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GENETIC DIVERSITY IN TWO AVIAN SPECIES FORMERLY ENDEMIC TO GUAM

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ABSTRACT.—We examine genetic diversity within endemic island avian species that recently went extinct in the wild and compare results with nonendangered, nonendemic, and non-island species. The Micronesian Kingfisher (*Halcyon cinnamomina cinnamomina*) and flightless Guam Rail (*Rallus owstoni*) once were endemic to the Pacific island of Guam and now survive only in captivity. Horizontal starch-gel electrophoresis and minisatellite DNA profiles were used to measure genetic diversity within and among the rails, kingfishers and closely related species. Allozyme analyses of Micronesian Kingfishers revealed no genetic diversity (29 enzymes screened). DNA profiles were variable, although similarity (S) among founders to the captive kingfisher population was high relative to nonendangered birds ($S = 0.56 \pm$ SE of 0.02; 89 bands scored/individual). Three other *Halcyon* species examined had average allozyme heterozygosity and DNA similarity. Guam Rails had average to high allozyme heterozygosity as measured by four polymorphic loci ($H = 0.03$ to 0.05) relative to four other rail species (3 *Rallus* spp., 1 *Porzana* sp.). However, S (0.62 ± 0.02) was higher than in Micronesian Kingfishers. Further analyses indicated a significant deviation from Hardy-Weinberg equilibrium in two of three polymorphic loci for captive Guam Rails. Our results suggest that caution be taken in making generalizations about genetic diversity in island species. The type and number of genetic techniques used, number of species or populations examined, and variance in life-history traits among species must be considered. *Received 14 March 1994, accepted 25 January 1995.*

ONE PREDICTION regarding island or small populations suggests that both founder effects and inbreeding due to population bottlenecks ought to reduce genetic diversity. The magnitude of the effect depends on factors such as severity of the bottleneck, population growth rate, and mutation rate (e.g. Wright 1931, 1940, Mayr 1963, Nei et al. 1975, Chakraborty and Nei 1977). This prediction has been studied in some detail for other taxa, but surprisingly few avian data are available (Boag 1986). Conclusions from avian research vary depending on the number of populations and measures of genetic diversity examined (Corbin et al. 1974, Yang and Patton 1981, Gyllensten et al. 1985, Parkin and Cole 1985, Baker and Moeed 1987, Baker et al. 1990a, b, Fleischer et al. 1991a, b, Triggs et al. 1992, Browne et al. 1993, Fleischer et al. 1994, Rave et al. 1994). Furthermore, many studies of island organisms have examined genetic diversity for species that were introduced by man and do not reflect natural founding events. Meanwhile, avian species on islands continue to decline. For instance, 93% of avian species that went extinct between 1600 and 1980 were island endemics (King 1980) and 73% of birds that went extinct in North America and U.S. territories from 1492–1987 were island species (Williams and Nowak 1987). Thus, understanding processes contributing to loss of population viability takes on immediate importance.

Introduction of the brown tree snake (*Boiga irregularis*) to the Northern Marianas island of Guam during World War II caused a precipitous decline or extinction for all of Guam's native forest birds, including Guam Rails (*Rallus owstoni*) and Micronesian Kingfishers (*Halcyon cinnamomina cinnamomina*; Savidge 1987, U.S. Fish and Wildlife Service 1984a, b). In this paper, we examine genetic diversity in these two recently extirpated endemic species. We then compare genetic diversity found in these island species with levels in mainland forms of similar species.

Guam Rails are flightless and have life-his-

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tory traits characteristic of a generalist. They are omnivorous and once occupied all habitats on Guam except wetlands (Jenkins 1979). They also have high fecundity; females reach early sexual maturity (three to four months of age) and are able to produce up to five clutches per year (three to five eggs per clutch) in captivity (Jenkins 1979, Derrickson 1995). The rails were once common on Guam (Jenkins 1979, 1983). In the early 1960s, their population numbered over 80,000 individuals. By 1983, less than 100 rails remained and were concentrated in the northern one-eight of the island (Engbring and Pratt 1985). Guam Rails were last seen in the wild in 1986. In 1983, 22 birds founded the captive population. As of March 1994, only 5 of 22 founders survived, although there were 173 birds in captivity (Derrickson 1995). Since Guam continues to be overrun with snakes (G. Rodda pers. comm., Rodda et al. 1992), a Guam Rail introduction program is now underway on the nearby island of Rota, where no other rails exist (U.S. Fish and Wildlife Service 1989, S. Derrickson pers. comm.).

