicating a number of populations may be present in the state. This is significant because it indicates that factors selecting for genetic characteristics in South Dakota pheasants are not uniform, but vary from one geographical location to another. Further sampling will have to be done to determine the number and extent of populations present, and whether gene frequencies change with time.

(3) The effect of stocking Pennsylvania pheasants in South Dakota cannot be determined because of the high degree of variation in the South Dakota populations and because of the lack of a difference in transferrin patterns in the Pennsylvania birds.

(4) Transferrin inheritance in South Dakota and Pennsylvania pheasants can best be described by the occurrence of three codominant alleles at a single locus.

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APPENDIX

Appendix Table A. Results of electrophoresis in wild pheasants, Brookings County, South Dakota.

Bird No.	Sex	Age[a]	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
1	M	J	8/13/70	0.4486	2	CC
2	M	A	8/18/70	0.4430	3	BC
3	M	J	8/18/70	0.4475	2	CC
4	M	A	8/20/70	0.4493	2	CC
5	M	A	8/24/70	0.5179	2	AA
6	M	A	8/24/70	0.4950	3	AB
7	M	J	8/25/70	0.4372	2	CC
8	F	J	8/25/70	0.4460	3	BC
9	M	J	8/25/70	0.4483	4	AC
10	M	J	8/25/70	0.4536	2	CC
11	M	J	8/25/70	0.4406	2	CC
12	F	A	9/19/70	0.5000	2	BB
13	M	A	9/22/70	0.4393	4	AC
14	F	A	5/12/71	0.4444	2	CC
15	M	A	5/12/71	0.4450	2	CC
16	M	A	5/12/71	0.4474	2	CC
17	F	A	5/12/71	0.4567	2	CC
18	M	A	5/28/71	0.4541	2	CC
19	F	A	9/30/71	0.5236	2	AA
20	F	A	9/30/71	0.4444	2	CC
21	F	A	6/17/70	0.5236	2	AA
22	F	A	8/4/71	0.5022	2	BB
23	M	A	8/4/71	0.4476	4	AC
24	M	A	8/4/71	0.4498	2	CC
25	M	A	8/4/71	0.4480	2	CC
26	F	J	8/4/71	0.4160	2	CC

[a]A-adult; J-juvenile.

Appendix Table B. Results of electrophoresis in wild pheasants, Charles Mix County, South Dakota, in 1970.

Bird No.	Sex	Age[a]	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
LA 1	M	J	9/3	0.4321	2	CC
LA 6	F	A	9/3	0.4515	2	CC
LA 7	?	?	9/3	0.4346	2	CC
LA 8	M	J	9/8	0.4455	2	CC
LA 9	F	A	9/8	0.4504	3	BC
LA 10	?	?	9/8	0.4491	2	CC
LA 11	M	J	9/9	0.4311	2	CC
LA 12	F	J	9/9	0.4523	2	CC
LA 13	M	J	9/9	0.4618	4	AC
LA 14	F	J	9/9	0.4506	2	CC
LA 15	M	A	9/9	0.4461	3	BC
LA 16	M	J	9/9	0.4554	2	CC
LA 17	M	J	9/9	0.4476	2	CC
LA 18	M	J	9/9	0.4562	2	CC
LA 19	F	J	9/10	0.4577	2	CC
LA 20	F	J	9/10	0.4340	3	BC
LA 21	M	J	9/10	0.4527	2	CC
LA 22	M	J	9/10	0.4626	2	CC
LA 23	F	J	9/10	0.4558	2	CC
LA 24	M	J	9/10	0.4318	2	CC
LA 25	F	A	9/10	0.4685	2	CC
LA 26	F	J	9/10	0.4571	2	CC
LA 27	F	A	9/10	0.4545	2	CC
LA 29	F	J	9/11	0.4516	2	CC
LA 30	F	J	9/11	0.4472	2	CC

[a]A-adult; J-juvenile.

Appendix Table C. Results of electrophoresis in wild, adult pheasants, Miner County, South Dakota.

