Wilson Bull., 107(2), 1995, pp. 235–250

MORPHOLOGICAL AND GENETIC DIVERGENCE AMONG ALASKAN POPULATIONS OF *BRACHYRAMPHUS* MURRELETS

JAY PITOCCHELLI,[1,2] JOHN PIATT,[3] AND MATTHEW A. CRONIN[3,4]

ABSTRACT.—We studied morphological and mitochondrial DNA (mtDNA) divergence among three populations of *Brachyramphus* Murrelets: Kittlitz's Murrelets (*B. brevirostris*), and tree-nesting and ground-nesting Marbled Murrelets (*B. marmoratus*). We found little morphological divergence in external and skeletal measurements among Marbled Murrelets, but both populations were easily distinguished from Kittlitz's Murrelets. Principal components analysis (PCA) of external measurements showed that Kittlitz's Murrelets occupied a distinct cloud in multivariate space separate from Marbled Murrelets. However, tree-nesting and ground-nesting Marbled Murrelets were indistinguishable. We obtained the same pattern from PCA of skeletal dimensions. Analysis of mtDNA revealed an estimate of sequence divergence of 4.4%–5.0% between Marbled Murrelets and Kittlitz's Murrelets, suggesting a divergence of about 2.2 MYBP. The difference between ground- and tree-nesting murrelets was 0.03%. This analysis suggests little divergence has occurred between tree- and ground-nesting populations of Marbled Murrelets. *Received 14 March 1994, accepted 1 Nov. 1994.*

The genus *Brachyramphus* currently contains two species of murrelets. Both are unique among the Alcidae in that they have cryptic alternate plumage and nest mostly inland instead of on predator-free, offshore islands. Kittlitz's Murrelets (*B. brevirostris*) nest on the ground in high-altitude alpine habitats throughout glaciated regions of Alaska (Van Vleit 1993). The North American subspecies of the Marbled Murrelet (*B. m. marmoratus*) breeds along the coast of the Pacific Ocean from central California to the Aleutian Islands (Carter and Morrison 1992). Over 65% of the North American population of Marbled Murrelets is found in Alaska (Piatt and Ford 1993). Throughout their range, most Marbled Murrelets nest on branches of trees in old-growth coastal forests. But in Alaska, at least 3% of the population nests on the ground in the Aleutian Islands, along the Alaskan Peninsula, and nonforested regions of coastal Alaska (Mendenhall 1992, Piatt and Ford 1993).

Tree-nesting populations of Marbled Murrelet are threatened by logging of old-growth forests, oil pollution, and gillnet fisheries through much of the breeding range (Piatt et al. 1990, Carter and Morrison 1992). Con-

[1] Biology Dept., Saint Anselm College, 100 Saint Anselm Drive, Manchester, New Hampshire 03102-1310.
[2] Research Associate, Ornithology Dept., American Museum of Natural History, Central Park West at 79th St., New York, New York 10024.
[3] National Biological Survey, Alaska Fish and Wildlife Research Center, 1011 E. Tudor Rd., Anchorage, Alaska 99503.
[4] Present address: LGL Alaska Research Associates, 4175 Tudor Centre Dr., Anchorage, Alaska 99508.

sequently, the U.S. Fish and Wildlife Service and the Canadian Wildlife Service have listed this species as "threatened" in California, Oregon, Washington, and British Columbia (Carter and Morrison 1992, Stein and Miller 1992). Despite these listings, there have been no thorough studies of genetic subdivision or geographic variation (*sensu* Zink and Remsen 1986) among Marbled Murrelet populations. Studies of morphological and genetic divergence among or within species provide crucial information about the existence of conservation units or interesting patterns within species such as clines, recent range extensions, or hybrid contact (Barrowclough 1992). Information on morphological divergence and genetic subdivision of Marbled Murrelet populations is essential to management decisions concerning the status of its widely distributed breeding populations. For example, the Asian subspecies of the Marbled Murrelet (*B. m. perdix*) has recently been elevated to a new species based on studies of mitochondrial DNA (mtDNA; Vicki Friesen, unpubl. data), effectively removing this population from management considerations for the North American populations of *B. m. marmoratus*. North American murrelets deserve special attention, especially disjunct breeding populations such as tree- and ground-nesting populations in Alaska. Results of these analyses can be used for comparisons with more contiguous southern populations experiencing recent declines.

In this paper, we examine the extent of morphological and genetic divergence between ground-nesting and tree-nesting populations of Marbled Murrelets from Alaska. These markedly different breeding behaviors may reflect invasion of new niches or adaptive zones. Genetic divergence often accompanies the exploitation of new niches. We investigated morphological divergence by comparing external measurements and skeletal dimensions of ground- and tree-nesting murrelets. Berger (1952) found that terrestrial species of cuckoos diverged from their arboreal relatives, especially in the distal elements of the hindlimb. Ground-dwelling species also tend to have stout hindlimbs for walking and running compared to their closest relatives. This is reflected among the Alcidae, in which puffins represent the extreme in ambulatory adaptation with heavy joints and long leg bones, whereas Marbled Murrelets have the shortest leg bones of any Alcids—presumably an adaptation for nesting in trees (Storer 1945). We paid close attention to variation in hindlimb measurements among ground- and tree-nesting Marbled Murrelets. We also used mtDNA to analyze genetic differences between these populations. Kittlitz's Murrelet was used as an outgroup for the morphological and genetic analyses.

