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Genetic differentiation of island spotted skunks, *Spilogale gracilis amphiala*

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The island spotted skunk (*Spilogale gracilis amphiala*) is endemic to the 2 largest California Channel Islands, Santa Cruz and Santa Rosa. Unlike the island fox (*Urocyon littoralis*) and island subspecies of the deer mouse (*Peromyscus maniculatus*), the island spotted skunk shows no morphological differentiation between islands and is differentiated only weakly from mainland subspecies, suggesting recent colonization. However, the islands have been isolated from each other and the mainland throughout the Quaternary Period. We used 8 microsatellite loci to investigate the distribution of genetic variation within and among populations of spotted skunks from 8 localities (the 2 islands and 6 mainland localities), representing 4 subspecies. Tissue samples were obtained from 66 fresh specimens collected from 2000 to 2002 and 142 museum specimens collected from 1906 to 1994. Allelic richness and heterozygosity in island spotted skunk populations was approximately 30% lower than that found in mainland localities or subspecies. All localities or subspecies were significantly differentiated (mean F_{ST} was 0.17 and 0.13 for localities and subspecies, respectively). Contrary to comparisons based on morphological data, genetic differentiation was especially strong between islands and between island and mainland localities or subspecies. Patterns of differentiation suggest that skunks colonized the Channel Islands shortly before rising sea levels separated Santa Cruz and Santa Rosa islands (11,500 years ago). Our results indicate that the taxonomic status of the island spotted skunk should be reconsidered and that both island populations might constitute evolutionarily significant units worthy of conservation.

Key words: California, Channel Islands, colonization, genetic differentiation, *Spilogale gracilis amphiala*, spotted skunks

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The Channel Islands off the coast of southern California support a depauperate mammalian fauna. The 8 islands in the group host a total of 6 species of native terrestrial mammals, of which 4 species—2 rodents and 2 carnivores—occur on multiple islands (von Bloeker 1965). Of these 4 species, 2 exhibit strong population differentiation in both genetic and morphologic characters. The island fox (*Urocyon littoralis*) is a species endemic to the Channel Islands, and it occurs on the 6 largest islands, with each island supporting a distinct subspecies (Collins 1993; Gilbert et al. 1990; Goldstein et al. 1999; Wayne et al. 1991). The deer mouse (*Peromyscus maniculatus*) is represented by a different endemic subspecies

on each of the 8 islands (Ashley and Wills 1987; Gill 1980; Pergams and Ashley 2000). In contrast, the western harvest mouse (*Reithrodontomys megalotis*) shows little or no differentiation in genetic or morphologic characters on the 2 islands where it is native, and it is suspected of having been introduced inadvertently by Native Americans (Ashley 1989; Collins and George 1990).



The island spotted skunk (*Spilogale gracilis amphiala*), an insular endemic subspecies restricted to the 2 largest islands, Santa Cruz (249 km²) and Santa Rosa (217 km²), has long been an enigma. Morphologic differentiation from mainland subspecies (based on a shorter tail and wider interorbital region) is so modest that the island spotted skunk is considered only a “weak” subspecies, and the 2 island populations are taxonomically indistinguishable (Van Gelder 1959). Such a lack of differentiation suggests a recent colonization; however, no land bridge to the Channel Islands existed throughout the Quaternary Period (Junger and Johnson 1980), and water is an effective barrier to skunk dispersal (Van Gelder 1965). Skunks might have colonized by rafting on floating debris washed out to sea (Johnson 1983; Wenner and Johnson 1980). Native Americans have been implicated in the transport of mammals to or among the Channel Islands (Ashley and Wills 1987; Collins and George 1990; Gilbert et al. 1990; Rick et al. 2009), but transport of skunks seems unlikely because of their chemical defense (Van Gelder 1965).

Morphologic affinities of island spotted skunks are also somewhat perplexing. Spotted skunk subspecies are distinguished on the basis of a combination of skull metrics, tail length, and coat color pattern (Van Gelder 1959). Based on tail length, interorbital breadth, and cranial height, *S. g. amphiala* is more closely aligned with *S. g. latifrons* of Oregon and Washington than with *S. g. phenax* of the California coast adjacent to the Channel Islands (Van Gelder 1959, 1965). Van Gelder (1959, 1965) hypothesized that skunks colonized the Channel Islands during a cool, moist period when coastal southern California supported conifer forests and a *latifrons*-like skunk that withdrew northward as the climate warmed. Island skunks might have retained the *latifrons*-like characters because they lacked sufficient genetic variation (Van Gelder 1959, 1965), which is required for populations to evolve in response to environmental change (Reed and Frankham 2003). Reduced genetic variation, although documented for the island fox (Wayne et al. 1991), has not been addressed in the island spotted skunk.

Relatively low genetic diversity on islands appears to be the rule for insular populations of nonvolant mammals (Eldridge et al. 2004; Frankham 1997; Paetkau et al. 1997; Telfer et al. 2003). The same is true for many flying birds despite their greater capacity for overwater dispersal (Boessenkool et al. 2007; Kretzmann et al. 2003; Wilson et al. 2009). Among grizzly bears (*Ursus arctos*) on Kodiak Island, which likely have been completely isolated from mainland Alaska for ~12,000 years, allelic diversity and heterozygosity of microsatellite loci were approximately 65% lower than in mainland populations (Paetkau et al. 1997). *Urocyon l. dickeyi*, an island fox subspecies endemic to San Nicolas Island in the southern Channel Islands, is genetically the most monomorphic mammal yet found, lacking variation at microsatellite loci and 3 other genetic markers (Aguilar et al. 2004; Gilbert et al. 1990; Goldstein et al. 1999). Reduced allelic diversity and heterozygosity are expected consequences of founder effects, genetic drift, and inbreeding, which are

associated with colonization and subsequent small population size (Frankham 1997, 1998). These genetic effects can hinder conservation efforts for island species (Frankham 1997, 1998). In some cases, however, island populations can contribute to the overall genetic variation of a species by harboring unique alleles or divergent allele frequencies (Wilson et al. 2009).

The island spotted skunk is classified as a “species of special concern” by the state of California, primarily because so little is known about it (Williams 1986). We used microsatellite loci as selectively neutral markers to study genetic variation in island spotted skunks. We compared the amount of genetic diversity between island and mainland populations, and we investigated patterns of differentiation among island and mainland populations. Our goal was to provide information on the genetic divergence and biogeography of island spotted skunks and to inform efforts for their conservation.

