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GENETIC VARIABILITY IN THE ENDEMIC VIREOS OF PUERTO RICO AND JAMAICA CONTRASTED WITH THE CONTINENTAL WHITE-EYED VIREO

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ABSTRACT.—To test the hypothesis that island species exhibit lower genetic variability than mainland relatives, genetic data from three species of vireos endemic to Puerto Rico and Jamaica were compared with the White-eyed Vireo (*Vireo griseus*), a closely related species that breeds in North America. Variability was examined using random amplified polymorphic DNA (RAPD) markers. The White-eyed Vireo had a significantly higher proportion of polymorphic bands (67% of 115 total bands) than the Puerto Rican Vireo (*V. latimeri*; 38% of 95 bands), Jamaican Vireo (*V. modestus*; 34% of 107 bands), or Blue Mountain Vireo of Jamaica (*V. osburni*; 32% of 111 bands). The mean genetic distance among conspecific individuals (Jaccard's index) was significantly higher in the White-eyed Vireo (0.293) than in any of the three endemic species (Puerto Rican Vireo, 0.115; Jamaican Vireo, 0.115; Blue Mountain Vireo, 0.106); mean genetic distance values did not differ among the three endemic species. Population substructuring assessed by analysis of molecular variance (Φ_{ST}) and Wright's F_{ST} values revealed that the two common endemic species (Puerto Rican and Jamaican vireos) had greater among-population genetic variation than did three populations of the White-eyed Vireo. Genetic substructuring was moderate to substantial among two Puerto Rican Vireo populations (Φ_{ST} = 0.103, F_{ST} = 0.141) and weak to moderate among two Jamaican Vireo populations (Φ_{ST} = 0.048, F_{ST} = 0.117). The migratory White-eyed Vireo had the lowest values for both statistics (Φ_{ST} = 0.015, F_{ST} = 0.109). Low among-individual, within-species genetic variation was related to island endemism but was not influenced by differences among endemics in habitat specificity or local abundance. *Received 19 August 1998, accepted 28 January 1999.*

EARLY SCIENTISTS working on theoretical foundations of speciation and population genetics recognized that the initial colonization of an island by a small group of individuals, combined with the cessation of gene flow between colonizers and their mainland counterparts, would produce a severe reduction in effective population size that would cause an increase in inbreeding relative to the continental population and an amplification of the effects of genetic drift (Wright 1951). Based on these principles, Mayr (1954) developed the theory of speciation via the "founder effect," proposing that the effects of drift and differential selection pressures will cause a genetic revolution in the island population and result in rapid reproductive isolation.

Although debate continues regarding the founder effect and its relative importance as a speciation process (Moya et al. 1995, Slatkin 1996), a great deal of attention has been given to its role in causing reduced genetic variation in island populations. Mathematical models have shown that severe reductions in effective population size (i.e. "bottlenecks") maintained over long periods of time cause the loss of rare alleles and fixation due to inbreeding (Nei et al. 1975). Experimental evidence, largely using laboratory populations of *Drosophila*, has been equivocal: some investigators have confirmed the loss of allozyme and quantitative genetic variation in small populations (e.g. Briscoe et al. 1992, Leberg 1992, Frankham 1996), whereas others have found no change, or even increases, in additive genetic variance following reductions in population size (Bryant and Meffert 1993, 1996; Willis and Orr 1993).

Whether or not a population bottleneck can cause reduced genetic variation, the large number of equivocal comparative studies stands in contrast to the scarcity of experimental evidence. Based on a hypothesis by Soulé (1976) relating genetic variability to population size,

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Frankham (1996) developed 10 predictions for a variety of taxonomic and biogeographical comparisons and determined that all of them are supported by a broad range of empirical data (most based on allozyme diversity but also including mtDNA and quantitative characters). In addition, Frankham (1997) found that island populations have less allozyme variation than mainland populations (more than 80% of 202 comparisons) and that insular endemic populations show reduced genetic variation (almost 90% of the cases).

Initial studies of the population genetics of insular species of birds only weakly supported the hypothesis of reduced genetic variation in these populations (Boag 1988). More recently, however, a growing body of supporting evidence has appeared (e.g. Johnson et al. 1989, Baker et al. 1990, Hare and Shields 1992, Peterson et al. 1992, Haig and Ballou 1995), but with some notable exceptions (e.g. Yang and Patton 1981, Seutin et al. 1993). These exceptions involve conspecific populations that may experience some degree of gene flow that would prevent loss of genetic variation and "rescue" the island population from the risk of extinction (Brown and Kodric-Brown 1977). Few studies have examined the level of genetic variation in endemic island species and compared them directly with closely related mainland congeners. One exception is the study of Haig and Ballou (1995) of captive populations of two endemic species extirpated from Guam, the Micronesian Kingfisher (*Halcyon c. cinnamomina*) and the Guam Rail (*Rallus owstoni*). The Micronesian Kingfisher had zero allozyme variability (compared with average levels in continental *Halcyon*); however, the Guam Rail had heterozygosity levels equivalent to *Rallus* in North America.

