ANATOMICAL SKETCH OF WILD SWANS

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Symposium participants were presented with copies of a 44-page booklet on this subject, richly illustrated by line drawings. The very quality and abundance of these illustrations make it impossible to reproduce the booklet within the confines of the Proceedings.

The booklet, in both English and Japanese, is split into five sections: bone structure, muscles, internal organs, feathers and sexing. The first four sections are illustrated with a general 'concept picture', as well as further enlarged drawings of details, and many measurements are given.

The section on sexing, as well as giving details of the cloacal method, suggests two techniques that can be used in the field based on general appearance and colour of the culmen. The back parts of the head are rounder and larger in the male than the female, and from the larynx down to the neck look rounder in the male — a character that can be seen well when a family is swimming together. On arrival in autumn bill colour in the two sexes does not differ, but just before departure in spring, the colour of the culmen in the male gets slightly orange.

Copies of the booklet can be obtained from the author.

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GENETIC COMPARISON OF *CYGNUS CYGNUS BUCCINATOR* POPULATIONS

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Introduction

The historical range of *Cygnus cygnus buccinator* encompassed a large area of the North American continent, from breeding areas in Alaska to wintering grounds along the Gulf of Mexico (Walker 1968) and east to Hudson's Bay (Alison 1975).

The expansion of civilization disturbed or destroyed *C. c. buccinator* habitat, pushing the species to near extinction. The population was reduced to 69 birds south of Canada by 1932 (Hansen 1973). Most of these were in the valleys of the Rocky Mountains in Idaho-Montana-Wyoming. The enactment of the Migratory Bird Conservation Act in 1929 began a reversal in the population trend by outlawing all hunting and trapping of *C. c. buccinator,* as well as authorizing the acquisition of land for waterfowl refuges (Walker 1968). Red Rock Lakes National Wildlife Refuge in Montana was established in 1935 (Hansen 1973) and is dedicated to the preservation of this swan. Today a stable population of about 300 occupies the Refuge, breeding and wintering there, moving only to open water nearby.

The swans at Red Rock Lakes have served as a source of birds for transplants to other refuges. Nine national wildlife refuges have breeding colonies emanating from the transplant programme (Fjetland 1974). All transplanted birds are essentially non-migratory.

In western Alberta, Canada, another small but stable population breeds. These swans, centred in the vicinity of Grande Prairie, Alberta, winter with birds from Red Rock Lakes (Hansen 1973), but limited neck-banding studies have not indicated an exchange of individuals between these populations (R Shea pers comm).

The existence of a previously unknown breeding population was reported by Monson (1956) in the lower Copper River Basin, Alaska. The discovery of this population of about 3000 swans (Hansen *et al* 1971) boosted the species' population status and resulted in its removal from the endangered species list in 1968 (Fjetland 1974).

Based on extensive analysis of the Red Rock Lakes and Alaskan populations, Hansen (1973) suggested that these two isolated populations may be distinct subspecies. Furthermore, he discouraged manipulation of the separate populations until the relationship was clarified.

We have used electrophoresis to study the protein differences between these populations and the Alberta population to determine if there may be a genetic basis for subspecies classifications.

Electrophoresis allows proteins to be screened for changes in amino acid composition. These changes can be extrapolated to the entire genome for comparative population genetics. In the case of *C. c. buccinator,* three populations can be identified with no known genetic exchange among them.

Blood samples from members of all three populations were collected, from both cygnets and adults. Enzymatic proteins from erythrocytes and plasma, and non-enzymatic plasma proteins have been identified by electrophoresis.

Materials and methods

Adult swans were captured on the water during the summer moult with airboats or motorboats. Cygnets were caught in the fall before flight. Three to five ml of blood were drawn from the tarsal vein with a 22 gauge needle on a heparin treated syringe. The blood was transferred to a 5 ml evacuated tube containing either heparin or sodium citrate and then placed on ice. At the end of the day's collecting, the samples were centrifuged to separate the cells from the plasma. Plasma was frozen immediately and stored at -60^{o}C. Erythrocytes were washed once in 0.85% sodium chloride and then frozen in one-two volumes of a glycerol-citrate solution, pH 8.4 (VandeBerg and Johnston 1977). Some lysis of cells and decrease of enzyme activity does occur with repeated freezing and thawing but there is little change with undisturbed storage.

