Adaptive Units for Conservation: Population Distinction and Historic Extinctions in the Island Scrub-Jay

KATHLEEN S. DELANEY* AND ROBERT K. WAYNE

University of California, Department of Ecology and Evolutionary Biology, 621 S. Charles E. Young Drive, Los Angeles, CA 90095–1606

Abstract: *The Island Scrub-Jay (*Aphelocoma insularis*) is found on Santa Cruz Island, California, and is the only insular bird species in the continental United States. We typed seven microsatellite loci and sequenced a portion of the mitochondrial DNA control region of Island Scrub-Jays and their closest mainland relative, the Western Scrub-Jay (*Aphelocoma californica*), to assess levels of variability and effective population size and to examine the evolutionary relationship between the two species. The estimated female effective population size,* N_{ef}*, of the Island Scrub-Jay was 1603 (90% confidence interval: 1481–1738) and was about 7.5% of the size of the mainland species. Island and Western Scrub-Jays have highly divergent control-region sequences, and the value of 3.14 ± 0.09% sequence divergence between the two species suggests a divergence time of approximately 151,000 years ago. Because the four northern Channel Islands were joined as one large island as recently as 11,000 years ago, extinctions must have occurred on the three other northern Channel islands, Santa Rosa, San Miguel, and Anacapa, highlighting the vulnerability of the remaining population. We assessed the evolutionary significance of four island endemics, including the Island Scrub-Jay, based on both genetic and adaptive divergence. Our results show that the Island Scrub-Jay is a distinct species of high conservation value whose history and adaptive potential is not well predicted by study of other island vertebrates.*

Key Words: *Aphelocoma*, Channel Islands, conservation genetics, endemic species, genetic diversity

Unidades Adaptativas para Conservación: Diferenciación de la Población y Extinciones Históricas de *Aphelocoma insularis*

Resumen: Aphelocoma insularis *se encuentra en la Isla Santa Cruz, California, y es la única especie de ave insular en Estados Unidos continental. Clasificamos siete locus microsatelitales y secuenciamos una porción de la región control del ADN mitocondrial de* A. insularis *y su pariente continental más cercano* A. californica *para evaluar niveles de variabilidad y tamaño poblacional efectivo y examinar las relaciones evolutivas entre las dos especies. El tamaño poblacional efectivo de hembras,* N_{eb}*, de* A. insularis *fue estimado en 1603 (90% CI: 1481-1738) y fue aproximadamente 7.5% del tamaño de la especie continental.* Aphelocoma insularis *y* A. californica *tienen secuencias muy divergentes en la región control, y el valor de divergencia secuencial de 3.14 ± 0.09% entre las dos especies sugiere un tiempo de divergencia de aproximadamente 151,000 años. Debido a que las cuatro Islas Channel estuvieron unidas en una sola isla tan recientemente como hace 11,000 años, deben haber ocurrido extinciones en las otras tres islas Channel, Santa Rosa, San Miguel y Anacapa, acentuando la vulnerabilidad de la población remanente. Evaluamos el significado evolutivo de cuatro especies insulares endémicas incluyendo* A. insularis *con base en la divergencia genética y adaptativa.*

**email ksemple@ucla.edu*
Paper submitted September 22, 2003; revised manuscript accepted June 22, 2004.

Nuestros resultados muestran que A. insularis *es una especie distinta de alto valor de conservación, cuya historia y potencial adaptativo no es pronosticado correctamente por el estudio de otros vertebrados insulares.*

Palabras Clave: *Aphelocoma*, diversidad genética especie, endémica, genética de conservación, Islas Channel

Introduction

The study of pattern and process on islands has played important roles in the development of evolutionary theory and conservation biology. Island species are often adaptively divergent in a wide variety of traits (Adler & Levins 1994). Compared with their mainland counterparts, island species tend to have higher densities and survivorship, lower reproductive output, and decreased dispersal (Stamps & Buechner 1985; Adler & Levins 1994). Aspects of their social behavior are affected, and island populations often show reduced aggressiveness, small territory sizes, relaxed territory boundaries, and occasional abandonment of territoriality (Stamps & Buechner 1985; Roemer et al. 2001). For many species, the island perimeter represents a hard boundary, and the absence of migration leads to high levels of interpopulation differentiation (e.g., Wayne et al. 1991; Roemer et al. 2001) and vulnerability to extinction (e.g., Roemer et al. 2002).

The Island Scrub-Jay (*Aphelocoma insularis*) is the only insular bird species in the continental United States and is found on Santa Cruz Island, the largest of the Channel Islands (Fig. 1). Island Scrub-Jays are the most morphologically distinct member of the Channel Islands' avifauna and exhibit substantial morphological divergence from Western Scrub-Jays (*A. californica*) (Table 1). In addition to morphological differences, life-history differences such as time to first breeding and adult survival exist between Island and Western Scrub-Jays (Table 1).

Peterson (1992) found substantial genetic divergence between *A. insularis* and *A. californica*. Because of mutation rate heterogeneity, however, he concluded that the relationship between Island Scrub-Jays and other *Aphelocoma* jays was unresolved. Migration of scrub jays between island and mainland habitats has not been observed, despite intensive searches for vagrant birds (Pitelka 1951). Both species are nonmigratory and are thought to have a relatively weak humerus that does not allow long flights (Pitelka 1951). Santa Rosa Island, which has suitable scrub jay habitat, lies 9 km from Santa Cruz but does not have an Island Scrub-Jay population. During the last glaciation between 11,000 and 18,000 years ago, sea levels were lower, and the four northern Channel Islands were joined as one large island named *Santarosae*. This large island would have only been 7 km from the mainland of California (Junger & Johnson 1980). Because there are no Island Scrub-Jays and no fossil evidence of them on Anacapa, Santa Rosa, or Santa Miguel islands, it is thought that colonization occurred after *Santarosae* was separated into the four islands, about 11,000 years ago (Johnson 1972). Scrub jays could have been on *Santarosae* much earlier, however, only to go extinct on all but Santa Cruz Island.

The majority of recent bird extinctions have been island species. Of the 108 bird species extinctions since 1600, 97 have occurred on islands (Johnson & Stattersfield 1990). The probability of an individual island species going extinct is approximately 40% higher than that of a continental species (Johnson & Stattersfield 1990). Island species generally are range-restricted and considered "ecologically naïve" and therefore vulnerable to mainland diseases, random demographic shifts, and competition or predation by exotic species (Milberg & Tyrberg 1993; Owens & Bennett 2000).