MATERIALS AND METHODS

Sample collection.—We obtained DNA from spotted skunks from Santa Cruz Island, Santa Rosa Island, and 6 mainland localities (Fig. 1). These include a total of 4 subspecies of spotted skunks (Hall 1981; Verts et al. 2001). *S. g. phenax* was represented by 4 localities: Santa Barbara County and adjacent portions of Ventura County (approximately 35 km to the north of Santa Cruz Island); Los Angeles County and adjacent portions of Orange and San Bernardino counties (approximately 100 km to the east); Central California (Alameda and Contra Costa counties); and Northern California (Humboldt and Trinity counties). *S. g. latifrons* was represented by Oregon (Clatsop, Tillamook, Lincoln, and Clackamas counties). *S. g. gracilis* was represented by Lake Tahoe (El Dorado, Placer, and Alpine counties, California; Lyon and Douglas counties, Nevada). For all mainland localities except Oregon, skunks were sampled from sites lying within a 60-km radius. We extended the radius for northwestern Oregon to 100 km to improve sample size.

For most samples from Santa Cruz Island and El Dorado County, some samples from Santa Rosa Island, and 1 sample from Santa Barbara County ($n = 66$), DNA was obtained from tissues (blood, hair follicles, skin, or muscle) removed from live or recently dead skunks during 2000–2002. Because of the difficulty in livetrapping spotted skunks in California (Carroll 2000; Crooks 1994), we augmented our samples with tissue obtained from 142 museum specimens collected during 1906–1990 (Appendix I) by shaving a thin sliver of epidermis off 1 toe pad of the specimen. The year of sample collection ranged from 1907 to 1943 for Central California, 1914 to 2000 for Lake Tahoe, 1919 to 1990 for Los Angeles, 1910 to 1994 for Northern California, 1921 to 1990 for Oregon, 1906 to 2000 for Santa Barbara, 1928 to 2001 for Santa Cruz Island, and 1927 to 2002 for Santa Rosa Island. All sampling of live skunks followed the guidelines of the American Society of Mammalogists (Gannon et al. 2007) and was approved by the Institutional Animal Care and Use Committee at the

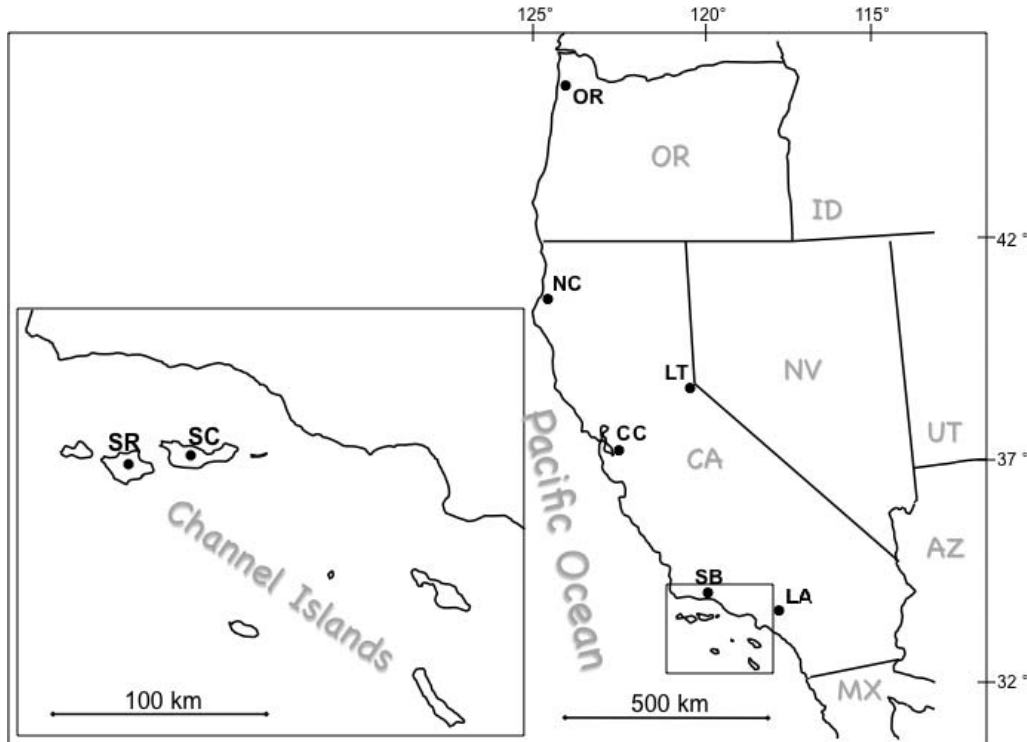


FIG. 1.—Localities (marked by black circles and capital letters) where 208 spotted skunks (*Spilogale gracilis*) were collected or livetrapped. Tissues were sampled for microsatellite DNA analysis from these individuals at the time of collection or trapping or later from archived or museum specimens. Circles indicate the approximate center of a sampling radius of 60 km (100 km in the case of OR). SR = Santa Rosa Island, SC = Santa Cruz Island, LA = Los Angeles County, SB = Santa Barbara County, CC = Central California (Alameda and Contra Costa counties), LT = Lake Tahoe (El Dorado, Placer, and Alpine counties, California; Lyon and Douglas counties, Nevada), NC = Northern California (Humboldt and Trinity counties), and OR = Oregon (Clatsop, Tillamook, Lincoln, and Clackamas counties). Four subspecies are represented by these samples: *S. g. amphiala* (SR and SC), *S. g. phenax* (LA, SB, CC, and NC), *S. g. gracilis* (LT), and *S. g. latifrons* (OR).

University of California. Island spotted skunks were trapped under Memoranda of Understanding between the California Department of Fish and Game and the University of California, Davis, and the Institute for Wildlife Studies.