STUDY AREA AND METHODS

Field work.—We used data from specimens collected during the Outer Continental Shelf Environmental Assessment Program (OCSEAP) of the 1970's and birds we collected from

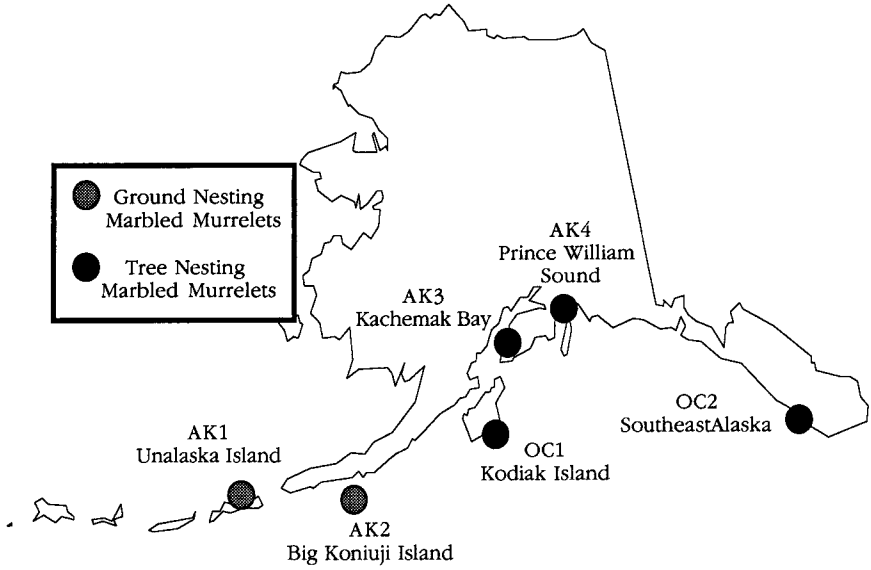

FIG. 1. Collection localities in Alaska: AK1—Unalaska Island, Captain's Bay (ground-nesting Marbled and Kittlitz's murrelets); AK2—Big Koniuji Island, Flying Eagle Harbor (ground-nesting Marbled and Kittlitz's murrelets); AK3—Kachemak Bay, Tutka Bay to Grewingk Glacier (tree-nesting Marbled and Kittlitz's murrelets); AK4—Prince William Sound, Unakwik Fjord (tree-nesting Marbled Murrelets); OC1—OCSEAP collections around the Kodiak Archipelago (tree-nesting Marbled Murrelets); OC2—collections from Glacier Bay and surrounding region (tree-nesting Marbled Murrelets). AK designations refer to collections made in the late 1980s and early 1990s while OC designations refer to OCSEAP collections of the 1970s.

1988–1992 (Fig. 1). All specimens were collected during the breeding season. Birds from the later collections were prepared as skeletal specimens in the field. Heart, liver, and breast muscle tissues from these birds were frozen in liquid nitrogen within four hours of collection. Tissues are currently stored in ultra-cold freezers at the American Museum of Natural History, New York, and at the National Biological Survey regional office in Anchorage, Alaska. Skeletal materials have been deposited at the American Museum of Natural History.

We made comparisons between three groups: tree-nesting Marbled Murrelets, ground-nesting Marbled Murrelets, and ground-nesting Kittlitz's Murrelets. Marbled Murrelet specimens collected during the breeding season from treeless areas (Aleutian and Shumagin Islands) were lumped into the ground-nesting category, whereas Marbled Murrelet specimens from the Kodiak Archipelago, Kachemak Bay, Prince William Sound, and southeast Alaska were presumed to belong to tree-nesting populations (Fig. 1). There are reported cases of ground-nesting Marbled Murrelets in southcentral and southeast Alaska, but the majority of Marbled Murrelets collected from Kodiak to southeast Alaska are assumed to be ground-nesters based on the observed distribution of known tree-nests in Alaska (Naslund et al. 1994). Breeding Kittlitz's Murrelets were collected during the OCSEAP surveys and recent collections we made in Kachemak Bay and the Aleutian and Shumagin islands.

We acknowledge that there may be different variance components, including sex, age,

and geography, that account for part of the measurement variation we observed (Baker 1985, Zink and Remsen 1986). Preliminary results of Student's *t*-tests (Pitocchelli et al., unpubl. data) revealed no significant sexual dimorphism for 13 of 18 measurements, so we pooled male and female specimens for these analyses. Only adult specimens were used. Lack of adequate skeletal materials in existing museum collections was an important factor limiting the geographic extent of our analyses.

Morphometrics.—We analyzed size variation in weight, external measurements and skeletal dimensions. We obtained data on weight and five external measurements from the OCSEAP surveys: WING—wing chord; TARS—tarsus length; CULMEN—culmen length; GAPE—gape; BDEPEXT—bill depth. Birds were weighed with Pesola scales to the nearest gram. External measurements were made with Vernier calipers to the nearest 0.1 mm.

We measured 17 skeletal dimensions from specimens collected from 1988–1992: six cranial dimensions, PRL—premaxillary length, SKW—skull width, SKL—skull length, BDEP—bill depth, MANDL—mandible length, DIAM—diameter of the sclerotic ring; 11 postcranial dimensions, CORL—coracoid length, STERL—sternum length, KEEL—keel length, KEED—keel depth, SYNMAX—maximum synsacrum width, FEL—femur length, TIBL—tibiotarsus length, TARL—tarsometatarsus length, HUML—humerus length, ULNL—ulna length, CARPL—carpometacarpus length. Measurements were entered directly into personal computers using Max-cal digital calipers and Lessoft (Marcus 1982). Measurements were made to the nearest 0.1 mm.

We carried out univariate and multivariate analyses to assess size differences among *Brachyramphus* populations. At the univariate level, we examined the contribution of species (Marbled versus Kittlitz's), sex, and individual variation to the total variance in each measurement using a Nested ANOVA (PROC NESTED, SAS 1985). We conducted an ANOVA (PROC GLM, SNK, SAS 1985) on each measurement to detect significant differences between ground-nesting Marbled Murrelets, tree-nesting Marbled Murrelets, and Kittlitz's Murrelets. A Student-Newman-Keuls post hoc comparisons test was used to determine which of the three groups were significantly different from each other.