Here, I examine relative levels of genetic variation in three species of the genus *Vireo* endemic to the islands of Puerto Rico and Jamaica contrasted with that of a continental species that is a Neotropical migrant. One endemic vireonid inhabits Puerto Rico (Puerto Rican Vireo [*Vireo latimeri*]), and two are restricted to Jamaica (Jamaican Vireo [*V. modestus*] and Blue Mountain Vireo [*V. osburni*]). These are well-differentiated species (Bond 1985, Raffaele 1989, Downer and Sutton 1990), suggesting minimal (if any) genetic exchange with other island species or with any continental species. The White-eyed Vireo (*V. griseus*) is a continental species that along with the three island species noted above is a member of the subgenus *Vireo*, a clade genetically distinct from the subgenus *Vireosylva* (Murray et al. 1994). White-eyed Vireos are presumed to be closely related to the island species based on morphological comparisons (Hamilton 1962, Barlow 1980) and molecular analysis of phylogenetic affiliations (Zwartjes 1997).

Using random amplified polymorphic DNA (RAPD) genetic markers (Williams et al. 1990), I tested the hypothesis that the island species exhibit lower genetic variability than their mainland relative. In addition, the Puerto Rican and Jamaican vireos are common habitat generalists on islands of roughly equivalent area, whereas the Blue Mountain Vireo is less abundant, has a more limited range, and generally is restricted to upper and mid-elevation wet forests (Lack 1976, Downer and Sutton 1990). This allowed me to test three additional hypotheses proposed by Frankham (1996) regarding comparative levels of genetic variation: (1) that genetic variation is directly related to population size across species within a taxonomic group, (2) that genetic variation is lower in species with greater habitat restriction or specialization, and (3) that genetic variation is lower in species with restricted ranges. I also examined the partitioning of genetic variance within the two common endemic species (Puerto Rican and Jamaican vireos) for evidence of population genetic structuring within their respective islands and compared with the partitioning of genetic variance among three populations of the continental White-eyed Vireo.

Methods

Data and blood sampling.—I captured at a variety of sites in Puerto Rico, Jamaica, and the eastern United States (Figs. 1A–C). Puerto Rican Vireos were sampled at two sites in 1993 and 1994: Guajataca State Forest located in northwestern Puerto Rico, characterized by wet limestone forest; and Guánica State Forest on the southwestern coast, which lies in the rain shadow of the Central Cordillera mountain range and consists of semiarid scrub forest. Jamaican and Blue Mountain vireos were captured at two localities in Jamaica during summer 1995. The eastern site was located in montane forest in the vicinity of Hardwar Gap in the Blue Mountains (elevation ca. 1,350 m); a northwestern site was located near Wind-

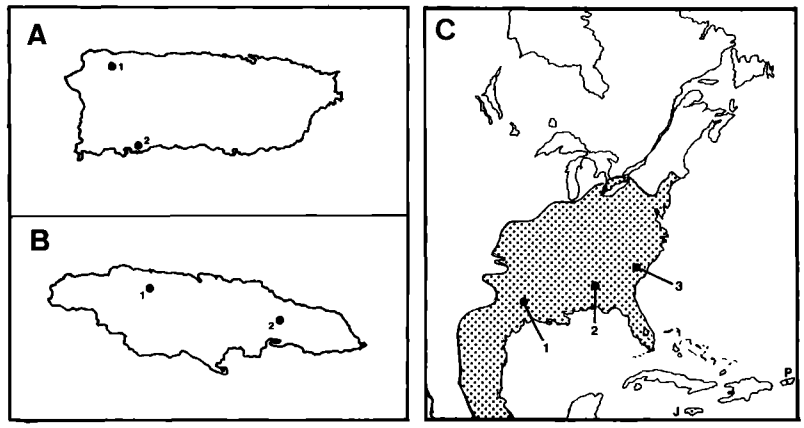

FIG. 1. Geographic localities for sampling sites. (A) Puerto Rican Vireo: 1, Guajataca State Forest; 2, Guánica State Forest. (B) Jamaican Vireo and Blue Mountain Vireo: 1, Windsor; 2, Blue Mountains. (C) White-eyed Vireo: 1, Nacogdoches, Texas; 2, Tuskegee National Forest, Alabama; 3, Congaree Swamp National Monument, South Carolina. Stippling denotes breeding range in North America

sor in wet limestone forests at about 300 m elevation. I obtained samples of White-eyed Vireos in 1995 from three sites: Nacogdoches County, Texas, near the city of Nacogdoches; Macon County, Alabama, in Tuskegee National Forest; and Richland County, South Carolina, in Congaree Swamp National Monument.

Birds were captured in mist nets following taped playback of song. After I obtained a blood sample and measurement data, each bird was banded with a numbered U.S. Fish and Wildlife Service band and released. Blood samples were obtained from the leg vein on the interior of the joint connecting the tibiotarsus and tarsometatarsus. Approximately 40 to 50 μL of blood were drawn into a heparinized hematocrit tube and stored in 1 mL of a cell lysis buffer (100 mM Tris, 100 mM EDTA, 10 mM NaCl and 1% SDS, pH 8.0); samples were kept at room temperature until DNA extraction was performed in the laboratory.

