Genetic Structure of Endangered Clapper Rail (*Rallus longirostris*) Populations in Southern California

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Abstract: *We assessed the genetic structure of two subspecies of endangered Clapper Rails (*Rallus longirostris*) in Southern California using DNA fingerprinting to uncover variation in minisatellite DNA. Minisatellite DNA variation in the Salton Sea population of the* R. l. yumanensis *subspecies was at a level typical of outbred avian species (average proportion of fragments shared, or S, was 0.33). Variation was extremely low (S from 0.63 to 0.77), however, within four coastal, salt-marsh populations of the subspecies* R. l. levipes *located along a transect extending about 260 km northwest from the Mexican border. Between-population similarity (S_{ij}) was also high for the four* levipes *populations, although individuals of the small, isolated population at Mugu Lagoon consistently clustered separately in phenograms constructed using neighbor-joining or other algorithms. Individuals of* yumanensis *always clustered as a sister group to all* levipes *individuals. The minisatellite data were contrasted with the extremely low mtDNA and RAPD variation we found in both subspecies. We propose that variation in these less-mutable markers was lost in a bottleneck that occurred at least 1000 years ago, thus allowing sufficient time for recovery of variation in the rapidly mutating ($\mu \sim 0.001$/gamete/generation) minisatellites ($t = 1/\mu$, or 1000 generations). A second, more-recent bottleneck, or series of bottlenecks within a metapopulation structure, likely resulted in the depauparate variation seen in* levipes *today. We suggest that translocations from large to small* levipes *populations could restore important genetic variation to the small populations and would not compromise genetic boundaries.*

Estructura genética de las poblaciones de *Rallus longirostris* en peligro de extinción en el sur de California

Resumen: *En el presente estudio evaluamos la estructura genética de dos subespecies de* Rallus longirostris *en peligro de extinción en el sur de California usando huellas digitales de ADN para revelar la variación del ADN de minisatélites. La variación del ADN de minisatélites en la población del mar de Salton de la subespecie* R. l. yumanensis *estuvo a un nivel típico de especies de aves exogámicas (la proporción promedio de los fragmentos compartidos, o S, fue de 0.33). Sin embargo, la variación fue extremadamente baja (de 0.63 a 0.77) dentro de cuatro poblaciones de marismas costeras de la subespecie* R. l. levipes *localizada a lo largo de un transecto que se extendía unos 260 km al noroeste de la frontera Mexicana. La similaridad entre poblaciones (Sij) también fue alta para las cuatro poblaciones de* levipes. *Sin embargo, los individuos de la pequeña población aislada de la Albufera Mugu se agruparon consistentemente en forma separada en los fenogramas construidos usando el algoritmo de union de vecinos u otros algoritmos. Los individuos de* yumanensis *se agruparon siempre como un grupo hermano al de todos los individuos de* levipes. *Los datos de minisatélites fueron contrastados con la variación en mtDNA y RAPD que encontramos para ambas especies y que es extremadamente baja. Proponemos que la variación en estos marcadores menos mutables, fue perdida en un cuello de botella que ocurrió hace por lo menos 1000 años atrás, lo que daría un período de tiempo suficiente como para recuperar la variación de los minisatélites que poseen una alta tasa de mutación ($\mu \sim 0.001$/gameta/generación, es decir $t = 1/\mu$ o 1000 generaciones). Un segundo cuello de botella*

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más reciente, o bién una serie de cuellos de botella dentro de una estructura metapoblacional, dió probablemente como resultado la empobrecida variación que hoy en día se observa en levipes. Sugerimos que la traslocación de individuos de levipes de poblaciones grandes a poblaciones pequeñas podría devolver la importante variación genética a las pequeñas poblaciones y no comprometería las fronteras genéticas.

Introduction

Population decline and range fragmentation can significantly modify the levels and patterns of genetic variation in natural populations. Heterozygosity and allelic diversity can be lost from generation to generation via increased consanguineous mating and genetic drift (Wright 1931; Nei et al. 1975; Maruyama & Fuerst 1985; Allendorf 1986), and these stochastic changes in allele frequencies can cause isolated or semi-isolated populations to diverge genetically, resulting in greater spatial genetic heterogeneity than prior to fragmentation (Wright 1931; Wright 1943; Gilpin 1987; Wade & McCauley 1988; Templeton et al. 1990). The monitoring of such genetic changes in response to population change is considered a major goal of both population genetics and conservation biology (Soulé 1980; Lande & Barrowclough 1987; Hedrick & Miller 1992; O'Brien 1994). In addition to the direct merit of assessing levels of genetic variation and inbreeding in endangered taxa, information about the distribution of genetic variation within and among populations often allows inference about important demographic variables, such as dispersal rates and patterns and trends in long-term population sizes (Fleischer et al. 1994; Moritz 1994). These variables may otherwise be difficult to estimate accurately without many years of intensive, expensive fieldwork.