Assessing the genetic variability and uniqueness of an island species is critical for its conservation. We sought to determine whether the Island Scrub-Jay lineage is genetically distinct by testing two hypotheses. First, because genetically effective gene flow may not be detectable in observational studies (Slatkin 1987), we assessed levels of gene flow between island and mainland populations. Gene flow between island and mainland populations is common in many nonendemic species found in the Channel Islands, although it is expected to be low in endemic taxa (Johnson 1972). Second, the colonization event that established scrub jays on Santa Cruz Island could have been recent or ancient. We tested the recentness-of-colonization hypothesis by assessing levels of genetic divergence between Island and Western Scrub-Jays. The ancient colonization hypothesis implies that the scrub jay existed on the other northern Channel Islands and recently went extinct, highlighting the vulnerability of the Island Scrub-Jay. Lastly, we examined a new comparative paradigm that incorporates data on both genetic and adaptive divergence to assess the relative evolutionary importance of taxa. We found that the Island Scrub-Jay should be regarded as an adaptively divergent and evolutionarily distinct island species of high conservation value. Our results imply that other taxa on Santa Cruz Island may also be highly distinct and that there is a need for additional phenotypic and genetic studies.

Methods

Samples

We sampled birds from mainland California and from Santa Cruz Island, located 31 km southwest of Ventura, California (Fig. 1). Western Scrub-Jay samples were

Figure 1. Map of California and Santa Cruz Island showing sampling locations of Island and Western Scrub-Jays. Control-region haplotypes and number of samples, respectively, are as follows: San Ramon, AC1 (4), AC2 (4); Mountain Springs Pass, AC2 (1); Bradley, AC4 (1), AC5 (1), AC7 (2), AC9 (1), AC11 (1); Bodfish, AC1 (4), AC3 (1), AC5 (3), AC15 (1); Los Angeles, AC4 (1), AC6 (1), AC13 (1), AC14 (1); Big Bear City, AC3 (1), AC5 (1), AC8 (1), AC10 (1); Starr Ranch, AC1 (1), AC2 (2), AC3 (1), AC5 (4), AC6 (2), AC8 (5), AC 12 (1); Christy Ranch, AI2 (2); Pines, AI1 (1); Central Valley, AI1 (19), AI2 (2); Prisoner's Harbor, AI1 (1).

collected from three subspecies and seven locations (*A. c. oocleptica*, San Ramon and Bodfish; *A. c. californica*, Bradley; *A. c. obscura*, Los Angeles, Big Bear City, Starr Ranch, and Mountain Springs Pass). We used walk-in traps to capture scrub jays. Birds were uniquely color-banded and fitted with a numbered metal band provided by the California Department of Fish and Game. A small sample (approximately 300 μL) of blood was taken from the brachial vein. Blood was stored in avian blood buffer (Seutin et al. 1991) at −70° C. Samples from San Ramon, Bradley, Bodfish, Big Bear City, and Mountain Springs Pass were collected by A. Townsend Peterson (Field Museum, Chicago).

DNA Extraction and Mitochondrial DNA Sequencing

We extracted DNA from blood or tissue with a standard proteinaseK/phenol/cholorform protocol (Sambrook et al. 1989). Genomic DNA was resuspended in TE buffer (10 mm Tris-Cl pH 8.0, 1 mm EDTA pH 8.0) for long-term storage.

We used the primers JCRO3 and H1248 and the polymerase chain reaction (PCR) to isolate and amplify the entire control region of the mitochondrial genome (mtDNA) (Tarr 1995; Saunders & Edwards 2000). We used approximately 10 ng total genomic DNA and standard PCR conditions to obtain double-stranded PCR products. Cycle sequencing (Beckman Coulter, Fullerton, California) was done with the amplification products and the JCRO3 primer. We used this primer so we could specifically sequence domain I at the 5′ end of the control region. Automated sequencing was performed with a Beckmann CEQ 2000 capillary sequencer. We also obtained sequences for the Unicolored Jay (*A. unicolor*: accession number AF218920) and Florida Scrub-Jay (*A. coerulescens*: accession number AF218918) from Genbank.

Microsatellite Genotyping

We used seven microsatellite loci to genotype Island and Western Scrub-Jays. Four loci (ApCo2, ApCo29, ApCo30, and ApCo37) were derived from the Florida Scrub-Jay (Stenzler & Fitzpatrick 2002), one (MSJ6) from the Mexican Jay (*A. ultramarina*) (Li et al. 1997), and one (Dpu05) from the Yellow Warbler (*Dendroica petechia*) (Dawson et al. 1997). We slightly modified PCR conditions from these published protocols. Additionally, we created a microsatellite library with a modified biotinylated capture protocol (Bardeleben et al. 2004) for Island Scrub-Jays, enriched for tetranucleotide repeats with the motif AAGG.

Table 1. Morphological and life-history differences between Island and Western Scrub-Jays (*A. c. obscura*).

Characteristic	*Western*	*Island*	*Reference*
Adult male mean weight (mm)	79.0	124.7	Pitelka 1951
Adult male mean wing length (mm ± SD)	121.1 ± 0.57	139.2 ± 0.31	Pitelka 1951
Adult male mean tarsus length (mm ± SD)	38.8 ± 0.19	46.1 ± 0.13	Pitelka 1951
Mean territory size (ha ± SD)	2.53 ± 0.4	1.35 ± 0.52	Kelsey & Collins 2000
Yearly breeder survival rate (%)	83	94	Atwood et al. 1990
Mean clutch size (±SD)	4.47 ± 0.13	3.71 ± 0.7	Atwood 1980; Carmen 1988
Breeding as a yearling	common	very rare	Carmen 1988; Collins & Corey 1994

From this library, one locus (AIAAGG13; F: TGGATGGTTCAGGGCTGTAT, R: GCCTCAAGGATGCTGTAGAGA, annealing temperature = 56° C, $MgCl_2$ concentration = 1.5 mm) was polymorphic in Island Scrub-Jays and was used for genotyping. After labeling microsatellite primers with florescent dyes, we scored loci on an ABI377 automated sequencer.

Analysis of MtDNA Sequences

Mitochondrial DNA control-region sequences were aligned with Sequencher version 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan). We used DnaSP version 3.51 (Rozas & Rozas 1999) to obtain measures of sequence polymorphism. These measures included (1) nucleotide diversity (π), which is the average number of nucleotide differences per site between two sequences; (2) haplotype or gene diversity (h), which is based on the frequency of different haplotypes in a sample; and (3) the average number of pairwise nucleotide differences (k) (Nei 1987). We also used DnaSP to test our sequences for selective neutrality (D^* and F^*) (Fu & Li 1993).

