COMPARATIVE GENETICS OF THREE TRUMPETER SWAN POPULATIONS

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ABSTRACT.—Proteins from 19 presumptive genetic loci in three populations of Trumpeter Swans (*Olor buccinator*) were compared by starch-gel electrophoresis. No significant differences were found among the populations in terms of mean heterozygosity or rare alleles. Trumpeter Swans have a low level of genetic variability when compared to other vertebrate taxa. *Received 18 September 1980, accepted 26 May 1981.*

THE historical range of Trumpeter Swans (*Olor buccinator*) extended across most of the North American continent from Alaska south 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 Trumpeter Swan habitat to the extent that the species was considered nearly extinct early in this century. Today trumpeter swans survive in three populations: a large Alaskan population of about 3,000 birds and two remenant populations in the interior of the continent, one localized around Red Rock Lakes National Wildlife Refuge in Montana and one in the vicinity of Grande Prairie, Alberta, Canada.

The Red Rock Lakes population was founded by birds that were endemic to the area (Hansen 1973) and that probably survived because of the relative isolation of the mountainous region. This population expanded rapidly from 69 birds in 1932 to a stable population of about 300 birds by the mid-1950's (Hansen 1973, Page 1976). As a result of a transplant program from the Red Rock Lakes refuge, nine other wildlife refuges in the United States now have breeding colonies of Trumpeter Swans (Fjetland 1974). Migration of Red Rock Lakes and transplanted birds is sharply curtailed: movement is limited to nearby open water during winter.

The Canadian swan population, which numbers about 200, breeds near Grande Prairie, Alberta and migrates south to share wintering grounds with swans from Red Rock Lakes. Swans, which are monogamous, form pair bonds at the wintering grounds, as do most waterfowl, but limited banding studies have failed to show an exchange of individuals between these two populations (R. Shea pers. comm.).

The third population of Trumpeter Swans breeds in Alaska and migrates south to winter along the coast of British Columbia and Washington. The great distance and imposing mountains separating them probably prohibit movement between the Alaskan population and the two interior populations.

Hansen (1973) observed physical differences between the Red Rock Lakes swans and the Alaskan swans, the primary one being that the Alaskan birds were larger throughout development, beginning with the egg. He suggested that these may be the result of genetic differences and recommended that managers refrain from manipulation of the populations until the basis of these physical differences was examined. To obtain information about the genetic basis of such differences, three populations were compared by electrophoretic examination of plasma and erythrocyte protein products of 19 presumptive gene loci.

MATERIALS AND METHODS

Swans were captured on the water from a floatplane, motorboat, or airboat during the summer molt of adults and prior to the autumn flight of cygnets, in 1979. We drew 3–5 ml of blood from the tarsal vein using a 22-gauge, 1½-inch needle on a heparinized syringe. The sample was immediately transferred to a 5-ml evacuated container (Vacutainer, Becton-Dickinson) with either sodium citrate or heparin as the anticoagulant, and the tube placed on ice. The birds were also weighed and sexed, and the length of the bill from the anterior edge of the nares to the tip of the nail was measured. Alaskan and Grande Prairie swans were neck- and leg-collared. Unbanded Red Rock Lake swans were leg-banded

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TABLE 1. Proteins analyzed by starch-gel electrophoresis in Trumpeter Swan populations.

Proteins	Abbreviation	E.C. number	Loci	Number of birds[a]
Erythrocyte proteins				
Hemoglobin	HB		Hb 1, 2, 3	43/26/128
Malate dehydrogenase	MDH	1.1.1.37	Mdh	43/26/128
Malate dehydrogenase	MDHm	1.1.1.37	Mdhm	26/26/68
Lactate dehydrogenase	LDH	1.1.1.27	Ldh 1, 2	41/26/115
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	G6pd	38/26/94
Phosphoglucose isomerase	PGI	5.3.1.9	Pgi	39/26/117
Phosphogluconate dehydrogenase	PGD	1.1.1.44	Pgd	42/26/82
Peptidase	PEP	3.4.11	Pep 1, 2, 3	40/26/107
Esterases	EST	3.1.1.1	Es 1, 2	43/26/128
Plasma proteins				
Prealbumin	PAB		Pab	41/58/123
Albumin	AB		Ab	43/58/128
Carotinoid binding	CAR		Car	43/58/124
Transferin	TF		Tf	43/58/128

[a] Number of bird samples examined from each population: Alaska/Grande Prairie/Red Rock.

and then sprayed with a blue dye to prevent recaptures.