Laboratory methods.—Genomic DNA was extracted using GenElute tissue kits (Sigma-Aldrich, St. Louis, Missouri). We followed the manufacturer's extraction procedure except that we used approximately 4 mm² of tissue per individual and digested the tissue at 55°C for 24 h while turning continuously. Polymerase chain reaction amplifications were performed in 10- μ l reactions containing 5–10 ng of DNA, 0.5 μ M of each primer, 175 μ M of deoxynucleoside triphosphates, 2–3 mM of MgCl₂, and 1 unit of FastStart Taq DNA polymerase (Roche Applied Science, Indianapolis, Indiana). Amplification conditions consisted of an initial 94°C step for 4 min, followed by 40 cycles of 94°C for 40 s, 52–55°C for 40 s, and 72°C for 1 min; and then a final extension of 5 min at 72°C. Polymerase chain reaction products were diluted in 98% formamide loading dye, separated on 5% denaturing polyacrylamide gels, stained with fluorescent dye, and visualized using a Fluorimager 595 (Molecular Dynamics, Sunnyvale, California).

We screened a total of 112 microsatellite loci for polymorphism in 8 individuals of *S. gracilis* (from the islands

and mainland) using primers originally developed for the following mustelids: *Taxidea taxus* (Davis and Strobeck 1998), *Meles meles* (Bijlsma et al. 2000; Carpenter et al. 2003; Domingo-Roura et al. 2003), *Gulo gulo* (Davis and Strobeck 1998; Walker et al. 2001), *Lontra canadensis* (Beheler et al. 2005), *Lutra lutra* (Dallas and Piertney 1998; Walker et al. 2001), *Mustela erminea* (Fleming et al. 1999), *Neovison vison* (Fleming et al. 1999; O'Connell et al. 1996), and *Martes americana* (Davis and Strobeck 1998); and for 1 mephitid: *Mephitis mephitis* (Dragoo et al. 2009). Only 3 of these loci showed clearly interpretable variation: Mell1 (Bijlsma et al. 2000), Meph22-70, and Meph22-76 (Dragoo et al. 2009). However, approximately 12 other loci showed microsatellite variation that was obscured by bands from nonspecific amplifications. To develop new primers specifically for the microsatellite loci we identified bands representing the locus of interest, cut out 1 of these bands from the gel, and soaked it overnight in 200 μ l of nanopure H₂O. Then we gently vortexed the solution, centrifuged it at 20,000 \times g for 3 min, drew off the top 10 μ l, and reamplified the DNA using our standard polymerase chain reaction method. Amplified DNA was extracted from the polymerase chain reaction solution using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and then sequenced. New primers then

were designed from the sequence using Primer Select software (DNASTAR Inc., Madison, Wisconsin). We screened 24 pairs of redesigned primers and found 5 pairs that clearly showed interpretable variation: rLut832 (primers: 5'-GTCAGTTT-CCTCACATTCAGA-3' and 5'-AACTTTGGGGGTCCTTT-TAT-3'—modified from Dallas and Piertney 1998), rLut818 (primers: 5'-TTTCAGGGCACAAGGATG-3' and 5'-CCG-CCAGGGGACACAGT-3'—modified from Dallas and Piertney 1998), rTt-2 (5'-TCCCTCATGTTACAGCAGTAT-3' and 5'-TTCAAGGATTCAAGGACCAT-3'—modified from Davis and Strobeck 1998), nRIO-01 (5'-CCTGCCAGGCC-TATTC-3' and 5'-TCTGAAAAGTGGATATCTGTCATC-3'—modified from Beheler et al. 2005), and nRIO-08 (5'-TGAGGTGTTGGTGTGTTTGTCTAT-3' and 5'-TTGCCTG-CTGACATTGAAGMT-3'—modified from Beheler et al. 2005).

We also sequenced 12 individuals from island and mainland localities at the cytochrome-*b* gene (approximately 1,000-base pair [bp] fragment) and D-loop region (approximately 500-bp fragment) of the mitochondrial genome. However, what little usable variation existed (only 3–5 base substitution sites in either fragment) failed to produce trees that could be resolved.

Analyses of microsatellite variation.—We quantified genetic diversity by calculating observed heterozygosity (H_O) and Nei's (1978) expected heterozygosity (H_E), using the program TFGA (version 1.3—Miller 1997). Allelic richness (A) was estimated using FSTAT (version 2.9.3, updated from Goudet 1995; <http://www2.unil.ch/popgen/softwares/fstat.htm>). To test whether spotted skunks on islands had significantly reduced genetic diversity relative to those on the mainland we used the permutation test in FSTAT (15,000 permutations), which compares the islands as a group to the mainland localities as a group.

To test for gametic disequilibrium we used the Markov chain approximation in GENEPOP (Raymond and Rousset 1995; <http://genepop.curtin.edu.au>), which estimates the probability of genotype independence for each pair of loci within each locality or subspecies. We used the Dunn–Sidak method ($\alpha = 0.05$ —Gotelli and Ellison 2004) to adjust significance level for multiple comparisons. To test for heterozygote deficiency, as might result from the presence of null (nonamplifying) alleles or inadvertent pooling of subpopulations (Wahlund 1928), we tested 1-tailed probabilities of departure from Hardy–Weinberg equilibrium, using the Markov chain exact test in GENEPOP.

Another factor that leads to underestimation of genetic diversity is allelic dropout, the stochastic nonamplification of an allele, which leads to a heterozygote being incorrectly called a homozygote. Allelic dropout becomes more likely with increasing age and associated decreasing quality of DNA samples (e.g., historical or museum specimens—Wandeler et al. 2007). As a rough test of whether allelic dropout was a problem in our study, we measured whether older samples tended to have lower apparent genetic diversity. First, we used linear regression to examine the relationship between sample age and homozygosity (arcsine-transformed proportion of

successfully amplified loci that were homozygous). Second, we compared genetic diversity (arcsine-transformed H_E and log-transformed A) in older and newer samples using 2-tailed Wilcoxon signed-rank tests (Dalggaard 2002; R Development Core Team 2008). Because we acquired our samples opportunistically—and, thus, the distribution of sample age varied greatly within and between localities—none of the localities provided the opportunity to compare 2 equal-sized groups sampled in the same locality at 2 different, widely spaced time periods. However, of the 55 samples from Santa Rosa, 18 were from 1927, 9 from 1941, and 26 from 2000. This allowed us to compare a fairly balanced pair of groups, 1 old (27 samples, 1927–1941) and 1 new (26 samples, 2000). As a 3rd test for reduced genetic diversity in older samples, we split the entire data set into an older (1906–1949, $n = 99$) and newer (1961–2000, $n = 113$) group and compared their genetic diversity as above. We chose this grouping because 1949–1961 was the only substantial gap in the sample dates with similar numbers of samples on either side. Finally, to examine the influence of sample age on sample quality (and, thus, the probability of allelic dropout) in our samples we used linear regression to measure the relationship between sample age and the proportion of loci that failed to amplify for that individual.

