Analysis of Mitochondrial DNA of Pacific Black Brant (*Branta bernicla nigricans*)

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Brant (*Branta bernicla*) have become the object of increased concern to managers of waterfowl populations. The numbers of wintering Brant have decreased markedly from a peak of 55,000 wintering birds in the United States in 1958 to approximately 5,000 in 1972 (Management Plan for Pacific Coast Brant 1981). Corresponding reductions in the numbers of breeding Brant in Alaska have also occurred (Lensink 1987). The numbers of wintering birds in traditional Brant areas in British Columbia, Washington, Oregon, and California have declined during periods when the numbers of wintering birds in Mexico have increased. Together, these observations imply that a variety of factors may influence Brant numbers in any locale throughout the year. Reductions in the number of Brant that traditionally winter in Canada and the western United States may simply be associated with habitat deterioration or disturbance, which forced the birds to winter farther south in Mexico.

Reductions in the sizes of breeding populations may be accompanied by a corresponding reduction in genetic diversity. Accordingly, we studied the mitochondrial DNA (mtDNA) of individuals from five separate locations across the breeding range of *B. bernicla*. We included four "gray-bellied" birds from an apparent distinct breeding population on Melville Island of the Canadian Arctic. Local populations characterized by unique mtDNA may suggest an older radiation followed by reductions in the numbers of individuals. Alternatively, homogeneity among mtDNA may indicate that the Brant have recently expanded their range dramatically or that the mutation rate in Brant mtDNA is low.

Because of its simplicity, haploid composition, lack of recombination, transmission through the maternal germ-line, and rapid rate of evolution, mtDNA has been useful in resolving questions about population dynamics and in making predictions about phylogenies (Wilson et al. 1985, Avise 1986). Analysis of mtDNA has been important to biologists who study waterfowl (Shields and Wilson 1987b, Van Wagner 1987) because unlike most other groups of vertebrates, female geese and ducks generally establish breeding sites near their natal sites and return to them year after year to reproduce (Greenwood and Harvey 1982, Rohwer and Anderson 1988). Thus, fidelity to natal sites and transmission of mtDNA through the germ-lines of these females provides a molecular record of the patterns of their dispersal and phylogenetic relationships. Moreover, effective rates of mitochondrial gene flow among populations should be approximately one quarter of those of nuclear genes (Birky et al. 1983), and historical reductions in the sizes of populations as well as founder events should be revealed more clearly by analysis of mtDNA than by analysis of nuclear DNA, which is diploid (Wilson et al. 1985). Finally, given the assumption that mtDNA approximates a molecular clock marking time via successive mutations occurring at somewhat regular intervals, one can use divergence values between DNAs to approximate both the extent and rate of separation from a common ancestor.

Nineteen individuals were available from five sites (Fig. 1). We purified mtDNA from the kidneys and spleens of all birds. Tissues were processed fresh, except for birds from Melville Island, which were banded on Melville as young of the year and then collected at Padilla Bay, Washington, in winter. Their kidneys were preserved in mannitol/sucrose/EDTA buffer on wet ice and sent to Fairbanks where mtDNA was purified upon receipt. Mitochondrial DNA of birds from the Yukon-Kuskokwim Delta, Anderson River, and Victoria Island was purified according to the methods of Lansman et al. (1981) and Cann (1982). We purified mtDNAs of samples from the North Slope of Alaska and from Melville Island according to the more efficient methods of Carr and Griffith (1987). We used *Ava*I, *Eco*RI, *Hinc*II, *Hin*dIII, *Hpa*I, *Pvu*II, *Ava*II, *Bst*UI, *Hha*I, *Hin*fI, and *Hpa*II to monitor DNA sequence variability in all birds of this study. Mitochondrial DNAs of the birds from the Yukon-Kuskokwim Delta, Anderson River, and Victoria Island were also digested with *Dde*I and *Rsa*I. Mitochondrial DNAs of birds from the North Slope of Alaska and Melville Island were also digested with *Bam*HI, *Ban*I, *Bgl*II, *Cla*I, *Nar*I, *Nci*I, *Nco*I, *Spe*I, and *Sty*I. Fragments of mtDNA were end-labeled with radioactive phosphorous, and their sizes were compared electrophoretically using either agarose (1.0–2.0%) or 5% polyacrylamide on long vertical gels. We used *Hin*dIII to digest DNA from phage lambda into fragments of known length that were then used as size standards on each gel. Statistics of homology from which the percentages of nucleotide sequence divergence were calculated were from Nei and Li (1979).