DNA extraction and primer selection.—Extraction of DNA from the blood samples was performed by first diluting the samples to approximately 4 μL of blood per 600 μL of lysis buffer, followed by an RNA-ase treatment at 37°C for 15 min. Proteins and other contaminants were removed either through the use of a 2 M NaCl solution or the protein-precipitation solution from the Purgene DNA Isolation kit (Gentra Systems, Inc.); the mixture was vortexed vigorously and centrifuged for 3 min at 14,000 rpm. The suspension was then added to 100% isopropanol, and the resulting DNA precipitate spun out by centrifugation. After washing with 70% ethanol, the resulting DNA pellet was air-dried overnight under a constant airflow hood and then resuspended in Tris-EDTA buffer. Samples were quantified by spectrofluorometer using a 100 ng/μL calf thymus DNA standard, followed by dilution to 20 ng/μL.

RAPD-PCR was performed using 25-μL reaction mixtures consisting of 10 mM Tris-HCl pH 8.3; 50 mM KCl; 2.0 mM $MgCl_2$ (Boehringer Mannheim PCR reaction buffer, with $MgCl_2$ added); 0.1 mM each of dATP, dTTP, dCTP, and dGTP (Pharmacia Biotech); 0.2 μM of primer (Operon); 0.5 units of *Taq* DNA polymerase (Boehringer Mannheim); and 20 ng of DNA sample. Variation between reactions in the concentrations of these ingredients was controlled by combining all ingredients except the DNA in a single large mixture, vortexing thoroughly, and pipetting an aliquot into each reaction tube. One microliter of DNA sample was added to each tube, centrifuged into the mixture, and then overlaid with 50 μL of mineral oil. Reactions were amplified on a Perkin-Elmer DNA thermal cycler by 45 cycles of 94°C for 30 s, 37°C for 1 min, 54°C for 30 s, and 72°C for 2 min, followed by one cycle of 72°C for 15 min, and then held at 4°C. Products were analyzed by electrophoresis using a 1.2% agarose gel, followed by staining with ethidium bromide, and then photographed with black-and-white Polaroid film while being fluoresced under ultraviolet light.

Primers designed for use in RAPD-PCR (Operon Technologies, Inc.) were surveyed for their ability to provide genetic marker information in all four species of interest; each primer consisted of 10 nucleotides with 60 to 70% G-C content. Thirteen primers were selected based on their ability to amplify several bands that were judged to be bright, distinct, and easily scored for presence or absence; these primers and their nucleotide sequences are listed in Table 1.

TABLE 1. The 13 primers selected for RAPD analysis in four species of vireos. Primer names are followed by their 10-nucleotide sequence, the number of bands scored per primer, and the number of polymorphic bands (in parentheses).

Primer	Nucleotide sequence	*V. griseus*	*V. latimeri*	*V. modestus*	*V. osburni*
OPA-16	5′-AGCCAGCGAA-3′	4 (2)	7 (2)	11 (6)	7 (3)
OPB-7	5′-GGTGACGCAG-3′	11 (10)	6 (0)	5 (1)	6 (1)
OPC-9	5′-CTCACCGTCC-3′	16 (14)	7 (5)	8 (2)	8 (0)
OPC-15	5′-GACGGATCAG-3′	11 (10)	7 (1)	6 (2)	8 (5)
OPD-3	5′-GTCGCCGTCA-3′	13 (8)	6 (3)	8 (6)	12 (6)
OPD-5	5′-TGAGCGGACA-3′	10 (6)	11 (7)	6 (2)	7 (1)
OPD-13	5′-GGGGTGACGA-3′	7 (3)	6 (1)	7 (2)	9 (1)
OPD-15	5′-CATCCGTGCT-3′	5 (4)	6 (3)	8 (1)	8 (3)
OPD-20	5′-ACCCGGTCAC-3′	7 (6)	11 (4)	11 (3)	8 (2)
OPE-2	5′-GGTGCGGGAA-3′	7 (5)	4 (1)	8 (2)	8 (4)
OPE-6	5′-AAGACCCCTC-3′	8 (5)	6 (1)	12 (3)	13 (3)
OPY-5	5′-GGCTGCGACA-3′	8 (6)	11 (4)	12 (4)	13 (4)
OPZ-3	5′-CAGCACCGCA-3′	8 (8)	7 (4)	5 (2)	4 (2)
Total		115 (87)	95 (36)	107 (36)	111 (35)

Scoring of RAPD-PCR products.—Not all RAPD-PCR products reveal accurate information about genetic structure. Typically, some RAPD bands are amplified strongly and consistently, whereas others are not reliably repeatable (Hadrys et al. 1992, Ellsworth et al. 1993). It is important to use a scoring protocol that rejects non-repeatable markers, but scoring error affects estimates of genetic distances less than sampling error, and a reduction in the number of RAPD bands used increases the relative importance of sampling error (Skroch and Nienhuis 1995).