Such assessments of genetic population structure can, however, be hampered by a lack of resolving power of the available genetic markers (Fleischer 1983; Fleischer et al. 1991). The ability to examine minisatellite and microsatellite DNA has been a major breakthrough because their exceptionally high mutation rates produce a level of variation that allows detection of variability and structure that often cannot be identified with less-variable genetic systems such as allozymes, RAPDs, or mitochondrial DNA (Gilbert et al. 1990; Degnan 1993; Ellegren et al. 1993; Haig et al. 1993; Parker & Whiteman 1993; Fleischer et al. 1994; Rave et al. 1994; Scribner et al. 1994). In addition, minisatellite DNA analysis can sometimes be used to resolve relationships among individuals and populations. This is especially true for populations containing somewhat limited variability (those that are not "saturated" by mutation; Gilbert et al. 1990; Triggs et al. 1992; Harding et al. 1993; Menotti-Raymond & O'Brien 1993). Many recent empirical studies have established calibrations between DNA fingerprint similarity and relatedness or inbreeding (Kuhnlein et al. 1990; Gilbert et al. 1991; Lehman et al. 1992; Piper & Rabenold 1992; Reeve et al. 1992; Haig et al. 1993; Haig et al., 1994; Rave et al. 1994), supporting theory that indicated such correlations were likely (Lynch 1988). Additional theoretical studies have supported use of DNA fingerprint data for population genetic analyses under certain assumptions likely to be met, such as Mendelian inheritance of fragments and linkage equilibrium (Lynch 1990, 1991; Shriver et al. 1993; Jin & Chakraborty 1994).

Three endangered subspecies of Clapper Rail, *Rallus longirostris*, occur in California. Two coastal subspecies, *R. l. levipes* and *R. l. obsoletus*, occur in salt marshes in southern California and northern coastal Baja California (Fig. 1), and northern California (mostly the San Francisco Bay area), respectively (Ripley 1977). Both coastal subspecies are similar morphologically (Ripley 1977; R. Fleischer, personal observation) and presently have very small and fragmented populations (Zembal 1993). Another more differentiated subspecies, *R. l. yumanensis*, occurs inland in California along the lower reaches of the Colorado River to northwestern Mexico and the Salton Sea (Eddleman 1989). This subspecies has a somewhat less fragmented range and overall larger population size than the coastal forms (Conway et al. 1993) and has populations of unknown number extending south along the Mexican Gulf of California coast to about Nayarit, San Blas (Ripley 1977). Analyses of mitochondrial DNA control-region sequences thus far have not resolved any differences among these three subspecies (Fleischer et al., in preparation).

Clapper Rail populations in coastal southern California (*R. l. levipes*) are currently very small (1992 census of 275 pairs), with individual populations ranging from as low as a single pair in some marshes to consistently over 100 pairs at Newport Bay (Fig. 2; Zembal 1993). During the past 15 years of censuses, the four marshes sampled in this study (Fig. 1) contained from 64% to 89% of the entire California *levipes* breeding population. The remaining birds reside in from 4 to 14 (depending upon the year) small populations in other marshes. Most smaller marshes, and even some intermediate-sized ones, have shown extreme fluctuations in numbers of rails (Fig. 2; Zembal 1993), including apparent local extirpations sometimes followed by recolonizations. Coastal populations have suffered largely from human-caused habitat destruction (an estimated 75% of southern California marshes have been destroyed since 1900; Speth 1971), but also from predation (especially by in-