To obtain the most likely nucleotide substitution model that would best fit our data, we used Modeltest (version 3.06; Posada & Crandall 1998). From this analysis, we determined that the Tamura-Nei and HKY+ Γ models were equally likely (Hasegawa et al. 1985; Tamura & Nei 1993). We then used PAUP* (version 4b8) to assess phylogenetic relationships of control-region sequences (Swofford 1998). We first used the Tamura-Nei distance and the neighbor-joining method to construct trees (Saitou & Nei 1987). We then performed a maximum-likelihood heuristic search with one random stepwise addition to determine empirical values for nucleotide frequencies, the gamma shape parameter (α), and transition/transversion ratios (Swofford 1998). With the values obtained from the maximum-likelihood tree, we performed statistical bootstrap analysis 100 times to obtain a 50% majority-rule consensus tree.

We used MEGA (version 2.1) (Kumar et al. 2001) to calculate Tamura-Nei distances between mtDNA haplotypes of Island and Western Scrub-Jay sequences. Divergence was calculated within (d_{AI} and d_{AC}) and between ($d_{AI/AC}$) each species. The between-species divergence was then adjusted for within-lineage polymorphism by calculating $d_{adjustedAI/AC} = d_{AI/AC} - 0.5(d_{AI} + d_{AC})$ (Kumar et al. 2001). We used Arlequin (version 2.0) and the Tamura-Nei distance to analyze the mtDNA sequence variation among and within taxa with analysis of molecular variance (AMOVA) (Excoffier et al. 1992; Schneider et al. 2000).

We compared the rates of evolution in Island and Western Scrub-Jay sequences with Tajima's test in MEGA (Tajima 1993). This test performs comparisons of Island and Western Scrub-Jay sequences to each other and an outgroup (*A. coerulescens* or *A. unicolor*). The molecular clock hypothesis can be rejected if the expected nucleotides per site are not similar between the focal species and the outgroup. Given the absence of heterogeneity, the substitution rate described above could be used to estimate divergence time between species with a fossil-calibrated molecular clock (Avise 1994).

For mtDNA, the mean number of generations since the common ancestor of two sequences is related to the number of mutations between sequences as $\theta = 2N_{ef}\mu$, where N_{ef} is the effective female population size and μ is the substitution rate per site per generation (Nei 1987). To calculate N_{ef} from our sequence data, we used θ_π, which is estimated from the infinite-site equilibrium relationship between the mean number of pairwise distances (π) and θ (Tajima 1983). We applied a substitution rate for avian control region I of 0.208 substitutions per site per 1 million years (substitutions/site/M years) (Quinn 1992) to calculate population size and divergence time. As a comparison to this substitution rate, we searched for rates that have been suggested for avian mtDNA control-region sequences in other species (see Discussion).

Analysis of Microsatellite Genotypes

We used GENEPOP (version 1.2) to calculate allele frequencies and expected heterozygosity (Raymond & Rousset 1995). We also tested microsatellite genotypes for deviations from Hardy-Weinberg equilibrium (Guo & Thompson 1992) and linkage disequilibrium. We applied a sequential Bonferroni correction and then evaluated the results for significance.

We observed alleles differing by less than the expected number of nucleotides (e.g., dinucleotide repeat alleles differing by one rather than two base pairs) and numerous

gaps in the distribution of alleles at all seven microsatellite loci. These results suggest that loci were not evolving according to a strictly step-wise mutation model. Consequently, we could not use a measure of genetic differentiation between Island and Western Scrub-Jays that assumes a step-wise model, such as R_{ST} (Slatkin 1995). In addition, R_{ST} measures have a high associated variance compared with other measures, such as F_{ST} (Balloux & Lugon-Moulin 2002). Therefore, we used Arlequin to calculate F_{ST} between Island and Western Scrub-Jays, which assumes an infinite-allele model of evolution (Weir & Cockerham 1984). We also calculated genetic differentiation between Island and Western Scrub-Jays with two genetic distances, D_A (Nei et al. 1983) and D_S (Nei 1987) in the program Populations (version 1.2.28; Langella 1999–2003). In addition, we used Structure (version 2.0), which uses a Bayesian clustering approach, to assign individuals to populations based on genetic similarity (Pritchard et al. 2000). We assumed a model of ancestry that has no admixture and in which allele frequencies among populations are correlated. We ran this analysis without information on species identification and used 30,000 burn-in and 1,000,000 MCMC repetitions. We calculated posterior probabilities from the ln $\Pr(X \mid K)$ estimates for 1–6 populations (K) within our data set (Pritchard et al. 2000).

We examined evidence of a recent population bottleneck using the program Bottleneck (version 1.2.02; Cornuet & Luikart 1996). A recent population bottleneck can be revealed as a correlation between a reduction in effective population size and a decrease in allele number and heterozygosity. We used Bottleneck to simulate, through the coalescent process, the distribution of expected heterozygosity under the infinite-allele model of microsatellite evolution. A sign test and a Wilcoxon test were used to assess the significance of excess heterozygosity.

We analyzed our microsatellite data with the program Migrate (version 1.7.3) to estimate the parameter θ (Beerli & Felsenstein 1999). Migrate uses a maximum-likelihood framework based on coalescent theory to estimate the parameter θ with a Metropolis Hastings algorithm. We assessed the likelihood of our final θ value by sampling 10 Markov chains of 10,000 steps and 3 chains of 100,000 steps. In addition, we used mean observed heterozygosity to calculate effective population size. Under an infinite-allele model of microsatellite evolution, we used the formula $N_e = H/4\mu(1 - H)$, where $\mu = 10^{-3}$ and H is the mean observed heterozygosity (Nei 1987).

Results

Genetic Variability

Only two mtDNA control-region haplotypes existed in 25 Island Scrub-Jays, whereas 15 haplotypes existed in 48 Western Scrub-Jays from California (Fig. 2). No deletions or insertions were observed. All measures of sequence diversity were an order of magnitude lower in Island Scrub-Jays (western: $\pi = 5.02 \times 10^{-3} \pm 4.5 \times 10^{-4}$, $h = 0.897 \pm 0.021$, $k = 1.94$, island: $\pi = 7.2 \times 10^{-4} \pm 2.6 \times 10^{-4}$, $h = 0.28 \pm 0.1$, $k = 0.28$). Tests of sequence neutrality were not significant (western: $D^* = -1.61$, $F^* = -1.79$; island: $D^* = 0.62$, $F^* = 0.55$).