Analyses of genetic structure.—We used GENEPOP and ARLEQUIN 3.01 (Excoffier et al. 2005) to quantify the magnitude of genetic differentiation (F_{ST}) among localities. Statistical significance was assessed via bootstrapping over loci (20,000 permutations) in ARLEQUIN. We used FSTAT to estimate overall F_{ST} and assessed statistical significance by jackknifing over loci. We tested for overall genetic structure (heterogeneity in microsatellite allelic frequencies) using the Markov chain approximation in GENEPOP to estimate the 1-tailed probability of allelic differentiation under the null hypothesis of no difference among localities. In addition, we analyzed genetic structure of the 4 subspecies (localities aggregated by subspecies) using the same methods described above.

To further characterize genetic divergence between localities and between subspecies we produced neighbor-joining trees using the program POPTREE2 (Takezaki et al. 2009), bootstrapping over loci (10^5 replicates). Three genetic distance measures were used: D_A (Nei et al. 1983), D_{ST} (Nei's standard genetic distance—Nei 1972), and F_{ST} (with sample size correction—Latter 1972).

We examined the relationship between genetic distance and geographic distance among localities using GENEPOP, which regresses genetic distance against geographic distance (Euclidean, ln km), calculates Spearman rank correlation coefficients (r_s), and uses Mantel (1967) permutation (10^6 permutations) to establish 95% confidence intervals (CIs) for r_s . As genetic distance measures we used $F_{ST}/1 - F_{ST}$ (Rousset 1997) and D_{LR} , the genotype likelihood ratio distance measure (Paetkau et al. 1997). Likelihood values for D_{LR} were calculated using GENECLASS2 (Piry et al. 2004; <http://www.ensam.inra.fr/URLB>).

TABLE 1.—Summary of microsatellite variation (8 loci) for 208 spotted skunks (*Spilogale gracilis*) sampled from 8 localities. SR = Santa Rosa Island, SC = Santa Cruz Island, LA = Los Angeles County, SB = Santa Barbara County, CC = Central California (Alameda and Contra Costa counties), LT = Lake Tahoe (El Dorado, Placer, and Alpine counties, California and Lyon and Douglas counties, Nevada), NC = Northern California (Humboldt and Trinity counties), and OR = Oregon (Clatsop, Tillamook, Lincoln, and Clackamas counties). *n* is number of individuals sampled, *A* is mean allelic richness, and *H_O* and *H_E* are mean observed and expected (Nei 1978) heterozygosity, respectively (range across 8 loci in parentheses). Asterisks indicate significant deviation from Hardy–Weinberg equilibrium after Dunn–Sidak correction for multiple comparisons (*P* < 0.006).

Localities	<i>n</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>
SR*	55	3.5	0.45 (0.22–0.77)	0.53 (0.36–0.76)
SC	35	2.9	0.46 (0.00–0.65)	0.49 (0.00–0.73)
LA*	18	5.0	0.56 (0.33–0.75)	0.71 (0.42–0.94)
SB*	26	5.1	0.65 (0.42–0.88)	0.75 (0.51–0.91)
CC*	19	4.6	0.65 (0.45–0.83)	0.69 (0.51–0.90)
LT	16	4.6	0.64 (0.38–0.93)	0.68 (0.41–0.84)
NC	22	5.1	0.74 (0.59–0.86)	0.76 (0.66–0.89)
OR*	17	5.3	0.60 (0.45–0.75)	0.75 (0.62–0.86)
	26	4.5	0.59	0.67

RESULTS

Microsatellite variation.—Allelic richness (estimated across loci) ranged from 2.9 to 5.3 per locality, and *H_O* and *H_E* ranged from 0.45 to 0.74 and 0.49 to 0.76, respectively (Table 1). The total number of alleles (across localities) per locus ranged from 4 to 21, and *H_O* and *H_E* per locus ranged from 0.49 to 0.65 and 0.62 to 0.88, respectively. Genetic diversity was approximately 30% lower on the islands (*H_O* = 0.45, *H_E* = 0.58, *A* = 3.17) compared to mainland localities (*H_O* = 0.65, *H_E* = 0.78, *A* = 4.93; permutation test *P* = 0.04). Of 79 total alleles, 19 were private (i.e., found only in 1 locality), and 2 of these were private to the islands, 1 on Santa Cruz and 1 on Santa Rosa.

When analyzed within localities, significant gametic disequilibrium was found in only 1 of the 224 locus-by-locus comparisons (Meph22-26 and Meph22-70; *P* = 0.001, adjusted α = 0.002). Analyzed across localities, significant gametic disequilibrium among loci was detected in only 1 of the 28 possible locus-by-locus comparisons (Mel1 \times nRIO-01; *P* = 0.002, adjusted α = 0.004). Significant heterozygote deficiency (adjusted α = 0.006) was found in 5 of the 8 loci (Mel1, rLut818, Meph22-70, Meph22-26, and nRIO-08; *P* < 0.006) and 5 of the 8 localities (Santa Rosa Island, Oregon, Santa Barbara, Los Angeles County, and Central California; *P* < 0.006).

The frequency of failed amplification increased slightly with sample age ($F_{1,210}$ = 31.86, adjusted R^2 = 0.13, *P* < 0.001); however, no relationship was found between sample age and homozygosity ($F_{1,210}$ = 0.33, adjusted R^2 = 0.00, *P* = 0.57). We found no significant difference in mean (\pm *SE*) genetic diversity between older (*H_O* = 0.40 \pm 0.06, *H_E* = 0.50 \pm 0.07, *A* = 4.25 \pm 0.69) and newer (*H_O* = 0.48 \pm 0.08,

TABLE 2.—Pairwise matrix of genetic distance (above diagonal; *F_{ST}*) and geographic distance (below diagonal; km) between localities where 208 spotted skunks (*Spilogale gracilis*) were collected or livetrapped and later genotyped at 8 microsatellite loci. Top numbers above the diagonal are *F_{ST}*-values calculated using all 8 loci; bottom numbers are *F_{ST}*-values calculated using a subset of the data. This subset excluded the 2 loci (Mel1 and Meph22-76) for which ≥ 0.05 of the individuals lacked allele information and the 39 individuals lacking allele information at ≥ 2 loci, thus allowing us to test whether the *F_{ST}*-values were significantly > 0 ; all of these *F_{ST}*-values were significant (*P* < 0.002) following Dunn–Sidak correction for multiple comparisons. See Table 1 for full names of localities.