At the end of the day's collecting, samples were centrifuged to separate the cells from the plasma. Cells were washed once in 0.85% sodium chloride and stored in an equal volume of glycolcitrate solution, pH 8.4 (VandeBerg and Johnston 1977). All samples were frozen and stored at −50°C for several weeks to 8 months. Horizontal starch-gel electrophoresis was performed after Selander et al. (1971) with staining procedures as described by Allendorf et al. (1977). Buffer systems for electrophoresis were adopted from Poulik (1957), Ridgeway et al. (1970), Selander et al. (1971), Clayton and Tretiak (1972), or Nelson et al. (1977). The proteins surveyed, with the presumed number of loci, and the number of birds examined at each locus are listed in Table 1.

RESULTS

MORPHOLOGICAL MEASUREMENTS

Hansen (1973) observed a size difference between Alaskan Trumpeter Swans and the swans of Red Rock Lakes. A comparison of the weight and bill length of adult swans in this study, however, shows no significant difference in the size of the birds of these two populations (Table 2). Measurements of Grande Prairie swans are not included in this table, because all but one of the birds captured at this site were cygnets.

ELECTROPHORESIS

The protein staining patterns observed on the starch gels after electrophoresis will be briefly described to present the rationale for the number of loci involved. The gel pattern will depend on the subunit structure and the physical and chemical properties of the protein molecule.

Erythrocyte proteins.—All Trumpeter Swan samples surveyed were monomorphic for the same form(s) of G6PD, LDH, PGI and PGD.

HB: Adult and juvenile Trumpeter Swan hemoglobins show a major band and a diffuse, minor band, with no variation. Examination of chicken (*Gallus gallus*) hemoglobin shows that the major (80%) and minor hemoglobins

TABLE 2. Morphological measurements of adult Trumpeter Swans.[a]

Site	Mean weight (kg ± SE)			Average bill length (mm ± SE)		
	Male	Female	Male and female	Male	Female	Male and female
Alaska	11.8 ± 0.24 (n = 7)	10.2 ± 0.17 (n = 15)	10.7 ± 0.21 (n = 22)	54 ± 0.97 (n = 7)	51 ± 1.31 (n = 8)	52 ± 0.85 (n = 15)
Red Rock	11.4 ± 0.14 (n = 27)	10.3 ± 0.18 (n = 47)	10.7 ± 0.14 (n = 74)	54 ± 0.38 (n = 27)	50 ± 0.53 (n = 47)	52 ± 0.29 (n = 74)

[a] n = number of birds measured.

TABLE 3. Mean heterozygosity and sampling variance (Nei and Roychoudhury 1975).

Locus	Alaska	Grande Prairie	Red Rock
Car	$h = 3/43 = 0.070$	$h = 6/58 = 0.103$	$h = 2/124 = 0.016$
Tf	$h = 5/43 = 0.115$	$h = 6/58 = 0.103$	$h = 13/124 = 0.105$
Mdh	$h = 1/43 = 0.023$		
Mdhm	$h = 1/26 = 0.038$		
Es 1	$h = 1/43 = 0.023$		
	$\bar{H} = 0.013$	$\bar{H} = 0.010$	$\bar{H} = 0.006$
	$V(\bar{H}) = 4.48 \times 10^{-5}$	$V(\bar{H}) = 5.03 \times 10^{-5}$	$V(\bar{H}) = 2.78 \times 10^{-5}$

have different physical properties (Huisman et al. 1964) and share one polypeptide chain (Manwell et al. 1966). On this basis we assume Trumpeter Swan hemoglobins to be the products of three loci.