I used a scoring protocol that maximizes the number of bands in the analysis while testing each fragment for scoring reliability and accuracy. Samples from all populations were amplified at least twice by each primer; these replications were organized into two sets of population samples arranged side by side on an agarose gel (Fig. 2). Identification of RAPD fragments of equivalent molecular weight among samples was facilitated by a reference "ladder" consisting of DNA in 100-base pair increments. Each replication set of a population was scored independently, with all readable fragments given a "1" for presence or a "0" for absence in each individual. Results were then compared between replications for all samples, and bands receiving a score of 1 in only one replication of a sample were identified as non-repeatable within that sample. Polymorphic bands

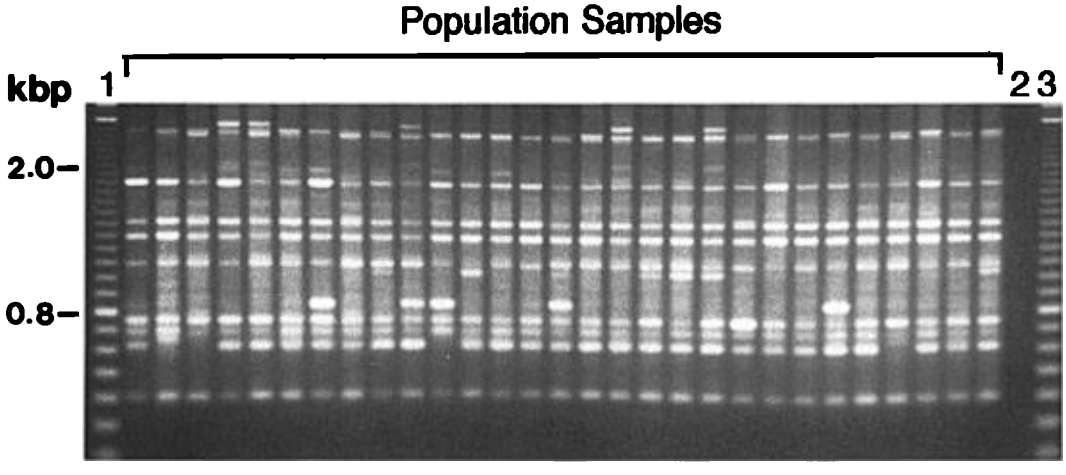

FIG. 2. Arrangement of DNA samples on an agarose gel after amplification by RAPD-PCR. Photo depicts *V. latimeri* samples from Guánica Forest amplified with primer OPY-5. Lanes 1 and 3 contain a DNA ladder with fragments in 100-bp increments; lane 2 is a negative control consisting of a sample of reaction mixture with no DNA. The reaction products are arranged with the longest base-pair sequences at the top, and the shortest at the bottom.

were retained for analysis only if 80% or more of its 1 scores were identified as being repeatable between replications (the use of 80% as an acceptable repeatability threshold was demonstrated by Peakall et al. 1995). This procedure allowed the retention of as many bands as possible while eliminating bands that were highly prone to scoring error.

Statistical analysis.—The patterns of RAPD bands within each species were first analyzed with the program of Armstrong et al. (1995) to calculate pairwise genetic distances between all samples within species. For each pair of samples, a metric based on Jaccard's index (J) was used:

$$J = (n_{01} + n_{10})/(n_{11} + n_{01} + n_{10}), \quad (1)$$

where for two samples A and B, n_{01} represents the number of RAPD bands present in A but absent in B, n_{10} is the number of bands present in B but absent in A, and n_{11} is the number of bands appearing in both samples. For each individual within a species, a value for J was calculated using every other individual in the sample population for comparison.

Jaccard's index is sensitive to the number of monomorphic bands within a population of samples and thus allows examination of the relative amount of allele fixation within a species' genome. However, statistical analysis is limited because of the lack of independence in each data set due to the multiple use of each sample in pairwise comparisons. For this reason, an estimate of the mean genetic distance among individuals within each species was generated using the jackknife resampling procedure (Shao and Tu 1995). The mean genetic distance is calculated repeatedly while sequentially removing one of the samples from the data set. The jackknife estimate is the mean of all the values calculated with excluded samples; a standard error is also calculated from this collection of values. To compare among species, confidence intervals were calculated using the jackknife mean, standard error, and critical values of the t distribution (Zar 1996). Intervals were calculated at both the 95% and 99% confidence levels (the latter being more appropriate for multiple comparisons).

