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Variation in plasma proteins among Mallards collected from three different geographic regions

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Abstract

Blood samples collected from Mallards in Quebec, Manitoba and British Columbia were analysed by starch-gel and cellulose-acetate electrophoresis. Polymorphs were detected. The only allele having a frequency which differed among locations appeared to be an esterase. There were statistically significant differences in relative and total protein density among samples from the three locations, but a more extensive sampling program is necessary before it can be decided that this technique is of practical value in identifying the origins of Mallard stocks. Observed differences in electrophoretic scans of plasma proteins may potentially serve as an indicator for comparing different habitats.

Résumé

L'analyse par électrophorèse au gel amylacé et à l'acétocellulose d'échantillons de sang recueillis chez des Canards malards au Québec, au Manitoba et en Colombie-Britannique a révélé des polymorphes. Il appert que l'unique allèle dont la fréquence ait varié selon le lieu du prélèvement était un estérase. Bien qu'il y ait eu, chez les échantillons tirés des trois lieux en cause, des différences statistiquement significatives pour ce qui est de la densité, tant absolue que relative, de la teneur en protéines, seule l'exécution préalable d'un programme d'échantillonage à plus grande envergure permettrait de juger s'il y aurait avantage à user de cette technique pour déceler l'origine d'une lignée donnée de Canards malards. La variation observée des résultats du balayage électrophorétique de protéines de plasma pourrait éventuellement servir d'indicateur aux fins de comparaison d'habitats différents.

Introduction

Banding data have been the traditional source (Bellrose 1972, Geis 1972) of information on distribution and movement of waterfowl within and among populations. Development of an alternative method to banding may be feasible if variations in waterfowl gene pools can be described and correlated with either geographic locations or environmental gradients. The purpose of this study was to determine whether or not differences in the frequency of certain alleles and their manifest protein densities could be readily detected among populations of Mallards (*Anas platyrhynchos*) from three geographic locations.

Methods

Adult Mallards were bait trapped at Thurso, Quebec (on the Ottawa River), Big Grass Marsh, Manitoba, and Delta, British Columbia during August, September, and October, 1974, respectively. A 1–5 ml blood sample was drawn from the wing vein of each bird, using either 5-ml heparinized syringes or 5-ml heparinized vacuum blood-collecting tubes fitted with 22-gauge needles. After collection the samples were centrifuged for 20 min, the plasma was drawn off using a 5¼ in. Pasteur pipette and divided into two small vials which were then sealed, labelled and frozen at −23°C.

Starch-gel electrophoresis

Starch-gel electrophoresis was conducted using modified methods of Kristajansson (1963).

Gels were prepared two at a time by suspending 60 g of hydrolysed potato starch (Connaught Lab. Ltd., Toronto) in 115 ml of gel buffer at 21°C in an aspirator flask; 385 ml of buffer at 100°C was then added to this mixture. The contents of the flask were swirled by hand under partial vacuum. When the starch was uniformly dissolved in the buffer it was poured into two moulds 12 x 21 x 0.5 cm. (Moulds were prepared by affixing plexiglass strips (12 x 1.5 x 0.5 cm and 24 x 0.5 cm) to a glass plate with vaseline.) Another glass plate was gently lowered on to the upper surface of the gel, avoiding any air bubbles between the gel and the upper plate. Gels were then placed in the refrigerator at 4°C for approximately 30 min to cool and set.

Once the gels had set, the upper plate was removed gently in order not to tear the gel. The gel, still in the mould, was covered with a piece of plastic film to prevent desiccation during the run and to enable us to mark the surface. Electrolyte lines were marked 2 cm from the top and bottom of the gel and an insert line was indicated 4 cm from the bottom. The plastic film was folded back from the bottom of the gel to the insert line. A pre-cut metal template was pushed perpendicularly into the gel to form eight evenly-spaced 1-cm insert slots.

Plasma samples were applied to 0.5 x 1 cm strips of Whatman No. 3 chromatography paper and placed into the insert slots. Electrophoresis was carried out under a continuous current of air at 4°C, to avoid heat distortion of the proteins.

Electrolyte trays with 250 ml of buffer were placed next to the top and bottom of the gel. The tray closest to the insert line was attached to the negative terminal (cathode) of the power source (Heathkit Regulated H.V. Power Supply model IP17), and the other tray was attached to the positive terminal (anode). The plastic film was folded back to the electrolyte lines exposing 2 cm at either end of the gel. An electrolyte wick (Whatman No. 1 chromatography paper, 7 sheets thick, 15 x 15 cm) was placed over each exposed end of the gel and draped into the electrolyte buffer.

Electrophoresis was continued either for a specified time or until the borate line migrated a specified distance, according to the nature of the sample. The borate line is a narrow, light brown line that migrates from the cathode to the anode.