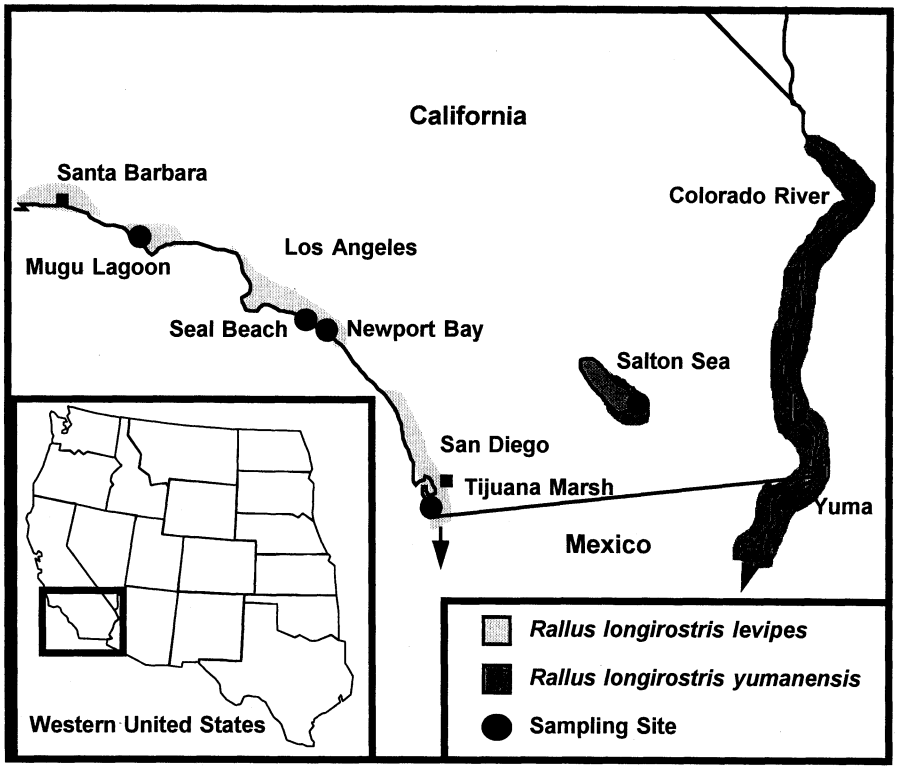

Figure 1. Map of southern California showing the locations of the populations of Clapper Rails sampled for this study and the historical California ranges of the subspecies levipes *and* yumanensis. *Inset shows location of study area in the western United States. (Note that both subspecies' ranges extend into Mexico in the south; these were not sampled in this study.)*

troduced and feral predators; U.S. Fish and Wildlife Service 1990), severe winter storms that deposit large loads of silt on the marshes, organophosphate and heavy metal pollution (Goodbred et al., unpublished data), and possibly from inbreeding depression or other genetic problems. Egg hatchability and fertility are exceedingly low in some of the northern coastal populations (Ledig 1990), characteristics commonly associated with inbreeding depression (Rave 1994).

We report on analyses of minisatellite DNA variation in four coastal populations from *R. l. levipes* and one inland population from *R. l. yumanensis* (Fig. 1). We used multilocus DNA fingerprinting to assess the levels of minisatellite DNA variation within each of these populations and the patterns of variation and relationships among the populations and subspecies. We found remarkably low levels of minisatellite DNA variation within the coastal *levipes* populations in comparison to more typical levels in the *yumanensis* population. In addition, only minor differences in minisatellite frequencies exist among the four *levipes* populations, whereas much larger differences occur between *levipes* and *yumanensis*.

Methods

Field Sampling and Laboratory Methods

Rails were caught in drop-door traps or dip nets from kayaks during high tides (Eddleman 1989). All 48 captured birds were banded with U. S. Fish and Wildlife Service bands, and some were fixed with radio-transmitters for another study. Every attempt was made to obtain a random sample from each population, or at least one consisting of unrelated individuals, by sampling several areas in each marsh. Up to 600 μl of blood was drawn into heparinized capillary tubes from the brachial vein. The blood samples were kept on ice in the field, fresh frozen, and then shipped on dry ice to the laboratory. Blood samples were kept frozen in the laboratory until DNA was extracted for genetic analyses.

Forty eight Clapper Rails were sampled from five populations in Southern California (Fig. 1, Table 1) during the breeding season of 1989. Four of these samples (Tijuana Marsh, San Diego County, Newport Bay and Seal Beach, Orange County, and Mugu Lagoon, Ventura County) were from salt-marsh populations located along a transect of about 260 km of the California coast and were within the range of the described subspecies *R. l. levipes*. The fifth sample was from the subspecies *R. l. yumanensis*. Samples were collected from the rail population at the Wister Unit within the Salton Sea National Wildlife Refuge, Imperial County.

DNA was isolated using standard methods of proteinase K digestion, phenol-chloroform extraction, and ethanol-salt precipitation (Fleischer et al. 1994; Rave et al. 1994). About 5 μg of clean, genomic DNA was digested with an excess (about 10 units per μg) of HaeIII restriction endonuclease and electrophoresed in a 1% agarose gel for about 40 hours. The digested genomic DNA was

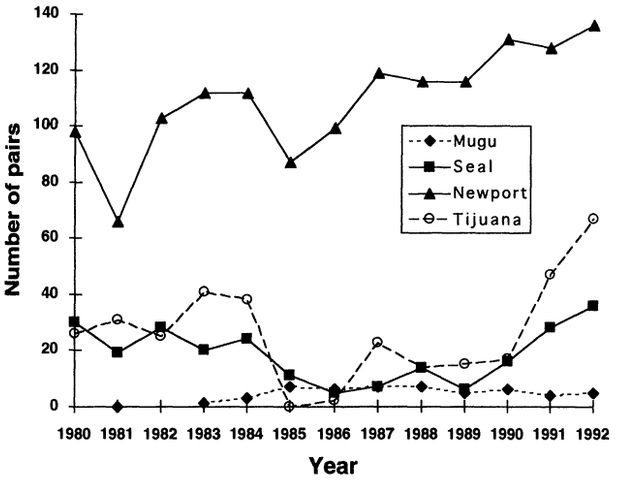

Figure 2. Census results for the four Light-footed Clapper Rail (R. l. levipes) populations sampled in this study. The Newport Bay population is significantly correlated to year in the 13-year census period ($r = 0.83, p < 0.001$), indicating a stable rise in census size. Data are summarized from Zembal (1993).

acid-depurinated, alkali-denatured, and transferred by vacuum to a nylon filter (MSI-Magnagraph). Jeffreys 33.15 and 33.6 probes (Jeffreys et al. 1985) were radiolabeled using the random priming method to specific activities above 5×10^8 CPM/μg and hybridized to the nylon filters following the protocols of Westneat et al. (1988) and Fleischer et al. (1994). Autoradiograph exposures varied from overnight to several days with intensifying screens at −70°C.

