The Auk 129(1):44–55, 2012

Printed in USA.

RANGEWIDE POPULATION GENETIC STRUCTURE OF XANTUS'S MURRELET (*SYNTHLIBORAMPHUS HYPOLEUCUS*)

Tim P. Birt,[1,4] Harry R. Carter,[2,5] Darrell L. Whitworth,[2] Anne McDonald,[1] Scott H. Newman,[3] Franklin Gress,[2] Eduardo Palacios,[2,6] Josh S. Koepke,[2] and Vicki L. Friesen[1]

[1]*Department of Biology, Queen's University, Kingston, Ontario K0H 2H0, Canada;*
[2]*California Institute of Environmental Studies, 3408 Whaler Avenue, Davis, California 95616, USA; and*
[3]*Animal Health Service, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, Rome 00153, Italy*

Abstract.—Population genetic structure was characterized in Xantus's Murrelet (*Synthliboramphus hypoleucus*) by analyzing variation in the mitochondrial control region (505 samples) and 12 microsatellite loci (428 samples) in birds captured at all 13 current breeding areas in southern California and northwestern Baja California, Mexico. The two types of molecular markers were consistent in revealing strong genetic differentiation between the two currently recognized subspecies (*S. h. hypoleucus* and *S. h. scrippsi*) and little or no differentiation among breeding areas within subspecies. Estimates of gene flow were essentially zero, and no evidence for admixture was found. Gene flow among breeding locations within subspecies, on the other hand, was seemingly high. Given these genetic results, as well as clear morphological differences between the subspecies and the apparent lack of interbreeding at breeding areas where the two forms are sympatric, we suggest that two species be recognized. Both forms are genetically distinct from Craveri's Murrelet (*S. craveri*), a closely related species whose breeding range partially overlaps that of Xantus's Murrelet. Taxonomic subdivision of Xantus's Murrelet introduces new conservation concerns, especially for *S. h. hypoleucus*, which urgently requires greater study and protective efforts. *Received 13 January 2011, accepted 27 September 2011.*

Key words: conservation genetics, control region, gene flow, microsatellite, mitochondrial DNA, mtDNA, population genetic structure, *Synthliboramphus hypoleucus*, Xantus's Murrelet.

Estructura Genética Poblacional en Toda la Distribución de(*Synthliboramphus hypoleucus*)

Resumen.—Se caracterizó la estructura genética de las poblaciones de *Synthliboramphus hypoleucus* mediante el análisis de la variación en la región control mitocondrial (505 muestras) y en 12 loci de microsatélites (428 muestras) en aves capturadas en las 13 áreas de reproducción actuales en el sur de California y el noreste de Baja California, México. Los dos tipos de marcadores moleculares fueron consistentes al revelar una fuerte diferenciación genética entre las dos subespecies reconocidas (*S. h. hypoleucus y S. h. scrippsi*) y poca o ninguna diferenciación entre las zonas de reproducción dentro de las subespecies. Los estimados de flujo genético fueron esencialmente cero y no se encontró evidencia de entrecruzamiento. Por otro lado, el flujo genético entre zonas de reproducción dentro de las subespecies fue aparentemente alto. Con base en estos resultados genéticos, y considerando las diferencias morfológicas claras entre las subespecies y la falta aparente de entrecruzamiento en las zonas reproductivas donde ambas subespecies son simpátricas, sugerimos que se reconozcan dos especies diferentes. Ambas formas son genéticamente distintas de *S. craveri*, una especie cercanamente relacionada cuya área de distribución reproductiva se sobrepone parcialmente con la de *S. hypoleucus*. La subdivisión taxonómica de *S. hypoleucus* introduce nuevas preocupaciones sobre la conservación, especialmente para *S. h. hypoleucus*, que requiere urgentemente de mayores estudios y esfuerzos para protegerla.

A current issue in avian taxonomy is the uncertain validity of many currently recognized subspecies (Collar 2003, James 2010). In particular, many bird species from Pacific Ocean islands appear to contain subspecies that are actually valid species (Pratt 2010). Atlantic island avifaunas also contain good species lumped as subspecies (Benkman 1993, Hazevoet 1995), as well as previously unrecognized cryptic species (e.g., Friesen et al. 2007, Bolton et al. 2008). Overlumping has serious consequences, not the least of

[4]E-mail: tim.birt@queensu.ca
[5]Present address: Carter Biological Consulting, 1015 Hampshire Road, Victoria, British Columbia V8S 4S8, Canada.
[6]Present address: Centro de Investigación Científica y de Educación, Superior de Ensenada, Baja California, Miraflores 334 Col. Bellavista, La Paz, Baja California Sur 23050, Mexico.

The Auk, Vol. 129, Number 1, pages 44–55. ISSN 0004-8038, electronic ISSN 1938-4254. DOI: 10.1525/auk.2011.11011

which is that it leads to skewed biodiversity estimates that affect conservation efforts. Recent genetic studies within the family Alcidae have helped to resolve some taxonomic and conservation issues, especially for murrelets in the genus *Brachyramphus*. For example, molecular data showed that one of the two designated subspecies of the Marbled Murrelet (*Brachyramphus marmoratus perdix*) is a different species, the Long-billed Murrelet (*B. perdix*; Zink et al. 1995, Friesen et al. 1996). Knowledge that the global range of the Marbled Murrelet simply does not include Asia has been critical for focusing conservation efforts in North America. In addition, concerns have been growing about conservation of the less common Long-billed Murrelet in northeast Asia.

Much past confusion also existed regarding taxonomic relationships within murrelets in the genus *Synthliboramphus*, and few samples were available for genetic analyses (Jehl and Bond 1975). To improve population knowledge in relation to conservation efforts, we undertook a study of molecular genetic variation in Xantus's Murrelet (*Synthliboramphus hypoleucus*), an at-risk seabird that is currently state-listed as threatened in California (Burkett et al. 2003) and endangered in Mexico (Secretaría de Medio Ambiente y Recursos Naturales 2002), with a geographically restricted global breeding distribution and small global numbers (<9,000 breeding pairs; Karnovsky et al. 2005). Surveys in 1991–2008 detected birds attending 13 breeding areas (all islands) extending from San Miguel Island (~34°N) in the northwestern Southern California Bight to the San Benito Islands (~28°N) off central-western Baja California. Two subspecies are currently recognized (American Ornithologists' Union 1998): *S. h. hypoleucus* (described by Xantus 1859), the sole subspecies breeding at Guadalupe Island; and *S. h. scrippsi* (described by Green and Arnold 1939), the primary or sole subspecies at 12 other breeding areas (Carter et al. 2005; Fig. 1). These subspecies are most easily distinguished by facial plumage, especially by the presence of white feathers above and in front of the eye in *S. h. hypoleucus* (vs. black feathers in this area in *S. h. scrippsi*), but differences also exist in bill length and depth, tarsus length, and back and wing lining plumage color (Green and Arnold 1939, Jehl and Bond 1975).

Numbers of Xantus's Murrelets declined at most or perhaps all breeding areas during the 19th and early 20th centuries, mainly because of introduced predators, loss and alteration of breeding habitats, and oil pollution (Drost and Lewis 1995, McChesney and Tershy 1998, Carter et al. 2000, Roth et al. 2005). Although numbers or reproductive success at certain breeding areas are still declining or apparently below (poorly known) historical levels (Burkett et al. 2003, H. Carter and D. Whitworth unpubl. data), much progress has occurred with conservation efforts (see below). Given incomplete knowledge of the status of Xantus's Murrelet, several studies were conducted in 1991–2008 to investigate current abundance and distribution across its entire breeding range (Drost and Lewis 1995, Keitt 2005, H. Carter and D. Whitworth unpubl. data). As part of that effort, blood samples were collected for an examination of rangewide population genetic structure. In the present study, our objectives were to estimate genetic differentiation and gene flow between *S. h. scrippsi* and *S. h. hypoleucus*, and among breeding areas within subspecies. We also examined the genetic relationship between both subspecies of Xantus's Murrelet and the closely related Craveri's Murrelet (*S. craveri*). Craveri's Murrelet breeds primarily in the Gulf of California (Jehl and Bond 1975, DeWeese and Anderson 1976), but historical records