For Puerto Rican and Jamaican vireos, sufficient samples were obtained at two localities to analyze the partitioning of genetic variance between localities. Although this study does not describe the complete genetic structuring of the continental White-eyed Vireo, samples from the three widely separated localities were analyzed as above to understand how variance is partitioned within and among these populations for comparison with the island species. Two methods were used for these analyses. First, variance in RAPD phenotypic patterns within and among populations were analyzed using the analysis of molecular variance (AMOVA) procedure of Excoffier et al. (1992). For this analysis, a distance metric (Armstrong et al. 1995) was first used to calculate pairwise distance indices between all individuals. The Euclidean metric of Excoffier et al. (1992) was used, and can be described as the sum of all bands that are present in only one sample of a pair; this metric differs from the Jaccard's index in that it is influenced only by polymorphic sites and is not affected by monomorphic bands. The AMOVA program then uses the phenotypic distances in performing a classical analysis of variance, partitioning the sums of squared deviations into variance components in different hierarchical levels (e.g. among populations; among individuals within populations). AMOVA also provides a measure of population subdivision (Φ_{ST}), which is analogous to Wright's (1951) F_{ST} measure. The AMOVA Φ_{ST} equals 0 when two populations have identical band frequencies, and 1 when two populations are monomorphic for presence and absence, respectively, at a single RAPD locus.

AMOVA also provides a test of the null hypothesis that the variance components and the Φ_{ST} measure are not significantly different from zero. Null distributions of the variance components and of the Φ_{ST} measure are created by 1,000 random reallocations of all individuals into different populations. Rejection of the null hypothesis is warranted when the probability that the random value is larger than the observed value is below the level of significance (0.05). For populations where Φ_{ST} differs significantly from zero, an estimate of the level of gene flow (Nm = number of individuals per generation) was calculated from a modified version of Wright's (1951) equation:

$$Nm = 0.25((1/F_{ST}) - 1), \quad (2)$$

with the AMOVA analog Φ_{ST} substituted for F_{ST}.

The second analysis of the partitioning of genetic variance between localities involved the calculation of Wright's F_{ST} by assuming that each RAPD marker represents a locus with two alleles (one causing amplification of a band, the other causing the band to be absent). Because the heterozygous genotype is not detectable, allele frequencies are calculated by assuming that each RAPD locus is in Hardy-Weinberg equilibrium; thus, the frequency of the "absent" phenotype for any particular marker is equal to the square of the "absent" allele frequency. From this information, all allele and genotype frequencies at this locus are calculated. Values of F_{ST} were calculated using the adjustments of Weir and Cockerham (1984) for unequal sample sizes. It should be noted that the use of traditional population genetic statistics on RAPD data is problematic, chiefly because it is impossible to determine whether the presence of a RAPD band represents the homozygous or heterozygous genotype at that locus, thereby preventing the verification of the assumption of Hardy-Weinberg equilibrium (see Lynch and Milligan 1994). However, if it can be assumed that the markers not in Hardy-Weinberg equilibrium are equally as likely to increase average F_{ST} as to reduce it, the overall val-

TABLE 2. Analysis of band polymorphism and genetic distance based on Jaccard's index in four species of vireos. Sample sizes are totals by species.

Locality	*n*	Band polymorphism	Jaccard's index	
			$\bar{x}$	Range
	***Vireo griseus* (*n* = 26)**			
Nacogdoches, Texas	10	0.63	0.300	0.231–0.394
Tuskegee National Forest, Alabama	11	0.60	0.290	0.183–0.379
Congaree Swamp, South Carolina	5	0.49	0.277	0.299–0.304
	***Vireo latimeri* (*n* = 43)**			
Guajataca State Forest, Puerto Rico	14	0.28	0.127	0.049–0.207
Guánica State Forest, Puerto Rico	29	0.38	0.109	0.036–0.195
	***Vireo modestus* (*n* = 31)**			
Blue Mountains, Jamaica	17	0.33	0.123	0.054–0.198
Windsor, Jamaica	14	0.24	0.100	0.034–0.165
	***Viero osburni* (*n* = 19)**			
Blue Mountains, Jamaica	16	0.29	0.101	0.040–0.163
Windsor, Jamaica	3	0.17	0.119	0.099–0.147

ue calculated should be a good approximation of the central tendency for these markers.

RESULTS

Amplification of RAPD bands.—The amplification of molecular markers by the 13 primers in the RAPD analysis produced a mean of 107 acceptable fragments per species (range 95 in Puerto Rican Vireo to 115 in White-eyed Vireo; Table 1). The continental White-eyed Vireo had a greater proportion of bands in which polymorphisms were detected than any of the endemic island species; these differences were significant by a Tukey-type test of multiple proportions ($q = 4.321$ vs. Puerto Rican Vireo, $P < 0.025$; $q = 4.640$ vs. Jamaican Vireo, $P < 0.01$; $q = 4.448$ vs. Blue Mountain Vireo, $P < 0.01$). None of the comparisons of proportions between pairs of endemic island species differed significantly (all values of $q < 0.70$; all *P*-values > 0.50). The percentage of polymorphic bands found within each population are reported in Table 2; differences among these populations appear to be driven largely by differences in sample size.