Data Analyses

Two types of gels were run: (1) three "mixed" gels that had 3–4 individuals from each locality so that interpopulation comparisons could be made and (2) three gels that contained all the individuals sampled from a locality in adjacent lanes so that intrapopulation variability could be assessed using all individuals of a locality sample. Each of the mixed gels included different individuals from a locality, such that all individuals were run on at least one gel, although three *yumanensis* individuals were run on two different gels.

All fragments above about 2 kb in size were scored for all individuals run on a gel; no comparisons were made between gels because gel-to-gel variation in fragment mobility curves is likely. Fragments of different sizes were numbered sequentially, beginning with the largest. Fragments of the same mobility in different lanes (individuals) were given the same number. Coefficients of band sharing or similarity (S of Lynch 1988, 1991) were calculated from the matrix of individuals-by-fragments for all pairwise comparisons. These pairwise values of S were averaged for each locality sample from the single-sample gels. For the mixed gels, S was averaged over individuals within and between localities. Mantel's test (Schnell et al. 1985; Rohlf 1990) was used to determine whether significant differences existed in band-sharing distributions, and thus variability, among samples at different localities. Mantel's test controls for the lack of independence among cells of a matrix.

Data matrices were also used to calculate values of heterozygosity (Gilbert et al. 1991; Jin & Chakraborty 1993) and average allele frequency (Jeffreys et al. 1985). Mean S for each population was used to estimate effective population size, N_e, under the assumption that the populations under study are in a state of mutation-drift equilibrium (Lynch 1991) and the mutation follows an infinite-alleles model (Harding et al. 1993). The calculations require an estimate of mutation rate (μ), so we determined the range of minisatellite mutation values from the literature (10^{-4} to 10^{-2} mutations/gamete/generation; Jeffreys et al. 1988, 1991; Westneat 1990; Fleischer et al. 1994; Scribner et al. 1994). Two estimates of N_e were made using a midpoint for $\mu = 0.001$, one that apparently underestimates N_e (equation 10 of Lynch 1991) and one that apparently overestimates it (equation 5 of

Table 1. Population designations, sample sizes, and mean band-sharing coefficients (S) for Jeffreys 33.15 and 33.6 minisatellite probes for Clapper Rails (*Rallus longirostris*) from Southern California.

Taxon and Population	*Individuals*	*Dyads*	*Mean S* ± Standard Deviation*		
			33.15	*33.6*	*Combined*
R. l. yumanensis					
Salton Sea	9	36	0.35 ± 0.12	0.32 ± 0.12	0.33 ± 0.10
R. l. levipes					
Tijuana Marsh	10	45	0.68 ± 0.09	0.71 ± 0.12	0.70 ± 0.09
Newport Bay	9	36	0.68 ± 0.09	0.68 ± 0.12	0.68 ± 0.08
Seal Beach	10	45	0.60 ± 0.09	0.66 ± 0.09	0.63 ± 0.08
Mugu Lagoon	10	45	0.77 ± 0.06	0.77 ± 0.09	0.77 ± 0.06
Total	48	207			

**These within-population comparisons of S were calculated from data scored from gels on which the entire sample from a population was run.*

Lynch 1991), and these were averaged for subsequent calculations.

Allele frequencies were compared among populations by calculating the corrected between-population similarity, S_{ij} (equation 16, Lynch 1991), for all pair-wise comparisons of populations. In addition, the inbreeding coefficient of interdemic differentiation, F_{ST} (equation 17, Lynch 1991), was calculated over all populations and among all *levipes* populations. These values of F_{ST} were used, along with the average N_e values calculated above, in an island model of genetic structure to estimate m, the long-term rate of gene flow among populations and subspecies (Hartl & Clark 1989). Distance matrices ($1 - S$) of all pairwise comparisons were constructed for each mixed gel using the combined data for both probes. These matrices were analyzed in the program MEGA (Kumar et al. 1993) to construct UPGMA and neighbor-joining phenograms indicating relationships among individuals and populations. Phylogenetic or parsimony trees for each gel were also constructed from matrices of fragment profiles using the heuristic algorithm of the computer program PAUP 3.1.1 (Swofford 1993).

Results

Intrapopulation Variation

All 48 individuals sampled were fingerprinted at least twice, once on a mixed gel and once on a population sample gel. The sizes for each population sample are presented in Table 1. Examples of DNA fingerprints for Salton Sea and Mugu Lagoon are shown in Fig. 3. The mean number of fragments ± standard deviation for both Jeffreys 33.15 and Jeffreys 33.6 probes combined was 39.1 ± 10.6.