At the multivariate level we used principal components analysis (PROC PRINCOMP, SAS 1985) to determine whether ground-nesting Marbled Murrelets, tree-nesting Marbled Murrelets, and Kittlitz's Murrelets occupy separate clouds in multivariate space. Separate PCA's were performed on the external and skeletal data. Specimens with broken bones were excluded from the PCA of skeletal measurements. We used 10 of the 17 skeletal dimensions for this analysis—SKW, CORL, STERL, KEEL, KEED, FEL, TIBL, TARL, ULNL, CARPL. We chose these characters in order to increase sample sizes while still sampling at least one bone from the skull, girdle, wing and leg complexes. Raw data were $\log_{10}$-transformed before entry into the PCA. We extracted PC scores from a variance-covariance matrix for the first two components and plotted specimens along these axes. We compared character loadings on the PC axes to determine which measurements contributed most to the separation of specimens. Character loadings are correlations between the skeletal measurements and the principal components generated by the PCA (Schnell et al. 1985).

mtDNA analysis.—We analyzed mtDNA from five Kittlitz's Murrelets, five tree-nesting Marbled Murrelets from Kachemak Bay, and nine ground-nesting Marbled Murrelets from the Shumagin Islands. We also analyzed mtDNA from two Black-legged Kittiwakes (*Rissa tridactyla*) as a distantly related outgroup. MtDNA restriction fragment patterns were compared visually and genotypes defined by composite fragment patterns (Lansman et al. 1981). MtDNA sequence divergence among genotypes (base substitutions per nucleotide, *P*) was estimated from the proportion of shared restriction fragments (F, Upholt 1977).

MtDNA variation was assessed with restriction enzymes. Genomic DNA was extracted from about 0.5 g muscle tissue using standard methods (Cronin et al. 1991a). About 0.5 μg

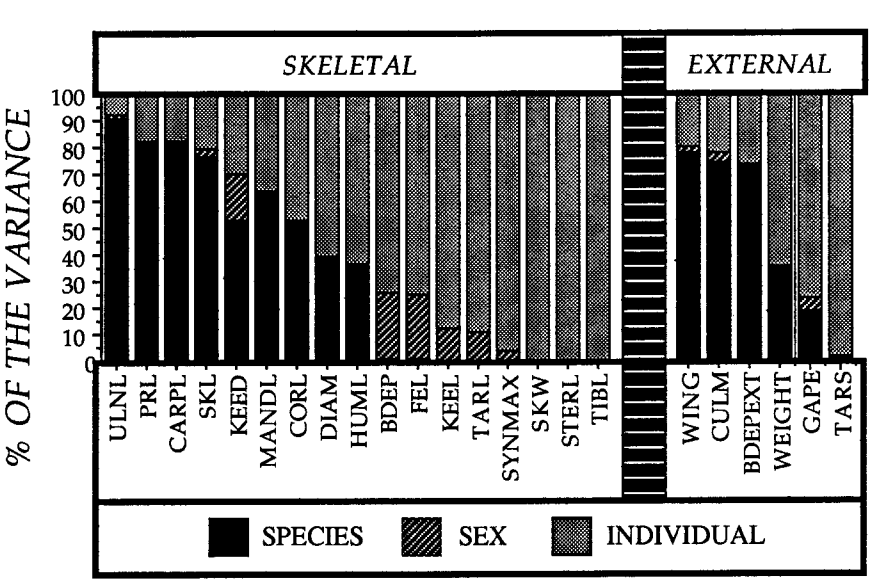

FIG. 2. Contribution of species, sex, and individual variation to character variance in a nested ANOVA of skeletal and external characters. Shaded areas represent the percentage of the total variance attributed to each variance component.

of DNA from each bird was digested with restriction enzymes (*Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sac*I, *Xba*I, *Eco*RV, *Bgl*I, and *Bcl*I). Digested DNA fragments were separated electrophoretically in 0.75% agarose gels with Tris-borate-EDTA buffer (Sambrook et al. 1989), transferred onto nylon membranes by southern blotting, and the filters were baked at 80° C for 2 h. Filtrates were prehybridized at 60–65° C in 5× SSC, 1% sodium sarkosyl, 1× Denhardt's solution, 0.025 M potassium phosphate, 0.025 mg/ml salmon sperm DNA for 1 h. A [P32]-labeled mtDNA probe was denatured at 100° C and added to the prehybridization solution. The probe consisted of Northern Pintail (*Anas acuta*) mtDNA isolated from brain (see Cronin et al. 1988) and further purified in low melting agarose (Sambrook et al. 1989). Hybridization was at 60–65° C for 12–48 h with constant shaking. After hybridization, filtrates were washed for 30 min at room temperature with 2× SSC, 0.2% SDS, 1× Denhardt's solution, and two hours at 37°C with 2× SSC, 0.1% SDS. Filtrates were air dried, covered in plastic wrap, and exposed to X-ray film for 12–170 h.