Micronesian Kingfishers are one of three subspecies of *Halcyon cinnamomina* (Fry et al. 1992). Two other subspecies occur in the Pacific on the Caroline islands of Pohnpei (*H. c. reichenbachii*) and Palau (*H. c. pelewensis*). Micronesian Kingfishers occurred throughout Guam in all habitats except pure savannah and wetlands (Marshall 1949, Baker 1951, Tubb 1966). They excavated nest cavities in decaying trees in limestone forests (Marshall 1989). Both sexes participated in the excavating, 23 days of incubation for their two-egg clutches, and care of young until fledged at approximately 33 days of age (Jenkins 1979).

Micronesian Kingfishers were common throughout Guam as recently as 1945 (Marshall 1949). They survived in northern Guam until 1986, but were last reported in southern Guam in the 1970s (Beck and Savidge 1985). By 1981 they occupied less that one-quarter of their original range and only 3,023 birds were counted (Engbring and Ramsey 1984). From that point the subspecies declined to extinction in the wild: 100 birds were seen in 1985, 7 to 8 males were seen in 1986, and the last 2 to 4 males were observed in 1988 (Beck and Savidge 1985, Shelton 1986, S. Derrickson pers. comm.).

From 1984 through 1986, 29 birds captured from Guam founded the captive kingfisher population. As of March 1994, only six founders remained and the living captive population had reached only 48 birds (Bahner 1995). While "snake-proofing" efforts are becoming more effective (G. Rodda pers. comm.), Micronesian Kingfishers must remain in captivity until their habitat on Guam has been secured from the brown tree snake.

METHODS

Sample collection.—Our choice of tissue to sample was determined by the status of the species we examined. We would have preferred to use multiple tissues (e.g. blood, liver, heart, and pectoralis) for all species as allozyme variability sampled from avian blood is generally lower than from other tissues. However, this option was clearly impossible for all but the nonendangered rails as it would result in killing the birds. Therefore, intraspecific comparisons for Guam Rails and Micronesian Kingfishers, as well as interspecific comparisons for the *Halcyon* kingfishers, were carried out using results from blood samples only. Interspecific rail comparisons incorporated results from analysis of heart, pectoralis, and liver tissue. Furthermore, sample sizes needed for statistical adequacy of various analyses differ (e.g. Nei 1978, Gorman and Renzi 1979, Archie et al. 1989); yet, in some cases, our sample sizes were limited due to difficulty in obtaining rare samples. Data from these birds, however, were helpful in general interspecific comparisons.

Blood (0.75 ml) was collected from all living Micronesian Kingfishers and three other species of *Halcyon* kingfishers held in captivity: White-throated Kingfishers (*H. smyrnensis*, south Asia), Woodland Kingfishers (*H. senegalensis*, Africa), and Black-capped Kingfishers (*H. pileata*, China; Fry et al. 1992)(Table 1). Blood (1.5 ml) also was collected from all living

TABLE 1. Resolution of samples for allozyme study of inter- and intraspecific comparisons in Guam Rails and Micronesian Kingfishers.

	Guam Rail		Micronesian Kingfisher	
	Intra-specific	Inter-specific	Intra-specific	Inter-specific
Initial enzymes screened	28	29	28	28
Enzymes included in study	23	29	23	24
Presumptive loci	26	36	31	31
Polymorphic loci	4	17	0	16
Number of samples	102	40	18	29
Species sampled	1	5	1	4
Tissue used[a]	B	L, H, P	B	B

[a] (B) blood; (L) liver; (H) heart; (P) pectoralis.

