AFFINITIES OF THE HAWAIIAN GOOSE BASED ON TWO TYPES OF MITOCHONDRIAL DNA DATA

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ABSTRACT.—We compared restriction fragments of mitochondrial DNA and sequences of portions of the cytochrome *b* gene of the following: the Hawaiian Goose (*Nesochen sandvicensis*), five subspecies of the Canada Goose (*Branta canadensis*), the Pacific Black Brant (*B. bernicla nigricans*), and the Emperor Goose (*Chen canagica*). Both comparisons support the association of the Hawaiian Goose and the Canada Goose as sister taxa as well as the association of all four large-bodied subspecies of Canada Geese as separate from the one small-bodied subspecies analyzed here. Bootstrap and winning-sites tests show these associations to be statistically significant. The amount of sequence divergence implies that Hawaiian and Canada Geese diverged from a common ancestor less than three million years ago and that this divergence preceded the divergence of large- and small-bodied Canada Geese only slightly, if at all. Therefore, we suggest a North American origin for the Hawaiian Goose. *Received 2 July 1990, accepted 27 December 1990.*

THE HAWAIIAN Goose (*Nesochen sandvicensis*), or Nene as it is called locally, is the only extant species of goose endemic to Hawaii, yet its ancestry and geographic origin have never been established. Within the present century, the goose subfamily Anserinae has routinely been divided into at least five genera (*Anser, Branta, Chen, Nesochen,* and *Philacte*) (Johnsgard 1978, Bellrose 1980, Palmer 1976). There is disagreement as to whether *Anser* should be split into several genera (e.g. *Anser, Chen,* and *Philacte*). Moreover, taxonomists have been equivocal regarding the allocation of the Hawaiian Goose. Some have emphasized morphological differences from the other geese by placing it in *Nesochen*. Others have emphasized obvious similarities by placing it in *Branta*. Berger (1972) has proposed that *Nesochen* is closely related to *Branta* and that it arose specifically from the Canada Goose (*Branta canadensis*) after one or more migration events from North America. This hypothesis has awaited testing with modern methods of phylogenetic analysis.

Information preserved in the DNA of this species may be used to infer its relatedness to other extant species of geese. Mitochondrial DNA (mtDNA) has been valuable for phylogenetic reconstructions because of its ease of isolation, rapid evolutionary change, maternal inheritance and apparent lack of recombination (Wilson et al. 1985). This approach has been particularly useful for studies of phylogeny of maternal lineages in waterfowl (Kessler and Avise 1984; Shields and Wilson 1987a, b; Van Wagner and Baker 1990; also see Discussion).

Both restriction and sequencing methods of characterizing mtDNA yield traits that can be subjected to parsimony analysis (e.g. Edwards and Wilson 1990), allowing branching orders to be tested without strict assumptions of clock-like rates of change. We compared restriction fragments of whole mtDNA and DNA sequences of portions of a specific mitochondrial gene of the Hawaiian Goose with those of other geese. Geographic considerations influenced our choice of the other geese. Because our intent was to determine the sister taxon of the Hawaiian Goose and to estimate when and where it may have arisen, we centered attention on those geese that inhabit western North America, the Aleutian Islands, and northeastern Asia, and that had not already been studied by Shields and Wilson (1987a, b).

MATERIALS AND METHODS

We obtained approximately 20 g of heart tissue from an adult Hawaiian Goose that had died at the Honolulu Zoo. The tissue was immediately minced and maintained in cold (4°C) buffer (mannitol, sucrose, Tris-EDTA) during subsequent transport to Fairbanks. The combined methods of Shields and Wilson (1987a) and Carr and Griffith (1987) were used to obtain purified mtDNA from this tissue and from hearts and kidneys of a single small-bodied Canada Goose (Taverner's Canada Goose, *Branta canadensis taverneri*); four large-bodied Canada Geese (the Lesser Canada Goose, *B. c. parvipes*; the Dusky Canada Goose, *B. c. occidentalis*; the Vancouver Canada Goose, *B. c. fulva*; the Western Canada Goose, *B. c. moffitti*); the Pacific Black Brant, *B. bernicla nigricans*; and the Emperor Goose, *Chen canagica* (also known as *Philacte canagica*). We