RESULTS AND DISCUSSION

Morphometrics.—A nested ANOVA of external and skeletal dimensions revealed that the percentage of total variance attributable to species, sex, and individual variation was different for each mensural character (Fig. 2). Species affinity or individual variance components were responsible for most of the variation in each measurement. Species affinity ac-

TABLE 1

RESULTS FROM ANOVA OF DIFFERENCES IN EXTERNAL MEASUREMENTS FROM TREE-NESTING MARBLED MURRELETS, GROUND-NESTING MARBLED MURRELETS AND GROUND-NESTING KITTLITZ'S MURRELETS[a]

Character	Marbled Murrelet (tree)	Marbled Murrelet (ground)	Kittlitz's Murrelet (ground)	Significance
WEIGHT	225.38	225.10	241.14	0.0033
(N, SD)	171, 23.59	56, 20.33	28, 25.29	
WING	128.35	132.35	142.00	0.0001
(N, SD)	140, 5.93	14, 6.99	17, 7.36	
TARS	17.92	17.40	17.70	ns
(N, SD)	138, 1.36	12, 0.85	18, 1.91	
CULMEN	16.00	15.08	11.85	0.0001
(N, SD)	134, 1.56	12, 1.44	20, 1.30	
GAPE	34.62	32.91	32.42	0.0028
(N, SD)	32, 1.86	12, 2.50	14, 2.20	
BDEPEXT	5.97	5.93	5.12	0.0001
(N, SD)	125, 0.38	14, 0.31	14, 0.22	

[a] Means that are underlined are not significantly different from each other according to SNK post hoc comparisons test.

counted for 73% or more of the variance in three of six external measurements. Individual variation contributed 64% or more of the variance in the other external dimensions. Results of the skeletal analyses showed the same pattern. Species affinity accounted for more than 35% of the variance in nine of 17 skeletal dimensions. Individual variation accounted for 29% or more of the variance in 13 skeletal measurements. We also performed a nested ANOVA on Marbled Murrelets only (not shown here). Nesting habits and sexual dimorphism played minor roles in measurement variance compared to individual variation which accounted for 90% or more of the total variance in each character.

Comparisons of external measurements showed that size differences between Kittlitz's Murrelets and both Marbled Murrelet populations are much more pronounced than differences between ground- and tree-nesting populations of Marbled Murrelets (Table 1). This trend was evident in three of the six external measurements, WEIGHT, CULMEN, and BDEPEXT. An ANOVA of WEIGHT revealed that Kittlitz's Murrelets were significantly heavier than Marbled Murrelets, but there were no significant differences between ground- and tree-nesting Marbled Murrelets. Kittlitz's Murrelets were significantly smaller than Marbled Murrelets for CULMEN and BDEPEXT, but no differences were found among the Marbled

TABLE 2
LOADINGS OF EXTERNAL MEASUREMENTS ON THE FIRST THREE PRINCIPAL COMPONENTS

Character	Prin1	Prin2	Prin3
WING	−0.228	−0.002	0.550
TARS	0.172	0.825	0.465
CULMEN	0.864	−0.369	0.340
BDEPEXT	0.412	0.427	−0.602
Percent of total variance	64.1%	18.7%	9.6%

Murrelet groups. Significant differences between ground- and tree-nesting populations existed for WING and GAPE. WING measurements were significantly different for all three groups, with Kittlitz's Murrelets having the largest WING and tree-nesting Marbled Murrelets the shortest. The shorter, rounded wings of tree-nesting birds may be associated with nesting in dense old-growth forests while the longer, pointed wings of the other two groups may be adaptations for open habitats like treeless talus slopes where the birds nest. The GAPE of ground-nesting Marbled Murrelet and Kittlitz's Murrelet populations were significantly different from those of tree-nesting Marbled Murrelets but not from each other. This difference is difficult to explain since the other external bill measurements (CULMEN, BDEPEXT) showed no significant differences between the Marbled Murrelet populations, but both were significantly different from Kittlitz's Murrelets. There were no differences in TARS among the three groups.

A PCA of external dimensions showed that specimens of both Marbled Murrelet populations clustered together to form a single cloud of points separate from those of Kittlitz's Murrelets but not from each other (Fig. 3). CULMEN and BDEPEXT had the highest loadings on PC1 and contributed the most to separation of murrelets along this axis (Table 2). PC1 accounted for 64.1% of the total variation. TARS and BDEPEXT had the highest loadings on PC2 which accounted for 18.7% of the total variation. PC3 explained only 9.6% of the variation. WING and TARS had the highest loading on PC3.

There were three trends in the ANOVA's of skeletal dimensions. (1) Both populations of Marbled Murrelets had larger skull measurements (PRL through DIAM) than Kittlitz's Murrelets (Table 3). Kittlitz's Murrelets were significantly smaller than both Marbled Murrelet populations for four of the six skull measurements. There were no significant differences ($P < 0.05$) between ground- and tree-nesting Marbled Murrelets for four of the six skull dimensions (Table 3). BDEP and MANDL dif-

TABLE 3

RESULTS FROM ANOVA OF DIFFERENCES IN SKELETAL DIMENSIONS BETWEEN TREE-NESTING MARBLED MURRELETS, GROUND-NESTING MARBLED MURRELETS AND GROUND-NESTING KITTLITZ'S MURRELETS[a]

Character	Marbled Murrelet (tree)	Marbled Murrelet (ground)	Kittlitz's Murrelet (ground)	Significance
PRL	29.18	29.41	26.22	0.0001
(N, SD)	23, 0.99	27, 1.09	8, 0.75	
SKW	20.11	20.10	19.84	ns
(N, SD)	24, 0.49	29, 050	12, 0.62	
SKL	60.60	61.71	57.55	0.0001
(N, SD)	23, 1.63	29, 1.27	7, 1.46	
BDEP	3.412	4.82	5.061	0.0001
(N, SD)	25, 1.50	29, 0.22	12, 0.28	
MANDL	48.46	49.38	46.56	0.0001
(N, SD)	23, 1.26	28, 1.20	11, 1.24	
DIAM	12.80	12.98	12.27	0.0003
(N, SD)	24, 0.45	21, 0.47	11, 0.30	
CORL	22.83	22.94	23.81	0.0002
(N, SD)	25, 0.64	29, 0.67	12, 0.63	
STERL	74.04	74.61	74.49	ns
(N, SD)	25, 2.05	29, 2.41	12, 1.65	
KEEL	81.53	82.27	83.35	ns
(N, SD)	25, 2.55	29, 3.17	12, 2.86	
KEED	25.64	26.13	27.34	0.0001
(N, SD)	25, 0.84	29, 0.84	12, 1.20	
SYNMAX	18.25	17.75	18.07	ns
(N, SD)	23, 0.63	27, 0.75	11, 0.76	
FEL	23.45	23.85	23.91	ns
(N, SD)	24, 0.52	29, 0.52	12, 0.87	
TIBL	44.94	45.24	44.44	ns
(N, SD)	25, 1.30	29, 1.20	12, 1.75	
TARL	16.91	17.38	16.95	0.0111
(N, SD)	25, 0.44	29, 0.54	12, 0.92	
HUML	48.84	49.18	50.56	0.0002
(N, SD)	25, 1.05	29, 1.09	11, 1.18	