Comparison of within-species genetic variation.—Jaccard indices calculated between samples revealed much greater genetic variability among individuals in the continental White-eyed Vireo than in any of the island species; this is the case even on the level of individual sample populations (Table 2). The jackknife estimate of the mean Jaccard value within White-eyed Vireos was more than twice that of any of the other species (Table 3, Fig. 3). The 95% confidence intervals for the Puerto Rican Vireo, Jamaican Vireo, and Blue Mountain Vireo all overlapped substantially and were outside the interval for the White-eyed Vireo. Because of the multiple comparisons involved, a more stringent 99% level of confidence was also used. These wider intervals also did not overlap between the White-eyed Vireo and any of the island endemics (Table 3). It should be noted that because of the lack of independence in the data, these are not rigorous statistical re-

TABLE 3. Values of Jaccard's index of genetic distance among individuals within four species of vireos. Mean, standard error, and confidence intervals estimated by jackknife procedure.

Species	$\bar{x} \pm$ SE	Range	95% CI	99% CI
Vireo griseus	0.293 ± 0.010	0.163–0.424	0.272–0.314	0.265–0.321
Vireo latimeri	0.115 ± 0.005	0.036–0.222	0.105–0.125	0.102–0.128
Vireo modestus	0.115 ± 0.005	0.075–0.182	0.105–0.125	0.101–0.129
Vireo osburni	0.106 ± 0.006	0.040–0.178	0.092–0.118	0.088–0.122

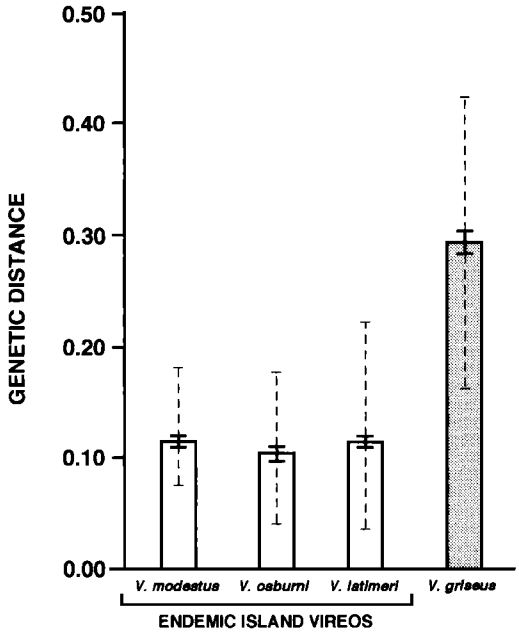

FIG. 3. Genetic distance among individuals within the three island endemic vireos (open bars) and the continental *Vireo griseus* (shaded bar) measured by Jaccard's index. Height of bars indicates means estimated by jackknife procedure. Solid brackets denote standard errors; dashed brackets indicate ranges.

sults but rather are indicative of an overall pattern.

Intraspecific genetic substructuring.—Weak but significant evidence existed for genetic subdivision among the two populations of Puerto Rican and Jamaican vireos (Table 4). The amount of differentiation within the Puerto Rican Vireo was somewhat greater than that of the Jamaican Vireo, as indicated by permutation tests. The value for the AMOVA statistic Φ_{ST} in each species was significantly different from zero, indicating low rates of migration between populations. The values of *Nm* for the Puerto Rican (2.18) and Jamaican (4.96) vireos were higher than the minimum amount of gene flow thought to prevent differentiation at neutral loci among populations by genetic drift (ca. one individual per generation; Wright 1931). Nevertheless, gene flow between sampled populations within these species appeared to be significantly restricted to some degree.

Although comparative analysis using Jaccard's index revealed that White-eyed Vireos had higher levels of within-species genetic variability, the AMOVA showed that this variation was not partitioned into the three areas from which individuals were sampled (Table 4). The value for Φ_{ST} was very low and did not differ significantly from zero; more than 98% of the variation measured was attributable to variation among individuals within populations.

Similar patterns were found in the analysis using Wright's F_{ST}. All values were higher than the Φ_{ST} calculated by AMOVA (Table 5), but the Puerto Rican Vireo and the White-eyed Vireo again had the highest and lowest values, respectively. The estimates of migration rates be-

TABLE 4. Results of analysis of molecular variance among intra-island populations of *Vireo latimeri* and *Vireo modestus* and three continental populations of *Vireo griseus*. Data are sums of squares (SS), mean squares (MS), variance components of the different hierarchies in the analysis (percent total variance in parentheses), and genetic distance (Φ_{ST}). Estimates of gene flow (N_m) are given when Φ_{ST} is significantly different from zero (NS = not significant).

Source	df	SS	MS	Variance component	Φ_{ST}	*P*	N_m
			Vireo latimeri				
Among populations	1	14.77	14.77	0.535 (10.3)	0.103	0.001	2.18
Among individuals/within populations	41	191.05	4.66	4.660 (89.7)			
			Vireo modestus				
Among populations	1	9.62	9.62	0.275 (4.8)	0.048	0.019	4.96
Among individuals/within populations	29	156.70	5.40	5.404 (95.2)			
			Vireo griseus				
Among populations	2	29.56	14.78	0.198 (1.5)	0.015	0.164	NS
Among individuals/within populations	23	302.25	13.14	13.141 (98.5)			

TABLE 5. Number of polymorphic markers used in each calculation, Wright's F_{ST} calculated from RAPD data assuming Hardy-Weinberg equilibrium for each marker, and estimated number of migrants per generation (N_m) among populations of vireos.

