SERUM PROTEINS OF CANADA GOOSE (*BRANTA CANADENSIS*) SUBSPECIES

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In North America, the major goose species is the Canada Goose (*Branta canadensis*), with total population estimates ranging around 3 million individuals (Bellrose 1976). A major problem in Canada Geese studies centers around population structure on the breeding grounds. Bellrose (1976) listed eleven subspecies (exhibiting tremendous morphological variation) of *B. canadensis*, but also criticized, based on recent work by Hansen (1968) and Hanson (1965), the current racial (= subspecies) classification. For example, Hansen (1968) reported seven races overwintering on the Pacific Coast, while Hanson (1965) noted a similar situation in Missouri. Bellrose (1976) delineated the twelve ranges of specific Canada Geese populations, with small races tending to breed further north than the medium and large races.

Baker and Hanson (1966) first attempted to use the electrophoretic method to differentiate geese species and subspecies. They worked with both *Anser* and *Branta* species including seven *B. canadensis* subspecies—*interior, maxima, parvipes, occidentalis, fulva, hutchinsii* and *minima*. Using vertical starch gel techniques, they were unable to distinguish among *Branta* subspecies or to differentiate between *Branta* and *Anser* at the generic level.

We initially questioned Baker and Hanson's 1966 paper after completing electrophoretic work on a few samples of serum collected from geese at Remington Farms, Chestertown, Maryland. J. W. Aldrich (pers. comm.) indicated that the samples were from three subspecies of *B. canadensis*: *canadensis, interior* and *maxima*, all of which were distinct as separated on gradient slab electrophoresis. This limited information suggested that it would be worthwhile to reexamine serum protein patterns in *B. canadensis*. Therefore we attempted to collect many serum samples from a wide number of subspecies for analyses.

Our findings in the examination of a number of individuals and subspecies are the subject of this paper. However, we must view some of our initial findings as tentative because: (1) identification of some individuals was not certain (not all samples collected on nesting grounds), and (2) sample sizes were sometimes small. This paper evaluates the serum protein patterns of nine subspecies of the Canada Goose using current improved electrophoretic techniques.

MATERIALS AND METHODS

Serum samples were collected from nine subspecies of *B. canadensis* (Table 1). We recognize that the classification of these subspecies basically comes from Delacour (1951, 1954) and Hansen and Nelson (1964). Therefore, we listed the collection locality when known. We also question the subspecific classification of geese collected at the Patuxent Wildlife Research Center. These birds are presumably *B. c. canadensis*; they should be compared to geese collected on or near the breeding grounds of that form. For this paper, we are following the names used in Bellrose (1976).

TABLE 1. Description of Canada Goose (*Branta canadensis*) samples studied.

Common name	Subspecies	Collection locality	Sample size
Atlantic	*canadensis*[1]	Laurel, Maryland	25
Vancouver	*fulva*	Prince William Sound, Alaska	4
Richardson's	*hutchinsii*[2]	Jamestown, North Dakota	3
Aleutian	*leucopareia*[1]	Laurel, Maryland	6
Giant	*maxima*[2]	Jamestown, North Dakota	7
Cackling	*minima*[2]	Jamestown, North Dakota	2
Western	*moffitti*	Brigham City, Utah	10
Western	*moffitti*	Flathead Lake, Montana	11
Dusky	*occidentalis*	Copper River Delta, Alaska	19
Dusky	*occidentalis*	Corvallis, Oregon	19
Taverner's	*taverneri*	Potter Marsh, Cook Inlet, Alaska	13

[1] Captive geese from Patuxent Wildlife Research Center.
[2] Captive geese from Northern Prairie Wildlife Research Center.

All serum was shipped frozen; all samples were stored at –20°C until analysis. Discontinuous column and gradient slab acrylamide electrophoresis followed the techniques of Chapman and Morgan (1973). A 4 μl aliquot of serum was used for both column and slab electrophoresis.