TABLE 1. Fragment patterns of mtDNAs from eight taxa of geese: *Nesochen sandvicensis, Branta canadensis taverneri, B. c. parvipes, B. c. occidentalis, B. c. fulva, B. c. moffitti, B. bernicla,* and *Chen canagica.*

Restriction enzyme	Fragment patterns[a]							
	N. sand.	*B. c. tav.*	*B. c. par.*	*B. c. occ.*	*B. c. ful.*	*B. c. moff.*	*B. bernicla*	*C. canagica*
*Ava*I	A	A	A	A	A	A	B	C
*Ava*II	A	A	A	A	A	A	B	C
*Bam*HI	A	A	A	A	A	A	A	B
*Ban*I	A	B	C/D/E	C	C	C	F	G
*Bgl*II	A	A	A	A	A	A	B	A
*Bst*UI	A	B	C	C	D	C	E	F
*Cla*I	A	A	A	A	A	A	B	C
*Eco*RI	A	A	A	A	A	A	B	C
*Hha*I	A	B	A	A	A	A	C	D
*Hin*cII	A	A	A	A	A	A	B	C
*Hin*dIII	A	B	A	A	A	A	C	A
*Hin*fI	A	B	B	B	B	B	C	D
*Nar*I	A	A	A/B	B	B	B	C	D
*Nci*I	A	A	A	A	A	A	B	C
*Nco*I	A	B	B	B	B	B	C	D
*Pvu*II	A	B	A	A	A	A	C	D
*Spe*I	A	B	B	B	B	B	C	D
*Sty*I	A	B	C	C	C	C	D	E
*Xba*I	A	A	B	B	B	B	A	C

[a] A complete list of all fragment sizes is available upon request.

were not concerned about larger sample sizes because intrasubspecific variation in Canada Geese and Brant is extremely low (Shields and Wilson 1987b; Shields 1990; Van Wagner and Baker 1990). Moreover, the mtDNA diversity among Hawaiian Geese must have been reduced drastically when its population crashed to only 17 individuals in the 1940s (Kear and Berger 1980). While two other extant geese of the genus *Branta* (the Barnacle Goose, *B. leucopsis,* and the Red-breasted Goose, *B. ruficollis*) could have been included in this study, they are poor candidates as ancestors of the Hawaiian Goose because their breeding ranges (northern Atlantic and Eurasia, respectively) and past distributions (never in eastern Asia) make it unlikely that they could have flown to Hawaii.

RESTRICTION FRAGMENT ANALYSIS

We compared fragment patterns of mtDNA of the Hawaiian Goose (Table 1) to those of the other taxa of geese in several ways. We first compared restriction fragments of mtDNA from the Hawaiian Goose in the same gels with those of the geese identified above. Then we compared these fragment patterns with those

TABLE 2. Extent of sequence difference (percent) for taxa of geese based on restriction fragment analysis of mtDNA.[a] Species: *Nesochen sandvicensis, Branta canadensis* ssp., *Branta bernicla,* and *Chen canagica.*

	1	2	3	4	5	6	7	8	9	10	11
1. *N. sandvicensis*	—	1.44[d]	1.44[d]	1.44	1.60	1.64	1.64	1.64	1.64	5.85	4.87
2. *B. c. leucopareia*		—	0.06[c]	0.11[c]	1.28[d]	1.31[d]	1.27	1.00[c]	1.30[c]	6.14	5.77
3. *B. c. minima*			—	0.05[c]	1.28[d]	1.31[d]	1.27	0.80[c]	1.10[c]	6.14	5.77
4. *B. c. taverneri*				—	1.28	1.31	1.27	0.90	1.12[c]	6.14	5.77
5. *B. c. parvipes*					—	0.08	0.20	0.16	0.26[d]	6.02	5.54
6. *B. c. occidentalis*						—	0.14	0.17	0.26[d]	6.05	5.33
7. *B. c. fulva*							—	0.14	0.26[d]	6.11	5.32
8. *B. c. moffitti*								—	0.26[c]	6.05	5.33
9. *B. c. maxima*									—	6.10[b]	5.33
10. *B. bernicla*										—	5.99
11. *C. canagica*											—

[a] Overall these values tend to be lower than comparable estimates (Van Wagner and Baker 1990), probably because the present analysis uses mainly 6-base cutters.