TABLE 3
CONTINUED

Character	Marbled Murrelet (tree)	Marbled Murrelet (ground)	Kittlitz's Murrelet (ground)	Significance
ULNL	37.18	37.39	41.55	0.0001
(N, SD)	24, 0.94	28, 0.92	12, 1.24	
CARPL	25.51	25.62	27.38	0.0001
(N, SD)	25, 0.74	29, 0.60	12, 0.78	

[a] Means that are underlined are not significantly different from each other according to SNK post hoc comparisons test.

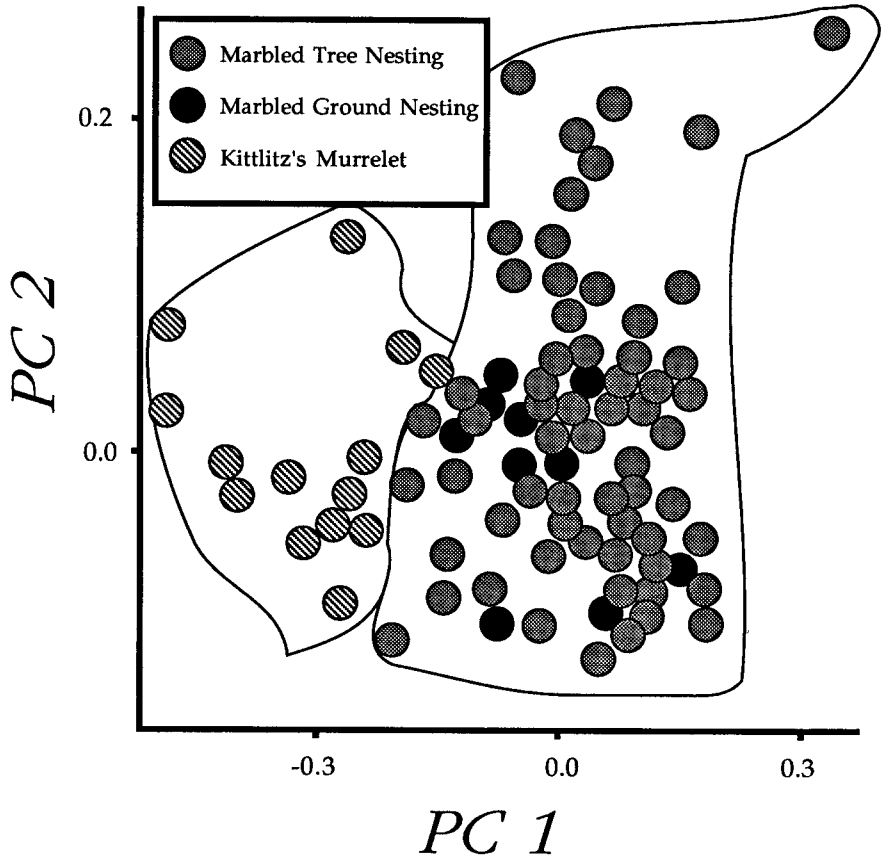

FIG. 3. Plots of PC scores based on external dimensions for each specimen for the first two PC axes.

fered significantly between ground-nesting and tree-nesting Marbled Murrelets ($P < 0.0001$). (2) Kittlitz's Murrelets were significantly larger than all Marbled Murrelets for five of the 11 postcranial dimensions (CORL through CARPL, Table 3). There were no significant differences between ground- and tree-nesting Marbled Murrelets for 10 of 11 postcranial dimensions. (3) TARL, from the hindlimb region, exhibited an interesting pattern of variation. The tree-nesting population was not significantly different from Kittlitz's Murrelets but both were significantly smaller than ground-nesting Marbled Murrelets. It is interesting because it suggests that Kittlitz's Murrelets are more similar to tree-nesting Marbled Murrelets versus ground-nesting Marbled Murrelets for TARL.

Results of the PCA of skeletal measurements were similar to the PCA of external measurements. Plots of specimens along the first two PC axes revealed that Kittlitz's Murrelets occupied a distinct cloud in multivariate space. Ground- and tree-nesting Marbled Murrelets clustered together in a second cloud of points, but they were not segregated from each other (Fig. 4).

The first three PC axes explained 80.3% of the total variance (Table 4). All of the skeletal characters except SKW had positive loadings on PC1. Although SKW's loading is very small compared to the other characters, PC1 should not be regarded as a *size* axis because of the negative loading. ULNL, KEED, and CARPL had the highest loadings on PC1. They contributed the most to the separation of murrelets along the PC1 axis. PC2 and PC3 each had characters with positive and negative loadings indicating that they are shape components. Wing and leg bones contributed most to the separation of specimens along the PC2 axis. TIBL and ULNL had the highest loadings on PC2. PC2 axis explained 17.2% of the total variation. KEEL, TARL, and STERL had the highest loadings on the PC3 axis which accounted for 11.9% of the variation.

