

GENETIC DIVERSITY IN BARRIER ISLAND CAROLINA WREN POPULATIONS

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INTRODUCTION

Island endemism is well documented in isolated island groups such as the Galapagos and Hawaiian Islands and has more recently been reported from younger, less isolated areas such as the California Channel Islands (Johnson 1972, Gilbert et al. 1990). The Mississippi barrier islands of Gulf Islands National Seashore are very young, and our best knowledge of their history suggests that their separation from the mainland occurred over the last several thousand years. These islands thus provide a model system for examination of the effects of time in isolation and island size on the genetic structure of island populations. These parameters may influence speciation rates for organisms inhabiting these islands.

The Mississippi barrier islands are estimated to be 4000 to 5000 years old (Waller and Marbrough, 1976; Otvos 1979). However, because they are migrating from east to west, the eastern-most islands have been the last to separate from the mainland; Petit Bois Island was connected to Dauphin Island, Alabama as recently as 1740 (Otvos 1979). Dauphin Island itself probably became separated from the mainland during the last 1000 years and currently lies 5 km off the Alabama shore (connected by a causeway). At the other extreme, western-most Ship Island was probably separated from the mainland several thousand years ago and now lies 60 km to the west of Dauphin Island and 20 km from the Mississippi Coast.

At least one vertebrate subspecies endemic to the Mississippi barrier islands has been identified, the Burleigh's Carolina wren (*Thryothorus ludovicianus burleighi*) (Lowery 1940). These birds are weak flyers and do not typically undertake long migrations, and barrier island populations have developed plumage and song characteristics that distinguish them from mainland forms (Burleigh 1945). The purpose of this project was to examine evidence for patterns of gene flow within and among island populations and between island and mainland populations to begin to evaluate Lowery's subspecific designation. More generally, we can begin to ask whether patterns of genetic variation in island Carolina wren populations can be explained in terms of island age, size, or degree of isolation. Because the Mississippi barrier islands of Gulf Islands National Seashore represent the best example of an undisturbed coastal ecosystem remaining along the North Gulf of Mexico, this represents an unusual opportunity to examine important constraints on gene flow in a system unaltered by human activity.

We used similarity of individuals at hypervariable minisatellite loci ("DNA fingerprinting"; Jeffreys 1985a,b) as an index of genetic diversity (Lynch 1990). These loci consist of tandem repetitive units of a common core sequence. Because there is extreme variation in copy number per allele, because alleles assort in Mendelian fashion, and because there are hundreds of these loci

per individual genome, dozens of which are screened simultaneously, the technique has been widely applied in studies of genetic parentage in natural populations (e.g., Burke and Bruford 1987; Rabenold et al. 1990). The extremely high mutation rates that produce multiallelic variation in copy number of core sequence per allele (Jeffreys et al. 1988) should lead to accumulation of differences in isolated populations at a rate detectable over ecological time. Recent applications of this technique to measure differentiation among populations (Gilbert et al. 1990; Rabenold et al. 1991a) are encouraging, and measurable loss of diversity at these loci is clearly associated with degree of isolation (Gilbert et al. 1990; Wayne et al. 1991). For measuring effects of recent disturbances on natural populations, these loci may be preferable over more conventional estimates of diversity derived from allozymes that are plagued by low levels of polymorphism in some taxa, particularly birds (Barrowclough 1983; Sherwin et al. 1991). Our aim here was to apply DNA fingerprinting techniques to quantify: (1) differences in genetic variability between island and mainland Carolina wren populations (if islands are isolated from mainland populations, they should show less diversity at these loci due to genetic drift acting in small isolated populations), and (2) to determine differences among populations on Horn, East Ship, and Petit Bois Islands as a function of their size and time in isolation. The second category of questions are of interest only if differences are found the first category, suggesting isolation of islands from mainland.

METHODS

Carolina wrens were captured and individually sampled for genetic analyses on three barrier islands (East Ship Island, Horn Island, and Petit Bois Island) and two mainland areas (one immediately surrounding the Gulf Islands National Seashore property and the other surrounding Pascagoula). Dates, locations and numbers of individuals sampled are summarized in Table 1.