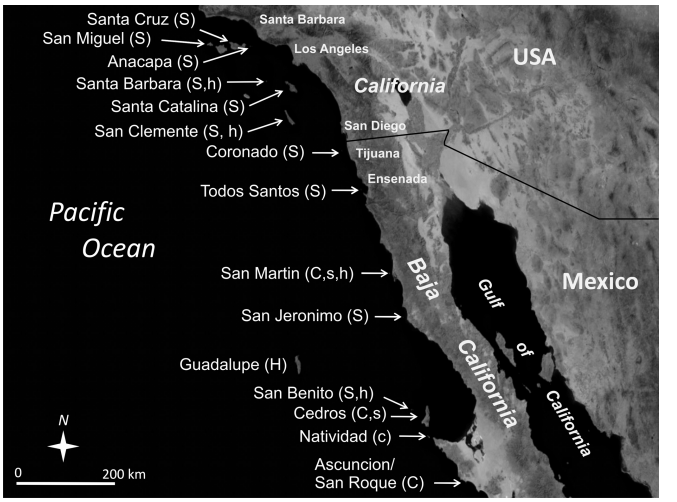

FIG. 1. Breeding areas of *Synthliboramphus* in southern California and northwestern Baja California, Mexico. Uppercase letters after island names indicate the most prevalent taxon breeding there: S = *S. hypoleucus scrippsi*, H = *S. h. hypoleucus*, and C = *S. craveri*. Lowercase letters indicate presence, occasional breeding, or historical breeding (see text).

and recent surveys indicate that small numbers breed sympatrically with Xantus's Murrelets at the San Benito Islands and possibly at nearby islands in central western Baja California (Jehl and Bond 1975, H. Carter and D. Whitworth unpubl. data).

Determination of species limits can be difficult when allopatric distributions complicate the identification of reproductive isolating mechanisms (Mayr 1963). However, both subspecies of Xantus's Murrelet currently breed sympatrically with Craveri's Murrelet at the San Benito Islands, a natural test of the distinctness of these taxonomic entities. In addition, individual Xantus's Murrelets with facial plumage intermediate between *S. h. hypoleucus* and *S. h. scrippsi* are present on these islands and have been regarded as evidence for genetic introgression among these subspecies (Jehl and Bond 1975).

METHODS

Sampling.—Breeding by Xantus's Murrelets has been confirmed (e.g., through discoveries of active breeding sites and nocturnal colony attendance) at 11 breeding areas in southern California and northwestern Baja California, and is suspected (e.g., through attendance) at Cedros and San Martín islands. Breeding by Craveri's Murrelets has been confirmed at Asuncíon–San Roque and Cedros islands and is suspected at San Benito and San Martín islands (Drost and Lewis 1995; Whitworth et al. 1997, 2000; Carter et al. 2005; H. Carter and D. Whitworth unpubl. data). Blood samples were obtained from after-hatch-year birds captured at these 14 current breeding areas between 1996 and 2008 (Table 1 and Fig. 1). No samples were obtained at Natividad Island, where historical breeding by Craveri's Murrelet only has been recorded and where current breeding is doubtful (Keitt 2005; see below). Following Carter et al. (2005), we define "breeding area" as a distinct island or a group of islands with clear geographic separation from other breeding areas. Some breeding areas are relatively closely spaced (e.g., Santa Cruz and Anacapa islands are only 7 km apart),

Table 1. Sampling information for Xantus's and Craveri's murrelets from breeding areas in southern California and northwestern Baja California, Mexico. Breeding areas are ordered from north to south within each species or subspecies. Subspecies were designated by morphology.

Species or subspecies	Breeding area	Collection year	Number of samples[a]	Number analyzed (CR/M)[b]	Collectors[c]
Synthliboramphus hypoleucus scrippsi	San Miguel	2007	10	10/10	CIES
	Santa Cruz	2008	15	15/15	CIES
	Anacapa	2000	6	6/6	HSU, UCD
	Anacapa	2002	34	29/30	HSU, USGS, UCD
	Santa Barbara	1996	80	79/79	UCD, USGS, HSU
	Santa Barbara	1997	105	95/17	UCD, USGS, HSU
	Santa Barbara	2000	20	18/0	HSU, UCD
	Santa Barbara	2002	35	0/0	HSU, UCD
	Santa Catalina	2008	12	12/12	CIES, HSU
	San Clemente	2008	6	6/6	CIES, HSU
	Coronado	2005	65	65/65	CIES, WT
	Todos Santos	2005	30	30/30	CIES, WT
	San Jerónimo	2007	16	16/16	CIES
	San Jerónimo	2008	50	16/32	CIES
	San Martín[d]	2008	2	2/2	CIES
	Cedros[d]	2007	2	2/2	CIES
	San Benito	2002	27	26/27	HSU, IC
	Subtotal		515	427/349	
S. h. hypoleucus	San Clemente[d]	2008	6	6/6	CIES
	San Martín[d]	2008	1	1/1	CIES
	San Benito	2002	13	12/13	HSU, IC
	Guadalupe	2004	6	6/6	IC
	Guadalupe	2007	50	50/50	CIES
	Subtotal		76	75/76	
S. h. intermediate	San Benito	2002	3	3/3	HSU, IC
	Subtotal		3	3/3	
S. craveri	San Martín	2008	5	5/5	CIES
	Cedros	2007	9	9/9	CIES
	Asuncíon/San Roque[e]	2007	6	6/6	CIES
	Subtotal		20	20/20	
Total			614	525/448	

[a]All blood samples collected at each breeding area, except for Santa Barbara Island, where additional samples were taken for several studies in 1994–1997, but many were not retained for genetic analyses.
[b]CR = control region and M = microsatellites.
[c]CIES = California Institute of Environmental Studies, HSU = Humboldt State University, UCD = University of California Davis, USGS = U.S. Geological Survey, WT = Wildlife Trust, and IC = Island Conservation.
[d]Breeding uncertain.
[e]All birds were captured at San Roque.

whereas others are more widely spaced (e.g., Guadalupe Island is >250 km from other breeding areas). Various surveys (H. Carter and D. Whitworth unpubl. data) indicate that murrelets apparently breed along only parts of coastlines of larger islands (>30 km long; e.g., Santa Cruz, Guadalupe, Cedros, San Clemente, and Santa Catalina islands) and that breeding appears to be fairly continuous around smaller islands (e.g., Anacapa, Santa Barbara, Coronado, Todos Santos, San Jerónimo, and San Benito islands). San Miguel Island is intermediate in size, with breeding only on parts of the north side. We reserve the use of "population" for genetic populations defined below.

Birds were captured at night during the pre-incubation or incubation periods (March–May) on the sea surface within 300 m of shore. Breeding status could not be confirmed because most captured birds did not have brood patches. However, at this time of year, nocturnal congregations of *Synthliboramphus* within ~500 m of breeding areas are almost certainly local breeders or future local breeders (Sealy 1975; Gaston 1992; Whitworth et al. 1997, 2000; Hamer et al. 2005). Note that accidental inclusion of birds that were not local breeders would inflate gene flow estimates. Thus, gene flow between subspecies of Xantus's Murrelets, between Xantus's and Craveri's murrelets, and among breeding areas within species or subspecies may be lower than we estimate.

Murrelets attending nocturnal at-sea congregations beside breeding areas were captured from a 4.3-m inflatable boat by focusing a continuous spotlight beam on them as we approached and dip netting them when close enough to reach (Whitworth et al. 1997). Captured murrelets were assigned to species or subspecies on the basis of facial plumage and coloration of wing lining (Jehl and Bond 1975). Blood samples were collected from metatarsal or brachial veins and either blotted on strips of filter paper and

dried (mainly 2007–2008 samples) or transferred to tubes, centrifuged, and the cell fraction separated and frozen (1996–2005 samples; Whitworth et al. 2000, Newman et al. 2005).

Three birds from the San Benito Islands had facial plumage intermediate between *S. h. scrippsi* and *S. h. hypoleucus* (score = 2; Jehl and Bond 1975). These were excluded from most population-level genetic analyses because their subspecies status was uncertain. In addition, samples of *S. h. hypoleucus* from San Clemente Island (n = 6) and San Martín Island (n = 1), and *S. h. scrippsi* from Cedros Island (n = 2) and San Martín Island (n = 2), were excluded from most population-level analyses because either the number of samples was too small for reliable estimation of allele frequencies or breeding at the capture area was uncertain (H. Carter and D. Whitworth unpubl. data). However, all samples were included in other analyses (e.g., construction of a haplotype tree and tests for genetic introgression) between subspecies.

DNA preparation.—DNA was prepared from blood cells using standard phenol-chloroform extraction (Sambrook et al. 1989, Friesen et al. 2005). For a small number of samples, DNA was isolated using the DNeasy method following the vendor's instructions (Qiagen, Mississauga, Ontario).