For analysis of column electropherograms (the protein pattern visualized after electrophoresis, staining and destaining on an electrophoretic medium), a model serum protein pattern was constructed for each subspecies set. All gels from that set were compared against the model gel. Proteins were coded for the presence (1) or absence (0) of the serum protein. If a protein occurred in a gel and not in the model, then that protein was added to the model pattern and all gels were reexamined for that protein. However, the model was scored as 0 for the new protein. Relative mobilities of serum proteins were measured with an Adams micro-hematocrit reader. Both Jaccard's coefficient, S_J, and the simple matching coefficient, S_{SM} (Sneath and Sokal 1973) were calculated for each set of protein characters within a subspecies; coefficients were not calculated for individual proteins, rather the entire set of matches was considered.

For gradient slab electropherograms, direct comparisons were made within a gel for mobility and distribution of proteins. Gels containing the same subspecies were initially analyzed, followed by more rigorous analysis through comparing different subspecies within an electrophoretic run.

Most of the preceding information deals with strictly quantitative characters such as the number of proteins, density, and position of proteins. In addition, there is a subjective qualitative sense, difficult to express at times, about serum protein patterns. Of these qualitative characters, the density of serum proteins relative to each other appears to be critical.

For cluster analysis, results from the column and slab work were combined and analyzed through the BMDP-PIM multivariate program (program revised 7

FIGURE 1. Column and slab acrylamide electropherograms from 9 subspecies of *Branta canadensis*. Direction of migration is from left to right.

February 1975, developed at the Health Sciences Computing Facility, UCLA, with support from NIH Special Research Resources Grant RR-3) by correlation between pairs of variables with average linkage.

RESULTS

Canada Geese serum proteins, separated by column slab electrophoresis techniques, are shown in Figure 1. The diagrammed pattern is the model observed in each set of subspecies electropherograms. For each set of samples, coefficients of similarity, S_{SM} and S_J, for each subspecies are generally above 0.85, indicating little variation within a subspecies group (Table 2). More variation is present in *hutchinsii* than the other subspecies, but this may be partially a reflection of the small sample size ($n = 3$). Better agreement (compared to the column work) within a subspecies occurs with the use of slab electrophoresis because serum proteins are compared readily. All S_J's are above 0.89 for the slab technique (Table 2). Numbers of serum proteins visualized for each subspecies by the two electrophoretic techniques varied (Table 2).

In all cases, the serum protein pattern of a subspecies is characterized by a large dominant albumin and either a single or double transferrin. Some distinguishing serum lipoproteins are also present in a few of the subspecies. Resolution of albumin and transferrin is better with the column technique than slab, however, extremely good resolution of the pre-transferrin serum proteins is possible with the slab technique. Except for the occurrence of a lipoprotein in three samples from *B. c. occidentalis*, no other proteins related to age, sex or maturity are apparent.

Of particular interest is the presence of a double albumin (conforming to a Hardy-Weinberg equilibrium) in *B. c. canadensis*. We did not find a polymorphic albumin in any of the other subspecies, but sample size for a few of the subspecies was inade-

TABLE 2. Coefficients and number of serum proteins observed in *Branta canadensis* subspecies.

Subspecies	Protein no.		Column		Slab
	Column	Slab	S_J	S_{SM}	S_J
canadensis	21	18	0.95	0.95	0.95
fulva	19	22	0.95	0.95	0.98
hutchinsii	20	25	0.79	0.80	1.00
leucopareia	16	27	1.00	1.00	0.95
maxima	16	20	0.90	0.90	0.91
minima	17	19	0.94	0.94	0.95
moffitti	18	22	0.97	0.98	0.96
occidentalis (Alaska)	20	17	0.92	0.92	0.98
occidentalis (Oregon)	20	17	0.95	0.95	0.97
taverneri	21	20	0.85	0.89	0.89

quate. In general, we found little serum protein polymorphism within each subspecies investigated.