TABLE 2. Enzymes, buffer systems, and loci examined in intraspecific and interspecific allozyme study of Micronesian Kingfishers and Guam Rails.

Enzyme	Locus	E.C. no.[a]	Buffer system[b]	Guam Rail		Micronesian Kingfisher	
				Intra-specific	Inter-specific	Intra-specific	Inter-specific
Adenosine deaminase	ADA	3.5.4.4	S, M, R	+[c]	+	+	*
Alcohol dehydrogenase	ADH	1.1.1.1	R		+		
Aspartate aminotransferase	AAT-1	2.6.1.1	C	+	*	+	+
	AAT-2				*		
Creatine kinase	CK-1	2.7.3.2	C	+	+	+	*
	CK-2			+	+	+	
Diaphorase	DIA	1.6.4.3	S	+	+	+	+
Esterase	EST-1	3.1.1.1	S, R	+	+	+	+
	EST-2				*	+	*
Galactoseaminidase	GAM		R		*		
Glucathione reductase	GR-1	1.6.4.2	S	+	*	+	+
	GR-2				+		
Glucosephosphate isomerase	GPI	5.3.1.9	S, R	*	*	+	*
Glutamic pyruvic transaminase	GPT	2.6.1.2	R		*		
Glyceraldehyde-3-phosphate dehydr.	GAPDH	1.2.1.12	C		+		
Glycerol-3-phosphate dehydrogenase	G3P	1.1.1.8	R		*		
Guanine deaminase	GDA		R		+		
Hemoglobin	HGB		M				*
Isocitrate dehydrogenase	IDH-1	1.1.1.42	C	+	+	+	*
	IDH-2				*		
Lactate dehydrogenase	LDH-1	1.1.1.29	C	+		+	+
	LDH-2			*		+	*
Malate dehydrogenase	MDH-1	1.1.1.37	S, C	*	*	+	+
	MDH-2			+	+	+	+
Malic enzyme	ME	1.1.1.40	C	+		+	+
Mannosidase	MAN		M	+	+	+	+
Mannosephosphate isomerase	MPI	5.3.1.8	R	+	*	+	*
Methylumbelliferyl phosphatase	MUP		M	+	+	+	+
Nucleoside phosphorylase	NP	2.4.2.1	S, R	+	*	+	+
Peptidase w/glycyl-leucine	PEP-GL-1	3.4.11/13	S	+	*	+	*
	PEP-GL-2		S				*
Peptidase w/phenyl-alanyl-proline	PEP-PAP-1	3.4.11/13	R	+	+	+	*
	PEP-PAP-2						*
Phosphoglucomutase	PGM-1	2.7.5.1	S, C, R		*	+	*
	PGM-2			*		+	*
Phosphogluconate dehydrogenase	PGD	1.1.1.44	C	+	*	+	*
Phosphoglycerate kinase	PGK	2.7.2.3	C	+	+	+	+
Protein	PRO-1		M, R	+		+	+
	PRO-2					+	
	PRO-3				*	+	*
	PRO-4					+	
Sorbitol dehydrogenase	SDH	1.1.1.14	S		+		
Superoxide dismutase	SOD	1.15.1.1	R	+	*	+	+
Triose phosphate isomerase	TPI	5.3.1.1	C	+	+	+	+

[a] Enzyme Commission number.
[b] (C) Clayton and Tretiak 1972; (M) Markert and Faulhaber 1965; (S) Selander et al. 1971; (R) Ridgeway et al. 1970.
[c] (+) monomorphic; (*) polymorphic.