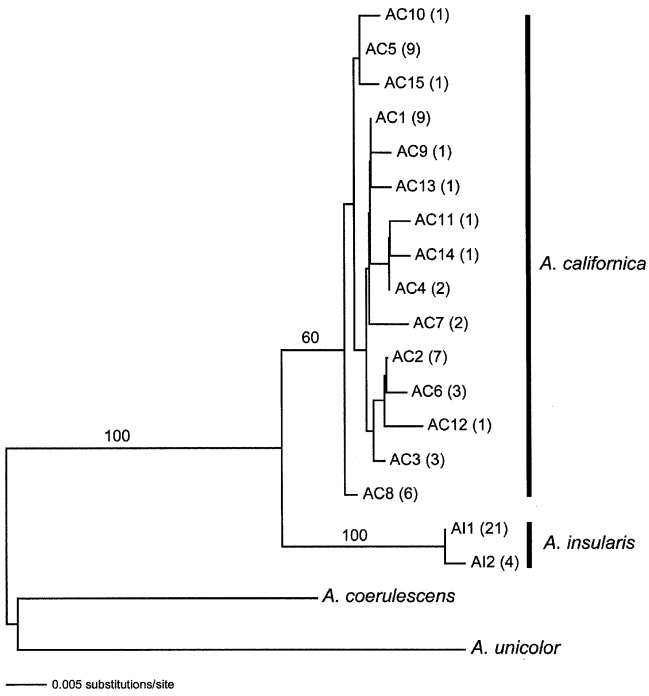

Figure 2. Maximum-likelihood tree on which each branch represents a haplotype with the corresponding number of individuals in parentheses. Bootstrap values are above nodes (100 replicates).

The Island Scrub-Jays had substantially less microsatellite variation than the Western Scrub-Jays. We obtained microsatellite genotypes from seven loci in 25 Island and 58 Western Scrub-Jays, a sample that included all those typed for mitochondrial control-region sequences. All seven microsatellite loci were in Hardy-Weinberg equilibrium in the Island Scrub-Jay. Loci Dpu05 and MSJ6 were not in Hardy-Weinberg equilibrium, however, because of a heterozygote deficiency in the Western Scrub-Jay. After Bonferonni correction, these two loci remained out of Hardy-Weinberg equilibrium; therefore, we performed subsequent analyses with five and seven loci. None of the microsatellite loci showed significant linkage disequilibrium. The average observed allelic diversity (A) (western: $A = 25.0$, SE = 4.0; island: $A = 6.7$, SE = 2.1) and heterozygosity (H) (western: $H = 0.93$, SE = 0.02; island: $H = 0.65$, SE = 0.08) of seven microsatellite loci were significantly lower in Island Scrub-Jays than in Western Scrub-Jays (Wilcoxon signed ranks test $Z_A = -2.375$, $p = 0.018$, $Z_{\mathrm{He}} = -2.366$, $p = 0.018$) (Table 2). With five loci,

Table 2. Summary of observed microsatellite allele number (*A*), sample size (*n*), and heterozygosity (*H*) of Island and Western Scrub-Jays.

	A. californica			A.insularis		
Locus	n	A	H	n	A	H
ApCo2	51	21	0.925	21	4	0.633
ApCo29	55	13	0.841	25	3	0.577
ApCo37	54	14	0.908	25	4	0.592
ApCo30	52	21	0.929	25	4	0.290
Dpμ05	47	40	0.970	23	12	0.889
MSJ6	42	37	0.974	23	17	0.942
AIAAGG13	53	29	0.955	25	3	0.603

heterozygosity and allele number remained significantly lower in the Island Scrub-Jay ($Z_A = -2.041$, $p = 0.041$; $Z_{\mathrm{He}} = -2.023$, $p = 0.043$). In the island population, heterozygosity values ranged from 34% to 97% and the number of alleles from 23% to 43% of their corresponding values in the mainland population.

Genetic Structure

Island and Western Scrub-Jays shared no haplotypes, implying an absence of gene flow. Moreover, haplotypes from Island Scrub-Jays were markedly divergent from all California Western Scrub-Jay haplotypes. Within each species, Tamura-Nei distances were $d_{\mathrm{AC}} = 0.0051 \pm 0.0017$ and $d_{\mathrm{AI}} = 0.00073 \pm 0.00071$ for mainland and island species, respectively. The net divergence between species, adjusted for within-species variability, was $d_{\mathrm{adjustedAI/AC}} = 0.0314 \pm 0.009$. Consequently, the two species differed in about 3.14% of control-region domain I. Genetic divergence between species was also evidenced by the AMOVA. The percentage of nucleotide variation observed among populations was 90.1% ($\phi_{ST} = 0.901$; $p < 0.0001$).

Island and Western Scrub-Jays differed significantly in allele frequencies (Fisher's method, $\chi^2 =$ infinity, df = 14, $p = 0.0$). Moreover, alleles unique to Island Scrub-Jays occurred at three microsatellite loci. In the Island Scrub-Jay, locus Dpu05 had 8 unique alleles of 12 (67%), MSJ6 had 10 of 17 unique alleles (59%), and AIAAGG13 had 3 of 3 unique alleles (100%). The F_{ST} between Island and Western Scrub-Jays was 0.184 and significantly different from zero ($p < 0.001$), and Nei's genetic distances were $D_A = 0.636$ and $D_S = 0.968$.

Phylogeography

A well-resolved maximum-likelihood tree (ln likelihood = −921.53) of control-region sequence data showed that Island and Western Scrub-Jays in California form reciprocally monophyletic groups (Fig. 2). Sequence phylogenies constructed by maximum-likelihood and parsimony algorithms were topologically identical. Similarly, using Structure on data from five microsatellite loci, we found the highest probability with two genetically distinct clusters ($K = 2$, ln = −1783). We calculated the posterior probabilities of each K given our data. The probability of $K = 2$ was 1.0 (Pr[$K = 2$] = 1.0), whereas for all other Ks the probabilities were approximately zero. The two clusters consisted of island and mainland populations, where 100% of the individuals were correctly assigned to their species of origin. Structure can overestimate K when microsatellite loci are used that are not in Hardy-Weinberg equilibrium (Pritchard et al. 2000). We did not observe this result when we used all seven microsatellite loci. Structure indicated that there were two clusters, one Island and one mainland, where 100% of individuals were correctly assigned ($K = 2$, ln = −2721, Pr[$K = 2$] = 1.0, probabilities for all other Ks were approximately zero).

Divergence Time

We used a molecular clock for domain I of the control region to estimate the divergence time between Island and Western Scrub-Jays. We tested for substitution-rate heterogeneity in scrub jay mtDNA control-region sequences because rate heterogeneity can lead to errors when divergence times and phylogenetic relationships are estimated. There were no differences in the rates of evolution in Island or Western Scrub-Jays when we used two of their close relatives as outgroups, Florida Scrub-Jays and Unicolored Jays (sequence divergence < 10%) (Tajima's [1993] test, $p > 0.05$).