Species	No. of polymorphic markers	F_{ST}	N_m
Vireo latimeri	38	0.141	1.52
Vireo modestus	37	0.117	1.89
Vireo griseus	86	0.109	2.04

tween populations (Nm) were lower than two individuals per generation in the island species, reflecting the finding that gene flow between the island populations was relatively low. Both analyses of genetic substructuring (AMOVA Φ_{ST} and F_{ST}) revealed greater structure among populations in each of the island species than in the migratory White-eyed Vireo, which exhibits a high level of genetic variation among individuals with little geographic structuring.

DISCUSSION

Continental versus island species.—The comparison of within-species genetic variation among the continental White-eyed Vireo and the three island species of *Vireo* supports the hypothesis that island endemics have less genetic variation than their mainland relatives (Frankham 1997). Indeed, the three island endemics had equivalent levels of genetic variability that were significantly lower than that of the continental White-eyed Vireo. The White-eyed Vireo also had a significantly higher proportion of polymorphic bands than the three endemics, all of which were similar to each other in this regard.

The pattern of reduced genetic variation in the three island species compared with a mainland congener parallels patterns observed in a wide variety of vertebrates (Frankham 1997), including a number of birds: Chaffinch (*Fringilla coelebs*; Baker et al. 1990), Song Sparrow (*Melospiza melodia*; Hare and Shields 1992 [but see Zink and Dittmann 1993]), Hawaiian Goose (*Branta sandvicensis*; Rave et al. 1994), Micronesian Kingfisher (Haig and Ballou 1995), and Savannah Sparrow (*Passerculus sandwichensis*; Freeman-Gallant 1996). Notable exceptions are the Galapagos finches (Yang and Patton 1981), Grey-crowned Babbler (*Pomatostomus temporalis*; Edwards 1993), Lesser Antillean Saltator (*Saltator albicollis*; Seutin et al. 1993), and Guam Rail (Haig and Ballou 1995). Island populations of the Bananaquit (*Coereba flaveola*) exhibit both high and low genetic variability (Seutin et al. 1994). Exceptions such as the babbler and Bananaquit may be explained by gene flow from mainland populations or other islands, because these birds are in close proximity to other populations of conspecifics. However, this fails to explain the substantial genetic variation in Galapagos finches, Lesser Antillean Saltator, and Guam Rail, which are highly differentiated and/or genetically isolated from their mainland relatives.

Does the reduced level of genetic variation in these endemic West Indian vireos result from a historical bottleneck at the time of colonization? Theoretical and empirical analyses have indicated that such reductions in genetic variation occur only after a severe reduction in population size, after which the population remains small for many generations (Nei et al. 1975, Rave et al. 1994, Bouzat et al. 1998). Estimates of time since divergence in these species (Zwartjes 1997) suggest that millions of generations have occurred since speciation, presumably enough to reestablish substantial levels of genetic variation through mutation. The reduced level of genetic variation in these endemic vireos may be due to the fact that their population sizes are substantially smaller than that of the White-eyed Vireo. Kimura (1983) suggested that most of the genetic variation within species is driven by neutral mutations. A large population has more sources of mutation (i.e. more individuals) than a small one, and in larger populations these mutations are less likely to become fixed because of genetic drift. Some species of vireo are considered to be among the most abundant migratory passerines (Barlow 1980), and reported densities for White-eyed Vireos have ranged from 24 per 40.5 ha to two to three breeding pairs per ha, depending on habitat (Hopp et al. 1995). Thus, the effective population sizes of the Puerto Rican and Jamaican vireos, although abundant on their respective islands, are certainly a small fraction of that of continental White-eyed Vireos.

If the effective population size of the Blue Mountain Vireo is smaller than those of the other two West Indian endemics (as appears to be the case), the similar within-species level of genetic variation among the three species fails to support the hypothesis that genetic variation is related to population size across species within a taxonomic group (Frankham 1996). However, the differences in effective population size among these three endemic species may not be large enough to create detectable differences in genetic variation. A relationship between population size and genetic variation may be evident only when a species with a much greater effective population size (e.g. White-eyed Vireo) is used in the comparison. In addition, the limitations of the RAPD method (including the random nature of band amplification and the limited number of bands usable for scoring) are likely to make it difficult to detect small but significant differences among populations.

Both the Puerto Rican Vireo and Jamaican Vireo inhabit a variety of vegetation types and are distributed over different elevations; in contrast, the Blue Mountain Vireo generally is restricted to upper and mid-elevation wet forests. The similar within-species genetic variation among these three endemic species fails to support any hypotheses regarding reduction in genetic variation owing to habitat restriction or specialization, or to restricted range within an island. Predicted differences based on levels of habitat specificity may not be detectable if most of the RAPD genetic variation between species is neutral. Furthermore, because RAPDs sample the entire DNA complement of an individual (i.e. coding and noncoding regions), much of the variation between species may not be heavily influenced by selection.