Guam Rails (Table 1). One-half of each blood sample was immediately centrifuged and separated into plasma, red blood cells, and white blood cells, then frozen at −70°C for allozyme analyses. The other half of each sample, used for DNA profiles, was placed in a cryogenic vial and stored at −70°C. Heart, liver, and pectoralis tissue was collected from 2 Guam Rails that had died in captivity, 1 King Rail (*Rallus elegans*) donated from the Lousiana State University Museum, 10 Clapper Rails (*R. longirostris*), 8 Virginia Rails (*R. limicola*), and 19 Soras (*Porzana carolina*). Samples from the last three species were collected by rail hunters

TABLE 3. Inter- and intraspecific genetic variability among Guam Rails and other rails as estimated using allozymes. Sample sizes differ for Guam Rails in inter- and intraspecific comparisons due to different tissues used.

	n	Alleles/locus ($\bar{x}$ ± SE)	Percent polymorphic loci[a]	Heterozygosity per locus ($\bar{x}$ ± SE)
Intraspecific				
Founders	13	1.1 ± 0.1	12 (GPI, LDH-2, MDH, PGM-2)	0.03 ± 0.02
Nonfounders	89	1.1 ± 0.1	12 (GPI, LDH-2, MDH, PGM-2)	0.03 ± 0.02
Total population	102	1.1 ± 0.1	12 (GPI, LDH-2, MDH, PGM-2)	0.03 ± 0.02
Interspecific				
Guam Rail	2	1.1 ± 0.0	6.5 (GPI, MDH-1)	0.05 ± 0.04
King Rail	1	1.0 ± 0.0	3.2 (GPI)	0.03 ± 0.03
Clapper Rail	10	1.1 ± 0.1	3.2 (GPI)	0.01 ± 0.01
Virginia Rail	8	1.1 ± 0.1	9.7 (AAT-2, GR-1, MPI)	0.02 ± 0.01
Sora	19	1.3 ± 0.1	16.1 (AAT-1, GPI, GR-1, MPI, PGD)	0.02 ± 0.01

[a] Using 0.99 criterion for polymorphism. Polymorphic loci in parentheses.

during the 1987 fall migration (15 September–10 October): on the United States Atlantic coast at Merrimeeting Bay, Maine (8 Soras); Parker River National Wildlife Refuge, Massachusetts (5 Soras and 8 Virginia Rails); Patuxent River Wildlife Management Area, Maryland (6 Soras); and Eastern Shore of Virginia National Wildlife Refuge, Virginia (10 Clapper Rails). Since blood could not be extracted from the carcasses, we used other tissue for analysis. The heart, liver, and pectoralis were immediately removed, placed on dry ice in the field, and then stored at −70°C until analysis.

Protein electrophoresis.—Horizontal starch-gel electrophoresis was carried out at the Cornell Laboratory of Ecological and Evolutionary Genetics using methodology outlined in May (1992). Sample resolution is summarized in Tables 1 and 2. Electromorphs were assumed to be products of different alleles. Loci were numbered sequentially with integers beginning with "1" for the most anodal form, alleles were designated alphabetically with "A" indicating the most common allele.

Genotypes scored as a result of electrophoresis were analyzed using the BIOSYS 1.7 computer program (Swofford and Selander 1989). Genetic differences among species were estimated for comparison with DNA profile data and other avian studies using Roger's (1972) distance coefficients. For inter- and intraspecific comparisons, heterozygosity at each locus was estimated by direct count of heterozygotes (i.e. observed heterozygosity, H_o) and, within Guam Rails, by calculating the frequency of heterozygotes expected at Hardy-Weinberg equilibrium (expected heterozygosity, $H_E = 1 - Ep_i^2$, where p_i is the estimated frequency of the ith allele in the population). Guam Rails also were examined for departures from Hardy-Weinberg equilibrium using a chi-square goodness-of-fit test and Fisher's exact-probability estimate. In these tests, a P-value of 1.00 indicate perfect fit to predictions, while a value of 0.00 indicates lack of fit. Throughout the paper, results were considered significantly different with a probability of 0.05 or less.

DNA profiles.—As part of another study, minisatellite DNA profiles were prepared for the *Halcyon* kingfishers and Guam Rails. Detailed methods, including probe description, are found in Haig et al. (1994) and Haig et al. (1995). Two independent multilocus probes were used to examine similarity (S = proportion of DNA bands shared between two individuals) among all Guam Rails and Micronesian Kingfishers. Mean similarity and mean number of DNA bands scored per individual within and among kingfisher species and within Guam Rails are presented for better understanding of relative genetic diversity.