MDH: Both MDH enzymes are dimeric in man (Harris and Hopkinson 1976) as well as in many reptiles and birds (Karig and Wilson 1971) and are presumed to be coded for by two separate autosomal genes (Kitto and Wilson 1966). MDH is very conservative as measured by electrophoretic mobility, because it shows little interspecific variation and still less intraspecific variation among 100 species of birds examined by Kitto and Wilson (1966). Typically, the Trumpeter Swan samples displayed a single band for both MDH and MDHm. One heterozygous three-banded phenotype for MDH and one two-banded phenotype for MDHm were observed. The two-banded MDHm phenotype is not easily explained, unless it is the product of a heterozygote with no active heterodimer formation or, alternatively, there is an active heterodimer that is the product of a null activity allele and the normal allele.

PEP: Three distinct invariable zones could be resolved on starch gels, at pH 6, and are assumed to represent three separate loci. Substitution of leucyl-alanine by two other substrates (alanyl-glycine, leucyl-naphthylamide) produced the same gel pattern and failed to reveal any other peptidases.

EST: The swan esterases are represented on starch gels as five bands with staining intensity varying among individuals and are the same in plasma and erythrocytes. A similar five-band complex observed for the esterases of the cricket frog (*Acris* sp.) was postulated to represent a tetrameric molecule composed of subunits from two loci (Dessaur and Nevo 1969). A single swan from the Alaskan population showed a consistently faster migrating band at the cathodal side of the zone and was considered to be homozygous for a rare allele at one locus. The close apposition of esterase bands might make the heterozygote difficult to identify; thus, the heterozygosity at this locus may be underestimated.

Plasma proteins.—The PAB and AB proteins of Trumpeter Swans show identical electrophoretic mobility for all samples.

CAR: A diffuse protein band, which stains in the post-albumin region of the gel, has been identified as a carotinoid-binding protein, because it has been observed as a yellow pigmented region on the unstained gel due to combination with xanthophyll (Baker et al. 1966, Bush 1967). This protein may also be associated with lipid (Common et al. 1953).

Baker et al. (1966) found that serum from an occasional pheasant had a carotinoid-binding zone considerably cathodal to the normal position on the gel. This condition was also observed in several Trumpeter Swans. The difference may be due to the carotinoid or to the protein portion of the molecule. In Trumpeter Swans we have observed two bands in this region. We suggest a genetic basis for this variation, with two-banded patterns representing the heterozygous state and an individual with a single slow band representing the alternative homozygote.

Tf: Tfs of Trumpeter Swans show two phenotypes. The presumed homozygotes have two bands, while heterozygotes show four (Baker and Hansen 1966). The homozygote for the rare allele was never observed.

HETEROZYGOSITY AND GENETIC DISTANCE

All populations share a common allele for all loci surveyed. The heterozygosity (h) per locus and the mean heterozygosity ($\bar{H}$) for each population are shown in Table 3.

The genetic distance (Nei 1972, 1975) calculated pairwise among the three populations was found to be an identical 0.001. This illus-

TABLE 4. Genetic distances between different taxonomic levels (Barrowclough and Corbin 1978).

Taxa	Local populations	Subspecies	Semispecies	Sibling species	Species	Genera
Drosophila	0.028	0.230	0.226	0.740	1.056	—
Sunfish	0.020	0.174	—	—	0.616	—
Minnows	0.031	—	—	—	—	0.528
Salamanders	0.051	0.174	—	—	0.462	1.170
Mammals[a]	0.056	0.219	—	—	0.302	0.651
Birds						
Aplonis	0.003	0.007	—	0.035	—	—
Zonotrichia	0.004	0.009	—	—	—	—
Icteridae	0.002	—	0.003	0.012	—	0.248
Parulidae	—	—	—	—	0.100	0.179

[a] Rogers index of genetic distance.

trates the close genetic relationship among the three populations.