Mitochondrial DNA.—The mitochondrial control region was initially amplified from one Xantus's Murrelet using general alcid polymerase-chain-reaction (PCR) primers BmaH600 and ND6 (Friesen et al. 2005), which amplify a DNA segment of ~650 base pairs (bp) between the genes for ND6 and the central conserved domain of the control region. Sequences derived from this segment were used to design primers xamu-L60 (5′-GCATACGAGCGGTGTGCGTA-3′) and xamu-H575 (5′-GGTGATATATAGCCGACCAGA-3′), which amplify a DNA segment (~500 bp) containing domain I and part of domain II of the control region (Baker and Marshall 1997). These primers were employed for amplifying and sequencing all remaining samples. Most samples were sequenced in one direction using primer H575, although in cases in which sequences were ambiguous, both strands were sequenced. Sequences were trimmed to a common fragment length of 478 bp prior to analysis. Amplifications were performed as described in Friesen et al. (2005) or using Multiplex Mix (Qiagen) according to the vendor's instructions. The PCR temperature profile consisted of 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 45 s. The PCR products were sequenced on a 3730XL DNA Analyzer at the Génome Québec Innovation Centre, Montreal.

Microsatellites.—Length variation was assayed at 12 microsatellite loci using PCR primers and protocols described by Birt et al. (2010). Amplifications were done in a volume of 10 μL containing Multiplex Mix, PCR primers (1 μM each), and a third primer, D4-labeled M13F (0.045 μM). A touchdown PCR temperature profile was employed with an initial annealing temperature of 60°C dropping by 0.5°C per cycle until a final annealing temperature of 52°C was achieved. Twenty-six additional cycles were done at this temperature. Alleles were sized on a Beckman CEQ 8000 automated DNA sequencer at the Core Genotyping Facility in the Department of Biology at Queen's University, Kingston, Ontario.

Tests of assumptions and variability.—Most uses of molecular markers for population genetic inference make several assumptions, especially that sequence variation is neutral to selection, that genotype frequencies do not deviate from Hardy-Weinberg or genotypic equilibrium, and that populations are in equilibrium between mutation and genetic drift (e.g., not growing or declining). Control region variation was tested for deviations from neutrality using the tests of Ewens-Watterson (Ewens 1972, Watterson 1978) and Chakraborty (1990), and microsatellite variation was tested for departures from Hardy-Weinberg and genotypic equilibrium using the software package ARLEQUIN, version 3.1 (Excoffier et al. 2005). Benjamini-Yekutieli (Narum 2006) corrections were used to reduce Type I errors when multiple tests of a single hypothesis were made.

We tested for evidence of historical population growth or decline in control region variation using FLUCTUATE, version 1.4 (Kuhner et al. 1998), which uses a maximum likelihood approach based on coalescent theory to estimate θ ($2N_f\mu$, where N_f is the long-term genetically effective population size in numbers of females and μ is the mutation rate site^{-1} generation^{-1}) and population growth rate (g; μ generation^{-1}). We used the empirical transition:transversion ratio (1.5) and base frequencies (see below), and Watterson's estimate for the initial value of θ. However, final results were robust even to large changes in these initial values. To test whether g differed from zero, the program was rerun with g constrained to zero, and a chi-square statistic was calculated as 2 (ln L[max] – ln L[0]), where ln L[max] is the maximum ln likelihood value found by the program and ln L[0] is the ln likelihood value at g = 0. This statistic was compared with the critical values of χ^2 at df = 1.

We also tested for evidence of recent population growth or decline in microsatellite variation using BOTTLENECK, version 1.2.02 (Cornuet and Luikart 1996), which tests for heterozygosity excess or deficiency across loci. Analyses were conducted using the stepwise mutation model, which is more conservative than the infinite allele or two-phase models when testing for population decline. Significance was assessed using the Wilcoxon test (implemented in BOTTLENECK), which is more sensitive than either the sign or standardized differences tests (Cornuet and Luikart 1996), using a two-tailed test (for heterozygosity excess or deficiency) and 1,000 iterations of the data.

Haplotype diversities (h), nucleotide diversities (π, control region data), and expected heterozygosities (H_E) were estimated using ARLEQUIN, and allelic richness was estimated using FSTAT, version 2.9.3.2 (Goudet 2001). Because genetic differentiation among breeding areas within subspecies (Xantus's Murrelet) or species (Craveri's Murrelet) was weak (see below), samples from different breeding areas were combined for each species or subspecies for all these analyses.

Population genetic structure.—To infer the extent of phylogeographic structure among control region haplotypes, a statistical parsimony tree was constructed using TCS, version 1.21 (Clement et al. 2000). The few (10) ambiguous connections (loops) in the tree were broken following the rules of Crandall and Templeton (1993). Analysis of molecular variance (AMOVA; Excoffier et al. 1992), as implemented in ARLEQUIN, was used to index the geographic distribution of variation both in control region sequences (Φ_{ST}) and in microsatellite loci (F_{ST}). Hierarchical AMOVAs also were conducted to estimate the proportion of variation due to subspecies (Φ_{CT} and F_{CT}) versus breeding area within subspecies (Φ_{SC} and F_{SC}). For the control region data, Kimura's two-parameter model of substitution (Kimura 1980) was applied with a gamma parameter of 0.45 (Baker and Marshall 1997). Significance was determined by randomization using 10,000 data permutations.

The program STRUCTURE, version 2.3.3 (Pritchard et al. 2000, Falush et al. 2003, Hubisz et al. 2009), was used to infer population structure based on microsatellite variation. This program estimates the number of genetic populations (*k*) within a sample, using a Bayesian approach to minimize deviations from Hardy-Weinberg and genotypic equilibrium, and then calculates the probabilities that each individual belongs to each genetic population. The program was run under both the admixture and no-admixture models with correlated allele frequencies, using sampling site as prior information. Use of alternative settings did not change the most likely value of *k*, although assignment probabilities for individual birds differed slightly. The most likely value of *k* was inferred both directly from the ln likelihood values (Pritchard et al. 2010) and using the method of Evanno et al. (2005). Because STRUCTURE can have difficulty detecting genetically differentiated populations in species with hierarchical population genetic structure, analyses were repeated for *S. h. hypoleucus*, *S. h. scrippsi*, and *S. craveri* individually. All runs involved a burn-in of 10,000 iterations followed by 100,000 iterations. Visual inspection of likelihood plots indicated that this run duration was sufficient for likelihood values to stabilize. The histogram of individual assignment probabilities (*Q* values) from the admixture analysis with the highest ln likelihood was redrawn for display using DISTRUCT, version 1.1 (Rosenberg 2004).

Gene flow.—Gene flow (hybridization and introgression) between *S. h. scrippsi* and *S. h. hypoleucus* was estimated using three approaches. Long-term female-mediated gene flow was estimated from control region variation using an isolation-with-migration model implemented in the software IMa (Hey and Nielsen 2007). IMa simultaneously estimates six parameters, all scaled by the neutral mutation rate, μ (in mutations $year^{-1}$ $locus^{-1}$): the initial divergence time (*t*), two gene flow parameters (m_{ij}, the effective forward-in-time female-mediated gene flow rate from population *j* to *i*), the genetically effective sizes of populations 1 and 2 (Θ_1 and Θ_2 respectively), and the ancestral population size (Θ_A). The IMa analysis included all 73 *S. h. hypoleucus* individuals, and 73 individual *S. h. scrippsi* that were randomly sampled from the full data set to equalize the sample sizes and reduce the run time. The analysis was run using the Hasegawa-Kishino-Yano nucleotide substitution model (HKY; Hasegawa et al. 1985). Bayesian priors were placed on each parameter such that posterior distributions peaked and descended again. The analysis was run for >100 million generations, sampling genealogies every 1,000 generations. The first 1 million generations were discarded as burn-in. We ensured convergence of the Markov chain Monte Carlo (MCMC) process by monitoring trend plots of all parameters, requiring that all effective sample sizes were at least 100. The analysis was run three times and results were consistent among runs.

Contemporary gene flow also was estimated from microsatellite variation using BAYESASS, version 1.3 (Wilson and Rannala 2003), which employs a Bayesian maximum likelihood analysis to conduct molecular assignments. The program was run with 3 million MCMC iterations with a burn-in of 1 million iterations and a sampling frequency of 2,000.