[b] Value computed in Shields and Wilson (1987a).

[c] Values computed in Shields and Wilson (1987b).

[d] Values averaged.

found by Shields and Wilson (1987a, b) for the Aleutian Canada Goose (*B. c. leucopareia*), the Cackling Canada Goose (*B. c. minima*), Taverner's Canada Goose, the Western Canada Goose, the Giant Canada Goose (*B. c. maxima*), and the Pacific Black Brant.

The 19 enzymes *Ava*I, *Ava*II, *Bam*HI, *Ban*I, *Bgl*II, *Bst*UI, *Cla*I, *Eco*RI, *Hha*I, *Hin*cII, *Hin*dIII, *Hin*fI, *Nar*I, *Nci*I, *Nco*I, *Pvu*II, *Spe*I, *Sty*I and *Xba*I were used in the first series of fragment comparisons. Restriction endonuclease digestions were carried out under the conditions specified by the vendor (New England Biolabs.). Five of these enzymes (*Bst*UI, *Hha*I, *Hin*dIII, *Pvu*II, and *Xba*I) unequivocally differentiate large-bodied from small-bodied Canada Geese (Shields and Wilson 1987b), and thus they were included here as potential phylogenetically informative enzymes relative to the Hawaiian Goose.

The 22 restriction enzymes used in the second comparison are listed in Shields and Wilson (1987a, b); 12 of these coincide with enzymes used in the first comparison. Divergence values for both data sets were computed from the fraction of shared fragments according to equation 20 of Nei and Li (1979). In cases where pairwise comparisons were not possible, we used average values among small-bodied Canada Geese (first comparison) and among large-bodied Canada Geese (second comparison) to account for missing data (Table 2). For phylogenetic analysis, we inferred restriction sites from the fragment data for the taxa studied here and used the exhaustive search algorithm of the PAUP (Phylogenetic Analysis Using Parsimony) computer program (Swofford 1989). Finally, bootstrap (Felsenstein 1985) and winning-sites tests were applied to the tree arrangements to test for significance (Shields and Wilson 1987b, Prager and Wilson 1988).

Gene Amplifications and Direct Sequencing

We used the methods of Gyllensten and Erlich (1988) and Kocher et al. (1989) to amplify and sequence portions of the cytochrome *b* genes of the Hawaiian Goose, Taverner's Canada Goose, the Lesser Canada Goose, the Dusky Canada Goose, the Vancouver Canada Goose, the Western Canada Goose, the Pacific Black Brant, and the Emperor Goose. Two primer pairs were used. The first pair (L14841 and H15149) were described by Kocher et al. (1989). The second pair are L15420 (5'-ATCCCATTCCACCCATACTACTC-3') and H15915 (5'-AACTGCAGTCATCTCCGGTTTACAAGAC-3'). In each case, L and H refer to the light and heavy strands, respectively, and the numbers describe the 3' base position according to the numbering system of Anderson et al. (1981). Products of the initial double-stranded amplifications were visualized by ethidium bromide staining of 2% NuSieve agarose gels, cut from the gels, diluted (with heating) in 1 ml of distilled water, and 1 μl of each was used as a source of template for single-stranded amplifications. The