RESULTS

Guam Rails.—Allozyme data indicated that Guam Rails had levels of genetic diversity similar to those of the other rails; no genetic diversity had been lost between founders and the subsequent population (Table 3). Among founders, 4 of 26 presumptive loci were polymorphic (Table 4). Allele frequencies among founders was not significantly different from Hardy-Weinberg predictions, however, nonfounders and the total population deviated significantly for two of the three polymorphic alleles (Table 5). Mean DNA similarity among Guam Rail founders was 0.62 ± SE of 0.02 compared with 0.64 ± 0.01 among nonfounders (Haig et al. 1994).

Interspecific comparisons indicate that among the five rail species sampled, 18 of 36 presumptive loci were polymorphic (Table 2 and 3). A

TABLE 4. Allele frequencies of polymorphic allozyme loci for Guam Rails.

Allele	Founder (n = 13)	Nonfounder (n = 89)	Total population (n = 102)
	GPI		
A	0.85	0.83	0.83
B	0.15	0.17	0.17
	LDH-2		
A	0.92	0.99	0.98
B	0.08	0.01	0.02
	MDH		
A	0.89	0.78	0.79
B	0.11	0.22	0.21
	PGM-2		
A	0.83	—	—
B	0.17	—	—

number of these alleles were unique for each species: for Guam Rails, PEPGL (C), GPI (D), LDH-2 (C), SOD (B), and MDH-1 (B); for Clapper Rails, GPI (E); for King Rails, IDH-2 (B); for Virginia Rails, G3P (B), SOD (C), and AAT-2 (B); and for Soras, PEPGL (B), GPI (F), LDH-2 (A), MPI (B,D), AAT-1 (B), PGD (A,D), GR-1 (D), and PRO-3 (A).

The number of alleles per locus did not vary significantly among species. The percent of polymorphic loci varied considerably with values for Guam Rails (6.5%), falling in the middle range of those species sampled. Guam Rails had the highest level of heterozygosity (H = 0.03 to 0.05) among all rail species examined. Genetic distance measures indicated similar distances between Guam Rails and the other species sampled including Soras, which are classified in the genus *Porzana* (Table 6). The other *Rallus* rails were more similar to each other than to the Sora. Not surprisingly, King Rails and Clapper Rails were most similar.

TABLE 5. Comparisons of polymorphic allele frequencies for deviation from Hardy-Weinberg predictions for Guam Rail founders (n = 13), nonfounders (n = 89), and the total number of birds sampled (n = 102). Significant ($P < 0.05$) departure from Hardy-Weinberg expectations indicated by asterisk (*).

Group	Fisher's exact test (P)	X^2
	GPI	
Founders	1.00	0.31
Nonfounders	0.02*	7.31*
Total	0.03*	5.40*
	LDH	
Founders	1.00	0.04
Nonfounders	1.00	0.01
Total	1.00	0.03
	MDH	
Founders	1.00	0.14
Nonfounders	0.06	4.34*
Total	0.40*	4.26*
	PGM-2	
Founders	1.00	0.35

TABLE 6. Roger's (1972) genetic distances based on rail allozyme data.

Species	Guam Rail	King Rail	Clapper Rail	Virginia Rail
King Rail	0.52			
Clapper Rail	0.49	0.20		
Virginia Rail	0.41	0.44	0.40	
Sora	0.47	0.80	0.82	0.77

Micronesian Kingfishers.—No genetic variability was found among the 18 Micronesian Kingfisher founders screened for 29 enzymes (Tables 1 and 2). Comparison of Micronesian Kingfishers with three other congenors indicated polymorphisms existed in the other species examined, despite small sample sizes (Tables 2 and 7). Conversely, DNA profiles were variable for all kingfishers (Table 7). The mean similarity among Micronesian Kingfisher founders was slightly higher than the nonendangered kingfishers. There was no difference in similarity between founders and nonfounders for Micronesian Kingfishers.