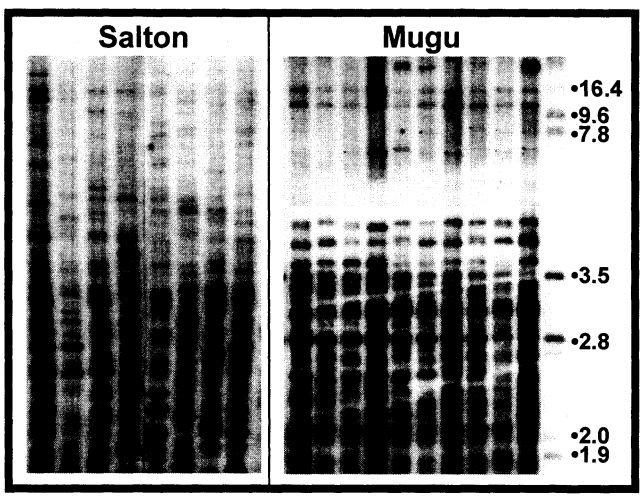

Figure 3. Examples of DNA fingerprints of yumanensis *(Salton Sea) and* levipes *(Mugu Lagoon) individuals. The gels were probed using the Jeffreys 33.15 minisatellite. Approximate sizes of marker fragments are noted on the Mugu gel in kilobases. The fragment range shown for the Salton gel is approximately the same as for the Mugu gel.*

Intrapopulation variation, assessed by S for each probe separately and combined, is presented in Table 1. Fragment similarity was very high for the coastal *levipes* populations (S ranged from 0.63 to 0.77 for both probes combined), especially in comparison to the inland *yumanensis* population ($S = 0.33$ for combined). Mean values of S were similar for each probe, and similarity was especially high for the sample from Mugu Lagoon (mean $S = 0.77$), which was the smallest population sampled (Fig. 2). Mantel's tests were performed for each pairwise comparison of populations (Table 2), and eight of the ten comparisons yielded a significant difference in the distribution of band-sharing coefficients between populations. The most significant differences ($p < 0.001$) in S occurred between each *levipes* sample and the *yumanensis* sample. Thus, *yumanesis* exhibited significantly more minisatellite variability than *levipes*. In addition, samples from within Mugu Lagoon had significantly higher band-sharing coefficients than the other three *levipes* samples (Table 2). Estimates of heterozygosity and average allele frequency parallel mean S (Table 3). The Mugu Lagoon population apparently contains only about one-third the heterozygosity of the Salton Sea population.

Values of effective population size for the *levipes* populations, as expected, were relatively small in comparison to the estimates for Salton Sea and *yumanensis* (Table 3). Estimates based on $\mu = 0.001$ ranged from about 75 to 420 individuals for the *levipes* populations; when $\mu = 0.01$, the values ranged an order of magnitude lower (7 to 42). It should be emphasized here that these estimates of N_e are obviously sensitive to mutation rate, for which we have no information for Clapper Rails. Analysis of fingerprints from a number of Clapper Rail

Table 2. Results of Mantel's tests comparing distributions of band-sharing coefficients (*S*) among all pairwise comparisons of populations of Clapper Rails.[a]

	Salton	*Tijuana*	*Newport*	*Seal*	*Mugu*
Salton	—	3.90[b]	3.96[b]	4.21[b]	3.97[b]
Tijuana		—	0.69	2.46[c]	2.77[d]
Newport			—	1.85	2.89[d]
Seal				—	3.71[b]
Mugu					—

[a] *Values of* t *(tested with infinite degrees of freedom) are above the diagonal. A significant* t *means that the distributions of* S *differed significantly between the two populations. See Table 1 for the means of the distributions that are being compared.*
[b] $p < 0.001$.
[c] $p < 0.05$.
[d] $p < 0.01$.

Table 3. Predicted heterozygosities (H), average allele frequency (q), and estimated effective population sizes (N_e) for each of the populations of Clapper Rails using both probes in a combined analysis.

Taxon and Population	H^a	q^b	N_e *(range)*^{c}
R. l. yumanensis			
Salton Sea	0.715	0.181	824 (507–1140)
R. l. levipes			
Tijuana Marsh	0.348	0.452	223 (107–339)
Newport Bay	0.394	0.434	239 (118–360)
Seal Beach	0.421	0.392	283 (147–419)
Mugu Lagoon	0.247	0.520	174 (75–274)

[a] *Heterozygosity calculated by the method of Gilbert et al. (1991).*
[b] *Average allele frequency calculated by the method of Jeffreys et al. (1985).*
[c] *Estimated effective population size calculated by the methods of Lynch (1991). The calculations of* N_e *assume a* μ *of 0.001; a* μ *of 0.01 would reduce the* N_e*'s by a factor of 0.1 (for example, from 824 to 82.4).*

families would allow a direct estimate of μ and thus a more accurate prediction of N_e. In addition, if minisatellite sequences are saturated by mutation or mutation does not conform to an infinite alleles expectation, the estimates may be biased (Harding et al. 1993; Shriver et al. 1993). This is especially true for small values of mean S (and values of $H > 0.8$), which may result in an underestimation of long-term N_e.

Interpopulation Differentiation

Interpopulation and intersubspecific differentiation was assessed by calculating the mean of pairwise band-sharing among individuals from different localities (Table 4) and subspecies. These means were corrected for the level of within-sample band-sharing (Lynch 1991), and the corrected values ranged from 0.79 to 0.96 (Table 4). In general, values for comparisons within *levipes* (mean of 0.91 ± 0.015) were higher than comparisons between *levipes* and *yumanensis* (0.81 ± 0.009; Mann-Whitney $U = 24.0$, $\chi^2 = 6.5$, $p = 0.011$). The corrected S_{ij} for the entire *levipes* sample versus the *yumanensis* sample was 0.84.