On average, 88 fragments were analyzed for each of the 19 individuals. This level of analysis is typical of comparisons of this type for birds (Ball et al. 1988, Shields and Helm-Bychowski 1988). All Brants studied fell into either of two types based on their mtDNAs. Birds from the Yukon-Kuskokwim Delta, North Slope of Alaska, Anderson River of the Yukon Territory, and Victoria Island had essentially identical mtDNAs. In birds from the Anderson River, one of the three

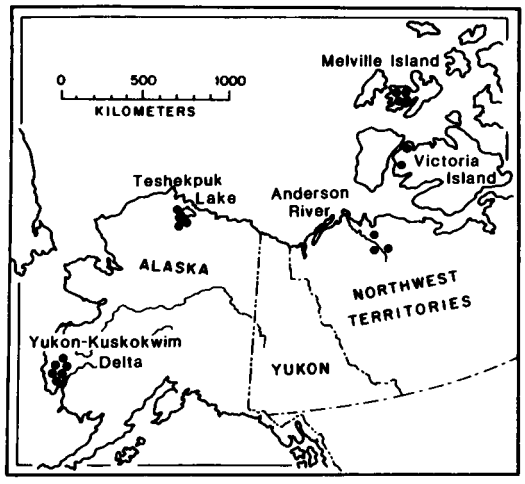

Fig. 1. Collection locations for Brant of this study.

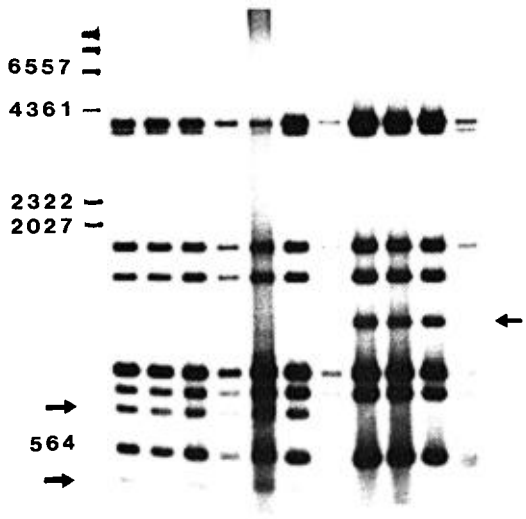

Fig. 2. Autoradiograph of Brant DNA digested with the restriction enzyme *Bst*UI. Numbers on the left refer to the length in nucleotides of lambda phage DNA digested with the enzyme *Hin*dIII and used as a size standard. Sample lanes: 1–4, North Slope; 5, Anderson River; 6–7 Victoria Island; and 8–11, Melville Island.

individuals lacked an *Rsa*I fragment of approximately 1,250 base pairs but possessed a unique fragment of approximately 1,200 base pairs, which all other individuals lacked.

The birds from Melville Island were distinct from all others of this study. These birds had unique fragment patterns for 7 (*Bst*UI, *Ava*II, *Pvu*II, *Ban*I, *Nco*I, *Sty*I, and *Nar*I) of the 20 restriction enzymes used to study them. Accordingly, they were 0.74% divergent from the birds at all other sites. The restriction fragment patterns for mtDNAs digested with the enzyme *Bst*UI emphasize the uniqueness of the Melville Island birds (Fig. 2). The four Melville birds (Fig. 2) possess a fragment of approximately 1,200 nucleotides in length (right arrow) which is replaced in all other Brant by two fragments of approximately 750 and 460 nucleotides (left arrows). Within the Melville birds, one individual (No. 825) differed from others in fragment patterns for two enzymes (Table 1).

The homogeneity of mtDNAs of Brant from the Yukon-Kuskokwim Delta, North Slope of Alaska, Anderson River, and Victoria Island could be caused either by a very recent radiation and movement into previously unoccupied territory, or by a decelerated rate of change in Brant mtDNA relative to other vertebrates. Decelerated rates seem unlikely because the rate of mtDNA evolution in geese (*Branta, Chen, Anser*) is ca. 2.0% per million years (Shields and Wilson 1987a), similar to most other vertebrates that have been studied in this way (Wilson et al. 1985).

Female Brants exhibit strong philopatry to their natal sites. Such reproductive behavior will eventually promote genetic differentiation among birds which are distributed as widely as Brant. However, with the exception of the Brant on Melville Island, the mtDNAs of birds of this study were essentially homogeneous. It seems likely that Brant have recently expanded their range to include the Yukon-Kuskokwim Delta, North Slope, Anderson River, and Victoria Island. The intermediacy of Melville Brant belly coloration and breeding distribution relative to *nigricans* and *hrota* might imply that Melville birds are hybrids. The 0.74% divergence in mtDNA of Melville birds from *nigricans* is significant and approximates the 0.8% divergence between the Ross Goose (*Chen rossii*) and the Snow Goose (*C. caerulescens*) (Shields and Wilson 1987a). This divergence makes a recent hybrid origin for Melville birds unlikely. The hybrid origin hypothesis can be tested in another way. Fortunately, data on restriction fragments of mtDNA of a single *hrota* are available (Van Wagner 1987). If Melville birds are recent hybrids, they should possess a mtDNA characteristic of the subspecies of female or females involved in the hybridization. Melville birds

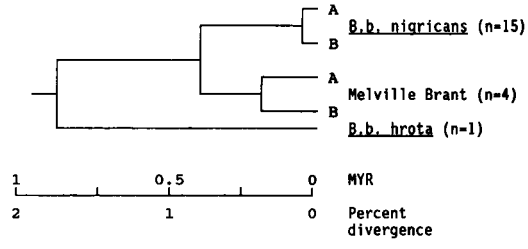

Fig. 3. Phylogenetic tree for Brant constructed by the midpoint method. We assume a 2.0% per million year rate of evolution for goose mtDNA.