Alleles unique to each species were found in three of the four species at the following loci: for Micronesian Kingfishers, CK (B), HGB (B), MPI (B), PEPGL (C), PEPPAP (A), PGD (D), PGM-1 (B), and PRO-3 (B); for Woodland Kingfishers, EST-1 (B), GPI (B), HGB (C), IDH (B), PEPGL (D), PEPPAP (B), and PGM-1 (C); and for White-throated Kingfishers, CK (B), and PEPPAP (A). Table 8 gives allozyme and DNA distances between Micronesian Kingfishers and the other species examined.

DISCUSSION

Not surprisingly, our results give variable support to the initial prediction that island species or small populations have reduced genetic

TABLE 7. Interspecific genetic variability among *Halcyon* kingfishers as estimated by allozymes and DNA profiles ($\bar{x} \pm$ SE).

Species	*n*	Alleles per locus	Percent polymorphic loci[a]	Heterozygosity per locus	DNA similarity	DNA bands per individuals
Micronesian Kingfisher	18	1.0	0.0	0.00 ± 0.00	0.49 ± 0.04	44.7 ± 1.2
Black-capped Kingfisher	3	1.1	6.9 (PEPGL)	0.02 ± 0.02	0.51 ± 0.02	38.5 ± 1.7
Woodland Kingfisher	1	1.0	3.4 (PGD)	0.03 ± 0.03		
White-throated Kingfisher	7	1.1	10.3 (PEPGL, PGD)	0.03 ± 0.02	0.51 ± 0.02	33.5 ± 1.9

[a] Polymorphic loci in parentheses.

diversity. Guam Rails have average or slightly higher levels of genetic diversity, as measured by allozymes, relative to other rails and other birds. Conversely, Micronesian Kingfishers have no genetic diversity as measured by allozymes. However, both species showed genetic variability as measured by DNA profiles. Differences in life-history traits and recent population history for Guam Rails and Micronesian Kingfishers discussed below will provide a perspective for these results.

Effects of population bottlenecks on genetic diversity.—Historically, and just prior to their rapid decline, Guam Rails had much higher population estimates and probably a higher effective population size compared to Micronesian Kingfishers. Thus, the rails may have had high allozyme diversity before the bottleneck, and the species declined so rapidly that not enough time or generations had passed for genetic changes to take place via inbreeding or drift. Similar results have been shown for the greater one-horned rhinoceros (*Rhinoceros unicornis*), which is near extinction, yet has not measureably lost genetic diversity (Dinerstein and McCracken 1990). Lack of detected allozyme variation, as was found in Micronesian Kingfishers, has been reported for four other avian species (all critically endangered): (1) Lesser Prairie Chickens (*Tympanuchus pallidicinctus*; Gutierrez et al. 1983); (2) six populations of Spotted Owls (*Strix occidentalis*; Barrowclough and Gutierrez 1990), (3) the Blue Duck (*Hymenolaimus malachorhynchos*; Triggs et al. 1992) endemic to New Zealand; and (4) one of two sampled populations for Bald Eagles (*Haliaeetus leucocephalus*; Knight et al. 1995).

TABLE 8. Genetic distances among *Halcyon* kingfishers. Mean DNA profile distance (1-similarity) estimate above diagonal and Roger's (1972) genetic distance of allozyme data below diagonal.

Species	Micronesian Kingfisher	Woodland Kingfisher	White-throated Kingfisher	Black-capped Kingfisher
Micronesian Kingfisher		0.44	0.52	0.51
Woodland Kingfisher	0.44		0.50	0.50
White-throated Kingfisher	0.34	0.36		0.60
Black-capped Kingfisher	0.33	0.32	0.08	

Minisatellite DNA profiles provided an additional perspective for evaluating relative genetic diversity. First, minisatellite loci typically have a mutation rate that is two to three orders of magnitude higher than that of typical genes like enzymes. Therefore, allozyme and minisatellite systems should not show the same pattern of variation nor reflect demographic events from the same time period. For example, allozyme variation in the Micronesian Kingfisher could have been reduced by a founder effect following colonization of Guam in the distant or recent past; it could take 10^6 or more generations to restore the lost allozyme variation (see Nei et al. 1975). On the other hand, 10^3 generations may be all that is required to regenerate minisatellite variability. Finally, bottlenecks have different effects on multilocus characters. Since many loci are contributing to any one character, reduction of populations to even a few individuals may not have much effect in reducing variation in polygenic characters such as morphological attributes.