Localities	SR	SC	LA	SB	CC	LT	NC	OR
SR	—	0.206	0.228	0.216	0.246	0.282	0.211	0.242
		0.186	0.301	0.277	0.313	0.318	0.240	0.257
SC	9	—	0.276	0.185	0.162	0.251	0.194	0.177
			0.342	0.198	0.219	0.253	0.191	0.194
LA	204	163	—	0.057	0.139	0.087	0.055	0.118
				0.103	0.207	0.128	0.092	0.174
SB	53	40	184	—	0.051	0.068	0.040	0.074
					0.092	0.056	0.047	0.082
CC	453	457	577	435	—	0.114	0.059	0.061
						0.146	0.087	0.104
LT	541	537	574	500	234	—	0.087	0.122
							0.076	0.116
NC	826	833	938	806	374	413	—	0.066
								0.070
OR	1,301	1,308	1,348	1,264	849	779	518	—

H_E = 0.51 \pm 0.05, *A* = 4.06 \pm 0.38) samples on Santa Rosa Island (*P* > 0.45). Also, no significant difference was detected in mean genetic diversity between older (*H_O* = 0.54 \pm 0.04, *H_E* = 0.75 \pm 0.03, *A* = 8.41 \pm 1.30) and newer (*H_O* = 0.57 \pm 0.03, *H_E* = 0.73 \pm 0.03, *A* = 8.67 \pm 1.62) samples in the overall data set (*P* > 0.54).

Genetic structure of localities.—Exact tests revealed statistically significant differentiation among localities over all 8 loci (*P* < 0.001). *F_{ST}*-values between localities averaged 0.17 (*SE* = 0.02, 95% *CI* = 0.13–0.21), ranging from 0.04 to 0.28 (Table 2). The degree of genetic differentiation among localities was especially strong for island–island and island–mainland comparisons (Table 2). The mean *F_{ST}* for island–mainland (approximately 0.22, *SE* = 0.04) and island–island (0.21) *F_{ST}* comparisons was almost 3 times that of mainland–mainland comparisons (approximately 0.08, *SE* = 0.03). Statistical significance of *F_{ST}*-values could be calculated for only a subset of the data that excluded the 2 loci (Mel1 and Meph22-26) for which $\geq 5\%$ of the individuals lacked allele information. The subset also excluded the 39 individuals lacking allele information at ≥ 2 loci. Most of these individuals were from 1949 or earlier and represented 7% of samples from Santa Rosa Island, 50% from Los Angeles, 19% from Santa Barbara, 58% from Central California, 13% from Lake Tahoe, and 47% from Oregon. The *F_{ST}*-values from the subset ranged from 0.05 to 0.34, all of which were significant (*P* < 0.002, adjusted α = 0.002; Table 2).

We found no significant relationship between geographic and genetic distance measured as $F_{ST}/1 - F_{ST}$; this was the

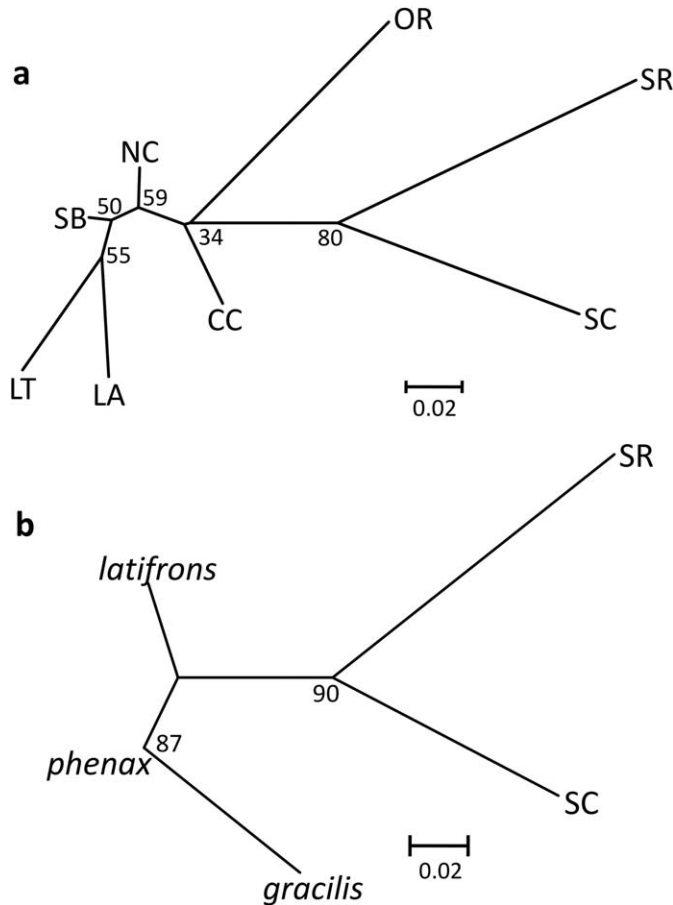


FIG. 2.—Neighbor-joining trees for spotted skunks (*Spilogale gracilis*) sampled from 2 island (SR and SC) and 6 mainland (LA, SB, LT, CC, NC, and OR) localities, based on F_{ST} (with sample size correction—Latter 1972). Trees were constructed for a) the 8 localities and b) the group composed of SR, SC, and the mainland subspecies. The number at a node represents the fraction of bootstrap replicates (10^5 replicates) supporting that node. Almost identical trees (not shown) were constructed using the D_A (Nei et al. 1983) and D_{ST} (Nei 1972) distance measures, although somewhat different clustering for mainland localities was found in D_A -based trees. See Fig. 1 for full names of localities and subspecies.

case for all samples considered together ($r_s = -0.26$, $n = 28$, $P = 0.69$) and for mainland samples only ($r_s = 0.04$, $n = 15$, $P = 0.45$). A similar lack of significant correlation was found when using D_{LR} (all samples: $r_s = -0.17$, $n = 28$, $P = 0.79$; mainland only: $r_s = 0.31$, $n = 15$, $P = 0.20$).