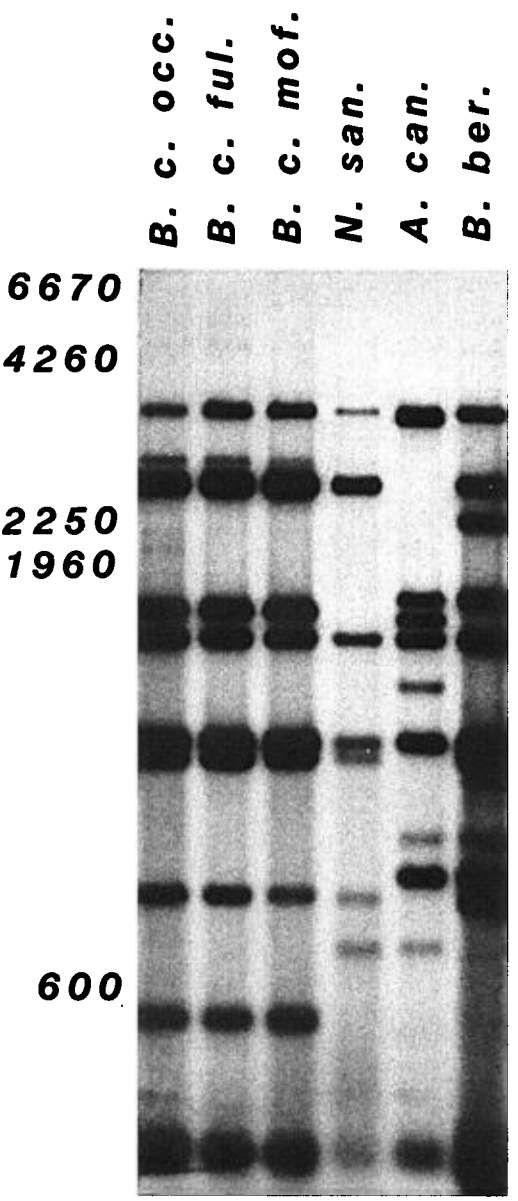

Fig. 1. Restriction fragment patterns of mtDNA (for 6 geese) digested with the restriction enzyme *Ban*I. Numbers on the left refer to the known lengths of phage lambda DNA fragments generated with the enzyme *Hin*dIII. The fragment patterns are identified in parentheses as follows: lanes 1–3 (C), lane 4 (A), lane 5 (G), lane 6 (F).

reaction conditions used to generate single-stranded template differed by having one primer reduced in concentration to 0.02 μM, increasing the total volume to 50 μl, and completing 32 cycles of the reaction. This product was then dialyzed centrifugally three times

TABLE 3. Diagnostic fragment patterns of mtDNA from eight subspecies of Canada Geese and the Nene. "Plus" symbols refer to the presence of the uncut fragment and "minus" symbols to its absence.

Enzyme	Nature of site change (fragment sizes)	Small-bodied[a]			Large-bodied[a]					
		leuc.	*min.*	*tav.*	*parv.*	*occ.*	*fulva*	*moff.*	*max.*	Nene
*Bst*UI (*Fnu*DII)	6,700 ↔ 4,000 + 2,700	+	+	+	−	−	−	−	−	+
*Hha*I	3,350 ↔ 2,700 + 650	+	+	+	−	−	−	−	−	−
*Hha*I′	1,170 ↔ 640 + 530	+	+	+	−	−	−	−	−	−
*Hin*dIII	6,000 ↔ 3,800 + 2,200	+	+	+	−	−	−	−	−	−
*Xba*I	16,500 ↔ 9,500 + 7,000	+	+	+	−	−	−	−	−	+
*Pvu*II	5,800 ↔ 4,700 + 1,100	−	−	−	+	+	+	+	+	+

[a] See Table 2 for full scientific names of subspecies.

in Centricon 30 (Amicon) or Ultrafree-mc (Millipore) tubes in a volume of 1 ml or 0.35 ml of distilled water, respectively. Approximately 50 μl of resuspended template remained in the Centricon 30 tubes, and the samples in the Ultrafree-mc tubes were resuspended in 40 μl d H_2O. We sequenced by the dideoxy method (Sanger et al. 1977) with Sequenase (United States Biochemical) kits and following the manufacturer's suggestions, starting with 7 μl of resuspended template, 2 μl reaction buffer, and 1 μl of the primer (10 μM), which was limiting in the single-stranded amplification. While some overlapping sequences were obtained from the L14841 and H15149 primers, in most cases sequences were determined in a single direction. Trees were built by the parsimony method and tested statistically as described above for restriction sites.

RESULTS

FRAGMENT ANALYSIS

Four fragment patterns were observed when mtDNAs from six geese were digested with the enzyme *Ban*I (Fig. 1). Fragment patterns observed when mtDNAs were digested with the 19 enzymes are listed (Table 1). The patterns of the Hawaiian Goose were often identical to those of Canada Geese. In only four cases (*Bam*HI, *Bgl*II, *Hin*dIII, and *Xba*I) was identity found between the Hawaiian Goose and either the Brant or the Emperor Goose. Together, the 19 enzymes produced an average of approximately 109 fragments per individual (Table 1), which means that we monitored, on average, approximately 109 cleavage sites per mtDNA. Because these sites range in size from four-base to six-base pairs, our methods surveyed approximately 4% of the mitochondrial genome. The mean genome size of the mtDNA of these geese (16.7 ± 0.3 kb) is similar to those of other birds analyzed in the same way (Quinn and White 1987, Shields and Helm-Bychowski 1988).