Starch gel electrophoresis was performed after Selander *et al* (1971) using 12.5% (w/v) hydrolyzed potato starch cooked in an appropriate buffer. The buffer systems used for electrophoresis were those previously published (Selander *et al* 1971; Clayton and Tretiak 1972; Nelson *et al* 1977). Samples were thawed and applied to the gel on a wick of no 3 Whatman filter paper. Electrophoresis was stopped when the marker dye (bromphenol blue) had migrated to the anodal end of the gel. This took 3 to 5 hours depending on the buffer system used. The gel was then sliced and the individual slices stained with a histochemical stain specific for a particular enzyme or with the general protein stain amido-black (Allendorf *et al* 1977). Slices were incubated in the stain at room temperature in the dark overnight, then fixed in methanol-water-acetic acid (5:5:1) to be scored and photographed later. Many slices were wrapped in plastic film and stored in the refrigerator.

Results and discussion

Erythrocyte enzymes

Malate dehydrogenase (MDH 1.1.1.37). Both cytoplasmic and mitochondrial forms of the enzyme are detectable. The cytoplasmic enzyme appears as a single band anodal to the origin. All birds were presumed homozygous except for a single individual from the Alaskan population which showed three distinct bands, presumably the phenotype of a heterozygote with a rare allele. This phenotype suggests a dimeric form of the enzyme (Kitto and Wilson 1966). The mitochondrial MDH migrates cathodally and is often pale but disappears altogether when samples are centrifuged a second time at 10 000 x g for 20 minutes. One sample consistently showed two bands for the mitochondrial enzyme which is considered to represent a heterozygote, without an active heterodimer.

Lactate dehydrogenase (LDH 1.1.1.27). The five banded pattern typical of vertebrates produced by the random association of four subunits from two loci

(Markert 1975) can be seen in all samples without variation.

Peptidase (PEP 3.4.11). Three bands of peptidase activity are seen after staining of starch gels of erythrocytes. Each band presumably represents the product of a separate locus (Harris and Hopkinson 1976). No consistent variation was observed in any of these peptidases.

Esterase (EST 3.1.1.1). This enzyme has a five banded pattern which is the same for plasma and erythrocyte samples. Esterases are a broad class of enzymes whose function *in vivo* is unclear (Shaw 1965). The staining intensity of esterase from individuals is highly variable. The sample from a single swan from the Alaskan population showed a consistently faster migrating esterase considered to be due to a rare allele at one locus.

Glucose-6-phosphate dehydrogenase (1.1.1.49). One invariant band of G-6-PD activity was seen in the swan samples. Without variation, the subunit structure of the swan enzyme cannot be determined.

Haemoglobin. Electrophoresis of samples shows a major band and a slower streaked minor haemoglobin. Examination of chicken haemoglobin shows that the major (80%) and minor haemoglobins interact with each other and may have one polypeptide chain in common (Manwell *et al* 1966). Thus three monomorphic loci were scored.

Plasma proteins

The conventions established by Baker *et al* (1966) and Baker and Hanson (1966) for the nomenclature of avian serum proteins was adopted for swan plasma proteins.

Albumin. The dominant plasma protein is albumin which migrates anodally ahead of the other plasma proteins except for a slightly faster migrating prealbumin protein. No variation has been seen in the large heavy band that is albumin but dilution of plasma samples with water (1:1) reveals two bands without unveiling any variation.

Carotinoid-binding protein. This protein is described by Baker *et al* (1966) as a protein which migrates in the region behind albumin. As in *Phasianus colchicus,* an occasional swan has a carotinoid-binding zone considerably cathodal to the normal position. The differences could be due either to the protein or the carotinoid. Samples with two bands may be presumed to be heterozygotes, which suggests that the variation is genetic.