The program NEW HYBRIDS, version 1.1 beta (Anderson and Thompson 2002), was used both to determine the ancestries of three birds with intermediate facial plumage and to detect potential genetic introgression between *S. h. scrippsi* and *S. h. hypoleucus*. The program placed individuals into one of six genotype frequency classes (categories): pure *S. h. scrippsi*, pure *S. h. hypoleucus*, F_1 hybrid, F_2 hybrid, F_1 back-crossed to *S. h. scrippsi*, and F_1 back-crossed to *S. h. hypoleucus*. The program was run twice (to check consistency of results) with Jeffery's-type priors, a burn-in of 30,000 cycles, and an additional 300,000 cycles after the burn-in (determined from test runs to be sufficient run lengths for parameters to stabilize). The program was also run with only three genotype classes (pure *S. h. scrippsi*, pure *S. h. hypoleucus*, and F_1 hybrid) in case the data did not provide enough power to detect second-generation hybrids.

Results

Tests of assumptions and variability.—Seventy-seven control region haplotypes were detected among 525 murrelets (GenBank accession nos. JN382251–JN382328). Haplotype frequencies are shown in Supplementary Table 1 (see Acknowledgments). Several lines of evidence indicated that the amplified fragment represented a true mitochondrial control region rather than a nuclear homolog ("numt"; Lopez et al. 1994, Kidd and Friesen 1998, Ibarguchi et al. 2006). The $tRNA^{glu}$ sequence flanking the control region folded into an appropriate cloverleaf (Desjardins and Morais 1990). F- and D-Boxes were invariant and identical to those of the Marbled Murrelet (Friesen et al. 2005). Variable sites were concentrated in Domain I, which is the most variable region in birds (Baker and Marshall 1997). Nucleotide composition was typical of avian mitochondrial DNA (mtDNA; T = 28.8%, C = 29.7%, A = 26.5%, G = 15%; Baker and Marshall 1997).

Ewens-Watterson and Chakraborty tests detected one deviation from neutrality: the number of haplotypes within *S. h. scrippsi* was higher than expected (Table 2). FLUCTUATE suggested that *S. h. scrippsi* has undergone slow but significant historical population growth ($P < 0.01$), but neither *S. h. hypoleucus* nor *S. craveri* have undergone detectable changes in numbers (Table 2). Estimates of haplotype and nucleotide diversity were high and similar among species and subspecies (Table 2).

Microsatellite locus 1A-39 had significant heterozygote excesses in all three taxa (*S. h. hypoleucus*, *S. h. scrippsi*, and *S. craveri*), and loci 1A-84 and 1A-102 showed significant heterozygote deficiencies in two taxa each. Significant departures from genotypic equilibrium were detected for two pairs of loci within *S. h. scrippsi* (1A-136/2A-153 and 2A-52/2A-153), and for one pair of loci within both *S. h. hypoleucus* and *S. craveri* (1A-39/1A-89). We therefore conducted all further analyses both with and without loci 1A-39, 1A-49, 1A-102, 1A-136, and 2A-41. Results were qualitatively similar (e.g., the significance of pairwise estimates of F_{ST} did not change, although the actual estimates differed slightly, and the number of genetic populations inferred using STRUCTURE was the same, although individual assignment probabilities differed slightly), and so only results from the 7-locus data set are presented. Results for the full data set are available on request.

After removal of loci with potential null alleles, BOTTLENECK indicated a significant heterozygosity excess for *S. h. hypoleucus*, which suggests the possibility of recent population decline (Table 2). Note that this approach has low power to detect population bottlenecks, especially with only 7 loci (Cornuet and Luikart 1996); thus, failure to detect a bottleneck in *S. h. scrippsi* and *S. craveri* may not mean that a bottleneck did not occur.

TABLE 2. Diversity estimates (± SD) and results of neutrality tests for subspecies of Xantus's Murrelets and for Craveri's Murrelets: haplotype diversity (h), nucleotide diversity (π), ratio of observed to expected F (F_O/F_E) from the Ewens-Watterson test of neutrality, ratio of observed to expected numbers of alleles (n_O/n_E) from Chakraborty's test of neutrality (θ) and population growth rate (g) from the program FLUCTUATE, expected and observed heterozygosities (H_E and H_O), allelic richness averaged across loci (A_R), and observed and expected numbers of loci with heterozygosity excesses (E_O/E_E) from the program BOTTLENECK.

	Synthliboramphus hypoleucus scrippsi	*S. h. hypoleucus*	*S. craveri*
Control region			
h	0.862 ± 0.011	0.896 ± 0.021	0.884 ± 0.042
π (%)	0.715 ± 0.406	0.596 ± 0.358	0.795 ± 0.467
F_O/F_E	0.145/0.088	0.116/0.087	0.160/0.0180
n_O/n_E	41/22.6***	24/18.3	9/9.5
θ	0.140 ± 0.007	0.125 ± 0.011	0.020 ± 0.006
g	378 ± 23.1**	726 ± 51.5	250 ± 107
Microsatellites[a]			
H_O	0.502 ± 0.186	0.533 ± 0.250	0.462 ± 0.227
H_E	0.518 ± 0.189	0.567 ± 0.245	0.501 ± 0.228
A_R	4.25 ± 3.12	5.48 ± 3.41	4.71 ± 2.98
E_O/E_E	5/3.8	7/3.8*	5/3.8

Note: *P < 0.05, **P < 0.01, ***P < 0.001.
[a] After removal of loci with potential null alleles.

Population genetic structure.—Other than the haplotype of one individual that may have been misclassified (96-35; see below), no mtDNA haplotypes were shared among *S. h. scrippsi*, *S. h. hypoleucus*, or *S. craveri*. Control region sequences of *S. hypoleucus* and *S. craveri* formed separate clades on the TCS gene tree and differed by a minimum of 7 substitutions (Fig. 2). Similarly, with the exception of two haplotypes (X23 and X65), haplotypes of *S. h. hypoleucus* and *S. h. scrippsi* were reciprocally monophyletic on the gene tree and differed by a minimum of 4 substitutions.

The AMOVA for *S. hypoleucus* indicated strong structuring of control region variation among breeding areas (Φ_{ST} = 0.37, P < 0.001). Variation due to subspecies was high (Φ_{CT} = 0.66, P < 0.001), whereas variation due to breeding area within subspecies was low and not significantly different from zero (Φ_{SC} = 0.004, P = 0.27; although power to detect genetic differentiation at this level using AMOVA may be weak). Differentiation among breeding areas of Craveri's Murrelet was also very low and not different from zero (Φ_{ST} = −0.03, P = 0.68), although sample sizes were small and most of the breeding range was not sampled.

Although statistical power was low for some comparisons because of small sample sizes, Φ_{ST} estimates between pairs of breeding areas were consistent with the global estimates and hierarchical AMOVAs (Supplementary Table 2). All comparisons representing different species or subspecies were significantly different from zero, whereas no pairwise Φ_{ST} estimates within species or subspecies were significantly different from zero. Control region sequence divergence between *S. h. hypoleucus* and *S. h. scrippsi* averaged 1.74 ± 0.14%, and sequence divergence between *S. hypoleucus* and *S. craveri* averaged 2.44 ± 0.18% (Supplementary Table 2).

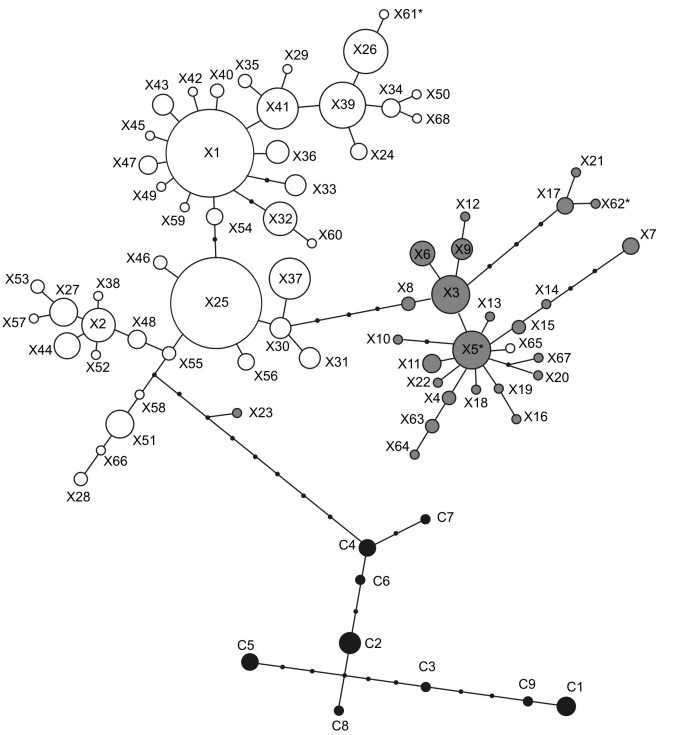

FIG. 2. Statistical parsimony tree showing substitutional relationships among control region haplotypes for Xantus's and Craveri's murrelets. Haplotype names beginning with "X" were found in Xantus's Murrelet, and names beginning with "C" were found in Craveri's Murrelet. Haplotypes represented by white were found in samples of *Synthliboramphus hypoleucus scrippsi,* haplotypes represented by gray were found in samples of *S. h. hypoleucus,* and haplotypes represented by black were found in samples of *S. craveri.* Asterisks represent haplotypes found in birds from San Benito Islands with facial plumage intermediate between *S. h. scrippsi* and *S. h. hypoleucus*. Dots represent hypothetical haplotypes not found in the samples analyzed. Haplotype frequency is proportional to symbol size.