Table 3 presents comparisons of various subspecies using Jaccard's coefficient based on the electrophoretic data from analyses of both column and slab electropherograms. Coefficients vary from 0.59 to 0.82, a variation of only 0.23 similarity units. The coefficients among subspecies (Table 3) are lower than those within subspecies (Table 2).

Analysis of the coefficient distribution indicates that S_J's below 0.62 signify distinctness whereas S_J's above 0.77 indicate similarity. Four S_J's fall below 0.62 with 5 S_J's above 0.77. The remaining 27 coefficients tend to display a normal distribution.

Very distinct pairs are *canadensis* and *taverneri*, *hutchinsii* and *leucopareia*, *minima* and *taverneri*, and *maxima* and *occidentalis*. All of these pairs have S_J's below 0.62. Very similar pairs include *hutchinsii* and *moffitti*, *canadensis* and *fulva*, *fulva* and *leucopareia*, *maxima* and *moffitti*, and *fulva* and *occidentalis*. All of these pairs have S_J's above 0.77.

Cluster analysis for the nine subspecies (Fig. 2) also points out some of the results described previously. *Taverneri* appears to be the most distinct subspecies based on quantitative characters. The most similar pairs are *occidentalis* and *fulva* plus *moffitti* and *maxima*. The majority of subspecies have similarity index values greater than 0.85.

Qualitative characters are also important in any consideration of these subspecies. Qualitatively, *leucopareia* appears to be the most distinct. Also, *maxima* and *moffitti* are very similar (as with the quantitative characters) for both column and slab electropherograms. Finally, the western subspecies tend to be more similar (as in the cluster analysis).

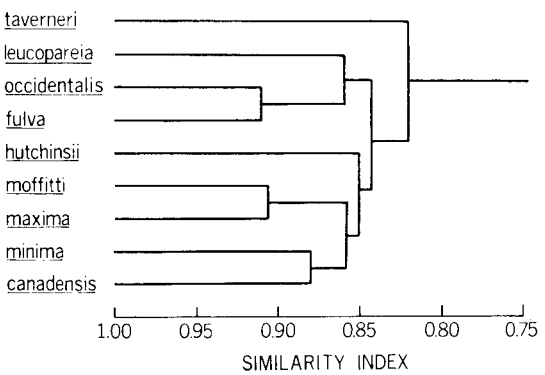

FIGURE 2. Similarity matrix for the 9 subspecies of *Branta canadensis*.

DISCUSSION

Although it is tempting to speculate on relationships among these subspecies based on the electrophoretic information, we must qualify the work described here. First, in all cases, we knew the identity of the subspecies before electrophoretic analysis. The creation of a data base for each subspecies with sample sizes adequate for discriminant analysis would be more appropriate. Subsequent samples would be analyzed "blind," i.e. without knowledge of sample source except for the collector. In this way the reliability of determining *Branta canadensis* subspecies could be improved. In addition, the development of discriminant analysis based only on serum protein information is inadequate. These data should be supplemented with serum enzyme information for each subspecies. Unless red blood cells are also used, serum lacks enzymes in sufficient quantities for extensive electrophoretic work. Tissues such as muscle or liver containing enzyme levels for additional electrophoretic information analyses could be biopsied and used with little injury to the geese. Birds killed by hunters may best be analyzed using their muscle or liver rather than serum.

An important finding was that in the serum protein pattern *leucopareia* is quite distinct from the other subspecies studied. This population is very restricted from other subspecies, breeding only on Buldir Island (Bellrose 1976). *Leucopareia*'s distinct pattern presumably results from long isolation and lack of gene exchange with the other Alaskan populations.

In Baker and Hanson's (1966) work, starch gel electrophoresis of hemoglobin and erythrocyte enzymes did not reveal any differences at generic, specific or individual levels. Electrophoresis of serum proteins did show some differences at the subspecific level, transferrins in *fulva*, *hutchinsii* and *minima* all having distinct phenotypes. Some variation also occurs in the prealbumin systems, but Baker and Hanson (1966) made no further inferences except to express caution on observing prealbumin systems. Also, a distinct albumin was seen in one *B. c. maxima*.