TABLE 1. Sizes in base pairs of fragments generated by digestion of Brant mtDNA with five restriction endonucleases.

	Fragment size (bp)							
*Ava*II								
Atlantic	7,900	—	—	2,800	1,800	1,500	900	800
Melville	—	—	6,500	—	1,800	1,500	900	—
Pacific	—	6,800	—	2,800	1,800	1,500	—	—
*Pvu*II								
Atlantic		16,200	—	—	—	—		
Melville (A)		—	11,800	—	—	4,800		
Melville (B)		—	—	11,000	5,800	—		
Pacific		—	11,800	—	—	4,800		
*Hha*I								
Atlantic		—	3,150	1,700	—	1,500		
Melville (A)		3,900	—	1,700	1,600	1,500		
Melville (B)		3,900	—	1,700	1,600	1,500		
Pacific (A)		—	3,150	1,700	—	1,500		
Pacific (B)		—	3,150	1,700	—	1,500		
Atlantic		1,250	1,175	—	1,050			
Melville (A)		1,250	1,175	1,100	—			
Melville (B)		—	1,175	1,100	—			
Pacific (A)		—	1,175	1,100	1,050			
Pacific (B)		1,250	1,175	1,100	—			
*Bst*UI								
Atlantic		4,300	—	—	2,700	1,700	1,500	
Melville		—	3,400	3,300	—	1,700	1,500	
Pacific		—	3,400	3,300	—	1,700	1,500	
Atlantic		1,200	960	870	—	550	—	
Melville		1,200	960	870	—	550	—	
Pacific		—	960	870	750	550	460	
*Hin*dIII								
Atlantic		4,300	4,000	3,900	3,100	2,300	—	—
Melville		—	4,000	3,900	3,100	2,300	1,500	1,000
Pacific		—	4,000	3,900	3,100	2,300	1,500	1,000

share no patterns with *hrota* and only two of the five patterns with *nigricans* (Table 1). The fact that Melville Brant have unique fragment patterns for most of these enzymes argues against a recent hybridization event. If mtDNA of geese evolves at a rate of 2.0% per million years, then Melville birds have been isolated reproductively from Pacific Black Brant for approximately 400,000 years (Fig. 3).

My observations on the mtDNA of Brant are complemented by banding studies. Boyd et al. (1988: 1) stated that "It used to be thought that the Brant of North America were of just two populations wintering on the Pacific and Atlantic coasts respectively: the former were dark-bellied (*B. b. nigricans*) and the latter were pale-bellied (part of *B. b. hrota*). During the 1970s and early 1980s, ringing and colour-marking of Brant in the Queen Elizabeth Islands and Foxe Basin showed that the situation was more complex. Pale-bellied Brant breeding in the northeastern Canadian arctic were found to winter in Ireland (Maltby-Prevett et al. 1975), whereas those wintering on the US Atlantic coast originated from Southhampton Island and Foxe Basin (T. Barry, K.L. Abraham, A. Reed, unpubl.). Another relatively pale-bellied form, breeding chiefly on Melville Island, was found to winter in Puget Sound, Washington; and the majority of dark-bellied western Brant from northwestern Canada and Alaska wintered farther south on the Pacific coast, principally in Mexico (see Bellrose 1980)." Thus, it appears that Melville Brant are morphologically distinct and generally separated from other Brant on their wintering territories in Puget Sound (Boyd et al. 1988). Their mtDNA is unique. A study of a larger sample of Atlantic Brant and pale-bellied Brant that breed in the northeastern Canadian Arctic and winter in Ireland seems justified.

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Song Features Birds Use to Identify Individuals

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The ability of birds to discriminate between individuals on the basis of song has been widely demonstrated (Falls 1982). Despite its prevalence, little is known about how the birds perform this discrimination. Only two relevant experiments have been performed (Brooks and Falls 1975, Nelson 1989), both of which examined only variation within a single song type. The ability of birds to discriminate between the songs of neighbors and strangers in playback experiments decreases as the repertoire size of the species increases (Falls 1982). This suggests that a repertoire of song types is less recognizable than a single song.

Male Great Tits (*Parus major*) have an average rep-

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