Neighbor-joining trees showed strong support (bootstrap values ranging from 0.80 to 0.95 over the 3 distance measures) for a division between island and mainland localities (Fig. 2a). Fairly weak (0.59–0.68) support was found for a group containing the Los Angeles, Lake Tahoe, Northern California, and Santa Barbara localities, and the Oregon and Central California localities fell in the middle (for all 3 distance measures). Divergence between island localities was greater than between any of the mainland localities (Fig. 2a).

Genetic structure of subspecies.— F_{ST} analyses revealed stronger levels of differentiation between the island (*S. g.*

amphiala) and mainland subspecies than between mainland subspecies (Table 3). The strongest level of genetic differentiation between subspecies was between island skunks and *S. g. gracilis*, and the weakest levels were between *S. g. phenax* and *S. g. latifrons* and between *S. g. phenax* and *S. g. gracilis* (Table 3). Island skunks (lumped into *S. g. amphiala* or separate island populations) were less differentiated from *S. g. phenax* than from either of the other 2 subspecies. Overall between-subspecies F_{ST} averaged 0.13 ($SE = 0.03$, 95% $CI = 0.09$ –0.18). Exact tests revealed statistically significant differentiation of subspecies over all 8 loci ($P < 0.001$). Significant heterozygote deficiency ($P < 0.006$) was found in 7 of the 8 loci (all but nRIO-01) and 3 of the 4 subspecies (all but *S. g. gracilis*; $P < 0.006$). When analyzed within subspecies, significant gametic disequilibrium was found in only 3 of the 112 locus-by-locus comparisons (rLut832 \times Mel1 and Meph22-76 \times Meph22-26 in *S. g. amphiala*, and nRIO-08 \times Meph22-26 in *S. g. gracilis*; $P < 0.0002$, adjusted $\alpha = 0.005$). Analyzed across subspecies, significant gametic disequilibrium among loci was detected in only 3 of the 28 possible pairwise locus-by-locus comparisons (rLut832 \times Mel1, nRIO-08 \times Meph22-70, and nRIO-08 \times Meph22-26; $P < 0.002$, adjusted $\alpha = 0.004$). Of 79 total alleles, 21 were private, and 2 of these were private to *S. g. amphiala*. Levels of genetic diversity were on par with those found when localities were not lumped by subspecies (e.g., allelic diversity and heterozygosity in *S. g. amphiala* approximately 30% less than in mainland subspecies).

When *S. g. amphiala* was split into the 2 island localities, the level of differentiation between islands was roughly intermediate to that between island localities and mainland subspecies but stronger than the levels of differentiation between any of the mainland subspecies (Table 3). Levels of genetic diversity and results of tests for Hardy–Weinberg deficiency, gametic disequilibrium, and overall differentiation were similar to those found when *S. g. amphiala* was not split into the 2 island localities.

When considering subspecies, neighbor-joining trees again showed strong support (bootstrap values ranging from 0.90 to 0.98 over the 3 distance measures) for an island–mainland division (Fig. 2b). The trees also all showed a weaker (0.60–0.87) grouping of *S. g. phenax* and *S. g. gracilis*, with *S. g. latifrons* falling in the middle. Divergence between islands was greater than that between the mainland subspecies (Fig. 2b).

DISCUSSION

Because our sampling was necessarily opportunistic, sample age extended over a 96-year period, which presented 2 potential problems, low quality of DNA samples and strong temporal heterogeneity of allelic frequencies within localities. With decreased quality of template DNA (such as that from historical samples) comes an increased probability of allelic dropout and, consequently, underestimation of genetic diversity in a population (Wandeler et al. 2007). We found heterozygote deficiency at most loci and localities. Further-

TABLE 3.—Microsatellite genetic distance (F_{ST}) between subspecies of spotted skunks (*Spilogale gracilis*), sampled from 8 localities: *S. g. amphiala* (SR and SC), *S. g. latifrons* (OR), *S. g. gracilis* (LT), *S. g. phenax* (LA, SB, CC, and NC). Also shown are separate entries for each island population (SR and SC, both considered to be *S. g. amphiala*). See Table 1 for names of localities. Top numbers are F_{ST} -values calculated using all 8 loci; bottom numbers are F_{ST} -values calculated using a subset of the data. This subset excluded the 4 loci (Me11, rLut818, Meph22-70, and Meph22-76) for which ≥ 0.05 of the individuals lacked allele information and the 39 individuals lacking allele information at ≥ 2 loci, thus allowing us to test whether the F_{ST} -values were significantly >0 ; all of these F_{ST} -values were significant ($P < 0.002$) following Dunn–Sidak correction for multiple comparisons.

Subspecies	SR	SC	<i>S. g.</i> <i>amphiala</i>	<i>S. g.</i> <i>latifrons</i>	<i>S. g.</i> <i>gracilis</i>
SC	0.206				
	0.182				
<i>S. g. latifrons</i>	0.242	0.177	0.170		
	0.257	0.194	0.191		
<i>S. g. gracilis</i>	0.282	0.251	0.225	0.122	
	0.318	0.253	0.256	0.116	
<i>S. g. phenax</i>	0.180	0.156	0.139	0.054	0.058
	0.218	0.168	0.174	0.064	0.053

more, polymerase chain reaction failure was slightly greater in older samples ($R^2 = 0.13$), suggesting that allelic dropout might have influenced our results. However, we found no association between sample age and homozygosity, as would be expected if the frequency of false homozygotes had been higher in older, presumably lower-quality samples. A more likely possibility is that heterozygote deficiency was due to inadvertent pooling of subpopulations (Wahlund effect), because our samples were not drawn randomly from the population represented by a locality.