The geographic distribution of microsatellite variation (Supplementary Table 3) was generally consistent with the pattern of control region variation. For Xantus's Murrelet, the global estimate of F_{ST} was low but significantly greater than zero (F_{ST} = 0.04, P < 0.001). Differentiation between subspecies was high (F_{CT} = 0.10, P = 0.016), and differentiation among breeding areas within subspecies was low but significantly greater than zero (F_{SC} = 0.059, P = 0.016), which indicates the existence of some genetic structure within subspecies. For Craveri's Murrelet, global F_{ST} also was small and not significantly different from zero (F_{ST} = 0.01, P = 0.39), although sample sizes were small and most of the breeding range was not sampled. Pairwise F_{ST} estimates were consistent with global estimates (Supplementary Table 2): all comparisons between species or subspecies but no comparisons within species or subspecies were significantly greater than zero.

STRUCTURE, run with either the admixture or the no-admixture model, distinguished three genetic populations (P > 0.9999; Fig. 3). Under both models, most *S. h. scrippsi* had probabilities >80% of assignment to genetic population 1, most *S. h. hypoleucus* had probabilities >80% of assignment to genetic population 2, and all *S. craveri* had probabilities >90% of

Table 3. Maximum likelihood estimates (MLEs) of IMa model parameters and 90% highest posterior densities (HPDs). Genetically effective population size estimates (Θ) are shown for *Synthliboramphus hypoleucus hypoleucus, S. h. scrippsi,* and the ancestral population. Estimates of gene flow (*m*) are interpreted forward in time (e.g., $m_{hypoleucus->scrippsi}$ is the migration rate from *S. h. hypoleucus* into *S. h. scrippsi*). The 90% HPD for *t* (initial divergence time) was undefined (see text).

Parameter	MLE	90% HPD
$\Theta_{scrippsi}$	5.68	3.43–9.08
$\Theta_{hypoleucus}$	9.58	6.33–14.43
$\Theta_{ancestral}$	1.88	0.00–41.53
t	4.25	Undefined
$m_{hypoleucus->scrippsi}$	0.11	0.02–0.33
$m_{scrippsi->hypoleucus}$	0	0.00–0.10

assignment to genetic population 3 (Fig. 3). One individual sampled at Santa Barbara Island (sample 96-35, within the range of *S. h. scrippsi*) had a 100% probability of belonging to genetic population 2, which otherwise represents *S. h. hypoleucus*, and had an mtDNA haplotype otherwise found only in *S. h. hypoleucus*. BAYESASS (below) also assigned this bird to *S. h. hypoleucus* with 100% probability. The plumage of this bird (captured 17 April 1996; no brood patch) was not recorded as different from *S. h. scrippsi*, but it had a culmen length (20.6 mm) above the range (15.6–20.3; n = 97) for *S. h. scrippsi* in the Channel Islands and Coronado Islands and above the mean (± SE) for males (19.32 ± 0.19; n = 27) and females (19.85 ± 0.16; n = 22) for *S. h. hypoleucus* at Guadalupe Island (Jehl and Bond 1975). We could not confirm that this individual was breeding at Santa Barbara Island and removed it from further analyses. In addition, one bird sampled as *S. h. scrippsi* from Cedros Island (XM-CD-07-09) had a probability of 50% (admixture model) to 80% (no-admixture model) of assignment to genetic population 3, otherwise represented by *S. craveri*. This bird (captured 7 April 2007; no brood patch) had facial plumage and morphometrics typical of *S. h. scrippsi*, but it also had a hint of a small neck collar as occurs in *S. craveri* (Jehl and Bond 1975, H. Carter and D. Whitworth unpubl. data). No other individuals with uncertain assignment probabilities from STRUCTURE had probabilities from NEWHYBRIDS of <80% of belonging to their correct (phenotypic) subspecies (see below). No genetic substructure was detected within *S. h. scrippsi*, *S. h. hypoleucus*, or *S. craveri* following hierarchical analysis with STRUCTURE.

Gene flow.—The posterior distribution of the gene flow parameter from *S. h. scrippsi* into *S. h. hypoleucus* from IMa peaked at zero (90% highest posterior density [HPD] = 0.00–0.10; Table 3 and Supplementary Fig. 1). The maximum likelihood estimate (MLE) of long-term gene flow from *S. h. hypoleucus* into *S. h. scrippsi* was low but significantly different from zero (peak = 0.11, 90% HPD = 0.02–0.33). The posterior distribution of the divergence time parameter (*t*) peaked at 4.25, but the right-hand tail of the posterior distribution for *t* did not descend to zero so we could not estimate the 90% HPD. Regardless, the MLE of *t* remained relatively unchanged if we modified the prior for this parameter (data not shown).

Initial analyses of microsatellite data with BAYESASS indicated nonmigration (i.e., residency) rates too low (less than ~83%) to enable reliable estimation of gene flow among breeding areas within species or subspecies (i.e., gene flow was too high to estimate reliably; Wilson and Rannala 2003). Samples were therefore combined within each subspecies or species for a subsequent analysis, resulting in residency rates high enough for reliable estimation of gene flow (Supplementary Table 4). Other than sample 96-35 (mentioned under results for STRUCTURE) and three individuals that could not be assigned with high probability to any population (probably because of incomplete data), all murrelets had a high probability (>87%) of originating within the species or subspecies from which they were sampled (i.e., no immigrants or birds with immigrant ancestry were detected. Results indicated that contemporary gene flow between these taxa is very low

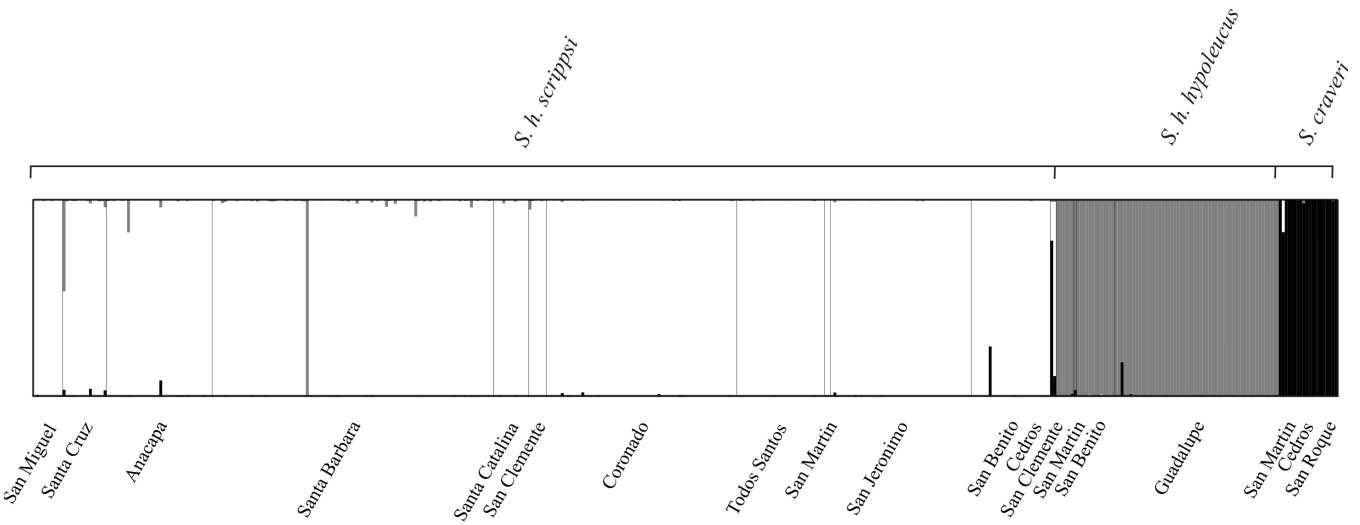

Fig. 3. Probabilities of assignment (generated by STRUCTURE) of individual Xantus's and Craveri's murrelets to each of three genetic populations. Vertical bars represent individuals; black vertical lines separate breeding areas, which are ordered from north (left) to south (right) within each subspecies or species. Genetic populations are represented by different shades of gray. Labels on the *x*-axis are breeding areas sampled.