A substitution rate of 0.208 substitutions/site/M years has been suggested for domain I of the avian control region (Quinn 1992). Although Island Scrub-Jays exhibit delayed breeding, to be conservative we chose a generation time of 1 year for both species. For example, when a generation time of 3 years was used, N_{ef} was three times lower and divergence time was three times longer. When we used 0.208 substitutions/site/M years and a generation time of 1 year, we obtained a divergence time of 150,961 ± 4327 years ago. This result suggests that Island Scrub-Jays colonized the northern Channel islands well before they were subdivided into four separate islands.

Effective Population Size

We used mtDNA sequences to calculate effective population size according to the relation $\theta = 2N_{\mathrm{ef}}\mu$. In this equation we used a mutation rate of 0.208 substitutions/site/M years, a generation time of 1 year, and θ values based on the nucleotide diversity of mtDNA control-region sequences for each species. For Western Scrub-Jays, $\theta = 1.98 \pm 1.27$; therefore, N_{ef} was 47,596 (±30,529). For Island Scrub-Jays, $\theta = 0.28 \pm 0.34$; therefore, $N_{\mathrm{ef}} = 6731$ (±8173).

Given the very large standard deviations of θ calculated from sequence data and to provide a perspective based on a biparentally inherited marker, we used the program Migrate to estimate population sizes from the microsatellite data. The Markov chain Monte Carlo estimates of the parameter θ (90% confidence interval [CI] in parentheses)

for Island Scrub-Jays was 6.41 (5.92–6.95) with seven loci and $\theta = 0.86$ (0.78–0.95) with five loci. For Western Scrub-Jays θ was 48.33 (45.14 and 51.86) with seven loci and 57.39 (49.35–67.15) with five loci.

We used two common microsatellite mutation rates, 10^{-3} and 10^{-4} mutations per locus per generation, to bracket our estimates of effective population size (Ellegren 2000 and references therein). Effective population sizes (N_e) for Island Scrub-Jays with a generation time of 1 year and the 10^{-3} mutation rate (90% CI) were 1603 (1481–1738) and 215 (195–238) with seven and five loci, respectively. Effective population size for Western Scrub-Jays was 12,082 (11,285–12,840) with seven loci and 14,348 (12,338–16,788) with five loci. Using the lower mutation rate yielded population size estimates 10 times higher, which is probably unrealistic, so we assumed that 10^{-3} was a more reasonable mean mutation rate. In addition, we used this mutation rate and the observed mean heterozygosity to calculate N_e, resulting in $N_e = 3264$ (SD = 12) for Western Scrub-Jays and $N_e = 457$ (SD = 69) for Island Scrub-Jays. With five loci, $N_e = 2578$ (SD = 11) for Western Scrub-Jays and $N_e = 292$ (SD = 41) for Island Scrub-Jays.

We used Bottleneck to test for excess heterozygosity in Island Scrub-Jays to assess the possibility of a population bottleneck. The expected and observed heterozygosity of the microsatellite loci under the infinite-allele model of evolution did not significantly differ in Island or Western Scrub-Jays. Wilcoxon (island $p = 0.30$, western $p = 0.69$) and sign tests (island $p = 0.15$, western $p = 0.56$) for heterozygosity excess were not significant for either Island or Western Scrub-Jays. Using only five microsatellite loci gave similar nonsignificant results, with the exception of the Western Scrub-Jay Wilcoxon test (island $p = 0.12$, western $p = 0.02$). Sign tests, however, were not significant (island $p = 0.26$, western $p = 0.56$). Therefore, we found no evidence of a recent population bottleneck on Santa Cruz Island.

Discussion

Gene Flow between Island and Western Scrub-Jays

Our results showed that gene flow between Island and Western Scrub-Jays is not occurring now nor did it occur in the distant past. The two species shared no control-region mtDNA haplotypes and instead defined reciprocally monophyletic clades indicative of historic isolation. Our estimate of divergence time between Island and Western Scrub-Jays of approximately 151,000 years ago suggests a long history of isolation. This degree of antiquity is remarkable considering the flux of bird species inhabiting the Channel Islands. Supporting the genetic evidence of no dispersal, however, is the observation that the Island Scrub-Jay has not colonized Santa Rosa Island, which would involve only a 9-km flight.

Comparison of Substitution Rate

To better determine whether a more recent colonization of Santa Cruz Island by scrub jays was feasible, we compiled a range of substitution rates of mtDNA control regions from other studies to conservatively bracket divergence times of Island and Western Scrub-Jays (Table 3). The value of 0.208 substitutions/site/M years is close to the medium rate of those published. The highest substitution rate (μ) of 0.93 substitutions/site/M years is 4–8 times higher than that of any other study and was estimated for Adélie Penguins (*Pygoscelis adeliae*) based on DNA from fossils and a genealogical sampling approach (Lambert et al. 2002). Even if this rate was used, however, the divergence time of approximately 33,763 ($\pm$968) years ago still precedes the division of the four northern Channel Islands about 11,000–18,000 years ago. Moreover, the higher substitution rate seems unlikely because it implies an effective population size for Island Scrub-Jays of only 151 ($\pm$183) individuals (see below).

Effective Population Size

All measures of gene and sequence diversity were lower in Island Scrub-Jays than in their mainland relatives (Tables 2 & 4). Only two haplotypes, differing by a single substitution, were found in Island Scrub-Jays. At equilibrium between mutation and drift, this level of diversity suggests an N_{ef} of 6731 ($\pm$8413). Because of the extreme lack of genetic diversity in control-region haplotypes of the Island Scrub-Jay, our error for this estimate of N_{ef} size was large. Therefore, more confidence can be placed in N_e estimates from the microsatellite loci with coalescent-based measures. Population size estimates for the Island

Table 3. Comparison of substitution rates used for avian control region mtDNA sequence data.*

Species name	*Common name*	*Substitution rate (μ)*	*Reference*
Parus major	Great Tit	0.02	Kvist et al. 1999
Alca torda	Razorbill	0.033	Moum & Arnason 2001
Uria aalge	Guillemot	0.033	Moum & Arnason 2001
Calidris alpine	Dunlin	0.148	Wenink et al. 1996
Chen caerulescens	Lesser Snow Goose	0.208	Quinn 1992
Dendroica petechia	Yellow Warbler	0.30	Milot et al. 2000
Pygoscelis adeliae	Adélie Penguin	0.93	Lambert et al. 2002

**Substitution rate is measured in substitutions per site per million years (substitutions/site/M years).*

Scrub-Jay based on microsatellite data were 1603 (90% CI: 1481–1738) with seven loci and 215 (90% CI: 195–238) with five loci. Presumably, because all seven microsatellite loci are in Hardy-Weinberg equilibrium in Island Scrub-Jays, the former value provides a more accurate estimate of effective population size.