Results similar to Micronesian Kingfishers were found in Blue Ducks, where no allozyme diversity was found but DNA profiles were diverse (Triggs et al. 1992). However, both Guam

Rails and Micronesian Kingfishers had high similarity relative to unrelated individuals in nonthreatened avian species such as Purple Martins (*Progne subis*, $S = 0.19$; Morton et al. 1990), Indigo Buntings (*Passerina cyanea*, $S = 0.23$; Westneat 1990), House Sparrows (*Passer domesticus*, $S = 0.15$; Wetton et al. 1992), Bull-headed Shrikes (*Lanius bucephalus*, $S = 0.30$; Yamagishi et al. 1992), Tree Swallows (*Tachycineta bicolor*, $S = 0.25$; Dunn and Robertson 1993), and European Starlings (*Sturnus vulgaris*, $S = 0.12$; Pinxten et al. 1993), but were similar to other species that had experienced a population decline, such as Puerto Rican Parrots (*Amazona ventralis*, $S = 0.51$; Brock and White 1992), Red-cockaded Woodpeckers (*Picoides borealis*, $S = 0.51$; Haig et al. 1994), and Nene (*Branta sandvicensis*, $S = 0.63$ to 0.77; Rave 1995). Exceptions are the Palila (*Loxioides bailleui*, $S = 0.26$), an endangered species now confined to a dormant volcano on the island of Hawaii (Fleischer et al. 1994), and the Blue Duck ($S = 0.17$ to 0.24; Triggs et al. 1992).

The significance of genetic diversity, as measured by allozymes or DNA profiles, remains difficult to assess. In some cases, species or populations with no or low measureable genetic diversity have functioned seemingly well (Bonnell and Selander 1974, Hoezel et al. 1993), whereas in others, lack of allozyme diversity was potentially symptomatic of serious problems (O'Brien et al. 1985, Packer et al. 1991, but see Caro and Laurenson 1994). As yet, no negative effects (e.g. reduced reproductive success) have been noted among Guam Rails or Micronesian Kingfishers due to genetic factors.

Current status of Guam Rails and Micronesian Kingfishers.—Events in the immediate future of a population that has just experienced a bottleneck can critically affect its ability to return to a higher effective population size (Nei et al. 1975, Carson and Templeton 1984). In the two Guam species, any loss of genetic diversity could hamper current conservation efforts to return these species to viable wild populations. Despite careful breeding plans that reduce matings among relatives and maximize retention of expected heterozygosity (Haig et al. 1990), DNA profiles and pedigree analyses both indicate that Guam Rails have lost some genetic diversity in the short time since they have been in captivity (Haig et al. 1990, 1994). DNA and allozyme analyses indicated an approximate 6% loss of genetic diversity between founders and the remaining population sampled. This loss may be partially attributed to difficulty among some founders in forming pair bonds in captivity. Of 22 founders, only 13 have bred in captivity and only four of five living founders have bred (Derrickson 1995). Thus, the entire captive population (387 Guam Rails have lived in captivity at some point) has descended from 13 birds, some of which most likely were closely related (Haig et al. 1994). Furthermore, because Guam Rails breed at a younger age and more often than Micronesian Kingfishers, our understanding of their pedigree is greater (three to four generations deep) than the Micronesian Kingfisher pedigree (two generations deep). Therefore, there is more opportunity to measure loss of genetic diversity among Guam Rails than Micronesian Kingfishers.