(<0.01), with all 95% confidence intervals overlapping zero (Supplementary Table 4).

For three individuals with intermediate facial plumage sampled from the San Benito Islands, NEW HYBRIDS assigned one (xamu 259) to *S. h. scrippsi* with 100% confidence and two (xamu 273 and xamu 286) to *S. h. hypoleucus* with 91% and 98% confidence, respectively. These results are consistent with mtDNA haplotypes. NEW HYBRIDS was not able to assign five individuals from Guadalupe Island to any genetic category with confidence, probably because of missing data. Otherwise, all individuals were assigned to the correct (phenotypic) species or subspecies with >80% confidence, and no individual was assigned to any hybrid category with >26% confidence. When the program was re-run with only three genotype classes (pure *S. h. scrippsi*, pure *S. h. hypoleucus*, and F_1 hybrid), all individuals were assigned to their phenotypic subspecies with >86% probability.

Discussion

Genetic relationship between S. h. scrippsi *and* S. h. hypoleucus.—We found little population genetic structure within either *S. h. scrippsi* or *S. h. hypoleucus*. By contrast, both mitochondrial and nuclear loci were strongly differentiated between these subspecies. Specifically, indices of population differentiation were high and significantly different from zero for both types of markers, the program STRUCTURE defined two genetic populations, and strong phylogeographic structure was apparent on the mtDNA gene tree. Furthermore, little evidence was found for hybridization or genetic introgression between *S. h. scrippsi* and *S. h. hypoleucus*, even where they breed sympatrically at the San Benito Islands. Although results from IMa suggest that low but significant female-mediated gene flow has occurred from *S. h. hypoleucus* into *S. h. scrippsi*, gene flow could represent shared ancestral variation, low-level gene flow since initial divergence, or recent secondary contact. Otherwise, none of the estimates of gene flow from IMa or BAYESASS differed from zero, and no evidence of hybridization or introgression was found using either BAYESASS or NEW HYBRIDS. Notably, birds with intermediate facial plumage, which occur regularly at the San Benito Islands, did not appear to represent F_1 or backcrossed hybrids. A similar result was found for Kittlitz's Murrelets (*B. brevirostris*) with unusual facial plumage in the Gulf of Alaska (Pacheco and Friesen 2002). The lack of genetic introgression, particularly in regard to birds with intermediate facial plumage, contradicts previous interpretations at the San Benito Islands (Jehl and Bond 1975). Mixed pairs of *S. h. hypoleucus*, *S. h. scrippsi*, or intermediate facial patterns constituted 30% (n = 20) of pairs examined at the San Benito Islands in 2003–2004 (Wolf et al. 2005), and a mixed pair of *S. h. hypoleucus* and an intermediate were recorded at Santa Barbara Island in 1977–1978 (Winnett et al. 1979). More work is needed to examine pairing behavior and to genetically characterize additional birds with intermediate facial plumage at the San Benito Islands.

The genetic differences separating *S. h. hypoleucus* and *S. h. scrippsi*, combined with their morphological differences (see above), strongly suggest that these taxa represent reproductively isolated species. We feel that the two currently recognized subspecies of Xantus's Murrelet were inappropriately lumped into the same species when *S. h. scrippsi* was first described in 1939, likely because of plumage similarity and overlap in morphological characters. Reproductive isolation may be maintained by prezygotic isolating mechanisms such as sexually selected traits (West-Eberhard 1983), geographic isolation of breeding areas, or differences in timing of reproduction (e.g., Friesen et al. 2007). For both taxa, little information is available about breeding behavior and mate choice preferences. The vocalizations of the two subspecies appear to be quite different, but a quantitative comparison is needed (H. Carter and D. Whitworth pers. obs.). Although both subspecies (and seabirds in general) are highly mobile and are capable of moving between breeding areas, the breeding area for almost all *S. h. hypoleucus* (i.e., Guadalupe Island) is ≥250 km distant and much farther offshore than the next-nearest breeding area (San Benito Islands) where *S. h. scrippsi* is the primary breeding subspecies. Breeding at sites in central-western Baja California also often occurs 1 to 2 months earlier than breeding by *S. h. scrippsi* in northwestern Baja California and southern California (Drost and Lewis 1995, Keitt 2005, Wolf et al. 2005, H. Carter and D. Whitworth unpubl. data). We also suspect greater preference for offshore foraging habitats during the breeding season by *S. h. hypoleucus*. Most Xantus's Murrelets (both subspecies combined) forage 26–150 km from the mainland (Karnovsky et al. 2005). However, *S. h. hypoleucus* probably forage farther offshore, on average, than *S. h. scrippsi* during the pre-incubation and incubation periods when they attend breeding habitats at Guadalupe Island (~250 km from the mainland), given potential foraging distances <150 km from colonies (Whitworth et al. 2000). Longer and thinner bills exhibited by *S. h. hypoleucus* could be adaptive for foraging in far offshore habitats while shorter and thicker bills of *S. h. scrippsi* might be better suited for more inshore foraging over the continental shelf. More work is needed to examine at-sea distribution, diet, and foraging habits of the two subspecies.

Although we argue that the two Xantus's Murrelet subspecies are almost certainly different species with isolating mechanisms other than allopatric breeding areas, two alternative hypotheses could result in an apparent absence of gene flow: (1) the two subspecies are sympatric at only one breeding area, with few individuals having the opportunity to interbreed; or (2) the subspecies were previously completely allopatric and only recently came into contact. Limited historical information is available to address these hypotheses, but sufficient evidence exists to discount them. Even though the first *S. h. hypoleucus* was found at the San Benito Islands in 1968, relatively large numbers of *S. h. hypoleucus* (42%; n = 48) were recorded in the early 1970s (Jehl and Bond 1975). Thus, substantial genetic differences between the two subspecies have been maintained despite potential for gene flow for at least 34 years of documented sympatry between 1968 and blood sampling in 2002.

Genetic relationship between S. hypoleucus *and* S. craveri.—Results from STRUCTURE as well as morphology suggested that one bird from Cedros Island (XM-CD-07-09) may represent a hybrid between *S. h. scrippsi* and *S. craveri*. Otherwise, analyses of both mitochondrial and nuclear DNA indicate that Xantus's and Craveri's murrelets are genetically distinct and that gene flow between them is essentially zero.

After initial descriptions of Xantus's Murrelet (i.e., *S. h. hypoleucus*) in 1859 (Xantus 1859) and Craveri's Murrelet in 1865 (Violani and Boano 1990), debate about their distinct species

status ensued (summarized in Jehl and Bond 1975, Carter et al. 2005). Until recently, the two species were generally considered to be allopatric, with Xantus's Murrelet breeding between southern California and central western Baja California and Craveri's Murrelet breeding only within the Gulf of California. However, Craveri's Murrelets had been reported off western Baja California during the breeding season since 1897 (Cooke 1916, Jehl and Bond 1975, DeWeese and Anderson 1976, Carter et al. 2005). Breeding was confirmed in central-western Baja California in 2007 when birds with brood patches were captured and eggshells were discovered at Cedros and Asuncíon–San Roque islands (H. Carter and D. Whitworth unpubl. data). In addition, a Craveri's Murrelet egg specimen collected in 1919 by H. A. Edwards at Natividad Island resurfaced (California Academy of Sciences no. 10043; R. A. Erickson pers. comm.), which gave new credence to earlier claims of breeding in this region. For example, the type specimen of the Craveri's Murrelet had reportedly been collected at Natividad Island, but Cooke (1916) later dismissed this collection locality as unlikely. Sympatry of the two species has probably been overlooked because of relatively small numbers of Craveri's Murrelets breeding in western Baja California, possible earlier or later breeding by Craveri's Murrelets compared with Xantus's Murrelets (DeWeese and Anderson 1976, H. Carter and D. Whitworth unpubl. data), and the relatively low level of research effort in this region.