Based on observations of territory size and available habitat on Santa Cruz Island, the number of Island Scrub-Jay breeders was estimated to be 7000 (Kelsey & Collins 2000). The disparity between census and N_e based on genetic measures is common (Avise 1994). One possible reason for this incongruity is the fact that Island Scrub-Jays exhibit delayed breeding and are long-lived, creating a reproductive skew that may be reflected in a lower N_e. Another possible reason for this disparity is past bottleneck events. We did not find evidence of a recent population bottleneck when looking for an excess of heterozygosity at the seven microsatellite loci. This method has low statistical power with our small data set, however, and will detect a bottleneck only within 2–4 N generations (Cornuet & Luikart 1996).

Recentness of Colonization

There was a high level of sequence divergence between Island and Western Scrub-Jays, implying a separation time of 150,961 (±4327) years ago. Other vertebrate species, including deer mice (*Peromyscus maniculatus* spp.) (Ashley & Wills 1987), island foxes (*Urocyon littoralis*) (Gilbert et al. 1990), and Loggerhead Shrikes (*Lanius ludovicianus* spp.) (Mundy et al. 1996), have only modest amounts of genetic divergence, consistent with an origin during low sea levels between 11,000 and 18,000 years ago. Our evidence of ancient colonization suggests that scrub jays were present on the larger landmass of *Santarosae* during periods of glacial maxima (Orr 1968). Consequently, extinction must have occurred on the three smaller northern Channel Islands, Santa Rosa, San Miguel, and Anacapa. Careful study of fossil evidence from middens on the northern Channel Islands could corroborate an ancient occupation. However, no records of fossil scrub jays from any of the Channel Islands have been published.

Native Chumash on the northern Channel Islands may have used scrub jays for food or feathers. Two feather bands found in Bower's Cave in Los Angeles County were made by the Chumash partially from Steller's Jay (*Cyanocitta stelleri*) and Western Scrub-Jay feathers (Elsasser & Heizer 1963). Many island endemic species have been driven to extinction by human overexploitation (Milberg & Tyrberg 1993), and this remains a possible explanation for extinction of jays on Santa Rosa and San Miguel islands.

The areas of Santa Rosa, San Miguel, and Anacapa islands, where Island Scrub-Jays have presumably gone extinct, are 86%, 15%, and 1% of the area of Santa Cruz Island, respectively. Further, because of ranch operations, habitat conversion, and invasive species on Santa Cruz, the habitat now available for Island Scrub-Jays is less than was available historically (Brumbaugh 1980). Therefore, assuming that scrub jay densities were similar on both Santa Cruz and Santa Rosa islands, the Santa Cruz Island population may be smaller than the population that went extinct on Santa Rosa Island. Consequently, Island Scrub-Jays may be threatened with extinction.

Evolutionary Significance

Our results support species status for the Island Scrub-Jay because they provide evidence for long-term isolation and because previous studies suggest divergence in fitness-related traits (Table 1). It has been proposed that units for conservation, including species, should be defended from an ecological and genetic perspective as testable hypotheses (Crandall et al. 2000). The hypotheses-testing format posits that, to be considered for separate management, two populations must be shown to be ecologically and genetically nonexchangeable. Evidence for the lack of ecological exchangeability include heritable differences in life-history traits, functional morphologic and behavioral traits, and surrogates for divergent selection such as habitat and climate differences. Evidence for the lack of genetic exchangeability comes primarily from molecular data showing an absence of gene flow. Finally, for both ecological and genetic exchangeability, a time dimension, recent or ancient, informs management action (Crandall et al. 2000). Rejection of any of these four hypotheses provides support for classification of the populations as distinct, a concept implemented by laws such as the U.S. Endangered Species Act of 1973.

We evaluated evolutionary divergence in a comparative framework by contrasting divergence data from the Island Scrub-Jay with three other island endemics. This comparative approach, in which a variety of management recommendations can be made, depending on genetic and ecological exchangeability, provides more options for management than the evolutionarily significant unit approach used in the past (Crandall et al. 2000). First, the San Clemente Loggerhead Shrike (*L. l. mearnsi*) is regarded as a distinct evolutionary unit (Mundy et al. 1996) and has been listed under the U.S. Endangered Species Act. Evidence of current genetic divergence of island and mainland loggerhead shrikes is weak. The F_{ST} values based on microsatellite loci are similar in magnitude to those of geographically proximate mainland populations (Table 4), and no unique markers are found in the San Clemente Island population. Moreover, a recent study of postjuvenile museum skins collected decades ago reveals that although there probably once was a separate subspecies of loggerhead shrike on San Clemente Island, current migratory and breeding patterns have swamped the genetic and morphological differences that may have existed (Patten

Table 4. A comparison of genetic differences between three Channel Island species and their mainland sister taxon (Ashley & Wills 1987; Gilbert et al. 1990; Mundy et al. 1996; Mundy et al. 1997; Roemer et al. 2001)*.

Characteristic (comparison to mainland sister taxon)	*Island Scrub-Jay (*A. insularis*)*	*San Clemente Island Loggerhead Shrike (*L. l. mearnsii*)*	*Island fox (*U. littoralis*)*	*Deer mouse (*P. m. santacruzae*)*
Number of shared mtDNA haplotypes (total number of sister-taxon haplotypes)	0 (15)	4 (4)	0 (12)	0 (16)
Percentage of unique microsatellite alleles	65.6	0	N/A	N/A
F_{ST} between island and mainland	0.901	0.54	0.68	N/A

**Unknown or unpublished data are noted with N/A.*

& Campbell 2000). Therefore, under the Crandall et al. (2000) system, this population would be regarded as genetically exchangeable but was perhaps not so in the past (Fig. 3). The recommendation for management would be to allow gene flow, consistent with past levels, rather than isolation. As implied by the genetic evidence, this level of gene flow is approximately one to two migrants per generation (see also Roemer & Wayne 2003).

Second, the island fox is morphologically, ecologically, and behaviorally divergent from the mainland gray fox (*U. cinereoargenteus*) (Roemer et al. 2001). Island foxes define a monophyletic group based on mtDNA restriction-site data and nuclear DNA mini- and microsatellite loci, and the level of divergence is consistent with a geographic isolation as recent as 15,000 years ago (Gilbert et al. 1990). Divergence in life-history traits, such as smaller home ranges, decreased territory size, and shorter dispersal distance, is suggested by a field study of foxes on Santa Cruz Island (Roemer et al. 2001). In addition, island foxes are 25% smaller and tend to be more diurnal and more insectivorous than their mainland counterparts (Collins 1982; Roemer et al. 2001). Under the criteria of Crandall et al. (2000), the island fox is a distinct species, with divergent and isolated island populations that require separate management (Fig. 3).