Both allozyme and DNA profile data suggest a genetic bottleneck may have occurred in Micronesian Kingfishers. Micronesian Kingfishers never were common; hence, they may have been more susceptible to suffer negative effects from a genetic bottleneck. Nei et al. (1975) and others have shown that founding populations or subsequent bottlenecks must be small or take place over a long period of time to significantly reduce heterozygosity. Founder effects will have a greater impact on loss of rare alleles due to random drift. Thus, genetic diversity measured in the kingfishers may reflect both founder effect and a subsequent bottleneck. Because no diversity could be found with allozymes, we were not able to measure differences between founders and the subsequent population with allozymes. However, pedigree analyses suggest that as much as 6% heterozygosity may have been lost (Bahner 1993), indicating that the severity of the bottleneck has increased since Micronesian Kingfishers were brought into captivity. Of the 29 founders, 20 have bred (Bahner 1995), but many of their offspring have proven inviable due to behavioral problems associated with hand-rearing. Furthermore, DNA profile analyses of founders suggested that six to seven founders were close relatives (probably siblings; Haig et al. 1995); hence, the effective population size in captivity is extremely low and not likely to increase in the near future.

Interspecific comparisons.—Levels of heterozygosity exhibited among all rail species except Guam Rails ($H = 0.01$ to 0.03) were slightly below average estimates for single avian populations ($H = 0.05$ in Barrowclough [1983]; $H = 0.07$ in Evans [1987]). It is difficult to make broad

generalizations about relative heterozygosity from these comparisons as some species that clearly were not in decline had low levels of heterozygosity. More relevant comparisons exist for Clapper and King rails in Louisiana, where Avise and Zink (1988) reported average heterozygosity as 0.04 ± 0.02 and 0.03 ± 0.01, respectively. Slight differences in heterozygosity in this study ($H = 0.01 \pm 0.01$) may be a result of the different Clapper Rail populations examined or of the allozyme sampling. We examined 18 of the enzymes used by Avise and Zink (1988). Among these common enzymes, ADA and MPI were polymorphic in the Avise and Zink study and not in ours. However, we identified variability in IDH-2 and GR-1, whereas Avise and Zink did not. Among those not commonly examined, we found polymorphism at GPI and Avise and Zink had variability at GPT-1, LEU-PEP-2, and PGI. It is possible that Clapper Rails on the east coast have experienced a population bottleneck or that the Louisiana Clapper Rails had hybridized to some extent with King Rails (Avise and Zink 1988), but more extensive sampling throughout the Atlantic Coast is necessary before an assessment is made.

The average to high heterozygosity observed in Guam Rails relative to the other rails may be a function of their recent large population size (i.e. just 35 years ago), whereas local populations of rails from the North American Atlantic Coast may not be as large and may have reduced effective sizes. Furthermore, if measures such as allelic diversity and percent polymorphic loci are more indicative of bottlenecks (Leberg 1992), Guam Rail results do not suggest measurable effects of a bottleneck.

Among kingfishers, the similar genetic distances among *Halcyon* congenors for DNA and allozyme data reflect results from an allozyme study by Knox (1980). He evaluated the hypothesis proposed by Fry (1980) that there was a close relationship among Woodland, White-throated and Black-capped kingfishers relative to six other Afrotropical *Halcyon* species. A future study should more extensively sample these and less-closely related kingfisher species to further examine these relationships.

Conclusions.—Our study illustrates how expectations of low genetic diversity in island species are difficult to assess. Interpretation of results may vary depending on a species' life history, the number and types of genetic techniques employed, and sampling intensity. The surprising paucity of genetic data for birds that naturally occur on islands makes comparison with other species difficult. In Guam Rails and Micronesian Kingfishers, there no longer are opportunities for gene flow from other populations. Thus, quantification and conservation of their current genetic diversity is critical. If suitable habitat in the wild can be found, this bottleneck may be short-lived and their respective effective population sizes may begin to recover. Certainly, other factors in addition to genetic diversity must be considered in recovering these species. However, if our genetic analyses are indicative of the status of these species, the situation is precarious at best.

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