DNA techniques: Multilocus DNA fingerprinting uses standard DNA technology to reveal a class of hypervariable loci common to all vertebrates examined (Jeffreys et al. 1985a, 1985b) to determine the genotypes of individual animals. Allelic variation among these loci is determined primarily by the number of tandem repetitive units of a core sequence found at each site. The methods involve using a restriction endonuclease (an enzyme) to cut DNA at specific base sequences along the DNA molecule. The DNA fragments produced by this enzymatic digestion are then separated by size along an electrical gradient in an agarose gel. The resulting smear of fragments is transferred out of the gel onto a stable nylon membrane, to which it is then permanently bonded. Those fragments among the total that carry a particular nucleotide sequence are identified via hybridization with a radioactively labeled "probe" molecule carrying the complementary nucleotide sequence (in the case

of fingerprinting, the core sequence of the tandem repetitive units). The positions at which the probe attaches are recorded by exposing X-ray film to the hybridized membrane. The resulting autoradiograph shows dozens of bands that correspond to the many different fragment sizes bearing the core sequence. This is the "fingerprint". The size (length) of the labelled fragments can be estimated by comparing their position within the smear to that of fragments of known size. Specific conditions for our protocol are given below:

Small blood samples (50 - 100 μ l) were collected from the brachial vein for each bird. Samples (or 50- μ l subsamples) were stored in 0.5 ml phosphate-buffered saline preservative.

DNA extraction: Blood samples were incubated overnight at 55C after adding SDS to 0.8% and 200 μ proteinase K. After incubation, samples were subjected to 2 phenol, 2 phenol:chloroform:isoamyl alcohol, and 1 chloroform:isoamyl alcohol extractions, then dialyzed against TNE₂.

Digestion and electrophoresis: Samples of each individual's genomic DNA (5 μ g) were digested with excess restriction endonuclease at 37 C. Resulting fragments were separated through a 0.8% agarose gel (22 cm) in TBE at 20 V for 65 h until all fragments smaller than 1,600 base pairs were run off, and then transferred to nylon by Southern blot in 10 X SSC buffer.

Hybridization: Jeffreys' multilocus probes 33.15 and 33.6 were radiolabelled by primer extension with [³²P]dCTP. Hybridizations were run overnight at 62 C in 1.5 X SSC, 0.1 % SDS, 5 X Denhardt's solution, and 6% w/v polyethylene glycol. Hybridized filters were washed 4 X 30 minutes at 62 C in 1.5 X SSC, 0.1% SDS, and exposed to X-ray film at -20C.

Scoring autoradiographs: The proportions of fragments visible on autoradiographs that were shared among dyads of individuals within and between populations or islands was calculated as an index of similarity (Lynch 1990). A fragment was judged to match one found in the comparison lane if the two bands were of identical mobility (indicating similar molecular weight) and intensity. When a faint fragment in one lane was matched by either a blurry "possible fragment" or an area of high background that might have obscured the presence of a band, it was eliminated from the analysis to avoid bias toward low band sharing due to artificially increasing the number of novel bands per lane (Waser and Rabenold 1990). The proportion of bands shared was calculated as $S = 2A/(2A+B+C)$, where A is the number of bands shared between two individuals, B is the number of bands unique to the first individual and C is the number of bands unique to the second (Wetton et al. 1987).

RESULTS

A total of seventy two Carolina wrens were captured on East Ship Island (11), Petit Bois Island (6), Horn Island (23), the mainland Park area (26) and the mainland Pascagoula area (8) by Ted Simons, Duane Baughman and park personnel. Table 1 summarizes numbers of birds captured at each site and their ages and sexes, when known.

TABLE 1: Captures of Carolina wrens on Mississippi barrier islands and adjacent mainland populations. Each individual reported was banded with USFWS aluminum bands and a small blood sample collected (see methods).