Third, the Channel Island deer mouse (*P. m. santacruzae*) is a subspecies that is morphologically and genetically distinct from mainland deer mice (*P. m. gambelii*) (Ashley & Wills 1987; Pergams & Ashley 2001). Estimated percent genetic divergence based on restriction fragments of mtDNA between mainland and Santa Cruz Island mice is 0.65%, suggesting a divergence over 10,000 years ago (Ashley & Wills 1987). *P. m. santacruzae* is approximately 20% larger than mainland *P. m. gambelii* (Gill 1980). Size differences in organs such as heart, kidney, and liver between mainland and island mice suggest different physiological adaptations to the island environment (Gill 1980). Divergence in genetic and morphological measurements suggest that deer mice on Santa Cruz Island are a distinct evolutionary lineage requiring separate management (Fig. 3).

Compared to these three species, the Island Scrub-Jay should be given high priority for conservation. The Island

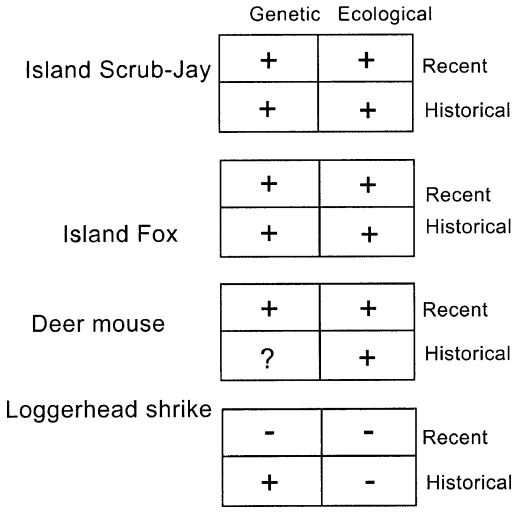

Figure 3. Null hypothesis of ecological and genetic exchangeability for two different time periods is rejected, as shown by a plus sign (+). If the null hypothesis cannot be rejected, a minus sign (−) appears. A question mark (?) appears if the hypothesis cannot be accepted or rejected given the published data. The null hypothesis of genetic and ecological exchangeability can be rejected in the Island Scrub-Jay, island fox, and deer mouse for both recent and historical times. Only weak support exists for the rejection of the genetic exchangeability in historical times for the San Clemente Loggerhead Shrike.

Scrub-Jay has a long history of isolation from its mainland conspecific and is divergent in phenotypic and life-history characters (Table 1). Island fox and deer mouse populations, although isolated, show lower levels of distinction from each other, and evidence for ecological divergence is less strong. Finally, the separate management of the San Clemente Loggerhead Shrike population is questionable considering genetic data suggesting a modest amount of gene flow from the mainland population and evidence that the introduced population is different from the form that existed there in the past. Currently, as much as U.S.$2 million is spent on captive breeding and reintroduction of

the San Clemente Loggerhead Shrike (Roemer & Wayne 2003).

Acknowledgements

We thank C. Collins, M. J. Elpers, P. W. Collins, D. Delaney, the University of California Genetic Resources Conservation Program, the Laguna Hills Audubon Society, L. Laughrin, P. and S. DeSimone, the Field Museum, A. T. Peterson, M. Ashley, and an anonymous reviewer.

Note added in Proof: For new arguments regarding the status of *L. I. Mearnsi* see Eggert et al. (2004).

Literature Cited

Adler, G. H., and R. Levins. 1994. The island syndrome in rodent populations. Quarterly Review of Biology **69:**473–490.

Ashley, M., and C. Wills. 1987. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. Evolution **41:**854–863.

Atwood, J. L. 1980. Breeding biology of the Santa Cruz Island Scrub Jay. Pages 675–688 in D. M. Power. The California islands: proceedings of a multidisciplinary symposium. Santa Barbara Museum of Natural History, Santa Barbara, California.

Atwood, J. L., M. J. Elpers, and C. Collins. 1990. Survival of breeders in Santa Cruz Island and mainland California Scrub Jay populations. Condor **92:**783–788.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman & Hall, New York.

Balloux, F., and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology **11:**155–165.

Bardeleben, C., V. Palchevskiy, R. Calsbeek, and R. K. Wayne. 2004. Isolation of polymorphic tetranucleotide microsatellite markers for the brown anole (*Anolis sagrei*). Molecular Ecology Notes **4:**176–178.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics **152:**763–773.

Brumbaugh, R. W. 1980. Recent geomorphic and vegetation dynamics on Santa Cruz Island, California. Pages 139–158 in D. M. Power. The California islands: proceedings of a multidisciplinary symposium. Santa Barbara Museum of Natural History, Santa Barbara, California.

Carmen, W. J. 1988. Behavioral ecology of the California Scrub Jay (*Aphelocoma coerulescens californica*): a noncooperative breeder with close cooperative relatives. Ph.D. dissertation. University of California, Berkeley.

Collins, C. T., and K. Corey. 1994. Delayed breeding in the Santa Cruz Island scrub jay: why not be cooperative? Pages 371–378 in W. L. Halverson and G. J. Maender. The fourth California islands symposium: update on the status of resources. Santa Barbara Museum of Natural History, Santa Barbara, California.

Collins, P. W. 1982. Origin and differentiation of the island fox: a study of evolution of insular populations. M.S. thesis. University of California, Santa Barbara.

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.

Dawson, R. J. G., H. L. Gibbs, K. A. Hobson, and S. M. Yezerinac. 1997. Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warbler), and their use in population studies. Heredity **79:** 506–514.

Eggert, L. S., N. I. Mundy, and D. S. Woodruff. 2004. Population structure of loggerhead shrikes in the California Channel Islands. Molecular Ecology **13:**2121–2133.

Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. Trends in Genetics **16:**551–558.

Elsasser, A. B., and R. F. Heizer. 1963. The archaeology of Bowers Cave, Los Angeles County, California. Archaeology survey report. University of California, Berkeley.

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

Fu, Y. X., and W. H. Li. 1993. Statistical tests of neutrality of mutations. Genetics **133:**693–709.

Gilbert, D. A., N. Nehman, S. J. O'Brien, and R. K. Wayne. 1990. Genetic fingerprinting reflects population differentiation in the California Channel-Island fox. Nature **344:**764–767.

