Microsatellite variation and microevolution in the critically endangered San Clemente Island loggerhead shrike (*Lanius ludovicianus mearnsi*)

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SUMMARY

Polymorphic nuclear microsatellite loci were used to characterize genetic variation in contemporary and historic populations of the San Clemente Island loggerhead shrike (*Lanius ludovicianus mearnsi*), an endangered bird with a current population of about 30 individuals, that is endemic to one of the California Channel Islands. We also compared the population of the shrike with two contemporary populations of the still abundant subspecies, *L. l. gambeli*, which live 120 km away on the adjacent mainland. The current population of *L. l. mearnsi* has 60% of the genetic variation of the mainland shrike populations and is strongly differentiated from them. Comparison of living birds with 19 birds collected in 1915 shows that most of the variation within the island population was lost before the recent 90% decline in population size, and the 20% decrease in variation this century is probably attributable to genetic drift. Mitochondrial DNA control region sequence data from 80-year-old specimens show that there may have been limited introgression to *L. l. mearnsi*, this century, from another island subspecies, *L. l. anthonyi*, found in the northern Channel Islands. Today, gene flow between *L. l. mearnsi* and mainland *L. l. gambeli* is very low, even though a few mainland birds visit the island annually. The island subspecies population has evolved sufficient genetic independence to justify ongoing conservation efforts to counter demographic collapse and genetic erosion; the course of genetic erosion can now be monitored non-invasively, as demonstrated by this study, based on DNA amplified from feathers.

1. INTRODUCTION

Bird populations on islands have featured prominently in studies of microevolution and speciation (Mayr 1963; Diamond 1975; Grant 1986), but there are few examples in which genetic variation in such populations has been measured directly, and none to our knowledge in which temporal change in genetic variation has been assayed over periods of decades. In this report on the San Clemente Island (SCI) loggerhead shrike, *Lanius ludovicianus mearnsi*, we show how the coupling of new methods, for the resolution of polymorphic nuclear markers by non-invasive genotyping, circumvent earlier technical impediments to studies of this type. The SCI loggerhead shrike is found only on San Clemente Island, one of the southern Californian Channel Islands lying about 120 km off San Diego (figure 1). This bird is a critically endangered subspecies whose numbers have declined from 100–300 individuals early this century to 5–10

birds in the years 1983–1988 (Scott & Morrison 1990). Endangerment was primarily due to habitat destruction by introduced goats, which have recently been removed, and since 1989, the SCI loggerhead shrike has been the subject of a recovery programme directed by the US Navy, which administers the Island. Two other subspecies of loggerhead shrike occur in coastal southern California, *L. l. gambeli*, which breeds on the mainland, and *L. l. anthonyi*, which breeds on three of the northern Channel Islands, including Santa Catalina Island. The two island subspecies are morphologically closer to each other than either is to *L. l. gambeli*, but field identification of these subspecies is generally not possible. A recent review of range and abundance of the entire species is provided by Cade & Woods (1997).

Research on microevolutionary change in avian populations has long been frustrated by a lack of sufficiently informative genetic markers (Rockwell & Barrowclough 1987; Haig & Avise 1996). The recent development of PCR-based methods for amplifying and electrophoretically sizing nuclear microsatellites (simple sequence repeats) has removed this obstacle, as such loci are hypervariable and abundant. Here, this

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Figure 1. Southern California and the south-eastern California Channel Islands, showing sampling sites in black. The San Clemente Island loggerhead shrike (*L. l. mearnsi*) only breeds on San Clemente Island. The range of *L. l. gambeli* (shaded) extends 1500 km north of this map to Washington state.

technology has been coupled with non-invasive DNA sampling methods based on plucked feathers and museum specimens (Woodruff 1990, 1993; Morin & Woodruff 1996; Mundy *et al.* 1997a). In this report, we demonstrate how seven nuclear microsatellite loci (probably unlinked) provided enough information to permit meaningful comparison of genetic variation between contemporary populations which were isolated in space, and also permit reconstruction of the genetic history of the island subspecies over an 80 year period. 200 bp of mitochondrial DNA (mtDNA) control region, described in contemporary populations in a previous study (Mundy *et al.* 1997b), was also determined in the island birds collected in 1915 and permits further resolution of temporal aspects of genetic change.