LOCATION	DATE	BAND #	AGE/SEX
1. EAST SHIP ISLAND	6/28/91	1481-11202	
	6/27/91	1381-15229	
	7/25/91	1381-15250	AHY-M
	7/25/91	1381-15251	AHY-M
	4/ /91	1481-11201	AHY-M
	4/2/91	1441-06135	AHY
	4/2/91	1421-17379	AHY
		74019	AHY-M
	6/12/91	11365	AHY-M
	6/11/91	15218	AHY-M
	4/16/91	1481-11235	
2. BELLE FOUNTAIN (MAIN)	7/23/91	1381-15241	AHY-M
	7/23/91	1381-15242	AHY-M
	7/23/91	1381-15243	HY-U
	7/23/91	1381-15244	AHY-F
	7/23/91	1381-15245	AHY-M
	7/23/91	1381-15246	AHY-M
	7/23/91	1381-15247	HY-U
GULF ISL. NP (MAIN)	6/12/91	1381-15103	AHY-M
	6/12/91	1381-15104	AHY-M
	6/12/91	1381-15105	AHY-M
	6/13/91	1381-15106	AHY-M
	6/13/91	1381-15107	AHY-F
	6/13/91	1381-15108	AHY-M
	6/13/91	1381-15109	AHY-M
	6/13/91	1381-15110	HY-U
	6/14/91	1381-15111	AHY-M
	6/14/91	1381-15112	AHY-U
	6/14/91	1381-15113	AHY-M
	6/14/91	1381-15114	AHY-M
	6/14/91	1381-15115	HY-U
	6/14/91	1381-15116	AHY-F
	6/15/91	1381-15219	HY-U
	6/15/91	1381-15220	HY-U
	6/15/91	1381-15222	AHY-U

	6/7/91	1381-15102	AHY-M
3. GAUTIER (MAINLAND)	7/18/91	1381-15234	HY-U
(continued)			
4. PASCAGOULA	7/18/91	1381-15235	AHY-F
	7/18/91	1381-15236	AHY-M
	7/18/91	1381-15237	AHY-F
	7/18/91	1381-15238	HY-U
	7/18/91	1381-15239	AHY-F
	7/18/91	1381-15240	AHY-M
5. PETIT BOIS ISLAND	7/2/91	1381-15232	
	7/1/91	1381-15231	
	7/1/91	1381-15230	
	7/2/91	1381-15233	
	7/24/91	1381-15248	AHY-M
	7/24/91	1381-15249	AHY-F
6. HORN ISLAND	5/28/91	1381-15201	
	5/29/91	1381-15202	AHY
	5/30/91	1381-15203	AHY
	5/30/91	1381-15204	HY-U
	5/30/91	1381-15205	HY-U
	5/30/91	1381-15206	AHY
	5/30/91	1381-15207	HY
	5/31/91	1381-15101	AHY
	5/31/91	1381-15208	HY
	5/31/91	1381-15209	HY
	6/2/91	1381-15210	AHY
	6/2/91	1381-15211	HY
	6/2/91	1381-15212	HY
	6/2/91	1381-15213	HY
	6/2/91	1381-15214	HY
	6/7/91	1381-15215	AHY-M
	6/8/91	1381-15216	AHY-M
	6/20/91	1381-15223	AHY-M
	6/20/91	1381-15224	HY
	6/20/91	1381-15225	AHY-M
	6/22/91	1381-15226	AHY-M
	6/22/91	1381-15227	AHY-M
	6/23/91	1381-15228	AHY-M

High molecular weight DNA was extracted from samples at Purdue University, yielding > 300 µg DNA/sample. Five electrophoretic gels containing 36 individuals each were run using genomic DNA from all individuals from all populations digested with the restriction endonuclease *Hae*III. Each gel was Southern transferred to nylon and probed sequentially with Jeffreys' multilocus minisatellite probes 33.6 and 33.15. Resulting autoradiographs were of excellent quality, yielding an average of 21.4 +/- 2.5 scorable fragments in *Hae*III/33.6 gels and 27.9 +/- 4.8 scorable fragments in *Hae*III/33.15 gels. All autoradiographs were scored by both Duane Baughman and Patricia Rabenold and genetic similarity among dyadic comparisons of individuals was calculated as the mean proportion of fragments shared across both scorers across both probe/enzyme combinations.

Gels were constructed so as to equalize numbers of comparisons within and between populations as much as possible, given that comparisons can only be made among individuals run on the same gel, and that scoring accuracy declines with number of intervening lanes even on within-gel comparisons (Piper and Rabenold, in review). To maintain accuracy, we scored only adjacent lanes and lanes separated by one intervening lane. This resulted in 61 within-population dyads scored for the 5 populations (mean per population = 12.2 ± 3.8) and 129 between-population dyads scored for 9 between-population comparisons (mean = 14.3 ± 2.1) (Table 2).

Table 2: Similarity indices within and between islands and mainland populations of Carolina wrens. D = mean proportion of bands shared among dyads, with standard deviation () and sample size (n).