Table 4. Results of Clapper Rail DNA fingerprints from the mixed population gels.*

	Salton	*Tijuana*	*Newport*	*Seal*	*Mugu*
Salton	**0.40 (18)**	0.38 (37)	0.35 (37)	0.33 (43)	0.36 (40)
Tijuana	0.83	**0.71 (12)**	0.62 (30)	0.61 (33)	0.59 (33)
Newport	0.81	0.94	**0.67 (9)**	0.64 (30)	0.63 (30)
Seal	0.79	0.90	0.96	**0.69 (12)**	0.57 (33)
Mugu	0.81	0.88	0.94	0.87	**0.71 (12)**

**Means of pairwise* S_{ij} *are above the bold-face diagonal. The number of comparisons are in parentheses. Means of pairwise within-sample* S *are on the diagonal (bold-faced type). Corrected interpopulational similarities (Lynch 1991) are below the diagonal.*

Corrected S_{ij} values were used to calculate estimates of F_{ST} (Lynch 1991) across only *levipes* populations (F_{ST} = 0.22) and all populations (F_{ST} = 0.26). Using these F_{ST} values, a μ of 0.001, and the means of the N_e values from above, we calculated an interpopulation migration rate of 0.0029 among *levipes* and 0.0003 between *levipes* and *yumanesis*.

Clustering relationships among individuals and populations were visualized by constructing trees using three algorithms. All three methods (parsimony, UPGMA clustering, and neighbor joining) gave mostly similar results; only those of the neighbor-joining analysis are provided here (Fig. 4). As expected from the corrected similarities above, individuals of the *yumanensis* population clustered together, and their cluster was somewhat distant from the cluster of individuals from the *levipes* popula-

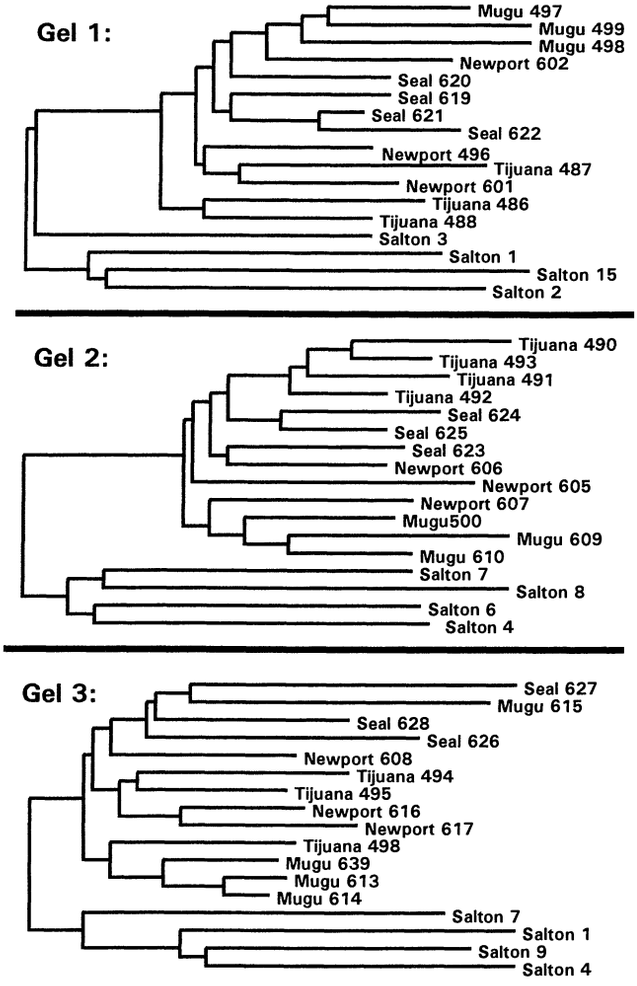

Figure 4. Neighbor-joining trees constructed from matrices of percentage difference (1 − S) between all pairwise combinations of individual Clapper Rails within each of three mixed gels. The number following each locality name indicates the identification number of the individual bird included on the gel.

tions. Structuring of the *levipes* populations was less apparent, but samples from Mugu Lagoon usually clustered together (except for Mugu 615 on gel 3 of Fig. 4, which may be a disperser from a more southern population). Individuals from Tijuana Marsh, Newport Bay, and Seal Beach usually showed only weak clustering, if any.