We estimated (Table 2) the percent nucleotide sequence difference for all taxa of this study as well as the additional subspecies of Canada Geese studied by Shields and Wilson (1987a, b). Based on these values, the Hawaiian Goose is more similar to Canada Geese (mean difference, 1.6%) than it is to either the Emperor Goose (4.9%) or to the Black Brant (5.9%). In fact, the Hawaiian Goose is only slightly more different from subspecies of Canada Geese than are the large-bodied subspecies from the small-bodied subspecies (mean difference, 1.2%).

As mentioned earlier, six restriction sites unequivocally separate large-bodied from small-bodied Canada Geese (Shields and Wilson 1987b: table 2 and Shields and Helm-Bychowski 1988: fig. 2). Hawaiian Goose mtDNA does not fall exclusively into either one or the other of these two groups (Table 3). At two of the diagnostic sites, this goose is identical to the small-bodied Canada Geese, whereas at the other four sites the opposite pattern is shown. Thus, based on sites that are phylogenetically informative for subspecies of Canada Geese, the Hawaiian Goose cannot be linked exclusively to either the small-bodied or the large-bodied types.

The most parsimonious tree based on analysis of the restriction sites links the Hawaiian Goose with the Canada Goose (Fig. 2: upper). Bootstrap analysis (Felsenstein 1985) showed that the Hawaiian Goose was always associated with the Canada Goose lineage (Fig. 2: upper).

SEQUENCES OF THE CYTOCHROME *b* GENE

We compared 612 nucleotides of portions of the cytochrome *b* genes for the eight taxa (Fig. 3). The considerable distance between primers L15420 and H15915 made it difficult to sequence across the entire interior region of the gene; hence sequence results are presented in three

parts (Fig. 3: a–c). All large-bodied Canada Geese had identical sequences across all regions. Among the codons across all taxa, there were 13 variable sites in the first position, 4 in the second position, and 69 in the third position. Changes at 16 of these sites cause amino acid replacements in at least one of the compared taxa.

Of the amino acid changes, a disproportionate number (10/14) occur in the transmembrane domains of cytochrome *b* depicted by Howell's (1989) structural model. Overall, 109/202 (54%) of the amino acids determined fall into that region. The transmembrane region is highly variable in amino acid sequence in mammals relative to most other parts of the molecule (Irwin et al. 1991), and the same appears to be true in birds. Of the remaining four variable sites (all in the outer membrane region), three are at hypervariable sites as described in mammals (Irwin et al. 1991). Percent sequence differences for every pair of these taxa, as well as transition/transversion ratios for all comparisons, are reported (Table 4).

Tree for the cytochrome b *gene.*—Parsimony analysis was performed on the 18 informative sites elucidated by the sequence analysis. The most parsimonious tree (Fig. 2: lower) associates the Hawaiian Goose with the small-bodied Canada Goose, and that pair in turn with the large-bodied Canada Geese. Two other trees just one step longer than the most parsimonious one maintain the same general topology except that the Hawaiian Goose is associated with the large-bodied geese in one, but the two types of Canada Geese are closer to each other than to the Hawaiian Goose in the other. From those three trees, the next most parsimonious tree is 10 steps longer.

Statistical testing.—Bootstrap analysis (Felsenstein 1985) showed that of 2,500 trials, the Hawaiian Goose was always associated with the Canada Goose lineage, and within that lineage, was sister to the small-bodied Canada Geese 72% of the time.