We performed several additional analyses (not shown here) using different combinations of skeletal characters. The results of these analyses revealed the same trend as above, separation of Kittlitz's Murrelets from Marbled Murrelets but no separation of ground- and tree-nesting Marbled Murrelets. Analyses (not shown here) of only Marbled Murrelet populations also did not show separation of ground- and tree-nesting populations.

mtDNA analysis.—The Northern Pintail mtDNA probe hybridized well to murrelet DNA, resulting in clear fragment patterns on the autoradiograms (Fig. 5). Murrelet mtDNA contains about 16,000 base pairs. This is similar to other species of birds (Kessler and Avise 1985). Our analysis of five Kittlitz's and 14 Marbled Murrelets shows that each species has distinct mtDNA. All Kittlitz's Murrelets had the same fragment patterns

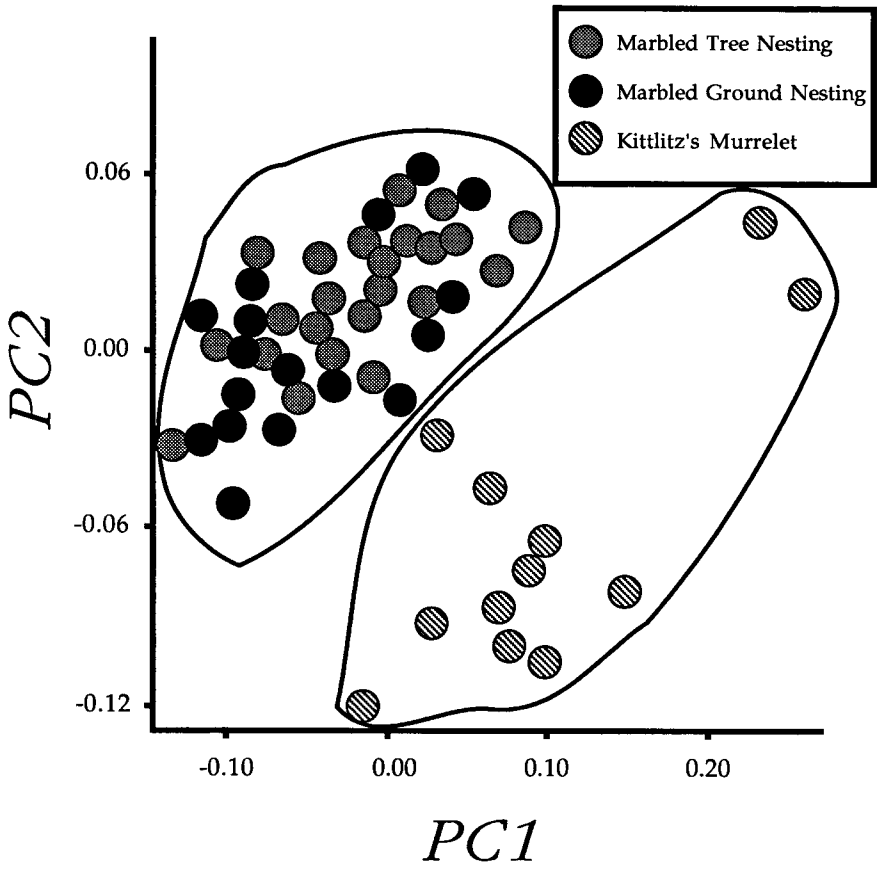

FIG. 4. Plots of PC scores based on skeletal dimensions for each specimen for the first two PC axes.

for nine restriction enzymes and hence one genotype designated K1 (Fig. 5). The Marbled Murrelets had different fragment patterns than Kittlitz's Murrelets for six (*Eco*RI, *Hin*dIII, *Pvu*II, *Sac*I, *Eco*RV, and *Bgl*I) of the restriction enzymes. With one exception, tree-nesting and ground-nesting Marbled Murrelets had identical fragment patterns for all nine enzymes and a mtDNA genotype designated M1 (Fig. 5). The exception is one ground-nesting Marbled Murrelet from the Shumagin Islands which had a variable pattern for *Bcl*I and a genotype designated M2. There were 27 restriction fragments comprising genotype K1, and 20 each in M1 and M2. Eleven fragments were shared between K1 and M1 ($F = 0.468$, $P = 0.044$), 10 fragments shared between K1 and M2 ($F = 0.426$, $P =$

TABLE 4
LOADINGS OF SKELETAL VARIABLES ON THE FIRST THREE PRINCIPAL COMPONENTS

Character	Prin1	Prin2	Prin3
SKW	−0.013	0.092	0.149
CORL	0.316	−0.047	0.197
STERL	0.184	0.317	−0.426
KEEL	0.290	0.300	−0.516
KEED	0.420	−0.001	−0.328
FEL	0.219	0.219	0.030
TIBL	0.169	0.442	0.264
TARL	0.197	0.530	0.499
ULNL	0.560	−0.473	0.116
CARPL	0.417	−0.218	0.219
Percent of total variance	52.1%	17.2%	11.0%

0.050), and 19 fragments shared between M1 and M2 ($F = 0.95$, $P = 0.003$). As expected, mtDNA of Black-legged Kittiwakes was quite divergent from that of Marbled Murrelets ($F = 0.24$, $P = 0.085$) and Kittlitz's Murrelets ($F = 0.25$, $P = 0.085$) compared to differences between *Brachyramphus* populations.

The divergence observed between Marbled and Kittlitz's Murrelet mtDNA is similar to that observed between other congeneric avian species. For example, Shields and Wilson (1987) reported $P = 0.027$ between the White-fronted Goose (*Anser albifrons*) and the Snow Goose (*A. caerulescens*), and $P = 0.061$ between Brant (*Branta bernicla*) and the Canada Goose (*B. canadensis*). Kessler and Avise (1985) reported a mean $F = 0.46$ for 55 interspecific comparisons of avian congeners. This is very similar to our mean $F = 0.447$ for K1 versus M1 and M2. However, the level of mtDNA divergence among congeneric species varies widely, as indicated by the wide range of F values (0.26–0.96) reported by Kessler and Avise (1985).