Discussion

Genetic Variation

Clapper Rails in southern California exhibit a contrasting pattern of genetic variability. The population at the Salton Sea, of the subspecies *yumanesis*, exhibits typical levels of minisatellite variability: The mean value of S over two probes for *yumanensis*, 0.33, is similar to other background, band-sharing estimates for a large number of avian and other taxa (Burke et a. 1989; Westneat 1990; Lynch 1991; Rabenold et al. 1991; Oring et al. 1992; Degnan 1993; Fleischer et al. 1994; Bereson et al. 1995). On the other hand, the four populations occupying coastal salt marshes, of the subspecies *levipes*, showed extremely high band-sharing and thus low levels of genetic variability. The values for the *levipes* populations (0.63–0.77) were considerably higher than those of most examinations to date, matching or nearly matching levels found for highly inbred species such as chicken strains (*Gallus gallus*; Kuhnlein et al. 1990), captive Nene (*Branta sandvicensis*; Rave et al. 1994) and Guam Rails (*Rallus owstoni*; Haig et al. 1994), colonies of naked mole rats (*Heterocephalus glaber*, Reeve et al. 1990), and other mammalian and avian species.

The four coastal *levipes* populations also showed remarkably high levels of among-population band sharing (S from 0.57 to 0.64). This is especially remarkable given the wide geographic range of the sampled populations (over 260 km, Fig. 1). In a typical outbred population, such high values would indicate first-order relatedness of individuals (siblings or parent-offspring). In fact, if we consider S from the *yumanensis* sample as the background level of band sharing for the species, then the expected value of S between first-order relatives (r = 0.5) is 0.67 (equation 22 of Lynch 1991).

If the *yumanesis* sample does contain the typical level of genetic variability for the species, then our data clearly indicate that considerable variation has been lost, presumably through inbreeding and drift, in each *levipes* population and, perhaps more interesting, among populations within the subspecies as a whole. Because of the relatively high mutation rate of minisatellite loci ($\mu \sim 0.001$/gamete/generation), their genetic diversity can recover fairly rapidly following a bottleneck (within $1/\mu$ or 1000 generations; Nei et al. 1975), especially in comparison to single-copy nuclear genes or mtDNA (for example, μ for allozymes is $\sim 10^{-6}$; Voelker et al. 1980). Thus, minisatellite data will usually provide an indication of events that have occurred over a much shorter time scale than more slowly evolving mtDNA, allozyme, or RAPD data. Our concurrent studies of mtDNA (Fleischer et al., in preparation) and RAPD variation (Nusser et al., unpublished manuscript) reveal extremely low variability within and between *yumanensis* and *levipes* populations. A restriction fragment-length polymorphism study of whole mtDNA by Avise and Zink (1988) revealed extremely low intra-population variation in a Louisiana sample of Clapper Rails, as did a study of allozymes in Clapper Rails of the eastern U.S. by Haig and Ballou (unpublished).

We suggest then, that a bottleneck in Clapper Rail populations that occurred over a 1000 years ago was responsible for loss of variation in mtDNA, allozyme, and most RAPD loci. The minisatellite variation for *yumanensis* is relatively high and typical of nonbottlenecked populations; it either was recovered by mutation following the bottleneck, or the bottleneck was not severe enough to reduce the minisatellite variability in the first place (although this seems less likely given the extremely low mtDNA and RAPD variability). A similar scenario was suggested by Menotti-Raymond and O'Brien (1993) to explain the discrepancy they found between very low mtDNA/scnDNA and typical minisatellite DNA variation in cheetahs (*Acinonyx jubatus*).

The interesting pattern of variation within *levipes* could potentially be explained by two historical scenarios. First, *levipes* populations recently could have been severely reduced to a single, small population that remained small long enough to reduce variability, and then recently expanded into its current range. Alternatively, but not exclusively, *levipes* could be structured like a classical metapopulation (Ehrlich 1983; Gilpin 1987), and many continuous cycles of subpopulations undergoing bottlenecks or extinction followed by gene flow or recolonization could result in exceedingly low variability within subpopulations and few differences accruing among subpopulations. The demographic data cited above and in Zembal (1993) certainly suggest that these populations are behaving like a classical metapopulation, with limited dispersal, local extirpations, and recolonizations. Even though band-sharing is very high among all *levipes* populations, however, genetic distances are not unity (Table 4), and individuals within the Mugu Lagoon subpopulation (and to a minor extent the Tijuana Marsh one) cluster in the mixed gel phenograms (Fig. 4). This suggests that gene flow is not so high that it can completely counteract the effects of founder events and genetic drift in some small populations.

We also found that the Mugu Lagoon sample had significantly lower levels of variability than any other population. This makes sense because the Mugu population was the smallest we sampled, averaging only 4.6 ± 2.4 pairs of birds over the past 15 years (and 6.5 pairs when

sampled in 1989), in comparison to 18.8 ± 10.0, 109.5 ± 19.1, and 26.6 ± 18.5 pairs of birds for Seal Beach, Newport Bay, and Tijuana River, respectively (Fig. 2; Zembal 1993). It is also interesting that Ledig (1990) found that egg success in eight clutches of eggs at Mugu was only 49%, whereas Massey et al. (1984) found the hatchability of eggs in 130 clutches at Seal Beach, Newport Bay, and Tijuana Marsh to be 81%. The difference, however, could relate to high contaminant levels in Mugu Lagoon rather than low genetic variation or inbreeding, and additional studies are needed to determine the source of these fitness differences.