Bird No.	Sex	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
28242	M	1/1/71	0.4529	3	BC
28243	F	5/7/71	0.4562	3	BC
28245	M	1/1/71	0.4512	3	BC
28246	M	7/1/70	0.4435	4	AC
28249	M	1/1/71	0.4554	2	CC
28250	M	6/15/71	0.4554	2	CC
28251	F	9/30/70	0.4471	2	CC
28252	F	9/30/70	0.4533	3	BC
28255	F	9/30/70	0.4450	2	CC
28256	F	6/16/70	0.4565	3	BC
28257	F	5/7/71	0.4709	2	CC
28258	M	6/25/70	0.4627	3	BC
28260	F	5/7/71	0.4450	2	CC
28261	F	6/15/71	0.4648	2	CC
28262	F	9/30/70	0.4537	2	CC
28264	M	1/1/71	0.4541	2	CC
28266	F	9/30/70	0.4523	2	CC
28268	F	9/30/70	0.4480	2	CC
28269	F	9/30/70	0.4410	2	CC
28270	M	9/30/70	0.4600	2	CC
28271	F	5/7/71	0.4461	3	BC
28272	M	1/1/71	0.4536	2	CC
28275	M	2/17/71	0.4605	3	BC
28276	F	4/7/70	0.4530	3	BC
28277	M	2/17/71	0.4459	3	BC
28278	M	2/17/71	0.4554	2	CC
28279	F	4/7/70	0.4537	2	CC
28280	F	4/7/70	0.4464	2	CC
28281	M	2/17/71	0.4639	3	BC
28282	M	2/17/71	0.4541	3	BC
28283	M	2/17/71	0.4578	2	CC
28284	F	4/7/70	0.4541	2	CC
28285	F	4/7/70	0.4545	2	CC
28286	F	4/7/70	0.4581	3	BC
28287	M	2/17/71	0.4663	3	BC
28288	F	4/7/70	0.4978	3	AB

Appendix Table C. Continued

Bird No.	Sex	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
28289	F	4/7/70	0.4495	3	BC
28290	F	4/7/70	0.4409	2	CC
28291	F	4/7/70	0.4524	2	CC
28292	F	4/7/70	0.4585	2	CC
28294	F	4/7/70	0.4485	4	AC
28295	M	2/17/71	0.4600	2	CC
28298	M	2/17/71	0.4697	3	BC
28299	F	4/7/70	0.4545	3	BC
28300	F	4/7/70	0.4509	2	CC
28302	F	4/7/70	0.4601	4	AC
28303	F	4/7/70	0.4563	2	CC
28305	M	2/17/71	0.4511	3	BC
28306	M	2/17/71	0.4550	2	CC
28307	M	2/17/71	0.4597	3	BC

Appendix Table D. Results of electrophoresis in wild, adult, female pheasants, Union County, Pennsylvania, sampled on February 3, 1971.

Bird No.	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
646	0.4605	3	BC
647	0.4884	2	BB
648	0.4444	2	CC
649	0.4558	4	AC
650	0.4643	3	BC
651	0.4619	2	CC
652	0.4660	3	BC
653	0.4608	2	CC
654	0.4515	2	CC
655	0.4588	2	CC
656	0.4450	2	CC
657	0.4718	3	BC
658	0.4646	2	CC
659	0.4581	2	CC
660	0.4489	2	CC
661	0.4563	3	BC
662	0.4571	2	CC
663	0.4722	3	BC
664	0.4505	2	CC
665	0.4566	2	CC
666	0.4645	2	CC
667	0.4757	3	BC
668	0.4429	2	CC
669	0.4519	3	BC

Appendix Table E. Results of electrophoresis in wild, adult, female pheasants, Montgomery County, Pennsylvania, sampled on February 9, 1971.

Bird No.	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
400	0.4430	2	CC
502	0.4477	2	CC
504	0.4484	3	BC
574	0.4487	4	AC
575	0.4518	3	BC
576	0.4502	2	CC
577	0.4402	3	BC
578	0.4537	4	AC
579	0.4317	2	CC
580	0.4372	3	BC
581	0.4430	2	CC
582	0.4912	3	AB
583	0.4486	2	CC
586	0.4473	4	AC
599	0.4430	2	CC
600	0.4357	3	BC
670	0.4449	2	CC
671	0.4435	3	BC
672	0.4458	2	CC
673	0.4426	2	CC
674	0.4372	3	BC
675	0.4417	2	CC

Appendix Table F. Results of electrophoresis of offspring from matings between South Dakota cocks and Pennsylvania hens.