	Islands			Mainland Areas	
	East Ship	Horn	Petit Bois	Park Area	Pascagoula
East Ship	0.19 (0.06) (n=16)	0.13 (0.06) (n=15)	0.19 (0.06) (n=17)	0.17 (0.06) (n=13)	0.09 (0.04) (n=12)
Horn		0.20 (0.06) (n=13)	0.21 (0.07) (n=15)	0.15 (0.08) (n=11)	not available
Petit Bois			0.21 (0.09) (n=9)	0.18 (0.04) (n=14)	0.10 (0.04) (n=15)
Park Area				0.16 (0.06) (n=15)	0.11 (0.05) (n=17)
Pascagoula					0.12 (0.05) (n=8)

Mean within-island similarity across all 3 islands is 0.20 (mean proportions of bands shared by random draws of individuals from within the 3 island populations). Mean within-population similarity in mainland populations is 0.14, suggesting slightly higher diversity within mainland populations. Mean among-island similarity is 0.18, while mean among-mainland population similarity is 0.11. This suggests that individuals in both areas (islands or mainland) are more similar within populations than between. Mean between-population similarity comparing individuals from islands with those from mainland populations is 0.14.

DISCUSSION

There is only the slightest evidence, using these genetic markers, for isolation effects in barrier island populations of Carolina wrens. While island populations show slight elevation in within-population similarity values compared to mainland within-population similarity (0.20 within islands vs. 0.14 within mainland populations), these differences are not great, and between-island values (mean 0.18) suggest mixing among island populations occurs more frequently than exchange between islands and mainland. Were island populations truly isolated, one would expect higher within-population values (here only 0.20) as diversity would be lost through drift, and significantly lower between-island values (here 0.18) as populations diverge in isolation. Island populations may interact more frequently with each other than with mainland populations (0.18 between-island vs 0.14 mainland-island), but this difference has yet to be subjected to statistical testing and is slight compared to findings from other island systems using the same approach.

Two recent studies of island endemics suggest that hypervariable minisatellite markers are excellent tools with which to approach the study of genetic differentiation of island forms. Channel Island foxes are endemic to the California Channel Islands. Foxes sampled from six islands (Gilbert et al. 1990) showed high levels of similarity within islands, with band-sharing proportions ranging from 0.75 to 1.0 (all individuals identical). Furthermore, the degree of similarity found within islands correlated well with the age of islands and their separation from the mainland and from each other, with the longest-isolated populations showing the greatest monomorphism. Between-island comparisons showed remarkable differences in light of the low levels of polymorphism found within islands; the 15 between-island comparisons made by dyadic comparisons of each of the 6 island populations produced similarity values ranging from 0.17 to 0.44.

Similar recent studies of endemic populations of Galapagos hawks *Buteo galapagoensis* (Rabenold and Faaborg, unpublished data) revealed band-sharing proportions of 0.82 and 0.90 within two island populations, with higher levels of between-island similarity (0.80) than found in the island foxes, perhaps owing to the greater mobility of the hawks. These values estimate the genetic similarity of presumably unrelated individuals at these loci, and are very high compared to values found from mainland populations of different bird species (range 0.23 - 0.40; Rabenold 1991b).

To our knowledge, only one recent study has used minisatellite loci to examine genetic variability in an island population for a species that also existed in widespread mainland populations. The gray wolves of Isle Royale had band-sharing similarity values of 0.72 (Wayne et al. 1991), indicating loss of variation compared to

those found in mainland populations (0.32), but not very different from wolves sampled from 2 captive breeding programs (0.68).

Our results suggest that Mississippi barrier island populations of Carolina wrens are therefore not significantly isolated from mainland populations. However, we emphasize that these are the most preliminary data, still to be subjected to statistical testing. The fox and wolf data presented earlier were not tested statistically, perhaps because the differences were so great that it was judged unnecessary. The very slight differences uncovered in the Mississippi barrier island Carolina wren populations require a statistical approach. Our ongoing analysis will include not only the development of appropriate tests for the results presented here, but their correlation with other genetic tests more readily interpretable and for which test statistics are well developed.

Specifically, we will use the samples already collected to examine patterns in mitochondrial DNA lineages within and among populations. Mitochondrial DNA is maternally inherited and thus provides information regarding matrilineal relationships among populations of birds. Application of this technique is especially appropriate in population genetic studies of birds, in which sex-biased dispersal makes females the primary agents of gene flow (Greenwood and Harvey 1982). Mitochondrial variation is more conservative than that of minisatellite loci, but greater than that of nuclear DNA in general (Quinn and White 1987). Application of mitochondrial DNA will allow us to calibrate the results presented here against a more conservative marker.

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