2. MATERIALS AND METHODS

(a) Samples

L. l. mearnsi: 30 individuals from SCI, Los Angeles Co., in 1991–1995 (designated 1995 herein); 19 specimens collected from SCI in 1915 (San Diego Natural History Museum and Department of Biology, University of California, LA). *L. l. gambeli*: 19 individuals from various sites within 20 km of San Diego, San Diego Co., in 1994–1995; 20 individuals from Perris, Riverside Co., in 1991–1994. All samples were collected during the breeding season except for contemporary samples from SCI, which were taken year-round. The relationships of all of the birds was unknown; no individuals known to be first degree relatives were included in this study. 37 out of the 39 mainland birds (San Diego and Perris populations) were local breeders occupying territories at the time they were trapped; one juvenile at each site was also included.

(b) Laboratory methods

Analyses from contemporary individuals were performed, with seven exceptions where whole carcasses were available, with feathers plucked from the flank of birds handled briefly in the context of captive propagation or field banding efforts.

Figure 2. Genetic similarity among birds in three populations. Within each population, for each individual the average fraction of microsatellite alleles it shared with the remaining individuals was calculated and plotted as a frequency distribution. MtDNA haplotype data from Mundy *et al.* (1997b), is also indicated. (a) San Clemente Island. The four mtDNA haplotype C individuals are significantly ($p < 0.01$) different from the remainder. (b) Mainland: San Diego. The figure includes the four haplotype C individuals from SCI. (c) Mainland: Perris. The figure includes the four haplotype C individuals from SCI. Data on the x-axis are in bins of 0.01. Full genotypes of the four SCI haplotype C birds at seven microsatellite loci (LS1, LS2, LS3, LS4, LTMR7, SJR4, STG4) are (alleles absent from remaining contemporary SCI sample in bold): #1(sb74): 212/212, **182**/186, **139**/143, 109/109, **152**/170, 124/**130**, **141**/141; #2(lm5): 212/212, **182**/186, **139**/143, 109/109, **154**/162, **130**/136, **137**/145; #3(lm17): **210**/212, 186/186, 143/**147**, 109/109, **168**/170, **130**/132, 139/139; #4(lm22): 212/212, 186/186, 143/145, 109/109, **168**/170, 132/**136**, **137**/141.

DNA extraction from feathers was performed using Chelex (Biorad) (Mundy *et al.* 1996) and from foot skin of museum specimens using the Qiagen QiaAmp extraction kit (Mundy *et al.* 1997a). Microsatellites were amplified by PCR in the presence of forward primer end-labelled with γ - 32 P-dATP, and were separated on denaturing polyacrylamide gels (Mundy & Woodruff 1996). Primers for microsatellite amplifications were as follows: LS1F/R, LS2F/R, LS3F2/R2, and LS4F/R or LS4F/R2 (Mundy & Woodruff 1996), SJR4F/R, LTMR7F/R (Macdonald & Potts, University of Florida, in preparation), and STG4A/B (Ellegren 1992). Primers for the mtDNA control region amplifications from

Table 1. *Genetic variation at seven microsatellite loci*

(Asterisks represent significantly greater expected heterozygosities (H_e) in pairwise comparisons; for SCI (1915), Perris (P) and San Diego (SD) populations, pairwise comparisons are with SCI (1995); for SCI (1995), comparison is with SCI (1915). Wilcoxon signed ranks test for differences in overall allelic diversity between pairs of populations gave the following significant results: SCI (1995) *vs.* San Diego, $T_s = 0$, $n = 7$, $p < 0.01$; SCI (1995) *vs.* Perris, $T_s = 1$, $n = 7$, $p < 0.05$; SCI (1915) *vs.* San Diego, $T_s = 0$, $n = 7$, $p < 0.01$; SCI (1915) *vs.* Perris, $T_s = 0$, $n = 7$, $p < 0.01$. Wilcoxon signed ranks test for differences in overall expected heterozygosities between pairs of populations gave the following significant result: SCI (1995) *vs.* San Diego, $T_s = 0$, $n = 7$, $p < 0.05$.)