Gill, A. E. 1980. Evolutionary genetics of California Islands *Peromyscus*. Pages 719–743 in D. M. Power. The California islands: proceedings of a multidisciplinary symposium. Santa Barbara Museum of Natural History, Santa Barbara, California.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test for Hardy-Weinberg proportion for multiple alleles. Biometrics **48:**361–372.

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 **21:**160–174.

Johnson, N. K. 1972. Origin and differentiation of the avifauna of the Channel Islands, California. Condor **74:**295–315.

Johnson, T. H., and A. J. Stattersfield. 1990. A global review of island endemic birds. Ibis **132:**167–180.

Junger, A., and D. L. Johnson. 1980. Was there a Quaternary land bridge to the northern Channel Islands? Pages 33–39 in D. M. Power. The California Islands: proceedings of a multidisciplinary symposium. Santa Barbara Natural History Museum, Santa Barbara, California.

Kelsey, R., and C. T. Collins. 2000. Estimated population size of the Island Scrub-Jay *Aphelocoma insularis*. Bird Conservation International **10:**137–148.

Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei. 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics **17:**1244–1245.

Kvist, L., M. Ruokonen, J. Lumme, and M. Orell. 1999. The colonisation history and present day colonisation structure of the European Great Tit (*Parus major*). Heredity **82:**495–502.

Lambert, D. M., P. A. Ritchie, C. D. Miller, B. Holland, A. J. Drummond, and C. Baroni. 2002. Rates of evolution in ancient DNA from Adelie Penguins. Science **295:**2270–2273.

Langella, O. 1999–2003. Populations, a free population genetic software. Centre National de la Recherche Scientifique, Paris. Available from http://www.pge.cnrs-gif.fr/bioinfo/populations (June 2004).

Li, S. H., Y. J. Huang, and J. L. Brown. 1997. Isolation of tetranucleotide microsatellites from the Mexican jay *Aphelocoma ultramarina*. Molecular Ecology **6:**499–501.

Milberg, P., and T. Tyrberg. 1993. Naive birds and noble savages: a review of man-caused prehistoric extinctions of island birds. Ecography **16:**229–250.

Milot, E., H. L. Gibbs, and K. A. Hobson. 2000. Phylogeography and genetic structure of northern populations of Yellow Warbler (*Dendroica petechia*). Molecular Ecology **9:**667–681.

Moum, T., and E. Arnason. 2001. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Molecular Ecology **10:**2463–2478.

Mundy, N. I., C. S. Winchell, and D. S. Woodruff. 1996. Genetic differences between the endangered San Clemente Loggerhead Shrike *Lanius ludovicianus mearnsi* and two neighbouring subspecies demonstrated by mtDNA control region and cytochrome *b* sequence variation. Molecular Ecology **6:**29–37.

Mundy, N. I., C. S. Winchell, T. Burr, and D. S. Woodruff. 1997. Microsatellite variation and microevolution in the critically endangered San Clemete Island Loggerhead Shrike (*Lanius ludovicianus mearnsi*). Proceedings of the Royal Society of London Series B **264:**869–875.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution **19:**153–170.

Orr, P. C. 1968. Prehistory of Santa Rosa Island. Santa Barbara Museum of Natural History, Santa Barbara, California.

Owens, I. P. F., and P. M. Bennett. 2000. Ecological basis of extinction risk in birds: habitat loss versus human persecution and introduced predators. Proceedings of the National Acadademy of Sciences of the United States of America **97:**12144–12148.

Patten, M. A., and K. F. Campbell. 2000. Typological thinking and the conservation of subspecies: the case of the San Clemente Island Loggerhead Shrike. Diversity and Distributions **6:**177–188.

Pergams, O. R. W., and M. V. Ashley. 2001. Microevolution in island rodents. Genetica **112–113:**245–256.

Pitelka, F. A. 1951. Speciation and ecological distribution in American jays of the genus *Aphelocoma*. University of California Press, Berkeley.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics **14:**817–818.

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

Quinn, T. W. 1992. The genetic legacy of Mother Goose–phylogeographic patterns of Lesser Snow Goose *Chen caerulescens caerulescens* maternal lineages. Molecular Ecology **1:**105–117.

Raymond, M., and F. Rousset. 1995. GENEPOP (ver 1.2): a population genetics software for exact test and ecumenicism. Journal of Heredity **86:**248–249.

Roemer, G. W., and R. K. Wayne. 2003. Conservation in conflict: the tale of two endangered species. Conservation Biology **17:**1251–1260.

Roemer, G. W., D. A. Smith, D. K. Garcelon, and R. K. Wayne. 2001. The behavioural ecology of the island fox (*Urocyon littoralis*). Journal of Zoology (London) **255:**1–14.

Roemer, G. W., J. C. Donlan, and F. Courchamp. 2002. Golden eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. Proceedings of the National Academy of Sciences of the United States of America **99:**791–796.

Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics **15:**174–175.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4:**406–425.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York.

Saunders, M. A., and S. A. Edwards. 2000. Dynamics and phylogenetic implications of mtDNA control region sequences in New World Jays (Aves: Corvidae). Journal of Molecular Evolution **51:** 97–109.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin version 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, Geneva.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. Canadian Journal of Zoology **69:**82–90.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science **236:**787–792.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:**457–462.

Stamps, J. A., and M. Buechner. 1985. The territorial defense hypothesis and the ecology of insular vertebrates. Quarterly Review of Biology **60:**155–182.

Stenzler, L. M., and J. W. Fitzpatrick. 2002. Isolation of microsatellite loci in the Florida Scrub-Jay *Aphelocoma coerulescens*. Molecular Ecology Notes **2:**547–550.

Swofford, D. L. 1998. PAUP*: phylogenetic analysis using parsimony. Sinauer Associates, Sunderland, Massachusetts.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics **105:**437–460.

Tajima, F. 1993. Simple method for testing the molecular evolutionary clock hypothesis. Genetics **135:**599–607.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution **10:**512–526.

Tarr, C. L. 1995. Primers for amplification and determination of mitochondrial control-region sequences in oscine passerines. Molecular Ecology **4:**527–529.

Wayne, R. K., S. B. George, D. Gilbert, P. W. Collins, S. D. Kovach, D. Girman, and N. Lehman. 1991. A morphological and genetic study of the island fox, *Urocyon littoralis*. Evolution **45:**1849–1868.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38:**1358–1370.

Wenink, P. W., A. J. Baker, H.-U. Rosner, and M. G. J. Tilanus. 1996. Global mitochondrial DNA phylogeography of holarctic breeding dunlins (*Calidris alpina*). Evolution **50:**318–330.