Another test which can be applied to this problem is the winning-sites method used by Shields and Wilson (1987b) and explicitly illustrated by Prager and Wilson (1988). To test whether the Hawaiian Goose is closer to the Black Brant than it is to the Canada Goose, we have included the Emperor Goose and a (large-bodied) Canada Goose. This test (Fig. 4) shows that 13 of the 16 informative sites support the

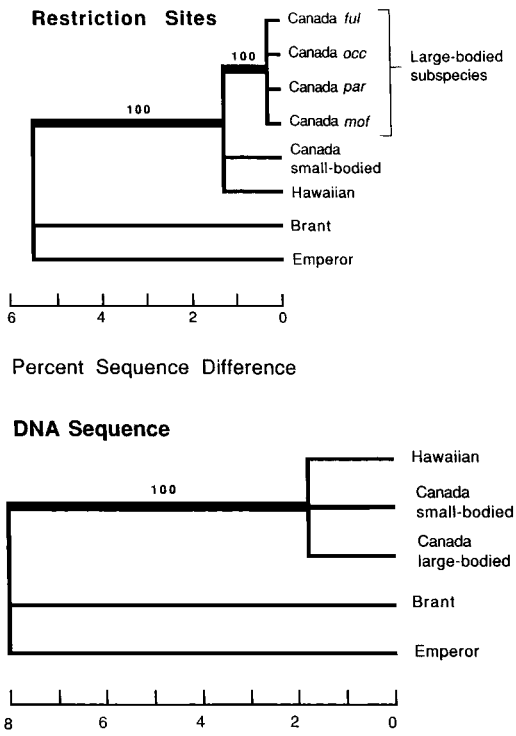

Fig. 2. Two trees showing order of branching and genetic distances among goose mtDNAs. Both the restriction tree (upper) and the sequence tree (lower) are based on a parsimony analysis (PAUP 3.0, Swofford 1989) with the Emperor Goose designated as an outgroup. In each case, a single most parsimonious tree resulted from an exhaustive search of all possible branching orders. Bootstrap values were averaged over 5 sets of 500 replicates calculated with the Swofford program. Only those values >95% are shown (thick lines). Internal branches with lower values were collapsed to produce polychotomies. In the precollapsed restriction tree, the Hawaiian Goose branched off before all Canada Geese with a bootstrap value of 90% on the stem leading to the common ancestor of Canada Geese. By contrast, in the sequence tree, the large-bodied Canada Goose branched off first with a bootstrap value of 72% on the stem leading to the common ancestor of the Hawaiian Goose and the small-bodied Canada Goose. Each node of the tree is plotted with respect to the distance scale, which is based on values in Tables 2 and 4. The restriction tree is based on inferred cleavage sites for the entire mtDNA and the consistency index for informative sites is 0.81. The sequence tree is based on those positions shown for that part of the cytochrome *b* gene (Fig. 3), and the consistency index is 0.83.