locus	number of alleles				expected heterozygosity			
	island		mainland		island		mainland	
	SCI ($n = 26$) (1995)	SCI ($n = 19$) (1915)	SD ($n = 19$)	P ($n = 20$)	SCI (1995)	SCI (1915)	SD	P
LS1	2	1	4	6	0.434*	0.000	0.431	0.655
LS2	2	2	3	3	0.292	0.444	0.437*	0.304
LS3	3	2	5	3	0.514	0.491	0.542	0.432
LS4	2	3	5	4	0.473	0.613*	0.481	0.350
LTMR7	3	5	12	13	0.377	0.627*	0.799*	0.921*
SJR4	2	3	4	5	0.317	0.667*	0.440	0.638*
STG4	1	2	5	5	0.000	0.053*	0.690*	0.735*
all loci	2.1 ± 0.26	2.6 ± 0.48	5.4 ± 1.13	5.6 ± 1.31	0.344 ± 0.065	0.414 ± 0.104	0.546 ± 0.055	0.576 ± 0.085

museum specimens were DLL1/DLH1 (Mundy *et al.* 1997b). Direct sequencing of double-stranded PCR product using α -³⁵S-dATP was performed as described in Mundy *et al.* (1997b); at least 200 bp of the sequence, including the three variable sites discovered previously, were obtained from each individual. For the results from museum specimens, the following evidence indicates that contamination was insignificant (see also Mundy & Woodruff (1997a)): extraction controls were negative, results were replicable (over 90% of microsatellite genotypes and all mtDNA haplotypes were duplicated, mostly from independent extractions), and only two out of 19 individuals had identical multilocus genotypes.

(c) Statistics

Unbiased expected heterozygosities (H_e) (Nei 1987), ϑ (Weir & Cockerham 1984), and R_{st} (Slatkin 1995) were computed using a program provided by T. Price, with a modified R_{st} computed across loci, and estimated as the fraction of variance in allele lengths among populations (Sokal & Rohlf 1981, p. 216). Significance tests of differences in heterozygosities for single loci between pairs of populations were performed with 1000 iterations of a randomization procedure using individual genotypes as replicates, with p values determined following the sequential Bonferroni procedure. Hardy-Weinberg tests were performed using GENEPOP (Raymond & Rousset 1995). Homogeneity tests were performed using the program described in Zaykin & Pudovkin (1993), with 1000 randomizations for Monte-Carlo approximation of the χ^2 test. Estimates of the number of migrants per generation (N_m) were derived by substituting ϑ or R_{st} for F_{st} in the following relation under the island model: $F_{st} = 1/(1 + 4N_m)$. The significance of outliers on the distribution of mean pairwise shared alleles (figure 2) was determined by 1000 randomizations using pairwise shared alleles as replicates.

Estimates of genetic effective population size (N_e) for the SCI population were calculated from the following results

from neutral theory: $H_t/H_o = (1 - 1/2N_e)^t = 1 - F_t$, where H_t is heterozygosity at time t , H_o is heterozygosity at time $t = 0$, t is the number of generations, and F_t is autozygosity at time t ; heterozygosities were estimated as average unbiased expected heterozygosities, and F was estimated as ϑ . Overlapping generations were taken into account (Hill 1972) by using the mean age at reproduction for loggerhead shrikes, estimated to be 4 years in both sexes (W. D. Everett, personal communication), thus giving $t \approx 20$, and assuming equal male and female genetic effective population sizes. To estimate the number of generations between the founding population on SCI and the population in 1915, we assumed that the expected heterozygosity of the founding population was equal to the average of that of the two current mainland populations (0.561), and that ϑ between the founding population and 1915 was equal to the average of the ϑ between mainland and 1915 island populations (0.26).

3. RESULTS

Multilocus genotypes were obtained for 30 birds representing the contemporary population of SCI and 39 birds representing two populations of the subspecies on the adjacent mainland. We genotyped an estimated 90% of the actual population on SCI during 1991–1995. Four individuals in the contemporary sample from SCI had divergent microsatellite genotypes and mtDNA haplotypes and are not considered to be a part of the resident breeding population; they are excluded from the following analysis which is based on the remaining 26 birds. The four exceptional island birds are described explicitly in the final paragraph of this section.

The amount of genetic variation in the contemporary population of the SCI loggerhead shrike is low (table 1). In spite of a larger sample size from the island, fewer alleles are present at six of seven loci compared with the mainland populations, and the

Table 2. *Allele and haplotype frequencies*

(Data are shown for seven microsatellite loci and mtDNA haplotypes (mtDNA data for three contemporary populations from Mundy *et al.* (1997*b*), with haplotype C birds on SCI excluded). All sample sizes as in table 1 except $n = 30$ for mtDNA data for SCI (1995). Homogeneity tests between SCI populations significant at the 0.05 level are as follows: Fisher's exact test: LS1, $p = 0.0001$; mtDNA, $p = 0.034$; Monte-Carlo simulation of χ^2 test; LS4, $p < 0.003$; SJR4, $p < 0.001$; LTMR7, $p < 0.012$.)