MtDNA sequence divergence is not, by itself, a reliable indicator of phylogeny or time of divergence of closely related species, and must be considered in light of morphology, natural history, and variation at other genetic loci (Avise et al. 1990, Cronin et al. 1991b). Because mtDNA is maternally inherited and separate from the chromosomal DNA, it gives a limited view of interspecific relationships. Despite the limitations of mtDNA analyses, our data indicate Marbled and Kittlitz's murrelet populations are genetically distinct whether they are sympatric or allopatric. However, little genetic divergence was observed between ground-nesting and tree-nesting Marbled Murrelets. Genotypes of tree- and ground-nest-

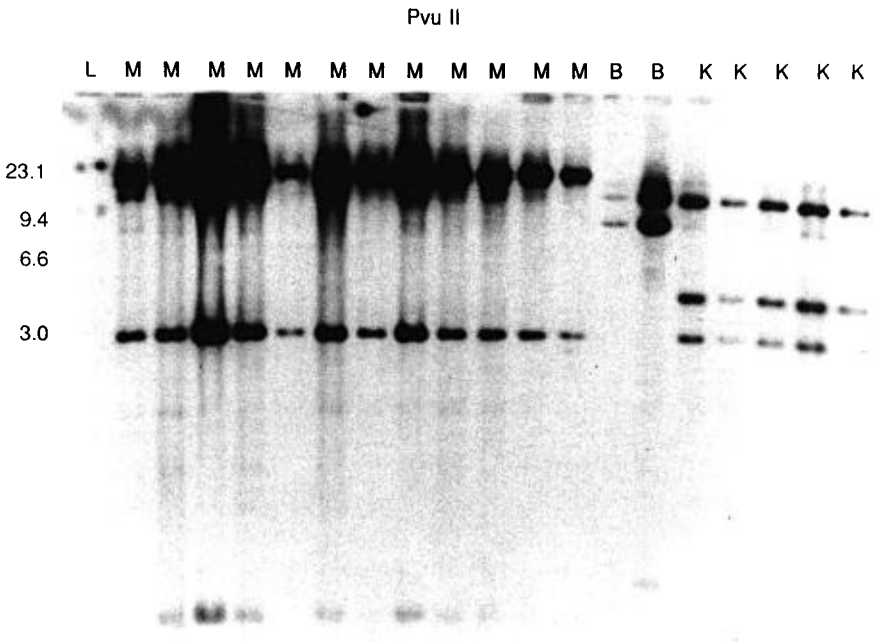

FIG. 5. Autoradiogram of Marbled (M) and Kittlitz's (K) murrelet mtDNA digested with the restriction enzyme *Pvu*II, run on 0.8% agarose gel, blotted onto nylon filter, and hybridized to radioactive Northern Pintail mtDNA probe. The lanes labeled B contain mtDNA of Black-legged Kittiwakes and the lane labeled L contains Lambda virus DNA digested with *Hin*dIII. Numbers along the left side of the figure indicate size of DNA fragments in kilobases. Note that all of the Marbled and Kittlitz's murrelets have distinct fragment patterns.

ing Marbled Murrelets were more similar to each other than either was to Kittlitz's Murrelet.

Our analyses of mtDNA agree with current taxonomic treatments of the species we surveyed. As expected, the largest amount of genetic divergence (P = 0.085) was observed between Black-legged Kittiwakes and *Brachyramphus* Murrelets. Analyses of mtDNA among *Brachyramphus* Murrelets, Marbled (M1 genotype) and Kittlitz's (K1) murrelets, revealed a genetic divergence of P = 0.044. Using Shields and Wilson's (1987) estimated rate of mtDNA divergence of 2% per million years, we estimate a divergence date for Marbled and Kittlitz mtDNA of approximately 2.2 MYBP. However, genetic divergence between ground- and tree-nesting Marbled Murrelet populations was minimal (P = 0.00–0.003). These results are consistent with similar intraspecific comparisons

between populations of *Ammodramus* sparrows ($P = 0.006$, Zink and Avise 1990) or Brown Towhees ($P = 0.001$, Zink and Dittman 1991).

Marbled Murrelets have evolved two vastly different breeding strategies: tree- and ground-nesting. Exploitation of new niches is often accompanied by morphological and genetic divergence. However, we observed little variation between tree- and ground-nesting populations of Marbled Murrelets for mensural and genetic characters. Only five of 23 measurements differed between tree- and ground-nesting Marbled Murrelets. Especially significant is the lack of divergence in leg measurements of ground- and tree-nesting Marbled Murrelets. Terrestrial species show marked divergence in skeletal elements of the hindlimbs when compared to their arboreal congeners (Berger 1952). Furthermore, the Marbled Murrelet is different from all other alcids in having an exceptionally short tibiotarsus and tarsometatarsus—presumably an adaptation for its arboreal nesting habits (Storer 1945). In our comparisons, TARS, FEL, and TIBL were not significantly different among the three murrelet groups (Tables 1, 3). The mean TARL of ground-nesting Kittlitz's Murrelets was closer to that of the tree-nesting Marbled Murrelet than to TARL of the ground-nesting Marbled Murrelet (Table 3). These results seem surprising considering that our data suggest that the ground-nesting Kittlitz's Murrelet may have diverged from the Marbled Murrelet 2 MYA, providing ample time for divergence among Marbled Murrelets to occur. Conservative variation in these characters may be related to adaptations to a marine environment where birds spend most of their time. Divergence between ground- and tree-nesting Marbled Murrelets may yet occur if isolation caused by different nesting behaviors prevents gene flow between them.