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Table 1
Observed and expected frequency of esterase band shown by an arrow in Figure 2. χ^2 at 2 degrees of freedom and 5% level is 5.99

Site	Condition	Observed	Expected	$\frac{(O-E)^2}{E}$
Quebec	Present	11	8.3	0.88
	Absent	21	23.7	0.31
Manitoba	Present	9	7.3	0.39
	Absent	19	20.7	0.14
British Columbia	Present	1	5.4	3.59
	Absent	20	15.6	1.24
Total		81	81	$\chi^2 = 6.55$

Figure 1
Photograph of a characteristic slice of starch-gel stained with Buffalo black. The arrows designate a characteristic polymorph. 'h' designates a human blood plasma control

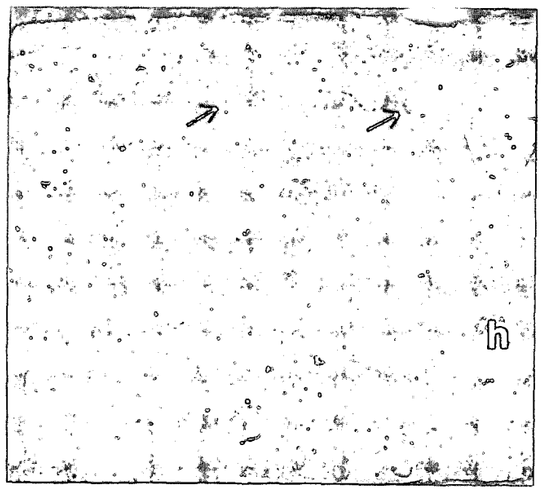
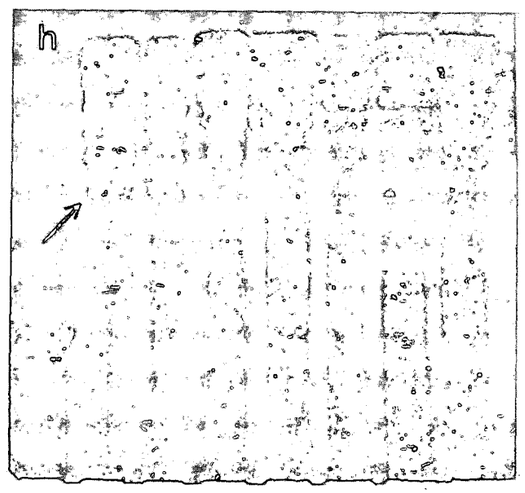


Figure 2
Photograph of a characteristic slice of starch-gel stained with alpha-*N*-butyrate. The arrow designates the esterase polymorph, the frequency of which differed among geographic locations. 'h' designates a human blood plasma control



When the run was complete, the plastic film and the plexiglass strips surrounding the gel were removed. The top and bottom ends of the gel (usually just above the borate line and just below the insert line) were cut off and discarded. The gel was sliced horizontally either in half or in three, and each piece was stained according to the methods described below.

Electrophoresis of plasma samples was continued until the borate line had migrated 10 cm anodal to the insert line. Boric acid – sodium hydroxide was the electrolyte buffer and *tris*-citric acid (p*H* 6.8) was the gel buffer. The voltage was 165 V for 30 min, then (after the inserts were removed) 300 V for the remainder of the run. Plasma esterases were stained with alpha-*N*-acetate and alpha-*N*-butyrate. General proteins were stained with Buffalo black.

After staining, the gels were soaked in an acetic acid – methanol – water solution to remove excess stain. They were then photographed.

During initial stages of the work, attempts were made to stain for leucine, aminopeptidase, hemoglobin, and glucosephosphate isomerase, but were discontinued because no differences among samples were found.

Cellulose-acetate electrophoresis of samples was conducted using the Beckman R-101 microzone cell (Beckman

1965), Beckman's buffer, B-2, at p*H* 9, and a 250 V current for 30 min. After electrophoresis, strips were stained with a fixative dye solution containing 0.5 g Ponceau-S stain, 7.5 g trichloroacetic acid and 7.5 g sulfosalicylic acid diluted to 250 ml in distilled water.

The strips were then scanned with the Beckman R-110 densitometer. The resulting graph was divided into 15 equal segments on the distance axis and the proportion of the area under the plotted curve in each segment was calculated. Each curve was then treated as a 15-variate observation on which multivariate analyses of variance (MANOVA) were used to test for differences between sexes and among geographic locations.