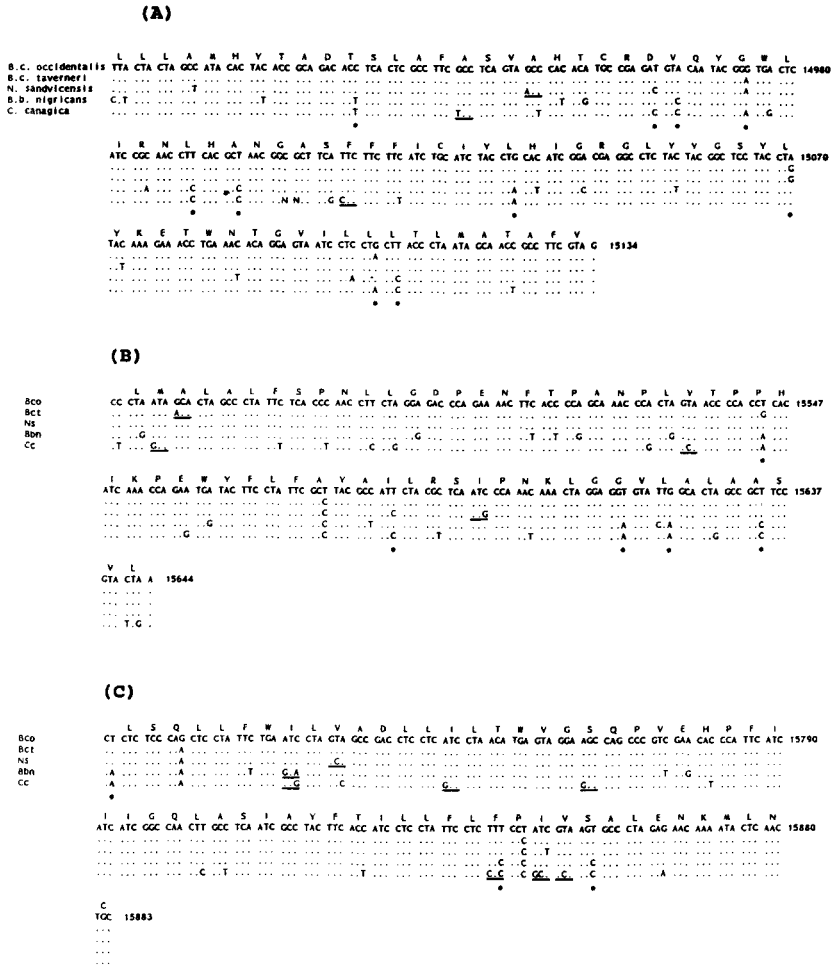

Fig. 3. Sequences for three parts of the cytochrome *b* gene. The second line shows the DNA sequence of a large-bodied goose (*Branta canadensis occidentalis*). The other three large-bodied goose sequences (*B. c. parvipes, B. c. fulva, B. c. moffitti*) were all identical to this and are not shown. Nucleotide sequences are numbered according to the human mtDNA sequence (Anderson et al. 1981). Below that line, dots indicate identity and "N" indicates unresolved bases. Among the large-bodied geese not shown, bases that could not be resolved include positions 15111–2, 15581–3 (*B. c. parvipes*) and positions 15468, 15736, and 15739 (*B. c. fulva*). We used the human mtDNA genetic code (Anderson et al. 1981) to translate the DNA sequence for *B. c. occidentalis*, and we obtained the amino acid sequence shown (top row). Base substitutions causing amino acid changes in other taxa are shown by underlining the affected codons. Solid circles mark 18 phylogenetically informative positions; 16 are informative for the test in Figure 4.

Hawaiian Goose–Canada Goose alliance, and that this support is statistically significant ($P < 0.02$). This test shows with statistical confidence that the Hawaiian Goose lineage is closer to the Canada Goose than to either the Pacific Black Brant or to the Emperor Goose. Within that lineage, however, whether the Hawaiian Goose split before or after the small- and large-bodied Canada Goose divergence is not statistically resolvable with this data set.

DISCUSSION

Our mitochondrial search for the nearest relatives of the Hawaiian Goose has considered four other taxa, namely the large- and small-bodied subspecies of Canada Geese, as well as the Brant and Emperor Goose. In addition, Shields and Wilson (1987b) provided relevant information about three other species, all relatively distant from the Canada Goose in the

TABLE 4. Percent sequence difference (above the diagonal) and transition/transversion ratios (below the diagonal) among 612 base pairs of cytochrome *b* genes of geese.[a]

Goose	Hawaiian	Small-bodied	Large-bodied	Brant	Emperor
Hawaiian	—	1.8	2.1	7.8	9.0
Small-bodied	9/2	—	1.3	6.9	8.4
Large-bodied	12/1	7/1	—	6.7	8.8
Brant	38/10	34/8	32/9	—	10.1
Emperor	47/8	45/6	47/7	56/6	—

[a] Small-bodied = Taverner's Canada Goose; large-bodied = Lesser Canada Goose, Dusky Canada Goose, Vancouver Canada Goose, and Western Canada Goose. Since all large-bodied subspecies of Canada geese tested had identical cytochrome *b* sequences, they are combined here under one category.

mtDNA tree. Only the Canada Goose had mtDNA closely related to the Hawaiian Goose. This link between the Hawaiian Goose and Canada Geese supports the assertion by Kear and Berger (1980: 23) that "Ornithologists believe that the Canada Goose (*Branta canadensis*) is the closest living relative of the Hawaiian Goose and, therefore, that its ancestors came from North America."

Canada Geese winter along the western coastal regions of the continental United States and return to Alaska and arctic Canada in spring to breed. Thus, it is not difficult to imagine that a small number of ancestors of these geese may have originally become disoriented during migration and arrived on Hawaii, where they survived without severe competition from other forms. During the past century alone, 27 waterfowl species from North America have been seen on the Hawaiian islands, 24 as stragglers or chance migrants, and three (including the Cackling Canada Goose) as regular winter residents (Berger 1972). In addition, two captive-reared Aleutian Canada Geese banded and released from a recovery program site on Agattu Island, Alaska (173°40'E, 52°30'N), on 6 September 1979 were seen 86 days later on Roi-Namur (167°30'E, 09°20'N) of the Marshall Islands (Springer et al. 1986). Clearly, Canada Geese are capable of long-distance movement over vast areas of open ocean.