Conservation implications.—Numbers of Xantus's Murrelets likely declined extensively at many colonies in the late 19th and early 20th centuries because of several factors, especially (1) introduced predators, particularly cats (*Felis catus*) and Black Rats (*Rattus rattus*); (2) loss and alteration of breeding habitats (because of ranching, introduced plants, etc.), which may have led to enhanced predation by natural predators, particularly Deer Mice (*Peromyscus maniculatus*) and Barn Owls (*Tyto alba*); and (3) factors at sea (e.g., oil pollution, bright lights, possible prey changes) (Drost and Lewis 1995, McChesney and Tershy 1998, Carter et al. 2000, Roth et al. 2005). Local extirpation was suspected at several breeding areas (e.g., Jehl and Bond 1975), and analyses using BOTTLENECK suggest recent declines in *S. h. hypoleucus*. However, no direct evidence of such extirpation has been found, with the possible exception of Natividad Island for Craveri's Murrelets; presumably, murrelets were able to continue breeding in areas inaccessible to predators at most islands (Hunt et al. 1980, Whitworth et al. 2005, H. Carter and D. Whitworth unpubl. data). Introduced predators have been removed from most breeding areas in the past 40 years (McChesney and Tershy 1998, Keitt 2005), and post-eradication increases have been documented at Santa Barbara and Anacapa islands (Hunt et al. 1980, Whitworth et al. 2005). It is unknown how small local numbers became at many breeding areas, especially in Baja California, because baseline surveys were not conducted prior to predator eradications. However, numbers of Xantus's or Craveri's murrelets currently breeding at San Miguel, San Clemente, San Martín, and Asuncíon–San Roque islands are very small (<200 pairs) (Burkett et al. 2003, Keitt 2005, H. Carter and D. Whitworth unpubl. data). Although these breeding areas are at risk of future extirpation, low genetic variation is unlikely to be contributing to low population sizes and is probably not preventing future recovery.

Long-term management of Xantus's Murrelet throughout its breeding and at-sea range from southwestern Baja California to central British Columbia (Drost and Lewis 1995, Karnovsky et al. 2005) should attempt to conserve ecological and evolutionary processes (Crandall et al. 2000). This study clearly supports recognition of the two subspecies as distinct species that represent evolutionary significant units (sensu Moritz 1994) that are evolving independently and adapting to different conditions, and therefore warrant separate management. Immediate efforts are needed to protect these distinct lineages, both of which have relatively small global numbers, have restricted breeding and at-sea ranges, and face significant multiple threats. Especially urgent efforts are needed to protect *S. h. hypoleucus*, whose small global numbers (<5,000 breeding individuals) are threatened by introduced cats on large portions of Guadalupe Island, although breeding also occurs extensively on offshore islets (Keitt 2005, H. R. Carter and D. L. Whitworth unpubl. data). Having only one major global breeding area makes *S. h. hypoleucus* highly vulnerable to extinction. To date, only limited studies have been conducted on this subspecies, and its breeding biology, behavior, reproductive success, vocalizations, and overall ecology urgently need study. The better-studied and relatively more widespread and numerous *S. h. scrippsi* may be more resilient to effects at any one breeding area because of apparently high dispersal between breeding areas, but this species is still vulnerable to a variety of threats because of its small global numbers, restricted breeding range, and reduced numbers over the past century.

Acknowledgments

Supplementary materials for this article are available at dx.doi.org/10.1525/auk.2011.11011. Funding for genetic analyses was provided primarily by ChevronTexaco Corporation (CTC; G. Minnery), Dames and Moore de Mexico (E. Sangines), the Natural Sciences and Engineering Research Council (Discovery Grant to V.L.F.), and California Institute of Environmental Studies (CIES), with supplementary funding by California Department of Fish and Game (CDFG; E. Burkett), Humboldt State University (HSU; R. Golightly), and the U.S. Fish and Wildlife Service (USFWS; R. McMorran). Many breeding areas were sampled in 2007–2008 during survey work also funded by CTC, with excellent research-vessel support, primarily aboard the *Alguita* (C. Moore) and additionally the *Miss Devin* (R. Fairbanks) and *Retreiver* (D. Carlson). Other samples were obtained between 1996 and 2005 during various studies conducted by H. Carter, D. Whitworth, S. Newman, J. Takekawa, R. Golightly, C. Hamilton, and B. Keitt with assistance or funding from the U.S. Navy (T. Keeney), U.S. Geological Survey (USGS; D. Gilmer and D. Orthmeyer), CDFG (E. Burkett), University of California Davis (UCD; J. Mazet), Island Conservation, USFWS (T. Zimmerman), and Wildlife Trust. Excellent research-vessel support was provided mainly by the *Instinct* (D. Christy), *Ballena*, and *Shearwater* (Channel Islands National Marine Sanctuary; E. Cassano and S. Fangman) and additionally from the *Blitzen* (R. Spight) and *Alguita*. Many individuals kindly assisted bird captures and blood sampling in 1996–2008, but we especially thank C. Hamilton, L. Harvey, P. Hébert, B. Keitt, G. McChesney, B. McIver, and R. Young for key help on many trips. Blood-sample collection in Baja California by CIES in 2005–2008 was conducted under permits issued to E.P. by Secretaría de Medio Ambiente y Recursos Naturales (Dirección

General de Vida Silvestre). Blood-sample collection in California by HSU, UCD, USGS, and CIES and transportation of samples from Baja California and California to Canada for analyses were conducted under permits issued to many individuals and organizations. R. A. Erickson kindly provided specimen information from the California Academy of Sciences, San Francisco. E. Kershner and R. McMorran provided comments on the manuscript. For expert technical assistance we thank personnel at the Génome Québec Innovation Centre, Montreal, and Z. Sun at the Core Genotyping Facility, Biology Department, Queen's University. S. Taylor kindly conducted analyses using NEW HYBRIDS, J. Morris-Pocock generously ran the IMa analyses, and both provided very useful comments and discussions.

Literature Cited

American Ornithologists' Union. 1998. Check-list of North American Birds, 7th ed. American Ornithologists' Union, Washington, D.C.

Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217–1229.

Baker, A. J., and H. D. Marshall. 1997. Mitochondrial control region sequences as tools for understanding evolution. Pages 51–82 *In* Avian Molecular Evolution and Systematics (D. P. Mindell, Ed.). Academic Press, London.

Benkman, C. W. 1993. The evolution, ecology, and decline of the Red Crossbill of Newfoundland. American Birds 47:225–229.

Birt, T. P., Z. Sun, H. R. Carter, D. L. Whitworth, S. H. Newman, and V. L. Friesen. 2010. Polymorphic microsatellite loci for the threatened Xantus's Murrelet (*Synthliboramphus hypoleucus scrippsi*). Conservation Genetics Resources 2:59–61.

Bolton, M., A. L. Smith, E. Gomez-Diaz, V. L. Friesen, R. Madeiros, J. Bried, J. L. Roscales, and R. W. Furness. 2008. Monteiro's Storm-petrel (*Oceanodroma monteiroi*): A new species from the Azores. Ibis 150:717–727.

Burkett, E. E., N. A. Rojek, A. E. Henry, M. J. Fluharty, L. Comrack, P. R. Kelly, A. C. Mahaney, and K. M. Fien. 2003. Report to the California Fish and Game Commission: Status review of Xantus's Murrelet (*Synthliboramphus hypoleucus*) in California. Status Report 2003-01. California Department of Fish and Game, Habitat Conservation Planning Branch, Sacramento.

Carter, H. R., S. G. Sealy, E. E. Burkett, and J. F. Piatt. 2005. Biology and conservation of Xantus's Murrelet: Discovery, taxonomy and distribution. Marine Ornithology 33:81–87.

Carter, H. R., D. L. Whitworth, J. Y. Takekawa, T. W. Keeney, and P. R. Kelly. 2000. At-sea threats to Xantus's Murrelets (*Synthliboramphus hypoleucus*) in the Southern California Bight. Pages 435–447 *in* Proceedings 5th California Islands Symposium, Camarillo, CA (D. R. Browne, K. L. Mitchell, and H. W. Chaney, Eds.). U.S. Department of the Interior, Minerals Management Service, Pacific OCS Region, Camarillo, California.

Chakraborty, R. 1990. Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. American Journal of Human Genetics 47:87–94.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: A computer program to estimate gene geneologies. Molecular Ecology 9:1657–1660.

Collar, N. J. 2003. How many bird species are there in Asia? Oriental Bird Club Bulletin 38:20–30.

Cooke, W. W. 1916. The type locality of *Brachyramphus craveri*. Auk 33:80.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15:290–295.

Crandall, K. A., and A. R. Templeton. 1993. Empirical tests of some predictions from coalescence theory with applications to intraspecific phylogeny reconstruction. Genetics 134:959–969.