Bird No.	Sex	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
			400 x 28242[a]		
5080	M	8/4/71	0.4459	2	CC
5081	F	8/4/71	0.4444	2	CC
5090	F	8/12/71	0.4434	2	CC
5112	F	8/19/71	0.4388	2	CC
5113	F	8/19/71	0.4426	3	BC
			574 x 28242		
5045	M	7/15/71	0.4525	4	AC
5084	M	8/4/71	0.4375	2	CC
5085	M	8/4/71	0.4375	2	CC
5091	M	8/12/71	0.4509	4	AC
			578 x 28272		
183	M	6/17/71	0.4439	2	CC
184	M	6/17/71	0.4450	2	CC
185	F	6/24/71	0.4370	2	CC
5036	F	7/15/71	0.4444	2	CC
			599 x 28272		
142	M	6/24/71	0.4414	2	CC
151	?	6/17/71	0.4470	2	CC
153	M	6/17/71	0.4434	2	CC
176	M	6/24/71	0.4369	2	CC
177	M	6/17/71	0.4525	2	CC
5049	M	7/15/71	0.4545	2	CC
5055	M	7/22/71	0.4424	2	CC
5056	M	7/22/71	0.4444	2	CC
5074	F	7/29/71	0.4420	2	CC
5075	F	7/29/71	0.4412	2	CC
5087	F	8/4/71	0.4481	2	CC
5088	M	8/4/71	0.4402	2	CC
5097	F	8/12/71	0.4532	2	CC
5100	F	8/12/71	0.4405	2	CC

[a]Mating of hen No. 400 with cock No. 28242.

Appendix Table F. Continued

Bird No.	Sex	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
			600 x 28272		
132	M	6/17/71	0.4512	3	BC
133	M	6/17/71	0.4393	3	BC
178	M	6/24/71	0.4601	2	CC
5030	M	7/15/71	0.4567	3	BC
5031	M	7/15/71	0.4460	2	CC
			648 x 28264		
134	F	6/24/71	0.4460	2	CC
164	M	6/24/71	0.4465	2	CC
165	F	6/24/71	0.4398	2	CC
189	F	6/17/71	0.4404	2	CC
5037	F	7/15/71	0.4495	2	CC
5043	M	7/15/71	0.4358	2	CC
5044	F	7/15/71	0.4500	2	CC
5058	M	7/22/71	0.4361	2	CC
5059	F	7/22/71	0.4558	2	CC
5061	F	7/22/71	0.4541	2	CC
5069	M	7/29/71	0.4516	2	CC
5070	F	7/29/71	0.4505	2	CC
5076	F	7/29/71	0.4388	2	CC
5079	M	8/4/71	0.4493	2	CC
5115	F	8/19/71	0.4222	2	CC
			653 x 28272		
155	?	6/17/71	0.4444	2	CC
156	?	6/17/71	0.4495	2	CC
158	F	6/17/71	0.4311	2	CC
186	F	6/17/71	0.4348	2	CC
187	M	6/17/71	0.4484	2	CC
5032	F	7/15/71	0.4449	2	CC
5033	M	7/15/71	0.4405	2	CC
5034	F	7/15/71	0.4372	2	CC
5051	F	7/22/71	0.4381	2	CC
5052	M	7/22/71	0.4408	2	CC
5053	M	7/22/71	0.4390	2	CC
5066	F	7/29/71	0.4454	2	CC
5067	M	7/29/71	0.4350	2	CC

Appendix Table F. Continued

Bird No.	Sex	Date Sampled	Transferrin: Albumin Ratio	No. of Bands	Transferrin Phenotype
5068	F	7/29/71	0.4426	2	CC
5082	M	8/4/71	0.4495	2	CC
5083	M	8/4/71	0.4529	2	CC
			656 x 28272		
179	F	6/17/71	0.4484	2	CC
180	?	6/17/71	0.4426	2	CC
181	M	6/17/71	0.4386	2	CC
5042	F	7/15/71	0.4440	2	CC
5063	F	7/29/71	0.4387	2	CC
5089	F	8/4/71	0.4367	2	CC
5093	F	8/12/71	0.4528	2	CC
5106	M	8/19/71	0.4478	2	CC
5107	F	8/19/71	0.4500	2	CC
5116	M	8/19/71	0.4369	2	CC
			668 x 28242		
5039	M	7/15/71	0.4420	2	CC
5078	F	8/4/71	0.4429	3	BC
5096	M	8/12/71	0.4562	3	BC
5102	F	8/12/71	0.4444	3	BC
5109	M	8/19/71	0.4480	2	CC
5110	M	8/19/71	0.4449	3	BC
5114	F	8/19/71	0.4317	3	BC
			670 x 28242		
135	F	6/17/71	0.4533	2	CC
137	M	6/24/71	0.4524	2	CC
144	F	6/24/71	0.4292	3	BC
170	M	6/24/71	0.4472	3	BC
5035	F	7/15/71	0.4545	3	BC
5062	M	7/22/71	0.4429	3	BC
			674 x 28272		
128	M	6/17/71	0.4460	3	BC
171	M	6/17/71	0.4537	3	BC