Transferrin. Transferrin is an iron-binding protein with a sialic acid side chain (Canham and Cameron 1972). Two phenotypes are seen in swan plasma, the homozygote shows two bands, apparently differing in the carbohydrate portion of the

molecule. Heterozygotes are seen with four bands. The homozygote for the rare allele was never observed.

Esterase (EST 3.1.1.1). Plasma esterases show the same pattern and migration distance as erythrocyte esterases but staining is much darker and appears almost immediately after application of the staining mixture, whereas the erythrocyte esterase is slow to appear. The plasma and erythrocyte enzymes are likely products of the same loci.

Population comparisons

The allele frequencies for 17 presumptive genetic loci can be compared for the three major populations of *C. c. buccinator* (Table 1). All populations share a

Table 1. Allele frequencies of presumptive loci in three populations of *Cygnus cygnus buccinator.*

Number of birds sampled in brackets.

		ALASKA	GRANDE PRAIRIE	RED ROCK
RBC				
	s-MDH	0.988 (43)	1.000 (26)	1.000 (122)
		0.014		
	m-MDH	0.981 (26)	1.000 (26)	1.000 (68)
		0.089		
	LDH-1	1.000 (41)	1.000 (26)	1.000 (115)
	LDH-2	1.000 (41)	1.000 (26)	1.000 (115)
	PEP-1	1.000 (40)	1.000 (26)	1.000 (107)
	PEP-2	1.000 (40)	1.000 (26)	1.000 (107)
	PEP-3	1.000 (40)	1.000 (26)	1.000 (107)
	Est-1	0.777 (43)	1.000 (26)	1.000 (128)
	Est-2	1.000 (43)	1.000 (26)	1.000 (128)
	G-6-PD	1.000 (43)	1.000 (26)	1.000 (94)
	Hb-1	1.000 (43)	1.000 (26)	1.000 (128)
	Hb-2	1.000 (43)	1.000 (26)	1.000 (128)
	Hb-3	1.000 (43)	1.000 (26)	1.000 (128)
PLASMA				
	Car	0.965 (43)	0.897 (58)	0.960 (124)
		0.035	0.103	0.040
	Tf	0.942 (43)	1.948 (58)	0.949 (128)
		0.058	0.052	0.052
	Alb	1.000 (43)	1.000 (58)	1.000 (128)
	Pre alb	1.000 (41)	1.000 (58)	1.000 (23)

common allele for each locus. Polymorphic loci also share the rare allele in all populations where the polymorphism occurs. In those instances where a locus is fixed for one allele, that same allele is either fixed or predominant in the other two populations. This preliminary screening indicates that there is probably very little genetic difference among the three populations analysed. The apparent size differences between the Alaskan and Red Rock Lakes swans (Hansen 1973) may reflect habitat rather than genetic differences. Alternatively, genetic differences may exist that have not been revealed by the limited number of proteins studied to date. The genetic data are even less complete for comparisons between the Alberta and the Red Rock Lakes populations. If the isolation of these two populations is real, sufficient genetic differences may not have arisen by drift or mutation to date to be identified by these techniques.

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Summary

Seventeen presumptive genetic loci have been examined in *Cygnus cygnus buccinator* to determine if the isolation of the three major populations, Alaska, Grande Prairie, Alberta and Red Rock Lakes, has resulted in genetic changes. No major differences in allele frequencies have been found among these populations.

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WEIGHTS OF *CYGNUS COLUMBIANUS COLUMBIANUS* AS AN INDICATOR OF CHANGING RESOURCES

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Review of data

The weights of 2368 *Cygnus columbianus columbianus,* processed and banded between 1967 and 1978, have been analysed. One hundred and two birds, all adults, were captured moulting on their Alaskan breeding grounds. The rest were adults and juveniles (first winter) trapped on their eastern wintering grounds, mostly at Mattamuskeet and Pungo National Wildlife Refuges, North Carolina (1308) and in Maryland (946). The mean weight for all adults was 6.7 kg (males averaging 7.1 kg, females 6.3 kg). The mean weight for all juveniles (first winter) was 5.8 kg with males averaging 6.0 kg and females 5.5 kg (Table 1).