Acrylamide electrophoresis has two advantages over vertical starch gel electrophoresis: (1) the background is clear after destaining, allowing serum proteins normally in very low concentrations to be seen and (2) the gradient slab acrylamide technique separates not only on total protein charge but also on molecular weight. The major differences between Baker and Hanson's

TABLE 3. Jaccard's coefficient for combined column and slab data for *Branta canadensis* subspecies.

	Subspecies								
Subspecies	*canadensis*	*fulva*	*hutchinsii*	*leucopareia*	*maxima*	*minima*	*moffitti*	*occidentalis*	*taverneri*
canadensis	—	.79	.68	.66	.68	.76	.71	.71	.59
fulva		—	.74	.80	.65	.75	.75	.82	.68
hutchinsii			—	.59	.63	.71	.78	.68	.64
leucopareia				—	.70	.71	.65	.68	.64
maxima					—	.74	.81	.61	.65
minima						—	.74	.64	.60
moffitti							—	.65	.63
occidentalis[1]								—	.67
taverneri									—

[1] Samples from Alaska and Oregon were combined for analysis. S_J for the two samples was greater than 0.95.

(1966) work and our study are the apparent resolution of the minor proteins through acrylamide electrophoresis and the increased sample size permitting a better assessment of intraracial variation.

Even though most of the subspecies are recognized readily from their breeding distribution (Hanson 1965), the biochemical relationship among subspecies is close. Baker and Hanson (1966) gave several explanations for the close biochemical relationships of *B. canadensis* subspecies including:

> "1. Geese are subjected to very strong selection pressures, keeping the blood proteins within very narrow limits of specificity. . . . 2. A lower rate of mutation for blood proteins exists in *Anser* and *Branta* in comparison with other groups of animals . . . . 3. The genus *Anser* dates back only to the Upper Miocene and the genus *Branta* to the Lower Pliocene; some of the present-day species probably evolved in Pleistocene times and, in the case of *B. canadensis*, some of the races are probably of very recent origin."

All these explanations are quite plausible, especially in view of the conclusion reached by Prager and Wilson (1975) who found that birds have a very slow evolutionary rate based on cytological and anatomical data and the slow rate at which birds have lost the potential for interspecific hybridization—a function of regulatory gene expression. This phenomenon may also in part account for the biochemical relationship among the subspecies of *B. canadensis*.

SUMMARY

Serum proteins from nine subspecies of Canada Geese (*Branta canadensis*) were analyzed through the use of column and slab acrylamide electrophoresis. Variation was minimal within a subspecies, although all the subspecies were closely related. *B. c. leucopareia* appeared to be the most distinct subspecies, while *maxima* and *moffitti* were the most similar. Our preliminary findings suggest that the electrophoresis techniques are sensitive enough to identify some of the subspecies; however, baseline data from breeding ranges of all subspecies are required.

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NORTHERN FINCHES FEEDING FROM FLOATING VEGETATION

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On 14 October 1972 I observed several flocks of Lapland Longspurs (*Calcarius lapponicus*) and Snow Buntings (*Plectrophenax nivalis*) feeding from floating vegetation in a large water-filled borrow pit along the southern edge of the Delta Marsh, about 22 km NW Portage la Prairie, Manitoba. Beginning at 1400 I watched for two hours while these finches flew low over the water occasionally skimming its surface and frequently landing for periods of up to four min on floating spike water-milfoil (*Myriophyllum exalbescens*). The birds apparently foraged while on the vegetation but I did not determine whether plant or animal matter from the water was being consumed. Some individuals preened while on the vegetation. The weather was sunny and generally calm.

On 16 October 1972, a windy day with light snowfall, I visited this borrow pit again but saw Snow Buntings foraging only along its shore and in open dry areas among clumps of bulrush (*Scirpus*) at the edge of the marsh. The choppy water that day may