DISCUSSION

The differences in morphological measurements discussed by Hansen (1973) are not supported by our results (Table 2). We found very little published data about the size of adult Red Rock Lake Swans. Banko (1960) gives minimum weight and bill-length data, but these are from an average of 8 male and 14 female "small" Trumpeters for a comparison between Trumpeter and Whistling (*Olor columbianus*) swans. Banko (1960) also reports one measurement of an adult male Red Rock Trumpeter that is slightly larger than comparable measurements for a male from the Kenai peninsula in Alaska. While a difference in egg size has been reported (Hansen et al. 1971, Banko 1960), there is apparently no basis for the statement that this size difference is maintained into the adult (Table 2). We suspect that the data reported by Banko (1960) on "small" Trumpeters were taken as representative of the entire population.

There were no significant differences in $\bar{H}$ among the three populations of Trumpeter Swans. However, the presence of alternate alleles, albeit at low frequency, at the Mdh, Mdhm, and Es-1 loci in the Alaskan population but not in the other two populations may indicate that some unique genetic variability exists in the Alaskan population. Heterozygosity was low in all populations, a trait not easily attributed to bottleneck phenomena (Bonnell and Selander 1974, Cameron and Vyse 1978) because neither the Alaskan nor Grande Prairie populations are known to have undergone severe population reductions.

The estimated $\bar{H}$ value of 0.01 for Trumpeter Swan populations is low relative to other vertebrates but within the range of $\bar{H}$ values found in other avian species (Tables 3, 4). The overall $\bar{H}$ compiled by Barrowclough and Corbin (1978) from all avian genetic surveys in the literature is 0.043 ± 0.005, which is at the low end of the range of $\bar{H}$ values for the other vertebrates and less than for invertebrates. This low level of genetic variability in birds was also reported by Baker and Fox (1978) and Powell (1975). Baker and Fox (1978) and Bock (1963, 1969) have attributed these low $\bar{H}$ values to selection imposed by flight morphology and/or the precision of homeothermy required for brooding. Because mammals are also homeothermic, however, this argument is unconvincing.

Alternatively, low $\bar{H}$ values may result from low mutation rates or small effective population sizes. Neutral allele arguments (Kimura and Crow 1964) may hold, but neither mutation rates nor pertinent population parameters have been measured in birds, so the low $\bar{H}$ values remain enigmatic.

In the course of this study, samples from 11 Whistling Swans, 7 Whistling-Trumpeter hybrids and one Mute Swan (*Cygnus olor*) were either collected or donated by Dr. W. Sladen. A comparison of these samples with the Trumpeter Swan samples revealed no difference between Trumpeter Swans and either the Whistling Swans or the Trumpeter-Whistling hybrids but did show electromorph variation for esterase and G6PD between the single Mute Swan and the Trumpeters. These findings support a close taxonomic relationship between Trumpeter and Whistling swans.

Compared with mammals, salamanders, and

fishes, local populations of birds are nearly an order of magnitude more similar to each other (Corbin 1977), and avian subspecies are about one hundred times more similar to each other than are subspecies of other animals (Table 4). This makes genera of birds as close genetically as subspecies of other vertebrates (Corbin 1977) or sibling species of *Drosophila* (Barrowclough and Corbin 1978). Trumpeter Swan populations are virtually identical based on the index of genetic distance.

The genetic identity data suggest that for management purposes the origin of individuals used to repopulate areas after local extinction is not critical (Corbin 1977). Consideration of ecological resemblance of the transplant area to the source area may be a more crucial factor, especially in the case of Trumpeter Swans, which are apparently very susceptible to alterations in habitat (Hansen 1973). Hansen (1973) suggested that the Grande Prairie population would be the best source of birds for stocking the interior of the U.S. We agree that ecologically it might be the best source but have no genetic reasons to favor the Grande Prairie populations over the other two populations.

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