Desjardins, P., and R. Morais. 1990. Sequence and organization of the chicken mitochondrial genome: A novel gene order in higher vertebrates. Journal of Molecular Biology 212:599–534.

DeWeese, L. R., and D. W. Anderson. 1976. Distribution and breeding biology of Craveri's Murrelet. Transactions of the San Diego Society of Natural History 18:155–168.

Drost, C. A., and D. B. Lewis. 1995. Xantus's Murrelet (*Synthliboramphus hypoleucus*). *In* The Birds of North America Online (A. Poole, Ed.). Cornell Lab of Ornithology, Ithaca, New York. [Online.] Available at bna.birds.cornell.edu/bna/species/164.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14:2611–2620.

Ewens, W. J. 1972. The sampling theory of selective neutral alleles. Theoretical Population Biology 3:87–112.

Excoffier, L., G. Laval, and S. Schneider. 2005. ARLEQUIN (version 3.01): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

Excoffier, L. G., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Friesen, V. L., T. P. Birt, J. F. Piatt, R. T. Golightly, S. H. Newman, P. N. Hébert, B. C. Congdon, and G. Gissing. 2005. Population genetic structure and conservation of Marbled Murrelets (*Brachyrhamphus marmoratus*). Conservation Genetics 6:607–614.

Friesen, V. L., J. F. Piatt, and A. J. Baker. 1996. Evidence from cytochrome *b* sequences and allozymes for a 'new' species of alcid: The Long-billed Murrelet (*Brachyramphus perdix*). Condor 98:681–690.

Friesen, V. L., A. L. Smith, E. Gómez-Díaz, M. Bolton, R. W. Furness, J. González-Solís, and L. R. Monteiro. 2007. Sympatric speciation by allochrony in a seabird. Proceedings of the National Academy of Sciences USA 104:18589–18594.

Gaston, A. J. 1992. The Ancient Murrelet: A Natural History in the Queen Charlotte Islands. T. & A. D. Poyser, London.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. [Online.] Available at www2.unil.ch/popgen/softwares/fstat.htm.

Green, J. E., and L. W. Arnold. 1939. An unrecognized race of murrelet on the Pacific coast of North America. Condor 41:25–29.

Hamer, T. E., S. M. Schuster, and D. Meekins. 2005. Radar as a tool for monitoring Xantus's Murrelet populations. Marine Ornithology 33:139–146.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

Hazevoet, C. J. 1995. The Birds of the Cape Verde Islands. British Ornithologists' Union, Tring, United Kingdom.

Hey, J., and R. Nielsen. 2007. Integrations within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proceedings of the National Academy of Sciences USA 104:2785–2790.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9:1322–1332.

Hunt, G. L., Jr., R. L. Pitman, and H. L. Jones. 1980. Distribution and abundance of seabirds breeding on the California Channel Islands. Pages 443–459 *in* The California Islands: Proceedings of a Multidisciplinary Symposium (D. M. Power, Ed.). Santa Barbara Museum of Natural History, Santa Barbara, California.

Ibarguchi, G., V. L. Friesen, and S. C. Lougheed. 2006. Defeating numts: Semi-pure mitochondrial DNA from eggs and simple purification methods for field-collected wildlife tissues. Genome 49:1438–1450.

James, F. C. 2010. Avian subspecies: Introduction. Pages 1–5 *in* Avian Subspecies (K. Winker and S. M. Haig, Eds.). Ornithological Monographs, no. 67.

Jehl, J. R., Jr., and S. I. Bond. 1975. Morphological variation and species limits in the genus *Endomychura*. Transactions of the San Diego Society of Natural History 18:9–23.

Karnovsky, N. J., L. B. Spear, H. R. Carter, D. G. Ainley, K. D. Amey, L. T. Ballance, K. T. Briggs, R. G. Ford, G. L. Hunt, Jr., C. Keiper, and others. 2005. At-sea distribution, abundance and habitat affinities of Xantus's Murrelets. Marine Ornithology 33:89–104.

Keitt, B. S. 2005. Status of Xantus's Murrelet and its nesting habitat in Baja California, Mexico. Marine Ornithology 33:105–114.

Kidd, M. G., and V. L. Friesen. 1998. Sequence variation in the guillemot (Alcidae: *Cepphus*) mitochondrial control region and its nuclear homolog. Molecular Biology and Evolution 15:61–70.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

Kuhner, M. K., J. Yamato, and J. Felsenstein. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429–434.

Lopez, J. V., N. Yuhki, R. Masuda, W. Modi, and S. J. O'Brien. 1994. *Numt*, a recent transfer and tandem amplification of mitochondrial DNA in the nuclear genome of the domestic cat. Journal of Molecular Evolution 39:171–190.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

McChesney, G. J., and B. R. Tershy. 1998. History and status of introduced mammals and impacts to breeding seabirds on the California Channel Islands and northwestern Baja California islands. Colonial Waterbirds 21:335–347.

Moritz, C. 1994. Defining "evolutionary significant units" for conservation. Trends in Ecology & Evolution 9:373–375.

Narum, S. R. 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conservation Genetics 7:783–787.

Newman, S. H., H. R. Carter, D. L. Whitworth, and J. G. Zinkl. 2005. Health assessments and stress response of Xantus's Murrelets to capture, handling, and radio-marking. Marine Ornithology 33:147–154.

Pacheco, N. M., and V. L. Friesen. 2002. An investigation of hybridization in *Brachyramphus* murrelets using nuclear introns. Conservation Genetics 3:175–182.

Pratt, H. D. 2010. Revisiting species and subspecies of island birds for a better assessment of biodiversity. Pages 79–89 *in* Avian Subspecies (K. Winker and S. M. Haig, Eds.). Ornithological Monographs, no. 67.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pritchard, J. K., X. Wen, and D. Falush. 2010. Documentation for *structure* software, version 2.3. [Online.] Available at pritch.bsd.uchicago.edu/structure.html.

Rosenberg, N. A. 2004. Distruct: A program for the graphical display of population structure. Molecular Ecology Notes 4:137–138.

Roth, J. E., W. J. Sydeman, and P. L. Martin. 2005. Xantus's Murrelet breeding relative to prey abundance and oceanographic conditions in the Southern California Bight. Marine Ornithology 33:115–121.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.

Sealy, S. G. 1975. Feeding ecology of the Ancient and Marbled murrelets near Langara Island, British Columbia. Canadian Journal of Zoology 53:418–433.

Secretaría de Medio Ambiente y Recursos Naturales. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001, Protección ambiental Especies nativas de México de flora y fauna silvestres. Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario Oficial de la Federacion 582:1–56.

Violani, C., and G. Boano. 1990. L'Uria di Craveri *Synthliboramphus craveri* (Aves, Alcidae). Rivista Piemontese di Storia Naturale 11:155–162.

Watterson, G. A. 1978. The homozygosity test of neutrality. Genetics 88:405–417.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Quarterly Review of Biology 58:155–183.

Whitworth, D. L., H. R. Carter, R. J. Young, J. S. Koepke, F. Gress, and S. Fangman. 2005. Initial recovery of Xantus's Murrelets following rat eradication on Anacapa Island, California. Marine Ornithology 33:131–137.

Whitworth, D. L., J. Y. Takekawa, H. R. Carter, and W. R. McIver. 1997. A night-lighting technique for at-sea capture of Xantus' Murrelets. Colonial Waterbirds 20:525–531.

Whitworth, D. L., J. Y. Takekawa, H. R. Carter, S. H. Newman, T. W. Keeney, and P. R. Kelly. 2000. Distribution of

Xantus' Murrelet *Synthliboramphus hypoleucus* at sea in the Southern California Bight, 1995–97. Ibis 142:268–279.

Wilson, G. A., and B. Rannala. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191.

Winnett, K. A., K. G. Murray, and J. C. Wingfield. 1979. Southern race of Xantus' Murrelet breeding on Santa Barbara Island, California. Western Birds 10:81–82.

Wolf, S., C. Phillips, J. A. Zepeda-Dominguez, Y. Albores-Barajas, and P. Martin. 2005. Breeding biology of Xantus's Murrelet at the San Benito Islands, Baja California, México. Marine Ornithology 33:123–129.

Xantus, J. 1859. Description of supposed new species of birds from Cape St. Lucas, Lower California. Proceedings of the Academy of Natural Sciences of Philadelphia 11:297–299.

Zink, R. M., S. Rohwer, A. V. Andreev, and D. L. Dittmann. 1995. Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. Condor 97:639–649.

Associate Editor: K. T. Scribner