Subspecies Differentiation

The two subspecies showed highly significant differences in variability (Tables 1 and 2), but they also showed differences in allele frequencies as assessed by relatively low corrected similarities, high F_{ST}, and the consistent structuring of the phenograms (Table 4; Fig. 4). These major differences conflict with our mtDNA control-region sequence data (Fleischer et al., unpublished data), which show virtually no differentiation between these two subspecies (and for that matter no or very low differentiation between these two subspecies and *R. l. obsoletus* of northern California, *R. l. crepitans* of the eastern U.S. coastal marshes, and *R. l. saturatus* of the Gulf Coast marshes). The divergences are matched to some extent by our RAPD data (Nusser et al., unpublished manuscript), in which the distance between *yumanensis* and *levipes* is about 2–3 times the distance among *levipes* populations, although the overall distance values are very small. The lack of divergence in mtDNA suggests that the two subspecies had a common ancestor sometime within the past 50,000 years (Fleischer et al., unpublished data), but the minisatellite and RAPD DNA differences suggest that the two subspecies have not been exchanging many genes recently (m = 0.0003/generation).

The consistent topologies of the neighbor-joining trees in placing *yumanensis* in a separate cluster from all *levipes* individuals, and in clustering the Mugu Lagoon population, are reminiscent of trees constructed from minisatellite data for Channel Island grey foxes (*Urocyon littoralis*; Gilbert et al. 1990) and cheetahs (Menotti-Raymond & O'Brien 1993). These results point out the potential utility of minisatellite data in reconstructing or inferring the similarity and relationships of individuals and populations. But minisatellite DNA may be less likely to reveal population structure and relationships if mutation saturates divergences to high variability and homoplasy.

Implications for Conservation Management

Our findings, in conjunction with those for mtDNA and RAPDs, allow us to make several recommendations for the management of California populations of Clapper Rails. First, genetic variation for all markers is extremely low in *levipes* populations. Although there is not always a strong link between genetic variation and fitness, there is a general consensus that high inbreeding and loss of genetic variability is not good for individual or mean population fitness or for future adaptive capability (Allendorf & Leary 1986; Ralls et al. 1988; Hedrick & Miller 1992; Lacy et al. 1993; O'Brien 1994).

It is also thought by some workers (Lande & Barrowclough 1987) that fitness is affected primarily by variation at quantitative trait loci. Thus, variation in molecular or biochemical markers, such as allozymes, scnDNA, RAPDs, mtDNA, or VNTRs, may not be as relevant to conservation management as it was hoped they would be. Quantitative trait loci are thought to mutate at an effective rate of about 10^{-3} (including effects due to pleiotropy and epistasis; Lande & Barrowclough 1987). Thus, like minisatellite variability, quantitative trait variation may regenerate more rapidly following a bottleneck and be affected to a lesser degree by a bottleneck than less-mutable loci. If minisatellite variability is greatly reduced, as we found for *levipes*, this might indicate that quantitative trait variation is also low and could be limiting fitness. We suggest, therefore, that some action be taken to increase genetic variability in *levipes* populations, especially those that are very small, such as the Mugu Lagoon population.

Most of the *levipes* populations (Newport Bay, Seal Beach, and Tijuana Marsh) are very similar genetically, which indicates that some natural gene flow has occurred among them recently (estimated at 0.3%/generation above). Thus, translocation of individuals among these larger *levipes* populations would not greatly modify overall genetic structure and would only add a small amount of novel variation to each. But translocations, and their associated additions of V_G, could be important to some of the smaller populations and would also provide unrelated individuals as mates to reduce the potential for inbreeding depression. Translocations would essentially increase the effective population size of these small populations, perhaps closer to levels that existed prior to the population declines caused by extreme destruction of salt-marsh habitats in southern California. Perhaps translocations should await confirmation that inbreeding depression is a significant problem for small populations of Clapper Rails. We suggest that studies be initiated to assess the relationship between reproductive success measures and the DNA fingerprint similarity of pair-mates and populations (Brock & White 1992; Rave 1994).

The overall high interpopulation similarities indicate that outbreeding depressions are not likely to occur, which also supports translocation of individuals among *levipes* populations. Finally, an increase in Clapper Rail population sizes within marshes via translocation would

have obvious demographic as well as genetic benefits. Thus, we recommend that pilot translocations be conducted among *levipes* populations.

Translocation from *yumanesis* to *levipes* populations might also be considered on the grounds that input of their higher minisatellite variability would greatly increase variation in *levipes*. The virtual identity in mtDNA haplotypes (Fleischer et al., in preparation) and similarities in RAPDs suggest that the two subspecies are not very differentiated and share a recent common ancestor. They are morphologically differentiated (mostly differences in coloration), but these differences may not be genetically based or important to local adaptation or sexual selection. The success of the subspecies at the Salton Sea, a highly saline inland body of water, suggests that they would have little or no problem coping with the sea or the brackish water of salt marshes. But we do not advise that translocations of *yumanensis* into *levipes* populations be made until pilot studies with captive individuals are carried out. Such studies would more clearly determine the roles that the morphological, ecological, or behavioral differences play in the absence of notable genetic ones.

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