The 96-year extent of our sample age also could have contributed to heterozygote deficiency by inadvertent pooling of temporally differentiated populations (i.e., populations from different time periods that had different allelic frequencies). Statistically significant allelic differentiation was found between older (1927 and 1941) and newer (2000) Santa Rosa samples ($\chi^2_{16} = 58.0$, $P < 0.001$); however, the magnitude of differentiation was small ($F_{ST} = 0.029$, 95% $CI = 0.01$ – 0.05). We were unable to assess the influence of temporal heterogeneity in the other localities because sample date ranged too widely and inconsistently. Nonetheless, we do not believe that temporal or genetic heterogeneity in our samples unduly influenced our results, because the temporal scale of our study's biogeographical context (approximately 20,000 years) is approximately 2 orders of magnitude larger than that of our sample period (96 years).

Genetic diversity in island spotted skunks was approximately 30% lower than in mainland spotted skunks. The relatively low level of genetic diversity in island skunks, along with the relatively strong island–island and island–mainland genetic differentiation, is consistent with long-term isolation of skunks on Santa Rosa and Santa Cruz islands. If gene flow among populations was rare or nonexistent, microsatellite

allelic frequencies would be expected to diverge due to genetic drift (Bohonak 1999). In addition to homogenizing genetic differences between populations, gene flow tends to replace alleles lost to genetic drift (Hartl 2000; Telfer et al. 2003) and alleviate inbreeding (Tallmon et al. 2004). Reduced genetic diversity on islands also could reflect founder effects associated with colonization by a small number of individuals (Dlugosch and Parker 2008). The allelic diversity sampled on the Channel Islands was mostly a subset of the diversity on the mainland, because only 2 of the 19 private alleles found in the study were private to the islands.

The patterns of genetic differentiation between localities in our study were not consistent with results from morphologic analyses (Van Gelder 1959). The level of differentiation between the 2 islands (currently considered the same subspecies, *S. g. amphiala*) was greater than between any of the mainland subspecies and also greater than that between *S. g. amphiala* and *S. g. latifrons* and between *S. g. amphiala* and *S. g. phenax*. The strongest level of differentiation among subspecies was between *S. g. amphiala* and *S. g. gracilis*. Contrary to the expectation of Van Gelder (1959), we found no evidence that *S. g. amphiala* was closely linked with *S. g. latifrons*, because the level of differentiation between *S. g. amphiala* and *S. g. latifrons* was greater than that between *S. g. amphiala* and *S. g. phenax*. However, these results must be interpreted with caution, because our sample sizes (number of samples per locality and number of loci) were small and most of the loci and localities exhibited heterozygote deficiency; the latter violates the assumptions of Hardy–Weinberg equilibrium in statistical tests of differentiation.

If geographic distance between populations restricts movement among them, we would expect to find an isolation-by-distance pattern in which more closely situated populations are less genetically differentiated than those farther apart (assuming equilibrium between gene flow and genetic drift at neutral loci—Hutchison and Templeton 1999). However, we found no such relationship. The lack of isolation by distance suggests that the effects of genetic drift were stronger than the effects of gene flow; that is, the frequencies of microsatellite alleles in each locality were allowed to drift independently with little relation to the intervening geographic distances (Hutchison and Templeton 1999). The effects of genetic drift would have been especially strong for the island localities, which are separated from the nearest source of migrants by 5–30 km of open water. Alternatively, the lack of isolation by distance could have been due to our use of simple Euclidean distance between localities. Distance measures that take into account habitat quality or dispersal barriers for skunks might provide a better test of the role of gene flow compared to genetic drift.

Results from our comparisons of genetic diversity in island and mainland spotted skunk populations correspond with results from studies of island and mainland populations of foxes (Aguilar et al. 2004; Wayne et al. 1991) and deer mice (Ashley and Wills 1987; Gill 1980). Levels of genetic diversity at allozyme, mitochondrial DNA (mtDNA), and

minisatellite markers were lower in island foxes than in the closely related gray fox (*Urocyon cinereoargenteus*) on the mainland (Aguilar et al. 2004; Wayne et al. 1991). Similarly, in island deer mice levels of diversity at mtDNA and allozyme markers were lower on the islands than on the mainland (Ashley and Wills 1987; Gill 1980).

Contrary to our results with island skunks, however, genetic differentiation among island populations of deer mice, measured at mtDNA markers, was substantially weaker than that between islands and the mainland (Ashley and Wills 1987). Likewise in island foxes, genetic differentiation between islands (measured at allozyme, mtDNA, and minisatellite markers) was substantially weaker than between islands and the mainland (Aguilar et al. 2004; Wayne et al. 1991). Information on island-mainland comparisons of microsatellite diversity and differentiation was unavailable for island foxes and deer mice.

Patterns of differentiation in extant mammals in the Channel Islands likely reflect their respective biogeographic histories. All 4 species probably reached the Channel Islands by rafting or transport by Native Americans (Johnson 1983; Wenner and Johnson 1980). Successful dispersal via rafting was most likely during the late Quaternary Period (~24,000–18,000 years ago) when sea level reached its lowest point (Johnson 1983), leaving the 4 northern islands (Santa Cruz, Santa Rosa, San Miguel, and Anacapa) joined as 1 superisland, Santarosae (Johnson 1983). At 2,331 km² Santarosae Island was 4 times larger than the present-day total of the 4 constituent islands and separated from the mainland by only 6 km (Johnson 1983), as opposed to 30 km separating Santa Cruz Island (the closest of the 4) from the mainland today. Rising sea levels separated Santa Cruz from Santa Rosa Island about 11,500 years ago, and Santa Rosa from San Miguel Island about 9,500 years ago (Johnson 1978). Native Americans colonized 13,000–11,000 years ago (Johnson et al. 2002; Rick et al. 2009) and established an active trade via seagoing canoes (Collins 1993).