Results

Variation in the presence and absence of certain proteins was demonstrated through protein separation resulting from starch-gel electrophoresis (Figs. 1 and 2). Assuming that this presence or absence resulted directly from the presence or absence of particular alleles, only one allele was found to differ significantly among geographic regions (Table 1). The protein band from this allele is indicated with an arrow in Figure 2. Its identity was not determined although it responded as an esterase in binding with alpha-*N*-butyrate and alpha-*N*-acetate.

Total plasma protein concentration was not estimated. However, assuming that the intensity of staining is constant among plasma samples, differences in total protein can be detected by comparing the area under the curves derived from the densitometric scan of cellulose-acetate strips (Fig. 3). Additionally, differences in the relative distribution of proteins within a curve can be compared by standardizing the area under each curve to equal 100%. The multivariate analyses of variance comparing total densities (Fig. 4) and relative densities (Fig. 5) revealed significant ($P \leq 0.05$) differences in both parameters among geographic locations.

The average density of plasma protein as measured by stain intensity was greatest in Quebec and least in British Columbia (Fig. 4). When comparing relatively higher concentrations of albumens, the samples from Manitoba displayed relatively greater amounts of betaglobulin but less fibrinogen and gammaglobulin.

No significant differences between sexes were detected in either total protein density or relative protein density.

Discussion

Frequency of certain alleles and intensity of their manifest proteins differed among Mallard populations from different geographic locations. If some of these differences are genetic in origin, gene flow between eastern and western birds is not sufficiently great to mask the results of certain selection pressures which favour some alleles over others. Should these genetic differences be real, systematic sampling of Mallard blood and development of a map describing distribution of alleles might be possible. Sampling of waterfowl blood during harvest could lead to statistical descriptions of the sources of the fall migration.

Many alleles were described only by electrophoresis. Random error will probably cause 5% of the alleles to appear to have statistically significant differences ($P \geq 0.05$) in frequency even when no real differences occur. However, since only one of many was found to demonstrate a significant difference, verification of these results with a more powerful and extensive sampling program would be an essential prerequisite to any anticipated management program.

Differences in the concentrations of proteins were evident from the cellulose-acetate results.

Perhaps the largest source of variation in protein density lies directly in the bird's response to habitat and its physiological state rather than in the genotype itself. For example, the density of pre-albumens and post-albumens is known to vary with time after laying in female chickens (Sturkie 1965). The absence of specific descriptions of habitat and physiological condition precludes detailed discussion of the biological significance of differences in protein density reported here.

Cellulose-acetate electrophoresis, although providing less distinct separations of individual proteins, was less costly, less time consuming, more repeatable, and easier to quantify. The equipment for cellulose-acetate electrophoresis is standardized and readily available in many medical laboratories. The ease with which differences can be detected suggests that cellulose-acetate electrophoresis of waterfowl plasma could provide a useful tool for the study of waterfowl populations.

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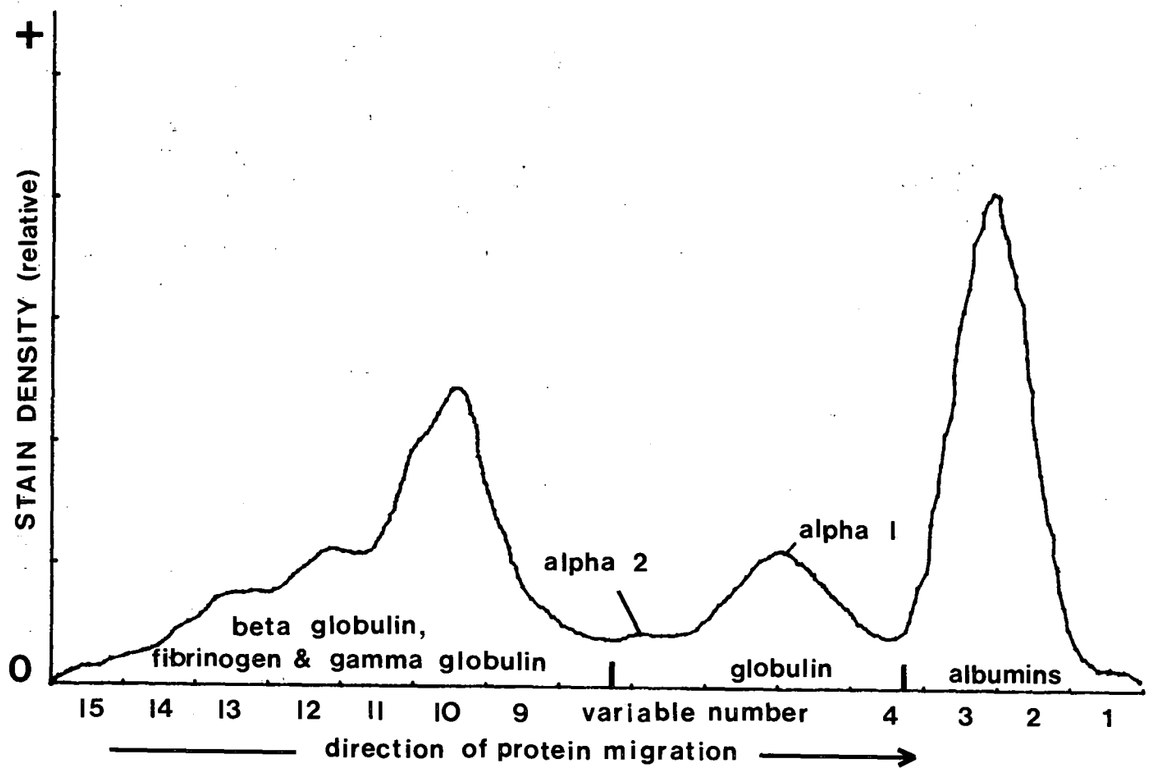
Figure 3
Characteristic chart recording from densitometric scan of cellulose-acetate strip after electrophoresis