Substructuring within islands.—Analyses of the partitioning of genetic variation within and among populations of Puerto Rican, Jamaican, and White-eyed vireos revealed moderate differentiation between the Puerto Rican populations and weak but detectable differentiation between the Jamaican Vireo populations. In contrast, the three continental populations of the White-eyed Vireo showed a high level of variation within populations but little variation among populations. This is consistent with the idea that a large effective population size generates a variety of mutations that are freely exchanged by a high level of gene flow throughout the species. This appears to be characteristic of North American temperate-zone bird species (Barrowclough and Johnson 1988), and the main migratory continental population of the White-eyed Vireo appears to conform to this generalization.

Comparison of among-population genetic substructuring statistics with previously published F_{ST} values in birds is complicated by the use of different types of molecular methods. Most studies of avian population substructuring have used protein electrophoresis to analyze allozyme variation (Barrowclough and Johnson 1988, Haig and Avise 1996), and interpretations of RAPD data are somewhat limited due to the dominant nature of the markers, which prevents direct assessment of heterozygosities (Lynch and Milligan 1994). The analysis of RAPD markers as phenotypes, as done in the AMOVA, allows the determination of variance structure within and among populations, which has been shown to be consistent with studies using allozymes (Haig et al. 1994, Peakall et al. 1995). In Haig et al.'s (1994) study of Red-cockaded Woodpeckers (*Picoides borealis*), F_{ST} values from allozyme analyses were roughly two-thirds the difference between the AMOVA Φ_{ST} and F_{ST} values calculated from RAPD data. Using this relationship to calculate an "allozyme F_{ST} estimator" for the data presented here, allozyme estimates of F_{ST} would be 0.128 for Puerto Rican Vireo, 0.094 for Jamaican Vireo, and 0.077 for White-eyed Vireo. The latter two estimates are relatively high for North American passerines (Barrowclough and Johnson 1988), whereas the value for the Puerto Rican Vireo would represent substantial allozyme genetic structure.

Because gene flow among animal populations is influenced by the dispersal abilities of individuals, gene flow in birds should be higher among migratory populations than among sedentary ones. Relative to species in temperate latitudes, it may be more typical for tropical birds to exhibit greater differentiation over short distances and to experience greater inhibition of dispersal by geographic barriers (Capparella 1988; Seutin et al. 1993, 1994), although genetic similarity tends to be relatively high throughout continuous habitat (Brawn et al. 1996). Puerto Rican and Jamaican vireos appear to be well characterized by both of these

descriptions. Puerto Rican Vireos are reported to have a low frequency of dispersal and short dispersal distances (Woodworth et al. 1998). Although Puerto Rican Vireos occur continuously in the forests across the western mountain range, gene flow between the two study populations may be restricted somewhat by the Central Cordillera mountain range that separates the two sites. Suitable vireo habitat on both islands also may be interrupted by fragmentation from cultivation and residential development (Downer and Sutton 1990, Woodworth 1997). In both species, estimates of gene flow were relatively low, suggesting that some differentiation by genetic drift occurs. Differences in allele frequencies can also be maintained by differential forces of selection, and recent studies suggest that strong local selection can overpower gene flow to cause evolutionary divergence among populations (Smith et al. 1997). However, because the vast majority of RAPD markers are likely to be from neutral sites in the genome, it is not known to what extent selection is contributing to the evolution of genetic differences between these populations.

Conservation implications.—This study contributes to the growing body of evidence showing that population size and genetic variation are related, and that species endemic to islands have reduced levels of genetic variation compared with their continental relatives (Frankham 1996, 1997). Nevertheless, inbreeding appears to be minimal in the Puerto Rican, Jamaican, and Blue Mountain vireos. All three of these endemic species are relatively abundant within their preferred habitats. The Blue Mountain Vireo is likely to be less numerous overall in part because of its greater habitat restriction, but it was readily captured during the course of this study in suitable habitat, indicating a sizable population.

It is difficult to determine whether low genetic variation in island species of vireos relative to a continental species, as indicated by RAPD markers, indicates an increased risk of extinction. Maintenance of genetic variation is important in providing the raw material for selection (Franklin 1980), but a causal link does not necessarily exist between genetic variability and the evolutionary status of a population (Haig and Avise 1996). Lande (1995) and Lynch et al. (1995) suggest that small effective population size ultimately will create conditions that increase the risk of extinction. Puerto Rican Vireos may be declining in Guánica Forest, probably due to brood parasitism by Shiny Cowbirds (*Molothrus bonariensis*; Faaborg et al. 1997, Woodworth 1997). It is likely that factors such as brood parasitism and habitat degradation, which can cause rapid declines in population size, may pose the greatest dangers to these birds in the near future.

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