Weak differentiation in western harvest mice, both among islands and between island and mainland, indicates their recent colonization of the Channel Islands, probably as stowaways in Native American canoes (Ashley 1989; Collins and George 1990). Deer mice are more strongly differentiated (Collins and George 1990), and mtDNA markers indicate that multiple colonization events occurred, in some cases to the same island (Ashley and Wills 1987). One such event was probably by rafting to Santarosae Island before that island broke up, as indicated by genetic similarity among deer mouse subspecies in the northern Channel Islands, and later colonizations could have resulted from unintentional transport by Native Americans (Ashley and Wills 1987). Examination of genetic and morphological data suggests a somewhat similar pattern for foxes. The gray fox is thought to have colonized Santarosae Island, probably by rafting but possibly by Native American transport, then rapidly evolved into the diminutive island fox, which further differentiated into the 3 northern island subspecies (at 3 km² Anacapa was too small to support

carnivores) after the breakup of Santarosae Island (Collins 1993; Goldstein et al. 1999; Rick et al. 2009; Wayne et al. 1991). Island foxes likely were subsequently transported intentionally by Native Americans to the 3 southern Channel Islands large enough to support carnivores, Santa Catalina, San Clemente, and San Nicolas (Collins 1993; Wayne et al. 1991).

For spotted skunks we found levels of genetic differentiation between the 2 island populations that were roughly equivalent to that between island and mainland localities, suggesting that island populations have been isolated from each other for about as long as they have been isolated from the mainland. If so, then spotted skunks might have colonized Santarosae Island via overwater dispersal shortly before the breakup of that island due to rising sea levels. Occupancy of Santarosae Island is supported by extant populations of island spotted skunks on 2 of the 3 constituent islands large enough to support carnivores, and evidence that skunks persisted on the 3rd, San Miguel Island (37 km²), before going extinct in historic times (Walker 1980). How skunks reached the Channel Islands has been debated (Van Gelder 1965; Wenner and Johnson 1980). Wenner and Johnson (1980) conjectured that skunks might have been valued by Native Americans and hence transported to the Channel Islands. If this relationship between humans and skunks is correct, it is unclear why skunk distribution was so limited. Island foxes were valued by Native Americans (Rick et al. 2009), resulting in their transport throughout the Channel Islands.

Despite their isolation, spotted skunks on the Channel Islands have maintained considerable genetic diversity at microsatellite loci. Although few of the alleles in the island skunks were unique, frequencies of the alleles on the islands were substantially different from those on the mainland. The relatively high level of genetic divergence between the 2 island skunk populations, and between *S. g. amphiala* and the 3 mainland subspecies, was contrary to comparisons based on morphological data, which suggests that the taxonomic status of the island spotted skunk should be reconsidered. Assuming that subspecies delineations for *S. gracilis* on the mainland are valid, our findings support the elevation of each island population of *S. g. amphiala* to the status of separate subspecies, or perhaps even species. Regardless of taxonomic classification, each island population might constitute an evolutionarily significant unit worthy of conservation. Skunk populations on both islands have been fluctuating in recent years, apparently in response to a complex dynamic involving island foxes and golden eagles (*Aquila chrysaetos*), and their future trajectories are uncertain (Jones et al. 2008). This uncertainty, coupled with an insular distribution and genetic distinctness, warrants heightened vigilance for both populations of island spotted skunks.

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APPENDIX I

Specimens examined.—Abbreviations refer to the Vertebrate Museum, Humboldt State University (HSU); University of Kansas Natural History Museum and Biodiversity Research Center (KUNHM); Natural History Museum of Los Angeles County (LACM); Museum of Wildlife and Fish Biology, University of California at Davis (MWFB); Museum of Vertebrate Zoology, University of California at Berkeley (MVZ); Mammal Collection, Department of Fisheries and Wildlife, Oregon State University (OSU); Santa Barbara Museum of Natural History (SBMNH); and Dickey Collection of Birds and Mammals, University of California at Los Angeles (UCLA). Year of collection is in parentheses.

Central California.—KUNHM 22217 (1928), 22218 (1928), 22222 (1928), 22224 (1928), 22225 (1928), 48050 (1926); MVZ 4076 (1907), 4077 (1907), 16579 (1911), 18480 (1912), 21915 (1915), 21916 (1915), 28731 (1918), 38298 (1927), 46403 (1921), 46404 (1922), 47169 (1931), 63502 (1932), 102288 (1943), 149009 (1927).

Lake Tahoe.—MVZ 21362 (1914), 34277 (1924), 84956 (1939), 88060 (1939), 90009 (1939); SBMNH 8797 (1961).

Los Angeles.—LACM 3772 (1970), 7634 (1941), 7811 (1941), 7812 (1941), 8337 (1946), 9338 (1946), 20619 (1942), 36764 (1970), 62830 (1974), 67342 (1933), 87481 (1989), 91118 (1990); MWFB 2965 (1966); UCLA 1737 (1919), 2207 (1919), 2742 (1920), 9967 (1924), 11799 (1926), 13062 (1927).

Northern California.—HSU 238 (1964), 694 (1967), 695 (1967), 830 (1968), 867 (1968), 886 (1968), 890 (1968), 966 (1966), 986 (1969), 1100 (1969), 4235 (1970), 4881 (1986), 7160 (1994); MVZ 11744–11746 (1910), 58872 (1933), 58873 (1933), 77324 (1937), 97418 (1942), 113063 (1949), 113064 (1949).

Oregon.—KUNHM 3348 (1921), 48047 (1929), 48048 (1929), 54327 (1939), 54328 (1947), 143990 (1990); MVZ 53804 (1926), 94205 (1940), 94206 (1940), 94956 (1940), 95406 (1940), 95407 (1940), 95942 (1941); OSU 1120 (1970), 1121 (1970), 2446 (1971), 8613 (1937).

Santa Barbara.—MVZ 3953–3956 (1906), 90624 (1939); SBMNH 18 (1936), 451 (1965), 1001 (1974), 1218 (1975), 1403 (1978), 1936 (1978), 2112 (1982), 2179 (1984), 2189–2191 (1984), 2207 (1985), 2256 (1984), 2503 (1985), 2504 (1986), 2505 (1987), 2875 (1988), 2970 (1990), 2973 (1990).

Santa Cruz Island.—UCLA 13937–13939 (1928), 15327 (1929), 15328 (1929).

Santa Rosa Island.—LACM 7870 (1941), 7872 (1941), 7874 (1941), 7876 (1941), 7878 (1941), 7880 (1941), 7882 (1941), 7884 (1941), 7885 (1941); SBMNH 1247 (1976), 1248 (1976); UCLA 13395 (1927), 13396 (1927), 13399 (1927), 13405 (1927), 13406 (1927), 13414 (1927), 13415 (1927), 13431–13436 (1927), 13438–13442 (1927), 13451 (1927).