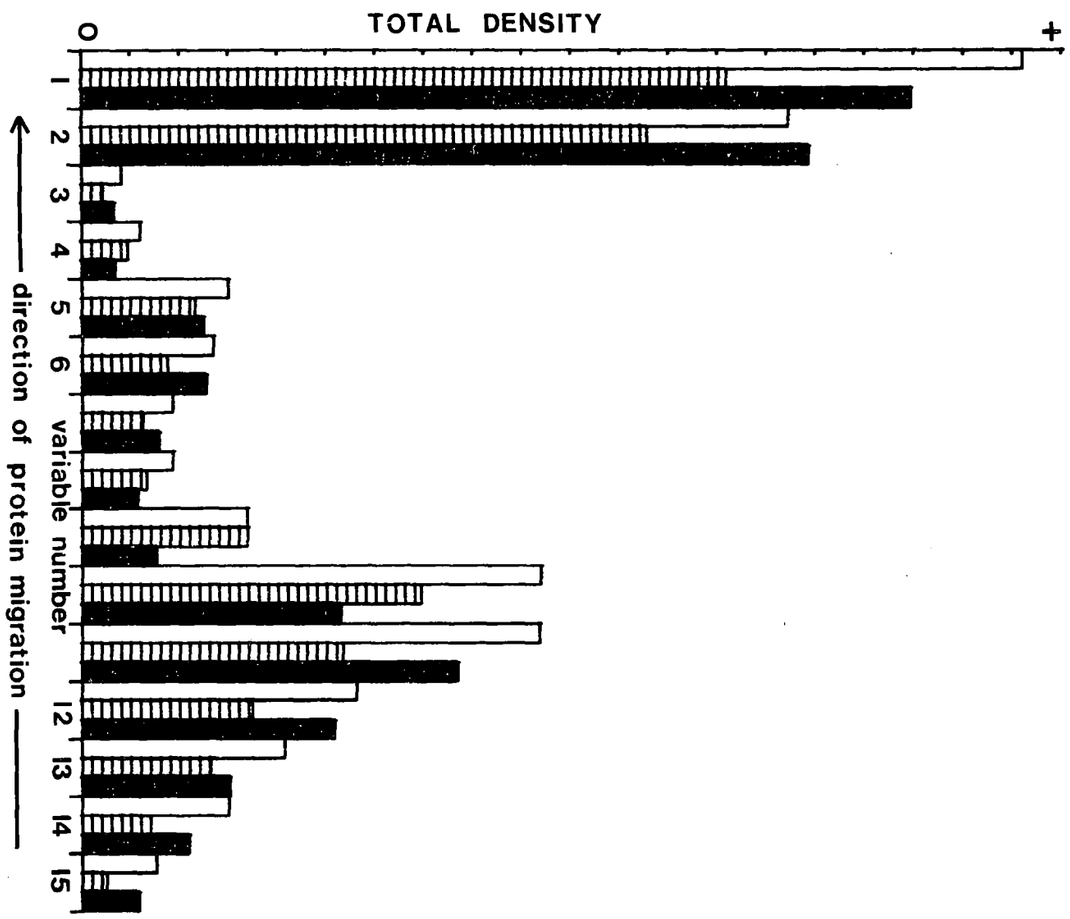


Figure 4
Distribution derived from MANOVA regression coefficients of total density of plasma proteins as measured by cellulose-acetate electrophoresis. The white, striped, and solid bars respectively designate samples taken from Quebec, British Columbia, and Manitoba

Figure 5
Distribution derived from MANOVA regression coefficients of relative density of plasma proteins as measured by cellulose-acetate electrophoresis. The white, striped, and solid bars respectively designate samples taken from Quebec, British Columbia, and Manitoba