locus	allele	SCI (1995)	SCI (1915)	SD	Perris
LS1	208	—	—	0.026	0.075
	210	—	—	—	0.100
	212	0.692	1.000	0.737	0.550
	214	—	—	0.184	0.200
	216	0.308	—	0.053	0.025
	222	—	—	—	0.050
LS2	182	—	—	0.026	0.025
	186	0.173	0.315	0.711	0.825
	188	0.827	0.685	0.263	0.150
LS3	139	—	—	0.026	—
	141	0.019	—	0.053	0.050
	143	0.577	0.395	0.632	0.725
	144	0.404	0.605	0.263	0.225
	147	—	—	0.026	—
LS4	105	0.635	0.368	0.079	0.050
	107	—	0.132	0.132	—
	109	0.365	0.500	0.711	0.800
	111	—	—	0.053	0.125
	113	—	—	0.026	0.025
SJR4	122	—	—	0.026	—
	124	0.192	0.421	0.132	0.075
	128	—	—	—	0.075
	130	—	0.237	0.737	0.525
	132	0.808	0.342	0.105	0.300
	136	—	—	—	0.025
LTMR7	152	—	—	0.026	0.075
	156	—	—	0.026	0.050
	158	0.038	0.026	0.053	0.075
	160	—	0.026	0.079	0.075
	162	—	—	0.026	0.100
	164	—	—	0.132	0.125
	166	0.192	0.316	0.105	—
	168	—	0.105	0.421	0.075
	169	—	—	0.053	0.050
	170	0.769	0.526	0.026	0.200
	172	—	—	—	0.025
	174	—	—	0.026	0.075
STG4	176	—	—	—	0.025
	178	—	—	0.026	0.050
	135	—	—	0.026	—
	137	—	—	0.053	0.075
	139	1.000	0.973	0.158	0.175
	141	—	0.027	0.447	0.350
	143	—	—	0.316	0.350
mtDNA	145	—	—	—	0.050
	A	0.767	1.000	0.105	—
	B	0.233	—	—	—
	C	—	—	0.842	1.000
	D	—	—	0.052	—

allele number is significantly lower in pairwise comparisons over all loci between the SCI and both mainland populations. Expected heterozygosity (H_E) is also significantly lower at three loci in pairwise comparisons between contemporary SCI and mainland populations. Average H_E over all loci is *ca.* 60% lower on SCI compared to both mainland populations, and this was significant for the comparison between the SCI and San Diego populations.

Based on genotyping 19 birds shot in 1915, genetic variation of the SCI loggerhead shrike has been low for at least the past 80 years. The 1915 population had a slightly higher average number of alleles and average H_E (20% in both cases) than the contemporary population, and these differences are small compared with the differences in genetic variation between the historical island and contemporary mainland populations (table 1).

Allele frequencies at five of seven microsatellite loci display obvious differences between the contemporary SCI population and the mainland populations (table 2). The two estimates of population differentiation, F_{st} , (as estimated by ϑ ; Weir & Cockerham 1984) and R_{st} (which takes allele size into account; Slatkin 1995), are both large in pairwise SCI and mainland population comparisons (table 3). Derived estimates of gene flow from these statistics are correspondingly low (table 3), and are likely to be overestimates as they assume equilibrium. In contrast, the estimates of population subdivision between the two mainland populations were small (ϑ) or negative (R_{st}), although the geographical distance between these populations is similar to their distance from SCI (*ca.* 120 km). Genetic differentiation between the contemporary and the historical SCI populations is significant. Genetic differences between the historical SCI and the mainland populations are lower than those between the contemporary SCI and the mainland populations. No loci in either SCI sample showed significant deviation from Hardy–Weinberg expectations (not shown).

Four microsatellite alleles showed significant frequency changes over time on SCI, and the pattern of allele change over time varied between loci (table 2). At three loci (LS4, LTMR7 and SJR4), one allele present at frequency > 0.1 was lost between 1915 and 1995, whereas locus LS1 shows the opposite: an allele (216) present at high frequency in the contemporary population was not detected in the 1915 population. Mt DNA control region sequences from the 1915 sample on SCI showed that all individuals were of haplotype A (table 2). This contrasts with the contemporary population on SCI in which haplotype A predominates, but a second haplotype, haplotype B, is also present at a frequency of *ca.* 0.25 (Mundy *et al.* 1997*b*).