Given the short time scale that we propose for the time of origin of the Hawaiian Goose (see below), it would appear that the founders of this species underwent relatively rapid morphological changes. Prominent among these changes were reduction in the webbing of the toes and increased size and strength of the legs as a response to the relatively dry upland habitats that the Hawaiian Goose now occupies (Miller 1937).

Relations between large- and small-bodied Canada Geese.—Our fragment analysis, and that of Van Wagner and Baker (1990), extend the work of Shields and Wilson (1987a, b) to the point where there is now restriction fragment information on the extents of divergence among all 11 of the generally recognized subspecies of Canada Geese. This includes some subspecific information on 9 of the 11 subspecies. These subspecies fall clearly into two sister groups, large-bodied and small-bodied, which share no mtDNA types. This result contrasts with that based on molecular studies of proteins encoded by nuclear genes (Van Wagner and Baker 1986, 1990). The nuclear evidence associates all the subspecies very closely, similar to the situation reported by Gelter (1989) in Old World flycatchers. Further, *B. c. hutchinsi*, a small-bodied subspecies that breeds and winters far to the east of other small-bodied subspecies, is closely akin (in its nuclear genes) to geographically neighboring subspecies with large bodies. This is probably the result of introgression mediated by large-bodied males from those neighboring subspecies.

Approximate molecular time scale.—The sequence comparisons make it possible to obtain rough estimates of the time of the initial mitochondrial radiation among Canada Geese and the time of mitochondrial divergence between the lineages leading to Hawaiian Geese and Canada Geese. Because of the tendency for transitions to accumulate so quickly that some sites have experienced multiple hits, we confine our analysis to transversions, which accumulate roughly 10 times more slowly (Kocher et al. 1989). The average number of transversions by which the large- and small-bodied subspecies differ is 1, while that by which the Canada Goose differs from the Brant and Emperor Goose is 7.5. Assuming strictly clock-like evolution during a period of 4–5 million years (Shields and Wilson 1987a), the large- and small-bodied subspecies

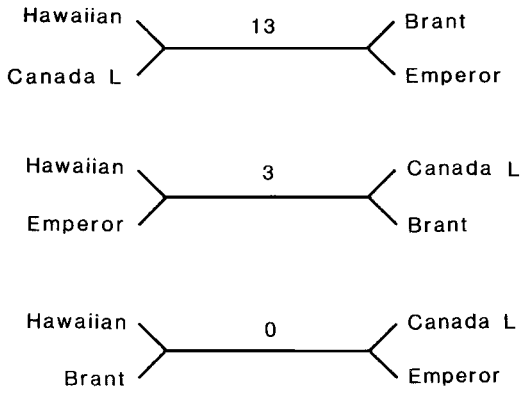

Fig. 4. Winning-sites test applied to the cytochrome *b* genes of four goose taxa.

of Canada Geese had a common mitochondrial ancestor 0.6 million years ago. Similarly, we estimate an apparent time of common ancestry of 0.9 million years for the Hawaiian Goose and Canada Goose. However, there are large stochastic errors in such estimates, which prevent us from ruling out times as great as 3 million years for these divergences. Nevertheless, these figures are well accommodated within the known geological record for Hawaii (McDougall 1964, Macdonald and Abbott 1970).

Taxonomic recommendations.—Although it may be tempting to reclassify the Hawaiian Goose as a member of the genus *Branta*, we feel that such an action would be premature. In the first place, the mitochondrial trees pertain only to the maternal lineage. When dealing with lineages that have diverged within the past few million years, the possibility exists that nuclear gene comparisons could reveal a more complex picture (cf. Shields and Wilson 1987b, Gelter 1989, Van Wagner and Baker 1990). Second, we do not wish to overlook the magnitude of the phenotypic differences between the Hawaiian Goose and Canada Geese. If quantitative morphometric comparisons of the sort done by Van Wagner and Baker (1990) on Canada Geese were extended to other geese, the extent of this phenotypic difference could be evaluated objectively and quantitatively. Such an evaluation is advisable before deciding whether cladistic considerations should take precedence in the classification of the Hawaiian Goose.

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