Although our sample sizes were small, a concordant pattern has emerged from three different data sets, external measurements, skeletal dimensions, and mtDNA. Ground-nesting and tree-nesting populations of Marbled Murrelets have evolved different breeding behaviors, but this behavioral divergence has not been accompanied by extreme morphological or genetic divergence. Our data suggest that these populations probably comprise the same genetic stock. However, larger sample sizes, analyses of populations from throughout the breeding range, and additional genes are needed to better characterize genetic structure of this species.

ACKNOWLEDGMENTS

This project was funded in part by a Frank M. Chapman Award from the American Museum of Natural History and Institutional Funding from the Kutztown University Research Committee. This manuscript benefited from the constructive comments of Barry Wicklow, Jim Rising, Dan Roby, and an anonymous reviewer. The Alaska Maritime National Wildlife Refuge and the M/V Tiglax provided logistical support for much of the field work. We thank the crew of the M/V Tiglax: Captain Al Bayer, Greg Snedgen, John Jameison,

Eric Nelson, Marcia Maccone, and especially Kevin Bell for their contributions. We also thank Cal Lensink and Kathy Kuletz for use of their facilities during field work in Kachemak Bay and Prince William Sound, respectively.

LITERATURE CITED

AVISE, J. C., C. D. ANKNEY, AND W. S. NELSON. 1990. Mitochondrial gene trees and the evolutionary relationship of mallard and black ducks. Evolution 44:1109–1119.

BAKER, A. J. 1985. Museum collections and the study of geographic variation. Pp. 55–77 *in* Museum collections: their roles and future in biological research (E. H. Miller, ed.). Br. Columbia Prov. Mus. No. 25, Occasional Papers Series. Ministry of Provincial Secretary and Government Services, Victoria, British Columbia.

BARROWCLOUGH, G. 1992. Systematics, biodiversity and conservation biology. Pp. 121–143 *in* Systematics, ecology and the biodiversity crisis (N. Eldredge, ed.). Columbia University Press, New York, New York.

BERGER, A. J. 1952. The comparative functional morphology of the pelvic appendage in three genera of Cuculidae. Amer. Midl. Nat. 47:513–605.

CARTER, H. R. AND M. L. MORRISON (EDS.). 1992. Status and conservation of the Marbled Murrelet in North America. Proc. West. Found. Vert. Zool. Vol. 15. No. 1.

CRONIN, M. A., D. A. PALMISCIANO, E. R. VYSE, AND D. G. CAMERON. 1991a. Mitochondrial DNA in wildlife forensic science: species identification of tissues. Wild. Soc. Bull. 19: 94–105.

———, S. C. AMSTRUP, G. W. GARNER, AND E. R. VYSE. 1991b. Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). Can. J. Zool. 69:2985–2992.

———, E. R. VYSE, AND D. G. CAMERON. 1988. Genetic relationships between mule deer and white-tailed deer in Montana. J. Wildl. Manage. 52:320–328.

KESSLER, L. G. AND J. C. AVISE. 1985. A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. Mol. Biol. Evol. 2:109–125.

LANSMAN, R. A., R. O. SHADE, J. F. SHAPIRA, AND J. C. AVISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. J. Molecular Evol. 17:214–226.

MARCUS, L. 1982. A portable, digital printing caliper. Curator 25:223–226.

MENDENHALL, V. 1992. Distribution, breeding records, and conservation problems of the Marbled Murrelet in Alaska. Pp. 5–16 *in* Status and conservation of the Marbled Murrelet in North America (H. R. Carter and M. L. Morrison, eds.). Proc. West. Found. Vert. Zool. Vol. 15. West. Found. Vert. Zool., Camarillo, California.

NASLUND, N. J., K. J. KULETZ, M. CODY, AND D. MARKS. 1994. Tree and habitat characteristics and behavior at fourteen Marbled Murrelet tree nests in Alaska. Northwestern Nat. (in press).

PIATT, J. F., C. J. LENSINK, W. BUTLER, M. KENDZIOREC, AND D. NYSEWANDER. 1990. Immediate impact of the Exxon Valdez oil spill on marine birds. Auk 107:387–397.

——— AND R. G. FORD. 1993. Distribution and abundance of Marbled Murrelets in Alaska. Condor 95:662–669.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning, a laboratory manual. Second ed. Cold Spring Harbor Lab. Press, Cold Spring Harbor, New York.

SAS INSTITUTE. 1985. SAS user's guide: statistics (version 6.06). SAS Institute Inc., Cary, North Carolina.

SCHNELL, G. D., G. L. WORTHEN, AND M. E. DOUGLAS. 1985. Morphometric assessment of sexual dimorphism in skeletal elements of California Gulls. Condor 87:484–493.

SHIELDS, G. F. AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. J. Mol. Evol. 24:212–217.

STEIN, J. L. AND G. S. MILLER. 1992. Endangered and threatened wildlife and plants: determination of threatened status for the Washington, Oregon, and California populations of the Marbled Murrelet. Fed. Reg. 57:45328–45337.

STORER, R. W. 1945. Structural modification of the hind limb of the Alcidae. Ibis 87:433–456.

UPHOLT, W. B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic Acids Res. 4:1257–1265.

VAN VLEIT, G. 1993. Status concerns for the global population of Kittlitz's Murrelet: is the "glacial murrelet" receding? Pacific Seabird Group Bull. 20:15–16.

ZINK, R. M. AND J. C. AVISE. 1990. Patterns of mitochondrial DNA and allozyme evolution in the avian genus *Ammodramus*. Syst. Zool. 39:148–161.

——— AND D. DITTMANN. 1991. Evolution of Brown Towhees: mitochondrial DNA evidence. Condor 93:98–105.

——— AND J. V. REMSEN, JR. 1986. Evolutionary processes and patterns of geographic variation in birds. Pp. 1–69 *in* Current ornithology, Vol. 4 (R. F. Johnston, ed.). Plenum Press, New York, New York.