Four of the 30 contemporary birds trapped on SCI in the 1990s appear to be visitors from the mainland and were excluded from the above characterization of *L. l. mearnsi*. They have divergent microsatellite genotypes: each individual has at least four alleles absent from the remaining island sample and they are outliers on a distribution of mean pairwise allele-

Table 3. *Population subdivision and gene flow*

(Estimates of N_m from ϑ are above the diagonal and from R_{st} below the diagonal. The negative R_{st} between San Diego and Perris indicates a value indistinguishable from zero, and N_m cannot be estimated (*).)

	ϑ (above diagonal) and R_{st} (below diagonal)					gene flow (N_{m})		
	SCI (1995)	SCI (1915)	SD	Perris		SCI (1995)	SD	Perris
SCI (1995)	—	0.12	0.39	0.35	SCI (1995)	—	0.39	0.46
SCI (1915)	0.12	—	0.26	0.26	SD	0.82	—	10.45
SD	0.23	0.14	—	0.02	Perris	0.94	*	—
Perris	0.21	0.16	−0.01	—				

sharing between all SCI birds (figure 2*a*). They also represent four out of the only six birds on SCI to have mitochondrial haplotype C, which is prevalent on the mainland (Mundy *et al.* 1997*b*; no microsatellite data could be obtained for the other two haplotype C individuals). The mean pairwise shared allele distributions of the mainland populations with the SCI haplotype C birds included are consistent with the possibility that they originated on the adjacent mainland (figures 2*b,c*). Furthermore, two of the four were caught in the northern half of SCI, where no shrikes have been reported as breeding for at least 16 years and there is no evidence that any of these four birds hatched or have bred on SCI.

4. DISCUSSION

Following in the tradition of Darwin, Grant & Grant (1989) and others in the study of evolution in birds on oceanic islands, we here report on the variation in a peripherally isolated population of a widespread North American species, and show how the processes of microevolutionary change (differentiation, genetic drift and gene flow) can be monitored. Many of the conclusions would have been impossible to reach without the historical data.

The degree of differentiation between the current *L. l. mearnsi* population and mainland *L. l. gambeli* populations is very high, as the F_{st} values are among the highest reported for avian populations at any spatial scale (Rockwell & Barrowclough 1987), although similar or higher values were found among some isolated Atlantic island chaffinch populations (Baker *et al.* 1990) and within one member of a pair of cryptic species of brown kiwi (Baker *et al.* 1995). These results concur with our previous mtDNA results in the same populations (Mundy *et al.* 1996; Mundy *et al.* 1997*b*), and show that there is a substantial barrier to gene flow in both sexes between *L. l. mearnsi* and *L. l. gambeli*. Of relevance here are the four mtDNA haplotype C birds observed on SCI for which microsatellite data were obtained. Migration of mainland shrikes to SCI had been suspected from field observations, as one to four previously unknown birds per year (1990–1995) have been seen on SCI, mostly during the winter, and the genetic evidence supports

this view. Thus the barrier to gene flow is not because shrikes rarely fly across the water to SCI, but lies in the reason why the visiting shrikes do not usually stay to breed. The visiting shrikes may migrate away from the island before the onset of the breeding season, a situation similar to the case of two Australian silvereyes on Heron Island (Degnan & Moritz 1992), where a wintering subspecies leaves the island before the resident subspecies breeds. Alternatively, the migrants may attempt to breed but fail to do so, either due to competition with the island residents for resources, or due to failure to mate with island residents through a problem with mate-recognition or preference to breed within kin groups, as occurs in the thick-billed murre (Friesen *et al.* 1996).

The low genetic variation in *L. l. mearnsi* provides one of the few clear examples of reduced variation in insular avian populations (Baker *et al.* 1990; Degnan 1993). In microsatellite surveys of other island vertebrate populations, low variation was found in black bears (Paetkau & Strobeck 1994) but not Soay sheep (Bancroft *et al.* 1995). The more consistent difference across loci in numbers of alleles, rather than in H_E , for comparisons between the contemporary SCI population and the mainland populations is as expected from theoretical (Nei *et al.* 1975) and experimental studies (Leberg 1992) of genetic drift in small populations. Genetic variation in the historical sample of *L. l. mearnsi* was already low before the mid-20th century population crash, and so the low genetic variation on SCI is attributable to events before the 20th century, such as founder effects and long term genetic drift. The 20% overall loss of genetic variation since 1915 can be attributed to genetic drift, as can the significant differentiation over time measured by F_{st} and R_{st} . Estimates of the long-term genetic effective population size (N_e) for *L. l. mearnsi* can be calculated from neutral theory (Kimura & Crow 1963) using either the change in heterozygosity over time ($N_e = 54$) or ϑ as an estimate of autozygosity for the two populations ($N_e = 78$). These represent upper bounds for N_e , as gene flow is ignored. With a population of 30 birds, the current N_e of *L. l. mearnsi* cannot be more than about 20, and if this value represented the long-term N_e since 1915, the expected loss of genetic variation would be 40% compared with the 20% observed.

Losses of individual microsatellite alleles between the 1915 and the 1990s' samples can be accounted for by genetic drift, as sampling error is unlikely to explain the failure to detect a missing microsatellite allele in the 1990s' samples (from Sjögren & Wyöni (1994), there is a 95% probability of detection if the frequency of the rare allele is > 0.03 for the contemporary sample and > 0.07 for the historical sample). Conversely, the first detection of allele LS1²¹⁶ in the 1990s' is probably due to introgression rather than mutation, given *ca.* 20 generations and typical microsatellite mutation rates (Bruford & Wayne 1993) of about 10^{-4} to 10^{-5} . The absence of mtDNA haplotype B in 1915 may be due to sampling error (there is a 95% probability of detection if the frequency of haplotype B was > 0.15). Alternatively, the haplotype may have been introduced from elsewhere (mutation is extremely unlikely as haplotype B differs from haplotype A by two unrelated transitional changes). If introgression has occurred, it could be from the subspecies *L. l. anthonyi*, which breeds on three of the northern Californian Channel Islands, including Santa Catalina, and in which haplotype B has been found in all nine birds examined to date (Mundy *et al.* 1997*b*); haplotype B was not found in the 39 mainland *L. l. gambeli* examined. Unfortunately, it has not yet been possible to genotype *L. l. anthonyi* at these microsatellite loci.

The 20 microsatellite alleles detected on SCI are a subset of the 45 found on the adjacent mainland (table 2), and the divergence (as measured by F_{st} and R_{st}) between the island and mainland populations has increased substantially in 80 years. Taken together this suggests that the SCI loggerhead shrike could have been founded from populations genetically similar to those that exist on the mainland today. The mtDNA haplotype data are consistent with this hypothesis as the island and mainland share haplotype A (table 2), and as explained above, the otherwise anomalous presence of haplotype B on the island may be due to recent introgression. Approximate bounds on the age of the SCI population in 1915 can be made depending on alternate gene flow assumptions. With low gene flow (N_m *ca.* 0.7), the island population could have remained in equilibrium with the mainland for long periods, but if the island population were $> 10^4$ years old, novel microsatellite alleles would be expected (not observed), and R_{st} should be greater than 0 (not observed) (Slatkin 1995; Forbes *et al.* 1995). Alternatively, with no gene flow, a founding population on SCI with the same genetic variation as the 1995 mainland samples and $N_e = 100$, then the expected number of generations, t , to 1915 is $t = 61$ (whether calculated from change in heterozygosity, or using 0 as an estimate of autozygosity). With a generation time of 4 years, this suggests a founding time *ca.* 250 years before 1915, or round about the year 1650. As SCI is an oceanic island of Pliocene origin (Vedder & Howell 1981) and shrikes invaded North America from Asia at least 100 000 years BP (Sibley & Ahlquist 1990), possibly long before, the genetic estimate of the population's age is surprisingly low.

Results from microsatellites and mtDNA (Mundy *et al.* 1997*b*) are concordant and provide a genetic basis

for the observed morphological divergence of SCI *L. l. mearnsi* and mainland *L. l. gambeli*. Although *L. l. mearnsi* may be a relatively young population, it is already strongly differentiated from *L. l. gambeli* and potentially has a different evolutionary future, although its distinctions from *L. l. anthonyi* on the northern Channel Islands require further attention. Conservation efforts have merit as the current SCI population retains a large proportion of the genetic variation that was present before the recent population crash, there is no evidence for mating system inbreeding (Templeton & Read 1994), and the subspecies is not being introgressed out of existence by more common relatives, as is the case with the Catalina Island mahogany (Rieseberg & Gerber 1996).

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